The present invention relates to novel donor-acceptor fluoranthenes of the general formula I which can be used potentially in developing fluorescent probes, and a process of preparing said novel compounds. More particularly, the present invention relates to amine or alkoxy group as donor and nitrile group as an acceptor attached to the fluoranthene skeleton, processes for preparing the said compounds and their uses as fluorescent probes in chemical and biological sciences such as cell imaging applications, diagnostics, fluorescent tags and other useful applications. The compounds are prepared by reacting 2H-pyran-2-ones in isolated or rigid conformations with cyclic ketones containing methylene carbonyl moiety in the presence of a base in an organic solvent.
The present invention relates to novel donor-acceptor fluoranthenes of the general formula I which can be used potentially in developing fluorescent probes, and a process of preparing said novel compounds. More particularly, the present invention relates to amine or alkoxy group as donor and nitrile group as an acceptor attached to the fluoranthene skeleton, processes for preparing the said compounds and their uses as fluorescent probes in chemical and biological sciences such as cell imaging applications, diagnostics, fluorescent tags and other useful applications.

The present invention more particularly relates to a compound of formula I:

![Chemical Structure](image)

Wherein $R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8$ are independently selected from the groups consisting of hydrogen, unsubstituted or substituted alkyl, unsubstituted or substituted alkenyl, unsubstituted or substituted alkoxy, unsubstituted or substituted aryl, unsubstituted or substituted cycloalkyl, unsubstituted or substituted heteroaryl, unsubstituted or substituted alkylthio, unsubstituted or substituted amino, unsubstituted or substituted acylamino, unsubstituted or substituted arylamino, unsubstituted or substituted acylthio, unsubstituted or substituted acyloxy, unsubstituted or substituted thioamido, halogens, nitriles, esters, hydroxy, mercapto, carbontrifluoride, nitro;
Wherein X is a donor group consisting of unsubstituted or substituted amino, unsubstituted or substituted alkoxyl, unsubstituted or substituted alkylthio group. Wherein CN is a nitrile, an acceptor group.

BACKGROUND OF THE INVENTION

The formation of cytoplasmic lipid droplets is a normal cellular process and found in almost all cells under physiological or pathological conditions. Number and size of lipid droplets vary from one cell type to another. Lipid droplets have two major compartments: neutral lipids, such triacylglycerides, diacylglycerol and cholesterol esters, and a surrounding phospholipid monolayer, which contains free cholesterol and proteins. Lipid droplets have been considered as a "great balls of fat". But in recent years lipid droplets regarded as complex subcellular organelles in adiposites of fat tissues (Walther et al. Annu. Rev. Biochem. 2012, 81, 687-714). An imbalance between energy intake and expenditure accumulation of the cytoplasmic lipid droplets occurs in a variety of pathological conditions and have been recognized as metabolic syndromes, such as obesity and diabetes.

In order to study lipid accumulation in living cells fluorescence imaging plays an important role in imaging of lipid regulation. Several imaging technologies on the basis of fluorescence are available to cell biologists. Lipid droplets form functionally and morphologically diverse subpopulations, no single marker protein exists that would stain the entire LDs. Therefore, the only reliable way to visualize lipid droplets in a living cell is by staining the LDs with lipophilic dyes. Small molecule organic fluorescent dyes with a variety of emission characteristics play a pivotal role in biological and material sciences. In biological perspective, these fluorescent dyes are being used as probes for imaging of specific intracellular organelles in living cells to understand intracellular events and elucidating various biological phenomena in cell biology and drug discovery. Commercially available, mainly two class dyes, Nile Red (Greenspan et al. J. Lipid Res. T985, 26, 781) and BODIPY derivatives (Spandl et al. Traffic 2009, 10, 1579; Chang, Y-T. et al. WO 2012/1 18444) have been used for this purpose. Fluorescent probes fluorescein, BODIPY, rhodamine and cyanine are representative skeletons popularly used for various biosensors, discovery of novel fluorescent probes remains highly challenging.
Significance of lipid droplet markers in the area of kinetoplastid biology

In fungi and Kinetoplastid protozoans including *Leishmania*, ergosterol is the major storage lipid, and synthesized via type-2 fatty acid biosynthesis pathway. The biosynthesis pathway being unique to these organisms are thus targets by some of the major antifungal and antitrypanosomal agents, such as ketoconazole, triclosan, miltefosine, amphotericin B. Studies have shown that Lysophospholipid analogues (LPAs) like edelfosine, ilmofosine and miltefosine interact with various sub-cellular structures and enzymes especially those associated with cellular membranes thus interfering with metabolism of membrane lipids. It has been reported that *Leishmania* infection induces lipid droplet accumulation in infected macrophages where Bodipy 493/503 has been used to visualize lipid droplet accumulation. *L. donovani* parasites, when undergoing stress tend to alter lipid metabolism and deposit more lipids in these lipid reservoirs. The size and number of LDs can be monitored using fluorescent markers. Recent findings linking LDs to the regulation and execution of immune responses in the context of host-pathogen interactions have been reviewed by Hector Alex Saka and Raphael Valdivia.

Markers can be used to visualize and quantify lipid droplets especially to study the effect of agents that interfere with lipid metabolism in kinetoplastid parasites like *Leishmania* and *Trypanosoma*.

Commercially available lipid markers:
Commercially available common lipid markers are BODIPY, Nile Red, LipidTOX Red, LipidTOX Green, LipidTOX deep red, Monodansylpentane. The most widely used Nile Red dye has wide spectral occupancy and cannot be used with ranges of FITC, TRITC, Texas Red. BODIPY 493/503 is a FITC range dye with greater affinity to Lipid droplets and smaller fluorescent spectral footprint than Nile Red. A new lipid marker LD540 is a near red BODIPY family dye with excellent specificity but not available commercially (Thiele C. WO 201 1/018184) Lipid TOX dyes from Invitrogen are FITC compatible but very expensive (-200 times that of BODIPY 493/503). 1,6-Diphenyl-1,3,5-hexatrine (DPH) is only fluorescent in hydrophobic environment used for LD visualization with fluorescence microscopy but has not been very useful since it shares its excitation and emission properties with 4',6'-diamino-2-phenylindole (DAPI) and Hoechst stains.
In the present Invention novel donor-acceptor fluoranthenes have been prepared and investigated as fluorescent marker for staining neutral lipid droplets. Examples of some of these derivatives are mentioned here.

**Synthesis of fluoranthene scaffolds:**


The present invention relates to a highly rapid novel synthesis of a new series of donor-acceptor fluoranthene systems.

**OBJECTS OF THE INVENTION**

Main object of the present invention is to provide novel donor-acceptor fluoranthenes, having the general formula I.

Another object of the invention is to provide a process for the preparation of the novel donor-acceptor fluoranthene compounds having the general formula I.

Further object of the invention is to provide the compounds having the general formula I which are useful in preparing fluorescent dyes for cell imaging applications and other useful biological applications such as developing diagnostic kits.
SUMMARY OF THE INVENTION

Accordingly, the present invention provides a novel Donor-acceptor fluoranthenes having general formula I, and derivatives thereof:

![Chemical structure](image)

wherein $R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8$ are independently selected from the groups comprising hydrogen, unsubstituted or substituted alkyl, unsubstituted or substituted alkenyl, unsubstituted or substituted alkoxy, unsubstituted or substituted aryl unsubstituted or substituted cycloalkyl, unsubstituted or substituted heteroaryl, unsubstituted or substituted alkythio, unsubstituted or substituted amino, unsubstituted or substituted acylamino, unsubstituted or substituted arylamino, unsubstituted or substituted acyl, unsubstituted or substituted acyloxy, unsubstituted or substituted thioamido, halogens, nitriles, esters, hydroxy, mercapto, carbontrifluoride, nitro;

wherein the word "substituted" means substitution with a group selected from halogen(s), nitrile(s), ester(s), hydroxy(s), alkoxy(s), mercapto(s), carbontrifluoride(s), nitro(s), amino group(s) or C1- to C20-units;

wherein $X$ is a donor group comprising unsubstituted or substituted amine group, unsubstituted or substituted alkoxy group, unsubstituted or substituted alkythio group;

wherein CN is a nitrile, an acceptor group.

In an embodiment of the present invention, the unsubstituted or substituted amine group is selected from amino, pyrrolidine, piperidine, methyl amine, ethyl amine, propyl amine, dimethylamine, N-alkylalkanolate, N,N-dialkanolate and pegylatedamine.
In another embodiment of the present invention, the representative compounds include:

i. 8-(dimethylamino)-10-isopropylfluoranthene-7-carbonitrile;
ii. 10-Phenyl-8-(pyrrolidin-1-yl)fluoranthene-7-carbonitrile;
iii. 10-(4-Methoxyphenyl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile;
iv. 8-(piperidin-1-yl)-10-(thiophen-2-yl)fluoranthene-7-carbonitrile;
v. 10-(benzo[cd][1,3]dioxol-5-yl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile;
vi. 8-(piperidin-1-yl)-10-(3,4,5-trimethoxyphenyl)fluoranthene-7-carbonitrile;

In another embodiment of the present invention, process for preparing donor-acceptor fluoranthene compounds of general formula I comprising the steps of;

(i) reacting a compound having general formula S-1 with a compound having general formula S-2 in an organic solvent in the presence of a base at a temperature ranging between -78°C to 100°C for a period ranging between 1 minute to 24 hr to obtain a reaction mixture wherein,
R₁, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸ are independently selected from the groups comprising hydrogen, unsubstituted or substituted alkyl, unsubstituted or substituted alkenyl, unsubstituted or substituted alkoxyl, unsubstituted or substituted aryl, unsubstituted or substituted cycloaryl, unsubstituted or substituted cycloalkyl, unsubstituted or substituted heteroaryl, unsubstituted or substituted alkylthio, unsubstituted or substituted amino, unsubstituted or substituted acylamino, unsubstituted or substituted arylthio, unsubstituted or substituted acyloxy, unsubstituted or substituted thioamido, halogens, nitriles, esters, hydroxy, mercapto, carbon trifluoride, nitro;

wherein the word "substituted" means substitution with a group selected from halogen(s), nitrile(s), ester(s), hydroxy(s), alkoxy(s), mercapto(s), carbon trifluoride(s), nitro(s), amino group(s) or C₁- to C₂₀-units;

wherein X is a donor group comprising unsubstituted or substituted amine, unsubstituted or substituted alkoxyl, unsubstituted or substituted alkylthio group;

(ii) isolating and purifying the compound of general formula I from the reaction mixture.

