



(51) International Patent Classification:

C07D 311/18 (2006.01) C07C 205/37 (2006.01)

C07C 217/54 (2006.01) C07C 37/20 (2006.01)

C07C 213/00 (2006.01) C07C 39/23 (2006.01)

C07C 201/12 (2006.01)

(21) International Application Number:

PCT/EP2017/064203

(22) International Filing Date:

09 June 2017 (09.06.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

16174002.2 10 June 2016 (10.06.2016) EP

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA,

SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

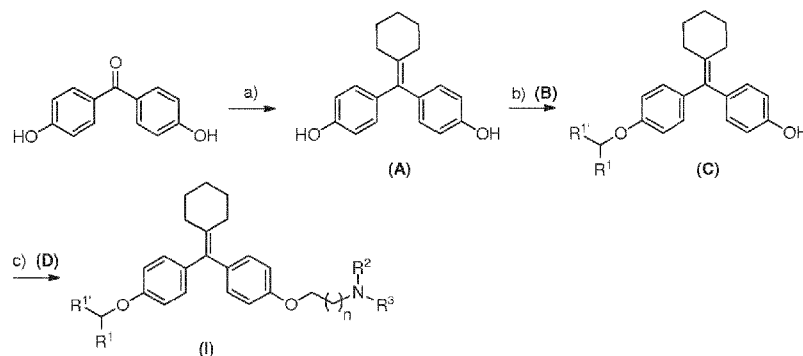
Declarations under Rule 4.17:

— of inventorship (Rule 4.17(iv))

Published:

— with international search report (Art. 21(3))

(54) Title: SYNTHESIS OF PHOTOACTIVABLE CAGED CYCLOFEN-OH AND DERIVATIVES THEREOF



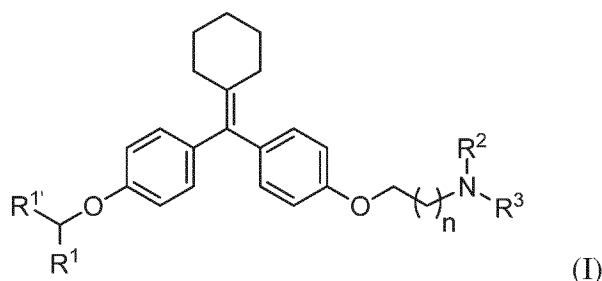
(57) Abstract: The present invention relates to a new process of manufacturing of caged cyclofen-OH and derivatives thereof, of formula (I) or salts thereof, wherein n, R¹, R^{1'}, R², and R³ are as defined in the claims.



SYNTHESIS OF PHOTOACTIVABLE CAGED CYCLOFEN-OH AND DERIVATIVES THEREOF

FIELD OF INVENTION

- 5 The present invention relates to a new process of manufacturing of photoactivable caged precursors, which are useful for understanding the spatiotemporal dynamics of biological cells. Especially, the invention relates to a process of manufacturing of caged cyclofen-OH and derivatives thereof, more specifically of compounds of formula (I):



- 10 or salts thereof, wherein n , R^1 , $R^{1'}$, R^2 , and R^3 are as defined below.

BACKGROUND OF INVENTION

- Cells respond to external signals by modifying their internal state and their environment. In multicellular organisms in particular, cellular differentiation and intra-cellular
15 signaling are essential for the coordinated development of the organism. Revealing and understanding the spatiotemporal dynamics of these complex interaction networks is a major goal in biology.

- While some of the most important players of these networks have been identified, much less is known of the quantitative rules that govern their interactions with one another and
20 with other cellular components. Investigating these interactions requires the development of means to control or interfere spatially and temporally with these processes.

To address these issues, various approaches have been introduced to control proteins activity. One of these strategies directly acts at the protein level: the protein of interest is

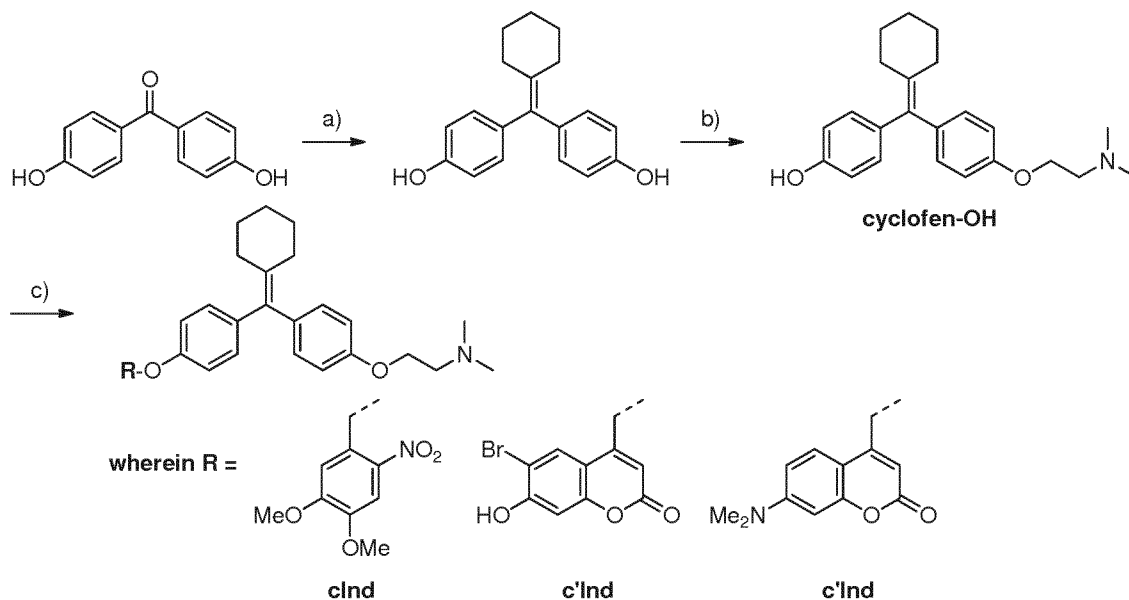
first inactivated and the activity of the protein is then restored with an appropriate stimulus in a controlled manner.

Photoactivation methods have proved particularly attractive for such control of protein's activity restoration due to their fast spatio-temporal dynamics.

- 5 For that purpose, genetically encoded photoactivable proteins were designed to intrinsically bear a non-photoactive site which can be activated by an inducer released upon photo-activation of a small lipophilic caged precursor. This method has been successfully implemented to photo-control gene expression in eukaryotic systems with one- and two-photon excitation.
- 10 Especially, the Applicant has developed such a system to photocontrol protein activity (Sinha et al., ChemBioChem, 2010, 11, 653-663). In this system, a steroid-related inducer was used since various proteins, such as for example Engrailed, Otx2, Gal4, p53, kinases such as Raf-1, Cre or Flp recombinases, fused to a steroid receptor were shown to be activated by binding of an appropriate inducer such as cyclofen-OH (Figure 1).
- 15 As represented on Figure 1, in absence of this appropriate inducer (Ind), the steroid receptor (ER^{T2}) forms a cytoplasmic assembly with a chaperone complex (cc), inactivating the fusion protein (Prot). The function of the protein is restored in the presence of the inducer which binds to the receptor and disrupts the complex. The photocontrol of the activity of the targeted protein is performed by fusing it to the
- 20 extensively used modified estrogen receptor ligand-binding domain (ER^{T2}) specific for the non-endogenous 4-hydroxy-cyclofen inducer, also called cyclofen-OH.

- The Applicant evidenced that photo-releasing cyclofen-OH from a caged precursor (cInd) is an efficient strategy to restore the function of a protein fused to the ER^{T2} receptor. The dynamical effects of this system were investigated in cultured cells and in live zebrafish
- 25 embryos. This method is compatible with a wide variety of proteins and may open up opportunities for the local spatio-temporal investigation of developmental pathways, the identification of stem cells and the study of cancer in a live organism.

