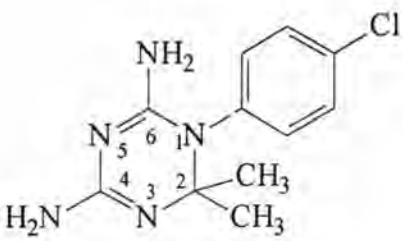
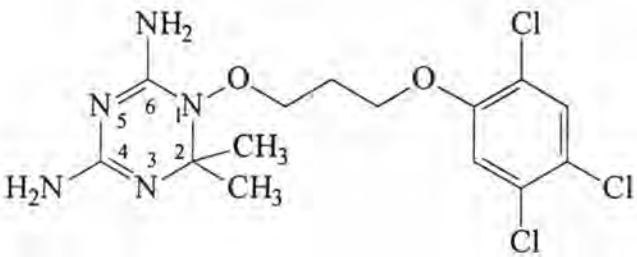
CHAPTER III
RESULTS AND DISCUSSION

The purpose of this study was to investigate the relationship between structure and biological activities of *Plasmodium falciparum* dihydrofolate reductase (DHFR) inhibitors belonging to the class 4,6-diamino-1,2-dihydro-1,3,5-triazine especially cycloguanil (Cyc) and WR99210 and their analogues.



Cycloguanil



WR99210

Scheme 3.1 The structures of cycloguanil and WR99210

The study focused on the synthesis of a library of Cyc and WR99210 derivatives by varying substituents at N-1 position of the dihydrotriazine ring including aryl (Ar), alkyl (R) and alkyloxy (OR) groups. At the same time, substituent effect at the C-2 position was investigated by replacing one or both methyl groups in cycloguanil and WR99210 with H or alkyl groups. These compounds were tested against both wild-type and A16V+S108T mutant *Plasmodium falciparum* dihydrofolate reductases (pfDHFRs) in order to find a better inhibitor for both enzymes and to study their structure-activity relationships. Details of which will be discussed below.

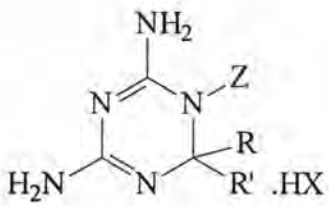
3.1 Synthesis of 4,6-diamino-1,2-dihydro-1,3,5-triazines

The derivatives of 4,6-diamino-1,2-dihydro-1,3,5-triazine synthesized in this study have been divided into three main classes (Scheme 3.2):

i. 1-Aryl-4,6-diamino-1,2-dihydro-1,3,5-triazines (Z = aryl)

ii. 1-Alkyl-4,6-diamino-1,2-dihydro-1,3,5-triazines (Z = alkyl)

iii. 1-Alkyloxy-4,6-diamino-1,2-dihydro-1,3,5-triazines (Z = *O*-alkyl)



Z : aryl, alkyl, alkyloxy

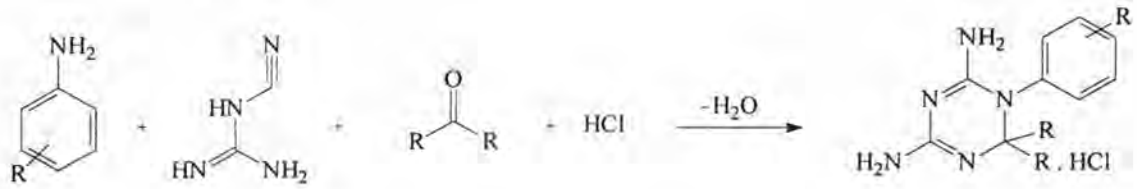
R, R' : H, alkyl, aryl

Scheme 3.2 The structure of 4,6-diamino-1,2-dihydro-1,3,5-triazine derivatives

1-Aryl and 1-alkyl-4,6-diamino-1,2-dihydro-1,3,5-triazine derivatives have been prepared by many methods such as three-component condensation,[31] two-component condensation[32] or the reaction of Schiff base with dicyanodiamide.[33] 1-Alkyloxy-4,6-diamino-1,2-dihydro-1,3,5-triazine derivatives may be prepared by two-component condensation[32] or by alkylation of 1-hydroxy-4,6-diamino-1,2-dihydro-1,3,5-triazine with alkyl halides.[17,37] Before these compounds can be successfully synthesized, we need to find an appropriate method and conditions for preparing them.

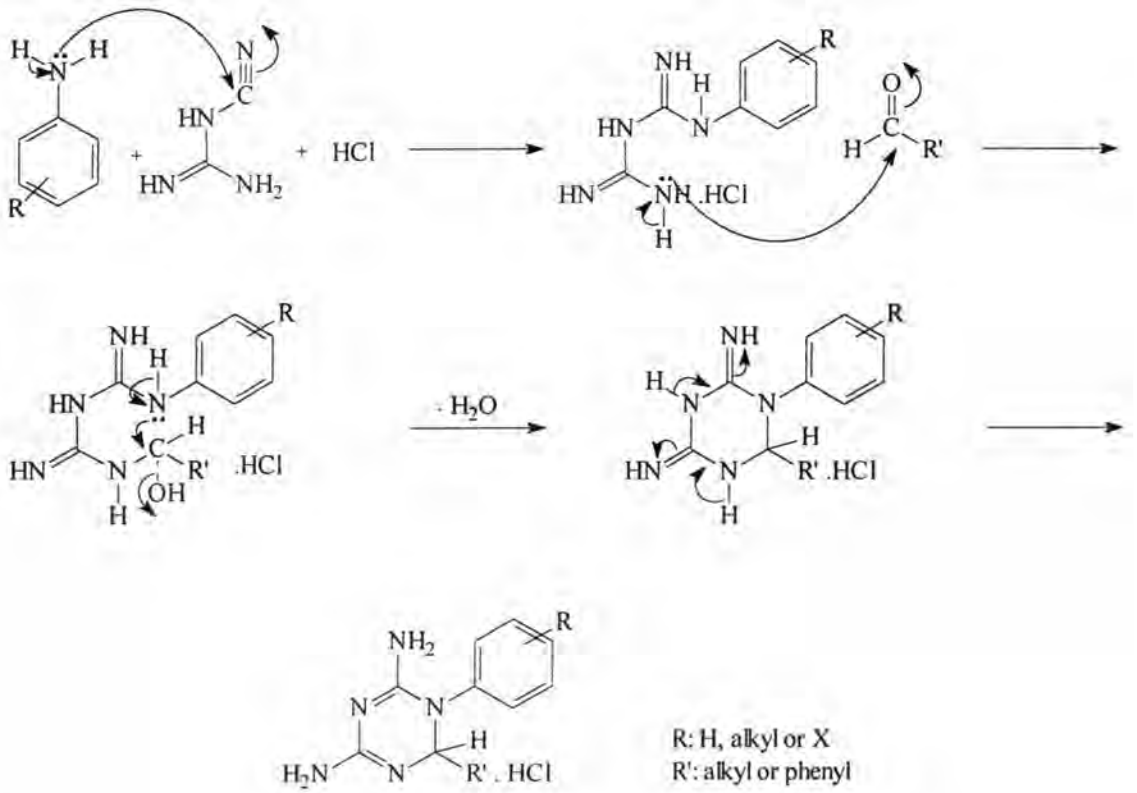
3.1.1 Synthesis of 1-aryl-4,6-diamino-1,2-dihydro-1,3,5-triazines

Compounds **II-1** to **II-4** were prepared by a classical three-component condensation,[31] which is a one-step condensation, between aromatic amine, dicyanodiamide and acetone in the presence of concentrated HCl (or *p*-toluenesulfonic acid) (Scheme 3.3).



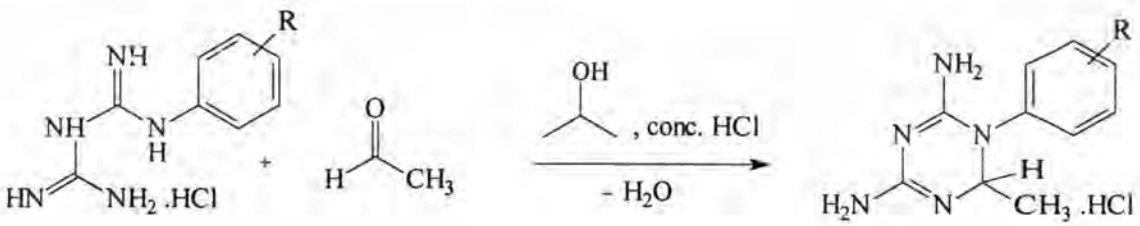
Scheme 3.3 The synthesis of 1-aryl-4,6-diamino-1,2-dihydro-1,3,5-triazines by three-component condensation

Compounds **II-6** to **II-65** were generally made in two steps. The aromatic amines were reacted in the presence of concentrated HCl to first generate arylbiguanides which were then reacted with the appropriate aldehydes in the presence of concentrated HCl as catalyst (two-component condensation)[32] to give compounds **II-6** to **II-65**. The method was, however, unsuccessful for compounds **II-20**, **II-41**, **II-46** and **II-47** (Scheme 3.4).



Scheme 3.4 The synthesis of 1-aryl-4,6-diamino-1,2-dihydro-1,3,5-triazines by two-component condensation

It was therefore necessary to find an appropriate condition to prepare them by variation of conditions such as solvent, reactant, temperature, equivalent of conc. HCl and addition of miscible water scavenger such as triethyl orthoacetate. It was found that addition of water scavenger is not necessary for reactions involving aldehydes, which is in sharp contrast to ketones indicating the difference in their reactivities, was studied by Vilaivan and Saesaengseerung.[41] The best conditions for synthesis of compounds **II-20** and **II-41**, 1-(4′-ethylphenyl)- and 1-(3′-chlorophenyl)-2-methyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride, were found to be: 1 eq arylbiguanide hydrochloride, 3 eq acetaldehyde, 5 mL absolute isopropanol and 0.069 mL conc. HCl at room temperature (Scheme 3.5).

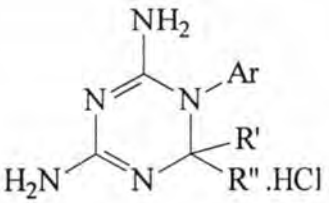


R: 4-ethyl, 3-chloro

Scheme 3.5 The reaction of 4-ethyl and 3-chlorophenylbiguanide hydrochloride with acetaldehyde

Compounds **II-46** and **II-47**, 1-(3′-chlorophenyl)-2-pentyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride and 1-(3′-chlorophenyl)-2-heptyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride, were similarly prepared using absolute isopropanol as a solvent. It was reasoned that isopropanol is a sterically hindered alcohol and is therefore less likely to form acetals with the aldehyde component, thus less likely to decrease the effective concentration of the aldehyde during the cyclization than methanol or ethanol.

Table 3.1 Tabulated percent yield and mass of 1-aryl-4,6-diamino-1,2-dihydro-1,3,5-triazine derivatives



Cmpd	Ar	R′	R″	Yield (%)	M.H$^+$
II-1[a]	3-$HO_2CC_6H_4$	CH_3	CH_3	79	262
II-2[a]	4-$HO_2CC_6H_4$	CH_3	CH_3	86	262
II-3	3-ClC_5H_4	CH_3	CH_3	79	252
II-4	4-Cl-3$NO_2C_6H_3$	CH_3	CH_3	75	297
II-6	Ph	H	CH_3	51	204
II-7	Ph	H	C_2H_5	37	218
II-8	Ph	H	*n*-C_3H_7	38	232
II-9	Ph	H	*i*-C_3H_7	32	232
II-10	Ph	H	*n*-C_4H_9	18	246
II-11	Ph	H	C_6H_{11}	60	272
II-12	Ph	H	Ph	78	266
II-13	4-$CH_3C_6H_4$	H	CH_3	27	218
II-14	4-$CH_3C_6H_4$	H	C_2H_5	77	232
II-15	4-$CH_3C_6H_4$	H	*n*-C_3H_7	83	246
II-16	4-$CH_3C_6H_4$	H	*i*-C_3H_7	85	246
II-17	4-$CH_3C_6H_4$	H	*n*-C_4H_9	59	260
II-18	4-$CH_3C_6H_4$	H	C_6H_{11}	59	286
II-19	4-$CH_3C_6H_4$	H	Ph	88	280
II-20	4-$C_2H_5C_6H_4$	H	CH_3	43	232
II-21	4-$C_2H_5C_6H_4$	H	C_2H_5	72	246
II-22	4-$C_2H_5C_6H_4$	H	*n*-C_3H_7	63	260
II-23	4-$C_2H_5C_6H_4$	H	*i*-C_3H_7	62	260
II-24	4-$C_2H_5C_6H_4$	H	*n*-C_4H_9	54	274

