A compound according to the general Formula (I).

Formula I
Title: Novel bis-chromone derivatives, methods for their preparation and uses thereof.

The invention relates to new pharmaceutical compounds, methods for their preparation and uses thereof. In particular, it relates to novel bis-chromone derivatives and their therapeutic application, especially in the treatment or prevention of diseases involving mast cell activation, such as allergic diseases.

The prevalence of allergic diseases has increased over the last decades and the search for suitable pharmacotherapeutical treatment is ongoing. Several options to treat allergic patients are available, including antihistamines, leukotriene inhibitors, corticoids and chromones. This last class of drugs has lost popularity, mainly due to their less pronounced effect and short duration of action. However, chromones show few side-effects as opposed to the commonly used corticoids, which renders them continuously interesting therapeutic agents. Furthermore, they seem to have an interesting, yet not fully understood, mechanism of action.

Chromone (or 1,4-benzopyrone) is a derivative of benzopyran with a keto group on the pyran ring. It is an isomer of coumarin. Derivatives of chromone are collectively known as chromones. Chromones stabilize mast cells, which are effector cells of immediate hypersensitivity reactions and therefore play a key role in allergic diseases. These cells can be found throughout the human body and they secrete granules upon activation. The granules contain inflammatory mediators, which induce the symptoms of an allergic reaction, like edema, warmth and itchiness. Activation of mast cells occurs as a result of cross-linking of high affinity IgE receptors on the cell surface by allergens. Chromones bind to the Cromolyn Binding Protein (CBP) on the surface of mast cells and inhibit this activation process. While the exact mechanism of their action is yet to be elucidated,
it is hypothesized that chromones interfere with the calcium influx which is necessary for degranulation. 16,17

Cromoglicic acid (INN) (also referred to as cromolyn (USAN), cromoglycate (former BAN), or cromoglicate) is described as a mast cell stabilizer, and is commonly marketed as the DiSodiumCromoGlycate (DSCG)18. DSCG inhibits mast-cell activation and its structure-activity relationships are well studied.19 The drug consists of two chromone moieties linked by a spacer (Fig. 1A). Altering the length of the spacer changes the inhibitory activity of the drug.19 See also US 3,419,578. Further reported therapeutic uses of DSCG include the treatment of pathological mineral resorptive states, such as osteoporosis (US 4,501,754)

Recognizing the advantages of chromone-based drugs as well as the need for new and more effective anti-allergic agents, the present inventors aimed at providing a new class of chromone derivatives which have higher potency than the currently available chromone-based drugs. Furthermore, they sought to develop mast cell activation inhibitors that allow for a high level of spatiotemporal control over events of the molecular level. This would facilitate studying mechanisms involved in allergic reactions in a novel manner.

It was surprisingly found that at least some of these goals could be met by the provision of a bis-chromone compound wherein the chromone groups are linked by a spacer containing an azo-moiety. In mast cell degranulation assays, these azo-chromone compounds possess a significantly greater potency than the standard chromone-based anti-allergic agent DSCG.

Accordingly, the invention provides a compound of the general formula I
or a pharmaceutically acceptable salt, ester or amide thereof, wherein 
n is 0 or 1;

Y1 is Xi-Ari or 0-Xi-Ari, and Y2 is X2-Ar2 or 0-X2-Ar2, wherein Ari and Ar2 are linked to N=N;

wherein Xi and X2 are the same or different and each is a substituted or unsubstituted straight hydrocarbon chain of 1 to 3 carbon atoms;

wherein Ari and Ar2 are the same or different and each is a 5- or 6-membered (hetero)aromatic ring, optionally substituted with one or more substituents selected from the group consisting of halogen, hydroxyl, (substituted) lower alkyl, (substituted) lower alkoxy; and

R1, R2, R3, R4, R5 and R6 are the same or different and each is selected from the group consisting of H, halogen, nitro, amino, cyano, hydroxyl, (substituted) lower alkyl, (substituted) lower alkoxy.

Azo-linked bis-chromonyl compounds according to Formula I are not known or suggested in the art. US 3,419,578 discloses compounds wherein the chromone groups are connected via the linker -O-X-O-, wherein X is a saturated or unsaturated, substituted or unsubstituted, straight or branched polymethylene chain. The chain may be interrupted by one or
more carbocyclic rings or oxygen containing heterocyclic rings, oxygen atoms or carbonyl groups.

US 3,673,218 discloses bis-chromonyl compounds wherein the linker is a carbon to carbon bond or a single atom through which the chromone residues are linked. Exemplary single atoms include oxygen, sulphur, or substituted sulphur or nitrogen.

WO2010088455 discloses cromolyn analogs useful as imaging agents for detecting atherosclerotic plaques and for treating atherosclerosis and Alzheimer's Disease. Like in US 3,419,578, the linker is based on a polymethylene chain.

US 4,105,781 relates to chromone compounds wherein the linker is hydroxyl (lower) alkylene, preferably having 1 to 4 carbon atoms.

In one embodiment, the invention provides a bis-chromonyl compound according to Formula I wherein n is 0, such that the azo-linker is directly bound to the chromone-moieties.

In another embodiment, the invention provides a bis-chromonyl compound according to Formula I wherein n is 1, such that the linker comprises an azo-group flanked on each side by an aromatic moiety. Bis-chromonyl compounds comprising an aromatic azo-linker were found to be very potent mast cell stabilizers. The aromatic moieties \( A_{R1} \) and \( A_{R2} \) are bound directly to the N=N, and bound to the chromonys via a lower alkyl (Xi; X2) or lower alkoxy spacer (O-Xi; O-X2) consisting of 1 to 3 carbon atoms. Thus, \( Y_1 \) is Xi-Ari or O-Xi-Ari and \( Y_2 \) is X2-Ari2 or 0 -X2-Ari2, wherein Xi and X2 are the same or different and each is a straight hydrocarbon chain of 1 to 3 carbon atoms. For example, at least one of Xi and X2 is \(-CH2-\) or \(-CH2-CH2-\). Preferably, Xi and X2 are the same, more preferably each of Xi and X2 is \(-CH2-\).

In one embodiment, at least one of the aromatic moieties is bound to the chromonyl via a lower alkoxy spacer, i.e. \( Y_1 \) is O-Xi-Ari and/or \( Y_2 \) is O-
X2-Ar2. For example, \( Y_i \) is O-CH2-An and/or \( Y_2 \) is 0-CH2-Ar2. As another example, \( Y_i \) is 0-CH2-CH2-Ari and/or \( Y_2 \) is 0-CH2-CH2-Ar2.

In another embodiment, at least one of the aromatic moieties is bound to the chromonyl via a lower alkyl spacer, i.e. \( Y_i \) is Xi-Ari and/or \( Y_2 \) is X2-Ar2. For example, \( Y_i \) is -CH2-An and/or \( Y_2 \) is -CH2-Ar2. As another example, \( Y_i \) is -CH2-CH2-Ari and/or \( Y_2 \) is -CH2-CH2-Ar2. In a specific aspect, both aromatic moieties Ar1 and Ar2 are bound to the respective chromonyls via a lower alkyl spacer. As will be understood, in case of an alkoxy spacer the ether bond is connected to the chromonyls and the X moieties to the aromatic moieties.

Preferably, both aromatic moieties An and Ar2 are bound to the chromonyl via a lower alkoxy spacer. Preferred compounds include

![Chemical Structure](image)

In a compound of the invention, the aromatic moiety is a 5- or 6-membered aromatic ring or heteroaromatic ring with one or two heteroatoms selected from the group of O, N and S. For example, An and Ar2 can be independently selected from the group consisting of benzene, pyridine, pyrrole, furan, thiophene, pyrazole, imidazole, isoxazole, oxazole, isothiazole, thiazole, pyridazine, pyrimidine and pyrazine. An and Ar2 may be the same or different. Preferably, at least one of An and Ar2 is benzene,
pyridine, pyrrole, furan or thiophene. Preferably, \( \text{Ar}_1 \) and \( \text{Ar}_2 \) are the same. Most preferably, \( \text{Ar}_1 \) and \( \text{Ar}_2 \) are benzene.

