



(86) Date de dépôt PCT/PCT Filing Date: 1997/01/29

(87) Date publication PCT/PCT Publication Date: 1997/08/07

(45) Date de délivrance/Issue Date: 2004/10/05

(85) Entrée phase nationale/National Entry: 1998/07/23

(86) N° demande PCT/PCT Application No.: US 1997/001728

(87) N° publication PCT/PCT Publication No.: 1997/028165

(30) Priorité/Priority: 1996/01/30 (08/594,235) US

(51) Cl.Int.⁶/Int.Cl.⁶ C07D 491/22, A61K 31/47

(72) Inventeurs/Inventors:

ZHISONG, CAO, US;
GIOVANELLA, BEPPINO C., US

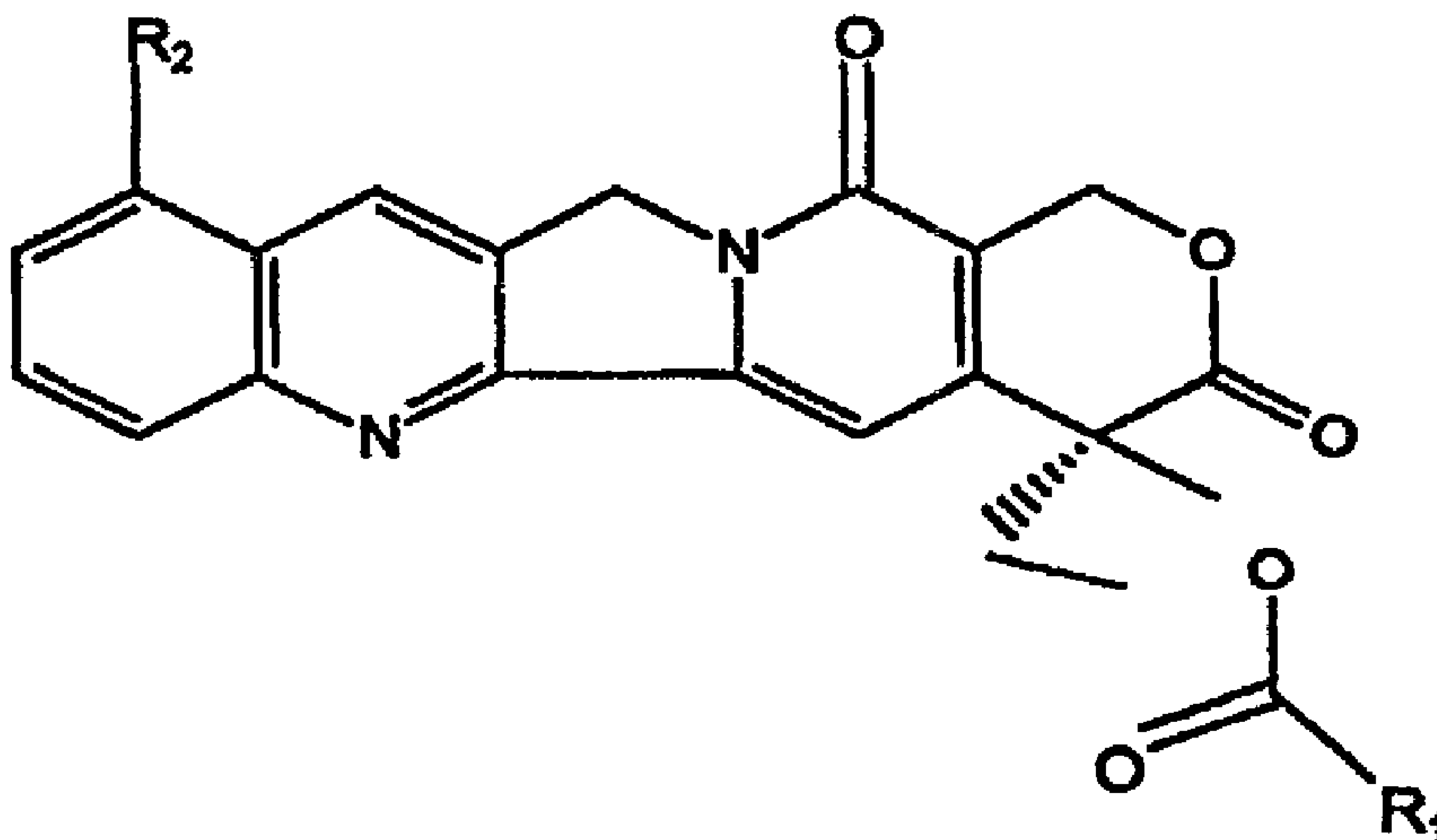
(73) Propriétaire/Owner:

THE STEHLIN FOUNDATION FOR CANCER
RESEARCH, US

(74) Agent: DIMOCK STRATTON CLARIZIO LLP

(54) Titre : DERIVES DE CAMPTOTHECINE UTILISES DANS LE TRAITEMENT DU CANCER

(54) Title: DERIVATIVES OF CAMPTOTHECIN FOR USE IN TREATING CANCER



(57) Abrégé/Abstract:

Derivatives of camptothecin are disclosed and are represented by general formula (I), wherein when R₂ is H, R₁ is a C₂-C₄ alkyl group, a C₆-C₁₅ alkyl group, a C₃-C₈ cycloalkyl group, a C₂-C₁₅ alkenyl group or a C₂-C₁₅ epoxy group; and when R₂ is a nitro group, R₁ is a C₁-C₁₅ alkyl group, a C₂-C₁₅ alkenyl group, a C₃-C₈ cycloalkyl group, or an epoxy group. Processes for making these derivatives and for using them in cancer treatment are also disclosed.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁶ : C07D 491/22, A61K 31/47</p>	A1	<p>(11) International Publication Number: WO 97/28165</p> <p>(43) International Publication Date: 7 August 1997 (07.08.97)</p>
<p>(21) International Application Number: PCT/US97/01728</p> <p>(22) International Filing Date: 29 January 1997 (29.01.97)</p> <p>(30) Priority Data: 08/594,235 30 January 1996 (30.01.96) US</p> <p>(60) Parent Application or Grant (63) Related by Continuation US 08/594,235 (CIP) Filed on 30 January 1996 (30.01.96)</p> <p>(71) Applicant (for all designated States except US): THE STEHLIN FOUNDATION FOR CANCER RESEARCH [US/US]; Suite 1818, 1315 Calhoun, Houston, TX 77002 (US).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): ZHISONG, Cao [CN/US]; 16123 Affirmed Way, Friendswood, TX 77546 (US). GIOVANELLA, Beppino, C. [US/US]; 1325 F Post Oak Park Drive, Houston, TX 77027 (US).</p>	<p>(74) Agents: ARMSTRONG, James, E., III et al.; Armstrong, Westerman, Hattori, McLeland & Naughton, Suite 1000, 1725 K Street, Washington, DC 20006 (US).</p> <p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</p>	
<p>(54) Title: DERIVATIVES OF CAMPTOTHECIN FOR USE IN TREATING CANCER</p>		
<p>(57) Abstract</p> <p>Derivatives of camptothecin are disclosed and are represented by general formula (I), wherein when R₂ is H, R₁ is a C₂-C₄ alkyl group, a C₆-C₁₅ alkyl group, a C₃-C₈ cycloalkyl group, a C₂-C₁₅ alkenyl group or a C₂-C₁₅ epoxy group; and when R₂ is a nitro group, R₁ is a C₁-C₁₅ alkyl group, a C₂-C₁₅ alkenyl group, a C₃-C₈ cycloalkyl group, or an epoxy group. Processes for making these derivatives and for using them in cancer treatment are also disclosed.</p>	<p style="text-align: right;">(I)</p>	

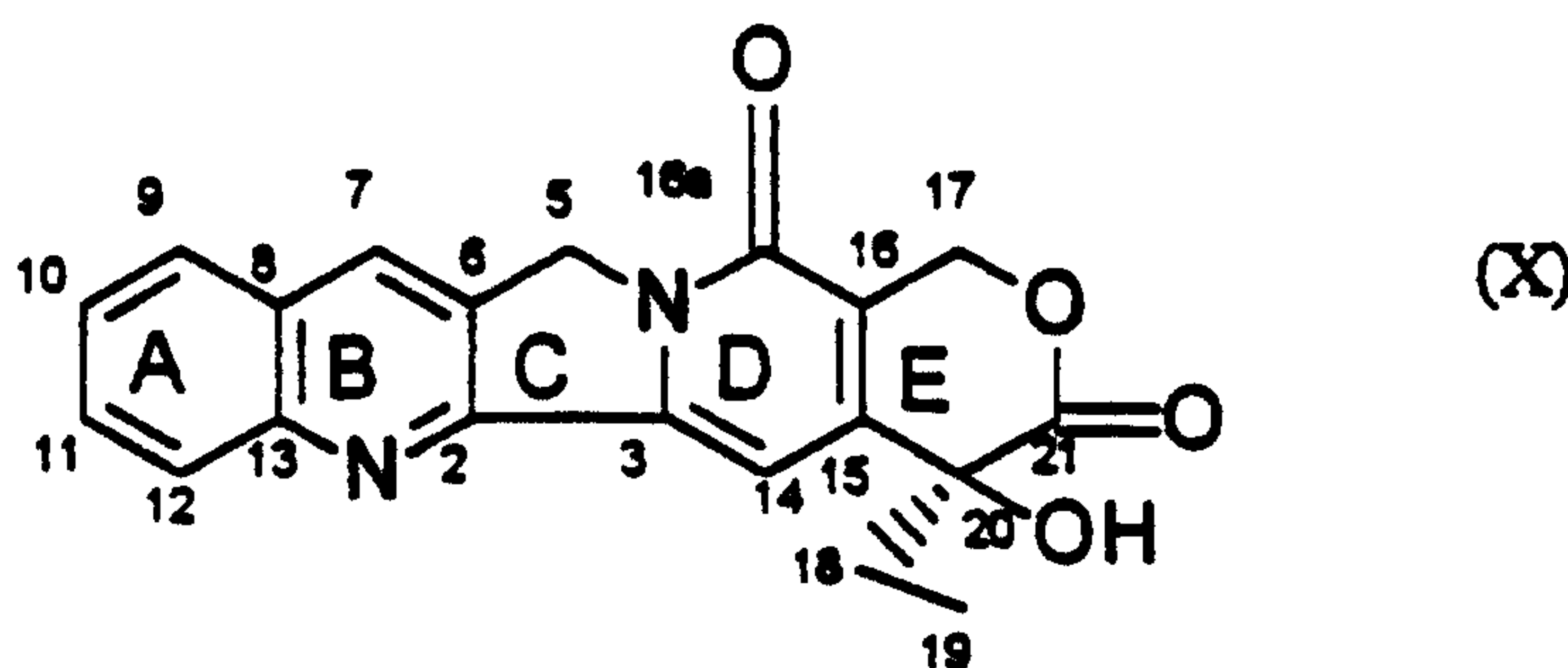
DERIVATIVES OF CAMPTOTHECIN FOR USE IN TREATING CANCER.

FIELD OF THE INVENTION

The present invention is directed to derivatives of camptothecin, preferably having low toxicity, and to the use of these derivatives for cancer treatment.

BACKGROUND OF THE INVENTION

Camptothecin, a cytotoxic alkaloid first isolated from the wood and bark of *Camptotheca Acuminata* (*Nyssaceae*) by Wall and his coworkers (*J. Am. Chem. Soc.* 88, 3888, 1966), was shown to have antitumor activity against the mouse leukemia L 1210 system. The structure of camptothecin, an alkaloid which has a commonly occurring indole alkaloid group (Heckendorf et al, *J Org. Chem.* 41, 2045, 1976), is shown below as Formula (X).



This compound has a pentacyclic ring system with only one asymmetrical center in ring E with a 20(S)-configuration. The pentacyclic ring system includes a pyrrolo [3, 4 - b] quinoline moiety (rings A, B and C), a conjugated pyridone (ring D), and a six-membered lactone (ring E) with an α -hydroxyl group. Camptothecin was of great interest from the time of its initial isolation due to its noteworthy activity in the mouse leukemia L 1210 system. Earlier data for the antitumor activity of camptothecin were obtained by employing experimentally transplanted malignancies such as leukemia L 1210 in mice, or Walker 256 tumor in rats (*Chem. Rev.* 23, 385, 1973. *Cancer Treat.*

-2-

Rep. 60, 1007, 1967). Subsequent clinical studies showed that this compound was not usable as an anticancer agent *in vivo* due to its high toxicity. Camptothecin itself is insoluble in water. Therefore, camptothecin was evaluated clinically as a water-soluble sodium carboxylate salt in the early times. This form of camptothecin produced severe toxicity and seemed devoid of anticancer activity (Gottlieb et al, *Cancer Chemother. Rep. 54, 461, 1970*, and *56, 103, 1972*, Muggia et al, *Cancer Chemother. Rep. 56, 515, 1972*, Moertel et al, *Cancer Chemother. Rep. 56, 95, 1972*, and Schaeppi et al, *Cancer Chemother. Rep. 5:25, 1974*). These results caused the discontinuation of phase II trials. Continued evaluation of this agent showed that the sodium carboxylate salt is only 10% as potent as the native camptothecin with the closed lactone ring intact (Wall et al, *In International Symposium on Biochemistry And Physiology of The Alkaloids, Mothes et al, eds, Academie - Verlag, Berlin, 77, 1969*, Giovanella et al, *Cancer res. 51, 3052, 1991*). In addition, important parameters for antitumor activity in the camptothecin family have been established (Wall et al, *Ann. Rev., Pharmacol. Toxicol. 17, 117, 1977*). These results indicate that intact lactone ring E and α -hydroxyl group are essential for antitumor activity.

In 1989, Giovanella et al. found that some of the non-water soluble derivatives of camptothecin have high antitumor activity against xenograft of human tumors (Giovanella et al., *Science, 246, 1046, 1989*). It has also been shown that administration of camptothecin with closed lactone ring is superior to injections of water-soluble carboxylate salt (Giovanella et al, *Cancer Res., 51, 3052, 1991*). These findings further confirmed the importance of the intact lactone ring.

Clearly, there is a need to modify 20(S)-camptothecin ("CPT") to enable the lactone form to stay longer in the body while retaining the structural elements (i.e. 20-hydroxyl and lactone ring E) which are essential for its antitumor activity.

Ring opening of CPT leads to much more potent anticancer activity in mice than in humans. In effect, CPT administered intramuscularly ("i.m."), subcutaneously ("s.c."), and intrastomach ("i.s.") has proved to be a very potent anticancer agent against human tumors in mice, *i.e.*, when growing as xenotransplants in nude mice (Giovanella *et al*, *Cancer Res. 51:3052, 1991*). However, when tumors were treated

-3-

with CPT in humans, a lower degree of anticancer activity in humans, than in mice, was exhibited (Stehlin *et al.*, In *Camptothecins: New Anticancer Agents*, 1995, CRC Press, pp. 59-65).

The same phenomenon was observed with other CPT derivatives. In mice, 9-nitrocamptothecin ("9NC") has proven to be 2-3 times more potent than CPT against human tumor xenografts causing the total eradication of all the human malignancies treated (Pantazis *et al.*, *Cancer Res.* 53:1577, 1993; Pantazis *et al.*, *Int. J. Cancer* 53:863, 1995).

Pharmacological studies demonstrated that the majority (57%) of the 9NC drug present in the plasma after i.s. administration is in the closed lactone form (Fig. 3). Pharmacological studies on the plasma levels of 9NC after oral administration to Phase I clinical trial patients demonstrate that, on average, only ~3% of the drug present is in the closed lactone form (Figs. 4 and 5).

In perfect agreement with such findings, the clinical responses in this group of patients, although higher than those obtained with CPT are still a far cry below the results obtained in mice (32/32 complete tumor regressions in mice versus 2/32 in humans). Clearly, again there is a pressing need for a modification which will slow and delay the lactone ring opening upon its entrance into the blood circulation.

A number of attempts have made to provide more active derivatives of camptothecin, but none of these compounds has been disclosed to be able to delay the opening of the lactone ring E.

