with H_2O; yield 2.67 g (96%), mp 206–207.5°. Recrystallization from *i*-PrOH gave 2.27 g (81%) of white spears with unchanged melting point. *Anal.* ($C_{13}H_{10}FNO_3S$) C, H, F.

Irreversible Enzyme Assays.—The velocity of the reaction with 0.1 m*M* 2′-deoxycytidine was found to be proportional to the cytosine nucleoside deaminase concentration. No spontaneous inactivation of the enzyme in 10% DMSO and pH 7.4 Tris buffer occurred after 1 hr at 37°. The incubation method for detection of irreversible inhibition was the same as described for trypsin.[17]

(17) B. R. Baker and E. H. Erickson, *J. Med. Chem.*, **11**, 245 (1968), paper CXV of this series.

Irreversible Enzyme Inhibitors. CLXIV.[1,2] Proteolytic Enzymes. XIV.[2] Inhibition of Guinea Pig Complement by *meta*-Substituted Benzamidines

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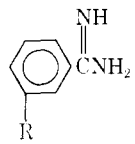
A series of 33 *meta*-substituted benzamidines were evaluated as inhibitors of guinea pig complement–sheep red blood cell–antibody system in order to determine the optimum substituents for inhibition. Since some of the benzamidines with *meta*-hydrocarbon substituents caused severe lysis of the red blood cells in the absence of complement, optimum hydrophobic interaction with minimal lysis was determined. The activity peaked at *n*-amyloxy (**15**) and isoamyloxy (**16**) which showed 50% inhibition at 0.5 m*M*; *n*-butoxy (**14**) showed less inhibition and *n*-hexyloxy (**17**) caused extensive lysis of the red blood cells. *m*-Alkyl substituents were less effective than *m*-alkoxy. Similar results were seen with phenylalkyl and phenylalkoxy substituents; *m*-phenylbutyl (**30**) and *m*-phenylbutyloxy (**20**) showed extensive lysis of the red blood cells, but *m*-phenylpropoxy (**19**) was an effective inhibitor at 0.5 m*M*. The best inhibitor showing no lysis was still the previously described *m*-phenoxypropyloxybenzamidine (**21**)[4] which at 0.5 m*M* showed 50% inhibition of complement.

Complement is a complex mixture of eleven serum proteins with proteolytic activity that has a number of biological actions including rejection of foreign cells.[3] Since complement has both "tryptic" and "chymotryptic" properties,[3] it can be inhibited by some compounds that inhibit trypsin[4] or chymotrypsin[2,5] when measured by the sheep red blood cell–hemolysin–serum method.[4,6] Effective "tryptic" type inhibitors were found in the benzamidine series (**1**), particularly when



R was isoamyloxy or phenoxypropoxy.[4] The hydrocarbon nature of the R group leading to enhanced activity suggested that further studies be performed to determine the nature and dimensions of this hydrophobic interaction, studies that have proved highly successful with other single enzyme systems.[7] Such a study with compounds of structure **1** is the subject of this paper.

Inhibition Results.—The inhibition of complement by a given concentration of compound is determined by comparison with a control lysis of sheep red blood cells (RBC) with no compound (Table I). In some cases acceleration of lysis occurred which is expressed as a minus amount of inhibition. The increase in rate of lysis can be due to one of two factors; the compound can cause lysis in the absence of complement, which is recorded as the percentage of total lysis possible (0.7 OD) (see **30**), or the compound can directly accelerate the complement system as shown by a negative inhibition or a decrease in inhibition as concentration is increased, but no lysis in the absence of complement[4] (see **19**).

A strict comparison of the effects of substitution on benzamidine for inhibition of complement is complicated by the multitude of enzymes involved in the complement system; with a single enzyme such as trypsin[8] or chymotrypsin,[9] good comparisons of substituent effects can be made. Nevertheless, the substitutions giving the best increments in inhibition of complement can be determined. Little variation in inhibition of complement was seen with single small *meta* substituents (**3**–**8**), except for NO_2 (**7**) where inhibition was poor; the effect of the NO_2 group does not appear to be electronic since CH_3 (**4**) and CF_3 (**6**) are nearly the same. The best inhibitor of this group was *m*-methoxybenzamidine (**5**); therefore higher alkoxy groups were studied as discussed below.

Five disubstituted benzamidines were investigated. The 3,4-Me_2 derivative (**10**) was slightly more effective than 3-Me (**4**), being equivalent to the parent benzamidine (**2**). The 3,5-Me_2 derivative (**11**) was three to four

(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 in this series, see B. R. Baker and J. L. Kelley, *J. Med. Chem.*, **12**, 1046 (1969).

