Central Cholinergic Agents. I. Potent Acetylcholinesterase Inhibitors, 2-[ω-[*N*-Alkyl-*N*-(ω-phenylalkyl)amino]alkyl]-1*H*-isoindole-1,3(2*H*)-diones, Based on a New Hypothesis of the Enzyme's Active Site

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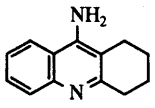
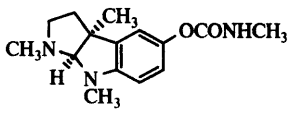
It has been suggested that the active site of acetylcholinesterase contains a hydrophobic binding site (HBS-1), which is closely adjacent to both the anionic and the esteratic sites. In this paper, we assumed that there exists another hydrophobic binding site (HBS-2), some distance removed from the anionic site. On this assumption, a new working hypothesis was proposed for the design of acetylcholinesterase inhibitors. A series of 2-[ω-[*N*-alkyl-*N*-(ω-phenylalkyl)amino]alkyl]-1*H*-isoindole-1,3(2*H*)-diones was designed based on this hypothesis and tested for its inhibitory activities on acetylcholinesterase. Some in this series were revealed to be more potent than physostigmine. Optimum activity was found to be associated with a five carbon chain length separating the benzylamino group from the 1*H*-isoindole-1,3(2*H*)-dione (phthalimide) moiety. Quantitative study of substitution effect on the phthalimide moiety revealed that hydrophilic and electron-withdrawing groups enhance the activity.

Keywords anticholinesterase; 1*H*-isoindole-1,3(2*H*)-dione; phthalimide; structure–activity relationship; Hansch–Fujita analysis; hydrophobic binding site

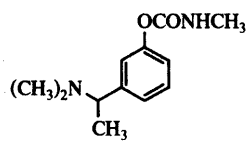
Senile dementia of the Alzheimer type (SDAT) has been shown to be closely associated with defects in the central cholinergic system. Many clinical and animal studies suggest that cholinergic dysfunction may be one of the causes of disturbances in learning and memory in SDAT patients.[1] Thus, pharmacological manipulation of the cholinergic system has been targeted as a viable approach for the treatment of SDAT. Cholinergic enhancement can be achieved by the use of acetylcholine precursors, muscarinic agonists, or acetylcholinesterase (AChE) inhibitors. Acetylcholine precursors such as choline or lecithin have failed to produce any obvious clinical effects.[2] Therapeutic benefits derived from muscarinic agonists such as arecoline or RS-86 are likely to be limited by peripheral cholinergic side effects.[3] On the other hand, clinical studies with various AChE inhibitors have shown the most success. After the report of clinical improvement with tetrahydroaminoacridine (THA),[4] much attention has been focused on AChE inhibitors such as THA, physostigmine, and their analogues (Chart 1),[5,6] which have been considered promising candidates as drugs to treat SDAT.



THA



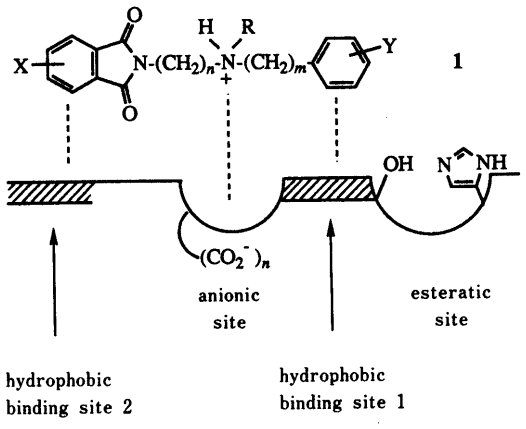
physostigmine



miotine

Chart 1

It has been shown that many AChE inhibitors interact with the active site of AChE, which contains an esteratic site and an anionic site. Physostigmine binds to the anionic site and carbamoylates the hydroxyl group of a serine residue in the esteratic site.[7] In addition, the existence of a hydrophobic binding site (HBS) has been suggested,[8] which is thought to be closely adjacent to both the anionic and the esteratic sites.[9,10] This will be referred to as HBS-1 in this paper. It has been suggested that THA binds at the anionic site as well as at HBS-1.[9] Quinn has given a stylized diagram of these subsites in his review,[11] which prompted us to assume that there may exist at least one more hydrophobic binding site (HBS-2) some distance away from the anionic site (Chart 2). Compounds which can interact with both hydrophobic binding sites as well as the anionic site seem to have provided us with a new type of AChE inhibitor. On the basis of this working hypothesis, we designed 2-[ω-[*N*-alkyl-*N*-(ω-phenylalkyl)amino]alkyl]-1*H*-isoindole-1,3(2*H*)-diones (**1**), some of which were revealed to be



a hypothetical diagram of the active site of AChE

Chart 2. Design of New AChE Inhibitors

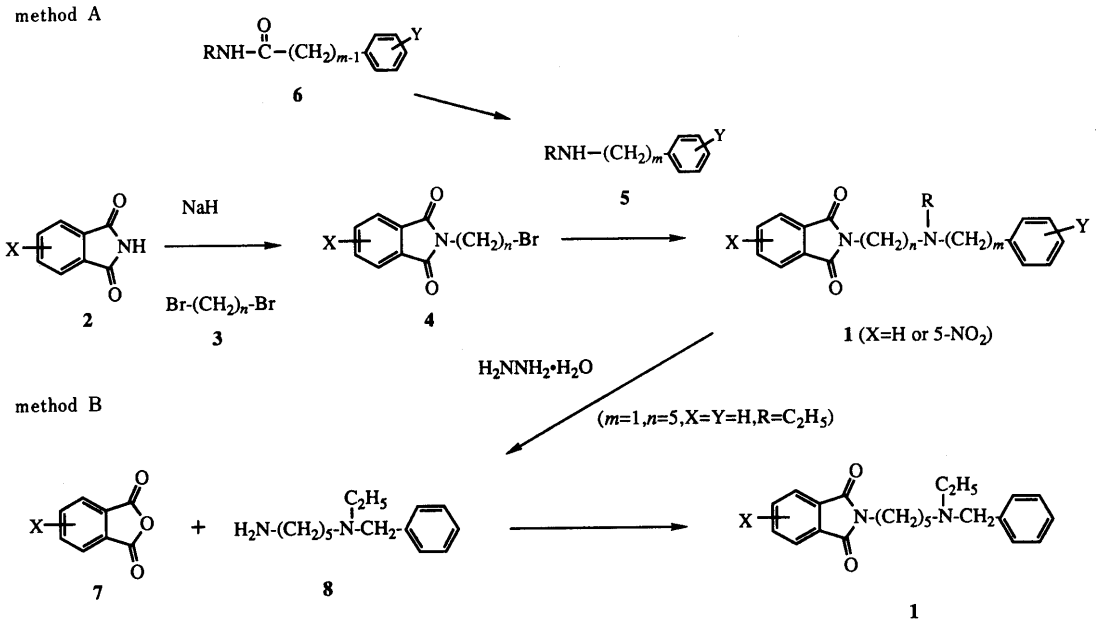


Chart 3

potent AChE inhibtors. For the following two reasons, 1*H*-isoindole-1,3(2*H*)-dione (phthalimide) was chosen as the moiety which could potentially interact with HBS-2. One is that the phthalimide moiety is useful for evaluating the working hypothesis since compounds having different carbon chain lengths (*n* in Chart 2) can be conveniently prepared. The other is that the phthalimide moiety has two carbonyl groups which may contribute to the enzyme inhibition by hydrogen bonding with the enzyme. The importance of the carbonyl groups is described in an accompanying paper[12] in this issue. The choice of an *N*-alkyl-*N*-(ω-phenylalkyl)amino moiety, especially the *N*-alkyl-*N*-benzylamino group, was based on its similarity to miotine which is known as a potent AChE inhibitor.[10] In this paper structure–activity relationships of the phthalimide derivatives (**1**) will be discussed in detail, demonstrating the effectiveness of the working hypothesis in the design of new AChE inhibitors.

Chemistry Synthesis of 2-[ω-[*N*-alkyl-*N*-(ω-phenylalkyl)amino]alkyl]-1*H*-isoindole-1,3(2*H*)-diones (**1**) was accomplished largely by methods A or B as described in the literature (Chart 3).[13] In method A, 1*H*-isoindole-1,3(2*H*)-dione (**2**) was first treated with sodium hydride, then allowed to react with 2 eq of 1,ω-dibromoalkanes (**3**) to afford 2-(ω-bromoalkyl)-1*H*-isoindole-1,3(2*H*)-diones (**4**). The desired compounds (**1**) were obtained by condensation of these bromides (**4**) with various *N*-(ω-phenylalkyl)-amines (**5**), which were prepared by $LiAlH_4$ reduction of the amides (**6**). The preparation of **1**, which has various substituents on the phthalimide moiety, was carried out chiefly by method B. Treatment of **1v** with hydrazine hydrate gave *N*-ethyl-*N*-(phenylmethyl)pentane-1,5-diamine (**8**), which led to **1** by reaction with 3- or 4-substituted phthalic anhydrides (**7**).

A variety of compounds were prepared by the usual transformations of substituents of compounds (**1**) obtained according to the procedure described above. Treatment of **1z** (X=5-OH) with sodium hydride, followed by reaction with methyl iodide or methanesulfonyl chloride yielded **1aa** (X=5-OMe) and **1bb** (X=5-OSO_2Me), respectively. Hydrogenation of a nitro group of **1j** over 10%Pd/C gave the amine (**1cc**), which was acylated to afford the acylamide (**1dd**). Sulfonamides (**1ee** and **1ff**) were obtained by reaction of **1cc** (X=5-NH_2) with sulfonyl chlorides. The acid (**1yy**: $m=1$, $n=5$, R=Et, X=5-COOH, Y=H) was converted into the esters (**1hh** and **1ii**) by treatment with a catalytic amount of concentrated HCl in the corresponding alcohol. Successive treatment of the acid (**1yy**) with thionyl chloride and amine gave the amides (**1jj** and **1kk**). The carbamate (**1uu**) was prepared by reaction of the phenol (**1zz**: $m=1$, $n=5$, R=Et, X=5-NO_2, Y=3-OH) with methyl isocyanate in the presence of triethylamine.

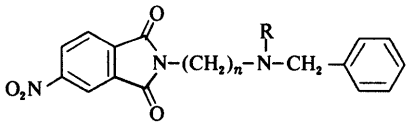
The *cis*-3a,4,5,6,7,7a-hexahydroisoindole-1,3(2*H*)-dione derivative (**9**) was prepared by condensation of *cis*-cyclohexane-1,2-dicarboxylic anhydride and the pentane-1,5-diamine (**8**). The bromide (**4c**) was allowed to react with *N*-(cyclohexylmethyl)-*N*-ethylamine (**10**) to give *N*-cyclohexylmethyl derivative (**11**). An *N*-acetyl derivative (**12**) was prepared by reaction of **1u** with acetic anhydride.

Biological Results and Discussion

The measurement of AChE inhibitory activity was carried out radiometrically *via* the method of Kleinberger and Yanai,[14] which is a slight modification of the method of Johnson and Russell.[15] The results were expressed as IC_{50} values (concentration required to inhibit control enzyme activity by 50%). The inhibitory activities of physostigmine and THA have been measured using the same technique: IC_{50}=220 nM (physostigmine), 300 nM (THA).

