Pharmaceutical Research, Vol. 6, No. 6, 1989

Report

Short-Chain Alkyl Esters of L-Dopa as Prodrugs for Rectal Absorption

Joseph A. Fix,[1,2] Jose Alexander,[1] Margot Cortese,[1] Karen Engle,[1] Paula Leppert,[1] and Arnold J. Repta[1]

Received July 1, 1988; accepted January 30, 1989

The bioavailability of L-dopa following rectal administration of a series of short-chain alkyl esters of L-dopa was determined in rats and dogs. The esters were stable (>360 min) to hydrolysis in physiological buffer. *In vitro* enzymatic hydrolysis of the esters in plasma was species dependent, with the hydrolytic rate being faster in rat plasma ($t_{1/2}$ < 5 min) than dog plasma ($t_{1/2}$ = 68–181 min) or human plasma ($t_{1/2}$ = 96–238 min). *In vivo* hydrolysis in dogs, as indicated by the L-dopa plasma profile following intravenous administration of the esters, was very rapid (high extravascular esterase activity). Significant L-dopa bioavailability was observed in rats following rectal administration of the methyl (46%), ethyl (14%), isopropyl (48%), butyl (100%), and 4-hydroxybutyl (13%) esters of L-dopa (rectal L-dopa absorption, <5%). In dogs, significant L-dopa bioavailability was also observed for the methyl (28%), isopropyl (30%), butyl (32%), and 4-hydroxybutyl (34%) esters of L-dopa in the presence of carbidopa. The data indicate that these highly water-soluble (>600 mg/ml) esters of L-dopa are potential candidates for controlled-release rectal delivery systems designed to provide more constant plasma L-dopa levels.

KEY WORDS: L-dopa esters; prodrugs; rectal absorption; carbidopa; L-dopa.

INTRODUCTION

Levodopa (L-dopa), in the presence of an aromatic amino acid decarboxylase inhibitor, is the treatment of choice in the management of Parkinson's disease (1,2). Although significant alleviation of the clinical symptoms is observed with this regimen, fluctuations in response are often observed following chronic treatment (3–5). Typically encountered responses include the "on–off phenomenon," the "end-of-dose effect," and "peak-dose dyskinesia" (6). Although these response fluctuations do not correlate well with plasma L-dopa levels (7,8), clinical evidence indicates that intravenous L-dopa infusion, which results in more constant plasma L-dopa levels, provides significant mobility improvement in many patients (9,10). A more constant absorption and systemic availability of L-dopa may, therefore, lead to better management and more consistent symptomatic control for Parkinson's disease patients. When orally administered as standard treatment for Parkinson's disease, the absorption of L-dopa can be influenced by several factors. Since animal studies indicate that L-dopa is absorbed in the small intestine via a specific transport mechanism (11,12), competition from food constituents and variations in intestinal transit time may affect the extent and hence, reproducibility of absorption. An absorbable form of L-dopa which did not utilize the specific L-dopa transport system of the small intestine might provide more constant plasma L-dopa levels independent of the gastrointestinal (GI) absorption site and the presence of competitive nutrient amino acids.

In this study, the rectal absorption of a series of simple, aliphatic esters of L-dopa was examined in both rats and dogs. As esters, these compounds should not utilize the amino acid transport system but, rather, cross the GI barrier by passive diffusion. If postabsorptive esterase activity rapidly converts the esters to L-dopa, then this approach may provide a potential avenue for achieving systemic plasma L-dopa levels independent of GI transit and amino acid competition.

MATERIALS AND METHODS

Materials

Carbidopa and L-dopa were obtained from Merck Sharp and Dohme Research Laboratories. 3,4-Dihydroxybenzylamine was obtained from Aldrich Chemical Company. All other chemicals were reagent grade.

Animal Studies

Male Sprague–Dawley rats (200–250 g) and Beagle dogs (10–15 kg) were used in all experiments. In the rat studies, animals were anesthetized with sodium pentobarbital (Nembutal, 5.0 mg i.p./100 g body weight). All dosing solutions

[1] INTERx Research Corporation, Subsidiary of Merck & Co., Inc., 2201 West 21st Street, Lawrence, Kansas 66046.
[2] To whom correspondence should be addressed.