(3) (a) H. J. Müller-Eberhard, *Advan. Immunol.*, **8**, 1 (1968); (b) P. H. Schur and K. R. Austen, *Ann. Rev. Med.*, **19**, 1 (1968).

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

(5) (a) B. R. Baker and J. A. Hurlbut, *ibid.*, **12**, 415 (1969), paper CLIII of this series; (b) B. R. Baker and J. A. Hurlbut, *ibid.*, **12**, 677 (1969), paper CLVI of this series.

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

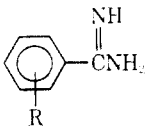
(7) For a review see B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," John Wiley and Sons, Inc., New York, N. Y., 1967.

(8) B. R. Baker and E. H. Erickson, *J. Med. Chem.*, **10**, 1123 (1967), paper CVI of this series.

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

Table I

Inhibition[a,b] of Guinea Pig Complement by



No.	R	Compd, mM	% inhibn[c]	% lysis[d]
2[e]	H	3	38	8
		1	15	
3[e]	*m*-OH	3	38	
4[e]	*m*-CH_3	3	25	0
		1	26	
5	*m*-OCH_3	3	50	0
		1	0	
6	*m*-CF_3	3	26	0
		1	10	
7[e]	*m*-NO_2	3	12	0
		1	10	
8	*m*-Br	3	44	0
		1	0	
9	*o*-CH_3	3	14	0
10	3,4-$(CH_3)_2$	3	41	0
		1	5	
11	3,5-$(CH_3)_2$	3	72	0
		1	39	
12	3,5-$(OCH_3)_2$	3	7	0
		1	0	
13[e]	*m*-OC_3H_7-*n*	1	36	4
		0.5	20	
14	*m*-OC_4H_9-*n*	1	35	0
		0.5	17	
15	*m*-OC_5H_{11}-*n*	1	62	0
		0.5	39	
16[e]	*m*-OC_5H_{11}-*i*	1	55	0
		0.5	46	
17	*m*-OC_6H_{13}-*n*	1	−81	82
		0.5	0	31
		0.25	20	7
18[e]	*m*-$OCH_2C_6H_5$	1	53	0
		0.5	21	
19[e]	*m*-$O(CH_2)_3C_6H_5$	1	12	0
		0.5	56	
20	*m*-$O(CH_2)_4C_6H_5$	1		100
		0.5	26	25
		0.25	51	15
		0.125	43	3
21[e]	*m*-$O(CH_2)_5OC_6H_5$	1	60	0
		0.5	54	
		0.25	27	
22	2,3-Benzo	3	0	0
		1	0	
23	3,4-Benzo	1	9	0
		0.5	~0	
24	*m*-C_4H_9-*n*	1	44	0
		0.5	20	
25	*m*-C_4H_{11}-*i*	1	37	8
		0.5	25	
26	*m*-C_6H_{13}	1	74	0
		0.5	15	
27	*m*-$CH_2C_6H_5$	1	24	
		0.5	13	
28	*m*-$(CH_2)_2C_6H_5$	1	55	7
		0.5	14	
29	*m*-$CH{=}CHC_6H_5$	1	40	0
		0.5	4	
30	*m*-$(CH_2)_4C_6H_5$	1	−52	100
		0.5	16	35
		0.25	40	0
31[e]	*m*-$(CH_2)_4C_6H_4NHAc$-*p*	1	59	0
		0.5	59	
		0.25	37	
32[g]	*m*-CH_2- 	0.25[h]	29	0
		0.125	4	
33[g]	*m*-CH_2- 	0.25[h]	31	5
		0.125	16	
34[g]	*m*-CH_2- 	1	87	4
		0.5	55	
		0.25	22	
35[g]	*m*-CH_2- 	0.25[h]	53	2
		0.125	25	

[a] The technical assistance of Sharon Lafler with these assays is acknowledged. [b] See ref 4 for the assay of inhibition of RBC lysis by complement. [c] A minus number indicates more lysis than the complement control without compound. [d] Lysis in the absence of complement corrected for 0–5% lysis in a control in the absence of compound; this is expressed as the per cent of the total lysis possible, 0.7 OD unit. [e] Data from ref 4. [f] Commercially available. [g] Dipicrate salt that was dissolved in 1:4 H_2O–MeOEtOH for assay. Picric acid was assayed as the Tris salt and at 2 m*M* showed no lysis of RBC in the absence of complement; picric acid at 2, 1, 0.5, and 0.25 m*M* showed 71, 32, 14, and 0% inhibition of complement. [h] Maximum solubility in assay medium.

