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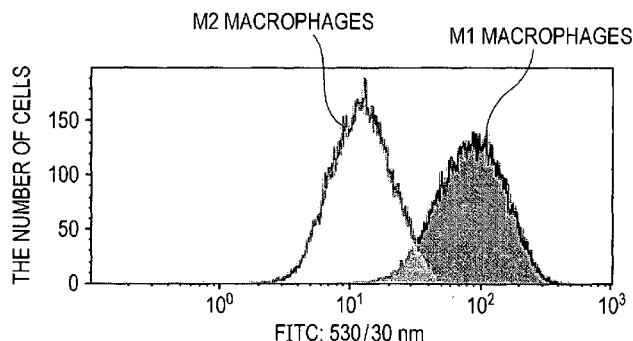(74) Agents: OKABE, Yuzuru et al.; 22F, Marunouchi Kitaguchi Bldg., 1-6-5 Marunouchi, Chiyoda-ku, Tokyo, 1000005 (JP).

[Continued on next page]

(54) Title: MACROPHAGE IDENTIFICATION AGENT, AND IDENTIFICATION METHOD, SORTING METHOD, EVALUATION METHOD, SCREENING METHOD AND KIT USING THE MACROPHAGE IDENTIFIER AGENT

FIG. 2

(57) Abstract: An object of the present invention is to provide an identification method for a macrophage subtype using an organic compound. Another object of the present invention is to provide a macrophage identification agent containing an organic compound in which a spectral characteristic obtained when the organic compound is added is different depending on a macrophage subtype. Still another object of the present invention is to provide an evaluation method for a macrophage subtype using a macrophage identification agent, an analysis method for correlation between a macrophage subtype and a test substance, a screening method for correlation between a macrophage subtype and a test substance, and a kit. An identification method for a macrophage subtype utilizing that a spectral characteristic obtained with an organic compound added is different depending on a macrophage subtype is provided.

WO 2014/192972 A1

DESCRIPTION
Title of Invention: MACROPHAGE IDENTIFICATION AGENT, AND IDENTIFICATION METHOD, SORTING METHOD, EVALUATION METHOD, SCREENING METHOD AND KIT USING THE MACROPHAGE IDENTIFIER AGENT

Technical Field

[0001] The present invention relates to a macrophage
identification agent that shows different staining
properties depending on a macrophage subtype, and an
identification method, a sorting method, an evaluation
method, a screening method and a kit for a macrophage
subtype by using the macrophage identification agent.

Background Art

[0002] As the advancement of the aging society in recent years,
the number of patients suffering from inflammatory
diseases such as cancers, lifestyle related diseases
and circulatory diseases is increasing, and as a result,
there arises a social problem of, for example, the
increase of medical expenses. In order to solve the
problem, there is a demand for a diagnostic method with
which an inflammatory disease can be detected at an
early stage or an effective treatment method with which
an inflammatory disease can be cured before becoming
severe. An inflammatory disease is considered to be
caused and become serious when inflammation becomes
chronic. There are, however, many unknown parts in the
chronic inflammation, and the mechanism has not been
clarified yet. Therefore, studies for purpose of
clarifying the mechanism of the chronic inflammation
are being earnestly prosecuted inside and outside Japan.

[0003] As one of cell types playing a significant role in the
chronic inflammation, attention is recently paid to
macrophage, that is, a type of leucocytes. It is
currently known that there are at least two subtypes of
macrophage, that is, one having a function to

accelerate inflammation (M1 macrophage) and one having
a function to inhibit inflammation (M2 macrophage).

[0004] It has been revealed that the macrophage subtypes
(hereinafter referred to merely as the subtypes; herein,
the term "subtype" refers to the macrophage subtype)
are significantly related to pathologic change in
inflammatory diseases such as cancer, type II diabetes
mellitus related to fatness, arterial sclerosis, and
nephritis. There are a large number of reports on the
relation between the subtype and the pathology. For
example, it is known that the number, the density, the
balance or the like of the subtypes reflects the
pathology. Accordingly, identification of a subtype is
expected to be applied to diagnosis and treatment.

[0005] As a method for identifying a subtype, a method in
which a fluorescent dye-labeled antibody is used for
identifying a protein marker specifically expressed on
cell surfaces of each subtype (hereinafter referred to
as the fluorescent antibody method) is known (NPL 1).

[0006] On the other hand, an exemplified method in which a
porphyrin compound is used to be specifically
incorporated into macrophages infiltrated into an
inflammatory site in a pancreatic island is disclosed
(PTL 1).

[0007] If identification and evaluation of a subtype, and
evaluation, analysis and screening of influence of a
substance on the subtype can be simply performed, the
roles of the subtype played in an inflammatory disease
will be understood in more detail. An effective
diagnostic method for an inflammatory disease can be
expected to be developed based on the identification
and evaluation of the subtype. Besides, if the subtype
is controlled in a disease site or a specific subtype
having been adjusted in vitro is administered based on
the identification, evaluation, analysis and screening
of the subtype, an early treatment method for the

inflammatory disease can be developed.

Citation List

Patent Literature

[0008] PTL 1: Japanese Patent Application Laid-Open No. 2003-12547

PTL 2: WO 2010/07432

Non Patent Literature

[0009] NPL 1: Angelo A Manfredi et al., Journal of Leukocyte Biology, pp. 779-787 (2009)

NPL 2: Yakugaku Zasshi, Vol., 69, pp. 237-239, 1949

NPL 3: Heterocyclic Communications, 15(4), 2009, pp. 245-250

NPL 4: Heteroatom Chemistry, Volume 1, Number 5, 1990, pp. 389-399

NPL 5: Angew. Chem., Int. Ed. Engl., 2005, 44, pp. 3694-3698

NPL 6: Angew. Chem., Int. Ed. Engl., 1997, 36, pp. 1333-1335

NPL 7: J. Neuroinflam., vol. 8, 2011, 58 (URL: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3123187)

NPL 8: Indian Journal of Chemistry, Vol. 6, pp. 136-139, 1968

NPL 9: J. Thorac. Oncol., Vol. 7, pp. 1091-1100, 2012

NPL 10: Mol. Cancer Res., Vol. 10, p. p.727-738, 2012

Summary of Invention

Technical Problem

[0010] The fluorescent antibody method disclosed in NPL 1 includes a large number of treatments for preventing a fluorescent antibody from non-specifically binding to a biological tissue sample, and a long time is necessary for staining. This method further has a problem of, for example, expensiveness because storage stability of the antibody is poor and a difference between lots largely affects.

[0011] On the other hand, if the porphyrin dye compound as disclosed in PTL 1 is used, there arises a problem in

which M1 macrophage and M2 macrophage cannot be
identified each other.

Solution to Problem

[0012] The present inventors made earnest studies for solving
the aforementioned problems, resulting in finding a
method for identifying the macrophage subtypes M1 and
M2 by using an organic compound.

[0013] Specifically, the present invention provides a method
for identifying macrophage subtypes M1 and M2, in which
the macrophages M1 and M2 are identified based on
spectral characteristics obtained with an organic
compound added thereto.

[0014] Besides, a macrophage identification agent of the
present invention is a macrophage identification agent
for identifying the macrophage subtypes M1 and M2, and
contains one or more organic compounds, and a spectral
characteristic obtained when added is different between
the macrophage M1 and the macrophage M2.

[0015] Moreover, the present inventors have developed, by
using the macrophage identification agent, a sorting
method for a subtype, an analysis method for
correlation between a subtype and a substance, a
screening method and a kit, and thus, the present
invention has been accomplished.

Advantageous Effects of Invention

[0016] According to the present invention, a subtype can be
identified by a simple and inexpensive method. As a
result, a subtype can be efficiently identified or
separated, the number, density, balance or the like of
a subtype can be evaluated, and a substance affecting a
subtype can be evaluated or screened.

[0017] Further features of the present invention will become
apparent from the following description of exemplary
embodiments with reference to the attached drawings.

Brief Description of Drawings

[0018] Fig. 1 illustrates a result of gene expression analysis

performed on M1 macrophage and M2 macrophage
differentiated from mouse bone marrow cells.
Fig. 2 illustrates a histogram observed in Example 1.
Fig. 3 illustrates a histogram observed in Comparative
Example 1.
Fig. 4 illustrates a histogram observed in Comparative
Example 2.
Figs. 5A, 5B, 5C, 5D and 5E illustrate histograms (Figs.
5A and 5B) and cytograms (Figs. 5C, 5D and 5E) observed
in Example 55. The cytogram illustrated in Fig.5C is
obtained from M1 macrophages stained with a compound
(1) and a compound (33). The cytogram illustrated in
Fig. 5D is obtained from M2 macrophages stained with
the compound (1) and the compound (33). The cytogram
illustrated in Fig.5E is obtained by overlapping the
cytograms illustrated in Figs. 5C and 5D.
Figs. 6A, 6B and 6C illustrate cytograms observed in
Example 56. The cytogram illustrated in Fig. 6A is
obtained from M1 macrophages stained with the compound
(1), the cytogram illustrated in Fig. 6B is obtained
from M2 macrophages stained with the compound (1), and
the cytogram illustrated in Fig.6C is obtained from a
sample of a mixture of the M1 macrophages and the M2
macrophages stained with the compound (1).
Figs. 7A and 7B illustrate histograms observed in
Example 57 and Comparative Example 3. The histogram
illustrated in Fig.7A is obtained from M1 macrophages
stained with the compound (1), and the histogram
illustrated in Fig.7B is obtained from M2 macrophages
stained with the compound (1).
Fig. 8 illustrates a result of the gene expression
analysis performed on M1 macrophage and M2 macrophage
differentiated from RAW264.7 cells.
Fig. 9 illustrates a histogram observed in Example 59.
Fig. 10 illustrates a result of the gene expression
analysis performed on M1 macrophage and M2 macrophage

differentiated from THP-1 cells.

Fig. 11 illustrates a histogram observed in Example 65.

Fig. 12 illustrates a histogram observed in Comparative
Example 6.

Figs. 13A, 13B and 13C illustrate cytograms observed in
Example 78. The cytogram illustrated in Fig.13A is
obtained from M1 macrophages stained with a compound
(86), the cytogram illustrated in Fig.13B is obtained
from M2 macrophages stained with the compound (86), and
the cytogram illustrated in Fig.13C is obtained from a
sample of a mixture of the M1 macrophages and the M2
macrophages stained with the compound (86).

Figs. 14A and 14B illustrate histograms observed in
Example 79 and Comparative Example 5. The cytogram
illustrated in Fig.14A is obtained from M1 macrophages
stained with a compound (113), and the cytogram
illustrated in Fig.14B is obtained from M2 macrophages
stained with the compound (113).

Fig. 15 illustrates a histogram observed in Example 81.

Fig. 16 illustrates a histogram observed in Comparative
Example 8.

Figs. 17A, 17B, 17C, 17D and 17E illustrate histograms
(Figs. 17A and 17B) and cytograms (Figs. 17C, 17D and
17E) observed in Example 91. The cytogram illustrated
in Fig. 17C is obtained from M1 macrophages stained
with a compound (116) and a compound (120). The
cytogram illustrated in Fig. 17D is obtained from M2
macrophages stained with the compound (116) and the
compound (120). The cytogram illustrated in Fig. 17E
is obtained by overlapping the cytograms illustrated in
Figs. 17C and 17D.

Figs. 18A, 18B and 18C illustrate cytograms observed in
Example 92. The cytogram illustrated in Fig. 18A is
obtained from M1 macrophages stained with the compound
(120), the cytogram illustrated in Fig. 18B is obtained
from M2 macrophages stained with the compound (120),

and the cytogram illustrated in Fig. 18C is obtained
from a sample of a mixture of the M1 macrophages and
the M2 macrophages stained with the compound (120).
Figs. 19A and 19B illustrate histograms observed in
Example 93 and Comparative Example 9. The histogram
illustrated in Fig. 19A is obtained from M1 macrophages
stained with the compound (120), and the histogram
illustrated in Fig. 19B is obtained from M2 macrophages
stained with the compound (120).
Fig. 20 illustrates a histogram observed in Example 95.
Fig. 21 illustrates a histogram observed in Example 96.
Fig. 22 illustrates a histogram observed in Example 97.
Fig. 23 illustrates a histogram observed in Example 98.
Figs. 24A, 24B, 24C, 24D and 24E illustrate histograms
(Figs. 24A and 24B) and cytograms (Figs. 24C, 24D and
24E) observed in Example 108. The cytogram illustrated
in Fig. 24C is obtained from M1 macrophages stained
with a compound (139) and a compound (161). The
cytogram illustrated in Fig. 24D is obtained from M2
macrophages stained with the compound (139) and the
compound (161). The cytogram illustrated in Fig. 24E
is obtained by overlapping the cytograms illustrated in
Figs.24C and 24D.
Figs. 25A, 25B and 25C illustrate cytograms observed in
Example 109. The cytogram illustrated in Fig. 25A is
obtained from M1 macrophages stained with the compound
(139), the cytogram illustrated in Fig. 25B is obtained
from M2 macrophages stained with the compound (139),
and the cytogram illustrated in Fig. 25C is obtained
from a sample of a mixture of the M1 macrophages and
the M2 macrophages stained with the compound (139).
Figs. 26A and 26B illustrate histograms observed in
Example 110 and Comparative Example 10. The histogram
illustrated in Fig. 26A is obtained from M1 macrophages
stained with the compound (139), and the histogram
illustrated in Fig. 26B is obtained from M2 macrophages

stained with the compound (139).

Fig. 27 illustrates a histogram observed in Example 112.

Fig. 28 illustrates a histogram observed in Example 113.

Fig. 29 illustrates a histogram obserbed in Example 114.

Figs. 30A, 30B, 30C, 30D and 30E illustrate histograms
(Figs. 30A and 30B) and cytograms (Figs. 30C, 30D and
30E) observed in Example 115. The cytogram illustrated
in Fig. 30C is obtained from M1 macrophages stained
with the compound (1) and the compound (113). The
cytogram illustrated in Fig. 30D is obtained from M2
macrophages stained with the compound (1) and the
compound (113). The cytogram illustrated in Fig. 30E
is obtained by overlapping the cytograms illustrated in
Figs. 30C and 30D.

Description of Embodiments

[0019] Preferred embodiments of the present invention will
now be described in detail in accordance with the
accompanying drawings.

[0020] The present invention provides a macrophage
identification agent for identifying macrophage
subtypes M1 and M2, containing one or more organic
compounds, in which a spectral characteristic obtained
when the compound is added is different between the
macrophage M1 and the macrophage M2.

[0021] Embodiments of the present invention will now be
described. It is noted that the individual embodiments
exemplarily describe a novel identification method for
macrophage subtypes, a macrophage identification agent,
a sorting method, an evaluation method, an analysis
method for correlation between a subtype and a
substance, a screening method and a kit of the present
invention, which do not limit the present invention.

[0022] Embodiment 1

A macrophage identification agent according to
Embodiment 1 of the present invention contains one or
more compounds represented by the following general
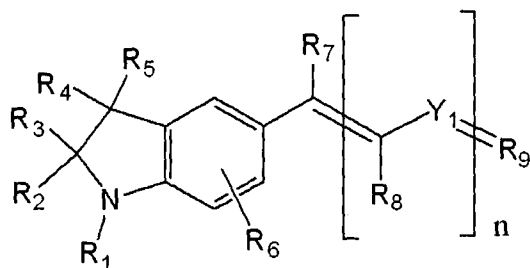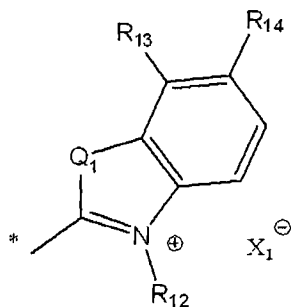
formula (1):

General formula (1)

[0023] In general formula (1), R_1 represents a hydrogen atom, an alkyl group, an aralkyl group, an alkenyl group, or an aryl group; and R_2 to R_5 each independently represent a hydrogen atom, an alkyl group, an aryl group, a carboxyl group, a carboxylalkyl group, or an alkylcarbonyl group, wherein R_2 and R_4 may be bound to each other to form an aliphatic ring. R_6 represents a hydrogen atom, an alkyl group, an alkoxy group, or a halogen atom; R_7 and R_8 each independently represent a hydrogen atom, an alkyl group, or an aryl group; R_9 represents a dicyanomethylene group, a cyanocarboxymethylene group, or any one of the following general formulas (2) to (4); Y_1 represents a carbon atom having aryl group, a cyclopentene ring, a cyclohexene ring or a carbon atom (-C=); and n represents an integer of 0 to 1.
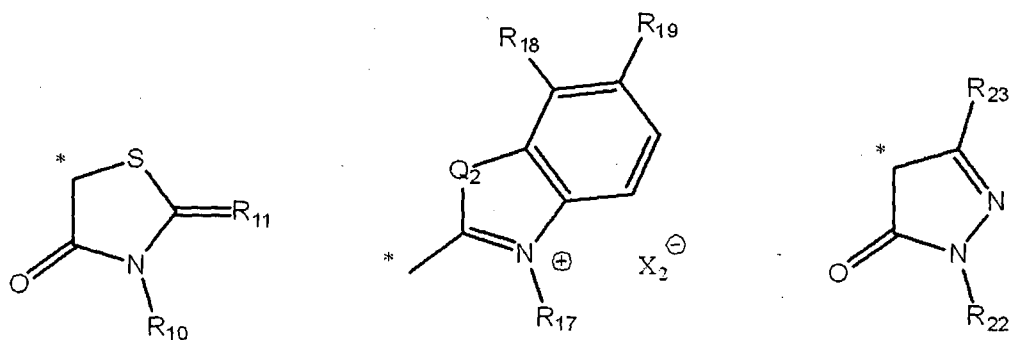
General formula (2)   General formula (3)   General formula (4)

[0024] In general formula (2), R_{10} represents an alkyl group, a carboxylalkyl group, an alkoxycarbonylalkyl group, or

an alkylcarbonyloxyalkyl group; and R_{11} represents a
sulfur atom, a 2-thioxothiazolidin-4-one group, a
thiazoline-2,4-one group, or the following general
formula (5). A sign "*" represents a binding site.

