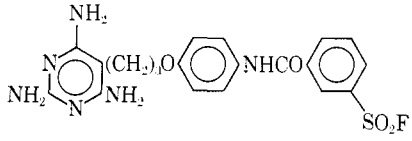

35

pigeon liver. Further studies led to compound **35** which showed little difference in irreversible inhibition of mammalian dihydrofolic reductases, but showed selective irreversible inhibition; a 1 μM concentration of **35** could rapidly inactivate the Walker 256 rat tumor enzyme and the L1210/FR8 mouse leukemia enzyme but did not inactivate the rat or mouse liver enzymes at this concentration.[17]

(17) B. R. Baker and R. B. Meyer, Jr., *J. Med. Chem.*, **11**, 480 (1968), paper CXIX of this series.

Irreversible Enzyme Inhibitors. CXVIII.[1,2] Hydrophobic Bonding to Dihydrofolic Reductase. XII.[2] Further Comparisons with the Enzyme from Walker 256 Rat Tumor and Rat Liver

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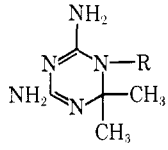
Fourteen *ortho*-substituted 1-phenyl-4,6-diamino-1,2-dihydro-*s*-triazines have been measured as inhibitors of the dihydrofolic reductase from Walker 256 rat tumor and rat liver: no appreciable difference in binding to the enzyme from the two sources was found. The 600-fold loss in binding when 4,6-diamino-2,2-dimethyl-1,2-dihydro-1-phenyl-*s*-triazine (**4**) is substituted with an *o*-chloro group (**5**) can be recouped by further substitution of a 3-chloro group (**16**) or 4-phenylbutyl group (**20**).

In the previous paper of this series a comparison of the binding to the hydrophobic bonding region of the dihydrofolic reductase from Walker 256 rat tumor and rat liver were made with 31 substituted 4,6-diamino-1,2-dihydro-*s*-triazines. The largest difference in binding between the two enzymes was observed[2] with **1** and **2**, the tumor enzyme being inhibited better by 100- and 40-fold, respectively. Even though *ortho* substituents on the 1-phenyl group of dihydro-*s*-triazines give a great loss in biological activity,[3,4] this area has now been further studied particularly since it was possible that

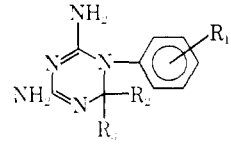


1, R = $(CH_2)_4C_6H_5$
2, R = p-$C_6H_4(CH_2)_4C_6H_3Cl_2$-2,4
3, R = o-R′C_6H_4

the lowered activity could be recouped by additional substituents on the benzene ring[5] and it was possible that tissue specificity might be uncovered. The results of this study on binding of **3** and related compounds to Walker 256 and liver dihydrofolic reductase of the rat is the subject of this paper.

The inhibition of dihydrofolic reductase from two sources with 14 *ortho*-substituted 1-phenyl-*s*-triazines and three related compounds are collated in Table I. No specificity in binding to the two enzymes was observed; nevertheless, some interesting correlations on the *ortho* effects can be made. For discussion purposes only the Walker 256 data will be used.

Table I
Inhibition of Dihydrofolic Reductase by *ortho*-Substituted 1-Phenyl-*s*-triazines



No.	R_1	R_2	R_3	μM concn for 50% inhib[a] Walker 256	Rat liver
4[b]	H	CH_3	CH_3	0.12	0.15
5[b]	o-Cl	CH_3	CH_3	70	77
6[c]	o-Br	CH_3	CH_3	56	87
7	o-I	CH_3	CH_3	24	26
8	o-F	CH_3	CH_3	18	15
9[c]	o-CH_3	CH_3	CH_3	100	93
10[c,d]	o-CH_3O	CH_3	CH_3	210	210
11[c]	H	C_6H_5	H	4.5	10
12	o-Cl	C_6H_5	H	150	130
13	o-F	C_6H_5	H	140	88
14	o-Br	C_6H_5	H	2500	2700
15[b]	m-Cl	CH_3	CH_3	0.017	0.030
16	2,3-Cl_2	CH_3	CH_3	0.30	0.41
17[c]	2,5-Cl_2	CH_3	CH_3	370	350
18[c]	2,4-Cl_2	CH_3	CH_3	150	150
19	2,4,5-Cl_3	CH_3	CH_3	42	90
20	o-Cl-p-$C_6H_5(CH_2)_4$	CH_3	CH_3	0.29	0.46

[a] Assayed with 6 μM dihydrofolate and 30 μM TPNH in pH 7.4 Tris buffer as previously described.[13] The technical assistance of Sharon Lafler, Jean Reeder, and Diane Shea is acknowledged. [b] Data from ref 2. [c] Prepared by the method of Modest.[3b] [d] See M. Furukawa, Y. Seto, and S. Toyoshima, *Chem. Pharm. Bull.* (Tokyo), **9**, 914 (1961).

