Irreversible Enzyme Inhibitors. CLX.[1,2] Some Factors in Cell Wall Transport of Active-Site-Directed Irreversible Inhibitors of Dihydrofolic Reductase Derived from 4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-(phenylalkylphenyl)-*s*-triazines

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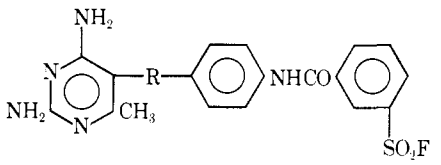
A series of nine derivatives of 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-phenyl-*s*-triazines bearing a phenylbutyl, phenethyl, or phenoxybutyl side chain on the *meta* or *para* position of the benzene ring were evaluated as inhibitors of L1210 cell culture; several of these compounds were excellent inhibitors that showed 50% cell kill in the 10^{-9}–10^{-10} *M* concentration range and had $I_{50} = 6K_i$ for L1210 dihydrofolic reductase of about 10^{-8} *M*. When these phenylalkyl derivatives were substituted with an SO_2F group, some potent irreversible inhibitors were obtained that could inactivate L1210 dihydrofolic reductase >80% in 2 min or less; for example, the *p*-fluorosulfonylphenylbutyl substituent on the *meta* position (**19**) had $I_{50} = 6K_i = 8 \times 10^{-9}$ *M*, and at 6×10^{-8} *M* gave 86% inactivation of the L1210 enzyme in <2 min, but also inactivated the mouse liver enzyme. It is estimated that the polar SO_2F moiety slows passive diffusion about 40-fold, but increases effectiveness on the target enzyme, dihydrofolic reductase, by about 200-fold.

Numerous active-site-directed irreversible inhibitors[4] of the dihydrofolic reductase from L1210 mouse leukemia have been reported from this laboratory; these were derived either from 2,4-diaminopyrimidines (**1**, **2**) or 4,6-diamino-1,2-dihydro-1-phenyl-*s*-triazines (**3**) with



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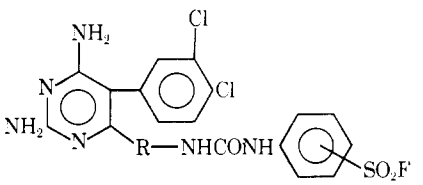

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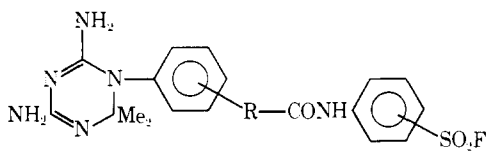

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a terminal sulfonyl fluoride group; these followed in rapid order after the discovery of the first irreversible inhibitor of the SO_2F type.[5] The main emphasis, after the initial irreversible inhibitor[5] was found, was to determine if irreversible specificity between L1210 and liver enzymes from the mouse could be achieved; this was indeed the case since completely selective irreversible inhibitors were designed that could operate at 10^{-8}–10^{-9} *M*.[6,7]

Most of the studies were with compounds containing an amide or urea bridge between the diamino heterocycle and the benzenesulfonyl fluoride moieties, since these were the easiest to synthesize to see if specificity could be achieved. The possible difficulty that the compounds could be ineffective *in vivo* due to poor cell wall transport was avoided in these earlier studies by use of broken cell systems containing dihydrofolic reductase as the target. Now that it has been demonstrated that irreversible specificity can be achieved, attention has been directed toward structures that would have good cell wall transport characteristics.

Although the amide bridges were easier to construct than ether or alkane bridges for the specificity studies, such amides could be expected to impair cell wall transport compared to the other two types; an amide bridge would be hydrogen bonded to water and these bonds would have to be broken in order for the compound to enter the lipid phase of a cell wall, a process which would slow transport since it requires energy.[8] Therefore, attention has now been directed toward synthesis of appropriate irreversible inhibitors without amide bridges that might have better cell wall transport characteristics.

Since it could be envisioned that there might be limitations on the bulkiness of the side chains, as well as their polarity, on transport, an initial survey of L1210 cell kill[9] was made with some previously described reversible inhibitors[10-13] with appropriate bridges; the results are listed in Table I.

