Irreversible Enzyme Inhibitors. CLXV.[1,2] Proteolytic Enzymes. XV.[2] Inhibition of Guinea Pig Complement by Derivatives of *m*-Phenoxypropoxybenzamidine

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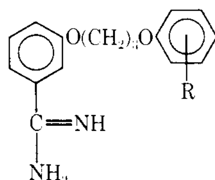
A series of 28 derivatives of *m*-phenoxypropoxybenzamidine (**2**) with substituents on the phenoxy moiety were synthesized, then evaluated as inhibitors of the guinea pig complement–sheep red blood cell–antibody system. Small substituents such as Cl, NO_2, OCH_3, NH_2, AcNH, or $COOCH_3$ give at best only fourfold more effective inhibition. Larger substituents such as substituted benzamido or substituted phenylureido gave 6–30-fold better inhibition. The best inhibitor of the series was *m*-[*m*-(*p*-nitrophenylureido)phenoxypropoxy]benzamidine (**29**) which at 62 μM showed 95% inhibition of guinea pig complement and 60% inhibition at 16 μM. Thus **29** was 30-fold more effective than **2** and about 400-fold more effective than benzamidine.

Inhibition of the serum complement system[3] could have a number of medicinal uses.[4] Such inhibition of complement is readily assayed by the sheep red blood cell–hemolysin–serum method.[5,6] Among the better inhibitors found in this laboratory is the "tryptic-type" inhibitor, *m*-phenoxypropoxybenzamidine (**1**, R = H).[2,5] A study has now been made to determine



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if substitution on the phenxoy moiety of **1** could enhance activity; this was indeed the case and the results are the subject of this paper.

Inhibition Results.—The inhibition of complement[5,6] by a compound is determined by comparison with a control lysis of sheep red blood cells (RBC) by complement and hemolysin (Table I). In some cases (see **11**) the compound could cause lysis of RBC in the absence of complement, which is recorded as a percentage of the total lysis (0.7 OD) possible.

The base-line compound with which this study was started was *m*-phenoxypropyloxybenzamidine (**2**), which is about tenfold more effective on a concentration basis than the parent benzamidine.[2,5] The effect of small substituents on the phenoxy moiety was studied first to determine if there were any electronic effects on inhibition by the phenoxy moiety.

In the *para* series, substitution of a *p*-nitro (**3**) or *p*-amino (**5**) group gave no change in inhibition, indicating that there were no electronic effects on the binding of the phenoxy moiety. Binding was enhanced twofold with a *p*-OCH_3 (**6**) or *p*-acetamido substituent (**7**). A fourfold enhancement of inhibition was seen with *p*-Cl (**4**) or *p*-$COOCH_3$ (**8**) substituents; measurements with the *p*-COOH (**9**) substituent were hampered by lack of solubility but it appeared that this substituent was slightly less effective than *p*-$COOCH_3$ (**8**).

In the *meta* series, NO_2 (**10**), NH_2 (**12**), CH_3O (**13**), and AcNH (**14**) gave about a fourfold increment in binding; the CF_3 substituent (**11**) was about as effective at 0.125 mM as the *m*-nitro (**10**); at higher concentration **11** gave substantial lysis in the absence of complement. The most effective small group in the *meta* series was $COOCH_3$ (**15**) which gave an eightfold increment in binding over the parent base-line compound (**2**). Again it is clear that these increments are not due to any electronic effects on the binding of the phenoxy moiety; also there did not appear to be any correlation with the relative hydrophobic character of the substituent.[7]

Only two compounds in the *ortho* series were investigated: *o*-nitro (**16**) gave a fourfold increment in binding and *o*-amino (**17**) twofold. Since these compounds were no more effective than *meta* or *para* substituents, no further compounds with small *ortho* substituents were synthesized for investigation.

Two compounds with two small substituents on the phenoxy moiety were investigated. 3-NO_2-4-CH_3 (**19**) was only slightly more effective than 3-NO_2 (**10**). Measurements with the 3,4-Cl_2 derivative (**18**) were hampered by the lack of solubility; however, **18** was about twofold less effective than 4-Cl (**4**).