The effects on AChE inhibition of variation of the chain length (*n*) as well as the *N*-alkyl substituents (R) were examined, and the results are shown in Table I. Among the compounds having an *N*-Me group (**1a**—**g**), optimum activity was found to be associated with a chain length of 6 carbon atoms (**1d**). Figure 1 illustrates the relationship between enzyme inhibition and the chain length (*n*).

TABLE I. Physicochemical and Biological Properties of 2-[ω-[*N*-Alkyl-*N*-(phenylmethyl)amino]alkyl]-1*H*-isoindole-1,3(2*H*)-diones (**1a—u**)



1a—u

Compd. No.	*n*	R	Yield (%)	mp (°C)	Formula	Analysis (%) Calcd C	Calcd H	Calcd N	Found C	Found H	Found N	Inhibition of AChE (IC_{50}, nM)
1a	3	Me	64	156—159	$C_{19}H_{19}N_3O_4 \cdot HCl$	58.54	5.17	10.78	58.28	4.92	10.93	93600
1b	4	Me	61	188—192	$C_{20}H_{21}N_3O_4 \cdot HCl$	59.48	5.49	10.40	59.23	5.38	10.53	8320
1c	5	Me	65	199—202	$C_{21}H_{23}N_3O_4 \cdot HCl$	60.36	5.79	10.06	60.17	5.55	10.26	1360
1d	6	Me	69	161—163	$C_{22}H_{25}N_3O_4 \cdot HCl$	61.18	6.07	9.73	61.08	6.10	9.86	474
1e	7	Me	71	97—99	$C_{23}H_{27}N_3O_4 \cdot HCl$	61.95	6.33	9.42	61.80	6.48	9.33	1780
1f	8	Me	71	84—87	$C_{24}H_{29}N_3O_4 \cdot HCl$	62.67	6.57	9.14	62.51	6.69	9.01	8250
1g	9	Me	68	88—93	$C_{25}H_{31}N_3O_4 \cdot HCl$	63.35	6.80	8.87	63.29	6.72	8.84	27900
1h	3	Et	78	143—145	$C_{20}H_{21}N_3O_4 \cdot HCl$	59.48	5.49	10.40	59.39	5.31	10.20	12300
1i	4	Et	64	147—150	$C_{21}H_{23}N_3O_4 \cdot HCl$	60.36	5.79	10.06	60.09	5.56	10.35	252
1j	5	Et	67	126—129	$C_{22}H_{25}N_3O_4 \cdot HCl$	61.18	6.07	9.73	60.93	6.11	9.78	151
1k	6	Et	72	134—137	$C_{23}H_{27}N_3O_4 \cdot HCl$	61.95	6.33	9.42	61.88	6.09	9.28	607
1l	7	Et	70	86—89	$C_{24}H_{29}N_3O_4 \cdot HCl$	62.67	6.57	9.14	62.59	6.53	9.11	4530
1m	3	iso-Pr	26	199—202	$C_{21}H_{23}N_3O_4 \cdot HCl$	60.36	5.79	10.06	60.21	5.83	10.01	56200
1n	4	iso-Pr	57	154—157	$C_{22}H_{25}N_3O_4 \cdot HCl$	61.18	6.07	9.73	60.98	6.03	9.82	495
1o	5	iso-Pr	67	185—188	$C_{23}H_{27}N_3O_4 \cdot HCl$	61.95	6.33	9.42	61.88	6.30	9.37	824
1p	3	Pr	43	204—206	$C_{21}H_{23}N_3O_4 \cdot HCl$	60.36	5.79	10.06	60.08	5.89	9.93	44000
1q	4	Pr	53	Amorphous	$C_{22}H_{25}N_3O_4 \cdot HCl$	61.18	6.07	9.73	61.10	5.99	9.69	1870
1r	5	Pr	57	Amorphous	$C_{23}H_{27}N_3O_4 \cdot HCl$	61.95	6.33	9.42	61.69	6.17	9.32	2650
1s[a]	4	Bu	69	Amorphous	$C_{23}H_{27}N_3O_4 \cdot HCl$	61.95	6.33	9.42	61.32	6.27	9.17	19600
1t	5	Bu	61	128—130	$C_{24}H_{29}N_3O_4 \cdot HCl$	62.67	6.57	9.14	62.54	6.72	9.04	13300
1u	4	H	55	244—246	$C_{19}H_{19}N_3O_4 \cdot HCl$	58.54	5.17	10.78	58.26	4.88	10.60	13800

a) MS *m/z*: 409 [M^+].

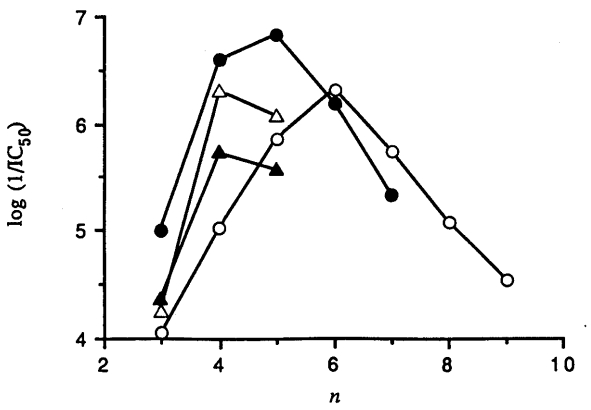


Fig. 1. Relationship between AChE Inhibition and Carbon Chain Length (*n*) of **1** with Various *N*-Alkyl Substituents (R) R=Me (○); Et (●); iso-Pr (△); Pr (▲)

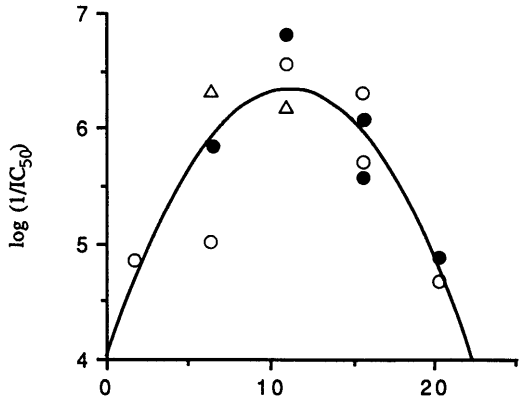


Fig. 2. Relationship between AChE Inhibition and MR Values of *N*-Alkyl Substituents (R) of **1** with Carbon Chain Lengths of 4 (○), 5 (●), and 6 (△)

The potency gradually decreased either by extending or reducing the chain length. A similar tendency was observed in the compounds having ethyl, propyl, or isopropyl groups on the benzylamino nitrogen. The number of carbon atoms (*n*) with optimum inhibitory activities were 5 (**1j**: R=Et) and 4 (**1q**: R=Pr or **1n**: R=iso-Pr). Interestingly, all these compounds have the same total of carbon atoms ($n+R=7$). This may suggest that a suitable size (and/or hydrophobicity) of molecule is required in order to access the catalytic surface of the enzyme.

The relationship between the potency of AChE inhibition and the size of *N*-alkyl substituents (R) is illustrated in Fig. 2. Molar refractivity (MR) of the substituent is used as a steric parameter. Quantitative analysis gave good correlation as shown in Eq. 1. The activity was related parabolically to the MR values. The optimum value was calculated to be 10.95, which corresponds to that of an ethyl group. Using a hydrophobic parameter (π), good correlation was also obtained (Eq. 2). Once again the activity was related parabolically to π with optimum value of 1.003. In each equation, *n*, *r*, *s*, and *F* represent the number of compounds used, correlation coefficient, standard deviation, and value in the *F* test, respectively. The number in parentheses is the 95%

confidence interval. The compounds used and their parameters are listed in Table II. Because of the known high collinearity between π and MR ($r=0.99$)[16] for the substituents (H, Me, Et, iso-Pr, Pr, and Bu), it is very hard to separate the hydrophobic and steric parameters definitely. Our data is not sufficient to determine which factor contributes more to the activity.[17]

$$\log(1/IC_{50}) = 0.416MR - 0.019MR^2 + 4.044 \quad (1)$$
$$(0.189) \quad (0.008) \quad (1.007)$$
$$(n=13,\ r=0.860,\ s=0.394,\ F_{10}^2=14.18)$$

$$\log(1/IC_{50}) = 3.205\pi - 1.597\pi^2 + 4.722 \quad (2)$$
$$(1.438) \quad (0.650) \quad (0.716)$$
$$(n=13,\ r=0.870,\ s=0.380,\ F_{10}^2=15.56)$$

TABLE II. Acetylcholinesterase Inhibitory Activity and Physicochemical Parameters[a] of 2-[ω-[*N*-Alkyl-*N*-(phenylmethyl)amino]alkyl]-5-nitro-1*H*-isoindole-1,3(2*H*)-diones (**1**)

Compd. No.	n	R	MR	π	log(1/IC$_{50}$) Obsd.	Eq. 1 Calcd (Δ)[b]	Eq. 2 Calcd (Δ)[b]
1u	4	H	1.68	0.00	4.86	4.69 (0.17)	4.72 (0.14)
1b	4	Me	6.34	0.50	5.08	5.93 (−0.85)	5.92 (−0.84)
1c	5	Me	6.34	0.50	5.87	5.93 (−0.06)	5.92 (−0.05)
1d	6	Me	6.34	0.50	6.32	5.93 (0.39)	5.92 (0.40)
1i	4	Et	11.00	1.00	6.60	6.36 (0.24)	6.33 (0.27)
1j	5	Et	11.00	1.00	6.82	6.36 (0.46)	6.33 (0.49)
1k	6	Et	11.00	1.00	6.22	6.36 (−0.14)	6.33 (−0.11)
1n	4	iso-Pr	15.66	1.37	6.31	5.98 (0.33)	6.11 (0.20)
1o	5	iso-Pr	15.66	1.37	6.08	5.98 (0.10)	6.11 (−0.03)
1q	4	Pr	15.66	1.50	5.73	5.98 (−0.25)	5.94 (−0.21)
1r	5	Pr	15.66	1.50	5.58	5.98 (−0.40)	5.94 (−0.36)
1s	4	Bu	20.32	2.00	4.71	4.79 (−0.08)	4.74 (−0.03)
1t	5	Bu	20.32	2.00	4.88	4.79 (0.09)	4.74 (0.14)

a) Taken from the literature.[16] *b*) Δ, the difference between observed and calculated values.

Among compounds (**1a—u**), **1j** ($n=5$, R=Et) showed the most potent activity, which was greater than that shown by either physostigmine or THA.