In above-referred study (Sinha et al., ChemBioChem, 2010, 11, 653-663), the Applicant used 3 caged precursors (cInd, c'Ind and c''Ind) obtained through the following route of synthesis (Scheme 1):



- 5 **Scheme 1.** Synthesis of caged precursors cInd, c'Ind and c''Ind: a) cyclohexanone, TiCl_4 , Zn, THF, reflux, 2 h; b) $\text{Cl}(\text{CH}_2)_2\text{N}(\text{CH}_3)_2 \cdot \text{HCl}$, K_2CO_3 , acetone/ H_2O , reflux, 18 h; c) caging alcohol, $\text{P}(\text{Ph})_3$, diisopropyl azo dicarboxylate, THF, sonication, 20 min.

In this route of synthesis, a first step of McMurry coupling enables to introduce the cyclohexyl ring on 4,4'-dihydroxybenzophenone. Cyclofen-OH is then obtained by phenol monosubstitution. Caging of cyclofen-OH is then performed by a Mitsunobu reaction enabling to introduce the caging moiety.

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Above route of synthesis presents the drawback to be not very reproducible.

Besides, the overall yields are respectively of 22%, 9.6% and 11% for cInd, c'Ind and c''Ind. These low yields are not compatible with synthesis at large scales.

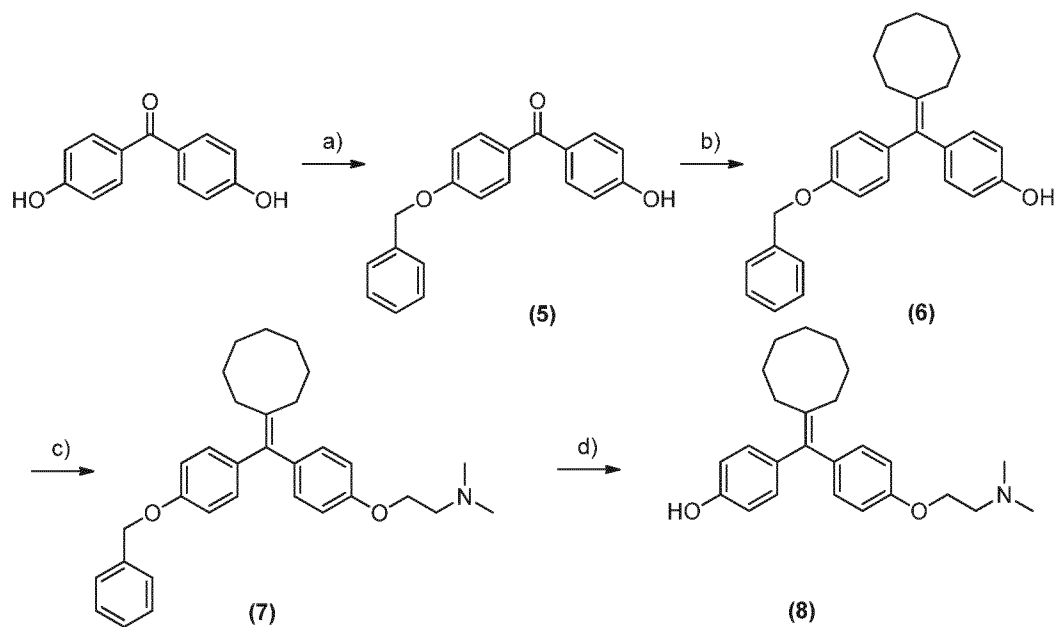
- 15 Moreover, the caged precursor is obtained from the inducer (cyclofen-OH). A careful purification of the caged precursor is thus required so that the amount of remaining inducer in the final product be lower than 2%. Otherwise, the inducer present as an impurity will directly activate the protein, without control by photo-activation. In order

to ensure such a low content of cyclofen-OH in the final product, a HPLC purification was needed. This is also not compatible with a scale-up process.

Above route of synthesis is thus not suitable for providing large amounts of caged precursors, which are required when dealing with demanded applications in animal models such as mice.

There is thus a need to provide a new process of synthesis of caged precursors of cyclofen-OH and derivatives thereof.

Patent application WO2008/030771 discloses modulators of estrogen receptor for therapeutic uses. Compounds of WO2008/030771 are cyclic alkylidene derivatives whose general formula encompasses cyclofen-OH. WO2008/030771 discloses three routes of synthesis, especially the route of synthesis of Scheme 2 below, leading to compound (8) via intermediate (7) (example 3 of WO2008/030771):



Scheme 2. Synthesis of estrogen modulator (8): a) benzylbromide, K_2CO_3 , DMF, $60^\circ C$, 48 h; b) cyclooctanone, $TiCl_4$, Zn, THF, $65^\circ C$, overnight; c) $Cl(CH_2)_2N(CH_3)_2 \cdot HCl$, Cs_2CO_3 , DMF, $70^\circ C$, overnight; d) H_2 , Pd/C, EtOAc, 2 h.

In this route of synthesis, a first step of phenol monoprotection is followed by a McMurry coupling to introduce the cycloalkyl ring. The second phenol is then substituted by alkylation and the final compound is released after deprotection.

With this route of synthesis, the overall yield for protected intermediate (7) is of 22%. As mentioned above with the other route of synthesis, such low yield is not suitable for scaling-up the process.

There is thus a need to provide an improved process of manufacturing of caged precursors of cyclofen-OH and derivatives thereof, being reproducible, providing an overall higher yield, not requiring sensible purification steps and being suitable for scale-up. The process of the present invention has such properties, as evidenced in the example part.

DEFINITIONS

In the present invention, the following terms have the following meanings:

- “**alkoxy**” refers to any group –O-alkyl, wherein alkyl is as herein defined. Suitable alkoxy groups include for example methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, t-butoxy, sec-butoxy, and n-pentoxy.
- “**alkyl**” refers to a hydrocarbyl radical of formula C_nH_{2n+1} wherein n is a number greater than or equal to 1. Generally, alkyl groups of this invention comprise from 1 to 12 carbon atoms, preferably from 1 to 6 carbon atoms. Alkyl groups may be linear or branched and may be substituted as indicated herein. Suitable alkyl groups include methyl, ethyl, propyl (n-propyl, i-propyl, n-butyl), butyl (i-butyl, s-butyl and t-butyl), pentyl and its isomers (e.g. n-pentyl, iso-pentyl), and hexyl and its isomers (e.g. n-hexyl, iso-hexyl).
- “**alkylamino**” refers to a group of formula –NH(alkyl), wherein alkyl is as herein defined.
- “**alkyloxyaryl**” refers to any group –aryl-O-alkyl, wherein aryl and alkyl are as herein defined.