The effect of larger substituents on the phenoxy moiety were then investigated. A *p*-C_6H_5 substituent (**20**) appeared to enhance activity compared to the base-line compound (**2**) at low concentration, but measurements were hampered by its low solubility of 0.03 mM. Therefore more polar amide substituents with greater water solubility were investigated.

The *p*-benzamido derivative (**21**) was about eight times as soluble as the *p*-phenyl derivative (**20**); furthermore, **21** gave about a sixfold enhancement in binding compared to **2**. When the benzamido group of **21** was substitued by *p*-NO_2 (**23**), *m*-NO_2 (**24**), or *p*-OCH_3 (**25**), inhibition was enhanced six- to eightfold; the *p*-chlorobenzamido derivative (**22**) at its maximum solubility was about eightfold more effective than the same concentration of **21**; however, **22** was too in-

(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 M. Cory, *J. Med. Chem.*, **12**, 1049 (1969).

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

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

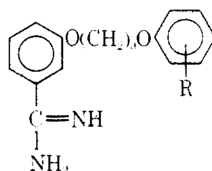
(5) B. R. Baker and E. H. Erickson, *ibid.*, **12**, 408 (1969), paper CLII of this series.

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

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

Table I

Inhibition[a,b] of Guinea Pig Complement by



No.	R	Compd, mM	% inhibn[c]	% lysis[d]
2[e]	H	1	60	0
		0.5	54	
		0.25	27	
3	*p*-NO_2	1	40	20
		0.5	53	0
		0.25	27	
4	*p*-Cl	0.125[f]	48	7
		0.062	27	
5	*p*-NH_2	1	79	3
		0.5	52	
6	*p*-OCH_3	1	92	5
		0.5	82	0
		0.25	59	
7	*p*-AcNH	1	92	0
		0.5	79	
		0.25	50	
		0.125	28	
8	*p*-$COOCH_3$	1	91	5
		0.5	93	
		0.25	87	
		0.125	66	
		0.062	38	
9	*p*-COOH	0.50[f]	16	0
		0.025	8	
10	*m*-NO_2	1	84	9
		0.5	91	0
		0.25	77	
		0.125	49	
		0.062	22	
11	*m*-CF_3	0.5		100
		0.25	40	18
		0.125	42	9
		0.062	38	0
		0.031	19	
12	*m*-NH_2	1	94	3
		0.5	86	
		0.25	67	
		0.125	40	
13[e]	*m*-OCH_3	0.25[f]	80	0
		0.125	50	
		0.062	17	
14	*m*-AcNH	1	88	0
		0.5	82	
		0.25	61	
		0.125	36	
15	*m*-$COOCH_3$	0.5	60	19
		0.25	88	6
15	*m*-$COOCH_3$	0.125	74	
		0.062	45	
16	*o*-NO_2	1	96	0
		0.5	91	
		0.25	77	
		0.125	49	
		0.062	23	
17	*o*-NH_2	1	93	2
		0.5	75	
		0.25	46	
18	3,4-Cl_2	0.125[f]	34	29
		0.062	23	
19	3-NO_2-4-CH_3	0.5	−67	100
		0.25	68	7
		0.125	73	
		0.062	39	
20	*p*-C_6H_5	0.031[f]	22	5
		0.015	0	
21	*p*-$NHCOC_6H_5$	0.25[f]	82	4
		0.125	65	
		0.062	44	
22	*p*-$NHCOC_6H_4Cl$-*p*	0.016[f]	20	0
		0.0080	0	
23	*p*-$NHCOC_6H_4NO_2$-*p*	0.050[f]	37	0
		0.025	14	
24	*p*-$NHCOC_6H_4NO_2$-*m*	0.062[f]	50	4
		0.031	26	
25	*p*-$NHCOC_6H_4OCH_3$-*p*	0.125[f]	70	0
		0.062	43	
		0.031	21	
26	*p*-$NHCONHC_6H_5$	0.062[f]	50	4
		0.031	26	
27	*m*-$NHCOC_6H_4NO_2$-*p*	0.25[f]	82	4
		0.125	93	
		0.062	82	
		0.031	65	
		0.016	43	
28[g]	*m*-$NHCOC_6H_4OCH_3$-*p*	0.125[f]	82	0
		0.062	61	
		0.031	30	
29[g]	*m*-$NHCONHC_6H_4NO_2$-*p*	0.062[f]	95	0
		0.031	78	
		0.016	60	
		0.0078	32	
		0.0039	13	
30	*o*-$NHCOC_6H_4NO_2$-*p*	0.062	48	0
		0.031	23	

