cedure described above failed. Reaction conditions were varied from ice temperature for 2 hr. to reflux temperature for 1 hr. In one run a small amount (*ca.* 10% by weight of the material subjected to the reaction) of crystalline substance, m.p. 171–173°, was obtained after elution chromatography of the oily residue. The infrared spectrum of this material showed bands at 3.0 (N–H), 6.03 (C=O), and 8.1 μ (Si-CH_3). The structure of this material was not determined.

Ultraviolet Spectra.—The sample was dissolved in 70% aqueous ethanol. Since the spectra of these spirobarbiturates change with time, the solutions were used within 1–2 hr. For the neutral solutions an aliquot was diluted to the proper volume with 70% ethanol. For the basic solutions, an aliquot was diluted to the proper volume using NaOH (70% ethanol solution; 0.01 *N* final volume). These latter solutions were run within 5 min. of mixing. The spectra were run from 3500 to 2150 Å. The ultraviolet data obtained are summarized in Table II. The data obtained for diethylbarbital were: neutral solution, end absorption; 0.01 *N* NaOH, λ_{max} 2400 Å. (ϵ_{max} 9650).

Infrared Spectra.—The infrared spectrum for each of the spirobarbiturates was run as a mineral oil mull. The bands observed in the N–H region (2.7 to 3.3 μ) and in the C=O region (5.5 to 6.2 μ) are summarized in Table II. For the spirothiobarbiturates, the bands in the 6.5-μ region (C=S) are also tabulated.

The Effect of Piperidinecarboxamide Derivatives on Isolated Human Plasma Cholinesterase. II. Variations in the Amide Function[1]

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A series of carbamoylpiperidine derivatives has been prepared. The inhibitory activity of these and similar derivatives[2,3] upon isolated human plasma cholinesterase was determined. The results are discussed primarily in terms of the effect on biochemical response elicited by variations in the amide function.

In previous investigations, we have explored the effect of compounds of several series of carbamoylpiperidines (piperidinecarboxamides) upon isolated human plasma pseudo-cholinesterase,[2] and the respective relationships with surface-active properties.[4] The very interesting results obtained in these studies prompted additional synthetic[3] and physicochemical[5,6] investigations designed to elucidate further the influence that structural variations in the inhibitor molecules have on isolated cholinesterase systems. We have prepared additional mono- and bis(carbamoylpiperidino)ethanes and -decanes, reported here and in an earlier paper,[3] for the evaluation of their inhibitory characteristics.

Our earlier work[2] yielded data reflecting responses effected by (1) the nature and degree of alkyl substitution on the amido function, (2) the mono- and the corresponding bis(carbamoylpiperidino) substitution on the alkane homologs, (3) the number and arrangement of the methylene units in the alkane component attached to the ring nitrogen(s), and (4) unsaturation in the piperidine ring.

This article deals primarily with the effect of structural variation in the amide function upon biochemical response. More specifically, we were interested in the effect such molecular modification might elicit on the latter response in terms of (1) the electron densities around the carbonyl function,[7] and (2) the lyophobic–lyophilic nature of substitution.[7]

Experimental

Synthetic Work.[8,9]—The chemistry of some of our piperidinecarboxamide derivatives has been reported previously.[3]

3-(Piperidinoformyl)pyridine (XXI).[10]—This compound was prepared from nicotinic acid. It distilled at 115–118° (0.15 mm.).

3-(Morpholinoformyl)pyridine (XXII).[10]—This compound was also prepared from nicotinic acid. It distilled at 138–140° (0.4 mm.).

The compounds described in Table I were prepared by the following procedures.

Procedure A.[11] **1-Decyl-3-(N-ethylcarbamoyl)piperidine Hydrobromide (III).**—N-Ethylnicotinamide (50 g., 0.332 mole) and 1-bromodecane (73.4 g., 0.332 mole) were heated at 150° for 7 hr. After cooling the reaction mixture, the solid product was dissolved in aqueous ethanol, and the solution was subjected to hydrogenation in the presence of 1 g. of platinum oxide at maximum pressures of 3.16–3.51 kg./cm.^{2} (45 to 50 p.s.i.). When absorption of hydrogen ceased, the catalyst was removed by filtration and the solvent was removed *in vacuo* utilizing a rotary evaporator. Residual traces of moisture were removed from the oily product by azeotropic distillation with absolute ethanol and/or benzene, and the product was purified by recrystallization.