The (hetero)aromatic ring may be substituted with one or more substituents selected from the group consisting of halogen (e.g. fluoro, chloro, bromo, or iodo), hydroxyl, (substituted) lower alkyl (preferably alkyl of 1 to 4 carbon atoms), and (substituted) lower alkoxy (preferably alkoxy of 1 to 4 carbon atoms). In one embodiment, the (hetero)aromatic ring is unsubstituted.

Preferred azo-bis-chromonyl compounds comprising a heteroaromatic ring include

In a compound of the invention, the substituents \( \text{R}_1, \text{R}_2, \text{R}_3, \text{R}_4, \text{R}_5 \) and \( \text{R}_6 \) on the chromone moieties may be the same or different, and each is selected from the group consisting of H, halogen, nitro, amino, cyano, hydroxyl, (substituted) lower (e.g. \( \text{Ci}-\text{Ce} \)) alkyl and (substituted) lower (e.g. \( \text{Ci}-\text{Ce} \)) alkoxy. In one embodiment, the substituents on the two chromone moieties
of the bis-chromonyl compound are different i.e. \( R_1 \), \( R_2 \) and \( R_3 \) are different from \( R_4 \), \( R_5 \) and \( R_6 \). Preferably, the two chromone moieties of the bis-chromonyl compound are identical i.e. \( R_1 \), \( R_2 \) and \( R_3 \) are the same as \( R_4 \), \( R_5 \) and \( R_6 \). In one embodiment, at least one of the \( R \) substituents on each ring is hydrogen, preferably at least two \( R \) substituents are hydrogen. Hence, provided are compounds wherein each of \( R_2 \), \( R_3 \), \( R_5 \) and \( R_6 \) is hydrogen. In a specific aspect, each of \( R_1 \), \( R_2 \), \( R_3 \), \( R_4 \), \( R_5 \) and \( R_6 \) is hydrogen.

The relative positioning of the bond attaching the azo-containing linker to the benzene ring of each of the chromonyl moiety can vary. For example, the azo-linker can be bound to one chromonyl moiety via position \( a \), \( b \), \( c \) or \( d \) and to the other chromonyl moiety via position to any one of position \( e \), \( f \), \( g \) or \( h \) in the Formula shown below.

As will be understood, the remaining positions of \( a \), \( b \), \( c \) and \( d \) are available for \( R_1 \), \( R_2 \) and \( R_3 \). Likewise, the remaining positions of \( e \), \( f \), \( g \) and \( h \) are available for \( R_4 \), \( R_5 \) and \( R_6 \).

Preferably, the azo-linker is bound to one chromonyl moiety via position \( a \), \( b \) or \( c \) and to the other chromonyl moiety via position to \( e \), \( f \) or \( g \).

In one embodiment, the azo-linker is bound to the respective chromonyl moieties via symmetrical (equivalent) or asymmetrical positions on the benzene ring. Exemplary compounds wherein the azo-linker is connected via asymmetrical positions include compounds E (positions \( b \) and \( e \)) and F.
(positions d and e). Preferably, the positions are symmetrical.

Symmetrical/equivalent positions are position pairs a and e, b and f, c and g, and d and h. Exemplary compounds wherein the azo-linker is connected via symmetrical positions include compounds A and B (positions a and e), C and G (positions b and f) and compound D (positions c and g). Preferred symmetrical position pairs for azo-linker attachment are a and e, and b and f.

In one embodiment, n=1 and the linker is attached to the chromonyl moieties via positions a and e. See for example compounds A and B, and compounds (E)-5,5'-'-(((diazone-1,2-diyl)bis(3,1-phenylene))bis(methylene))bis(oxy))bis(4-oxo-4H-chromene-2-carboxylate) (MAC) and (E)-5,5'-(((diazone-1,2-diyl)bis(4,1-phenylene))bis(methylene))bis(oxy))bis(4-oxo-4H-chromene-2-carboxylate) (PAC; see Fig. 1).

In a preferred aspect, the invention provides a compound wherein n=1 and wherein aromatic moieties Ar1 and Ar2 are bound to the chromonyl via a lower alkoxy spacer and wherein Y1 and Y2 are attached to the chromonyl moieties via positions a and e.

In another embodiment, n=0 and the azo-linker is attached to the chromonyl moieties via positions b and f. See for example compound (E)-6,6'-((diazone-1,2-diyl)bis(4-oxo-4H-chromene-2-carboxylate)(DAC; see Fig. 1).

Other preferred compounds include (£J)-5,5'-'-(((5,5'-(diazone-1,2-diyl)bis(pyridine-5,3-diyl))bis(methylene))bis(oxy))bis(4-oxo-4H-chromene-2-carboxylic acid) (Compound A of Fig. 5), (E)-5,5'-(((4,4'-((diazone-1,2-diyl)bis(thiazole-4,2-diyl))bis(methylene))bis(oxy))bis(4-oxo-4H-chromene-2-carboxylic acid) (Compound B of Fig. 5), (£J)-6-(2-(4-((3-(2-carboxy-4-oxo-4H-chromen-5-yl)phenyl)diazenyl)thiophen-2-yl)ethyl)-4-oxo-4H-chromene-2-carboxylic acid (Compound C of Fig. 5) and (£J)-6-((2-carboxy-4-oxo-4H-chromen-6-yl)diazenyl)furan-2-yl)oxy)-4-oxo-4H-chromene-2-carboxylic acid (Compound G of Fig. 5).
Pharmaceutically acceptable salts, esters or amides thereof include salts, esters and amides of one or more of the carboxylic acid functions present and esters of any hydroxylic functions present. Salts of the bis-chromononyl compounds include salts with physiologically acceptable cations, for example, ammonium salts, metal salts such as alkali metal salts (e.g. sodium, potassium and lithium salts) and alkaline earth metal salts (e.g. magnesium and calcium salts) and salts with organic bases, e.g. amine salts such as piperidine, triethanolamine and diethylaminoethylamine salts.

Esters which may be mentioned include simple alkyl esters (e.g. methyl, ethyl, propyl, isopropyl, butyl and tertiary butyl esters) and amides which include simple amides and more complex amides with amino acids, such as glycine.

The compounds MAC and PAC show an almost 100 times higher potency than the chromone drug DSCG, basing on β-hexosaminidase release assays (Fig. 3). This large increase in potency makes MAC and PAC very interesting anti-allergic agents. Thus, the novel compounds as disclosed herein are a highly attractive alternative to known chromone-based drugs.

In one embodiment, the invention provides a pharmaceutical composition comprising a compound according to the invention and a pharmaceutically active carrier, diluent or excipient. Preferred compounds include MAC, PAC, DAC (see Fig. 1) and compounds A through G shown in Figure 5. Most preferred are compounds A through G wherein each of R₁-R₆ is hydrogen.

The composition comprises a therapeutically effective dose of the chromone-derivative. The skilled person will be able to determine for each application the therapeutically effective dose, taking into account e.g. the nature and severity of the disease, age and/or weight of the subject to be treated, and the dose regimen. For example, for the treatment of asthma, a
dose up to 20 mg (e.g. 1-15 mg) may be administered at 4-12 hour intervals by inhalation.

The pharmaceutical composition can be formulated in any desirable form, depending on the intended use and route of administration. For example, it is formulated as a nasal spray, inhalator, eye drops, oral formulation, nebulizer solution or topical formulation, like a solution, gel, paste, ointment or cream. Also provided is the use of a compound according to the invention as therapeutic or prophylactic agent.