In order to normalize differences in the reversible binding to L1210 dihydrofolic reductase, the concentra-

(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 and W. A. Ashton, *J. Med. Chem.*, **12**, 894 (1969).

(3) N. M. J. V. wishes to thank the Council of Scientific and Industrial Research, Republic of South Africa, for a tuition fellowship.

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

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

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

(7) B. R. Baker and R. B. Meyer, Jr., *ibid.*, **12**, 108 (1969), paper CXLIII of this series.

(8) W. D. Stein, "The Movement of Molecules across Cell Membranes," Academic Press, Inc., New York, N. Y., 1967.

(9) We wish to thank Dr. Florence White of CCNSC for these results obtained by Dr. Philip Himmelfarb of Arthur D. Little, Inc.

(10) B. R. Baker and B. T. Ho, *J. Heterocycl. Chem.*, **2**, 72 (1965).

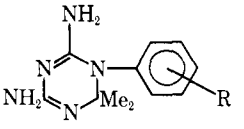
(11) B. R. Baker, B. T. Ho, and G. J. Lourens, *J. Pharm. Sci.*, **56**, 737 (1967), paper LXXXVI of this series.

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

(13) G. J. Lourens, Ph.D. thesis, University of California at Santa Barbara, 1968.

TABLE I

INHIBITION OF L1210 DIHYDROFOLIC REDUCTASE AND L1210 CELL CULTURE BY



No.	R	I_{50},[a,b] μM	ED_{50},[c] μM	ED_{50}/I_{50}
4[d]	3,4-Cl_2	0.0064	0.0002	0.03
5[e]	*m*-$(CH_2)_2C_6H_5$	0.0065	0.002	0.3
6[f]	*m*-$(CH_2)_4C_6H_5$	0.0045	0.0004	0.09
7[f]	*m*-$(CH_2)_4C_6H_3Cl_2$-2,4	0.0093	0.0009	0.1
8[g]	*m*-$(CH_2)_4OC_6H_5$	0.0045	0.002	0.4
9[f]	*p*-$(CH_2)_2C_6H_5$	0.013	0.002	0.2
10[f]	*p*-$(CH_2)_4C_6H_5$	0.010	0.01	1
11[f]	*p*-$(CH_2)_4C_6H_3Cl_2$-2,4	0.012	0.0007	0.06
12[h]	*p*-$(CH_2)_4OC_6H_5$	0.0058	0.0007	0.1
13[f]	*m*-Cl-*p*-$(CH_2)_4C_6H_5$	0.012	0.007	0.6

[a] The technical assistance of Diane Shea with these assays is acknowledged. [b] I_{50} = concentration for 50% inhibition of L1210/DF8 dihydrofolic reductase when assayed with 6 μM dihydrofolate and 0.15 M KCl in pH 7.4 Tris buffer as previously described.[6] [c] Concentration for 50% kill of L1210 cell culture. [d] Obtained from Dr. H. B. Wood, Jr., CCNSC. [e] See ref 10 for synthesis. [f] See ref 11 for synthesis. [g] See ref 12 for synthesis. [h] See ref 13 for synthesis.

tion for 50% cell kill (ED_{50}) can be converted to ED_{50}/I_{50} for comparison;[14] actually this series in Table I differed in I_{50} only by a factor of two so that this normalization procedure is not as important as in some previous cases. The compounds in Table I are derived from 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-phenyl-*s*-triazine by placing substituents on the 1-phenyl moiety. The 3,4-dichloro derivative **4** shows excellent cell wall transport as approximated by the $ED_{50} = 2 \times 10^{-10}$ M; this compound (**4**) was used as a base line for comparison of other compounds.

