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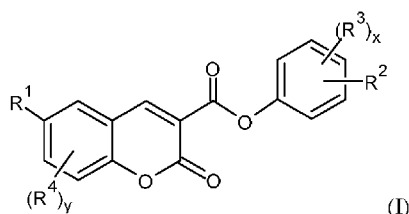
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(54) Title: SUBSTITUTED ARYL ESTERS OF COUMARIN-3-CARBOXYLIC ACID AND THEIR USE AS HOST CELL PROTEASES INHIBITORS



(57) Abstract: Compound of Formula (I) wherein R¹ is a substituted linear or branched alkyl chain of 1 to 6 carbon atoms; R² is a basic group bearing a positive charge at physiological pH or a precursor thereof, such basic group or precursor thereof being optionally preceded by a linear or branched alkyl chain of 1 to 6 carbon atoms and R² being in the ortho, meta or para position of the ester group on the aryl ring; R³ is a halogen with x being an integer between 0 and 4; R⁴ is a halogen with y being an integer between 0 and 3; or a prodrug thereof, and/or a salt thereof, for its use in the treatment of respiratory diseases and compounds of formula I wherein R² is a basic group bearing a positive charge at physiological pH or a precursor thereof, such basic group or precursor thereof being preceded by a linear or branched alkyl chain of 1 to 6 carbon atoms, preferably of 1 to 2 carbon atoms for use as a medicament.

Title: SUBSTITUTED ARYL ESTERS OF COUMARIN-3-CARBOXYLIC
ACID AND THEIR USE AS HOST CELL PROTEASES INHIBITORS

TECHNICAL FIELD OF THE INVENTION

5 The present invention relates to substituted aryl esters of coumarin-3-carboxylic acid and their use for the treatment of respiratory diseases, in particular as transmembrane serine protease 2 inhibitors. The present invention also relates to pharmaceutical compositions comprising substituted aryl esters of coumarin-3-carboxylic acid.

BACKGROUND OF THE INVENTION

10 There is an urgent need to identify new therapies to prevent acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and improve the outcome of COVID-19 patients. SARS-CoV-2 utilizes the membrane ectopeptidase angiotensin-converting enzyme 2 (ACE2) as its receptor to initiate binding and entry
15 in host cells (Hoffmann et al., *Cell* 2020, 181, 271). The virus binds to ACE2 through the receptor binding domain of its spike glycoprotein (S protein). To facilitate fusion with host cell membranes, the S protein undergoes proteolytic cleavage events. In respiratory epithelia, the predominant mode of viral entry appears to be membrane
20 fusion at the cell surface, where a cell surface-expressed serine protease, transmembrane serine protease 2 (TMPRSS2), executes the appropriate cleavage of the viral S protein. TMPRSS2 is also known to be implicated in the activation of influenza A, influenza B, and other coronaviruses to drive efficient infection of the lungs (Matsuyama et al., *J. Virol.* 2010, 84, 12658 and Limburg et al., *J. Virol.* 2019,
25 93, 1). As a result, TMPRSS2 is an attractive target for new antiviral agents, as inhibiting its proteolytic activity blocks efficiently viral entry (Hoffmann et al., *Cell* 2020, 181, 271, Singh et al., *Eur. J. Pharm. Sci.* 2020, 153, 105495). It should be specified that TMPRSS2 alone does not mediate viral infection. However, co-expression of TMPRSS2 with ACE2 results in enhanced infectivity, inducing S
30 protein cleavage and exposing the fusion peptide for efficient viral entry (Chavez-Medina et al., *Arch. Microbiol.* 2022, 204, 77).

According to recent literature, TMPRSS2 appears to be a druggable anti-SARS-CoV-2 host protein target for the following reasons. The host protein is not

subject to mutation. Moreover, TMPRSS2 being used by other viruses (i.e. SARS-CoV, MERS-CoV and influenza viruses) for the activation of surface glycoproteins, TMPRSS2 inhibitors may be used to treat a wide class of diseases caused by different pathogens (Cannalire et al., *Int. J. Mol. Sci.* 2020, 21, 5707), including SARS-CoV-2
5 variants. Lastly, in TMPRSS2-knockout mice, TMPRSS2 appeared to be useless for normal development and organ function (Stopsack et al., *Cancer Discov.* 2020, 10, 779). As a result, TMPRSS2 inhibition may have few on-target side effects (Huang et al., *Int. J. Mol. Sci.* 2021, 22, 7060).

Since TMPRSS2 constitutes an attractive therapeutic target, many recent works
10 were dedicated to the evaluation of existing TMPRSS2 inhibitors as possible antiviral agents. The irreversible serine protease inhibitors camostat and nafamostat, currently on the Japanese pharmaceutical market for therapeutic indications other than antivirals, were found to be effective at preventing host cell entry and replication of SARS-CoV-2 in Calu-3 cells through a TMPRSS2-dependent
15 mechanism (Hoffmann et al., *Cell* 2020, 181, 271, Hoffmann et al., *Antimicrob. Agents Chemother.* 2020, 64, e00754-20). A recent publication also reports the discovery of small-molecule ketobenzothiazole TMPRSS2 inhibitors with improved activity over the existing known inhibitors (Mahoney et al., *Proc. Natl. Acad. Sci.* 2021, 118, 43 e2108728118). A clinical trial is ongoing to assess the efficacy of
20 nafamostat as a novel treatment for SARS-CoV-2 infection (Zhuravel et al., *E Clinical Medicine* 2021, 41, 101169). However, this drug lacks high selectivity for TMPRSS2 and presents poor bioavailability (rapid metabolization after parenteral administration) (Quinn et al, *medRxiv* 2021, 10.06.21264648).

There is thus still a need to develop potent and highly selective TMPRSS2
25 inhibitors. There is also a need to develop potent and highly selective inhibitors against other serine protease involved in respiratory diseases such as human airways trypsin-like protease TMPRSS11D (HAT), cathepsin B/L or furin protease. An inhibitor being able to target both TMPRSS2 and HAT can have an effect both on infection and inflammation. A combined inhibition may thus be of interest in the
30 treatment and/or the prevention of respiratory diseases.

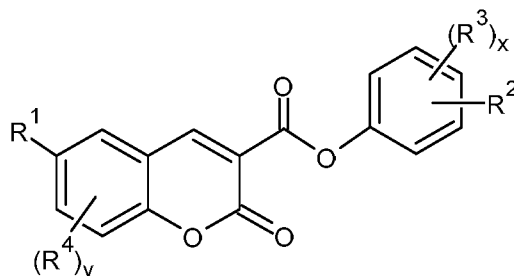
In previous work, some coumarin derivatives were described with their activity as protease inhibitors (Reboud-Ravaux et al. WO98/55472). However, while some coumarin derivatives were found to be potent and selective inhibitors of α -

chymotrypsin (Pochet et al., *J. Med. Chem.* 1996, 39, 2579), human leukocyte elastase (Doucet et al., *J. Med. Chem.* 1999, 42 4161 and Pochet et al., *Bioorg. Med. Chem.* 2000, 8, 1489), thrombin (Frederick et al., *J. Med. Chem.* 2005, 48, 7592) or disease-related kallikreins (WO2013/010963, Tan et al., *J. Med. Chem.* 2015, 58, 598; Hanke et al., *J. Med. Chem.* 2020, 63, 5723), no activity was yet reported towards the inhibition of TMPRSS2.

It is an aim of the present invention to provide compounds for use as selective inhibitors of host cell proteases such as TMPRSS2, HAT, cathepsin B/L, furin and/or other enzymes involved in respiratory diseases. It is also an aim of the present invention to provide compounds that heal or relief from pulmonary infections, in particular viral infections. It is also an aim of the present invention to provide new substituted aryl esters of coumarin-3-carboxylic acid.

SUMMARY OF THE INVENTION

The invention pertains to compounds of Formula I as presented below:



Formula I

wherein R¹ is a substituted linear or branched alkyl chain of 1 to 6 carbon atoms; R² is a basic group bearing a positive charge at physiological pH or a precursor thereof, such basic group or precursor thereof being optionally preceded by a linear or branched alkyl chain of 1 to 6 carbon atoms and R² being in the ortho, meta or para position of the ester group on the aryl ring; R³ is a halogen with x being an integer between 0 and 4; R⁴ is a halogen with y being an integer between 0 and 3; or a prodrug thereof, and/or a salt thereof, for its use in the treatment of respiratory diseases.

Such compounds of Formula I present an inhibitory activity against enzymes such as host cell proteases. Host cell proteases may be transmembrane

serine protease 2, generally referred to as TMPRSS2, human airways trypsin-like protease TMPRSS11D (HAT), another major serine protease involved in respiratory diseases, cathepsin B/L or furin protease. By inhibitory activity, one means a decrease of the activity of the enzyme. A test to show the inhibitory activity consists of incubating the enzyme, optionally a buffer, and a compound as recited above and then determining the activity of the enzyme by comparison of the initial rates to those obtained in control experiments without the tested compounds. The IC₅₀ values (inhibitor concentration giving 50% inhibition) express the inhibitory activity.

Advantageously, the coumarin derivatives are designed to present a basic group (R²), which bears a positive charge at physiological pH. By bearing a positive charge is also meant having a cationic group at physiological pH. A physiological pH is a pH of about 7 to about 8. Examples of such basic group are an amine, an amidine or a guanidine group, providing an aminium, an amidinium or a guanidinium group at physiological pH. These may be directly or not directly linked to the aromatic ring and introduced in the ortho, meta or para position of the ester group on the aryl group. When not directly linked, these groups are linked through a short linear or branched alkyl chain such a linear or branched alkyl chain with 1 to 6 carbon atoms. Such alkyl chain is preferably a linear alkyl chain. This cationic group enables to target the anionic carboxylate function of Asp435 of the enzymatic catalytic site of TMPRSS2 and to promote the establishment of a salt bridge between the enzyme and its inhibitor.

The basic group may advantageously be chosen among a relatively strong basic group such as amidine or guanidine or a mild basic group such as a primary amine.

Advantageously, the coumarine derivative are low molecular weight non peptidic molecules. Advantageously, their modular structure allows structural variations to obtain selective inhibitors of TMPRSS2 or other host cell proteases of interest (furin, cathepsin B/L, TMPRSS11D) to combat respiratory diseases. This may also lead to either dual inhibitors or use of a combination of inhibitors, both strategies being susceptible to increase antiviral potency. Advantageously they may have a dual inhibition against TMPRSS2 and HAT.

Alternatively, compounds may be designed as prodrugs. By prodrug is meant a compound that, after administration, is metabolized into a pharmacologically active drug. Hence, by prodrug of Formula I is meant any

compound that can be metabolized into a compound of Formula I. A prodrug form can be advantageous to improve the oral bioavailability. As an illustration, when the basic group of Formula I is an amidine or a guanidine-bearing group, it might exhibit poor oral bioavailability due to the permanent cationic form of these groups in biological media. It is for example known that the antithrombotic agent dabigatran bearing an amidine function is used as a prodrug (dabigatran etexilate) suitable for oral administration.

In some embodiments, the precursor of the basic group may bear a carbamate function or a pseudocarbamate function. A carbamate function refers to -NH-COOR while a pseudocarbamate function may refer to -(=NH)NH-COOR or -NH-C(=NH)NH-COOR, or -NH-C(=NH)NH-COOR. Such a (pseudo)carbamate function may be metabolized into a basic group after administration to a subject. Other precursors of amine function or other precursors of other basic functions are also possible.

In some embodiments, R^2 is chosen from the group consisting of $-(CH_2)_n-NH_2$, $-(CH_2)_n-C(=NH)NH_2$, $-(CH_2)_n-NH-C(=NH)NH_2$ or $-(CH_2)_n-NH-COOR^5$, $-(CH_2)_n-C(=NH)NH-COOR^5$, $-(CH_2)_n-NH-C(=NH)NH-COOR^5$ with R^5 being a linear or branched alkyl chain with 1 to 6 carbon atoms and with n being an integer between 0 and 6.

Advantageously, n may be 1 or 2 to facilitate the target of the anionic carboxylate function of Asp435 of the enzymatic catalytic site of TMPRSS2 and to promote the establishment of a salt bridge between the enzyme and its inhibitor.

Advantageously, the aryl ring linked to the ester function of the coumarin derivative is substituted by one or more halogen atoms (R^3). A halogen atom may be fluorine, chlorine, bromine or iodine. Preferably a halogen atom is chlorine or bromine. Preferably the aryl ring bears one or two or three halogen atoms, to favour the inhibitory activity on the enzyme. It will be easily understood by a person skilled in the art that the aryl ring bears a hydrogen in each position where no halogen or other substituent is present. Alternatively, the aryl ring linked to the ester function of the coumarin derivative does not bear a halogen atom (R^3). In other words, x in Formula I may be equal to 0.

Advantageously the coumarin core also bears one or more halogen atoms (R^4), preferably 1 or 2. It will also be easily understood by a person skilled in the art

that the coumarin bears a hydrogen in each position where no halogen or other substituent is present. Alternatively, the coumarin core does not bear a halogen atom (R^4). In other words, y in Formula I may be equal to 0.

An alkyl chain of 1 to 6 carbon atom may be a methyl, an ethyl, a propyl,
5 a butyl, a pentyl, a hexyl, a tert-butyl.

A substituent on the R^1 linear or branched alkyl chain of 1 to 6 carbon atoms may be one or more suitable substituent(s) to favour interaction with the host cell protease. Suitable substituents may be a halogen. Suitable substituents may be chosen among chloride, bromide, iodide, mesylate, triflate, tosylate, acetate or
10 trifluoroacetate. The one or more substituent(s) may be positioned on any carbon atom of the said R^1 alkyl chain. Advantageously, the substituent may be a leaving group.

By leaving group is meant an atom or a group of atoms that is able to depart upon heterolysis taking with it the bonding electrons. Chloride and bromide
15 are preferred leaving groups. Preferably a leaving group is linked to the aryl ring with a short linear or branched alkyl chain, such as an alkyl chain with 1 to 6 carbon atoms, such as a linear or branched alkyl chain of 1 to 6 carbon atoms e.g. a methyl. In preferred embodiments, R^1 is $-CH_2Cl$ or $-CH_2Br$.

A salt of the compound of Formula I may have as counterion for example
20 chloride, bromide, iodide or trifluoroacetate. A compound of Formula I may also be in the form of a conjugate acid base, such as in the form of a hydrochloride for example.

By respiratory disease one means a pathological condition affecting the organs, and/or the tissues and/or the cells thereof, that enable to breathe, making
25 such breathing difficult. Such organs or tissues include the lungs, the trachea, the bronchi, the bronchioles, the alveoli, the pleura and pleural cavity, the nerves and muscles of respiration. Respiratory diseases include but are not limited to the cold, influenza, pharyngitis, bronchitis, pneumonia, tuberculosis, asthma, lung cancer, Covid-19 or other severe acute respiratory syndromes (SARS). Targeted cells may be
30 cells expressing transmembrane serine protease 2 such as for example lung epithelial cells. Treatment may also include a reduction of the inflammation observed in such cells.

The respiratory disease may be a viral infection or another type of infection affecting the respiratory system.

A compound of Formula I or a prodrug thereof and/or a salt thereof, may also be used in the prevention of respiratory diseases.

5 The invention also pertains to a compound of Formula I or a prodrug thereof and/or a salt thereof, wherein R^2 is a basic group bearing a positive charge at physiological pH or a precursor thereof, such basic group or precursor thereof being preceded by a linear or branched alkyl chain of 1 to 6 carbon atoms, preferably of 1 to 2 carbon atoms.

