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(54) Titre : METHODES THERAPEUTIQUES ET D'ADMINISTRATION D'AGONISTES DE PROSTAGLANDINE EP4
(54) Title: THERAPEUTIC AND DELIVERY METHODS OF PROSTAGLANDIN EP4 AGONISTS

(57) Abrégé/Abstract:
A compound comprising a prodrug of a prostaglandin EP_4 agonist, wherein said prodrug is an ester, ether, or amide of a carbohydrate; or said prodrug is an ester, ether, or amide of an amino acid is disclosed herein. Maintenance of the colonic mucosal barrier by method comprising administering a therapeutically effective amount of a prostaglandin EP_4 agonist to a colon of a mammal is also disclosed herein. Dosage forms, medicaments, and compositions, related thereto are also disclosed.

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(54) Title: THERAPEUTIC AND DELIVERY METHODS OF PROSTAGLANDIN EP_4 AGONISTS

(57) Abstract: A compound comprising a prodrug of a prostaglandin EP_4 agonist, wherein said prodrug is an ester, ether, or amide of a carbohydrate; or said prodrug is an ester, ether, or amide of an amino acid is disclosed herein. Maintenance of the colonic mucosal barrier by method comprising administering a therapeutically effective amount of a prostaglandin EP_4 agonist to a colon of a mammal is also disclosed herein. Dosage forms, medicaments, and compositions, related thereto are also disclosed.

WO 2006/047476 A3

THERAPEUTIC AND DELIVERY METHODS OF PROSTAGLANDIN EP_4 AGONISTS

By Inventors

Wha Bin Im, Yariv Donde, Mark Holoboski, David W. Old, Karen M. Kedzie, Daniel W. Gil, John E. Donello, Robert M. Burk, and Todd S. Gac

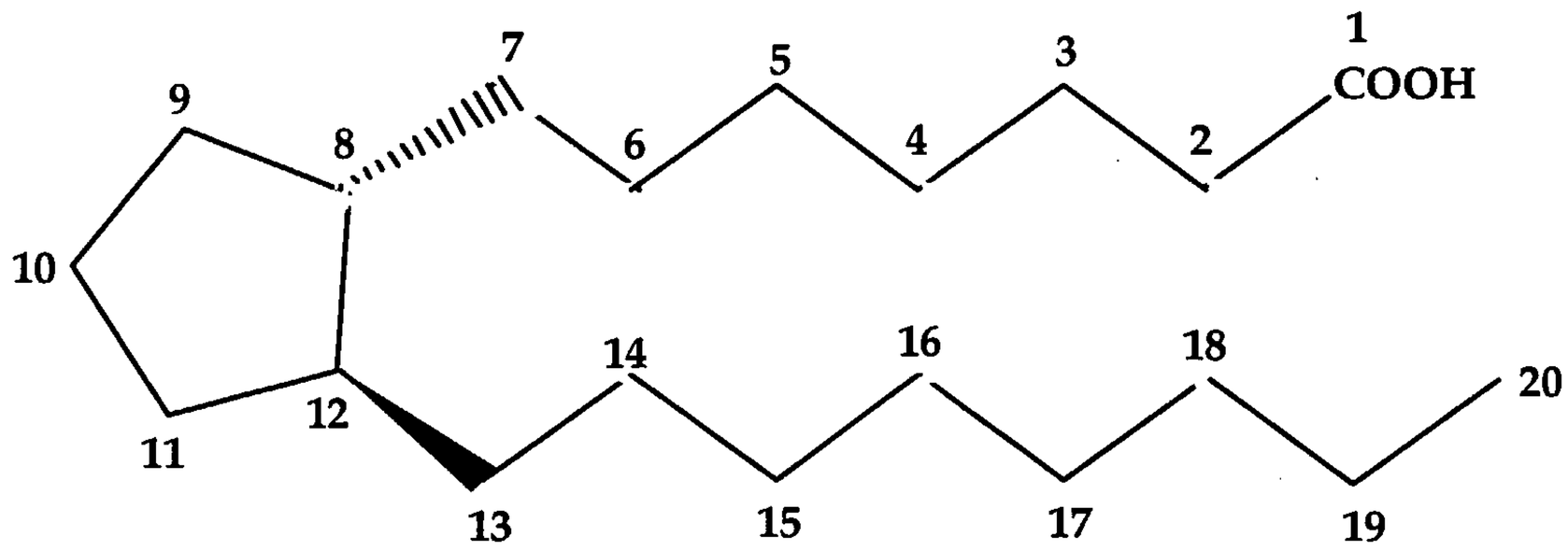
FIELD OF THE INVENTION

This invention relates to therapeutically active compounds and their delivery and use. Particularly, this invention relates to the delivery and use of prostaglandin EP_4 agonists.

BACKGROUND OF THE INVENTION

Description of Related Art

Prostaglandins can be described as derivatives of prostanoic acid which have the following structural formula:



Various types of prostaglandins are known, depending on the structure and substituents carried on the alicyclic ring of the prostanoic acid skeleton. Further classification is based on the number of unsaturated bonds in the side chain indicated by numerical subscripts after the generic type of prostaglandin [e.g. prostaglandin E_1 (PGE_1), prostaglandin E_2 (PGE_2)], and on the configuration of the substituents on the alicyclic ring indicated by α or β [e.g. prostaglandin $F_{2\alpha}$ ($PGF_{2\beta}$)].

Certain 10,10-dimethyl prostaglandins are known. These are described in documents such as the following:

Donde, in United States Patent No. Patent Application Publication No. 20040157901;

Pernet *et al* in US Patent 4,117,014;

Pernet, Andre G. et al., Prostaglandin analogs modified at the 10 and 11 positions, Tetrahedron Letters, (41), 1979, pp. 3933-3936;

Plantema, Otto G. et al., Synthesis of (.+-.)-10.10-dimethylprostaglandin E1 methyl ester and its 15-epimer, Journal of the Chemical Society, Perkin Transactions 1: Organic and Bio-organic Chemistry (1972-1999), (3), 1978, pp. 304-308;

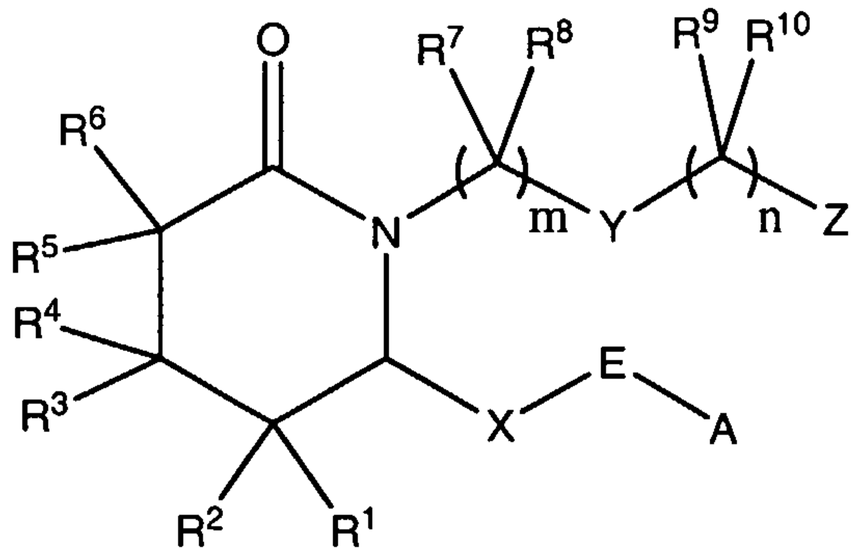
Plantema, O. G. et al., Synthesis of 10,10-dimethylprostaglandin E1, Tetrahedron Letters, (51), 1975, 4039;

Hamon, A., et al., Synthesis of (+-)- and 15-EPI(+-)-10,10-Dimethylprostaglandin E1, Tetrahedron Letters, Elsevier Science Publishers, Amsterdam, NL, no. 3, January 1976, pp. 211-214; and

Patent Abstracts of Japan, Vol. 0082, no. 18 (C-503), June 10, 1988 & JP 63 002972 A (Nippon Iyakuhin Kogyo KK), 7 January 1988;

the disclosures of these documents are hereby expressly incorporated by reference.

United States Patent Application Publication 2004/0142969 A1, expressly incorporated by reference herein, discloses compounds according to the formula below

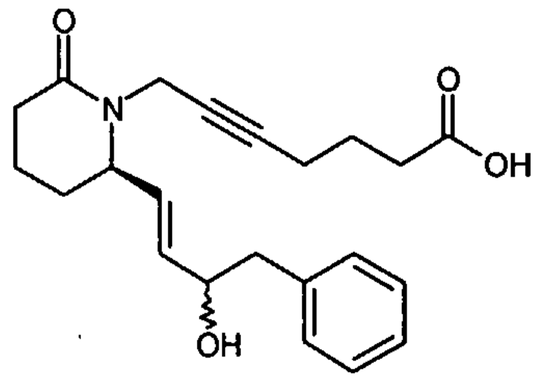


the application discloses the identity of the groups as follows.

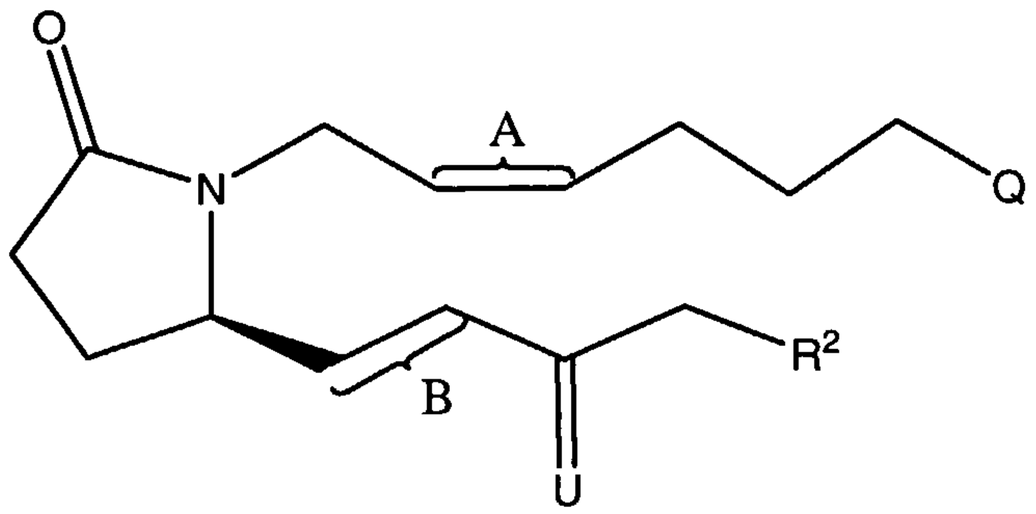
m is from 1 to 4; n is from 0 to 4; A is alkyl, aryl, heteroaryl, arylalkyl, arylcycloalkyl, cycloalkylalkyl, or aryloxyalkyl; E is -CHOH- or -C(O)-; X is $-(CH_2)_2-$ or –CH=CH-; Y is $–CH_2-$, arylene, heteroarylene, -CH=CH-, -O-, $-S(O)_p-$ where p is from 0 to 2, or $-NR^a-$ where R^a is hydrogen or alkyl;

Z is $–CH_2OH$, -CHO, tetrazol-5-yl, or $–COOR^b$ where R^b is hydrogen or alkyl; and R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} each independently are hydrogen or alkyl.

United States Patent No. 6,747,037, expressly incorporated by reference herein, discloses prostaglandin EP_4 agonists such as



United States Patent No. 6,610,719, expressly incorporated by reference herein, discloses EP_4 selected agonists having the structure



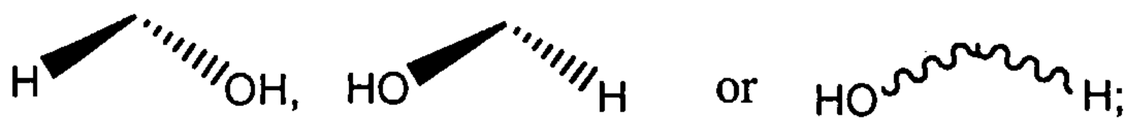
the patent describes the identity of the groups as follows:

Q is $COOR^3$, $CONHR^4$ or tetrazol-5-yl;

A is a single or cis double bond;

B is a single or trans double bond;

U is



R^2 is α-thienyl, phenyl, phenoxy, monosubstituted phenyl or monosubstituted phenoxy, said substituents being selected from the group consisting of chloro, fluoro, phenyl, methoxy, trifluoromethyl and (C_1 – C_3)alkyl;

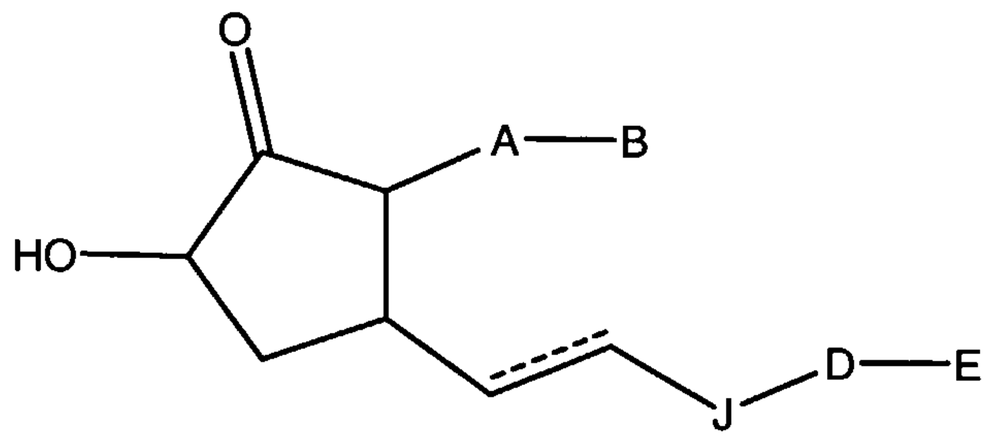
R.sup.3 is hydrogen, (C_1 –C_5)alkyl, phenyl or *p*-biphenyl;

R^4 is COR^5 or SO_2R^5 ; and

R^5 is phenyl or (C_1 –C_5)alkyl.

10-Hydroxyprostaglandin analogues, that is natural prostaglandin E compounds where the hydroxide is present on carbon 10 rather than carbon 11, are known in several patent documents including U.S. Patent No. 4,171,375; U.S. Patent No. 3,931,297; FR 2408567; DE 2752523, JP 53065854, DE 2701455, SE 7700257, DK 7700272, NL 7700272, JP 52087144, BE 850348, FR 2338244, FR 2162213, GB 1405301, and ES 409167; all of which are expressly incorporated by reference herein.

United States Patent Application Serial No. 821,705, filed April 9, 2004, expressly incorporated by reference herein, discloses compounds having the following structure

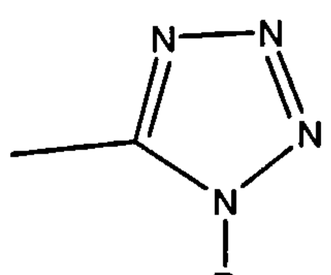


the groups are identified as follows

J is C=O or CHOH;

A is $-(CH_2)_6$-, or *cis* $-CH_2CH=CH-(CH_2)_3$-, wherein 1 or 2 carbons may be substituted with S or O;

B is CO_2H, or CO_2R, $CONR_2$, $CONHCH_2CH_2OH$, $CON(CH_2CH_2OH)_2$, CH_2OR, $P(O)(OR)_2$, $CONRSO_2R$, $SONR_2$, or



,

R is H, $C_{1\text{-}6}$ alkyl;

D is $-(CH_2)_n$-, $-X(CH_2)_n$, or $-(CH_2)_nX$-, wherein n is from 0 to 3 and X is S or O; and

E is an aromatic or heteroaromatic moiety having from 0 to 4 substituents, said substituents each comprising from 1 to 6 non-hydrogen atoms is disclosed herein.

Other compounds of interest are disclosed in United States Patent No. 6,670,485; United States Patent No. 6,410,591; United States Patent No. 6,538,018; WO 2004/065365; WO 03/074483; WO 03/009872; WO 2004/019938; WO 03/103664; WO 2004/037786; WO 2004/037813; WO 03/103604; WO 03/077910; WO 02/42268; WO 03/008377 WO 03/053923; WO 2004/078103; and WO 2003/035064, all of which are expressly incorporated by reference herein.

Prostaglandin EP_4 selective agonists are believed to have several medical uses. For example, U.S. Patent No. 6,552,067 B2, expressly incorporated by reference herein, teaches the use of prostaglandin EP_4 selective agonists for the treatment of "methods of treating conditions which present with low bone mass, particularly osteoporosis, frailty, an osteoporotic fracture, a bone defect, childhood idiopathic bone loss, alveolar bone loss, mandibular bone loss, bone fracture, osteotomy, bone loss associated with periodontitis, or prosthetic ingrowth in a mammal."

U.S. Patent No. 6,586,468 B1, expressly incorporated by reference herein, teaches that prostaglandin EP_4 selective agonists "are useful for the prophylaxis and/or treatment of immune diseases (autoimmune diseases (amyotrophic lateral sclerosis (ALS), multiple sclerosis, Sjoegren's syndrome, arthritis, rheumatoid arthritis, systemic lupus erythematosus, etc.), post-transplantation graft rejection, etc.), asthma, abnormal bone formation, neurocyte death, pulmopathy, hepatopathy, acute hepatitis, nephritis, renal insufficiency, hypertension, myocardial ischemia, systemic inflammatory syndrome, pain induced by ambustion, sepsis, hemophagocytosis syndrome, macrophage activation syndrome, Still's diseases, Kawasaki diseases, burn, systemic granuloma, ulcerative colititis, Crohn's diseases, hypercytokinemia at dialysis, multiple organ failure, shock, etc. They are also connected with sleeping disorders and platelet coagulations, and therefore they are thought to be useful for these diseases."

Inflammatory bowel disease (IBD) is a group of disease characterized by inflammation in the large or small intestines and is manifest in symptoms such as diarrhea, pain, and weight loss. Nonsteroidal anti-inflammatory drugs have been shown to be associated with the risk of developing IBD, and recently Kabashima and colleagues have disclosed that "EP_4 works to keep mucosal integrity, to suppress the innate immunity, and to downregulate the proliferation and activation of CD4+ T cells. These findings have not only elucidated the mechanisms of IBD by NSAIDs, but also indicated the therapeutic potential of EP_4-selective agonists in prevention and treatment of IBD." (Kabashima, et. al., *The Journal of Clinical Investigation*, April 2002, Vol. 9, 883-893)

BRIEF DESCRIPTION OF THE INVENTION

A compound comprising a prodrug of a prostaglandin EP_4 agonist, wherein said prodrug is an ester, ether, or amide of a carbohydrate; or said prodrug is an ester, ether, or amide of an amino acid is disclosed herein.

Maintenance of the colonic mucosal barrier by method comprising administering a therapeutically effective amount of a prostaglandin EP_4 agonist to a colon of a mammal is also disclosed herein.

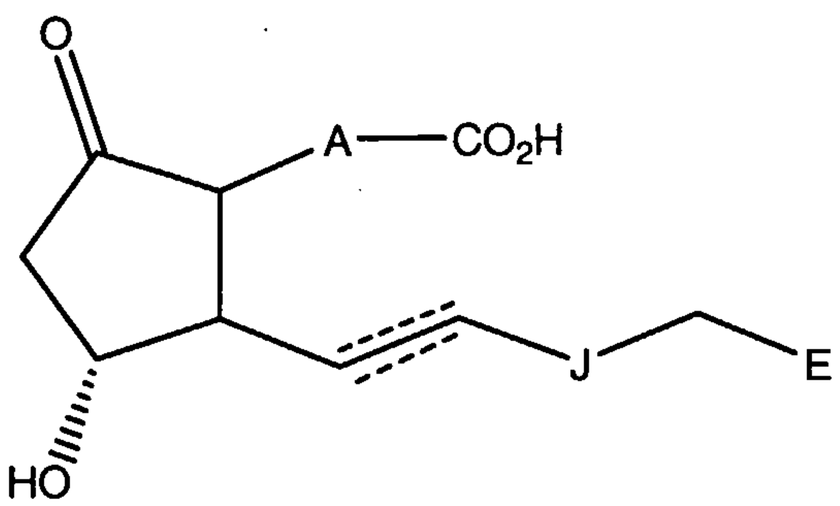
Dosage forms, medicaments, and compositions, related thereto are also disclosed.

DETAILED DESCRIPTION OF THE INVENTION

A prostaglandin EP_4 agonist is broadly defined as a compound which an ordinary person in the art reasonably believes agonizes a prostaglandin EP_4 receptor according to any one or more of numerous assays for determination of the EP_4 activity that are well known to those of ordinary skill in the art. While not intending to be limiting, one such assay is given in the example below.

In one embodiment, the prostaglandin EP_4 agonist is selective for a prostaglandin EP_4 receptor relative to other prostaglandin receptor subtypes. In another embodiment, the prostaglandin EP_4 agonist is at least 10 times more active at the EP_4 receptor than at any other prostaglandin receptor subtype. In another embodiment, the prostaglandin EP_4 agonist is at least 100 times more active at the EP_4 receptor than at any other prostaglandin receptor subtype. In another embodiment, the prostaglandin EP_4 agonist is at least 1000 times more active at the EP_4 receptor than at any other prostaglandin receptor subtype. While not intending to be limiting, typical assays for the other receptor subtypes are also given in examples below.

While not intending to limit the scope of the invention in any way, compounds according to the structures below are examples prostaglandin EP_4 agonists:



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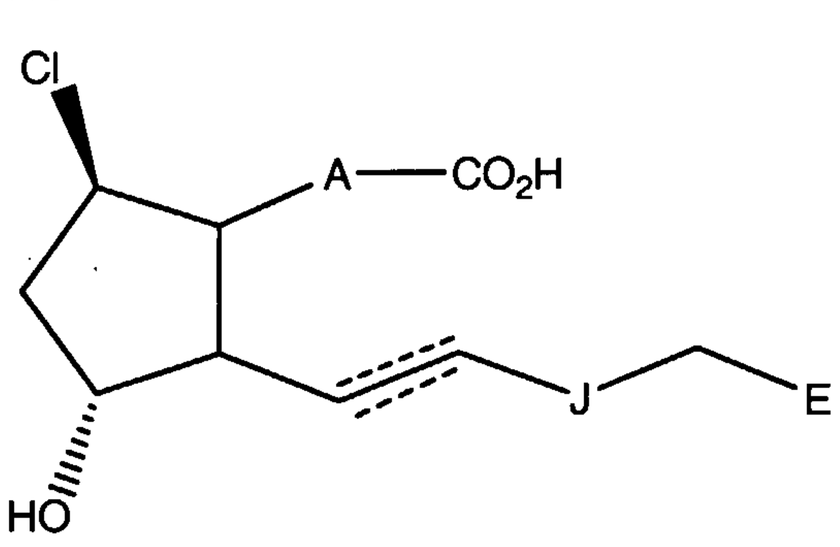

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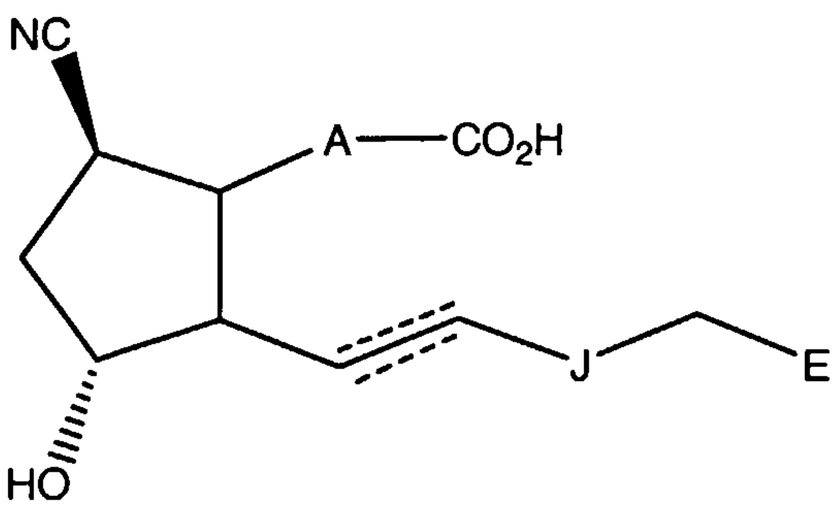

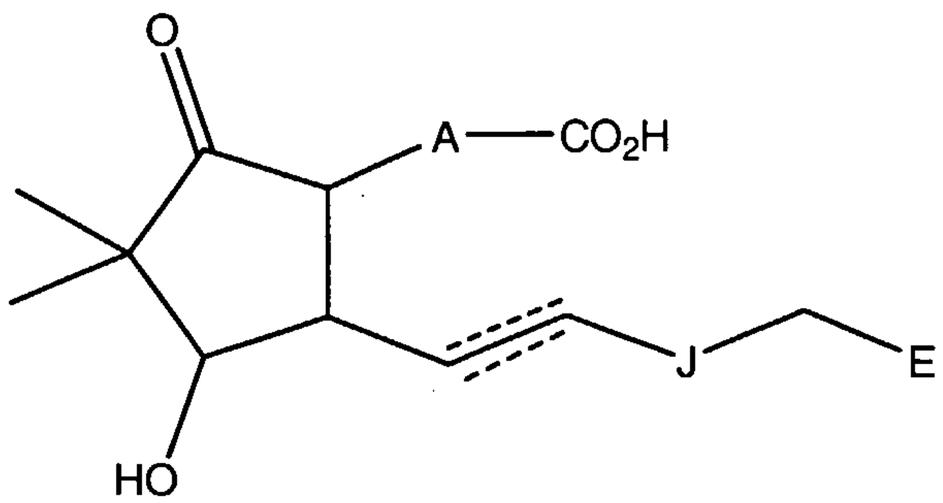

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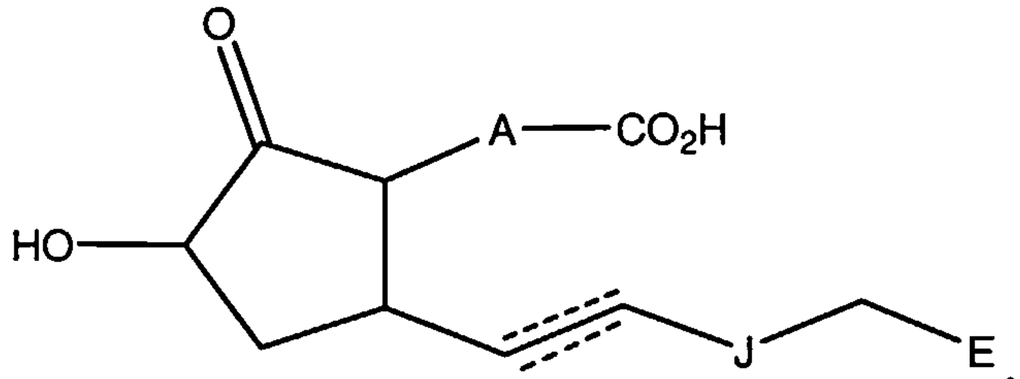

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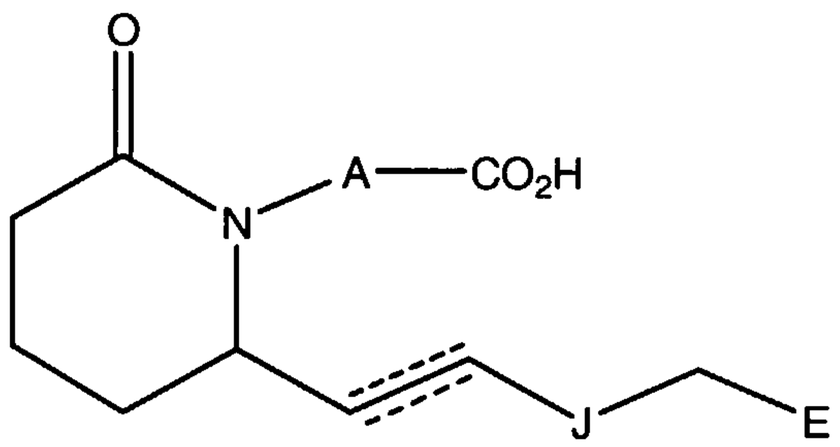

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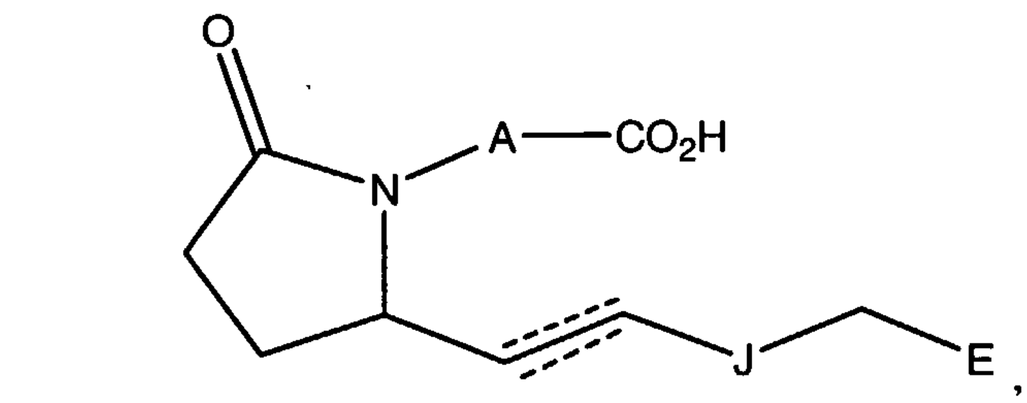

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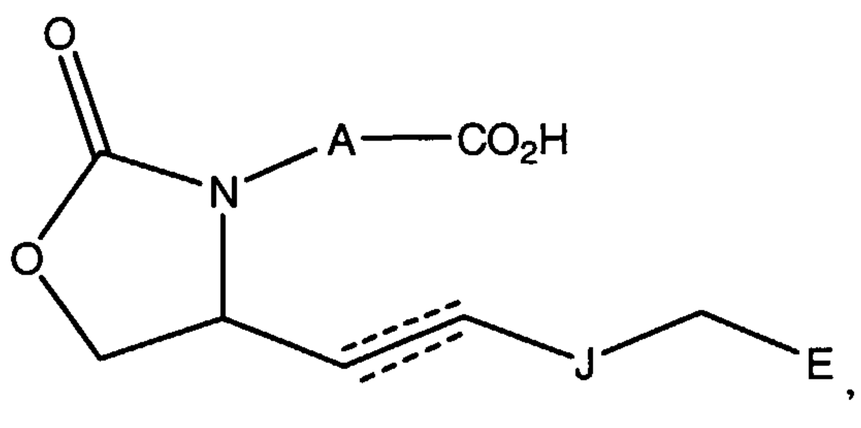

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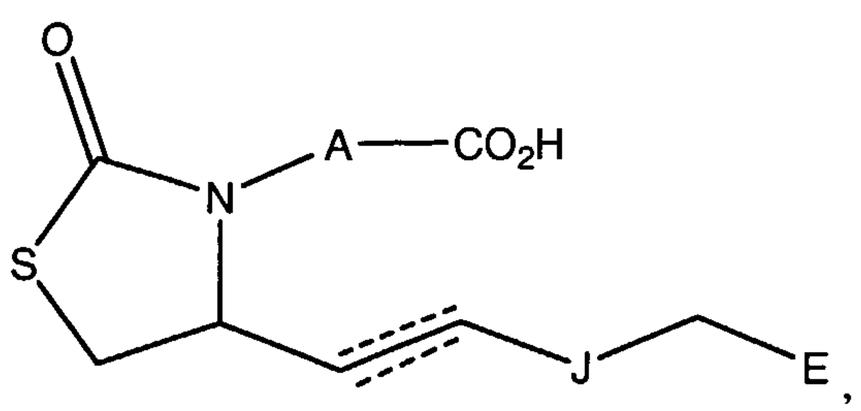

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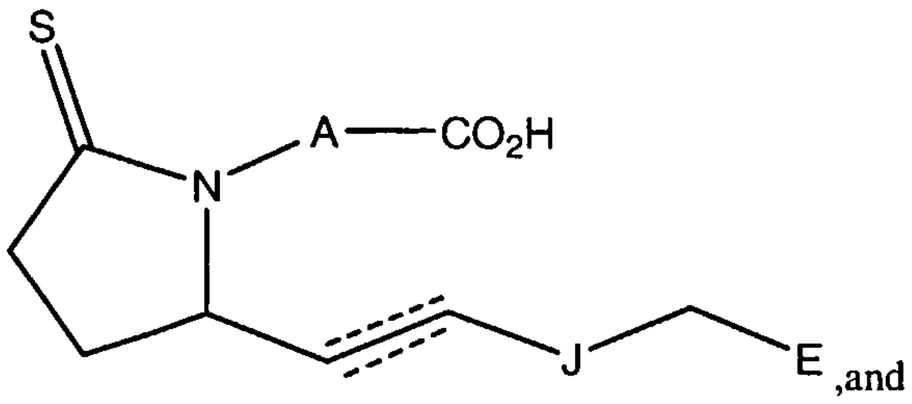
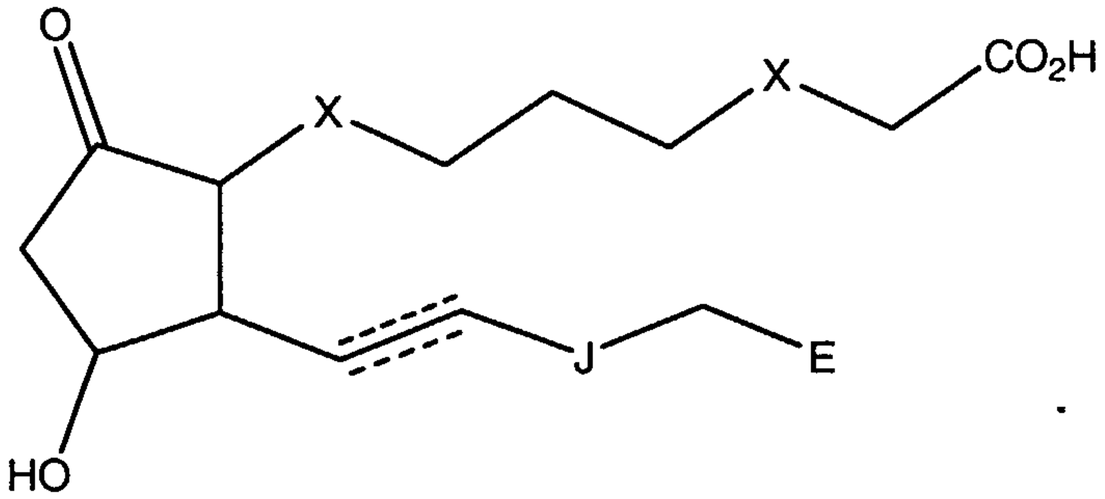


,and



;

or a pharmaceutically acceptable salt or a prodrug thereof,

wherein a dashed line represents the presence of absence of a bond;

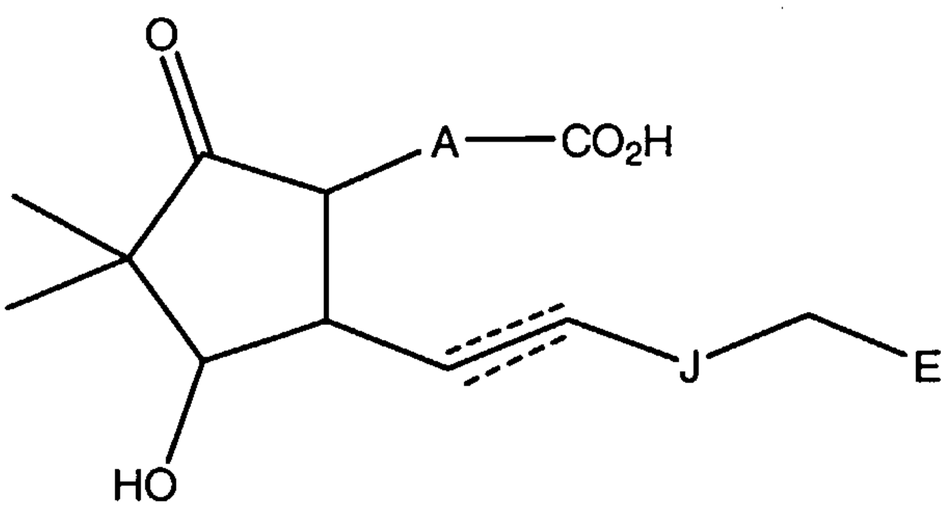
A is $-(CH_2)_6-$, *cis* $-CH_2CH=CH-(CH_2)_3-$, or $-CH_2C\equiv C-(CH_2)_3-$, wherein 1 or 2 carbon atoms may be substituted with S or O; or A is $-(CH_2)_m-Ar-(CH_2)_o-$ wherein Ar is interarylene or heterointerarylene, the sum of m and o is from 1 to 4, and wherein one CH_2 may be substituted with S or O;

X is S or O;

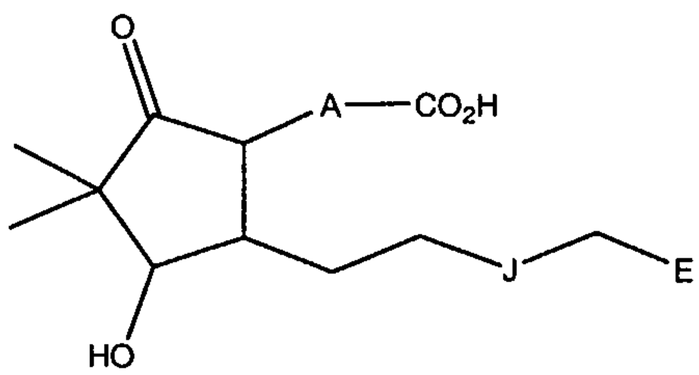
J is C=O, CHOH, or CH_2CHOH; and

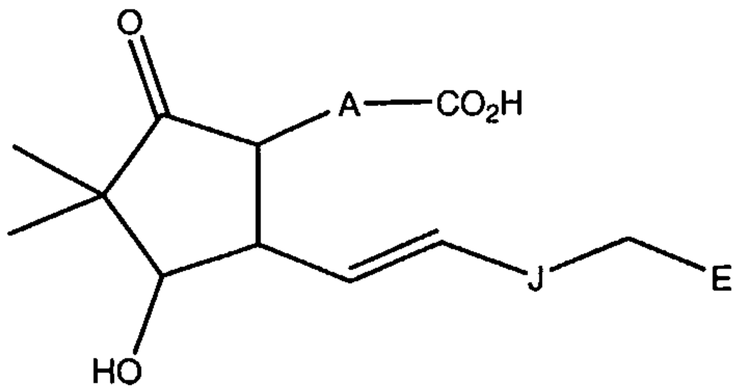
E is C_{1-12} alkyl, R^2, or $-Y-R^2$ wherein Y is CH_2, S, or O, and R^2 is aryl or heteroaryl.

