R_f 0.48; UV (EtOH) 280, 287, 273 nm; ^{1}H NMR ($CDCl_3$) δ 0.80 (s, 3 H, 18-CH_3), 0.91–2.36 (br m, 31 H, chain and steroid), 2.70–2.92 (m, 2 H, 6-CH_2), 3.74 (t, 1 H, J = 8.3 Hz, 17-H), 4.05–4.23 (m, 3 H, CH=CH_2), 6.63 (d, 1 H, J = 2.7 Hz, 4-H), 6.73 (dd, 1 H, J = 8.5, 2.7 Hz, 2-H), 7.18 (d, 1 H, J = 8.6 Hz, 1-H); MS m/z 424 (100, M^+), 271 (1), 253 (2), 171 (9), 157 (26), 145 (16). Anal. (HRMS) Calcd for $C_{29}H_{44}O_2$ 424.3341, found 424.3348. Anal. C, H.

Estrogen Receptor Binding Assay. The relative binding affinity (RBA) of the estradiol derivatives for estrogen receptors was determined by the displacement of 17β-[^{3}H]estradiol. Competitive binding assays were done as previously described,[7,13,15] with some minor modifications. Calf uterine cytosol was prepared by centrifugation (105000g, 60 min, 4 °C) of homogenates in phosphate buffer (5 mM sodium phosphate, pH 7.5, 10 mM monotioglycerol, 10% glycerol) and diluted with phosphate buffer to 0.85 mg of protein/mL (0.68 mg of protein in the final incubation mixture). Cytosols were incubated with a constant concentration (10^{-9} M) of 17β-[^{3}H]estradiol (Amersham, 140 Ci/mmol) and nine different concentrations (3×10^{-12}–10^{-6} M) of test compounds at 4 °C for 2, 12, and 20 h in the absence of DMF, and 20 h with the addition of 7% DMF. Each concentration was performed in triplicate. Incubations were stopped by adding 500 μL of a dextran-coated-charcoal slurry (0.25% charcoal Norit A, 0.025% dextran, in 0.01 M tris(hydroxymethyl)aminomethane (pH 8.0), and the mixture was agitated at 4 °C for 30 min. After centrifugation, the radioactivity of a 500-μL supernatant aliquot was counted. The RBA value of a competitor was established by using the ratio of unlabeled 17β-estradiol concentration required for 50% receptor displacement of the corresponding 17β-[^{3}H]estradiol and the competitor concentration required for the same effect, multiplied by 100. The RBA reported are the average values of at least three experiments.

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Registry No. 1, 434-22-0; **2**, 55592-09-1; **3**, 123266-46-6; 4, 105089-28-9; **5**, 123266-47-7; **6**, 105075-33-0; **7**, 123266-48-8; **8**, 123266-49-9; **9**, 123266-50-2; phenol, 108-95-2; *p*-iodophenol, 540-38-5.

Antifolate and Antibacterial Activities of 5-Substituted 2,4-Diaminoquinazolines

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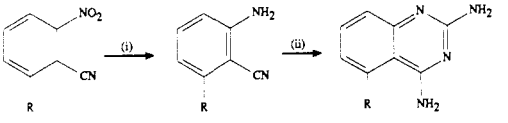
A series of 5-substituted 2,4-diaminoquinazolines (**3**) has been synthesized and evaluated as inhibitors of the enzyme dihydrofolate reductase (DHFR) from both bacterial and mammalian sources. The best compounds (e.g. **53**) show good activity against *Escherichia coli* DHFR, but there is no significant selectivity for the bacterial over the mammalian enzyme. The structure–activity relationships for enzyme inhibition appear to be complex and not amenable to simple analysis; a hypothesis to explain the observed qualitative structure–activity relationships is proposed. The inhibitory activities of the compounds against the growth of intact bacterial cells in vitro closely parallel those for the inhibition of the isolated bacterial enzymes, suggesting that their antifolate action is responsible for their antibacterial effects. Five of the compounds were tested for their ability to cure a systemic *E. coli* infection in the mouse, but they showed no therapeutic effects at their maximum tolerated doses.

Inhibitors of the enzyme dihydrofolate reductase (DHFR; tetrahydrofolate:NADP$^+$ oxidoreductase, EC 1.5.1.3) constitute an important class of therapeutic agents which have found application in anticancer (e.g. methotrexate),[1] antibacterial (e.g. trimethoprim),[2] and antimalarial (e.g. pyrimethamine)[3] chemotherapy. Since the discovery of the mode of action of methotrexate, DHFR inhibitors have been studied intensively and exhaustively. Interest in the development of new molecules of this class remains high and has been further stimulated by the recent publication of the X-ray crystallographic coordinates of various enzyme–inhibitor complexes.[4] Effective inhibitors of DHFR are limited to those compounds having the 2,4-diamino-1,3-diaza pharmacophore,[5] as exemplified by the 2,4-diaminopteridines (especially as anticancer agents), the 2,4-diaminopyrimidines (antibacterial, anticancer, and antimalarial), and the 2,4-diaminotriazines (anticancer and antimalarial). There are reviews available on all aspects of DHFR and its inhibitors.[5,6]

2,4-Diaminoquinazoline-based inhibitors of DHFR have also shown some antifolate properties that might make them useful in the treatment of malaria[7] and some cancers.[8] However, despite some promising early reports in the literature,[9] little attention has been paid to their antibacterial properties. Therefore, we decided to assess their potential as antibacterial agents. In order to do this a number of 2,4-diaminoquinazolines bearing carefully se-

Scheme I[a]



1 2 3

[a] (i) reduction, (ii) chloroformamidine hydrochloride/diglyme.

† University of Essex.

(1) Bleyer, W. A. *Cancer Treat. Rev.* **1978**, *41*, 36–51.
(2) Finland, M., Kass, E. H., Eds.; *J. Infect. Dis.* **1973**, *128*, S425–816.
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(4) (a) Matthews, D. A.; Bolin, J. T.; Burridge, J. M.; Filman, D. J.; Volz, K. W.; Kaufman, B. T.; Beddell, C. R.; Champness, J. M; Stammers, D. K.; Kraut, J. *J. Biol. Chem.* **1985**, *260*, 381–91. (b) Lai, P. H.; Pan, Y.-C. E.; Gleisner, J. M.; Peterson, D. L.; Williams, K. R.; Blakely, R. L. *Biochemistry* **1982**, *21*, 3284–3294. (c) Volz, K. W.; Matthews, D. A.; Alden, R. A.; Freer, S. T.; Hansch, C.; Kaufman, B. T.; Kraut, J. *J. Biol. Chem.* **1982**, *257*, 2528–36.
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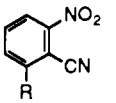
Table I. Preparation of 6-Substituted 2-Nitrobenzonitriles (1)



	R	method	% yield	mp, °C	recryst[a] solvent	anal.	ref
7	NO_2	*b*	82	145–147	*c*	C, H, N	12
8	CH_2CH_3	*b*	37	88–89	EtOH	C, H, N	
9	CO_2CH_3	*b*	61	140–141	CELL	C, H, N	13
10	Br	*b*	61	143	EtOH-H_2O	C, H, N, Br	
11	I	*b*	41	183–185	*c*	C, H, N, I	
12	OCH_3	A	49	173–174	EtOH	C, H, N	11
13	OCH_2CH_3	A	62	135–136	EtOH-H_2O	C, H, N	
14	$OCH(CH_3)_2$	*b*	48	100–102	CHEX	H, N; C[d]	
15	$OCH_2CH_2CH_3$	A	72	109–110	EtOH-H_2O	C, H, N	
16	$O(CH_2)_3CH_3$	A	66	103	EtOH-H_2O	C, H, N	
17	$O(CH_2)_5CH_3$	A	78	78	CHEX	C, H, N	
18	$OCH_2C_6H_5$	A	53	142–143	EtOH	C, H, N	
19	OC_6H_5	*b*	50	106–107	CCl_4	H, N; C[e]	
20	SCH_3	*b*	81	106–107	CELL-H_2O	C, H, N, S	13
21	SCH_2CH_3	*b*	87	125–126	EtOH	C, H, N, S	
22	$SCH_2CH_2CH_3$	*b*	55	95	EtOH	C, H, N, S	
23	SC_6H_5	*b*	59	107–108	CHEX	H, N; C[f]	
24	$N(CH_3)_2$	*b*	47	115–117	CHEX	C, H, N	13
25	$N(CH_2CH_2)_2O$	*b*	43	150–154	EtOH	C, H, N	13
26	$N(CH_2CH_2)_2CH_2$	*b*	56	117	EtOH	C, H, N	13

[a] CHEX = cyclohexane; CELL = 2-ethoxyethanol. [b] See the Experimental Section for details. [c] Purified by preparative column chromatography. [d] C: calcd, 58.3; found, 57.8. [e] C: calcd, 65.0; found, 64.4. [f] C: calcd, 60.9; found, 61.4.

lected substituents were synthesized and screened against isolated DHFR enzyme in vitro. The activities of the compounds against enzymes from both bacterial and mammalian sources were measured to determine both potential antibacterial efficacy and host toxicity. The compounds were also tested in vitro and, in a limited number of cases, for their ability to cure experimental bacterial infections in vivo. The purpose of this was to attempt to ascertain whether a useful drug can be identified solely on the basis of receptor binding studies, or whether other factors are dominant in determining activity in vivo. Recently Hansch's group have undertaken a similar program using anticancer diaminotriazine-based antifolates.[10]

A great many diaminoquinazolines have been tested as DHFR inhibitors (Hansch's compilation lists 198).[5] Previous workers in this field have tended to concentrate on the 6-substituted analogues because of their structural resemblance to dihydrofolic acid, the natural substrate of DHFR; usually substituents in the 5-position have been restricted to methyl or chloro groups.[5] As a result of this work some general structure–activity relationships are apparent. For neither series of compounds, however, has a set of analogues been specifically designed for the purpose of structure–activity analysis. In our work the primary criterion governing the choice of compounds to be prepared was their suitability for attempting such analyses. In this paper we describe the results of our studies on the antifolate properties of 5-substituted 2,4-diaminoquinazolines (**3**); the results on the 6-substituted series will be reported in a subsequent paper.