General formula (5)

[0025] In general formula (5), R_{12} represents an alkyl group,
a carboxylalkyl group, an alkoxycarbonylalkyl group, or
an alkylcarbonyloxyalkyl group; and R_{13} and R_{14} each
represent a hydrogen atom and may be bound to each
other to form a benzene ring. Q_1 represents an oxygen
atom, a sulfur atom, a N-alkyl nitrogen atom, or
$-C(R_{15})(R_{16})-$, wherein R_{15} and R_{16} each independently
represent an alkyl group and may be bound to each other
to form a ring; and X_1^- represents an anionic group. A
sign "*" represents a binding site.

[0026] In general formula (3), R_{17} represents an alkyl group,
a carboxylalkyl group, an alkoxycarbonylalkyl group, or
an alkylcarbonyloxyalkyl group; and R_{18} and R_{19} each
represent a hydrogen atom and may be bound to each
other to form a benzene ring. Q_2 represents an oxygen
atom, a sulfur atom, a N-alkyl nitrogen atom, or
$-C(R_{20})(R_{21})-$, wherein R_{20} and R_{21} each independently
represent an alkyl group and may be bound to each other
to form a ring; and X_2^- represents an anionic group. A
sign "*" represents a binding site. In general formula
(4), R_{22} represents an alkyl group or an aryl group; and
R_{23} represents an alkyl group or a carboxyl group. A
sign "*" represents a binding site.

[0027] In general formula (1), the alkyl group used as R_1 is

not especially limited, and examples include linear,
branched or cyclic alkyl groups having 1 to 20 carbon
atoms, such as a methyl group, an ethyl group, a propyl
group, a butyl group, an ethylhexyl group, and a
cyclohexyl group.

[0028] In general formula (1), the aralkyl group used as R_1 is
not especially limited, and examples include a benzyl
group and a phenethyl group.

[0029] In general formula (1), the alkenyl group used as R_1 is
not especially limited, and examples include alkenyl
groups having 2 to 20 carbon atoms, such as a vinyl
group, a 2,2-diphenylvinyl group, a 3-butenyl group,
and a cyclohexenyl group.

[0030] In general formula (1), the aryl group used as R_1 is
not especially limited, and examples include 6- to 14-
membered monocyclic or polycyclic aryl groups, such as
a phenyl group, a naphthyl group, a phenanthryl group,
and an anthracenyl group.

[0031] In general formula (1), R_1 may further have a
substituent and the substituent is not especially
limited as long as the storage stability of the dye
compound is not largely inhibited, and examples include
alkyl groups such as a methyl group, an ethyl group, a
n-propyl group, an isopropyl group, a n-butyl group, an
isobutyl group, a sec-butyl group and a tert-butyl
group; aryl groups such as a phenyl group and a
naphthyl group; alkoxy groups such as a methoxy group,
an ethoxy group and a butoxy group; aryloxy groups such
as a phenoxy group and a naphthyloxy group;
alkylsulfanyl groups such as a thiomethyl group, a
thioethyl group, a thiopropyl group, a thiobutyl group
and a thiophenyl group; di-substituted amino groups
such as a dimethylamino group, a N-ethyl-N-phenylamino
group, and a diphenylamino group; acyl groups such as
an acetyl group and a benzoyl group; a sulfonyl group;
a carboxyl group; a carboxylalkyl group; a carbamoyl

group; a sulfamoyl group; hetero ring groups such as a pyridyl group, a triazinyl group, and a benzothiazolyl group; a nitro group; and halogen atoms such as a fluorine atom, a chlorine atom, a bromine atom and an iodine atom.

[0032] In general formula (1), R_1 can be arbitrarily and independently selected from the aforementioned substituents, and in a preferred aspect, R_1 represents an alkyl group, an aryl group or the like because a subtype can be easily identified in this case. Specifically, R_1 represents preferably a methyl group, an ethyl group, a propyl group, a butyl group, a phenyl group, a bromophenyl group, a benzyl group, a bromobenzyl group, a methylthiophenyl group, a methoxyphenyl group, a methoxynaphthyl group, a benzylphenyl group, a 2,2-diphenylvinyl group or a 2,2-diphenylvinylphenyl group. More preferably, R_1 represents a phenyl group, a bromophenyl group, a benzyl group, a methylthiophenyl group, a methoxyphenyl group or a methoxynaphthyl group, and R_1 particularly preferably represents a methylthiophenyl group because a subtype can be easily identified based on a large stokes shift in this case.

[0033] In general formula (1), the alkyl group used as R_2 to R_5 is not especially limited, and examples include alkyl groups having 1 to 4 carbon atoms, such as a methyl group, an ethyl group, a propyl group and a butyl group.
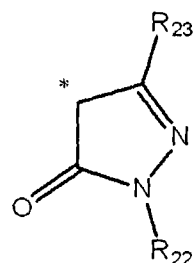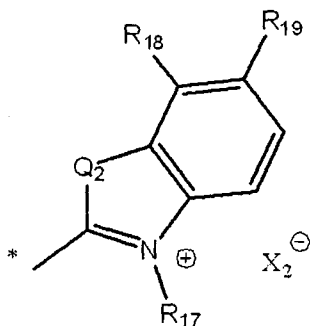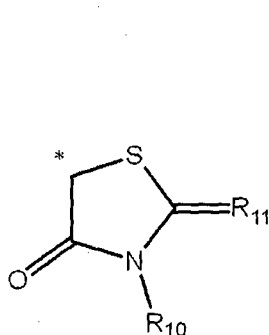
[0034] In general formula (1), the aryl group used as R_2 to R_5 is not especially limited, and examples include a phenyl group and a naphthyl group.

[0035] In general formula (1), the carboxylalkyl group used as R_2 to R_5 is not especially limited, and examples include a methyl carboxylate group, an ethyl carboxylate group, a propyl carboxylate group and a butyl carboxylate group.

[0036] In general formula (1), the alkylcarbonyl group used as R_2 to R_5 is not especially limited, and examples include a methylcarbonyl group, an ethylcarbonyl group, a propylcarbonyl group, a butylcarbonyl group, a phenylcarbonyl group, a naphthylcarbonyl group, and a phenanthrylcarbonyl group.

[0037] In general formula (1), the aliphatic ring formed when R_2 and R_4 are bound to each other is not especially limited, and examples include saturated aliphatic rings such as a cyclooctane ring, a cycloheptane ring, a cyclohexane ring, a cyclopentane ring, and a cyclobutane ring; and partially saturated aliphatic rings such as a cyclopentene ring and a cyclohexene ring. The ring may further have a substituent and the substituent is not especially limited as long as the identification of a subtype is not largely inhibited. Examples of such a substituent include alkyl groups such as a methyl group, an ethyl group, a n-propyl group, an isopropyl group and a butyl group; aryl groups such as a phenyl group; alkoxy groups such as a methoxy group, an ethoxy group and a butoxy group; aryloxy groups such as a phenoxy group; di-substituted amino groups such as a dimethylamino group, a N-ethyl-N-phenylamino group, and a diphenylamino group; acyl groups such as an acetyl group and a benzoyl group; a sulfonyl group, a carboxyl group; a carboxylalkyl group; a carbamoyl group; a sulfamoyl group; hetero ring groups such as a pyridyl group, a triazinyl group, and a benzothiazolyl group; a nitro group; and halogen atoms such as a fluorine atom, a chlorine atom, a bromine atom and an iodine atom; a carboxyl group, and a sulfo group.

[0038] In general formula (1), in a preferred aspect, R_2 to R_5 each independently represent a hydrogen atom, an alkyl group or an aryl group and R_2 and R_4 are bound to each other to form an aliphatic ring, and in a more

preferred aspect, R_2 and R_4 are bound to each other to
form an aliphatic ring, because a subtype can be easily
identified in such a case. Specifically, the aliphatic
ring can be a cyclooctane ring, a cycloheptane ring, a
cyclohexane ring, a cyclopentane ring or a cyclobutane
ring. More preferably, the aliphatic ring is a
cyclopentane ring.

[0039] In general formula (1), the alkyl group used as R_6 is
not especially limited, and examples include a methyl
group, an ethyl group, a propyl group and a butyl group.

[0040] In general formula (1), the alkoxy group used as R_6 is
not especially limited, and examples include a methoxy
group, an ethoxy group, a propoxy group and a butoxy
group.

[0041] In general formula (1), examples of the halogen atom
used as R_6 include a fluorine atom, a chlorine atom, a
bromine atom and an iodine atom.

[0042] In general formula (1), R_6 preferably represents a
hydrogen atom, a halogen atom or an alkoxy group, and
more preferably represents a hydrogen atom or a bromine
atom.

[0043] In general formula (1), the alkyl group used as R_7 and
R_8 is not especially limited, and examples include
alkyl groups having 1 to 4 carbon atoms, such as a
methyl group, an ethyl group, a propyl group and a
butyl group.

[0044] In general formula (1), the aryl group used as R_7 and
R_8 is not especially limited, and examples include a
phenyl group and a methylphenyl group.

[0045] In general formula (1), the carbon atom having aryl
group used as Y_1 is not especially limited, and
examples include phenyl-substituted carbon,
methylphenyl-substituted carbon, methoxyphenyl-
substituted carbon, and chlorophenyl-substituted carbon.

[0046] In general formula (1), R_9 represents a
dicyanomethylene group, a cyanocarboxymethylene group,

or any one of the following general formulas (2) to (4),
and the sign "*" represents a binding site.

[0047] R_9 preferably represents any one of general formulas
(2) to (4), and particularly preferably represents
general formula (2) because a subtype can be easily
identified in this case.

General formula (2)   General formula (3)   General formula (4)

[0048] In general formula (2), the alkyl group used as R_{10} is
not especially limited, and examples include linear,
branched or cyclic alkyl groups having 1 to 20 carbon
atoms, such as a methyl group, an ethyl group, a propyl
group, a butyl group, an ethylhexyl group, and a
cyclohexyl group.

[0049] In general formula (2), the carboxylalkyl group used as
R_{10} is not especially limited, and examples include a
carboxymethyl group, a carboxyethyl group, a
carboxypropyl group, a carboxybutyl group, a
carboxypentyl group, a carboxyhexyl group, a
carboxyheptyl group and a carboxyoctyl group.

[0050] In general formula (2), the alkoxycarbonylalkyl group
used as R_{10} is not especially limited, and examples
include a methoxycarbonylmethyl group, a
methoxycarbonylethyl group, an ethoxycarbonylethyl
group, a butoxycarbonylethyl group, a
methoxycarbonylpropyl group, and a methoxycarbonylhexyl
group.

[0051] In general formula (2), the alkylcarbonyloxyalkyl group
used as R_{10} is not especially limited, and examples

include a methylcarbonyloxymethyl group, an
ethylcarbonyloxymethyl group, an ethylcarbonyloxyethyl
group, ethylcarbonyloxybutyl, and a
proxycarbonyloxymethyl group.

[0052] In general formula (2), the 2-thioxothiazolin-4-one
group used as R_{11} is not especially limited, and
examples include the following compounds.
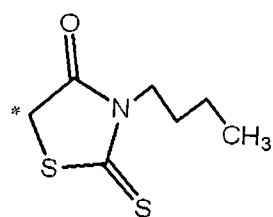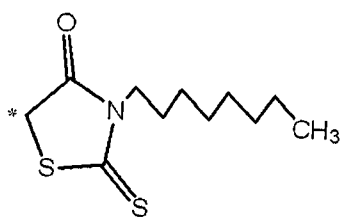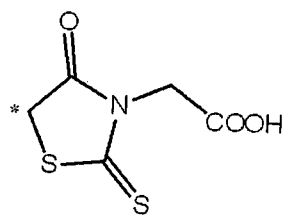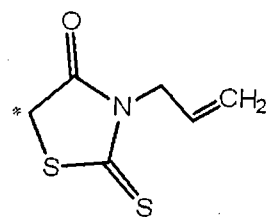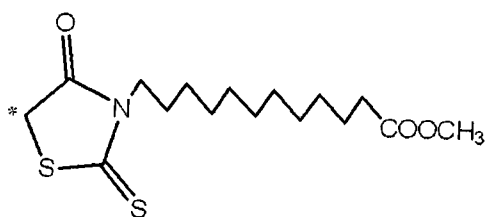The sign "*" represents a binding site.

[0053]
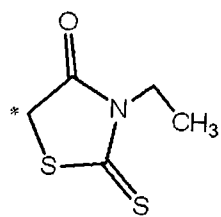
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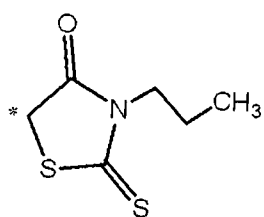
A2

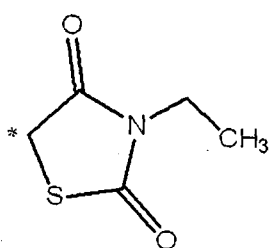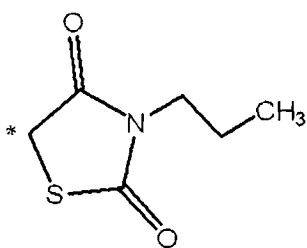A3

A4

A5

A6
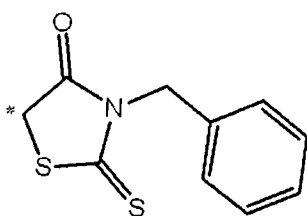
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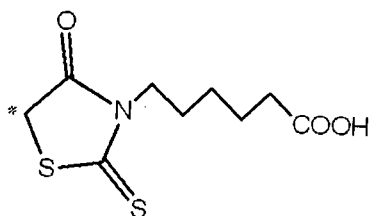
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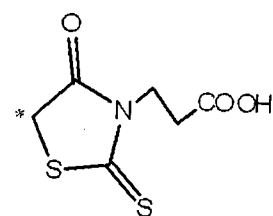
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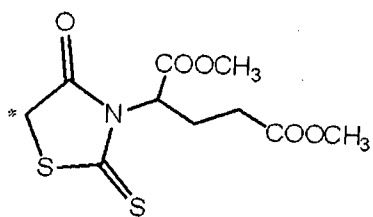
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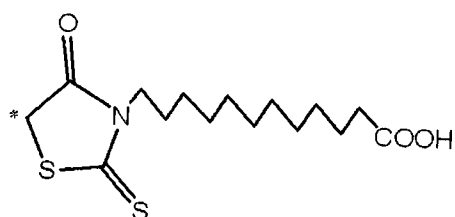
A11

A12

[0054] In general formula (2), the thiazoline-2,4-dione group used as R_{11} is not especially limited, and examples include the following compounds.
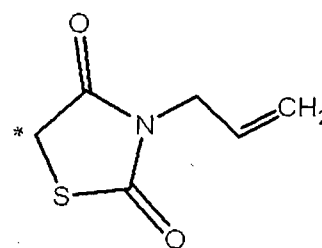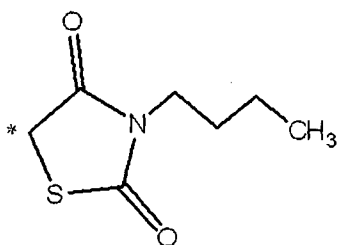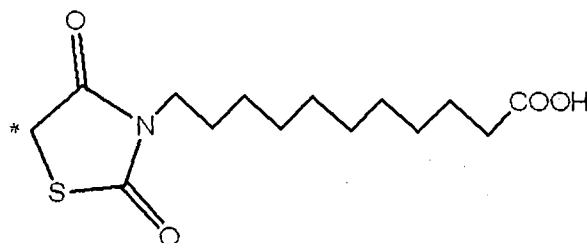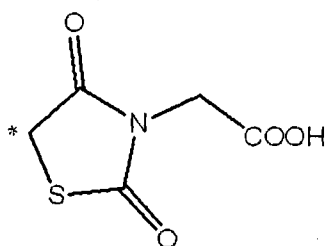The sign "*" represents a binding site.

B1   B2   B3
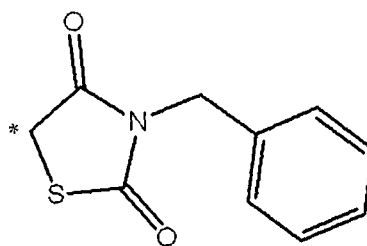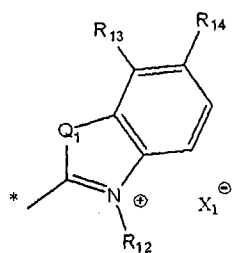
B4   B5

B6   B7

[0055] In general formula (2), R_{11} can be represented by the following general formula (5):

General formula (5)

[0056] In general formula (5), the alkyl group used as R_{12} is not especially limited, and examples include linear, branched or cyclic alkyl groups having 1 to 20 carbon atoms, such as a methyl group, an ethyl group, a propyl group, a butyl group, an ethylhexyl group and a cyclohexyl group.

[0057] In general formula (5), the carboxylalkyl group used as R_{12} is not especially limited, and examples include a carboxymethyl group, a carboxyethyl group, a carboxypropyl group, a carboxybutyl group, a carboxypentyl group, a carboxyhexyl group, a carboxyheptyl group and a carboxyoctyl group.

[0058] In general formula (5), the alkoxycarbonylalkyl group used as R_{12} is not especially limited, and examples include a methoxycarbonylmethyl group, a methoxycarbonylethyl group, an ethoxycarbonylethyl group, a butoxycarbonylethyl group, a methoxycarbonylpropyl group, and a methoxycarbonylhexyl group.

[0059] In general formula (5), the alkylcarbonyloxyalkyl group used as R_{12} is not especially limited, and examples include a methylcarbonyloxymethyl group, an ethylcarbonyloxymethyl group, an ethylcarbonyloxyethyl group, ethylcarbonylbutyl, and a proxycarbonyloxymethyl group.

[0060] In general formula (5), R_{13} and R_{14} are bound to each other to form a benzene ring, and the ring may further have a substituent.

[0061] In general formula (5), the N-alkyl nitrogen atom used as Q_1 is not especially limited, and examples include a N-methyl nitrogen atom, a N-ethyl nitrogen atom, a N-propyl nitrogen atom, and a N-butyl nitrogen atom.

[0062] In general formula (5), the alkyl group used as R_{15} and R_{16} of Q_1 is not especially limited, and examples include alkyl groups having 1 to 4 carbon atoms, such as a methyl group, an ethyl group, a propyl group and a

butyl group.

[0063] In general formula (5), the ring formed when R_{15} and R_{16} are bound to each other is not especially limited, and examples include a cyclopentane ring and a cyclohexane ring.