[a] The technical assistance of Sharon Lafler with these assays is acknowledged. [b] See ref 5 for 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 without complement and compound; this is expressed as the per cent of total lysis possible, 0.7 OD unit. [e] Data from ref 5. [f] Maximum solubility in buffer. [g] Picrate dissolved in 1:4 H_2O-MeOEtOH for assay; Tris picrate shows no inhibition or lysis at 0.25 m*M*.[2]

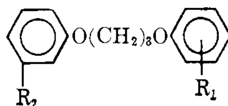
soluble to reach a concentration showing 50% inhibition. When the *p*-benzamido moiety of **21** was replaced by a *p*-phenylureido moiety (**26**), inhibition was enhanced only slightly.

Substituted benzamido substituents on the *meta* position of the phenoxy moiety of **2** were then investigated. The *p*-methoxybenzamido (**28**) derivatives were eightfold more effective than the parent **2**, while the *p*-nitrobenzamido derivative (**27**) was 16-fold more effective. The *p*-nitrophenylurea derivative (**29**) was the most potent compound in Table I, being 30-fold more effective than the parent **2**.

When the *p*-nitrobenzamido group of **27** was moved to the *ortho* position of the phenoxy moiety (**30**), about

TABLE II

PHYSICAL CONSTANTS OF



No.	R1	R2	Method[a]	% yield	Mp, °C	Formula[b]
3	p-NO_2	$C(NH_2)$=NH·HCl	B	37[c]	94–95	$C_{16}H_{18}ClN_3O_4 \cdot H_2O$
4	p-Cl	$C(NH_3)$=NH·HCl	B	73[c]	146–149	$C_{16}H_{18}Cl_2N_2O_2 \cdot H_2O$
5	p-NH_2	$C(NH_2)$=NH·2TsOH	C	43[c]	191–192	$C_{30}H_{35}N_3O_8S_2$
6	p-OCH_3	$C(NH_2)$=NH·TsOH	B	66[c]	129–131	$C_{24}H_{28}N_2O_6S$
7	p-NHAc	$C(NH_2)$=NH·HCl	D, B[d]	30[c]	131–133	$C_{18}H_{22}ClN_3O_3 \cdot H_2O$
8	p-CO_2CH_3	$C(NH_2)$=NH·TsOH	B	87[c]	162–164	$C_{25}H_{28}N_2O_7S$
10	m-NO_2	$C(NH_2)$=NH·HCl	B	82[e]	142–144	$C_{16}H_{18}ClN_3O_4$
11	m-CF_3	$C(NH_2)$=NH·TsOH	A, B[d]	34[c]	120–122	$C_{24}H_{25}F_3N_2O_5S$
12	m-NH_2	$C(NH_2)$=NH·2HCl	C	85[c]	106–107	$C_{16}H_{21}Cl_2N_3O_2$
13	m-OCH_3	$C(NH_2)$=NH·Picrate	A, B[d]	53[f]	140–142	$C_{23}H_{23}N_5O_{10}$
14	m-NHAc	$C(NH_2)$=NH·TsOH	B	28[c]	111–113	$C_{26}H_{28}N_3O_6S \cdot H_2O$
15	m-CO_2CH_3	$C(NH_2)$=NH·HCl	A, B[d]	67[c]	108–111	$C_{18}H_{21}ClN_2O_4$
16	o-NO_2	$C(NH_2)$=NH·HCl	B	66[c]	82–84	$C_{16}H_{18}ClN_3O_4 \cdot H_2O$
17	o-NH_2	$C(NH_2)$=NH·2TsOH	B	89[c]	124–126	$C_{30}H_{35}N_3O_8S_2$
