Synthesis and Evaluation of 6-Arylacetamido-2,4-diaminoquinazolines and Related Compounds as Folic Acid Antagonists†

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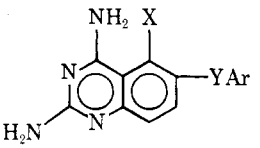
A series of 2,4-diaminoquinazolines bearing an aryl function attached to the 6 position through an acetamido or related linkage was synthesized. Each compound was evaluated as an inhibitor of rat liver dihydrofolate reductase as well as for suppressive antimalarial effects against *Plasmodium berghei* in mice. Significant *in vivo* activity was found to reside primarily with 5-chloro-6-arylacetamido derivatives. Most of these compounds were also tested for prophylactic activity against sporozoite-induced *Plasmodium gallinaceum* in chicks. Thirteen compounds, each of which possesses a 5-Cl or 5-CH_3 group, displayed curative activity in this test system. Since several of these showed markedly greater potency against the avian infection, selective inhibitory action upon preerythrocytic forms of the malaria parasite is thus implied.

With the emergence of chloroquine-resistant strains of *Plasmodium falciparum*, interest was rekindled in the development of new folic acid antagonists as potential antimalarial agents. The enzyme dihydrofolate reductase has served as the principal target for compounds of this class although other folate-dependent enzymes are often involved. Recently, quinazoline derivatives have received considerable attention in the quest for new antiprotozoan agents.[1] With respect to antimalarial activity, the following structure–activity patterns have evolved for compounds of this type: (1) as in the case for pyrimidines and pteridines, the 2,4-diamino configuration affords optimal activity;[2,3] (2) an aromatic substituent is usually necessary for potent activity and it should be attached to the 6 position of the quinazoline ring rather than being located at positions 5, 7, or 8;[3,4] (3) in some instances, significant activity enhancement can be realized by the introduction of a small hydrophobic group (Cl or CH_3) at position 5;[2] (4) a variety of spacers may be employed in bridging the aryl group to the quinazoline nucleus as exemplified in structure **1**.[5-7]



1, X = H, Cl, CH_3
Y = S, SO, SO_2, CH_2NH, $NHCH_2$, O

It was of interest, therefore, to learn that 6-arylcarboxamido-2,4-diaminoquinazolines such as **31** and **32** (*cf.* Table I) display only modest if any antimalarial effects.[8] Since such arylamides possess a high degree of structural ridigity, it was of interest to investigate the effect of adding methylene, oxymethylene, or thiomethylene units to the amide bridge upon antimalarial activity and upon inhibitory potency against rat liver dihydrofolate reductase. The greater flexibility thereby introduced might be expected to facilitate interaction of the aromatic moiety and the hydrophobic bonding region of this enzyme.

Chemistry. The target amides were prepared by reacting the appropriate acid chloride with 2,4,6-triaminoquinazoline,[9] 5-methyl-2,4,6-triaminoquinazoline,[9] 5-chloro-2,4,6-triaminoquinazoline,[9] or 6-aminoethyl-2,4-diaminoquinazoline.[10] The physical properties of these compounds are summarized in Table I. It was generally necessary to use an excess of acid chloride in order to obtain a reasonably complete reaction, in particular when 5-chloro-2,4,6-triaminoquinazoline was employed. The use of hydrogen chloride acceptors such as triethylamine or AW-500 molecular sieves did not significantly improve the yields of several of these reactions. However, triethylamine was effective in the case of the more basic substrate 6-aminomethyl-2,4-diaminoquinazoline.