Procedure B. 1-Decyl-3-(piperidinoformyl)piperidine Hydrobromide (XII).—3-(Piperidinoformyl)pyridine (25 g., 0.131 mole) and 1-bromodecane (73 g., 0.330 mole) were dissolved in 200 ml. of anhydrous benzene, and the solution was refluxed for 53 hr. The oily precipitate produced in the reaction was dissolved in 150 ml. of water, and the solution was washed with benzene, treated with charcoal, and filtered. The solution was

(1) This investigation is being supported by grants from the National Institute of Mental Health (USPHS MY-2072/MH-04379), the Geschickter Fund for Medical Research, Inc., and the National Science Foundation (GB-2381/B-15989).

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TABLE I

PROPERTIES OF CARBAMOYLPIPERIDINE DERIVATIVES

No.	Method of prepn.	Yield, %[a]	Salt	Recrystn. solvent[b]	M.p., °C.	Formula	C, % Calcd.	C, % Found	H, % Calcd.	H, % Found	Br, % Calcd.	Br, % Found	N, % Calcd.	N, % Found
I	A	100	HBr	E	164.4–164.6	$C_{16}H_{33}BrN_2O$	55.01	54.86	9.52	9.65	22.87	22.90	8.02	8.08
II	B	75.6	HBr	E	77.2–78.3	$C_{17}H_{35}BrN_2O$	56.19	56.01	9.71	9.54	21.99	22.05	7.71	7.84
III	A	83.6	HBr	EA	102.5–103.0	$C_{18}H_{37}BrN_2O$	57.28	57.00	9.88	10.07	21.17	21.20	7.42	7.62
IX	B	78.3	HBr	E–EA	167.3–167.8	$C_{13}H_{25}BrN_2O$	51.15	51.16	8.26	8.27	26.18	26.18	9.18	9.35
X	B	53.8	HBr	E–EA	186.2–187.1	$C_{12}H_{23}BrN_2O_2$	46.91	46.83	7.55	7.47	26.01	25.84	9.12	9.17
XII	B	90.9	HBr	E–EA	174.8–175.3	$C_{21}H_{41}BrN_2O$	60.42	60.39	9.90	9.71	19.14	19.18	6.71	6.69
XIII	B	65.3	HBr	E–EA	133.2–134.5	$C_{20}H_{39}BrN_2O_2$	57.26	57.18	9.37	9.48	19.05	18.92	6.68	6.54
XX	C	80.3	2HBr	E-EA	209.0–209.1	$C_{32}H_{60}Br_2N_4O_2$	55.49	55.31	8.73	8.65	23.07	22.85	8.09	7.83

[a] Crude yield. [b] E, ethanol; EA, ethyl acetate.

then subjected to hydrogenation and subsequent purification as described under procedure A.

Procedure C. 1,10-Bis[3-(piperidinoformyl)piperidino]decane Dihydrobromide (XX).—3-(Piperidinoformyl)pyridine (50.6 g., 0.266 mole) and 1,10-dibromodecane (39.9 g., 0.133 mole) were dissolved in 200 ml. of anhydrous benzene and refluxed for 55 hr. The precipitate formed during the reaction was then treated as described under procedure B.

While the inhibitory properties of the following compounds are still in the process of being determined, their synthesis is reported at the present time.

Procedure D.[14] **1-Methyl-3-(pyrrolidinoformyl)-1,2,5,6-tetrahydropyridine Hydrochloride (XXIII).**—1-Methyl-1,2,5,6-tetrahydropyridine-3-carboxylic acid[15] (39 g., 0.276 mole) was covered with 250 ml. of anhydrous benzene, and thionyl chloride (164.2 g., 1.380 moles) was added gradually. The reaction mixture was refluxed for 3 hr. after which excess thionyl chloride and benzene were removed by distillation *in vacuo*. The residue was dispersed in 250 ml. of anhydrous benzene, and pyrrolidine (98.2 g., 1.380 moles) was added gradually. The reaction mixture was refluxed for 6 hr., cooled, treated with 400 ml. of cold, aqueous 40% potassium hydroxide solution, and extracted with benzene. The combined benzene extracts were dried ($MgSO_4$) and filtered, and the benzene was removed by distillation under reduced pressure. Distillation of the residual dark oil gave a yellow oil, b.p. 122° (0.17 mm.), which was dissolved in dry ether and converted to the hydrochloride by the addition of a solution of dry hydrogen chloride in dry ether. The hydrochloride (55 g., 86.4%) was then purified by recrystallization from absolute ethanol–ethyl acetate. It melted at 194.6–195.4°.

