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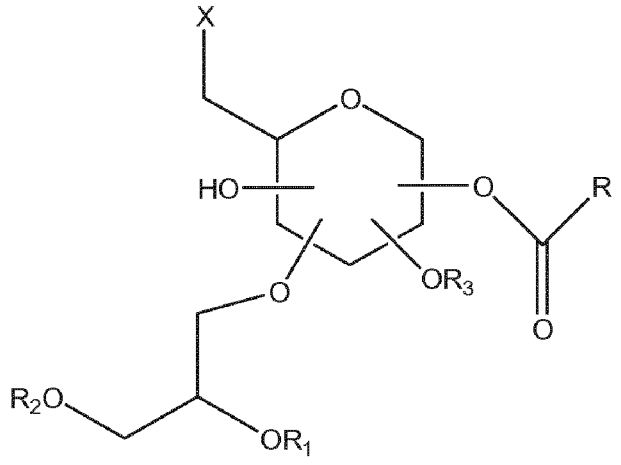
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(54) Title: COMPOUND



(I)

(57) Abstract: A compound of formula (I) wherein R is a C9-23 polyunsaturated alkenyl group; X is SO_3R_3, SO_2R_3, PO_3R_3, PO_2R_3, $COCHal_3$, or $COOR_3$; R_1 and R_2 are each independently H, a C1-6 a iky I group optionally substituted with OH, C6-10 aryl group optionally substituted with OH, C7-12 arylalkyl group optionally substituted with OH, COC1-6 alkyl group optionally substituted with OH, COC6-10 aryl group optionally substituted with OH, COC7-12 arylalkyl group optionally substituted with OH, or CO-$CHal_3$ group; hal is halide, preferably F; each R_3 is independently H, C1-6 alkyl, C6-10 aryl, or C7-12arylalkyl; or a salt thereof.

WO 2014/173957 A2

Compound

This invention relates to a class of new compounds which have been found to exhibit anti-inflammatory properties. The invention also relates to a series of known compounds which have been surprisingly found to possess anti-inflammatory properties by affecting the activity of PLA2 enzyme in the early stage of the inflammation cascade.

In particular, the invention relates to a new compound isolated from *Sclerochloa dura* (L.)P. Beauv and analogues thereof which is an anti-inflammatory agent. The invention also relates to a pharmaceutical composition comprising the compounds of the invention as well as the uses thereof in the treatment of inflammation and conditions associated with inflammation.

Background of invention

It is generally known that plants produce many chemical compounds during their life time such as alkaloids, flavonoids, proteins, amino acids, bioactive peptides, sugars, bio pesticides and others. Plants have always been a centre of attention in the history of traditional medicine, because they produce vital and complex molecules, which can be used either directly or in modified form to cure a number of diseases.

For example, berberine is well known for its anti-inflammatory activity. It is present in a number of plants such as *Hydrastis canadenis, Berberis vulgaris, Barberis aristata* and *Rhizoma coptidis*. Similarly, quercetin belongs to flavonoid class of compounds and is also anti-inflammatory in nature. It can be isolated from many plants, for instance *Helminthostachys zeylanica.* Some other classes of compounds showing anti-inflammatory activity are terpenoids, phenols, polyphenols and some sulphur containing compounds.

The present inventors have developed a class of new anti−inflammatory drugs with an improved mode of action compared to existing drugs. In particular, the compounds of the invention are inhibitors of cPLA2. Most non-steroidal anti-inflammatory drugs (NSAIDs) act on specific enzymes late in the inflammation

reaction pathway (e.g. COX-inhibitors). The problem with acting at such a late stage in the process is that it causes an imbalanced inflammation reduction due to over-activation of parallel pathways (Figure 1). Such an imbalance can cause severe side-effects such as heart attack, stroke and liver failure. The use of existing NSAIDs, therefore, has been notoriously linked with unwanted and serious side-effects.

For example, the NSAID Rofecoxib (sold under the name Vioxx, Merck) was shown to give serious cardiovascular problems in patients having been prescribed the drug in the period 1999-2004 (between 88 000 and 139 000 heart attacks in US only, 30 to 40 % of which were probably fatal). The drug was withdrawn from the market in 2004. These deaths could have been prevented if the drug had targeted enzymes earlier in the inflammation process.

In 2011, a British study reported that another NSAID, Diclofenac increases the probability of experiencing cardiovascular problems with 40%. Voltaren was in 2010 prescribed to 500 000 patients in Norway alone. The drug is still on the market worldwide. Other NSAIDs that have been withdrawn from the market due to serious side effects include Valdecoxib (Bextra, Pfizer, 2005), Lumiracoxib (Prexige, Novartis, 2007), Etoricoxib (Arcoxia, Merck,) and Parecoxib (Dynastat, Pfizer).

The long term use of most of the NSAIDs on the market today (e.g. COX inhibitors) are related to quite severe side-effects. Therefore, there is a need for NSAIDs with an improved mode of action.

The compounds of the presence invention affect (reduce) the activity of PLA2 enzyme although a complete mechanism and exact point of action has yet to be elucidated.

The core invention is the use of the compounds of the invention to interrupt the anti-inflammatory cascade by inhibiting PLA_2. Moreover, the invention relates to the characterisation of a new anti-inflammatory compound from a biological source and with that knowledge the design of a new class of anti-inflammatory agents.

The preferred compound of the present invention derives from *Sclerochloa dura* which has previously been identified as having medicinal uses. This plant has a traditional use in small communities in South-East Serbia for treatment of menstrual

disorders characterised by excessive bleeding and pain. Oral intake of a boiled water extract of the plant allegedly results in pain relief and significantly reduces bleeding.

The present inventors have identified a series of chemical compounds present in *S. dura* which have anti-inflammatory properties and based on this knowledge designed the claimed subject matter to achieve a new class of anti-inflammatory drugs with an improved mode of action and fewer side-effects.

Crude extracts and subsequent fractions were tested on human synovial sarcoma cell line SW982 (stimulated with interleukin-1β) to determine the inhibition of arachidonic acid (AA) release, which corresponds to the activation level of phospholipase A_2 (PLA_2) enzymes. The results prove that the crude extract and subsequent methanolic fraction of *S. dura* inhibits IL-1 induced release of AA in a time- and dose-dependent manner. Thus, they contain active ingredient which can inhibit the activity of the PLA_2 cascade.

The PLA_2 enzymes hydrolyze fatty acids such as AA from the *sn*-2 ester bond of membrane glycerophospholipids. These enzymes are sorted into five main categories, namely cytosolic PLA_2s ($cPLA_2$), secretory PLA_2s ($sPLA_2$), Ca^{2+}-independent PLA_2s ($iPLA_2$), PAF acetyl hydrolases (PAF-AH), and lysosomal PLA_2s. Apart from their role in normal physiological functions, the different isotypes are implicated in a variety of diseases and thus there is currently great interest in the development of inhibitors that target specific PLA_2 subtypes. The Ω6 fatty acid (AA) is the substrate for eicosanoids such as prostaglandins and leukotrienes, which are potent lipid mediators of inflammation. As $cPLA_2$ group IVa ($cPLA_2$-IVa) is the only PLA_2 with a preference for AA in the *sn*-2 position, it is a central enzyme of eicosanoid production and thus considered to be a main contributor in inflammatory processes.

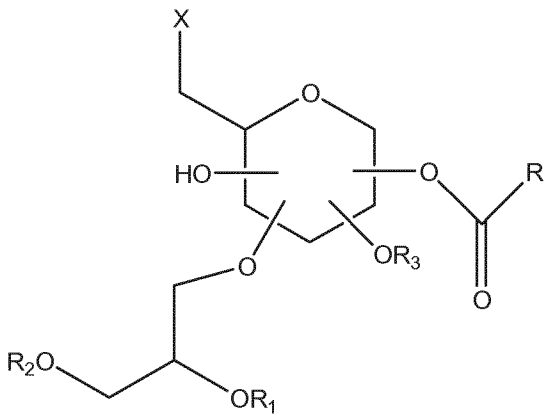
Most chronic diseases, such as atherosclerosis, rheumatoid arthritis, diabetes and chronic respiratory diseases are characterized by a considerable inflammatory component. These are the leading cause of global death (WHO, 2011). Inflammation is an acute response to harmful stimuli that normally abates after the challenge is cleared. In chronic inflammatory diseases, the response is not attenuated and the inflammation in the tissue is not completely resolved. Instead, a prolonged signaling ensures a persistent inflammatory environment. Pro-inflammatory cytokines, such as

tumor necrosis factor (TNF) and interleukin 1β (IL-1β), are abundant and activate $cPLA_2$-IVa, which results in the subsequent release of AA and generation of eicosanoids, creating a self-perpetuating process. Several studies using knockout mice or $cPLA_2$-IVa inhibitors have highlighted the potential for targeting this enzyme in order to quench the inflammation and ameliorate disease. The *S. dura* derived compounds that inhibit AA release exert an overall anti-inflammatory action.

The present inventors have isolated and elucidated compounds from *Sclerochloa dura.* The compounds expressed anti-inflammatory activity by showing the inhibition of $cPLA_2$ in an *in vitro* AA release assay. The invention further relates to the use of the information gleaned to devise a new group of anti-inflammatory agents as well as the possible organic synthesis of these molecules.

Summary of Invention

Thus, viewed from one aspect the invention provides a compound of formula (I)



(I)

wherein R is a C9-23 polyunsaturated alkenyl group;

X is SO_3R_3, SO_2R_3, PO_3R_3, PO_2R_3, $COCHal_3$, or $COOR_3$;

R_1 and R_2 are each independently H, a C1-6 alkyl group optionally substituted with OH, C6-10 aryl group optionally substituted with OH, C7-12 arylalkyl group optionally substituted with OH, COC1-6 alkyl group optionally

substituted with OH, COC6-10 aryl group optionally substituted with OH, COC7-12 arylalkyl group optionally substituted with OH, or $COCHal_3$ group;

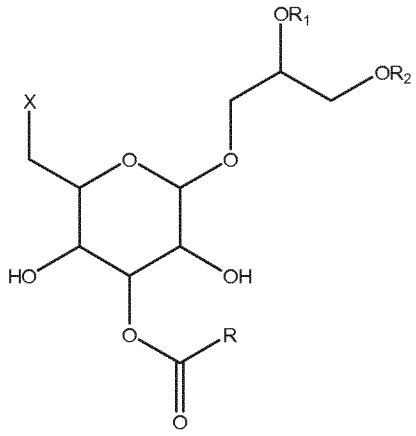
hal is halide, preferably F;

each R_3 is independently H, C1-6 alkyl, C6-10 aryl, or C7-12arylalkyl;

or a salt thereof.

For the avoidance of doubt each substituent in formula (I) must bind a separate carbon atom in the six membered ring. It is not therefore possible for two substituents to bind the same carbon atom. The substituents can otherwise attach in any order.

Preferably the compound of formula (I) is a compound of formula (V)

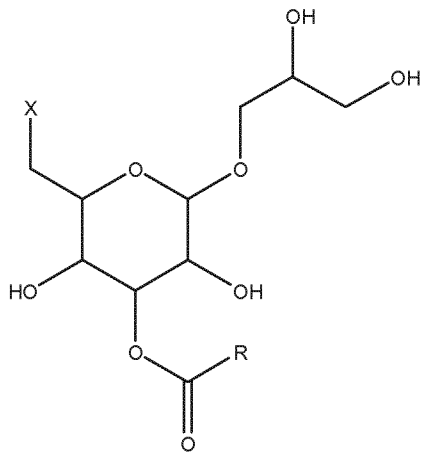


(V)

where X, R, and R_1-R_3 are as hereinbefore defined;

or a salt thereof.

