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(71) Applicant *(for all designated States except US)*: **PLIVA-ISTRAZIVACKI INSTITUT D.O.O.** [HR/HR]; Prilaz baruna Filopovica 29, 10000 Zagreb (HR).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **MERCEP, Mladen** [HR/HR]; Majstora Radonje 10, 10000 Zagreb (HR). **MESIC, Milan** [HR/HR]; Slavenskog 8, 10000 Zagreb (HR). **BOSKA, Hrvacic** [HR/HR]; Medvescak 72, 10000 Zagreb (HR). **IVAYLO, Jivkov, Elenkov** [HR/HR]; Ulica grada Chicaga 21, 10000 Zagreb (HR). **IVICA, Malnar** [HR/HR]; Snjeznicka 40, 51304 Gerovo (HR).

(74) Common Representative: **PLIVA-ISTRAZIVACKI INSTITUT D.O.O.**; Pravni Patentni Poslovi, Prilaz baruna Filopovica 29, 10000 Zagreb (HR).

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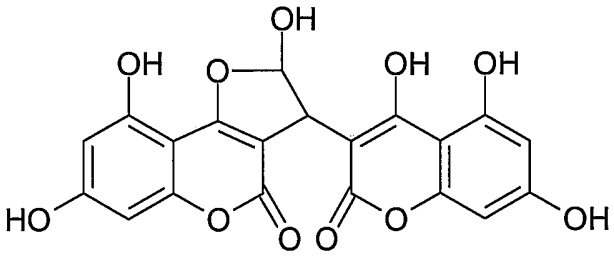


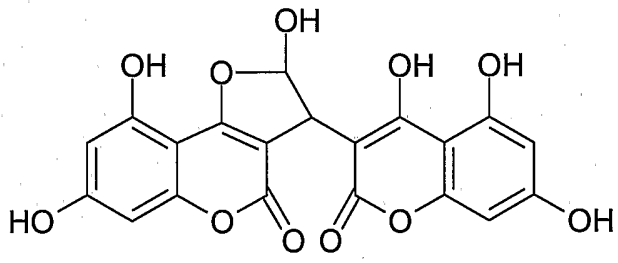
(I)

(57) Abstract: This invention relates to the novel compound of formula (I): and pharmaceutically acceptable salts and solvates thereof; to the processes and reactive intermediates for the preparation of this compound, pharmaceutical compositions which contain this compound, and the use of this compound in prophylaxis and therapeutic treatment of asthma as well as other diseases, disorders or conditions of human immune system.

FUROCHROMENE DERIVATIVE WITH ANTI-INFLAMMATORY ACTIVITY

Field of the Invention

The present invention relates to novel compound of the formula I,



(I)

and pharmaceutically acceptable salts and solvates thereof; to processes and reactive intermediates for the preparation of this compound, pharmaceutical compositions which contain this compound, and the use of this compound in prophylaxis and therapeutic treatment of asthma as well as other diseases, disorders or conditions of the human immune system.

Prior Art

Asthma is a chronic inflammatory disease of respiratory tract in humans, characterized by airway obstruction, edema, mucus secretion, and increased bronchial responsiveness to specific and non-specific challenges. Clinically, in oversensitive persons inflammation causes periodic attacks of cough, heavy breathing, piping, tightening and pain in the chest. Asthma can be triggered by allergens, chemical irritants, tobacco smoke, cold air, viral infections and/or exercise.

Pathogenesis of asthma is complex and comprises interaction of inflammatory cells, mediators, tissues and cells of the respiratory system. In the asthmatic process there are two phases of response, known as early and late phase. Immediately after antigen inhalation (dust mite, pollen, cockroach, cat allergen), airway mast cells are triggered by binding of antigen to the IgE receptor on their surface, they rapidly release mediators of inflammation stored in their granules (such as histamine, leukotrienes, prostaglandines) and start production of IL-4 and IL-5. This cascade of events results in vasodilatation, acute bronchospasm, and edema as an early phase response. The late response comprises permanent obstruction of the airways, bronchial hyperresponsiveness, and development of other inflammation processes beginning with influx of cells such as neutrophiles, eosinophils, T lymphocytes and macrophages in the respiratory tract. This accumulation of inflammatory cells is the result of concerted interaction of lymphokines (TNF-α, IL-4, IL-5), adhesion molecules on the surface of leucocytes (integrines) and endothelial cells (selectines), as well as chemokines (eotaxin, RANTES). The role and importance of T lymphocytes in asthma are suggested by the increased number of activated $CD4^+$ T-cells, found in bronchoalveolar lavage (BAL) fluid and bronchial biopsy specimens from asthmatic patients. Two subpopulations of $CD4^+$ T cells have been described, based on the profile of cytokines that they produce. Th1 cells produce IL-2, IL-3, GM-CSF, INF-γ. Activation of Th1 cells is important in defence of the host against intracellular organisms, viruses and neoplasms. However, Th2 type of response is suggested to be the most important in asthma, with elevated expression of IL-5, which is crucial in inception of eosinophil infiltration typical for "allergic" inflammation.

