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(54) **AMPHIPHILIC SQUARAINE DYES, PROCESS FOR PREPARATION THEREOF AND USE THEREOF**

(75) Inventors: **Ramaiah Danaboyina**, Kerala (IN); **Thazhathveetil Arun Kalliat**, Kerala (IN); **Kuthanapillil Jyothish**, Kerala (IN)

Correspondence Address:
FULBRIGHT & JAWORSKI L.L.P.
600 CONGRESS AVE., SUITE 2400
AUSTIN, TX 78701 (US)

(73) Assignee: **COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH**, New DELHI (IN)

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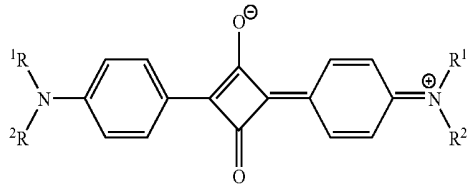
(57) **ABSTRACT**

The present invention relates to amphiphilic squaraine dyes of the general formula (1) as shown below Formula (1) wherein, R^1=—$(CH_2$—CH_2—$O)_n$—CH_3, n=4-8, or —$(CH_2)_n$—CO_2X, n=3-6, X=H, succinamide and R^2=—CH_3 or —$(CH_2$—CH_2—$O)_n$—CH_3, n=4-8 and pharmaceutically acceptable derivatives thereof, for use as near infrared fluorescence probes in photodynamic diagnostic and biological, biochemical and industrial applications.

(1)



