



(86) Date de dépôt PCT/PCT Filing Date: 2002/10/11

(87) Date publication PCT/PCT Publication Date: 2003/04/17

(85) Entrée phase nationale/National Entry: 2004/04/13

(86) N° demande PCT/PCT Application No.: CA 2002/001519

(87) N° publication PCT/PCT Publication No.: 2003/031400

(30) Priorité/Priority: 2001/10/12 (60/328,428) US

(51) Cl.Int.⁷/Int.Cl.⁷ C07C 401/00, A61K 31/593, A61P 3/14

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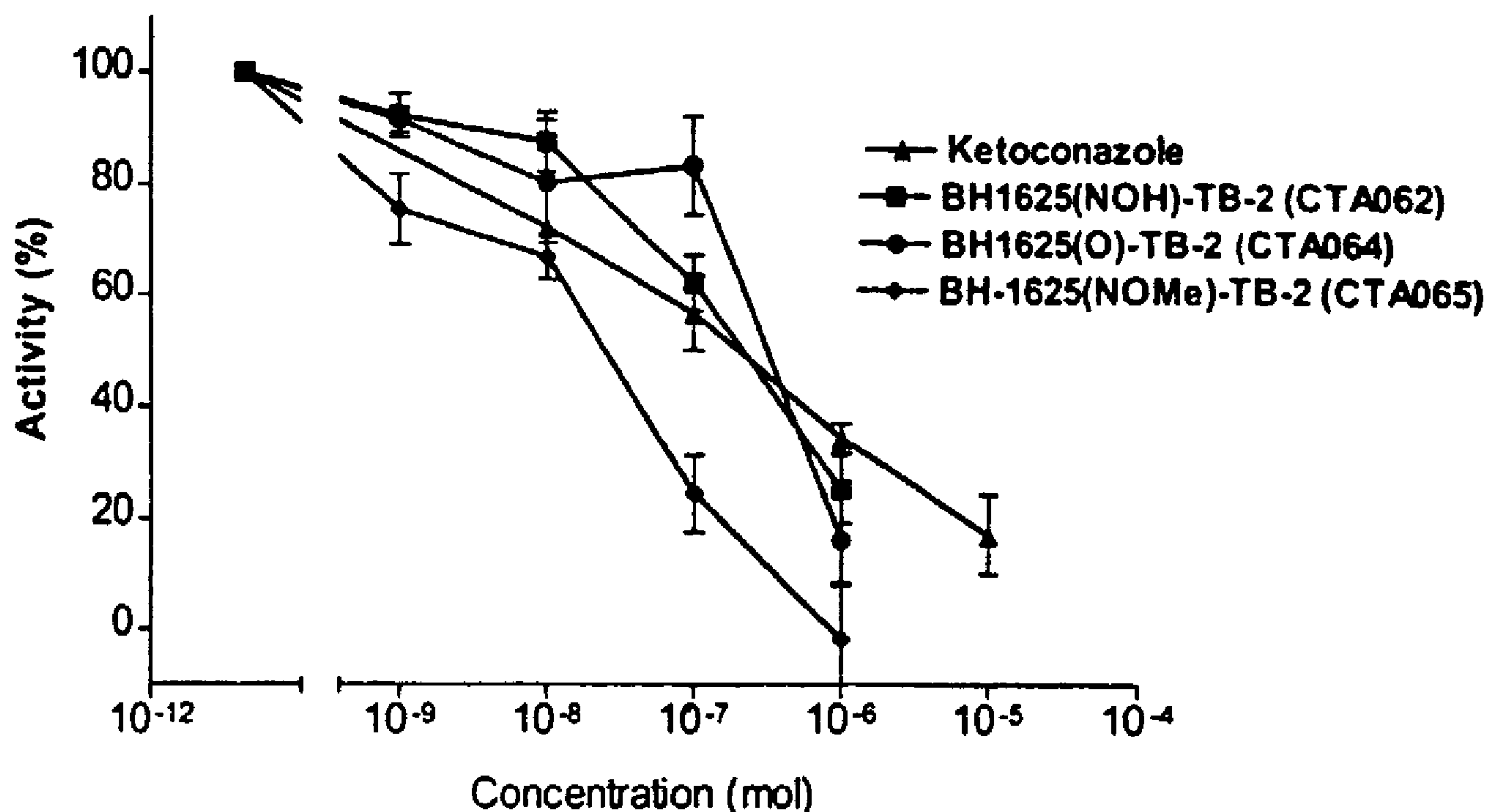
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(54) Titre : ANALOGUES D'OXIMES A CALCEMIE BASSE DE 1ALPHA, 25-DIHYDROXY-VITAMINE D3

(54) Title: LOW-CALCEMIC OXIME ANALOGS OF 1 α , 25-DIHYDROXY VITAMIN D₃

Inhibition of CYP24



(57) Abrégé/Abstract:

The present invention provides novel 16-ene-C25-oxime and 16-ene-C-25-oxime ether analogs of 1 α ,25-dihydroxy vitamin D₃, compositions comprising these compounds and methods of using these compounds as inhibitors of CYP24. In particular, the compound of Formula I are useful for treating diseases which benefit from a modulation of the levels of 1 α ,25-dihydroxy vitamin D₃, for example, cell-proliferative disorders.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
17 April 2003 (17.04.2003)

PCT

(10) International Publication Number
WO 03/031400 A1(51) International Patent Classification⁷: **C07C 401/00**,
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(21) International Application Number: PCT/CA02/01519

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(22) International Filing Date: 11 October 2002 (11.10.2002)

(25) Filing Language: English

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,
SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ,
VC, VN, YU, ZA, ZM, ZW.

(26) Publication Language: English

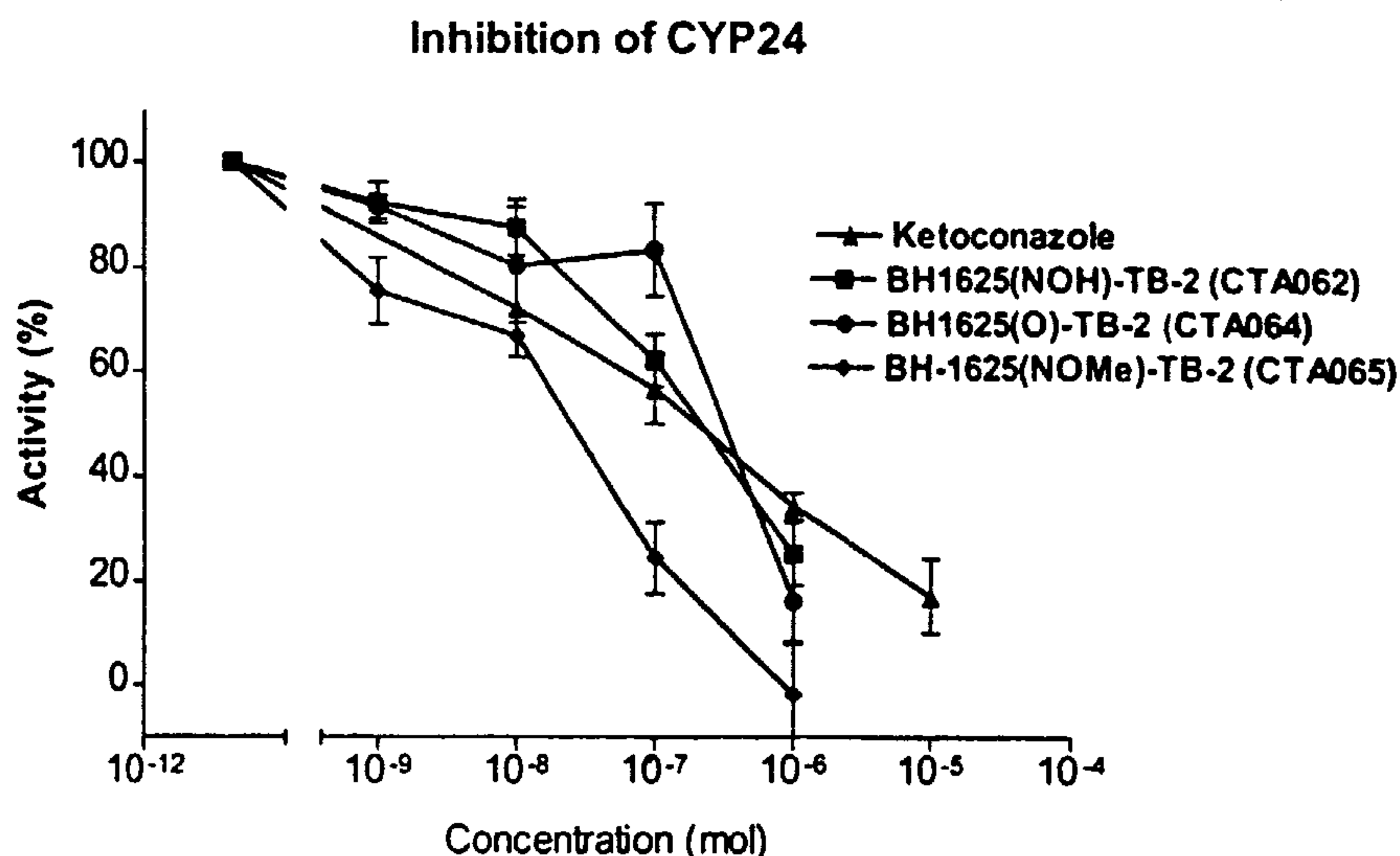
(30) Priority Data:
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KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK,
TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, ML, MR, NE, SN, TD, TG).

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- with international search report
- before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments

[Continued on next page]

(54) Title: LOW-CALCEMIC OXIME ANALOGS OF 1 α , 25-DIHYDROXY VITAMIN D₃(57) Abstract: The present invention provides novel 16-ene-C25-oxime and 16-ene-C-25-oxime ether analogs of 1 α ,25-dihydroxy vitamin D₃, compositions comprising these compounds and methods of using these compounds as inhibitors of CYP24. In particular, the compound of Formula I are useful for treating diseases which benefit from a modulation of the levels of 1 α ,25-dihydroxy vitamin D₃, for example, cell-proliferative disorders.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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TITLE: Low-calcemic Oxime Analogs of 1 α ,25-Dihydroxy Vitamin D₃

This invention was made with government support under NIH Grant Number CA 44530. The government has certain rights in the invention.

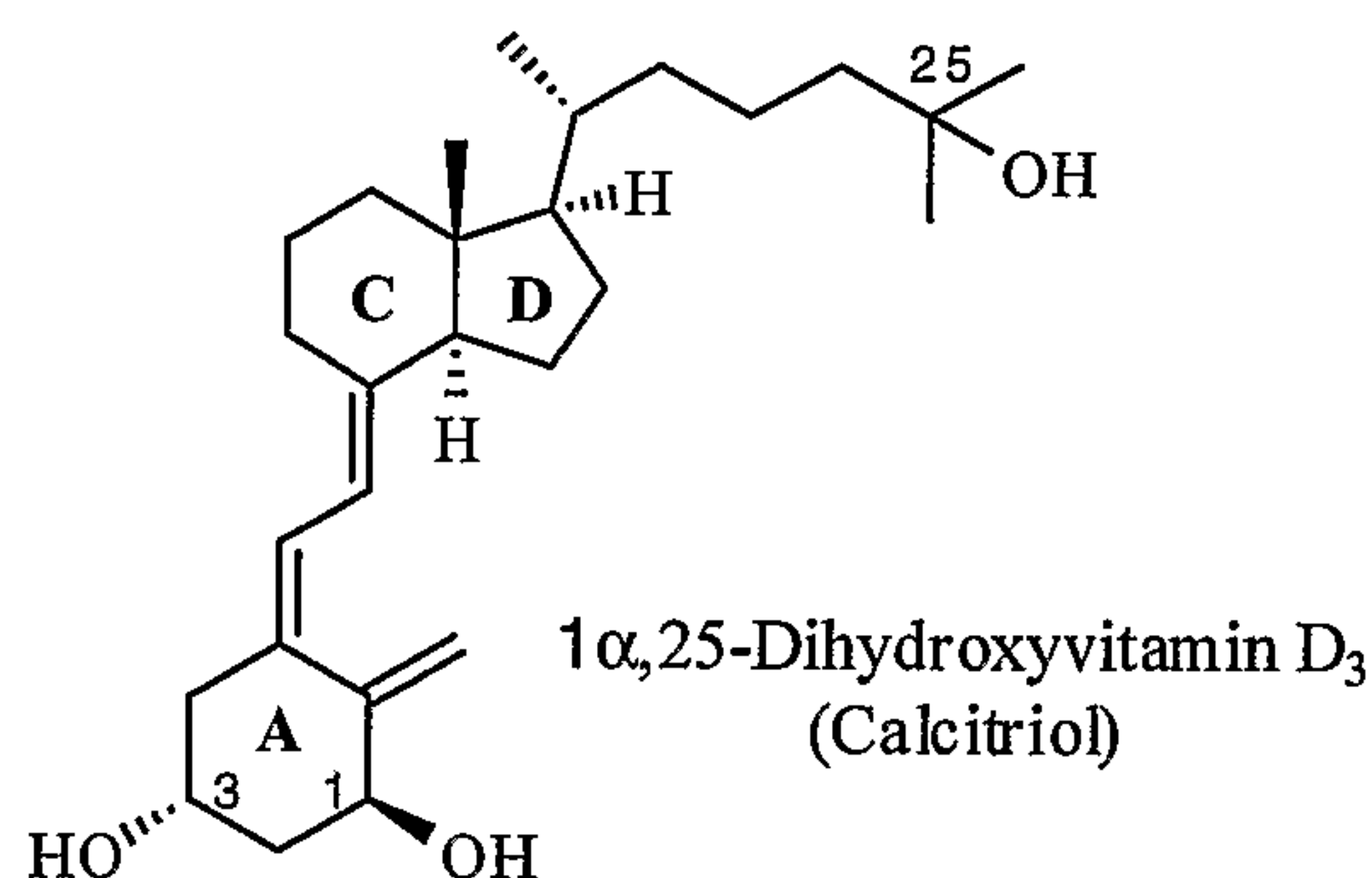
5 FIELD OF THE INVENTION

The present invention relates to novel analogs of the hormone 1 α ,25-dihydroxy vitamin D₃ that show selective inhibition of the enzyme CYP24 and which are low-calcemic and anti-proliferative, to pharmaceutical and diagnostic compositions containing them and to their medical use, particularly in the treatment
10 and/or prevention of cancer, dermatological disorders, bone disorders, thyroid disorders, wound healing and osteoporosis.

BACKGROUND OF THE INVENTION

The vitamin D metabolic pathway is part of a vital endocrine system that is highly regulated at certain stages and produces metabolites that control the
15 secretion of the parathyroid gland hormones (Beckman, M., and DeLuca, H. (1997) *Methods in Enzymol.* **282**, 200-223; Jones, G., Strugnell, S., and DeLuca, H. (1998) *Physiol. Rev.* **78**, 1193-1231). 1 α ,25-Dihydroxy vitamin D₃, also known as calcitriol (see below), a hormone produced in the vitamin D pathway, regulates phosphate and calcium levels in the blood which in turn control bone mass, the state of bones, and
20 affects cellular differentiation in the skin and the immune system (Armbrecht, H.J., Okuda, K., Wongsurawat, N., Nemani, R., Chen, M., and Boltz, M. (1992) *J. Steroid Biochem. Molec. Biol.* **43**, 1073-1081). In the vitamin D pathway, cytochrome P450s are enzymes that introduce functional groups by hydroxylation, usually at positions 1, 25, and 24, of vitamin D₃ (Beckman, M., and DeLuca, H. (1997) *Methods in Enzymol.*
25 **282**, 200-223).

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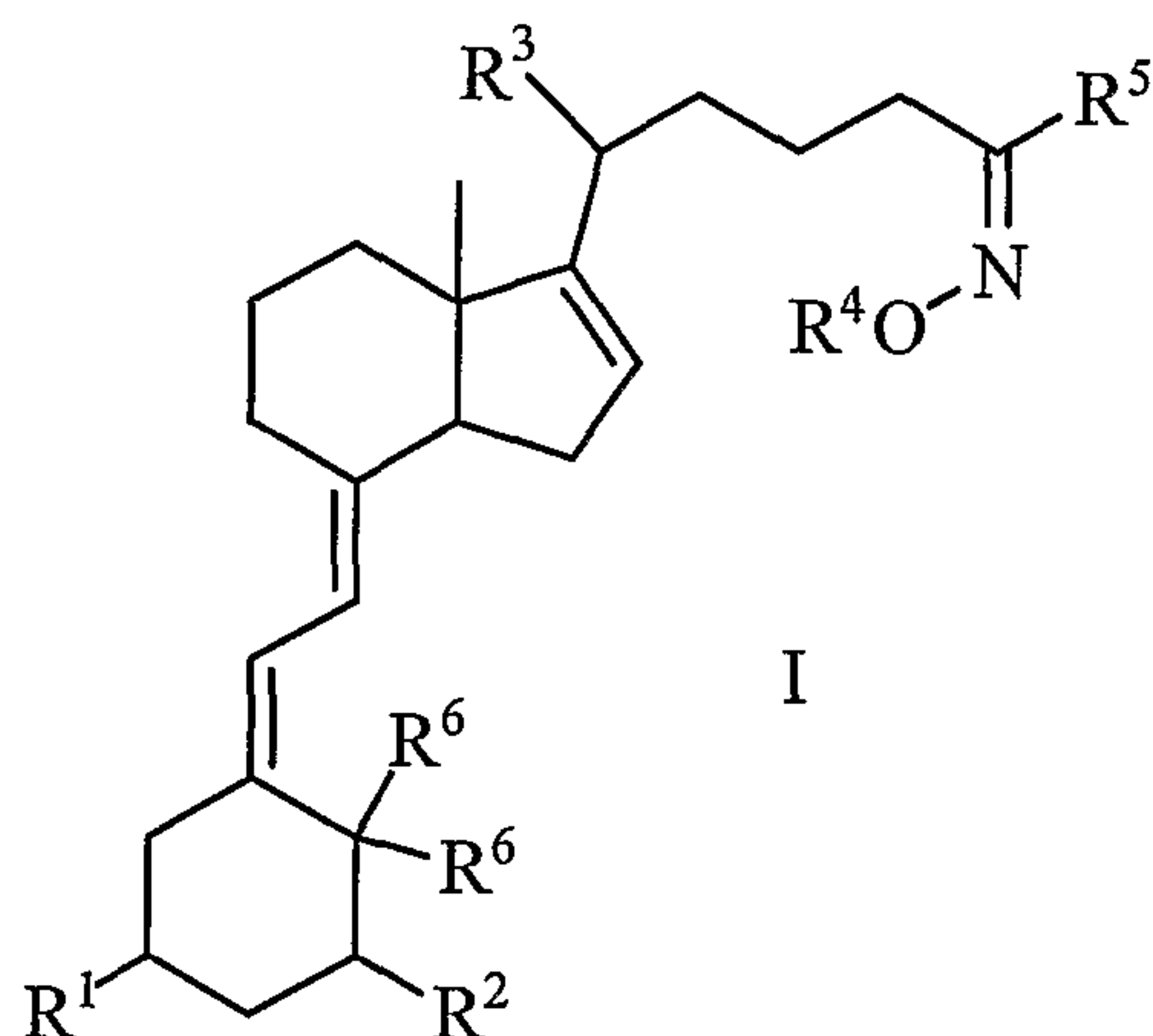
1 α ,25-Dihydroxy vitamin D₃ is converted to 1 α ,24,25-trihydroxy-D₃ by a mitochondrial P450 known as CYP 24 (Bell, N.H., (1998) *J. Bone Miner. Res.* **13**, 350- 35211). CYP 24 is induced by 1 α ,25-dihydroxy-D₃ and is found in the kidney as well as other vitamin D target tissues such as the parathyroid cells, keratinocytes, osteoblasts, and enterocytes (Jones, G., Strugnell, S., and DeLuca, H. (1998) *Physiol. Rev.* **78**, 1193-1231). 1 α ,25-Dihydroxy vitamin D₃ (1,25-D₃) has an important role in the antiproliferative and growth regulatory effects on normal and neoplastic cells (for e.g. prostate cancer cells). Clinical use of 1,25-D₃ analogs as effective drugs requires antiproliferative and pro-differentiating activities. There is a continuing need for synthetic analogs of 1 α ,25-dihydroxy vitamin D₃ that selectively exhibit desirable pharmacological activities but do not exhibit hypercalcemic and other undesirable activities.

15 SUMMARY OF THE INVENTION

Novel 16-ene-25-oxime and 16-ene-25-oxime ether analogs of 1 α ,25-dihydroxy vitamin D₃ have been prepared that show selective inhibition of the enzyme CYP24, anti-proliferative activity and are low-calcemic.

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The present invention therefore provides compounds of Formula I, and pharmaceutically acceptable salts, hydrates, solvates, and prodrugs thereof:



5 wherein

R^1 and R^2 are independently selected from the group consisting of OH, OC_{1-6} alkyl, and halo;

R^3 is C_{1-6} alkyl;

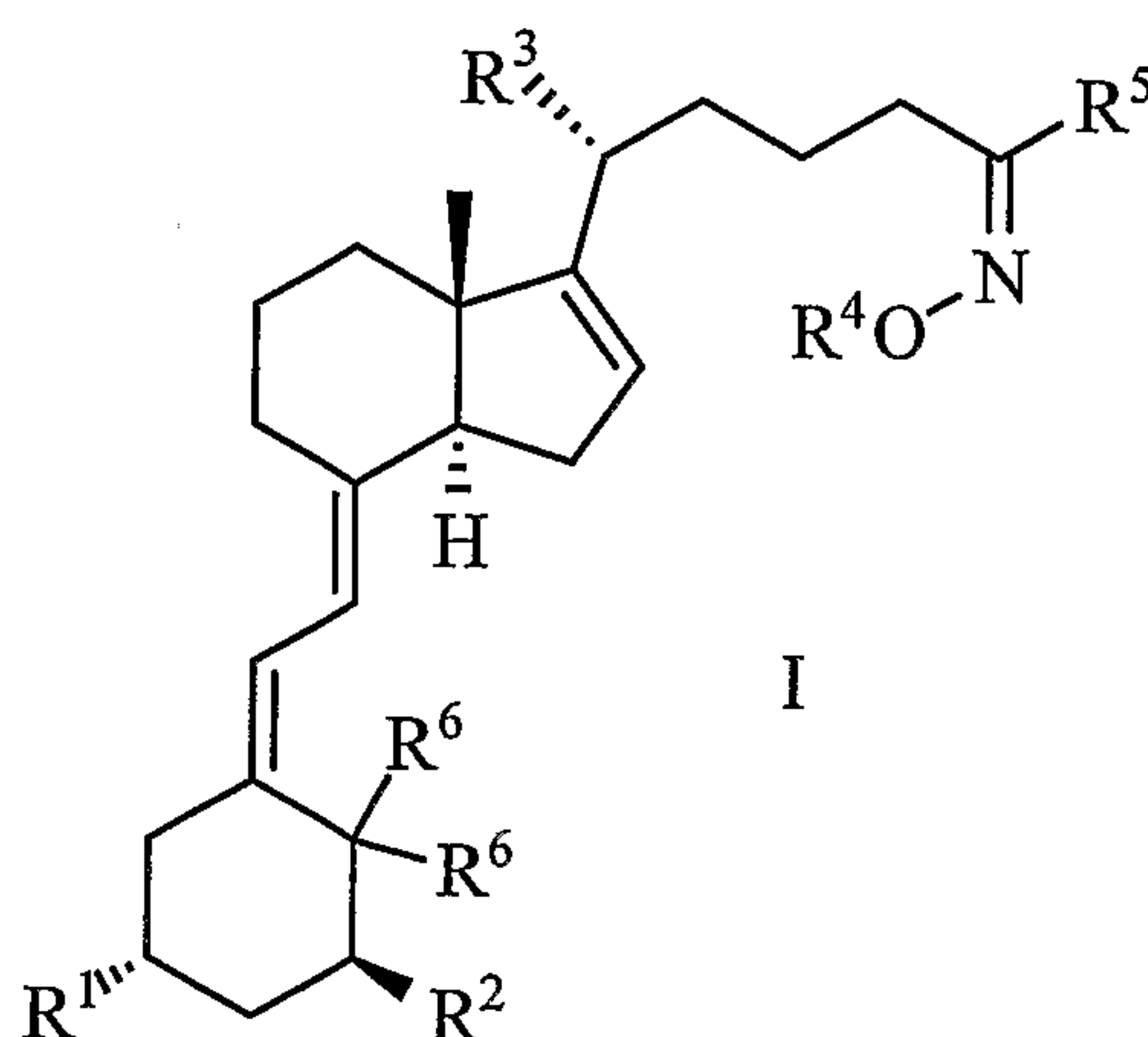
R^4 is selected from the group consisting of H, C_{1-6} alkyl, aryl and heteroaryl, with C_{1-6} alkyl being unsubstituted or substituted with 1-4 groups independently selected from C_{1-4} alkyl, OC_{1-4} alkyl, OH, halo, NH_2 , NHC_{1-4} alkyl and $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, and with aryl and heteroaryl being unsubstituted or substituted with 1-5 groups independently selected from C_{1-4} alkyl, OC_{1-4} alkyl, OH, CF_3 , OCF_3 , halo, SH, SC_{1-4} alkyl, NH_2 , NHC_{1-4} alkyl, $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, CN, $C(O)OH$, $C(O)OC_{1-4}alkyl$, $C(O)NHC_{1-4}alkyl$, $NHC(O)C_{1-4}alkyl$, $OC(O)C_{1-4}alkyl$, $SOC_{1-4}alkyl$, $SO_2C_{1-4}alkyl$, $SO_2NHC_{1-4}alkyl$ and SO_2NH_2 ;

R^5 is selected from the group consisting of C_{1-6} alkyl, cyclo(C_3-C_6)alkyl, aryl, heteroaryl, aryl- C_{1-6} alkyl and heteroaryl- C_{1-6} alkyl, with C_{1-6} alkyl being unsubstituted or substituted with 1-4 groups independently selected from C_{1-4} alkyl, OC_{1-4} alkyl, OH, halo, NH_2 , $NHC_{1-4}alkyl$ and $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, and with cyclo(C_3-C_6)alkyl, aryl, heteroaryl, aryl- C_{1-6} alkyl and heteroaryl- C_{1-6} alkyl, being unsubstituted or substituted with 1-5 groups independently selected from C_{1-4} alkyl, OC_{1-4} alkyl, OH, CF_3 , OCF_3 , halo, SH, $SC_{1-4}alkyl$, NH_2 , $NHC_{1-4}alkyl$, $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, CN, $C(O)OH$, $C(O)OC_{1-4}alkyl$, $C(O)NHC_{1-4}alkyl$, $NHC(O)C_{1-4}alkyl$, $OC(O)C_{1-4}alkyl$, $SOC_{1-4}alkyl$, $SO_2C_{1-4}alkyl$, $SO_2NHC_{1-4}alkyl$ and SO_2NH_2 ; and

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R^6 are either both H or together form $=CH_2$.

Preferably, the compounds of the invention have the stereochemistry of natural $1\alpha,25$ -dihydroxy vitamin D_3 . Therefore, in a preferred embodiment, the present invention provides compounds of Formula I, and pharmaceutically acceptable salts, hydrates, solvates, and prodrugs thereof, as shown below:



wherein

10 $R^1 - R^6$ are as defined above.

According to another aspect of the present invention, there is provided a pharmaceutical composition comprising a compound of Formula I and a pharmaceutically acceptable carrier or diluent.

By selectively modulating CYP24, the enzyme that metabolizes $1\alpha,25$ -dihydroxy vitamin D_3 , the levels of $1\alpha,25$ -dihydroxy vitamin D_3 are also modulated. Diseases that benefit from a modulation of the levels of $1\alpha,25$ -dihydroxy vitamin D_3 can therefore be treated using a modulator of CYP24. By acting preferentially on CYP24, side effects caused by interaction with other enzymes and receptors will be reduced. Accordingly, the present invention provides a method for treating diseases which benefit from a modulation of the levels of $1\alpha,25$ -dihydroxy vitamin D_3 comprising administering an effective amount of a compound of Formula I to a cell or animal in need thereof. The invention also includes the use of a compound of Formula I to modulate the levels of $1\alpha,25$ -dihydroxy vitamin D_3 . Further, the invention

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includes a use of a compound of Formula I to prepare a medicament to modulate the levels of $1\alpha,25$ -dihydroxy vitamin D_3 .

Inhibition of CYP24, inhibits the catabolism of $1\alpha,25$ -dihydroxy vitamin D_3 which will lengthen the biological lifetime of this hormone and thus allow smaller amounts of it to be used for effective disease treatment. Such smaller dosing will avoid, or at least minimize, the hypercalcemic toxicity associated with medicinal use of $1\alpha,25$ -dihydroxy vitamin D_3 (calcitriol). Therefore, in an embodiment, the present invention provides a method for treating diseases which benefit from inhibiting the catabolism of $1\alpha,25$ -dihydroxy vitamin D_3 comprising administering an effective amount of a compound of Formula I to a cell or animal in need thereof. The invention also includes the use of a compound of Formula I to inhibit the catabolism of $1\alpha,25$ -dihydroxy vitamin D_3 . Further, the invention includes a use of a compound of Formula I to prepare a medicament to inhibit the catabolism of $1\alpha,25$ -dihydroxy vitamin D_3 .

Diseases which may benefit for a modulation in the levels of $1\alpha,25$ -dihydroxy vitamin D_3 include, but are not limited to:

- (i) in the parathyroid - hyper- and hypo-parathyroidism, Pseudohypoparathyroidism, Secondary hyperparathyroidism;
- (ii) in the pancreas - diabetes;
- (iii) in the thyroid - medullary carcinoma;
- (iv) in the skin - psoriasis, wound healing;
- (v) in the lung - sarcoidosis and tuberculosis;
- (vi) in the kidney - chronic renal disease, hypophosphatemic VDDR, vitamin D dependent rickets;
- (vii) in the bone - anticonvulsant treatment, fibrogenesis imperfecta ossium, osteitis fibrosa cystica, osteomalacia, osteoporosis, osteopenia, osteosclerosis, renal osteodystrophy, rickets;
- (viii) in the intestine - glucocorticoid antagonism, idiopathic hypercalcemia, malabsorption syndrome, steatorrhea, tropical sprue.

The compounds of Formula I, or salts, solvates, hydrates or prodrugs thereof, can be used alone or in combination with other agents that modulate CYP24

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activity or in combination with other types of treatment (which may or may not modulate CYP24) for cell proliferative disorders or other disorders that benefit from a modulation in the levels of $1\alpha,25$ -dihydroxy vitamin D_3 and/or an inhibition of the catabolism of $1\alpha,25$ -dihydroxy vitamin D_3 . Preferably the compounds of Formula I
5 are administered in combination with $1\alpha,25$ -dihydroxy vitamin D_3 (calcitriol) or other vitamin D receptor agonists. The present invention therefore provides a method of increasing the efficacy of a vitamin D receptor agonist, preferably $1\alpha,25$ -dihydroxy vitamin D_3 (calcitriol), comprising co-administering an effective amount of a compound of Formula I and an effective amount of the vitamin D receptor agonist,
10 preferably $1\alpha,25$ -dihydroxy vitamin D_3 (calcitriol). Further the invention includes a use of a compound of Formula I to increase the efficacy of a vitamin D receptor agonist, preferably $1\alpha,25$ -dihydroxy vitamin D_3 (calcitriol), and a use of a compound of Formula I to prepare a medicament to increase the efficacy of a vitamin D receptor agonist, preferably $1\alpha,25$ -dihydroxy vitamin D_3 (calcitriol).

15 In accordance with a further aspect of the present invention, there is provided a method for modulating cell proliferation, preferably inhibiting cell proliferation, comprising administering an effective amount of a compound of Formula I to a cell or animal in need thereof. The invention also includes a use of a compound of Formula I to modulate cell proliferation, preferably to inhibit cell
20 proliferation. The invention further includes a use of a compound of Formula I to prepare a medicament to modulate cell proliferation, preferably to inhibit cell proliferation.

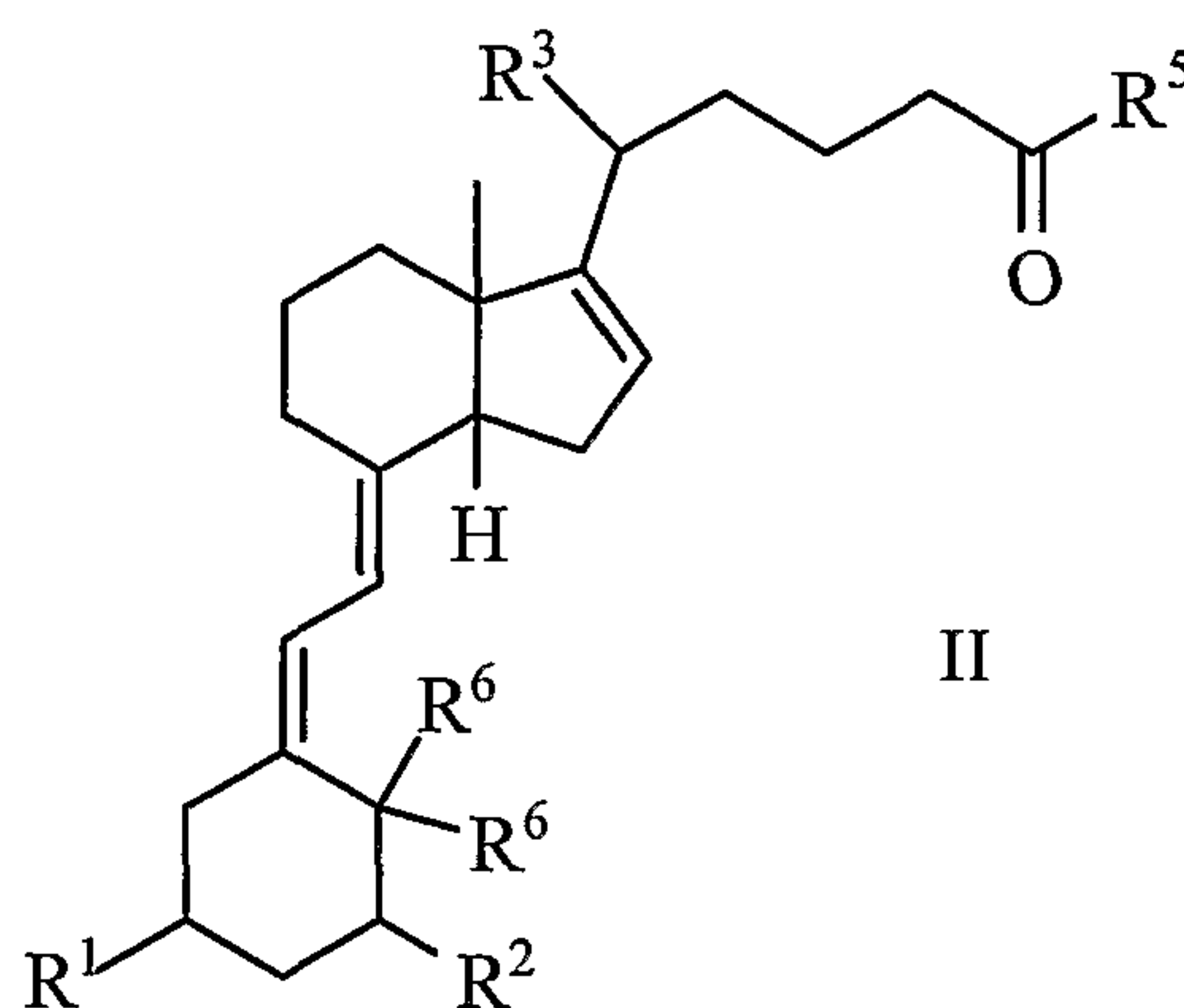
In an embodiment, the present invention provides a method of inhibiting the proliferation of a cancer cell comprising administering an effective
25 amount of a compound of Formula I to a cell or animal in need thereof. The invention also includes a use of a compound of Formula I to inhibit cancer cell proliferation. The invention further includes a use of a compound of Formula I to prepare a medicament to inhibit cancer cell proliferation.

In another aspect, the invention provides a method of modulating
30 CYP24 activity in a cell or animal by administering an effective amount of a compound of Formula I. In a further aspect, the invention provides a method of

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modulating CYP24 activity, preferably inhibiting CYP24 activity by administering an effective amount of a compound of Formula I to a cell or animal in need thereof. The present invention also provides a use of a compound of Formula I to modulate, preferably to inhibit, CYP24 activity. The present invention further provides a use of
 5 a compound of Formula I to prepare a medicament to modulate CYP24 activity, preferably to inhibit CYP24 activity. It is appreciated that the inhibition of cell growth by the compounds of the invention may be effected by other mechanisms.

The present invention further provides novel compounds useful in the preparation of the compounds of Formula I. Therefore the present invention further
 10 provides compounds of Formula II, and salts, hydrates and solvates thereof:



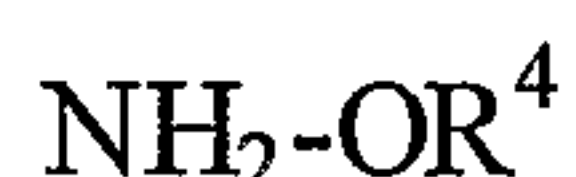
wherein

- 15 R^1 and R^2 are independently selected from the group consisting of OH, $OC_{1-6}alkyl$, OPG and halo;
 PG is a protecting group;
 R^3 is $C_{1-6}alkyl$;
 R^5 is selected from the group consisting of $C_{1-6}alkyl$, $cyclo(C_3-C_6)alkyl$, aryl,
 20 heteroaryl, $aryl-C_{1-6}alkyl$ and $heteroaryl-C_{1-6}alkyl$, with $C_{1-6}alkyl$ being unsubstituted or substituted with 1-4 groups independently selected from $C_{1-4}alkyl$, $OC_{1-4}alkyl$, OH, halo, NH_2 , $NHC_{1-4}alkyl$ and $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, and with $cyclo(C_3-C_6)alkyl$, aryl, heteroaryl, $aryl-C_{1-6}alkyl$ and $heteroaryl-C_{1-6}alkyl$, being unsubstituted or substituted with 1-5 groups independently selected from $C_{1-4}alkyl$, $OC_{1-4}alkyl$, OH, CF_3 , OCF_3 ,
 25 halo, SH, $SC_{1-4}alkyl$, NH_2 , $NHC_{1-4}alkyl$, $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, CN, $C(O)OH$,

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C(O)OC₁₋₄alkyl, C(O)NHC₁₋₄alkyl, NHC(O)C₁₋₄alkyl, OC(O)C₁₋₄alkyl, SOC₁₋₄alkyl, SO₂C₁₋₄alkyl, SO₂NHC₁₋₄alkyl and SO₂NH₂; and R⁶ are either both H or together form =CH₂.

Further, the present invention provides a method for preparing a compound of Formula I comprising reacting a compound of Formula II, or a salt, hydrate or solvate thereof, with a compound of Formula III, or a salt hydrate of solvate thereof:



III ,

wherein R⁴ is selected from the group consisting of H, C₁₋₆alkyl, aryl and heteroaryl, with C₁₋₆alkyl being unsubstituted or substituted with 1-4 groups independently selected from C₁₋₄alkyl, OC₁₋₄alkyl, OH, halo, NH₂, NHC₁₋₄alkyl and N(C₁₋₄alkyl)(C₁₋₄alkyl), and with aryl and heteroaryl being unsubstituted or substituted with 1-5 groups independently selected from C₁₋₄alkyl, OC₁₋₄alkyl, OH, CF₃, OCF₃, halo, SH, SC₁₋₄alkyl, NH₂, NHC₁₋₄alkyl, N(C₁₋₄alkyl)(C₁₋₄alkyl), CN, C(O)OH, C(O)OC₁₋₄alkyl, C(O)NHC₁₋₄alkyl, NHC(O)C₁₋₄alkyl, OC(O)C₁₋₄alkyl, SOC₁₋₄alkyl, SO₂C₁₋₄alkyl, SO₂NHC₁₋₄alkyl and SO₂NH₂,

in the presence of non-nucleophilic amine, followed by removal of any protecting groups, if present.

