



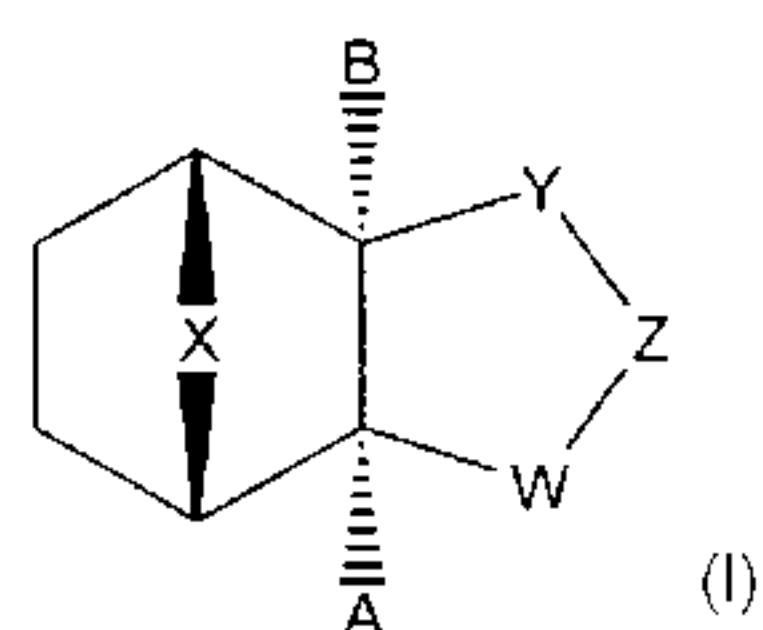
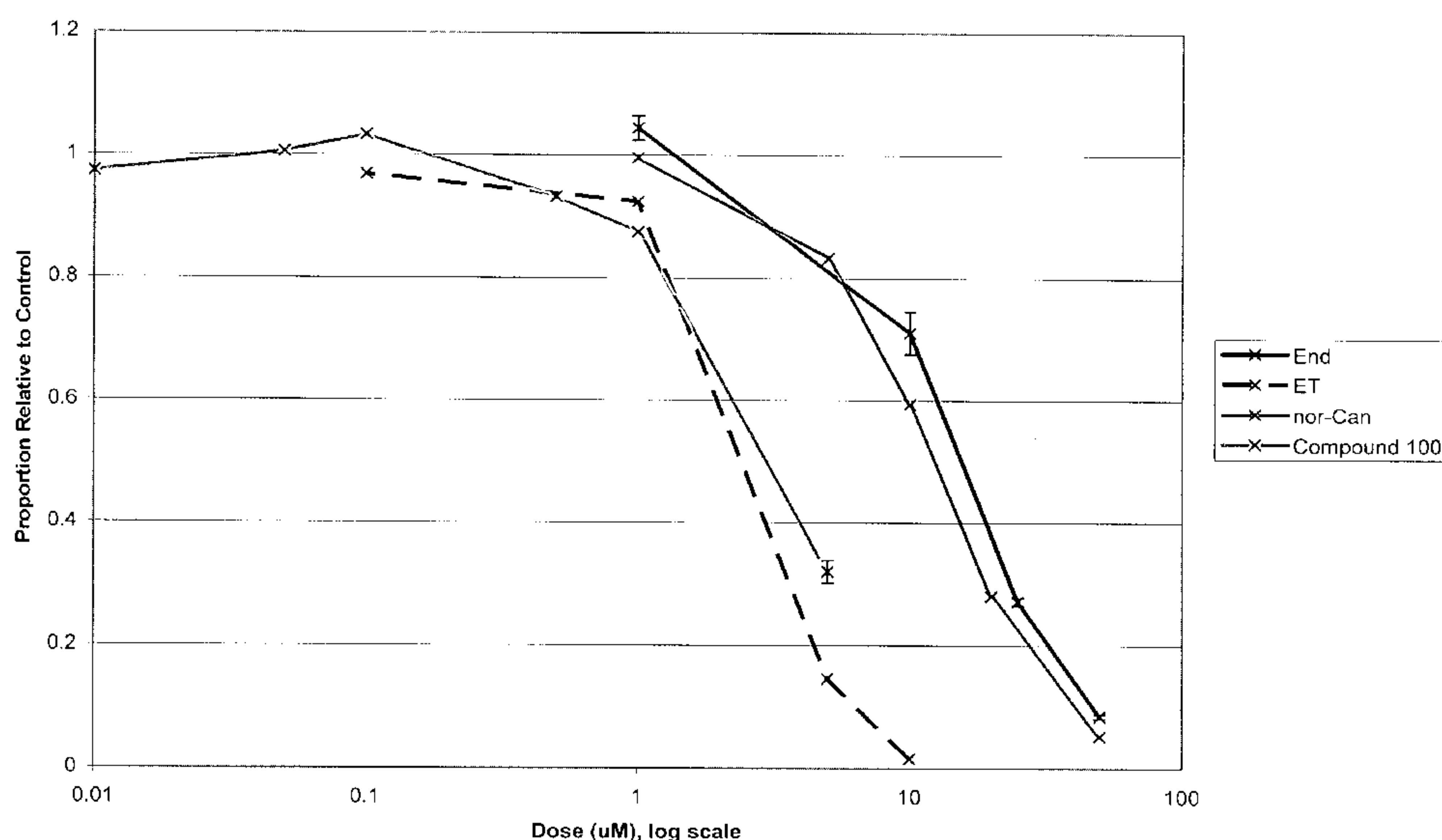
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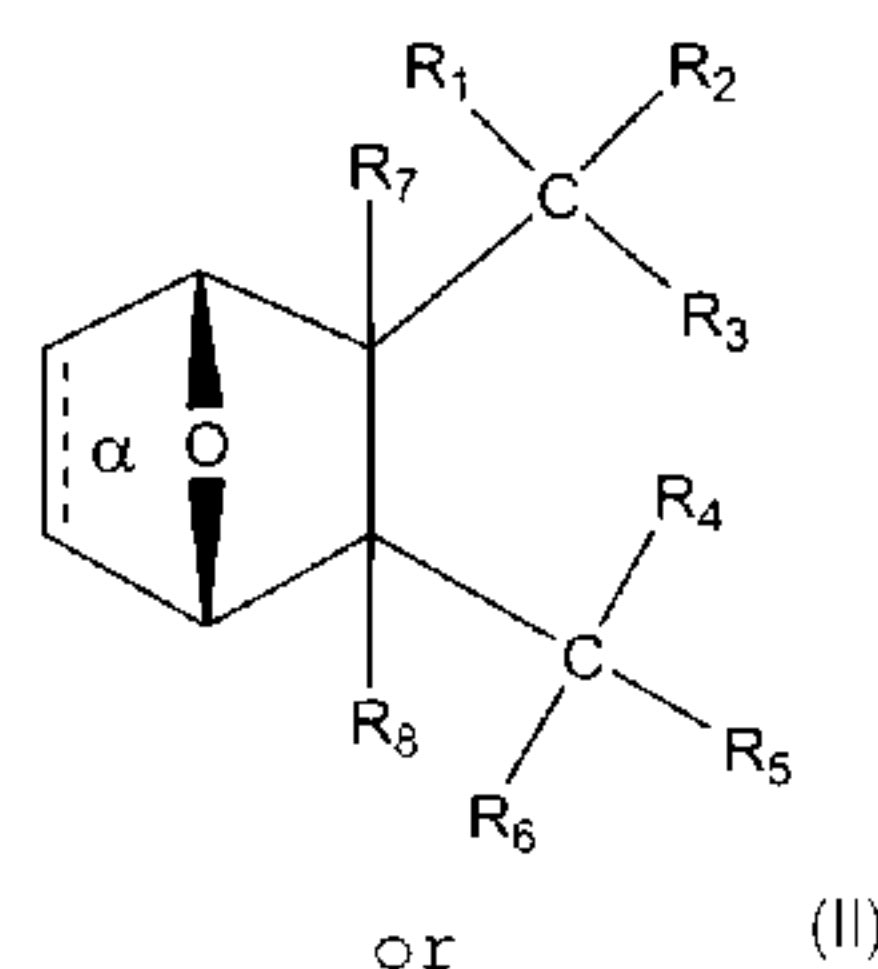
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(54) Title: OXABICYCLOHEPTANES AND OXABICYCLOHEPTENES, THEIR PREPARATION AND USE

Fig. 1

Inhibition of U373 by Four Cantharidin Analogs



(I)



(II)

(57) Abrégé/Abstract:

This invention provides compounds having the structure which may be used for the treatment of tumors.



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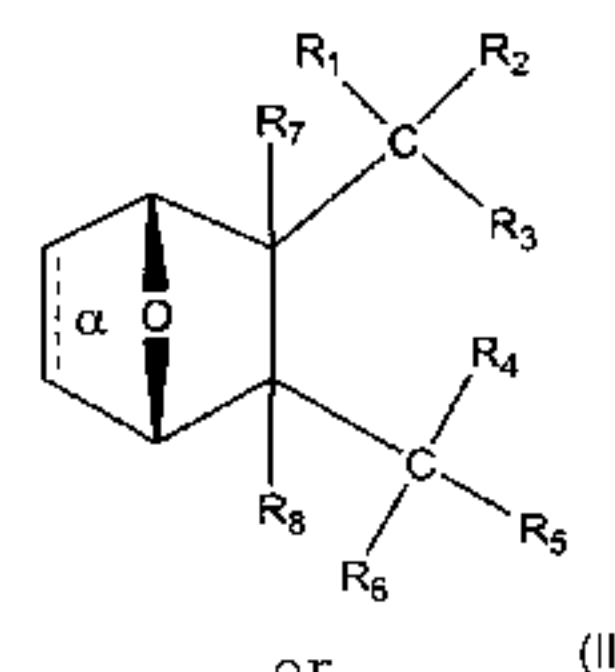
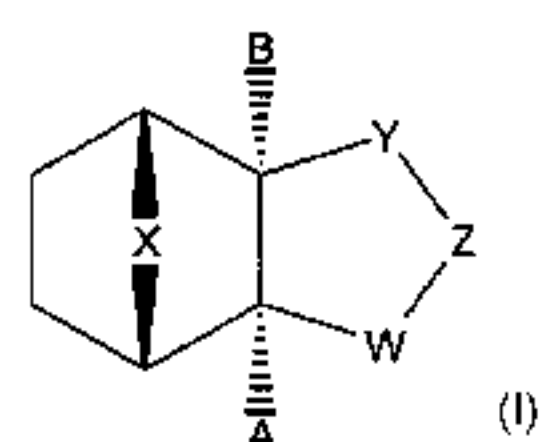
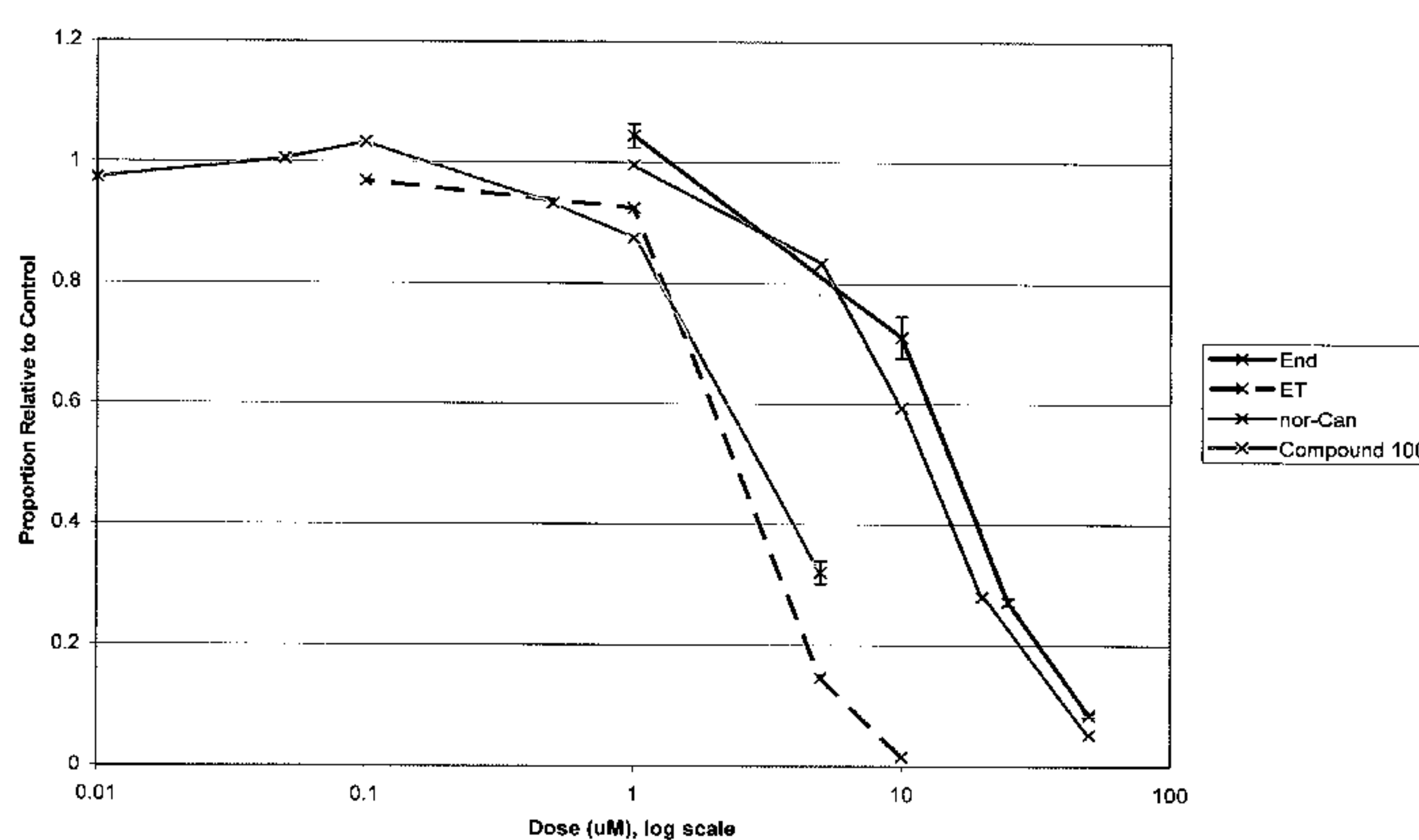
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FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL,  
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(54) Title: OXABICYCLOHEPTANES AND OXABICYCLOHEPTENES, THEIR PREPARATION AND USE

Fig. 1

Inhibition of U373 by Four Cantharidin Analogs



(57) Abstract: This invention provides compounds having the structure which may be used for the treatment of tumors.

Dkt. No. 4101/77449-A-PCT/JPW/YC

**OXABICYCLOHEPTANES AND OXABICYLCOHEPTENES,**  
**THEIR PREPARATION AND USE**

5

Throughout this application, certain publications are referenced. Full citations for these publications may be found immediately preceding the claims. The disclosures of these publications in their entireties are hereby incorporated by  
10 reference into this application in order to describe more fully the state-of-the art to which this invention relates.

**Background of the Invention**

15 Retinoids, metabolites of vitamin A, have been examined therapeutically against a variety of tumors, including gliomas. (Yung *et al.* (1996)) Nuclear receptor co-repressor (N-CoR) is closely associated with the retinoid receptor and is released upon ligand binding to the receptor. (Bastien *et al.* (2004)) By  
20 preventing the action of protein phosphatase-1 and protein phosphatase-2A, anti-phosphatases increase the phosphorylated form of N-CoR and promotes its subsequent cytoplasmic translocation. (Hermanson *et al.* (2002))

25 The phosphatase inhibitor, Cantharidin, has anti-tumor activity against human cancers of the liver (hepatomas) and of the upper gastrointestinal tract but is toxic to the urinary tract (Wang, 1989).

The publication of a report that cantharidin acts as a protein  
30 phosphatase inhibitor prompted a more general interest in compounds with this type of chemical structure (Li and Casida, 1992). Previously, it had been found that the simpler congener and its hydrolysis product (commercially available as the

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herbicide, Endothall) are hepatotoxic (Graziani and Casida, 1997). Binding studies have shown that the action of certain cantharidin homologs is direct on protein phosphatase-2A and indirect on protein phosphatase-1 (Honkanen *et al.*, 1993; Li *et al.*, 1993).

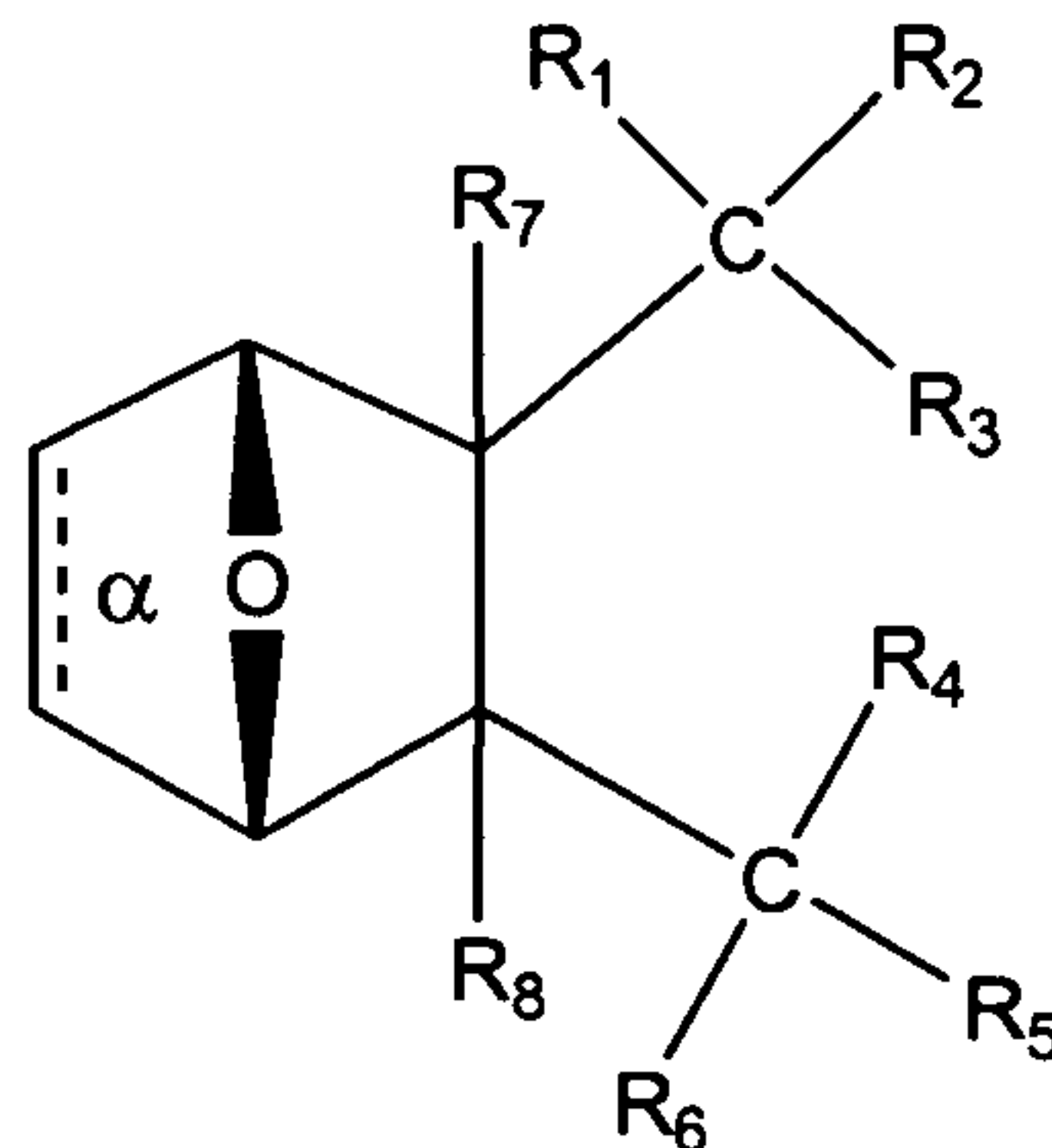
Of the known congeners of this type of compound, only the parent, cantharidin and its bis(normethyl)-derivative, norcantharidin, have seen any use as anti-cancer drug substances and only norcantharidin is used as an anti-neoplastic agent (Tsauer *et al.*, 1997).

Despite these successes, few compounds of this type have been screened for anti-tumor or cytotoxic activity. Currently, there is a significant need to develop inhibitors of protein phosphatases that are more active, less toxic and more specific in action than the known substances mentioned above.

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**Summary of the Invention**

A compound having the structure



5

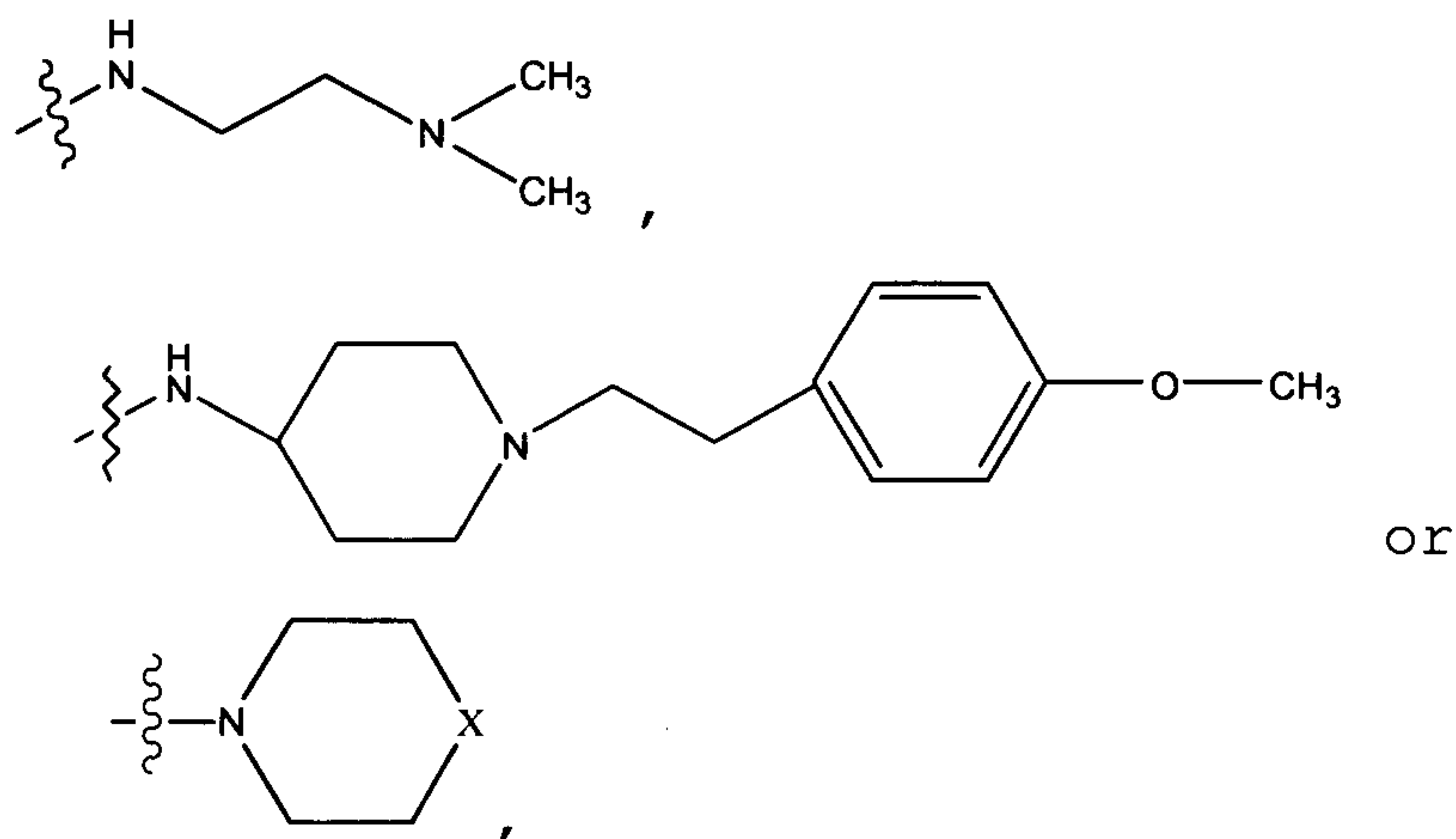
wherein

bond  $\alpha$  is present or absent;

$R_1$  and  $R_2$  is each independently H,  $O^-$ , or  $OR_9$ ,

10 where  $R_9$  is H, alkyl, alkenyl, alkynyl or aryl,  
or  $R_1$  and  $R_2$  together are  $=O$ ;

$R_3$  and  $R_4$  are each different and each is OH,  $O^-$ ,  $OR_9$ , SH,  $S^-$ ,  
15  $SR_9$



or

where X is O, S,  $NR_{10}$ , or  $N^+R_{10}R_{10}$ ,

20 where each  $R_{10}$  is independently alkyl, substituted  
 $C_2$ - $C_{12}$  alkyl, alkenyl, substituted  $C_4$ - $C_{12}$  alkenyl,

- 4 -

alkynyl, substituted alkynyl, aryl, substituted aryl where the substituent is other than chloro when  $R_1$  and  $R_2$  are =O,



5

$-\text{CH}_2\text{CN}$ ,  $-\text{CH}_2\text{CO}_2\text{R}_{11}$ ,  $-\text{CH}_2\text{COR}_{11}$ ,  $-\text{NHR}_{11}$  or  $-\text{NH}^+(\text{R}_{11})_2$ , wherein  $\text{R}_{11}$  is alkyl, alkenyl or alkynyl, each of which is substituted or unsubstituted, or H;

10

$\text{R}_5$  and  $\text{R}_6$  is each independently H, OH, or  $\text{R}_5$  and  $\text{R}_6$  taken together are =O; and

15

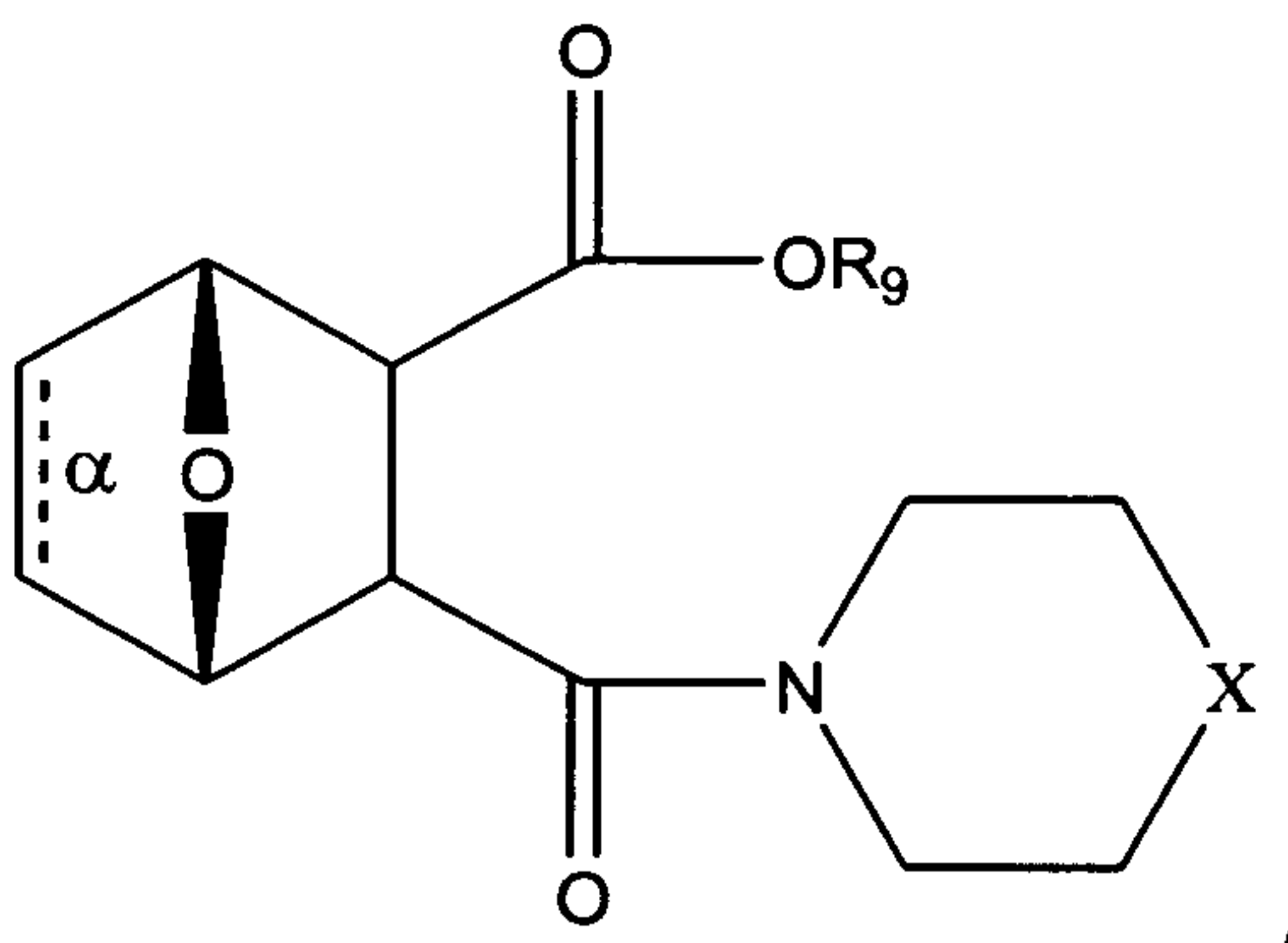
$\text{R}_7$  and  $\text{R}_8$  is each independently H, F, Cl, Br,  $\text{SO}_2\text{Ph}$ ,  $\text{CO}_2\text{CH}_3$ , or  $\text{SR}_{12}$ ,

where  $\text{R}_{12}$  is H, aryl or a substituted or unsubstituted alkyl, alkenyl or alkynyl,

or a salt, enantiomer or zwitterion of the compound.

20

A compound having the structure



wherein

25

bond  $\alpha$  is present or absent;

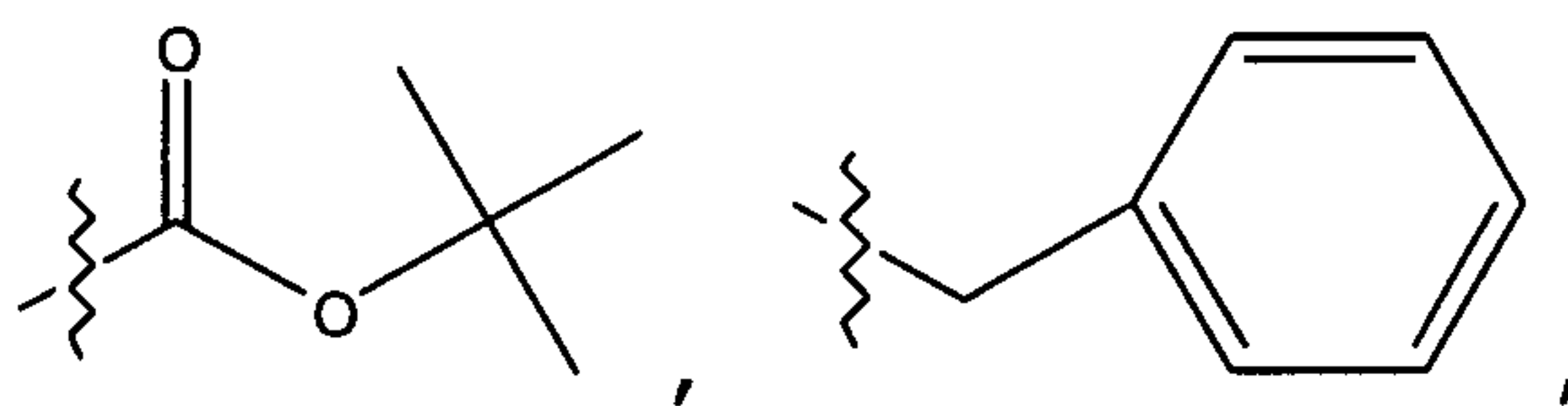
- 5 -

$R_9$  is present or absent and when present is H, alkyl, alkenyl, alkynyl or phenyl; and

X is O,  $NR_{10}$ , or  $NH^+R_{10}$ ,

5 where each  $R_{10}$  is independently H, alkyl, substituted  $C_2$ - $C_{12}$  alkyl, alkenyl, substituted  $C_4$ - $C_{12}$  alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl where the substituent is other than chloro,

10



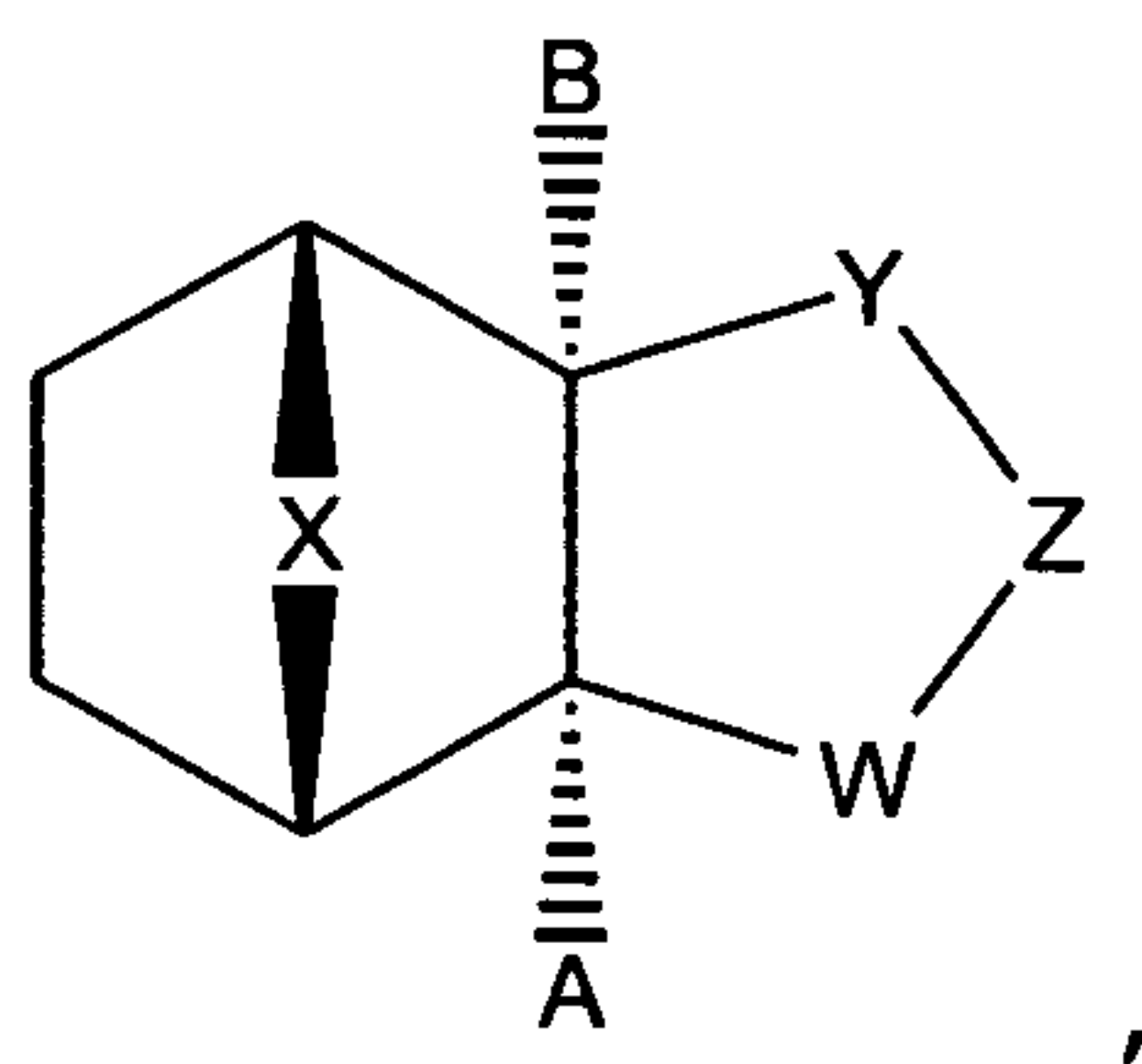
$-CH_2CN$ ,  $-CH_2CO_2R_{12}$ , or  $-CH_2COR_{12}$ , where  $R_{12}$  is H or alkyl,

15

or a salt, zwitterions, or enantiomer of the compound.

A compound having the structure

20



wherein

A and B is each independently H, F, Cl, Br,  $SO_2Ph$ ,  $CO_2CH_3$ , CN,  $COR_{14}$  or  $SR_{14}$ ,

25

where  $R_{14}$  is H or aryl or a substituted or unsubstituted alkyl, alkenyl or alkynyl;

- 6 -

X is O, NH or S;

Z is O, S, SR<sub>15</sub>, NH, NR<sub>15</sub>, CH<sub>2</sub>OH, CH<sub>2</sub>OR<sub>15</sub>,

where R<sub>15</sub> is an aryl or a substituted or unsubstituted  
alkyl, alkenyl or alkynyl; and

Y and W is each independently CH<sub>2</sub>, CHOH, C=O, or C=S,

or a salt of the compound.

10

The invention further contemplates a method of controlling undesired vegetation comprising contacting the vegetation or its environment with a herbicidally effective amount of the compounds of the invention.

15

The invention further contemplates a method of inhibiting plant phosphatase activity comprising contacting the plant or its environment with a herbicidally effective amount of the compounds of the invention.

20

This invention further contemplates a method of preventing or treating fungal infections in a subject comprising administering to the subject an effective amount of the compounds of this invention.

25

This invention further contemplates a method of treating cancer in a subject comprising administering to the subject an effective amount of the compounds of the invention.

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**Brief Description of the Figures**

Figure 1: Logarithmic curve of gliomal cell line U373  
5 treated with endothal (End), endothal  
thioanhydride (ET), nor-canthalidin (nor-  
Can) or Compound 100. Increasing dosages  
demonstrate a greater inhibition of growth.  
Error bars indicate SD.

10 Figure 2: Inhibition of gliomal cell line U373 treated  
with endothal (End) or Compound 100 or each  
with and without all-trans Retinoic acid  
(ATRA) for 7 days. Individual treatment with  
15 endothal and Compound 100 shows modest  
inhibition of growth. Combination of End or  
Compound 100 with ATRA shows synergistic  
reduction in cell growth. Error bars  
indicate SD.

20 Figure 3: Inhibition of growth of kidney cancer cell  
line, UMRC by endothal thioanhydride (ET),  
endothal (End), all-trans Retinoic Acid  
(ATRA), Trichostatin A (TSA) and  
25 norcanthalidin (nor-Can) for 7 days. Error  
bars indicate SD.

Figure 4: Inhibition of growth of gliomal cell line  
30 U373 by endothal thioanhydride (ET),  
endothal (End), all-trans Retinoic Acid  
(ATRA), Trichostatin A (TSA) and  
norcanthalidin (nor-Can) for 7 days.  
Individual treatment with endothal

- 8 -

thioanhydride showed the greatest inhibition of growth. Error bars indicate SD.

Figure 5:

5 Inhibition of growth of breast cancer cell line, MCF-7 by Inhibition of UMRC by endothal thioanhydride (ET), endothal (End), all-trans Retinoic Acid (ATRA), Trichostatin A (TSA) and norcantharidin (nor-Can) for 7 days. Treatment with individual doses of  
10 endothal, ATRA and TSA showed an inhibition in growth. Error bars indicate SD.

Figure 6:

15 Logarithmic curve of gliomal cell line U373 treated with 1-(3-Exocarboxy-7-oxabicyclo[2.2.1]heptane-2-exocarbonyl)-4-ethylpiperazine (Compound 105) after three days. Increasing dosages demonstrate a greater inhibition of growth.

Figure 7:

20 Logarithmic curve of human GBM cell line U373 treated with 1-(3-Exocarboxy-7-oxabicyclo[2.2.1]heptane-2-exocarbonyl)-4-ethylpiperazine (Compound 105) after seven days. Increasing dosages demonstrate a  
25 greater inhibition of growth.

Figure 8:

30 Inhibition of growth of human GBM cell line U373 treated with Compound 105 for seven days. Graph shows inhibition of growth over seven days at the following dosages: 1, 2, 5, 10, 50 and 100  $\mu$ M of compound 105, 10  $\mu$ M of compound 100, and a control dosage.

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Figure 9: Inhibition of growth of gliomal cell line U373 by Compound 105 over 7 days.

Figure 10: Inhibition of growth of gliomal cell line U373 by Compound 102 over 7 days.

Figure 11: Inhibition of growth of gliomal cell line U373 by Compound 101 over 7 days.

Figure 12: Inhibition of growth of gliomal cell line U373 by Compound 103 over 7 days.

Figure 13: Inhibition of growth of gliomal cell line U373 by Compound 104 over 7 days.

Figure 14: Inhibition of growth of gliomal cell line U373 by Compound 106 over 7 days.

Figure 15: Inhibition of growth of gliomal cell line U373 by Compound 100 over 7 days.

Figure 16: Inhibition of growth of medulloblastoma cell line DAOY by compound 100 over 7 days.

Figure 17: Effect of compound 100 on U87 xenograft tumor volume over 26 days.

Figure 18: Effect of compound 100 and compound 102 on DAOY xenograft tumor volume over 23 days.

Figure 19A: Graphical representation (I) and curve fit with  $IC_{50}$  value (II) of data obtained following exposure of MDA-MB-231 cells to

- 10 -

compound 100 and compound 102 using the CellTiter-Glo assay. The effects of 10 $\mu$ M doxorubicin that was used as a positive control are also shown in I. Each point represents the mean  $\pm$  SD of at least triplicate samples.

Figure 19B:

Graphical representation (I) and curve fit with IC<sub>50</sub> value (II) of data obtained following exposure of HT-29 cells to compound 100 and compound 102 using the CellTiter-Glo assay. The effects of 10 $\mu$ M doxorubicin that was used as a positive control are also shown in I. Each point represents the mean  $\pm$  SD of at least triplicate samples.

Figure 19C:

Graphical representation (I) and curve fit with IC<sub>50</sub> value (II) of data obtained following exposure of NCI-H460 cells to compound 100 and compound 102 using the CellTiter-Glo assay. The effects of 10 $\mu$ M doxorubicin that was used as a positive control are also shown in I. Each point represents the mean  $\pm$  SD of at least triplicate samples.

Figure 19D

Graphical representation (I) and curve fit with IC<sub>50</sub> value (II) of data obtained following exposure of NCI-H522 cells to compound 100 and compound 102 using the CellTiter-Glo assay. The effects of 10 $\mu$ M doxorubicin that was used as a positive

- 11 -

control are also shown in I. Each point represents the mean  $\pm$  SD of at least triplicate samples.

- 5     Figure 19E     Graphical representation (I) and curve fit  
with IC<sub>50</sub> value (II) of data obtained  
following exposure of NCI-H69 cells to  
compound 100 and compound 102 using the  
CellTiter-Glo assay. The effects of 10 $\mu$ M  
doxorubicin that was used as a positive  
10     control are also shown in I. Each point  
represents the mean  $\pm$  SD of at least  
triplicate samples.
- 15     Figure 19F:     Graphical representation (I) and curve fit  
with IC<sub>50</sub> value (II) of data obtained  
following exposure of GXF-209 cells to  
compound 100 and compound 102 using the  
CellTiter-Glo assay. The effects of 10 $\mu$ M  
doxorubicin that was used as a positive  
20     control are also shown in I. Each point  
represents the mean  $\pm$  SD of at least  
triplicate samples.
- 25     Figure 19G:     Graphical representation (I) and curve fit  
with IC<sub>50</sub> value (II) of data obtained  
following exposure of HepG2 cells to  
compound 100 and compound 102 using the  
CellTiter-Glo assay. The effects of 10 $\mu$ M  
doxorubicin that was used as a positive  
30     control are also shown in I. Each point  
represents the mean  $\pm$  SD of at least  
triplicate samples.

- 12 -

Figure 19H: Graphical representation (I) and curve fit with  $IC_{50}$  value (II) of data obtained following exposure of OVCAR-3 cells to compound 100 and compound 102 using the CellTiter-Glo assay. The effects of 10 $\mu$ M doxorubicin that was used as a positive control are also shown in I. Each point represents the mean  $\pm$  SD of at least triplicate samples.

Figure 19I: Graphical representation (I) and curve fit with  $IC_{50}$  value (II) of data obtained following exposure of PANC-1 cells to compound 100 and compound 102 using the CellTiter-Glo assay. The effects of 10 $\mu$ M doxorubicin that was used as a positive control are also shown in I. Each point represents the mean  $\pm$  SD of at least triplicate samples.

Figure 19J: Graphical representation (I) and curve fit with  $IC_{50}$  value (II) of data obtained following exposure of DU-145 cells to compound 100 and compound 102 using the CellTiter-Glo assay. The effects of 10 $\mu$ M doxorubicin that was used as a positive control are also shown in I. Each point represents the mean  $\pm$  SD of at least triplicate samples.

Figure 19K: Graphical representation (I) and curve fit with  $IC_{50}$  value (II) of data obtained

- 13 -

following exposure of LNCAP cells to compound 100 and compound 102 using the CellTiter-Glo assay. The effects of 10 $\mu$ M doxorubicin that was used as a positive control are also shown in I. Each point represents the mean  $\pm$  SD of at least triplicate samples.

Figure 19L:

Graphical representation (I) and curve fit with IC<sub>50</sub> value (II) of data obtained following exposure of HL-60 cells to compound 100 and compound 102 using the CellTiter-Glo assay. The effects of 10 $\mu$ M doxorubicin that was used as a positive control are also shown in I. Each point represents the mean  $\pm$  SD of at least triplicate samples.

Figure 19M:

Graphical representation (I) and curve fit with IC<sub>50</sub> value (II) of data obtained following exposure of K-562 cells to compound 100 and compound 102 using the CellTiter-Glo assay. The effects of 10 $\mu$ M doxorubicin that was used as a positive control are also shown in I. Each point represents the mean  $\pm$  SD of at least triplicate samples.

Figure 19N:

Graphical representation (I) and curve fit with IC<sub>50</sub> value (II) of data obtained following exposure of MOLT-4 cells to compound 100 and compound 102 using the CellTiter-Glo assay. The effects of 10 $\mu$ M

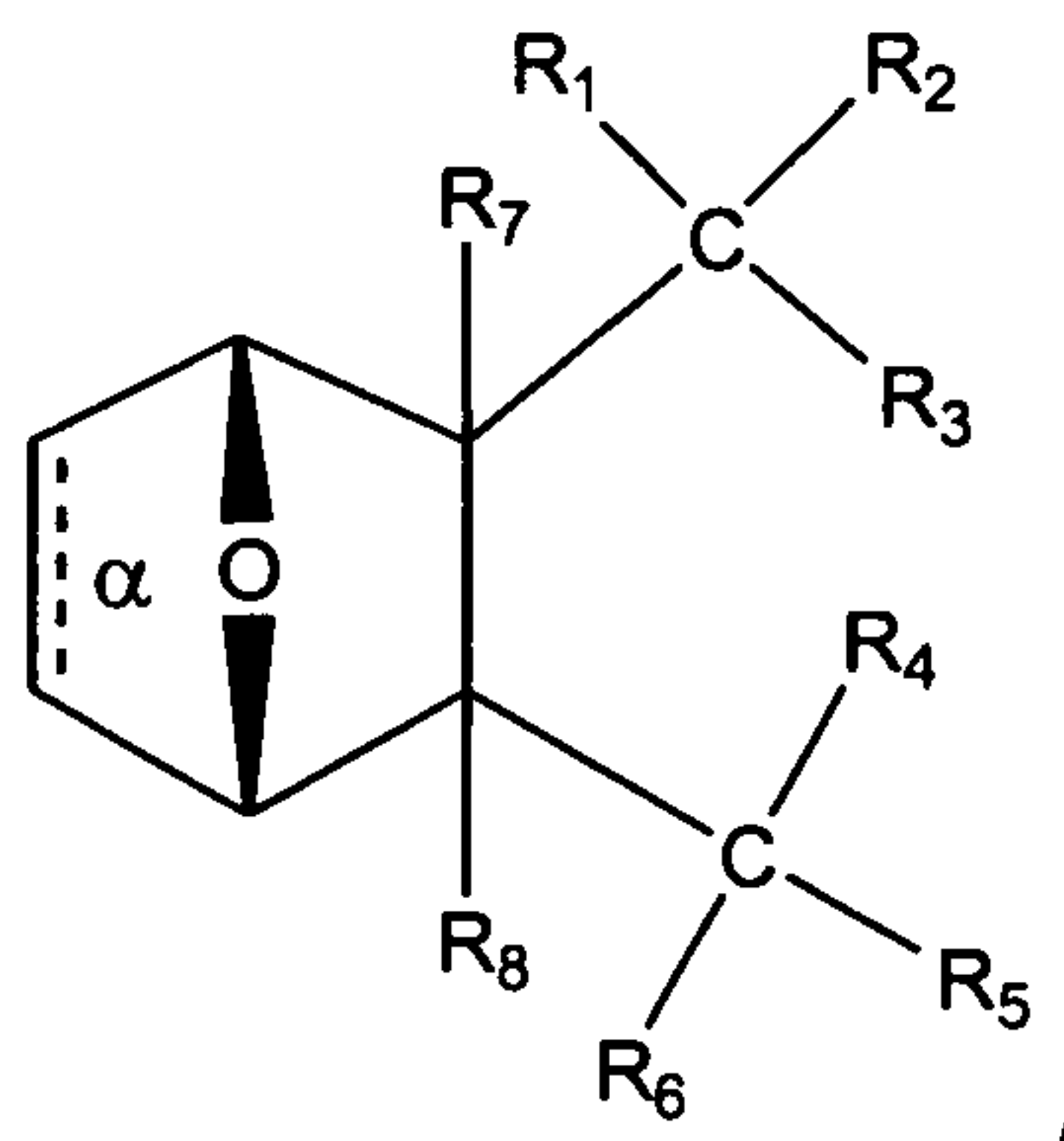
- 14 -

doxorubicin that was used as a positive control are also shown in I. Each point represents the mean  $\pm$  SD of at least triplicate samples.

- 15 -

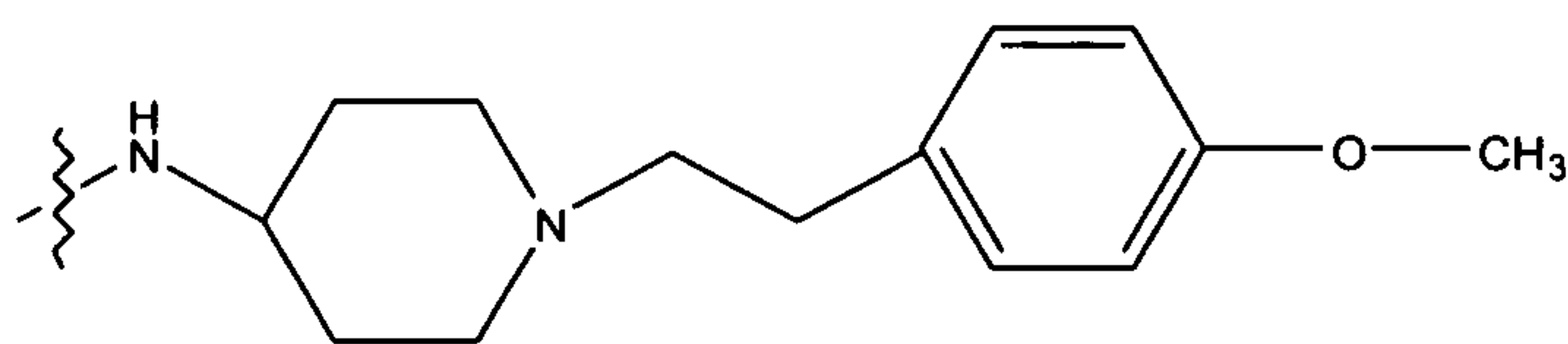
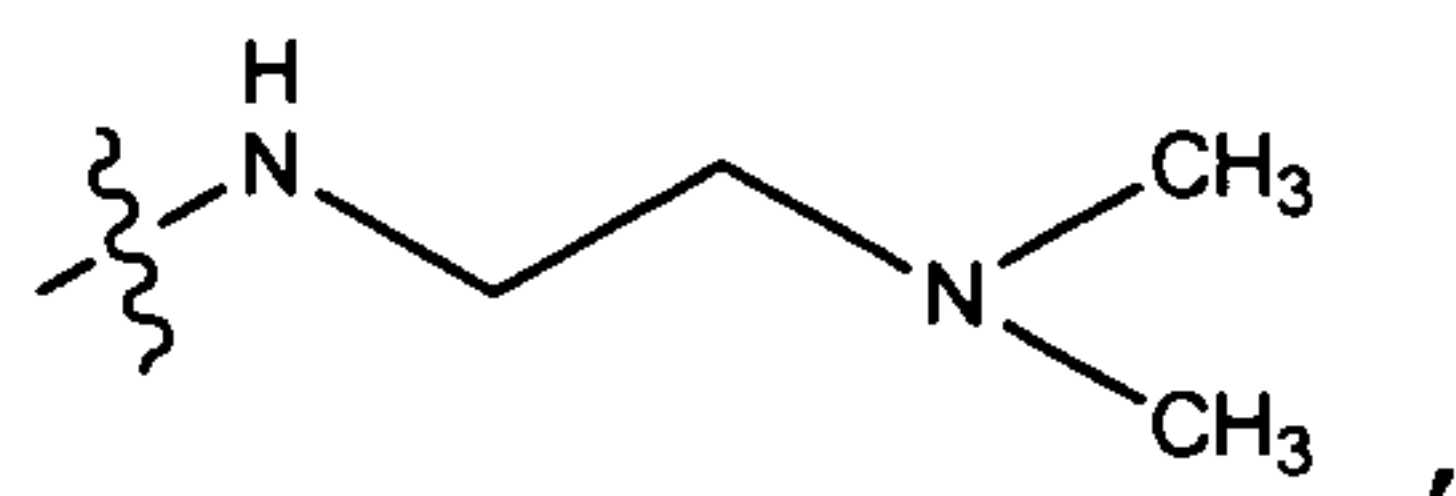
**Detailed Description of the Invention**

A compound having the structure



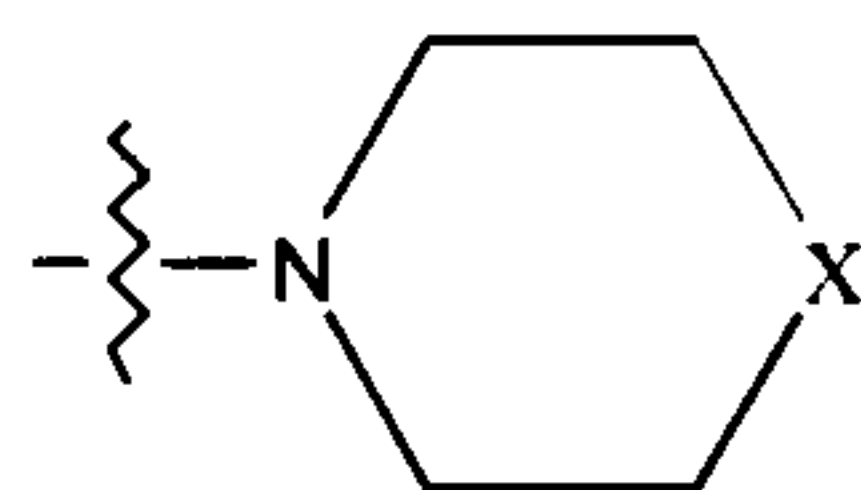
5

wherein bond  $\alpha$  is present or absent;  $R_1$  and  $R_2$  is each  
 10 independently H,  $O^-$ ,  $OR_9$ , where  $R_9$  is H, alkyl, alkenyl, alkynyl  
 or aryl, or  $R_1$  and  $R_2$  together are  $=O$ ;  $R_3$  and  $R_4$  are each  
 different and each is OH,  $O^-$ ,  $OR_9$ , SH,  $S^-$ ,  $SR_9$



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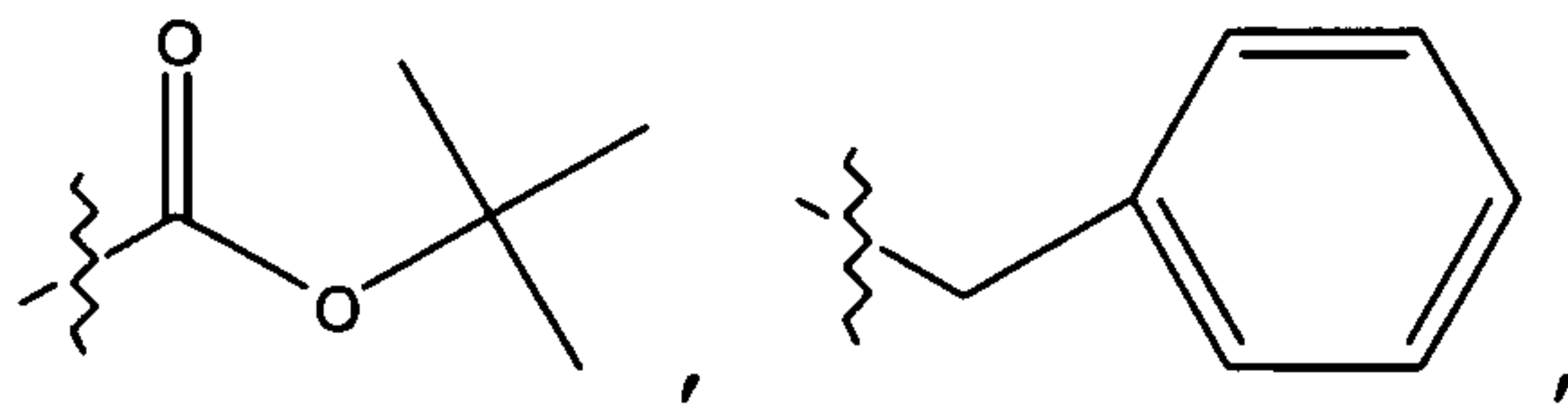
or



, where X is O, S,  $NR_{10}$ ,  $N^+R_{10}R_{10}$ ,

where each  $R_{10}$  is independently alkyl, substituted  
 $C_2$ - $C_{12}$  alkyl, alkenyl, substituted  $C_4$ - $C_{12}$  alkenyl,  
 alkynyl, substituted alkynyl, aryl, substituted  
 20 aryl where the substituent is other than chloro  
 when  $R_1$  and  $R_2$  are  $=O$ ,

- 16 -

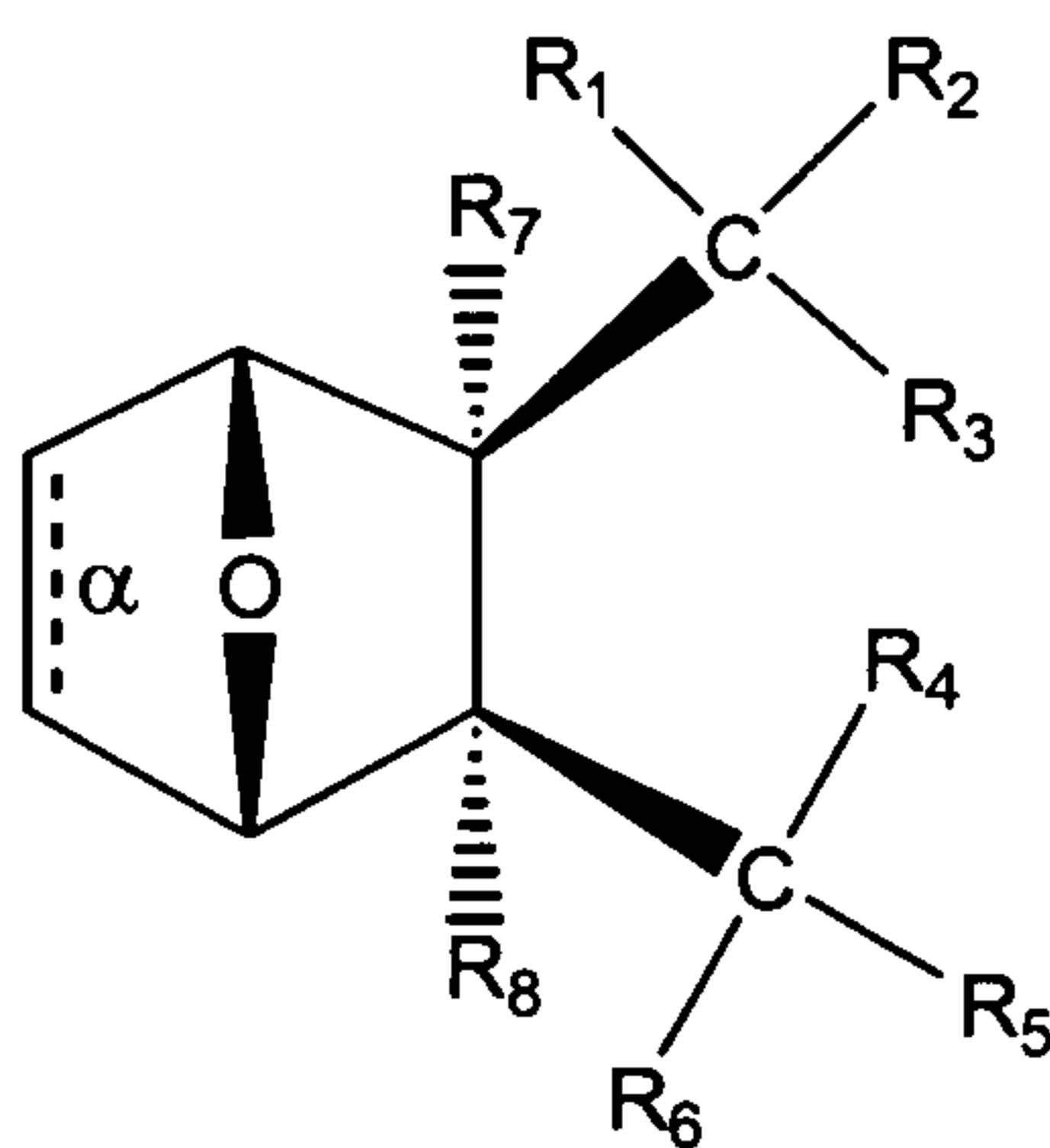


-CH<sub>2</sub>CN , -CH<sub>2</sub>CO<sub>2</sub>R<sub>11</sub>, -CH<sub>2</sub>COR<sub>11</sub>, -NHR<sub>11</sub>, -NH<sup>+</sup>(R<sub>11</sub>)<sub>2</sub>

5 wherein each R<sub>11</sub> is independently alkyl, alkenyl or alkynyl, each of which is substituted or unsubstituted, or H;

10 R<sub>5</sub> and R<sub>6</sub> is each independently H, OH, or R<sub>5</sub> and R<sub>6</sub> taken together are =O; and R<sub>7</sub> and R<sub>8</sub> is each independently H, F, Cl, Br, SO<sub>2</sub>Ph, CO<sub>2</sub>CH<sub>3</sub>, CN, COR<sub>12</sub>, or SR<sub>12</sub>, where R<sub>12</sub> is H, aryl or a substituted or unsubstituted alkyl, alkenyl or alkynyl, or a salt, enantiomer or zwitterion of the compound.

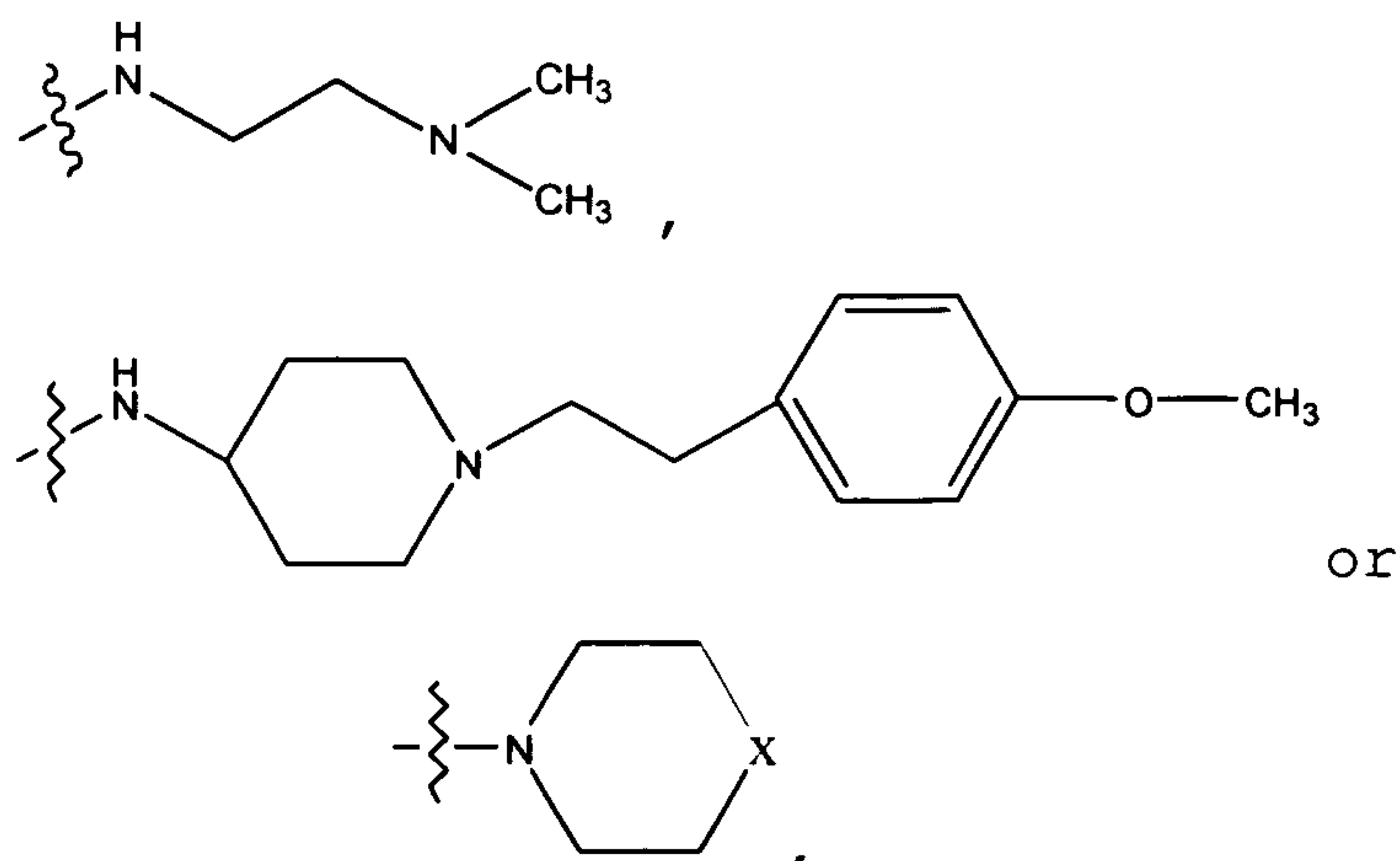
15 An embodiment of the invention provides a compound of the structure



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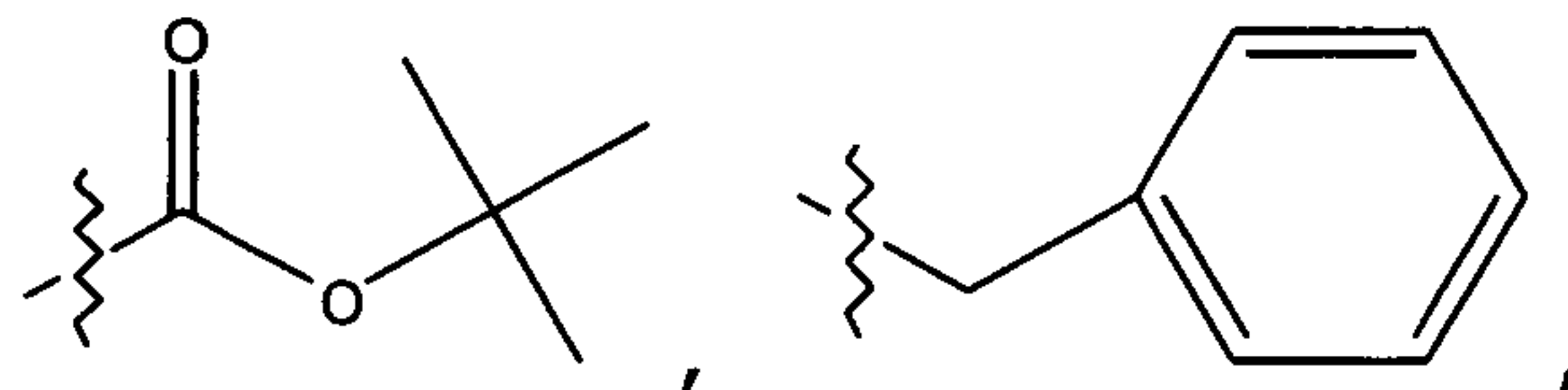
The invention further provides the instant composition, wherein R<sub>1</sub> and R<sub>2</sub> together are =O; R<sub>3</sub> is O<sup>-</sup> or OR<sub>9</sub>, where R<sub>9</sub> is H, methyl, ethyl or phenyl; R<sub>4</sub> is

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where X is O, S, NR<sub>10</sub> or N<sup>+</sup>R<sub>10</sub>R<sub>10</sub>,

where each R<sub>10</sub> is independently H, alkyl, substituted C<sub>2</sub>-C<sub>12</sub> alkyl, alkenyl, substituted C<sub>4</sub>-C<sub>12</sub> alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl where the substituent is other than chloro when R<sub>1</sub> and R<sub>2</sub> are =O,



-CH<sub>2</sub>CN, -CH<sub>2</sub>CO<sub>2</sub>R<sub>11</sub>, -CH<sub>2</sub>COR<sub>11</sub>, -NHR<sub>11</sub>, -NH<sup>+</sup>(R<sub>11</sub>)<sub>2</sub>

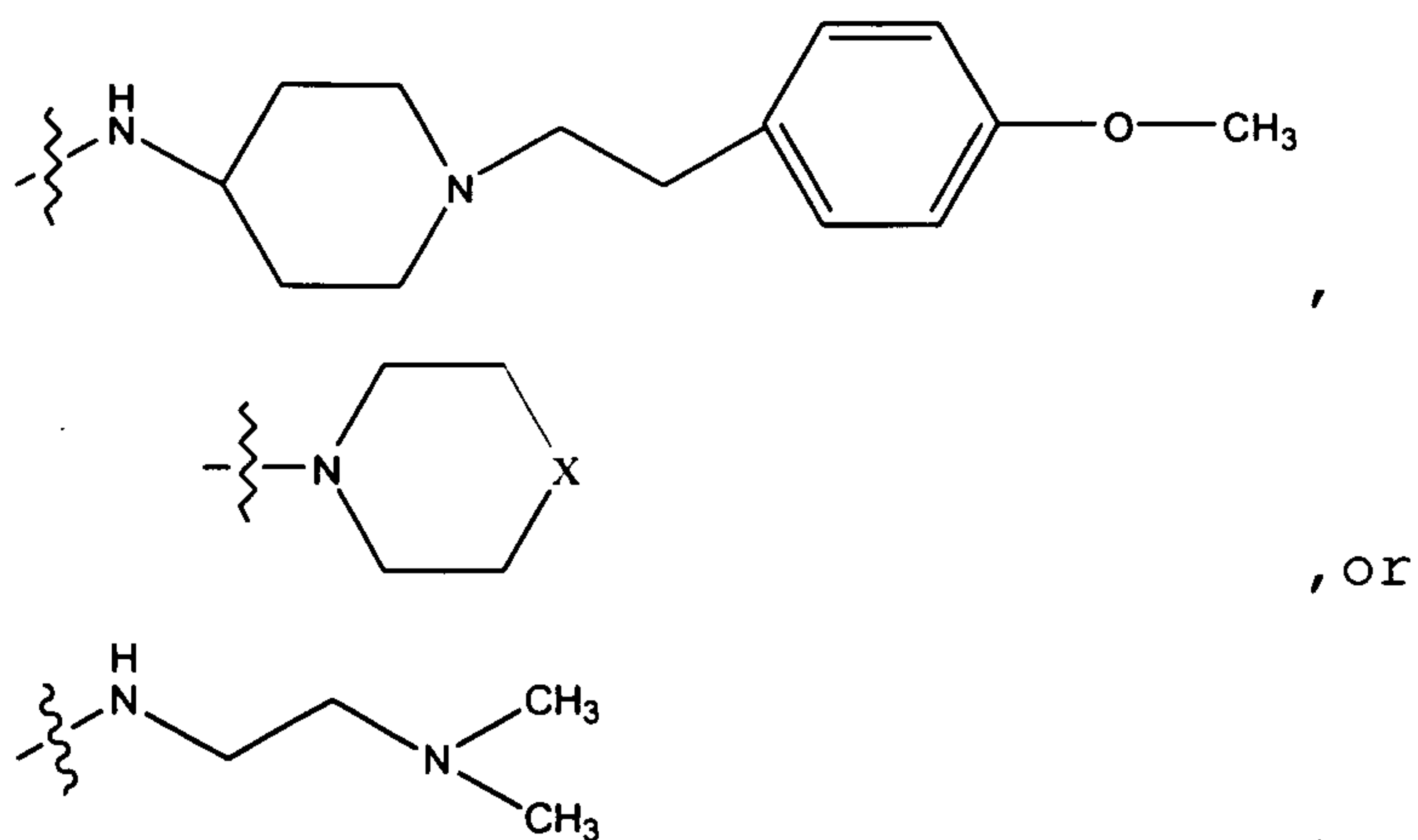
where R<sub>11</sub> is alkyl, alkenyl or alkynyl, each of which is substituted or unsubstituted, or H;

R<sub>5</sub> and R<sub>6</sub> taken together are =O; and R<sub>7</sub> and R<sub>8</sub> is each independently H, F, Cl, Br, SO<sub>2</sub>Ph, CO<sub>2</sub>CH<sub>3</sub>, CN, COR<sub>12</sub>, or SR<sub>12</sub>, where R<sub>12</sub> is H or a substituted or unsubstituted alkyl, alkenyl or alkynyl.

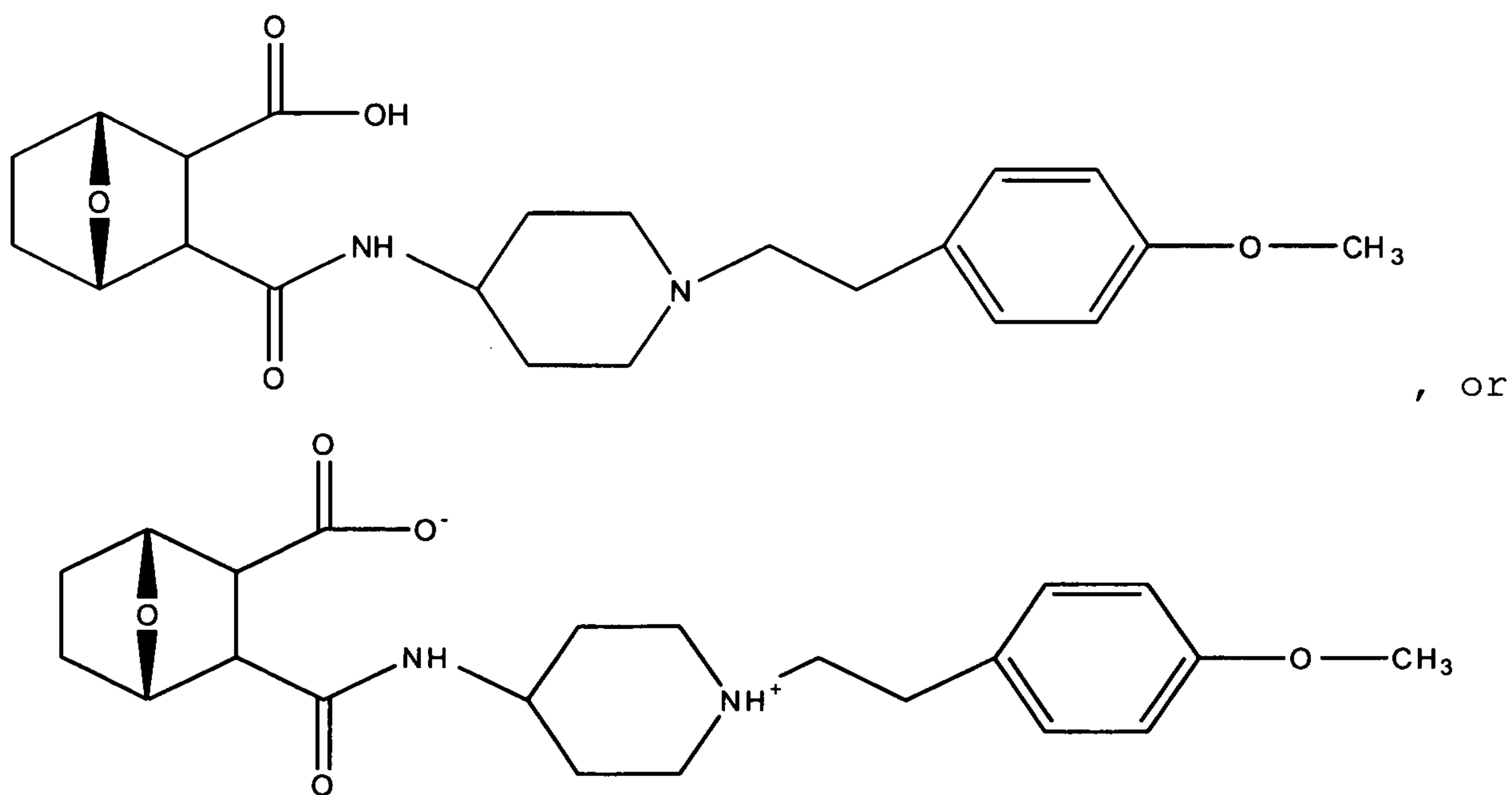
The invention further provides the instant composition, wherein R<sub>3</sub> is O<sup>-</sup>.

The invention further provides the instant composition, wherein R<sub>4</sub> is

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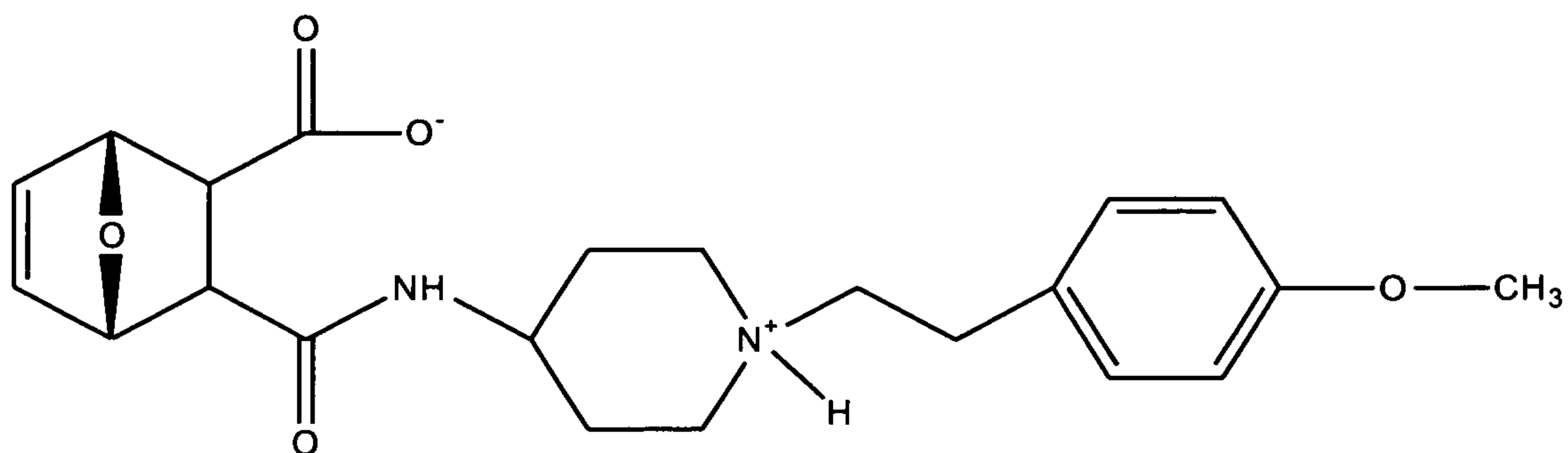
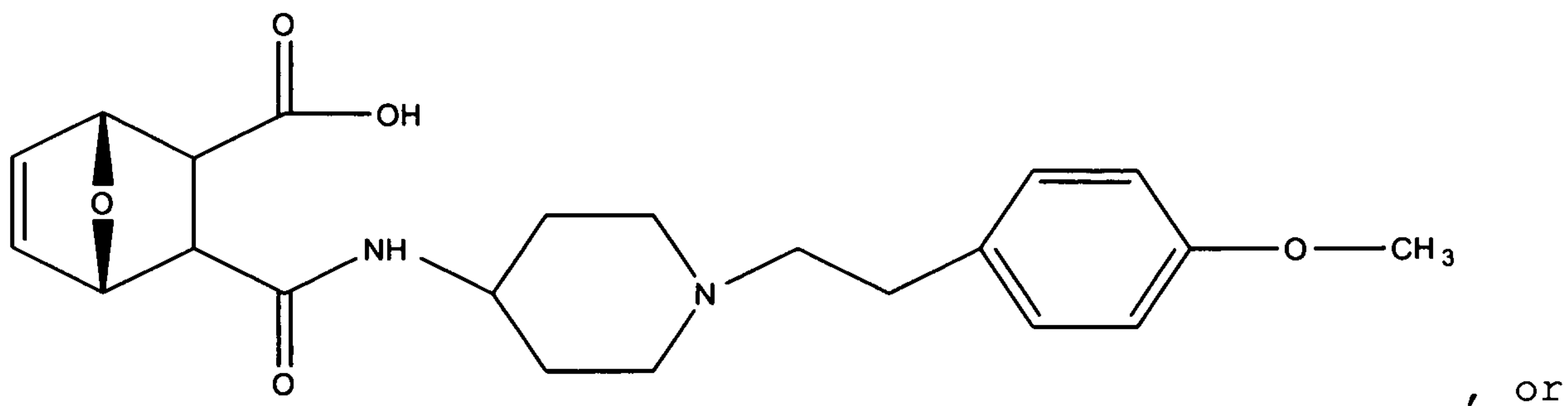
5 In an embodiment, the invention provides a compound having the structure (Compound 104)



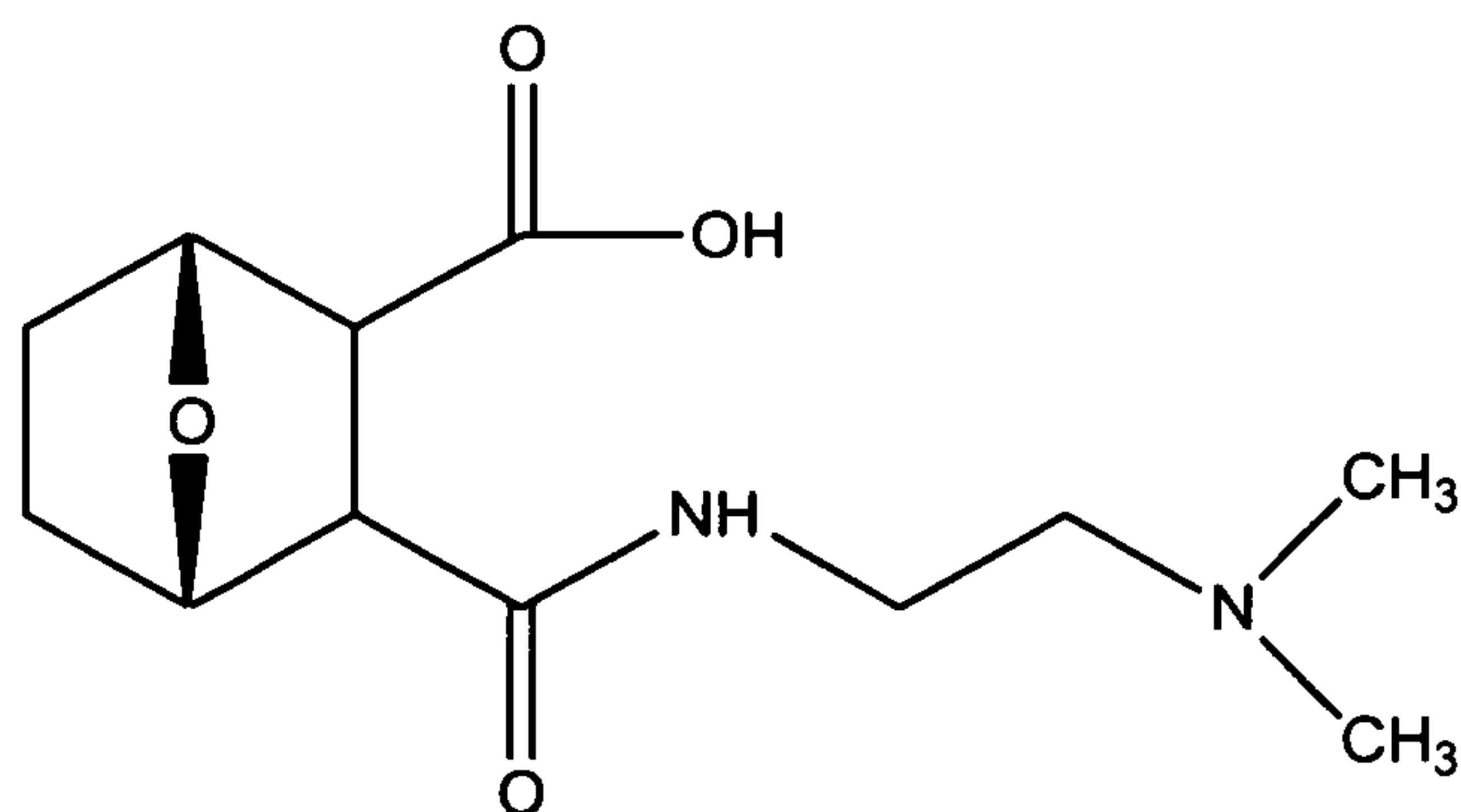
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In an embodiment, the invention provides a compound having the structure (Compound 104E)

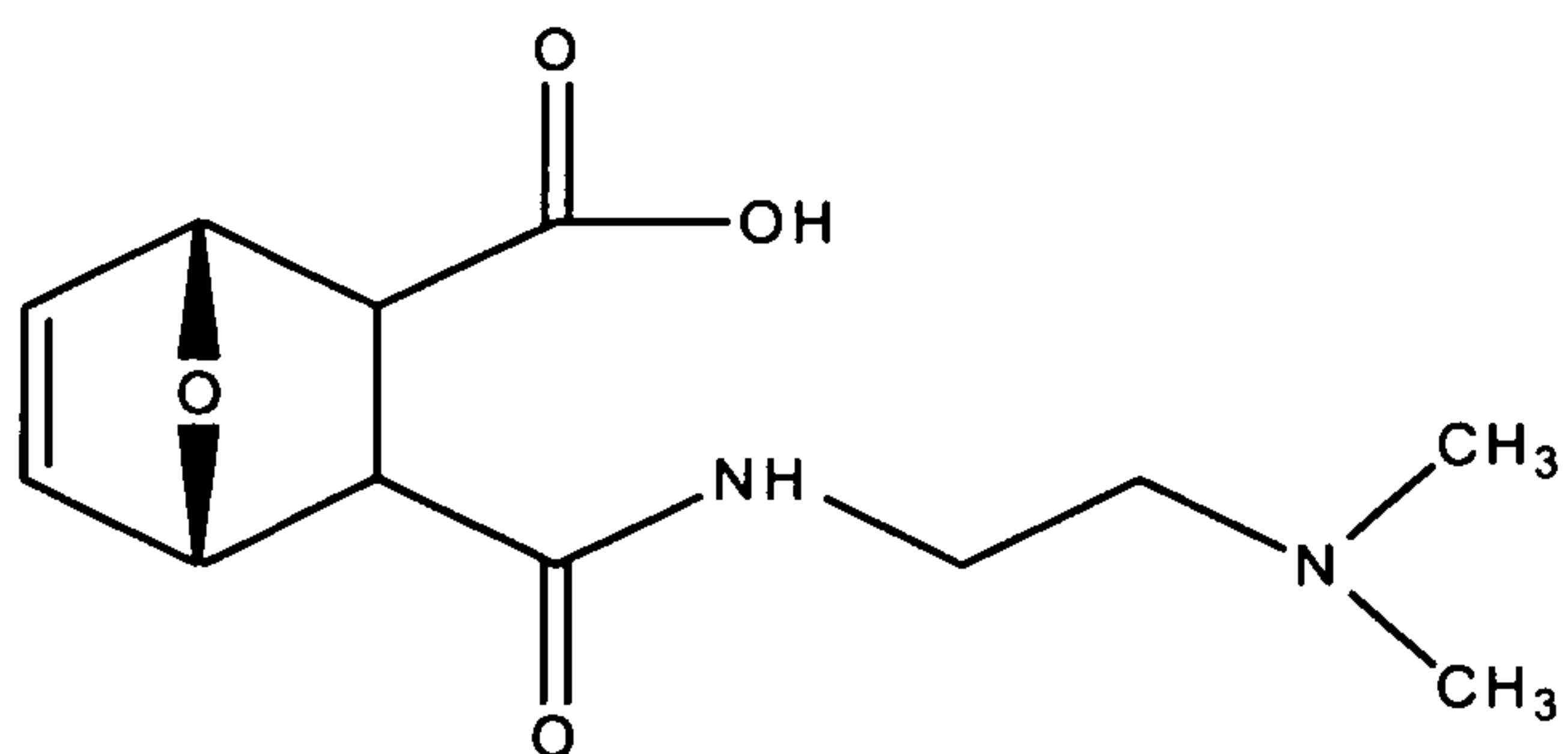
- 19 -



5 In an embodiment, the invention provides a compound having the structure (Compound 106)

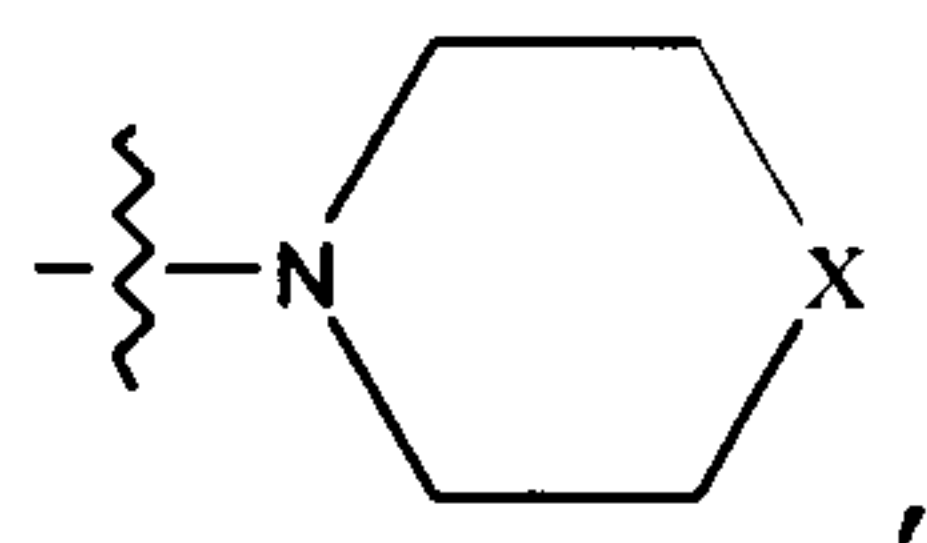


10 In an embodiment, the invention provides a compound having the structure (Compound 106E)

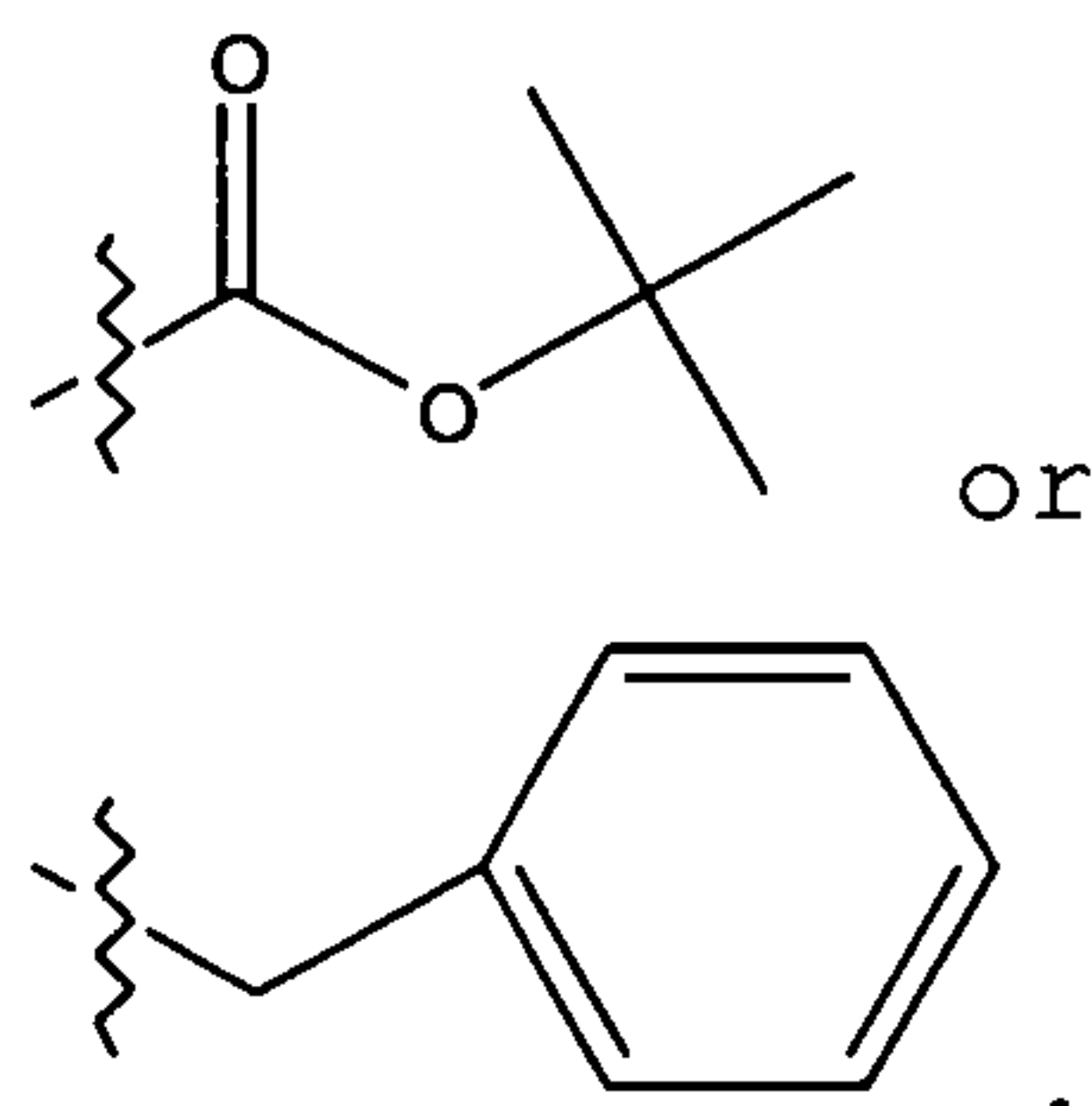


- 20 -

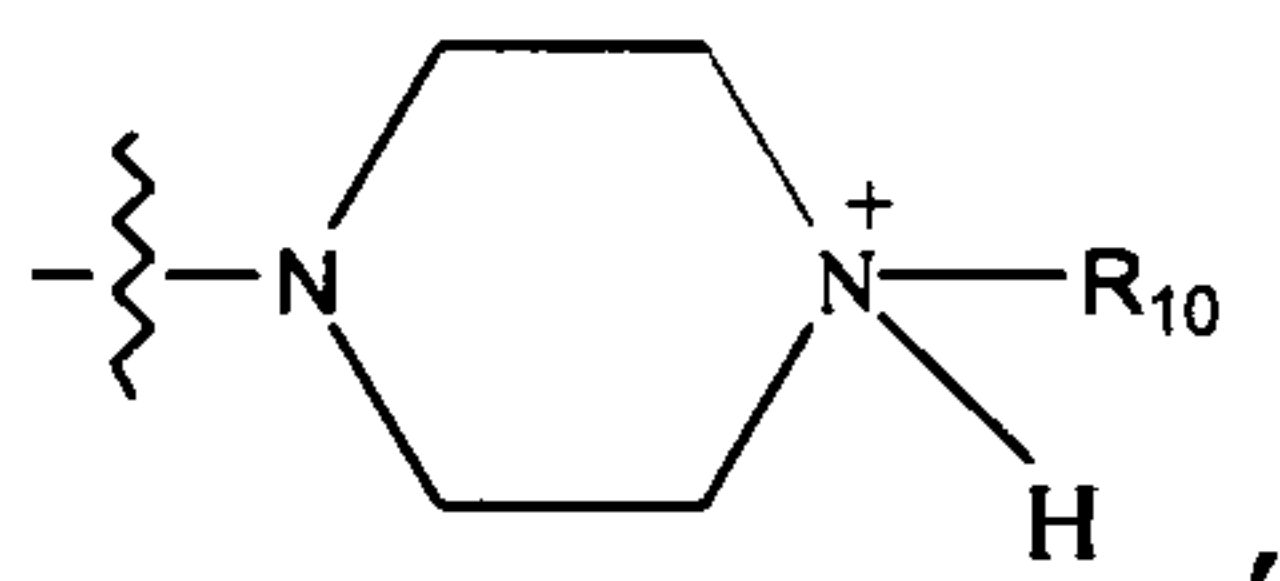
The invention further provides the instant composition, wherein  $R_4$  is



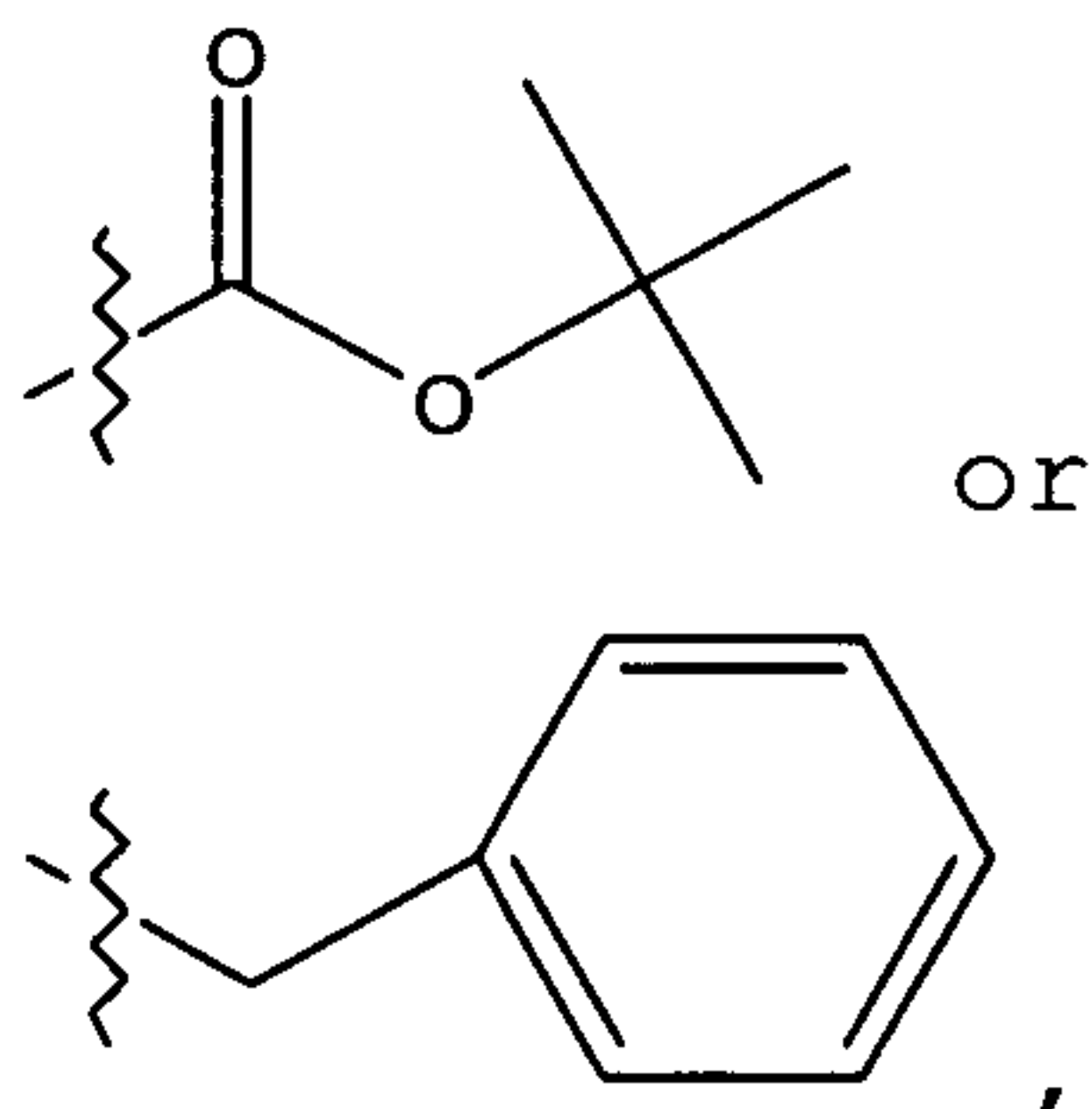
where X is O,  $NR_{10}$ , or  $N^+R_{10}R_{10}$ , where each  $R_{10}$  is independently H,  
 5 alkyl, substituted  $C_2$ - $C_{12}$  alkyl, alkenyl, substituted  $C_4$ - $C_{12}$   
 alkenyl, alkynyl, substituted alkynyl,



10 The invention further provides the instant composition, wherein  $R_4$  is



where  $R_{10}$  H, alkyl, substituted  $C_2$ - $C_{12}$  alkyl, alkenyl, substituted  
 $C_4$ - $C_{12}$  alkenyl, alkynyl, substituted alkynyl, substituted aryl  
 15 where the substituent is other than chloro when  $R_1$  and  $R_2$  are =O,

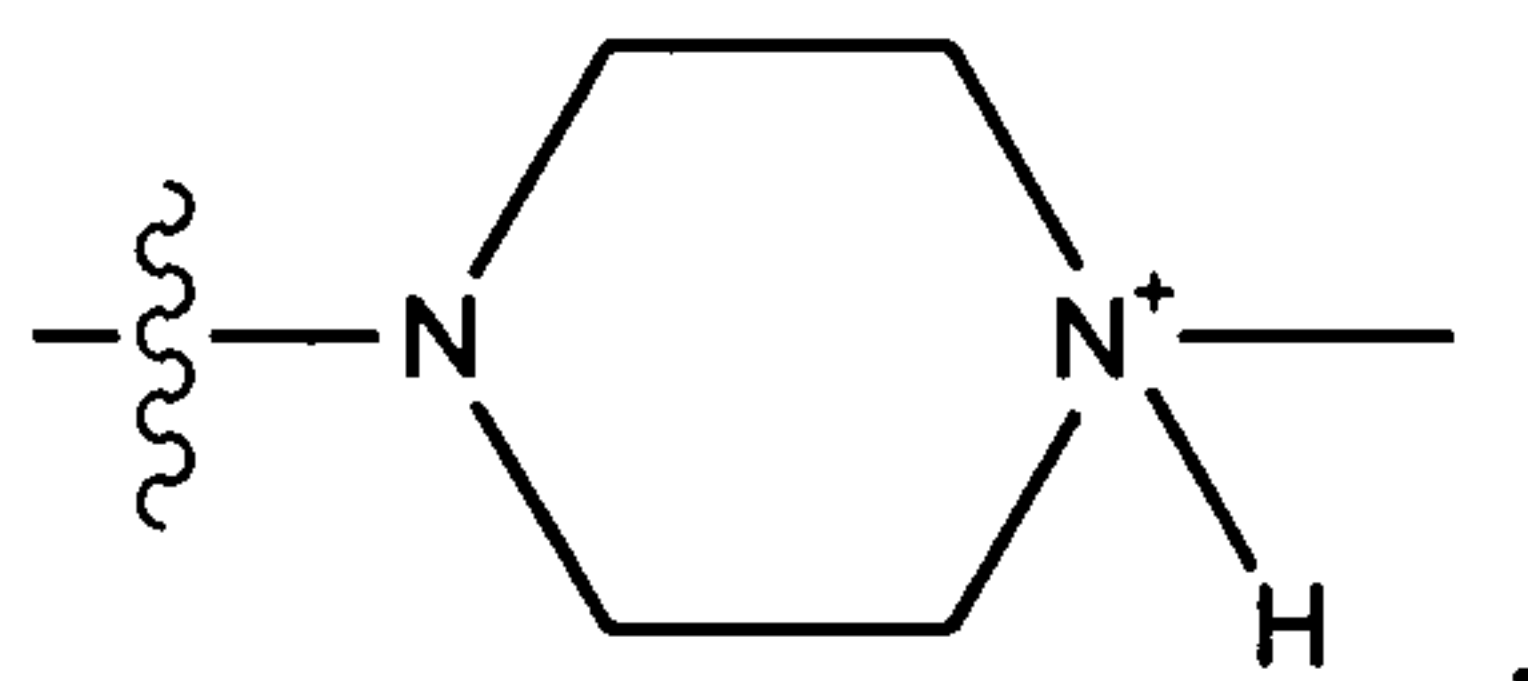


- $CH_2CN$ , - $CH_2CO_2R_{11}$ , - $CH_2COR_{11}$ , - $NHR_{11}$ , or  $NH^+(R_{11})_2$ , where  
 $R_{11}$  is H or alkyl.