10 Examples of a basic group are an amine, an amidine or a guanidine group, providing an aminium, an amidinium or a guanidinium group at physiological pH.

An example of precursor of a basic group is a carbamate function or a pseudocarbamate function.

15 The invention also pertains to a compound of Formula I wherein R^2 is -
(CH_2)_n-NH-COOR⁵ or -(CH_2)_n-C(=NH)NH-COOR⁵ or -(CH_2)_n-NH-C(=NH)NH-COOR⁵
with R⁵ being a linear or branched alkyl chain with 1 to 6 carbon atoms and with n being an integer between 0 and 6, preferably between 1 and 6.

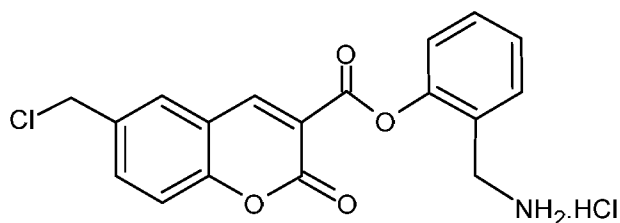
20 The invention also pertains to a compound of Formula I or a prodrug thereof and/or a salt thereof, wherein R^2 is a basic group bearing a positive charge at physiological pH or a precursor thereof, such basic group or precursor thereof being preceded by a linear or branched alkyl chain of 1 to 6 carbon atoms, preferably of 1 to 2 carbon atoms for use as a medicament. Such compounds present an inhibitory activity against serine proteases, as shown in the examples.

25 The invention also pertains to a pharmaceutical composition comprising a compound of Formula I or a prodrug thereof and/or salt thereof, wherein R^2 is a basic group bearing a positive charge at physiological pH or a precursor thereof, such basic group or precursor thereof being preceded by a linear or branched alkyl chain of 1 to 6 carbon atoms, preferably of 1 to 2 carbon atoms and a pharmaceutically acceptable carrier.

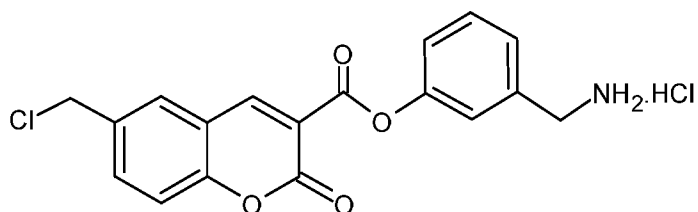
30 The invention particularly pertains to a compound of Formula I wherein R^1 is a methyl substituted by a chloride, R^2 is an amine or a guanidine or an amidine preceded by a methyl or an ethyl, R^2 being in ortho, meta or para position of the ester group on the aryl ring and wherein x and y are both 0, or a prodrug thereof and/or a

salt thereof. By a prodrug is particularly meant that the amine, guanidine or amidine may be present as a carbamate or a pseudocarbamate function bearing a methyl, ethyl, propyl, butyl, pentyl, hexyl or tertbutyl function. By a salt is particularly meant that the amine, guanidine or amidine may be conjugated with a hydrochloride. The invention also particularly pertains to pharmaceutical compositions of those compounds and their use as a medicament.

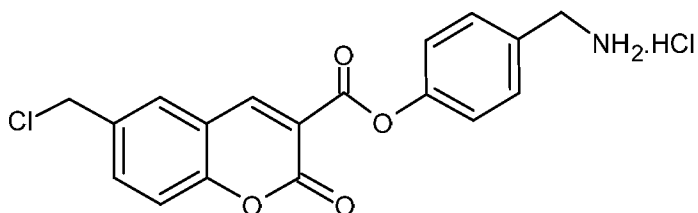
Examples of compounds according to the invention are:



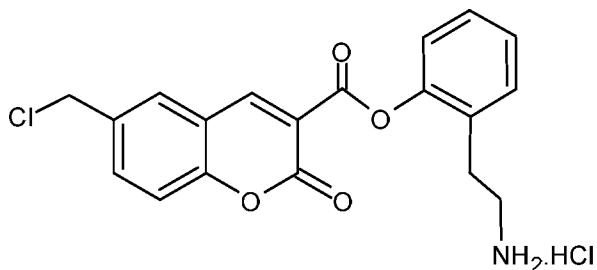
10 Compound a



Compound b



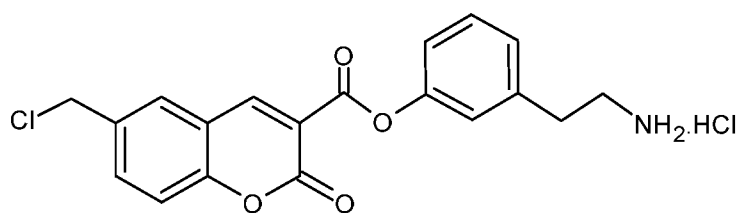
Compound c



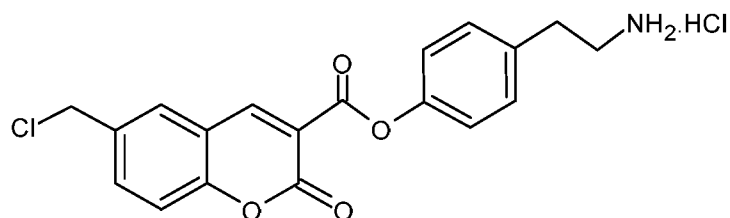
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Compound d

9



Compound e



Compound f

5

The invention also pertains to the preparation of such compounds. A general method of synthesis may imply the following steps:

- Preparation of a phenol intermediate bearing the desired R^2 group in ortho, meta or para position and optionally one or more R^3 groups. The basic group comprised in R^2 is advantageously protected by a tert-butyloxycarbonyl group or any other suitable protecting group
- Preparation of a 2-oxo-2H-1-benzopyran-3-carbonyl chloride bearing the desired R^1 group and optionally one or more R^4 groups from a salicylic aldehyde according to the process described in the literature (Pochet et al. J. Med. Chem. 1996, 39, 2579-252585)
- Adding the obtained 2-oxo-2H-1-benzopyran-3-carbonyl chloride to the phenol intermediate bearing the desired protected R^2 group in ortho, meta or para position and optionally one or more R^3 groups.
- Optionally deprotecting the protected basic group of the R^2 group in acidic conditions.

20

The invention also pertains to the preparation of such pharmaceutical composition.

The term "pharmaceutically acceptable carrier" is intended to mean a medium that is compatible with the respiratory system, such as for example the pulmonary system.

25

The pharmaceutical composition according to the invention can be administered through any type of administration, such as an oral administration, a cutaneous administration, an injection, an intravenous administration, a subcutaneous administration, a systemic administration, a parenteral, digestive, 5 rectal or transcutaneous administration, a transmucosal administration, a pulmonary, nasal or sublingual administration, an inhalation or a nebulization or an aerosol administration. Preferably, the composition is administered orally, by injection or by an aerosol. A pulmonary or aerosol administration can be through a gaseous composition, a volatile composition or through a composition that is a 10 suspension in a gaseous vector.

Advantageously, the pharmaceutical composition may be in a form suitable for an aerosol administration.

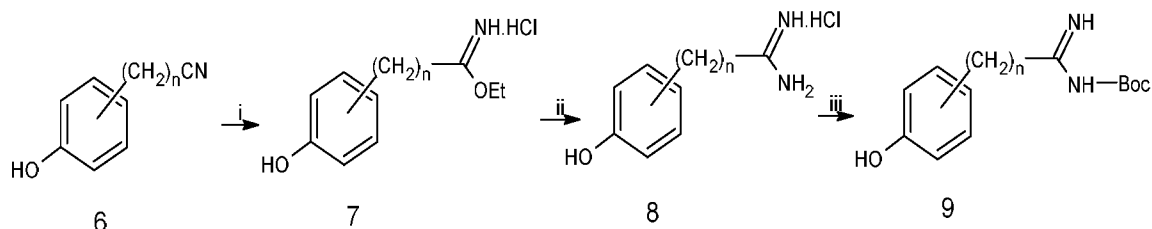
Compounds as recited above are also referred to as coumarin derivatives.

Possible routes of synthesis of the coumarin derivatives are described in 15 the examples. It will however be understood that such synthetic routes do not limit the present invention.

EXAMPLES

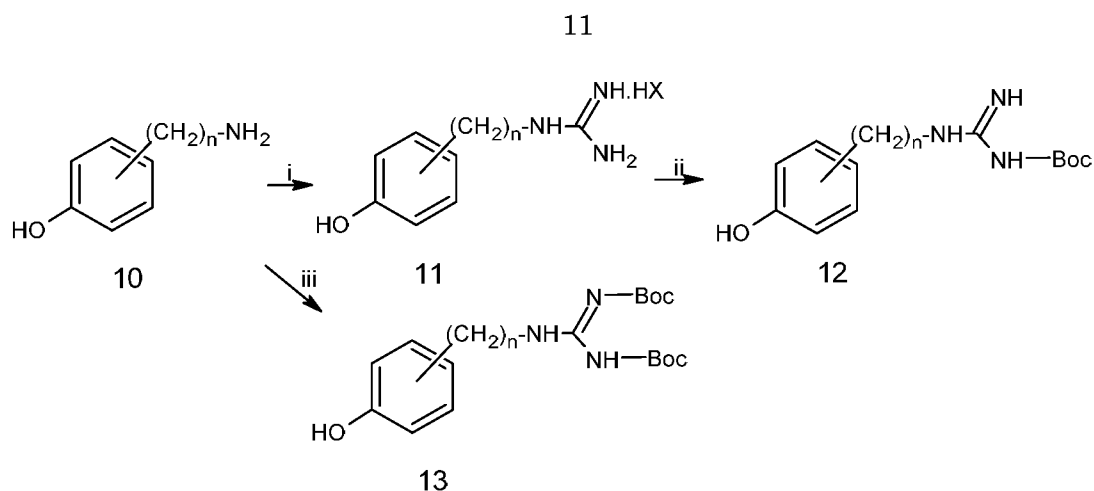
Example 1: Organic synthesis of the aryl esters of coumarin-3-carboxylic acid with 20 amidine or guanidine function.

One of the possible pathways to access the compounds of formula I bearing an amidine or a guanidine function is reported in schemes 1-3. It should be understood that present invention is not limited to the below synthesis strategies.



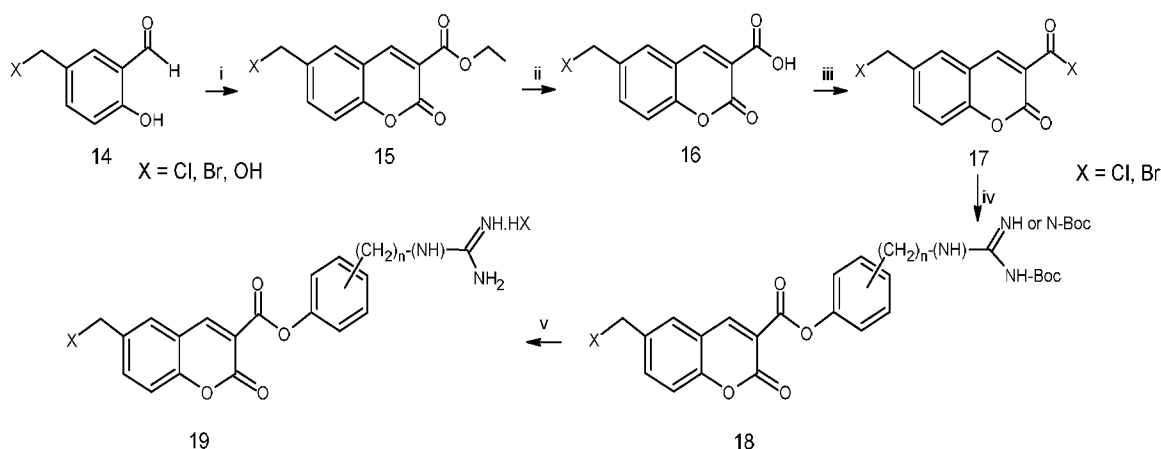
Scheme 1

Reagents are for step i: EtOH, HCl, for step ii: NH₃, MeOH, for step iii: di-*tert*-butyl dicarbonate (Boc)₂O, NaOH, dioxane/H₂O, Boc is *tert*-butyloxycarbonyl protecting group.



Scheme 2

Reagents are for step i: S-methyl isothiuronium salt ($\text{CH}_3\text{S}-\text{C}(=\text{NH})\text{NH}_2\cdot\text{HX}$), CH_3CN ; for step ii: $(\text{Boc})_2\text{O}$, NaOH , dioxane/ H_2O ; for step iii: S-methyl bis(*tert*-butoxycarbonyl)thiopseudourea ($\text{CH}_3\text{S}-\text{C}(=\text{N}-\text{Boc})\text{NH}-\text{Boc}$), CH_3CN .



Scheme 3

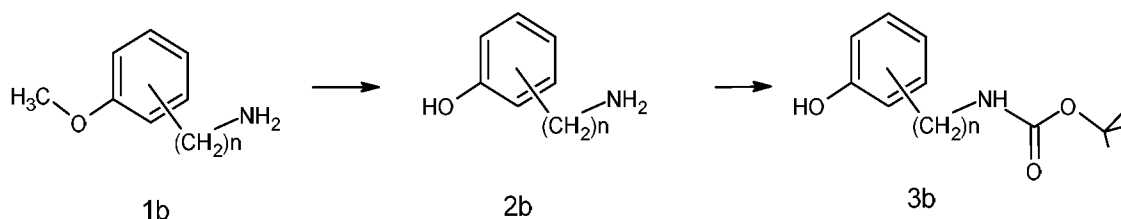
Reagents are for step i: diethyl malonate, piperidine, HOAc , EtOH ; for step ii: HOH , H^+ , EtOH ; for step iii: SOCl_2 or SOBr_2 ; for step iv: intermediate **9**, **12** or **13**, pyridine, dioxane; for step v: HX , methylene chloride.

The phenol intermediates bearing the *tert*-butoxycarbonyl (Boc)-protected amidines (**9**) or guanidines (**12,13**) are prepared according to schemes 1 and 2. Intermediate **17** obtained according to a well-known classical synthetic process (Pochet et al., *J. Med. Chem.* 1996, 39, 2579) reacts with the protected intermediates **9**, **12** or **13** to provide intermediates **18**. In the last step, deprotection of the *tert*-butoxycarbonyl group in acidic conditions provides the final compounds. According to the literature, the phenol hydroxyl group does not need to be protected

before reaction with (Boc)₂O (Frederick et al., *Bioorg. Med. Chem. Lett.* 2006, 16, 2017).

5 *Example 2a: Organic synthesis of the aryl esters of coumarin-3-carboxylic acid with amine function*

Synthetic pathway to intermediates **3b** is first shown in scheme 4.