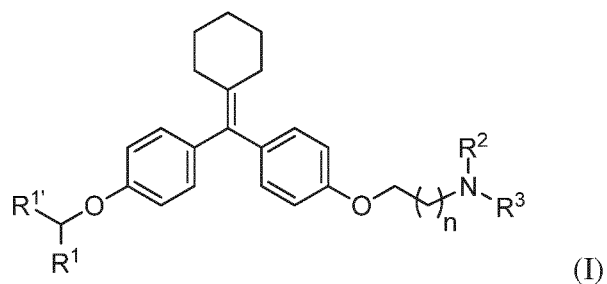
- **“alkyloxycarbonylalkyloxy”** refers to any group $\text{--O-alkyl-(CO)-O-alkyl}$, wherein alkyl is as herein defined.
- **“amino”** refers to the --NH_2 group.
- **“aryl”** refers to a polyunsaturated, aromatic hydrocarbyl group having a single ring (i.e. phenyl) or multiple aromatic rings fused together (e.g. naphthyl) or linked covalently, typically containing 5 to 12 atoms; preferably 6 to 10, wherein at least one ring is aromatic. The aromatic ring may optionally include one to two additional rings (either cycloalkyl, heterocyclyl or heteroaryl) fused thereto. Aryl is also intended to include the partially hydrogenated derivatives of the carbocyclic systems enumerated herein. Non-limiting examples of aryl comprise phenyl, biphenyl, biphenyl, biphenyl, 5- or 6- tetralinyl, naphthalen-1- or -2-yl, 4-, 5-, 6 or 7-indenyl, 1- 2-, 3-, 4- or 5- acenaphthylenyl, 3-, 4- or 5-acenaphthenyl, 1- or 2-pentalenyl, 4- or 5-indanyl, 5-, 6-, 7- or 8-tetrahydronaphthyl, 1,2,3,4-tetrahydronaphthyl, 1,4-dihydronaphthyl, 1-, 2-, 3-, 4- or 5-pyrenyl.
- **“arylethynyl”** refers to a group of formula $\text{--C}\equiv\text{C-aryl}$, wherein aryl is as herein defined.
- **“arylvinyl”** refers to a group of formula --CH=CH-aryl , wherein aryl is as herein defined.
- **“carbamimidoyl”** refers to the moiety --C(=NH)-NH_2 .
- **“dialkylamino”** refers to a group of formula --N(alkyl)(alkyl) , wherein alkyl is as herein defined.
- **“halo”** refers to fluoro, chloro, bromo, or iodo.
- **“haloalkoxy”** refers to any group alkoxy group substituted by one or more halo group. An example haloalkoxy group is --OCF_3 .
- **“haloalkyl”** refers to any group alkyl group substituted by one or more halo group. Examples of preferred haloalkyl groups are CCl_3 and CBr_3 .
- **“salt”** refers to the acid addition and base salts thereof. Suitable acid addition salts are formed from acids which form preferably non-toxic salts. Non-limiting examples

- include the acetate, trifluoroacetate, adipate, aspartate, benzoate, besylate, bicarbonate/carbonate, bisulphate/sulphate, borate, tetrafluoroborate, camsylate, citrate, cyclamate, edisylate, esylate, formate, fumarate, gluceptate, gluconate, glucuronate, hexafluorophosphate, hibenzate, hydrochloride/chloride, hydrobromide/bromide, hydroiodide/iodide, isethionate, lactate, malate, maleate, malonate, mesylate, methylsulphate, naphthylate, 2-napsylate, nicotinate, nitrate, orotate, oxalate, palmitate, pamoate, phosphate/hydrogen phosphate/dihydrogen phosphate, pyroglutamate, saccharate, stearate, succinate, tannate, tartrate, tosylate, trifluoroacetate and xinofoate salts. Suitable base salts are formed from bases which form preferably non-toxic salts. Non-limiting examples include the aluminium, arginine, benzathine, calcium, choline, diethylamine, diolamine, glycine, lysine, magnesium, meglumine, olamine, potassium, sodium, tromethamine, 2-(diethylamino)ethanol, ethanolamine, morpholine, 4- (2- hydroxyethyl)morpholine and zinc salts. Hemisalts of acids and bases may also be formed, for example, hemisulphate and hemicalcium salts. Preferred, pharmaceutically acceptable salts include hydrochloride/chloride, hydrobromide/bromide, bisulphate/sulphate, nitrate, citrate, and acetate.
- “**sulfonate**” refers to group of formula $-\text{OSO}_2\text{-R}$, wherein R represents alkyl, haloalkyl, optionally substituted aryl. Examples of preferred sulfonate groups are mesylate, triflate and tosylate.
 - “**vinylcarboxy**” refers to a group of formula $-\text{CO-CH=CH}_2$.

DETAILED DESCRIPTION

Compounds

- This invention relates to a process of manufacturing of caged cyclofen-OH and derivatives thereof, more specifically of compounds of formula (I):



or salts thereof, wherein

R^1 represents Ar^1 , $-CO-Ph$, $-CH_2-(o-COR)Ph$, $-CH_2-(o-NO_2)Ph$ or $-CO-CO-NR_2$, wherein

5 Ar^1 represents a phenyl or coumarin group, optionally substituted by one or more group selected from nitro, alkoxy, halo, hydroxyl, amino, alkylamino, dialkylamino, alkyloxycarbonylalkyloxy, alkyloxyaryl, aryl, arylvinyl, arylethynyl and cyano;

10 Ph represents a phenyl group, optionally substituted by one or more group selected from alkoxy, halo, hydroxyl, alkyl, haloalkyl, haloalkoxy, amino, alkylamino, dialkylamino, alkyloxycarbonylalkyloxy, alkyloxyaryl, aryl, arylvinyl, arylethynyl and cyano; and

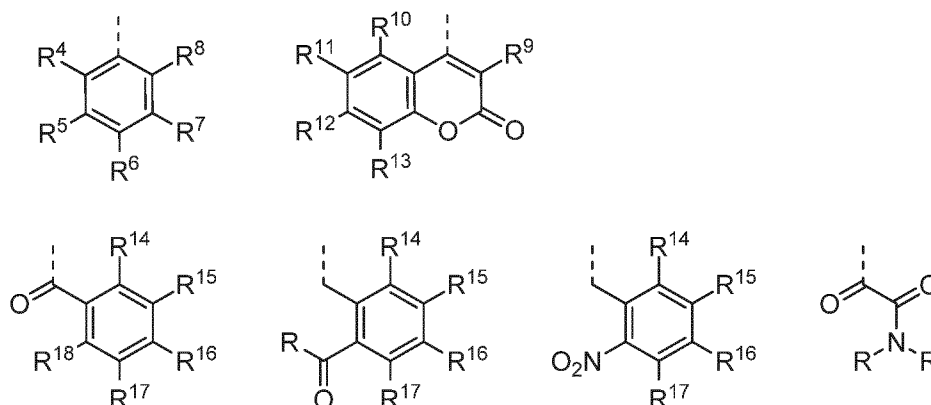
15 R represents aryl, alkyl or vinylcarboxy; wherein the aryl group is optionally substituted by one or more group selected from alkoxy, halo, hydroxyl, alkyl, haloalkyl, haloalkoxy, amino, alkylamino, dialkylamino, alkyloxycarbonylalkyloxy, alkyloxyaryl, aryl, arylvinyl, arylethynyl and cyano;

$R^{1'}$ represents H, alkyl, haloalkyl, cyano or aryl;

20 R^2 and R^3 represent each independently hydrogen, alkyl, carbomimidoyl; or R^2 and R^3 form together with the nitrogen atom to which they are attached an heterocyclic ring preferably selected from pyrrolidinyl, piperidinyl, N-methyl-piperazinyl, morpholinyl and pyrrolyl; and

n is an integer ranging from 1 to 6, preferably n is 1, 2, 3 or 4.

According to one embodiment, R^1 represents a moiety selected from:



wherein

the dotted line represents the point of attachment to the rest of the molecule,

R is as defined in formula (I); and

- 5 R^4 , R^5 , R^6 , R^7 , R^8 , R^9 , R^{10} , R^{11} , R^{12} and R^{13} represent each independently H, nitro, alkoxy, halo, hydroxyl, amino, alkylamino, dialkylamino, alkyloxycarbonylalkyloxy, alkyloxyaryl, aryl, arylvinyl, arylolefinyl or cyano; and

- 10 R^{14} , R^{15} , R^{16} , R^{17} and R^{18} represent each independently H, alkoxy, halo, hydroxyl, alkyl, haloalkyl, haloalkoxy, amino, alkylamino, dialkylamino, alkyloxycarbonylalkyloxy, alkyloxyaryl, aryl, arylvinyl, arylolefinyl and cyano.

According to one embodiment, $R^{1'}$ represents H or methyl, preferably $R^{1'}$ represents H.

According to one embodiment, $R^{1'}$ represents haloalkyl, preferably $-CCl_3$ or $-CBr_3$.