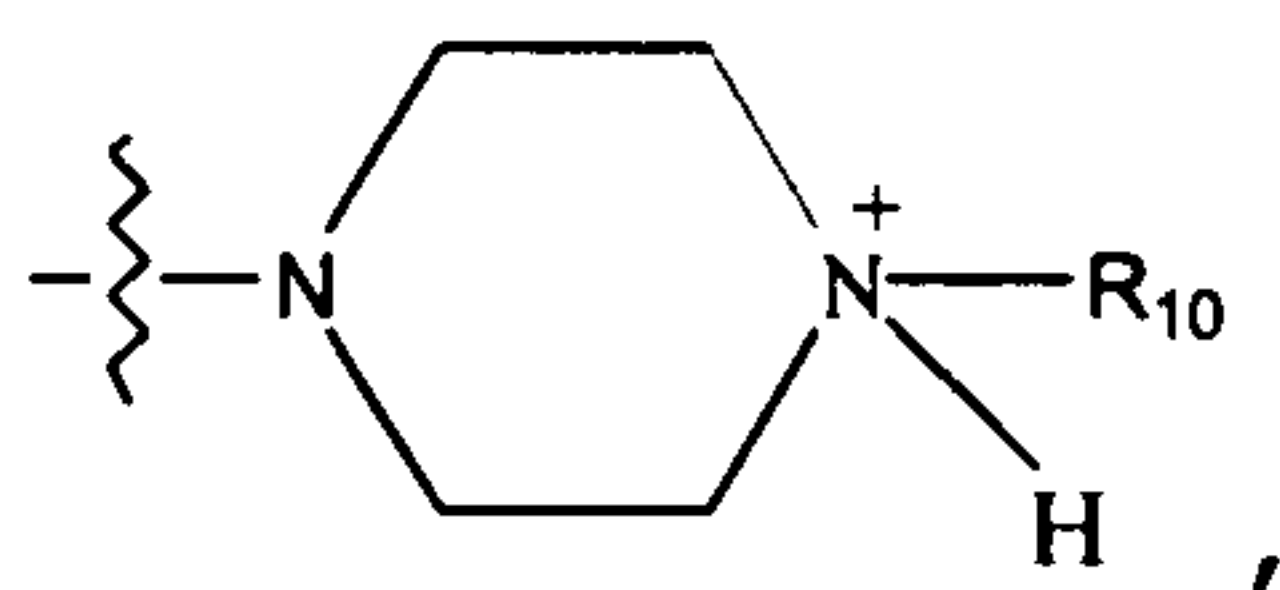
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The invention further provides the instant composition, wherein  $R_4$  is

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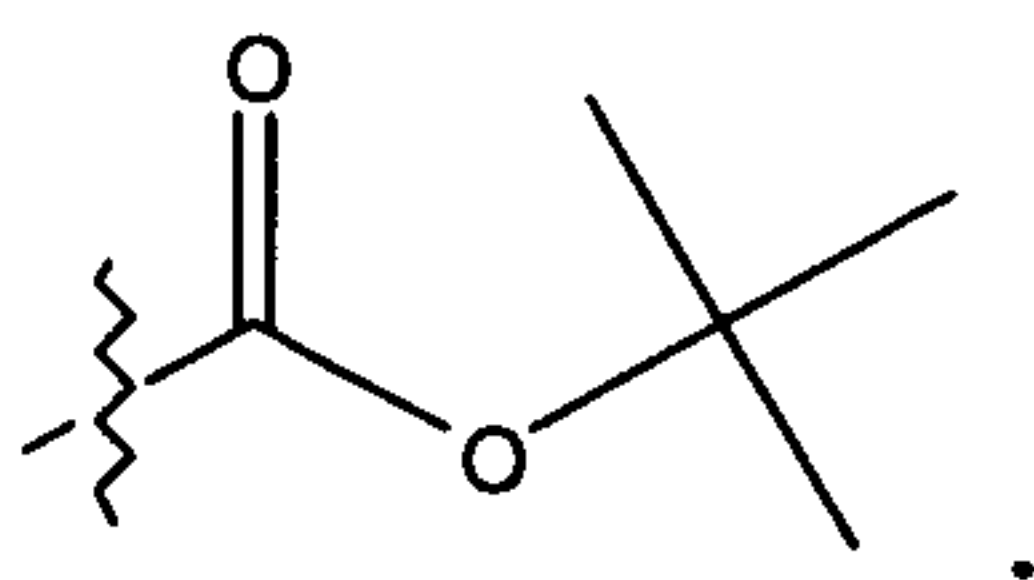


The invention further provides the instant composition, wherein  $R_4$  is



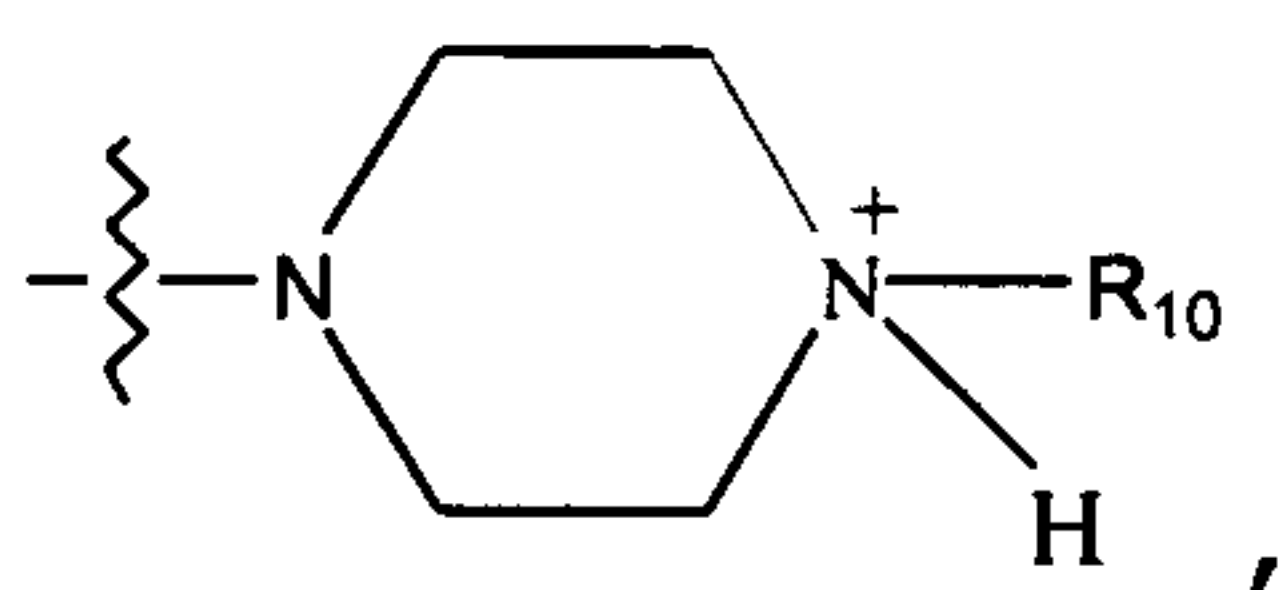
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where  $R_{10}$  is

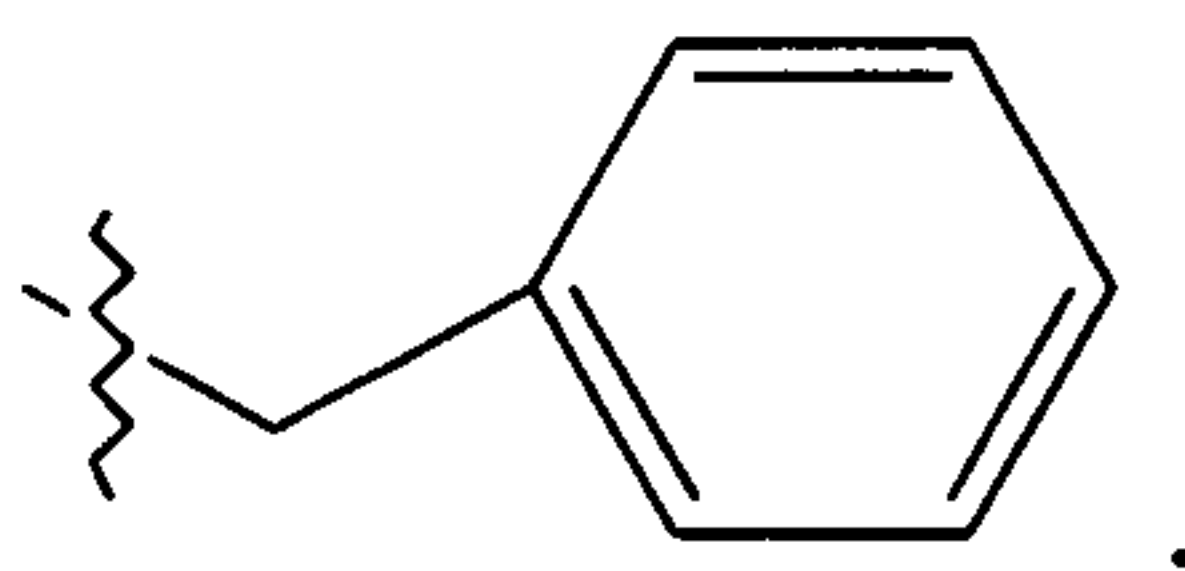


The invention further provides the instant composition, wherein  $R_4$  is

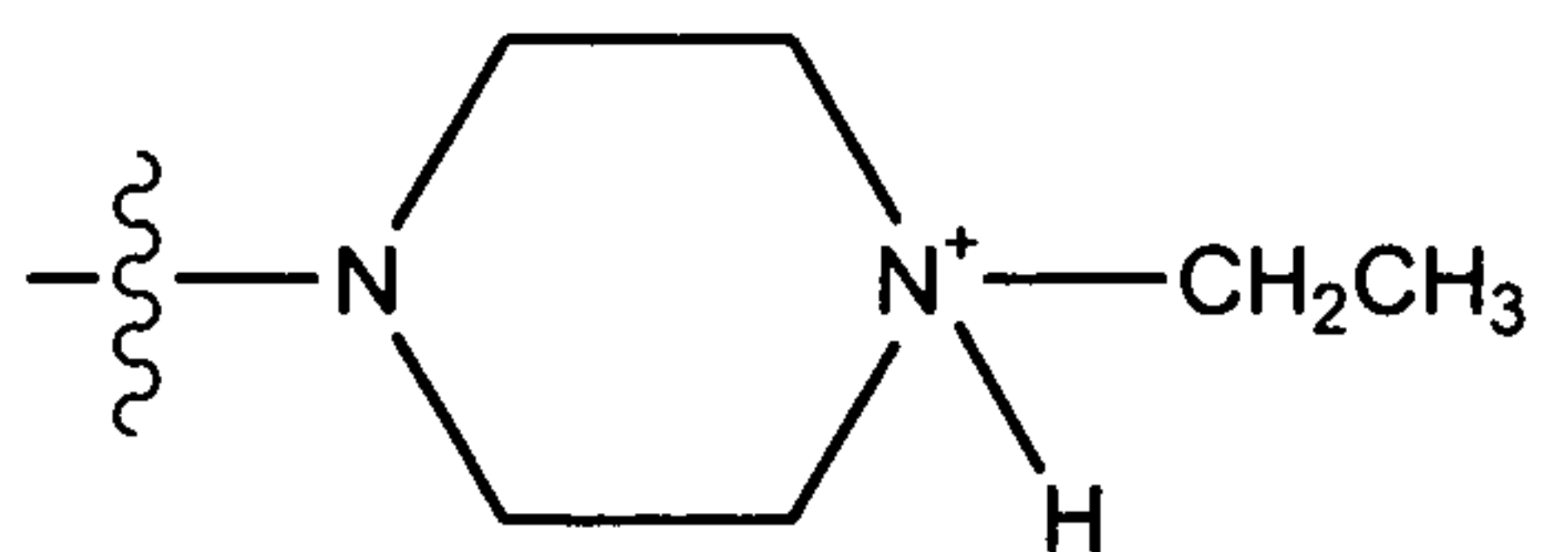
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where  $R_{10}$  is



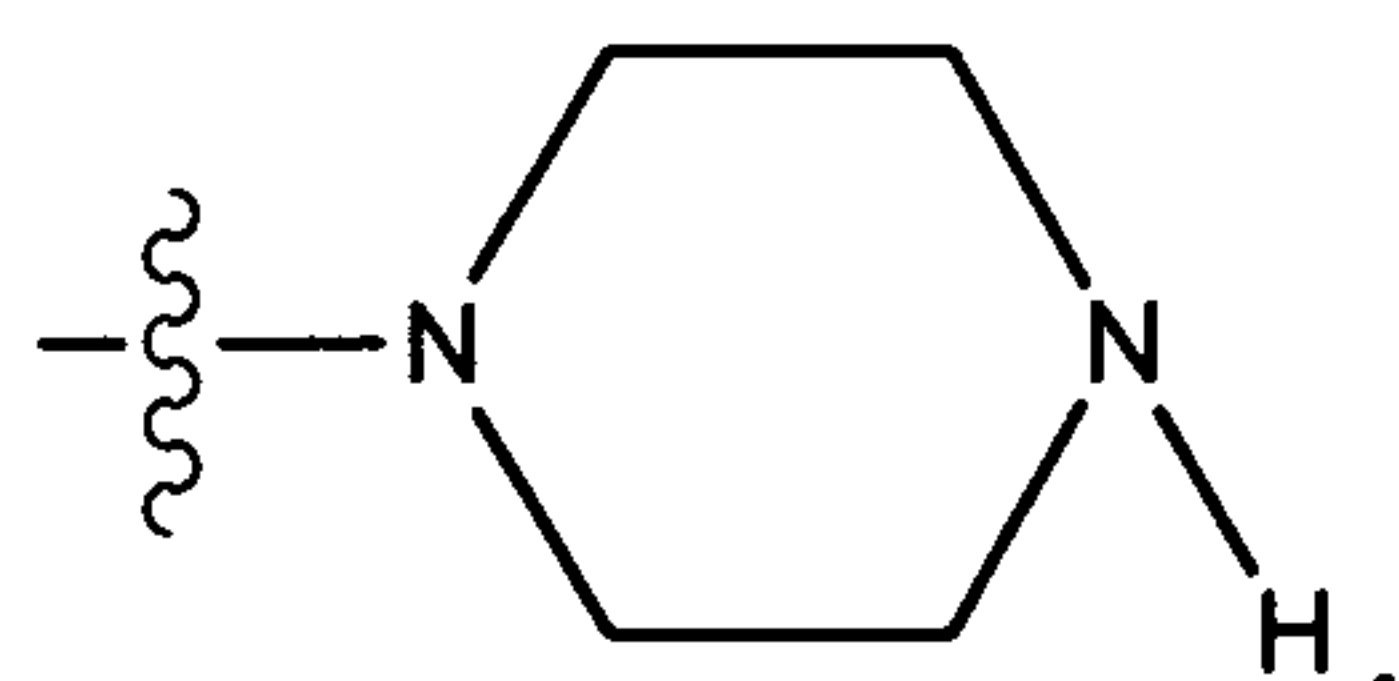
.5 The invention further provides the instant composition, wherein  $R_4$  is



The invention further provides the instant composition, wherein  $R_4$  is

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- 22 -



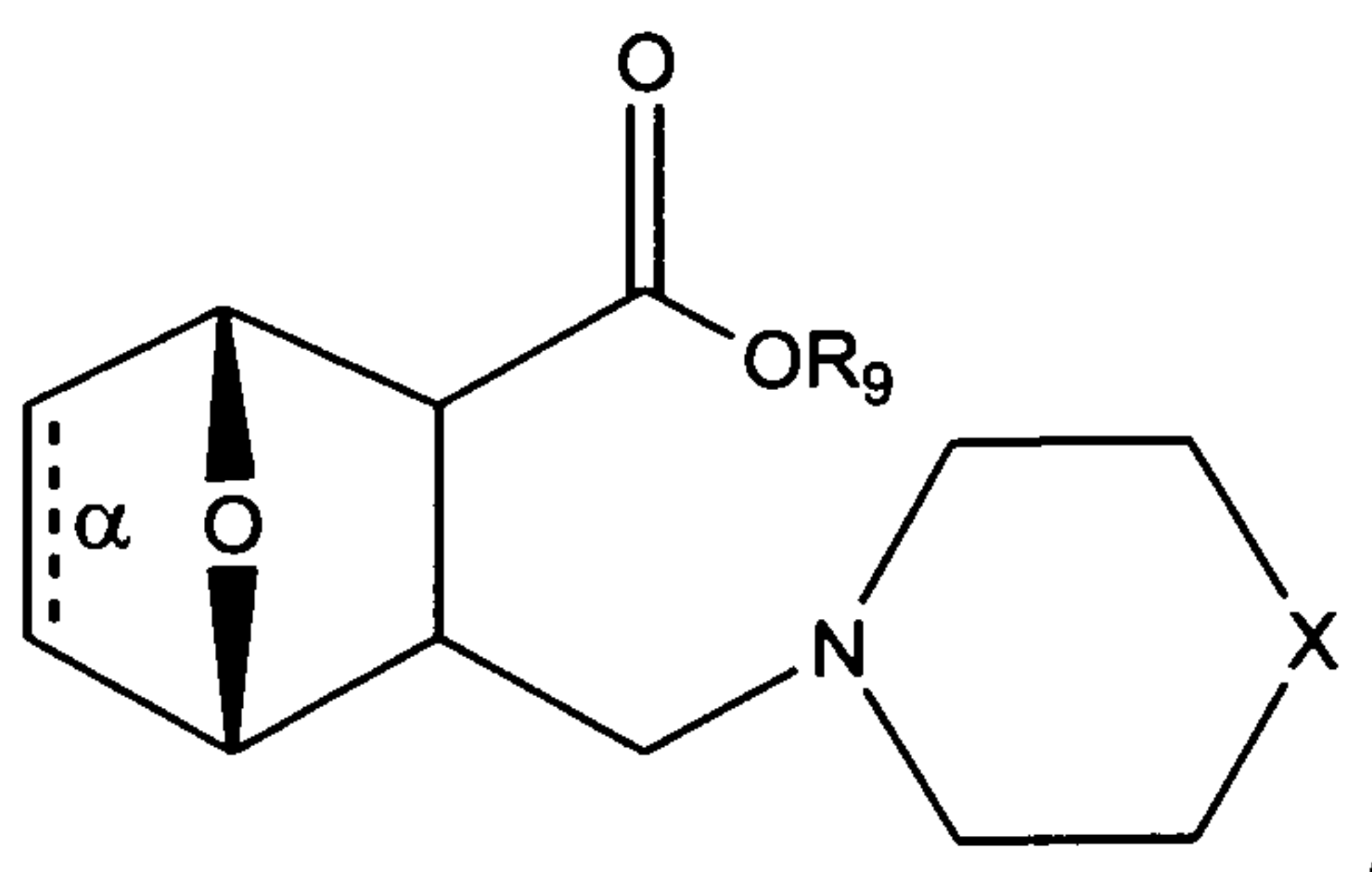
The invention further provides the instant composition, wherein  $R_5$  and  $R_6$  together are =O.

5

The invention further provides the instant composition, wherein  $R_7$  and  $R_8$  are each H.

The invention also provides a compound having the structure

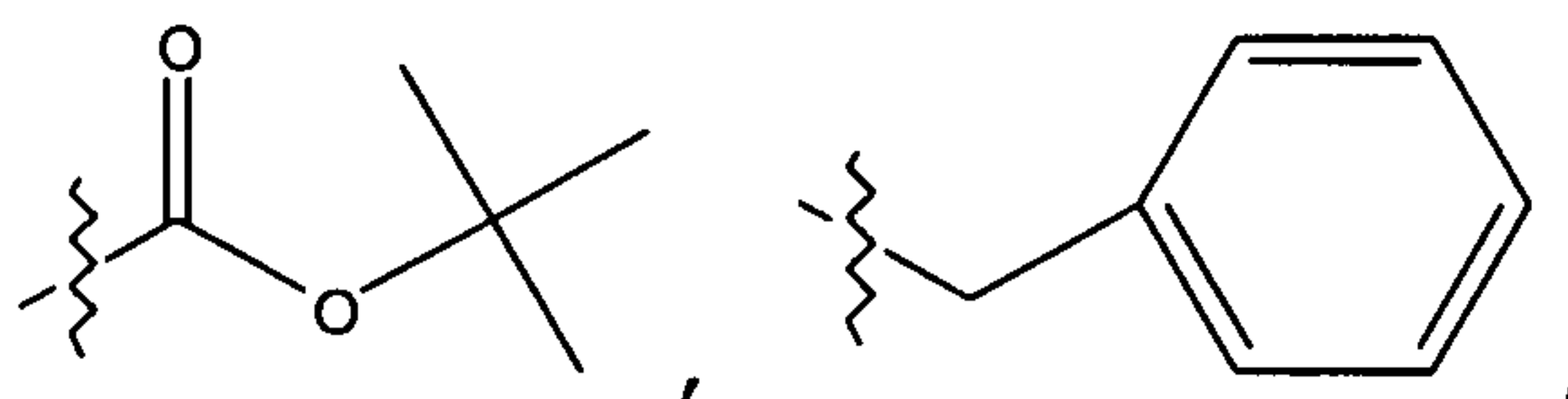
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wherein bond  $\alpha$  is present or absent;  $R_9$  is present or absent and when present is H,  $C_1$ - $C_{10}$  alkyl,  $C_2$ - $C_{10}$  alkenyl or phenyl; and X is O, S,  $NR_{10}$  or  $N^+R_{10}R_{10}$ , where each  $R_{10}$  is independently alkyl,  $C_2$ - $C_{12}$  substituted alkyl, alkenyl,  $C_4$ - $C_{12}$  substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl where the substituent is other than chloro,

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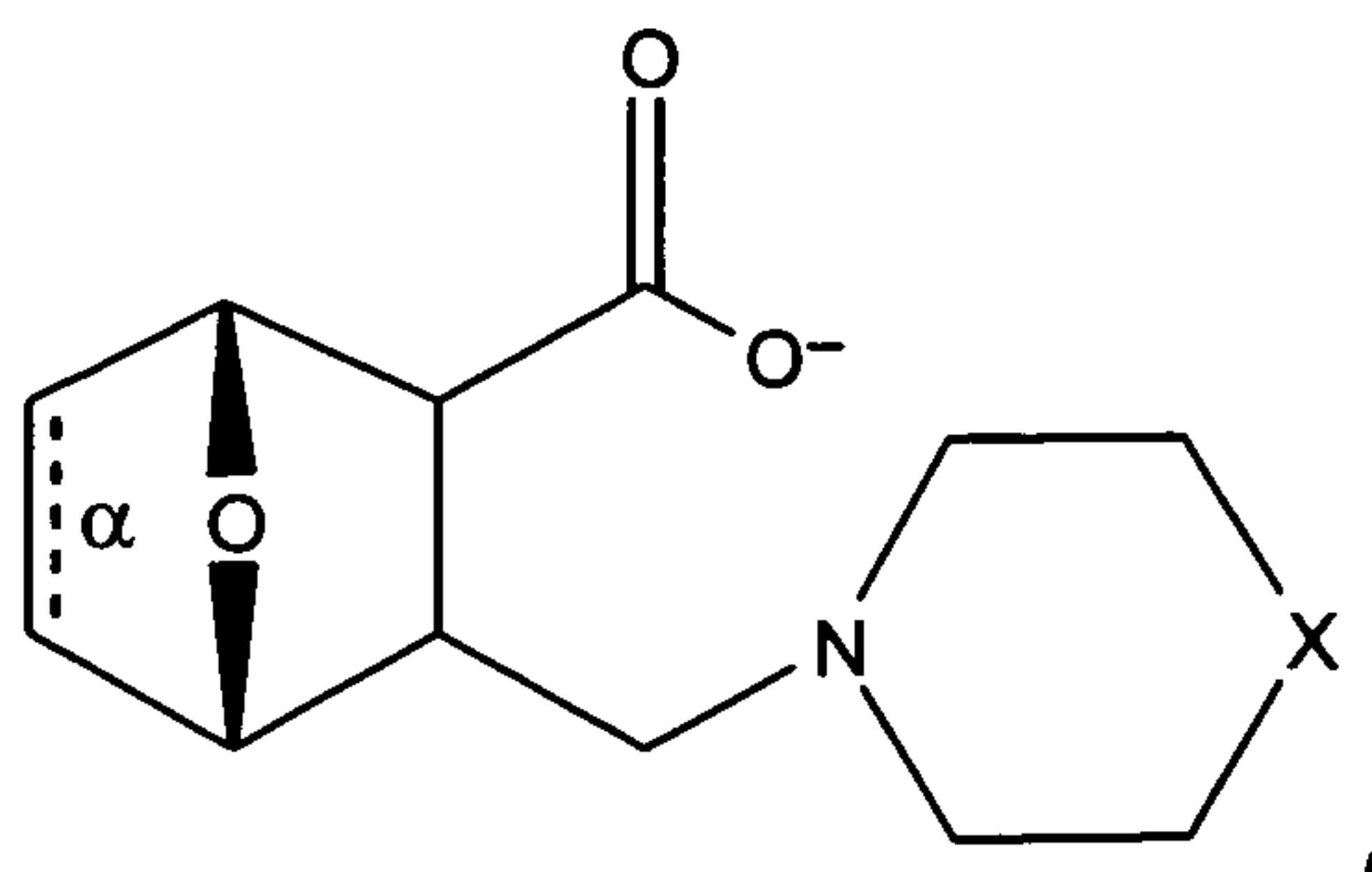


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-CH<sub>2</sub>CO<sub>2</sub>R<sub>11</sub>, -CH<sub>2</sub>COR<sub>11</sub>, -CH<sub>2</sub>CN, or -CH<sub>2</sub>CH<sub>2</sub>R<sub>16</sub>, where R<sub>11</sub> is H or alkyl, and where R<sub>16</sub> is any substituent that is a precursor to an aziridinyll intermediate, or a salt, enantiomer or zwitterion of the compound.

5

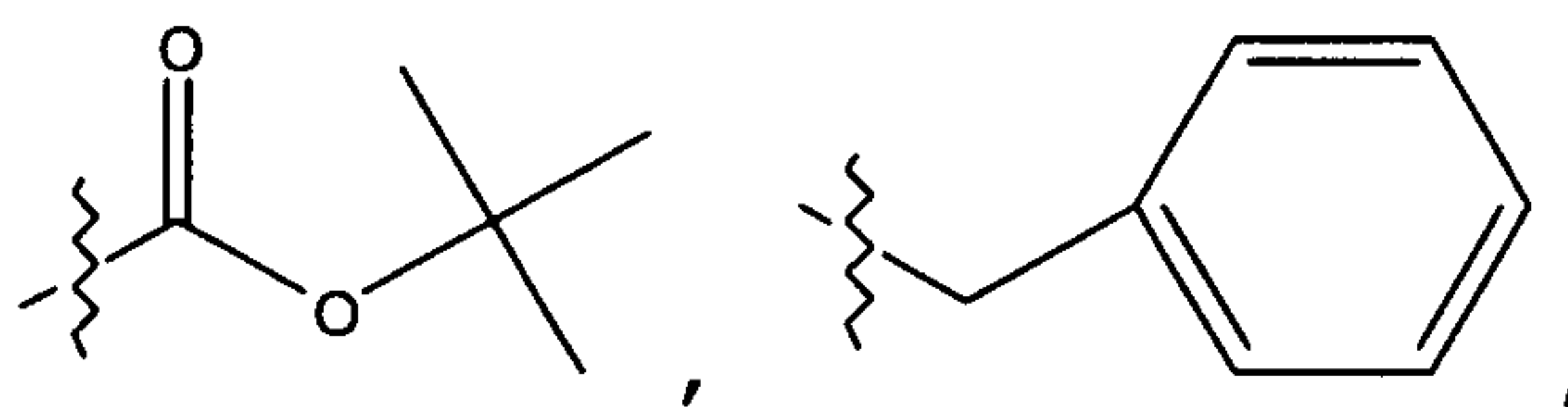
In an embodiment, the invention provides the structure



wherein, bond  $\alpha$  is present or absent; X is O, S, NR<sub>10</sub> or N<sup>+</sup>R<sub>10</sub>R<sub>10</sub>, where each R<sub>10</sub> is independently H, alkyl, C<sub>2</sub>-C<sub>12</sub> substituted alkyl, alkenyl, C<sub>4</sub>-C<sub>12</sub> substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl where the substituent is other than chloro,

10

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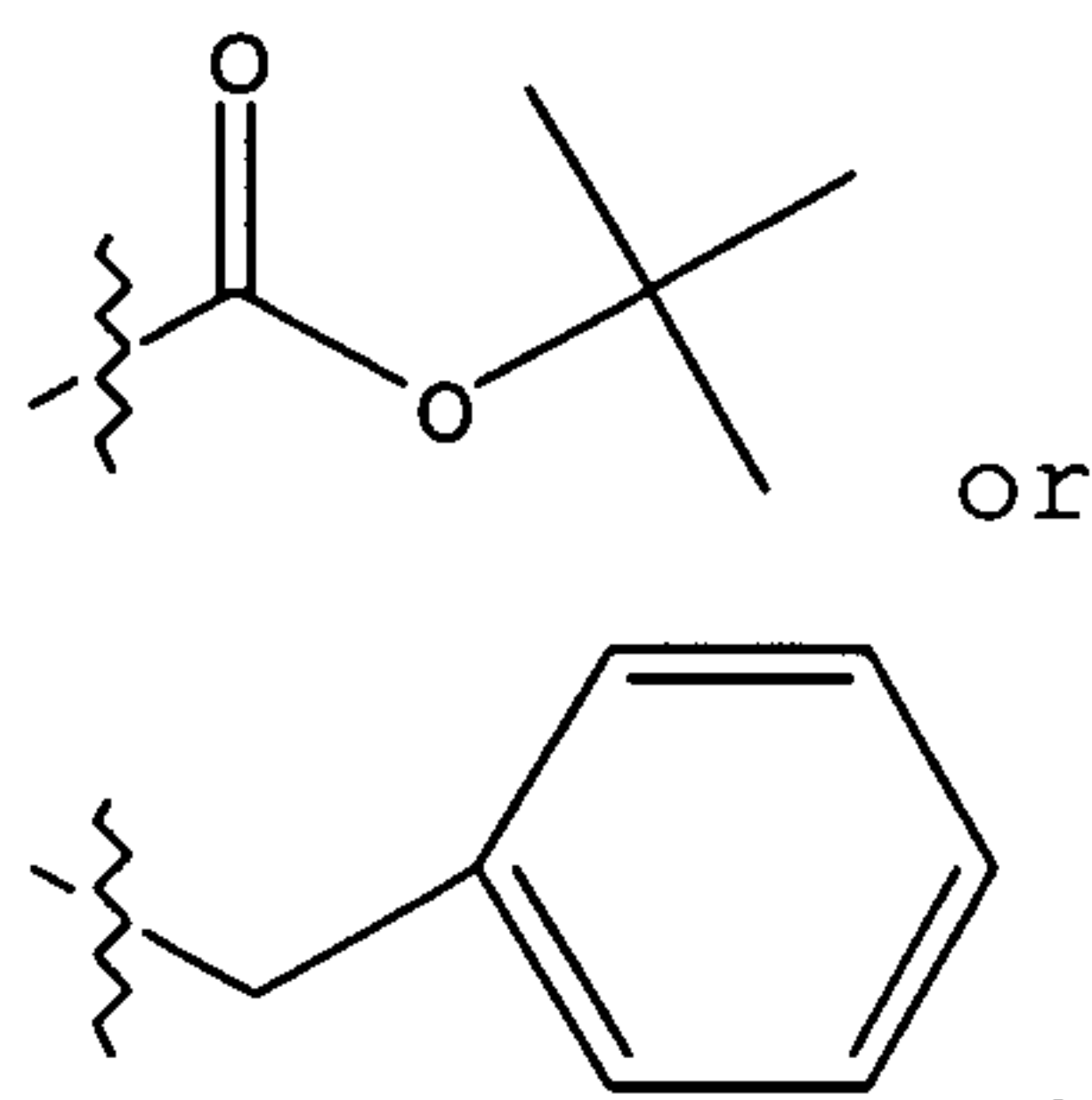
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-CH<sub>2</sub>CO<sub>2</sub>R<sub>11</sub>, -CH<sub>2</sub>COR<sub>11</sub>, -CH<sub>2</sub>CN, or -CH<sub>2</sub>CH<sub>2</sub>R<sub>16</sub>, where R<sub>11</sub> is H or alkyl, and where R<sub>16</sub> is any substituent that is a precursor to an aziridinyll intermediate,

25 or a salt, enantiomer or zwitterion of the compound.

- 24 -

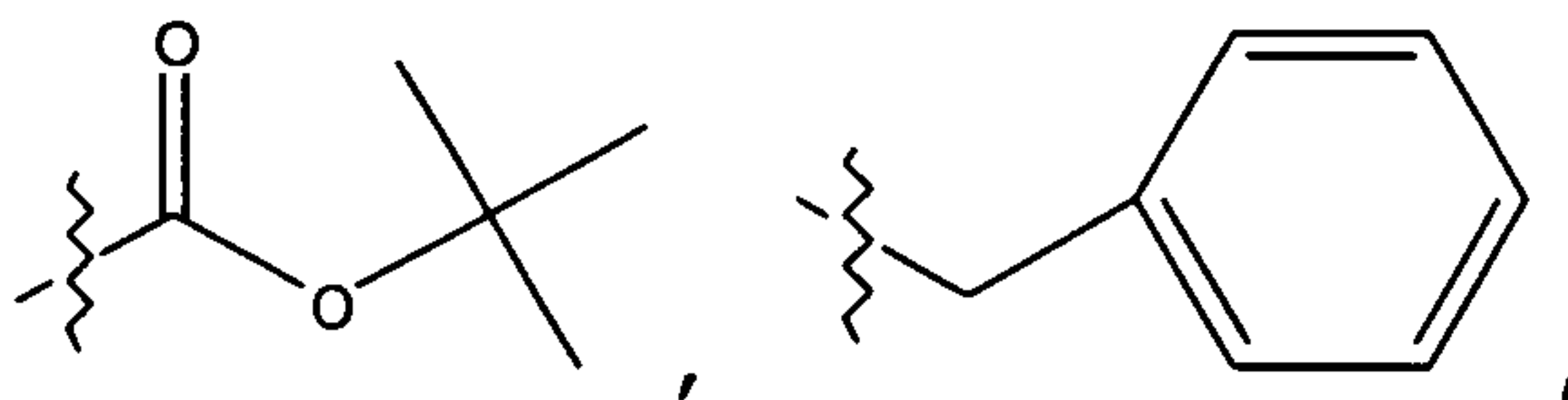
The invention further provides an embodiment wherein X is O or  $\text{NH}^+\text{R}_{10}$ , where  $\text{R}_{10}$  is H, alkyl,  $\text{C}_2\text{-C}_{12}$  substituted alkyl, alkenyl,  $\text{C}_4\text{-C}_{12}$  substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl where the substituent is other than chloro,



10 The invention further provides an embodiment wherein X is O.

The invention further provides an embodiment wherein X is  $-\text{CH}_2\text{CH}_2\text{R}_{16}$ , where  $\text{R}_{16}$  is any substituent is a precursor to an aziridinyll intermediate.

15 The invention further provides an embodiment wherein X is  $\text{NH}^+\text{R}_{10}$ , where  $\text{R}_{10}$  is H, alkyl, substituted  $\text{C}_2\text{-C}_{12}$  alkyl, alkenyl, substituted  $\text{C}_4\text{-C}_{12}$  alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl where the substituent is other than chloro,



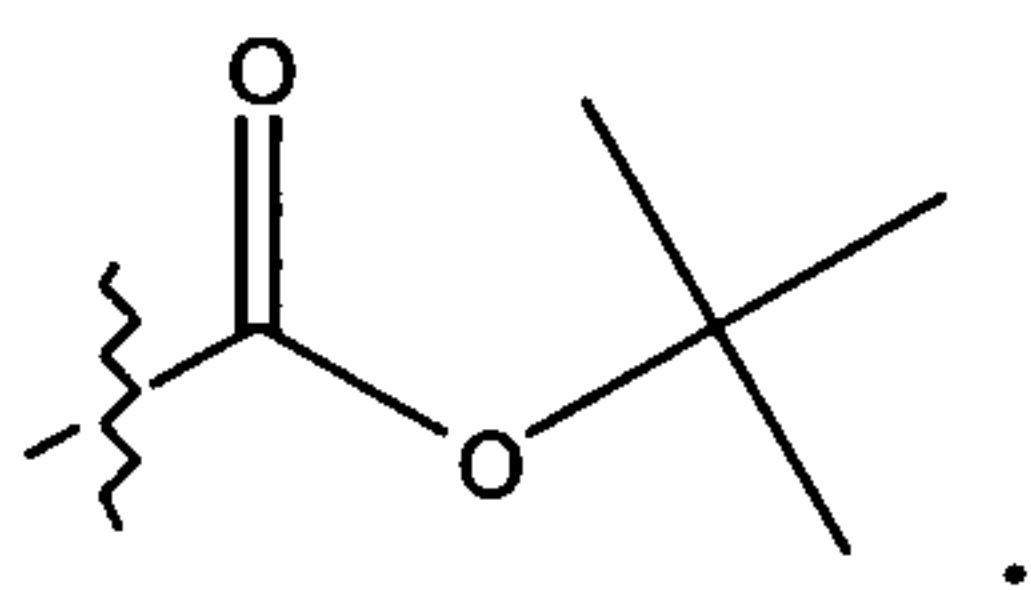
20 25  $-\text{CH}_2\text{CO}_2\text{R}_{11}$ ,  $-\text{CH}_2\text{COR}_{11}$ ,  $-\text{CH}_2\text{CN}$ , or  $-\text{CH}_2\text{CH}_2\text{R}_{16}$ , where  $\text{R}_{11}$  is H or alkyl, and where  $\text{R}_{16}$  is any

- 25 -

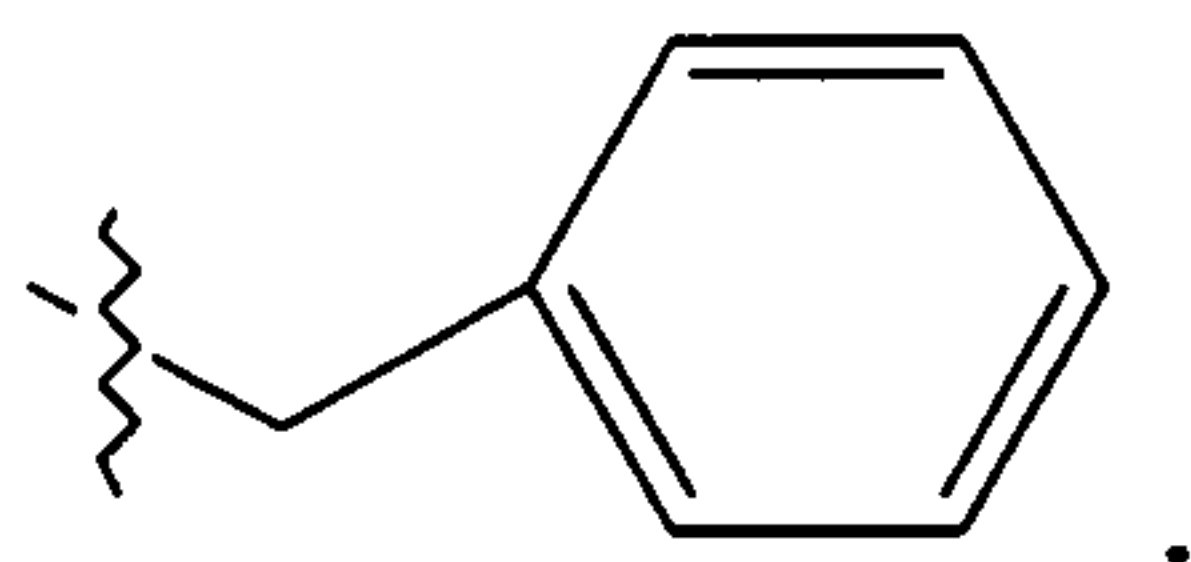
substituent that is a precursor to an aziridinyl intermediate.

The invention further provides an embodiment, wherein  $R_{10}$  is  
5 methyl.

The invention further provides an embodiment, wherein  $R_{10}$  is



10 The invention further provides an embodiment, wherein  $R_{10}$  is

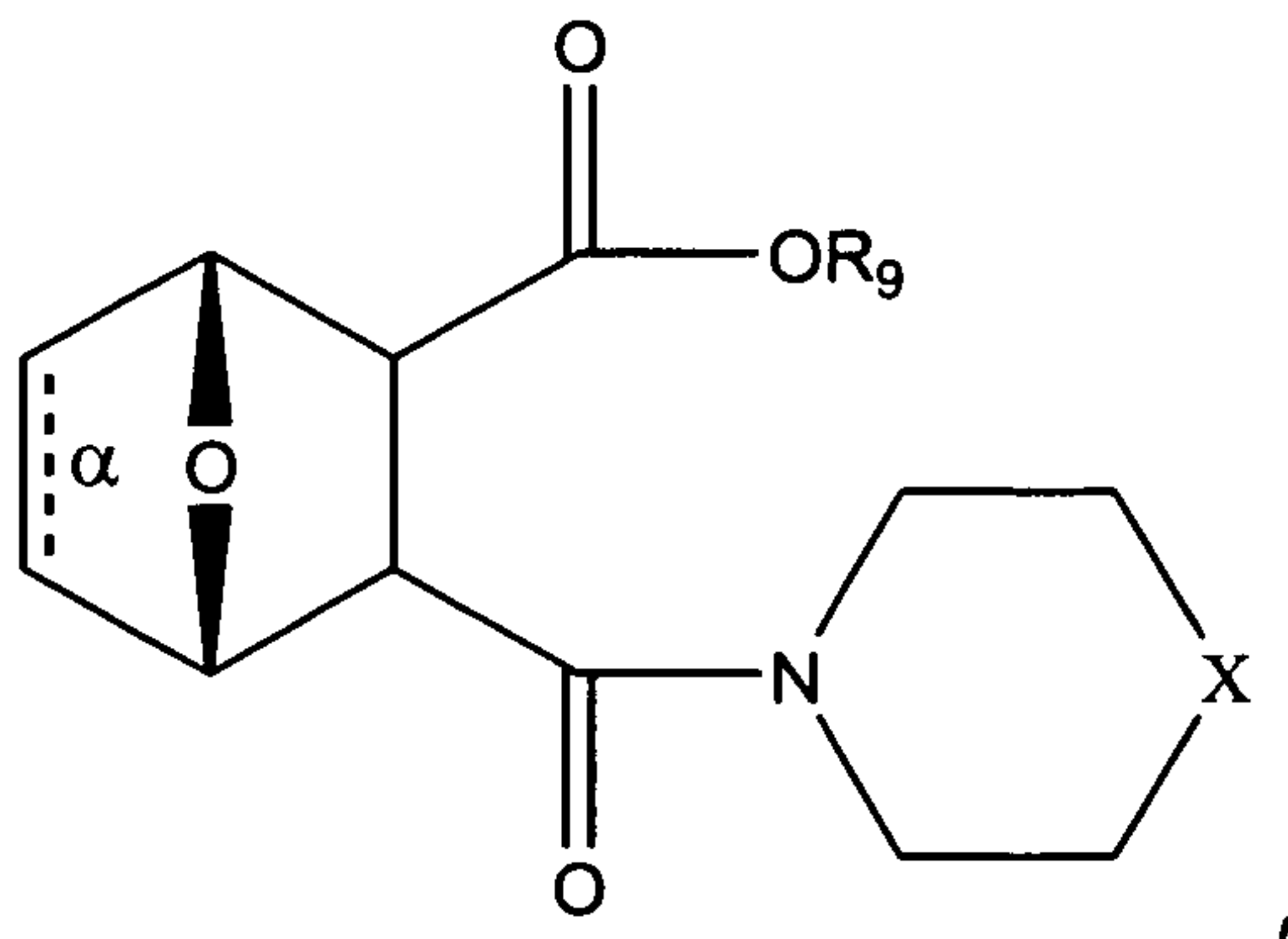


The invention further provides an embodiment, wherein  $R_{10}$  is  
ethyl.

15

The invention further provides an embodiment, wherein  $R_{10}$  is  
absent.

In another embodiment, the invention provides the structure



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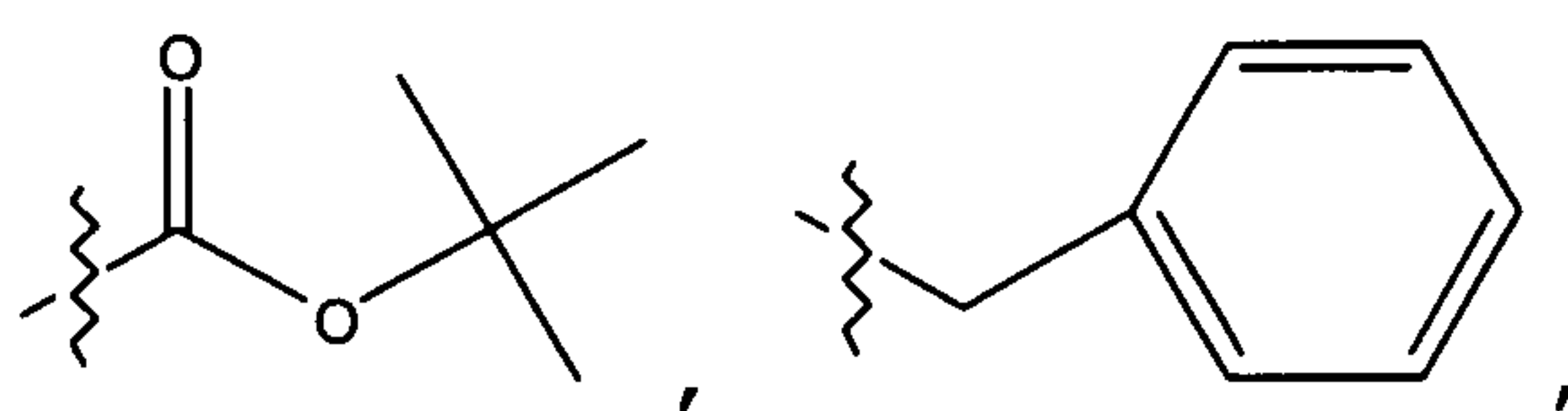
wherein  $R_9$  is present or absent and when present is H, alkyl, alkenyl, alkynyl or phenyl; and X is O,  $NR_{10}$  or

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$N^+R_{10}R_{10}$ , where each  $R_{10}$  is independently alkyl, C2-C12 substituted alkyl, alkenyl, C4-C12 substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl where the substituent is other than

5

chloro,



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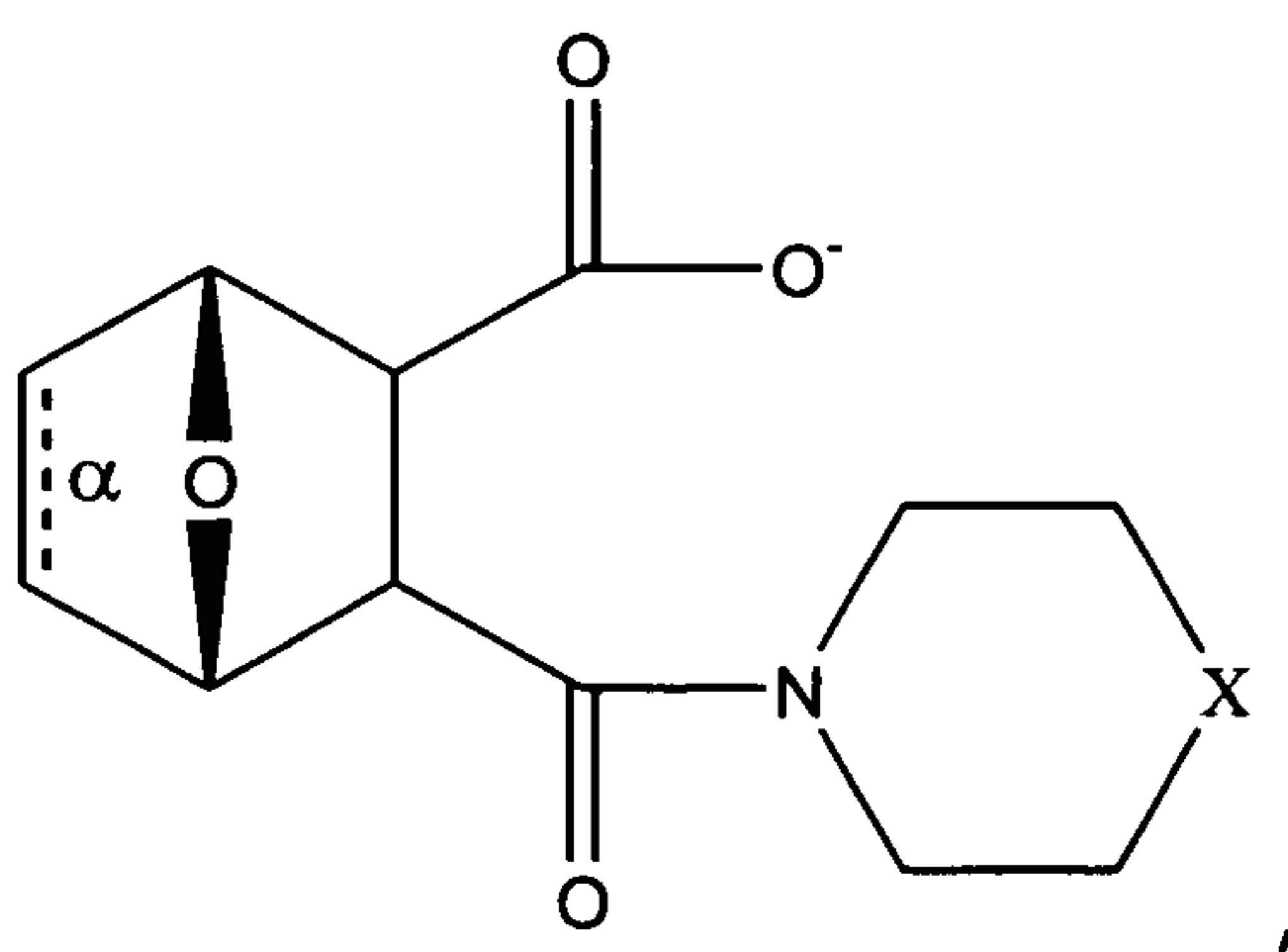
$-CH_2CO_2R_{11}$ ,  $-CH_2COR_{11}$ , or  $-CH_2CN$ , where  $R_{11}$  is H or alkyl,

or a salt, zwitterion or enantiomer of the compound.

15

In another embodiment of the invention,  $R_{10}$  is cyclopropyl.

In another embodiment, the invention provides the structure

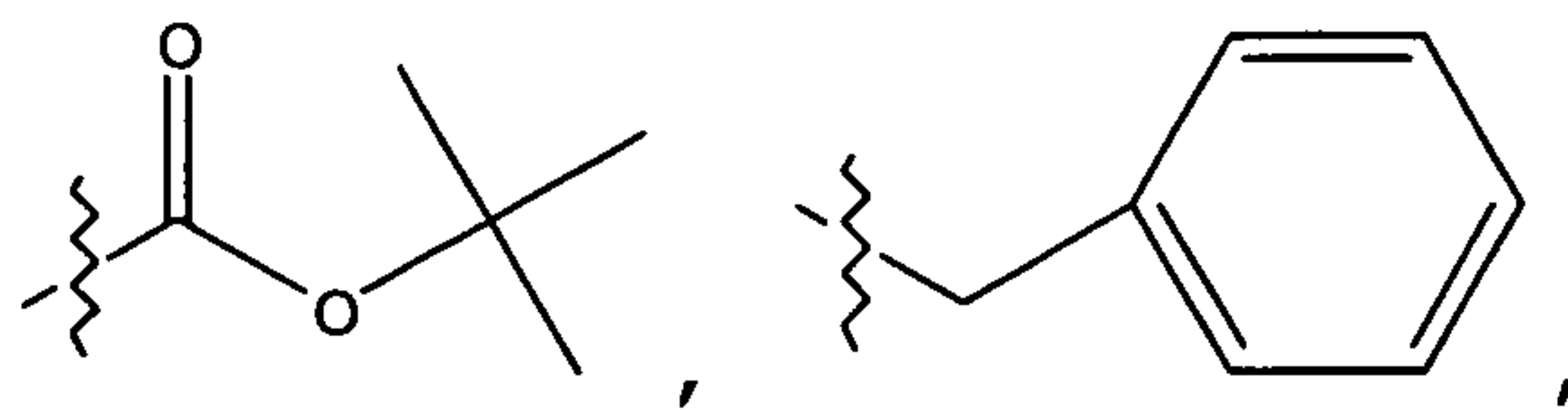


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wherein X is O or  $NH^+R_{10}$ , where  $R_{10}$  is present or absent and when present is alkyl, alkenyl or alkynyl, each of which is substituted or unsubstituted,

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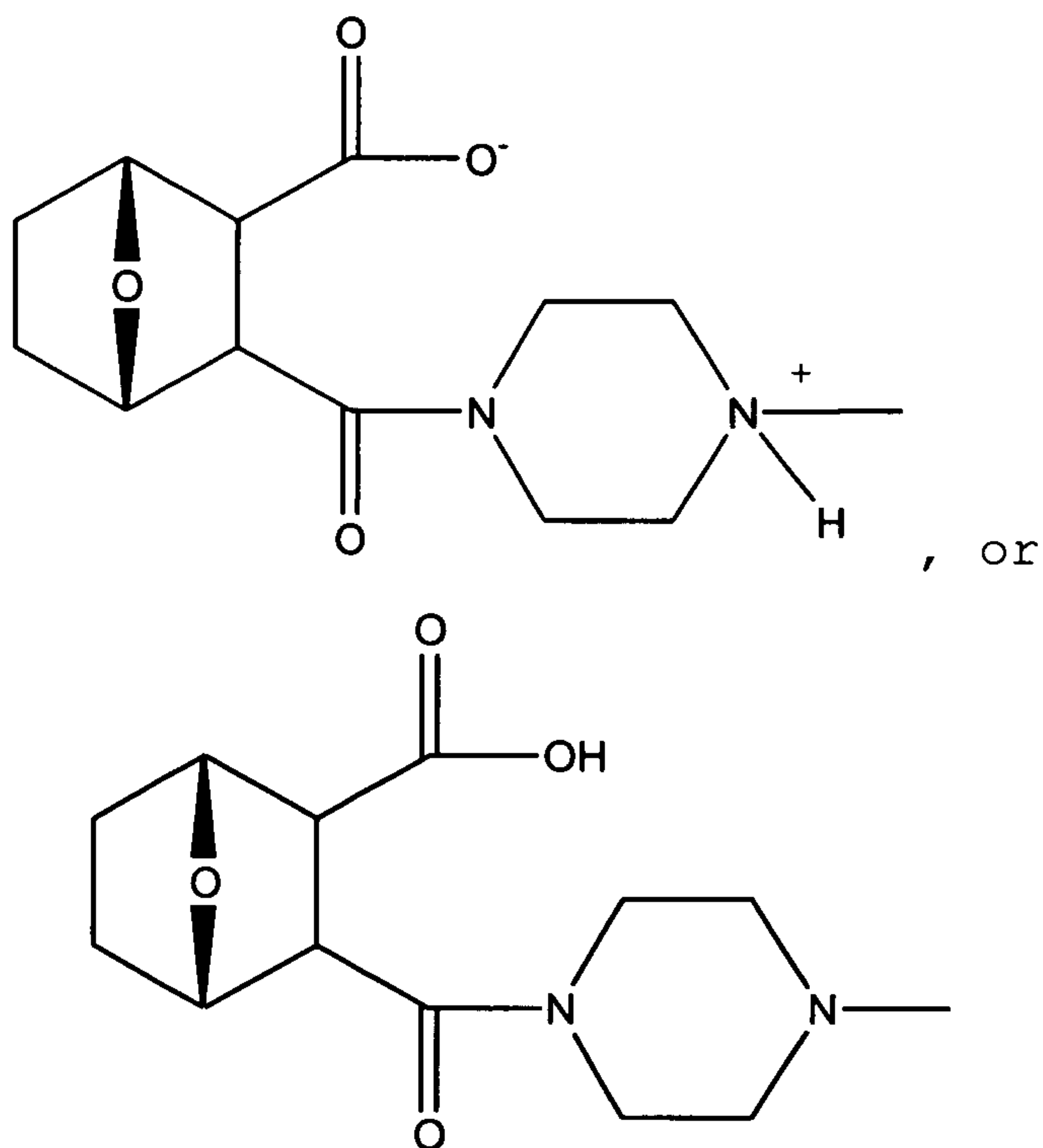


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$-\text{CH}_2\text{CO}_2\text{R}_{11}$ ,  $-\text{CH}_2\text{COR}_{11}$ , or  $-\text{CH}_2\text{CN}$ , where  $\text{R}_{11}$  is H or alkyl.

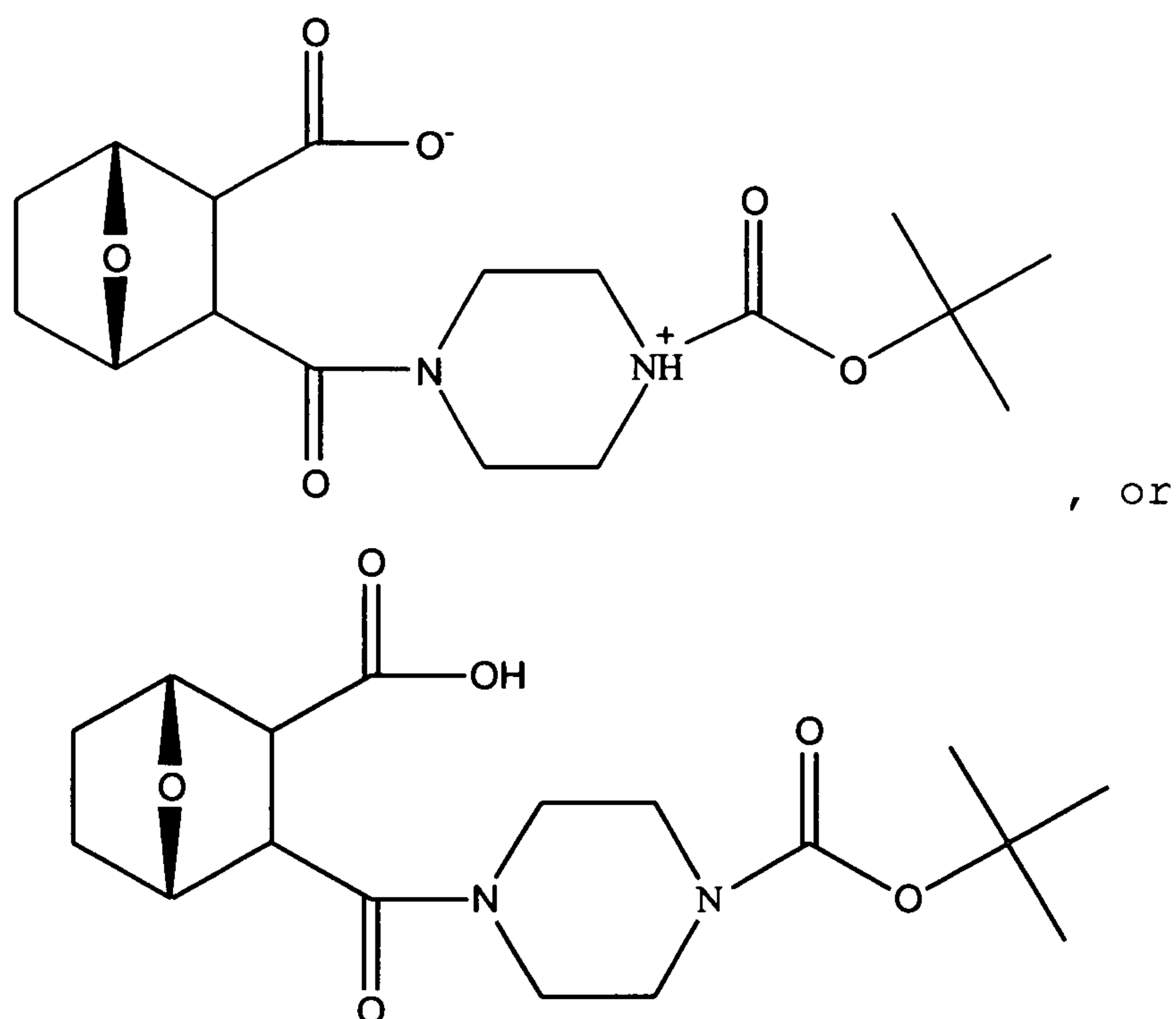
In an embodiment, the invention provides a compound having the structure (Compound 100)

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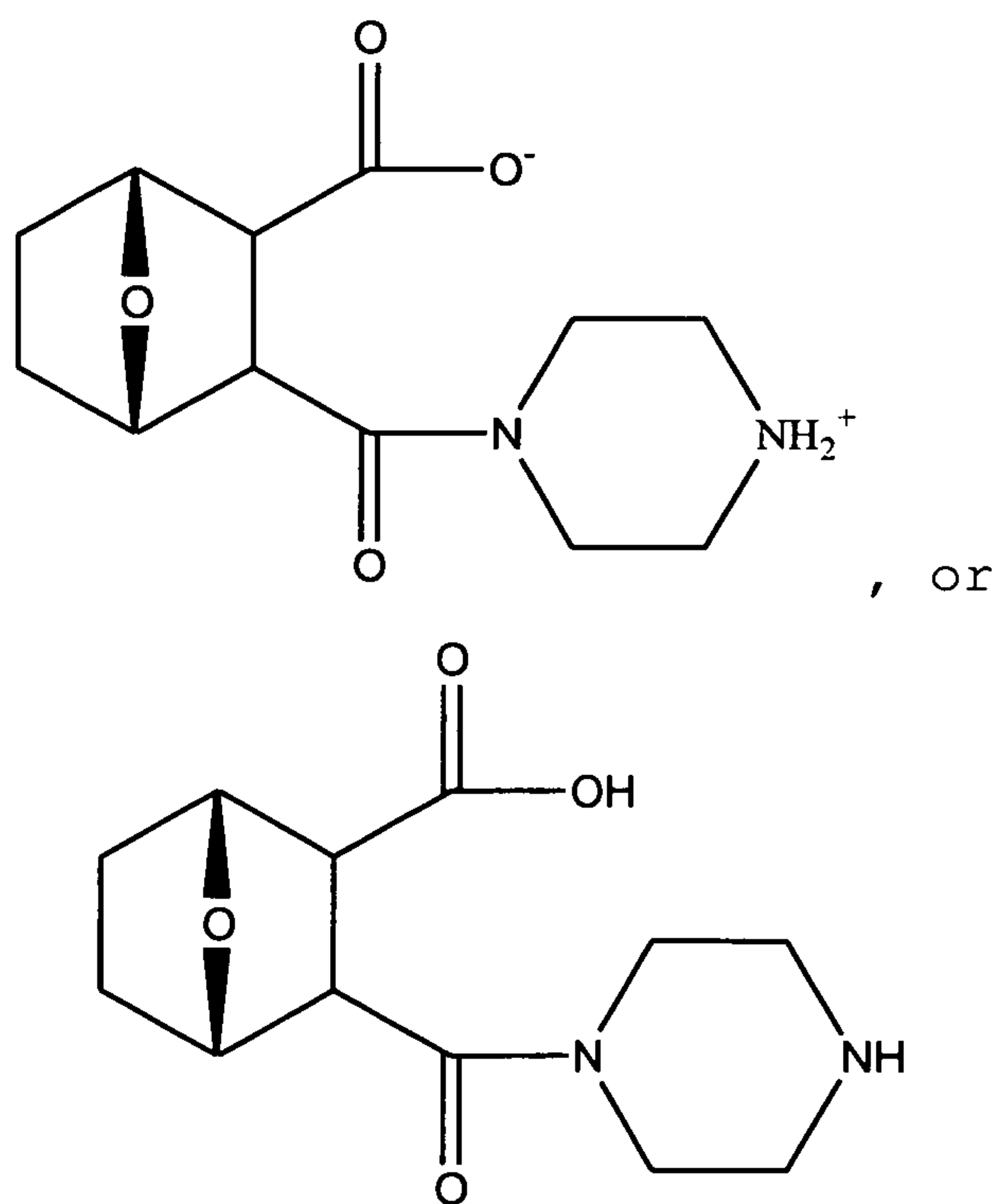


In an embodiment, the invention provides a compound having the structure (Compound 102)

- 28 -

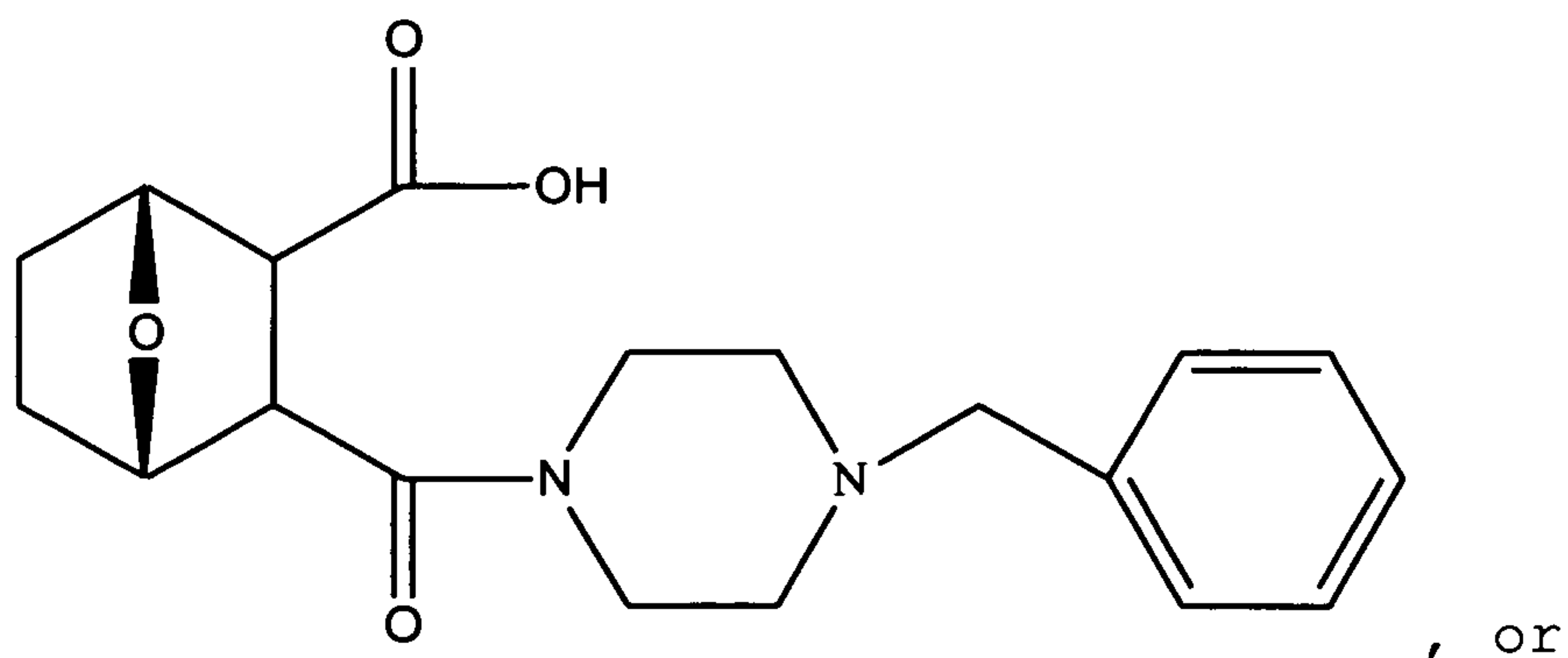


5 In an embodiment, the invention provides a compound having the structure (Compound 101)

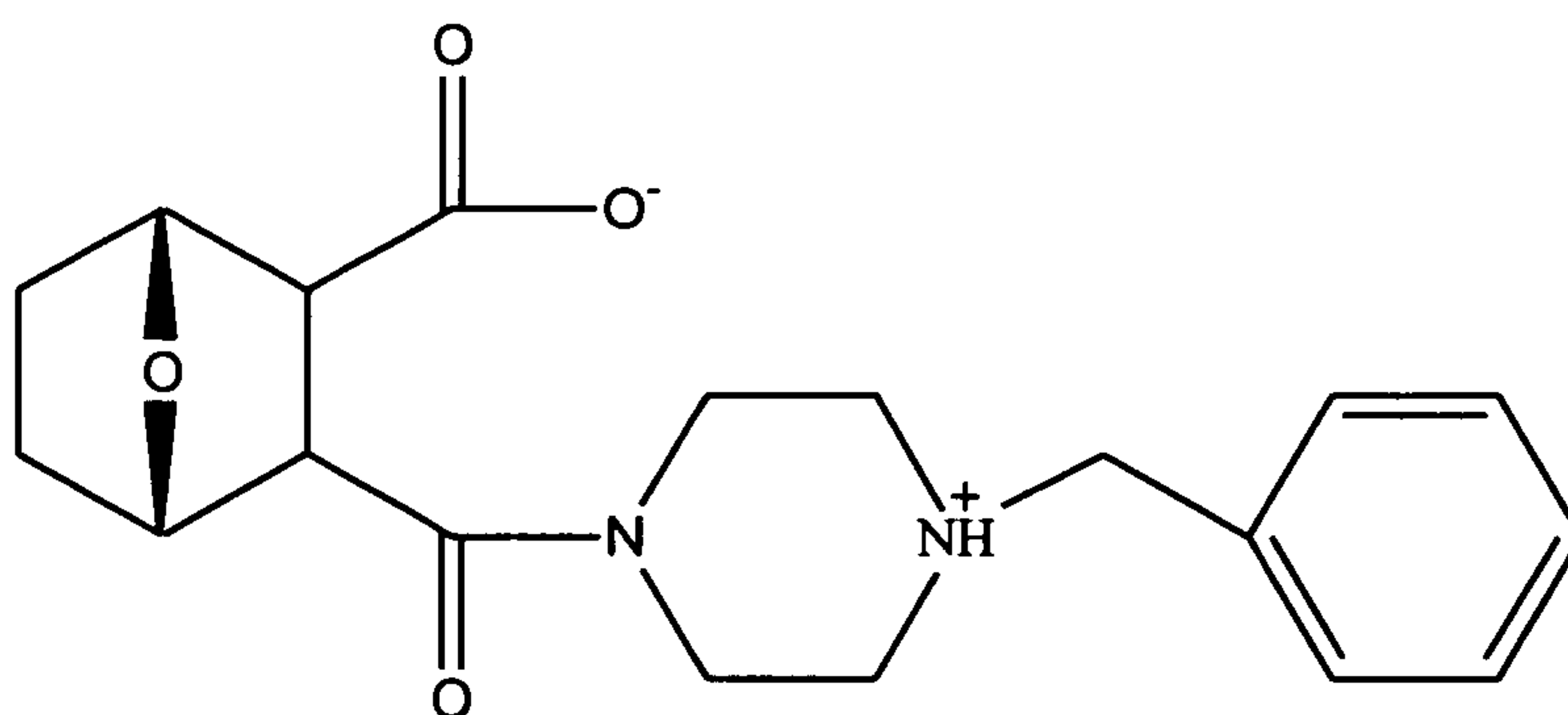


- 29 -

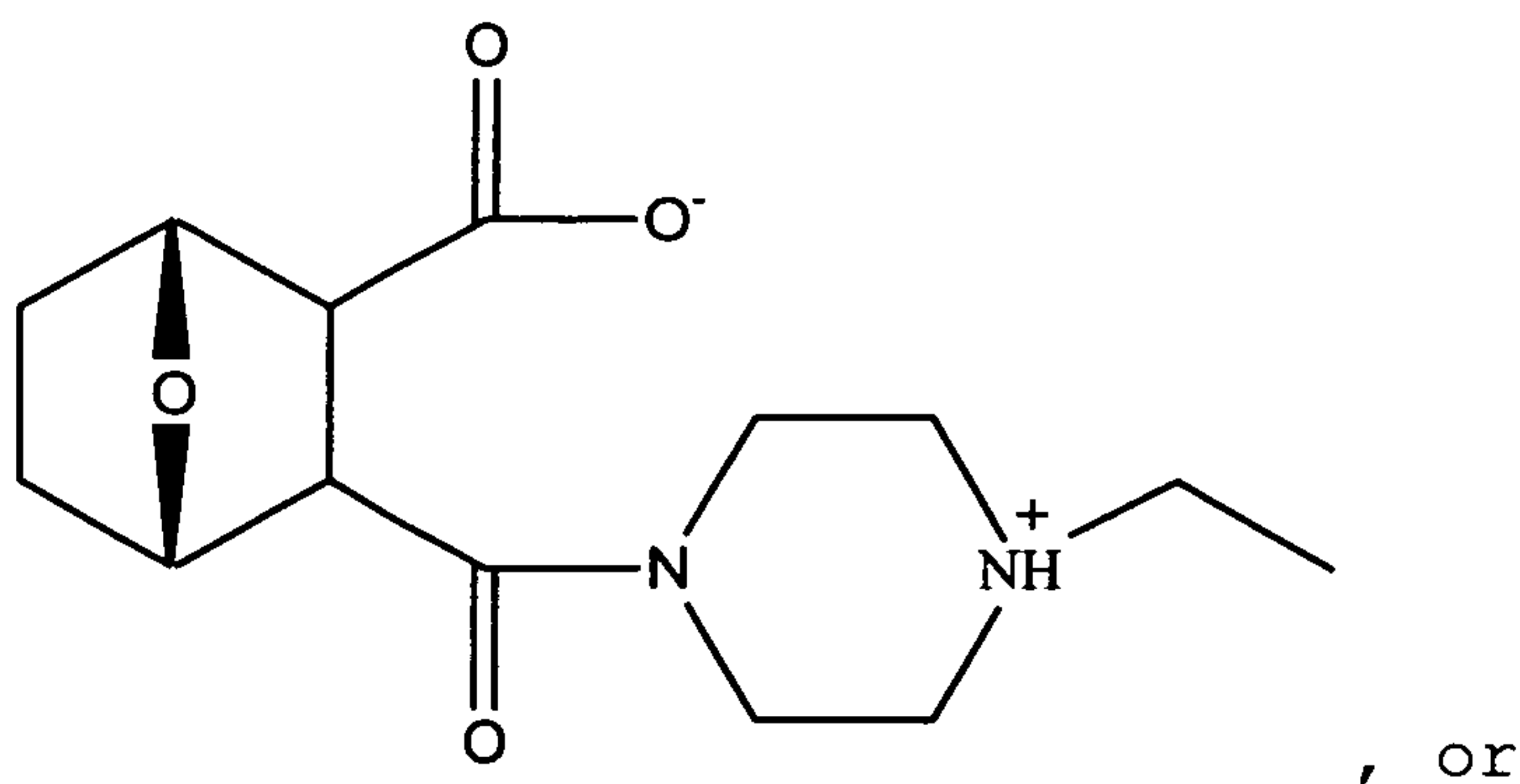
In an embodiment, the invention provides a compound having the structure (Compound 103)



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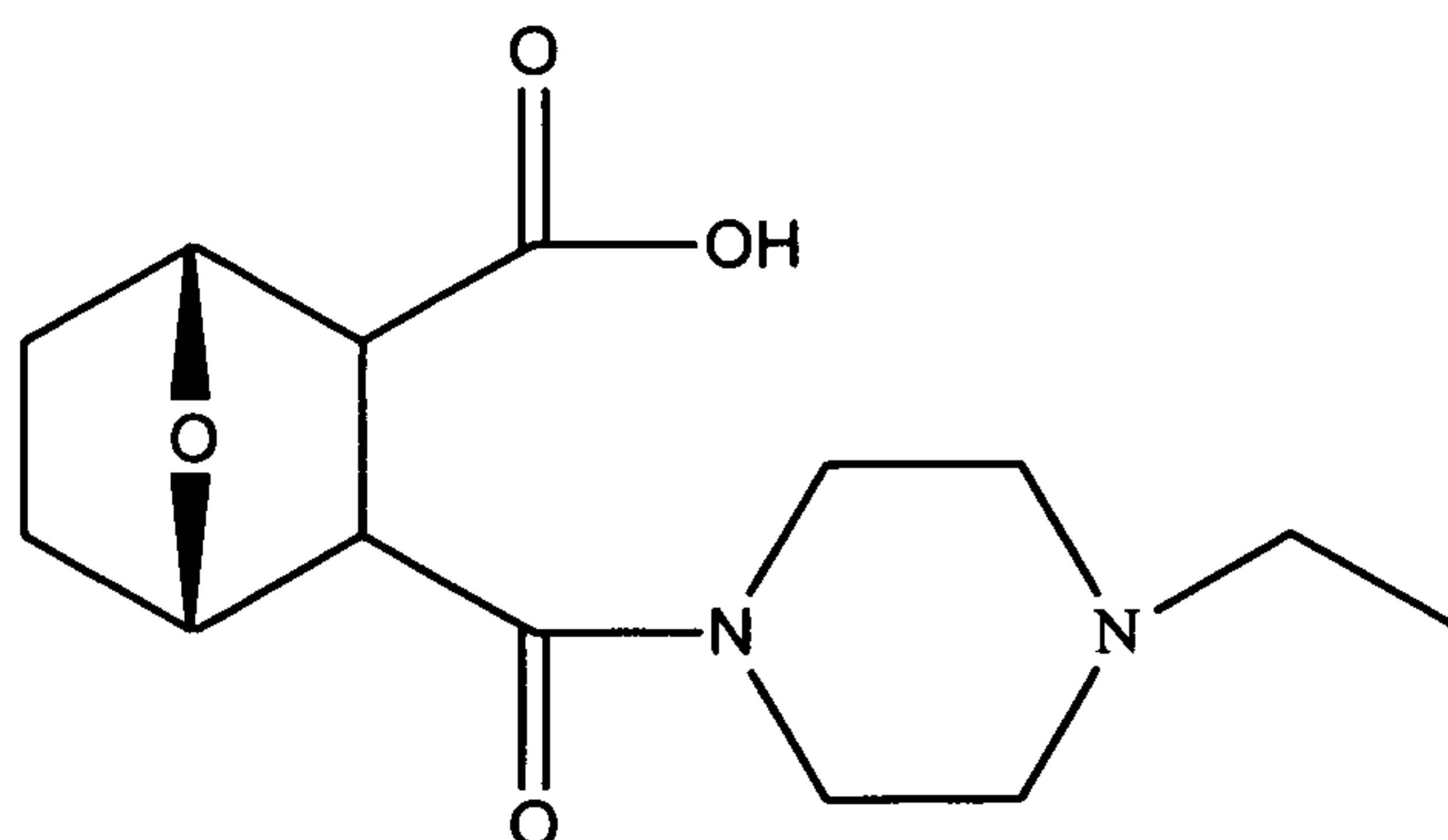


In an embodiment, the invention provides a compound having the structure (Compound 105)

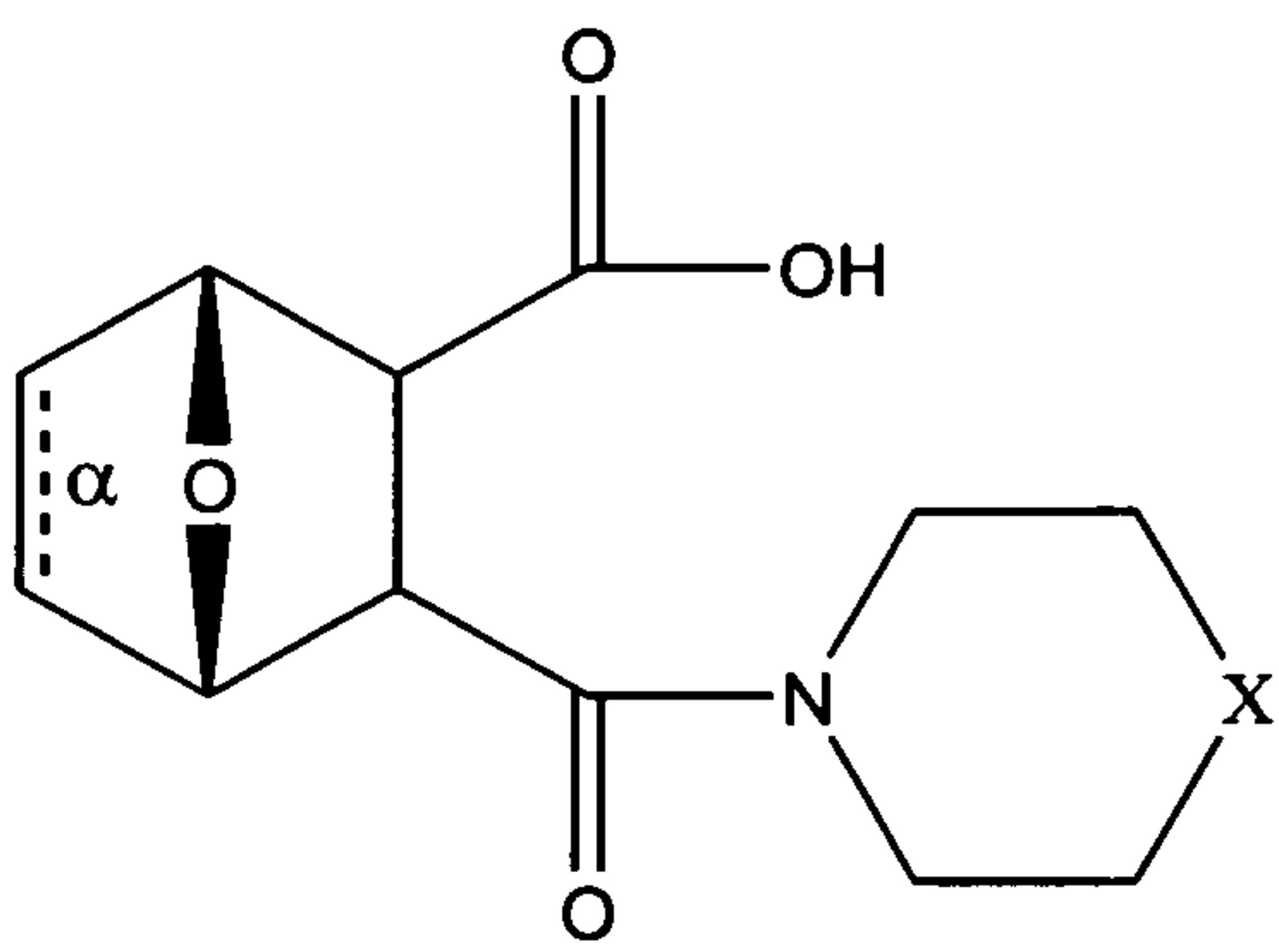


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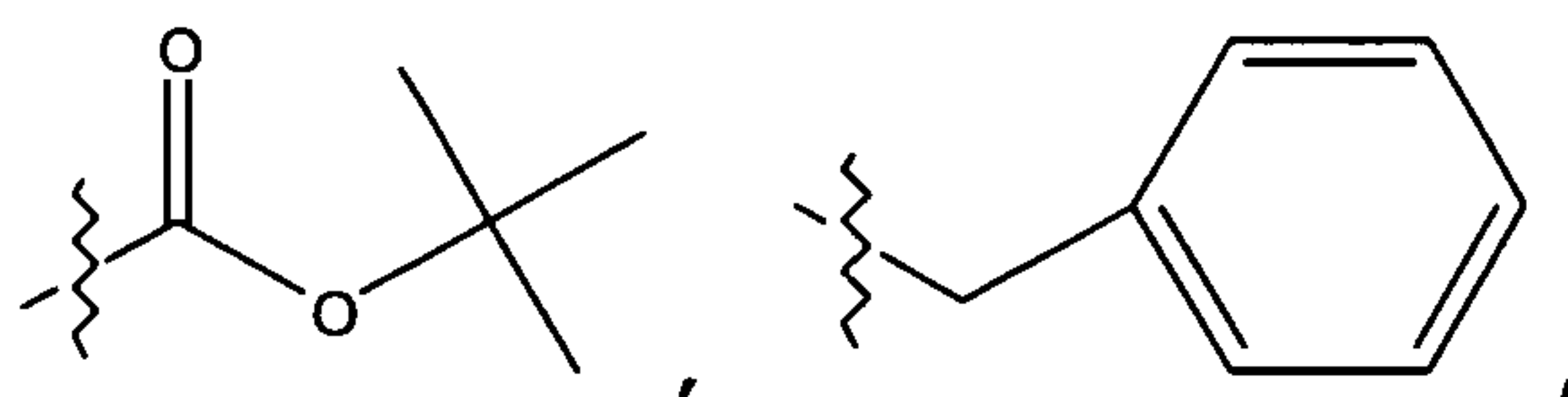
In another embodiment, the invention provides the structure



wherein X is O, NR<sub>10</sub> or NH<sup>+</sup>R<sub>10</sub>,

where R<sub>9</sub> is alkyl, alkenyl, alkynyl or aryl, and

where R<sub>10</sub> is present or absent and when present is alkyl, alkenyl or alkynyl, each of which is substituted or unsubstituted,

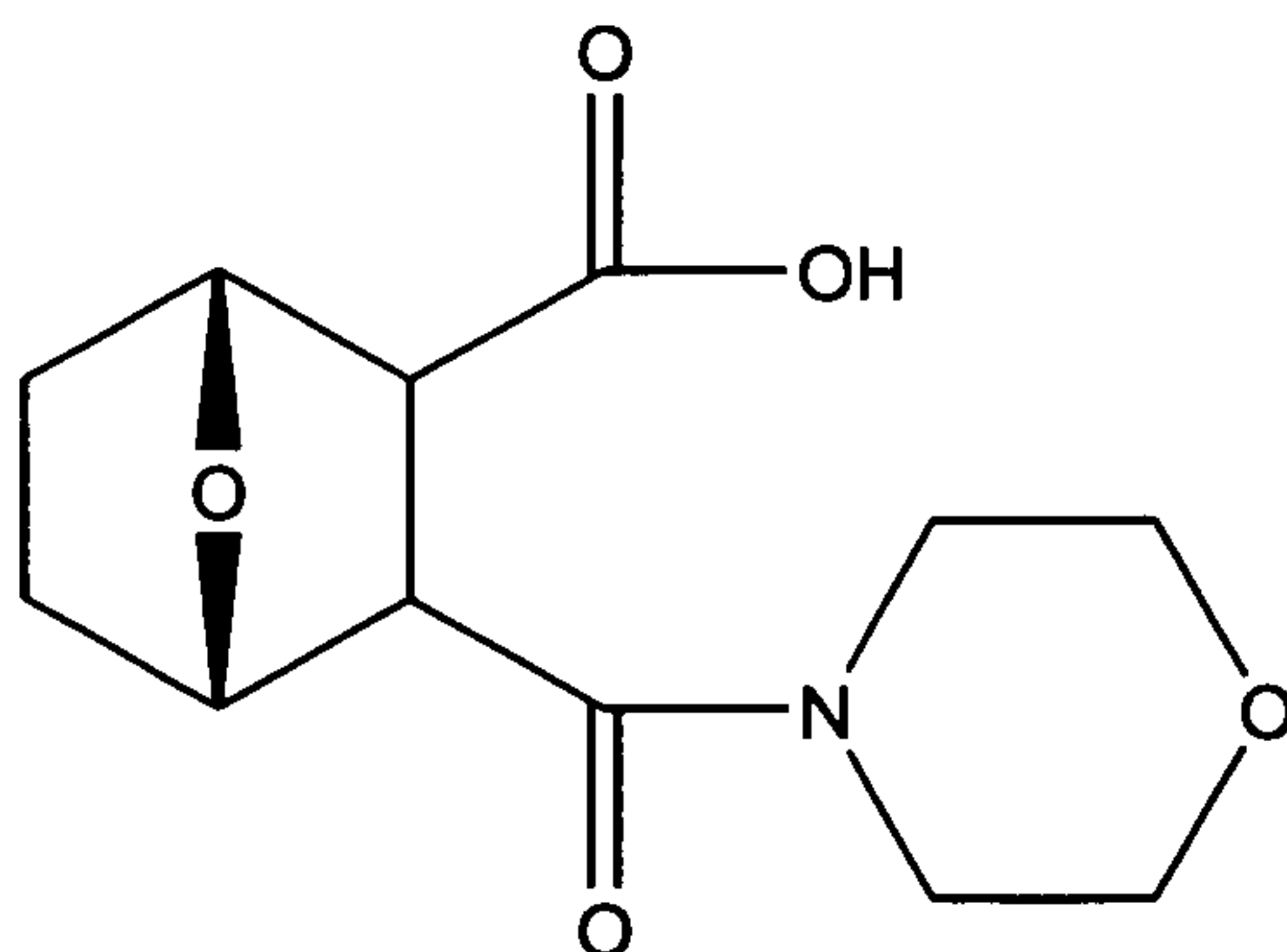


-CH<sub>2</sub>CO<sub>2</sub>R<sub>11</sub>, -CH<sub>2</sub>COR<sub>11</sub>, or -CH<sub>2</sub>CN, where R<sub>11</sub> is H or alkyl,.

In another embodiment of the invention, R<sub>10</sub> is cyclopropyl.

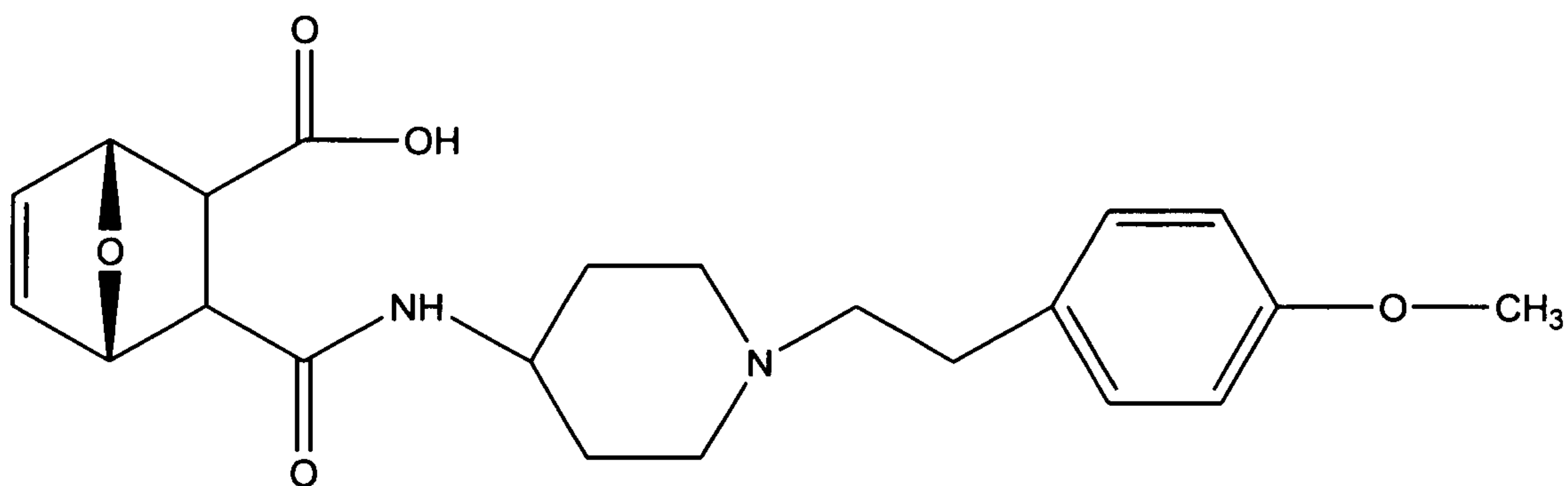
- 31 -

In an embodiment, the invention provides a compound having the structure (Compound 111)



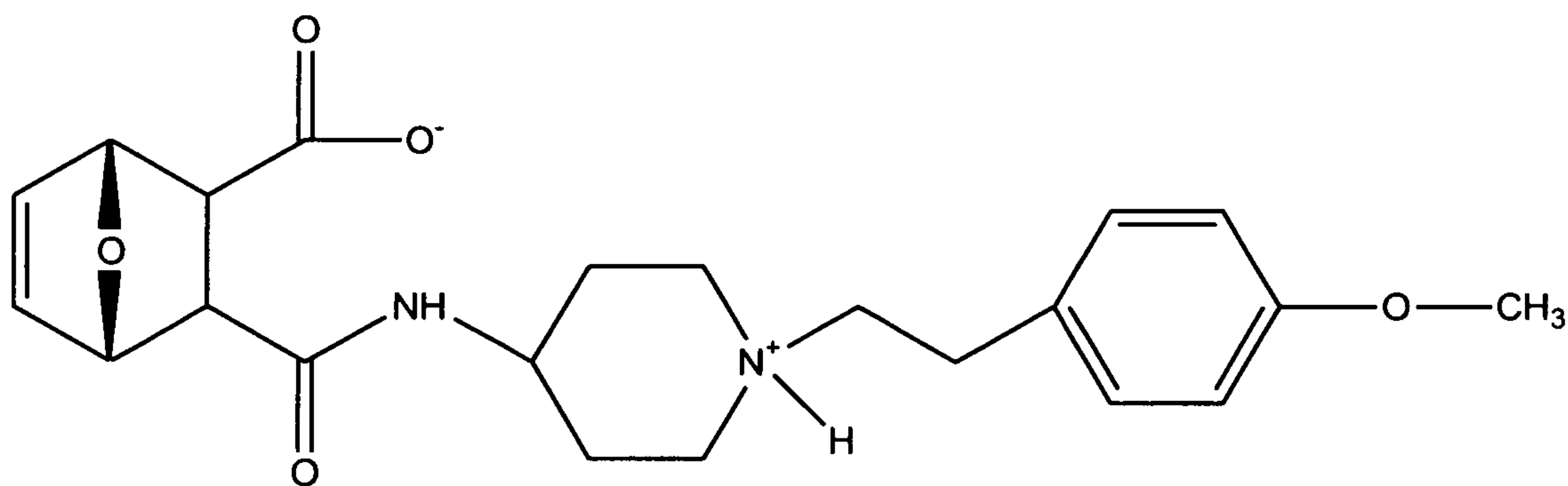
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In an embodiment, the invention provides a compound having the structure (Compound 104E)



or,

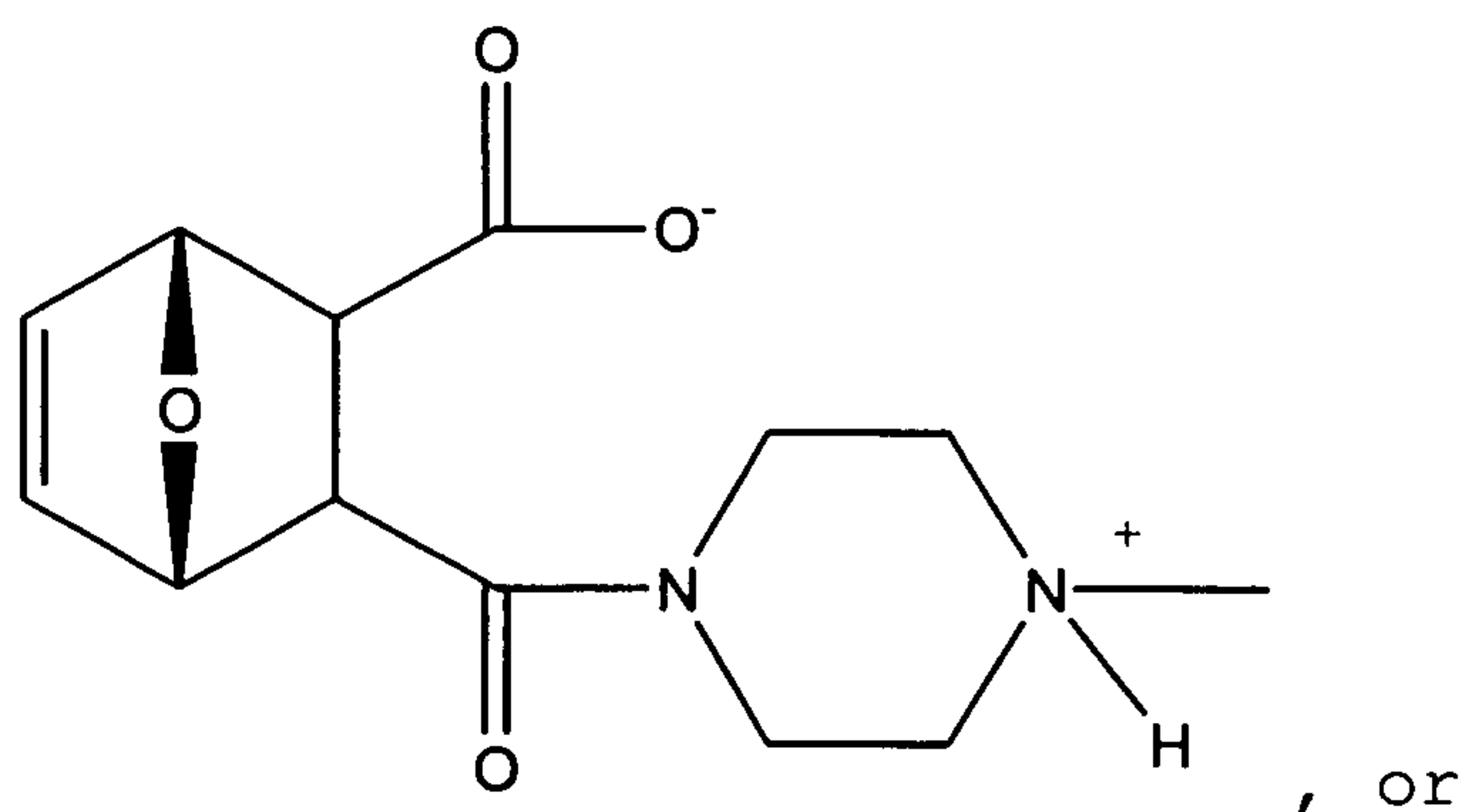
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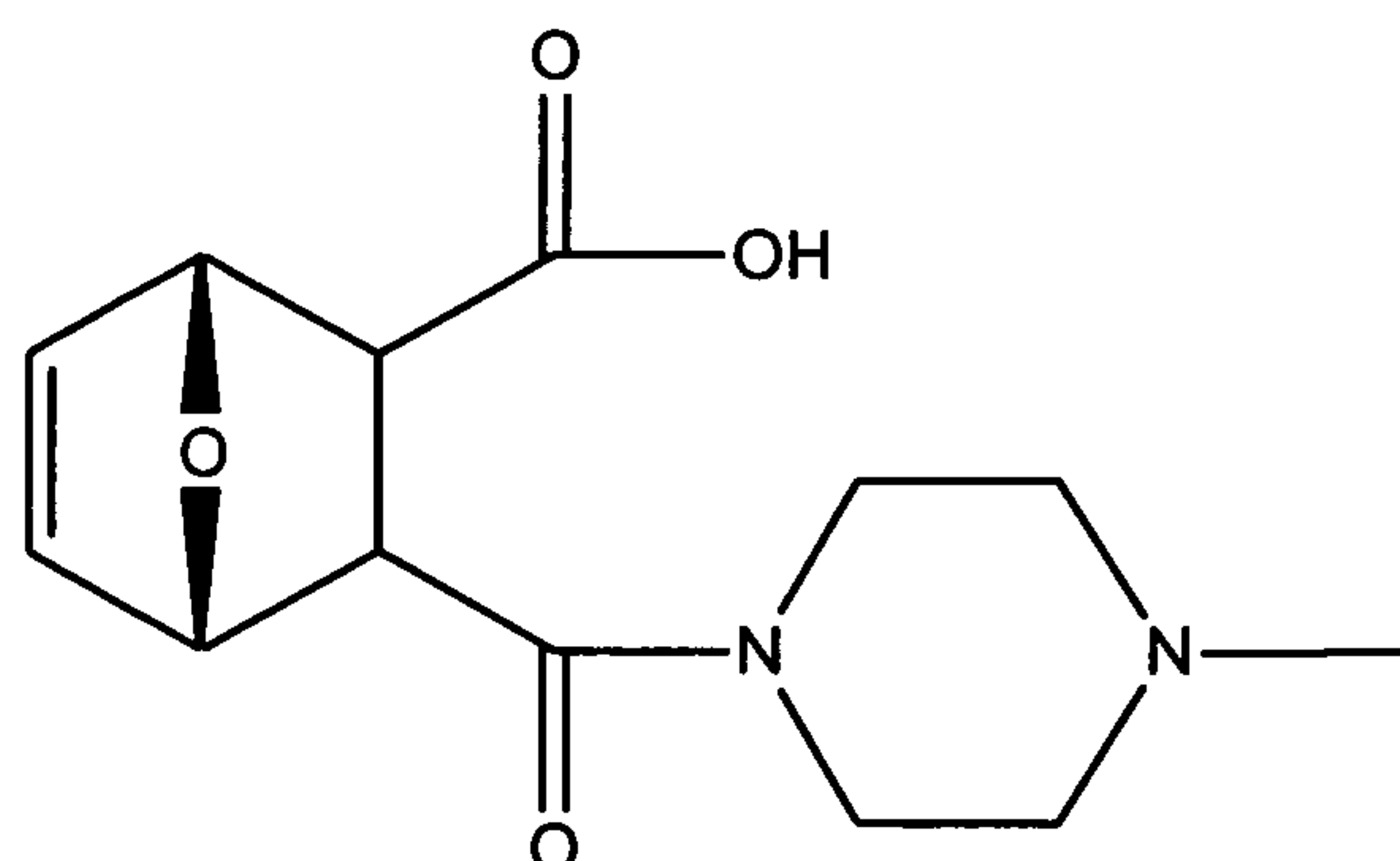
In an embodiment, the invention provides a compound having the structure (Compound 100E)

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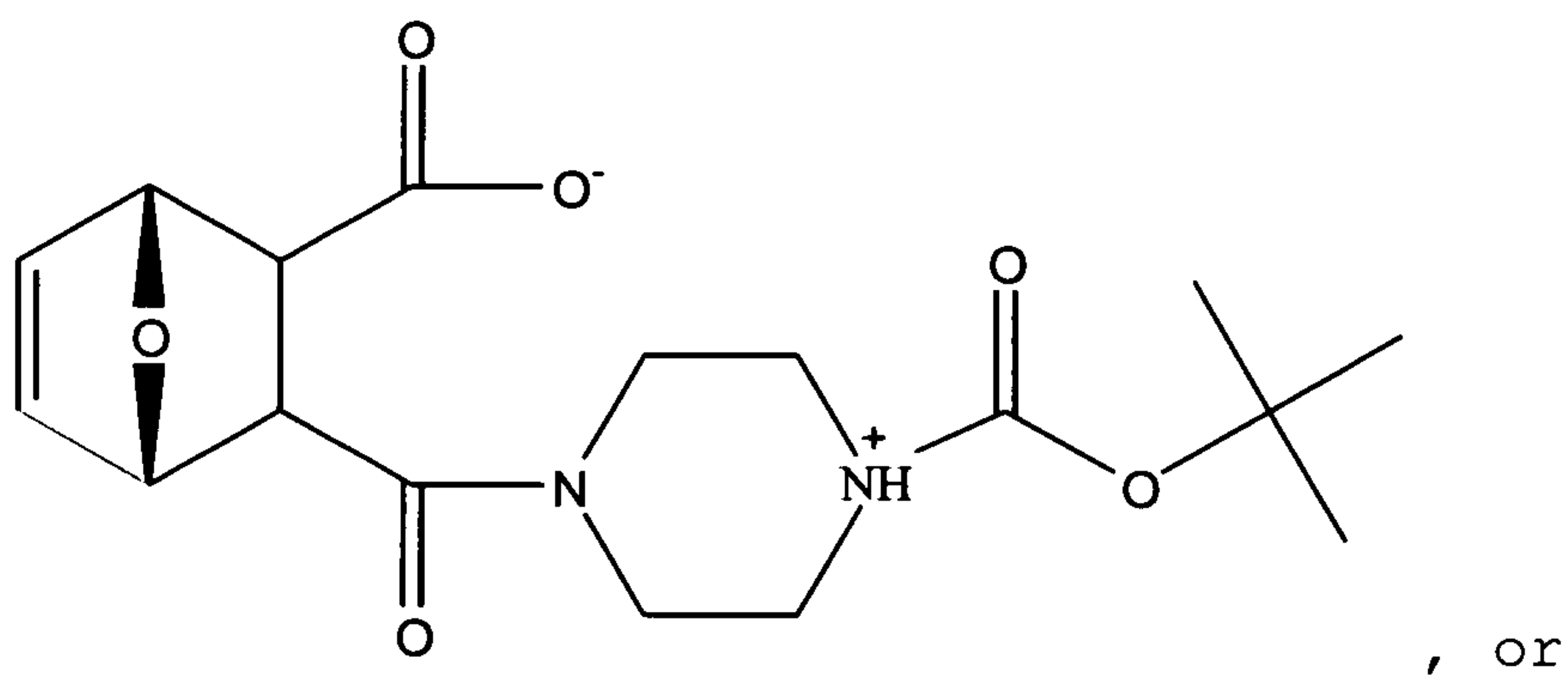
- 32 -