Three *meta* substituents on the 1-phenyl-*s*-triazine were evaluated. The *m*-phenethyl (**5**) and *m*-phenoxybutyl (**8**) substituents were tenfold less effective than 3,4-Cl_2 (**4**). The phenylbutyl substituent (**6**) was only threefold less effective than 3,4-Cl_2 (**4**); thus the poorer two were still extremely potent, with $ED_{50} = 2 \times 10^{-9}$ M. When **6** was further substituted on the terminal phenyl by 2,4-Cl_2 (**7**), no change in the ED_{50}/I_{50} ratio occurred. Therefore **5** and **6** were selected for initial studies on conversion to irreversible inhibitors.

The same three substituents on the *para* position were investigated, namely phenethyl (**9**), phenylbutyl (**10**), and phenoxybutyl (**12**); the best of these three was **12**. When the terminal phenyl group of the phenylbutyl derivative (**10**) was substituted by 2,4-Cl_2, the resultant **11** showed a 16-fold better ED_{50}/I_{50} ratio; however, little change occurred when **10** was substituted by Cl on the inside phenyl to give **13**. Therefore **9**, **10**, and **13** were selected for conversion to irreversible inhibitors.

In Table II are the reversible and irreversible inhibition results with some sulfonyl fluoride derivatives of **5**, **6**, **9**, and **10**. It was previously reported from this laboratory[15] that **14** was a good reversible inhibitor of dihydrofolic reductase, but showed no irreversible inhibition of the L1210 mouse leukemia enzyme. When the $(CH_2)_2$ bridge of **14** was extended to $(CH_2)_4$ (**15**) or if a chlorine atom was introduced on the inside phenyl *ortho* to the bridge (**16**), then good, but not complete, irreversible inhibition of the L1210 enzyme was seen.[15] These two structural changes have now been incorporated into the same molecule to give **17**; **17** at 0.05 μM was an excellent irreversible inhibitor of the L1210/DF8 enzyme, showing 82% inactivation of the enzyme in 2 min at 37° and total inactivation in further time. However, **17** showed poor selectivity since the liver enzyme was inactivated 41% under the same conditions.

The $(CH_2)_2$ and $(CH_2)_4$ bridges were then moved to the *meta* position of the inside phenyl to give **18** or **19**, respectively; reversible inhibition was enhanced three- to fivefold compared to the *para*-bridged compounds **14** and **15**, as might be anticipated from the data in Table I. In contrast to the *para* compound **14**, the *meta*-bridged $(CH_2)_2$ derivative **18** showed fair irreversible inhibition of the L1210 enzyme, but no irreversible inhibition of the mouse liver enzyme. The *meta*-bridged $(CH_2)_4$ derivative **19** at 0.066 μM was an excellent irreversible inhibitor of L1210 dihydrofolic reductase, showing 86% inactivation in <2 min at 37°; unfortunately, the mouse liver enzyme was inactivated extensively, but not completely.

When the *p*-SO_2F moiety of **19** was moved to the *meta* position, the resulting **20** was still an excellent irreversible inhibitor of the L1210 enzyme, and the inactivation of the mouse liver enzyme was decreased.

Four of the better irreversible inhibitors of L1210 dihydrofolic reductase were evaluated with the enzymes from Walker 256 rat tumor and rat liver; **16**, **17**, **19**, and **20** were all good to excellent irreversible inhibitors of the enzyme from both sources and no selectivity was seen.

The compounds in Table II were then evaluated for cell kill of L1210 cell culture, the concentration for 50% cell kill being expressed as ED_{50}.[9] If it is assumed that the lethality target in the cell is dihydrofolic reductase, then it must also be assumed that the compounds were transported through the cell wall; inhibition of this target would lead to death due to the lack of thymidylate and the resultant cessation of DNA synthesis.[16] Given this assumption, the ED_{50} is then dependent upon the following factors with a candidate inhibitor: (a) rate of transport, (b) concentration required for 50% reversible inhibition (I_{50}), (c) effectiveness as an irreversible inhibitor in inactivating the enzyme, and (d) intracellular destruction of the inhibitor by metabolism.

Factor b can be eliminated by comparing the ratios of ED_{50}/I_{50} instead of comparing the ED_{50}'s of two compounds.[14] Factor d can most probably be ignored if the inhibitor shows rapid inactivation of the enzyme; therefore, as a first approximation the ED_{50}/I_{50} ratio is mainly dependent upon the rate of transport and the effectiveness of the compound as an irreversible inhibitor.