In still an embodiment of the present invention, the organic solvent is selected from the group comprising DMF, THF, DMSO, DCM and isopropanol.

In yet another embodiment of the present invention, the base is selected from the group comprising KOH, NaOH, NaH, KH, K₂CO₃ and Cs₂CO₃.

In one another embodiment of the present invention, use of donor-acceptor fluoranthenes having the general formula I alone or in combination with other fluorescent compounds and derivatives thereof for fluorescence-based cell imaging;
wherein $R^1$, $R^2$, $R^3$, $R^4$, $R^5$, $R^6$, $R^7$, $R^8$ are independently selected from the groups comprising hydrogen, unsubstituted or substituted alkyl, unsubstituted or substituted alkenyl, unsubstituted or substituted alkoxyl, unsubstituted or substituted aryl unsubstituted or substituted cycloary, unsubstituted or substituted cycloalkyl, unsubstituted or substituted heteroaryl, unsubstituted or substituted alkylthio, unsubstituted or substituted amino, unsubstituted or substituted acylamino, unsubstituted or substituted arylamino, unsubstituted or substituted acylthio, unsubstituted or substituted acyl, unsubstituted or substituted acyloxy, unsubstituted or substituted thioamido, halogens, nitriles, esters, hydroxy, mercapto, carbontrifluoride, nitro; wherein the word "substituted" means substitution with a group selected from halogen(s), nitrile(s), ester(s), hydroxy(s), alkox(y)s), mercapto(s), carbontrifluoride(s), nitro(s), amino group(s) or C1- to C20-units;

wherein $X$ is a donor group comprising unsubstituted or substituted amine, unsubstituted or substituted alkoxyl, unsubstituted or substituted alkylthio group;

wherein CN is an acceptor group.

In still yet another embodiment of the present invention, aid fluorescence-based cell imaging or analysis is either a monocolour or a multi-colour imaging or analysis.

In another embodiment of the present invention, a method for fluorescence-based imaging or analysis of cells and/or cellular components, comprising the following steps:

i) staining cellular components with a donor-acceptor fluoranthenes having the general formula I, and derivatives thereof
wherein R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈ are independently selected from the groups comprising of hydrogen, unsubstituted or substituted alkyl, unsubstituted or substituted alkenyl, unsubstituted or substituted alkoxy, unsubstituted or substituted alkoxyl, unsubstituted or substituted aryl unsubstituted or substituted cycloaryl, unsubstituted or substituted cycloalkyl, unsubstituted or substituted heteroaryl, unsubstituted or substituted alkylthio, unsubstituted or substituted amino, unsubstituted or substituted acylamino, unsubstituted or substituted arylamino, unsubstituted or substituted acylthio, unsubstituted or substituted acyl, unsubstituted or substituted aroyl, unsubstituted or substituted acyloxy, unsubstituted or substituted thioamido, halogens, nitriles, esters, hydroxy, mercapto, carbon trifluoride, nitro;

wherein the word "substituted" means substitution of the moiety with a group selected from one or more halogen(s), nitrile(s), ester(s), hydroxy(s), alkoxy(s), mercapto(s), carbon trifluoride(s), nitro(s), amino group(s) or C₁ to C₂₀-units;

wherein X is a donor group comprising of unsubstituted or substituted amino, unsubstituted or substituted alkoxyl, unsubstituted or substituted alkylthio group;

wherein CN is an acceptor group;

ii) exciting the fluoranthene dye of step i) with laser light in the wave length range from 405 nm to 665 nm;

iii) detecting the light emitted in step (ii);

iv) optionally, generating images with the emission data obtained in step iii);

v) optionally performing an analysis with the data obtained in step iii) or the images obtained in step iv).
In one another embodiment of the present invention, the cellular components stained with the fluoranthene dye of formula I in step i) are lipid droplets.

In still one another embodiment of the present invention, 1, 2 or 3 other excitable fluorescent dye(s) are used, resulting in 2-color, 3-color or 4-color imaging.

In yet another embodiment of the present invention, living or fixed cells are subjected to said fluorescence-based imaging or analysis of cells and/or cellular components.

In still another embodiment of the present invention, a confocal laser scanning microscope, a spinning disc microscope, a two-photon microscope, a conventional epifluorescence microscope, a structured illumination microscope, or a single plane illumination microscope is used.

Even furthermore embodiment of the present invention relates to the compounds are prepared by reacting 2H-pyran-2-ones in isolated or rigid conformations with cyclic ketones containing methylene carbonyl moiety in the presence of a base in an organic solvent.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention may be more clearly understood by reference to the following Table/Figures:

Table 1 represents the photophysical properties of the compounds of the invention.
Table 2 represents the solvatochromic properties of compound 8.
Figure 1 illustrates the reaction sequence resulting in the preparation of various fluoranthene derivatives.
Figure 2 illustrates absorption, excitation and emission spectra of compound 1 in Water.
Figure 3 illustrates absorption, excitation and emission spectra of compound 2 in Water.
Figure 4 illustrates absorption, excitation and emission spectra of compound 3 in Water.
Figure 5 illustrates absorption, excitation and emission spectra of compound 4 in Water.
Figure 6 illustrates absorption, excitation and emission spectra of compound 5 in Water.
Figure 7 illustrates absorption, excitation and emission spectra of compound 6 in Water.
Figure 8 illustrates absorption, excitation and emission spectra of compound 8 in Water.
Figure 9 illustrates absorption, excitation and emission spectra of compound 9 in Water.

Figure 10 illustrates absorption, excitation and emission spectra of compound 10 in Water.

Figure 11 illustrates absorption, excitation and emission spectra of compound 11 in Water.

Figure 12 illustrates absorption, excitation and emission spectra of compound 12 in Water.

Figure 13 illustrates absorption, excitation and emission spectra of compound 13 in Water.

Figure 14 illustrates absorption, excitation and emission spectra of compound 14 in Water.

Figure 15 illustrates absorption, excitation and emission spectra of compound 15 in Water.

Figure 16 Solvatochromism spectra of compound 8 in solvent of varying polarity.

Figure 17 illustrates excitation spectra of compound 8 with BODIPY and Nile Red dyes in water.

Figure 18 illustrates emission spectra of compound 8 with BODIPY and Nile Red dyes in water.

Figure 19 illustrates L. donovani promastigotes stained with 300nM Nile Red in PBS for 10 min. in room temperature.

Figure 20 illustrates L. donovani promastigotes stained with 100nM compound 8 in PBS for 2h in room temperature.

Figure 21 illustrates dual staining of L. donovani promastigotes with Nile Red and compound 8.

Figure 22 illustrates 3T3-L1 pre-adipocytes stained with 300nM Nile Red for 10 min at 37°C / 5% CO₂ in medium (DMEM).

Figure 23 illustrates 3T3-L1 pre-adipocytes were stained with 100nm of compound 8 for 20 min. at 37°C / 5% CO₂ in medium (DMEM) on confocal dishes and directly analyzed by confocal microscopy.

Figure 24 illustrates dual staining of 3T3-L1 pre-adipocytes with Nile Red and compound 8.

Figure 25 illustrates dual staining of 3T3-L1 pre-adipocytes with Hoeschst and compound 8.

Figure 26 shows cell viability assessment of compound 8 in 3T3-L1 pre-adipocytes.

Figure 27 shows cell viability assessment of compound 8 in L. donovani promastigotes.

ABBREVIATIONS

LD: Lipid Droplets

BODIPY: Boron-dipyrromethene

FITC Flourescein isothiocyanate

DPH 1,6-Diphenyl-1,3,5-hexatrine
The present invention relates to novel donor-acceptor fluoranthenes of the general formula I which can be used potentially in developing fluorescent probes, and a process of preparing said novel compounds. More particularly, the present invention relates to amine or alkoxy group as donor and nitrile group as an acceptor attached to the fluoranthene skeleton, processes for preparing the said compounds and their uses as fluorescent probes in chemical and biological sciences such as cell imaging applications, diagnostics, fluorescent tags and other useful applications.

The term 'fluorescent probe' refers to a fluorophore, which may be used to localize within a specific region of a biological specimen or to respond to a specific analyte/substance.

The term 'fluorescent tag' refers to a fluorescent molecule that is attached chemically to aid in the labeling and detection of a biomolecule such as a protein, antibody, or amino acid.

