Irreversible Enzyme Inhibitors. CL.[1,2] Proteolytic Enzymes. IX.[3] (3,4-Dichlorophenoxyacetamido)pyridines Bridged by a Quaternary Salt to a Terminal Sulfonyl Fluoride. Potent Irreversible Inhibitors of α-Chymotrypsin

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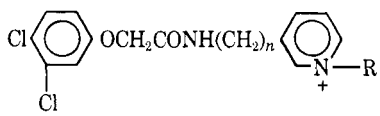
Six candidate irreversible inhibitors (**18**–**23**) for α-chymotrypsin were synthesized by quaternization of N-(β-pyridyl)- (**25**) and N-(β-pyridylmethyl)-3,4-dichlorophenoxyacetamide (**26**) with *m*- and *p*-bromomethylbenzenesulfonyl fluoride and N-bromoacetylsulfanilyl fluoride (**29**); all six were excellent irreversible inhibitors at a K_i concentration, inactivating the enzyme with a half-life of <2 min. One of the best inhibitors was the quaternary salt (**20**) formed between **26** and **29**; at a K_i = 7 μ*M* concentration, **20** showed 89% inactivation of the enzyme in 2 min and 100% in 4 min at 24°.

Derivatives of N-phenyl- (**1**) and N-benzylphenoxyacetamide (**2**) have been found to be good reversible inhibitors of α-chymotrypsin.[5,6] Optimum binding of the Ar group of **1** was achieved with the naphthyl, 3-chlorophenyl, or 3,4-dichlorophenyl moieties.[6] If the R group of **1** and **2** ended in an SO_2F moiety, a series of

1, *n* = 0
2, *n* = 1

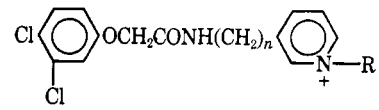


3, *n* = 0
4, *n* = 1

irreversible inhibitors emerged;[7,8] however, many of these could not be evaluated as irreversible inhibitors at a K_i concentration due to insolubility.[3,8] Solubilization was achieved by introducing a carboxyl group *ortho* to the ether linkage of **1** and **2** or replacement of the $ArOCH_2$ moiety by a quaternized 2-pyridylethyl group;[9] use of the *o*-COOH group led to a useful group of SO_2F-type irreversible inhibitors of α-chymotrypsin.[3] Another possibility for solubilization was to use a quaternized N-pyridyl group in the amide moiety (**3**, **4**); if such compounds were well complexed reversibly to the enzyme, they would also have the advantage that the R group could terminate with an SO_2F moiety to give a new class of active-site-directed irreversible inhibitors[10] of α-chymotrypsin. The results of studies with compounds of type **3** and **4** are the subject of this paper.

TABLE I

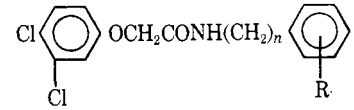
INHIBITION[a,b] OF α-CHYMOTRYPSIN BY



No.	*n*	R	I_{50},[c] μ*M*
7	0	CH_3	340
8	0	$CH_2C_6H_5$	120
9	0	$CH_2C_6H_4NO_2$-*p*	85
10	0	$CH_2C_6H_4NO_2$-*m*	150
11	0	$CH_2C_6H_4CH_3$-*p*	110
12	0	$CH_2C_6H_3Cl_2$-3,4	78
13	0	$CH_2C_6H_4OCH_3$-*p*	90
14	1	CH_3	540
15	1	$CH_2C_6H_5$	380
16	1	$CH_2C_6H_4NO_2$-*p*	470
17	1	$CH_2C_6H_4OCH_3$-*p*	400

[a] The technical assistance of Diane Shea and Marlene Dean is acknowledged. [b] Assayed with 200 μ*M* N-glutaryl-L-phenylalanine-*p*-nitroanilide in 0.05 *M* Tris buffer (pH 7.4) containing 10% DMSO as previously described.[6] [c] I_{50} = concentration for 50% inhibition which is about equivalent to K_i.[7]