Biological Results. Each of the compounds was evaluated as an inhibitor of rat liver dihydrofolate reductase and the results are presented in Table I. It will be seen that the two benzamides **31** and **32** are particularly ineffective in this regard being even less inhibitory than 2,4-diaminoquinazoline itself.[11] The interjection of a methylene group adjacent to the quinazoline ring enhances activity affording moderately effective inhibitors (**28–30**). The addition of a second methylene on the opposite side of the amide linkage produces a significantly less inhibitory compound, **27**. Conversely, the arylacetamido configuration (**2–17**) led to a group of compounds containing some remarkably effective inhibitors. In fact, several of these are as potent as any nonclassical quinazolines evaluated thus far in this laboratory.[12] That compounds of this type are superior inhibitors compared with their benzamidomethyl counterparts is fortified by the fact that **2** and **5** are approximately threefold more inhibitory than their isomers **28** and **29**, while **8** is some 20-fold more effective than **30**. Further extension of the bridge on the aryl side with methylene, oxygen, or sulfur (**18–25**) either has a negligible effect or causes a modest dimunition in activity. For example, **18**, **19**, and **20** are only slightly less inhibitory than their lower homologs **2**, **3**, and **4**. Similarly, **3** and **25** which differ only in the presence of an oxygen atom have virtually identical I_{50}'s. Likewise, the inclusion of a sulfur atom produces little change for the 2-naphthyl derivatives (**8** *vs.* **23**), while the oxygen counterpart **22** is threefold less effective.

In general, the presence of hydrophobic group at **5** causes a modest enhancement of inhibitory potency but there is no definite pattern as to whether chloro or methyl is more effective. Unfortunately, not all modifications containing each aryl moiety were prepared after it became apparent that the 5-chloro derivatives were of prime interest as potential antimalarial agents.

With the exception of **31** and **32**, each of the compounds in Table I was tested for suppressive antimalarial action against *Plasmodium berghei* in mice.[13,‡] It is noteworthy that none of these compounds produced toxic deaths at any dose level employed. Data obtained for compounds displaying significant activity are presented in Table II. Interestingly, with the exception of **13**, which bears a 5-CH_3 group and displays only a modest level of activity, each of

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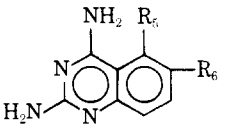
‡Testing of all compounds was carried out by the Rane Laboratory, University of Miami.