SUMMARY OF THE INVENTION

Accordingly, it is an object of the present invention to provide new CPT derivatives which are effective antitumor agents, preferably useful for the oral and intramuscular routes of drug administration.

It is another object of the present invention to provide new active CPT derivatives which sustain the opening of the lactone ring E, which makes the antitumor activity last longer than its mother analog, CPT.

-4-

It is still another object of the present invention to provide new CPT derivatives which retain significant antitumor activity as does the mother compound, CPT, and have much lower toxicity than its mother compound.

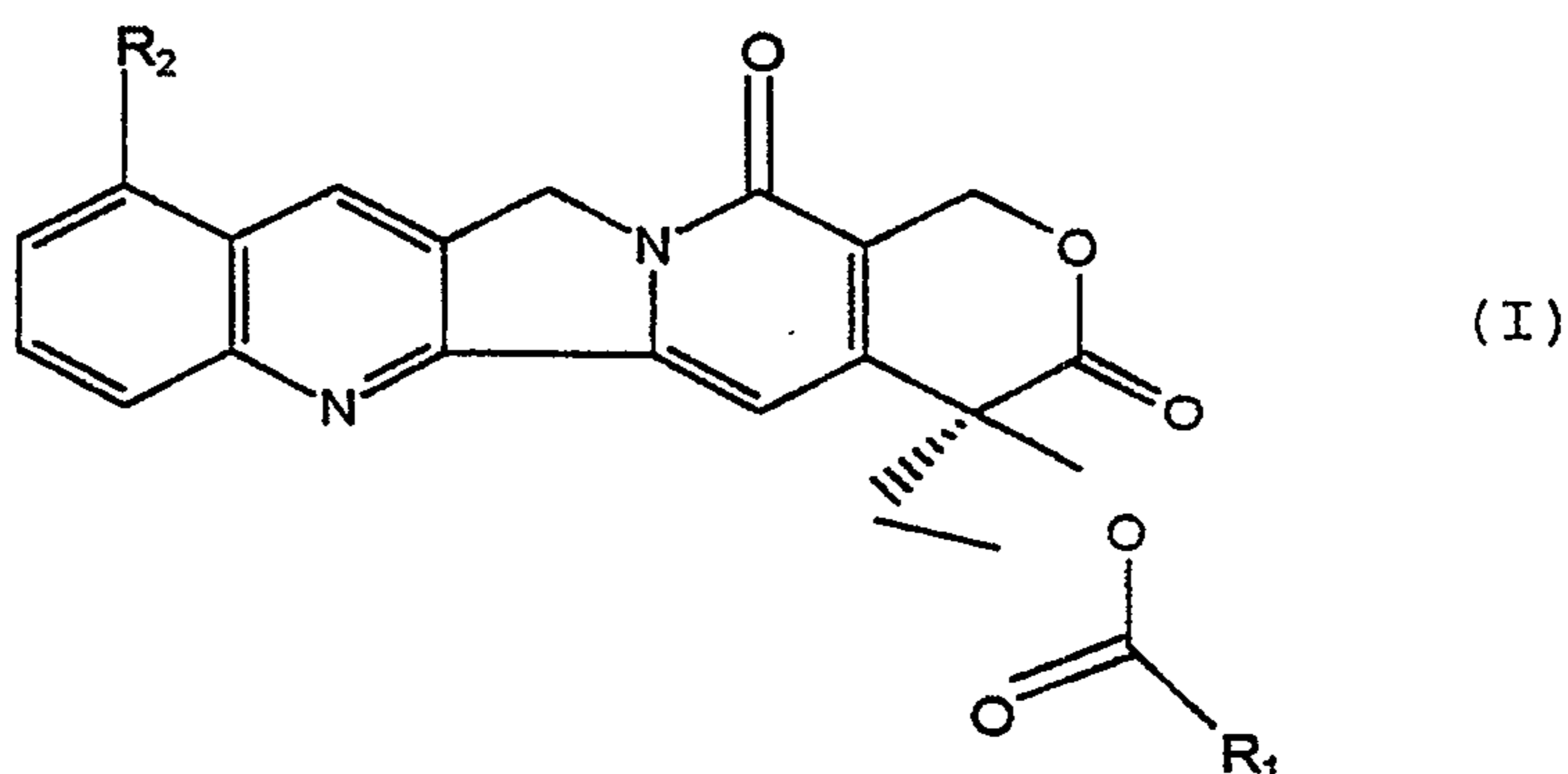
It is still another object of the present invention to provide new CPT derivatives possessing good absorbability in the living body.

It is a further object of the present invention to provide new CPT derivatives which retain the lactone ring E and the 20 - hydroxyl group intact, which are important for antitumor activity.

It is still a further object of the present invention to provide a method for preparing CPT derivatives.

Additional objects and advantages of the present invention will be set forth in part in the description which follows, and in part will be apparent from the description, or may be learned by practice of the present invention. The objects and advantages of the present invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims.

To achieve the objects and in accordance with the purpose of the present invention, as embodied and broadly described herein, the present invention relates to a compound of formula (I):



wherein R_2 is H or NO_2 , and R_1 is a $\text{C}_2\text{-C}_4$ alkyl group, a $\text{C}_6\text{-C}_{15}$ alkyl group, a $\text{C}_3\text{-C}_8$ cycloalkyl group, a $\text{C}_2\text{-C}_{15}$ alkenyl group, or an epoxy group when R_2 is H; and R_1 is a

-5-

C₁-C₁₅ alkyl group, a C₁-C₁₅ alkenyl group, a C₃-C₈ cycloalkyl group, or an epoxy group when R₂ is NO₂.

The invention also relates to a method for treating malignant tumors in a mammal and comprises administering an effective amount of one or more of the above compounds.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph showing the presence of CPT in the closed lactone form in mice after intrastomach administration.

Fig. 2 is a graph showing the amount of CPT and its closed lactone form in a human after oral administration.

Fig. 3 is a graph of the amount of 9-nitrocamptothecin and its closed lactone ring form in a mouse after oral administration.

Figs. 4 and 5 show the amount of 9-nitrocamptothecin and its closed lactone ring in clinical trial patients who received the dosage by oral administration.

Fig. 6 is a graph representing the antitumor activity of camptothecin 20(S) propionate in an implanted human breast carcinoma CLO in nude mice.

Fig. 7 is a graph showing the presence of camptothecin 20(S) propionate and its closed lactone ring form in human plasma after oral administration.

Fig. 8 is a graph showing the presence of 9-nitrocamptothecin-20-O-propionate in a patient who received the compound orally.

DETAILED DESCRIPTION OF THE INVENTION

The metabolism studies of camptothecin in human plasma carried out in the laboratory showed that the only metabolite detected is the ring-opened sodium carboxylate salt which is toxic and inactive. The measurement of pharmacokinetics for CPT in human plasma indicates that the half-life time of the drug with lactone intact is 30 min. These results implies that the drug will lose 90% of its activity and produce a lot of toxicities in very short time after the patients take it.

-6-

Comparative pharmacological studies in mice and humans have demonstrated that in mice the majority of the CPT present in the plasma after intrastomach administration is of the closed lactone form (Fig. 1), approximately 54% of the area under the curve. In humans, on the contrary, only about 0.4% of the area under the curve after oral administration of CPT is in the form of closed ring lactone (Fig. 2).

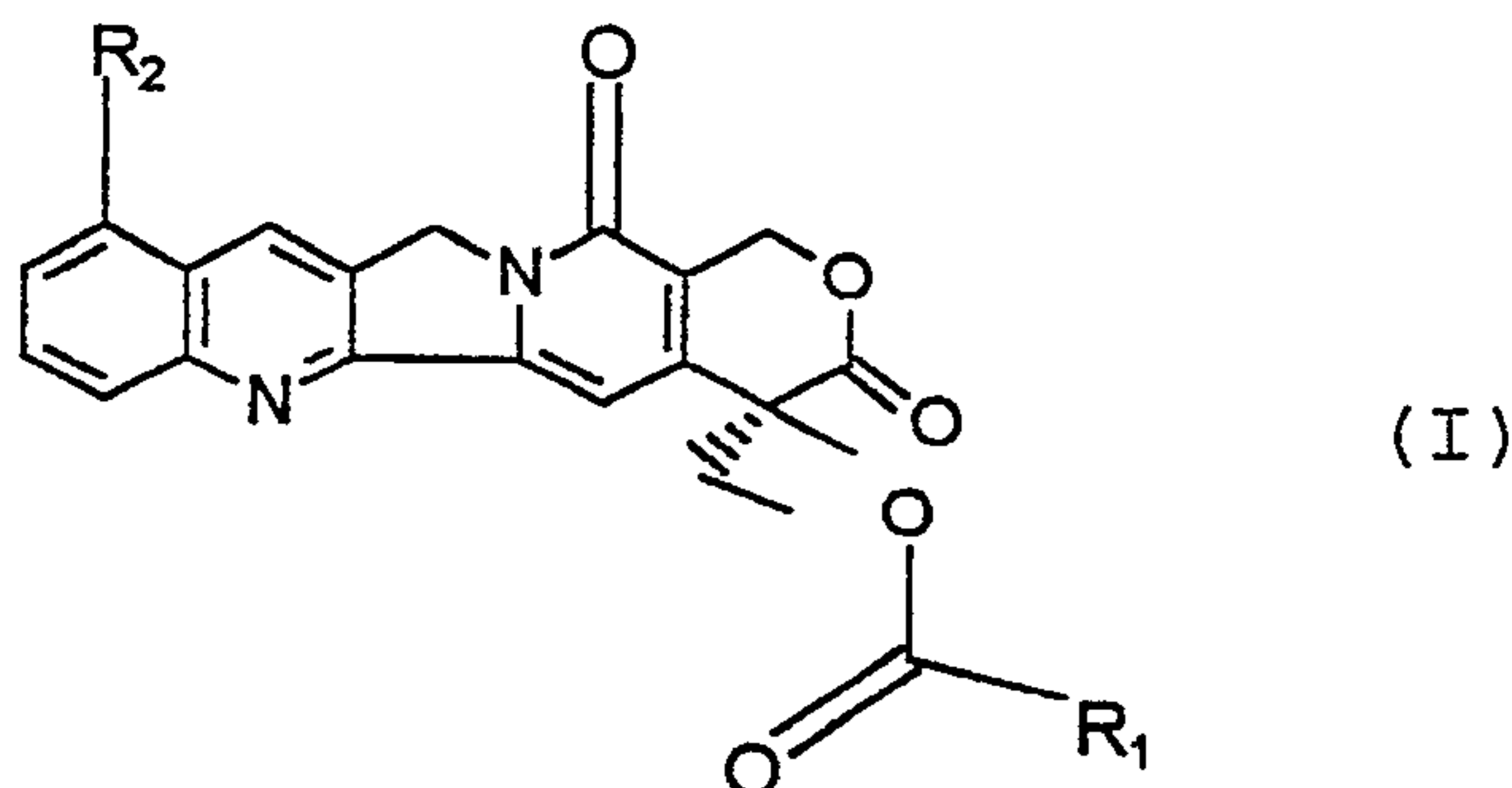
This difference between a mouse and a human is caused by the fact that although the blood pH of the mouse and human are the same, *i.e.*, 7.4, the human albumin, which catalyzes the conversion of CPT into its sodium salt is ~ 100 times more efficient in this process than mouse albumin (Mi and Burke, *Biochem.* 33:12540, 1994).

According to the present invention, CPT is converted into more lipo-soluble molecules, hereinafter, also called prodrugs. When taken orally by patients, the prodrugs are rapidly introduced into the bloodstream of the patients and are readily converted to the parent compound in the body.

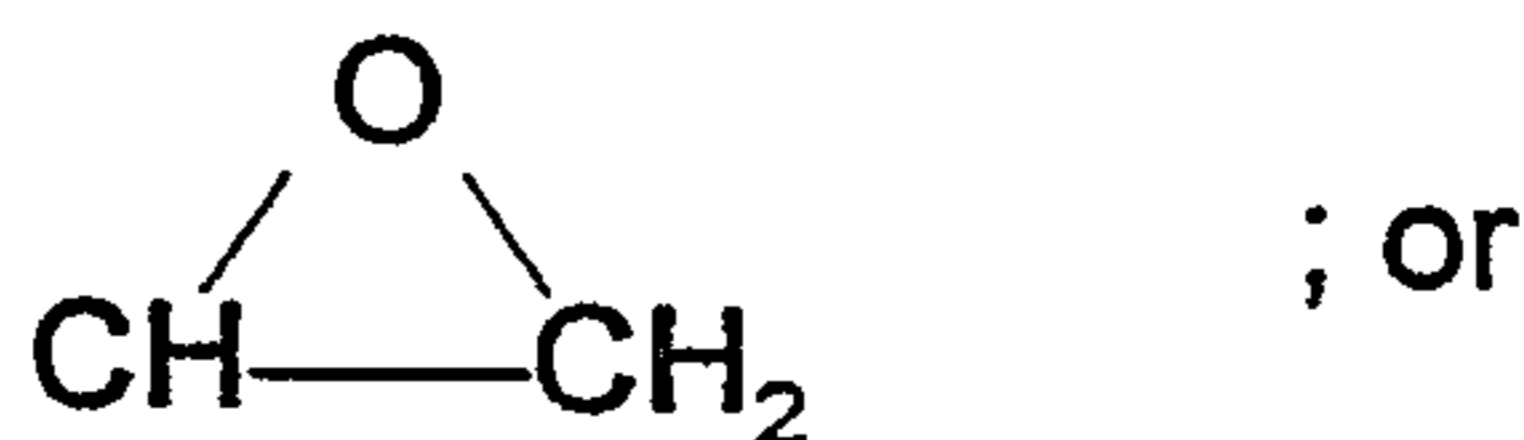
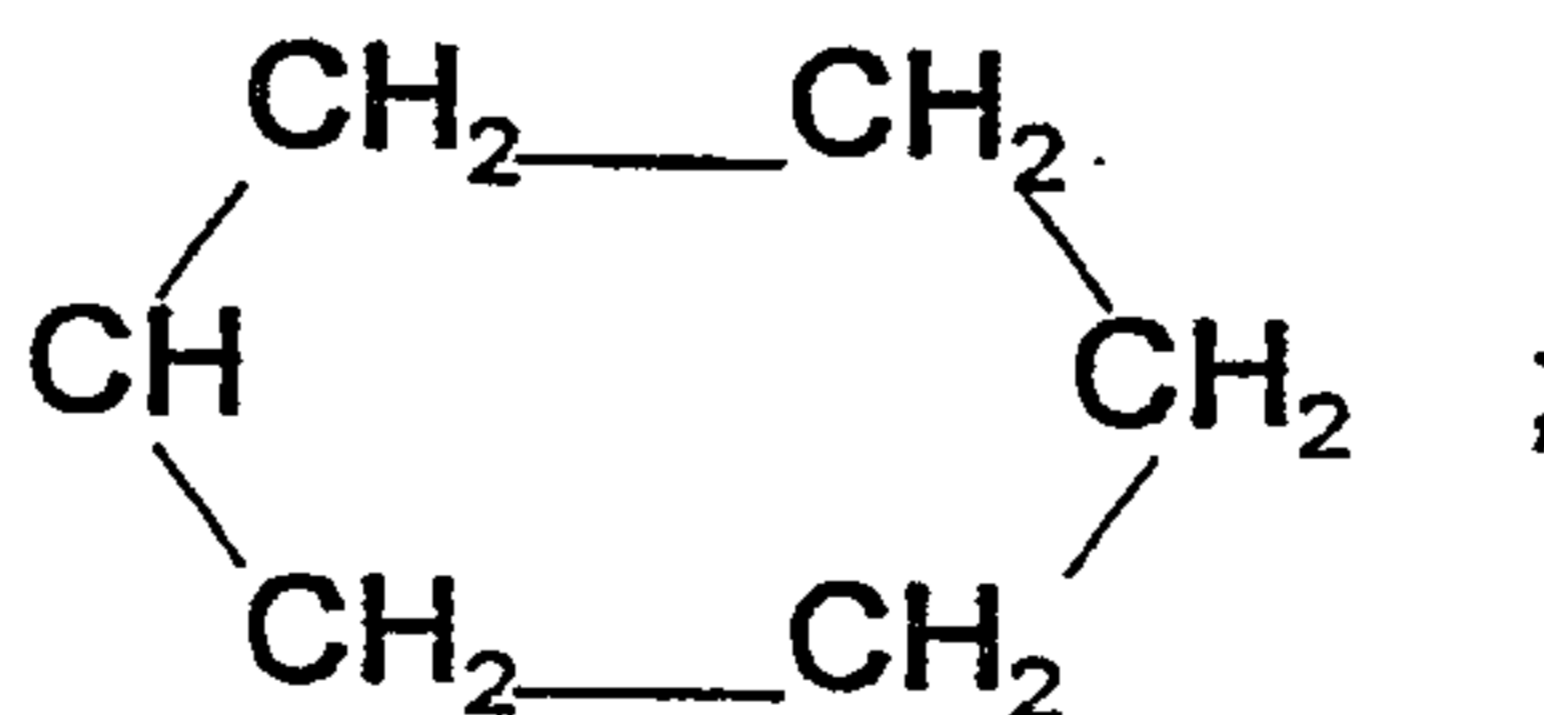
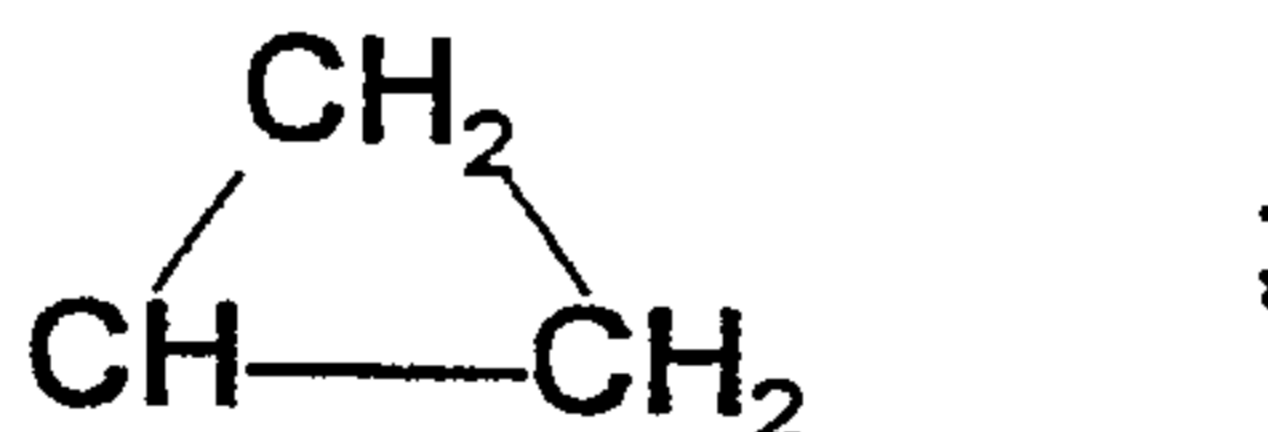
Conversion of the prodrugs to the mother compound, CPT, is mediated by a group of enzymes called esterases present in the blood of many animals, including humans. Since the prodrugs are rapidly distributed throughout the body in a short period of time after delivery, these compounds exist at a very low concentration at the time they undergo enzymatic hydrolysis by which the mother camptothecin is released, which prevents CPT from precipitating in the bloodstream.