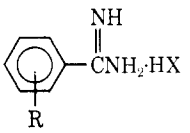
times more effective than 3-Me; in contrast, the 3,5-$(OMe)_2$ derivative (**12**) was ineffective, which should be compared to the 3-OMe (**5**), the most effective of the small single groups. Although the naphthcarboxamidines (**22**, **23**) can be classified as disubstituted derivatives of benzamidine, these compounds showed no activity.

It was reported earlier from this laboratory[4] that *m*-isoamyloxybenzamidine (**16**) was about sixfold more effective than the parent benzamidine (**2**) and about twofold more effective than *m*-propoxybenzamidine (**13**); this posed the question of what was the optimum alkoxy group for hydrophobic interaction.[4] This activity peaks at *n*-amyloxy (**15**) and isoamyloxy (**16**) since *n*-butoxy (**14**) is less effective and *n*-hexyloxy (**17**) causes severe RBC lysis in the absence of complement; inhibition by **17** at 0.25 m*M* can be detected, but higher concentrations lysed the RBC.

m-Phenylpropyloxybenzamidine (**19**) was reported earlier[4] to be an inhibitor of complement; good inhibition was seen at 0.5 m*M*, but at 1 m*M* **19** was less effective. The higher homolog (**20**) was a more effective inhibitor than **19**, since **20** showed 43% inhibition at 0.125 m*M*; however, at higher concentrations of **20**, RBC lysis in the absence of complement became severe. Replacement of the benzyl group of **20** by phenoxy (**21**)[4] gave a slightly less effective inhibitor, but lysis by the compound was eradicated; in the paper that follows,[10]

[10] B. R. Baker and M. Cory, *J. Med Chem.*, **12**, 1053 (1969), paper CLXV of this series.

TABLE II
PHYSICAL CONSTANTS OF



No.	R	HX	Method[a]	% yield	Mp, °C	Formula[b]
5	*m*-OCH_3	HCl	A, B[c]	20[d]	162–164[e]	
6	*m*-CF_3	TsOH	B	65[f]	202–204	$C_{15}H_{15}F_3N_2O_3S$
8	*m*-Br	TsOH	B	40[g]	188–190	$C_{14}H_{15}BrN_2O_3S$
9	*o*-CH_3	TsOH	C	9[g]	168–170[h]	
10	3,4-$(CH_3)_2$	TsOH	B	29	194–197[h]	
11	3,5-$(CH_3)_2$	TsOH	C	14[f]	174–176	$C_{16}H_{20}N_2O_3S$
12	3,5-$(OCH_3)_2$	TsOH	B	7[i]	135–137	$C_{16}H_{20}N_2O_5S$
14	*m*-OC_4H_9-*n*	TsOH	A, B[c]	60[d,f]	171–173	$C_{18}H_{24}N_2O_4S$
15	*m*-OC_5H_{11}-*n*	TsOH	A, B[c]	25[d,f]	164–166	$C_{19}H_{26}N_2O_4S$
17	*m*-OC_6H_{13}-*n*	TsOH	A, B[c]	44[d,f]	154–156	$C_{20}H_{28}N_2O_4S$
20	*m*-$O(CH_2)_4C_6H_5$	TsOH	A, B[c]	20[d,f]	147–148	$C_{24}H_{28}N_2O_4S$
22	2,3-Benzo	TsOH	C	8[f,j]	210–213	
23	3,4-Benzo	TsOH	B	65[f,k]	201–203	
24	*m*-C_4H_9-*n*	TsOH	D	17[f,m]	90–93	$C_{18}H_{24}N_2O_3S$
25	*m*-C_5H_{11}-*i*	HCl	D	51[f,m]	92–94	$C_{12}H_{19}ClN_2 \cdot 0.5H_2O$
26	*m*-C_6H_5	TsOH	B	16[f,n]	215–217	$C_{20}H_{20}N_2O_3S$
27	*m*-$CH_2C_6H_5$	TsOH	B	22[f,o]	177–179	$C_{21}H_{22}N_2O_3S$
28	*m*-$(CH_2)_2C_6H_5$	TsOH	D	55[m]	176–179	$C_{22}H_{24}N_2O_3S$
29	*m*-$CH{=}CHC_6H_5$	TsOH	D[p]	18[f,m]	110–112	$C_{22}H_{22}N_2O_3S$
30	*m*-$(CH_2)_4C_6H_5$	TsOH	D	23[f,m]	151–153	$C_{24}H_{28}N_2O_3S$
32	*m*-$(CH_2)_2$- 	Dipicrate	D	11[f,m]	220–232	$C_{26}H_{21}N_9O_{14}$
33	*m*-$(CH_2)_2$- 	Dipicrate	D	28[f,m]	241–244	$C_{26}H_{21}N_9O_{14}$
34	*m*-$(CH_2)_2$- 	Dipicrate	D	10[f,m]	236–238	$C_{26}H_{21}N_9O_{14}$
35	*m*-$(CH_2)_4$- 	Dipicrate	D[r]	25[f,m]	197–199	$C_{28}H_{25}N_9O_{14}$