Table I. Properties of 6-Substituted Quinazolines Synthesized



No.	R_5	R_6	Mp, °C	Rxn solvent[a]	Recrystn medium and no.[b]	Yield, %	Formula[c]	I_{50},[d] μM
2	H	3,4-$Cl_2C_6H_3CH_2CONH$–	238–240 dec	A	I	55	$C_{16}H_{13}Cl_2N_5O \cdot H_2O$	0.031
2·HCl	H	3,4-$Cl_2C_6H_3CH_2CONH$–	321–323 dec		II		$C_{16}H_{14}Cl_3N_5O \cdot 1.5H_2O$	
3	Cl	3,4-$Cl_2C_6H_3CH_2CONH$–	248–250 dec	A	I	48	$C_{16}H_{12}Cl_3N_5O$	0.024
4	CH_3	3,4-$Cl_2C_6H_3CH_2CONH$–	247–249	A	IV	64	$C_{17}H_{15}Cl_2N_5O \cdot 0.75H_2O$	0.017
5	H	3-$(CF_3)C_6H_4CH_2CONH$–	220–221	A	I	59	$C_{17}H_{14}F_3N_5O$	0.096
6	Cl	3-$(CF_3)C_6H_4CH_2CONH$–	241–242	A	I	39	$C_{17}H_{13}ClF_3N_5O$	0.023
7	CH_3	3-$(CF_3)C_6H_4CH_2CONH$–	232–233	A	I	53	$C_{18}H_{16}F_3N_5O \cdot 0.5H_2O$	0.059
8	H	2-$C_{10}H_7CH_2CONH$–	252–253	A	I	72	$C_{20}H_{17}N_5O$	0.0050
9	Cl	2-$C_{10}H_7CH_2CONH$–	252–253	A	I	53	$C_{20}H_{16}ClN_5O$	0.0050
10	CH_3	2-$C_{10}H_7CH_2CONH$–	253–253.5	A	I	73	$C_{21}H_{19}N_5O$	0.0042
11	H	4-$ClC_6H_4CH_2CONH$–	259–260	A	I	57	$C_{16}H_{14}ClN_5O$	0.023
12	Cl	4-$ClC_6H_4CH_2CONH$–	273–274	A	I	46	$C_{16}H_{13}Cl_2N_5O$	0.012
13	CH_3	4-$ClC_6H_4CH_2CONH$–	269–271 dec	A	III	59	$C_{17}H_{16}ClN_5O \cdot H_2O$	0.033
14	Cl	4-$BrC_6H_4CH_2CONH$–	277–279 dec	A	IV	56	$C_{16}H_{13}BrClN_5O$	0.013
15	CH_3	4-$BrC_6H_4CH_2CONH$–	276–279 dec	A	III	69	$C_{17}H_{16}BrN_5O$	0.032
16	Cl	3-$BrC_6H_4CH_2CONH$–	251–252	B	III	52	$C_{16}H_{14}BrClN_5O$	0.0075
17	CH_3	3-$BrC_6H_4CH_2CONH$–	238.5–240	A	III, I	47	$C_{17}H_{16}BrN_5O \cdot H_2O$	0.034
18	H	4-$ClC_6H_4(CH_2)_2CONH$–	258–261 dec	A	I	26	$C_{17}H_{16}ClN_5O$	0.047
19	Cl	4-$ClC_6H_4(CH_2)_2CONH$–	247.5–248.5	B	III	36	$C_{17}H_{15}Cl_2N_5O$	0.036
20	CH_3	4-$ClC_6H_4(CH_2)_2CONH$–	271.5–273 dec	A	III	48	$C_{18}H_{18}ClN_5O$	0.033
21	Cl	2-$C_{10}H_7OCH_2CONH$–	262–265 dec	A	III (2)	38	$C_{20}H_{14}ClN_5O_2$	0.014
22	CH_3	2-$C_{10}H_7OCH_2CONH$–	246–249 dec	A	III	52	$C_{21}H_{19}N_5O_2 \cdot 0.5H_2O$	0.015
23	CH_3	2-$C_{10}H_7SCH_2CONH$–	247–249 dec	A	III	36	$C_{21}H_{19}N_5OS$	0.0054
24	CH_3	2-$C_{10}H_7SCH(CH(CH_3)_2)CONH$–	232–234	A	I (2)[e]	26	$C_{24}H_{25}N_5OS \cdot 0.75H_2O$	0.021
25	Cl	3,4-$Cl_2C_6H_3OCH_2CONH$–	295 dec	A	I	53	$C_{16}H_{12}Cl_3N_5O_2$	0.023
26	Cl	$C_6H_5CH(CH_3)CONH$–	207–209	A	I	29	$C_{17}H_{16}ClN_5O \cdot 0.5H_2O$	0.017
27	H	3,4-$Cl_2C_6H_3CH_2CONHCH_2$–	254–256	C	I	36	$C_{17}H_{15}Cl_2N_5O$	1.5
28	H	3,4-$Cl_2C_6H_3CONHCH_2$–	256–256.5	C	I	45	$C_{16}H_{13}Cl_2N_5O$	0.10
29	H	3-$(CF_3)C_6H_4CONHCH_2$–	244–246	C	V, I	50	$C_{17}H_{14}F_3N_5O$	0.26
30	H	2-$C_{10}H_7CONHCH_2$–	272–272.5	C	V, I	59	$C_{20}H_{17}N_5O$	0.10
31	H	3,4-$Cl_2C_6H_3CONH$–	289–291	A	I	51	$C_{15}H_{11}Cl_2N_5O \cdot 1.5H_2O$	>20
32	H	4-ClC_6H_4CONH–	277–278	A	I	46	$C_{15}H_{12}ClN_5O \cdot 0.75H_2O$	>20

[a] A, DMF; B, THF; C, DMF-Et_3N. [b] I, 2-methoxyethanol-H_2O containing excess concentrated NH_4OH; II, MeOH; III, DMF-H_2O containing excess concentrated NH_4OH; IV, 2-methoxyethanol-DMF-H_2O containing excess concentrated NH_4OH; V, AcOH. [c] *Anal.* C, H, and N. Several of these compounds analyzed correctly as hydrates or fractional hydrates even after drying *in vacuo* at 100°. Similar findings have been reported with related 2,4-diaminoquinazolines.[2,5] [d] Concentration required to produce 50% inhibition of rat liver dihydrofolate reductase. Assayed spectrophotometrically (340 mμ) with 9 μM dihydrofolate, 30 μM NADPH, and 0.15 M KCl in 0.05 M Tris buffer (pH 7.4). Under these conditions the value for pyrimethamine was 0.07 μM.[11] [e] Reprecipitation.

the active compounds is a 5-chloro-6-arylacetamidoquinazoline. Four of these (**6, 12, 14,** and **26**) produced cures at one or more dose levels.