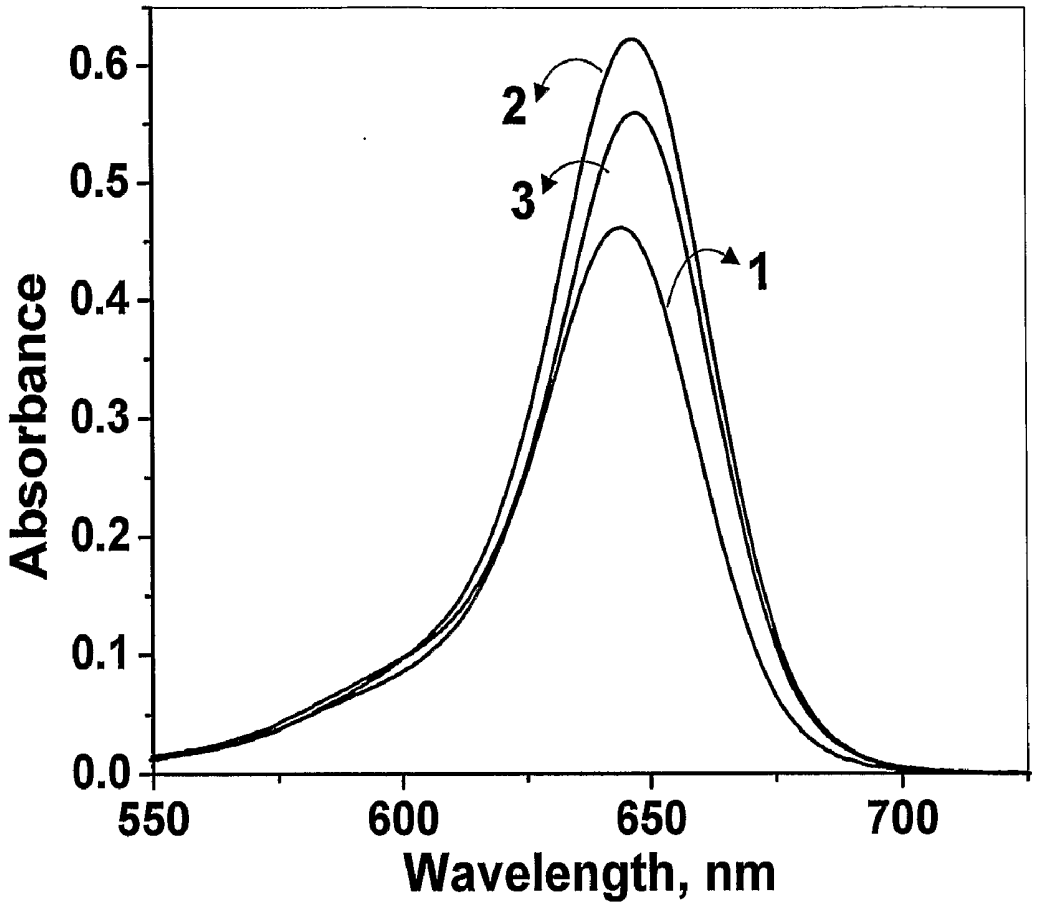

Absorbance
0.0
0.1
0.2
0.3
0.4
0.5
0.6
550
600
650
700
Wavelength, nm
1
2
3

FIG. 1

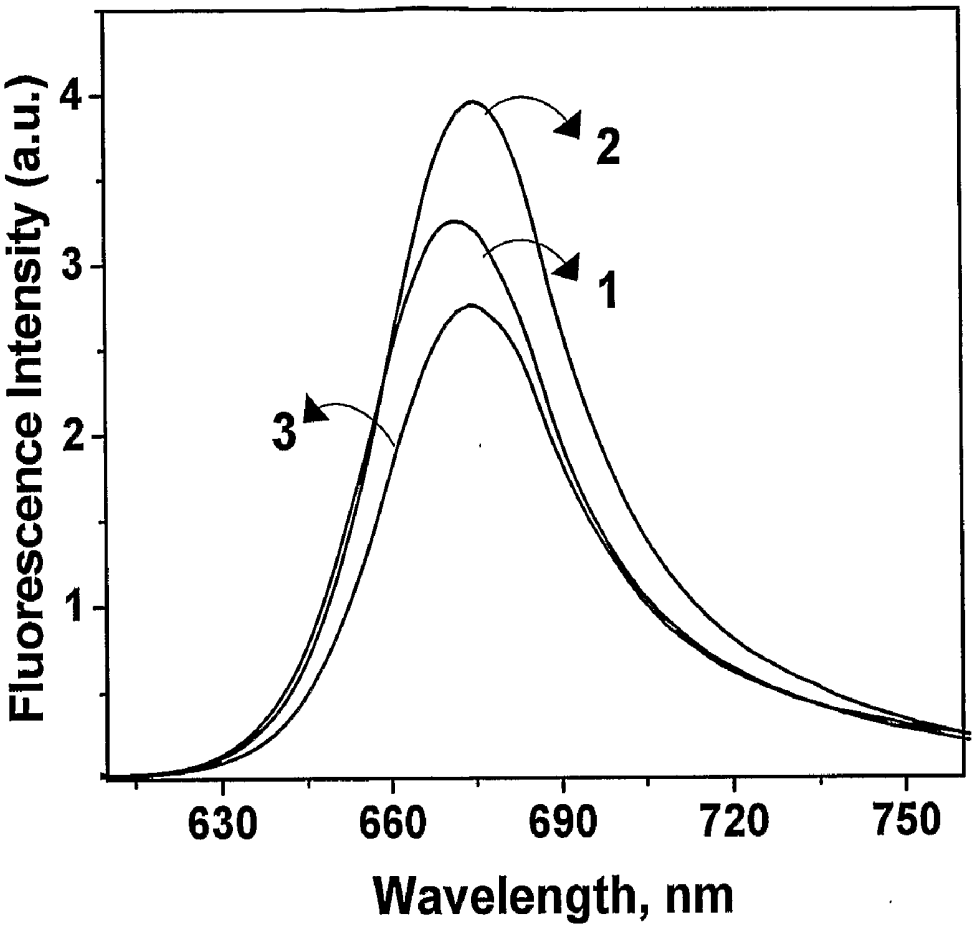

Fluorescence Intensity (a.u.)
1
2
3
4
630
660
690
720
750
Wavelength, nm
1
2
3

FIG. 2

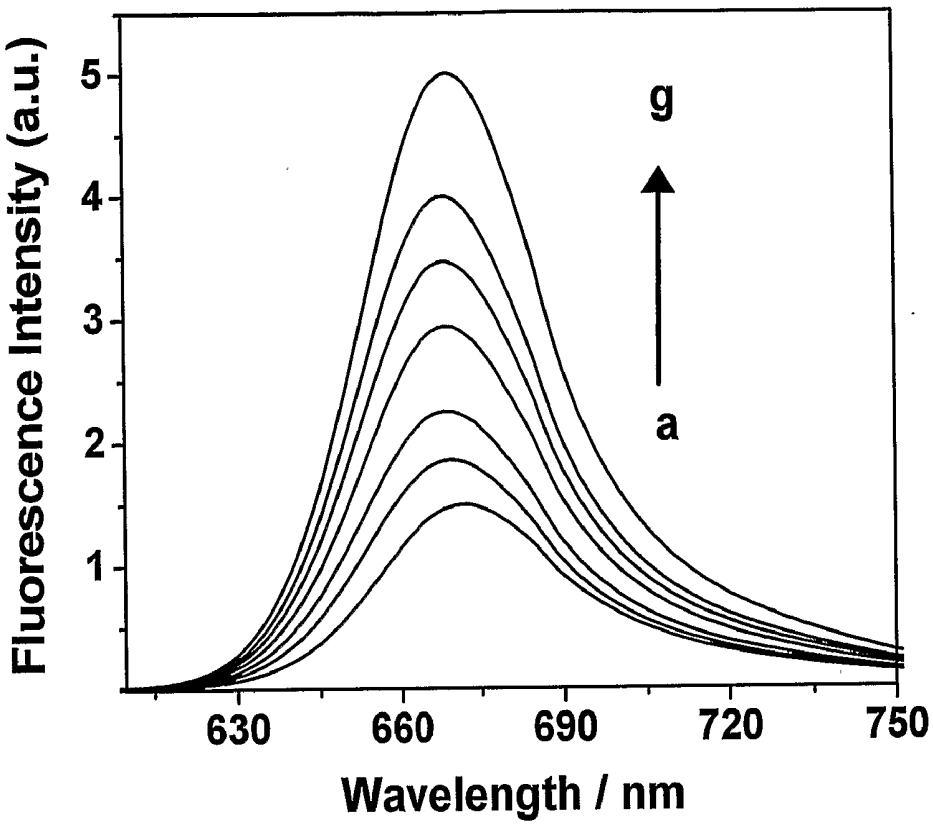

Fluorescence Intensity (a.u.)
1
2
3
4
5
630
660
690
720
750
Wavelength / nm
g
a

FIG. 3

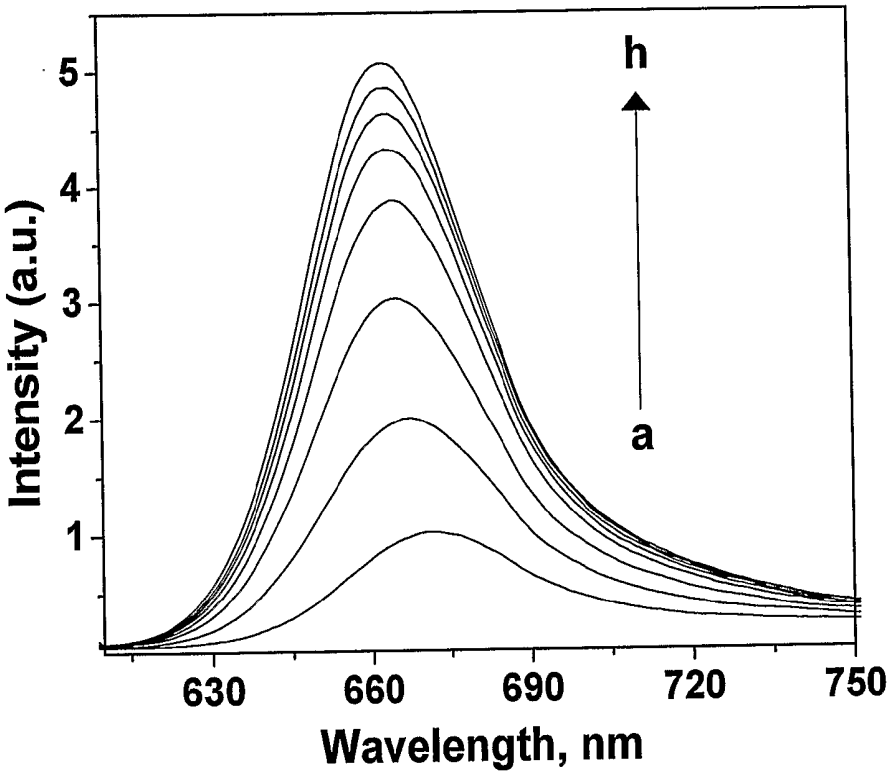


FIG. 4

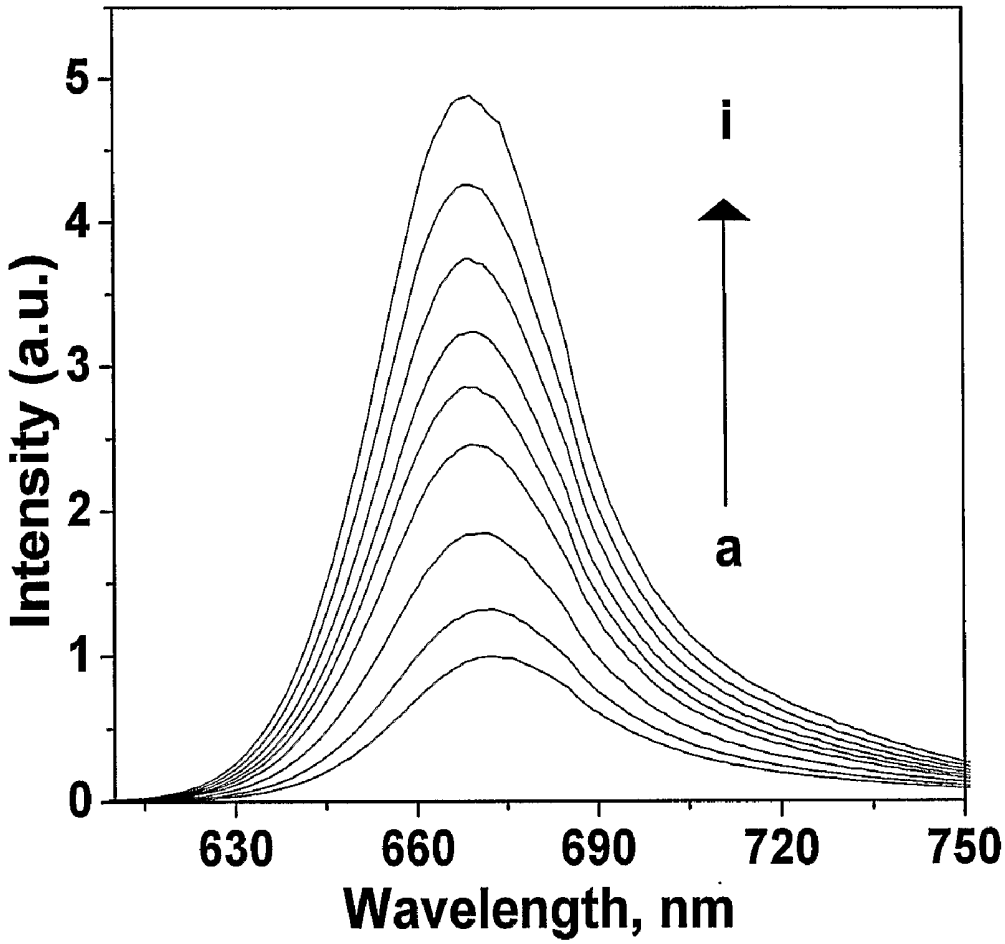


FIG. 5

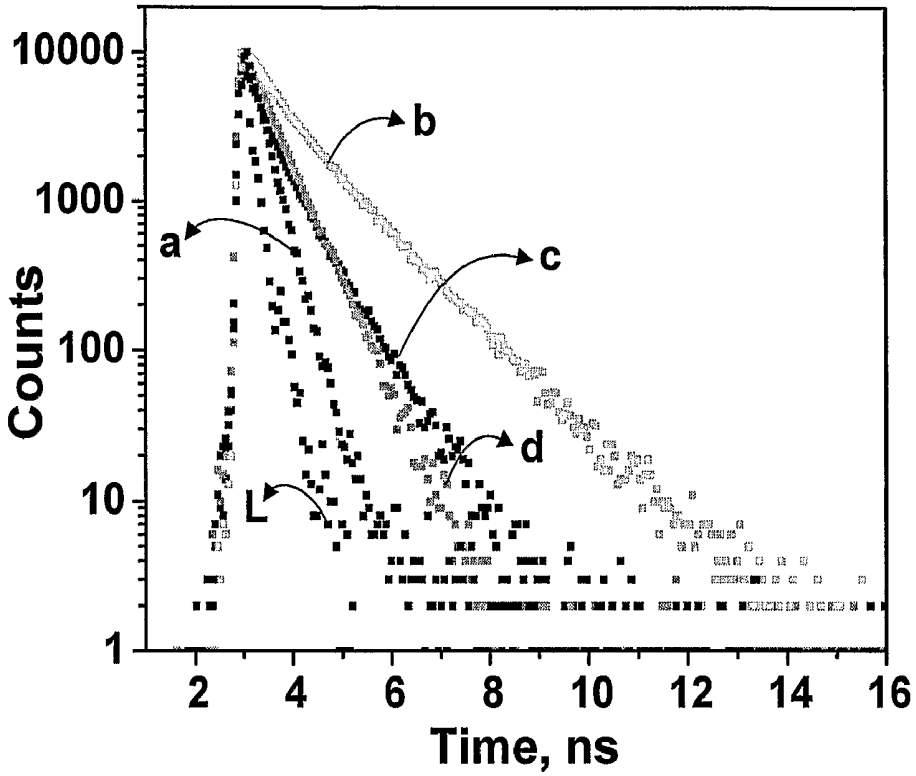


FIG. 6

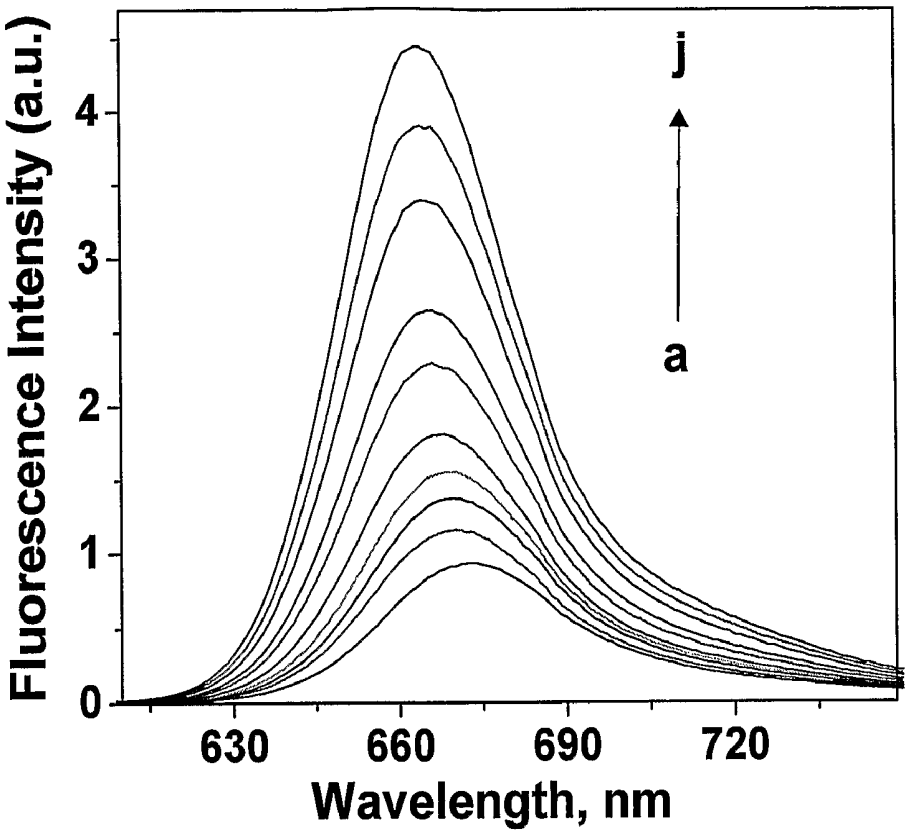

Fluorescence Intensity (a.u.)
0
1
2
3
4
630
660
690
720
Wavelength, nm
j
a

FIG. 7

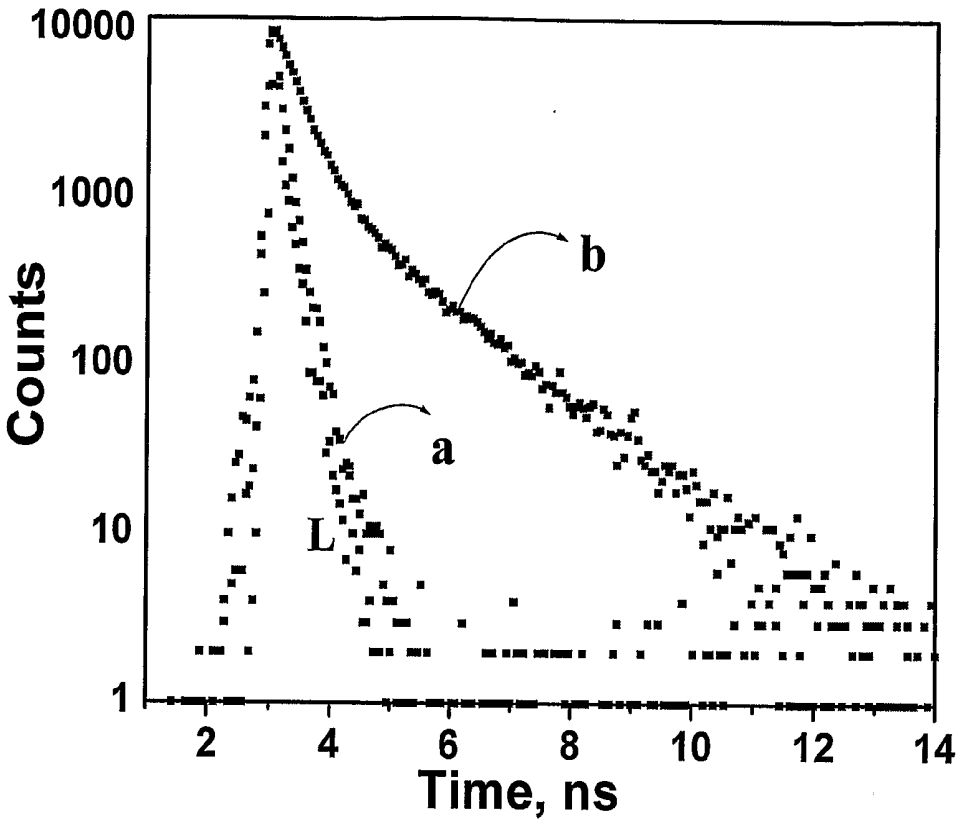


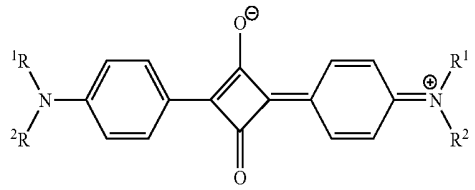
FIG. 8

AMPHIPHILIC SQUARAINE DYES, PROCESS FOR PREPARATION THEREOF AND USE THEREOF

FIELD OF INVENTION

[0001] The present invention relates to amphiphilic squaraine dyes of the general formulae 1 as shown below

Formula 1



[0002] wherein, R^1=—$(CH_2$—CH_2—$O)_n$—CH_3, n=4-8, or —$(CH_2)_n$—CO_2X, n=3-6, X=H, succinamide and R^2=—CH_3 or —$(CH_2$—CH_2—$O)_n$—CH_3, n=4-8 and pharmaceutically acceptable derivatives thereof, for use as near infrared fluorescence probes in photodynamic diagnostic and biological, biochemical and industrial applications.

[0003] The present invention also relates to a process for the preparation of squaraine dyes of the general formula 1 and use of such sensitizers as near infrared fluorescence probes in photodynamic, diagnostic and biological, biochemical and industrial applications.

[0004] The present invention also relates to squaraine dyes of the general formula 1 or pharmaceutically acceptable derivatives thereof, for use as near infrared fluorescence probes in photodynamic applications for the detection of cancer and other diseases in human beings or animals.