18	3,4-Cl_2	$C(NH_2)$=NH·HCl	B	75[c]	101–103	$C_{16}H_{17}Cl_3N_2O_2$
19	3-NO_2-4-CH_3	$C(NH_2)$=NH·TsOH	B	30[c]	101–102	$C_{24}H_{27}N_3O_7S \cdot H_2O$
20	p-C_6H_5	$C(NH_2)$=NH·HCl	B	54[f]	226–229	$C_{22}H_{23}ClN_2O_3$
21	p-$NHCOC_6H_5$	$C(NH_2)$=NH·TsOH	B	27[c]	184–187	$C_{30}H_{31}N_3O_6S$
22	p-$NHCOC_6H_4Cl$-p	$C(NH_2)$=NH·TsOH	E	40[c]	143–145	$C_{30}H_{30}ClN_3O_6S$
23	p-$NHCOC_6H_4NO_2$-p	$C(NH_2)$=NH·TsOH	E	32[c]	218–220	$C_{30}H_{30}N_4O_8S$
24	p-$NHCOC_6H_4NO_2$-m	$C(NH_2)$=NH·TsOH	E	47[c]	91–93	$C_{30}H_{30}N_4O_8S$
25	p-$NHCOC_6H_4OCH_3$-p	$C(NH_2)$=NH·TsOH	E	41[g]	152–155	$C_{31}H_{33}N_3O_7S$
26	p-$NHCONHC_6H_5$	$C(NH_2)$=NH·TsOH	F	72[c]	134–135	$C_{30}H_{32}N_3O_6S$
27	m-$NHCOC_6H_4NO_2$-p	$C(NH_2)$=NH·TsOH	E	53[c]	135–137	$C_{30}H_{30}N_4O_8S \cdot H_2O$
28	m-$NHCOC_6H_4OCH_3$-p	$C(NH_2)$=NH·Picrate	E	10[f]	198–202	$C_{30}H_{28}N_6O_{11}$
29	m-$NHCONHC_6H_4NO_2$-p	$C(NH_2)$=NH·Picrate	F	36[c]	181–183	$C_{29}H_{26}N_8O_{12} \cdot H_2O$
30	o-$NHCOC_6H_4NO_2$-p	$C(NH_2)$=NH·TsOH	E	35[c]	191–194	$C_{30}H_{30}N_4O_8S$
31	p-NO_2	CN	A	52[h]	100–102	$C_{16}H_{14}N_2O_4$
32	p-Cl	CN	A	84[h]	84–86	$C_{16}H_{14}ClNO_2$
33	p-OCH_3	CN	A	42[h]	63–65	$C_{17}H_{17}NO_3$
34	p-CO_2CH_3	CN	A	80[h]	81–82	$C_{18}H_{17}NO_4$
35	m-NO_2	CN	A	35[i]	73–74	$C_{16}H_{14}N_2O_4$
36	m-$NHCOCH_3$	CN	A	55[h]	81–82	$C_{18}H_{18}N_2O_3$
37	o-NO_2	CN	A	80[h]	81–83	$C_{16}H_{14}N_2O_4$
38	3,4-Cl_2	CN	A	47[h]	78–80	$C_{16}H_{13}Cl_2NO_2$
39	3-NO_2-4-CH_3	CN	A	70[h]	103–105	$C_{17}H_{16}N_2O_4$
40	p-C_6H_5	CN	A	64[h]	99–101	$C_{22}H_{19}NO_2$
41	p-$NHCOC_6H_5$	CN	D	65[j]	150–153	$C_{23}H_{26}N_2O_3$

[a] Methods: A,[4] alkylation of *m*-cyanophenol with 1,3-dibromopropane, then further alkylation of the bromopropyl ether; B,[8] CN → imino ether → amidine; C,[8] reduction of NO_2; D–F, see Experimental Section. [b] Analyzed for C, H, N. [c] Recrystallized from H_2O. [d] The intermediate nitrile was an oil that was not purified. [e] Recrystallized from Me_2CO–H_2O. [f] Recrystallized from 50% EtOH. [g] Recrystallized from EtOH. [h] Recrystallized from petroleum ether (60–120°)–C_6H_6. [i] Sublimed *in vacuo* for analysis. [j] Recrystallized from C_6H_6.

a fourfold loss in inhibition occurred; **30** was still about eightfold more effective than the parent **2**.