Other features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

25 **BRIEF DESCRIPTION OF THE DRAWINGS**

The invention will now be described in relation to the drawings in which:

Figure 1A is a graph showing the inhibition of CYP24 activity by compounds I(a) and I(c) (indicated as BH1625(NOH)-TB-2 (CTA062) and BH-1625(NOMe)-TB-2- (CTA065) respectively) compared to ketoconazole.

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Figure 1B is a graph showing the inhibition of CYP27B1 activity by compounds I(a) and I(c) (indicated as BH1625(NOH)-TB-2 (CTA062) and BH-1625(NOMe)-TB-2-(CTA065) respectively) compared to ketoconazole.

Figure 1C is a graph showing the inhibition of CYP27A1 activity by compounds I(a) and I(c) (indicated as BH1625(NOH)-TB-2 (CTA062) and BH-1625(NOMe)-TB-2-(CTA065) respectively) compared to ketoconazole.

Figure 2 is a graph showing the binding of compounds I(a) and I(c), (indicated as BH-1625(NOH)-TB-2 (CTA62) and BH-1625(NOMe)-TB-2-(CTA65) respectively) to transporter D protein (DBP) compared to $1\alpha,25$ -dihydroxy vitamin D₃ and 25-hydroxy vitamin D₃.

Figure 3 is a graph showing the activity of compounds I(a) and I(c) (indicated as BH1625(NOH)-TB-2 (CTA62) and BH-1625(NOMe)-TB-2-(CTA65) respectively) in the vitamin D transcription assay compared to $1\alpha,25$ -dihydroxy vitamin D₃.

Figure 4 is a graph showing the activity of compounds I(a) and I(c) (indicated as BH-1625(NOH)-TB-2 (CTA62) and BH-1625(NOMe)-TB-2-(CTA65) respectively) in the vitamin D receptor (VDR) binding assay compared to $1\alpha,25$ -dihydroxy vitamin D₃.

Figure 5 is a graph showing the dose response effects of compounds I(a) and I(c) on keratinocyte proliferation in comparison to $1\alpha,25$ -Dihydroxy vitamin D₃ or calcitriol.

Figure 6 is a graph showing the effect of compounds I(a) (indicated as BH 1625(NOH)) on calcium levels in rat urine in comparison to calcitriol ($1\alpha,25$ -Dihydroxy vitamin D₃).

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

The term "C₁₋₆alkyl" as used herein means straight and/or branched chain, saturated or unsaturated alkyl radicals containing from one to six carbon atoms and includes methyl, ethyl, propyl, isopropyl, s-butyl, t-butyl, neopentyl, vinyl, allyl, butenyl and the like.

The term "C₁₋₆alkoxy" as used herein means straight and/or branched chain, saturated or unsaturated alkoxy radicals containing from one to six carbon atoms and includes methoxy, ethoxy, propyloxy, isopropyloxy, t-butoxy and the like.

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The term "cyclo(C₃-C₆)alkyl" as used herein means saturated or unsaturated, non-aromatic cyclic alkyl radicals containing from three to six carbon atoms and includes cyclopropyl, cyclopentyl, cyclohexyl, cyclopentenyl, cyclohexenyl and the like.

5 The term "C₁₋₄alkyl" as used herein means straight and/or branched chain, saturated or unsaturated, alkyl radicals containing from one to four carbon atoms and includes methyl, ethyl, propyl, isopropyl, s-butyl, t-butyl and the like.

 The term "C₁₋₄alkoxy" as used herein means straight and/or branched chain, saturated or unsaturated alkoxy radicals containing from one to four carbon
10 atoms and includes methoxy, ethoxy, propyloxy, isopropoxy, t-butoxy and the like.

 The term "aryl" as used herein means unsubstituted or substituted mono- or bicyclic aromatic radicals containing from 6 to 10 carbon atoms and includes phenyl and naphthyl and the like.

 The term "heteroaryl" as used herein means unsubstituted or
15 substituted mono- or bicyclic heteroaromatic radicals containing from 5 to 10 atoms, of which 1-3 atoms may be a heteroatom selected from the group consisting of S, O and N, and includes furanyl, thienyl, pyrrolo, pyridyl, indolo, benzofuranyl and the like.

 The term "aryl-C₁₋₆alkyl" as used herein means unsubstituted or
20 substituted mono- or bicyclic aromatic radicals containing from 6 to 10 carbon atoms attached to the compounds of the invention *via* branched or unbranched alkylene radicals contain from 1-6 carbons atoms, the alkylene radicals being, saturated or unsaturated and unsubstituted or substituted with 1-4 groups independently selected from C₁₋₄alkyl, OC₁₋₄alkyl, OH, halo, NH₂, NHC₁₋₄alkyl and N(C₁₋₄alkyl)(C₁₋₄alkyl),
25 and includes Ph-C(CH₃)₂-, naphthylmethyl, benzyl and the like.

 The term "heteroaryl-C₁₋₆alkyl" as used herein means unsubstituted or substituted mono- or bicyclic heteroaromatic radicals containing from 5 to 10 atoms, of which 1-3 atoms may be a heteroatom selected from the group consisting of S, O and N attached to the compounds of the invention *via* branched or unbranched
30 alkylene radicals contain from 1-6 carbons atoms, the alkylene radicals being saturated or unsaturated and unsubstituted or substituted with 1-4 groups independently selected from C₁₋₄alkyl, OC₁₋₄alkyl, OH, halo, NH₂, NHC₁₋₄alkyl and

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N(C₁₋₄alkyl)(C₁₋₄alkyl), and includes thienyl-CH₂-, pyridyl-CH₂-, indolo-CH₂- and the like.

The term "halo" as used herein means halogen and includes chloro, fluoro, bromo, iodo and the like.

5 The term "solvate" as used herein means a compound of the invention, or a salt of a compound of the invention, wherein molecules of a suitable solvent are incorporated in the crystal lattice. A suitable solvent is physiologically tolerable at the dosage administered. Examples of suitable solvents are ethanol, water and the like. When water is the solvent, the molecule is referred to as a "hydrate".

10 The term "compound(s) of the invention" as used herein means compound(s) of Formulae I and II, and salts, hydrates, solvates and prodrugs thereof.

The term "pharmaceutically acceptable salt" means an acid addition salt or a basic addition salt which is suitable for or compatible with the treatment of patients.

15 The term "pharmaceutically acceptable acid addition salt" as used herein means any non-toxic organic or inorganic salt of any base compound of the invention, or any of its intermediates. Basic compounds of the invention that may form an acid addition salt include, for example, those where aryl, heteroaryl and/or the C₁₋₆alkyl group of R⁴ and/or R⁵ is substituted with a group having a basic
20 nitrogen, for example NH₂ and NHC₁₋₄alkyl. Illustrative inorganic acids which form suitable salts include hydrochloric, hydrobromic, sulfuric and phosphoric acids, as well as metal salts such as sodium monohydrogen orthophosphate and potassium hydrogen sulfate. Illustrative organic acids that form suitable salts include mono-, di-, and tricarboxylic acids such as glycolic, lactic, pyruvic, malonic, succinic, glutaric,
25 fumaric, malic, tartaric, citric, ascorbic, maleic, benzoic, phenylacetic, cinnamic and salicylic acids, as well as sulfonic acids such as p-toluene sulfonic and methanesulfonic acids. Either the mono or di-acid salts can be formed, and such salts may exist in either a hydrated, solvated or substantially anhydrous form. In general, the acid addition salts of the compounds of the invention are more soluble in water
30 and various hydrophilic organic solvents, and generally demonstrate higher melting points in comparison to their free base forms. The selection of the appropriate salt will be known to one skilled in the art. Other non-pharmaceutically acceptable acid

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addition salts, e.g. oxalates, may be used, for example, in the isolation of the compounds of the invention, for laboratory use, or for subsequent conversion to a pharmaceutically acceptable acid addition salt.

The term "pharmaceutically acceptable basic addition salt" as used
5 herein means any non-toxic organic or inorganic base addition salt of any acid compound of the invention, or any of its intermediates. Acidic compounds of the invention that may form a basic addition salt include, for example, those where aryl and/or heteroaryl is substituted with a group having acidic hydrogen, for example C(O)OH. Illustrative inorganic bases which form suitable salts include lithium,
10 sodium, potassium, calcium, magnesium or barium hydroxide. Illustrative organic bases which form suitable salts include aliphatic, alicyclic or aromatic organic amines such as methylamine, trimethylamine and picoline or ammonia. The selection of the appropriate salt will be known to a person skilled in the art. Other non-pharmaceutically acceptable basic addition salts, may be used, for example, in the
15 isolation of the compounds of the invention, for laboratory use, or for subsequent conversion to a pharmaceutically acceptable acid addition salt.

The term an "effective amount" or a "sufficient amount" of an agent as used herein is that amount sufficient to effect beneficial or desired results, including clinical results, and, as such, an "effective amount" depends upon the
20 context in which it is being applied. For example, in the context of administering an agent that modulates CYP24 activity, an effective amount of an agent is, for example, an amount sufficient to achieve such a modulation in CYP24 activity as compared to the response obtained without administration of the agent.

As used herein, and as well understood in the art, "treatment" is an
25 approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of extent of disease, stabilized (i.e. not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state,
30 and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment.

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“Palliating” a disease or disorder means that the extent and/or undesirable clinical manifestations of a disorder or a disease state are lessened and/or time course of the progression is slowed or lengthened, as compared to not treating the disorder.

5 The term "modulate" as used herein includes the inhibition or suppression of a function or activity (such as CYP24 activity) as well as the enhancement of a function or activity.

To “inhibit” or “suppress” or “reduce” a function or activity, such as CYP24 activity, is to reduce the function or activity when compared to otherwise
10 same conditions except for a condition or parameter of interest, or alternatively, as compared to another conditions.

The term “animal” as used herein includes all members of the animal kingdom including human. The animal is preferably a human.

The term “a cell” as used herein includes a plurality of cells.
15 Administering a compound to a cell includes *in vivo*, *ex vivo* and *in vitro* treatment.

The term “cancer cells” as used herein includes all forms of cancer or neoplastic disease.

The term “catabolism” as used herein refers to the metabolic process by which organisms convert substances into compounds for excretion.

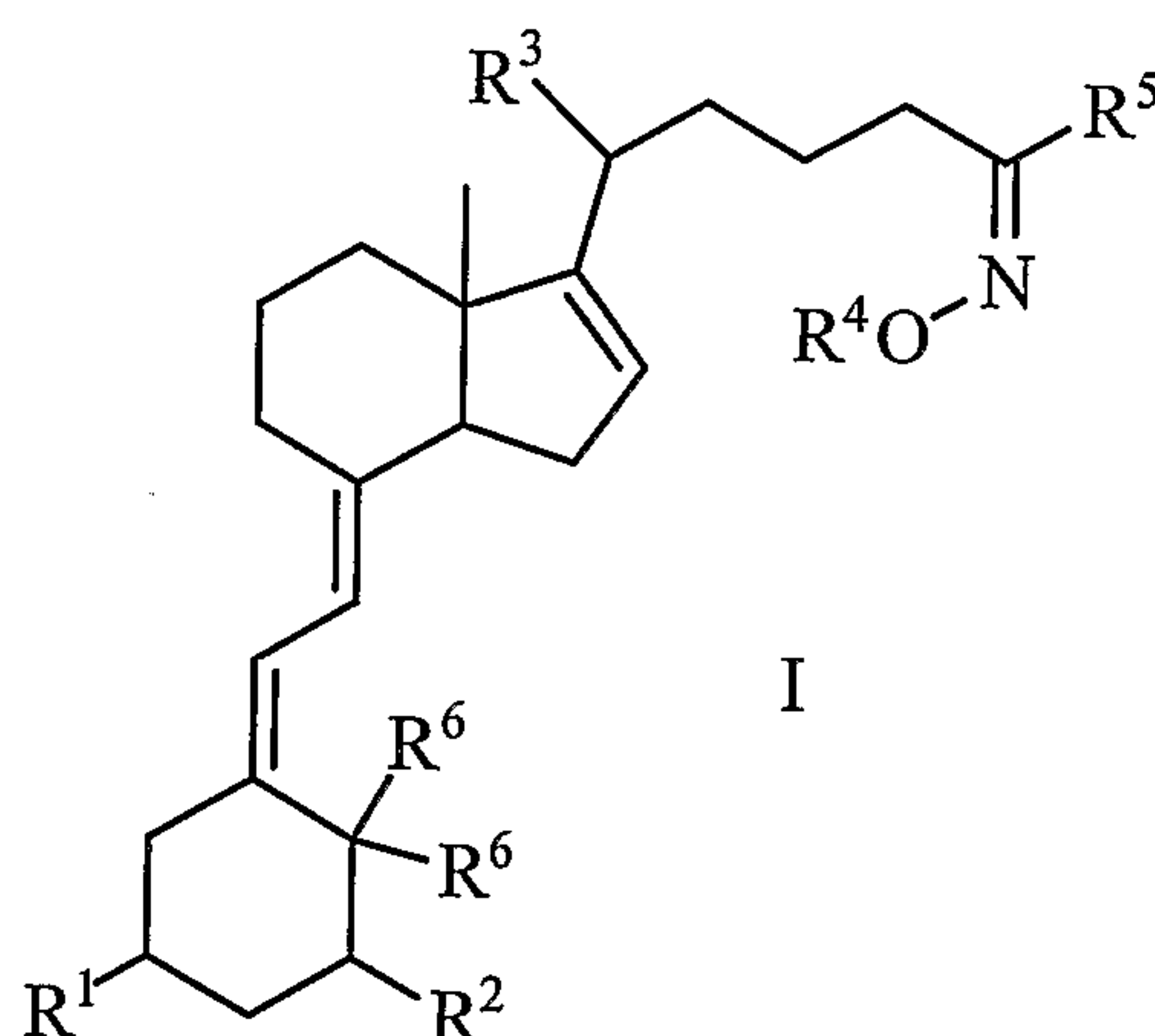
20 The term " $1\alpha,3\beta$ -stereochemistry" as used herein refers to the relative configuration of the groups , R^1 and R^2 , in which R^2 is above the plane of the page, and the R^1 is below the plane of the page. The term " $1\beta,3\alpha$ -stereochemistry" as used herein refers to the relative configuration of the groups , R^1 and R^2 , in which R^1 is above the plane of the page, and the R^2 is below the plane of the page.

25 II. Compounds of the Invention

Novel compounds showing selective inhibition of the enzyme CYP24, antiproliferative activity and that are low-calcemic have been prepared. As such, the compounds of the invention are useful for treating cell proliferative diseases, such as cancer.

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Accordingly, the present invention provides compounds of Formula I, and pharmaceutically acceptable salts, hydrates, solvates, and prodrugs thereof:



5 wherein

R^1 and R^2 are independently selected from the group consisting of OH, OC_{1-6} alkyl, and halo;

R^3 is C_{1-6} alkyl;

R^4 is selected from the group consisting of H, C_{1-6} alkyl, aryl and heteroaryl, with C_{1-6} alkyl being unsubstituted or substituted with 1-4 groups independently selected from C_{1-4} alkyl, OC_{1-4} alkyl, OH, halo, NH_2 , NHC_{1-4} alkyl and $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, and with aryl and heteroaryl being unsubstituted or substituted with 1-5 groups independently selected from C_{1-4} alkyl, OC_{1-4} alkyl, OH, CF_3 , OCF_3 , halo, SH, SC_{1-4} alkyl, NH_2 , NHC_{1-4} alkyl, $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, CN, $C(O)OH$, $C(O)OC_{1-4}alkyl$, $C(O)NHC_{1-4}alkyl$, $NHC(O)C_{1-4}alkyl$, $OC(O)C_{1-4}alkyl$, $SOC_{1-4}alkyl$, $SO_2C_{1-4}alkyl$, $SO_2NHC_{1-4}alkyl$ and SO_2NH_2 ;

R^5 is selected from the group consisting of C_{1-6} alkyl, cyclo(C_3-C_6)alkyl, aryl, heteroaryl, aryl- C_{1-6} alkyl and heteroaryl- C_{1-6} alkyl, with C_{1-6} alkyl being unsubstituted or substituted with 1-4 groups independently selected from C_{1-4} alkyl, OC_{1-4} alkyl, OH, halo, NH_2 , NHC_{1-4} alkyl and $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, and with cyclo(C_3-C_6)alkyl, aryl, heteroaryl, aryl- C_{1-6} alkyl and heteroaryl- C_{1-6} alkyl, being unsubstituted or substituted with 1-5 groups independently selected from C_{1-4} alkyl, OC_{1-4} alkyl, OH, CF_3 , OCF_3 , halo, SH, SC_{1-4} alkyl, NH_2 , NHC_{1-4} alkyl, $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, CN, $C(O)OH$, $C(O)OC_{1-4}alkyl$, $C(O)NHC_{1-4}alkyl$, $NHC(O)C_{1-4}alkyl$, $OC(O)C_{1-4}alkyl$, $SOC_{1-4}alkyl$, $SO_2C_{1-4}alkyl$, $SO_2NHC_{1-4}alkyl$ and SO_2NH_2 ; and

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R^6 are either both H or together form $=CH_2$.

The compounds of Formula I include those in which R^1 and R^2 are independently selected from the group consisting of OH, $OC_{1-6}alkyl$, and halo. In embodiments of the invention, R^1 and R^2 are independently selected from the group
 5 consisting of OH, OCH_3 , and fluoro. In a further embodiment, R^1 and R^2 are both OH.

The present invention also includes compounds of Formula I wherein R^3 is $C_{1-6}alkyl$. In an embodiment of the invention, R^3 is $C_{1-4}alkyl$. In further embodiments, R^3 is CH_3 .

10 The present invention includes compounds of Formula I wherein R^4 is selected from the group consisting of H, $C_{1-6}alkyl$, aryl and heteroaryl, with $C_{1-6}alkyl$ being unsubstituted or substituted with 1-4 groups independently selected from $C_{1-4}alkyl$, $OC_{1-4}alkyl$, OH, halo, NH_2 , $NHC_{1-4}alkyl$ and $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, and with aryl and heteroaryl being unsubstituted or substituted with 1-5 groups independently
 15 selected from $C_{1-4}alkyl$, $OC_{1-4}alkyl$, OH, CF_3 , OCF_3 , halo, SH, $SC_{1-4}alkyl$, NH_2 , $NHC_{1-4}alkyl$, $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, CN, $C(O)OH$, $C(O)OC_{1-4}alkyl$, $C(O)NHC_{1-4}alkyl$, $NHC(O)C_{1-4}alkyl$, $OC(O)C_{1-4}alkyl$, $SOC_{1-4}alkyl$, $SO_2C_{1-4}alkyl$, $SO_2NHC_{1-4}alkyl$ and SO_2NH_2 . In embodiments of the invention, R^4 is selected from the group consisting of H, $C_{1-4}alkyl$, and phenyl, with $C_{1-4}alkyl$ being unsubstituted or
 20 substituted with 1-2 groups independently selected from $C_{1-4}alkyl$, $OC_{1-4}alkyl$, OH, halo, NH_2 , $NHC_{1-4}alkyl$ and $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, and with phenyl being unsubstituted or substituted with 1-3 groups independently selected from $C_{1-4}alkyl$, $OC_{1-4}alkyl$, OH, CF_3 , OCF_3 , halo, SH, $SC_{1-4}alkyl$, NH_2 , $NHC_{1-4}alkyl$, $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, CN, $C(O)OH$, $C(O)OC_{1-4}alkyl$, $C(O)NHC_{1-4}alkyl$, $NHC(O)C_{1-4}alkyl$,
 25 $OC(O)C_{1-4}alkyl$, $SOC_{1-4}alkyl$, $SO_2C_{1-4}alkyl$, $SO_2NHC_{1-4}alkyl$ and SO_2NH_2 . In further embodiments, R^4 is selected from the group consisting of H, phenyl and $C_{1-4}alkyl$. In still further embodiments, R^4 is selected from the group consisting of H, phenyl, allyl and CH_3 .

The present invention includes compounds of Formula I wherein R^5 is
 30 selected from the group consisting of $C_{1-6}alkyl$, cyclo(C_3-C_6)alkyl, aryl and heteroaryl, aryl- $C_{1-6}alkyl$ and heteroaryl- $C_{1-6}alkyl$, with $C_{1-6}alkyl$ being unsubstituted or substituted with 1-4 groups independently selected from $C_{1-4}alkyl$, $OC_{1-4}alkyl$, OH,

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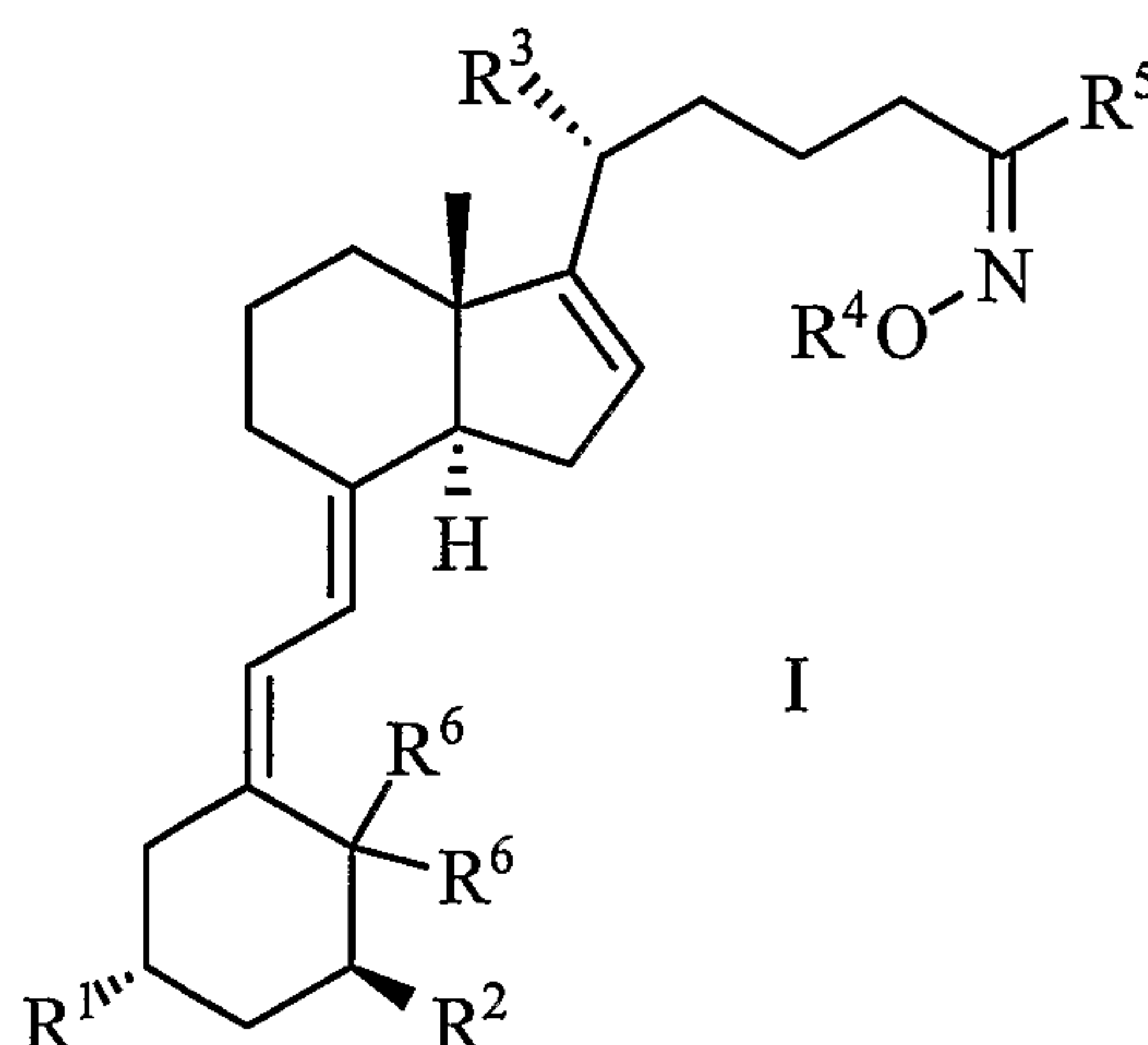
halo, NH₂, NHC₁₋₄alkyl and N(C₁₋₄alkyl)(C₁₋₄alkyl), and with cyclo(C₃₋₆)alkyl, aryl, heteroaryl, aryl-C₁₋₆alkyl and heteroaryl-C₁₋₆alkyl, being unsubstituted or substituted with 1-5 groups independently selected from C₁₋₄alkyl, OC₁₋₄alkyl, OH, CF₃, OCF₃, halo, SH, SC₁₋₄alkyl, NH₂, NHC₁₋₄alkyl, N(C₁₋₄alkyl)(C₁₋₄alkyl), CN, C(O)OH, C(O)OC₁₋₄alkyl, C(O)NHC₁₋₄alkyl, NHC(O)C₁₋₄alkyl, OC(O)C₁₋₄alkyl, SOC₁₋₄alkyl, SO₂C₁₋₄alkyl, SO₂NHC₁₋₄alkyl and SO₂NH₂. In embodiments of the invention, R⁵ is selected from the group consisting of C₁₋₄alkyl, phenyl, and phenyl-C₁₋₆alkyl with C₁₋₄alkyl being unsubstituted or substituted with 1-2 groups independently selected from C₁₋₄alkyl, OC₁₋₄alkyl, OH, halo, NH₂, NHC₁₋₄alkyl and N(C₁₋₄alkyl)(C₁₋₄alkyl), and with phenyl and phenyl-C₁₋₆alkyl being unsubstituted or substituted with 1-3 groups independently selected from C₁₋₄alkyl, OC₁₋₄alkyl, OH, CF₃, OCF₃, halo, SH, SC₁₋₄alkyl, NH₂, NHC₁₋₄alkyl, N(C₁₋₄alkyl)(C₁₋₄alkyl), CN, C(O)OH, C(O)OC₁₋₄alkyl, C(O)NHC₁₋₄alkyl, NHC(O)C₁₋₄alkyl, OC(O)C₁₋₄alkyl, SOC₁₋₄alkyl, SO₂C₁₋₄alkyl, SO₂NHC₁₋₄alkyl and SO₂NH₂. In further embodiments, R⁵ is selected from the group consisting of C₁₋₄alkyl, phenyl, and phenyl-C₁₋₄alkyl with C₁₋₄alkyl being unsubstituted or substituted with 1-2 groups independently selected from C₁₋₂alkyl, OC₁₋₂alkyl, OH, halo, NH₂, NHC₁₋₂alkyl and N(C₁₋₂alkyl)(C₁₋₂alkyl), and with phenyl and phenyl-C₁₋₄alkyl being unsubstituted or substituted with 1-3 groups independently selected from C₁₋₄alkyl, OC₁₋₄alkyl, OH, CF₃, OCF₃, halo, NH₂, NHC₁₋₄alkyl, N(C₁₋₄alkyl)(C₁₋₄alkyl), and CN. In still further embodiments, R⁵ is selected from isopropyl, s-butyl, t-butyl, neopentyl.

The present invention also includes compounds of Formula I, wherein R⁶ are either both H or together form =CH₂. In embodiments of the invention, R⁶ are both H.

All of the compounds of Formula I have more than one asymmetric centre. Where the compounds according to the invention possess more than one asymmetric centre, they may exist as diastereomers. It is to be understood that all such isomers and mixtures thereof in any proportion are encompassed within the scope of the present invention. Further, the invention extends to all geometric isomers of the present invention. For example, where there is a double bond in a compound of the invention, there may exist geometric isomers, such as cis and trans

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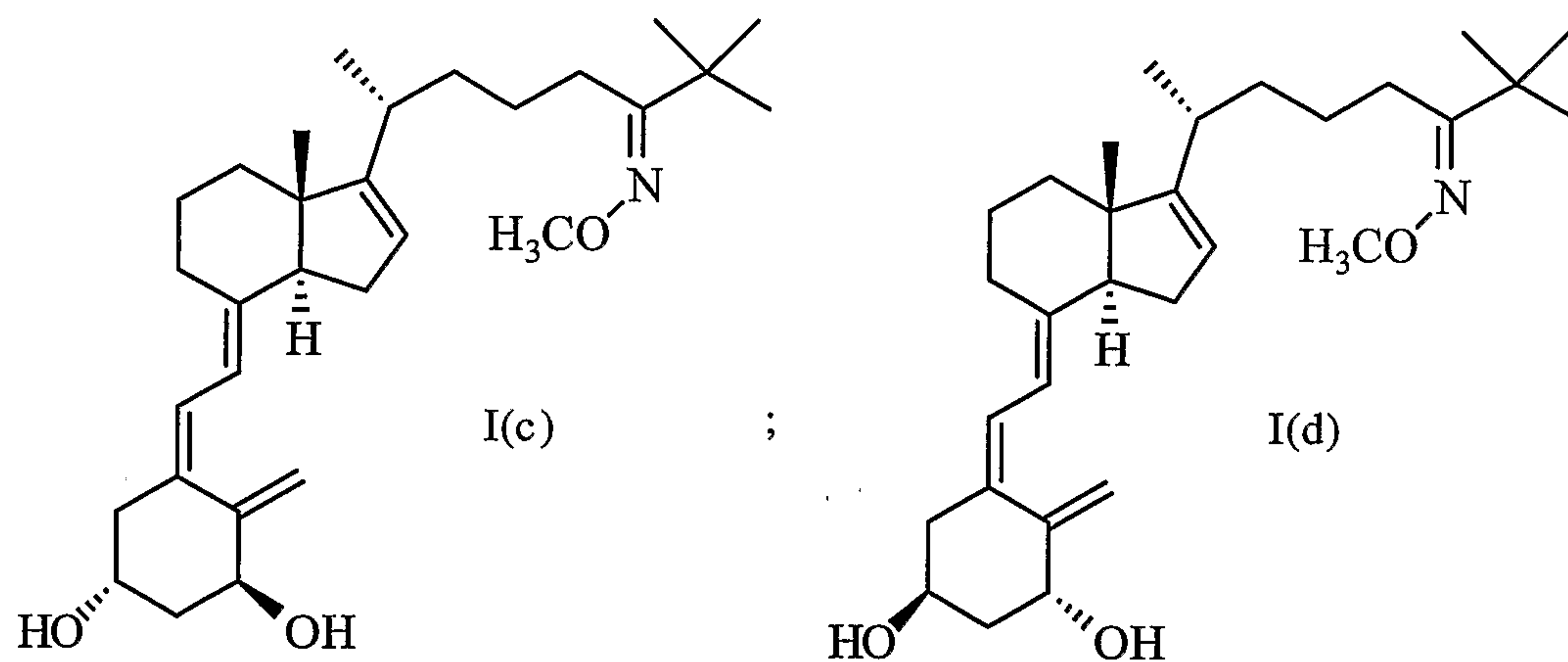
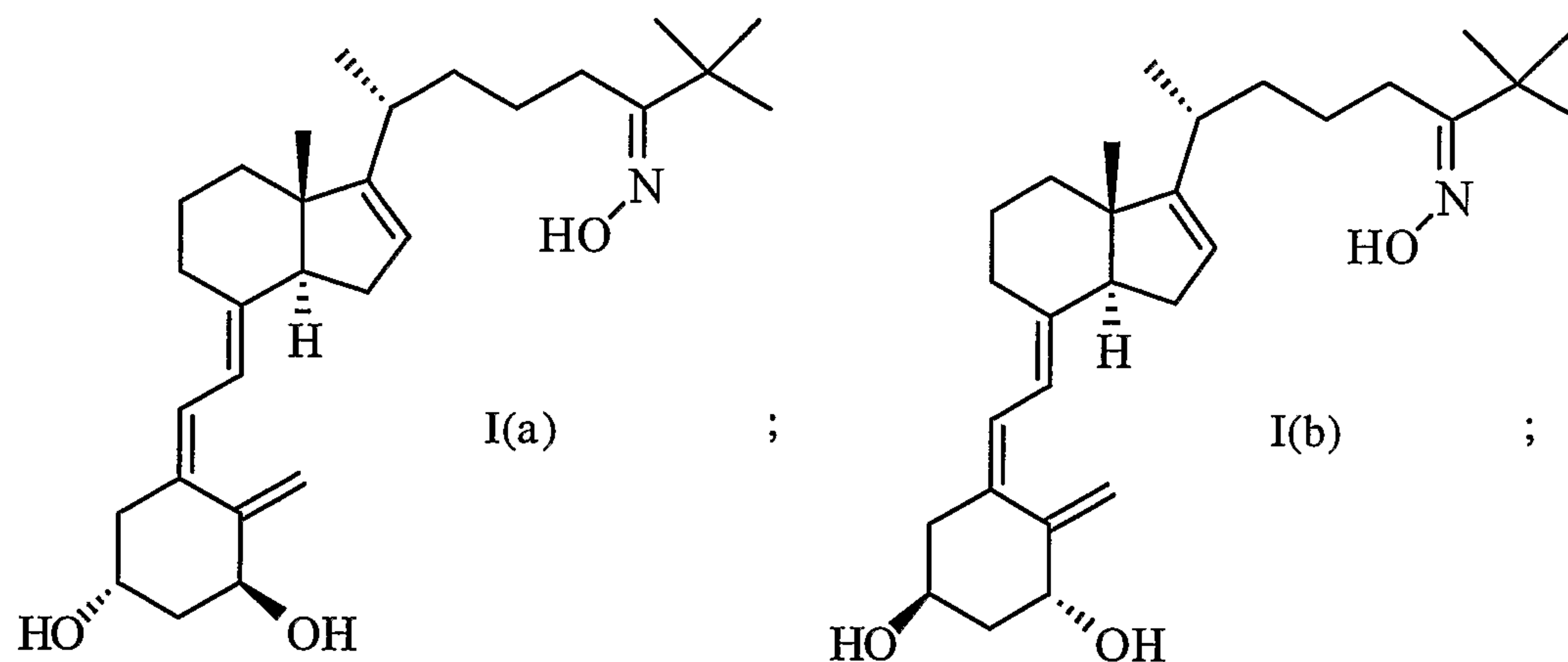
(also known as Z and E) isomers. The stereochemistry of the compounds of the invention is preferably that of natural $1\alpha,25$ -dihydroxy vitamin D_3 . Therefore, in a preferred embodiment, the present invention provides compounds of Formula I with the relative stereochemistry as shown below, and pharmaceutically acceptable salts, hydrates, solvates and prodrugs thereof:



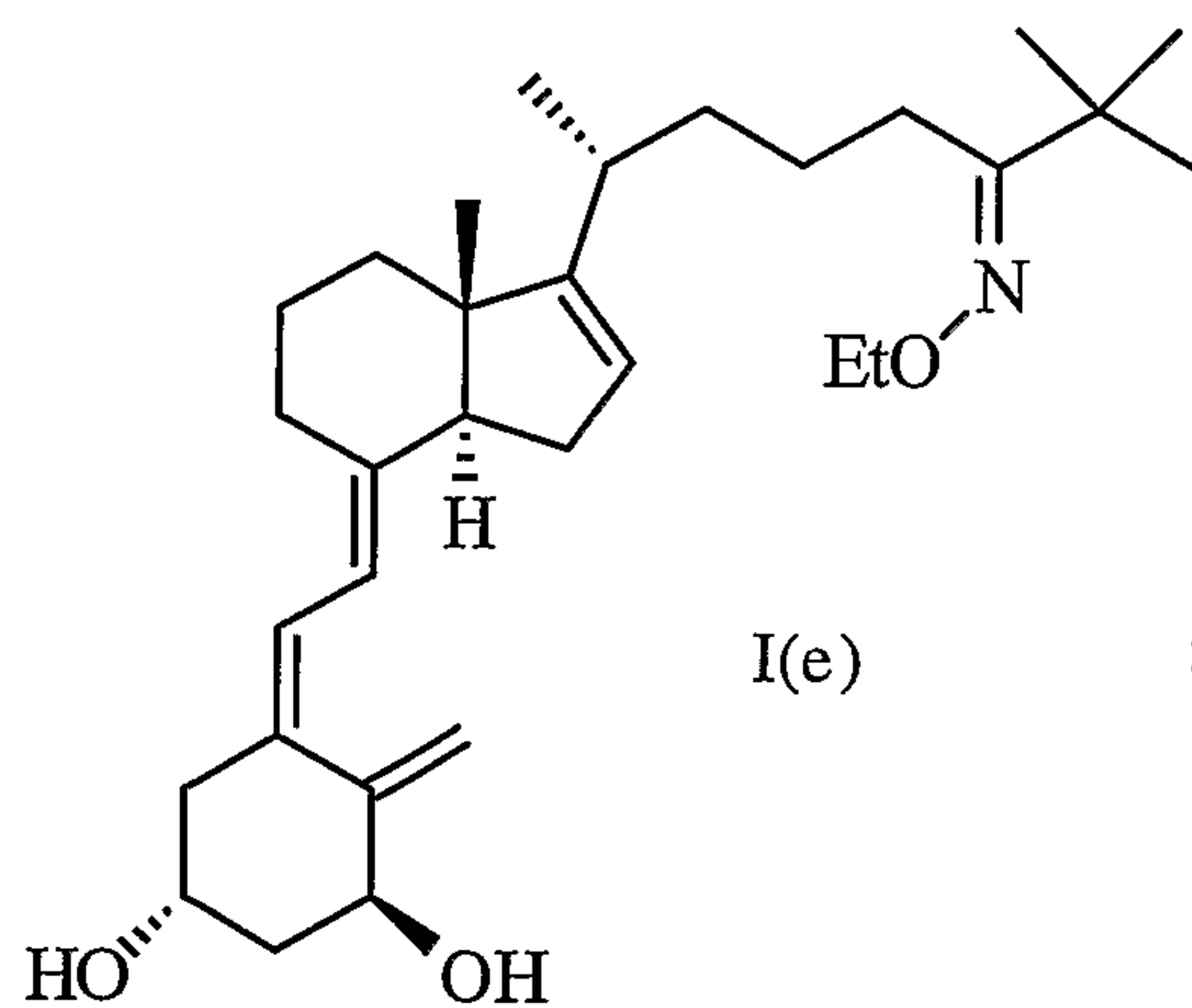
wherein $R^1 - R^6$ are as previously defined. It is to be understood that while, the relative stereochemistry of the compounds of Formula I is preferably as shown above, such compounds of Formula I may also contain certain amounts (e.g. less than 20%, preferably less than 10%, more preferably less than 5%) of compounds of Formula I having alternate stereochemistry. For example, a compound of Formula I having the $1\alpha,3\beta$ -stereochemistry of natural $1\alpha,25$ -Dihydroxy Vitamin D_3 , shown above, may contain less than 20%, preferably less than 10%, more preferably less than 5%, of a compound of Formula I having the unnatural $1\beta,3\alpha$ -stereochemistry.

