Irreversible Enzyme Inhibitors. 183.[1,2] Proteolytic Enzymes. 18.[3] Inhibitors of Guinea Pig Complement Derived by Quaternization of Phenylalkylpyridines with α-Bromomethylbenzenesulfonyl Fluorides

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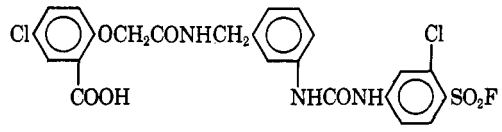
A series of 31 compounds derived from hydrocarbon-substituted pyridines by quaternization with $PhCH_2Br$ containing 4-SO_2F, 2-SO_2F, or 6-Cl-2-SO_2F were synthesized and evaluated as inhibitors of guinea pig complement and its C'1a component. The most active compound was 3-(3,4-dichlorophenylbutyl)-*N*-(*o*-fluorosulfonylbenzyl)pyridinium bromide (**7**) which showed about 60% inhibition at 31 μ*M*; three other compounds (**8**, **10**, **35**) showed about 50% inhibition at 62 μ*M*. The most effective irreversible inhibitor of the C'1a component in this series was *N*-(6-chloro-2-fluorosulfonylbenzyl)quinolinium bromide which showed 55% inhibition at 16 μ*M* and 88% inhibition at 31 μ*M*.

The serum complement system is a mixture of 11 distinct proteins[4-6] which has protease activity that is both "tryptic" and "chymotryptic." The function of the serum complement system is to kill foreign bacteria and protozoa; it can also lyse foreign mammalian cells thus causing rejection of organ and tissue transplants. Certain arthritic states are also caused by malfunction of the complement system. Thus inhibitors of the complement system have potential medical use for controlling transplant rejection and treatment of complement-induced arthritis.[7]

The complement system and its inhibition[7] is readily measured by the lysis of sheep red blood cells (RBC) by guinea pig complement and antibody.[7,8] Powerful tryptic-type inhibitors of complement derived from benzamidine[7,9] have emerged from this laboratory. Similarly, two types of chymotryptic-type inhibitors of complement have resulted from studies in this laboratory, as exemplified by **1**[10] and **2**.[11,12] Only side chains



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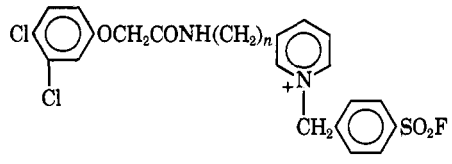

2, $n = 0,1$

at the 3 position of the pyridine ring could be made, the 2- or 4- amino group on pyridine (**2**, $n = 0$) being too inert to acylate and the 2- or 4-aminomethylpyridines (**2**, $n = 1$) giving unstable products;[13] as a result structure–activity relationships were incomplete. There appeared to be 2 possible solutions to this dilemma. The first was to increase n to two in **2**; inhibition of complement was lost when the side chain was at the 2 position on the pyridine, but was maintained when the side chain was on the 4 position.[14]

The second solution would be to replace the CONH part of the bridge between the pyridine and dichlorophenyl rings of **2** with CH_2CH_2; such a structural change would cause little change in binding to chymotrypsin[15] and presumably to other "chymotryptic" proteases. Furthermore, replacement of the O in the bridge by CH_2 causes no change in binding to chymotrypsin.[16] Since $(CH_2)_n$ bridges in **2** are easier to synthesize than $O(CH_2)_{n-1}$ bridges, a series of compounds related to **2** were synthesized with hydrocarbon bridges to the 2, 3, or 4 positions of the pyridine. These compounds were then evaluated as inhibitors of whole guinea pig complement, irreversible inhibitors of the C'1a component, chymotrypsin, and acetylcholine esterase. The results are the subject of this paper.

Assay Results.—When the OCH_2CONH bridge of **3** was replaced by $(CH_2)_4$, the resultant **6** showed such enhanced lysis of red blood cells in the absence of complement that **6** could not be evaluated as an inhibitor of the complement system; this result was not too surprising since the $(CH_2)_4$ bridge of *m*-(phenylbutyl)-benzamidine gave much more compound-induced lysis than *m*-(phenoxypropyloxy)benzamidine.[9] This dilemma was avoided by quaternization with *o*-benzylsulfonyl fluorides to give **7** and **8** since these *o*-sulfonyl fluorides showed considerably enhanced activity with the OCH_2CONH bridge.[11] The *o*-benzylsulfonyl fluoride quaternary salt of the compound with a $(CH_2)_4$ bridge (**7**) was just as effective as the corresponding compound with the OCH_2CONH bridge (**4**); similarly the benzyl quaternary with 6-chloro-2-fluorosulfonyl substituent with a $(CH_2)_4$ bridge (**8**) was just as effective as the corresponding compound with a OCH_2CONH bridge (**5**).

That the 3,4-dichlorophenylbutyl side chain at the 3

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(2) For the previous paper in this series see B. R. Baker, *Ann. N. Y. Acad. Sci.*, in press.

(3) For the previous paper is this subseries see B. R. Baker and M. Cory, *J. Med. Chem.*, **14**, 119 (1971).

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

(5) P. H. Schur and K. F. Austen, *Annu. Rev. Med.*, **19**, 1 (1968).

(6) *Complement, Ciba Found. Symp.*, **1964** (1965).

(7) B. R. Baker and E. H. Erickson, *J. Med. Chem.*, **12**, 408 (1969).

(8) E. A. Kabat and M. M. Mayer, "Experimental Immunochemistry," 2nd ed, Charles C Thomas Co., Springfield, Ill., 1967, pp 149–153.