The present invention more particularly relates to a compound of formula I:

\[
\begin{align*}
\text{I} & \\
R^1 & \\
\text{R}^2 & \\
\text{R}^3 & \\
\text{R}^4 & \\
\text{R}^5 & \\
\text{R}^6 & \\
\text{R}^7 & \\
\text{R}^8 & \\
\end{align*}
\]

(a) wherein \( R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8 \) are independently selected from the groups consisting of hydrogen, unsubstituted or substituted alkyl, unsubstituted or substituted alkenyl, unsubstituted or substituted alkoxy, unsubstituted or substituted aryl unsubstituted
or substituted cycloaryl, unsubstituted or substituted cycloalkyl, unsubstituted or substituted heteroaryl, unsubstituted or substituted alkylthio, unsubstituted or substituted amino, unsubstituted or substituted acylamino, unsubstituted or substituted arylamino, unsubstituted or substituted acyl, unsubstituted or substituted acyloxy, unsubstituted or substituted thioamido, halogens, nitriles, esters, hydroxy, mercapto, carbontrifluoride, nitro;

(b) wherein X is a donor group consisting of unsubstituted or substituted amino, unsubstituted or substituted alkoxy, unsubstituted or substituted alkylthio group

(c) wherein the word "substituted" means substitution with one or more halogen(s), nitrile(s), ester(s), hydroxy(s), alkoxy(s), mercapto(s), carbontrifluoride(s), nitro(s), amino group(s) or C₁₋₂₀-units.

In another embodiment of the invention wherein the representative compounds comprising:

i. 8-(Dimethylamino)-10-isopropylfluoranthene-7-carbonitrile (1).
ii. 10-Phenyl-8-(pyrrolidin-1-yl)fluoranthene-7-carbonitrile (2).
iii. 10-(4-Methoxyphenyl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile (3).
iv. 8-(Piperidin-1-yl)-10-(thiophen-2-yl)fluoranthene-7-carbonitrile (4).
v. 10-(Benzo[d][1,3]dioxol-5-yl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile (5).
vi. 8-(Piperidin-1-yl)-10-(3,4,5-trimethoxyphenyl)fluoranthene-7-carbonitrile (6).
vii. 10-(4-Nitrophenyl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile (7).
viii. 10-(4-Hydroxyphenyl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile (8).
ix. 10-(3,4-Dihydroxyphenyl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile (9).
x. 8-(Piperidin-1-yl)-10-(3,4,5-trihydroxyphenyl)fluoranthene-7-carbonitrile (10).
xı. 10-(4-Aminophenyl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile (11).
xii. 8-(Bis(2-hydroxyethyl)amino)-10-phenylfluoranthene-7-carbonitrile (12).
xiii. 8-((2-Hydroxyethyl)(methyl)amino)-10-(4-methoxyphenyl)fluoranthene-7-carbonitrile (13).
xiv. 8-((2-Hydroxyethyl)(methyl)amino)-10-(4-methoxyphenyl)fluoranthene-7-carbonitrile (14).
xv. 8-((2-Bromoethyl)(methyl)amino)-10-(4-methoxyphenyl)fluoranthene-7-carbonitrile (15).
Accordingly the present invention provides a process for the preparation of novel donor-acceptor fluoranthene of the general formula I as shown in drawing accompanying the specification represents a preferred embodiment of this process:

- Reacting a compound having general formula S-1 with a compound having general formula S-2 to furnish a compound having the general formula I
  - R1, R2, R3, R4, R5, R6, R7, R8 are independently selected from the groups consisting of hydrogen, unsubstituted or substituted alkyl, unsubstituted or substituted alkenyl, unsubstituted or substituted alkoxy, unsubstituted or substituted aryl, unsubstituted or substituted cycloalkyl, unsubstituted or substituted heteroaryl, unsubstituted or substituted alkylthio, unsubstituted or substituted amino, unsubstituted or substituted acylamino, unsubstituted or substituted arylamino, unsubstituted or substituted acyloxy, unsubstituted or substituted thioamido, halogens, nitriles, esters, hydroxy, mercapto, carbon trifluoride, nitro;
  - Wherein X is a donor group consisting of unsubstituted or substituted amino, unsubstituted or substituted alkoxy, unsubstituted or substituted alkylthio group

- Reaction may proceed in a common organic solvent particularly DMF, THF, DMSO, DCM, isopropanol in the presence of a base particularly KOH, NaOH, NaH, KH, K2CO3, Cs2CO3 at a temperature ranging between -78°C to 100°C for a period ranging between 1 minute to 24 hr.,

- Isolating the compound of general formula I from the reaction mixture and purifying by chromatographic techniques.
The starting material of the general formula S-1 and S-2 are known and the compound of the general formula S-1 has been prepared by the reaction of methyl 2-cyano/methoxycarbonyl-3, 3-di (methylsulfanyl) acrylate with substituted acetophenones under alkaline conditions in dry DMSO in high yields.

Accordingly the present invention is to provide the compounds having the general formula I which are useful in preparing fluorescent probes for cell imaging applications and other useful biological applications such as developing diagnostic kits.

Synthesis:

EXAMPLES:

Following examples are given by way of illustration and should not construe the scope of the present invention.

General procedure for the synthesis of compounds (Example 1-7, 12, 14, 15): Synthesis of fluoranthenes dyes with an amine donor and a nitrile acceptor substituents is an independent reaction of methyl 2-cyano-3,3-bis(dimethylsulfanyl)acrylate with various ketone were carried out to afford 6-aryl-3-cyano-4-methylsulfanyl-2H-pyran-2-ones in good yields. A good leaving methylsulfanyl group was replaced with an amine to furnish 6-aryl-2-oxo-4-(amin-1-yl)-2H-pyran-3-carbonitriles in good yields. Further stirring an equimolar mixture of 6-aryl-2-oxo-4-(amin-1-yl)-2H-pyran-3-carbonitriles and 2H-acenaphthylen-1-one in the presence of NaH in dry THF for 10-20 min. at 25-27°C yielded 10-alkyl/aryl 8-(amin-1-yl)fluoranthene-7-carbonitrile in good yields.

General procedure for the synthesis of compounds (Example 8-10): Compound 10-(4-methoxyphenyl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile (Example 3) or 10-(benzo[d][1,3]dioxol-5-yl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile (Example 5) or 8-(piperidin-1-yl)-10-(3,4,5-trimethoxyphenyl)fluoranthene-7-carbonitrile (Example 6) (1 mmol, lequiv.) was dissolved in dry CH2Cl2 (10 ml) and BBr3 (2 equiv. or 4 equiv. or 6 equiv. respectively) was added to the solution at -78°C. The reaction mixture was stirred for 50 min at -78°C and then 10-15 hours at room temperature. After completion, the reaction mixture was quenched with ice-cooled water and neutralized with 10% HCl. The reaction mixture was then extracted with CH2Cl2 and the organic layer separated, washed with brine, dried over
anhydrous Na₂SO₄, and concentrated. The residue was purified by silica gel column chromatography using methanol in chloroform as the eluent to afford good yields of 10-(4-hydroxyphenyl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile (Example 8) or 10-(3,4-dihydroxyphenyl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile (Example 9) or 8-(piperidin-1-yl)-10-(3,4,5-trihydroxyphenyl)fluoranthene-7-carbonitrile (Example 10) respectively.

**General procedure for the synthesis of compounds (Example 11):** 10-(4-nitrophenyl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile (431 mg, 1 mmol, 1 equiv.) in isopropanol (5 mL) was reflux at 80°C for 1 hr. with Sn/HCl. The progress of the reaction was monitored by TLC and on completion solvent was evaporated and the reaction mixture was poured onto crushed ice with vigorous stirring and finally neutralized with dilute sodium bicarbonate solution. The precipitate obtained was filtered and purified on a silica gel column with 5% methanol in chloroform as the eluent to afford 318 mg (79%) as a yellow solid.

**EXAMPLE-1**

8-(Dimethylamino)-10-isopropylfluoranthene-7-carbonitrile (1)

A mixture of 4-(dimethylamino)-6-isopropyl-2-oxo-2H-pyran-3-carbonitrile (206 mg, 1 mmol, 1 equiv.),acenaphthylene-1(2H)-one (168 mg, 1 mmol, 1 equiv.), and NaH(60% dispersion in oil, 60 mg, 1.5 mmol, 1.5 equiv.) in dry THF (5 mL) was stirred at 25°C for 15 min. The progress of the reaction was monitored by TLC and on completion solvent was evaporated and the reaction mixture was poured onto crushed ice with vigorous stirring and finally neutralized with 10% HCl. The precipitate obtained was filtered and purified on a silica gel column with 2% ethyl acetate in hexane as the eluent to afford 250 mg (80%) as a yellow solid; Rf = 0.51 (n-hexane/ethyl acetate, 9:1, v/v); mp (n-hexane/ethyl acetate) 208-210 °C; MS (ESI) 313 [M + H⁺]; H NMR (300 MHz, CDC13) □ □ □ = 1.46 (d, J = 6.8 Hz, 6H, 2CH₃), 3.14 (s, 6H, 2CH₃), 3.80-3.95 (m, 1H, CH), 6.86 (s, 1H, ArH), 7.57-7.74 (m, 2H, ArH), 7.81 (d, J = 8.2 Hz 1H, ArH), 7.88-7.99 (m, 2H, ArH), 8.65 (d, J = 7.1 Hz, 1H, ArH) ppm.
EXAMPLE-2