Table 3.1 Continued

Cmpd	Ar	R′	R″	Yield (%)	$M.H^+$
II-25	4-$C_2H_5C_6H_4$	H	C_6H_{11}	24	300
II-26	4-$C_2H_5C_6H_4$	H	Ph	78	294
II-27	4-ClC_6H_4	H	CH_3	49	238
II-28	4-ClC_6H_4	H	C_2H_5	92	252
II-29	4-ClC_6H_4	H	*n*-C_3H_7	86	266
II-30	4-ClC_6H_4	H	*i*-C_3H_7	98	266
II-31	4-ClC_6H_4	H	*n*-C_4H_9	89	280
II-32	4-ClC_6H_4	H	C_6H_{11}	95	306
II-33	4-ClC_6H_4	H	Ph	85	300
II-34	4-BrC_6H_4	H	CH_3	53	282
II-35	4-BrC_6H_4	H	C_2H_5	93	296
II-36	4-BrC_6H_4	H	*n*-C_3H_7	74	310
II-37	4-BrC_6H_4	H	*i*-C_3H_7	71	310
II-38	4-BrC_6H_4	H	*n*-C_4H_9	87	324
II-39	4-BrC_6H_4	H	C_6H_{11}	90	350
II-40	4-BrC_6H_4	H	Ph	93	344
II-41	3-ClC_6H_4	H	CH_3	76	238
II-42	3-ClC_6H_4	H	C_2H_5	96	252
II-43	3-ClC_6H_4	H	*n*-C_3H_7	78	266
II-44	3-ClC_6H_4	H	*i*-C_3H_7	79	266
II-45	3-ClC_6H_4	H	*n*-C_4H_9	66	280
II-46	3-ClC_6H_4	H	*n*-C_5H_{11}	56	294
II-47	3-ClC_6H_4	H	*n*-C_7H_{15}	39	322
II-48	3-ClC_6H_4	H	C_6H_{11}	48	306
II-49	3-ClC_6H_4	H	Ph	99	300
II-50	2,4-$Cl_2C_6H_3$	H	CH_3	41	272
II-51	2,4-$Cl_2C_6H_3$	H	C_2H_5	93	286
II-52	2,4-$Cl_2C_6H_3$	H	*n*-C_3H_7	75	300
II-53	2,4-$Cl_2C_6H_3$	H	*i*-C_3H_7	92	300
II-54	2,4-$Cl_2C_6H_3$	H	*n*-C_4H_9	50	314

Table 3.1 Continued

Cmpd	Ar	R′	R″	Yield (%)	M.H$^+$
II-55	2,4-$Cl_2C_6H_3$	H	C_6H_{11}	90	340
II-56	2,4-$Cl_2C_6H_3$	H	Ph	75	334
II-57	3,4-$Cl_2C_6H_3$	H	CH_3	76	272
II-58	3,4-$Cl_2C_6H_3$	H	C_2H_5	91	286
II-59	3,4-$Cl_2C_6H_3$	H	*n*-C_3H_7	82	300
II-60	3,4-$Cl_2C_6H_3$	H	*i*-C_3H_7	79	300
II-61	3,4-$Cl_2C_6H_3$	H	*n*-C_4H_9	97	314
II-62	3,4-$Cl_2C_6H_3$	H	*n*-C_5H_{11}	84	328
II-63	3,4-$Cl_2C_6H_3$	H	*n*-C_7H_{15}	36	356
II-64	3,4-$Cl_2C_6H_3$	H	C_6H_{11}	81	340
II-65	3,4-$Cl_2C_6H_3$	H	Ph	100	334

[a]*p*-TsOH salt.

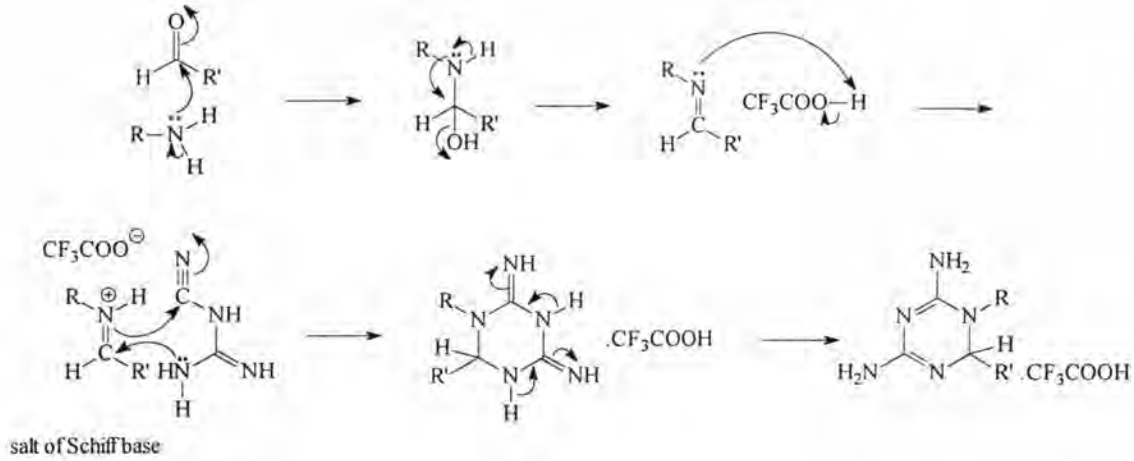
Thus compounds **II-1** to **II-65** have been successfully prepared in the form of crystalline hydrochloride or *p*-toluenesulfonate salts (Table 3.1). All compounds were purified by recrystallization from aqueous alcohol or alcohol-ether. ^{1}H NMR and mass spectra confirmed that the compounds have been successfully prepared. UV spectra of some representative compounds including 1-(4′-chlorophenyl)-2-butyl- **(II-31)**, 1-(3′-chlorophenyl)-2-methyl- **(II-41)**, 1-(4′-ethylphenyl)-2-isopropyl- **(II-23)** and 1-(3′,4′-dichlorophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(II-65)** showed a maximum absorption at 243, 244, 247 and 252 nm respectively, characteristic for N-1 substituted diaminodihydro-1,3,5-triazine.[31] It is therefore reasonable to conclude that the all compounds prepared are in the correct N-1 substituted form and not the isomeric N-4 substituted form.

3.1.2 Synthesis of 1-alkyl-4,6-diamino-1,2-dihydro-1,3,5-triazines

1-Benzyl-2-alkyl or 2-phenyl or 2,2-dimethyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride has been prepared by two-component condensation between benzylbiguanide hydrochloride **(I-13)** and the appropriate carbonyl compound at 153-165 °C according to the method of Shapiro et al.,[42-43] in the presence of concentrated

HCl. However, the reaction was reported to give poor yield and we confirmed that this is true. Variation of solvents, temperature, equivalent of conc. HCl and addition of a miscible water scavenger such as triethyl orthoacetate did not give the required products.

Another literature method to synthesize dihydrotriazine involves a condensation reaction between Schiff bases hydrochloride with dicyanodiamide in DMF.[33] Generally gaseous HCl is required to generate the iminiun salt and thus are not practical for our purposes. Many acid catalyst was tried including conc. HCl, methane sulfonic acid and trifluoroacetic acid, and it was found that trifluoroacetic acid gave the best results. Thus a reaction of *N*-benzylidinebenzylamine with dicyanodiamide[33] in the presence of trifluoroacetic acid in DMF-MeCN mixture gave 1-benzyl-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine **(II-98)** as trifluoroacetate salt in 62% yield. The structure of the product was confirmed by ^{1}H NMR, MALDI-TOF MS and x-ray crystallography. Compounds **II-73** to **II-113** and **II-120** to **II-121** were then prepared in the same way. They were generally made in two steps from alkyl amines and appropriate aromatic aldehydes in dichloromethane containing $MgSO_4$ as water scavenger to first generate the imines or Schiff bases. The imines were then treated with trifluoroacetic acid in acetonitrile to generate the protonated Schiff bases which were reacted with dicyanodiamide in dimethylformamide at room temperature to give 1-alkyl-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate (Scheme 3.6). It is interesting to note that the reaction works well only with aromatic aldehydes. Imines derived from simple aliphatic aldehydes gave no desired product after condensation with dicyanodiamide under the same conditions. Therefore only derivatives bearing C_2-phenyl groups were synthesized in this study.

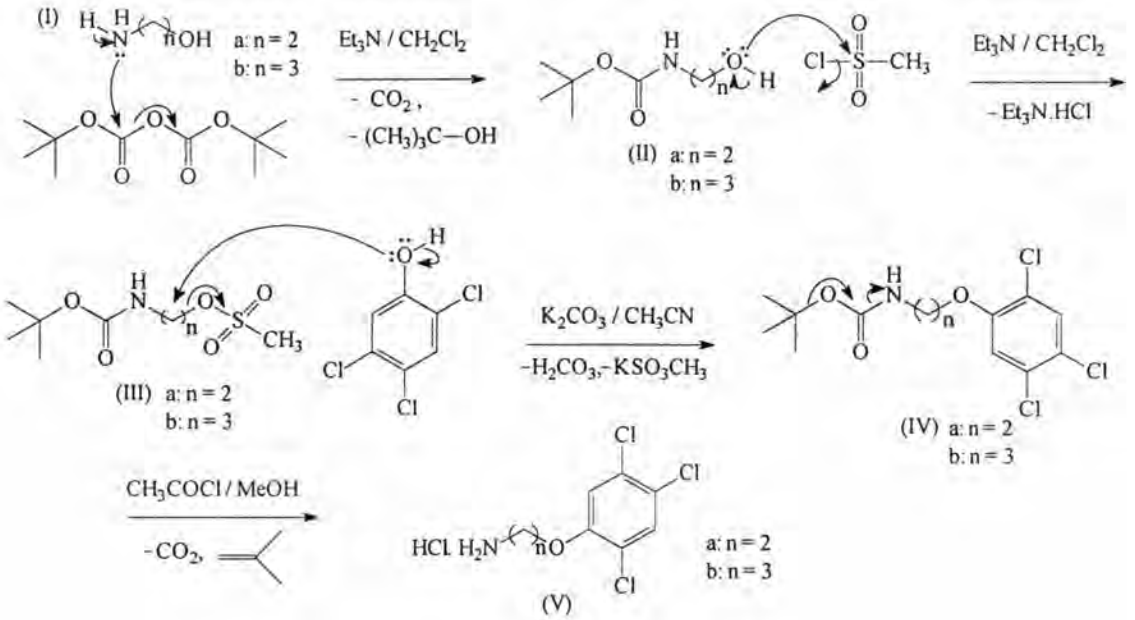


Scheme 3.6 Reaction mechanism for the synthesis of 1-alkyl-4,6-diamino-1,2-dihydro-1,3,5-triazines by the reaction of Schiff bases with dicyanodiamide

Compound **II-119**, bis-(2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine-1-yl) ethane bistrifluoroacetate, was prepared from ethylenediamine by first reacting with 2 equivalents of benzaldehyde in dichloromethane to generate the bis-imine, the structure of which was confirmed by ^{1}H NMR. The bis-imine was then reacted with 2 equivalents of trifluoroacetic acid in acetonitrile followed by addition of 2 equivalents of dicyanodiamide in dimethylformamide at room temperature. The structure of product was confirmed by ^{1}H NMR, which indicated double doublet signals of the methylene proton at 3.19 and 3.92 ppm and a singlet signal of C-2 proton at 5.87 ppm in addition to the aromatic signals. If the product were not the expected bis-compound, four triplet of doublet signals due to the presence of 4 inequivalent methylene protons would have been observed.

Since 2,4,5-trichlorophenoxypropyloxy side chain of WR99210 gave interesting activities, it is therefore of interest to mimic that side chain to study the role of the oxygen atom directly attached to N-1 in WR99210. For the synthesis of compounds **II-120d** and **II-121d**, 2-(2′,4′,5′-trichlorophenoxy)ethylamine and 3-(2′,4′,5′-trichlorophenoxy)propylamine were required as starting materials. Aminoalcohols (I) reacted with di-*tert*-butyl dicarbonate and triethylamine in dichloromethane at room temperature in order to protect the amino group with *t*-butoxycarbonyl (Boc) group (Scheme 3.7). The N-Boc amino alcohols (II) formed were then reacted with methanesulfonyl chloride and triethylamine in CH_2Cl_2 to activate the hydroxy group towards nucleophilic substitution by converting it into

methanesulfonyl derivatives (III). N-Boc methanesulfonylamines (III) were then reacted with 2,4,5-trichlorophenol and potassium carbonate in acetonitrile to give 2,4,5-trichlorophenoxyalkyl carbamic acid *tert*-butyl esters (IV), which were then reacted with methanolic HCl at room temperature to cleave the Boc-protecting group to give 2,4,5-trichlorophenoxylamine hydrochloride (V). Reactions of the amine hydrochloride with benzaldehyde/Et_3N gave the imines, which were further condensed with dicyanodiamide to give **II-120** and **II-121**, again as trifluoroacetate salts.