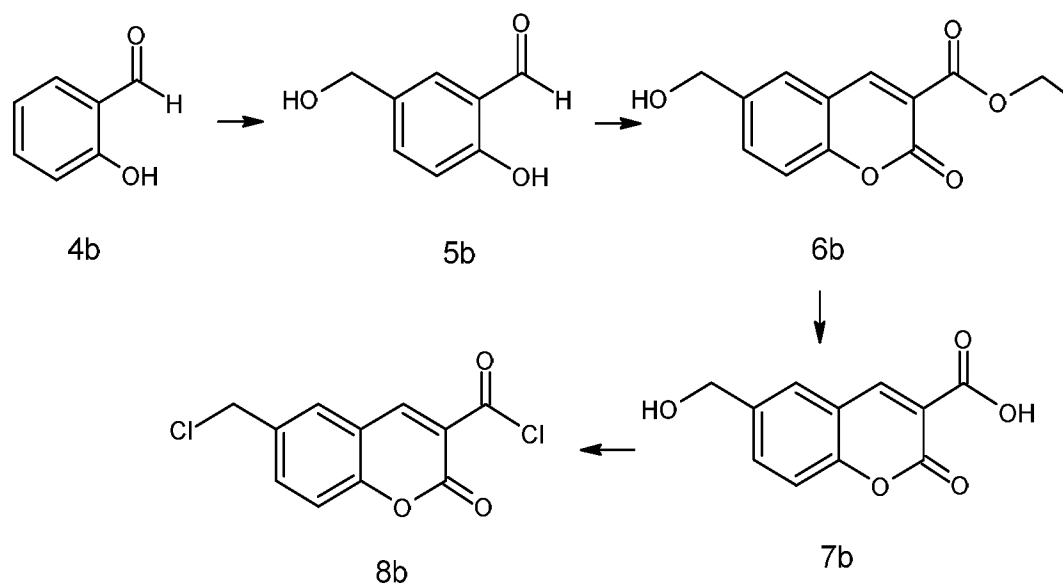
Scheme 4

To obtain the aminoalkylphenol **2b**, the appropriate methoxyphenylalkylamine **1b** (6.6 mmol) was dissolved in acetic acid (10 mL) and concentrated HBr (11 mL) and heated to reflux for 4 hours under stirring. The reaction mixture was cooled to room temperature and the solvent and HBr were removed by distillation under reduced pressure. The title compound **2b** as the hydrobromide was obtained as a solid in good yields and used in the next step without further purification.

To obtain the tert-butyl hydroxyphenylalkylcarbamate **3b**, the solution of compound **2b** (6.6 mmol) in DMF (2 mL) and dioxane (20 mL) was supplemented with triethylamine (6.6 mmol) and stirred for 15 minutes. Di-tert-butyl dicarbonate (6.6 mmol) was added and the reaction mixture was stirred at room temperature for 18 hours. The solvents were removed by distillation under reduced pressure and the resulting residue was taken up in water (20 mL) and extracted trice with ethyl acetate (3 x 25 mL). The organic layers were combined, dried over MgSO₄, and filtered. The filtrate was concentrated under reduced pressure and the resulting residue was purified by silica gel chromatography to afford the title compound **3b** as a white solid.

Synthetic pathway to intermediate **8b** is shown in scheme 5.

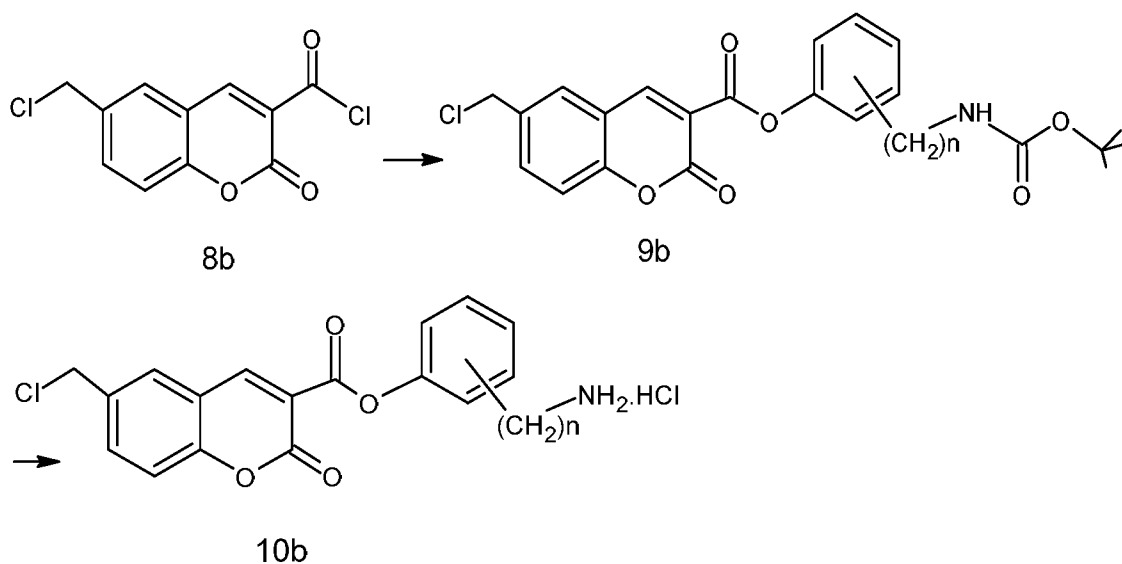
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Scheme 5

6-Chloromethyl-2-oxo-2H-1-benzopyran-3-carbonyl chloride (intermediate **8b**) was obtained as described in the literature (Pochet et al. *J. Med. Chem.* **1996**, 39, 2579-2585).

Synthetic pathway to final compounds **10b** is shown in scheme 6.



Scheme 6

(*Tert*-butoxycarbonyl)aminoalkylphenyl 6-(chloromethyl)-2-oxo-2H-1-benzopyran-3-carboxylate **9b** was obtained as follows. Compound **8b** (4.5 mmol) was dissolved in anhydrous dioxane (3 mL) and added with stirring to a solution of the appropriate compound **3b** (5.0 mmol) and of anhydrous pyridine (5.0 mmol) in

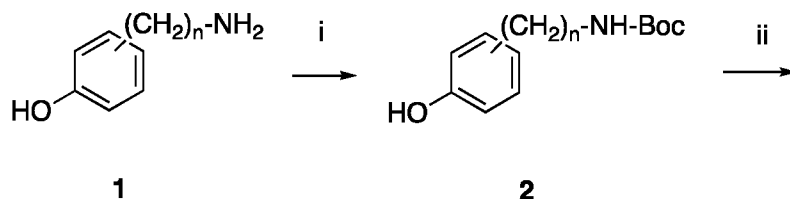
anhydrous dioxane (7 mL). After 2 hours at room temperature, the solvent was removed by evaporation under reduced pressure. The residue was purified by silica gel chromatography to afford the title compound **9b** as a white solid.

Aminoalkylphenyl 6-(chloromethyl)-2-oxo-2*H*-1-benzopyran-3-carboxylate hydrochloride **10b** was obtained as follows. Compound **9b** (4 mmol) was dissolved in anhydrous diethyl ether (10 mL) or another appropriate solvent (10 mL) and gaseous HCl was bubbled for 10 minutes. The reaction mixture was stirred for 1 hour and then the solvent was removed by distillation under reduced pressure. The residue of the final compound **10b** as the hydrochloride was recrystallized in the appropriate solvent.

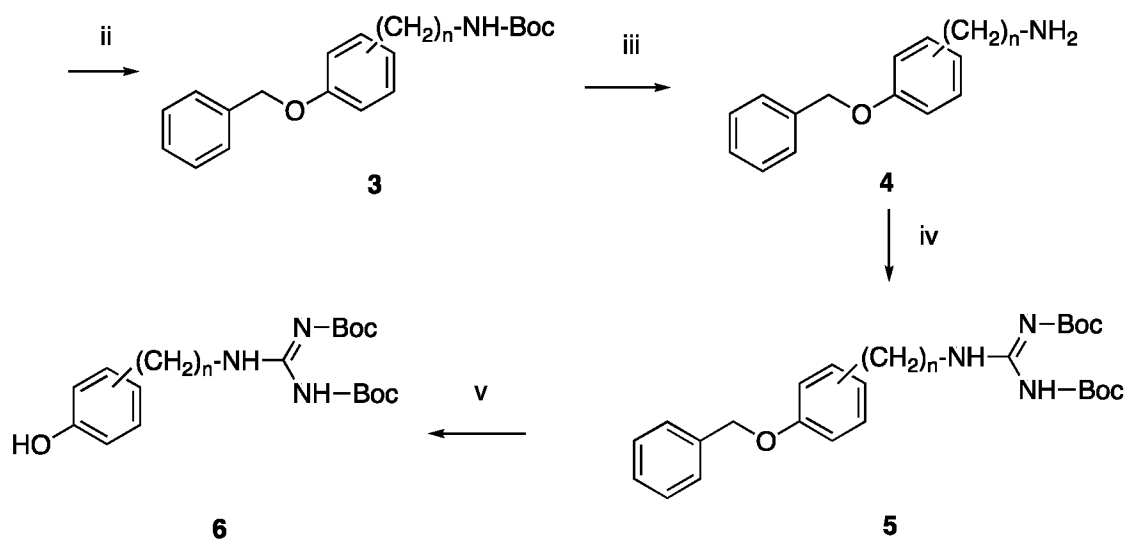
Example 2b: alternative organic synthesis of the aryl esters of coumarin-3-carboxylic acid with amine, amidine or guanidine function yielding final products 16 of scheme 9 through intermediates 2 or 6 of scheme 7 or intermediate 12 of scheme 8.

An alternative synthesis route is hereafter described with reference to schemes 7, 8 and 9. Each obtained compound was analysed by ¹H NMR and ¹³C NMR with main peaks listed. Melting temperatures of intermediates and of final products are also presented.

Table 1 summarizes the obtained compounds with their formulae and molecular weight (Mw) expressed in g/mol. The formulae also include an indication of the potential presence of crystallisation water molecules. This was deduced by elemental analysis. Compounds 15a-15j, 19a bearing carbamate or pseudocarbamate groups can be considered as prodrugs. Compounds 16a-16j, 20a are positively charged at physiological pH and are here presented as examples in the form of hydrochloride salts.

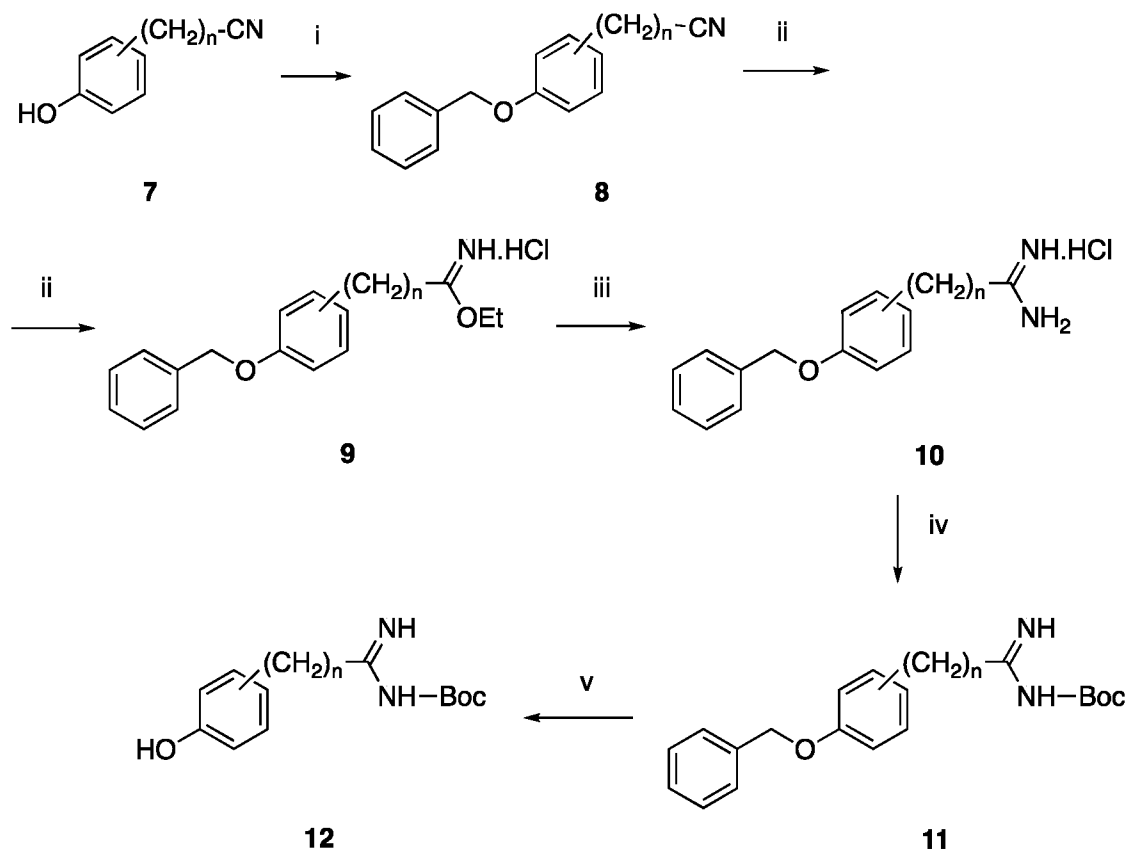


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Scheme 7

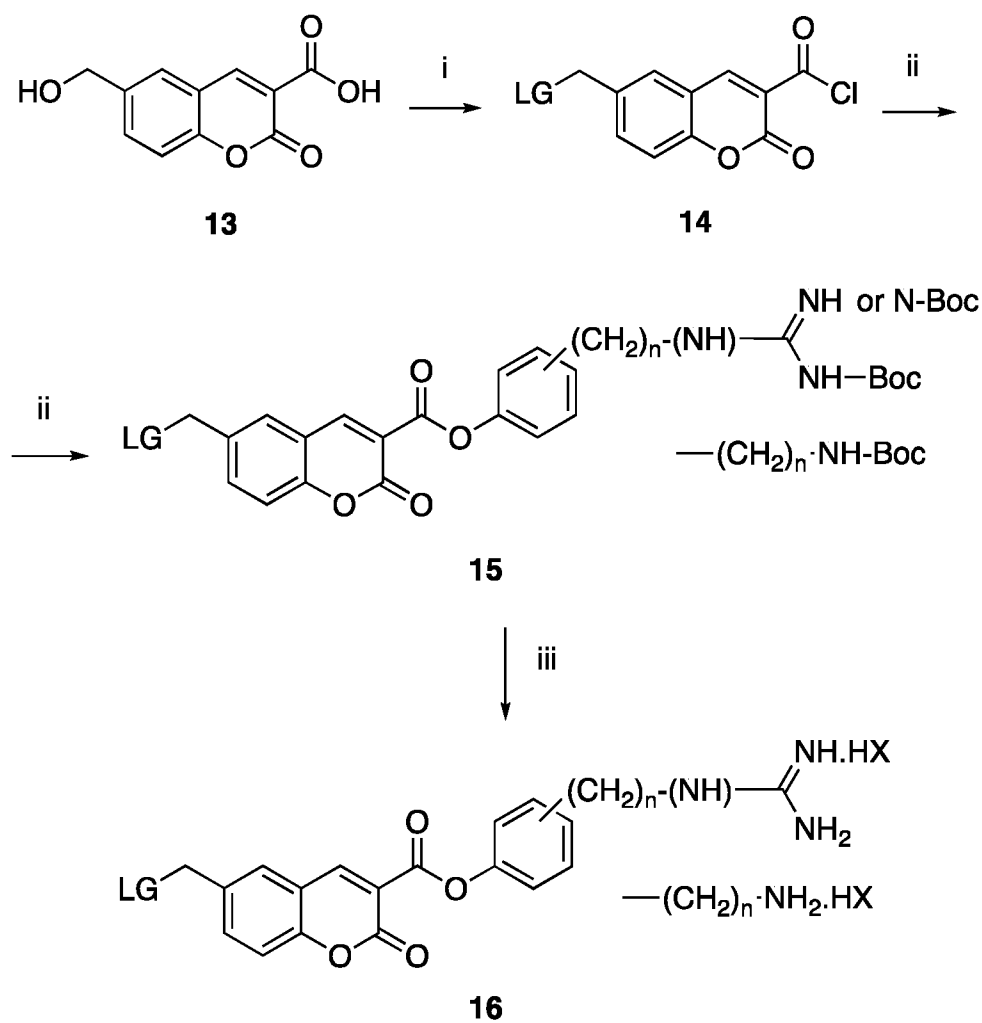
- Reagents of scheme 7 are for step i: (Boc)₂O, NaHCO₃, MeOH; for step ii: benzyl bromide, K₂CO₃, CH₃CN; for step iii: HCl 12N, MeOH; for step iv: S-methyl bis(*tert*-butoxycarbonyl)thiopseudourea (CH₃S-C(=N-Boc)NH-Boc), CH₂Cl₂; for step v: H₂, Pd/C, EtOH.