[a] Methods: A,[11] alkylation of *m*-cyanophenol; B,[11] CN → imino ether → amidine; C, amide and $(EtO)_3BF_4$ → imino ether → amidine; D,[4] Wittig reaction, reduction, then method B. [b] Previously prepared by an alternate procedure by F. H. S. Curd and F. L. Rose, *J. Chem. Soc.*, 343 (1946), mp 165–166°. [c] The intermediate nitrile was an oil that was not purified. [d] Over-all yield from *m*-cyanophenol. [e] Recrystallized from EtOH–Et_2O. [f] Recrystallized from H_2O. [g] Picrate, mp 231–233°; lit.[12] 235–236°. [h] Previously prepared by R. J. Clark, A. Isaacs, and J. Walker, *Brit. J. Pharmacol.*, **13**, 424 (1958), mp 195–196°. [i] Recrystallized from Me_2CO. [j] Previously prepared by an alternate procedure by P. Oxley and W. F. Short, *J. Chem. Soc.*, 147 (1946), mp 210–211°. [k] Previously prepared by an alternate procedure by P. Oxley and W. F. Short, *ibid.*, 147 (1946), mp 203°. [l] Recrystallized from Me_2CO–H_2O. [m] Over-all yield from *m*-cyanobenzyltriphenylphosphonium bromide; the intermediates were oils. [n] Over-all yield from 3-aminobiphenyl; nitrile prepared according to R. L. Dannley and E. C. Gregg, *J. Am. Chem. Soc.*, **76**, 2997 (1954). [o] Over-all yield from *m*-cyanobenzyl bromide; nitrile prepared according to C. R. Hauser, W. Q. Beard, and F. N. Jones, *J. Org. Chem.*, **26**, 4790 (1961). [p] Analyzed for C, H, N. [q] Reduction step omitted. [r] 3-(3-Pyridyl)acrolein, mp 62–66°, was prepared from 3-pyridylcarboxyaldehyde by the procedure described for *p*-nitrocinnamaldehyde by B. R. Baker and J. H. Jordaan, *J. Med. Chem.*, **8**, 35 (1965).

the effect of substitution on the phenoxy moiety of **21** is reported which greatly improved inhibition.

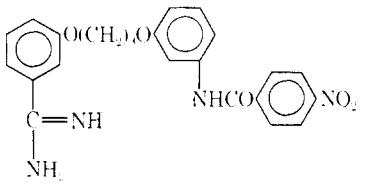
Benzamidines with an *m*-alkyl group were then studied. *n*-Butyl (**24**) and *n*-amyl (**25**) were about equally effective, being about threefold more effective than the parent benzamidine (**2**); since **24** and **25** were equipotent, no higher alkyl groups were investigated.

Attention was then turned to a series of *m*-$C_6H_5(CH_2)_n$ groups. The *m*-phenylbenzamidine (**26**) was three to six times as effective as the parent benzamidine (**2**); 1 m*M* of **26** showed 74% inhibition with no lysis of RBC. The benzyl (**27**), phenethyl (**28**), and styryl (**29**) derivatives were less effective than phenyl (**26**). *m*-Phenylbutylbenzamidine (**30**) at 1 m*M* caused 100% lysis of the RBC in the absence of complement; however, at 0.25 m*M* no lysis by **30** was seen and complement was inhibited by 40%. When the phenylbutyl moiety of **30** was substituted by the polar *p*-AcNH group (**31**),[4] lysis did not occur in the absence of complement, but inhibition at 0.25 m*M* was maintained; although inhibition increased to 59% with 0.5 m*M* of **31**, there was no further increase in inhibition at 1 m*M* indicating some other factor such as acceleration of one of the complement components was occurring simultaneously.