Chemistry

The general route used to prepare 5-substituted 2,4-diaminoquinazolines **3** is outlined in Scheme I. 2-Nitrobenzonitriles substituted at the 6-position by alkoxy, alkylthio, and dialkylamino groups (1, R = OR^1, SR^1, NR^1_2) were prepared from the selective displacement[11] by the appropriate nucleophile of one of the nitro groups in 2,6-dinitrobenzonitrile (**7**) (see 1, R = NO_2, Scheme I). Compound **7**, prepared by the method of Beck,[12] may also be partially reduced to 2-amino-6-nitrobenzonitrile (**27**),[13] and

(6) (a) Roth, B.; Bliss, E.; Beddell, C. R. *Moleular Aspects of Anticancer Drug Action*; Macmillan: New York, 1983; pp 363–393. (b) Hitchings, J. H.; Burchall, J. J. *Adv. Enzymol.* **1965**, *27*, 417–68. (c) Blakely, R. L. *The Biochemistry of Folic Acid and Related Pteridines*; North-Holland: Amsterdam, 1969; pp 219–223. (d) Beddell, C. R. *X-ray Crystallography and Drug Action*; Oxford Press: London, 1984; pp 169–93. (e) Roth, B.; Cheng, C. C. *Prog. Med. Chem.* **1982**, *19*, 270–331. (f) McCormack, J. J. *Med. Res. Rev.* **1981**, *1*, 303–31. (g) Roberts, G. C. K. *Chemistry and Biology of Pteridines*; Walter de Gruyter: Berlin, 1983; pp 197–214. (h) Hitchings, G. H.; Smith, S. L. *Adv. Enzyme Regul.* **1980**, *18*, 349–71. (i) Gready, J. E. *Adv. Pharmacol. Chemother.* **1980**, *17*, 37–102. (j) Blakely, R. L. *Molecular Actions and Targets for Cancer Chemotherapeutic Agents*; Academic Press: New York, 1980; pp 303–32. (k) Roberts, G. C. K. *Drug Action at the Molecular Level*; University Park Press: Baltimore, 1977; pp 127–50. (l) Albert, A. *Selective Toxicity*, 7th ed.; Chapman and Hall: London, 1985; pp 347–55. (m) Hitchings, G. H. *Inhibition of Folate Metabolism in Chemotherapy*; Springer-Verlag: Berlin, 1983.

(7) (a) Elslager, E. F.; Jacob, P.; Johnson, J.; Werbel, L. M.; Worth, D. F. *J. Med. Chem.* **1978**, *21*, 1059–70. (b) Elslager, E. F.; Hutt, M. P.; Jacob, P.; Johnson, J.; Temporelli, B.; Werbel, L. M.; Worth, D. F.; Rane, L. *J. Med. Chem.* **1979**, *22*, 1247–57. (c) Elslager, E. F.; Clarke, J.; Johnson, J.; Werbel, L. M.; Davoll, J. *J. Heterocycl. Chem.* **1972**, *9*, 759–73.

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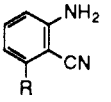
(12) Beck, J. R. *J. Org. Chem.* **1972**, *37*, 3224–6.

(13) Klaubert, D. H.; Sellstedt, J. H.; Guinosso, C. J.; Capetola, R. J.; Bell, S. C. *J. Med. Chem.* **1981**, *24*, 742–8.

Table II. Preparation of 6-Substituted 2-Aminobenzonitriles (2)



no.	R	method	% yield	mp, °C	recryst[a] solvent	anal.	ref
27	NO_2	*b*	71	190–191	EtOH–EA	C, H, N	13
28	CH_2CH_3	D	71	96–98	CHEX	C, H, N	
29	CO_2CH_3	D	64	113–114.5	EtOH–H_2O	C, H, N	13
30	OCH_3	D	60	139–141	BENZ	C, H, N	33
31	OCH_2CH_3	D	62	94–96	BENZ–CHEX	C, H, N	
32	$OCH(CH_3)_2$	D	65	156–157	*c*	C, H, N, Cl	
33	$OCH_2CH_2CH_3$	D	92	154–157	*c*	C, H, N; Cl[d]	
34	$O(CH_2)_3CH_3$	C	81	54–58	PET	C, H, N	
35	$O(CH_2)_5CH_3$	D	63	69–70	CHEX–PET	C, H, N	
36	$OCH_2C_6H_5$	B	74	99–100	EtOH	C, H, N	
37	OC_6H_5	C	37	65	CCl_4–CHEX	C, H, N	
38	SCH_3	B	54	81–82	BENZ–PET	C, H, N, S	13
39	SCH_2CH_3	C	63	88	CHEX–TOL	C, H, N, S	
40	$SCH_2CH_2CH_3$	C	46	66–67	CHEX	C, H, N, S	
41	SC_6H_5	B	76	75–78	CHEX	H, N, S; C[e]	
42	$N(CH_3)_2$	C	56		*f*		
43	$N(CH_2CH_2)_2O$	*b*	65	143–153	BENZ	H, N; C[g]	13
44	$N(CH_2CH_2)_2CH_2$	*b*	35		*h*		
45	CH_3	D	69	127–129	EtOH	C, H, N	33
46	Br	E	78	149–150		C, H, N, Br	13
47	I	E	90	136–137		C, H, N, I	
48	$C{\equiv}CC_6H_5$	*b*	88	107	CHEX	H, N; C[i]	
49	$CH_2CH_2C_6H_5$	*b*	93	75–76	CHEX–PET	C, H, N	

[a] BENZ = benzene; CHEX = cyclohexane; PET = petroleum ether, bp 60–80°; TOL = toluene; EA = ethyl acetate. [b] See the Experimental Section for details. [c] Purified by flash chromatography (chloroform), then converted to the hydrochloride salt by treating a solution in methanol with ethereal HCl. [d] Cl: calcd, 15.3; found, 15.9. [e] C: calcd, 69.0; found, 68.5. [f] Purified by column chromatography (0–5% ethanol in chloroform) and used in the next stage without characterization. [g] C: calcd, 65.0; found, 64.4. [h] Not characterized; used in the next stage without purification. [i] C: calcd, 82.6; found, 81.8.

this intermediate was used to prepare the halides (**10** and **11**) by Sandmeyer reactions. A Sandmeyer reaction was also used to prepare compound **8** from 2-ethyl-6-nitroaniline (**4**).[14] Methyl 2-bromo-3-nitrobenzoate (**6**, prepared by the esterification of the acid (**5**)[15]) was treated with copper(I) cyanide in 1-methyl-2-pyrrolidone[16] to give compound **9**. The key step in the preparation of the 2-phenylethyl derivative (**49**) was the Heck reaction[17] of the iodide (**47**) with phenylacetylene; the resulting acetylene (**48**) was hydrogenated to compound **49**. The properties and methods of preparation of the 6-substituted 2-nitrobenzonitriles (**1**) are summarized in Table I.

Apart from compound **49** all 6-substituted 2-aminobenzonitriles **2** were obtained by the reduction of the corresponding nitro compound **1** (Scheme I). Three methods of reduction were used: (1) tin(II) chloride or tin(0) in acid,[7a] (2) transfer hydrogenation from cyclohexene catalyzed by palladium on charcoal,[13] and (3) iron powder in acid[13] (Table II).

Cyclization of 6-substituted 2-aminobenzonitriles (**2**) to 5-substituted 2,4-diaminoquinazolines (**3**) was achieved by heating with chloroformamidine hydrochloride in diglyme (Scheme I).[7a,c] Most reactions were performed at 130–140 °C. Temperature control was not usually critical for this reaction, and temperatures up to 160 °C were acceptable in many cases. For compounds **53** and **59**, however, it was important that the temperature be maintained at or below 130 °C during the reaction, higher temperatures leading to dark, tarry impurities (possibly arising from acid-catalyzed dealkylation). The 5-nitro analogue (**50**) was reduced to the amine (**72**) by catalytic hydrogenation in an acidic medium (under neutral conditions only dark tars were isolated). Catalytic hydrogenation was also used to prepare the hydroxy analogue (**73**) by the reductive dealkylation of the benzyl ether (**59**). Three of the four quinazolines substituted with a thioether group were oxidized to the corresponding sulfone by potassium permanganate in aqueous acetic acid. Two amides (**77**, **78**) were obtained from the ester (**52**) by treatment with the appropriate amine. The methods of preparation and physical properties of the 5-substituted 2,4-diaminoquinazolines (**3**) are summarized in Table III.

Biology and Results

Thirty-one 5-substituted 2,4-diaminoquinazolines (**3**) were tested for their ability to inhibit the action of DHFR from three sources: (1) *Staphylococcus aureus* (a representative Gram-positive bacterium), (2) *E. coli* (a representative Gram-negative bacterium), and (3) bovine liver cells (a representative mammalian system); the results are summarized in Table IV. There is a wide range (≥1000-fold) in anti-DHFR activity with this class of inhibitor, although the best analogues are less active against the bacterial enzymes than trimethoprim. All but two of these compounds were also tested for their ability to prevent the growth of intact *E. coli* and *S. aureus* cells in vitro (Table IV). A comparison of these results with those for the inhibition of the activity of the isolated enzymes shows that there is a good correlation between them; that is, the greater the inhibition of the action of isolated DHFR, the greater the ability to prevent the growth of intact bacterial cells in vitro. Again, however, the best analogues are less active than trimethoprim. Five of the compounds (**53**, **54**, **68**, **80**, **81**) most active in these two primary screens were also assessed for their ability to protect mice against an experimental systemic *E. coli* infection. Each compound was administered as a single dose at its maximum tolerated dose, 100–200 mg kg^{-1} orally (po) or 10–25 mg kg^{-1} by

(14) Leidholm, B. *Acta Chem. Scand.* **1976**, *B30*, 141–9.
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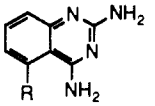
(16) Freidman, L.; Schehter, H. *J. Org. Chem.* **1961**, *26*, 2522–4.
(17) Heck, R. F. *Org. React.* **1982**, *27*, 345–90.