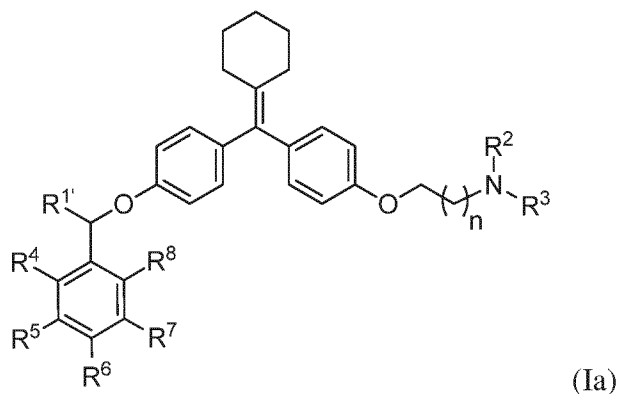
According to one embodiment, $R^{1'}$ represents aryl, preferably phenyl.

- 15 According to one embodiment, n is equal to 1 or 2.

According to one embodiment, R^2 and R^3 represent both methyl or R^2 and R^3 represent both ethyl. According to one embodiment, R^2 represents H and R^3 represents a carbomimidoyl group.

- 20 According to one embodiment, R^2 and R^3 form together with the nitrogen atom to which they are attached a heterocyclic ring selected from pyrrolidinyl, piperidinyl, N-methyl-piperazinyl, morpholinyl and pyrrolyl.

According to one embodiment, compounds of formula (I) are of formula (Ia)



or salts thereof, wherein

n , R^1 , R^2 and R^3 are as defined in formula (I); and

- 5 R^4 , R^5 , R^6 , R^7 and R^8 represent each independently H, nitro, alkoxy, halo, hydroxyl, amino, alkylamino, dialkylamino, alkyloxycarbonylalkoxy, alkyloxyaryl, aryl, arylvinyl, arylethynyl or cyano.

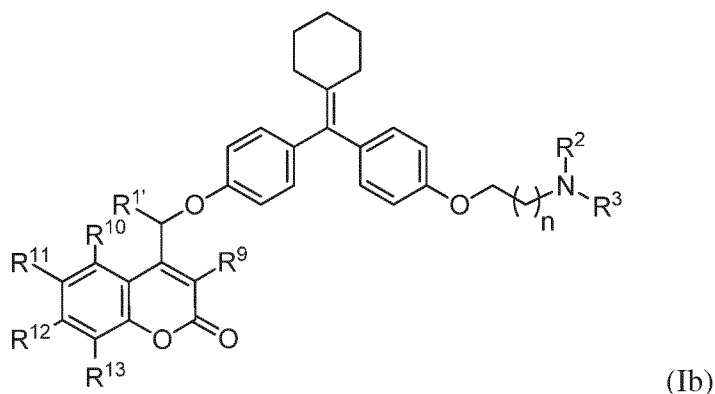
According to a specific embodiment, R^4 is nitro. According to a specific embodiment, R^4 is nitro and R^8 is H. According to another specific embodiment, R^4 and R^8 are both nitro.

- 10 According to a specific embodiment, R^5 is H.

According to a specific embodiment, R^6 is an alkoxy, preferably methoxy. According to another specific embodiment, R^6 is H.

- 15 According to a specific embodiment, R^7 is alkoxy, alkyloxycarbonylalkoxy or alkyloxyaryl, preferably R^7 is methoxy, ethoxy, ethyloxycarbonylmethoxy or methoxyphenyl.

According to one embodiment, compounds of formula (I) are of formula (Ib)



or salts thereof, wherein

n , R^1 , R^2 and R^3 are as defined in formula (I); and

R^9 , R^{10} , R^{11} , R^{12} and R^{13} represent each independently H, nitro, alkoxy, halo, hydroxyl, amino, alkylamino, dialkylamino, alkyloxycarbonylalkoxy, alkoxyaryl, aryl, arylvinyl, arylethynyl or cyano.

According to a specific embodiment, R^9 is H. According to a specific embodiment, R^{10} is H. According to a specific embodiment, R^{13} is H. According to a specific embodiment, R^9 , R^{10} and R^{13} are H.

According to a specific embodiment, R^{11} is H, alkoxy or halo, preferably R^{11} is H, methoxy or bromo.

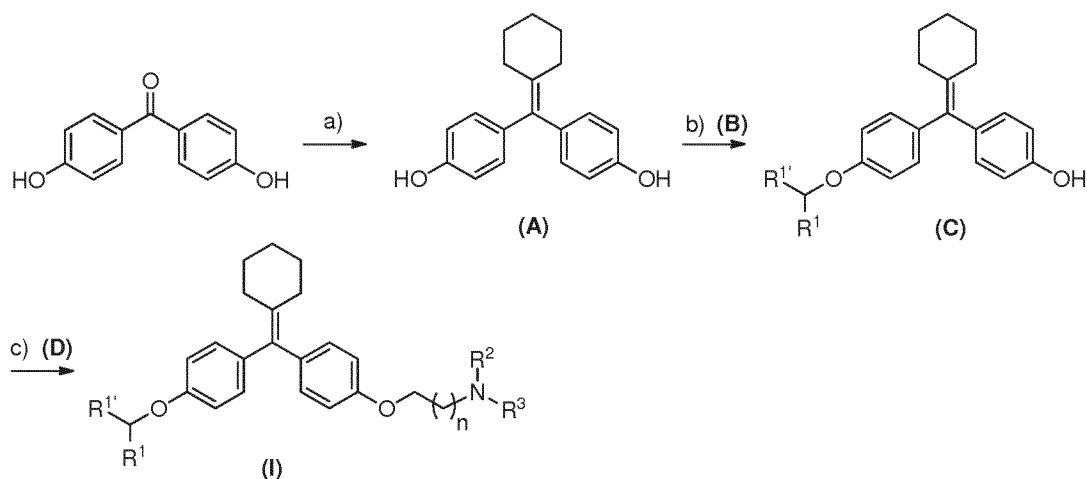
According to a specific embodiment, R^{12} is alkoxy, hydroxyl or dialkylamino, preferably R^{12} is methoxy, hydroxyl or dimethylamino.

In the present application, when it is referred to compounds of formula (I), it includes references to all subformulae thereof and to specific embodiments relative to substituents.

Process

The process of the invention comprises a first step of McMurry coupling on 4,4'-dihydroxybenzophenone, enabling to introduce the cyclohexyl ring. The second step introduces the caging arylalkyl moiety by phenol monosubstitution. The second phenol

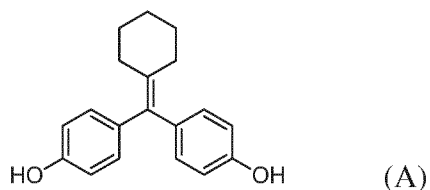
5 group is then alkylated. This route of synthesis is summarized in Scheme 3 below:



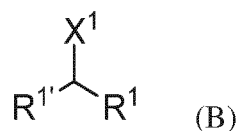
Scheme 3. Route of synthesis of the present invention.

According to one embodiment, the process of the invention is a process of manufacturing a compound of formula (I) as defined above, comprising the following steps:

- 10 a) performing a McMurry coupling between 4,4'-dihydroxybenzophenone and cyclohexanone to form intermediate (A):

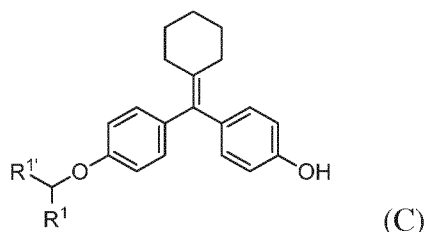


- b) performing a phenol monosubstitution on intermediate (A) with caging halide (B):

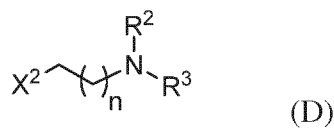


wherein R^1 and $R^{1'}$ are as defined in formula (I) and X^1 represents halo, preferably X^1 represents Br or Cl, more preferably X^1 represents Br,

to form intermediate (C):



- 5 wherein R^1 and $R^{1'}$ are as defined in formula (I),
 wherein step b) is performed in the dark, in a solvent in which intermediate (A) and caging halide (B) are soluble and in which intermediate (C) is not soluble; and
 c) alkylating, in the dark, the remaining phenol of intermediate (C) with derivative (D):



- 10 wherein n , R^2 and R^3 are as defined in formula (I) and X^2 represents halo or sulfonate, preferably X^2 represents Cl, Br, I, mesylate, triflate or tosylate, more preferably X^2 represents Cl;
 to afford compound of formula (I).