(9) B. R. Baker and M. Cory, *J. Med. Chem.*, **12**, 1053 (1969).

(10) B. R. Baker and J. A. Hurlbut, *ibid.*, **12**, 415 (1969).

(11) B. R. Baker and J. A. Hurlbut, *ibid.*, **12**, 902 (1969).

(12) B. R. Baker and J. A. Hurlbut, *ibid.*, **12**, 677 (1969).

(13) B. R. Baker and J. A. Hurlbut, *ibid.*, **12**, 221 (1969).

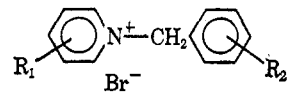
(14) J. A. Hurlbut, Ph.D. thesis, University of California at Santa Barbara, Santa Barbara, Calif., 1969.

(15) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," Wiley, New York, N. Y., 1967, pp 56–57.

(16) B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **10**, 1129 (1967).

TABLE I

INHIBITION[a] OF GUINEA PIG COMPLEMENT AND IRREVERSIBLE INHIBITION OF THE C'1A COMPONENT BY



No.	R_1	R_2	m*M* inhib	—Whole complement— % inhibn[b]	% lysis[c]	C'1a, % inactn[d]
3[e]	3-(3,4-$Cl_2C_6H_3OCH_2CONH$)	4-SO_2F	1.0		45	67
			0.50	45	4	
4[e]	3-(3,4-$Cl_2C_6H_3OCH_2CONH$)	2-SO_2F	0.50	93	0	95
			0.25	89		96
			0.125	84		70
			0.062	63		37
			0.031	27		
5[e]	3-(3,4-$Cl_2C_6H_3OCH_2CONH$)	6-Cl-2-SO_2F	0.50	94	8	98
			0.25	96		98
			0.125	96		73
			0.062	84		55
			0.031	45		
6	3-[3,4-$Cl_2C_6H_3(CH_2)_4$]	4-SO_2F	0.25[f]		100	66
			0.125	16	10	36
7	3-[3,4-$Cl_2C_6H_3(CH_2)_4$]	2-SO_2F	0.50		100	97
			0.25		100	96
			0.125	85		75
			0.062	72		37
			0.031	59		
			0.015	21		
8	3-[3,4-$Cl_2C_6H_3(CH_2)_4$]	6-Cl-2-SO_2F	0.50		100	95
			0.25		59	98
			0.125	94		97
			0.062	76		67
			0.031	20		39
9	3-$C_6H_5(CH_2)_4$	4-SO_2F	1.0	56	3	85
			0.50	30		64
			0.25			35
10	3-$C_6H_5(CH_2)_4$	6-Cl-2-SO_2F	0.50	88	7	95
			0.25	95		93
			0.125	82		73
			0.062	51		38
11	H	4-SO_2F	3.0	54	0	71
			1.0	24		29
12	H	2-SO_2F	1.0	67	0	79
			0.50	39		50
13	H	6-Cl-2-SO_2F	0.50	64	2	78
			0.25	39		54
14	2-[3,4-$Cl_2C_6H_3(CH_2)_4$]	4-SO_2F	0.50		100	84
			0.25	16	12	51
			0.125	3	0	27
15	2-[3,4-$Cl_2C_6H_3(CH_2)_4$]	2-SO_2F	0.50		100	77
			0.25	53	32	45
			0.125	34	0	
16	4-[3,4-$Cl_2C_6H_3(CH_2)_4$]	4-SO_2F	0.50		100	69
			0.25		100	38
			0.125	22	9	
17	4-[3,4-$Cl_2C_6H_3(CH_2)_4$]	2-SO_2F	0.50		100	95
			0.25		41	86
			0.125	64		52
			0.062	23		
18	4-$C_6H_5(CH_2)_4$	4-SO_2F	0.50[f]	20	0	35
19	4-[3,4-$Cl_2C_6H_3O(CH_2)_3$]	4-SO_2F	0.25[f]		29	49
			0.125	20	0	22
20	4-$C_6H_5(CH_2)_4$	6-Cl-2-SO_2F	0.50	98	0	96
			0.25	81		91
			0.125	40		61
			0.062			38
21	3-(3,4-$Cl_2C_6H_3CH_2CH_2$)	4-SO_2F	1.0		94	90
			0.50	53	5	72
			0.25	22		39
22	3-(3,4-$Cl_2C_6H_3CH_2CH_2$)	2-SO_2F	0.50	87	0	82
			0.25	68		57
			0.125	37		21

TABLE I (*Continued*)

No.	R_1	R_2	mM inhib	Whole complement: % inhibn[b]	Whole complement: % lysis[c]	C'1a, % inactn[d]
23	2-(3,4-$Cl_2C_6H_3CH_2CH_2$)	4-SO_2F	1.0	63	18	73
			0.50	43	0	43
24	2-(3,4-$Cl_2C_6H_3CH_2CH_2$)	2-SO_2F	1.0	64	14	95
			0.50	85	0	94
			0.25	66		82
			0.125	32		58
25	4-(3,4-$Cl_2C_6H_3CH_2CH_2$)	4-SO_2F	1.0		100	93
			0.50	56	0	74
			0.25	22		32
26	4-(3,4-$Cl_2C_6H_3CH_2CH_2$)	2-SO_2F	0.50	88	0	91
			0.25	65		78
			0.125	33		52
27	3-$C_6H_5CH_2CH_2$	6-Cl-2-SO_2F	0.50	93	0	93
			0.25	77		69
			0.125	40		44
28	4-$C_6H_5CH_2CH_2$	4-SO_2F	1.0	45	3	71
			0.50	23		39
29	4-$C_6H_5(CH_2)_3$	4-SO_2F	1.0	52	3	79
			0.50	33		61
			0.25			34
30	4-$C_6H_5(CH_2)_3$	6-Cl-2-SO_2F	0.50	89	0	94
			0.25	78		83
			0.125	42		48
31	4-$C_6H_5CH_2$	4-SO_2F	0.50[f]	21	0	17
32	4-C_6H_5	4-SO_2F	1.0	41	0	78
			0.50	22		65
			0.25	12		37
33	4-CH_3	4-SO_2F	3.0	46	0	84
			1.0	17		40
34	2,3-Benzo	4-SO_2F	1.0	21	0	22
35	2,3-Benzo	6-Cl-2-SO_2F	0.50	88	0	94
			0.125	83		95
			0.062	45		90
			0.031	11		88
			0.0156			55
			0.0078			28
36	3,4-Benzo	6-Cl-2-SO_2F	0.50	90	0	100
			0.25	74		93
			0.125	33		75
			0.062			41