A pharmaceutical composition may of course contain one or more additional therapeutically active ingredients. In one embodiment, it comprises at least one further ingredient capable of inhibiting the release of mediators from mast cells. The further ingredient(s) may be a novel azochromone compound of the invention and/or a previously described mast cell stabilizer. Examples of other compounds that inhibit the release of mediators from mast cells include olopatadine ((Z)-11-(3-(dimethylamino)propylidene)-6, 11-dihydrodibenz(b,e)oxepine-2-acetic acid hydrochloride), amlexanox (C16 H14 N2 O4 ; 2-Amino-7-(1-methylethyl)-5-oxo-5H-[l]benzopyrano(2,3-b)pyridine-3-carboxylic acid), ketotifen (C19 H19 NOS; 4,9-Dihydro-4-(1-methyl-4-piperidin-ylidene)-10H-benzo[4,5]cyclohepta[1,2-b]thiophen-10-one) and ketotifen fumarate (C23 H23 N5 O5 S), lodoxamide tromethamine (N,N'-2-chloro-5-cyano-m-phenylene)dioxamic acid tromethamine salt), minocromil (6-Methylamino-4-oxo-10-propyl-4H-pyran[3,2-g]quinoline-2,8-dicarboxylic acid) and its sodium salt, repirinast (C2O H21 N05 ; 5,6-Dihydro-7,8-dimethyl-4,5-dioxo-4H-pyran[3,2-c]quinoline-2-carboxylic acid 3-methyl-butylester), suplatast tosylate ((+-)-(2-{p-(3-Ethoxy-2-hydroxypropoxy)phenyl}[carbamoyl]ethyl) dimethyl sulphonium p-toluene sulphonate), tiacrilast ((E)-6-(methylithio)-4-oxo-3(4H)-quinazolineacrylic acid) and its sodium salt, tranilast (C18 H17 NO5 ; 2[3-(3,4-Dimethoxyphenyl)-l-oxo-2-propenyl]amino]benzoic acid), taxanox (C13
H6CIN502; 9-Chloro-7-(1H-tetrazol-5-yl)-5H-[1]benzopyrano[2,3-b]pyridin-5-one) and its sodium salt, and zaprinast (1,4-Dihydro-5-(2-propoxyphenyl)-1,2,3-triazolo[4,5-d]pyrimidin-7-one.

Mast cells play a role in immediate hypersensitivity and other inflammatory reactions by releasing a variety of chemical mediators upon activation. Mast cells play a key role in the inflammatory process. Mast cells can be stimulated to degranulate by crosslinking of surface IgE attached to FCEpsilonRI, by allergens, chemokines, cytokines, or by activated complement proteins. When activated, a mast cell rapidly releases its characteristic granules and various hormonal mediators into the interstitium. These mediators, some of which are stored inside granules in the cytoplasm, include biogenic amines such as histamine, lipid mediators such as leukotrienes, prostaglandins, and platelet-activating factor, cytokines and enzymes. As exemplified herein below, the azochromomonone compounds of the invention are very potent inhibitors of mast cell degranulation invoked by an established promoter of mast cell degranulation. IC50 values below 300 µM and even below 30 µM were observed under conditions wherein the reference bis-chromonyl compound DSCG did not show any inhibitory activity (IC50 > 1 mM).

Thus, a further aspect of the invention provides a bis-chromonyl compound for use in a method of treatment or prophylaxis of a disease involving unwanted or excessive mast cell activation, typically resulting in an excessive or undesirable release of mediators from mast cells.

In one embodiment, the disease is an allergic disease. The term "allergic disease" as used herein refers to any hypersensitivity condition of the immune system. Exemplary conditions to be treated with a compound of the invention include asthma, allergic rhinitis, mastocytosis, dermatographic...
urticaria, eczema, psoriasis, ulcerative colitis, food allergy and vulvar vestibulitis. For example, oral formulations can be used to treat the diarrhea, flushing, headaches, vomiting, urticaria, abdominal pain, nausea, and itching of mastocytosis, which is an accumulation of mast cells in the tissues. Opthalmic and nasal solutions can be used to treat the itching, redness, swelling, sneezing, tearing, and discharge of allergic conjunctivitis and allergic rhinitis. An inhalation aerosol is useful as prophylactic agents in the management of asthma.

An azo-linked bis-chromonyl compound of the invention, which inhibits the release of histamine and other mediators from mast cells that have been sensitized by specific antigens, is preferably used pharmacologically as an anti-asthmatic /anti-allergic.

Mast cells have also been implicated in the pathology associated with the autoimmune disorders rheumatoid arthritis, bullous pemphigoid, and multiple sclerosis. They have been shown to be involved in the recruitment of inflammatory cells to the joints (e.g. rheumatoid arthritis) and skin (e.g. bullous pemphigoid) and this activity is dependent on antibodies and complement components. Hence, symptoms associated with these conditions may also be alleviated upon treatment with a mast cell stabilizing compound as disclosed in the present invention.

In yet another embodiment, the compound is used in a method for treatment of pathological mineral resorptive states. For example, a composition is formulated for treating bone resorption and comprises one or more known anti-osteoporotic agents, e.g. selected from anabolic steroids, various phosphorus-containing agents, vitamin D and related substances, estrogenic steroids, and calcitonin. Also, certain aromatic carboxylic acids have been described as useful anti-osteoporotic agents. For a detailed review and discussion of such anti-osteoporotic agents, see U.S. Pat. Nos. 4,125,621 or 4,101,688.
A further aspect of the invention relates to the use of an azo-linked bis-chromonyle compound for use as in vitro mast cell stabilizer. For example, provided is a method for investigating in vitro the mechanism of mast cell activation, comprising contacting a mast cell culture with a mast cell stabilizing azo-containing bis-chromonyle compound as provided herein, wherein said compound is able to switch reversibly between an active state and an inactive state when irradiated with light. The light of a first wavelength to switch the compound from the inactive state to an active state and irradiated with the light of a second wavelength is different to the first wavelength to switch the compound from the active state to the inactive state. Preferably, the compound is \((E)-5,5'-(\text{diazene-1,2-diyldiylbis(4,1-phenylene)})\text{bis(methylene)}\) \(\text{bis(oxy)}\text{bis}(4\text{-oxo-4H-chromene-2-carboxylate})\).

Azo-linked bis-chromonyle compounds of the invention can be synthesized using standard organic chemistry using commercially available starting materials. The skilled person will be able to select the starting compounds and the appropriate synthetic schemes.

In one embodiment, a method for preparing a compound wherein \(n=0\) comprises the steps of (i) providing chromone ethyl ester from hydroxyacetophenone and diethyloxalate (ii) converting chromone ethyl ester into nitroso chromone via sequential nitration, reduction and oxidation; (hi) azobenzene formation from nitroso chromone and amino chromone; and (iv) saponification. See for example Scheme 1 herein below.

In another embodiment, the invention provides a method for preparing a compound according to Formula I wherein \(n=1\) comprising the steps of (i) reacting \(m/p\)-toluidine and \(m/p\)-nitroso toluene to afford methylated azobenzene (ii) subjecting methylated azobenzene to radical bromination (iii) introducing -5-hydroxychromone methyl ester to both methylene groups and (iv) saponification. See for example Scheme 2 herein below.
LEGEND TO THE FIGURES

**Figure 1:** Molecular structures of inhibitors. (A) Molecular structure of the prior art mast cell activation inhibitor DiSodiumCromoglycate (DSCG) and the three exemplary novel AzoChromone compounds MAC, PAC and DAC. (B) Principle of photoswitchable mast cell activation inhibitors Envisioned mode of action of MAC and PAC. When two chromone groups (illustrated as spheres) of the inhibitor in one of the photoisomeric forms are bound to the cromolyn binding protein (CBP) mast cell activation is inhibited. When the molecule photoisomerizes to the other form, only one chromone group can bind and mast cell activation is not inhibited.