[0005] The present invention also relates to squaraine dyes of general formula 1 or pharmaceutically acceptable derivatives thereof for use as near-infrared fluorescence probes for biological applications. The present investigation also relates to squaraine dyes of the general formula 1 that can be used as near infrared fluorescent probes for protein labeling. The present investigation also relates to squaraine dyes of the general formula 1 that can be used as near infrared fluorescent labels in immunoassays. The present invention also relates to a process for the preparation of squaraine dyes of the general formula 1 and/or their derivatives for photodynamic industrial applications such as sterilization of fluids and water and related other applications.

BACKGROUND OF THE INVENTION

[0006] Photodynamic therapy (PDT) is an emerging modality for the diagnosis and treatment of cancer and various diseases, which involves the combined action of light and a photosensitizer. References may be made to Lane, N. *Scientific American* 2003, 38-45; Bonnett, R. *Chem. Soc. Rev.* 1995, 24, 19; Dougherty, T. J. *Photochem. Photobiol.* 1987, 45, 879; Kessel, D.; Dougherty, T. J. Phorphyrin Photosensitization; Plenum Publishing Corp. New York, 1983. The process requires the presence of a photosensitizing agent, which is capable of being taken up by target tissues and which, on irradiation by light of a particular wavelength, generates species which are toxic to those tissues. Photodynamic therapy has advantages over many other conventional therapies due to the selectivity of the photodynamic process. There