Morphological changes that occur in asthma comprise infiltration of bronchi by inflammatory cells (mast cells, T-lymphocytes and eosinophils are the key executive cells), airway obstruction by mucus secretion, intersticial edema, and increased permeability of microcirculation. On the basis of pathohistological

findings it was evidently recognized that eosinophilic infiltration is specific, which differentiates asthma from other types of inflammation.

Current therapy of asthma includes two types of medications, symptomatic and basic. Symptomatic drugs include short acting bronchodilatators, such as selective β2-agonists, anticholinergics, xanthine derivatives, which rapidly relax constricted airways and relieve acute symptoms. Basic drugs include antiinflammatories and long acting bronchodilatators. Antiinflammatory drugs relieve and prevent inflammattion reaction that causes the asthma attack. This type of drugs consists of inhaled (topical) and systemic corticosteroids, inhalations of sodium chromoglycate and nedocromil sodium.

Steroid antiinflammatory drugs are still recognized as most effective and most widely used ones in the treatment of inflammatory diseases and conditions like asthma.

In addition to their excellent potency and effectiveness, medicaments of this type also possess numerous unfavourable side-effects, for example on carbohydrate metabolism, calcium resorption, secretion of endogenous corticosteroids as well as on the physiological functions of the pituitary gland, adrenal cortex and thymus. Patent applications WO 94/13690, WO 94/14834, WO 92/13873 and WO 92/13872 describe the so-called "soft" steroids or hydrolysable corticosteroids designed for topical application on the inflammation site, whereas their systemic side-effects are diminished due to instability of "soft" steroids in the serum, wherein the active steroid very rapidly hydrolyses into the inactive form. An ideal steroid, however, without unfavourable effects in a long-term and continuous treatment as required for the control of diseases such as asthma has yet to be found.

Some coumarin-class compounds (US 4,200,577; US 4,263,299; US 4,731,375; US 5,428,038) exhibit antiallergic effect in prevention and treatment of various allergic diseases, such as allergic asthma, allergic dermatitis, allergic rhinitis or enteritis, allergic conjunctivitis or allergic eczema.

More complex dimeric and tetrameric derivatives of 4-hydroxycoumarin asymetricaly connected with the central linker, which possess anti-HIV activity, are also known (Zhao, H. et al. *J. Med. Chem.* **1997**, *40*, 242-249). In addition, similar anti-HIV activity show numerous condensation products of hydroxycoumarins, which bear more than one hydroxy group per coumarin unit, with aromatic or aliphatic mono- or dialdehydes (U.S. 6,100,409; WO 03/029237 and EP 0906909).

In our recent patent applications (WO 2005/010006), hydroxycoumarin derivatives with various substituents on the coumarin part of the molecule have been described. It is believed that these compounds having inhibitory effect on degranulations of mast cells and which also reduce airway hyperresponsiveness, may be useful in prophylaxis and therapeutic treatment of asthma, as well as other inflammatory diseases and conditions.

Technical Solution

Hitherto, 3-(4,7-dihydroxy-2-oxo-2*H*-chromen-3-yl)-7-hydroxy-2,3-dihydro-furo[3,2-*c*]chromen-4-ones with methoxy or ethoxy substituted C2 position of furan ring and structurally akin to the compound of the present invention have been described in the literature (WO 03/029237). Those compounds are obtained by condensation of corresponding hydroxycoumarins and glyoxal carried in alcohol-water media at high temperatures. During the reaction alcohol is simultaneously attached with the formation of adequate substituent.

If the condensation reaction of hydroxycoumarin and glyoxal is carried out at lower temperatures in non-alcoholic media, undesired formation of alkoxy substituent at C2 position of the furan ring is prevented and hydroxy substituent is formed at the same position as an essential constitutive component of the compound of the formula I.

According to our knowledge and established prior art, however, neither compound represented by the formula I possessing hydroxy group at C2

position of the furan ring along with additional five hydroxy groups at coumarin part of the molecule, its pharmaceutically acceptable salts nor pharmaceutical compositions containing such a compound have been described so far. It is also not known either that the compound of the present invention has been depicted as substance of strong antiinflammatory activity and as effective remedy in prophylaxis and therapeutic treatment of asthma as well as other inflammatory diseases, disorders or conditions.

Applied *in vivo* models properly illustrate patophysiological incidents present in asthma and other inflammatory processes, and it is believed that compound which is effective in these models may be also useful therapeutic agent in the treatment of human body.