0724-8741/89/0600-0501$06.00/0

contained 0.1 mg/ml ascorbic acid and were adjusted to pH 5.5 to prevent oxidation of the catechol moiety. Gavage administration was used for oral dosing. For determination of the intravenous (i.v.) L-dopa plasma profile, each rat received a 250-μl i.v. injection containing 0.5 mg L-dopa and plasma samples were collected by repeated venipuncture from the external jugular vein at t = 0, 10, 20, 30, 45, 60, and 90 min. In the dog i.v. studies, each animal received a 1.0-ml i.v. injection containing 10 mg L-dopa and plasma samples were collected by repeated venipuncture from the saphenous vein at t = 0, 10, 20, 30, 60, 90, 120, 180, 240, and 300 min. In rectal studies, 250-μl solutions adjusted to pH 5.5 were administered with a 1-cm^3 syringe at an intrarectal depth of 2.5 cm. A spring clamp was employed to prevent leakage of the dosing solution from the rectal compartment. Following dosing, blood samples were collected at t = 15, 30, 60, 90, and 120 min by repeated venipuncture from the external jugular vein, and the plasma was isolated and frozen for high-pressure liquid chromatographic (HPLC) assay of L-dopa or L-dopa ester. When the plasma was to be analyzed for the esters, 3 mg/ml of sodium fluoride (NaF) was included in the blood collection tube to inhibit *ex vivo* esterase activity. In the dog studies, animals were not anesthetized. Rectal solutions (1 ml) were administered at an intrarectal depth of 4.5 cm. Blood samples were collected at t = 0, 15, 30, 60, 90, 120, 180, 240, 300, and 360 min by repeated venipuncture from the saphenous vein and processed as described for the rat studies.

Synthesis of L-Dopa Esters

The ethyl, isopropyl, hydroxypropyl, butyl, and 4-hydroxybutyl esters of L-dopa were prepared according to published procedures (13–15). The esters were isolated as crystalline hydrochloride salts (except the L-dopa ethyl ester HCl, which was an amorphous solid). The esters were characterized by NMR, melting point (except the amorphous ethyl ester), and microanalysis, and their purities verified by HPLC. Since the L-dopa hydroxypropyl ester HCl was synthesized from 1,2-propanediol, the product was a mixture of four isomers [diastereomers of (1-hydroxy)propyl ester contaminated with varying proportions of (2-hydroxy)propyl ester]. L-Dopa methyl ester HCl was purchased from Sigma Chemical Co. (St. Louis, Mo.).

In Vitro Hydrolysis of L-Dopa Esters

The time required for conversion of the L-dopa esters to L-dopa and the corresponding alcohols was determined by *in vitro* hydrolysis studies. Each ester was incubated at 10 μg/ml in 5 ml freshly isolated plasma (rat, dog, or human used within 30 min of collection). Incubation tubes were maintained at 37°C in a shaking water bath. The hydrolysis of the ethyl ester of L-dopa in phosphate buffer, pH 7.4, was also determined under identical experimental conditions. Aliquots (100 μl) were removed at 3-min intervals and assayed by HPLC for L-dopa and the L-dopa ester. The half-life for conversion of each L-dopa ester to L-dopa was calculated by a linear regresson fit of the L-dopa ester versus incubation time curve. These data and calculations were confirmed by determining the appearance rate of L-dopa in the hydrolysate.

Analysis of L-Dopa and L-Dopa Esters

L-Dopa and L-dopa esters were analyzed in buffer and plasma using HPLC with 3,4-dihydroxybenzylamine (3.0–7.7 μg/ml) as an internal standard. Assay variability was less than 5%. Samples (100 μl) of buffer or plasma were mixed with 100 μl of 0.1% H_3PO_4 and 200 μl of acetonitrile, then vortexed, and a centrifugation supernatant was isolated for HPLC analysis. A mobile phase of 10–30 mM $NaClO_4$, 0.1 g/liter ethylenediaminetetraacetic acid, and 20% methanol was used at a flow rate of 1.0–2.0 ml/min. The flow rate and $NaClO_4$ concentration were adjusted for optimization of the separation for each ester. Compounds were separated on a Analytichem SCX column (4.6 × 100 mm) with electrochemical detection (Environmental Science Associates 5100A detector). The sensitivity limit for L-dopa in plasma samples was 0.01 μg/ml. Plasma samples for L-dopa analysis were frozen immediately after plasma isolation and assayed within 2 weeks. L-Dopa degradation was not observable during this procedure. Samples for L-dopa ester determination were assayed immediately after blood collection and plasma isolation (samples contained 3 mg/ml NaF to inhibit *ex vivo* esterase activity).