Step a)

- 15 According to one embodiment, cyclohexanone is used at a proportion ranging from 1 to 10 equivalents compared to 4,4'-dihydroxybenzophenone, preferably at a proportion of 3 to 6 equivalents, more preferably at a proportion of about 4.2 equivalents.

According to one embodiment, step a) is performed in presence of $TiCl_4$ and zinc. Advantageously, zinc is used under the form of powder. Advantageously, zinc is activated
 20 zinc.

According to one embodiment, TiCl_4 is used at a proportion ranging from 2 to 15 equivalents compared to 4,4'-dihydroxybenzophenone, preferably at a proportion of 4 to 10 equivalents, more preferably at a proportion of about 6.2 equivalents.

According to one embodiment, zinc is used at a proportion ranging from 2 to 30 equivalents compared to 4,4'-dihydroxybenzophenone, preferably at a proportion of 6 to 20 equivalents, more preferably at a proportion of about 14 equivalents.

According to one embodiment, the solvent used in step a) is selected from tetrahydrofuran (THF), 1,2-dimethoxyethane (DME), dioxane and tetrahydropyran; preferably the solvent is THF, more preferably dry THF.

According to one embodiment, the McMurry coupling of step a) is performed at a temperature ranging from 20 to 100°C, preferably at reflux of the solvent.

According to one embodiment, the McMurry coupling of step a) is performed for a duration ranging from 0.5 to 24 hours, preferably from 1 to 12 hours, more preferably for 2 hours.

Intermediate (A) obtained in step a) may be purified by flash chromatography or by precipitation. According to one embodiment, intermediate (A) is purified by flash chromatography on silica gel, using preferably a mixture cyclohexane/ethyl acetate as solvent. According to another embodiment, (A) is purified by precipitation and the solvent of precipitation is selected from dichloromethane, cyclohexane and a mixture ethyl acetate/cyclohexane.

Step b)

According to one embodiment, caging halide (B) is used at a proportion ranging from 0.5 to 2 equivalents compared to intermediate (A), preferably at a proportion of 0.8 to 1.1 equivalents, more preferably at a proportion of about 0.95 equivalents.

According to one embodiment, the caging halide (B) is a bromide, i.e. X^1 represents Br. According to another embodiment, caging halide (B) is a chloride, i.e. X^1 represents Cl.

According to one embodiment, step b) is performed in presence of a base, preferably a base selected from K_2CO_3 and Cs_2CO_3 , more preferably the base is K_2CO_3 .

According to one embodiment, K_2CO_3 is used at a proportion ranging from 0.5 to 2 equivalents compared to intermediate (A), preferably at a proportion of 0.8 to 1.1 equivalents, more preferably at a proportion of about 0.95 equivalents.

According to one embodiment, NaI or KI may be added to perform step b) if the conversion rate is too low.

According to one embodiment, the solvent used in step b) is a solvent in which starting products are soluble and resulting compound is not soluble. In other words, the solvent used in step b) is a solvent in which intermediate (A) and caging halide (B) are soluble and in which intermediate (C) is not soluble. Such solvent enables to easily recover intermediate (C) as a precipitate by filtration. According to a preferred embodiment, the solvent used in step b) is a mixture of water and of a miscible organic solvent. Examples of organic solvents miscible in water are acetonitrile, acetone, dioxane, THF, alcohol (such as for example methanol, ethanol or butanol). According to one embodiment, the solvent used in step b) is selected from water, acetonitrile, acetone, dioxane, THF, alcohol (such as for example methanol, ethanol or butanol) or a mixture thereof; preferably the solvent is a mixture of acetonitrile and water, more preferably acetonitrile/water 2/1 (v/v).

According to one embodiment, step b) is performed at a temperature ranging from 0 to 100°C, preferably at room temperature.

According to one embodiment, step b) is performed for a duration ranging from 1 to 72 hours, preferably from 4 to 48 hours, more preferably for 16 to 24 hours.

Step b) is performed in the dark in order to avoid degradation of resulting intermediate (C) which is photosensitive.

Intermediate (C) obtained in step b) forms a precipitate in the conditions of the reaction. Advantageously, intermediate (C) does not need to be purified to be used in step c).

Step c)

According to one embodiment, derivative (D) is used at a proportion ranging from 1 to 10 equivalents compared to intermediate (C), preferably at a proportion of 1 to 3 equivalents, more preferably at a proportion of about 2 equivalents.

- 5 According to one embodiment, step c) is performed in presence of a base, preferably a base selected from Cs_2CO_3 and K_2CO_3 , more preferably the base is Cs_2CO_3 .

According to one embodiment, Cs_2CO_3 is used at a proportion ranging from 2 to 11 equivalents compared to intermediate (C), preferably at a proportion of 2 to 4 equivalents, more preferably at a proportion of about 3 equivalents.

- 10 According to one embodiment, the solvent used in step c) is selected from acetone, acetonitrile and dimethylformamide; preferably the solvent is acetone.

According to one embodiment, step c) is performed at a temperature ranging from 20 to 155°C, preferably at reflux of the solvent.

- 15 According to one embodiment, step c) is performed for a duration ranging from 1 to 48 hours, preferably from 6 to 24 hours, more preferably for 18 hours.

Step c) is performed in the dark in order to avoid degradation of final compound of formula (I) which is photosensitive.

- 20 Final compound (I) obtained in step c) is purified by flash chromatography. According to one embodiment, compound (I) is purified by flash chromatography on silica gel, using preferably a mixture dichloromethane and methanol as solvent.

The process of the invention presents the advantage to have an overall yield of more than 30%, preferably of more than 40%, even more preferably of more than 45%. This overall yield is twofold compared to process disclosed in the prior art.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a scheme representing the strategy to photo-activate properly engineered proteins: a protein (Prot) fused to the ER^{T2} receptor (ER^{T2}) is inactivated by the assembly formed with a chaperone complex (cc). Upon photoactivation of a caged precursor (cInd), a non-endogenous inducer (Ind) is released, which binds to the ER^{T2} receptor and sets the protein fusion free from its assembly with the chaperone complex. The inducer (Ind) may be 4-hydroxy-cyclofen (also called cyclofen-OH).

EXAMPLES

The present invention is further illustrated by the following examples.

Abbreviations

ACN: acetonitrile;
DMF: dimethylformamide;
DMSO: dimethylsulfoxide;
EtOAc: ethyl acetate;
HPLC: high performance liquid chromatography;
HRMS: high resolution mass spectroscopy;
MS: mass spectroscopy;
NMR: nuclear magnetic resonance;
TFH: tetrahydrofurane.

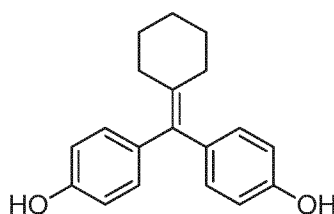
Material

Commercially available reagents were used as obtained. Microanalyses were performed by the Service de Microanalyses de Gif sur Yvette. The ¹H and ¹³C NMR spectra were recorded at room temperature on Bruker AM 250, 300 or 400 spectrometers; chemical shifts are reported in ppm with protonated solvent as internal reference (¹H, CHCl₃ in CDCl₃ 7.26 ppm, CHD₂SOCD₃ in CD₃SOCD₃ 2.49 ppm, CHD₂COCD₃ in CD₃COCD₃ 2.05 ppm; ¹³C, ¹³CDCl₃ in CDCl₃ 77.0, ¹³CD₃SOCD₃ in CD₃SOCD₃ 39.7 ppm,

$^{13}\text{CD}_3\text{COCD}_3$ in CD_3COCD_3 29.9 ppm); coupling constants (J) are given in Hz. Mass spectra (chemical ionization and high resolution with NH_3 or CH_4) were performed by the Service de Spectrométrie de Masse de Chimie ParisTech or by the Service de Spectrométrie de Masse de l'ICOA (Orléans). Column chromatography was performed
5 on silica gel 60 (0.040–0.063 nm; Merck). Analytical thin-layer chromatography (TLC) was conducted on Merck silica gel 60 F254 precoated plates.