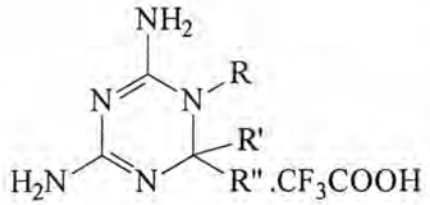


Scheme 3.7 Synthesis of 2-(2′,4′,5′-trichlorophenoxy)ethylamine hydrochloride **(II-120d)** and 3-(2′,4′,5′-trichlorophenoxy)propylamine hydrochloride **(II-121d)**

Compounds **II-73** to **II-121** have been successfully prepared in the form of crystalline trifluoroaectate salts (Table 3.2). All compounds were purified by recrystallization from aqueous alcohol or alcohol-diethyl ether. ^1H NMR and MALDI-TOF mass spectra confirmed that the desired compounds have been successfully prepared. Some derivatives, although formed as shown by TLC analysis, failed to crystallize on addition of ether. These compounds include 1-benzyl-2-(3′-hydroxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate **(II-114)**, 1-benzyl-2-(4′-hydroxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate **(II-115)**, 1-benzyl-2-(2′-hydroxy-3′-methoxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-

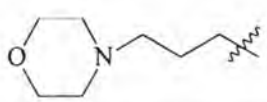
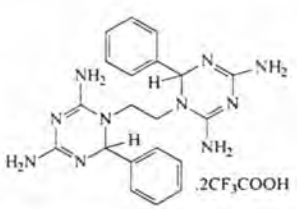
triazine trifluoroacetate **(II-116)** and 1-benzyl-2-(4′-hydroxy-3′-methoxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-triznine **(II-117)**. Attempts to synthesize 1-benzyloxy-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate **(II-118)** by the same method starting from *O*-benzylbenzaldoxime failed to give the desired product.

Table 3.2 Tabulated percent yield and mass of 1-alkyl-4,6-diamino-1,2-dihydro-1,3,5-triazine derivatives



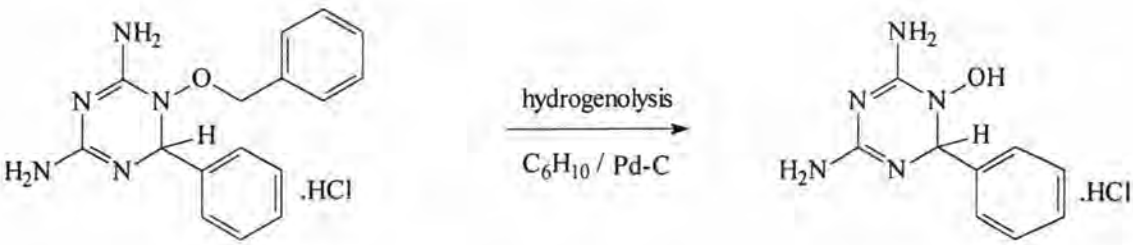
Cmpd	R	R′	R″	Yield (%)	M.H$^+$
II-73	$PhCH_2$-	H	2-$CH_3OC_6H_4$	76	310
II-74	$PhCH_2$-	H	3-$CH_3OC_6H_4$	74	310
II-75	$PhCH_2$-	H	4-$CH_3OC_6H_4$	64	310
II-76	$PhCH_2$-	H	2,4-$(CH_3O)_2C_6H_3$	50	340
II-77	$PhCH_2$-	H	2,5-$(CH_3O)_2C_6H_3$	74	340
II-78	$PhCH_2$-	H	3,4-$(CH_3O)_2C_6H_3$	57	340
II-79	$PhCH_2$-	H	3,5-$(CH_3O)_2C_6H_3$	74	340
II-80	$PhCH_2$-	H	3,4,5-$(CH_3O)_3C_6H_2$	65	370
II-81	$PhCH_2$-	H	3-FC_6H_4	71	298
II-82	$PhCH_2$-	H	4-FC_6H_4	52	298
II-83	$PhCH_2$-	H	2-ClC_6H_4	58	314
II-84	$PhCH_2$-	H	3-ClC_6H_4	54	314
II-85	$PhCH_2$-	H	4-ClC_6H_4	68	314
II-86	$PhCH_2$-	H	2,4-$Cl_2C_6H_3$	57	348
II-87	$PhCH_2$-	H	2,6-$Cl_2C_6H_3$	66	348
II-88	$PhCH_2$-	H	3,4-$Cl_2C_6H_3$	66	348
II-89	$PhCH_2$-	H	2-BrC_6H_4	45	358
II-90	$PhCH_2$-	H	4-BrC_6H_4	64	358
II-91	$PhCH_2$-	H	3-$NO_2C_6H_4$	80	325

Table 3.2 Continued

Cmpd	R	R′	R″	Yield (%)	M.H$^+$
II-92	$PhCH_2$-	H	4-$NO_2C_6H_4$	71	325
II-93	$PhCH_2$-	H	4-CNC_6H_4	82	305
II-94	$PhCH_2$-	H	2-Cl-5-$NO_2C_6H_3$	71	359
II-95	$PhCH_2$-	H	4-Cl-3-$NO_2C_6H_3$	73	359
II-96	$PhCH_2$-	H	2-Cl-6-FC_6H_3	100	332
II-97	$PhCH_2$-	H	4-^{t}BuC_6H_4	68	336
II-98	$PhCH_2$-	H	Ph	60	280
II-99	$PhCH_2CH_2$-	H	Ph	61	294
II-100	$PhCH_2CH_2CH_2$-	H	Ph	46	308
II-101	$CH_3OCH_2CH_2CH_2$-	H	Ph	51	262
II-102	$CH_3CH_2CH_2$-	H	Ph	57	232
II-103	$(CH_3)_2CH$-	H	Ph	9	232
II-104	$(CH_3)_2CHCH_2$-	H	Ph	53	246
II-105	$CH_3(CH_2)_6$-	H	Ph	82	288
II-106	$CH_3(CH_2)_9$-	H	Ph	30	330
II-107	$CH_3(CH_2)_{13}$-	H	Ph	26	386
II-108	$CH_3(CH_2)_{17}$-	H	Ph	59	442
II-109	C_6H_{11}-	H	Ph	30	272
II-110	$PhCH_2$-	H	3-$PhOC_6H_4$	53	372
II-111	$PhCH_2CH_2CH_2$-	H	3-$PhOC_6H_4$	61	400
II-112	$CH_3(CH_2)_9$-	H	3-$PhOC_6H_4$	46	422
II-113		H	Ph	63	317
II-119		-	-	20	406
II-120	2,4,5-$Cl_3C_6H_2O(CH_2)_2$-	H	Ph	100	412
II-121	2,4,5-$Cl_3C_6H_2O(CH_2)_3$-	H	Ph	27	426

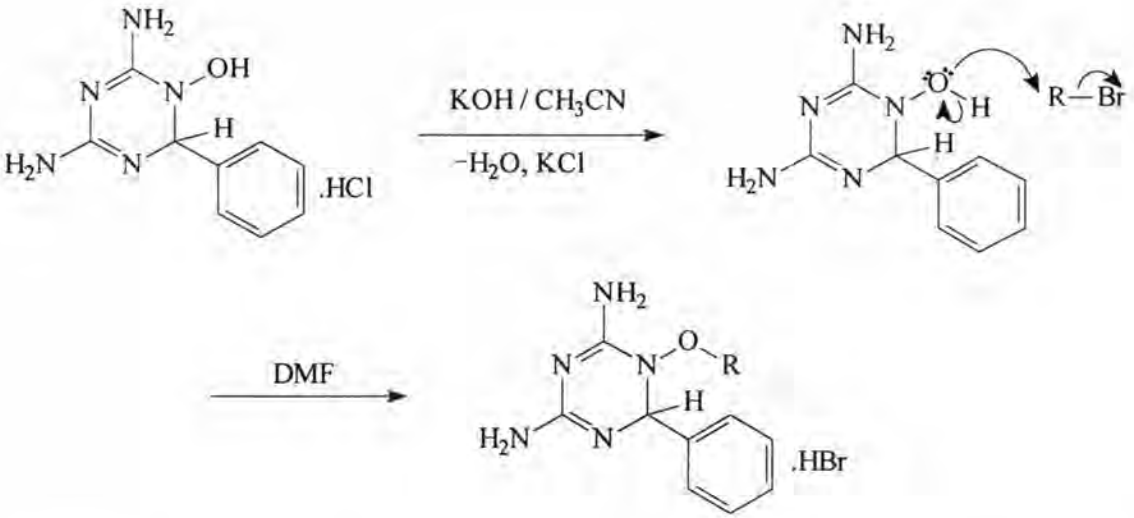
3.1.3 Synthesis of 1-alkyloxy-4,6-diamino-1,2-dihydro-1,3,5-triazines

Compounds **II-124** to **II-127** were prepared by two-component condensation between 1-benzyloxybiguanide **(I-14)**, which was generated from *o*-benzylhydroxylamine hydrochloride and dicyanodiamide,[42] and appropriate carbonyl compounds (aldehydes or ketones) in the presence of concentrated HCl.[16,34,40] Compound **II-128**, 1-hydroxy-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride, which is a common intermediate for all subsequent compounds in this series, was prepared by catalytic transfer hydrogenolysis of 1-benzyloxy-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(II-127)** in absolute methanol using Pd/C as catalyst and cyclohexene as a hydrogen donor (Scheme 3.8).



Scheme 3.8 Hydrogenolysis of 1-benzyloxy-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(II-127)**

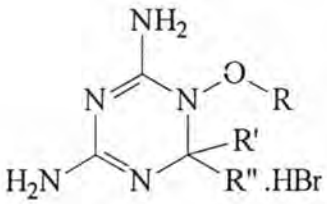
Compounds **II-129** to **II-151** were prepared by alkylation of 1-hydroxy-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine **(II-128)** free base, which could be prepared from the hydrochloride by treatment with methanolic NaOH, using an appropriate alkyl bromide in dimethylformamide[17,37] as shown in Scheme 3.9.



Scheme 3.9 Alkylation of 1-hydroxy-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(II-128)** and alkyl bromide

According to the method outlined above, compounds **II-124** to **II-151** have been successfully prepared in the form of crystalline hydrochloride or hydrobromide salt (Table 3.3). All compounds were purified by recrystallization from aqueous alcohol or alcohol-ether. ^{1}H NMR and mass spectra confirmed that the compounds have been successfully prepared. Some compounds not successfully prepared by this method include 1-(1′-Phenylcarbonylmethoxy)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrobromide **(II-152)**, 1-(2′-Hydroxy-2′-phenylethoxy)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrobromide **(II-153)**, 1-(3′-Cyanopropyloxy)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrobromide **(II-154)**, 1-(2′-Hydroxyethoxy)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrobromide **(II-155)**, *bis*-(2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine-2-yl)propoxy bishydrobromide **(II-156)**.

Table 3.3 Tabulated percent yield and mass of 1-alkyloxy-4,6-diamino-1,2-dihydro-1,3,5-triazine derivatives



Cmpd	R	R′	R″	Yield (%)	M.H$^+$
II-124[a,b]	$PhCH_2$-	H	CH_3CH_2-	39	248
II-125[a,b]	$PhCH_2$-	H	$CH_3(CH_2)_6$-	26	318
II-126[a,b]	$PhCH_2$-	-	-$(CH_2)_5$-	53	288
II-127[a,b]	$PhCH_2$-	H	Ph	59	296
II-128[a,c]	H	H	Ph	96	206
II-129	$PhCH_2CH_2$-	H	Ph	36	310
II-130	$Ph(CH_2)_3$-	H	Ph	56	324
II-131	2-$BrC_6H_4CH_2$-	H	Ph	25	374
II-132	3-$BrC_6H_4CH_2$-	H	Ph	69	374
II-133	4-$BrC_6H_4CH_2$-	H	Ph	64	374
II-134	4-$CH_3C_6H_4CH_2$-	H	Ph	61	310
II-135	2-NaphthylCH_2-	H	Ph	56	346
II-136	CH_3OCOCH_2-	H	Ph	68	278
II-137	CH_2=$CHCH_2$-	H	Ph	58	246
II-138	$CH_3CH_2CH_2$-	H	Ph	50	248
II-139	$CH_3(CH_2)_4$-	H	Ph	45	276
II-140	$CH_3(CH_2)_9$-	H	Ph	47	346
II-141	$(CH_3)_2CHCH_2$-	H	Ph	33	262
II-142	$(CH_3)_2CHCH_2CH_2$-	H	Ph	55	275
II-143	$C_6H_{11}CH_2$-	H	Ph	37	302
II-144	$BrCH_2CH_2CH_2$-	H	Ph	38	326
II-145	$PhOCH_2CH_2$-	H	Ph	75	326
II-146	$PhOCH_2CH_2CH_2$-	H	Ph	72	340
II-147	4-$ClC_6H_4OCH_2CH_2CH_2$-	H	Ph	83	374

Table 3.3 Continued

Cmpd	R	R′	R″	Yield (%)	M.H$^+$
II-148	4-$CH_3OCOC_6H_4O(CH_2)_3$-	H	Ph	53	398
II-149	4-$CH_3CONHC_6H_4O(CH_2)_3$-	H	Ph	82	397
II-150	4-$PhC_6H_4OCH_2CH_2CH_2$-	H	Ph	58	416
II-151	$PhSCH_2CH_2CH_2$-	H	Ph	77	356

[a]Hydrochloride salt. [b]By two-component condensation. [c]By hydrogenolysis.