Enzyme Results.—Reversible inhibition of α-chymotrypsin by quaternary salts of types **3** and **4** is shown in Table I. It was previously observed[6] that **5** (R = *p*-Cl) had I_{50} = 210 μ*M*; note that **7** has I_{50} = 340 μ*M*. Thus the pyridyl quaternary (**7**) binds about as



5, *n* = 0
6, *n* = 1

well as **5** (R = *p*-Cl) which in turn binds three to ten times better than **5** (R = H). Replacement of the N-methyl group of **7** by N-benzyl (**8**) gave a threefold

(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, *Accounts Chem. Res.*, in press.

(3) For paper VIII of the subseries see B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **11**, 118 (1969).

(4) NDEA predoctoral fellow.

(5) For a discussion of the utility of inhibitors of proteolytic enzymes in the cardiovascular disease and organ transplantation areas see B. R. Baker and E. H. Erickson, *J. Med. Chem.*, **10**, 1123 (1967), paper CVI of this series.

(6) B. R. Baker and J. A. Hurlbut, *ibid.*, **10**, 1129 (1967), paper CVII of this series.

(7) B. R. Baker and J. A. Hurlbut, *ibid.*, **11**, 233 (1968), paper CXIII of this series.

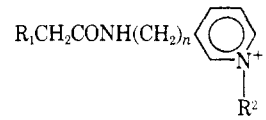
(8) B. R. Baker and J. A. Hurlbut, *ibid.*, **11**, 241 (1968), paper CXIV of this series.

(9) B. R. Baker and J. A. Hurlbut, *ibid.*, **11**, 1054 (1968), paper CXXXII of this series.

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

Table II

Irreversible Inhibition[a,b] of α-Chymotrypsin by



No.	n	R_1	R_2	I_{50},[c] μM	Inhib., μM	Time, min	% inactvn[d]
18	1	3,4-$Cl_2C_6H_3O$	$CH_2C_6H_4SO_2F$-*p*	16	16	0.5, 4, 8	50, 96, 100[e]
					4	60	100
19	1	3,4-$Cl_2C_6H_3O$	$CH_2C_6H_4SO_2F$-*m*	96	96	2, 4	98, 100[e]
20	1	3,4-$Cl_2C_6H_3O$	$CH_2CONHC_6H_4SO_2F$-*p*	7.4	7	2, 4	89, 100[e]
21	0	3,4-$Cl_2C_6H_3O$	$CH_2C_6H_4SO_2F$-*p*	15	15	2, 8, 30	81, 89, 100[e]
22	0	3,4-$Cl_2C_6H_3O$	$CH_2C_6H_4SO_2F$-*m*	51	51	2, 4, 8, 30	50, 73, 90, 100[e]
23	0	3,4-$Cl_2C_6H_3O$	$CH_2CONHC_6H_4SO_2F$-*p*	10	10	2, 4	98, 100
24	0	H	$CH_2C_6H_4SO_2F$-*p*	96	96	2, 4	99, 100
					15	2, 7, 16, 30	34, 53, 71, 85

[a-c] See corresponding footnotes in Table I. [d] Inactivation performed with ~1 μM enzyme at 24° in 0.05 M Tris (pH 7.4) containing 10% DMSO, then the remaining enzyme concentration was assayed with N-benzoyl-L-tyrosine ethyl ester in pH 8.1 Tris buffer containing 0.1 M $CaCl_2$ as previously described.[3] [e] From a six-point time study.[3,7]

increase in binding, indicating that this phenyl moiety was interacting weakly with the enzyme; however, this phenyl interaction was not appreciably influenced by substitution (**9**–**13**). In the pyridylmethyl series, the pyridyl quaternary **14** was only one fourth as effective as **6** (R = *m*-Cl);[6] furthermore, there was little change in binding with benzyl quaternaries (**15**–**17**).