Example 1: Synthesis of intermediate (A)

Step a): synthesis of 4-(Cyclohexylidene(4-hydroxyphenyl)methyl)phenol (A)

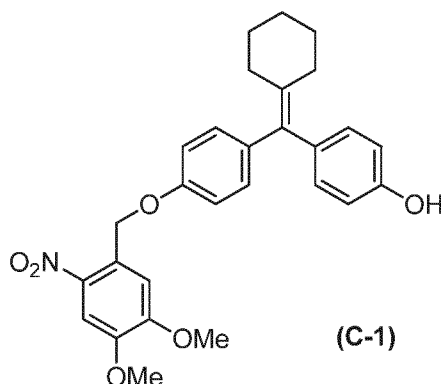


(A)

10 Titanium chloride (6.2 mL, 56 mmol) was added dropwise under argon to a stirred suspension of activated zinc powder (8.20 g, 126 mmol) in dry tetrahydrofuran (80 mL) at -10°C . When the addition was complete, the mixture was warmed to room temperature and then refluxed for 2 h. A solution of 4,4'-dihydroxybenzophenone (2.0 g, 9 mmol) and cyclohexanone (4 mL, 38 mmol) in dry tetrahydrofuran (120 mL) was added to the cooled
15 suspension of the titanium reagent at $0-5^\circ\text{C}$ and the mixture was refluxed for 2 h. After being cooled to room temperature, the reaction mixture was quenched with 10% (w/v) aqueous potassium carbonate (30 mL), filtered and extracted with ethyl acetate. The organic layer was washed with brine, dried over MgSO_4 , and concentrated *in vacuo*. Flash column chromatography (cyclohexane/ethyl acetate, 3:1, v/v) or precipitation in
20 dichloromethane afforded (A) as a white powder respectively with (2.15 g, 85%) and (2.1 g, 81%); precipitation can also be carried out in cyclohexane or in a EtOAc/cyclohexane mixture. ^1H NMR (250 MHz, DMSO-d_6 , δ): 9.28 (s, 2H), 6.82 (d, 4 H, $J=8$ Hz), 6.65 (d, 4H, $J=8$ Hz), 2.14 (m, 4 H), 1.52 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6 , δ): 155.5 (2C), 136.2, 133.8, 133.6 (2C), 130.3 (4C), 114.6 (4C),
25 31.9 (2C), 28.1(2C), 26.3.

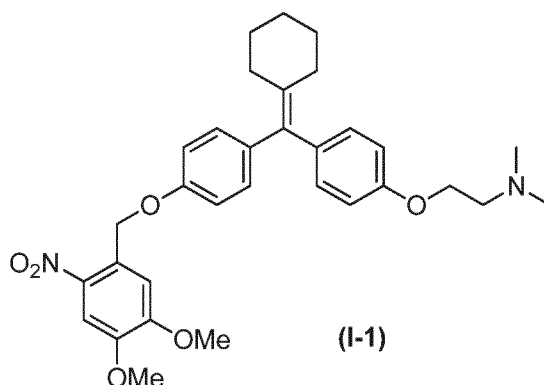
Example 2: Synthesis of compound (I-1)

Step b): synthesis of intermediate 4-((4-(4,5-dimethoxy-2-nitrobenzyloxy)phenyl)(cyclohexylidene)methyl)phenol (C-1)



- 5 To a mixture of **(A)** (1 g, 3.5 mmol) and K_2CO_3 (470 mg, 3.4 mmol, 0.95 eq) in acetonitrile (ACN)/ H_2O (60 mL, 2/1, v/v) was added portionwise 4,5-dimethoxy-2-nitrobenzyl bromide **(B-1)** (940 mg, 3.4 mmol, 0.95 eq) over a period of 1 h. The resulting mixture was stirred in the dark for 16 h. The precipitate was filtered, washed with ACN/ H_2O and then dried *in vacuo* over P_2O_5 . A white powder was obtained (1.4 g,
- 10 **(A)/(C-1)/disubstituted by-product: $\epsilon/96/4$**) and used without purification in the next step. 1H NMR (ppm, 300 MHz, $CDCl_3$, δ): 7.77 (s, 1H), 7.35 (s, 1H), 7.04 (AA'XX', 2H, $J=9$ Hz), 6.97 (AA'XX', 2H, $J=9$ Hz), 6.91 (AA'XX', 2H, $J=9$ Hz), 6.73 (AA'XX', 2H, $J=9$ Hz), 5.48 (s, 2H), 4.68 (s, 1H), 3.97 (s, 3H), 3.95 (s, 3H), 2.23 (m, 4H), 1.57 (m, 6H). ^{13}C NMR (75 MHz, DMSO- d_6 , δ): 156.4, 155.9, 153.3, 148.0, 139.9, 137.1, 136.2, 133.7, 133.5, 130.7 (2C), 130.5 (2C), 122.5, 114.9 (2C), 114.5 (2C), 111.5, 108.4, 66.7, 56.3, 56.2, 32.1 (2C), 28.3 (2C), 26.4; MS (CI, NH_3): m/z 493.30 (calculated average mass for $[C_{28}H_{29}NO_6 + NH_4]^+$: 493.23; HRMS (TOF MS ES $^+$): m/z 476.2077 (calculated mass for $[C_{28}H_{29}NO_6 + H]^+$: 476.2073.
- 15

Step c): synthesis of 2-(4-((4-(4,5-Dimethoxy-2-nitrobenzyloxy)phenyl)cyclohexylidene)methyl)phenoxy)-N,N-dimethylethanamine (I-1)

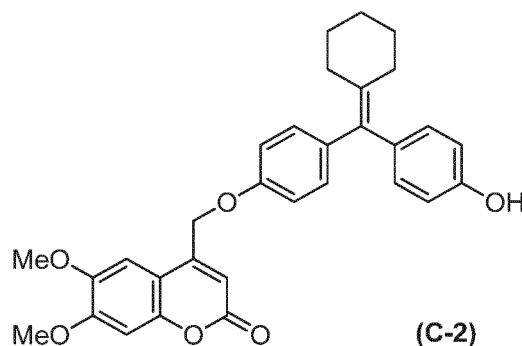


A solution of **(C-1)** (800 mg, 1.68 mmol) in acetone (50 mL) was treated with Cs₂CO₃ (1.64 g, 5 mmol, 3 eq.). 2-(Dimethylamino)ethylchlorid hydrochloride **(D-1)** (484 mg, 3.36 mmol, 2 eq.) was added portionwise over a period of 30 min and the resulting suspension was stirred at reflux temperature in the dark for 18 h. After being cooled to room temperature, the reaction mixture was filtered. The precipitate was washed with dichloromethane and the solvent was evaporated. Flash chromatography (CH₂Cl₂/MeOH: 9/1) afforded **(I-1)** (640 mg, 70% yield). ¹H NMR (400 MHz, CDCl₃, δ): 7.76 (s, 1H), 7.35 (s, 1 H), 7.04 (AA'XX', 2H, J=9 Hz), 7.00 (AA'XX', 2H, J=9 Hz), 6.90 (AA'XX', 2H, J=9 Hz), 6.82 (AA'XX', 2H, J=9 Hz), 5.47 (s, 2H), 4.03 (t, 2H, J= 6 Hz), 3.96 (s, 3 H), 3.94 (s, 3 H), 2.70 (t, 2H, J= 6 Hz), 2.32 (s, 6 H), 2.23 (m, 4H), 1.58 (m, 6H); ¹³C NMR (100 MHz, CDCl₃, δ): 157.0, 156.2, 153.8, 147.7, 138.9, 138.5, 136.8, 137.7, 133.2, 131.0 (2C), 130.7 (2C), 129.6, 114.3 (2C), 113.8 (2C), 109.4, 107.9, 67.0, 65.8, 58.3, 56.3, 56.3, 45.8 (2C), 32.4 (2C), 28.6 (2C), 26.8.