Table III. Preparation of 5-Substituted 2,4-Diaminoquinazolines (3)



no.	R	method	% yield	mp, °C	recryst[a] solvent	anal.	ref
50	NO_2	F	13	237–239	EtOH	C, H, N	34
51	CH_2CH_3	F	60	162–163	H_2O	C, H, N	-
52	CO_2CH_3	F	47	>300	EtOH-H_2O[b]	C, H, N, Cl	-
53	OCH_3	F	28	255–257	EtOH[b]	C, H, N, Cl	9b
54	OCH_2CH_3	F	48	255–258	EtOH-CELL	C, H, N	-
55	$OCH(CH_3)_2$	F	67	185–186	EtOH-H_2O	C, H, N	-
56	$OCH_2CH_2CH_3$	F	54	222–223	EtOH	C, H, N	-
57	$O(CH_2)_3CH_3$	F	22	187–190	EtOH	C, H, N	-
58	$O(CH_2)_5CH_3$	F	53	155–156	EtOH	C, H, N	-
59	$OCH_2C_6H_5$	F	29	207–208	EtOH	C, H, N	-
60	OC_6H_5	F	50	244–245	EtOH	H, N; C[c]	-
61	SCH_3	F	38	170–171	CELL	C, H, N, S	-
62	SCH_2CH_3	F	54	159	EtOH-H_2O	C, H, N, S	-
63	$SCH_2CH_2CH_3$	F	54	126	MEK	C, H, N, S	-
64	SC_6H_5	F	40	241–243	CELL-H_2O	C, H, N, S	-
65	$N(CH_3)_2$	F	17	282–284	*d*		-
66	$N(CH_2CH_2)_2O$	F	48	269–271	H_2O-EtOH	C, H, N	-
67	$N(CH_2CH_2)_2CH_2$	F	47	248–249	EtOH	C, H, N	-
68	CH_3	F	60	210–211	H_2O	C, H, N	33
69	Br	F	55	197–198	EtOH-H_2O	C, H, N; Br[e]	-
70	I	F	46	192–193	CELL-H_2O	C, H, N, I	-
71	$CH_2CH_2C_6H_5$	F	27	dec >282	EtOH[b]	C, H, N, Cl	-
72	NH_2	*d*	87	dec >292	*f*	C, H, N, Cl	34
73	OH	*d*	71	>300	*f*	C, H, N, Cl	-
74	SO_2CH_3	G	34	252–254	*f*	C, H, N, S	-
75	$SO_2CH_2CH_3$	G	37	203	*f*	C, H, N, S	-
76	$SO_2C_6H_5$	G	60	290–291	*f*	C, H, N, S	-
77	$CONH_2$	*d*	38	274–276	DMF-H_2O	C, H, N	-
78	$CONHCH_2CH_2CH_3$	*d*	37	257–259	DMF-H_2O	C, H, N	-
79	H	*d*	46	255–257	*f*	C, H, N	35
80	F	F	35	247–249	H_2O	C, H, N, F	-
81	Cl	F	40	184–187	H_2O	C, H, N, Cl	35

[a] CELL = 2-ethoxyethanol; MEK = 2-butanone; DMF = dimethylformamide. [b] Containing sufficient concentrated HCl to make the crystallization medium strongly acidic. [c] C: calcd, 66.7; found, 66.2. [d] See the Experimental Section for details. [e] Br: calcd, 33.4; found, 32.7. [f] Not recrystallized.

intraperitoneal injection (ip). All of the compounds tested provided some degree of protection for 24 h (by which time all of the infected controls had died), but there were no survivors after 7 days. Trimethoprim, in contrast, afforded complete protection for 7 days with a single dose of 500 mg kg^{-1}. Higher doses of the quinazolines invariably proved toxic. In order to assess this problem, the acute toxicity to mice of a small number of the compounds was determined by an LD_{50} test (Table V).

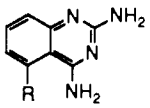
Discussion

Previous attempts have been made to generate quantitative structure–activity relationships for the inhibition of DHFR by 5-substituted 2,4-diaminoquinazolines (see below for a critical discussion). The data available in the literature, on which these studies have drawn, is limited to only a very restricted range of 5-substituents: H, Cl, CH_3, XC_6H_3-3,4-Cl_2 (X = S, SO, SO_2), X-2-$C_{10}H_7$ (X = S, SO_2, *Z*-CH=CH, *E*-CH=CH), and CH_2SR (R = C_6H_4-4-Cl, 2-$C_{10}H_7$).[5] It was a primary purpose of our work to synthesize a series of analogues whose 5-substituents were well-suited to the development of quantitative structure–activity relationships, and the set of 2,4-diaminoquinazolines (3) we have prepared possesses a good range of lipophilic (log *P* = −0.33 to > 3), bulk (MR = 1.03 to 34.65), and electronic (σ_p = −0.83 to +0.78) properties at the 5-position. Disappointingly, all our attempts to derive a general QSAR using the Hansch approach (including the use of indicator variables and physicochemical properties such as ^1H NMR chemical shifts) for the DHFR data in Table IV failed. The structure–activity relationships for the inhibition of DHFR by this type of compound appear to be complex. In order to facilitate the discussion of *qualitative* structure–activity relationships, we have divided the results in Table IV into four groups, based on the type of 5-substituent: (1) electron-withdrawing groups, (2) amino-substituted compounds, (3) the ethers, and (4) the remainder (halogens, alkyls, and thioethers). Within each group the compounds are arranged in order of their experimentally determined partition coefficients to provide a common reference scale for the discussion.

DHFR Inhibition. The most active quinazoline listed in Table IV (compound **53**) binds about 100–1000 times more tightly to DHFR than the parent unsubstituted compound (**79**), showing that a worthwhile increment in activity can be obtained by substituting at the 5-position in this system. However, compound **53** is less active (~ 5–10 times) than methotrexate, a "classical" inhibitor, which binds tightly to all DHFRs,[5] and trimethoprim, which binds tightly to bacterial enzyme but only very poorly to mammalian DHFRs.[18] Also, unlike trimethoprim, none of the quinazolines tested shows any preference for binding to the bacterial DHFRs rather than the mammalian enzyme; indeed some compounds, such as the more lipophilic ethers, show a "reverse" selectivity, binding more tightly to the bovine liver enzyme than to either of the bacterial enzymes.

(18) (a) Dietrich, S. W.; Blaney, J. M.; Reynolds, M. A.; Jow, P. Y. C.; Hansch, C. *J. Med. Chem.* **1980**, *23*, 1205–12. (b) Roth, B.; Aig, E.; Rauckman, B. S.; Strelitz, J. Z.; Philips, A. R.; Ferone, R.; Bushby, S. R. M.; Sigel, C. *J. Med. Chem.* **1981**, *24*, 933–41.

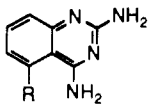
Table IV. DHFR Inhibition and Antibacterial Activity of 5-Substituted 2,4-Diaminoquinazolines (3)



no.	R	log P[a]	DHFR activity: 10^{-6} log $(1/I_{50})$, M SA[b]	DHFR activity: 10^{-6} log $(1/I_{50})$, M EC[b]	DHFR activity: 10^{-6} log $(1/I_{50})$, M BL[b]	antibact activity: 10^{-6} log (1/MIC), M SA[b]	antibact activity: 10^{-6} log (1/MIC), M EC[b]
77	$CONH_2$	−0.33	<0.0	<0.0	<0.0	<−0.7	−0.6
74	SO_2CH_3	0.63	<0.0	<0.0	<0.0	<−0.6	<−0.6
78	$CONHCH_2CH_2CH_3$	0.83	<0.0	<0.0	<0.0	<−0.6	<−0.6
79	H	1.01[c]	0.0	−1.0	−1.7	1.3	0.7
52	CO_2CH_3	*d*	<0.0	−1.7	−1.0	−0.6	−0.1
50	NO_2	1.19	<0.0	−2.0	−2.0	0.0	<0.5
76	$SO_2C_6H_5$	2.14	<0.0	<−2.0	<−2.0	<−0.5	<−0.5
72	NH_2	0.30	<0.0	<0.0	<0.0	<0.2	0.3
79	H	1.01	0.0	−1.0	−1.7	1.3	0.7
66	$N(CH_2CH_2)_2O$	1.11	<0.0	<0.0	<0.0	−0.6	−0.6
65	$N(CH_3)_2$	1.65	<0.0	−1.0	0.0	−0.5	−0.4
67	$N(CH_2CH_2)_2CH_2$	2.66	<0.0	<0.0	0.3	*e*	*e*
73	OH	*d*	<0.0	<0.0	<0.0	−0.7	<−0.7
79	H	1.01	0.0	−1.0	−1.7	1.3	0.7
53	OCH_3	1.18	0.0	1.3	1.0	1.0	2.1
54	OCH_2CH_3	1.69	<0.0	0.2	1.3	0.2	1.7
55	$OCH(CH_3)_2$	2.05	<0.0	<0.0	1.2	−0.1	0.8
56	$OCH_2CH_2CH_3$	2.26	<0.0	−0.2	1.3	0.2	1.1
60	OC_6H_5	2.78	*e*	0.3	1.3	0.0	0.2
57	$O(CH_2)_3CH_3$	2.81	<0.0	−0.4	1.0	0.4	0.6
59	$OCH_2C_6H_5$	2.82	*e*	<0.0	1.0	1.0	0.8
58	$O(CH_2)_5CH_3$	*d*	<0.0	0.3	0.3	1.1	0.0
79	H	1.01	0.0	−1.0	−1.7	1.3	0.7
80	F	1.19	0.2	0.2	<0.0	1.4	1.7
68	CH_3	1.26	*e*	0.3	0.0	0.5	1.7
51	CH_2CH_3	1.69	<0.0	0.8	1.0	0.6	2.6
81	Cl	1.72	0.0	1.0	0.2	1.2	2.1
61	SCH_3	1.81	<0.0	1.0	0.3	0.0	2.2
69	Br	1.91	<0.0	0.8	0.3	1.5	2.2
70	I	2.12	0.0	0.2	0.0	0.4	1.7
62	SCH_2CH_3	2.27	<0.0	0.2	0.2	−0.1	2.0
63	$SCH_2CH_2CH_3$	2.85	<0.0	0.0	1.0	*e*	*e*
71	$CH_2CH_2C_6H_5$	*d*	<0.0	0.2	0.8	1.3	1.3
64	SC_6H_5	*d*	<0.0	0.3	1.0	<−0.6	<−0.6
trimethoprim		−	1.8	2.0	−2.2	3.1	3.1
methotrexate		−	*e*	*e*	2.1		

[a] See the Experimental Section. [b] SA = *S. aureus*, EC = *E. coli*, BL = bovine liver. [c] Literature values: 1.00,[27] 0.88[36]. [d] Not measured. [e] Not tested.