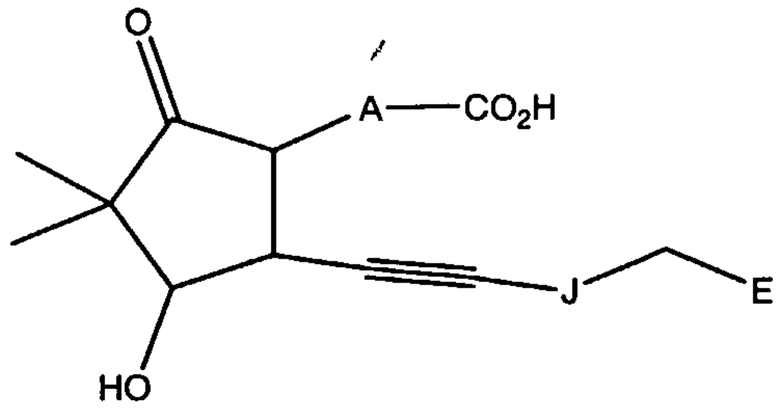
In these structures, a dashed line represents the presence or absence of a bond. Thus, a structure such as the one below,



represents three different structures, depicted as follows.



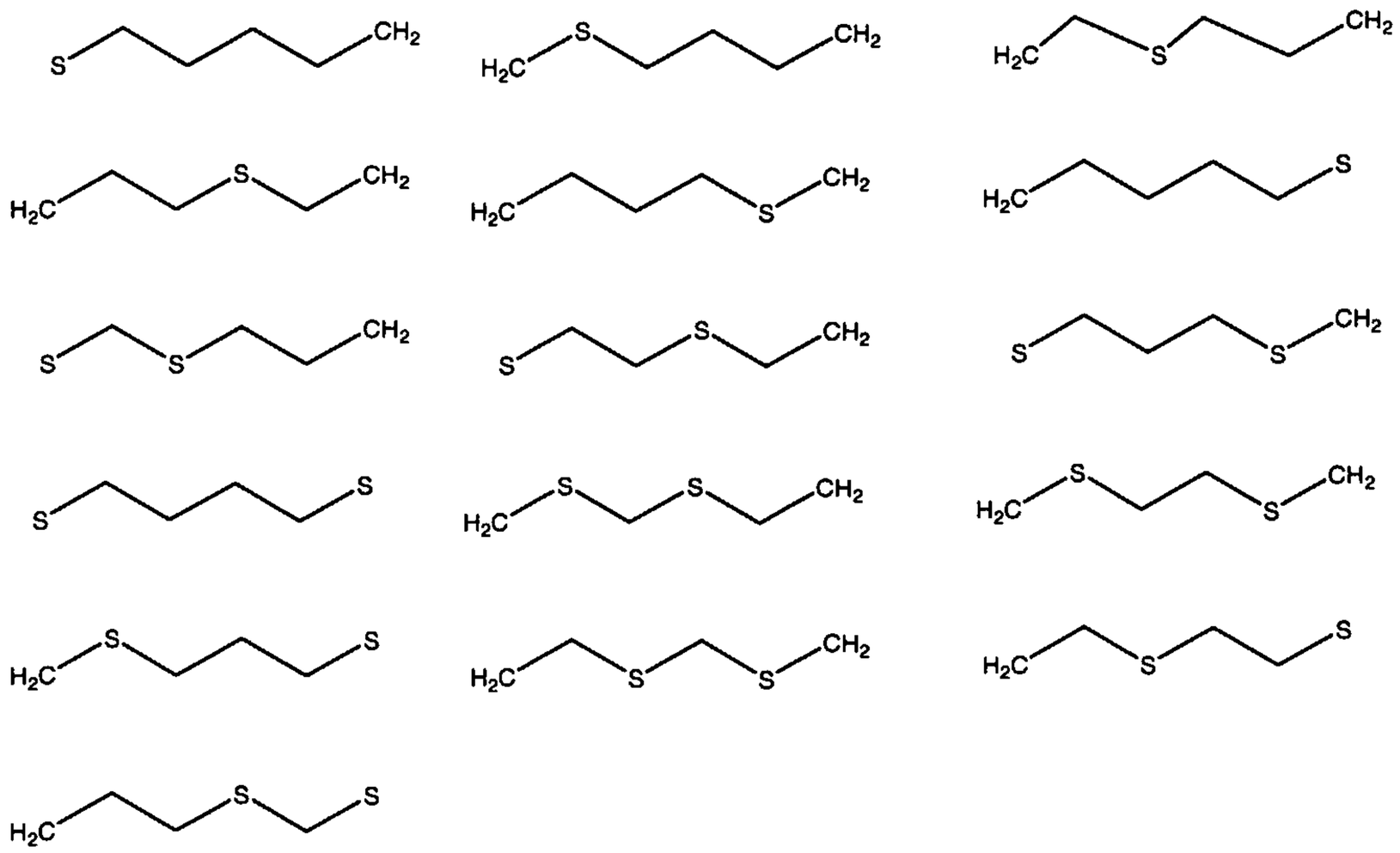


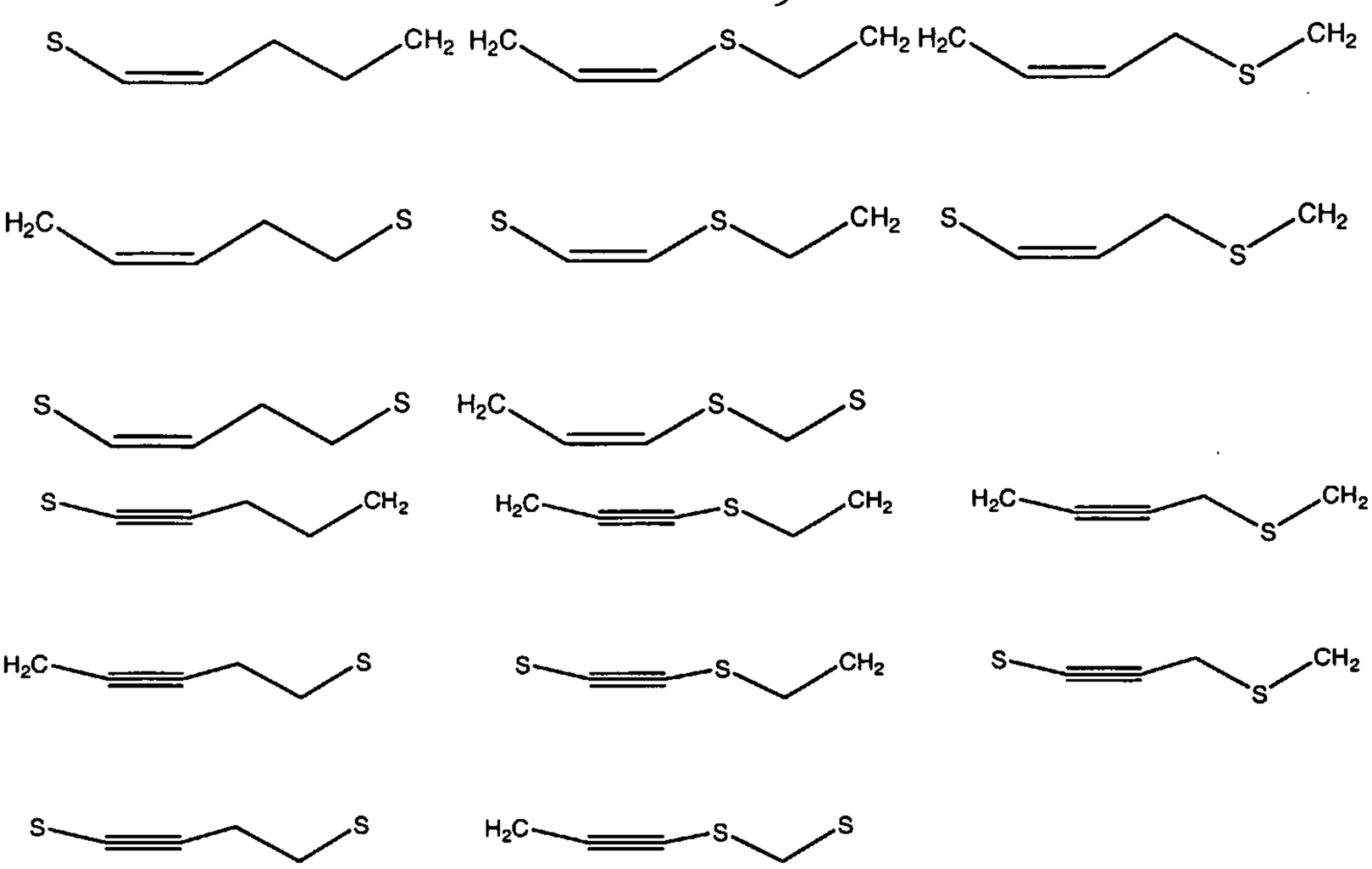


In relation to the identity of A disclosed in the chemical structures presented herein, in the broadest sense, A is $-(CH_2)_6-$, *cis* $-CH_2CH=CH-(CH_2)_3-$, or $-CH_2C\equiv C-(CH_2)_3-$, wherein 1 or 2 carbon atoms may be substituted with S or O; or A is $-(CH_2)_n-Ar-(CH_2)_o-$ wherein Ar is interarylene or heterointerarylene, the sum of m and o is from 1 to 3, and wherein one CH_2 may be substituted with S or O.

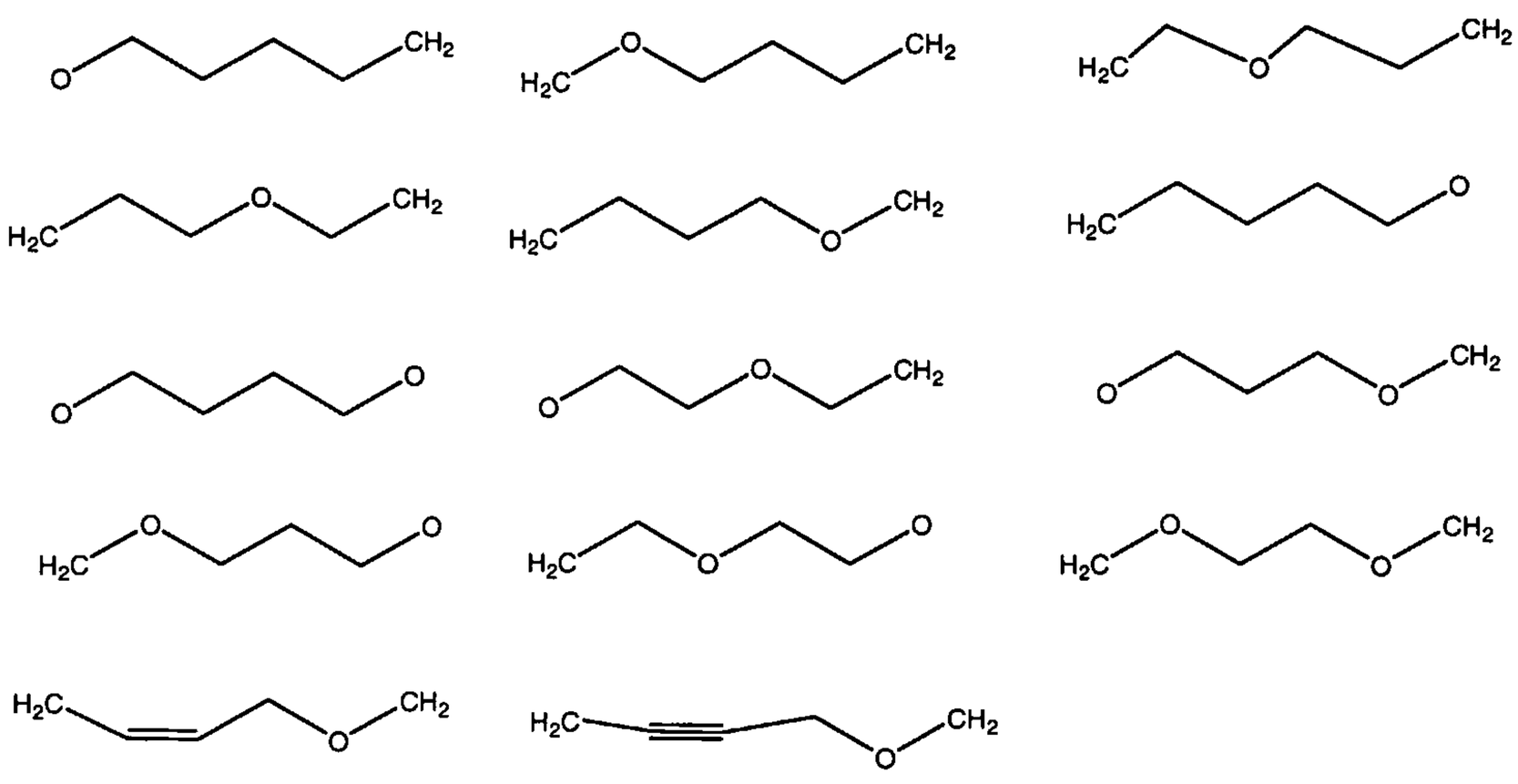
While not intending to be limiting, A may be $-(CH_2)_6-$, *cis* $-CH_2CH=CH-(CH_2)_3-$, or $-CH_2C\equiv C-(CH_2)_3-$.

Alternatively, A may be a group which is related to one of these three moieties in that any carbon is substituted with S and/or O. For example, while not intending to limit the scope of the invention in any way, A may be an S substituted moiety such as one of the following or the like.





Alternatively, while not intending to limit the scope of the invention in any way, A may be an O substituted moiety such as one of the following or the like.



Alternatively, while not intending to limit the scope of the invention in any way, A may have both an O and an S substituted into the chain, such as one of the following or the like.

Alternatively, while not intending to limit the scope of the invention in any way, in certain embodiments A is $-(CH_2)_n$-Ar-$(CH_2)_o$- wherein Ar is interarylene or heterointerarylene, the sum of m and o is from 1 to 4, and wherein one CH_2 may be substituted with S or O. In other words, while not intending to limit the scope of the invention in any way,

in one embodiment A comprises from 1 to 4 CH_2 moieties and Ar, e.g. -CH_2-Ar-, -$(CH_2)_2$-Ar-, -CH_2-ArCH_2-, -$CH_2Ar(CH_2)_2$-, -$(CH_2)_2$-Ar$(CH_2)_2$-, and the like; or

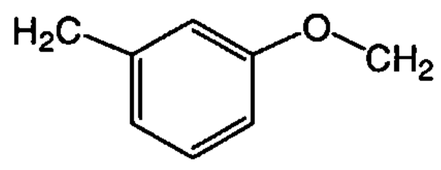
A comprises O, from 0 to 3 CH_2 moieties, and Ar, e.g., -O-Ar-, Ar-CH_2-O-, -O-Ar-$(CH_2)_2$-, -O-CH_2-Ar-, -O-CH_2-Ar-$(CH_2)_2$, and the like; or

A comprises S, from 0 to 3 CH_2 moieties, and Ar, e.g., -S-Ar-, Ar-CH_2-S-, -S-Ar-$(CH_2)_2$-, -S-CH_2-Ar-, -S-CH_2-Ar-$(CH_2)_2$, and the like.

Interarylene or heterointerarylene refers to an aryl ring or ring system or a heteroaryl ring or ring system which connects two other parts of a molecule, i.e. the two parts are bonded to the ring in two distinct ring positions. Interarylene or heterointerarylene may be substituted or unsubstituted. Thus, an unsubstituted interarylene has 4 potential positions where a substituent could be attached. In one embodiment, Ar is substituted or unsubstituted interphenylene, interthienylene, interfurylene, or interpyridinylene. In another embodiment Ar is interphenylene (Ph). In another embodiment A is –$(CH_2)_2$-Ph-. While not intending to limit scope of the invention in any way, substituents may have 4 or less heavy atoms, or in other words, non hydrogen atoms. Any number of hydrogen atoms required for a particular substituent will also be included. Thus, the substituent may be hydrocarbyl having up to 4 carbon atoms, including alkyl up to C_4, alkenyl, alkynyl, and the like; hydrocarbyloxy up to C_3; CF_3; halo, such as F, Cl, or Br; hydroxyl; NH_2 and alkylamine functional groups up to C_3; other N or S containing substituents; and the like.

In one embodiment A is –$(CH_2)_m$-Ar-$(CH_2)_o$- wherein Ar is interphenylene, the sum of m and o is from 1 to 3, and wherein one CH_2 may be substituted with S or O.

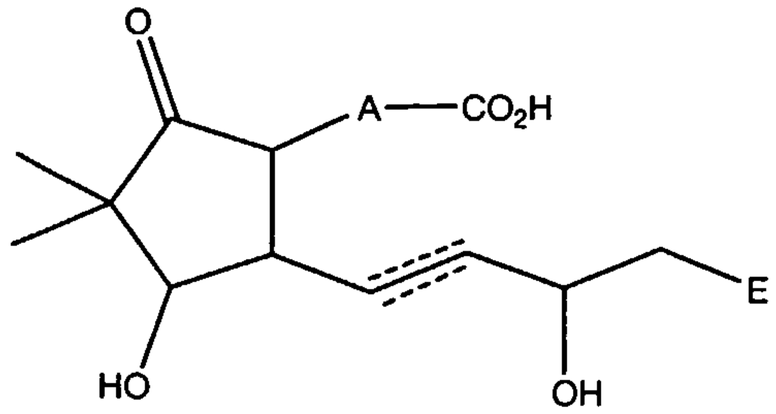
In another embodiment A is -CH_2-Ar-OCH_2-. In another embodiment A is –CH_2-Ar-OCH_2- and Ar is interphenylene. In another embodiment, Ar is attached at the 1 and 3 positions, such as when A has the structure shown below.

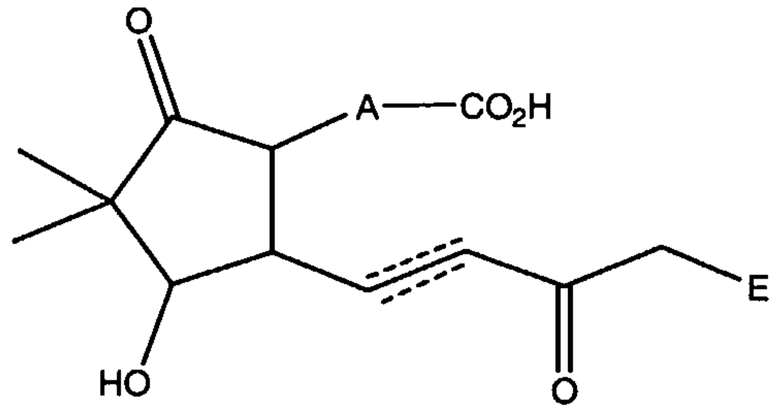


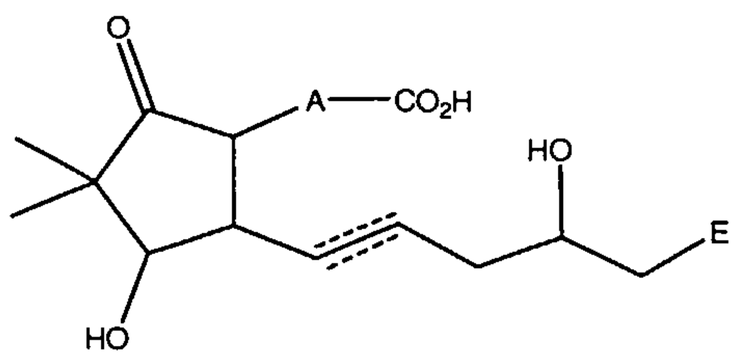
In another embodiment A is –$(CH_2)_6$-, *cis* –CH_2CH=CH-$(CH_2)_3$-, or –$CH_2C\equiv$C-$(CH_2)_3$-, wherein 1 or 2 carbon atoms may be substituted with S or O; or A is –$(CH_2)_2$-Ph- wherein one CH_2 may be substituted with S or O.

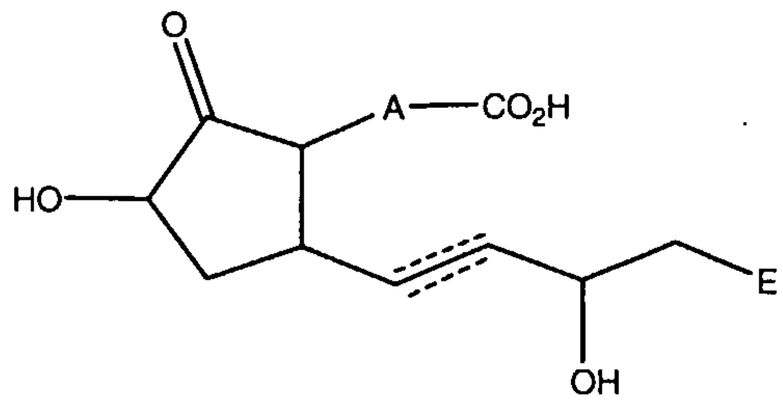
In another embodiment A is –$(CH_2)_6$-, *cis* –CH_2CH=CH-$(CH_2)_3$-, or –$CH_2C\equiv$C-$(CH_2)_3$-, wherein 1 or 2 carbon atoms may be substituted with S or O; or A is –$(CH_2)_2$-Ph-.

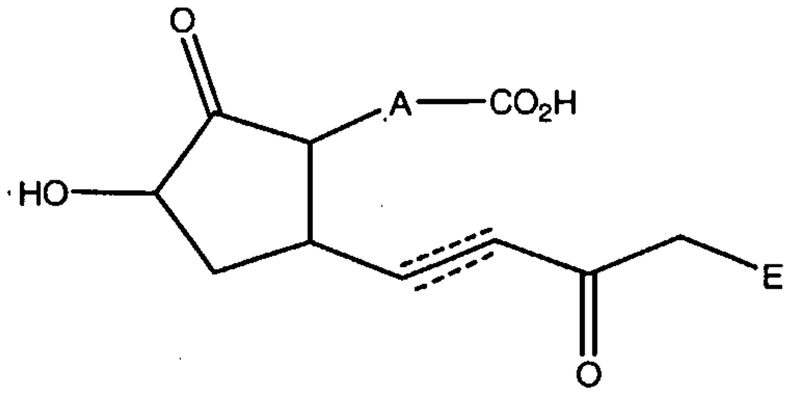
J is C=O, CHOH, or CH_2CHOH. Thus, while not intending to limit the scope of the invention in any way. Compounds such as the ones below are useful as the prostaglandin EP_4 agonists.

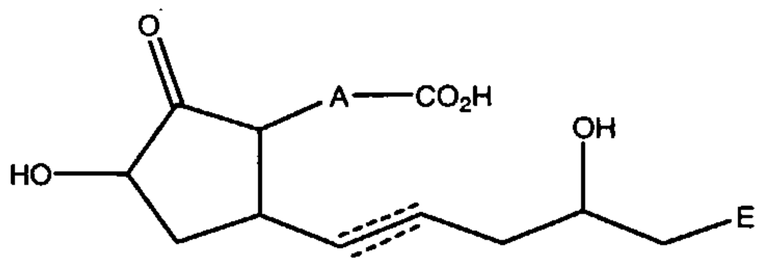


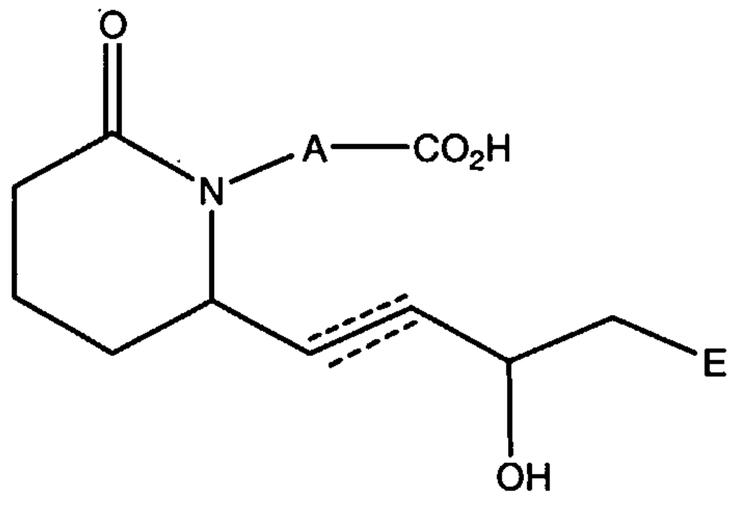


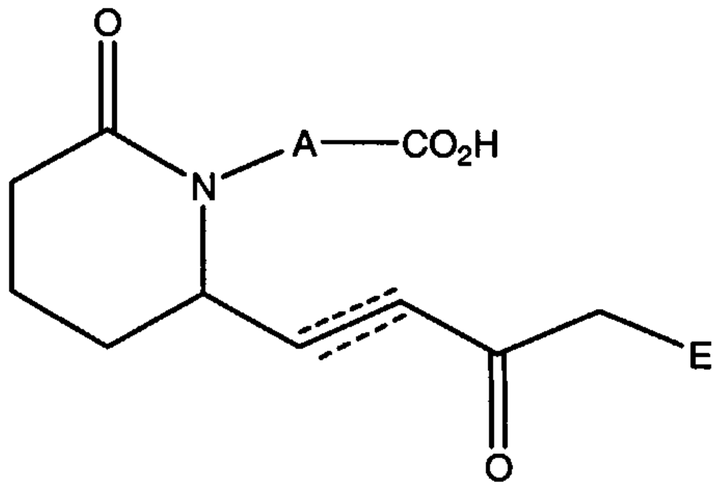


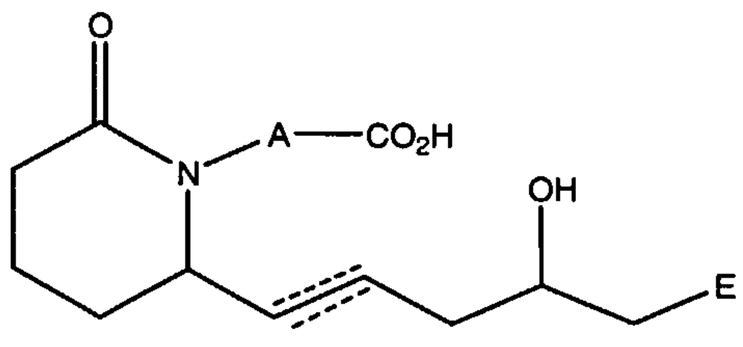


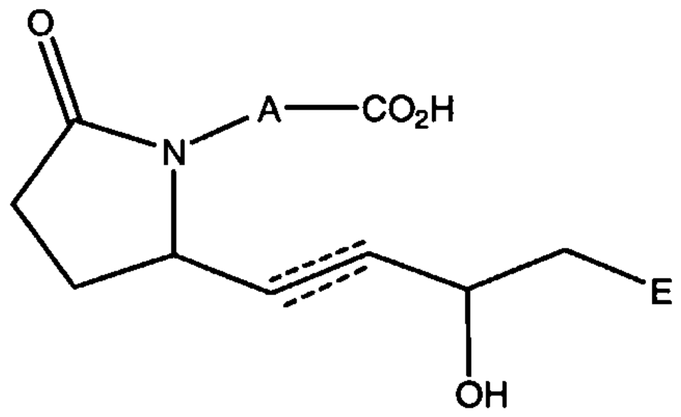


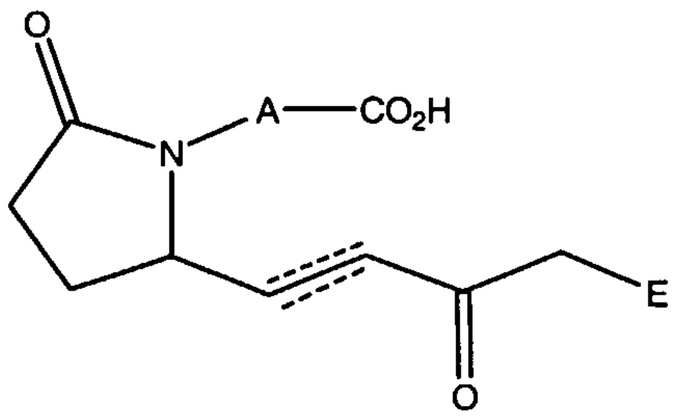


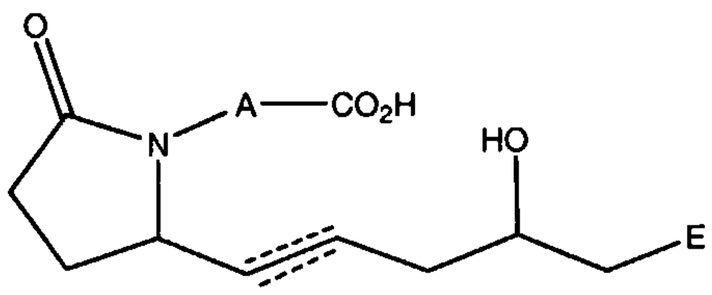


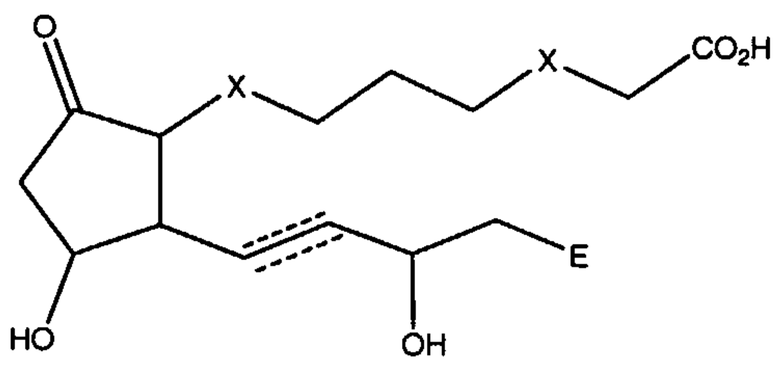


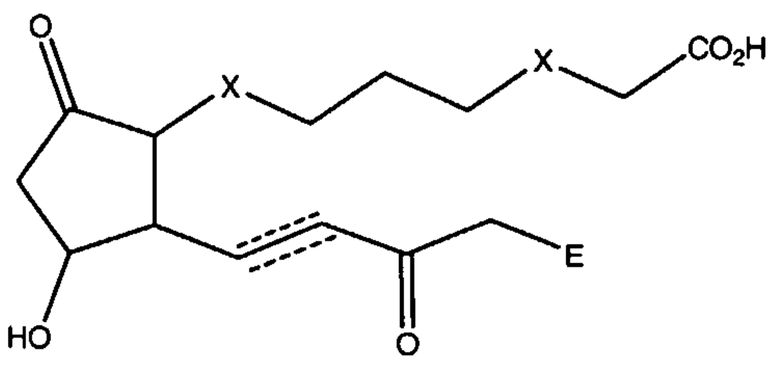


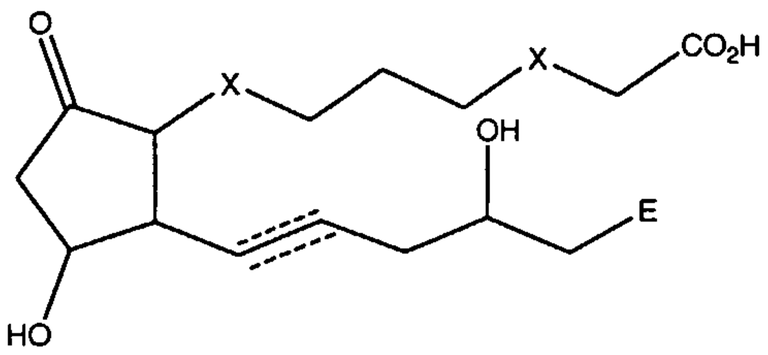












C_{1-12} alkyl is alkyl having from 1 to 12 carbon atoms, including:

linear alkyl, such as methyl, ethyl, n-propyl, n-butyl, etc.;

branched alkyl, such as iso-propyl, iso-butyl, t-butyl, isopentyl, etc.;

cyclic alkyl, such as cyclopropyl, cyclobutyl, cyclohexyl, etc.; including substituted cycloalkyl, such as methylcyclohexyl, ethylcyclopropyl, dimethylcycloheptyl, etc, and including moieties such as CH_2-cyclohexyl, where the cyclic group is not the point of attachment to the rest of the molecule; and any combination of the other types of alkyl groups listed above.

Thus, E may be any of these groups. In particular, linear alkyl of C_{1-6} is contemplated herein, especially butyl. Other particularly useful groups are cyclohexyl, cyclopentyl, and substituted cyclohexyl and cyclobutyl having less than 9 carbon atoms.

E may also be R^2 or Y-R^2 wherein Y is CH_2, S or O and R^2 is aryl or heteroaryl. Thus, E may be aryl, heteroaryl, –CH_2-aryl, -S-aryl, -O-aryl,–CH_2-heteroaryl, -S-heteroaryl, or -O-heteroaryl.

Aryl is defined as an aromatic ring or ring system as well as a substituted derivative thereof, wherein one or more substituents are substituted for hydrogen. While not intending to limit the scope of the invention in any way, phenyl, naphthyl, biphenyl, terphenyl, and the like are examples of aryl.

Heteroaryl is defined as aryl having at least one non-carbon atom in an aromatic ring or ring system. While not intending to limit the scope of the invention in any way, in many cases one or more oxygen, sulfur, and/or nitrogen atoms are present. While not intending to limit the scope of the invention in any way, examples of heteroaryl are furyl, thienyl, pyridinyl, benzofuryl, benzothienyl, indolyl, and the like.

The substituents of aryl or heteroaryl may have up to 12 non-hydrogen atoms each and as many hydrogens as necessary. Thus, while not intending to limit the scope of the invention in any way, the substituents may be:

hydrocarbyl, such as alkyl, alkenyl, alkynyl, and the like, and combinations thereof;

hydrocarbyloxy, meaning O-hydrocarbyl such as OCH_3, OCH_2CH_3, O-cyclohexyl, etc, up to 11 carbon atoms;

hydroxyhydrocarbyl, meaning hydrocarbyl-OH such as CH_2OH, $C(CH_3)_2OH$, etc, up to 11 carbon atoms;

nitrogen substituents such as NO_2, CN, and the like, including

amino, such as NH_2, $NH(CH_2CH_3OH)$, $NHCH_3$, and the like up to 11 carbon atoms;

carbonyl substituents, such as CO_2H, ester, amide, and the like;

halogen, such as chloro, fluoro, bromo, and the like

fluorocarbonyl, such as CF_3, CF_2CF_3, etc.;

phosphorous substituents, such as PO_3^{2-}, and the like;

sulfur substituents, including S-hydrocarbyl, SH, SO_3H, SO_2-hydrocarbyl, SO_3-hydrocarbyl, and the like.

In certain embodiments, the number of non-hydrogen atoms is 6 or less in a substituent. In other embodiments, the number of non-hydrogen atoms is 3 or less in a substituent. In other embodiments, the number of non-hydrogen atoms on a substituent is 1.

In certain embodiments, the substituents contain only hydrogen, carbon, oxygen, halo, nitrogen, and sulfur. In other embodiments, the substituents contain only hydrogen, carbon, oxygen, and halo.

In certain embodiments A is $-(CH_2)_6-$, *cis* $-CH_2CH=CH-(CH_2)_3-$, or $-CH_2C\equiv C-(CH_2)_3-$, wherein 1 or 2 carbon atoms may be substituted with S or O; and E is C_{1-6} alkyl, R^2, or $-Y-R^2$ wherein Y is CH_2, S, or O, and R^2 is aryl or heteroaryl.

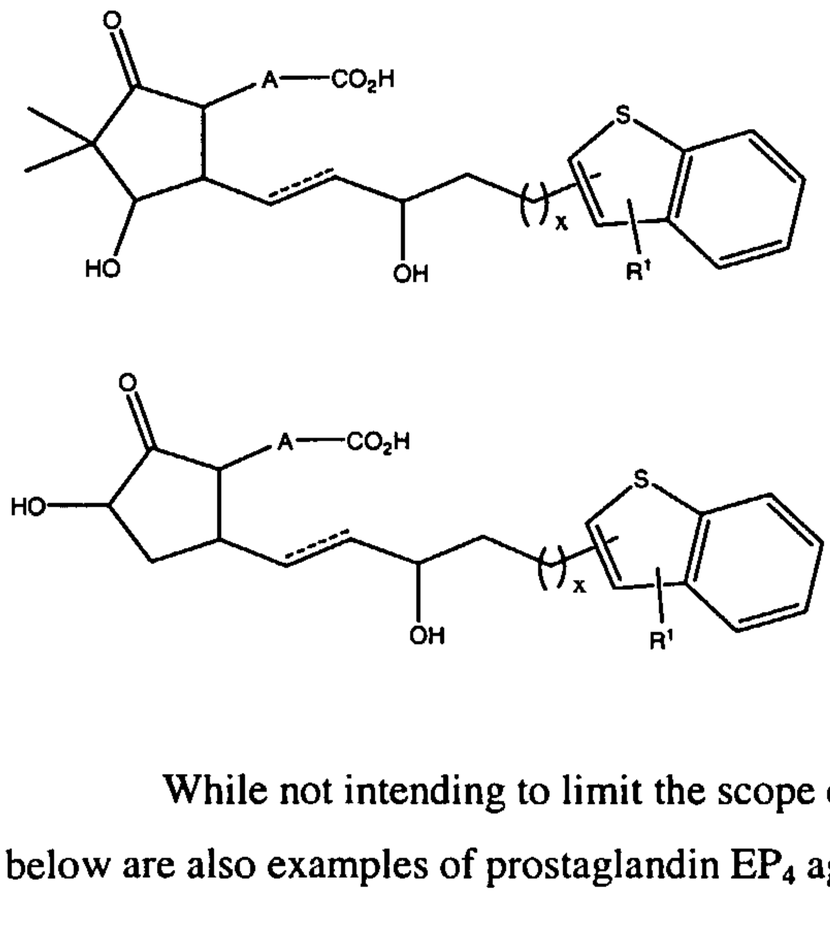
In one embodiment R^1 is H, chloro, or fluoro. In another embodiment R^1 is H. In another embodiment, R^1 is chloro.