Scheme 8

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Reagents of scheme 8 are for step i: benzyl bromide, K_2CO_3 , CH_3CN ; for step ii: HCl , $EtOH$; for step iii: NH_4OH , $EtOH$; for step iv: $(Boc)_2O$, pyridine, DMAP, CH_2Cl_2 ; for step v: H_2 , Pd/C , $EtOH$.

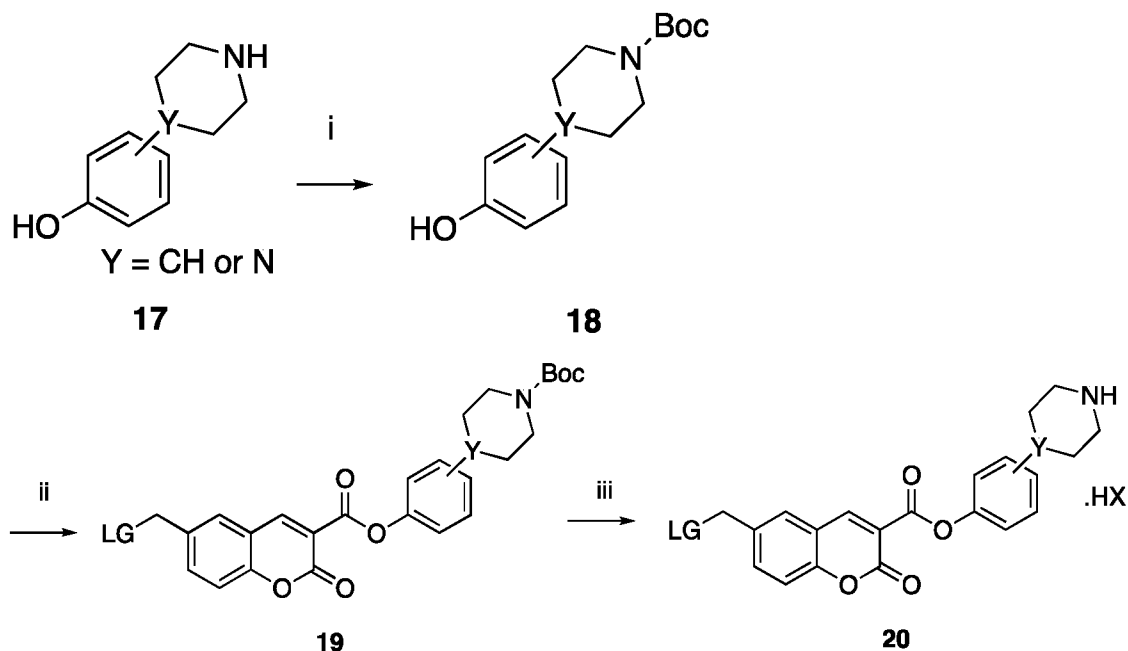


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Scheme 9

Reagents of scheme 9 are in step i: $-LG = -Cl$: $SOCl_2$; $-LG = -OCOR$, 1: $(RCO)_2O$, 2: $SOCl_2$; $-LG = -OSO_2R$, 1: RSO_2Cl , 2: $SOCl_2$; in step ii: intermediate **2**, **6** or **12**, pyridine, dioxane; in step iii: HX , CH_3CN .

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Scheme 10

Reagents of scheme 10 are in step i: (Boc)₂O, NaHCO₃, MeOH; in step ii:
 5 intermediate 14, pyridine, dioxane; in step iii: HX, CH₃CN

Synthesis of intermediates 2

A mixture of compound 1 of scheme 7 (7.29 mmol), di-tert-butyl dicarbonate (1.75 g, 8.02 mmol) and sodium bicarbonate (1.29 g, 15.31 mmol) in
 10 methanol (25 mL) was refluxed for 16 h. After cooling, the reaction mixture was evaporated under reduced pressure. The residue was taken up in water (25 mL) and the resulting suspension was extracted thrice with dichloromethane (3 x 15 mL). The organic phases were combined, washed twice with distilled water (3 x 10 mL), dried over MgSO₄ and filtered. The filtrate was evaporated to dryness and the resulting
 15 residue (compound 2 of scheme 7) was used in the next step without further purification.

Tert-butyl (4-hydroxyphenethyl)carbamate (**2a**)

White solid (m.p.: 73.7-75.1 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 9.14 (s, 1H, OH), 6.96 (d, *J* = 8.4 Hz, 2H, 2-*H*/6-*H*), 6.81 (t, *J* = 5.7 Hz, 1H, NH), 6.66 (d, *J* = 8.4 Hz,
 20 2H, 3-*H*/5-*H*), 3.05 (q, *J* = 15.2, 5.8 Hz, 2H, CH₂NH), 2.59 – 2.52 (m, 2H, CH₂), 1.36

(s, 9H, C(CH₃)₃). ¹³C NMR (DMSO-*d*₆) δ 155.6, 155.5, 129.4, 129.4, 115.0, 77.4, 41.9, 34.7, 28.3.

Tert-butyl (3-hydroxyphenethyl)carbamate (**2b**)

Liquid. ¹H NMR (DMSO-*d*₆) δ 1.37 (s, 9H, C(CH₃)₃), 2.58 (t, *J*=7.6 Hz, 2H, PhCH₂),
5 3.08 (dt, *J*=8.2 Hz/6.1 Hz, 2H, CH₂N), 6.58 (m, 3H, 2-*H*/4-*H*/6-*H*), 6.84 (t, *J*=5.7 Hz, 1H, NH), 7.05 (t, *J*=7.3 Hz, 1H, 5-*H*), 9.25 (s, 1H, OH). ¹³C NMR (DMSO-*d*₆) δ 28.3, 35.6, 41.6, 77.5, 113.0, 115.5, 119.2, 129.2, 140.8, 155.5, 157.3.

Tert-butyl (4-hydroxybenzyl)carbamate (**2c**)

Liquid. ¹H NMR (DMSO-*d*₆) δ 1.38 (s, 9H, C(CH₃)₃), 3.99 (d, *J*=6.2 Hz, 2H, CH₂), 6.68
10 (d, *J*=8.2 Hz, 2H, 3-*H*/5-*H*), 7.02 (d, *J*=8.1 Hz, 2H, 2-*H*/6-*H*), 7.23 (t, *J*=6.2 Hz, 1H, NH), 9.23 (s, 1H, OH). ¹³C NMR (DMSO-*d*₆) δ 28.2, 42.8, 77.5, 114.8, 128.2, 130.3, 155.6, 156.0.

Tert-butyl (3-hydroxybenzyl)carbamate (**2d**)

Liquid. ¹H NMR (DMSO-*d*₆) δ 1.39 (s, 9H, C(CH₃)₃), 4.03 (d, *J*=6.2 Hz, 2H, CH₂), 6.58-
15 6.64 (m, 3H, 2-*H*/4-*H*/6-*H*), 7.08 (t, *J*=8.0 Hz, 1H, 5-*H*), 7.31 (t, *J*=6.3 Hz, 1H, NH), 9.30 (s, 1H, OH). ¹³C NMR (DMSO-*d*₆) δ 28.8, 43.7, 78.2, 114.0, 114.2, 117.9, 129.6, 142.1, 156.3, 157.8.

Synthesis of intermediates 3

20 A mixture of intermediate **2** of scheme 7 (24.65 mmol), benzyl bromide (3 mL, 25.26 mmol) and potassium carbonate (1.50 g, 35.24 mmol) in acetone (100 mL) was refluxed for 20 h. After cooling, the reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was dissolved in ethyl acetate and then supplemented with hexane. The resulting precipitate of intermediate **3** of
25 scheme 7 was collected by filtration, washed with hexane and dried.

Tert-butyl (4-(benzyloxy)phenethyl)carbamate (**3a**)

White solid (m.p.: 80.7-82.8 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.43 (d, *J* = 6.7 Hz, 2H, 2'-*H*, 6'-*H*), 7.38 (t, *J* = 7.6 Hz, 2H, 3'-*H*/5'-*H*), 7.32 (t, *J* = 7.2 Hz, 1H, 4'-*H*), 7.09
(d, *J* = 8.6 Hz, 2H, 2-*H*/6-*H*), 6.92 (d, *J* = 8.6 Hz, 2H, 3-*H*/5-*H*), 6.83 (t, *J* = 5.7 Hz,
30 1H, NH), 5.06 (s, 2H, CH₂O), 3.08 (q, 2H, CH₂NH), 2.61 (t, 2H, CH₂), 1.36 (s, 9H, C(CH₃)₃). ¹³C NMR (DMSO-*d*₆) δ 156.7, 155.5, 137.3, 131.6, 129.6, 128.4, 127.7, 127.6, 114.6, 77.4, 69.1, 41.7, 34.6, 28.3.

Tert-butyl (4-(benzyloxy)benzyl)carbamate (**3b**)

White solid. ^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 9.22 (s, 1H, NH), 7.44 (d, J = 7.3 Hz, 2H, arom. H), 7.39 (t, J = 7.5 Hz, 1H, arom. H), 7.32 – 7.28 (m, 2H, arom. H), 7.15 (d, J = 8.4 Hz, 2H, arom. H), 6.95 (d, J = 8.5 Hz, 2H, arom. H), 5.08 (s, 2H, CH_2O), 4.04 (d, J = 6.1 Hz, 2H, CH_2), 1.39 (s, 9H, $\text{C}(\text{CH}_3)_3$).

5

Synthesis of intermediates 4

To a solution of intermediate **3** of scheme 7 (11.54 mmol) in methanol (40 mL) was added a concentrated aqueous solution of HCl (30 mL) under stirring and on an ice bath. After 5 h, the reaction mixture was evaporated under reduced pressure. The residue was taken up in an aqueous solution of sodium hydroxide 10% m/v (25 mL) to obtain an aqueous solution at pH 12-14. The resulting suspension was extracted thrice with ethyl acetate (3 x 20 mL). The organic phases were combined, washed twice with distilled water (2 x 15 mL) dried over MgSO_4 , and filtered. The filtrate was evaporated to dryness and the resulting residue of **4** of scheme 7 was used in the next step without further purification.

15

In some cases, intermediate **4** was obtained as the hydrochloride after treatment of intermediate **3** in methanol with concentrated HCl. The resulting precipitate was collected by filtration, washed with methanol and dried (ex: **4b**).

2-(4-(Benzyloxy)phenyl)ethan-1-amine (**4a**)

White solid (m.p.: 122.2-124.5 °C). ^1H NMR (500 MHz, CDCl_3) δ 7.43 (d, J = 7.5 Hz, 2H, 2'- $H/6'$ - H), 7.38 (t, J = 7.5 Hz, 2H, 3'- $H/5'$ - H), 7.32 (t, J = 7.3 Hz, 1H, 4'- H), 7.12 (d, J = 8.0 Hz, 2H, 2- $H/6$ - H), 6.92 (d, J = 8.0 Hz, 2H, 3- $H/5$ - H), 5.05 (s, 2H, CH_2O), 2.96 (t, J = 6.9 Hz, 2H, CH_2NH_2), 2.72 (t, J = 6.9 Hz, 2H, CH_2), 2.06 (s, 2H, NH_2). ^{13}C NMR (CDCl_3) δ 157.4, 137.1, 131.7, 129.8, 128.6, 127.9, 127.5, 115.0, 70.1, 43.2, 38.5.

25

(4-(Benzyloxy)phenyl)methanamine hydrochloride (**4b**)

White solid (m.p.: 240.4-241.7 °C). ^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 8.06 (s, 3H, NH_3^+), 7.44 (d, J = 7.2 Hz, 2H, 2- $H/6$ - H), 7.42 – 7.37 (m, 4H, 2'- $H/3'$ - $H/5'$ - $H/6'$ - H), 7.35 – 7.31 (m, 1H, 4'- H), 7.07 – 7.04 (m, 2H, 3- $H/5$ - H), 5.14 (s, 2H, CH_2O), 3.94 (s, 2H, CH_2). ^{13}C NMR ($\text{DMSO-}d_6$) δ 158.1, 136.7, 130.2, 128.2, 127.6, 127.3, 126.0, 114.7, 68.9, 41.6.

30

Synthesis of intermediates 5

A mixture of intermediate 4 of scheme 7 as the base (7.70 mmol) and 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea (2.19 g, 7.54 mmol) in dichloromethane (35 mL) was stirred at room temperature for 28 h. The reaction mixture was then evaporated under reduced pressure. The residue (intermediate 5 of scheme 7) was purified by flash column chromatography using hexane/ethyl acetate.

N1-(4-(Benzyloxy)phenethyl)-*N2,N3*-di(*tert*-butoxycarbonyl)guanidine
(5a)

White solid (m.p.: 99.2-101.5 °C). ¹H NMR (500 MHz, CDCl₃) δ 11.47 (s, 1H, *NHCO*), 8.36 (t, *J* = 5.2 Hz, 1H, CH₂NH), 7.43 (d, *J* = 6.8 Hz, 2H, 2'-*H*/6'-*H*), 7.38 (t, *J* = 7.4 Hz, 2H, 3'-*H*/5'-*H*), 7.32 (t, *J* = 7.2 Hz, 1H, 4'-*H*), 7.13 (d, *J* = 8.6 Hz, 2H, 2-*H*/5-*H*), 6.91 (d, *J* = 8.6 Hz, 2H, 3-*H*/5-*H*), 5.04 (s, 2H, CH₂O), 3.66 – 3.61 (m, 2H, CH₂NH), 2.81 (t, *J* = 7.3 Hz, 2H, CH₂), 1.50 (s, 9H, C(CH₃)₃), 1.48 (s, 9H, C(CH₃)₃). ¹³C NMR (CDCl₃) δ 163.6, 157.5, 156.1, 153.2, 137.1, 130.9, 129.8, 128.6, 127.9, 127.5, 115.0, 83.0, 79.2, 70.1, 42.5, 34.4, 28.3, 28.1.

N1-(4-(Benzyloxy)benzylamino)-*N2,N3*-di(*tert*-butoxycarbonyl)guanidine
(5b)

White solid (m.p.: 113.6-114.7 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.50 (s, 1H, *NHCO*), 8.55 (t, *J* = 5.5 Hz, 1H, NHCH₂), 7.44 (d, *J* = 7.4 Hz, 2H, 2-*H*/6-*H*), 7.39 (t, *J* = 7.5 Hz, 2H, 2'-*H*/6'-*H*), 7.32 (t, *J* = 7.2 Hz, 1H, 4'-*H*), 7.24 (d, *J* = 8.5 Hz, 2H, 3'-*H*/5'-*H*), 6.98 (d, *J* = 8.6 Hz, 2H, 3-*H*/5-*H*), 5.09 (s, 2H, CH₂O), 4.42 (d, *J* = 5.6 Hz, 2H, CH₂NH), 1.43 (s + s, 18H, C(CH₃)₃). ¹³C NMR (DMSO-*d*₆) δ 164.5, 158.9, 156.6, 153.4, 138.5, 131.7, 130.2, 129.8, 129.2, 129.0, 116.2, 84.4, 79.6, 70.6, 44.5, 29.4, 29.0.