A series of candidate irreversible inhibitors (**18**–**23**) of types **3** and **4** were then synthesized for enzymic evaluation (Table II); all six compounds were excellent irreversible inhibitors at a K_i concentration with a half-life of inactivation of 2 min or less. However, the *p*-fluorosulfonylbenzyl quaternary salts (**18**, **21**) were considerably better reversible inhibitors than the *meta* isomers (**19**, **22**). Since there did not appear to be any effect on binding by an electron-withdrawing (**10**, **16**) or an electron-donating group (**13**, **17**) on the benzyl moiety, this strong reversible binding by the *p*-SO_2F-substituted compounds may be due to a point interaction of this group to the enzyme by an F:→H-enzyme hydrogen bond.

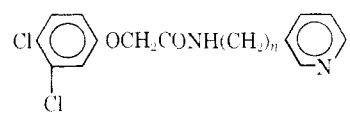
The question was then posed as to whether or not these quaternary salts (**18**–**23**) inactivated α-chymotrypsin by the active-site-directed mechanism or by a random bimolecular attack.[11] The compounds were such fast, irreversible inhibitors, it would be difficult to use the usual "rate-saturation" criterion, where comparison of the rates of inactivation by K_i and $3K_i$ concentration would give only a 1.5-fold increase in rate.[11] Therefore, the alternate approach of removing a reversible binding group on the inhibitor was employed.[12]

Since the 3,4-dichlorophenoxymethyl moiety of inhibitors of types **1** and **2** is reversibly complexed to the enzyme, this group in **21** was replaced by H (**24**). A sixfold loss in reversible binding occurred. When a K_i concentration (96 μM) of **24** was incubated with α-chymotrypsin, the rate of inactivation was as fast or faster than that with a K_i concentration (15 μM) of **21**. However, when **21** and **24** were compared at 15 μM, **24** was considerably slower than **21**. If **21** had inactivated the enzyme by a random bimolecular mechanism, then the same concentration of **24** should inactivate the enzyme even more rapidly than **21** due to the "self-protection" that **21** could afford.[11] However, if both **21** and **24** operated by the active-site-directed mechanism, then the rate of inactivation would depend on the amount of enzyme reversibly complexed (E···I).[11] Since 15 μM of **21** forms 50% EI complex and 15 μM of **24** forms only 13% EI complex, then **24** should inactivate the enzyme at about one-fourth the rate of **21** as noted in Table II; this calculation requires the logical assumption that the rate of reaction of the SO_2F moiety with α-chymotrypsin is not influenced by the structural change between **21** and **24** that is relatively remote from the SO_2F moiety.

An unlikely possibility existed that the irreversible inhibition by the compounds in Table II occurred by alkylation of the enzyme with the pyridinium moiety acting as the leaving group; this possibility was readily eliminated since I_{50} concentrations of **7**, **11**, and **16** showed no inactivation of the enzyme after 60-min incubation. Thus the SO_2F moiety is the only moiety left that could form a covalent bond with the enzyme.

If the irreversible inhibitors in Table II have their phenoxyacetamide moieties complexed in the same way to α-chymotrypsin, it is unlikely that the same amino acid (serine?) in the enzyme is covalently linked by **18**–**23**; such a study would be worthwhile. Furthermore, the "chymotryptic" component of the complement system[13] is inhibited by some of the members of Table II.

Chemistry.—The inhibitors in Tables I and II have the general structures **3** and **4**; they were prepared by quaternization of **25** or **26** with the appropriate alkyl



25, *n* = 0
26, *n* = 1

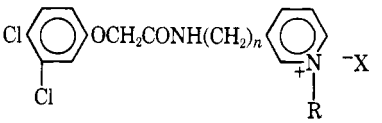
(11) For the kinetics of irreversible inhibition see ref 10, pp 122–129.

(12) (a) Reference 10, pp 170–171; (b) B. R. Baker, W. W. Lee, and E. Tong, *J. Theoret. Biol.*, **3**, 459 (1962).