Table V. Toxicities of 5-Substituted 2,4-Diaminoquinazolines (3)



no.	R	LD_{50}, mg kg^{-1} ip[a]	LD_{50}, mg kg^{-1} po[a]
53	OCH_3	137–291	~500
68	CH_3	~100	
81	Cl	~100	
54	OCH_2CH_3	~100	
80	F	100–215	489–1290
69	Br		271–500
70	I		~500
52	CO_2CH_3	68	
	4-aminoquinazoline	205–488	584

[a] Ip = by intraperitoneal injection; po = orally.

***S. aureus* DHFR.** Of the 28 compounds tested against this enzyme, only five (**53**, **70**, **79**, **80**, and **81**) have a log $(1/I_{50})$ greater than or equal to 0.0 μM, the most active being the 5-fluoro compound (**80**). This enzyme is particularly resistant to this class of inhibitors. Of the five best compounds, four have single atom substituents at the 5-position, suggesting that the active site pocket of this DHFR may be of very limited size at this point; a similar conclusion has been reached previously, based on more limited data.[6b]

***E. coli* DHFR.** Against the *E. coli* DHFR enzyme, by contrast, a number of compounds show good activity. Taking each group of compounds in turn, the following qualitative structure–activity relationships are apparent: (1) all compounds with an electron-withdrawing group at the 5-position are less active than 5-H (**79**); (2) replacing 5-H with an amino substituent produces little or no increase in activity; (3) the 5-OCH_3 analogue is about 500 times more active than 5-H, but then the level of activity drops very rapidly as log P increases; the remaining ethers, which span a log P range of approximately 2 log units, vary little in their activities; (4) the remaining compounds show a rough parabolic dependence of activity on log P, maximum activity occurring at log $P \approx 1.7$ with the 5-Cl (**81**) and 5-CH_3 (**68**) analogues, which are about 100 times more active than the 5-H analogue.

Mammalian (Bovine Liver) DHFR. Against the bovine liver enzyme the structure–activity relationships may be summarized thus: (1) replacing 5-H by an electron-withdrawing group, irrespective of its log P value, yields little or no increase in activity; (2) the more lipophilic

amino-based substituents produce a 50–100 times increase in activity relative to 5-H; (3) replacing 5-H with the almost isolipophilic methoxy group (**53**) yields an approximately 500 times increase in binding to the enzyme. As the series of ether-based substituents is ascended, activity remains roughly constant, falling off only with the hexyloxy analogue (**58**); (4) replacing 5-H with 5-CH_3 (**68**), which is also approximately isolipophilic with both 5-H and 5-OCH_3, produces only a 50-fold increase in activity. As this series is ascended, activity remains roughly constant until log P reaches 2.85 (5-$SCH_2CH_2CH_3$, **63**), when a further 5–10 times increase is achieved. (Note that the result for 5-CH_2CH_3 (**51**) does not fit this pattern and may be anomalous.)

Structure–Activity Relationships. It seems reasonable to divide the 5-substituents into three classes: "inactive", "moderately active", and "active". Inactive substituents are those which produce little or no improvement in binding over the parent unsubstituted molecule, irrespective of the log P of the group. This class comprises the electron-withdrawing groups. The active class consists of those substituent types that can produce a significant increase in activity relative to 5-H (up to 1000-fold). This class comprises the ethers, thioethers, alkyls, and halogens. Those compounds with substituted amino groups at the 5-position fall into an intermediate category: they are not significantly more active against the bacterial DHFRs than the parent 5-H molecule (thereby paralleling the behavior of those analogues substituted with electron-withdrawing groups), but they do show an approximately 50-fold increase in binding relative to 5-H on the bovine liver enzyme. We conclude that the binding of 5-substituted 2,4-diaminoquinazolines to DHFR does not depend initially on a single gross property of the 5-substituent, such as lipophilicity or electronic character (or on a simple combination of such properties), but rather on the structural type of the group.

Similar anomalies in the binding of some 5-substituted 2,4-diaminoquinazolines to DHFR have been observed previously, and various attempts have been made to explain them.[19-21] For example, in two studies[19] which used the Hansch approach for the generation of structure–activity relationships the unexpectedly low activities of some analogues led the authors to postulate that the binding pocket available to the 5-substituents of the inhibitors at the enzyme active site was of limited size in both of the enzymes studied (rat liver and *Streptococcus faecalis*). The correction factor for the steric inhibition of binding in the correlation equations was applied to all large groups; that is, the effect on activity was perceived to be due solely to the size of the 5-substituent. In our work there is some evidence for this in two of the enzymes studied: those from *S. aureus*, where only very small substituents appear to show any activity, and from *E. coli*, where activity drops off as the size of the 5-substituent increases. For the enzyme which corresponds most closely to one of the examples in the literature (the bovine liver enzyme), the evidence is rather for a binding pocket which is either large or open to the surrounding solvent, large 5-substituents being as active or more active than smaller ones.

Crippen, using his distance geometry approach, in contrast, has identified only 5-$SOAr$[21b,22] and 5-SO_2Ar[23] groups as producing anomalously low DHFR activities. He suggested that the powerful electron-withdrawing character of these groups may reduce the basicity of the quinazoline nucleus to such an extent that they bind to the active site of the enzyme in the unprotonated form. (It has been shown that inhibitors which bind tightly to DHFR, such as trimethoprim and methotrexate, are protonated at N(1), unlike the natural substrate, dihydrofolic acid, which binds to the enzyme in the unprotonated form; the interaction between $N(1)H^+$ and a carboxylate residue at the active site stabilizes the enzyme–inhibitor complex.)[24] Again, there is some evidence in our results to support this hypothesis. Consider, for example, the 5-NO_2 (**50**) and 5-OCH_3 (**53**) analogues: these two compounds have similar hydrophobic and general bulk properties, differing significantly only in their electronic character; their inhibition of *E. coli* and bovine liver DHFRs differs by a factor of about 1000. In order to test Crippen's hypothesis, we measured the acid–base dissociation constants of a representative set of the quinazolines (Table VI). The least basic of these compounds, the 5-NO_2 analogue (**50**) (pK_a = 6.24) is significantly protonated at physiological pH and is more basic than methotrexate (pK_a = 5.71),[25] which binds very tightly to DHFR and is certainly protonated at N(1) when bound to the enzyme active site.[24b] We conclude that the (relatively) low basicity of compounds bearing an electron-withdrawing group at the 5-position in not an important factor in their poor DHFR activity.

In Hopfinger's Molecular Shape Analysis of the binding of diaminoquinazolines to DHFR, the derived correlation equation includes a term dependent upon the torsional angle of the 4-amino group of the quinazoline nucleus.[20] This term arises from the proximity of the 5-substituent and the 4-amino group in 5-substituted 2,4-diaminoquinazolines. Such an interaction (a peri interaction) is likely to be complex, comprising many superimposed effects (intramolecular hydrogen bonding, direct through-space steric effects, through-ring resonance interactions, etc.). That such peri interactions do indeed operate in 5-substitued 2,4-diaminoquinazolines is shown by studying the chemical shifts (obtained from ^1H NMR spectra in DMSO-d_6 solution) of the 2- and 4-amino groups in these molecules (a representative set of values is listed in Table VI). Equations 1 and 2 show that, in both the free base

$$\delta_{FB} = 0.55\ (0.09)\sigma_1 + 0.46\ (0.10)\sigma^0{}_R + 5.97 \quad (1)$$

$$n = 8,\ s = 0.06,\ r = 0.97,\ F = 39\ (p = 0.0009)$$

$$\delta_{HCl} = 0.32\ (0.07)\sigma_1 + 0.49\ (0.07)\sigma^0{}_R + 7.83 \quad (2)$$

$$n = 8,\ s = 0.04,\ r = 0.97,\ F = 38\ (p = 0.0009)$$

(δ_{FB}) and hydrochloride (δ_{HCl}) forms, the chemical shifts of the 2-amino groups are well correlated[26] with the elec-

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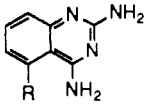
(25) Poe, M. *J. Biol. Chem.* **1977**, *252*, 3724–8.
(26) Equations 1–4 were derived by multiple linear regression; n is the number of data points, s the standard error of estimate, r the multiple correlation coefficient, and F the F statistic. The number in parentheses after each regression coefficient is its standard error.