Most preferably, it is a compound of formula (VI)

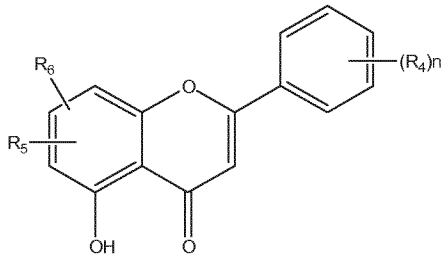


(VI)

where X and R, are as hereinbefore defined;

or a salt thereof.

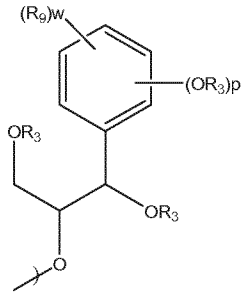
Viewed from another aspect the invention provides a compound of formula (II)



(II)

wherein

each R_4 is H, OH, OR_3, C1-6alkyl, C6-10 aryl, C7-12 arylalkyl or the group:



wherein each R_3 is as hereinbefore defined;

p is 0-3;

n is 1 to 3;

w is 0 to 2;

R_9 is C_{1-6} alkyl;

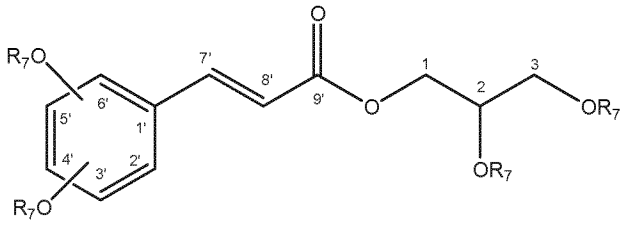
R_5 is a monosaccharide linked to the phenyl ring via a C-C bond or linked via a C-O bond at the 1-position of the saccharide, and

R_6 is H, OH, C1-6 alkyl or OR_3;

or a salt thereof;

for use in the treatment of inflammation.

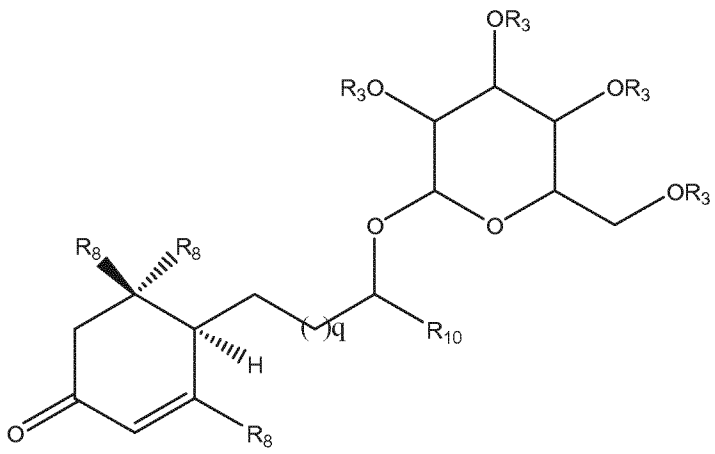
Viewed from another aspect the invention provides a compound of formula (III)



(III)

wherein R_7 is H or C1-6 alkyl; or salt thereof for use in the treatment of inflammation.

Viewed from another aspect the invention provides a compound of formula (IV)



(IV)

wherein each R_8 is H or a C1-6-alkyl;

R_{10} is H or C1-6 alkyl;

q is 0 to 3, such as 1 or 2;

each R_3 is as hereinbefore defined with at least one OR_3 group being OH; or a salt thereof; for use in the treatment of inflammation.

Viewed from another aspect the invention provides a pharmaceutical composition comprising a compound of formula (I) as hereinbefore defined and at least one carrier or excipient.

Viewed from another aspect the invention provides a compound of formula (I) for use as a medicament.

Viewed from another aspect the invention provides a compound of formula (I) for use in the treatment of inflammation.

Viewed from another aspect the invention provides a compound of formula (I) to (IV) for use as an inhibitor of $cPLA_2$.

Viewed from another aspect the invention provides a compound of formula (I) to (IV) for use in the treatment of a chronic inflammatory disease such as acne vulgaris, asthma, autoimmune diseases, celiac disease, chronic prostatitis, glomerulonephritis, hypersensitivities, inflammatory bowel diseases, pelvic inflammatory disease, reperfusion injury, rheumatoid arthritis, sarcoidosis, transplant rejection, and vasculitis.

Viewed from another aspect the invention provides a compound of formula (I) to (IV) for use in the treatment of hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, and cancer.

Viewed from another aspect the invention provides the use of a compound of formula (I) to (IV) as hereinbefore defined in the manufacture of a medicament for the treatment of inflammation.

Viewed from another aspect the invention provides a method of treating inflammation comprising administering to a patient in need thereof, such as a human, a therapeutically effective amount of a compound of formula (I) to (IV) as hereinbefore defined.

Detailed Description of Invention

This invention relates to a group of compounds which possess anti-inflammatory properties. The compounds are defined by formula (I) to (IV).

Compounds of formula (I) are new. Compounds of formula (II) to (IV) are new for use in the treatment of inflammatory conditions, e.g. via the inhibition of $cPLA_2$.

Definitions

In compounds of formula (I), for the avoidance of doubt each substituent must bind a separate carbon atom. It is not therefore possible for two substituents to bind the same carbon atom on the ring.

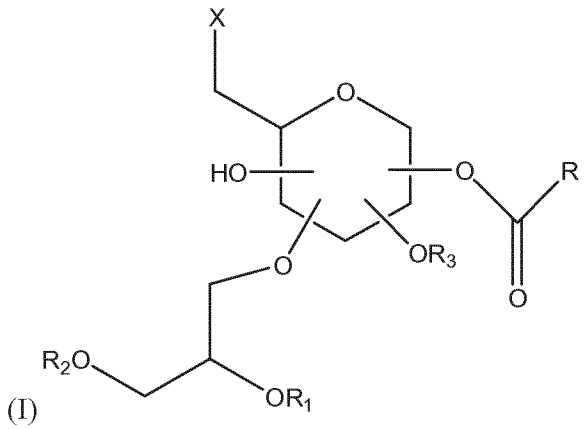
The group "arylalkyl" comprises an alkyl portion and an aryl portion. It may link to another group either via the aryl portion or alkyl portion. Thus, this term covers the groups tolyl and benzyl for example.

In compounds of formula (III), the monosaccharide is linked to the phenyl ring directly either via a C-C link from a saccharide carbon ring atom to a phenyl ring atom. It can also be linked via a C-O link at the 1-position of the saccharide directly to a carbon atom of the phenyl ring.

In general R_3 is preferably H or C_{1-6}-alkyl, such as C_{1-4} alkyl, especially methyl, especially H.

Compounds (I)

In a preferred embodiment, the invention relates to compounds of formula



(I) (I)

with substituents definitions as hereinbefore defined. As will be noted below, the specific compound (X), can be isolated from *Sclerochloa dura* (L.)P. Beauv but the invention covers analogues of this isolated compound as well.

Whilst the polyunsaturated long chain hydrocarbon substituent in the isolated molecule of formula (X) as defined herein is based on linolenic acid (C18, 3 double

bonds), it will be appreciated that other polyunsaturated long chain side groups would also be suitable.

In preferred R groups of the invention therefore, there are at least 3, such as 3 to 6 double bonds, preferably 3 to 5 double bonds. Such double bonds are preferably not conjugated.

The last double bond (i.e. furthest from the 6 membered sugar ring) is preferably 3 carbons from the end of the chain, i.e. the side chain is preferably based on an omega-3 type structure.

The total number of carbons present in group R may range from 11 to 23, preferably 13 to 21, such as 15 to 19. Preferably, there are an even number of carbon atoms in the side chain R when you include the CO carbon atom in the calculation. The use of a linoleic side chain is preferred (when including the CO carbon atom - i.e. 18 carbon atoms).

The sugar ring is preferably in the alpha configuration at the anomeric carbon. It should also be in the pyranose configuration. Ideally, the hexose is based on a 6-deoxyglucose moiety and ideally it is a D-glucopyranosyl ring. It will be appreciated that the glucose moiety is 6-deoxy in that the group at the 6-position is not OH but a group X as defined herein.

It is preferred if the -OCOR group lies equatorially on the ring. Ideally that group is opposite the O atom (i.e. on the para position to that O atom).

It is preferred if the CH_2X group lies equatorially on the ring. Ideally, the ring is based on glucose and all substituents other than the anomeric carbon are equatorial.

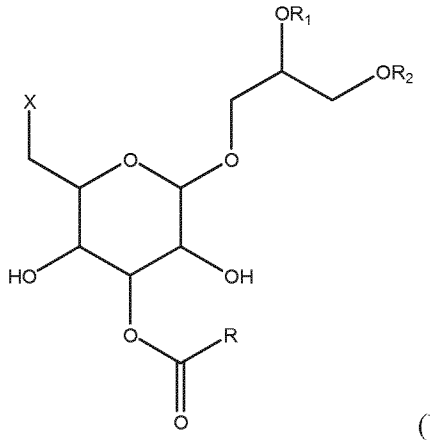
The X group is preferably SO_3R_3, SO_2R_3, $COCHal_3$, or $COOR_3$. More preferably it is SO_3R_3, especially SO_3H. The R_3 group in the definition of X is preferably H or Me.

It is preferred if at least one of R_1 and R_2 is H. Ideally both are H. If not H, it is preferred if R_1 or R_2 is a C1-6 alkyl, such as a C1-4 alkyl especially methyl group.

The group OR_3 in the compounds of formula (I) is preferably OH. If not OH, OR_3 is preferably OC1-6 alkyl such as OMe.

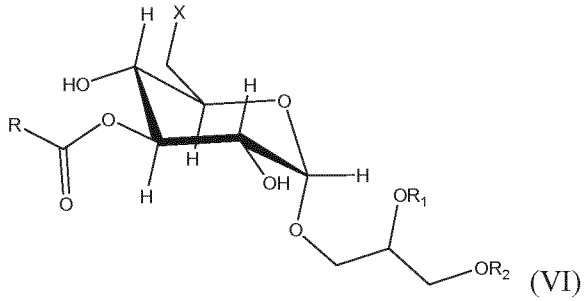
The glycerol based substituent group ideally sits axially on the ring.

Preferably the compound of formula (I) is a compound of formula (V)



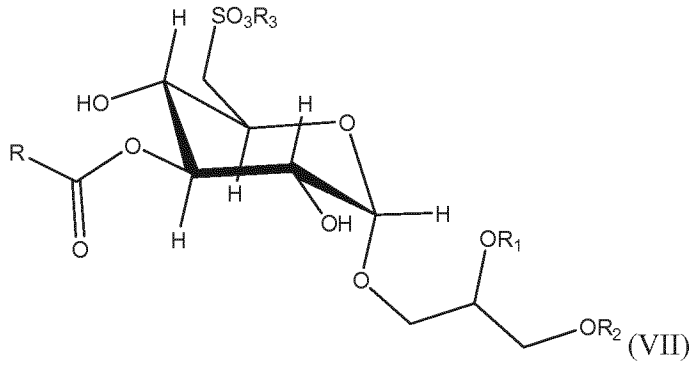
(V)

such as a compound of formula (VI)



(VI)

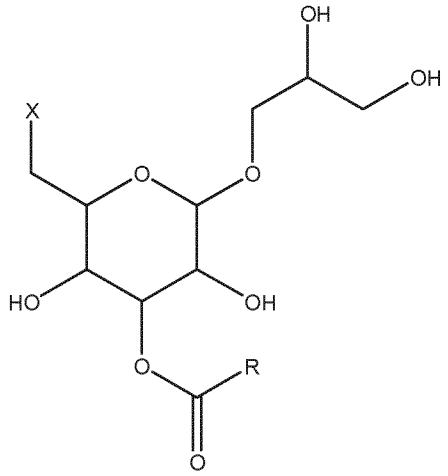
where X, R, and R_1-R_3 are as hereinbefore defined; or a salt thereof. More preferably, it is a compound of formula (VII)



(VII)

wherein R, and R_1-R_3 are as hereinbefore defined; or a salt thereof, e.g. where SO_3R_3 is SO_3H.