All but five compounds (**2, 11, 20, 31,** and **32**) were also tested against sporozoite-induced *Plasmodium gallinaceum* in chicks.[‡] Since this infection is introduced by intrajugular injection of sporozoites followed by subcutaneous administration of the test drug on the same day, activity in this system is indicative of prophylactic effects.[14] Thirteen compounds displayed curative activity and the results obtained are summarized in Table III. With the exception of **19** and **22,** which display only modest activity, each is a member of the 6-arylacetamido class possessing a chlorine or methyl in the 5 position. With respect to the 5-Cl derivatives, it is of interest to note that compounds **3, 9,** and **16** produce no cures against the murine infection while **19** was totally inactive. Nevertheless, each of these was curative at two or more dose levels in the avian system with the results obtained for **16** being particularly impressive. The contrast is even more striking in the case of the active 5-CH_3 compounds (**10, 13, 15, 17,** and **22**), since only **13** showed even a modicum of activity against the *P. berghei* infection. Selective action against preerythocytic forms of the malaria parasite is thus implied for certain members of this series and, therefore, additional testing appears warranted. It should be noted, however, that certain other antifols share this peculiar activity pattern.[§]

Experimental Section

All analytical samples were vacuum dried at 100° (P_2O_5) and gave combustion values for C, H, and N within ±0.4% of the theo-

[§] E. A. Steck, personal communication.

Table II. Compounds Displaying Activity against *Plasmodium berghei* in Mice

Compd	ΔMST (days) after single sc dose (mg/kg)[a,b]					
	20	40	80	160	320	640
3	0.3	0.7	1.1	3.9	7.1	8.7
6	1.7	4.1	6.9	10.5	9.9 (3C)	17.9 (4C)
9	0.3	0.5	0.7	2.1	3.3	5.1
12	2.9	8.5	10.1	13.1	12.9 (2C)	13.9 (2C)
13	0.3	0.3	0.5	1.1	1.3	5.7
14	0.5	1.7	4.9	5.1	9.3	15.9 (4C)
16	0.5	1.7	2.1	3.9	6.7	13.5
26	4.1	5.1	5.5	9.2 (1C)	9.6 (2C)	9.9 (4C)

[a] ΔMST represents the increase in mean survival time *vs.* controls. Mean survival time for controls, 6.1 days. [b] Mice surviving for 60 days are considered cured and are designated C. Testing of all compounds was carried out by the Rane Laboratory, University of Miami.

retical values. Melting points were determined with a Fisher-Johns or a Mel-Temp apparatus and are uncorrected. All compounds had ir spectra (Beckman IR-8) in agreement with their assigned structures and appeared free of significant impurities by tlc (Gelman SAF). Each of the requisite carboxylic acids or acid chlorides was obtained from commercial sources with the exception of 2-(β-naphthylthio)isovaleric acid, which was prepared as described below. Representative examples of the preparation of target amides are also presented.

6-(4-Bromophenylacetamido)-5-chloro-2,4-diaminoquinazoline (14). A mixture of 12.5 g (0.058 mol) of 4-bromophenylacetic acid, 11 ml of $SOCl_2$, and 24 ml of benzene was refluxed under protection from atmospheric moisture until HCl evolution had ceased (3.5 hr). The solvent and excess $SOCl_2$ were then removed *in vacuo* and the resulting acid chloride was used without further purification. To a solution of 3.0 g (0.0143 mol) of 5-chloro-2,4,6-triaminoquinazoline[9] in 10 ml of DMF was added dropwise 4.0 g (0.017 mol) of 4-bromophenylacetyl chloride in 5 ml of DMF. After stirring in the absence of moisture for 48 hr, the reaction was found to be incomplete by tlc (DMF–EtOAc 1:1). However, an additional 0.67 g (0.0028 mol) of acid chloride failed to improve the conversion. Therefore, the solid was separated by filtration and washed with DMF and then Me_2CO. Recrystallization from DMF–2-methoxyethanol–H_2O made basic with concentrated NH_4OH (charcoal) yielded a cream-colored solid which was separated on a filter and washed with 2-methoxyethanol, Me_2CO, MeOH, and Et_2O. After vacuum drying at 100° there was obtained 3.25 g (56%) of light yellow crystals, mp 277–279° dec.