In an attempt to synthesize new CPT derivatives with extremely reduced toxicity, while maintaining the inherent antitumor activity, the present inventors have performed an acylation reaction of camptothecin with various organic acids, organic acid chlorides, and organic acid anhydrides. A number of new camptothecin derivatives have been obtained. They are characterized by the formula I as shown below:

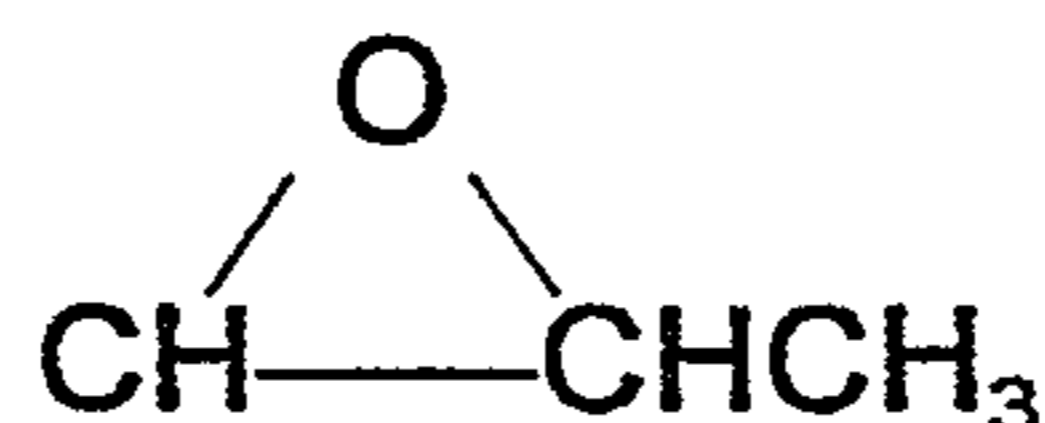
-7-



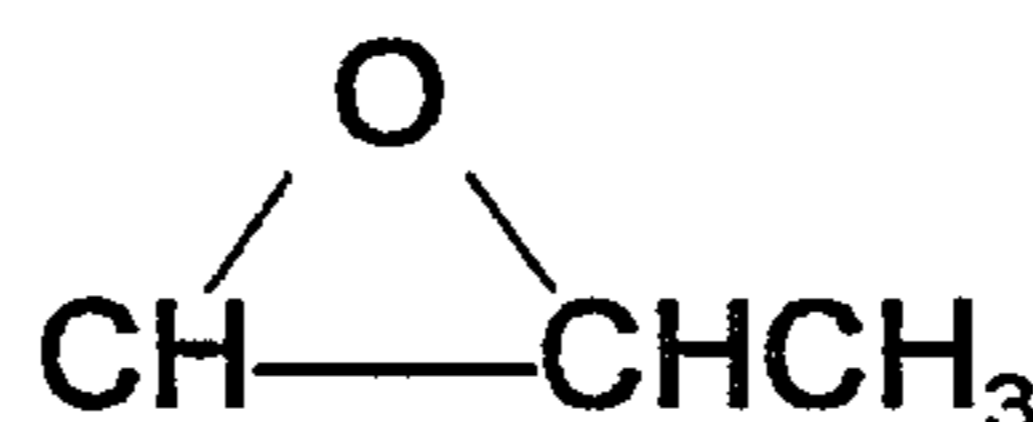
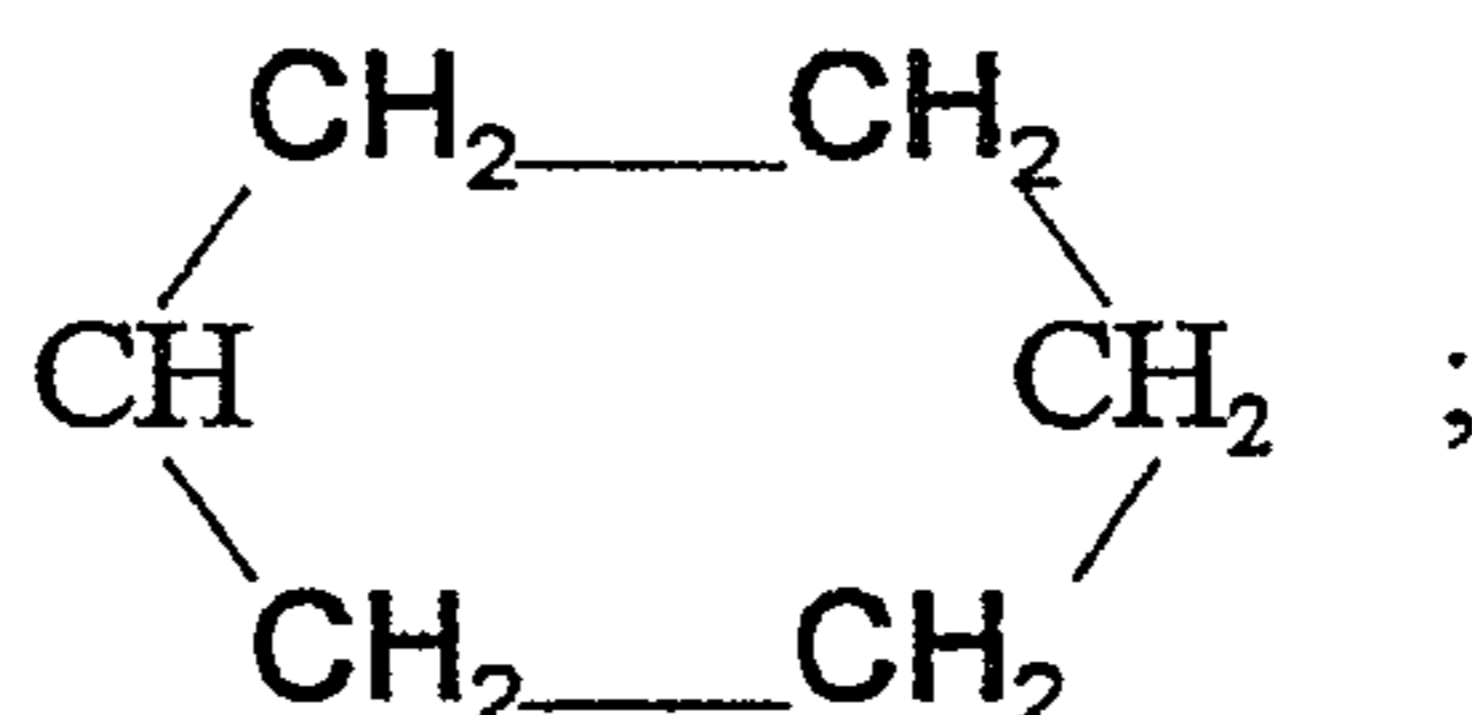
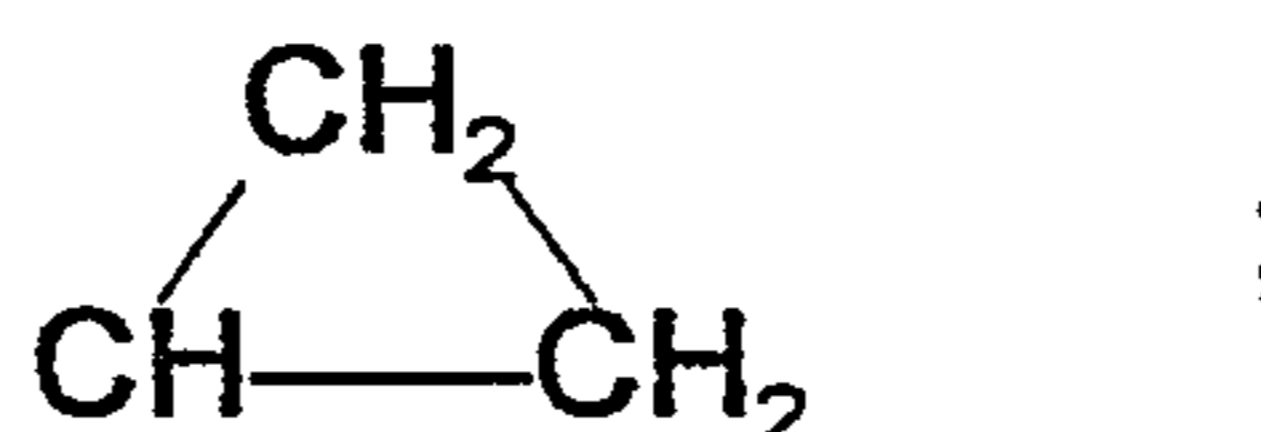
wherein R_2 is H, or NO_2 . R_1 in formula I represents a $\text{C}_2\text{-C}_4$ alkyl group, a $\text{C}_6\text{-C}_{15}$ alkyl group, a $\text{C}_3\text{-C}_8$ cycloalkyl group, a $\text{C}_2\text{-C}_{15}$ alkenyl group or a $\text{C}_2\text{-C}_{15}$ epoxy group when R_2 is H. When R_2 is NO_2 , R_1 is a $\text{C}_1\text{-C}_{15}$ alkyl group, a $\text{C}_3\text{-C}_8$ cycloalkyl group, a $\text{C}_2\text{-C}_{15}$ alkenyl group or a $\text{C}_2\text{-C}_{15}$ epoxy group. Preferably, when R_2 is H, R_1 is CH_2CH_3 ; $\text{CH}_2\text{CH}_2\text{CH}_3$; $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$; $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$; $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$; $\text{CH}=\text{CH}_2$; $\text{CH}=\text{CHCH}_3$ (trans);



-8-



Also, when R₂ is NO₂, R₁ is preferably CH₃; CH₂CH₃; CH₂CH₂CH₃; CH=CH₃; CH=CHCH₃ (trans);



The analogues of 9-nitrocamptothecins are prepared by the acylation of 9-nitrocamptothecin with organic anhydrides. All epoxy derivatives can be prepared by the reactions of the corresponding alkenyl esters of the present invention with m-chloroperoxy benzoic acid in benzene at room temperature.

The preferred derivatives display significant antitumor activity with much lower toxicity than their parent camptothecin, CPT. The animal experimental data for these new derivatives were collected. Camptothecin 20(S) propionate (where R₁ is ethyl and R₂ is H) is designated as Prodrug 2 and taken as an example to disclose some *in vivo* experimental data. Fig. 6 represents the antitumor activity of this compound in different doses against the implanted human breast carcinoma (CLO) in nude mice. Table 1 represents the toxicity of this compound against nude mice with different doses. The

-9-

change of body weight of mice is recorded with the time. There were no losses of mice body weights during the test time period.

Table 1
The Changes of Body Weights of Mice during The Test Time Period

Dose (mg/kg)	Time (days) / Body weight (gms)					
Control	21 / 32.7	27 / 33.5	34 / 34.4	41 / 35.2	48 / 35.0	56 / 36.7
5	21 / 33	27 / 33.7	34 / 34.3	41 / 33.5	48 / 32.9	56 / 33.2
6	21 / 32.9	27 / 33.4	34 / 33.8	41 / 33.5	48 / 34.0	56 / 32.2
7	21 / 30.8	27 / 31.7	34 / 30.6	41 / 31.1	48 / 31.6	56 / 31.5
8	21 / 32.9	27 / 34.1	34 / 34.0	41 / 33.4	48 / 33.9	56 / 33.2

The measurement of pharmacokinetics for Prodrug 2, camptothecin 20(S) propionate, shows that the lactone ring remained intact in human plasma much longer than its mother camptothecin, CPT. Table 2 represents this result.

Table 2
Comparison of Lactone % of 20(S) Propionate and Lactone % of Camptothecin in Human Plasma

Time (Hr.)	0	1	2	4	6
Lactone % for prodrug 2	100	86	77	68	56
Lactone % for camptothecin	100	12	0.5	0	0

As reflected in the tables, augmenting the biological life of the closed lactone ring has been achieved. As shown in Fig. 2, the % of CPT present as closed lactone form in human blood after oral administration is 0.4%. Its analogue, 20-(S) propionate, under similar conditions reaches 2.8% (Fig. 6), an increase of sevenfold. This compound exhibits antitumor activity against human tumor xenografts and an exceptional lack of toxicity, even at enormous doses in mice. Even more striking are the results obtained with 9NC. As shown in Figs. 4 and 5, after oral administration of this compound, only ~ 3% of it is present in the plasma as lactone. When its analogue, Prodrug 11 of the present invention, (where R₁ is ethyl and R₂ is NO₂) was prepared and administered orally to a patient, the lactone form constituted 68.7% of the total, an

-10-

increase of more than twentyfold (Fig. 8). This compound also exhibited antitumor activity against xenografts of human tumors in nude mice, even higher than the camptothecin analogue, Prodrug 2, where R_1 is ethyl and R_2 is H and with very low toxicity.