(13) (a) Ciba Foundation Symposium, Complement, G. E. W. Wolstenholme and J. Knight, Ed., Little, Brown and Co., Boston, Mass., 1965; (b) H. J. Müller-Eberhard, *Advan. Immunol.*, **8**, 1 (1968).

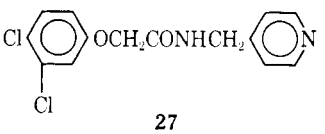
TABLE III

PHYSICAL PROPERTIES OF



No.	n	X	R	Method	Yield, %	Mp, °C dec	Formula	Analyses
7	0	I	CH_3	B	80[a]	206–209	$C_{14}H_{13}Cl_2IN_2O_2$	C, H, N
8	0	Br	$CH_2C_6H_5$	A	79[a]	195–196	$C_{20}H_{17}BrCl_2N_2O_2$	C, H, N
9	0	Br	$CH_2C_6H_4NO_2$-*p*	A	67[b]	218–219	$C_{20}H_{16}BrCl_2N_3O_4$	C, H, N
10	0	Cl	$CH_2C_6H_4NO_2$-*m*	A	46[b]	230–232	$C_{20}H_{16}Cl_3N_3O_4$	C, H, N
11	0	Br	$CH_2C_6H_4CH_3$-*p*	A	94[a]	209–210	$C_{21}H_{19}BrCl_2N_2O_2$	C, H, N
12	0	Cl	$CH_2C_6H_3Cl_2$-3,4	A	90[b]	228–229	$C_{20}H_{15}Cl_5N_2O_2$	C, H, N
13	0	Br	$CH_2C_6H_4OCH_3$-*p*	A[c]	55[a]	196–198	$C_{21}H_{19}BrCl_2N_2O_3$	C, H, N
14	1	TsO	CH_3	A	67[i]	132–134	$C_{22}H_{22}Cl_2N_2O_5S$	C, H, N
15	1	Br	$CH_2C_6H_5$	A[d]	57[e,a]	156–158	$C_{21}H_{19}BrCl_2N_2O_2$	C, H, N
16	1	Br	$CH_2C_6H_4NO_2$-*p*	A	58[b]	111–120	$C_{21}H_{18}BrCl_2N_3O_4$	C, H, N
17	1	Br	$CH_2C_6H_4OCH_3$-*p*	A[c]	65[a]	178–180	$C_{22}H_{21}BrCl_2N_2O_3$	C, H, N
18	1	Br	$CH_2C_6H_4SO_2F$-*p*	B[f]	61[a]	189–190	$C_{21}H_{18}BrCl_2FN_2O_4S$	C, H, F
19	1	Br	$CH_2C_6H_4SO_2F$-*m*	A[d]	24[g]	155–157	$C_{21}H_{18}BrCl_2FN_2O_4S$	C, H, N
20	1	Br	$CH_2CONHC_6H_4SO_2F$-*p*	A	74[h]	196–197	$C_{22}H_{19}BrCl_2FN_3O_5S$	C, H, F
21	0	Br	$CH_2C_6H_4SO_2F$-*p*	B[f]	17[i,a]	190–191	$C_{20}H_{16}BrCl_2FN_2O_4S$	C, H, F
22	0	Br	$CH_2C_6H_4SO_2F$-*m*	B	44[a]	212–213	$C_{20}H_{16}BrCl_2FN_2O_4S$	C, H, F
23	0	Br	$CH_2CONHC_6H_4SO_2F$-*p*	A	80[a]	178–180	$C_{21}H_{17}BrCl_2FN_3O_5S$	C, H, F

[a] Recrystallized from EtOH. [b] Recrystallized from Me_2CO–MeOEtOH. [c] The α-bromo-*p*-methoxytoluene was made by the method of S. Feng and K. Chiu, *Hua Hsüeh Hsüeh Pao*, **25**, 277 (1959), and used immediately due to its instability. [d] The compound was precipitated out of the reaction mixture with petroleum ether (60–110°). [e] Recrystallized from EtOH–petroleum ether (60–110°). [f] See B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **11**, 666 (1968), for the preparation of the required starting bromide. [g] Recrystallized from EtOH–Me_2CO. [h] Recrystallized from MeOEtOH–EtOH. [i] Recrystallized from Me_2CO.



