United States Patent [19]

Biller

[11] **Patent Number: 4,871,721**

[45] **Date of Patent: Oct. 3, 1989**

[54] **PHOSPHORUS-CONTAINING SQUALENE SYNTHETASE INHIBITORS**

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[73] Assignee: **E. R. Squibb & Sons, Inc.**, Princeton, N.J.

[21] Appl. No.: **141,744**

[22] Filed: **Jan. 11, 1988**

[51] **Int. Cl.**[4] **A61K 31/66**; C07F 9/40

[52] **U.S. Cl.** **514/102**; 558/134; 558/155; 562/21

[58] **Field of Search** ..... 260/302.4 P; 558/155; 514/102

[56] **References Cited**

PUBLICATIONS

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Faust, J. R. et al. *Proc. Nat. Acad Sci., U.S.A., "Squalene Synthetase Activity in Human Fibroblasts: Regulation via the Low Density Lipoprotein Receptor", 1979, 76, 5018–5022.*

de Montellano, P. Ortiz et al., *J. Med. Chem.*, "Inhibition of Squalene Synthetase by Farnesyl Pyrophosphate Analogues", 1977, 20, 243–249.

Corey and Volante, *J. Am. Chem. Cos.* "Application of Unreactive Analogs of Terpenoid Pyrophosphates to Studies of Multistep Biosynthesis, Demonstration that 'Presqualene Pyrophosphate' is an Essential Intermediate on the Path to Squalene", 1976, 98, 1291–3.

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Bertolino, A. et al., *Biochim. Biophys. Acta.*, 1978, 530, 17–23, "Polyisoprenoid Amphiphilic Compounds as Inhibitors of Squalene Synthesis and Other Microsomal Enzymes".

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McClard, R. W. et al., *J. Am. Chem. Soc.*, 1987, 109, 5544–5545, "Novel Phosphonylphosphinyl (P–C–P–C) Analogues of Biochemically Interesting Diphosphates, Syntheses and Properties of P–C–P–C Analogues of Isopentenyl Diphosphate and Dimethylallyl Diphosphate".

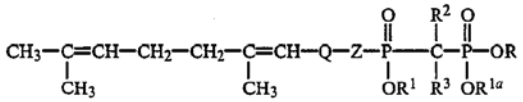
Capson, Phd Dissertation, Jun. 1987, Dept. of Med. Chem. Univ., Utah, Abstract, Table d Contents, pp. 16, 17, 40–43, 48–51, Summary.

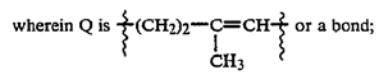
Primary Examiner—Anton H. Sutto

Attorney, Agent, or Firm—Burton Rodney

[57] **ABSTRACT**

Compounds which are useful as inhibitors of cholesterol biosynthesis and thus as hypocholesterolemic agents are provided which have the structure





Z is $-(CH_2)_n-$ or $-(CH_2)_p-CH=CH-(CH_2)_m-$, wherein n is 1 to 5; p is 0, 1 or 2; m is 0, 1 or 2;

R, R^1 and R^{1a} are the same or different and are H, lower alkyl or a metal ion; and

R^2 and R^3 may be the same or different and are H or halogen.

New intermediates used in preparing the above compounds and method for preparing same, pharmaceutical compositions containing such compounds and a method for using such compounds to inhibit cholesterol biosynthesis are also provided.

18 Claims, No Drawings

PHOSPHORUS-CONTAINING SQUALENE SYNTHETASE INHIBITORS

FIELD OF THE INVENTION

The present invention relates to new phosphorus-containing compounds which inhibit the activity of squalene synthetase and thus are useful in inhibiting cholesterol biosynthesis, to hypocholesterolemic compositions containing such compounds, to a method of using such compounds for inhibiting cholesterol biosynthesis, to new intermediates formed in the preparation of such compounds and to a method for preparing such compounds.