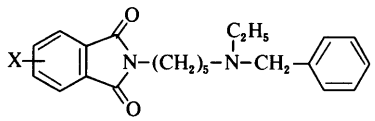
The effects of substituent (X) on the phthalimide moiety have been examined and the results are shown in Table III. A nitro group at the 5-position on the phthalimide moiety can be seen to enhance inhibitory activity (**1j** *vs.* **1v**). The 4-nitro derivative (**1w**) was less active than **1j**. The effects of substitution at the 5-position on the phthalimide moiety were quantitatively analyzed by the Hansch–Fujita method. Good correlation was obtained by regression analysis as shown in Eq. 3. The compounds used and their parameters are listed in Table IV. Correlations between the parameters used are insignificant, as shown in Table V.

$$\log(1/IC_{50}) = -0.279\pi + 0.926((\sigma_m+\sigma_p)/2) + 5.873 \quad (3)$$
$$(0.150) \quad (0.418) \quad (0.141)$$
$$(n=16,\ r=0.844,\ s=0.209,\ F_{13}^2=16.11)$$

Initial studies using either σ_m or σ_p gave similar results (Eqs. 4 and 5). This indicates that the two carbonyls of the phthalimide moiety make almost equal contributions to AChE inhibition. Therefore, since substituents located meta to one carbonyl are also para to the other carbonyl of the phthalimide moiety, an average value of the electronic parameters, $(\sigma_m+\sigma_p)/2$, is used in Eq. 3. According to Eq. 3, hydrophilic and electron-withdrawing substituents enhance AChE inhibitory activity. In this study, satisfactory correlation was not obtained using steric parameters such as MR and Es.

$$\log(1/IC_{50}) = -0.244\pi + 1.240\sigma_m + 5.750 \quad (4)$$
$$(0.144) \quad (0.538) \quad (0.173)$$
$$(n=16,\ r=0.852,\ s=0.204,\ F_{13}^2=17.26)$$

TABLE III. Physicochemical and Biological Properties of 2-[5-[*N*-Ethyl-*N*-(phenylmethyl)amino]pentyl]-1*H*-isoindole-1,3(2*H*)-diones (**1v—kk**)



1v—kk

Compd. No.	X	Yield (%)	mp (°C)	Formula	Analysis (%) Calcd C	Calcd H	Calcd N	Found C	Found H	Found N	Inhibition of AChE (IC$_{50}$, nM)
1v	H	72	92—94	$C_{22}H_{26}N_2O_2\cdot HCl$	68.29	7.03	7.24	68.11	6.91	7.31	3370
1w[a]	4-NO_2	67	Amorphous	$C_{22}H_{25}N_3O_4\cdot HCl$	61.18	6.07	9.73	60.72	6.11	9.66	637
1x	5-Me	71	Amorphous	$C_{23}H_{28}N_2O_2\cdot HCl$	68.90	7.29	6.99	68.81	7.25	6.91	1750
1y	5-Cl	69	Amorphous	$C_{22}H_{25}ClN_2O_2\cdot HCl$	62.71	6.22	6.65	62.64	6.09	6.48	1330
1z[b]	5-OH	70	Amorphous	$C_{22}H_{26}N_2O_3\cdot HCl$	65.58	6.75	6.95	65.02	6.79	6.94	2450
1aa[c]	5-OMe	77	Amorphous	$C_{23}H_{28}N_2O_3\cdot HCl$	66.26	7.01	6.72	65.71	6.93	6.53	701
1bb	5-OSO_2Me	63	Amorphous	$C_{23}H_{28}N_2O_5S\cdot HCl$	57.43	6.08	5.82	57.33	6.01	5.75	379
1cc[d]	5-NH_2	76	Amorphous	$C_{22}H_{27}N_3O_2\cdot 2HCl$	60.28	6.67	9.59	59.82	6.79	9.43	1070
1dd	5-NHAc	81	120—121	$C_{24}H_{29}N_3O_3$	70.74	7.17	10.31	70.68	7.01	10.19	328
1ee	5-$NHSO_2Me$	63	94—96	$C_{23}H_{29}N_3O_4S$	62.28	6.59	9.47	62.03	6.44	9.41	467
1ff	5-$NHSO_2$-  -Me	68	109—112	$C_{29}H_{33}N_3O_4S$	67.03	6.40	8.09	66.93	6.33	7.92	2190
1gg	5-COPh	69	Amorphous	$C_{29}H_{30}N_2O_3\cdot HCl$	70.94	6.36	5.71	70.91	6.33	5.64	1070
1hh	5-CO_2Me	89	Amorphous	$C_{24}H_{28}N_2O_4\cdot HCl$	64.79	6.57	6.30	64.67	6.56	6.25	569
1ii	5-CO_2Et	49	Amorphous	$C_{25}H_{30}N_2O_4\cdot HCl$	65.42	6.81	6.10	65.31	6.63	6.03	679
1jj	5-CONHMe	48	93—95	$C_{24}H_{29}N_3O_3$	70.74	7.17	10.31	70.59	7.02	10.22	380
1kk[e]	5-$CONEt_2$	61	Amorphous	$C_{27}H_{35}N_3O_3\cdot HCl$	66.72	7.47	8.65	66.64	7.41	8.57	980

a) MS *m/z*: 395 [M$^+$]. *b*) MS *m/z*: 366 [M$^+$]. *c*) MS *m/z*:380 [M$^+$]. *d*) MS *m/z*: 365 [M$^+$]. *e*) MS *m/z*: 449 [M$^+$].

$$\log(1/IC_{50}) = -0.294\pi + 0.696\sigma_p + 5.953 \quad (5)$$
$$(0.162)\quad (0.347)\quad (0.132)$$
$$(n=16,\ r=0.822,\ s=0.222,\ F_{13}^2=13.53)$$

The effects of substitution on the phenyl ring of the benzylamino moiety are shown in Table VI. In general, inhibitory activity decreased with the position of substituent in the order *ortho*>*meta*>*para*. Among the substituents examined, a methoxy group at either *meta* or *ortho* position (**1pp** and **1qq**) increased the activity, whereas 2,3-dimethoxy substitution (**1tt**) resulted in a reduction of inhibitory potency. Incorporation of a $MeNHCO_2$-group at the *meta* position (**1uu**), which is expected to contribute to the activity by carbamoylating the esteratic site, resulted in a sharp enhancement of inhibitory potency. The IC_{50} value has been measured as about 1000 times stronger than that of physostigmine and THA.

Table VI also shows the effects of chain length (*m*) of the *N*-(ω-phenylalkyl)amino moiety. Among the compounds examined (**1j** and **1vv**—**xx**), **1j** (*m*=1) was the most potent inhibitor. Extension of the chain length caused a gradual decrease in the activity. Surprisingly, **1ww** was as potent as **1j**. The reason is not clear, but one explanation could be that, by bending the propyl chain, the phenyl ring and the nitrogen of the (3-phenylpropyl)amino moiety could be

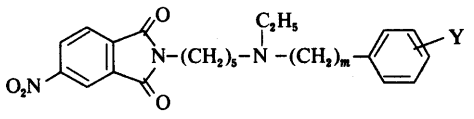
TABLE IV. Acetylcholinesterase Inhibitory Activity and Physicochemical Parameters[a] of 5-Substituted-2-[5-[*N*-ethyl-*N*-(phenylmethyl)amino]pentyl]-1*H*-isoindole-1,3(2*H*)-diones (**1**)

Compd. No.	X	π	$(\sigma_m+\sigma_p)/2$	log(1/IC50) Obsd.	Calcd[b]	(Δ)[c]
1v	H	0.00	0.00	5.47	5.87	(−0.40)
1j	NO_2	−0.28	0.745	6.82	6.64	(0.18)
1x	Me	0.56	−0.12	5.76	5.61	(0.15)
1y	Cl	0.71	0.30	5.88	5.95	(−0.07)
1z	OH	−0.67	−0.125	5.61	5.94	(−0.33)
1aa	OMe	−0.02	−0.075	6.15	5.81	(0.34)
1bb	OSO_2Me	−0.88	0.375	6.42	6.47	(−0.05)
1cc	NH_2	−1.23	−0.41	5.97	5.84	(0.13)
1dd	NHAc	−0.97	0.105	6.48	6.24	(0.24)
1ee	$NHSO_2Me$	−1.18	0.115	6.33	6.31	(0.02)
1ff	$NHSO_2$-C6H4-Me	0.95[d]	0.085[d]	5.66	5.69	(−0.03)
1gg	COPh	1.05	0.385	5.97	5.94	(0.03)
1hh	CO_2Me	−0.01	0.41	6.24	6.26	(−0.02)
1ii	CO_2Et	0.51	0.41	6.17	6.11	(0.06)
1jj	CONHMe	−1.27	0.355	6.42	6.56	(−0.14)
1kk	$CONEt_2$	0.23[d]	0.355[d]	6.01	6.14	(−0.13)

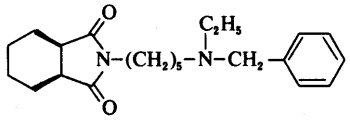
a) Taken from the literature.[20] *b*) Calculated from Eq. 3. *c*) Δ, the difference between observed and calculated values. *d*) Estimated from data for closely related substituents.

TABLE V. Simple Correlation Matrix for the Parameters of Eq. 3

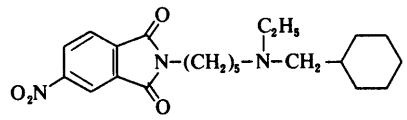
	π	$(\sigma_m+\sigma_p)/2$
π	1.000	
$(\sigma_m+\sigma_p)/2$	0.214	1.000



1ll—**xx**



9



11

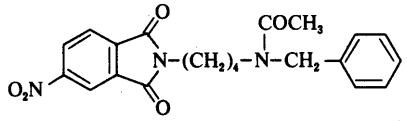


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TABLE VI. Physicochemical and Biological Properties of 2-[5-[*N*-Ethyl-*N*-(ω-phenylalkyl)amino]pentyl]-1*H*-isoindole-1,3(2*H*)-diones (**1ll**—**xx**) and Their Analogues (**9**, **11**, **12**)

Compd. No.	*m*	Y	Yield (%)	mp (°C)	Formula	Analysis (%) Calcd C	Calcd H	Calcd N	Found C	Found H	Found N	Inhibition of AChE (IC_{50}, nM)
1ll	1	4-Cl	55	138—142	$C_{22}H_{24}ClN_3O_4\cdot HCl$	56.66	5.40	9.01	56.59	5.32	8.97	240
1mm	1	3-Cl	55	148—152	$C_{22}H_{24}ClN_3O_4\cdot HCl$	56.66	5.40	9.01	56.61	5.21	8.88	244
1nn	1	2-Cl	57	Amorphous	$C_{22}H_{24}ClN_3O_4\cdot HCl$	56.66	5.40	9.01	56.45	5.36	8.92	129
1oo	1	4-OMe	60	Amorphous	$C_{23}H_{27}N_3O_5\cdot HCl$	59.80	6.11	9.10	59.68	6.01	9.06	269
1pp[a]	1	3-OMe	61	113—114	$C_{23}H_{27}N_3O_5\cdot HCl$	59.80	6.11	9.10	58.39	6.09	8.97	44.8
1qq	1	2-OMe	58	84—87	$C_{23}H_{27}N_3O_5\cdot HCl$	59.80	6.11	9.10	59.77	6.05	9.02	23.9
1rr[b]	1	2-F	57	Amorphous	$C_{22}H_{24}FN_3O_4\cdot HCl$	58.73	5.60	9.34	58.34	5.63	9.21	282
1ss[c]	1	2-Me	60	Amorphous	$C_{23}H_{27}N_3O_4\cdot HCl$	61.95	6.33	9.42	61.49	6.31	9.36	98.8
1tt	1	2,3-diOMe	57	Amorphous	$C_{24}H_{29}N_3O_6\cdot HCl$	58.59	6.15	8.54	58.41	6.02	8.44	274
1uu	1	3-OCONHMe	49	Amorphous	$C_{24}H_{28}N_4O_6\cdot HCl$	57.09	5.79	11.10	56.91	5.77	11.07	0.380
1vv	2	H	48	152—154	$C_{23}H_{27}N_3O_4\cdot HCl$	61.95	6.33	9.42	61.92	6.10	9.29	770
1ww	3	H	48	156—158	$C_{24}H_{29}N_3O_4\cdot HCl$	62.67	6.57	9.14	62.39	6.54	9.01	161
1xx	4	H	62	123—124	$C_{25}H_{31}N_3O_4\cdot HCl$	63.35	6.80	8.87	63.32	6.78	8.61	1750
9	—	—	46	135—137	$C_{22}H_{32}N_2O_2\cdot HCl$	67.24	8.46	7.13	67.11	8.52	6.98	18500
11[d]	—	—	43	Amorphous	$C_{22}H_{31}N_3O_4\cdot HCl$	60.33	7.36	9.59	60.09	7.43	9.56	1090
12[e]	—	—	95	Oil	$C_{21}H_{21}N_3O_5$	—	—	—	—	—	—	>100000

a) MS *m/z*: 425 [M^+]. *b*) MS *m/z*: 413 [M^+]. *c*) MS *m/z*: 409 [M^+]. *d*) MS *m/z*: 401 [M^+]. *e*) The structure was confirmed by IR, NMR and MS spectra (see Experimental).

placed in a similar position to the position they occupy in the benzylamino moiety.