The compounds of the present invention can be made in the following manner. 20(S)-camptothecin or 9-nitrocamptothecin can be reacted with organic anhydrides in pyridine for example. In particular, the anhydride is mixed with pyridine in about a 1:1 ratio to provide a homogeneous solution to which the 20(S)-camptothecin or 9-nitrocamptothecin is added all at once. The mixture is stirred under atmosphere for 24 to 48 hours. At the end of the reaction time, the mixture is poured onto a certain amount of petroleum ether while stirring. The product, precipitated from petroleum ether, is collected by filtration and washed with petroleum ether several times. The purity of the product by this procedure is usually 98% (analyzed by HPLC). This procedure is applicable to all of the compounds of the present invention except where R_1 is C_7 - C_{15} alkyl or alkenyl, or an epoxy group in Formula (I). The derivative where R_1 is a C_7 - C_{15} alkyl is obtained by the reaction of camptothecin with nonanoyl chloride in methylene chloride under reflux. The derivative where R_1 contains an epoxy moiety is obtained by epoxidation of the compound of the present invention, where R_1 is an alkenyl group in Formula (I).

The compounds of the present invention are effective in treating malignant tumors in a mammal. The compounds of the present invention can be administered in any fashion known to those skilled in the art. Preferably, the compounds are administered intramuscularly, orally, or transdermally.

As used herein, the term "malignant tumor" is intended to encompass all forms of human carcinomas, sarcomas and melanomas which occur in the poorly differentiated, moderately differentiated, and well differentiated forms.

In treating or retarding malignant tumors in mammals in accordance with the present invention, the aforescribed CPT derivatives of the present invention are administered by means known to those skilled in the art, and are preferably administered intramuscularly, transdermally, or orally, using commonly known methods,

-11-

for example, gelatin capsules for oral administration, as well as formulations such as microsuspensions in lipid and in lipid-like emulsions (e.g. - Intralipid™ 20, cottonseed oil and peanut oil) for intramuscular administration and inclusion in cholesterol pellets for subcutaneous long-term administration.

As used herein, an "effective amount" of CPT derivatives of the present invention is intended to mean that amount of the compound which will inhibit the growth of, or retard cancer, or kill malignant cells, and cause the regression and palliation of malignant tumors, i.e., reduce the volume or size of such tumors or eliminate the tumor entirely.

With mammals, including humans, the effective amounts can be administered on the basis of body surface area. The interrelationship of dosages varies for animals of various sizes and species, and for humans (based on mg/M² of body surface) is described by E.J. Freireich et al., Cancer Chemother. Rep., 50(4) :219 (1966). Body surface area may be approximately determined from the height and weight of an individual (see, e.g., Scientific Tables, Geigy Pharmaceuticals, Ardsley, N.Y. pp. 537-538 (1970). An effective amount of the camptothecin compounds in the present invention can range from about 12.5 to about 31.3 mg/m² of body surface per day.

The preferred effective amounts or dosages of CPT derivatives or prodrugs of the present invention in mice are about 1 to about 4 mg Prodrug/kg of body weight twice a week for an intramuscular route and about 0.75 to about 1.5 mg Prodrug/kg/day for the oral route. Effective amounts or dosages of CPT derivatives or Prodrugs of the present invention in mice are, for instance, about 1.5 mg/kg/week to about 10 mg/kg/week of the Prodrug for the transdermal route. For all of the administering routes, the exact timing of administration of the dosages can be varied to achieve optimal results. Generally, when using Intralipid™ 20 the carrier for the CPT derivative, the actual dosage of CPT derivative reaching the patient will be less. This is due to some loss of the CPT derivative on the walls of the syringes, needles and preparation vessels, which is prevalent with the Intralipid™ 20 suspension. When a carrier, such as cottonseed oil is used, this above-described loss is not so prevalent because the CPT derivative does not adhere as much to the surfaces of syringes, etc...

-12-

For instance and preferably, it has been found that generally about 2.5 mg Prodrug/kg of body weight twice per week using cottonseed oil, administered by an intramuscular route, will deliver the same amount to the patient as 4.0 mg Prodrug/kg of body weight twice per week using Intralipid 20 as a carrier. Generally, about 1 mg to about 4 mg of CPT derivative is added to about 0.1 ml to about 1 ml of carrier.

Another important feature of the method provided by the present invention relates to the relatively low or no apparent overall toxicity of the CPT derivatives administered in accordance with the teachings herein. Overall toxicity can be judged using various criteria. For example, loss of body weight in a subject over 10% of the initially recorded body weight (i.e., before treatment) can be considered as one sign of toxicity. In addition, loss of overall mobility and activity and signs of diarrhea or cystitis in a subject can also be interpreted as evidence of toxicity. In one of the examples which follow, the overall toxicity of the camptothecin compounds of the present invention was evaluated.

The compounds of the present invention may be administered in combination with pharmaceutically acceptable carriers or diluents, such as Intralipid 10 or 20 or natural oils, or other suitable emulsifiers for lipophilic compounds.

Another method of administering the compounds of the present invention is by a transdermal or transcutaneous route. One example of such an embodiment is the use of a patch. In particular, a patch can be prepared with a fine suspension of a compound disclosed in the present application in, for example, dimethylsulfoxide (DMSO), or a mixture of DMSO with cottonseed oil and brought into contact with the skin of the tumor carrying mammals away from the tumor location site inside a skin pouch. Other mediums or mixtures thereof with other solvents and solid supports would work equally as well. The patch can contain the CPT derivative of the present invention in the form of a solution or a suspension. The patch can then be applied to the skin of the patient, for example, by means of inserting it into a skin pouch of the patient formed by folding and holding the skin together by means of stitches, clips or other holding devices. This pouch should be employed in such a manner so that continuous contact with the skin is assured without the interference of the mammal. Besides using a skin pouch, any device

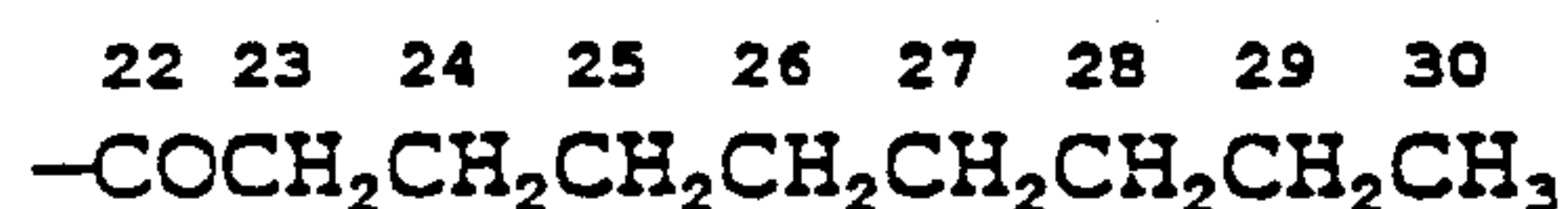
-13-

can be used which ensures the firm placement of the patch in contact with the skin. For instance, an adhesive bandage could be used to hold the patch in place on the skin.

The present invention will be further clarified by the following example, which is intended to be purely exemplary of the present invention.

EXAMPLES

All glassware referenced in the examples was baked at 80-100°C for a minimum of 2 hours before being used. Melting points were obtained with a MEL-TEMP melting point apparatus and were uncorrected. The ¹H and ¹³C NMR spectra were obtained at 270.05 MHz with a JEOL™ GX-270 WB NMR spectrometer. Chemical shifts are reported in parts per million (δ scale), employing tetramethylsilane as an internal standard. In reporting the NMR data, the following abbreviations are used: coupling constants in Hertz (J), singlet (s), doublet (d), triplet (t), broad singlet (brs), multiplet (m), and etc. Mass Spectra were recorded using a VG ZAB - SEQ mass spectrometer (VG Analytical Co., England) with a resolution of 10000. The numbering system used to depict NMR for camptothecin portion is shown in formula (X). The numbering for the side chain is shown as below:



EXAMPLE 1--Camptothecin 20 - O - propionate

In a 100 ml round - bottomed flask were mixed 25 ml propionic acid anhydride and 20 ml pyridine. A homogeneous solution was obtained after shaking for 30 s. To this solution, 2.0 g of starting camptothecin was suspended. The mixture was stirred at 40 ± 5°C for 48 h. After cooling to room temperature, the reaction mixture was poured onto 400 ml petroleum ether while stirring. The product precipitated from petroleum ether was collected by filtration and washed with 150 ml petroleum ether (50 ml x 3). After drying under air for 1 h, a white powder, 2.17 g, was obtained. The purity of the product which was measured by HPLC was 98%. Yield 94%, mp 250 - 252°C (dec.). ¹HNMR in CDCl₃: 0.98 (3H, t, J = 7.5 Hz, 19 - methyl protons), 1.17 (3H, t, J 7.51 Hz,

-14-

24 - methyl protons), 2.12 - 2.34 (2H, m, 18 - methylene protons), 2.48 - 2.58 (2H, m, 23 - methylene protons), 5.29 (2H, s, 5 - methylene protons), 5.39 - 5.72 (2H, dd, J = 17.12, 17.12 Hz, 17 - methylene protons), 7.23 (1H, s, 14 - H), 7.68 (1H, t, J = 6.96 Hz, 10 - H), 7.84 (1H, t, J = 6.96 Hz, 11 - H), 7.95 (1H, d, J = 8.43 Hz, 9 - H), 8.23 (1H, d, J = 8.06 Hz, 12 - H), 8.40 (1H, s, 7 - H); ¹H NMR in TFA: 1.18 (3H, t, J = 7.5 Hz, 19 - methyl protons), 1.32 (3H, t, J = 7.30 Hz, 24 - methyl protons), 2.30 - 2.80 (4H, m, 18 - and 23 - methylene groups), 5.60 - 6.10 (4H, s + dd, s at 5.86 for 5 - methylene protons, dd with J = 18.96, 18.32 Hz for 17 - methylene protons), 7.99 (1H, s, 14 - H), 8.19 (1H, t, J = 8.06 Hz, 10 - H), 8.20 - 8.46 (2H, m, 9 - H and 11 - H), 8.54 (1H, d, J = 8.79 Hz, 12 - H), 9.43 (1H, s, 7 - H); ¹³C NMR (TFA): 7.42 (C19), 8.55 (C24), 28.61 (C18), 33.07 (C23), 53.14 (C5), 68.77 (C17), 78.31 (C20), 105.68, 113.19, 117.35, 125.87, 131.23, 131.36, 132.59, 133.40, 139.30, 139.89, 140.78, 144.62, 147.00, 149.43 (C2, C3, C6 - C16, C16a), 172.50, 179.76 (C21, C22); mass m/e (relative intensity): 405 [(M+H)⁺, 100%], 404 (M⁺, 15%), 331 [(M - CH₃CH₂COO), 17%], 317 [(M - C₂H₅COO - CH₃ + H), 10%], 303 [(M - C₂H₅COO - CO), 15%], 287 [(M - C₂H₅COO - CO₂), 9%], 261(9%).

EXAMPLE 2--Camptothecin 20 - O - butyrate

Using 20 ml butyric anhydride, 18 ml pyridine, and 1.61 g camptothecin, the reaction is carried out in the same manner as in Example 1 whereby 1.77 g of the title compound was obtained as a brownish powder, yield 92%, mp 225 - 227°C (dec.). ¹H NMR in CDCl₃: 0.98 (6H, t, J = 7.51 Hz, 19 - and 25 - methyl groups), 1.65 - 1.74 (2H, m, 24 - methylene protons), 2.14 - 2.30 (2H, m, 18 - methylene protons), 2.44 - 2.51 (2H, m, 23 - methylene protons), 5.29 (2H, s, 5 - methylene protons), 5.38 - 5.71 (2H, dd, J = 17.59, 17.59 Hz, 17 - methylene protons), 7.23 (1H, s, 14 - H), 7.68 (1H, t, J = 8.06 Hz, 10 - H), 7.84 (1H, t, J = 7.96 Hz, 11 - H), 7.95 (1H, d, J = 6.96 Hz, 9 - H), 8.23 (1H, d, J = 8.06 Hz, 12 - H), 8.40 (1H, s, 7 - H); ¹H NMR in TFA: 0.75 - 1.15 (6H, m, 19 - and 25 - methyl groups), 1.70 - 1.80 (2H, m, 24 - methylene protons), 2.10 - 2.80 (4H, m, 18 - and 23 - methylene groups), 5.50 - 6.00 (4H, s + dd, s at 5.73 for 5 - methylene protons, dd for 17 - methylene protons), 7.86 (1H, s, 14 - H), 8.05 (1H, s, 10 - H), 8.30

-15-

(2H, brs, 9 - H and 11 - H), 8.40 (1H, s, 12 - H), 9.30 (1H, s, 7 - H); ^{13}C NMR (TFA): 7.23 (C19), 13.20 (C25), 19.20 (C24), 32.91 (C18), 36.91 (C23), 52.96 (C5), 68.58 (C17), 78.00 (C20), 105.56, 113.40, 113.50, 117.00, 117.10, 131.00, 132.40, 133.16, 139.06, 139.15, 140.00, 144.36, 146.90, 149.40 (C2, C3, C6 - C16, C16a), 172.50, 178.00 (C12, C22); mass m/e (relative intensity): 419 [(M+H)⁺, 100%], 331 [(M - C₃H₇COO), 17%], 303 [(M - C₃H₇COO - CO), 13%], 287 [(M - C₃H₇COO - CO₂), 8%], 273 (2%), 261(3%).

EXAMPLE 3--Camptothecin 20 - O - valerate

Using 15 ml valeric anhydride, 14 ml pyridine, and 1.51 g starting camptothecin, the reaction was carried out in the same manner as in Example 1 whereby 1.68 g of the title compound was obtained as a gray - white powder, yield 90%, mp 265°C (dec.). ^1H NMR in CDCl₃: 0.92 (3H, t, J = 7.33 Hz, 26 - methyl protons), 0.98 (3H, t, J = 7.51, 19 - methyl protons), 1.37 - 2.00 (4H, m, 24 - and 25 - methylene protons), 2.10 - 2.28 (2H, m, 18 - methylene protons), 2.46 - 2.53 (2H, m, 23 - methylene protons), 5.30 (2H, s, 5 - methylene protons), 5.38 - 5.71 (2H, dd, J = 17.22, 17.21 Hz, 17 - methylene protons), 7.23 (1H, s, 14 - H), 7.70 (1H, t, J = 6.96 Hz, 10 - H), 7.82 (1H, t, J = 6.96 Hz, 11 - H), 7.95 (1H, d, J = 7.32 Hz, 9 - H), 8.22 (1H, d, J = 8.42 Hz, 12 - H), 8.40 (1H, s, 7 - H); ^1H NMR In TFA: 0.83 (3H, brs, 26 - methyl protons), 0.99 (3H, brs, 19 - methyl protons), 1.32 (2H, m, 25 - methylene protons), 1.60 (2H, m, 24 - methylene protons), 2.19 - 2.58 (4H, m, 18 - and 23 - methylene protons), 5.49 - 5.82 (4H, s + dd, s at 5.67 for 5 - methylene protons, dd with J = 17.58, 18.68 Hz for 17 - methylene protons), 7.80 (1H, s, 14 - H), 7.99 (1H, s, 10 - H), 8.23 (2H, brs, 9 - H and 11 - H), 8.33 (1H, s, 12 - H), 9.24 (1H, s, 7 - H); ^{13}C NMR(TFA): 4.48 (C26), 10.37 (C19), 20.23 (C24), 24.98 (C25), 30.15 (C18), 32.03 (C23), 50.20 (C5), 65.82 (C17), 75.36 (C20), 102.84, 109.89, 110.24, 114.06, 114.39, 128.25, 128.39, 129.65, 130.41, 136.30, 137.00, 141.62, 148.57, 149.28 (C2, C3, C6 - C16, C16a), 169.00, 176.80 (C21, C22); mass m/e (relative intensity): 433 [(M+H)⁺, 100%], 331, [(M - C₄H₉COO), 17%], 303 [(M - C₄H₉COO - CO), 13%], 287 [(M - C₄H₉COO - CO₂), 7%], 273 (2%), 261(4%).