Table VI. Physiochemical Properties of 5-Substituted 2,4-Diaminoquinazolines (3)



			chemical shifts of the amino group protons[b]					
			2-NH_2		4-NH_2			
no.	R	pK_a[a]	δ_{FB}[c]	δ_{HCl}[d]	δ_{FB}[e]	δ_{HCl}[f]	σ_1[g]	σ^0_R[g]
79	H	8.08 ± 0.03	6.04	7.88	7.29	8.98	0.00	0.00
80	F	7.44 ± 0.04	6.10	–	7.00	8.67	0.50	–0.31
81	Cl	7.22 ± 0.04	6.19	7.92	7.32	–	0.46	–0.18
68	CH_3	8.19 ± 0.02	5.83	7.72	6.62	–	–0.04	–0.13
62	SCH_2CH_3	7.87 ± 0.06	–	–	–	–	0.25	–0.19
75	$SO_2CH_2CH_3$	6.44 ± 0.05	6.35	8.10	–	9.32	0.60	0.18
50	NO_2	6.24 ± 0.04	6.40	–	6.70	8.70	0.65	0.15
77	$CONH_2$	7.22 ± 0.03	–	7.88	–	–	0.27	0.01
53	OCH_3	8.32 ± 0.06	5.88	7.73	7.22	8.73	0.27	–0.42
60	OC_6H_5		6.09	–	7.22	–	0.38	–0.32
74	SO_2CH_3		–	–	7.77	–	0.60	0.12
70	I		–	7.88	–	8.70	0.39	–0.19
73	OH		–	7.68	–	8.72	0.29	–0.43
71	$CH_2CH_2C_6H_5$		–	–	–	8.43	–0.05	–0.12

[a] See the Experimental Section for details. [b] From the ^1H NMR spectra acquired in DMSO-d_6 solution; δ = chemical shift in ppm on the δ scale from internal tetramethylsilane as standard; FB = freebase, HCl = hydrochloride salt; the choice of compounds for deriving eq 1–4 was governed by Taft's[37] recommendations for a data set in which the variation in electronic properties is maximized with the minimum number of data points. [c] Used to derive eq 1. [d] Used to derive eq 2. [e] Used to derive eq 3. [f] Used to derive eq 4; in some of these compounds (e.g. **53**, **79**) the resonances of the 4-amino group protons are split into two one-proton singlets because of restricted conformational mobility of the 4-amino group; in these cases the midpoint of the two peaks has been taken. [g] Values taken from ref 27.

tronic parameters σ_1 and σ^0_R.[27] The corresponding correlations for the 4-amino group shifts (eq 3 and 4), where

$$\delta_{FB} = 0.36\ (0.63)\sigma_1 + 0.01\ (0.77)\sigma^0_R + 7.02 \quad (3)$$

$$n = 8,\ s = 0.42,\ r = 0.26,\ F = 0.17\ (p = 0.84)$$

$$\delta_{HCl} = 0.24\ (0.40)\sigma_1 + 0.49\ (0.43)\sigma^0_R + 8.77 \quad (4)$$

$$n = 8,\ s = 0.26,\ r = 0.56,\ F = 1.1\ (p = 0.40)$$

the peri effect should be important, are meaningless, showing that the through-ring resonance interactions between the 5-substituent and the 4-amino group are disrupted by some other effects.

Recently, Matthews et al.[28] have published the results of an elegant investigation into the reasons for the selective DHFR binding exhibited by trimethoprim. By comparing crystal structures of trimethoprim's ternary complexes with both *E. coli* and chicken liver DHFR, they identified four differences in the binding of trimethoprim to the two enzymes. A comparison with the binding of other inhibitors led to the conclusion that only one of these differences is important in explaining the selectivity of trimethoprim: "the crystallographic evidence strongly suggests that the loss of this hydrogen bond [between the 4-amino group of trimethoprim and the carbonyl oxygen of a valine residue at the active site] is a significant factor in trimethoprim's low affinity for chicken liver DHFR".[28] In considering our results we find it significant that those substituent types which we classify as "inactive" or "moderately active" are just those groups (e.g. sulfones, amides, nitro, dialkylamines) which are most likely to cause steric congestion at the 4-position by peri interactions and so possibly prevent the 4-amino group from attaining a conformation in which it could form a strong hydrogen bond to the appropriate residue at the enzyme active site. If the substituent is sufficiently "narrow" not to interfere with the conformation of the 4-amino group, then it appears that the 5-substituent occupies a pocket at the active site, the size of which varies with the source of the enzyme (small for the bacterial enzymes, large or open to solvent for the mammalian enzymes), the occupation of which can provide a significant increase in binding energy.

In Vitro Antibacterial Activity. All but two of the quinazolines were also subjected to in vitro antibacterial testing (Table IV). These results generally parallel those obtained for the inhibition of isolated DHFR (Table IV). No compound is significantly more active against *S. aureus* than the parent 5-H analogue (**79**). Those compounds inactive against isolated *E. coli* DHFR are also poor inhibitors of the growth of this bacterium. For the other compounds the variations in antibacterial activity roughly parallel those observed for anti-DHFR activity. The log (1/MIC) for growth inhibition is generally between 1–2 log units higher than the log $(1/I_{50})$ for enzyme inhibition (only some of the more lipophilic compounds such as analogues **58** and **64** appear to violate this relationship). The observation that the concentration of compound required to prevent the growth of intact bacteria in vitro (log (1/MIC) is 10–100 times less than the concentration required to inhibit 50% of the activity of isolated DHFR (log $(1/I_{50})$ is surprising in the light of the claim[29] that only a small fraction (<5%) of the total intracellular DHFR is necessary to maintain cell viability, but Khwaja et al.[10] have noted a similar effect with a DHFR extracted from murine leukaemia cells, and these workers have concluded that: "considerably less than 50% of the DHFR in living cells is inhibited to cause an overall reduction of 50% in the growth rate".[10] The close parallels between the degree of inhibition of the activity of isolated DHFR enzyme and the restriction of the growth of intact bacterial cells in vitro strongly suggest that these compounds owe their anti-

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bacterial effects to antifolate activity.

In Vivo Antibacterial Activity. As reported above, none of the quinazolines tested showed any significant in vivo activity at their maximum tolerated dose against murine *E. coli* infections, and all of the compounds submitted to the LD_{50} test showed significantly toxicity (Table V). The level of toxicity does not appear to be directly related to the ability of the compounds to inhibit the action of isolated mammalian (bovine liver) DHFR. For example, the 5-CO_2CH_3 (**52**) and 5-OCH_3 (**53**) analogues are equitoxic to mice, whereas their binding to bovine liver DHFR differs by a factor of 100 (Table IV). The last entry in table V is notable: 4-aminoquinazoline is almost as toxic as the 2,4-diaminoquinazolines, even though it lacks the 2,4-diamino-1,3-diazo pharmacophore considered necessary for tight binding to DHFR. The inhibition constant for the binding of this compound to a mammalian (rat liver) DHFR is log $(1/I_{50}) = -2.9$ μM;[30] this value compares very well with that for trimethoprim on bovine liver DHFR (log $(1/I_{50}) = -2.2$ μM), but trimethoprim is well tolerated by mice at doses of 500 mg kg^{-1}.[31] The quinazolines may, therefore, have pharmacological effects in the mouse unrelated to their inhibition to DHFR.

Conclusions

Our results show that a significant increase in antifolate potency can be obtained in the 2,4-diaminoquinazoline system by making an appropriate substitution at the 5-position, although the most active compounds in this series are less active than antifolates like trimethoprim and methotrexate. The best 5-substituent is a methoxy group, which generally produces better anti-DHFR activity than the commonly used methyl and chloro groups.[5] The particularly good activity as a class of the 5-alkoxy-substituted analogues against the mammalian enzyme may be of interest in the development of diaminoquinazolines with anticancer activity. There is no evidence for any selective binding to the bacterial enzymes. Sufficient data are available for us to develop some qualititative structure–activity relationships. These show that the relationships between structure and activity in this series are complex and do not appear to depend primarily on some generalized bulk property or properties of the 5-substituent, but rather on the structural type of the group. We postulate that the effect of the peri interaction between the adjacent 5-substituent and the 4-amino group is very important in determining the DHFR binding of a particular analogue, possibly because of its influence on the hydrogen bond formed between the 4-amino group and a residue at the enzyme's active site. Such effects, being noncontinuous, are very difficult to include in the Hansch approach to the study of structure–activity relationships (although Hopfinger's Molecular Shape Analysis, with the addition of a term dependent on the torsional angle of the 4-amino group, shows some promise—see above). The technique of combined X-ray crystallography and molecular graphics, as developed by Hansch for example,[32] may be more appropriate. Our results show a strong correspondence between activity against isolated DHFR and the inhibition of bacterial growth in vitro, encouraging the view that receptor studies are a useful means of identifying potential new drugs. The quinazolines, however, show no useful in vivo antibacterial activity in our test system, which might possibly have been predicted from the lack of any species specificity at the enzyme level. The evidence for possible nonantifolate toxic effects also makes the development of a therapeutically useful antibacterial agent from this series unlikely.

Experimental Section

Biological Methods. Dihydrofolate reductase was extracted from *E. coli* (B41), *S. aureus* (NTCT6571A), and bovine liver cells. The method used to determine enzyme inhibition was the standard one, in which changes in enzyme activity were measured by monitoring the changes in the UV absorbance at 340 nm of a mixture containing enzyme, inhibitor, mercaptoethanol, dihydrofolic acid, and NADPH in a phosphate buffer (pH 7.2).[38] The activity of each compound was expressed by its I_{50} value, where I_{50} is the concentration in units of 10^{-6} M of inhibitor which reduces the catalytic activity of the enzyme by 50%. Each determination was performed to a precision of approximately ±50%. The growth inhibitory properties of the quinazolines against intact bacterial cells were determined by a two-fold serial dilution method: for each compound a series of solutions was prepared whose concentrations differed by a factor of two; each solution was added to a fixed amount of a previously prepared test culture, and the mixture was incubated at 37 °C overnight. The antibacterial activity of each compound was then expressed as its minimum inhibitory concentration (MIC) in units of 10^{-6} M; that is, the lowest concentration that just inhibited bacterial growth. Each determination was performed in triplicate automatically by a "Microtitre" machine, and the MIC was taken as the average of the three readings. All compounds were tested on the same day to minimize random variations, and a standard antibacterial antifolate (trimethoprim) was included for reference.