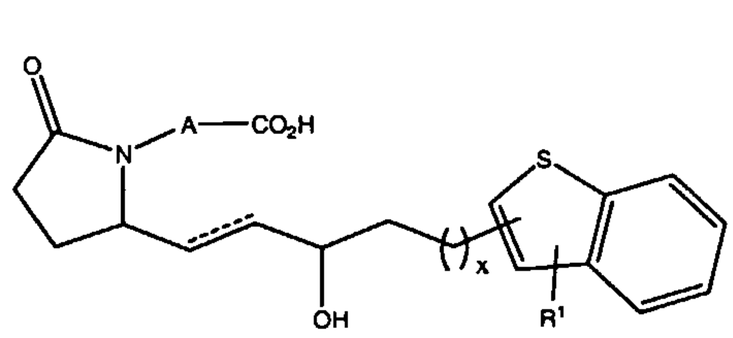
In other embodiments R^2 is phenyl, naphthyl, biphenyl, thienyl, or benzothienyl having from 0 to 2 substituents selected from the group consisting of F, Cl, Br, methyl, methoxy, and CF_3.

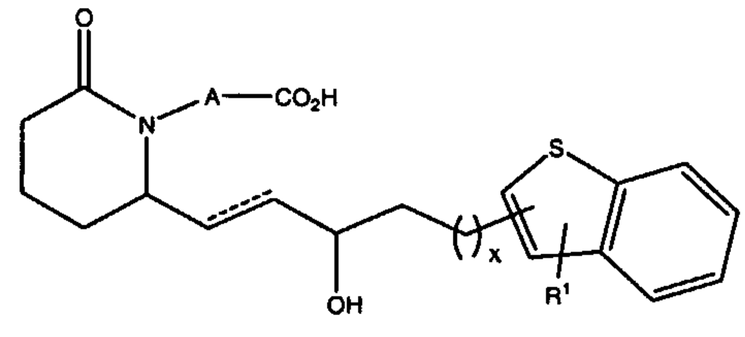
In other embodiments R^2 is CH_2-naphthyl, CH_2-biphenyl, CH_2-(2-thienyl), CH_2-(3-thienyl), naphthyl, biphenyl, 2-thienyl, 3-thienyl, CH_2-(2-(3-chlorobenzothienyl)), CH_2-(3-benzothienyl), 2-(3-chlorobenzothienyl), or 3-benzothienyl.

In other embodiments R^2 is CH_2-(2-thienyl), CH_2-(3-thienyl), 2-thienyl, 3-thienyl, CH_2-(2-(3-chlorobenzothienyl)), CH_2-(3-benzothienyl), 2-(3-chlorobenzothienyl), or 3-benzothienyl.

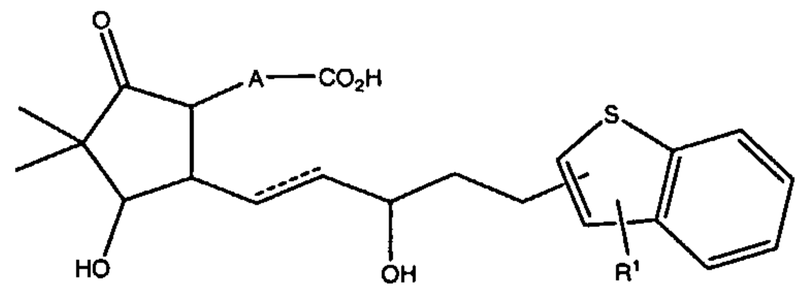
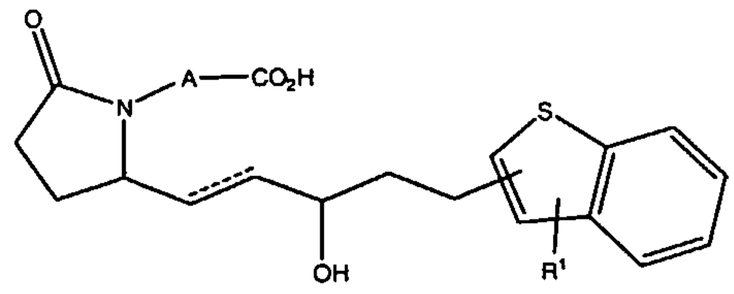
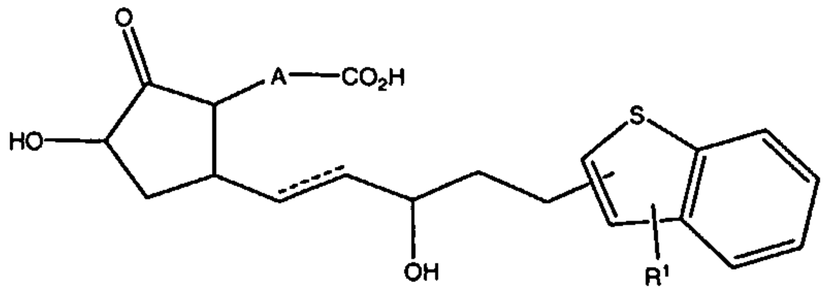
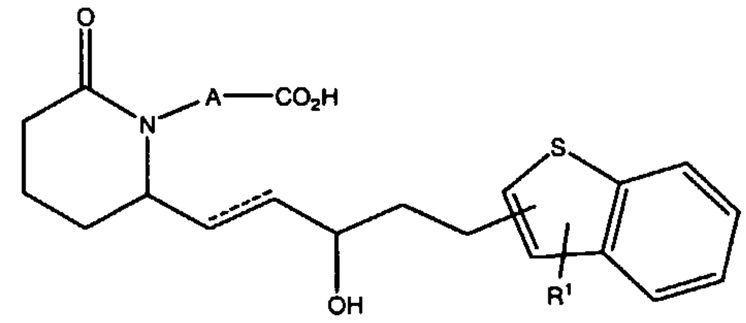
While not intending to limit the scope of the invention in any way, compounds according to the structures below, wherein x is 0 or 1 and R^1 is H, chloro, fluoro, bromo, methyl, methoxy, or CF_3, are also examples of prostaglandin EP_4 agonists.



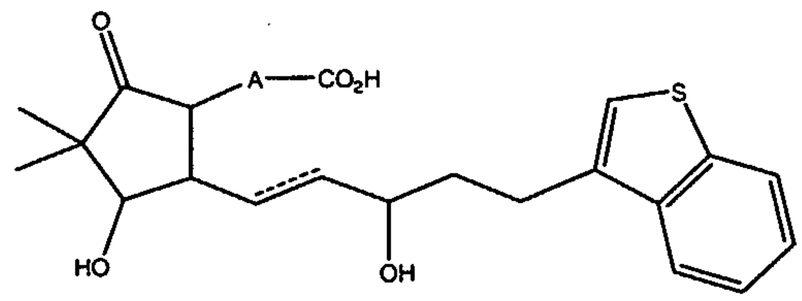
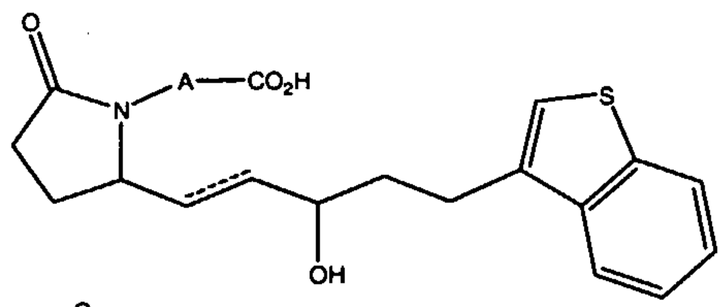
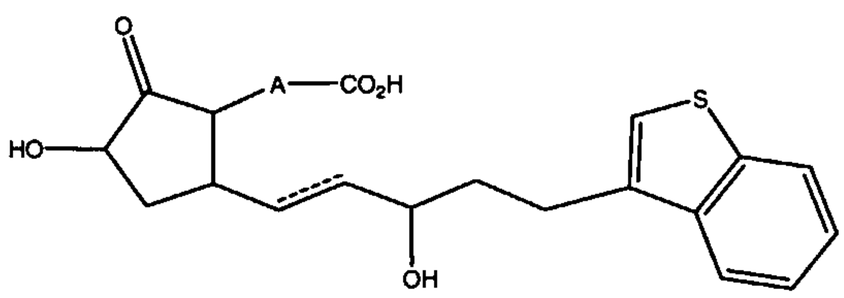
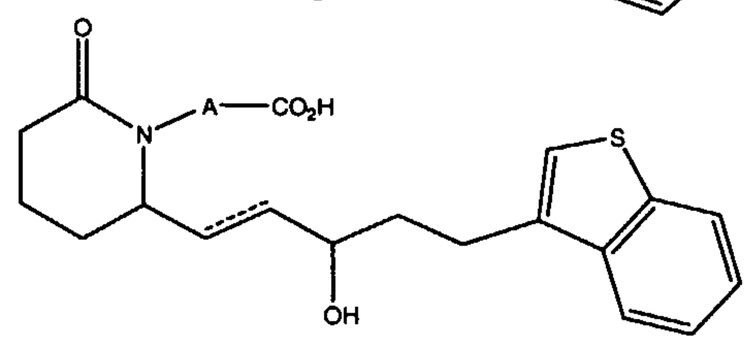




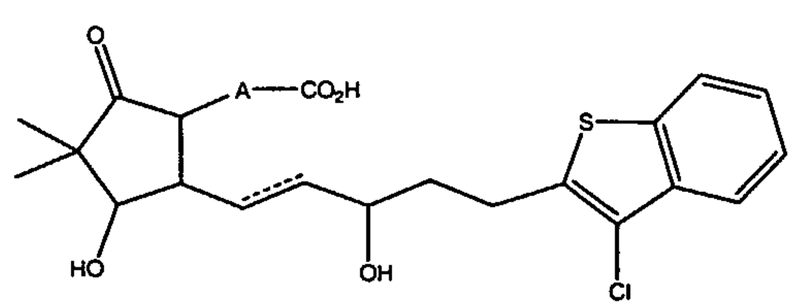
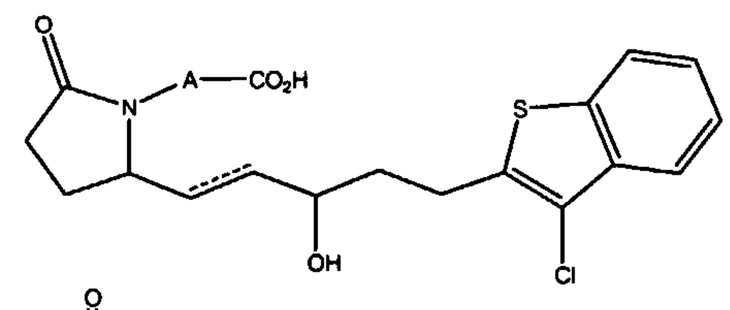
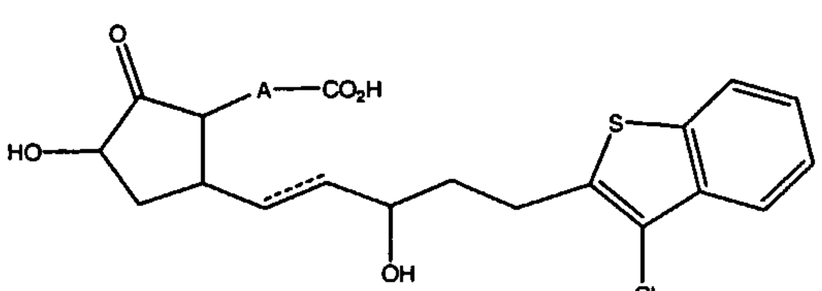
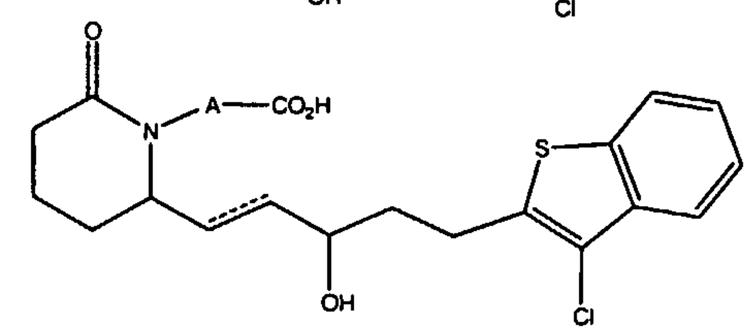
While not intending to limit the scope of the invention in any way, compounds according to the structures below are also examples of prostaglandin EP_4 agonists.

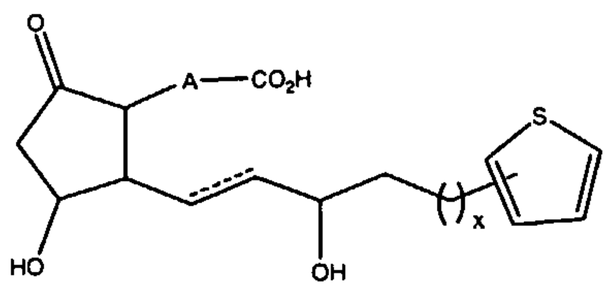
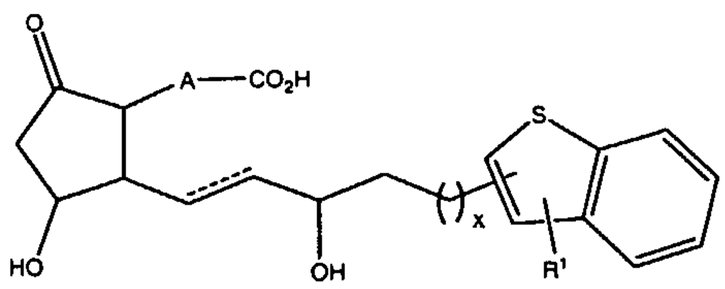
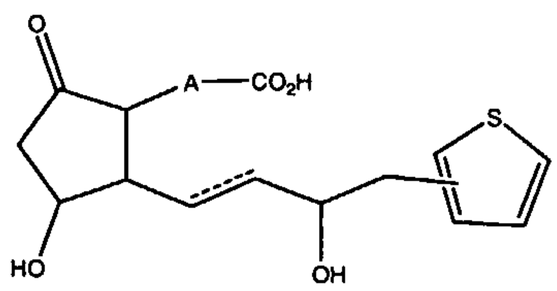
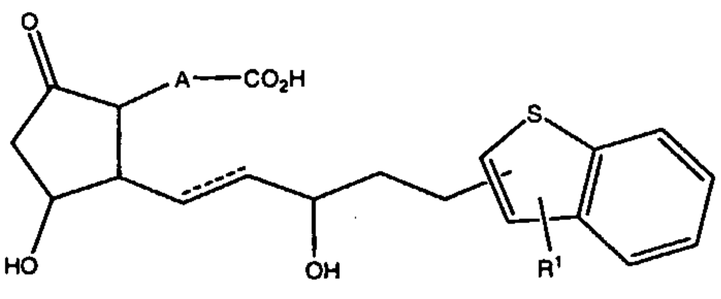
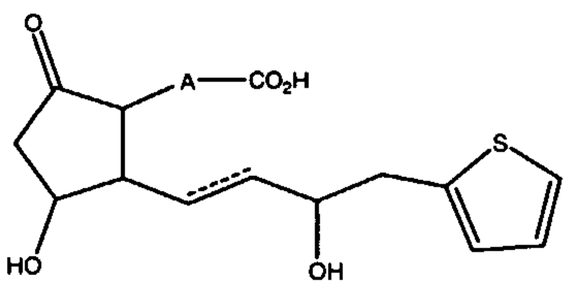
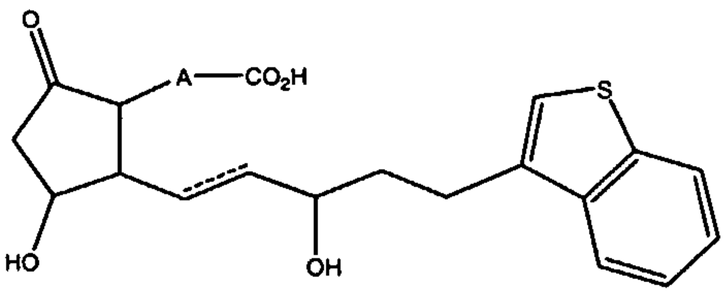
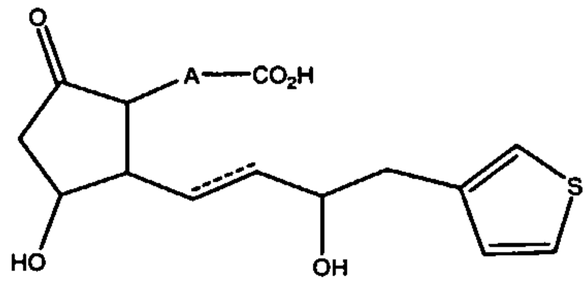
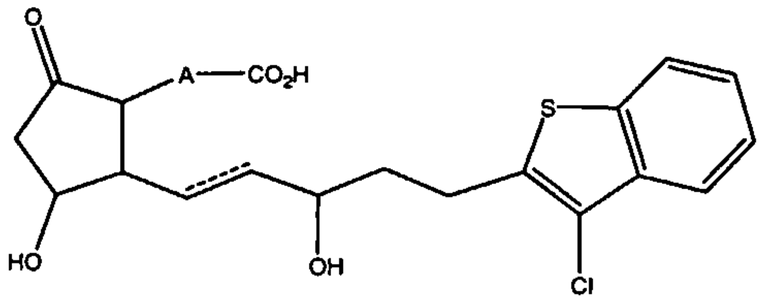
While not intending to limit the scope of the invention in any way, compounds according to the structures below are also examples of prostaglandin EP_4 agonists.

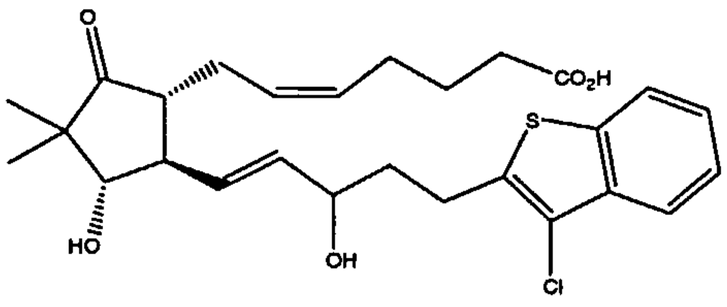
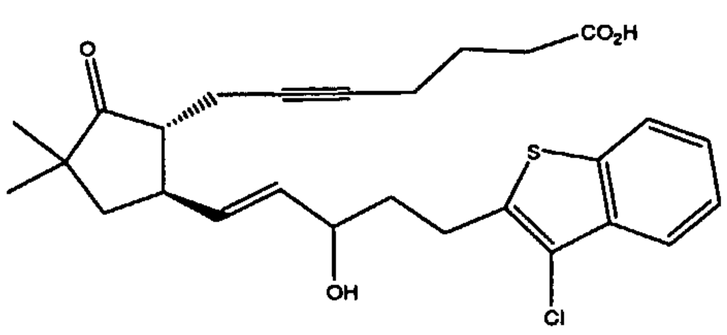
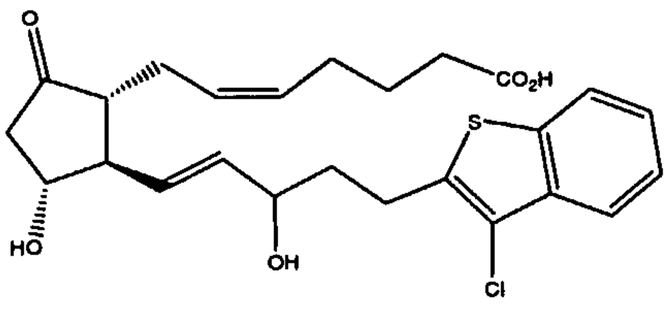
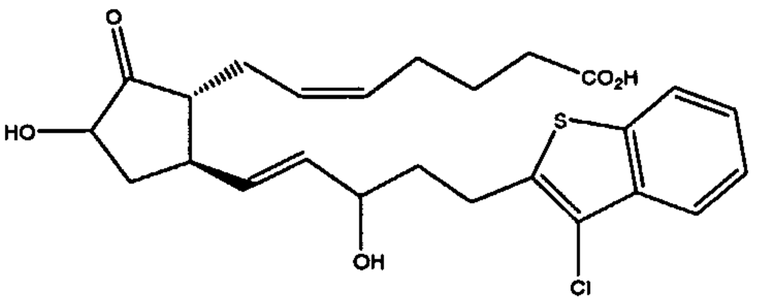
While not intending to limit the scope of the invention in any way, compounds according to the structures below are also examples of prostaglandin EP_4 agonists.

While not intending to limit the scope of the invention in any way, compounds according to the structures below, wherein x is 0 or 1 and R^1 is H, chloro, fluoro, bromo, methyl, methoxy, or CF_3, are also examples of prostaglandin EP_4 agonists.

While not intending to limit the scope of the invention in any way, compounds according to the structures below are also examples of prostaglandin EP_4 agonists.

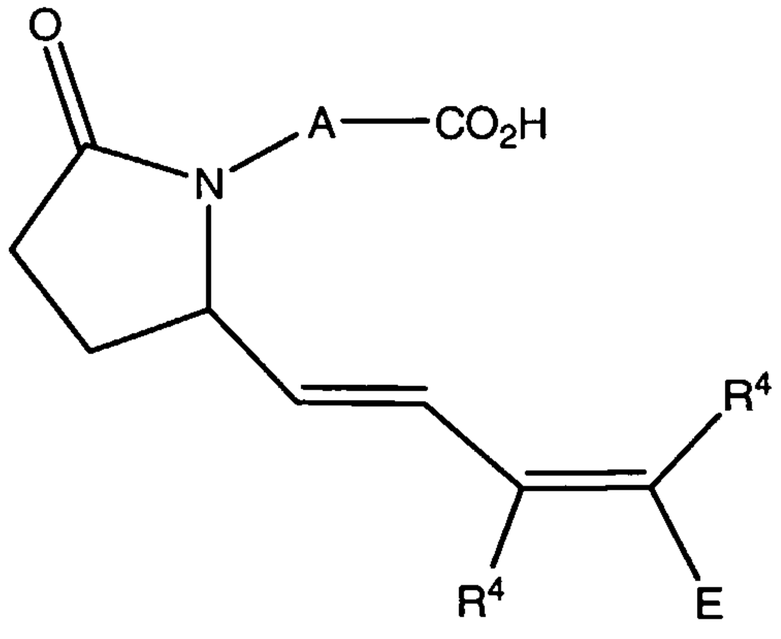





Furthermore, the following United States Patent Applications or Patents, all of which are expressly incorporated by reference herein, disclose compounds which are prostaglandin EP_4 agonists: United States Patent No. 6,552,067; United States Patent No. 6,747,054; United States Patent Application Publication No. 20030120079; and United States Patent Application Publication No. 20030207925; United States Patent Application Publication

No. 20040157901; United States Patent No. 4,117,014; United States Patent Application Publication No. 2004/0142969; United States Patent No. 6,747,037; United States Patent No. 6,610,719; U.S. Patent No. 4,171,375; U.S. Patent No. 3,931,297; United States Patent Application Serial No. 821,705, filed April 9, 2004; United States Patent No. 6,670,485; United States Patent No. 6,410,591; and United States Patent No. 6,538,018.

Methods and prodrugs related to all of these prostaglandin EP4 agonists are specifically contemplated herein.

Prodrugs of prostaglandin EP_4 agonists comprising



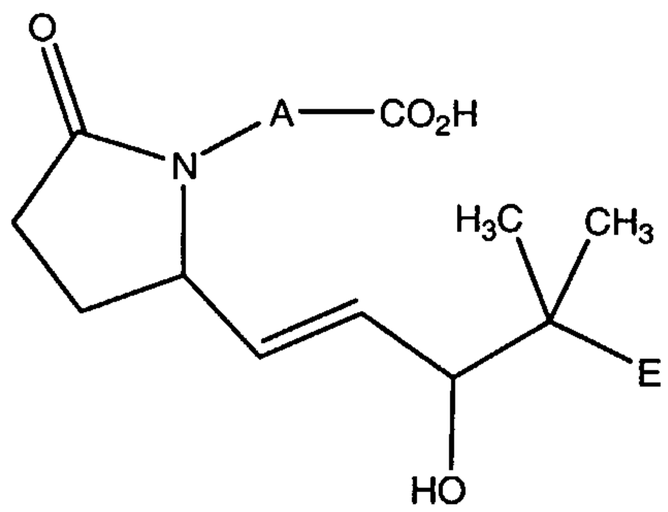
are also contemplated herein;

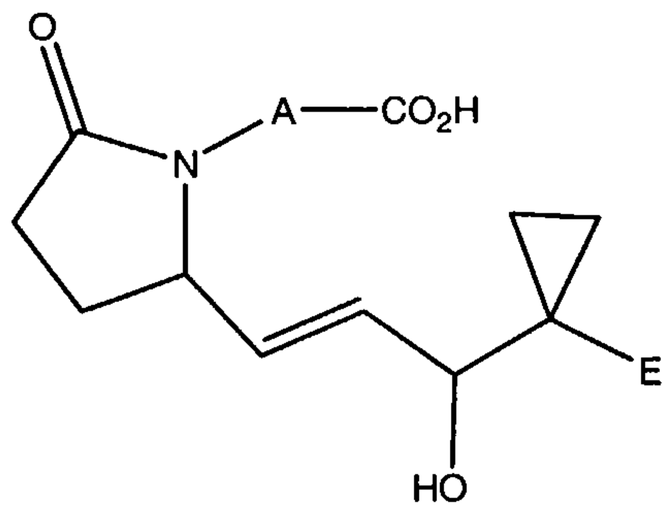
wherein R^4 is H, halo or $C_{1\text{-}6}$ alkyl.

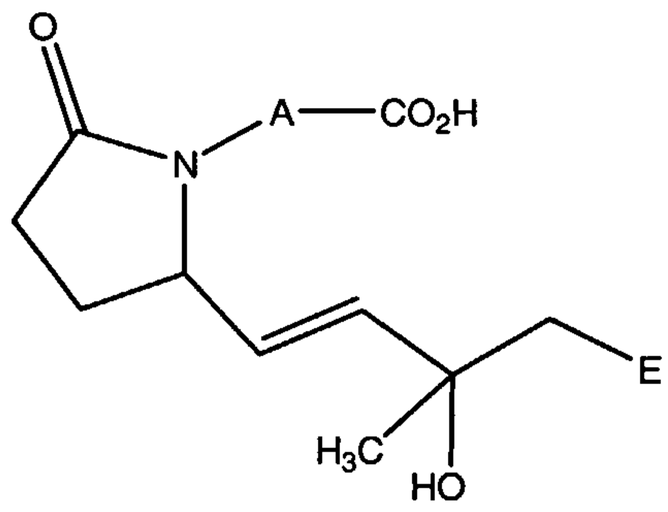
Halo is a group 7 atom such as fluoro, chloro, bromo, iodo, and the like.

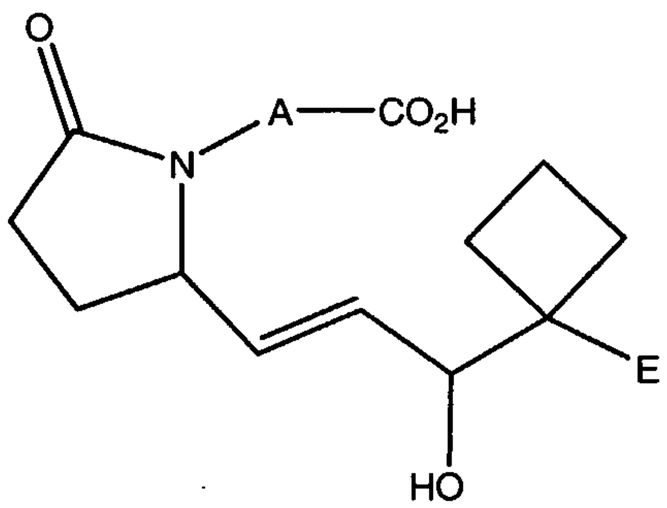
$C_{1\text{-}6}$ alkyl is linear, branched, or cyclic alkyl having from 1 to 6 carbons including, but not limited to, methyl, ethyl, propyl isomers, butyl isomers, pentyl isomers, hexyl isomers, cyclopropyl, cylobutyl, cyclohexyl, and the like.

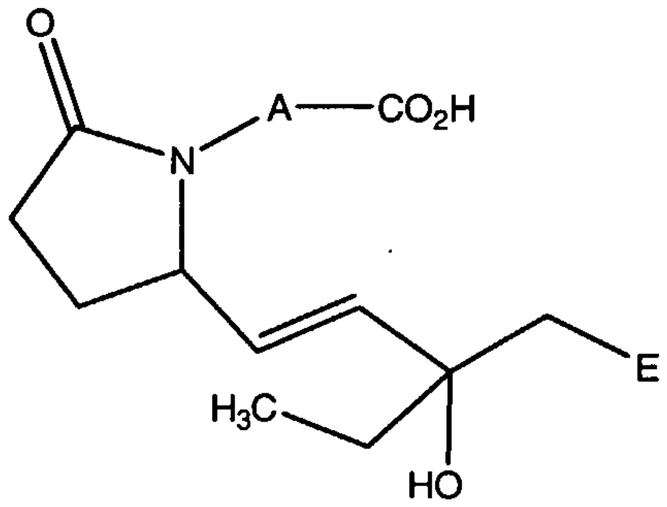
Prodrugs of prostaglandin EP4 agonists according to the structures below are also contemplated.