Most preferably, it is a compound of formula (VIII)

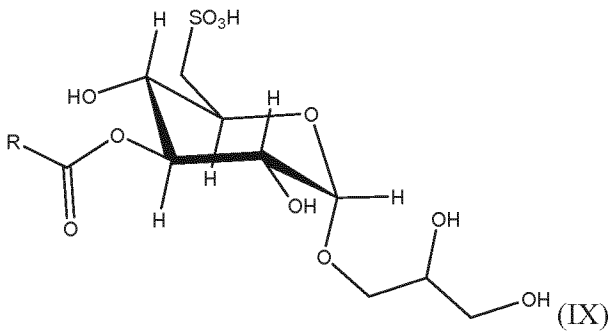


(VIII)

where X and R, are as hereinbefore defined;

or a salt thereof.

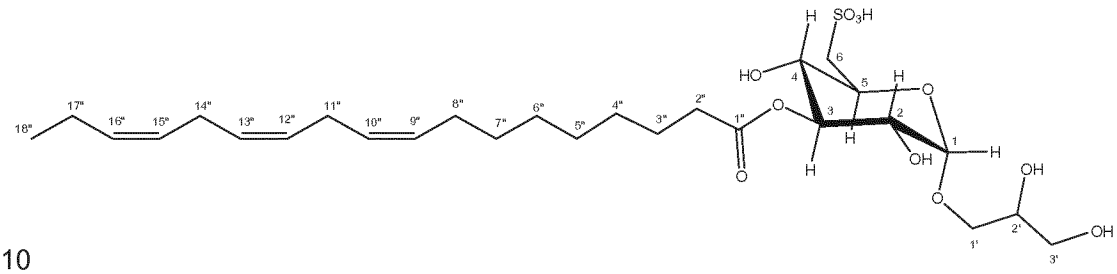
Ideally, the compound of the invention is one of formula (IX)



(IX)

where R is as hereinbefore defined.

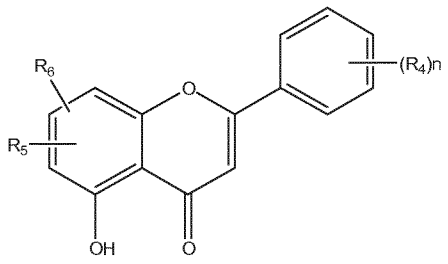
Most especially, the compound of formula (I) is a compound of formula (X) as herein described or a salt thereof:



Compounds (II)

In a second embodiment, the invention provides a compound of formula (II)

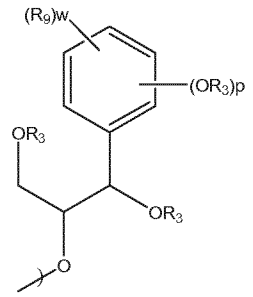
(II)



(II)

wherein

each R_4 is H, OH, OR_3, C1-6alkyl, C6-10 aryl, C7-12 arylalkyl or the group:



wherein each R_3 is as hereinbefore defined;

p is 0-3;

n is 1 to 3;

w is 0 to 2;

R_9 is C_{1-6} alkyl;

R_5 is a monosaccharide linked to the phenyl ring via a C-C bond or linked via a C-O bond at the 1-position of the saccharide, and

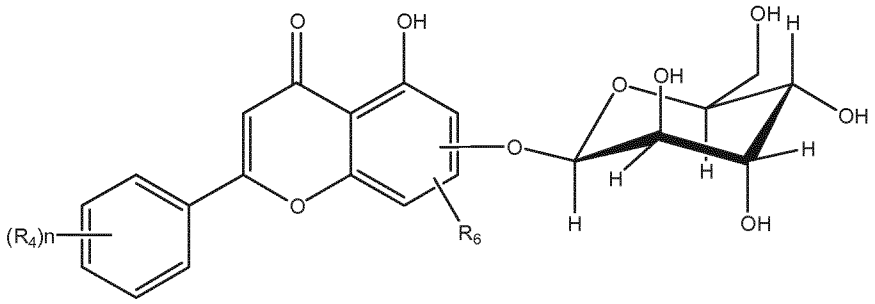
R_6 is H, OH, C1-6 alkyl or OR_3;

or a salt thereof;

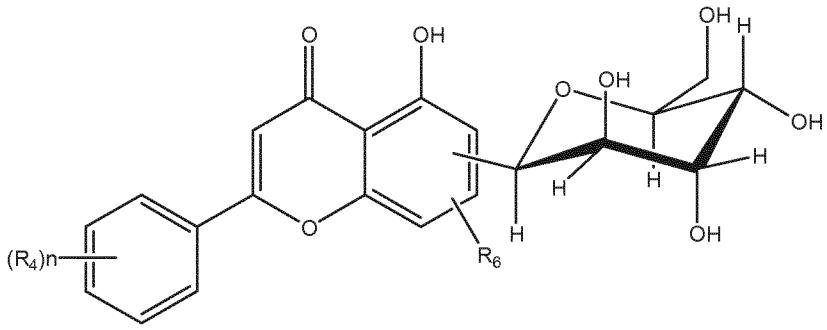
for use in the treatment of inflammation.

It is preferred if the saccharide employed in compounds of formula (II) as substituent R_5 is glucose or deoxy glucose. It may also be a 1-deoxy monosaccharide such as 1-deoxyglucose. In particular, the saccharide may be bound to the phenyl of the bicyclic ring via the anomeric oxygen of the sugar or via a direct C-C link, preferably at the 1-position of the ring.

A further preferred compound of formula (II) is therefore of formula (XI) or (XII)



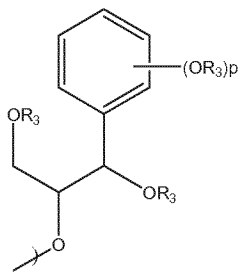
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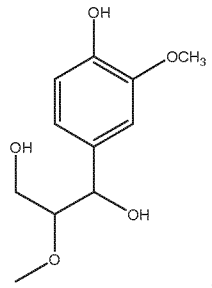
(XII)

It is preferred if the sugar is bound at the position adjacent the OH group (ortho) or meta to the OH group.

The subscript n is preferably 1 or 3. The R_4 group may independently be OH or OC_{1-6}alkyl such as OMe. The group R_4 may also preferably represent:

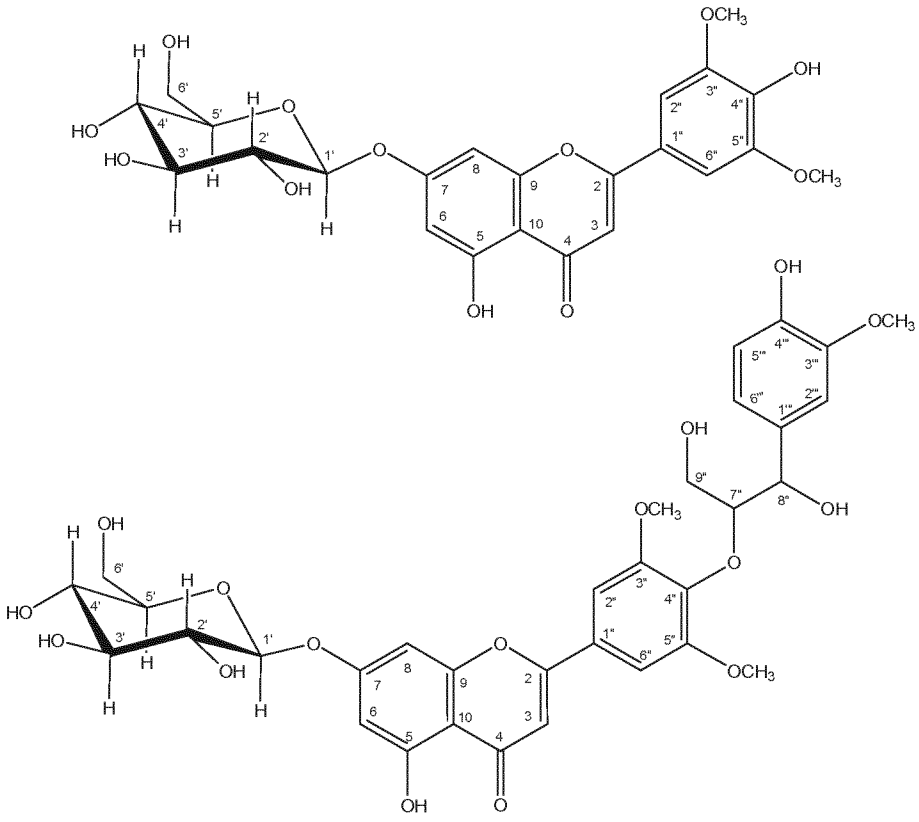


In this group each R_3 is preferably H or C1-6 alkyl and p can be 1 or 2. The R_3 groups outside the ring are ideally H. Ideally the group is

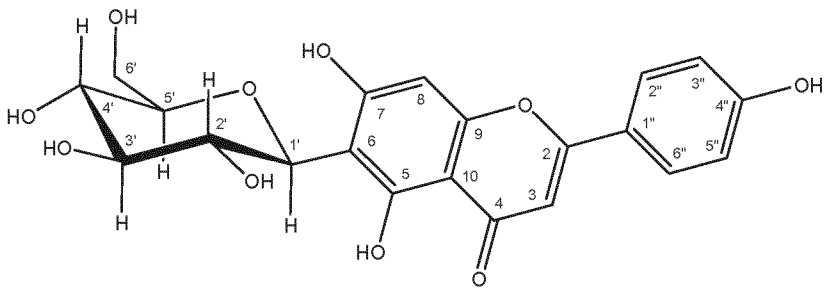


.

Highly preferred compounds of formula (II) are XIII, XIV and XV:



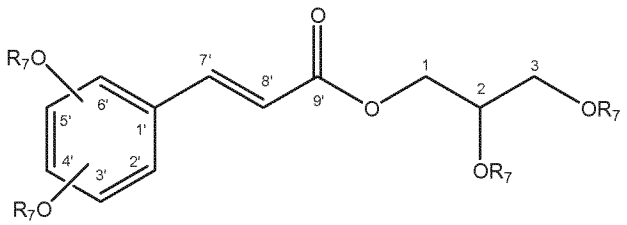
(XIV)



(XV)

Compounds (III)

The invention also relates to a compound of formula (III)

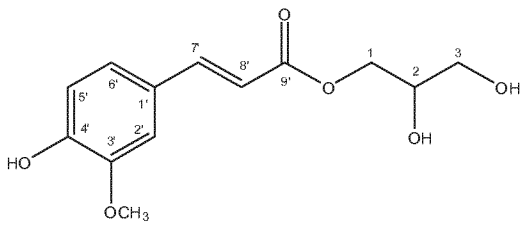


(III)

wherein R_7 is H or C1-6 alkyl; or salt thereof for use in the treatment of inflammation.

It is preferred if at least two R_7 groups are H, preferably at least 3 R_7 groups are H. Preferably, one of the R_7 groups on the Ph ring is C1-6 alkyl, ideally methyl.