**Figure 2:** Photochemical properties of novel AzoChromone compounds DAC, MAC and PAC. (A-C) UV-Vis absorption spectra of DAC (125 µM), MAC (128 µM) and PAC (100 µM) in water. The black lines represent the spectra of the non-irradiated forms and the grey lines of the 365 nm irradiated forms. (D-F) Reversible photochromism of photoswitchable inhibitors.

**Figure 3:** Concentration response curves of mast cell activation inhibitors (n=3). Degranulation was evoked by 1.0 µM of Compound 48/80. The novel mast cell activation inhibitors MAC and PAC show inhibitory activity in the same concentration range with IC50 values of 10 µM and 28 µM respectively. DAC shows to be a less potent inhibitor with an IC50 value of 272 µM, but is still more potent than the original drug DSCG, which did not show any inhibitory activity under these conditions (IC50>1 mM).

**Figure 4:** Inhibitory activity of the non-irradiated and 365 nm light-irradiated forms PAC. A difference in activity was observed between the
two forms of PAC. Non-irradiated PAC was 25% more active than irradiated PAC at a concentration of 10 \( \mu \text{M} \).

**Figure 5**: Exemplary Azo-chromone compounds of the invention.

**EXPERIMENTAL SECTION**

The invention is exemplified by the design and synthesis of three analogs of DSCG, with an azobenzene photoswitch incorporated in their structure. The inhibitory capacity of the molecules, named DAC, MAC and PAC (Figure 1A), in both isomeric forms was tested on human mast cell cultures. Remarkably, the molecules showed to have a considerably higher inhibitory effect on an evoked degranulation in mast cell cultures as compared to DSCG. Furthermore, PAC showed a difference in inhibitory capacity in two isomeric forms. It is anticipated that external control of mast cell activation can be achieved by switching between the two forms with light.

**Materials and Methods**

**LAD2 Cell culture** The human mast cell line LAD2 was kindly provided by Dr. A. Kirshenbaum (National Institute of Allergy and Infectious Diseases, Bethesda, MD). LAD2 cells were cultured in suspension at 37 °C, 95% relative humidity and 5% CO\(_2\) in StemPro-34 Serum-free medium (Invitrogen) supplemented with 2 mM L-glutamine (Sigma Aldrich), 100 IU/mL penicillin (Sigma Aldrich), 100 \( \mu \text{g/mL} \) streptomycin (Sigma Aldrich) and 100 ng/ml rhSCF (Invitrogen). Cells were not allowed to grow beyond a density of 5.0 \( \times \) 10\(^5\) cells/mL and fresh medium was supplied once a week.

**\( \beta \)-Hexosaminidase Assay** \( \beta \)-Hexosaminidase release was measured according to a previously published method.\(^{28}\) In short, 50 \( \mu \text{l} \) of supernatant was transferred to a 96-well plate. To this 100 \( \mu \text{l} \) of a 2 mg/mL solution of p-
nitrophenyl- N-acetyl-D-glucosaminidine in citrate buffer pH 4.5 was added. The plate was incubated at 37 °C for 1 h. The reaction was stopped by adding 100 µL of 0.4 M glycine solution and the absorbance was measured on a plate reader at a wavelength of 405 nm. Subsequently, the percentage released β-hexosaminidase was determined by comparison with the absorption of cell lysate of the same cells using the following formula: 

$$\frac{\text{supernatant}}{\text{supernatant} \times \text{lysate}} \times 100 = \% \text{Released } \beta-\text{Hexosaminidase.}$$

**Inhibitory Activity Tests** LAD2 cells were suspended in HEPES buffer at a concentration of 50,000 cells/mL. 80 µL of cell suspension was transferred to a microreaction cup to which 10 µL of inhibitor solution was added. This was incubated at 37 °C for 30 min after which 10 µL, of a Compound 48/80 solution was added. Next, the cell suspension was incubated for 30 min at 37 °C and the β-hexosaminidase release was assayed.

**Photoswitching Experiments** Irradiation experiments were performed with a Spectroline ENB-280C/FE UV lamp (365 nm) and Thor Labs OSL1-EC Fiber Illuminator (white light).

**Synthesis. General.** For synthesis all chemicals were obtained from commercial sources and used as received unless stated otherwise. Solvents were reagent grade. Thin-layer chromatography (TLC) was performed using commercial Kieselgel 60, F254 silica gel plates. Flash chromatography was performed on silica gel (Silicycle Siliaflash P60, 40-63 m, 230-400 mesh). Drying of solutions was performed with MgSO₄ and solvents were removed with a rotary evaporator. Chemical shifts for NMR measurements were determined relative to the residual solvent peaks. The following abbreviations are used to indicate signal multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; brs, broad signal; app’t, apparent triplet. HRMS (ESI) spectra were obtained on a Thermo scientific LTQ Orbitrap XL. Melting points were recorded using a Buchi melting point B-545 apparatus. UV/Vis absorption spectra were recorded on an Agilent 8453 UV-Visible
Spectrophotometer using Uvasol grade solvents. All compounds whose IC50 values were determined had purities ≥ 95%. Purities were determined with analytical RP-HPLC performed on a JASCO PU-980 chromatography system using a GRACE Alltima HP C18 5µ column. Compounds were detected with a JASCO UV-1575 UV-Vis intelligent detector.

**Ethyl 4-oxo-4H-chromene-2-carboxylate** (1) 2-Hydroxy acetophenone (2.04 g, 15 mmol) and diethyl oxalate (5.12 g, 35 mmol) were dissolved in ethanol (10 mL) and added to a solution of sodium (1.49 g, 65 mmol) in ethanol (100 mL). The reaction mixture was heated at reflux for 1 h, after which it was cooled down and acidified with concentrated HCl until a white precipitate was formed. The precipitate was filtered off and the filtrate was concentrated and extracted with ethyl acetate, washed with brine and dried (MgSO4). After evaporation a solid was obtained. This was recrystallized from methanol/diisopropylether (4:1) to yield 2.45 g (75%) of a white solid. Mp. 66-68°C.

^H-NMR (400 MHz, CDCl3): δ 8.21 (dd, J = 7.9, 1.7 Hz, 1H), 7.75 (ddd, J = 8.7, 7.1, 1.7 Hz, 1H), 7.62 (dd, J = 8.6, 1.0 Hz, 1H), 7.46 (ddd, J = 8.1, 7.1, 1.1 Hz, 1H), 7.12 (s, 1H), 4.47 (q, J = 7.1 Hz, 4H), 1.41 (t, J = 7.2 Hz, 3H). ^H-NMR spectrum in agreement with published data. 22

**Ethyl 6-nitro-4-oxo-4H-chromene-2-carboxylate** (2) Compound 1 (200 mg, 0.92 mmol) was suspended in 65% nitric acid (0.12 mL) and the mixture was cooled on ice. Concentrated sulfuric acid (1.5 mL) was added and the mixture was stirred for 2 h at room temperature, poured on ice-cooled water and the formed precipitate was filtered off to yield 195 mg (81%) of a white solid. Mp. 178-179 °C. ^H-NMR (400 MHz, CDCl3): δ 9.07 (d, J = 2.8 Hz, 1H), 8.57 (dd, J = 9.2, 2.8 Hz, 1H), 7.78 (d, J = 9.2 Hz, 1H), 7.19 (s, 1H), 4.50 (q, J = 7.2 Hz, 2H), 1.46 (t, J = 7.1 Hz, 3H). ^C-NMR (100 MHz, CDCl3): δ 176.8, 159.7, 158.7, 152.8, 145.2, 128.9, 124.4, 122.5, 120.6, 115.0, 63.5, 14.1. HR-MS (ESI, [M+H]+): Calcd. for C12H10NO6: 264.0508; Found: 264.0502.
Ethyl 6-amino-4-oxo-4H-chromene-2-carboxylate (3) Compound 2

(300 mg, 1.14 mmol) was dissolved in benzene (100 mL) and stirred overnight with 10% palladium on charcoal (15 mg) under a hydrogen atmosphere (balloon) at room temperature. The mixture was filtered over celite. The filtrate was evaporated yielding 300 mg of orange needles. Mp. 177-178 °C. $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 7.45 (d, $J =$ 9.0 Hz, 1H), 7.35 (d, $J =$ 2.9 Hz, 1H), 7.11 - 7.03 (m, 2H), 4.45 (q, $J =$ 7.1 Hz, 2H), 3.92 (brs, 2H), 1.43 (t, $J =$ 7.1 Hz, 3H). $^1$C-NMR (100 MHz, CDCl$_3$): $\delta$ 178.4, 160.8, 151.7, 149.6, 144.6, 125.4, 123.2, 119.8, 113.6, 107.4, 62.8, 14.1. HR-MS (ESI, [M+H]$^+$): Calcd. for C12H12NO4: 234.0766; Found: 234.0762.