[0064] In general formula (5), the anionic group represented by X_1^- is not especially limited, and examples include a chloride ion, a bromide ion, an iodide ion, a sulfate ion, a nitrate ion, a methanesulfonate ion, a p-toluenesulfonate ion, a tetrafluoroborate ion, and a hexafluorophosphate ion.

[0065] In general formula (3), the alkyl group used as R_{17} is not especially limited, and examples include linear, branched or cyclic alkyl groups having 1 to 20 carbon atoms, such as a methyl group, an ethyl group, a propyl group, a butyl group, an ethylhexyl group and a cyclohexyl group.

[0066] In general formula (3), the carboxylalkyl group used as R_{17} is not especially limited, and examples include a carboxymethyl group, a carboxyethyl group, and a carboxypropyl group.

[0067] In general formula (3), the alkoxycarbonylalkyl group used as R_{17} is not especially limited, and examples include a methoxycarbonylmethyl group, a methoxycarbonylethyl group, an ethoxycarbonylethyl group, a butoxycarbonylethyl group, and a methoxycarbonylpropyl group.

[0068] In general formula (3), the alkylcarbonyloxyalkyl group used as R_{17} is not especially limited, and examples include a methylcarbonyloxymethyl group, an ethylcarbonyloxymethyl group, an ethylcarbonyloxyethyl group, ethylcarbonylbutyl, and a proxycarbonyloxymethyl group.

[0069] In general formula (3), R_{18} and R_{19} are bound to each other to form a benzene ring, and the ring may further have a substituent.

[0070] In general formula (3), the N-alkyl nitrogen atom used
       as Q_2 is not especially limited, and examples include a
       N-methyl nitrogen atom, a N-ethyl nitrogen atom, a N-
       propyl nitrogen atom, and a N-butyl nitrogen atom.

[0071] In general formula (3), the alkyl group used as R_{20} and
       R_{21} of Q_2 is not especially limited, and examples
       include alkyl groups having 1 to 4 carbon atoms, such
       as a methyl group, an ethyl group, a propyl group and a
       butyl group.

[0072] In general formula (3), the ring formed when R_{20} and R_{21}
       are bound to each other is not especially limited, and
       examples include a cyclopentane ring and a cyclohexane
       ring.

[0073] In general formula (3), the anionic group represented
       by X_2^- is not especially limited, and examples include a
       chloride ion, a bromide ion, an iodide ion, a sulfate
       ion, a nitrate ion, a methanesulfonate ion, a p-
       toluenesulfonate ion, a tetrafluoroborate ion, and a
       hexafluorophosphate ion.

[0074] In general formula (4), the alkyl group used as R_{22} is
       not especially limited, and examples include linear,
       branched or cyclic alkyl groups having 1 to 20 carbon
       atoms, such as a methyl group, an ethyl group, a propyl
       group, a butyl group, an ethylhexyl group and a
       cyclohexyl group.

[0075] In general formula (4), the aryl group used as R_{22} is
       not especially limited, and examples include 6- to 14-
       membered monocyclic or polycyclic aryl groups, such as
       a phenyl group, a naphthyl group, a phenanthryl group,
       and an anthracenyl group.

[0076] In general formula (4), the aryl group used as R_{22} may
       further have a substituent, and the substituent is not
       especially limited as long as the identification of a
       subtype is not largely inhibited. Examples include
       alkyl groups such as a methyl group, an ethyl group, a
       n-propyl group, an isopropyl group, a n-butyl group, an

isobutyl group, a sec-butyl group and a tert-butyl
group; aryl groups such as a phenyl group and a
naphthyl group; alkoxy groups such as a methoxy group,
an ethoxy group and a butoxy group; aryloxy groups such
as a phenoxy group and a naphthyloxy group;
alkylsulfanyl groups such as a thiomethyl group, a
thioethyl group, a thiopropyl group, a thiobutyl group
and a thiophenyl group; di-substituted amino groups
such as a dimethylamino group, a N-ethyl-N-phenylamino
group, and a diphenylamino group; acyl groups such as
an acetyl group and a benzoyl group; a sulfonyl group;
a carboxyl group; a carboxylalkyl group; a carbamoyl
group; a sulfamoyl group; hetero ring groups such as a
pyridyl group, a triazinyl group, and a benzothiazolyl
group; a nitro group; and halogen atoms such as a
fluorine atom, a chlorine atom, a bromine atom and an
iodine atom.

[0077] In general formula (4), the alkyl group used as R_{23} is
not especially limited, and examples include linear,
branched or cyclic alkyl groups having 1 to 20 carbon
atoms, such as a methyl group, an ethyl group, a propyl
group, a butyl group, an ethylhexyl group and a
cyclohexyl group.

[0078] <Regarding compounds>

The compounds represented by general formula (1) of the
present embodiment can be easily synthesized by a
method similar to known methods (PTL 2 and the like).

[0079] Suitable specific examples of the compounds represented
by general formula (1) of the present invention include,
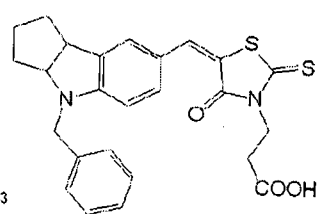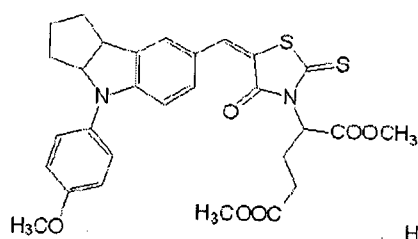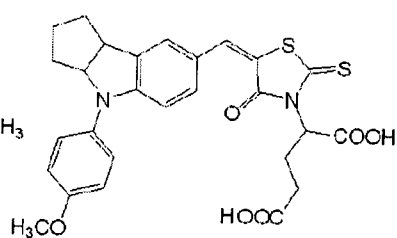but are not limited to, the following compounds (1) to
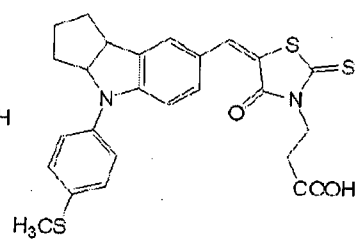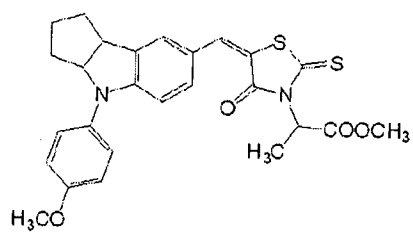(85):

[0080]
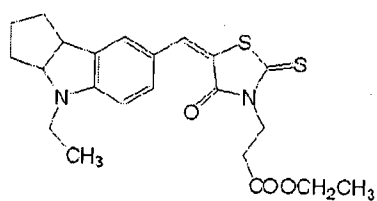
Compound (1)
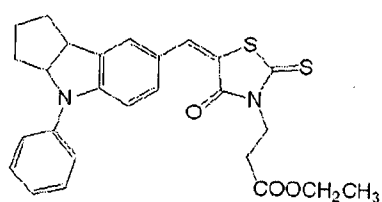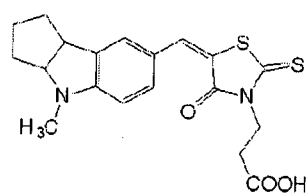
Compound (2)

Compound (3)

Compound (4)

Compound (5)

Compound (6)

Compound (7)

Compound (8)

Compound (9)

[0081]

Compound (10)   Compound (11)   Compound (12)

Compound (13)   Compound (14)   Compound (15)
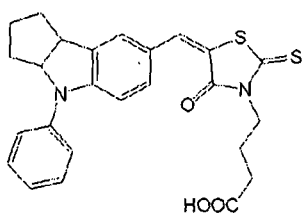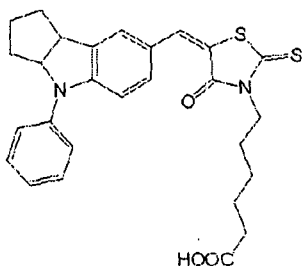
Compound (16)   Compound (17)   Compound (18)
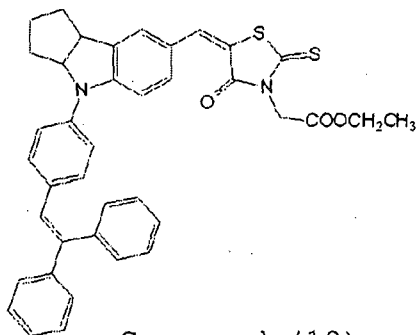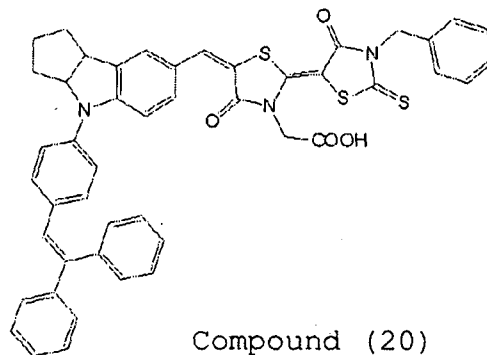
Compound (19)   Compound (20)
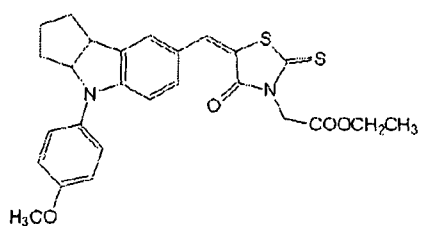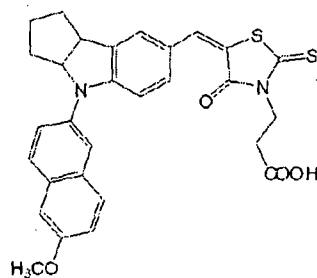
Compound (21)   Compound (22)

[0082]
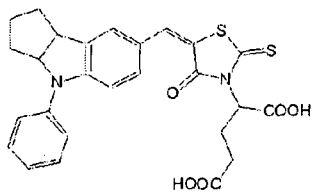
Compound (23)

Compound (24)

Compound (25)

Compound (26)

Compound (27)

Compound (28)

Compound (29)

Compound (30)

Compound (31)

Compound (32)
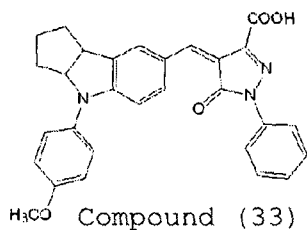
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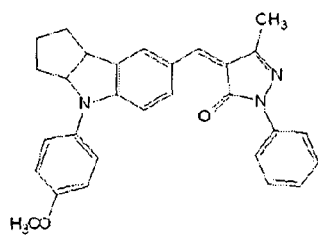
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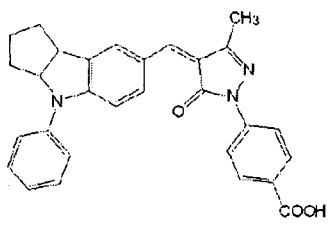
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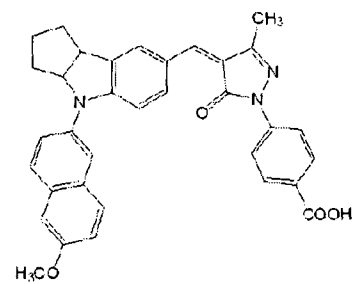
Compound (36)

26

[0083]
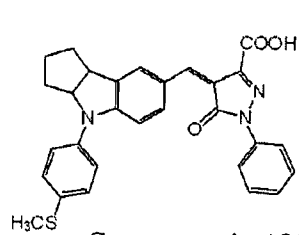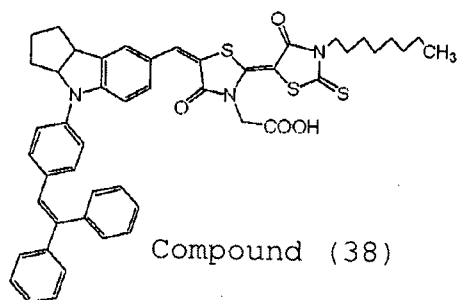
Compound (37)

Compound (38)
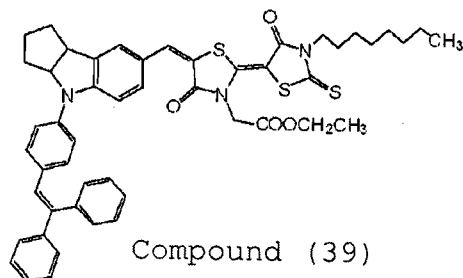
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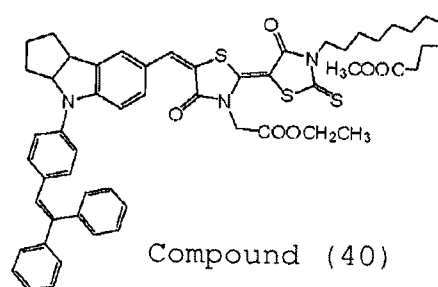
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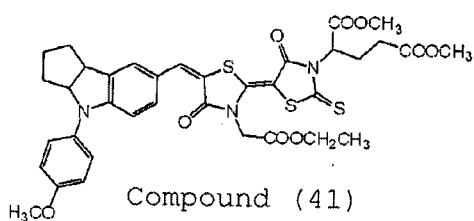
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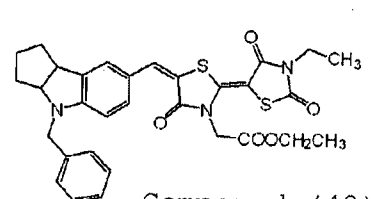
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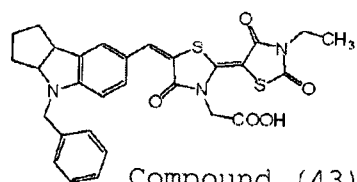
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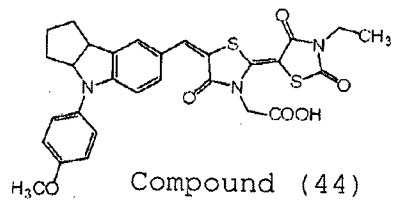
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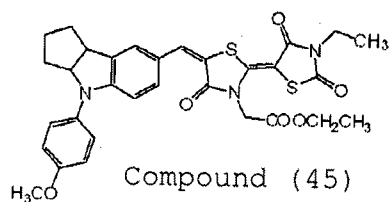
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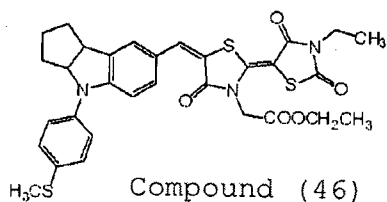
Compound (46)

Compound (47)

Compound (48)

27

[0084]

Compound (49)

Compound (50)
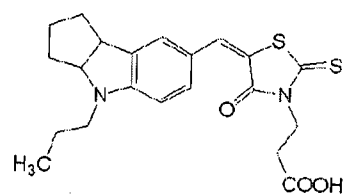
Compound (51)

Compound (52)

Compound (53)

Compound (54)

Compound (55)

Compound (56)

Compound (57)

Compound (58)

Compound (59)

Compound (60)

28

[0085]

Compound (61)

Compound (62)

Compound (63)
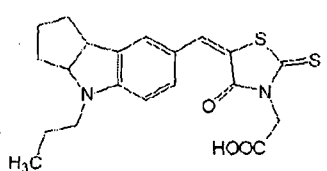
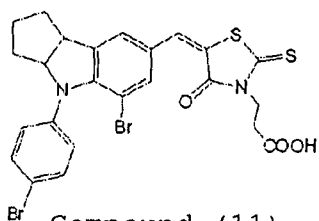Compound (64)

Compound (65)