27

bromide or toluenesulfonate in hot Me_2CO or $CHCl_3$. Attempts to quaternize **27** led to blue pigments which could be decolorized by addition of HBr; however, pure quaternary salts could not be isolated from **27**. The required pyridines (**25–27**) were prepared by condensation of the appropriate pyridine with 3,4-dichlorophenoxyacetic acid, the carboxyl group being activated by a mixed anhydride for **26** and **27** and an acid chloride for **25**.

Experimental Section

Each analytical sample had an appropriate ir spectrum and moved as a single spot on tlc on Brinkmann silica gel GF; Brinkmann MN-polyamide was used for the quaternary salts. Combustion values within 0.4% of theoretical for C, H, and N or F were obtained. Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected.

N-(β-Pyridylmethyl)-3,4-dichlorophenoxyacetamide (26).—To a stirred solution of 4.42 g (20 mmoles) of 3,4-dichlorophenoxyacetic acid in 50 ml of THF containing 2.02 g (20 mmoles) of Et_3N was added 2.18 g (20 mmoles) of ethyl chloroformate in 10 ml of THF over a period of 3 min maintaining the temperature at −5 to 0°. After being stirred by an additional 45 min at −5 to 0°, 2.16 g (20 mmoles) of 3-(aminomethyl)pyridine in 50 ml of ice-cold THF was added dropwise (5 min). The mixture was allowed to warm slowly to room temperature over 12 hr, then it was refluxed for 5 min. After filtration the THF solution was evaporated *in vacuo*, and the resulting oil was crystallized from aqueous EtOH. Recrystallization from petroleum ether (bp 60–110°) gave 3.5 g (57%) of white solid, mp 96–99°. *Anal.* ($C_{14}H_{12}Cl_2N_2O_2$) C, H, N.

Similarly, **27** was prepared and recrystallized from EtOH; yield 2.35 g (38%), mp 145–147°. *Anal.* ($C_{14}H_{12}Cl_2N_2O_2$) C, H, N.

N-(3-Pyridyl)-3,4-dichlorophenoxyacetamide (25).—A mixture of 5.5 g (25 mmoles) of 3,4-dichlorophenoxyacetic acid and 10 ml of $SOCl_2$ was refluxed until gas evolution ceased (45 min). The excess $SOCl_2$ was evaporated *in vacuo*, and the resulting acid chloride in 20 ml of $CHCl_3$ was added dropwise over 30 min to an ice-cooled solution of 1.88 g (20 mmoles) of 3-aminopyridine and 2.0 g (20 mmoles) of Et_3N in 40 ml of $CHCl_3$. After being stirred 30 min at ambient temperature and 5 min on a steam bath, the solution was washed with three 200-ml portions of H_2O and dried ($MgSO_4$), then the product was isolated by precipitation with petroleum ether (60–110°). Recrystallization from toluene yielded 3.3 g (56%) of white needles, mp 134–136°, which gave a negative Bratton–Marshall test for aromatic amine.[14] *Anal.* ($C_{13}H_{10}Cl_2N_2O_2$) C, H, N.

α-Bromo-*m*-toluenesulfonyl Fluoride (28).—*m*-Toluenesulfonyl fluoride[15] was prepared from the corresponding chloride.[16] A mixture of 17.4 g (0.1 mole) of the fluoride, 17.8 g (0.1 mole) of NBS, 0.3 g of benzoyl peroxide, and 100 ml of CCl_4 was refluxed for 7 hr when the succinimide had risen to the surface. The cooled CCl_4 was treated with charcoal, filtered, and evaporated *in vacuo* leaving 24.6 g (97%) of a yellow oil; this gave one major spot and one minor spot on tlc using petroleum ether (60–110°)–EtOAc (4:1). Both spots gave a positive active halide test.[14] The oil was used without further purification. *This compound is a skin irritant* that should be handled with care.