Calculations and Statistics

The area under the plasma L-dopa concentration versus

Table I. Properties of L-Dopa Esters[a]

L-Dopa ester	MW	Yield (%)	m.p. (°C)	log P_{app}[b]	Aqueous solubility (mg/ml)
Methyl	247	—[c]	168–171	−0.20	790
Ethyl	261	—[d]	—[d]	—[e]	—[e]
Hydroxypropyl	291	57	200–201	—[e]	—[e]
Isopropyl	275	91	162–165	+0.57	782
Butyl	289	50	181–182	+1.19	674
4-Hydroxybutyl	305	46	145–147	−0.46	816

[a] HCL salts.
[b] Determined in octanol/0.05 M Tris–HCl buffer, pH 7.4.
[c] Purchased from Sigma Chemical Co., St. Louis, Missouri.
[d] Amorphous glassy solid.
[e] Not determined.

Table II. *In Vitro* Plasma Hydrolysis of L-Dopa Esters[a]

Plasma source	Half-life of conversion (min)[b]			
	Ethyl ester	Isopropyl ester	Butyl ester	4-Hydroxybutyl ester
Rat	5	4	<1	4
Dog	50	95	68	181
Human	100	238	96	105

[a] Starting concentration of L-dopa ester HCl = 10 μg/ml.
[b] Based on loss of esters as determined by HPLC.

time curve (AUC) was calculated by the trapezoid summation method with the plasma drug profile truncated at the last data point. Percentage L-dopa bioavailability was determined by comparison to intravenous (i.v.) administration of L-dopa. Since the volume of plasma required precluded using rats for more than one study, a mean i.v. AUC was used for rat studies. Individual i.v. AUCs were used in the dog studies. Statistical comparisons utilized Student's *t* test and analysis of variance (16).

RESULTS AND DISCUSSION

The synthetic yield and selected properties of the L-dopa esters are shown in Table I. With the exception of the ethyl ester, all compounds were isolated as crystalline HCl salts. Formation of the HCl salts of the L-dopa esters resulted in aqueous solubilities much greater than the solubility of L-dopa itself (1.65 mg/ml). An approximate 45-fold range in apparent partition coefficients was obtained via esterification of L-dopa with the various alkyl groups.

In order to be effective in delivering a therapeutic agent to the target system, the prodrug must be cleaved to the active parent drug in a timely manner. The conversion of four L-dopa esters to L-dopa was examined in an *in vitro* plasma hydrolysis experiment and the results are shown in Table II. A significant species difference in hydrolytic rate was observed. The enzymatic hydrolysis of the L-dopa esters to L-dopa was much faster in rat plasma than dog or human plasma. A similar pattern of species difference in plasma esterase activity has been reported with the succinimidoethyl ester of methyldopa as the substrate (17). In the present studies, a conversion half-life of >360 min was observed for the ethyl ester of L-dopa in isotonic phosphate buffer, pH 7.4, indicating that chemical instability cannot account for the rapid conversion seen in the plasma hydrolysis experiments.

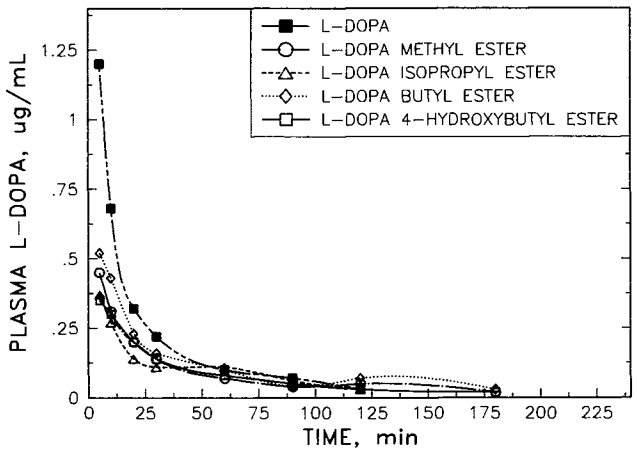

Fig. 1. Plasma L-dopa levels in dogs following intravenous administration of 10-mg L-dopa equivalents per animal. Dogs were not pretreated with carbidopa. The results are the mean plasma L-dopa profile from three dogs.

Since the L-dopa esters were converted to L-dopa very rapidly in rat plasma, analysis of L-dopa in rat plasma during *in vivo* experiments should accurately reflect absorption of the L-dopa esters, provided at least one of two conditions are met. If the L-dopa esters are not converted to L-dopa prior to absorption or L-dopa itself is not significantly absorbed, then analysis of plasma L-dopa can be employed to estimate L-dopa ester absorption. In rats, the rectal absorption of L-dopa, in either the presence or the absence of carbidopa, was less than 5%. Therefore, in the rat model, plasma L-dopa following rectal administration of L-dopa esters reflects absorption of the esters themselves.