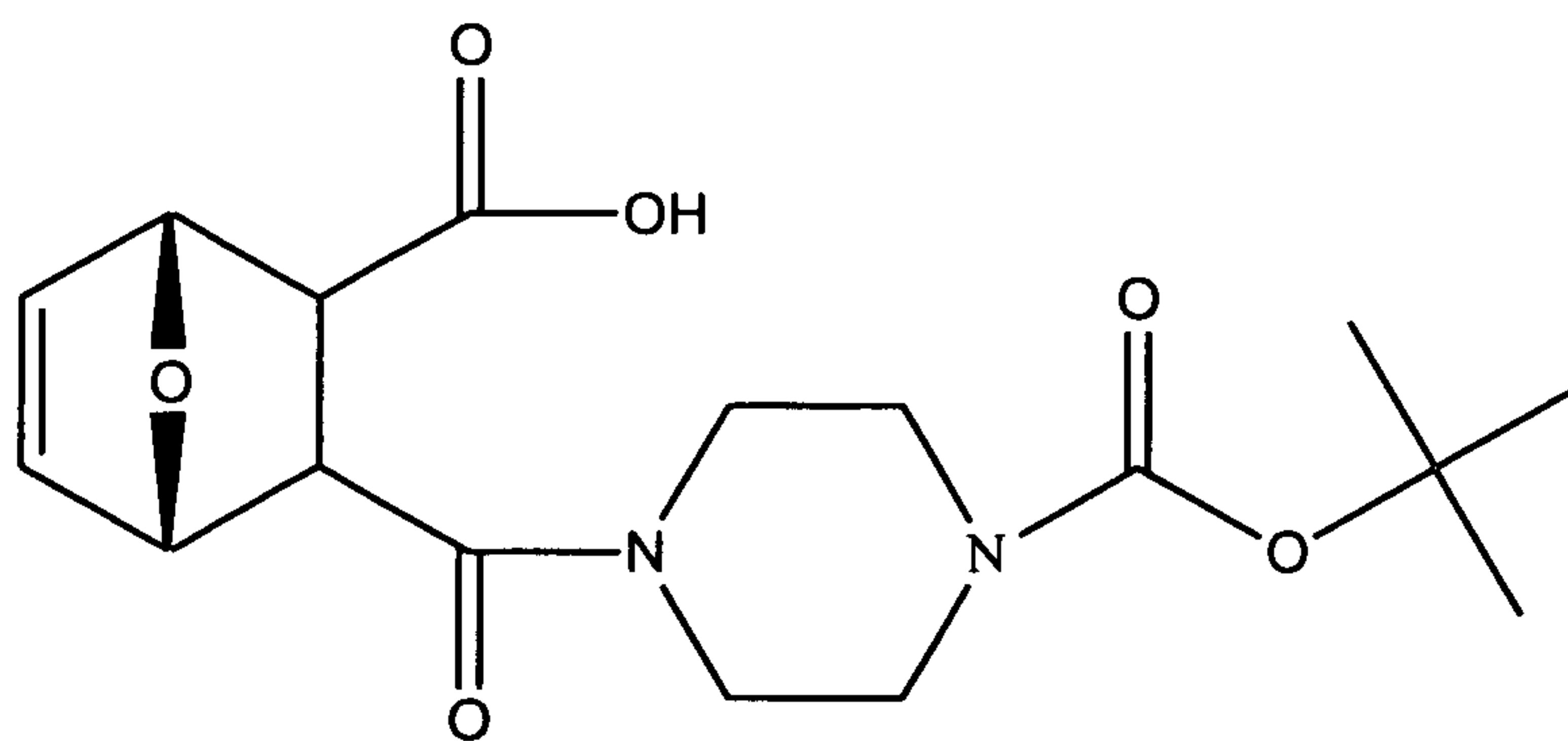
, or



In an embodiment, the invention provides a compound having the  
5 structure (Compound 102E)

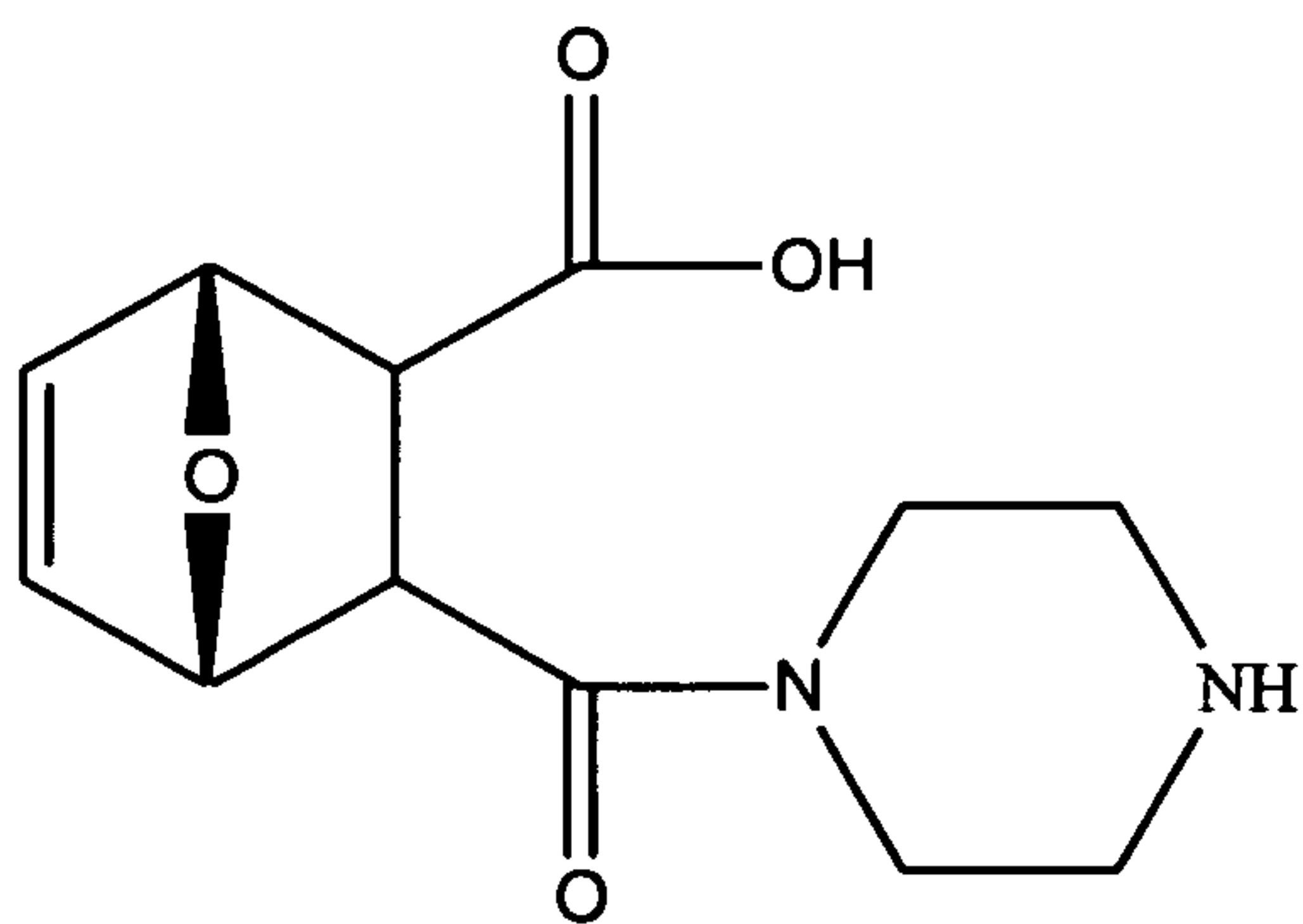
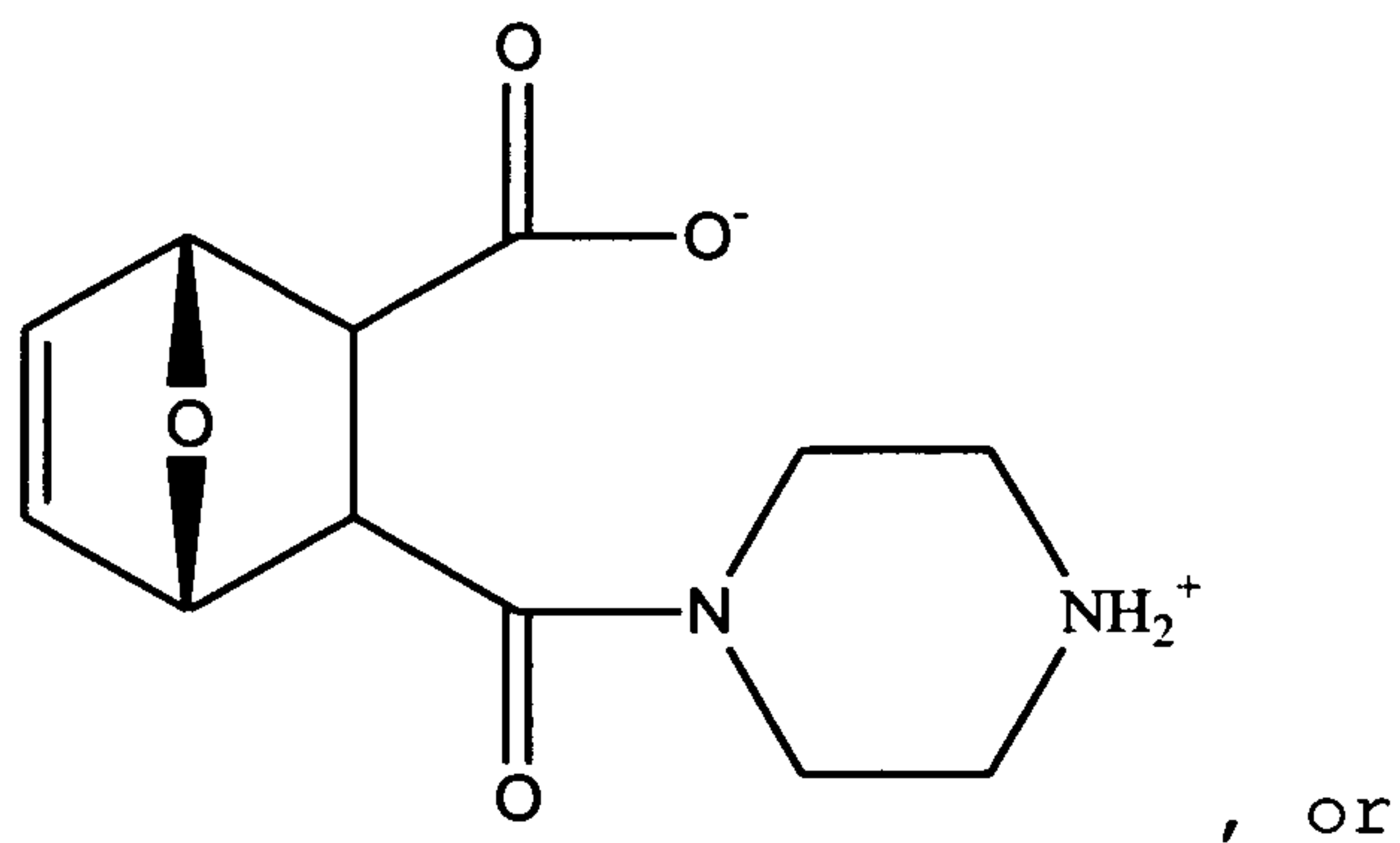


, or



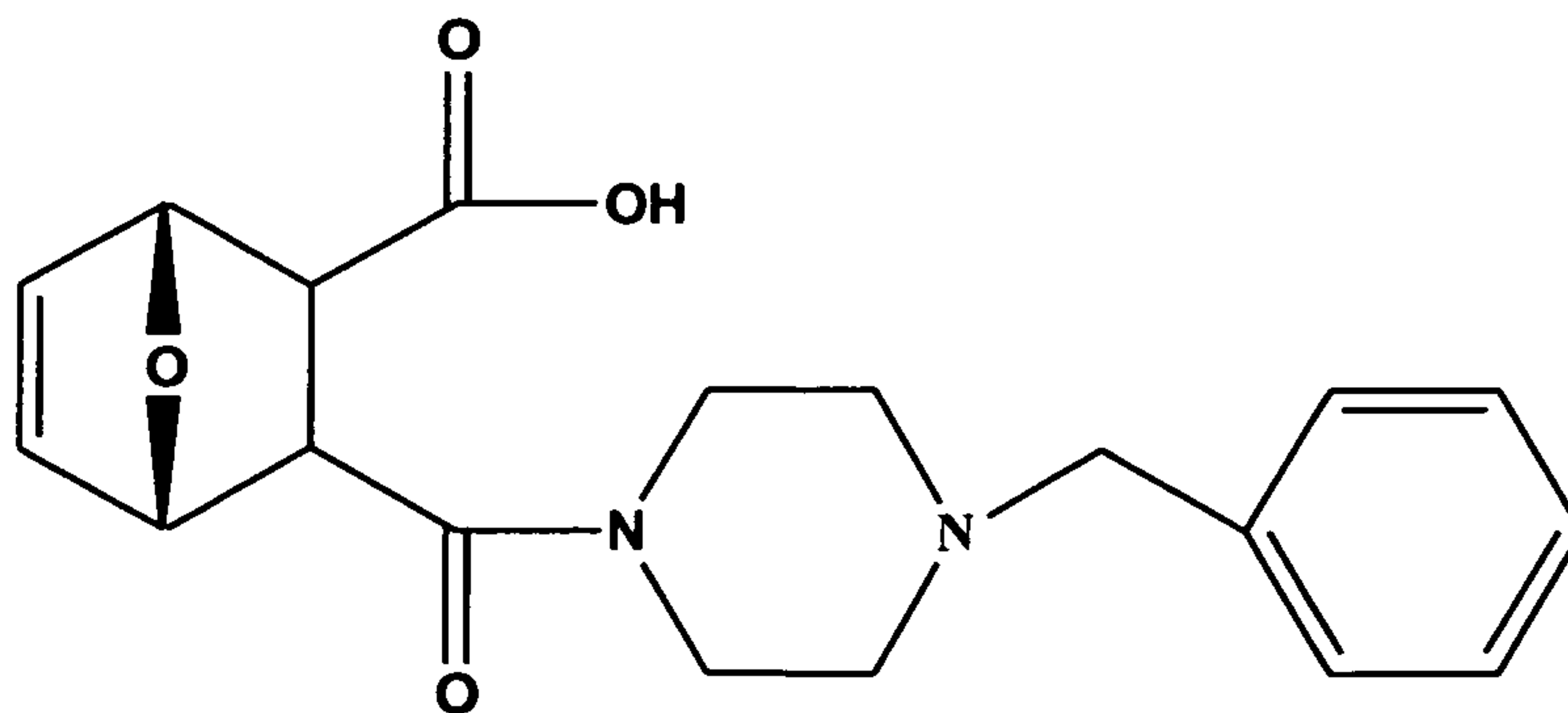
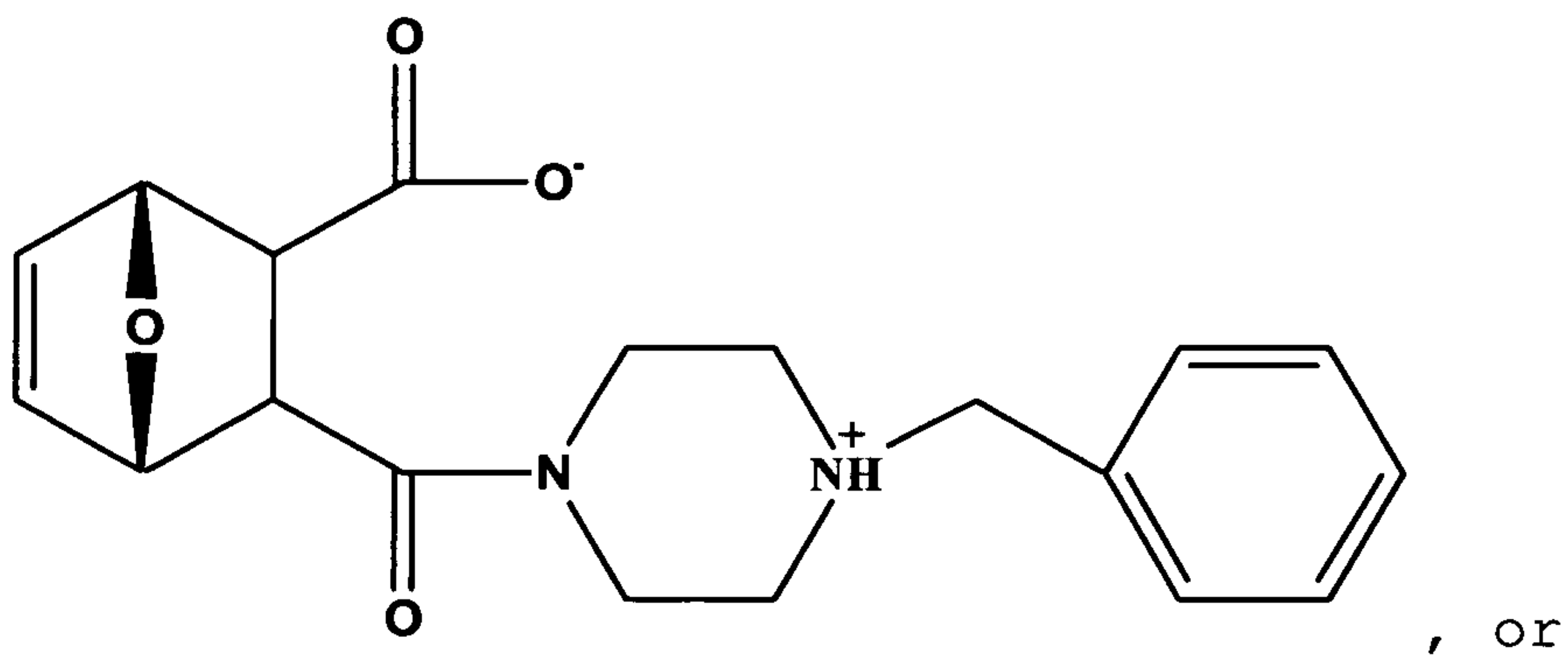
- 33 -

In an embodiment, the invention provides a compound having the structure (Compound 101E)



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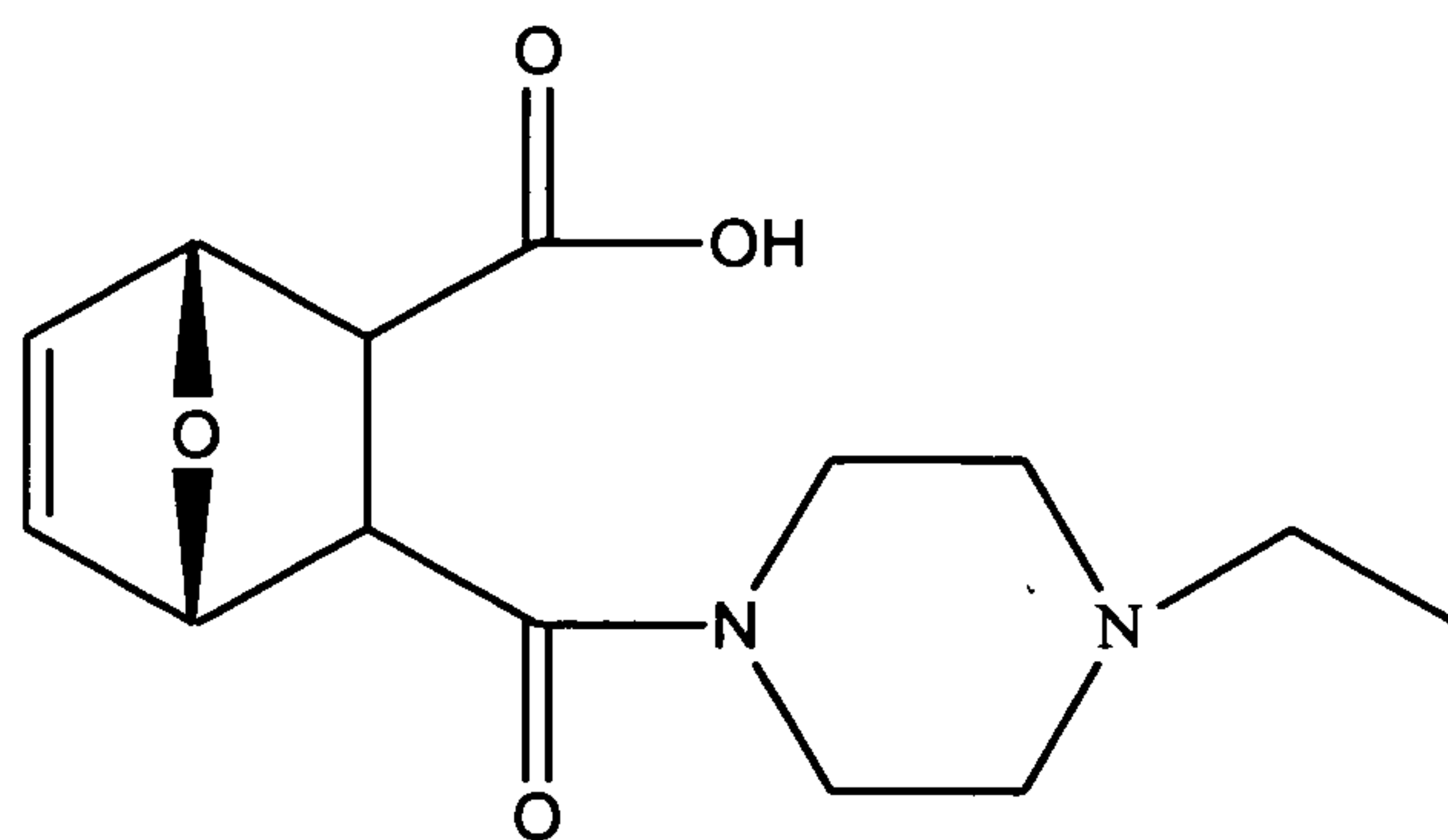
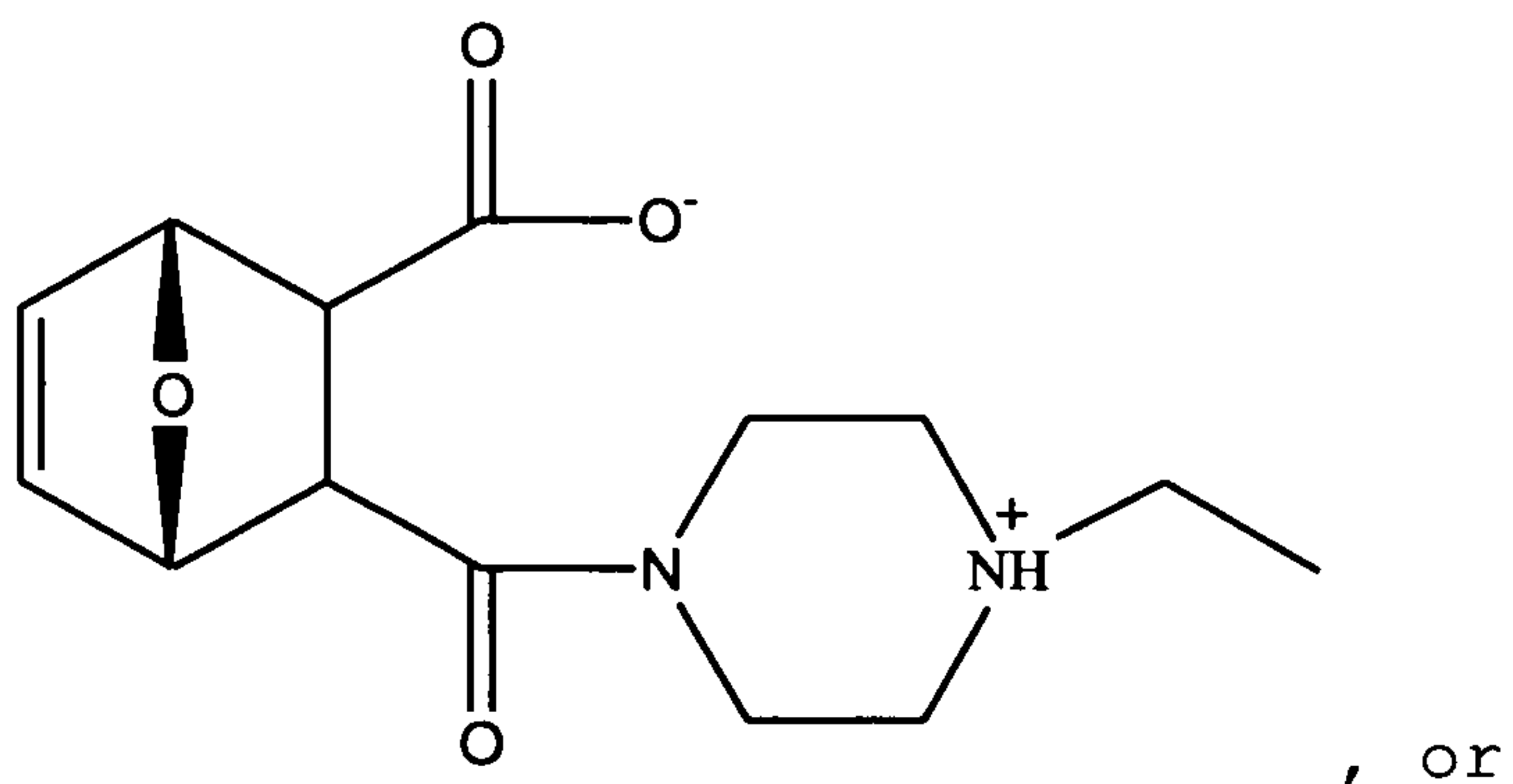
In an embodiment, the invention provides a compound having the structure (Compound 103E)



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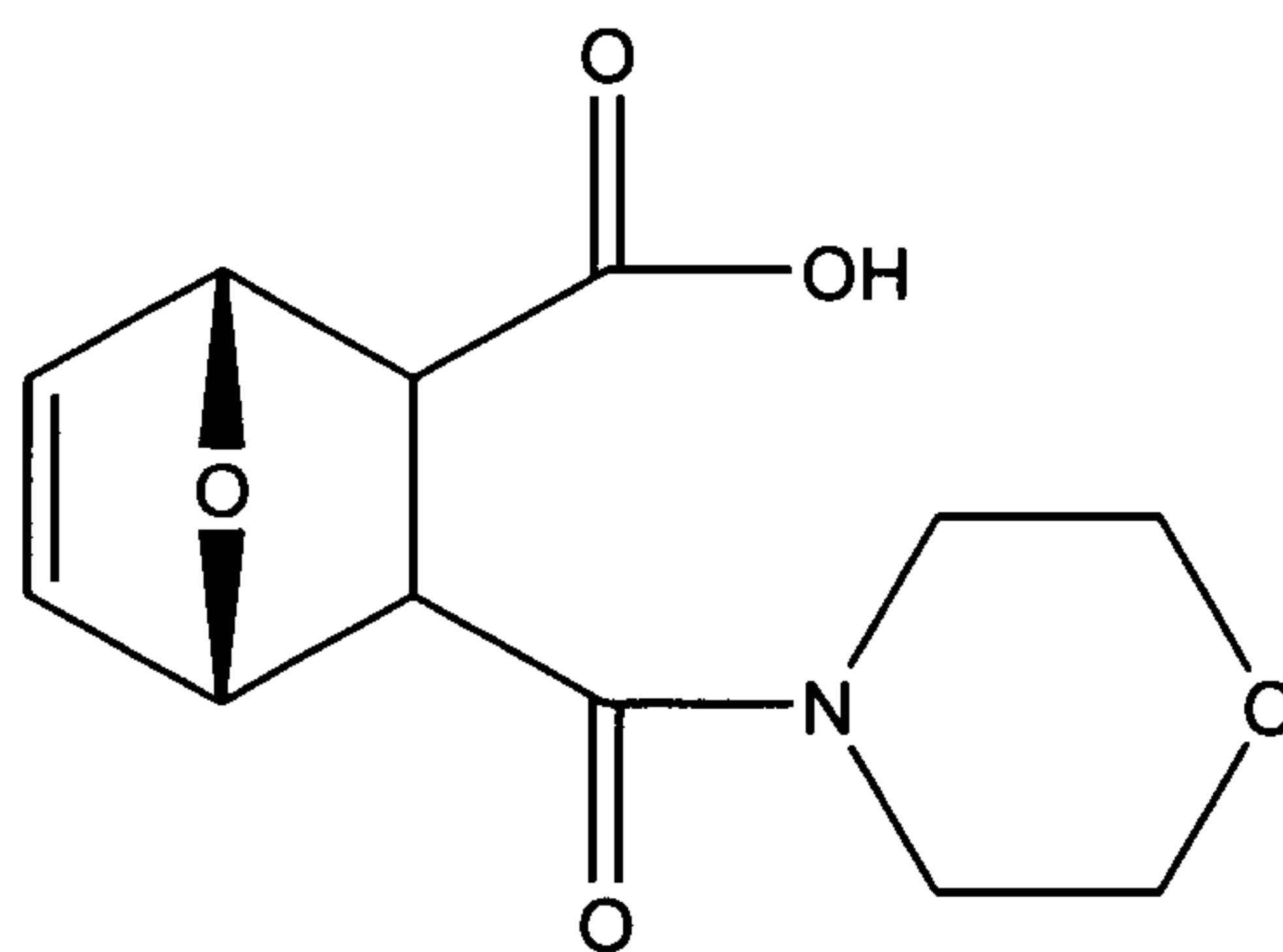
- 34 -

In an embodiment, the invention provides a compound having the structure (Compound 105E)



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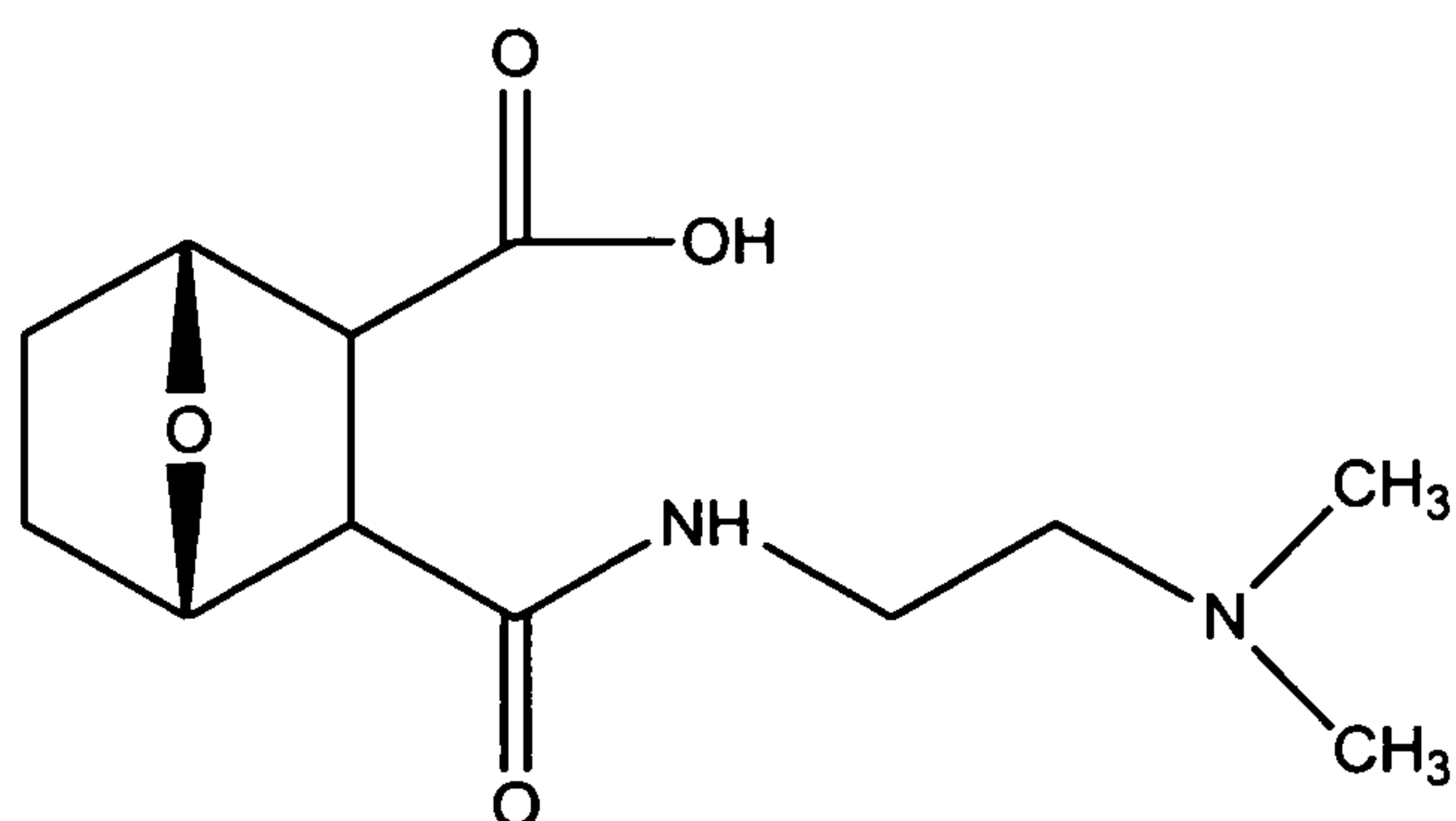
In an embodiment, the invention provides a compound having the structure (Compound 111E)



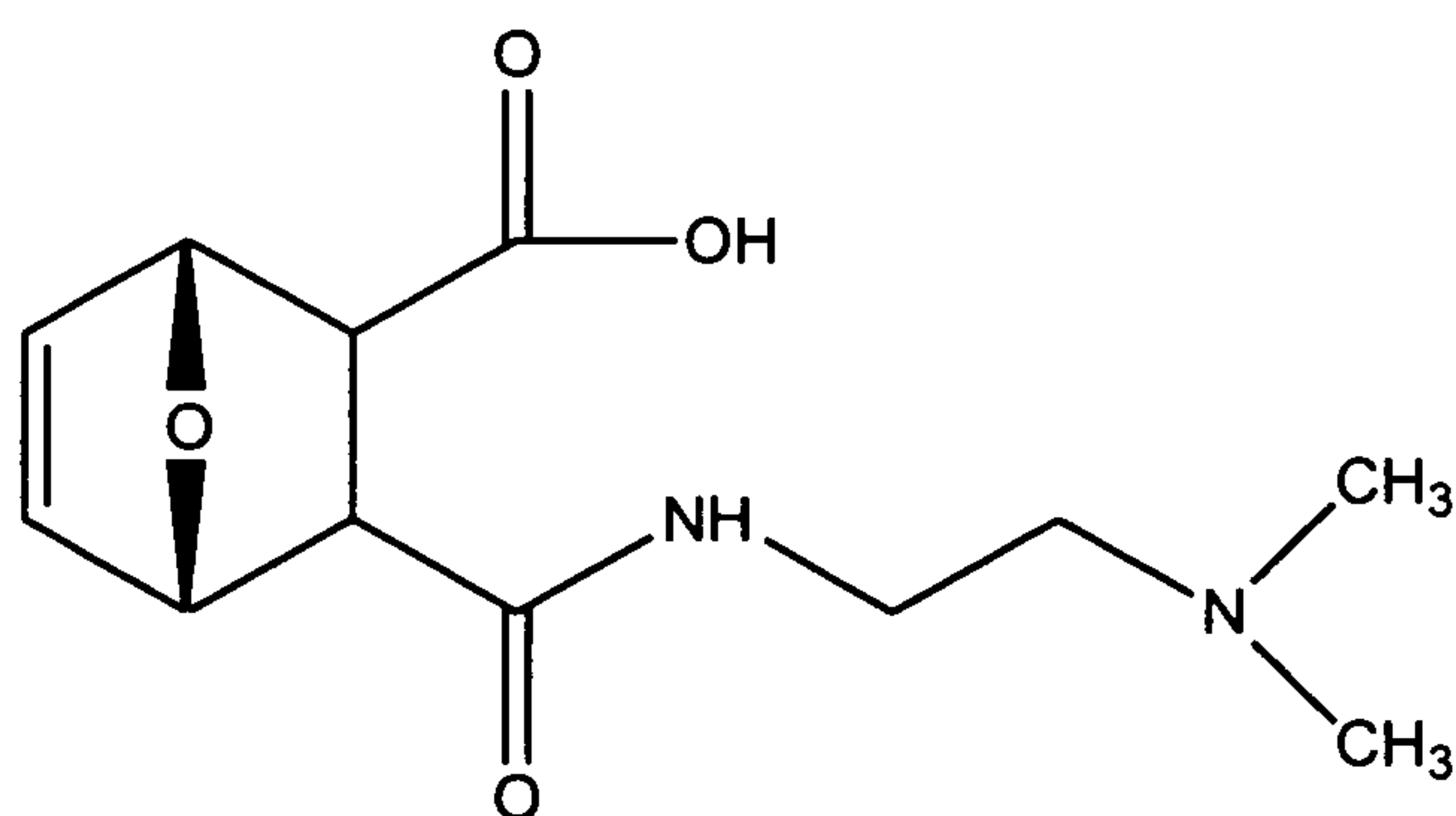
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This invention provides a compound having the structure (compound 106)

- 35 -

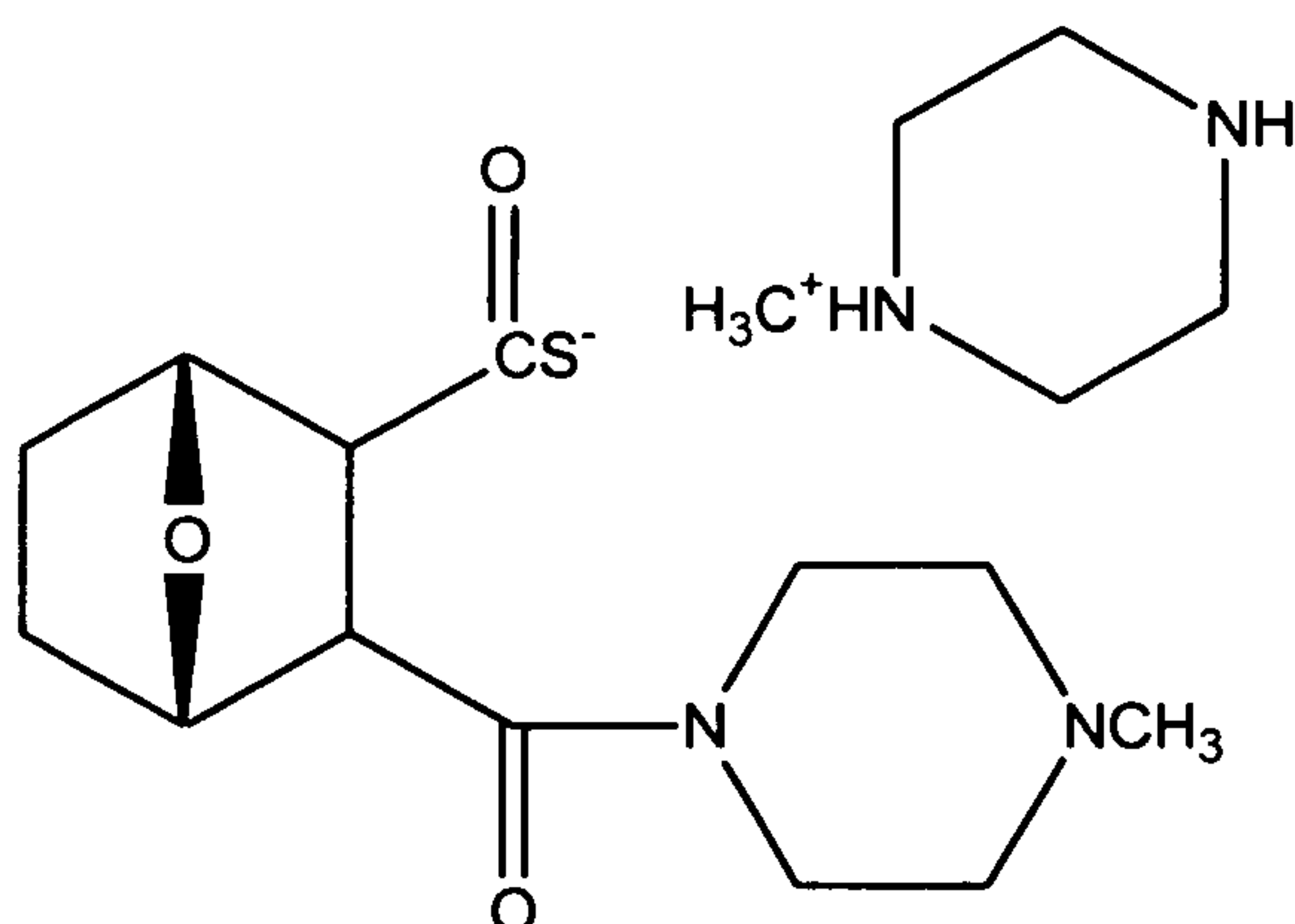


This invention provides a compound having the structure  
(compound 106E )



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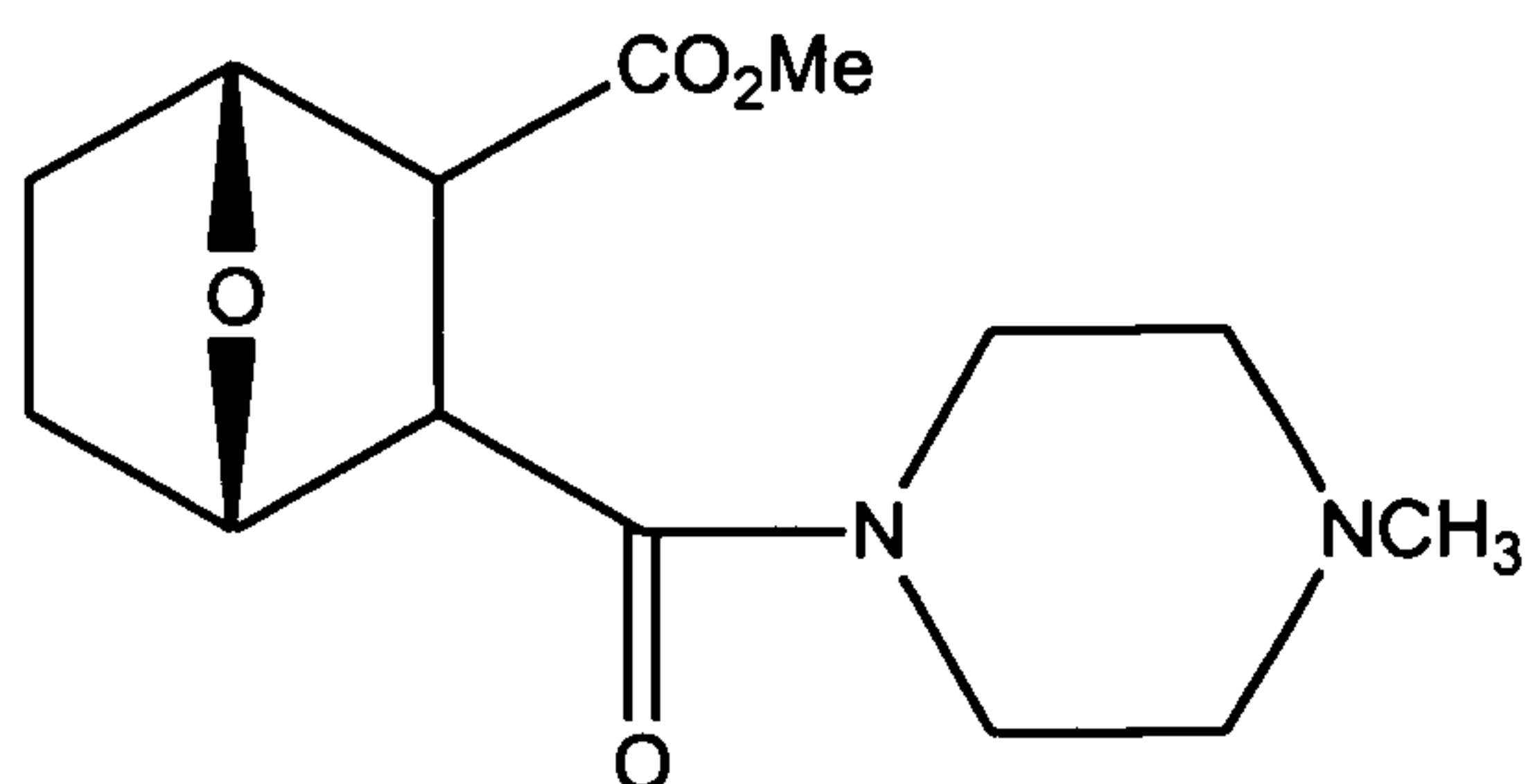
This invention provides a compound having the structure  
(compound 108)



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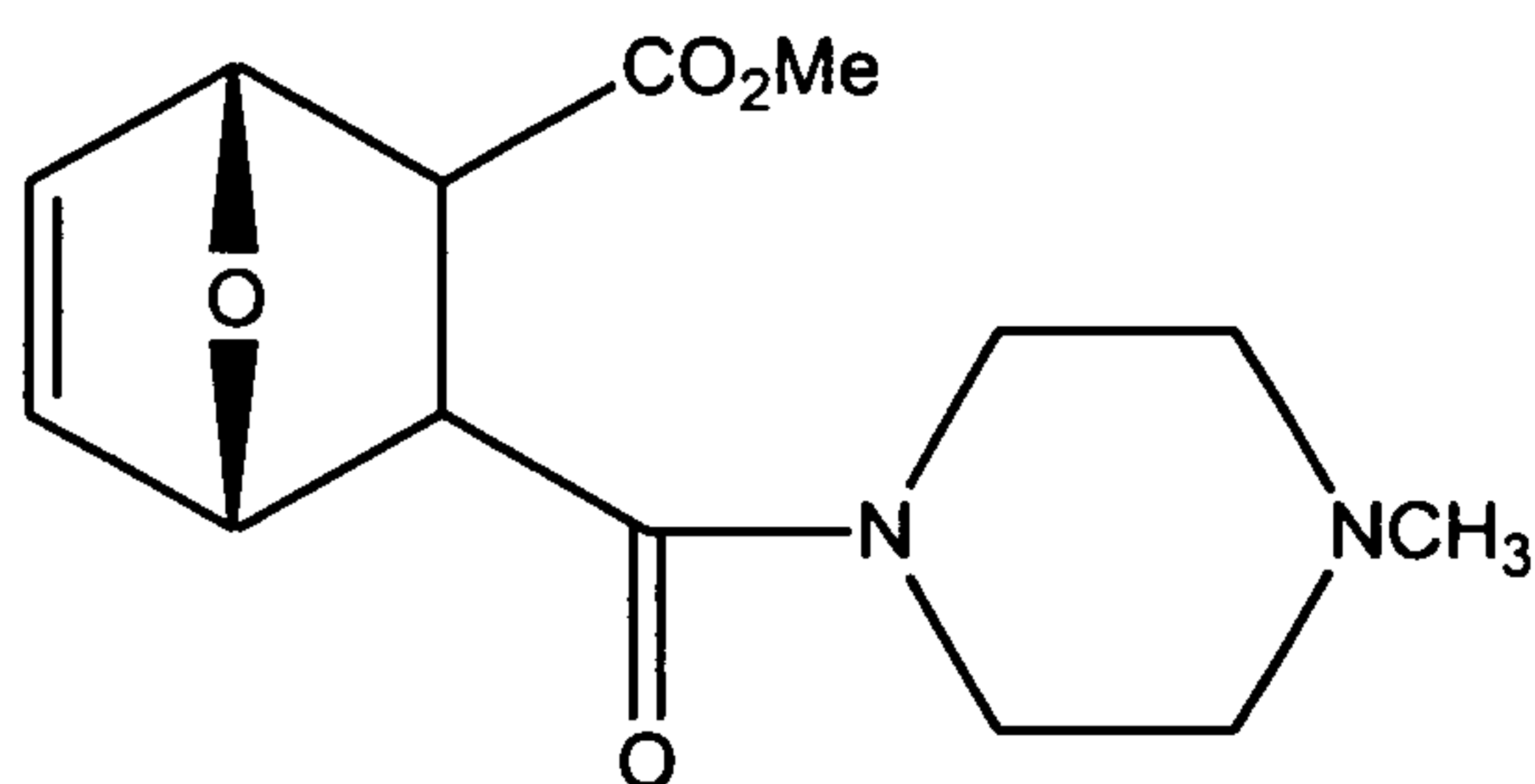
This invention provides a compound having the structure  
(compound 107)

- 36 -



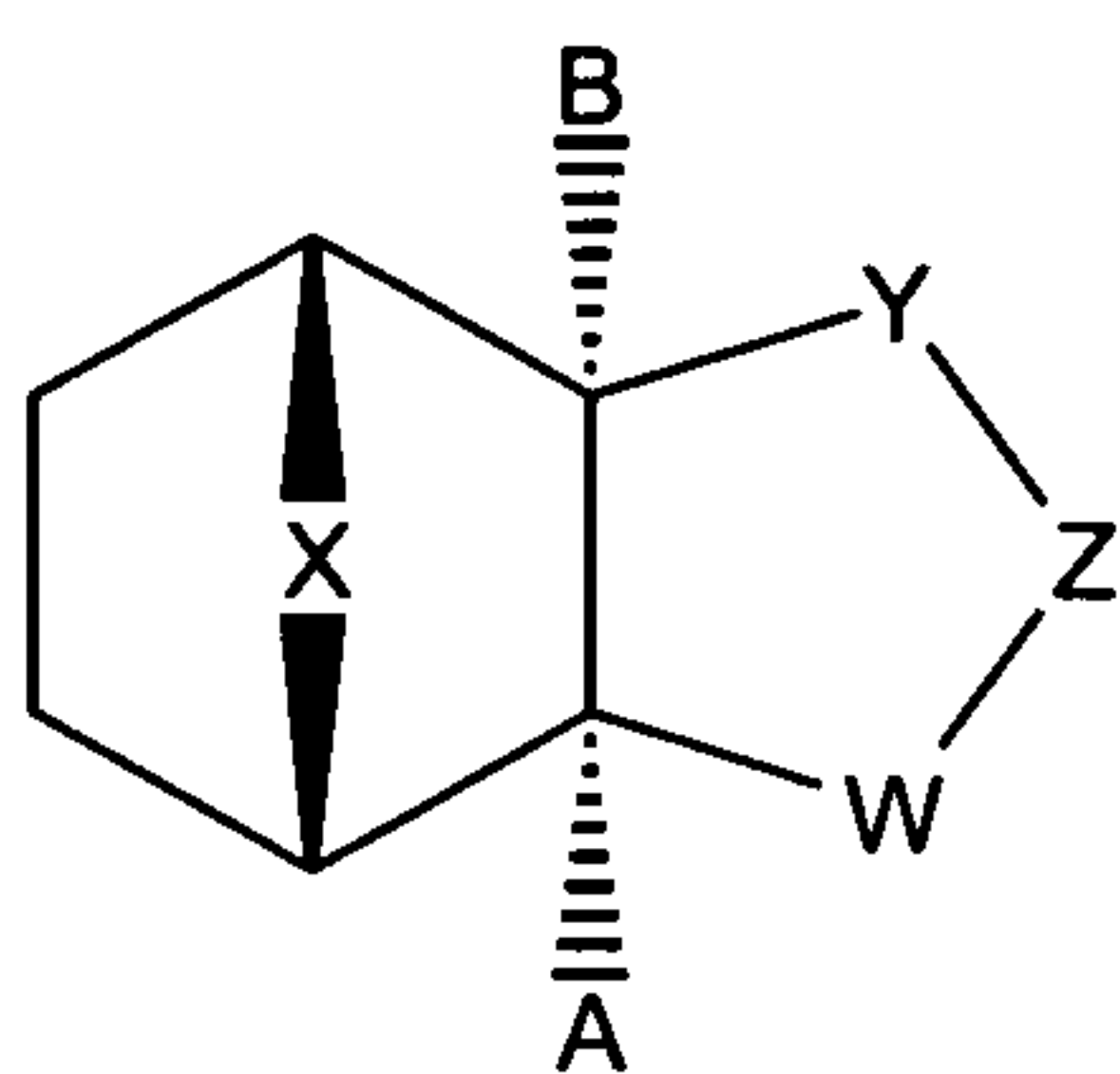
This invention provides a compound having the structure

5 (compound 107E)



In another embodiment, the invention provides a compound having the structure

10



wherein A and B is each independently H, F, Cl, Br, SO<sub>2</sub>Ph, CO<sub>2</sub>CH<sub>3</sub>, COR<sub>14</sub>, SR<sub>14</sub>, where R<sub>14</sub> is H or aryl or a substituted or unsubstituted alkyl, alkenyl or alkynyl; X is O, NH or S; Z is  
 15 O, S, SR<sub>15</sub>, NH, NR<sub>15</sub>, CH<sub>2</sub>OH, CH<sub>2</sub>OR<sub>15</sub>, where R<sub>15</sub> is an aryl or a substituted or unsubstituted alkyl, alkenyl or alkynyl; and Y and W is each independently CHOH CH<sub>2</sub>, C=S or C=O, or a salt of the compound.

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In an embodiment of the foregoing invention, Y and W are each independently CH<sub>2</sub> or C=S.

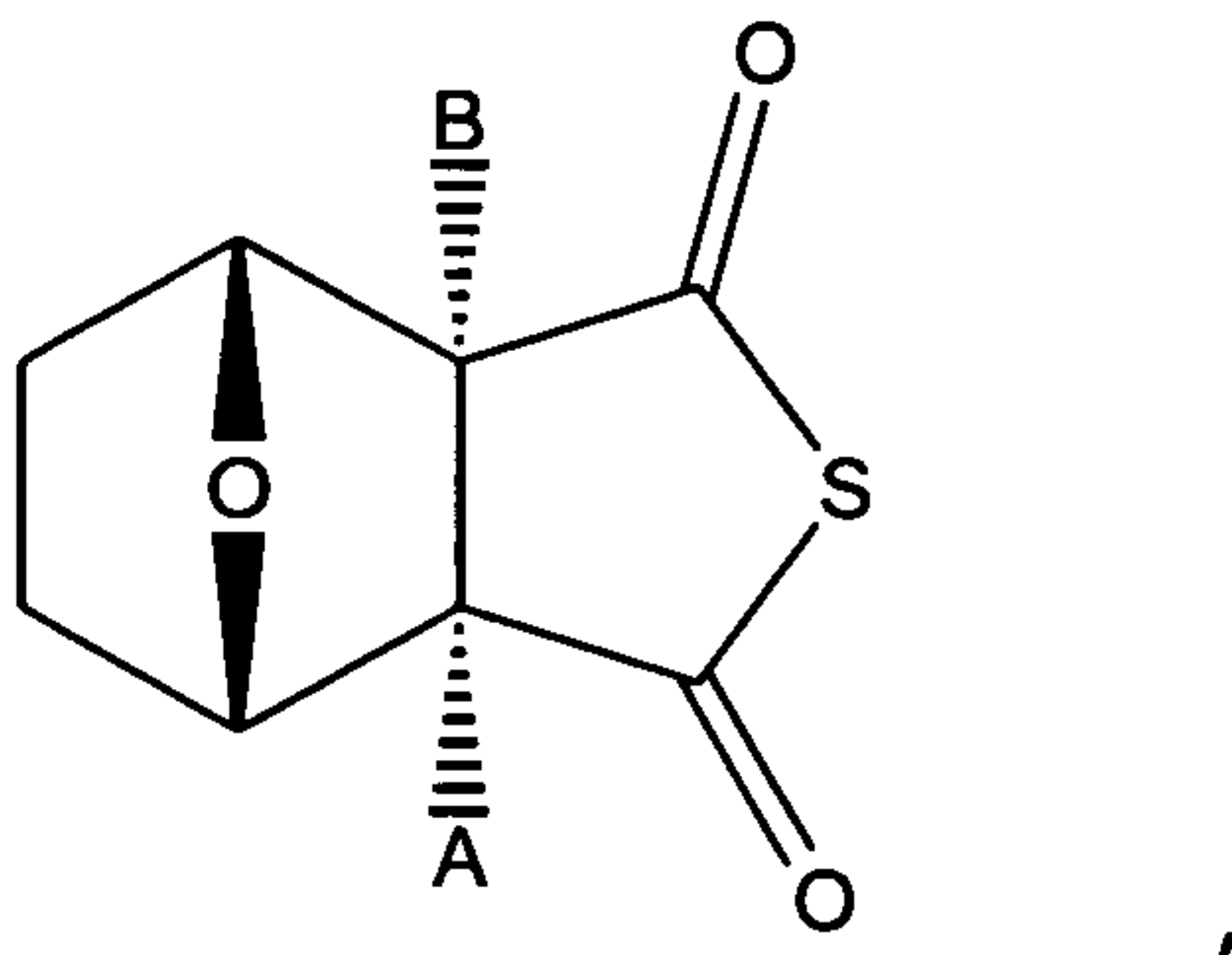
The invention further provides an embodiment wherein X is O; Z  
5 is S; and Y and W are each C=S.

The invention further provides an embodiment wherein A and B are each F.

10 The invention further provides an embodiment wherein A is H; and B is F.

In another embodiment, the invention provides a compound having the structure

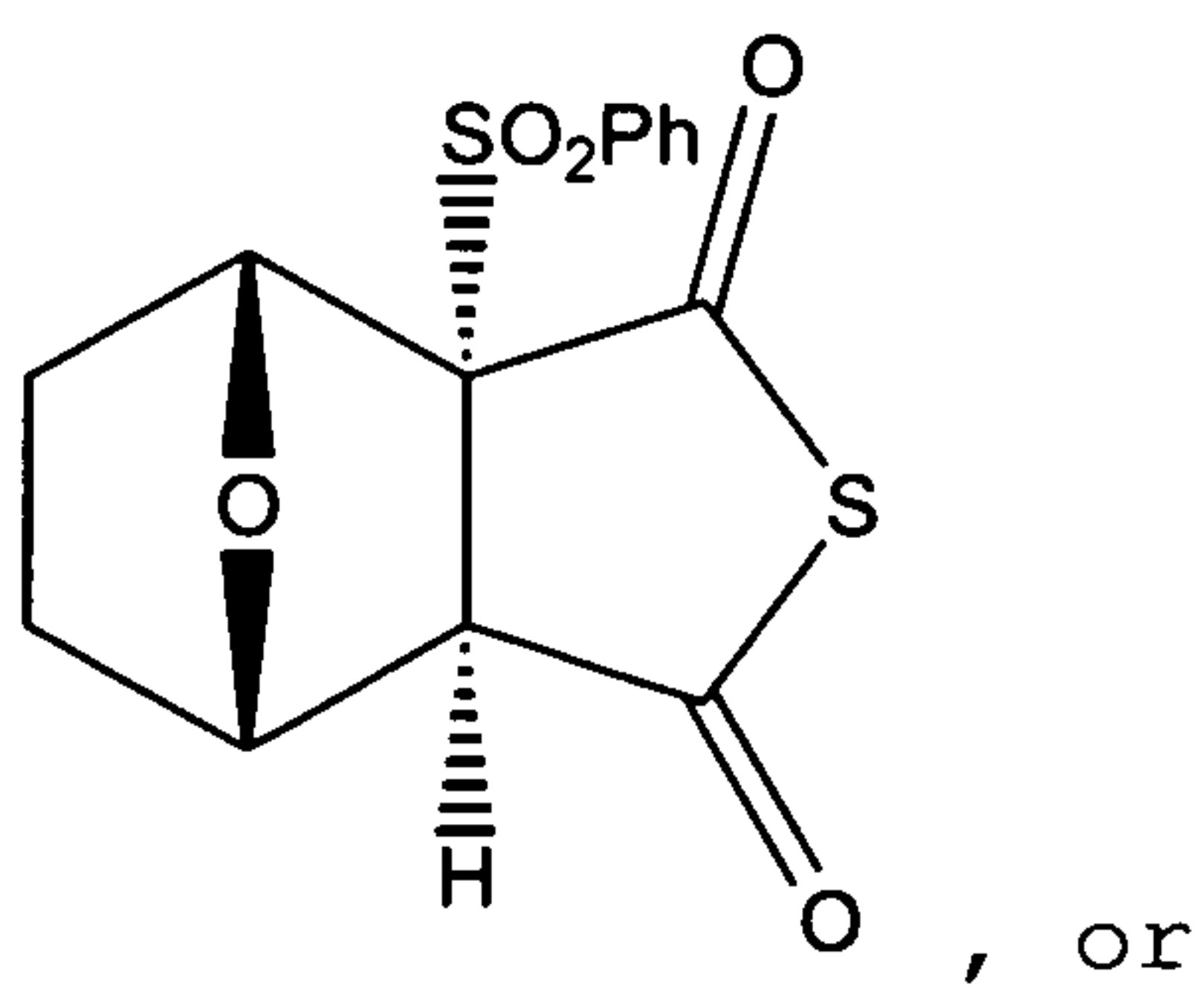
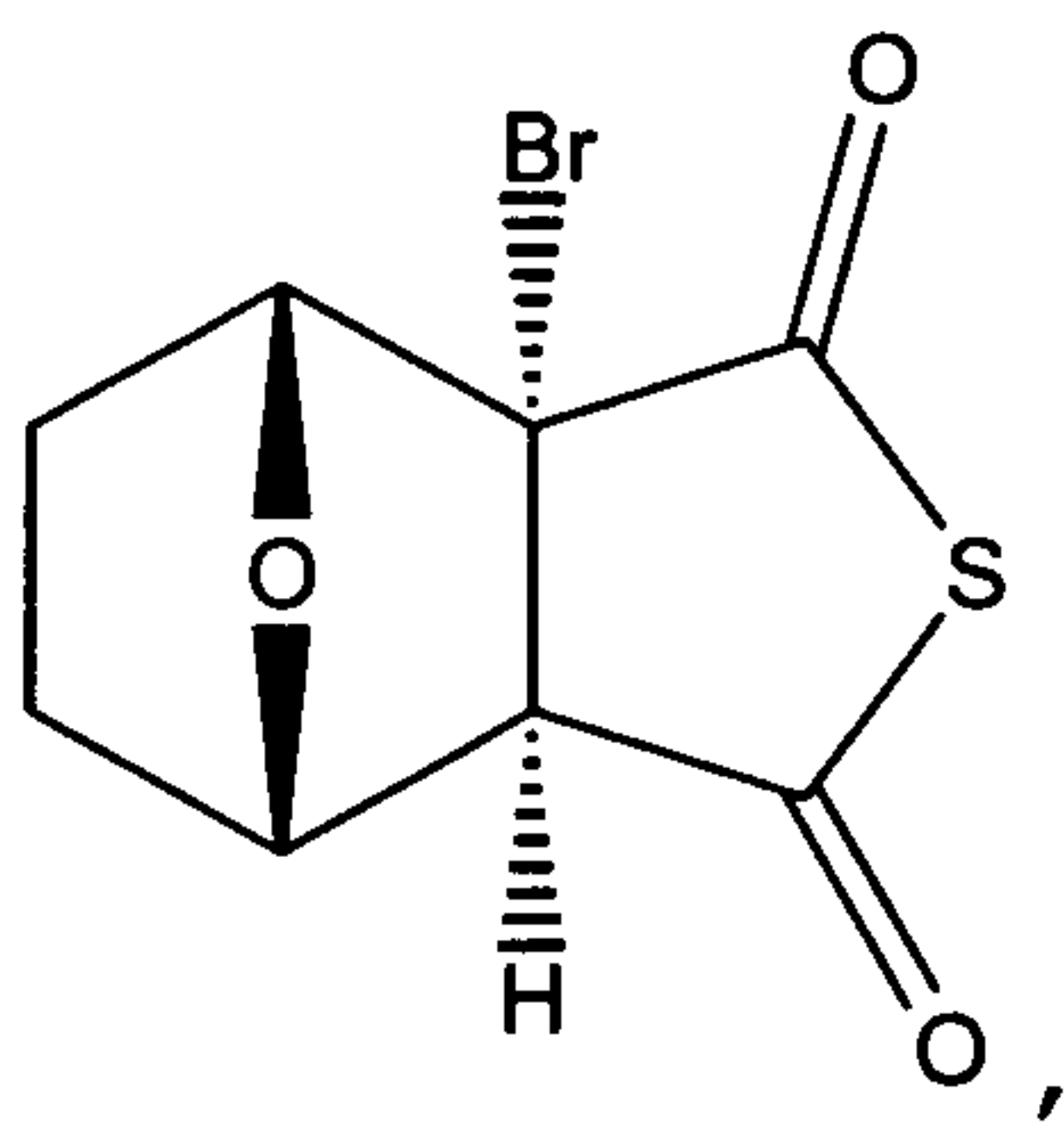
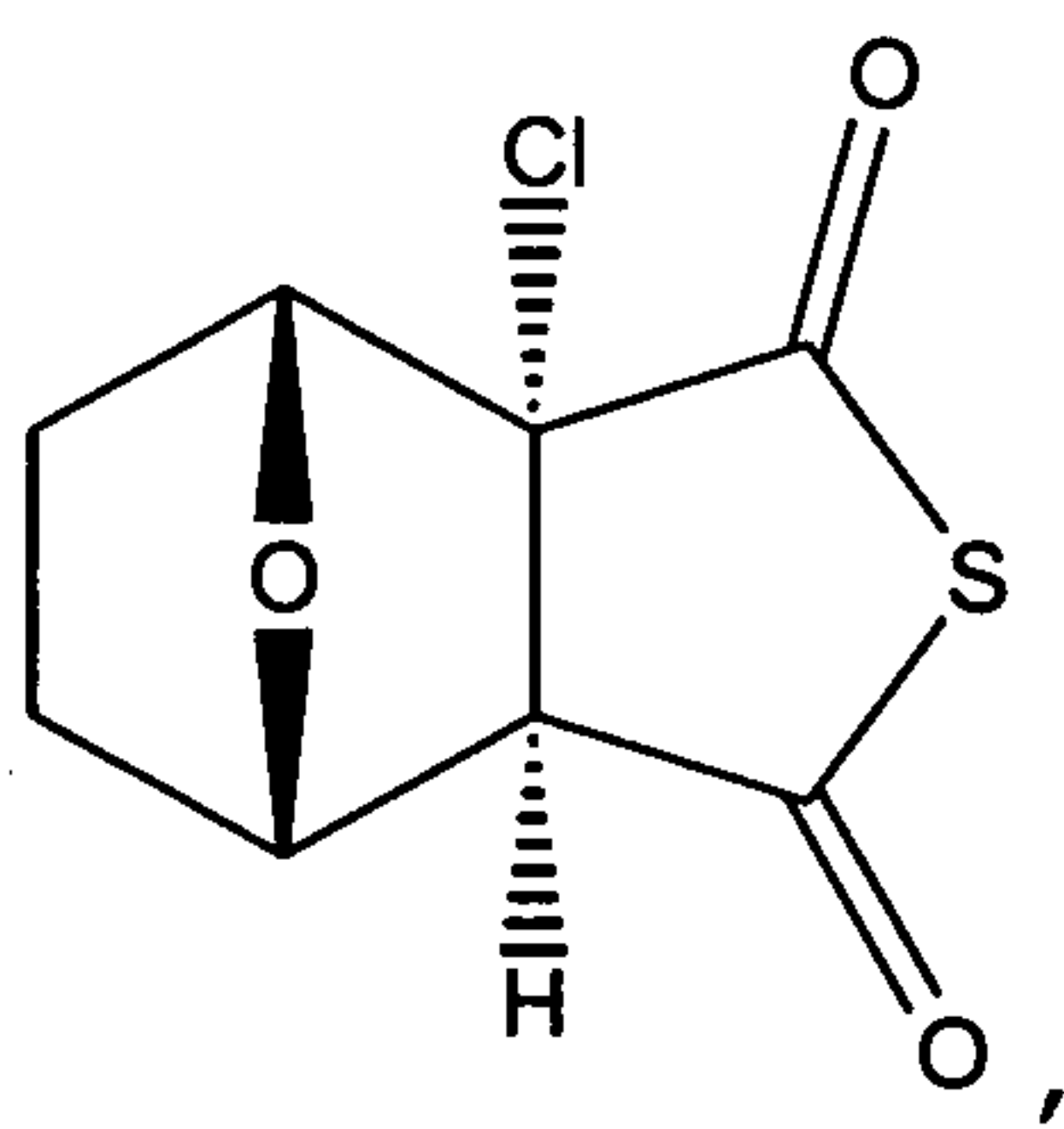
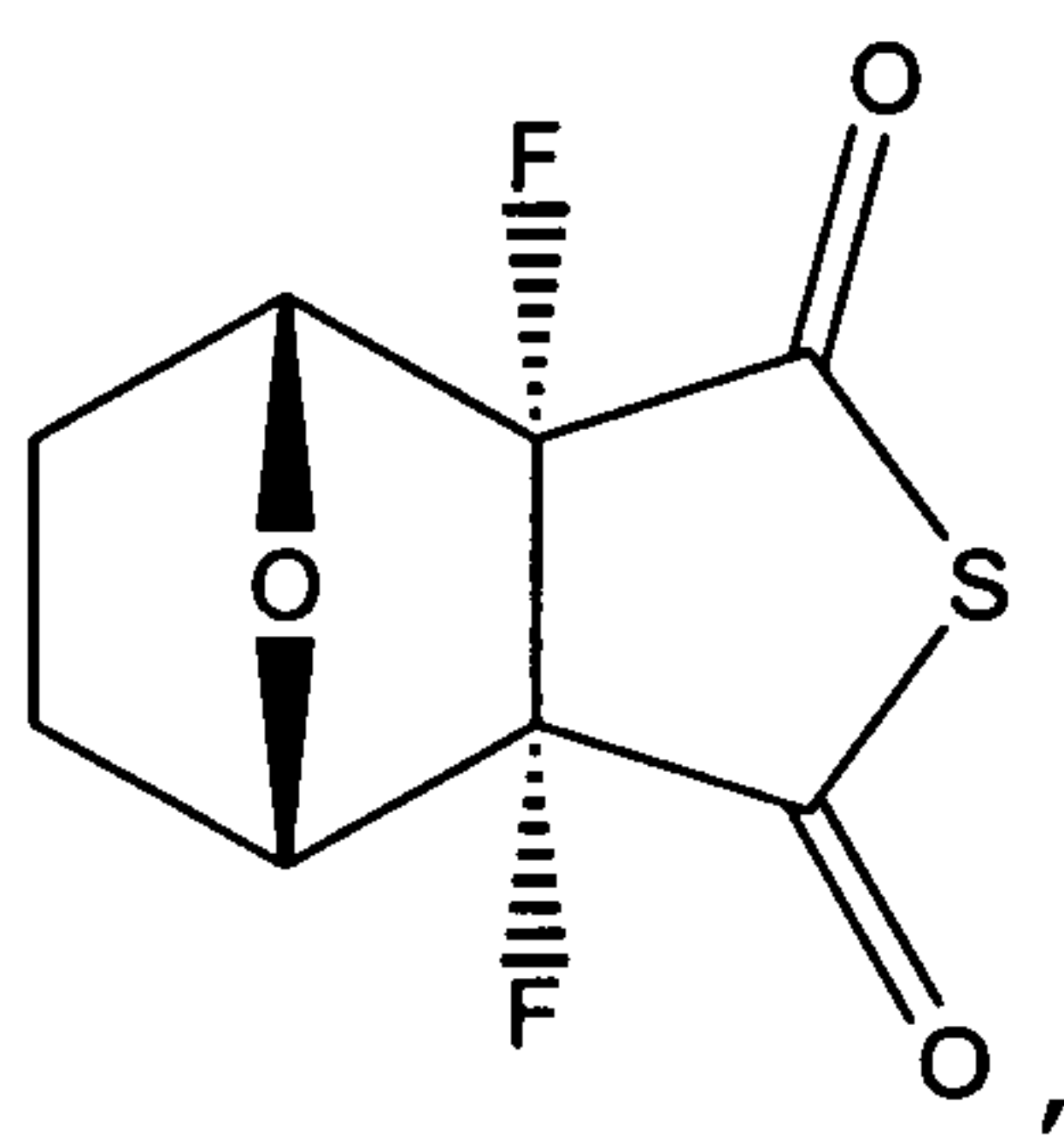
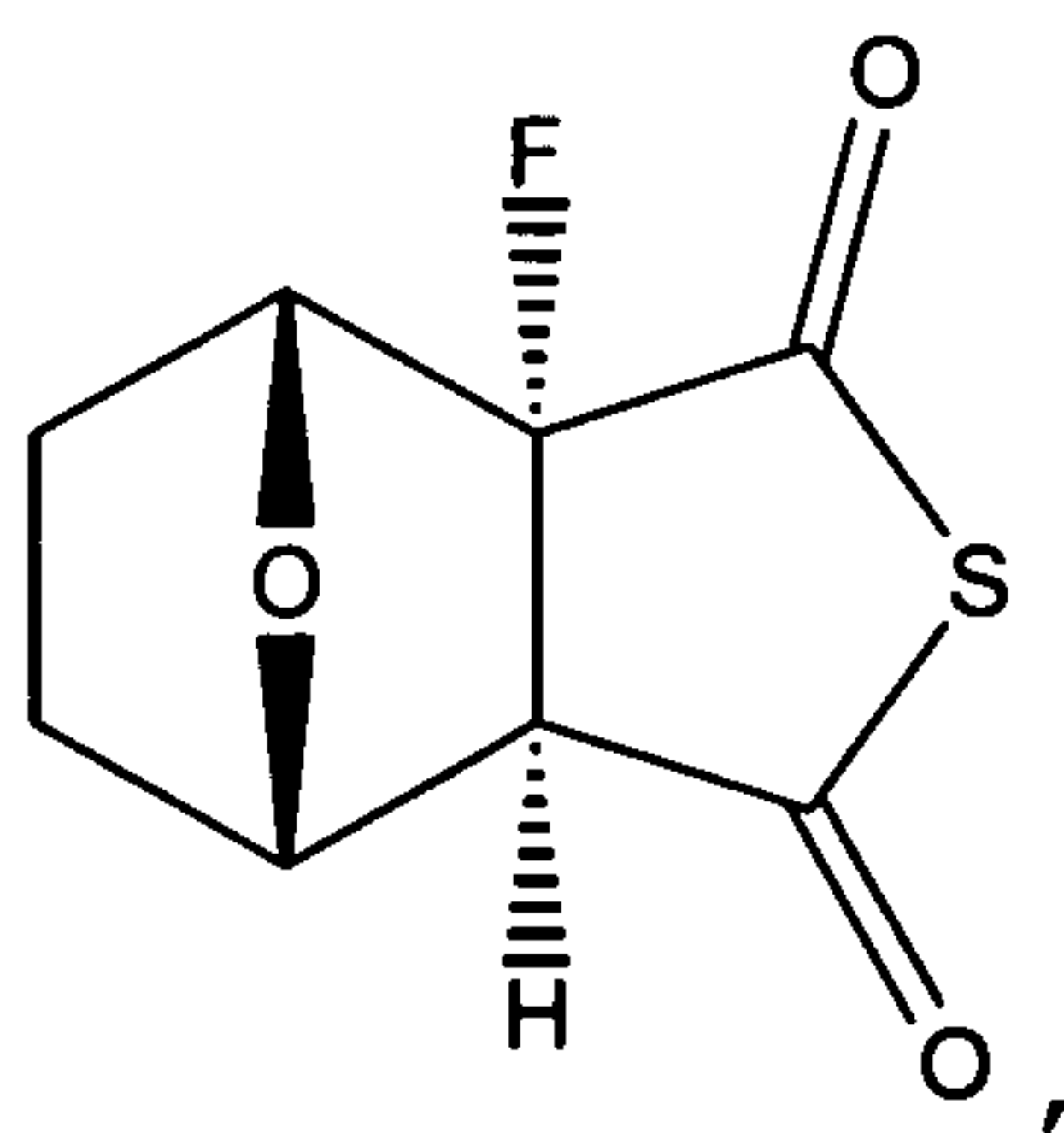
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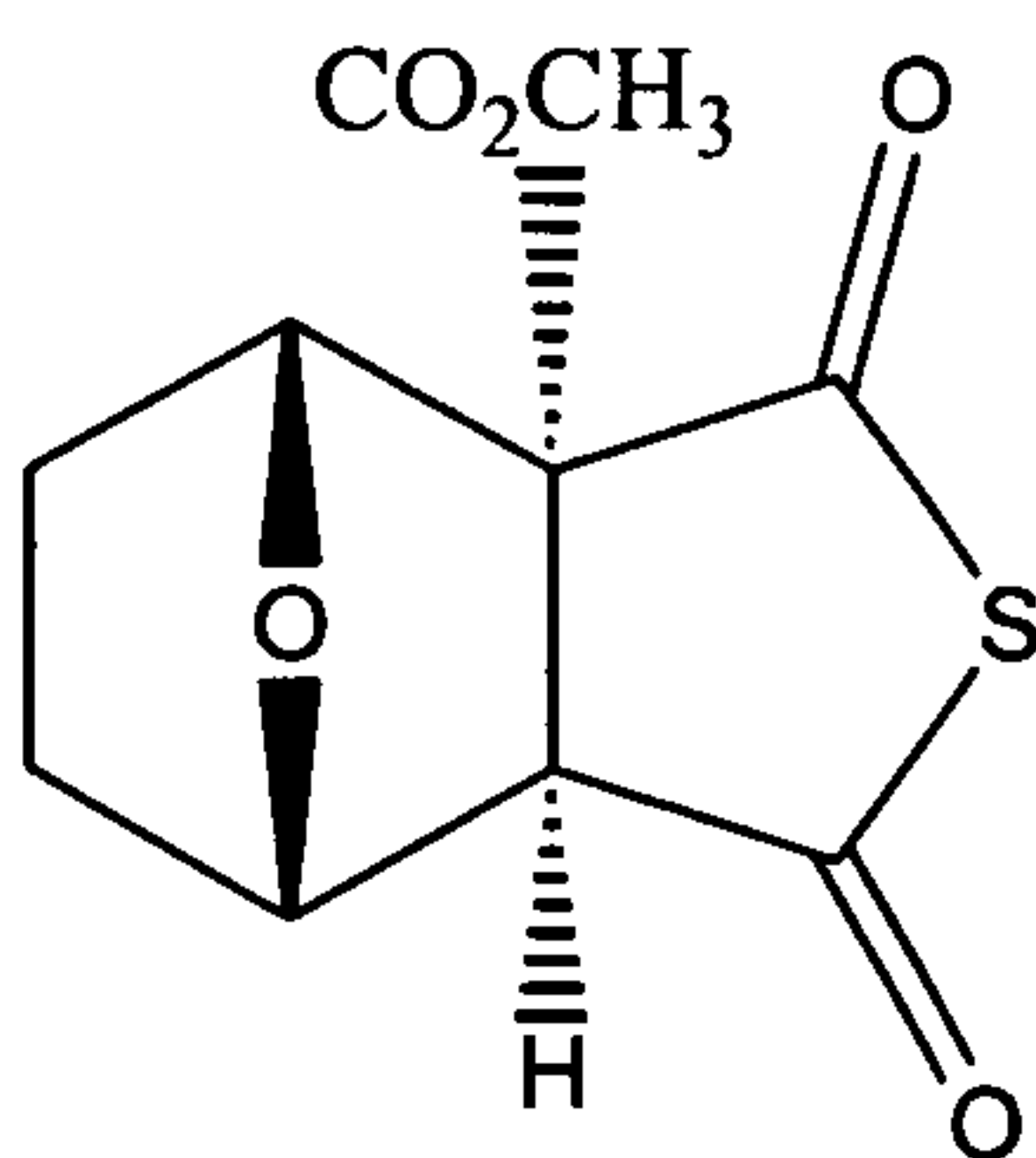
wherein A is F or SR<sub>14</sub>, where R<sub>14</sub> is aryl or a substituted or unsubstituted alkyl, alkenyl or alkynyl; and B is F, Cl, Br,  
20 SO<sub>2</sub>Ph, CO<sub>2</sub>CH<sub>3</sub>, or SR<sub>14</sub>, where R<sub>14</sub> is aryl or a substituted or unsubstituted alkyl, alkenyl or alkynyl.

In a further embodiment, the invention provides the structures

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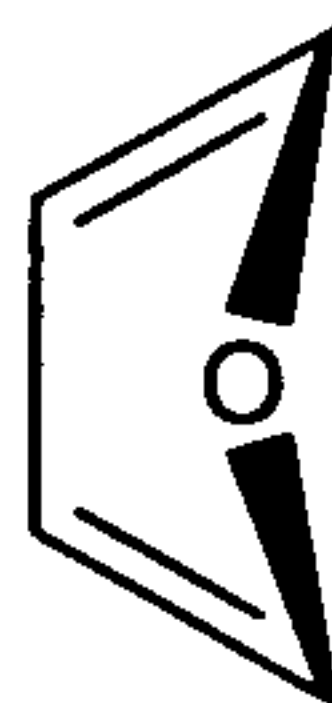


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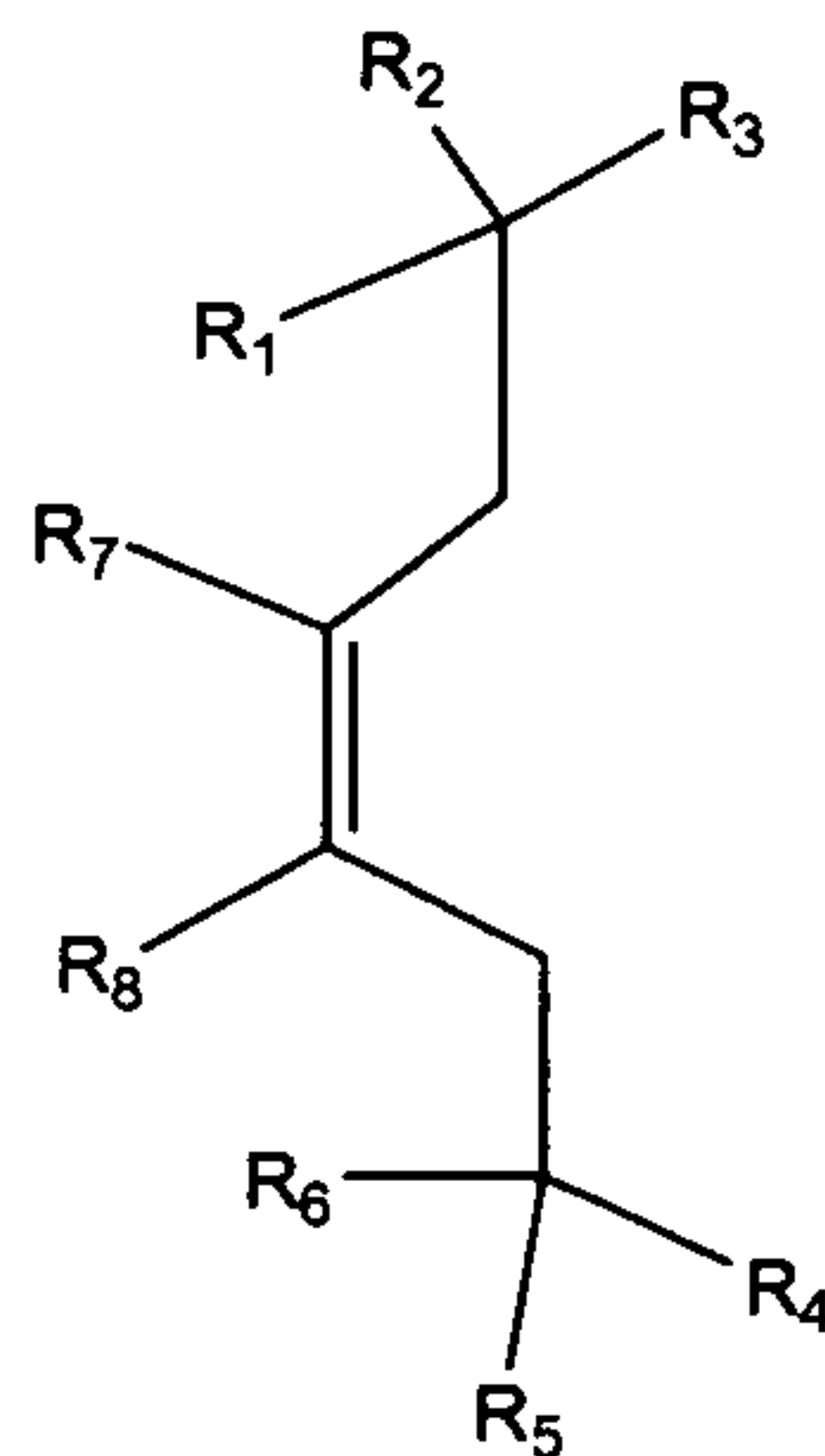


The invention provides a pharmaceutical composition comprising  
the compounds of the invention and a pharmaceutically acceptable  
5 carrier.

The invention also provides a process for making a compound  
comprising reacting a compound having the structure

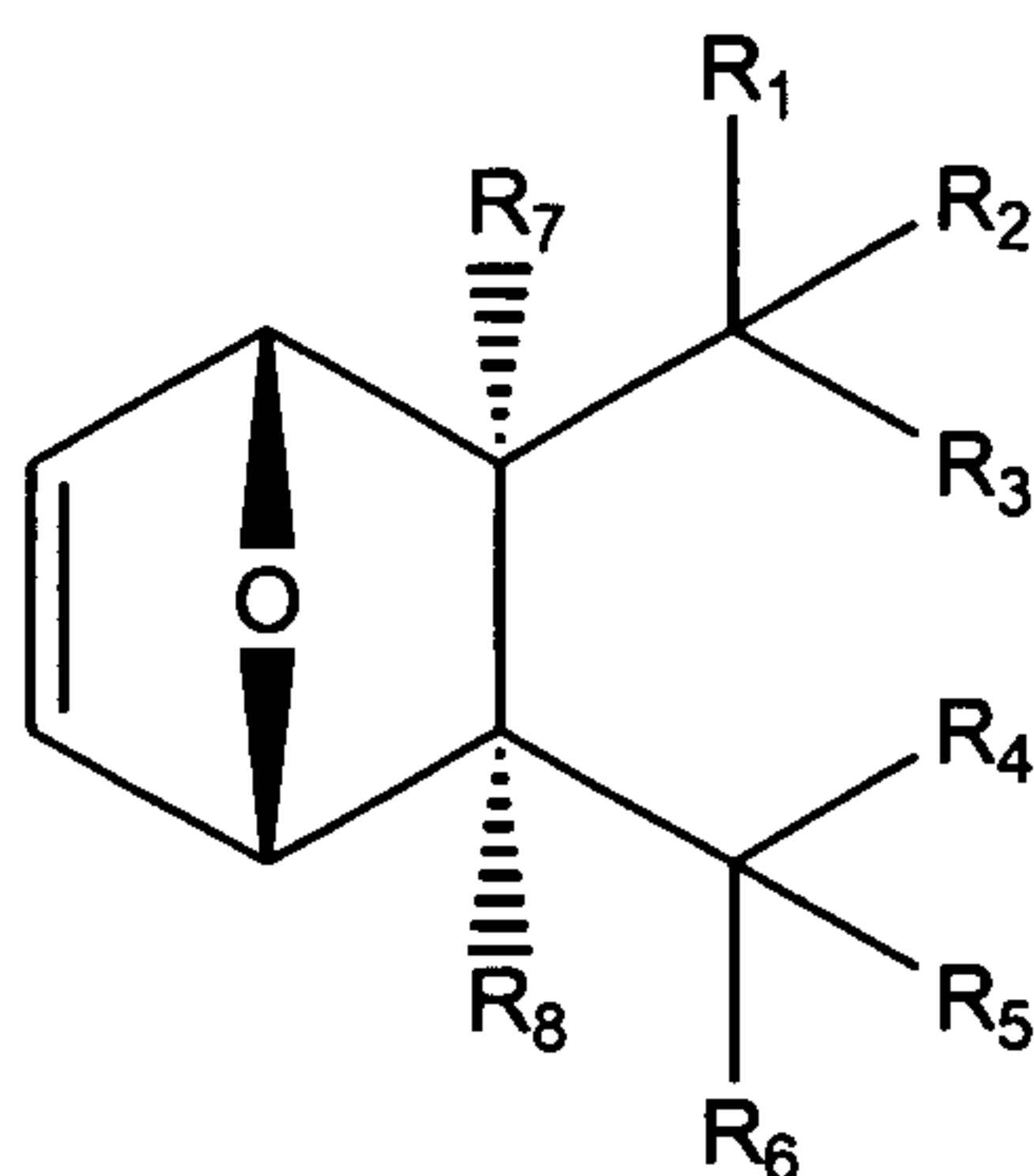


10 with a compound having the structure

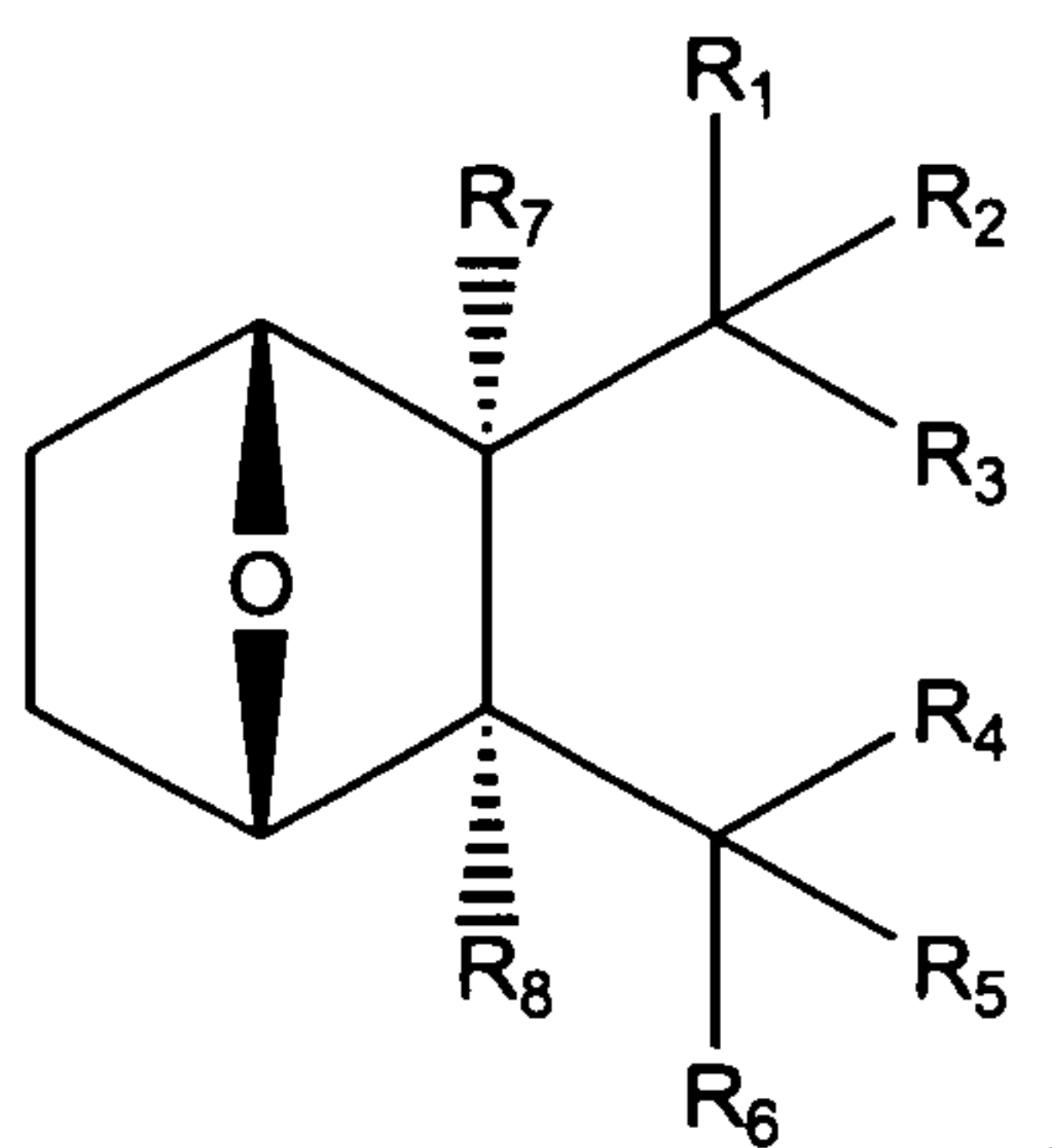


to form a compound having the following structure

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reacting a compound having the above structure with hydrogen in the presence of a catalyst to form



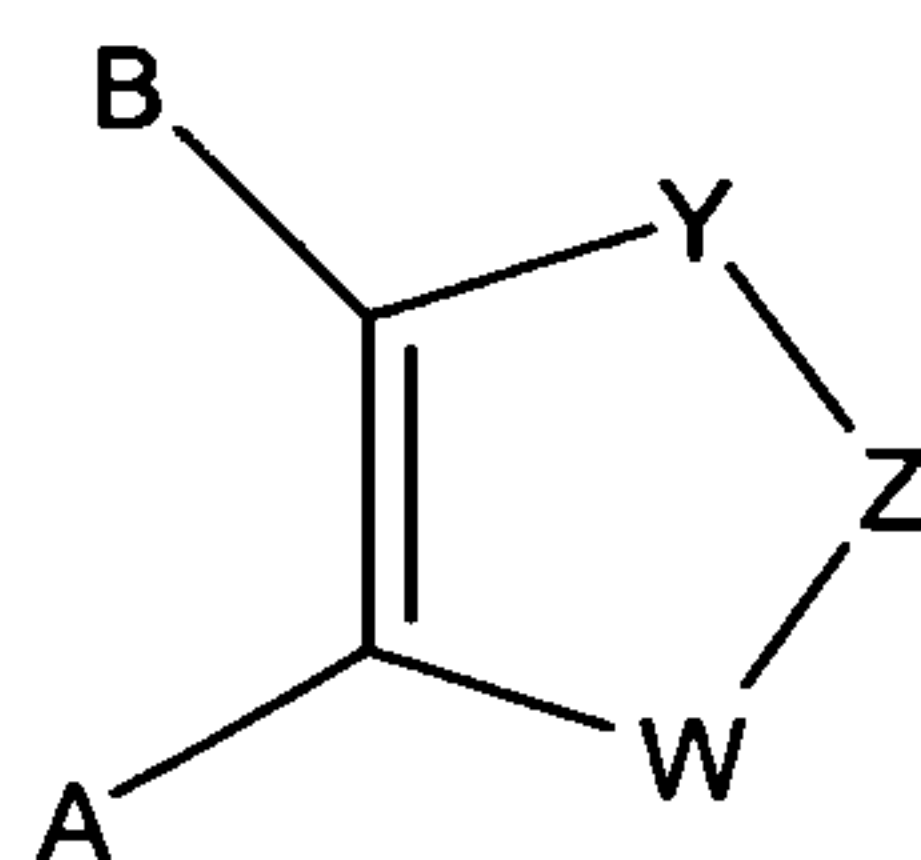
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The invention also provides a process for making a compound comprising reacting a compound having the structure



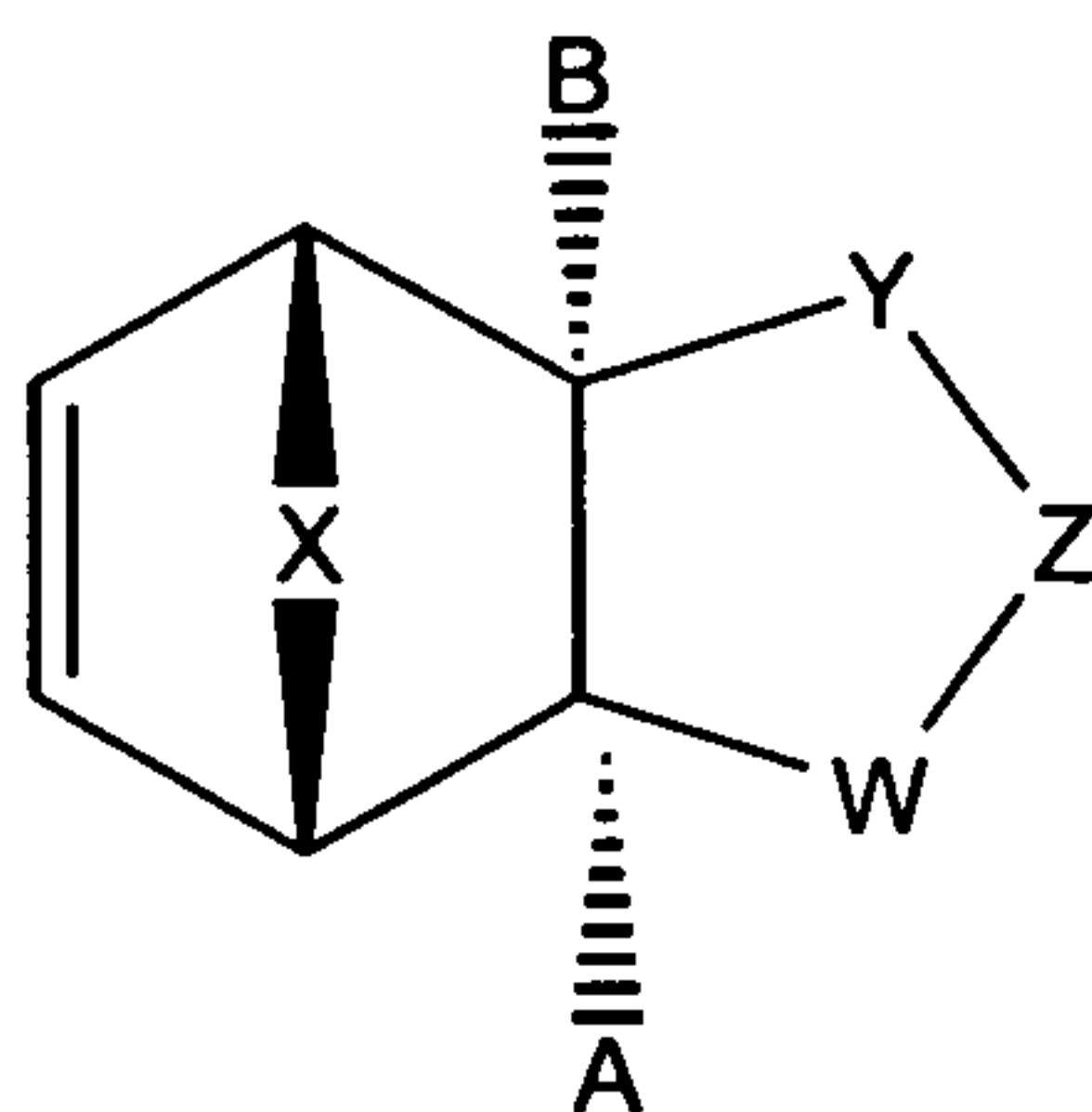
with a compound having the structure

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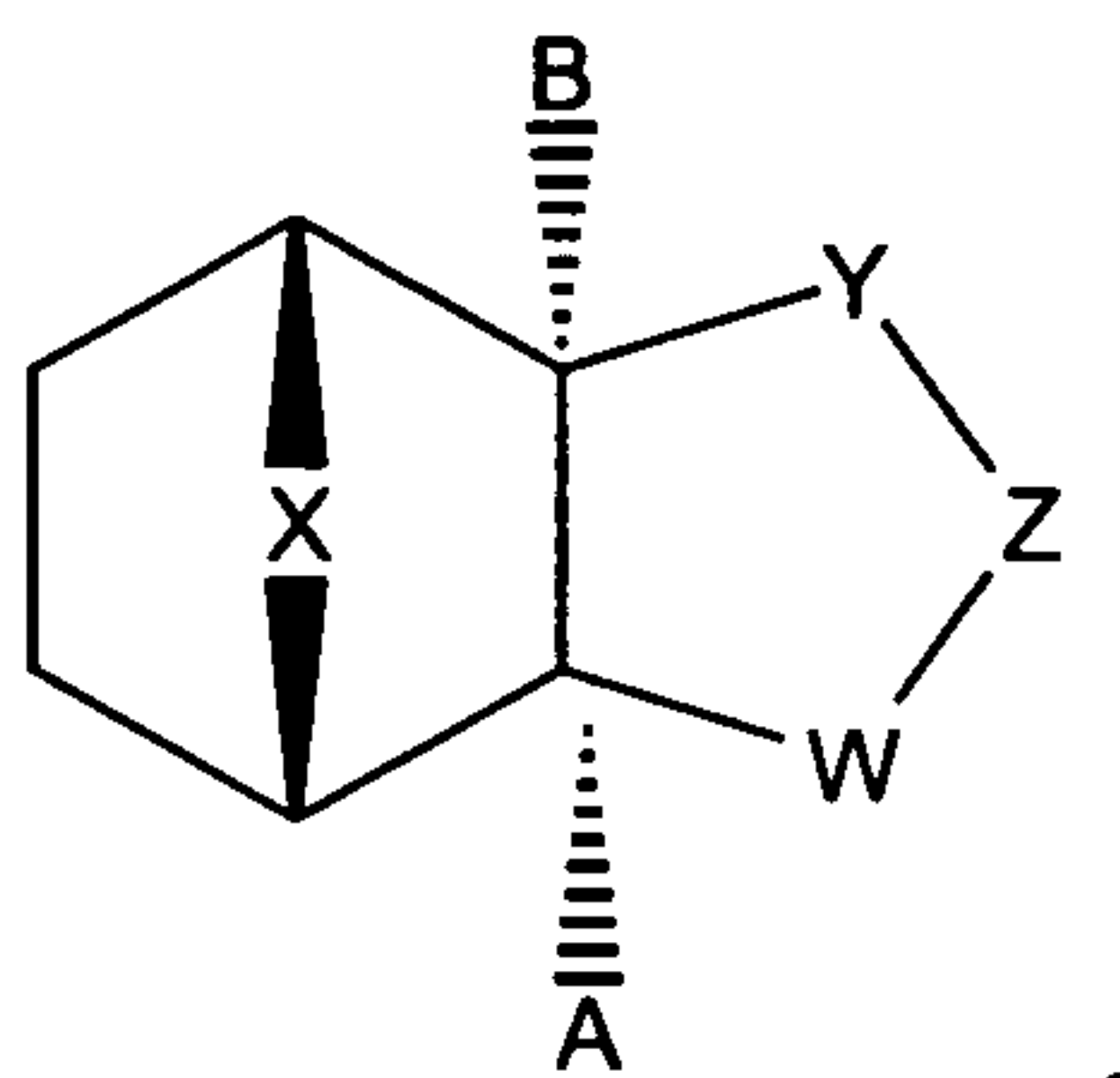


to form a compound having the following structure

- 41 -

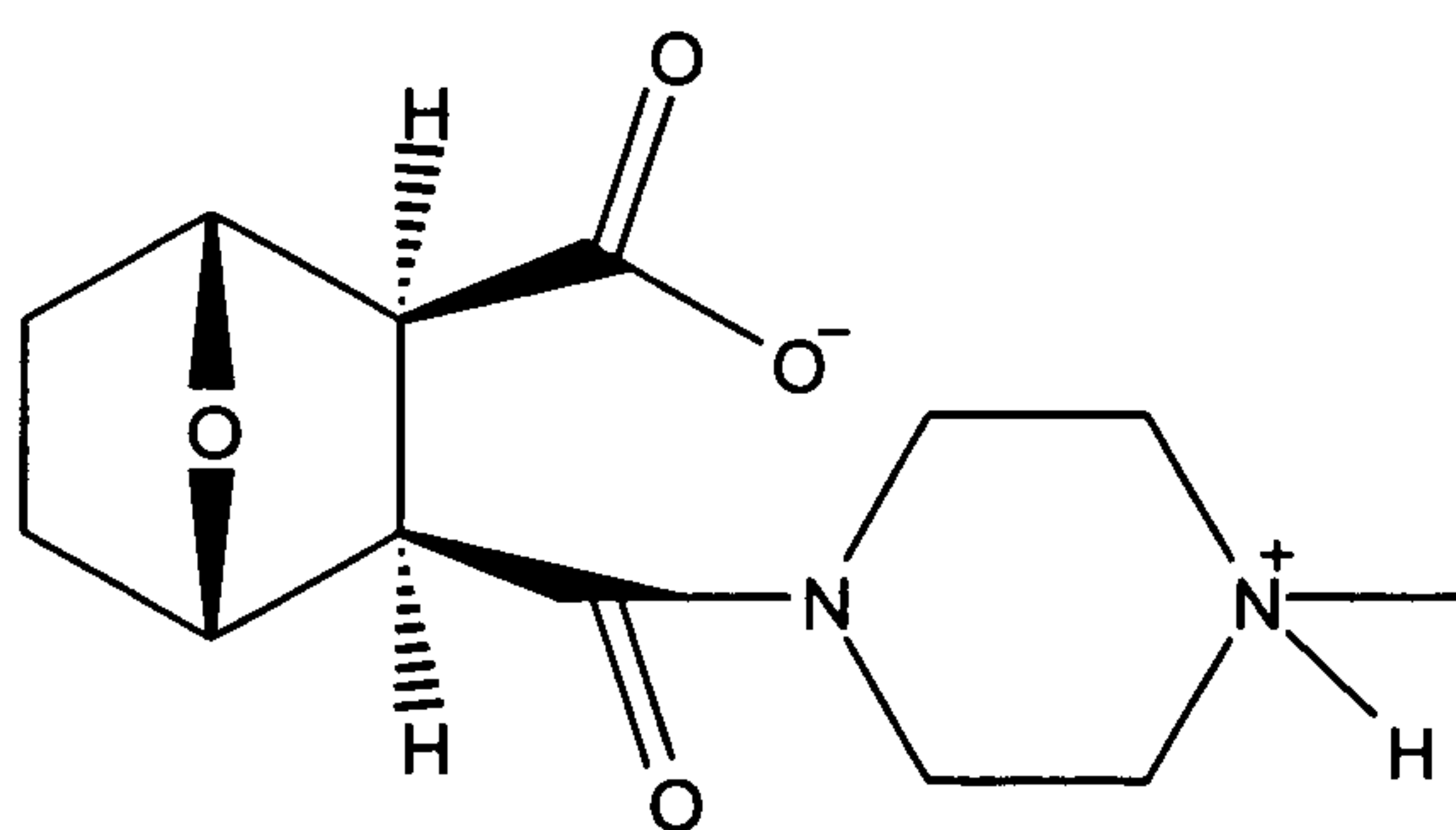


reacting a compound having the above structure with hydrogen in the presence of a catalyst a catalyst to form



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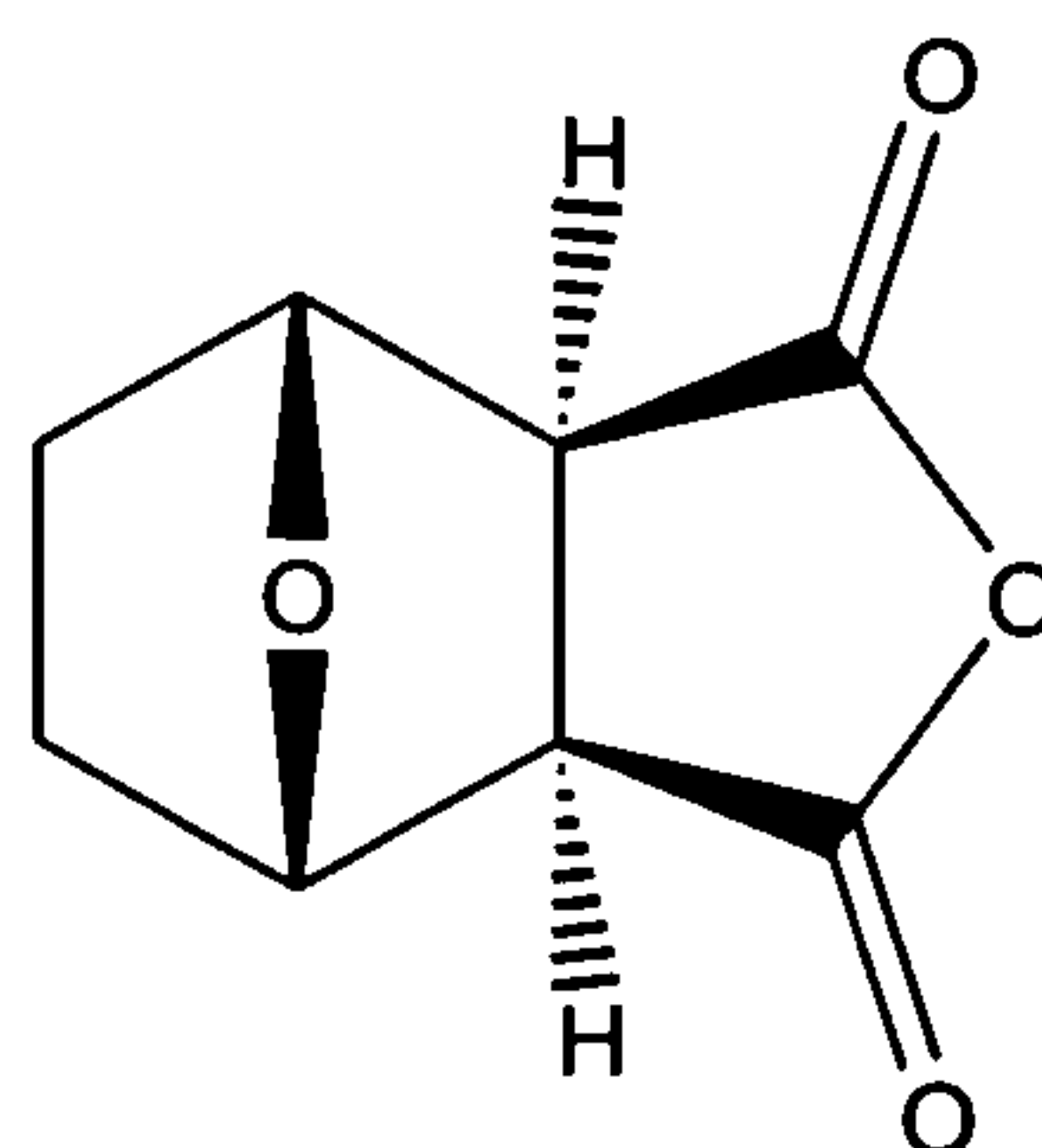
The invention also provides a process for making a compound having the structure



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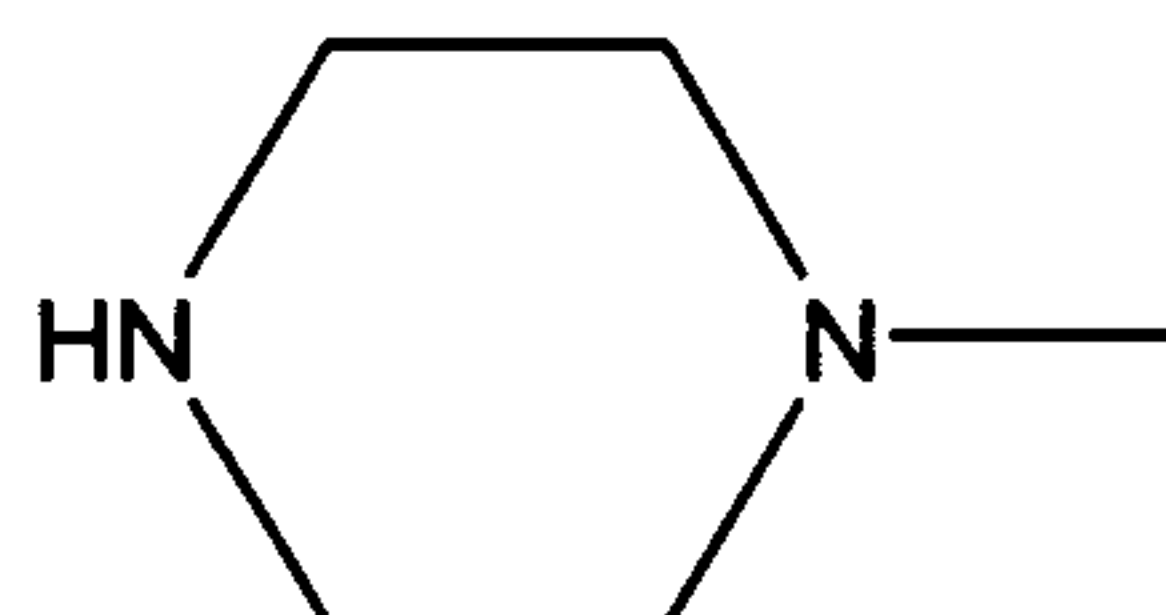
comprising

dissolving a compound having the following structure



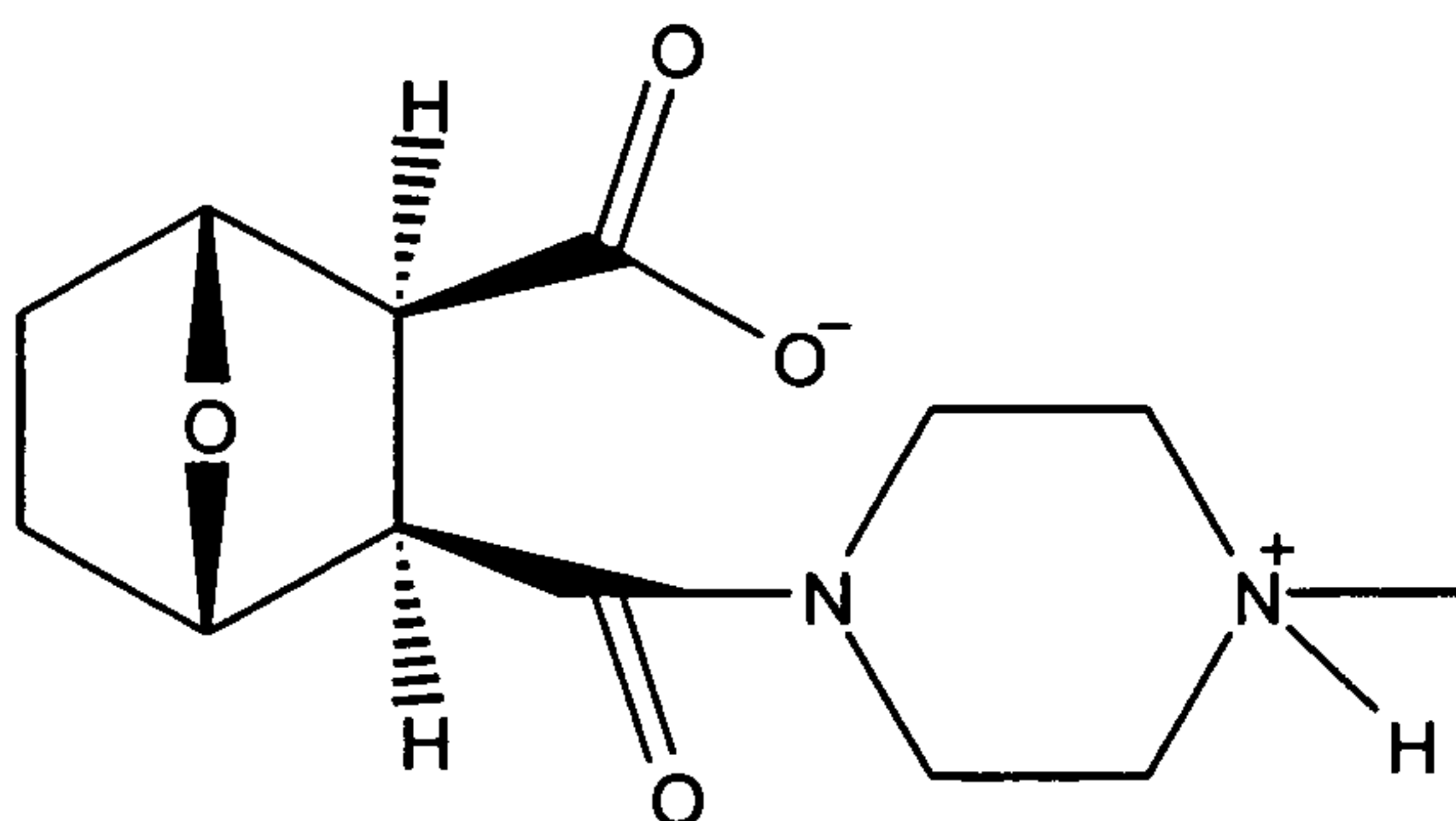
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in benzene and adding a compound having the structure



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at room temperature to produce a compound having the structure



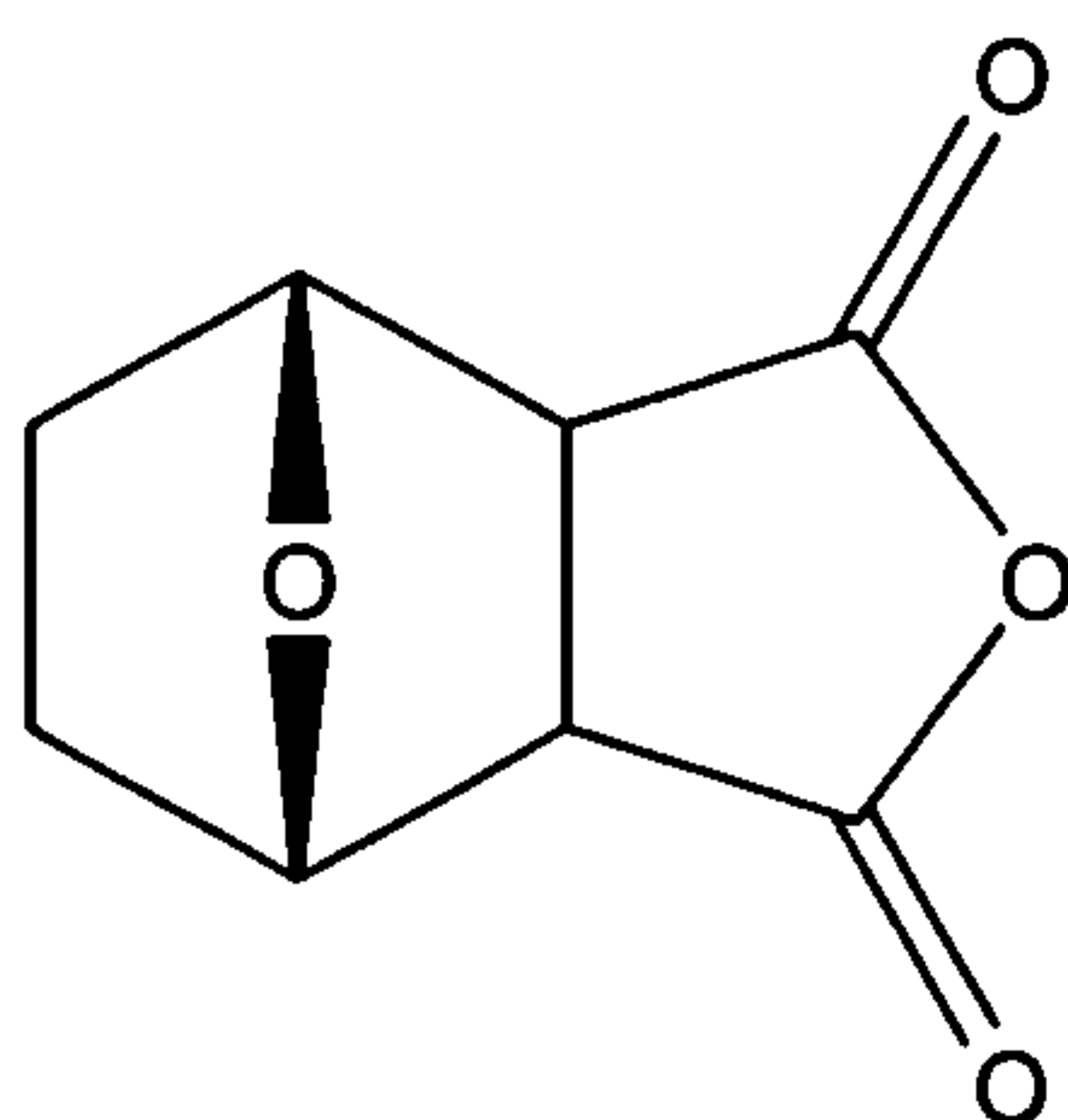
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and recrystallizing the compound produced from a hot solvent.