3.2 Bioactivities of 4,6-diamino-1,2-dihydro-1,3,5-triazines

We have synthesized a number of Cyc and WR99210 derivatives and these were tested against both wild-type and A16V+S108T mutant pfDHFRs.

Table 3.4 summarizes the inhibition constant (K_i) values of Cyc and analogues, in which the substituents at the N-1 (aryl group) as well as the substituents at C-2 were modified. In recent years, Cyc **(1)** was reported[28] to bind with the wild-type pfDHFR with the K_i value of ~ 1.5 nM, but bound approximately 876-fold less tightly to the A16V+S108T mutant enzyme. According to our hypothesis based on molecular modeling,[29] we surmised that the poor binding of Cyc to A16V+S108T enzyme might be attributed to the 4-Cl atom of phenyl group at the N-1 position and to one of the methyl substituent at the C-2 position of Cyc. So we have designed and synthesized a number of Cyc derivatives, in which the 4-chlorophenyl substituent at N-1 was fixed while one substituent at C-2 was H and the other varied from methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, cyclohexyl and phenyl. The effects of the substituents on binding to both wild-type and A16V+S108T mutant pfDHFRs, were assessed by determination of the ratios of the K_i values for the A16V+S108T mutant enzyme and the wild-type enzyme (K_i-mut./K_i-wt) as well as their K_i values relative to Cyc.

The data in Table 3.4 shows that the monomethyl analogue of cycloguanil **(II-27)** showed improved binding affinity (~10-fold) for the A16V+S108T pfDHFR, but was about 2.7-fold less effective than Cyc for the wild-type enzyme. The K_i values of monoethyl **(II-28)**, mono *n*-propyl **(II-29)** and mono *n*-butyl **(II-31)** analogues for the wild-type and A16V+S108T pfDHFRs were comparable to that of the monomethyl analogue **(II-27)**. This implied that the C-2 substituents of these analogues did not

appreciably decrease or improve the binding affinities of the inhibitors compared with analogue **II-27**. This is in accord with the proposed model[29] and suggested that there is only one alkyl group at C-2 having non-bonding interaction with the Val16 side chain of the A16V+S108T mutant enzyme. However, the K_i values for both wild-type and A16V+S108T pfDHFRs were greatly increased when the C-2 substituents were branched (and therefore bulkier) alkyl groups, as observed with analogues **II-30** and **II-32**. Interestingly, analogue **II-33**, in which one substituent at the C-2 was H and the other a phenyl group, inhibited the A16V+S108T mutant pfDHFR with the K_i value of 49.3 nM, a value which was 37.5-fold better than that observed for Cyc **(1)** suggesting that additional stabilizing interaction such as aromatic π-stacking may operate since if one consider from the steric ground, the C-2 mono phenyl derivative should bind less effectively than the monomethyl analogue. Nevertheless the above results illustrated the crucial role of residue 16 at the for Cyc binding and emphasized the importance of the substituents at position C-2 especially phenyl group for achieving effective inhibition of the A16V+S108T pfDHFR.

To explore the significance of the 4-chloro group on the N-1 of Cyc, four series of Cyc analogues were synthesized in which the N-1 4′-chlorophenyl group was replaced by 4-bromophenyl, 4-ethylphenyl, 4-methylphenyl and phenyl groups. Inhibition constants for the wild-type and A16V+S108T mutant pfDHFRs by 4-bromophenyl (**II-34** to **II-40**), 4-ethylphenyl (**II-20** to **II-26**), 4-methylphenyl (**II-13** to **II-19**) and phenyl (**II-6** to **II-12**) analogues indicated a trend similar to those when the N-1 substituent is 4-chlorophenyl. The replacement of the 4-chloro group with H (**II-6** to **II-12**) did not effect the K_i values for the A16V+S108T mutant DHFR but substantially increased the K_i values for the wild-type enzyme. It is evidenced that the presence of a substituent at the 4′ position was necessary for locking with the binding site of the wild-type enzyme. Replacement of the chlorine by bromine atom, ethyl and methyl groups had relatively little effect on the binding of the inhibitor to both wild-type and A16V+S108T mutant pfDHFRs.

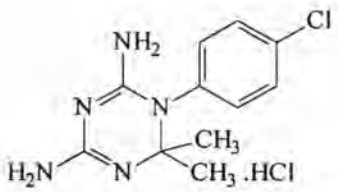
Next, we investigated the Cyc analogues in which an additional chlorine atom was placed at the 2-position and 3-positon of the 4-chlorophenyl substitute. The three series of Cyc analogues were synthesized in which the 4-chlorophenyl group was replaced by 2,4-dichlorophenyl, 3-chlorophenyl and 3,4-dichlorophenyl. The results in Table 3.4 indicated that replacement of the 4-chlorophenyl group of Cyc with 2,4-

dichlorophenyl group (**II-50** to **II-56**) increased the K_i values both the wild-type and A16V+S108T mutant pfDHFRs. This result illustrated that the substituent at the ortho position of benzene ring was not good for binding to both the wild-type and A16V+S108T mutant enzymes. While the Cyc analogue with 3-chlorophenyl group **(II-3)** inhibited the wild-type enzyme less effectively than Cyc **(1)** with the K_i value 2.5 times higher, it was about 3.8-fold more effective than Cyc against the A16V+S108T mutant pfDHFR. Replacement of the 4-chlorophenyl group of Cyc derivatives with 3-chlolrophenyl group (**II-41** to **II-49**) resulted in a significant decrease in the K_i values against the A16V+S108T mutant pfDHFR, especially compound **II-47** binds to the mutant with the K_i value of as low as 3.75 nM which means that it is nearly as effective against mutant enzyme as wild-type enzyme. Replacement of the 4-chlorophenyl group of Cyc with the 3,4-dichlorophenyl group yielded analogues **II-57**, **II-58**, **II-59**, **II-62**, **II-63** and **II-65** which were about as effective as Cyc against the wild-type DHFR but they inhibited the A16VS108T mutant enzyme about 73.4, 56.8, 45.1, 61.4, 215.4 and 119.4 times better than Cyc, respectively. Analogue **II-63** with a flexible *n*-heptyl substituent at C-2 was about 2.3-fold more effective than Cyc against the wild-type enzyme and was about 215-fold more effective than Cyc against the A16VS108T mutant pfDHFR.

In the case of dihydrotriazine bearing 3-chlorophenyl substituent at N-1, if the alkyl groups at C-2 position were small (**II-41** to **II-43**) or being the dimethyl groups **(II-3)**, the K_i values against wild-type enzyme were considerably higher than that of cycloguanil. This indicated that the substituent at the 4-position of benzene ring helped the binding to the wild-type enzyme but the substituent at 3-position of benzene ring helped to bind to A16V+S108T mutant enzyme. Consequently, derivatives bearing N-1 3,4-dichlorophenyl substituent gave good K_i values to both wild-type and A16V+S108T mutant pfDHFRs. However, when the N-1 substituent was 3-ClC_6H_4 and the alkyl groups at C-2 position was a long chain hydrocarbon, good K_i value to both wild-type enzyme and A16V+S108T mutant were observed. Therefore, 1-(3′-chlorophenyl)-2-heptyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(II-47)** appeared to be an interesting lead compound which may be developed to be anti-malarial drugs.

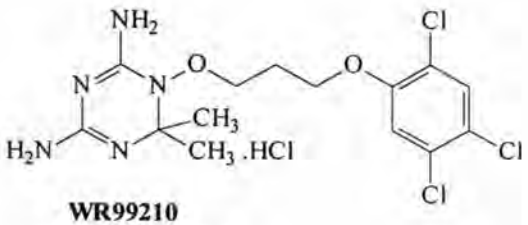
The above results indicated that the substituents at C-3 of the benzene ring were important to binding with A16V+S108T mutant. Substituent at C-4 position

appeared to be important in binding to wild-type but inhibit binding to A16V+S108T mutant which agree with the model where non-bonding interaction between the 4-substituent and the S108T side chain was observed.[29] With long chain alkyl groups or appropriately substituted phenyl group at C-2 position, the effect of the 4-substituent in binding to both wild-type and mutant DHFR was less pronounced. Among 65 compounds tested, 1-(3′-chlorophenyl)-2-heptyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(II-47)** was the most effective compound against both wild-type and A16V+S108T mutant DHFR. 1-(3′,4′-Dichlorophenyl)-2-heptyl- and 1-(3′-chlorophenyl)-2-pentyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride (**II-63** and **II-46**) were the second and third most effective compounds. 1-(3′,4′-Dichlorophenyl)- and 1-(3′-chlorophenyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride (**II-65** and **II-49**) were the most effective compounds bearing phenyl substituent. Their structures and K_i were presented in Scheme 3.10.



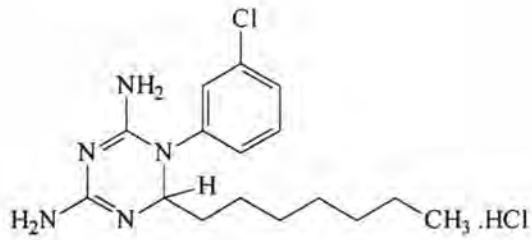
Cyc

K_i (wt) = 1.5 nM
K_i (mut.) = 1314 nM



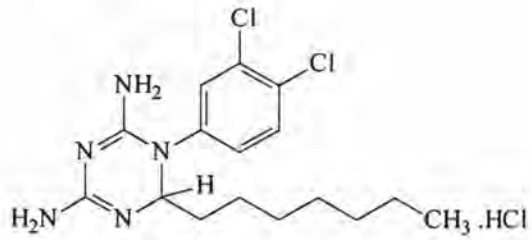
WR99210

K_i (wt) = 0.5 nM
K_i (mut.) = 2.4 nM



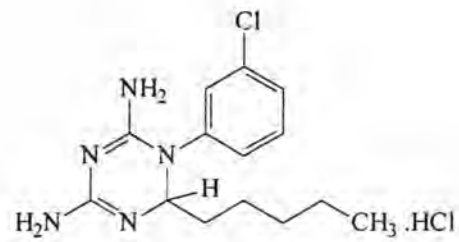
(II-47)

K_i (wt) = 2.7 nM
K_i (mut.) = 3.75 nM



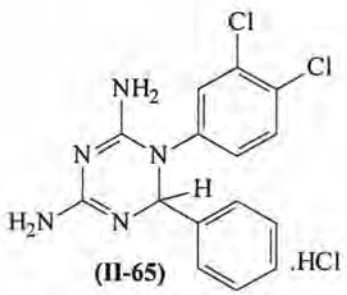
(II-63)

K_i (wt) = 0.65 nM
K_i (mut.) = 6.08 nM



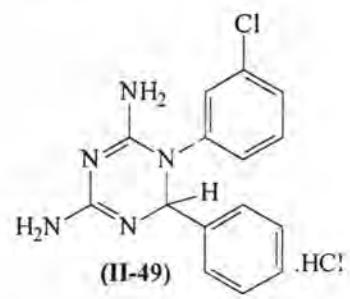
(II-46)

K_i (wt) = 9.8 nM
K_i (mut.) = 10.9 nM



(II-65)

K_i (wt) = 1.6 nM
K_i (mut.) = 11.0 nM

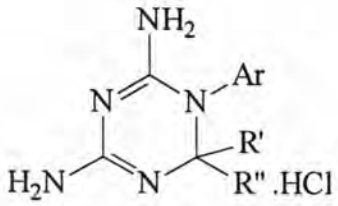


(II-49)