Anal. Calcd. for $C_{11}H_{19}ClN_2O$: C, 57.26; H, 8.30; Cl, 15.37; N, 12.14. Found: C, 57.25; H, 8.37; Cl, 15.20; N, 12.11.

1-Methyl-3-(morpholinoformyl)-1,2,5,6-tetrahydropyridine Hydrochloride (XXIV).—This compound was prepared by procedure D. The free base distilled at 150° (0.7 mm.). The hydrochloride (40.5 g., 59.0%), recrystallized from absolute ethanol-ethyl acetate, melted at 227.0–227.8°.

Anal. Calcd. for $C_{11}H_{19}ClN_2O_2$: C, 53.55; H, 7.76; Cl, 14.37; N, 11.35. Found: C, 53.80; H, 7.92; Cl, 14.15; N, 11.21.

1,10-Bis[3-(morpholinoformyl)piperidino]decane Dihydrobromide (XXV).—This compound was prepared by procedure C. The product (35.2 g., 51.8%), recrystallized from absolute ethanol–ethyl acetate, melted at 241.0–242.0°.

Anal. Calcd. for $C_{30}H_{56}Br_2N_4O_4$: C, 51.72; H, 8.10; Br, 22.94; N, 8.04. Found: C, 51.80; H, 8.20; Br, 22.80; N, 7.97.

1,2-Bis[3-(morpholinoformyl)piperidino]ethane Dihydrobromide (XXVI).—This compound was prepared by procedure C. The product (11.2 g., 18.4%), recrystallized from methanol, melted at 278.1–278.6° dec.

Anal. Calcd. for $C_{22}H_{40}Br_2N_4O_4$: C, 45.21; H, 6.90; Br, 27.35; N, 9.59. Found: C, 45.28; H, 7.11; Br, 27.20; N, 9.45.

1,2-Bis[3-(piperidinoformyl)piperidino]ethane Dihydrobromide (XXVII).—This compound was prepared by procedure C. The product (12.7 g., 19.0%), recrystallized from absolute ethanol–ethyl acetate, melted at 269.4–269.7° dec.

Anal. Calcd. for $C_{24}H_{44}Br_2N_4O_2$: C, 49.66; H, 7.64; Br, 27.54; N, 9.65. Found: C, 49.45; H, 7.69; Br, 27.42; N, 9.66.

Biochemical Evaluation.—Manometric determinations were carried out on a GME-Lardy RWB-3 Warburg instrument, using 15-ml. flasks. Predominantly, acetylcholine iodide was used as a substrate (8.22×10^{-3} *M* concentration in the final reaction mixture). A Krebs–Ringer bicarbonate buffer, consisting of 2.3×10^{-2} *M* $NaHCO_3$, 7.5×10^{-2} *M* NaCl, 7.5×10^{-2} *M* KCl, and 4.0×10^{-2} *M* $MgCl_2 \cdot 6H_2O$ was prepared according to Cohen[16a]; the final reaction mixture was calculated[16b] to yield a pH of 7.6 with the indicated $NaHCO_3$ concentration, in a gas phase of 5% CO_2 and 95% N_2, at 37° (740 mm.). The displacement of air in the reaction vessels with the above gas mixture was carried out by means of a gas-exchange technique[16c] under reduced pressure. The reaction volume totaled 3.20 ml., with 0.60 ml. of substrate solution in the side arm and all other reaction components in the main compartment. The compounds were introduced in 0.08 ml. of aqueous solution appropriately concentrated to yield the desired dilution of the compounds in the final reaction mixture. Equilibration was initiated at −60 min. with readings every 5 min. beginning at −25 min. Dumping was effected at 0 min., with the side arm being washed twice with the reaction mixture. Each manometer was read precisely at electrically timed 10.0-min. intervals during the reaction period of 0 to +60 min. The rate is expressed as $V = \{[(\mu l.\text{ of } CO_2 \text{ at } 30 \text{ min.}) - (\mu l.\text{ of } CO_2 \text{ at } 10 \text{ min.})]/20\} \times 60$, where V signifies $\mu l.$ of CO_2/hr. evolved within the reaction interval of +10 to +30 min., during which time the rate is linear in all instances. The percentages of inhibition are reported as $I = [(V_c - V_i)/V_c] \times 100$, where V_c represents the control rate and V_i the inhibited rate.