It is most probable that structures of the type in Tables I and II are transported through the cell wall

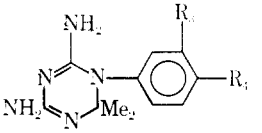
(14) B. R. Baker and R. B. Meyer, Jr., *J. Med. Chem.*, **12**, 668 (1969), paper CLIV of this series.

(15) B. R. Baker and G. J. Lourens, *ibid.*, **12**, 95 (1969), paper CXL of this series.

(16) (a) D. Roberts and I. Wodinsky, *Cancer Res.*, **28**, 1955 (1968); (b) T. H. Jukes and H. P. Broquist in "Metabolic Inhibitors," R. M. Hochster and J. H. Quastel, Ed., Academic Press, Inc., New York, N. Y., 1963, pp 481–534.

Table II

Inhibition[a] of Dihydrofolic Reductase and L1210 Cell Culture by



No.	R_3	R_4	Tissue	I_{50},[b] μM	Inhib., μM	Time, min	% inactvn[c]	ED_{50},[d] μM	ED_{50}/I_{50}
14	H	$(CH_2)_2C_6H_4SO_2F$-*p*	L1210/DFS	0.039[e]	0.16	60	0	0.27[f]	8
15	H	$(CH_2)_4C_6H_4SO_2F$-*p*	L1210/DFS	0.020	0.10	<2, 30	75, 75	0.18	9
			Mouse liver		0.10	60	27		
16	Cl	$(CH_2)_2C_6H_4SO_2F$-*p*	L1210/DFS	0.014	0.070	2, 60	71, 93[g]	0.03	2
			Mouse liver		0.070	60	18		
			Walker 256		0.050	60	86[f]		
			Rat liver		0.050	60	86[f]		
17	Cl	$(CH_2)_4C_6H_4SO_2F$-*p*	L1210/DFS	0.0072	0.050	2, 60	82, 100[g]	0.0009	0.1
			Mouse liver		0.050	60	41		
			Walker 256		0.050	60	95		
			Rat liver		0.050	60	97		
18	$(CH_2)_2C_6H_4SO_2F$-*p*	H	L1210/DFS	0.0080	0.050	2, 16, 30	33, 53, 53[g]	2.9	400
			Mouse liver		0.50	60	0		
19	$(CH_2)_4C_6H_4SO_2F$-*p*	H	L1210/DFS	0.0080	0.066	<2, 30	86, 86[g]	0.0002	0.03
			Mouse liver		0.066	60	54		
			Walker 256		0.050	60	85		
			Rat liver		0.050	60	95		
20	$(CH_2)_4C_6H_4SO_2F$-*m*	H	L1210/DFS	0.0080	0.050	60	94	0.35	10
			Mouse liver		0.050	60	25		
			Walker 256		0.050	60	70		
			Rat liver		0.050	60	80		

[a] The technical assistance of Diane Shea and Sharon Lafler with these assays is acknowledged. [b] I_{50} = concentration for 50% inhibition when measured with 6 μM dihydrofolate, 12 μM TPNH, and 0.15 M KCl in pH 7.4 Tris buffer as previously described.[6] [c] Enzyme incubated with inhibitor at 37° in pH 7.4 Tris buffer containing 60 μM TPNH, then the remaining enzyme was assayed as previously described.[6] [d] *Concentration for 50% inhibition of L1210 cell culture.*[9] [e] Assayed with L1210/FR8 enzyme. [f] New datum; other data on this compound from ref 15. [g] From six-point time study.[6]

by passive diffusion.[17] Among the factors which may be important for passive diffusion are the following: (a) any group on the inhibitor that can hydrogen bond to water will slow the rate of transport since energy is required to break the hydrogen bonds when the inhibitor is transferred from the aqueous medium to the lipid phase of the cell wall;[8] (b) a proper balance between hydrophobic and hydrophilic character should be maintained,[18] probably because the compound must also be transferred from the lipid phase of the cell wall to the aqueous phase inside the cell; and (c) the rate of passive diffusion might be influenced by the shape or bulkiness of an inhibitor due to steric factors.