Compound (66)
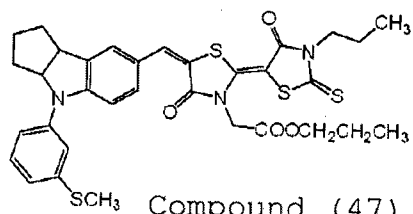
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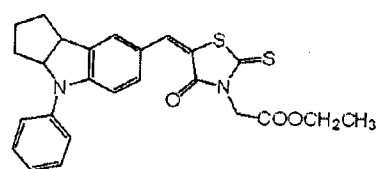
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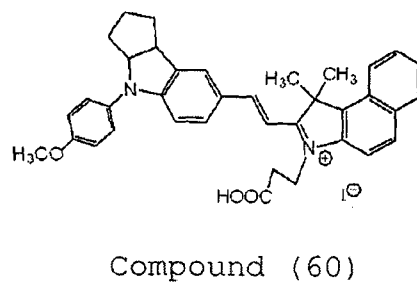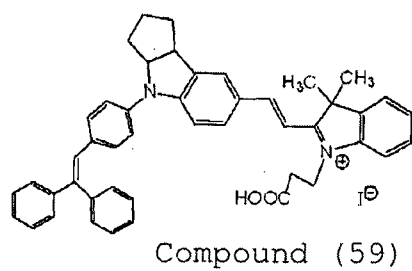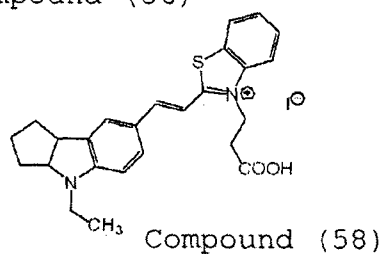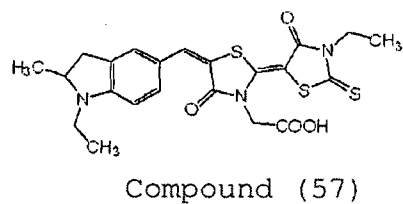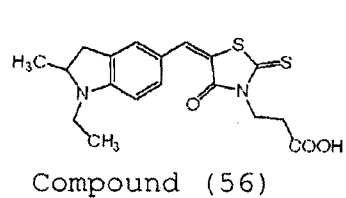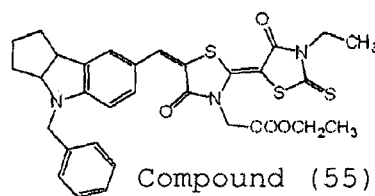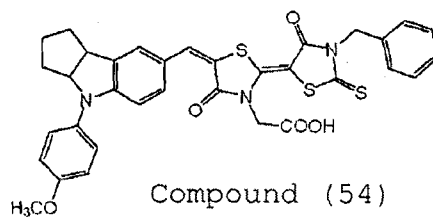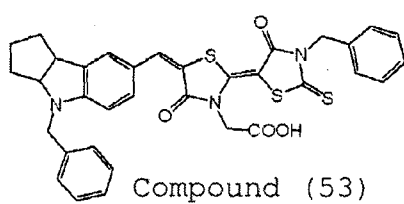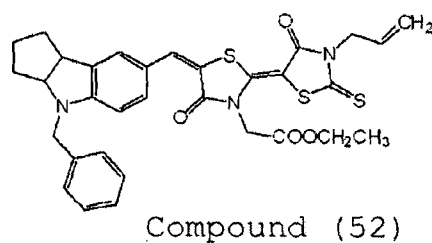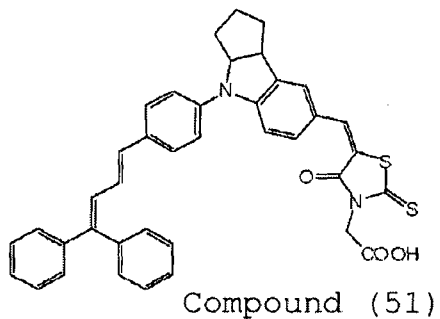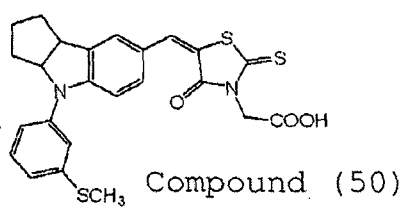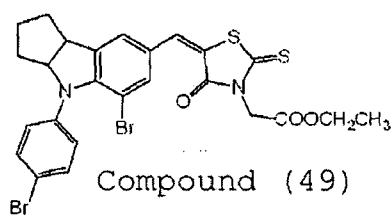
Compound (69)
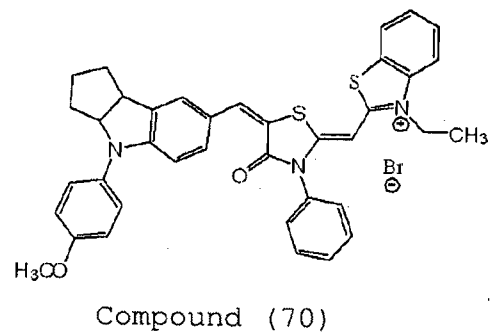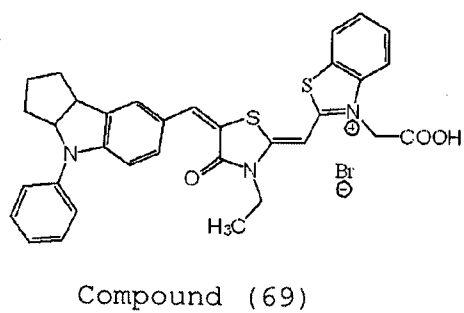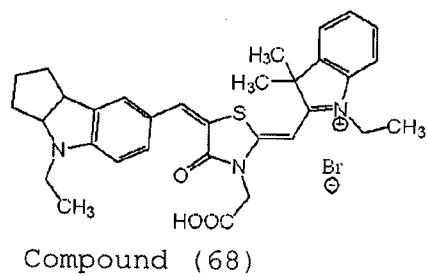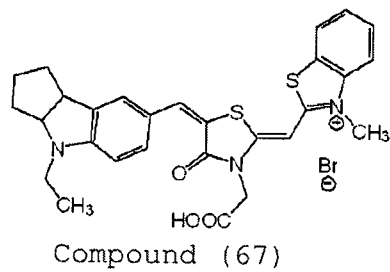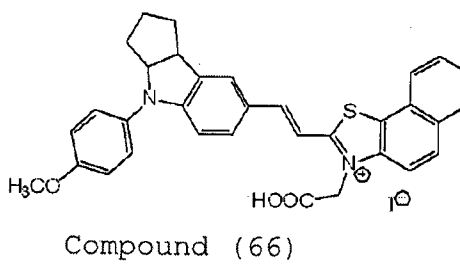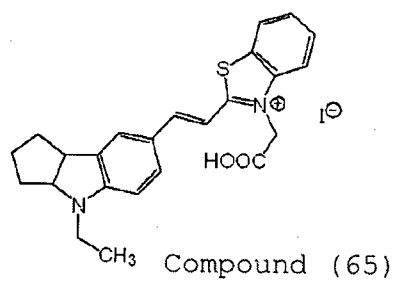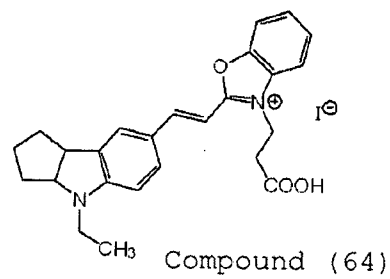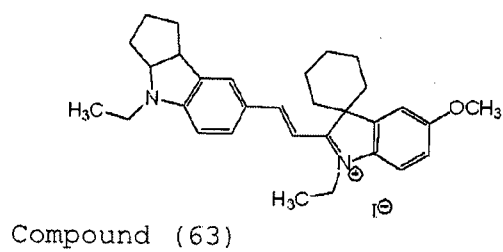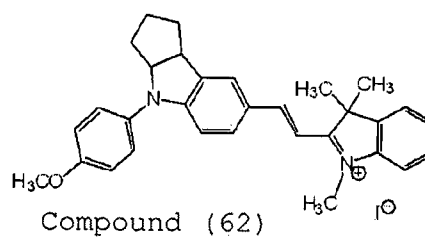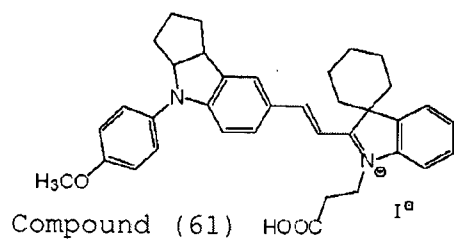
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[0086]
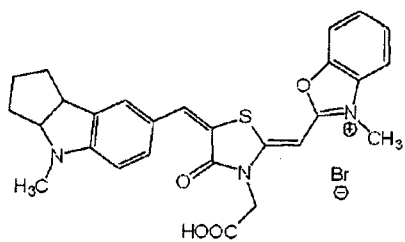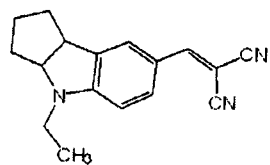
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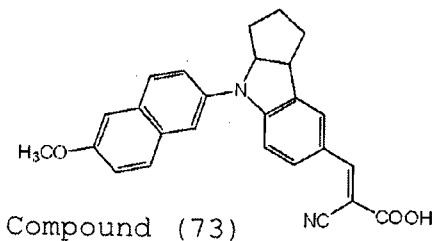
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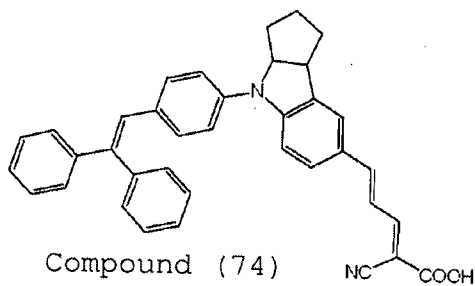
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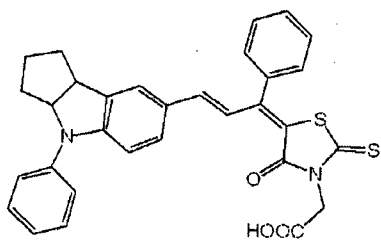
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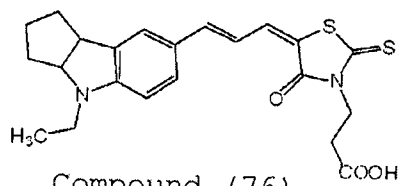
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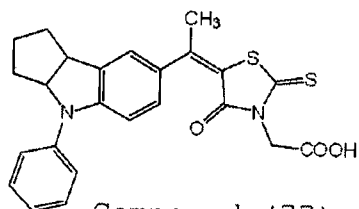
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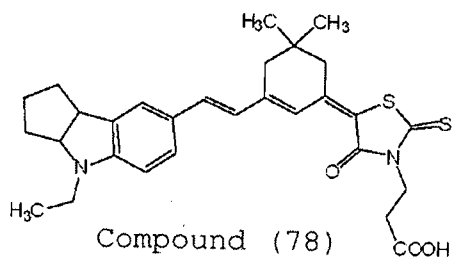
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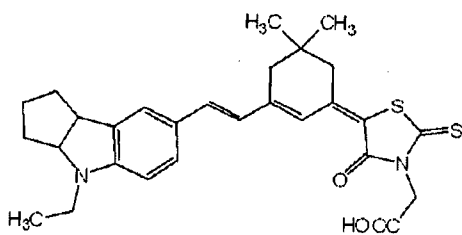
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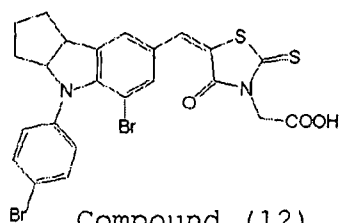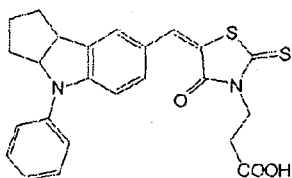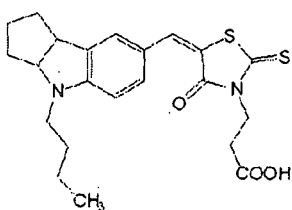Compound (80)
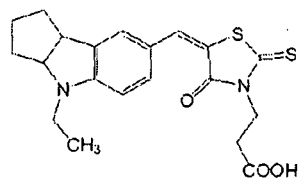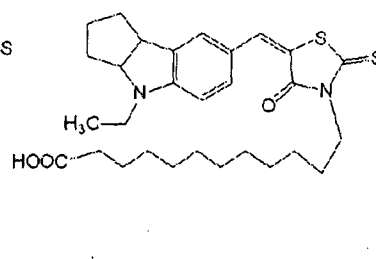
[0087]

Compound (81)

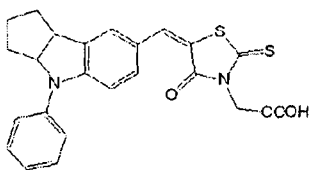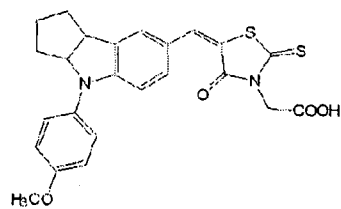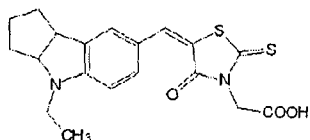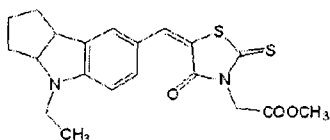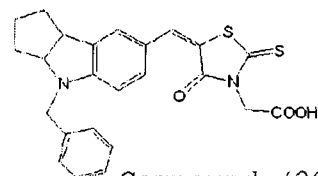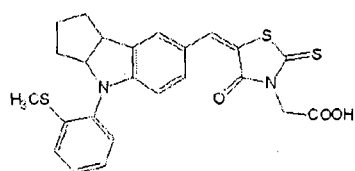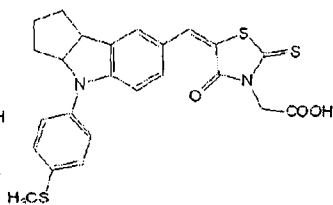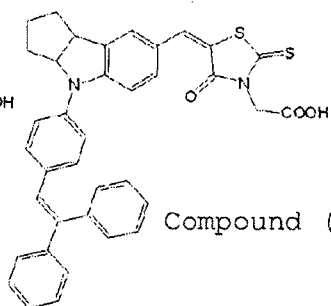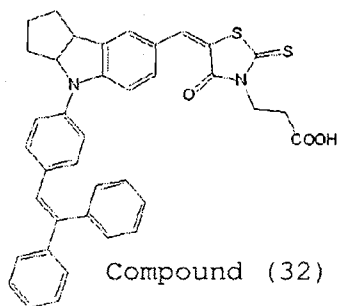Compound (82)
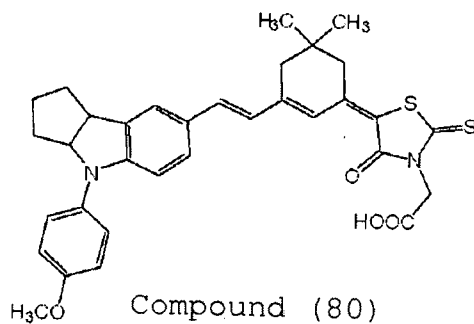
Compound (83)

Compound (84)
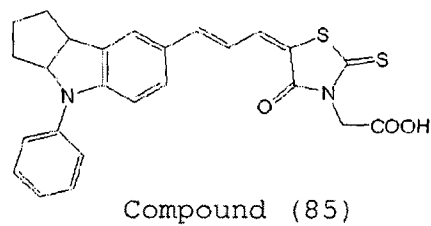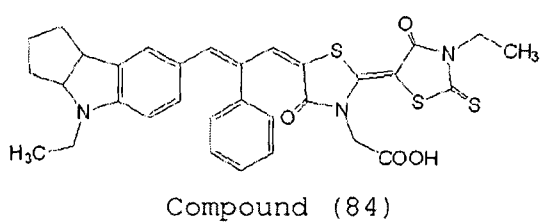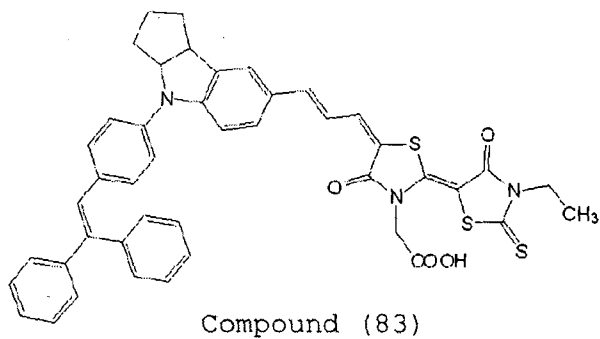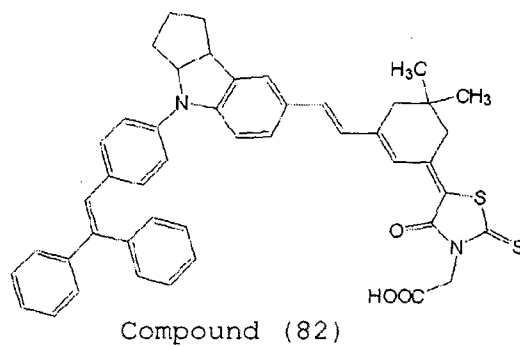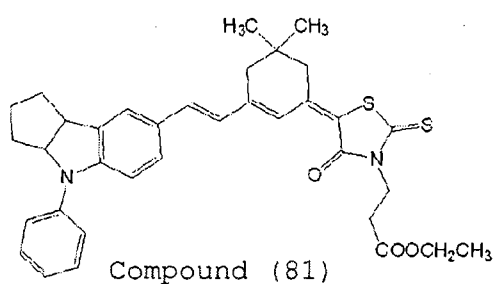
Compound (85)

Substituents used in general formula (1) of the compounds (1) to (85) are shown in Table 1.

[0088]Table 1

Compound	R1	R2	R3	R4	R5	R6	R7	R8	Y1	n	R9
(1)	Alkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(2)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(3)	Aralkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(4)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(5)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(6)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(7)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(8)	Alkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(9)	Alkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(10)	Alkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(11)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(12)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(13)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(14)	Alkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(15)	Alkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(16)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(17)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(18)	Alkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(19)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(20)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(21)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(22)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(23)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(24)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)

Table 1 (continued)

Compound	R1	R2	R3	R4	R5	R6	R7	R8	Y1	n	R9
(25)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(26)	Alkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(27)	Alkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(28)	Aralkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(29)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(30)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(31)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(32)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(33)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (4)
(34)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (4)
(35)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (4)
(36)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (4)
(37)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (4)
(38)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(39)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(40)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(41)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(42)	Aralkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(43)	Aralkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(44)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(45)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(46)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(47)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(48)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)

Table 1 (continued)

Compound	R1	R2	R3	R4	R5	R6	R7	R8	Y1	n	R9
(49)	Aryl group	Aliphatic ring				Halogen atom	Hydrogen atom	-	-	0	General formula (2)
(50)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(51)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(52)	Aralkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(53)	Aralkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(54)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(55)	Aralkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(56)	Alkyl group	Alkyl group		Hydrogen atom		Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(57)	Alkyl group	Alkyl group		Hydrogen atom		Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(58)	Alkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (3)
(59)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (3)
(60)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (3)
(61)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (3)
(62)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (3)
(63)	Alkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (3)
(64)	Alkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (3)
(65)	Alkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (3)
(66)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (3)
(67)	Alkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(68)	Alkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(69)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(70)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(71)	Alkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	General formula (2)
(72)	Alkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	Dicyanomethylene group
(73)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	-	-	0	Cyanocarboxymethylene group

Table 1 (continued)

Compound	R1	R2	R3	R4	R5	R6	R7	R8	Y1	n	R9
(74)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	Hydrogen atom	-CH=	1	Cyanocarbox ymethylene group
(75)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	Hydrogen atom	Cyclohexene ring	1	General formula (2)
(76)	Alkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	Hydrogen atom	-CH=	1	General formula (2)
(77)	Aryl group	Aliphatic ring				Hydrogen atom	Alkyl group	-	-	0	General formula (2)
(78)	Alkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	Hydrogen atom	Cyclohexene ring	1	General formula (2)
(79)	Alkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	Hydrogen atom	Cyclohexene ring	1	General formula (2)
(80)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	Hydrogen atom	Cyclohexene ring	1	General formula (2)
(81)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	Hydrogen atom	Cyclohexene ring	1	General formula (2)
(82)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	Hydrogen atom	Cyclohexene ring	1	General formula (2)
(83)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	Hydrogen atom	-CH=	1	General formula (2)
(84)	Alkyl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	Aryl group	-CH=	1	General formula (2)
(85)	Aryl group	Aliphatic ring				Hydrogen atom	Hydrogen atom	Hydrogen atom	-CH=	1	General formula (2)