[a] The technical assistance of Pauline Minton with these assays is acknowledged. [b] Inhibition of lysis of sheep red blood cells by guinea pig complement and antibody detd as previously described.[7] [c] Lysis by the compd in the absence of complement expressed as per cent of total lysis possible.[7] [d] Inhibitor incubated 10 min at 37° with C'1a, then whole complement restored and assayed as previously described.[3] [e] Data from ref 3 and 11. [f] Maximum solubility.

position (**8**) made a contribution to inhibition of the complement system was shown by replacement of this side chain by H (**13**); **13** was 8-fold less effective than **8**.

When the side chain of **7** was moved to the 2 position of the pyridine ring, the resultant **15** was 8-fold less effective than **7**. When the side chain of **7** was moved to the 4 position of the pyridine ring, the resultant **17** was also about 4-fold less effective than **7** on the whole complement system.

Removal of the two Cl's from **8** gave **10** which was almost as effective as **8**; thus the contribution of the two Cl's to inhibition of whole complement was small, but lysis of the red blood cells by **10** was considerably reduced compared to **8**. When the 3-phenylbutyl side chain of **10** was moved to the 4 position of the pyridine ring, the resultant **20** was again 4-fold less effective than the 3 isomer.

When the 3,4-dichlorophenylbutyl side chain of **7** was reduced in length to phenethyl, the resultant **22** was 8-fold less effective than **7**. Shifting the side chain of **22** from the 3 position to the 2 position of the pyridine ring (**24**) gave no change in binding; similar results were observed when the side chain of **22** was moved to the 4 position of the pyridine ring (**26**). When **27** was compared to **22**, the inhibition by phenethyl and 3,4-dichlorophenethyl appeared to be equivalent.

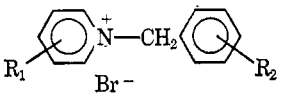
Since the contribution of a phenylbutyl group at the 4 position of the pyridine ring (**17**) was 4-fold less effective than the contribution by the phenylbutyl group at the 3 position (**7**), the contribution of a $C_6H_5(CH_2)_n$ group at the 4 position was investigated where n = 0–4. The contribution was only 2-fold where n = 0–4; this conclusion can be arrived at by comparing **13** *vs.* **20**, **20** *vs.* **27** and **30**, **18** *vs.* **31** and **32**.

Replacement of the pyridine ring of **13** with quinoline gave about a 6-fold increment in better inhibition with the resultant **35**. Replacement in **13** with an isoquinoline ring (**36**) gave only a 2-fold increment in inhibition.

The compounds in Table I were then investigated as irreversible inhibitors of the C'1a component of complement by the previously described assay;[3] in general