The two best compounds in Table II for L1210 cell kill were **17** and **19**. Surprisingly, these two compounds were only three- to sixfold more effective than the corresponding reversible inhibitors, **13** and **6**, without an SO_2F moiety when ED_{50}/I_{50}'s were compared. We anticipated that irreversible inhibitors should be considerably more potent than a reversible inhibitor since the irreversible inhibitors are destroying the target enzyme; however, the SO_2F group could have slowed transport due to its polarity.[19] That an SO_2F could slow transport was supported by comparison of the ED_{50}/I_{50}'s of **9** and **14**, neither of which is an irreversible inhibitor; **9** was 40 times as effective as **14**. If the SO_2F group slows transport by a factor of about 40, then it can be estimated that the irreversible inhibitor, **17**, is over 200-fold more effective on the target enzyme in the intact cell than is the corresponding reversible inhibitor **13**.

When the SO_2F group of **19** was moved from the *para* to the *meta* position, the resultant **20** still showed the same irreversible inhibition of dihydrofolic reductase; however, **19** was 1200 times as effective as **20** in L1210 cell culture. A likely rationalization of this difference is the greater two-dimensional bulk of **20** due to the *meta-meta* substitution pattern.

Since the irreversible inhibitors **17** and **19** show good L1210 cell kill in the range of 10^{-10} M, the following questions arise.

(a) Can **17** and **19**, as well as fluorosulfonyl derivatives of **8** and **12**, be further modified to give irreversible specificity for the L1210 dihydrofolic reductase with no inactivation of this enzyme in normal mouse tissues, an accomplishment achieved earlier[6,7] with dihydro-*s*-triazines and diaminopyrimidines connected to an SO_2F moiety by an amide bridge?

(b) Will further correlations on steric factors that slow transport emerge from the studies in the preceding question which will be useful for design of effective *in vivo* compounds?

(c) What is the proper balance of hydrophobic and hydrophilic groups[18] to give maximum rate of transport? Such measurements should be made by determining

(17) (a) See ref 4, pp 263–266; (b) R. C. Wood and G. H. Hitchings, *J. Biol. Chem.*, **234**, 2377, 2381 (1959); (c) B. R. Baker, D. V. Santi, P. I. Almanla, and W. C. Werkheiser, *J. Med. Chem.*, **7**, 24 (1964).

(18) C. Hansch, A. R. Steward, J. Iwasa, and E. W. Deutsch, *Mol. Pharmacol.*, **1**, 205 (1965).

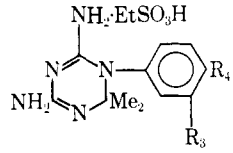
(19) The SO_2F group should have a Hansch π constant between −1.8 for SO_2NH_2 and −1.26 for SO_2CH_3;[20] actually the SO_2F group had a π constant = −1.6 when measured[20] with *p*-fluorosulfonylphenoxyacetic acid.[21]

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

(21) B. R. Baker and W. F. Wood, *J. Med. Chem.*, **12**, 211 (1969).

TABLE III

PHYSICAL PROPERTIES OF



No.[a]	R_3	R_4	Yield,[b] %	Mp, °C dec	Formula[c]
17	Cl	$(CH_2)_4C_6H_4SO_2F$-*p*	66[d]	204–206	$C_{21}H_{23}ClFN_5O_2S \cdot C_2H_5SO_3H$
18	$(CH_2)_2C_6H_4SO_2F$-*p*	H	30[e]	191–193	$C_{19}H_{22}FN_5O_2S \cdot C_2H_5SO_3H$
19	$(CH_2)_4C_6H_4SO_2F$-*p*	H	22[f]	176–177	$C_{21}H_{26}FN_5O_2S \cdot C_2H_5SO_3H$
20	$(CH_2)_4C_6H_4SO_2F$-*m*	H	42[f,g]	194–195	$C_{21}H_{26}FN_5O_2S \cdot C_2H_5SO_3H$

[a] Synthesized by method E.[21] [b] Yield of analytically pure material. [c] Compounds analyzed correctly for C, H, F. [d] Recrystallized from *i*-PrOH–H_2O. [e] Recrystallized from *i*-PrOH–EtOH–H_2O. [f] Recrystallized from *i*-PrOH. [g] Pure **24c** employed as starting material.