Saturation of the benzene ring of the phthalimide or the phenyl ring of the benzylamino moiety decreased inhibitory activity 5- (**9** *vs.* **1v**) and 7-fold (**11** *vs.* **1j**), respectively. Replacement of the *N*-ethyl group of **1i** with an *N*-acetyl (**12**) reduced the potency remarkably.

The results obtained in this work can be summarized as follows.

1. Optimum activity was closely associated with a five carbon chain length ($n=5$) separating the phthalimide moiety from the nitrogen atom of the benzylamino moiety. 2. The most potent activity was observed when the *N*-alkyl substituent (R) was an ethyl group. 3. The compounds (**1d, j, n, q**) showed optimum activities of the *N*-Me, Et, iso-Pr, and Pr derivatives, each having the same total of carbon atoms ($n+R=7$). 4. Saturation of the benzene ring of the phthalimide moiety caused an approximately 5-fold decrease in activity. 5. Quantitative study of the substituents (X) on the phthalimide moiety revealed that hydrophilic and electron-withdrawing groups enhance the potency. 6. Reduction of the basicity of the benzylamino nitrogen significantly decreased the activity. 7. As an *N*-(ω-phenylalkyl)amino moiety, the benzylamino group was best for potent inhibition. 8. Methoxy substitution on the phenyl ring of the benzylamino moiety increased the activity. 9. Incorporation of a 3-$MeNHCO_2$-group on the phenyl ring of the benzylamino moiety greatly enhanced the inhibitory potency. 10. Saturation of the phenyl ring of the benzylamino moiety led to an approximately 7-fold loss in enzyme inhibition.

The above results seem to support the working hypothesis which has been the basis of this work. This hypothesis includes the following two points. The first, which may be supported by results 6, 9, and 10, is that the benzylamino moiety of **1** binds to the anionic site as well as to the hydrophobic binding site (HBS-1) which is closely adjacent to the anionic and esteratic sites. These interactions correspond to those postulated for binding in the case of miotine.[10] Results 1 and 4 may support the second point, which is that the phthalimide moiety interacts with another hydrophobic binding site (HBS-2) some distance removed from the anionic site. The distance can be estimated at a seven carbon chain length or less.[18] Hydrophobic interactions with both HBS-1 and HBS-2 seem to be mainly π–π interactions.[19] In addition, result 5 may provide us with some information about the nature of HBS-2. The enzyme may demand particular hydrophobic requirements with regard to the substituents (X). It is not clear how the electron-withdrawing character of the substituents (X) contributes to an enhancement of activity. One explanation may be that, after withdrawing the electron, the substituents interact with the enzyme electrostatically.

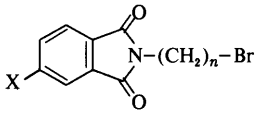
In conclusion, the working hypothesis proposed in this paper led to the discovery of several new potent AChE inhibitors, demonstrating its effectiveness in the design of such inhibitors. Detailed studies of structure–activity relationships may confirm the existence of a hydrophobic binding site (HBS-2) along with its location relative to the anionic site. Further studies concerning the nature of HBS-2 are described in an accompanying paper[12] in this issue.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were taken on a Hitachi 260-10 spectrophotometer using KBr disks for solids and liquid films for oils. Mass spectra (MS) were measured on a JOEL JMS-01SC spectrometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Varian EM-390 NMR spectrometer with tetramethylsilane as an internal standard. The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad. Chromatographic purifications were carried out on silica gel columns (Kieselgel 60, 0.063—0.200 mm, Merck).

Preparation of 2-[ω-[*N*-Alkyl-*N*-(ω-phenylalkyl)amino]alkyl]-1*H*-isoindole-1,3(2*H*)-diones (1). Method A. 2-(5-Bromopentyl)-5-nitro-1*H*-isoindole-1,3(2*H*)-dione (4c) Sodium hydride (1.38 g, oil free) was added portionwise to a solution of 5-nitro-1*H*-isoindole-1,3(2*H*)-dione (10.5 g) in *N*,*N*-dimethylformamide (DMF, 50 ml). The mixture was stirred at 60 °C for 30 min, cooled to 0 °C, and a solution of 1,5-dibromopentane (25.0 g) in acetone (50 ml) was added. The mixture was refluxed for 16 h, cooled to room temperature, and the resulting precipitate was removed by filtration. The filtrate was concentrated to give colorless crystals, which were recrystallized from methanol–ether to afford colorless cubes (17.3 g). IR (KBr): 3466, 3110, 3056, 2942, 2868, 1772, 1711, 1624, 1543 cm^{-1}.

TABLE VII. Physicochemical Properties of 2-(ω-Bromoalkyl)-1*H*-isoindole-1,3(2*H*)-diones (**4a—h**)



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Compd. No.	X	*n*	Yield (%)	mp (°C)	Formula	Analysis (%) Calcd C	Calcd H	Calcd N	Found C	Found H	Found N
4a	NO_2	3	94	103—104	$C_{11}H_9BrN_2O_4$	42.20	2.90	8.95	42.05	2.79	8.81
4b	NO_2	4	90	95—96	$C_{12}H_{11}BrN_2O_4$	44.06	3.39	8.56	44.01	3.20	8.42
4c	NO_2	5	93	78—79	$C_{13}H_{13}BrN_2O_4$	45.77	3.84	8.21	45.58	3.78	7.99
4d	NO_2	6	92	83—85	$C_{14}H_{15}BrN_2O_4$	47.34	4.26	7.89	47.09	4.22	7.63
4e	NO_2	7	94	73—74	$C_{15}H_{17}BrN_2O_4$	48.80	4.64	7.59	48.96	4.60	7.76
4f	NO_2	8	90	84—85	$C_{16}H_{19}BrN_2O_4$	50.14	5.00	7.31	50.08	4.93	7.31
4g	NO_2	9	94	81—83	$C_{17}H_{21}BrN_2O_4$	51.40	5.33	7.05	51.66	5.22	7.10
4h	H	5	79	60—61[a]	$C_{13}H_{14}BrNO_2$	52.72	4.76	4.73	52.84	4.68	4.89

a) Lit.[21] mp 58—60 °C.

TABLE VIII. Spectral Data of **1a—i, k—t, v, x—z, bb, ee—gg, ii, kk—tt, vv—xx, 4a, b, d—h, 9,** and **11**