The invention also provides a process for making a compound  
comprising

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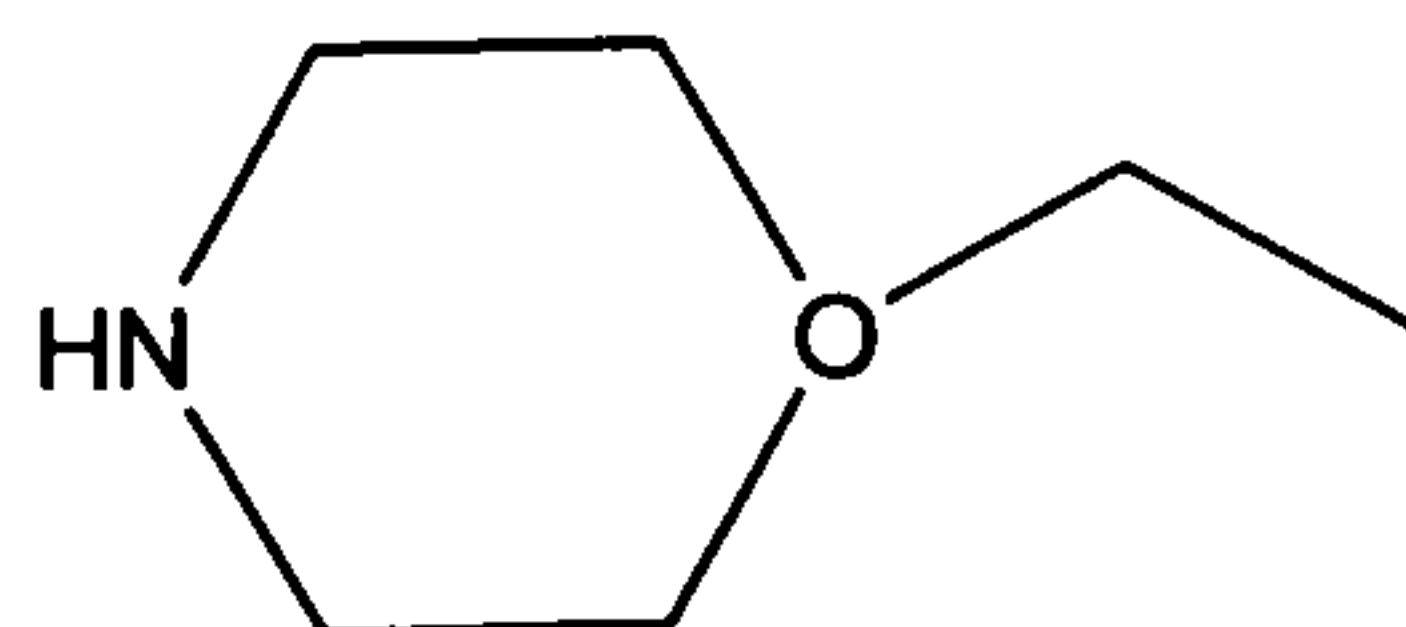
dissolving a compound having the following structure



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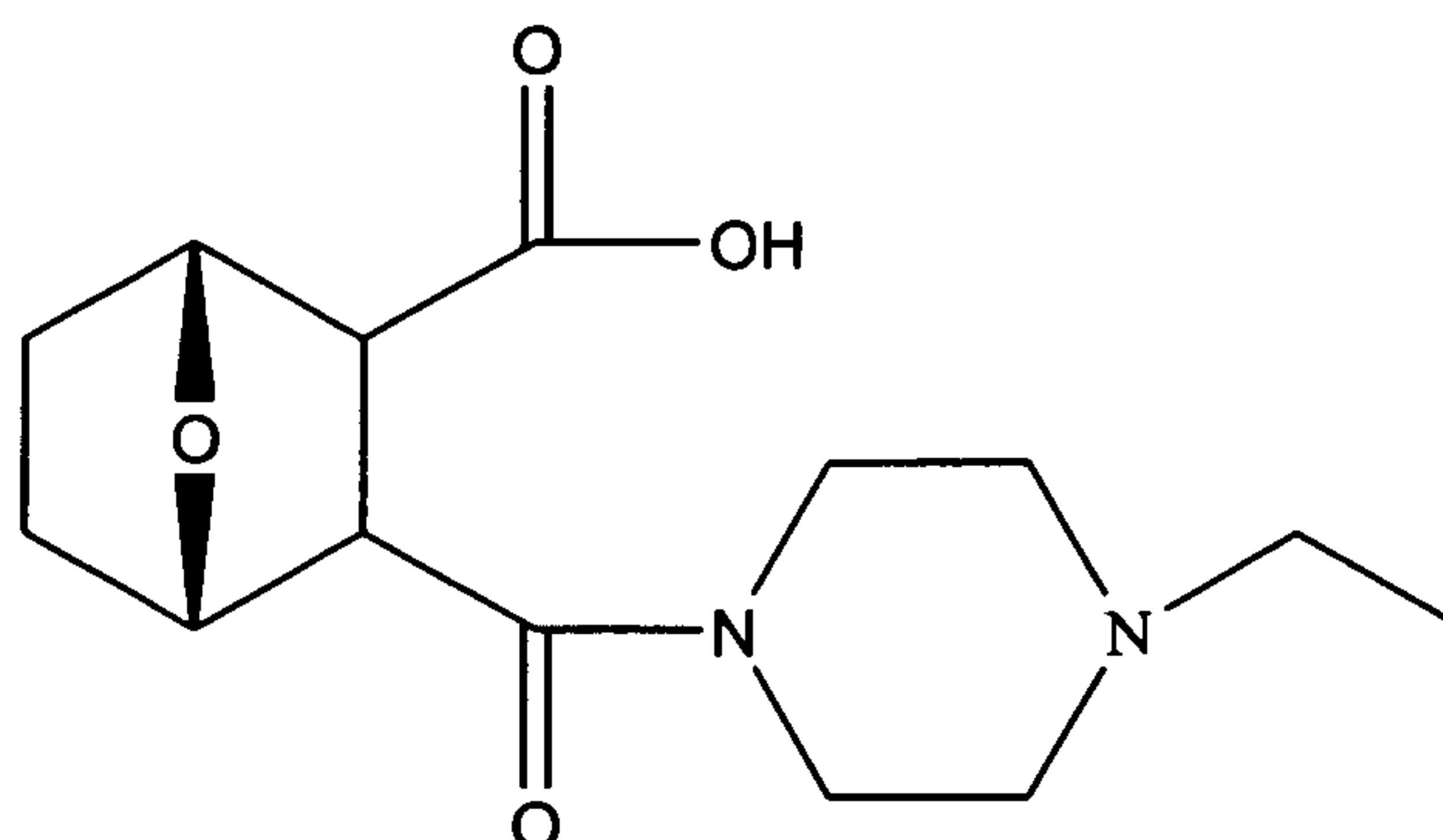
in benzene and adding a compound having the structure

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at room temperature to produce a compound having the structure

5



recrystallizing the compound produced from a hot solvent.

In an embodiment, the solvent is dimethylformamide (DMF) or ethanol.

The invention provides a method of treating a patient suffering from a tumor overexpressing N-CoR comprising administering to the patient one or more of the compounds of this invention, alone or in combination with one or more retinoid receptor ligand, or one or more histone deacetylase ligands, or both, in each case in an amount effective to treat the patient.

In an embodiment, the compound is selected from Compound 100 and Compound 105.

In the method of the invention, the histone deacetylase ligand may be an inhibitor, e.g. the histone deacetylase inhibitor of HDAC-3 (histone deacetylase-3). The histone deacetylase ligand may also be selected from the group consisting of 2-amino-8-oxo-9,10-epoxy-decanoyl, 3-(4-aryl-1H-pyrrol-2-yl)-N-hydroxy-2-

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propenamide, APHA Compound 8, apicidin, arginine butyrate, butyric acid, depsipeptide, depudecin, HDAC-3, m-carboxycinnamic acid bis-hydroxamide, N-(2-aminophenyl)-4-[N-(pyridin-3-ylmethoxycarbonyl) aminomethyl] benzamide, MS 275, oxamfiatin, phenylbutyrate, pyroxamide, scriptaid, sirtinol, sodium butyrate, suberic bishydroxamic acid, suberoylanilide hydroxamic acid, trichostatin A, trapoxin A, trapoxin B and valproic acid.

10 The compounds of this invention may be used in combination with compounds which inhibit the enzyme histone deacetylase (HDAC). These HDAC enzymes post-translationally modify histones (U.S. Patent Publication No. 2004/0197888, Armour *et al.*) Histones are groups of proteins which associate with DNA in eukaryotic cells  
15 to form compacted structures called chromatin. This compaction allows an enormous amount of DNA to be located within the nucleus of a eukaryotic cell, but the compact structure of chromatin restricts the access of transcription factors to the DNA. Acetylation of the histones decreases the compaction of the  
20 chromatin allowing transcription factors to bind to the DNA. Deacetylation, catalysed by histone deacetylases (HDACs), increases the compaction of chromatin, thereby reducing transcription factor accessibility to DNA. Therefore, inhibitors of histone deacetylases prevent the compaction of chromatin,  
25 allowing transcription factors to bind to DNA and increase expression of the genes.

In the methods of the invention, an assessment of the percentage of cells with N-CoR in the cytoplasm relative to the percentage  
30 of cells with N-CoR in the nucleus is representative of the ratio of the number of more-differentiated cells to the number of less-differentiated cells in a given tissue.

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In the method of the invention, tumors that overexpress N-CoR may include glioblastoma multiforme, breast cancer, colorectal cancer, small cell lung cancer or ovarian cancer.

5 This invention also provides a method of inhibiting growth of a tumor overexpressing N-CoR in a patient, comprising administering to the patient one or more of the compounds of this invention, alone or in combination with one or more retinoid receptor ligand, one or more histone deacetylase  
10 ligand, or both, in each case in amounts effective to affect N-CoR so as to thereby induce differentiation of cells of the tumor overexpressing N-CoR and inhibit growth of the tumor in the patient.

15 In an embodiment, the compound is selected from Compound 100 and Compound 102.

In the methods of the invention, an assessment of the percentage of cells with N-CoR in the cytoplasm relative to the percentage  
20 of cells with N-CoR in the nucleus is representative of the ratio of the number of more-differentiated cells to ratio of less-differentiated cells in a given tissue.

In the method of the invention, tumors that overexpress N-CoR  
25 may include glioblastoma multiforme, breast cancer, colorectal cancer, small cell lung cancer or ovarian cancer.

The invention further contemplates a method of controlling undesired vegetation comprising contacting the vegetation or its  
30 environment with a herbicidally effective amount of the compounds of the invention.

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The invention further contemplates a method of inhibiting plant phosphatase activity comprising contacting the plant or its environment with a herbicidally effective amount of the compounds of the invention.

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This invention further contemplates a method of preventing or treating fungal infections in a subject comprising administering to the subject an effective amount of the compounds of this invention.

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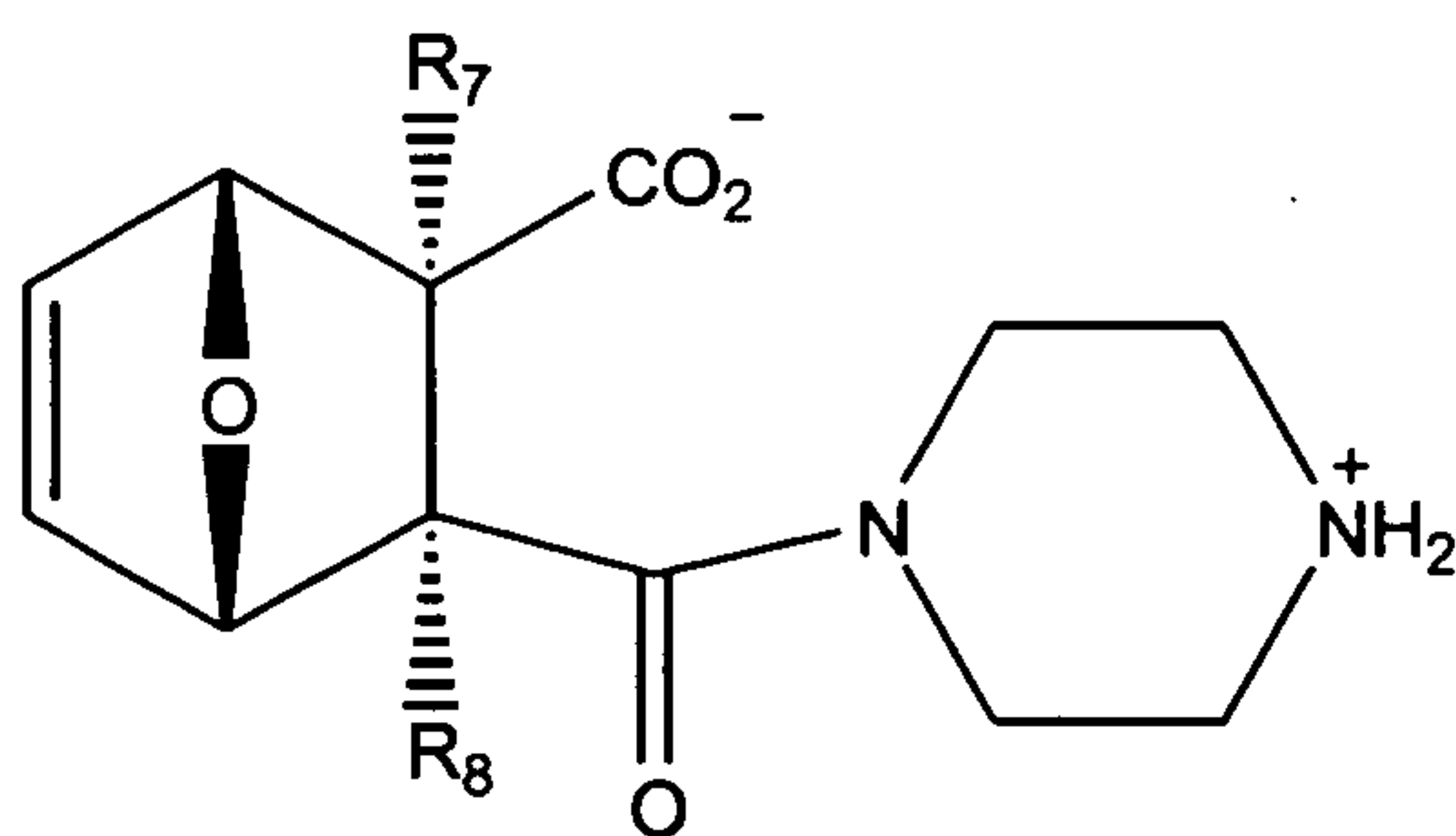
This invention further contemplates a method of treating cancer in a subject comprising administering to the subject an effective amount of the compounds of the invention.

15 This invention further contemplates a method of treating a subject afflicted with breast cancer, colon cancer, large cell lung cancer, adenocarcinoma of the lung, small cell lung cancer, stomach cancer, liver cancer, ovary adenocarcinoma, pancreas carcinoma, prostate carcinoma, promyelocytic leukemia, chronic  
20 myelocytic leukemia, or acute lymphocytic leukemia comprising administering to the subject a therapeutically effective amount of the compounds of the invention, thereby treating the subject.

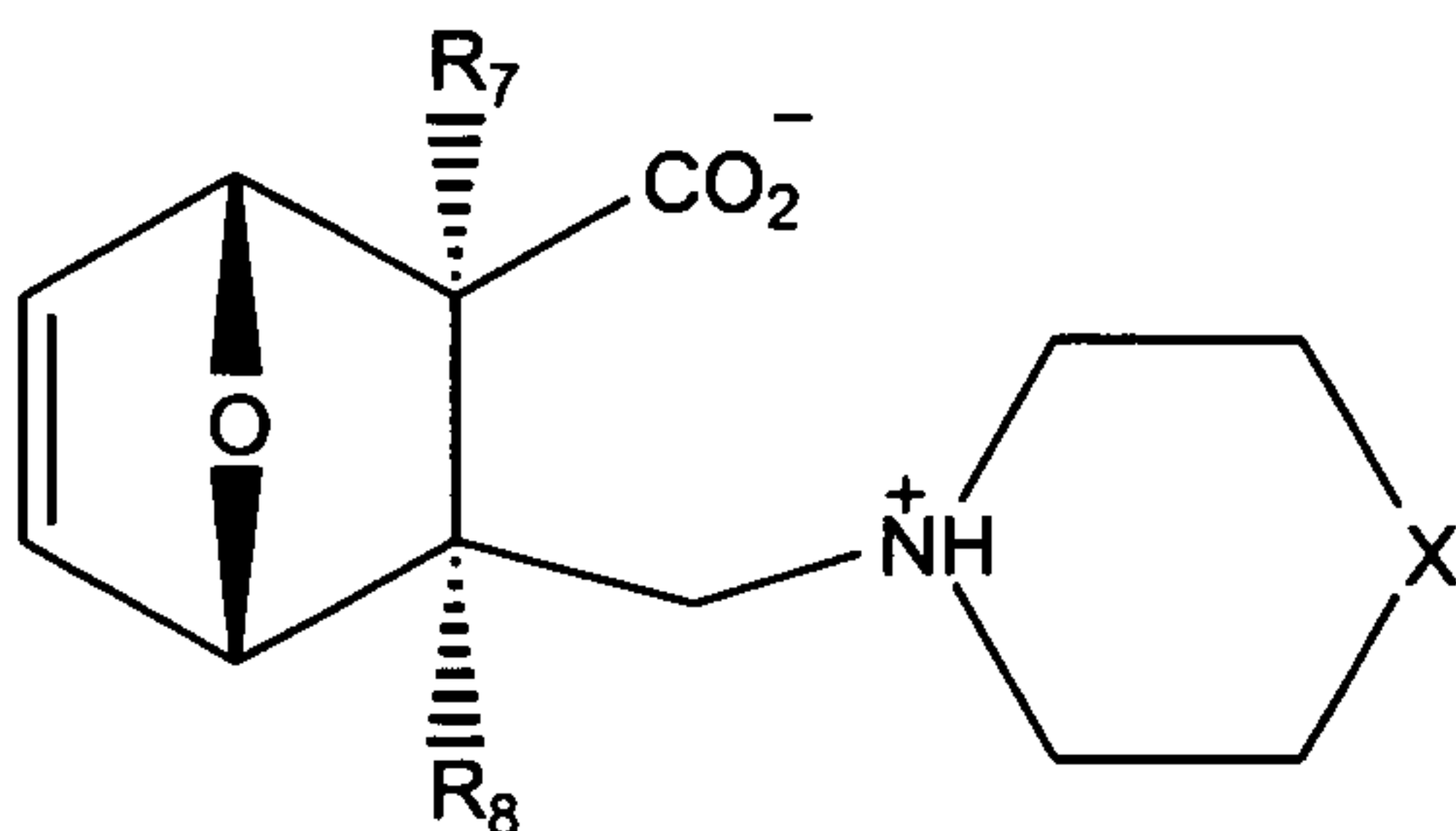
The invention further contemplates the use of prodrugs which are  
25 converted *in vivo* to the compounds of the invention (see, e.g., R.B. Silverman, 1992, "The Organic Chemistry of Drug Design and Drug Action", Academic Press, Chapter 8, the entire contents of which are hereby incorporated by reference). Such prodrugs can be used to alter the biodistribution (e.g., to allow compounds  
30 which would not typically enter a reactive site) or the pharmacokinetics of the compound.

In an embodiment of the invention the compound has the structure

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In an embodiment of the invention the compound has the structure



5

As used herein, "zwitterion" means a compound that is electrically neutral but carries formal positive and negative charges on different atoms. Zwitterions are polar, have high solubility in water and have poor solubility in most organic solvents.

10

Aziridines are organic compounds sharing the aziridine functional group which is a three membered heterocycle with one amine group and two methylene groups. Precursors to aziridinyl intermediates include compounds known to those skilled in the art which readily provide arizidinyl intermediates under suitable conditions.

15

The compounds described in the present invention are in racemic form or as individual enantiomers. The enantiomers can be separated using known techniques, such as those described, for example, in Pure and Applied Chemistry 69, 1469-1474, (1997) IUPAC.

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As used herein, "overexpressing N-CoR" means that the level of the Nuclear receptor co-repressor (N-CoR) expressed in cells of the tissue tested are elevated in comparison to the levels of N-CoR as measured in normal healthy cells of the same type of tissue under analogous conditions. The nuclear receptor co-repressor (N-CoR) of the subject invention may be any molecule that binds to the ligand binding domain of the DNA-bound thyroid hormone receptor (T<sub>3</sub>R) and retinoic acid receptor (RAR). (U.S. Patent No. 6,949,624, Liu et al.) Examples of tumors that overexpress N-CoR may include glioblastoma multiforme, breast cancer (Myers et al.), colorectal cancer (Giannini and Cavallini), small cell lung carcinoma (Waters et al.) or ovarian cancer (Havrilesky et al.).

"Solvent" as used herein is intended to include compounds such as, hexanes, benzene, toluene, diethyl ether, chloroform, methylene chloride, ethyl acetate, 1,4-dioxane, water, THF, acetone, acetonitrile, DMF, DMSO, acetic acid, n-butanol, isopropanol, n-propanol, ethanol, methanol, formic acid, carbon tetrachloride, benzenethiol, chlorobenzene, cyclohexanethiol, 1-diethylaminoethanol, ethylene dichloride, ethylene glycol, xylene, 1,1,2,2-tetrachloroethane, phenol, acetic acid, 1-butanol, 2-butanol, 2-butanone, diglyme, dimethylether, dioxane, petroleum ether, (NMP) N-methyl-2-pyrrolidinone, heptane, glycerin, HMPA (Hexamethylphosphorus triamide), MTBE (methyl t-butyl ether), nitromethane, pyridine, 1-propanol, 2-propanol, and triethylamine.

Certain embodiments of the disclosed compounds can contain a basic functional group, such as amino or alkylamino, and are thus capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable acids, or contain an acidic

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functional group and are thus capable of forming pharmaceutically acceptable salts with bases. The instant compounds therefore may be in a salt form. As used herein, a "salt" is a salt of the instant compounds which has been modified by making acid or base salts of the compounds. The salt may be pharmaceutically acceptable. Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as phenols. The salts can be made using an organic or inorganic acid. Such acid salts are chlorides, bromides, sulfates, nitrates, phosphates, sulfonates, formates, tartrates, maleates, malates, citrates, benzoates, salicylates, ascorbates, and the like. Phenolate salts are the alkaline earth metal salts, sodium, potassium or lithium. The term "pharmaceutically acceptable salt" in this respect, refers to the relatively non-toxic, inorganic and organic acid or base addition salts of compounds of the present invention. These salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or by separately reacting a purified compound of the invention in its free base or free acid form with a suitable organic or inorganic acid or base, and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like. For a description of possible salts, see, e.g., Berge et al. (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19.

As used herein, "therapeutically effective amount" means an amount sufficient to treat a subject afflicted with a disease

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(e.g. tumors overexpressing N-CoR) or to alleviate a symptom or a complication associated with the disease.

As used herein, "herbicidally effective" means an amount  
5 sufficient to adversely affect plant growth, particularly through inhibition of plant phosphatase 2 A activity.

As used herein, "treating" means slowing, stopping or reversing the progression of a disease, particularly tumors overexpressing  
10 N-CoR.

As used herein, "alkyl" is intended to include both branched and straight-chain saturated aliphatic hydrocarbon groups having the specified number of carbon atoms. Thus,  $C_1-C_n$  as in " $C_1-C_n$  alkyl" is defined to include groups having 1, 2, ...,  $n-1$  or  $n$  carbons  
15 in a linear or branched arrangement, and specifically includes methyl, ethyl, propyl, butyl, pentyl, hexyl, and so on. An embodiment can be  $C_1-C_{12}$  alkyl. "Alkoxy" represents an alkyl group as described above attached through an oxygen bridge.

20 The term "alkenyl" refers to a non-aromatic hydrocarbon radical, straight or branched, containing at least 1 carbon to carbon double bond, and up to the maximum possible number of non-aromatic carbon-carbon double bonds may be present. Thus,  $C_2-C_n$  alkenyl is defined to include groups having 1, 2, ...,  $n-1$  or  $n$   
25 carbons. For example, " $C_2-C_6$  alkenyl" means an alkenyl radical having 2, 3, 4, 5, or 6 carbon atoms, and at least 1 carbon-carbon double bond, and up to, for example, 3 carbon-carbon double bonds in the case of a  $C_6$  alkenyl, respectively. Alkenyl groups include ethenyl, propenyl, butenyl and cyclohexenyl. As  
30 described above with respect to alkyl, the straight, branched or cyclic portion of the alkenyl group may contain double bonds and

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may be substituted if a substituted alkenyl group is indicated.  
An embodiment can be C<sub>2</sub>-C<sub>12</sub> alkenyl.

The term "alkynyl" refers to a hydrocarbon radical straight or  
5 branched, containing at least 1 carbon to carbon triple bond,  
and up to the maximum possible number of non-aromatic carbon-  
carbon triple bonds may be present. Thus, C<sub>2</sub>-C<sub>n</sub> alkynyl is  
defined to include groups having 1, 2, ....., n-1 or n carbons.  
For example, "C<sub>2</sub>-C<sub>6</sub> alkynyl" means an alkynyl radical having 2 or  
10 3 carbon atoms, and 1 carbon-carbon triple bond, or having 4 or  
5 carbon atoms, and up to 2 carbon-carbon triple bonds, or  
having 6 carbon atoms, and up to 3 carbon-carbon triple bonds.  
Alkynyl groups include ethynyl, propynyl and butynyl. As  
described above with respect to alkyl, the straight or branched  
15 portion of the alkynyl group may contain triple bonds and may be  
substituted if a substituted alkynyl group is indicated. An  
embodiment can be a C<sub>2</sub>-C<sub>n</sub> alkynyl.

As used herein, "aryl" is intended to mean any stable monocyclic  
20 or bicyclic carbon ring of up to 10 atoms in each ring, wherein  
at least one ring is aromatic. Examples of such aryl elements  
include phenyl, naphthyl, tetrahydro-naphthyl, indanyl,  
biphenyl, phenanthryl, anthryl or acenaphthyl. In cases where  
the aryl substituent is bicyclic and one ring is non-aromatic,  
25 it is understood that attachment is via the aromatic ring. The  
substituted aryls included in this invention include  
substitution at any suitable position with amines, substituted  
amines, alkylamines, hydroxys and alkylhydroxys, wherein the  
"alkyl" portion of the alkylamines and alkylhydroxys is a C<sub>2</sub>-C<sub>n</sub>  
30 alkyl as defined hereinabove. The substituted amines may be  
substituted with alkyl, alkenyl, alkynyl, or aryl groups as  
hereinabove defined.

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The alkyl, alkenyl, alkynyl, and aryl substituents may be unsubstituted or unsubstituted, unless specifically defined otherwise. For example, a (C<sub>1</sub>-C<sub>6</sub>) alkyl may be substituted with one or more substituents selected from OH, oxo, halogen, alkoxy, dialkylamino, or heterocyclyl, such as morpholinyl, piperidinyl, and so on.

In the compounds of the present invention, alkyl, alkenyl, and alkynyl groups can be further substituted by replacing one or more hydrogen atoms by non-hydrogen groups described herein to the extent possible. These include, but are not limited to, halo, hydroxy, mercapto, amino, carboxy, cyano and carbamoyl.

The term "substituted" as used herein means that a given structure has a substituent which can be an alkyl, alkenyl, or aryl group as defined above. The term shall be deemed to include multiple degrees of substitution by a named substituent. Where multiple substituent moieties are disclosed or claimed, the substituted compound can be independently substituted by one or more of the disclosed or claimed substituent moieties, singly or plurally. By independently substituted, it is meant that the (two or more) substituents can be the same or different.

As used herein, "administering" an agent may be performed using any of the various methods or delivery systems well known to those skilled in the art. The administering can be performed, for example, orally, parenterally, intraperitoneally, intravenously, intraarterially, transdermally, sublingually, intramuscularly, rectally, transbuccally, intranasally, liposomally, via inhalation, vaginally, intraocularly, via local delivery, subcutaneously, intraadiposally, intraarticularly, intrathecally, into a cerebral ventricle, intraventricularly,

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intratumorally, into cerebral parenchyma or intraparenchymally.

5 The following delivery systems, which employ a number of routinely used pharmaceutical carriers, may be used but are only representative of the many possible systems envisioned for administering compositions in accordance with the invention.

10 Injectable drug delivery systems include solutions, suspensions, gels, microspheres and polymeric injectables, and can comprise excipients such as solubility-altering agents (e.g., ethanol, propylene glycol and sucrose) and polymers (e.g., polycaprylactones and PLGA's).

15 Implantable systems include rods and discs, and can contain excipients such as PLGA and polycaprylactone.

Oral delivery systems include tablets and capsules. These can contain excipients such as binders (e.g.,  
20 hydroxypropylmethylcellulose, polyvinyl pyrrolidone, other cellulosic materials and starch), diluents (e.g., lactose and other sugars, starch, dicalcium phosphate and cellulosic materials), disintegrating agents (e.g., starch polymers and cellulosic materials) and lubricating agents (e.g., stearates  
25 and talc).

Transmucosal delivery systems include patches, tablets, suppositories, pessaries, gels and creams, and can contain excipients such as solubilizers and enhancers (e.g., propylene  
30 glycol, bile salts and amino acids), and other vehicles (e.g., polyethylene glycol, fatty acid esters and derivatives, and hydrophilic polymers such as hydroxypropylmethylcellulose and hyaluronic acid).

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Dermal delivery systems include, for example, aqueous and nonaqueous gels, creams, multiple emulsions, microemulsions, liposomes, ointments, aqueous and nonaqueous solutions, lotions, aerosols, hydrocarbon bases and powders, and can contain excipients such as solubilizers, permeation enhancers (e.g., fatty acids, fatty acid esters, fatty alcohols and amino acids), and hydrophilic polymers (e.g., polycarbophil and polyvinylpyrrolidone). In one embodiment, the pharmaceutically acceptable carrier is a liposome or a transdermal enhancer.

Solutions, suspensions and powders for reconstitutable delivery systems include vehicles such as suspending agents (e.g., gums, xanthans, cellulose and sugars), humectants (e.g., sorbitol), solubilizers (e.g., ethanol, water, PEG and propylene glycol), surfactants (e.g., sodium lauryl sulfate, Spans, Tweens, and cetyl pyridine), preservatives and antioxidants (e.g., parabens, vitamins E and C, and ascorbic acid), anti-caking agents, coating agents, and chelating agents (e.g., EDTA).

It is understood that substituents and substitution patterns on the compounds of the instant invention can be selected by one of ordinary skill in the art to provide compounds that are chemically stable and that can be readily synthesized by techniques known in the art, as well as those methods set forth below, from readily available starting materials. If a substituent is itself substituted with more than one group, it is understood that these multiple groups may be on the same carbon or on different carbons, so long as a stable structure results.

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Discussion

We found that one protein found to have increased expression in glioblastoma multiforme (GBMs) as compared to normal brain is nuclear receptor co-repressor (N-CoR), a regulator of the normal neural stem cell pool. Expression of N-CoR in GBM was confirmed by immunohistochemistry and Western blotting.

N-CoR is expressed in the nucleus of neural stem cells (NSCs). (Hermanson et al. (2002)) Following phosphatidyl-inositol-3-OH kinase/Akt1 kinase-dependent phosphorylation, N-CoR translocates to the cytoplasm and leads to astrocytic differentiation of NSCs. The nuclear retention of N-CoR, therefore, is essential for the maintenance of NSCs in the undifferentiated state (Hermanson et al.). Analogous to CD133+ NSC found within the developing brain, brain tumor stem cells (BTSC) bearing CD133 have been identified within GBM. (Uchida et al. (2000); and Singh et al. (2003)) BTSC are capable of proliferation, self-renewal, and differentiation. BTSC, but not CD133- differentiated tumor cells, are able to recapitulate tumors upon xenograft transplantation. (Singh et al. (2004)).

Retinoids, metabolites of vitamin A, have been examined therapeutically in a variety of tumors, including gliomas. (Yung et al. (1996)) N-CoR is closely associated with the retinoid receptor and is released upon ligand binding to the receptor. (Bastien et al. (2004)) We hypothesized that one effect of retinoids on malignant gliomas may be the induction of differentiation by the binding of retinoids to the retinoid receptor followed by dissociation of the N-CoR/retinoid receptor complex and translocation of N-CoR to the cytoplasm. This idea would explain the previous observation of increase GFAP expression in a glioma cell line (U343 MG-A) treated with

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retinoids. (Rudka *et al.* (1988)) To test this we targeted two different sites, individually or simultaneously, within the N-CoR pathway by treating the GBM cell line U343 MG-A with 50  $\mu$ M of retinoic acid (RA) and/or 10 nM of okadaic acid (OA), a protein phosphatase-1 and protein phosphatase-2A inhibitor. By preventing the action of protein phosphatase-1 and protein phosphatase-2A, okadaic acid increases the phosphorylated form of N-CoR and promotes its subsequent cytoplasmic translocation. (Hermanson *et al.* (2002))

10

Cell line U343 MG-A is available from the University of California at San Francisco (UCSF) Brain Tumor Research Center Tissue Bank. (University of California, San Francisco, Health Sciences West building, San Francisco, California 94143-0520.)

15 In addition, cell lines U343 and U87 are commercially available from EPO-GmbH, Robert-Rössle-Str.10, 13092 Berlin-Buch, Germany.

Several molecules that have anti-PP2A activity synergize with retinoic acids in inhibiting the growth of cells of tumors that overexpress N-CoR in vitro. The most effective group of phosphatase inhibitors synergizing with retinoic acids that have been evaluated are analogues of the ancient therapeutic agent, mylabris, derived from the crushed bodies of the blister beetle, in which the principal active agent is cantharidin, a known potent inhibitor of PP2A (Wang, 1989; Peng *et al.*, 2002).

Cantharidin has anti-tumor activity against human cancers of the liver (hepatomas) and of the upper gastrointestinal tract but is toxic to the urinary tract (Wang, 1989). Norcantharidin, a demethylated cantharidin, maintains antitumor activity of cantharidin against hepatomas and cancers of the stomach and esophagus, but has little or no urinary tract toxicity. Norcantharidin also stimulates white blood cell production in

30

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patients and mice, a phenomenon not understood mechanistically, but a pharmacological effect of potential benefit as an anticancer agent (Wang *et al.*, 1986; Wang, 1989).

The publication of a report that cantharidin acts as a protein phosphatase inhibitor prompted a more general interest in compounds with this type of chemical structure (Li and Casida, 1992). Previously, it had been found that the simpler congener and its hydrolysis product (commercially available as the herbicide, Endothall) are hepatotoxic (Graziano and Casida, 1997). The primary targets in liver appear to be the protein phosphatases PP2A and PP1, all of the compounds showing ED<sub>50</sub> values at the micromolar level. Binding studies have shown that the action of certain cantharidin homologs is direct on PP2A and indirect on PP1 (Honkanen *et al.*, 1993; Li *et al.*, 1993). Phosphatase PP1B is affected only at millimolar levels of these compounds, whereas the enzyme PP2C is not influenced at all.

In the past, several cantharidin analogues had been synthesized and evaluated for anti-phosphatase activity and for their ability to inhibit the growth of cancer cells in culture (Sakoff and McCluskey, 2004; Hart *et al.*, 2004). Some of the previously evaluated modified norcantharidin molecules inhibited the growth of several human tumor cell lines. The activity of norcantharidin analogues against cells of tumors overexpressing N-CoR or the activity of norcantharidins combined with other potential anti-tumor agents was not analyzed. Further studies included 16 "modified norcantharidins" evaluated for activity against four human tumor cell lines including ovarian, kidney, colorectal and lung as well as a mouse leukemia line. None were as active as single agents as cantharidin or norcantharidin and none were evaluated for activity in combination with another antitumor agent (McCluskey *et al.*, US Patent Application Publication No. 2006/0030616, 2006).

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A different series of cantharidin analogues had been previously synthesized and evaluated as pesticides and for antitumor activity against cancer cell lines. Forty-three analogues of endothal and cantharidin have been developed and assessed for their activity as herbicides and their lethality to mice (Matsuzawa *et al.*, 1987). Endothal thioanhydride was shown to be a more potent herbicide than endothal but was toxic to the liver of mice (Matsuzawa *et al.*, 1987; Kawamura *et al.*, 1990).

More recently, it has been found that endothal thioanhydride is an active agent against PP2A and PP1 *in vivo* (Erdodi *et al.*, 1995). Endothal and endothal thioanhydride, like cantharidin, inhibit the activity of PP2A and to some extent, the activity of PP1 (Erdodi *et al.*, 1995). In the liver, the principal target appears to be PP1. In fibroblasts, only endothal thioanhydride caused marked morphological changes whereas cantharidin and endothal did not (Erdodi *et al.*, 1995). The enhanced activity of endothal thioanhydride *in vivo* is thought to be related to its enhanced lipophilicity resulting in increased diffusion across the plasmalemma (Essers *et al.*, 2001). A more recent publication has described the synthesis of the mono-, and the di-fluoro analogues of Endothal and also the corresponding anhydrides, however no pharmacological data accompanied this synthetic work (Essers *et al.*, 2001).

Of the known congeners of this type of compound, only the parent, cantharidin and its bis(normethyl)-derivative, norcantharidin, have seen any use as anti-cancer drug substances and only norcantharidin is used as an anti-neoplastic agent (Tsauer *et al.*, 1997).

In pursuing the development of new drug substances in this area, we have found it essential to develop inhibitors that have

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greater specificity, especially towards those enzymes which display high activity against the replication processes of cancer cells. High specificity also holds out the possibility of avoiding targets important to normal cell function. From the point of view of the physical characteristics of any newly-developed drug substance, it must preeminently have good membrane permeability (*i.e.*, has a log P value of between 2 and 4 units).

10 The compounds described herein have an antagonistic effect on phosphatase-2A and phosphatase 1. We have confirmed that at least Compounds 100, 105, and 102 are effective in inhibiting the growth of cells of tumors overexpressing N-CoR. Compounds 100 and 105 are advantageous over other cantharidin homologs as  
15 they exist as zwitterions, which renders them water soluble and stable at acid pH, features desirable for orally effective drugs. Compound 102 is lipid soluble, which affords a greater ability than Compounds 100 and 105 to cross the blood-brain barrier. This is especially important when treating such tumors  
20 as glioblastoma multiforme.

Compound 100, norcantharidin (nor-Can), endothal (End), and endothal thioanhydride (ET) were evaluated for their ability to inhibit the growth of GBMs in a dose dependent manner *in vivo* as  
25 shown in Figure 1.

From graphic plots of the glioblastoma multiforme GBM cell line U373 (available from the National Institute of Neurological Disorders and Stroke Molecular Pathogenesis Unit, Building 10,  
30 Room 5 D37, National Institutes of Health, 900 Rockville Pike, Bethesda, Maryland, 20892) as a function of exposure to different doses of drug for 7 days, the concentration of each compound that inhibited brain tumor cell proliferation by 50%

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(IC50) was estimated. The IC50s expressed in micro-molarity ( $\mu\text{M}$ ), were: 2.5, 3.0, 12.0, and 15.0 for endothal thioanhydride, Compound 100, norcantharidin, and endothal respectively as seen in Figure 1. As shown, on a molar basis, Compound 100 was a  
5 potent inhibitor of GBMs *in vitro*.

In combination with anti-phosphatases, retinoids synergistically inhibit the proliferation of glioblastoma multiforme. Synergism (potentiation) of the inhibitory activity of two drugs in  
10 combination is said to be present when the percent survival in the presence of two drugs is greater than the sum of the percent survivals of the two drugs used alone at the same doses in the combination.

15 The inhibitory activity of Compound 100 was further evaluated in combination with retinoids as well as individually. As shown in Figure 2, the combination of Compound 100 with all-trans retinoic acid (ATRA) demonstrated a synergistic reduction in cell growth.

20 The expected percent survival of U373 cells exposed to the combination of ATRA and End is 50% ( $77\% \text{ by ATRA} \times 65\% \text{ by End} = 50\%$ ) whereas the observed survival was 32%. The expected percent survival in the presence of the combination of ATRA and Compound  
25 100 is 60% ( $77\% \text{ by ATRA} \times 78\% \text{ by Compound 100} = 60\%$ ), whereas the observed survival was 53%.

To determine whether there is tumor type specificity of the inhibitory properties of PP2A inhibitors, retinoic acid and  
30 Trichostatin A we measured their inhibitory effects as single agents against the GBM line U373, a breast cancer line, MCF-7 (obtained from ATCC) and a kidney cancer cell line, UMRC (UMRC obtained by Dr. Zhuang, NINDS, NIH from the Intramural Research

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Support Program, SAIC, National Cancer Institute, Frederick Cancer Research and Development Center).

5 The kidney cancer cell line, UMRC (Figure 3) was less sensitive than the brain tumor line, U373 (Figure 4) whereas the breast cancer line, MCF-7 (Figure 5) was as sensitive as U373 to all-trans retinoic acid, endothal thioanhydride, norcantharidin, endothal, and Trichostatin A. There is some cell type  
10 specificity of these drugs for GBMs. The activity of the drugs against MCF-7 cells indicates that regimens being developed for brain tumor treatment are likely also useful against breast cancer and other tumors that overexpress N-CoR.

We have shown that Compound 100 will have similar inhibitory  
15 effects in the treatment of breast cancer and other tumors that overexpress N-CoR due to the structural similarities of Compound 100 to endothal as well as their similar effects in the treatment of glioblastoma multiforme.

20 Endothal is also known as an active defoliant and potent contact herbicide used in many agricultural situations. It is considered effective as a pre-harvest desiccant and as a selective pre-emergence herbicide (Crafts, 1953).

25 Endothal, norcantharidins and cantharidin are all well known inhibitors of mammalian protein phosphatase as well as potent herbicides (Matsuzawa *et al.*, 1987). The mechanism by which endothal and other homologs exert their potent herbicidal activity has not been studied extensively despite the widespread  
30 use of endothal internationally in agriculture. It should be noted that endothal is water soluble where cantharidin and norcantharidin are not.

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It was assumed that the activity of endothal as a contact herbicide and defoliant is related to the known irritating toxicity of its parent compound, norcantharidin. However, more recent studies suggest that the herbicidal activity of endothal may be a function primarily of its anti-plant protein phosphatase (PP2A) activity. Li *et al.* (1993) showed that cantharidin and endothal inhibit spinach leaf PP2A and PP1 and inhibit the activation of nitrate reductase by light in the intact spinach leaf, a process mediated by PP2A. Smith *et al.* (1994) demonstrate that the structurally unrelated protein phosphatase inhibitors okadaic acid and calyculin-A are potent inhibitors at nanomolar concentrations of the growth of certain plants. The activity of okadaic acid and calyculin-A strongly suggest that the activity of endothal as an herbicide is due to its anti-phosphatase activity.

Baskin and Wilson (1997) showed inhibitors of serine-threonine protein phosphatases including cantharidin inhibit organization of plant microtubules. Ayaydin *et al.* (2000) show that endothal inhibited PP2A activity causing alteration of cell division in cultured alfalfa cells. They noted that endothal was cell permeable.

Like endothal, Compounds 100 and 105 are water soluble. But unlike endothal, which is a diacid, Compounds 100 and 105 may be zwitterions. The fact that compounds disclosed herein such as Compound 100 are more potent than endothal against mammalian cancer cell growth may be due to greater cell permeability of the zwitterions than the diacid. Compounds 100 and 105 are more potent herbicides on a molar basis because of better cell penetration. Compounds 100 and 105, as zwitterions, are also less toxic to distributors and applicators of the herbicide as well as to the public inadvertently exposed to the agent.

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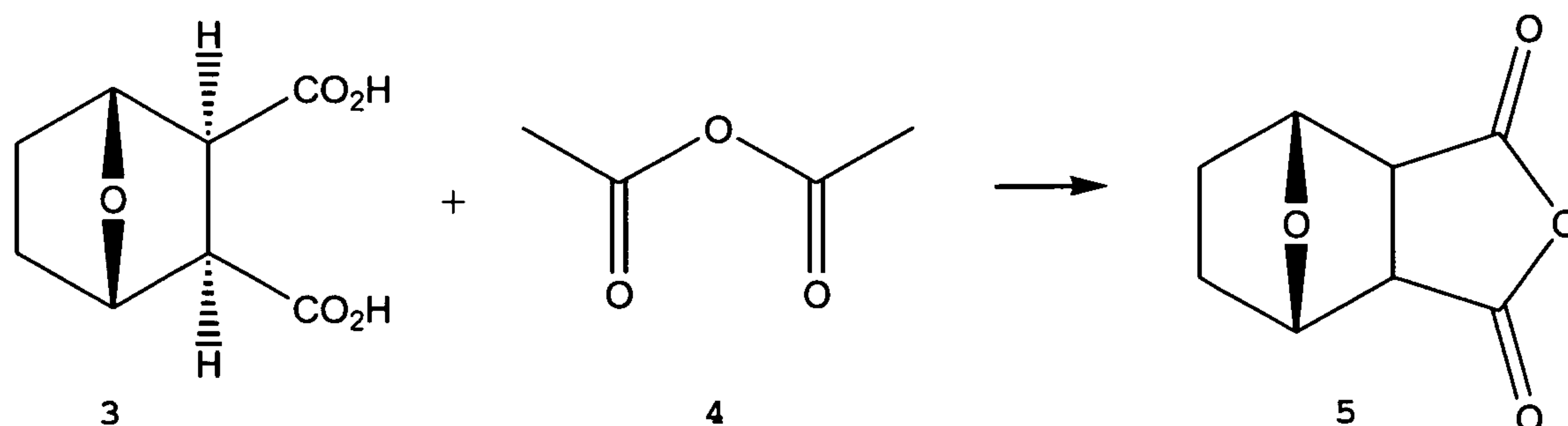
Compounds 100 and 105 do not have the acidic character of endothal.

The compounds herein, including Compounds 100 and 105, therefore, are useful, commercially feasible, and safer herbicides both with respect human exposure and to the environment.

### Experimental Details

#### Methods and Materials

##### *Preparation of Endothall Anhydride*

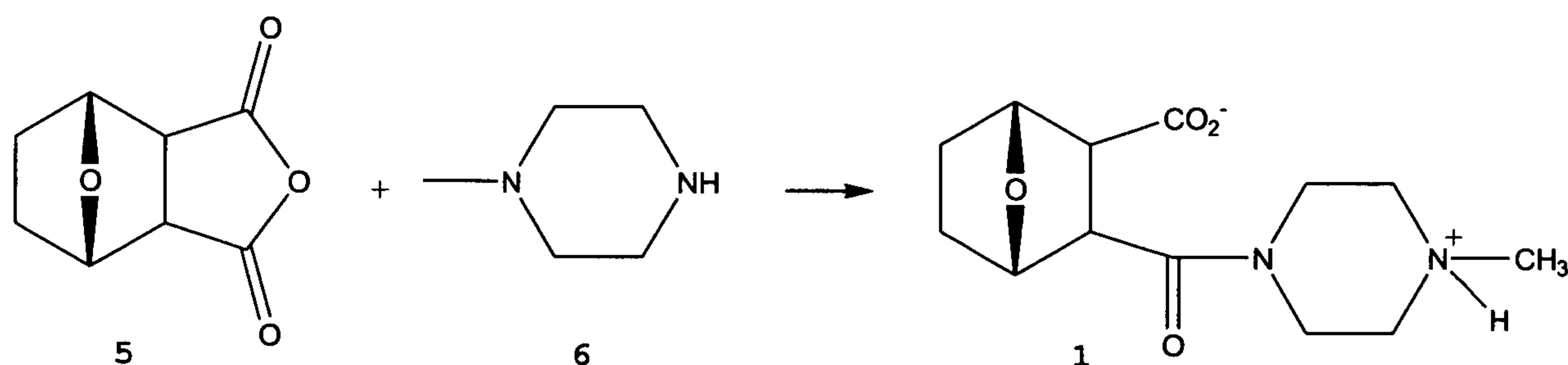


**Scheme 1**

As shown in scheme 1, we prepared endothal anhydride by adding acetic anhydride (0.5 mL) (**4**) to a suspension of Endothall (186 mg) (**3**) in benzene (3 mL) and the mixture was then stirred until the solid had gone into solution (2 hours). The solution was heated under vacuum to remove the benzene and the residue was then heated at 80°C for 30 min. Petroleum ether (5 mL) was then added and the desired anhydride spontaneously crystallized. The product was removed by filtration washed with a little petroleum ether to give the pure product (85 mg) which was used immediately in the following two preparations.

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*Preparation of Endothall 4-Methylpiperazine Monoamide (EMPM)  
(Compound 100)*



**Scheme 2**

5 Endothall anhydride (85 mg) (**5**) prepared as described above was dissolved in benzene (2 mL) and N-methylpiperazine (60 mg) (**6**) was added in one portion at room temperature. Almost immediately a white crystalline appeared. The mixture was left at room temperature overnight and the product was then removed by  
10 filtration washed with a little benzene then dried (145 mg). A negative ion mass spectrum showed a parent ion at m/z 267 (theo. 267) confirming the molecular weight as 268 mass units. The product was recrystallized from hot DMF to give 95 mg of the pure monoamide, Compound 100 (**1**) m.p. 226-227°C with some  
15 decomposition beginning at 224°C. The <sup>1</sup>H NMR spectrum of the sodium salt of compound 100 confirms the structure. Sodium salt. <sup>1</sup>H NMR: (D<sub>2</sub>O): 1.41-1.70 (m, 4H), 2.18 (s, 3H), 2.21-2.55 (m, 4H), 2.92 (d, 1H), 3.17 (d, 1H), 3.22-3.40 (m, 2H), 3.45 (m, 2H), 4.60 (q, 1H), 4.78 (q, 1H). Mass spectrometry data  
20 measuring the mass to charge ratio of compound 100 showed peaks at 141 m/z, 167 m/z, 254 m/z 199 m/z and 185 m/z corresponding to negatively charged fragments (Table 1).

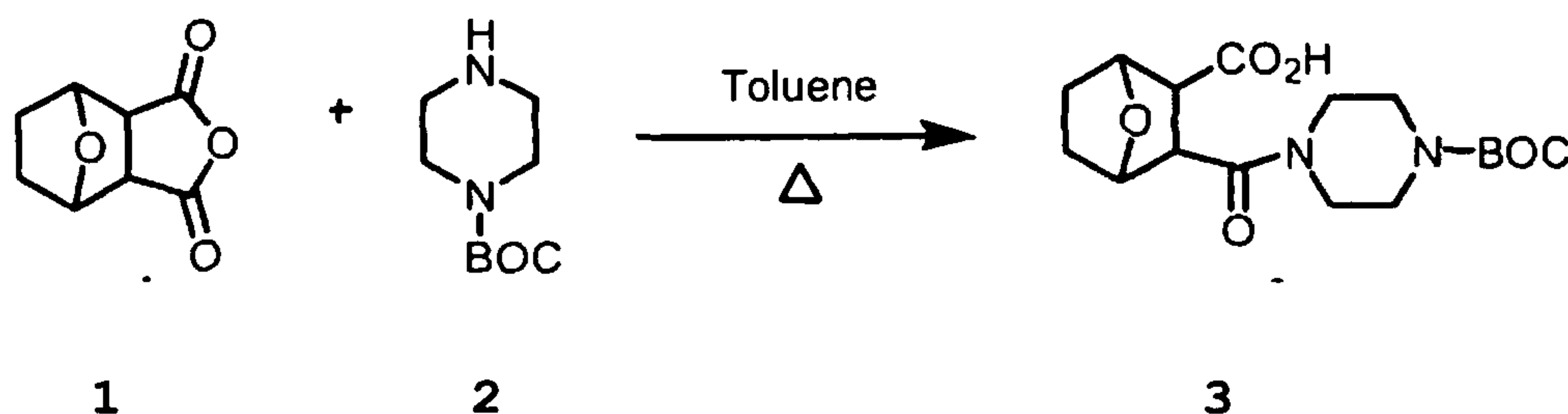
25 *Preparation of Endothall 4-Ethylpiperazine Monoamide (EPPM) (Compound 105)*

Endothal anhydride (1.68g; 10mmol) and N-ethylpiperazine (3.42g; 30mmol) were added to 10 mL of toluene and heated under reflux

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for 18h. The solvent was then evaporated under reduced pressure and the residue was crystallized from methyl t-butyl ether to give the crude product (1.8g). Recrystallization from the same solvent gave a total of 1.2g (42.5% yield) in two crops. m.p. 215-218°C (with decomposition). The  $^1\text{H}$  NMR of the sodium salt in  $\text{D}_2\text{O}$  and the MS negative ion spectrum confirm the structure and molecular weight ( $m/z$  282.2 ams), respectively. Sodium salt.  $^1\text{H}$  NMR: ( $\text{D}_2\text{O}$ ): 0.95 (t, 3H), 1.42-1.65 (m, 4H), 2.20-2.42 (m, 4H), 2.43-2.55 (m, 2H), 2.93 (d, 1H), 3.08 (d, 1H), 3.20-3.33 (m, 2H), 3.42-3.58 (m, 2H), 4.45 (q, 1H), 4.75 (q, 1H). The MS spectrum indicates the presence of the association dimer at  $m/z$  563.3 ams, which could be expected for a zwitterion. In addition, a trace of Endothal (MS  $m/z$  185 ams) is present but this is not evident in the NMR spectrum. A negative ion mass spectrum showed a parent ion at 282.2  $m/z$  (theo. 282.2) confirming molecular weight as 282.2 mass units.

Preparation of 4-(3-Carboxy-7-oxa-bicyclo[2.2.1]heptane-2-carbonyl)piperazine-1-carboxylic acid tert-butyl ester (Compound 102):

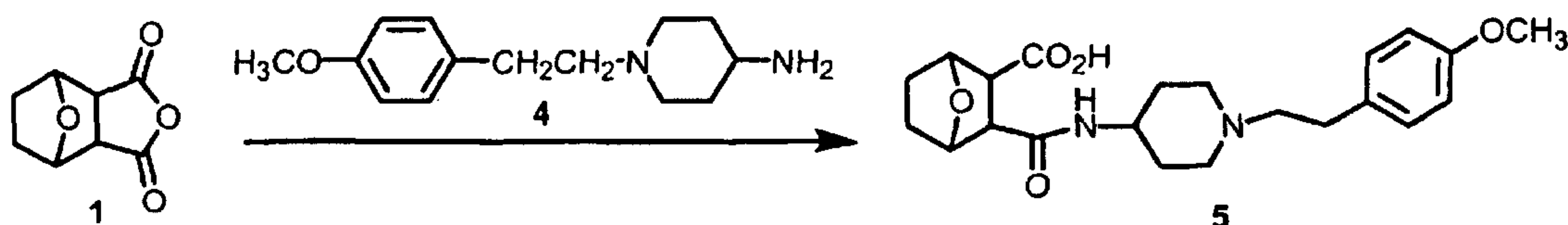


Endothal anhydride (**1**) (500 mg, 3 mmole) and N-BOC piperazine (**2**) (1.86g, 10 mmole) were added to dry toluene (8 mL) and heated at 100-110 C° for 8h. The solvent was removed on a rotary evaporator and to the residue was added a mixture of 10% aqueous citric acid (20 mL) and ethyl acetate (20 mL). The separated solid (compound 102) (**3**) was filtered, washed with hexane and

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dried under vacuum. Yield 500mg (47%). mp 206-208° C. The mass spectrum and the <sup>1</sup>H NMR data confirmed the identity of compound 102. Mass spectrometry data measuring the mass to charge ratio of compound 102 showed peaks at 141.1 m/z, 185 m/z, and 354 m/z. <sup>1</sup>H NMR: (DMSO-d<sub>6</sub>): 1.42 (s, 9H), 1.45-1.72 (m, 4H), 3.02-3.15 (d, 1H), 3.20-3.55 (m, 9H), 4.65-4.70 (m, 2H).

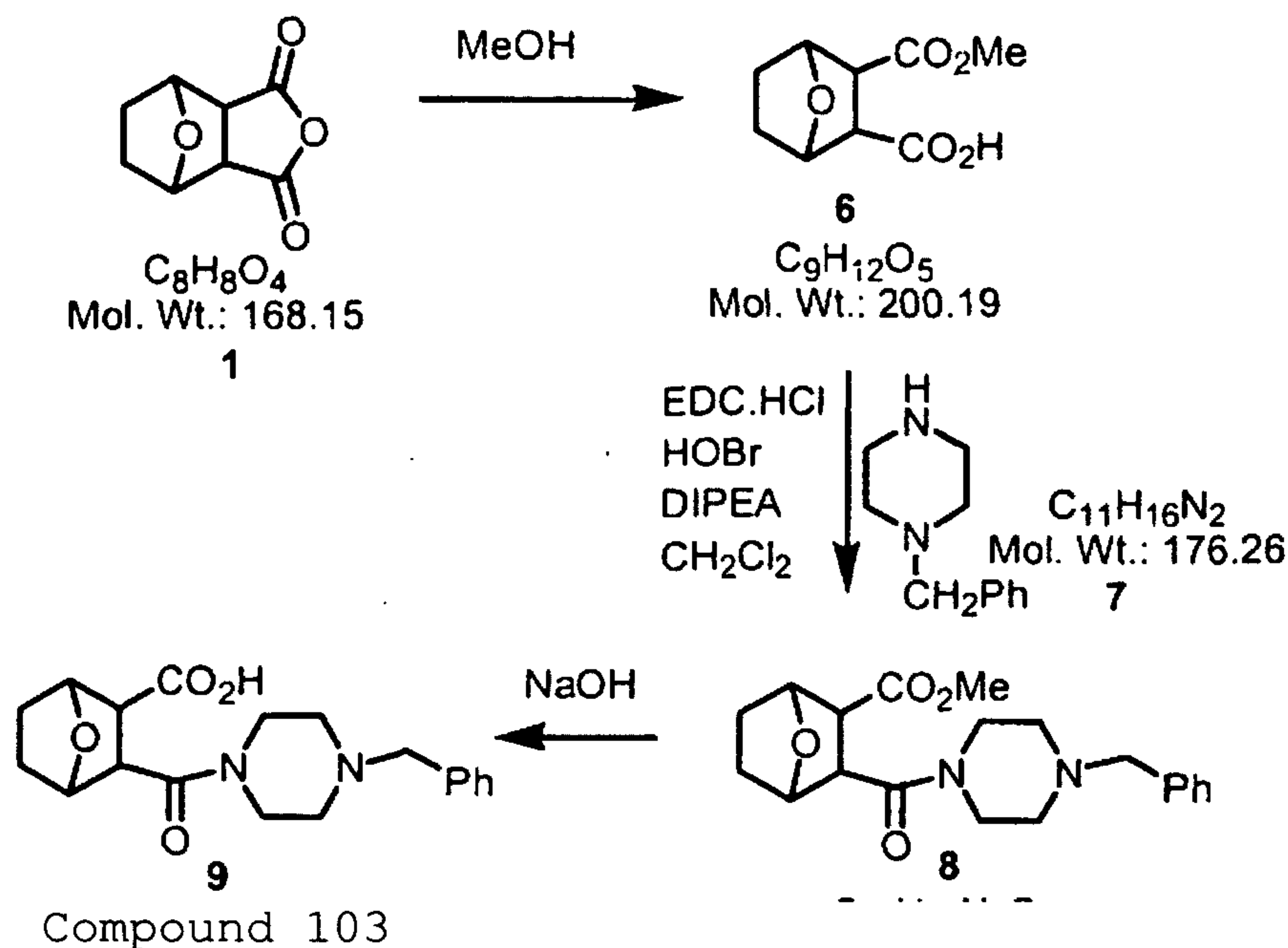
Preparation of 3-{1-[2-(4-Methoxy-phenyl)-ethyl]piperidin-4-ylcarbamoyl}-7-oxa-bicyclo[2.2.1]-heptane-2-carboxylic acid (Compound 104):



Endothal anhydride (**1**) (500 mg, 3 mmole) and amine (**4**) (2.34 g, 10 mmole) were added to dry toluene (8 mL) and heated at 100°C for 20 h. The solvent was then evaporated under reduced pressure and the residue was taken in water and the solution was acidified to pH to 5.5 to 6 with dilute HCl. The solid which was filtered and recrystallized from methanol to give pure (**5**) (compound 104). Yield 450 mg (36%). Mp 140-142° C. The mass spectrum and the <sup>1</sup>H NMR data confirmed the identity of compound 104. Mass spectrometry data measuring the mass to charge ratio of compound 104 showed peaks at 385. <sup>1</sup>H NMR: (CDCl<sub>3</sub>): 1.48-1.65 (m, 4H), 1.78-1.95 (m, 2H), 1.98-2.15 (t, 2H), 2.40-2.60 (m, 4H), 2.68-2.78 (m, 2H), 2.80 (s, 2H), 3.02-3.15 (d, 2H), 3.78 (s, 3H), 3.82-3.98 (m, 1H), 4.82-4.88 (m, 2H), 6.78-6.82 (d, 2H), 7.09-7.12 (d, 2H).

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Preparation of 3-(4-Benzylpiperazine-1-carbonyl)-7-oxa-bicyclo[2.2.1]heptane-2-carboxylic acid (Compound 103): This compound was prepared in 3 steps starting from Endothal anhydride (1) as shown below in Scheme-1.



SCHEME-1

Step 1: Preparation of 7-Oxa-bicyclo[2.2.1]heptane-2,3-dicarboxylic acid monomethyl ester:

Endothal anhydride (1) (4g, 24 mmole) was heated under reflux in dry methanol (20 mL) for 3 h. The reactions mixture was cooled to room temperature and the separated solid (6) was filtered and crystallized from methanol. Yield 4.6 g (96%). Mp 114-146°C.

Step 2: Preparation of 3-(4-Benzyl-piperazine-1-carbonyl)-7-oxa-bicyclo[2.2.1]heptane-2-carboxylic acid methyl ester (8):

To the mixture of acid derivative (6) (2.6 g, 13 mmole) in methylene chloride (40 mL) was added EDC.HCL (2.75 g, 15 mmole) followed by HOBT (150 mg). The mixture was stirred for 10 minutes at room temperature before adding N-benzyl piperazine (7) (1.76 g.) followed by DIPEA (3.5 mL, 20 mmole). The reaction mixture was stirred at room temperature overnight.

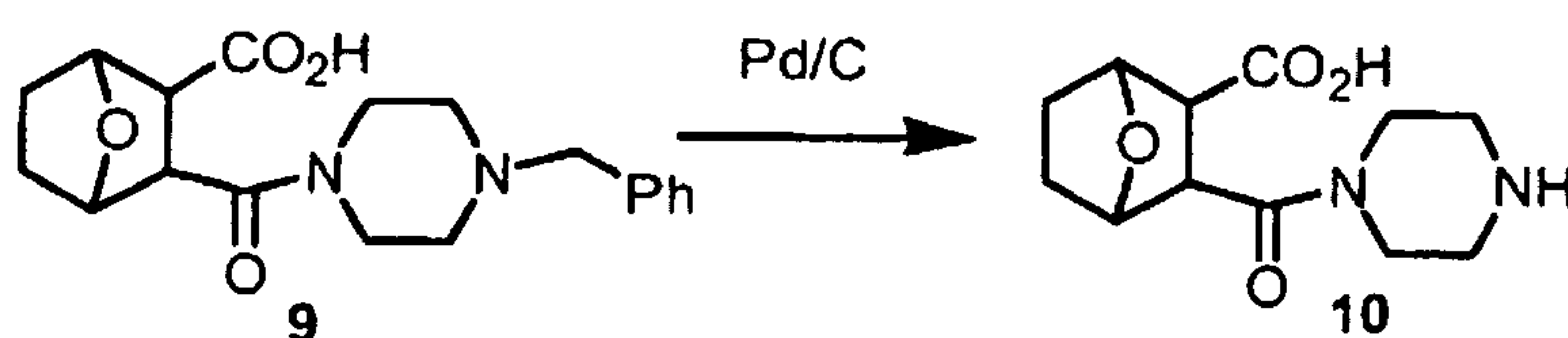
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Water was added to the reaction mixture, the methylene chloride layer was separated, washed once with aqueous  $\text{NaHCO}_3$ , then dried over  $\text{NaSO}_4$ , filtered and concentrated. The crude residue was purified by column chromatography using 1-2% methanol in methylene chloride as the eluant to give the required pure material **(8)**. Yield 2.7g. (58%).

*Step 3: Preparation of 3-(4-Benzyl-piperazine-1-carbonyl)-7-oxa-bicyclo[2.2.1]heptane-2-carboxylic acid (Compound 103):*

To a solution of ester **(8)** (2.50g, 7 mmole) in methanol (20 mL) was added aqueous NaOH (360 mg dissolved in 5 mL of water) and was stirred at room temperature overnight. The solvent was then evaporated to dryness, water (20 mL) was added and the pH of the solution was then adjusted to pH 5 using 6N HCL. It was then evaporated to dryness, and methylene chloride (30 mL) was added. The residual solid NaCl was removed by filtration and the filtrate was concentrated. The resulting solid was triturated with isopropyl ether to give the pure acid **(9)** (compound 103). Yield 1.8g (75%). mp  $> 190^\circ\text{C}$  (dec). The mass spectrum and the  $^1\text{H}$ NMR data confirmed the identity of compound 103.  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ ): 1.45-1.84 (m, 4H), 2.45-2.68 (m, 2H), 2.75-2.95 (m, 2H), 3.12-3.35 (m, 2H), 3.38-3.55 (m, 2H), 3.60-3.80 (m, 2H), 3.95-4.20 (m, 2H), 4.75-4.85 (m, 2H), 7.40 (s, 5H). Mass spectrometry data measuring the mass to charge ratio of compound 103 showed peaks at 177.2 m/z, 345 m/z and 711 m/z.

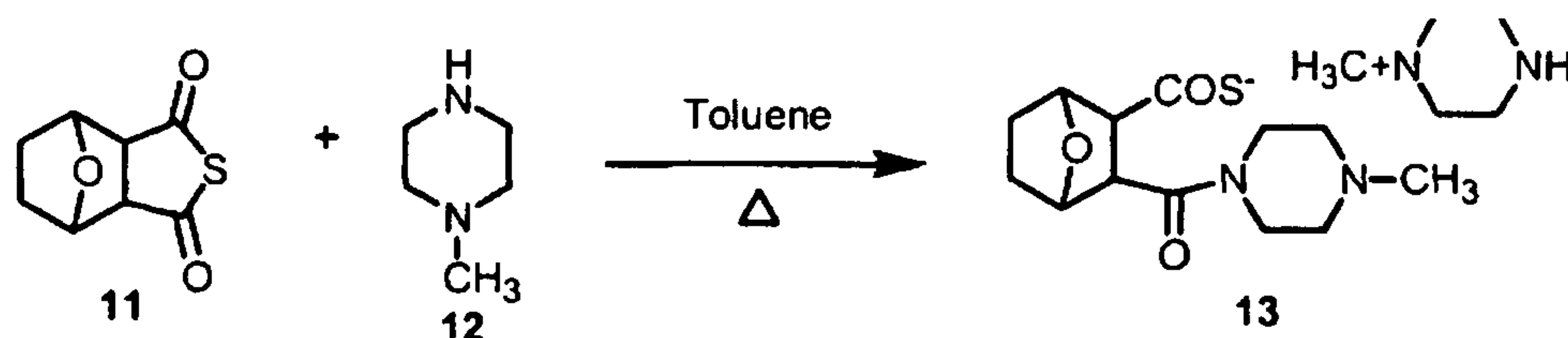
*Preparation of 3-(Piperazine-1-carbonyl)-7-oxa-bicyclo[2.2.1]heptane-2-carboxylic acid (Compound 101):*



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The N-Benzylprotected acid derivative **(9)** (500 mg, 1.45 mmole) in methanol was hydrogenated using hydrogen balloon over a Pd/C (5%, 50 mg) catalyst at room temperature overnight. The reaction mixture was filtered from the catalysts and concentrated to dryness. The crude residue was crystallized from 2-propanol to give amine **(10)** (Compound 101) as pure white solid. Yield 215 mg (58%) mp > 240°C (dec). The mass spectrum and the <sup>1</sup>H NMR data confirmed the identity of Compound 101. <sup>1</sup>H NMR: (CDCl<sub>3</sub>-CD<sub>3</sub>OD): 1.42-1.78 (m, 4H), 2.92-3.15 (m, 8H), 3.52-3.82 (m, 2H), 4.58 (q, 1H), 4.78 (q, 1H). Mass spectrometry data measuring the mass to charge ratio of compound 101 showed peaks at 177.2 m/z, 255.2 m/z, 277.2 m/z and 318.2 m/z.

Preparation of 3-(Piperazine-1-carbonyl)-7-oxa-bicyclo[2.2.1]heptane-2-carbothioic acid salt of N-methyl piperazine (Compound 108):

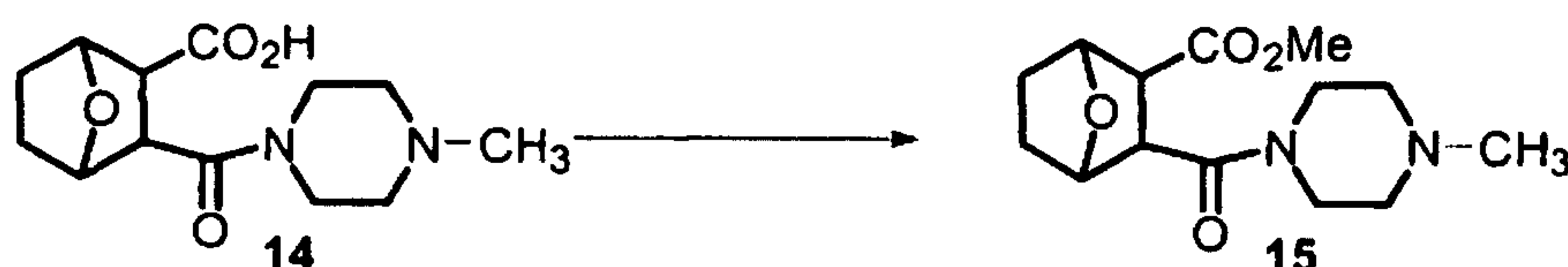


Endothal thioanhydride **(11)** (552 mg, 3 mmole) and N-methyl piperazine **(12)** (1 g, 10 mmole) were added to dry toluene (10 mL) and heated under reflux for 2.5 h. The reaction mixture was cooled to room temperature and the solid which had separated was filtered and crystallized from methylene chloride/ethyl acetate to give pure required **(13)** (Compound 108). Yield 585 mg (51%) mp >180°C (dec). The mass spectrum and the <sup>1</sup>H NMR data confirmed the identity of Compound 108. <sup>1</sup>H NMR: (CDCl<sub>3</sub>): 1.52-1.56 (m, 2H), 1.82-1.85 (m, 2H), 2.27 (s, 3H), 2.28-2.42 (m, 11H), 3.01 (s, 3H), 3.28-3.36 (m, 2H), 3.42-3.49 (m, 4H), 3.60-3.78 (m, 2H), 4.89-4.91 (m, 2H). Mass spectrometry data measuring the

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mass to charge ratio of compound 108 showed peaks at 217 m/z, 251 m/z and 351 m/z.

Preparation of 3-(4-Methyl-piperazine-1-carbonyl)-7-oxa-bicyclo[2.2.1]2-carboxylic acid methyl ester (Compound 107):



To a suspension of acid derivative (**14**) (536 mg, 2 mmole) in methylene chloride was added thionyl chloride (0.5 mL) followed by 2 drops DMF. The reaction mixture was stirred at room temperature overnight. It was a suspension of acid chloride hydrochloride salt. Added dry methanol (10 mL) and stirred for 30 minutes and evaporated to dryness using rotary evaporator. To the residue added water (20 mL) and extracted with ethyl acetate (20 mL). The aqueous layer pH was adjusted to 4.5 using aqueous NaHCO<sub>3</sub> (10%) and evaporated to dryness. The residue was azeotroped with acetonitrile. It was again dissolved in acetonitrile and the separated NaCl was removed by filtration. The filtrate was evaporated to dryness and triturated with ethyl acetate to get a sticky solid, which was dried in a vacuum oven to get the required compound as a colorless solid (**15**) (Compound 107). Yield 140 mg (25%, mp 105-107°C). The <sup>1</sup>H NMR data were compatible with the expected spectrum for compound 107. <sup>1</sup>H NMR: (D<sub>2</sub>O): 1.49-1.53 (m, 2H), 1.60-1.64 (m, 2H), 2.8 (s, 3H), 2.96-3.36 (m, 10H), 3.52 (s, 3H), 4.84-4.86 (m, 2H). Mass spectrometry data measuring the mass to charge ratio of compound 107 showed peaks at 123 m/z, 183 m/z, 251 m/z and 283 m/z.

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*Preparation of 1-{N-(3-Exocarboxy-7-oxabicyclo[2.2.1]heptane-2-exocarbonyl)amino-2-(N,N-dimethyl)aminoethane (Compound 106)}*

Endothal anhydride (1.68g; 10 mmole) and unsym-N,N-  
5 dimethylethylene diamine (2.64g; 30 mmole) were added to toluene  
(10 mL) and heated under reflux for 2 h. The solvent was then  
removed by evaporation under reduced pressure and the residue  
was triturated with a little di-isopropyl ether to cause  
crystallization. The product was then recrystallized twice from  
10 hexane by cooling to -20°C. The pure product (1.9g; 74% yield)  
melted at 48-50°C. The <sup>1</sup>H NMR spectrum is in perfect agreement  
with the structure which is zwitterionic and undergoes an  
exchange of two protons when treated with D<sub>2</sub>O. The mass spectrum  
also confirmed the identity of Compound 106. <sup>1</sup>H NMR: (CDCl<sub>3</sub>):  
15 1.62-1.75 (m, 2H), 1.85-1.95 (m, 2H), 2.2 (s, 6H), 2.45 (t, 2H),  
2.92 (s, 2H), 3.60 (t, 2H), 4.85-4.95 (m, 2H). Mass  
spectrometry data measuring the mass to charge ratio of compound  
106 showed peaks at 239.2 m/z, 257 m/z and 513 m/z.

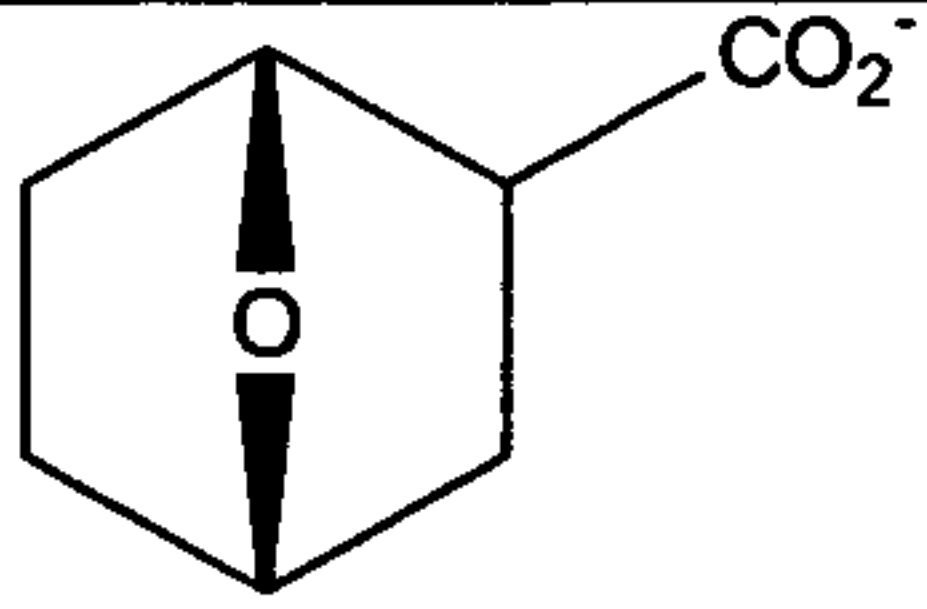
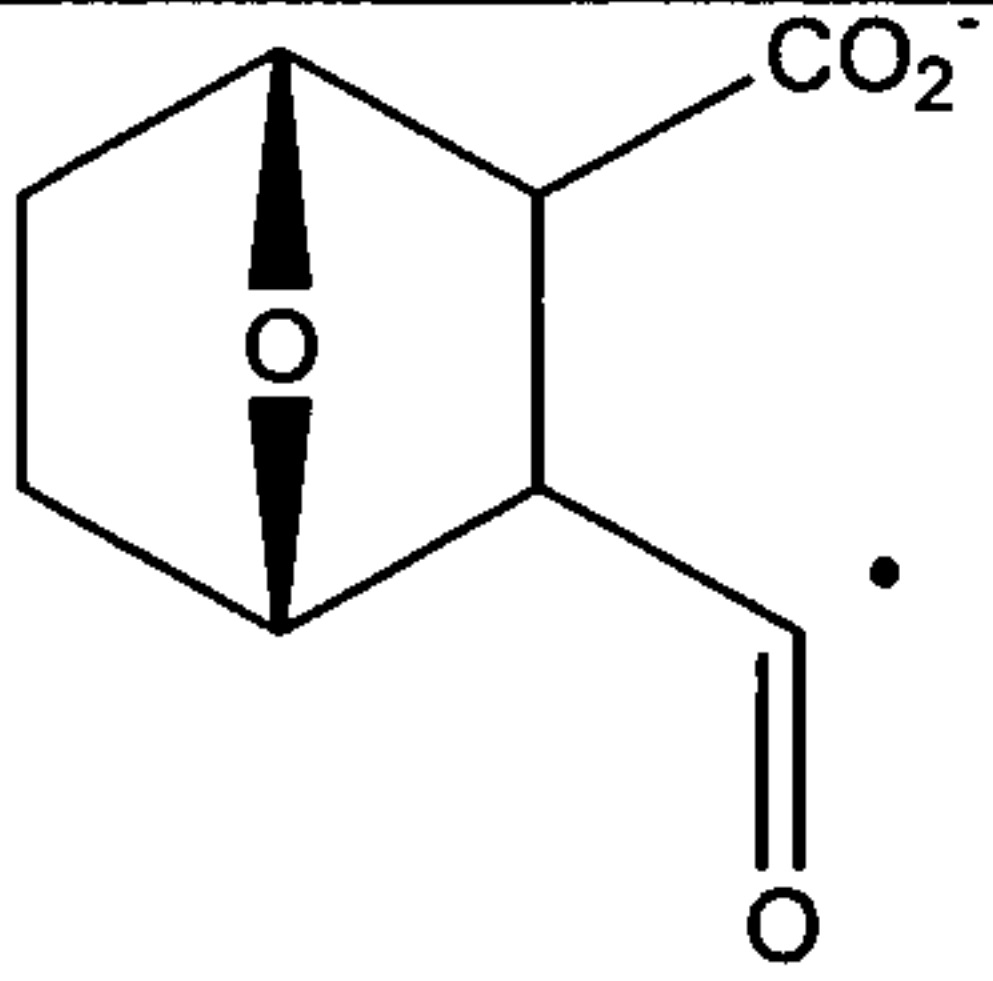
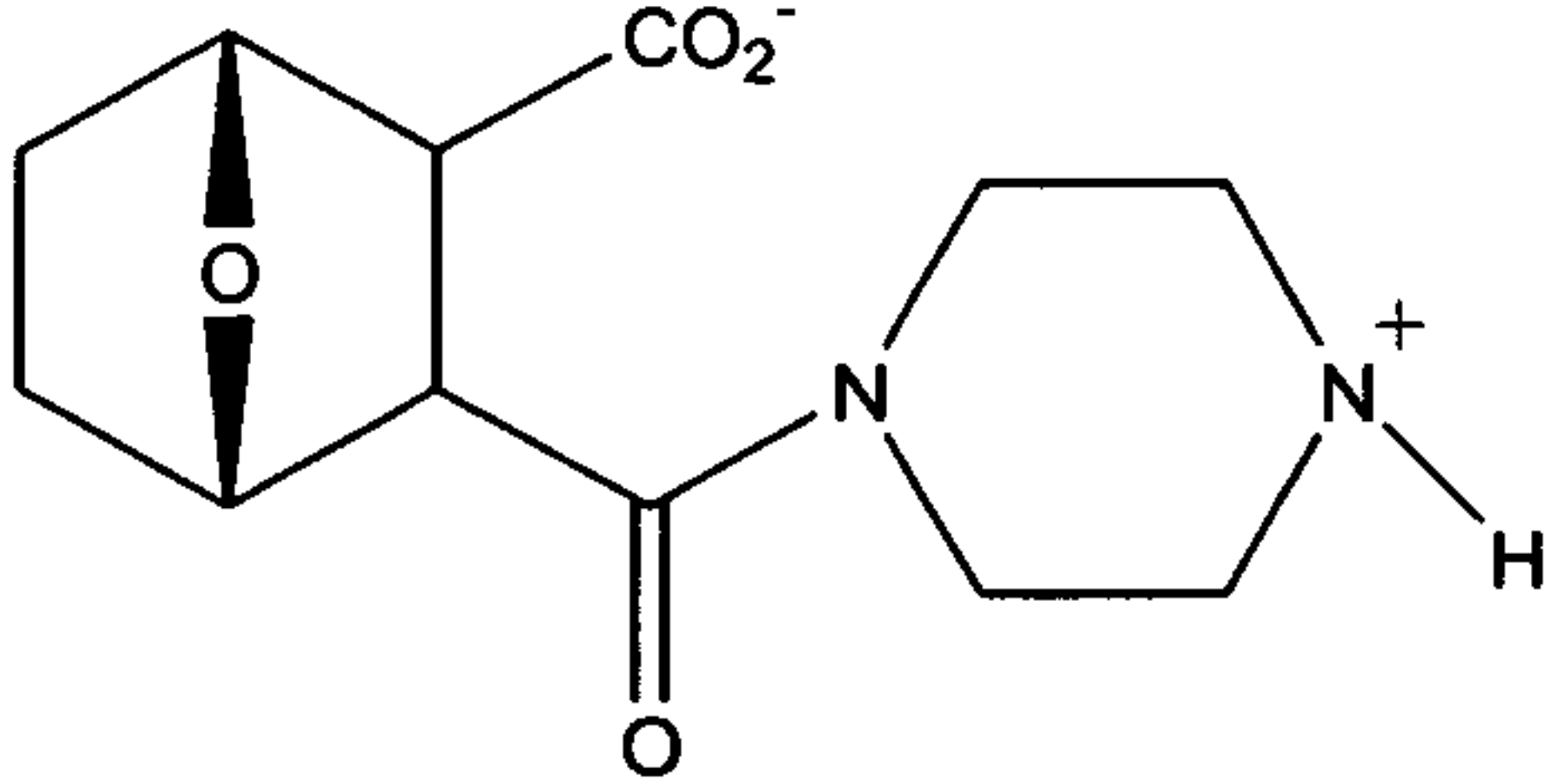
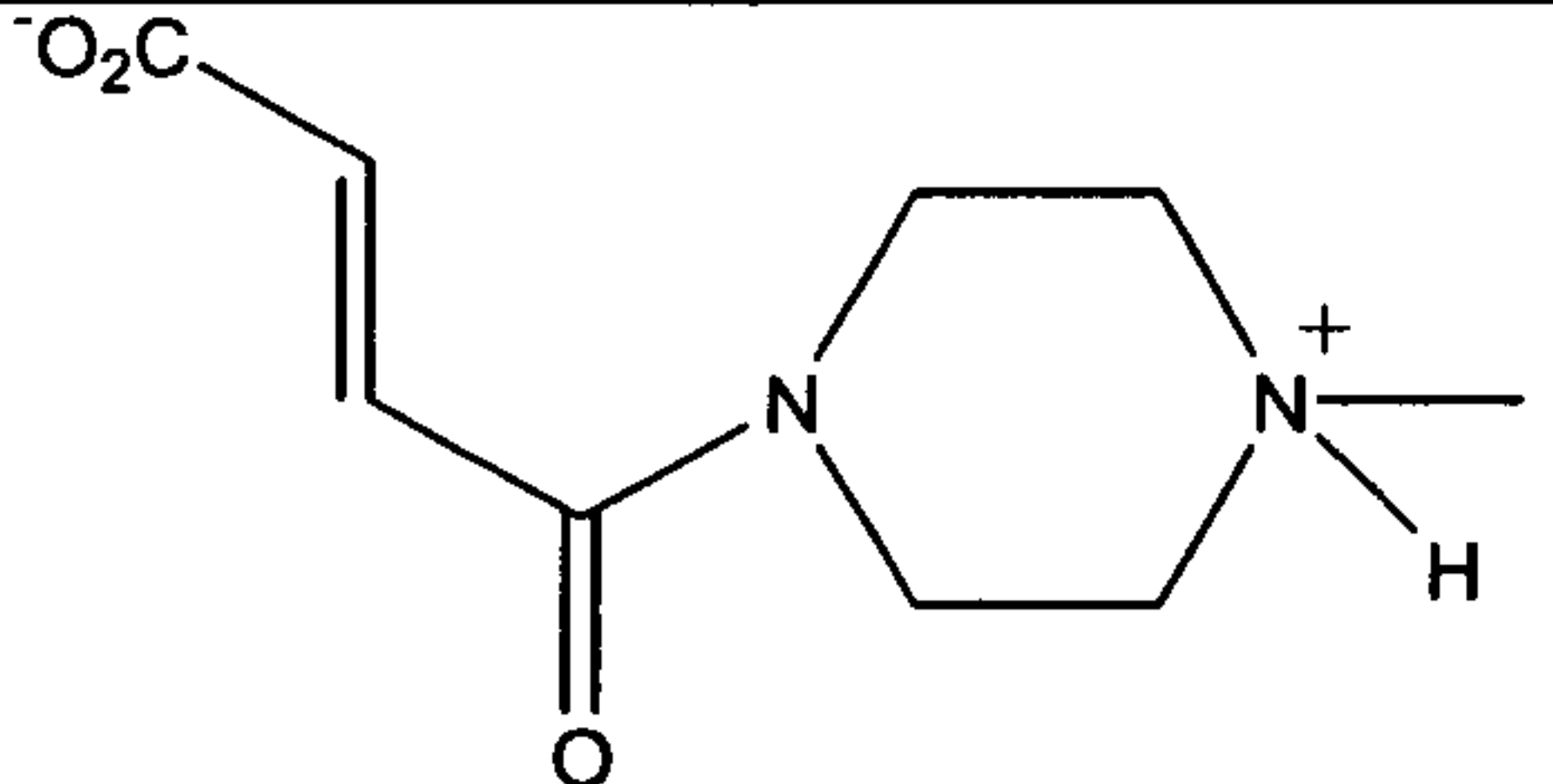
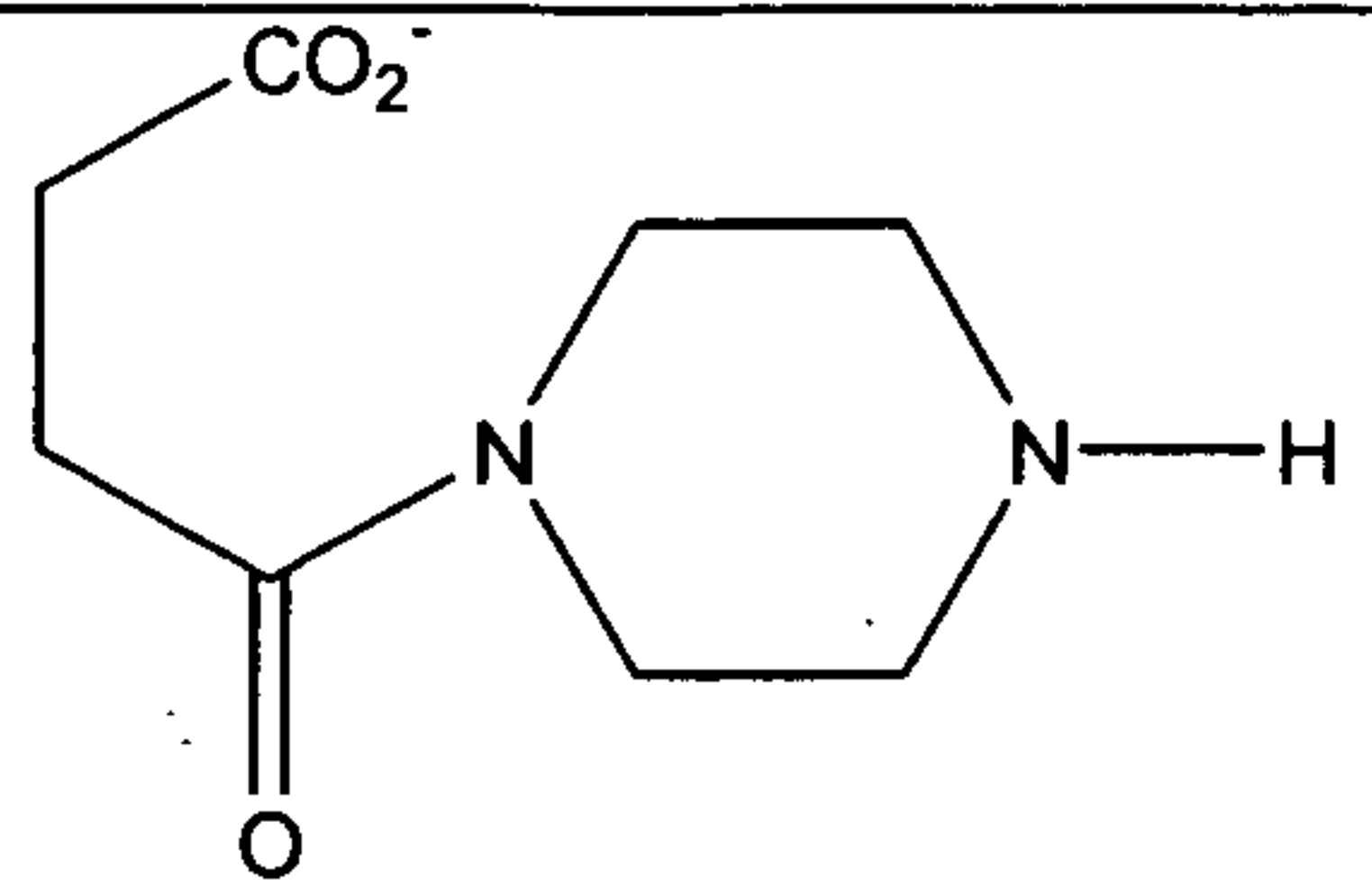
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Mass Spectrometry

Table 1 shows the correlation between the mass to charge ratio of the ions and corresponding structures. A sample of the compound 100 was ionized in the mass spectrometer. Ions of different masses were separated and their relative abundance was measured

Table 1: Mass to Charge Ratio of Ions

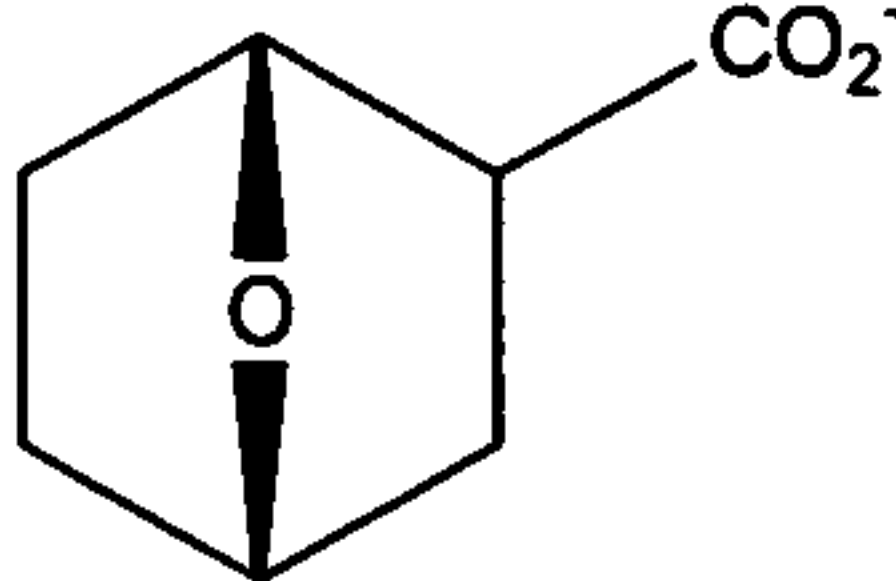
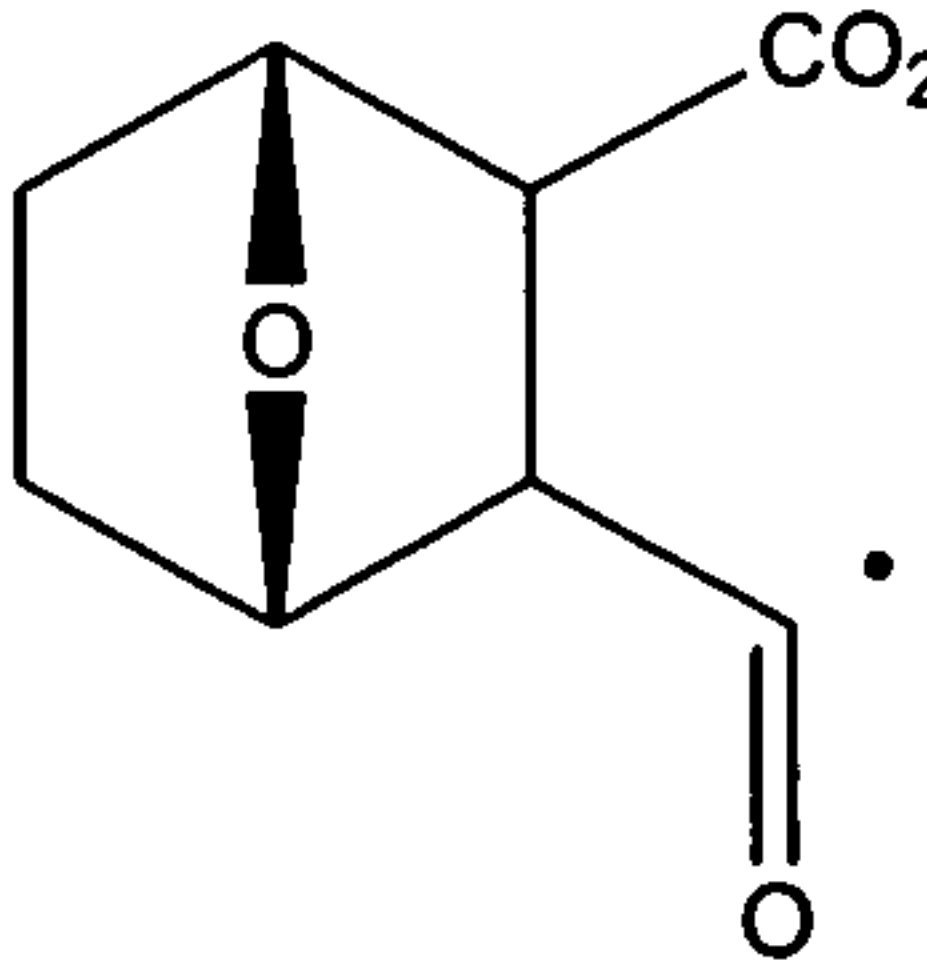
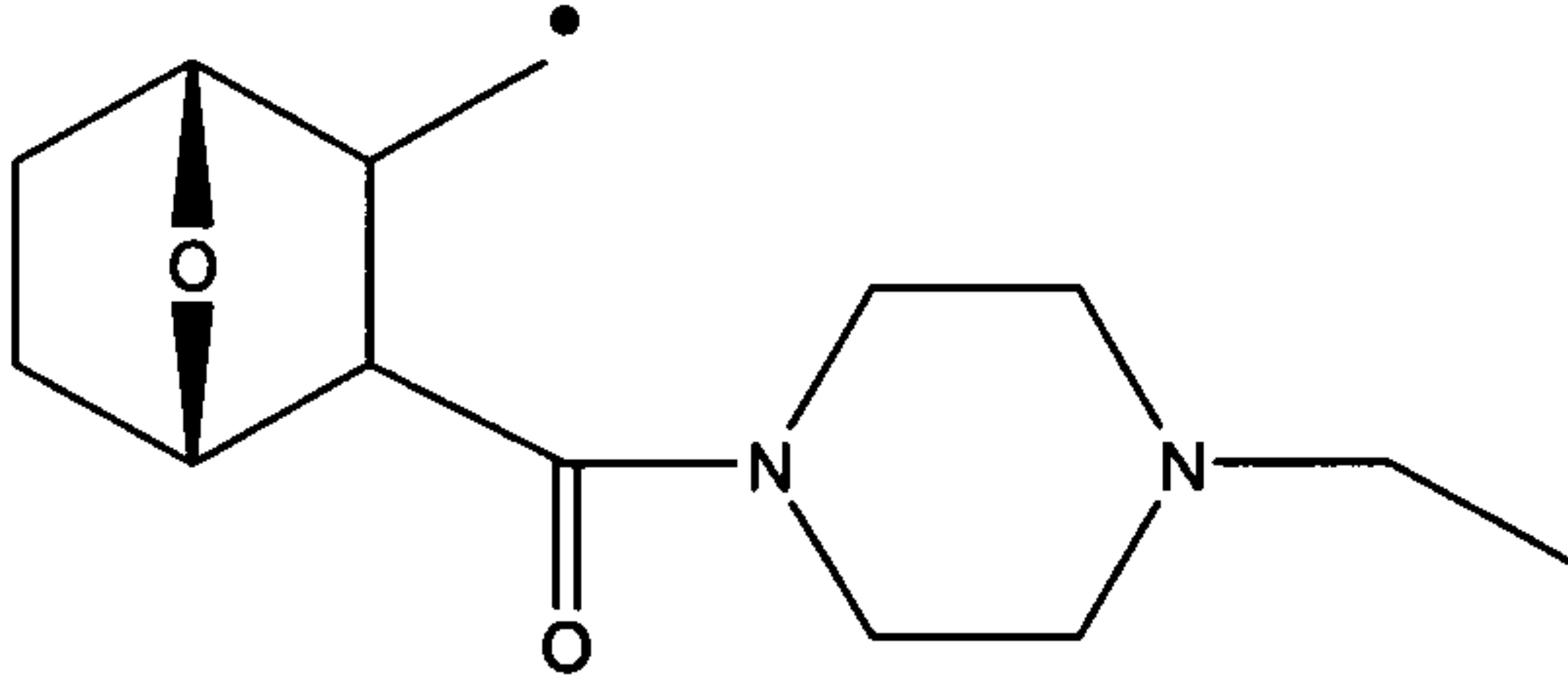
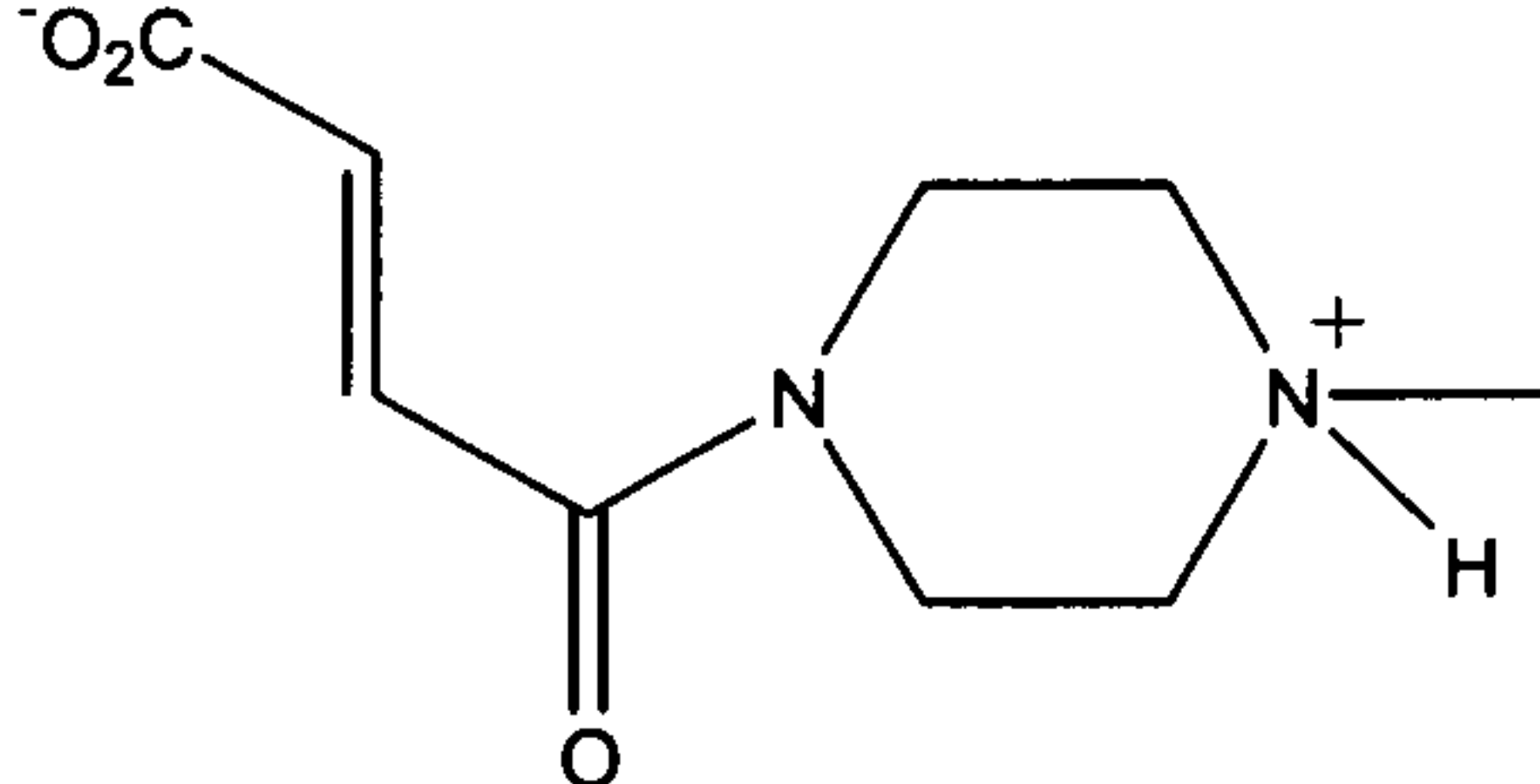
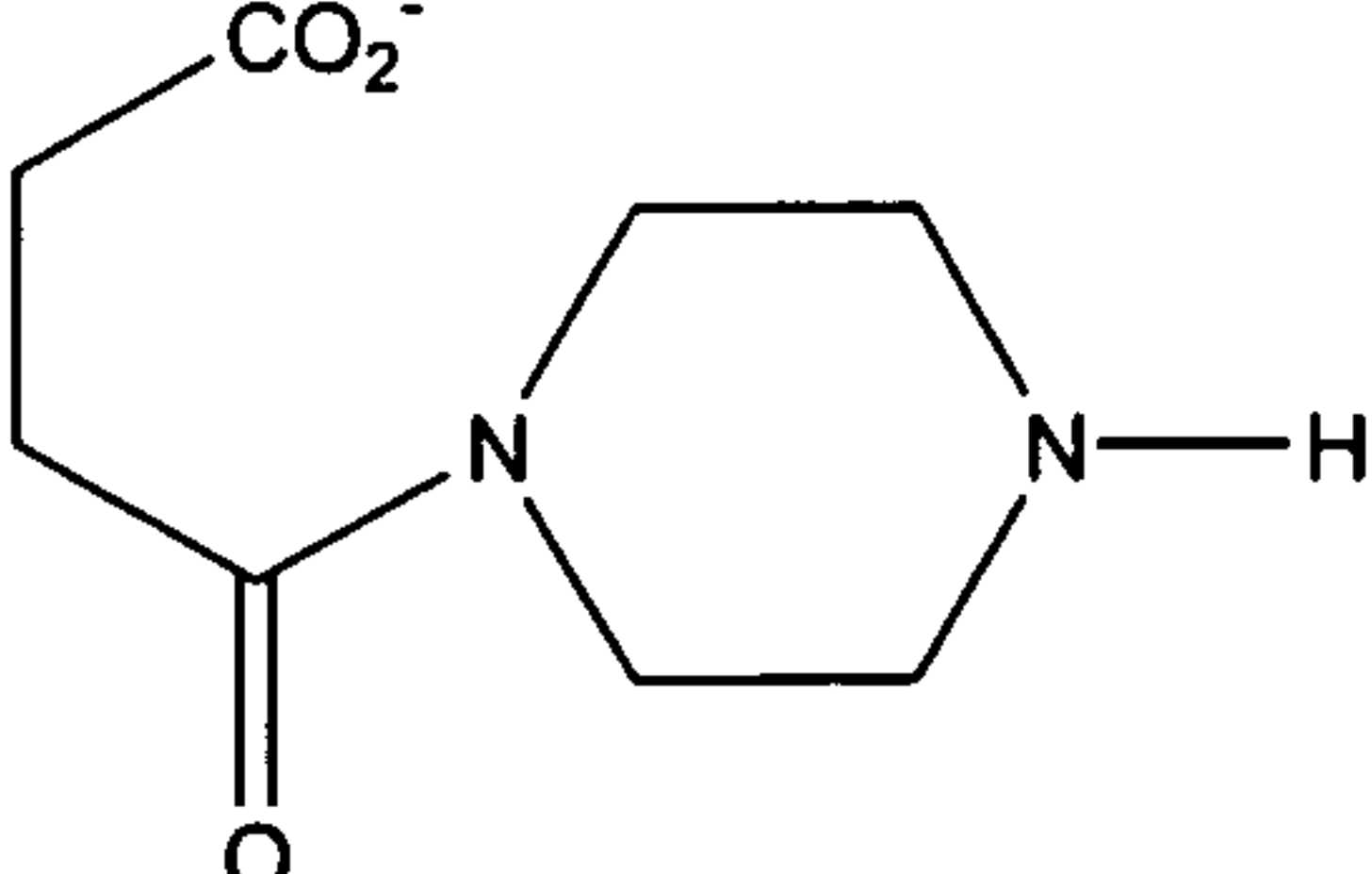
10

| <u>Mass to Charge Ratio (m/z)</u> | <u>Theoretical Structure of Ions</u>                                                  |
|-----------------------------------|---------------------------------------------------------------------------------------|
| 141                               |  |
| 167                               |  |
| 254                               |  |
| 199                               |  |
| 185                               |  |

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Table 2 shows the correlation between the mass to charge ratio of the ions and corresponding structures. A sample of the compound 105 was ionized in the mass spectrometer. Ions of different masses were separated and their relative abundance was measured

Table 2: Mass to Charge Ratio of Ions

| <u>Mass to Charge Ratio (m/z)</u> | <u>Theoretical Structure of Ions</u>                                                  |
|-----------------------------------|---------------------------------------------------------------------------------------|
| 141                               |  |
| 167                               |  |
| 249                               |  |
| 199                               |  |
| 185                               |  |

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**Example 1: Effect of Compound 100 and related analogues on GBM cells**

To identify novel therapeutic targets for the treatment of glioblastoma multiforme (GBM), cantharidin analogues were evaluated for their ability to inhibit growth of glioblastoma multiforme cells. Specifically, GBM cell line U373 was used in evaluations.