TABLE IV

PHYSICAL PROPERTIES OF INTERMEDIATE STILBENES AND DIPHENYLBUTADIENES

No.	Compound	Yield,[a] %	Mp, °C	Formula	Analyses
23a	2-Cl-4-$NO_2C_6H_3(CH{=}CH)_2C_6H_4SO_2F$-*p*[b]	72[c]	166–170	$C_{16}H_{11}ClFNO_4S$	C, H, F
23b	*m*-$NO_2C_6H_4CH{=}CHC_6H_4SO_2F$-*p*[b]	37[d]	60–110[e]	$C_{14}H_{10}FNO_4S$	C, H, N
23c	*m*-$NO_2C_6H_4(CH{=}CH)_2C_6H_4SO_2F$-*p*[b]	67[f]	137–139	$C_{16}H_{12}FNO_4S$	C, H, N
23d	*m*-$NO_2C_6H_4(CH{=}CH)_2C_6H_4SO_2F$-*m*	33[d]	169–171	$C_{16}H_{12}FNO_4S$	C, H, N

[a] Analytically pure; yield is a minimum value prepared by method A. [b] See ref 22 for Wittig reagent. [c] Recrystallized from EtOH–THF. [d] Recrystallized from EtOH. [e] *cis–trans* mixture: pure *cis*, mp 64–67°; pure *trans*, mp 143–147°. Both reduced to the same saturated amine. [f] Recrystallized from EtOH, then $CHCl_3$.

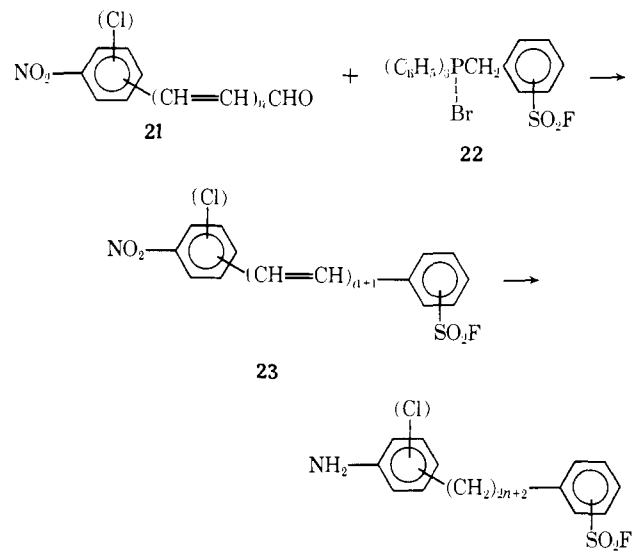
the partition coefficient of the dihydro-*s*-triazine moiety of compounds related to those in Tables I and II.

(d) Can all of these factors be properly balanced to achieve high specificity for L1210 cell kill in the mouse?

Studies on these important factors are continuing.

Chemistry.—The candidate irreversible inhibitors in Table II were synthesized by the general route described earlier for **14–16**.[15,22] Wittig condensation of the appropriate benzaldehyde or cinnamaldehyde (**21**) with **22** afforded the stilbenes and diphenylbutadienes (**23**) (Scheme I). Hydrogenation with a PtO_2 catalyst afforded the desired substituted anilines (**24**) which were condensed with cyanoguanidine and acetone to the dihydro-*s*-triazines in Table II by the general method of Modest.[23]

SCHEME I



Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Each analytical sample had ir and uv spectra compatible with its structure and moved as a single spot on tlc on Brinkmann silica gel GF_{254} or polyamide MN, and gave combustion analyses for C, H, and N or F within 0.4% of theory. The physical properties of **17–20** are given in Table III.