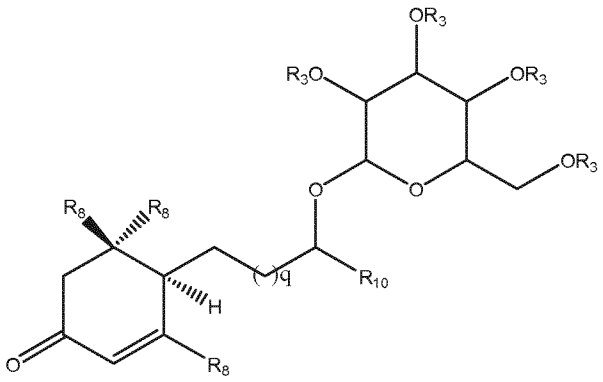
Ideally, the compound is:



(XVI)

Compounds IV

The invention also provides a compound of formula (IV)



(IV)

wherein each R_8 is H or a C1-6-alkyl;

R_{10} is H or C1-6 alkyl;

q is 0 to 3, such as 1 or 2;

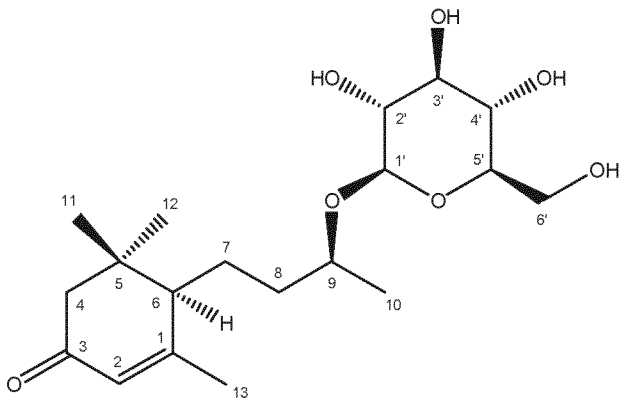
each R_3 is as hereinbefore defined with at least one OR_3 group being OH; or a salt thereof; for use in the treatment of inflammation.

Preferred R_8 groups are C1-4 alkyl groups, such as methyl. R_{10} is preferably ethyl or especially methyl.

It is preferred if at least two OR_3 groups in formula (IV) are OH, such as at least three groups OR_3 are OH. Ideally, all OR_3 groups are OH in formula (IV).

The hexose ring is again preferably based on glucose.

In a preferred embodiment, the compound is of formula (XVII)



(XVII)

Synthesis

As we noted below compounds of formula (I) to (IV) can be isolated from natural sources. In order to make analogues of these compounds as described in the general formulae above, it may be easiest to start with the isolated compounds and effect transformations from those compounds using well known chemistry. Conversion of OH to Oalkyl groups is trivial and so on.

It is also possible however to synthesize the compounds of the invention, and in particular those of formula (I). The compounds of formula (I) can be made synthetically using known protocols. In general, any synthesis of a compound of formula (X) and related molecules of formula (I) may start from the saccharide, e.g. glucose. Introduction of the SO_3H group at C-6 may be accomplished via conversion of the OH group to a good leaving group (Br, Cl, tosylate, mesylate, triflate); reaction with thioacetate followed by oxidation.

Introduction of the glycerol substituent at C-1 might involve an acetonide of glycerol.

The polyunsaturated fatty acid chain required is a readily available product which can be coupled to the sugar ring using known chemistry with protection if required. Nucleophilic attack of the 3-position hydroxyl group onto to an acid chloride modified fatty acid chain allows the side chain to be introduced.

Esterfication of the 3-position-OH might occur via a mixed anhydride and there is also a set of methods which can be used on the linolenic acid directly. Possibly enzymatic reactions could have some benefit in offering high regioselectivity, mild conditions and easy clean up.

Protocols discussed in the following papers can be employed:

Kvrno, Lisbet, Werder, Moritz, Hauser, Helmut, and Carreira, Erick M. Synthesis and in Vitro Evaluation of Inhibitors of Intestinal Cholesterol Absorption. J.Med.Chem. 48[19], 6035-6053. 2005;

Kvrno, Lisbet, Werder, Moritz, Hauser, Helmut, and Carreira, Erick M. Carbohydrate Sulfonyl Chlorides for Simple, Convenient Access to Glycoconjugates. Org.Lett. 7[6], 1145-1148. 2005;

Yamazaki, Takayuki, Aoki, Satoko, Ohta, Keisuke, Hyuma, Shinji, Sakaguchi, Kengo, and Sugawara, Fumio. Synthesis of an immunosuppressant

SQAG9 and determination of the binding peptide by T7 phage display. Bioorg.Med.Chem.Lett. 14[16], 4343-4346. 2004;

Liptak, Andras, Balla, Edit, Janossy, Lorant, Sajtos, Ferenc, and Szilagyi, Laszlo. The first synthesis of secondary sugar sulfonic acids by nucleophilic displacement reactions. Tetrahedron Lett. 45[4], 839-842. 2004;

Sahara, Hiroeki, Hanashima, Shinya, Yamazaki, Takayuki, Takahashi, Shunya, Sugawara, Fumio, Ohtani, Seiji, Ishikawa, Masako, Mizushina, Yoshiyuki, Ohta, Keisuke, Shimozawa, Kumiko, Gasa, Shinsei, Jimbow, Kouichi, Sakaguchi, Kengo, Sato, Noriyuki, and Takahashi, Nobuaki. Anti-tumor effect of chemically synthesized sulfolipids based on sea urchin's natural sulfonoquinovosylmono-acylglycerols. Jpn.J.Cancer Res. 93[1], 85-92. 2002;

Sugawara, Fumio, Sakaguchi, Kengo, Yamazaki, Takayuki, Murata, Hiroshi, Ohta, Keisuke, and Masaki, Kazuyoshi. Novel 6-deoxy-6-carbonylthioglucopyranosyl acetimidates, novel intermediates for production thereof and processes for production of both. (Toyo Suisan Kaisha, Ltd. Japan. 2001-JP1607[2001064699], 60. 20010907. WO. 2-3-2001;

Hanashima, S., Mizushina, Y., Yamazaki, T., Ohta, K., Takahashi, S., Sahara, H., Sakaguchi, K., and Sugawara, F. Synthesis of sulfoquinovosylacylglycerols, inhibitors of Eukaryotic DNA Polymerase Î± and Î². Bioorg.Med.Chem. 9[2], 367-376. 2001; and

Hanashima, Shinya, Mizushina, Yoshiyuki, Yamazaki, Takayuki, Ohta, Keisuke, Takahashi, Shunya, Koshino, Hiroyuki, Sahara, Hiroeki, Sakaguchi, Kengo, and Sugawara, Fumio. Structural determination of sulfoquinovosyldiacylglycerol by chiral syntheses. Tetrahedron Lett. 41[22], 4403-4407. 2000;

The specific compound of formula (X) can, however be isolated from *Sclerochloa dura* (Linneaus) P Beauvois, also known as common hard grass. The experimental section contains a detailed description of the isolation procedure. In particular, a compound of formula (X) can be isolated from the aerial parts of the plant. Compounds of formula (II) to (IV) are also isolable from this plant.

Thus viewed from another aspect the invention provides a process for the isolation of a compound of formula (X) comprising extracting said compound from *Sclerochloa dur.*

Applications

The compounds of the invention have surprisingly been found to have potential as anti-inflammatory agents. They have been found to be potent inhibitors of PLA_2 in particular $cPLA_2$ The compounds of the invention are therefore of use as possible therapies for chronic inflammatory conditions that can generally be associated with PLA_2 inhibition.

It is a particular feature of the invention that the compounds of the invention are able to reduce inflammation via inhibition of cPLA2 as opposed to some enzyme later in the inflammation reaction pathway.

Conditions of interest therefore include acne vulgaris, asthma, autoimmune diseases, celiac disease, chronic prostatitis, glomerulonephritis, hypersensitivities, inflammatory bowel diseases, pelvic inflammatory disease, reperfusion injury, rheumatoid arthritis, sarcoidosis, transplant rejection, and vasculitis.

The compounds of the invention may also have utility in the treatment of hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, and cancer.

The animal being treated will typically be a human although these compounds could also be used by a vet field to treat domestic, farm or even wild animals.

As used herein, the expression "pharmaceutically acceptable salts" refers to derivatives of the disclosed compounds wherein the parent compound is modified by making acid or base salts thereof. The pharmaceutically acceptable salts include conventional non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. Any pharmaceutically acceptable salt can be used in the invention such as a rifluoroacetate, sulphate, phosphate, acetate, fumarate, maleate, citrate, pyruvate, succinate, oxalate, methanesulphonate, p-toluenesulphonate, bisulphate, benzenesulphonate, ethanesulphonate, malonate, xinafoate, ascorbate, oleate, nicotinate, saccharinate, adipate, formate, glycolate, L-lactate, D-lactate, aspartate,

malate, L-tartrate, D-tartrate, stearate, 2-furoate, 3-furoate, napadisylate (naphthalene-1,5-disulfonate or naphthalene-1-(sulfonic acid)-5-sulfonate), edisylate (ethane-1,2-disulfonate or ethane-1-(sulfonic acid)-2-sulfonate), isethionate (2-hydroxyethylsulfonate), 2-mesitylenesulphonate, 2-naphthalenesulphonate, D-mandelate or L-mandelate.

Of course the salt may well be a base salt such as ammonium salts such as tromethamine, meglumine, epolamine, etc., metal salts such as sodium, potassium, calcium, zinc or magnesium.

By treating or treatment is meant at least one of:

(i). preventing or delaying the appearance of clinical symptoms of the disease developing in a mammal;

(ii). inhibiting the disease i.e. arresting, reducing or delaying the development of the disease or a relapse thereof or at least one clinical or subclinical symptom thereof, or

(iii). relieving or attenuating one or more of the clinical or subclinical symptoms of the disease.

The benefit to a subject to be treated is either statistically significant or at least perceptible to the patient or to the physician. In general a skilled man can appreciate when "treatment" occurs. It is particularly preferred if the compounds of the invention are used therapeutically, i.e. to treat a condition which has manifested rather than prophylactically. It may be that the compounds of the invention are more effective when used therapeutically than prophylactically.

The compounds of the invention can be used on any animal subject, in particular a mammal and more particularly to a human or an animal serving as a model for a disease (e.g., mouse, monkey, etc.).

In order to treat a disease an effective amount of the active agent needs to be administered to a patient. A "therapeutically effective amount" means the amount of a compound that, when administered to an animal for treating a state, disorder or condition, is sufficient to effect such treatment. The "therapeutically effective

amount" will vary depending on the compound, the disease and its severity and the age, weight, physical condition and responsiveness of the subject to be treated and will be ultimately at the discretion of the attendant doctor.

It may be that to treat an inflammatory condition according to the invention that the compound of formula (I) has to be reapplied at certain intervals. Suitable dosage regimes can be prescribed by a physician.

While it is possible that, for use in the methods of the invention, a compound may be administered as the bulk substance, it is preferable to present the active ingredient in a pharmaceutical formulation, for example, wherein the agent is in admixture with a pharmaceutically acceptable carrier selected with regard to the intended route of administration and standard pharmaceutical practice.

The term "carrier" refers to a diluent, excipient, and/or vehicle with which an active compound is administered. The pharmaceutical compositions of the invention may contain combinations of more than one carrier. Such pharmaceutical carriers are well known in the art.. The pharmaceutical compositions may also comprise any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), and/or solubilizing agent(s) and so on. The compositions can also contain other active components, e.g. other drugs for the treatment of inflammation.

It will be appreciated that pharmaceutical compositions for use in accordance with the present invention may be in the form of oral, parenteral, transdermal, sublingual, topical, implant, nasal, or enterally administered (or other mucosally administered) suspensions, capsules or tablets, which may be formulated in conventional manner using one or more pharmaceutically acceptable carriers or excipients. The compositions of the invention could also be formulated as nanoparticle formulations.

For some inflammatory conditions, the compositions of the invention will preferably be applied topically. They may be in the form of ointments, gels, syrups, creams and so on. Any conventional topical formulation vehicle may be used.