K_i (wt) = 11.7 nM
K_i (mut.) = 10.5 nM

Scheme 3.10 The structures of 5 most effective inhibitors of DHFR in the 1-aryl-4,6-diamino-1,2-dihydro-1,3,5-triazine series and WR99210

Table 3.4 Inhibition Constants (K_i) of Cycloguanil and Its Analogues (.HCl) against the Wild-Type and A16V+S108T Mutant DHFRs of *P. falciparum*



Cmpd	Ar	R′	R″	K_i (wt)[a] (nM)	Rel. to Cyc	K_i (mut.)[b] (nM)	Rel. to Cyc	K_i (mut.)/K_i (wt)
1	4-ClC_6H_4	CH_3	CH_3	1.5 ± 0.3[c]	1.0	1,314.0 ± 16.0[c]	1	876.0
II-1[d,e]	3-$CO_2HC_6H_4$	CH_3	CH_3	-	-	-	-	-
II-2[d]	4-$CO_2HC_6H_4$	CH_3	CH_3	Not dissolved				
II-3	3-ClC_6H_4	CH_3	CH_3	3.7 ± 0.6	2.5	340.1 ± 37.9	0.3	91.9
II-4	4-Cl-3$NO_2C_6H_3$	CH_3	CH_3	1.0 ± 0.0	0.7	814.8 ± 120.3	0.6	814.8
II-6	Ph	H	CH_3	113.1 ± 14.8	75.4	456.3 ± 49.0	0.3	4.0
II-7	Ph	H	C_2H_5	144.2 ± 16.7	96.1	252.6 ± 37.6	0.2	1.7
II-8	Ph	H	*n*-C_3H_7	112.3 ± 11.3	74.8	205.9 ± 23.6	0.2	1.8
II-9	Ph	H	*i*-C_3H_7	1,181.1 ± 160.6	787.8	1,508.8 ± 226.3	1.1	1.3
II-10	Ph	H	*n*-C_4H_9	92.4 ± 16.4	61.6	307.3 ± 28.8	0.2	3.0

Table 3.4 Continued

Cmpd	Ar	R′	R″	K_i (wt)[a] (nM)	Rel. to Cyc	K_i (mut.)[b] (nM)	Rel. to Cyc	K_i (mut.)/K_i (wt)
II-11	Ph	H	C_6H_{11}	299.1 ± 61.6	199.4	3,983.3 ± 515.4	3.0	13.3
II-12	Ph	H	Ph	49.1 ± 3.5	32.7	61.8 ± 15.6	0.05	1.3
II-13	4-$CH_3C_6H_4$	H	CH_3	23.4 ± 1.9	15.6	185.5 ± 22.4	0.1	7.9
II-14	4-$CH_3C_6H_4$	H	C_2H_5	5.9 ± 0.2	3.9	127.9 ± 3.9	0.1	21.7
II-15	4-$CH_3C_6H_4$	H	*n*-C_3H_7	13.7 ± 0.8	9.1	188.3 ± 11.8	0.1	13.7
II-16	4-$CH_3C_6H_4$	H	*i*-C_3H_7	166.9 ± 10.6	111.3	1,460.1 ± 161.3	1.1	8.7
II-17	4-$CH_3C_6H_4$	H	*n*-C_4H_9	14.4 ± 4.7	9.6	405.8 ± 55.9	0.3	28.2
II-18	4-$CH_3C_6H_4$	H	C_6H_{11}	1,476.9 ± 118.5	984.6	12,291.6 ± 2,024.7	9.4	8.3
II-19	4-$CH_3C_6H_4$	H	Ph	7.7 ± 2.2	5.1	170.4 ± 13.8	0.1	22.1
II-20	4-$C_2H_5C_6H_4$	H	CH_3	11.0 ± 3.0	7.3	322.1 ± 56.4	0.2	29.3
II-21	4-$C_2H_5C_6H_4$	H	C_2H_5	17.3 ± 1.0	11.5	225.3 ± 40.2	0.2	13.0
II-22	4-$C_2H_5C_6H_4$	H	*n*-C_3H_7	16.7 ± 1.7	11.1	299.0 ± 49.1	0.2	17.9
II-23	4-$C_2H_5C_6H_4$	H	*i*-C_3H_7	2,470.4 ± 181.2	1,646.9	11,524.2 ± 1,478.8	8.8	4.7
II-24	4-$C_2H_5C_6H_4$	H	*n*-C_4H_9	13.6 ± 2.5	9.1	246.6 ± 17.8	0.2	18.1
II-25	4-$C_2H_5C_6H_4$	H	C_6H_{11}	198.7 ± 9.7	132.5	256.3 ± 33.4	0.2	1.3
II-26	4-$C_2H_5C_6H_4$	H	Ph	12.1 ± 2.3	8.1	86.6 ± 10.8	0.07	7.2

Table 3.4 Continued

Cmpd	Ar	R′	R″	K_i (wt)[a] (nM)	Rel. to Cyc	K_i (mut.)[b] (nM)	Rel. to Cyc	K_i (mut.)/K_i (wt)
II-27	4-ClC_6H_4	H	CH_3	4.1 ± 0.0	2.7	127.0 ± 14.0	0.8	245.6
II-28	4-ClC_6H_4	H	C_2H_5	3.6 ± 0.0	2.4	189.0 ± 37.0	0.9	325.0
II-29	4-ClC_6H_4	H	*n*-C_3H_7	4.6 ± 0.2	3.1	106.7 ± 31.7	0.08	23.2
II-30	4-ClC_6H_4	H	*i*-C_3H_7	60.5 ±10.1	40.3	1,538.0 ± 345.0	1.2	25.4
II-31	4-ClC_6H_4	H	*n*-C_4H_9	3.7 ± 0.1	2.5	167.3 ± 6.4	0.1	45.2
II-32	4-ClC_6H_4	H	C_6H_{11}	125.1 ± 12.5	83.4	968.3 ± 45.4	0.7	7.7
II-33	4-ClC_6H_4	H	Ph	4.5 ± 0.2	3.0	49.3 ± 3.3	0.04	11.0
II-34	4-BrC_6H_4	H	CH_3	5.7 ± 0.5	3.8	201.7 ± 16.9	0.2	35.4
II-35	4-BrC_6H_4	H	C_2H_5	2.7 ± 0.3	1.8	99.2 ± 7.4	0.08	36.7
II-36	4-BrC_6H_4	H	*n*-C_3H_7	2.6 ± 0.8	1.7	127.1 ± 10.7	0.1	48.9
II-37	4-BrC_6H_4	H	*i*-C_3H_7	30.0 ± 4.3	20.0	1,195.5 ± 53.3	0.9	39.8
II-38	4-BrC_6H_4	H	*n*-C_4H_9	2.3 ± 0.5	1.5	297.7 ± 37.5	0.2	129.4
II-39	4-BrC_6H_4	H	C_6H_{11}	208.0 ± 13.8	15.1	8,694.9 ± 879.8	6.6	41.2
II-40	4-BrC_6H_4	H	Ph	2.9 ± 1.2	1.9	90.3 ± 11.4	0.07	31.1
II-41	3-ClC_6H_4	H	CH_3	10.2 ± 0.6	6.8	38.7 ± 2.9	0.03	3.8
II-42	3-ClC_6H_4	H	C_2H_5	11.7 ± 0.7	7.8	33.4 ± 5.5	0.03	2.8

Table 3.4 Continued

Cmpd	Ar	R′	R″	K_i (wt)[a] (nM)	Rel. to Cyc	K_i (mut.)[b] (nM)	Rel. to Cyc	K_i (mut.)/K_i (wt)
II-43	3-ClC_6H_4	H	*n*-C_3H_7	19.7 ± 2.2	13.1	38.3 ± 3.7	0.03	1.9
II-44	3-ClC_6H_4	H	*i*-C_3H_7	140.8 ± 8.2	93.9	228.7 ± 15.8	0.2	1.6
II-45	3-ClC_6H_4	H	*n*-C_4H_9	10.7 ± 1.8	7.1	39.1 ± 1.3	0.03	3.6
II-46	3-ClC_6H_4	H	*n*-C_5H_{11}	9.8 ± 0.2	6.5	10.9	0.008	1.1
II-47	3-ClC_6H_4	H	*n*-C_7H_{15}	2.7 ± 0.1	1.8	3.8 ± 0.05	0.003	1.4
II-48	3-ClC_6H_4	H	C_6H_{11}	125.1 ± 12.5	83.4	968.3 ± 45.4	0.7	7.7
II-49	3-ClC_6H_4	H	Ph	11.7 ± 2.5	7.8	10.5 ± 7.4	0.008	0.9
II-50	2,4-$Cl_2C_6H_3$	H	CH_3	817.8 ± 183.5	545.2	972.7 ± 7.31	13.3	1.2
II-51	2,4-$Cl_2C_6H_3$	H	C_2H_5	419.7 ± 67.3	279.8	416.8 ± 51.9	0.3	1.0
II-52	2,4-$Cl_2C_6H_3$	H	*n*-C_3H_7	678.3 ± 99.2	118.9	938.1 ± 29.4	0.7	1.4
II-53	2,4-$Cl_2C_6H_3$	H	*i*-C_3H_7	3,540.9 ± 717.9	2360.6	3,166.1 ± 655.3	2.4	0.9
II-54	2,4-$Cl_2C_6H_3$	H	*n*-C_4H_9	1,061.1 ± 88.2	707.4	696.2 ± 57.0	0.5	0.7
II-55	2,4-$Cl_2C_6H_3$	H	C_6H_{11}	>10,000	>6,000	12,247.0 ± 2,413.7	9.3	<1.2
II-56	2,4-$Cl_2C_6H_3$	H	Ph	135.9 ± 14.9	90.6	262.9 ± 35.6	0.2	1.9
II-57	3,4-$Cl_2C_6H_3$	H	CH_3	1.4 ± 0.2	0.9	17.8 ± 0.8	0.01	12.7
II-58	3,4-$Cl_2C_6H_3$	H	C_2H_5	1.3 ± 0.1	0.9	23.1 ± 2.7	0.02	17.7

Table 3.4 Continued

Cmpd	Ar	R′	R″	K_i (wt)[a] (nM)	Rel. to Cyc	K_i (mut.)[b] (nM)	Rel. to Cyc	K_i (mut.)/K_i (wt)
II-59	3,4-$Cl_2C_6H_3$	H	*n*-C_3H_7	1.1 ± 0.2	0.7	29.1 ± 2.1	0.02	26.5
II-60	3,4-$Cl_2C_6H_3$	H	*i*-C_3H_7	13.1 ± 0.7	8.7	141.4 ± 0.0	0.1	10.2
II-61	3,4-$Cl_2C_6H_3$	H	*n*-C_4H_9	0.9 ± 0.1	0.6	16.0 ± 3.2	0.01	16.5
II-62	3,4-$Cl_2C_6H_3$	H	*n*-C_5H_{11}	1.1 ± 0.5	0.8	21.1 ± 3.3	0.02	18.1
II-63	3,4-$Cl_2C_6H_3$	H	*n*-C_7H_{15}	0.6 ± 0.2	0.4	6.1 ± 1.5	0.004	9.4
II-64	3,4-$Cl_2C_6H_3$	H	C_6H_{11}	111.6 ± 30.9	74.4	940.5 ± 96.9	0.7	8.4
II-65	3,4-$Cl_2C_6H_3$	H	Ph	1.6 ± 0.2	1.1	11.0 ± 1.8	0.008	6.9

[a]Wild-type pfDHFR. [b]A16V+S108T mutant pfDHFR. [c]Data from ref 28. [d] salt of *p*-TsOH. [e]The activity is being tested.