-16-

EXAMPLE 4--Camptothecin 20 - O - heptanoate

Using 15 ml heptanoic anhydrides, 13 ml pyridine, and 1.55 g starting camptothecin, the reaction was carried out in the same manner as in Example 1 whereby 2.0 g of the title compound was obtained as a gray - white powder, yield 98%, mp 270°C (deformed at 210°C). ¹H NMR in CDCl₃: 0.82 (3H, t, J = 7.51 Hz, 28-methyl protons), 0.98 (3H, t, J = 7.01 Hz, 19 -methyl protons), 1.20 - 1.80 (8H, m, 24-, 25-, 26-, and 27 - methylene protons), 2.10 - 2.30 (2H, m, 18 - methylene protons), 2.40 - 2.60 (2H, m, 23 - methylene protons), 5.29 (2H, s, 5 -methylene protons), 5.38 - 5.72 (2H, dd, J = 17.69, 17.22 Hz, 17 - methylene protons), 7.23 (1H, s, 14-H), 7.68 (1H, t, J=7.30 Hz, 10-H), 7.84 (1H, t, J = 7.42 Hz, 11-H), 7.95 (1H, d, J= 8.06 Hz, 9-H), 8.22 (1H, d, J=8.32Hz, 12-H), 8.40(1H, s, 7-H); ¹H NMR in TFA: 0.74 (3H, s, 28 - methyl protons), 0.99 (3H, s, 19 - methyl protons), 1.21 (6H, brs, 25-, 26-, and 27 -methylene protons), 1.62 (2H, s, 24 - methylene protons), 2.10 - 2.30 (4H, m, 18 - and 23 - methylene groups), 5.50 - 6.00 (4H, s + dd, s at 5.67 for 5 - methylene protons, dd for 17 -methylene protons), 7.80 (1H, s, 14 - H), 7.99 (1H, s, 10 - H), 8.23 (2H, s, 9 - H and 11 - H), 9.24 (1H, s, 7 - H); ¹³C NMR(TFA): 8.63 (C19), 14.99 (C28), 24.66 (C27), 27.14 (C26), 31.07 (C24), 33.68 (C25), 34.29 (C18), 36.45 (C23), 54.34 (C5), 69.98 (C17), 79.50 (C20), 106.97, 114.39, 118.55, 127.11, 132.41, 133.79, 134.55, 140.46, 141.11, 142.00, 145.79, 148.14, 150.62, 153.00 (C2, C3, C6 - C16, C16a), 180.57, 193.10 (C21, C22); mass m/e (relative intensity): 461 [(M+H)⁺, 100%], 331 [(M- C₆H₁₃COO), 20%], 317 [(M - C₆H₁₃COO - CH₃ + H), 10%], 303 [(M- C₆H₁₃COO - CO), 15%], 287 [(M - C₆H₁₃COO - CO₂), 8%], 273 (2%), 261 (2%).

EXAMPLE 5--Camptothecin 20 - O - nonanoate

To 40 ml methylene chloride in a 100 ml round - bottomed flask were added 5 ml nonanoyl chloride and 2 g starting camptothecin. The mixture was stirred under reflux for 48 h. After the solvent was evaporated by a rotary evaporator, the residue was chromatographically separated with methylene chloride - THF as eluent. The title compound, 169 mg, was obtained as a pale yellow powder, yield 6%, mp 180°C. ¹H NMR in CDCl₃: 0.84 (3H, t, J = 6.60 Hz, 30 -methyl protons), 1.02 (3H, t, J = 7.69 Hz,

-17-

19 - methyl protons), 1.20 - 1.80 (12H, m, 24 - 29 methylene protons), 2.10 - 2.38 (2H, m, 18 - methylene protons), 2.40 - 2.60 (2H, m, 23 -methylene protons), 5.33 (2H, s, 5 - methylene protons), 5.40 - 5.80 (2H, dd, $J = 17.22, 17.22$ Hz, 17 - methylene protons), 7.26 (1H, s, 14 - H), 7.71 (1H, t, $J = 8.06$ Hz, 10 - H), 7.88 (1H, t, $J = 8.43$ Hz, 11 -H), 7.99 (1H, d, $J=7.33$ Hz, 9-H), 8.26 (1H, d, $J = 8.79$ Hz, 12-H), 8.44 (1H, s, 7 -H); ^1H NMR in TFA: 0.96 (3H, s, 30 - methyl protons), 1.24 (3H, s, 19 - methyl protons), 1.38 (10H, brs, 25 - 29 - methylene protons), 1.87 (2H, m, 24 - methylene protons), 2.40 - 2.90 (4H, m, 18 - and 23 - methylene protons), 5.74 - 6.07 (4H, s + dd, s at 5.91 for 5 - methylene protons, dd with $J = 17.90, 18.21$ Hz for 17 - methylene protons), 8.05 (1H, s, 14 - 11), 8.24 (1H, t, 10 -H), 8.48 (2H, m, 9 -H and 11-11), 8.57(1H, d, 12-H), 9.48 (1H, s, 7-11); ^{13}C NMR (TFA): 4.99 (C30), 11.45 (C19), 21.17 (C29), 23.53 (C28), 27.82 (C24, C26 - 27), 30.52 (C25), 30.63 (C18), 32.80 (C23), 103.28, 110.73, 114.91, 123.47, 128.79, 128.90, 130.14, 130.93, 136.84, 137.46, 138.33, 142.17, 144.47, 146.94 (C2, C3, C6 - C16, C16a), 169.98, 176.92 (C21, C22); mass m/e (relative intensity): 489 [(M+H)⁺, 100%], 331 [(M - C₈H₁₇COO, 23%], 317 [(MC₈H₁₇COO - CH₃ + 11), 13%], 303 [(M- C₈H₁₇COO - CO), 17%], 287 [(M- C₂H₁₇COO - CO₂), 8%], 273 (3%), 216 (2%).

EXAMPLE 6--Camptothecin 20 - O crotonate

Crotonic anhydride (40 ml) and pyridine (30 ml) were mixed in a 100 ml round - bottomed flask. To this solution, the starting camptothecin (8 g) was added. The mixture was stirred at $90 \pm 10^\circ\text{C}$ for 15 h. After cooling to room temperature, the crude product was precipitated in 1000 ml petroleum ether and collected by filtration. After column chromatography, the compound (3 g) was obtained, yield 31%, mp $218 - 220^\circ\text{C}$ (deformed at 155°C). ^1H NMR(CDCl₃): 1.00 (3H, t, $J= 7.51$ Hz, 19 - methyl protons), 1.94 (3H, dd, $J_{\text{H25 H24}}=6.96\text{HZ}$, $J_{\text{H25 - H23}}= 1.89\text{Hz}$, 25 -methyl protons), 2.15 - 2.35 (2H, m, 18 -methylene protons), 5.28 (2H, s, 5 - methylene protons), 5.38 - 5.74 (2H, dd, $J = 17.22, 17.22$ Hz, 17 -methylene protons), 5.99 (1H, d, $J = 13.92$ Hz, 23 - H), 7.05 - 7.14 (1H, dq, $J_{\text{H24 - H23}} = 15.38$ Hz, $J_{\text{H24 - H25}} = 6.96$ Hz, 24 - H), 7.24 (1H, s, 14 - H), 7.67 (1H, t, $J = 6.96$ Hz, 10-H), 7.83 (1H, t, $J = 6.96\text{Hz}$, 11 -H), 7.94 (1H, d, $J = 7.70$ Hz, 9-

-18-

H), 8.21 (1H, d, $J = 8.43$ Hz, 12-H), 8.39 (1H, s, 7 - H); mass m/e (relative intensity): 416 (M^+ , 20%), 330 [(M - $\text{CH}_3\text{CH}=\text{CHCOOH}$), 100%], 315 [(M - $\text{CH}_3\text{CH}=\text{CHCOOH} - \text{CH}_3$), 40%], 302 [(M - $\text{CH}_3\text{CH}=\text{CHCOOH} - \text{CO}$), 73%], 287 [(M - $\text{CH}_3\text{CH}=\text{CHCOOH} - \text{CO}_2 + 11$), 30%], 274 (10%), 259 (9%), 246 (9%), 234 (3%), 218 (5%), 205 (4%), 191(3%); precise mass: found 416.137, $\text{C}_{24}\text{H}_{20}\text{N}_2\text{O}_5$ requires 416.137.

EXAMPLE 7--Camptothecin 20 - O - 2', 3' - epoxy - butyrate

To 50 ml benzene in a 100 ml round - bottomed flask were added 160 mg of the starting compound of Example 6 and 150 mg m - chloroperoxybenzoic acid (57-86%, Aldrich Chemical Co., Milwaukee, WI). The mixture was stirred at room temperature for a week. The solvent was removed by a rotary evaporator. The residue was chromatographically separated. The compound (120 mg) was obtained as white powder, yield 72%, mp: the compound deformed at 175°C and decomposed at $210 - 213^\circ\text{C}$. ^1H NMR (CDCl_3): 0.99 (3H, t, $J = 7.51$ Hz, 19 - methyl protons), .90 - 1.94 (3H, dd, $J_{\text{H}25 - \text{H}24} = 6.96\text{Hz}$, $J_{\text{H}25 - \text{H}23} = 1.84\text{Hz}$, 25-methyl protons), 2.07-2.33 (2H, m, 18 -methylene protons), 5.30 (2H, s, 5 - methylene protons), 5.38 - 5.72 (2H, dd, $J = 17.59, 17.95$ Hz, 17 - methylene protons), 5.95 - 6.02 (1H, dd, $J_{\text{H}23 - \text{H}24} = 15.75$ Hz, $J_{\text{H}23 - \text{H}25} = 1.83$ Hz, 23 -H), 7.04 - 7.12 (1H, dq, $J_{\text{H}24 - \text{H}25} = 6.59$ Hz, $J_{\text{H}24 - \text{H}23} = 15.38$ Hz, 24 - H), 7.22 (1H, s, 14 - H), 7.75 - 8.01 (4H, m, 9 - 12 aromatic protons), 8.78 (1H, d, $J = 8.06$ Hz, 7 - H); mass m/e (relative intensity): 432 (M^+ , 28%), 416 [(M - O), 12%], 346 [(M - $\text{C}_4\text{H}_6\text{O}_2$), 100%], 331 [(M - $\text{C}_4\text{H}_5\text{O}_3$), 53%], 318 [(M - $\text{C}_4\text{H}_6\text{O}_2 - \text{CO}$), 75%], 303 [(M - $\text{C}_4\text{H}_5\text{O}_3 - \text{CO}$), 54%], 287 [(M - $\text{C}_4\text{H}_5\text{O}_3 - \text{CO}_2$), 27%], 275 (15%), 259 (7%), 246 (8%), 231 (5%), 218 (8%), 205 (10%), 191 (5%); precise mass: found 432.132, $\text{C}_{24}\text{H}_{20}\text{N}_2\text{O}_6$ requires 432.132.

EXAMPLE 8--9 - Nitrocamptothecin 20 - O - acetate

Acetic anhydride (3 ml) and pyridine (2 ml) were mixed in a 50 ml round-bottomed flask in which 140 mg 9-nitrocamptothecin was placed. The mixture was stirred at room temperature for 24 h. The mixture was then separated by chromatotron. The title compound, 70 mg, was obtained as a yellow powder, yield

-19-

45%, mp 195°C (deformed at 165°C). ¹H NMR (CDCl₃): 1.02 (3H, t, J = 7.51 Hz, 19 - methyl protons), 2.10 - 2.40 (5H, s + m, s at 2.26 for 23 - methyl protons, m for 18 - methylene protons), 5.40 (2H, s, 5 - methylene protons), 5.41 - 5.75 (2H, dd, J = 17.59, 17.95 Hz, 17 - methylene protons), 7.23 (1H, s, 14 - H), 7.96 (1H, t, J = 6.96 Hz, 11 - H), 8.53 (1H, d, J 10.99 Hz, 10 - H), 8.58 (1H, d, J = 9.98 Hz, 12 - H), 9.31 (1H, s, 7 - H); mass m/e (relative intensity): 435 (M⁺, 25%), 375 [(M- CH₃COOH), 100%], 360 [(MCH₃COOH - CH₃), 40%], 347 [(M- CH₃COOH - CO), 87%], 332 [(M- CH₃COOH - CO -CH₃), 37%], 319 (13%), 302 (11%), 291 (10%), 286 (11%), 274 (10%), 258 (4%), 246 (5%), 216 (8%); precise mass: found 435.107, C₂₂H₁₇N₃O₇ requires 435. 107.

EXAMPLE 9--9 - Nitrocamptothecin - 20 - O - propionate

Using 6 ml propionic anhydride, 5 ml pyridine, and 600 mg starting 9-nitrocamptothecin, the reaction was carried out in the same manner as in Example 8. After the reaction, the crude product was allowed to precipitate from 200 ml petroleum ether, collected by filtration, separated by column chromatography, and purified by reprecipitation from 200 ml petroleum ether. The pure compound (500 mg) was obtained as a yellow powder, yield 73%, mp 163°C (deformed at 155°C). ¹H NMR(CDCl₃): 0.99 (3H, t, J 7.51 Hz, 19 - methyl protons), 1.18 (3H, t, J= 7.46 Hz, 24 - methyl protons), 2.10 - 2.30 (2H, m, 18- methylene protons), 2.52 - 2.70 (2H, m, 23 - methylene protons), 5.37 (2h, s, 5 - methylene protons), 5.39 - 5.73 (2H, dd, J = 17.58, 17.58 Hz, 17 - methylene protons), 7.22 (1H, s, 14 - H), 7.93 (1H, t, J = 8.06 Hz, 11 - H), 8.50 (1H, d, J = 10.60 Hz, 10 - H), 8.54 (1H, d, J = 8.43 Hz, 12 - H), 9.28 (1H, s, 7 - H); mass m/e (relative intensity): 449 (M⁺, 28%), 375 [(M- C₂H₅COOH), 100%], 360 [(M - C₂H₅COOH - CH₃), 35%], 347 [(M- C₂H₅COOH - CO), 82%], 332 [(M - C₂H₅COOH - CO - CH₃), 26%], 319 (9%), 302 (8%), 291(7%), 274 (7%), 258 (2%), 245 (2%), 216 (2%); precise mass: found 449.122, C₂₃H₁₉N₃O₇ requires 449.122.butyrate.

-20-

EXAMPLE 10--9 - Nitrocamptothecin 20 - O - butyrate

Using 2 ml butyric anhydride, 2 ml pyridine, and 60 mg starting 9 - nitrocamptothecin, the procedure for preparation of the compound was the same as Example 8. The title compound (40 mg) was obtained as a yellow powder, yield 56%, mp 182°C. ¹H NMR (CDCl₃): 0.98 (6H, m, 19 - and 25 - methyl groups), 1.65 - 1.70 (2H, m, 24 - methylene protons), 2.10 - 2.40 (2H, m, 18 -methylene protons), 2.41 - 2.60 (2H, m, 23 - methylene protons), 5.36 (2H, s, 5 - methylene protons), 5.38 - 5.72 (2H, dd, J = 17.59, 17.96 Hz, 17 - methylene protons), 7.22 (1H, s, 14 -H), 7.92 (1H, t, J = 7.52 Hz, 11 - H), 8.49 (1H, d, J = 10.80 Hz, 10 - H), 8.53 (1H, d, J = 9.53 Hz, 12 - H), 9.27 (1H, s, 7 - H); mass m/e (relative intensity): 463 (M⁺, 14%), 375 [(M-C₃H₇COOH), 100%], 360 [(M- C₃H₇COOH - CH₃), 32%], 347 [(M - C₃H₇COOH - CO), 78%], 332 [(M- C₃H₇COOH - CO - CH₃), 25%], 319 (9%), 302 (8%), 291(7%), 274 (7%), 258 (2%), 245 (3%), 216 (5%); precise mass: found 463.137, C₂₄H₂₁N₃O₇.