is more sensitizer in the tumor tissues than in the normal tissues; this reduces the potential for destruction of normal tissues. In addition the ability to direct light specifically onto the target cells and tissues by the use of fiberoptic technology further increased the selectivity of this process. Also, use of photosensitizing agents, which produce no response until irradiated with light, significantly reduces the potential for side effects.

[0007] In PDT, the detection of tumor tissue (diagnosis) is equally important when compared to the necrosis of tumor cells (treatment). Near-infrared (NIR) dyes are presently attracting considerable interest as fluorescence probes for the detection of cancer. References may be made to Lin, Y.; Weissleder, R.; and Tung, C. H. *Bioconjugate Chem.* 2002 13, 605-610; Achilefu, S.; Jimenez, H. N.; Dorshow, R. B.; Bugaj, J. E.; Webb, E. G.; Wilhelm, R. R.; Rajagopalan, R.; Johler, J.; Erion, J. L. *J. Med. Chem.* 2002 45, 2003-2015; Mujumdar, S. R.; Mujumdar, R. B.; Grant, C. M.; Waggoner, A. S. *Bioconjugate Chem.* 1996, 7, 356-362. Since tissue is relatively transparent to NIR light, NIR fluorescence imaging (NIRF) and PDT are capable of detecting and treating, respectively, even subsurface tumors. In this context the present invention aims at the development of efficient near infrared absorbing fluorescent probes based on squaraine dyes for biological applications. We have synthesized dyes based on squaraine moiety which exhibit absorption and emission in the near infrared region and have substituents like carboxyl and glycolic groups, which would render them amphiphilicity thereby increasing their solubility, fluorescence intensity and accelerating their cellular uptake.