2,4-Diamino-6-(2-naphthamidomethyl)quinazoline (30). To a stirred suspension of 1.88 g (0.01 mol) of 6-aminomethyl-2,4-diaminoquinazoline[10] in 1.36 g (0.0135 mol) of triethylamine and 10 ml of DMF was added slowly a solution of 2.29 g (0.012 mol) of 2-naphthoyl chloride in 4 ml of DMF. The mixture was then stirred at ambient temperature in a sealed vessel. After 2.25 hr, the product was precipitated by the addition of 60 ml of 2 *N* HCl. After stirring overnight, the solid was collected on a filter, washed with H_2O, and recrystallized from glacial AcOH. The purified salt thus obtained was washed with Me_2CO and then recrystallized from 2-methoxyethanol–H_2O containing excess concentrated NH_4OH. There was obtained, after drying *in vacuo* at 100°, 2.0 g (59%) of cream-colored crystals, mp 272° with preliminary softening.

2-(β-Naphthylthio)isovaleric Acid. Equimolar quantities (0.072) of ethyl 2-bromovalerate, β-naphthalenethiol, and K_2CO_3 were allowed to react at ambient temperature in 70 ml of DMF. After 2 hr the mixture was added to H_2O and the resulting suspension extracted twice with EtOAc. The extracts were combined, washed with 10% $NaHCO_3$, 0.5 *N* HCl, and H_2O, treated with charcoal, and dried over $MgSO_4$, and then the solvent was removed *in vacuo*. The resulting oil was extracted with MeOH and some insoluble material was removed by filtration. The MeOH was then removed *in vacuo* to yield a yellow oil which was saponified by refluxing in a mixture of 100 ml of EtOH and 35 ml of 2 *N* NaOH for 3 hr. Acidification with 2 *N* HCl and dilution with H_2O caused the product to separate as a gummy oil which gradually solidified after scratching. The solid was collected on a filter, washed with H_2O, and dried *in vacuo* over P_2O_5. There was obtained 17.05 g (90% overall) of white crystalline solid, mp 77–79° (lit.[15] mp 78.5–79.5°).

Table III. Prophylactic Antimalarial Effects of Compounds Active against Sporozoite-Induced *Plasmodium gallinaceum* in Chicks

Compd	ΔMST (days) after single sc dose (mg/kg)[a-c]					
	15	30	60	120	240	480
3	4.6	7.0	5C	5C	5C	5C
	1.5 (3C)		2.5 (4C)		5C	
6[d]	2.0 (3C)[e]	5C		5C		5C
9		3C		3C		4C
10		3C		5C		5C
	3C		4C		5C	
12	1.0 (3C)		1.5 (4C)		2.5 (4C)	
13		5C		5C		5C
14	2C	3C	3C	3C	4C	5C
15		2C		5C		5C
16[f,g]	11.7 (4C)	5C	5C	5C		5C
	3.4 (4C)	5C	5C	5C		
17		5C		5C		5C
19	1.1		2.2 (2C)		3.0 (3C)	
22		0		1.7 (2C)		6.9 (3C)
26		5C		5C		5C

[a] ΔMST is the mean survival time increase; mean survival time for controls, 7.5–8.3 days. [b] Chicks surviving for 30 days are considered cured and are designated C. Testing of all compounds was carried out by the Rane Laboratory, University of Miami. [c] Each entry at each dose level represents results with a five-bird group. [d] Inactive at 5 mg/kg. [e] 20 mg/kg. [f] 0.7 (2C) at 3.75 mg/kg. [g] 2.7 (3C) at 7.5 mg/kg.

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