Determination of Acid–Base Dissocation Constants. Acid–base dissociation constants were measured by the UV method.[39] A stock solution of the diaminoquinazoline (2–3mg) in all-glass-distilled water (10 mL) was prepared. Two solutions were prepared by diluting 0.10 mL of the stock solution to 10.0 mL with 0.01 M sodium hydroxide (pH ~12) and 0.001 M hydrochloride acid (pH ~3). The UV difference spectrum of these two solutions was obtained in order to determine an analytical wavelength, and then the optical density (d_3 and d_{12}) of each solution versus distilled water was measured at the analytical wavelength. (All absorbencies were measured in a 1.0-cm cell thermostated at 25.0 °C.) A series of buffer solutions (between five and seven), whose pHs bracketed the estimated pK_a of the compound under consideration, were prepared by mixing appropriate volumes of 0.1 M KH_2PO_4 and 0.05 M borax.[40] The pH of each buffer solution was measured at 25 °C with a Corning pH meter Model 109 (standardized on borax). Then a series of test solutions was made up by diluting 0.10 mL of the stock solution to 10.0 mL with the buffers. The optical density (d_x) of each solution was measured against the appropriate buffer solution and the pK_a was calculated from eq 5.[39] The separate values were averaged, and the standard deviation was calculated, to give the concentration pK_a at 25 °C.

$$pK_a = pH + \log [(d_{12} - d_x)/(d_x - d_3)] \quad (5)$$

Determination of Octanol–Water Partition Coefficients. A stock solution of the quinazoline (~1 mg) in 0.01 M sodium

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hydroxide saturated with 1-octanol (100 mL) was prepared. A second stock solution was obtained by diluting 50 mL of this solution with 50 mL of fresh octanol–saturated sodium hydroxide solution. An analytical wavelength was determined from the UV absorbance spectrum of the first solution. Two aliquots of different volume were accurately measured out from each stock solution, and each was shaken with an accurately measured amount of 1-octanol previously saturated with 0.01 M sodium hydroxide solution. (The volume ratios chosen depended on the estimated log P of the compound under consideration.) Small volumes (<1.0 mL) of octanol were measured out by syringe; larger amounts were weighed out and the measured weight was converted to a volume with an experimentally determined value for the density of the batch of alkali-saturated octanol (a typical value was 0.8363 ± 0.0003 g mL^{-1}, n = 4). The mixtures were gently inverted in stoppered test tubes for 10 min (minimum of 100 inversions) and then centrifuged for 15 min to ensure complete separation of the layers. The upper octanol layer was removed and the absorbencies of the two stock and four test solutions at the analytical wavelength were measured. The partition coefficients were calculated from eq 6, where d_x is the optical density

$$P = [(d_x - d_0)/d_0](V_{H_2O}/V_{oct}) \quad (6)$$

of the test solution and d_0 is the optical density of the appropriate stock solution. The four values were averaged and the standard deviation was calculated. For all but compounds **57** (6%), **63** (6%), **67** (12%), **70** (8%), and **72** (7%) the standard deviation expressed as a percentage of the average was less than 5%.

The structure of all compounds prepared was confirmed by spectroscopic analysis: ^{1}H NMR spectra were recorded at ambient temperature on DMSO-d_6 solutions with a Varian CFT-20 machine operating at 80 MHz; infrared spectra were obtained on a Pye-Unicam SP3-200 spectrometer on potassium bromide dispersions. Preparative column chromatography was performed by using Still's method of flash chromatography;[41] the stationary phase used was Merck silica gel, mesh size 40–63 μm (Art no. 9385).

Hydrochloride salts, required for the NMR studies, were prepared in the following fashion: The free base (10–25 mg) was dissolved in hot ethanol (~10 mL) and this solution was made acid with concentrated hydrochloric acid (~0.2 mL). Evaporation gave the hydrochloride as a white or cream powder.

Preparation of 6-Substituted 2-Nitrobenzonitriles (1).

Methyl 2-Bromo-3-nitrobenzoate (6). A mixture of 2-bromo-3-nitrobenzoic acid (**5**,[15] 2.0 g, 8 mmol) and thionyl chloride (4 mL) was refluxed for 1 h. Excess thionyl chloride was evaporated off and the residue was refluxed with anhydrous methanol (20 mL) for 1 h. Evaporation of the clear solution and crystallization of the residue from cyclohexane gave compound **6** as a fine white powder: 1.74 g (83%); mp 76–77 °C. Anal. ($C_8H_6BrNO_4$) C, H, N, Br.

2,6-Dinitrobenzonitrile (7). A mixture of 2,6-dinitro-1-chlorobenzene (100 g, 0.49 mol) and copper(I) cyanide (53 g, 0.59 mol) in DMF (500 mL) was stirred at 140–150 °C for 2 h. After cooling to room temperature, the reaction mixture was poured into water (1500 mL) and the olive precipitate was filtered, washed with water, and dried. The residue was extracted with boiling ethyl acetate (2 × 750 mL). The combined extracs were treated with decolorizing charcoal, filtered, and evaporated. The residue was suspended in boiling ethanol (150 mL), and the insoluble product was filtered, washed with hot ethanol (150 mL), and dried to give compound **7** as an olive powder: 77.7 g (82%); mp 143–147 °C. An analytical sample was prepared by flash chromatography (0–40% ethyl acetate in toluene); mp 145–147 °C (lit.[12] mp 149–151 °C). Anal. ($C_7H_3N_3O_4$) C, H, N.

2-Ethyl-6-nitrobenzonitrile (8). A solution of potassium cyanide (25.7 g, 0.40 mol) in water (100 mL) was added to a vigorously stirred suspension of copper(I) cyanide (15.3 g, 0.16 mol) in water (30 mL). The mixture was covered by a layer of ethyl acetate and cooled in an ice bath. 2-Ethyl-6-nitroaniline (**4**,[14] 10.0 g, 60 mmol) was dissolved in glacial acetic acid (18 mL) and a mixture of concentrated hydrochloric acid (14.6 mL) and concentrated sulfuric acid (14.6 mL) was added with stirring. The mixture was cooled to −10 °C and diazotized with a solution of sodium nitrite (4.56 g, 56 mmol) in water (15 mL). When diazotization was complete, the mixture was covered with a layer of toluene, cooled to −15 °C, and taken to pH 5 by the careful addition of solid sodium hydrogen carbonate. The neutralized solution was added to the vigorously stirred cyanide solution as quickly as possible. When the evolution of nitrogen had ceased, the mixture was warmed to 70 °C to complete the reaction. After cooling to room temperature, the mixture was shaken with ethyl acetate (100 mL), and the insoluble material was filtered and washed with fresh ethyl acetate. The filtrate plus washings were combined, the layers were separated, and the organic solution was dried and evaporated. Crystallization of the residue from ethanol gave compound **8** as large sandy needles: 3.97 g (37%); mp 88–89 °C. Anal. ($C_9H_8N_2O_2$) C, H, N.

Methyl 2-Cyano-3-nitrobenzoate (9). A mixture of compound **6** (30.3 g, 0.12 mol) and copper(I) cyanide (12.9 g, 0.15 mol) in 1-methyl-2-pyrrolidone (40 mL) was stirred at 120–130 °C for 3 h. After cooling to room temperature, the mixture was poured into water (600 mL) and the brick-red precipitate was filtered, washed with water, and dried. This solid was extracted with boiling ethyl acetate (2 × 500 mL); the brick-red color was discharged. The beige insolubles were filtered off and the combined extracts were evaporated to dryness. The residue was boiled with ethanol (150 mL) and the off-white powder was filtered and washed with ethanol to give compound **9**: 14.5 g (61%); mp 140–142 °C (lit.[13] mp 143–144 °C). Anal. ($C_9H_6N_2O_4$) C, H, N.

2-Bromo-6-nitrobenzonitrile (10). Compound **27** (2.0 g, 12 mmol) was added in small portions to a stirred solution of sodium nitrite (940 mg, 14 mmol) in concentrated sulfuric acid (10 mL) and glacial acetic acid (10 mL) at 40 °C. During this addition the internal temperature did not exceed 43 °C. After 30 min at 40 °C, the almost clear, pale orange solution was poured into a cold solution of copper(I) bromide (2.64 g, 19 mmol) in 48% hydrochloric acid (10 mL): the internal temperature rose to 65 °C. When the evolution of nitrogen had ceased, the mixture was warmed to 90 °C to complete the reaction. The thick, dark mixture was poured into iced water (100 mL) and the resulting red brown precipitate was filtered and washed with water. After purification by flash chromatography (chloroform), crystallization from aqueous ethanol afforded compound **10** as very fine, pale orange needles: 1.70 g (61%); mp 143 °C. Anal. ($C_7H_3BrN_2O_2$) C, H, N, Br.

2-Iodo-6-nitrobenzonitrile (11). Compound **27** (20.0 g, 0.12 mol) was added in small portions over 20 min to a stirred solution of sodium nitrite (9.34 g, 0.13 mol) in concentrated sulfuric acid (100 mL) and glacial acetic acid (100 mL) at 40 °C. The mixture was stirred at 40 °C for a further 30 min and then the clear pale orange solution was poured into a solution of potassium iodide (30.6 g, 0.19 mol) in 1 M sulfuric acid (100 mL). A vigorous evolution of nitrogen occurred, accompanied by a rise in temperature to 80 °C and the liberation of some iodine. This mixture was allowed to stand at room temperature for 60 min and then was poured into a large volume of water. The dark product was filtered, washed with water, and stirred in a solution of sodium metabisulfite (20 g) in water (500 mL). When the violet color due to iodine had been discharged, the red-brown product was filtered, washed with water, and dried. Purification by flash chromatography (chloroform) gave **11** as a fawn powder: 13.8 g (41%); mp 183–185 °C. Anal. ($C_7H_3IN_2O_2$) C, H, N, I.

2-Methoxy-6-nitrobenzonitrile (12). Method A. A solution of sodium (2.2 g, 0.114 mol) in methanol (45 mL) was added dropwise over 45 min to a stirred solution of compound **7** (20.0 g, 0.10 mol) in DMF (150 mL) with ice cooling. After stirring at room temperature for 2 h, the mixture was poured into water (1000 mL). The beige precipitate was filtered, washed with water, and crystallized from ethanol to give compound **12** as translucent plates: 9.1 g (49%); mp 173–174 °C (lit.[11] mp 175–177 °C). Anal. ($C_8H_6N_2O_3$) C, H, N.