The results in Table 3.4 revealed that the long chain of alkyl or phenyl groups at C-2 position gave the good K_i values both wild-type and A16V+S108T mutant pfDHFRs. In the next step, we had the idea to study and synthesize the other series of Cyc analogue bearing a flexible alkyl side chain at N-1. The data in Table 3.5 presented the K_i values of such Cyc analogues (**II-73** to **II-121**) in which the substituents at N-1 were varied by increasing the flexibility of the side chain by replacing the 4-chlorophenyl group with *n*-alkyl or branched alkyl or aralkyl groups. One of the 2,2-dimethyl groups at C-2 was replaced with an H atom and the other was replaced with phenyl or substituted phenyl groups. Unfortunately, derivatives bearing non-aromatic substituent could not be made by the present synthetic method therefore the results could not be compared. 1-Benzyl-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate **(II-98)** poorly bound to both wild-type and A16V+S108T pfDHFRs, the K_i values being 735.9 and 6,688.2 nM, respectively. Compared to Table 3.4, the K_i values of 1-phenyl-2-phenyl-4,6-diamino-1,2-diamino-1,3,5-triazine hydrochloride **(II-12)** for the wild-type and mutant pfDHFRs were 49.1 and 61.8 nM respectively. Therefore, the poor binding with wild-type and A16V+S108T mutant enzymes of **II-98** was due to the presence of additional methylene group. Not surprisingly, inhibition constants for the wild-type and A16V+S108T mutant pfDHFRs by mono-, di- and tri- methoxyphenyl (**II-73** to **II-80**), mono-fluorophenyl (**II-81** to **II-82**), mono- and di-chlorophenyl (**II-83** to **II-88**), mono-bromophenyl (**II-89** to **II-90**), mono-nitrophenyl (**II-91** to **II-92**), mono-cyanophenyl **(II-93)**, mono-chloro-mono-nitrophenyl (**II-94** to **II-95**), mono-chloro-mono-fluorophenyl **(II-96)** and mono-*tert*-butylphenyl **(II-97)** analogues at C-2 exhibited a high K_i values to both wild-type and mutant enzymes similar to **II-98**. Interestingly, analogue **II-110**, in which one substituent at the C-2 was H and the other was 3-phenoxyphenyl group, inhibited the wild-type and A16V+S108T mutant enzymes quite effectively with the K_i values of 9.1 and 185.2 nM, a values which were 81- and 36-folds better than that observed for **II-98** and was comparable to many 1-aryl-4,6-diamino-1,2-dihydro-1,3,5-triazines. Thus (Table 3.4) 3-phenoxyphenyl group at C-2 was the most suitable substituent which conferred good activity to both wild-type and mutant enzyme.

To investigate the significance of the flexible side chain at N-1 substituent of **II-98**, analogues **II-99** and **II-100** were synthesized in which the N-1 were phenylethyl and phenylpropyl groups respectively. The K_i values of **II-99** against the wild-type and A16V+S108T mutant pfDHFRs were even higher than **II-98**. While **II-**

100 inhibited the wild-type and mutant enzymes with the K_i values ~47 and ~87 times better than **II-98**. The result indicated that the length of side chain of aryl alkyl had effect on binding with wild-type and mutant enzymes, and with longer side-chain, the binding becomes better.

Replacement of the benzyl group at N-1 with long chain *n*-alkyl group (**II-105** to **II-108**) resulted in a significant decrease in the K_i values for the wild-type and A16V+S108T pfDHFRs. Notably, the analogue **II-106**, with the *n*-decyl side-chain, bound to wild-type and mutant enzymes with the K_i values of 10.9 and 16.9 (nM), which were 67.4 times and 43.5 times better than **II-98** respectively. In case of too short **(II-102)** or too long *n*-alkyl substituents at N-1 (**II-107** and **II-108**), the K_i values became high to both wild-type and A16V+S108T pfDHFR mutants (>100 nM). The K_i values for both wild-type and A16V+S108T mutant pfDHFRs were also greatly increased when the N-1 substituents were small, polar or branched such as *n*-methoxypropyl, *n*-propyl, isopropyl, isobutyl and cyclohexyl groups (**II-101** to **II-104** and **II-109**).

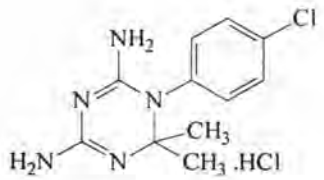
Based on the above results, we synthesized the analogues **II-111** and **II-112**, in which the substituents at N-1 were phenylpropyl and *n*-decyl groups, and one substituent at C-2 was 3-phenoxyphenyl group and the other substituent was the H atom. Analogue **II-111** was about 108- and 735-folds more effective than analogue **II-98** against the wild-type and A16V+S108T mutant enzymes. While analogue **II-112** was about 91-fold and 614-fold more effective than analogue **II-98** against both enzymes. These two compounds are about as effective as compound **II-46** and **II-49** from the 1-aryl-4,6-diamino-1,2-dihydro-1,3,5-triazine series.

Bis-(2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine-1-yl)ethane bistrifluoroacetate **(II-119)** was not very effective against both wild-type and A16V+108T pfDHFRs. The K_i values of 1-[2'-(2'',4'',5''-trichlorophenoxy)ethyl]-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate **(II-120)** against the wild-type and A16V+S108T mutant enzymes were higher than 1-[3'-(2'',4'',5''-trichlorophenoxy)propyl]-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate **(II-121)**. Analogue **II-121** inhibited the wild-type and mutant enzymes with the K_i values of 1.2 and 7.3 nM, a values which are 613- and 916-fold better than that observed for analogue **II-98** but still nearly an order of magnitude poorer than the analogous WR99210. The results indicated that the increasing of the

methylene group of side chain from 2 to 3 would be help the binding of inhibitor to both wild-type and mutant pfDHFRs which is in accord with the results obtained from the series of phenylalkyl analogues **II-98**, **II-99** and **II-100**. Activity of compound 1-(3′-morpholin-4′-ylpropyl)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate **(II-113)** has not yet been tested but it is expected to poorly inhibited the enzyme due to the presence of polar side-chain.

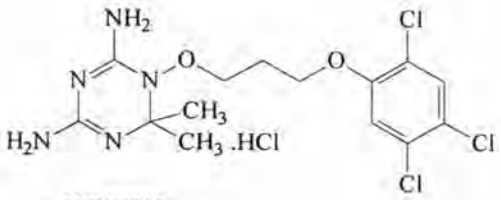
The above results emphasizes that the flexible side chain at N-1 with suitable length was important to binding with wild-type and A16V+S108T pfDHFRs and the most interesting substituent groups at N-1 were 2,4,5-trichlorophenoxypropyl, phenylpropyl and *n*-decyl groups. When C-2 substituents were considered, the 3-phenoxyphenyl group was the only interesting substituent at C-2 which significantly increase binding to wild-type and A16V+S108T mutant pfDHFRs. From these 1-alkyldihydrotriazine series, 1-[3′-(2″,4″,5″-trichlorophenoxy)propyl]-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate **(II-121)** showed the best K_i values to both wild-type and A16V+S108T mutant pfDHFR. 1-Phenylpropyl-2-(3′-phenoxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate **(II-111)** and 1-decyl-2-(3′-phenoxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine trifluoroacetate **(II-112)** were the second and the third best inhibitors in this series respectively.

Interestingly, almost all compounds in this series showed not much different in K_i values to wild-type and A16V+S108T enyzmes and thus they are interesting lead compounds to be developed for as effective drugs against both wild-type and resistant DHFR enzymes. Unfortunately, it appeared that these "flexible" -*N*-alkyl dihydrotriazine are less effective antifolate when compared to 1-aryl dihydrotriazine therefore more diverse substituents should be explored in order to find compounds in this series with better K_i values. Nevertheless, the most effective compound in this series, ie **(II-121)** inhibited wild-type and A16V+S108T mutant pfDHFR 1.25 times and 180 times better than cycloguanil respectively.



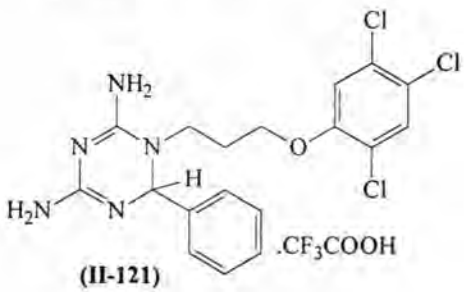
Cyc

K_i (wt) = 1.5 nM
K_i (mut.) = 1314 nM



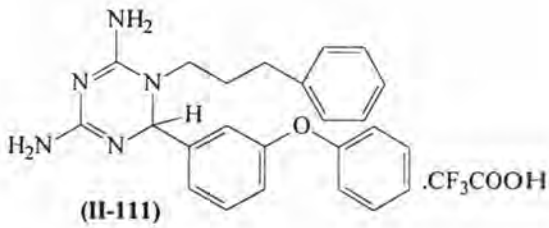
WR99210

K_i (wt) = 0.5 nM
K_i (mut.) = 2.4 nM



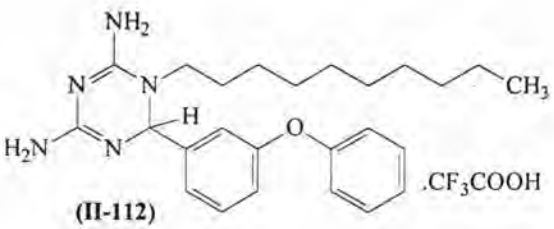
(II-121)

K_i (wt) = 1.2 nM
K_i (mut.) = 7.3 nM



(II-111)

K_i (wt) = 6.8 nM
K_i (mut.) = 9.1 nM

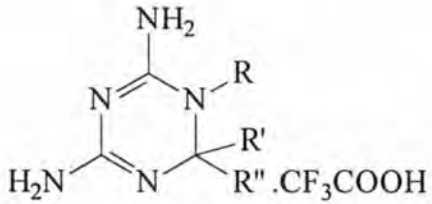


(II-112)

K_i (wt) = 8.1 nM
K_i (mut.) = 10.9 nM

Scheme 3.11 Structures of 3 most effective inhibitors of DHFR in the 1-alkyl-4,6-diamino-1,2-dihydro-1,3,5-triazine series, Cyc and WR99210

Table 3.5 Inhibition Constants (K_i) of Cycloguanil Analogues (.CF_3COOH) against the Wild-Type and A16V+S108T Mutant DHFRs of *P. falciparum*



Cmpd	R	R′	R″	K_i (wt)[a] (nM)	Rel. to Cyc	K_i (mut.)[b] (nM)	Rel. to Cyc	K_i (mut.)/ K_i (wt)
II-73	$PhCH_2$-	H	2-$CH_3OC_6H_4$	>10,000	>6,000	very high	-	-
II-74	$PhCH_2$-	H	3-$CH_3OC_6H_4$	>10,000	>6,000	14,208.0 ± 4,913.5	10.8	<1.4
II-75	$PhCH_2$-	H	4-$CH_3OC_6H_4$	>10,000	>6,000	10,779.8 ± 2,510.5	8.2	<1.1
II-76	$PhCH_2$-	H	2,4-$(OCH_3)_2C_6H_3$	very high	-	very high	-	-
II-77	$PhCH_2$-	H	2,5-$(OCH_3)_2C_6H_3$	very high	-	very high	-	-
II-78	$PhCH_2$-	H	3,4-$(OCH_3)_2C_6H_3$	>10,000	>6,000	19,144.2 ± 5,895.3	14.6	<1.9
II-79	$PhCH_2$-	H	3,5-$(OCH_3)_2C_6H_3$	very high	-	very high	-	-
II-80	$PhCH_2$-	H	3,4,5-$(OCH_3)_3C_6H_2$	very high	-	very high	-	-
II-81	$PhCH_2$-	H	3-FC_6H_4	5,354.0 ± 1,212.1	3,569.9	1,406.2 ± 78.4	1.1	0.3
II-82	$PhCH_2$-	H	4-FC_6H_4	5,194.0 ± 540.0	3,462.7	2,037.5 ± 366.6	1.5	0.04

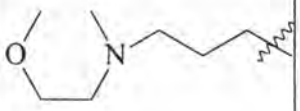
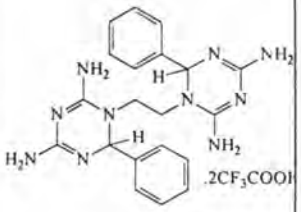
Table 3.5 Continued

Cmpd	R	R′	R″	K_i (wt)[a] (nM)	Rel. to Cyc	K_i (mut.)[b] (nM)	Rel. to Cyc	K_i (mut.)/ K_i (wt)
II-83	$PhCH_2$-	H	2-ClC_6H_4	7,546.8 ± 1,200.2	5,031.2	2,451.6 ± 398.1	1.9	0.3
II-84	$PhCH_2$-	H	3-ClC_6H_4	6,284.6 ± 2,590.0	4,189.7	1,201.8 ± 590.0	0.9	0.2
II-85	$PhCH_2$-	H	4-ClC_6H_4	>10,000	>6,000	14,865.4 ± 1,392.6	11.3	<1.5
II-86	$PhCH_2$-	H	2,4-$Cl_2C_6H_3$	>10,000	>6,000	10,539.9 ± 2,893.9	8.0	<1.1
II-87	$PhCH_2$-	H	2,6-$Cl_2C_6H_3$	>10,000	>6,000	13,249.0 ± 3,297.1	10.1	<1.3
II-88	$PhCH_2$-	H	3,4-$Cl_2C_6H_3$	>10,000	>6,000	very high	-	-
II-89	$PhCH_2$-	H	2-BrC_6H_4	>10,000	>6,000	very high	-	-
II-90	$PhCH_2$-	H	4-BrC_6H_4	>10,000	>6,000	very high	-	-
II-91	$PhCH_2$-	H	3-$NO_2C_6H_4$	>10,000	>6,000	>10,000	-	<1.0
II-92	$PhCH_2$-	H	4-$NO_2C_6H_4$	>10,000	>6,000	>10,000	-	<1.0
II-93	$PhCH_2$-	H	4-CNC_6H_4	very high	-	very high	-	-
II-94	$PhCH_2$-	H	2-Cl-5-$NO_2C_6H_3$	>10,000	>6,000	>10,000	>7.6	<1.0
II-95	$PhCH_2$-	H	4-Cl-3-$NO_2C_6H_3$	>10,000	>6,000	>10,000	>7.6	<1.0
II-96	$PhCH_2$-	H	2-Cl-6-FC_6H_3	>10,000	>6,000	19,302.9 ± 3,964.7	14.7	<19.3
II-97	$PhCH_2$-	H	4-^{t}BuC_6H_4	3,757.1 ± 830.1	2,504.7	2,681.9 ± 909.0	2.0	0.7
II-98	$PhCH_2$-	H	Ph	735.9 ± 304.7	490.6	6,688.2 ± 705.3	5.1	9.1