The overall yield for the synthesis of compound (I-1) from 4,4'-dihydroxybenzophenone is of 47%.

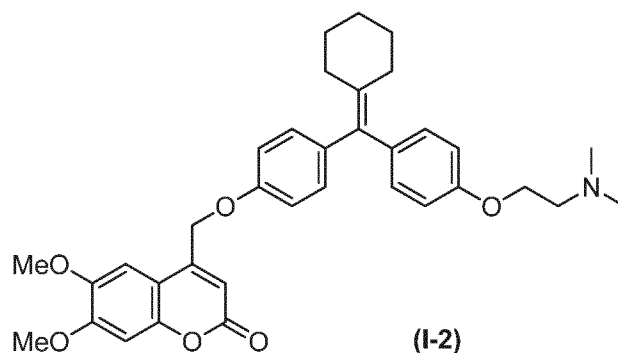
Example 3: Synthesis of compound (I-2)

Step b): synthesis of intermediate 4-((4-(6,7-dimethoxycoumarin)phenyl)(cyclohexylidene)methyl)phenol (C-2)



- 5 To a mixture of **(A)** (100 mg, 0.35 mmol) and K₂CO₃ (47 mg, 0.34 mmol, 0.95 eq) in ACN/H₂O (7 mL, 2/1, v/v) was added 4-bromomethyl-6,7-dimethoxycoumarin **(B-2)** (97 mg, 0.34 mmol, 0.95 eq). The resulting mixture was stirred in the dark for 24 h. The precipitate was filtered, washed with ACN/H₂O and then dried *in vacuo* over P₂O₅. A white powder was obtained (120 mg, **(A)/(C-2)/disubstituted by-product**: ε/90/10) and
- 10 used without purification in the next step. ¹H NMR (ppm, 300 MHz, acetone-d₆, δ): 7.30 (s, 1H), 7.08 (m, 4H), 6.99 (s, 1H), 6.93 (AA'XX', 2H, J= 9 Hz), 6.73 (AA'XX', 2H, J= 9 Hz), 6.45 (s, 1H), 5.41 (s, 2H), 3.97 (s, 3H), 3.987 (s, 3H), 2.24 (m, 4H), 1.59 (m, 6H). ¹³C NMR (75 MHz, CDCl₃, δ): 160.3, 156.1, 155.9, 152.7, 151.6, 149.0, 145.8, 136.9, 136.4, 133.5, 132.9, 130.5 (2C), 130.3 (2C), 114.8 (2C), 114.5 (2C), 109.1,
- 15 108.7, 105.7, 100.3, 65.4, 56.1, 31.9 (2C), 28.1 (2C), 26.2; MS (ESI): m/z 499.5 (calculated mass for [C₃₁H₃₀O₆ + H]⁺: 499.2, m/z 521.5 (calculated mass for [C₃₁H₃₀O₆ + Na]⁺: 521.1).

Step c): synthesis of 2-(4-((4-(4,5-Dimethoxy-2-nitrobenzyloxy)phenyl)cyclohexylidene)methyl)phenoxy)-N,N-dimethylethanamine (I-2)

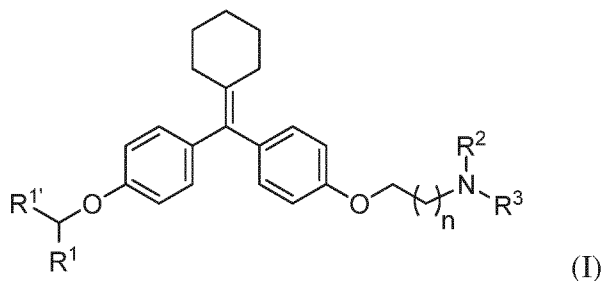


To a mixture of **(C-2)** (80 mg, 0.16 mmol) and Cs₂CO₃ (157 mg, 0.48 mmol, 3 eq.) in
 5 acetone (5 mL) was added 2-(dimethylamino)ethylchlorid hydrochloride **(D-1)** (46 mg, 0.32 mmol, 2 eq.). The resulting suspension was stirred at reflux temperature in the dark for 18 h. After being cooled to room temperature, the reaction mixture was filtered. The precipitate was washed with dichloromethane and the solvent was evaporated. Flash chromatography (CH₂Cl₂/MeOH: 9/1 v/v) afforded **(I-2)** (60 mg, 66% yield). ¹H NMR
 10 (300 MHz, CDCl₃, δ): 7.26 (s, 1H), 7.07 (m, 4 H), 7.01 (AA'XX', 2H, J=9 Hz), 6.95 (s, 1H), 6.85 (AA'XX', 2H, J=9 Hz), 6.44 (s, 1H), 5.38 (s, 2H), 4.09 (t, 2 H, J= 6 Hz), 3.93 (s, 3 H), 3.86 (s, 3 H), 2.73 (t, 2 H, J= 6 Hz), 2.30 (s, 6 H), 2.23 (m, 4H), 1.59 (m, 6H); ¹³C NMR (75 MHz, CDCl₃, δ): 161.1, 158.3, 157.5, 154.3, 152.0, 150.7, 147.4, 138.9, 137.7, 136.6, 134.7, 131.7 (2C), 131.6 (2C), 115.4 (2C), 114.8 (2C), 110.4, 110.4, 106.5,
 15 101.1, 66.9, 66.7, 58.9, 56.8, 56.6, 46.1 (2C), 33.1 (2C), 29.4 (2C), 27.6. MS (ESI): m/z 570.5 (calculated mass for [C₃₅H₃₉O₆ + H]⁺: 570.2).

The overall yield for the synthesis of compound (I-1) from 4,4'-dihydroxybenzophenone is of 42%.

CLAIMS

1. A process of manufacturing of compounds of formula (I)



5 or salts thereof, wherein

R^1 represents Ar^1 , $-CO-Ph$, $-CH_2-(o-COR)Ph$, $-CH_2-(o-NO_2)Ph$ or $-CO-CO-NR_2$, wherein

10 Ar^1 represents phenyl or coumarin groups, optionally substituted by a group selected from nitro, alkoxy, halo, hydroxyl, amino, alkylamino, dialkylamino, alkyloxycarbonylalkyloxy, alkyloxyaryl, aryl, arylvinyl, arylethynyl, cyano;

15 Ph represents a phenyl group, optionally substituted by one or more group selected from alkoxy, halo, hydroxyl, alkyl, haloalkyl, haloalkoxy, amino, alkylamino, dialkylamino, alkyloxycarbonylalkyloxy, alkyloxyaryl, aryl, arylvinyl, arylethynyl and cyano; and

20 R represents aryl, alkyl or vinylcarboxy; wherein the aryl group is optionally substituted by one or more group selected from alkoxy, halo, hydroxyl, alkyl, haloalkyl, haloalkoxy, amino, alkylamino, dialkylamino, alkyloxycarbonylalkyloxy, alkyloxyaryl, aryl, arylvinyl, arylethynyl and cyano;

R^1 represents H, alkyl, haloalkyl, cyano, aryl;