The cantharidin homologs that were evaluated were norcantharidin (nor-Can), which is a bis(normethyl) cantharidin; endothal (End), which is a dicarboxylic acid derivative of norcantharidins; endothal thioanhydride (ET); and Compounds 100 and 105, prepared as described above.

Cells were plated in triplicate on day one with and without different amounts of each drug dissolved in media (Compound 100, Compound 105, and endothal) or in dimethylsulfoxide (endothal thioanhydride and norcantharidin). The total number of cells is counted in the triplicate cultures at each dose and in controls after 7 days and the average number of cells and the standard deviation is determined.

The amount of inhibition of GBM cell growth is expressed as the proportion of the number of cells in the experimental dishes compared to the number of cells in control dishes containing only the drug vehicle and culture medium. The average percent of control is plotted and bracketed by one standard deviation calculated from the triplicate measurements.

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Results

Each of the norcantharidin analogues inhibited the growth of GBMs in a dose dependent manner in vivo as shown in Figure 1.

5

From graphic plots of the GBM cell line U373 as a function of exposure to different doses of drug for 7 days, the concentration of each compound that inhibited brain tumor cell proliferation by 50% (IC50) was estimated. The IC50s expressed in micro-molarity (uM), were: 2.5, 3.0, 12.0, and 15.0 for endothal thioanhydride, Compound 100, norcantharidin, and endothal respectively as seen in Figure 1.

In addition, logarithmic curves of gliomal cell line U373 treated with different doses of Compound 105 for three (Figure 6) and seven days (Figure 7) indicates that increasing dosages demonstrate a greater inhibition of growth. Further, Figure 8 is a dose-response curve for compound 105 against the human GBM cell growth over seven days at various concentrations of compound 105. It should be noted that Compound 100 was included at a single concentration of 10µM and that at this concentration the two compounds have identical inhibitory values.

25

**Example 2: Effect of Compound 100 combined with Retinoic Acid**

To identify the effect of combinations of PP2A anti-phosphatases and retinoids affecting nuclear complexes, we focused on water soluble cantharidin derivatives that have been shown to be active against human GBMs in vitro, endothal and Compound 100.

30

To observe the effects Compound 100 in combination with retinoic acids, Compound 100 was combined with all-trans retinoic acid.

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Cells were plated in triplicate on day one with and without different amounts of each drug dissolved in media (Compound 100 and endothal). The total number of cells is counted in the triplicate cultures at each dose and in controls after 7 days  
5 and the average number of cells and the standard deviation is determined.

The amount of inhibition of GBM cell growth is expressed as the proportion of the number of cells in the experimental dishes  
10 compared to the number of cells in control dishes containing only the drug vehicle and culture medium. The average percent of control is plotted and bracketed by one standard deviation calculated from the triplicate measurements.

## 15 Results

Figure 2 shows that Compound 100 as well as Endothal, each in combination with ATRA, synergistically inhibited proliferation of GBM cell line U373. Synergism (potentiation) of the  
20 inhibitory activity of two drugs in combination is said to be present when the percent survival in the presence of two drugs is less than the product of the percent survivals of the two drugs used alone at the same doses in the combination. The extent of synergism of Compound 100 and endothal (end) in  
25 combination with ATRA is quantified below in Table 3.

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Table 3. Endothal and Compound 100 +/- ATRA Inhibition of U373 Cells.

|                               | Percent of Control |                      |
|-------------------------------|--------------------|----------------------|
|                               | Observed           | Expected if Additive |
| ATRA 25 uM                    | 77%                | —                    |
| END 10 uM                     | 65%                | —                    |
| ATRA 25 uM + END 10 uM        | 32%                | 50%                  |
| Compound 100 1 uM             | 78%                | —                    |
| ATRA 25 uM + Compound 100 1uM | 53%                | 60%                  |

The expected percent survival of U373 cells exposed to the combination of ATRA and Compound 1 was 60% (77% by ATRA x 78% by Compound 100 = 60%), whereas the observed survival was 53%. The expected percent survival in the presence of the combination of ATRA and End was 50% (77% by ATRA x 65% by End = 50%) whereas the observed survival was 32%.

10

Compound 100, when combined with Trichostatin A or when combined with 13-cis retinoic acid synergistically inhibited the growth of GBM cell line U373 as shown below in Table 4.

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Table 4. Compound 100 +/- 13-cis Retinoic Acid (CIS-RA) and Compound 1 +/- Trichostatin A (TSA) Inhibition of GBM Cell Line U373

|                                              | Percent of Control |                      |
|----------------------------------------------|--------------------|----------------------|
|                                              | Observed           | Expected If Additive |
| Cis-RA 50 $\mu$ M                            | 93.3 +/- 2.2       |                      |
| TSA 0.033 $\mu$ M (0.01 $\mu$ g/ml)          | 71.6 +/- 0.4       |                      |
| Compound 100 - 1 $\mu$ M                     | 97.9 +/- 1.0       |                      |
| Compound 100 - 5 $\mu$ M                     | 52.5 +/- 2.9       |                      |
| Cis-RA 50 $\mu$ M + Compound 100 - 1 $\mu$ M | 79.3 +/- 3.2       | 91.3                 |
| Cis-RA 50 $\mu$ M + Compound 100 - 5 $\mu$ M | 31.6 +/- 2.0       | 49.0                 |
| TSA 0.033 $\mu$ M + Compound 100 - 1 $\mu$ M | 65.7 +/- 2.0       | 70.1                 |
| TSA 0.033 $\mu$ M + Compound 100 - 5 $\mu$ M | 13.9 +/- 1.0       | 37.6                 |

5

The two drugs were synergistic in their inhibition of the growth of U373 cells. The percent survival of the cells after exposure to two drugs in combination was less than would be expected from the percent survival of the cells when exposed to each of the two drugs at the same doses used in the combination.

10

### **Example 3: Determination of tumor type specificity.**

To determine whether there is tumor type specificity of the inhibitory properties of analogues of Compound 100, retinoic acid and Trichostatin A, we measured their inhibitory effects as single agents against the GBM line U373, a breast cancer line, MCF-7 (obtained from ATCC) and a kidney cancer cell line, UMRC (UMRC obtained by Dr. Zhuang, NINDS, NIH from the Intramural Research Support Program, SAIC, National Cancer Institute, Frederick Cancer Research and Development Center).

15

20

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Results:

The kidney cancer cell line, UMRC (Figure 3) was less sensitive than the brain tumor line, U373 (Figure 4) whereas the breast cancer line, MCF-7 (Figure 5) was as sensitive as U373 to all-trans retinoic acid, endothal thioanhydride, norcantharidin, endothal, and Trichostatin A. There is some cell type specificity of these drugs for GBMs. The activity of the drugs against MCF-7 cells indicates that regimens being developed for brain tumor treatment may also be useful against breast cancer as well as other tumors that overexpress N-CoR.

Example 4:

In addition to the inhibitory activities at low micromolar concentrations of compound 100, compound 105, like compound 100, is also a zwitterion, and is highly active as shown in Figure 9. The lipid soluble compound 102 is also highly potent in inhibiting glioma cell line, U373 (Figure 10). Other derivatives of nocantharidin, compounds 101, 103, 104 and 106 have less activity (Figures 11-14).

For all cell culture experiments, cells are allowed to grow in the presence or absence of different concentrations of drug or in the presence of the vehicle used to dissolve the drug, PBS for compound 100 and DMSO for compound 102. Cell counts are done in triplicate on day 3 and day 7 and inhibition of growth is expressed as a percentage of the number of cells present in the experimental well divided by the number of cells in a control well.

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There is dose dependent inhibition by compound 100 of the GBM cell line U373 (Figure 15) and dose dependent inhibition of the medulloblastoma cell line DAOY (Figure 16).

5 For in vivo experiments, subcutaneously implanted tumor cells are allowed to grow over 7 days to a size of 5-7mm. On day 7, drug administration is begun ip daily for 20 days. The maximum perpendicular diameters of the tumor masses are measured every 2 to 5 days. On the 21<sup>st</sup> day after initiation of treatment, the  
10 animals are sacrificed and dissected free of the subcutaneous tissues and measured.

It is shown the compound 100 supresses the growth of the GBM cell line U87 growing subcutaneously in SCID mice (Figure 17).  
15 It is also demonstrated the compound 100 and compound 102 both inhibit the proliferation of DAOY cells when implanted subcutaneously in SCID mice (Figure 18).

20 **Example 5: Drug Activity Against Human Cancer Cell Lines Other Than Glioblastomas and Medulloblastomas.**

**Materials and Methods**

MDA-MB-231, HT-29, NCI-H460, NCI-H522, NCI-H69, GXF-209, HepG2,  
25 OVAR-3, PANC-1, DU-145, LNCAP, HL-60, K-562, and MOLT-4 cell lines were either obtained from the American Type Culture Collection (ATCC; Manassas, VA), or from the National Cancer Institute (NCI; Frederick, MD). RPMI-1640 media, L-glutamine dipeptide (HyQ SG-200), and HEPES were obtained from Hyclone  
30 (Logan, UT).

Fetal bovine serum (FBS) was obtained from Sigma-Aldrich, (St. Louis, MO). DMSO was purchases from Fisher Chemicals (Fair

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Lawn, NJ). The CellTiter-Glo Luminescent Cell Viability Assay reagent was obtained from Promega Corporation (Madison, WI). All tissue culture plasticware was obtained from Corning Incorporated (New York, NY). Compound 100 and Compound 102 were  
5 provided by Lixte Biotechnology Holdings, Inc. (East Setauket, NY).

All cell lines were routinely cultured twice weekly in RPMI-1640 medium supplemented with 2 mM L-glutamine dipeptide, 10 mM  
10 HEPES, and 10% FBS.

The adherent cell lines MDA-MB-231, HT-29, NCI-H460, NCI-H522, GXF-209, HepG2, OVAR-3, PANC-1, DU-145, and LNCAP cells were each seeded into two 96-well plates at 2,500 cells per well in a  
15 total volume of 50 uL and incubated in a 37° C humidified 5% CO<sub>2</sub> cell culture incubator overnight. The suspension cell lines NCI-H69, HL-60, K-562, and MOLT-4 were each seeded into two 96-well plates at 10,000 cell per well in a total volume of 50 uL and incubated in a 37° C humidified 5% CO<sub>2</sub> incubator overnight.

20

A 20mM stock of water soluble drug Compound 100 was made in sterile water, and 20 mM stocks of Compound 102 were made in DMSO. This was followed by making 2X stocks of the final concentrations required in RPMI-1640 medium. 50uL of the 2X  
25 stock solutions were added to the appropriate wells, which contained 50 uL of cells and medium to give the final concentrations outlined in the Appendix. The top concentration of Compound 100 was filter sterilized before use. 50 uL of media were added to media and cell control wells and 50 uL of a  
30 mock 2X DMSO stock solution were added to vehicle control wells. At the same time that the drugs were added to the cells, one of the plates from each cell line was used for the CellTiter-Glo assay as described below in order to obtain Day 0 values for

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each cell line. Following a 72 hr incubation period, the CellTiter-Glo assay was performed on the remaining plate.

#### CellTiter-Glo Assay

5

The assay was performed as per the manufacturer's instructions. Briefly, plates were removed from the incubator and placed on the bench at room temperatures for 30 minutes. Plates were not stacked. Following the 30 min incubation at room temperature,  
10 100 uL of CellTiter-Glo reagent were added to each well on the plate and mixed for 2 minutes, followed by further 10 minute incubation at room temperature. Luminescence was then recorded using the PerkinElmer Microbeta scintillation and luminescence counter (Trilux).

15

#### Results and Discussion

These studies were performed as described in Materials and Methods, with the raw data and plate set-up outlined in the  
20 Appendix. The IC50 values obtained for each drug in each cell line are outlined in Table 5. The graphical representation of the effect of the compounds on each cell line, along with the associated curve fits, is illustrated in Figures 19A-N.

25 The majority of cell lines tested were sensitive to all drugs in the low uM range (Table 5). Both Compound 100 and Compound 102 have significant activity (approximately equal for all lines to the positive comparator clinically used anti-cancer drug, doxorubicin). The drugs were active against cell lines of:  
30 breast cancer; colon cancer; the three major types of lung cancer, large cell, adenocarcinoma and small cell; stomach cancer; liver cancer (hepatoma); ovary adenocarcinoma; pancreas carcinoma, two types of prostate carcinoma; and three types of

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leukemia, promyelocytic, chronic myelocytic, and acute lymphocytic (Table 5).

TABLE 5: The Concentration of Compound 100 and 102 that Results in 50% Inhibition of Proliferation (IC<sub>50</sub>) Obtained from the Curve Fits of Fourteen Human Cancer Cell Lines (Figures 19A-N)

5

| Cell Line                          | IC <sub>50</sub> $\mu$ M |              |  |
|------------------------------------|--------------------------|--------------|--|
| Cancer Type                        | Compound-100             | Compound-102 |  |
| MDA-MB-231<br>Breast               | 9.5                      | 6.6          |  |
| HT-29<br>Colon                     | 5.3                      | 3.6          |  |
| NCI-H460-<br>Lung:large<br>cell    | 24.3                     | 17.5         |  |
| NCI-H522 Lung<br>adenoca           | 3.4                      | 2.2          |  |
| NCI-H69<br>Lung small<br>cell      | 23.9                     | 24.6         |  |
| GXF-209<br>Stomach                 | 7.9                      | 5.3          |  |
| HepG2<br>Liver                     | 31.6                     | 22.1         |  |
| OVCAR-3 Ovary<br>adenoca           | 2.9                      | 5.1          |  |
| PANC-1<br>Pancreas                 | 25.1                     | 20.7         |  |
| DU-145<br>Prostate                 | 16.4                     | 13.0         |  |
| LNCAP<br>Prostate                  | 1.5                      | 0.48         |  |
| HL-60<br>Leukemia<br>promyelocytic | 7.7                      | 6.5          |  |
| K-562<br>Leukemia<br>chronic myelo | 10.7                     | 13.7         |  |
| MOLT-4<br>acute lympho             | 5.7                      | 5.6          |  |

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**Example 6: Antifungal Activity**

The growing population of immunocompromised patients due to transplantation, HIV/AIDS and cancer, primarily leukemia, has  
5 resulted in an increase in severe fungal infections. The fungi most often recovered from infections in these patients are *Aspergillus* spp. and *Candida* spp. Effective therapies are available for the treatment of *Candida* spp. but there remains a concern about the treatment of infections caused by *Aspergillus*  
10 spp., which are associated with high mortality in the immunocompromised host. Such infections are difficult to clear in this group of patients thus increasing the need for agents with good activity against these fungi. In addition, less serious but chronic troublesome fungal infections of the nails  
15 and skin of the feet and hands, the dermatophytoses, affect millions of people worldwide. As a result of the changing scene of fungal infections and the lack of an overall cure for these infections, Compounds 100 and 102 are undergoing testing for possible future development.

20

**Materials And Methods**

Anti-fungal testing was completed with a common lot for compounds 100 and 102.

25

A 10 mg portion of each powder was weighed out and added to 1 ml of sterile distilled water for compound 100 and DMSO for compound 102. The resulting concentration of 10 µg/ml was diluted to a working concentration of 640 µg/ml for each  
30 compound. All subsequent dilutions were also made using the respective diluents. Final testing concentrations ranged from 0.125-64 µg/ml.

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Results

A total of 23 isolates were tested to include 3 *Candida albicans*, 3 *Candida glabrata*, 3 *Cryptococcus neoformans*, 3  
5 *Aspergillus fumigatus*, 3 *Rhizopus oryzae*, 3, *Fusarium solani*, 3  
*Pseudallescheria boydii*, and 2 *Trichosporon rubrum*. All  
isolates were clinical isolates submitted to the Fungus Testing  
Laboratory for evaluation. Antifungal susceptibility testing  
was accomplished according to the methods outlined in the  
10 National Committee for Clinical Laboratory Standards, M-27A2,  
Reference Method for Broth Dilution Antifungal Susceptibility  
Testing of Yeasts; Approved Standard, and M38-A "Reference  
Method for Broth Dilution Antifungal Susceptibility Testing of  
Conidium-Forming Filamentous Fungi; Approved Standard". This  
15 includes testing in RPMI-1640 with glutamine and without  
bicarbonate, an inoculum size of  $0.5-2.5 \times 10^3$  for yeasts or  $1-5$   
 $\times 10^4$  for moulds, and incubation at 35°C for 24 and 48 hours.  
The minimum inhibitory concentration (MIC) was defined as the  
lowest concentration that resulted in an 50% reduction in  
20 turbidity as compared to a drug-free control tube for the yeast  
and 80% inhibition for the moulds.

While no activity was noted for compound 100 at the levels  
tested, significant activity was found for compound 102 against  
25 *T. rubrum* (Table 6).

Conclusion

Depending on achievable levels of this compound in human  
30 subjects, safety profiles and other pertinent factors, this  
compound may be a viable contender for dermatophytic infections  
caused by *Trichosporon rubrum*.

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Table 6

|         |                      | Compound<br>100 / 24<br>Hours | Compound<br>100/48<br>Hours | Compound<br>102/24<br>Hours | Compound<br>102/48<br>Hours |
|---------|----------------------|-------------------------------|-----------------------------|-----------------------------|-----------------------------|
| CP      | Control              | >64                           | >64                         | >64                         | >64                         |
| 07-3006 | <i>C. albicans</i>   | >64                           | >64                         | >64                         | >64                         |
| 07-3011 | <i>C. albicans</i>   | >64                           | >64                         | >64                         | >64                         |
| 07-3012 | <i>C. albicans</i>   | >64                           | >64                         | >64                         | >64                         |
| 07-2964 | <i>C. glabrata</i>   | >64                           | >64                         | >64                         | >64                         |
| 07-2965 | <i>C. glabrata</i>   | >64                           | >64                         | >64                         | >64                         |
| 07-3013 | <i>C. glabrata</i>   | >64                           | >64                         | >64                         | >64                         |
| 07-2665 | <i>C. neoformans</i> | >64                           | >64                         | >64                         | >64                         |
| 07-2737 | <i>C. neoformans</i> | >64                           | >64                         | >64                         | >64                         |
| 07-2829 | <i>C. neoformans</i> | >64                           | >64                         | >64                         | >64                         |
| 07-1870 | <i>R. arrhizus</i>   | >64                           | >64                         | >64                         | >64                         |
| 07-2044 | <i>R. arrhizus</i>   | >64                           | >64                         | >64                         | >64                         |
| 07-2078 | <i>R. arrhizus</i>   | >64                           | >64                         | >64                         | >64                         |
| 07-1399 | <i>F. solani</i>     | >64                           | >64                         | >64                         | >64                         |
| 07-1755 | <i>F. solani</i>     | >64                           | >64                         | >64                         | >64                         |
| 07-1867 | <i>F. solani</i>     | >64                           | >64                         | >64                         | >64                         |
| 07-1333 | <i>P. boydii</i>     | >64                           | >64                         | >64                         | >64                         |
| 07-1502 | <i>P. boydii</i>     | >64                           | >64                         | >64                         | >64                         |
| 07-1601 | <i>P. boydii</i>     | >64                           | >64                         | >64                         | >64                         |
| 05-388  | <i>A. fumigatus</i>  | >64                           | >64                         | >64                         | >64                         |
| 06-4126 | <i>A. fumigatus</i>  | >64                           | >64                         | >64                         | >64                         |
| 07-2039 | <i>A. fumigatus</i>  | >64                           | >64                         | >64                         | >64                         |
| 07-1743 | <i>T. rubrum</i>     | >64                           | >64                         | 2                           | 2                           |
| 07-2055 | <i>T. rubrum</i>     | >64                           | >64                         | 2                           | 2                           |

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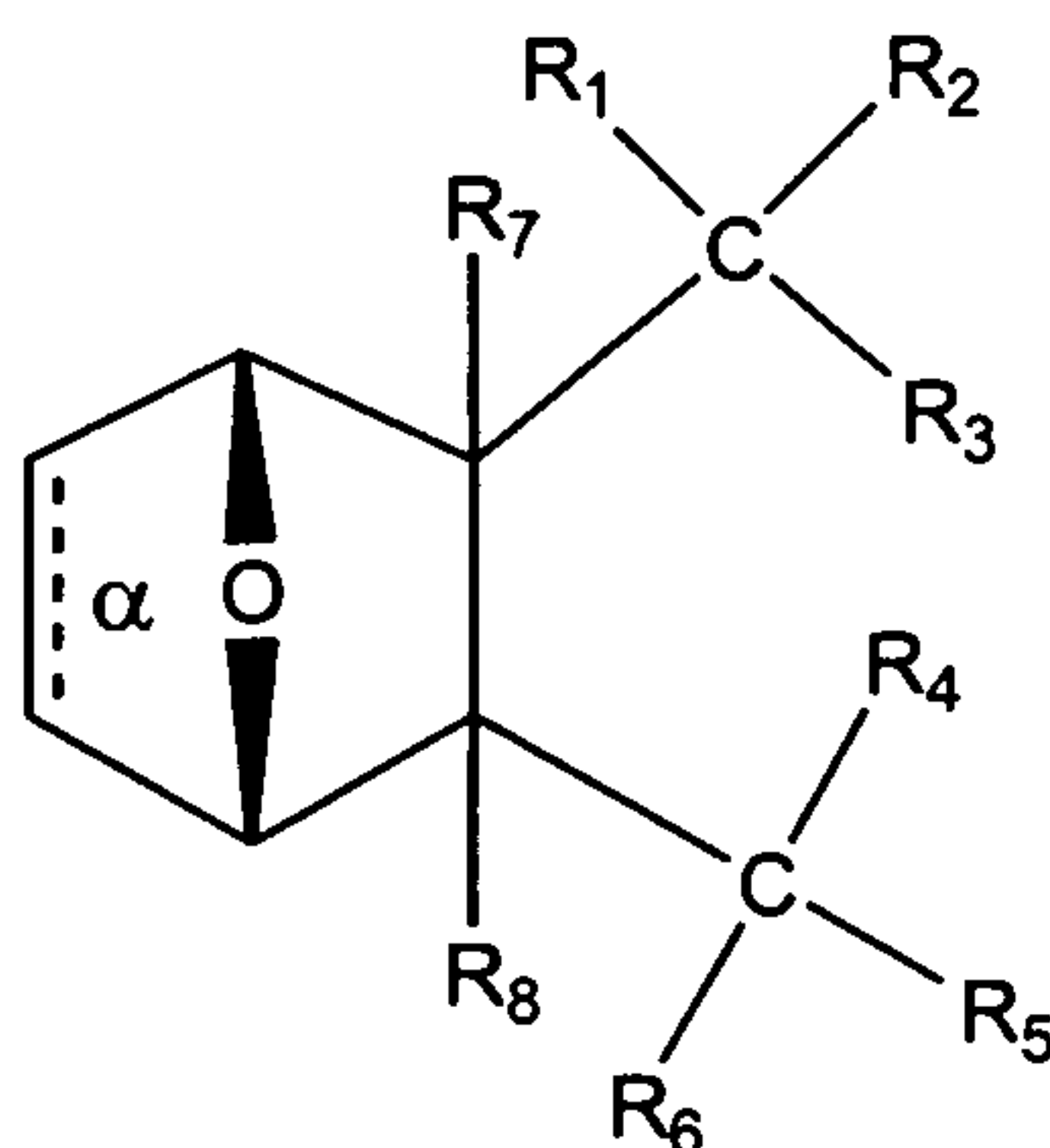
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What is claimed is:

1. A compound having the structure



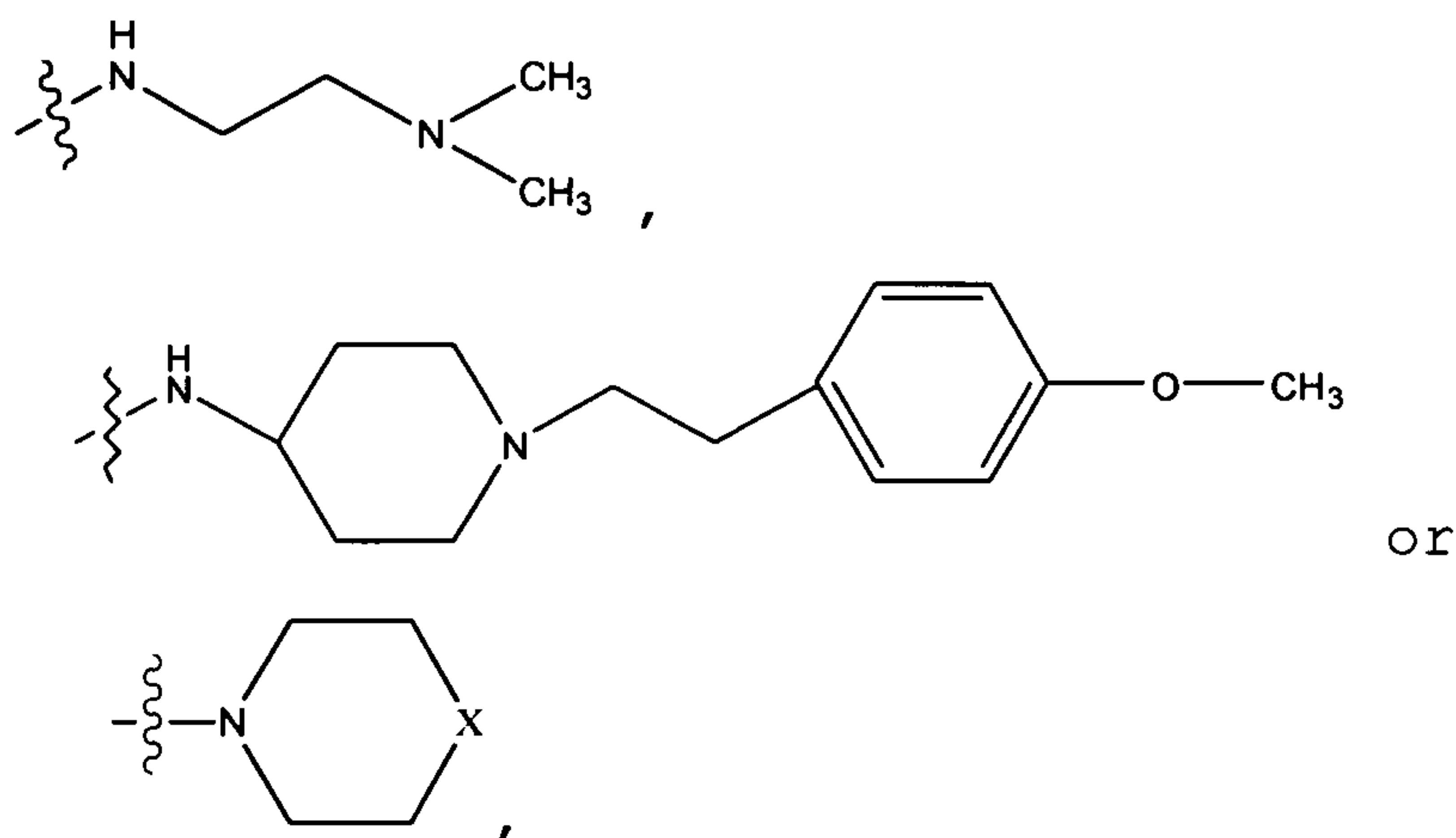
wherein

bond  $\alpha$  is present or absent;

$R_1$  and  $R_2$  is each independently H,  $O^-$  or  $OR_9$ ,

where  $R_9$  is H, alkyl, alkenyl, alkynyl or aryl,  
or  $R_1$  and  $R_2$  together are  $=O$ ;

$R_3$  and  $R_4$  are each different, and each is OH,  $O^-$ ,  $OR_9$ , SH,  $S^-$ ,  $SR_9$ ,



where X is O, S,  $NR_{10}$ , or  $N^+R_{10}R_{10}$ ,

where each  $R_{10}$  is independently H, alkyl,

substituted  $C_2$ - $C_{12}$  alkyl, alkenyl, substituted  $C_4$ -

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C<sub>12</sub> alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl where the substituent is other than chloro when R<sub>1</sub> and R<sub>2</sub> are =O,



-CH<sub>2</sub>CN, -CH<sub>2</sub>CO<sub>2</sub>R<sub>11</sub>, -CH<sub>2</sub>COR<sub>11</sub>, -NHR<sub>11</sub> or -NH<sup>+</sup>(R<sub>11</sub>)<sub>2</sub>,  
 where each R<sub>11</sub> is independently alkyl, alkenyl or alkynyl, each of which is substituted or unsubstituted, or H;

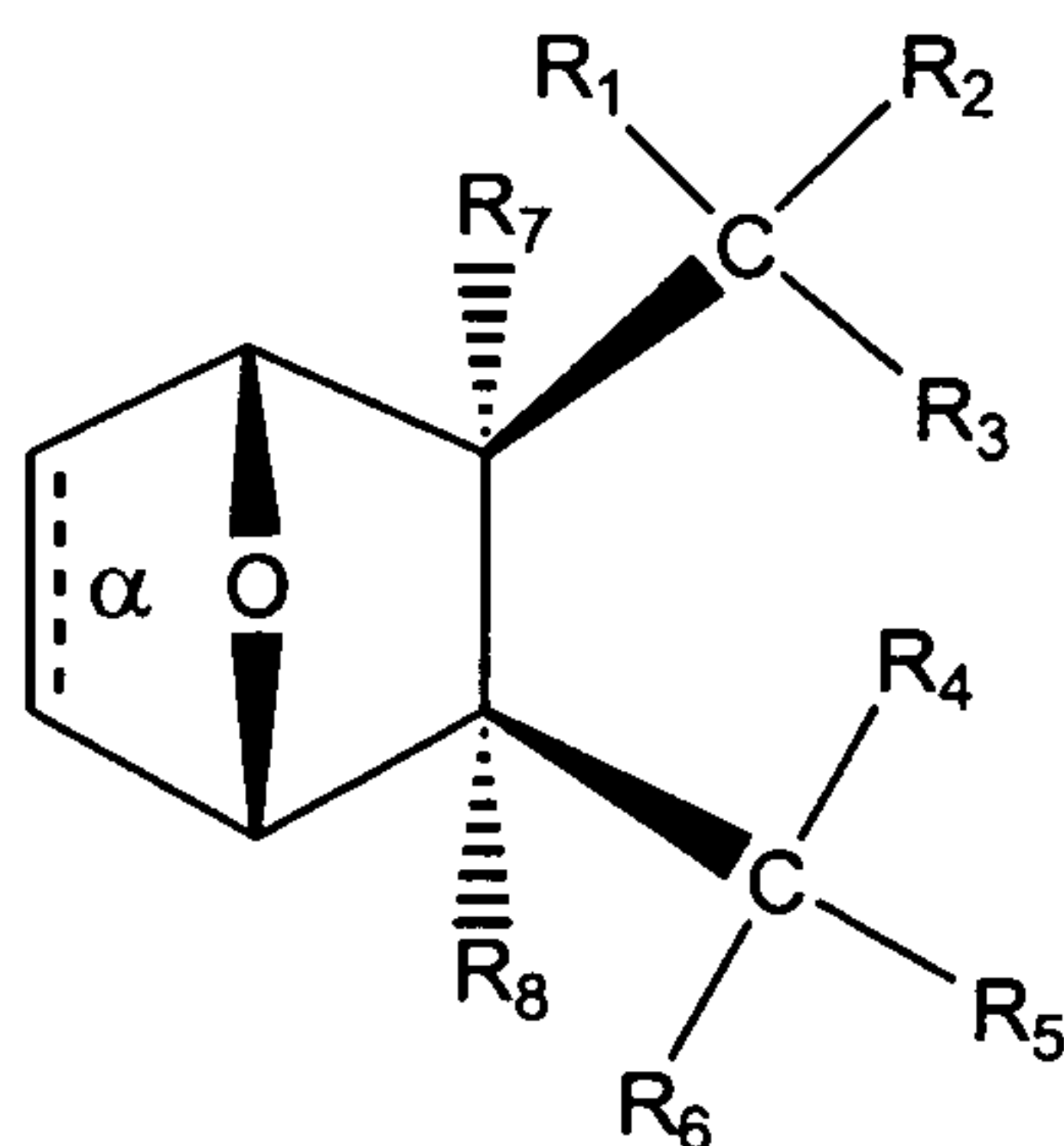
R<sub>5</sub> and R<sub>6</sub> is each independently H, OH, or R<sub>5</sub> and R<sub>6</sub> taken together are =O; and

R<sub>7</sub> and R<sub>8</sub> is each independently H, F, Cl, Br, SO<sub>2</sub>Ph, CO<sub>2</sub>CH<sub>3</sub>, or SR<sub>12</sub>,

where R<sub>12</sub> is H, aryl or a substituted or unsubstituted alkyl, alkenyl or alkynyl,

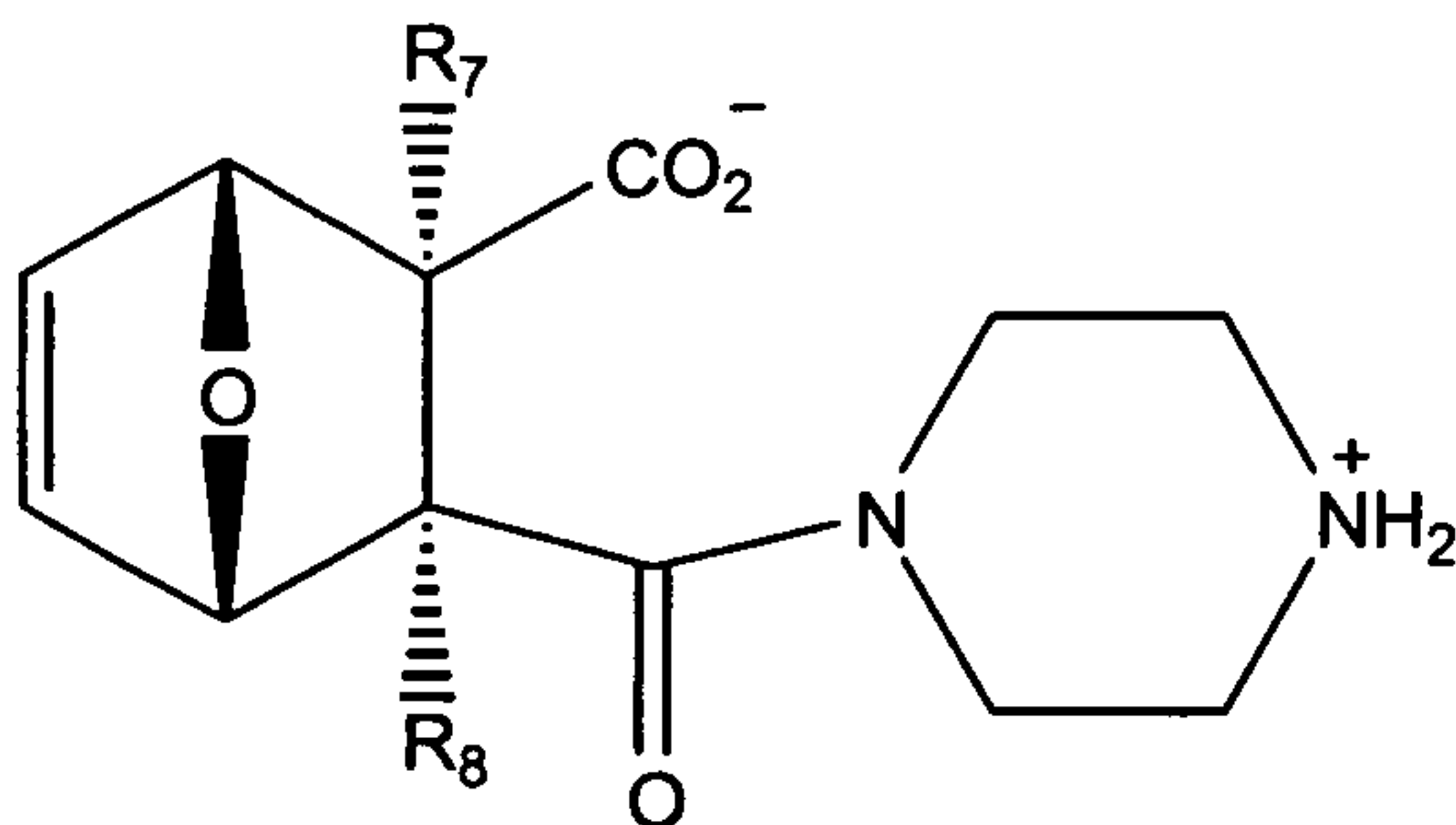
or a salt, enantiomer or zwitterion of the compound.

2. The compound of claim 1, wherein the compound has the structure

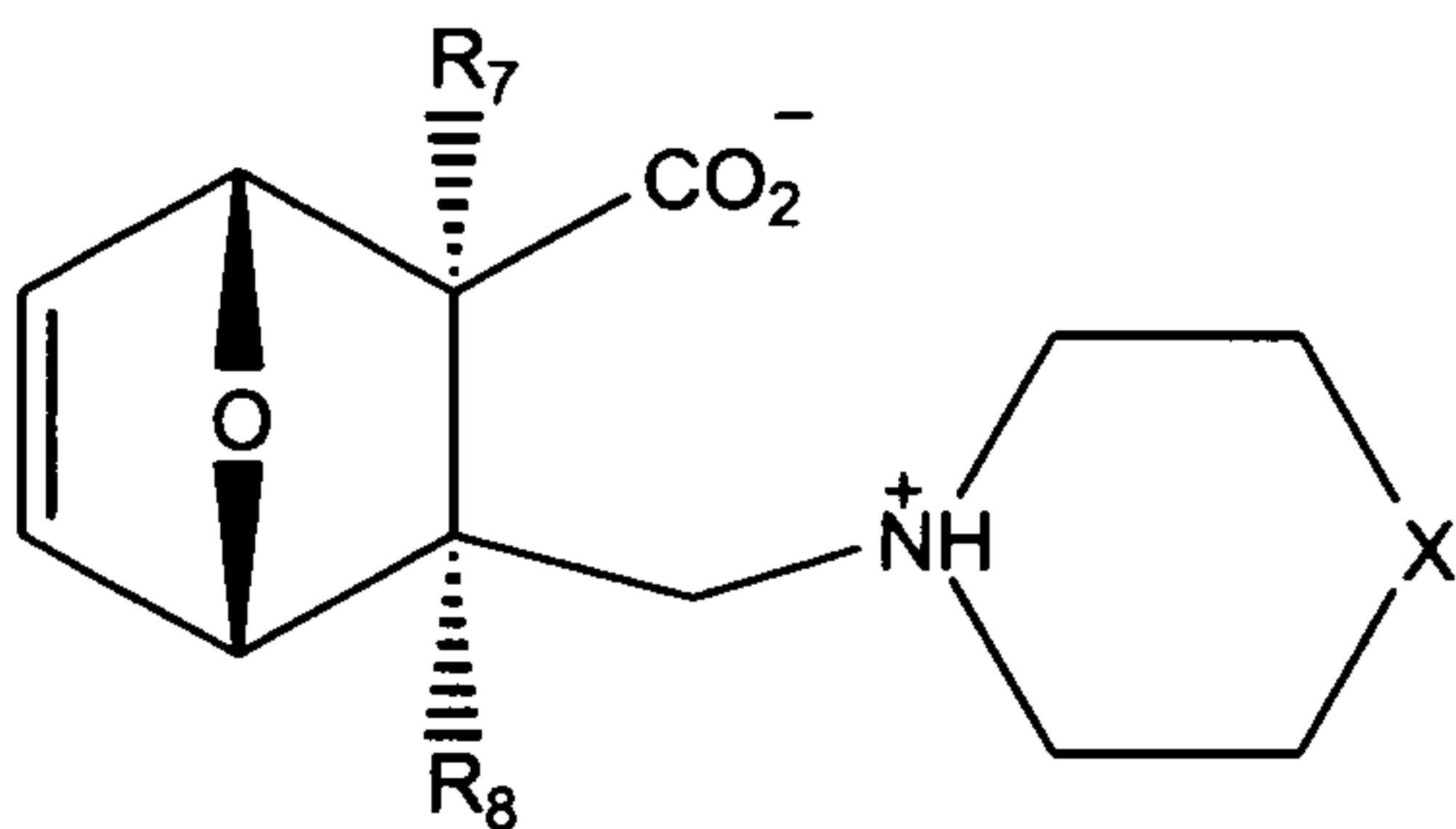


- 95 -

3. The compound of claim 2, wherein the compound has the structure



4. The compound of claim 2, wherein the compound has the structure



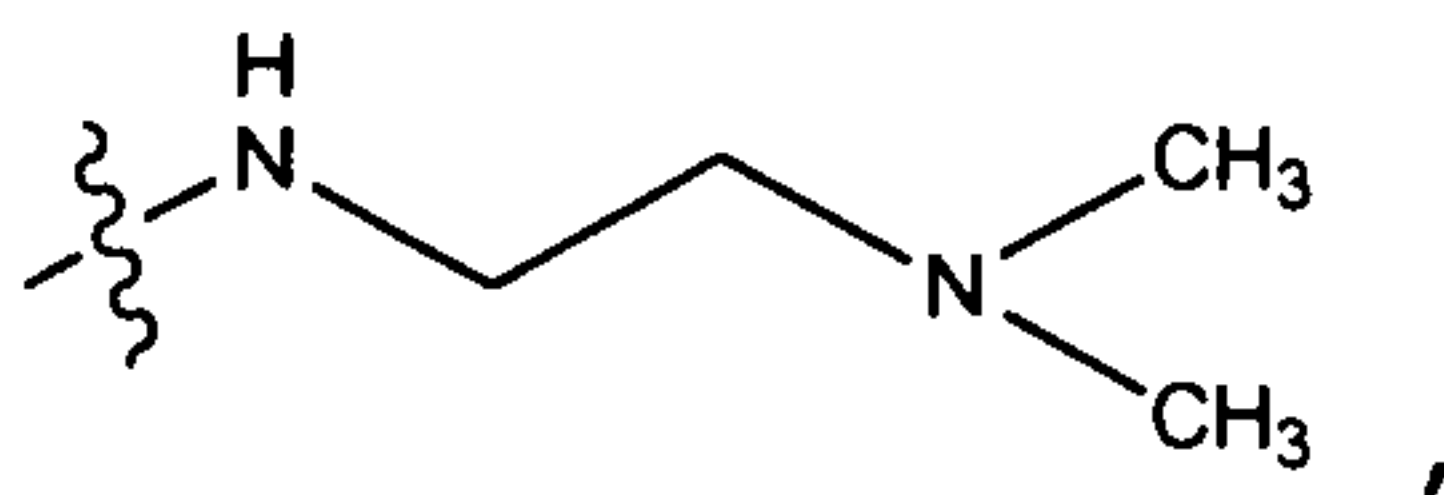
5. The compound of claim 1 or 2, wherein bond  $\alpha$  is present.
6. The compound of claim 1 or 2, wherein bond  $\alpha$  is absent.
7. The compound of claim 5 or 6, wherein

$R_1$  and  $R_2$  together are  $=O$ ;

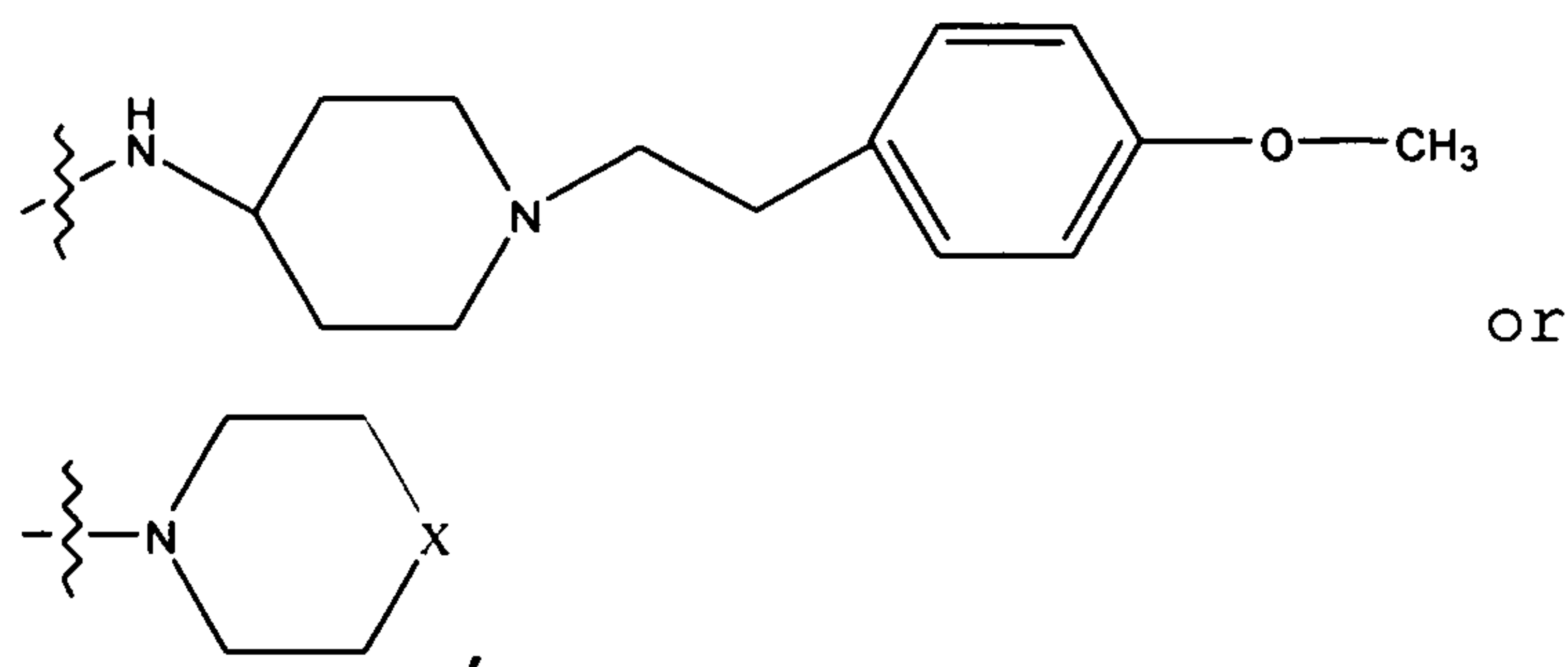
$R_3$  is  $O^-$  or  $OR_9$ ,

where  $R_9$  is H, methyl, ethyl or phenyl;

$R_4$  is



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where X is O, S, NR<sub>10</sub>, or N<sup>+</sup>R<sub>10</sub>R<sub>10</sub>,

where each R<sub>10</sub> is independently H, alkyl, substituted C<sub>2</sub>-C<sub>12</sub> alkyl, alkenyl, substituted C<sub>4</sub>-C<sub>12</sub> alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl where the substituent is other than chloro,



-CH<sub>2</sub>CN, -CH<sub>2</sub>CO<sub>2</sub>R<sub>11</sub>, -CH<sub>2</sub>COR<sub>11</sub>, -NHR<sub>11</sub> or -NH<sup>+</sup>(R<sub>11</sub>)<sub>2</sub>,

where R<sub>11</sub> is alkyl, alkenyl or alkynyl, each of which is substituted or unsubstituted, or H;

R<sub>5</sub> and R<sub>6</sub> taken together are =O; and

R<sub>7</sub> and R<sub>8</sub> is each independently H, F, Cl, Br, SO<sub>2</sub>Ph, CO<sub>2</sub>CH<sub>3</sub>, or SR<sub>12</sub>,

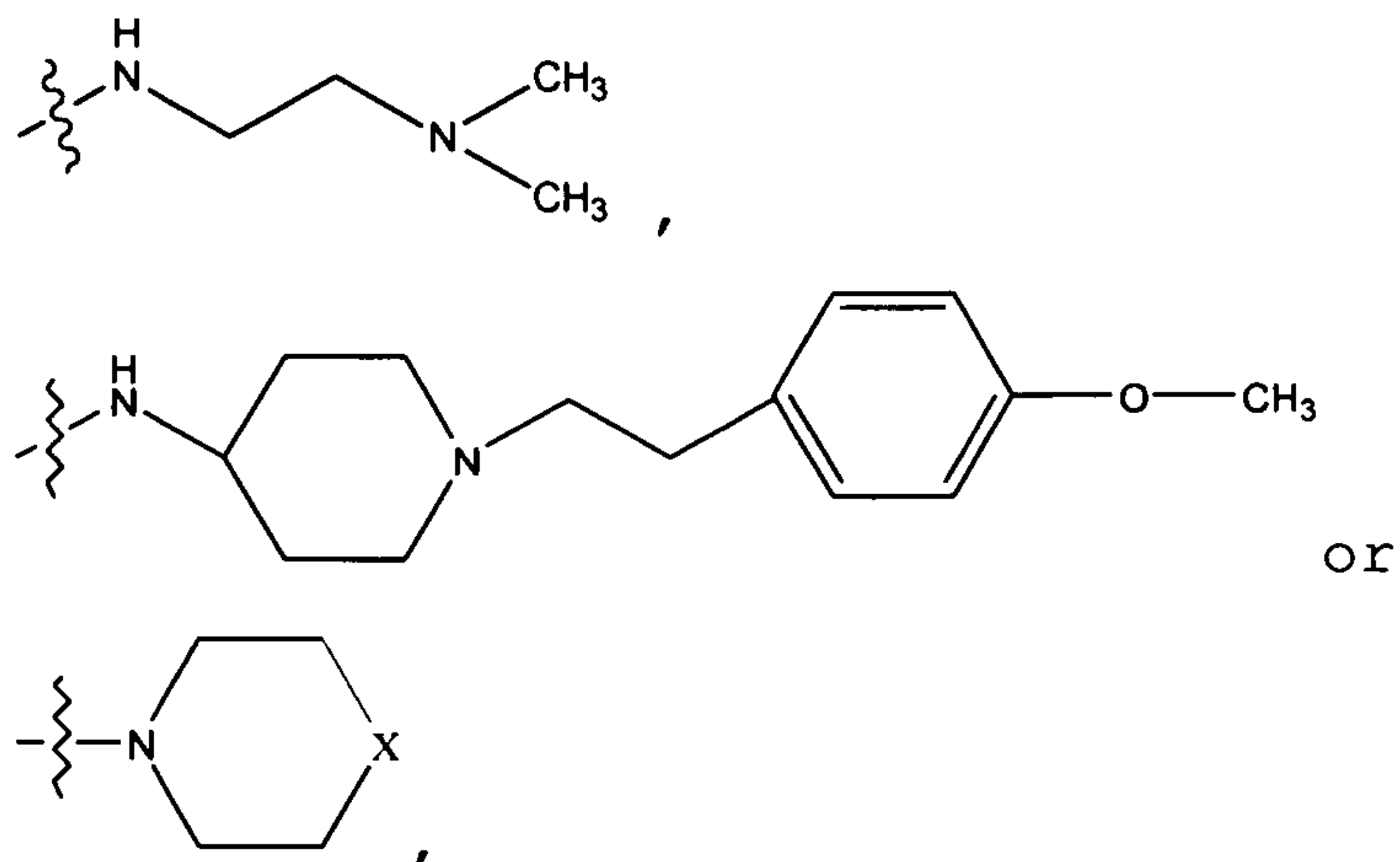
where R<sub>12</sub> is a substituted or unsubstituted alkyl, alkenyl or alkynyl.

8. The compound of any of claims 1-7, wherein R<sub>3</sub> is O<sup>-</sup>.

9. The compound of any of claims 1-8,

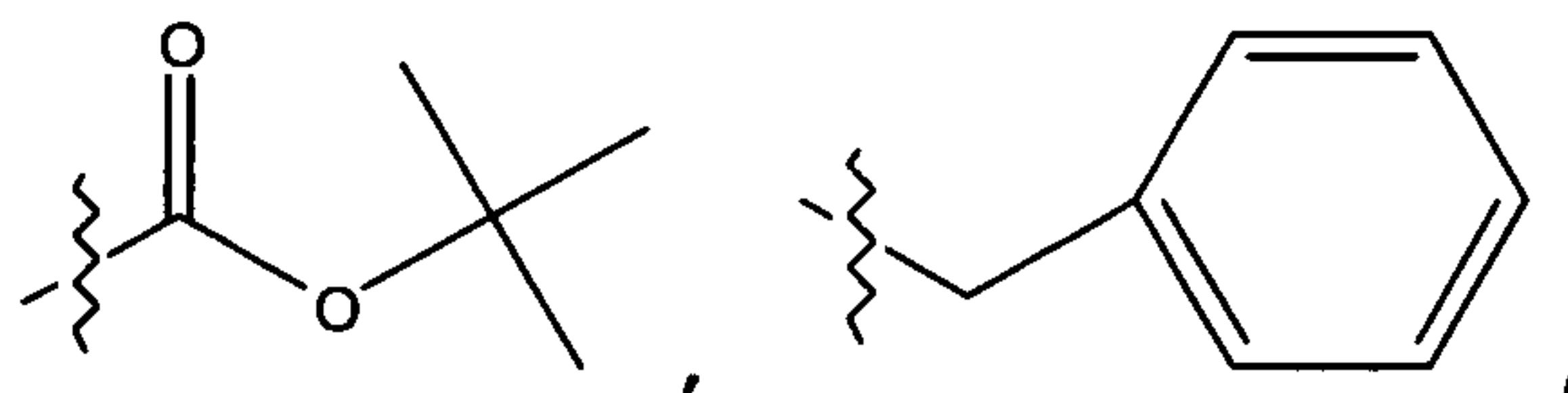
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wherein  $R_4$  is



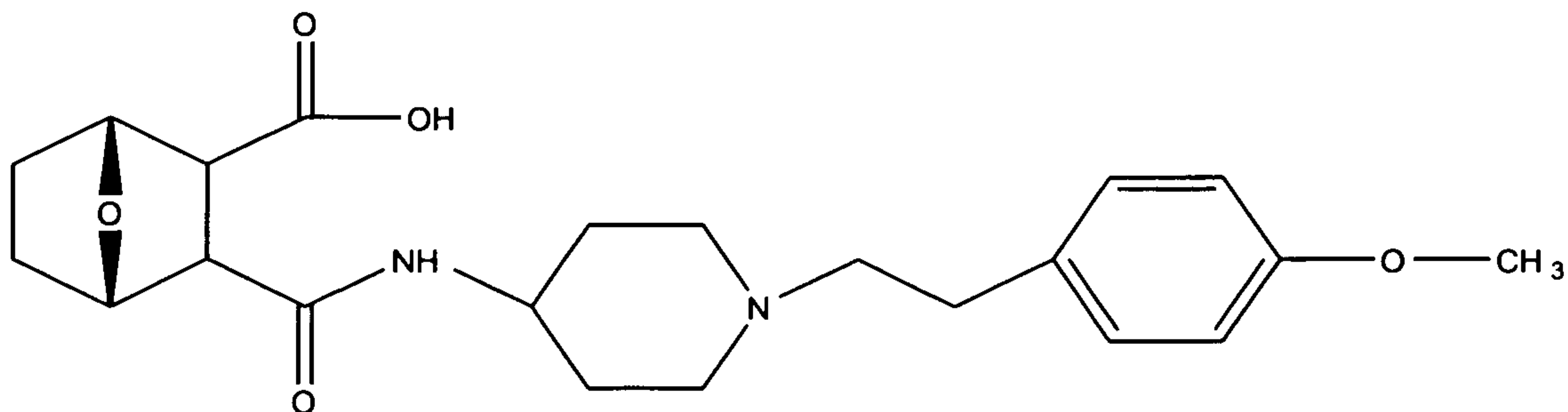
where X is O,  $NR_{10}$ ,  $N^+R_{10}R_{10}$

where each  $R_{10}$  is independently H, alkyl, substituted  $C_2$ - $C_{12}$  alkyl, alkenyl, substituted  $C_4$ - $C_{12}$  alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl where the substituent is other than chloro when  $R_1$  and  $R_2$  are =O,

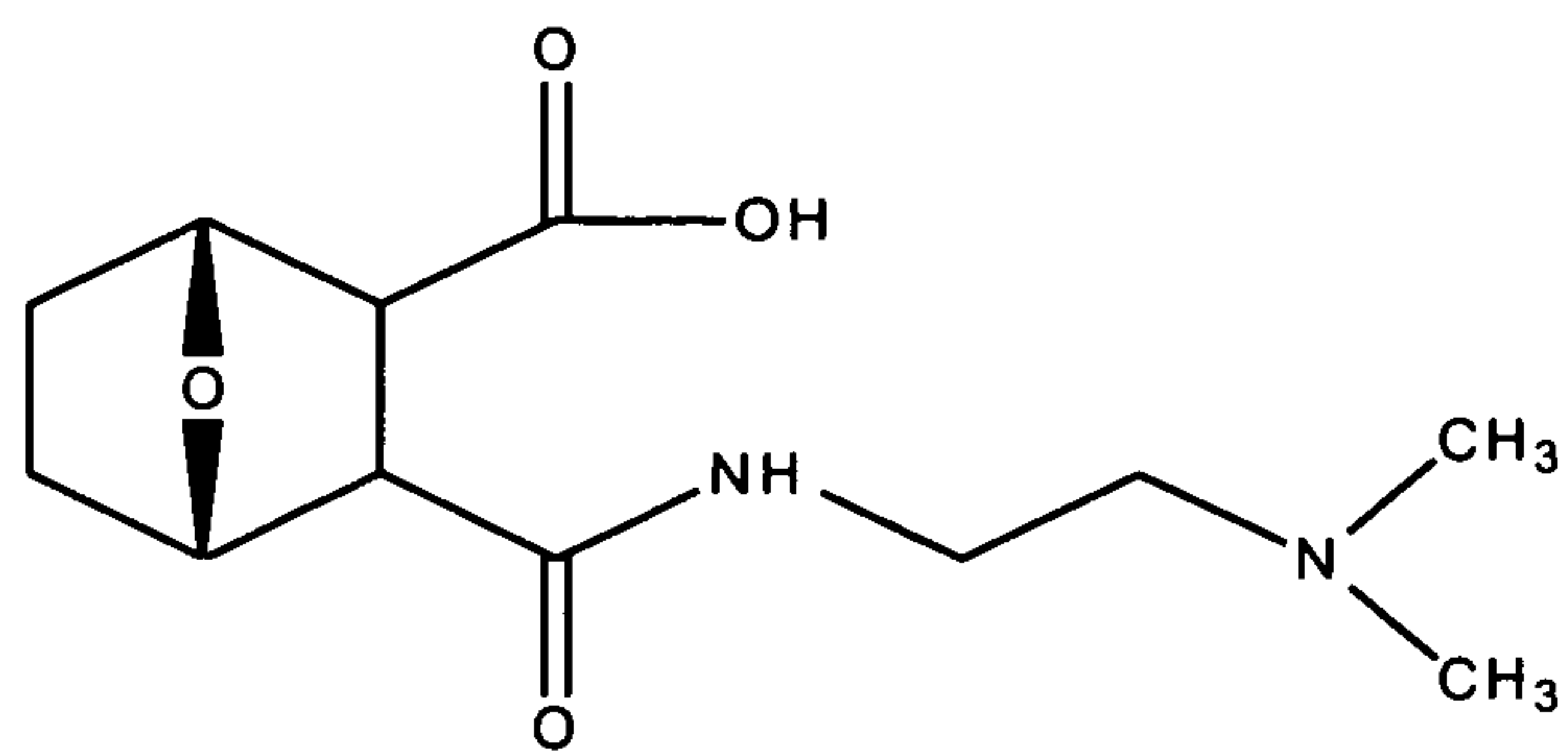
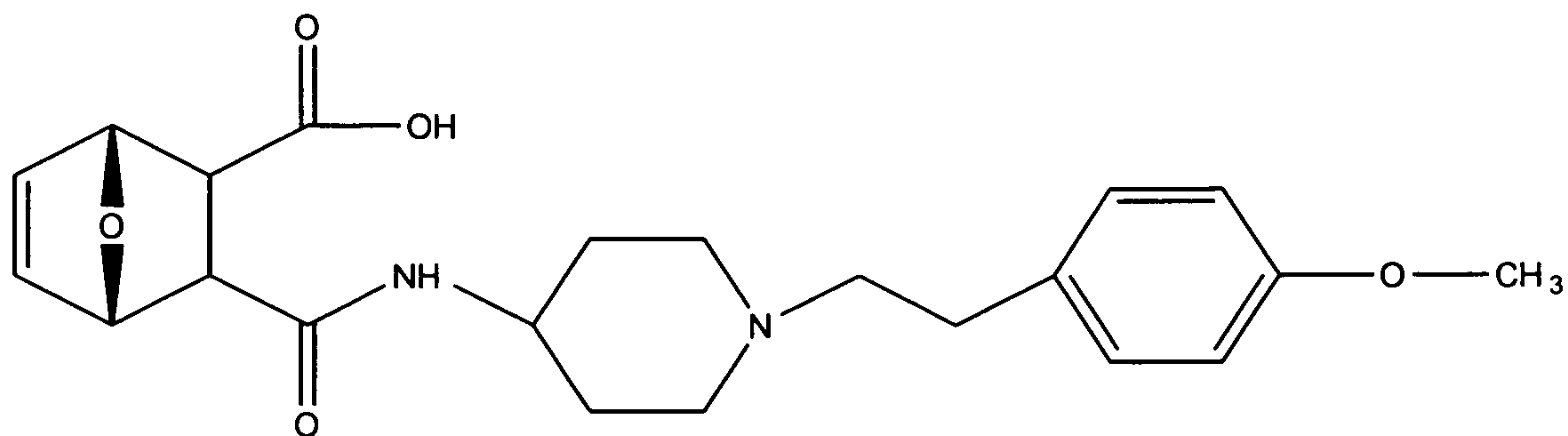


$-CH_2CN$ ,  $-CH_2CO_2R_{11}$ ,  $-CH_2COR_{11}$ ,  $-NHR_{11}$  or  $-NH^+(R_{11})_2$ ,  
where  $R_{11}$  is H or alkyl.

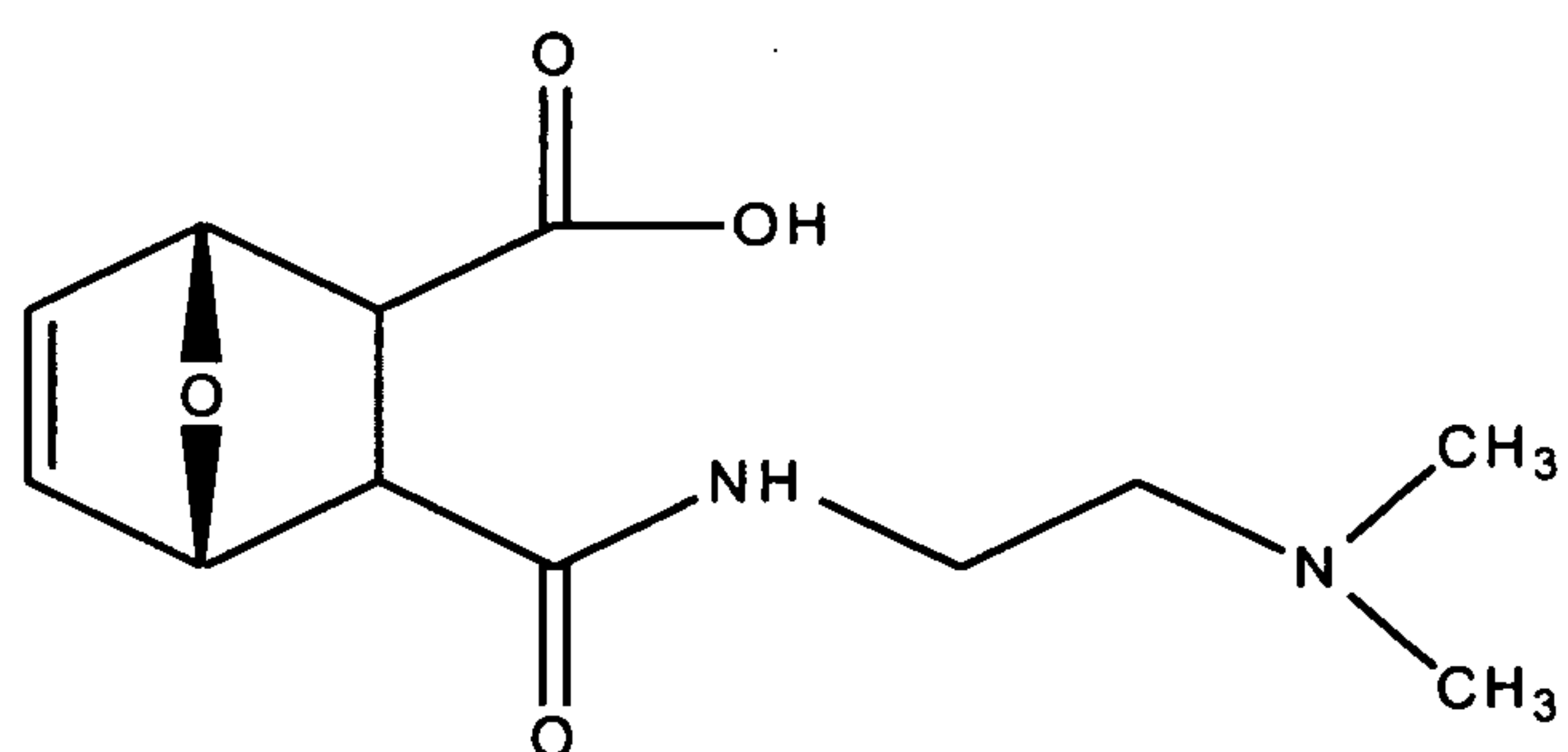
10. The compound of claim 9, having the structure



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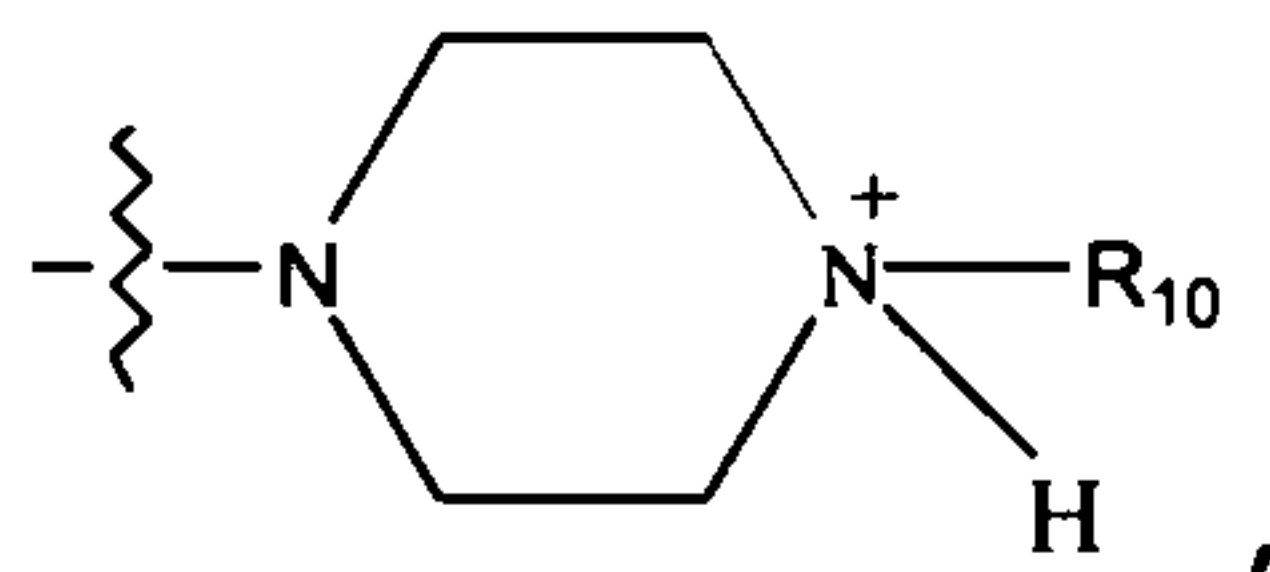


, or



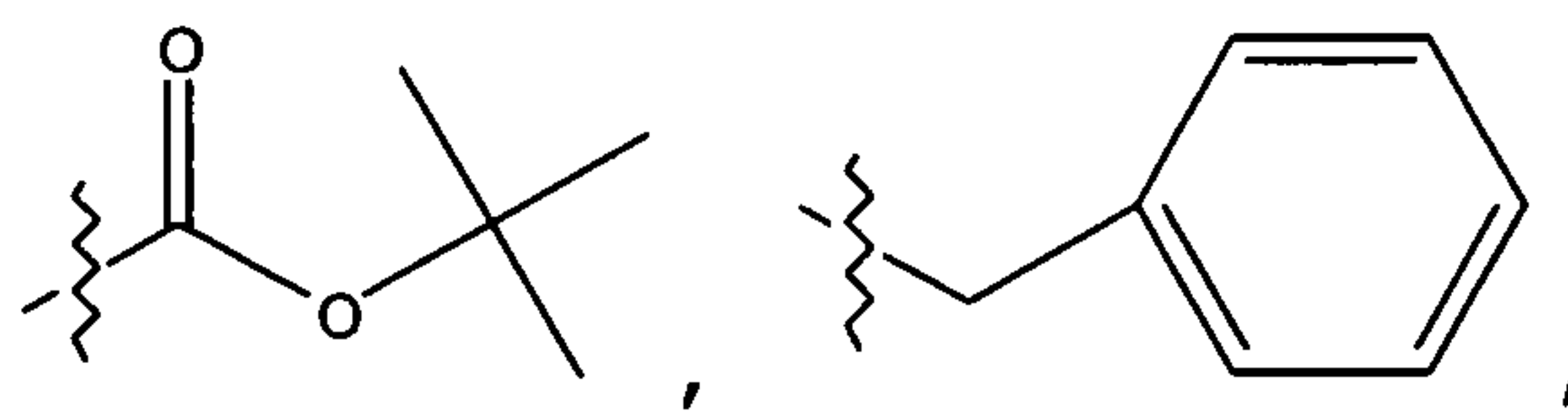
11. The compound of any of claims 1-9,

wherein R<sub>4</sub> is



where R<sub>10</sub> is H, alkyl, substituted C<sub>2</sub>-C<sub>12</sub> alkyl, alkenyl, substituted C<sub>4</sub>-C<sub>12</sub> alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl where the substituent is other than chloro when R<sub>1</sub> and R<sub>2</sub> are =O,

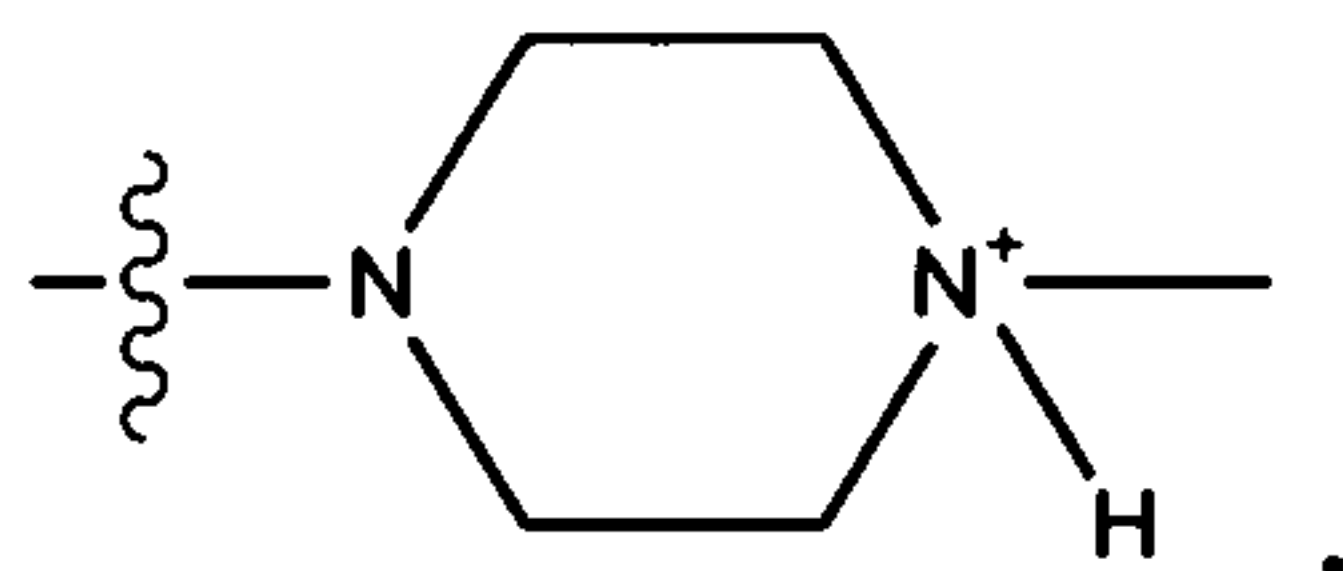
- 99 -



-CH<sub>2</sub>CN, -CH<sub>2</sub>CO<sub>2</sub>R<sub>11</sub>, -CH<sub>2</sub>COR<sub>11</sub>, -NHR<sub>11</sub> or -NH<sup>+</sup>(R<sub>11</sub>)<sub>2</sub>,  
where R<sub>11</sub> is H or alkyl.

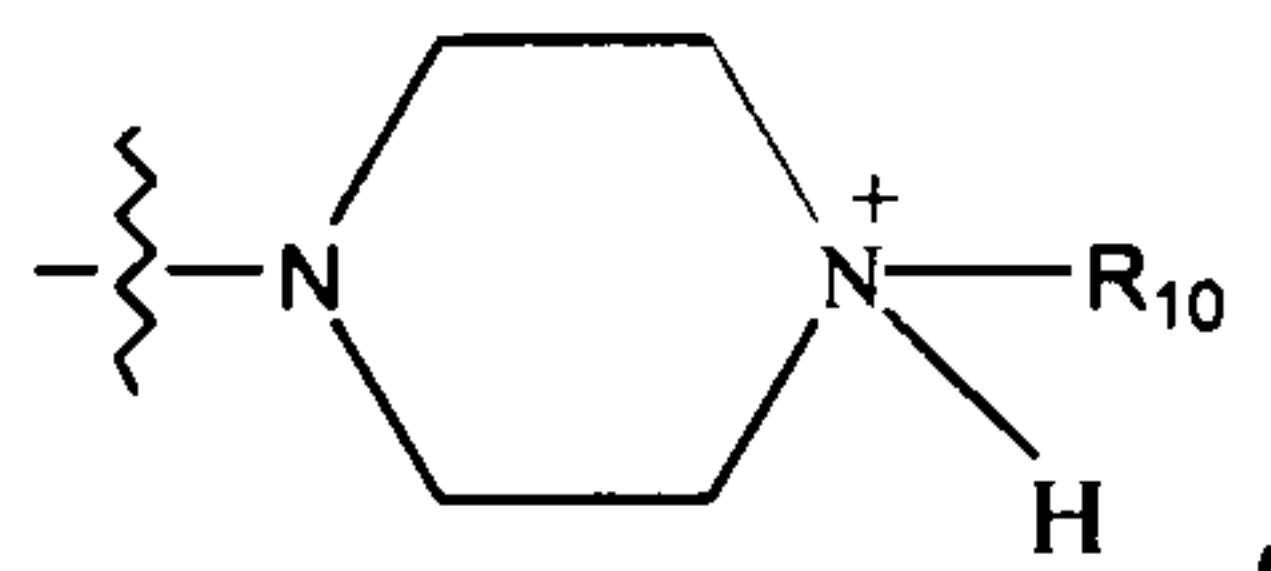
12. The compound of any of claims 1-9 or 11,

wherein R<sub>4</sub> is

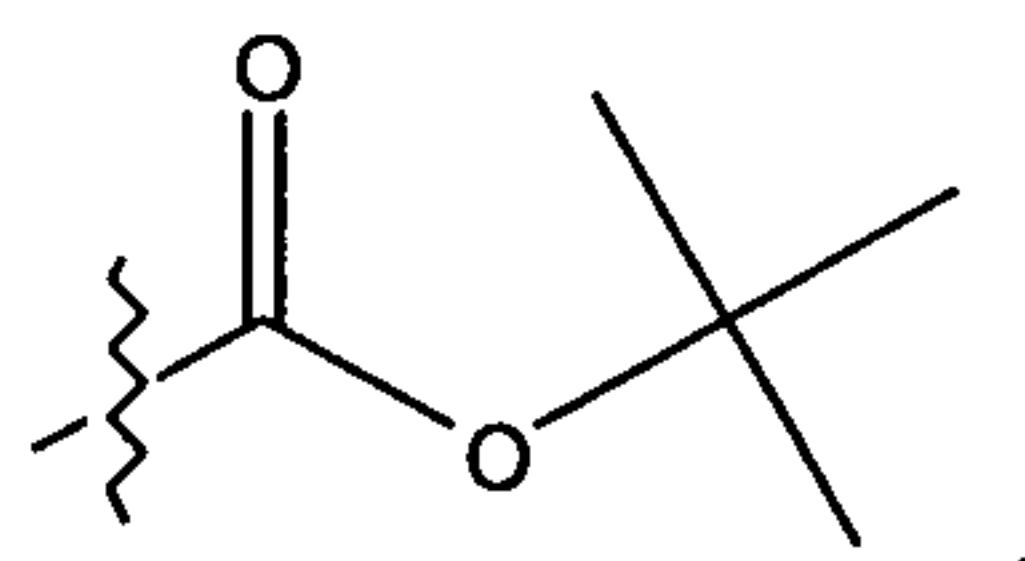


13. The compound of any of claims 1-9 or 11,

wherein R<sub>4</sub> is

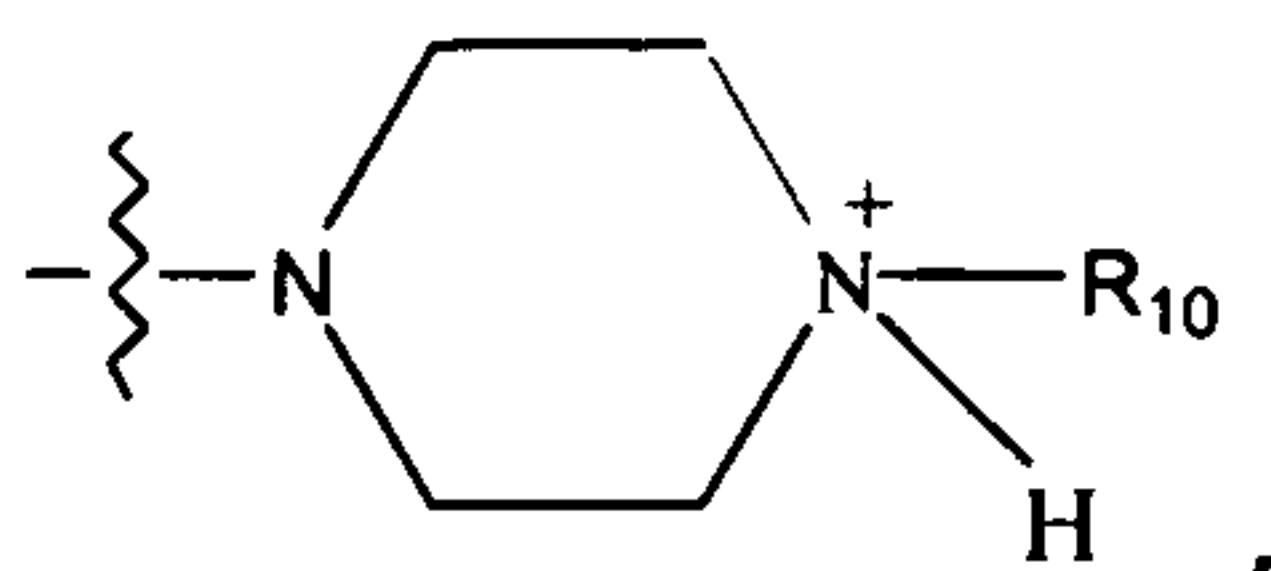


where R<sub>10</sub> is



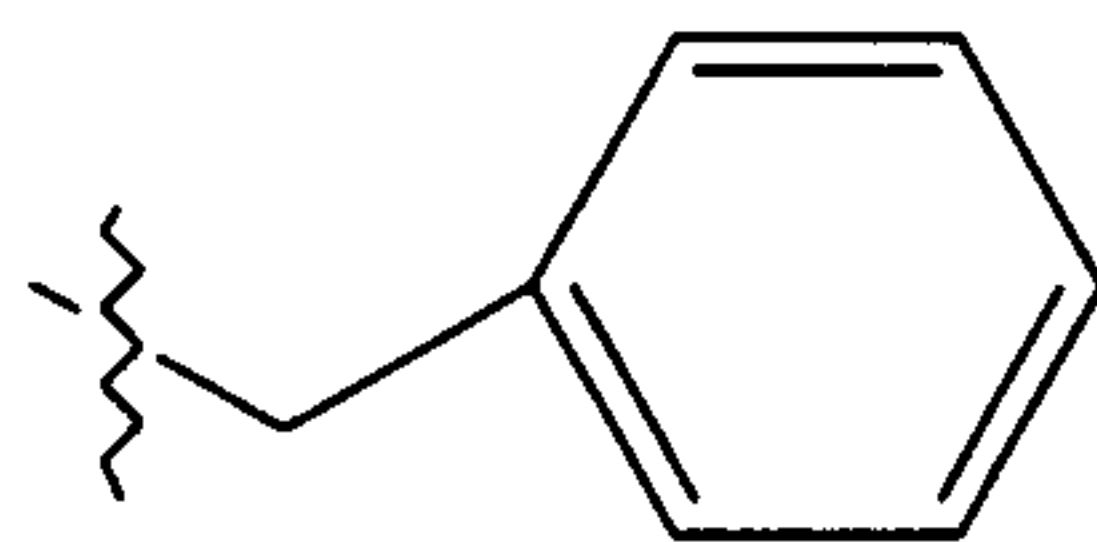
14. The compound of any of claims 1-9 or 11,

wherein R<sub>4</sub> is



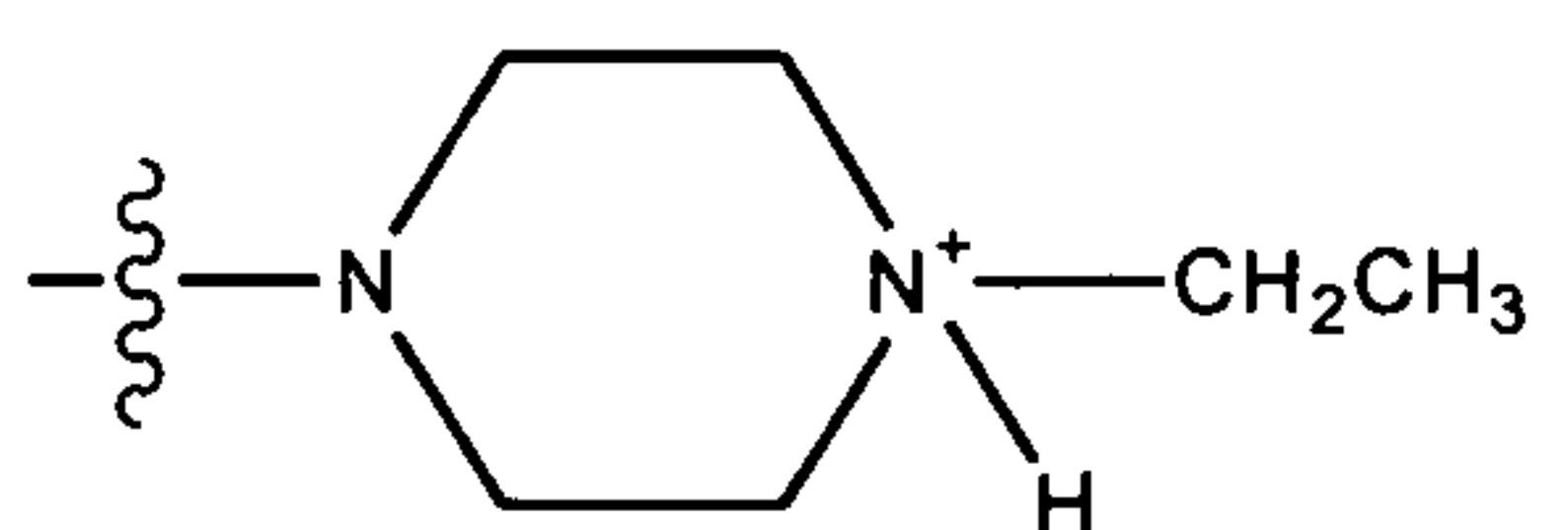
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where  $R_{10}$  is



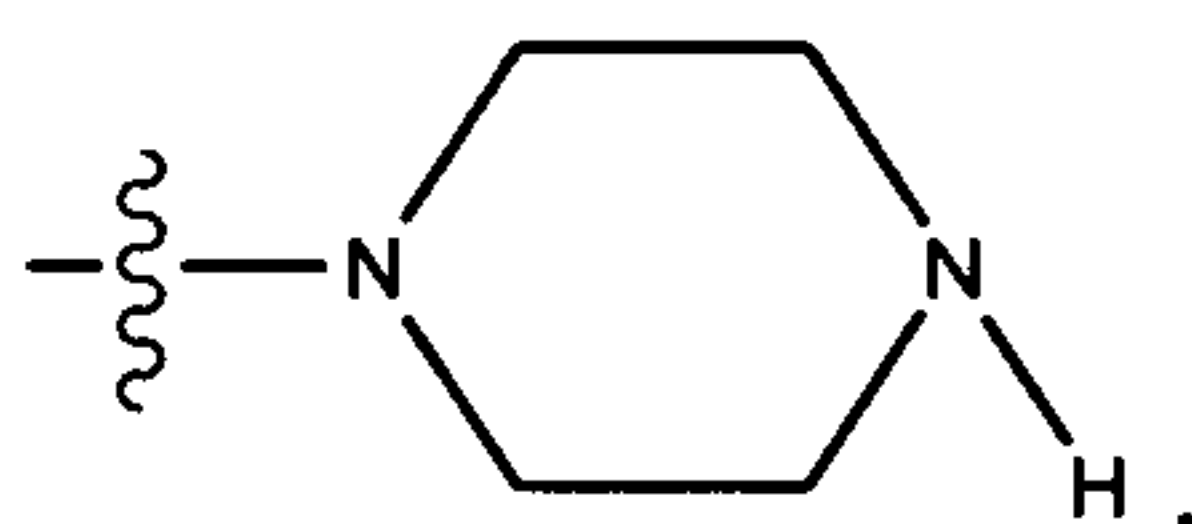
15. The compound of any of claims 1-9 or 11,

wherein  $R_4$  is



16. The compound of any of claims 1-9 or 11,

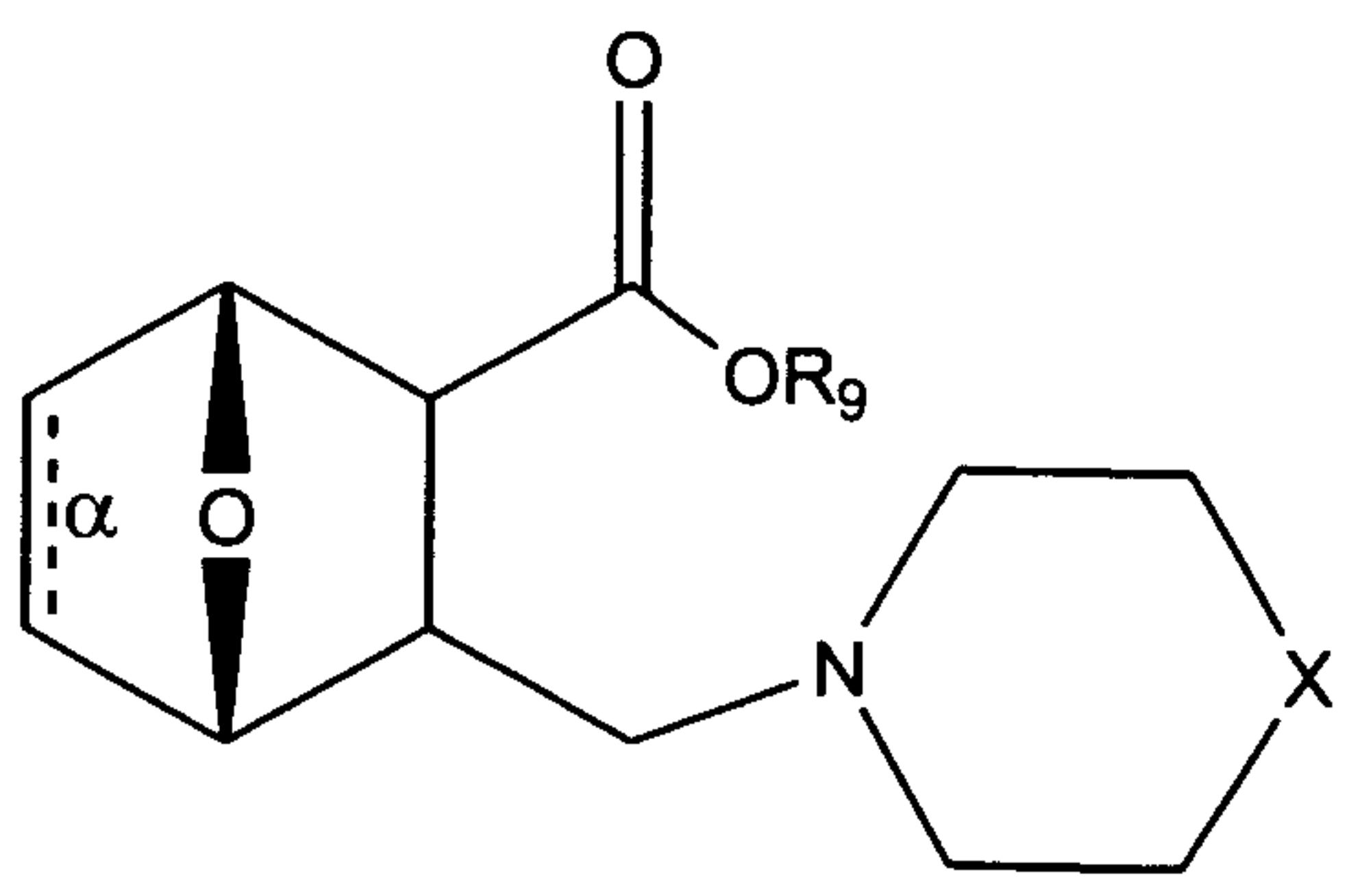
wherein  $R_4$  is



17. The compound of any one of claims 1-9 or 11-16, wherein  $R_5$  and  $R_6$  together are =O.

18. The compound of any one of claims 1-9 or 11-17, wherein  $R_7$  and  $R_8$  are each H.

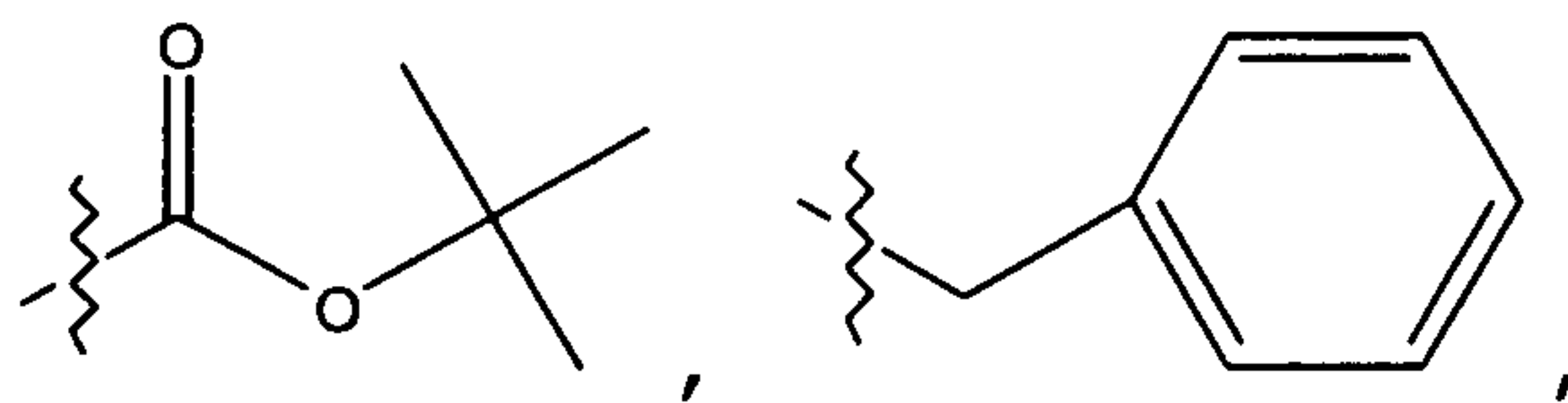
19. The compound of claim 1,



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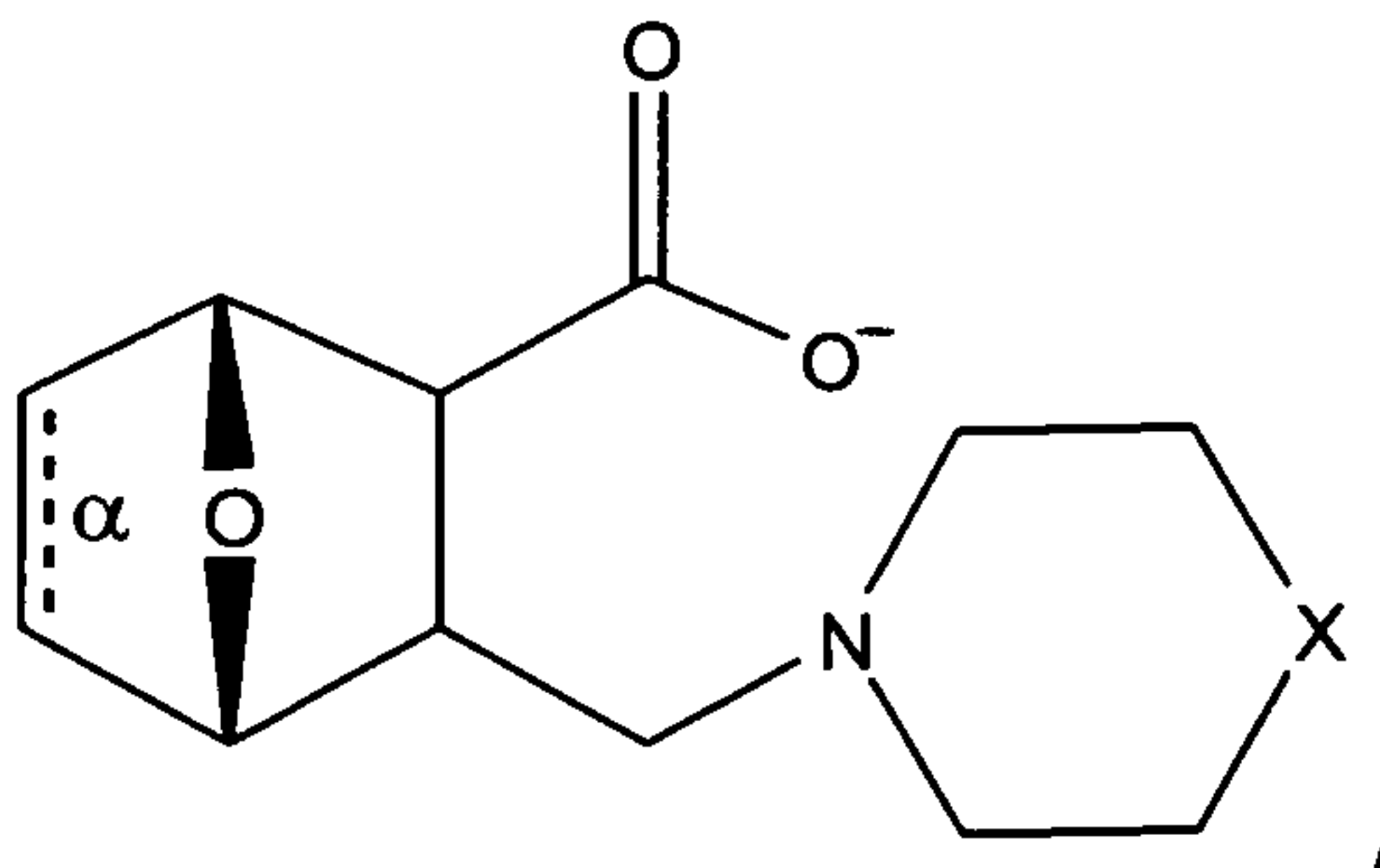
wherein bond  $\alpha$  is present or absent;  $R_9$  is present or absent and when present is H,  $C_1$ - $C_{10}$  alkyl,  $C_2$ - $C_{10}$  alkenyl or phenyl; and X is O, S,  $NR_{10}$  or  $N^+R_{10}R_{10}$ ,

where each  $R_{10}$  is independently H, alkyl, substituted  $C_2$ - $C_{12}$  alkyl, alkenyl, substituted  $C_4$ - $C_{12}$  alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl where the substituent is other than chloro,



$-CH_2CO_2R_{11}$ ,  $-CH_2COR_{11}$ ,  $-CH_2CN$ , or  $-CH_2CH_2R_{16}$ , where  $R_{11}$  is H or alkyl, and where  $R_{16}$  is any substituent that is a precursor to an aziridinyl intermediate, or a salt, zwitterion or enantiomer of the compound.