Summary of the Invention

Subjects of present invention are:

a) Compound of the formula (I);

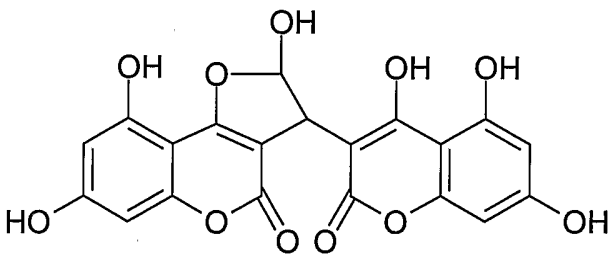
b) Processes and reactive intermediates for the preparation thereof;

c) Composition of the obtained compound in sufficient amounts for keeping down inflammatory processes or conditions;

d) Methods of usage of the prepared compound in the treatment of diseases, disorders or conditions caused by inflammatory processes.

Detailed Description of the Invention

The present invention relates primarily to the compound of the formula I.



(I)

A further object of the present invention relates to pharmaceutically acceptable salts of the compounds of the formula (I). The compounds representing an object of the present invention comprise at least one acidic hydroxy group on the coumarin nucleus and thus can form salts with pharmaceutically acceptable bases. Examples of such salts formed on hydroxy substituent are e.g. aluminum salts, corresponding salts of alkali metals such as sodium or potassium, salts of earth alkali metals such as calcium or magnesium, pharmaceutically acceptable salts of transient metals such as zinc and copper, salts with ammonia or salts with lower organic amines such as cyclic amines, mono-, di- or trisubstituted lower alkyl amines, further lower hydroxyalkyl amines such as lower mono-, di- or trihydroxyalkyl amines, lower (hydroxyalkyl)alkyl amines or lower polyhydroxyalkyl amines and salts with amino acids. Examples of cyclic amines are morpholine, thiomorpholine, piperidine or pyrrolidine. Suitable lower monoalkylamines are e.g. ethylamine and *tert*-butylamine, suitable lower dialkyamines are e.g. diethylamine and diisopropylamine, and suitable lower trialkylamines are e.g. trimethylamine and triethylamine. Corresponding lower hydroxylalkyamines are e.g. mono-, di- and triethanolamine, lower (hydroxyalkyl)alkyamines are e.g. *N,N*-dimethylaminoethanol and *N,N*-diethylaminoethanol. Amino acids are e.g. lysine, arginine, methylglutamine, alanine or serine. These salts can be prepared *in situ* during the final isolation and purification of the compounds of the present invention or separately in a reaction with a suitable inorganic or organic base in a manner known to the one skilled in the art.

In the context of the present invention, the general terms used preferably have the following meanings:

The prefix "lower" designates a radical having up to and including 7, and in particular up to and including 4, carbon atoms. Lower alkyl is, for example, *n*-

propyl, isopropyl, *n*-butyl, isobutyl, *sec*-butyl, *tert*-butyl, *n*-pentyl, neopentyl, *n*-hexyl or *n*-heptyl, preferably ethyl or methyl.

In view of the close connection between free forms and salt forms of the compounds represented by the formula (I), it should be understood that in the present invention the free forms of compounds represented by the formula (I) and their salts are identical forms and in the corresponding context it is suitable to consider the free forms of the compounds of the present invention and their corresponding salts as synonymous.

Compound of the formula I and its salts may also exist in the form of solvates, for example hydrates, and the present invention includes each solvate and mixtures thereof.

Compound of the formula I and its salts may exist in more than one physical form (for example different crystal forms) and the present invention includes each physical form (for example each crystal form) of compound of the formula I and mixtures thereof.

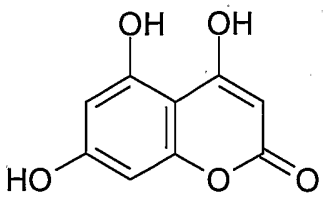
The present invention further includes all prodrugs forms of the compound of the formula I, *i.e.* compounds which after *in vivo* application in mammals release the active compound of the formula I. Prodrug forms may be prepared by modification of any functional group present in the compound of the formula I in a way that such modified functional group may be easily disconnected *in vivo*, with simultaneous release of the starting active compound. Hydroxy functional groups are suitable positions for preparation of the prodrug form of the compound of the formula I.