Synthesis of intermediates 6

Intermediate 5 of scheme 7 (4.17 mmol) dissolved in ethanol (40 mL) was introduced in a hydrogenator in the presence of 10% wt Pd/C (0.2 g, 1.88 mmol). After removing the air inside the reactor, the reaction mixture was pressurized to 10 bar H₂ and stirred for 48 h. The catalyst was then removed by filtration and the filtrate was evaporated to dryness under reduced pressure. The residue (intermediate 6 of scheme 7) was used in the next step without further purification.

N1-(4-Hydroxyphenethyl)-*N2,N3*-di(*tert*-butoxycarbonyl)guanidine (**6a**)

White solid (m.p.: 175.8 °C (dec.)). ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.48 (s, 1H, OH), 9.19 (s, 1H, NH), 8.28 (t, *J* = 5.6 Hz, 1H, CH₂NH), 7.01 (d, *J* = 8.4 Hz, 2H, 2-*H*/6-*H*), 6.68 (d, *J* = 8.4 Hz, 2H, 3-*H*/5-*H*), 3.49-3.41 (m, 2H, CH₂NH), 2.69 (t, *J* = 7.4 Hz, 2H, CH₂), 1.46 (s, 9H, C(CH₃)₃), 1.40 (s, 9H, C(CH₃)₃). ¹³C NMR (DMSO-*d*₆) δ 163.0, 155.7, 155.1, 151.9, 129.4, 128.7, 115.1, 82.8, 78.0, 41.9, 33.5, 27.9, 27.5.

Synthesis of intermediates 8

A mixture of intermediate **7** of scheme 8 (7.66 mmol), benzyl bromide (0.9 ml, 7.58 mmol) and potassium carbonate (1.50 g, 10.85 mmol) in acetone (40 mL) was refluxed for 12 h. After cooling, the reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was dissolved in ethyl acetate and then supplemented with hexane under stirring. The resulting precipitate of intermediate **8** of scheme 8 was collected by filtration, washed with hexane and dried.

2-(4-(Benzyloxy)phenyl)acetonitrile (**8a**)

White solid (m.p.: 67.8-69.1 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.44 (d, *J* = 6.8 Hz, 2H, 2'-*H*/6'-*H*), 7.39 (t, *J* = 7.4 Hz, 2H, 3'-*H*/5'-*H*), 7.33 (t, *J* = 7.3 Hz, 1H, 4'-*H*), 7.26 (d, *J* = 8.7 Hz, 2H, 2-*H*/6-*H*), 7.03 (d, *J* = 8.7 Hz, 2H, 3-*H*/5-*H*), 5.10 (s, 2H, CH₂O), 3.93 (s, 2H, CH₂CN). ¹³C NMR (DMSO-*d*₆) δ 157.7, 137.0, 129.3, 128.4, 127.8, 127.7, 123.3, 119.5, 115.3, 69.2, 21.5.

4-(Benzyloxy)benzonitrile (**8b**)

White solid (m.p.: 93.7-95.1 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.77 (d, 2H, 2-*H*/6-*H*), 7.45 (d, *J* = 7.1 Hz, 2H, 2'-*H*/6'-*H*), 7.40 (t, 2H, 3'-*H*/5'-*H*), 7.37 – 7.32 (m, 1H, 4'-*H*), 7.18 (d, 2H, 3-*H*/5-*H*), 5.20 (s, 2H, CH₂O). ¹³C NMR (DMSO-*d*₆) δ 160.0, 134.4, 132.5, 126.8, 126.4, 126.1, 117.3, 114.1, 101.2, 67.9.

Synthesis of intermediates 9

HCl gas was bubbled during 30 minutes under stirring and argon atmosphere in a solution of intermediate **8** of scheme 8 (13.39 mmol) in ethanol (30 mL) cooled on an ice bath. The reaction mixture was then stirred at room temperature for 16 h. The solvent was partly removed under reduced pressure and

diethyl ether (20 mL) was added. The resulting solid (intermediate 9 of scheme 8) in suspension was collected by filtration, washed with diethyl ether and dried.

Ethyl 2-(4-(benzyloxy)phenyl)acetimidate hydrochloride (**9a**)

White solid (m.p.: 194.8-196.6 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 7.44 (d, *J* = 6.9 Hz, 2H, 2'-*H*,6'-*H*), 7.39 (t, *J* = 7.4 Hz, 2H, 3'-*H*/5'-*H*), 7.33 (t, *J* = 7.2 Hz, 1H, 4'-*H*), 7.28 (d, *J* = 8.7 Hz, 2H, 2-*H*/6-*H*), 7.02 (d, *J* = 8.7 Hz, 2H, 3-*H*/5-*H*), 5.10 (s, 2H, CH₂O), 4.38 (q, *J* = 7.0 Hz, 2H, CH₂CH₃), 3.89 (s, 2H, CH₂), 1.28 (t, *J* = 7.0 Hz, 3H, CH₂CH₃). ¹³C NMR (DMSO-*d*₆) δ 177.6, 158.0, 136.9, 130.4, 128.5, 127.9, 127.7, 124.2, 115.2, 69.2, 68.9, 37.7, 13.3.

Ethyl 4-(benzyloxy)benzimidate hydrochloride (**9b**)

White solid (m.p. : 185.5-187.2 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.05 (s, 2H, NH₂⁺), 8.07 (d, 2H, 2-*H*/6-*H*), 7.46 (d, 1H, 2'-*H*/6'-*H*), 7.40 (t, 2H, 3'-*H*/5'-*H*), 7.37 – 7.33 (m, 1H, 4'-*H*), 7.26 (d, 2H, 3-*H*/5-*H*), 5.26 (s, 2H, CH₂O), 4.56 (q, *J* = 7.0 Hz, 2H, CH₂CH₃), 1.47 (t, *J* = 7.0 Hz, 3H, CH₂CH₃). ¹³C NMR (126 MHz, DMSO-*d*₆) δ 170.1, 163.9, 136.1, 131.4, 129.3, 128.5, 128.5, 128.2, 127.9, 127.8, 117.8, 115.4, 114.2, 69.8, 68.9, 13.5.

Synthesis of intermediates 10

To a solution of intermediate **9** of scheme 8 (3.34 mmol) in ethanol (20 mL) was added a concentrated aqueous solution of ammonia (7 mL) under stirring and on an ice/acetone bath. After addition, the reaction mixture was stirred at room temperature for 5 h. The reaction mixture was evaporated under reduced pressure. The residue was taken up by ethanol (10 mL) and the suspension was heated to boiling point. The hot suspension was filtered, and the filtrate was evaporated under reduced pressure. The residue of intermediate **10** of scheme 8 was used in the next step without further purification.

2-(4-(Benzyloxy)phenyl)acetimidamide hydrochloride (**10a**)

White solid (m.p.: 130.2-132.5 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 9.15 (s, 2H, NH₂⁺), 8.67 (s, 2H, NH₂), 7.43 (d, *J* = 7.3 Hz, 2H, 2'-*H*/6'-*H*), 7.41 – 7.31 (m, 5H, 3'-*H*/5'-*H*/4'-*H*/2-*H*/6-*H*), 7.01 (d, *J* = 8.2 Hz, 2H, 3-*H*/5-*H*), 5.10 (s, 2H, CH₂O), 3.62 (s, 2H, CH₂). ¹³C NMR (DMSO-*d*₆) δ 169.6, 158.0, 137.2, 130.3, 128.6, 128.0, 127.8, 126.3, 115.2, 69.4, 37.0.

4-(Benzyloxy)benzimidamide hydrochloride (**10b**)

White solid (m.p.: 191.9-193.6 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 9.00 (s, 4H, C(NH₂)NH₂⁺), 7.83 (d, *J* = 8.9 Hz, 2H, 2-*H*/6-*H*), 7.46 (t, *J* = 9.1 Hz, 2H, 2'-*H*/6'-*H*), 7.41 (t, *J* = 7.4 Hz, 2H, 3'-*H*/5'-*H*), 7.35 (t, *J* = 7.2 Hz, 1H, 4'-*H*), 7.24 (d, *J* = 8.9 Hz, 2H, 3-*H*/5-*H*), 5.25 (s, 2H, CH₂O). ¹³C NMR (DMSO-*d*₆) δ 163.9, 161.8, 135.5, 129.4, 127.7, 127.3, 127.0, 118.9, 114.4, 68.8.

Synthesis of intermediates 11

A mixture of intermediate **10** of scheme 8 (3.25 mmol), di-tertbutyl dicarbonate (2.00 g, 9.16 mmol), 4-dimethylaminopyridine (1.03 g, 8.43 mmol), pyridine (10 mL) and dichloromethane (20 mL) was refluxed under stirring for 20 h. The reaction mixture was then evaporated under reduced pressure. The residue was taken up in an aqueous solution of NaHCO₃ 10 % m/v (25 mL) and the resulting suspension was extracted with dichloromethane (3 x 15 mL). The organic phases were combined, washed twice with an aqueous solution of NaHCO₃ 10% m/v (2 x 15 mL), dried over MgSO₄, and filtered. The filtrate was evaporated to dryness and the residue (intermediate 11 of scheme 8) was purified by flash column chromatography using hexane/ethyl acetate.

Tert-butyl (2-(4-(benzyloxy)phenyl)-1-iminoethyl)carbamate (**11a**)

White solid (m.p.: 82.5-84.4 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 10.37 (s, 1H, NH), 7.43 (d, *J* = 6.9 Hz, 2H, 2'-*H*/6'-*H*), 7.38 (t, *J* = 7.6 Hz, 2H, 3'-*H*/5'-*H*), 7.32 (t, *J* = 7.3 Hz, 1H, 4'-*H*), 7.15 (d, *J* = 8.7 Hz, 2H, 2-*H*/6-*H*), 6.94 (d, *J* = 8.6 Hz, 2H, 3-*H*/5-*H*), 5.08 (s, 2H, CH₂O), 3.60 (s, 2H, CH₂), 1.43 (s, 9H, C(CH₃)₃). ¹³C NMR (DMSO- *d*₆) δ 170.9, 157.2, 150.2, 137.2, 130.4, 128.4, 127.8, 127.6, 127.0, 114.6, 80.6, 69.1, 41.8, 27.7.

Tert-butyl ((4-(benzyloxy)phenyl)(imino)methyl)carbamate (**11b**)

White solid. ¹H NMR (500 MHz, DMSO-*d*₆) δ 9.30 (s, 2H, NH), 7.91 (d, *J* = 8.6 Hz, 2H, 2-*H*/6-*H*), 7.46 (d, *J* = 7.3 Hz, 2H, 2'-*H*/6'-*H*), 7.40 (t, *J* = 7.4 Hz, 2H, 3'-*H*/5'-*H*), 7.34 (t, *J* = 7.1 Hz, 1H, 4'-*H*), 7.10 (d, *J* = 8.7 Hz, 2H, 3-*H*/5-*H*), 5.19 (s, 2H, CH₂O), 1.45 (s, 9H, C(CH₃)₃).

Synthesis of intermediates 12

In a hydrogenator were introduced intermediate **11** of scheme 8 (1.38 mmol), 10% wt Pd/C (0.047 g, 0.44 mmol) and ethanol (20 mL). After removing the

inside air, the reactor was pressurized to 10 bar H₂ and the reaction mixture was stirred for 24 h. The catalyst was then removed by filtration and the filtrate was evaporated to dryness under reduced pressure. The residue (intermediate 12 of scheme 8) was used in the next step without further purification.

5 *Tert*-butyl (2-(4-hydroxyphenyl)-1-iminoethyl)carbamate (**12a**)

White solid (m.p.: 134.2 °C (dec.)). ¹H NMR (500 MHz, CDCl₃) δ 7.15 (d, *J* = 8.4 Hz, 2H, 2-*H*/6-*H*), 6.79 (d, *J* = 8.5 Hz, 2H, 3-*H*/5-*H*), 5.30 (s, 1H, OH), 3.96 (s, 2H, CH₂), 1.49 (s, 9H, C(CH₃)₃). ¹³C NMR (DMSO-*d*₆) δ 171.0, 156.1, 150.2, 130.3, 125.0, 115.0, 80.5, 41.8, 27.7.

10

Synthesis of intermediates 15

A mixture of 6-(hydroxymethyl)-2-oxo-2*H*-1-benzopyran-3-carboxylic acid **13** (0.50 g, 2.27 mmol) (obtained according to Pochet et al. *J. Med. Chem.* **1996**, 39, 2579–2585) and thionyl chloride (5 mL, 68.92 mmol) was refluxed for 3 h. The
15 reaction mixture was cooled and evaporated under vacuum. After adding anhydrous toluene (5 mL) to the residue, the solvent was removed under reduced pressure. This last operation was carried out three times. Without further purification, the acyl chloride **14** of scheme 9 (-LG = -Cl) was stirred for 18 h at room temperature under argon atmosphere with intermediate **2** of scheme 7 (or **6** of scheme 7 or compound **12**
20 of scheme 9) (2.27 mmol) and anhydrous pyridine (0.3 mL, 3.72 mmol) in anhydrous dioxane (7 mL). The reaction mixture was then evaporated under reduced pressure. The residue was purified by flash column chromatography using dichloromethane/methanol.

Starting from compound **13** of scheme 9, intermediate **14** of scheme 9 with
25 -LG = -OCOCH₃ was obtained according to the literature (Doucet et al. *J. Med. Chem.* **1999**, 42, 4161-4171). Then, the above-mentioned protocol was followed to obtain the corresponding 6-acetoxymethyl-substituted intermediates **15** of scheme 9.

3-(2-((*Tert*-butoxycarbonyl)amino)ethyl)phenyl 6-(chloromethyl)-2-oxo-2*H*-chromene-3-carboxylate (**15a**)

30 White solid (m.p.: 145-157 °C). ¹H NMR (DMSO-*d*₆) δ 1.37 (s, 9H, C(CH₃)₃), 2.75 (t, *J*=7.4 Hz, 2H, PhCH₂), 3.17 (dt, *J*=7.9 Hz/6.1 Hz, 2H, CH₂N), 4.88 (s, 2H, CH₂Cl), 6.92 (t, *J*=5.7 Hz, 1H, NH), 7.11 (m, 2H, 2'-*H*/4'-*H*), 7.16 (d, *J*=7.6 Hz, 1H, 6'-*H*), 7.40 (t, *J*=8.1 Hz, 1H, 5'-*H*), 7.52 (d, *J*=8.6 Hz, 1H, 8-*H*), 7.85 (dd, *J*=8.6

Hz/2.2 Hz, 1H, 7-*H*), 8.05 (d, *J*=2.2 Hz, 1H, 5-*H*), 9.05 (s, 1H, 4-*H*). ¹³C NMR (DMSO-*d*₆) δ 28.3, 35.1, 41.2, 45.0, 77.6, 116.8, 117.1, 117.8, 119.4, 121.8, 126.5, 129.4, 130.5, 134.6, 135.5, 141.4, 149.9, 150.3, 154.5, 155.5, 155.8, 161.0.