20. The compound of claim 19 having the structure



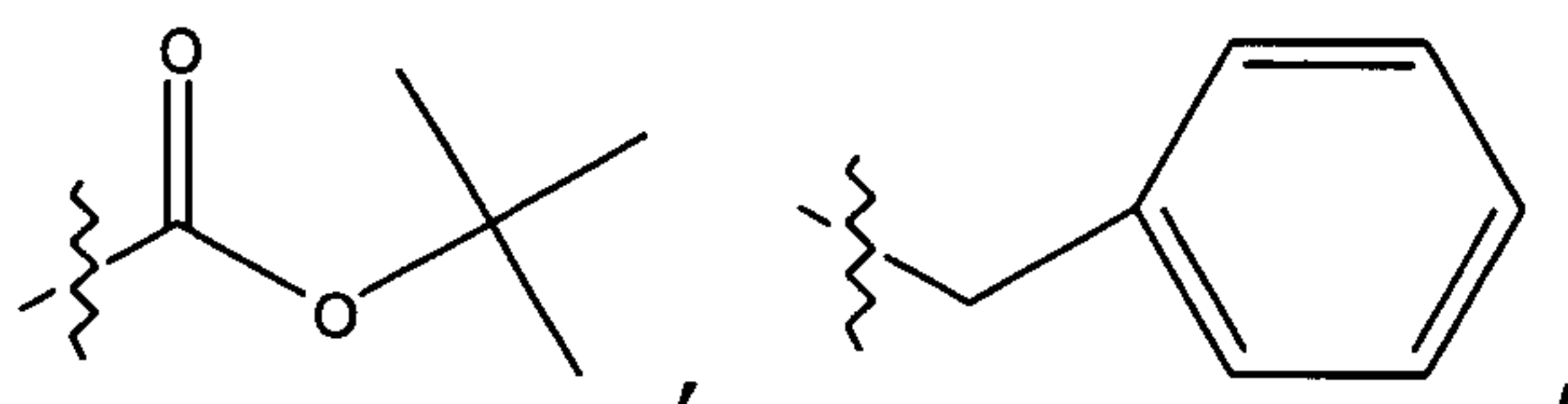
wherein,

bond  $\alpha$  is present or absent;

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X is O, S, NR<sub>10</sub> or N<sup>+</sup>R<sub>10</sub>R<sub>10</sub>,

where each R<sub>10</sub> is independently H, alkyl, substituted C<sub>2</sub>-C<sub>12</sub> alkyl, alkenyl, substituted C<sub>4</sub>-C<sub>12</sub> alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl where the substituent is other than chloro,



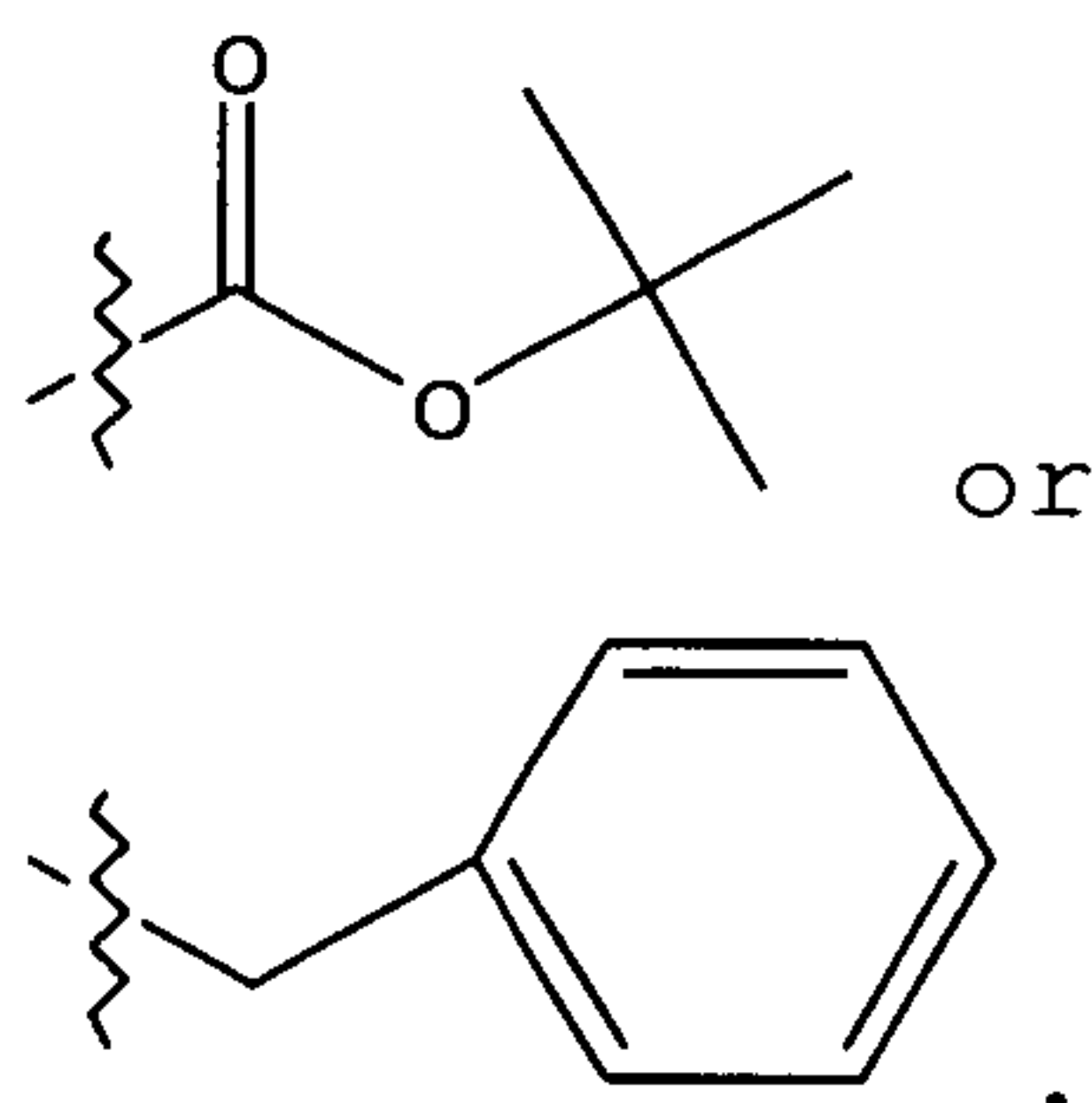
-CH<sub>2</sub>CO<sub>2</sub>R<sub>11</sub>, -CH<sub>2</sub>COR<sub>11</sub>, -CH<sub>2</sub>CN, or -CH<sub>2</sub>CH<sub>2</sub>R<sub>16</sub>, where R<sub>11</sub> is H or alkyl, and where R<sub>16</sub> is any substituent that is a aziridinyll intermediate,

or a salt, zwitterion or enantiomer of a compound.

21. The compound of claim 20, wherein

X is O or NH<sup>+</sup>R<sub>10</sub>,

where R<sub>10</sub> is H, alkyl, substituted C<sub>2</sub>-C<sub>12</sub> alkyl, alkenyl, substituted C<sub>4</sub>-C<sub>12</sub> alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl where the substituent is other than chloro,



22. The compound of claim 20, wherein X is -CH<sub>2</sub>CH<sub>2</sub>R<sub>16</sub>, where R<sub>16</sub> is any substituent that is a precursor to an aziridinyll intermediate.

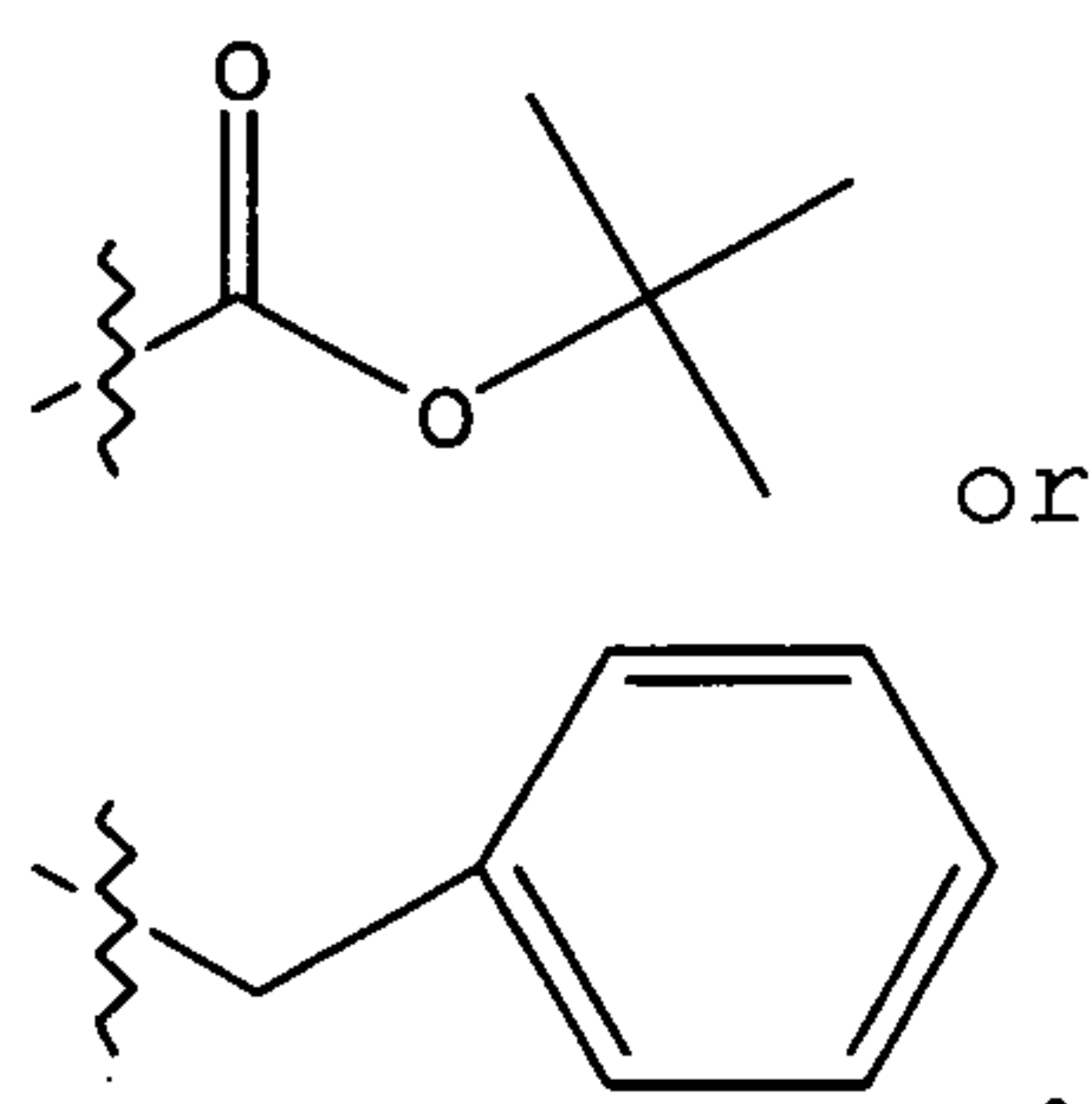
- 103 -

23. The compound of claim 20 or 21, wherein X is O.

24. The compound of claim 20 or 21, wherein

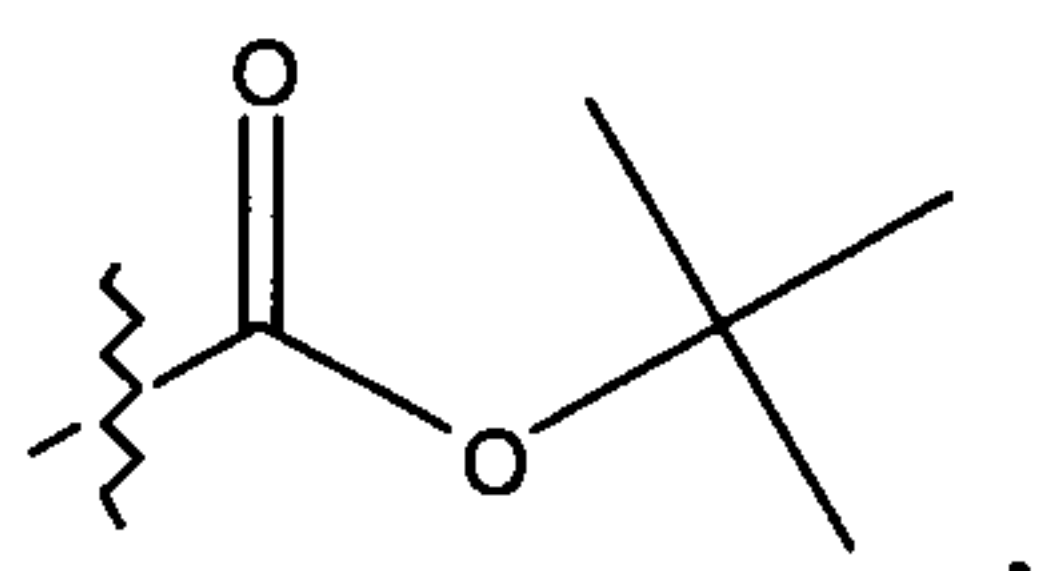
X is  $\text{NH}^+\text{R}_{10}$ ,

where  $\text{R}_{10}$  H, alkyl, substituted  $\text{C}_2\text{-C}_{12}$  alkyl, alkenyl, substituted  $\text{C}_4\text{-C}_{12}$  alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl where the substituent is other than chloro,

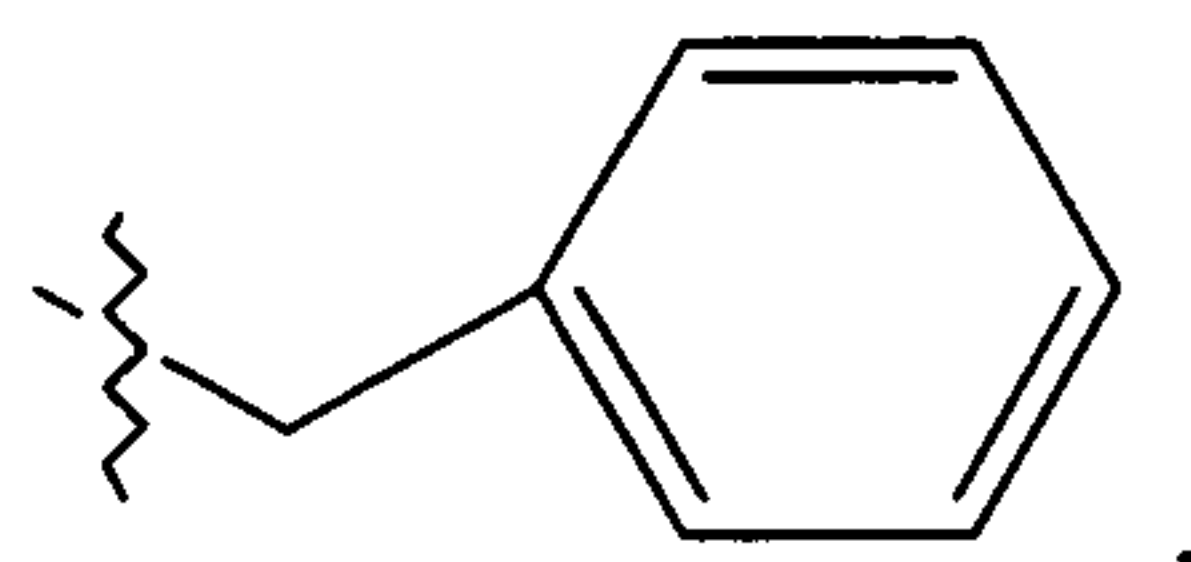


25. The compound of claim 20, 21 or 24, wherein  $\text{R}_{10}$  is methyl.

26. The compound of claim 20, 21 or 24, wherein  $\text{R}_{10}$  is



27. The compound of claim 20, 21 or 24, wherein  $\text{R}_{10}$  is

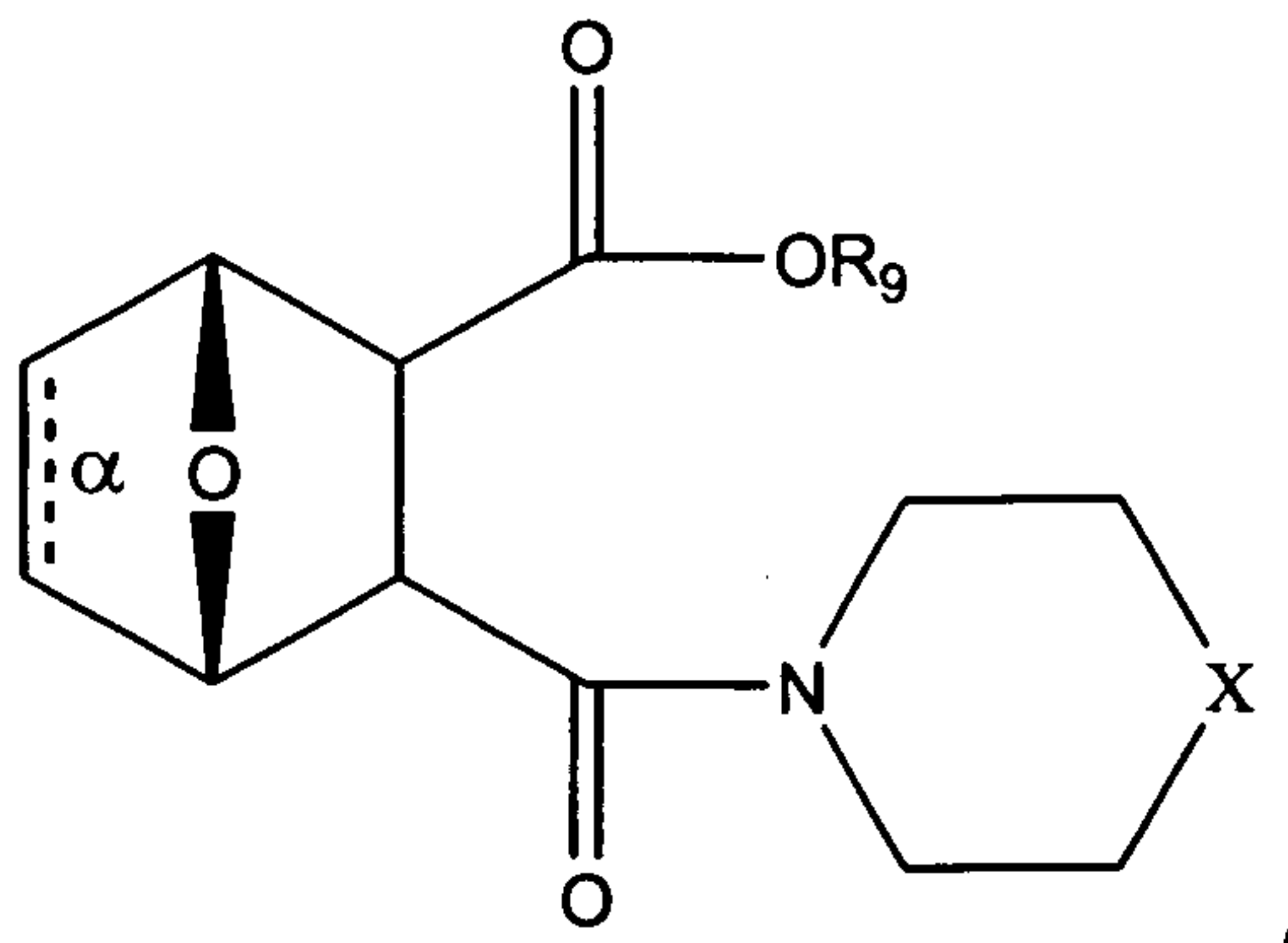


28. The compound of claim 20, 21 or 24, wherein  $\text{R}_{10}$  is ethyl.

29. The compound of claim 20, 21 or 24, wherein  $\text{R}_{10}$  is absent.

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30. A compound having the structure



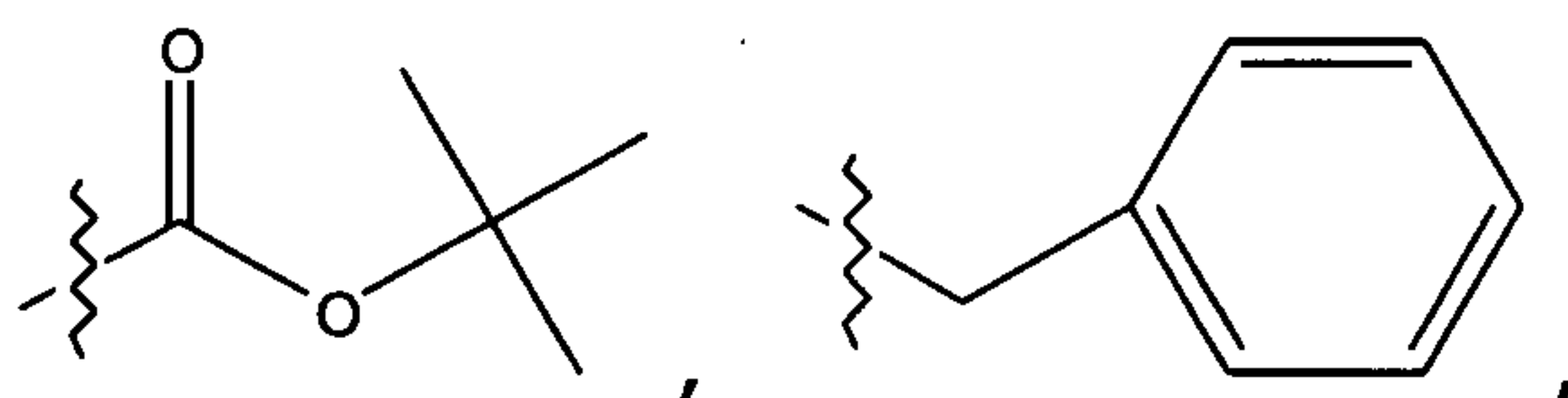
wherein

bond  $\alpha$  is present or absent;

$R_9$  is present or absent and when present is H, alkyl, alkenyl, alkynyl or phenyl; and

$X$  is O,  $NR_{10}$ , or  $N^+R_{10}R_{10}$ ,

where each  $R_{10}$  is independently H, alkyl, substituted  $C_2$ - $C_{12}$  alkyl, alkenyl, substituted  $C_4$ - $C_{12}$  alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl where the substituent is other than chloro,

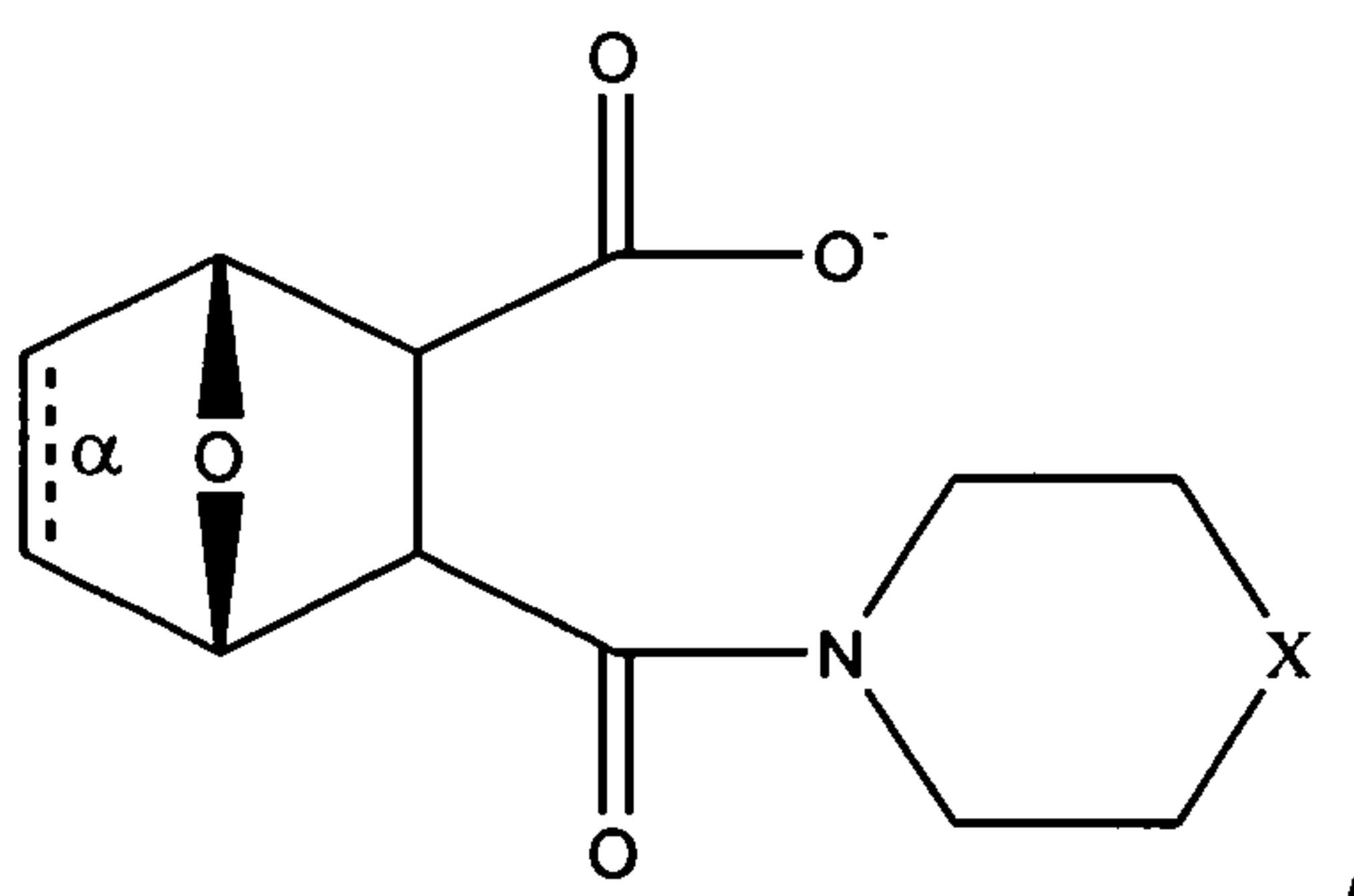


$-CH_2CN$ ,  $-CH_2CO_2R_{12}$ , or  $-CH_2COR_{12}$ , where  $R_{12}$  is H or alkyl,

or a salt, zwitterion, or enantiomer of the compound.

31. The compound of claim 30, having the structure

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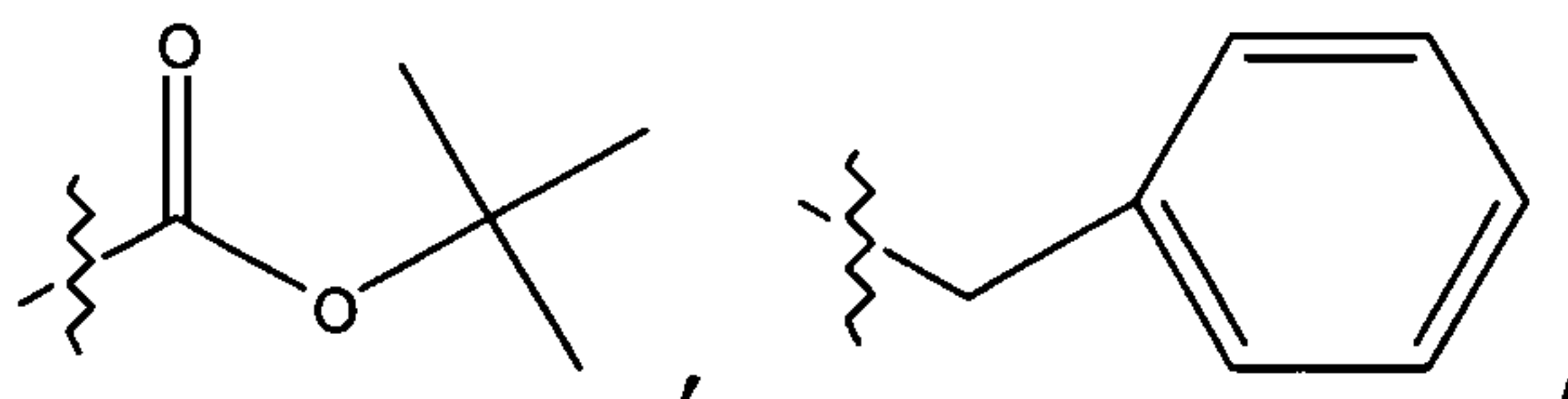


wherein

bond  $\alpha$  is present or absent;

$\text{X}$  is  $\text{O}$  or  $\text{NH}^+\text{R}_{10}$ ,

where  $\text{R}_{10}$  is  $\text{H}$ , alkyl, substituted  $\text{C}_2\text{-C}_{12}$  alkyl, alkenyl, substituted  $\text{C}_4\text{-C}_{12}$  alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl where the substituent is other than chloro,

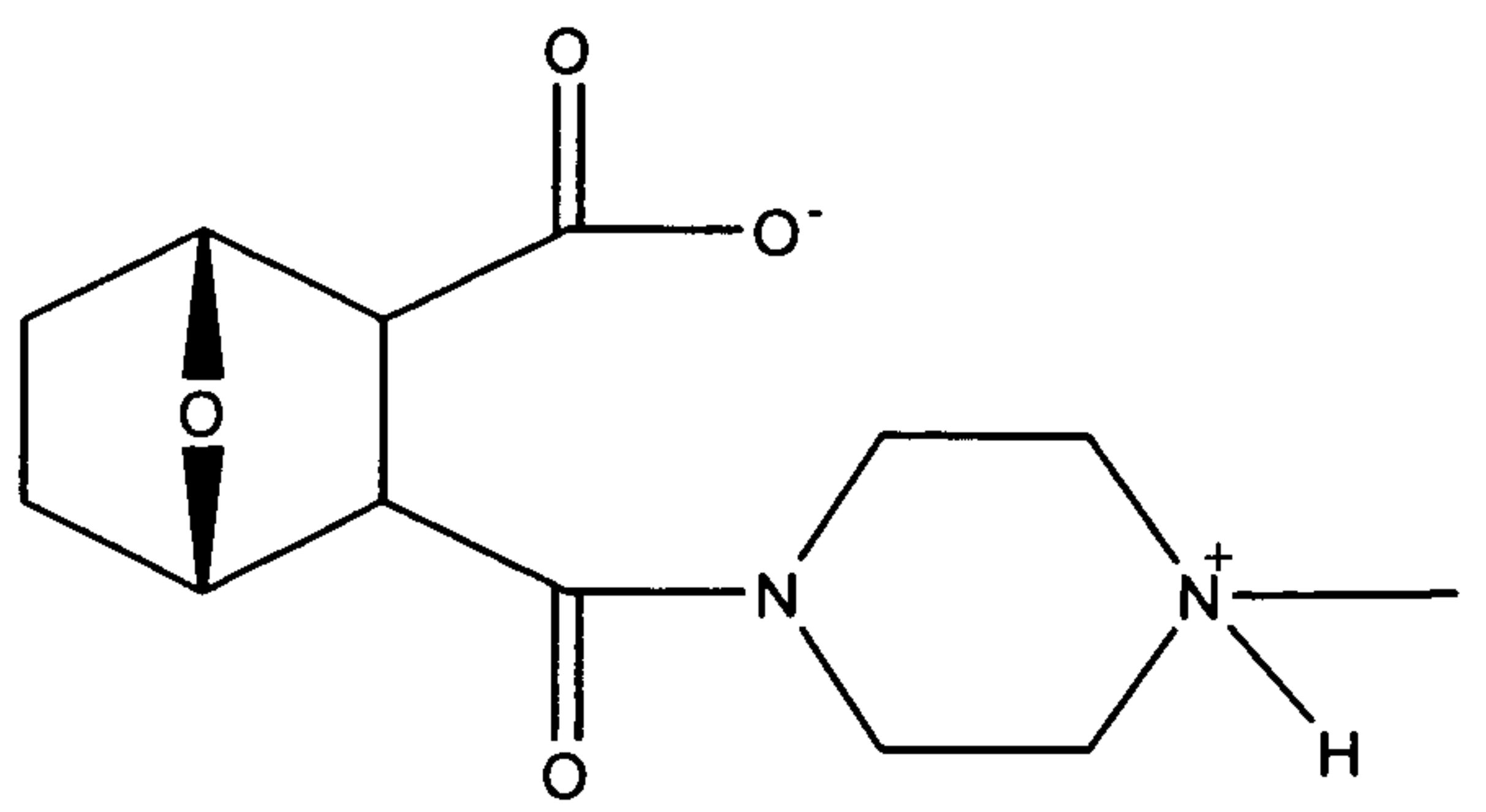


$-\text{CH}_2\text{CN}$ ,  $-\text{CH}_2\text{CO}_2\text{R}_{12}$ , or  $-\text{CH}_2\text{COR}_{12}$ , where  $\text{R}_{12}$  is  $\text{H}$  or alkyl.

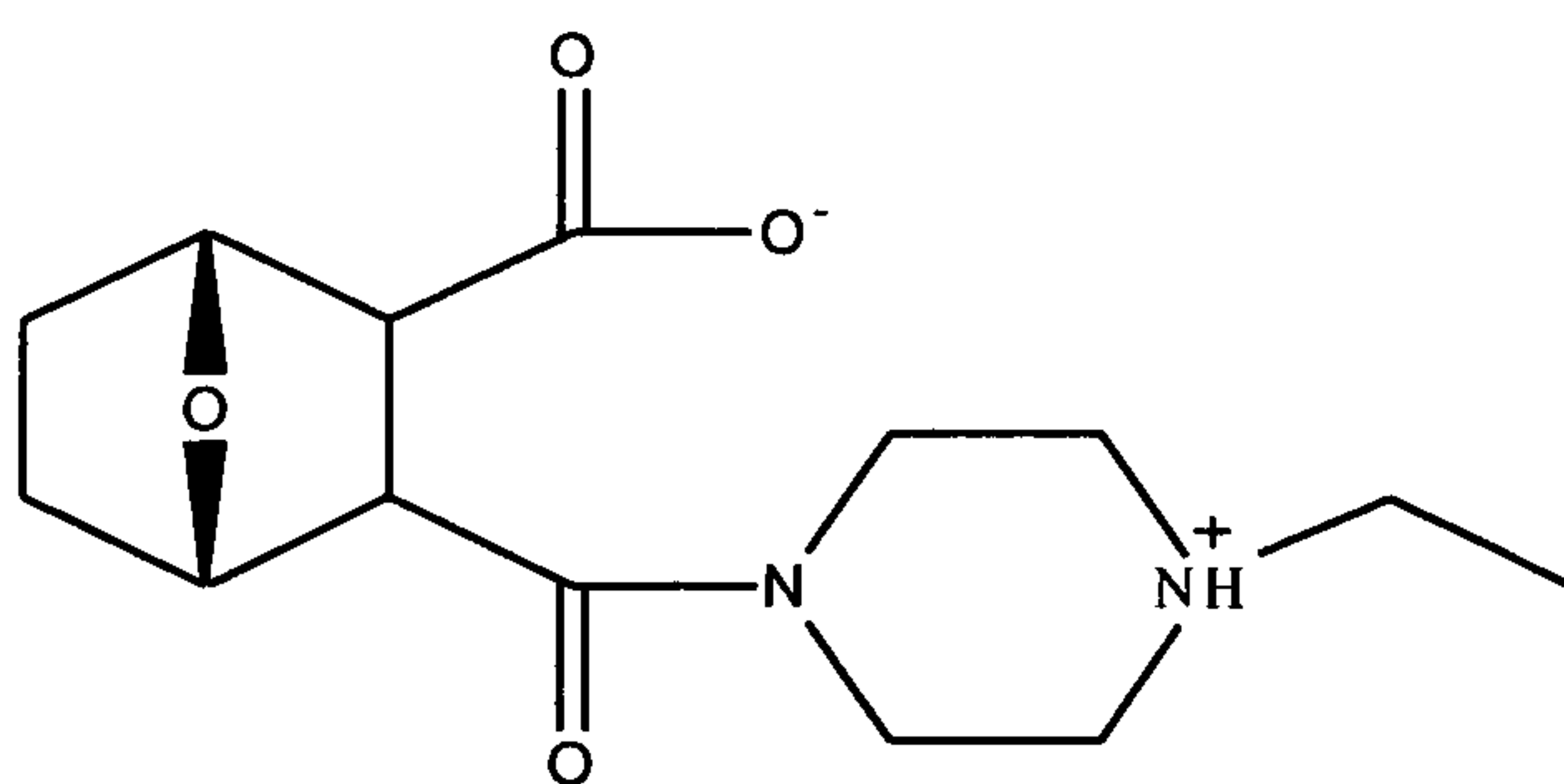
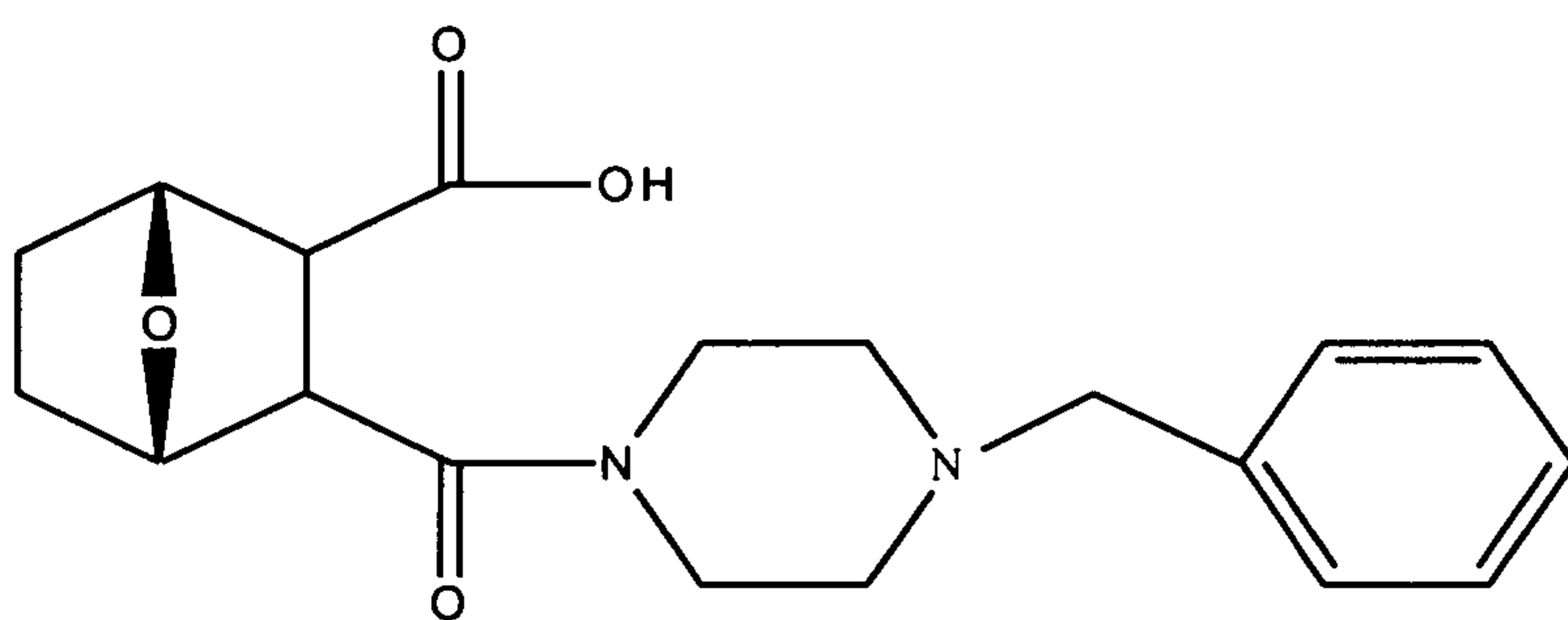
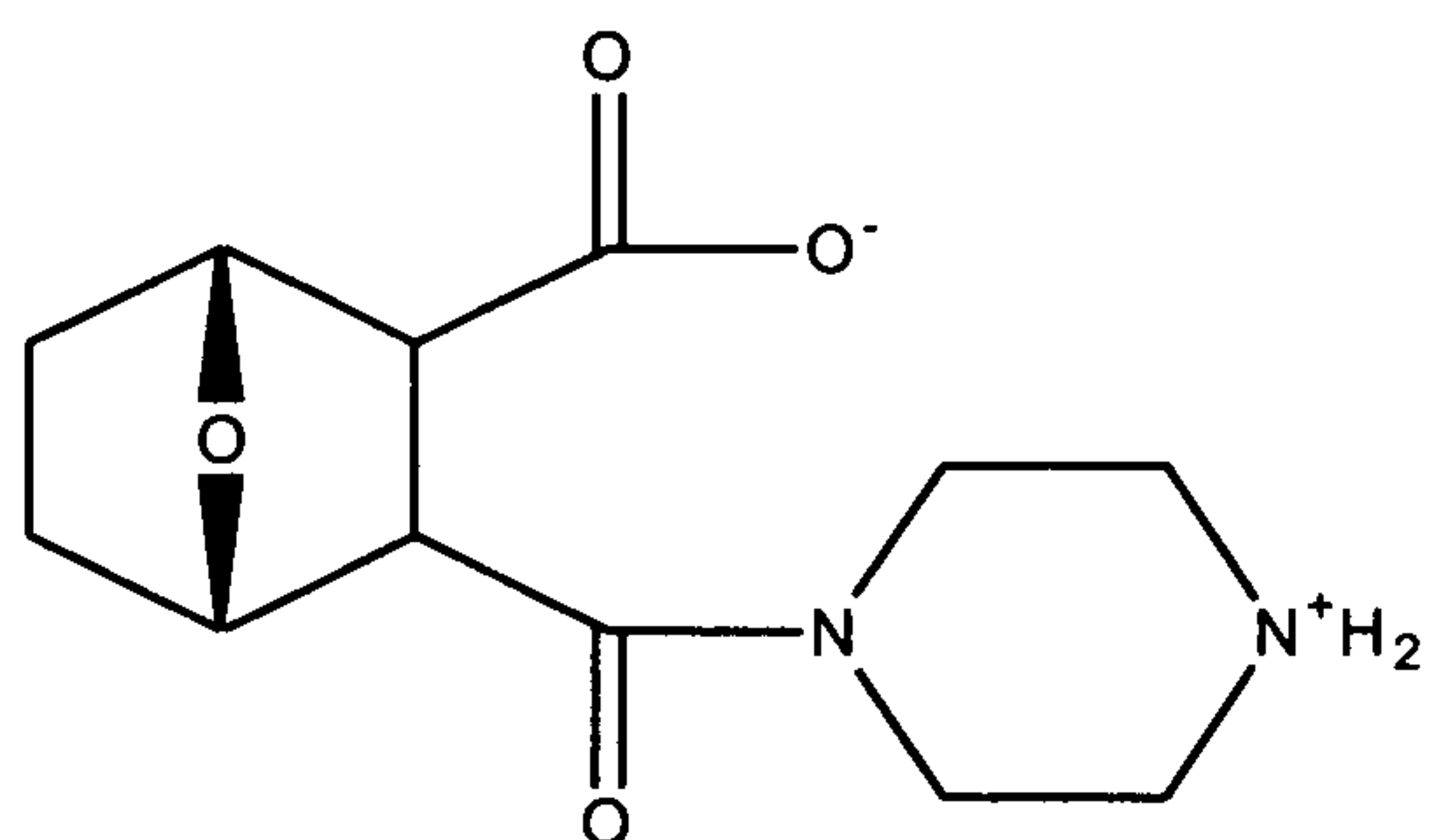
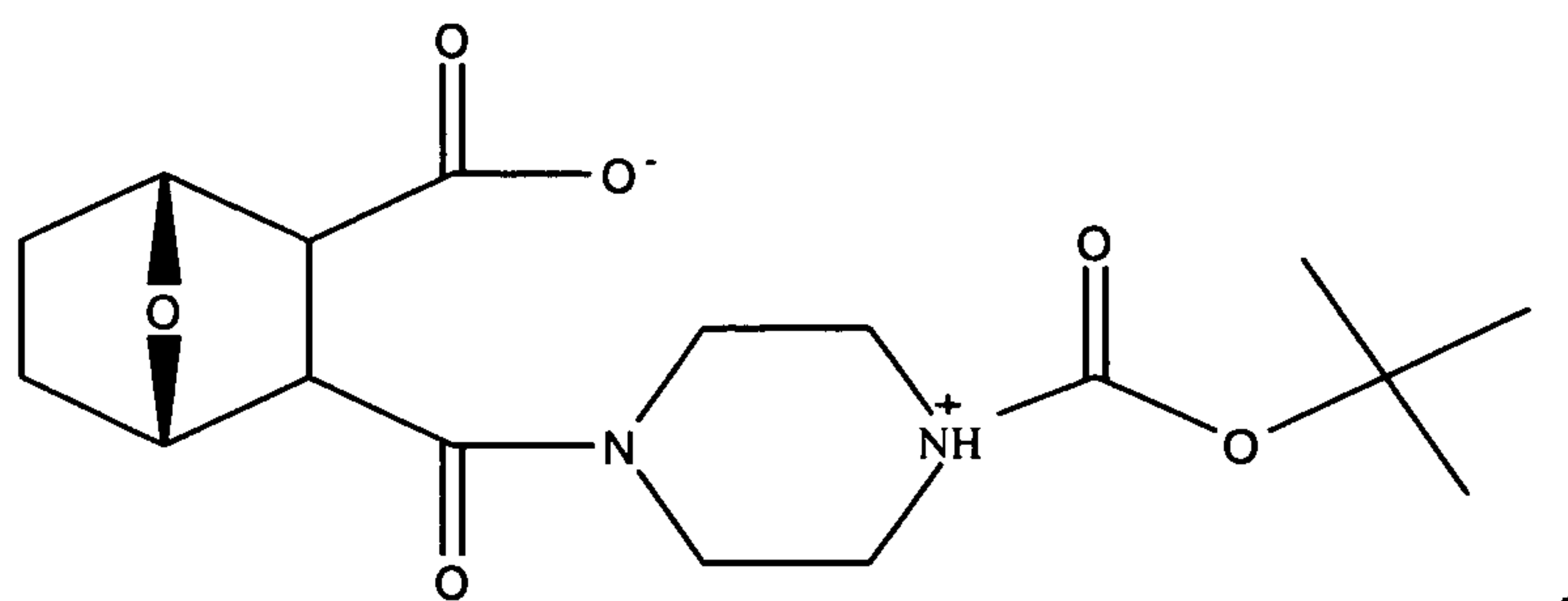
32. The compound of claim 30, wherein bond  $\alpha$  is present.

33. The compound of claim 30, wherein bond  $\alpha$  is absent.

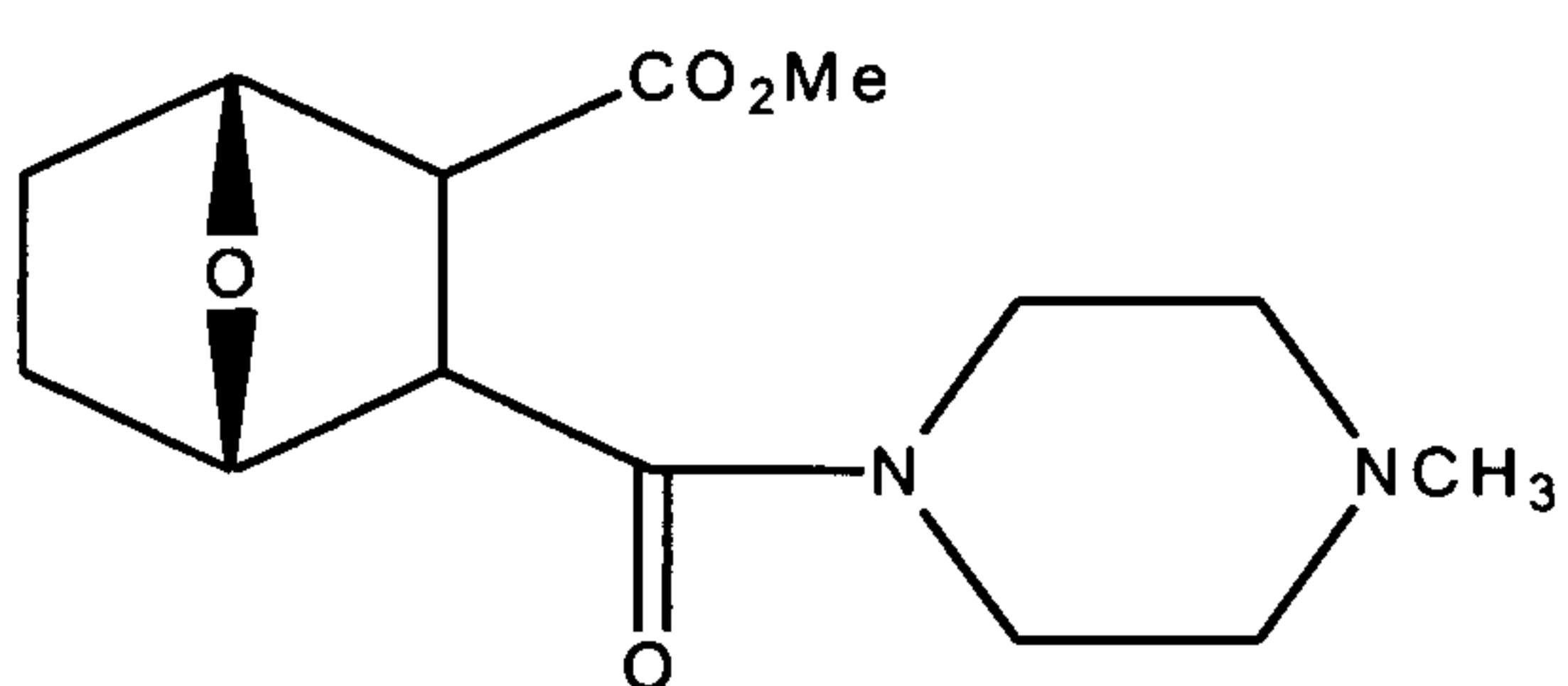
34. The compound of claim 33, having the structure



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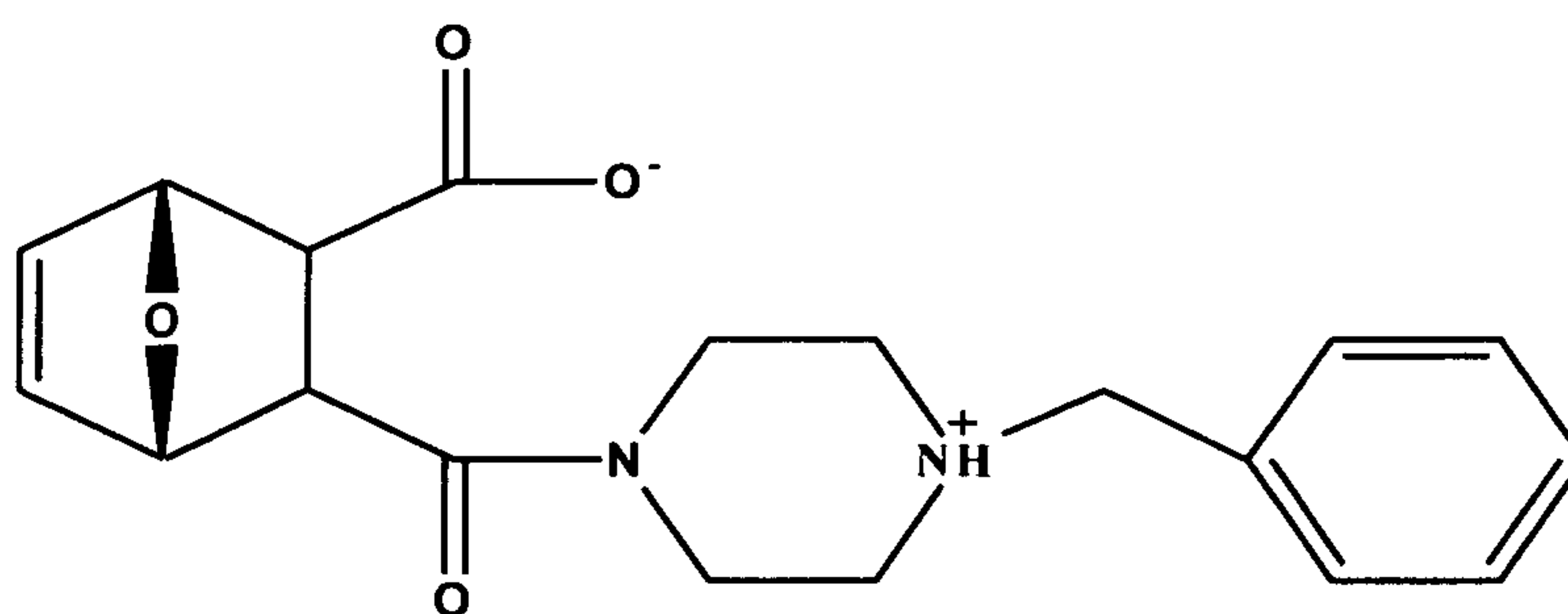
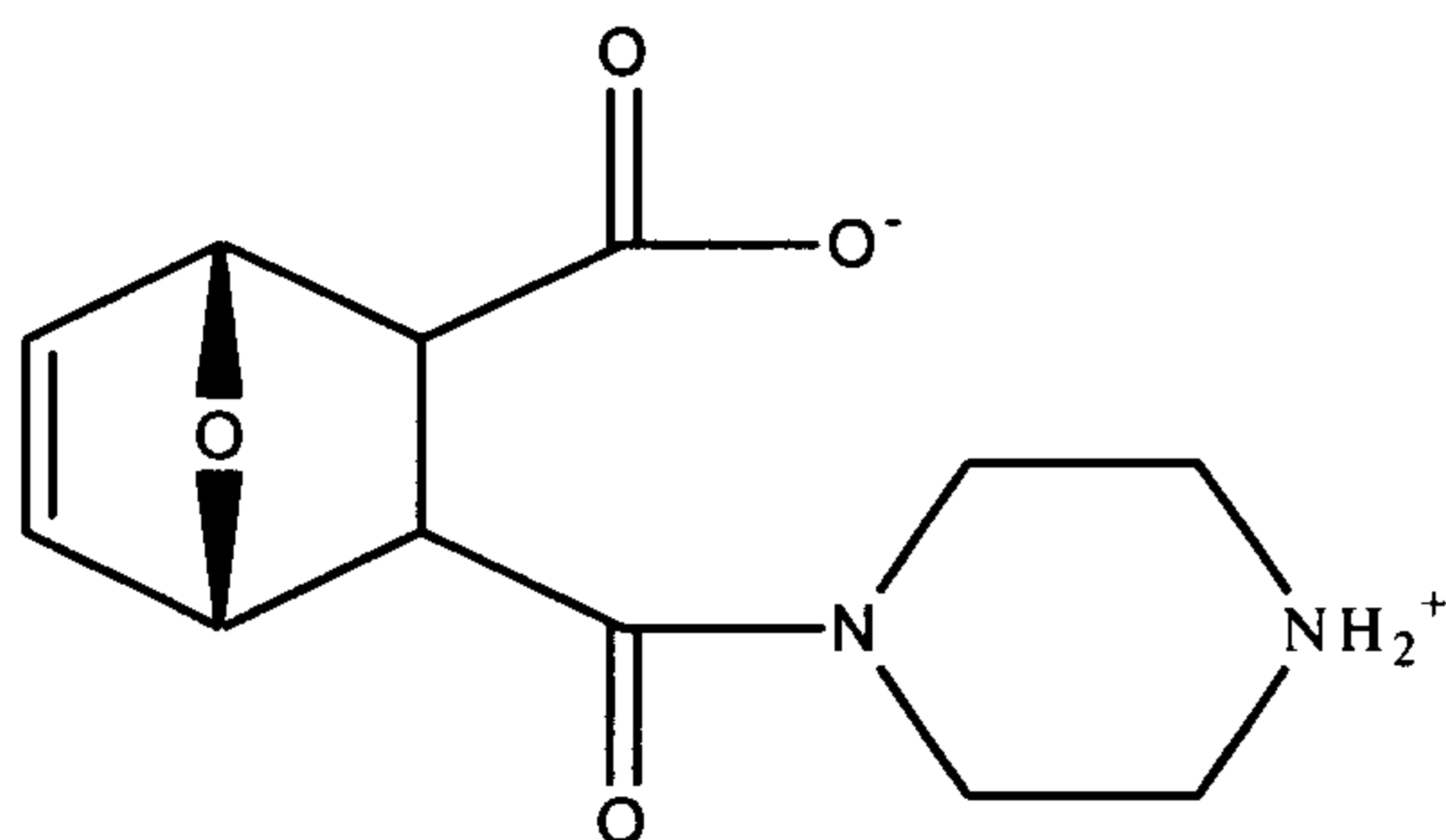
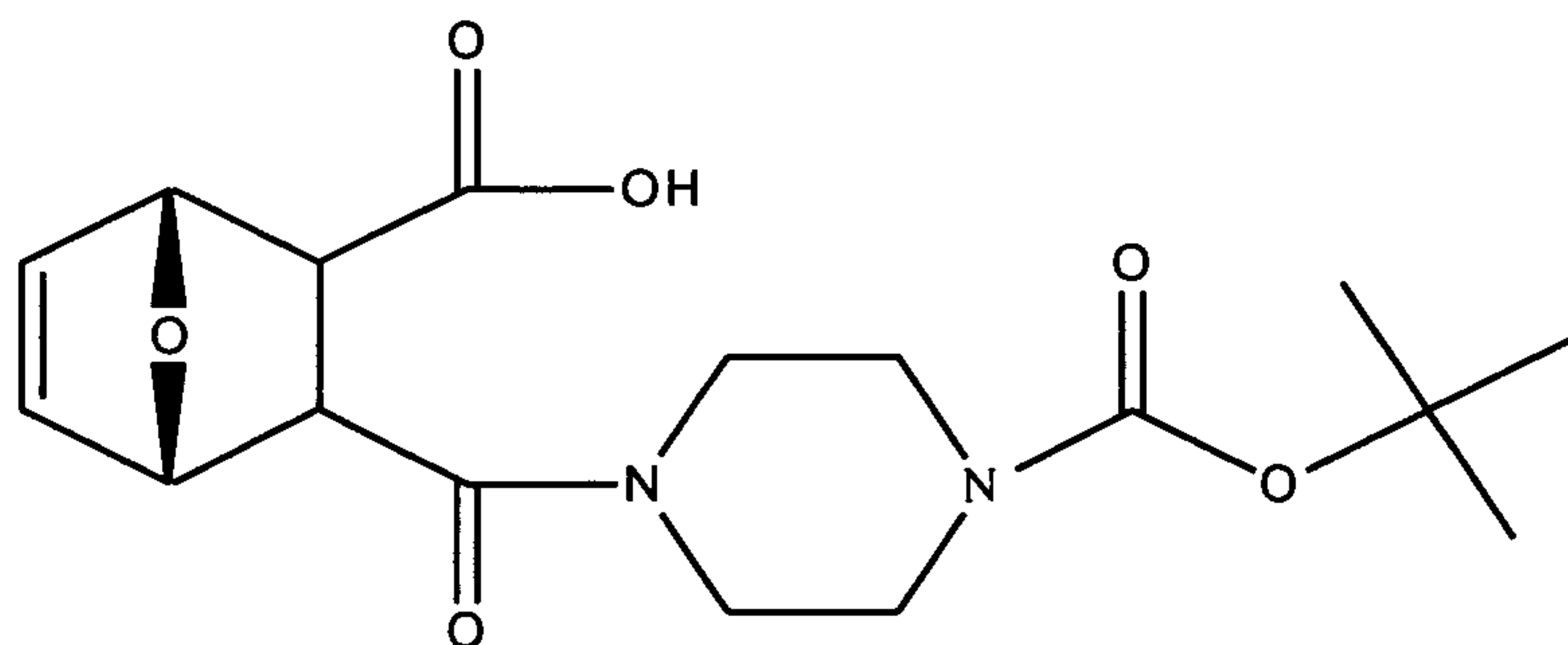
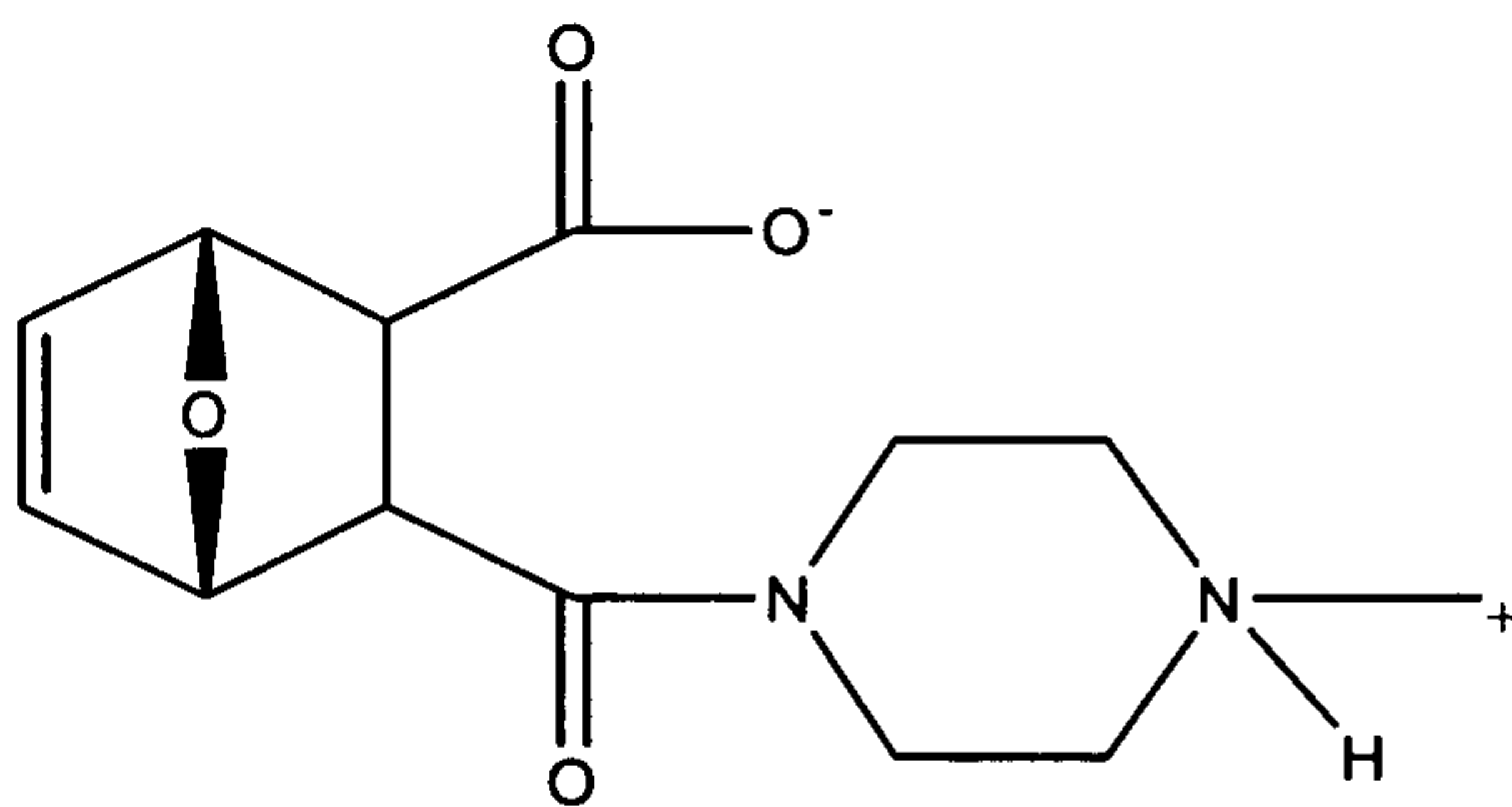


, or

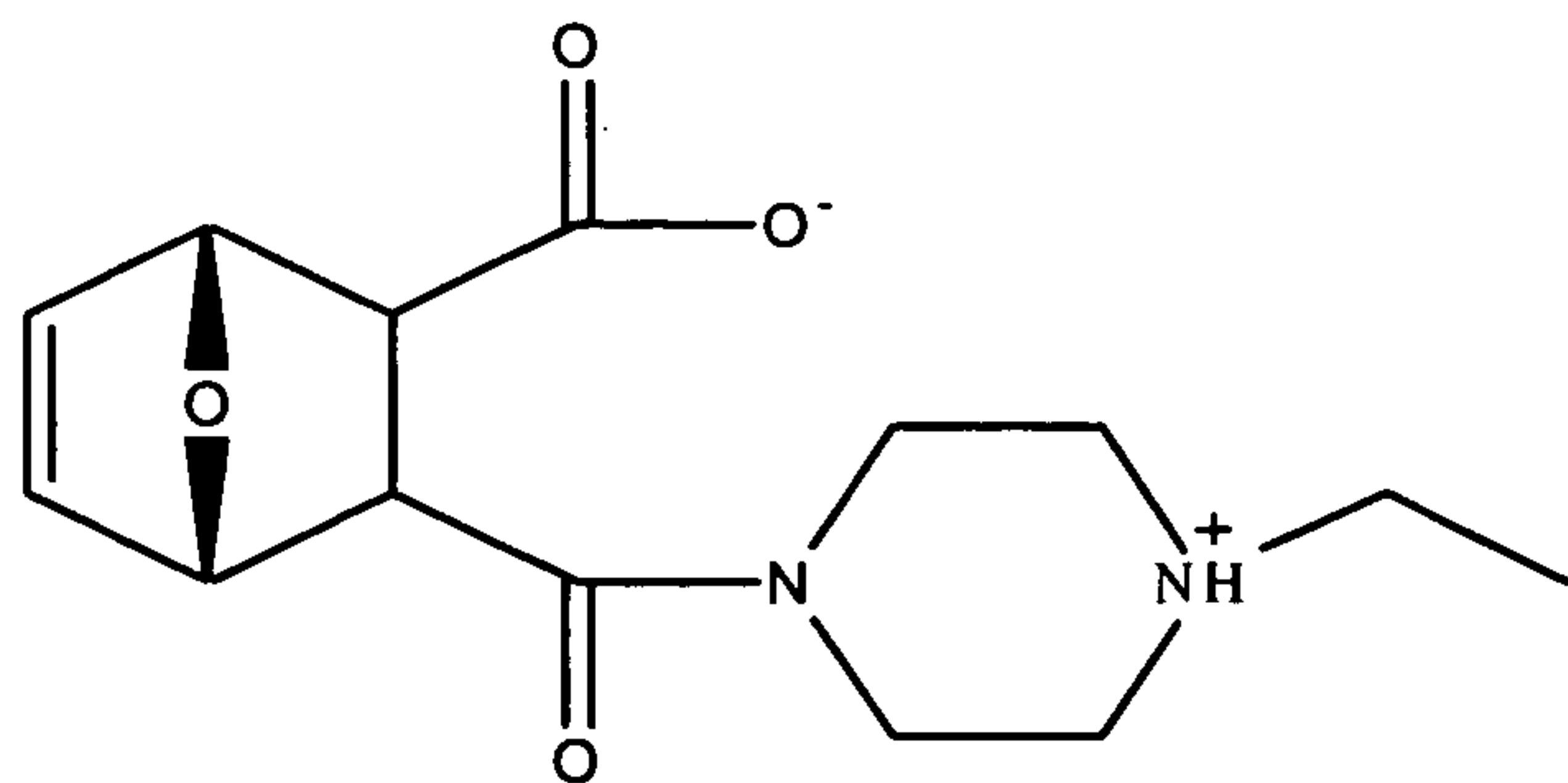


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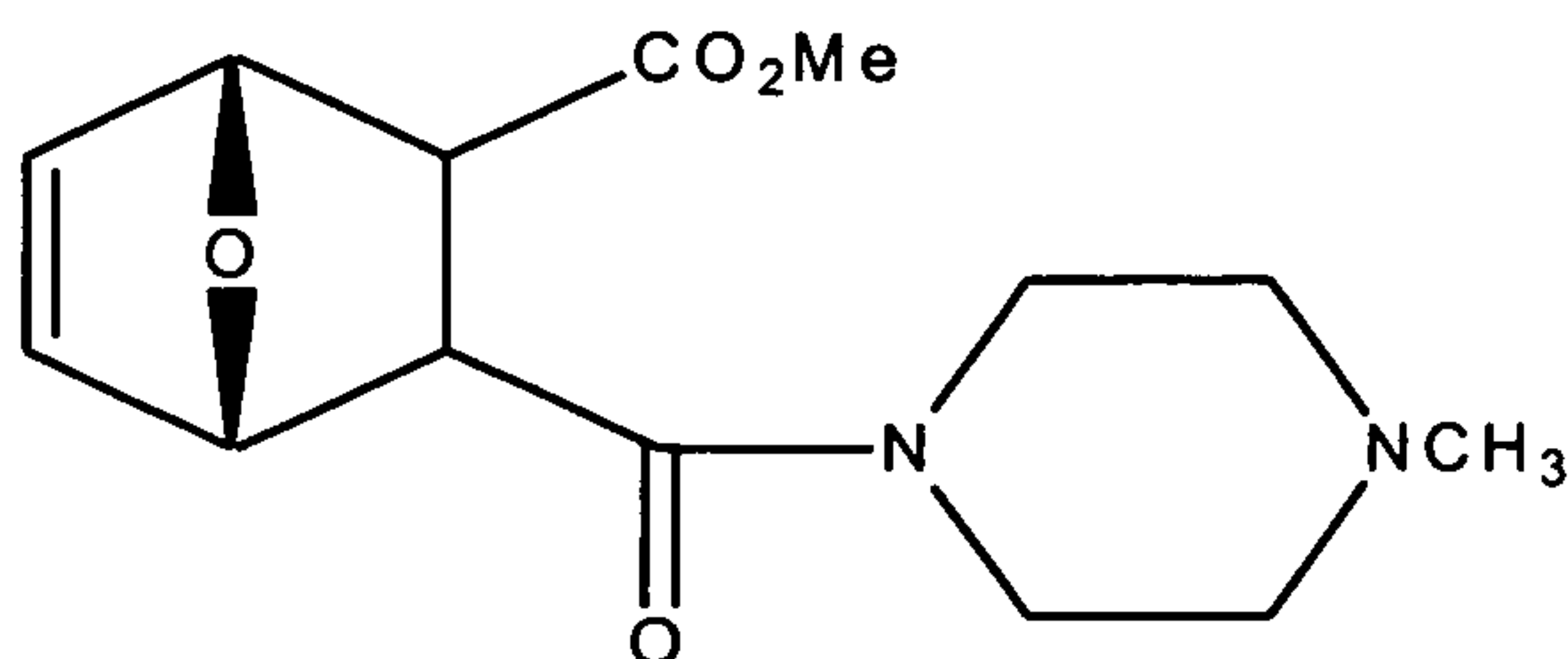
35. The compound of claim 32, having the structure



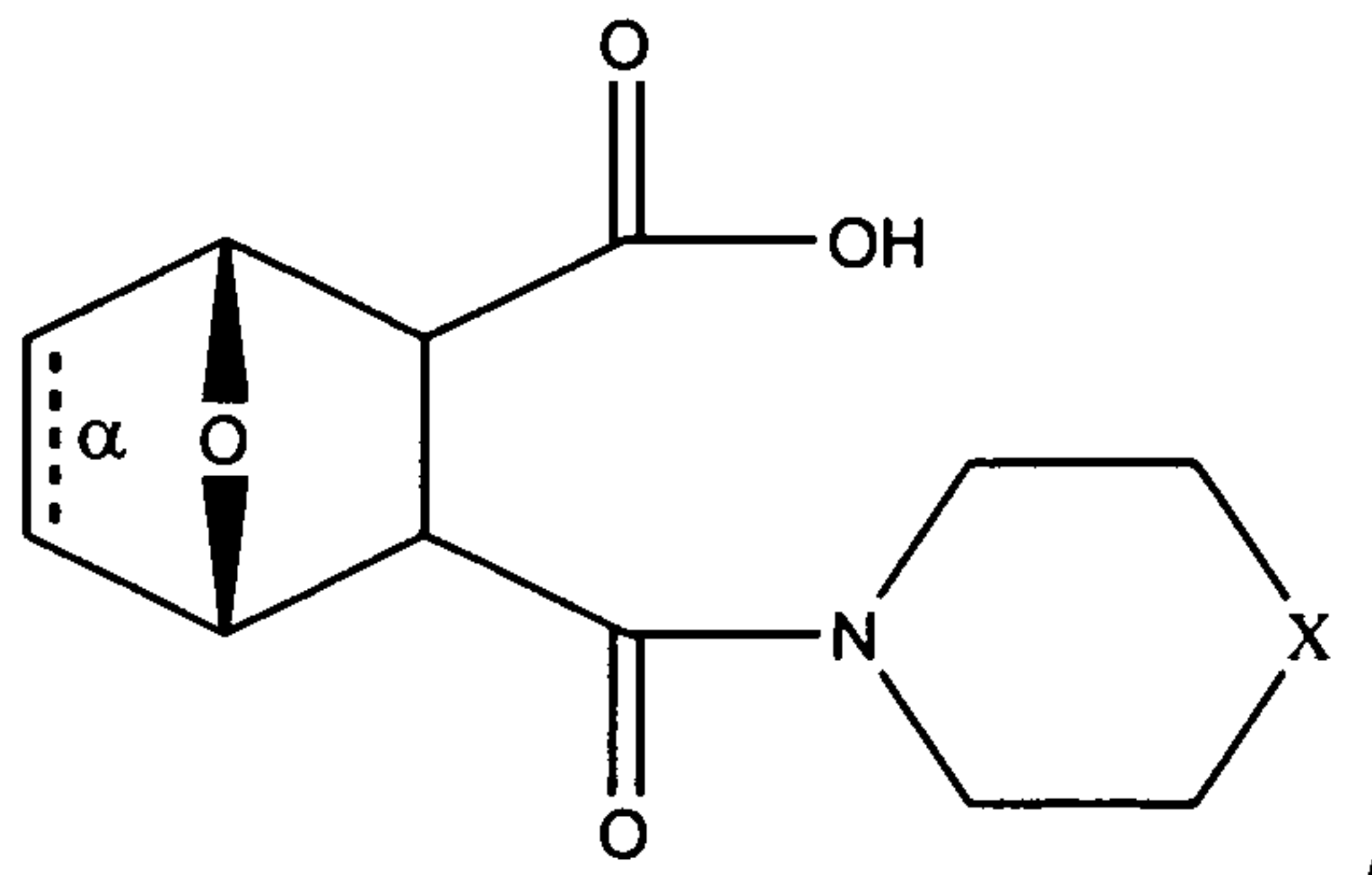
- 108 -



, or



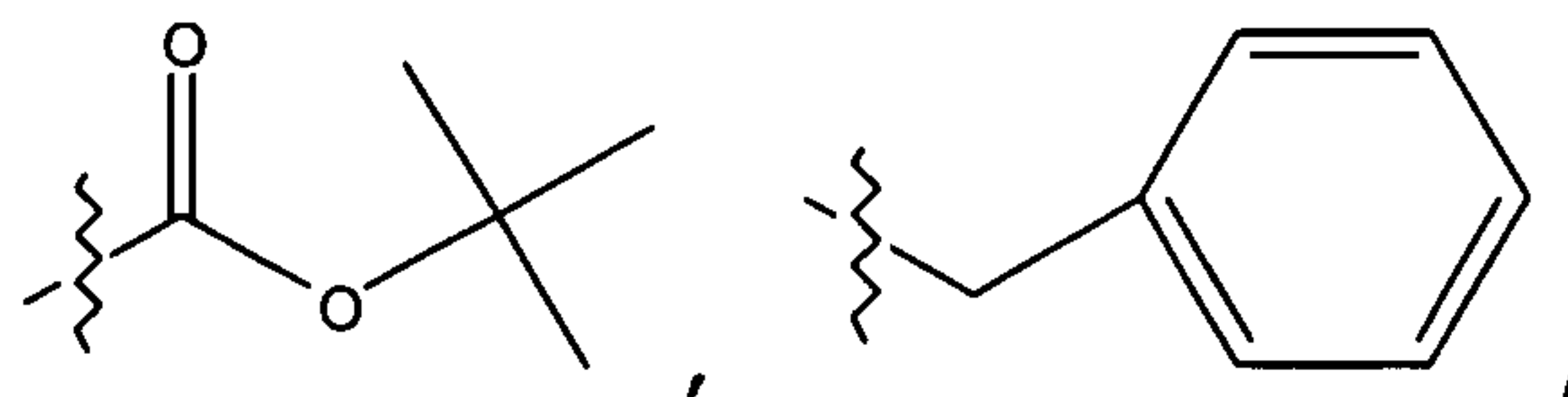
36. The compound of claim 30, having the structure



wherein

bond  $\alpha$  is present or absent; X is  $\text{NH}^+\text{R}_{10}$ ,

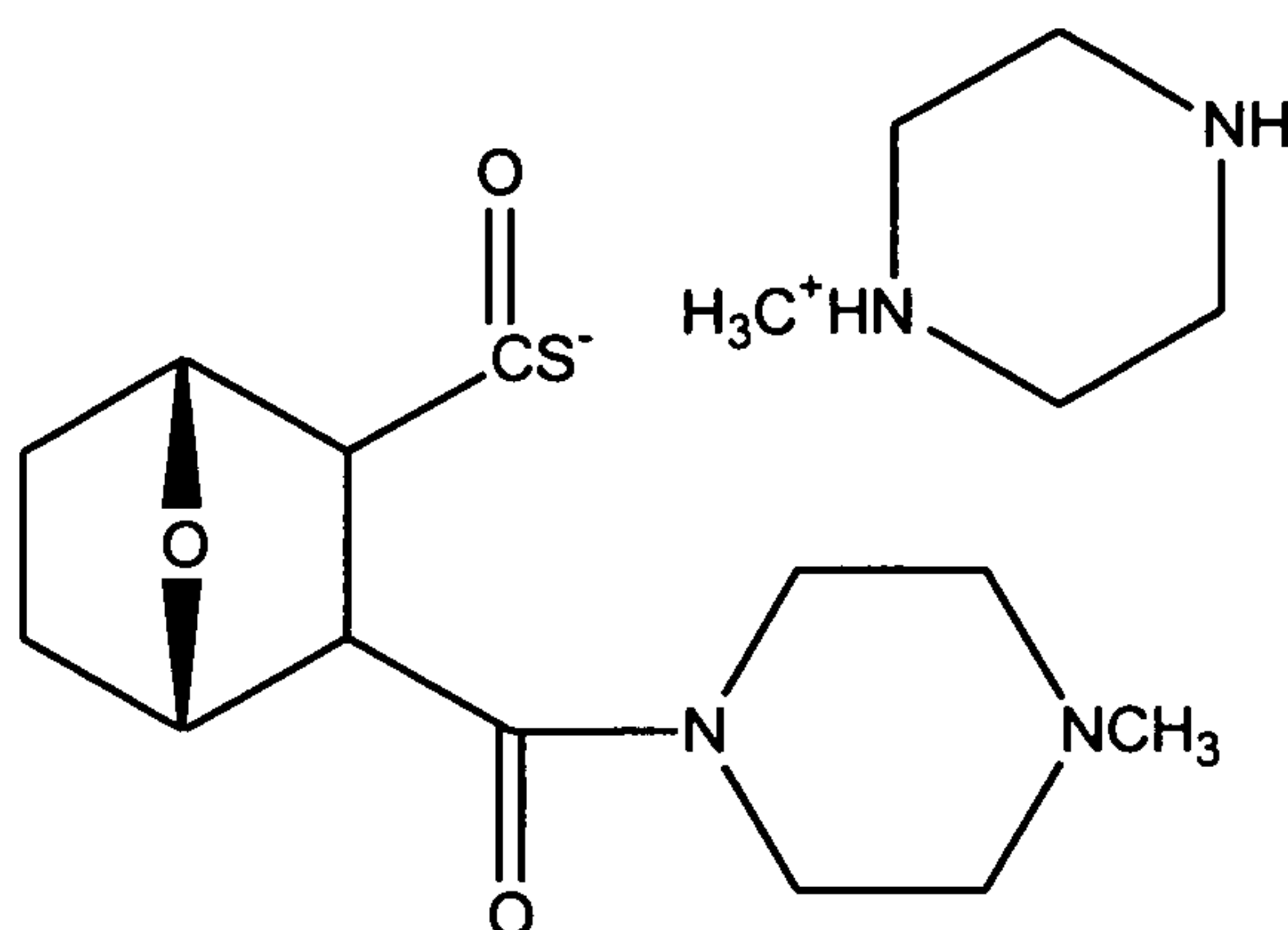
where  $\text{R}_{10}$  is present or absent and when present  $\text{R}_{10}$  is alkyl, substituted C2-C12 alkyl, alkenyl, substituted C4-C12 alkenyl,



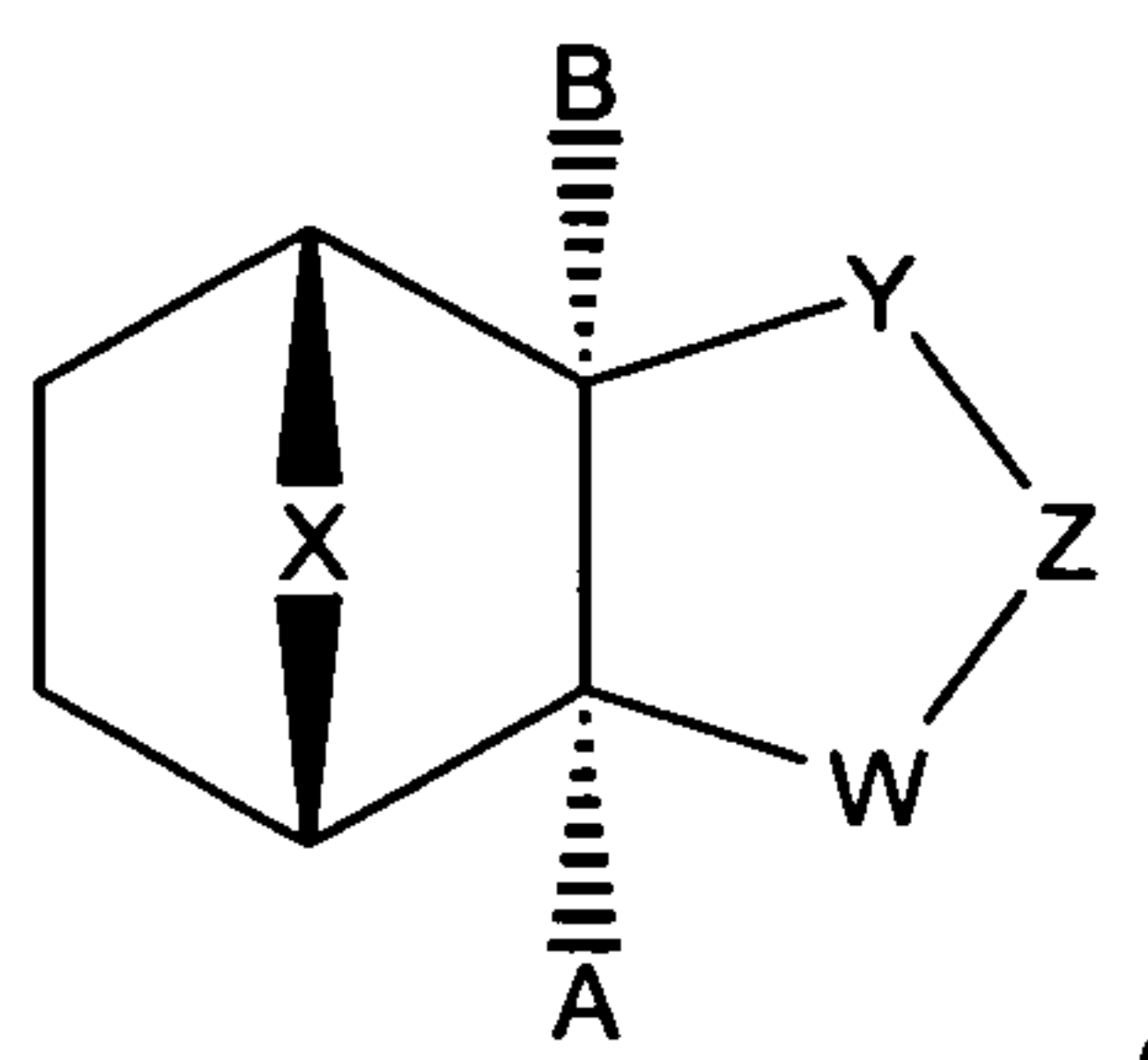
$-\text{CH}_2\text{CN}$ ,  $-\text{CH}_2\text{CO}_2\text{R}_{12}$ , or  $-\text{CH}_2\text{COR}_{12}$ , where  $\text{R}_{12}$  is H or alkyl.

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37. The compound of claim 1, wherein the compound has the structure



38. A compound having the structure



wherein

A and B is each independently H, F, Cl, Br, SO<sub>2</sub>Ph, COR<sub>14</sub>, CO<sub>2</sub>CH<sub>3</sub>, SR<sub>14</sub>,

where R<sub>14</sub> is H or aryl or a substituted or unsubstituted alkyl, alkenyl or alkynyl;

X is O, NH or S;

Z is O, S, SR<sub>15</sub>, NH, NR<sub>15</sub>, CH<sub>2</sub>OH, CH<sub>2</sub>OR<sub>15</sub>,

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where  $R_{15}$  is an aryl or a substituted or unsubstituted alkyl, alkenyl or alkynyl; and

Y and W is each independently CHOH, CH<sub>2</sub>, C=S or C=O,  
or a salt of the compound.

39. The compound of claim 38 wherein,

A and B is each independently H or F;

X is O;

Z is S; and

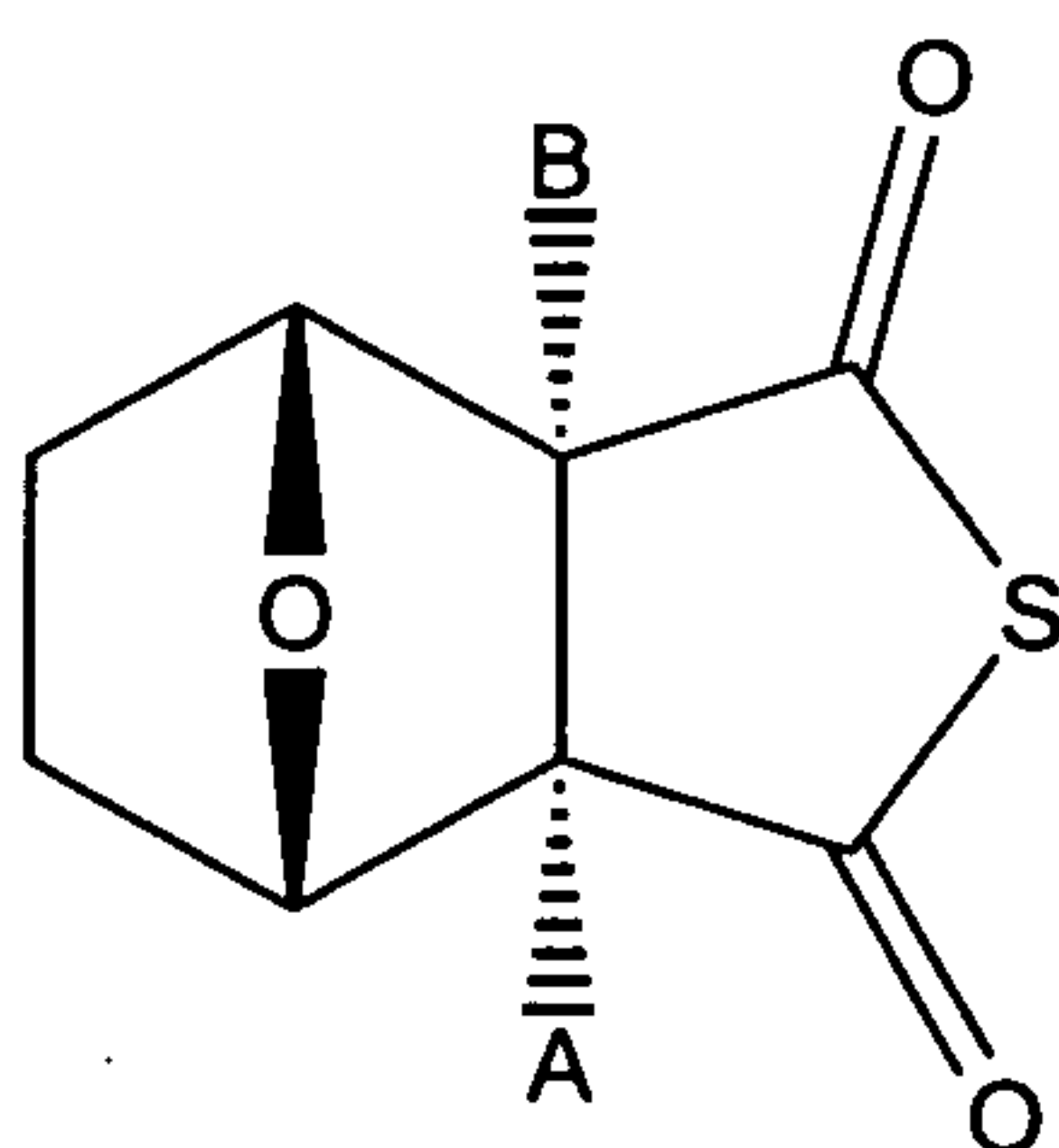
Y and W are each C=S.

40. The compound of claim 38 or 39 wherein,  
A and B are each F.

41. The compound of claim 38 or 39 wherein,  
A is H; and

B is F.

42. A compound of claim 38 having the structure



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wherein

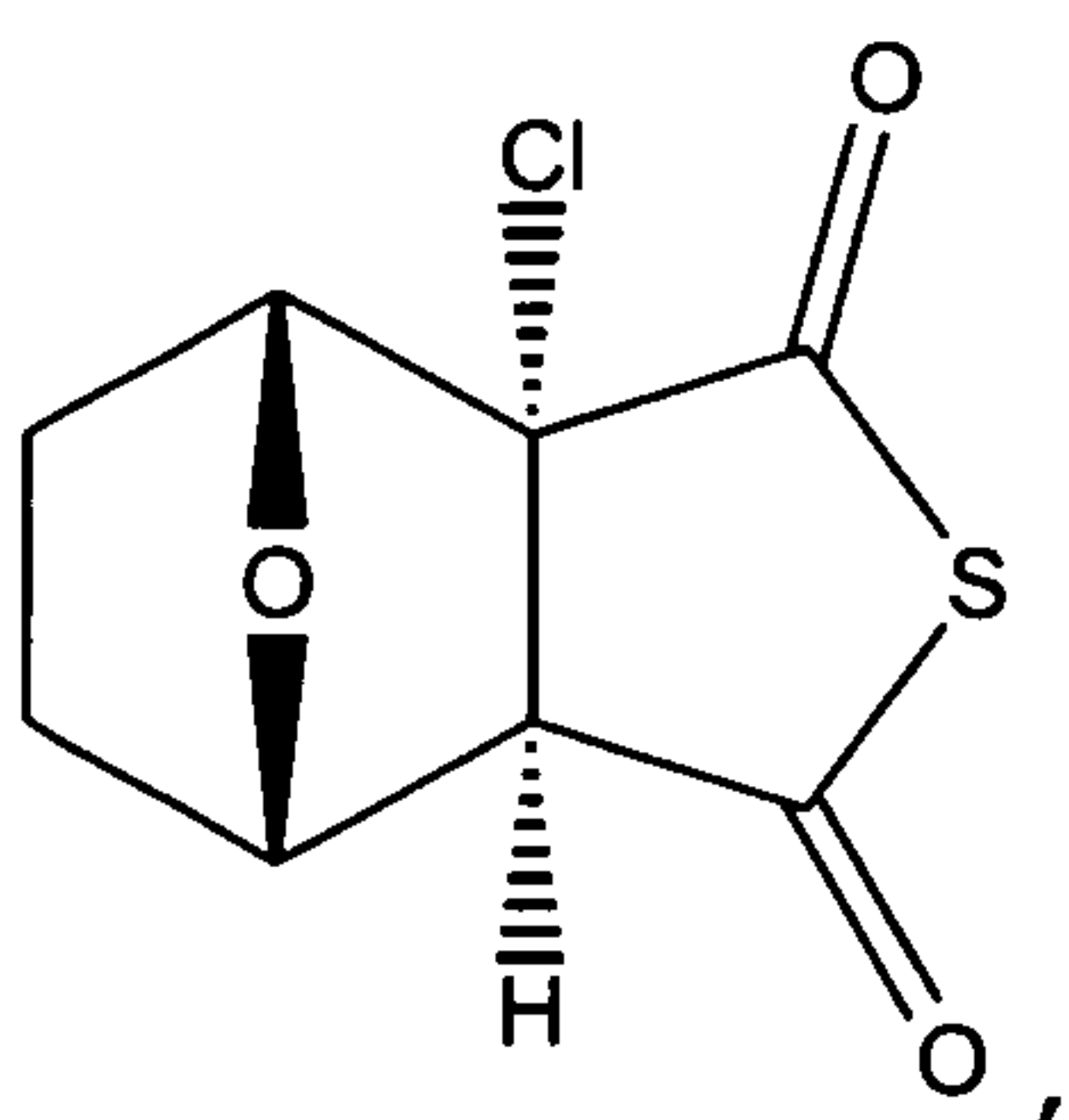
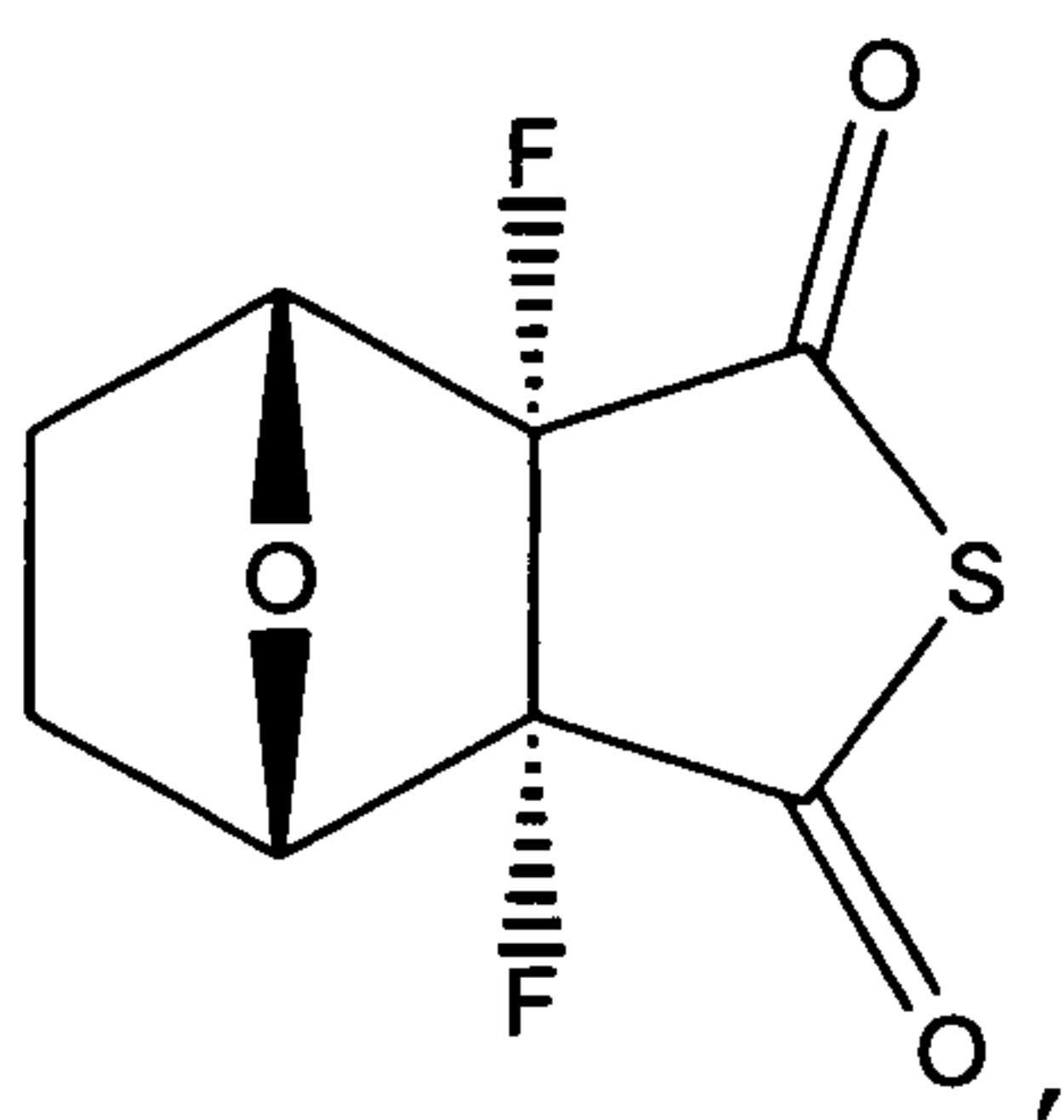
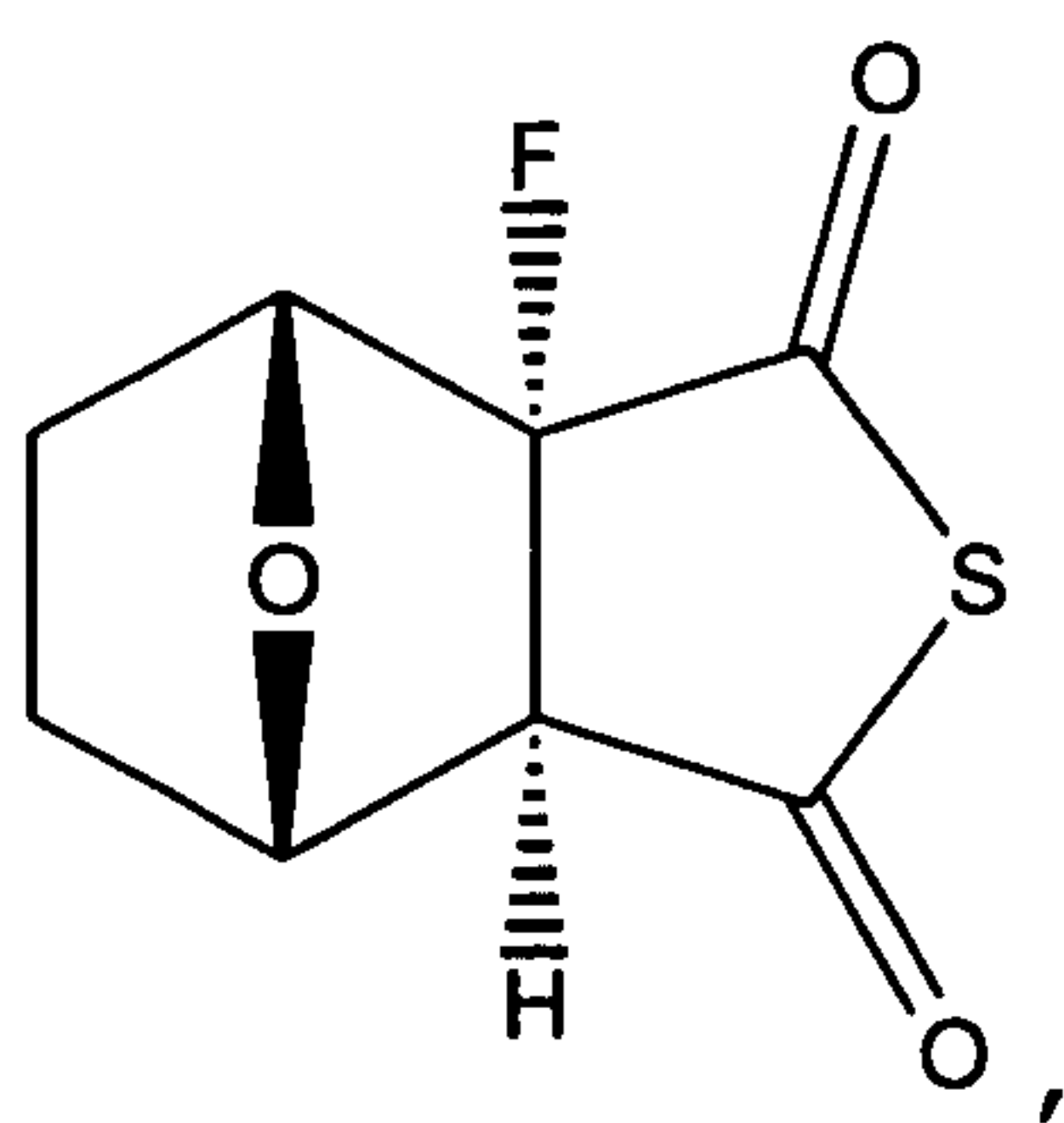
A is F or  $\text{SR}_{14}$ ,

where  $\text{R}_{14}$  is aryl or a substituted or unsubstituted alkyl, alkenyl or alkynyl; and

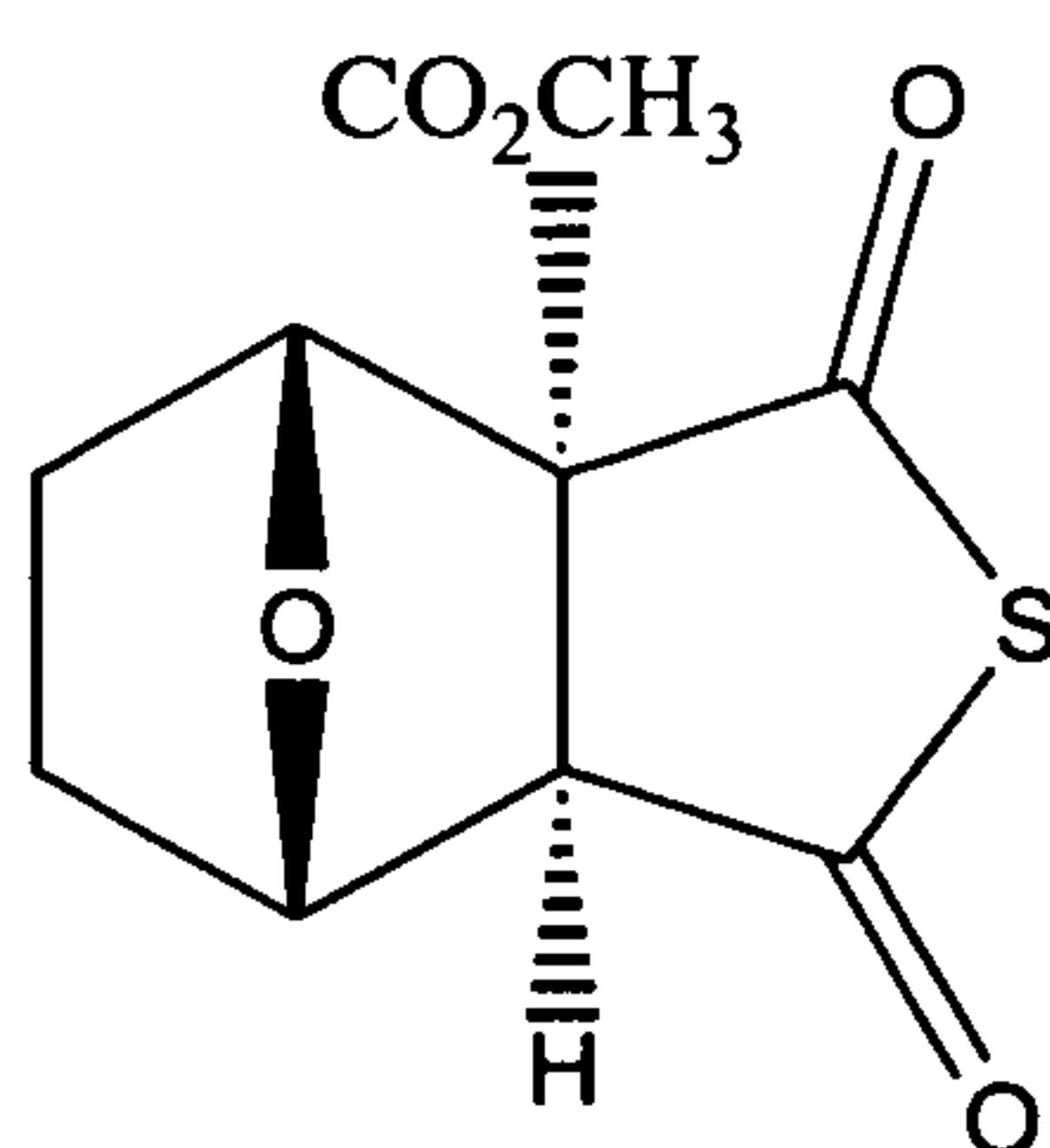
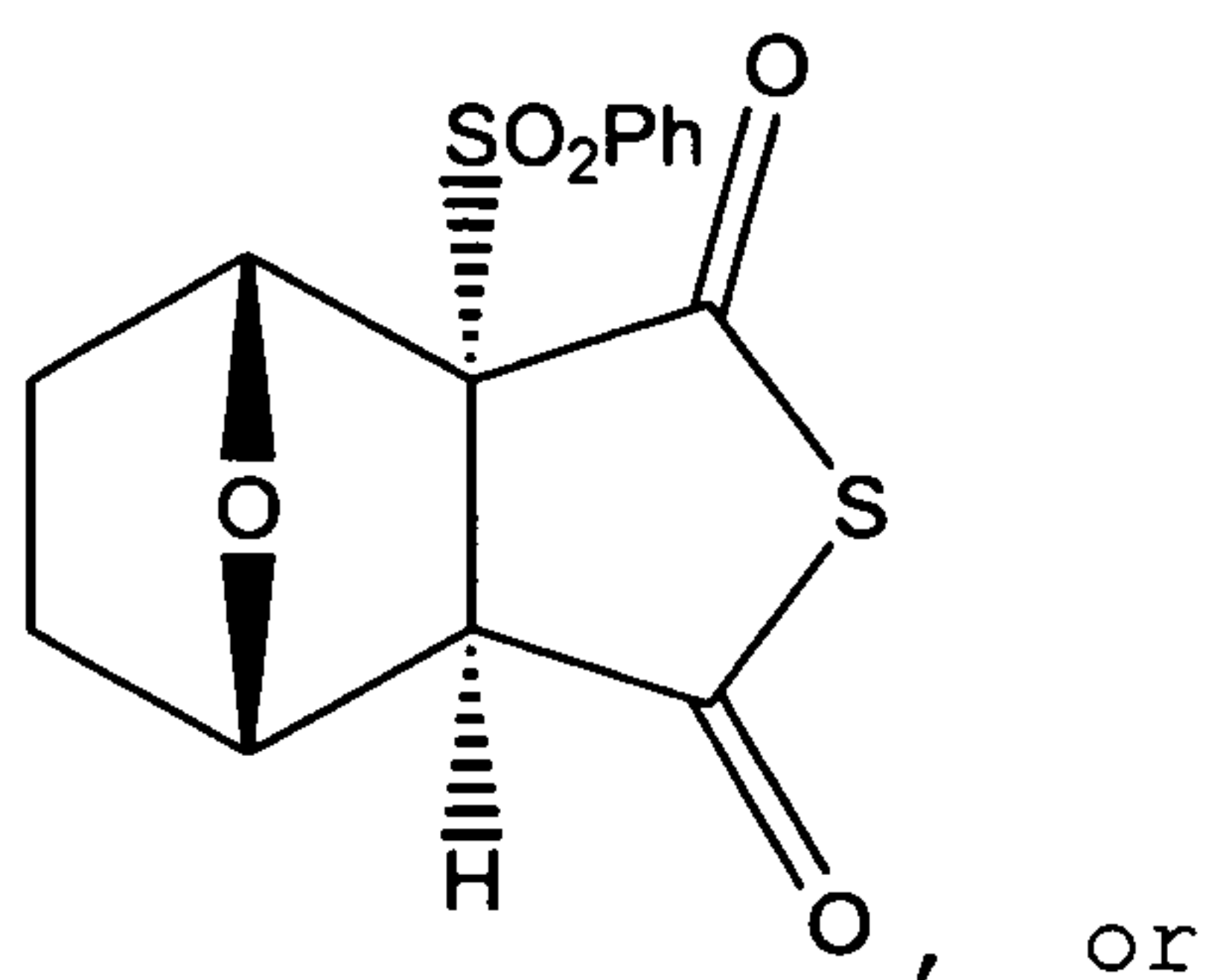
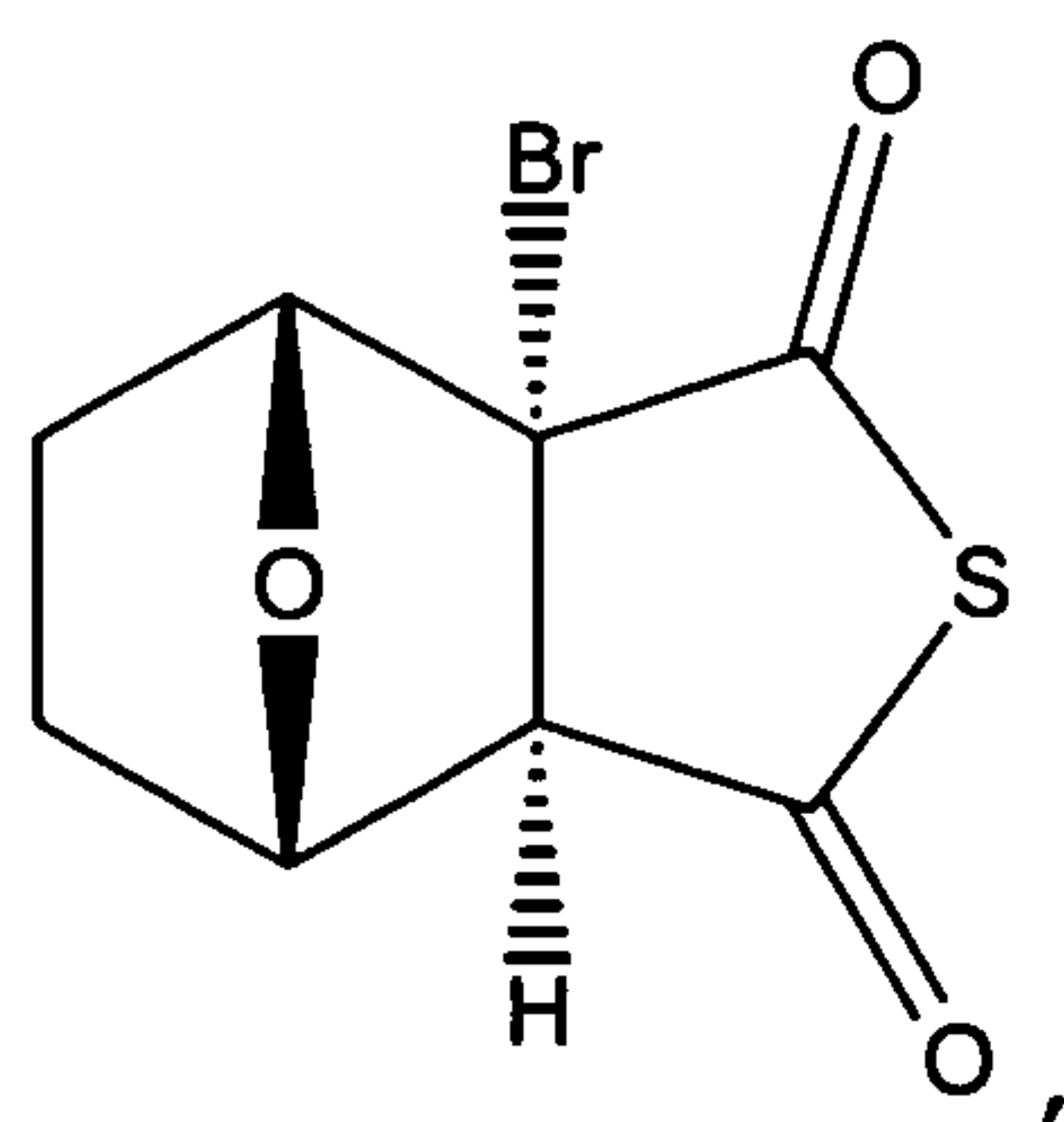
B is F, Cl, Br,  $\text{SO}_2\text{Ph}$ ,  $\text{CO}_2\text{CH}_3$ ,  $\text{COR}_{14}$ , or  $\text{SR}_{14}$ ,

where  $\text{R}_{14}$  is aryl or a substituted or unsubstituted alkyl, alkenyl or alkynyl.