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In specific embodiments of the present invention, the compounds of the invention include:

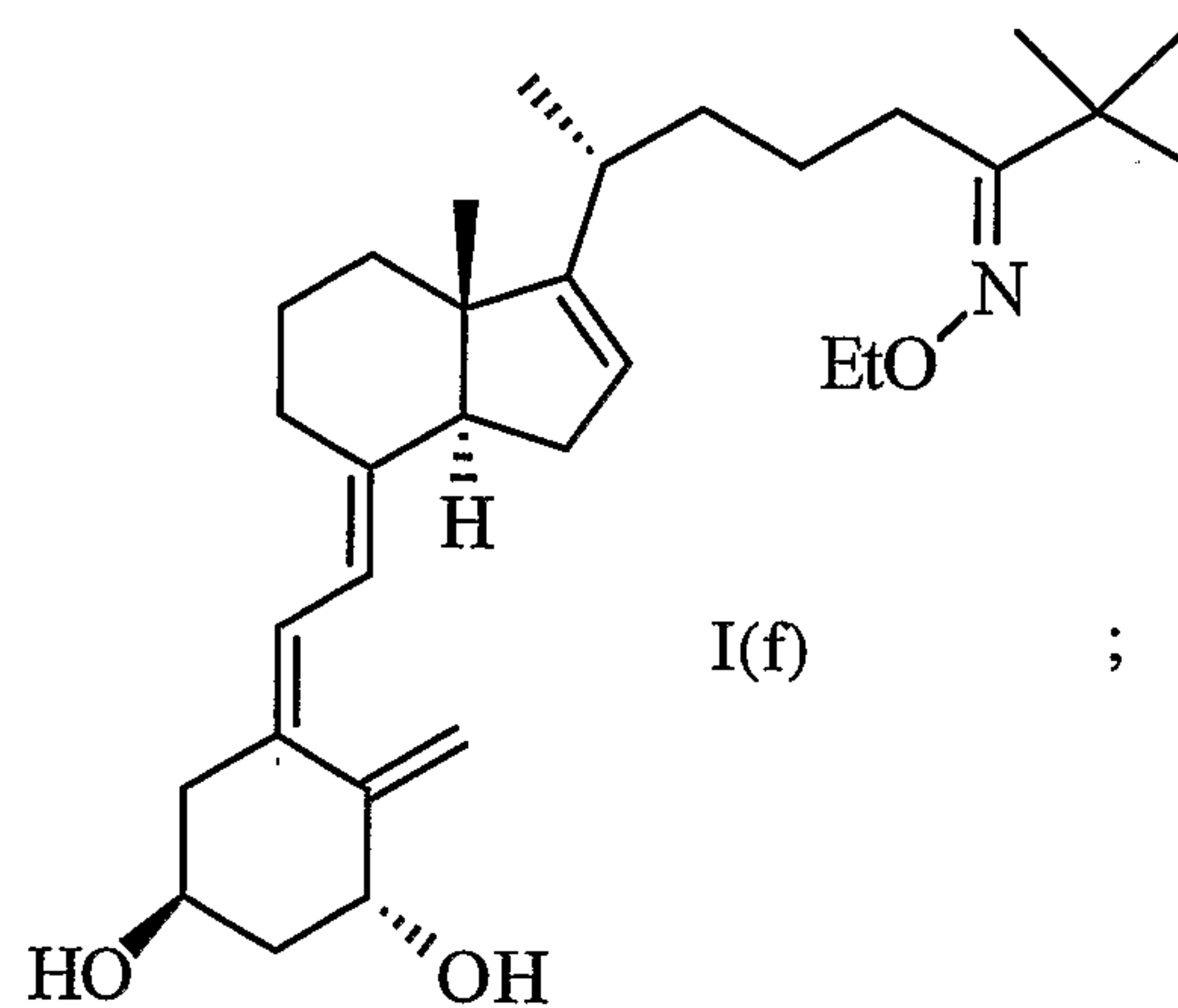


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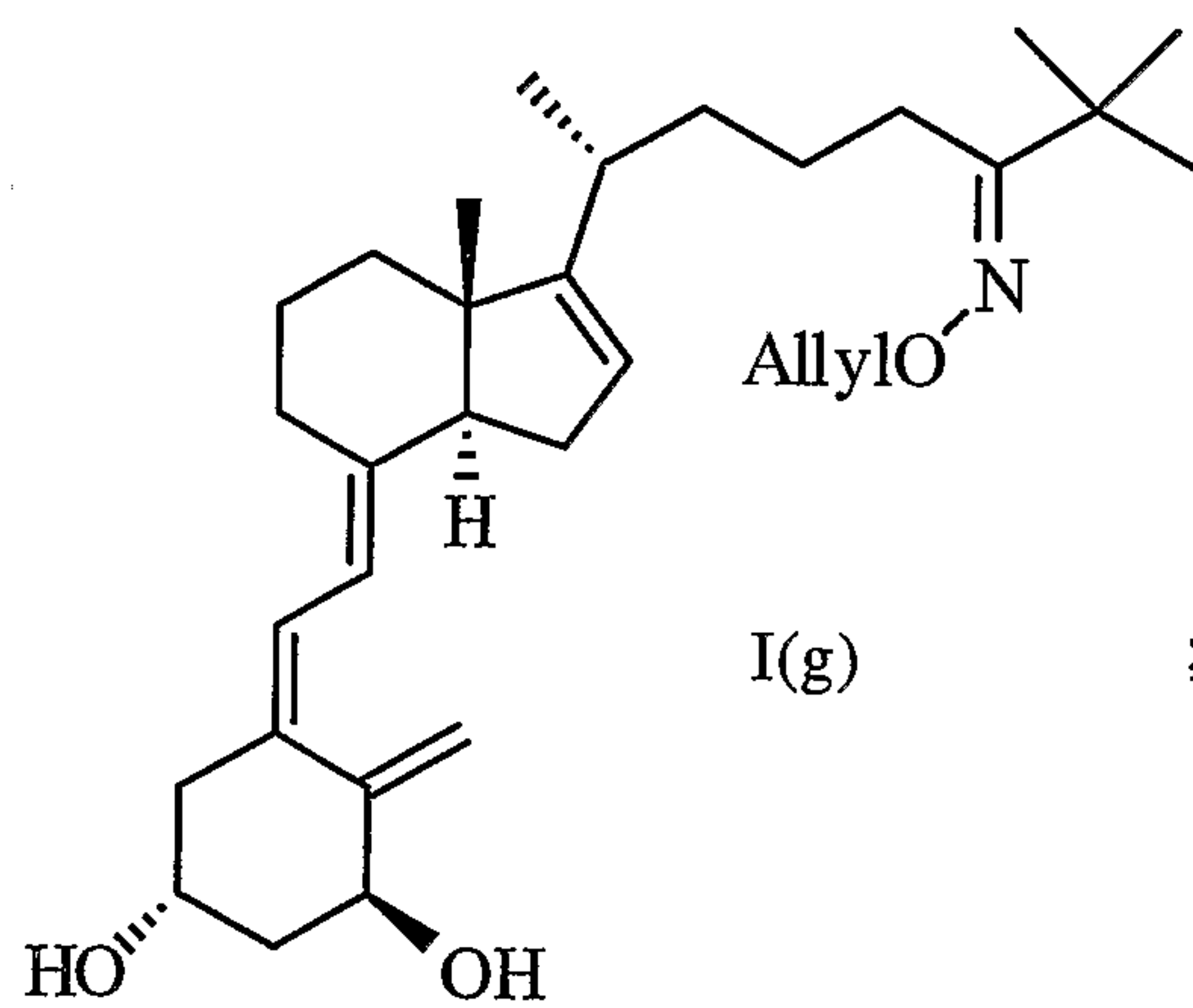
I(e)

;



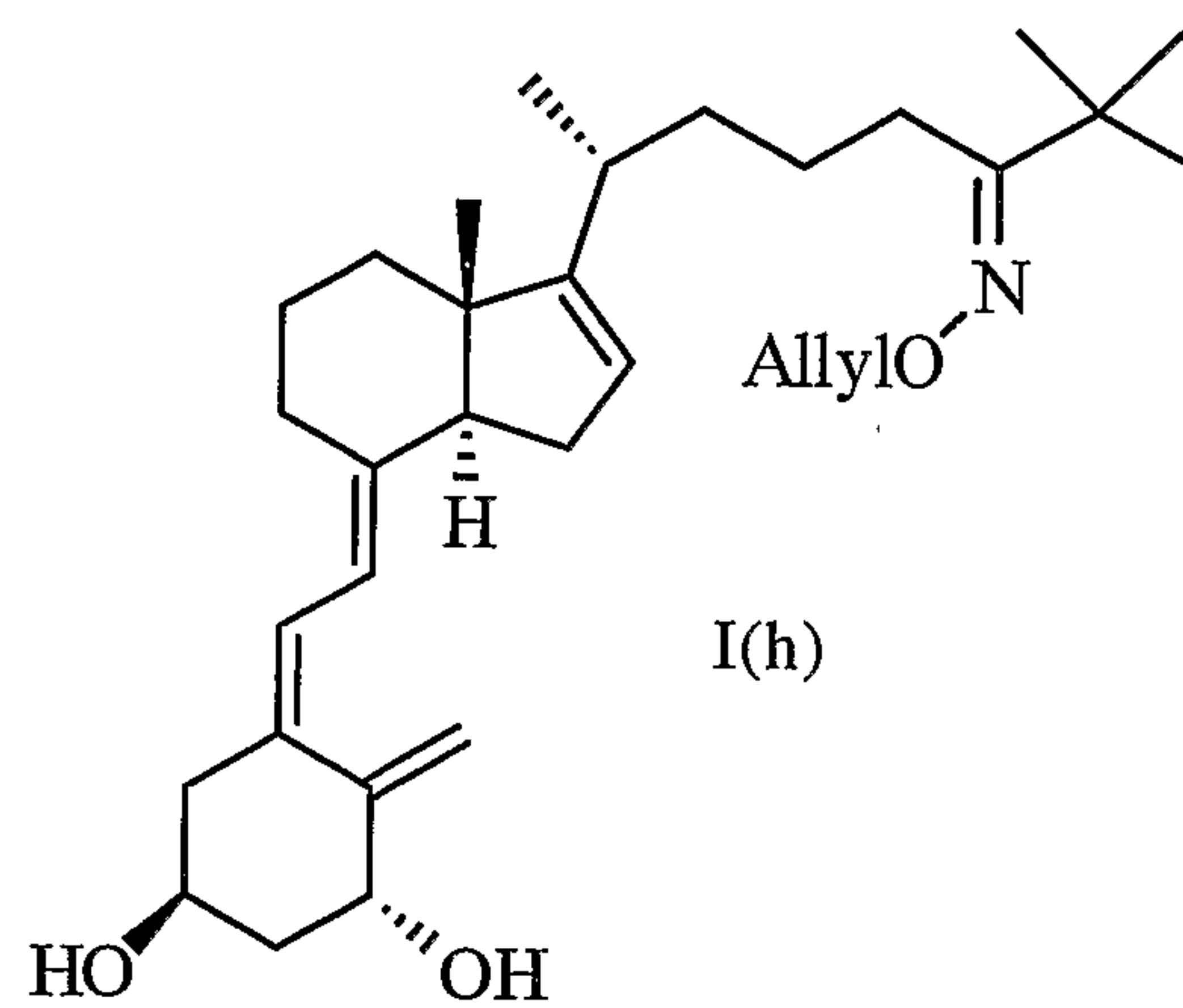
I(f)

;

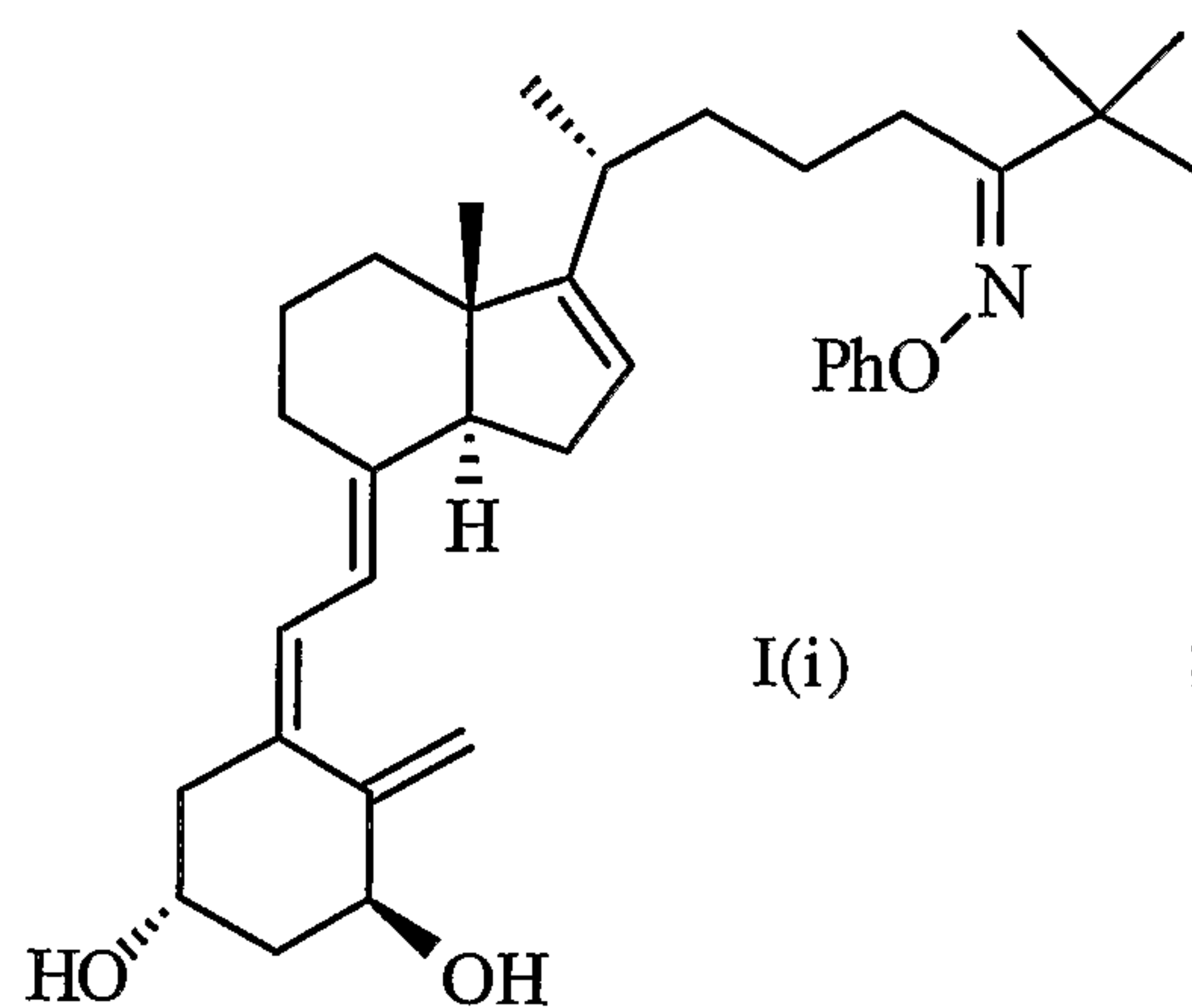


I(g)

;

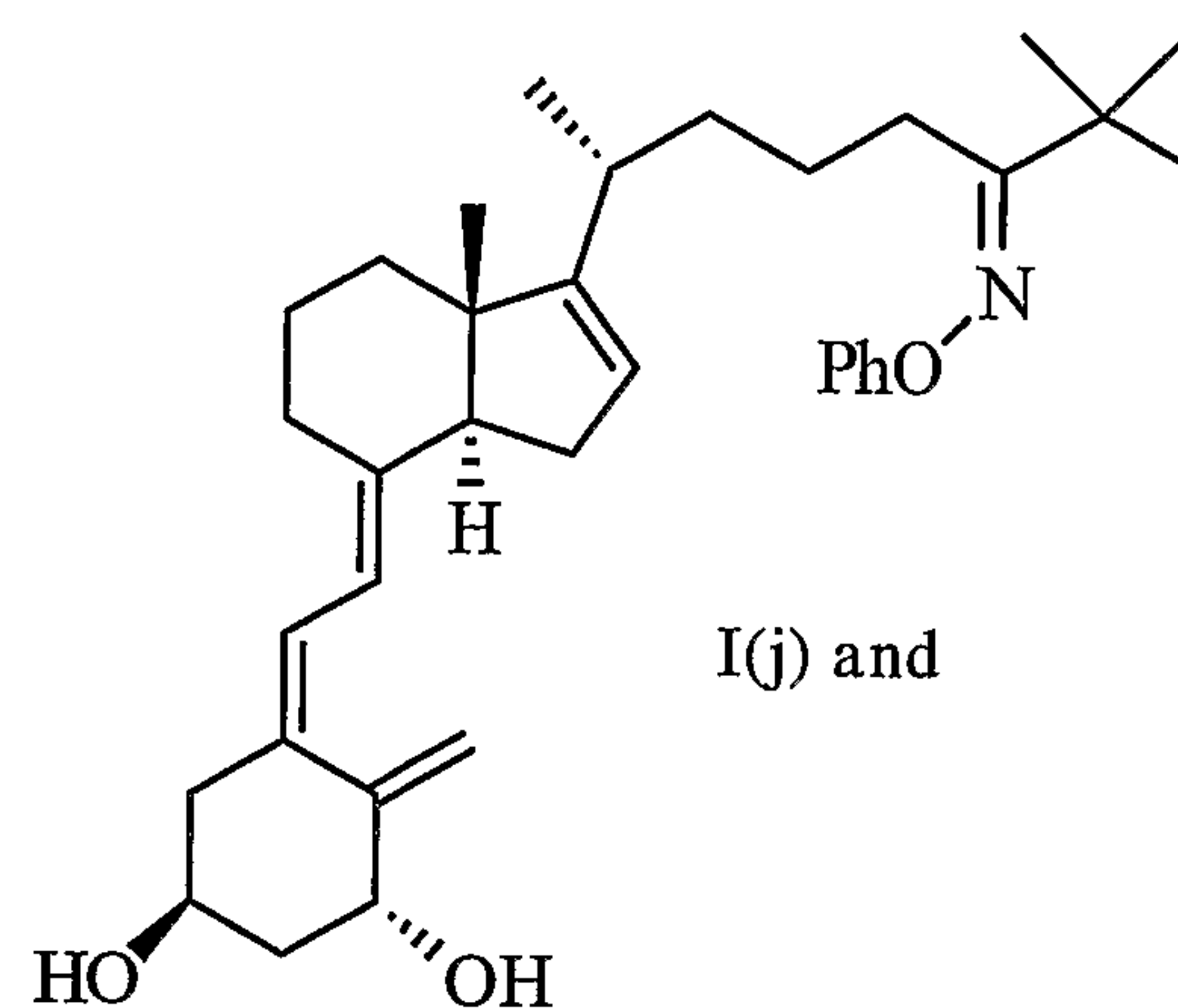


I(h)



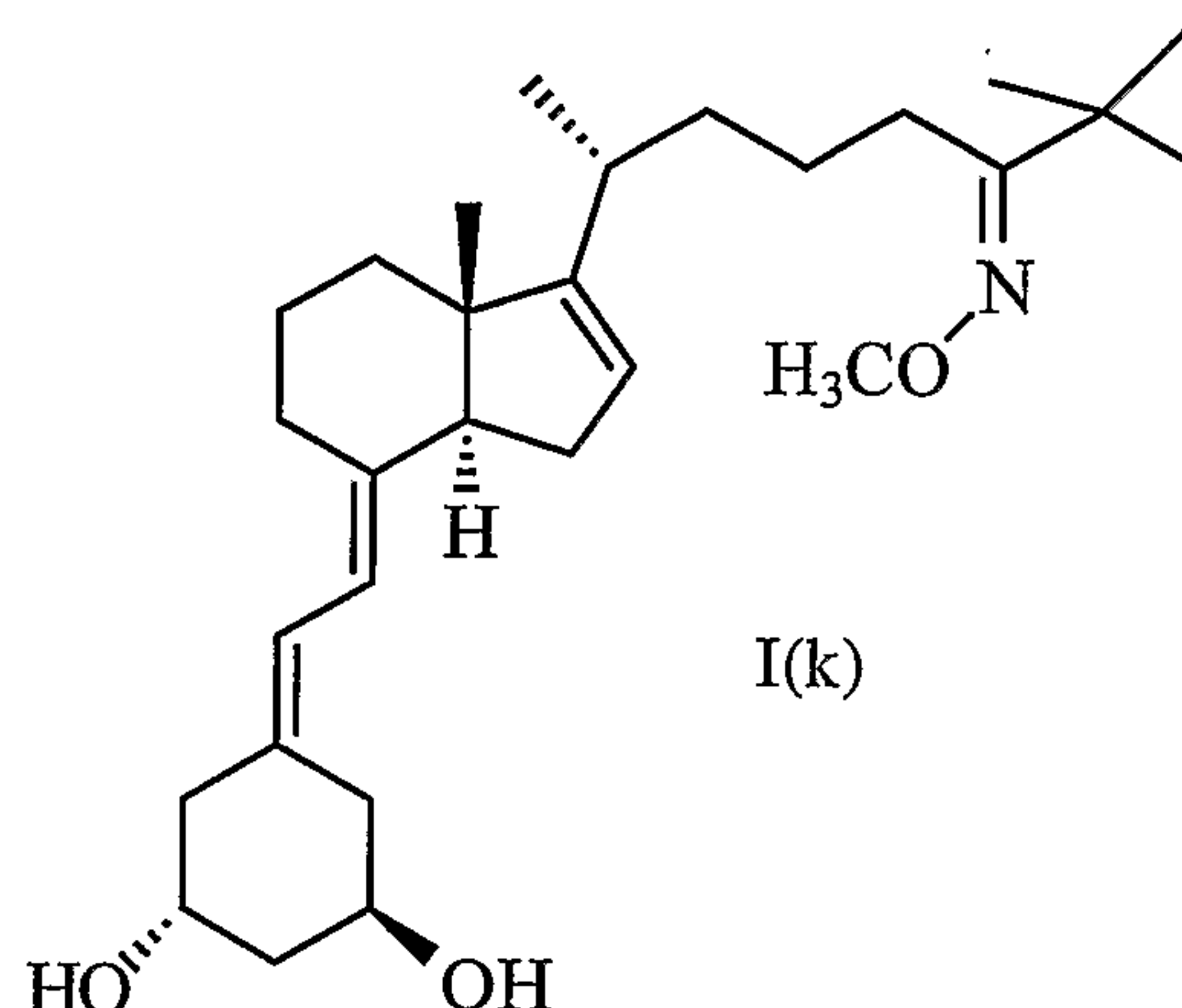
I(i)

;



I(j) and

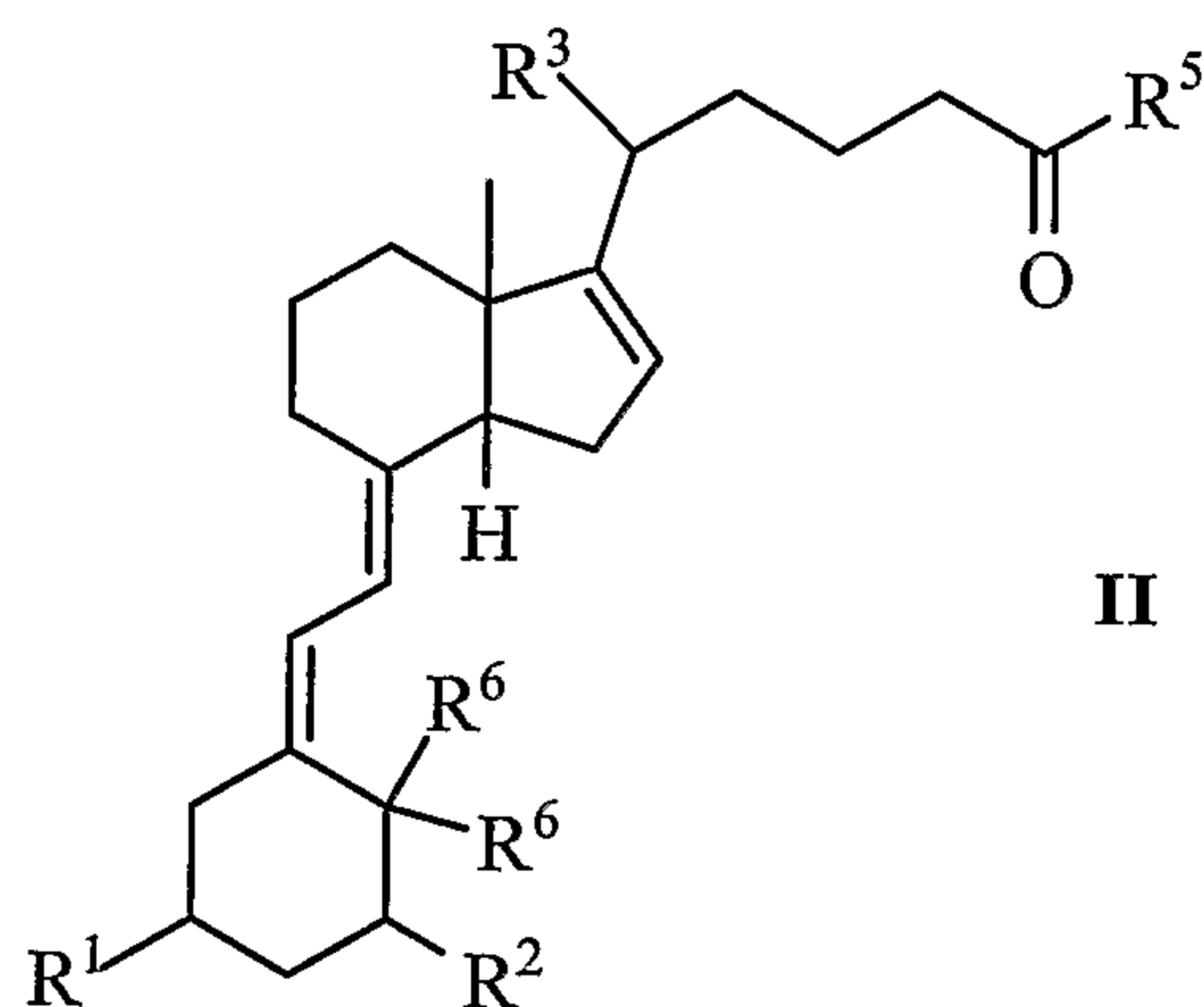
- 20 -



and pharmaceutically acceptable salts, hydrates, solvates and prodrugs thereof. Preferred compounds of the invention include compounds I(a), I(c), I(e), I(g), I(i) and
 5 I(k) as shown above, and pharmaceutically acceptable salts, hydrates, solvates and prodrugs thereof.

The present invention further provides novel compounds useful in the preparation of the compounds of Formula I. Therefore the present invention further provides compounds of Formula II, and salts, hydrates and solvates thereof:

10



wherein

- R^1 and R^2 are independently selected from the group consisting of OH, $OC_{1-6}alkyl$,
 15 OPG and halo;
 PG is a protecting group;
 R^3 is $C_{1-6}alkyl$;

- 21 -

- R⁵ is selected from the group consisting of C₁₋₆alkyl, cyclo(C₃₋₆)alkyl, aryl and heteroaryl, aryl-C₁₋₆alkyl and heteroaryl-C₁₋₆alkyl, with C₁₋₆alkyl being unsubstituted or substituted with 1-4 groups independently selected from C₁₋₄alkyl, OC₁₋₄alkyl, OH, halo, NH₂, NHC₁₋₄alkyl and N(C₁₋₄alkyl)(C₁₋₄alkyl), and with cyclo(C₃₋₆)alkyl, aryl, heteroaryl, aryl-C₁₋₆alkyl and heteroaryl-C₁₋₆alkyl, being unsubstituted or substituted with 1-5 groups independently selected from C₁₋₄alkyl, OC₁₋₄alkyl, OH, CF₃, OCF₃, halo, SH, SC₁₋₄alkyl, NH₂, NHC₁₋₄alkyl, N(C₁₋₄alkyl)(C₁₋₄alkyl), CN, C(O)OH, C(O)OC₁₋₄alkyl, C(O)NHC₁₋₄alkyl, NHC(O)C₁₋₄alkyl, OC(O)C₁₋₄alkyl, SOC₁₋₄alkyl, SO₂C₁₋₄alkyl, SO₂NHC₁₋₄alkyl and SO₂NH₂; and
- 10 R⁶ are either both H or together form =CH₂.

The compounds of Formula II include those in which R¹ and R² are independently selected from the group consisting of OH, OC₁₋₆alkyl, OPG and halo. In embodiments of the invention, R¹ and R² are independently selected from the group consisting of OH, OCH₃, OPG and fluoro. In a further embodiment, R¹ and R² are both OH or OPG. Further, PG is meant to include any protecting group that protects the free OH groups of R¹ and /or R² in the compounds of Formula I from unwanted side reactions during the conversion of compounds of Formula II to compounds of Formula I, and that can be removed under conditions that do not cause unwanted side reactions with other functional groups on the molecule. Suitable protecting groups include trialkylsilyl groups, such as t-butyldimethylsilyl.

The present invention further includes compounds of Formula II wherein R³ is C₁₋₆alkyl. In embodiments of the invention, R³ is C₁₋₄alkyl. In further embodiments, R³ is CH₃.

The present invention also includes compounds of Formula II wherein

25 R⁵ is selected from the group consisting of C₁₋₆alkyl, cyclo(C₃₋₆)alkyl, aryl and heteroaryl, aryl-C₁₋₆alkyl and heteroaryl-C₁₋₆alkyl, with C₁₋₆alkyl being unsubstituted or substituted with 1-4 groups independently selected from C₁₋₄alkyl, OC₁₋₄alkyl, OH, halo, NH₂, NHC₁₋₄alkyl and N(C₁₋₄alkyl)(C₁₋₄alkyl), and with cyclo(C₃₋₆)alkyl, aryl, heteroaryl, aryl-C₁₋₆alkyl and heteroaryl-C₁₋₆alkyl, being unsubstituted or substituted

30 with 1-5 groups independently selected from C₁₋₄alkyl, OC₁₋₄alkyl, OH, CF₃, OCF₃, halo, SH, SC₁₋₄alkyl, NH₂, NHC₁₋₄alkyl, N(C₁₋₄alkyl)(C₁₋₄alkyl), CN, C(O)OH, C(O)OC₁₋₄alkyl, C(O)NHC₁₋₄alkyl, NHC(O)C₁₋₄alkyl, OC(O)C₁₋₄alkyl, SOC₁₋₄alkyl,

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SO₂C₁₋₄alkyl, SO₂NHC₁₋₄alkyl and SO₂NH₂. In embodiments of the invention, R⁵ is selected from the group consisting of C₁₋₄alkyl, phenyl, and phenyl-C₁₋₆alkyl with C₁₋₄alkyl being unsubstituted or substituted with 1-2 groups independently selected from C₁₋₄alkyl, OC₁₋₄alkyl, OH, halo, NH₂, NHC₁₋₄alkyl and N(C₁₋₄alkyl)(C₁₋₄alkyl), and

5 with phenyl and phenyl-C₁₋₆alkyl being unsubstituted or substituted with 1-3 groups independently selected from C₁₋₄alkyl, OC₁₋₄alkyl, OH, CF₃, OCF₃, halo, SH, SC₁₋₄alkyl, NH₂, NHC₁₋₄alkyl, N(C₁₋₄alkyl)(C₁₋₄alkyl), CN, C(O)OH, C(O)OC₁₋₄alkyl, C(O)NHC₁₋₄alkyl, NHC(O)C₁₋₄alkyl, OC(O)C₁₋₄alkyl, SOC₁₋₄alkyl, SO₂C₁₋₄alkyl, SO₂NHC₁₋₄alkyl and SO₂NH₂. In further embodiments, R⁵ is selected from the group

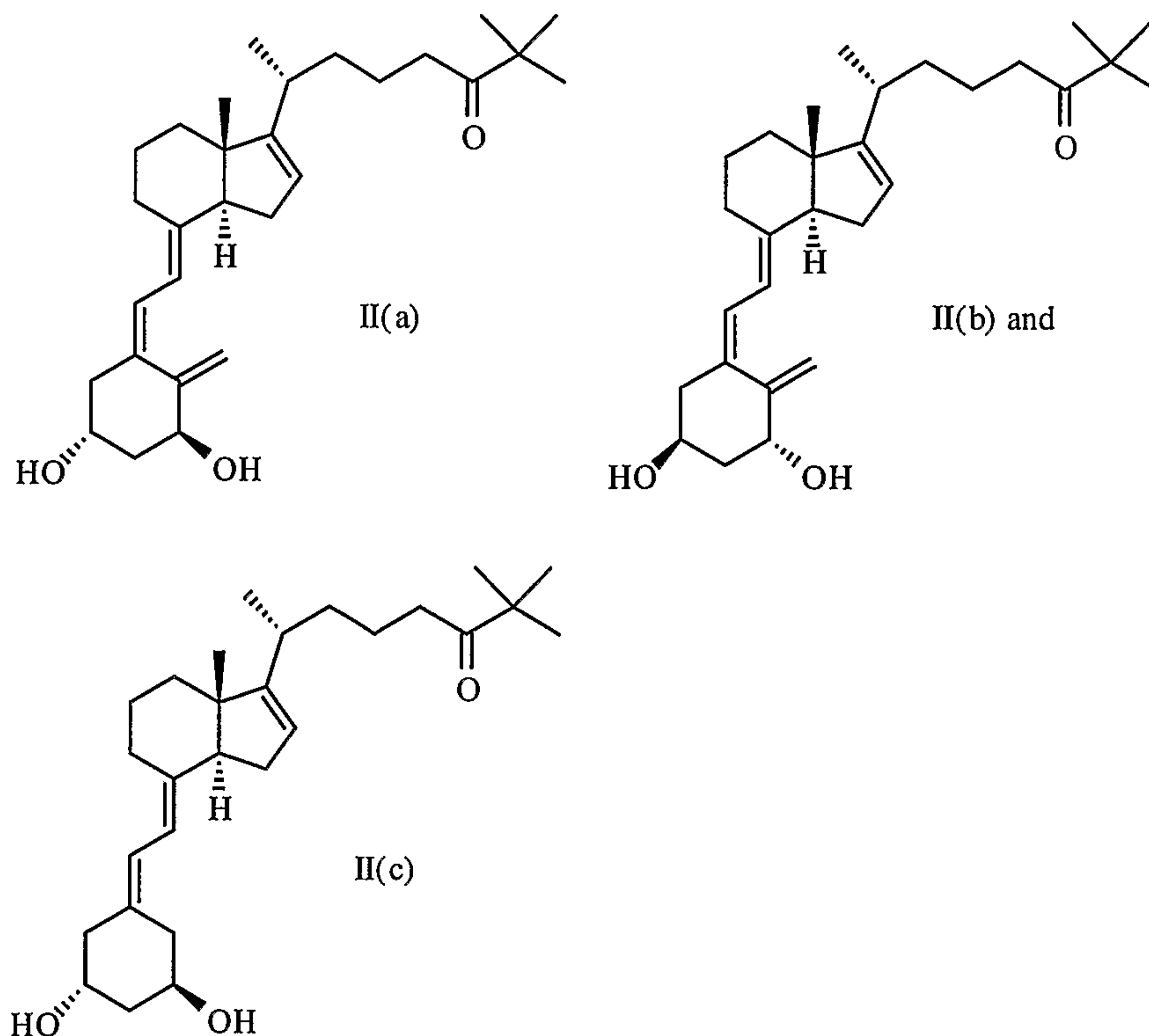
10 consisting of C₁₋₄alkyl, phenyl, and phenyl-C₁₋₄alkyl with C₁₋₄alkyl being unsubstituted or substituted with 1-2 groups independently selected from C₁₋₂alkyl, OC₁₋₂alkyl, OH, halo, NH₂, NHC₁₋₂alkyl and N(C₁₋₂alkyl)(C₁₋₂alkyl), and with phenyl and phenyl-C₁₋₄alkyl being unsubstituted or substituted with 1-3 groups independently selected from C₁₋₄alkyl, OC₁₋₄alkyl, OH, CF₃, OCF₃, halo, NH₂, NHC₁₋₄alkyl, N(C₁₋

15 4alkyl)(C₁₋₄alkyl), and CN. In still further embodiments, R⁵ is selected from isopropyl, s-butyl, t-butyl, neopentyl.

The present invention also includes compounds of Formula I, wherein R⁶ are either both H or together form =CH₂. In embodiments of the invention, R⁶ are both H.

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In specific embodiments of the present invention, the compounds of the Formula II include:



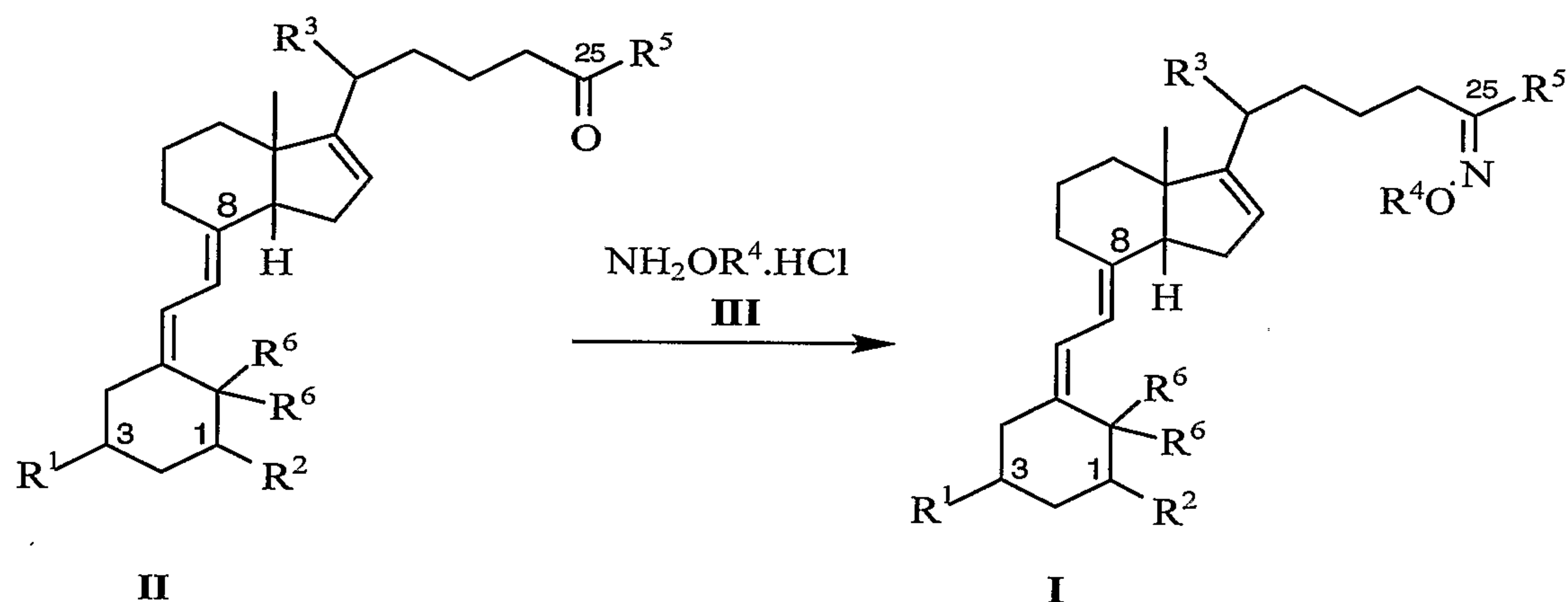
and salts, hydrates and solvates thereof. Preferred compounds of Formula II are
 5 compounds II(a) and II(c), as shown above, and salts, hydrates and solvates thereof.