A slightly different situation exists in dogs, where the plasma conversion of L-dopa esters to L-dopa was much slower ($t_{1/2}$ = 50–181 min) than that seen in rats ($t_{1/2}$ < 5 min). If the *in vivo* conversion rate of L-dopa esters to L-dopa in the dog is much slower than the absorption rate, then the plasma profile obtained following rectal administration would be a composite of both absorption and conversion contributions. To clarify this point, several L-dopa esters were administered by intravenous (i.v.) injection and the resultant L-dopa plasma profile compared to i.v. L-dopa administration (Fig. 1). The similar plasma L-dopa profiles for i.v. L-dopa ester and L-dopa administration indicated that, even though the *in vitro* ester hydrolysis is relatively slow in dog plasma ($t_{1/2}$ = 50–181 min), the *in vivo* breakdown to

Table III. Systematic Bioavailability of L-Dopa Following Intravenous Administration of L-Dopa Esters to Beagle Dogs[a]

L-Dopa ester	Percentage systematic bioavailability ($\bar{X}$ ± SD; N = 3)[b]
Methyl	66 ± 15.4
Isopropyl	82 ± 15.4
Hydroxypropyl	61 ± 8.8
Butyl	58 ± 12.7
4-Hydroxybutyl	44 ± 3.8

[a] Dogs not pretreated with carbidopa. Each dog received 10-mg equivalents of L-dopa as the ester prodrug in an injection volume of 1 ml.
[b] Determined relative to intravenous L-dopa administration.

Table IV. L-Dopa Bioavailability in Rats Following Rectal Administration of L-Dopa and L-Dopa Esters in the Presence or Absence of Carbidopa[a]

L-Dopa or L-dopa ester[b]	L-Dopa bioavailability (%) ($\bar{X}$ ± SD)	
	No carbidopa[c]	With carbidopa[d]
L-Dopa[e]	3 ± 2	4 ± 6
L-Dopa methyl ester	46 ± 12	17 ± 7
L-Dopa ethyl ester	14 ± 3	27 ± 14
L-Dopa isopropyl ester	48 ± 17	28 ± 13
L-Dopa hydroxypropyl ester	8 ± 3	7 ± 1
L-Dopa butyl ester	100 ± 13	51 ± 45
L-Dopa 4-hydroxybutyl ester	13 ± 6	9 ± 1

[a] Each animal received 2 mg L-dopa or L-dopa equivalents in the presence or absence of 0.5 mg carbidopa.
[b] Esters as HCl salts.
[c] Bioavailabilities calculated relative to intravenous administration of 2 mg L-dopa.
[d] Bioavailabilities calculated relative to intravenous administration of 2 mg L-dopa and 0.5 mg carbidopa.
[e] Bioavailabilities for L-dopa represent $N = 8$ determinations without carbidopa and $N = 12$ determinations with carbidopa. All other values represent $N = 3$ determinations.

L-dopa is very rapid. If this were not the case, the shape of the plasma L-dopa profile following i.v. L-dopa ester administration would be significantly prolonged due to a slow conversion of the esters to L-dopa. An additional line of evidence supporting a very rapid *in vivo* hydrolysis of the esters to L-dopa is that L-dopa esters were not detectable by HPLC in plasma samples taken 10 min after i.v. L-dopa ester administration. This rapid conversion is most likely due to ester cleavage at nonvascular sites, notably the liver and small intestine, which are rich in esterase activity (18). This rapid rate of conversion of L-dopa esters to L-dopa in the dog permits estimation of ester absorption on the basis of plasma L-dopa levels (the plasma L-dopa level at any time will accurately reflect the total amount of L-dopa ester absorbed).

Although *in vivo* ester hydrolysis appeared rapid in dogs, some elimination or metabolism of the esters apparently occurs prior to complete conversion of the esters to L-dopa. This was evident from examining the systemic bioavailability of L-dopa following i.v. administration of the L-dopa esters (Table III). None of the esters afforded systemic L-dopa bioavailabilities near 100% compared to i.v. L-dopa administration. The elimination half-life for L-dopa following these i.v. injections remained constant, ranging from 21 ± 1.2 min (mean ± SD) for the 4-hydroxybutyl ester to 25 ± 5.4 min for the hydroxypropyl ester. The facts that similar L-dopa elimination rates were observed following various i.v.