10-Phenyl-8-(pyrrolidin-1-yl)fluoranthene-7-carbonitrile (2)

A mixture of 2-oxo-6-phenyl-4-(pyrrolidin-1-yl)-2H-pyran-3-carbonitrile (266 mg, 1 mmol, 1 equiv.), acenaphthylene-1(2H)-one (168 mg, 1 mmol, 1 equiv.), and NaH (60% dispersion in oil, 60 mg, 1.5 mmol, 1.5 equiv) in dry THF (5 mL) was stirred at 27°C for 12 min. The progress of the reaction was monitored by TLC and on completion solvent was evaporated and the reaction mixture was poured onto crushed ice with vigorous stirring and finally neutralized with 10% HCl. The precipitate obtained was filtered and purified on a silica gel column with 2% ethyl acetate in hexane as the eluent to afford 305 mg (82%) as a yellow solid; Rf = 0.48 (n-hexane/ethyl acetate, 9:1, v/v); mp (n-hexane/ethyl acetate) 188-190 °C; MS (ESI) 373[M + H+]; 1H NMR (300 MHz, CDC13): δ = 2.00-2.11 (m, 4H, 2CH2), 3.71-3.83 (m, 4H, 2CH2), 6.52 (s, 1H, ArH), 6.98 (d, J = 7.0 Hz, 1H, ArH), 7.32 (t, J = 7.7 Hz, 1H, ArH), 7.50-7.59 (m, 5H, ArH), 7.65-7.75 (m, 2H, ArH), 7.90 (d, J = 8.1 Hz, 1H, ArH), 8.73 (d, J = 7.1 Hz, 1H, ArH).

EXAMPLE-3

10-(4-Methoxyphenyl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile (3)

A mixture of 6-(4-methoxyphenyl)-2-oxo-4-(piperidin-1-yl)-2H-pyran-3-carbonitrile (310 mg, 1 mmol, 1 equiv.), acenaphthylene-1(2H)-one (168 mg, 1 mmol, 1 equiv.), and NaH (60% dispersion in oil, 60 mg, 1.5 mmol, 1.5 equiv) in dry THF (5 mL) was stirred at 25°C for 15 min. The progress of the reaction was monitored by TLC and on completion solvent was evaporated and the reaction mixture was poured onto crushed ice with vigorous stirring and...
finally neutralized with 10% HCl. The precipitate obtained was filtered and purified on a silica gel column with 3% ethyl acetate in hexane as the eluent to afford 352 mg (84%) as a yellow solid; \( R_f = 0.49 \) (n-hexane/ethyl acetate, 9:1, v/v); mp (n-hexane/ethyl acetate) 242-244 °C; MS (ESI) 417 [M + H⁺]; \(^1\)H NMR (300 MHz, CDCl₃) \( \delta = 1.59-1.68 \) (m, 2H, CH₂), 1.82-1.92 (m, 4H, 2CH₂), 3.22-3.33 (m, 4H, 2CH₂), 3.94 (s, 3H, CH₃), 6.82 (s, 1H, ArH), 7.08 (d, \( J = 8.4 \) Hz, 2H, ArH), 7.20-7.31 (m, 1H, ArH), 7.25 (t, \( J = 7.6 \) Hz, 1H, ArH), 7.52 (d, \( J = 8.4 \) Hz, 2H, ArH), 7.67-7.80 (m, 2H, ArH), 7.92 (d, \( J = 8.1 \) Hz, 1H, ArH), 8.66 (d, \( J = 7.1 \) Hz, 1H, ArH) ppm.

**EXAMPLE-4**

8-(Piperidin-1-yl)-10-(thiophen-2-yl)fluoranthene-7-carbonitrile (4)

A mixture of 2-oxo-4-(piperidin-1-yl)-6-(thiophen-2-yl)-2H-pyran-3-carbonitrile (286 mg, 1 mmol, 1 equiv.), acenaphthylen-1(2H)-one (168 mg, 1 mmol, 1 equiv.), and NaH (60% dispersion in oil, 60 mg, 1.5 mmol, 1.5 equiv.) in dry THF (5 mL) was stirred 27°C for 15 min. The progress of the reaction was monitored by TLC and on completion solvent was evaporated and the reaction mixture was poured onto crushed ice with vigorous stirring and finally neutralized with 10% HCl. The precipitate obtained was filtered and purified on a silica gel column with 2% ethyl acetate in hexane as the eluent to afford 305 mg (78%) as a yellow solid; \( R_f = 0.53 \) (n-hexane/ethyl acetate, 9:1, v/v); mp (n-hexane/ethyl acetate) 246-248 °C; MS (ESI) 393 [M + H⁺]; \(^1\)H NMR (300 MHz, CDCl₃) \( \delta = 1.60-1.70 \) (m, 2H, CH₂), 1.80-1.91 (m, 4H, 2CH₂), 3.24-3.32 (m, 4H, 2CH₂), 6.94 (s, 1H, ArH), 7.20-7.27 (m, 2H, ArH), 7.34-7.40 (m, 1H, ArH), 7.41-7.47 (m, 1H, ArH), 7.50-7.55 (m, 1H, ArH), 7.69-7.83 (m, 2H, ArH), 7.94 (d, \( J = 8.2 \) Hz, 1H), 8.67 (d, \( J = 7.1 \) Hz, 1H, ArH) ppm.

**EXAMPLE-S**

10-(Benzo[d][1,3]dioxol-5-yl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile (5)
A mixture of 6-(benzo[1,3]dioxol-5-yl)-2-oxo-4-(piperidin-1-yl)-2H-pyran-3-carbonitrile (324 mg, 1 mmol, 1 equiv.), acenaphthylen-l(2 H)-one (168 mg, 1 mmol, 1 equiv.), and NaH(60% dispersion in oil, 60 mg, 1.5 mmol, 1.5 equiv.) in dry THF (5 mL) was stirred at 25°C for 12 min. The progress of the reaction was monitored by TLC and on completion solvent was evaporated and the reaction mixture was poured onto crushed ice with vigorous stirring and finally neutralized with 10% HCl. The precipitate obtained was filtered and purified on a silica gel column with 3% ethyl acetate in hexane as the eluent to afford 318 mg (74%) as a yellow solid; Rf = 0.48 (n-hexane/ethyl acetate, 9:1, v/v); mp (n-hexane/ethyl acetate) 244-246 °C; MS (ESI) 431 [M + H+]; 1H NMR (300 MHz, CDCl3) □□ = 1.62-1.68 (m, 2H, CH2), 1.83-1.88 (m, 4H, 2CH2), 3.23-3.32 (m, 4H, 2CH2), 6.10 (s, 2H, CH2), 6.81 (s, 1H, ArH), 6.97-7.10 (m, 1H, ArH), 7.26-7.35 (m, 2H, ArH), 7.68-7.78 (m, 1H, ArH), 7.42 (t, J = 7.6 Hz, 1H, ArH), 7.66-7.81 (m, 2H, ArH), 7.93 (d, J = 8.1 Hz, 1H, ArH), 8.67 (d, J = 7.1 Hz, 1H, ArH) ppm.

**EXAMPLE-6**

8-(Piperidin-l-yl)-10-(3,4,5-trimethoxyphenyl)fluoranthene-7-carbonitrile (6)

A mixture of 2-oxo-4-(piperidin-1-yl)-6-(3,4,5-trimethoxyphenyl)-2H-pyran-3-carbonitrile (370 mg, 1 mmol, 1 equiv.), acenaphthylen-l(2 H)-one (168 mg, 1 mmol, 1 equiv.), and NaH(60% dispersion in oil, 60 mg, 1.5 mmol, 1.5 equiv.) in dry THF (5 mL) was stirred at 25°C for 10 min. The progress of the reaction was monitored by TLC and on completion solvent was evaporated and the reaction mixture was poured onto crushed ice with vigorous...
stirring and finally neutralized with 10% HCl. The precipitate obtained was filtered and purified on a silica gel column with 5% ethyl acetate in hexane as the eluent to afford 348 mg (73%) as a yellow solid; \( R_f = 0.43 \) (n-hexane/ethyl acetate, 9:1, v/v); mp (n-hexane/ethyl acetate) 240-245 °C; MS (ESI) 477 [M + H\(^+\)]; \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta = 1.62\)-1.67 (m, 2H, \( \text{C}_2\text{H}_2 \)), 1.84-1.88 (m, 4H, 2CH\(_2\)), 3.25-3.32 (m, 4H, 2CH\(_2\)), 3.88 (s, 6H, 2CH\(_3\)), 3.99 (s, 3H, \( \text{C}_3\text{H}_3 \)), 6.79 (s, 1H, ArH), 6.86 (s, 1H, ArH), 7.14 (t, \( J = 7.6 \text{ Hz} \), 1H, ArH), 7.68-7.78 (m, 2H, ArH), 7.94 (d, \( J = 8.1 \text{ Hz} \), 1H, ArH) ppm.

EXAMPLE-7
10-(4-Nitrophenyl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile (7).