Compd.	IR (KBr) cm^{-1}	^{1}H-NMR (DMSO-d_6) δ[a]
1a	3444, 1778, 1716, 1624, 1539	1.89—2.33 (2H, m), 2.58 (3H, br s), 2.88—3.29 (2H, m), 3.67 (2H, t, J=6 Hz), 4.00—4.50 (2H, m), 7.23—7.75 (5H, m), 8.15 (1H, d, J=9 Hz), 8.51 (1H, d, J=1.5 Hz), 8.67 (1H, dd, J=1.5, 9 Hz), 11.4 (1H, br s)
1b	3436, 1775, 1717, 1543	1.37—2.03 (4H, m), 2.58 (3H, d, J=5 Hz), 2.80—3.77 (4H, m), 3.98—4.37 (2H, m), 7.30—7.73 (5H, m), 8.12 (1H, d, J=9 Hz), 8.46 (1H, d, J=1.5 Hz), 8.62 (1H, dd, J=1.5, 9 Hz), 11.4 (1H, br s)
1c	3446, 1773, 1716, 1625, 1544	1.05—2.00 (6H, m), 2.59 (3H, br s), 2.78—3.76 (4H, m), 4.03—4.47 (2H, m), 7.30—7.76 (5H, m), 8.12 (1H, d, J=9 Hz), 8.48 (1H, d, J=1.5 Hz), 8.63 (1H, dd, J=1.5, 9 Hz), 11.45 (1H, br s)
1d	3436, 1774, 1717, 1624, 1543	1.15—1.93 (8H, m), 2.58 (3H, d, J=5 Hz), 2.75—3.13 (2H, m), 3.59 (2H, t, J=6 Hz), 4.05—4.45 (2H, m), 7.32—7.75 (5H, m), 8.13 (1H, d, J=8 Hz), 8.48 (1H, d, J=1.5 Hz), 8.64 (1H, dd, J=1.5, 8 Hz), 11.4 (1H, br s)
1e	3430, 2938, 1777, 1717, 1624, 1541	1.00—1.97 (10H, m), 2.59 (3H, d, J=5 Hz), 2.75—3.13 (2H, m), 3.60 (2H, t, J=6.5 Hz), 4.00—4.50 (2H, m), 7.26—7.80 (5H, m), 8.13 (1H, d, J=8 Hz), 8.48 (1H, d, J=1.5 Hz), 8.66 (1H, dd, J=1.5, 8 Hz), 11.45 (1H, br s)
1f	3436, 2934, 1777, 1718, 1624, 1540	1.03—1.97 (12H, m), 2.63 (3H, d, J=5 Hz), 2.75—3.13 (2H, m), 3.60 (2H, t, J=6.5 Hz), 4.00—4.50 (2H, m), 7.30—7.77 (5H, m), 8.14 (1H, d, J=8 Hz), 8.49 (1H, d, J=1.5 Hz), 8.66 (1H, dd, J=1.5, 8 Hz), 11.43 (1H, br s)
1g	3430, 2932, 1776, 1716, 1624, 1541	1.03—2.00 (14H, m), 2.62 (3H, d, J=5 Hz), 2.73—3.12 (2H, m), 3.60 (2H, t J=6.5 Hz), 4.05—4.50 (2H, m), 7.27—7.77 (5H, m), 8.11 (1H, d, J=8 Hz), 8.46 (1H, d, J=1.5 Hz), 8.63 (1H, dd, J=1.5, 8 Hz), 11.26 (1H, br s)
1h	3436, 2948, 1782, 1716, 1625, 1540	1.26 (3H, t, J=6 Hz), 1.85—2.30 (2H, m), 2.70—3.20 (4H, m), 3.64 (2H, t, J=6 Hz), 4.26 (2H, d, J=5 Hz), 7.22—7.74 (5H, m), 8.13 (1H, d, J=8 Hz), 8.47 (1H, d, J=1.5 Hz), 8.66 (1H, dd, J=1.5, 8 Hz), 11.43 (1H, br s)
1i	3432, 1778, 1714, 1624, 1540	1.26 (3H, t, J=7.5 Hz), 1.43—2.00 (4H, m), 2.70—3.20 (4H, m), 3.50—3.85 (2H, m), 4.25 (2H, d, J=5 Hz), 7.30—7.80 (5H, m), 8.12 (1H, d, J=9 Hz), 8.47 (1H, d, J=1.5 Hz), 8.63 (1H, dd, J=1.5, 9 Hz), 9.55 (1H, br s)
1k	3432, 2946, 1775, 1715, 1623, 1543	1.10—1.93 (11H, m), 2.66–3.20 (4H, m), 3.60 (2H, t, J=6.5 Hz), 4.26 (2H, d, J=5 Hz), 7.26—7.83 (5H, m), 8.14 (1H, d, J=8 Hz), 8.49 (1H, d, J=1.5 Hz), 8.66 (1H, dd, J=1.5, 8 Hz), 11.37 (1H, br s)
1l	3386, 2938, 1778, 1721, 1623, 1541	1.10—1.95 (13H, m), 2.70—3.26 (4H, m), 3.60 (2H, t, J=6 Hz), 4.30 (2H, d, J=6 Hz), 7.30—7.80 (5H, m), 8.12 (1H, d, J=8 Hz), 8.46 (1H, d, J=1.5 Hz), 8.63 (1H, dd, J=1.5, 8 Hz), 10.87 (1H, br s)
1m[b]	2966, 2822, 1778, 1718, 1620, 1542[c]	1.03 (6H, d, J=6.5 Hz), 1.70—1.87 (2H, m), 2.50 (2H, t, J=6.5 Hz), 2.91—3.06 (1H, m), 3.54 (2H, s), 3.73 (2H, t, J=7.5 Hz), 7.12—7.41 (5H, m), 8.01 (1H, d, J=8 Hz), 8.58 (1H, dd, J=2, 8 Hz), 8.63 (1H, d, J=2 Hz)[d]
1n	3444, 1782, 1709, 1621, 1544	1.32 (6H, d, J=6 Hz), 1.45—1.93 (4H, m), 2.78—3.77 (4H, m), 3.98—4.40 (3H, m), 7.26—7.85 (5H, m), 8.12 (1H, d, J=8 Hz), 8.47 (1H, d, J=1.5 Hz), 8.65 (1H, dd, J=1.5, 8 Hz), 10.83 (1H, br s)
1o	3436, 1777, 1718, 1623, 1541	1.15—1.93 (12H, m), 2.67—3.10 (2H, m), 3.57 (2H, t, J=6 Hz), 3.95—4.46 (3H, m), 7.30—7.87 (5H, m), 8.11 (1H, d, J=8 Hz), 8.46 (1H, d, J=1.5 Hz), 8.63 (1H, dd, J=1.5, 8 Hz), 11.4 (1H, br s)
1p[b]	2958, 2802, 1779, 1719, 1623, 1540[c]	0.88 (3H, t, J=7 Hz), 1.42—1.57 (2H, m), 1.77—1.93 (2H, m), 2.39 (2H, t, J=7 Hz), 2.49 (2H, t, J=7 Hz), 3.54 (2H, s), 3.76 (2H, t, J=7 Hz), 7.13—7.36 (5H, m), 8.02 (1H, d, J=8 Hz), 8.59 (1H, dd, J=2, 8 Hz), 8.64 (1H, d, J=2 Hz)[d]
1q	3432, 1778, 1714, 1624, 1540	0.90 (3H, t, J=6 Hz), 1.45—2.03 (6H, m), 2.55—3.17 (4H, m), 3.63 (2H, t, J=6 Hz), 4.15—4.40 (2H, m), 7.23—7.80 (5H, m), 8.12 (1H, d, J=8 Hz), 8.48 (1H, d, J=1.5 Hz), 8.65 (1H, dd, J=1.5, 8 Hz), 9.60 (1H, br s)
1r	3436, 1778, 1718, 1623, 1540	0.87 (3H, t, J=7 Hz), 1.18—1.38 (2H, m), 1.53—1.87 (6H, m), 2.82—3.08 (4H, m), 3.63 (2H, t, J=7.5 Hz), 4.31 (2H, d, J=5 Hz), 7.40—7.63 (5H, m), 8.13 (1H, d, J=8 Hz), 8.50 (1H, d, J=2 Hz), 8.64 (1H, dd, J=2, 8 Hz), 9.95 (1H, br s)
1s	3440, 1779, 1719, 1623, 1540	0.85 (3H, t, J=6 Hz), 0.97—2.00 (8H, m), 2.64—3.18 (4H, m), 3.61 (2H, t, J=6 Hz), 4.26 (2H, br d, J=4.5 Hz), 7.28—7.80 (5H, m), 8.12 (1H, d, J=8 Hz), 8.46 (1H, d, J=1.5 Hz), 8.63 (1H, dd, J=1.5, 8 Hz), 11.2 (1H, br s)
1t	3428, 2948, 1777, 1722, 1617, 1537	0.88 (3H, t, J=7 Hz), 1.14—1.37 (4H, m), 1.53—1.85 (6H, m), 2.83—3.07 (4H, m), 3.63 (2H, t, J=7.5 Hz), 4.30 (2H, d, J=5 Hz), 7.40—7.52 (3H, m), 7.55—7.68 (2H, m), 8.13 (1H, d, J=8 Hz), 8.50 (1H, d, J=2 Hz), 8.64 (1H, dd, J=2, 8 Hz), 10.37 (1H, br s)
1v[b]	2936, 1772, 1712[c]	1.00 (3H, t, J=7 Hz), 1.12—1.84 (6H, m), 2.23—2.61 (4H, m), 3.51 (2H, s), 3.64 (2H, t, J=7 Hz), 7.27 (5H, s), 7.60—7.92 (4H, m)[d]
1x[b]	2936, 1769, 1709,[c] 1618	1.00 (3H, t, J=7.5 Hz), 1.13—1.85 (6H, m), 2.28—2.64 (4H, m), 2.49 (3H, s), 3.52 (2H, s), 3.63 (2H, t, J=6 Hz), 7.24 (5H, s), 7.45 (1H, d, J=8 Hz), 7.60 (1H, s), 7.68 (1H, d, J=8 Hz)[d]
1y	3424, 2930, 2612, 1771, 1712, 1610	1.07—2.00 (9H, m), 2.76—3.23 (4H, m), 3.57 (2H, t, J=6.5 Hz), 4.26 (2H, d, J=5 Hz), 7.32—7.97 (8H, m), 11.05 (1H, br s)
1z[b]	3450, 2942, 2626, 1764, 1709, 1605[c]	0.96—1.96 (9H, m), 2.53—3.06 (4H, m), 3.54 (2H, t, J=6 Hz), 3.94 (2H, s), 6.86—7.70 (8H, m), 9.55 (1H, s)[d]
1bb[b]	2936, 1774, 1716, 1396, 1370, 1182[c]	1.03—1.95 (9H, m), 2.47—3.00 (4H, m), 3.25 (3H, s), 3.66 (2H, t, J=7 Hz), 3.85 (2H, s), 7.10—8.03 (8H, m)[d]
1ee	3290, 1764, 1700, 1623, 1340, 1160	1.03 (3H, t, J=7 Hz), 1.10—1.85 (6H, m), 2.30—2.70 (4H, m), 3.07 (3H, s), 3.47—3.76 (4H, m), 6.13 (1H, br s), 7.25 (5H, s), 7.40—7.82 (3H, m)[d]
1ff	3438, 2934, 1759, 1700, 1610, 1300, 1125	0.75—1.70 (9H, m), 1.97 (3H, s), 2.13—2.60 (4H, m), 3.13—3.65 (4H, m), 4.25 (1H, br s), 6.57—7.63 (12H, m)[d]
1gg[b]	2938, 1774, 1717, 1666[c]	0.99 (3H, t, J=7.5 Hz), 1.14—1.87 (6H, m), 2.23—2.63 (4H, m), 3.51 (2H, s), 3.66 (2H, t, J=6 Hz), 7.93—8.22 (13H, m)[d]
1ii[b]	2938, 1775, 1721[c]	1.02 (3H, t, J=7 Hz), 1.13—1.85 (6H, m), 1.43 (3H, t, J=7.5 Hz), 2.30—2.64 (4H, m), 3.56 (2H, s), 3.66 (2H, t, J=7 Hz), 4.43 (2H, q, J=7.5 Hz), 7.28 (5H, s), 7.88 (1H, d, J=8 Hz), 8.39 (1H, d, J=8 Hz), 8.46 (1H, s)[d]
1kk[b]	2972, 2938, 1774, 1713, 1638[c]	0.90—1.87 (15H, m), 2.33—2.73 (4H, m), 3.03—3.83 (8H, m), 7.30 (5H, s), 7.61—7.94 (3H, m)[d]
1ll[b]	2938, 1778, 1720, 1541[c]	1.00 (3H, t, J=7 Hz), 1.10—1.84 (6H, m), 2.27—2.61 (4H, m), 3.47 (2H, s), 3.70 (2H, t, J=7 Hz), 7.27 (4H, m), 8.01 (1H, d, J=8 Hz), 8.58 (1H, d, J=8 Hz), 8.64 (1H, s)[d]

TABLE VIII. (continued)