All reagents were prepared using redistilled water, registering at least 73 dynes/cm. (uncorrected) in surface tension measurements on a Kahlsico TE03 du Nouy interfacial tensiometer. Cholase (Cutter Laboratories, Berkeley, Calif.), a lyophilized cholinesterase preparation obtained through extensive alcoholic fractionation of large pools of normal human plasma, was the enzyme used. It was stored at −25° with the temperature being continuously registered by a spring-activated temperature recorder.

As reported previously,[17] our system was kinetically characterized[18] and the system's response checked against established inhibitors.[2]

All compounds were screened first for inhibitory properties at, 1.0×10^{-3} *M* concentration. For purposes of our evaluation,

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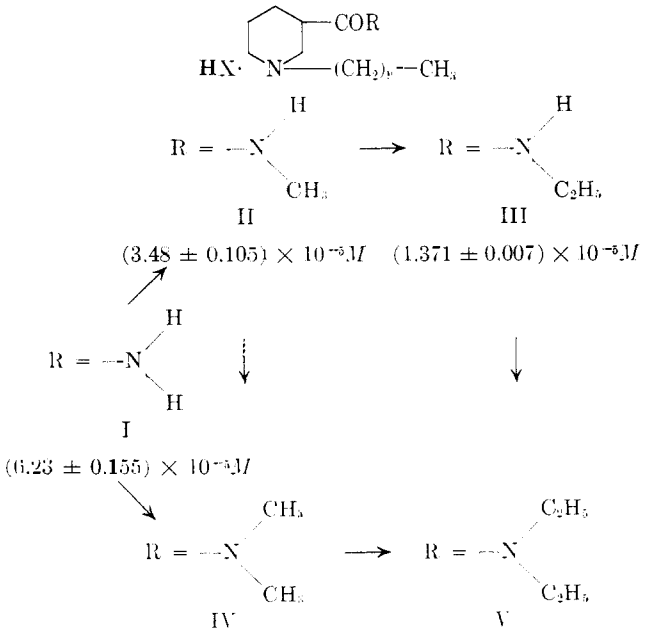
TABLE II

THE INFLUENCE OF ALKYL SUBSTITUTION[a] IN THE AMIDE FUNCTION UPON THE CHOLINESTERASE INHIBITION (I_{50} ± S.E.) OF CARBAMOYLPIPERIDINODECANES



[a] Taft's polar substituent constants (σ^*)[20]: H = +0.49, CH_3 = 0.00, C_2H_5 = −0.10. [b] The chemistry of this compound has been previously reported.[3] [c] The chemistry[14] and biochemical evaluation[2] of this compound have been previously reported.

Snedecor and Cochran[19] was less than 5% and it never exceeded 7%.

Whenever the evaluated base was associated with a previously untested salt-forming acid component, the specific effect of the anion on the enzyme system was carefully checked.

Results and Discussion

The results of our biochemical evaluation of the member compounds of the several subject series are presented in Tables II and III.

Wilson and his associates[7] have advanced an explanation for cholinesterase inhibition of compounds containing the O=C—R function based on the "effect of the substituting (R) group upon the electrophilic character of the carbonyl carbon."[7a] They show, for example, in going from nicotinamide (3-carbamoylpyridine) to nikethamide [3-(N,N-diethylcarbamoyl)-pyridine], that the electrophilicity of the carbonyl carbon should increase and, thus, nikethamide should be the stronger inhibitor; this was shown experimentally to be the case.