[0008] In a diagnostic technique, a dye is administered and allowed to distribute in the body as in the case of the treatment technique. However, in addition to the tumor selectivity, the sensitizer in the diagnostic technique should exhibit significant fluorescence yields under physiological conditions. Hence the development of photosensitizers, which have strong absorption in the long wavelength region, non-toxic to normal tissues, soluble in buffer at physiological pH, and exhibit higher therapeutic efficacy are still desired. Also the design of functional molecules that can target specific cancer cells are extremely important because of the biochemical and biomedical applications.

[0009] Our interest in this area originated from the idea of utilizing the squaraine dyes for photodynamic applications. Squaraines form a class of dyes possessing sharp and intense absorption bands in the red to near infra red region. The photophysical and photochemical properties of these have been studied extensively, because their absorption and photochemical characteristics make them highly suitable for a number of industrial applications. References may be made to U.S. Pat. Nos. 6,001,523; 5,552,253; 5,444,463; Law, K.-Y. *Chem. Rev.* 1993, 93, 449; Piechowski, A P; Bird, G. R.; Morel, D L.; Stogryn, E. L. *J. Phy. Chem.* 1984, 88, 934. Preliminary investigations by us indicated that substitution of the squaraine dyes with heavy atoms like bromine and iodine results in their increased solubility in the aqueous medium and enhanced intersystem crossing efficiency, when compared to the parent squaraine dye. These dyes exhibited absorption in the range from 600-620 nm and showed quantum yields of triplet excited states (Φ_T=0.22-0.5) and singlet oxygen ($\Phi(^1O_2)$=0.13-0.47), depending on the nature of the halogen atoms. The cytotoxicity and mutagenicity studies using mammalian cell lines and bacterial strains indicated that these dyes exhibit significant cytotoxicity upon excitation with visible light and the mechanism of their biological

activity could be attributed to the in vitro generation of singlet oxygen. References may be made to Ramaiah, D.; Arun, K. T.; Das, S, and Epe, B. U.S. Pat. No. 6,770,787B2 (2004), Ramaiah, D.; Arun, K. T.; Das, S, and Epe, B. Indian patent No. 193540 (2004). Ramaiah, D.; Joy, A.; Chandrasekhar, N; Eldho, N. V.; Das, S.; George, M. V. *Photochem. Photobiol.* 1997, 65, 783; Arun, K. T.; Ramaiah, D.; Epe, B. *J. Phys. Chem. B* 2002, 107, 11622, Ramaiah, D.; Eckert, I; Arun, K. T.; Weidenfeller, L.; Epe, B. *Photochem. Photobiol.* 2002, 76, 672; Ramaiah, D.; Eckert, I; Arun, K. T.; Weidenfeller, L.; Epe, B. *Photochem. Photobiol.* 2004, 79, 99. However, these heavy atom substituted dyes possesses very low fluorescence quantum yields ($\Phi_F \leqq 0.0003$) in aqueous medium, thereby limiting their use for the detection of tumors (diagnosis) by the fluorescence emission of the dyes that can localize selectively in tumor tissues.

[0010] In the present invention novel amphiphilic dyes based on squaraine moiety have been synthesized and their potential as near infrared fluorescent probes for biological, biochemical and industrial applications has been demonstrated.

OBJECTIVES OF THE INVENTION

[0011] The main objective of the present invention is to provide efficient squaraine based dyes and/or pharmaceutical acceptable derivatives thereof, for use as near infrared sensitizers in photodynamic diagnostic, biochemical and industrial applications.

[0012] Another objective of the present invention is to provide efficient squaraine based dyes and/or pharmaceutical acceptable derivatives thereof, for use as near infrared fluorescence probes in photodynamic diagnostic applications for the detection of tumors.

[0013] Another objective of the present invention is to provide efficient squaraine based dyes and/or pharmaceutical acceptable derivatives thereof, for use as near-infrared fluorescence sensors for biological, biochemical and industrial applications.

[0014] Yet another objective of the present investigation is to provide squaraine-based dyes that can be used as near infrared fluorescent probes for protein labeling.

[0015] Yet another objective of the present investigation is to provide squaraine dyes of the general formula 1 that can be used as near infrared fluorescent labels in immunoassays.

BRIEF DESCRIPTION OF THE ACCOMPANYING DRAWINGS

[0016] In the drawings accompanying the specifications

[0017] FIG. **1** Absorption spectra of squaraine dyes of the general formula 1 wherein, R^1=—$(CH_2—CH_2—O)_n$—CH_3, n=4 and R^2=—CH_3, general formula 2 wherein R^1, R^2=—$(CH_2—CH_2—O)_n$—CH_3, n=4 and general formula 3, wherein R^1=—$(CH_2)_n$—CO_2X, n=3, X=H, and R^2=—CH_3, in 10% vol/vol ethanol water mixtures.

[0018] FIG. **2** Fluorescence emission spectra of squaraine dyes of general formula 1, wherein, R^1=—$(CH_2—CH_2—O)_n$—CH_3, n=4 and R^2=—CH_3, general formula 2 wherein R^1, R^2=—$(CH_2—CH_2—O)_n$—CH_3, n=4 and general formula 3, wherein R^1=—$(CH_2)_n$—CO_2X, n=3, X=H, and R^2=—CH_3, in 10% vol/vol ethanol water mixtures.

[0019] FIG. **3** Emission spectra of squaraine dyes of general formula 1, wherein R^1=—$(CH_2—CH_2—O)_n$—CH_3, n=4 and R^2=—CH_3, in the presence of varying concentration of [CTAB] a) 0 and g) 129 mM. Excitation wavelength, 600 nm.

[0020] FIG. **4** Emission spectra of squaraine dyes of general formula 1, wherein R^1=—$(CH_2—CH_2—O)_n$—CH_3, n=4 and R^2=—CH_3, in the presence of varying concentration of [SDS] a) 0 and e) 21 mM. Excitation wavelength, 600 nm.

[0021] FIG. **5** Emission spectra of squaraine dyes of general formula 1, wherein R^1=—$(CH_2—CH_2—O)_n$—CH_3, n=4 and R^2=—CH_3, in the presence of varying concentration of [Triton X-100] a) 0 and g) 118 mM. Excitation wavelength, 600 nm.