From these studies, it is clear that substitution of benzamido or phenylureido groups on the *meta* position of the phenoxy moiety of **2** gives the best enhancement of activity. The most potent inhibitor of complement to date is the *m*-(*p*-nitrophenylureido) derivative (**29**) on the phenoxy moiety of *m*-phenoxypropyloxybenzamidine (**2**); not only is **29** 30-fold more effective than **2**, but activity has been enhanced about 400-fold over benzamidine, the inhibitor of complement with which these studies were started.[5]

Under investigation is the synthesis of candidate irreversible inhibitors of complement such as replacement of the NO_2 group of **27** and **29** with SO_2F.[8]

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

Chemistry.—The necessary substituted *m*-(phenoxypropoxy)benzonitriles were prepared by the previously described alkylation of *m*-cyanophenol (method A);[4] these were converted to the amidines through the imino ether hydrochlorides (method B).[8] Catalytic reduction of the nitro group of **3**, **10**, or **16** with 5% Pd–C (method C)[8] gave crystalline aminoamidine salts which could be acylated to the desired amides or ureas (methods E and F). Another route was reduction of **31** followed by acylation to **41**, which could be converted to the amidine **21**; this route was used for **7** and not pursued further.

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 NM_{254}; each gave combustion values for C, H, and N within 0.4% of theory.

***m*-(*m*-Aminophenoxypropoxy)benzamidine (12) Dihydrochloride (Method C).**—To a solution of 3.5 g (10.0 mmoles) of **10** in 100 ml of EtOH containing 200 mg of 5% Pd-C was added 0.83 ml (10.0 mmoles) of 12 *N* HCl. The resulting mixture was shaken with H_2 at 2-3 atm; reduction was complete in 2 hr. The filtered solution was evaporated *in vacuo* and the residue recrystallized; yield 3.1 g (85%) of white crystals. See Table II for additional data.

***m*-(*p*-Carboxyphenoxypropoxy)benzamidine Toluenesulfate (9).** Solution of 0.511 g (10.0 mmoles) of **8** in 5 ml of 6 *N* HCl and 5 ml of HOAc was stirred at reflux for 16 hr, then evaporated *in vacuo*. Two recrystallizations from H_2O gave 0.210 g (43%) of white crystals, mp 213-215°. *Anal.* ($C_{24}H_{26}N_2O_7S$) C, H, N.

***m*-(*p*-Benzamidophenoxypropoxy)benzonitrile (41) (Method D).**—A solution of 2.85 g (9.55 mmoles) of **31** in 200 ml of EtOH containing 0.30 g of 5% Pd-C was shaken with H_2 at 2-3 atm; reduction was complete in 1 hr. The filtered solution was evaporated *in vacuo* to yield 2.6 g (100%) of a colorless oil suitable for the next reaction.

To 2.0 g (7.45 mmoles) of the crude oil was added 20 ml of $CHCl_3$ and 1.1 ml (8.0 mmoles) of Et_3N followed by 0.92 ml (8.0 mmoles) of benzoyl chloride. The resulting solution was stirred at ambient temperature for 24 hr, then washed successively with three 30-ml portions of 1 *N* HCl, three 30-ml portions of 1 *N* NaOH, and three 30-ml portions of H_2O. The dried solution was evaporated *in vacuo*. Two recrystallizations from C_6H_6 afforded 1.8 g (65%) of white crystals, mp 150-153°. *Anal.* ($C_{23}H_{20}N_2O_3$) C, H, N.