Since the lysis of RBC cells by *m*-phenylbutylbenzamidine (**30**) was removed by substitution of the polar acetamido group on the terminal phenyl to give **31**, but inhibition of complement was maintained, the phenyl group of **30** was replaced by the more polar 3-pyridyl (**35**). None of the usual salts of **35** could be crystallized, but **35** could be isolated as the dipicrate; this complicated the assay since Tris picrate itself shows 71, 32, 14, and 0% inhibition of complement when assayed at

2, 1, 0.5, and 0.25 m*M*, respectively. At its maximum solubility of 0.25 m*M*, **35** dipicrate showed 53% inhibition of complement; when this was corrected for the 14% inhibition by 0.5 m*M* picric acid, **35** had the same order of inhibition of complement as shown by **30** and **31**. Although the question of whether or not 1 m*M* of **35** could lyse RBC cells was not answered, it was clear that **35** was not more effective ininhibiting complement than **30** or **31**; therefore, more detailed studies were not pursued. The same complications with the dipicrate salts of the three pyridylethylbenzamidines (**32**–**34**) arose; since these compounds appeared to have the same order of activity of *m*-phenethylbenzamidine (**28**) when corrected for the inhibition by the picrate present, further studies were not made.

The best *meta* side chains on benzamidine (**2**) giving inhibition of complement with little lysis of RBC are phenoxypropyloxy (**21**) and *p*-acetamidophenylbutyl (**31**). A study of effects of substituents on the phenoxy moiety of **21** has led to considerable enhancement of activity;[10] for example, **36** was about 20-fold more effective than **21**, since **36** showed 65% inhibition at



36

0.031 m*M*.

Chemistry.—As noted in Table I, the synthesis of some of the compounds tested had been previously described.[4] The new benzamidines in Table I were prepared from the corresponding nitrile *via* the imino ether procedure,[11] except for **9**, **11**, and **22** where the method of Weintraub[12] was used; this involves reaction of an appropriately substituted benzamide with triethyloxonium fluoroborate, then conversion of the resultant imino ether fluoroborate with ammonia in ethanol to the amidine.

The required alkoxy- or arylalkoxybenzonitriles were prepared by the alkylation of *m*-cyanophenol with the appropriate bromide;[5] the resulting crude oils were converted to the amidines. The required alkyl- or arylalkylbenzonitriles were prepared by a Wittig reaction using *m*-cyanobenzyltriphenylphosphonium bromide[4] and the required aldehyde, followed by catalytic reduction; the intermediate nitriles were again oils.

Experimental Section

Melting points were determined in capillary tubes on a Mel-Temp block and are uncorrected. All analytical samples had ir spectra compatible with their assigned structures and moved as a single spot on tlc on Brinkmann silica gel GF or polyamide MN_{254}; each gave combustion values for C, H, and N within 0.4% of theory.

3,5-Dimethylbenzamidine Toluenesulfonate (11) (Method C).[12] To a rapidly stirred solution of 9.88 g (40 mmoles) of $BF_3{\cdot}OEt_2$ in 20 ml of dry Et_2O protected from moisture was slowly added 5.60 g (30 mmoles) of epichlorohydrin at such a rate that the solution maintained a slow reflux (very exothermic reaction). The resulting mixture was stirred at reflux for 18 hr and cooled to room temperature. The crystalline product was washed with three 60-ml portions of dry Et_2O by decantation, then dissolved in 100 ml of CH_2Cl_2. This CH_2Cl_2 solution was added to a solution of 1.49 g (10 mmoles) of 3,5-dimethylbenzamide in 50 ml of CH_2Cl_2 at 0°. The resulting solution was stirred at ambient temperature for 15 hr, then evaporated *in vacuo*. The residue was dissolved in 20 ml of EtOH, and 35 ml of EtOH previously saturated with NH_3 at 0° was added. The mixture was stirred 15 hr, then filtered. Evaporation of the filtrate *in vacuo* gave a residue that was partitioned between C_6H_6 and 50% aqueous NaOH solution. The C_6H_6 layer was washed with H_2O, dried with $MgSO_4$, then evaporated *in vacuo*. The TsOH salt was prepared by dissolving the residue in 60 ml of hot H_2O containig 0.23 g of TsOH. The solution was cooled and the crystalline product was collected. See Table II for additional data and other compounds prepared by this procedure.

(11) B. R. Baker and E. H. Erickson, *J. Med. Chem.*, **11**, 245 (1968), paper CXV of this series.

(12) L. Weintraub, S. R. Oles, and N. Kalish, *J. Org. Chem.*, **33**, 1679 (1968).