[0089] Embodiment 2

A macrophage identification agent according to
Embodiment 2 of the present invention contains one or
more compounds represented by the following general
formula (6):
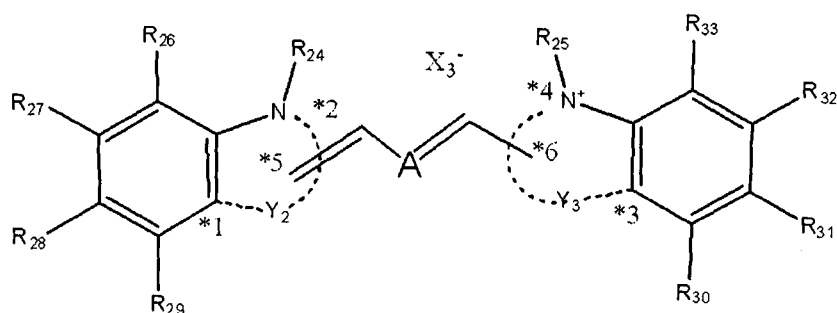
General formula (6)

[0090] In general formula (6), R_{24} and R_{25} each independently
represent an alkyl group, a carboxylalkyl group, an
alkoxycarbonylalkyl group, or an alkylcarbonyloxyalkyl
group; R_{26} to R_{33} each independently represent a
hydrogen atom, an alkyl group, an aryl group, an alkoxy
group, a halogen atom, an alkoxysulfonyl group, a N-
alkylsulfamoyl group, an alkyloxycarbonyl group, a
carbamoyl group, or a N-alkylcarbamoyl group, wherein
R_{26} and R_{27}, R_{28} and R_{29}, R_{30} and R_{31}, and R_{32} and R_{33} may
be independently cyclized to form a benzene ring; X_1^-
represents an anionic group; Y_2 represents a group
containing *1, *2 and *5, and containing an oxygen atom,
a sulfur atom, a N-alkyl nitrogen atom or $-C(R_{34})(R_{35})-$,
or a group containing *1, *2 and *5 and represented by
*1-C*5-CH=CH-*2 or *1-CH=CH-*5-*2; Y_3 represents a group
containing *3, *4 and *6 and containing an oxygen atom,
a sulfur atom, a N-alkyl nitrogen atom or $-C(R_{34})(R_{35})-$,
or a group containing *3, *4 and *6 and represented by
*3-Y_3-*6-*4, *3-C*6-CH=CH-*4, or *3-CH=CH-*6-*4, wherein
R_{34} and R_{35} each independently represent an alkyl group
and may be bound to each other to form a ring; and A
represents a 3-oxocyclobutenolate ring or the following
general formula (7) or (8):
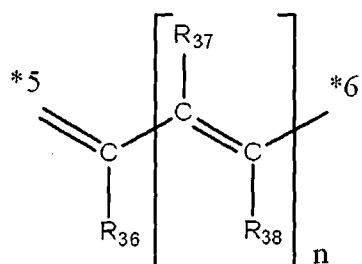
General formula (7)

General formula (8)

In general formula (7), R_{36} to R_{38} each independently represent a hydrogen atom, an alkyl group or an aryl group; and n represents a value of 0 to 2. In general formula (8), R_{39} represents a hydrogen atom, a phenyl group, a thiol group, an alkoxy group, an aryloxy group, or a halogen atom; and R_{40} and R_{41} each independently represent a hydrogen atom, an alkyl group or an alkyloxycarbonyl group.

[0091]<Regarding compounds>

The compounds represented by general formula (6) of the present embodiment will now be described.

[0092]In general formula (6), the alkyl group used as R_{24} and R_{25} is not especially limited, and examples include a methyl group, an ethyl group, a propyl group, a butyl group, a pentyl group and a hexyl group.

[0093]In general formula (6), the carboxylalkyl group used as R_{24} and R_{25} is not especially limited, and examples include a carboxylmethyl group, a carboxylethyl group, and a carboxylpropyl group.

[0094]In general formula (6), the alkoxycarbonylalkyl group

used as R_{24} and R_{25} is not especially limited, and
examples include a methoxycarbonylmethyl group, a
methoxycarbonylethyl group, an ethoxycarbonylethyl
group, a butoxycarbonylethyl group, and a
methoxycarbonylpropyl group. The alkylcarbonyloxyalkyl
group used as R_{24} and R_{25} is not especially limited, and
examples include a methylcarbonyloxymethyl group, an
ethylcarbonyloxymethyl group, an ethylcarbonyloxyethyl
group, ethylcarbonylbutyl, and a proxycarbonyloxymethyl
group.

[0095] In general formula (6), the alkyl group used as R_{26} to
R_{33} is not especially limited, and examples include a
methyl group, an ethyl group, a propyl group, a butyl
group, a pentyl group and a hexyl group.

[0096] In general formula (6), the aryl group used as R_{26} to
R_{33} is not especially limited, and examples include a
phenyl group, a 2-bromophenyl group, a 3-bromophenyl
group, a 4-bromophenyl group, a 2-methoxyphenyl group,
a 3-methoxyphenyl group, a 4-methoxyphenyl group, a 2-
thiomethylphenyl group, a 3-thiomethylphenyl group, a
4-thiomethylphenyl group and a naphthyl group.

[0097] In general formula (6), the alkoxy group used as R_{26} to
R_{33} is not especially limited, and examples include a
methoxy group, an ethoxy group a propoxy group and a
butoxy group.

[0098] In general formula (6), examples of the halogen atom
used as R_{26} to R_{33} include a fluorine atom, a chlorine
atom, a bromine atom and an iodine atom.

[0099] In general formula (6), the alkoxysulfonyl group used
as R_{26} to R_{33} is not especially limited, and examples
include a methoxysulfonyl group and an ethoxysulfonyl
group.

[0100] In general formula (6), N-alkylsulfamoyl used as R_{26} to
R_{33} is not especially limited, and examples include a N-
methylsulfamoyl group, a N-ethylsulfamoyl group, a N,N-
dimethylsulfamoyl group and a N,N-ethylsulfamoyl group.

[0101] In general formula (6), the alkyloxycarbonyl group used as R_{26} to R_{33} is not especially limited, and examples include a methyloxycarbonyl group, an ethyloxycarbonyl group, a propyloxycarbonyl group and a butyloxycarbonyl group.

[0102] In general formula (6), the N-alkylcarbamoyl group used as R_{26} to R_{33} is not especially limited, and examples include a N-methylcarbamoyl group, a N-ethylcarbamoyl group, a N,N-dimethylcarbamoyl group and a N,N-diethylcarbamoyl group.

[0103] In general formula (6), R_{26} to R_{33} each independently represent preferably a hydrogen atom, a halogen atom, a phenyl group or an alkoxy group, and more preferably a hydrogen atom or a phenyl group.

[0104] In general formula (6), the anionic group represented by X_3^- is not especially limited, and examples include a chloride ion, a bromide ion, an iodide ion, a sulfate ion, a nitrate ion, a methanesulfonate ion, a p-toluenesulfonate ion, a tetrafluoroborate ion, a hexafluorophosphate ion, and an ammonium ion.

[0105] In general formula (6), the alkylene group used as Y_2 and Y_3 is not especially limited, and examples include a methylene group, an ethylene group, a propylene group and a butylene group.

[0106] In general formula (6), the alkyl group used as R_{34} and R_{35} of Y_2 and Y_3 is not especially limited, and examples include a methyl group, an ethyl group, a propyl group, a butyl group, a pentyl group, a hexyl group, and a 2-ethylhexyl group. R_{34} and R_{35} are preferably the same but may be different from each other.

[0107] In general formula (6), R_{34} and R_{35} may be bound to each other to form an aliphatic ring, and examples of the ring include a cyclohexane ring and a cyclopentane ring.

[0108] In general formula (7), the alkyl group used as R_{36} to R_{38} is not especially limited, and examples include a methyl group, an ethyl group, a propyl group, a butyl

group, a pentyl group and a hexyl group.

[0109] In general formula (7), the aryl group used as R_{36} to R_{38} is not especially limited, and examples include a phenyl group, a 2-bromophenyl group, a 3-bromophenyl group, a 4-bromophenyl group, a 2-methoxyphenyl group, a 3-methoxyphenyl group, a 4-methoxyphenyl group, a 2-thiomethylphenyl group, a 3-thiomethylphenyl group, and a 4-thiomethylphenyl group.

[0110] In general formula (8), examples of the thiol group used as R_{39} include a mercaptomethyl group, a mercaptobutyl group and a mercaptophenyl group.

[0111] In general formula (8), examples of the alkoxy group used as R_{39} include a methoxy group, an ethoxy group, a propoxy group and a butoxy group.

[0112] In general formula (8), examples of the aryloxy group used as R_{39} include a phenoxy group and a phenoxy group that may have a substituent.

[0113] In general formula (8), examples of the halogen atom used as R_{39} include a fluorine atom, a chlorine atom, a bromine atom and an iodine atom.

[0114] In general formula (8), the alkyl group used as R_{40} and R_{41} is not especially limited, and examples include alkyl groups, such as a methyl group, an ethyl group, a propyl group, a butyl group, a pentyl group and a hexyl group.

[0115] In general formula (8), the alkyloxycarbonyl group used as R_{40} and R_{41} is not especially limited, and examples include a methyloxycarbonyl group, an ethyloxycarbonyl group, a propyloxycarbonyl group and a butyloxycarbonyl group.

[0116] <Regarding compounds represented by general formula (9)>
Suitable examples of the compound of the present embodiment include compounds represented by the following general formula (9):
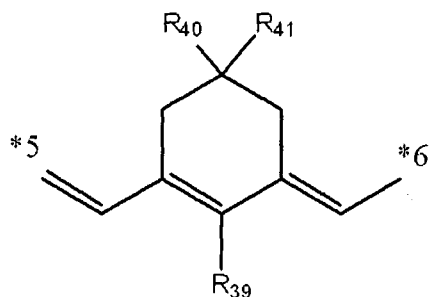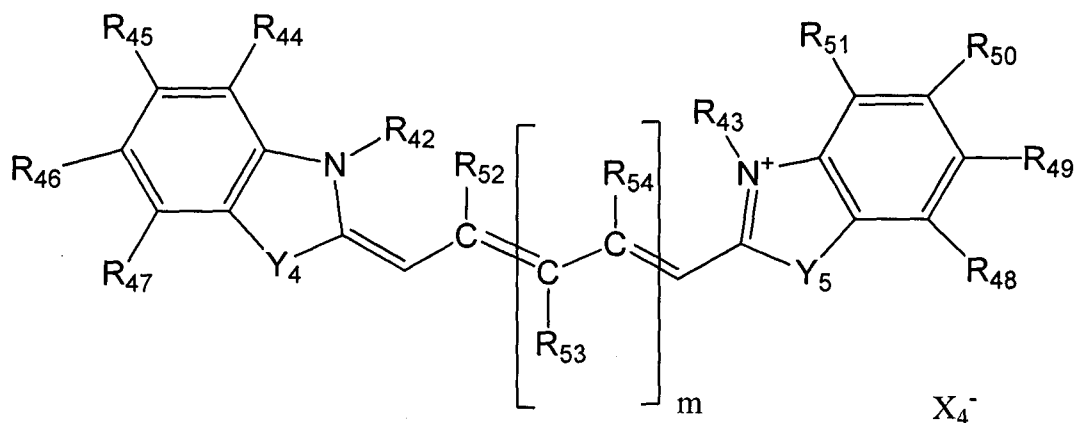
General formula (9)

[0117] In general formula (9), R_{42} and R_{43} each independently represent an alkyl group, a carboxylalkyl group, an alkylcarbonyloxyalkyl group, or an alkoxycarbonyl alkyl group; R_{44} to R_{51} each independently represent a hydrogen atom, an alkyl group, an aryl group, an alkoxy group, a halogen atom, an alkoxysulfonyl group, a N-alkylsulfamoyl group, an alkyloxycarbonyl group, or a N-alkylcarbamoyl group, wherein R_{44} and R_{45}, R_{46} and R_{47}, R_{48} and R_{49}, and R_{50} and R_{51} may be independently cyclized to form a benzene ring; R_{52} to R_{54} each independently represent a hydrogen atom, an alkyl group or an aryl group; m represents a value of 0 to 2; X_2^- represents an anionic group; Y_4 and Y_5 each represent an oxygen atom, a sulfur atom or an alkylene group, wherein the alkylene group may have a substituent, the substituent used in this case is an alkyl group, and the substituents may be bound to each other to form an aliphatic ring.

[0118] In general formula (9), the alkyl group used as R_{42} and R_{43} is not especially limited, and examples include alkyl groups such as a methyl group, an ethyl group, a propyl group, a butyl group, a pentyl group, and a hexyl group.

[0119] In general formula (9), the carboxylalkyl group used as R_{42} and R_{43} is not especially limited, and examples include a carboxylmethyl group, a carboxylethyl group

and a carboxylpropyl group.

[0120] In general formula (9), the alkoxycarbonylalkyl group used as R_{42} and R_{43} is not especially limited, and examples include a methoxycarbonylmethyl group, a methoxycarbonylethyl group, an ethoxycarbonylethyl group, a butoxycarbonylethyl group, and a methoxycarbonylpropyl group.

[0121] In general formula (9), the alkylcarbonyloxyalkyl group is not especially limited, and examples include a methylcarbonyloxymethyl group, an ethylcarbonyloxymethyl group, an ethylcarbonyloxyethyl group, ethylcarbonylbutyl, and a proxycarbonyloxymethyl group.

[0122] In general formula (9), the alkyl group used as R_{44} to R_{48} is not especially limited, and examples include a methyl group, an ethyl group, a propyl group, a butyl group, a pentyl group and a hexyl group.

[0123] In general formula (9), the aryl group used as R_{44} to R_{48} is not especially limited, and examples include a phenyl group, a 2-bromophenyl group, a 3-bromophenyl group, a 4-bromophenyl group, a 2-methoxyphenyl group, a 3-methoxyphenyl group, a 4-methoxyphenyl group, a 2-thiomethylphenyl group, a 3-thiomethylphenyl group, a 4-thiomethylphenyl group and a naphthyl group.

[0124] In general formula (9), the alkoxy group used as R_{44} to R_{48} is not especially limited, and examples include a methoxy group, an ethoxy group, a propoxy group and a butoxy group.

[0125] In general formula (9), examples of the halogen atom used as R_{44} to R_{51} include a fluorine atom, a chlorine atom, a bromine atom and an iodine atom.

[0126] In general formula (9), the alkoxysulfonyl group used as R_{44} to R_{51} is not especially limited, and examples include a methoxysulfonyl group and an ethoxysulfonyl group.

[0127] In general formula (9), the N-alkylsulfamoyl group used

as R_{44} to R_{51} is not especially limited, and examples include a N-methylsulfamoyl group, a N-ethylsulfamoyl group, a N,N-dimethylsulfamoyl group and a N,N-ethylsulfamoyl group.

[0128] In general formula (9), the alkyloxycarbonyl group used as R_{44} to R_{51} is not especially limited, and examples include a methyloxycarbonyl group, an ethyloxycarbonyl group, a propyloxycarbonyl group and a butyloxycarbonyl group.

[0129] In general formula (9), the N-alkylcarbamoyl group used as R_{44} to R_{51} is not especially limited, and examples include a N-methylcarbamoyl group, a N-ethylcarbamoyl group, a N,N-dimethylcarbamoyl group and a N,N-diethylcarbamoyl group.

[0130] In general formula (9), R_{44} to R_{51} each independently represent preferably a hydrogen atom, a halogen atom, a phenyl group or an alkoxy group, and more preferably a hydrogen atom or a phenyl group.

[0131] In general formula (9), the alkyl group used as R_{52} to R_{51} is not especially limited, and examples include a methyl group, an ethyl group, a propyl group, a butyl group, a pentyl group and a hexyl group.

[0132] In general formula (9), the aryl group used as R_{52} to R_{51} is not especially limited, and examples include a phenyl group, a 2-bromophenyl group, a 3-bromophenyl group, a 4-bromophenyl group, a 2-methoxyphenyl group, a 3-methoxyphenyl group, a 4-methoxyphenyl group, a 2-thiomethylphenyl group, a 3-thiomethylphenyl group, and a 4-thiomethylphenyl group.

[0133] In general formula (9), the anionic group represented by X_4^- is not especially limited, and examples include a chloride ion, a bromide ion, an iodide ion, a sulfate ion, a nitrate ion, a methanesulfonate ion, a p-toluenesulfonate ion, a tetrafluoroborate ion, a hexafluorophosphate ion, and an ammonium ion.

[0134] In general formula (9), Y_4 and Y_5 each represent an

oxygen atom, a sulfur atom or an alkylene group, the alkylene group may have a substituent, the substituent used in this case is an alkyl group, and the substituents may be bound to each other to form an aliphatic ring.

[0135] Here, the alkylene group is not especially limited, and examples include a methylene group, an ethylene group, a propylene group, a butylene group, a pentylene group, a hexylene group, and a 2-ethylhexylene group. The aliphatic ring formed by the substituents is not especially limited, and examples include a cyclohexane ring and a cyclopentane ring.

[0136] Many of the compounds represented by general formula (9) of the present embodiment are put on the market and can be available. Alternatively, these compounds can be synthesized by a method similar to known methods (NPL 2 and the like).