Table 3.5 Cointinued

Cmpd	R	R′	R″	K_i (wt)[a] (nM)	Rel. to Cyc	K_i (mut.)[b] (nM)	Rel. to Cyc	K_i (mut.)/ K_i (wt)
II-99	$PhCH_2CH_2$-	H	Ph	>10,000	>6,000	19,478.9 ± 4,227.5	14.8	<1.9
II-100	$PhCH_2CH_2CH_2$-	H	Ph	15.5 ± 2.6	10.4	77.12 ± 7.4	0.06	4.9
II-101	$CH_3OCH_2CH_2CH_2$-	H	Ph	504.5 ± 113.2	336.4	9,068.7 ± 1,101.3	6.9	17.9
II-102	$CH_3CH_2CH_2$-	H	Ph	>10,000	>6,000	15,205.9 ± 2,153.5	11.6	<1.5
II-103	$(CH_3)_2CH$-	H	Ph	>10,000	>6,000	very high	-	-
II-104	$(CH_3)_2CHCH_2$-	H	Ph	very high	-	very high	-	-
II-105	$CH_3(CH_2)_6$-	H	Ph	45.5 ± 5.4	30.3	68.9 ± 18.3	0.05	1.5
II-106	$CH_3(CH_2)_9$-	H	Ph	10.9 ± 3.6	7.2	16.9 ± 1.2	0.01	1.5
II-107	$CH_3(CH_2)_{13}$-	H	Ph	221.8 ± 18.0	147.9	207.4 ± 37.6	0.2	0.9
II-108	$CH_3(CH_2)_{17}$-	H	Ph	271.1 ± 11.4	180.7	210.9 ± 45.4	0.2	0.5
II-109	C_6H_{11}-	H	Ph	>10,000	>6,000	6,700.4 ± 1,493.6	5.1	<0.7
II-110	$PhCH_2$-	H	3-$PhOC_6H_4$	9.1 ± 3.5	6.1	185.2 ± 67.0	0.1	20.3
II-111	$PhCH_2CH_2CH_2$-	H	3-$PhOC_6H_4$	6.8 ± 0.7	4.5	9.1 ± 0.6	0.007	1.3
II-112	$CH_3(CH_2)_9$-	H	3-$PhOC_6H_4$	8.1 ± 2.6	5.4	10.9 ± 4.3	0.008	1.3

Table 3.5 Continued

Cmpd	R	R′	R″	K_i (wt)[a] (nM)	Rel. to Cyc	K_i (mut.)[b] (nM)	Rel. to Cyc	K_i (mut.)/ K_i (wt)
II-113[c]		H	Ph	-	-	-	-	-
II-119		-	-	269.8 ± 12.8	179.8	8,605.5 ± 2,030.2	6.5	31.8
II-120	2,4,5-$Cl_3C_6H_2O$ $(CH_2)_2$-	H	Ph	21.8 ± 9.0	14.50	84.2 ± 6.3	0.06	3.8
II-121	2,4,5-$Cl_3C_6H_2O$ $(CH_2)_3$-	H	Ph	1.2 ± 0.3	0.8	7.3 ± 2.8	0.006	6.1

[a]Wild-type pfDHFR. [b]A16V+S108T mutant pfDHFR. [c]The activity is being tested.

WR99210 **(2)** was the best inhibitor to both wild-type and A16V+S108T mutant pfDHFR. In fact, most mutant pfDHFRs are susceptible to WR99210, but it was very toxic and the process of production must use chlorinated phenol which was also toxic and posed environmental problems. As a result, even though it was very effective inhibitor, it could not be developed to be antimalarial drug. Therefore, it is necessary to find the new compound in these series.

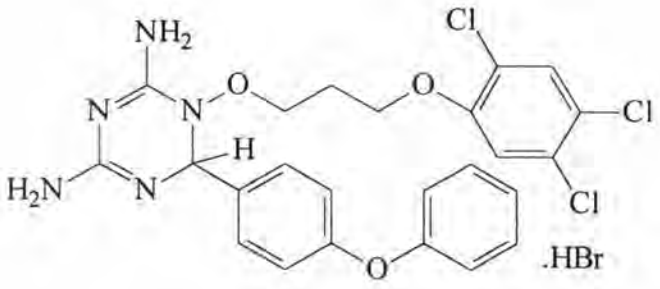
As reported previously,[29] WR99210 **(2)** binds to the wild-type and A16V+S108T pfDHFRs with the K_i values of 0.5 and 2.4 nM, the values which are 3 and 548 times better than cycloguanil. Molecular modeling studies[29] explained that WR99210 **(2)** was highly effective inhibitor for both wild-type and A16V+S108T mutant enzymes due to its flexible side chain at N-1 even though it possess the geminal 2,2-dimethyl substituents at C-2. This seems to be contradicted with the steric constraint model, which was used to explain the loss of activity of cycloguanil against A16V+S108T. There is, however, explanation to this. According to Rastelli's model,[29] the flexible side-chain of WR99210 **(2)** does not lock itself in the same position as cycloguanil, therefore the gem-dimethyl group can be comfortably accommodated in the binding site. However, little as it is, steric effect at C-2 may still play a minor role as seen by when one methyl group in WR99210 **(2)** was replaced by one hydrogen atom **(3)**, a slightly better K_i against A16V+S108T was observed while K_i to wild-type remained the same. It is therefore interesting to study the effect of varying substituent at both N-1 and C-2 position further to compare the result with the previous two series. So, we designed and synthesized a number of 1-alkyloxy-4,6-diamino-1,2-dihydro-1,3,5-triazine derivatives (**II-124** to **II-151**) in which one substituent at C-2 was H and the other was phenyl group or alkyl group, while the group at N-1 was modified. Table 3.6 summarized the inhibition constant values of WR99210 and analogues in which the substituents at N-1 and C-2 were varied. 1-Benzyl-2,2-cyclohexylidene-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrochloride **(II-126)** exhibited very poor binding with both wild-type and A16V+S108T mutant enzyme. When the C-2 substituents were hydrogen and phenyl group. The K_i values of benzyloxy **(II-127)**, phenethyloxy **(II-129)** and 3-phenylpropyloxy **(II-130)** analogues for the wild-type DHFR were not much different and were in the order of 1.4-2.2 nM which is comparable to 1-aryl-4,6-diamino-1,2-dihydro-1,3,5-triazine but the K_i values for the A16V+S108T mutant pfDHFRs of analogues **II-127**, **II-129** and **II-130** were decreased when the length of methylene spacers increased, being 53.2,

8.3 and 7.0 nM, respectively. Interestingly, the alkyloxytriazine series gave much better K_i against A16V+S108T mutant pfDHFR when compared to 1-aryl-4,6-diamino-1,2-dihydro-1,3,5-triazine in general.

The significance of substitution at 2-, 3-, and 4-position on the benzyl group were studied in a series of bromobenzyl substituted triazine (**II-131** to **II-133**). 1-(3′-Bromobenzyloxy)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrobromide **(II-132)** inhibited the wild-type and mutant enzymes with the K_i values of 1.0 and 5.2 nM respectively. The *ortho*-bromo substituent gave slightly higher K_i values and the *para*-substituent gave highest K_i in these series. Similarly, 1-(4′-methylbenzyloxy)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrobromide **(II-134)** inhibited the wild-type enzyme with the K_i value of ~23.7 nM, yet it bound approximately 1.6-fold more tightly to the A16V+S108T mutant enzyme when compared to **II-132**.

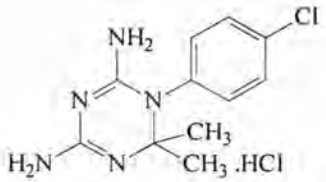
The K_i values for the wild-type and mutant enzymes of naphthalen-2-ylmethoxy analogue **(II-135)** showed a similar trend to **II-132** where the N-1 substituent was 3-bromobenzyloxy. Small alkyl side chain in allyloxy **(II-137)** and propoxy groups **(II-138)** at N-1 inhibited relatively poorly to both wild-type and A16V+S108T mutant enzymes with the K_i values nearly 10-times higher than the corresponding benzyl analogue **(II-127)**. The presence of double bond in the side chain did not have significant effect to the binding. 1-Pentyloxy-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrobromide **(II-139)** binds considerably more effective to both enzymes than the propyloxy **(II-137)** and allyloxy **(II-138)** analogues. The activity test for a higher homologue, 1-decyloxy-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrobromide **(II-140),** has not yet been done. However, based on the data above, it can be predicted that analogue **II-140** that should be even more effective inhibitor than **II-139**. The K_i values for both wild-type and A16V+S108T pfDHFRs were not greatly effected when the N-1 substituents were branched (and therefore bulkier) alkyloxy groups, as observed with analogues **II-141** to **II-143**. Their K_i values against both enzymes were similar to the K_i values of **II-139**, suggesting that steric effect at N-1 play a little role in binding of these flexible analogues which is in sharp contrast to the 1-alkyl series. Polar substitutents at N-1 such as 1-methoxycarbonylmethoxy derivative **(II-136)** gave relatively high K_i values to both wild-type and mutant pfDHFRs.

Data in Table 3.7 showed the inhibition constant values of WR99210 analogues in which the subtituent at N-1 was benzyloxy and C-2 was varied, one substituent being H atom and the other being aryl group with different substitutents on the benzene ring. This was obtained by Vilaivan[44] and was taken with permission for reference purpose. The K_i values of 1-benzyloxy-2-(3′-phenoxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrobromide **(5)** and 1-benzyloxy-2-(4′-phenoxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrobromide **(6)** were among the best with K_i values in the range of 0.8-0.9 and 5.3-5.4 nM for wild-type and A16V+S108T mutant enzymes respectively. The K_i values of 4-benzyloxyphenyl **(7)**, 3-benzyloxyphenyl **(8)** and biphenyl-4-yl **(9)** analogues for the wild-type were not much different from phenyl analogues **(II-127)** but the K_i values for A16V+S108T mutant enzyme of analogues **7**, **8** and **9** were much better, being 6.9, 8.7 and 6.4 nM, respectively. Analogues **10** and **14** bearing 4-hydroxyphenyl and 4-fluorophenyl substituents at C-2 were about as effective as analogue **II-127** against the wild-type and A16V+S108T mutant pfDHFRs. While the 4-methoxyphenyl **(11)**, 4-chlorophenyl **(12)** and 4-bromophenyl **(13)** substituents at C-2 inhibited wild-type enzyme similar to **II-127** but inhibited the mutant pfDHFR with K_i values of 2.5-2.7-fold lower than that observed for **II-127**. Analogue **15**, 1-benzyloxy-2-(3′,4′,5′-trimethoxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrobromide, showed a high K_i values to both wild-type and mutant enzymes. 4-Methylphenyl **(16)** substituent at C-2 inhibited wild-type and A16V+S108T mutant enzymes with K_i values of ~5.3 and ~250.6 nM, a value which were 3.3- and 4.7-fold higher than **II-127**. The above result showed that 3-phenoxyphenyl and 4-phenoxyphenyl groups were the best substituents at C-2. So, there was an attempt to combine the best substituents at N-1 of WR99210, ie, the 2,4,5-trichlorophenoxypropoxy group, and at C-2 ie, the 4-phenoxyphenyl group. Therefore 1-[3′-(2′′,4′′,5′′-trichlorophenoxy) propoxy)-2-(4′-phenoxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrobromide (Scheme 3.12) have been synthesized by Vilaivan.[44] Disappointly, the K_i values for the wild-type and A16V+S108T mutant pfDHFRs of 1-[3′-(2′′,4′′,5′′-trichlorophenoxy)propoxy)-2-(4′-phenoxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrobromide were 14.6 and 21.0 nM respectively, which were not better than either WR99210 or **6**. This result indicated that the effect of substituents at N-1 and C-2 are probably synergistic and they must be considered together.