A mixture of 6-(4-nitrophenyl)-2-oxo-4-(piperidin-1-yl)-2\(H\)-pyran-3-carbonitrile (325 mg, 1mmol, eq.), acenaphthylene-1(2\(H\))-one (168 mg, 1 mmol, 1 equiv.), and NaH(60% dispersion in oil, 60 mg, 1.5 mmol, 1.5 equiv.) in dry THF (5 mL) was stirred at 25°C for 15 min. The progress of the reaction was monitored by TLC and on completion solvent was evaporated and the reaction mixture was poured onto crushed ice with vigorous stirring and finally neutralized with 10% HCl. The precipitate obtained was filtered and purified on a silica gel column with 2% ethyl acetate in hexane as the eluent to afford 348 mg (81%) as an orange solid; \( R_f = 0.53 \) (n-hexane/ethyl acetate, 9:1, v/v); mp (n-hexane/ethyl acetate) >250°C; MS (ESI) 432 [M + H\(^+\)]; \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta = 1.62\)-1.73 (m, 2H, \( \text{C}_2\text{H}_2 \)), 1.80-1.93 (m, 4H, 2CH\(_2\)), 3.30 (t, \( J = 5.3 \text{ Hz} \), 4H, 2CH\(_2\)), 6.80 (s, 1H, ArH), 7.06 (d, \( J = 7.1 \text{ Hz} \), ArH), 7.39 (t, \( J = 7.7 \text{ Hz} \), 1H, ArH), 7.71-7.84 (m, 4H, ArH), 8.00 (d, \( J = 8.2 \text{ Hz} \), 1H, ArH), 8.43 (d, \( J = 8.6 \text{ Hz} \), 2H, ArH), 8.70 (d, \( J = 7.1 \text{ Hz} \), 1H, ArH) ppm.

EXAMPLE-8
10-(4-Hydroxyphenyl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile (8)
10-(4-Methoxyphenyl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile (416mg, 1 mmol, lequiv.) was dissolved in dry \( \text{CH}_2\text{Cl}_2 \) (10 ml) and BBr\(_3\) (192 DL, 2 mmol, 2 equiv.) was added to the solution at -78°C. The reaction mixture was stirred for 50 min at -78°C and then 10 hours at 25°C. After completion, the reaction mixture was quenched with ice-cooled water and neutralized with 10% HCl. The reaction mixture was then extracted with \( \text{CH}_2\text{Cl}_2 \) and the organic layer separated, washed with brine, dried over anhydrous Na\(_2\)SO\(_4\), and concentrated. The residue was purified by silica gel column chromatography using 1% methanol in chloroform as the eluent to afford 312mg (78%) as yellow solid; R\(_f\) = 0.59 (chloroform/methanol, 9:1, v/v); mp (chloroform/methanol) >250 °C; MS (ESI) 403 [M + H\(^+\)]; \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) = 1.62-1.68 (m, 2H, CH\(_2\)), 1.79-1.91 (m, 4H, 2CH\(_2\)), 3.23-3.31 (m, 4H, 2CH\(_2\)), 5.10 (s, 1H, OH), 6.82 (s, 1H ArH), 7.02 (d, \( J = 8.5 \) Hz, 2H, ArH), 7.20-7.24 (m, 1H, ArH), 7.39 (t, \( J = 8.1 \) Hz, 1H, ArH), 7.47 (d, \( J = 8.5 \) Hz, 2H, ArH), 7.70-7.79 (m, 2H, ArH), 7.93 (d, \( J = 8.2 \) Hz, 1H, ArH), 8.66 (d, \( J = 7.2 \) Hz, 1H, ArH) ppm.

EXAMPLE-9

10-(3,4-dihydroxyphenyl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile (9)

10-(benzo[d][1,3]dioxol-5-yl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile (430mg, 1 mmol, lequiv.) was dissolved in dry \( \text{CH}_2\text{Cl}_2 \) (10 ml) and BBr\(_3\) (384 DL, 4 mmol, 4 equiv.) was added to the solution at -78°C. The reaction mixture was stirred for 50 min at -78°C and then 12 hours at 25°C. After completion, the reaction mixture was quenched with ice-cooled water and neutralized with 10% HCl. The reaction mixture was then extracted with \( \text{CH}_2\text{Cl}_2 \) and the organic layer separated, washed with brine, dried over anhydrous Na\(_2\)SO\(_4\), and concentrated.
The residue was purified by silica gel column chromatography using 1% methanol in chloroform as the eluent to afford 297mg (72%) yield as yellow solid; \( R_f = 0.51 \) (chloroform/methanol, 9:1, v/v); mp (chloroform/methanol) 244-246 °C; MS (ESI) 419 [M + H⁺]; \(^1\)H NMR (300 MHz, DMSO-d$_6$) \( \delta = 1.59-164 \) (m, 2H, CH$_2$), 1.74-1.78 (m, 4H, 2CH$_2$), 3.24-3.27 (m, 4H, 2CH$_2$), 6.85 (s, 4H, ArH), 7.40 (d, \( J = 6.9 \) Hz, IH, ArH), 7.56 (t, \( J = 7.6 \) Hz, IH, ArH), 7.85 (t, \( J = 7.7 \) Hz, IH, ArH), 7.93 (d, \( J = 8.1 \) Hz, IH, ArH), 8.11 (d, \( J = 8.2 \) Hz, IH, ArH), 8.53 (d, \( J = 7.1 \) Hz, IH, ArH), 9.23 (s, IH, -OH), 9.29 (s, IH, OH) ppm.

**EXAMPLE-10**

8-(Piperidin-1-yl)-10-(3,4,5-trihydroxyphenyl)fluoranthene-7-carbonitrile (10)

![Chemical Structure](image)

Compound 8-(piperidin-1-yl)-10-(3,4,5-trimethoxyphenyl)fluoranthene-7-carbonitrile (476mg, 1 mmol,leq.) was dissolved in dry CH$_2$C$_2$ $\text{L}$ (10 ml) and BBr$_3$ (576DL, 6 mmol, 6 equiv) was added to the solution at -78°C. The reaction mixture was stirred for 50 min at -78°C and then 15 hours at 25°C. After completion, the reaction mixture was quenched with ice-cooled water and neutralized with 10% HCl. The reaction mixture was then extracted with CH$_2$C$_2$ $\text{L}$ and the organic layer separated, washed with brine, dried over anhydrous Na$_2$SO$_4$, and concentrated. The residue was purified by silica gel column chromatography using 5% methanol in chloroform as the eluent to afford 292 mg (69%) as yellow solid; \( R_f = 0.47 \) (chloroform/methanol, 9:1, v/v); mp (chloroform/methanol) 238-240 °C; MS (ESI) 435 [M + H⁺]; \(^1\)H NMR (300 MHz, DMSO-d$_6$) \( \delta = 1.58-1.78 \) (m, 2H, CH$_2$), 1.74-1.78 (m, 4H, 2CH$_2$), 3.23-3.27 (m, 4H, 2CH$_2$), 6.53 (s, 2H, ArH), 6.87 (s, IH, ArH), 7.45-7.51 (m, IH, ArH), 7.58 (t, \( J = 7.3 \) Hz, IH, ArH), 7.85 (t, \( J = 7.5 \) Hz, IH, ArH), 7.94 (d, \( J = 8.0 \) Hz, IH, ArH), 8.12 (d, \( J = 7.7 \) Hz, IH, ArH), 8.47 (s, IH, -OH) 8.52 (d, \( J = 6.9 \) Hz, IH, ArH), 9.16 (s, 2H, -20H) ppm.

**EXAMPLE-11**

10-(4-Aminophenyl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile (11)
10-(4-nitrophenyl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile (431 mg, 1 mmol, 1 equiv.) in isopropanol (5 mL) was reflux at 80°C for 1 hr. with Sn/HCl. The progress of the reaction was monitored by TLC and on completion solvent was evaporated and the reaction mixture was poured onto crushed ice with vigorous stirring and finally neutralized with dilute sodium bicarbonate solution. The precipitate obtained was filtered and purified on a silica gel column with 5% methanol in chloroform as the eluent to afford 318 mg (79%) as a yellow solid; R_f = 0.51 (chloroform/methanol, 9:1, v/v); mp (chloroform/methanol) 232-234 °C; MS (ESI) 402 [M + H]+; 1H NMR (300 MHz, DMSO-d_6) □□□ = 1.51-1.58 (m, 2H, CH_2), 1.67-1.71 (m, 4H, CH_2), 3.14-3.21 (m, 4H, CH_2), 5.42 (s, 2H, NH_2), 6.69 (d, J = 8.4 Hz, 2H, ArH), 6.80 (s, 1H, ArH), 7.27 (d, J = 8.3 Hz, 2H, ArH), 7.38-7.53 (m, 2H, ArH), 7.77 (t, J = 7.7 Hz, 1H, ArH), 7.86 (d, J = 7.9 Hz, 1H, ArH), 8.03 (d, J = 8.1 Hz, 1H, ArH), 8.45 (d, J = 7.1 Hz, 1H, ArH) ppm.

**EXAMPLE-12**

8-(Bis(2-hydroxyethyl)amino)-10-phenylfluoranthene-7-carbonitrile (12)

A mixture of 4-(bis(2-hydroxyethyl)amino)-2-oxo-6-phenyl-2H-pyran-3-carbonyl (300 mg, 1 mmol, 1 equiv.), 2H-acenaphthylene-1-one (168 mg, 1 mmol, 1 equiv.), and NaH (60% dispersion in oil, 60 mg, 1.5 mmol, 1.5 equiv.) in dry DMF (5 mL) was stirred at 22°C for 12 min. The progress of the reaction was monitored by TLC and on completion solvent was evaporated and the reaction mixture was poured onto crushed ice with vigorous stirring and finally neutralized with 10% HCl. The precipitate obtained was filtered and purified on a
silica gel column using 5% methanol in chloroform as the eluent to afford 276 mg (68%) as yellow solid; \( R_f = 0.48 \) (chloroform/methanol, 9:1, v/v); mp (chloroform/methanol) 110-112 °C; MS (ESI) 407 [M + H\(^+\)]; \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \( \delta = 3.53-3.71 \) (m, 8H, 4CH\(_2\)), 4.68 (t, \( J = 4.9 \) Hz, 2H, OH), 6.92 (s, 1H, ArH), 7.00 (d, \( J = 7.1 \) Hz, 1H, ArH), 7.39 (t, \( J = 7.7 \) Hz, 1H, ArH), 7.53 (s, 5H, ArH), 7.71-7.92 (m, 2H, ArH), 8.01 (d, \( J = 8.1 \) Hz, 1H, ArH), 8.50 ppm.