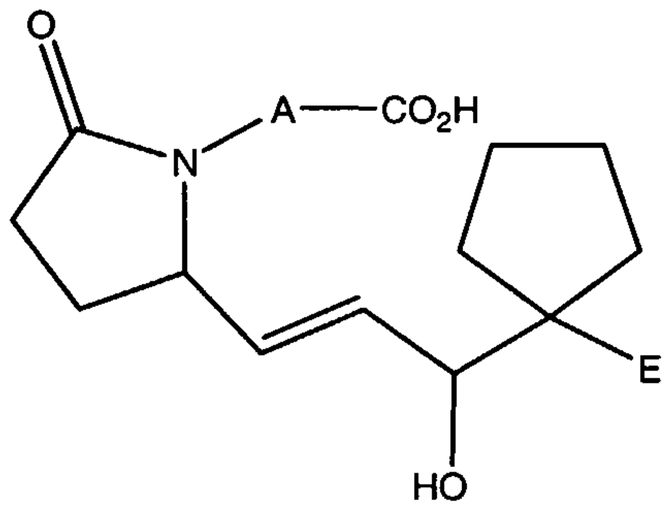


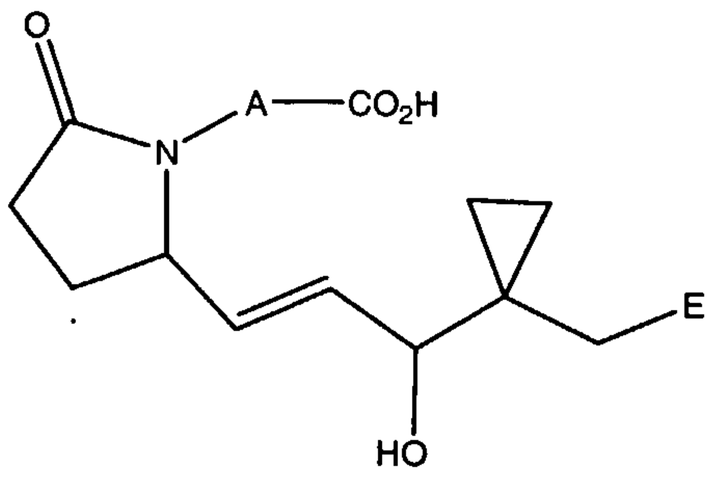


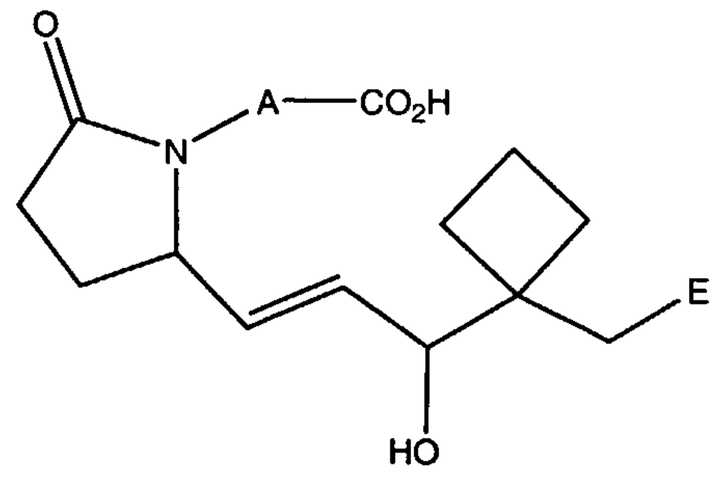


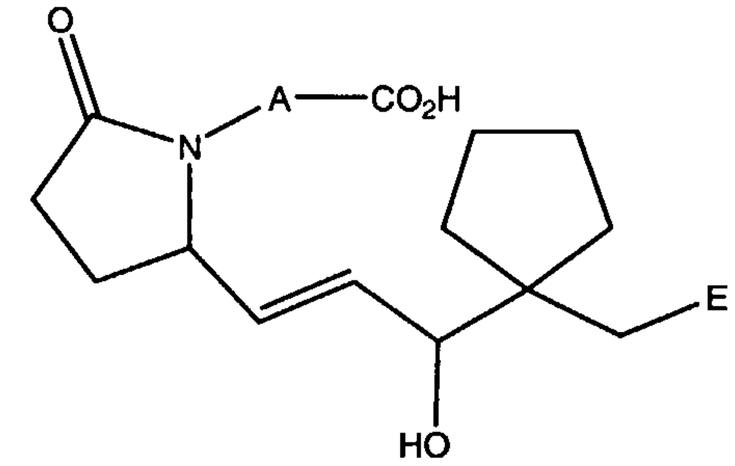


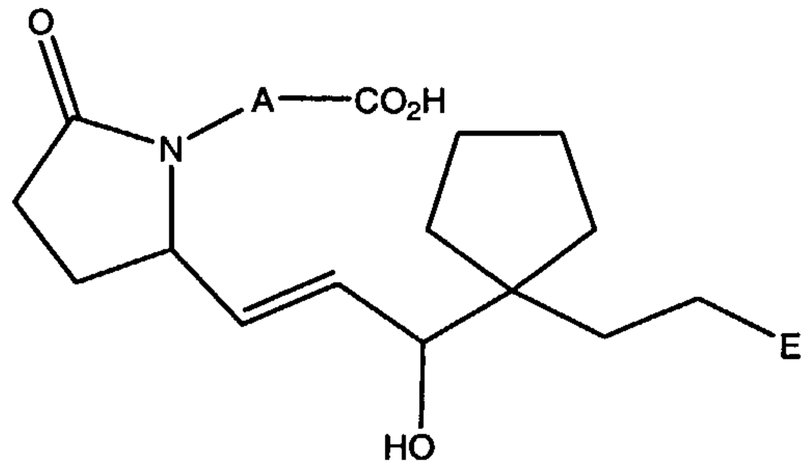


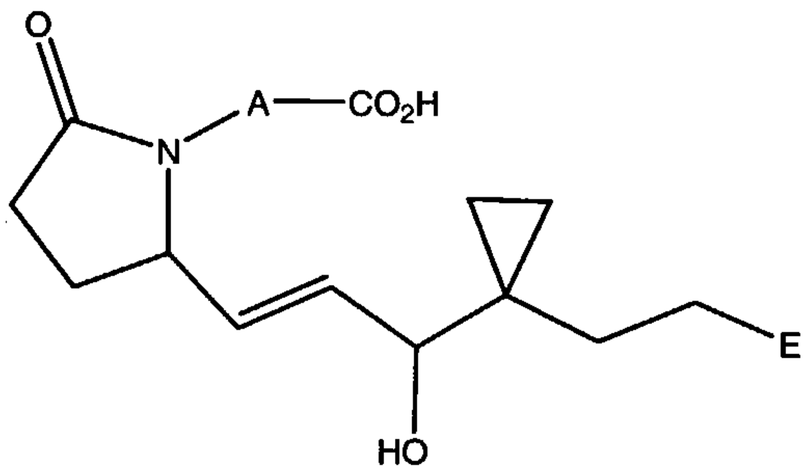


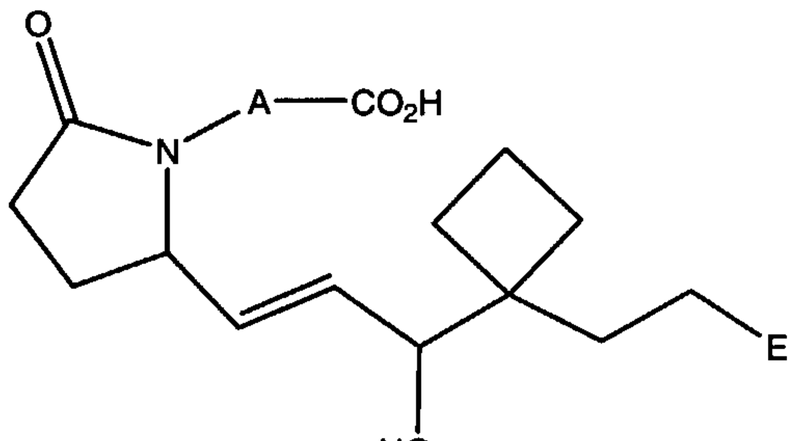


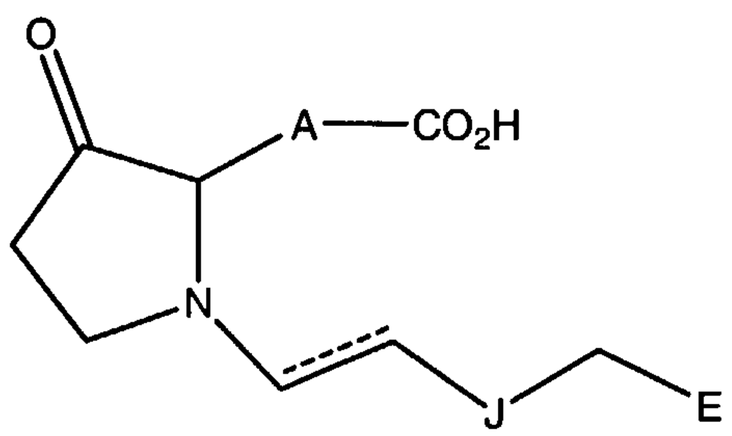


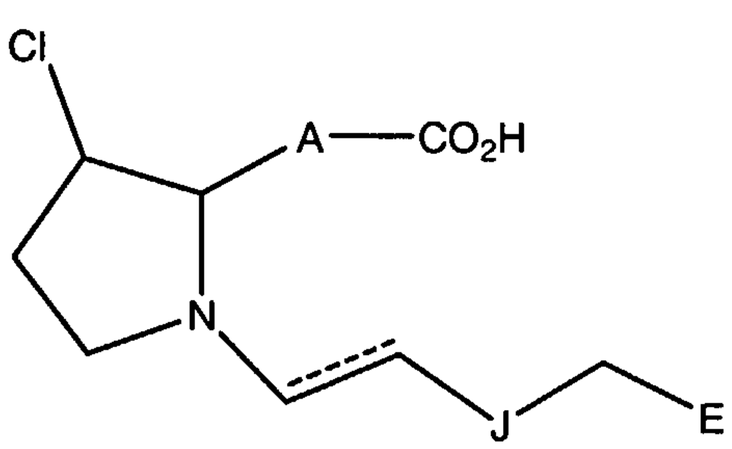


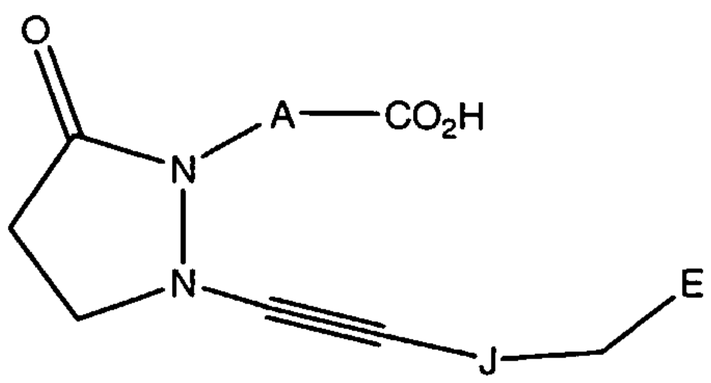


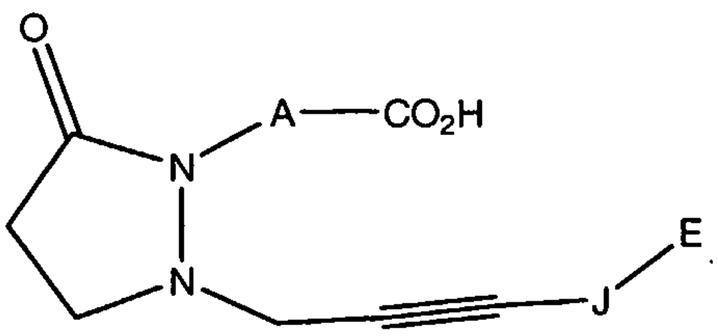


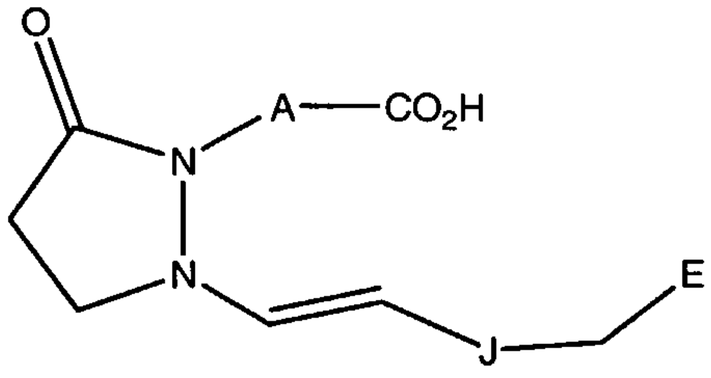


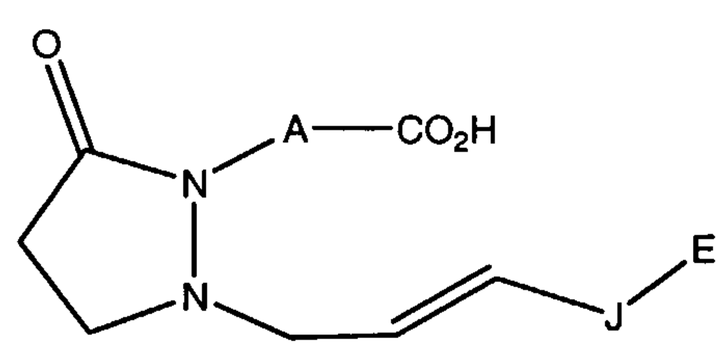


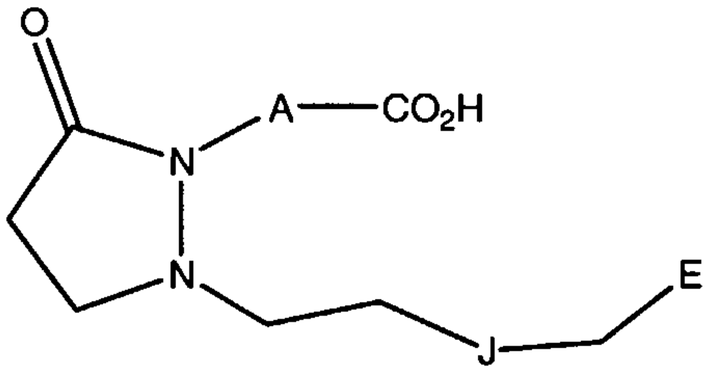


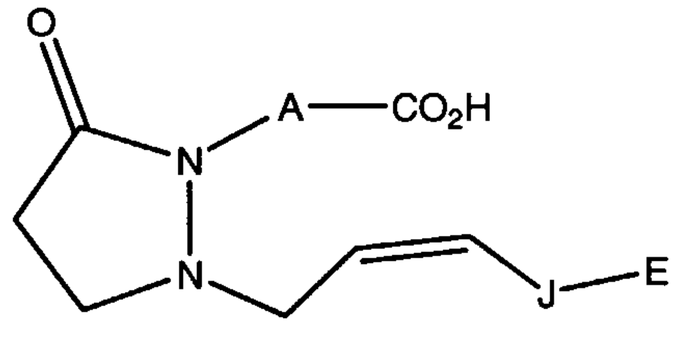


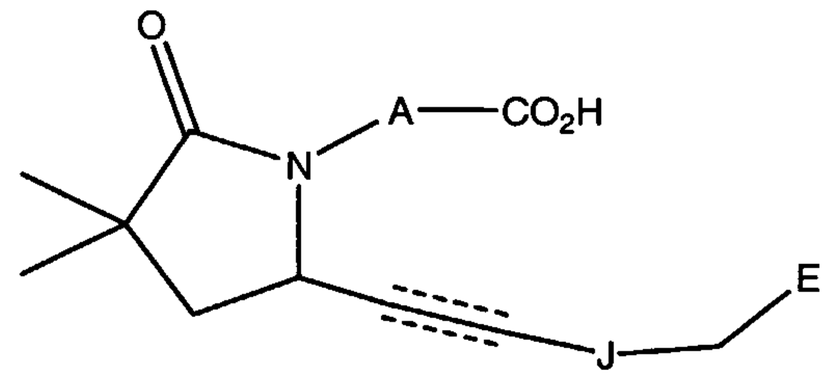


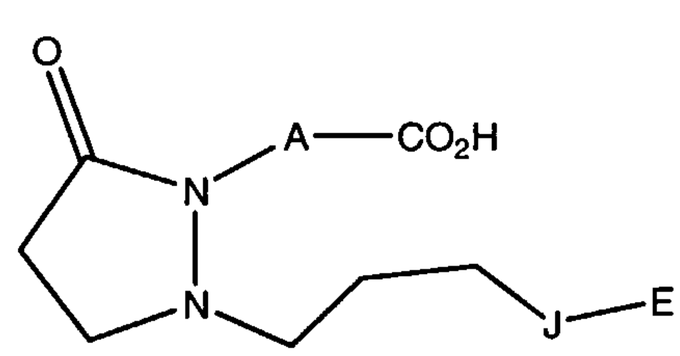












The term carbohydrate should be defined broadly to encompass simple sugars, disaccharides, oligosaccharides, polysaccharides, starches, and the like, whether linear, branched or macrocyclic. The term carbohydrate also refers to one of the foregoing classes of compounds having up to one amine functional group present for every six carbon atoms.

The esters, ethers, or amide prodrugs herein may incorporate either a direct bond to the carbohydrate or amino acid, or may alternatively incorporate a spacer group including, but not limited to,

polyols such as ethylene glycol, glycerine, and the like, or oligomers or polymers thereof;

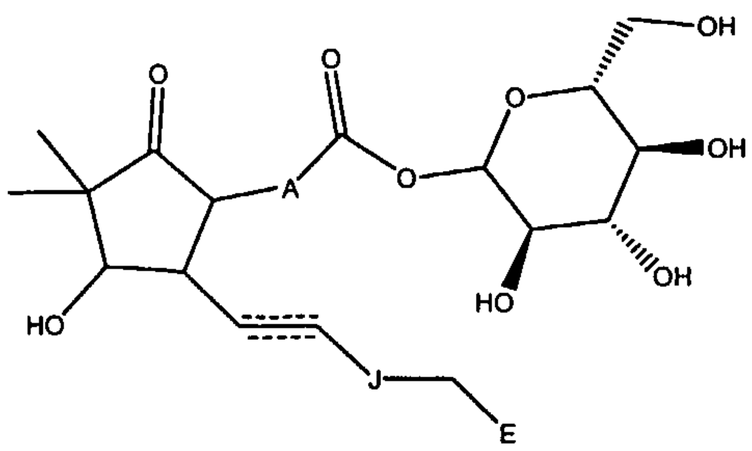
dicarboxylic acids such as succinic acid, maleic acid, malonic acid, azelaic acid, and the like;

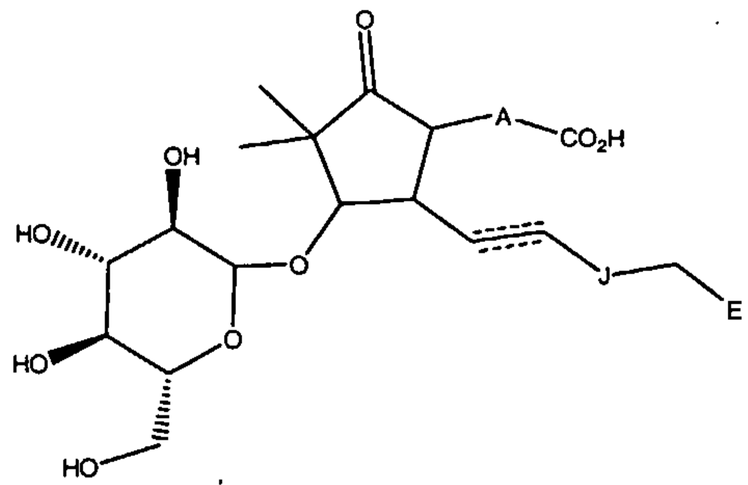
hydroxycarboxylic acids such as lactic acid, hydroxyacetic acid, citric acid, and the like;

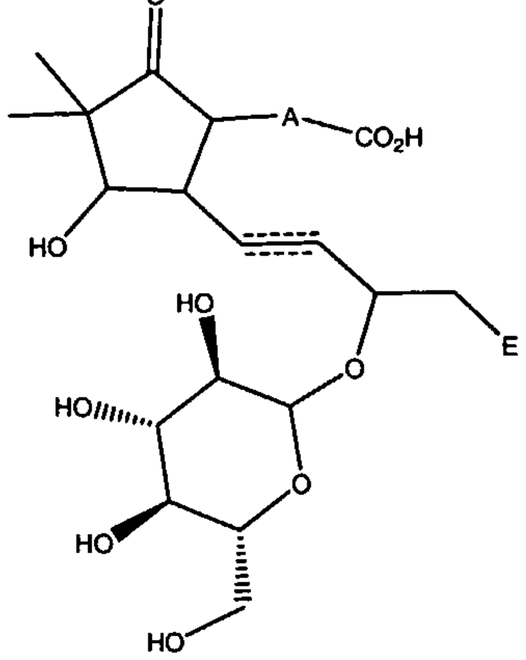
polyamines such as ethylene diamine and the like; and

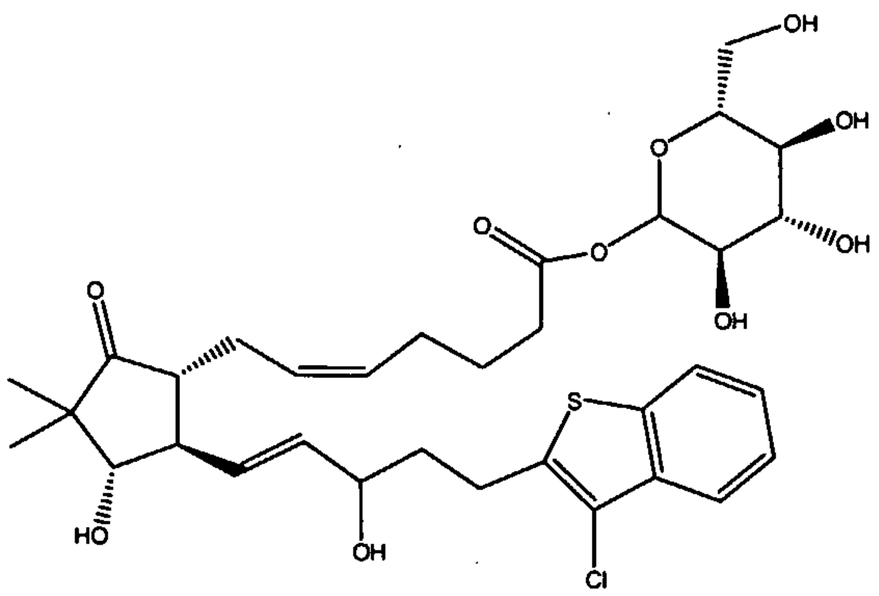
esters, amides, or ethers to form combinations of any of the above.

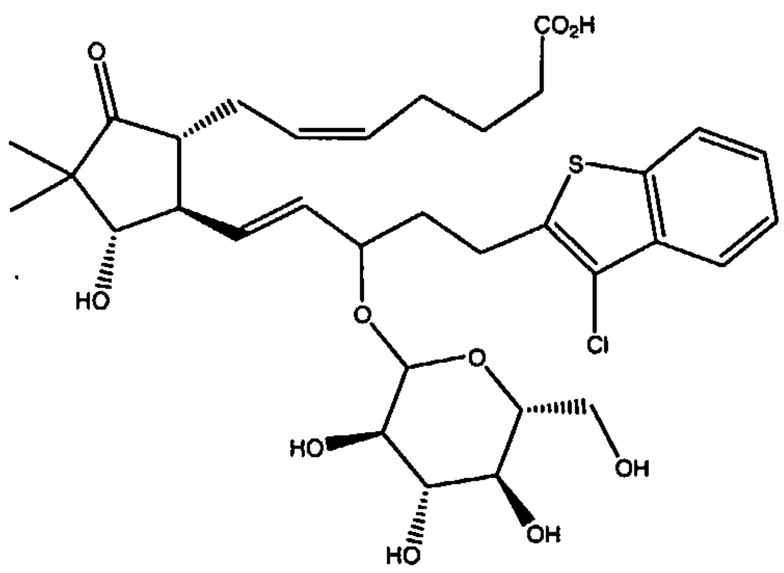
In certain embodiments, the prodrug is a glucoside ester or ether. Thus, compounds like those shown below, or pharmaceutically acceptable salts thereof, are useful.

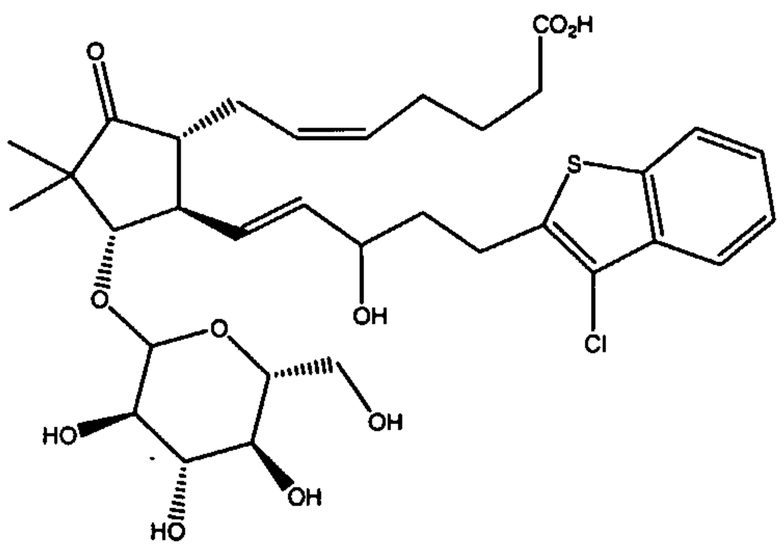


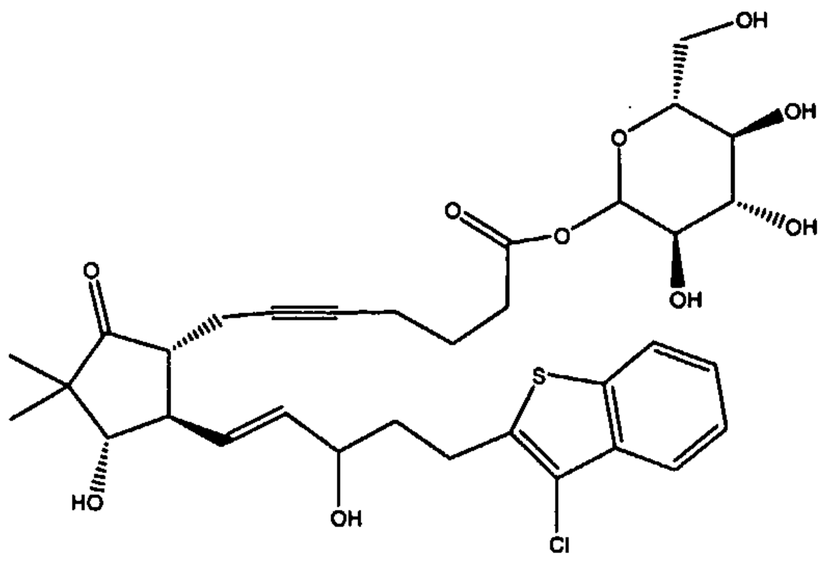


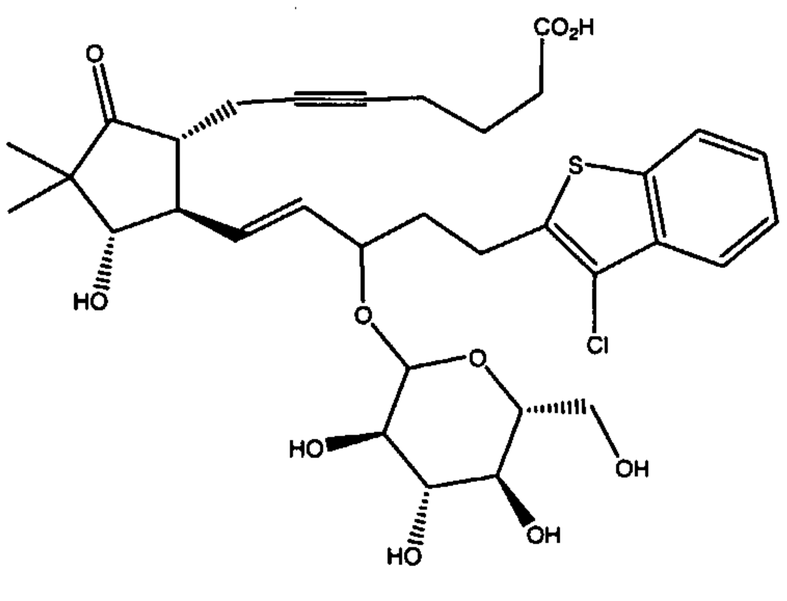


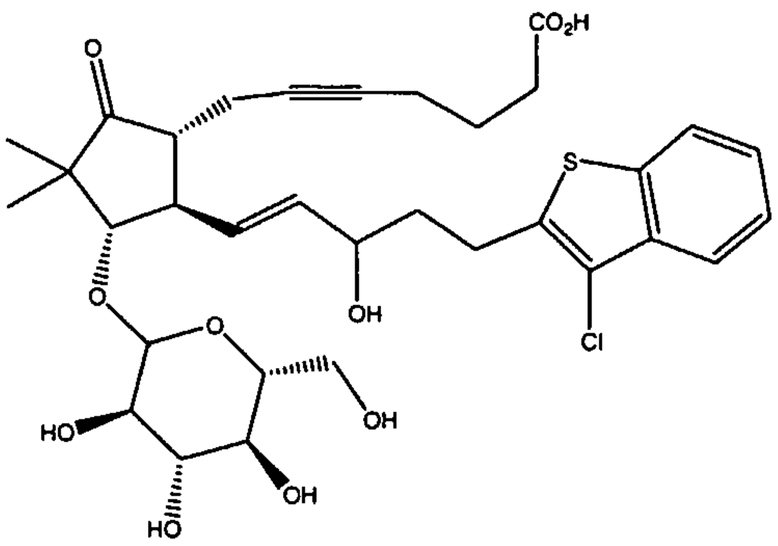


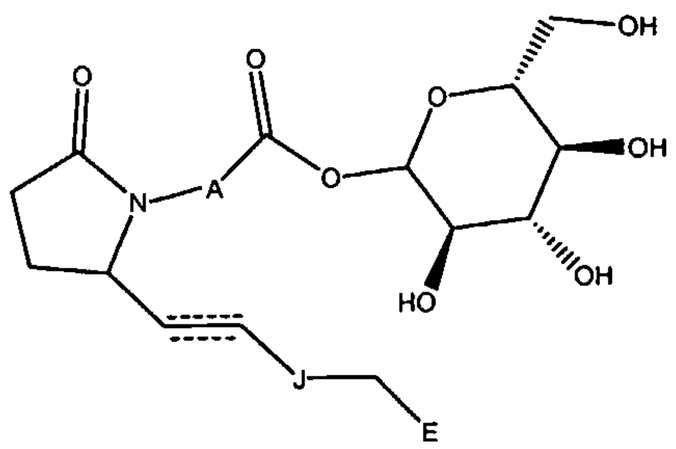


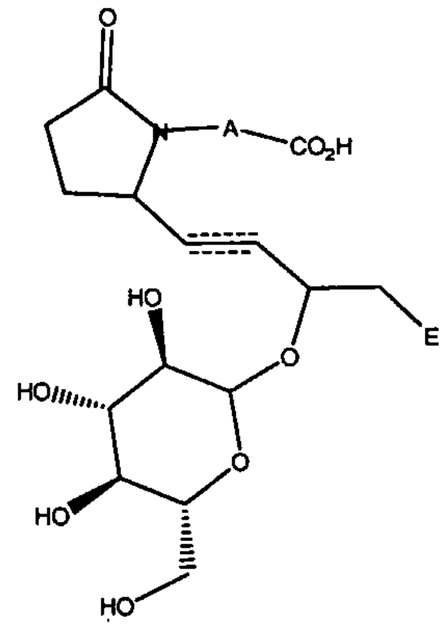


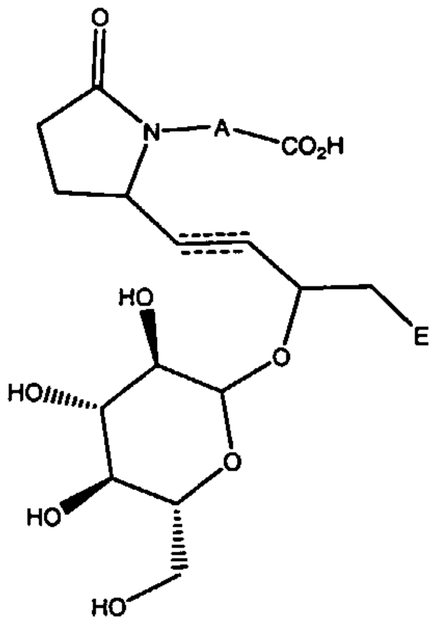


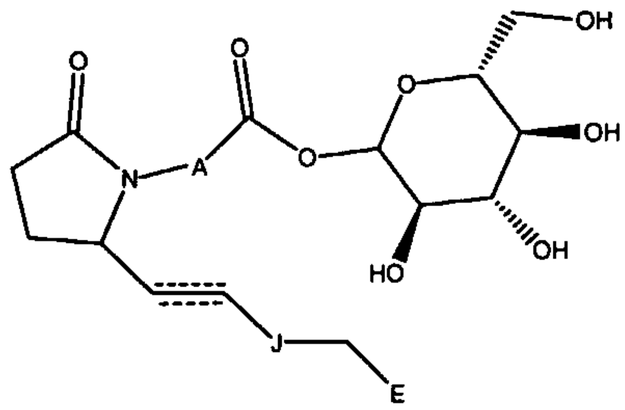


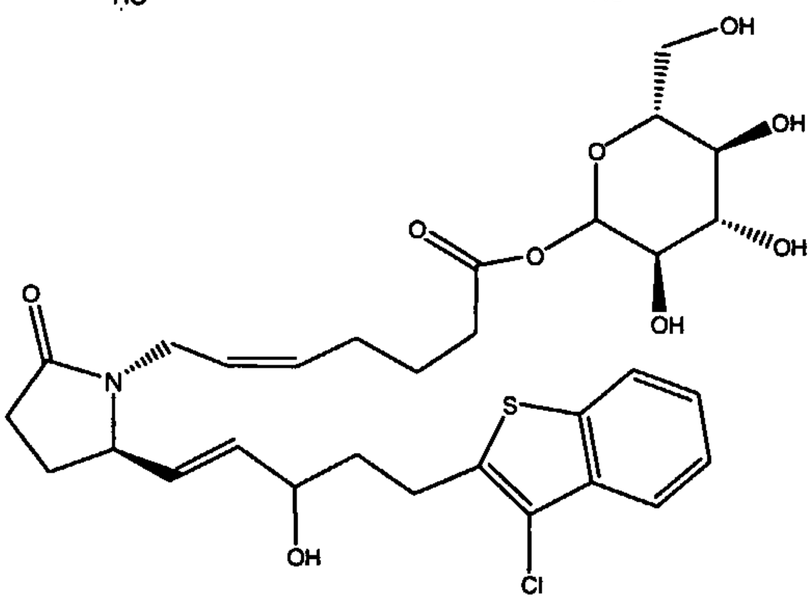


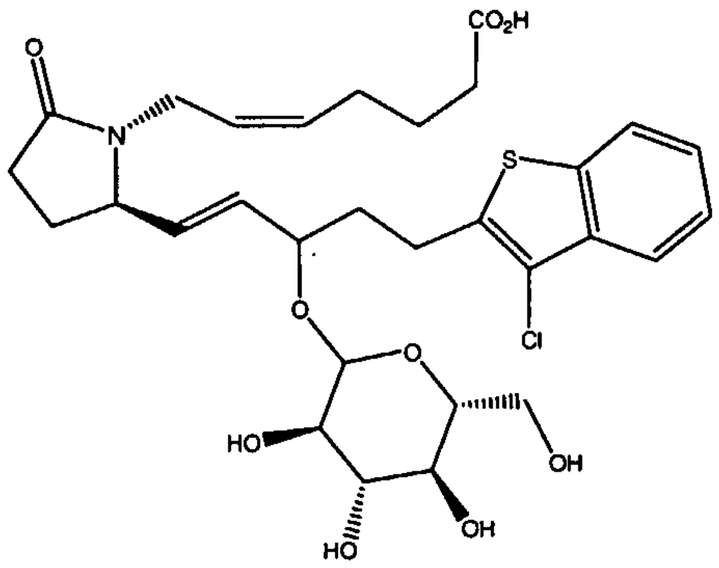












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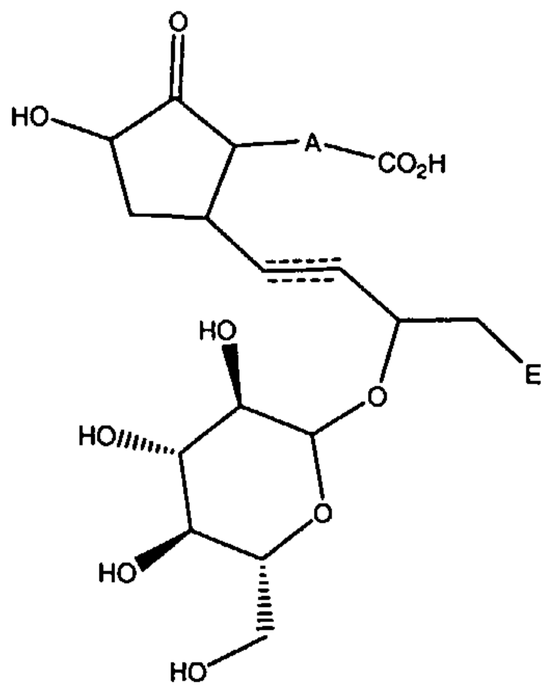

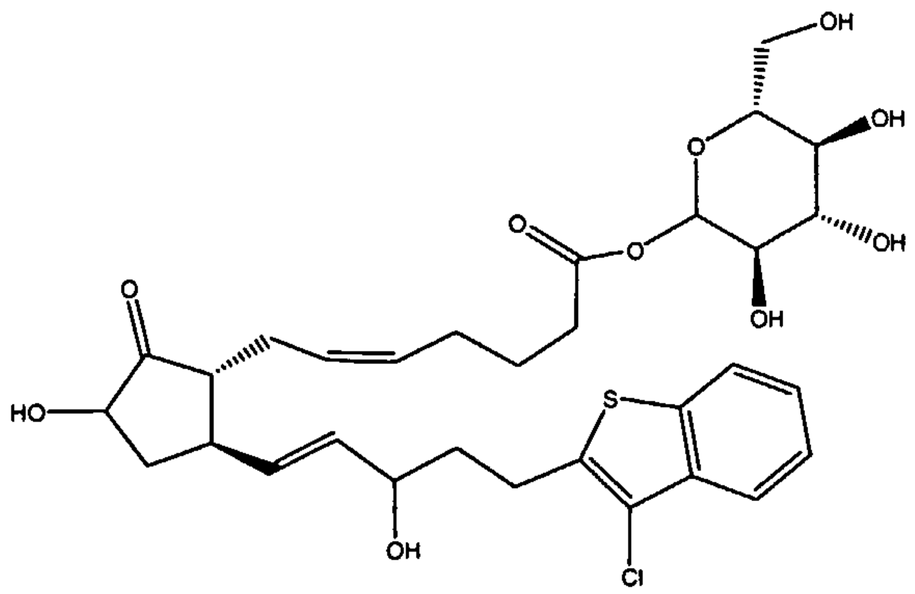

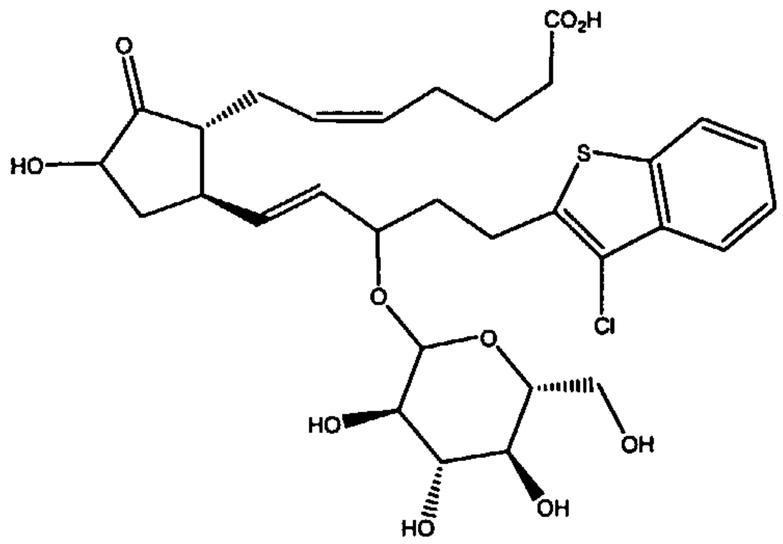

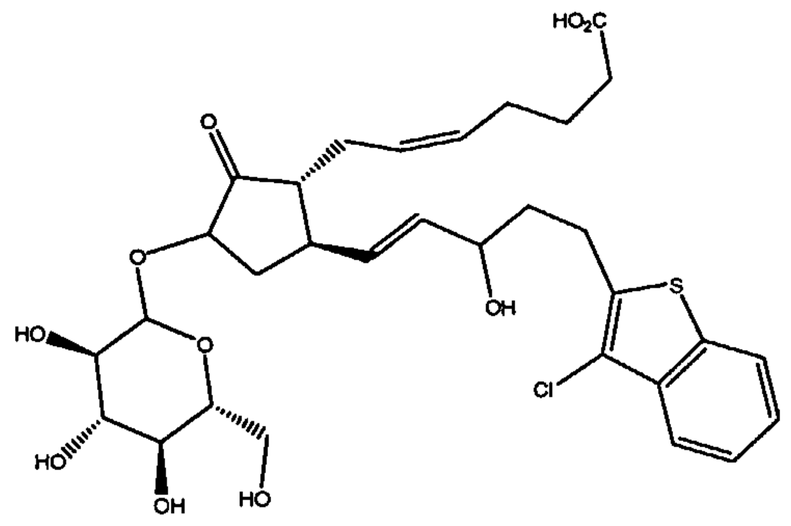

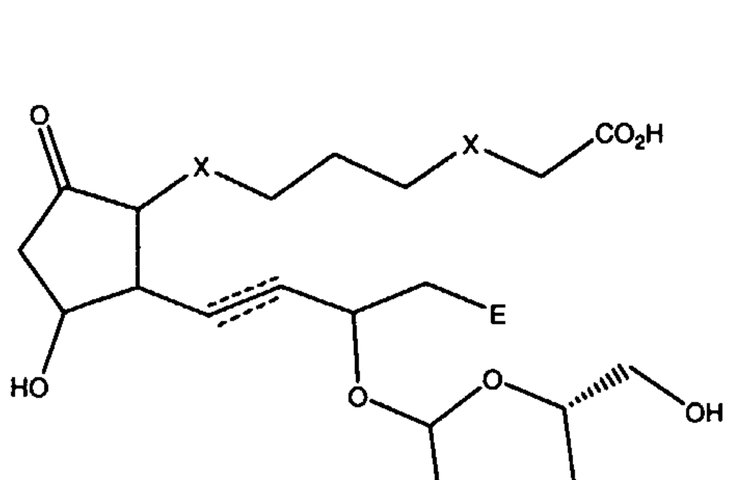

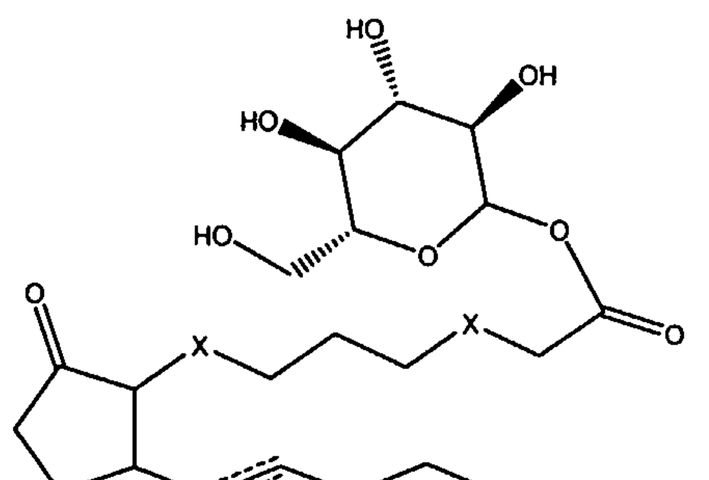

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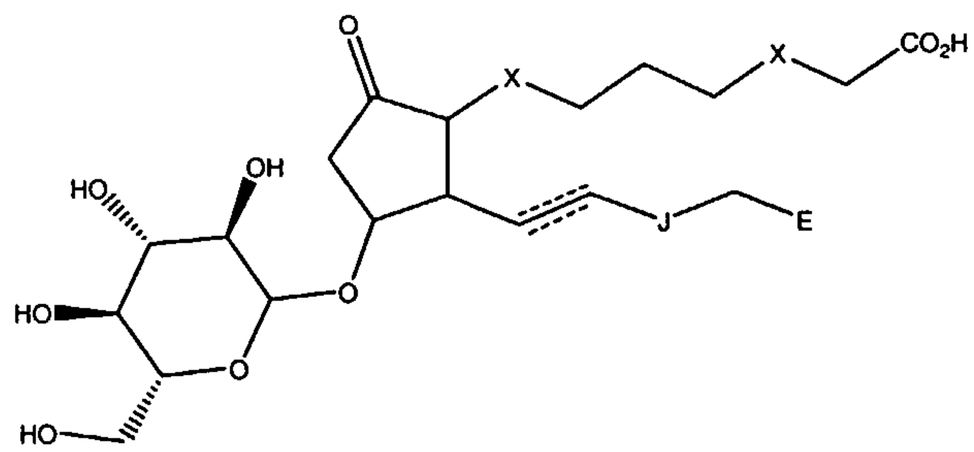
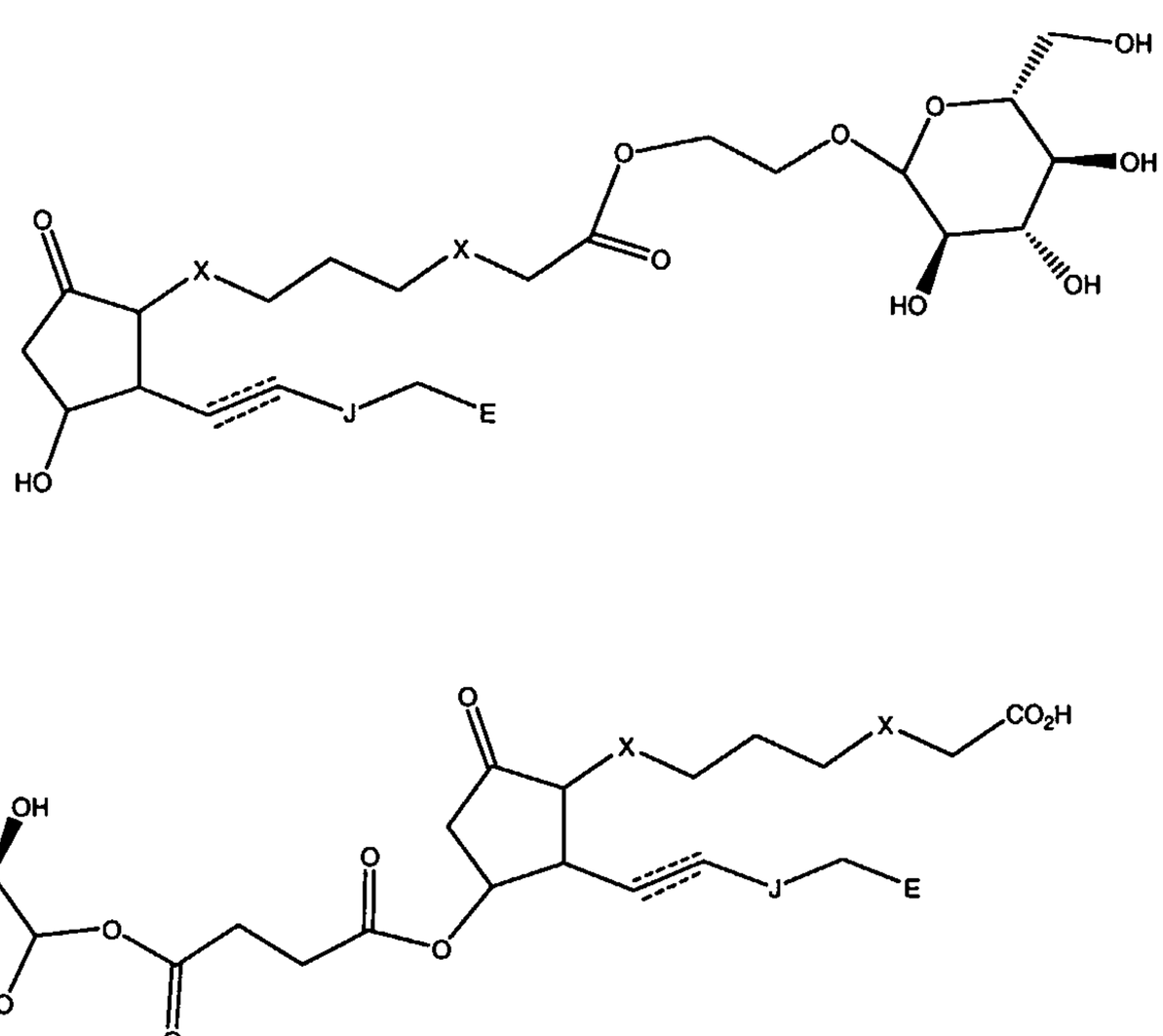
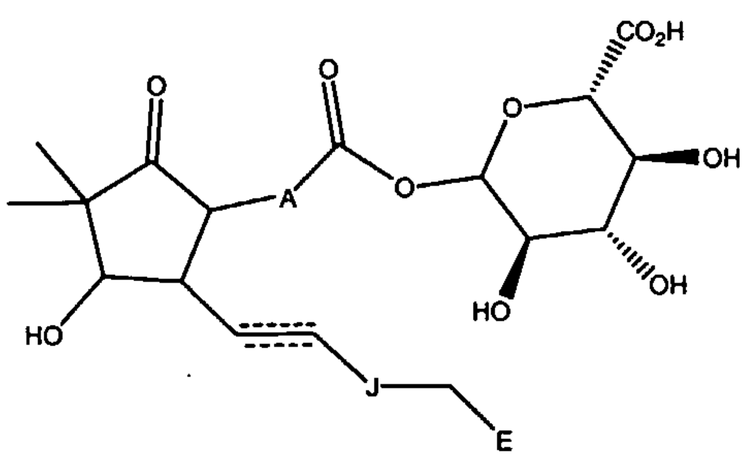
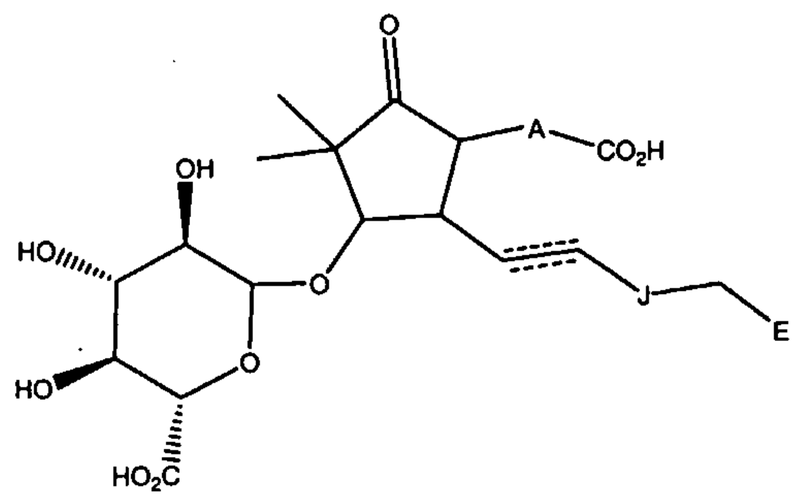