The o-chloro substituent of **5** causes a 600-fold loss in binding to the enzyme; this loss corresponds to the total increment in 1-phenyl binding compared to 1-methyl,[2] indicating that the phenyl ring of **5** is now out-

(1) This work was generously supported by Grant CA-08695 from the National Cancer Institute, U. S. Public Health Service.

(2) For the previous paper of this series, see B. R. Baker, *J. Med. Chem.*, **11**, 483 (1968).

(3) (a) E. J. Modest, S. Farber, and G. E. Foley, *Proc. Am. Assoc. Cancer Res.*, **1**, 33 (1954); (b) E. J. Modest, *J. Org. Chem.*, **21**, 1 (1956).

(4) B. R. Baker and B.-T. Ho, *J. Pharm. Sci.*, **53**, 1137 (1964).

(5) B. R. Baker, B.-T. Ho, and G. J. Lourens, *ibid.*, **56**, 737 (1967), paper LXXXVI of this series.

of-plane with the *s*-triazine ring, as previously proposed.[3] This lack of coplanarity with **5** is further supported by the observation with the 2-phenyl-*s*-triazines **11** and **12**. When phenyl groups are present at both the 1 and 2 positions of the *s*-triazine (**11**), a 37-fold loss in binding occurs compared to **4**; this loss in binding can also be attributed to some restricted rotation where one of two phenyl groups must be out-of-plane. If both the *o*-chloro substituent of **5** and the 2-phenyl substituent of **11** forces the 1-phenyl out-of-plane, then no further loss in binding should occur if both structural changes are made in the same molecule as in **12**; note that **12** and **5** differ only twofold in binding. However, **11** is a 15-fold better inhibitor than **5**, indicating that other factors are also involved.

One of the uninterpretable factors that may be involved is electronic, but such electronic factors as the Hammett σ value are unreliable with *ortho* substituents. For example, the *o*-fluoro substitution on **8** causes a 150-fold loss in binding compared to **4**; since the fluorine atom is not much larger than hydrogen, this result cannot be due to restricted rotation forcing the 1-phenyl group out-of-plane as in the case of **5**, but must be electronic in nature. That the fluorine has some electronic effect is also indicated by comparison of **11–13**; **12** and **13** are complexed equally, both being about 30-fold less effective than the corresponding compound without an *o*-halogen (**11**).

That restricted rotation is not the complete story is also indicated by comparison of **5–10**. If the size of the *ortho* atom and its resultant effect on copolanarity of the 1-phenyl group were the only effect, then the order of effective binding should parallel decreasing size, that is, F > Cl > Br, CH_3, CH_3O > I. The observed order is F > I > Br > Cl > CH_3 > CH_3O. At least two more effects should be considered, namely, electronic effects and hydrophobic interaction of the group with the enzyme.[6] If it is assumed that Cl, Br, and I have similar electronic effects, then the order of binding can be accounted for by the order of hydrophobicity of I > Br > Cl.[7]

It has been previously observed that the *m*-chloro substituent of **15**, compared to **4**, gives a 7–15-fold increment to binding to the enzyme.[2,4] Recently, evidence has been accumulated to indicate that the interaction of this *m*-chloro with the enzyme is direct, perhaps through a dipole donor to the enzyme, and not due to a hydrophobic interaction since *m*-methyl gives a poorer interaction.[8,9] A pleasant surprise was the observation that the *m*-chloro substituent of the 2,3-dichloro derivative (**16**) gives a 240-fold increment in binding compared to the *o*-chloro derivative (**5**); that the *m*-chloro group of **16** most probably directly interacted with the enzyme is indicated by the fact that the 4- and 5-chloro of **17–18** did not give a gain but a two- to five-fold loss in binding. If the *m*-chloro group has a point interaction with the enzyme, then one must reconcile the probability that the phenyl group of **5** and **16** should be equally out-of-plane in the ground state. One possibility is that the phenyl group of **16** is in-plane when complexed to the enzyme and that energy required to be in-plane is derived from binding energy. This explanation would then require that the *m*-chloro of **16** be complexed to the enzyme considerably stronger than the *m*-chloro of **15**; this might be feasible if the 2-chloro group could augment the supposed dipole interaction of the 3-chloro group with the enzyme. Regardless of mechanism, the important point remains that almost all of the binding lost when an *o*-chloro group (**5**) is introduced on **4** is regained with the second chloro of **16**.