EXAMPLE 11--Camptothecin 20 - O - Acrylate

A 100 ml, one-necked flask equipped with a calcium chloride drying tube was charged with 1 g (0.0029 mol) of camptothecin, 50 ml of dry methylene chloride, 0.42 g (0.0058 mol) of acrylic acid, and 0.5 g (0.0041 mol) of 4-dimethylaminopyridine. The solution was stirred and cooled to 0°C while 1.2 g (0.0058 mol) of dicyclohexylcarbodiimide was added over a 5 minute period. After an additional 5 minutes at 0°C, the ice bath was removed and the dark reaction mixture was stirred for 15 hours at room temperature. After the dicyclohexylurea was removed by filtration, the organic solution was dried over anhydrous sodium sulfate for 4 hours. After the solvent was removed by a rotary evaporator, the residue was chromatographically separated. The product was obtained in 30% yield. Mass m/e (relative intensity): 403 [(M+H⁺, 100%], 331 [(M-C₃H₃COO), 17%], 303 [(M-C₃H₃COO-CO), 13%], 287 [M-C₃H₃COO-CO₂], 8%], 273 (2%), 261 (3%).

-21-

EXAMPLE 12--Camptothecin 20 - O - 2',3'-epoxy-propionate

To 50 ml benzene in a 100 ml round-bottomed flask were added 100 mg of starting camptothecin 20-O-acrylate and 150 mg m-chloroperoxybenzoic acid (57-86%, Aldrich Chemical Co., Milwaukee, WI). The mixture was stirred at room temperature for a week. The solvent was removed by a rotary evaporator. The residue was chromatographically separated. The compound (80 mg) was obtained as white powder, yield 77%. Mass m/e (relative intensity): 418 (M^+ , 22%), 402 [(M-O), 10%], 347 [(M-C₃H₃O₂), 100%], 331 [(M-C₃H₃O₃), 53%], 319 [(M-C₃H₃O₂-CO), 65%], 303 [(M-C₃H₃O₃-CO), 50%], 287 [(M-C₃-H₃O₃-CO₂), 20%], 275 (10%), 259 (7%), 246 (8%), 231 (5%), 218 (8%), 205 (10%), 191 (5%).

EXAMPLE 13--9-nitrocamptothecin 20 - O - cyclopropionate

The starting 9-nitrocamptothecin (0.5 g, 0.0013 mol) and cyclopropanecarboxylic acid chloride (8 ml) were added to 20 ml acetone in a 100 ml round-bottomed flask equipped with a magnetic stirrer. To this mixture 7 ml pyridine was added dropwise while stirring. After stirring at room temperature for 15 hours, the mixture was poured onto 750 ml of 5% hydrochloric acid solution in water while stirring. The yellow suspension obtained was extracted with 400 ml methylene chloride (100 ml x 4). The combined extracts were washed with 200 ml distilled water and dried over anhydrous sodium sulfate for 4 hours. After filtration, the solvent was removed by a rotary evaporator. The residue was chromatographically separated with methanol-chloroform as eluent. The pure product was obtained as yellow powder, yield 30%, purity 99% (HPLC), mp 274°C. ¹H NMR (CDCl₃): δ 0.62-1.07 (7H, m, C19-methyl, C24- and C25-methylene groups), 1.70-1.80 (1H, m, C23-tertiary proton), 2.10-2.70 (2H, m, C18-methylene protons), 5.23 (2H, s, C5-methylene protons), 5.33-5.65 (2H, dd, J=17.35, 17.35 Hz, C17-methylene protons), 7.23 (1H, s, C14-H), 7.88 (1H, t (d+d), J=8.03, 8.28 Hz, C11-H), 8.44 (1H, d, J=7.51 Hz, C10-H), 8.51 (1H, d, J=8.55 Hz, C12-H), 9.23 (1H, s, C7-H); ¹³C NMR (CDCl₃), δ 7.60 (C19), 9.19, 9.36 (C24, C25), 12.78, (C23), 31.90 (C18), 50.20 (C5), 67.00 (C17), 75.64 (C20), 96.99, 120.99, 121.59, 125.81, 127.46, 128.61, 131.55, 136.51, 144.94, 146.04, 148.92,

-22-

153.86, 157.21 (C2, C3, C6-C16, C16a), 167.00, 173.75 (C21, C22); mass m/e (relative intensity): 461 (M^+ , 13%), 375 (M-cyclopropanecarboxylic acid, 100%), 360 (M-cyclopropanecarboxylic acid-methyl group, 30%), 347 (M-cyclopropanecarboxylic acid-CO, 66%), 332 (M-cyclopropanecarboxylic acid-CO-CH₃, 27%), 319 (9%), 302 (8%), 291 (4%), precise mass (C₂₄H₁₉N₃O₇): Found 461.123.

EXAMPLE 14--9-nitrocamptothecin 20-O-cyclohexanate

The starting 9-nitrocamptothecin (0.38 g, 0.0010 mol) and cyclohexanecarboxylic acid chloride were added to 25 ml acetone in a 100 ml round-bottomed flask equipped with a magnetic stirrer. To this mixture 5 ml pyridine was added dropwise. The mixture was stirred at 40±5°C for 15 hours. After the workup and separation which was the same as in Example 13, a yellow powder was obtained, yield 63%, purity 99% (HPLC), mp 186°C. ¹H NMR (CDCl₃), 0.80-1.15 (5H, m, C19-methyl, and C26-methylene protons), 1.20-2.40 (10H, m, C18-, C24-, C25-, C27-, and C28-methylene groups), 2.42-2.60 (1H, m, C23-tertiary proton), 5.36 (2H, s, C5-methylene protons), 5.37-5.72 (2H, dd, J=17.34, 17.34 Hz, C17-methylene protons), 7.23 (1H, s, C14-H), 7.92 (1H, t(d+d), J=8.02, 8.28 Hz, C11-H), 8.48 (1H, d, J=7.77 Hz, C10-H), 8.56 (1H, d, J=8.54 Hz, C12-H), 9.28 (1H, s, C7-H; ¹³C NMR (CDCl₃): 7.63 (C19), 25.23 (C25, C27), 26.65 (C24, C28), 28.58 (C26), 31.84 (C18), 42.49 (C23), 50.20 (C5), 66.98 (C17), 75.32 (C20), 96.87, 120.99, 121.54, 125.75, 127.42, 128.51, 131.54, 136.56, 144.96, 146.02, 146.23, 148.96, 153.83, 157.21, (C2, C3, C6-C16, C16a), 167.23, 174.77 (C21, C22); mass m/e (relative intensity): 503 (M^+ , 20%), 375 (M-cyclohexanecarboxylic acid, 100%), 360 M-cyclohexanecarboxylic acid-methyl group, 33%), 347 (M-cyclohexanecarboxylic acid-CO, 90%), 332 (M-cyclohexanecarboxylic acid-CO-CH₃, 26%), 319 (10%), 302 (10%), 291 (4%); precise mass (C₂₇H₂₅N₃O₇): Found 503.170.

EXAMPLE 15--Camptothecin 20 - O - cyclopropionate

By using cyclopropionic acid anhydride as an acylating agent, the product camptothecin 20-O-cyclopropionate was prepared by the procedure described in

-23-

Example 13 and was obtained in 35% yield. Mass m/e (relative intensity): 416 (M^+ , 30%), 330 (M-cyclopropanecarboxylic acid, 100%), 316 (M-cyclopropanecarboxylic acid-methyl group, 20%), 302 (M-cyclopropanecarboxylic acid-CO, 68%), 287 (M-cyclopropanecarboxylic acid-CO- CH_3 , 17%).

EXAMPLE 16--Camptothecin 20 - O - cyclohexanate

By using cyclohexane carboxylic acid anhydride as an acylating agent, the product camptothecin 20-O-cyclohexanate was prepared by the procedure described in Example 14 and was obtained in 28% yield. Mass m/e (relative intensity): 458 (M^+ , 30%), 330 (M-cyclohexanecarboxylic acid, 100%), 315 (M-cyclohexanecarboxylic acid-methyl group, 23%), 302 (M-cyclohexanecarboxylic acid-CO, 85%), 237 (M-cyclohexanecarboxylic acid-CO- CH_3 , 36%).

EXAMPLE 17--9-Nitrocamptothecin - 20 - O - acrylate

Using 9-nitrocamptothecin as starting material and acrylic acid as an acylating agent, the product 9-nitrocamptothecin 20-O-acrylate was prepared by the procedure described in Example 11. Yield 38%. Mass m/e (relative intensity): 447 (M^+ , 25%), 375 (M-acrylic acid, 100%), 360 (M-acrylic acid-methyl group, 28%), 357 (M-acrylic acid-CO, 78%), 342 (M-acrylic acid-CO-methyl group, 34%).

EXAMPLE 18--9-Nitrocamptothecin 20 - O - 2',3'-epoxy-propionate

Using 9-nitrocamptothecin 20-O-acrylate as starting material and m-chloroperoxybenzoic acid as an oxidizing agent, the product 9-nitrocamptothecin 20-O-2',3'-epoxy-propionate was prepared by the procedure described in Example 12. Yield 76%. Mass m/e (relative intensity): 463 (M^+ , 16%), 447 (M-O, 12%), 392 [(M- $C_3H_3O_2$), 100%], 376 [(M- $C_3H_3O_3$), 43%], 364 [(M- $C_3H_3O_2$ -CO), 52%], 348 [(M- $C_3H_3O_3$ -CO), 45%], 332 [(M- $C_3H_3O_3$ - CO_2), 18%].

-24-

EXAMPLE 19--9-Nitrocamptothecin 20 - O - crotonate

Using 9-nitrocamptothecin as starting material and crotonic anhydride as an acylating agent, the product 9-nitrocamptothecin 20-O-crotonate was prepared by following the procedure described in Example 6. Yield 46%. Molecular ion peak was observed at m/e 461.

EXAMPLE 20--9-Nitrocamptothecin 20-O-2',3'-epoxy-butyrate

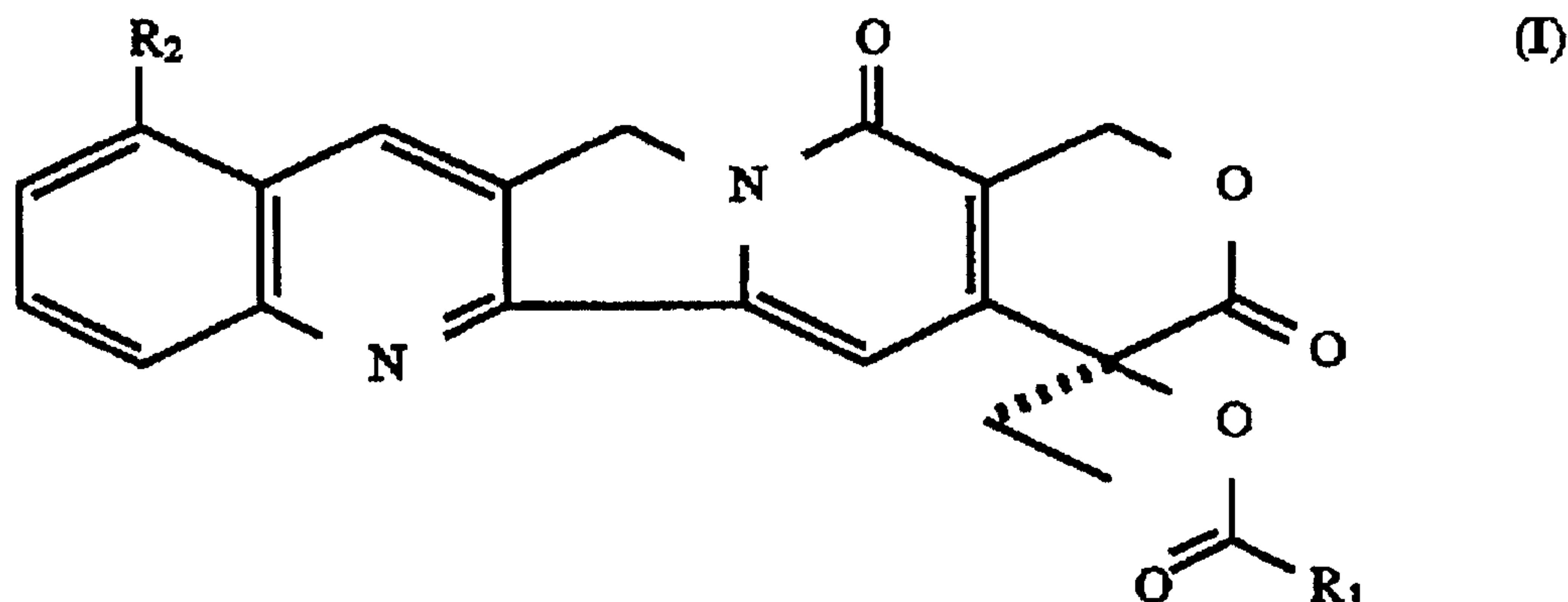
Using 9-nitrocamptothecin 20-O-crotonate as starting material and m-chloroperoxybenzoic acid as oxidizing reagent, the product 9-nitrocamptothecin 20-O-2',3'-epoxy-butyrate was prepared by following the procedure described in Example 7. Yield 76%. The molecular ion peak was observed at m/e 477.

The camptothecin derivatives of the present invention can be orally administered to a patient suffering from cancer at a dosage rate to provide a dose of camptothecin of from about 1 to 20 mg / kg of body weight. The compounds can be administered in a sequence of doses, e.g., one dose every three weeks. Therapy can be repeated until definite disease progression is halted.

Other embodiments of the present invention will be apparent to those skilled in the art from consideration of the specification and practice of the present invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

What is claimed is:

1. A compound formula of (I):



- 5 wherein R_2 is H or NO_2 ; and R_1 is a $\text{C}_2\text{-C}_4$ alkyl group, a $\text{C}_6\text{-C}_{15}$ alkyl group, a $\text{C}_3\text{-C}_8$ cycloalkyl group, a $\text{C}_2\text{-C}_{15}$ alkenyl group or a $\text{C}_2\text{-C}_{15}$ epoxy group when R_2 is H, and R_1 is a $\text{C}_1\text{-C}_{15}$ alkyl group, a $\text{C}_3\text{-C}_8$ cycloalkyl group or a $\text{C}_2\text{-C}_{15}$ epoxy group when R_2 is NO_2 .
- 10 2. A compound of claim 1, wherein R_1 is a linear $\text{C}_2\text{-C}_4$ alkyl group or $\text{C}_6\text{-C}_{15}$ alkyl group when R_2 is H and R_1 is a linear $\text{C}_1\text{-C}_{15}$ alkyl group when R_2 is NO_2 .
3. A compound of claim 1, wherein R_1 is a linear $\text{C}_2\text{-C}_{15}$ alkenyl group.
4. A compound of claim 1, wherein R_1 is a $\text{C}_2\text{-C}_{15}$ epoxy group.
5. A compound of claim 2, wherein R_2 is H.
6. A compound of claim 2, wherein R_2 is NO_2 .
7. A compound of claim 3, wherein R_2 is H.
- 15 8. A compound of claim 3, wherein R_2 is NO_2 ,
9. A compound of claim 4, wherein R_2 is H.
10. A compound of claim 4, wherein R_2 is NO_2 .
11. A compound of claim 2, wherein R_1 is a linear ethyl, propyl, butyl, hexyl or octyl group, and R_2 is H.
- 20 12. A compound of claim 11, wherein R_1 is ethyl.

13. A compound of claim 2, wherein R₁ is a linear methyl, ethyl or propyl group and R₂ is NO₂.
14. The compound of claim 13, wherein R₁ is ethyl.
15. The compound of claim 1, wherein R₁ is a C₃–C₈ cycloalkyl group.
- 5 16. The compound of claim 15, wherein R₂ is H.
17. The compound of claim 15, wherein R₂ is NO₂.
18. The use of a composition for treating a malignant tumour in a patient, wherein the composition comprises an effective amount of the compound of claim 1 and a pharmaceutically acceptable carrier or diluent, and wherein said malignant tumour
10 is responsive to said composition.
19. The use of claim 18, wherein R₁ is a linear C₂-C₄ alkyl group or a C₆-C₁₅ alkyl group when R₂ is H and R₁ is a linear C₁-C₁₅ alkyl group when R₂ is NO₂.
20. The use of claim 18, wherein R₁ is a linear C₂-C₁₅ alkenyl group.
21. The use of claim 18, wherein R₁ is a C₂-C₁₅ epoxy group.
- 15 22. The use of claim 19, wherein R₂ is H.
23. The use of claim 19, wherein R₂ is NO₂.
24. The use of claim 20, wherein R₂ is H.
25. The use of claim 20, wherein R₂ is NO₂.
26. The use of claim 21, wherein R₂ is H.
- 20 27. The use of claim 21, wherein R₂ is NO₂.
28. The use of claim 19, wherein R₁ is a linear ethyl, propyl, butyl, hexyl or octyl and R₂ is H.
29. The use of claim 28, wherein R₁ is ethyl.
30. The use of claim 19, wherein R₁ is a linear methyl, ethyl or propyl group and R₂ is
25 NO₂.
31. The use of claim 30, wherein R₁ is ethyl.