[0022] FIG. **6** Fluorescence decay profile of squaraine dyes of general formula 1 wherein R^1=—$(CH_2—CH_2—O)_n$—CH_3, n=4 and R^2=—CH_3, in 10% (vol/vol) ethanol-water mixtures. (a) 10% (vol/vol) ethanol-water, (b) triton X-100, (c) CTAB, (d) SDS and (L) lamp profile. Excitation wavelength, 635 nm. Emission wavelength, 670 nm.

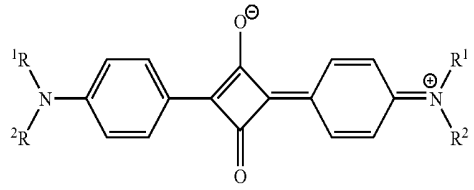
[0023] FIG. **7** Emission spectra of squaraine dyes of general formula 1, wherein R^1=—$(CH_2—CH_2—O)_n$—CH_3, n=4 and R^2=—CH_3, in the presence of varying concentration of [β-CD] a) 0 and g) 25 mM. Excitation wavelength, 600 nm.

[0024] FIG. **8** Fluorescence decay profile of squaraine dyes of general formula 1, wherein R^1=—$(CH_2—CH_2—O)_n$—CH_3, n=4 and R^2=—CH_3, in 10% (vol/vol) ethanol-water mixtures. [β-CD] (a) 0 (b) 12 mM and (L) lamp profile.

SUMMARY OF THE INVENTION

[0025] Accordingly, the present invention relates to squaraine dyes of the general formula 1 and pharmaceutically acceptable derivatives thereof.

Formula 1



[0026] wherein, R^1=—$(CH_2—CH_2—O)_n$—CH_3, n=4-8, or —$(CH_2)_n$—CO_2X, n=3-6, X=H, succinamide and R^2=—CH_3 or —$(CH_2—CH_2—O)_n$—CH_3, n=4-8.

[0027] In one embodiment of the invention, the N-methyl-N-substituted or N,N-disubstituted aniline and squaric acid in the ratio 2:1 in a mixture of benzene and n-butanol (1:1) is refluxed at (90-110° C.) for a time period of 18-24 h. Removal of the solvent gave a residue, which was then subjected to column chromatography over silica gel to obtain compounds of the general formula 1.

[0028] Another embodiment of the present invention is to provide efficient squaraine based dyes and/or pharmaceutical acceptable derivatives thereof, for use as near-infrared fluorescence sensors for biological, biochemical and industrial applications.

[0029] In yet another embodiment of the invention, the compounds of the formula 1 are used in photodynamic therapy as near infrared fluorescent sensors for the diagnosis of cancer.

[0030] Yet another embodiment of the present investigation is to provide squaraine-based dyes that can be used as near infrared fluorescent probes for protein labeling.

[0031] Another embodiment of the present investigation is to provide squaraine dyes of the general formula 1 that can be used as near infrared fluorescent labels in immunoassays.

[0032] Yet another embodiment elates to the use of compounds of formula 1 as sensitizers in the sterilization of fluids, water and related other industrial applications.

DETAILED DESCRIPTION OF THE INVENTION

[0033] In the present investigation, squaraine dyes of the general formula 1 have been synthesized and their photophysical properties in the presence and absence of membrane mimics like micelles and drug carrier systems like β-cyclodextrin were investigated. In the preparation of the compounds of the general formula 1, the amino protons of the aniline moiety are replaced with methyl, glycol and aliphatic carboxylic acid moieties. Modification with glycol and carboxylic acid moieties is expected to render amphiphilicity to these dyes and hence increase the cell permeability and to bring about target specificity.

[0034] The following examples are given by way of illustration and therefore should not be construed to limit the scope of present investigation.

[0035] Examples 1-3 represent typical synthesis of compounds of the general formula 1 and examples 4 and 5 represent the photophysical properties of compounds of general formula 1 and in the absence and presence of membrane mimics like neutral, anionic and cationic micelles and drug carriers like β-cyclodextrin.

EXAMPLE 1

[0036] Preparation of the squaraine dye of general formula 1, wherein R^1=—$(CH_2$—CH_2—$O)_n$—CH_3, n=4 and R^2=—CH_3. A solution of N-methyl-N-(3,6,9,12-tetraoxamideca) aniline (400 mg, 1.35 mmol) and squaric acid (77 mg, 0.67 mmol) in a mixture of n-butanol and benzene (1:3) was refluxed by azeotropic distillation of water for 18 h. The solvent was distilled off under reduced pressure and the residue obtained was chromatographed over silica gel. Elution of the column with a mixture of methanol and chloroform (1:49) gave 110 mg (15%) of the squaraine dye of the general formula 1, wherein, R^1=—$(CH_2$—CH_2—$O)$, —CH_3, n=4 and R^2=—CH_3, mp 100-102° C.; ^{1}H NMR (300 MHz, $CDCl_3$, 30° C., TMS): δ=3.21 (s, 6H, —NCH_3), 3.37 (s, 6H, —OCH_3), 3.72-3.52 (m, 32H, —OCH_2), 6.81 (d, 4H, J=8.96, Ar—H), 8.39 (d, 4H, J=8.95 Hz, Ar—H); ^{13}C NMR (75 MHz, $CDCl_3$, 30° C., TMS): δ=39.68, 52.29, 58.97, 68.53, 70.42, 70.52, 70.56, 70.81, 71.83, 112.43, 119.90, 133.17, 154.41, 183.32, 188.58; IR (Neat): ν_{max} 2877, 1610, 1584, 1140, 1098 cm^{-1}; elemental analysis calcd (%) for $C_{36}H_{52}N_2O_{10}$: C, 64.27; H, 7.79; N, 4.16. found: C, 64.51; H, 7.69; N, 3.88.

EXAMPLE 2

[0037] Preparation of the squaraine dye of the general formula 2, wherein R^1, R^2=—$(CH_2$—CH_2—$O)_n$—CH_3, n=4. A solution of bis-(N,N-(3,6,9,12-tetraoxamideca)aniline (350 mg, 0.74 mmol) and squaric acid (42 mg, 0.37 mmol) in a mixture of n-butanol and benzene (1:3) was refluxed by azeotropic distillation of water for 18 h. The solvent was distilled off under reduced pressure and the residue obtained was chromatographed over silica gel. Elution of the column with a mixture (1:99) of methanol and chloroform gave 40 mg (5%) of the squaraine dye of the general formula 2, wherein R^1, R^2=—$(CH_2$—CH_2—$O)_n$—CH_3, n=4, mp 78-80° C.; ^{1}H NMR (300 MHz, $CDCl_3$, 30° C., TMS): δ=3.37 (s, 12H, —OCH_3), 3.77-3.55 (m, 64H, —OCH_2), 6.84 (d, 4H, J=8.96, Ar—H), 8.37 (d, 4H, J=8.95 Hz, Ar—H); ^{13}C NMR (75 MHz, $CDCl_3$, 30° C., TMS): δ=50.73, 58.91, 68.31, 70.38, 70.49, 70.53, 71.80, 111.54, 115.85, 129.15, 147.60, 183.32, 188.58; IR (Neat): ν_{max} 2918, 2867, 1610, 1584, 1114 cm^{-1}; Elemental analysis calcd (%) for $C_{52}H_{84}N_2O_{18}$: C, 60.92; H, 8.26; N, 2.73. found: C, 61.20; H, 7.98; N, 2.49.