Compd.	IR (KBr) cm^{-1}	^{1}H-NMR (DMSO-d_6) δ[a]
1mm[b]	2966, 2938, 1779, 1720, 1541[c]	1.00 (3H, t, J=7 Hz), 1.13—1.87 (6H, m), 2.23—2.62 (4H, m), 3.48 (2H, s), 3.71 (2H, t, J=6.5 Hz), 7.03—7.33 (4H, m), 8.00 (1H, d, J=8 Hz), 8.58 (1H, d, J=8 Hz), 8.61 (1H, s)[d]
1nn[b]	2936, 1778, 1719, 1541[c]	1.01 (3H, t, J=7 Hz), 1.13—1.90 (6H, m), 2.30—2.67 (4H, m), 3.61 (2H, s), 3.70 (2H, t, J=6 Hz), 7.03—7.57 (4H, m), 8.01 (1H, d, J=9 Hz), 8.60 (1H, d, J=9 Hz), 8.64 (1H, s)[d]
1oo	2936, 1778, 1720, 1541	1.25 (3H, t, J=7.5 Hz), 1.10—2.00 (6H, m), 2.68—3.17 (4H, m), 3.60 (2H, t, J=6 Hz), 3.75 (3H, s), 4.18 (2H, d, J=5 Hz), 6.93 (2H, d, J=9 Hz), 7.58 (2H, d, J=9 Hz), 8.10 (1H, d, J=8 Hz), 8.45 (1H, d, J=1.5 Hz), 8.62 (1H, dd, J=1.5, 8 Hz), 11.03 (1H, br s)
1pp[b]	2938, 1778, 1719, 1541[c]	1.00 (3H, t, J=7 Hz), 1.13—1.90 (6H, m), 2.25—2.63 (4H, m), 3.49 (2H, s), 3.72 (2H, t, J=7 Hz), 3.78 (3H, s), 6.62—6.96 (3H, m), 7.17 (1H, dd, J=8, 8 Hz), 8.01 (1H, d, J=8 Hz), 8.58 (1H, d, J=8 Hz), 8.63 (1H, s)[d]
1qq[b]	2940, 1778, 1717, 1539[c]	1.01 (3H, t, J=7 Hz), 1.16—1.90 (6H, m), 2.33—2.65 (4H, m), 3.54 (2H, s), 3.62 (2H, br t, J=6 Hz), 3.80 (3H, s), 6.73—7.44 (4H, m), 8.00 (1H, d, J=8 Hz), 8.58 (1H, d, J=8 Hz), 8.63 (1H, s)[d]
1rr[b]	2938, 1778, 1720, 1541[c]	1.01 (3H, t, J=7 Hz), 1.13—1.90 (6H, m), 2.30—2.64 (4H, m), 3.57 (2H, s), 3.71 (2H, t, J=7 Hz), 6.80—7.50 (4H, m), 8.01 (1H, d, J=8 Hz), 8.58 (1H, d, J=8 Hz), 8.62 (1H, s)[d]
1ss[b]	2938, 1779, 1719, 1541[c]	0.99 (3H, t, J=7 Hz), 1.13—1.87 (6H, m), 2.31 (3H, s), 2.26—2.61 (4H, m), 3.46 (2H, s), 3.67 (2H, t, J=7 Hz), 6.98—7.40 (4H, m), 7.98 (1H, d, J=8 Hz), 8.56 (1H, d, J=8 Hz), 8.60 (1H, s)[d]
1tt[b]	2936, 1778, 1719, 1541[c]	1.00 (3H, t, J=7 Hz), 1.13—1.96 (6H. m), 2.30—2.63 (4H, m), 3.54 (2H, s), 3.71 (2H, t, J=7 Hz), 3.80 (3H, s), 3.84 (3H, s), 6.67—7.07 (3H, m), 8.02 (1H, d, J=8 Hz), 8.60 (1H, d, J=8 Hz), 8.65 (1H, s)[d]
1vv[b]	2936, 1777, 1719, 1541[c]	1.03 (3H, t, J=7 Hz), 1.16—2.10 (6H, m), 2.33—2.93 (8H, m), 3.73 (2H, t, J=7 Hz), 7.24 (5H, s), 8.05 (1H, d, J=9 Hz), 8.53—8.77 (2H, m)[d]
1ww	3472, 2966, 2476, 1778, 1708, 1626, 1540	1.10—1.41 (5H, m), 1.52—1.73 (4H, m), 1.85—2.05 (2H, m), 2.64 (2H, t, J=7.5 Hz), 2.88—3.07 (6H, m), 3.63 (2H, t, J=7 Hz), 7.20—7.36 (5H, m), 8.13 (1H, d, J=8 Hz), 8.49 (1H, d, J=2 Hz), 8.64 (1H, dd, J=2, 8 Hz), 10.1 (1H, br s)
1xx[b]	2934, 1778, 1719, 1619, 1540[c]	0.98 (3H, t, J=7 Hz), 1.24—1.87 (10H, m), 2.33—2.67 (8H, m), 3.73 (2H, t, J=7.5 Hz), 7.11—7.33 (5H, m), 8.02 (1H, d, J=8 Hz), 8.59 (1H, dd, J=2, 8 Hz), 8.65 (1H, d, J=2 Hz)[d]
4a	3470, 2950, 1773, 1712, 1623, 1538	2.13—2.50 (2H, m), 3.43 (2H, t, J=6 Hz), 3.92 (2H, t, J=6 Hz), 8.08 (1H, d, J=9 Hz), 8.67 (1H, d, J=9 Hz), 8.72 (1H, s)[d]
4b	3460, 2954, 1779, 1711, 1625, 1541	1.53—2.10 (4H, m), 3.43—3.80 (4H, m), 8.12 (1H, d, J=9 Hz), 8.46 (1H, d, J=1.5 Hz), 8.63 (1H, dd, J=1.5, 9 Hz)
4d	3452, 1774, 1706, 1625, 1543	1.20—2.04 (8H, m), 3.37 (2H, t, J=6 Hz), 3.73 (2H, t, J=6 Hz), 8.05 (1H, d, J=8 Hz), 8.62 (1H, d, J=8 Hz), 8.67 (1H, s)[d]
4e	3450, 2934, 2860, 1772, 1708, 1624, 1542	1.15—2.06 (10H, m), 3.38 (2H, t, J=7 Hz), 3.73 (2H, t, J=7 Hz), 8.06 (1H, d, J=8 Hz), 8.63 (1H, d, J=8 Hz), 8.70 (1H, s)[d]
4f	3466, 2932, 2856, 1773, 1708, 1625, 1544	1.16—2.05 (12H, m), 3.37 (2H, t, J=7 Hz), 3.73 (2H, t, J=7 Hz), 8.05 (1H, d, J=8 Hz), 8.63 (1H, d, J=8 Hz), 8.68 (1H, s)[d]
4g	3466, 2924, 2854, 1772, 1709, 1624, 1543	1.07—2.03 (14H, m), 3.37 (2H, t, J=7 Hz), 3.72 (2H, t, J=7 Hz), 8.05 (1H, d, J=8 Hz), 8.62 (1H, d, J=8 Hz), 8.67 (1H, s)[d]
4h	2942, 1772, 1714, 1616	1.34—2.10 (6H, m), 3.37 (2H, t, J=6.5 Hz), 3.67 (2H, t, J=6.5 Hz), 7.62—7.95 (4H, m)[d]
9[b]	2936, 2858, 1773, 1702, 1644[c]	0.90—2.03 (17H, m), 2.28—2.90 (6H, m), 3.44 (2H, t, J=7 Hz), 3.66 (2H, s), 7.33 (5H, s)[d]
11[b]	2924, 1779, 1720, 1622, 1542[c]	0.69—0.90 (2H, m), 0.96 (3H, t, J=7 Hz), 1.04—1.55 (7H, m), 1.56—1.83 (8H, m), 2.12 (2H, d, J=7 Hz), 2.35 (2H, t, J=7.5 Hz), 2.44 (2H, q, J=7 Hz), 3.75 (2H, t, J=7.5 Hz), 8.05 (1H, d, J=8 Hz), 8.59 (1H, dd, J=2, 8 Hz), 8.66 (1H, d, J=2 Hz)[d]

a) Chemical shifts are given with proton numbers, absorption patterns and coupling constants in parentheses. *b*) Spectral data of free base were measured. *c*) Taken as liquid film. *d*) Measured in $CDCl_3$.

^{1}H-NMR ($CDCl_3$) δ: 1.30—2.13 (6H, m), 3.40 (2H, t, J=6 Hz), 3.77 (2H, t, J=6 Hz), 8.08 (1H, d, J=9 Hz), 8.66 (1H, d, J=9 Hz), 8.71 (1H, s). The yield, melting point, and analytical data of this sample are shown in Table VII.

The following compounds (**4a, b, d—h**) listed in Tables VII and VIII were prepared in the same manner as described above.

***N*-Ethyl-*N*-[(2-methoxyphenyl)methyl]amine (5f)** A mixture of 70% ethylamine solution (5 ml) and $NaHCO_3$ (4.9 g) in dioxane–water (50/50 ml) was stirred for 20 min at room temperature. A solution of 2-methoxybenzoyl chloride (6.6 g) in dioxane (5 ml) was added dropwise to the mixture, which was stirred for an additional 1 h. Water (100 ml) and CH_2Cl_2 (100 ml) were added to the mixture and the organic phase was separated. Aqueous phase was extracted with CH_2Cl_2, and the combined organic phase was washed with water and dried over Na_2SO_4. The solvent was removed *in vacuo* to give a residue, which was dissolved in tetrahydrofuran (THF, 100 ml). $LiAlH_4$ (2.2 g) was added portionwise to the solution. The mixture was refluxed for 30 min, cooled to room temperature, and treated successively with water (4.4 ml) and 10% NaOH (3.6 ml). The resulting solid was removed by filtration and the filtrates were concentrated to give a residue. The residue was taken up in 5% HCl, washed with ether twice, made basic with 10% NaOH, and extracted with CH_2Cl_2. The extracts were dried over Na_2SO_4, and concentrated to give a pale yellow oil (5.63 g), which was used in the next reaction without further purification. The yield, IR and NMR data of this sample are given in Table IX.

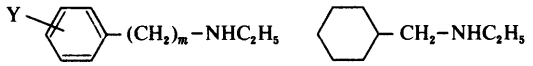
N-Ethyl-*N*-(ω-phenylalkyl)amines (**5a—e, g—l**) and *N*-(cyclohexylmethyl)-*N*-ethylamine (**10**) listed in Table IX were obtained in the same manner as described for **5f**.

***N*-Ethyl-*N*-[(3-hydroxyphenyl)methyl]amine (5m)** Ethylamine solution (70%, 10 ml) was added rapidly to a solution of 3-hydroxybenzaldehyde (12.2 g) in ethanol (12 ml) at about 40 °C. The mixture was allowed to stand at 0—5 °C for 2 h. The resulting precipitate was collected by filtration, washed successively with 40% ethanol and water, and dried *in vacuo* to give the Schiff base (13.1 g, 88%) as colorless needles, mp 127—129 °C.

Sodium borohydride (4.8 g) was added portionwise to a solution of the Schiff base (12.5 g) in methanol (190 ml) at 0—5 °C. The mixture was stirred at 0—5 °C until gas evolution stopped (about 30 min), then refluxed for 15 min, cooled, and quenched with water. Methanol was evaporated and the remaining aqueous solution was washed with CH_2Cl_2. The aqueous solution was made acidic with 10% HCl, washed with ether, neutralized with 10% NaOH, and extracted with 1-butanol. Removal of the solvent *in vacuo* gave a residue, which was dissolved in CH_2Cl_2. The solution was dried over Na_2SO_4 and the solvent was evaporated to afford a viscous oil. Crystallization from ether gave colorless cubes (11.7 g, 77% from the starting aldehyde), mp 107—111 °C. Spectral data of this sample are given in Table IX.