III. Methods of Preparing Compounds of the Invention

In accordance with another aspect of the present invention, the
 compounds of the invention can be prepared by processes analogous to those
 established in the art. Therefore, compounds of this invention may be prepared, for
 10 example, by the reaction sequence shown in Scheme 1:

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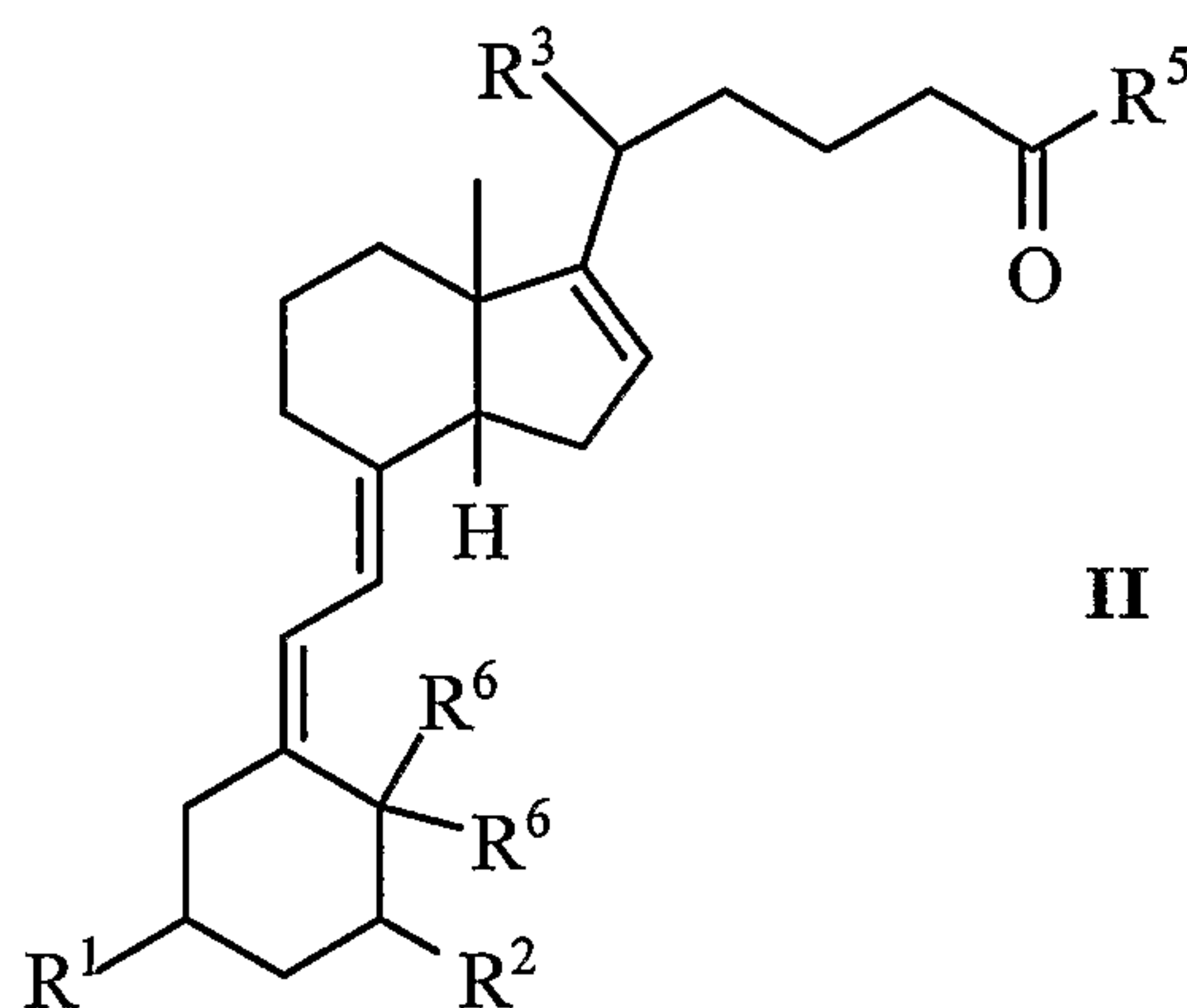
Scheme 1



Therefore, compounds of Formula I, may be prepared by reacting compounds of
 5 Formula II, wherein R^1 - R^3 , R^5 and R^6 are as defined in Formula II, with reagents of
 Formula III, wherein R^4 is as defined in Formula I, preferably in the presence of a
 non-nucleophilic amine, at temperatures in the range of about 0°C to about 40°C ,
 suitably at room temperature, followed by removal of any protecting groups (if
 present). The non-nucleophilic amine may be any tertiary aromatic or aliphatic
 10 amine, for example pyridine, and is preferably present in excess amounts. When
 pyridine is the non-nucleophilic amine, it is preferably also used as a solvent for the
 transformation of compounds of Formula II to compounds of Formula I. This oxime
 installation step proceeds without destroying the acid-sensitive conjugated triene unit
 of compounds of Formula II and indicates that such an oximation step will be useful
 15 in producing a library of new and diverse 25-oxime ether analogs. The E-oxime alkyl
 ether of the Formula I is predominately obtained due to the strongly unfavourable
 steric congestion that would be present in the corresponding Z-oxime alkyl ether (see
 Hawkes, G. E. and Herwig, K.; Roberts, J. D. *J. Org. Chem.* **1974**, *39*, 1017-1028).

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Accordingly, the present invention provides a method for preparing a compound of Formula I comprising reacting a compound of Formula II, or a salt, hydrate or solvate thereof:



5

wherein

R^1 and R^2 are independently selected from the group consisting of OH, OC_{1-6} alkyl, OPG and halo;

10 PG is a protecting group;

R^3 is C_{1-6} alkyl;

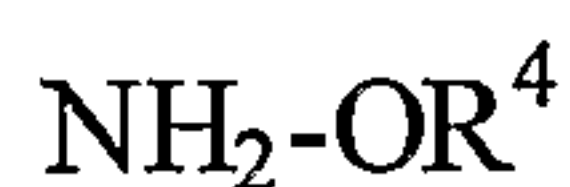
R^5 is selected from the group consisting of C_{1-6} alkyl, cyclo(C_3-C_6)alkyl, aryl and heteroaryl, aryl- C_{1-6} alkyl and heteroaryl- C_{1-6} alkyl, with C_{1-6} alkyl being unsubstituted or substituted with 1-4 groups independently selected from C_{1-4} alkyl, OC_{1-4} alkyl, OH,

15 halo, NH_2 , NHC_{1-4} alkyl and $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, and with cyclo(C_3-C_6)alkyl, aryl, heteroaryl, aryl- C_{1-6} alkyl and heteroaryl- C_{1-6} alkyl, being unsubstituted or substituted with 1-5 groups independently selected from C_{1-4} alkyl, OC_{1-4} alkyl, OH, CF_3 , OCF_3 , halo, SH, SC_{1-4} alkyl, NH_2 , NHC_{1-4} alkyl, $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, CN, $C(O)OH$, $C(O)OC_{1-4}alkyl$, $C(O)NHC_{1-4}alkyl$, $NHC(O)C_{1-4}alkyl$, $OC(O)C_{1-4}alkyl$, $SOC_{1-4}alkyl$,

20 $SO_2C_{1-4}alkyl$, $SO_2NHC_{1-4}alkyl$ and SO_2NH_2 ; and

R^6 are either both H or together form $=CH_2$,

with a compound of Formula III, or a salt hydrate or solvate thereof:



III ,

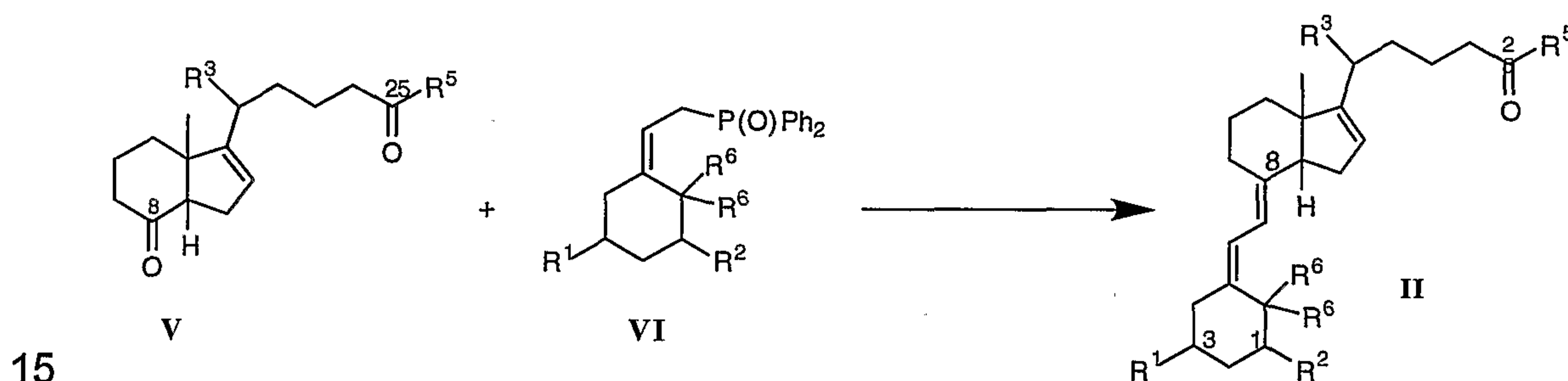
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wherein R^4 is selected from the group consisting of H, C_{1-6} alkyl, aryl and heteroaryl, with C_{1-6} alkyl being unsubstituted or substituted with 1-4 groups independently selected from C_{1-4} alkyl, OC_{1-4} alkyl, OH, halo, NH_2 , NHC_{1-4} alkyl and $N(C_{1-4}$ alkyl)(C_{1-4} alkyl), and with aryl and heteroaryl being unsubstituted or substituted with 1-5 groups independently selected from C_{1-4} alkyl, OC_{1-4} alkyl, OH, CF_3 , OCF_3 , halo, SH, SC_{1-4} alkyl, NH_2 , NHC_{1-4} alkyl, $N(C_{1-4}$ alkyl)(C_{1-4} alkyl), CN, $C(O)OH$, $C(O)OC_{1-4}$ alkyl, $C(O)NHC_{1-4}$ alkyl, $NHC(O)C_{1-4}$ alkyl, $OC(O)C_{1-4}$ alkyl, SOC_{1-4} alkyl, SO_2C_{1-4} alkyl, SO_2NHC_{1-4} alkyl and SO_2NH_2 ;

in the presence of non-nucleophilic amine, followed by removal of any protecting groups, if present.

Ketones of Formula II, wherein R^1 - R^3 , R^5 and R^6 are as defined in Formula I, may be prepared, for example, as shown in Scheme 2:

Scheme 2



Ketones of Formula V, wherein R^3 and R^5 are as defined in Formula I, may be chemospecifically mono-olefinated at C-8 (due to steric hindrance at C-25) with phosphine oxides of Formula VI, wherein R^1 , R^2 and R^6 are as defined in Formula I, under standard Horner-Wadsworth-Emmons (HWE) coupling conditions (see Posner, G. H. et al. *J. Org. Chem.* **1997**, *62*, 3299-3314). Therefore phosphine oxides VI are treated with a strong base, for example an alkyl lithium such as phenyl lithium, under anhydrous conditions in an inert atmosphere and solvent, for example tetrahydrofuran (THF), at temperatures in the range of about -60°C to about -90°C , suitably at about -78°C . To the resulting intermediate ylide is added a cold, preferably at about -78°C , solution of a ketone V in an inert solvent such as THF while maintaining the

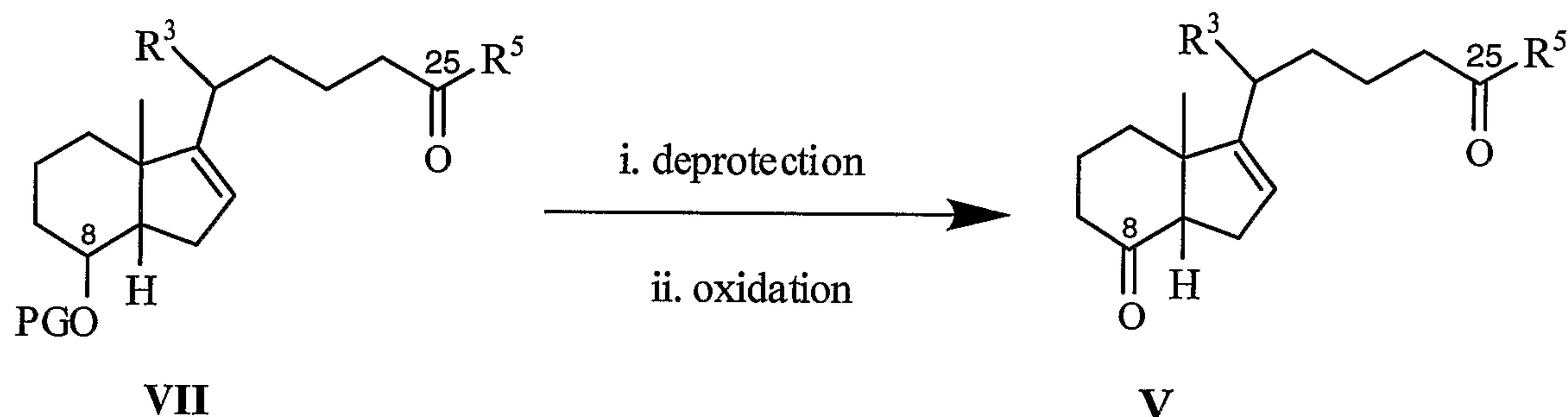
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anhydrous conditions. After removal of any protecting groups using standard chemistries (if needed), compounds of Formula II may be obtained.

Ketones of Formula V, wherein R^3 and R^5 are as defined in Formula I, may be prepared, for example, as shown in Scheme 3:

5

Scheme 3



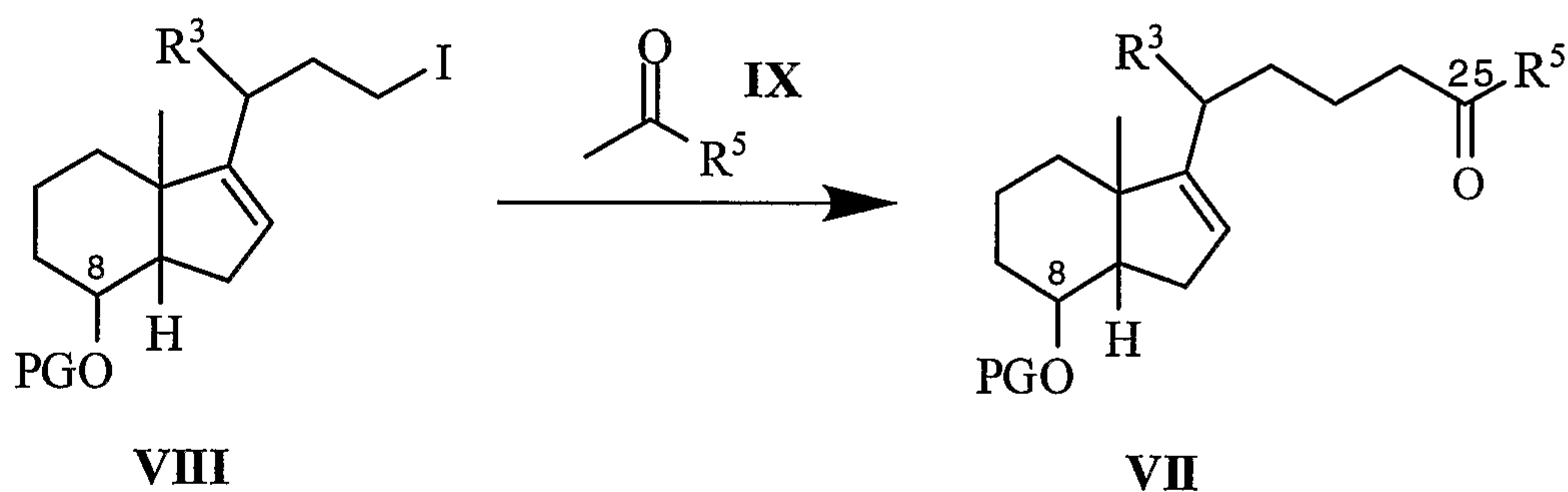
- 10 Suitably protected compounds of the Formula VII, wherein R^3 and R^5 are as defined in Formula I and PG is a suitable protecting group, are first deprotected and then oxidized to provide ketones V. For example, when PG is trialkyl silyl, such as triethyl silyl, deprotection may be affected by reacting compounds of Formula VII with tetrabutylammonium fluoride (TBAF) in an inert solvent, such as THF, and in an
- 15 inert atmosphere, suitably at about room temperature. Oxidation of the resulting alcohol may be performed, for example, using 4-methylmorpholine-N-oxide (NMO), or any other suitable oxidizing agent, in an inert solvent such as methylene chloride, under standard conditions.

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Compounds of Formula VII, wherein R^3 and R^5 are as defined in Formula I and PG is a suitable protecting group, may be obtained, for example, as shown in Scheme 4:

Scheme 4

5

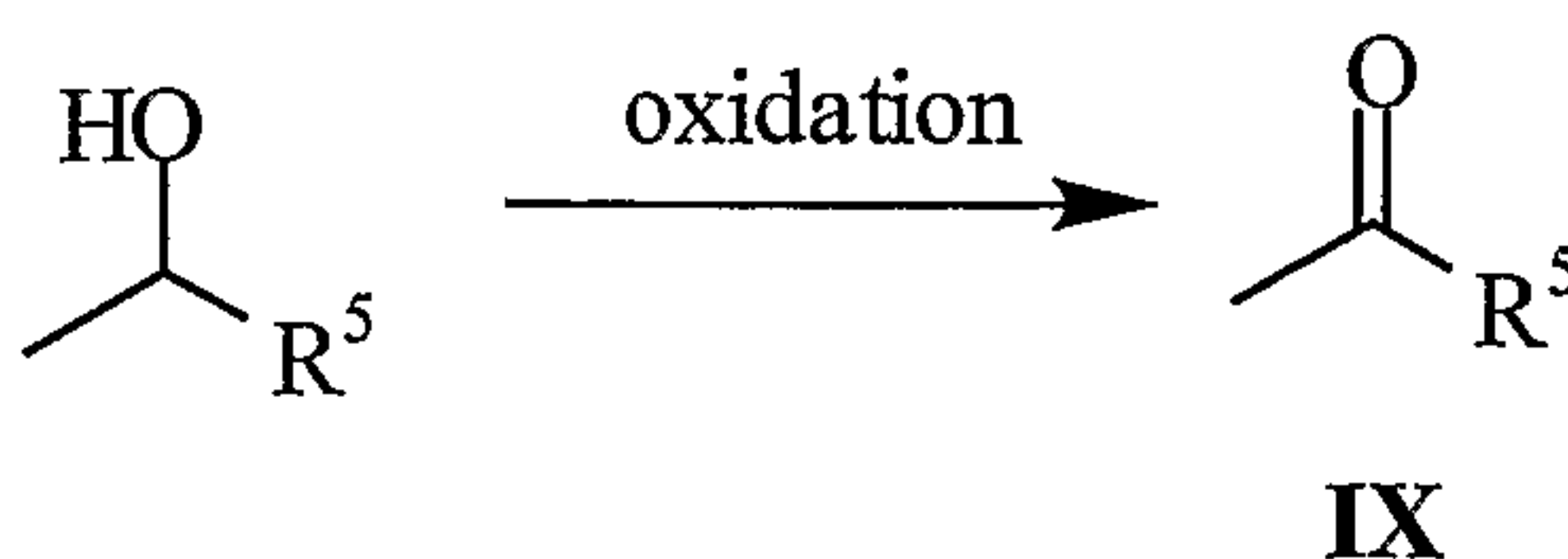


Compounds of Formula VIII, wherein R^3 is as defined in Formula I and PG is a suitable protecting group, may be reacted with the anion of compounds of Formula IX, wherein R^5 is as defined in Formula I under anhydrous conditions at temperatures in the range of about -60°C to about -90°C , suitably at about -78°C . The anions of compounds of Formula IX may be prepared by treating compounds of Formula IX with a strong base, for example an alkyl lithium such as n-butyl lithium or lithium diisopropylamide (LDA), under inert conditions and, in the presence of hexamethylphosphoramide (HMPA), for example, or N_1, N, N^1, N^1 -tetramethylethylenediamine (TMEDA).

Compounds of Formula IX, wherein R^5 is as defined in Formula I are either commercially available or may be prepared, for example, by the oxidation of the corresponding alcohols as shown in Scheme 5:

20

Scheme 5



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Examples of oxidizing agents include pyridium dichromate (PDC), m-chloroperbenzoic acid (mCPBA) and manganese dioxide.

The preparation of compounds of Formula VIII, wherein R^3 is as defined in Formula I and PG is a suitable protecting group, is known in the art.
5 Therefore compounds of Formula VIII may be prepared as described in Posner, G. H. *et al. J. Med. Chem.* **1999**, 42, 3425-3435.

The preparation of compounds of Formula VI, wherein R^1 , R^2 and R^6 are as defined in Formula I is known in the art. Therefore compounds of Formula VI may be prepared as described in Posner, G. H. *et al. J. Med. Chem.* **1992**, 35, 3280-
10 3287, the contents of which are incorporated herein by reference.

The preparation of enantiomerically pure compounds of Formula I and or II, may be accomplished by using enantiomerically pure compounds of Formula V and VI in the reaction shown in Scheme 2. In this reaction, a mixture of the $1\alpha,3\beta$ and $1\beta,3\alpha$ diastereomers is typically obtained, with the $1\alpha,3\beta$ diastereomer as the
15 major product. These diastereomers may be separated using chromatography, for example using high performance liquid chromatography (HPLC).

In some cases the chemistries outlined above may have to be modified, for instance by use of protective groups, to prevent side reactions due to reactive groups, such as reactive groups attached as substituents. This may be achieved by
20 means of conventional protecting groups, for example as described in "Protective Groups in Organic Chemistry" McOmie, J.F.W. Ed., Plenum Press, 1973 and in Greene, T.W. and Wuts, P.G.M., "Protective Groups in Organic Synthesis", John Wiley & Sons, 1991.

The formation of a desired compound salt is achieved using standard
25 techniques. For example, the neutral compound is treated with an acid or base in a suitable solvent and the formed salt is isolated by filtration, extraction or any other suitable method.

The formation of solvates of the compounds of the invention will vary depending on the compound and the solvate. In general, solvates are formed by
30 dissolving the compound in the appropriate solvent and isolating the solvate by cooling or using an antisolvent. The solvate is typically dried or azeotroped under ambient conditions.

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Prodrugs of the compounds of Formula I may be, for example, conventional esters formed with available hydroxy, thiol, amino or carboxyl group. For example, when R¹ and/or R² is OH it may be acylated using an activated acid in the presence of a base, and optionally, in inert solvent (e.g. an acid chloride in pyridine). Some common esters which have been utilized as prodrugs are phenyl esters, aliphatic (C₈-C₂₄) esters, acyloxymethyl esters, carbamates and amino acid esters.

A radiolabeled compound of the invention may be prepared using standard methods known in the art. For example, tritium may be incorporated into a compound of the invention using standard techniques, for example by hydrogenation of a suitable precursor to a compound of the invention using tritium gas and a catalyst. Alternatively, a compound of the invention containing radioactive iodo may be prepared from the corresponding trialkyltin (suitably trimethyltin) derivative using standard iodination conditions, such as [¹²⁵I] sodium iodide in the presence of chloramine-T in a suitable solvent, such as dimethylformamide. The trialkyltin compound may be prepared from the corresponding non-radioactive halo, suitably iodo, compound using standard palladium-catalyzed stannylation conditions, for example hexamethylditin in the presence of tetrakis(triphenylphosphine) palladium (0) in an inert solvent, such as dioxane, and at elevated temperatures, suitably 50-100°C.

IV. Uses

As hereinbefore mentioned, novel compounds of the Formulae I and II have been prepared. Accordingly, the present invention includes all uses of the compounds of the invention including their use in therapeutic methods and compositions for modulating cell proliferation, their use in diagnostic assays and their use as research tools and as starting materials and/or intermediates in the preparation of other chemical entities.

Inhibiting catabolism of calcitriol will lengthen the biological lifetime of this hormone and thus allow smaller amounts of it to be used for effective human chemotherapy; such smaller dosing will avoid, or at least minimize, the hypercalcemic toxicity associated with medicinal use of calcitriol. Selectively inhibiting the cytochrome P450 enzymatic pathway, through which calcitriol is

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catabolized (mainly via C-24 hydroxylation), is one important way to prolong the lifetime of this hormone. Therefore, the compounds of Formula I were tested *in vitro*, using a standard protocol, for their ability to inhibit specifically CYP24, responsible for 24-hydroxylation of calcitriol. Antimycotic ketoconazole, a drug used clinically
5 for chemotherapy of human prostate cancer (Trachtenberg, J. *et al.* J. Urol. **1984**, J32, 61-63), was used as a control standard for inhibition of CYP24. Selected compounds of Formula I were more potent than ketoconazole in inhibiting CYP24 activity. These compounds showed little to no inhibition of the enzymes CYP27A1 and CYP27B1, indicating that they can selectively inhibit CYP24 activity.

10 Selected compounds of Formula I have also been shown to have *in vitro* antiproliferative activity in murine keratinocytes. Also, in standard hypercalcemia assays, selected compounds of Formula I did not increase the levels of calcium in the urine of a rat after they were administered orally to the rats daily for one week. At similar doses, calcitriol causes a significant increase in calcium levels
15 in the urine.

The compounds of Formula I are CYP24 modulators and are useful in modulating CYP24 activity, including the inhibition of CYP24 activity, for the treatment of various conditions such as cell proliferative disorders. Accordingly, the invention provides a method of modulating CYP24 activity by administering an
20 effective amount of a compound of Formula I to a cell or animal in need thereof. In a further aspect, the invention provides a method of inhibiting CYP24 activity by administering an effective amount of a compound of Formula I to a cell or animal in need thereof. The present invention also includes the use of a compound of Formula I to modulate, preferably to inhibit, CYP24 activity and a use of a compound of
25 Formula I to prepare a medicament to modulate, preferably to inhibit, CYP24 activity.

By selectively modulating CYP24, the enzyme that metabolizes $1\alpha,25$ -dihydroxy vitamin D_3 , the levels of $1\alpha,25$ -dihydroxy vitamin D_3 will be modulated. Diseases that benefit from a modulation of the levels of $1\alpha,25$ -dihydroxy vitamin D_3
30 can therefore be treated using a modulator of CYP24. By acting preferentially on CYP24, side effects caused by interaction with other enzymes and receptors will be reduced. Accordingly, the present invention provides a method for treating diseases

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which benefit from a modulation of the levels of $1\alpha,25$ -dihydroxy vitamin D_3 comprising administering an effective amount of a compound of Formula I to a cell or animal in need thereof. The invention also includes the use of a compound of Formula I to modulate the levels of $1\alpha,25$ -dihydroxy vitamin D_3 . Further, the invention includes a use of a compound of Formula I to prepare a medicament to modulate the levels of $1\alpha,25$ -dihydroxy vitamin D_3 .

Inhibition of CYP24, will inhibit the catabolism $1\alpha,25$ -dihydroxy vitamin D_3 which will lengthen the biological lifetime of this hormone and allow smaller amounts of it to be used for effective disease treatment. Such smaller dosing will avoid, or at least minimize, the hypercalcemic toxicity associated with medicinal use of $1\alpha,25$ -dihydroxy vitamin D_3 (calcitriol). Therefore, in an embodiment, the present invention provides a method for treating diseases which benefit from inhibiting the catabolism of $1\alpha,25$ -dihydroxy vitamin D_3 comprising administering an effective amount of a compound of Formula I to a cell or animal in need thereof. The invention also includes the use of a compound of Formula I to inhibit the catabolism of $1\alpha,25$ -dihydroxy vitamin D_3 . Further, the invention includes a use of a compound of Formula I to prepare a medicament to inhibit the metabolism of $1\alpha,25$ -dihydroxy vitamin D_3 .

Diseases which may benefit for a modulation in the levels of $1\alpha,25$ -dihydroxy vitamin D_3 include, but are not limited to:

- (i) in the parathyroid - hyper- and hypo-parathyroidism, Pseudohypoparathyroidism, Secondary hyperparathyroidism;
- (ii) in the pancreas - diabetes;
- (iii) in the thyroid - medullary carcinoma;
- (iv) in the skin - psoriasis, wound healing;
- (v) in the lung - sarcoidosis and tuberculosis;
- (vi) in the kidney - chronic renal disease, hypophosphatemic VDDR, vitamin D dependent rickets;
- (vii) in the bone - anticonvulsant treatment, fibrogenesis imperfecta ossium, osteitis fibrosa cystica, osteomalacia, osteoporosis, osteopenia, osteosclerosis, renal osteodystrophy, rickets;

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(viii) in the intestine - glucocorticoid antagonism, idopathic hypercalcemia, malabsorption syndrome, steatorrhea, tropical sprue.

In one aspect, the present invention provides a method for modulating cell proliferation comprising administering an effective amount of a compound of Formula I to a cell or animal in need thereof. Preferably, the invention provides a method of inhibiting cell proliferation comprising administering an effective amount of a compound of Formula I to a cell or animal in need thereof. The present invention also includes a use of a compound of Formula I in order to modulate, preferably to inhibit, cell proliferation. The present invention further includes a use of a compound of Formula I to prepare a medicament to modulate, preferably to inhibit, cell proliferation. In particular, the method of the invention is useful in inhibiting the proliferation of abnormal but not normal cells. Abnormal cells include any type of cell that is causative of or involved in a disease or condition and wherein it is desirable to modulate or inhibit the proliferation of the abnormal cell to treat the disease or condition. Examples of abnormal cells include malignant or cancerous cells as well as cell that over-proliferate in inflammatory conditions such as psoriasis. In an embodiment of the invention, the cell proliferative disorder is cancer, in particular cancer of the breast, prostate and lung.

While the compounds of the invention may act by modulating CYP24 activity, one of skill in the art will appreciate that other modes or mechanisms of action for the compounds of Formula I are possible.

One skilled in the art can determine which compounds of Formula I would have therapeutic utility, for example, in inhibiting cell proliferation in any type of cancer or cell proliferative disorder. Compounds may be examined for their efficacy in inhibiting cell growth in cell proliferation assays such as inhibition of growth of murine keratinocyte cells (cell line PE) as described in Example 13 herein, and for the inhibition of TPA-induced ornithine decarboxylase (ODC) activity as described in US. Patent No. 5,830,885, the contents of which are incorporated herein by reference. The compounds of Formula I may also be screened for their propensity to cause hypercalcemia using the method described in Example 14 herein. Compounds showing hypercalcemia are not desirable.

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In addition to cancer, the compounds of Formula I are useful in treating other conditions involving aberrant or abnormal cell proliferation. Other cell proliferative disorders that may be treated by the present invention include inflammatory diseases, allergies, autoimmune disease, graft rejection, psoriasis, restenosis, arteriosclerosis, and any other disorder wherein it is desirable to inhibit, prevent or suppress cell growth. Compounds of Formula I may be tested for their efficacy in a particular cell proliferation disorder using assays and techniques known to those of skill in the art. For example, the following references provide assays for various conditions: Rheumatoid Arthritis: "Regulation of IL-15 - Simulated TNF-alpha Production by Rolipram", Journal of Immunology (1999) volume 163 page 8236 by C. S. Kasyapa et al.; Allergy: "A novel Lyn-Binding Peptide Inhibitor Blocks Eosinophil Differentiation, Survival, and Airway eosinophilic inflammation". Journal of Immunology (1999) volume 163 page 939 by T. Adachi et al.; Psoriasis: Journal of Immunology (2000) volume 165 page 224 "Inhibition of Keratinocyte apoptosis by IL-15: a new parameter in the pathogenesis of psoriasis" by R. Üchert; and Psoriasis: International Archives of allergy and Immunology (2000) Volume 123 page 275. "T-cell receptor mimic peptides and their potential application in T-cell mediated disease" by A. H. Enk.

The compounds of Formula I are preferably formulated into pharmaceutical compositions for administration to human subjects in a biologically compatible form suitable for administration *in vivo*. Accordingly, in another aspect, the present invention provides a pharmaceutical composition comprising a compound of Formula I, or a pharmaceutically acceptable salt, hydrate, solvate or prodrug thereof, in admixture with a suitable diluent or carrier.

The compositions containing the compounds of Formula I can be prepared by known methods for the preparation of pharmaceutically acceptable compositions which can be administered to subjects, such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985). On this basis, the compositions include, albeit not exclusively, solutions of the substances in association with one or more pharmaceutically

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acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

The compounds of Formula I may be used pharmaceutically in the form of the free base, in the form of salts, solvates and as hydrates. All forms are within the scope of the invention. Acid and basic addition salts may be formed with the compounds of the invention (i.e. compounds of Formulae I and II) for use as sources of the free base form even if the particular salt per se is desired only as an intermediate product as, for example, when the salt is formed only for the purposes of purification and identification. All salts that can be formed with the compounds of the invention are therefore within the scope of the present invention.

In accordance with the methods of the invention, the described compounds of Formula I, or salts, solvates, hydrates or prodrugs thereof, may be administered to a patient in a variety of forms depending on the selected route of administration, as will be understood by those skilled in the art. The compounds of Formula I may be administered, for example, by oral, parenteral, buccal, sublingual, nasal, rectal, patch, pump or transdermal administration and the pharmaceutical compositions formulated accordingly. Parenteral administration includes intravenous, intraperitoneal, subcutaneous, intramuscular, transepithelial, nasal, intrapulmonary, intrathecal, rectal and topical modes of administration. Parenteral administration may be by continuous infusion over a selected period of time.

A compound of Formula I may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsules, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the compound of Formula I may be incorporated with excipient and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like.

A compound of Formula I may also be administered parenterally. Solutions of a compound of Formula I can be prepared in water suitably mixed with a surfactant such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, DMSO and mixtures thereof with or without alcohol, and in oils. Under ordinary conditions of storage and use, these preparations

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contain a preservative to prevent the growth of microorganisms. A person skilled in the art would know how to prepare suitable formulations. Conventional procedures and ingredients for the selection and preparation of suitable formulations are described, for example, in Remington's Pharmaceutical Sciences (1990 - 18th edition) and in The United States Pharmacopeia: The National Formulary (USP 24 NF19) published in 1999.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersion and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists.

Compositions for nasal administration may conveniently be formulated as aerosols, drops, gels and powders. Aerosol formulations typically comprise a solution or fine suspension of the active substance in a physiologically acceptable aqueous or non-aqueous solvent and are usually presented in single or multidose quantities in sterile form in a sealed container, which can take the form of a cartridge or refill for use with an atomizing device. Alternatively, the sealed container may be a unitary dispensing device such as a single dose nasal inhaler or an aerosol dispenser fitted with a metering valve which is intended for disposal after use. Where the dosage form comprises an aerosol dispenser, it will contain a propellant which can be a compressed gas such as compressed air or an organic propellant such as fluorochlorohydrocarbon. The aerosol dosage forms can also take the form of a pump-atomizer.

Compositions suitable for buccal or sublingual administration include tablets, lozenges, and pastilles, wherein the active ingredient is formulated with a carrier such as sugar, acacia, tragacanth, or gelatin and glycerine. Compositions for rectal administration are conveniently in the form of suppositories containing a conventional suppository base such as cocoa butter.

The compounds of Formula I, or salts, solvates, hydrates or prodrugs thereof, may be administered to an animal alone or in combination with pharmaceutically acceptable carriers, as noted above, the proportion of which is determined by the solubility and chemical nature of the compound, chosen route of administration and standard pharmaceutical practice.

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The dosage of the compounds of Formula I and/or compositions of the invention can vary depending on many factors such as the pharmacodynamic properties of the compound, the mode of administration, the age, health and weight of the recipient, the nature and extent of the symptoms, the frequency of the treatment
5 and the type of concurrent treatment, if any, and the clearance rate of the compound in the animal to be treated. One of skill in the art can determine the appropriate dosage based on the above factors. The compounds of Formula I may be administered initially in a suitable dosage that may be adjusted as required, depending on the clinical response. For *ex vivo* treatment of cells over a short period,
10 for example for 30 minutes to 1 hour or longer, higher doses of compound may be used than for long term in vivo therapy.

The compounds of Formula I, or salts, solvates, hydrates or prodrugs thereof, can be used alone or in combination with other agents that modulate CYP24 activity or in combination with other types of treatment (which may or may not
15 modulate CYP24) for cell proliferative disorders or other disorders that benefit from a modulation in the levels of $1\alpha,25$ -dihydroxy vitamin D_3 and/or an inhibition of the catabolism of $1\alpha,25$ -dihydroxy vitamin D_3 . Preferably the compounds of Formula I are administered in combination with $1\alpha,25$ -dihydroxy vitamin D_3 (calcitriol) or other vitamin D receptor agonists. Inhibiting catabolism of vitamin D receptor agonists
20 will lengthen the biological lifetime or efficacy of these therapies and thus to allow smaller amounts of the drug to be used for effective human chemotherapy; such smaller dosing will avoid, or at least minimize, the hypercalcemic toxicity associated with medicinal use of calcitriol or other vitamin D receptor agonists. The present invention therefore provides a method of increasing the efficacy of a vitamin D
25 receptor agonist, preferably $1\alpha,25$ -dihydroxy vitamin D_3 (calcitriol), comprising co-administering an effective amount of a compound of Formula I and an effective amount of the vitamin D receptor agonist, preferably $1\alpha,25$ -dihydroxy vitamin D_3 (calcitriol). Further the invention includes a use of a compound of Formula I to increase the efficacy of a vitamin D receptor agonist, preferably $1\alpha,25$ -dihydroxy
30 vitamin D_3 (calcitriol), and a use of a compound of Formula I to prepare a

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medicament to increase the efficacy of a vitamin D receptor agonist, preferably $1\alpha,25$ -dihydroxy vitamin D_3 (calcitriol).

In a further aspect of the present invention, the compounds of Formula I, or salts, solvates, hydrates or prodrugs thereof, may be used in combination with
5 other therapies and therapeutics to treat dermatological disorders, bone disorders, thyroid disorders, wound healing and osteoporosis.

In addition to the above-mentioned therapeutic uses, the compounds of the invention are also useful in diagnostic assays, screening assays and as research tools.

10 In diagnostic assays the compounds of the invention (including compounds of Formula II, which have also been shown to inhibit CYP24, but are calcemic) may be useful in identifying or detecting a cell proliferative disorder. In such an embodiment, the compounds of the invention may be radiolabelled (as hereinbefore described) and contacted with a population of cells. The presence of the
15 radiolabelled on the cells may indicate a cell proliferative disorder.

In screening assays, the compounds of the invention (including compounds of Formula II) may be used to identify other compounds that modulate cell proliferation or CYP24 activity. As research tools, the compounds of the invention may be used in receptor binding assays and assays to study the localization
20 of CYP24. In such assays, the compounds may also be radiolabelled.