4-(((*Tert*-butoxycarbonyl)amino)methyl)phenyl 6-(chloromethyl)-2-oxo-
5 2*H*-chromene-3-carboxylate (**15b**)

White solid (m.p.: > 260 °C). ¹H NMR (DMSO-*d*₆) δ 1.40 (s, 9H, C(CH₃)₃), 4.16 (d, *J*=6.2 Hz, 2H, CH₂N), 4.88 (s, 2H, CH₂Cl), 7.21 (d, *J*=8.2 Hz, 2H, 2'-*H*/6'-*H*), 7.33 (d, *J*=8.2 Hz, 2H, 3'-*H*/5'-*H*), 7.44 (t, *J*=6.3 Hz, 1H, NH), 7.51 (d, *J*=8.6 Hz, 1H, 8-*H*), 7.85 (dd, *J*=8.7 Hz/2.2 Hz, 1H, 7-*H*), 8.05 (d, *J*=2.1 Hz, 1H, 5-*H*), 9.06 (s, 1H, 4-
10 *H*). ¹³C NMR (DMSO-*d*₆) δ 28.4, 43.0, 45.1, 78.0, 116.9, 118.0, 121.7, 128.2, 130.7, 134.7, 135.6, 138.3, 149.1, 150.0, 154.6, 155.9, 155.9, 161.2.

4-(2-(((*Tert*-butoxycarbonyl)amino)ethyl)phenyl 6-(chloromethyl)-2-oxo-
2*H*-chromene-3-carboxylate (**15c**)

White solid (m.p.: 184 °C (dec)). ¹H NMR (DMSO-*d*₆) δ 1.37 (s, 9H, C(CH₃)₃), 2.73 (t, *J*=7.4 Hz, 2H, PhCH₂), 3.17 (q, *J*=6.8 Hz, 2H, CH₂N), 4.88 (s, 2H, CH₂Cl), 6.91 (t, *J*=5.7 Hz, 1H, NH), 7.18 (d, *J*=8.1 Hz, 2H, 2'-*H*/6'-*H*), 7.29 (d, *J*=8.4 Hz, 1H, 3'-*H*/5'-*H*), 7.51 (d, *J*=8.6 Hz, 1H, 8-*H*), 7.85 (dd, *J*=8.6 Hz/2.2 Hz, 1H, 7-*H*), 8.05 (d, *J*=2.1 Hz, 1H, 5-*H*), 9.04 (s, 1H, 4-*H*). ¹³C NMR (DMSO-*d*₆) δ 28.3, 34.8, 41.4, 45.0, 77.5, 116.8, 117.1, 117.8, 121.5, 129.8, 130.5, 134.5, 135.4, 137.4, 148.6, 149.8,
20 155.0, 155.5, 155.7, 161.1.

3-(((*Tert*-butoxycarbonyl)amino)methyl)phenyl 6-(chloromethyl)-2-oxo-
2*H*-chromene-3-carboxylate (**15d**)

White solid (m.p.: > 260 °C). ¹H NMR (DMSO-*d*₆) δ 1.40 (s, 9H, C(CH₃)₃), 4.18 (d, *J*=6.2 Hz, 2H, CH₂N), 4.88 (s, 2H, CH₂Cl), 7.13 (m, 2H, 2'-*H*/4'-*H*), 7.19 (d, *J*=7.7 Hz, 1H, 6'-*H*), 7.43 (t, *J*=7.8 Hz, 1H, 5'-*H*), 7.46 (t, *J*=6.3 Hz, 1H, NH), 7.51 (d, *J*=8.6 Hz, 1H, 8-*H*), 7.85 (dd, *J*=8.6 Hz/2.2 Hz, 1H, 7-*H*), 8.05 (d, *J*=2.2 Hz, 1H, 5-*H*), 9.05 (s, 1H, 4-*H*). ¹³C NMR (DMSO-*d*₆) δ 28.2, 40.3, 45.0, 77.9, 116.8, 117.1, 117.8, 120.0, 124.7, 129.5, 130.5, 134.5, 135.5, 142.3, 149.9, 150.3, 154.5, 155.8, 155.8, 161.1.

4-(2-(2,3-Bis(*tert*-butoxycarbonyl)guanidino)ethyl)phenyl 6-
30 (chloromethyl)-2-oxo-2*H*-chromene-3-carboxylate (**15e**)

White solid (m.p.: > 250 °C). ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.50 (s, 1H, NH), 9.05 (s, 1H, 4-*H*), 8.37 (t, *J* = 5.6 Hz, 1H, CH₂NH), 8.05 (d, *J* = 2.1 Hz, 1H, 5-*H*), 7.85 (dd, *J* = 8.6, 2.2 Hz, 1H, 7-*H*), 7.51 (d, *J* = 8.6 Hz, 1H, 8-*H*), 7.34 (d, *J* = 8.3

Hz, 2H, 3'-H/5'-H), 7.21 (d, $J = 8.4$ Hz, 2H, 2'-H/6'-H), 4.88 (s, 2H, CH₂Cl), 3.56 (q, $J = 6.3$ Hz, 2H, CH₂NH), 2.87 (t, $J = 7.4$ Hz, 2H, CH₂), 1.47 (s, 9H, C(CH₃)₃), 1.41 (s, 9H, C(CH₃)₃). ¹³C NMR (DMSO-*d*₆) δ 163.0, 160.9, 155.7, 155.1, 154.4, 151.9, 149.8, 148.6, 136.8, 135.4, 134.5, 130.4, 129.7, 121.6, 117.7, 117.0, 116.7, 82.9, 78.1, 44.9,
 5 41.5, 33.7, 27.9, 27.5.

4-(2-((*Tert*-butoxycarbonyl)amino)ethyl)phenyl 6-(acetoxymethyl)-2-oxo-2H-chromene-3-carboxylate (**15f**)

White solid (m.p.: 181-184 °C). ¹H NMR (DMSO-*d*₆) δ 1.37 (s, 9H, C(CH₃)₃), 2.10 (s, 3H, OCOCH₃), 2.73 (t, $J = 7.4$ Hz, 2H, PhCH₂), 3.17 (m, 2H, CH₂N),
 10 5.17 (s, 2H, CH₂O), 6.91 (t, $J = 5.7$ Hz, 1H, NH), 7.18 (d, $J = 8.4$ Hz, 2H, 3'-H/5'-H), 7.29 (d, $J = 8.6$ Hz, 2H, 2'-H/6'-H), 7.50 (d, $J = 8.6$ Hz, 1H, 8-H), 7.78 (dd, $J = 8.6$ Hz/2.1 Hz, 1H, 7-H), 7.98 (d, $J = 2.1$ Hz, 1H, 5-H), 9.05 (s, 1H, 4-H). ¹³C NMR (DMSO-*d*₆) δ 20.7, 28.3, 32.8, 41.4, 64.3, 77.5, 116.5, 116.9, 117.7, 121.5, 129.6, 129.7, 133.1, 134.6, 137.4, 148.6, 150.0, 154.4, 155.5, 155.8, 161.1, 170.2.

15 3-(2-((*Tert*-butoxycarbonyl)amino)ethyl)phenyl 6-(acetoxymethyl)-2-oxo-2H-chromene-3-carboxylate (**15g**)

White solid (m.p.: 159-161.5°C). ¹H NMR (DMSO-*d*₆) δ 1.36 (s, 9H, C(CH₃)₃), 2.10 (s, 3H, OCOCH₃), 2.75 (t, $J = 7.4$ Hz, 2H, PhCH₂), 3.17 (dt, $J = 8.0$ Hz/6.1 Hz, 2H, CH₂N), 5.17 (s, 2H, CH₂O), 6.92 (t, $J = 5.6$ Hz, 1H, NH), 7.11 (m, 2H, 2'-H/4'-H),
 20 7.15 (d, $J = 7.8$ Hz, 1H, 6'-H), 7.39 (t, $J = 8.2$ Hz, 1H, 5'-H), 7.50 (d, $J = 8.6$ Hz, 1H, 8-H), 7.78 (dd, $J = 8.6$ Hz/2.2 Hz, 1H, 7-H), 7.98 (d, $J = 2.2$ Hz, 1H, 5-H), 9.05 (s, 1H, 4-H). ¹³C NMR (DMSO-*d*₆) δ 20.7, 28.2, 35.1, 41.2, 64.3, 77.6, 116.5, 116.9, 117.7, 119.4, 121.8, 126.5, 129.4, 129.6, 133.2, 134.6, 141.4, 150.1, 150.3, 154.4, 155.5, 155.8, 161.0, 170.2.

25 4-(((*Tert*-butoxycarbonyl)amino)methyl)phenyl 6-(acetoxymethyl)-2-oxo-2H-chromene-3-carboxylate (**15h**)

White solid (m.p.: 164-167°C). ¹H NMR (DMSO-*d*₆) δ 1.40 (s, 9H, C(CH₃)₃), 2.10 (s, 3H, OCOCH₃), 4.16 (d, $J = 6.2$ Hz, 2H, CH₂N), 5.17 (s, 2H, CH₂Cl), 7.21 (d, $J = 8.5$ Hz, 2H, 3'-H/5'-H), 7.33 (d, $J = 8.5$ Hz, 2H, 2'-H/6'-H), 7.44 (t, $J = 6.3$ Hz, 1H, NH),
 30 7.50 (d, $J = 8.6$ Hz, 1H, 8-H), 7.78 (dd, $J = 8.6$ Hz/2.2 Hz, 1H, 7-H), 7.98 (d, $J = 2.1$ Hz, 1H, 5-H), 9.06 (s, 1H, 4-H). ¹³C NMR (DMSO-*d*₆) δ 20.7, 28.3, 42.8, 64.3, 77.9, 116.5, 116.8, 117.7, 121.6, 128.1, 129.6, 133.1, 134.6, 138.2, 149.0, 150.1, 154.4, 155.8, 161.1, 170.2.

3-(((*Tert*-butoxycarbonyl)amino)methyl)phenyl 6-(acetoxymethyl)-2-oxo-2*H*-chromene-3-carboxylate (**15i**)

White solid (m.p.: 122.5-126°C). ¹H NMR (DMSO-*d*₆) δ 1.40 (s, 9H, C(CH₃)₃), 2.10 (s, 3H, OCOCH₃), 4.18 (d, *J*=6.2 Hz, 2H, CH₂N), 5.17 (s, 2H, CH₂Cl), 7.14 (m, 2H, 2'-*H*/4'-*H*), 7.19 (d, *J*=7.6 Hz, 1H, 6'-*H*), 7.43 (t, *J*=7.8 Hz, 1H, 5'-*H*), 7.46 (t, *J*=6.1 Hz, 1H, *NH*), 7.50 (d, *J*=8.6 Hz, 1H, 8-*H*), 7.78 (dd, *J*=8.6 Hz/2.2 Hz, 1H, 7-*H*), 7.98 (d, *J*=2.1 Hz, 1H, 5-*H*), 9.06 (s, 1H, 4-*H*). ¹³C NMR (DMSO-*d*₆) δ 20.7, 28.2, 43.0, 64.3, 77.9, 116.5, 116.9, 117.7, 120.0, 124.7, 129.5, 129.6, 133.1, 134.6, 142.3, 150.1, 150.3, 154.4, 155.8, 155.8, 161.1, 170.2.

4-(2-(2,3-Bis(*tert*-butoxycarbonyl)guanidino)ethyl)phenyl 6-(acetoxymethyl)-2-oxo-2*H*-chromene-3-carboxylate (**15j**)

White solid (m.p.: 222.4 °C (dec)). ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.50 (s, 1H, *NH*), 9.06 (s, 1H, 4-*H*), 8.37 (t, *J* = 5.7 Hz, 1H, CH₂*NH*), 7.98 (d, *J* = 2.1 Hz, 1H, 5-*H*), 7.78 (dd, *J* = 8.6, 2.1 Hz, 1H, 7-*H*), 7.50 (d, *J* = 8.6 Hz, 1H, 8-*H*), 7.34 (d, *J* = 8.6 Hz, 2H, 3'-*H*/5'-*H*), 7.21 (d, *J* = 8.5 Hz, 2H, 2'-*H*/6'-*H*), 5.17 (s, 2H, CH₂OCO), 3.56 (q, *J* = 7.8, 7.1 Hz, 2H, CH₂*NH*), 2.87 (t, *J* = 7.3 Hz, 2H, CH₂), 2.10 (s, 3H, CH₃), 1.47 (s, 9H, C(CH₃)₃), 1.41 (s, 9H, C(CH₃)₃). ¹³C NMR (DMSO-*d*₆) δ 163.0, 161.0, 155.7, 155.1, 154.3, 151.9, 150.0, 148.6, 136.8, 134.5, 133.1, 129.7, 129.5, 121.6, 117.6, 116.8, 116.4, 82.9, 78.1, 64.2, 41.5, 33.7, 27.9, 27.5, 20.6.

20

Synthesis of the target compounds 16

Intermediate **15** of scheme 9 (1.12 mmol) was dissolved in acetonitrile saturated with HCl gas (10 mL) (HX = HCl). The reaction mixture was stirred for 16-96 h at room temperature. The formed precipitate of compound **16** of scheme 9 as the hydrochloride was collected by filtration, washed with acetonitrile and dried. Alternatively, trifluoroacetic acid can be used instead of HCl (HX = CF₃COOH) to obtain the target compounds **16** of scheme 9 as the trifluoroacetate salts.

25

3-(2-Aminoethyl)phenyl 6-(chloromethyl)-2-oxo-2*H*-chromene-3-carboxylate hydrochloride (**16a**)

White solid (m.p.: 220 °C (dec)). ¹H NMR (DMSO-*d*₆) δ 2.94 (m, 2H, PhCH₂), 3.08 (m, 2H, CH₂N), 4.89 (s, 2H, CH₂Cl), 7.19 (d, *J*=7.8 Hz, 1H, 4'-*H*), 7.21 (t, *J*=1.7 Hz, 1H, 2'-*H*), 7.24 (d, *J*=7.9 Hz, 1H, 6'-*H*), 7.46 (t, *J*=7.8 Hz, 1H, 5'-*H*), 7.53 (d, *J*=8.6 Hz, 1H, 8-*H*), 7.86 (dd, *J*=8.6 Hz/2.2 Hz, 1H, 7-*H*), 7.92 (bs, 3H, *NH*₃⁺), 8.06

30

(d, $J=2.2$ Hz, 1H, 5-*H*), 9.05 (s, 1H, 4-*H*). ^{13}C NMR (DMSO- d_6) δ 32.6, 44.9, 116.7, 116.9, 117.7, 120.1, 121.8, 126.5, 129.7, 130.4, 134.5, 135.5, 139.1, 149.9, 150.3, 154.4, 155.7, 160.9.

4-(Aminomethyl)phenyl 6-(chloromethyl)-2-oxo-2*H*-chromene-3-
5 carboxylate hydrochloride (**16b**)

White solid (m.p.: 250 °C (dec)). ^1H NMR (DMSO- d_6) δ 4.07 (s, 2H, CH_2N), 4.89 (s, 2H, CH_2Cl), 7.34 (d, $J=8.6$ Hz, 2H, 2'-*H*/6'-*H*), 7.52 (d, $J=8.6$ Hz, 1H, 8-*H*), 7.58 (d, $J=8.5$ Hz, 2H, 3'-*H*/5'-*H*), 7.86 (dd, $J=8.6$ Hz/2.2 Hz, 1H, 7-*H*), 8.06 (d, $J=2.2$ Hz, 1H, 5-*H*), 8.15 (bs, 3H, NH_3^+), 9.08 (s, 1H, 4-*H*). ^{13}C NMR (DMSO- d_6) δ 41.6, 44.9,
10 116.7, 116.7, 117.7, 122.0, 130.3, 130.5, 134.5, 135.5, 150.1, 150.2, 154.4, 155.6, 160.9.