43. The compound of claim 42 having the structure



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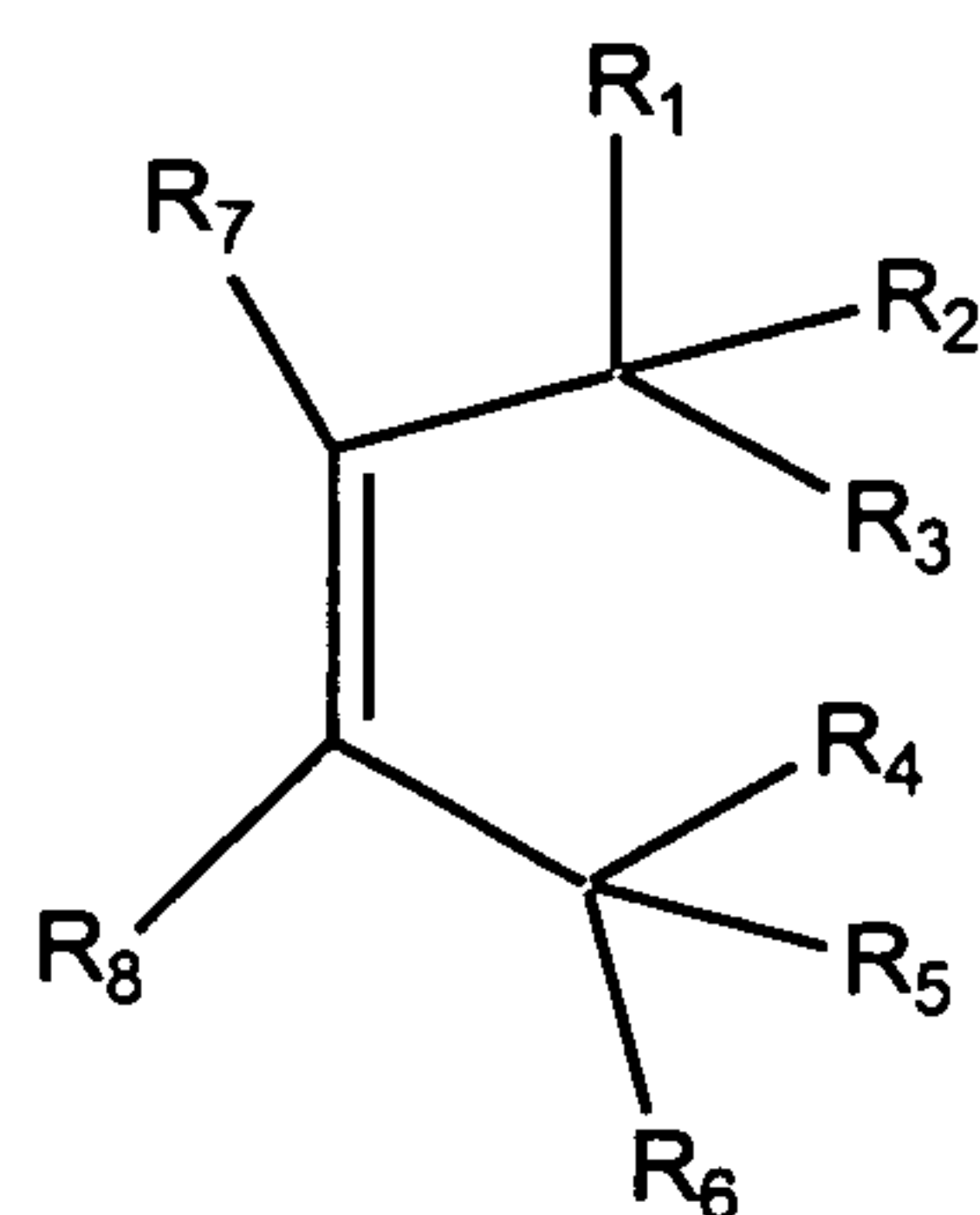


44. A pharmaceutical composition comprising the compound of any one of claims 1-37 and a pharmaceutically acceptable carrier.
45. A process for preparing a compound having the structure of claim 1 or 2 comprising
- a) reacting a compound having the structure

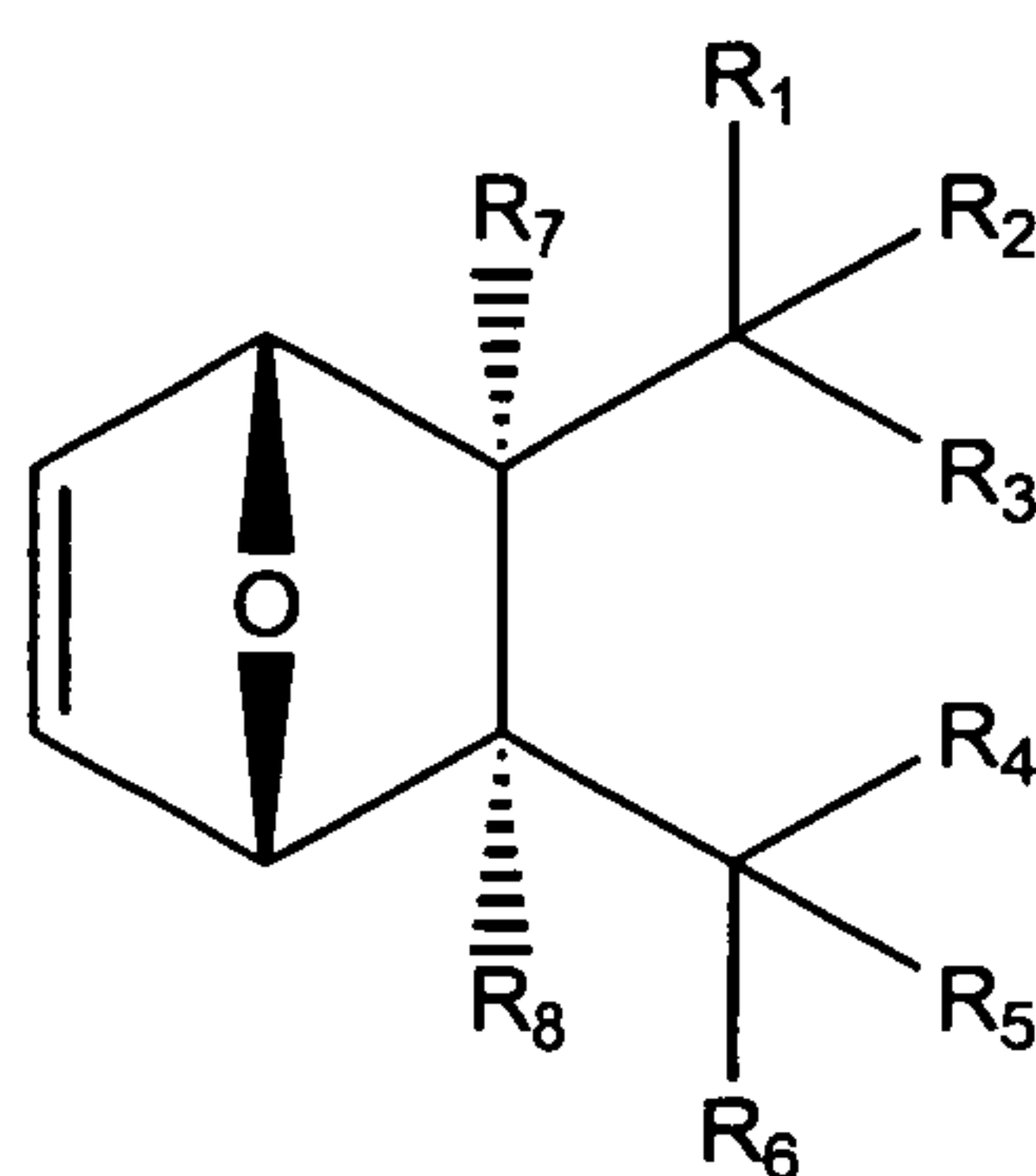


with a compound having the structure

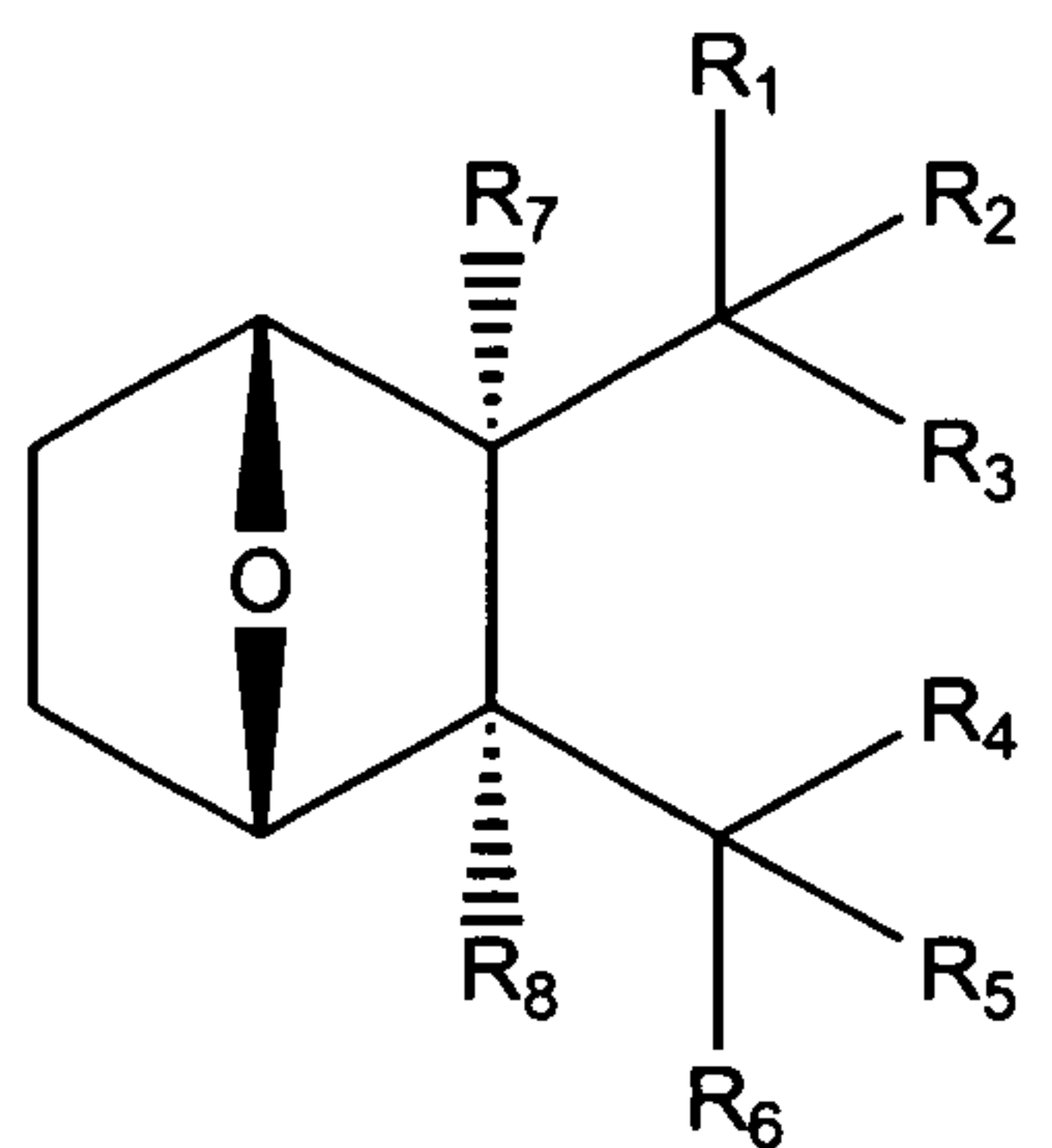
- 113 -



to form a compound having the following structure



b) reacting a compound having the above structure with hydrogen in the presence of a catalyst to form



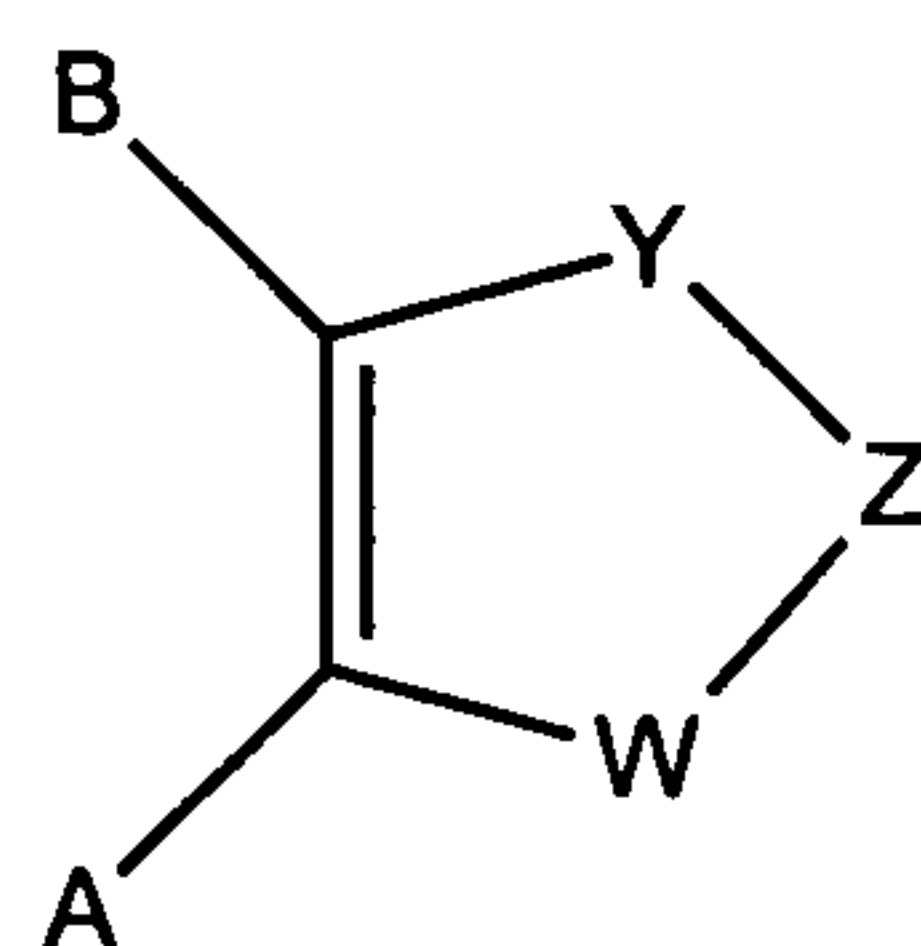
46. A process for preparing a compound having the structure of claim 38 comprising

a) reacting a compound having the structure

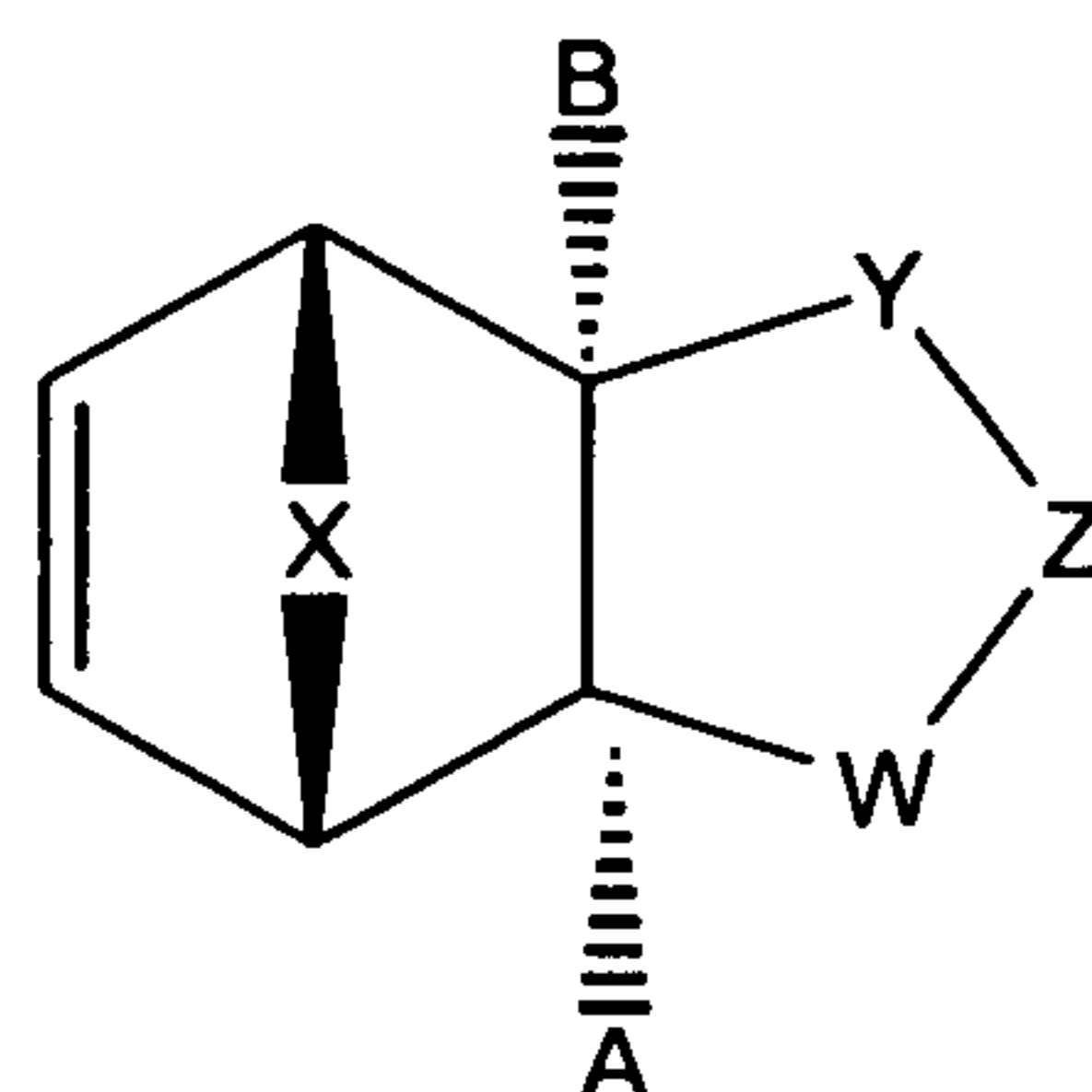


with a compound having the structure

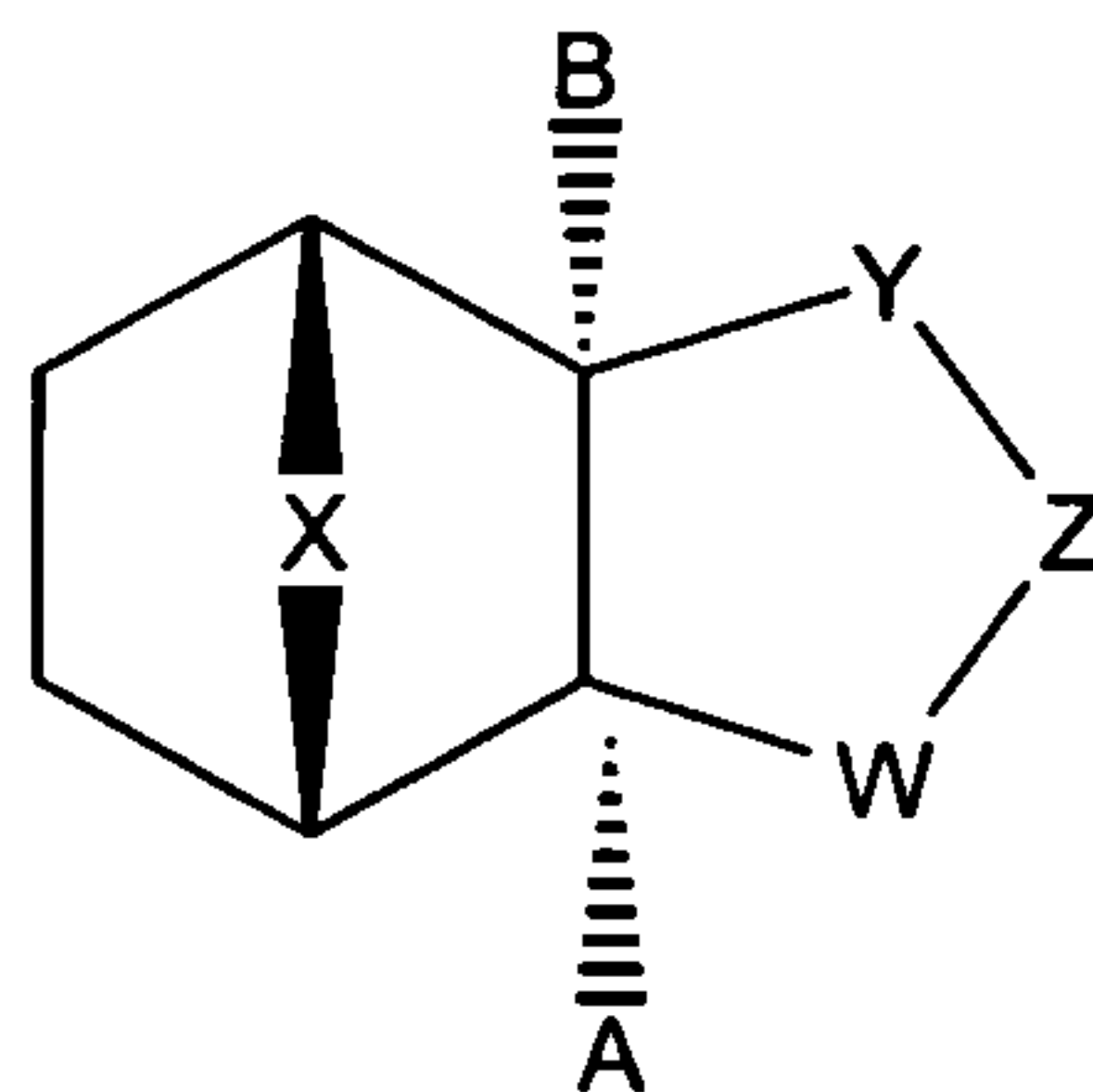
- 114 -



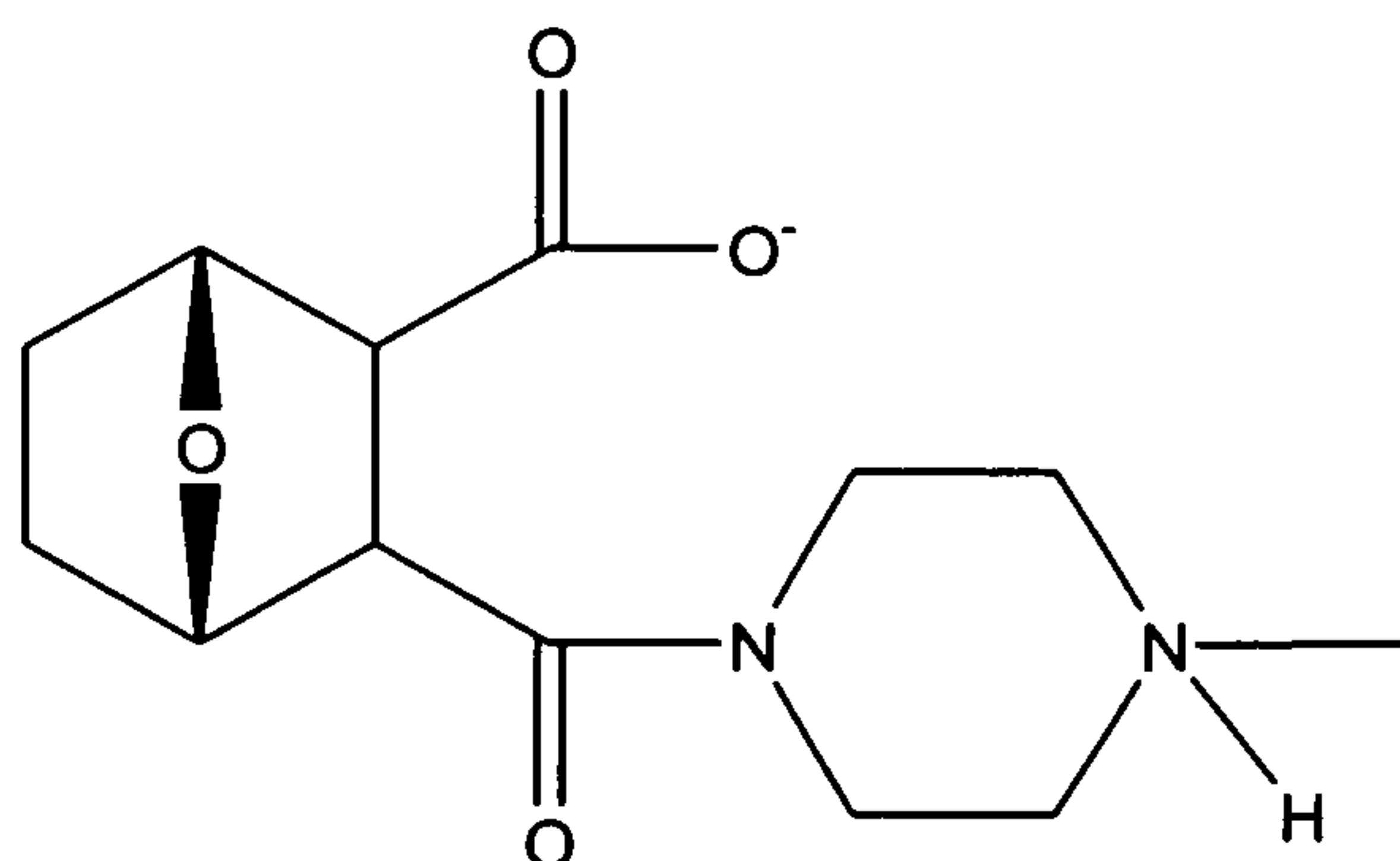
to form a compound having the following structure



b) and reacting a compound having the above structure with hydrogen in the presence of a catalyst to form



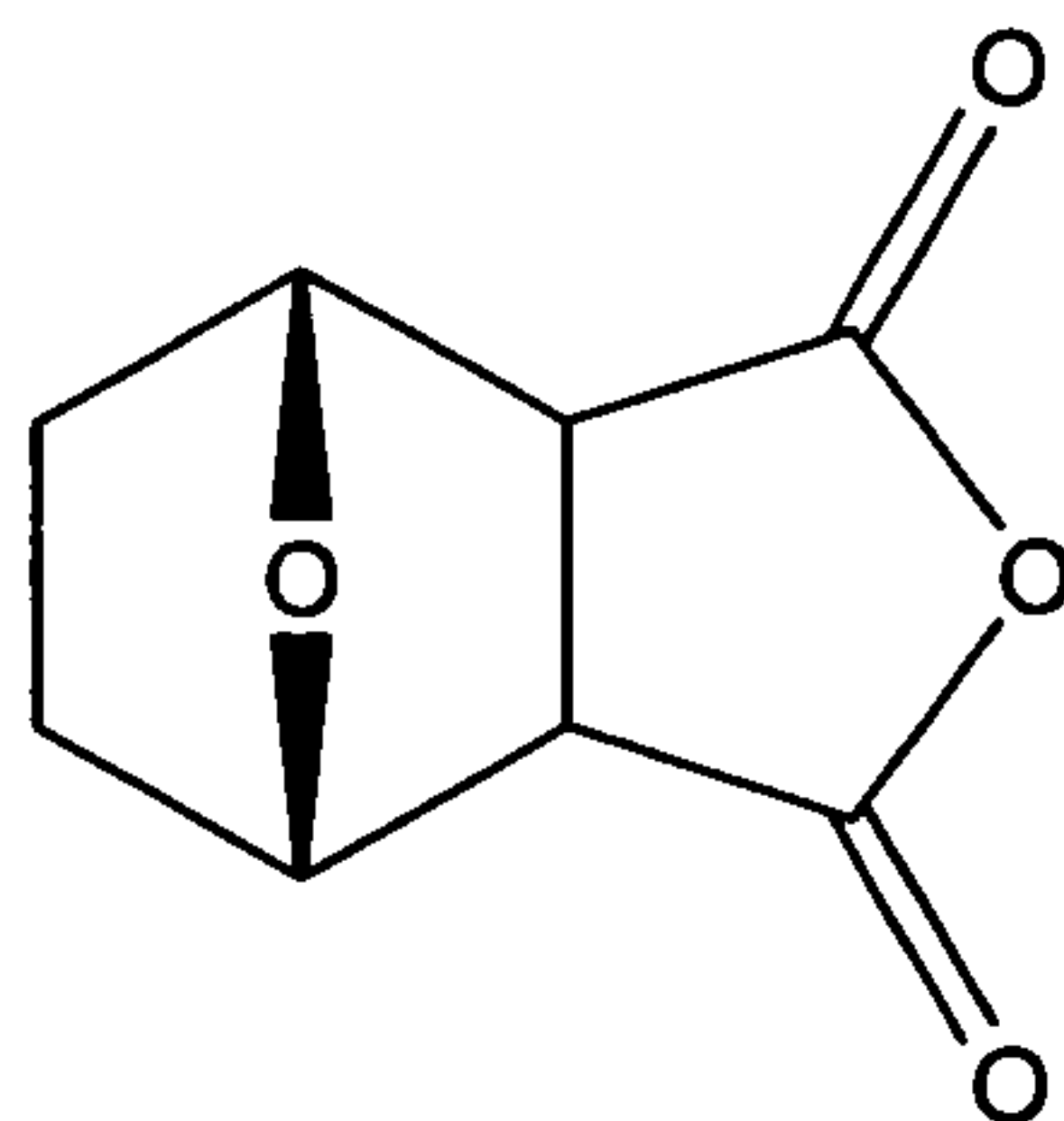
47. A process for preparing the compound having the structure



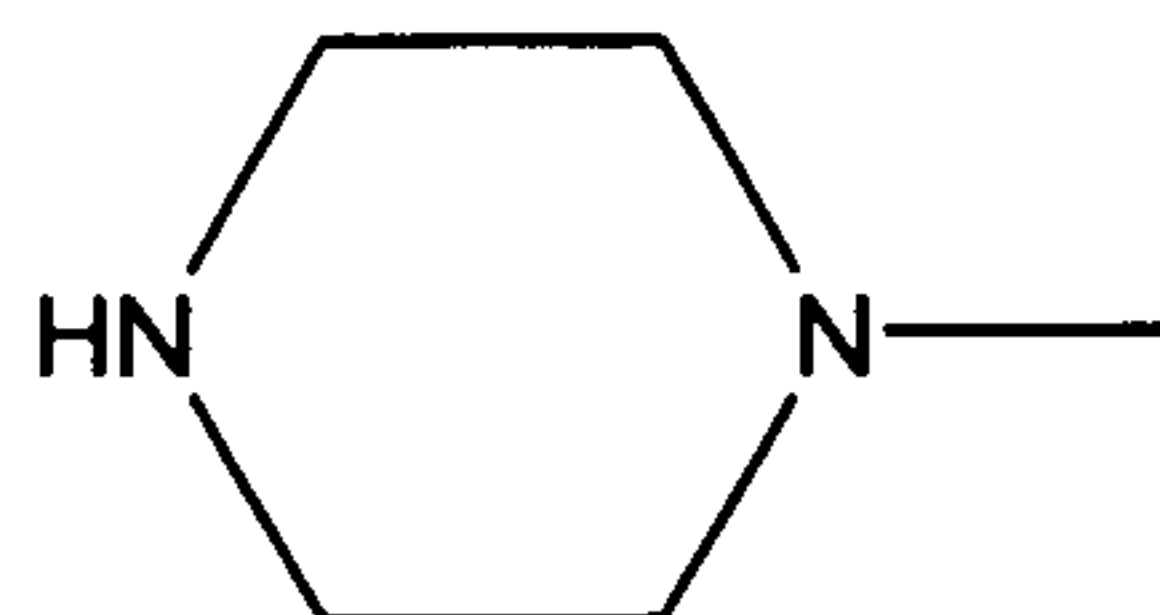
comprising

a) dissolving a compound having the following structure

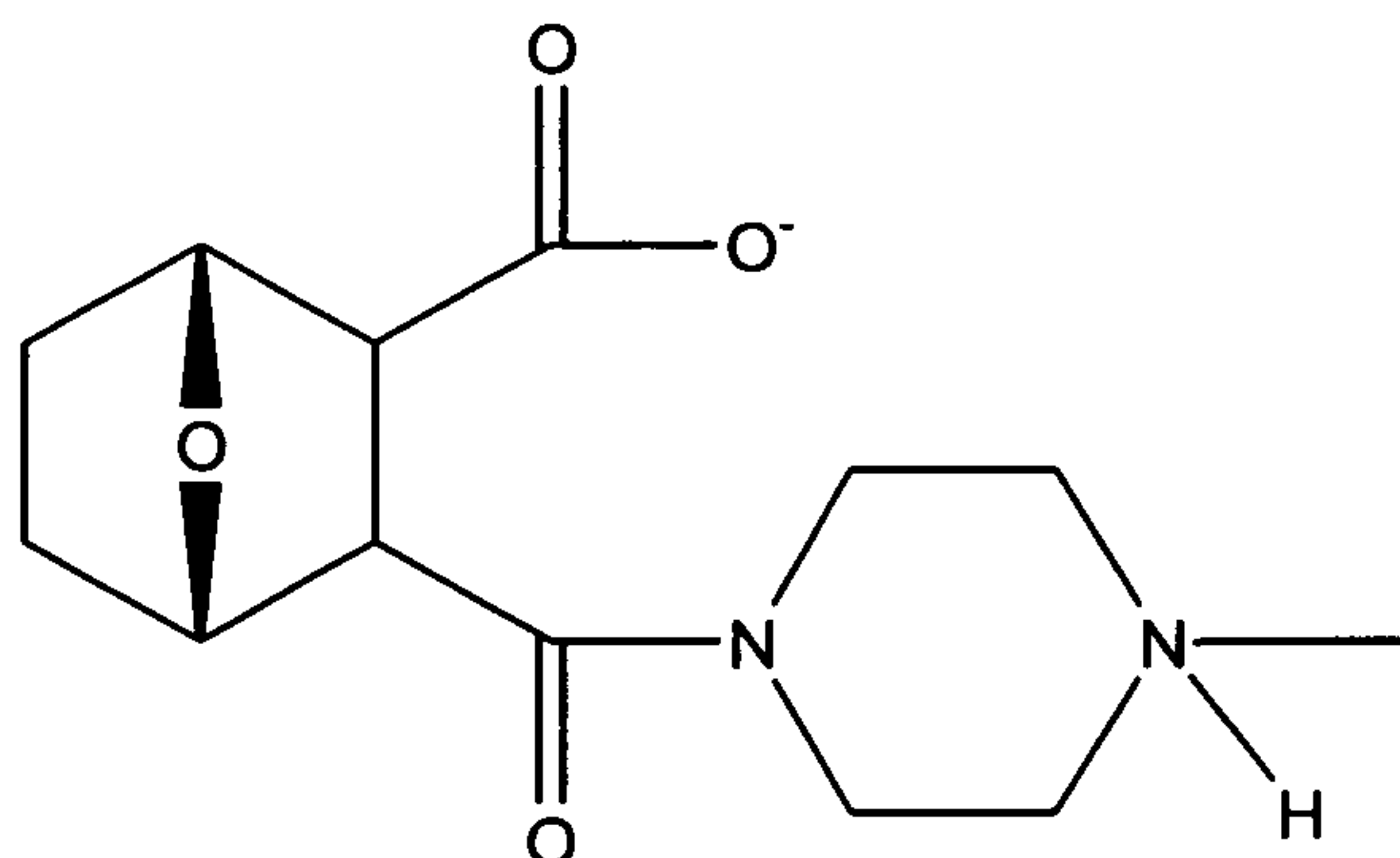
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in benzene and adding a compound having the structure



to produce a compound having the structure



b) recrystallizing the compound following step a), to produce the compound.

48. The process of either claim 43 or 44, wherein the solvent is dimethylformamide.

49. A method of controlling undesired vegetation comprising contacting the vegetation or its environment with a herbicidally effective amount of the compounds of any one of claims 1-37.

50. A method of inhibiting plant phosphatase activity comprising contacting the plant or its environment with a herbicidally effective amount of the compounds of any one of claims 1-37.

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51. The method of claim 49 or 50, wherein the compound is Compound 100, 100E, 101, 101E, 102, 102E, 103, 103E, 104, 104E, 105, 105E, 106, 106E, 107, 107E, 108, or 111.
52. A method of preventing or treating a fungal infection in a subject comprising administering to the subject an effective amount of the compounds of any one of claims 1-37 to treat the fungal infection, thereby treating the fungal infection.
53. The method of claim 52, wherein the compound is Compound 100, 100E, 101, 101E, 102, 102E, 103, 103E, 104, 104E, 105, 105E, 106, 106E, 107, 107E, 108 or 111.
54. A method of treating a subject afflicted with breast cancer, colon cancer, large cell lung cancer, adenocarcinoma of the lung, small cell lung cancer, stomach cancer, liver cancer, ovary adenocarcinoma, pancreas carcinoma, prostate carcinoma, promyelocytic leukemia, chronic myelocytic leukemia, or acute lymphocytic leukemia, comprising administering to the subject a therapeutically effective amount of the compounds of any one of claims 1-37, thereby treating the subject.
55. The method of claim 54, wherein the compound is 100, 100E, 101, 101E, 102, 102E, 103, 103E, 104, 104E, 105, 105E, 106, 106E, 107, 107E, 108 or 111.

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Fig. 1

## Inhibition of U373 by Four Cantharidin Analogs

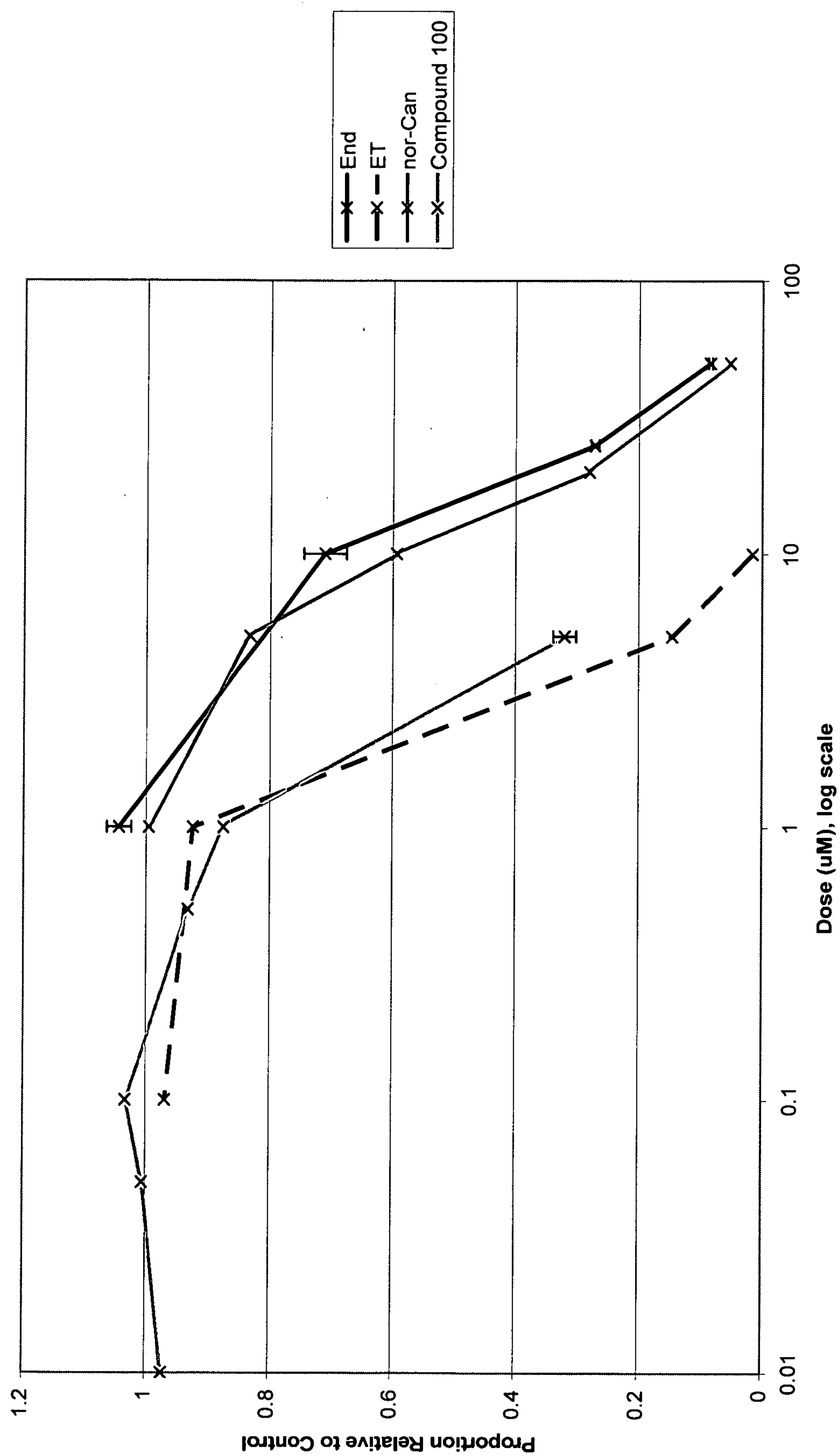


Fig. 2

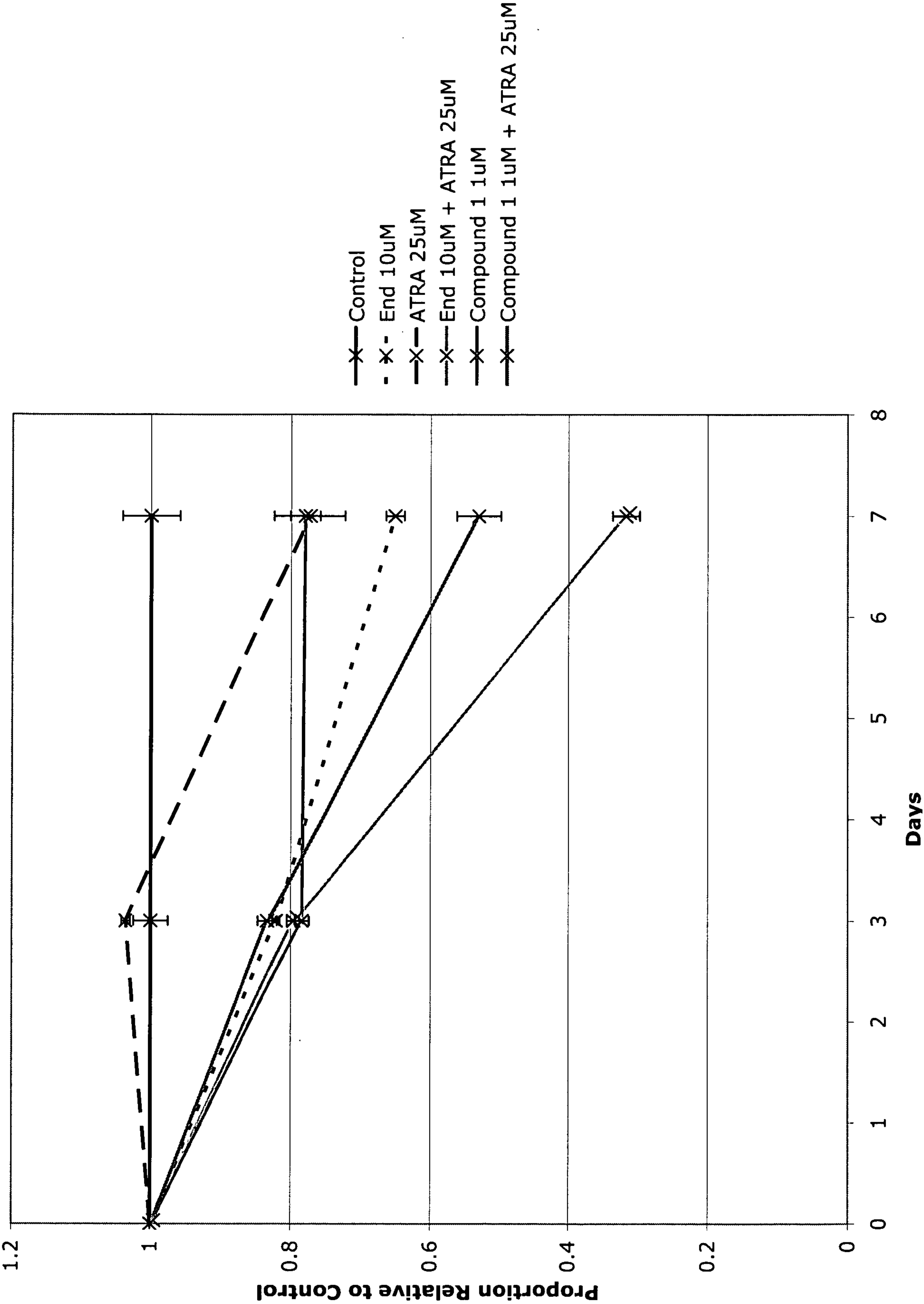


Fig. 3 Inhibition of Kidney Cancer Cell Line, UMRC

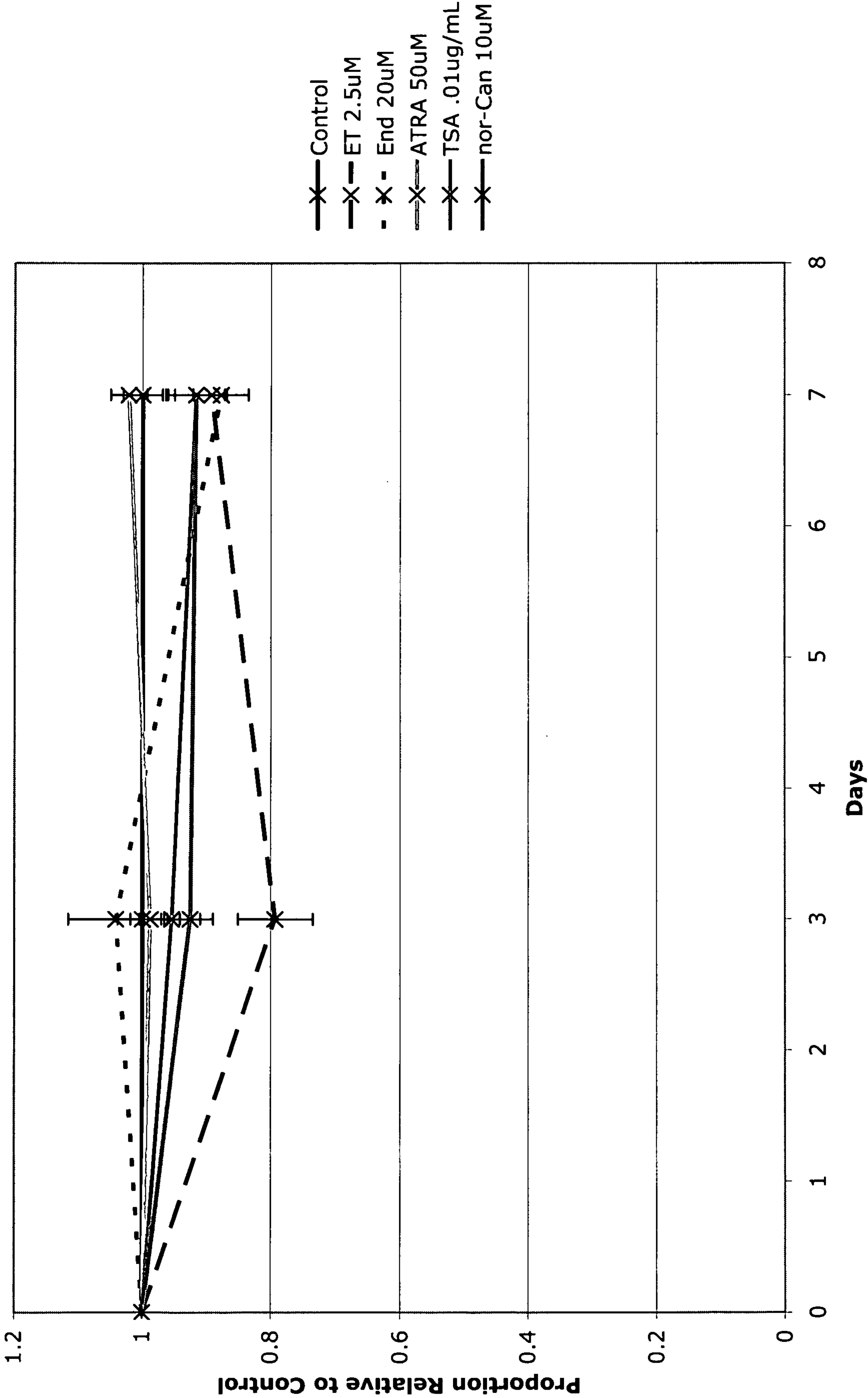
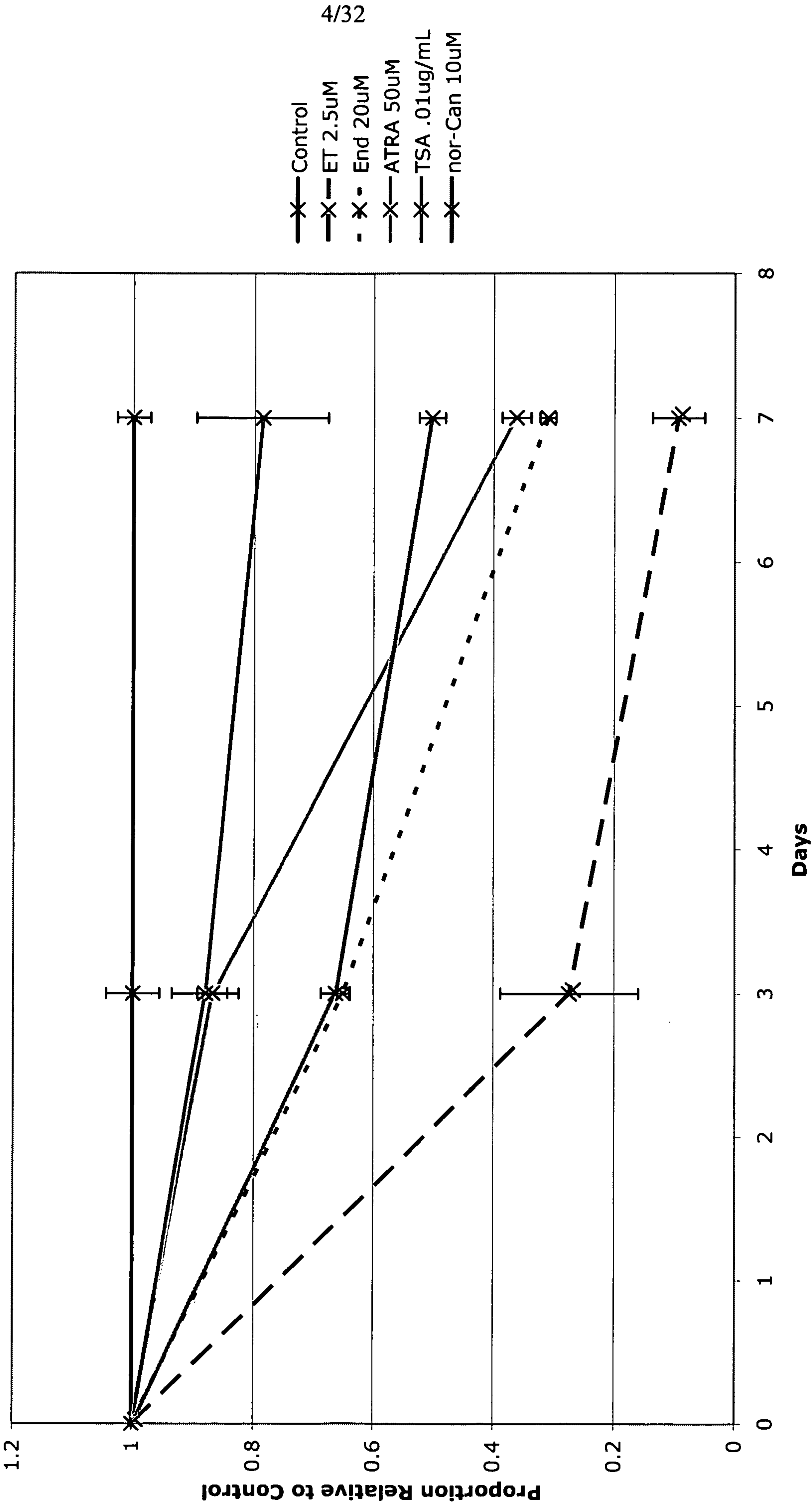


Fig. 4

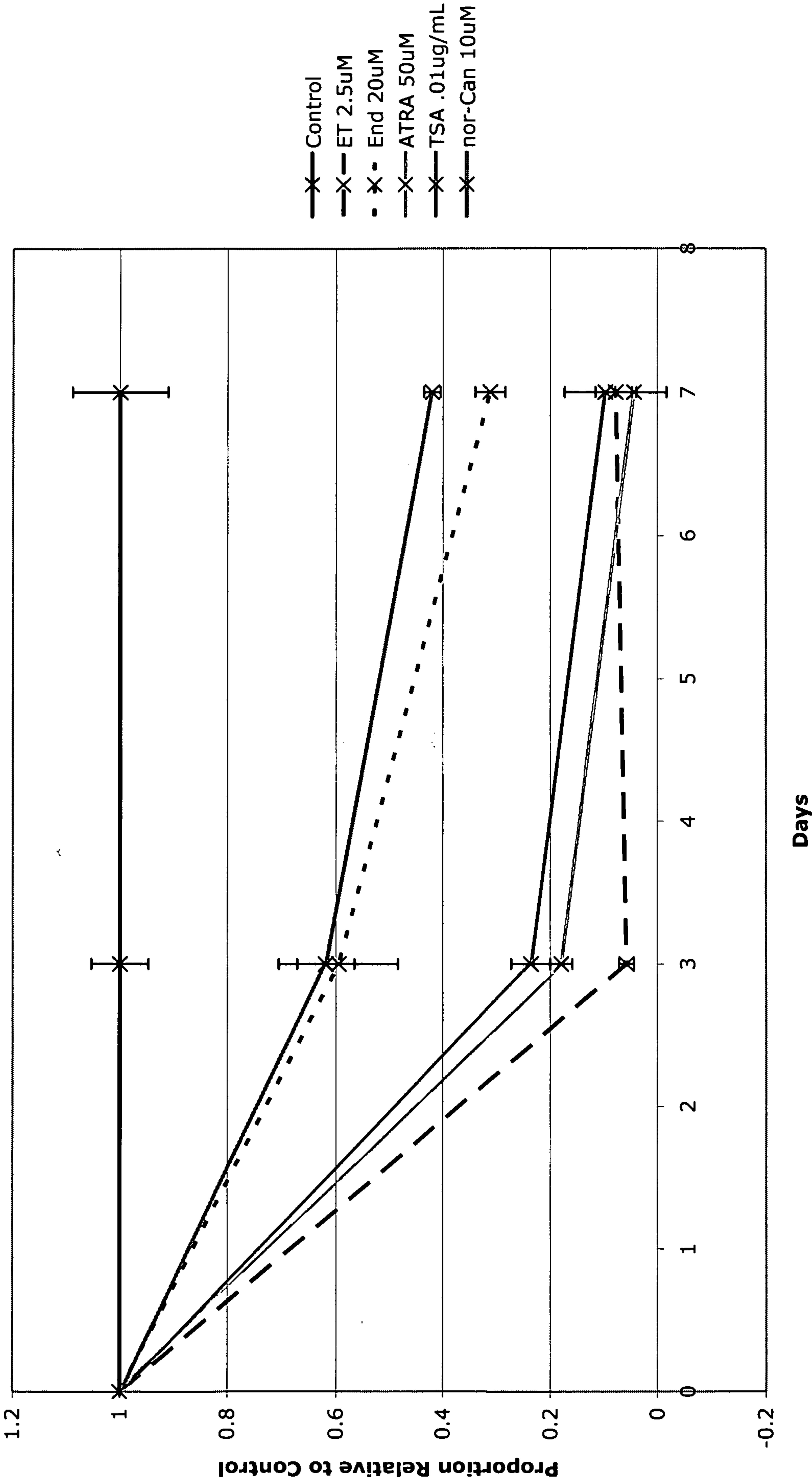
Inhibition of Glimoa Cell Line, U373



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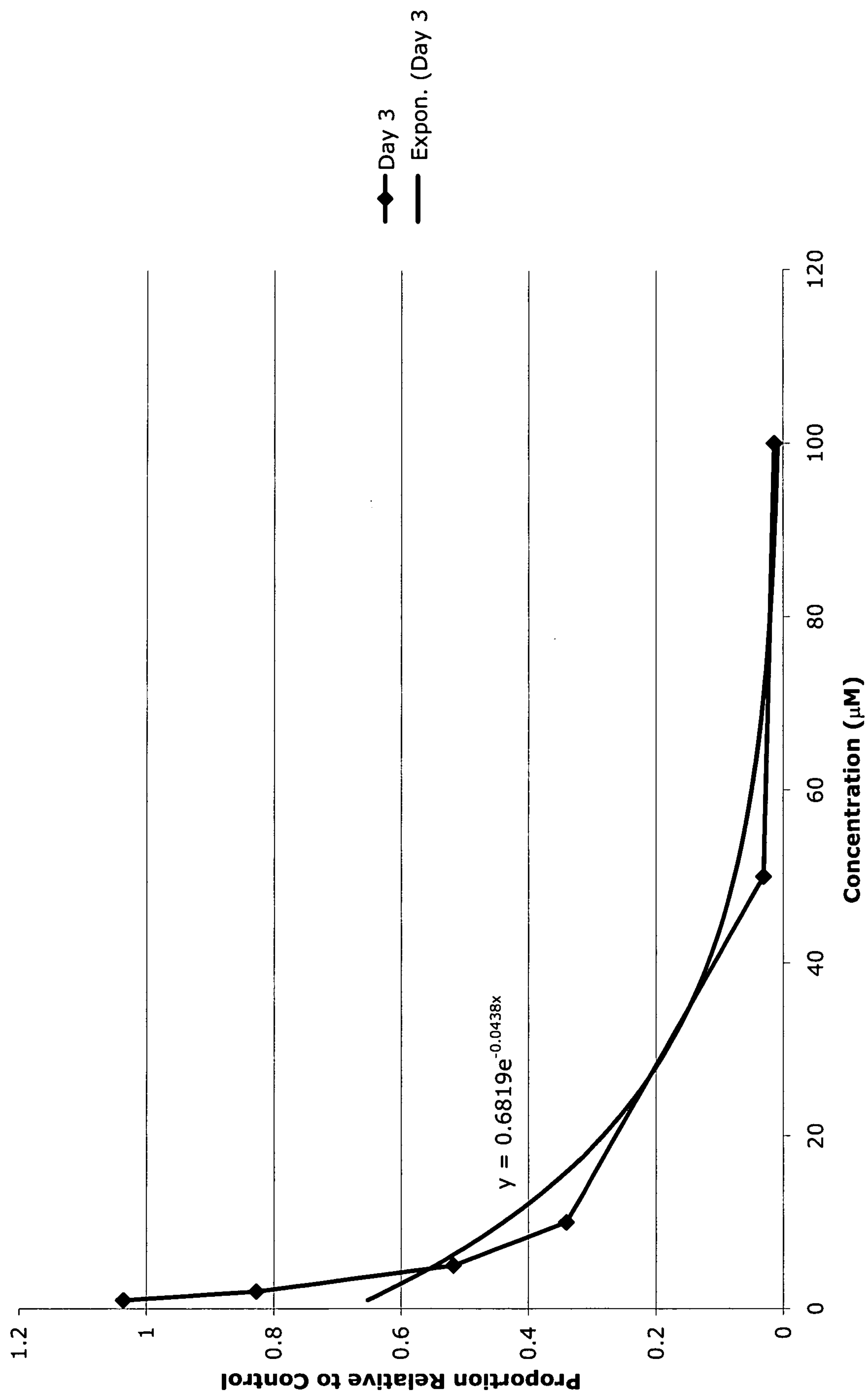
Fig. 5

Inhibition of Breast Cancer Cell Lines, MCF-7



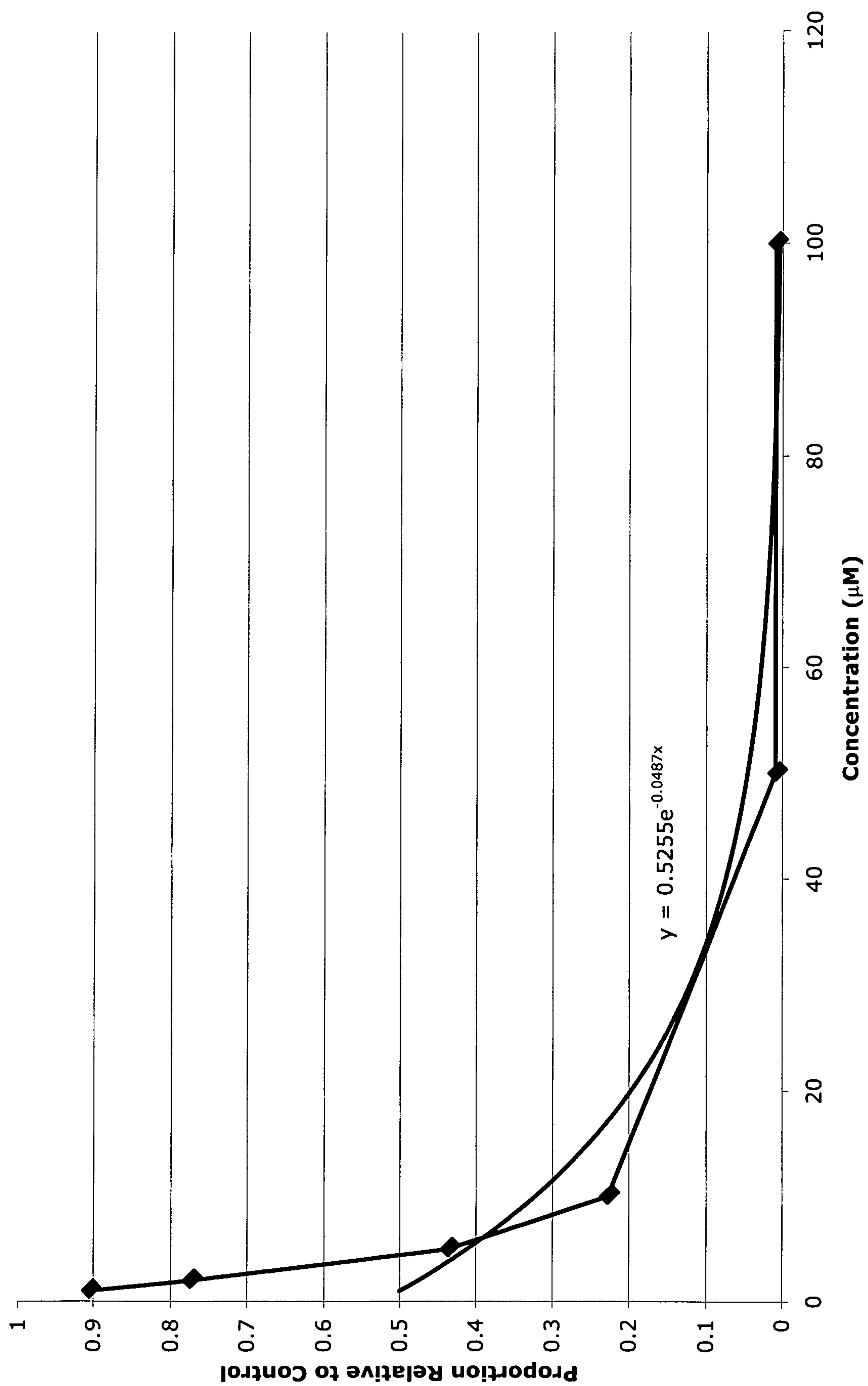
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Fig. 6 Glioblastoma Multiforme: Line U373



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Fig. 7 Glioblastoma Multiforme: Line U373



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Fig. 8  
Glioblastoma Multiforme: Line U373

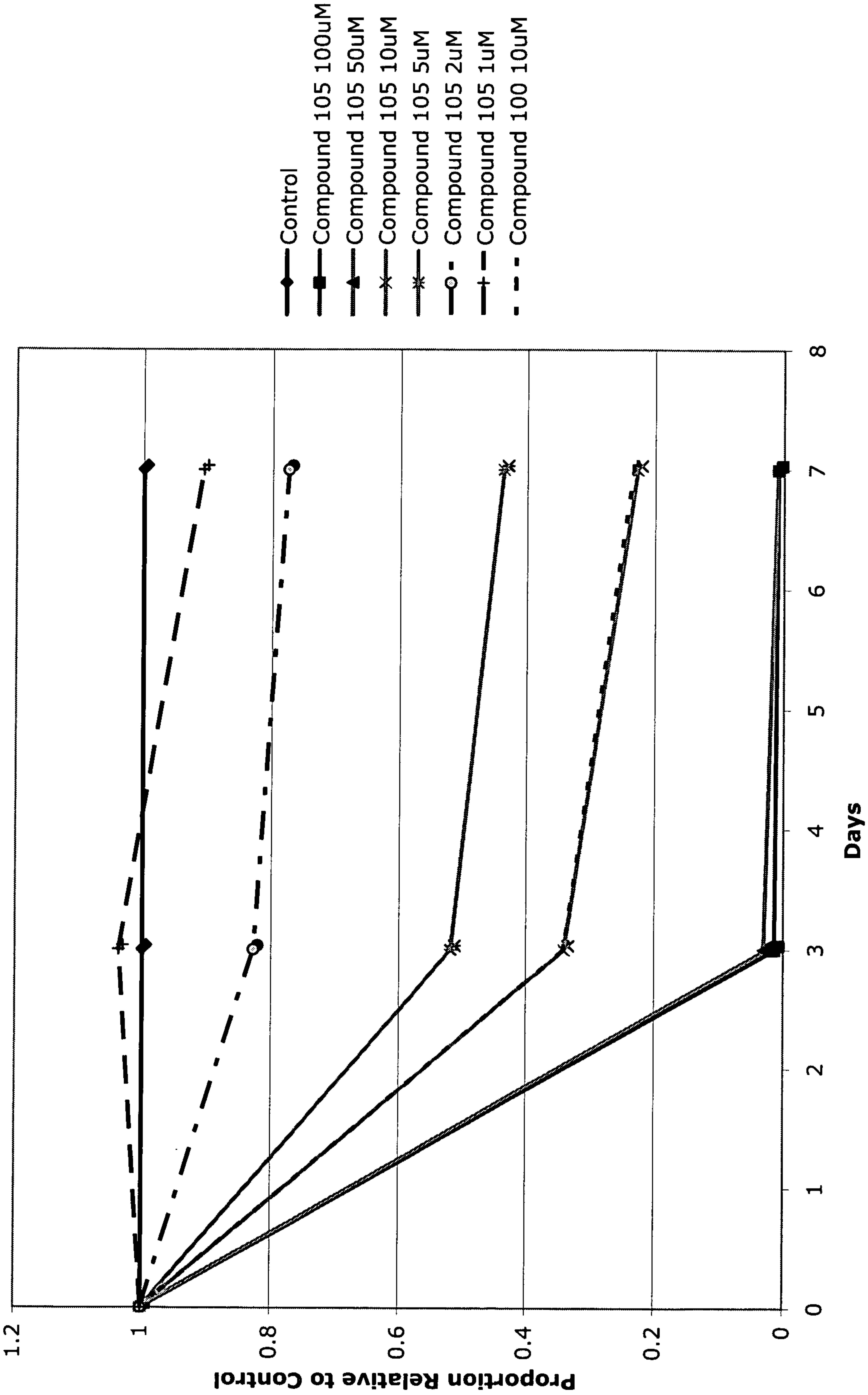
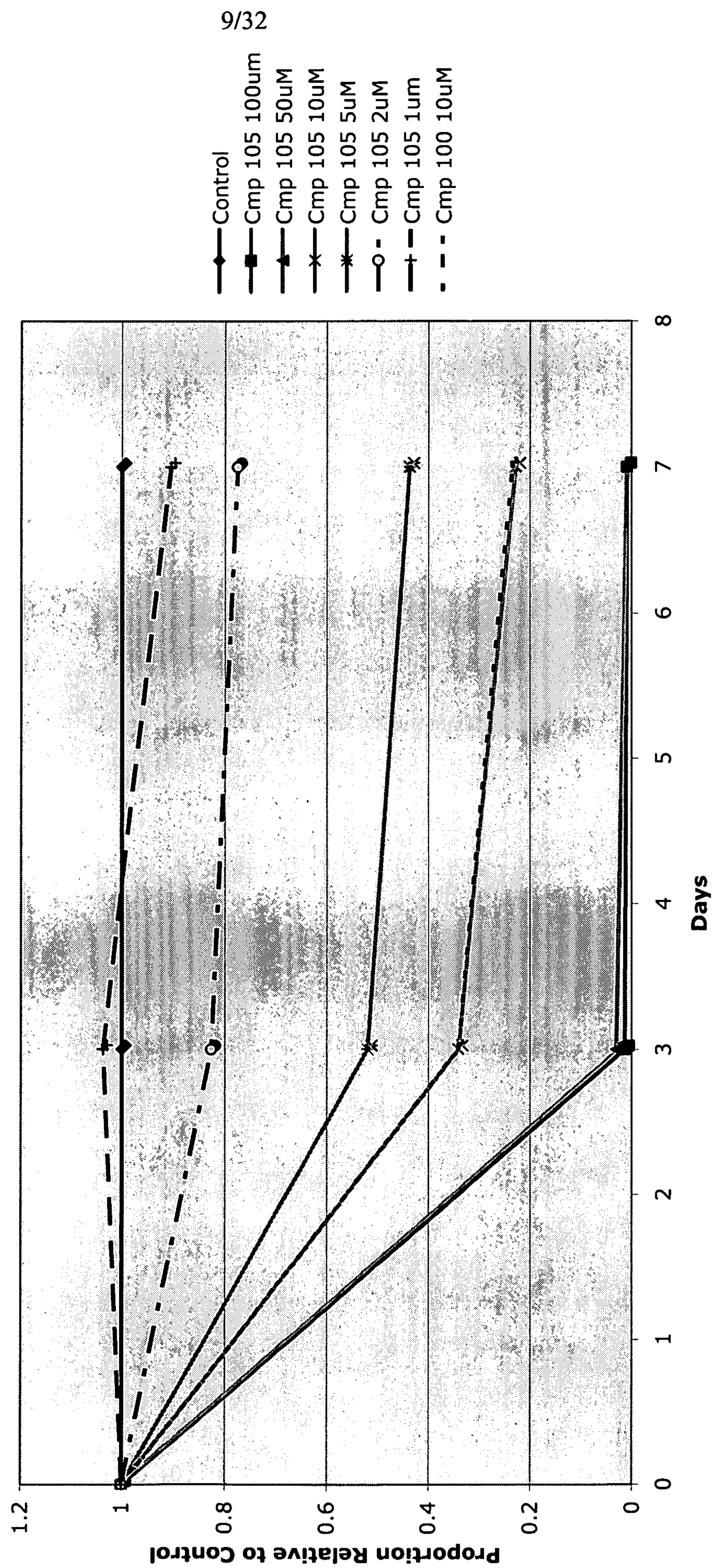


Fig. 9

## FIGURE 9

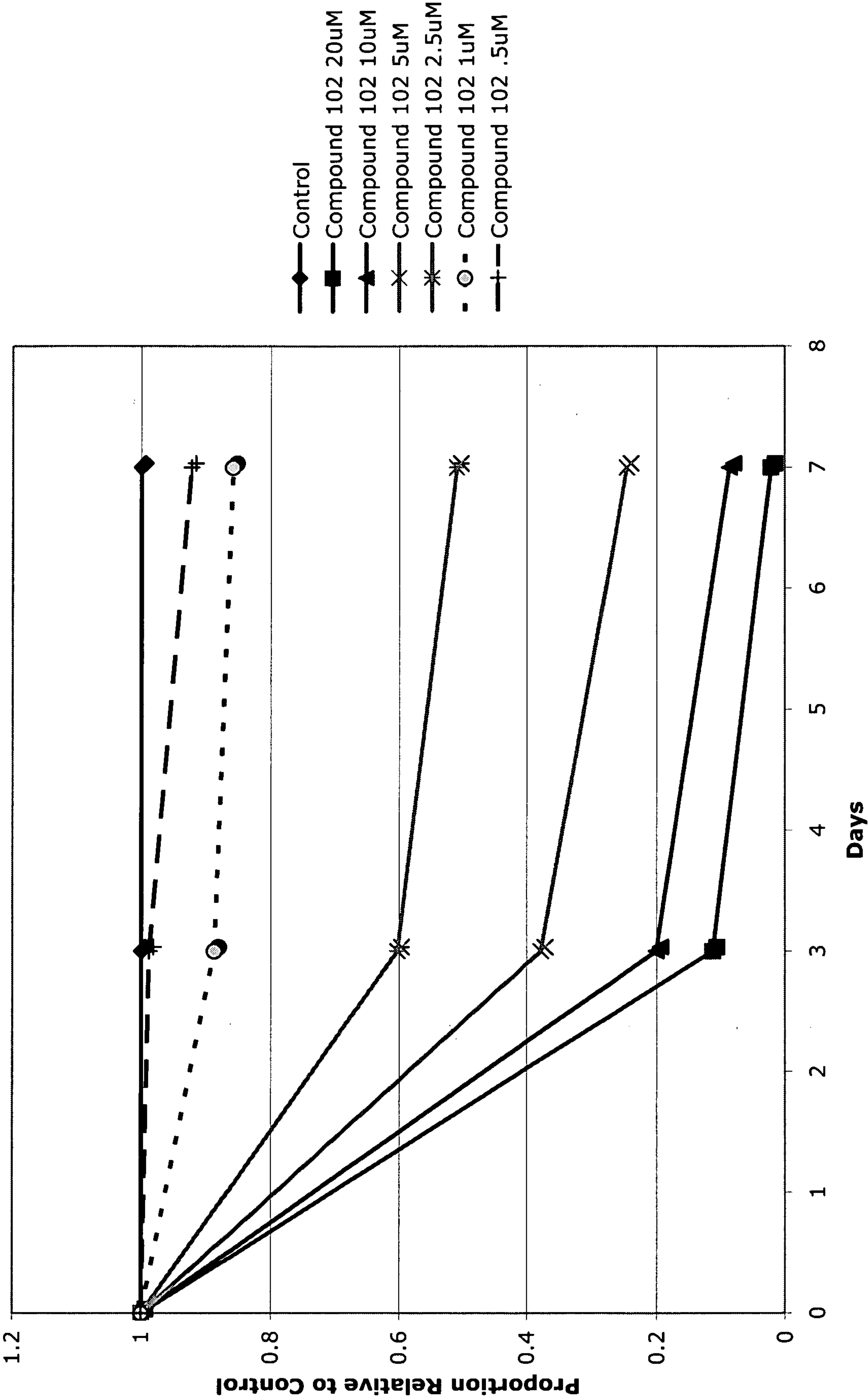
## Glioblastoma Multiforme: Line U373

### Dose Response to Compound 105 Compared to Compound 100 at 10 $\mu$ M



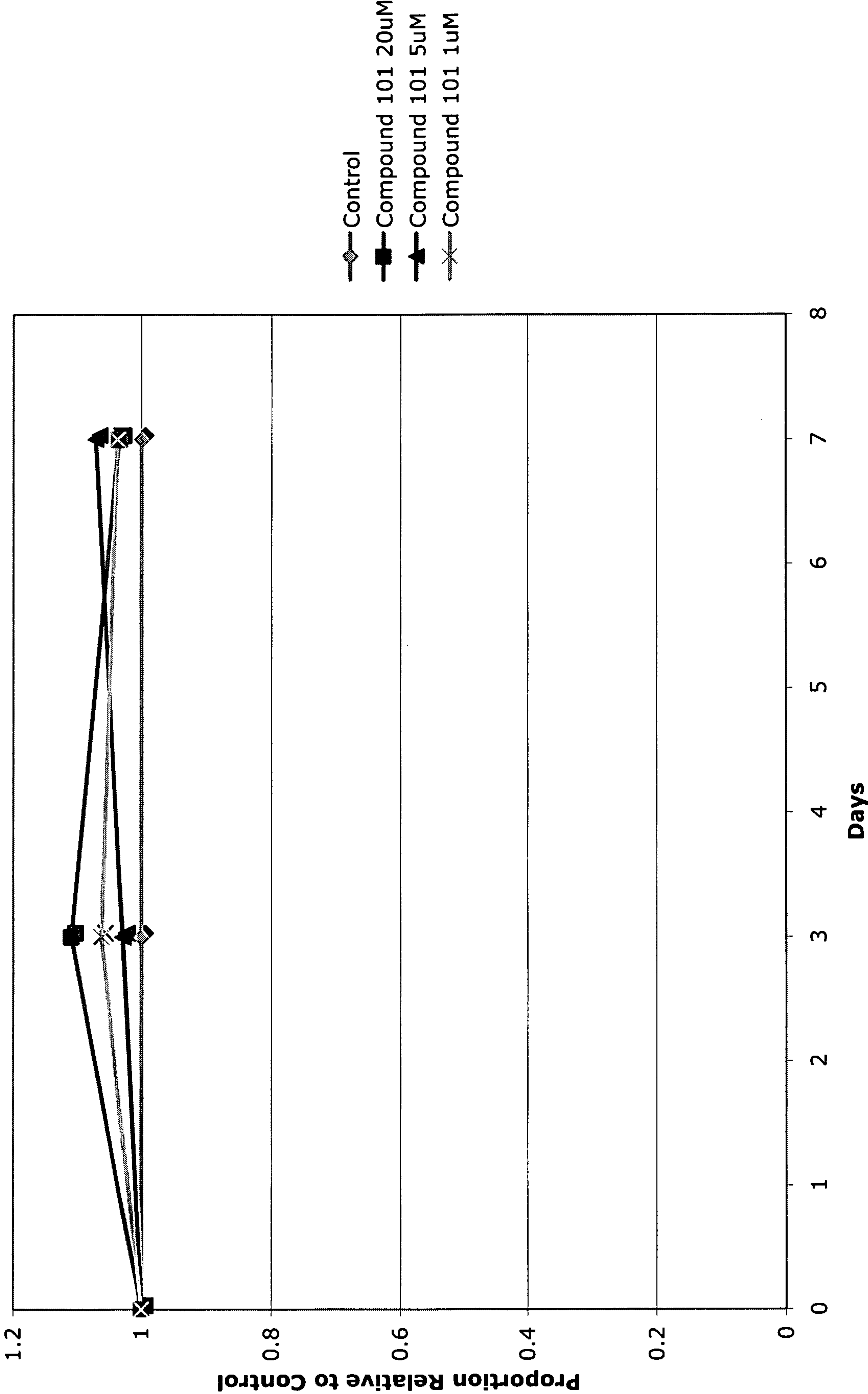
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Fig. 10  
Glioblastoma Multiforme: Line U373



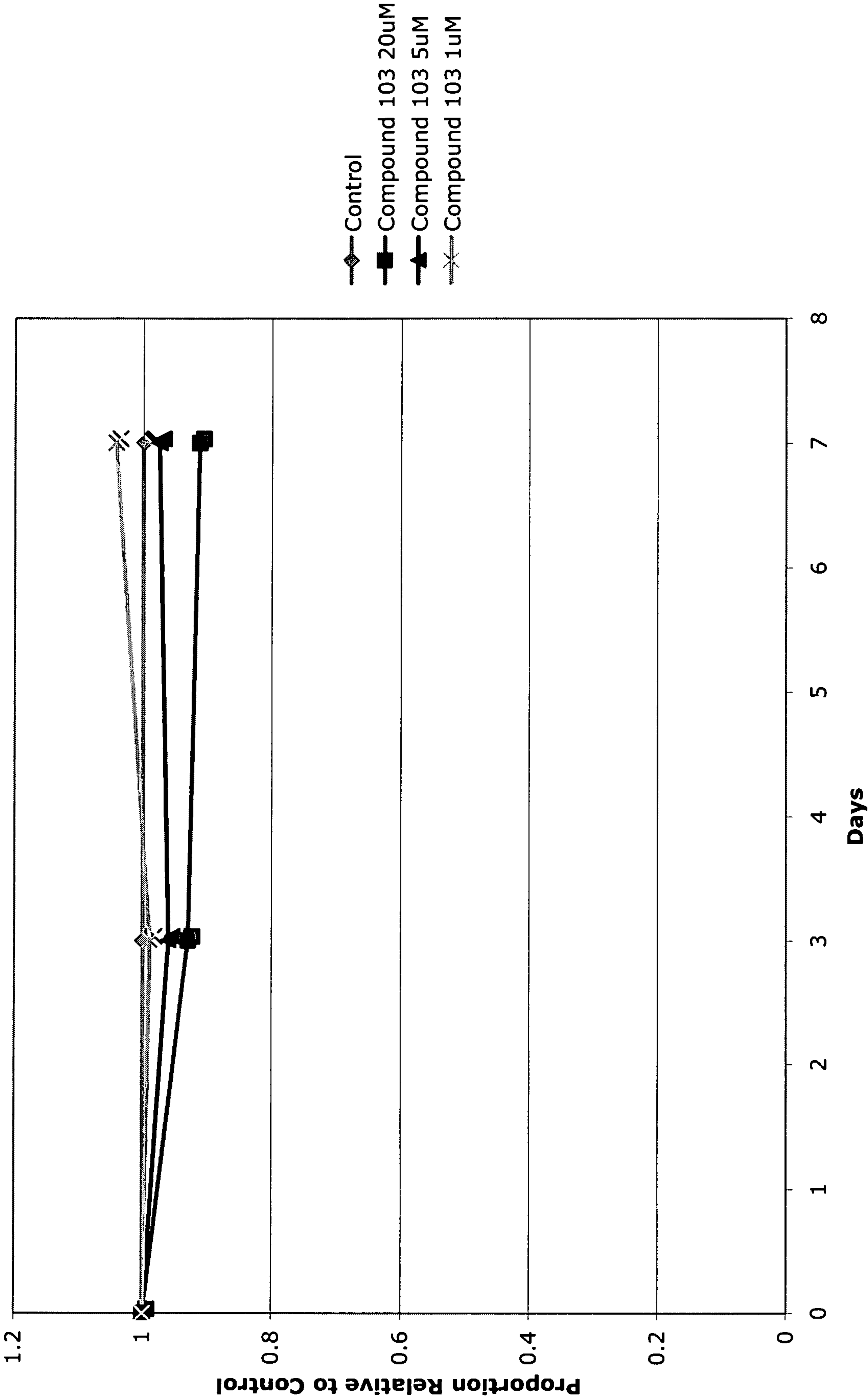
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Fig. 11 Glioblastoma Multiforme: Line U373



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Fig. 12  
Glioblastoma Multiforme: Line U373



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Fig. 13 Glioblastoma Multiforme: Line U373

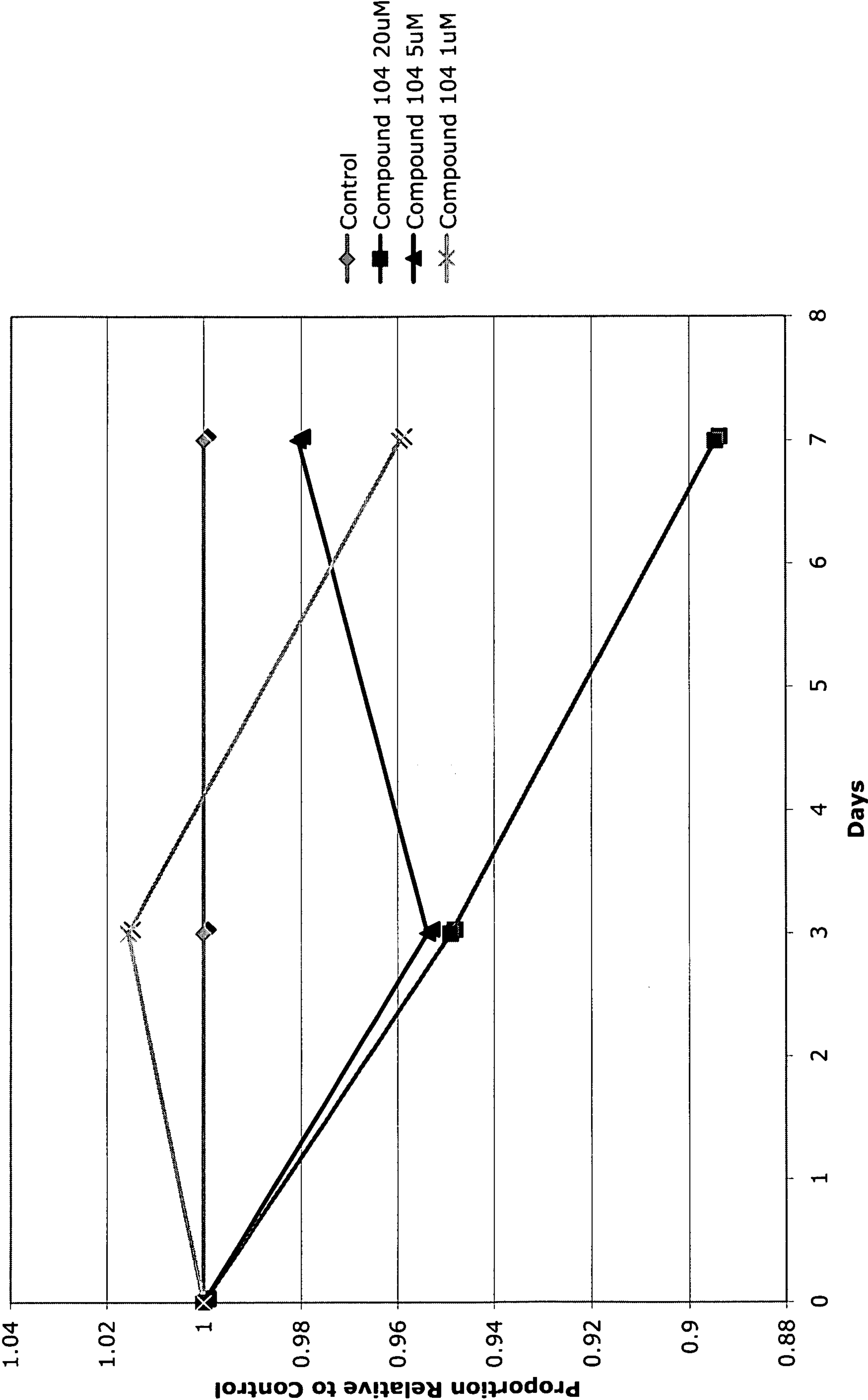


Fig. 14 Glioblastoma Multiforme: Line U373

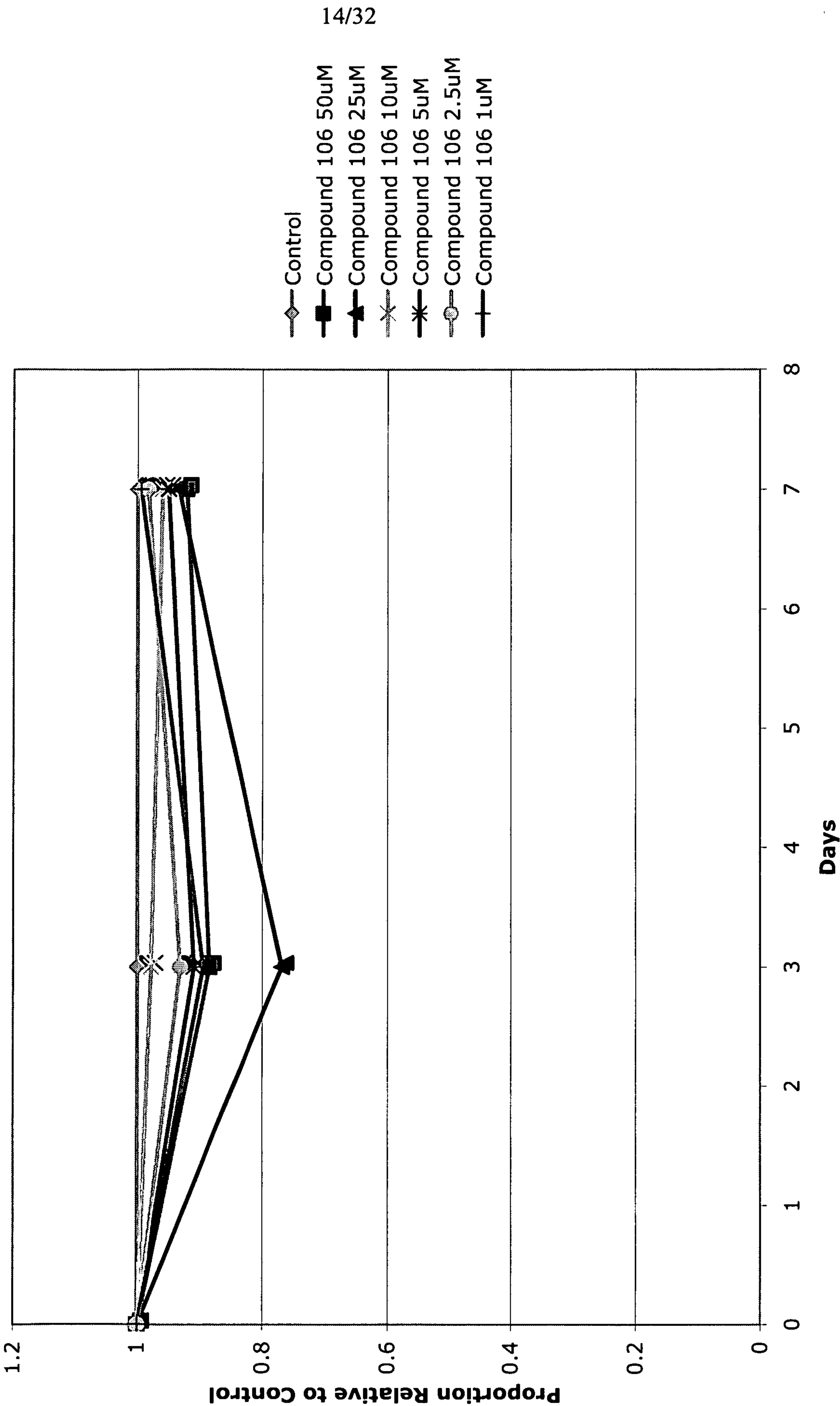
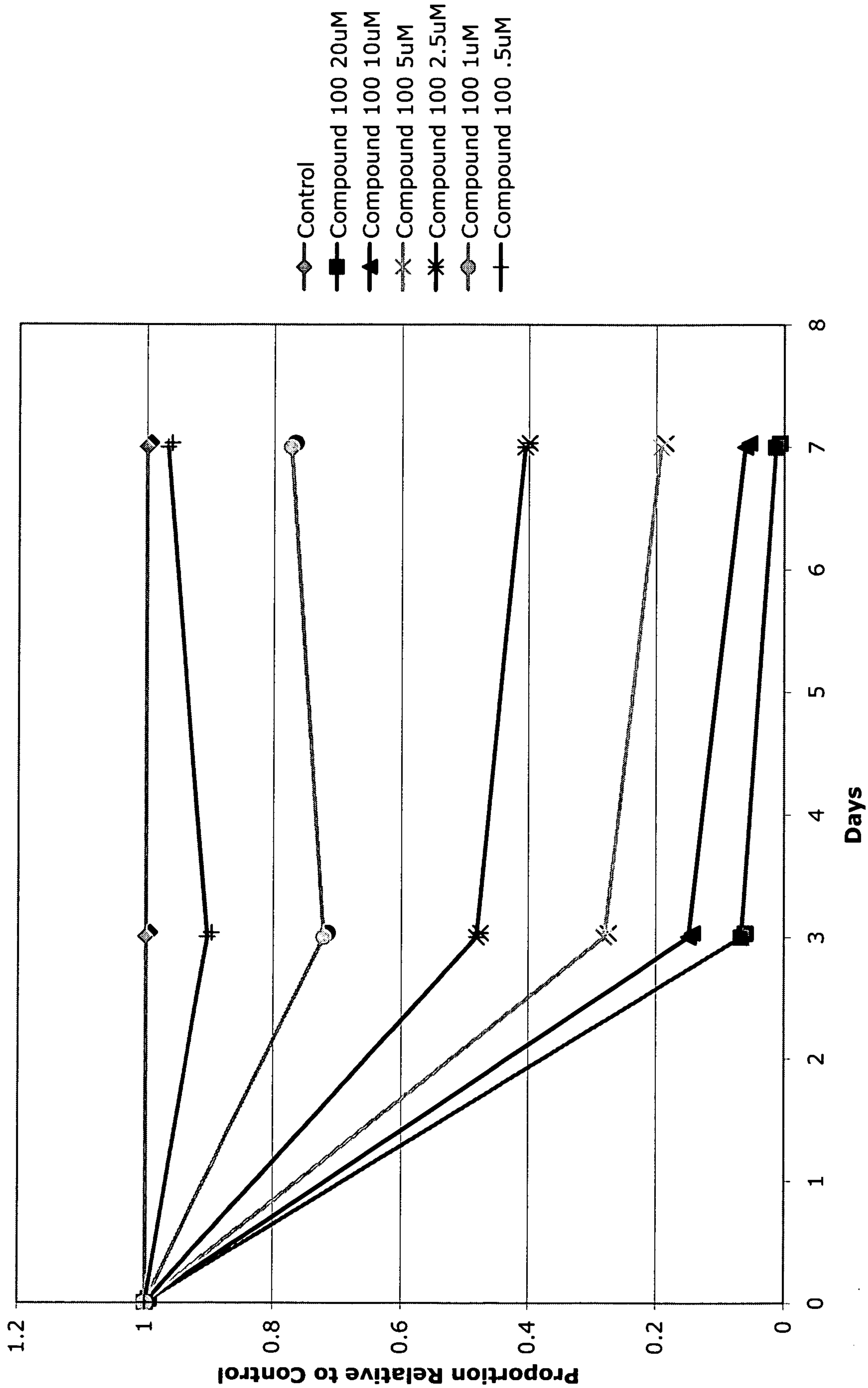


Fig. 15 Glioblastoma Multiforme: Line U373



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**FIGURE 16**  
**Medulloblastoma: Line DAOY**