32. The use of claim 18, wherein R_1 is a C_2-C_8 cycloalkyl group.
33. The use of claim 32, wherein R_2 is H.
34. The use of claim 32, wherein R_2 is NO_2 .

Mouse CPT Plasma Levels

2 MG/KG, I.S.

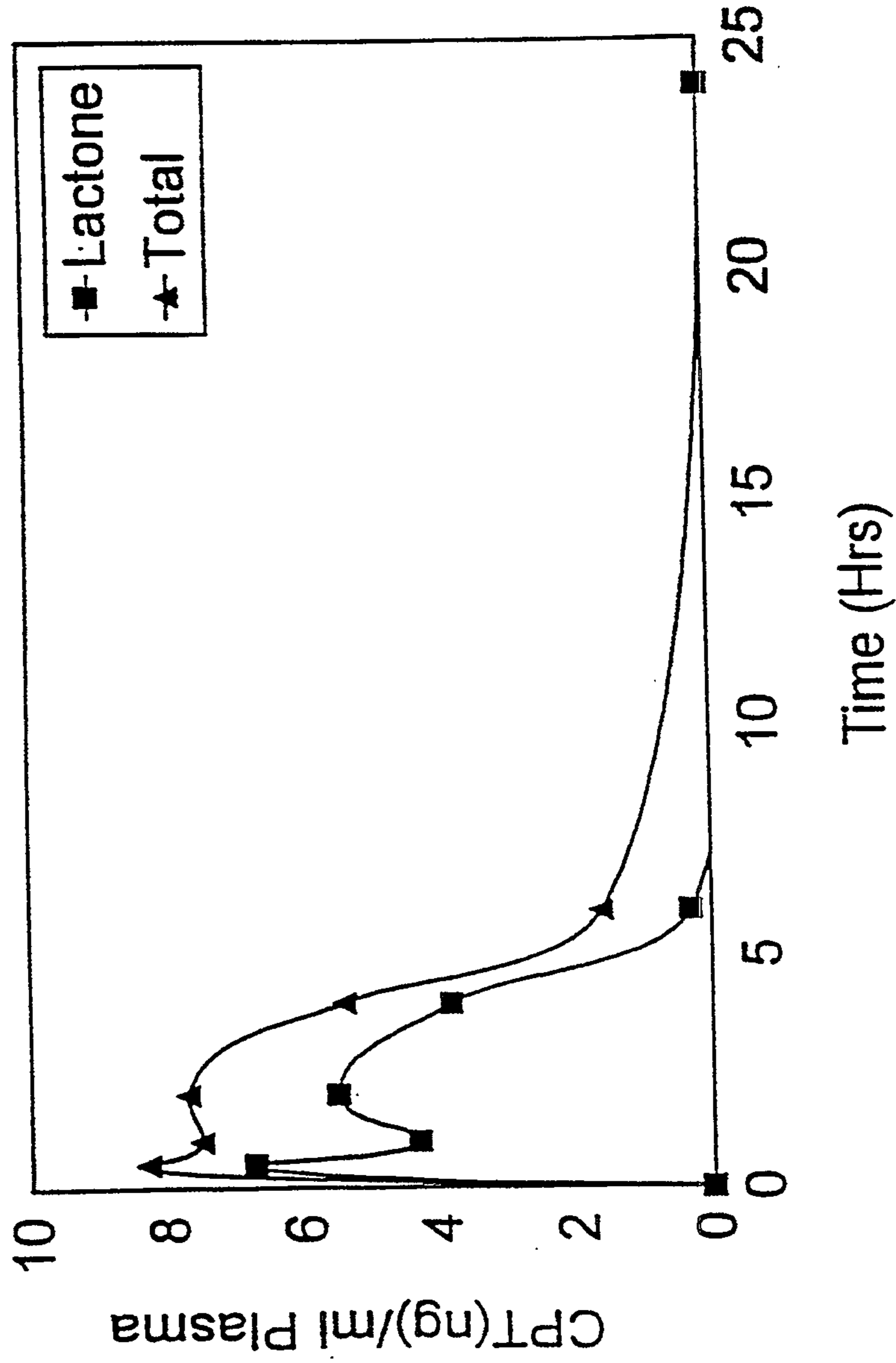


FIG. 1

Human CPT Plasma Levels

4 MG, P.O.

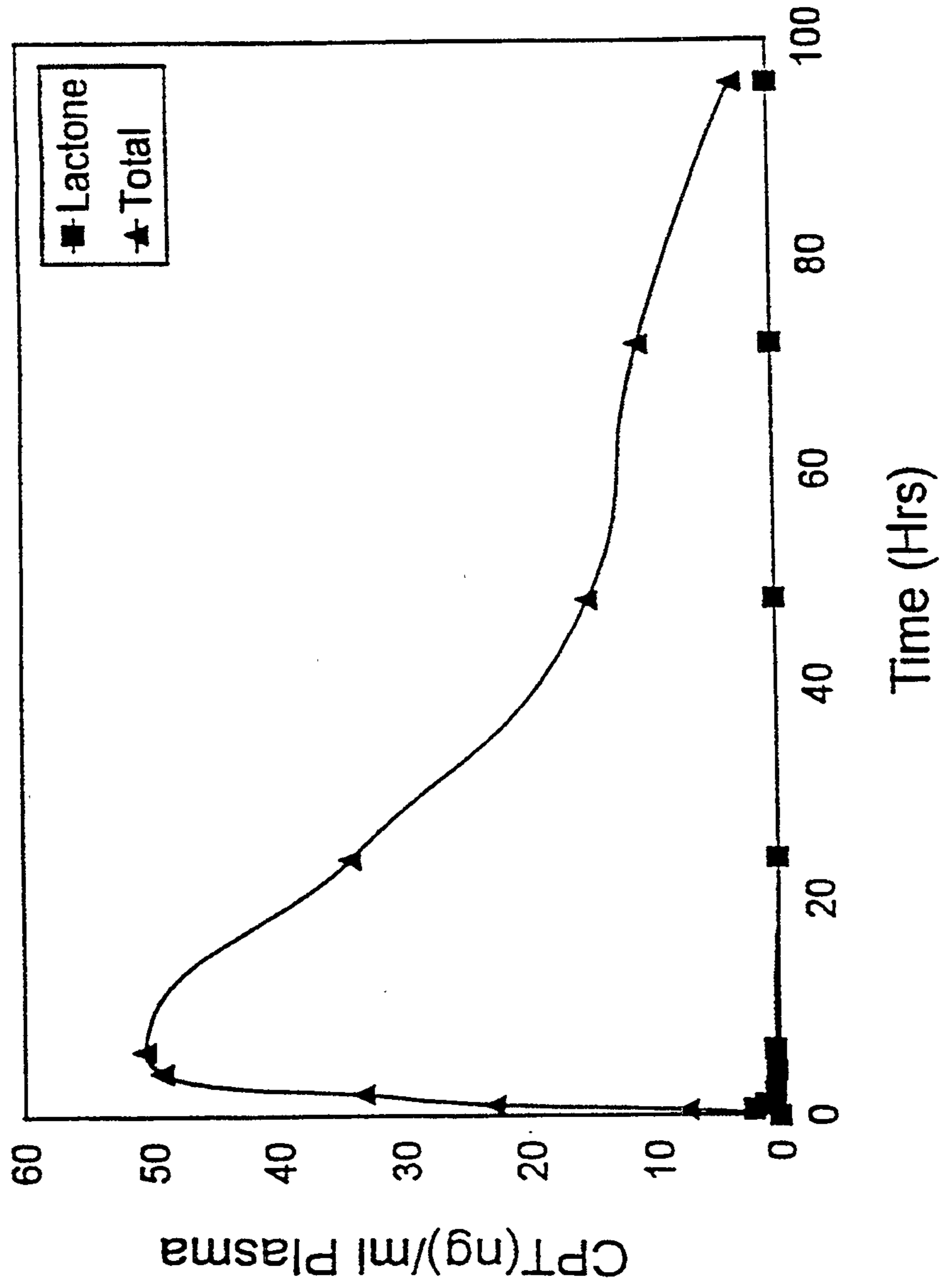


FIG. 2

Mouse 9 - NC Plasma Levels Day 1

1 Mg/kg, I.S.

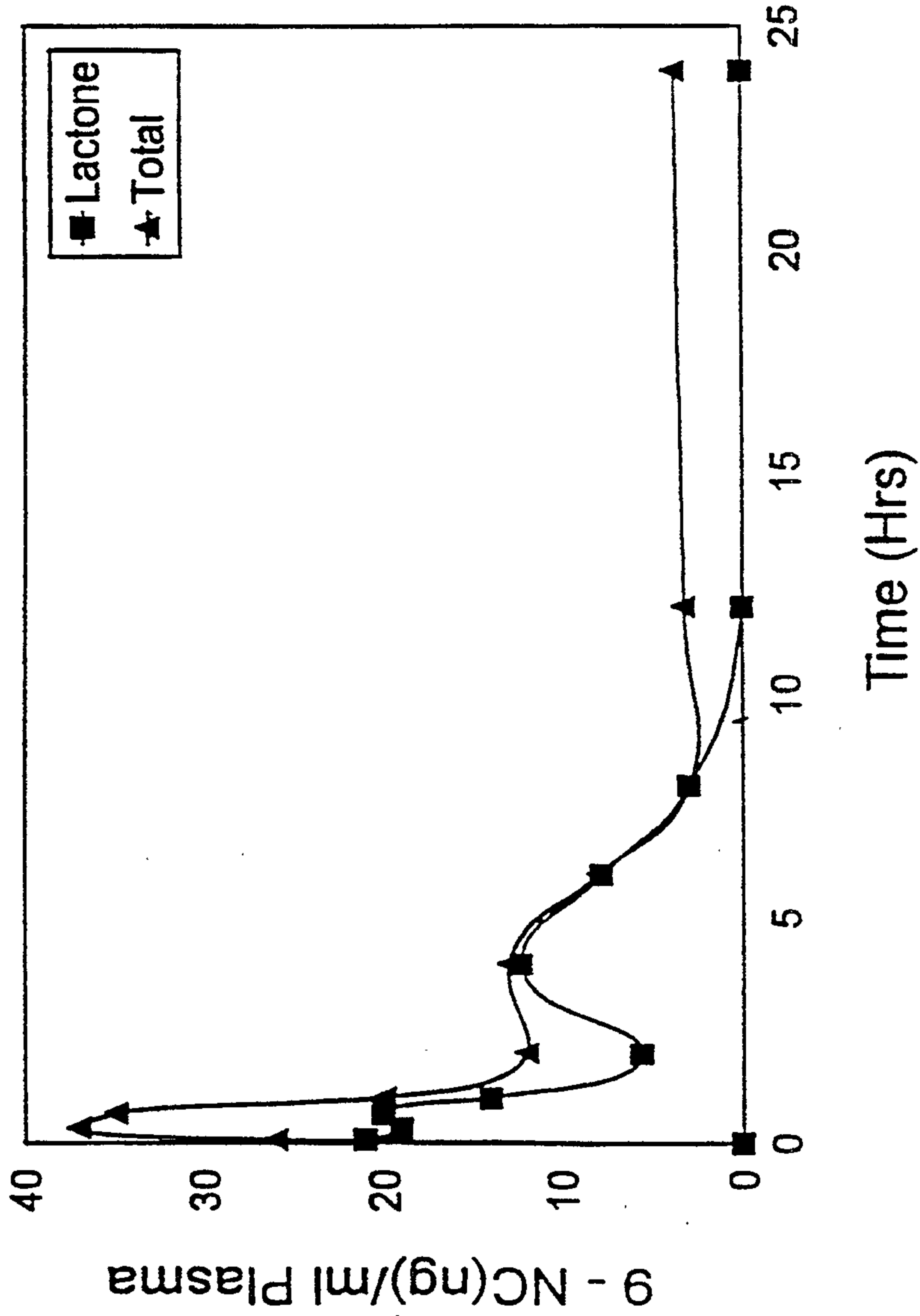


FIG. 3