EXAMPLE-13

10-(4-(Heptyloxy)phenyl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile (13)

A mixture of 10-(4-hydroxyphenyl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile (402 mg, 1mmol, eq.), 1-bromohexane (178 mg, 1 mmol, 1 equiv.), and NaH(60% dispersion in oil, 60 mg, 1.5 mmol, 1.5 equiv.) in DMF (5 mL) was stirred at 25°C for 1 hr. The progress of the reaction was monitored by TLC and on completion solvent was evaporated and the reaction mixture was poured onto crushed ice with vigorous stirring and finally neutralized with 10% HC1. The precipitate obtained was filtered and purified on a silica gel column with 2% ethyl acetate in hexane as the eluent to afford 390 mg (78%) as yellow solid; \( R_f = 0.59 \) (n-hexane/ethyl acetate, 9:1, v/v); mp (n-hexane/ethyl acetate) >250 °C; MS (ESI) 501 [M + H\(^+\)]; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta = 0.92 \) (t, \( J = 6.1 \) Hz, 3H, CH\(_3\)), 1.24-1.62 (m, 10H, 5CH\(_2\)), 1.84-1.87 (m, 6H, 3CH\(_2\)), 3.27 (t, \( J = 4.9 \) Hz, 4H, 2CH\(_2\)), 4.07 (t, \( J = 6.5 \) Hz, 2H, CH\(_2\)), 6.82 (s, 1H, ArH), 7.1(d, \( J = 8.4 \) Hz, 2H, ArH), 7.23-7.26 (m, 1H, ArH), 7.38(t, \( J = 7.6 \) Hz, 1H, ArH), 7.67-7.78(m, 2H, ArH), 7.92 (d, \( J = 8.1 \) Hz, 1H, ArH), 8.7 (d, \( J = 7.1 \) Hz, 1H, ArH) ppm.

EXAMPLE-14

8-((2-Hydroxyethyl)(methyl)amino)-10-(4-methoxyphenyl)fluoranthene-7-carbonitrile (14)
A mixture of 4-((2-hydroxyethyl)(methyl)amino)-6-(4-methoxyphenyl)-2-oxo-2H-pyran-3-carbonitrile (300 mg, 1 mmol, 1 equiv.), acenaphthylen-1(2H)-one (168 mg, 1 mmol, 1 equiv.), and NaH(60% dispersion in oil, 60 mg, 1.5 mmol, 1.5 equiv.) in dry DMF (5 mL) was stirred at 25°C for 20 min. The progress of the reaction was monitored by TLC and on completion solvent was evaporated and the reaction mixture was poured onto crushed ice with vigorous stirring and finally neutralized with 10% HCl. The precipitate obtained was filtered and purified on a silica gel column with 5% ethyl acetate in hexane as the eluent to afford 280 mg (68%) as a yellow solid; R_f = 0.45 (n-hexane/ethyl acetate, 9:1, v/v); mp (n-hexane/ethyl acetate) 242-244 °C; MS (ESI) 407 [M + H]^+; ^1H NMR (400 MHz, CDCl_3) □□ = 3.09 (s, 3H, CH_3), 3.55 (t, J = 5.7 Hz, 2H, CH_2), 3.92-4.01 (m, 5H, CH_2, OCH_3), 6.90 (s, IH, ArH), 7.03-7.11 (m, 2H, ArH), 7.22-7.28 (m, 2H, ArH), 7.39 (t, J = 7.6 Hz, fIH, ArH), 7.49-7.55 (m, 2H, ArH), 7.69-7.78 (m, 2H, ArH), 7.94 (d, J = 8.1 Hz, IH, ArH), 8.66 (d, J = 7.0 Hz, IH, ArH) ppm.

**EXAMPLE-15**

8-((2-Bromoethyl)(methyl)amino)-10-(4-methoxyphenyl)fluoranthene-7-carbonitrile (15)

A mixture of 8-((2-hydroxyethyl)(methyl)amino)-10-(4-methoxyphenyl)fluoranthene-7-carbonitrile (406 mg, 1 mmol, 1 equiv.) and POBr_3 (339 mg, 1.2 mmol) was refluxed in the presence of Cs_2CO_3 in dry toluene for 1-3h. The progress of the reaction was monitored by TLC and after completion; the excess toluene was removed under vacuum. Crude product was purified by silica gel column chromatography using 2% ethyl acetate in hexane as the eluent to afford 335 mg (71%) as a yellow solid; R_f = 0.56 (n-hexane/ethyl acetate, 9:1, v/v); mp (n-
hexane/ethyl acetate) 242-244 °C; MS (ESI) 469 [M + H⁺]; ¹H NMR (400 MHz, CDCl₃) δ = 3.13 (s, 3H, CH₃), 3.66-3.72 (m, 2H, CH₂), 3.87 (t, J= 7.4 Hz, 2H, CH₂), 3.94 (s, 3H, CH₃), 6.83 (s, 1H, ArH), 7.06-7.1 (m, 2H, ArH), 7.22-7.27 (m, 1H, ArH), 7.38-7.43 (m, 1H, ArH), 7.49-7.55 (m, 2H, ArH), 7.69-7.78 (m, 2H, ArH), 7.94 (d, J= 8.0 Hz, 1H, ArH), 8.68 (d, J= 7.0 Hz, 1H, ArH).

Photophysical studies of the compounds of general formula I

The photophysical properties of all the synthesized compounds 1-15 were examined by UV-vis and fluorescence techniques (Figure 2-15). Table 1 showed the □max of their UV, excitation and fluorescence spectral data along with stoke's shift. These compounds produced different color emissions depending upon the nature and position of electron donor-acceptor substituents and chromophores attached on fluoranthene scaffolds (Table 1).

Table 1. Photophysical properties of fluoranthene (Examples 1-15) in 0.2% DMSO in Water.

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<th>□max, em (nm)ᵇ</th>
<th>Ass (nm)ᶜ</th>
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</table>
Solvatochromism: Due to the difference in the dipole moments of the donor-acceptor molecules in the ground and excited states and their stabilization by polar or non-polar solvent molecules through various noncovalent interactions like hydrogen bonding, solvation or dipole-dipole interactions, novel donor-acceptor fluoranthenes exhibit solvatochromic behaviour as shown in Figure 16. The absorption and PL spectra of compound 8 were examined using solvents of varying polarity as depicted in Table 2.

Table 2. Photophysical analysis of compound 8 in various solvents.

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<th>$\lambda_{\text{max;abs}}$ (nm)</th>
<th>$\lambda_{\text{max;em}}$ (nm)</th>
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<tr>
<td>DMSO</td>
<td>333</td>
<td>580</td>
<td>12790</td>
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Excitation and emission spectra of compound 8 with Nile Red and BODIPY dyes.

Excitation and emission spectra of compound 8 were examined with commercially available lipid droplet probes (Nile Red and BODIPY) in 0.2 % DMSO in water as shown in drawing accompanying the specification (Figure 17 and Figure 18). An excitation maximum of compound 8 was observed at 332 nm and 435 nm. Excitation maxima of BODIPY and Nile Red were observed at 493 and 597 nm respectively while emission maxima of these fluorescent probes were 550, 503 and 657 nm respectively. On comparison of Ex/Em range of these dyes with compound 8, novel fluoranthene dye showed different emission window and high stokes shift compare to the commercially available BODIPY and Nile Red dyes and therefore, it can be used in multicolor live cell imaging without background noise.

<table>
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<th>$\lambda_{\text{max; abs}}$ (nm)</th>
<th>$\lambda_{\text{max; ex}}$ (nm)</th>
<th>$\lambda_{\text{max; em}}$ (nm)</th>
<th>Stokes Shifts (nm)</th>
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<tr>
<td>Compound 8</td>
<td>330, 430</td>
<td>332, 435</td>
<td>550</td>
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<tr>
<td>BODIPY</td>
<td>491</td>
<td>493</td>
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<td>Nile Red</td>
<td>585</td>
<td>597</td>
<td>657</td>
<td>72</td>
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</table>

Dyes | Ex/Em max | Application                      |
Nile Red | 485/525-636 | Staining of neutral lipid       |
Lipid TOX Red | 577/609 | Neutral lipid staining in HCS |
Lipid TOX Green | 495/505 | Neutral lipid staining in HCS |
Lipid TOX deep red | 637/655 | Neutral lipid staining in HCS |
BODIPY     | 493/503    | LD staining                     |
Monodansylpentane | 405/480 | Neutral Lipid droplet Staining |
compound 8 | 405/550    | Neutral LD staining             |

**Biological Evaluation:**

**Cell Culture of 3T3-L1 Preadipocytes**

3T3-L1 pre-adipocyte cells were obtained earlier from American Type Culture Collection, Manassas, VA, Cat. No. CL-173 and maintained in the tissue and cell culture facility at CDRI, Lucknow. 3T3-L1 pre-adipocytes were cultured in DMEM with 10% heat-inactivated
FBS at 37°C. After reaching 70% confluence, cells were seeded two days before starting experiments. *Leishmania donovani* (strain MHOM/IN/80/Dd8) promastigotes were cultured as described previously (Nayak, R. C.; Sahasrabuddhe, A. A.; Bajpai, V. K.; Gupta, C. M.; Mol. Biochem. Parasitol. 2005, 143, 152.). Briefly, cells were cultured in Dulbecco's Modified Eagles Medium (DMEM) with phenol red, L-glutamine and 4.8g/L D-glucose supplemented with 10% heat inactivated FBS and sodium pyruvate. Cell were checked for their health periodically and maintained in standard tissue culture flasks. After reaching an approximate density of 10^6 cells/ml they were prepared for incubation.