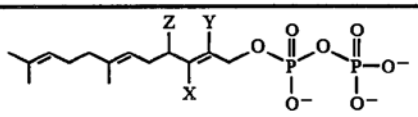
BACKGROUND OF THE INVENTION

Squalene synthetase is a microsomal enzyme which catalyzes the reductive dimerization of two molecules of farnesyl pyrophosphate (FPP) in the presence of nicotinamide adenine dinucleotide phosphate (reduced form) (NADPH) to form squalene (Poulter, C. D.; Rilling, H. C., in "Biosynthesis of Isoprenoid Compounds", Vol. I, Chapter 8, pp. 413–441, J. Wiley and Sons, 1981 and references therein). This enzyme is the first committed step of the de novo cholesterol biosynthetic pathway. The selective inhibition of this step should allow the essential pathways to isopentenyl tRNA, ubiquinone, and dolichol to proceed unimpeded. Squalene synthetase, along with HMG-CoA reductase has been shown to be down-regulated by receptor mediated LDL uptake (Faust, J. R.; Goldstein, J. L.; Brown, M. S. *Proc. Nat. Acad. Sci. USA*, 1979, 76, 5018–5022), lending credence to the proposal that inhibiting squalene synthetase will lead to an up-regulation of LDL receptor levels, as has been demonstrated for HMG-CoA reductase, and thus ultimately should be useful for the treatment and prevention of hypercholesterolemia and atheroschlerosis.

One approach to inhibitors of squalene synthetase is to design analogs of the substrate FPP. It is clear from the literature that the pyrophosphate moiety is essential for binding to the enzyme. However, such pyrophosphates are unsuitable as components of pharmacological agents due to their chemical and enzymatic liability towards allylic C-O cleavage, as well as their susceptibility to metabolism by phosphatases.

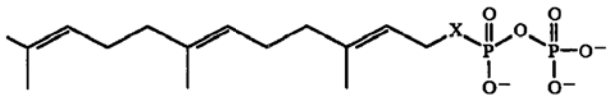
P. Ortiz de Montellano et al in *J. Med. Chem.*, 1977, 20, 243–249 describe the preparation of a series of substituted terpenoid pyrophosphate (Table A), and have shown these to be competitive inhibitors of the squalene synthetase enzyme. These substances retain the unstable allylic pyrosphosphate moiety of FPP.

TABLE A

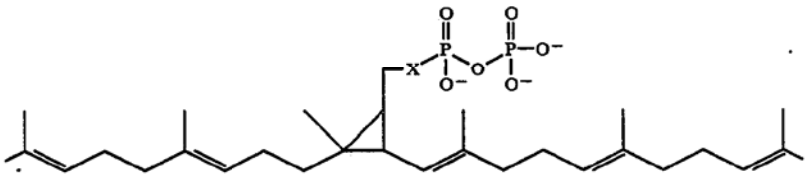


No.	X	Y	Z
1	CH_3	CH_3	H
2	H	H	H
3	C_2H_5	H	H
4	I	H	H
5	H	I	H
6	CH_3	H	SCH_3

Corey and Volante, *J. Am. Chem. Soc.* 1976, 98, 1291–3, have prepared FPP analog A and presqualene pyrophosphate (PSQ-PP) analog B as inhibitors of squalene biosynthesis. (Presqualene pyrophosphate is an intermediate in the conversion of FPP to squalene). These inhibitors possess methylene groups in place of the allylic oxygen moiety of FPP and PSQ-PP, but still retain the chemically and enzymatically unstable pyrophosphate linkage.

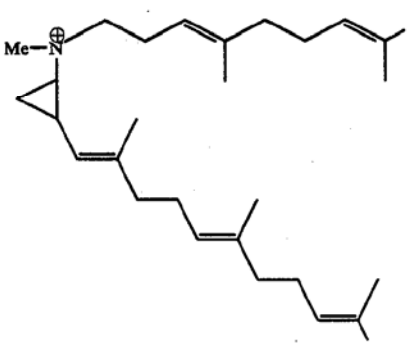


A X = CH_2
FPP X = O



B X = CH_2
PSQ—PP X = O

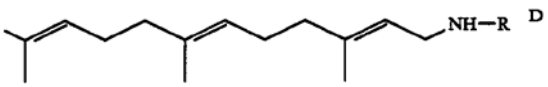
Poulter and co-workers have prepared cyclopropane C (Sandifer, R. M., et al., *J. Am. Chem. Soc.* 1982, 104, 7376–8) which in the presence of inorganic pyrophosphate is an intermediate analog inhibitor of the enzyme squalene synthetase.



C

Altman and co-workers, Bertolino, A., et al., *Biochim. Biophys. Acta.* 1978, 530, 17–23, reported that farnesyl amine and related derivatives D inhibit squalene synthetase, but provide evidence that this inhibition

is non-specific and probably related to membrane disruption.