4-(2-(Aminoethyl)phenyl) 6-(chloromethyl)-2-oxo-2*H*-chromene-3-
carboxylate hydrochloride (**16c**)

White solid (m.p.: 253 °C (dec)). ^1H NMR (DMSO- d_6) δ 2.92 (m, 2H, PhCH_2), 3.08 (m, 2H, CH_2N), 4.88 (s, 2H, CH_2Cl), 7.25 (d, $J=8.5$ Hz, 2H, 2'-*H*/6'-*H*),
15 7.38 (d, $J=8.5$ Hz, 2H, 3'-*H*/5'-*H*), 7.52 (d, $J=8.6$ Hz, 1H, 8-*H*), 7.86 (dd, $J=8.6$ Hz/2.2 Hz, 1H, 7-*H*), 7.92 (bs, 3H, NH_3^+), 8.06 (d, $J=2.2$ Hz, 1H, 5-*H*), 9.05 (s, 1H, 4-*H*). ^{13}C NMR (DMSO- d_6) δ 32.4, 45.0, 116.8, 117.0, 117.8, 121.9, 129.9, 130.5, 134.6, 135.3, 135.5, 149.1, 150.0, 154.5, 155.8, 161.1.

3-(Aminomethyl)phenyl 6-(chloromethyl)-2-oxo-2*H*-chromene-3-
20 carboxylate hydrochloride (**16d**)

White solid (m.p.: 217 °C (dec)). ^1H NMR (DMSO- d_6) δ 4.08 (s, 2H, CH_2N), 4.88 (s, 2H, CH_2Cl), 7.31 (d, $J=8.1$ Hz, 1H, 4'-*H*), 7.43 (t, $J=2.0$ Hz, 1H, 2'-*H*), 7.47 (d, $J=8.1$ Hz, 1H, 6'-*H*), 7.53 (m, 2H, 5'-*H*/8-*H*), 7.86 (dd, $J=8.6$ Hz/2.2 Hz, 1H, 7-*H*), 8.07 (d, $J=2.2$ Hz, 1H, 5-*H*), 8.50 (bs, 3H, NH_3^+), 9.06 (s, 1H, 4-*H*). ^{13}C NMR (DMSO- d_6) δ
25 41.8, 45.0, 116.8, 116.9, 117.8, 122.0, 122.2, 126.7, 130.0, 130.6, 134.6, 135.6, 135.9, 150.1, 150.3, 154.6, 155.8, 161.1.

1-(4-((6-(Chloromethyl)-2-oxo-2*H*-chromene-3-
carbonyl)oxy)phenethyl)guanidinium chloride dihydrate (**16e**)

White solid (m.p.: 208 °C (dec)). ^1H NMR (500 MHz, DMSO- d_6) δ 9.05 (s, 1H, 4-*H*), 8.05 (d, $J=2.2$ Hz, 1H, 5-*H*), 7.86 (dd, $J=8.6, 2.2$ Hz, 1H, 7-*H*), 7.55 – 7.50 (m, 2H, 8-*H*/NH), 7.39 (d, $J=8.5$ Hz, 2H, 3'-*H*/5'-*H*), 7.23 (d, $J=8.5$ Hz, 2H, 2'-*H*/6'-*H*), 4.88 (s, 2H, CH_2Cl), 3.44 – 3.36 (m, 2H, CH_2NH), 2.83 (t, $J=7.4$ Hz, 2H, CH_2).

^{13}C NMR (DMSO- d_6) δ 161.1, 156.7, 155.8, 154.5, 149.9, 148.9, 136.3, 135.5, 134.6, 130.5, 130.0, 121.7, 117.8, 117.0, 116.8, 45.0, 41.9, 33.8.

4-(2-(Aminoethyl)phenyl) 6-(acetoxymethyl)-2-oxo-2*H*-chromene-3-carboxylate hydrochloride hemihydrate (**16f**)

5 White solid (m.p.: 229-232 °C). ^1H NMR (DMSO- d_6) δ 2.10 (s, 3H, CH_3), 2.91 (dd, $J=9.3$ Hz/6.6 Hz, 2H, PhCH_2), 3.08 (dd, $J=9.3$ Hz/6.6 Hz, 2H, CH_2N), 5.17 (s, 2H, CH_2O), 7.24 (d, $J=8.5$ Hz, 2H, 2'-*H*/6'-*H*), 7.38 (d, $J=8.5$ Hz, 2H, 3'-*H*/5'-*H*), 7.51 (d, $J=8.6$ Hz, 1H, 8-*H*), 7.79 (dd, $J=8.6$ Hz/2.2 Hz, 1H, 7-*H*), 7.88 (bs, 3H, NH_3^+), 7.98 (d, $J=2.1$ Hz, 1H, 5-*H*), 9.06 (s, 1H, 4-*H*). ^{13}C NMR (DMSO- d_6) δ 20.7, 32.4, 39.8, 10 64.3, 116.5, 116.8, 117.7, 121.9, 129.6, 129.9, 133.2, 134.7, 135.3, 149.1, 150.2, 154.4, 155.8, 161.1, 170.2.

3-(2-(Aminoethyl)phenyl) 6-(acetoxymethyl)-2-oxo-2*H*-chromene-3-carboxylate hydrochloride hemihydrate (**16g**)

White solid (m.p.: 198.5-200.5 °C). ^1H NMR (DMSO- d_6) δ 2.10 (s, 3H, OCOCH_3), 2.95 (dd, $J=9.6$ Hz/6.3 Hz, 2H, PhCH_2), 3.07 (dd, $J=9.4$ Hz/6.5 Hz, 2H, CH_2N), 5.17 (s, 2H, CH_2Cl), 7.18 (dd, $J=7.9$ Hz/1.8 Hz, 1H, 4'-*H*), 7.20 (t, $J=2.0$ Hz, 2'-*H*), 7.23 (dd, $J=7.7$ Hz/1.4 Hz, 1H, 6'-*H*), 7.45 (t, $J=7.8$ Hz, 1H, 5'-*H*), 7.51 (d, $J=8.6$ Hz, 1H, 8-*H*), 7.79 (dd, $J=8.6$ Hz/2.1 Hz, 1H, 7-*H*), 7.99 (d, $J=2.1$ Hz, 1H, 5-*H*), 8.05 (bs, 3H, NH_3^+), 9.06 (s, 1H, 4-*H*). ^{13}C NMR (DMSO- d_6) δ 20.7, 32.6, 39.6, 64.3, 116.5, 15 116.8, 117.7, 120.2, 121.9, 126.6, 129.6, 129.8, 133.2, 134.7, 139.3, 150.2, 150.4, 154.4, 155.8, 161.0, 170.2.

4-(Aminomethyl)phenyl 6-(acetoxymethyl)-2-oxo-2*H*-chromene-3-carboxylate hydrochloride hemihydrate (**16h**)

White solid (m.p.: 243 °C (dec)). ^1H NMR (DMSO- d_6) δ 2.10 (s, 3H, OCOCH_3), 4.07 (s, 2H, CH_2N), 5.17 (s, 2H, CH_2Cl), 7.34 (d, $J=8.5$ Hz, 2H, 3'-*H*/5'-*H*), 7.51 (d, $J=8.6$ Hz, 1H, 8-*H*), 7.59 (d, $J=8.6$ Hz, 2H, 2'-*H*/6'-*H*), 7.79 (dd, $J=8.6$ Hz/2.2 Hz, 1H, 7-*H*), 7.99 (d, $J=2.1$ Hz, 1H, 5-*H*), 8.32 (bs, 3H, NH_3^+), 9.09 (s, 1H, 4-*H*). ^{13}C NMR (DMSO- d_6) δ 20.7, 41.7, 64.3, 116.5, 116.6, 117.7, 122.1, 129.6, 130.5, 132.0, 133.2, 134.8, 150.3, 150.4, 154.4, 155.8, 161.0, 170.2.

30 3-(Aminomethyl)phenyl 6-(acetoxymethyl)-2-oxo-2*H*-chromene-3-carboxylate hydrochloride hemihydrate (**16i**)

White solid (m.p.: 228 °C). ^1H NMR (DMSO- d_6) δ 2.10 (s, 3H, OCOCH_3), 4.09 (s, 2H, CH_2N), 5.17 (s, 2H, CH_2Cl), 7.32 (ddd, $J=8.2$ Hz/2.4 Hz/1.0 Hz, 1H, 4'-*H*),

7.41 (t, $J=2.0$ Hz, 2'-*H*), 7.44 (dd, $J=7.8$ Hz/1.4 Hz, 1H, 6'-*H*), 7.51 (d, $J=8.6$ Hz, 1H, 8-*H*), 7.55 (t, $J=7.9$ Hz, 1H, 5'-*H*), 7.80 (dd, $J=8.6$ Hz/2.1 Hz, 1H, 7-*H*), 7.99 (d, $J=2.1$ Hz, 1H, 5-*H*), 8.29 (bs, 3H, NH_3^+), 9.07 (s, 1H, 4-*H*). ^{13}C NMR (DMSO- d_6) δ 20.7, 41.8, 64.3, 116.5, 116.7, 117.7, 122.0, 122.2, 126.7, 129.7, 130.0, 133.2, 134.8, 135.9, 150.3, 150.3, 154.4, 155.8, 161.1, 170.2.

1-(4-((6-(Acetoxymethyl)-2-oxo-2*H*-chromene-3-carbonyl)oxy)phenethyl)guanidinium chloride hemihydrate (**16j**)

White solid (m.p.: 205.9-206.9 °C). 1H NMR (500 MHz, DMSO- d_6) δ 9.06 (s, 1H, 4-*H*), 7.98 (d, $J = 2.0$ Hz, 1H, 5-*H*), 7.79 (dd, $J = 8.6, 2.1$ Hz, 1H, 7-*H*), 7.57 (t, $J = 5.8$ Hz, 1H, CH_2NH), 7.51 (d, $J = 8.6$ Hz, 1H, 8-*H*), 7.39 (d, $J = 8.3$ Hz, 2H, 3'-*H*/5'-*H*), 7.23 (d, $J = 8.2$ Hz, 2H, 2'-*H*/6'-*H*), 5.17 (s, 2H, CH_2OCO), 3.40 (q, $J = 6.4$ Hz, 2H, CH_2NH), 2.83 (t, $J = 7.4$ Hz, 2H, CH_2), 2.10 (s, 3H, CH_3). ^{13}C NMR (DMSO- d_6) δ 170.2, 161.1, 156.7, 155.8, 154.4, 150.1, 148.9, 136.3, 134.7, 133.2, 130.0, 129.6, 121.7, 117.7, 116.8, 116.5, 64.3, 41.9, 33.8, 20.7.

Synthesis of target compounds 20 of scheme 10

Target compounds 20 of scheme 10 are compounds with R2 being a cyclic compound. The synthesis enables to obtain piperidinyll compounds (Y in compound 20 of scheme 10 = CH) and piperazinyll compounds (Y in compound 20 of scheme 10 = N). The position of R2 may be in ortho, meta or para.

An example is illustrated as **20a** in Table 1. The appropriate piperidinyll- or piperazinyll-substituted phenol **17** of scheme 10 was converted into the *N*-*tert*-butoxycarbonyl-substituted intermediate **18** of scheme 10 according to the conditions described for the synthesis of intermediates **2** of scheme 7. Subsequently, intermediate **19** of scheme 10 was obtained by following the conditions described for the synthesis of intermediates **15** of scheme 9. Deprotection of the *tert*-butoxycarbonyl group of **19** of scheme 10 was finally performed in acidic conditions as described for the synthesis of the target compounds **16** of scheme 9.

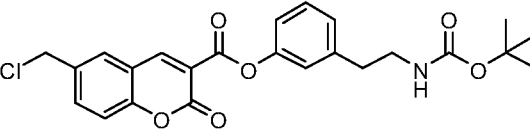
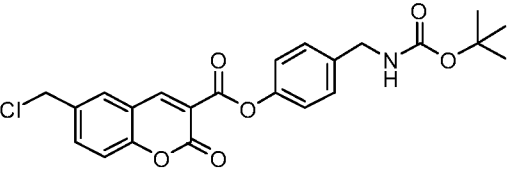
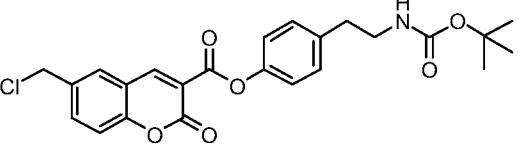
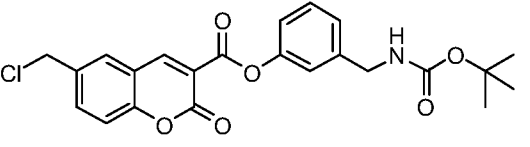
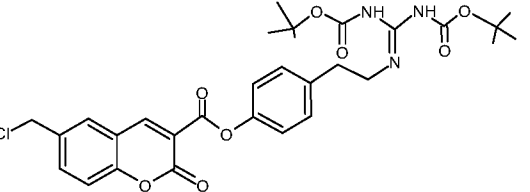
tert-butyl 4-(4-((6-(chloromethyl)-2-oxo-2*H*-chromene-3-carbonyl)oxy)phenyl)piperazine-1-carboxylate **19a**

White solid (m.p.: 171.5-173.5°C). 1H NMR (DMSO- d_6) δ 1.43 (s, 9H, $C(CH_3)_3$), 3.12 (t, $J=5.2$ Hz, 4H, 2''-*H*₂/6''-*H*₂), 3.48 (t, $J=5.1$ Hz, 4H, 3''-*H*₂/5''-*H*₂), 4.88 (s, 2H, CH_2Cl), 7.04 (d, $J=9.0$ Hz, 2H, 3'-*H*/5'-*H*), 7.14 (d, $J=9.0$ Hz, 2H, 2'-*H*/6'-*H*),

7.51 (d, J=8.6 Hz, 1H, 8-*H*), 7.85 (dd, J=8.6 Hz/2.2 Hz, 1H, 7-*H*), 8.05 (d, J=2.1 Hz, 1H, 5-*H*), 9.03 (s, 1H, 4-*H*). ¹³C NMR (DMSO-*d*₆) δ 28.5, 45.5, 49.1, 79.5, 117.2, 117.3, 117.7, 118.3, 122.5, 131.0, 135.0, 135.9, 143.5, 149.5, 150.1, 154.3, 154.9, 156.3, 161.9.

4-(piperazin-1-yl)phenyl 6-(chloromethyl)-2-oxo-2*H*-chromene-3-carboxylate dichlorhydrate dihydrate **20a**

White solid (m.p.: 60-62 °C). ¹H NMR (DMSO-*d*₆) δ 3.23 (m, 4H, 2''-*H*₂/6''-*H*₂), 3.38 (m, 4H, 3''-*H*₂/5''-*H*₂), 4.88 (s, 2H, CH₂Cl), 7.08 (m, 2H, 3'-*H*/5'-*H*), 7.17 (m, 2H, 2'-*H*/6'-*H*), 7.51 (d, J=8.6 Hz, 1H, 8-*H*), 7.85 (dd, J=8.7 Hz/2.2 Hz, 1H, 7-*H*), 8.05 (d, J=2.2 Hz, 1H, 5-*H*), 9.04 (s, 1H, 4-*H*), 9.15 (bs, 2H, NH₂⁺). ¹³C NMR (DMSO-*d*₆) δ 42.5, 44.9, 45.6, 116.7, 116.8, 117.0, 117.7, 122.1, 130.4, 134.4, 135.3, 143.4, 148.0, 149.6, 154.4, 155.7, 161.2.