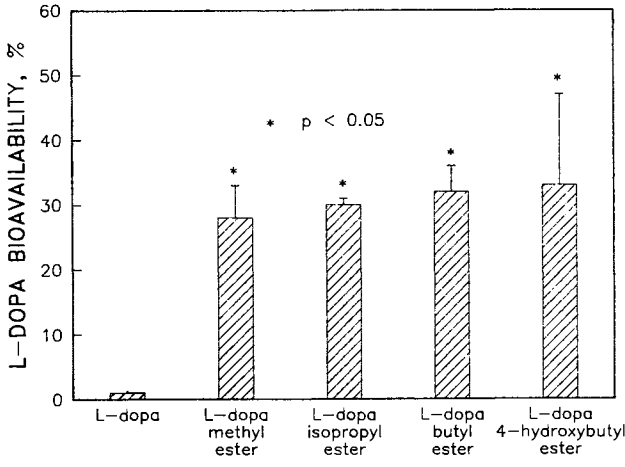

Fig. 2. Percentage L-dopa bioavailability in dogs following rectal administration of 50 mg L-dopa equivalents in the presence of 12.5 mg carbidopa. Dogs were treated for 3 days prior to the study and on the study day with oral carbidopa (25 mg b.i.d.). The results are the means ± SD for three determinations. Asterisks indicate statistically significant differences at the $P < 0.05$ level compared to L-dopa administration (analysis of variance).

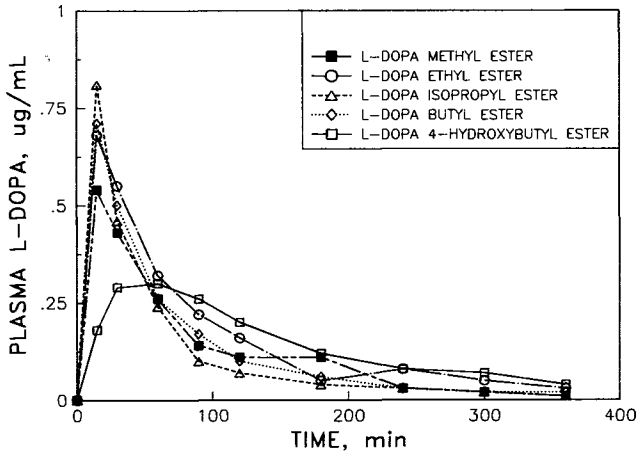

Fig. 3. Plasma L-dopa profile in dogs following rectal administration of 50-mg L-dopa equivalents in the presence of 12.5 mg carbidopa. Dogs were treated for 3 days prior to the study and on the study day with oral carbidopa (25 mg b.i.d.). The results are the mean profiles for three determinations.

ester injections and that all the esters were very rapidly converted to L-dopa following i.v. administration indicated that differences in L-dopa AUC and bioavailability were likely due to varying metabolic disposition or tissue distribution of the esters.

Initial bioavailability studies examined the rectal absorption in rats of a series of L-dopa esters in the presence and absence of carbidopa. With the exception of the hydroxypropyl ester, all the L-dopa esters resulted in L-dopa bioavailabilities significantly greater than that obtained with L-dopa itself (Table IV). The fact that the calculated L-dopa bioavailability is generally less in the presence of carbidopa probably reflects the relative level of decarboxylase activity in rectal tissue and plasma and poor rectal absorption of carbidopa (19). With low decarboxylase activity in rectal tissue and poor rectal carbidopa absorption, rectal administration of carbidopa should not significantly increase the rectal L-dopa area under the curve (AUC) determination. In contrast, including carbidopa in the i.v. administration of L-dopa will inhibit systemic decarboxylase activity and significantly increase the i.v. L-dopa AUC. These relative effects would cause an apparent decrease in rectal L-dopa bioavailability in the presence of carbidopa since the calculation of bioavailability compares rectal AUC with i.v. AUC. It is clear, however, from these initial studies that esterification of L-dopa with short-chain alkyls provides a prodrug with significantly enhanced rectal absorption.