The pharmaceutical compositions of the invention may contain from 0.01 to 99% weight of the active compound.

A therapeutically effective amount of the compound of the present invention can be determined by methods known in the art. The therapeutically effective

quantities will depend on the age and on the general physiological condition of the patient, the route of administration and the pharmaceutical formulation used. The therapeutic doses will generally be between about 10 and 2000 mg/day and preferably between about 30 and 1500 mg/day. Other ranges may be used, including, for example, 50-500 mg/day, 50-300 mg/day, 100-200 mg/day.

Administration may be once a day, twice a day, or more often, and may be decreased during a maintenance phase of the disease or disorder, e.g. once every second or third day instead of every day or twice a day. The dose and the administration frequency will depend on the clinical signs, which confirm maintenance of the remission phase, with the reduction or absence of at least one or more preferably more than one clinical signs of the acute phase known to the person skilled in the art.

The compounds of the invention may be used to treat inflammation in combination with other known pharmaceuticals for said purpose and this forms a further aspect of the invention. Other useful pharmaceuticals include corticosteroids and anti-histamines.

The invention is described further below with reference to the following non-limiting examples and figures.

Figure 1: The inflammation process showing point of action for both the compounds of the invention and most NSAIDs. Inhibition of one of the three parallel pathways late in the process gives an over-expression of the two other pathways, thus an imbalanced inhibition.

Figure 2: describes the IC_{50} value of compound **1**.

Examples

For extraction, VWR ultrasound cleaner, BÜCHI rotavapor R-200, BÜCHI rotavapor RII and isomantle of KEBO-GRAVE isopadisomantle were used. MPLC system used was Kronlab GmbH with data system Prepcon 4.47. Stationary phase used in MPLC was polygoprep 60-50 RP-18 (Macherey & Nagel) and mobile phase used was distilled water, methanol and isopropanol. For preparative and semi preparative HPLC, the system used was SEPBOX (Fa. SEPIAtec), the mobile phase

components were water, methanol and acetonitrile. Detection had been made by ELSD (Sedex 75) and UV (Merck, 250nm) detectors. For preparative HPLC Kromasil C18 250x50 mm, 10 μm was used as separation phase. For semi preparative HPLC Kromasil C18 250x16 mm, 10 μm and Nucleodur C18 Isis, 100x10; 5 μm were used as separation phases. The human synovial sarcoma cell line SW982 was purchased from ATCC (UK). Recombinant human IL-1β was purchased from Roche (UK); phosphate-buffered saline solution (PBS) was from Oxoid (UK); Dulbecco's Modified Eagle Medium (DMEM), foetal bovine serum (FBS), fatty acid-free bovine serum albumin (fBSA), gentamicin and L-glutamine were from Sigma-Aldrich (USA). Labelled (5,6,8,9,11,12,14,15-^{3}H)-AA (specific activity 180-240 Ci/mmol) and liquid scintillation cocktail OptiPhase Supermix were from NEN Perkin Elmer (USA). The ^{1}H-NMR, ^{13}C-NMR, HMBC, COSY, NOESY, HSQC and HSQC-TOCSY experiments for structure elucidation of the isolated compounds were run on a Bruker AVANCE II instrument, operating at a proton frequency of 600.18MHz, with a 5-mm triple-resonance cryo probe equipped with a z-gradient.

Sclerochloa dura (Linnaeus) P. Beauvois, known also as common hardgrass, belongs to family Poaceae Barnhart, which involves more than 700 genera and almost 50,000 species. *Sclerochloa dura* is an annual plant. It has:

flat leaves and procumbent or erect steam up to 16 cm;

inflorescence 1-4 cm, rigid, more or less second;

pedicels stout, up to 1 mm;

spikelets 6-10 mm, narrowly oblong, crowded;

glumes somewhat asymmetrical, glabrous, oblong ovate, with wide hyaline margins;

lower glume 2-3 mm, obtuse to emarginated, with (1) 3 (5) veins;

upper glume 3-5 mm, rounded to emarginated, with (5) 7-9 veins;

lowest lemma 4.8-6 mm, the upper much shorter, glabrous, rounded to emarginated, sometimes very shortly mucronate;

anthers 0.8-1.5; and

lowest rhachilla segments 2-2.5 mm, the upper shorter. 2n=14.

The plant is a common inhabitant of areas with heavy traffic, e.g. along dirty roads, on play yards and walking pathways. It is widely spread in the moderate climate zone on almost all the continents on North Hemisphere and introduced in Australia as well.

S. dura was collected in the city of Nis, Serbia, near Gabrovacka reka creek. The plant was air dried in the shade for ten days. The dry plant material was packed in plastic bags and kept in a coolish and dark place until extraction.

Extraction

Aerial plant parts were crushed into smaller pieces. Clean plant material weighing 1.4 kg was exhaustively extracted by soaking in methanol for 24 hours at room temperature twice. The extraction was aided by 90 minute of sonication. The obtained extracts were filtered (Blue ribbon filter paper from Schleicher and Schuell) and combined. The volume was reduced on rotavapour and the concentrated solution transferred into separation funnels. Extraction with n-hexane (three times) was used for defatting and the separated methanolic phase dried on rotavapour. The dry methanolic extract yielded 170g (12.14%) and was used for further work.

Bioassay guided isolation

Isolation was done by using a multistage fractionation approach by liquid chromatography. The crude extract was subjected to pre fractionation by MPLC using a double injection, 85 g per injection. The method used for MPLC is given in Table 1. The obtained pre-fractions were tested on human synovial sarcoma cell line SW982 to determine any effect on PLA2 activity by measuring AA release. Three pre-fractions found very active were subsequently fractionated by 3 respective runs on preparative HPLC into 259 fractions. The method used for preparative HPLC is given in Table 2. All the obtained fractions were tested for inhibition of PLA2 activity by measuring AA release. Based on the similarity of chromatograms and bio-activity, some of the fractions of pre-fraction F were pooled into groups, e.g.

F40-F44 as one group and F70-F74 as the other. These groups were subjected to semi-preparative HPLC runs. The method used for semi preparative runs is given in Table 3. Based on TLC, fractions G50-G51 of pre-fraction G were pooled together and subjected to semi-preparative HPLC run. The method used for semi-preparative run is given in Table 3.

TABLE 1 Method 1. Used for MPLC

MPLC system	Kronlab GmbH
Data System	Prepcon 4.47
Stationary Phase	Polygoprep 60-50 RP-18 (Macherey & Nagel)
Mobile Phase	A: distilled Water B: Methanol C: Isopropanol
Injected amounts	2 x 85 gram

Gradient	Time (min)	Flow rate (ml/min)	% A	% B	% C
Individual gradient	0.0	100	100	0	0
	5.0	100	100	0	0
	5.1	130	100	0	0
	10.0	130	100	0	0
	10.1	100	100	0	0
	18.0	100	100	0	0
	51.0	100	30	70	0
	61.0	100	10	90	0
	61.1	150	0	100	0
	66.0	150	0	100	0
	66.1	30	0	0	100
	70.0	30	0	0	100
	70.1	75	0	0	100
	74.0	75	0	0	100

Nine fractions were collected starting at 10 min, 8 min collection time each.

Table 2 Method 2. Used for preparative HPLC

HPLC	SEPBOX (Fa. SEPIAtec)

system	
Separation phase	Kromasil C18 250x50 mm,10 µm
Amounts injected	2.3638 g "E", 0.7078 g "F" and 1.0822 g "G"
Flow rate	109 ml/min
Detection	ELSD (Sedex 75), UV (Merck, 250 nm)
Mobile phase	A: water B: Methanol/Acetonitrile 1:1

Gradients	Time (min)	% A	% B
Fraction E	00.0	85	15
	57.7	58	42
	58.0	0	100
	63.0	0	100
Fraction F	00.0	77	23
	57.7	43	57
	58.0	0	100
	63.0	0	100
Fraction G	00.0	45	55
	57.7	9	91
	58.0	0	100
	63.0	0	100

A total of 80 fractions from pre-fraction E, 90 fractions from pre-fraction F and 89 fractions from pre-fraction G were obtained. All fractionations were time based: 18 to 20 second per fraction

5

Table 3 Method 3. Used for semi preparative HPLC

HPLC system	SEPBOX (Fa. SEPIAtec)
Separation phase	Kromasil C18 250x16 mm,10 µm (Group G50-G51); all other separations: Nucleodur C18 Isis, 100x10; 5 µm
Amounts injected	34.3 mg "Group F40-F44", 39.6 mg "Group F70-F74" and 45.0 mg "Group G50-G51"
Flow rate	15 ml/min (Group G50-G51); all other separations: 5 ml/min

Detection	ELSD (Sedex 75), UV (Merck, 250 nm)
Mobile phase	A: water B: Methanol/Acetonitrile 1:1

Gradients	Time (min)	% A	% B
Group F40-F44	00.0	100	0
	57.7	94	6
	58.0	0	100
	63.0	0	100
Group F70-F74	00.0	94	6
	57.7	86	14
	58.0	0	100
	63.0	0	100
Group G50-G51	00.0	37	63
	25.0	20	80
	25.1	0	100
	30.0	0	100

Five sub fractions from group F40-F44, 7 sub fractions from group F-70-74 and 2 sub fractions from group G50-G51 were obtained. All sub fractionations were time based: 18 to 20 second per sub fraction

Cell culture and treatment of SW982 cells

The human synovial sarcoma cell line SW982 is used as a model system to determine $cPLA_2$ activity by measuring AA release. SW982 cells are widely used as a model for rheumatoid synovial inflammation and express $cPLA_2$-IVa and cytokines after inflammatory stimulation by e.g., IL-1β. Cells were sub-cultured bi-weekly by routine trypsin detachment and kept in a sub-confluent state. The cells were cultured in DMEM supplemented 10% FBS, 0.1 mg/mL gentamicin and 0.3 mg/mL L-glutamine in a humidified 10% CO_2 atmosphere at 37°C. For AA release experiments, $5*10^5$ cells/well were seeded in a 48-well/plate format. At two days post-confluency, the cells were labelled with ^{3}H-AA in serum-free DMEM overnight and processed at day three post-confluence to ensure differentiation and synchronization of the cells. The experiments were performed in serum-free DMEM in triplicates of wells. Untreated cells without inducing agents or plant extract were included for unstimulated control; distilled water was added to these for vehicle

control. Following treatments, cells were routinely observed by microscopy to ensure unaltered cell morphology, integrity and viability.

AA release assay

The AA release assay determines the amount of AA released from cells under inflammatory conditions. The enzyme $cPLA_2$ is the only arachidonyl-specific PLA_2 enzyme and thus the AA release mainly corresponds to the activity of $cPLA_2$ that cleaves off AA in the sn-2 position of phospholipids. In the presence of inhibiting compound(s), the AA release is reduced, which is taken as evidence that the compound(s) target some level in the arachidonyl cascade, such as the $cPLA_2$ enzyme.

At two days post-confluency, SW982 cells were serum-starved and labelled overnight with ^{3}H-AA (0.4 µCi/ml). Prior to the addition of S. dura fractions, the cells were washed with PBS containing fBSA (2 mg/ml) to remove unincorporated radioactivity. Cells were pre-treated for 1 h with various dilutions of fractions of the plant extract, followed by addition of IL-1β (10 ng/mL) to induce an inflammatory state. Following 24 h of IL-1β stimulation, the supernatants were cleared of detached cells by centrifugation (13000 rpm, 10 min). Adherent cells were dissolved in 1M NaOH in order to measure the amount of labelled AA incorporated into the cells. The activity in the supernatants and cellular fractions was assessed by liquid scintillation counting in LS 6500 Multi-Purpose Scintillation Counter (Beckman Coulter, Inc USA). The results are given as released ^{3}H-AA in the supernatants relative to total ^{3}H-AA incorporated into the cells. Inhibition was calculated as AA release in inhibited relative to stimulated cells, normalized to control, subtracted by 100.