Ref	Code	Structure	Formula	MW
15a	EG4164		C ₂₄ H ₂₄ ClNO ₆	457.91
15b	EG4170		C ₂₃ H ₂₂ ClNO ₆	443.88
15c	EG4175		C ₂₄ H ₂₄ ClNO ₆	457.91
15d	EG4177		C ₂₃ H ₂₂ ClNO ₆	443.88
15e	CL047		C ₃₀ H ₃₄ ClN ₃ O ₈	600.06

15f	EG4249		$C_{26}H_{27}NO_8$	481.50
15g	EG4250		$C_{26}H_{27}NO_8$	481.50
15h	EG4251		$C_{25}H_{25}NO_8$	467.47
15i	EG4253		$C_{25}H_{25}NO_8$	467.47
15j	CL057		$C_{32}H_{37}N_3O_{10}$	623.66
19a	EG4189		$C_{26}H_{27}ClN_2O_6$	498.96
16a	EG4173		$C_{19}H_{17}Cl_2NO_4$	394.25
16b	EG4185		$C_{18}H_{15}Cl_2NO_4$	380.22
16c	EG4186		$C_{19}H_{17}Cl_2NO_4$	394.25

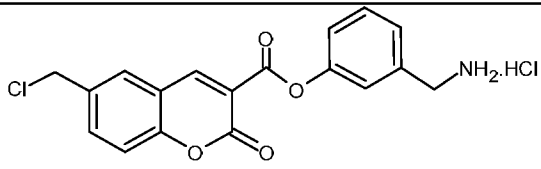
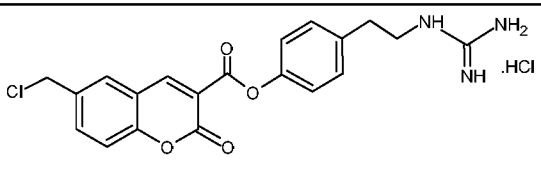
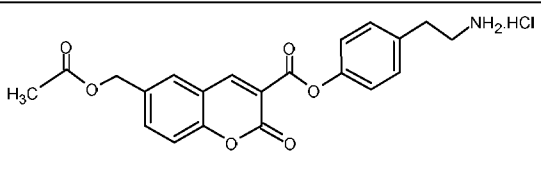
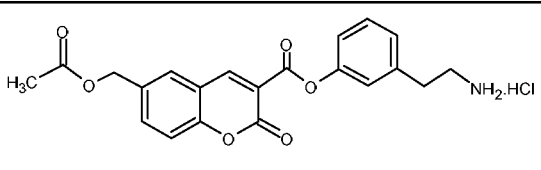
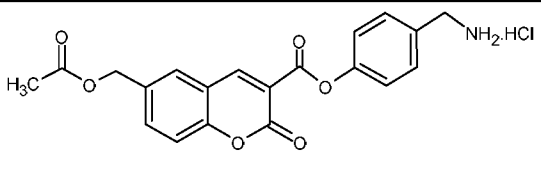
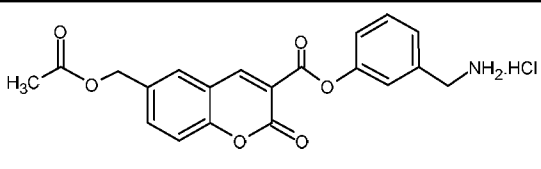
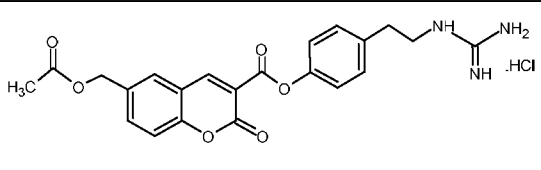
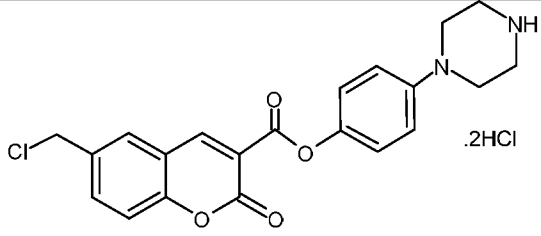
16d	EG4192		$C_{18}H_{15}Cl_2NO_4$	380.22
16e	CL048		$C_{20}H_{19}Cl_2N_3O_4 \cdot 2H_2O$	468.33
16f	EG4252		$C_{21}H_{20}ClNO_6 \cdot 1/2H_2O$	426.83
16g	EG4254		$C_{21}H_{20}ClNO_6 \cdot 1/2H_2O$	426.83
16h	EG4255		$C_{20}H_{18}ClNO_6 \cdot 1/2H_2O$	412.83
16i	EG4256		$C_{20}H_{18}ClNO_6 \cdot 1/2H_2O$	412.83
16j	CL058		$C_{22}H_{22}ClN_3O_6 \cdot 1/2H_2O$	468.89
20a	EG4193		$C_{21}H_{21}Cl_3N_2O_4 \cdot 2H_2O$	507.79

Table 1: Compounds obtained in example 2b

Example 3: In vitro enzymatic assays and inhibition mechanistic studies

The compounds were evaluated *in vitro* for their inhibitory potential on the human recombinant TMPRSS2 and HAT (TMPRSS11D. The enzyme activities

are measured at 25°C in the presence of appropriate commercially available fluorogenic peptide substrates, Boc-QAR-AMC for TMPRSS2 and, Boc-VPR-AMC for HAT using a fluorescence microplate reader (BMG Fluostar Optima). The experimental conditions are summarized in Table 2.

5

Protease	Fluorogenic substrate	Buffer	$\lambda_{\text{Excitation}}$ $\lambda_{\text{Emission}}$	Temperature
TMPRSS2 18,5 nM	Boc-QAR-AMC (100 μ M)	Tris 50 mM, NaCl 150 mM, 0.01% Tween-20, pH 8	360 nm 460 nm	25°C
HAT 20 pM	Boc-VPR-AMC (100 μ M)	Tris 50 mM, 0.05% Brij-35, pH 9.5	360 nm 460 nm	25°C

Table 2. Experimental conditions used for inhibition studies of TMPRSS2 and HAT.

Compounds (5-100 μ M) were tested in triplicate for each inhibitor to detect its inhibitory potential. The tested compounds were previously incubated with
 10 TMPRSS2 or HAT in the appropriate buffer for 15 min at 25°C. The enzymatic reactions were triggered by the addition of the fluorogenic substrate and the kinetics were monitored for 30 min. Initial rates (V_0) determined in control experiments (no inhibitor) were considered to be 100% of the proteinase activity; initial rates V_i that were below 100% in the presence of a tested compound were considered to be
 15 inhibitions. The same percentage of co-solvent was used in both cases. The inhibitory activity of compounds was expressed as IC₅₀ (inhibitor concentrations giving 50% inhibition). The values of IC₅₀ were calculated by fitting the experimental data to equation: % Inhibition = $100(1 - V_i/V_0) = 100[I]/(IC_{50} + [I])$.

Compounds displaying an IC₅₀ value below 10 μ M were retained for
 20 structure-activity analysis (Table 3).

Cpd	Code	TMPRSS2		HAT	
		% Inhibition at 10 μ M	IC ₅₀ (μ M)	% Inhibition at 10 μ M	IC ₅₀ (μ M)
16a	EG4173	63.9	8.1 $\pm$ 0.3	39.9	-
16b	EG4185	82.1	3.9 $\pm$ 0.1	90.2	0.7 $\pm$ 0.1
16c	EG4186	89.8	2.0 $\pm$ 0.1	73.5	4.0 $\pm$ 0.2
16d	EG4192	28.4	-	55.3	-
16j	CL058	100	0.27 $\pm$ 0.03	-	-
20a	EG4193	25.4	-	8.7	-

Table 3. Inhibition of TMPRSS2 and HAT by compounds **16a-d**, **16j** and **20a** at 25°C.

Several compounds displayed a noticeable inhibitory activity against
 5 TMPRSS2 such as **16a**, **16b**, **16c**, **16d**, **16j** and **20a**. Compounds **16a**, **16b**, **16c** and
16d also inhibited HAT. Compound **16b** was more active against HAT than
 TMPRSS2 (factor 5.6). A similar inhibitory activity on the two enzymes was observed
 for compound **16c**. The alkyl chain length or its position (4' or 5') was not
 discriminative (**16a** versus **16d**). The inhibition of TMPRSS2 and HAT was favored
 10 by the presence of a positive charge (factor of 74 for TMPRSS2 and of 10 for HAT) by
 comparing compounds **16c** and **15c**. The charged compounds **16f**, **16e** and **16i**
 displayed about 100% inhibition at 10 μ M inhibitor concentration. The nature of the
 charged group R² (-(CH₂)_n-NH₂ or -(CH₂)₂-C(=NH)NH₂) for example) had a poor
 influence on the inhibitory activity whereas the cyclic charged group of **20a** was
 15 unfavorable.

The selectivity spectrum was studied using a panel of representative
 proteases including several trypsin-like kallikrein-related peptidases (KLK5, KLK6,
 KLK8, KLK13), plasma-blood serine proteases (plasmin, thrombin, tPA)
 transmembrane serine proteases (matriptase) (Table 4).

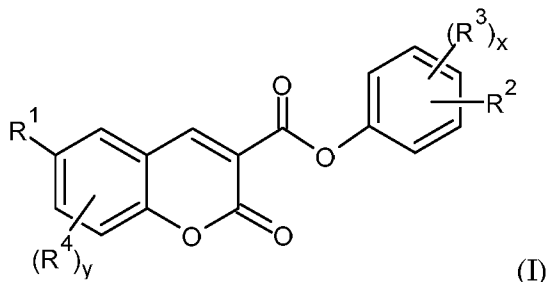
	TMPR SS2	HAT	KLK 5	KLK 6	KLK 8	KLK 13	Plasmin	Thrombin	tPA	Matri -ptase
16a	63.9	39.9	NI	NI	21	NI	36	NI	NI	29
16b	82.1	90.2	NI	32	70	NI	65	44	87	21
16c	89.8	73.5	NI	30	23	NI	38	NI	NI	40
16d	28.4	55.3	NI	38	38	NI	22	NI	20	NI

Table 4. Effect of selected compounds on a panel of serine proteases showing the percentage of inhibition at 10 μ M (at 30 min and 37°C). NI: < 20% inhibition. Each inhibitor was tested in triplicate.

- 5 From results summarized in Table 4, it can be observed that compound **16c** has a good selectivity for TMPRSS2 and HAT in comparison with all other tested proteases. Compound **16a** inhibited in a quite similar way TMPRSS2, HAT and plasmin. Compound **16b** inhibited in a quite similar way TMPRSS2, HAT and tPA whereas compound **16d** was less selective.

CLAIMS

1. Compound of Formula I:



wherein R¹ is a substituted linear or branched alkyl chain of 1 to 6 carbon atoms;

5 R² is a basic group bearing a positive charge at physiological pH or a precursor thereof, such basic group or precursor thereof being optionally preceded by a linear or branched alkyl chain of 1 to 6 carbon atoms and R² being in the ortho, meta or para position of the ester group on the aryl ring; R³ is a halogen with x being an integer between 0 and 4; R⁴ is a halogen with y being an integer between 0 and 3;
 10 or a prodrug thereof, and/or a salt thereof,
 for its use in the treatment of respiratory diseases.

2. Compound for use according to claim 1 wherein the basic group bears an amine function, an amidine function or a guanidine function.

3. Compound for use according to claim 1 or 2 wherein the precursor of the
 15 basic group bears a (pseudo)carbamate function.

4. Compound for use according to claim 2 wherein R² is chosen from the group consisting of $-(CH_2)_n-NH_2$, $-(CH_2)_n-C(=NH)NH_2$, $-(CH_2)_n-NH-C(=NH)NH_2$, $-(CH_2)_n-NH-COOR^5$, $-(CH_2)_n-C(=NH)NH-COOR^5$, $-(CH_2)_n-NH-C(=NH)NH-COOR^5$ with R⁵ being a linear or branched alkyl chain with 1 to 6 carbon atoms and with n being
 20 an integer between 0 and 6.

5. Compound for use according to claim 4 wherein n is 1 or 2.

6. Compound for use according to any of previous claims wherein x is 1 or 2.

7. Compound for use according to any of previous claims wherein y is 0, 1 or 2.

8. Compound for use according to any of previous claims wherein R¹ is $-CH_2Cl$
 25 or $-CH_2Br$.

9. Compound for use according to any of previous claims wherein said respiratory disease is a viral infection.

10. Compound of Formula I or a prodrug thereof, and/or a salt thereof for its use in the prevention of respiratory diseases.
11. Compound of Formula I or a prodrug thereof, and/or a salt thereof wherein R^2 is a basic group bearing a positive charge at physiological pH or a precursor thereof, such basic group or precursor thereof being preceded by a linear or
5 branched alkyl chain of 1 to 6 carbon atoms, preferably of 1 to 2 carbon atoms.
12. Compound according to claim 11 wherein R^2 is $-(CH_2)_n-NH-COOR^5$, $-(CH_2)_n-C(=NH)NH-COOR^5$, $-(CH_2)_n-NH-C(=NH)NH-COOR^5$ with R^5 being a linear or branched alkyl chain with 1 to 6 carbon atoms and with n being an integer between
10 1 and 6.
13. Compound according to any of claims 11 to 12 for use as a medicament.
14. A pharmaceutical composition comprising a compound as defined in any of claim 11 or 12 and a pharmaceutically acceptable carrier.
15. A pharmaceutical composition according to claim 14 in a form suitable for an aerosol administration.

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2023/061344

A. CLASSIFICATION OF SUBJECT MATTER INV. A61K31/37 A61K9/00 A61K31/496 A61P11/00 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) 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		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BISSENETTE E Y ET AL: "Coumarinic derivatives show anti-inflammatory effects on alveolar macrophages, but their anti-elastase activity is essential to reduce lung inflammation in vivo", INTERNATIONAL IMMUNOPHARMACOLOGY, ELSEVIER, AMSTERDAM, NL, vol. 9, no. 1, 1 January 2009 (2009-01-01), pages 49-54, XP025868950, ISSN: 1567-5769, DOI: 10.1016/J.INTIMP.2008.09.009 [retrieved on 2009-01-01]	1-4, 6-11
A	the whole document ----- -/-	5, 12-15
☒ Further documents are listed in the continuation of Box C. ☐ See patent family 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 application or patent 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 21 July 2023		Date of mailing of the international search report 31/07/2023
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Trifilieff, Sylvie

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2023/061344

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	POCHET L ET AL: "ESTERS AND AMIDES OF 6-(CHLOROMETHYL)-2-OXO-2H-1-BENYOPYRAN-3-CARBOXYLIC ACID AS INHIBITORS OF ASPIRE-CHYMOTRYSPIN: SIGNIFICANCE OF THE "AROMATIC" NATURE OF THE NOVEL ESTER-TYPE COUMARIN FOR STRONG INHIBITORY ACTIVITY", JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY, US, vol. 39, 1 January 1996 (1996-01-01), pages 2579-2585, XP002053886, ISSN: 0022-2623, DOI: 10.1021/JM960090B the whole document -----	1-15
A	I KEMPEN ET AL: "3-Bromophenyl 6-acetoxymethyl-2-oxo-2H-1-benzopyran-3-carboxylate inhibits cancer cell invasion in vitro and tumour growth in vivo", BRITISH JOURNAL OF CANCER, vol. 88, no. 7, 7 April 2003 (2003-04-07), pages 1111-1118, XP55038116, ISSN: 0007-0920, DOI: 10.1038/sj.bjc.6600856 the whole document -----	1-15