2-Chloro-4-nitrocinnamaldehyde (21a).—To a stirred suspension of 8.0 g (43 mmoles) of 2-chloro-4-nitrobenzaldehyde[11] in 30 ml of freshly distilled acetaldehyde cooled in an ice bath was added 0.50 ml of 25% NaOH in MeOH over about 5 min. After 30 min in the ice bath, the mixture was treated with 25 ml of Ac_2O. The mixture was heated in a bath at 120° for 1 hr, then cooled and carefully diluted with 60 ml of H_2O. After addition of 25 ml of 5 *N* HCl, the mixture was refluxed for 30 min, then cooled and filtered. The product was recrystallized from C_6H_6; yield 6.0 g (66%), mp 133–134°. *Anal.* ($C_9H_6ClNO_3$) C, H, N.

m-**Fluorosulfonylbenzyltriphenylphosphonium bromide (22a)** was synthesized from *m*-tolylsulfonyl fluoride[24] as described for the *para* isomer;[22] the yield of pure material after recrystallization from MeOH–Et_2O was 49%, mp 253–254°. *Anal.* ($C_{25}H_{21}BrFO_2PS$) C, H.

1-(2-Chloro-4-nitrophenyl)-4-(4-fluorosulfonylphenyl)butadiene (23a) (Method A).—To a stirred mixture of 2.1 g (10 mmoles) of **21a**, 5.15 g (10 mmoles) of *p*-fluorosulfonylbenzyltriphenylphosphonium bromide (**22b**),[24] and 25 ml of DMF was added 1.24 g (10 mmoles) of 1,5-diazabicyclo[4.3.0]non-5-ene (DBN).[25] After 16 hr at ambient temperature protected from moisture, the mixture was diluted to 50 ml with H_2O. The prod-

(22) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **11**, 666 (1968), paper CXXVII of this series.

(23) E. J. Modest, *J. Org. Chem.*, **21**, 1 (1956).

(24) (a) F. E. Jenkins and A. N. Hambly, *Aust. J. Chem.*, **6**, 318 (1953); (b) B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **12**, 221 (1969), paper CL of this series.

(25) H. Oediger, H. Kabbe, F. Möller, and K. Eiter, *Chem. Ber.*, **99**, 2012 (1966).

met was collected on a filter and washed with H_2O. Recrystallization from EtOH–THF gave 2.63 g (72%) of yellow crystals, mp 166–170°.

See Table IV for additional data and other compounds prepared by this method.

1-(*m*-Aminophenyl)-4-(*p*-fluorosulfonylphenyl)butane Ethanesulfonate (24c).—A solution of 1.05 g (3.2 mmoles) of **23c** (Table III), 0.35 g (3.2 mmoles) of $EtSO_3H$, 100 ml of 95% EtOH, and 100 mg of PtO_2 was shaken with H_2 at 2–3 atm until the uv of the solution no longer showed a double band conjugated with the ring. The filtered solution was evaporated to a thin syrup *in vacuo*, then stored at 0° until crystallization started. The mixture was slightly thinned with *i*-PrOH, then filtered. The product was washed with cold *i*-PrOH, then recrystallized from C_6H_6–petroleum ether (bp 30–60°): yield 0.50 g (38%), mp 114–116°. *Anal.* ($C_{18}H_{24}FNO_5S_2 \cdot C_2H_6SO_3H$) C, H, N.

Irreversible Enzyme Inhibitors. CLXI.[1,2] Proteolytic Enzymes. XIII.[3] Inhibitors of Guinea Pig Complement Derived by Quaternization of 3-Acylamidopyridines with α-Bromomethylbenzenesulfonyl Fluorides. II