Compound of the present invention may exist in different isomeric forms, such as different tautomeric forms or as different geometric isomers, such as conformational isomers, and since it contains two chiral centers, it may also exist in different optically active forms, *i.e.* as different stereoisomers. Isomers that have their atoms connected in the same order but have different three-dimensional arrangements around asymmetric center (stereogenic center) are called stereoisomers. Stereoisomers that are not mirror images of each other are

called diastereomers, while stereoisomers that have a mirror-image relationship, *i.e.* that are mirror images of each other are called enantiomers. Each stereoisomer may be characterized by determining absolute configuration around stereogenic center, specified verbally by *R,S* convention using Cahn-Ingold-Prelog sequence rules. Next method for determination of stereoisomers is to measuring rotation of the plane of polarization of plane-polarized light that passes through the molecule, and designating chiral molecules to be dextrorotatory (+) or levorotatory (-) isomers. Chiral molecules may exist in a form of single enantiomer or in a mixture of enantiomers. A mixture consisting of equal parts (+) and (-) enantiomers of a chiral substance is called racemic mixture. The present invention relates to each stereoisomer that may be shown by the formula I, either isolated as separate enantiomers, diastereomers or existing in racemic or any other mixture thereof. Methods for determination of stereochemical configuration, resolution and separation of stereoisomers are well known from the literature.

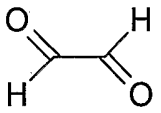
Compound of the formula I may exist in a number forms of structural isomers that may be formed as a result of tautomerism, and may exist in different ratios at equilibrium. Due to dynamic equilibrium such isomers (tautomers) are rapidly interconvertible from one isomeric form to another. The most common isomerism is keto-enol tautomerism, but equilibrium between open chain and cyclic forms are also known. The isomeric forms predominant for a particular compound of formula I are dependent on the nature of the substituent, whether the compound exists in the free form or in the form of any of its salts, type of the salt, solvent in which the compound is dissolved, as well as pH value of the solution. It is to be understood that whenever in the present invention we refer to the compound of formula I we mean to include tautomeric forms thereof, keto-enol tautomeric, open chain-cyclic, isolated as separate isomers or existing in any other mixture of different ratios at equilibrium.

Further aspect of the present invention relates to the processes for the preparation of compound of formula I, and salts thereof by condensation of hydroxycoumarin of formula II or salts thereof:



(II)

with glyoxal of formula (III)



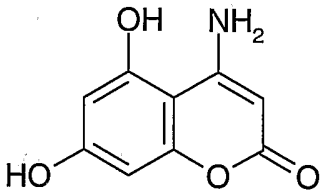
(III)

in aqueous-organic media protected from light at strictly controlled pH range; and/or, if desired, converting a resulting salt into the free compound or into another salt, and/or, if desired, converting a resulting free compound of the formula I having salt-forming properties into a salt.

Reaction is based on the modified process described in EP 0906909 B1, primarily performed in the presence of solvent inert to utilized chemical reagents. Such solvents are aromatic solvents, for example toluene, xylene or dipolar aprotic solvents such as aliphatic or cyclic ethers, dialkylamides of carboxylic acids, for example *N,N*-dimethylformamide, or dimethyl sulfoxide. Especially suitable are solvents with low boiling point miscible with water, for example buffered mixture of acetonitrile and water. Reaction may be performed in a pH range from 2 to 7, preferably at pH=3. Commercially available 40% aqueous glyoxal solution may be employed in the reaction, which may be used in substantial excess in respect to hydroxycoumarin, preferably in the ratio 2:1. Glyoxal derivative such as 2,3-dihydroxy-1,4-dioxane may be utilized instead.

Reaction may be carry out at a temperature in the range of 0-80 °C, preferably at room temperature, during 1-24 hours, preferably 3-6 hours.

Hydroxycoumarin of formula II may be prepared from corresponding enamine of formula IV:



(IV)

by methods known to those skilled in the art, for example by hydrolysis in strong acidic medium such as 50% aqueous sulphuric (Desai, N. J. et al. *J. Org. Chem.* **1957**, *22*, 388-390) or 25% aqueous hydrochloric (Sonn, A. *Ber.* **1917**, *50*, 1292-1305). Enamine of formula IV may be prepared by methods known to those skilled in the art, for example, by condensation of commercially available phloroglucinol with either cyanoacetic acid (see, for example, A. Sonn, Ber. 1917, 50, 1292-1305) or its alkyl esters such as ethyl cyanoacetate (see, for example, N. J. Desai et al., J. Org. Chem. 1957, 22, 388-390).

It will be appreciated by those skilled in the art that in the cases where competing side-reactions may occur that functional groups such as certain hydroxy groups of hydroxycoumarins or one of aldehyde groups of glyoxal will require protection before the process is undertaken, followed by deprotection after the process. Examples of suitable protecting groups and methods for their addition and removal may be found in the textbook "Protective Groups in Organic Organic Synthesis" by T. W. Greene and P. G. Wuts, John Wiley and Sons, 1999). Selection, processes for their addition and removal are common and well known to those skilled in the art.