Meek Plasma Levels

9 - NC

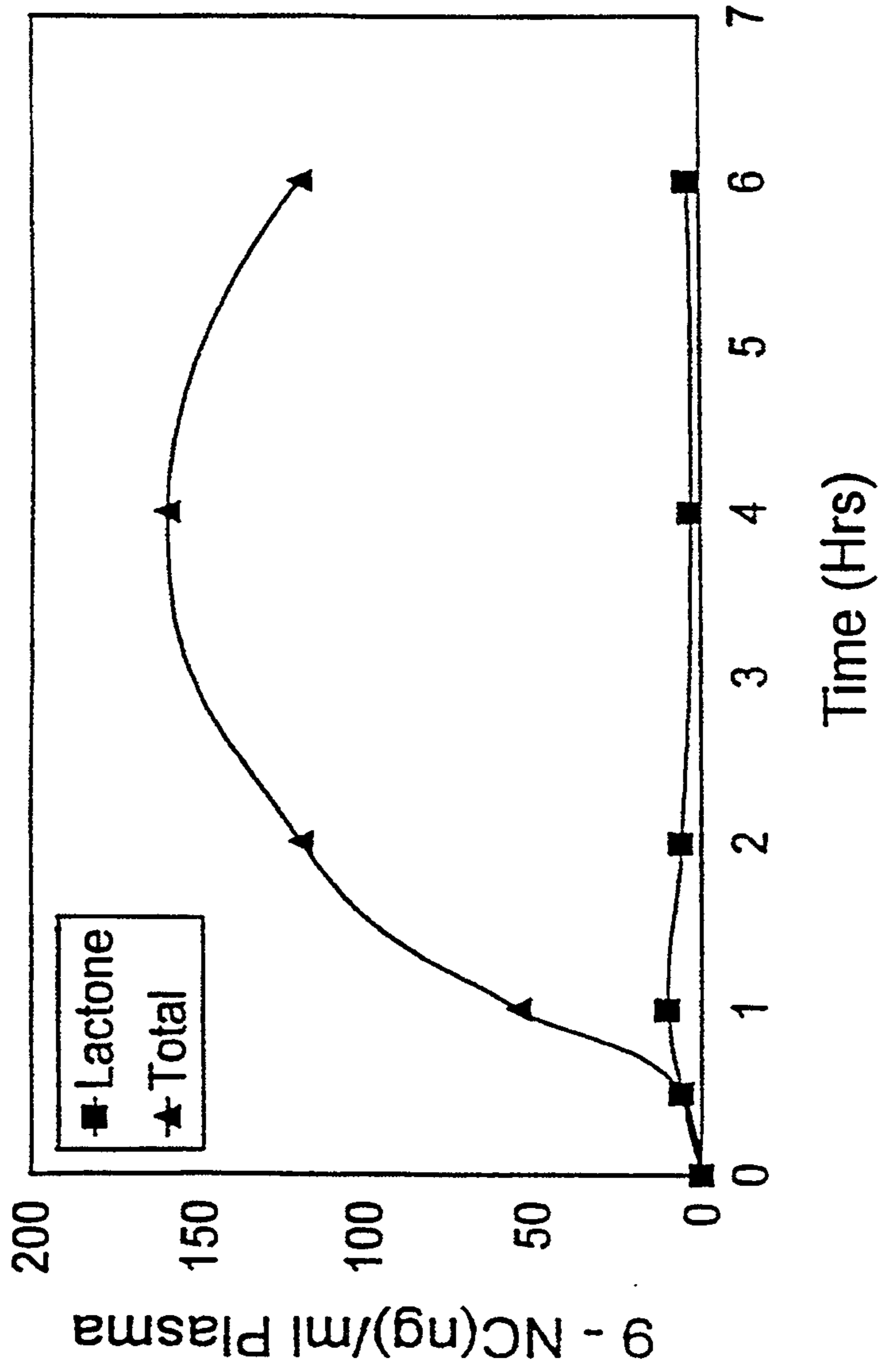


FIG. 4

Moore Plasma Levels

9 - NC

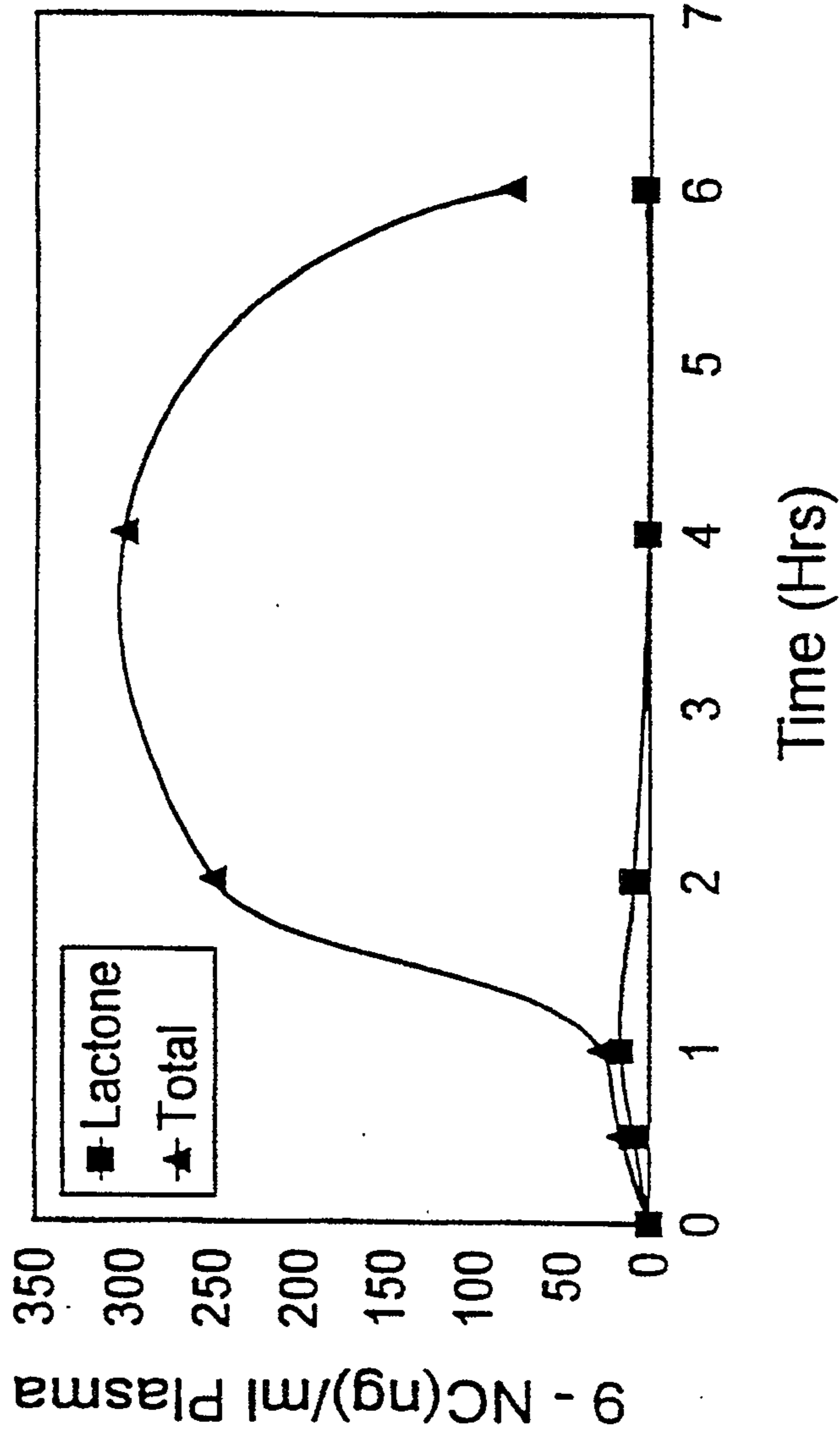


FIG. 5

The Activity of Prodrug 2 against
Human Breast Carcinoma

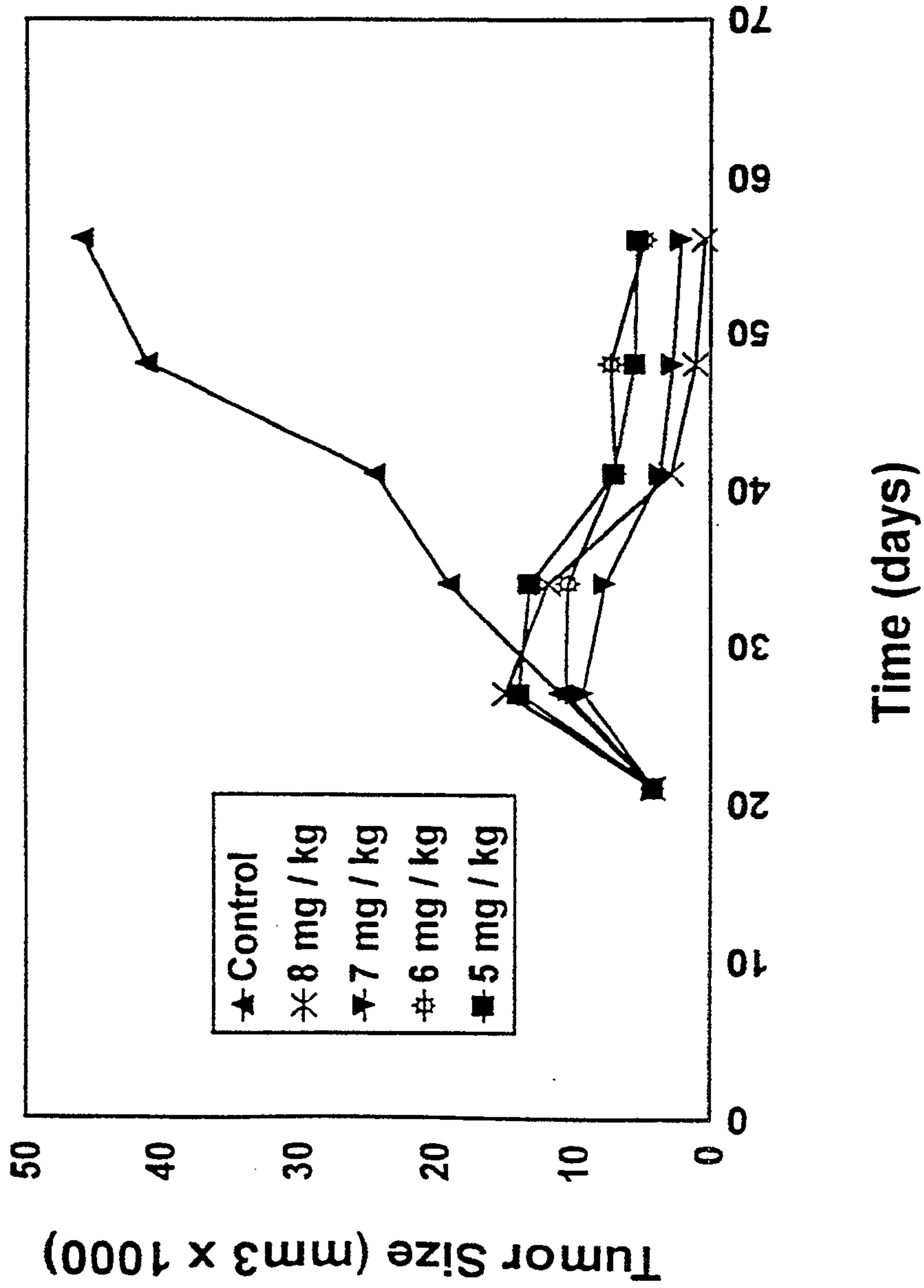


FIG. 6

Prodrug 2 in Human Plasma

4 Mg, P.O.

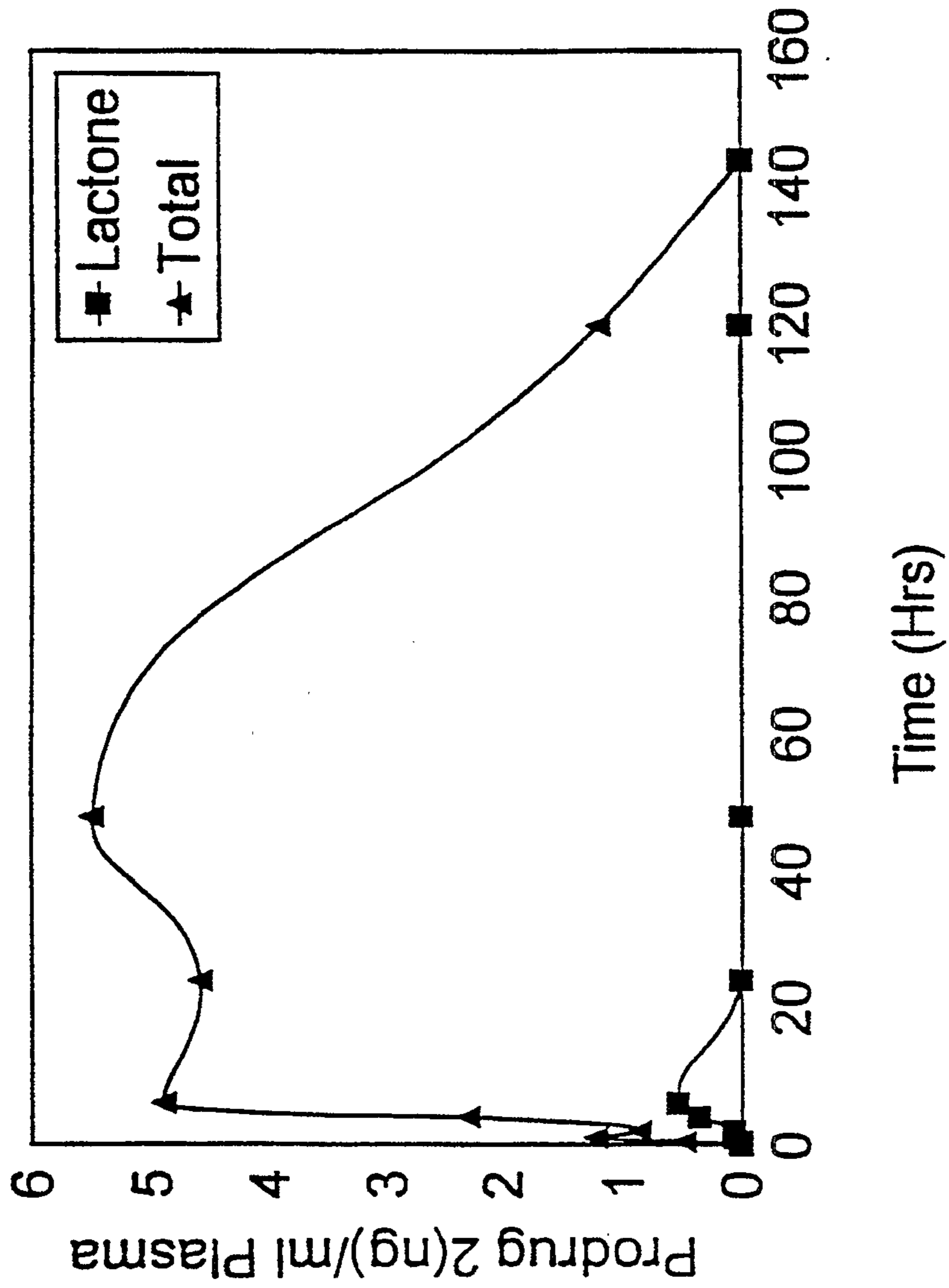


FIG. 7

Prodrug 11 in Human Plasma
10 Mg, P.O.

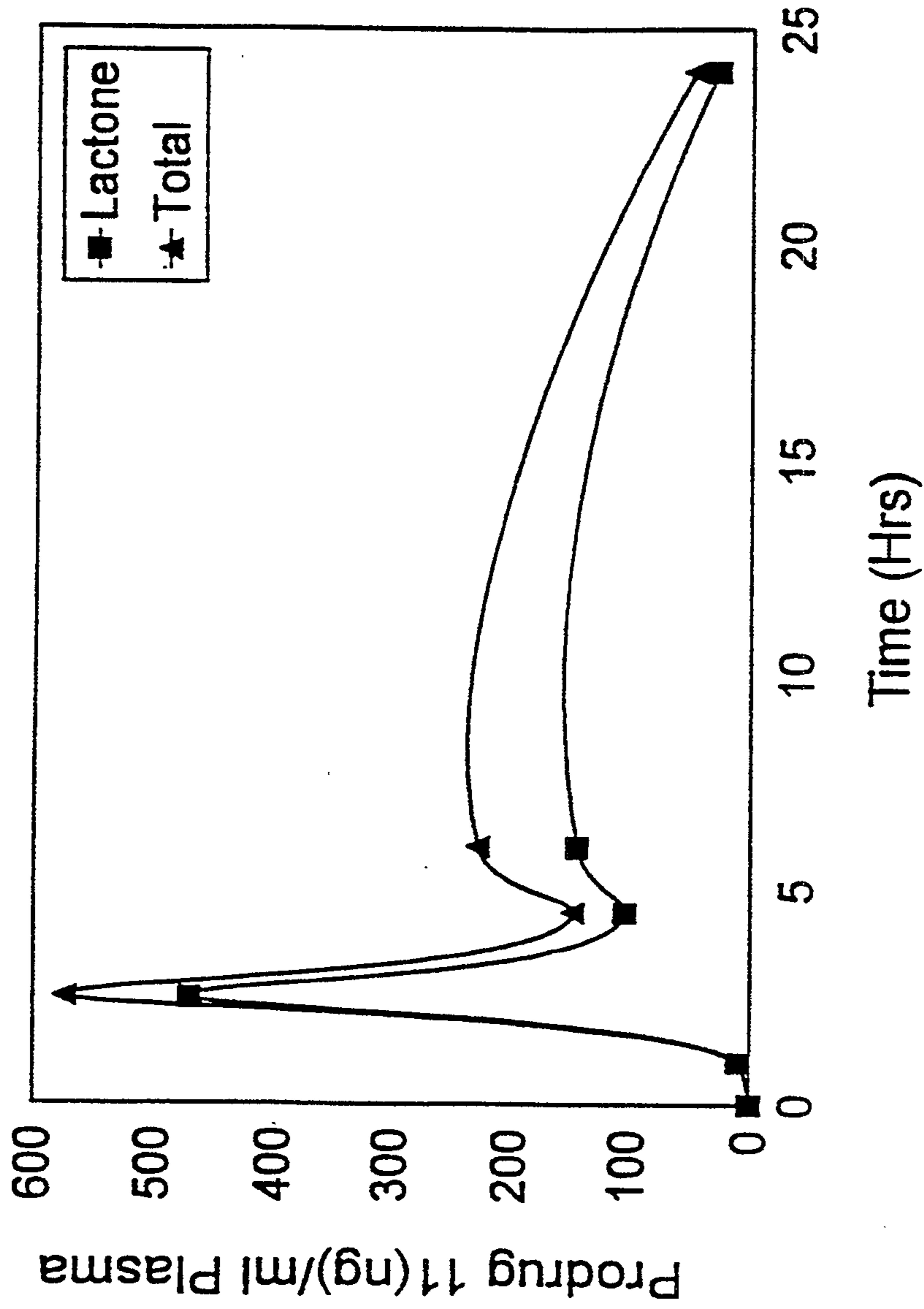


FIG. 8