By means of MPLC, the crude extract was separated into 9 pre fractions (A-I), which were tested for inhibitory effects in AA-release assay. Out of these, E, F and G were found to exert the highest inhibitory effect, as shown in Table 4.

The results of inhibition activity of the three most active pre-fractions E, F and G are shown in the Table 4.

Table 4. Comparison of activity of fractions E, F and G. Experiments were done in triplicates. Inhibition and IC_{50} values are based on two independent experiments. IC_{50} could not be calculated for fraction F as inhibition did not go below 50 % in any concentration tested.

Fraction	Concentration (mg/ml)	% Inhibition (mean ± S.D.)	IC_{50}
E	0.10	63.1 ± 18.4	0.10
F	0.10	68.3 ± 4.8	
G	0.08	63.5 ± 25.5	0.60

By the bioactivity guided study, from pre fraction F and G a total of six compounds with pronounced anti-inflammatory activity were identified.

Compound 1

Compound **1** was obtained by semi-preparative HPLC run of group G50-G51 (Table 3). The analysis of spectral data revealed that compound **1** is a novel compound. HRMS pointed out to a molecular formula $C_{27}H_{46}O_{11}S$ (ESI -) with a molecular mass 578.2751 (theoretical value 578.2761). The analysis of 1D and 2D NMR spectra with homo- and hetero- nuclear direct or long range correlations allowed structure elucidation and a complete ^{1}H and ^{13}C assignment, as listed in Table 5. The carbons 1-6, belonging to glucose moiety, had shift values for ^{13}C ranging from δ 54.4 to 100.3 ppm and for protons from δ 2.92 to 4.77 ppm. The shift values of both ^{13}C and protons at C-6 indicated a direct attachment to an SO_3H group, which was also confirmed by MS analysis. The 18 C-atoms with shift values ranging from δ 14.8 to 176.2 ppm with attached protons from δ 0.98 to 5.37 ppm belong to linolenoyl chain of the molecule. An HMBC correlation peak of H-3 (at δ 3.65 ppm) of glucose and carbon C-1"(δ 176.2 ppm) of the linolenoyl chain revealed the attachment position. Glycerol moiety exhibited shift values for carbons ranging from δ 64.4 to 72.7 ppm and protons from δ 3.36 to 4.05 ppm. The correlation peaks between carbon C-1 of glucose and protons $H_{a,b}$-1' of glycerol in HMBC unambiguously revealed connectivity between glucose and glycerol. Both the molecular formula and NMR data indicated that **1** was a derivative of glycerol with the chemical name 1-*O*-(3-*O*-linolenoyl-6-deoxy-6-sulfo-α-D-glucopyranosyl)-glycerol.

Compound 2

Compound **2** was identified as isovitexin. It was isolated as the main compound in fraction F-07 obtained by preparative HPLC run of pre fraction F (Table 2). HRMS (ESI +) was 432.1055 which corresponded to molecular formula $C_{21}H_{20}O_{10}$, (theoretical value 432.1056). The compound was identified by a series of 1D and 2D NMR experiments. The signal assignments for carbons and protons can be seen in Table 5. The NMR data were in agreement with those already published (Leong et al., 2010).

Compound 3

Compound **3** was isolated as the main compound in fraction F-51. This fraction was obtained by preparative HPLC run of pre fraction F (Table 2). Compound **3** was identified as tricin 4'-*O*-(erythro-β-guaiacylglyceryl) ether 7-*O*-β-glucopyranoside. HRMS (ESI +) found was 688.2003 (theoretical value 688.2003) and it corresponded to the molecular formula $C_{33}H_{36}O_{16}$. The NMR data of the compound (Table 5) fits the published ones (Bouaziz et al., 2002).

Compound 4

Semi preparative HPLC run of group F-40-F44 (Table 3) yielded a fraction containing **4** as the main compound. HRMS (ESI +) for **4** was 372.2147 compared to the theoretical value of 372.2148 and matches the molecular formula $C_{19}H_{32}O_7$. Compound **4** was identified as byzantionoside B. The NMR data (Table 5) are in agreement with those published earlier (Matsunami et al., 2010).

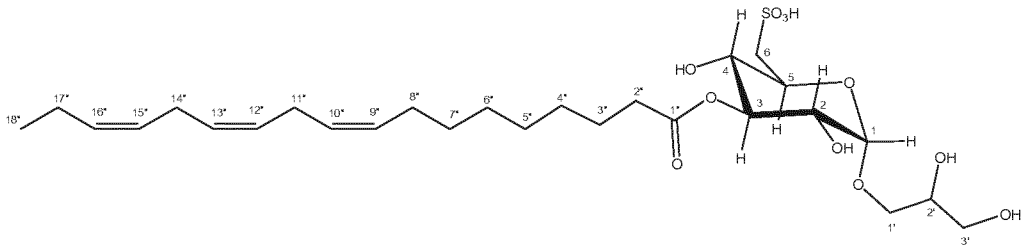
Compound 5

The semi preparative HPLC run of group F-70-F74 (Table 3) gave **5** as the main compound in the resulted sub fractions. It was identified as 1-*O*-feruloyl glycerol. HRMS (ESI +) was found to be 268.0952 (corresponds to molecular formula $C_{13}H_{16}O_6$), with theoretical value 268.0947. . The NMR resonances of ^{1}H and ^{13}C for **5** are in agreement with those reported previously (Table 5) (Luo et al., 2012).

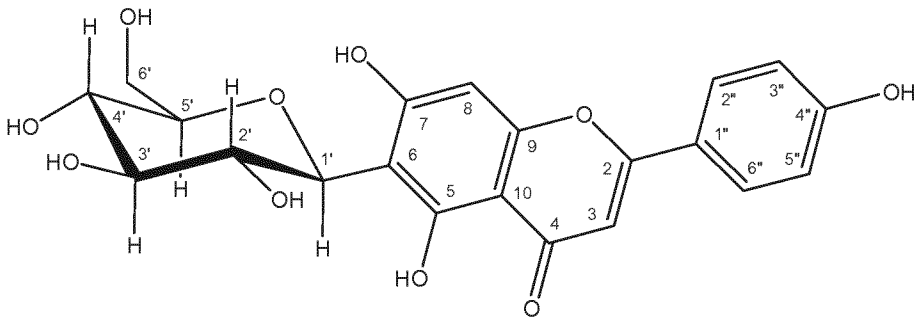
Compound 6

Compound **6** was the main compound in fraction F-32, obtained by preparative HPLC run of pre fraction F (Table 2). It was identified as tricin 7-glucoside. HRMS (ESI +) 492.1268 for $C_{23}H_{24}O_{12}$, found 492.1263. The NMR data of **6** (Table 5) fits to those reported earlier (Awaad et al., 2008).

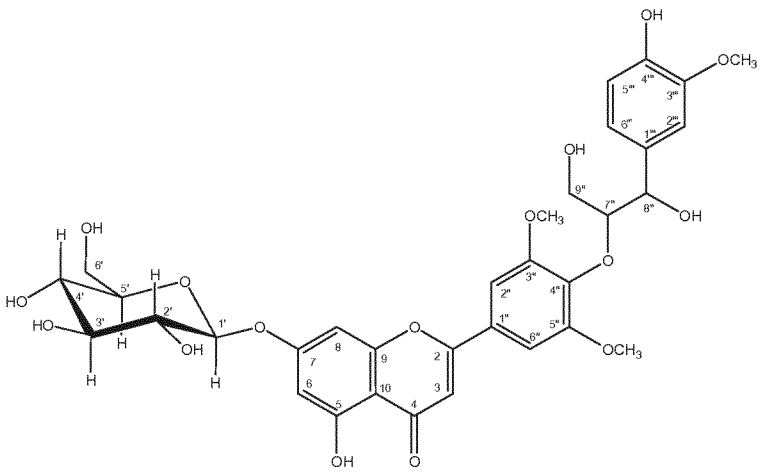
The structures of compounds 1 to 6 are shown below:



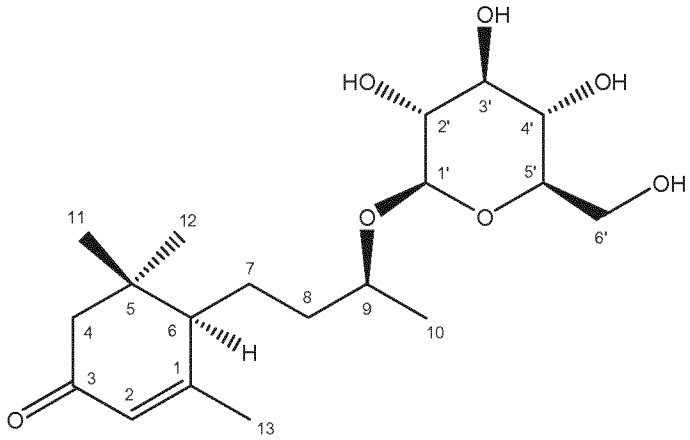
1 = (formula X)



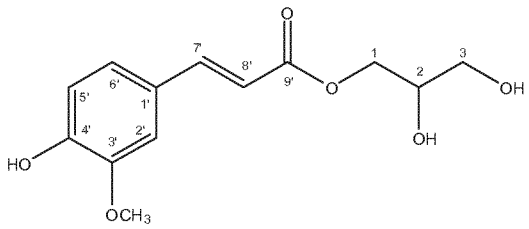
2 = formula (XV)



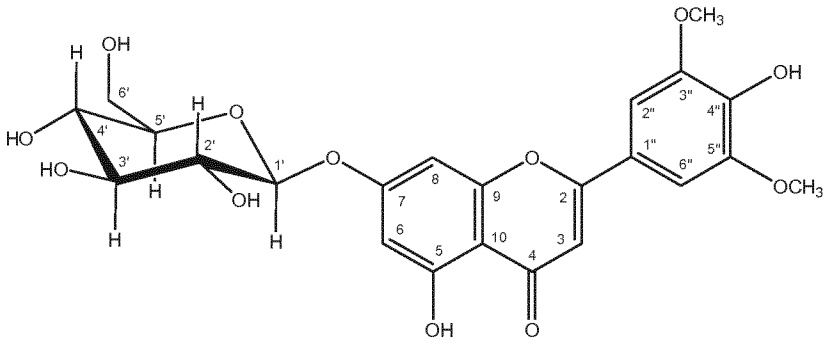
3 = **formula (XIV)**



4 = formula (XVII)



5 = formula (XVI)



6 = formula (XIII)

The structures of identified compounds from methanolic extract of *S. dura*. Compound **1**: 1-*O*-(3-*O*-linolenoyl-6-deoxy-6-sulfo-α-D-glucopyranosyl)-glycerol; Compound **2**: Isovitexin; Compound **3**: Tricin 4'-*O*-(erythro-β-guaiacylglyceryl) ether 7-*O*-β-glucopyranoside; Compound **4**: Byzantionoside B; Compound **5**: 1-*O*-feruloyl glycerol; Compound **6**: Tricin 7-glucoside.

Table 5. Assignment of ^{1}H and ^{13}C resonances of compounds **1-6**.