**Cell Culture of Leishmania donovani:**

*Leishmania donovani* (strain MHOM/IN/80/Dd8) cells were obtained from Dr Sahasrabudhe, Molecular and Structural Biology division of the CDRI, Lucknow. These cells were cultured in Dulbecco's Modified Eagles Medium (DMEM) with phenol red, L-glutamine and 4.8g/L D-glucose supplemented with 10% heat inactivated FBS and sodium pyruvate. Cells were checked for their health periodically and maintained in standard tissue culture flasks. After reaching an approximate density of 10^6, they were prepared for incubation. 3T3-L1 pre-adipocytes were cultured in DMEM with 10% heat-inactivated FBS at 37°C. After reaching 70% confluence, cells were seeded and cultured for 2 days in confocal dishes before commencing experiments.

**Confocal Microscopy:**

*L. donovani* cells, briefly after harvesting, were incubated with 100nM compound 8 for 1 hour (empirically determined) at room temperature. Afterwards, they were washed once in PBS (lx) and further incubated with either 300 nM Nile Red for 10 minutes. Cells were then adhered to poly-L-lysine coated coverslips and immediately processed for microscopic examination. 3T3-L1 pre-adipocytes adhered to confocal microscopic dishes were incubated with the dyes in media for 30 min. (empirically determined) at 37°C. They were washed and stained with Hoechst 33342 (Invitrogen) for 10 min. at room temperature before analyzing directly under the confocal microscope LSM 510 META (Carl Zeiss, Jena, Germany). Objective used was a Plan Apochromat 60X or a 40* Ph/DIC objective.
Analysis:
The images saved from the microscope were analyzed with the LSM Image Examiner software (Carl Zeiss).

Fluorescence staining in *Leishmania* promastigotes with Nile Red:
*L. donovani* promastigotes stained with 300nM Nile Red in PBS for 10 min. at room temperature. Lipid droplets are indicated by distinct red spots scattered throughout the cell as indicated by arrows in Figure 19. Cells were imaged with a 63x Plan Apochromat Oil DIC 1.4 NA objective. Imaging experiments were performed at Excitation wavelength at 514 nm with Emission filter of 575nm Long Pass.

Fluorescence staining in *Leishmania* promastigotes with compound 8:
*L. donovani* promastigotes stained with 100nM compound 8 in PBS for 2h at room temperature. Lipid droplets are indicated by orange spots as indicated by arrows in Figure 20 scattered throughout the cell. Bar indicates 5µm. Imaging experiments were performed at Excitation wavelength at 405 nm with Emission filter of 530nm Long Pass.

Dual staining of *Leishmania* promastigotes with Nile Red and compound 8.
Figure 16 represents Dual staining of *L. donovani* promastigotes with Nile Red and compound 8. Cells were first incubated with compound 8 for 2h, then stained with Nile Red for 15 min at room temperature. Bars indicate 5µm. Imaging experiments were performed at Excitation wavelength at 514 nm for Nile Red and 405 nm for compound 8. Staining pattern is similar with Nile Red and compound 8 as indicated by arrows in Figure 21.

Fluorescence staining in 3T3-L1 pre-adipocytes with Nile Red:
3T3-L1 pre-adipocytes stained with 300nM Nile Red for 10 min. at 37°C/5%C0₂ in medium (DMEM). Cells were imaged with a 63x PlanApochromat Oil DIC 1.4 NA objective. Lipid droplets are distinctly stained as red dots throughout the cytoplasm as shown in Figure 22. Imaging experiments were performed at Excitation wavelength at 514 nm with Emission filter of 575nm Long Pass. Bars indicate 5µm.
Fluorescence staining in 3T3-L1 pre-adipocytes with compound 8:

3T3-L1 pre-adipocytes were stained with 100 nm of compound 8 for 20 min. at 37°C/5%CO₂ in medium (DMEM) on confocal dishes and directly analyzed by confocal microscopy. Lipid droplets are distinctly stained as orange dots throughout the cytoplasm as shown in Figure 23. Imaging experiments were performed at Excitation wavelength at 405 nm with Emission filter of 505-550 nm. Bars indicate 5 μm.

Dual staining of 3T3-L1 pre-adipocytes with Nile Red and compound 8.

Figure 24 represents Dual staining of 3T3-L1 pre-adipocytes with Nile Red and compound 8. Cells were first incubated with compound 8 for 2h, then stained with Nile Red for 15 min at room temperature. Bars indicate 5 μm. Imaging experiments were performed at Excitation wavelength at 514 nm for Nile Red and 405 nm for compound 8. Staining pattern is similar with Nile Red.

Dual staining of 3T3-L1 pre-adipocytes with Hoechst 33342 and compound 8.

Figure 25 represents Dual staining of 3T3-L1 pre-adipocytes with Hoechst 33342 and compound 8. Cells were first incubated with compound 8 for 2h, then stained with Hoechst 33342 for 10 min at room temperature. Bars indicate 5 μm. Imaging experiments were performed at Excitation wavelength at 405 nm for Hoechst 33342 and compound 8.

Toxicity Assessment in 3T3-L1 pre-adipocytes and L. donovani promastigotes.

3T3-L1 cells were seeded a day before experiment in confocal coverglass bottom dishes (105 cells/dish). Afterwards, cells were treated with compound 8 at concentrations of 1 μM, 500 nM, 250 nM and 100 nM for 24 hrs before staining with SYTOX® Green (500 nM) for 15 min. Samples were analyzed under the confocal microscope. Cells that had taken up the stain were counted in a random population of >500 cells and were considered dead. Viability from these concentrations of the fluorophore was compared with vehicular control (DMSO).

Figure 26 represents cell viability assessment of compound 8 in 3T3-L1 pre-adipocytes.

L. donovani promastigotes were incubated with the compound 8 at different working concentrations (50 nM, 100 nM, 500 nM and 1 μM) for the required incubation period of 3 hrs.

Toxicity was determined by the resazurin assay (PrestoBlue® Cell Viability Reagent), as described previously (Fernandez-Checa, J. C.; Biochem. Biophys. Res. Commun. 2003, 304, 471.). Resazurin is a blue non-fluorescent dye that is reduced to highly fluorescent resorufin.
upon activity of mitochondrial enzymes in healthy cells. After incubation with the dye a 10% solution of PrestoBlue was added to the cells (10^6 cells/ml) and allowed to incubate further for 2 hrs away from light. Fluorescence was measured with a TECAN M1000 monochromator based microplate reader. Figure 27 represents cell viability assessment of compound 8 in L. donovani promastigotes.

Results:

Staining pattern with compound 8 in 3T3-L1 pre-adipocytes and Leishmania promastigotes strongly suggested localization in lipid droplets similar to Nile Red staining (known to target lipid droplets). To confirm this observation, dual staining experiments were also performed which clearly showed co-localization of the Nile Red and compound 8 within LDs. No labeling was found in nucleus and other organelles. In summary, a new class of live cell permeant, solvatochromic bioprobes compound 8 was identified, which exhibited strong and selective staining of intracellular lipid droplets in in vitro live 3T3-L1 pre-adipocytes, and in parasitic protozoa Leishmania donovani promastigotes. The live cell imaging studies clearly demonstrated that dye 8 is a specific and non-toxic lipid molecular bioprobe for quantification of lipid droplets with high brightness at a low concentration of 100 nM without background noise and is better than Nile red. Cell viability experiments revealed that Compound 8 showed no cytotoxicity in 3T3-L1 preadipocytes up to a concentration of 1DM, where 93.5% cells were viable and in Leishmaniadonovani 99% of the cells were viable. Dye 8 exhibited clearly distinct PL from the commercially available BODIPY and Nile Red dyes and it may be used as a fluorescent probe alone or in combination with other fluorophores for multi-color imaging applications.
We claim:

1. Donor-acceptor fluoranthenes having general formula I, and derivatives thereof;

$$\text{I}$$

$$\begin{array}{c}
\text{X} \\
\text{R}^1 \\
\text{R}^2 \\
\text{R}^3 \\
\text{R}^4 \\
\text{R}^5 \\
\text{R}^6 \\
\text{CN} \\
\text{R}^7 \\
\text{R}^8 \\
\end{array}$$

wherein $R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8$ are independently selected from the groups comprising hydrogen, unsubstituted or substituted alkyl, unsubstituted or substituted alkenyl, unsubstituted or substituted alkoxy, unsubstituted or substituted aryl unsubstituted or substituted cycloaryl, unsubstituted or substituted cycloalkyl, unsubstituted or substituted heteroaryl, unsubstituted or substituted alkylthio, unsubstituted or substituted amino, unsubstituted or substituted acylamino, unsubstituted or substituted arylamino, unsubstituted or substituted acyl, unsubstituted or substituted acyloxy, unsubstituted or substituted thioamido, halogens, nitriles, esters, hydroxy, mercapto, carbontrifluoride, nitro;

wherein the word "substituted" means substitution with a group selected from halogen(s), nitrile(s), ester(s), hydroxy(s), alkoxy(s), mercapto(s), carbontrifluoride(s), nitro(s), amino group(s) or $\text{Cl}^-$ to $\text{C}_{20}$-units;

wherein $X$ is a donor group comprising unsubstituted or substituted amine group, unsubstituted or substituted alkoxy group, unsubstituted or substituted alkylthio group;

wherein $\text{CN}$ is a nitrile, an acceptor group.