TABLE II

INHIBITION[a] OF CHYMOTRYPSIN AND ACETYLCHOLINE ESTERASE BY



			Chymotrypsin				
				Irreversible[d]			AChE,[e]
No.	R_1	R_2	I_{50},[b,c] μM	Inhib, μM	Time, min	% inactvn	I_{50},[b] μM
3[f]	3-(3,4-$Cl_2C_6H_3OCH_2CONH$)	4-SO_2F	15	15	2, 8, 30[g]	81, 89, 100	15
4[h]	3-(3,4-$Cl_2C_6H_3OCH_2CONH$)	2-SO_2F	13	13	<2[g]	100	3.7
5[h]	3-(3,4-$Cl_2C_6H_3OCH_2CONH$)	6-Cl-2-SO_2F	5.7	5.7	2, 4[g]	98, 100	5.0
6	3-[3,4-$Cl_2C_6H_3(CH_2)_4$]	4-SO_2F	4.2	4.2	60	94	26
7	3-[3,4-$Cl_2C_6H_3(CH_2)_4$]	2-SO_2F	3.3	3.3	60	100	4.2
8	3-[3,4-$Cl_2C_6H_3(CH_2)_4$]	6-Cl-2-SO_2F	3.3	3.3	60	100	8.2
9	3-$C_6H_5(CH_2)_4$	4-SO_2F	6.5	6.5	60	89	40
10	3-$C_6H_5(CH_2)_4$	6-Cl-2-SO_2F	3.4	3.4	60	86	12
11	H	4-SO_2F	110	110	60	93	71
12	H	2-SO_2F	170	170	60	100	38
13	H	6-Cl-2-SO_2F	28	28	60	97	65
14	2-[3,4-$Cl_2C_6H_3(CH_2)_4$]	4-SO_2F	6.2	6.2	60	82	22
15	2-[3,4-$Cl_2C_6H_3(CH_2)_4$]	2-SO_2F	3.4	3.4	60	100	9.2
16	4-[3,4-$Cl_2C_6H_3(CH_2)_4$]	4-SO_2F	20	20	60	85	19
17	4-[3,4-$Cl_2C_6H_3(CH_2)_4$]	2-SO_2F	2.8	2.8	60	96	9.8
18	4-$C_6H_5(CH_2)_4$	4-SO_2F	37	37	60	95	25
19	4-[3,4-$Cl_2C_6H_3O(CH_2)_3$]	4-SO_2F	13	13	60	90	23
20	4-$C_6H_5(CH_2)_4$	6-Cl-2-SO_2F	16	16	60	100	8.3
21	3-[3,4-$Cl_2C_6H_3(CH_2)_2$]	4-SO_2F	23	23	60	92	44
22	3-[3,4-$Cl_2C_6H_4(CH_2)_2$]	2-SO_2F	3.0	3.0	60	83	20
23	2-[3,4-$Cl_2C_6H_3(CH_2)_2$]	4-SO_2F	17	17	60	85	44
24	2-[3,4-$Cl_2C_6H_3(CH_2)_2$]	2-SO_2F	6.6	6.6	60	100	19
25	4-[3,4-$Cl_2C_6H_3(CH_2)_2$]	4-SO_2F	22	22	60	90	32
26	4-[3,4-$Cl_2C_6H_3(CH_2)_2$]	2-SO_2F	33	33	60	87	17
27	3-$C_6H_5CH_2CH_2$	6-Cl-2-SO_2F	7.2	7.2	60	89	17
28	4-$C_6H_5CH_2CH_2$	4-SO_2F	66	66	60	94	62
29	4-$C_6H_5(CH_2)_3$	4-SO_2F	59	59	60	94	13
30	4-$C_6H_5(CH_2)_3$	6-Cl-2-SO_2F	48	48	60	92	10
31	4-$C_6H_5CH_2$	4-SO_2F	50	50	60	94	100
32	4-C_6H_5	4-SO_2F	11	11	60	94	62
33	4-CH_3	4-SO_2F	98	98	60	95	130
34	2,3-Benzo	4-SO_2F	50	50	60	88	12
35	2,3-Benzo	6-Cl-2-SO_2F	5.6	5.6	60	91	15
36	3,4-Benzo	6-Cl-2-SO_2F	19	19	60	96	4.8
37	H	H	>5000				66
38	2,3-Benzo	H					15
39	H	3,4-Benzo					90
40	H	2,3-Benzo					50
41	2,3-Benzo	3,4-Benzo					75

[a] The technical assistance of Julie Beardslee, Nancy Middleton, and Pauline Minton with these assays is acknowledged. [b] I_{50} = concn for 50% inhibition. [c] Assayed with 200 μM *N*-glutaryl-L-phenylalanine *p*-nitroanilide in 0.05 *M* Tris buffer (pH 7.4) contg 10% DMSO as previously described.[16] [d] Inactivation performed with $\simeq$1 μM enzyme at 24° in 0.05 *M* Tris buffer (pH 7.4) containing 10% DMSO, then the remaining enzyme assayed with *N*-benzoyl-L-tyrosine Et ester in pH 8.1 Tris buffer containing 0.1 *M* $CaCl_2$ as previously described [B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **12**, 118 (1969)]. [e] AChE (acetylcholine esterase) from rabbit brain was assayed with 1000 μM [^{14}C]ACh as previously described [B. R. Baker and R. E. Gibson, *J. Med. Chem.*, **14**, 315 (1971)]. [f] Chymotrypsin data from ref 13. [g] From a 6-point time study. [h] Data from ref 11.

the results paralleled the inhibition of the whole complement system with the following exception: the phenylbutyl group was equally effective when attached to the 3 or 4 position of the pyridine ring. In cases where inhibition of whole complement system and irreversible inhibition of its C'1a component run parallel, the results have been interpreted to indicate that the main blockade of the whole system is that of C'1a.[3]

In summary, the following important conclusions are reached from the data in Table I on inhibition of the whole complement system.

(1) The $(CH_2)_4$ bridge at the 3 position of the pyridine ring is just as effective as the OCH_2CONH bridge, although lysis by the compounds is increased with the nonpolar $(CH_2)_4$ bridge.

(2) The $C_6H_5(CH_2)_4$ substituent gives maximum inhibition when at the 3 position, being less effective at the 4 or 2 positions of the pyridine ring.

(3) The $C_6H_5(CH_2)_2$ substituent is less effective at the 3 position than $C_6H_5(CH_2)_4$.

Current studies are focused on synthesis of other substituents with hydrocarbon bridges at the 3 position of the pyridine ring to see if inhibition of the whole complement system can be further maximized.

The amides **3–5** are good reversible and irreversible inhibitors of chymotrypsin;[11] therefore **6–36** were

examined for their inhibition of chymotrypsin, and the results are recorded in Table II. The I_{50}'s for reversible inhibition were determined with *N*-glutaryl-L-phenylalanine *p*-nitroanilide[17] as previously described;[16] in this assay the I_{50} is approximately equal to K_i.[18] Irreversible inhibition was performed by incubation at 24°, then assay of the remaining enzyme with *N*-benzoyl-L-tyrosine ethyl ester as previously described.[19] The reversible I_{50}'s of the inhibitors with a 3-(3,4-dichlorophenylbutyl) side chain (**6**–**8**) were 2- to 4-fold better than the I_{50}'s of inhibitors with the corresponding compounds with 3-(3,4-dichlorophenoxyacetamido) side chain (**3**–**5**). Removal of the phenyl chlorines (**9**, **10**) from **6** and **8** gave no appreciable change in I_{50}. That the 3-phenylbutyl side chains did contribute to binding was shown by comparison of **6**–**8** with **11**–**13**; the binding increments were 9- to 50-fold.