[0137] <Compounds represented by general formula (12)> Suitable examples of the compound of the present embodiment include compounds represented by the following general formula (12):
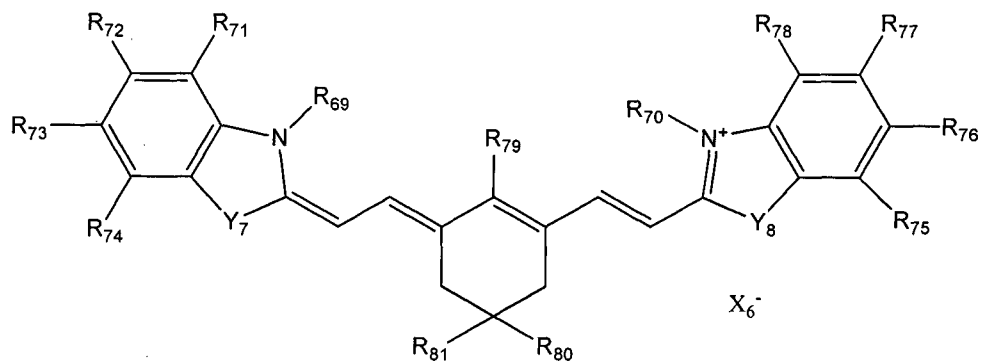
General formula (12)

[0138] In general formula (12), R_{69} and R_{70} each independently represent an alkyl group, a carboxylalkyl group, or an alkoxycarbonylalkyl group; and R_{71} to R_{78} each independently represent a hydrogen atom, an alkyl group, an aryl group, an alkoxy group, a halogen atom, an

alkoxysulfonyl group, a N-alkylsulfamoyl group, an alkyloxycarbonyl group, or a N-alkylcarbamoyl group, wherein R_{71} and R_{72}, R_{73} and R_{74}, R_{75} and R_{76}, and R_{77} and R_{78} may be independently cyclized to form a benzene ring.

[0139] R_{79} represents a hydrogen atom, a phenyl group, a thiol group, an alkoxy group, an aryloxy group, or a halogen atom; R_{80} and R_{81} each independently represent a hydrogen atom, an alkyl group or an alkylcarbonyloxy group; X_6^- represents an anionic group; Y_7 and Y_8 each represent an oxygen atom, a sulfur atom or an alkylene group, wherein the alkylene group may have a substituent, the substituent used in this case is an alkyl group, and the substituents may be bound to each other to form an aliphatic ring.

[0140] In general formula (12), the alkyl group used as R_{69} and R_{70} is not especially limited, and examples include alkyl groups such as a methyl group, an ethyl group, a propyl group, a butyl group, a pentyl group, and a hexyl group.

[0141] In general formula (12), the carboxylalkyl group used as R_{69} and R_{70} is not especially limited, and examples include an acetic acid group, a propionic acid group and a butanoic acid group.

[0142] In general formula (12), the alkoxycarbonylalkyl group used as R_{69} and R_{70} is not especially limited, and examples include a methoxycarbonylmethyl group, a methoxycarbonylethyl group, an ethoxycarbonylethyl group, a butoxycarbonylethyl group, and a methoxycarbonylpropyl group.

In general formula (12), the alkyl group used as R_{71} to R_{78} is not especially limited, and examples include a methyl group, an ethyl group, a propyl group, a butyl group, a pentyl group, and a hexyl group.

[0143] In general formula (12), the aryl group used as R_{71} to R_{78} is not especially limited, and examples include a phenyl group, a 2-bromophenyl group, a 3-bromophenyl

group, a 4-bromophenyl group, a 2-methoxyphenyl group, a 3-methoxyphenyl group, a 4-methoxyphenyl group, a 2-thiomethylphenyl group, a 3-thiomethylphenyl group, a 4-thiomethylphenyl group, and a naphthyl group.

[0144] In general formula (12), the alkoxy group used as R_{71} to R_{78} is not especially limited, and examples include a methoxy group, an ethoxy group, a propoxy group and a butoxy group.

[0145] In general formula (12), examples of the halogen atom used as R_{71} to R_{78} include a fluorine atom, a chlorine atom, a bromine atom and an iodine atom.

[0146] In general formula (12), the alkoxysulfonyl group used as R_{71} to R_{78} is not especially limited, and examples include a methyl sulfonate group and an ethyl sulfonate group.

[0147] In general formula (12), the alkylsulfamoyl group used as R_{71} to R_{78} is not especially limited, and examples include a sulfonic acid monomethyl amide group, a monoethyl amide sulfonate group, a dimethyl amide sulfonate group, and a diethyl amide sulfonate group.

[0148] In general formula (12), the alkylcarbonyloxyalkyl group used as R_{71} to R_{78} is not especially limited, and examples include a carboxylic acid methyl ester group, a carboxylic acid ethyl ester group, a carboxylic acid propyl ester group, and a carboxylic acid butyl ester group.

[0149] In general formula (12), the N-alkylcarbamoyl group used as R_{71} to R_{78} is not especially limited, and examples include a N-methylcarbamoyl group, a N-ethylcarbamoyl group, a N,N-dimethylcarbamoyl group and a N,N-diethylcarbamoyl group.

[0150] In general formula (12), R_{71} to R_{78} represent preferably a hydrogen atom, a halogen atom, a phenyl group or an alkoxy group, and more preferably a hydrogen atom or a phenyl group.

[0151] In general formula (12), examples of the thiol group

used as R_{79} include a mercaptomethyl group, a
mercaptobutyl group and a mercaptophenyl group.

[0152] In general formula (12), examples of the alkoxy group
used as R_{79} include a methoxy group, an ethoxy group, a
propoxy group and a butoxy group.

[0153] In general formula (12), examples of the aryloxy group
used as R_{79} include a phenoxy group, or a phenoxy group
that may have a substituent.

[0154] In general formula (12), examples of the halogen atom
used as R_{79} include a fluorine atom, a chlorine atom, a
bromine atom and an iodine atom.

[0155] In general formula (12), the alkyl group used as R_{80}
and R_{81} is not especially limited, and examples include
alkyl groups such as a methyl group, an ethyl group, a
propyl group, a butyl group, a pentyl group, and a
hexyl group.

[0156] In general formula (12), the alkyloxycarbonyl group
used as R_{80} and R_{81} is not especially limited, and
examples include a methyloxycarbonyl group, an
ethyloxycarbonyl group, a propyloxycarbonyl group and a
butyloxycarbonyl group.

[0157] In general formula (12), the anionic group represented
by X_6^- is not especially limited, and examples include a
chloride ion, a bromide ion, an iodide ion, a sulfate
ion, a nitrate ion, a methanesulfonate ion, a p-
toluenesulfonate ion, a tetrafluoroborate ion, a
hexafluorophosphate ion, and an ammonium ion.

[0158] In general formula (12), the alkylene group used as Y_7
and Y_8 is not especially limited, and examples include
a methylene group, an ethylene group, a propylene group,
a butylene group, a pentylene group, a hexylene group,
and a 2-ethylene hexyl group. The alkyl group used as
a substituent of the alkylene group is not especially
limited, and examples include a methyl group, an ethyl
group, a propyl group and a butyl group.

[0159] Many of the compounds represented by general formula

(12) of the present invention are put on the market and can be easily available. Alternatively, these compounds can be easily synthesized by a method similar to known methods (for example, NPL 8).

[0160] Suitable specific examples of the compounds of the present embodiment include, but are not limited to, the following compounds (86) to (115):

[0161]

Compound (86)

Compound (87)

Compound (88)

Compound (89)

Compound (90)

Compound (91)

Compound (92)

Compound (93)

Compound (94)
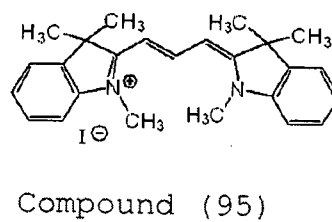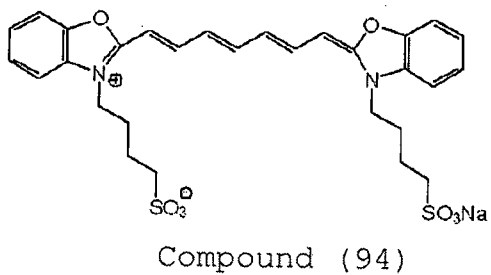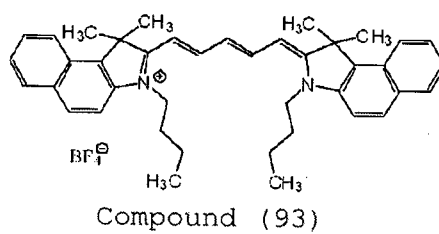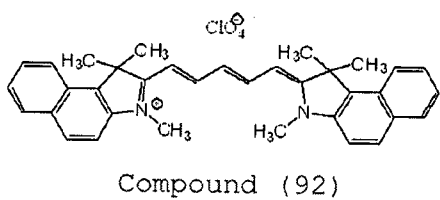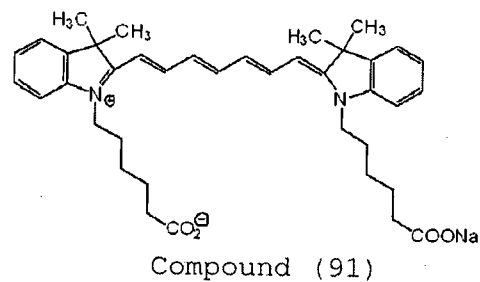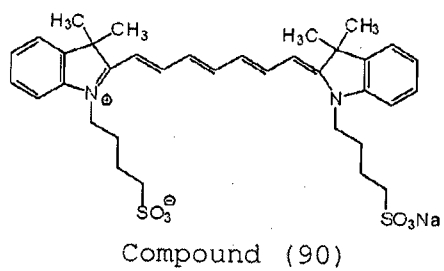
Compound (95)

[0162]
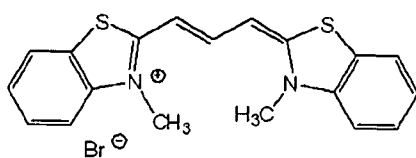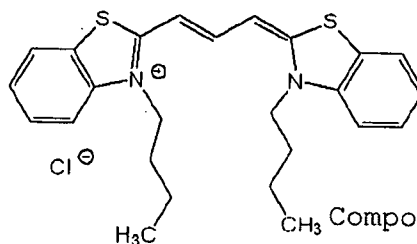
Compound (96)

Compound (97)
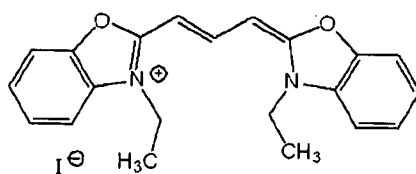
Compound (98)
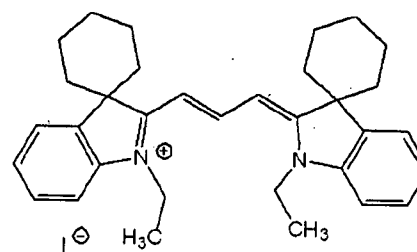
Compound (99)
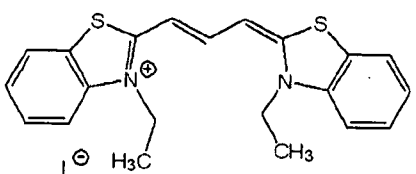
Compound (100)
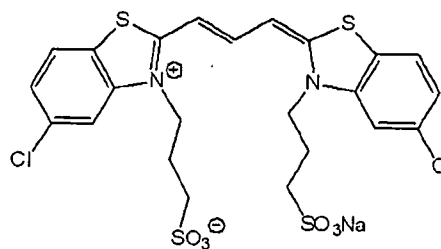
Compound (101)
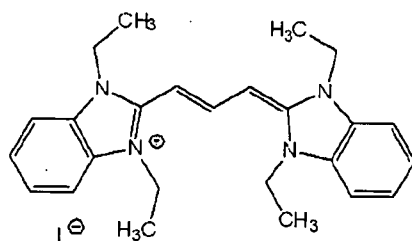
Compound (102)
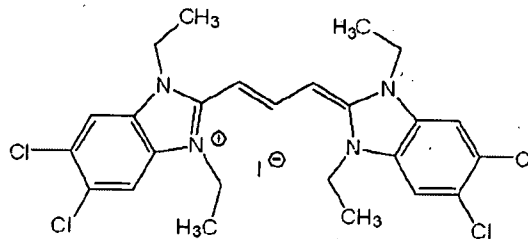
Compound (103)
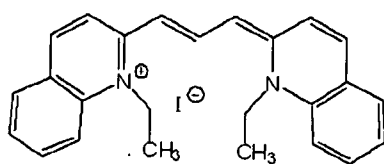
Compound (104)

Compound (105)

50
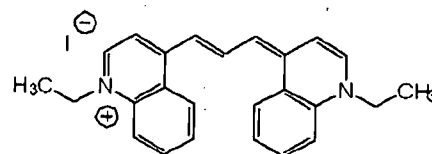
[0163]
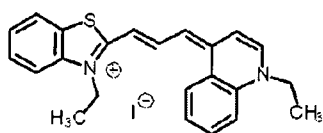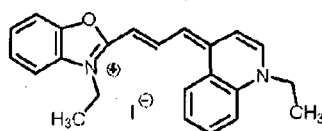
Compound (106)   Compound (107)   Compound (108)
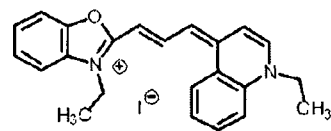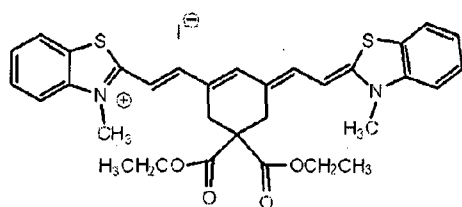
Compound (109)
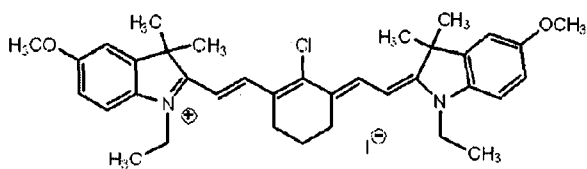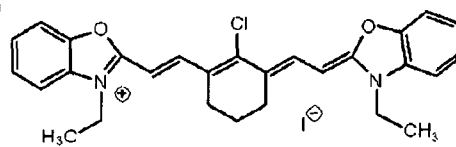
Compound (110)   Compound (111)
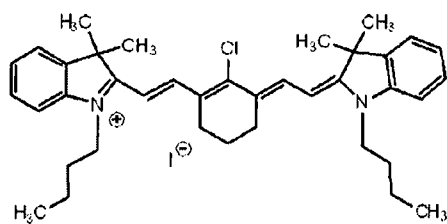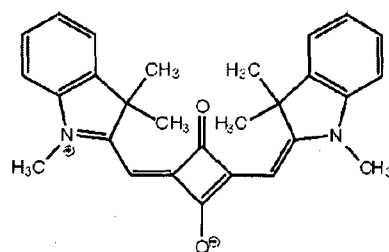
Compound (112)   Compound (113)
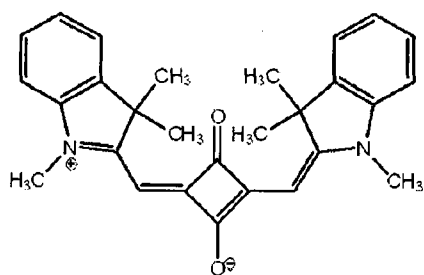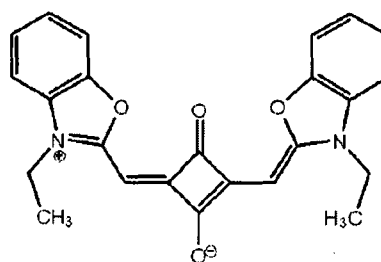
Compound (114)   Compound (115)

[0164] Embodiment 3

A macrophage identification agent according to
Embodiment 3 of the present invention contains one or
more compounds represented by the following general
formula (10):
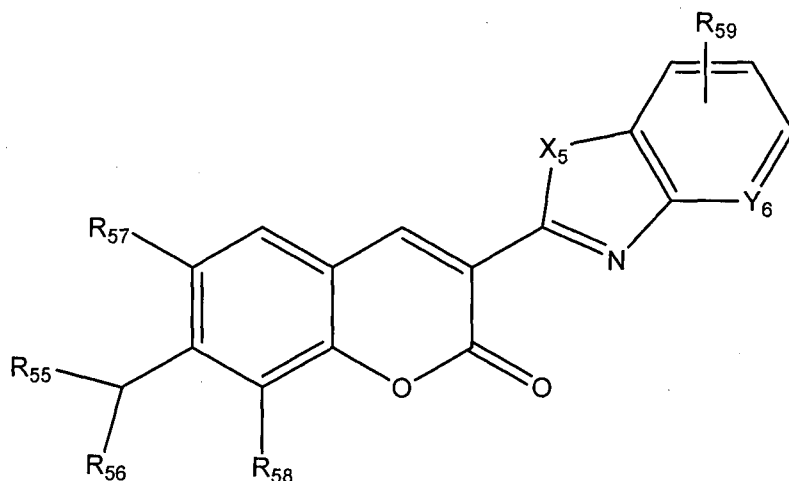
General formula (10)

[0165] In general formula (10), R_{55} and R_{56} each independently
represent an alkyl group; R_{57} and R_{58} each independently
represent a hydrogen atom or an alkyl group, wherein R_{55}
and R_{57}, and R_{56} and R_{58} may be independently bound to
each other to form a ring; R_{59} represents a hydrogen
atom, an alkyl group, an aryl group, an alkoxy group or
a halogen atom; X_5 represents a sulfur atom, an oxygen
atom or $-NR_6-$; and Y_6 represents a carbon atom or a
nitrogen atom.

[0166] <Regarding compounds>

The compounds represented by general formula (10) will
be described. In general formula (10), the alkyl group
used as R_{55} and R_{56} is not especially limited, and
examples include linear or branched alkyl groups having
1 to 12 carbon atoms, such as a methyl group, an ethyl
group, a propyl group, a butyl group and an ethylhexyl
group. R_{55} and R_{56} can be a methyl group or an ethyl
group.

[0167] In general formula (10), the alkyl group used as R_{57}

and R_{58} is not especially limited, and examples include linear or branched alkyl groups having 1 to 12 carbon atoms, such as a methyl group, an ethyl group, a propyl group, a butyl group and an ethylhexyl group.

[0168] In general formula (10), the ring formed when R_{55} and R_{57} or R_{56} and R_{58} are bound to each other is not especially limited, and examples include a tetrahydropyridine ring or a piperidine ring.

[0169] In general formula (10), the alkyl group used as R_{59} is not especially limited, and examples include linear or branched alkyl groups having 1 to 12 carbon atoms, such as a methyl group, an ethyl group, a propyl group, a butyl group and an ethylhexyl group.

[0170] In general formula (10), the aryl group used as R_{59} is not especially limited, and examples include a phenyl group, a methylphenyl group, a bromophenyl group and a methoxyphenyl group.

[0171] In general formula (10), the alkoxy group used as R_{59} is not especially limited, and examples include a methoxy group, an ethoxy group, a propoxy group and a butoxy group.