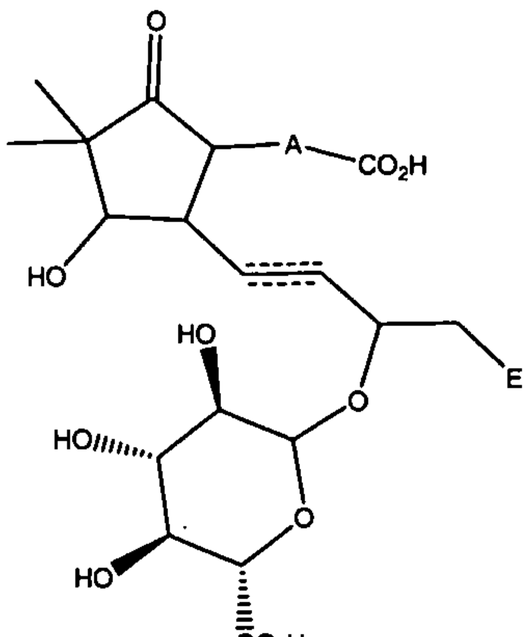


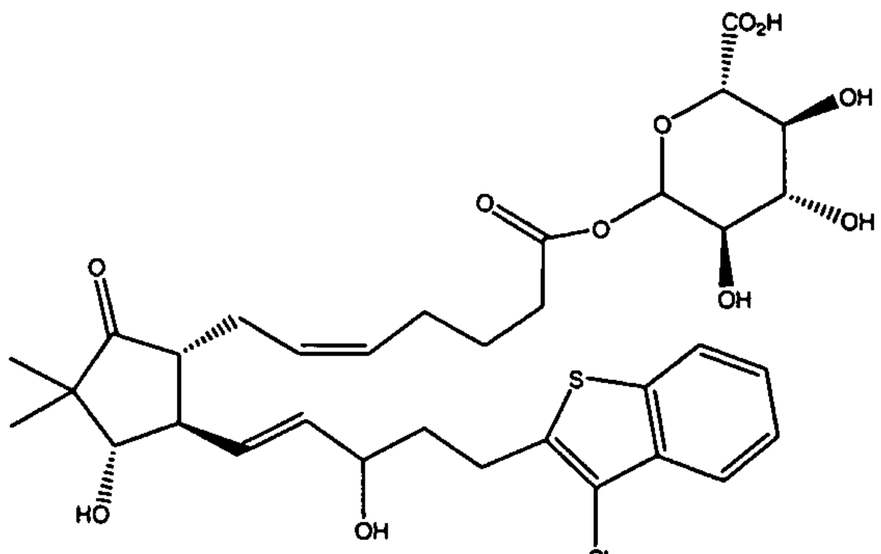
Alternatively, the ester or ether bond may occur at a different position on the sugar; i.e. the oxygen of one of the other hydroxyl groups is the oxygen of the ester or ether bond.

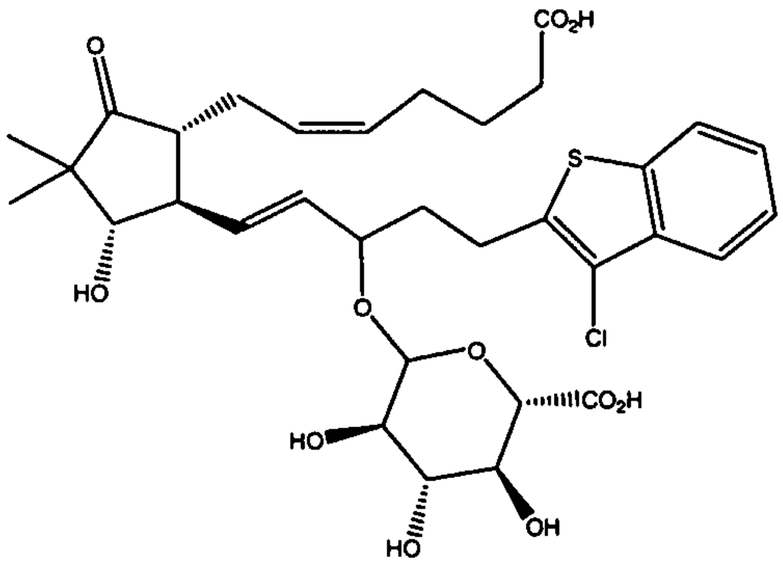
In other embodiments, the prodrug is a glucuronide ester or ether. Thus, compounds like those shown below, or pharmaceutically acceptable salts thereof, are useful.

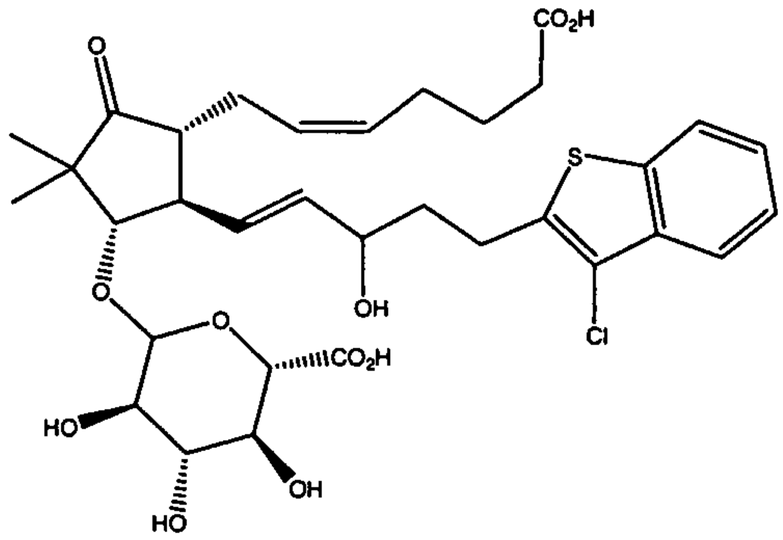


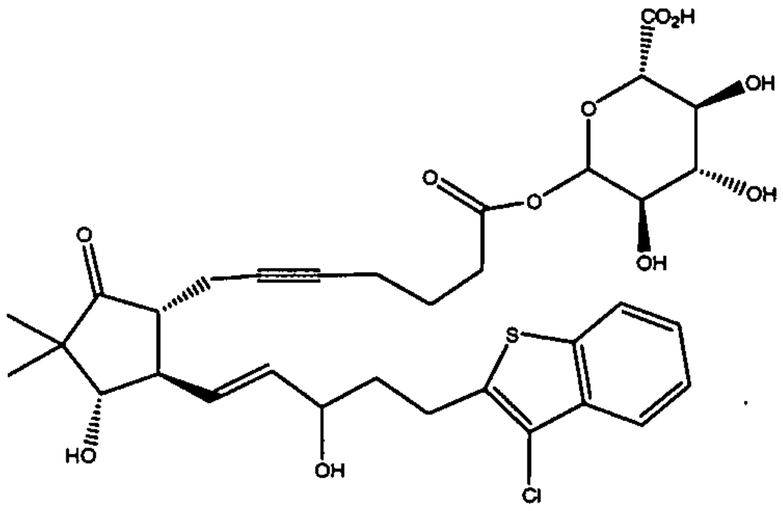


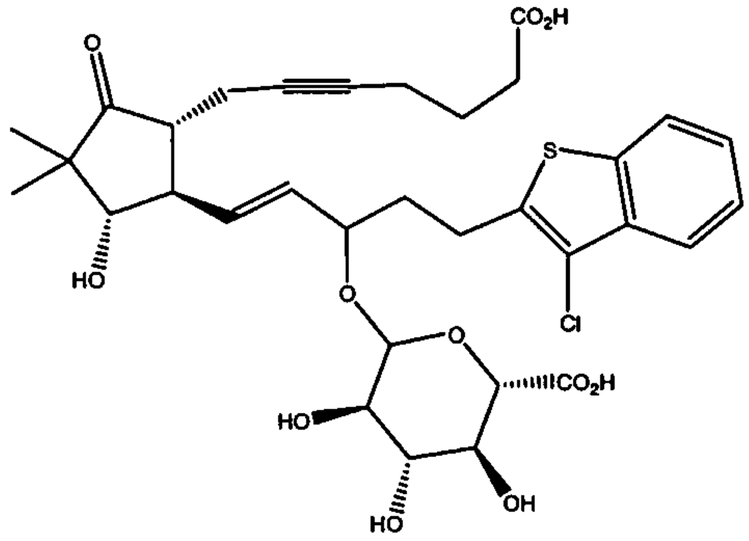


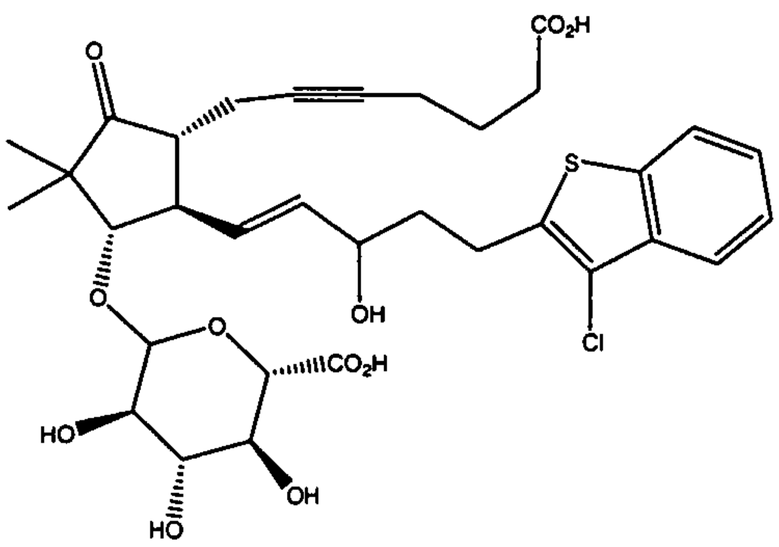


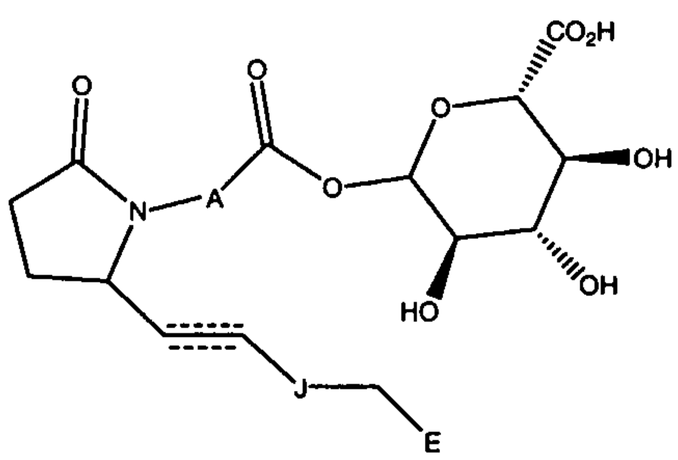


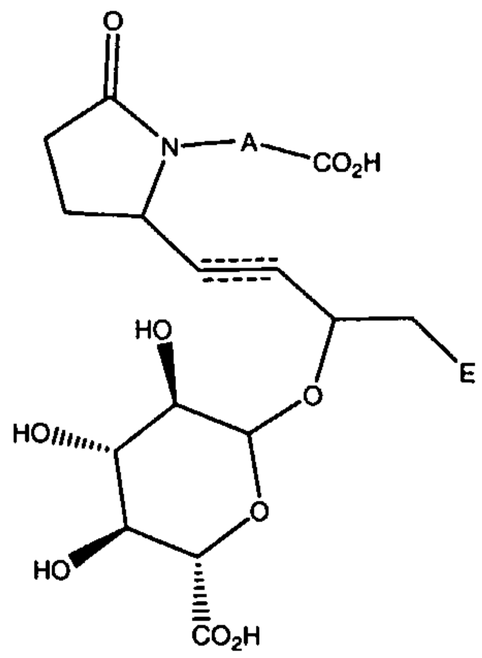


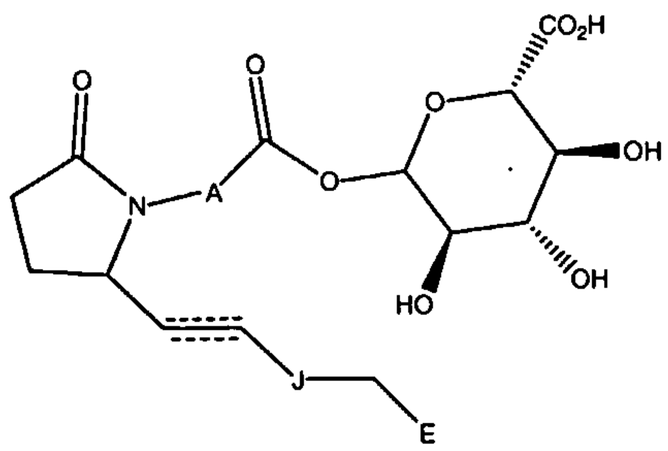


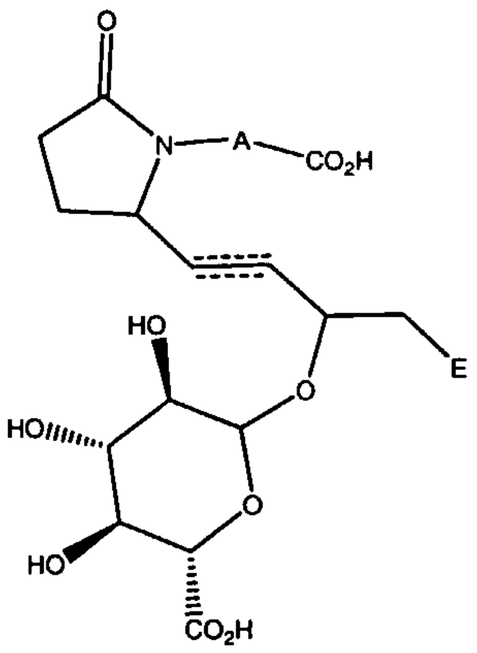


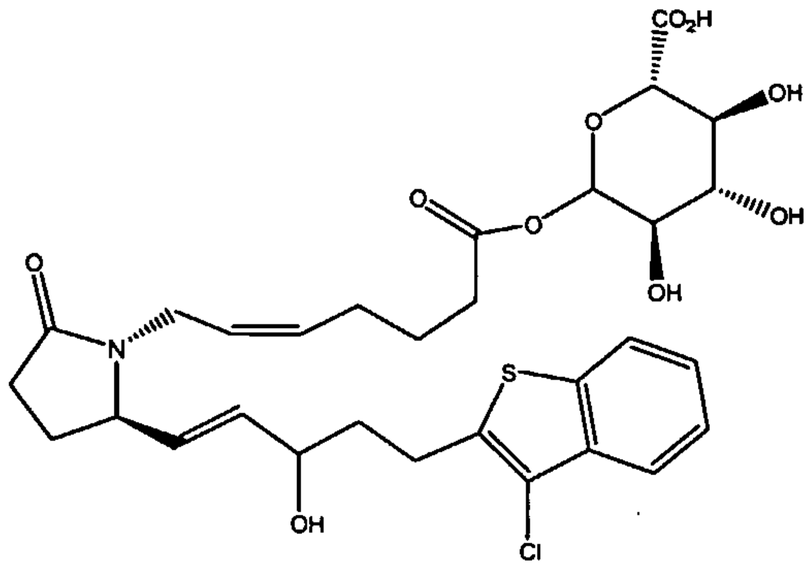


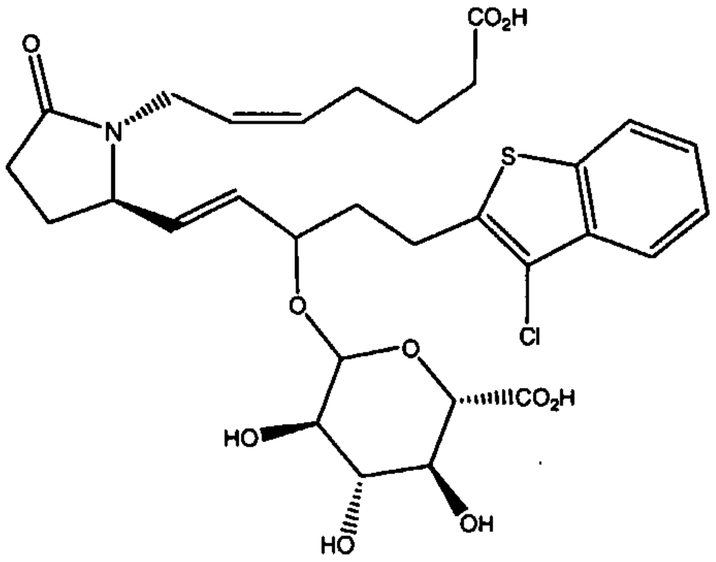


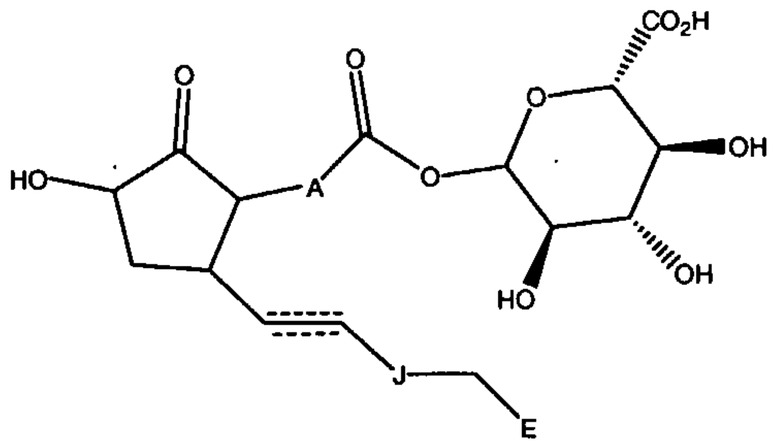


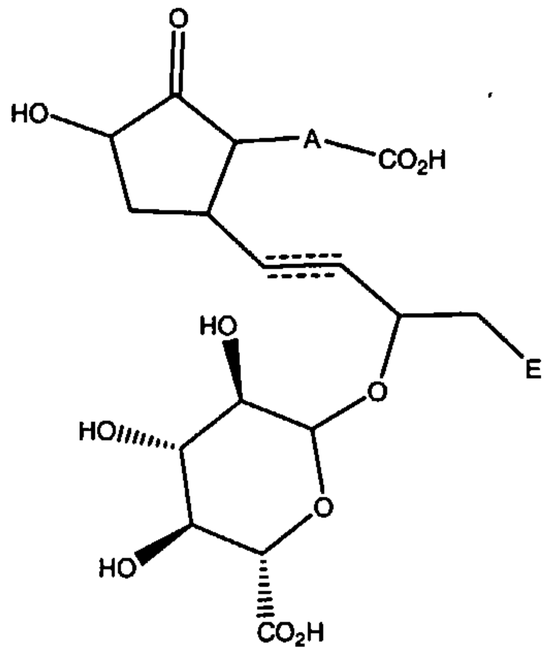


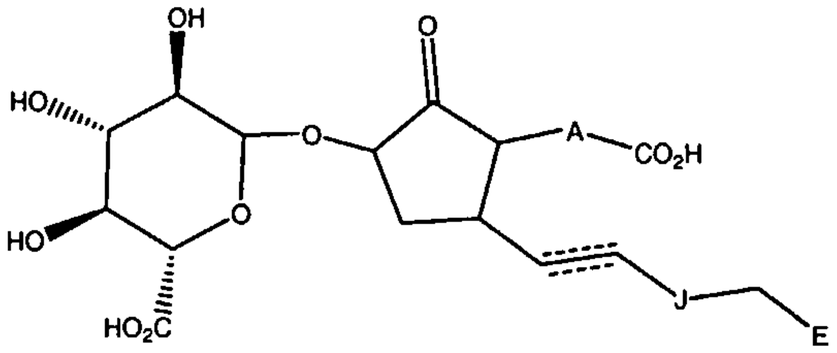


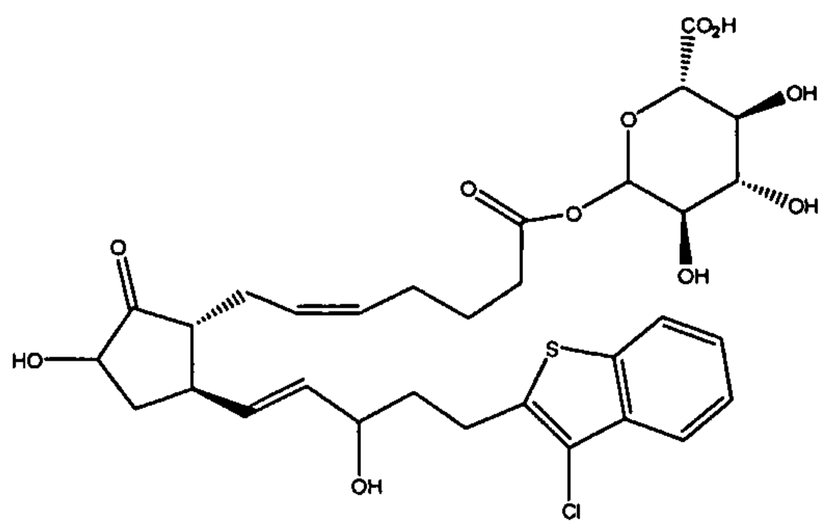


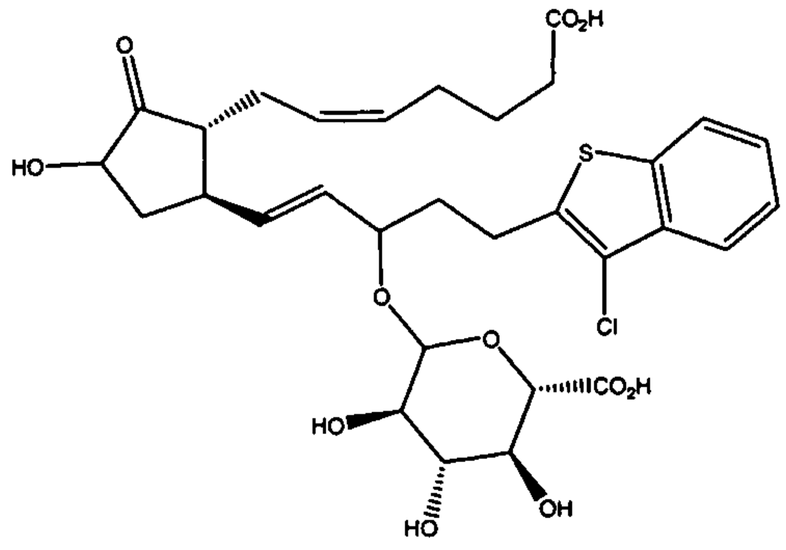


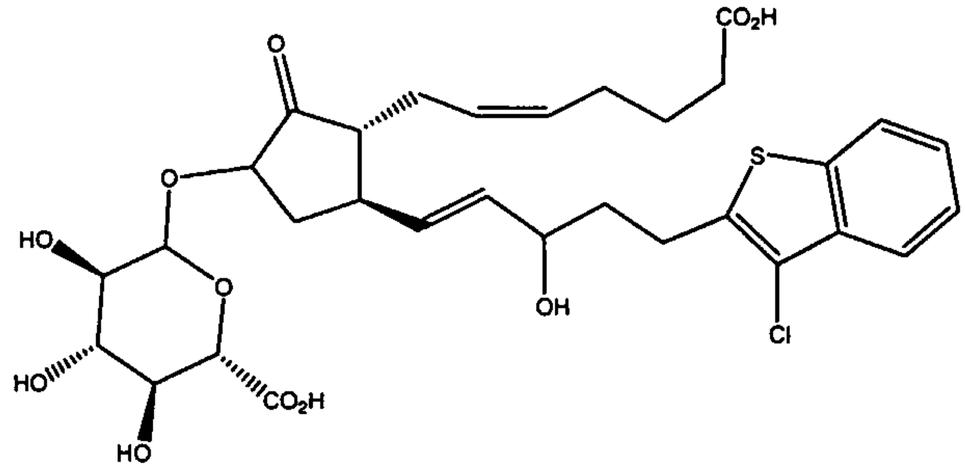


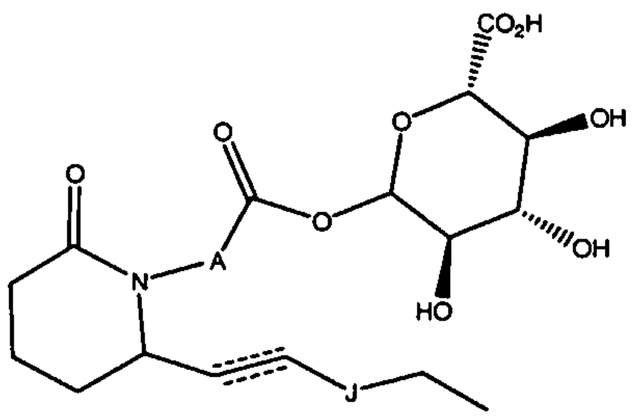


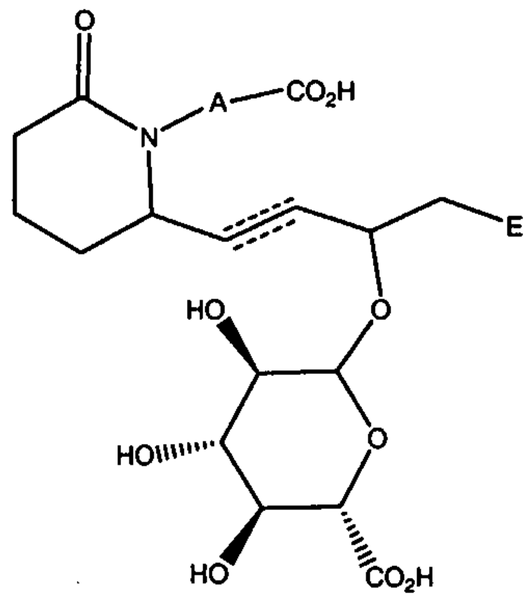


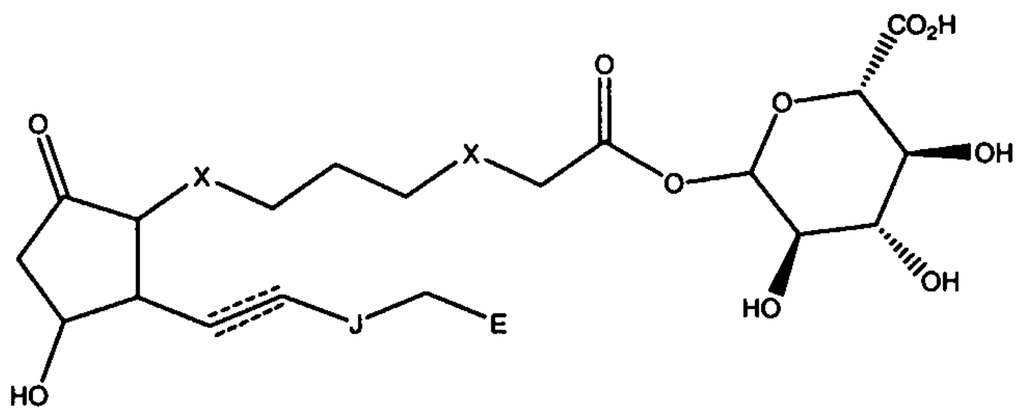


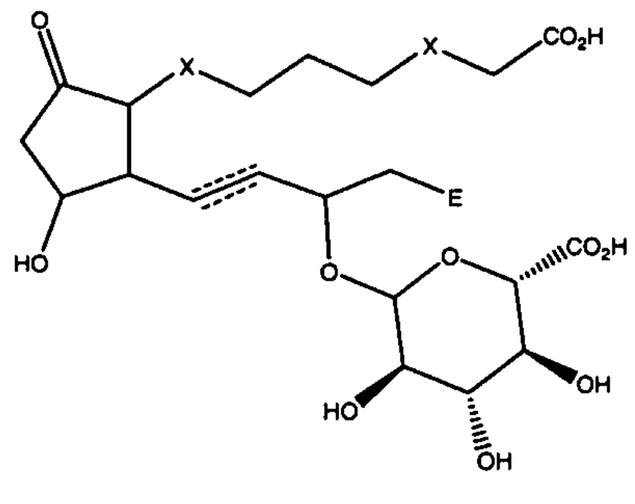


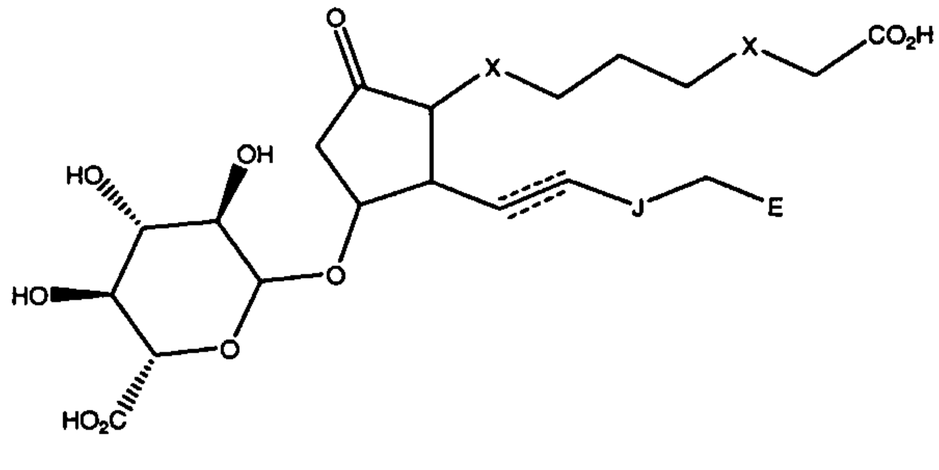


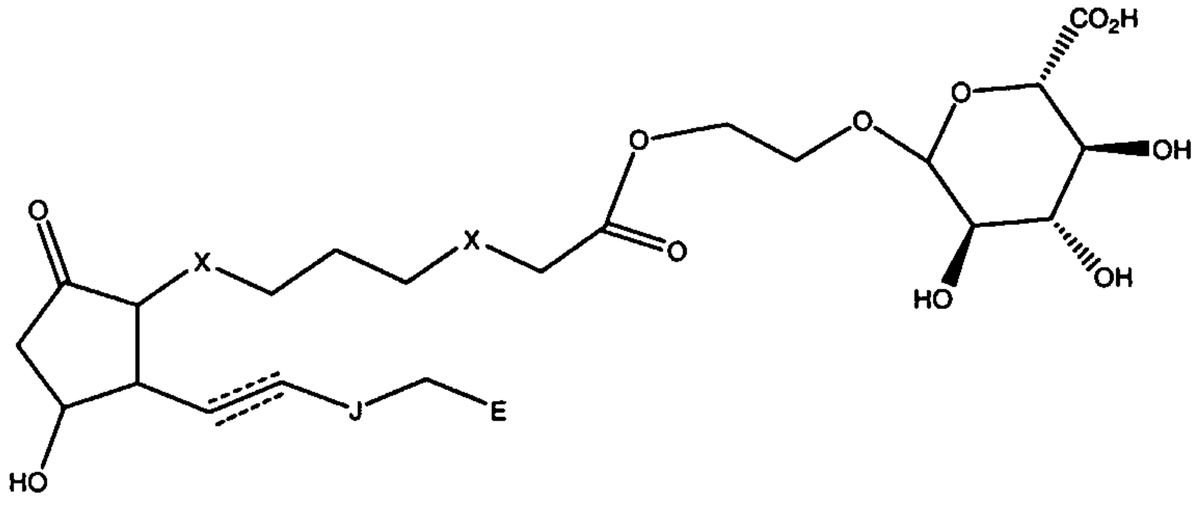


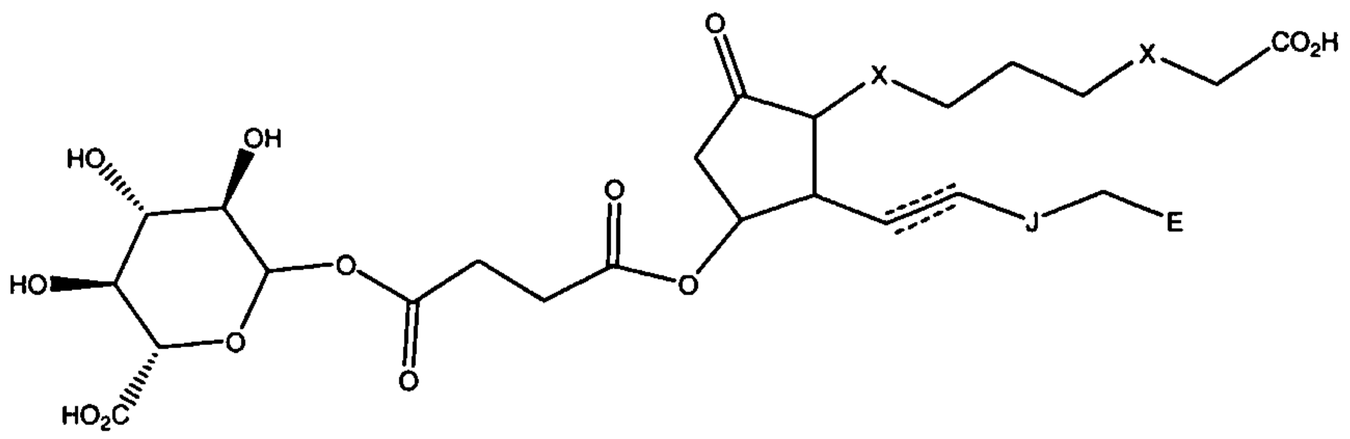






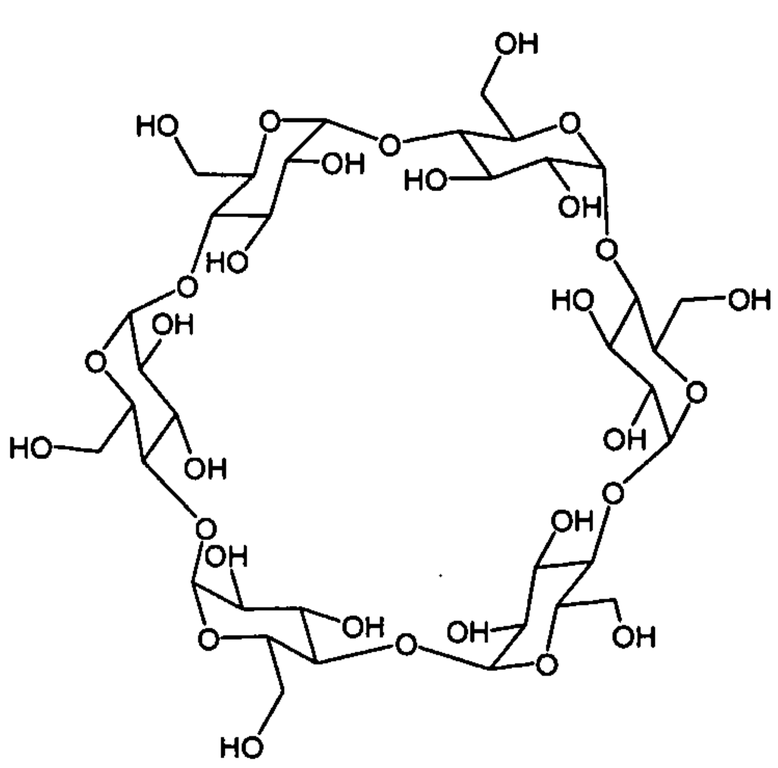




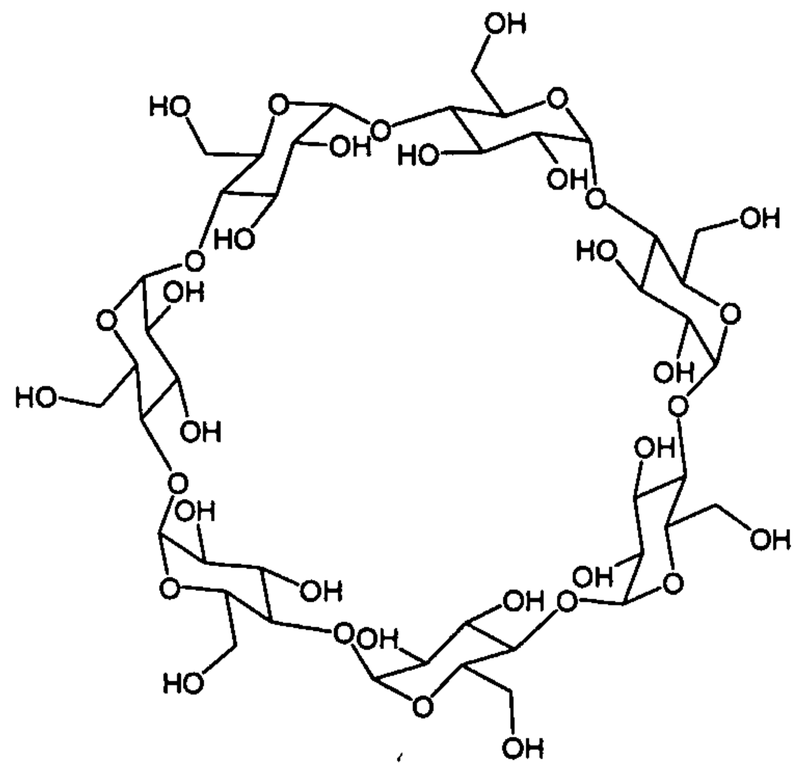


Alternatively, the ester or ether bond may occur at a different position on the sugar; i.e. the oxygen of one of the other hydroxyl groups is the oxygen of the ester or ether bond.