	1(MeOD)		2 (MeOD)		3(MeOD)	
C	δ C (ppm)	δ H (ppm)	δ C (ppm)	δ H (ppm)	δ C (ppm)	δ H (ppm)
1	100.3	4.77, d, 3.7Hz	-	-	-	-
2	73.9	3.41, dd, 3.7/9.6Hz	166.0	-	165.9	-
3	75.3	3.65, m	103.6	6.53, s	106.0	6.77, m
4	75.1	3.08, dd, 8.8/9.6Hz	183.7	-	184.0	-
5	70.0	4.10, m	162.2	-	163.1	-
6	54.4	2.92,dd, 9.3/14.3Hz; 3.36, m	109.8	-	101.1	6.50, s
7	-	-	167.8	-	164.8	-
8	-	-	96.0	6.42, s	96.1	6.87, s
9	-	-	159.1	-	158.7	-
10	-	-	104.5	-	107.1	-
1'	71.0	3.36, m 4.05, m	75.5	4.90 (overlapped by solvent)	101.7	5.08, d, 6.6 Hz
Table 5. Continuation						
2'	72.7	3.89, m	72.6	4.24, tr, 9.2 Hz	77.8	3.51, m
3'	64.4	3.59, m	80.4	3.48, m	74.7	3.51, m
4'	-	-	71.9	3.48, m	71.2	3.40, m
5'	-	-	82.7	3.42, m	78.4	3.55, m
6'	-	-	63.0	3.74, dd, 5.3/12.2 Hz; 3.87, dd, 2.2/12.2 Hz	62.2	3.68, m
1"	176.2	-	123.1	-	127.5	-
2"	34.9	2.31, t, 7.5Hz	129.5	7.80, d 8.8 Hz	105.1	7.23, s
3"	26.2	1.60, m	117.0	6.90, d 8.8 Hz	154.8	-
4"	30.3	1.33, m	163.3	-	140.6	-
5"	30.4[a]	1.33, m	117.0	6.90, d 8.8 Hz	154.8	-
6"	30.5[a]	1.33, m	129.5	7.80, d 8.8 Hz	105.1	7.23, s
7"	30.6[a]	1.33, m	-	-	87.4	4.46, m
8"	28.4	2.07, m	-	-	74.1	4.92, m
9"	131.3	5.37, m	-	-	62.2	3.70, m 3.94, m
10"	128.4[b]	5.29, m	-	-	-	-
11"	26.5	2.81, m	-	-	-	-
12"	129.3[c]	5.34, m	-	-	-	-
13"	129.4[c]	5.34, m	-	-	-	-
14"	26.5	2.81, m	-	-	-	-
15"	128.9[b]	5.31, m	-	-	-	-
16"	132.9	5.37, m	-	-	-	-
17"	21.6	2.09, m	-	-	-	-

18"	14.8	0.98, t 7.5 Hz	-	-	-	-
1"'	-	-	-	-	133.4	-
2"'	-	-	-	-	111.5	7.00, m
3"'	-	-	-	-	148.6	-
4"'	-	-	-	-	146.8	-
5"'	-	-	-	-	115.6	6.73, m
6"'	-	-	-	-	120.5	6.81, m
O Me						
3", 5"	-	-			57.0	3.92
3"'					56.5	3.83

	4(MeOD)		5(MeOD)		6 (DMSO)	
C	δ C (ppm)	δ H (ppm)	δ C (ppm)	δ H (ppm)	δ C (ppm)	δ H (ppm)
1	170.1	-	66.3	4.17, m 4.26, m	-	-
2	125.2	5.81, s	71.1	3.88, m	164.5	-
3	202.1		63.9	3.59, m	102.1	6.94, s
Table 5. Continuation						
4	48.2	1.96, m, α 2.47, d, 17.2Hz, β	-	-	181.6	-
5	37.1	-	-	-	161.0	-
6	52.2	1.99, m, β	-	-	99.3	6.43, d, 1.8 Hz
7	27.0	1.50, m 1.97, m	-	-	162.8	-
8	37.5	1.62, m 1.68, m	-	-	95.1	6.89, d, 1.8 Hz
9	75.6	3.88, m	-	-	156.8	-
10	20.0	1.18, d, 6.2 Hz	-	-	105.2	-
11	29.2	1.01, s, β	-	-		
12	27.6	1.09, s, α	-	-		
13	25.1	2.05, s	-	-		
1'	102.3	4.32, d 7.8Hz	126.1	-	100.1	5.04, d, 7.5 Hz
2'	75.3	3.14, dd 7.8/9.2H	111.4	7.20, m	73.1	3.27, m
3'	78.3	3.35, m	148.8	-	76.5	3.31, m
4'	72.0	3.27, m	145.8	-	69.6	3.16, t, 8.9 Hz
5'	78.0	3.25, m	116.5	6.81, m	77.3	3.45, m
6'	63.0	3.65, m 3.86, m	123.9	7.08, m	60.6	3.46, m 3.72, m
1"	-	-	146.8	7.66, m	123.6[§]	-
2"	-	-	115.0	6.39, m	104.9	7.30
3"	-	-	-	-	148.8	-
4"	-	-	-	-	135.8[†]	-
5"	-	-	-	-	148.8	-

6"	-	-	-	-		104.9	7.30
OMe							
3'			56.3	3.90			
3", 5"						56.2	3.84

[a,b...] Assignments marked with the same letter are interchangeble

[§, †] Tentative assignments

Comparison of bioactivity of the identified compounds from *S. dura*

The respective fractions containing each of these compounds were again tested for bioactivity, confirming inhibitory effects on PLA_2 enzyme activity. IC_{50} values of inhibition were estimated to be 0.11 mg/ml both for compound **1** and **3** (Table 6). The identified compounds from *S. dura* inhibited IL-1 induced release of AA in SW982 cells in this order of decreasing efficiency:

Compound **5** > Compound **4** > Compound **1** and **3** > Compound **6** > Compound **2**.

Table 6. Comparison of activity of compounds **1 - 6** based on experiments in triplicates. Inhibition and IC_{50} values are based on at least three independent experiments, unless otherwise mentioned.
*Based on one experiment **Based on two independent experiments

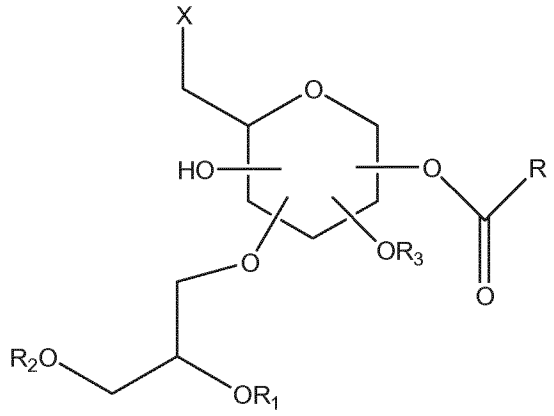
Compound	Concentration (mg/ml)	% Inhibition (mean ± S.D.)	IC_{50}
1	0.15	92.8 ± 46.52	0.11
2	0.30	26.8 ± 35.60**	--
3	0.15	67.3 ± 7.10	0.11
4	0.12	96.9*	--
5	0.09	99.5*	--
6	0.30	54.9± 41.40	--

The graph showing IC_{50} value of compound **1** is given in Figure 1.

The mole percentage of compound **1** in the active fraction was estimated to be 60%, based on integral ratio in ^{1}H-NMR spectrum. Therefore, the IC_{50} value of **1** from three independent experiments corresponds to 114.1 ± 31.1 µM. The obtained IC_{50} value shows that **1** has the potential to be used as a lead compound for the development of anti-inflammatory drugs. Furthermore, the spectroscopic study of **1** shows unambiguous presence of an $-SO_3H$ group. The presence of sulfonyl group in **1** might be important for its bioactivity.

Claims

1. A compound of formula (I)



(I)

wherein R is a C9-23 polyunsaturated alkenyl group;

X is SO_3R_3, SO_2R_3, PO_3R_3, PO_2R_3, $COCHal_3$, or $COOR_3$;

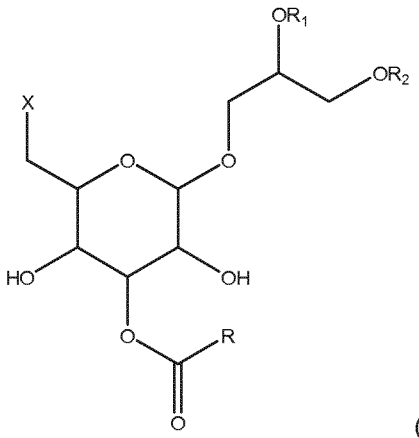
R_1 and R_2 are each independently H, a C1-6 alkyl group optionally substituted with OH, C6-10 aryl group optionally substituted with OH, C7-12 arylalkyl group optionally substituted with OH, COC1-6 alkyl group optionally substituted with OH, COC6-10 aryl group optionally substituted with OH, COC7-12 arylalkyl group optionally substituted with OH, or $COCHal_3$ group;

hal is halide, preferably F;

each R_3 is independently H, C1-6 alkyl, C6-10 aryl, or C7-12arylalkyl;

or a salt thereof.

2. A compound as claimed in any preceding claim being a compound of formula (V)

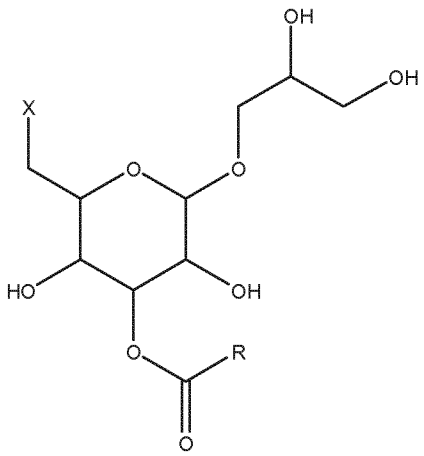


(V)

where X, R, and R_1-R_3 are as hereinbefore defined;

or a salt thereof.

3. A compound as claimed in any preceding claim being a compound of formula (VI)



(VI)

where X and R, are as hereinbefore defined;

or a salt thereof.

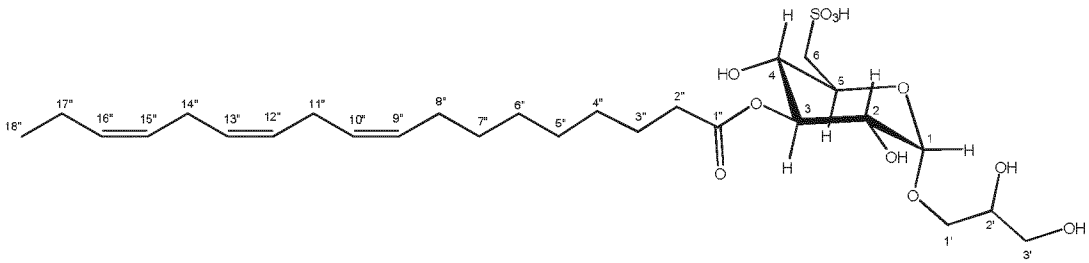
4. A compound as claimed in any preceding claim wherein X is SO_3H

5. A compound as claimed in any preceding claim wherein -COR is linoleic acid derivative.

6. A compound as claimed in any preceding claim wherein the hexose is 6-deoxyglucose.

7. A compound as claimed in any preceding claim wherein the hexose is alpha 6-deoxyglucopyransoyl.

8. The compound:



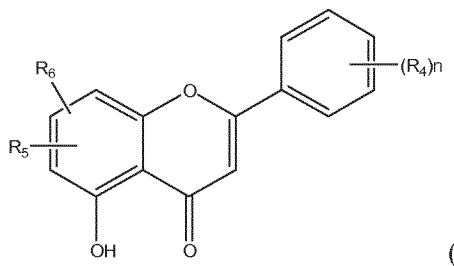
or a salt thereof.

9. A pharmaceutical composition comprising a compound of formula (I) as hereinbefore defined and at least one carrier or excipient.