2-(1-Methylethoxy)-6-nitrobenzonitrile (14). Sodium hydride (100%, 273 mg, 11.4 mmol) was dissolved in propan-2-ol (6 mL). When the evolution of hydrogen had subsided, DMF (15 mL) was added, followed by compound **7** (2.0 g, 11 mmol). The very dark purple solution was stirred at 90 °C for 1 h and then poured into water (100 mL). The red-brown precipitate was filtered, washed with water, and dried. Crystallization of the

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residue from cyclohexane gave compound 14 as pale orange needles: 1.04 g (48%); mp 100–102 °C. Anal. ($C_{10}H_{10}N_2O_3$) H, N; C: calcd, 58.3; found, 57.8.

2-Nitro-6-phenoxybenzonitrile (19). A mixture of compound 7 (3.0 g, 16 mmol), phenol (2.1 g, 23 mmol), and anhydrous potassium carbonate (2.15 g, 16 mmol) in DMF (30 mL) was stirred at room temperature for 24 h. The mixture was poured into water (100 mL) and extracted with ethyl acetate (150 mL). The organic phase was separated, washed with water (50 mL), and dried. Evaporation and crystallization of the residue from tetrachloromethane gave compound 19 as off-white plates: 1.86 g (50%); mp 106–107 °C. Anal. ($C_{13}H_8N_2O_3$) H, N; C: calcd 65.0; found, 64.4.

2-(Methylthio)-6-nitrobenzonitrile (20). A solution of compound 7 (1.0 g, 5.2 mmol) in DMF (10 mL) was cooled in an ice bath. The addition of triethylamine (550 mg, 5.4 mmol) produced a green coloration. Methanethiol was bubbled through the solution until the green color had been discharged and a fawn precipitate was produced. The mixture was diluted with water (50 mL) and filtered. After washing with water, the residue was crystallized from aqueous 2-ethoxyethanol to give compound 20 as bright yellow needles: 820 mg (81%); mp 183–184 °C (lit.[13] mp 179–181 °C). Anal. ($C_8H_6N_2O_2S$) C, H, N, S. Compounds 21 and 22 were prepared in a similar manner, except that the thiol (1.05 equiv) was added as a solution in DMF to the mixture of compound 7 and triethylamine in DMF.

2-Nitro-6-(phenylthio)benzonitrile (23). A mixture of compound 7 (10.0 g, 52 mmol), thiophenol (6.28 g, 57 mmol), and powdered anhydrous potassium carbonate (7.20 g, 52 mmol) in DMF (50 mL) was stirred in an ice bath for 90 min and then poured into water (300 mL). The resulting yellow solid was filtered, washed with water, dried, and crystallized from cyclohexane to give compound 23 as fine yellow needles: 7.8 g (59%); mp 107–108 °C. Anal. ($C_{13}H_8N_2O_2S$) H, N; C: calcd, 60.9; found, 61.4.

2-(Dimethylamino)-6-nitrobenzonitrile (24). Dimethylamine (30% w/w in ethanol, 5 mL) was added in small portions over 30 min to a stirred solution of compound 7 (2.0 g, 11 mmol) in DMF (10 mL) with stirring in an ice–salt bath. The mixture was kept at 0 °C overnight and then poured into water (50 mL). The bright orange precipitate was filtered, washed with water, dried, and crystallized from a large volume of cyclohexane to give compound 24 as bright orange needles: 970 mg (47%); mp 111–113 °C (lit.[13] mp 115–117 °C). Anal. ($C_9H_9N_3O_2$) C, H, N.

2-(4-Morpholinyl)-6-nitrobenzonitrile (25). A mixture of compound 7 (8.7 g, 45 mmol) and morpholine (11.8 g, 0.14 mol) in DMF (135 mL) was stirred at 90 °C for 1 h. After cooling to room temperature, the dark red mixture was diluted with water (1000 mL) and the bright orange precipitate was filtered, washed with water, and crystallized from ethanol (charcoal) to give compound 25 as small bright orange crystals: 4.5 g (43%); mp 150–154 °C (lit.[13] mp 152–155 °C). Anal. ($C_{11}H_{11}N_3O_3$) C, H, N. Compound 26 was prepared in a similar manner by reaction at 70 °C for 30 min.

Preparation of 6-Substituted 2-Aminobenzonitriles (2).

2-Amino-6-nitrobenzonitrile (27). Concentrated hydrochloric acid (30 mL) was added slowly to a stirred solution of compound 7 (9.64 g, 60 mmol) in refluxing methanol (200 mL) and dioxane (125 mL). Iron powder (9.0 g, 0.16 mol) was added to the stirred mixture at such a rate that reflux was maintained; the time required for addition was about 15 min. The dark mixture was stirred at reflux for a further 30 min and then evaporated and the residue shaken with water. The insoluble material was filtered, washed with water, and dried. Purification by flash chromatography (chloroform) gave compound 27 as an orange-brown powder; 5.77 g (71%); mp 189–193 °C. An analytical sample was prepared by crystallization from ethanol–ethyl acetate; mp 190–191 °C (lit.[13] mp 192–196 °C). Anal. ($C_7H_5N_3O_2$) C, H, N.

2-Amino-6-(methylthio)benzonitrile (38). Method B. A solution of tin(II) chloride hydrate (27.2 g, 0.12 mol) in concentrated hydrochloric acid (80 mL) was added dropwise over 30 min to a stirred suspension of compound 20 (7.5 g, 39 mmol) in diglyme (190 mL). The temperature was kept below 25 °C by external cooling as necessary. The mixture was stirred at room temperature for 90 min and then poured onto a mixture of potassium hydroxide (130 g), water (130 mL), and ice (500 g). The mixture was filtered and the residue was washed with water and dried to give compound 38 as a cream solid: 3.4 g (54%); mp 81–82 °C. An analytical sample was obtained by crystallization from benzene–petroleum ether (bp 60–80 °C); mp 81–83 °C (lit.[13] mp 84–86 °C). Anal. ($C_8H_8N_2S$) C, H, N, S.

2-Amino-6-(ethylthio)benzonitrile (39). Method C. Small portions of compound 21 (13.8 g, 66 mmol) were added over 15 min to a stirred solution of tin(II) chloride dihydrate (52.5 g, 0.23 mmol) in glacial acetic acid (210 mL) and concentrated hydrochloric acid (140 mL); the internal temperature was maintained at 20 °C by external cooling as necessary. The mixture was stirred at room temperature for 4.5 h and then poured into a mixture of potassium hydroxide (500 g), water (500 mL), and ice (1000 g). The product was extracted into ether, and this solution was washed thoroughly with water, dried, and evaporated. Crystallization of the residue from a mixture of cyclohexane (100 mL) and toluene (35 mL) gave compound 39 as white microcrystals: 7.4 g (63%); mp 88 °C. Anal. ($C_9H_{10}N_2S$) C, H, N, S.

2-Amino-6-(4-morpholinyl)benzonitrile (43). A suspension of granulated tin (23.3 g, 0.195 mol) in concentrated hydrochloric acid (50 mL) and water (50 mL) was brought to 50 °C with stirring. Compound 25 (5.7 g, 25 mmol) was added in one portion. An exotherm was observed and the temperature rose to 55 °C. After stirring in the water bath at 45 °C for 1 h, the clear supernatant liquors were decanted from the unreacted tin. This solution was made strongly alkaline with 50% potassium hydroxide while the temperature of the mixture was maintained below 20 °C. The oily product was extracted into dichloromethane, and this solution was washed thoroughly with water, dried, and evaporated. Crystallization of the residue from benzene gave compound 43 as white crystals: 3.2 g (65%); mp 143–153 °C (lit.[13] mp 154–157 °C). Anal. ($C_{11}H_{13}N_3O$) H, N; C: calcd, 65.0; found, 64.4. Compound 44 was prepared in a similar manner from compound 26.

2-Amino-6-methylbenzonitrile (45). Method D. A mixture of 2-methyl-6-nitrobenzonitrile[42] (13.8 g, 85 mmol), cyclohexene (28 mL), and palladium on charcoal (1.5 g) in ethanol (280 mL) was stirred at reflux for 3 h. After cooling to room temperature, the mixture was diluted with its own volume of ethanol and filtered through Filter-aid. Evaporation of the filtrate and crystallization of the residue from ethanol (charcoal) gave compound 45 as off-white needles: 7.8 g (69%); mp 127–129 °C (lit.[33] mp 126.5–128 °C). Anal. ($C_8H_8N_2$) C, H, N.

2-Amino-6-indobenzonitrile (47). Method E. Iron powder (11.0 g, 20 mmol) was added in small portions over 30 min to a stirred suspension of compound 11 (12.1 g, 20 mmol) in methanol (23 mL) and concentrated hydrochloric acid (30 mL). The resulting dark mixture was stirred for a further 10 min and then poured into water (300 mL). The grey precipitate was filtered, washed with water, dried, and dissolved in boiling ethanol (300 mL). After charcoal treatment, this solution was filtered and evaporated to dryness to give compound 47 as a white powder: 9.65 g (90%); mp 136–137 °C. Anal. ($C_7H_5IN_2$) C, H, N, I.

2-Amino-6-(phenylethynyl)benzonitrile (48). A mixture of palladium(II) acetate (110mg, 0.5 mmol), tri(2-tolyl)phosphine (295 mg, 1.0 mmol), and copper(I) iodide (98 mg) was added in one portion to a stirred solution of compound 47 (6.0 g, 25 mmol) and phenylacetylene (3.0 g, 30 mmol) in piperidine (30 mL) under argon at room temperature. Almost immediately the clear solution darkened, there was heavy precipitation, and an exotherm was observed. The mixture was stirred at room temperature for 90 min and diluted with ethyl acetate (150 mL), and the precipitated piperidine hydrochloride was removed by filtration. Evaporation of the filtrate gave a dark semisolid, which was purified by flash chromatography (chloroform) to give compound 48 as a light brown powder: 4.7 g (88%); mp 107–108 °C. An analytical sample was obtained by crystallization from cyclohexane; mp 109 °C. Anal. ($C_{15}H_{10}N_2$) H, N; C: calcd, 82.6; found, 81.8.