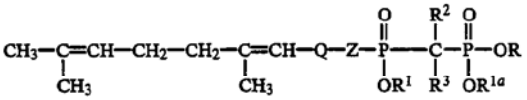


D

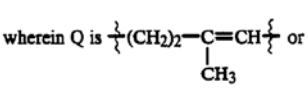
R = H, CH_2CH_2OH, $CH_2CH_2OCH_3$

DESCRIPTION OF THE INVENTION

In accordance with the present invention, there is provided phosphorus-containing compounds which inhibit the enzyme squalene synthetase and thus are useful as hypocholesterolemic agents and have the following structure



I.

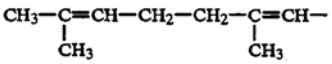
wherein Q is  or a bond;

Z is $-(CH_2)_n-$ or $-(CH_2)_p-CH=CH-(CH_2)_m-$, wherein n is 1 to 5; p is 0, 1 or 2; m is 0, 1 or 2;

R, R^1 and R^{1a} may be the same or different and are H, lower alkyl or a metal ion; and

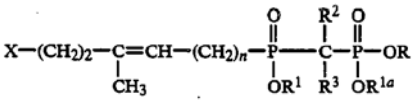
R^2 and R^3 may be the same or different and are H or halogen.

Hereinafter the moiety

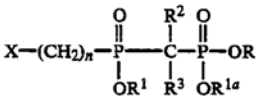


will be expressed as "X" in the structural formulae set out below.

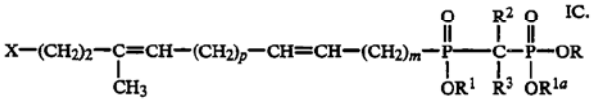
Thus, the following types of compounds are included within the scope of the present invention.



IA.



IB.



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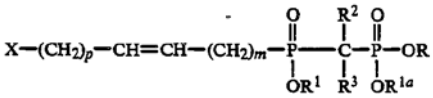


ID.

The term "lower alkyl" or "alkyl" as employed herein alone or as part of another group includes both straight and branched chain hydrocarbons, containing 1 to 12 carbons in the normal chain, preferably 1 to 7 carbons, such as methyl, ethyl, propyl, isopropyl, butyl, t-butyl, isobutyl, pentyl, hexyl, isohexyl, heptyl, 4,4-dimethylpentyl, octyl, 2,2,4-trimethylpentyl, nonyl, decyl, undecyl, dodecyl, the various branched chain isomers thereof, and the like. The lower alkyl or alkyl group may be substituted with a substituent including a halo-substituent, such as F, Br, Cl or I or CF_3, an alkoxy substituent, an aryl substituent, an alkyl-aryl substituent, a haloaryl substituent, a cycloalkyl substituent, an alkylcycloalkyl substituent, hydroxy, and alkylamino substituent, an alkanoylamino substituent, an arylcarbonylamino substituent, a nitro substituent, a cyano substituent, a thio substituent or an alkylthio substituent.

The term "cycloalkyl" as employed herein alone or as part of another group includes saturated cyclic hydrocarbon groups containing 3 to 12 carbons, preferably 3 to 8 carbons, which include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclodecyl and cyclododecyl, any of which groups may be substituted with 1 or 2 halogens, 1 or 2 lower alkyl groups, 1 or 2 alkoxy groups, 1 or 2 hydroxy groups, 1 or 2 alkylamino groups, 1 or 2 alkanolyamino groups, 1 or 2 arylcarbonylamino groups, 1 or 2 amino groups, 1 or 2 nitro groups, 1 or 2 cyano groups, 1 or 2 thiol groups, and/or 1 2 alkylthio groups.

The term "aryl" or "Ar" as employed herein refers to monocyclic or bicyclic aromatic groups containing from 6 to 10 carbons in the ring portion, such as phenyl, naphthyl, substituted phenyl or substituted naphthyl wherein the substituent on either the phenyl or naphthyl may be 1, 2 or 3 lower alkyl groups, halogens (Cl, Br or F), 1, 2 or 3 lower alkoxy groups, 1, 2 or 3 hydroxy groups, 1, 2 or 3 phenyl groups, 1, 2 or 3 alkanoyloxy groups, 1, 2 or 3 benzoyloxy groups, 1, 2 or 3 haloalkyl groups, 1, 2 or 3 halophenyl groups, 1, 2 or 3 allyl groups, 1, 2 or 3 cycloalkylalkyl groups, 1, 2 or 3 adamantylalkyl groups, 1, 2 or 3 alkylamino groups, 1, 2 or 3 alkanoylamino groups, 1, 2 or 3 arylcarbonylamino groups, 1, 2 or 3 amino groups, 1, 2 or 3 nitro groups, 1, 2 or 3 cyano groups, 1, 2 or 3 thiol groups, and/or 1, 2 or 3 alkylthio groups with the aryl group preferably containing 3 substituents.