Ethyl 6-nitroso-4-oxo-4H-chromene-2-carboxylate (4) Compound 3

(100 mg, 0.43 mmol) was dissolved in DCM (2 mL), a solution of ozone (422 mg, 0.69 mmol) in H2O (8 mL) was added to this and the biphasic mixture was stirred at room temperature for 2 h. The organic layer was separated and the aqueous layer was extracted twice with DCM. The combined organic layers were washed with 1 M aq. HCl, saturated NaHCO$_3$ and brine and dried (MgSO$_4$). Evaporation of the solvent yielded 75 mg (70%) of a green solid. Mp. 144-145 °C. $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 9.31 (d, $J =$ 2.1 Hz, 1H), 7.77 - 7.65 (m, 2H), 7.21 (s, 1H), 4.50 (q, $J =$ 7.1 Hz, 2H), 1.46 (t, $J =$ 7.1 Hz, 3H). $^1$C-NMR (100 MHz, CDCl$_3$): $\delta$ 177.9, 161.6, 159.9, 159.2, 152.6, 125.6, 124.9, 120.8, 120.2, 115.0, 63.4, 14.1. HR-MS (ESI, [M+H]$^+$): Calcd. for C12H10NO5: 248.0559; Found: 248.0552.

Diethyl 6,6’-(diazenyl-1,2-diyl)bis(4-oxo-4H-chromene-2-carboxylate) (5) Compounds 3 (70.8 mg, 0.304 mmol) and 4 (75.0 mg, 0.304 mmol) were dissolved in glacial acetic acid (2.5 mL) and stirred for 2 d. The solution was diluted with water and extracted with DCM. The organic phase was washed with water (4x) and brine and dried (MgSO$_4$). Recrystallization from DCM yielded 60 mg (43%) of a light orange solid. Mp. 254-255 °C (dec). $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 8.79 (d, $J =$ 2.4 Hz, 2H), 8.35 (dd, $J =$ 9.0, 2.4 Hz, 2H), 7.77 (d, $J =$ 9.0 Hz, 2H), 7.18 (s, 2H), 4.50 (q, $J =$ 7.2 Hz,
4H), 1.46 (t, J = 7.1 Hz, 6H). $^1$H-NMR (100 MHz, CDC1$_3$): δ 178.1, 160.3, 157.5, 152.4, 149.4, 127.2, 125.0, 122.8, 120.2, 114.9, 63.2, 14.1. $^1$C-NMR (100 MHz, CDC1$_3$): δ 178.1, 160.3, 157.5, 152.4, 149.4, 127.2, 125.0, 122.8, 120.2, 114.9, 63.2, 14.1. HR-MS (ESI, [M+H]$^+$): Calcd. for C$_{25}$H$_{18}$N$_2$O$_8$: 463.1136; Found: 463.1131.

Sodium (E)-6,6′-(diazene-1,2-diyl)bis(4-oxo-4H-chromene-2-carboxylate) (6) (DAC) To a solution of compound 5 (50 mg, 0.11 mmol) in ethanol (2 mL) was added aq. NaOH (2.5 M, 0.128 mL) dropwise at 0 °C. The reaction mixture was heated at reflux for 2 h after which the insoluble product was filtered off and purified by recrystallization from methanol yielding 45 mg (91%) of a red solid. H -NMR (400 MHz, Methanol-d$_4$): δ 8.71 (d, J = 2.4 Hz, 2H), 8.43 (dd, J = 9.0, 2.5 Hz, 2H), 7.90 (d, J = 9.0 Hz, 2H), 7.04 (s, 2H). $^1$H-NMR (400 MHz, Methanol-d$_4$): δ 180.2, 164.2, 160.3, 157.9, 149.3, 126.7, 124.2, 121.5, 120.3. $^1$C-NMR (100 MHz, Methanol-d$_4$): δ 180.2, 164.2, 160.3, 157.9, 149.3, 126.7, 124.2, 121.5, 120.3. HR-MS (ESI, [M+Na]$^+$): Calcd. for C$_{20}$H$_{19}$N$_2$O$_8$Na: 451.0154; Found: 451.0149.

Methyl 5-hydroxy-4-oxo-4H-chromene-2-carboxylate (7) 2,6-Dihydroxyacetophenone (1.50 g, 9.90 mmol) and dimethyloxalate (5.80 g, 49.3 mmol) were dissolved in 0.5 M MeONa/MeOH (100 mL) and heated at reflux overnight. The solvent was removed in vacuo and the slurry was dissolved in water (100 mL) and subsequently acidified with concentrated HCl. The precipitate was filtered off and dissolved in MeOH (50 mL) and concentrated HCl (10 mL). This solution was heated at reflux for 2 h after which the crude product was purified by flash chromatography (Silicagel, 40-63 μη, pentane/AcOEt, 9:1, v/v) yielding 762 mg (33%) of a yellow powder. H -NMR (400 MHz, CDCl3): δ 7.61 (appt, J=8.4 Hz, 1H), 7.03-7.06 (m, 2H), 6.85 (d, J=8.3 Hz, 1H), 4.02 (s, 3H). H -NMR spectrum in agreement with published data. 24

l-Methyl-3-nitrosobenzene (8a) 3-Methylaniline (708 mg, 6.61 mmol) was dissolved in DCM (20 mL), a solution of oxone (8.10 g, 13.2 mmol) in water (80 mL) was added to this and the resulting biphasic mixture was stirred at room temperature for 30 min. The organic layer was separated and the aqueous layer was extracted twice with DCM. The combined organic
layers were washed with 1 M aq. HC1, saturated NaHCOe and brine and
dried (MgSO\(_4\)). The crude product was purified by flash chromatography
(Silicagel, 40-63 µm, pentane/AcOEt, 4:1, v/v), yielding 430 mg (54%) of a
light green solid. \(\text{H}^1\)-NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.77 (d, \(J=6.4\) Hz, 1H), 7.63
(s, 1H), 7.48-7.54 (m, 2H), 2.50 (s, 3H). \(\text{H}^1\)-NMR spectrum in agreement with
published data. \(^{32}\)

1-Methyl-4-nitrosobenzene (8b) 4-Methylaniline (5.00 g, 33.1 mmol) was dissolved in DCM (100 mL), a solution of oxone (40.7 g, 66.2 mmol) in
water (400 mL) was added to this and the resulting biphasic mixture was
stirred at room temperature for 30 min. The organic layer was separated
and the aqueous layer was extracted twice with DCM. The combined organic
layers were washed with 1 M aq. HC1, saturated NaHCOe and brine and
dried (MgSO\(_4\)). Evaporation of the solvent yielded 2.34 g (59%) of a light
green solid. \(\text{H}^1\)-NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.81 (d, \(J=8.2\) Hz, 2H), 7.39 (d,
\(J=8.0\) Hz, 2H), 2.44 (s, 3H). \(\text{H}^1\)-NMR spectrum in agreement with published
data. \(^{33}\)