Classically, alkyl groups have been accepted in organic chemistry as electron donors. In classifying hydrogen and alkyl groups as electron donors according to Taft's σ^*-values[20] (see Table II), the order of increasing electron-release potential is H < CH_3 < C_2H_5. Thus, the relative electron contribution to the carbonyl function in a series of N-substituted amides can be estimated accordingly. With the increasing electron-releasing potential of the respective substi-

TABLE III

RELATIONSHIPS BETWEEN THE MOLECULAR CONSTITUTION OF SUBSTITUTED CARBAMOYLPIPERIDINES AND THEIR EFFECTS UPON ISOLATED HUMAN PLASMA CHOLINESTERASE

	n = 1		n = 9		n = 1		n = 9	
—NR_1R_2	Compd.	(I_{50} ± S.E.)[g] × 10^5	Compd.	(I_{50} ± S.E.)[g] × 10^5	Compd.	(I_{50} ± S.E.)[g] × 10^5	Compd.	(I_{50} ± S.E.)[g] × 10^5
—$N(CH_3)_2$	VI	(4.53 ± 10.5)[c]	IV	(2.17 ± 0.10)[b]	XIV	(123.3 ± 1.75)[d]	XVII	(10.71 ± 0.085)[e]
—$N(C_2H_5)_2$	VII	(118.5 ± 0.5)[b,f]	V	(0.527 ± 0.011)[b,c]	XV	(17.5 ± 1.0)[b]	XVIII	(2.59 ± 0.05)[b]
—N⟨	VIII	(197 ± 3.0)[c]	XI	(0.766 ± 0.0085)[c]	XVI	(9.46 ± 0.29)[c]	XIX	(2.80 ± 0.11)[c]
—N⟨	IX	(62.2 ± 1.80)	XII	(0.318 ± 0.0195)			XX	(0.662 ± 0.017)
—N⟨O	X	Inhib. not sig. at 100 × 10^{-5} M	XIII	(2.57 ± 0.125)				

[a] The chemistry of this compound has been previously reported.[3] [b] The chemistry[14] and biochemical evaluation[2] of this compound have been previously reported. [c] The I_{50} ± S.E. value of the corresponding 3,4-unsaturated derivative [1-ethyl-3-(N,N-diethylcarbamoyl)-1,2,5,6-tetrahydropyridine hydrochloride,[3] XXVIII] is (197 ± 12.0) × $10^{-5}M$. [d] The I_{50} ± S.E. value of the corresponding 4-carbamoyl-substituted derivative[3] [1-ethyl-4-(N,N-diethylcarbamoyl)piperidine hydrobromide, XXIX] is (283 ± 14.5) × $10^{-5}M$. [e] The I_{50} ± S.E. value of the corresponding 3,4-unsaturated derivative[3] [1-decyl-3-(N,N-diethylcarbamoyl)-1,2,5,6-tetrahydropyridine hydrochloride, XXX] is (0.670 ± 0.003) × $10^{-5}M$. [f] The I_{50} ± S.E. value for the corresponding 4-carbamoyl-substituted derivative[3] [1-decyl-4-(N,N-diethylcarbamoyl)piperidine hydrobromide, XXXI] is (2.65 ± 0.18) × $10^{-5}M$. [g] The I_{50} value is defined as the molar concentration of the compound effecting 50% inhibition.

compounds exhibiting less than 15% inhibition under these conditions were not considered further; at least two independent duplicate determinations were run to confirm responses of less than 15% inhibitory action. Conversely, an observed inhibition of 15% or higher at 1.0×10^{-3} M concentration was deemed sufficient to warrant further evaluation.

The effect of such compounds was evaluated at four appropriate concentrations, with at least two independent duplicate determinations for each concentration, and the I_{50} (molarity of compound effecting 50% inhibition) was graphically determined. In most instances, the standard error computed according to

tution, a more effective inhibition is elicited, indicating that the affinity of the enzyme's esteratic site for the correspondingly substituted carbonyl function is increasing.

By applying these principles to the amide functions of these cholinesterase inhibitors, one may arrive at an

(19) G. W. Snedecor and W. G. Cochran, "Statistical Methods," 5th Ed., Iowa State College Press, Ames, Iowa, 1956, pp. 42–45.

(20) R. W. Taft, Jr., in "Steric Effects in Organic Chemistry," M. S. Newman, Ed., John Wiley and Sons, Inc., New York, N. Y., 1956, p. 591.

expected order of activity, expressed in terms of the $-NR_1R_2$ segment, paralleling that of the respective I_{50} values (see Table II). Indeed, the significant parallel of these relationships holds true when it is considered in three additional sets of compounds listed in Table III (VI and VII, XIV and XV, and XVII and XVIII).