When the 3-(dichlorophenylbutyl) side chain of **6** and **7** was shifted to the 2 position, the resultant **14** and **15** were just as good reversible and irreversible inhibitors; these results should be contrasted with the irreversible inhibition of C'1a (Table I) by these 4 compounds, particularly where **7** is considerably more effective than **15** on C'1a. The side chain at the 4 position of **17** was as effective as having it at the 3 position (**7**), but **16** was about 5-fold less effective than **6**. The dichlorophenethyl side chain at the 3 position of the pyridine ring (**22**) was as effective as the dichlorophenylbutyl side chain (**7**); however, the ethyl bridge (**26**) was less effective at the 4 position when compared with the butyl bridge of **17**.

All of the compounds (**6**–**36**) were excellent irreversible inhibitors of chymotrypsin when incubated at their $I_{50} \simeq K_i$ concn with the enzyme.

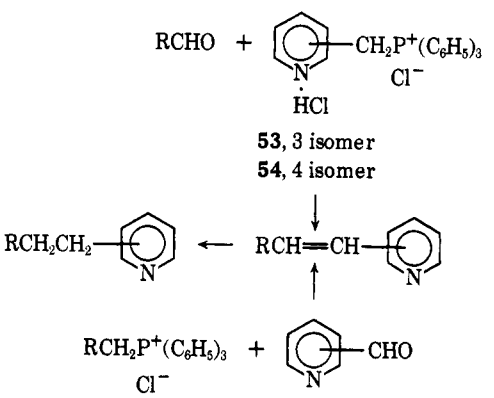
Since quaternary salts are known to be inhibitors of AChE,[20] and since the assay for this enzyme from rabbit brain was available from another project in this laboratory,[21] **3**–**36** were assayed as inhibitors of this enzyme; **37**–**41** were prepared and assayed for comparison. These compounds showed reversible inhibition with I_{50}'s in the range of 3–130 μM; these I_{50}'s should be considered minimum values since no effort was made to separate possible irreversible inhibition which may have occurred in the 15–30 min assay period.

The baseline compound for AChE inhibition is benzylpyridinium ion (**37**)(Table II) which had I_{50} = 66 μM. The corresponding quinoline (**38**) showed 4-fold better binding, but the naphthylmethylpyridinium ions (**39**, **40**) showed little change in binding compared to **37**. The SO_2F moiety of **11**–**13** gave no enhancement in binding compared to **37**, indicating that these 3 compounds were not showing irreversible inhibition. The largest contribution to binding by a side chain occurred in **4** where the increment was about 10-fold compared to **12**.

It is unlikely that these quaternary compounds (**3**–**36**) would give CNS disturbances *in vivo* since they most probably cannot pass the blood–brain barrier.[22] However, it is possible that some effects on the peripheral nervous system could occur.

Chemistry.—The quaternary salts in Tables I and II were prepared by reaction of the appropriate benzyl bromides[11] or chlorides with the appropriate pyridines. The substituted pyridines necessary for **29**–**36** were commercially available. The remainder of the substituted pyridines were prepared by one of the following general routes (see Scheme I).

SCHEME I



Compounds **42**–**46** and **48**–**51** were prepared by a Wittig reaction on the appropriate benzyl- or cinnamyltriphenylphosphonium chloride with the 2, 3, or 4 isomer of pyridinecarboxaldehyde, followed by catalytic reduction. Compound **47** was prepared by oxidation of 3-(3,4-dichlorophenoxy)-1,2-propanediol to 3,4-dichlorophenoxyacetaldehyde[23,24] followed by a Wittig reaction with 4-picolyltriphenylphosphonium chloride hydrochloride (**54**) and catalytic reduction. The precursors to **42** and **45** were also prepared by treating 3-picolyltriphenylphosphonium chloride (**53**) or **54** with 3,4-dichlorocinnamaldehyde (**55**). Compound **52** was prepared by catalytic reduction of commercial 4-stilbazole.

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Each anal. sample had an ir spectrum compatible with its structure and moved as a single spot on tlc on Brinkmann silica gel GF. All anal. samples gave combustion values for C, H or C, H, N within 0.4% of theoretical.

3,4-Dichlorocinnamaldehyde (**55**).—This compd was prepd from 3,4-dichlorobenzaldehyde and AcH by the general method of Baker, *et al.*[25] and was recrystd from EtOH in 48% yield, mp 93–95°. *Anal.* ($C_9H_6Cl_2O$) C, H, N.

3-Picolyltriphenylphosphonium Chloride·HCl (**53**).—A soln of 15.0 g (91.5 mmoles) of 3-picolyl chloride·HCl and 24.0 g (91.5 mmoles) of PPh_3 in 100 ml of DMF was stirred and heated at 80° for 45 min. The product crystd as the soln cooled to room temp; it was collected by filtration, washed with PhMe, and recrystd from PhMe–EtOH; yield, 15.9 g (41%) of white amorphous solid, mp 310–315°. *Anal.* ($C_{24}H_{22}Cl_2NP \cdot H_2O$) C, H, N.

4-Picolyltriphenylphosphonium chloride·HCl (**54**) was prepd by the same method as **53** using 4-picolyl chloride·HCl and PPh_3. Recrystn from PhMe–MeOH gave 23% of white amorphous solid, mp 259–261° *Anal.* ($C_{24}H_{22}Cl_2NP \cdot 0.5H_2O$) C, H, N.