3,3'-Dimethylazobenzene (9a) Compound 8a (500 mg, 4.13 mmol) and 3-methylaniline (369 mg, 3.44 mmol) were dissolved in glacial acetic
acid (33 mL) and the mixture was stirred overnight. The solution was
diluted with water and extracted with ethyl acetate. The organic phase was
washed four times with water and once with brine and dried (MgSO\(_4\)). The
crude product was filtered through silica yielding 400 mg (55%) of an orange
solid. \(\text{H}^1\)-NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.70-7.74 (m, 4H), 7.41 (appt, \(J=8.0\) Hz, 2H), 7.29 (d, \(J=7.6\) Hz, 2H), 2.46 (s, 6H). \(\text{H}^1\)-NMR spectrum in agreement
with published data. \(^{34}\)

4,4'-Dimethylazobenzene (9b) Compound 8b (300 mg, 2.48 mmol) and 4-methylaniline (292 mg, 2.73 mmol) were dissolved in glacial acetic
acid (20 mL) and stirred overnight. The solution was diluted with water and
extracted with ethyl acetate. The organic phase was washed four times with
water and once with brine and dried (MgSO\(_4\)). The crude product was
purified by flash chromatography (Silicagel, 40-63 µm, pentane/Et20, 9:1, v/v) yielding 450 mg (86%) of an orange solid. H-NMR (400 MHz, CDCl3): δ 7.81 (d, J=8.4 Hz, 4H), 7.30 (d, J=8.4 Hz, 4H), 2.43 (s, 6H). H-NMR spectrum in agreement with published data. 35

3,3'-Bis(bromomethyl)azobenzene (10a) To a solution of compound 9a (1.30 g, 6.18 mmol) in 60 mL of CC14 was added NBS (2.50 g, 14.2 mmol) and AIBN (80 mg, 0.48 mmol). The resultant solution was stirred overnight at 70°C, then filtered and the filtrate was washed with hot water and brine and dried (MgSO4). After evaporation the product was recrystallized from acetonitrile yielding 800 mg (35%) of an orange solid. Mp. 140-141 °C. H-NMR (400 MHz, CDCl3): δ 7.95 (m, 2H), 7.87 (m, 2H), 7.52 (m, 4H), 4.59 (s, 4H). H-NMR spectrum in agreement with published data. 34

4,4'-Bis(bromomethyl)azobenzene (10b) To a solution of compound 9b (300 mg, 1.43 mmol) in 20 mL of CC14 was added NBS (584 mg, 3.30 mmol) and AIBN (18 mg, 0.10 mmol). The resultant solution was stirred overnight at 70 °C, then filtered and the filtrate was washed with hot water and brine and dried (MgSO4). After evaporation of the solvent, the product was recrystallized from acetonitrile yielding 220 mg (42%) of an orange solid. Mp. 183-185 °C. H-NMR (400 MHz, CDCl3): δ 7.89 (d, J=8.4 Hz, 4H), 7.54 (d, J=8.4 Hz, 4H), 4.56 (s, 4H). H-NMR spectrum in agreement with published data. 35

(E)-dimethyl5,5'-(((diaze-ne-1,2-diylbis(3,1-phenylene))bis(methylene))bis(ox y))bis(4-oxo-4H-chromene-2-carboxylate) (11a) To a solution of compounds 7 (638 mg, 2.73 mmol) and 10a (400 mg, 1.09 mmol) in 80 mL acetonitrile was added K2CO3 (451.93 mg, 3.27 mmol) and the resulting mixture was stirred overnight at 65 °C. The solution was concentrated in vacuo and the crude product was purified using flash chromatography (Silicagel, 40-63 µm, DCM/Methanol, 95:5, v/v) yielding 520 mg (70%) of a light orange solid. H-NMR (400 MHz, CDCl3): δ 8.06 (s, 2H), 7.90-7.85 (m, 4H), 7.56-7.61 (m, 4H), 7.18 (d, J = 8.5 Hz, 2H), 7.02 (s, 2H),
6.91 (d, J = 8.3 Hz, 2H), 5.38 (s, 4H), 4.00 (s, 6H). ^13C NMR (100 MHz, CDC1₃): δ 177.6, 161.1, 158.4, 158.0, 152.7, 150.1, 137.5, 134.7, 129.7, 129.4, 122.6, 120.7, 116.7, 113.8, 111.1, 108.9, 70.6, 53.4. HR-MS (ESI, [M+H]^+): Calcd. for C₃₆H₂₇N₂O₁₀: 641.1167; Found: 641.1160.

(E)-dimethyl5,5’-(((diazenyl-1,2-diylbis(4,1-phenylene))bis(methylene))bis(oxy))bis(4-oxo-4H-chromene-2-carboxylate) (lib) To a solution of compounds 7 (100 mg, 0.45 mmol) and 10b (75.8 mg, 0.21 mmol) in acetonitrile (25 mL) was added CS₂CO₃ (201 mg, 0.62 mmol) and the resulting mixture was stirred at 65 °C for 3 h. The solution was concentrated in vacuo and the residue was dissolved in DCM, washed with 1 M aq. HCl, saturated NaHCO₃ and brine and dried (MgSO₄). After evaporation the product was recrystallized from DCM yielding 27 mg of a light orange solid. Mp. 235-237 °C (dec). ^1H-NMR (400 MHz, CDC1₃): δ 7.96 (d, J = 8.0 Hz, 4H), 7.76 (d, J = 8.1 Hz, 4H), 7.58 (t, J = 8.0 Hz, 2H), 7.18 (d, J = 8.2 Hz, 2H), 7.02 (s, 2H), 6.90 (d, J = 8.3 Hz, 2H), 5.36 (s, 4H), 4.00 (s, 6H). ^13C-NMR (100 MHz, CDC1₃): δ 177.7, 161.1, 158.4, 158.0, 152.2, 150.1, 139.3, 134.6, 127.2, 123.2, 116.6, 113.8, 111.1, 108.7, 70.5, 53.4. HR-MS (ESI, [M+H]^+): Calcd. for C₃₆H₂₇N₂O₁₀: 647.1485; Found: 647.1480.

Sodium (E)-5,5’-(((diazenyl-1,2-diylbis(3,1-phenylene))bis(methylene))bis(oxy))bis(4-oxo-4H-chromene-2-carboxylate) (12a) (MAC) To a solution of compound 11a (100 mg, 0.15 mmol) in ethanol (5 mL) was added aq. NaOH (2.5 M, 186 µL) dropwise at 0°C. The reaction mixture was heated at reflux for 2 h after which the insoluble product was filtered off yielding 85 mg (86%) of an orange solid.