[0172] In general formula (10), the halogen atom used as R_{59} is not especially limited, and examples include a fluorine atom, a chloro atom, a bromo atom and an iodine atom.

[0173] In general formula (10), X_5 can be a sulfur atom or an oxygen atom because a subtype can be more easily identified in such a case.

[0174] The compounds represented by general formula (10) of the present embodiment can be easily synthesized by a method similar to known methods (NPL 3 and the like).

[0175] Suitable specific examples of the compounds represented by general formula (10) of the present embodiment include, but are not limited to, the following compounds (116) to (138):

53

[0176]
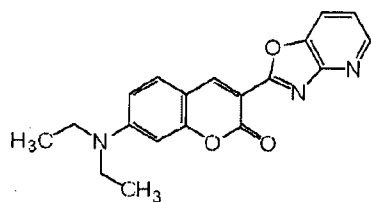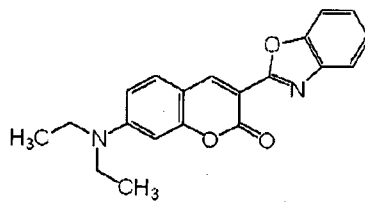
Compound (116)

Compound (117)
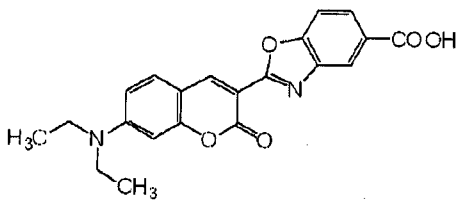
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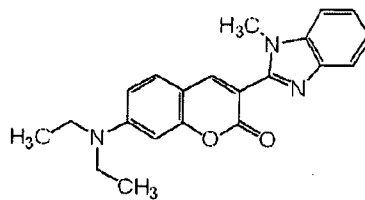
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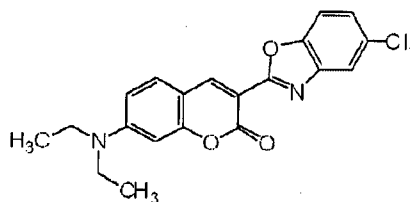
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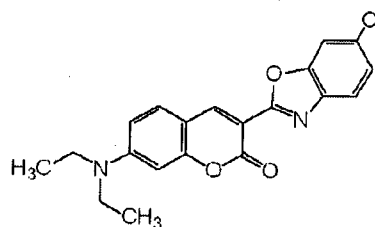
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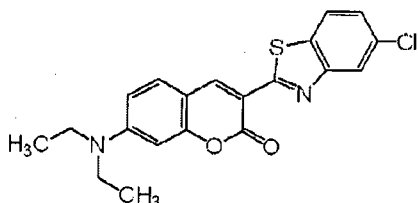
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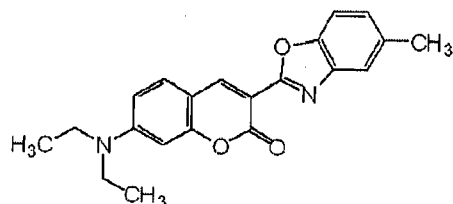
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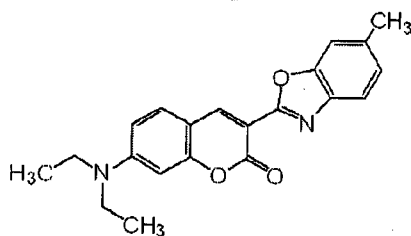
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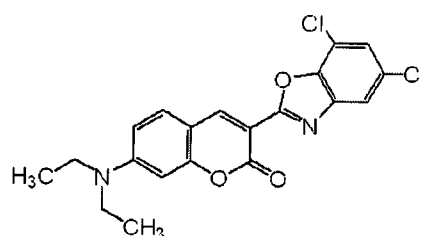
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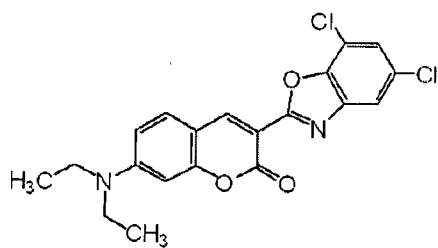
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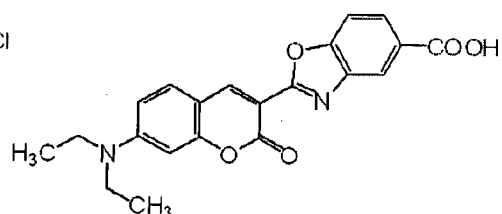
Compound (127)

[0177]
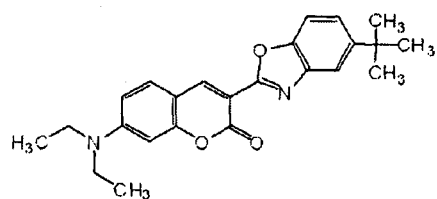
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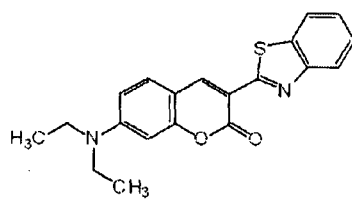
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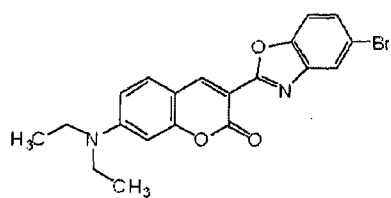
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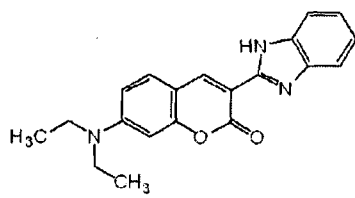
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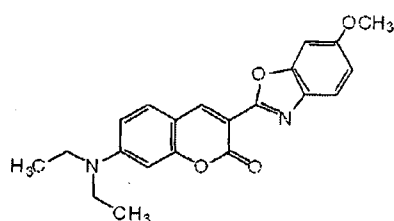
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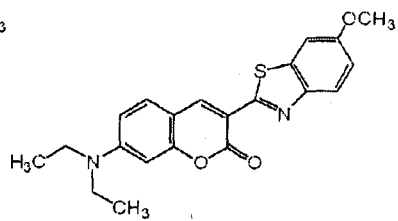
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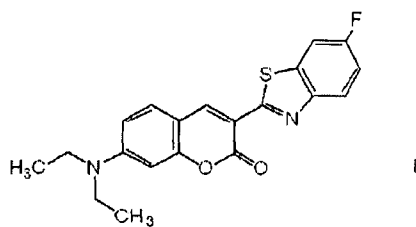
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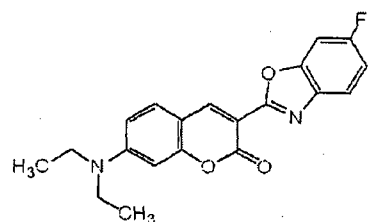
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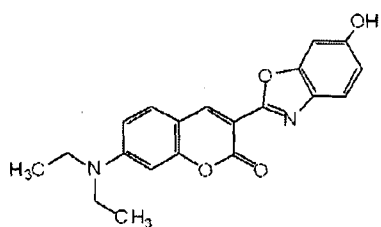
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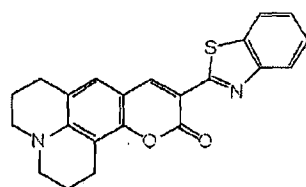
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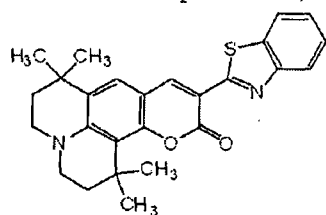
Compound (138)

[0178] Embodiment 4

A macrophage identification agent according to Embodiment 4 of the present invention contains one or more compounds represented by the following general formula (11):
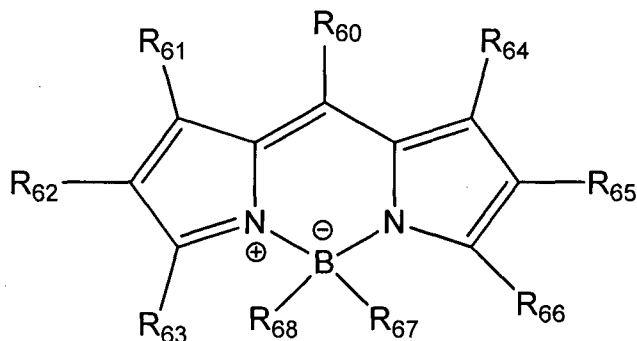
General formula (11)

[0179] In general formula (11), R_{60} represents a hydrogen atom, an alkyl group, an aryl group, a thioalkyl group, an amino group, a hetero ring group, an alkenyl group, a hydroxyl group, a halogen atom, or an alkoxy group; R_{61} to R_{66} each independently represent a hydrogen atom, an alkyl group, an aryl group, a hetero ring group, an aralkyl group, or a sulfonyl group, wherein R_{62} and R_{63}, and R_{65} and R_{66} may be bound to each other to form a hetero ring; and R_{67} and R_{68} each independently represent a fluorine atom or an alkynyl group.

[0180] <Regarding compounds>
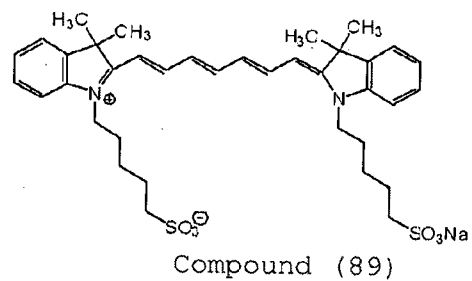
The compounds represented by general formula (11) will be described. In general formula (11), the alkyl group used as R_{60} is not especially limited, and examples include linear or branched alkyl groups having 1 to 12 carbon atoms, such as a methyl group, an ethyl group, a propyl group, a butyl group and an ethylhexyl group, and alkyl halide groups such as trifluoromethyl.
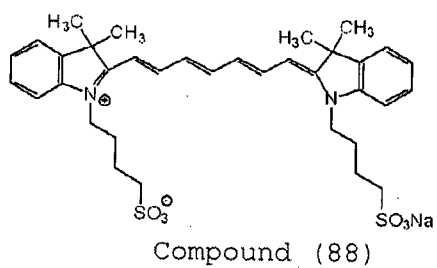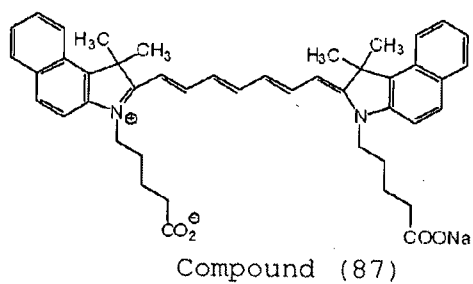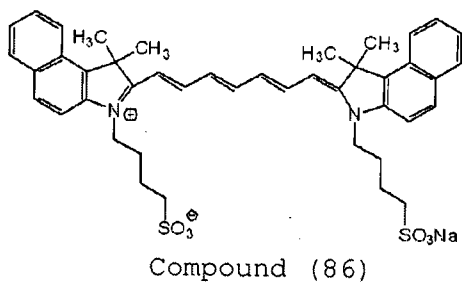
[0181] In general formula (11), the aryl group used as R_{60} is not especially limited, and examples include a phenyl group, a methylphenyl group, a methoxyphenyl group, a thiomethylphenyl group, a chlorophenyl group, a

bromophenyl group, a fluorophenyl group, a butylphenyl group, a dimethylphenyl group, a trimethylphenyl group and a naphthyl group.

[0182] In general formula (11), the thioalkyl group used as R_{60} is not especially limited, and examples include linear or branched thioalkyl groups having 1 to 12 carbon atoms, such as a thiomethyl group, a thioethyl group, a thiopropyl group, a thiobutyl group, and a thioethylylhexyl group.

[0183] In general formula (11), the amino group used as R_{60} is not especially limited, and examples include an amino group, a methylamino group, a dimethylamino group, a butylamino group, a dibutylamino group and a phenylamino group.

[0184] In general formula (11), the hetero ring group used as R_{60} is not especially limited, and examples include a pyridine ring, a thiophene ring, and a furan ring.

[0185] In general formula (11), the alkenyl group used as R_{60} is not especially limited, and examples include a vinyl group and a 3-butenyl group.

[0186] In general formula (11), the halogen atom used as R_{60} is not especially limited, and examples include a fluorine atom, a chloro atom, a bromo atom and an iodine atom.

[0187] In general formula (11), the alkoxy group used as R_{60} is not especially limited, and examples include a methoxy group, an ethoxy group, a propoxy group and a butoxy group.

[0188] In general formula (11), R_{60} represents preferably an alkyl group or an aryl group, and particularly preferably a methyl group, an ethyl group, a propyl group, a butyl group or a phenyl group because a subtype can be more easily identified in such a case.

[0189] In general formula (11), the alkyl group used as R_{61} to R_{66} is not especially limited, and examples include linear or branched alkyl groups having 1 to 12 carbon

atoms, such as a methyl group, an ethyl group, a propyl group, a butyl group and an ethylhexyl group.

[0190] In general formula (11), the aryl group used as R_{61} to R_{66} is not especially limited, and examples include a phenyl group, a methylphenyl group, a methoxyphenyl group, a thiomethylphenyl group, a chlorophenyl group, a bromophenyl group, a fluorophenyl group, a butylphenyl group, a dimethylphenyl group, a trimethylphenyl group and a naphthyl group.

[0191] In general formula (11), the hetero ring group used as R_{61} to R_{66} is not especially limited, and examples include a pyridine ring, a thiophene ring, and a furan ring.

[0192] In general formula (11), the aralkyl group used as R_{61} to R_{66} is not especially limited, and examples include a benzyl group or a phenethyl group.

[0193] In general formula (11), the sulfonyl group used as R_{61} to R_{66} is not especially limited, and examples include a sulfo group and a salt such as sodium sulfonate.

[0194] In general formula (11), the hetero ring formed when R_{62} and R_{63} or R_{65} and R_{66} are bound to each other is not especially limited, and an example includes a furan ring.

[0195] In general formula (11), R_{60} to R_{66} each may further have a substituent, and the substituent is not especially limited as long as the identification of a subtype is not largely inhibited. Examples include alkyl groups such as a methyl group, an ethyl group, a n-propyl group, an isopropyl group, a n-butyl group, an isobutyl group, a sec-butyl group and a tert-butyl group; aryl groups such as a phenyl group and a naphthyl group; alkoxy groups such as a methoxy group, an ethoxy group, and a butoxy group; aryloxy groups such as a phenoxy group and a naphthyloxy group; di-substituted amino groups such as a dimethylamino group, a N-ethyl-N-phenylamino group and a diphenylamino

group; acyl groups such as an acetyl group and a benzoyl group; a sulfonyl group; a carbamoyl group; a sulfamoyl group; hetero ring groups such as a pyridyl group, a triazinyl group, and a benzothiazolyl group; a nitro group; and halogen atoms such as a fluorine atom, a chlorine atom, a bromine atom and an iodine atom.

[0196] In general formula (11), the alkynyl group used as R_{67} and R_{67} is not especially limited, and examples include a vinyl group and a 3-butenyl group.

[0197] The compounds represented by general formula (11) of the present embodiment can be easily synthesized by a method similar to known methods (for example, NPL 4, NPL 5 and NPL 6).

[0198] Suitable specific examples of the compounds represented by general formula (11) of the present invention include, but are not limited to, the following compounds (139) to (169). In the following, TMS stands for a trimethyl silyl group ($-Si(CH_3)_3$).