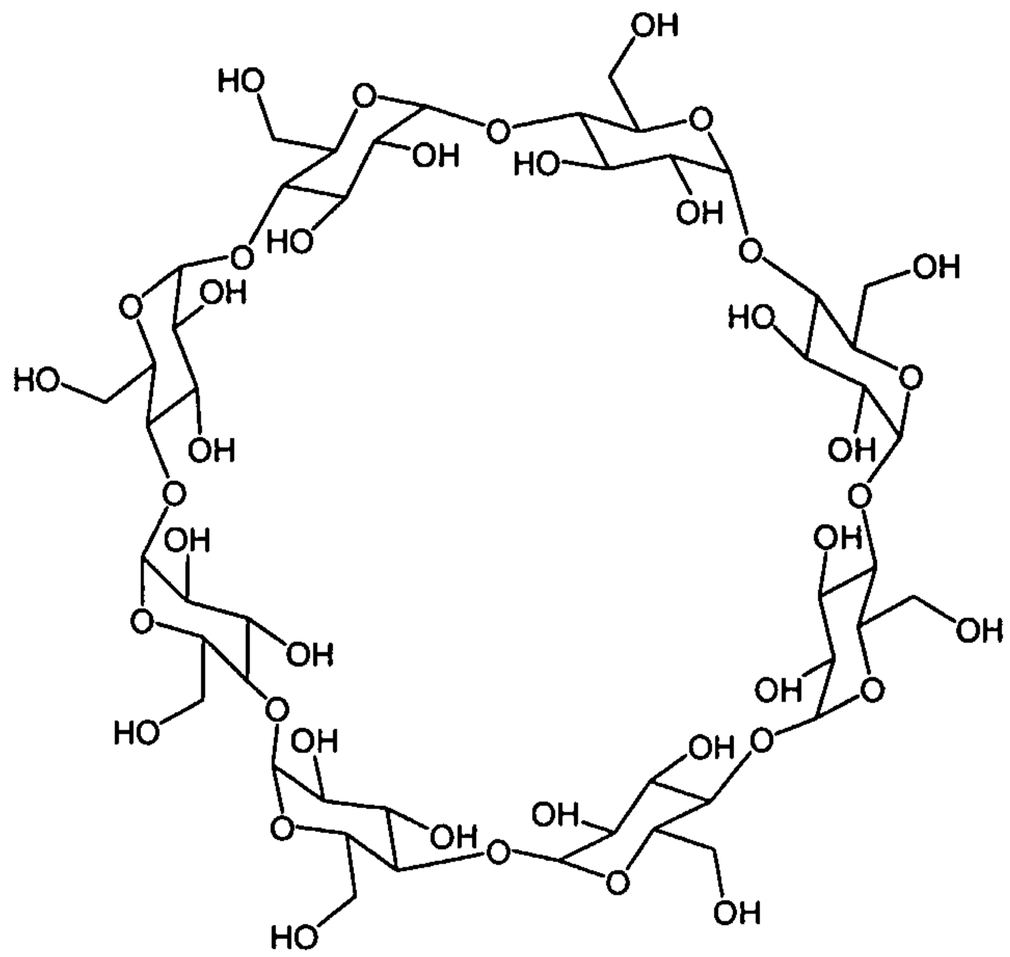
Other prodrugs are cyclodextrin esters. Cyclodextrins are cyclic oligosaccharides containing 6, 7, or 8 glucopyranose units, referred to as α-cyclodextrin , β-cyclodextrin, or γ-cyclodextrin respectively (structures depicted below).



α-cyclodextrin

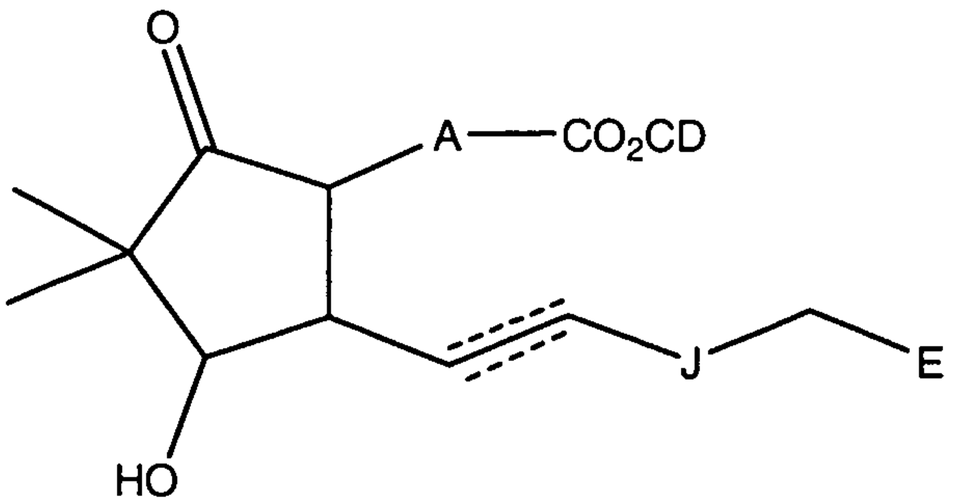


β-cyclodextrin



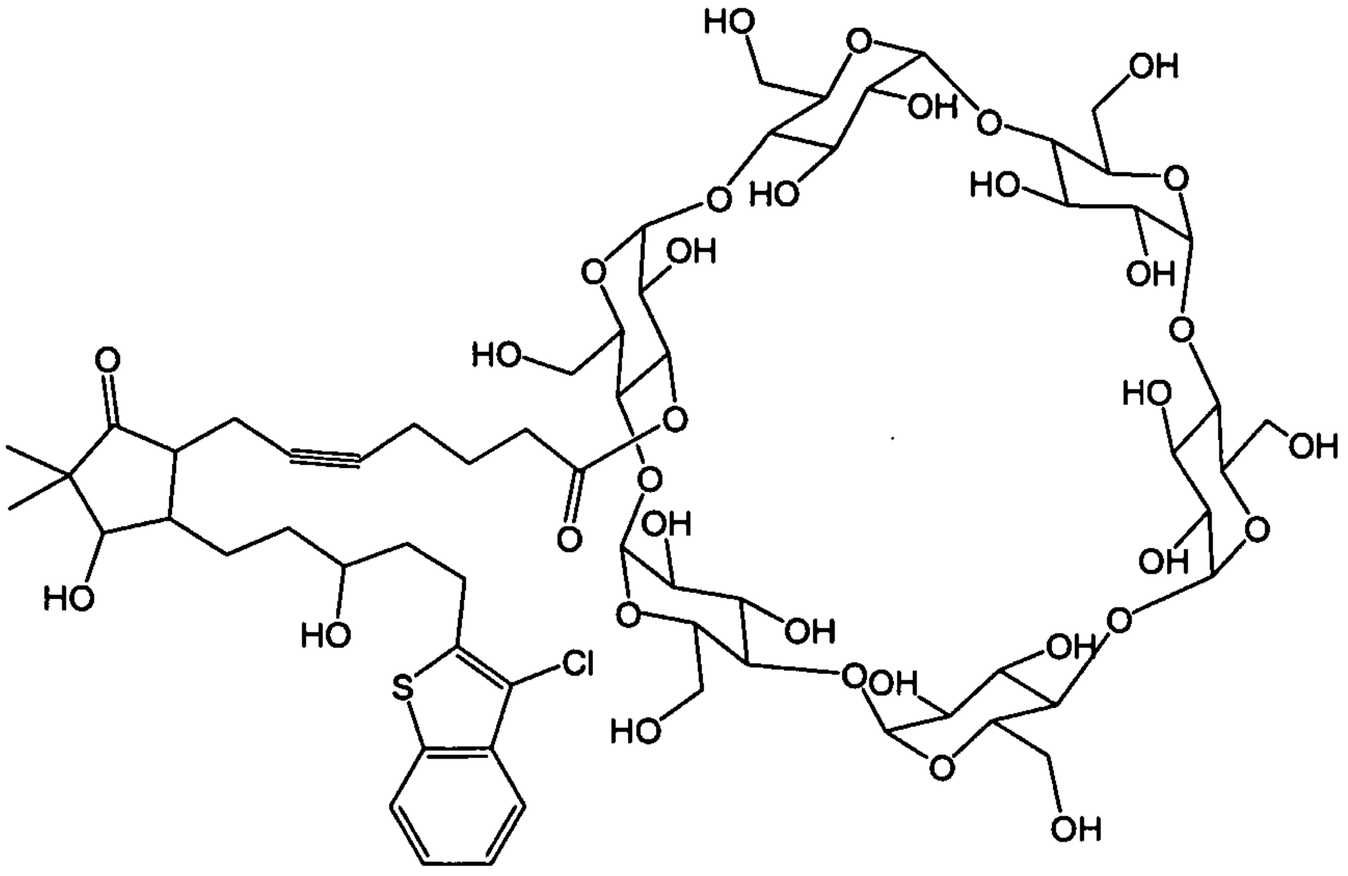
γ-cyclodextrin

Thus, compounds like those shown below, or pharmaceutically acceptable salts thereof, are useful.

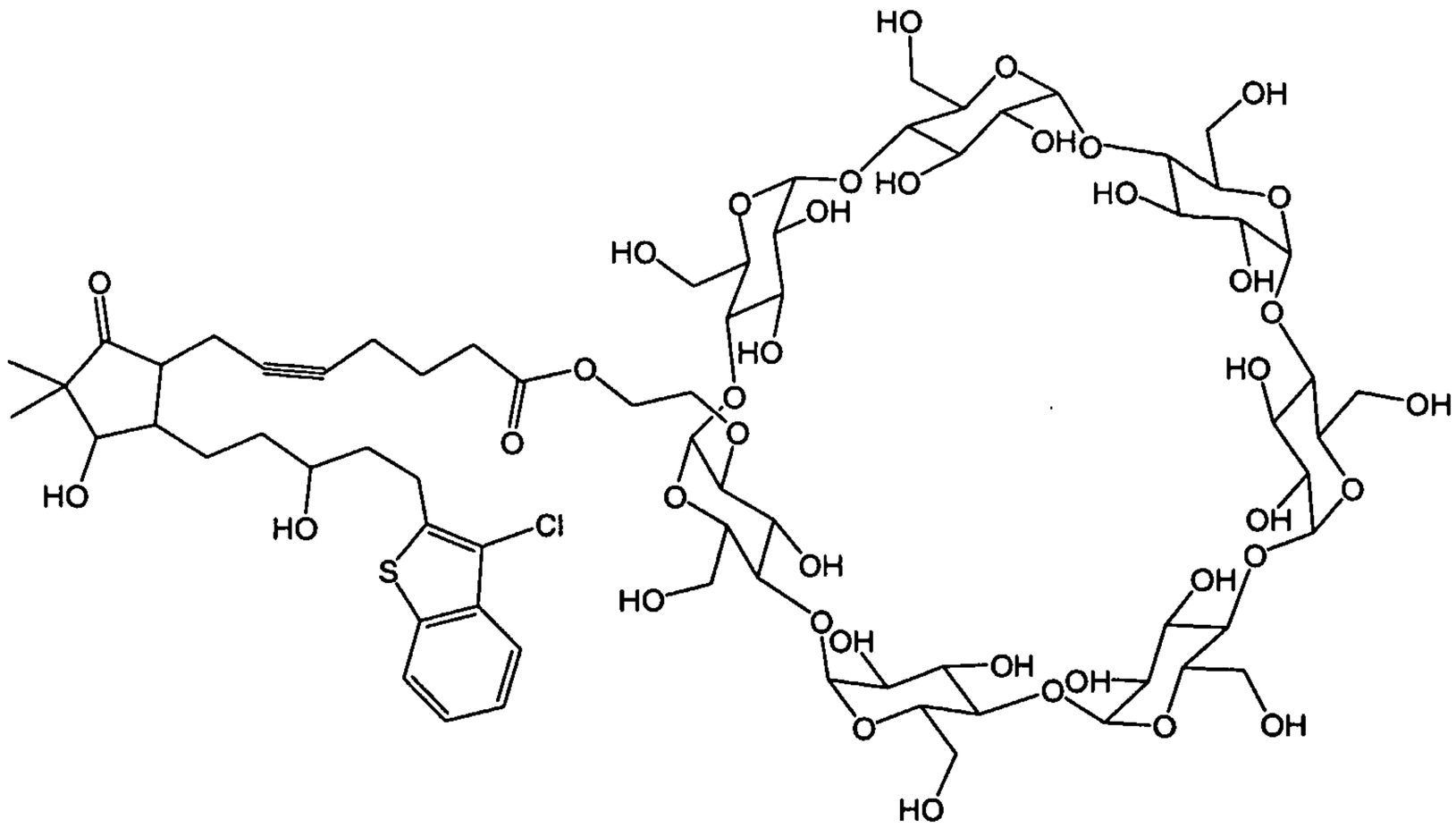


In any structure disclosed herein, CD indicates a cyclodextrin or a spacer-cyclodextrin, including α-, β-, and γ-cyclodextrin, which may be attached at a 2-, 3-, or 6- hydroxyl group. A 2-, 3-, or 6-hydroxyl group refers to the position on the glucose monomer where the anomeric carbon is 1 and the terminal carbon (in the chain form) is 6. The following examples illustrate this nomenclature.

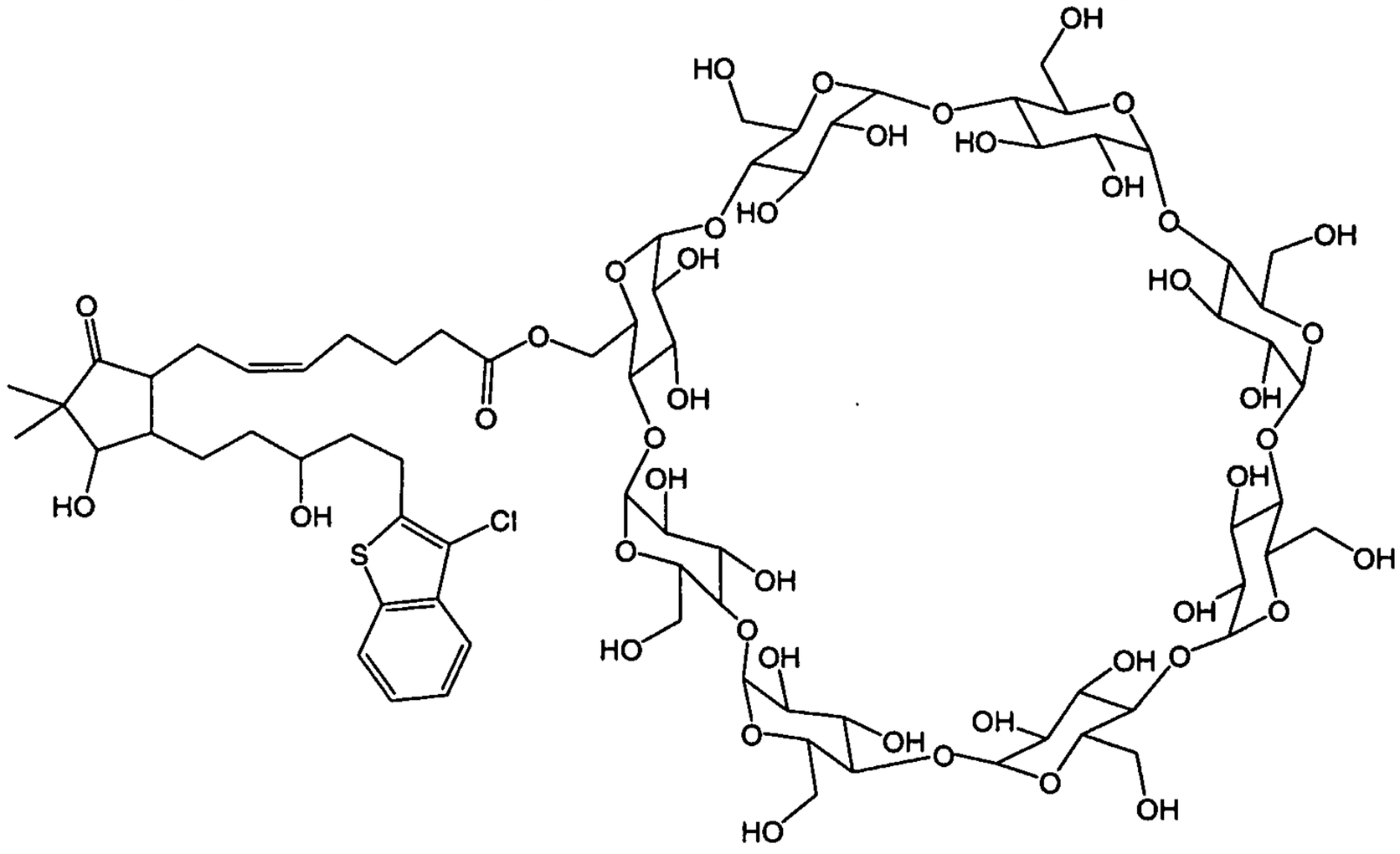
For the compound below, CD is α-cyclodextrin linked at a 3-hydroxyl group.



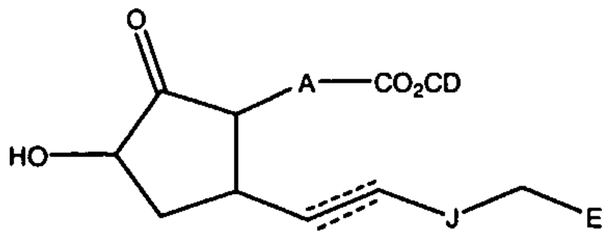
For the compound below, CD is an ethylene glycol-β-cyclodextrin linked at a 2-hydroxyl group.

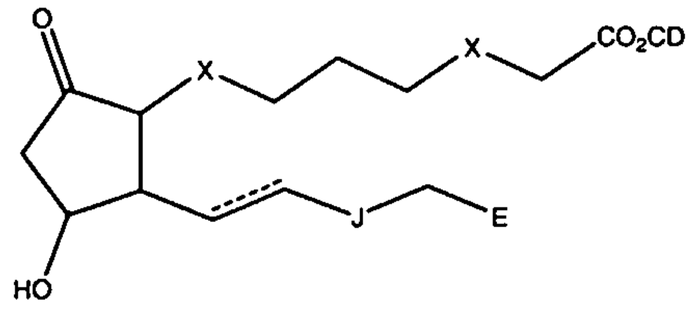


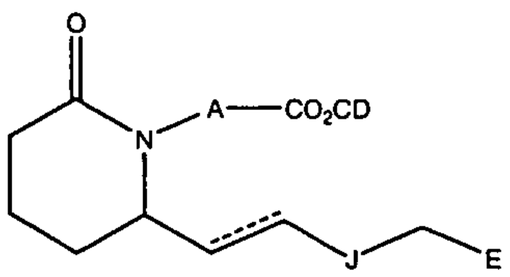
For the compound below, CD is a γ-cyclodextrin linked at a 6-hydroxyl group.

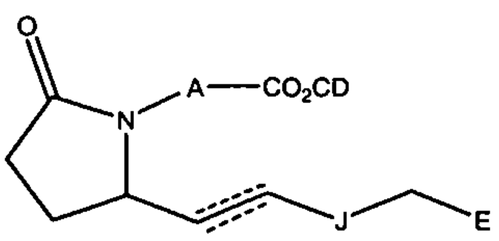


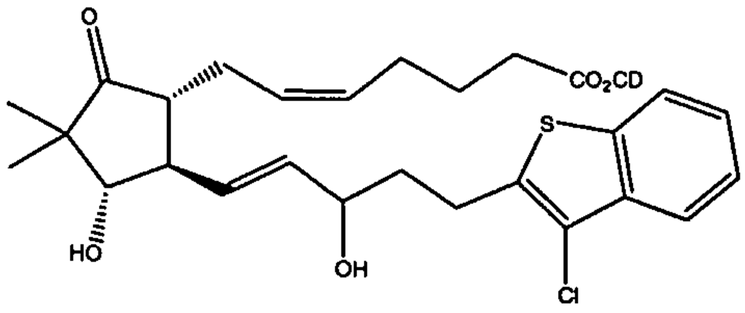
The CD esters shown below are also useful prodrug compounds, as well as pharmaceutically acceptable salts thereof.

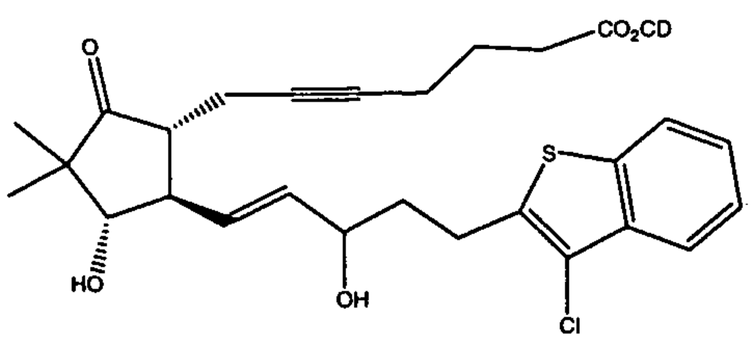


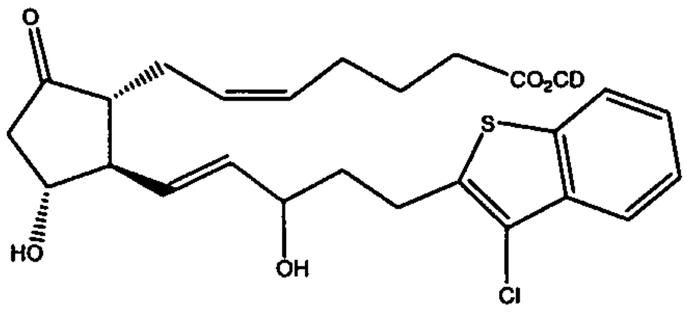


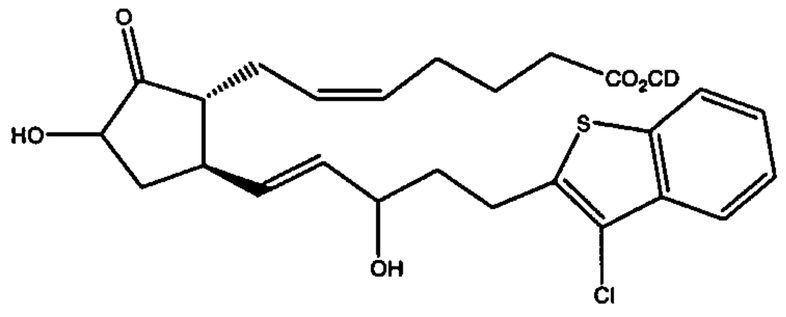






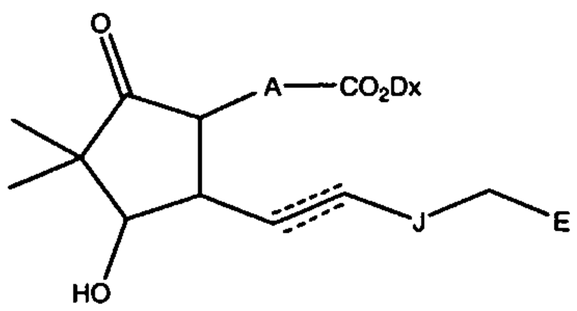


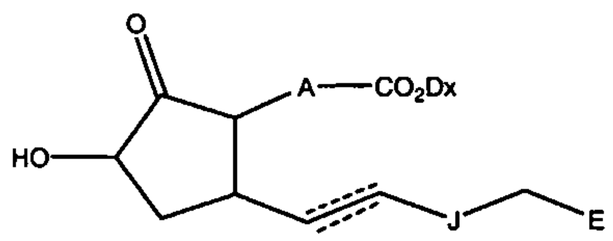


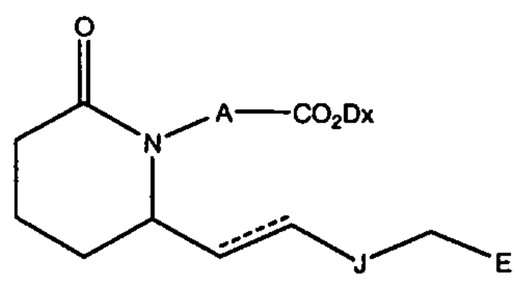


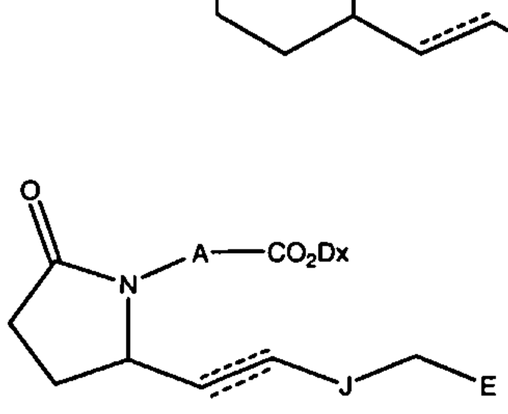
Dextran esters are also useful prodrugs. Dextran is a polymer of glucose primarily linked of α-D(1→6), i.e. D-glucose units are linked by a bond between an α-hydroxyl group at the anomeric (position 1) carbon and the hydroxyl group at carbon 6.

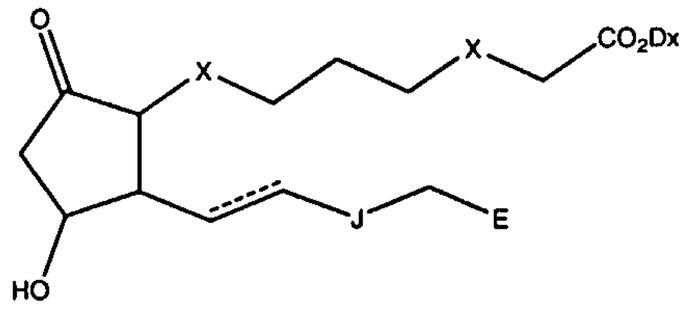
The dextran esters shown below are especially useful as prodrugs, as well as their pharmaceutically acceptable salts. Dx is dextran or spacer-dextran, where the O in CO_2 comes from a dextran hydroxyl group or from a spacer bonded to a dextran hydroxyl group, analogous to the structures shown for cyclodextrin esters.

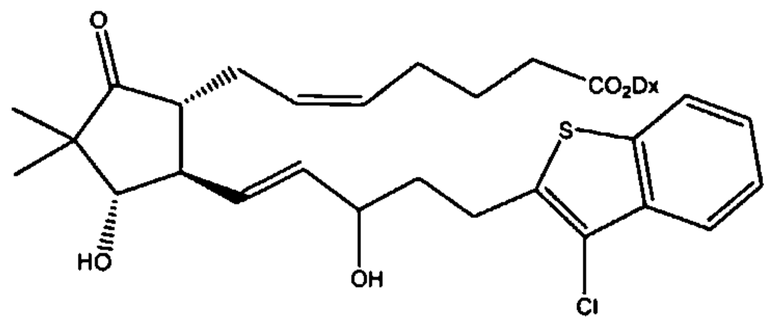


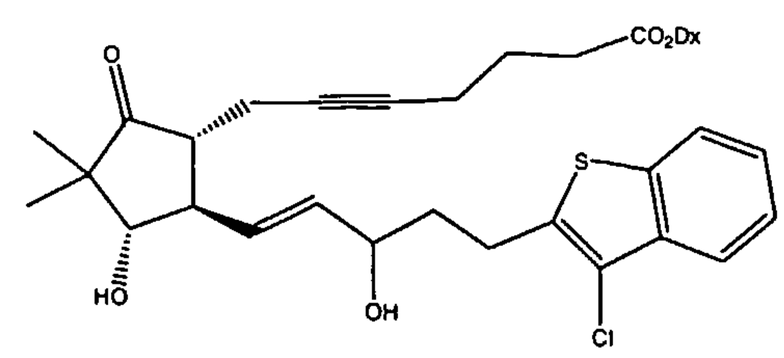


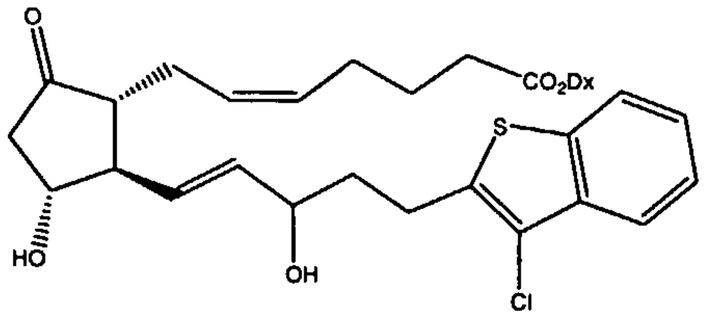


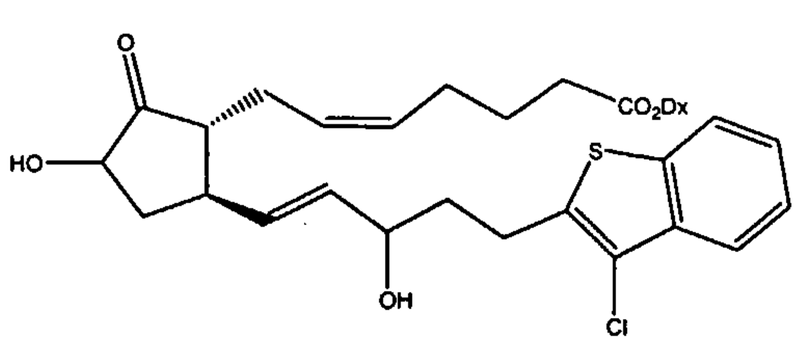




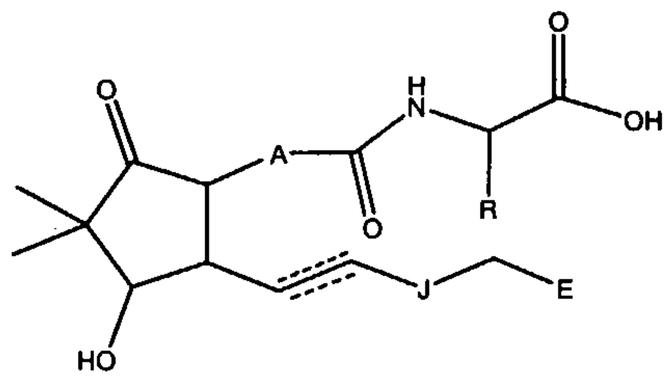


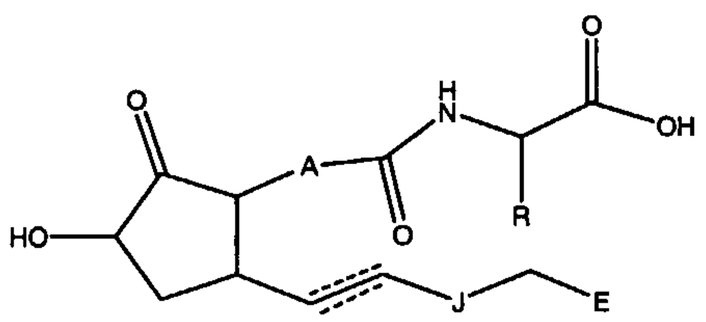


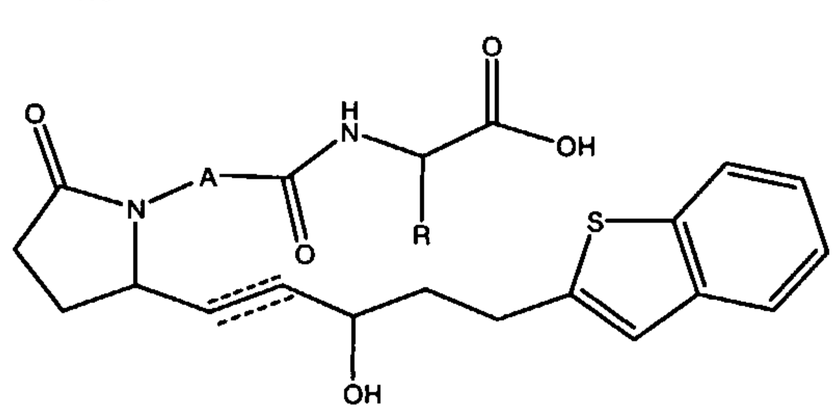


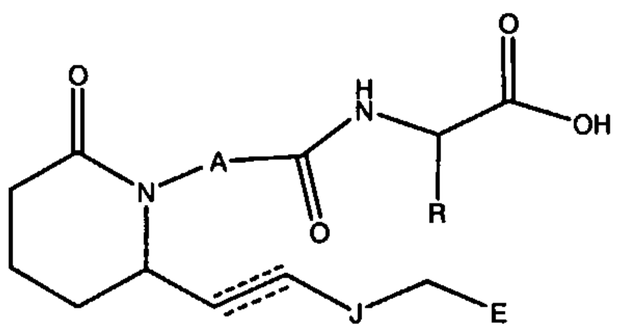


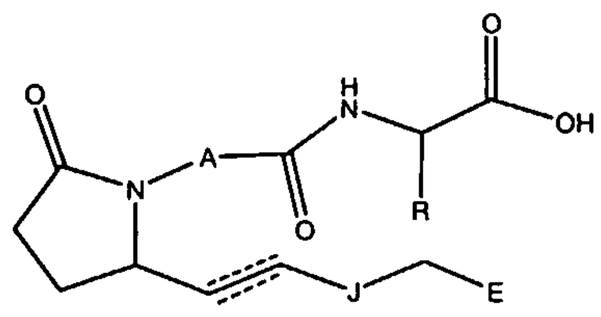
Amino acid prodrugs are also contemplated, such as in the structures shown below, where R represents the side chain characteristic of a natural amino acid, and where R and the amide nitrogen may be connected as per proline. Pharmaceutically acceptable salts of compounds of these structures, whether anionic, cationic, or zwitterionic, are also useful.