The term "aralkyl", "aryl-alkyl" or "aryl-lower alkyl" as used herein alone or as part of another group refers to lower alkyl groups as discussed above having an aryl subsitutent, such as benzyl.

The term "lower alkoxy", "alkoxy", or "aryloxy" or "aralkoxy" as employed herein alone or as part of an-

other group includes any of the above lower alkyl, alkyl, aralkyl or aryl groups linked to an oxygen atom.

The term "lower alkylthio", "alkylthio", "arylthio" or "aralkylthio" as employed herein alone or as part of another group includes any of the above lower alkyl, alkyl, aralkyl or aryl groups linked to a sulfur atom.

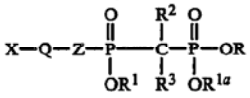
The term "lower alkylamino", "alkylamino", "arylamino", "arylalkylamino" as employed herein alone or as part of another group includes any of the above lower alkyl, alkyl, aryl or arylalkyl groups linked to a nitrogen atom.

The term "alkanoyl" as used herein as part of another group refers to lower alkyl linked to a carbonyl group.

The term "halogen" or "halo" as used herein refers to chlorine, bromine, fluorine, iodine and CF_3, with chlorine or fluorine being preferred.

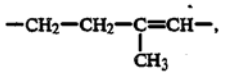
The term "metal ion" refers to alkali metal ions such as sodium, potassium or lithium and alkali earth metal ions such as magnesium.

Preferred are those compounds of formula I which have the following structure



IE.

wherein Q is

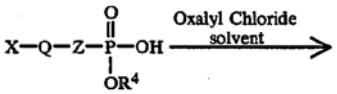


Z is $-CH_2CH_2-$ or

$-CH=CH-$; R^2 and R^3 are each H or each F; R, R^1 and R^{1a} are OH or metal ions.

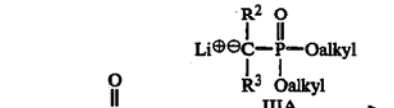
The compounds of formula I of the invention may be prepared according to the following reaction sequence and description thereof.

A. Preparation of Compounds of Invention

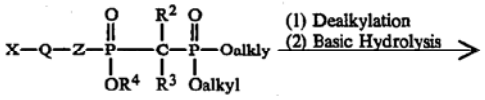


(where R^4 is alkyl) (Acid Cl Formation)

II



III (Phosphinyl-Phosphonate Formation)



IF.

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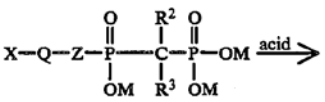
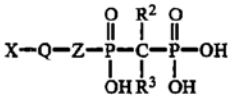


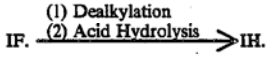
(where M is a metal ion)

IG.



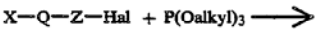
IH.

A(1) Alternative Preparation of Compounds of Invention



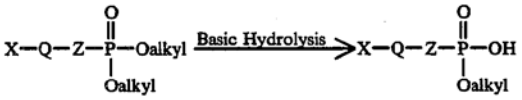
B. Preparation of Starting Materials

(1) Where Z is $(CH_2)_n$ and n is 1 to 5 or Z is $(CH_2)_p-CH=CH-(CH_2)_m-$ and p is 0 to 2 and m is 1 or 2



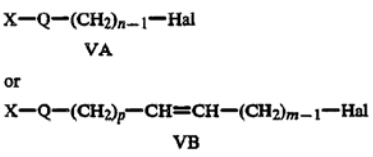
V (wherein Hal is a halogen such as Cl, Br or I)

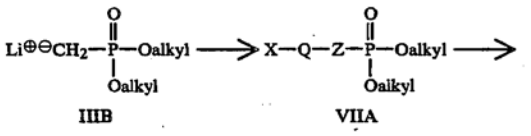
VI



VII II

(2) Where Z is $(CH_2)_n$ and n is 2 to 5 or Z is $-(CH_2)_p-CH=CH-(CH_2)_m-$ and p is 0 to 2 and m is 2