N-Bromoacetylsulfanilyl Fluoride (29).—To 12.1 g (60 mmoles) of bromoacetyl bromide in 100 ml of $CHCl_3$ was added dropwise (15 min) with stirring and ice cooling 8.75 g (50 mmoles) of sulfanilyl fluoride in 50 ml of $CHCl_3$. To this ice-cold mixture was then added (10 min) 5.35 g (50 mmoles) of 2,6-lutidine in 25 ml of $CHCl_3$. After being stirred an additional 15 min at room temperature, the mixture was filtered and the filtrate was washed successively with two 200-ml portions of H_2O, two 100-ml portions of 5% HCl, 100 ml of 5% $NaHCO_3$, and two 200-ml portions of H_2O. Dried with $MgSO_4$, the solution was diluted with 100 ml

(14) B. R. Baker, D. V. Santi, J. K. Coward, H. S. Shapiro, and J. H. Jordaan, *J. Heterocycl. Chem.*, **3**, 425 (1966).

(15) F. E. Jenkins and A. N. Hambly, *Aust. J. Chem.*, **6**, 318 (1953).

(16) *m*-Toluenesulfonyl chloride was prepared from *m*-toluidine by the general method of H. Meerwein, G. Dittmar, R. Gollner, K. Hafner, F. Mensch, and O. Steinfort, *Chem. Ber.*, **90**, 841 (1957).

of petroleum ether (60–110°) and the product was collected on a filter. Recrystallization from toluene yielded 5.05 g (34%) of white crystals, mp 141–144°, which gave a positive active halide test[14] and a negative Bratton–Marshall test for aromatic amine.[14] *Anal.* ($C_{15}H_{17}BrFNO_3S$) C, H, F.

This compound should be handled with caution since it is an extreme skin irritant.

α-[3-(3,4-Dichlorophenoxyacetamido)pyridinium]-p-fluorosulfonylacetanilide Bromide (23) (Method A).—A solution of 0.89 g (3.0 mmoles) of **25** and 0.97 g (3.3 mmoles) of **29** in 20 ml of Me_2CO was refluxed for 18 hr. The warm Me_2CO was decanted from the yellow oil, and the oil was rubbed with fresh Me_2CO until it solidified. The product was collected, washed (Me_2CO), and recrystallized from EtOH; yield, 1.43 g (80%) of white solid, mp 178–180°.

See Table III for additional compounds prepared by this method. Similarly, **24** was prepared from 3-acetamidopyridine;[17] yield, 0.70 g (36%) of tan crystals, mp 208–210°. *Anal.* ($C_{14}H_{14}BrFN_2O_3S$) C, H, F.

Method B was the same as A, but $CHCl_3$ was employed as solvent.

(17) W. Herz and D. Murty, *J. Org. Chem.*, **25**, 2242 (1960).

Irreversible Enzyme Inhibitors. CLI.[1,2] Active-Site-Directed Irreversible Inhibitors of Dihydrofolic Reductase[3] Derived from 5-(*p*-Aminophenylbutyl)-2,4-diaminopyrimidines with a Terminal Sulfonyl Fluoride