2-Amino-6-(2-phenylethyl)benzonitrile (49). A mixture of compound 48 (1.0 g, 4.6 mmol) and 10% palladium on charcoal (200 mg) in ethanol (40 mL) was hydrogenated at room temperature and pressure; hydrogen uptake ceased after 20 min. The

(42) Commercially available, e.g. from Lancster Synthesis Ltd., Lancaster, England.

spent catalyst was removed by filtration and evaporation of the filtrate gave a clear viscous oil, which crystallized on standing to give compound **49** as an off-white solid: 960 mg (93%); mp 73 °C. An analytical sample was obtained by crystallization from cyclohexane–petroleum ether (bp 60–80 °C); mp 75–76 °C. Anal. ($C_{15}H_{14}N_2$) C, H, N.

Preparation of 5-Substituted 2,4-Diaminoquinazolines (3). Chloroformamidine Hydrochloride. A solution of cyanamide in ether (~0.05 g mL^{-1}) was treated with an excess of ethereal hydrogen chloride with ice cooling. The white precipitate was filtered off, washed thoroughly with ether, and dried. This material is stable for a month at room temperature in an anhydrous atmosphere.

2,4-Diamino-5-(dimethylamino)quinazoline (65). Compound **42** (3.30 g, 21 mmol) was cyclized with chloroformamidine hydrochloride (2.9 g, 25 mmol) in diglyme (13 mL) (method F). The crude hydrochloride was converted to the free base and purified by flash chromatography (chloroform–ethanol–triethylamine, 85:15:0.5), followed by crystallization from ethanol–2-propanol to give a very pale green powder. This was dissolved in anhydrous methanol (20 mL) and the solution was treated with ethereal hydrogen chloride (60 mL). After stirring for 1 h, filtration gave compound **65** as a bright yellow powder: 1.0 g (17%); mp 282–284 °C dec. Anal. ($C_{10}H_{13}N_5 \cdot 1.8HCl \cdot H_2O$) C, H, N, Cl.

2,4-Diamino-5-fluoroquinazoline (80). Method F. A mixture of 2-amino-6-fluorobenzonitrile[42] (5.0 g, 37 mmol) and chloroformamidine hydrochloride (5.30 g, 46 mmol) in diglyme (25 mL) was stirred at 130–140 °C for 3 h. During the reaction period complete solution occurred, accompanied by the evolution of hydrogen chloride, subsequently followed by heavy precipitation. The mixture was cooled to room temperature, diluted with dioxane (75 mL), and filtered. The crude hydrochloride was crystallized from dilute aqueous ammonia to give compound **80** as large white crystals: 2.30 g (35%); mp 247–249 °C. Anal. ($C_8H_7FN_4$) C, H, N, F.

2,4,5-Triaminoquinazoline Dihydrochloride (72). A suspension of compound **50** (200 mg, 1 mmol) and 10% palladium on charcoal (20 mg) in 2 M hydrochloric acid (2 mL) and ethanol (3 mL) was hydrogenated at room temperature and pressure. The theoretical quantity of hydrogen was taken up in 45 min. The thick, grey suspension was diluted with water (15 mL), warmed on the steam bath, and filtered through Filter-aid. Evaporation of the straw filtrate gave compound **72** as a light brown, crystalline solid: 230 mg (87%); mp >292 °C dec. Anal. ($C_8H_9N_5 \cdot 2HCl \cdot 1.2H_2O$) C, H, N, Cl.

2,4-Diamino-5-hydroxyquinazoline Hydrochloride (73). A mixture of compound **59** (1.50 g, 5.6 mmol) and 5% palladium on charcoal (150 mg) in ethanol (50 mL) was hydrogenated at room temperature and pressure (3 h). The mixture was diluted with ethanol (50 mL), brought to reflux, and filtered through Filter-aid. Evaporation of the filtrate gave compound **73** as a free base: 700 mg (71%); mp ~250 °C dec. This was dissolved in hot ethanol (100 mL) and concentrated hydrochloric acid (1.5 mL) was added. Evaporation gave compound **73** as a light grey solid; mp >300 °C. Anal. ($C_8H_8N_4O \cdot 1.05HCl \cdot 0.25H_2O$) C, H, N, Cl.

2,4-Diamino-5-(methylsulfonyl)quinazoline (74). Method G. A solution of potassium permanganate (1.4 g, 8.7 mmol) in water (35 mL) was added in small portions over 1 h at room temperature to a stirred solution of compound **61** (0.90 g, 4.4 mmol) in glacial acetic acid (55 mL). The mixture was stirred at room temperature overnight and the filtered through Filter-aid. The dark brown filtrate was made strongly basic with concentrated ammonia (ice cooling). The resulting dark brown precipitate was filtered, washed with water, and dried. The residue was extracted with boiling DMF (30 mL) and the hot extract was treated with charcoal. The mixture was filtered hot and the residue was washed with a further portion of hot DMF (20 mL). The combined filtrates were treated with twice their own volume of hot water and cooled in an ice bath to give compound **74** as fine off-white needles: 360 mg (34%); mp 252–254 °C. Anal. ($C_9H_{10}N_4O_2S$) C, H, N, S.

5-(Aminocarbonyl)-2,4-diaminoquinazoline (77). A mixture of compound **52** (1.0 g, 3.9 mmol) and concentrated ammonia (20 mL) was stirred at room temperature overnight. The pale yellow solid was filtered and washed with water. Crystallization from aqueous DMF gave **77** as a fine white powder: 300 mg (38%); mp 274–276 °C. Anal. ($C_9H_9N_5O$) C, H, N. Compound **78** was prepared in a similar fashion from compound **52** and 1-aminopropane.

2,4-Diaminoquinazoline (79). A mixture of 2-aminobenzonitrile (47.2 g, 0.40 mol) and dicyanodiamide (33.6 g, 0.40 mol) in 2 M hydrochloric acid (200 mL) was stirred at reflux for 2 h. The solution was diluted with 0.5 M sodium hydroxide (1750 mL) and the resulting precipitate was redissolved by warming. The clear solution was treated with charcoal and filtered hot. On cooling, white crystals of compound **79** separated: 29.3 g (46%); mp 255–257 °C (lit.[35] mp 248–252 °C). Anal. ($C_8H_8N_4$) C, H, N.

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Registry No. **4**, 59816-94-3; **5**, 573-54-6; **6**, 5337-09-7; **7**, 35213-00-4; **8**, 58580-00-0; **9**, 77326-46-6; **10**, 79603-02-4; **11**, 79603-05-7; **12**, 38469-85-1; **13**, 123241-32-7; **14**, 123241-33-8; **15**, 123241-34-9; **16**, 123241-35-0; **17**, 123241-36-1; **18**, 1591-33-9; **19**, 123241-37-2; **20**, 63920-80-9; **21**, 123241-38-3; **22**, 123241-39-4; **23**, 123241-40-7; **24**, 63140-76-1; **25**, 63365-42-4; **26**, 63365-13-9; **27**, 63365-23-1; **28**, 123241-41-8; **29**, 77326-43-3; **30**, 1591-37-3; **31**, 123241-42-9; **32**, 77326-42-2; **33**, 123241-43-0; **34**, 123241-44-1; **35**, 123241-45-2; **36**, 123241-46-3; **37**, 123241-47-4; **38**, 77326-38-6; **39**, 123241-48-5; **40**, 123264-69-7; **41**, 123241-49-6; **42**, 63365-11-7; **43**, 63005-74-3; **44**, 63365-14-0; **45**, 56043-01-7; **46**, 77326-62-6; **47**, 123241-50-9; **48**, 123241-51-0; **49**, 123241-52-1; **50**, 123241-89-4; **50**·HCl, 123241-53-2; **51**, 90870-69-2; **51**·HCl, 123241-54-3; **52**, 123241-90-7; **52**·HCl, 123241-55-4; **53**, 27018-21-9; **53**·HCl, 123241-56-5; **54**, 119584-81-5; **54**·HCl, 123241-57-6; **55**, 123241-91-8; **55**·HCl, 123241-58-7; **56**, 123241-92-9; **56**·HCl, 123241-59-8; **57**, 123241-93-0; **57**·HCl, 123241-60-1; **58**, 123241-94-1; **58**·HCl, 123241-61-2; **59**, 123241-95-2; **59**·HCl, 123241-62-3; **60**, 123241-96-3; **60**·HCl, 123241-63-4; **61**, 119584-80-4; **61**·HCl, 123241-64-5; **62**, 123241-97-4; **62**·HCl, 123241-65-6; **63**, 123241-98-5; **63**·HCl, 123241-66-7; **64**, 123241-99-6; **64**·HCl, 123241-67-8; **65**, 119584-83-7; **65**·2HCl, 123241-68-9; **66**, 123242-00-2; **66**·HCl, 123241-69-0; **67**, 123242-01-3; **67**·HCl, 123241-70-3; **68**, 27018-14-0; **68**·HCl, 123241-71-4; **69**, 119584-75-7; **69**·HCl, 123241-72-5; **70**, 119584-76-8; **70**·HCl, 123241-73-6; **71**, 123242-02-4; **71**·HCl, 123241-74-7; **72**, 123242-03-5; **72**·2HCl, 123241-75-8; **73**, 123241-85-0; **73**·HCl, 123241-76-9; **74**, 123241-86-1; **74**·HCl, 123241-77-0; **75**, 123242-04-6; **75**·HCl, 123241-78-1; **76**, 123242-05-7; **76**·HCl, 123241-79-2; **77**, 123241-87-2; **77**·HCl, 123241-80-5; **78**, 123241-88-3; **78**·HCl, 123241-81-6; **79**, 1899-48-5; **79**·HCl, 123241-82-7; **80**, 119584-70-2; **80**·HCl, 123241-83-8; **81**, 17511-21-6; **81**·HCl, 123241-84-9; PhC≡CH, 536-74-3; 2,6-dinitro-1-chlorobenzene, 606-21-3; 2-methyl-6-nitrobenzonitrile, 1885-76-3; chloroformamidine hydrochloride, 29671-92-9; 2-amino-6-fluorobenzonitrile, 77326-36-4.