Salts of the compound of formula I may be prepared by methods known to those skilled in the art, for example in the reaction of the compound of formula I with corresponding base in an adequate solvent or mixture of solvents such as

ethers (diethyl ether) or alcohols (ethanol, propanol or isopropanol) or by mixing equivalent amounts of corresponding compounds followed by lyophilization and purification of the reaction mixture.

The present invention relates also to the reactive intermediates obtained during the preparation of the compound of formula I, and its pharmaceutically acceptable salts. Such intermediates may be isolated and defined or without isolation employed in the next step of chemical synthesis.

Next aspect of the present invention relates to the use of the compound of formula I, and pharmaceutically acceptable salts thereof in prophylaxis and therapeutic treatment of diseases, disorders or conditions which may occur as a result of disturbance of human immune system, primarily inflammatory diseases, disorders and conditions, asthma in paricular, in therapeutically effective amounts.

A further object of the present invention relates to the use of compound of the formula (I) and their pharmaceutically acceptable salts as antiinflammatory, antianaphylactic and immunomodulating agents, which - depending on the site of disease - can be differently administered, e.g. per os, parenterally, percutaneously, subcutaneously, buccally, rectally or by inhalation in case of local application in respiratory system.

A further object of the present invention relates to the preparation of pharmaceutical forms of the present compound formulated in such a manner as to achieve an optimal bioavailability of the active compound of the formula (I). For percutaneous application the compound of the formula (I) can be formulated in the form of an ointment, cream, gel or lotion. Ointments, creams and gels can be formulated with a water base or an oil base under the addition of a suitable emulsifier or gelling agent when gel is formulated. The formulation is especially important for the use by inhalation, wherein compound of the formula (I) can be in the form of aerosol under pressure. For all forms of aerosol formulations there is suggested a micronization of the compound of the formula (I) being previously homogenized in lactose, glucose, higher fatty acids, sodium salt of

dioctylsulfosuccinic acid or most preferably in carboxymethylcellulose, so that the majority of the particles have the size of 5 μm. For the inhalation formulation the aerosol can be mixed with a propellant intended for the spraying of the active substance.

For the inhalation application the compound of the formula (I) can be used in the form of a dry powder with micronized particles.

Suitable preparations of the compound of the present invention can be used in the prophylaxis and treatment of several inflammatory diseases and pathological allergical conditions. Examples of such conditions and diseases are, without limitation, asthma, chronic obstructive pulmonary disease, inflammatory nasal diseases (allergic rhinitis, nasal polyps), dermatological inflammations (eczemas, psoriasis, allergic dermatitis, neurodermatitis, pruritis, conjunctivitis), inflammatory bowel diseases (Crohn's disease, colitis and ulcerative colitis), rheumatoid arthritis, further autoimmune thyroiditis, lupus erythematosus, multiple sclerosis, Raynaud's disease, rheumatoid spondylitis, septic arthritis, polyarthritis, insulin-dependent diabetes, inflammatory brain diseases (meningitis and encephalitis), conditions induced by acute trauma (brain, miocard and lung lesions), inflammations accompanying infections (sepsis, glomerulonephritis).

The compound of the formula (I) can be used individually or in combination with any other commercial product suitable for treating said diseases and/or conditions.

The compound represented by the formula (I) possesses useful pharmacological properties supported by *in vitro* and *in vivo* investigations disclosed in the continuation of the present invention.

Inhibition of lymphocytes proliferation

In a 96 well plate, dilutions of the 10 mM stock solution (final 30 μM concentration) of the compound of formula I in RPMI medium (Imunološki

zavod) with addition of 10% of FBS (Invitrogen) are made in triplicate. 50 000 of peripheral mononuclear cells per well are added to each solution, followed by mitogen (PHA or PMA + ionomycin), and incubation is carried out at 37 °C overnight in the atmosphere with 5% of CO_2. 48 hours later 1 μCi [^{3}H]thymidine (Pharmacia) per well is added and incubation is continued additional 16-18 hours. Cells are harvested using cell harvester, on GF/C filter (Packard) and dried at 65 °C. Microscynt O scintillation fluid (30μL, Packard) is added on each well, and incorporated radioactivity is measured on β-counter (Top Count, Packard).

Compound of formula I does not show inhibition of lymphocytes proliferation in 30 μM concentration in comparison to the positive control.

Croton Oil Induced Ear Edema in Rats

Male Sprague-Dawley rats with a body weight of 250-300 g are randomly divided into groups. Initial ear thicknesses are measured with a digital calliper. To the control group, then, vehicle (25 μL of acetone) is applied to both surfaces of the ears. To other groups, compound of formula I or standard substance respectively, is applied to both surfaces of the ears, in the same solvent and volume (50μL per ear). Thirty minutes later, 20% croton oil (Sigma) is applied in the same manner, in the same solvent and volume.