The following non-limiting examples are illustrative of the present invention:

EXAMPLES

25 Materials and Methods

Unless otherwise noted, all reactions were performed in oven-dried glassware stirred under an atmosphere of ultra-high-purity argon. THF was distilled from Na/benzophenone ketyl and CH_2Cl_2 distilled from CaH_2 immediately prior to use. Organolithiums were titrated prior to use following known methods (Suffert, J.
30 *J. Org. Chem.* **1989**, 54, 509–510). Methylene chloride (CH_2Cl_2) and triethylamine (Et_3N) were distilled from calcium hydride prior to use. All other reagents were used as received from commercial suppliers. Analytical TLC analysis was conducted on

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precoated glass-backed silica gel plates (Merck Kieselgel 60 F₂₅₄, 250 mm thickness) and visualized with *p*-anisaldehyde or KMnO₄ stains. Column chromatography was performed using short path silica gel (particle size < 230 mesh) or flash silica gel (particle size 230–400 mesh). Preparative-plate chromatography was performed using silica-gel-coated glass preparative plates (500–1000 μm) from Analtech and analyzed by UV. High-performance liquid chromatography (HPLC) was carried out using a Rainin HPLX system equipped with two 25-mL/min preparative pump heads using Rainin Dynamax 10-mm x 250-mm (semipreparative) columns packed with 60 Å silica gel (8 μm pore size) as C-18-bonded silica and a Rainin Dynamax UV-C dual-beam variable-wavelength detector set at 265 nm. Yields are reported for pure products (>95% based on their chromatographic and spectroscopic homogeneity) and are unoptimized. Optical rotations were measured at the Na line using a Perkin-Elmer 141 Polarimeter. Nuclear magnetic resonance (NMR) spectra were obtained on a Varian XL-400 spectrometer operating at 400 MHz for ¹H, and 100 MHz for ¹³C. Chemical shifts are reported in ppm (δ) and are referenced to CDCl₃ (7.26 ppm for ¹H and 77.0 ppm for ¹³C), and tetramethylsilane (TMS, 0.00 ppm for ¹H). Ultraviolet (UV) spectra were obtained using a Cary Bio 400 spectrophotometer at ambient temperature. Infrared spectra (IR) spectra were obtained using a Perkin Elmer 1600 Series FT-IR instrument. Absorption bands are reported in wavenumbers (cm⁻¹). Low and high resolution mass spectra (LRMS and HRMS) were obtained with electronic of chemical ionization (EI or CI) at the mass spectrometry facility at the Ohio State University on a Micromass QTOF Electrospray mass spectrometer.

Example 1: Preparation of t-Butyl Ketone VII (R³ = CH₃, R⁵ = t-Butyl, PG = TES)

A 15 mL round-bottom flask was charged with triisopropylamine (42 mg, 0.41 mmol, 7.4 eq. - distilled over calcium hydride prior to use) and 2mL distilled THF. This solution was cooled to -78°C, and *n*-butyllithium (250 μL of 1.6M solution, 0.43 mmol, 7.2 eq.) was added *via* syringe. Pinacolone (**IX**, R⁵ = t-Butyl) (39 mg, 0.39 mmol, 7.0 eq. - dried over potassium carbonate and activated molecular sieves for 24 hours immediately prior to use) was dissolved in 1 mL of distilled THF and cooled to -78°C at which point it was added to the reaction flask *via* cannula. The reaction was left to stir for 30 minutes. Hexamethylphosphoramide (HMPA, 250 μL) was then

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added *via* syringe and the reaction mixture was allowed to stir for an additional 15 minutes. A solution of iodide (-)-**VIII** ($R^3 = \text{CH}_3$, PG = triethylsilyl (TES)) (25 mg, 0.06 mmol) in 1 mL THF was cooled to -78°C and added to the reaction mixture *via* cannula. The reaction mixture was stirred at -78°C for two hours and then warmed to -41°C in a dry ice/acetonitrile bath where it was allowed to warm to room temperature over the course of two hours and to stir for an additional 6 hours. The resulting yellow solution was quenched with 2 mL water, extracted with ethyl acetate (3 x 25 mL), dried over MgSO_4 , concentrated, and purified using silica gel column chromatography (0-20% ethyl acetate/ petroleum ether) to give a colorless oil (18 mg, 76%). ^1H NMR (400 MHz, CDCl_3) δ 5.26 (t, $J = 1.4$ Hz, 1H), 4.11 (d, $J = 2.0$ Hz, 1H), 2.46-1.25 (m, 16H), 1.13 (s, 9H), 1.00 (s, 3H), 0.98-0.96 (d, $J = 6.8$ Hz, 3H), 0.97-0.93 (t, $J = 8$ Hz, 9H), 0.59-0.53 (q, $J = 7.8$ Hz, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 216.1, 160.2, 119.7, 69.0, 55.1, 46.7, 44.1, 36.6, 36.2, 35.8, 35.0, 31.7, 30.7, 26.4, 22.3, 22.0, 18.7, 18.1, 6.9, 4.9; IR (neat) 2956, 1708, 1607, 1456, 1366, 1235, 1143, 1082, 1029, 972, 725 cm^{-1} ; $[\alpha]_D = +19.4$; HRMS calcd for $\text{C}_{26}\text{H}_{48}\text{O}_2\text{SiNa}$ $[\text{M}+\text{Na}]$: 443.3321, found: 443.3318.

Example 2: Preparation of CD-ring Ketone V ($R^3 = \text{CH}_3$, $R^5 = \text{t-Butyl}$)

A 15 mL round-bottom flask was charged with *tert*-butyl ketone **VII** ($R^3 = \text{CH}_3$, $R^5 = \text{t-Butyl}$, PG = TES) (18 mg, 0.4 mmol) dissolved in 5 mL distilled THF. Tetrabutylammonium fluoride hydrate (TBAF, 112 mg, 10 eq.) and 4Å molecular sieves (100 mg) were added to the reaction flask and this solution was left to stir at reflux for four hours. Additional portions of TBAF and sieves were added every four hours until starting material was no longer visible by analytical thin layer chromatography (TLC). The reaction solution was filtered through a plug of silica gel using ethyl acetate as the eluent to remove excess TBAF and molecular sieves. This solution was concentrated and a 10 mL round-bottom flask was charged with the resulting material dissolved in 5 mL distilled dichloromethane (CH_2Cl_2). To this solution was added 4Å molecular sieves (20 mg), 4-methylmorpholine-N-oxide (NMO, 10 mg, 0.09 mmol, 2 eq.) and a catalytic amount of tetrapropylammonium perruthenate (TPAP). After stirring for 1 hour, TLC showed complete consumption

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of starting material. The reaction solution was filtered through a plug of silica gel using ethyl acetate as the eluent to remove TPAP and molecular sieves. This solution was concentrated and purified using silica gel column chromatography (20% ethyl acetate/ petroleum ether) to give a colorless oil (9 mg, 71%). ^1H NMR (400 MHz, CDCl_3) δ 5.29-5.28 (m, $J = 1.6$ Hz, 1H), 2.87-2.82 (dd, $J = 10.6, 6.6$ Hz, 1H), 2.47-1.31 (m, 16H), 1.12 (s, 9H), 1.05-1.04 (d, $J = 6.8$ Hz, 3H), 0.80 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 215.9, 211.1, 157.8, 120.3, 63.1, 53.8, 44.1, 40.5, 36.5, 36.0, 34.4, 32.9, 27.1, 26.4, 24.0, 21.9, 21.7, 17.25; IR (neat) 2955, 1702, 1461, 1367 cm^{-1} ; $[\alpha]_{\text{D}} = 15.6$; HRMS calcd for $\text{C}_{20}\text{H}_{32}\text{O}_2\text{Na}$ $[\text{M}+\text{Na}]$: 327.2300, found: 327.2302.

10 **Example 3: Preparation of II(a) and II(b)**

Anhydrous phosphine oxide ($\pm$)-VI ($\text{R}^1, \text{R}^2 = \text{O}-t\text{-Butyldimethylsilyl}$ (OTBDMS)) (79 mg, 0.14 mmol, 1.4 eq.) was dissolved in 2.5 mL distilled THF and cooled to -78°C . Phenyllithium (88 μL of a 1.7M solution in cyclohexane-ether, 0.15 mmol, 1.5 eq.) was added dropwise *via* syringe resulting in a deep red color. This solution was left to stir for 20 minutes. Anhydrous CD-ring ketone (+)-V ($\text{R}^3 = \text{CH}_3$, $\text{R}^5 = t\text{-Butyl}$) (31 mg, 0.10 mmol) was dissolved in 1.5 mL distilled THF and cooled to -78°C . This solution was then added to the reaction mixture *via* cannula, and the red color persisted. This solution was stirred at -78°C in the dark for 7 hours at which point it was quenched with saturated potassium carbonate (1 mL) and potassium sodium tartrate (2 mL of a 2M solution). The product was extracted with ethyl acetate (4 x 60 mL), dried using MgSO_4 , filtered, concentrated and purified using silica gel column chromatography (3-10% ethyl acetate/hexanes buffered with 1% Et_3N) to give a colorless oil. A 5 mL round-bottom flask was charged with this oil dissolved in 2.5 mL THF, TBAF hydrate (241 mg, 0.92 mmol, 14 eq.), 4Å molecular sieves (100 mg), and 3 drops of Et_3N sequentially. This solution was left to stir in the dark at room temperature for 8 hours. The reaction mixture was purified directly using silica gel column chromatography (99% ethyl acetate buffered with 1% Et_3N) to give II(a) and II(b) (15 mg, 38%) as a mixture of diastereomers (1:3.5) which were separated using normal phase HPLC chromatography (90% ethyl acetate/hexanes, buffered with 1% Et_3N) to give 2 mg (5%, 3% overall) of the natural A-ring isomer II(b). II(a) (1 β ,

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3 α): ^1H NMR (400 MHz, CDCl_3) δ 6.41-6.38 (d, $J = 11.2$ Hz, 1H), 6.11-6.08 (m, $J = 11.2$ Hz, 1H), 5.32 (m, 1H), 5.30 (m, 1H), 5.02 (m, 1H), 4.44 (m, 1H), 4.22 (m, 1H), 2.83-2.80 (dm, $J = 11.6$ Hz, 1H), 2.65-1.33 (m, 19H), 1.13 (s, 9H), 1.03-1.01 (d, $J = 6.8$ Hz, 3H), 0.67 (s, 3H); $[\alpha]_D^{25} = 4.3$; HRMS calcd for $\text{C}_{29}\text{H}_{44}\text{O}_3\text{Na}$ $[\text{M}+\text{Na}]$:

5 463.3188, found: 463.3163; **II(b)** (1 α , 3 β) ^1H NMR (400 MHz, CDCl_3) δ 6.39-6.36 (d, $J = 11.2$ Hz, 1H), 6.12-6.09 (m, $J = 11.2$ Hz, 1H), 5.34-5.33 (t, $J = 1.6$ Hz, 1H), 5.30-5.29 (t, $J = 1.4$ Hz, 1H), 5.01 (m, 1H), 4.45 (m, 1H), 4.24 (m, 1H), 2.83-2.79 (dm, $J = 12$ Hz, 1H), 2.62-2.58 (dm, $J = 12.8$ Hz, 1H), 2.47-1.33 (m, 18H), 1.12 (s, 9H), 1.03-1.01 (d, $J = 6.8$ Hz, 3H), 0.68 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ
 10 216.1, 159.6, 147.7, 142.6, 132.9, 124.9, 120.3, 116.8, 111.6, 70.6, 66.9, 58.3, 50.1, 45.2, 42.8, 36.6, 36.1, 35.3, 32.7, 29.7, 29.4, 28.8, 26.4, 23.6, 21.9, 21.6, 16.9; IR (neat) 3855, 3752, 3677, 3386, 2924, 2846, 1702, 1654, 1561, 1432, 1362, 1209, 1051, 1010, 846 cm^{-1} ; UV (MeOH) λ_{max} 264 nm (ϵ 8157); $[\alpha]_D^{25} = +17.2$; HRMS calcd for $\text{C}_{29}\text{H}_{44}\text{O}_3\text{Na}$ $[\text{M}+\text{Na}]$: 463.3188, found: 463.3175. It was possible to
 15 separate the diastereomers using normal phase HPLC although optimal separation was obtained only by using very small injections ($\sim 200\mu\text{g}$ or less).

Example 4: Preparation of Compounds I(a) and I(b)

Anhydrous phosphine oxide ($\pm$)-**VI** ($\text{R}^1, \text{R}^2 = \text{O-OTBDMS}$) (89 mg, 0.15 mmol, 2 eq.) was dissolved in 2.5 mL distilled THF and cooled to -78°C . Phenyllithium (93 μL of
 20 a 1.8M solution in cyclohexane-ether, 0.17 mmol, 2.2 eq.) was added dropwise *via* syringe resulting in a deep red color. This solution was left to stir for 20 minutes. Anhydrous CD-ring ketone (+)-**II** ($\text{R}^3 = \text{CH}_3$, $\text{R}^5 = \text{t-Butyl}$) (23 mg, 0.08 mmol) was dissolved in 1.5 mL distilled THF and cooled to -78°C . This solution was then added to the reaction mixture *via* cannula, and the red color persisted. This solution was
 25 stirred at -78°C in the dark for 7 hours at which point it was quenched with saturated potassium carbonate (1 mL) and potassium sodium tartrate (2 mL of a 2M solution). The product was extracted with ethyl acetate (4 x 60 mL), dried using MgSO_4 , filtered, concentrated and purified using silica gel column chromatography (3-10% ethyl acetate/hexanes buffered with 1% Et_3N) to give a colorless oil. A 5 mL round-
 30 bottom flask was charged with a portion of this material (27 mg, 0.04 mmol)

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dissolved in 2 mL of anhydrous pyridine. Hydroxylamine hydrochloride (**III**, $R^4 = H$) (51 mg, 0.74 mmol, 20 eq.) was added and the reaction was allowed to stir in the dark at room temperature for 24 hours at which point TLC analysis showed complete consumption of starting material and the appearance of a new, more polar product.

5 This material was purified directly using silica gel column chromatography (10% ethyl acetate/hexanes buffered with 1% Et₃N) to give a colorless oil. A 5 mL round-bottom flask was charged with this oil dissolved in 2.5 mL THF, TBAF hydrate (135 mg, 0.52 mmol, 14 eq.), 4Å molecular sieves (60 mg), and 3 drops of Et₃N sequentially. This solution was left to stir in the dark at room temperature for 8 hours.

10 The reaction mixture was purified directly using silica gel column chromatography (99% ethyl acetate buffered with 1% Et₃N) to **I(a)** and **I(b)** (14 mg, 61%) as a mixture of diastereomers (1:3.5) which were separated using reverse phase HPLC chromatography (38% H₂O/acetonitrile) to give 2.3 mg (16%, 5% overall) of **I(a)** and 4.0 mg (29%, 10% overall) of **I(b)**. **I(a)** (1β, 3α): ¹H NMR (400 MHz, CDCl₃) δ

15 6.40-6.37 (d, $J = 11.2$ Hz, 1H), 6.10-6.07 (m, $J = 11.2$ Hz, 1H), 5.32 (m, 1H), 5.29 (m, 1H), 5.02 (m, 1H), 4.45 (m, 1H), 4.22 (m, 1H), 2.83-2.80 (dm, $J = 11.6$ Hz, 1H), 2.64-2.60 (dd, $J = 12.8$ Hz, $J = 3.4$ Hz, 1H), 2.38-1.24 (m, 18H), 1.11 (s, 9H), 1.03-1.01 (d, $J = 6.8$ Hz, 3H), 0.68 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 167.7, 159.7, 147.1, 142.7, 132.7, 125.0, 120.3, 116.8, 112.7, 71.4, 66.7, 58.4, 50.0, 45.4, 42.8,

20 37.4, 37.2, 35.3, 32.6, 29.4, 28.8, 27.7, 26.1, 24.3, 23.6, 21.4, 17.0; $[\alpha]_D = -3.7$; HRMS calcd for C₂₉H₄₅NO₃Na [M+Na]: 478.3297, found: 478.3336; **I(b)** (1α, 3β) ¹H NMR (400 MHz, CDCl₃) δ 6.39-6.36 (d, $J = 11.6$ Hz, 1H), 6.12-6.09 (m, $J = 11.6$ Hz, 1H), 5.34-5.33 (t, $J = 1.6$ Hz, 1H), 5.30 (m, 1H), 5.02-5.01 (m, $J = 1.6$ Hz, 1H), 4.44 (m, 1H), 4.24 (m, 1H), 2.83-2.79 (dm, $J = 12$ Hz, 1H), 2.63-2.59 (dm, $J = 13.6$ Hz, 1H), 2.38-1.69 (m, 18H), 1.11 (s, 9H), 1.03-1.02 (d, $J = 6.8$ Hz, 3H), 0.69 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 167.7, 159.7, 147.6, 142.7, 132.9, 125.0, 120.3, 116.8, 111.7, 70.7, 66.8, 58.4, 50.0, 45.2, 42.8, 37.4, 37.2, 35.3, 32.6, 29.4, 28.8, 27.7, 26.1, 24.3, 23.6, 21.4, 17.0; IR (neat) 3331, 2955, 2919, 2837, 1661, 1461, 1367,

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1349, 1049, 932, 797, 756 cm^{-1} ; UV (MeOH) λ_{max} 266 nm (ϵ 8502); $[\alpha]_{\text{D}} = +4.8$; HRMS calcd for $\text{C}_{29}\text{H}_{45}\text{NO}_3\text{Na}$ $[\text{M}+\text{Na}]$: 478.3297, found: 478.3336.

Example 5: Preparation of Compounds I(c) and I(d)

Anhydrous phosphine oxide ($\pm$)-**VI** ($\text{R}^1, \text{R}^2 = \text{OTBDMS}$, $\text{R}^6 = =\text{CH}_2$) (89 mg, 0.15 mmol, 2 eq.) was dissolved in 2.5 mL distilled THF and cooled to -78°C . Phenyllithium (93 μL of a 1.8M solution in cyclohexane-ether, 0.17 mmol, 2.2 eq.) was added dropwise *via* syringe resulting in a deep red color. This solution was left to stir for 20 minutes. Anhydrous CD-ring ketone (+)-**II** ($\text{R}^3 = \text{CH}_3$, $\text{R}^5 = \text{t-Butyl}$) (23 mg, 0.08 mmol) was dissolved in 1.5 mL distilled THF and cooled to -78°C . This solution was then added to the reaction mixture *via* cannula, and the red color persisted. This solution was stirred at -78°C in the dark for 7 hours at which point it was quenched with saturated potassium carbonate (1 mL) and potassium sodium tartrate (2 mL of a 2M solution). The product was extracted with ethyl acetate (4 x 60 mL), dried using MgSO_4 , filtered, concentrated and purified using silica gel column chromatography (3-10% ethyl acetate/hexanes buffered with 1% Et_3N) to give a colorless oil. A 5 mL round-bottom flask was charged with a portion of this material (12 mg, 0.02 mmol) dissolved in 1.5 mL of anhydrous pyridine. Methoxylamine hydrochloride (**III**, $\text{R}^4 = \text{CH}_3$) (27 mg, 0.33 mmol, 20 eq.) was added and the reaction was allowed to stir in the dark at room temperature. Additional portions of methoxylamine were added (60 eq. Total) and the reaction was stirred for a total of 36 hours. The starting material and the product were very similar in polarity and TLC analysis was not particularly useful for following the reaction. This material was purified directly using silica gel column chromatography (3% ethyl acetate/hexanes buffered with 1% Et_3N) to give a colorless oil. A 5 mL round-bottom flask was charged with this oil dissolved in 2mL THF, TBAF hydrate (59 mg, 0.22 mmol, 14 eq.), 4Å molecular sieves (60 mg), and 1 drop of Et_3N sequentially. This solution was left to stir in the dark at room temperature for 24 hours. The reaction mixture was purified directly using silica gel column chromatography (99% ethyl acetate buffered with 1% Et_3N) to give **I(c)** and **I(d)** (6 mg, 59%) as a mixture of diastereomers (1:3.5) which were separated using reverse phase HPLC chromatography (15% H_2O /acetonitrile) to give 1.4 mg (19%, 6% overall) of **I(c)** and

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2.8 mg (37%, 12% overall) of **I(d)**. **I(c)** (1 β , 3 α): ^1H NMR (400 MHz, CDCl_3) δ 6.41-6.38 (d, J = 11.2 Hz, 1H), 6.11-6.08 (m, J = 11.6 Hz, 1H), 5.32 (m, 1H), 5.29 (t, J = 1.2 Hz, 1H), 5.02 (m, 1H), 4.46-4.44 (m, 1H), 4.24-4.20 (m, 1H), 3.78 (s, 3H), 2.84-2.80 (dm, J = 11.8 Hz, 1H), 2.65-2.60 (dd, J = 13.0 Hz, 3.8 Hz, 1H), 2.38-1.35 (m, 18H), 1.09 (s, 9H), 1.03-1.01 (d, J = 76.8 Hz, 3H), 0.69 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 166.8, 159.7, 147.1, 142.7, 132.7, 125.0, 120.3, 116.8, 112.5, 71.4, 66.9, 60.9, 58.3, 50.0, 45.2, 42.8, 37.1, 35.3, 32.6, 29.4, 28.8, 27.8, 26.5, 24.6, 23.6, 21.3, 17.0; $[\alpha]_{\text{D}}$ = -2.4; HRMS calcd for $\text{C}_{30}\text{H}_{48}\text{NO}_3$ $[\text{M}+\text{H}]$: 470.3634, found: 470.3623; **I(d)** (1 α , 3 β) ^1H NMR (400 MHz, CDCl_3) δ 6.39-6.37 (d, J = 11.2 Hz, 1H), 6.12-6.09 (m, J = 11.2 Hz, 1H), 5.34-5.33 (t, J = 1.6 Hz, 1H), 5.29 (t, J = 1.2 Hz, 1H), 5.02 (m, 1H), 4.46-4.43 (m, 1H), 4.26-4.23 (m, 1H), 3.78 (s, 3H), 2.84-2.80 (dm, J = 11.8 Hz, 1H), 2.63-2.58 (dd, J = 13.6 Hz, 3.6 Hz, 1H), 2.38-1.35 (m, 18H), 1.09 (s, 9H), 1.03-1.01 (d, J = 7.2 Hz, 3H), 0.69 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 166.8, 159.7, 147.6, 142.6, 132.9, 124.9, 120.3, 116.8, 111.7, 70.7, 66.8, 60.9, 58.4, 50.0, 45.2, 42.8, 37.1, 35.3, 32.6, 29.7, 29.4, 28.8, 27.8, 26.5, 24.6, 23.6, 21.3, 16.9; IR (neat) 3331, 2955, 2919, 2849, 1461, 1361, 1049, 885 cm^{-1} ; UV (MeOH) λ_{max} 264 nm (ϵ 6923); $[\alpha]_{\text{D}}$ = +6.0; HRMS calcd for $\text{C}_{30}\text{H}_{48}\text{NO}_3$ $[\text{M}+\text{H}]$: 470.3634, found: 470.3636.

In a like manner, the following additional compounds were prepared:

- 20 **Compounds I(e) and I(f):** By replacing methoxylamine hydrochloride (**III**, $\text{R}^4 = \text{CH}_3$) with *O*-ethylhydroxylamine hydrochloride (**III**, $\text{R}^4 = \text{Et}$). The crude reaction product was purified by a column chromatography eluted with 99% ethyl acetate in the presence of 1% triethylamine to afford 4.8 mg of a mixture of diastereomers **I(e)** (1 α , 3 β) and **I(f)** (1 β , 3 α) in 63% yield and in a ratio of 1.8:1, respectively. This
- 25 diastereomeric mixture was then separated by HPLC (Phenomenex Luna column, reserve phase, 3 mL/min) eluted with 15% water in acetonitrile to afford 1.2 mg **I(e)** (1 α , 3 β) and 0.6 mg **I(f)** (1 β , 3 α) in 16% and 8% yields, respectively. Retention time for **I(e)** (1 α , 3 β) is 55.48 min. and for **I(f)** (1 β , 3 α) is 53.23 min. Data for **I(e)** (1 α , 3 β): $[\alpha]_{\text{D}}^{25} = +4.66$ ($c=0.01$, CHCl_3) ^1H NMR (CDCl_3 , 400 MHz): δ 6.38 (d, 1H, $J=10.8$ Hz), 6.11 (d, 1H, $J=11.6$ Hz), 5.34-5.30 (m, 2H), 5.02 (d, 1H, $J=1.2$ Hz), 4.44
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(m, 1H), 4.24 ((m, 1H), 4.03 (q, 2H, J=7.2 Hz), 2.84-2.79 (m, 1H), 2.62-2.59 (m, 1H), 2.39-2.30 (m, 2H), 2.23-1.87 (m, 7H), 1.79-1.66 (m, 4H), 1.56-1.38 (m, 6H), 2.21 (t, 3H, J=7.2 Hz), 1.09 (s, 9H), 1.02 (d, 3H, J=6.8 Hz), 0.69 (s, 3H). ¹³C NMR (CDCl₃, 100 MHz): δ 166.40, 159.78, 147.65, 142.70, 132.88, 124.99, 120.27, 116.84, 111.61, 70.68, 68.49, 66.88, 58.44, 50.03, 45.17, 42.86, 37.18, 37.15, 35.35, 32.55, 29.37, 28.79, 27.85, 26.52, 24.59, 23.63, 21.34, 16.97, 14.64. IR (Thin Film) 3345 (br, m), 2928 (s), 1666 (w), 1462 (w), 1365 (w), 1092 (w), 1053 (s), 916 (w), 873 (w), 801 (w) cm⁻¹. HRMS: calculated for C₃₁H₄₉NO₃Na⁺ [M+Na]: 506.3604 Found: 506.3604. Data for **I(f)** (1β,3α) was not obtained due to insufficient amount of compound.

- 10 **Compounds I(g) and I(h):** By replacing methoxylamine hydrochloride (**III**, R⁴ = CH₃) with *O*-allylhydroxylamine hydrochloride (**III**, R⁴ = allyl). The crude reaction product was flash column chromatography eluted with 99% ethyl acetate in the presence of 1% triethylamine afforded 6.1 mg of a mixture of diastereomers **I(g)** (1α, 3β) and **I(h)** (1β,3α) in 73% yield and in a ratio of 2.0:1, respectively.
- 15 This diastereomeric mixture was then separated by HPLC (Phenomenex Luna column, reserve phase, 3 mL/min) eluted with 15% water in acetonitrile to afford 1.1 mg **I(g)** (1α, 3β) and 0.53 mg **I(h)** (1β,3α) in 13% and 6% yields, respectively. Retention time for **I(g)** (1α, 3β) is 55.55 min. and for **I(h)** (1β,3α) is 53.19 min. Data for **I(g)** (1α, 3β) MK-1625 (NOAll)-TB-2: [α]_D²⁵ = +4.0 (c=0.01, CHCl₃) ¹H NMR (CDCl₃, 400 MHz): δ 6.38 (d, 1H, J=11.2 Hz), 6.11 (d, 1H, J=11.2 Hz), 6.02-5.92 (m, 1H), 5.34-5.29 (m, 1H), 5.26-5.22 (m, 1H), 5.16-5.13 (m, 1H), 5.02 (br, 1H), 4.50-4.48 (m, 2H), 4.44 (br, 1H), 4.24 (br, 1H), 3.50 (br, 2H), 2.83-2.79 (m, 1H), 2.62-2.59 (m, 1H), 2.38-2.30 (m, 2H), 2.24-1.87 (m, 9H), 1.79-1.76 (m, 3H), 1.52-1.30 (m, 2H), 2.21 (t, 3H, J=7.2 Hz), 1.09 (s, 9H), 1.02 (d, 3H, J=6.8 Hz), 0.68 (s, 3H). ¹³C NMR (CDCl₃, 100 MHz): δ 166.88, 159.74, 147.66, 142.72, 134.90, 132.87, 124.99, 120.28, 116.82, 116.36, 111.60, 74.07, 70.69, 66.89, 54.43, 50.03, 45.18, 42.87, 37.17, 35.35, 32.58, 29.70, 29.36, 28.79, 27.81, 26.61, 24.62, 23.63, 21.40, 16.96. IR (Thin Film) 3353 (br, m), 2926 (s), 1668 (sh, w), 1462 (m), 1365 (m), 1261 (w), 1092 (w), 1048 (br, m), 915 (m), 802 (w), cm⁻¹. HRMS: calculated for C₃₂H₄₉NO₃Na⁺ [M+Na]: 518.3604 Found:
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- 25
- 30

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518.3572. Data for **I(h)** (1 β ,3 α) MK-1625 (NOAll)-TB-1 was not obtained due to insufficient amount of compound.

Compounds I(i) and I(j): By replacing methoxylamine hydrochloride (**III**, R⁴ = CH₃) with *O*-phenylhydroxylamine hydrochloride (**III**, R⁴ = phenyl). The crude
5 reaction product was flash column chromatography eluted with 99% ethyl acetate in the presence of 1% triethylamine afforded 11.8 mg of a mixture of diastereomers **I(i)** (1 α , 3 β) and **I(j)** (1 β ,3 α) in 83% yield and in a ratio of 1.8:1, respectively. This diastereomeric mixture was then separated by HPLC (Phenomenex Luna column, reserve phase, 3 mL/min) eluted with 15% water in
10 acetonitrile to afford 5.3 mg **I(i)** (1 α , 3 β) and 2.8 mg **I(j)** (1 β ,3 α) in 37% and 20% yields, respectively. Retention time for **I(i)** (1 α , 3 β) is 67.86 min. and for **I(j)** (1 β ,3 α) is 64.85 min. Data for **I(i)** (1 α , 3 β): $[\alpha]^{25}_D = +0.31$ (c=0.25, CHCl₃) ¹H NMR (CDCl₃, 400 MHz): δ 7.30-7.26 (m, 2H), 7.16-7.13 (m, 2H), 6.98-6.94 (m, 1H), 6.37 (d, 1H, J=11.2 Hz), 6.09 (d, 1H, J=11.2 Hz), 5.34 (dd, 1H, J=1.6 Hz, J=1.6 Hz), 5.31-5.30 (m, 1H), 5.02-5.01 (m, 1H), 4.45-4.44 (m, 1H), 4.24-4.23 (m, 1H), 2.80 (dd, 1H, J=4 Hz, J=12 Hz), 2.60 (dd, 1H, J=3.6 Hz, J=13.6 Hz), 2.37-2.30 (m, 4H), 2.20-2.13 (m, 2H), 2.07-1.87 (m, 3H), 1.78-1.44 (m, 9H), 1.20 (s, 9H), 1.03 (d, 3H, J=6.8 Hz), 0.68 (s, 3H). ¹³C NMR (CDCl₃, 100 MHz): δ 170.34, 159.75, 159.57, 147.62, 132.89, 129.13, 124.95, 121.36, 120.41, 116.83, 114.33, 111.63, 70.69, 66.88, 58.42, 50.03, 45.17, 42.85, 37.99, 37.13, 35.32, 32.55, 29.34, 28.76, 27.73, 27.03, 24.87, 23.61, 21.42, 17.00. IR (Thin Film) 3357 (br, m), 2928 (s), 2856 (sh, m), 1590 (s), 1489 (sh, s), 1394 (m), 1219 (br, s), 1158 (w), 1052 (br, m), 1023 (w), 956 (m), 910 (s) cm⁻¹. HRMS: calculated for C₃₅H₄₉NO₃Na⁺ [M+Na]: 554.3604 Found: 554.3601.
20
25 Data for **I(j)** (1 β ,3 α): $[\alpha]^{25}_D = -24.35$ (c=0.27, CHCl₃) ¹H NMR (CDCl₃, 400 MHz): δ 7.30-7.26 (m, 2H), 7.16-7.13 (m, 2H), 6.98-6.94 (m, 1H), 6.39 (d, 1H, J=11.2 Hz), 6.08 (d, 1H, J=11.6 Hz), 5.32-5.30 (m, 2H), 5.01 (d, 1H, J=1.6 Hz), 4.45 (br, 1H), 4.23-4.21 (m, 1H), 2.81 (dd, 1H, J=4.4 Hz, J=12 Hz), 2.63 (dd, 1H, J=3.6 Hz, J=13.2 Hz), 2.37-2.27 (m, 3H), 2.19-2.13 (m, 2H), 2.06-1.89 (m, 3H),
30 1.78-1.75 (m, 3H), 1.64-1.44 (m, 7H), 1.20 (s, 9H), 1.04 (d, 3H, J=6.8 Hz), 0.68 (s, 3H). ¹³C NMR (CDCl₃, 100 MHz): δ 170.34, 159.75, 159.57, 147.08,

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142.68, 132.71, 129.13, 124.99, 121.37, 120.41, 116.83, 114.33, 112.82, 71.51, 66.76, 58.41, 50.03, 45.51, 42.77, 37.99, 37.13, 35.30, 32.55, 29.38, 28.74, 27.73, 27.03, 24.86, 23.59, 21.39, 17.02. IR (Thin Film) 3350 (br, m), 2926 (s), 2850 (m), 1590 (m), 1490 (m), 1220 (br, s), 1158 (w), 1050 (br, m), 910 (s) cm^{-1} .

5 HRMS: calculated for $\text{C}_{35}\text{H}_{49}\text{NO}_3\text{Na}^+$ [M+Na]: 554.3604 Found: 554.3578.

Example 6: Preparation of Compound I(k)

A solution of 53 mg (0.094 mmol) of 19-nor-phosphine oxide VI ($\text{R}^1, \text{R}^2 = \text{OTBDMS}, \text{R}^6 = \text{H}$) in 2.0 mL of anhydrous THF was cooled to -78°C and treated with 59 μL (0.094 mmol, 1.6 M in hexanes) of n-BuLi under argon atmosphere. The mixture turned deep reddish and was stirred for 15 min at -78°C . To the solution was added dropwise a precooled (-78°C) solution of 10 mg (0.031 mmol) of the C,D-ring ketone V ($\text{R}^3 = \text{CH}_3, \text{R}^5 = \text{t-butyl}$, see Example 2) in 1.5 mL of anhydrous THF via cannula. The reaction kept going until the reddish orange color faded to yellow (about 2 hr). The reaction was quenched by adding 1.0 mL of pH 7 buffer at -78°C , then warmed to room temperature, extracted with EtOAc (20 mL x 2), washed with brine, dried over MgSO_4 , concentrated. The residue was subjected to column chromatography with EtOAc/hexanes (1/15) as eluent to afford 11 mg (52 %) of the coupled product as a colorless oil.

The coupled product (10 mg, 0.015 mmol) was dissolved in 1.0 mL of anhydrous pyridine, and to this solution was added *O*-ethylhydroxylamine hydrochloride (26 mg, 20 eq.) and 4A powdered molecular sieves (10 mg) at room temperature. The mixture solution was then stirred at room temperature for 20 hr. Reaction was monitored by TLC. This reaction mixture was then directly subjected to column chromatography with EtOAc/hexanes (1/15) as eluent to afford 10 mg (97 %) of oxime product as a colorless oil.

The oxime product (8.9 mg, 0.013 mmol) was dissolved in 2 mL of anhydrous THF, and to the solution was added 0.19 mL (0.19 mmol) of a 1.0 M solution of TBAF in THF. The resulting mixture was stirred overnight at room temperature, then quenched with 2 mL of water. The solution was extracted with EtOAc (20 mL x 3), washed with brine, dried over MgSO_4 , concentrated. The residue was subjected to column chromatography with EtOAc as eluent to give 5.6 mg (94 %) of the crude product of (-)-1(k) as a colorless oil. The crude product (4 mg out of 5.6 mg) was purified by

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reverse-phase HPLC (C-18 semipreparative column, 18 % H₂O in MeCN, 3 ml/min) to afford 2.5 mg of (-)-1(k) (1 α , 3 β , t_R = 38.7 min). : $[\alpha]^{24}_D$ = -47.2 (c = 0.023; CHCl₃). ¹H NMR (400 MHz, CDCl₃) δ 6.31 (d, J = 11.2 Hz, 1H), 5.95 (d, J = 11.2 Hz, 1H), 5.31 (m, 1H), 4.13 (m, 1H), 4.06 (m, 1H), 3.78 (s, 3H), 2.75-2.81 (m, 2H),
 5 2.49 (dd, J = 13.6, 3.6 Hz, 1H), 2.38 (dd, J = 11.2, 5.6 Hz, 1H), 2.10-2.29 (m, 6H), 1.94-2.06 (m, 2H), 1.36-1.82 (m, 12H), 1.09 (s, 9H), 1.02 (d, J = 6.8 Hz, 3H), 0.69 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 166.8, 159.9, 142.6, 131.1, 123.8, 120.3, 115.1, 67.4, 67.2, 61.0, 58.4, 49.9, 44.6, 42.2, 37.2, 37.1, 35.3, 32.6, 29.4, 28.6, 27.8, 26.5, 24.6, 23.5, 21.3, 27.1. IR (neat, cm⁻¹) 3355, 2930, 1668, 1463, 1364, 1054, 976, 886,
 10 810. HRMS ([M+Na]⁺) calcd. 480.3448, found 480.3427.

Example 7: CYP24 Enzyme Assay (Induced KPK1A-ras Cells)

(i) Material and reagents:

1,25(OH)₂D₃ 10⁻⁵ M
 [³H]- 1,25(OH)₂D₃ 25,000 CPM/ μ L
 15 HPK1A-ras cells
 48-well plate
 Methanol
 Dichloromethane
 Saturated KCl: KCl 30g, H₂O 400 ml

20 (ii) Procedure:

1. Induction of HPK1A-ras cells (The day before assay)

When the HPK1A-ras cells were 80-90% confluent, add 1 μ L 10⁻⁵ M 1,25(OH)₂D₃ to 1 mL medium in the plate (final concentration is 10⁻⁸ M).

2. Preparation of cell suspension

25 After 18 to 20 hours induction, removed the medium and washed the cell twice with PBS. Then trypsinized the cells from plate, centrifuge (2,000 rpm, 5 min) and suspended cells pellet in DMEM medium+1%BSA.

Counted the cells and adjust cells density to 250,000/150 μ L, add 150 μ L cell suspension to each well in 48-well plate. (including 3 wells as no cell control,
 30 and 3 well cells without drug or inhibitor as control).

3. Added 25 μ L ketoconazole (final concentration 10⁻⁵ M, 10⁻⁶ M, 10⁻⁷ M, 10⁻⁸ M) or drugs into each designated well. Keep the plate in 37°C for 10 min.

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4. Preparation of substrate

Took certain amount of DMEM+1%BSA medium (25*Well number+200) μ L to a tube, added certain amount of ^3H -1,25(OH) $_2$ D $_3$ (well number+2) μ L and certain amount of 100mM DPPD (well number/5) μ L and mixed them by vortex.

5. Incubation

Added 25 μ L substrate to each well, incubated the plate at 37°C for 3 hour.

Added 25 μ L substrate to counting plate (2 well) as a total count.

6. Lipid extraction and counting

Added 500 μ L methanol to each well to stop the reaction, transferred them to tube

Added 250 μ L dichloromethane and vortex.

Added 250 μ L dichloromethane and 250 μ L saturated KCl, and vortexed.

Centrifuged at 4000 rpm for 5 min.

Transferred 100 μ L of aqueous phase (upper phase) to counting plastic counting plate. Added 600 μ L of scintillation fluid to each well. Counted the plate in scintillation counter.

7. Calculation enzyme activity

CPM of cell control after subtraction of CPM of NCC is as 100% enzyme activity.

Enzyme activity = (CPM in test compounds well – CPM in NCC well)/(CPM in Cell control - CPM in NCC well) * 100%

Dilution of Ketoconazole

Stock 10^{-2} M

Concentration (final)	From previous step	DMEM + 1%BSA	Concentration (actual)
10^{-5} M	4	496	$8*10^{-5}$ M
10^{-6} M	12.5	112.5	$8*10^{-6}$ M
10^{-7} M	12.5	112.5	$8*10^{-7}$ M
10^{-8} M	12.5	112.5	$8*10^{-8}$ M

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Dilution of test compounds

Stock 10^{-3} M

Concentration (final)	From previous step (μ L)	DMEM + 1%BSA (μ L)	Concentration (actual)
10^{-5} M	10	115	8×10^{-5} M
10^{-6} M	12.5	112.5	8×10^{-6} M
10^{-7} M	12.5	112.5	8×10^{-7} M
10^{-8} M	12.5	112.5	8×10^{-8} M

(iii) Results:

5 Compounds of Formula I(a), I(c), I(e), I(g), I(i) and I(k) showed significantly greater inhibition of CYP24 than ketoconazole. A graph showing inhibition of CYP24 activity by compounds I(a) and I(c) (indicated as BH1625(NOH)-TB-2 (CTA062) and BH-1625(NOMe)-TB-2-(CTA065) respectively) compared to ketoconazole is shown in Figure 1A.