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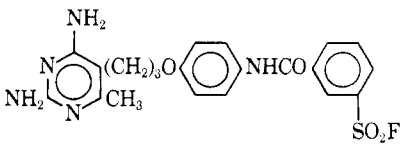
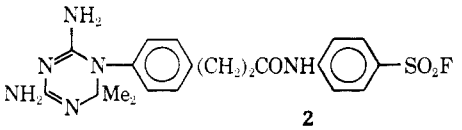
Wittig condensation of 2-acetamido-4-hydroxy-6-methylpyrimidine-5-propionaldehyde (**5**) with *p*-nitrobenzyl triphenylphosphonium bromide (**6**) proceeded in 69% yield to 2-acetamido-6-methyl-5-[4-(*p*-nitrophenyl)-3-buten-1-yl]-4-pyrimidinol (**7**) in DMF by use of 1,4-diazabicyclo[4.3.0]non-5-ene (DBN) as the base. By further transformations, **7** was converted to the key intermediate, 5-(*p*-aminophenylbutyl)-2,4-diamino-6-methylpyrimidine (**11**). 2,4-Diacetamidopyrimidine-5-carboxaldehyde (**14**) condensed smoothly with *p*-nitrocinnamyltriphenylphosphonium bromide (**15**) with DBN as the base to give 2,4-diacetamido-5-[4-(*p*-nitrophenyl)-1,3-butadien-1-yl]pyrimidine (**16**) in 84% yield. Catalytic reduction of **16** and hydrolysis afforded the key intermediate, 5-(*p*-aminophenylbutyl)-2,4-diaminopyrimidine (**17**) in 56% yield. Acylation of the arylamino group of **11** and **17** with *m*- or *p*-fluorosulfonylbenzoyl chloride gave a series of candidate irreversible inhibitors of dihydrofolic reductase. Two of these were excellent irreversible inhibitors of the enzyme from L1210 mouse leukemia and showed good specificity by showing poor inactivation of the mouse liver enzyme; however, these compounds showed poor diffusion through the L1210 cell wall.

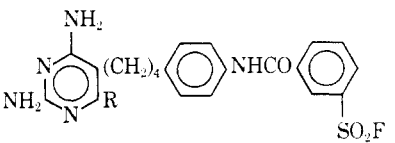
In a previous study, the 5-phenoxypropylpyrimidine (**1**) was found to be an excellent active-site-directed irreversible inhibitor[4] of dihydrofolic reductase from L1210 mouse leukemia when assayed at a $K_i = 3 \times 10^{-9}$ *M* concentration; furthermore, at 60–100K_i, **1** showed no inactivation of this enzyme from the liver, intestine, or spleen of the mouse.[3,5] Unfortunately, **1** showed poor penetration of the L1210 cell wall and as a result was inactive *in vivo*.[3] In contrast, **2** was an active-site-directed irreversible inhibitor of dihydrofolic reductase with little selectivity;[5] however, **2** penetrated the cell quite effectively and as a result **2** showed reproducible *in vivo* activity against L1210.[3,5] Presumably, both **1** and **2** penetrate the L1210 cell wall by passive diffusion;[6] therefore, the difference in the ability of **1** and **2** to diffuse through the cell wall was surprising. Little is known about effect of variation of structure on cell wall diffusion since assays are usually performed with intact cells or with isolated target enzymes, but seldom both; without both assays, one cannot differentiate poor inhibition from poor diffusion. A recent study on dihydrofolic reductase inhibitors for *Escherichia coli*[7] indicated that structural change could change diffusion by a factor of 2700 after the effect on the enzyme is normalized by comparing the ED_{50}/I_{50} ratio.



1



2



3, R = CH_3
4, R = H

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(2) For the previous paper in this series see B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **12**, 221 (1969).

(3) For the previous paper on this enzyme see B. R. Baker and R. B. Meyer, Jr., *ibid.*, **12**, 108 (1969), paper CXLIII of this series.

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

(5) B. R. Baker, G. J. Lourens, R. B. Meyer, Jr., and N. M. J. Vermeulen, *ibid.*, **12**, 67 (1969), paper CXXXIII of this series.

(6) (a) R. C. Wood and G. H. Hitchings, *J. Biol. Chem.*, **234**, 2381 (1959); (b) R. C. Wood and G. H. Hitchings, *ibid.*, **234**, 2377 (1959); (c) B. R. Baker, D. V. Santi, P. I. Almaula, and W. C. Werkheiser, *J. Med. Chem.*, **7**, 24 (1964).

(7) B. S. Hurlbert, R. Ferone, T. A. Herrmann, G. H. Hitchings, M. Barnett, and S. R. M. Bushby, *ibid.*, **11**, 711 (1968).