Five hours after application of croton oil, at peak inflammation, ear thicknesses are remeasured. Percent inhibition of ear edema formation is determined by comparison of the ear thicknesses of animals treated with the standard substance and the compound of formula I respectively, with that of control animals. Percent inhibition of ear edema in animals treated with prednisolone (in a dose of 0.5 mg/animal) is 80%, while in animals treated with the compound of the present invention (in a dose of 2 mg/animal) is 67% in comparison with the control group.

Model of Lung Eosinophilia in Mice

Male Balb/C mice (Charles River) with a body weight of 20-25 g are randomly divided into groups, and sensitized by an *i.p.* application of ovalbumin (OVA, Sigma) on day zero and day fourteen. On the twentieth day, the mice are subjected to a challenge test by *i.n.* (intranasal) application of OVA (positive control or test groups) or PBS (negative control). 48 hours after *i.n.* application of OVA, the animals are anaesthetized and the lungs are rinsed with 1 mL of PBS (bronchoalveolar lavage – BAL). The cells are separated on Cytospin 3 cytocentrifuge (Shandon). The cells are then stained in Diff-Quick (Dade) and the percentage of eosinophils are determined by differential counting of at least 100 cells.

Beclomethasone (Pliva) is used as a standard anti-inflammatory substance, and it is administered daily by *i.n.* application in a dose of 1mg/kg two days before the challenge until the end of the study, together with the positive and the negative control. The compound of formula I is administered daily by *i.p.* or *i.n.* application, or by inhalation (aerosol) in different doses, preventively before or therapeutically after the provocative test, up to the completion of the study.

The compound of formula I significantly (t-test, $p<0.001$) reduces the number of eosinophils in BAL fluid, and histological preparations of lungs in comparison with the positive control group, similarly to a standard steroid compound – beclomethasone.

Immunohistochemistry

Slices of formalin fixed lung tissue of mice sacrificed at the end of the study are used for immunohistochemistry. After deparrafinization, slices are labeled with specific antibodies (R&D), according to manufacturer's recommendation. Levels of IL-5 and chemokines, eotaxin and RANTES, are determined using light microscopy. Expressions of IL-5, eotaxin and RANTES are significantly decreased in the group treated with the compound of formula (I).

Bronchial hyperresponsiveness

Bronchial hyperresponsiveness is measured using a whole body pletismography method (Buxco pletismograph), based on pressure differences. The study is carried out on conscious and unrestrained mice. Effect of the substance on bronchial hyperresponsiveness provoked by methacholine (Sigma), applied by aerosol in different concentrations, is expressed in percentage increase of enhanced pause above the baseline. Baseline is measured without a cholinergic stimulus. The compound of formula (I) is applied *i.n.* in a dose of 2 mg/kg one day before the provocative test, up to the completion of the study, three days in total. Measurement of the bronchial hyperresponsiveness by pletismography method is performed 24 hours after the provocative test. Compound of formula I significantly reduces (t-test, $p<0.001$) bronchial hyperresponsiveness of upper airways in comparison to the control group, in applied dose of methacholine at 50 mg/mL.

Toxicology

Acute toxicity study was performed for the compound of formula (I) after single dose peroral administration. Applied doses were 500 mg and 2000 mg/kg. LD_{50} was determined to be more than 2000 mg/kg.

Preparational Processes With Examples

Processes for the preparation of the compound of formula I form a further aspect of the present invention. These processes are preferably carried out at atmospheric pressure and room temperature. The present invention is illustrated but in no way limited by the following Examples. The final product of each of these Examples was characterized by one or more of the following procedures: high performance liquid chromatography (HPLC) and/or HPLC-mass spectrometry (HPLC-MS) and/or high resolution mass spectrometry (HR-MS), and nuclear magnetic resonance spectroscopy (NMR). Temperatures are given

in degrees Celsius; DMF = *N,N*-dimethylformamide; RE = rotary evaporator; RT = room temperature; h = hour(s).

2,7,9-Trihydroxy-3-(4,5,7-trihydroxy-2-okso-2*H*-chromen-3-yl)-2,3-dihydro-4*H*-furo[3,2-*c*]chromen-4-one

Example 1

4,5,7-Trihydroxycoumarin (0.195 g; 1 mmol) was dissolved in acetonitrile (16 mL) with addition of phosphate buffer (4 mL; pH=7.0; 0.1 M) and water (2 mL). In clear reaction solution (pH=3) protected from light 40% aqueous glyoxal solution (0.23 mL; 2 mmol) was added dropwise, and the reaction mixture was left to stir at RT additional 4-5 h. When the reaction was completed (monitored by HPLC), 2.5% aqueous solution of sodium chloride (10 mL) was added, and then the resulting mixture concentrated in a RE. Amorphous pinkish precipitates gradually separated in several fractions, filtered off, washed with cold water, and dried to obtain 0.130 g (60%) of the product:

MALDI-TOF-MS: 429.0313 (calcd. MH^+ 429.0458);

ESI-MS: 429.1 (calcd. MH^+ 429.0);

^{1}H-NMR (500 MHz, DMF-d_7, 277 K) δ / ppm: 4.65 (d, 1H, J = 3,0 Hz), 6.29 (bs, 2H), 6.30 (s, 1H), 6.40 (s, 1H), 6.42 (s, 1H), 10.6-11.1 (bs, 3H);

^{13}C-NMR (76 MHz, DMF-d_7, 277 K) δ / ppm: 41.9, 93.9, 94.4, 95.0, 96.0, 96.3, 96.8, 98.1, 108.7, 154.8, 156.0, 156.3, 157.8, 158.5, 161.0, 161.8, 161.9, 163.7, 166.1;

^{1}H-NMR (500 MHz, acetone-d_6, 277 K) δ / ppm: 5.46 (s, 1H), 6.17 (s, 2H), 6.23 (s, 2H), 8.95-9.10 (bs, 1H), 9.56 (s, 1H), 9.40-9.95 (bs, 3H), 10.80-11.20 (bs, 2H);

^{13}C-NMR (126 MHz, acetone-d_6, 277 K) δ / ppm: 46.1, 94.6, 97.2, 99.1, 99.9, 156.1, 160.0, 162.6, 166.8, 172.0, 198.9.

Example 2

4,5,7-Trihydroxycoumarin (1.95 g; 10 mmol) was dissolved in acetonitrile (160 mL) with addition of phosphate buffer (40 mL; pH=7.0; 0.1 M) and water (20 mL). In clear reaction solution (pH=3) protected from light 40% aqueous glyoxal solution (2.3 mL; 20 mmol) was added dropwise, and the reaction mixture was left to stir at RT additional 4-5 h. When the reaction was completed (monitored by HPLC), 20% aqueous solution of sodium chloride (20 mL) was added, phases were separated, aqueous phase was extracted with acetonitrile, and combined organic phases washed with brine. Organic phase was then concentrated in a RE, viscous sticky gum that precipitated was removed, and 10% aqueous solution of sodium chloride was added to the remaining solution followed by addition of 1M hydrochloric acid at +4 °C until the pH=2. After additional cooling at +4 °C amorphous pinkish precipitate was filtered off, washed with cold water, and dried to obtain 1.65 g (77%) of the product.

Example 3

4,5,7-Trihydroxycoumarin (1.0 g; 5.15 mmol) was dissolved in DMF (3 mL) and cooled to +4 °C. In clear reaction solution protected from light 40% aqueous glyoxal solution (0.89 mL; 7.7 mmol) was added dropwise, and the reaction mixture was left to stir at +5 °C additional 20-24 h. Reaction mixture was placed at -18 °C, and crystalline product usually separated after 6 days. It was filtered off, washed with cold acetonitrile, and dried to obtain 0.19 g (17%) of the product.

Monosodium salt of 2,7,9-Trihydroxy-3-(4,5,7-trihydroxy-2-okso-2*H*-chromen-3-yl)-2,3-dihydro-4*H*-furo[3,2-*c*]chromen-4-one

Example 4

2,7,9-Trihydroxy-3-(4,5,7-trihydroxy-2-okso-2*H*-chromen-3-yl)-2,3-dihydro-4*H*-furo[3,2-*c*]chromen-4-one (10 mg, 0.0233 mmol) was dissolved in ice cold 0.01 M sodium hydroxide (2.4 mL, 0.024 mmol). Resulting solution was lyophilized to obtain 10 mg of the product.

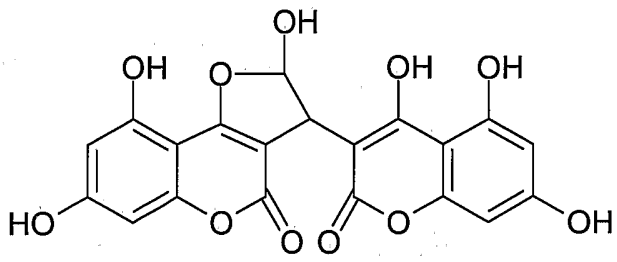
Disodium salt of 2,7,9-Trihydroxy-3-(4,5,7-trihydroxy-2-okso-2*H*-chromen-3-yl)-2,3-dihydro-4*H*-furo[3,2-*c*]chromen-4-one

Example 5

2,7,9-Trihydroxy-3-(4,5,7-trihydroxy-2-okso-2*H*-chromen-3-yl)-2,3-dihydro-4*H*-furo[3,2-*c*]chromen-4-one (10 mg, 0.0233 mmol) was dissolved in ice cold 0.01 M sodium hydroxide (4.7 mL, 0.047 mmol). Resulting solution was lyophilized to obtain 10 mg of the product.