(iv) References:

- 10 Ray S, Ray R, Holick M. Metabolism of ^{3} H-1 α , 25-dihydroxyvitamin D₃ in the cultured human keratinocytes (1995) 59:117-122
- Dilworth F J, Scott I, Green A, Strugnell S, Guo Y D, Roberts E A, Kremer R, Calverley, M J, Makin H L J, Jones G. Different mechanisms of hydroxylation site selection by liver and kidney cytochrome P450 species
- 15 (CYP27 and CYP24) involved in Vitamin D metabolism. (1995) J Biochem 270(28):16766-16774

Example 8: Assay of CYP1-alpha hydroxylase (Using Transfected COS-1 Cells)**(A) Transit transfection**

- 20 (i) Reagent and material
1. COS-1 cells (50-80% confluent)
 2. FuGene 6 Transfection Reagent
 3. PcDNA vector containing CYP-1alpha hydroxylase cDNA(1 μ g/ μ l)
 4. DMEM Medium + 10% FCS
 - 25 5. DMEM Medium (serum-free)
 6. 6-well plate

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(ii) Transfection cocktail preparation (The amount depended on how many wells transfected)

1. To a sterile tube, added serum-free medium (100 µl per well), then added FuGene 6 Reagent (3 µl per well). Tapped gently to mix. Payed attention to the order. Added FuGene 6 Reagent directly to medium, did not allow undiluted Fugene 6 Reagent to come in contact with plastic surfaces other than the pipette tip.
2. Added DNA solution (1 µg per well) to the prediluted FuGene 6 Reagent from step 2
3. Gently tapped the tube to mix the contents. Did not vortex. Incubated for 15 min at room temperature (no more than 45 min).

(iii) Cells preparation

1. Trypsinized Cos-1 cells, centrifuged cell suspension, suspended cells pellet in DMEM medium +10% FCS.
2. Diluted the cells suspension to 750,000 cell/ml (75cell/square),

(iv) Transfection.

1. Added 1.7 ml DMEM medium+10%FCS to each well of 6 well plate.
2. Transferred the correct volume of the cell suspension (200 µl/well) to the transfection cocktail. Mixed gently.
3. Added 0.3 ml of the mixture to each well. Made sure that the same amount cells were added to each well. Swirled the wells to ensure even dispersal.
4. Incubated the cells for 24 hours at 37°C, 5% CO₂ until enzyme activity assay.

25 (B) Enzyme Activity Assay

(i) Reagent and materials

DMEM medium +1% BSA

PBS

[³H-26,27]-25(OH)D₃

30 DPPD 100mM

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(ii) Procedure

1. Washed cells once with PBS. Took care no to disturb the attached cells.
2. Added 0.55 ml medium (DMEM+1%BSA) each well.
- 5 3. Added 0.025 ml medium containing test compounds.
4. Incubated the cells for 10 minutes.
5. Added 0.025ml medium containing [³H-26,27]-25(OH)D₃ (50,000 CPM) and DPPD (0.6 µl stock).
6. Incubated the cells for 2 hour.
- 10 7. Added 1.5 ml Methanol to stop reaction.
8. Added internal standard.
9. Transferred the medium to labeled tube.
10. Added 0.75 ml dichloromethane, vortexed and kept in room temperature for 15 minutes.
- 15 11. Added 0.75 ml dichloromethane and 0.75 ml saturated KCl.
12. Vortexed and centrifuged.
13. Removed upper phase and dried the lower phase in Speed-Vac.
14. Added 110 µl mobile phase, vortexed and centrifuged for 5 min.
15. Transferred 105 µl to the insert in HPLC vial.
- 20 16. HPLC analysis conditions:
Solvent: Hexane/isopropanol/methanol (91/7/2)
Column: SIL 3µm column
Flow rate: 2 ml/min
Detector: UV detector and radioactive detector.

25 (C) Results:

Compounds of Formula I(a), I(c), I(e), I(g), I(i) and I(k) showed little to no (IC₅₀ > 10,000 nM) inhibition of CYP27B1. A graph showing the inhibition of CYP27B1 activity by compounds I(a) and I(c) (indicated as BH1625(NOH)-TB-2 (CTA062) and BH-1625(NOMe)-TB-2-(CTA065) respectively) compared to

30 ketoconazole is shown in Figure 1B.

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(D) References

- Shink T, Shimada H, Wakino S, Anazawa H, Hayashi M, Saruta T, Deluca H, Suda T. Cloning and expression of rat 25-hydroxyvitamin D₃-1- α -hydroxylase cDNA. (1997) *Proc. Natl Acad Sci* 94:12920-12925
- 5 Muralidharan K R, Rowland-goldsmith M, Lee S A, Park G, Norman A W, Henry H L, Okamura W H. Inhibitors of 25-hydroxyvitamin D₃-1 α -hydroxylase: Thiavitamin D analogues and biological evaluation. (1997) *J Steroid Biochem. Molec. Biol.* 62(1):73-78.

Example 9: CYP27A1 Enzyme Assay**10 (A) Procedure:**

As described in:

- Dilworth F J, Black S M, Guo Y D, Miller W L, Jones G. Construction of a P450c27 fusion enzyme: a useful tool for analysis of vitamin D₃ 25-hydroxylase (1996) *Biochem J* 320:267-271
- 15 Sawada N, Sakaki T, Ohta M, Inouye K. Metabolism of vitamin D (3) by human CYP27A1 (2000) *Biochem Biophys Res Commun* 273(3):977-84

(B) Results:

- Compounds of Formula I(a), I(c), I(e), I(g), I(i) and I(k) showed little to no (IC₅₀ > 10,000 nM) inhibition of CYP27A1. A graph showing the inhibition of CYP27A1
- 20 activity by compounds I(a) and I(c) (indicated as BH1625(NOH)-TB-2 (CTA062) and BH-1625(NOMe)-TB-2-(CTA065) respectively) compared to ketoconazole is shown in Figure 1C.

Example 10: VDR Binding Assay**(A) Reagent and materials**

- 25 1. VDR 9.3 pmol/ μ l (human, recombinant, Biomol).
2. [³H]-1,25(OH)₂D₃ in ethanol
3. 1,25(OH)₂D₃ in ethanol
4. TEK₃₀₀
- | | | |
|----|---------------------------|--------|
| | Tris-HCl | 50 mM |
| 30 | EDTA | 1.5 mM |
| | KCl | 300 mM |
| | Adjusted pH to 7.4 (25°C) | |

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5. TEDK₃₀₀
TEK₃₀₀
DTT (dithiothreitol) 10 mM (MW 154.24)
6. Tris buffer
 - 5 22.50 g Tris-HCl
 - 500 ml H₂O
 - 13.25 g Tris-base
 - 500 ml H₂O
 - Kept in 4°C
- 10 7. Dextran-T70 (Mol 70,000) Pharmacia
8. Charcoal (carbon decolorizing neutral, norit) Fishery
9. Gelatin (G-2625 Sigma)

(B) Reagent Preparation

1. Charcoal dextran solution
 - 15 (1) Tris buffer
Mixed equal amount of Tris-HCl and Tris-base.
 - (2) Norit decolorizing neutral charcoal 2.0 g
Tris buffer 150 mL
Stirred
 - 20 (3) Dextran T – 70 0.2 g
Tris buffer 50 ml.
 - (4) Slowly dripped the suspended dextran into charcoal solution with stirring.
Kept in refrigerator overnight.
 - 25 Thirty minute before use, stored on ice with continuous mixing
2. TEK₃₀₀/Gelatin solution
 - 50 mg swine gelatin
 - 5 ml TEDK₃₀₀ solution
 - heated, stirred then cooled to 4°C.
 - 30 5 ml TEDK₃₀₀ solution

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3. Preparation of 1,25(OH)₂D₃ and test compounds in ethanol

1,25(OH)₂D₃: 125, 250, 500, 1000, 2000, 4000 pg/25µl. (stock 10⁻⁵ M/25µL = 100,000pg/25µL)

Test compounds: 12,500, 25,000, 50,000, 100,000, 200,000 and 400,000 pg/25

5 µL. (4*10⁻⁵M/25µL = 400,000 pg/25µL)

Label	Concentration (ng/mL)	Amount (pg/50µL)
	5.0	125
Std F	10.0	250
Std G	20.0	500
Std H	40.0	1000
	80.0	2000
Std I	160.0	4000

4. Dilution of VDR:

1 µl stock VDR in 2.5 ml TEDK₃₀₀/Gelatin solution (500µl/tube), (kept on ice)

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(C) Assay:

<u>label</u>	<u>Standards</u>	<u>NSB</u> <u>buffer</u>	<u>VDR</u>	<u>1 h</u> <u>RT</u>	<u>³H-</u> <u>1.25(OH)₂D₃</u>	<u>1 h</u> <u>RT</u>	<u>Reagent C -</u> <u>charcoal</u>	<u>On ice</u> <u>30 min</u>	<u>Spin at 4°C</u>	
<u>TC</u> (Total)	<u>25 μL</u> <u>reagent D</u>	<u>100 μL</u> <u>reagent</u> <u>L</u>	<u>500 μL</u> <u>reagent A</u>		<u>50 μL</u> <u>reagent B</u>		<u>100 μL</u> <u>reagent C</u>		<u>2000 rpm,</u> <u>10 min</u>	
<u>NSB</u> (non-specific b)		<u>500 μL</u> <u>reagent</u> <u>L</u>			mixed all tubes		mixed all tubes		Added 100 μl to counting rack	
<u>Max b₀</u> <u>binding</u>			<u>500 μL</u> <u>reagent A</u>							Counted 5- 10 min
<u>Standard</u>		<u>25 μL</u> <u>of each</u> <u>standard</u>								
<u>Test</u>	<u>25 μL</u> <u>of each</u> <u>concentrat</u> <u>ion of</u> <u>sample</u>		mixed all tubes							

(D) Calculations:

- 5 The amount of 1,25(OH)₂D₃ to displace 50 percent [³H]-1,25(OH)₂D₃ from VDR is calculated as B₅₀ for 1,25(OH)₂D₃. The VDR binding of other compounds is calculated as B₅₀ relative to a value of 1 for 1,25(OH)₂D₃.

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Dilution of 1,25(OH)D₃

Concentration (pg/25ul)	Final concentration M	10 ⁻⁵ M	Ethanol (ul)
4,000	2*10 ⁻⁸	6	144
2,000	10 ⁻⁸	70	70
1,000	5*10 ⁻⁹	70	70
500	2.5*10 ⁻⁹	70	70
250	1.25*10 ⁻⁹	70	70
125	6.25*10 ⁻¹⁰	70	70

Dilution of test compounds

Concentration (pg/50ul)	Final concentration M	10 ⁻³ M	Ethanol
400,000	2*10 ⁻⁶	6	144
200,000	10 ⁻⁶	70	70
10,000	5*10 ⁻⁷	70	70
5,000	2.5*10 ⁻⁷	70	70
25,000	1.25*10 ⁻⁷	70	70
12,500	6.25*10 ⁻⁸	70	70

5

(E) Results:

A graph showing the binding of compounds I(a) and I(c), (indicated as BH-1625(NOH)-TB-2 (CTA62) and BH-1625(NOMe)-TB-2-(CTA65) respectively) to transporter D protein (DBP) compared to 1 α ,25-dihydroxy vitamin D₃ and 25-hydroxy vitamin D₃ is shown in Figure 2.

10

(F) References:

1. Ross T K, Prah J M, DeLuka H. Overproduction of rat 1,25-dihydroxyvitamin D₃ receptor in insect cells using the baculovirus expression system. (1991) Proc Natl Acad Sci USA 88:6555-6559

- 59 -

2. Wecksler W R, Norman A W. An hydroxylapatite batch assay for the quantitation of 1 α , 25-dihydroxyvitamin D₃-receptor complexes (1979)
Anal Biochem 92:314-323

Example 11: Transcriptional Activity Assay

5 (A) Reagent and materials:

- pSG5-hVDR1/3 from DRs. Mark Haussler and Kerr Whitfield, (University of Arizona, Tucson, AZ); hVDR1/3 DNA inserted into the EcoRI site of pSG5vector (CT4)⁴TKGH from DRs. Mark Haussler and Kerr Whitfield, (University of arizona,Tucson, AZ); Four copies of the CT4 synthetic rat osteocalcin VDRE
 10 ligated and annealed into pTKGH vector which has a thymidine promoter linked to the human GH gene.
 hGH ELISA kit. Boehringer Mannheim
 Fugene 6 transfection reagent
 COS-1 cells
 15 DMEM medium and DMEM medium+10%FCS
 1,25(OH)₂D₃ and test compounds

(B) Transfection:

1. Subcultured COS cells into 24-well plate (5,000 cell/well) one day before transfection.
- 20 2. Cocktail preparation (the amount depended on how may wells transfected).
 - (1) To a sterile tube, added serum-free medium (100 μ l per well), then added FuGene 6 Reagent (0.6 μ l per well). Tapped gently to mix. Payed attention to the order. Added FuGene 6 Reagent directly to medium, did not
 25 allow undiluted Fugene 6 Reagent to come in contact with plastic surfaces other than the pipette tip.
 - (2) Added DNA solution (pSG5-hVDR1/3 and (CT4)⁴TKGH vectors) (0.1 μ g each per well) to the prediluted FuGene 6 Reagent from step 2
 - (3) Gently tapped the tube to mix the contents. Did not vortex.
 - 30 Incubated for 15 min at room temperature (no more than 45 min).

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3. Removed the medium and replaced by 0.4 ml fresh medium

4. Added the 100µl cocktail to each well in drop-wise manner.

(C) Treatment of transfected cells with different concentrations of 1,25(OH)₂D₃ and test compounds:

- 5 30 min to 1 hour after transfection, 1,25(OH)₂D₃ (as control) and test compounds were added to the medium in 20 µl medium. The concentration range for 1,25(OH)₂D₃ was 10⁻¹⁰ to 10⁻⁸ M (10⁻¹⁰, 3*10⁻⁹, 10⁻⁹, 3*10⁻⁸, 10⁻⁸ M) and for test compounds was from 3*10⁻⁹ M to 10⁻⁷ M (3*10⁻⁹, 10⁻⁹, 3*10⁻⁸, 10⁻⁸, 3*10⁻⁸, 10⁻⁷ M). Incubation continued for 24 hours.

10 **(D) Measurement of GH content in medium:**

After 24 hour incubation, 200 µL diluted aliquots of medium (dilution of 20-50 times) were used for human GH determination. Sample was assayed according to instruction of hGH ELISA kit.

(E) Results:

- 15 A graph showing the activity of compounds I(a) and I(c) (indicated as BH1625(NOH)-TB-2 (CTA62) and BH-1625(NOMe)-TB-2-(CTA65) respectively) in the vitamin D transcription assay compared to 1α,25-dihydroxy vitamin D₃ is shown in Figure 3.

(F) References

- 20 Hashimoto Y, Ikeda I, Ikeda M, Takahashi Y, Hosaka M, Uchida H, Kono N, Fukui H, Makino T, Honjo M. Construction of a specific and sensitive sandwich enzyme immunoassay for 20 KD human growth hormone (1998) J Immunol Methods 221:77-85
- Jone G, Byford V, Makin H L J, Kremer R, Rice R H, deGraffenried L A, Knutson J C, Bishop C W. Anti-proliferative activity and target cell catabolism of the vitamin D analogue 1alpha, 24(OH)2D2 in normal and immortalized human epidermal cells (1996) Biochem Pharmacol 52:133-140

Example 12: DBP Binding Assay (Human Plasma)

(A) Reagents:

30

1. Tris buffer:
22.50 g Tris-HCl

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500 ml H₂O

2. 13.25 g Tris-base

500 ml H₂O

Kept in 4°C

5 3. Dextran-T70 (Mol 70,000) Pharmacia

4. Charcoal (carbon decolorizing neutral, norit) Fishery

5. DBP (vitamin D binding protein) (human plasma)

6. [³H] 25(OH)D₃

7. Gelatin (G-2625 Sigma)

10 **(B) Reagent preparation:**

1. Tris buffer

Mixed equal volume of two Tris buffer.

2. Dextran coated charcoal solution

(1) preparation of charcoal solution

15 Norit decolorizing neutral charcoal 2.0 g

Tris buffer 150 mL

Stirring

(2) preparation of dextran solution

Dextran T – 70 0.2 g

20 Tris buffer 50 ml

(3) preparation of dextran coated charcoal solution

Slowly dripped the dextran solution into charcoal solution with stirring.

Kept in refrigerator overnight.

Thirty minutes before use, kept it on ice with continuous mixing.

25 This solution could be kept in 4°C for 2 month.

3. Tris buffer/Gelatin solution

250 mg swine gelatin

50 ml Tris buffer

heated, stirred and cooled on ice.

30 Prepared just before use.

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4. DBP solution

Human plasma was diluted to 1:5000 with Tris buffer/gelatin solution

5. Dilution of Standard 25(OH)D₃

Stock 10,000pg/50 µl

5 Diluted to 0, 62.5, 125, 250, 500, 750, 1000, 10,000 pg/50 µl with ethanol

6. Dilution of Standard 1,25(OH)₂D₃

Stock 200,000 pg/50 µl (10⁻⁵ M/50 ul)

Diluted to 6,250, 12,500, 25,000, 50,000, 100,000, 200,000 pg/50 µl with ethanol

10 7. Dilution of test compounds

Stock 200,000pg /50 µl (10⁻³ M)

Diluted to 12,500, 25,000, 50,000, 100,000, 200,000 and 400,000pg/50 µl with ethanol

8. [³H-26,27]-25(OH)₂D₃ solution

15 The stock solution was diluted in Tris buffer, 20,000 CPM/50µl.

(C) Assay

Label	25(OH) D ₃	Test compounds (µl)	3H- 25(OH) D ₃ (µl)	DBP (µl)	Super mix	Incubation (Rm T)	Charcoal dextran (µl)	On ice	Centrifuge	Counting
1-3 (total)	—	—	50	—	600 600	—	—	—	—	—
4-8	—	—	50	500	—	—	—	—	—	—
STD 5-35	50	—	50	—	—	4 h	200	1 h	2000rpm 15min, 4°C	200 µl Super + 600 µl Supermix
Test 36-	—	50	50	—	—	—	—	—	—	—

20

(D) Calculation:

The amount of 25(OH)D₃ to displace 50 percent [³H]-25(OH)D₃ is calculated as B₅₀ for 25(OH)D₃ DBP binding. The DBP binding of other compounds is calculated as B₅₀ relative to a value of 1 for 25(OH)D₃.

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(E) Dilution of 25(OH)D₃:

Amount (mol/50ul)	From previous steps (μl)	Ethanol (μl)
2.5×10^{-11} (5×10^{-7} M)	5×10^{-7} M	
2.5×10^{-12}	40	360
1.875×10^{-12}	90	30
1.25×10^{-12}	130	130
6.25×10^{-13}	130	130
3.125×10^{-13}	130	130
1.5625×10^{-13}	130	130

5

(F) Dilution of 1, 25(OH)D₃

Amount (mol in 50μl)	From previous steps (μl)	Ethanol (μl)
5×10^{-10} (10^{-5} M)		
2.5×10^{-10}	130	130
1.25×10^{-10}	130	130
6.25×10^{-11}	130	130
3.215×10^{-11}	130	130
1.625×10^{-11}	130	130

(G) Dilution of test compounds:

10

Amount (mol in 50μl)	From previous steps (μl)	Ethanol (μl)
Stock (10^{-3} M)		
1.0×10^{-9}	5	245
5.0×10^{-10}	130	130
2.5×10^{-10}	130	130
1.25×10^{-10}	130	130
6.25×10^{-11}	130	130
3.125×10^{-11}	130	130

(H) Results:

15 A graph showing the activity of compounds I(a) and I(c) (indicated as BH-1625(NOH)-TB-2 (CTA62) and BH-1625(NOMe)-TB-2-(CTA65) respectively) in the vitamin D receptor (VDR) binding assay compared to 1α,25-dihydroxy vitamin D₃ is shown in Figure 4.

- 64 -

(I) References:

Bouillon R, van Baelen H, Moor P D. Comparative study of the affinity of the serum vitamin D –binding protein. (1980) J Steroid Biochem 13:1029-44.

- 5 Jones L, Byrnes B, Palma F, Segev D, Mazur E. Displacement potency of vitamin D₂ analogue in competitive protein-binding assay for 25-hydroxyvitamin D₃, 24,25-dihydroxyvitamin D₃ and 1,25-dihydroxyvitamin D₃ (1980) J Clin Endocrinol Metab 50:773-775

Example 13: Keratinocyte Proliferation

- 10 Compounds of Formula I(a) and I(c) were assayed *in vitro* for antiproliferative activity in murine keratinocytes using a standard protocol (Posner, G.H. *et al.* J. med. Chem. 1992, 35, 3280-3287). A graph showing the dose response effects of compounds I(a) and I(c) on keratinocyte proliferation in comparison to 1 α ,25-dihydroxy vitamin D₃ or calcitriol is shown in Figure 5.

Example 14: Calcium Excretion

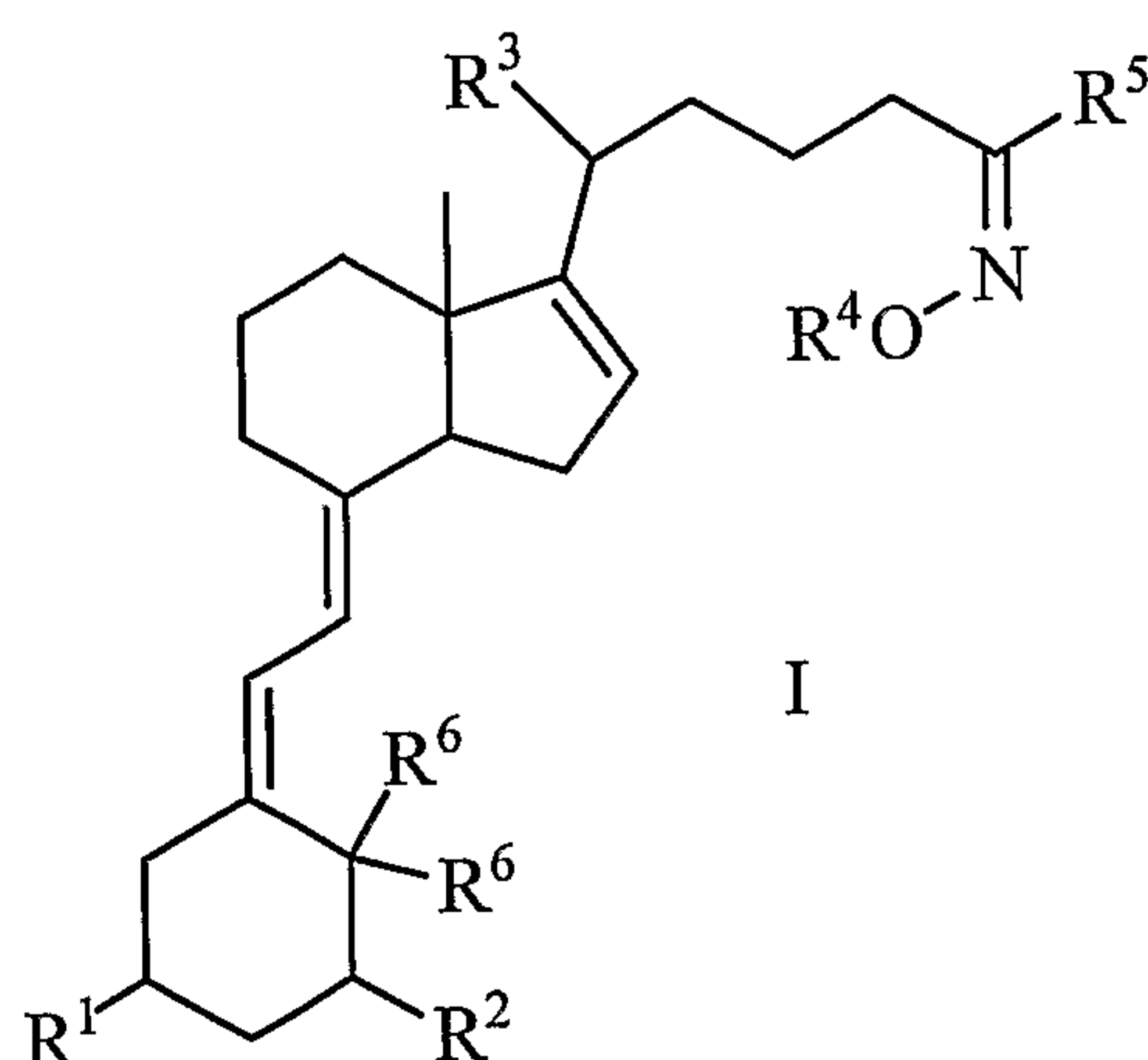
- 15 As a measure of their safety in animals, compound of Formula I(a) was administered orally to rats daily for 1 week at a similar dose (0.5 microgram/Kg body weight) to calcitriol, using a procedure described previously (Posner G.H. *et al.* J. Med. Chem. 1999, 42, 3425-3435). A graph showing the effect of compound I(a) (indicated as BH 1625(NOH)) on calcium levels in rat urine in comparison to calcitriol (1 α ,25-Dihydroxy vitamin D₃) is shown in Figure 6.

- 25 While the present invention has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the invention is not limited to the disclosed examples. To the contrary, the invention is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

WE CLAIM:

1. A compound of Formula I, and pharmaceutically acceptable salts, hydrates,
 5 solvates, and prodrugs thereof:



wherein

- 10 R^1 and R^2 are independently selected from the group consisting of OH, OC_{1-6} alkyl, and halo;
- R^3 is C_{1-6} alkyl;
- R^4 is selected from the group consisting of H, C_{1-6} alkyl, aryl and heteroaryl, with C_{1-6} alkyl being unsubstituted or substituted with 1-4 groups independently selected from
- 15 C_{1-4} alkyl, OC_{1-4} alkyl, OH, halo, NH_2 , NHC_{1-4} alkyl and $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, and with aryl and heteroaryl being unsubstituted or substituted with 1-5 groups independently selected from C_{1-4} alkyl, OC_{1-4} alkyl, OH, CF_3 , OCF_3 , halo, SH, SC_{1-4} alkyl, NH_2 , NHC_{1-4} alkyl, $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, CN, $C(O)OH$, $C(O)OC_{1-4}alkyl$, $C(O)NHC_{1-4}alkyl$, $NHC(O)C_{1-4}alkyl$, $OC(O)C_{1-4}alkyl$, $SOC_{1-4}alkyl$, $SO_2C_{1-4}alkyl$,
- 20 $SO_2NHC_{1-4}alkyl$ and SO_2NH_2 ;
- R^5 is selected from the group consisting of C_{1-6} alkyl, cyclo(C_3-C_6)alkyl, aryl and heteroaryl, aryl- C_{1-6} alkyl and heteroaryl- C_{1-6} alkyl, with C_{1-6} alkyl being unsubstituted or substituted with 1-4 groups independently selected from C_{1-4} alkyl, OC_{1-4} alkyl, OH, halo, NH_2 , NHC_{1-4} alkyl and $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, and with cyclo(C_3-C_6)alkyl, aryl,
- 25 heteroaryl, aryl- C_{1-6} alkyl and heteroaryl- C_{1-6} alkyl, being unsubstituted or substituted

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with 1-5 groups independently selected from C₁₋₄alkyl, OC₁₋₄alkyl, OH, CF₃, OCF₃, halo, SH, SC₁₋₄alkyl, NH₂, NHC₁₋₄alkyl, N(C₁₋₄alkyl)(C₁₋₄alkyl), CN, C(O)OH, C(O)OC₁₋₄alkyl, C(O)NHC₁₋₄alkyl, NHC(O)C₁₋₄alkyl, OC(O)C₁₋₄alkyl, SOC₁₋₄alkyl, SO₂C₁₋₄alkyl, SO₂NHC₁₋₄alkyl and SO₂NH₂; and

5 R⁶ are either both H or together form =CH₂.

2. The compound according to claim 1, wherein R¹ and R² are independently selected from the group consisting of OH, OCH₃, and fluoro.

10 3. The compound according to claim 2, wherein R¹ and R² are both OH.

4. The compound according to any of claims 1-3, wherein R³ is CH₃.

15 5. The compound according to any of claims 1-4, wherein R⁴ is selected from the group consisting of H, phenyl and C₁₋₄alkyl.

6. The compound according to claim 5, wherein R⁴ is selected from the group consisting of H, phenyl, allyl and CH₃.

20 7. The compound according to any of claims 1-6, wherein R⁵ is selected from the group consisting of isopropyl, s-butyl, t-butyl, and neopentyl.

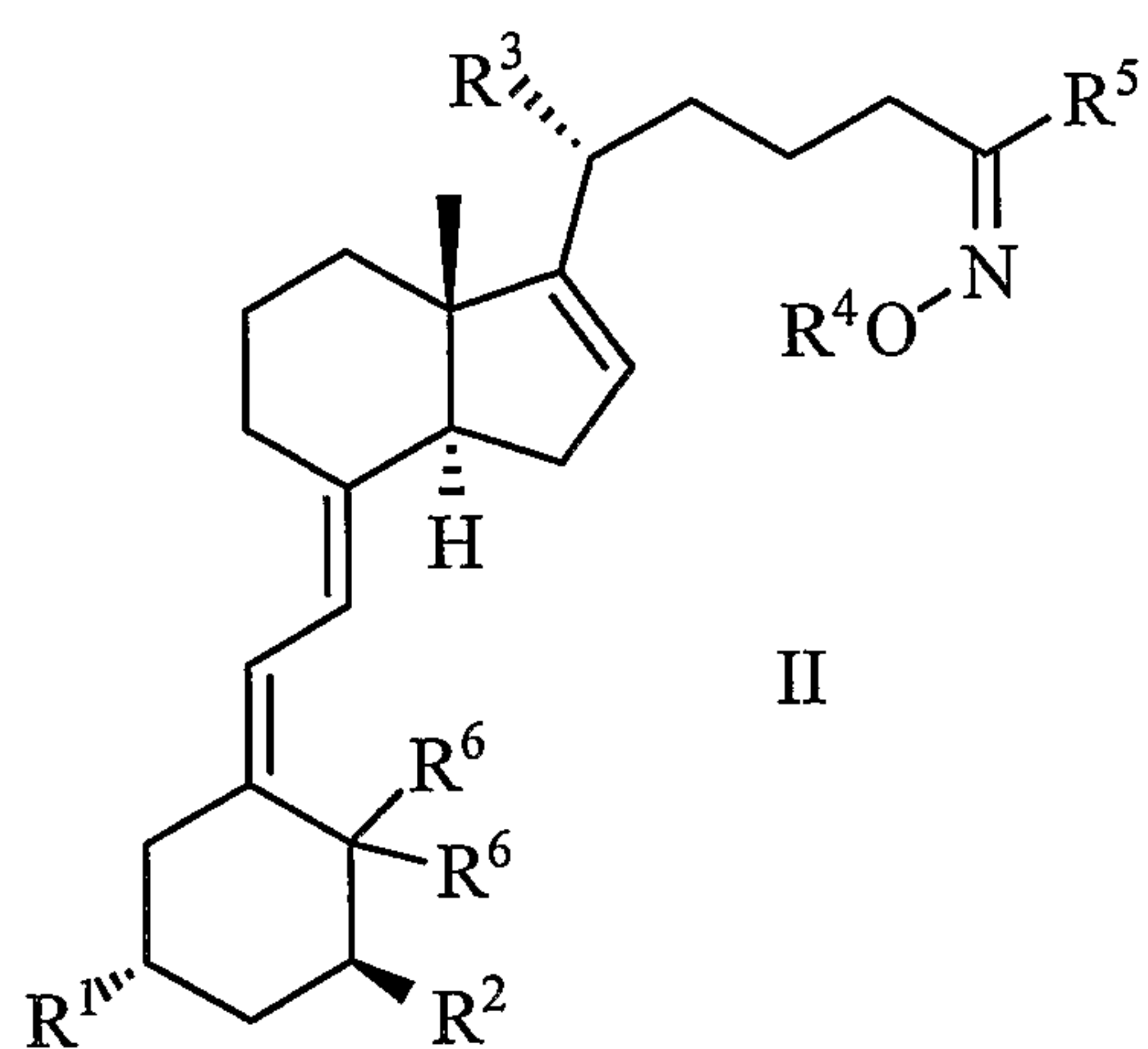
8. The compound according to claim 7, wherein R⁵ is *t*-butyl.

25 9. The compound according to any of claims 1-8, wherein the geometry about the C=N double bond of the oxime is trans.