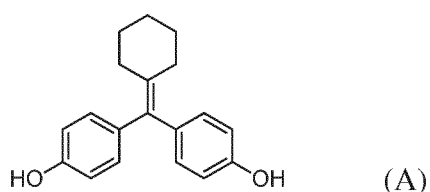
R^2 and R^3 represent each independently hydrogen, alkyl, carbomimidoyl; or R^2 and R^3 form together with the nitrogen atom to which they are attached an

heterocyclic ring preferably selected from pyrrolidinyl, piperidinyl, N-methyl-piperazinyl, morpholinyl, pyrrolyl;

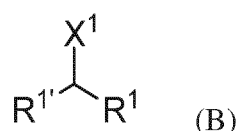
n is an integer ranging from 1 to 6, preferably n is 1, 2, 3 or 4, more preferably n is 1 or 2;

5 comprising the following steps:

a) performing a McMurry coupling between 4,4'-dihydroxybenzophenone and cyclohexanone to form intermediate (A):

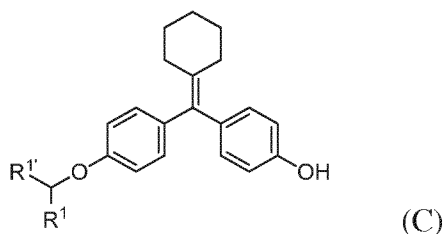


b) performing a phenol monosubstitution on intermediate (A) with caging halide (B):



wherein R^1 and $R^{1'}$ are as defined in formula (I) and X^1 represents halo, preferably X^1 represents Br or Cl, more preferably X^1 represents Br,

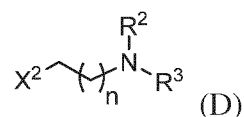
to form intermediate (C):



wherein R^1 and $R^{1'}$ are as defined in formula (I),

wherein step b) is performed in the dark, in a solvent in which intermediate (A) and caging halide (B) are soluble and in which intermediate (C) is not soluble; and

c) alkylating, in the dark the remaining phenol of intermediate (C) with derivative (D):



wherein n, R² and R³ are as defined in formula (I) and X² represents halo or sulfonate, preferably X² represents Cl, Br, I, mesylate, triflate or tosylate, more preferably X² represents Cl,

- 5 to afford compound of formula (I).
2. The process according to claim 1, wherein step a) is performed in presence of TiCl₄ and zinc.
3. The process according to claim 1 or claim 2, wherein cyclohexanone is used at a proportion ranging from 1 to 10 equivalents compared to 4,4'-
10 dihydroxybenzophenone, preferably at a proportion of 3 to 6 equivalents, more preferably at a proportion of about 4.2 equivalents.
4. The process according to anyone of claims 1 to 3, wherein the solvent used in step a) is selected from tetrahydrofuran, 1,2-dimethoxyethane, dioxane and tetrahydropyran; preferably the solvent is tetrahydrofuran, more preferably dry
15 tetrahydrofuran.
5. The process according to anyone of claims 1 to 4, wherein intermediate (A) is purified by precipitation and the solvent of precipitation is selected from dichloromethane, cyclohexane and a mixture ethyl acetate/cyclohexane.
6. The process according to anyone of claims 1 to 5, wherein step b) is performed in
20 presence of a base, preferably a base selected from K₂CO₃ and Cs₂CO₃, more preferably the base is K₂CO₃.
7. The process according to anyone of claims 1 to 6, wherein caging halide (B) is used at a proportion ranging from 0.5 to 2 equivalents compared to intermediate (A), preferably at a proportion of 0.8 to 1.1 equivalents, more preferably at a proportion
25 of about 0.95 equivalents.

8. The process according to anyone of claims **1** to **7**, wherein the solvent used in step b) is selected from acetonitrile, water, acetone, dioxane, tetrahydrofuran, alcohol or a mixture thereof; preferably the solvent is a mixture of acetonitrile and water, more preferably acetonitrile/water 2/1 (v/v).
- 5 9. The process according to anyone of claims **1** to **8**, wherein step c) is performed in presence of a base, preferably a base selected from Cs_2CO_3 and K_2CO_3 , more preferably the base is Cs_2CO_3 .
- 10 10. The process according to anyone of claims **1** to **9**, wherein derivative (D) is used at a proportion ranging from 1 to 10 equivalents compared to intermediate (C), preferably at a proportion of 1 to 3 equivalents, more preferably at a proportion of about 2 equivalents.
11. The process according to anyone of claims **1** to **10**, wherein the solvent used in step c) is selected from acetone, acetonitrile and dimethylformamide; preferably the solvent is acetone.
- 15 12. The process according to anyone of claims **1** to **11**, wherein compound (I) is purified by flash chromatography.

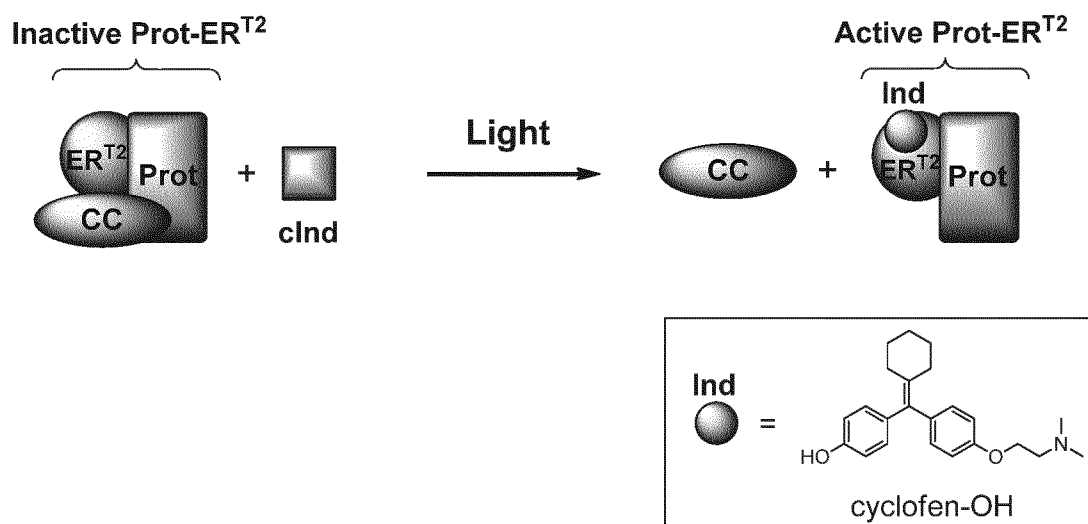


FIG. 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/064203

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07D311/18 C07C217/54 C07C213/00 C07C201/12 C07C205/37
C07C37/20 C07C39/23

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 C07C

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, WPI Data, 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.
A	DEEPAK KUMAR SINHA ET AL: "Photocontrol of Protein Activity in Cultured Cells and Zebrafish with One- and Two-Photon Illumination", CHEMBIOCHEM - A EUROPEAN JOURNAL OF CHEMICAL BIOLOGY., vol. 11, no. 5, 24 February 2010 (2010-02-24), pages 653-663, XP055313167, DE ISSN: 1439-4227, DOI: 10.1002/cbic.201000008 cited in the application Scheme 2 ----- -/--	1-12



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

7 July 2017

Date of mailing of the international search report

19/07/2017

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer

Österle, Carmen

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/064203

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2008/030771 A1 (SMITHKLINE BEECHAM CORP [US]; ZUERCHER WILLIAM JOHN [US]; HEYER DENNIS) 13 March 2008 (2008-03-13) cited in the application Scheme 1; page 22	1-12
A	----- LUDOVIC FOURNIER ET AL: "A Blue-Absorbing Photolabile Protecting Group for in Vivo Chromatically Orthogonal Photoactivation", ACS CHEMICAL BIOLOGY, vol. 8, no. 7, 19 July 2013 (2013-07-19), pages 1528-1536, XP055212890, ISSN: 1554-8929, DOI: 10.1021/cb400178m Scheme 1 -----	1-12

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2017/064203

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
W0 2008030771	A1	13-03-2008	NONE