^1H-NMR (400 MHz, Methanol-d₄): δ 8.12 (s, 2H), 7.86 (d, J = 8.0 Hz, 2H), 7.81 (d, J = 8.0 Hz, 2H), 7.63 (m, 4H), 7.26 (d, J = 8.4 Hz, 2H), 7.06 (d, J = 8.4 Hz, 2H), 6.88 (s, 2H), 5.42 (s, 4H). ^13C-NMR (100 MHz, Methanol-d₄): δ 180.4, 164.7, 158.3, 158.1, 157.9, 152.7, 138.3, 134.5, 129.1, 121.2, 113.8, 112.5, 110.9, 108.5, 69.9. HR-MS (ESI, [M+Na]^+): Calcd. for C₃₄H₂₂N₂O₁₀Na: 641.1167; Found: 641.1140.
Sodium (E)-5,5'-(((diazene-1,2-diylbis(4,1-phenylene))bis(methylene))bis(oxy))bis(4-oxo-4H-chromene-2-carboxylate) (12b) (PAC) To a solution of compound **lib** (25 mg, 0.04 mmol) in ethanol (3 mL) was added aq. NaOH (2.5 M, 50 µL) dropwise at 0 °C. The reaction mixture was heated at reflux for 2 h after which the insoluble product was filtered off yielding 23 mg (91%) of an orange solid. 1H-NMR (400 MHz, Methanol^): δ 7.96 (d, J = 8.3 Hz, 4H), 7.80 (d, J = 8.2 Hz, 4H), 7.67 (t, J = 8.4 Hz, 2H), 7.28 (d, J = 8.5 Hz, 2H), 7.05 (d, J = 8.3 Hz, 2H), 6.89 (s, 2H), 5.41 (s, 4H). 13C-NMR (100 MHz, Methanol^): δ 180.3, 164.6, 158.3, 158.1, 158.0, 155.8, 152.1, 140.2, 134.4, 127.3, 122.5, 112.5, 110.9, 108.4, 69.8. HR-MS (ESI, [M+Na]^+): Calcd. for C_{34}H_{22}N_{2}O_{10}Na: 641.1167; Found: 641.1155.

**Design of photoswitchable inhibitors** The structure of photoswitchable inhibitors DAC, MAC and PAC was based on the known mast cell activation inhibitor DSCG. This drug consists of two chromone groups linked by a spacer (Fig. 1A). The length of the spacer influences the inhibitory activity of the drug. We chose an azobenzene unit as the photoswitch, since the difference in structure and length between the two forms (the trans and cis isomer) of this photoswitch is relatively large.

We hypothesized that both chromone groups need to bind to the CBP for the drug to have an optimal inhibitory effect (Figure IB). With this in mind we designed three photoswitchable inhibitors called Di-AzoChromone (DAC), Meta-AzoChromone (MAC) and Para-AzoChromone (PAC) (Fig. 1A). The first photoswitchable inhibitor, DAC, was designed in such a way that the azobenzene photoswitch integrates two chromone groups in its structure. This reduced the length of the spacer as compared to DSCG and rendered the molecule more rigid, which could result in a larger difference in inhibitory activity between the two photoisomeric forms. DAC seemed to be a more potent mast-cell activation inhibitor than DSCG (vide infra, Fig.
3), however, a significant change in inhibitory activity after photoisomerization was not observed (Fig. 4). Therefore two second-generation photoswitchable inhibitors were designed. Compounds MAC and PAC (Figure 1A) consist of an azobenzene moiety with two chromone groups linked to it in the meta and para position, respectively. A methylene group was placed in-between the azobenzene and chromone groups to introduce more flexibility in the molecule. This flexibility might be favorable when two chromone groups need to interact with the CBP at the same time (Fig. 1B).

**Synthesis**

The photoswitchable inhibitor DAC was synthesized starting from chromone ethyl ester 1 (Scheme 1), which was obtained from hydroxyacetophenone and diethyl oxalate. Compound 1 was converted into nitroso chromone 4 via sequential nitration, reduction and oxidation reactions. Azobenzene formation from precursors 3 and 4 and a sequential saponification step afforded the photoswitchable inhibitor DAC 6.

Compounds MAC and PAC were prepared in six steps as depicted in Scheme 2. The key features of these syntheses include a formation of a diazo compound by a reaction between m/p-toluidine and m/p-nitroso toluene (8a and 8b) to afford methylated azobenzenes (9a and 9b), which were subjected to radical bromination to yield compound 10a and 10b. Via a Williamson reaction, 5-hydroxycromone methyl ester 7, obtained from 1,2-dihydroxyacetophenone and dimethyl oxalate, was introduced to both methylene groups of the azobenzenes 10a and 10b resulting in 11a and 11b. A saponification step afforded the sodium salts of the photoswitchable inhibitors MAC (12a) and PAC (12b).
Scheme 1

Reagents and conditions: (i) diethyl oxalate, NaOEt/EtOH, H2SO4 (75%); (ii) H2SO4, HNO3, rt, 1 h (81%); (iii) H2, Pd/C, benzene, rt, 16 h (75%); (iv) Oxone, DCM/water, rt, 2 h (70%); (v) compound, glacial AcOH, rt, 2 d (43%); (vi) EtOH, NaOH, reflux, 2 h (91%).

Scheme 2

Reagents and conditions: (i) dimethyl oxalate, NaOMe/MeOH, HCl (33%); (ii) Oxone, DCM/water, rt, 1 h (8a: 54%; 8b: 25%); (iii) m/p-toluidine, glacial AcOH, rt, 16 h (9a: 55%; 9b: 86%); (iv) NBS, AIBN, CC14, reflux, 16 h (10a: 35%; 10b: 42%); (v) Cs2CO3, MeCN, reflux, 5 h (11a: 70%; 11b: 35%); (vi) EtOH, NaOH, reflux, 2 h (12a: 86%; 12b: 91%).
Photoswitchable behavior of designed mast cell activation inhibitors

The photoswitchable behavior of DAC, MAC and PAC was studied using UV-VIS spectroscopy and RP-HPLC. The trans-isomers of DAC, MAC and PAC have a characteristic absorbance maximum between 300 and 400 nm in water. This absorbance maximum decreases when the molecules are photoswitched to their cis-isomers and a new absorbance maximum appears around 430 nm (Fig. 2A-C). Isosbestic points at 405 nm (DAC), 270 nm (MAC) and 270 nm (PAC) clearly illustrate the selective isomerization and reversibility of the photoswitching process. Using RP-HPLC, the ratio of photoisomers was determined for both the non-irradiated and 365 nm-irradiated forms of DAC, MAC and PAC (Table 1). Higher photostationary states were obtained when the photoswitchable inhibitors were irradiated with 365 nm light in DMSO as compared to water (Table 1). Therefore, the compounds were switched at a high concentration in DMSO and diluted in water to obtain a maximum concentration of 1% DMSO (v/v). Thus obtained samples were used for inhibitory activity studies. The inhibitors could be photoisomerized by alternating irradiation at 365 nm and 400-700 nm, for at least 5 times showing little fatigue (Fig. 2D-F).

Table 1 Ratio of trans and cis isomers of the photoswitchable inhibitors under different conditions. Ratios are determined at the isosbestic point (DAC=405 nm, MAC=270 nm, PAC=270 nm) with RP-HPLC (C18, flow 1 mL/min, MeCN and H$_3$PO$_4$ pH=3.0).

<table>
<thead>
<tr>
<th></th>
<th>DAC (trans.xis)</th>
<th>MAC (trans.xis)</th>
<th>PAC (trans.xis)</th>
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<tr>
<td>Non-Irradiated</td>
<td>99:1</td>
<td>88:12</td>
<td>91:9</td>
</tr>
<tr>
<td>Irradiated (water)</td>
<td>59:41</td>
<td>45:55</td>
<td>56:44</td>
</tr>
<tr>
<td>Irradiated (DMSO)</td>
<td>48:52</td>
<td>38:62</td>
<td>19:81</td>
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</table>
Inhibitory activity  The inhibitory activity of DAC, MAC and PAC was determined on the LAD2 mast cell line using a β-hexosaminidase release assay. β-Hexosaminidase is an enzyme that is secreted by mast cells upon activation and therefore its activity is a reliable measure for the degree of mast cell degranulation. LAD2 cells were incubated with different concentrations of DSCG and non-irradiated DAC, MAC and PAC for 30 min after which degranulation was evoked by the secretagogue Compound 48/80. From Fig. 3, it is apparent that DAC, MAC and PAC show significantly greater inhibitory activity than the original drug DSCG. MAC and PAC seem to have an almost 100 times greater inhibitory potency than DSCG. Furthermore, MAC and DAC show greater efficacy than PAC. At the concentration of Compound 48/80 (1.0 µM) that was used in this assay, DSCG did not show any inhibitory activity. At lower concentrations of Compound 48/80 (0.1 µM), DSCG was able to inhibit mast cell degranulation (data not shown).