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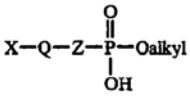
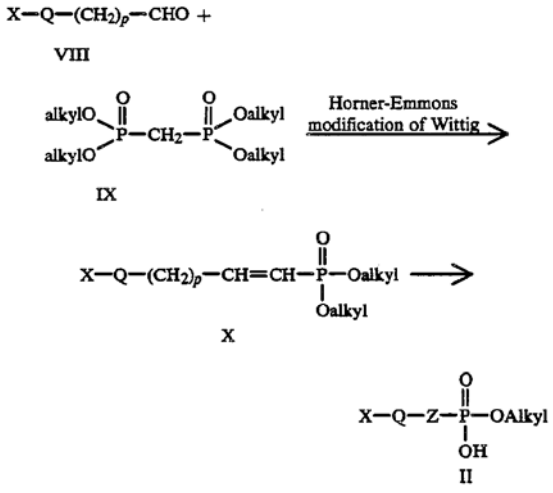


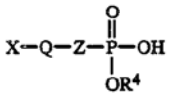
II

(wherein Hal is a halogen such as Br or I)

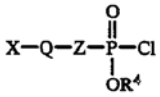
(3) Where Z is $(CH_2)_p-CH=CH-(CH_2)_m-$, p is 0 to 2 and m is o



As seen in reaction sequence "A", compounds of Formula I may be prepared by treating monoacid II

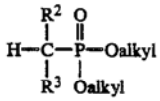


(wherein R^4 is an alkyl group) in an aromatic solvent such as benzene or toluene, preferably containing dimethylformamide, or other appropriate inert organic solvent, with oxalyl chloride, and then evaporating the reaction mixture to give acid chloride III



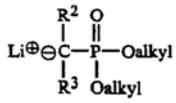
III

To a stirred solution of an optionally substituted dialkyl methyl phosphonate



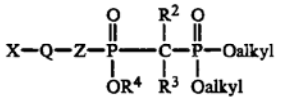
IIIA'

in an inert organic solvent such as tetrahydrofuran cooled to a temperature within the range of from about −90° C. to about 0° C. is added a lithium source, such as n-butyl lithium or lithium diisopropylamide in a hexane or other inert organic solvent under an inert atmosphere such as argon to form the lithium salt IIIA



IIIA

The lithium salt IIIA is maintained at a reduced temperature as described above and acid chloride III in an inert organic solvent such as tetrahydrofuran or ethyl ether is added to form the phosphinyl-phosphonate IF.



IF.

The lithium salt IIIA will be employed in a molar ratio to acid chlorida III of within the range of from about 1.8:1 to about 2.5:1 and preferably from about 2.0:1 to about 2.4:1. Ester IF, in an inert organic solvent such a methylene chloride, may then be subjected to dealkylation by treating with bromotrimethylsilane or iodotrimethylsilane in the presence of 2,4,6-collidine or bis(-trimethyl)silyltrifluoroacetamide and then treating with a strong inorganic base such as aqueous NaOH, KOH, LiOH or $Mg(OH)_2$, optionally in the presence of an alcohol such as methyl alchol, to form the salt IG which may be separted out by chromatography. Salt IG may be treated with a strong acid such as HCl to form acid IH.

Intermediates II and III are novel compounds and thus form a part of the present invention.

As seen in reaction sequence "B", the starting materials II may be prepared as follows.

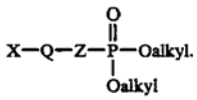
As seen in reaction scheme B(1), where Z is $(CH_2)_n$ and n is 1 to 5 or Z is $—(CH_2)_p—CH=CH—(CH_2)_m—$ and p is 0, 1 or 2 and m is 1 or 2, starting material II may be prepared by treating halide V

V X-Q-Z-Hal

with trialkyl phosphite VI

VI $P(Oalkyl)_3$

under an inert atmosphere, such as argon, at an elevated temperature of within the range of from about 120° to about 165° C. for a period of from about 1 to about 30 hours to form the phosphonic ester compound VII



VII

The reaction of phosphite VI and halide V is carried out employing a molar ratio of VI:V of within the range of from about 2:1 to about 50:1.

Phosphonic ester VII is subjected to basic hydrolysis such as by treatment with alkali metal hydroxide such as aqueous KOH or NaOH, optionally in the presence of an alcohol such as methanol, ethanol or isopropanol under an inert atmosphere, such as argon, at reflux temperature to form the phosphonic acid II which is a novel compound and thus forms a part of the present invention.

The starting halides V are either known or are prepared from farnesol or geraniol by conventional means.

As seen in reaction scheme B(2), where in starting material II, Z is $(CH_2)_n$ and n is 2 to 5 or Z is $—(CH_2)_p—CH=CH—(CH_2)_m—$ and p is 0 to 2 and m is 2, II may be prepared by treating halide VA or VB with the salt IIIB such as

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