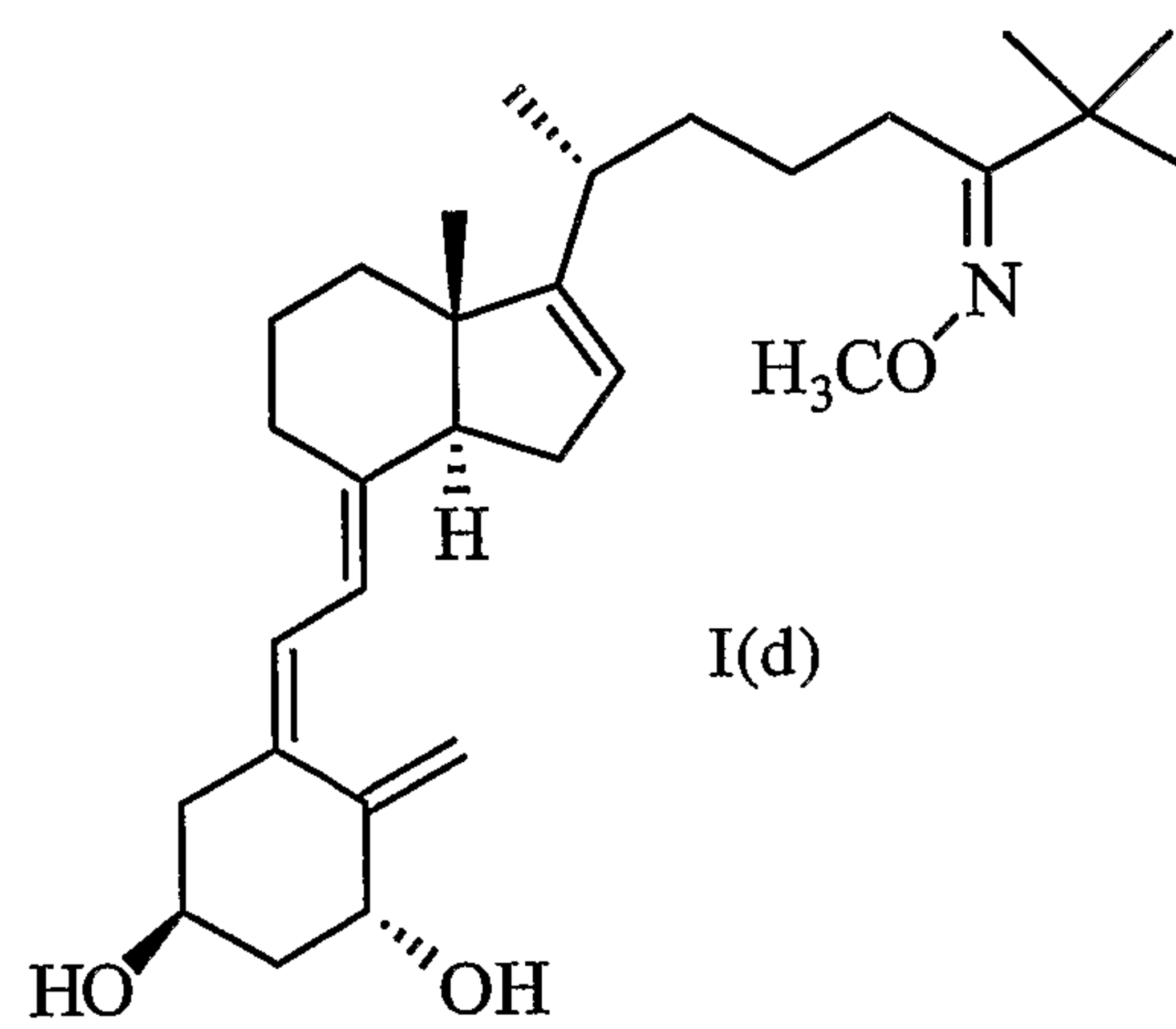
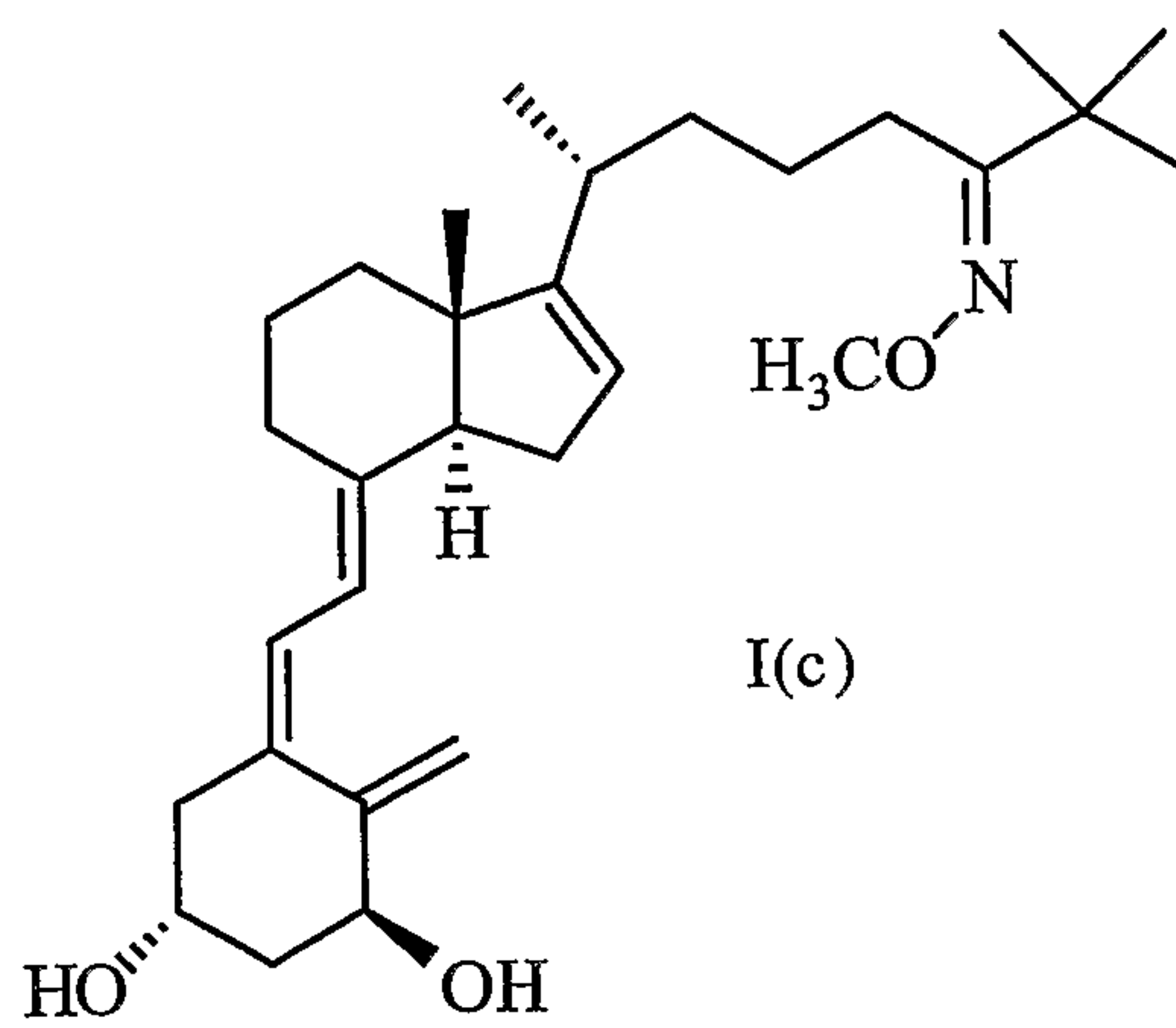
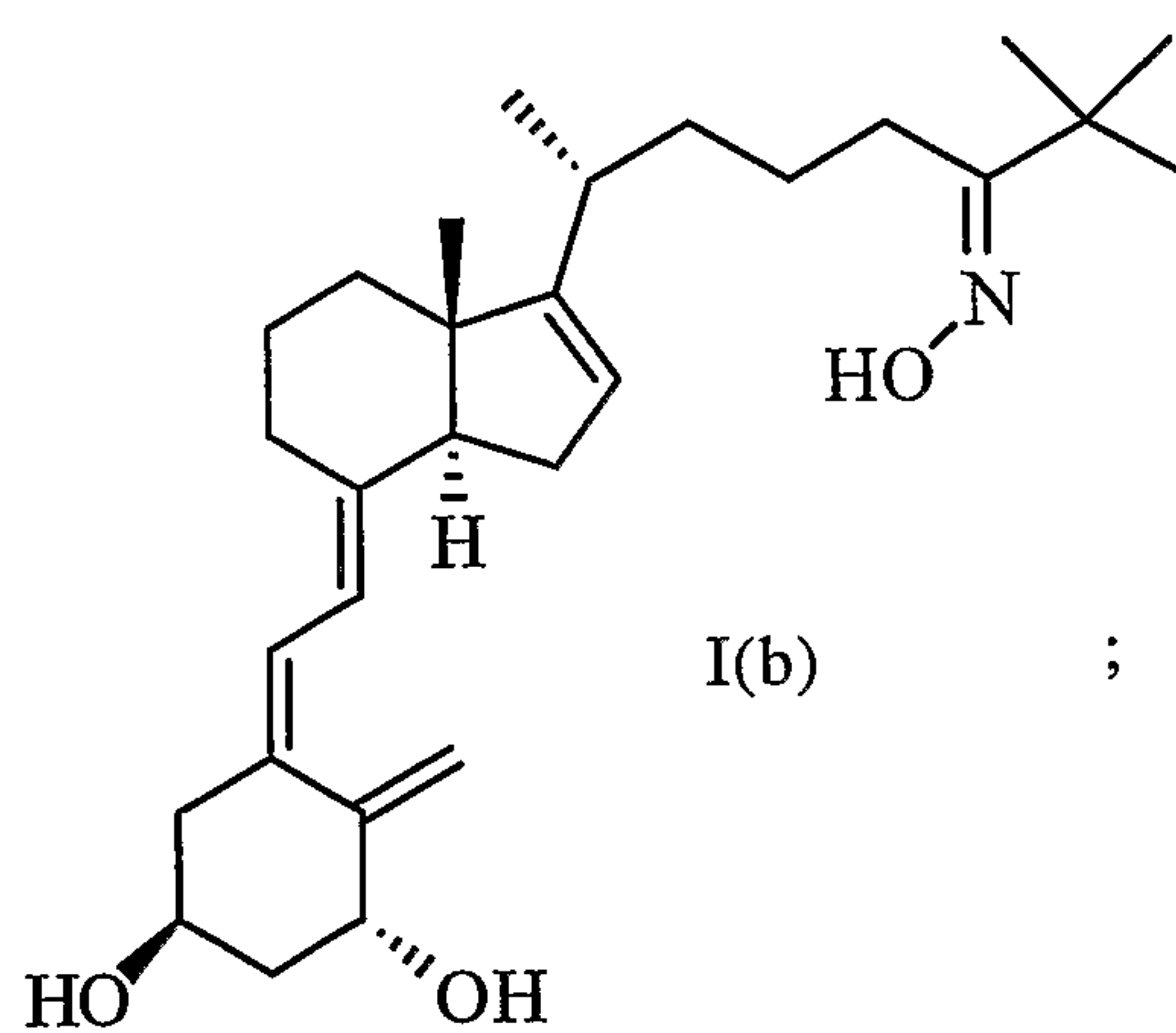
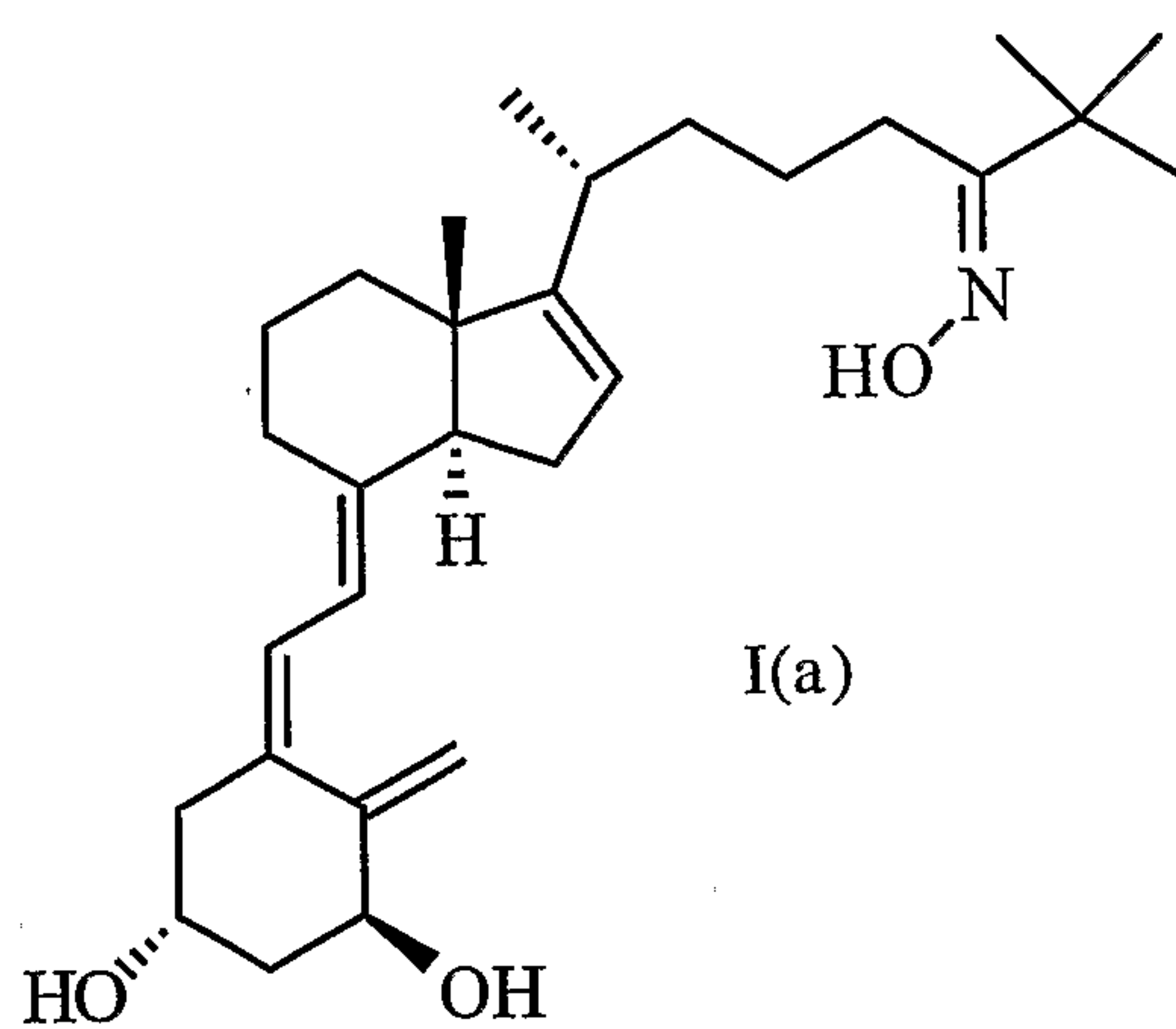
10. The compound according to claim 1, wherein both R⁶ are H.

30 11. The compound according to claim 1, having a relative stereochemistry as shown below:

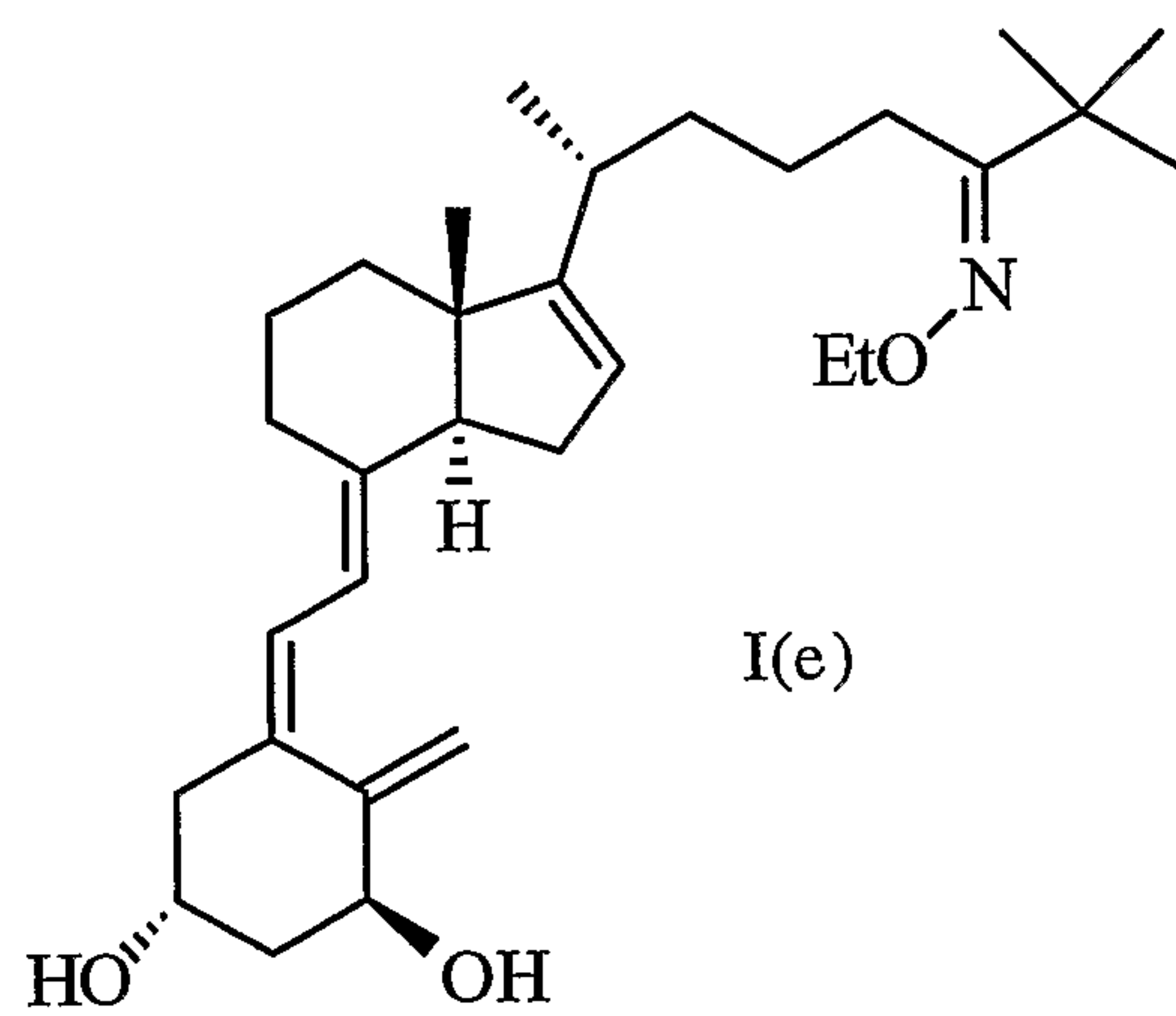
- 67 -



12. The compound according to claim 1 that is selected from the group consisting of:

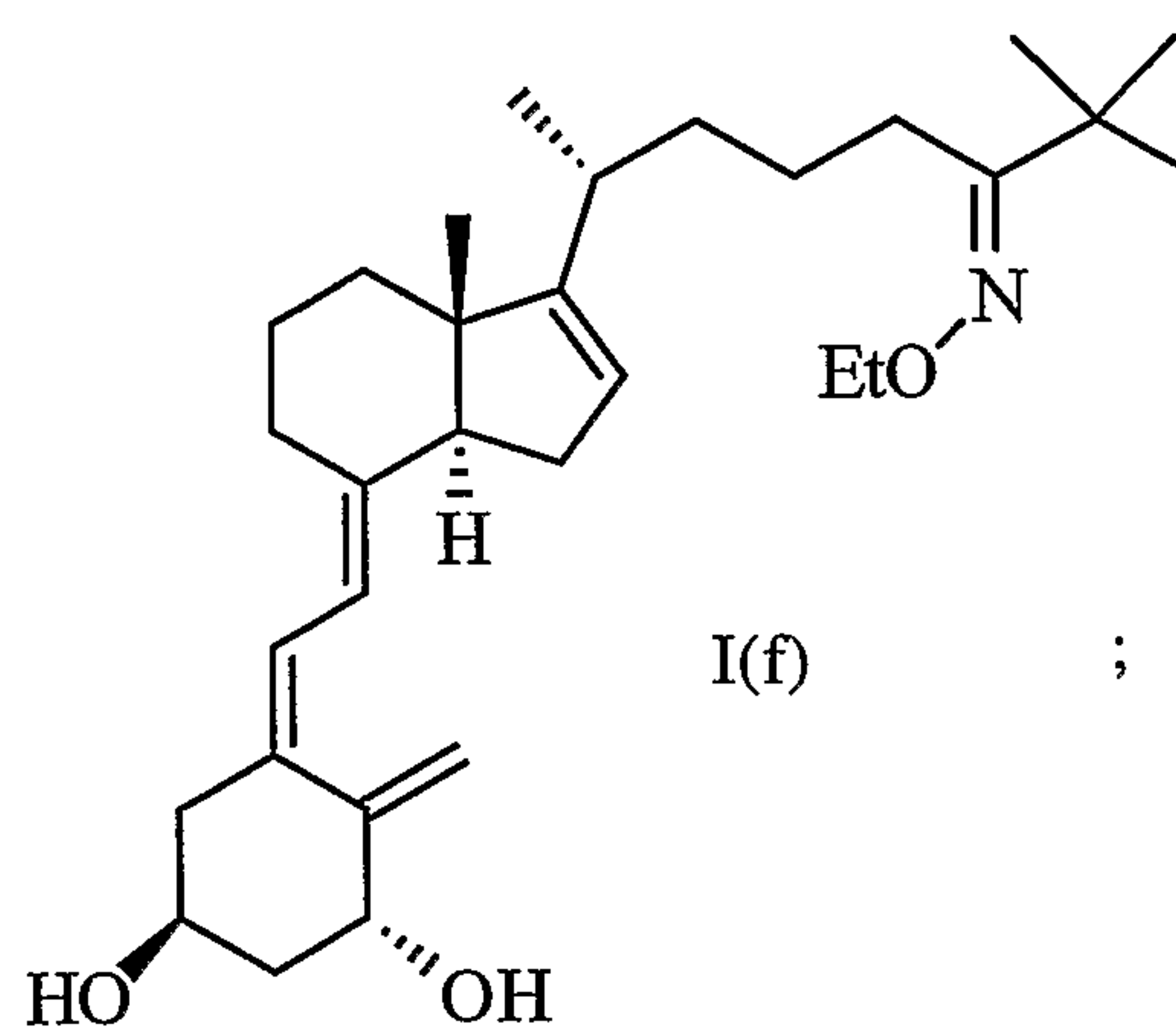


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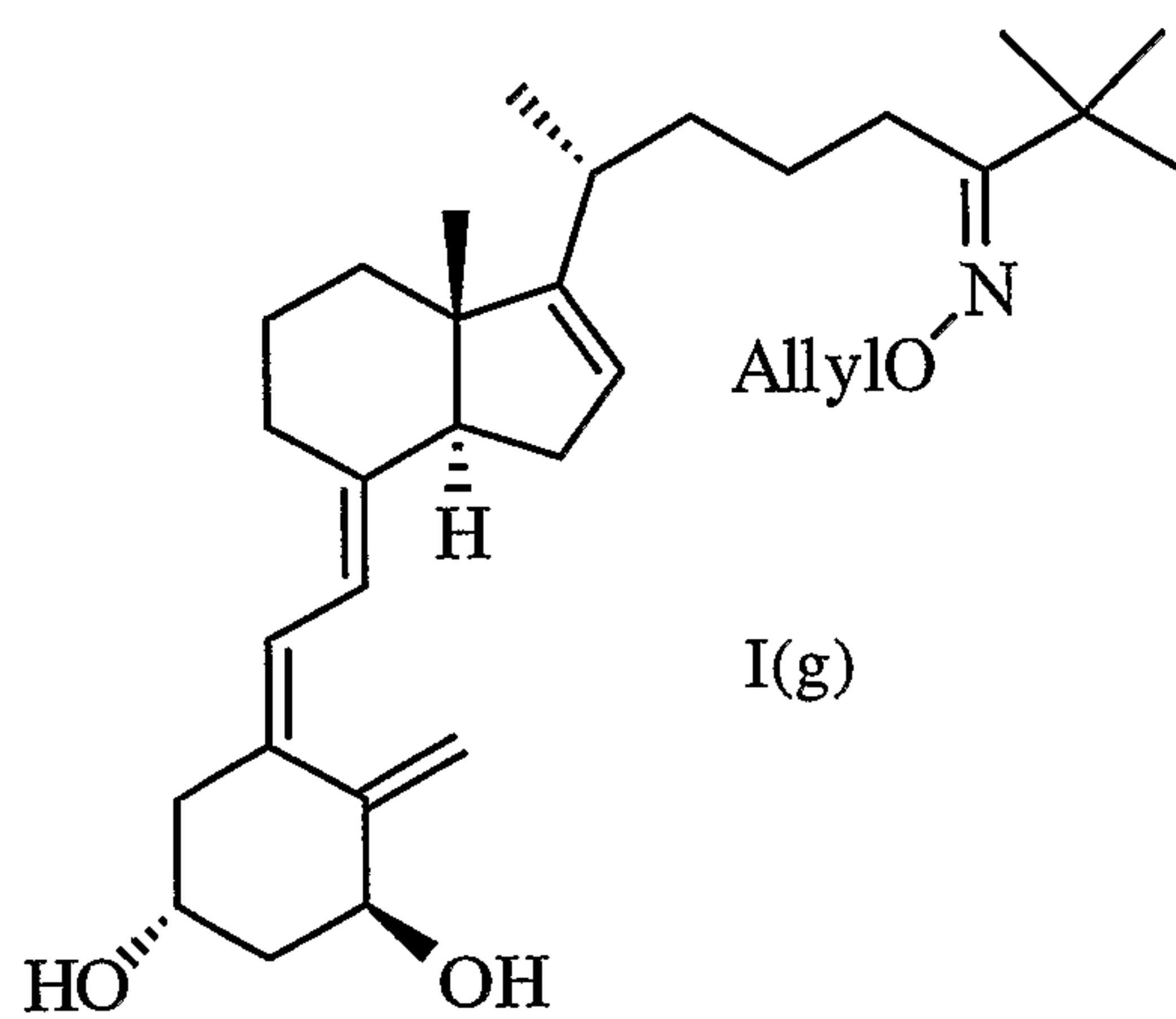
I(e)

;



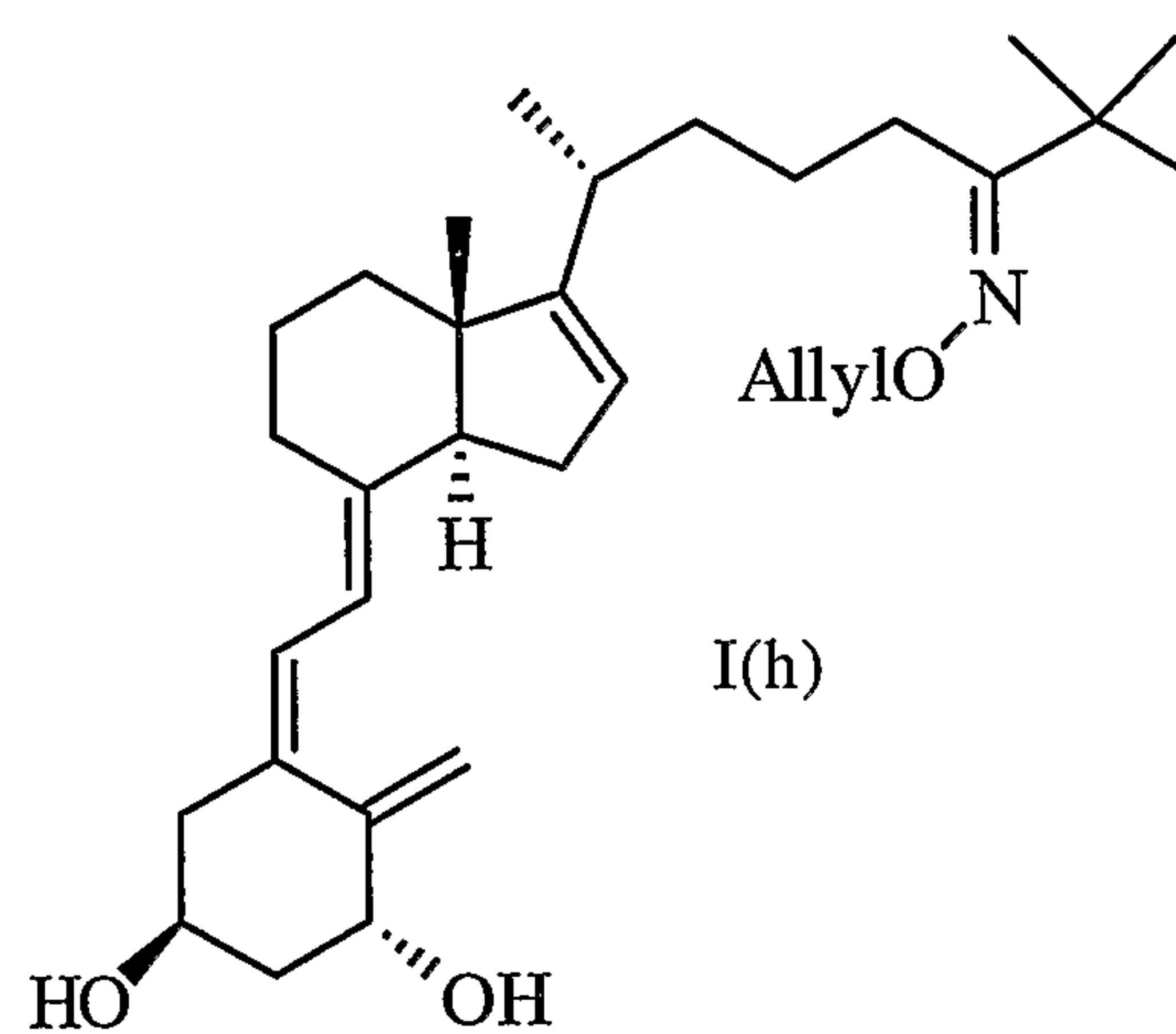
I(f)

;

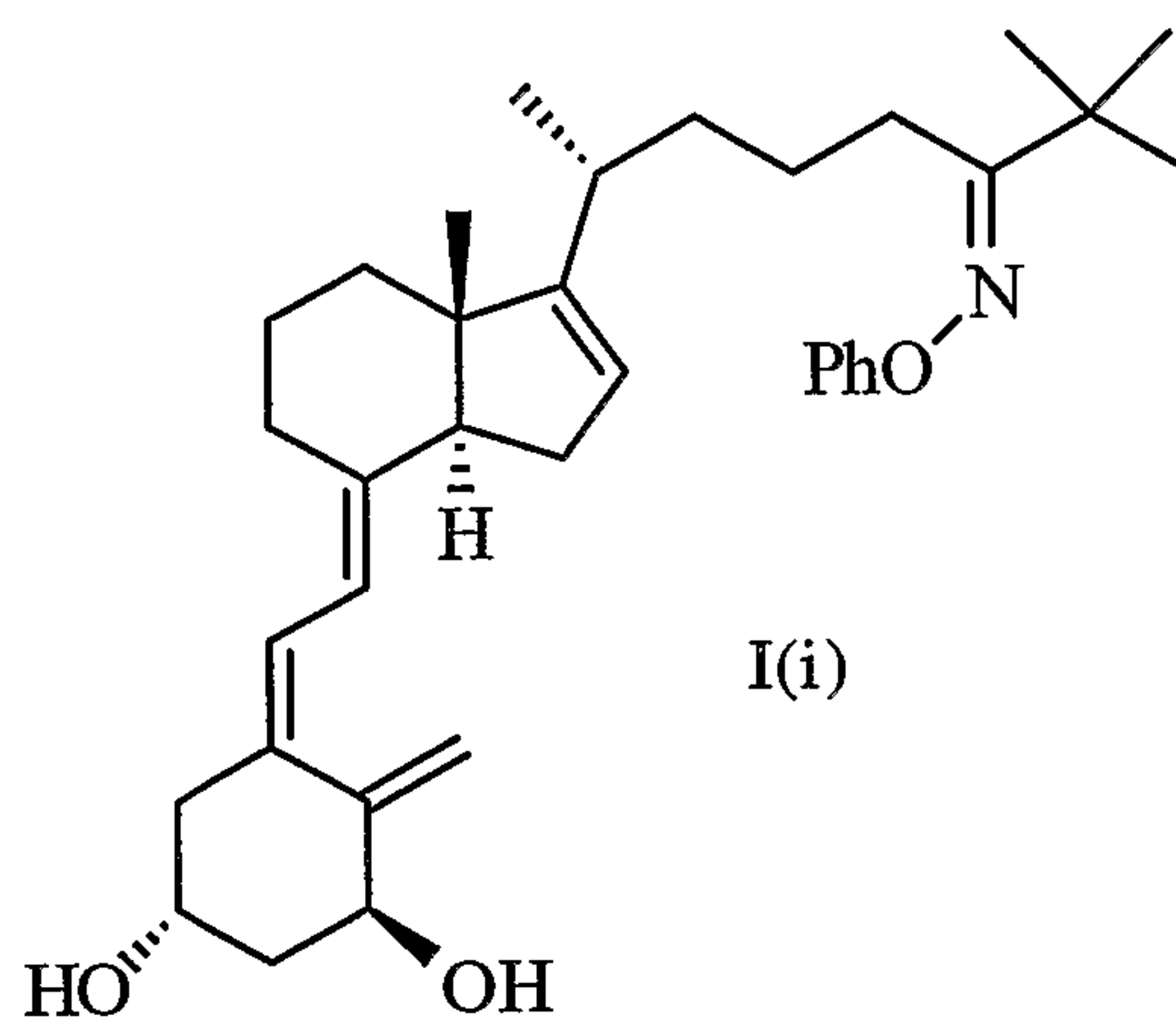


I(g)

;

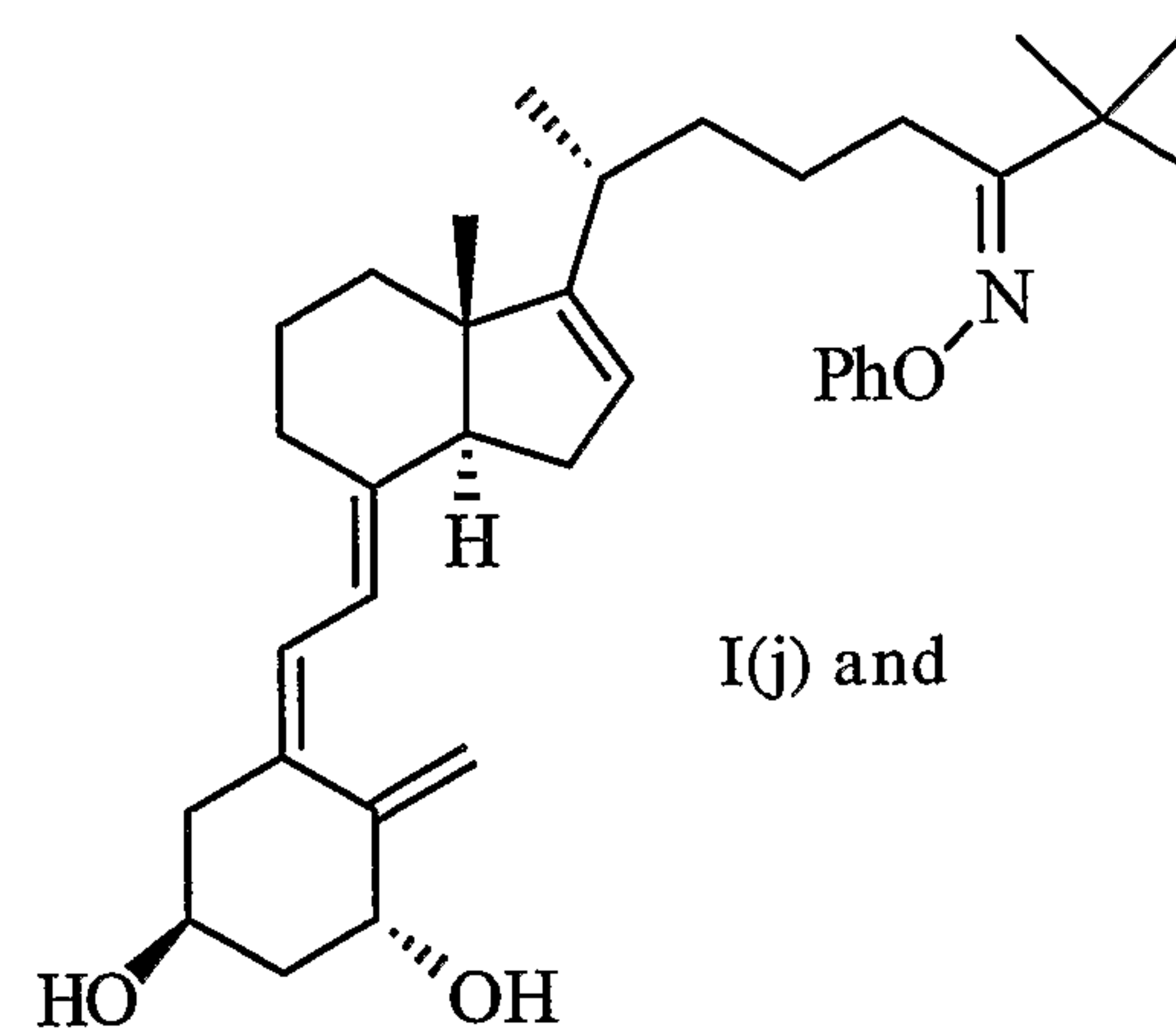


I(h)



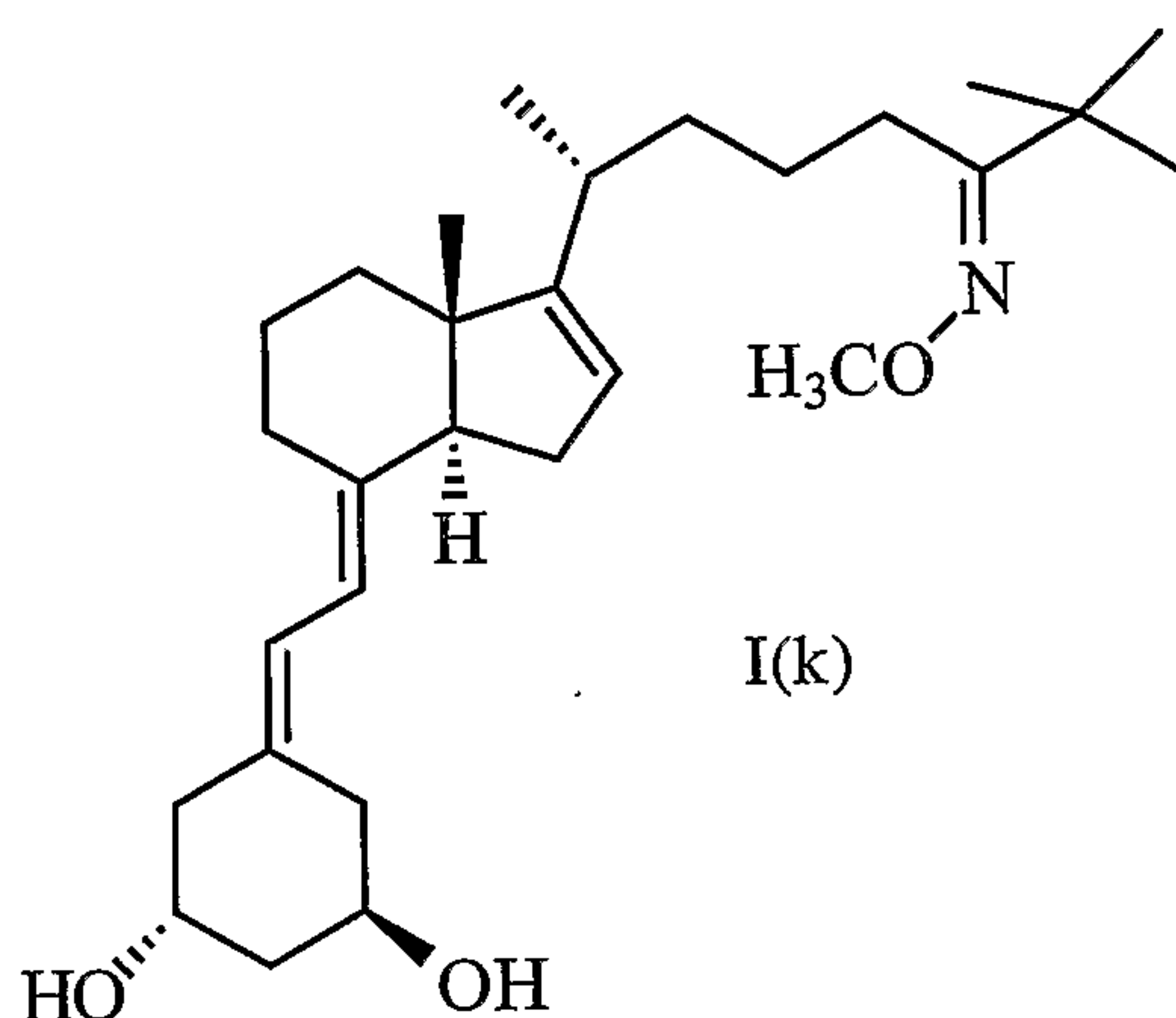
I(i)

;



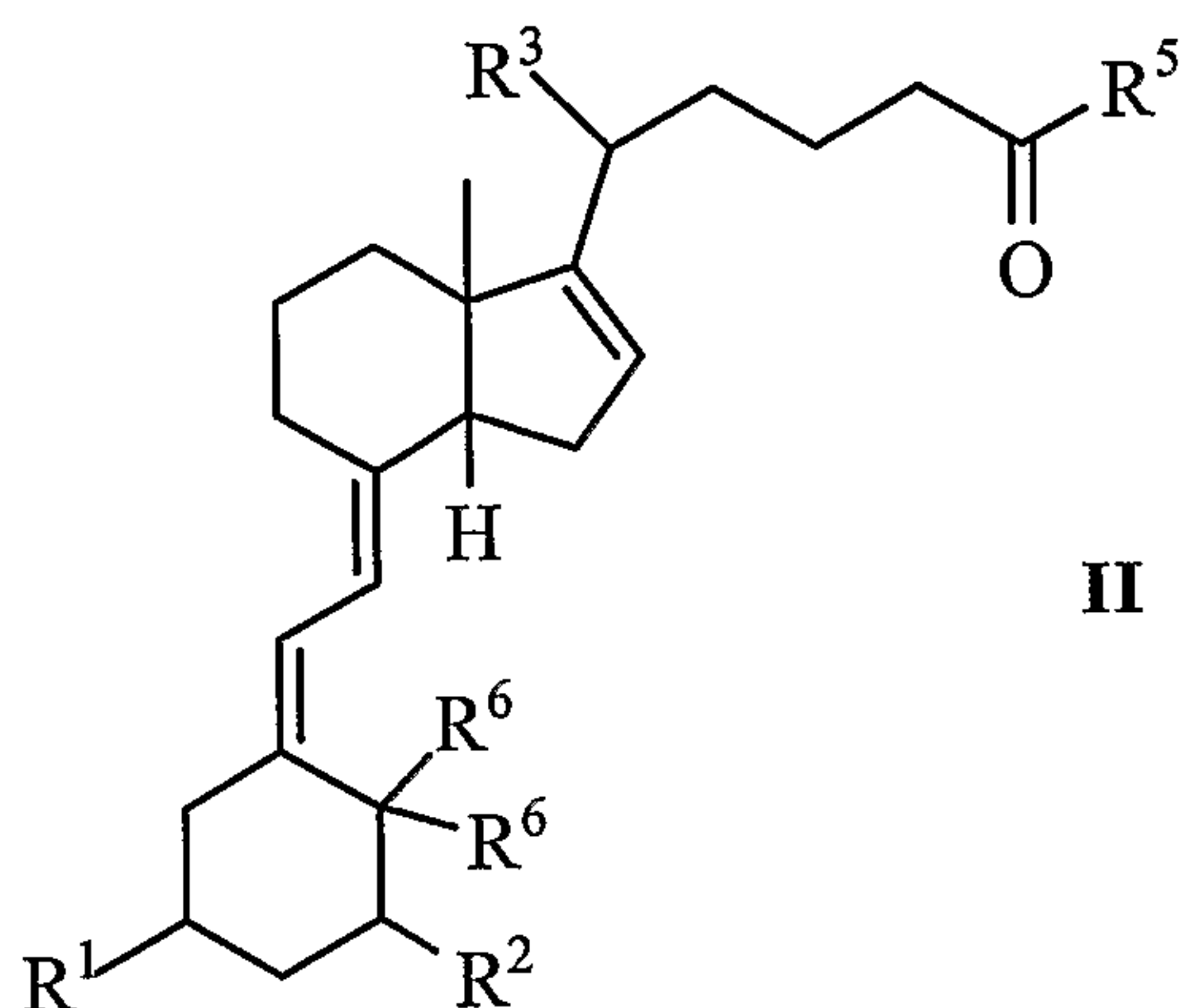
I(j) and

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13. The compound according to claim 11 that is selected from the group consisting of compound I(a), I(c), I(e), I(g), I(i) and I(k).