3-Styrylpyridine (**Method B**).—A soln of 7.8 g (20 mmoles) of benzyltriphenylphosphonium chloride, 2.2 g (20 mmoles of 3-

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(19) B. R. Baker and J. A. Hurlbut, *ibid.*, **12**, 118 (1969).
(20) F. Bergmann, I. B. Wilson, and D. Nachmansohn, *Biochim. Biophys. Acta*, **6**, 217 (1950).
(21) B. R. Baker and R. E. Gibson, *J. Med. Chem.*, **14**, 315 (1971).
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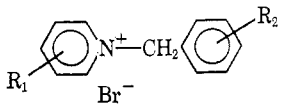
(24) R. Speer and H. Mahler, *ibid.*, **71**, 1133 (1949).
(25) B. R. Baker, E. E. Janson, and N. M. J. Vermeulen, *J. Med. Chem.*, **12**, 898 (1969).

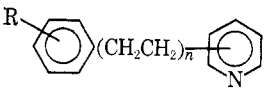
TABLE III
PHYSICAL PROPERTIES OF



No.	R_1	R_2	Method[a,b]	Yield, %	Mp, °C	Formula[c]
6	3-[3,4-$Cl_2C_6H_3(CH_2)_4$]	4-SO_2F	A	33	120–121	$C_{22}H_{21}BrCl_2FNO_2S$
7	3-[3,4-$Cl_2C_6H_3(CH_2)_4$]	2-SO_2F	A	23	129–130	$C_{22}H_{21}BrCl_2FNO_2S$
8	3-[3,4-$Cl_2C_6H_3(CH_2)_4$]	6-Cl-2-SO_2F	A	21	135–137	$C_{22}H_{20}BrCl_3FNO_2S$
9	3-$C_6H_5(CH_2)_4$	4-SO_2F	A	46	62–64[d]	$C_{22}H_{23}BrFNO_2S \cdot H_2O$
10	3-$C_6H_5(CH_2)_4$	6-Cl-2-SO_2F	A	46	149–151	$C_{22}H_{22}BrClFNO_2S$
11	H	4-SO_2F	A	38	221–222	$C_{12}H_{11}BrFNO_2S$
12	H	2-SO_2F	A	13	184–186	$C_{12}H_{11}BrFNO_2S$
13	H	6-Cl-2-SO_2F	A	41	139–142	$C_{12}H_{10}BrClFNO_2S$
14	2-[3,4-$Cl_2C_6H_3(CH_2)_4$]	4-SO_2F	A	19	122–124	$C_{22}H_{21}BrCl_2FNO_2S$
15	2-[3,4-$Cl_2C_6H_3(CH_2)_4$]	2-SO_2F	A	9	190–192	$C_{22}H_{21}BrCl_2FNO_2S$
16	4-[3,4-$Cl_2C_6H_3(CH_2)_4$]	4-SO_2F	A	36	184–185	$C_{22}H_{21}BrCl_2FNO_2S$
17	4-[3,4-$Cl_2C_6H_3(CH_2)_4$]	2-SO_2F	A	25	158–159	$C_{22}H_{21}BrCl_2FNO_2S$
18	4-$C_6H_5(CH_2)_4$	4-SO_2F	A	32	175–176	$C_{22}H_{23}BrFNO_2S$
19	4-[3,4-$Cl_2C_6H_3O(CH_2)_3$]	4-SO_2F	A	40	196–198	$C_{21}H_{19}BrCl_2FNO_3S$
20	4-$C_6H_5(CH_2)_4$	6-Cl-2-SO_2F	A	29	149–151	$C_{22}H_{22}BrClFNO_2S$
21	3-[3,4-$Cl_2C_6H_3(CH_2)_2$]	4-SO_2F	A	5	174–177	$C_{20}H_{17}BrCl_2FNO_2S$
22	3-[3,4-$Cl_2C_6H_3(CH_2)_2$]	2-SO_2F	A	16	184–185	$C_{20}H_{17}BrCl_2FNO_2S$
23	2-[3,4-$Cl_2C_6H_3(CH_2)_2$]	4-SO_2F	A	17	175–176	$C_{20}H_{17}BrCl_2FNO_2S$
24	2-[3,4-$Cl_2C_6H_3(CH_2)_2$]	2-SO_2F	A	45	192–193	$C_{20}H_{17}BrCl_2FNO_2S$
25	4-[3,4-$Cl_2C_6H_3(CH_2)_2$]	4-SO_2F	A	37	182–183	$C_{20}H_{17}BrCl_2FNO_2S$
26	4-[3,4-$Cl_2C_6H_3(CH_2)_2$]	2-SO_2F	A	15	203–204	$C_{20}H_{17}BrCl_2FNO_2S$
27	3-$C_6H_5CH_2CH_2$	6-Cl-2-SO_2F	A	29	168–170	$C_{20}H_{18}BrClFNO_2S$
28	4-$C_6H_5CH_2CH_2$	4-SO_2F	A	47	168–170	$C_{20}H_{19}BrFNO_2S$
29	4-$C_6H_5CH_2CH_2CH_2$	4-SO_2F	A	44	99–102	$C_{21}H_{21}BrFNO_2S$
30	4-$C_6H_5CH_2CH_2CH_2$	6-Cl-2-SO_2F	A	62	122–125	$C_{21}H_{20}BrClFNO_2S$
31	4-$C_6H_5CH_2$	4-SO_2F	A	49	180–181	$C_{19}H_{17}BrFNO_2S$
32	4-C_6H_5	4-SO_2F	A	25	224–226	$C_{18}H_{15}BrFNO_2S$
33	4-CH_3	4-SO_2F	A	27	183–185	$C_{13}H_{13}BrFNO_2S$
34	2,3-Benzo	4-SO_2F	A	21	210–212	$C_{16}H_{13}BrFNO_2S$
35	2,3-Benzo	6-Cl-2-SO_2F	A	53	209–211	$C_{16}H_{12}BrClFNO_2S$
36	3,4-Benzo	6-Cl-2-SO_2F	A	57	236–238	$C_{16}H_{12}BrClFNO_2S$
37	H	H	A	50	94–96[e]	
38	2,3-Benzo	H	A	70	185–186[f]	
39	H	3,4-Benzo	A	75	155–157[g]	
40[h]	H	2,3-Benzo	A	75	167–177[i]	
41	2,3-Benzo	3,4-Benzo	A	80	203–204[j]	