2-[5-[*N*-Ethyl-*N*-(phenylmethyl)amino]pentyl]-5-nitro-1*H*-isoindole-1,3(2*H*)-dione Hydrochloride (1j) A mixture of **4c** (1.0 g) and *N*-ethylbenzylamine (0.79 g) in toluene (15 ml) was refluxed for 6 h, cooled to room temperature, and the resulting precipitate was removed by

TABLE IX. Physicochemical Properties of *N*-Ethyl-*N*-(ω-phenylalkyl)amines (**5**) and *N*-(Cyclohexylmethyl)-*N*-ethylamine (**10**)



5 **10**

Compd. No.	*m*	Y	Yield (%)	IR (film) cm^{-1}	^1H-NMR ($CDCl_3$) δ[a]
5a	1	4-Cl	91	3316, 2966, 2820, 1491, 1458, 1089, 1014, 800	1.12 (3H, t, *J*=7 Hz), 1.31 (1H, br s), 2.66 (2H, q, *J*=7 Hz), 3.75 (2H, s), 7.27 (4H, s)
5b	1	3-Cl	90	3282, 2966, 2818, 1597, 1574, 1121, 1074, 777	1.13 (3H, t, *J*=7 Hz), 1.34 (1H, br s), 2.67 (2H, q, *J*=7 Hz), 3.77 (2H, s), 7.13—7.35 (4H, m)
5c	1	2-Cl	92	3314, 2964, 2818, 1470, 1442, 1124, 1049, 1035, 749	1.14 (3H, t, *J*=7 Hz), 1.55 (1H, br s), 2.68 (2H, q, *J*=7 Hz), 3.89 (2H, s), 7.14—7.28 (2H, m), 7.30—7.41 (2H, m)
5d	1	4-OMe	91	3308, 2962, 2834, 1612, 1512, 1245, 1174, 1035, 820	1.12 (3H, t, *J*=7 Hz), 1.45 (1H, br s), 2.67 (2H, q, *J*=7 Hz), 3.72 (2H, s), 3.79 (3H, s), 6.86 (2H, d, *J*=8.5 Hz), 7.23 (2H, d, *J*=8.5 Hz)
5e	1	3-OMe	89	3306, 2962, 2832, 1599, 1488, 1457, 1261, 1153, 1048, 779	1.13 (3H, t, *J*=7 Hz), 1.39 (1H, br s), 2.68 (2H, q, *J*=7 Hz), 3.77 (2H, s), 3.81 (3H, s), 6.79 (1H, dd, *J*=2, 8 Hz), 6.86—6.92 (2H, m), 7.22 (1H, dd, *J*=8, 8 Hz)
5f	1	2-OMe	88	3354, 2962, 2836, 1600, 1588, 1491, 1463, 1240, 1048, 1029, 752	1.12 (3H, t, *J*=7 Hz), 1.90 (1H, br s), 2.65 (2H, q, *J*=7 Hz), 3.79 (2H, s), 3.84 (3H, s), 6.83—6.96 (2H, m), 7.18—7.28 (2H, m)
5g	1	2-F	76	3300, 2968, 2870, 1594, 1539, 1490, 1454, 1227, 757	1.13 (3H, t, *J*=7 Hz), 1.55 (1H, br s), 2.67 (2H, q, *J*=7 Hz), 3.85 (2H, s), 6.97—7.46 (4H, m)
5h	1	2-Me	85	3318, 2964, 2814, 1456, 1377, 1127, 1104, 741	1.15 (3H, t, *J*=7 Hz), 1.30 (1H, br s), 2.35 (3H, s), 2.72, (2H, q, *J*=7 Hz), 3.77 (2H, s), 7.12—7.34 (4H, m)
5i	1	2,3-diOMe	67	3306, 2936, 2810, 1587, 1485, 1274, 1221, 1075, 1007, 748	1.03 (3H, t, *J*=7 Hz), 1.45 (1H, br s), 2.53 (2H, q, *J*=7 Hz), 3.67 (2H, s), 3.72 (3H, s), 3.78 (3H, s), 6.94 (3H, s)
5j	2	H	93	3298, 2932, 1635, 1495, 1454, 697	1.07 (3H, t, *J*=7 Hz), 2.00 (1H, br s), 2.67 (2H, q, *J*=7 Hz), 2.75—2.93 (4H, m), 7.23 (5H, s)
5k	3	H	92	3300, 2932, 1653, 1495, 1132, 746, 698	1.10 (3H, t, *J*=7 Hz), 1.27 (1H, br s), 1.82 (2H, tt, *J*=7, 7 Hz), 2.55—2.72 (6H, m), 7.13—7.34 (5H, m)
5l	4	H	90	3288, 2930, 1495, 1453, 1132, 746, 697	1.09 (3H, t, *J*=7 Hz), 1.13 (1H, br s), 1.45—1.75 (4H, m), 2.57—2.71 (6H, m), 7.12—7.33 (5H, m)
5m	1	3-OH	77	3420, 3280, 2898, 2562, 1609, 1582	1.13 (3H, t, *J*=7 Hz), 2.70 (2H, q, *J*=7 Hz), 3.67 (2H, s), 6.16 (2H, br s), 6.60—6.87 (3H, m), 7.10 (1H, dd, *J*=8, 8 Hz)
10	—	—	73	3294, 2922, 2852, 1651, 1539, 1447, 1132	0.78—1.57 (8H, m), 1.10 (3H, t, *J*=7 Hz), 1.59—1.82 (4H, m), 2.44 (2H, d, *J*=6.5 Hz), 2.62 (2H, q, *J*=7 Hz)

a) Chemical shifts are given with proton numbers, absorption patterns and coupling constants in parentheses.

filtration. The filtrate was concentrated to give an oily residue, which was chromatographed on silica gel eluting with ethyl acetate to afford an oil. Treatment of the oil with ethanolic HCl (1 eq) gave colorless cubes (0.85 g). IR (KBr): 3434, 2946, 1778, 1717, 1625, 1541 cm^{-1}. ^1H-NMR (dimethylsulfoxide-d_6 (DMSO-d_6)) δ: 1.10—2.00 (9H, m), 2.65—3.45 (4H, m), 3.60 (2H, t, *J*=6 Hz), 4.24 (2H, br d, *J*=4 Hz), 7.20—7.80 (5H, m), 8.10 (1H, d, *J*=8 Hz), 8.45 (1H, s), 8.61 (1H, d, *J*=8 Hz), 11.17 (1H, br s). The yield, melting point, and analytical data of this sample are shown in Table I.

The following compounds (**1a—i, k—t, v, ll—tt, vv—xx**, and **11**) listed in Tables I, III, VI, and VIII were similarly prepared by reaction of the bromides (**4a—h**) with the amines (**5a—l** or **10**).

Method B. *N*-Ethyl-*N*-(phenylmethyl)pentane-1,5-diamine (8) A mixture of **1v** (18 g, free base) and hydrazine hydrate (10 ml) in ethanol (300 ml) was refluxed for 1 h, cooled to room temperature, and the resulting precipitate was filtered off. The filtrate was concentrated and the resulting precipitate was removed again. This procedure was repeated until no precipitate appeared, giving an oil (10.35 g, 91%), which was used in the next reaction without further purification. IR (film): 3350, 2932, 2858, 2798, 1494, 1452, 1369, 734, 697 cm^{-1}. ^1H-NMR ($CDCl_3$) δ: 1.04 (3H, t, *J*=7 Hz), 1.21—1.58 (6H, m), 1.88 (2H, br s), 2.44 (2H, t, *J*=7.5 Hz), 2.52 (2H, q, *J*=7 Hz), 2.68 (2H, t, *J*=7 Hz), 3.57 (2H, s), 7.26 (5H, s). MS *m/z*: 220 [M^+].

2-[5-[*N*-Ethyl-*N*-(phenylmethyl)amino]pentyl]-4-nitro-1*H*-isoindole-1,3(2*H*)-dione Hydrochloride (1w) A mixture of **8** (0.5 g) and 3-nitrophthalic anhydride (0.44 g) in dioxane (15 ml) was refluxed for 8 h, cooled to 0 °C, diluted with water, and extracted with CH_2Cl_2. The extracts were dried over Na_2SO_4 and the solvent was removed *in vacuo* to give a residue, which was chromatographed on silica gel eluting with CH_2Cl_2–ethanol (20:1) to afford an oil. Treatment of the oil with ethanolic HCl (1 eq) gave a hygroscopic powder (0.66 g). IR (KBr): 3434, 2946, 2620, 1779, 1717, 1619, 1541 cm^{-1}. ^1H-NMR (DMSO-d_6) δ: 1.06—2.00 (9H, m), 2.72—3.20 (4H, m), 3.56 (2H, t, *J*=6 Hz), 4.26 (2H, d, *J*=5 Hz), 7.30—7.53 (3H, m), 7.53—7.80 (2H, m), 7.95—8.36 (3H, m), 11.13 (1H, br s). The yield and analytical data of this sample are shown in Table III.

The following compounds (**1x—z, gg**, and **9**) listed in Tables III, VI, and VIII were prepared in the same manner as described for **1w** by reaction of **8** with commercially available substituted phthalic anhydrides or *cis*-cyclohexane-1,2-dicarboxylic anhydride.

5-Nitro-2-[4-[*N*-(phenylmethyl)amino]butyl]-1*H*-isoindole-1,3(2*H*)-dione Hydrochloride (1u) A mixture of 4-chlorobutyronitrile (4.0 g), benzylamine (4.14 g), KI (7.1 g), and K_2CO_3 (5.9 g) in 1-butanol (40 ml) was refluxed for 8 h, cooled to room temperature, and insoluble salts were filtered off. Removal of the solvent *in vacuo* gave an oil, which was treated with ethanolic HCl (1 eq) to afford colorless crystals. Recrystallization from ethanol gave *N*-(3-cyanopropyl)benzylamine hydrochloride as colorless plates (3.5 g, 43%), mp 165—168 °C. IR (KBr): 3434, 2940, 2778, 2420, 2244, 1439, 746, 697 cm^{-1}. ^1H-NMR (DMSO-d_6) δ: 2.03 (2H, tt, *J*=7.5, 7.5 Hz), 2.67 (2H, t, *J*=7.5 Hz), 2.95 (2H, t, *J*=7.5 Hz), 4.11 (2H, s), 7.30—7.73 (5H, m), 9.80 (2H, br s). *Anal.* Calcd for $C_{11}H_{14}N_2 \cdot HCl$: C, 62.70; H, 7.18; N, 13.30. Found: C, 62.44; H, 6.96; N, 13.24.

A solution of the amine (2.9 g) in THF (50 ml) was added dropwise to a suspension of $LiAlH_4$ (1.05 g) in THF (50 ml). The mixture was refluxed for 15 min, cooled to 0 °C, and the resulting mixture was treated successively with water (2.2 ml) and 10% NaOH (1.8 ml). The precipitated solid was removed by filtration and the filtrate was concentrated to give

a residue. The residue was dissolved in 5% HCl, washed with ether twice, made basic with 10% NaOH, and extracted with CH_2Cl_2. The extracts were dried over Na_2SO_4 and the solvent was removed *in vacuo* to give *N*-(phenylmethyl)butane-1,4-diamine (1.7 g, 69%) as a pale yellow oil, which was used in the next step without further purification.

The diamine was allowed to react with 4-nitrophthalic anhydride in the same manner as described for **1w** to give the desired product **1u** as colorless cubes. IR (KBr): 3438, 1778, 1710, 1626, 1546 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.53—1.85 (4H, m), 2.70—3.07 (2H, m), 3.46—3.76 (2H, m), 3.94—4.16 (2H, m), 7.30—7.70 (5H, m), 8.13 (1H, d, J=8 Hz), 8.49 (1H, d, J=1.5 Hz), 8.65 (1H, dd, J=1.5, 8 Hz), 9.5 (2H, br s). The yield, melting point and analytical data of this sample are given in Table I.