Next, the difference in inhibitory activity between the two photoisomers of DAC, MAC and PAC was determined. The inhibitory activity was determined for each compound at two concentrations that were on the steep part of the concentration response curve, because at these concentrations a possible difference in inhibitory activity is best detectable. LAD2 cells were incubated with either non-irradiated solutions of DAC, MAC and PAC or with solutions that were irradiated with 365 nm light prior incubation. After evoking a degranulation with Compound 48/80, β-hexosaminidase release was determined. A significant difference was observed between the irradiated and non-irradiated form of PAC. At a concentration of 10 µM the relative difference in inhibitory activity between the two forms of PAC is 25% (see Figure 4). The non-irradiated form, which consists of 91% trans-
PAC in the photo stationary state (PSS), is more active than the irradiated form which is 81% cis-PAC in the PSS.
REFERENCES


(14) Klemm, S.; Ruland, J. *Immunobiology* 2006, 211, 815.


Claims

1. A bis-chromonyl compound of the general formula

$$\text{Formula I}$$

or a pharmaceutically acceptable salt, ester or amide thereof,
wherein

- $n$ is 0 or 1;
- $Y_1$ is $X_1$-$Ari$ or $O$-$X_1$-$Ari$ and $Y_2$ is $X_2$-$Ari$ or $O$-$X_2$-$Ari$; wherein Ari and Ari are bound to N=N;
- wherein $X_1$ and $X_2$ are the same or different and each is a substituted or unsubstituted straight hydrocarbon chain of 1 to 3 carbon atoms;
- wherein Ari and Ari are the same or different and each is a 5- or 6-membered aromatic or heteroaromatic ring, optionally substituted with one or more substituents selected from the group consisting of halogen, hydroxy, substituted or unsubstituted C1-C4 alkyl, substituted or unsubstituted C1-C4 alkoxy; and
- $R_1$, $R_2$, $R_3$, $R_4$, $R_5$ and $R_6$ are the same or different and each is selected from the group consisting of H, halogen, nitro, amino, cyano, hydroxyl, substituted or unsubstituted C1-C4 alkyl, substituted or unsubstituted C1-C4 alkoxy.
2. Bis-chromonyl compound according to claim 1, wherein Ari and Ar2 are independently selected from the group consisting of benzene, pyridine, pyrrole, furan, thiophene, pyrazole, imidazole, isoxazole, oxazole, isothiazole, thiazole, pyrazine, pyridazine, pyrimidine and pyrazine.

3. Bis-chromonyl compound according to claim 1 or 2, wherein Yi is O-Xi-Ari and/or Y2 is O-X2-Ar2, preferably wherein Yi is O-Xi-An and Y2 is O-X2-Ar2.

4. Bis-chromonyl compound according to any of claims 1 to 3, wherein Yi and Y2 are the same.

5. Bis-chromonyl compound according to claim 4, wherein Yi and Y2 are O-X-Ar, wherein Ar is selected from the group consisting of benzene, pyridine, pyrrole, furan, thiophene, preferably benzene.

6. Bis-chromonyl compound according to any of the preceding claims, wherein each of R2, R3, R5 and R6 is hydrogen, preferably wherein each of R1, R2, R3, R4, R5 and R6 is hydrogen.

7. Bis-chromonyl compound selected from

(¾)-6,6'-(diazene-1,2-diyl)bis(4-oxo-4H-chromene-2-carboxylate) (DAC)

(E)-5,5'-((((diazene-1,2-diylbis(3,1-phenylene))bis(methylene))bis(oxy))bis(4-oxo-4H-chromene-2-carboxylate) (MAC)

(¾)-5,5'-((((diazene-1,2-diylbis(4,1-phenylene))bis(methylene))bis(oxy))bis(4-oxo-4H-chromene-2-carboxylate)(PAC)
(£J)-5,5’-(((5,5’-(diazene-1,2-diyl)bis(pyridine-5,3-diyl))bis(methylene))bis(oxy))bis(4-oxo-4H-chromene-2-carboxylic acid)
(¾-5,5’-(((4,4’-(diazene-1,2-diyl)bis(thiazole-4,2-diyl))bis(methylene))bis(oxy))bis(4-oxo-4H-cliromene-2-carboxylic acid)

(£T)-6-(2-(4-((3-(2-carboxy-4-oxo-4H-chromen-5-yl)phenyl)diazenyl)thiophen-2-yl)ethyl)-4-oxo-4H-chromene-2-carboxylic acid; and

(£J)-6-((5-((2-carboxy-4-oxo-4H-chromen-6-yl)diazenyl)furan-2-yl)oxy)-4-oxo-4H-chromene-2-carboxylic acid

8. A pharmaceutical composition comprising a bis-chromonyl compound according to any of claims 1-7 and a pharmaceutically active carrier, diluent or excipient.

9. Pharmaceutical composition according to claim 8 formulated as nasal spray, inhalator, eye drops, oral formulation, nebulizer solution or topical formulation.

10. Pharmaceutical composition according to claim 9, comprising at least one further ingredient capable of inhibiting the release of mediators from mast cells.

11. A bis-chromonyl compound according to any of claims 1 to 7 for use as a therapeutic or prophylactic agent.

12. Bis-chromonyl compound according to any one of claims 1-7 for use in a method of treatment or prophylaxis of a disease involving unwanted or excessive mast cell activation.
13. Bis-chromonyl compound for use according to claim 12, wherein the
disease is an allergic disease.

14. Bis-chromonyl compound for use according to claim 12 or 13, wherein
the disease is selected from the group consisting of asthma, allergic rhinitis,
mastocytosis, dermatographic urticaria, eczema, psoriasis, ulcerative colitis,
food allergy, vulvar vestibulitis.

15. Bis-chromonyl compound according to any one of claims 1-7 for use as
in vitro mast cell stabilizer.

16. A method of treatment or prophylaxis of a disease involving
unwanted or excessive mast cell activation, comprising administering to a
mammalian subject in need thereof a therapeutically effective amount of a
bis-chromonyl compound according to any one of claims 1-7.

17. Method according to claim 16, wherein the disease is an allergic
disease.

18. Method according to claim 16 or 17, wherein the disease is selected
from the group consisting of asthma, allergic rhinitis, mastocytosis,
dermatographic urticaria, eczema, psoriasis, ulcerative colitis, food allergy
and vulvar vestibulitis.

19. A method for alleviating symptoms associated with an autoimmune
disorder, preferably rheumatoid arthritis, bullous pemphigoid or multiple
sclerosis, comprising administering to a mammalian subject in need thereof
an effective dose of a bis-chromonyl compound according to any one of
claims 1-7.
20. A method for treatment of a pathological mineral resorptive state, comprising administering to a subject in need thereof a bis-chromonyl compound according to any one of claims 1-7.

21. Method according to claim 20, wherein said bis-chromonyl compound is administered in combination with one or more anti-osteoporotic agents, preferably wherein said one or more anti-osteoporotic agent is selected from anabolic steroids, various phosphorus-containing agents, vitamin D and related substances, estrogenic steroids, calcitonin and aromatic carboxylic acids.
**INTERNATIONAL SEARCH REPORT**

**International application No**
PCT/NL2013/050930

**A. CLASSIFICATION OF SUBJECT MATTER**

**INVENTION** C07D407/12 A61K31/34 A61P11/06 A61P37/08

**ADD.**

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)

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 practicable, search terms used)

EPO-Internal, CHEM ABS Data, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<td>A</td>
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Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
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**Date of the actual completion of the international search**

30 January 2014

**Date of mailing of the international search report**

17/02/2014

**Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2**

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