[a] A, the appropriate pyridine free bases were quaternized by the previously described general procedure[11] except that Et_2O was added to ppt the product. [b] Products were recrystd by dissolving in boiling Me_2CO contg about 2% MeOH followed by the addn of petr ether (bp 60–110°) to cloudiness; yield of anal. pure material is a minimum. [c] Anal. for C, H, N. [d] Amorphous solid. [e] J. Willems and J. Nys, *Bull. Soc. Chim. Belg.*, **66**, 502 (1957), reported mp 98–100°. [f] F. Kroehnke, H. Dickhauser, and I. Vogt, *Justus Liebigs Ann. Chem.*, **644**, 93 (1961), reported mp 184°. [g] R. Corral and O. Orazi, *An. Asoc. Quim. Argent.*, **42**, 169 (1954), reported mp 163.5–165°. [h] Cl^- salt. [i] J. V. Braun, J. Nelles, and A. May, *Ber. Deut. Chem. Ges., A*, **70**, 1767 (1937), reported mp 162°. [j] R. Corral and O. Orazi, *An. Asoc. Quim. Argent.*, **42**, 169 (1954), reported mp 194–196°.

TABLE IV
PHYSICAL PROPERTIES OF



No.	Pyridine bridge position	R	n	Method[a]	Yield,[b] %	Mp, °C	Formula	Analysis
42	3	3,4-Cl_2	2	B or C + D	42	150–151[c,d]	$C_{15}H_{15}Cl_2N \cdot HCl$	C, H, N
43	3	H	2	B + D	58	49–51[e]	$C_{15}H_{17}N$	C, H
44	2	3,4-Cl_2	2	B + D	49	141–142[c,d]	$C_{15}H_{15}Cl_2N \cdot HCl$	C, H, N
45	4	3,4-Cl_2	2	B or C + D	53	142–143[c,d]	$C_{15}H_{15}Cl_2N \cdot HCl$	C, H N
46	4	H	2	B + D	49	45–47[e,f]	$C_{15}H_{17}N$	
48	3	3,4-Cl_2	1	B + D	63	140–141[c,d]	$C_{13}H_{11}Cl_2N \cdot HCl$	C, H, N
49	2	3,4-Cl_2	1	B + D	60	149–150[c,d]	$C_{13}H_{11}Cl_2N \cdot HCl$	C, H, N
50	4	3,4-Cl_2	1	B + D	60	227–229[c,d]	$C_{13}H_{11}Cl_2N \cdot HCl$	C, H, N
51	3	H	1	C + D	27	32–34[e]	$C_{13}H_{13}N$	C, H, N
52	4	H	1	D	80	69–71[e,g]	$C_{13}H_{13}N$	

[a] See Experimental Section. [b] Overall yield from pyridinecarboxaldehyde except **52**. [c] HCl salt. [d] Recrystd from Me_2CO. [e] HCl salt purified, then base regenerated. [f] F. Bergstrom, T. Norton, and R. Seibert, *J. Org. Chem.*, **10**, 452 (1945), reported mp 47–49°. [g] F. Bergstrom, T. Norton, and R. Seibert, *ibid.*, **10**, 452 (1945), reported mp 70–71°.

pyridinecarboxaldehyde, and 2.5 g (20 mmoles) of DBN[26] in 75 ml of anhyd DMF was allowed to stand at ambient temp for 24 hr, then poured into 125 ml of H_2O. The resulting soln was decanted from the oil which formed. The oil was dissolved in EtOH, and the soln was clarified with charcoal, then treated by method D without further purification.

4-[3-(3,4-Dichlorophenoxy)-1-propenyl]pyridine (method C) was prepd by the above procedure from **54** and 3,4-dichlorophenoxyacetaldehyde[23,24] and treated by method D without further purification.

4-[3-(3,4-Dichlorophenoxy)propyl]pyridine (47) (Method D).—A soln of 0.50 g (1.8 mmoles) of 4-[3-(3,4-dichlorophenoxy)-1-propenyl]pyridine in 100 ml of EtOH was shaken with 2–3 atm of H_2 and 50 mg of 10% Pd/C until the theor amt was used. The soln was treated with charcoal, filtered, and evapd to give 0.45 g (21% from **54**) of an oil which was dissolved in Et_2O and treated with HCl gas. The resulting HCl salt was recrystd from Me_2CO–Et_2O; yield, 0.40 g, mp 164–166°. *Anal.* ($C_{14}H_{13}Cl_2NO \cdot HCl$) C, H, N.

3-(3,4-Dichlorophenoxy)-1,2-propanediol (57).—A soln of 16.3 g (0.10 mole) of 3,4-dichlorophenol and 35.0 g (0.32 mole) of 3-chloro-1,2-propanediol in 70 ml of DMF was heated at 70° with 13.8 g (0.10 mole) of K_2CO_3 for 3 days, then poured into H_2O. The crude product was collected on a filter, washed with H_2O, triturated in 2 *M* NaOH, then washed with H_2O, and finally recrystd from H_2O; yield, 13.7 g (55%) white solid, mp 105–107°. *Anal.* ($C_9H_{10}Cl_2O_3$) C, H, Cl.