5 14. A compound of Formula II and salts, hydrates and solvates thereof:



wherein:

R¹ and R² are independently selected from the group consisting of OH, OC₁₋₆alkyl, OPG and halo;

10 PG is a protecting group;

R³ is C₁₋₆alkyl;

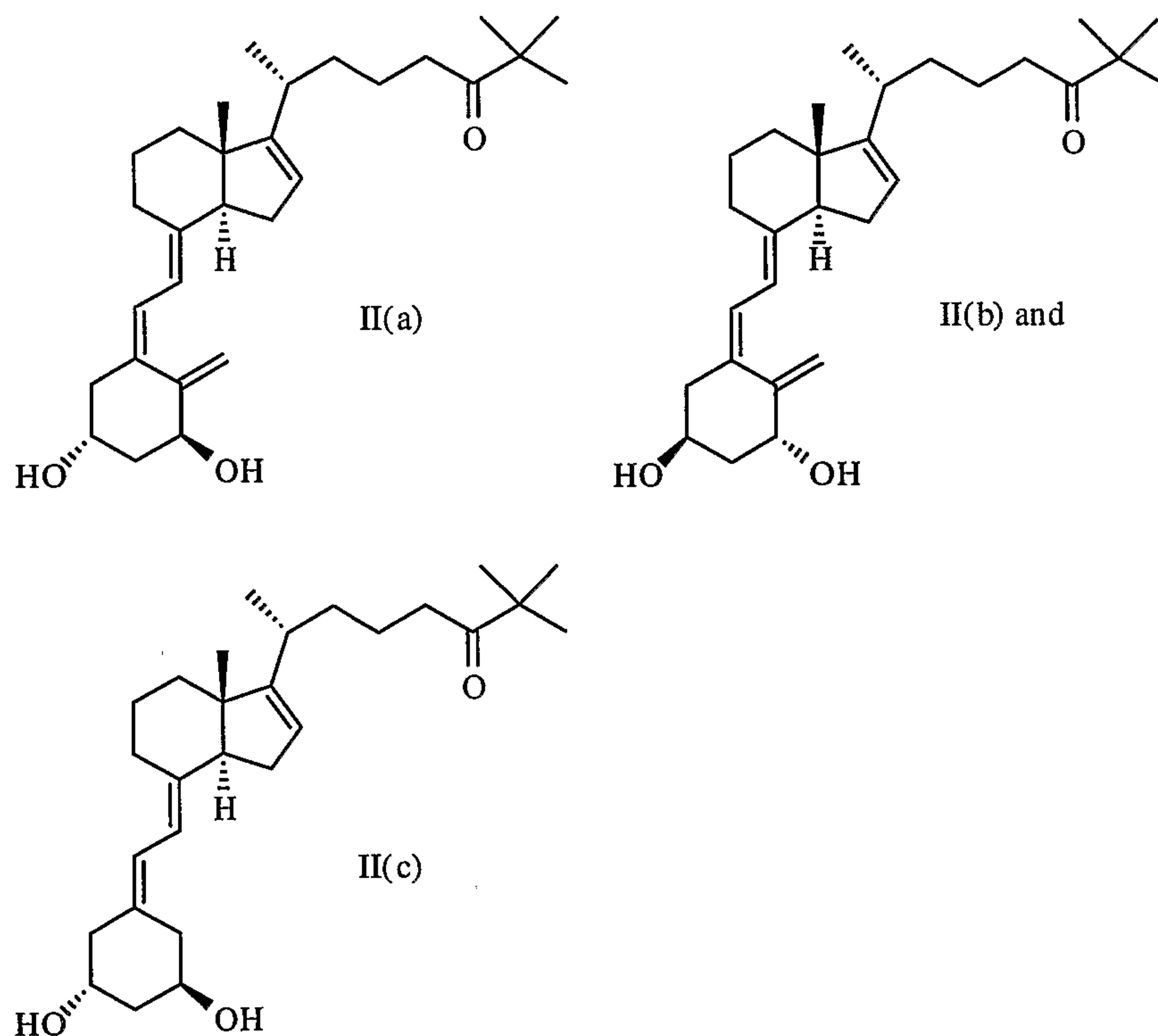
R⁵ is selected from the group consisting of C₁₋₆alkyl, cyclo(C₃₋₆)alkyl, aryl and heteroaryl, aryl-C₁₋₆alkyl and heteroaryl-C₁₋₆alkyl, with C₁₋₆alkyl being unsubstituted or substituted with 1-4 groups independently selected from C₁₋₄alkyl, OC₁₋₄alkyl, OH,

15 halo, NH₂, NHC₁₋₄alkyl and N(C₁₋₄alkyl)(C₁₋₄alkyl), and with cyclo(C₃₋₆)alkyl, aryl, heteroaryl, aryl-C₁₋₆alkyl and heteroaryl-C₁₋₆alkyl, being unsubstituted or substituted with 1-5 groups independently selected from C₁₋₄alkyl, OC₁₋₄alkyl, OH, CF₃, OCF₃, halo, SH, SC₁₋₄alkyl, NH₂, NHC₁₋₄alkyl, N(C₁₋₄alkyl)(C₁₋₄alkyl), CN, C(O)OH,

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C(O)OC₁₋₄alkyl, C(O)NHC₁₋₄alkyl, NHC(O)C₁₋₄alkyl, OC(O)C₁₋₄alkyl, SOC₁₋₄alkyl, SO₂C₁₋₄alkyl, SO₂NHC₁₋₄alkyl and SO₂NH₂; and R⁶ are either both H or together form =CH₂.

- 5 15. The compound according to claim 14, wherein R¹ and R² are independently selected from the group consisting of OH and OPG.
16. The compound according to any of claims 14-15, wherein R⁴ is C₁₋₄alkyl
- 10 17. The compound according to any of claims 14-16, wherein R⁵ is selected from the group consisting of isopropyl, s-butyl, t-butyl, and neopentyl.
18. The compound according to claim 14, selected from the group consisting of:



19. A pharmaceutical composition comprising a compound according to any of claims 1-13 and a pharmaceutically acceptable carrier.

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20. A method for treating diseases which benefit from a modulation of the levels of $1\alpha,25$ -dihydroxy vitamin D_3 comprising administering an effective amount of a compound according to any one of claims 1-13 to a cell or animal in need thereof.
- 5 21. A method for treating diseases which benefit from an inhibition of the catabolism of $1\alpha,25$ -dihydroxy vitamin D_3 comprising administering an effective amount of a compound according to any one of claims 1-13 to a cell or animal in need thereof.
- 10 22. The method according to claims 19-21, wherein the disease is selected from the group consisting of cancer, dermatological disorders and bone disorders.
23. The method according to claim 22, wherein the disease is selected from the group consisting of cancer, psoriasis and osteoporosis.
- 15 24. A method for inhibiting cell proliferation comprising administering an effective amount of a compound according to any of claims 1-13 to a cell or animal in need thereof.
- 20 25. The method according to claims 24, wherein the cell is a cancer cell.
26. The method according to claim 25, wherein the cancer is selected from breast cancer, lung cancer and prostate cancer.
- 25 27. A method of inhibiting CYP24 activity in a cell by administering an effective amount of a compound according to any of claims 1-13.
28. A use of a compound according to any of claims 1-13 to modulate the levels of $1\alpha,25$ -dihydroxy vitamin D_3 .
- 30 29. A use of a compound according to any of claims 1-13 to inhibit the catabolism of $1\alpha,25$ -dihydroxy vitamin D_3 .

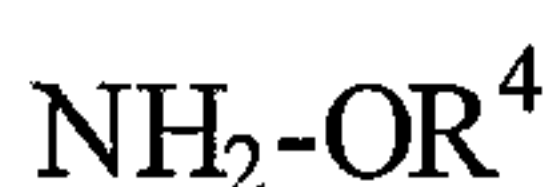
- 72 -

30. A use of a compound according to any of claims 1-13 to prepare a medicament to modulate the levels of $1\alpha,25$ -dihydroxy vitamin D_3 .
- 5 31. A use of a compound according to any of claims 1-13 to prepare a medicament to inhibit the catabolism of $1\alpha,25$ -dihydroxy vitamin D_3 .
32. A use of a compound according to any of claims 1-13 to inhibit cell proliferation.
- 10 33. A use of a compound according to any of claims 1-13 to prepare a medicament to inhibit cell proliferation.
- 15 34. A use of a compound according to any of claims 1-13 to inhibit CYP24 activity.
35. A use of a compound according to any of claims 1-13 to prepare a medicament to inhibit CYP24 activity.
- 20 36. A method for increasing the efficacy of a vitamin D receptor agonist comprising co-administering an effective amount of a compound according to any of claims 1-13 and an effective amount of the vitamin D receptor agonist.
- 25 37. The method according to claim 36, wherein the vitamin D receptor agonist is $1\alpha,25$ -dihydroxy vitamin D_3 (calcitriol).
38. A use of a compound according to any of claims 1-13 to increase the efficacy of a vitamin D receptor agonist.
- 30 39. A use of a compound according to any of claims 1-13 to prepare a medicament to increase the efficacy of a vitamin D receptor agonist.

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40. The use according to any of claims 38-39, wherein the vitamin D receptor agonist is $1\alpha,25$ -dihydroxy vitamin D₃ (calcitriol).

41. A method for preparing a compound of Formula I comprising reacting a
5 compound according to any of claims 14-18, with a compound of Formula III, or a salt hydrate of solvate thereof:



III

wherein R⁴ is selected from the group consisting of H, C₁₋₆alkyl, aryl and heteroaryl,
10 with C₁₋₆alkyl being unsubstituted or substituted with 1-4 groups independently selected from C₁₋₄alkyl, OC₁₋₄alkyl, OH, halo, NH₂, NHC₁₋₄alkyl and N(C₁₋₄alkyl)(C₁₋₄alkyl), and with aryl and heteroaryl being unsubstituted or substituted with 1-5 groups independently selected from C₁₋₄alkyl, OC₁₋₄alkyl, OH, CF₃, OCF₃, halo, SH, SC₁₋₄alkyl, NH₂, NHC₁₋₄alkyl, N(C₁₋₄alkyl)(C₁₋₄alkyl), CN, C(O)OH, C(O)OC₁₋₄alkyl,
15 C(O)NHC₁₋₄alkyl, NHC(O)C₁₋₄alkyl, OC(O)C₁₋₄alkyl, SOC₁₋₄alkyl, SO₂C₁₋₄alkyl, SO₂NHC₁₋₄alkyl and SO₂NH₂, in the presence of a non-nucleophilic amine; and removal of any protecting groups, if present.

42. The method according to claim 41, wherein the amine is pyridine.

Inhibition of CYP24

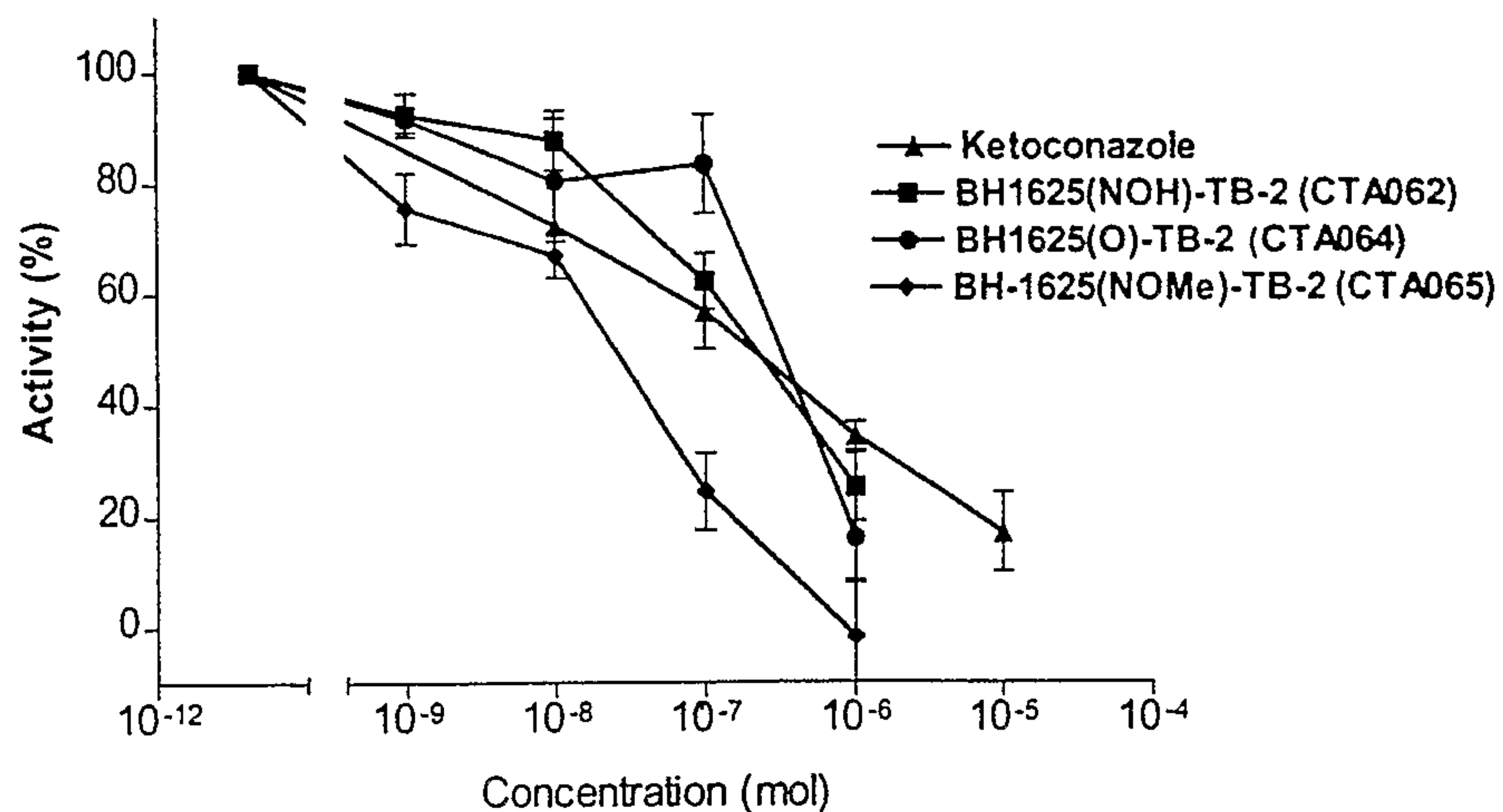


FIGURE 1A

Inhibition of CYP27B1

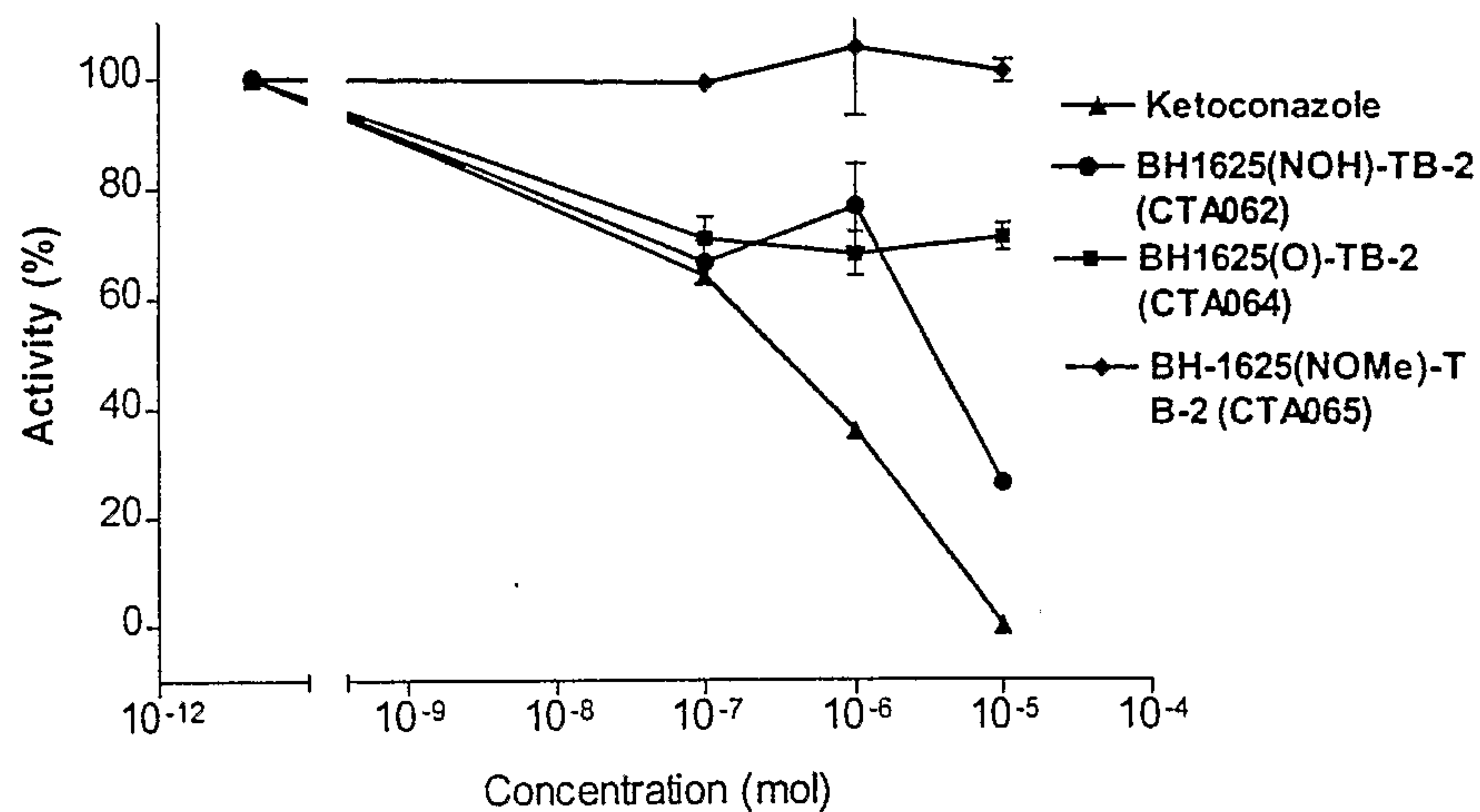


FIGURE 1B

Inhibition of CYP27A1

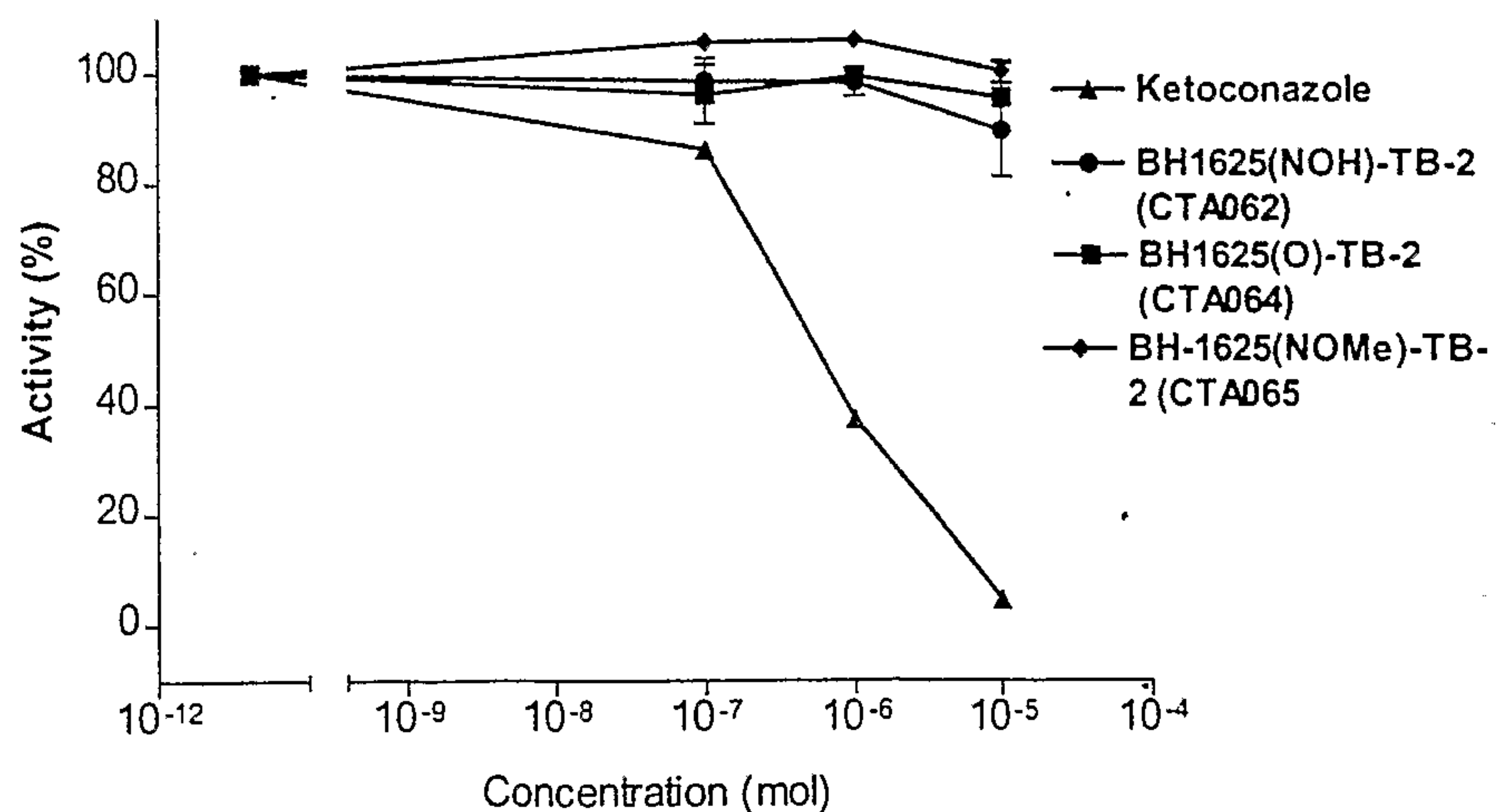


FIGURE 1C

FIGURE 2

DBP Binding Curve

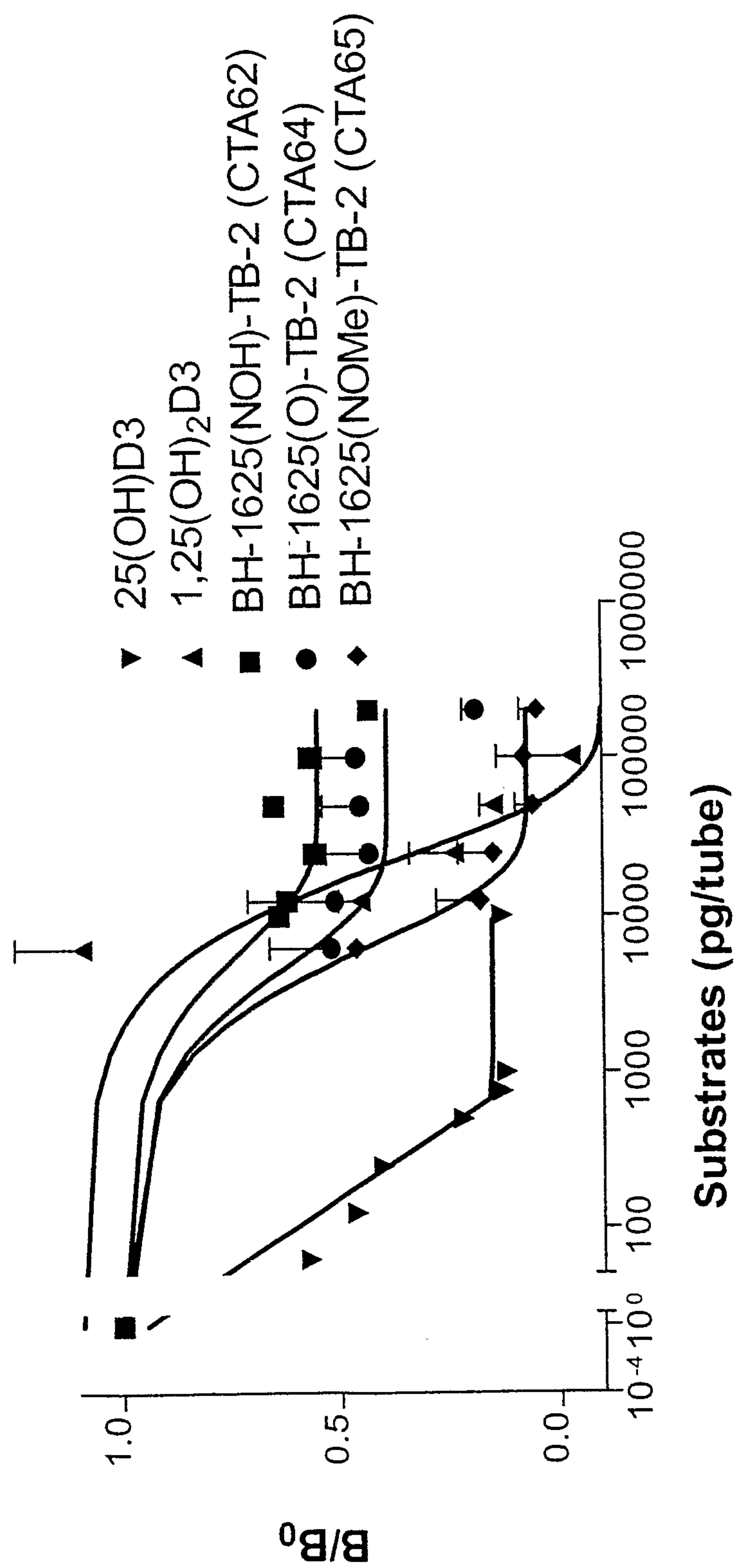


FIGURE 3
Transcription Assay

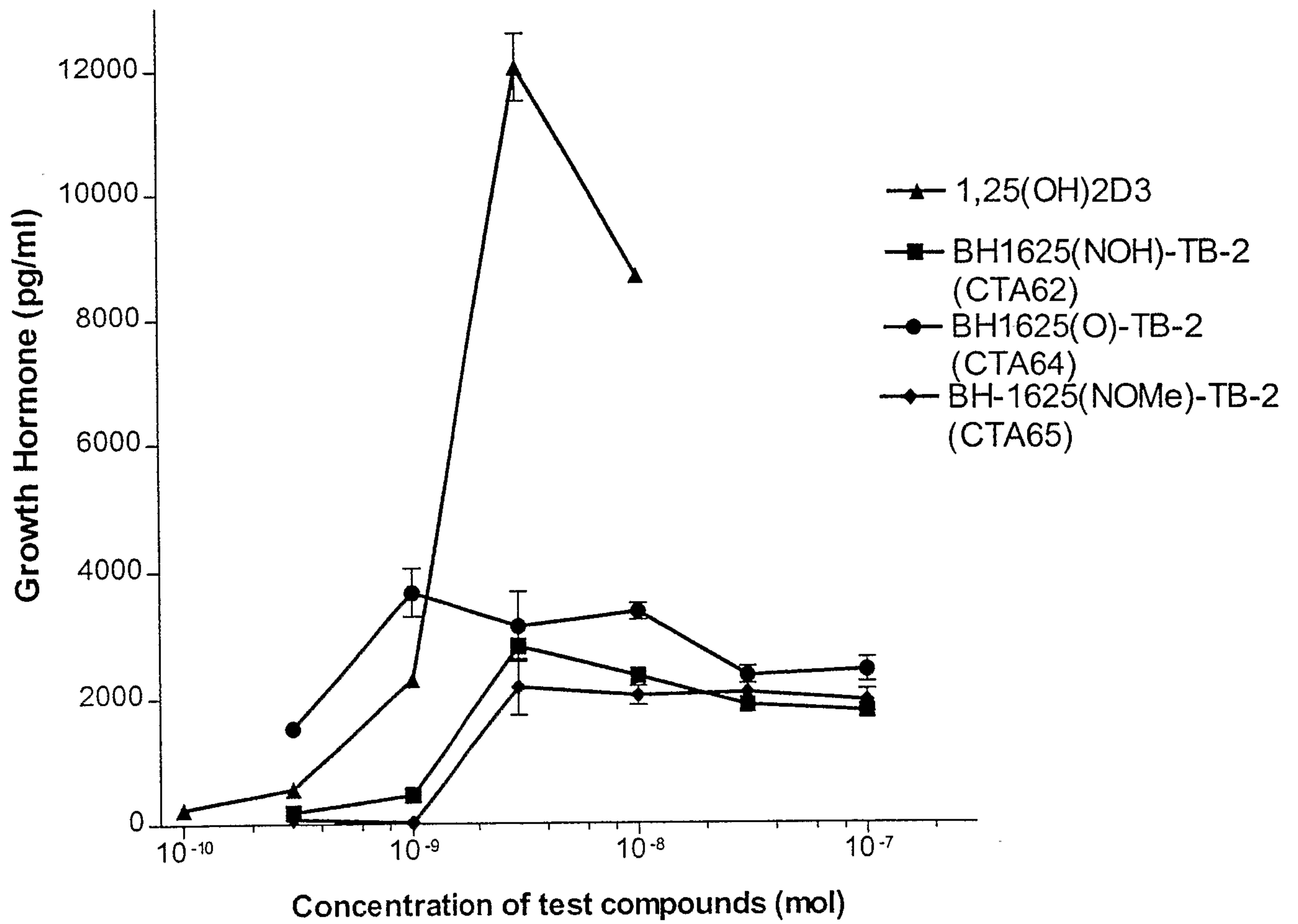


FIGURE 4

VDR Binding Assay

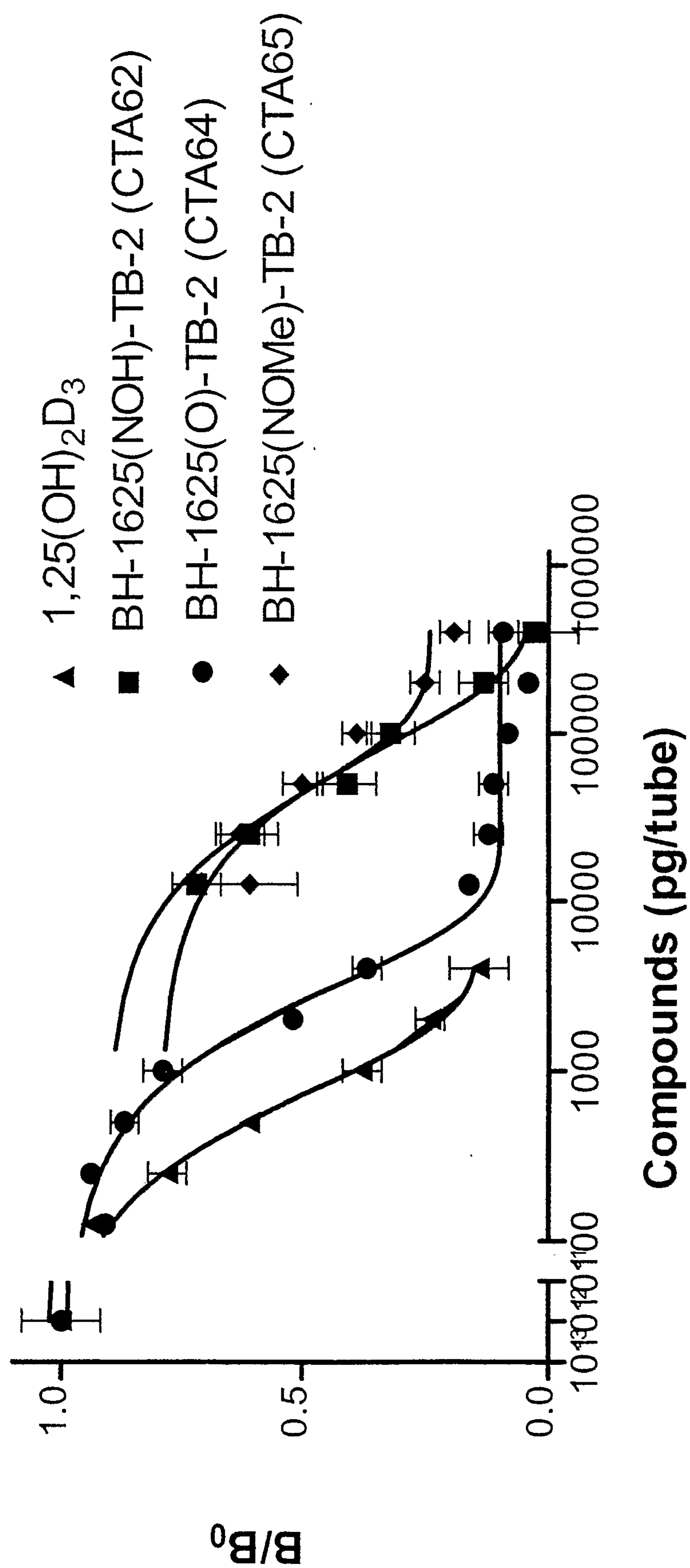


FIGURE 5
DOSE RESPONSE EFFECTS OF ANALOGS ON
KERATINOCYTE PROLIFERATION (96 HR.)

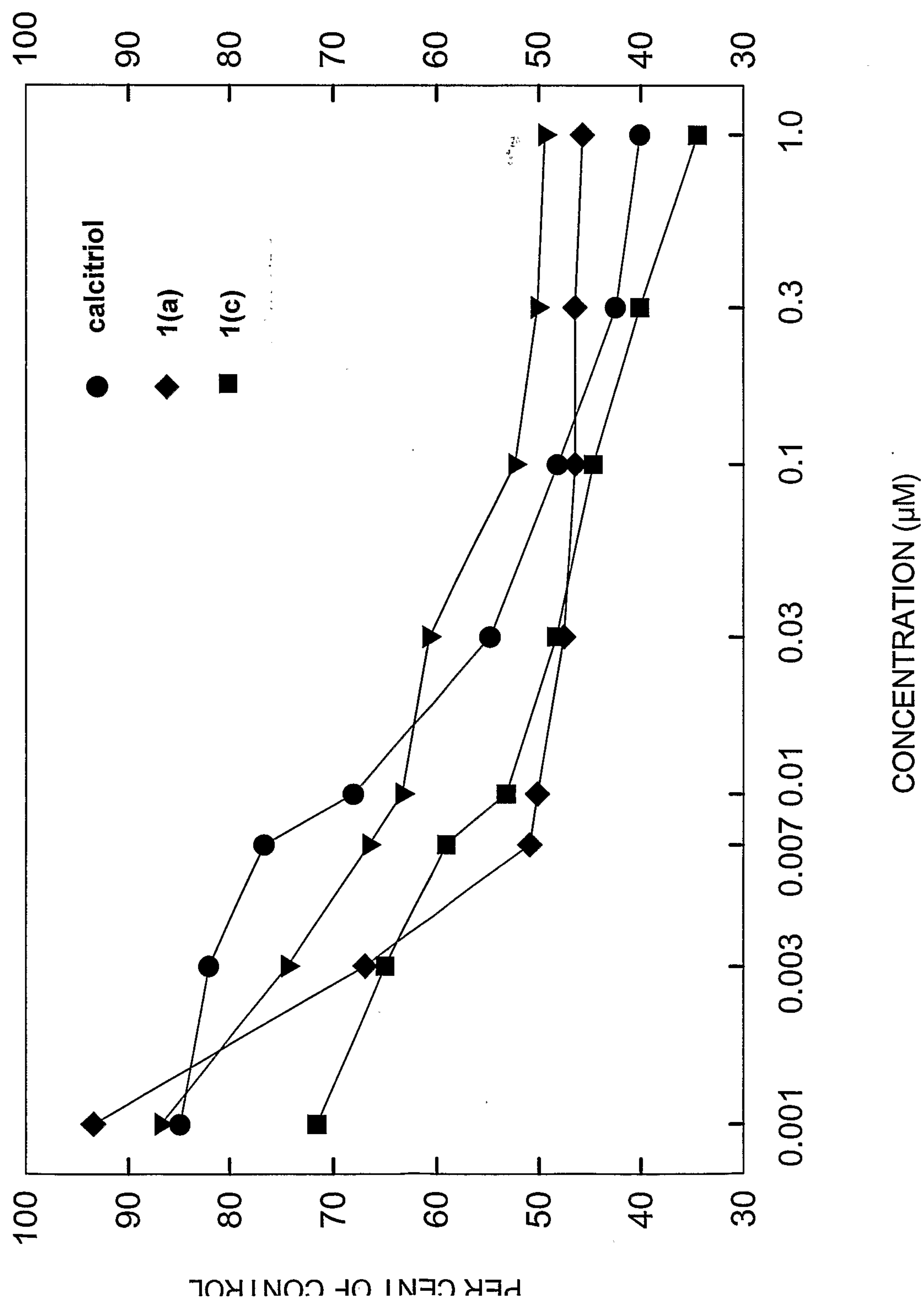
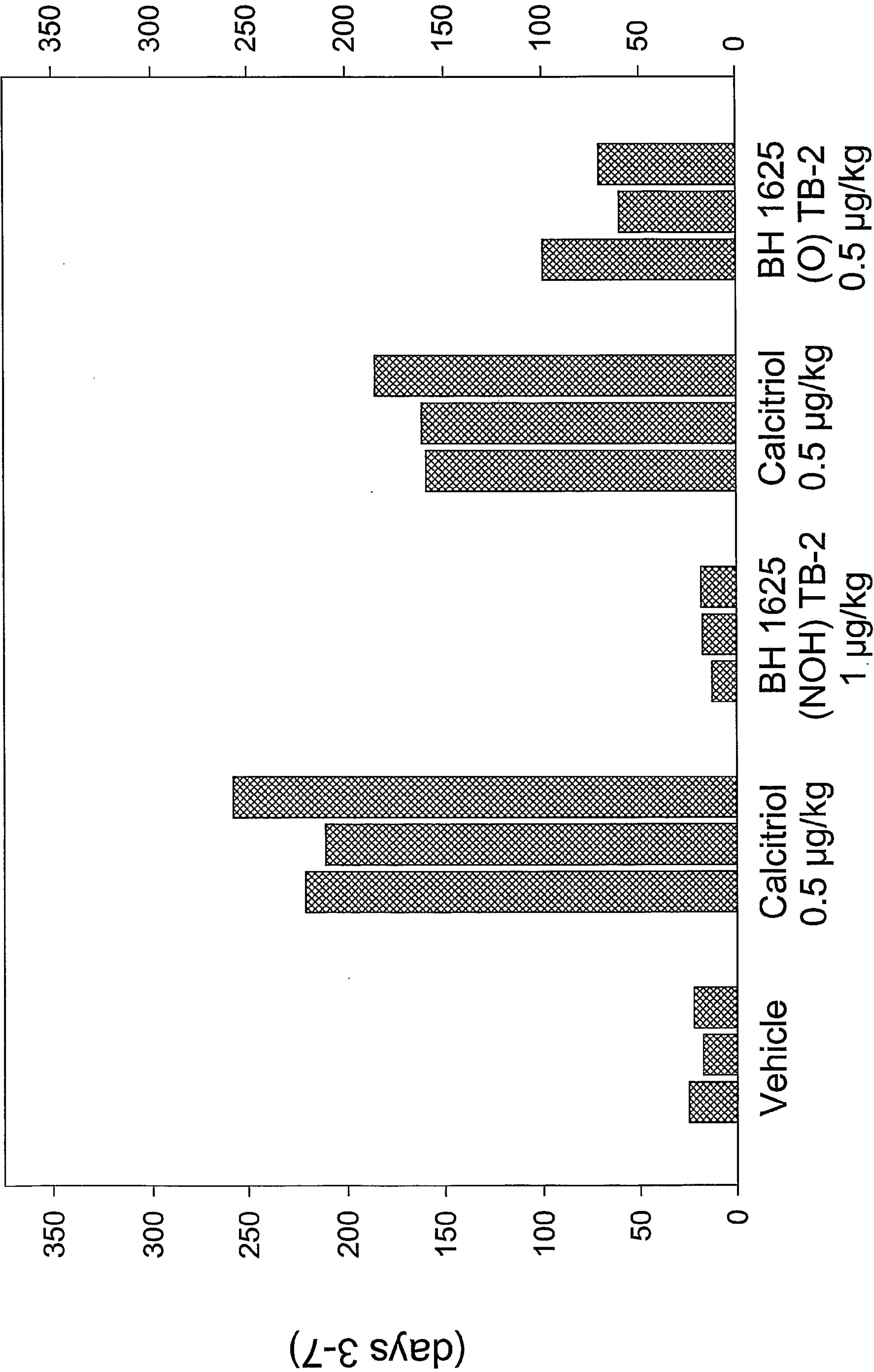


FIGURE 6
EFFECT OF VITAMIN D₃ ANALOGS ON CALCIUM
LEVELS IN RAT URINE



Inhibition of CYP24