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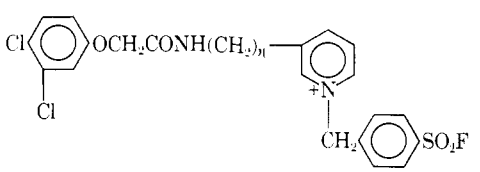
Twenty quaternary salts (**26**) derived from N-(3-pyridyl)- or N-(3-pyridylmethyl)-3,4-dichlorophenoxyacetamide (**24**) by reaction with substituted fluorosulfonylbenzyl bromides (**25**) were evaluated as inhibitors of the lysis of sheep red blood cells by hemolysin and complement. The most effective compound was 3-(3,4-dichlorophenoxyacetamido)-N-(6-chloro-2-fluorosulfonylbenzyl)pyridinium bromide (**16**); at 62 and 31 μM, **16** showed 84 and 45% inhibition, respectively. A number of these compounds were excellent irreversible inhibitors of α-chymotrypsin; for example, **16** had an $I_{ai} \sim K_i$ of 5.7 μM and at this concentration gave 98% inactivation in 2 min.

Inhibition of the serum complement system has potential medical use for organ transplantation and in certain arthritic states.[5,6] One of the normal functions of the complement system, a complex mixture of at least eleven serum proteins, is for rejection of foreign cells by lysis.[6,7] Since some of the proteins of the complement system are proteases with "tryptic" or "chymotryptic" properties,[6,7] this system can be inhibited with inhibitors of trypsin[3] or chymotrypsin[3,8] when measured by complement-antibody-mediated lysis of sheep red blood cells (RBC).[5,9]

Among the inhibitors of guinea pig complement found in this laboratory are the pyridine quaternaries, **1** and **2**;[3] it was also established that the SO_2F moiety was necessary for activity.[3] For example, 0.5 mM **1**



1, $n = 0$
2, $n = 1$

showed 45% inhibition of complement when measured by RBC lysis. This observation has been verified by Becker;[10] 0.4 mM **1** could inhibit one out of two complement units in his assay system.[11] Furthermore, he observed that **1** at 0.4 mM was an irreversible inhibitor of the C'1a component with a half-life of 18 min.[10]

Sixteen additional variants of **1** and **2** with changes in the fluorosulfonylbenzyl moiety have now been synthesized for evaluation as inhibitors of the complement system; these have also been evaluated as irreversible inhibitors of chymotrypsin. Some of these variants are 25 times as effective as **1** or **2** as inhibitors of the complement system.

Complement Inhibition.—The data in Table I indicates the effect of a given concentration of compound on lysis of RBC catalyzed by complement, compared to a control with no compound. Any lysis of RBC by the compound in the absence of complement is expressed as a percentage of the total lysis possible, 0.7 OD unit, corrected for 0–5% lysis in the absence of compound and complement.[5]

The *p*-SO_2F quaternaries (**1** and **2**) were previously reported[3] from this laboratory to give about 50% inhibition of complement when assayed at 0.5 and 1 mM, respectively (Table I); when the SO_2F group was moved to the *meta* position, activity was improved less than twofold.[3] The *o*-SO_2F isomers (**5** and **6**) have now been synthesized for comparison. Activity was considerably enhanced, being about tenfold with **5** and about 25-fold with **6**; the two compounds showed 50% inhibition somewhere between 0.031 and 0.062 mM.

The effect of chloro substitution on the fluorosulfonylbenzyl moiety was then studied. There are two possi-

(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, E. E. Janson, and N. M. J. Vermeulen, *J. Med. Chem.*, **12**, 898 (1969).

(3) For the previous paper on complement see B. R. Baker and J. A. Hurlbut, *ibid.*, **12**, 677 (1969), paper CLVI of this series.

(4) NDEA predoctoral fellow.

(5) B. R. Baker and E. H. Erickson, *J. Med. Chem.*, **12**, 408 (1969), paper CLII of this series.

(6) H. J. Müller-Eberhard, *Advan. Immunol.*, **8**, 1 (1968).

(7) (a) Ciba Foundation Symposium, "Complement," G. E. W. Wolstenholme and J. Knight, Ed., Little, Brown and Co., Boston, Mass., 1965; (b) P. H. Schur and K. F. Austen, *Ann. Rev. Med.*, **19**, 1 (1968).

(8) B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **12**, 115 (1969), paper CLIII of this series.

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(10) Private communication from Dr. E. L. Becker, Walter Reed Army Medical Center.

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