Transformation of Substituents of 1. 2-[5-[*N*-Ethyl-*N*-(phenylmethyl)-amino]pentyl]-5-methoxy-1*H*-isoindole-1,3(2*H*)-dione Hydrochloride (1aa) A mixture of sodium hydride (20 mg) and **1z** (0.25 g) in DMF (5 ml) was stirred at room temperature for 2 h. Methyl iodide (0.1 g) was added to the mixture, which was then stirred at room temperature for 1 h, quenched with water, and extracted with CH_2Cl_2. The extracts were dried over Na_2SO_4 and the solvent was removed *in vacuo* to afford a residue, which was chromatographed on silica gel eluting with CH_2Cl_2–ethanol (20 : 1) to give an oil. IR (film): 2938, 2800, 1769, 1709, 1618, 1491 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.02 (3H, t, J=7.5 Hz), 1.10—1.86 (6H, m), 2.25—2.66 (4H, m), 3.55 (2H, s), 3.62 (2H, t, J=6 Hz), 3.89 (3H, s), 7.00—7.47 (7H, m), 7.71 (1H, d, J=8 Hz).

Treatment of the oil with ethanolic HCl (1 eq) gave a hygroscopic powder (0.22 g). The yield and analytical data of this sample are shown in Table III.

The compound (**1bb**) was prepared as described for **1aa** (Tables III and VIII).

5-Amino-2-[5-[*N*-ethyl-*N*-(phenylmethyl)amino]pentyl]-1*H*-isoindole-1,3(2*H*)-dione Dihydrochloride (1cc) A suspension of **1j** (4.4 g) and concentrated HCl (2 ml) in ethanol (150 ml) was hydrogenated over 10% Pd/C (0.3 g) under atmospheric pressure at room temperature. After removal of the catalyst by filtration, the filtrate was concentrated to give a residue, which was dissolved in 10% NaOH and extracted with CH_2Cl_2. The extracts were dried over Na_2SO_4 and the solvent was evaporated to afford a residue. The residue was chromatographed on silica gel eluting with ethyl acetate to give an oil, which was treated with ethanolic HCl (2 eq) to afford a hygroscopic powder (3.1 g). IR (KBr): 3438, 2942, 1774, 1757, 1709, 1617 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.00—2.00 (6H, m), 1.26 (3H, t, J=7.5 Hz), 2.70—3.20 (4H, m), 3.48 (2H, t, J=6 Hz), 4.26 (2H, d, J=5 Hz), 6.67—7.05 (4H, m), 7.30—7.80 (6H, m), 11.2 (2H, br s). The yield and analytical data of this sample are shown in Table III.

5-Acetylamino-2-[5-[*N*-ethyl-*N*-(phenylmethyl)amino]pentyl]-1*H*-isoindole-1,3(2*H*)-dione (1dd) A mixture of **1cc** (0.4 g) and acetyl chloride (0.09 g) in pyridine (4 ml) was stirred at room temperature for 1 h, diluted with water, and extracted with CH_2Cl_2. The extracts were dried over Na_2SO_4 and the solvent was removed *in vacuo* to give a viscous oil, which was triturated with hexane to give a powder. Recrystallization from CH_2Cl_2–hexane gave colorless cubes (0.33 g). IR (KBr): 3444, 3358, 2934, 1770, 1696, 1627, 1612, 1557 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.99 (3H, t, J=7 Hz), 1.10—1.86 (6H, m), 2.05—2.63 (4H, m), 2.24 (3H, s), 3.51 (2H, s), 3.63 (2H, t, J=6 Hz), 7.25 (5H, s), 7.76 (1H, d, J=9 Hz), 7.97 (1H, d, J=1.5 Hz), 8.07 (1H, dd, J=1.5, 9 Hz), 8.25 (1H, s). The yield, melting point, and analytical data of this sample are shown in Table III.

The sulfonamides (**1ee** and **1ff**) listed in Tables III and VIII were prepared in the same manner as described for **1dd**.

Methyl 2-[5-[*N*-Ethyl-*N*-(phenylmethyl)amino]pentyl]-1,3(2*H*)-dioxo-1*H*-isoindole-5-carboxylate Hydrochloride (1hh) 2-[5-[*N*-Ethyl-*N*-(phenylmethyl)amino]pentyl]-1,3-dioxo-1*H*-isoindole-5-carboxylic acid hydrochloride (**1yy**, a very hygroscopic powder) was prepared by reaction of trimellitic anhydride with **8** in the same manner as described for **1w** in 74% yield. IR (KBr): 3422, 2400—3200, 2946, 1774, 1716, 1656 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.27 (3H, t, J=7 Hz), 1.07—2.03 (6H, m), 2.67—3.23 (4H, m), 3.60 (2H, t, J=6 Hz), 4.28 (2H, d, J=5 Hz), 7.27—7.82 (5H, m), 7.95 (1H, d, J=8 Hz), 8.22 (1H, s), 8.35 (1H, d, J=8 Hz), 11.33 (2H, br s).

A mixture of the acid (**1yy**, 0.7 g) and concentrated HCl (2 drops) in methanol (30 ml) was refluxed for 30 min. The mixture was concentrated, diluted with CH_2Cl_2, washed successively with 10% NaOH and water, and dried over Na_2SO_4. The solvent was removed *in vacuo* to give a residue. Chromatographic purification of the residue on silica gel eluting with CH_2Cl_2–ethanol (20 : 1) gave an oil. IR (film): 2940, 1776, 1721 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.02 (3H, t, J=7 Hz), 1.10—1.87 (6H, m), 2.23—2.65 (4H, m), 3.54 (2H, s), 3.68 (2H, t, J=7 Hz), 4.00 (3H, s), 7.30 (5H, s), 7.90 (1H, d, J=7.5 Hz), 8.40 (1H, d, J=7.5 Hz), 8.47 (1H, s).

Treatment of the oil with ethanolic HCl (1 eq) afforded **1hh** (0.64 g) as an amorphous powder. The yield and analytical data of this sample are shown in Table III.

The ethyl ester (**1ii**) was prepared in the same manner as described above (Tables III and VIII).

2-[5-[*N*-Ethyl-*N*-(phenylmethyl)amino]pentyl]-5-methylaminocarbonyl-1*H*-isoindole-1,3(2*H*)-dione (1jj) A mixture of **1yy** (0.3 g) and thionyl chloride (0.55 ml) was heated at 70 °C for 30 min. After excess thionyl chloride was removed *in vacuo* the remaining residue was dissolved in CH_2Cl_2. A mixture of methylamine hydrochloride (53 mg) and Et_3N (0.22 ml) in CH_2Cl_2 (8 ml) was added to the solution. The resulting mixture was stirred at room temperature for 30 min. The mixture was concentrated and extracted with CH_2Cl_2. The extracts were washed successively with 5% NaOH and water, dried over Na_2SO_4, and the solvent was evaporated to give a residue. The residue was chromatographed on silica gel eluting with CH_2Cl_2–ethanol (20 : 1) to afford crystals. Recrystallization from CH_2Cl_2–ether gave colorless cubes (0.15 g). IR (KBr): 3428, 2932, 1773, 1702, 1677, 1534 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.00 (3H, t, J=7 Hz), 1.13—1.84 (6H, m), 2.27—2.65 (4H, m), 3.01 (3H, d, J=5 Hz), 3.52 (2H, s), 3.63 (2H, t, J=7 Hz), 7.08—7.40 (6H, m), 7.81 (1H, d, J=8 Hz), 8.10—8.30 (2H, m). The yield, melting point, and analytical data of this sample are given in Table III.

N,N-Diethylamide (**1kk**) was prepared in the same manner as described for **1jj** (Tables III and VIII).

2-[5-[*N*-Ethyl-*N*-[[3-(methylaminocarbonyloxy)phenyl]methyl]amino]-pentyl]-5-nitro-1*H*-isoindole-1,3(2*H*)-dione Hydrochloride (1uu) In the same manner as described for **1j**, **4c** (0.9 g) was allowed to react with **5m** (0.8 g) to give 2-[5-[*N*-ethyl-*N*-[(3-hydroxyphenyl)methyl]amino]pentyl]-5-nitro-1*H*-isoindole-1,3(2*H*)-dione (**1zz**, 0.6 g, 55%) as an oil, which was used in the next step without further purification. IR (film): 3200—3600, 3418, 2940, 1777, 1719, 1588, 1540 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.98 (3H, t, J=7 Hz), 1.20—1.93 (6H, m), 2.23—2.66 (4H, m), 3.47 (2H, s), 3.74 (2H, t, J=7 Hz), 5.54 (1H, br s), 6.55—7.22 (4H, m), 8.04 (1H, d, J=8 Hz), 8.51—8.70 (2H, m).

Methyl isocyanate (0.06 ml) and Et_3N (1 drop) were added successively to a solution of **1zz** (0.35 g) in CH_2Cl_2 (3 ml) at 0—5 °C. The mixture was stirred at room temperature for 10 min, quenched with water, and extracted with CH_2Cl_2. The extracts were washed with water, dried over Na_2SO_4, and the solvent was removed *in vacuo* to give a residue. Chromatographic purification of the residue on silica gel eluting with ethyl acetate–methanol (20 : 1) afforded a colorless oil, which was treated with ethanolic HCl (1 eq) to give a hygroscopic powder (0.38 g). IR (KBr): 3420, 2940, 1777, 1716 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.10—2.00 (9H, m), 2.59—3.23 (7H, m), 3.62 (2H, t, J=7 Hz), 3.81 (1H, br s), 4.27 (2H, d, J=*ca.* 5 Hz), 7.07—7.77 (5H, m), 8.14 (1H, d, J=8 Hz), 8.50 (1H, d, J=1.5 Hz), 8.67 (1H, dd, J=1.5, 8 Hz). The yield and analytical data of this sample are shown in Table VI.

2-[4-[*N*-Acetyl-*N*-(phenylmethyl)amino]butyl]-5-nitro-1*H*-isoindole-1,3(2*H*)-dione (12) A mixture of **1u** (0.5 g, free base) and acetic anhydride (0.15 g) in CH_2Cl_2 was refluxed for 10 min, cooled to room temperature, washed successively with 5% NaOH and water, dried over Na_2SO_4, and the solvent was removed *in vacuo* to give an oil. The oil was chromatographed on silica gel eluting with ethyl acetate to afford a colorless oil (0.53 g, 95%). IR (film): 2940, 1778, 1716, 1642, 1540 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.49—1.85 (4H, m), 2.11, 2.18 (3H, each s), 3.26, 3.40 (2H, each t, J=7 Hz), 3.72, 3.74 (2H, each t, J=6.5 Hz), 4.53, 4.59 (2H, each s), 7.14—7.42 (5H, m), 8.04, 8.06 (1H, each d, J=8 Hz), 8.58—8.68 (2H, m). MS *m/z*: 395 [M^+].

Biological Method and Materials The cerebral cortex of male Wistar rats was homogenized with 20 volumes of ice-cooled 0.32 M sucrose, and centrifuged at 1000 × *g* for 10 min. The supernatant (S1) was preincubated in a scintillation vial with test compound for 15 min at room temperature, and then [acetyl-^{3}H]-acetylcholine (final 200 μM) was added and the incubation was continued for 30 min. The reaction was terminated by adding a 1 M solution of chloroacetic acid, followed by a toluene-based scintillant, and the vials were capped and shaken to transfer the produced [^{3}H]-acetic acid to the toluene phase. Radioactivity in the toluene phase was then counted by liquid scintillation spectrometry (Aloka LSC-903 or LSC-1000). The inhibitory activities were expressed as the 50%-inhibitory concentration (IC_{50}), which was calculated by probit analysis. The inhibitory activities of physostigmine and THA were measured using the same technique.

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