3,4-Dichlorocinnamyltriphenylphosphonium Chloride (56).—A soln of 23.0 g (10.6 mmoles) of 3,4-dichlorocinnamic acid in 100 ml of $SOCl_2$ was refluxed for 20 min and then evapd *in vacuo* to a solid which was suspended in 100 ml of dioxane and added over 30 min to a stirred suspension of 25.0 g (65.7 mmoles) of $NaBH_4$ in 250 ml of dioxane. The soln was stirred at room temp for 30 min then cooled to and maintained at 20° while 60 ml of ice water and then 125 ml of 8 *M* HCl were slowly added over 1 hr. The contents of the flask were poured into 800 ml of H_2O and twice extd with $CHCl_3$. The combined org layers were washed with three 400-ml portions of satd aq $NaHCO_3$, two 400-ml portions of H_2O, dried (Na_2SO_4), and evapd to an orange liquid. This alcohol was treated with 100 ml of $SOCl_2$ as above and then evapd to a dark liquid (19.8 g, 89.5 mmoles) which was dissolved in 150 ml of PhMe contg 24.0 g (90 mmoles) of PPh_3. The resulting soln was heated overnight on a steam bath, cooled, and filtered. The collected product was recrystd from PhH–MeOH; yield, 25.0 g (57%) of white amorphous solid, mp 144–146°. *Anal.* ($C_{27}H_{22}Cl_3P \cdot 0.5H_2O$) C, H.

(26) 1,5-Diazabicyclo[4.3.0]non-5-ene; see H. Oediger, H. Kabbe, F. Moller, and E. Eiter, *Chem. Ber.*, **99**, 2012 (1966).

Irreversible Enzyme Inhibitors. 184.[1,2] An Affinity Column for Purification of Rat Liver Guanine Deaminase and Xanthine Oxidase

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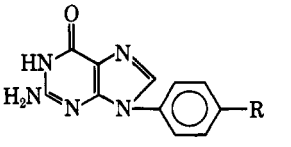
An affinity column for guanine deaminase and xanthine oxidase was prepared by coupling 9-(*p*-aminoethoxyphenyl)guanine, an inhibitor of both enzymes, to BrCN-activated Sepharose-4B. Xanthine oxidase from a 0–45% $(NH_4)_2SO_4$ fraction of rat liver was purified 230-fold with 90% recovery by absorption on the affinity column and elution with 1 m*M* substrate, hypoxanthine. The xanthine oxidase could also be eluted with an inhibitor, 2-benzylthiohypoxanthine; the latter was readily removed by 2-octanol extraction, and the recovery of purified enzyme was 68%. Guanine deaminase from a 45–90% $(NH_4)_2SO_4$ fraction of rat liver was purified 200-fold with 87% recovery by elution with 0.5 m*M* guanine.

The design, synthesis, and enzymic evaluation of active-site-directed irreversible inhibitors[3] of guanine deaminase[4] and xanthine oxidase[5–10] have been previously reported from this laboratory. 9-Phenylguanine (**1**) was found to be a good reversible inhibitor of guanine deaminase, being complexed slightly better than the substrate;[11] it was then established that the 28-fold binding increment by the Ph group compared to Me was due to hydrophobic bonding to the enzyme.[12] The nature and dimensions of the hydrophobic region of the enzyme were then mapped;[13] then an appropriate leaving group with the proper dimensions for formation of a covalent bond with a polar group on the enzyme outside the active site (exo mechanism) could be placed on an inhibitor such as **3**.[4]



1, R = H
2, R = $O(CH_2)_2NH_2$
3, R = $O(CH_2)_2NHCO$
4, R = NH_2
5, R = $O(CH_2)_2NH$—Sepharose

An affinity column for enzyme purification consists of a solid polymeric support to which is attached by covalent linkage an inhibitor relatively specific for the enzyme to be purified.[14] The solid support must be attached to a position on the inhibitor that does not interfere with complex formation with the enzyme. Such a position on the inhibitor is not obvious until sufficient information becomes available with reversible inhibitors as to where a large group can be placed on the inhibitor without interfering with complex formation;[14] this is the same information needed to design an active-

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

(2) For the previous paper in this series see B. R. Baker and M. H. Doll, *J. Med. Chem.*, **14**, 793 (1971).

(3) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," Wiley, New York, N. Y., 1967.

(4) B. R. Baker and W. F. Wood, *J. Med. Chem.*, **12**, 216 (1969).

(5) B. R. Baker and W. F. Wood, *ibid.*, **12**, 214 (1969).

(6) B. R. Baker and W. F. Wood, *ibid.*, **12**, 211 (1969).

(7) B. R. Baker and J. A. Kozma, *ibid.*, **11**, 656 (1968).

(8) B. R. Baker and J. A. Kozma, *ibid.*, **11**, 652 (1968).

(9) B. R. Baker and W. F. Wood, *ibid.*, **11**, 650 (1968).

(10) B. R. Baker and W. F. Wood, *ibid.*, **10**, 1106 (1967).

(11) B. R. Baker and D. V. Santi, *ibid.*, **10**, 62 (1967).

(12) B. R. Baker and W. F. Wood, *ibid.*, **10**, 1107 (1967).

(13) B. R. Baker and W. F. Wood, *ibid.*, **11**, 644 (1968).

(14) Ref 3, Chapter XIII.