The rectal absorption of L-dopa esters was also examined in dogs. Since the synthesis of the hydroxypropyl ester of L-dopa resulted in a combination of four isomers, this compound was excluded from the dog studies. It is apparent from the data in Fig. 2 that rectal administration of all four L-dopa esters resulted in L-dopa bioavailabilities greater than that observed for L-dopa itself ($P < 0.05$ compared to L-dopa itself by analysis of variance). Examination of the L-dopa plasma profiles following rectal administration of the esters in dogs (Fig. 3) indicated a somewhat unusual pattern following 4-hydroxybutyl ester absorption. With the exception of the 4-hydroxybutyl ester, the plasma C_{max} occurred at 15 min (earliest plasma sample obtained) for all the rectally administered esters. The plasma L-dopa profile for the 4-hydroxybutyl ester was clearly more protracted, with peak L-dopa plasma levels occurring at 60 min. Since it was previously shown that the 4-hydroxybutyl ester was rapidly converted to L-dopa *in vivo* (i.v. administration; Fig. 1), the prolonged elevation of L-dopa levels in the rectal studies probably suggests a slower absorption rate than that observed for the other esters. A slower absorption rate for the 4-hydroxybutyl ester might prove to be a useful advantage in terms of designing a sustained-release formulation to achieve more constant circulating L-dopa levels.

The data reported here indicate that short-chain alkyl esters of L-dopa are characterized by significant rectal absorption and rapid conversion of the ester prodrug to the parent compound in the systemic circulation. Given the high level of esterase activity in the small intestine (18), the utility of these compounds as candidates for nonparenteral administration is limited to the rectal route. Cooper *et al.* have shown in animal studies that oral administration of a series of L-dopa esters results in behavioral activity which is not markedly greater than after oral administration of L-dopa itself (20). These results likely reflect rapid hydrolysis of the L-dopa esters to L-dopa in the small intestine. The use of rectal L-dopa esters may provide a distinct advantage in terms of designing a sustained-release system for delivering L-dopa to the systemic circulation in therapeutic doses, while minimizing elevated plasma peak values which are often implicated in the side effects of L-dopa therapy.

REFERENCES

1. M. E. Jaffe. *Adv. Neurol.* 2:161–172 (1973).
2. K. Ghose. *Drugs Today* 10:463–471 (1985).
3. R. D. Sweet and F. H. McDowell. *Neurology* 24:953–956 (1974).
4. M. D. Yahr. *Neurology* 24:431–437 (1974).
5. E. S. Tolosa, W. E. Martin, H. P. Cohen, and R. L. Jacobson. *Neurology* 25:177–183 (1975).
6. U. K. Rinne. *Acta Neurol. Scand. (Suppl.)* 95:19–26 (1983).
7. M. D. Muenter and G. M. Tyce. *Mayo Clin. Proc.* 46:231–239 (1971).
8. J. B. Pilling, J. Baker, L. L. Iversen, S. D. Iversen, and T. J. Robbings. *Neurol. Neurosurg. Psychiat.* 38:129–135 (1975).
9. I. R. A. Shoulson, G. A. Glaubiger, and T. N. Chase. *Neurology* 25:1144–1148 (1975).
10. N. Quinn, C. D. Marsden, and J. D. Parkes. *Lancet* 2:412–415 (1982).
11. H. Shindo, T. Komai, and K. Kawai. *Chem. Pharm. Bull. (Tokyo)* 21:2031–2038 (1973).
12. D. N. Wade, P. T. Mearrick, and J. Morris. *Nature* 242:463–465 (1973).
13. C. M. Lai and W. D. Mason. *J. Pharm. Sci.* 62:510–511 (1973).
14. C. Marrel, G. Boss, H. Van de Waterbeemd, and B. Testa. *Eur. J. Med. Chem.* 20:459–465 (1985).
15. C. Marrel, G. Boss, B. Testa, H. Van de Waterbeemd, D. Cooper, P. Jenner, and C. D. Marsden. *Eur. J. Med. Chem.* 20:467–470 (1985).
16. J. E. Freund. *Mathematical Statistics,* Prentice-Hall, Englewood Cliffs, N.J., 1962.
17. S. Vickers, C. A. Duncan, S. D. White, G. O. Breault, R. B. Boyds, P. J. De Schepper, and K. F. Tempero. *Drug Metab. Disp.* 6:640–646 (1978).
18. M. Inoue, M. Morikawa, M. Tsuboi, and M. Sugiura. *Jap. J. Pharmacol.* 29:9–16 (1979).
19. P. S. Leppert, M. Cortese, and J. A. Fix. *Pharm. Res.* 5:587–591 (1988).
20. D. R. Cooper, C. Marrel, H. Van De Waterbeemd, B. Testa, P. Jenner, and C. D. Marsden. *J. Pharm. Pharmacol.* 39:627–635 (1987).