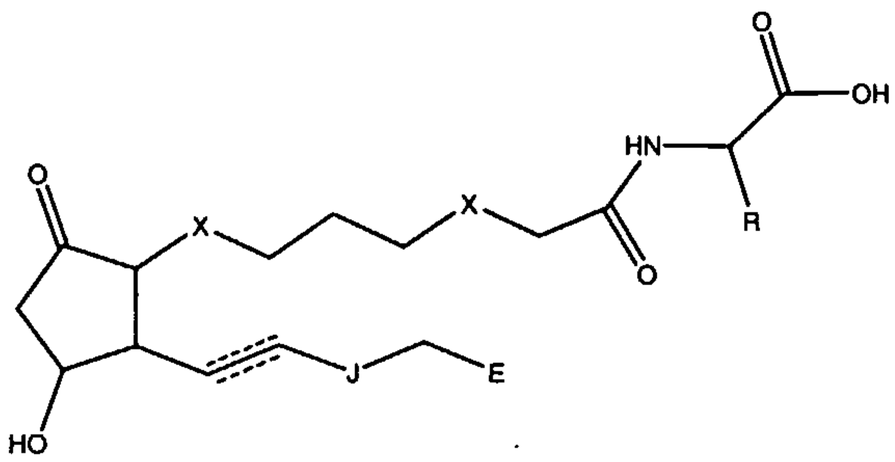


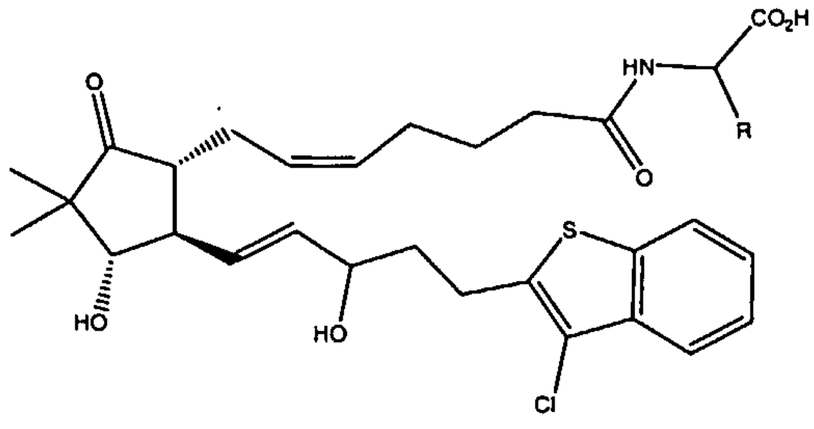


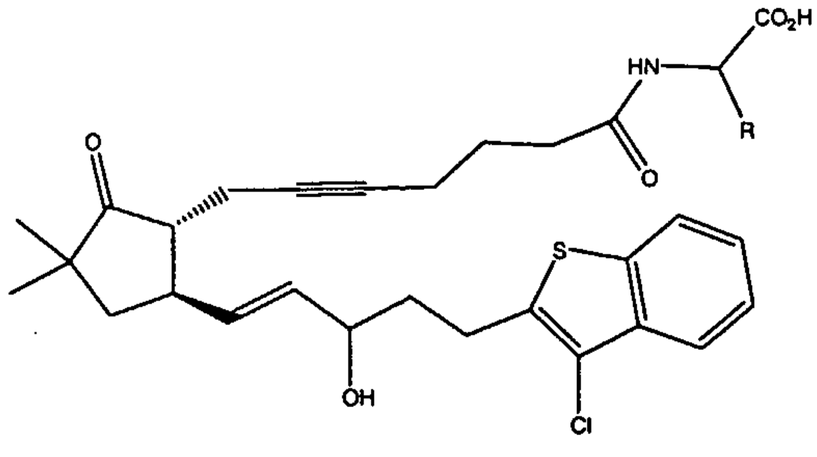


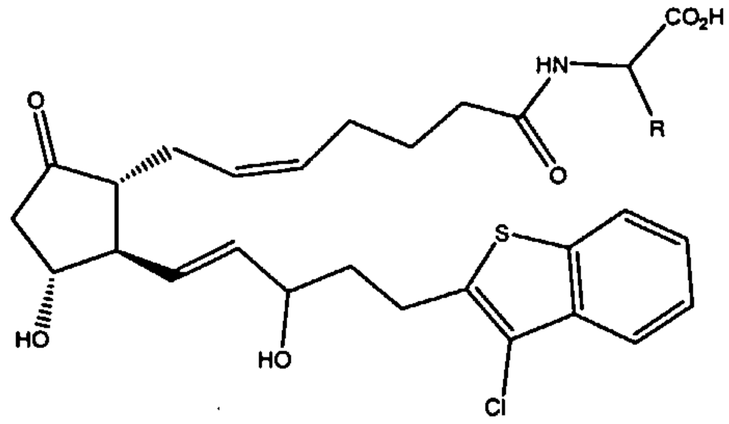


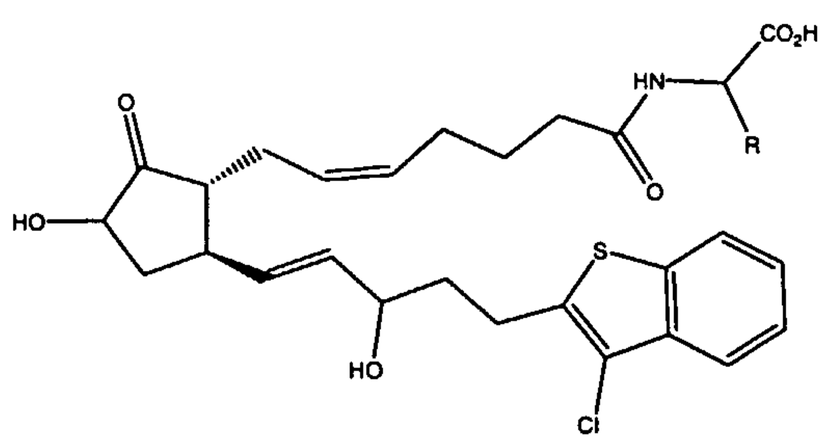






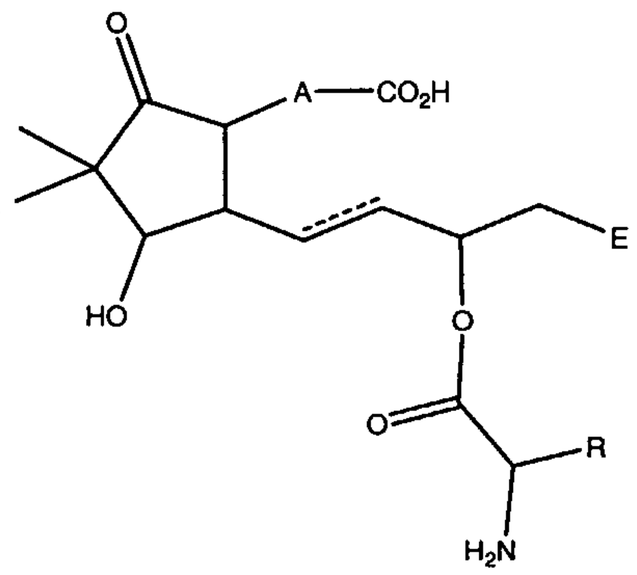


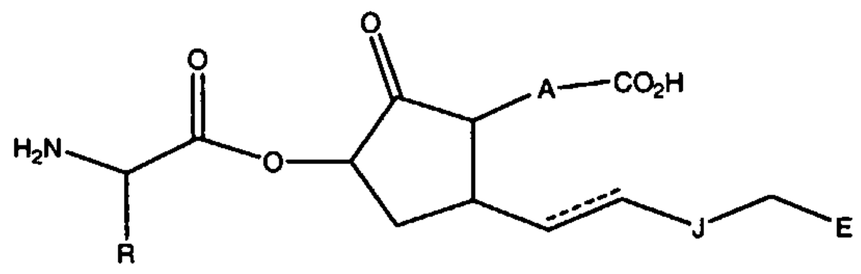




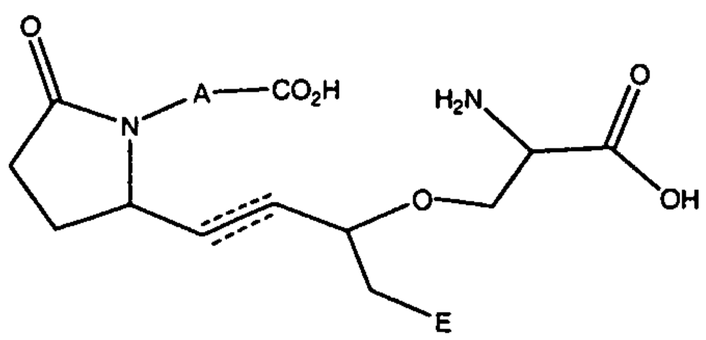
In certain embodiments, R is selected from the group consisting of H, methyl, iso-propyl, sec-butyl, benzyl, indol-3-ylmethyl, hydroxymethyl, $CHOHCH_3$, CH_2CONH_2, *p*-hydroxybenzyl, CH_2SH, $(CH_2)_4NH_2$, $(CH_2)_3NHC(NH_2)_2^+$, methylimidizol-5-yl, CH_2CO_2H, or $(CH_2)_2CO_2H$.

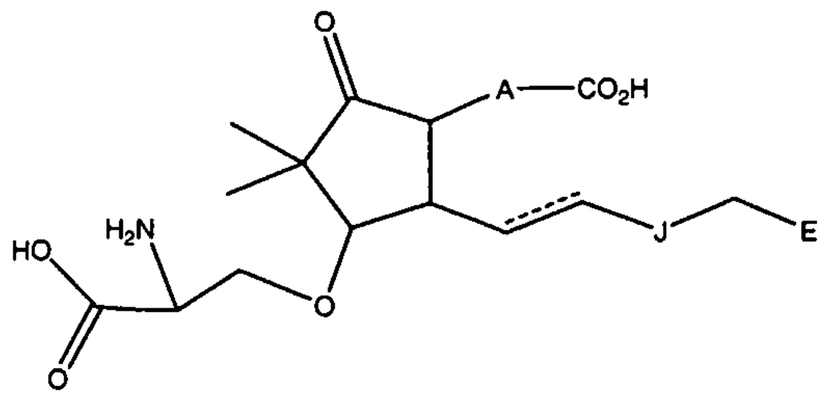
Esters prodrugs of EP_4 agonists may also be based upon amino acids, as demonstrated by the examples shown below. Pharmaceutically acceptable salts of compounds of these structures, whether anionic, cationic, or zwitterionic, are also useful.





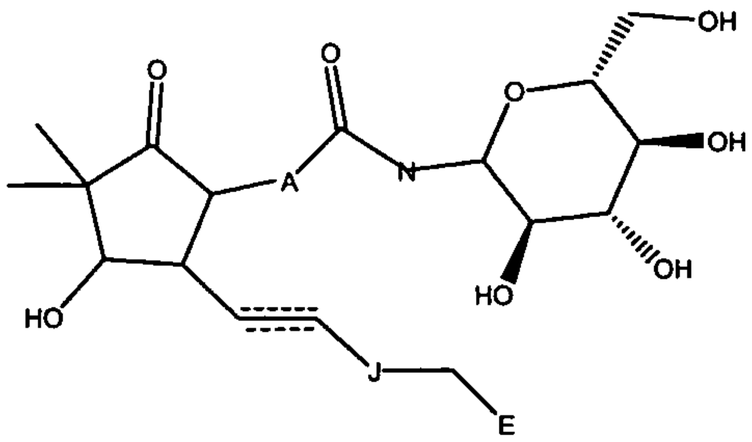
Since amino acids such as serine, threonine, and tyrosine have hydroxyl functional groups in their side chains, ether prodrugs of EP_4 agonists based upon amino acids are also possible, as demonstrated in the examples below. Pharmaceutically acceptable salts of compounds of these structures, whether anionic, cationic, or zwitterionic, are also useful.

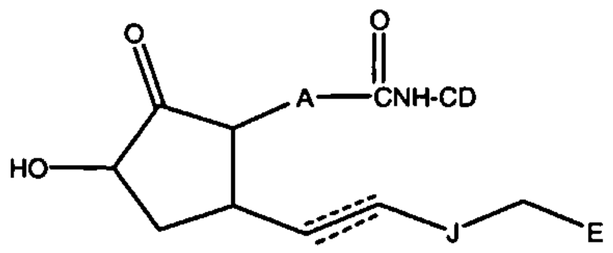


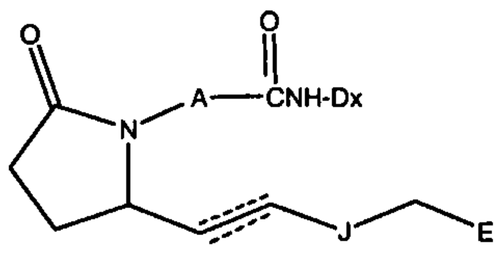


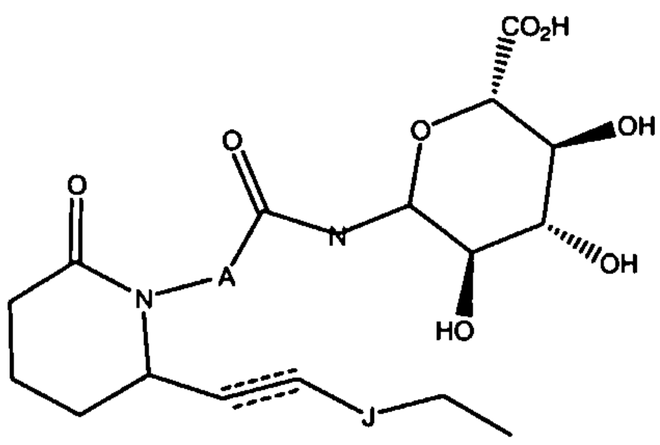
In addition, the spacers illustrated herein may be applied to amino acids to further increase the number kinds of amino acid prodrugs available.

Since a carbohydrate according to the definition given herein may have a limited amount of amine functional groups, carbohydrate amides are also possible such as the ones depicted below.



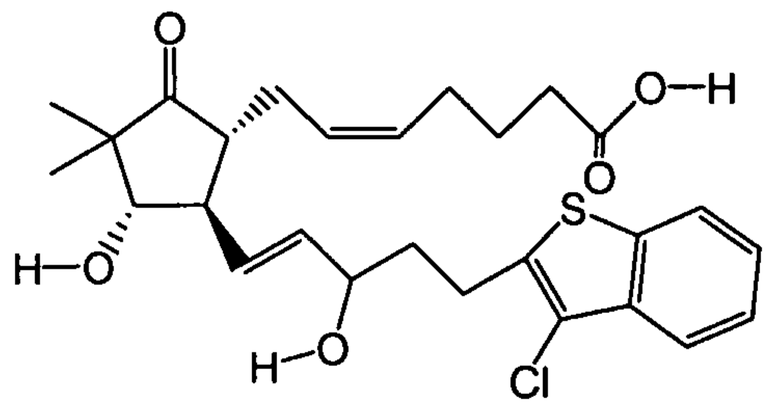


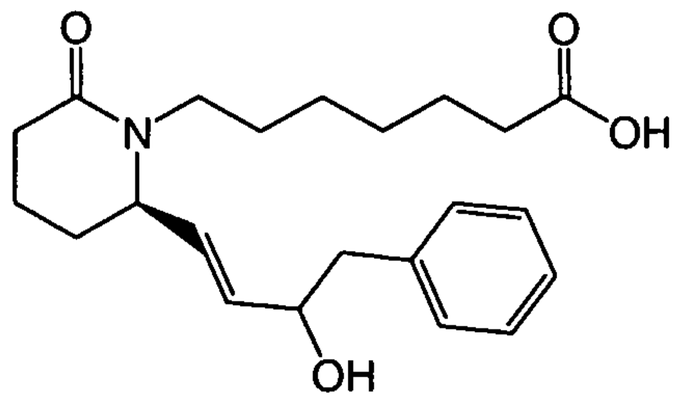




Analogous structures could also be drawn with any of the carbohydrate esters shown herein, making a large variety of carbohydrate amides possible for use in the methods disclosed herein. Further, since the prodrugs may incorporate an amine spacer, the number of carbohydrate amides contemplated is further diversified.

Prodrugs of the compounds shown below, and use of the compounds, or salts or prodrugs thereof, for any method, composition, or treatment disclosed herein, are specifically contemplated herein.

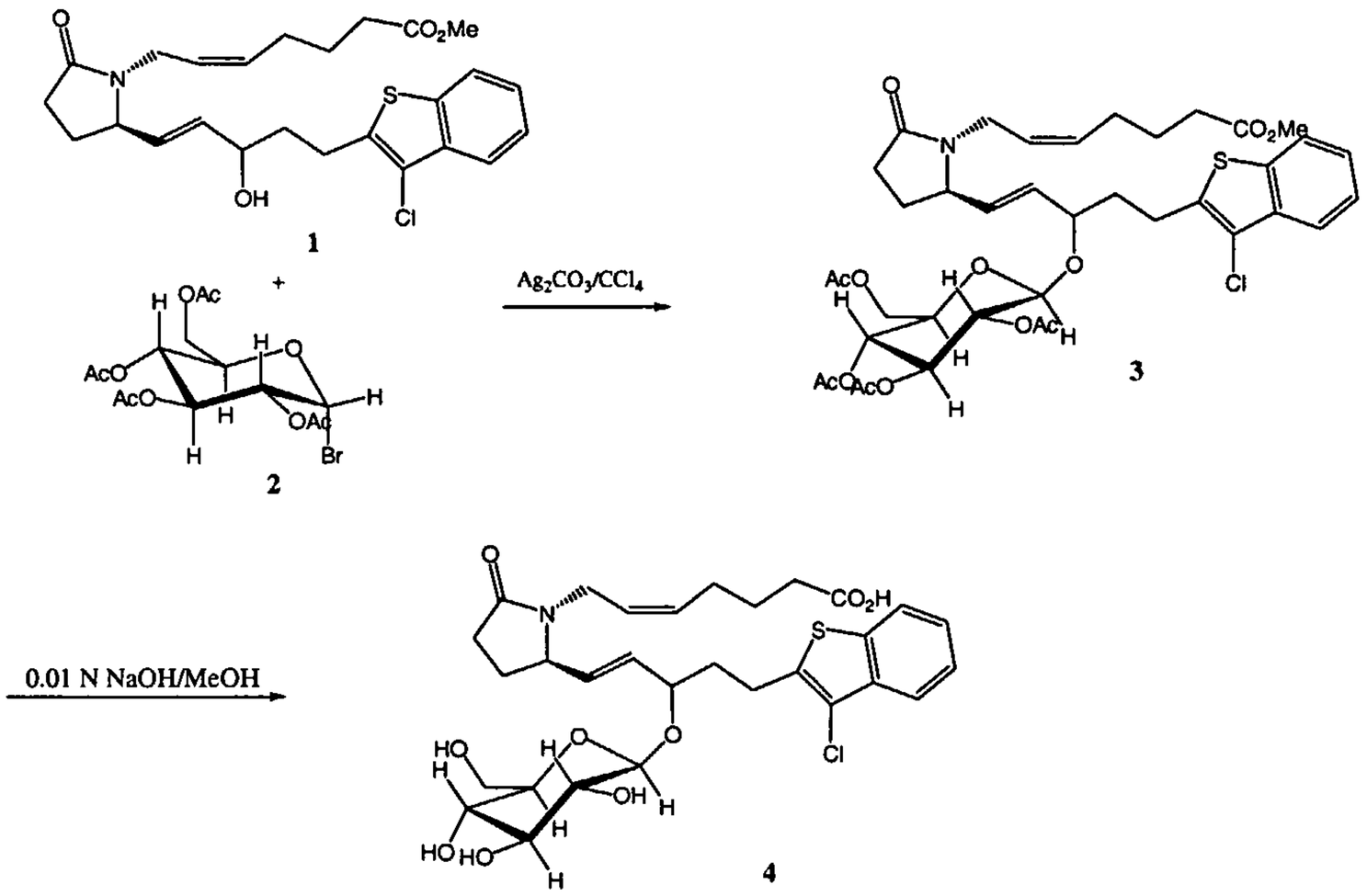




Unless indicated by a wedge or a dash, a carbon which has a chiral center can be construed to include the S isomer, the R isomer, or any mixture of isomers including a 50:50 R/S mixture. In particular, the pure isomers of each of the structures above, and any possible isomeric mixtures, including the 50:50 R/S mixtures, are

contemplated. Methods of preparing these compounds are in United States Patent No. 6,747,037 and United States Patent No. 6,875,787.

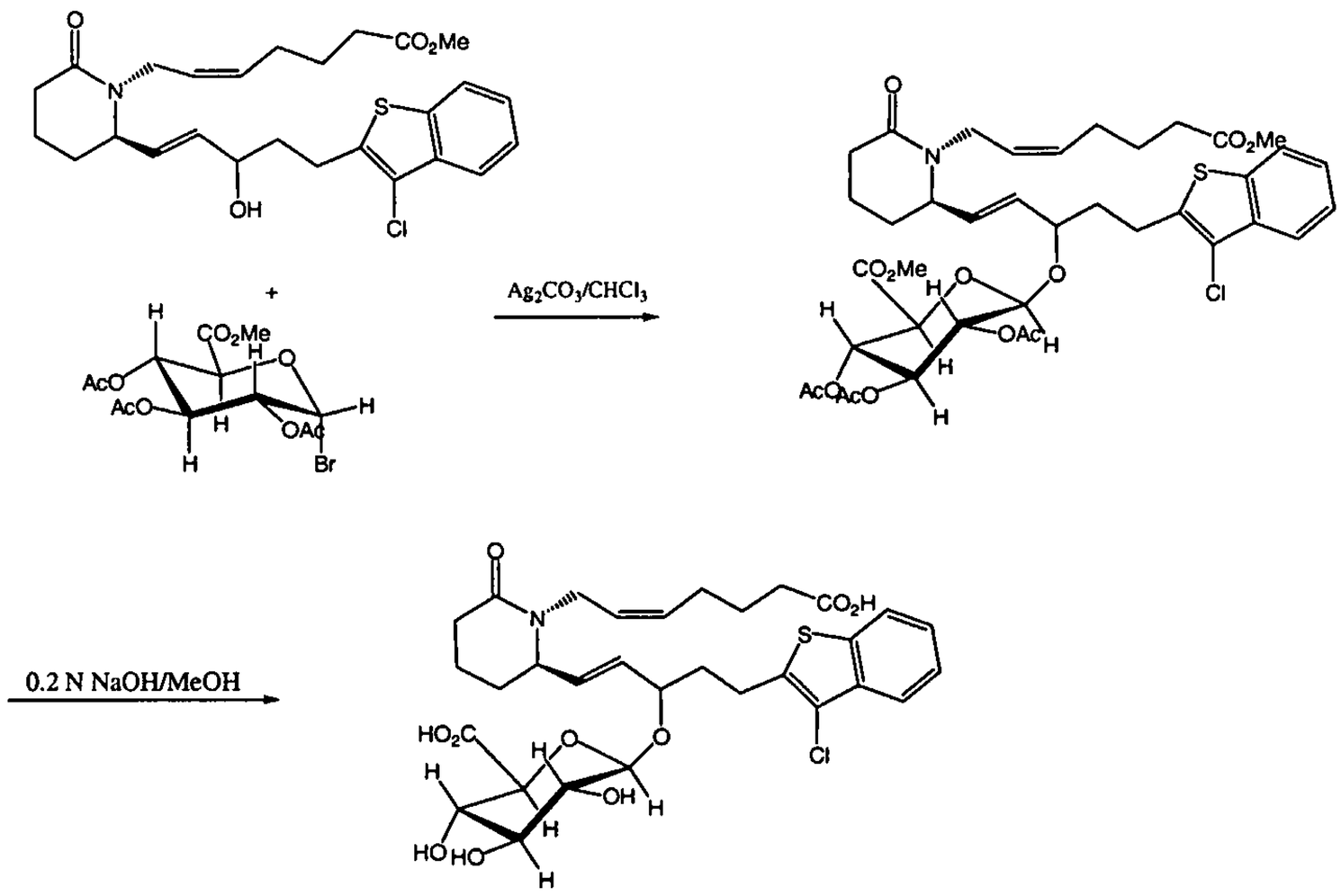
There are a number of methods of preparing the prodrug compounds disclosed herein. While not intending to limit the scope of the invention in any way, a glucoside ether of a prostaglandin EP_4 agonist may be prepared from commercially available (Sigma Chemical Co.) 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (**2**) by coupling the two in CCl_4 in the presence of silver carbonate, followed by hydrolysis of the ester protecting groups using a procedure adapted from Friend and Chang (*J. Med. Chem.* **1984**, *27*, 261-266; *J. Med. Chem.* **1985**, *28*, 51-57).



In this method, compound **1** is dissolved in dry CCl_4 or another suitable solvent, and freshly prepared Ag_2CO_3 (about 4.5 equivalents) is added. Compound **2** (about 2.7 equivalents) is then added dropwise while protecting the reaction mixture from light, and continuously distilling the solvent. The distilled solvent is replaced with fresh solvent during the course of the reaction. When the reaction is complete, the solution is worked up according to standard methods and purified by flash chromatography on RP-18 or another suitable purification method to yield compound **3**. The ester groups of compound **3** are then saponified according to an art acceptable procedure such as NaOH in MeOH, and worked up and purified according to standard procedures.

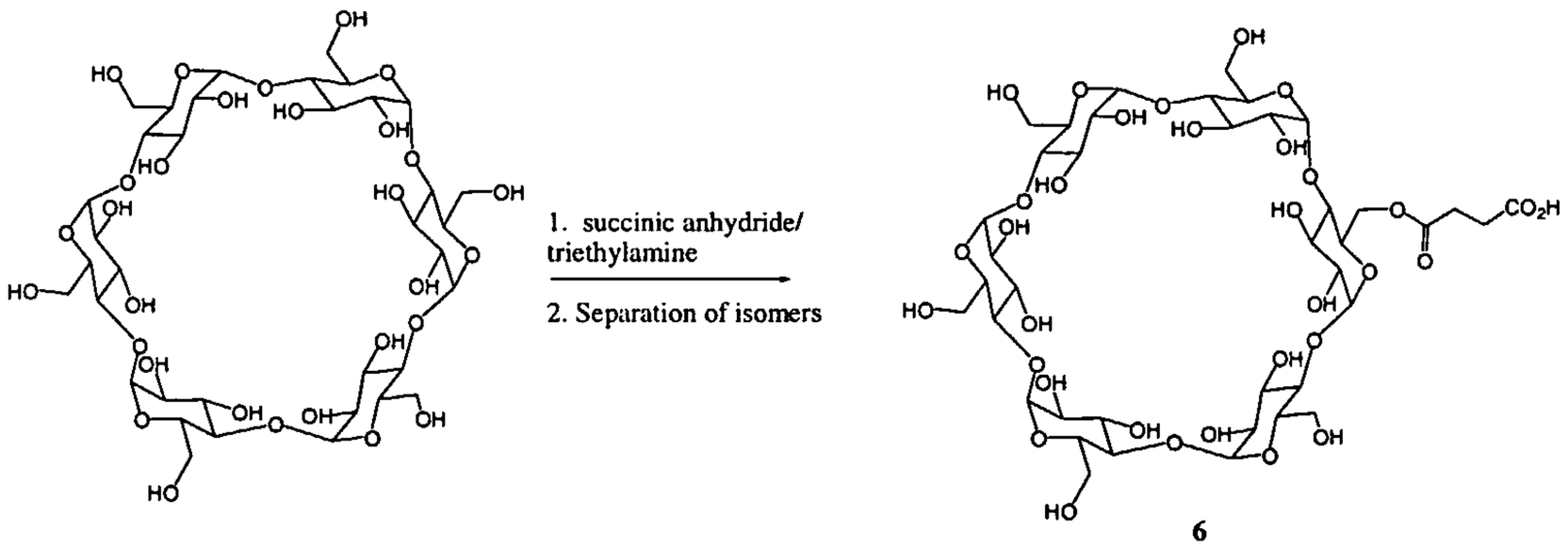
This procedure may be used for prostaglandin EP_4 agonists having a single hydroxyl group. Alternatively, prodrugs for prostaglandin EP_4 agonists having more than 1 hydroxyl group may be prepared by protection of the hydroxyl groups with different groups, so that one may be removed for preparation of a prodrug. Generally, the ring, the α-chain, and the ω-chain are prepared separately and coupled toward the end of the synthetic procedure, so protection with distinct groups for each part is within the ability of a person of ordinary skill in the art.

A similar procedure may be used to prepare glucouronide ethers. Haeberlin et. al. (*Pharmaceutical Research* **1993**, *10*, 1553-1562) discloses such a procedure which may be adapted here.

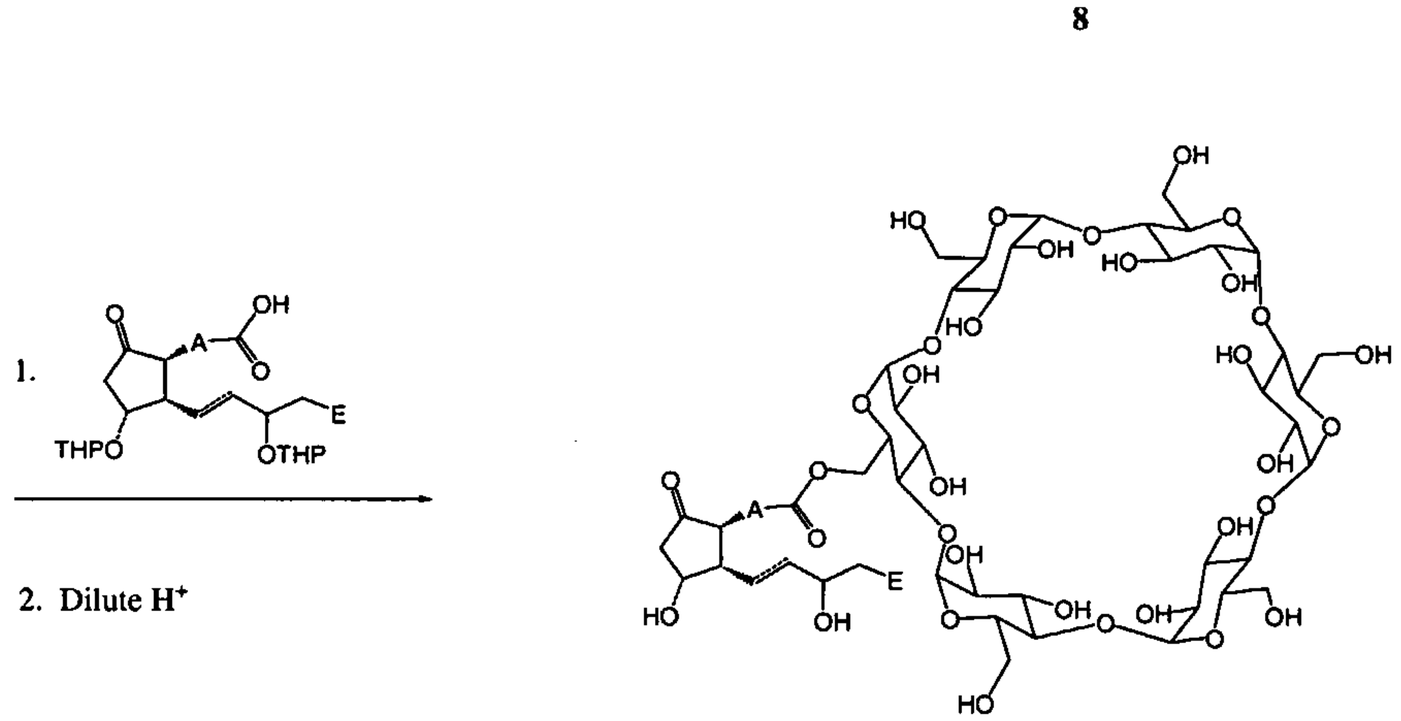


The procedure shown below may be used to link prostaglandin EP_4 agonists to cyclodextrin or to another carbohydrate. Coupling of the succinic acid to cyclodextrin is carried out as described by Tanaka et. al. (*Journal of Antibiotics* **1994**, *47*, 1025-1029), by suspending cyclodextrin in DMF, dissolving the mixture in pyridine, adding 1.2 equivalents of succinic anhydride, and stirring for 18 hours at room temperature. The mixture is poured into chloroform to precipitate the succinate ester product, which is filtered, washed with chloroform and methanol, and purified by an ODS column. Tanaka showed that reaction occurs preferentially at the 6 OH by a ratio of 4.6/1 for succinic anhydride. The preference reaction at the 6-OH is even greater for phthalic anhydride (13.6/1), naphthalene dicarboxylic anhydride (14.0/1), and cyclohexane dicarboxylic anhydride (14.7/1).

The hydroxyl group of the prostaglandin EP_4 agonist is activated by reacting with *p*-toluenesulfonyl chloride, and the tosylate **7** is reacted with the cyclodextrin derivative **6** to obtain the prodrug product.



Alternatively, cyclodextrin may be attached directly to the carboxylic acid of a prostaglandin EP_4 agonist as shown below. This procedure is an adaptation of one disclosed by Uekama and coworkers (*J. Med. Chem.* **1997**, *40*, 2755-2761 and *Pharm. Pharacol.* **1996**, *48*, 27-31) which described preparing cyclodextrin prodrugs of anti-inflammatory carboxylic acids such as 4-biphenylacetic acid. This procedure is readily adapted to prostaglandin EP_4 agonists. In this procedure, the cyclodextrin is reacted with *p*-toluensulfonyl chloride to form the tosylate **8**, which is purified ion exchange chromatography followed by recrystallization from water. The hydroxyl groups of the prostaglandin are protected with THP by reaction with THPCl. Alternatively, a THP protected prostaglandin EP_4 agonist ester, which is frequently a late stage synthetic intermediate in the preparation of a prostaglandin EP_4 agonist, is saponified to give a THP protected free prostaglandin EP_4 agonist acid. The acid is then reacted with the cyclodextrin tosylate to give the desired prodrug, which is worked up and purified according to methods known in the art.



The procedure shown below may be used to line prostaglandin EP4 agonist analogs to dextran or to another carbohydrate. A procedure for the coupling of dexamethasone to dextran via a succinate linkage (McLeod et. al., *Int J. Pharm.* **1993**, 92, 105-114) is readily adapted to the compounds herein. While not intending to limit the scope of the invention in any way, this procedure is most conveniently carried out with a prostaglandin EP_4 agonist having no free carboxylic acid (e.g. an ester) and 1 unprotected hydroxyl group. Connection to dextran to form the prodrug occurs at the free hydroxyl group. In this procedure, a hemisuccinate is formed from a hydroxyl group of a prostaglandin EP_4 agonist by adding it to succinic anhydride to form the hemisuccinate ester. The prostaglandin EP_4 agonist hemisuccinate is then reacted with 2 equivalents of 1,1-carbonyldiimidizole for 30 minutes under nitrogen. Dextran and a base such as triethylamine is added and the reaction is stirred for about 21 hours at room temperature. Any protecting groups on other hydroxyl groups may then be removed by stirring in dilute acid or another method appropriate to the protecting group being used. The carboxylic acid need not be deprotected because the ester will readily hydrolyze in vivo.

The carbohydrates used in the procedures described above are easily varied or interchanged by a person of ordinary skill in the art. For example, glucoside and glucouronide esters of the carboxylic acid of the prostaglandin EP_4 agonist may be prepared using the tosylate of the carbohydrate in a procedure analogous to that described for cyclodextrin.

Amino acid prodrugs are readily obtained by many methods. For example, while not intending to be limiting, one of several procedures used for the coupling of salicylic acid to a methyl ester of alanine, glycine, methionine, or tyrosine (Nakamura et. al. *J. Pharm. Pharmacol.* **1992**, *44*, 295-299, and Nakamura et. al. *Int. J. Pharm.* **1992**, *87*, 59-66) can be adapted for use with prostaglandin EP_4 agonists. In this procedure, an equimolar amount of dicyclohexylcarbodiimide is added at or below 0°C to a prostaglandin EP_4 agonist carboxylic acid and

stirred about 30 minutes. An equimolar amount of the methyl ester of the amino acid is then added and stirred overnight at room temperature to form the amide. Deprotection of any hydroxyl group can then be carried out by using dilute aqueous acid or another method, depending on the protecting group.

While not intending to be bound by theory, it is commonly believed by those skilled in the art that the colonic mucosal barrier is central to protecting the inner layers of the colon from irritants such as foods, oxidizing agents, bacterial metabolites, and intestinal flora. While not intending to be bound in any way by theory, it is believed that impaired and/or leaky epithelial layers lead to various inflammations of the colon including immunogenic inflammatory bowel diseases and subsequent secondary inflammations. While not intending to be bound by theory, it is believed that prostaglandin EP_4 receptors mediate two cellular signaling pathways using either the 2^{nd} messenger cAMP or activation of phosphoinositide 3-kinases. It is believed that the latter pathway is particularly prominent in epithelial cells. While not intending to be bound by theory, it is believed that activation of the signaling pathways promotes cell proliferation, cell growth, cell metabolism and the inhibition of apoptosis. Thus, while not intending to be bound in any way by theory, EP_4 agonists applied to the colon should recognize the prostaglandin EP_4 receptor and thus activate one or more of these signaling pathways. This should thus promote epithelial cell growth, proliferation, inhibition of apoptosis, and increases in mucus secretion, reducing permeability to intestinal antigens and irritants. Thus, while not intending to be bound by theory, this enhancement and maintenance of the colonic mucosal barrier by prostaglandin EP_4 agonists should be prophylactic and therapeutic for colitis, amebic colitis, collagenous colitis, colitis cystica profunda, colitis cystica superficialis, granulomatous colitis, hemorrhagic colitis, mucous colitis, Crohn's disease, and ulcerative colitis.