***m*-[*m*-(*p*-Nitrobenzamido)phenoxypropoxy]benzamidine Toluenesulfonate (27) (Method E).**—To a solution of 0.310 g (0.86 mmole) of **12** in 2 ml of DMF was added 1.0 g of 4A Molecular Sieves (Linde) followed by 0.245 ml (1.75 mmoles) of Et_3N. To the resulting mixture was added a solution of 0.209 g (1.11 mmoles) of *p*-nitrobenzoyl chloride in 2 ml of DMF. The mixture was stirred 1 hr at ambient temperature, then poured into 30 ml of H_2O containing 0.380 g (2.0 mmoles) of *p*-toluenesulfonic acid. The crystalline product was collected and recrystallized from H_2O; yield 0.271 g (53%), mp 135-137°. See Table II for additional data.

***m*-(*p*-Phenylureido)phenoxypropoxybenzamidine Toluenesulfonate (26) (Method F).**—To a solution of 0.30 g (0.48 mmole) of **5** in 2 ml of DMF was added 0.077 ml (0.48 mmole) of Et_3N followed by 0.129 g (0.50 mmole) of O-(*p*-nitrophenyl) N-phenylcarbamate.[9] The resulting solution was stirred at room temperature for 16 hr, then poured into 30 ml of H_2O, and the product was collected. Recrystallization from H_2O gave 0.20 g (72%), mp 134-135°. See Table II for additional data.

(9) B. R. Baker and N. M. J. Vermeulen, *J. Med. Chem.*, **12**, 74 (1969); paper CXXXIV of this series.

Synthesis and Biological Activity of Some New N^6-Substituted Purine Nucleosides

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The synthesis of N^6-2-phenoxyethyl-, N^6-benzyl-, N^6-*n*-hexyl-, N^6-*n*-pentyl-, N^6-phenyl-, N^6-2-thienyl-, and N^6-2-ethoxyethyladenosines was carried out by quaternization of the N^1 of adenosine with the appropriate halide, followed by rearrangement of the product in aqueous NH_3, or by nucleophilic substitution of 6-chloropurine riboside with the appropriate amine. Also synthesized were the N^6-(Δ^2-isopentenyl) and N^6-allyl derivatives of the antibiotic tubercidin (7-deazaadenosine). The compounds were examined for biological activity in a number of test systems. All of the adenosine derivatives examined showed cytokinin activity in the tobacco pith bioassay. Similarly, at low concentrations (10^{-8} to 10^{-6} *M*), the N^6-substituted adenosines tested stimulated the growth of a human leukemic cell line (6410). At higher concentrations, they decreased the viability of this line of leukemic myeloblasts of line HR1K of Burkitt's lymphoma, and line LK1D of leukemic lymphoblasts, whereas they were all ineffective against a culture of normal leukocytes. The N^6-substituted tubercidins on the other hand inhibited the normal leukocytes, but were variably effective against the tumor lines. Most of the compounds interfered with the growth of *Escherichia coli* and some with the growth of Sarcoma 180 cells *in vitro*. A moderate but significant increase in survival time of mice bearing leukemia L1210 was produced by four of the adenosine derivatives.

N^6-(3-Methyl-2-butenyl)adenosine or N^6-(Δ^2-isopentenyl)adenosine (IPA) occurs in sRNA[1] and was originally synthesized by Leonard, *et al.*[2,3] This nucleoside has high cytokinin activity.[1c,3] It also inhibits the growth of human myelogenous leukemic cells and certain mouse tumors[4,5] and has undergone preliminary clinical trials.[6] Because of these findings, several other N^6-substituted adenosines were synthesized and found to have biological activity.[7a] Additional N^6-substituted adenosines have now been prepared[7b] and their biological properties have been studied. The new series of compounds reported in this paper are the N^6-β-D-ribofuranosyl derivatives of those N^6-substituted adenine bases which have shown potent cytokinin activity as reported by Strong[8] and by Skoog, *et al.*[9a] They include the N^6-2-phenoxyethyl- (I), N^6-benzyl- (II), N^6-*n*-hexyl- (III), N^6-*n*-pentyl- (IV), N^6-phenyl- (V), N^6-thienyl- (VI), and N^6-2-

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