2. The compound as claimed in claim 1, wherein the unsubstituted or substituted amine group is selected from amino, pyrrolidine, piperidine, methyl amine, ethyl amine, propyl amine, dimethylamine, $N$-alkylalkanolamine, $N,N$-dialkanolamine and pegylatedamine.

3. The compound as claimed in claim 1, wherein the representative compounds include;
i. 8-(dimethylamino)-10-isopropylfluoranthene-7-carbonitrile;
ii. 10-Phenyl-8-(pyrrolidin-1-yl)fluoranthene-7-carbonitrile;
iii. 10-(4-Methoxyphenyl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile;
iv. 8-(piperidin-1-yl)-10-(thiophen-2-yl)fluoranthene-7-carbonitrile;
v. 10-(benzo[d][1,3]dioxol-5-yl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile;
vi. 8-(piperidin-1-yl)-10-(3,4,5-trimethoxyphenyl)fluoranthene-7-carbonitrile;
vii. 10-(4-nitrophenyl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile;
viii. 10-(4-hydroxyphenyl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile;
ix. 10-(4-hydroxyphenyl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile;
x. 8-(piperidin-1-yl)-10-(3,4,5-trihydroxyphenyl)fluoranthene-7-carbonitrile;
x. 10-(4-nitrophenyl)-8-(piperidin-1-yl)fluoranthene-7-carbonitrile;
xii. 8-(bis(2-hydroxyethyl)amino)-10-phenylfluoranthene-7-carbonitrile;
xiii. 8-((2-hydroxyethyl)(methyl)amino)-10-(4-methoxyphenyl)fluoranthene-7-carbonitrile;
xiv. 8-((2-hydroxyethyl)(methyl)amino)-10-(4-methoxyphenyl)fluoranthene-7-carbonitrile;
xv. 8-((2-bromoethyl)(methyl)amino)-10-(4-methoxyphenyl)fluoranthene-7-carbonitrile.

4. A process for preparing donor-acceptor fluoranthene compounds of general formula I comprising the steps of:

(i) reacting a compound having general formula S-1 with a compound having general formula S-2 in an organic solvent in the presence of a base at a temperature ranging between -78°C to 100°C for a period ranging between 1 minute to 24 hr to obtain a reaction mixture wherein,
R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈ are independently selected from the groups comprising hydrogen, unsubstituted or substituted alkyl, unsubstituted or substituted alkenyl, unsubstituted or substituted alkoxy, unsubstituted or substituted aryl, unsubstituted or substituted cycloaryl, unsubstituted or substituted cycloalkyl, unsubstituted or substituted heteroaryl, unsubstituted or substituted alkylthio, unsubstituted or substituted -amino, unsubstituted or substituted acylamino, unsubstituted or substituted arylamino, unsubstituted or substituted acylthio, unsubstituted or substituted acyl, unsubstituted or substituted aryl, unsubstituted or substituted acyloxy, unsubstituted or substituted thioamido, halogens, nitriles, esters, hydroxy, mercapto, carbontiifluoride, nitro;

wherein the word "substituted" means substitution with a group selected from halogen(s), nitrile(s), ester(s), hydroxy(s), alkoxy(s), mercapto(s), carbontrifluoride(s), nitro(s), amino group(s) or C₁- to C₂₀-units;

wherein X is a donor group comprising unsubstituted or substituted amine, unsubstituted or substituted alkoxy, unsubstituted or Substituted alkylthio group;

(ii) isolating and purifying the compound of general formula I from the reaction mixture.

5. The process as claimed in claim 4, wherein the organic solvent is selected from the group comprising DMF, THF, DMSO, DCM and isopropanol.

6. The process as claimed in claim 4, wherein the base is selected from the group comprising KOH, NaOH, NaH, KH, K₂C₀₃ and Cs₂C₀₃.

7. Use of donor-acceptor fluoranthenes having the general formula I alone or in combination with other fluorescent compounds and derivatives thereof for fluorescence-based cell imaging:
wherein \( R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 \) are independently selected from the groups comprising hydrogen, unsubstituted or substituted alkyl, unsubstituted or substituted alkenyl, unsubstituted or substituted alkoxy, unsubstituted or substituted aryl unsubstituted or substituted cycloaryl, unsubstituted or substituted cycloalkyl, unsubstituted or substituted heteroaryl, unsubstituted or substituted alkylthio, unsubstituted or substituted amino, unsubstituted or substituted acylamino, unsubstituted or substituted arylamino, unsubstituted or substituted acyl, unsubstituted or substituted acyloxy, unsubstituted or substituted thioamido, halogens, nitriles, esters, hydroxy, mercapto, carbontrifluoride, nitro; wherein the word "substituted" means substitution with a group selected from halogen(s), nitrile(s), ester(s), hydroxy(s), alkoxy(s), mercapto(s), carbontrifluoride(s), nitro(s), amino group(s) or C\(_{1-20}\)-units;

wherein \( X \) is a donor group comprising unsubstituted or substituted amine, unsubstituted or substituted alkoxy, unsubstituted or substituted alkylthio group;

wherein CN is an acceptor group.

8. The use of a donor-acceptor fluoranthenes according to claim 7, wherein said fluorescence-based cell imaging or analysis is either a monocolour or a multi-color imaging or analysis.

9. A method for fluorescence-based imaging or analysis of cells and/or cellular components, comprising the following steps:

i) staining cellular components with a donor-acceptor fluoranthenes having the general formula I, and derivatives thereof
wherein R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈ are independently selected from the groups comprising of hydrogen, unsubstituted or substituted alkyl, unsubstituted or substituted alkenyl, unsubstituted or substituted alkoxyl, unsubstituted or substituted aryl unsubstituted or substituted cycloaryl, unsubstituted or substituted cycloalkyl, unsubstituted or substituted heteroaryl, unsubstituted or substituted amino, unsubstituted or substituted acylamino, unsubstituted or substituted arylamino, unsubstituted or substituted aroyl, unsubstituted or substituted acyloxy, unsubstituted or substituted thioamido, halogens, nitriles, esters, hydroxy, mercapto, carbontrifluoride, nitro;

wherein the word "substituted" means substitution of the moiety with a group selected from one or more halogen(s), nitrile(s), ester(s), hydroxy(s), alkoxy(s), mercapto(s), carbontrifluoride(s), nitro(s), amino group(s) or C₁ to C₂₀-units;

wherein X is a donor group comprising of unsubstituted or substituted amino, unsubstituted or substituted alkoxy, unsubstituted or substituted alkylthio group;

wherein CN is an acceptor group;

ii) exciting the fluoranthene dye of step i) with laser light in the wavelength range from 405 nm to 665 nm;

iii) detecting the light emitted in step (ii);

iv) optionally, generating images with the emission data obtained in step iii);

v) optionally performing an analysis with the data obtained in step iii) or the images obtained in step iv).
10. The method according to claim 9, wherein the cellular components stained with the fluoranthene dye of formula I in step i) are lipid droplets.

11. The method according to any one of claims 9-10, wherein 1, 2 or 3 other excitable fluorescent dye(s) are used, resulting in 2-color, 3-color or 4-color imaging.

12. The method according to any one of claims 9-11, wherein living or fixed cells are subjected to said fluorescence-based imaging or analysis of cells and/or cellular components.

13. The method according to any one of claims 9-12, wherein a confocal laser scanning microscope, a spinning disc microscope, a two-photon microscope, a conventional epifluorescence microscope, a structured illumination microscope, or a single plane illumination microscope is used.
Figure 4

Figure 5

Figure 6
Figure 7

Figure 8

Figure 9
Figure 13

Figure 14

Figure 15
Figure 16

Figure 17

Figure 18
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

| INV. | C07C255/58 C07C255/59 GO1N1/30 G01N33/92 | C07D405/10 C07D409/10 C07D295/155 |

**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)

C07C  C07D  G01N

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

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

EPO-Internal , CHEM ABS Data, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<td>ATUL GOEL ET AL: &quot;Synthesis, Electrochemical and Optical Properties of Stably Yel low Fluorescent Fluoranthenes&quot;, THE JOURNAL OF ORGANIC CHEMISTRY, vol. 75, no. 11, 6 May 2010 (2010-05-06), pages 3656-3662, XP055130639, ISSN: 0022-3263, DOI: 10.1021/jo00420x</td>
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Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:
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  - "E" earlier application or patent but published on or after the international filing date
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**Date of the actual completion of the international search**

22 July 2014

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

28/07/2014

**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**

Mooren, Ni colai

Form PCT/ISA/210 (second sheet) (April 2000)
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