EXAMPLE 3

[0038] Preparation of squaraine dye of the general formula 3, wherein R^1=—$(CH_2)_n$—CO_2X, n=3, X=H, and R^2=—CH_3. N-methyl-N-(carboxypropyl) aniline (319 mg, 1.74 mmol) and squaric acid (100 mg, 0.87 mmol) were refluxed in a mixture of n-butanol and benzene (1:3) by azeotropic distillation of water for 24 h. The solvent was distilled off under reduced pressure to obtain a residue which was chromatographed over silica gel. Elution of the column with a mixture (1:9) of methanol and chloroform gave 100 mg (13%) of the squaraine dye of the general formula 3, wherein R^1=—(CH_2), —CO_2X, n=3, X=H, and R^2=—CH_3, mp 238-240° C. (d); ^{1}H NMR (300 MHz, [D_6]DMSO, 30° C., TMS): δ=1.91 (p, 4H, —CH_2), 2.40 (t, 4H, J=7.2 Hz, —CH_2), 2.91 (s, 6H, —NCH_3), 3.35 (t, 4H, J=7.3 Hz, —CH_2), 6.99 (d, 4H, J=9.07 Hz, Ar—H), 8.05 (d, 4H, J=8.97 Hz, Ar—H); ^{13}C NMR (75 MHz, [D_6]DMSO, 30° C., TMS): δ=21.82, 31.41, 38.46, 52.03, 112.64, 116.73, 129.17, 149.13, 179.23; IR (KBr): ν_{max} 3420, 2924, 1729, 1590, 1439, 1130 cm^{-1}; elemental analysis calcd (%) for $C_{26}H_{28}N_2O_6$: C, 67.23; H, 6.08; N, 6.03. found: C, 67.50; H, 5.81; N, 5.80.

EXAMPLE 4

[0039] Enormous interest has been generated in the development of water soluble near infrared probes which have absorption in the longer wavelength region where biological chromophores do not absorb. FIG. **1**. shows the absorption spectra of dyes of the general formula 1 in 10% vol/vol ethanol-water mixtures. As seen from the figures, these dyes showed sharp absorption with absorption maxima around 640-645 nm in aqueous solutions, whereas they showed a bathochromic shift in alcoholic solvents like ethanol with maxima around 640-645. FIG. **2** shows the emission spectra of these dyes in 10% vol/vol ethanol-water mixtures. These dyes exhibited fluorescence emission maxima in the region 670-676 nm in aqueous solutions while the emission maximum was found to be in the region 660-665 nm in alcoholic solvents. These dyes exhibited fluorescence quantum yield in the range 0.007 to 0.021 in aqueous media while they exhibited fluorescence quantum yield in the range 0.18 to 0.21 in ethanol. The absorption and emission maxima, and fluorescence quantum yields of these dyes in aqueous and alcoholic solvents are listed in Table 1. The long wavelength absorption and emission maxima and good fluorescence quantum yields in aqueous media make these dyes ideal candidates for application as fluorescence probes.

TABLE 1

For-	Ethanol λ_{max}, nm			10% v/v Ethanol/water λ_{max}, nm			Water λ_{max}, nm		
mula	abs	ems	Φ_f	abs	ems	Φ_f	abs	ems	Φ_f
1	637	660	0.19	645	674	0.018	644	671	0.013
2	638	662	0.21	646	675	0.026	646	676	0.021
3	636	662	0.18	647	673	0.015	645	673	0.007

EXAMPLE 5

[0040] Study of the stability and photophysical properties of a photosensitizer under physiological conditions is important during its evaluation for various in vitro and in vivo applications. Particularly, the influence of membrane mimics like cetyltrimethylammonium bromide and carrier systems like β-cyclodextrin will provide information on the sensitizer behaviour under physiological conditions. Moreover, such studies are also useful to clarify points such as whether a particular dye forms aggregates or not and whether it generates cytotoxic agents or not in such environments. These media are unique in their properties, since β-CD forms inclusion complexes with the guest molecules, while others form micellar structures thereby provide in them both the hydrophobic and hydrophilic environment.

[0041] FIGS. **3**, **4**, and **5** show the emission spectra of dye of the general formula 1, wherein, R^1=—$(CH_2$—CH_2—$O)_n$—CH_3, n=4 and R^2=—CH_3 in the presence of cationic micelle CTAB, anionic micelle SDS and neutral micelle Triton X-100, respectively. As the concentration of micelles increases, the fluorescence intensity of the dye increases showing an effective interaction between the micelles and the dye. To have a better understanding of the effect of micellar media we have analyzed the picosecond time-resolved fluorescence lifetimes of dyes of the general formula 1. These dyes showed a mono exponential decay in the absence of micellar media but biexponential decay in the presence of micelles. This biexponential decay in the presence of micelles indicates the existence of two spectroscopically distinct species, one arising from the encapsulated dye molecules and the other arising from unbound dye molecules. FIG. **6** shows the fluorescence decay profiles of squaraine dye of the general formula 1 wherein, R^1=—$(CH_2$—CH_2—$O)_n$—CH_3, n=4 and R^2=—CH_3 in 10% vol/vol ethanol-water mixtures and in the presence of cationic micelle CTAB, anionic micelle SDS and neutral micelle Triton X-100. Tables 2, 3 and 4

TABLE 2

	In the presence of CTAB			
Formula	Abs λ_{max}, nm	Ems λ_{max}, nm	Φ_f	Lifetimes, ps
1	642	663	0.12	560 (45%) 920 (55%)
2	644	667	0.096	410 (25%) 1140 (75%)
3	644	667	0.14	440 (4%) 860 (96%)

lists the absorption and emission maxima, fluorescence quantum yield and lifetimes of dyes of the general formula 1 wherein, R^1=—$(CH_2$—CH_2—$O)_n$—CH_3, n=4 and R^2=—CH_3, general formula 2, wherein R^1, R^2=—$(CH_2$—CH_2—$O)_n$—CH_3, n=4 and general formula 3, wherein R^1=—$(CH_2)_n$—CO_2X, n=3, X=H, and R^2=—CH_3, in the absence and presence of cationic micelle CTAB, anionic micelle SDS and neutral micelle Triton X-100. These results demonstrate that these dyes are ideal candidates as near infrared sensors in biological applications where they would effectively interact with cell membrane structures.