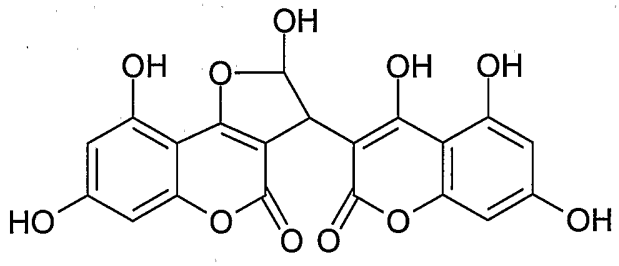
CLAIMS

1. Compound of formula I including pharmaceutically acceptable salts and solvates thereof:



(I)

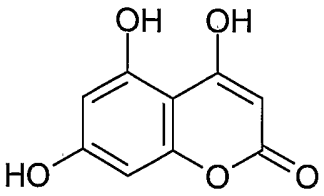
2. Process for the preparation of the compound of formula I including pharmaceutically acceptable salts and solvates thereof



(I)

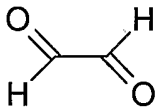
characterized in that the process comprises:

condensation of hydroxycoumarin of formula II or salts thereof



(II)

with glyoxal of formula III:



(III)

in aqueous-organic media protected from light at strictly controlled pH range.

3. Process for the preparation as defined in claim **2** characterized in that an organic solvent is acetonitrile.

4. Process for the preparation as defined in claim **2** characterized in that pH of a reaction mixture is less than 7, preferably between 2 and 4 and most preferably 3.

5. Pharmaceutical compositions containing a therapeutically effective amount of the compound of formula I or a salt or a solvate thereof according to claim **1** together with a pharmaceutically acceptable diluent and carrier.

6. The use of the compound of formula I as described in claim **1** for the manufacture of medicament for prophylaxis and therapeutic treatment of patients that suffer from inflammatory diseases, disorders or conditions.

7. Use of the compound of formula I as defined in claim **6** characterized in that inflammatory diseases, disorders and conditions are selected from: asthma; chronic obstructive pulmonary disease, allergic rhinitis, adenoids; eczema, psoriasis, allergic dermatitis, neurodermatitis, pruritis, conjunctivitis; Crohn's disease, colitis, ulcerative colitis, rheumatoid arthritis, autoimmune thyroiditis; lupus erythematosus; multiple sclerosis, Raynaud's disease, rheumatoid spondylitis, septic arthritis, polyarthritis, insulin-dependent diabetes, inflammatory brain disorders such as meningitis and encephalitis, conditions associated with acute trauma such as cerebral injury, heart tissue injury and lung injury, inflammation accompanying infections such as sepsis and glomerulonephritis.

8. The use according to claim 6 characterized in that the therapeutically effective amount of a compound of formula I is administered orally, parenterally or topically.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/HR2005/000022

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D493/04 A61K31/37 A61P11/06 A61P29/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, INSPEC, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 2005/010007 A (PLIVA- ISTRAZIVACKI INSTITUT D.O.O; MERCEP, MLADEN; MESIC, MILAN; HRVA) 3 February 2005 (2005-02-03) abstract; claims 1-19; examples 1-32 in particular example 13	1-8
P,X	WO 2005/010006 A (PLIVA-ISTRAZIVACKI INSTITUT D.O.O; MERCEP, MLADEN; MESIC, MILAN; HRVAC) 3 February 2005 (2005-02-03) abstract; claims 1-14; examples 1-50	1-8
P,X	JP 2005 053829 A (UNIV NIHON) 3 March 2005 (2005-03-03) abstract; compound III	1-8

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 August 2005	10/08/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Papathoma, S

INTERNATIONAL SEARCH REPORT

International Application No
PCT/HR2005/000022

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/029237 A (PLIVA, FARMACEUTSKA INDUSTRIJA, DIONICKO DRUSTVO; IVEZIC, ZRINKA; TRKO) 10 April 2003 (2003-04-10) cited in the application abstract; claims 1-4,6,7,22-25; examples 1-3,5,6	1-8
X	US 2 810 730 A (FUCIK KAREL ET AL) 22 October 1957 (1957-10-22) the whole document	1-8
A	MIDDLETON E ET AL: "EFFECTS OF FLAVONOIDS ON IMMUNE AND INFLAMMATORY CELL FUNCTIONS" BIOCHEMICAL PHARMACOLOGY, PERGAMON, OXFORD, GB, vol. 43, no. 6, 1992, pages 1167-1179, XP000886668 ISSN: 0006-2952 the whole document	1-8

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Information on patent family members

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