It was previously noted[5] that introduction of a *p*-phenylbutyl substituent on the 1-phenyl group of **4** gave about a 30-fold increment in binding to the enzyme from pigeon liver. Introduction of the *p*-phenylbutyl substituent (**20**) on the *o*-chlorophenyl-*s*-triazine **5** gave an even larger increment in binding, namely 240-fold. This greater effect on introduction of the phenylbutyl group on **5** than on **4** is difficult to reconcile, but may be due to the stronger hydrophobic interaction of a phenylbutyl group to the rat tumor enzyme[2] than the pigeon liver enzyme;[5] nevertheless, the fact remains that nearly all of the binding lost in substituting an *o*-chloro group (**5**) on **4** is regained by further substitution of a *p*-phenylbutyl group to give **20**. Since some species seem to be able to bind an out-of-plane phenyl group to their dihydrofolic reductases,[10] the observations with **16** and **20** may be useful to increase potency.

Although none of the compounds in Table I showed appreciably greater inhibition of the rat tumor enzyme than the rat liver enzyme, it is still theoretically possible, though unlikely, that compounds with the 50,000-fold difference in binding needed for chemotherapy[11] can be found However, it is clear that active-site-directed irreversible inhibitors with tissue specificity[12] for dihydrofolic reductase[13,14] may be easier to find than specific reversible inhibitors.

Chemistry.—All of the dihydro-*s*-triazines (**22**) in Table I were prepared by the three-component method of Modest;[3b] with $R_2 = R_3 = CH_3$, Me_2CO was the reactant and solvent, but with $R_1 = H$ and $R_3 = C_6H_5$, benzaldehyde was the reactant and EtOH was the solvent. All of the arylamines **21** except **26** were commercially available. This amine (**26**) was prepared by Wittig condensation of cinnamyltriphenylphosphonium chloride[15] with the commercially available aldehyde, **24**, followed by hydrogenation with a PtO_2 catalyst (Scheme I).

Experimental Section

All analytical samples had ir and uv spectra and combustion analyses (±0.3) in agreement with their assigned structures; each moved as a single spot on tlc on Brinkmann polyamide MN except for **25** and **26** where silica gel GF was employed. Melting points were determined in capillary tubes on a Mel-Temp block and those below 230° are corrected.

(6) B. R. Baker, B.-T. Ho, and D. V. Santi, *J. Pharm. Sci.*, **54**, 1415 (1965).

(7) T. Fujita, J. Iwasa, and C. Hansch, *J. Am. Chem. Soc.*, **86**, 5175 (1964).

(8) B. R. Baker and G. J. Lourens, *J. Pharm. Sci.*, **56**, 871 (1967), paper LXXXVII of this series.

(9) H. R. Baker and B.-T. Ho, *J. Heterocycl. Chem.*, **2**, 335 (1965).

(10) B. Roth, R. B. Burrows, and G. H. Hitchings, *J. Med. Chem.*, **6**, 370 (1963).

(11) (a) G. H. Hitchings and J. J. Burchall, *Advan. Enzymol.*, **27**, 417 (1965); (b) J. J. Burchall and G. H. Hitchings, *Mol. Pharmacol.*, **1**, 126 (1965).

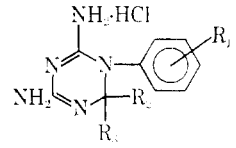
(12) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors. The Organic Chemistry of the Active-Site," John Wiley and Sons, Inc., New York, N. Y., 1967, Chapter IX.

(13) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **10**, 1113 (1967), paper CV of this series.

(14) B. R. Baker and R. B. Meyer, Jr., *ibid.*, **11**, 489 (1968), paper CXIX of this series.

(15) R. N. McDonald and T. W. Campbell, *J. Org. Chem.*, **24**, 1969 (1959).