10. A compound of formula (I) for use as a medicament.

11. A compound of formula (I) for use in the treatment of inflammation, e.g. via the inhibition of cPLA2.

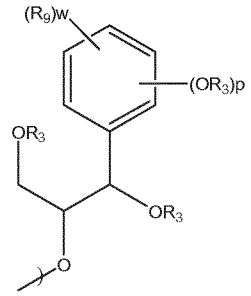
12. A compound of formula (II)



(II)

wherein

each R_4 is H, OH, OR_3, C1-6alkyl, C6-10 aryl, C7-12 arylalkyl or the group:



wherein each R_3 is as hereinbefore defined;

p is 0-3;

n is 1 to 3;

w is 0 to 2;

R_9 is C_{1-6} alkyl;

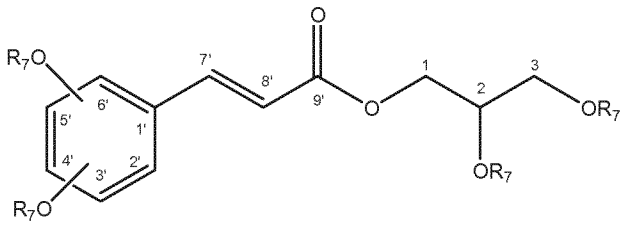
R_5 is a monosaccharide linked to the phenyl ring via a C-C bond or linked via a C-O bond at the 1-position of the saccharide, and

R_6 is H, OH, C1-6 alkyl or OR_3;

or a salt thereof;

for use in the treatment of inflammation.

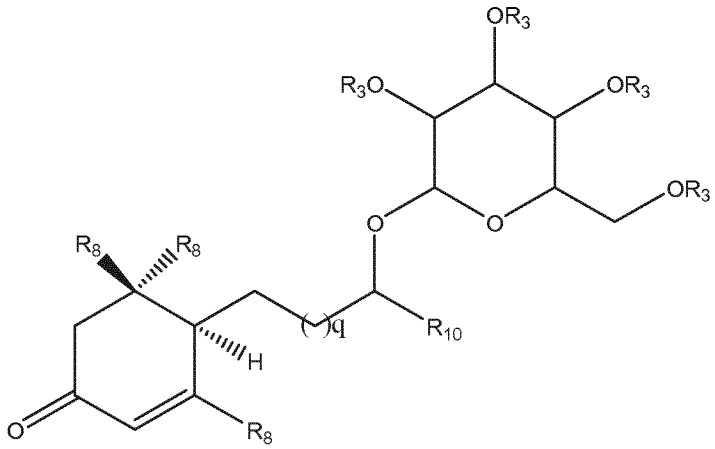
13. A compound of formula (III)



(III)

wherein R_7 is H or C1-6 alkyl; or salt thereof for use in the treatment of inflammation.

14. A compound of formula (IV)



(IV)

wherein each R_8 is H or a C1-6-alkyl;

R_{10} is H or C1-6 alkyl;

q is 0 to 3, such as 1 or 2;

each R_3 is as hereinbefore defined with at least one OR_3 group being OH; or a salt thereof; for use in the treatment of inflammation.

15. A compound of formula (I) to (IV) as claimed in claim 1 to 14 for use as an inhibitor of $cPLA_2$.

16. A compound of formula (I) to (IV) as claimed in claim 1 to 14 for use in the treatment of a chronic inflammatory disease such as acne vulgaris, asthma, autoimmune diseases, celiac disease, chronic prostatitis, glomerulonephritis, hypersensitivities, inflammatory bowel diseases, pelvic inflammatory disease, reperfusion injury, rheumatoid arthritis, sarcoidosis, transplant rejection, and vasculitis.

17. A compound of formula (I) to (IV) as claimed in claim 1 to 14 for use in the treatment of hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, and cancer.

18. Use of a compound of formula (I) to (IV) as claimed in claim 1 to 14 in the manufacture of a medicament for the treatment of inflammation.

19. A method of treating inflammation comprising administering to a patient in need thereof, such as a human, a therapeutically effective amount of a compound of formula (I) to (IV) as claimed in claim 1 to 14.

5

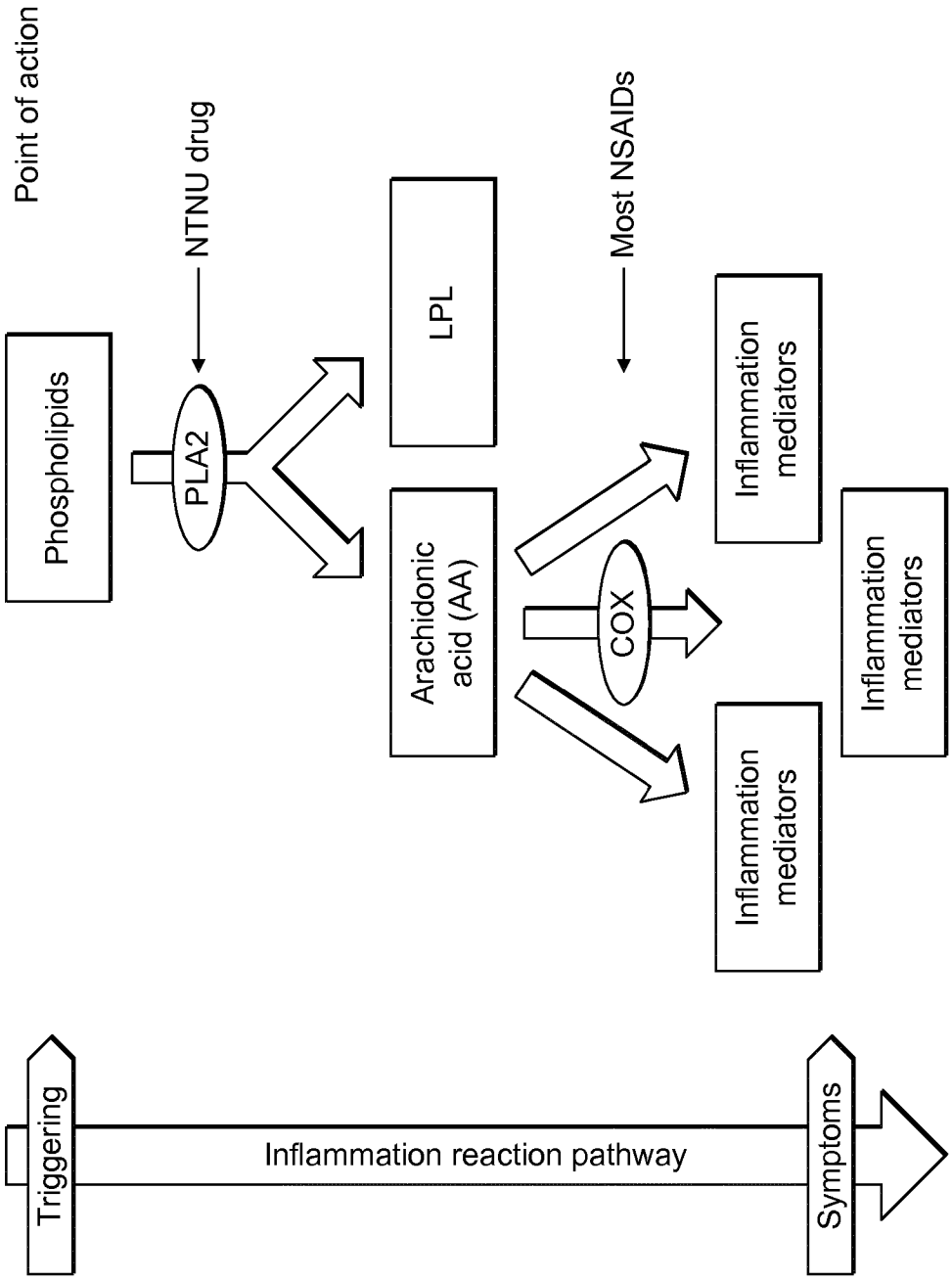
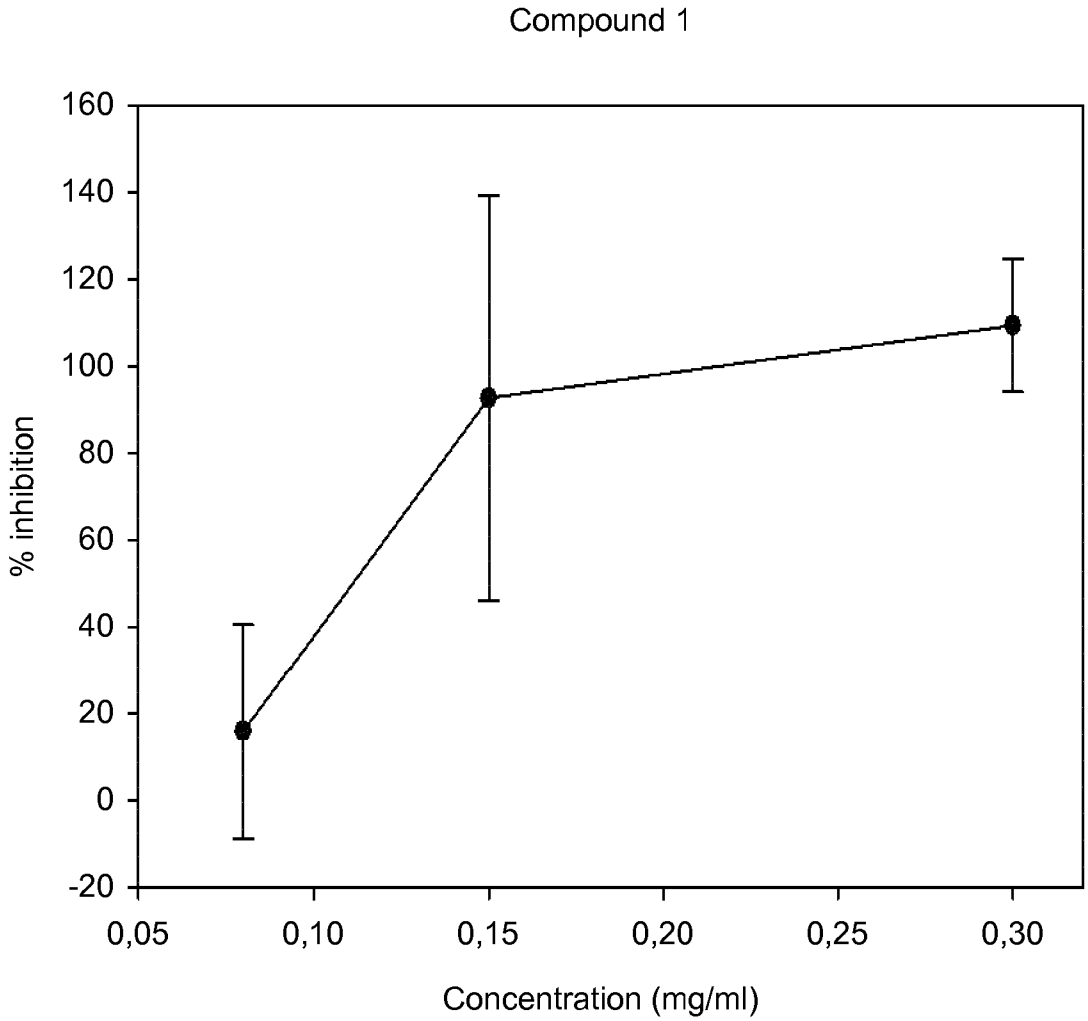

Point of action
NTNU drug
Most NSAIDs
Phospholipids
PLA2
LPL
Arachidonic acid (AA)
COX
Inflammation mediators
Inflammation mediators
Inflammation mediators
Triggering
Inflammation reaction pathway
Symptoms

FIG. 1



The inhibitory activity of compound 1. Activity on AA release is dose-dependent. Results are based on three independent experiments, except for 0.3 mg/ml (two experiments)

FIG. 2