A number of methods of delivering a drug to the colon via oral dosage forms are known in the art, and are reviewed by Chourasia and Jain in J Pharm Pharmaceut Sci 6 (1): 33-66, 2003. These include 1) administration of a prodrug, including an azo or a carbohydrate based prodrug; 2) coating the drug with, or encapsulating or impregnating the drug into a polymer designed for delivery to the colon, 3) time released delivery of the drug, 4) use of a bioadhesive system; and the like. Intestinal microflora are capable of reductive cleavage of an azo bond leaving the two nitrogen atoms as amine functional groups. Bacteria of the lower GI also have enzymes which can digest glycosides, glucuronides, cyclodextrins, dextrans, and other carbohydrates, and ester prodrugs formed from these carbohydrates have been shown to deliver the parent active drugs selectively to the colon. This prodrug approach has been used to deliver 5-aminosalicylic acid to humans. In vivo and in vitro studies on rats and guinea pigs with prodrugs of dexamethasone, prednisolone, hydrocortisone, and fludrocortisone, suggest that glycoside conjugates may be useful for the delivery of steroids to the human colon. Other in vivo studies have suggested that glucouronide, cyclodextrin, and dextran prodrugs of steroids or non-steroidal anti-inflammatory drugs are useful for delivery of these drugs to the lower GI tract. Similarly, carbohydrate polymers such as amylase, arabinogalactan, chitosan, chondroiton sulfate, dextran, guar gum, pectin, xylin, and the like, can be used to coat a drug compound, or a drug may be impregnated or encapsulated in the polymer. An amide of salicylic acid and glutamic acid has been shown to be useful for the delivery of salicylic acid to the colon of rabbit and dog. After oral administration, the polymers remain stable in the upper GI tract, but are digested by the microflora of the lower GI thus releasing the drug for treatment. Polymers which are sensitive to pH may also be used since the colon has a higher pH than the upper GI tract. Such polymers are commercially available. For example, Rohm Pharmaceuticals, Darmstadt, Germany, markets pH dependent methacrylate based polymers and copolymers which have varying solubilities over

different pH ranges based upon the number of free carboxylate groups in the polymer under the tradename Eudragit®. Several Eudragit® dosage forms are currently used to deliver salsalazine for the treatment of ulcerative colitis and Crohn's disease. Time release systems, bioadhesive systems, and other delivery systems have also been studied.

Coadministration of prostaglandin EP4 agonists, either in a single composition or in separate dosage forms, is also contemplated. While not intending to limit the scope of the invention in any way, drugs which may be included in combination therapies with EP4 agonists and their prodrugs include, but are not limited to:

1. Anti-inflammatory drugs such as aminosalicylates and their prodrugs, Sulfasalazine, and the like;
2. Steroids, including corticosteroids, and the like;
3. Immunomodulators such as azathioprine, 6-mercaptopurine, cyclosporine, and the like; and
4. Humanized monoclonal antibodies against pro-inflammatory cytokines such as infliximab, etanercept, onercept, adalimumab, CDP571, CDP870, natalizumab, MLN-02, ISIS 2302, cM-T412, BF-5, vasilizumab, daclizumab, basiliximab, Anti-CD40L, and the like.

One useful assay for determining prostaglandin EP_4 activity and selectivity of compounds is described below.

HUMAN RECOMBINANT EP_1, EP_2, EP_3, EP_4, FP, TP, IP and DP RECEPTORS: STABLE TRANSFECTANTS.

Plasmids encoding the human EP_1, EP_2, EP_3, EP_4, FP, TP, IP and DP receptors are prepared by cloning the respective coding sequences into the eukaryotic expression vector $pCEP_4$ (Invitrogen). The $pCEP_4$ vector contains an Epstein Barr virus (EBV) origin of replication, which permits episomal replication in primate cell lines expressing EBV nuclear antigen (EBNA-1). It also contains a hygromycin resistance gene that is used for eukaryotic selection. The cells employed for stable transfection are human embryonic kidney cells (HEK-293) that are transfected with and express the EBNA-1 protein. These HEK-293-EBNA cells (Invitrogen) are grown in medium containing Geneticin (G418) to maintain expression of the EBNA-1 protein. HEK-293 cells are grown in DMEM with 10% fetal bovine serum (FBS), 250 $\mu g\ ml^{-1}$ G418 (Life Technologies) and 200 $\mu g\ ml^{-1}$ gentamicin or penicillin/streptomycin. Selection of stable transfectants is achieved with 200$\mu g\ ml^{-1}$ hygromycin, the optimal concentration being determined by previous hygromycin kill curve studies.

For transfection, the cells are grown to 50-60% confluency on 10 cm plates. The plasmid $pCEP_4$ incorporating cDNA inserts for the respective human prostanoid receptor (20 μg) is added to 500 μl of 250 mM $CaCl_2$. HEPES buffered saline x 2 (2 x HBS, 280 mM NaCl, 20 mM HEPES acid, 1.5 mM Na_2HPO_4, pH 7.05 – 7.12) is then added dropwise to a total of 500 μl, with continuous vortexing at room temperature. After 30 min, 9 ml DMEM are added to the mixture. The DNA/DMEM/calcium phosphate mixture is then added to the cells, which is previously rinsed with 10 ml PBS. The cells are then incubated for 5 hr at 37° C in humidified 95% air/5% CO_2. The calcium phosphate solution is then removed and the cells are treated with 10% glycerol in DMEM for 2 min. The glycerol solution is then replaced by DMEM with 10% FBS. The cells are incubated overnight and the medium is replaced by DMEM/10% FBS containing 250 $\mu g\ ml^{-1}$ G418 and penicillin/streptomycin. The following day hygromycin B is added to a final concentration of 200 $\mu g\ ml^{-1}$.

Ten days after transfection, hygromycin B resistant clones are individually selected and transferred to a separate well on a 24 well plate. At confluence each clone is transferred to one well of a 6 well plate, and then expanded in a 10 cm dish. Cells are maintained under continuous hygromycin selection until use.

RADIOLIGAND BINDING

Radioligand binding studies on plasma membrane fractions prepared from cells are performed as follows. Cells washed with TME buffer are scraped from the bottom of the flasks and homogenized for 30 sec using a Brinkman PT 10/35 polytron. TME buffer is added as necessary to achieve a 40 ml volume in the centrifuge tubes. TME is comprised of 50 mM TRIS base, 10 mM $MgCl_2$, 1 mM EDTA; pH 7.4 is achieved by adding 1 N HCl. The cell homogenate is centrifuged at 19,000 rpm for 20-25 min at 4°C using a Beckman Ti-60 or Tι-70 rotor. The pellet is then resuspended in TME buffer to provide a final protein concentration of 1 mg/ml, as determined by Bio-Rad assay. Radioligand binding assays are performed in a 100 µl or 200 µl volume.

The binding of [^{3}H] PGE_2 (specific activity 165 Ci/mmol) is determined in duplicate and in at least 3 separate experiments. Incubations are for 60 min at 25° C and are terminated by the addition of 4 ml of ice-cold 50 mM TRIS-HC1 followed by rapid filtration through Whatman GF/B filters and three additional 4 ml washes in a cell harvester (Brandel). Competition studies are performed using a final concentration of 2.5 or 5 nM [^{3}H] PGE_2 and non-specific binding is determined with 10^{-5} M unlabelled PGE_2.

For all radioligand binding studies, the criteria for inclusion are >50% specific binding and between 500 and 1000 displaceable counts or better.

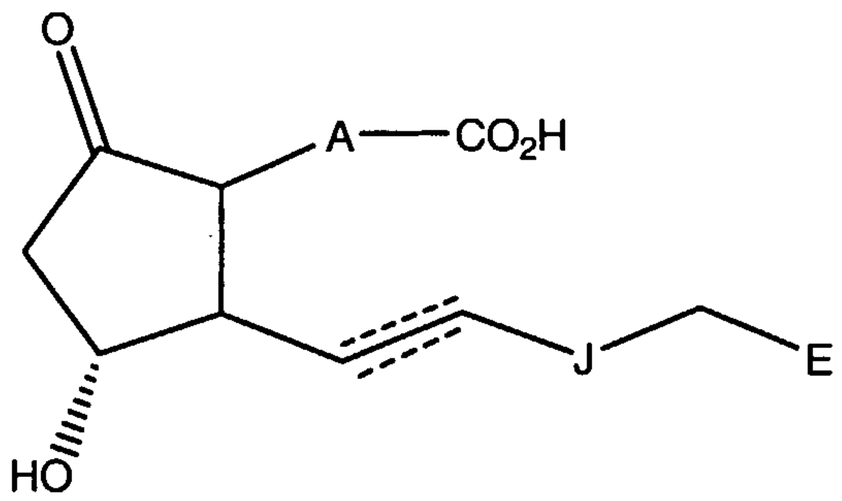
CLAIMS

What is claimed is:

1. A compound comprising a prodrug of a prostaglandin EP_4 agonist, wherein said prodrug is an ester, ether, or amide of a carbohydrate; or said prodrug is an ester, ether, or amide of an amino acid.

2. The compound of claim 1 comprising a glucoside ester, ether, or amide; a glucuronide ester, ether, or amide; a cyclodextrin ester, ether, or amide; or a dextran ester, ether, or amide.

3. The compound of claim 2 wherein said prostaglandin EP_4 agonist is a compound selected from the group consisting of



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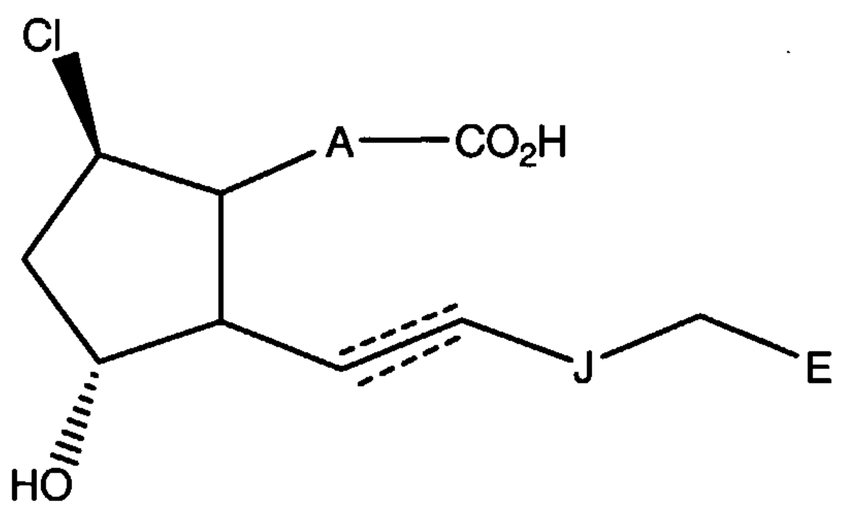

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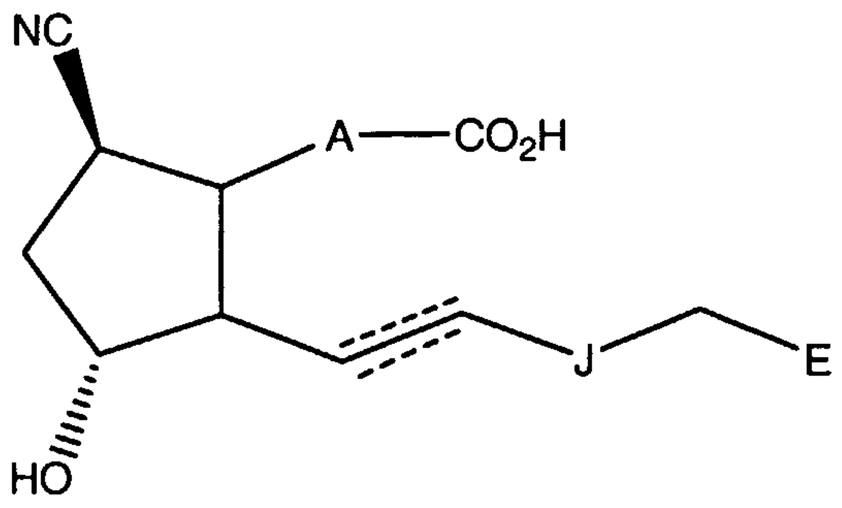

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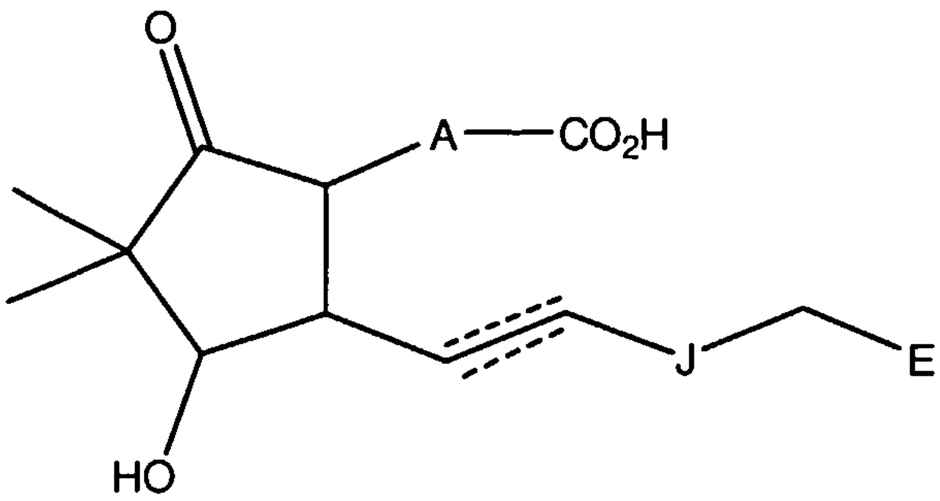

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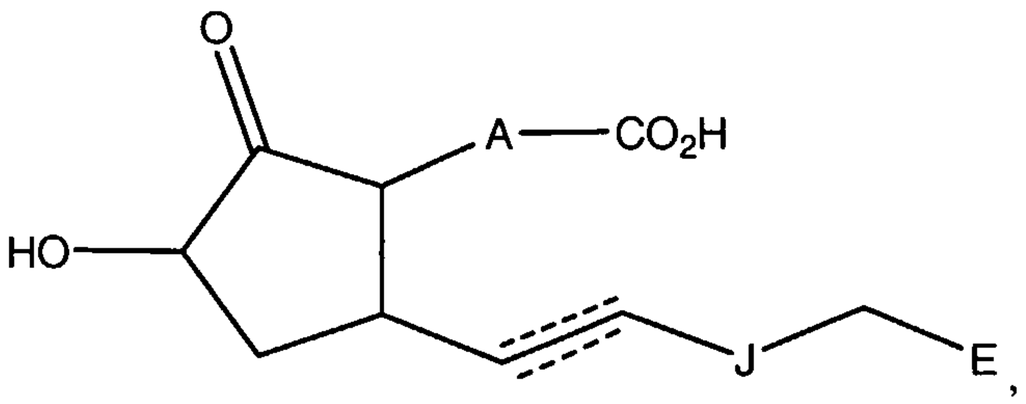

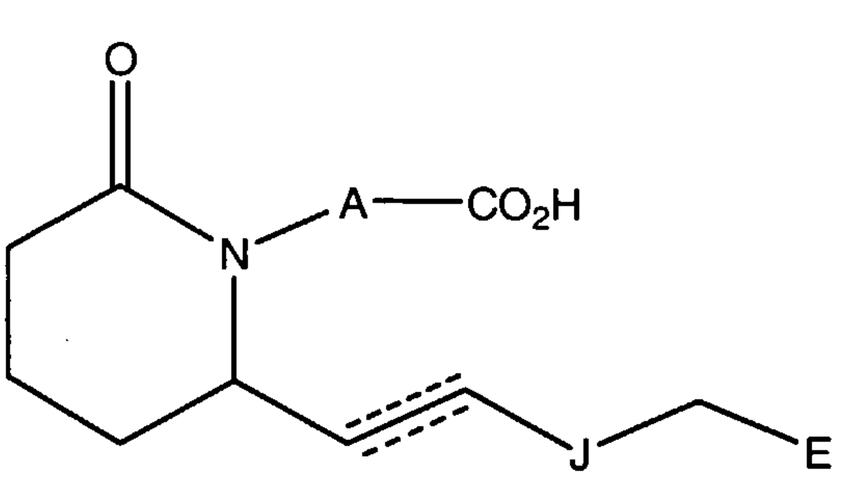

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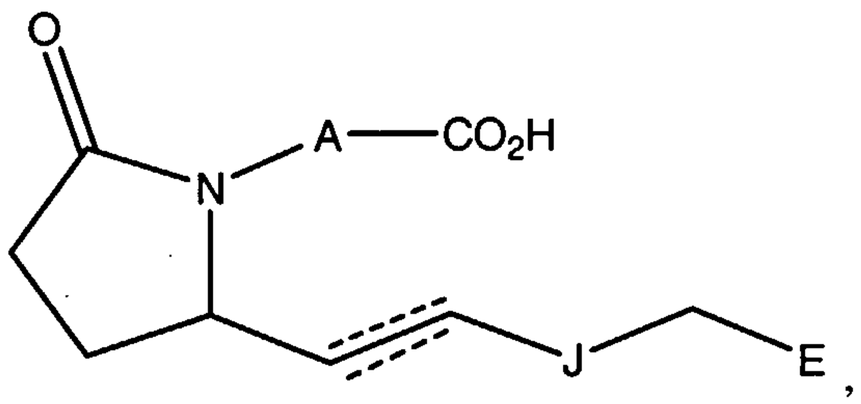

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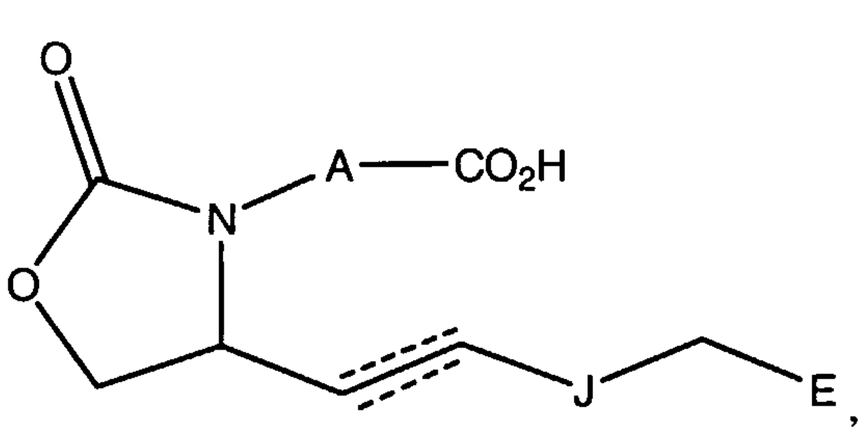

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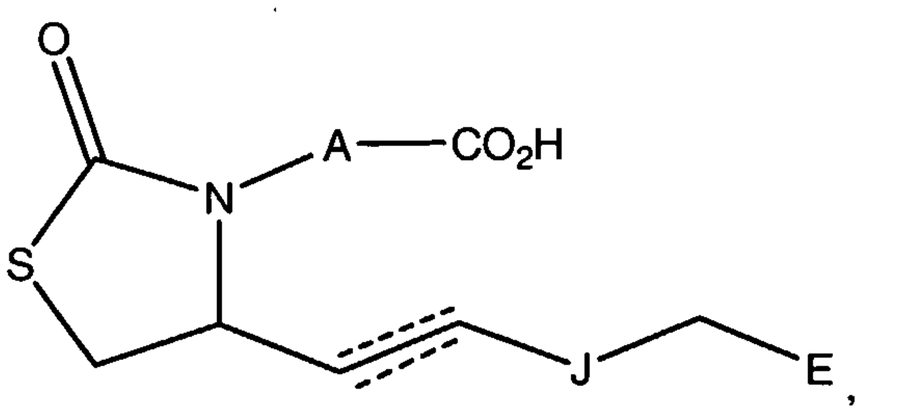

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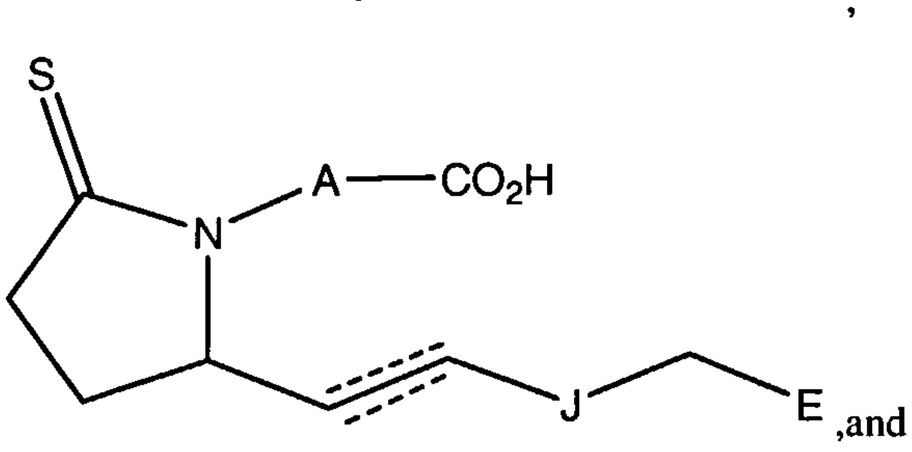

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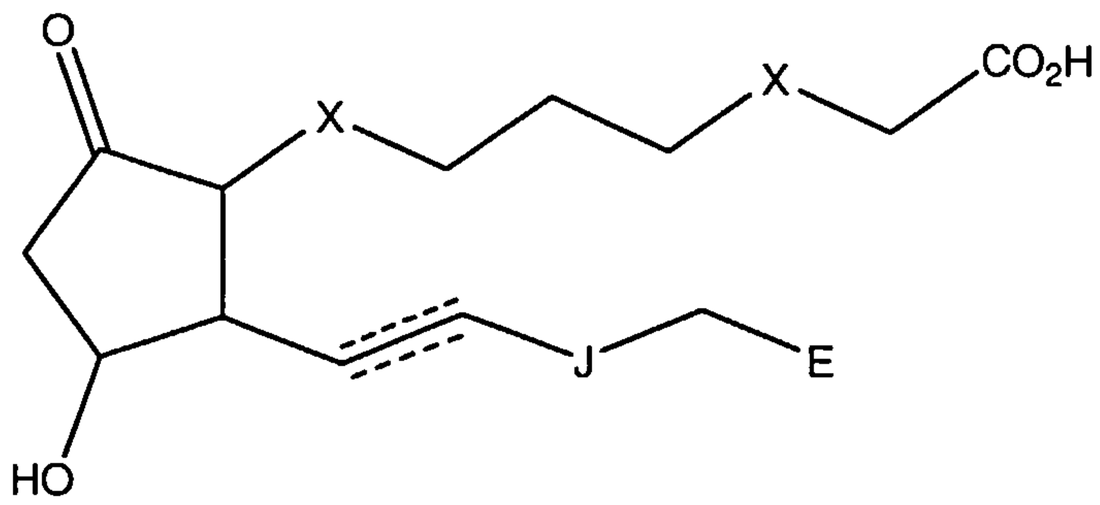


;

or a pharmaceutically acceptable salt or a prodrug thereof,

wherein a dashed line indicates the presence or absence of a bond;

A is $-(CH_2)_6-$, *cis* $-CH_2CH=CH-(CH_2)_3-$, or $-CH_2C\equiv C-(CH_2)_3-$, wherein 1 or 2 carbon atoms may be substituted with S or O; or A is $-(CH_2)_m-Ar-(CH_2)_o-$ wherein Ar is interarylene or heterointerarylene, the sum of m and o is from 1 to 4, and wherein one CH_2 may be substituted with S or O;

X is S or O;

J is C=O, CHOH, or CH_2CHOH; and

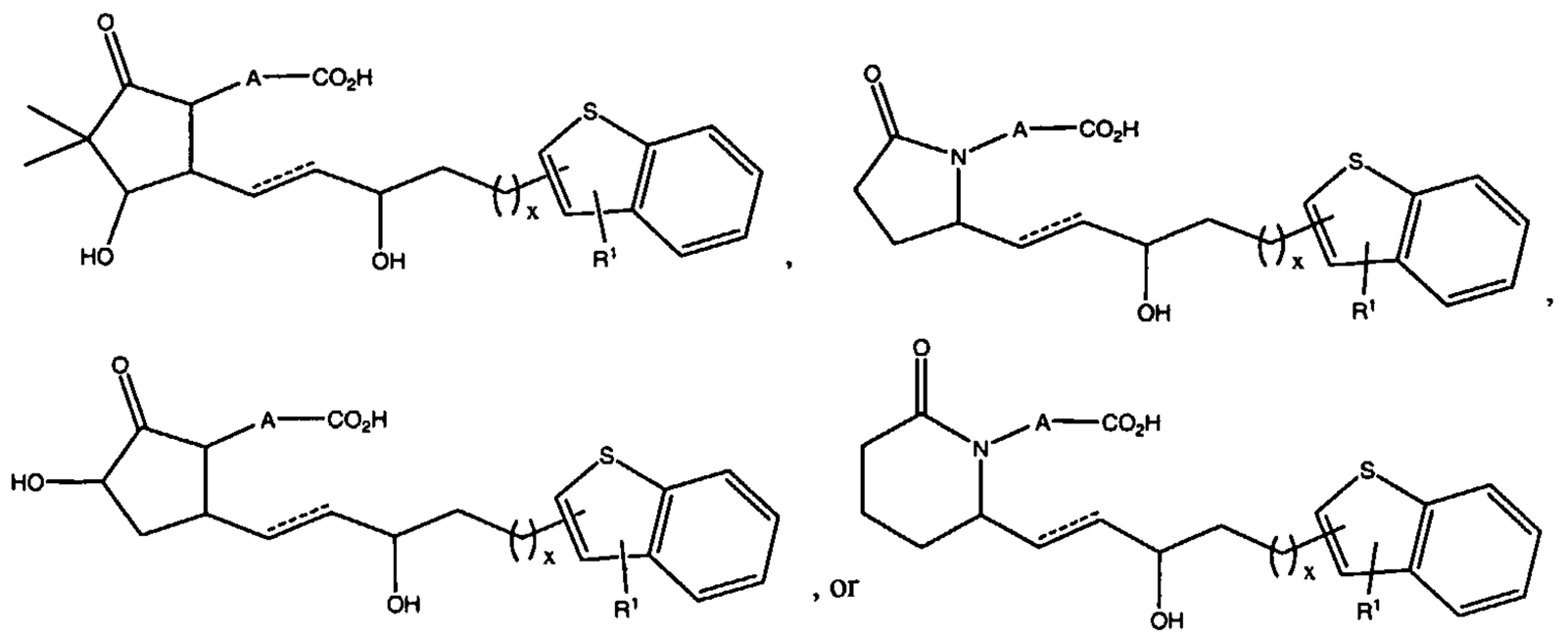
E is C_{1-12} alkyl, R^2, or $-Y-R^2$ wherein Y is CH_2, S, or O, and R^2 is aryl or heteroaryl.

4. The compound of claim 3 wherein A is $-(CH_2)_6-$, *cis* $-CH_2CH=CH-(CH_2)_3-$, or $-CH_2C\equiv C-(CH_2)_3-$, wherein 1 or 2 carbon atoms may be substituted with S or O; and E is C_{1-6} alkyl, R^2, or $-Y-R^2$ wherein Y is CH_2, S, or O, and R^2 is aryl or heteroaryl.

5. The compound of claim 4 wherein R^2 is phenyl, naphthyl, biphenyl, thienyl, or benzothienyl having from 0 to 2 substituents selected from the group consisting of F, Cl, Br, methyl, methoxy, and CF_3.

6. The compound of claim 5 wherein R^2 is CH_2-naphthyl, CH_2-biphenyl, CH_2-(2-thienyl), CH_2-(3-thienyl), naphthyl, biphenyl, 2-thienyl, 3-thienyl, CH_2-(2-(3-chlorobenzothienyl)), CH_2-(3-benzothienyl), 2-(3-chlorobenzothienyl), or 3-benzothienyl.

7. The compound of claim 5 wherein the prostaglandin EP_4 agonist comprises

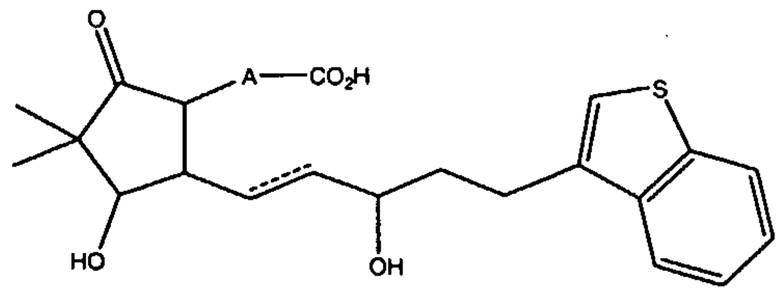
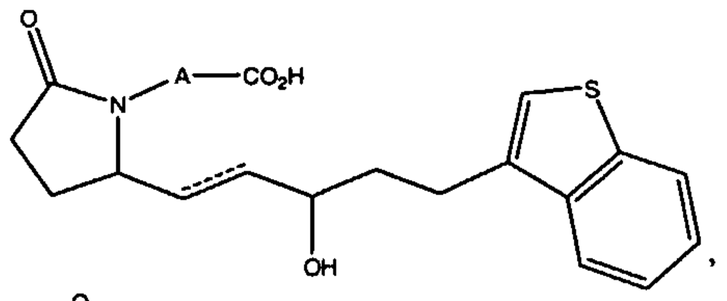
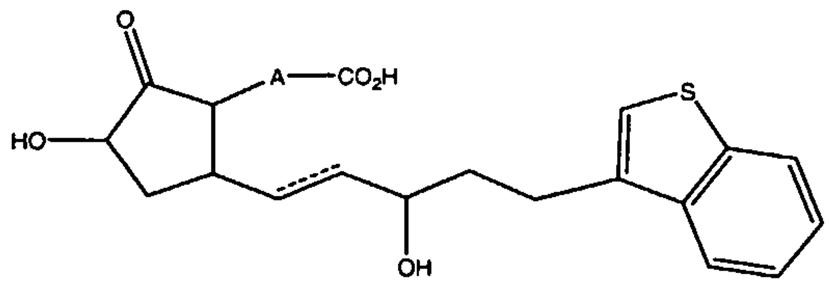
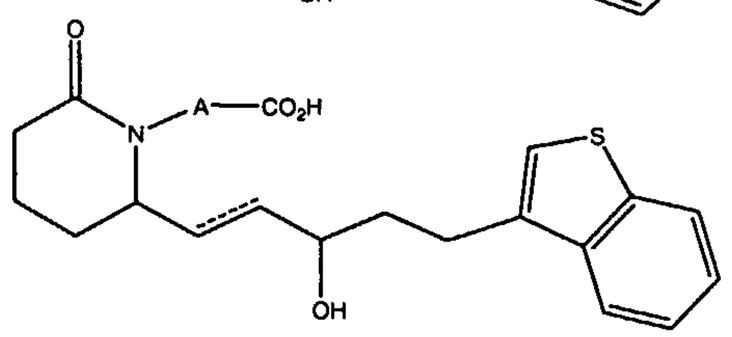
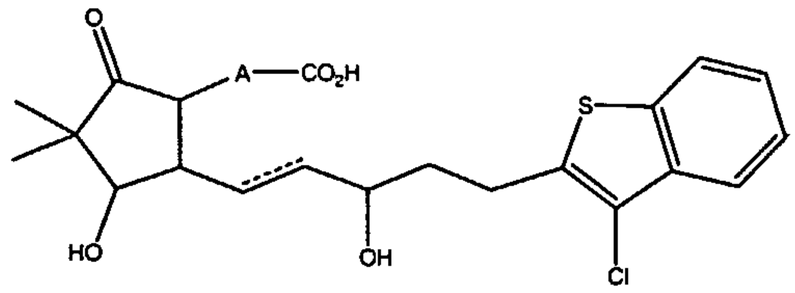
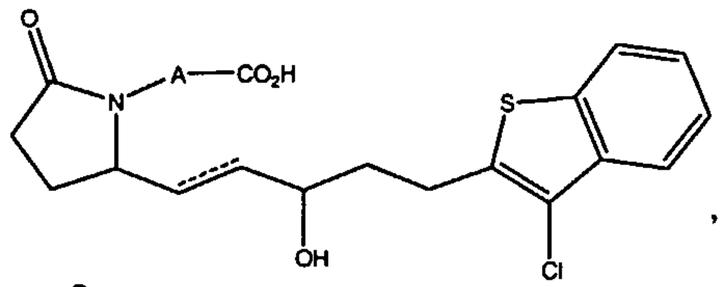
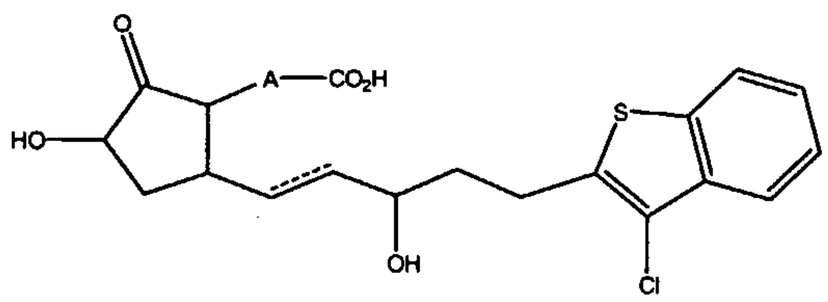
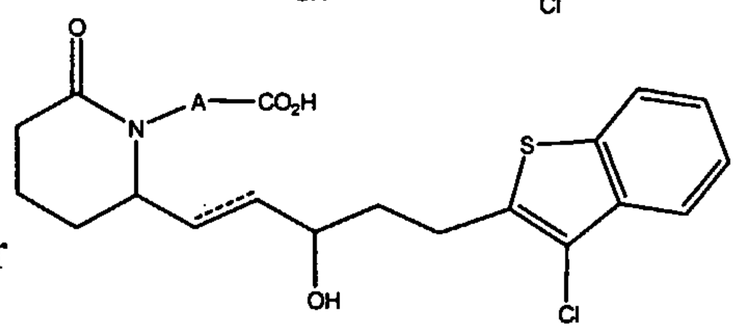


, or

;

wherein x is 0 or 1, and R^1 is H, chloro, fluoro, bromo, methyl, methoxy, or CF_3.

8. The compound of claim 7 wherein the prostaglandin EP_4 agonist comprises

9. A method comprising administering a therapeutically effective amount of a prostaglandin EP_4 agonist to a colon of a mammal, said method being effective in maintaining the colonic mucosal barrier.

10. The method of claim 9 wherein said method is effective in the treatment or prevention of one or more diseases or conditions selected from the group consisting of colitis, amebic colitis, collagenous colitis, colitis cystica profunda, colitis cystica superficialis, granulomatous colitis, hemorrhagic colitis, mucous colitis, Crohn's disease, and ulcerative colitis.

11. The method of claim 10 wherein said disease or condition is Crohn's disease.

12. The method of claim 10 wherein said disease or condition is ulcerative colitis.

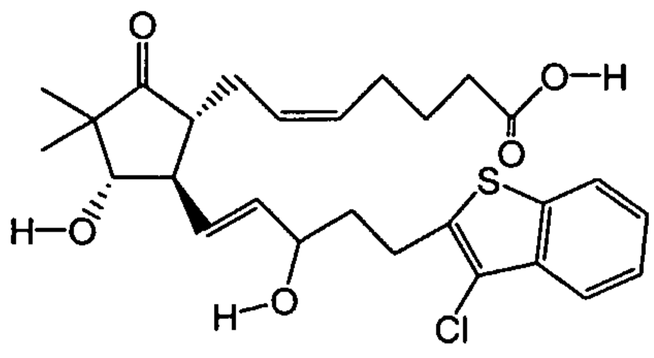
13. A method comprising administering a therapeutically effective amount of a prostaglandin EP_4 agonist to a colon of a mammal, wherein said method is effective in the treatment or prevention of one or more diseases or conditions selected from the group consisting of colitis, amebic colitis, collagenous colitis, colitis cystica profunda, colitis cystica superficialis, granulomatous colitis, hemorrhagic colitis, mucous colitis, Crohn's disease, and ulcerative colitis.

14. The method of claim 13 wherein said disease or condition is Crohn's disease.

15. The method of claim 13 wherein said disease or condition is ulcerative colitis.

16. The compound of claim 1 wherein the prodrug is an amide, ester, or ether of an amino acid.

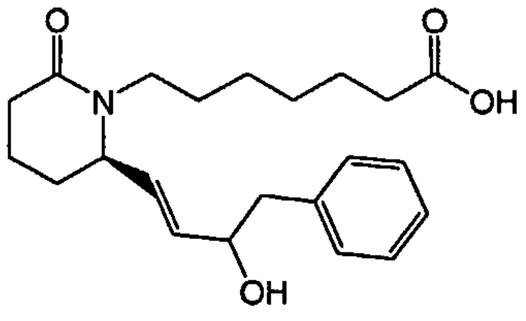
17. The compound of claim 1, which is a prodrug of



;

or a pharmaceutically acceptable salt thereof.

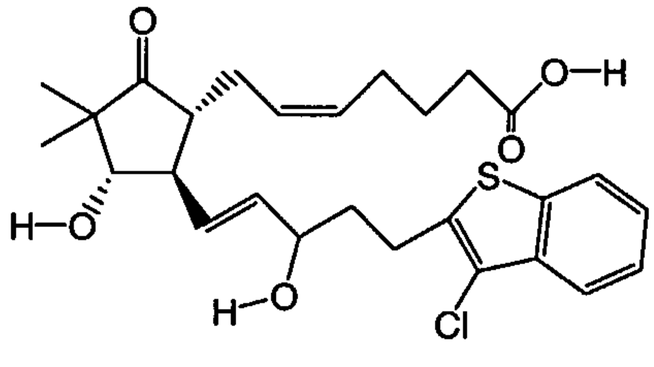
18. The compound of claim 1, which is a prodrug of



;

or a pharmaceutically acceptable salt thereof.

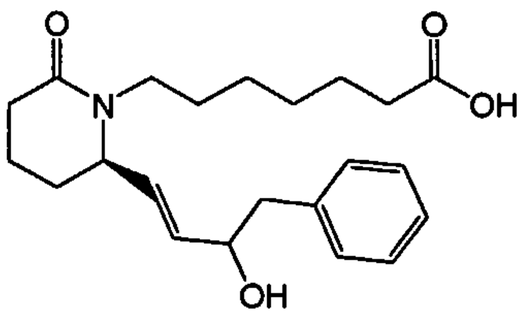
19. The method of claim 13, wherein the prostaglandin EP4 agonist is at least one of



,

a pharmaceutically acceptable salt thereof, and a prodrug thereof.

20. The method of claim 13, wherein the prostaglandin EP4 agonist is at least one of



,

a pharmaceutically acceptable salt thereof, and a prodrug thereof.