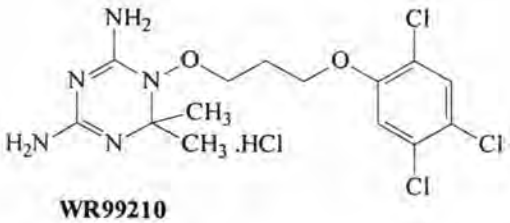
Scheme 3.12 The structure of 1-[3′-(2″,4″,5″-trichlorophenoxy)propoxy]-2-(4′-phenoxyphenyl)-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrobromide

The above result revealed that the alkyloxy flexible side chain at N-1 had significant effect to binding with wild-type and A16V+S108T mutant pfDHFRs. Moreover, the length of alkyloxy flexible side chain was one important contribution factor to good binding. In addition to WR99210-type side-chain, the 3-(biphenyl-4-yloxy)propoxy, 3-bromobenzyloxy and naphthalen-2-ylmethoxy groups were N-1 substituents which confers good binding activities to both wild-type and mutant enzymes. 1-[3′-(Biphenyl-4″-yloxy)propoxy]-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrobromide **(II-150)** was the most effective compound in this series. While 1-(3′-bromobenzyloxy)- and 1-(naphthalen-2′-ylmethoxy)-2-phenyl-4,6-diamino-1,2-dihydro-1,3,5-triazine hydrobromide (**II-132** and **II-135**) were second and third most effective compounds. This structures were illustrated in Scheme 3.13.



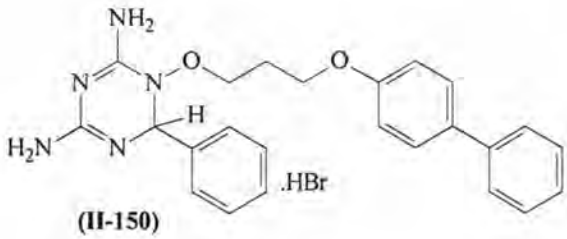
Cyc

K_i (wt) = 1.5 nM
K_i (mut.) = 1314 nM



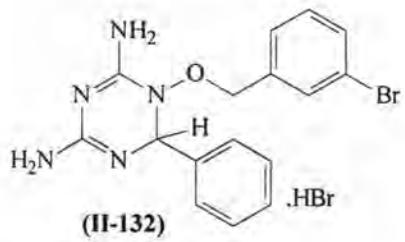
WR99210

K_i (wt) = 0.5 nM
K_i (mut.) = 2.4 nM



(II-150)

K_i (wt) = 1.2 nM
K_i (mut.) = 2.1 nM



(II-132)

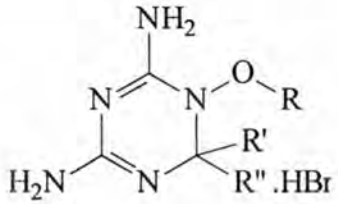
K_i (wt) = 1.0 nM
K_i (mut.) = 5.2 nM

(II-135)

K_i (wt) = 1.3 nM
K_i (mut.) = 5.7 nM

Scheme 3.13 The structures of 3 most effective inhibitors of DHFR in the 1-alkyloxy-4,6-diamino-1,2-dihydro-1,3,5-triazine series and Cyc

Table 3.6 Inhibition Constants (K_i) of WR99210 and Its Analogues (.HBr) against the Wild-Type and A16V+S108T Mutant DHFRs of *P. falciparum*



Cmpd	R	R′	R″	K_i (wt)[a] (nM)	Rel. to Cyc	K_i (mut.)[b] (nM)	Rel. to Cyc	K_i (mut.)/K_i (wt)
2[c]	2,4,5-$Cl_3C_6H_2O(CH_2)_3$-	CH_3	CH_3	0.5 ± 0.1[d]	1.0	2.4 ± 0.4[d]	1.0	4.8
3[c,e]	2,4,5-$Cl_3C_6H_2O(CH_2)_3$-	H	CH_3	0.3 ± 0.0	0.2	0.8 ± 0.1	0.0006	2.7
4[c,e]	2,4,5-$Cl_3C_6H_2O(CH_2)_3$-	H	Ph	0.6 ± 0.0	0.4	2.2 ± 0.4	0.002	3.7
II-124[c,f]	$PhCH_2$-	H	CH_3CH_2	-	-	-	-	-
II-125[c,f]	$PhCH_2$-	H	$CH_3(CH_2)_6$	-	-	-	-	-
II-126[c]	$PhCH_2$-	-	-$(CH_2)_5$-	53.9 ± 20.0	35.9	very high	-	-
II-127[c]	$PhCH_2$-	H	Ph	1.6 ± 0.4	1.1	53.2 ± 3.4	0.04	32.7
II-128[c,f]	H	H	Ph	-	-	-	-	-
II-129	$PhCH_2CH_2$-	H	Ph	1.4 ± 0.2	0.9	8.3 ± 2.8	0.006	5.9
II-130	$Ph(CH_2)_3$-	H	Ph	2.2 ± 1.0	1.5	7.0 ± 3.7	0.005	3.2

Table 3.6 Continued

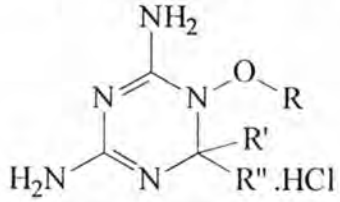
Cmpd	R	R′	R″	K_i (wt)[a] (nM)	Rel. to Cyc	K_i (mut.)[b] (nM)	Rel. to Cyc	K_i (mut.)/K_i (wt)
II-131	2-$BrC_6H_4CH_2$-	H	Ph	6.3 ± 1.3	4.2	7.7 ± 2.3	0.006	1.2
II-132	3-$BrC_6H_4CH_2$-	H	Ph	1.0 ± 0.2	0.7	5.2 ± 0.9	0.004	5.2
II-133	4-$BrC_6H_4CH_2$-	H	Ph	46.0 ± 4.1	30.7	52.1 ± 11.2	0.04	1.1
II-134	4-$CH_3C_6H_4CH_2$-	H	Ph	23.7 ± 2.4	15.8	3.2 ± 5.4	0.02	1.3
II-135	2-Naphthyl CH_2-	H	Ph	1.3 ± 0.3	0.9	5.7 ± 1.3	0.004	4.3
II-136	CH_3OCOCH_2-	H	Ph	842.9 ± 250.3	561.9	572.3 ± 300.9	0.4	0.7
II-137	CH_2=$CHCH_2$-	H	Ph	86.1 ± 5.7	57.4	98.1 ± 11.0	0.08	1.1
II-138	$CH_3CH_2CH_2$-	H	Ph	76.2 ± 2.3	50.8	53.6 ± 17.3	0.04	0.7
II-139	$CH_3(CH_2)_4$-	H	Ph	5.3 ± 1.8	3.5	5.9 ± 1.7	0.005	1.1
II-140[f]	$CH_3(CH_2)_9$-	H	Ph	-	-	-	-	-
II-141	$(CH_3)_2CHCH_2$-	H	Ph	7.5 ± 2.1	5.0	4.9 ± 0.5	0.004	0.6
II-142	$(CH_3)_2CH(CH_2)_2$-	H	Ph	7.6 ± 2.9	5.1	40.2 ± 13.6	0.03	5.3
II-143	$C_6H_{11}CH_2$-	H	Ph	4.2 ± 2.0	2.8	9.8 ± 2.0	0.007	2.3
II-144[f]	$Br(CH_2)_3$-	H	Ph	-	-	-	-	-
II-145[f]	$PhO(CH_2)_2$-	H	Ph	-	-	-	-	-

Table 3.6 Continued

Cmpd	R	R′	R″	K_i (wt)[a] (nM)	Rel. to Cyc	K_i (mut.)[b] (nM)	Rel. to Cyc	K_i (mut.)/K_i (wt)
II-146[f]	$PhO(CH_2)_2$-	H	Ph	-	-	-	-	-
II-147[f]	4-$ClC_6H_4O(CH_2)_3$-	H	Ph	-	-	-	-	-
II-148[f]	4-$CH_3OCOC_6H_4O(CH_2)_3$-	H	Ph	-	-	-	-	-
II-149[f]	4-$CH_3CONHC_6H_4O(CH_2)_3$-	H	Ph	-	-	-	-	-
II-150	4-$PhC_6H_4OCH_2CH_2CH_2$-	H	Ph	1.2 ± 0.3	0.8	2.1 ± 0.3	0.0008	1.7
II-151[f]	$PhS(CH_2)_3$-	H	Ph	-	-	-	-	-

[a]Wild-type pfDHFR. [b]A16V+S108T mutant pfDHFR. [c]Hydrochloride salt. [d]Data from ref 29. [e]Study by Vilaivan, T. [f]The activity is being tested.

Table 3.7 Inhibition constants (K_i) of WR99210 Analogues (.HCl) against the wild-type and A16V+S108T mutant DHFRs of *P. falciparum*



Cmpd	R	R′	R″	K_i (wt)[a] (nM)	Rel. to Cyc	K_i (mut.)[b] (nM)	Rel. to Cyc	K_i (mut.)/K_i (wt)
5[c]	$PhCH_2$-	H	3-$PhOC_6H_4$	0.9 ± 0.05	0.6	5.4 ± 0.6	0.004	6.2
6[c]	$PhCH_2$-	H	4-$PhOC_6H_4$	0.8 ± 0.07	0.6	5.3 ± 1.4	0.004	6.4
7[c]	$PhCH_2$-	H	4-$PhCH_2OC_6H_4$	1.5 ± 0.5	1.0	6.9 ± 0.7	0.005	4.7
8[c]	$PhCH_2$-	H	3-$PhCH_2OC_6H_4$	1.4 ± 0.4	0.9	8.7 ± 1.4	0.007	6.4
9[c]	$PhCH_2$-	H	4-PhC_6H_4	1.6 ± 0.7	1.0	6.4 ± 1.8	0.005	4.1
10[c]	$PhCH_2$-	H	4-HOC_6H_4	1.9 ± 0.3	1.2	41.8 ± 3.4	0.03	22.5
11[c]	$PhCH_2$-	H	4-$CH_3OC_6H_4$	1.6 ± 0.3	1.1	21.3 ± 5.8	0.02	13.3
12[c]	$PhCH_2$-	H	4-ClC_6H_4	1.4 ± 0.1	1.0	19.8 ± 1.5	0.02	13.8
13[c]	$PhCH_2$-	H	4-BrC_6H_4	1.8 ± 0.5	1.2	19.6 ± 4.7	0.02	10.6
14[c]	$PhCH_2$-	H	4-FC_6H_4	1.9 ± 0.2	1.2	41.1 ± 7.9	0.03	22.2
15[c]	$PhCH_2$-	H	3,4,5-$(OCH_3)_3C_6H_2$	50.4 ± 7.2	33.6	1,505.1 ± 396.5	1.2	29.9
16[c]	$PhCH_2$-	H	4-$CH_3C_6H_4$	5.3 ± 1.4	3.5	250.6 ± 4.0	0.2	47.3

[a]Wild-type pfDHFR. [b]A16V+S108T mutant pfDHFR. [c]Study by Vilaivan, T., obtained with permission.

In the present study, the relationship between the structure and biological activity of derivatives of 4,6-diamino-1,2-dihydro-1,3,5-triazine have been investigated. A number of Cyc and WR99210 derivatives were synthesized and K_i values for the wild-type and the A16V+S108T mutant pfDHFRs measured. The K_i values showed that the substituents at 4- and 3-position of the benzene ring at N-1 were important for binding to the wild-type and A16V+S108T pfDHFRs respectively. While the substituent at 2-position of benzene ring at N-1 decrease the binding affinity to both the wild-type and mutant enzymes. Replacement of the chlorine at the 4-position by bromine and smaller alkyl groups had relatively little effect on the binding of the inhibitor to both wild-type and A16V+S108T mutant enzymes while substitution at 3-position improve binding to A16V+S108T mutant enzyme. Furthermore, the presence of flexible side chain of *n*-alkyl or alkyloxy substituents at N-1 showed improved binding to the A16V+S108T mutant pfDHFRs. The length of at lest more than a few carbon atoms of the side chain was important factor for the binding. As for the C-2 substituent, best inhibitors against both wild-type and A16V+S108T were found to possess one H atom and a long chain, phenyl, 3- or 4-phenoxyphenyl groups. These information lead to more understanding about the structural basis of resistance for the cycloguanil-resistant malaria strain. It is also very important for development of new active antimalarial againsts, which are effective against resistant malarial strain as well as the wild-type strain in the future.