TABLE 3

	In the presence of SDS			
Formula	Abs λ_{max}, nm	Ems λ_{max}, nm	Φ_f	Lifetimes, ps
1	640	663	0.15	590
2	643	668	0.16	690 (4%) 1240 (96%)

TABLE 4

	In the presence of Triton X-100			
Formula	Abs λ_{max}, nm	Ems λ_{max}, nm	Φ_f	Lifetimes, ps
1	645	669	0.12	130 (21%) 1120 (90%)
2	646	671	0.14	450 (7%) 1450
3	649	672	0.12	430 (31%) 1140 (69%)

[0042] FIG. **7** shows the emission spectra of dye of the general formula 1, wherein, R^1=—$(CH_2$—CH_2—$O)_n$—CH_3, n=4 and R^1=—CH_3 in the presence of β-CD. Similar to what was observed with micelles, as the concentration of β-CD increases the emission intensity of the squaraine dyes of general formula 1 increases and the emission maxima shows a blue shift revealing effective interaction between the dyes and β-CD cavity. FIG. **8** shows the fluorescence decay profile of squaraine dye of general formula 1 wherein R^1=—$(CH_2$—CH_2—$O)_n$—CH_3, n=4 and R^2=—CH_3 in the absence and presence of β-CD, it showed a mono exponential decay in the absence of β-CD. Biexponential decay was observed in the presence of β-CD indicating the presence of two species, one arising from the dye molecules encapsulated inside the β-CD cavity and the other arising from free dye molecules. Table 5 summarises the absorption and emission maxima, fluorescence quantum yields and lifetimes of squaraine dyes of the general formula 1 in the presence of β-CD. These results clearly indicate that these dyes can be localized on at specific targets using drug delivery systems.

TABLE 5

	In the presence of β-CD			
Formula	Abs λ_{max}, nm	Ems λ_{max}, nm	Φ_f	Lifetime, ps
1	644	663	0.10	380 (58%) 1470 (42%)
2	645	668	0.07	610 (83%) 1920 (17%)
3	649	666	0.19	470 (34%) 1600 (66%)

[0043] The squaraine dyes of the present invention possess satisfactory properties of a near infrared fluorescent probe in photodynamic, diagnostic and biological, biochemical and industrial applications.

[0044] The main advantages of these systems include:

[0045] 1. Squaraines dyes represented by formulae 1, 2 and 3 are novel and pure single substances.

[0046] 2. Their synthetic methodology is very economical.

[0047] 3. Squaraine dyes represented by formulae 1, 2 and 3 possess absorption in the near-infrared region (600-700 nm).

[0048] 4. Squaraine dyes represented by formulae 1, 2 and 3 possess fluorescence emission in the near-infrared region (620-720 nm).

[0049] 5. Symmetrical squaraine dyes represented by formulae 1, 2 and 3 possess emission quantum yields in the range 0.015-0.03 in aqueous media and increased nearly 10-fold (Φ_F=0.09-0.2) in the presence membrane mimics and drug carriers.

[0050] 6. They can be used for photodynamic applications such as sterilization of fluids etc.

[0051] 7. The squaraine-based dyes can be used as near infrared fluorescent probes for protein labeling.

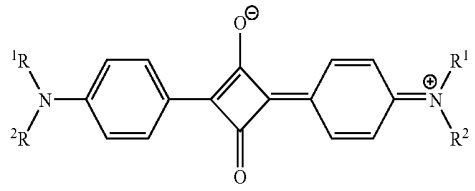
[0052] 8. The squaraine dyes of the general formula 1 can be used as near infrared fluorescent labels in immunoassays.

[0053] 9. They can be used for the detection of biologically important metal ions under physiological conditions.

[0054] 10. These novel dyes can be used as near-infrared fluorescence sensors in biological, biochemical and industrial applications.

1. A Squaraine dye composition having the general formula 1

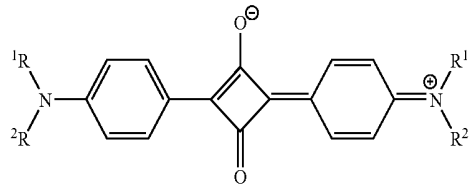
Formula 1



and/or pharmaceutically acceptable derivatives thereof, wherein, R^1=—$(CH_2$—CH_2—$O)_n$—CH_3, n=4-8, or —$(CH_2)_n$—CO_2X, n=3-6, X=H, succinamide and R^2=—CH_3 or —$(CH_2$—CH_2—$O)_n$—CH_3, n=4-8

2. A process for the preparation Squaraine dyes of the general formula 1

Formula 1



and/or pharmaceutically acceptable derivatives thereof, wherein, R^1—$(CH_2$—CH_2—$O)_n$—CH_3, n=4-8, or —$(CH_2)_n$—CO_2X, n=3-6, X=H, succinamide and R^2=—CH_3 or —$(CH_2$—CH_2—$O)_n$—CH_3, n=4-8, the process comprising the steps of

reacting N-methyl-N-substituted or N,N-disubstituted aniline with squaric acid in a mixture of benzene and n-butanol, and

evaporating the solvent, and purifying the residue to give compounds of the formula 1.

3. The process of claim **2** wherein the mixture of benzene and n-butanol is 1:1.

4. The process of claim **2** wherein the temperature of the reaction is in the range of 90-110° C.

5. The process of claim **2** wherein the reaction is carried out for a period of 18-24 hours.

6. The process of claim **2** wherein the purification is effected by column chromatography over silica gel to obtain compounds of the general formula 1.

7. A method of analyzing a material by near-infrared fluorescence comprising the steps of:

combining the material with the compound of formula 1 to form a derivative material; and,

measuring a near-infrared fluorescence signal.

8. The method of claim **7**, wherein cancer and/or other diseases are detected in human beings or animals.

9. The method of claim **7**, wherein the material is a protein.

10. The method of claim **7**, wherein the derivative material is analyzed in an immunoassay.

11. A method of sterilizing a fluid or water comprising the step of combining the fluid or water with the compound of formula 1.

* * * * *

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当前申请(专利权)人(译)	科学理事会和工业研究		
[标]发明人	DANABOYINA RAMAIAH KALLIAT THAZHATHVEETIL ARUN JYOTHISH KUTHANAPILLIL		
发明人	DANABOYINA, RAMAIAH KALLIAT, THAZHATHVEETIL ARUN JYOTHISH, KUTHANAPILLIL		
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摘要(译)

本发明涉及如下式（1）所示的通式（1）的两亲性方酸菁染料，其中，R 1 = -（CH 2 -CH 2 -O）n-CH 3，n = 4-8，或 -（CH 2）n -CO2X，n = 3-6，X = H，琥珀酰胺和R2 = -CH3或 -（CH2-CH2-O）n-CH3，n = 4-8及其药学上可接受的衍生物，用作近红外荧光探针在光动力诊断和生物，生物化学和工业应用中。