An additional factor which should be considered in predicting comparative anticholinesterase activities of alkyl-substituted amides is the lipophilic–lipophobic nature of the substituent groups. As the N-substituted group becomes more fat soluble, the lipophilic–lipophobic ratio for the substituent should increase in the order $H < CH_3 < C_2H_5$. This leads to the same order of activity suggested earlier.

Our study of variations in the amide structure was extended to include cyclic pyrrolidide, piperidide, and morpholide substituents. As expected, the piperidides (Table III, compounds IX, XII, and XX) were in all instances more powerful inhibitors than the corresponding pyrrolidides (VIII, XI, and XIX). Likewise, as anticipated, the morpholides (X and XIII) were weaker inhibitors than the similarly constituted pyrrolidides or piperidides. Thus, replacement of a methylene group in the 4-position of the piperidine ring by an electronegative oxygen (or introduction of oxygen into the pyrrolidine ring) results in a marked lessening of activity. This may be due to two factors: (1) the ring oxygen competes with the electron-attracting part of the amide function for the electrons of the morpholide's alkylene units, and/or (2) the decreased lipophilic character of the inhibitor molecule resulting from replacement of the piperidide with the more polar morpholide.

Isomers containing the amide function in the 4-position (Table III, footnotes *d* and *f*, compounds XXIX and XXXI) were less effective inhibitors than their 3-substituted counterparts (V and VII); this is in accordance with Wilson and Quan's conclusion,[21] relative to interatomic distances between the pertinent reactive functions.

The 3,4-unsaturated analogs of derivatives V and VII (Table III, footnotes *c* and *e*, compounds XXVIII and XXX) were less effective than the corresponding saturated compounds, confirming our earlier findings[2] concerning the introduction of 3,4-unsaturation into the piperidine moiety.

An important objective of this study was to substantiate further the data obtained in our preceding experiments.[2] We consider it an important observation that, in each instance, (1) among the decanes the monosubstituted one is the more powerful inhibitor, and (2) among the ethanes the bis-substituted derivative has the more potent inhibitory action.

A more comprehensive interpretation of this work and that currently in progress will be reported at a later date.

Acknowledgment.—We wish to acknowledge valuable discussions with Dr. Andrew Lasslo which contributed to the successful completion of this phase of our study.

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Thyroxine Analogs. XI.[1] Structural Isomers of 3,5,3′-Triiodo-DL-thyronine

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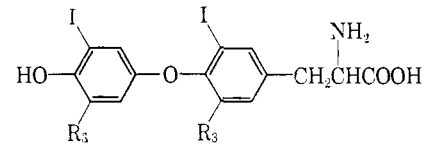
The synthesis of two structural isomers of 3,5,3′-triiodo-DL-thyronine is described, namely, 3-[2-(3-iodo-4-hydroxyphenoxy)-3,5-diiodophenyl]-DL-alanine (VIIa) and 3-[5-(3-iodo-4-hydroxyphenoxy)-2,4-diiodophenyl]-DL-alanine (VIIb). Both isomers were found to be inactive in an antigoiter assay in the rat which would have determined activity greater than 1.5% that of L-thyroxine.

The L-alanine side chains of the thyroid hormones, thyroxine (Ia) and triiodothyronine (Ib), have been replaced by a variety of ionizable groups with retention of thyroxine-like activity.[2] However, no such side-chain-substituted analog has proven more potent than the naturally occurring hormones with respect to the major physiological effects in the intact animal. Unusually high activity found for the aliphatic carboxylic acid side-chain congeners in inducing metamorphosis in tadpoles appears to be related to the absorption of the analogs from test solutions in which the tadpoles were immersed.[3] No analogs have been reported in which the side chain is moved to other positions on the same ring, although an approach to the synthesis of such isomers has been reported,[4] as have analogs with alanine side chains in both phenyl rings.[5]



Ia, $R_3 = R_3' = I$
b, $R_3 = I$; $R_3' = H$
c, $R_3 = R_3' = H$
d, $R_3 = H$; $R_3' = I$

The nature and position of substituents in the ring bearing the side chain ("inner ring") is important to

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