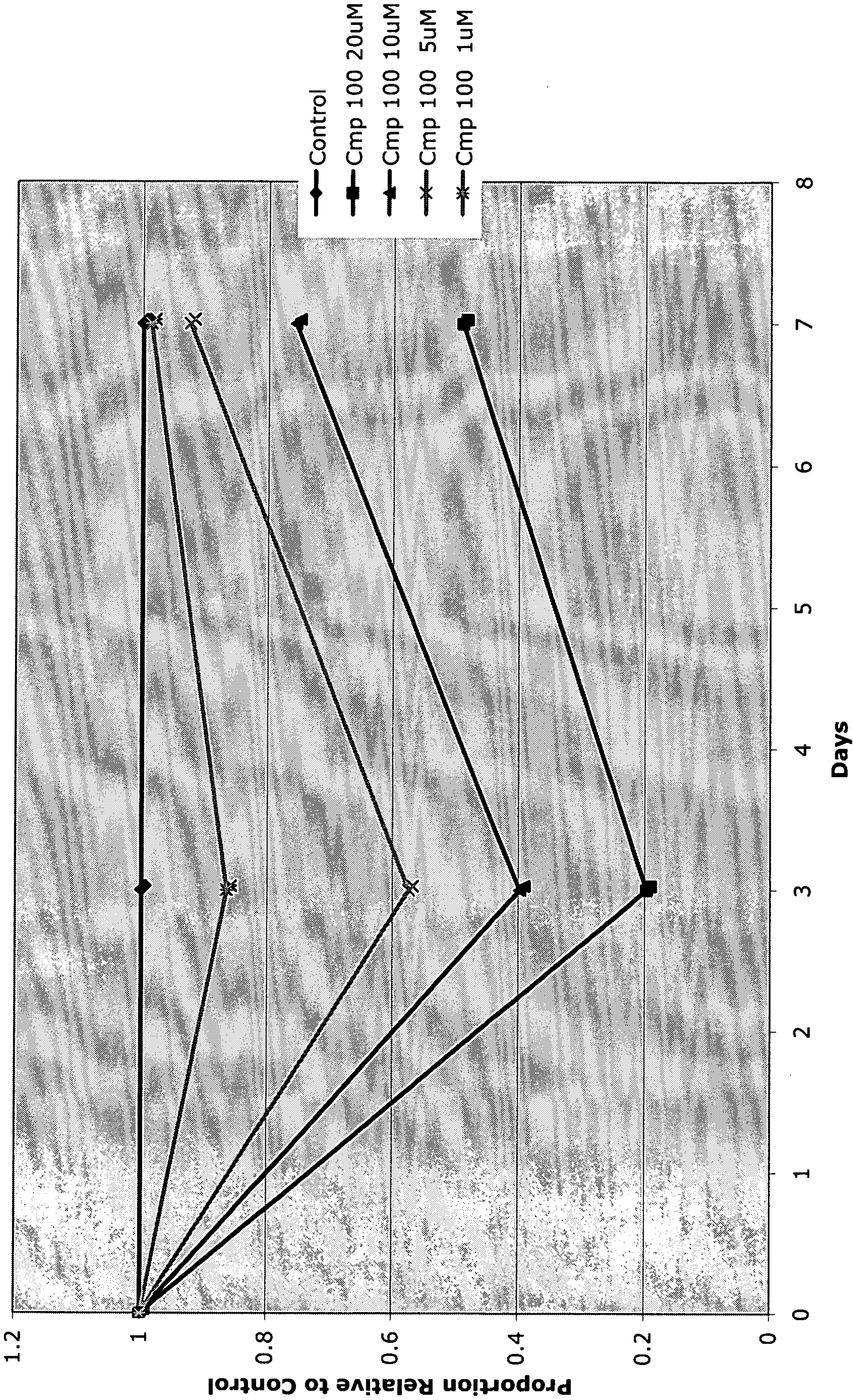


Fig. 17

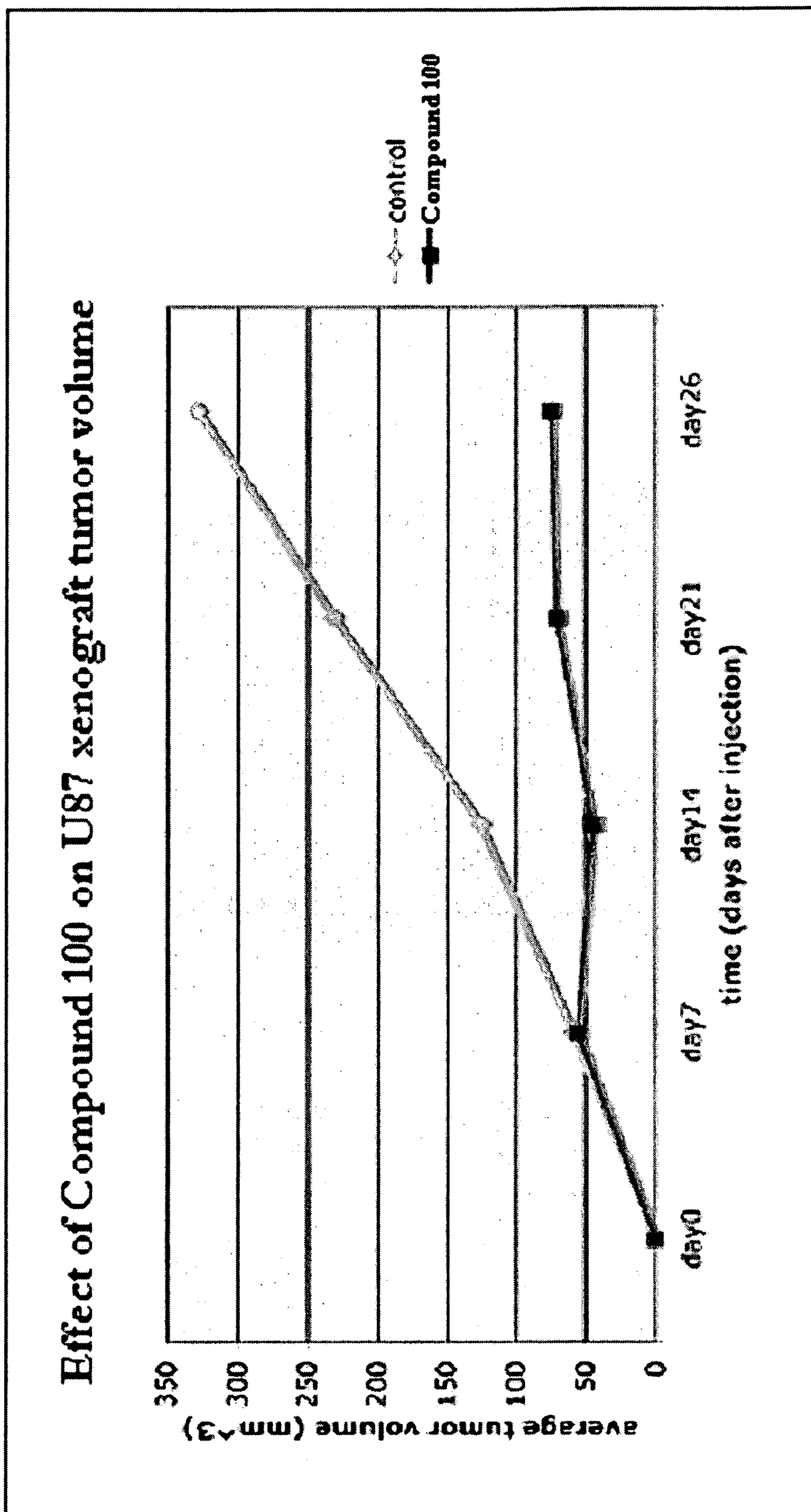


Fig. 18

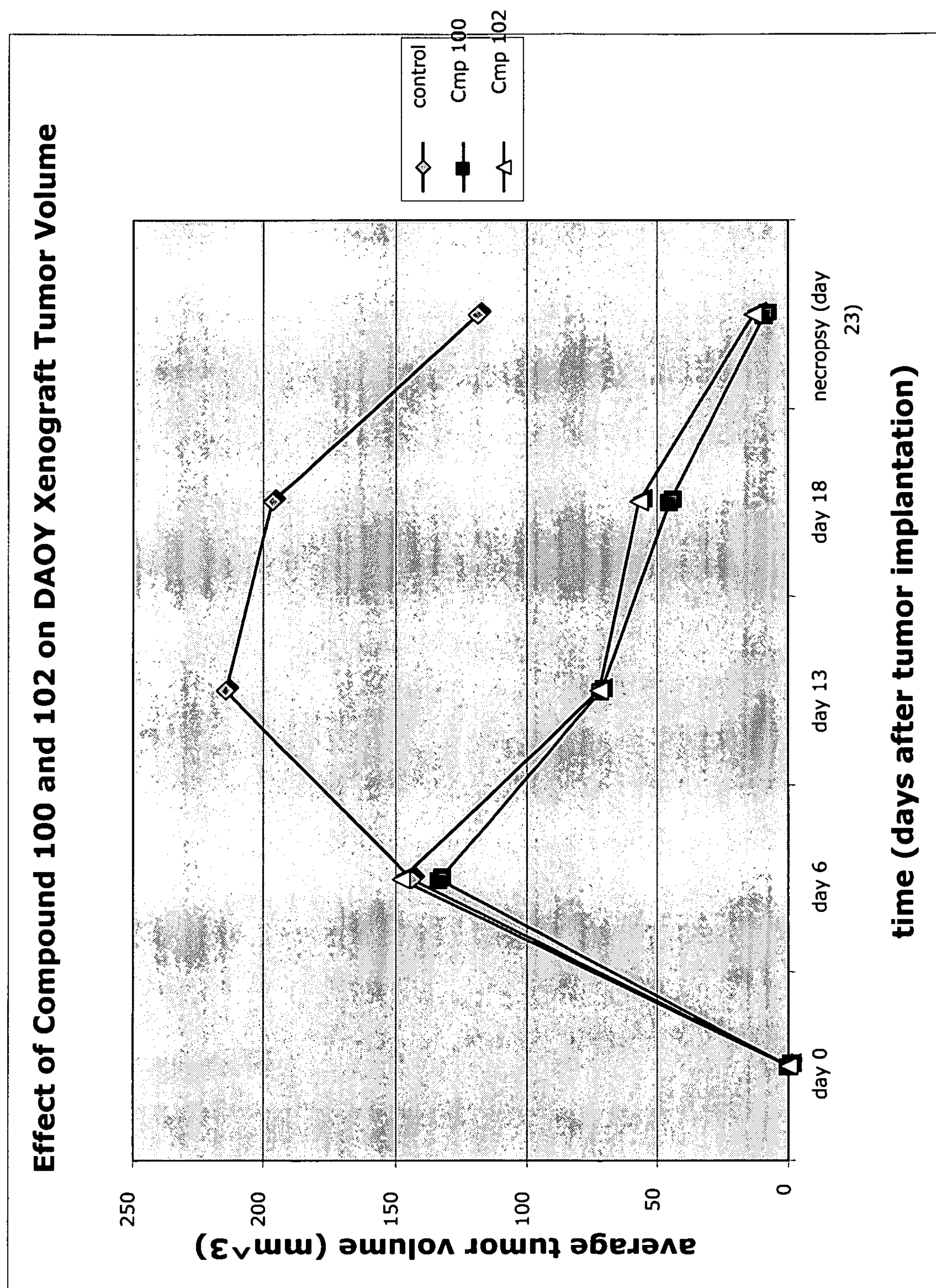


Fig. 19A

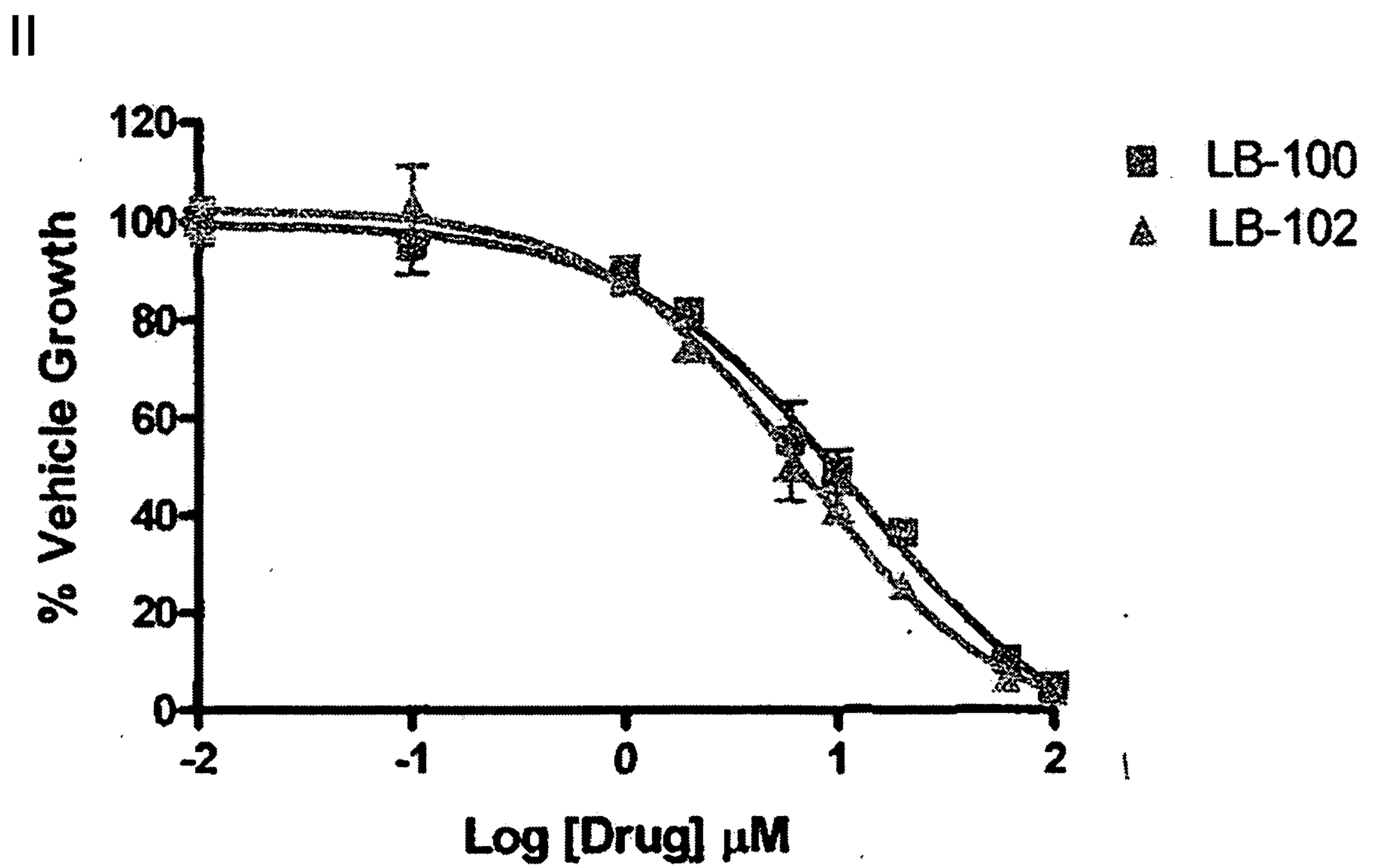
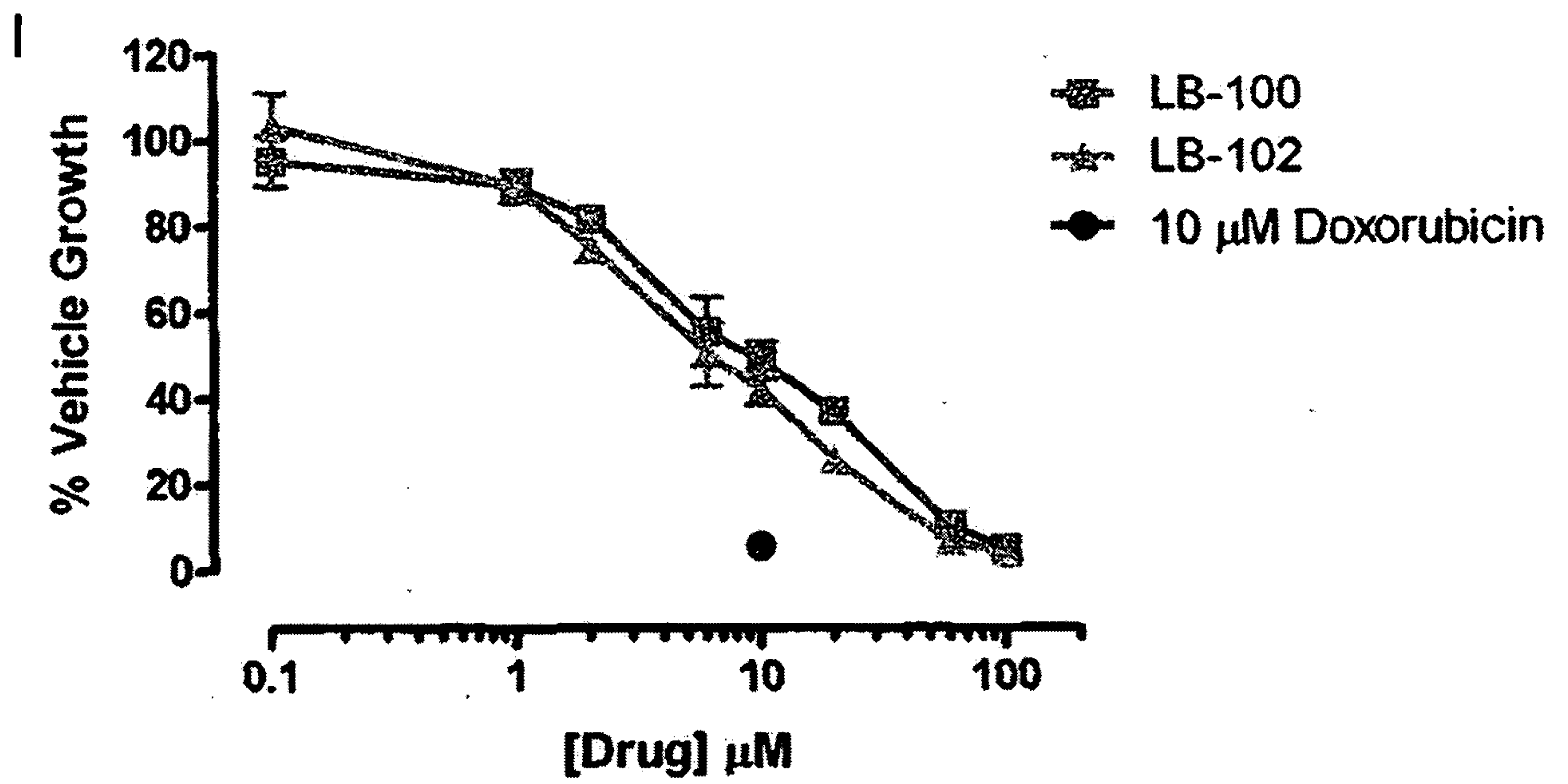


Fig. 19B

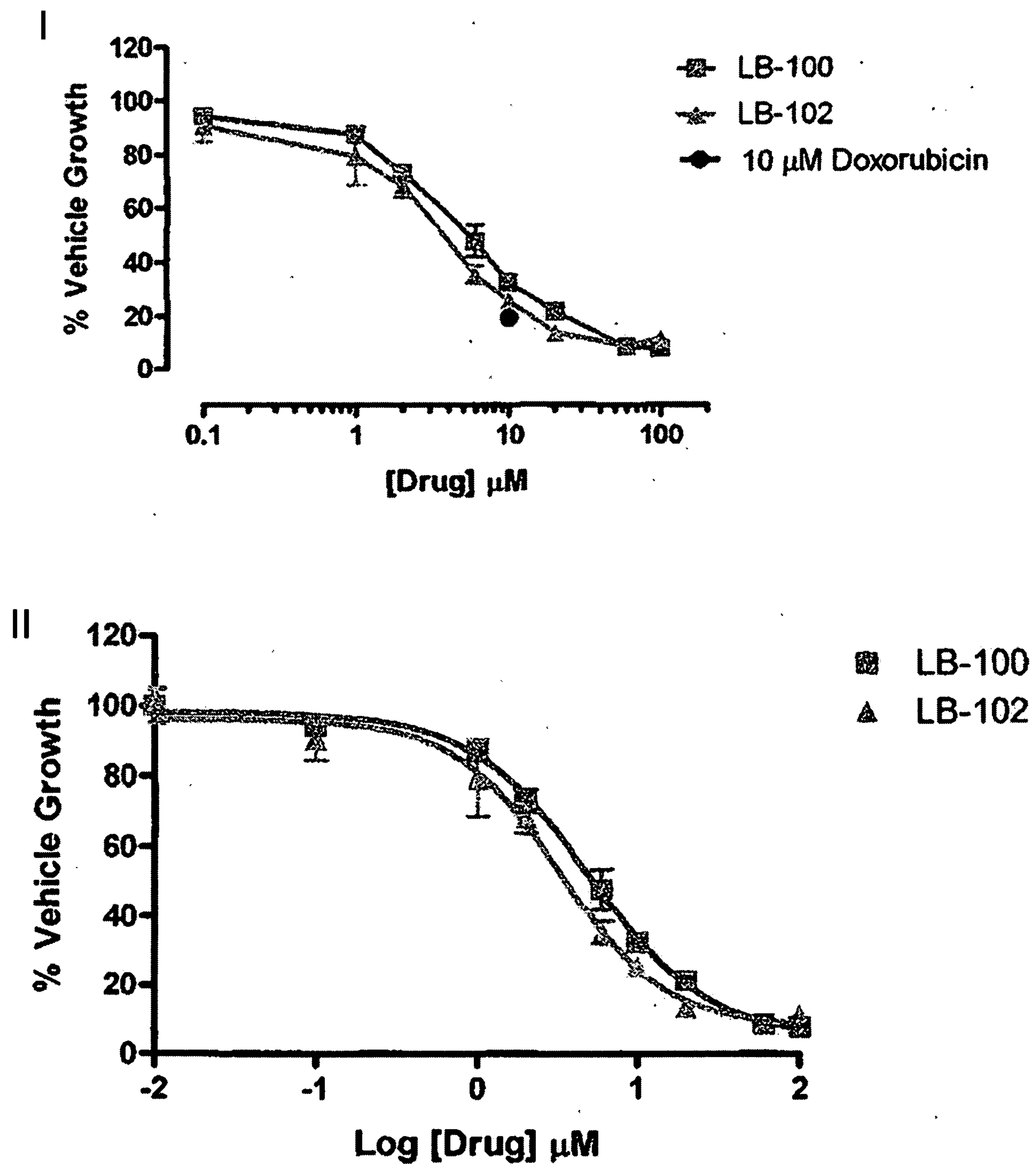


Fig. 19C

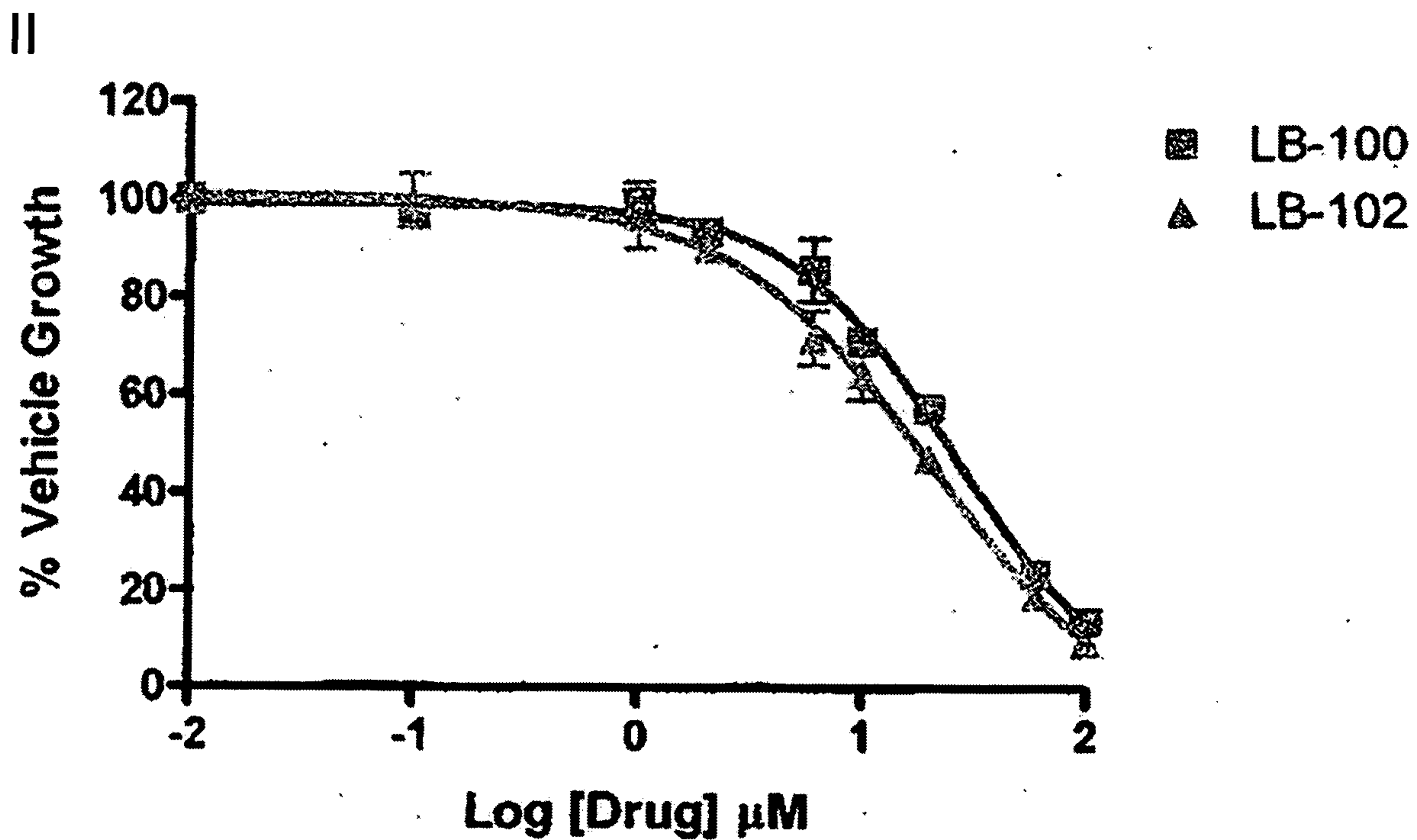
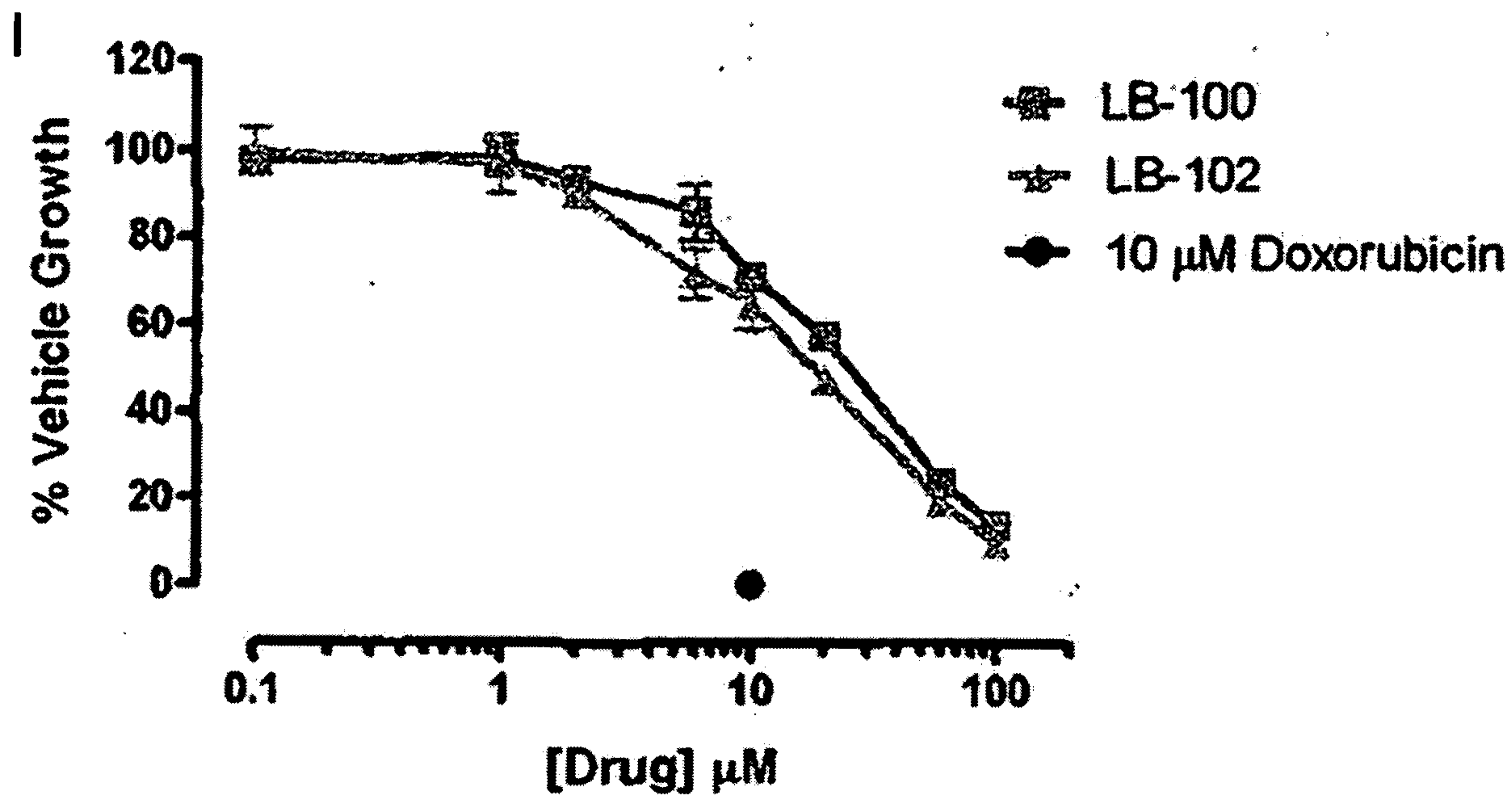


Fig. 19D

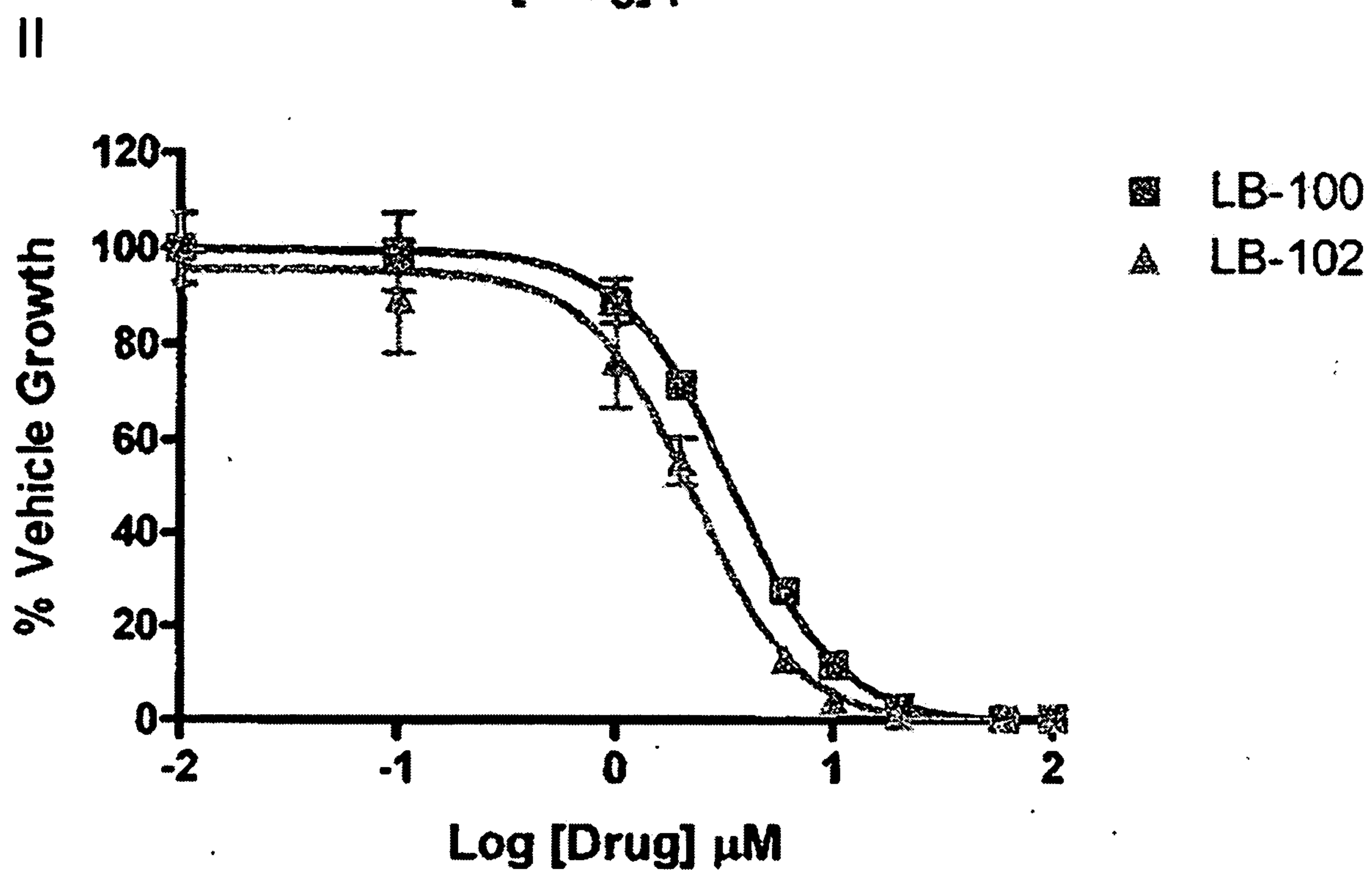
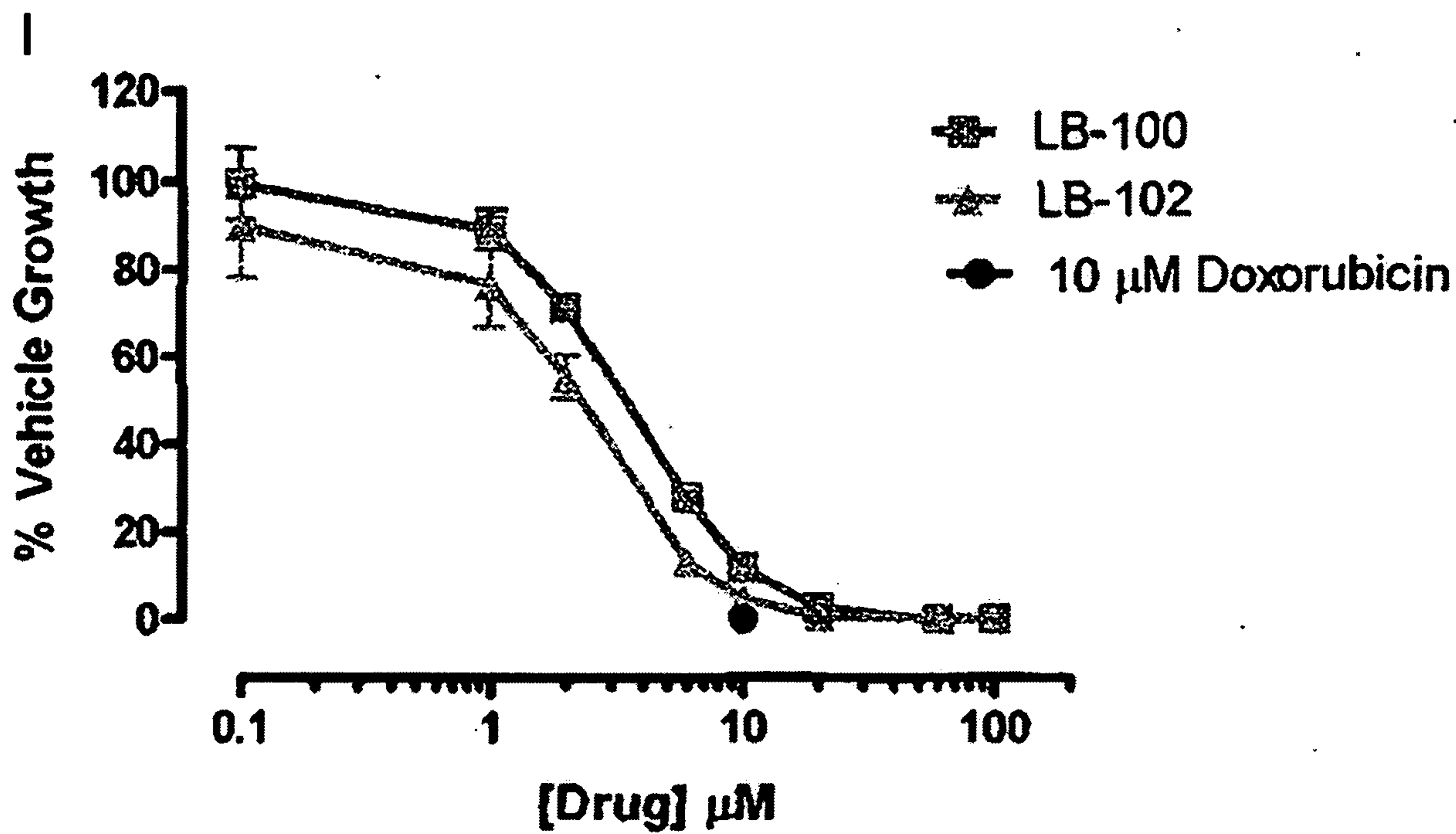
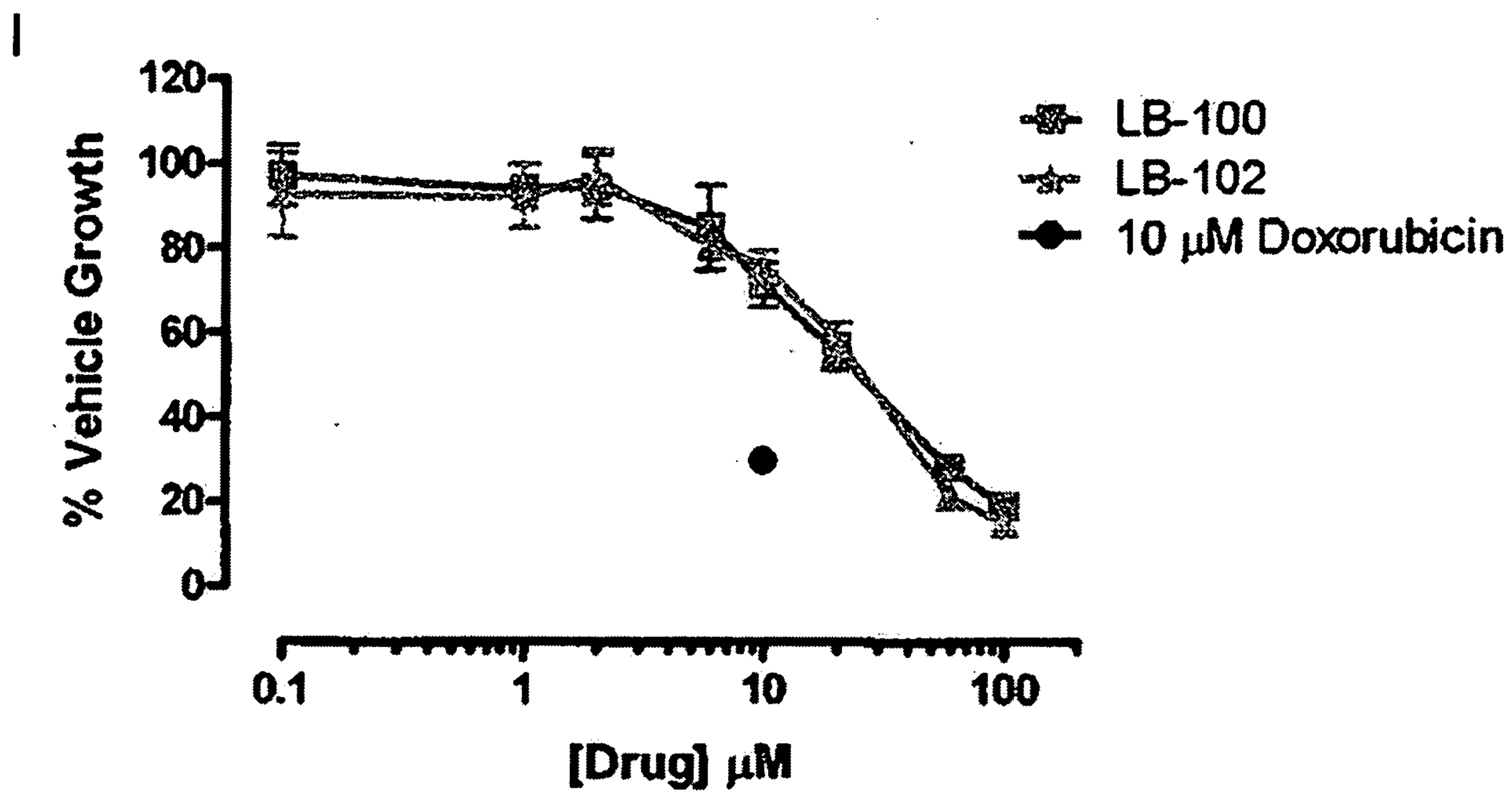


Fig. 19E



II

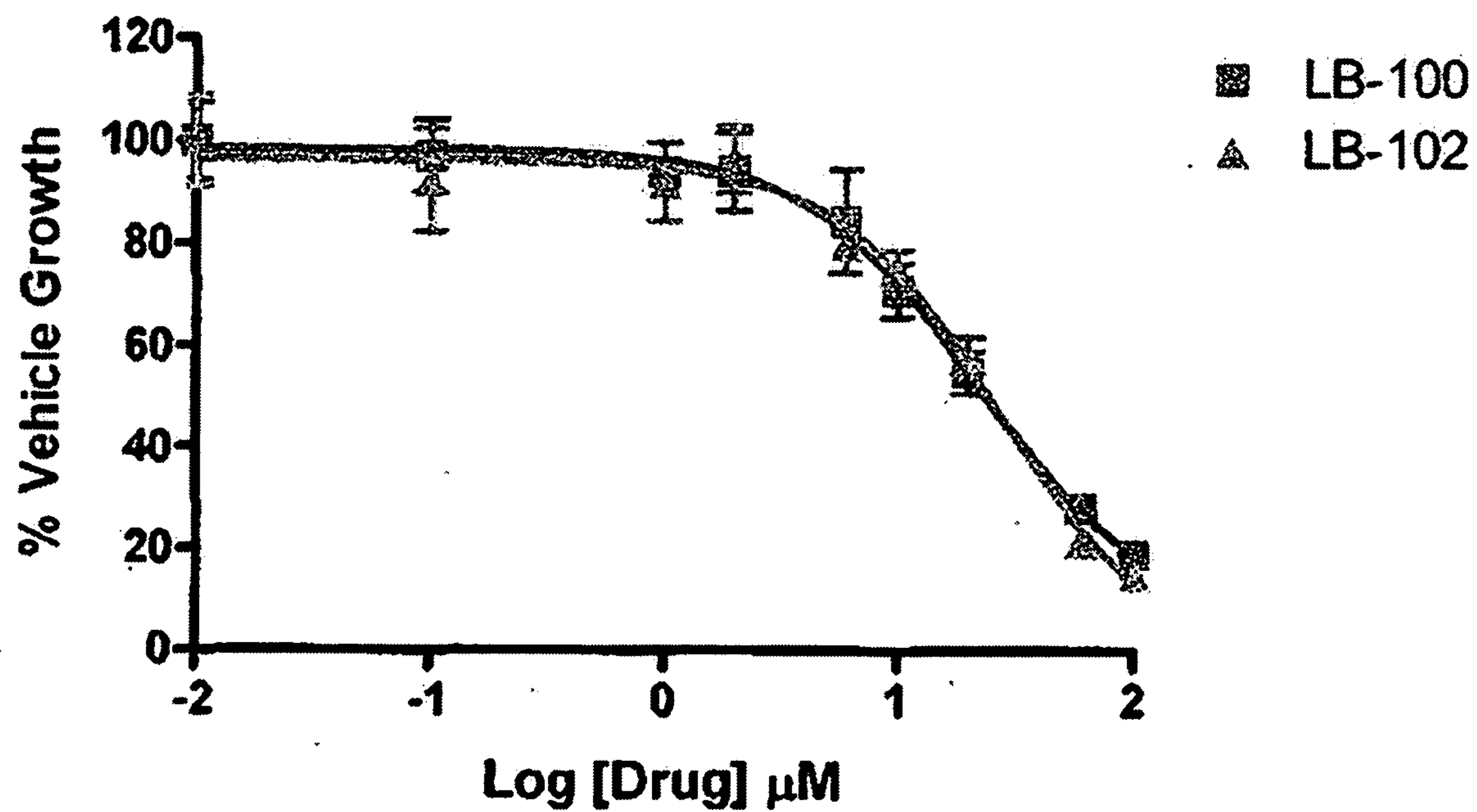
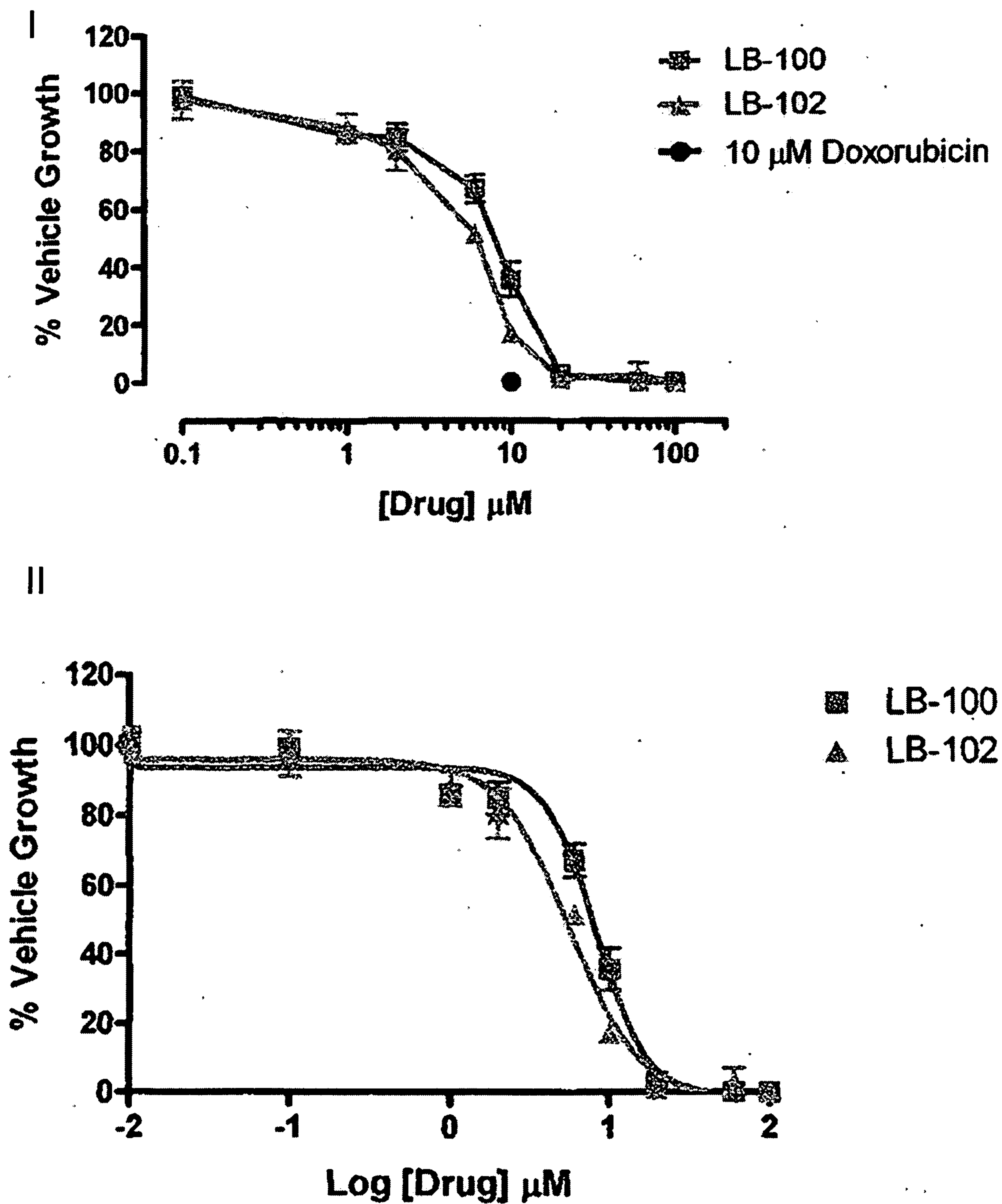


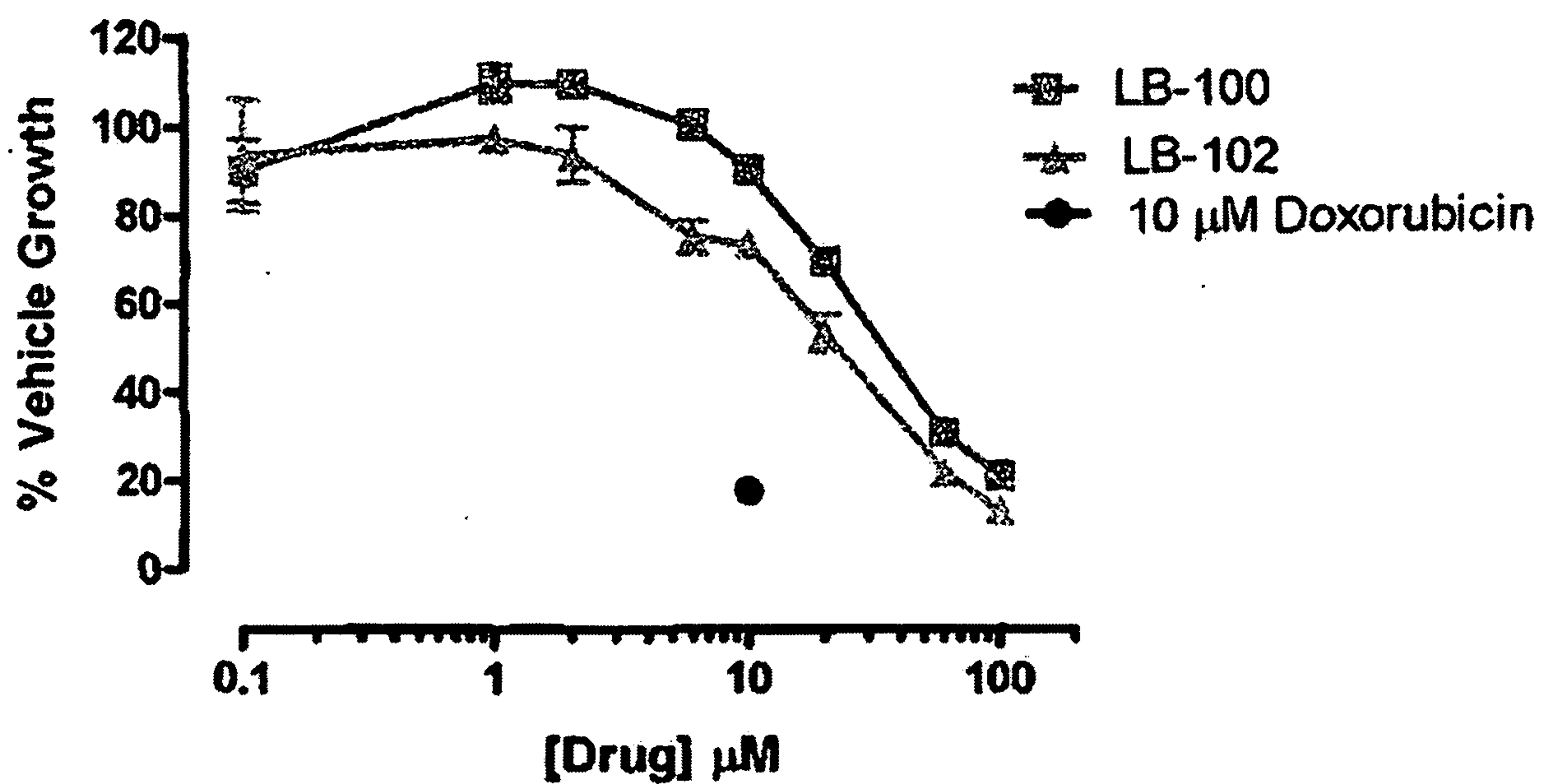
Fig. 19F



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Fig. 19G

I



II

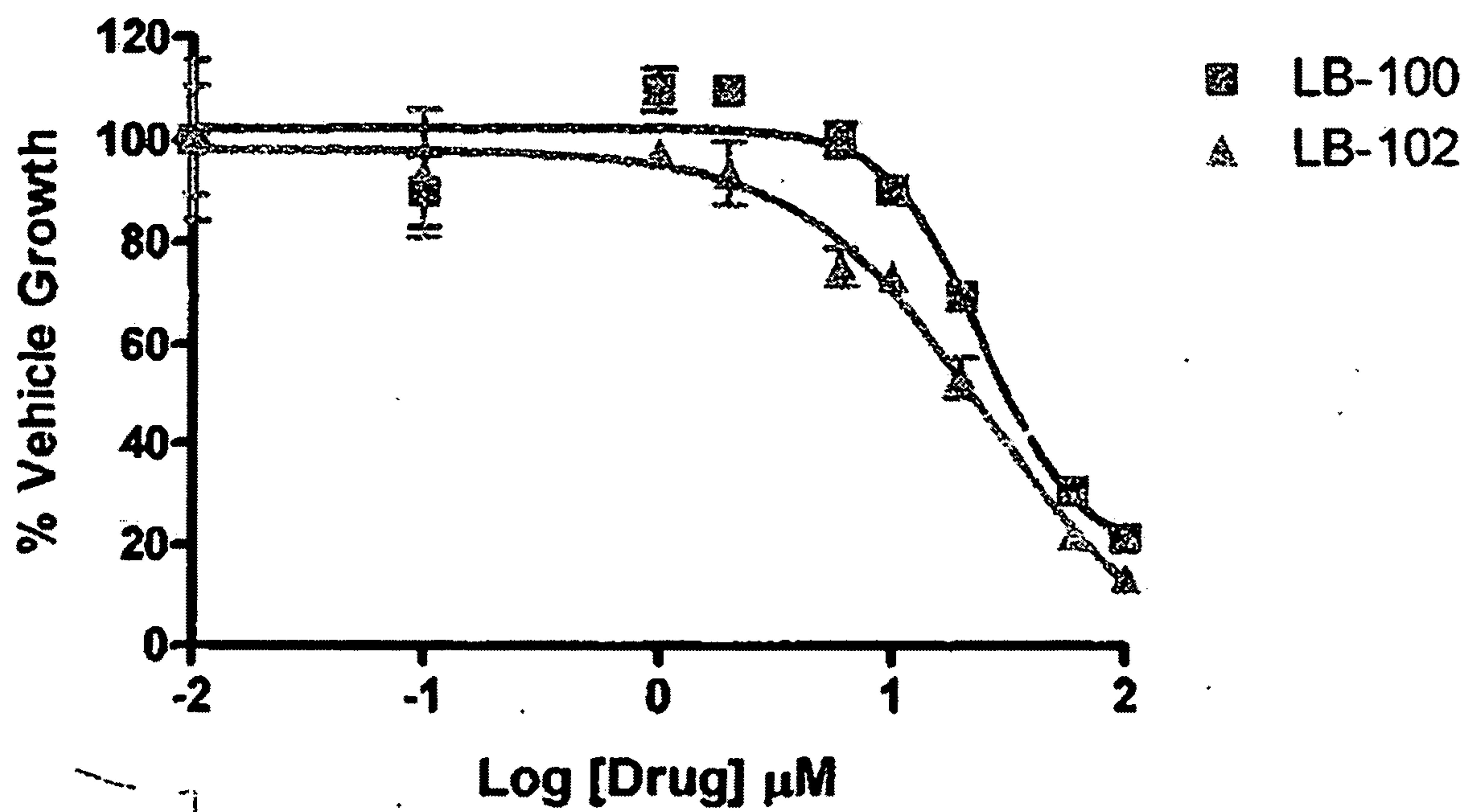
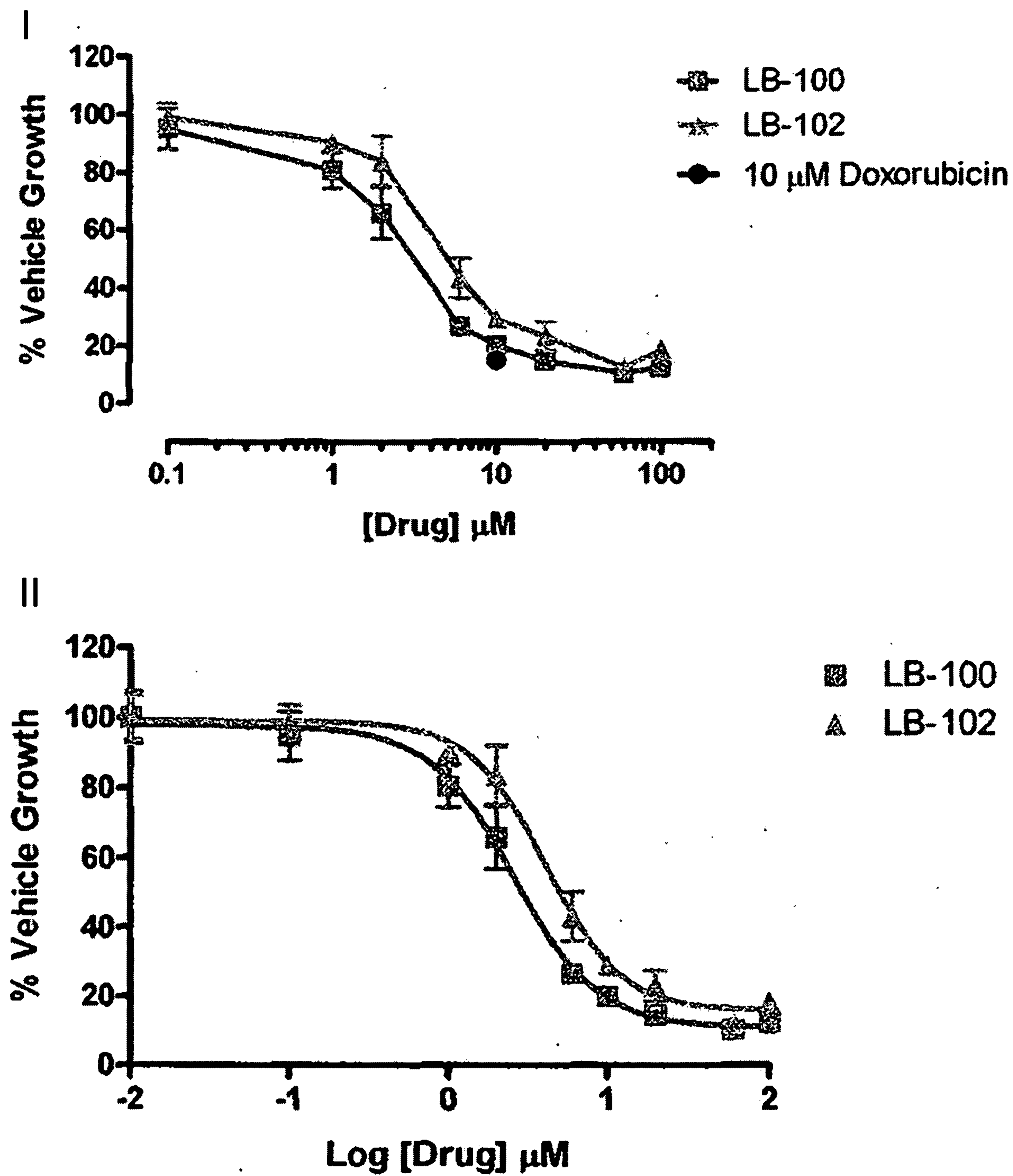


Fig. 19H



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Fig. 19I

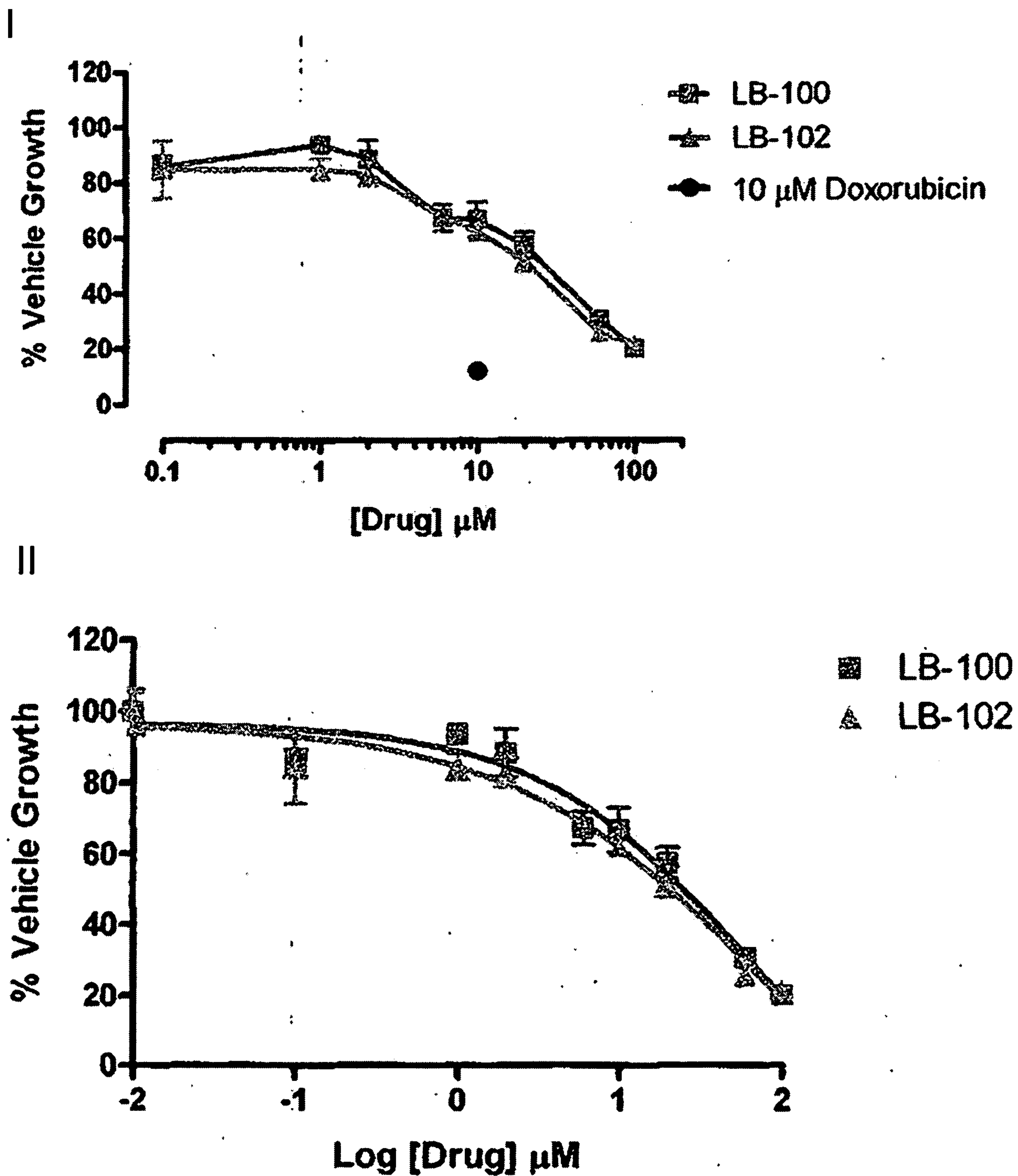
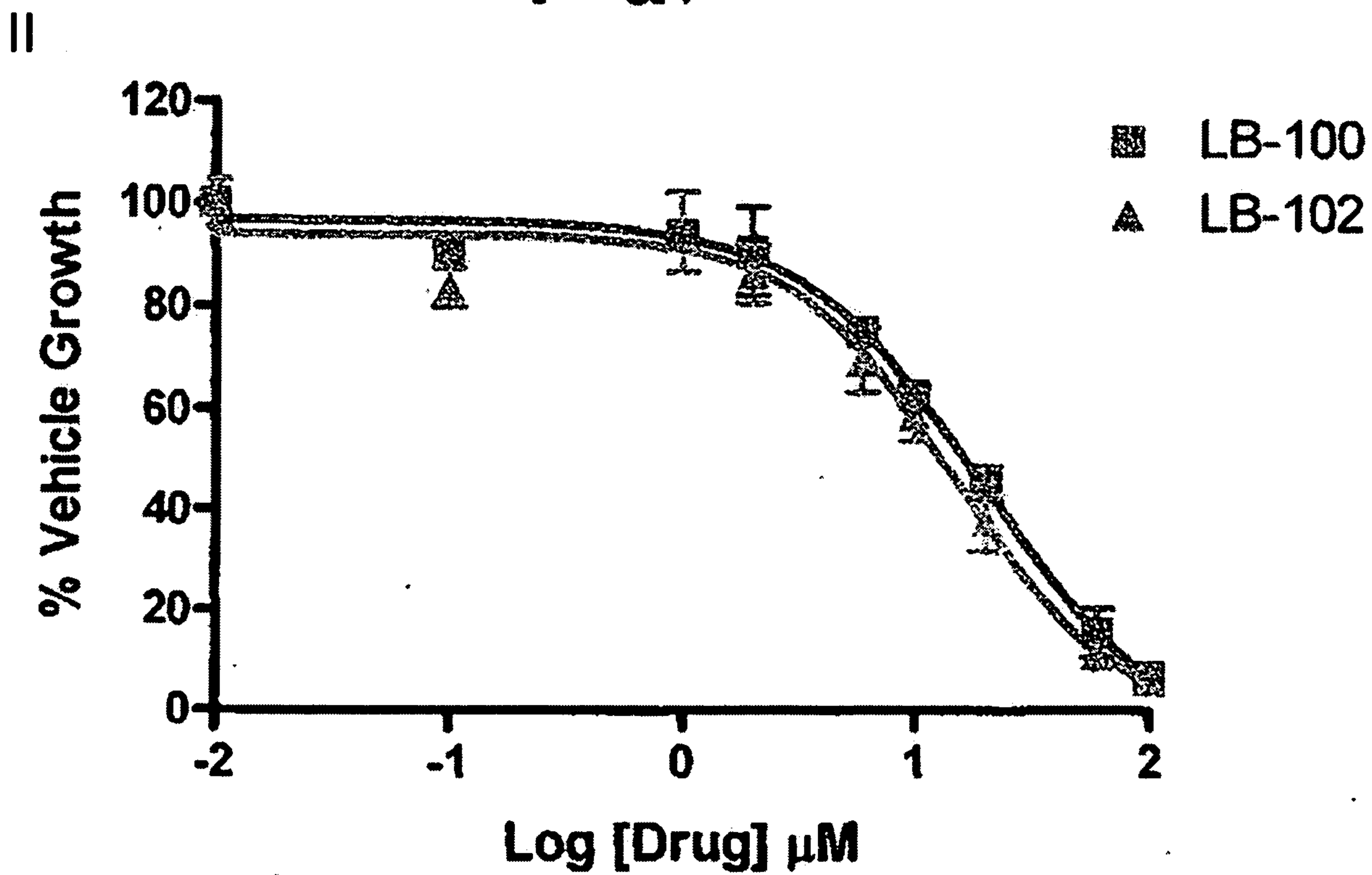
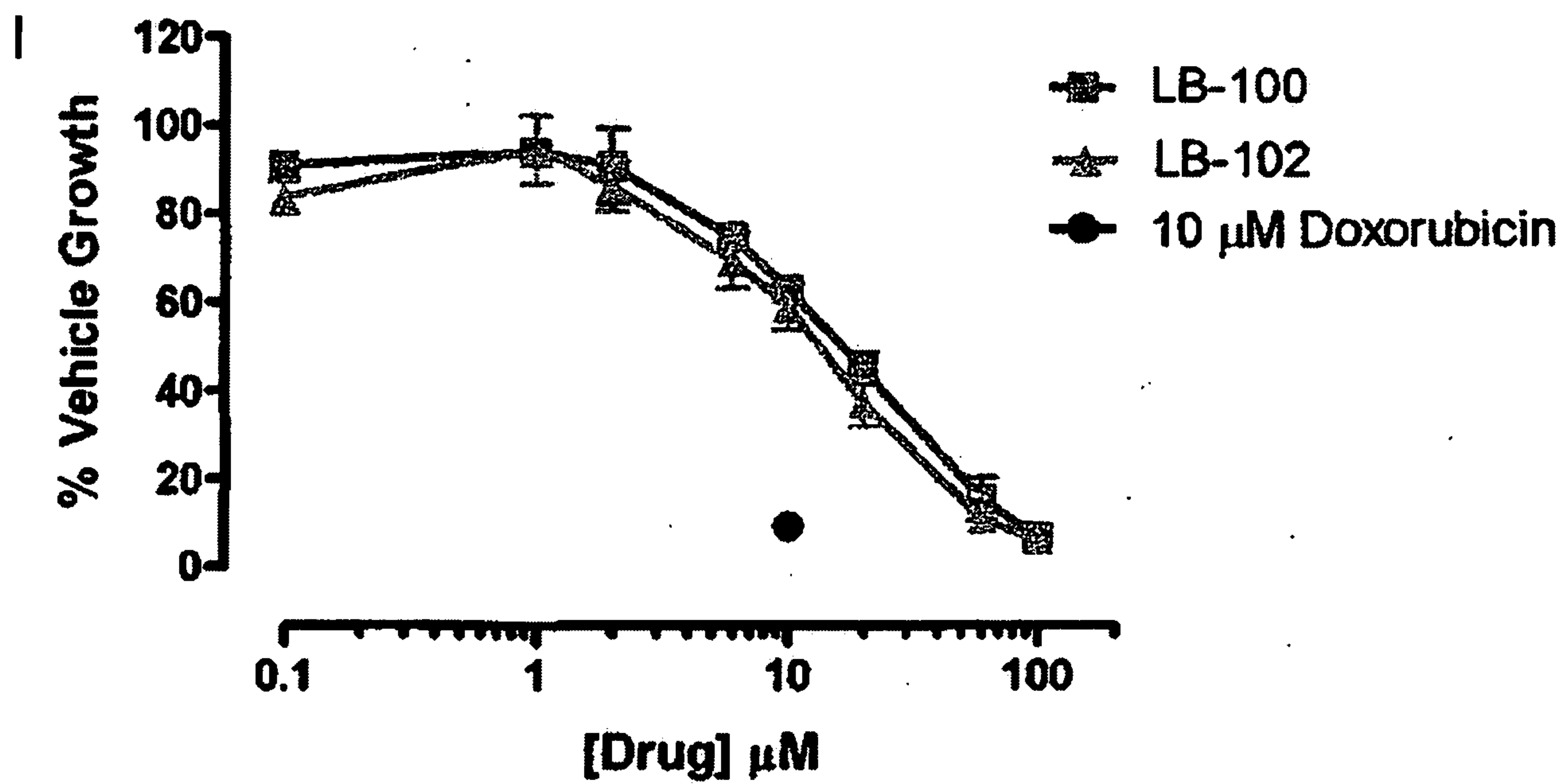


Fig. 19J



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Fig. 19K

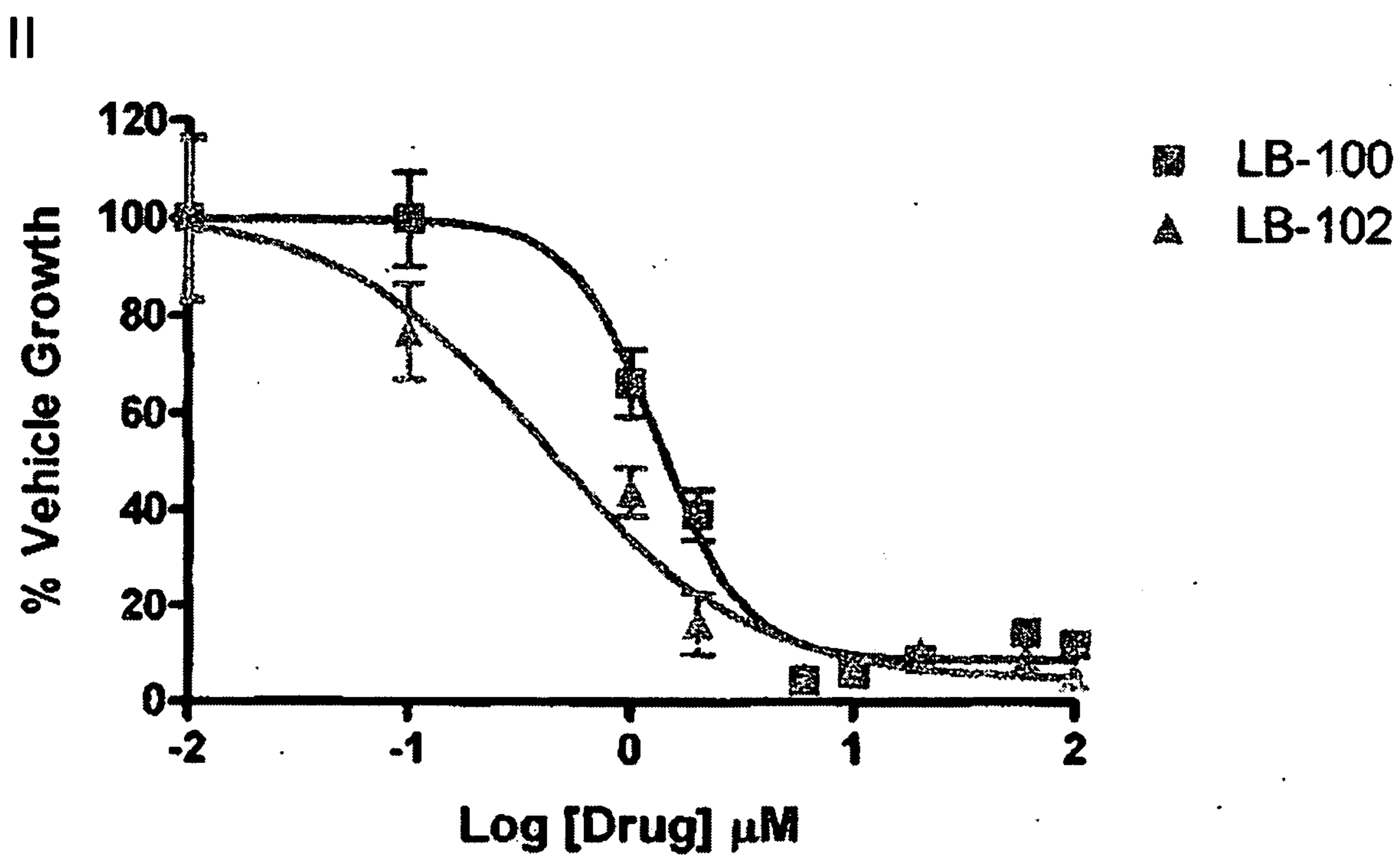
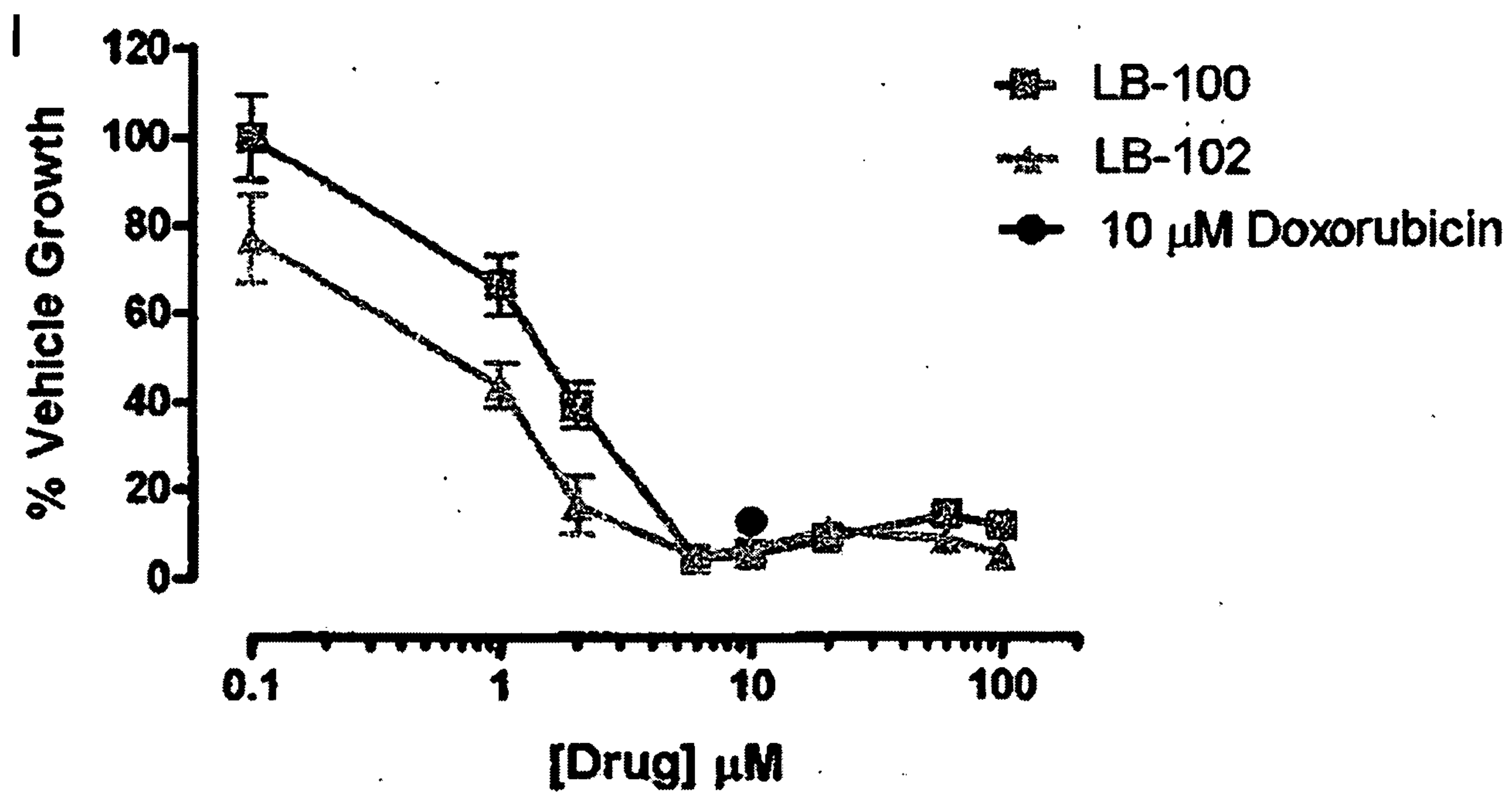


Fig. 19L

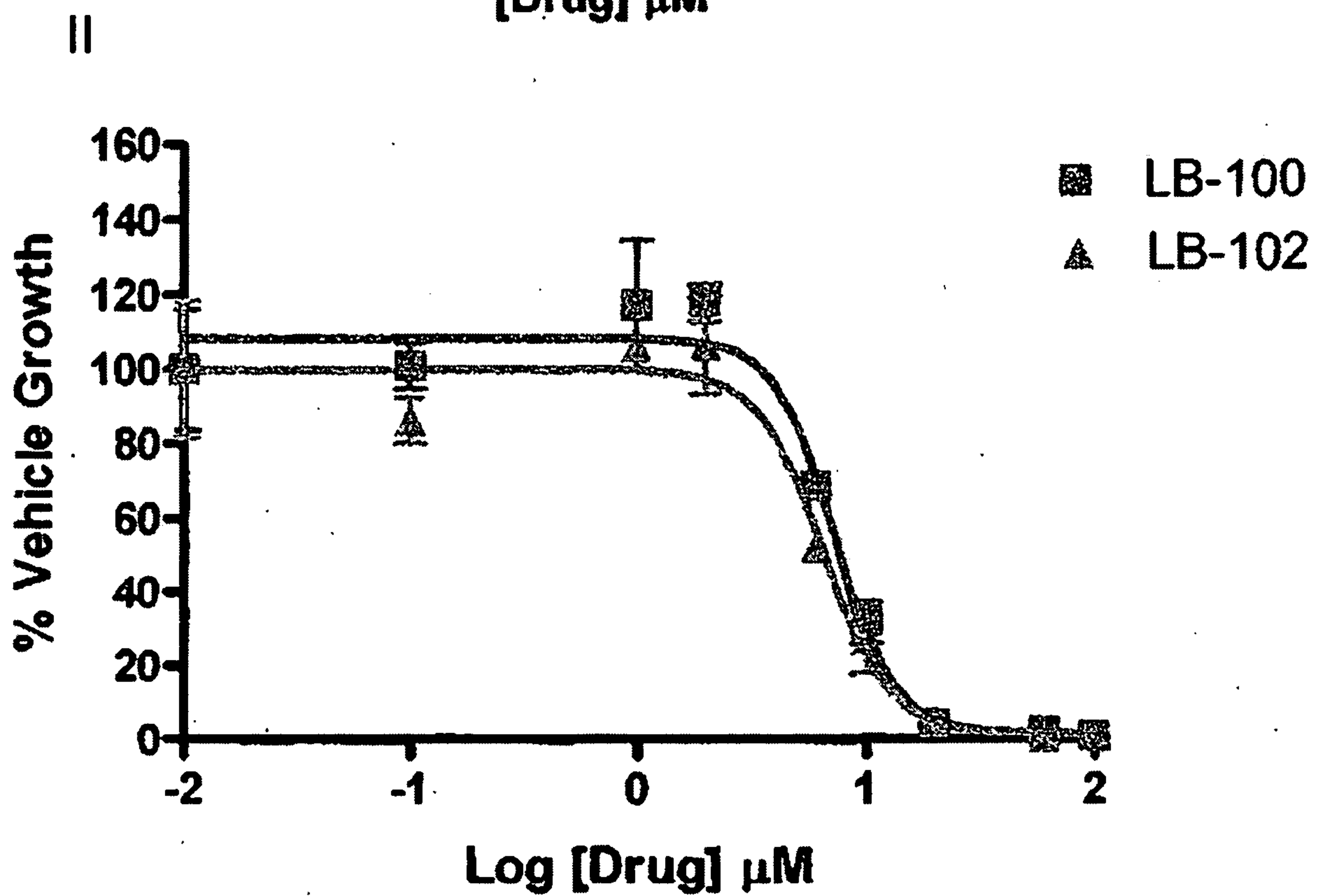
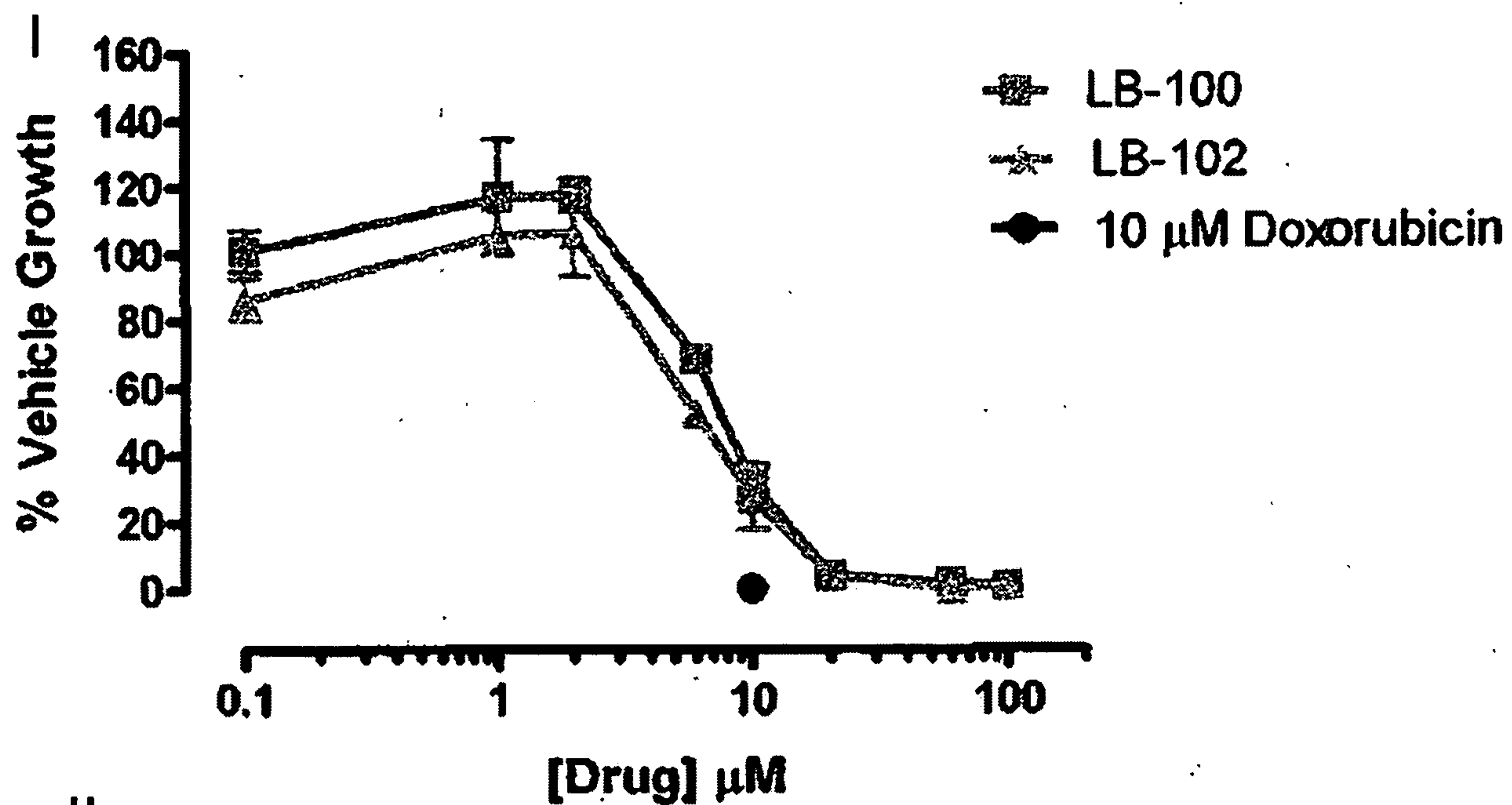


Fig. 19M

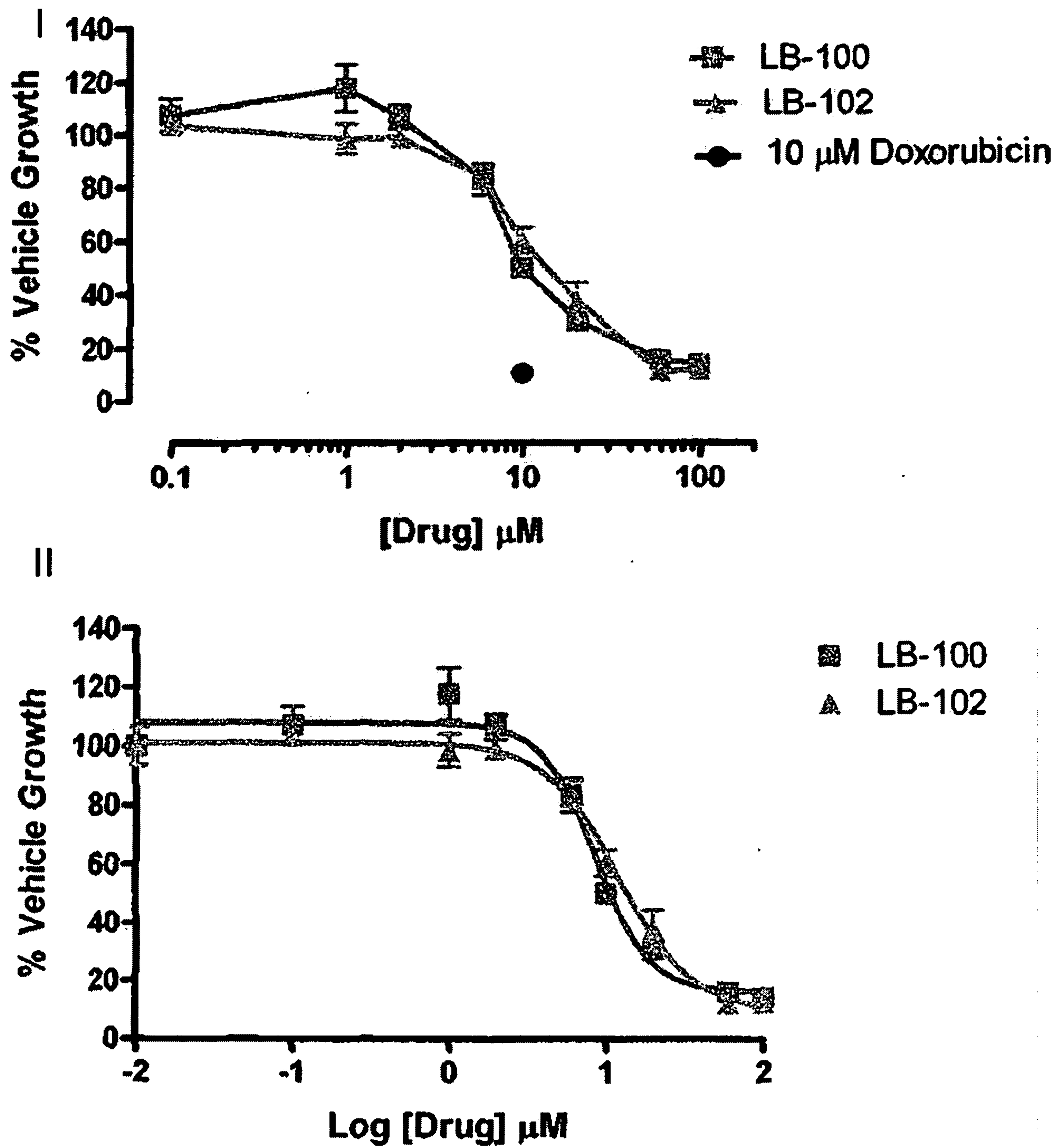


Fig. 19N

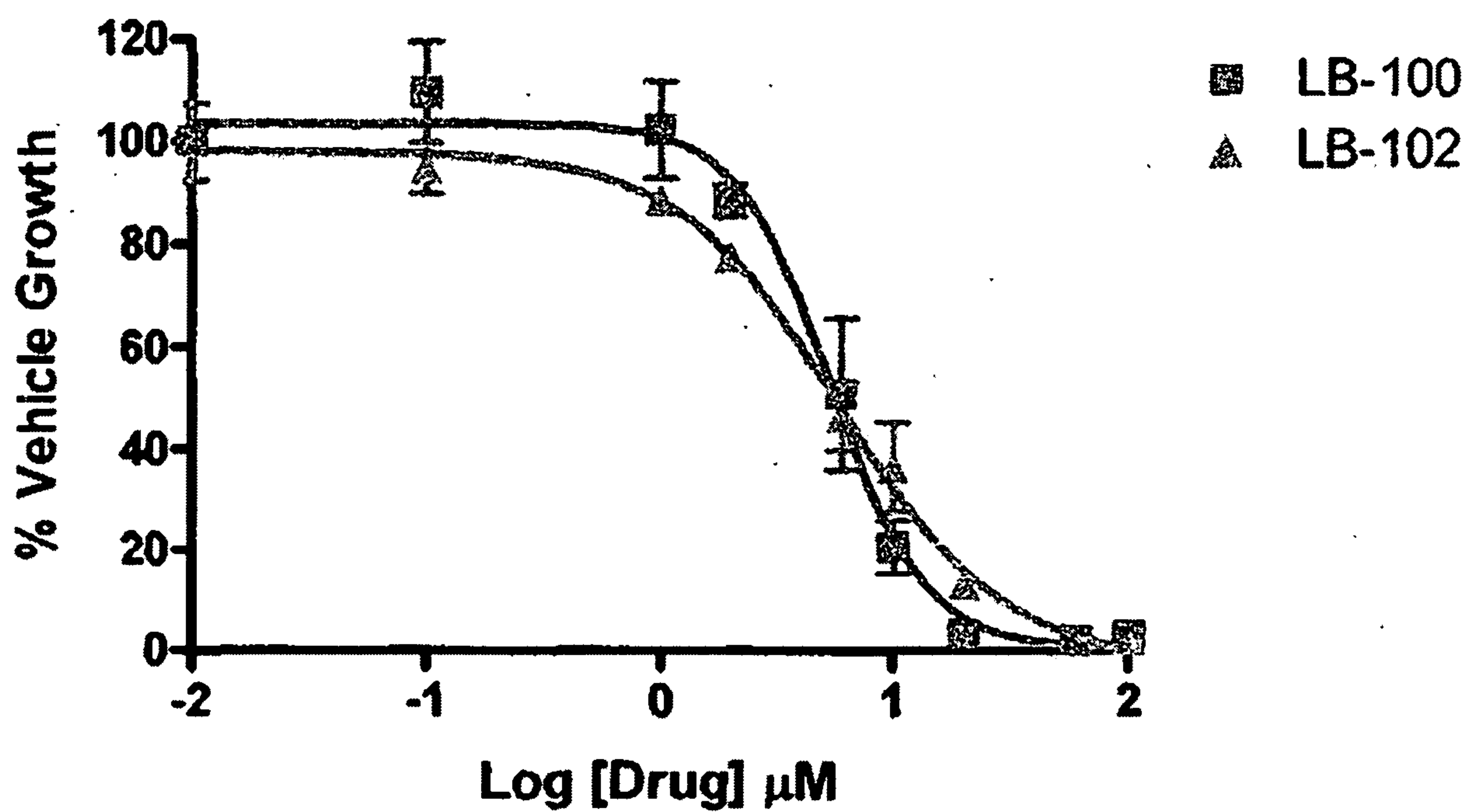
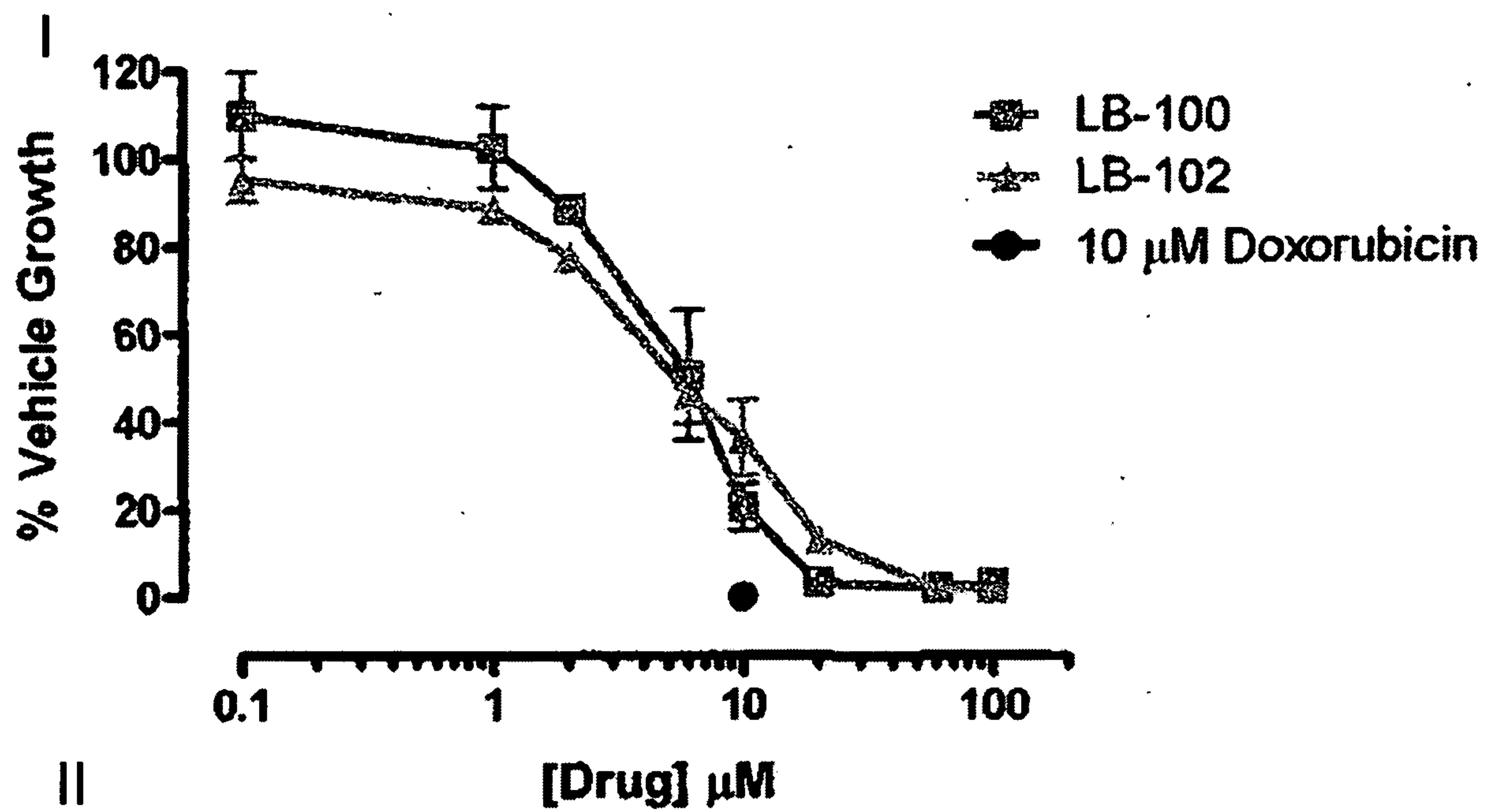
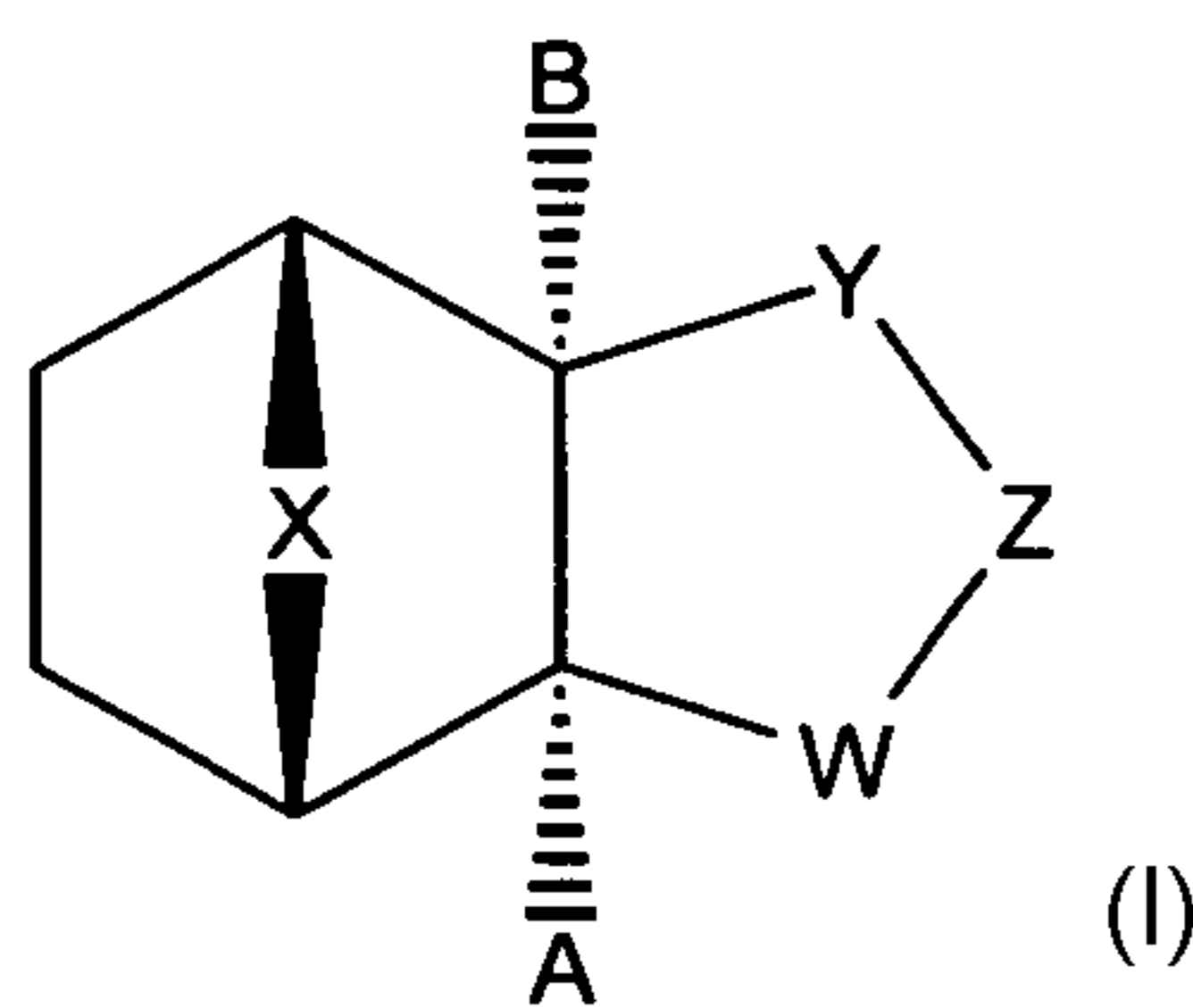
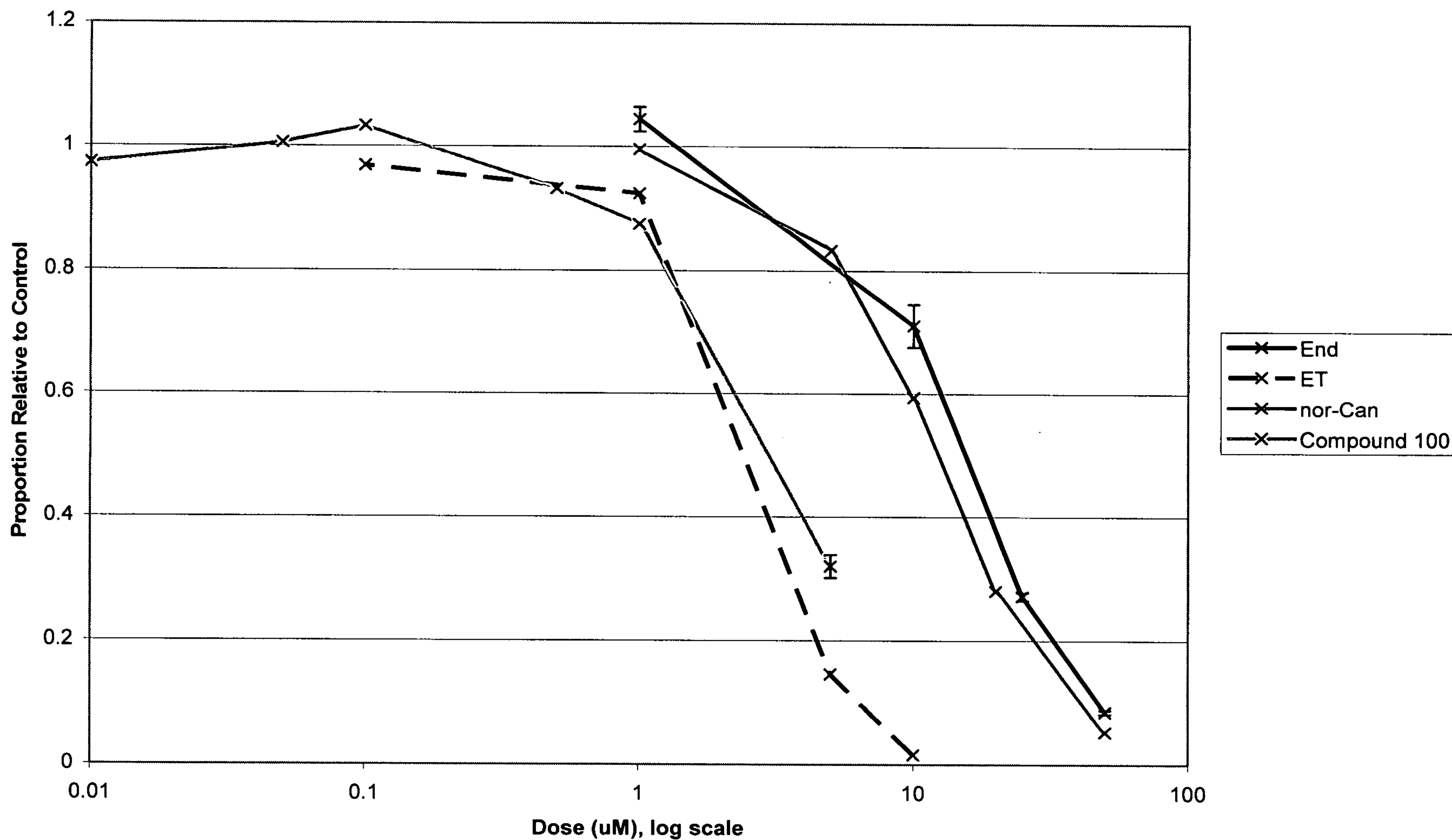
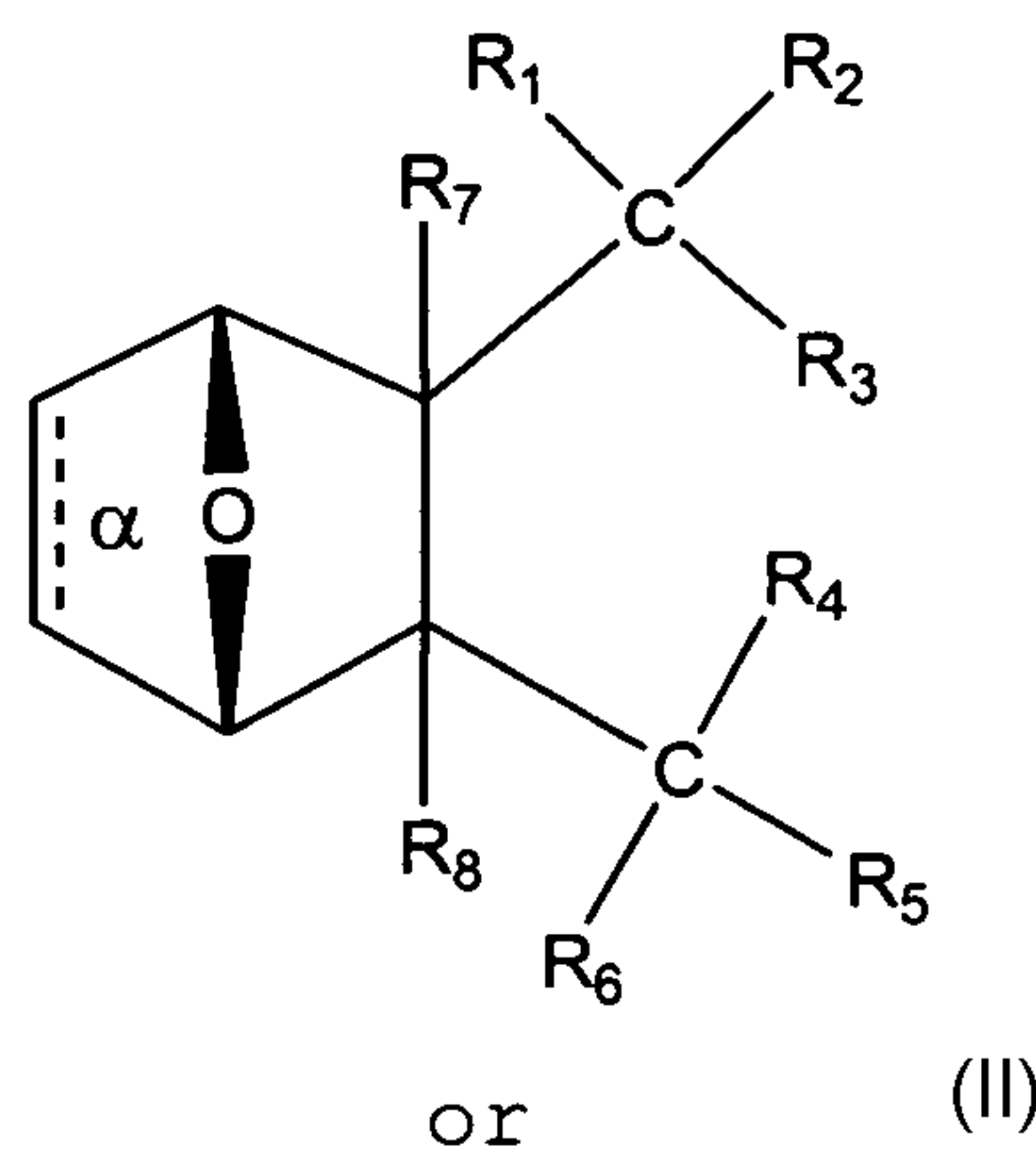


Fig. 1

# Inhibition of U373 by Four Cantharidin Analogs



(I)



(II)