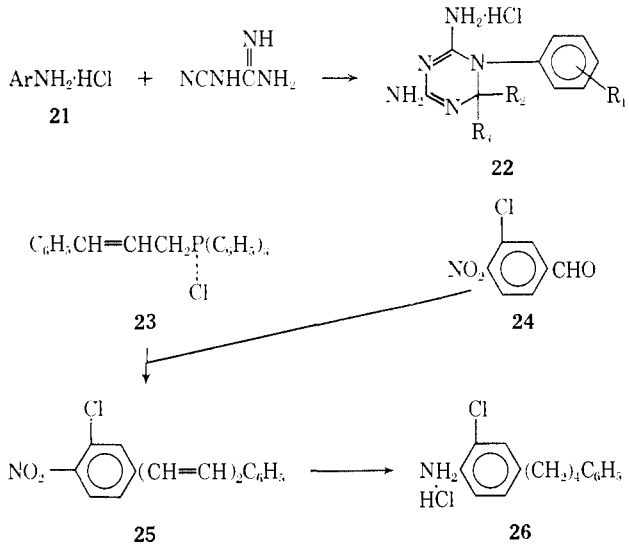
Table II
Physical Properties of



No.	R	R_2	R_3	Method	Yield, %	Mp, °C	Formula	Analyses
7	*o*-I	CH_3	CH_3	A	85	224–228[a]	$C_{11}H_{14}IN_5 \cdot HCl$	C, H, N
8	*o*-F	CH_3	CH_3	A	89	220–225[b]	$C_{11}H_{14}FN_5 \cdot HCl$	C, H, N
10	*o*-Cl	C_6H_5	H	B	32	219–223[c]	$C_{15}H_{14}ClN_5 \cdot HCl$	C, H, N
11	*o*-F	C_6H_5	H	B	33	204–206[c]	$C_{15}H_{14}FN_5 \cdot HCl$	C, H, N
12	*o*-Br	C_6H_5	H	B	34	215–220[d]	$C_{15}H_{14}BrN_5 \cdot HCl$	C, H, N
16	2,3-Cl_2	CH_3	CH_3	A	66	213–218[c]	$C_{11}H_{13}Cl_2N_5 \cdot HCl$	C, H, N
19	2,4,5-Cl_3	CH_3	CH_3	A	26	213–222[e]	$C_{11}H_{12}Cl_3N_5 \cdot HCl$	C, H, N
20	*o*-Cl-*p*-$C_6H_5(CH_2)_4$	CH_3	CH_3	A	66	186–187[c]	$C_{21}H_{26}ClN_5 \cdot HCl$	C, H, N

[a] Recrystallized from H_2O. [b] Recrystallized from EtOH. [c] Separated from reaction mixture in analytical purity. [d] Recrystallized from EtOH–MeOEtOH–petroleum ether (bp 60–110°). [e] Recrystallized from H_2O–MeOEtOH.

Scheme I



1-(3-Chloro-4-nitrophenyl)-4-phenylbutadiene (25).—To a stirred mixture of 2.63 g (14 mmoles) of **24** (Aldrich) and 6.40 g (15 mmoles) of **23**[15] in 40 ml of MeOH was added a solution of 1.57 g (23 mmoles) of NaOMe in MeOH. After being stirred at ambient temperature protected from moisture for 20 hr, the mixture was filtered. The yellow product was washed successively with 25 ml of cold MeOH, 25 ml of cold 50% H_2O–CH_3OH, and 25 ml of cold MeOH; yield 1.75 g, mp 120–123°. An additional 0.1 g (total 46%) was isolated from the combined filtrate and washings. Recrystallization from EtOAc gave yellow crystals, mp 122–124°.

Anal. ($C_{16}H_{12}ClNO_2$) C, H, N.

2-Chloro-4-phenylbutylaniline Hydrochloride (26).—A solution of 1.42 g (5 mmoles) of **25** in 100 ml of EtOH and 0.50 ml of 12 *N* HCl was shaken with H_2 at 2–3 atm in the presence of 50 mg of PtO_2 until 25 mmoles of H_2 was consumed. The filtered solution was spin-evaporated *in vacuo* to about 15 ml when the product began to crystallize. After 2 hr at −15°, the mixture was filtered and the product was washed with cold EtOH; yield 0.45 g, mp 168–172° dec. The combined filtrate and washings were evaporated *in vacuo*. Recrystallization of the residue from *i*-PrOH–petroleum ether (bp 60–110°) gave an additional 0.65 g (total 74%) of crude product, mp 153–163°. An analytical sample, mp 160–163°, was prepared by recrystallization from the same solvent.

Anal. ($C_{16}H_{18}ClN \cdot HCl$) C, H, N.

4,6-Diamino-1-(2,3-dichlorophenyl)-1,2-dihydro-2,2-dimethyl-*s*-triazine Hydrochloride (16) (Method A).—A mixture of 8.10 g (50 mmoles) of 2,3-dichloroaniline, 4.60 g (60 mmoles) of cyanoguanidine, 100 ml of Me_2CO, and 5.0 ml of 12 *N* HCl was refluxed with magnetic stirring for about 18 hr. The product was collected on a filter and washed with acetone; yield 9.92 g, mp 218–223°. An additional 0.15 g (total 66%) was isolated from the filtrate. Recrystallization from water with the aid of decolorizing carbon gave white crystals, mp 213–218°. See Table II for additional data.

1-(*o*-Bromophenyl)-4,6-diamino-1,2-dihydro-2-phenyl-*s*-triazine Hydrochloride (14) (Method B).—A mixture of 172 mg (1 mmole) of *o*-bromoaniline, 0.50 ml of EtOH, 0.090 ml of 12 *N* HCl, 84 mg (1 mmole) of cyanoguanidine, and 106 mg (1 mmole) of benzaldehyde was refluxed with magnetic stirring for 21 hr. The cooled reaction mixture was filtered and the product was washed with cold ethanol. See Table II for additional data.