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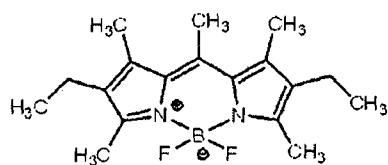
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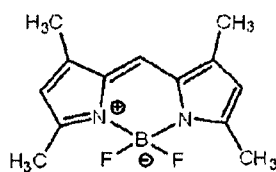
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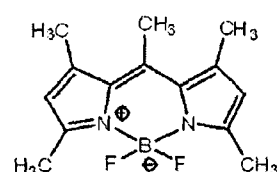
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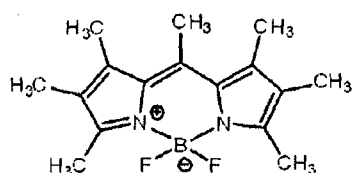
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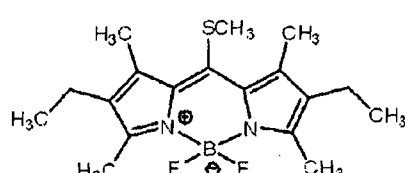
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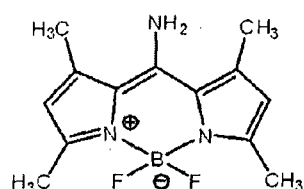
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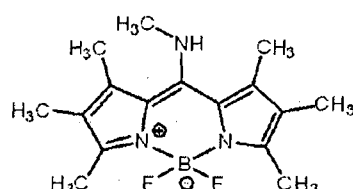
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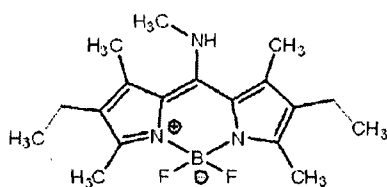
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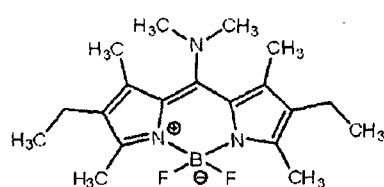
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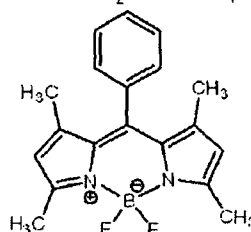
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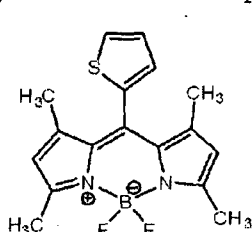
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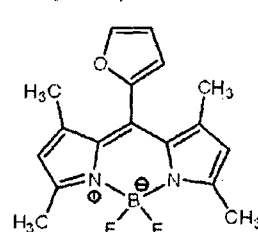
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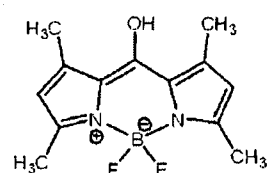
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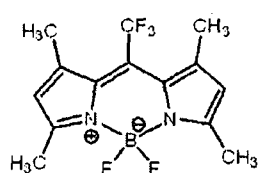
Compound (152)

Compound (153)

60
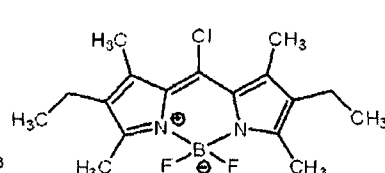
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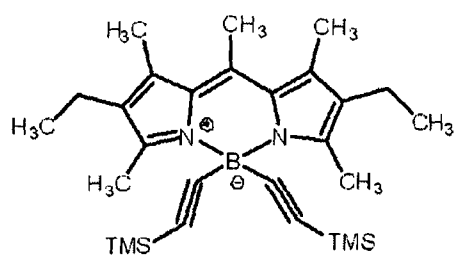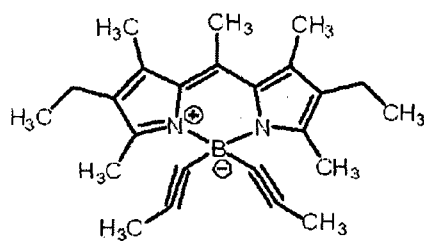
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Compound (155)
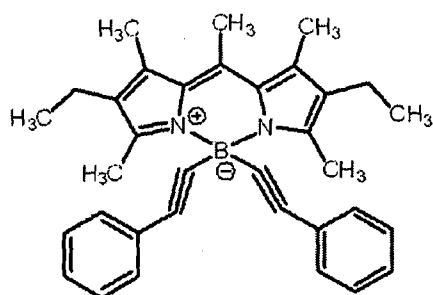
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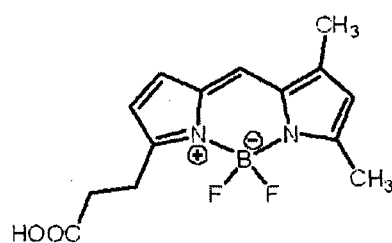
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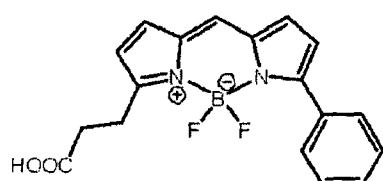
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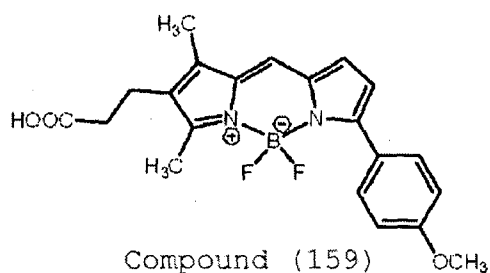
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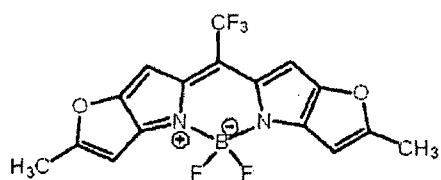
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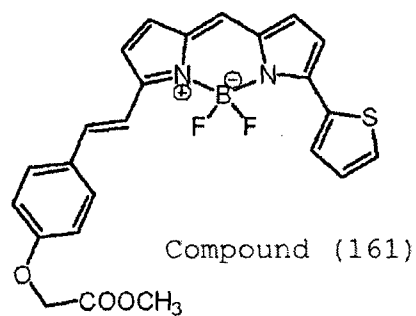
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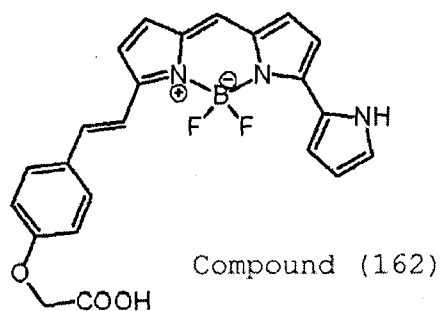
Compound (162)

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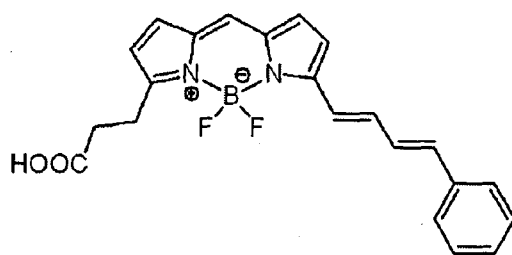
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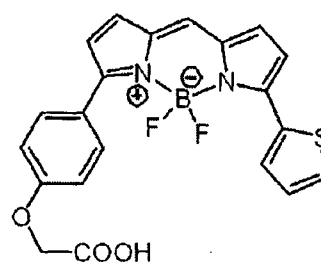
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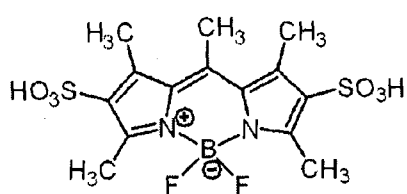
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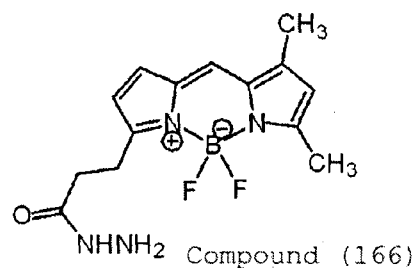
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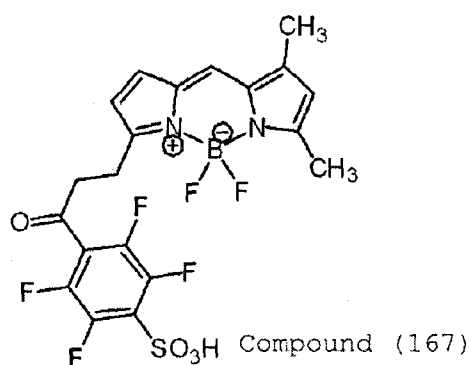
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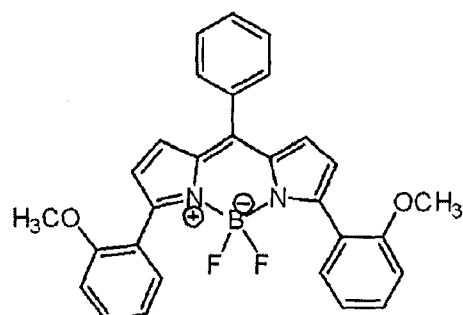
Compound (168)
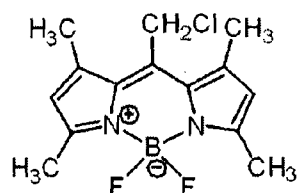
Compound (169)

[0202] The organic compounds of the present invention can be organic compounds having a molecular weight less than 10,000, preferably 5,000 or less, and more preferably 2,000 or less. Besides, the organic compounds of the present invention are preferably dye compounds, and

more preferably fluorescent compounds having
fluorescent characteristics. Since a fluorescent
compound has high sensitivity, the compound can be used
for staining at a low concentration, and hence, the
necessary amount of compound can be relatively reduced.

[0203] The wavelength for exciting each of the organic
compounds represented by general formulas (1), (6),
(10) and (11) of the present invention is not
especially limited, and a wavelength of 200 to 1400 nm
is generally used because of the limit of a laser
wavelength for generating excitation light and a range
of a wavelength that can be easily transmitted through
a living body. The wavelength can be 340 to 800 nm
because such a wavelength falls in to a wavelength
range in which fluorescence can be detected by various
types of apparatuses. In particular, if merely one
type of compounds represented by general formulas (1),
(6), (10) and (11) is used, a compound having an
excitation wavelength in the vicinity of 455 to 530 nm
can be suitably used because an inexpensive versatile
apparatus containing a 488 nm laser can be used.

[0204] Although it has been revealed that the macrophage
subtypes largely affect the pathologic change of an
inflammatory disease, the detailed mechanism has not
been clarified yet. When the identification agent of
the present invention is used, macrophage subtypes can
be identified to be visualized, and therefore, the
identification agent of the present invention can be
used as a research reagent for clarifying the
relationship between the pathologic change of an
inflammatory disease and a subtype. Furthermore, since
there are a large number of reports that a subtype
reflects the pathologic change of an inflammatory
disease, the identification agent of the present
invention can be used as a labeling agent for a subtype
to be used for determining or diagnosing the pathology

of an inflammatory disease in the research or clinical
field. The identification agent of the present
invention can be used as a labeling agent for making
pathological diagnosis in vitro or ex vivo, or a
labeling agent for making diagnosis in vivo with an
endoscope or the like.

[0205]<Regarding macrophages>
    In the present invention, subtypes are not especially
    limited as long as the subtypes are those different in
    the function, the expression gene, the expression
    protein, the cell producing substance such as a protein
    or a low-molecular weight substance, or the like, but
    herein refer to the M1 macrophage and the M2 macrophage.

[0206]The M1 macrophage herein is not especially limited as
    long as the M1 macrophage has functions of immune
    activation, promotion of inflammation, disinfection,
    antitumor and the like. Examples include macrophages
    having a large amount of proteins, expressed on cell
    surfaces, such as cluster of differentiation
    (hereinafter referred to as CD) 16, CD32, CD64, CD80,
    CD169 and lymphocyte antigen 6C, macrophages producing
    a large amount of inflammatory proteins such as tumor
    necrosis factor-α (hereinafter referred to as TNF-α),
    interleukin-6 (hereinafter referred to as IL-6),
    interleukin-12, interleukin-23 and interleukin-1β,
    macrophages producing a small amount of anti-
    inflammatory proteins such as interleukin-10
    (hereinafter referred to as the IL-10), macrophages
    producing a large amount of proteins such as CC
    chemokine ligand 2, CC chemokine ligand 3, CC chemokine
    ligand 4, CC chemokine ligand 5, CC chemokine ligand 9,
    CC chemokine ligand 10, CC chemokine ligand 11, CXC
    chemokine ligand 9, CXC chemokine ligand 10, and CXC
    chemokine ligand 11, macrophages producing a large
    amount of reactive nitrogen species such as nitrogen
    monoxide or reactive oxygen species, and macrophages

expressing, at high expression level, genes of any of
the aforementioned proteins, inducible nitric monoxide
synthase (hereinafter referred to as the iNOS) or the
like.

[0207] The M2 macrophage herein is not especially limited as
long as the M2 macrophage has functions of suppression
of immune or inflammation, healing of wound, tissue
remodeling, vascularization, promotion of tumor growth
and the like. Examples include macrophages having a
large amount of proteins, expressed on cell surfaces,
such as CD163, CD206, CD204, CD209, CD301, Dectin-1 and
Galectin-3, macrophages producing a large amount of
anti-inflammatory proteins such as IL-10, interleukin-
1RA and a decoy receptor for interleukin-1, macrophages
producing a small amount of inflammatory proteins such
as interleukin-12, macrophages producing a large amount
of proteins such as CC chemokine ligand 1, CC chemokine
16, CC chemokine ligand 17, CC chemokine ligand 18, CC
chemokine ligand 22, CC chemokine ligand 24, CXC
chemokine ligand 3, CXC chemokine ligand 4, CXC
chemokine ligand 23 and transforming growth factor β
(hereinafter referred to as the TGF-β), macrophages
producing a large amount of polyamine, and macrophages
expressing, at high expression level, genes of any of
the aforementioned proteins, arginase-1 (hereafter
referred to as the Arg1), Yml, FIZZ1 and the like.

[0208] <Regarding biological sample>
A biological sample used in the present invention is
not especially limited, and the biological sample can
be, for example, a biont, a biological tissue, a
biological tissue section, a biological tissue block,
human cells, animal cells, human cultured cells, or
animal cultured cells. The biological sample may be
precedently fixed with formalin or the like.

[0209] Examples of species for the biological sample of, for
example, vertebrates include a teleost such as tiger

puffer, grass puffer, green spotted puffer, killifish, or zebra fish; an amphibian such as a xenopus; a bird such as a chicken or a quail; a small animal such as a rat, a mouse or a hamster; a large animal such as a goat, a pig, a dog, a cat, a rabbit, a bovine or a horse; and a primate such as a monkey, a chimpanzee or a human.

[0210] Examples of species for the biological sample of invertebrates include a fruit fly and a roundworm.

[0211] Examples of the biological sample of cultured cells include cultured cells derived from normal tissues of any of the aforementioned species, or derived from various diseased tissues.

[0212] In some cases, cells of a specific subtype may be produced by culture, so as to be used for screening or the like.

[0213] A method for producing cells by culture is not especially limited as long as precursor cells of macrophages of monocytes or the like are stimulated by a specific cytokine or the like to be differentiated into a desired subtype.

[0214] The M1 macrophage can be produced, for example, by adding a macrophage colony stimulating factor (hereinafter referred to as the M-CSF), a granulocyte macrophage colony stimulating factor or phorbol myristate acetate (hereinafter referred to as the PMA) to a medium for culturing precursor cells of macrophages collected from a bone marrow, a spleen, a tissue, a blood or the like. Alternatively, in addition to the administration of the M-CSF, the granulocyte macrophage stimulating factor or the PMA, either of protein combinations of interferon-γ and lipopolysaccharide, and interferon-γ and a tumor necrosis factor may be added for producing the M1 macrophage.

[0215] The M2 macrophage can be produced, for example, by

adding an M-CSF, a granulocyte macrophage stimulating factor or the PMA to a medium for culturing precursor cells of macrophages collected from a bone marrow, a spleen, a tissue, a blood or the like. Alternatively, in addition to the administration of the M-CSF, the granulocyte macrophage stimulating factor or the PMA, any of proteins and protein combinations out of interleukin-4 (hereinafter referred to as the IL-4), interleukin-13(hereinafter referred to as the IL-13), IL-4 and IL-13, IL-10, an immune complex and interleukin-1β, an immune complex and lipopolysaccharide, an immune complex and a toll-like receptor, and an immune complex and interleukin-1 receptor ligand may be added for producing the M2 macrophage.

[0216] On the other hand, for purpose of developing a treatment method, in a case where, for example, a subtype present in a biological sample or in vivo is to be changed to another subtype, or macrophage having been changed in the subtype in vitro is intravitally administered to find the effect of the subtype change, the subtype can be changed by a method similar to that described above.

[0217] Identification method

Embodiment 5 of the present invention provides a method for identifying the macrophage subtypes M1 and M2, specifically, a method for identifying the macrophage M1 and the macrophage M2 based on spectral characteristics obtained with an organic compound added. The organic compound is not especially limited but can be a dye compound having a molecular weight of 2000 or less.

[0218] In the identification method of the present embodiment, the M1 macrophage or the M2 macrophage contained in a biological sample is identified preferably by utilizing a difference in staining properties between the M1

macrophage and the M2 macrophage.

[0219] In the present embodiment, the organic compound can be a macrophage identification agent of the present invention.

[0220] The identification method of the present embodiment can be performed, after staining a biological sample by exposing the macrophage identification agent of the present invention to the biological sample, by a flow cytometry method or by using an identifying apparatus such as a fluorescence activated cell sorting (hereinafter referred to as FACS). The subtypes can be identified by using such an apparatus by utilizing a difference in the staining properties depending upon the subtypes.

[0221] The difference in the staining properties is not especially limited, but can be, for example, a difference in the amount or speed for import of the macrophage identification agent into cells, a difference in the amount or speed for extracellularly export of the macrophage identification agent having been imported into cells, a difference in the interaction of the macrophage identification agent on cell surfaces of the subtypes, and a difference in the fluorescence intensity of the macrophage identification agent depending upon the subtypes.

[0222] Alternatively, a stained biological sample may be observed with a fluorescence microscope, so as to make identification in an image based on a difference in the fluorescence intensity derived from a difference in the abundance of the macrophage identification agent in cells or on cell surfaces depending upon the subtypes.

[0223] Alternatively, a plurality of excitation lights can be used for irradiation and a plurality of types of fluorescence can be detected, so that fluorescence derived from the macrophage identification agent can be detected by using a combination of a plurality of

excitation light and fluorescence wavelengths. When a plurality of excitation light and fluorescence wavelengths are thus combined, useful information for identifying the subtypes can be obtained.

[0224] In performing the identification by the flow cytometry method or by using the identifying apparatus such as an FACS, optical characteristics such as forward scattering and side scattering can be simultaneously used for removing, from signals detected by the identifying apparatus, signals derived from particles other than cells. Besides, the identification can be performed merely on living cells by staining dead cells with a dead cell detection reagent. As the dead cell detection reagent, a commercially available reagent can be suitably used.

[0225] In employing the observation with a fluorescence microscope, if an image of a biological sample is captured while allowing the compound used as the macrophage identification agent to emit light in cells or on cell surfaces by irradiating the biological sample with excitation light, a light emitting portion and a non-light emitting portion can be easily detected. Besides, if a bright field image obtained by irradiation with visible light and a fluorescent image obtained by irradiation with excitation light are combined with each other by using image processing means, distributions of the subtypes in the biological sample can be observed in more details. Furthermore, a confocal microscope can be used because an optical section image can be thus obtained. Besides, a multiphoton excitation fluorescence microscope can be suitably used for observing the inside of a biological sample because this type of microscope has deep tissue penetration and high spatial resolution.

[0226] The macrophage subtypes can be identified by the identification method of the present invention, and

therefore, the identification method of the present invention can be used for clarifying the relationship between the pathologic change of an inflammatory disease and the subtypes. Besides, the identification method of the present invention can be used for determining or diagnosing the pathology of an inflammatory disease in the research or clinical field.

[0227]<Staining>

In staining cells by exposing the macrophage identification agent of the present invention to the biological sample, the compound represented by general formula (1) may be directly used, or may be dissolved in a suitable solvent before use.

[0228]The solvent used in the present invention is not especially limited, and examples of the solvent include water, physiological saline, a buffer such as a phosphate buffered saline (hereinafter referred to as PBS) or a Tris buffer, a cell culture medium such as a Dulbecco's Modified Eagle Medium (hereinafter referred to as D-MEM), an Iscove's Modified Dulbecco's Medium (hereinafter referred to as IMDM), a Hanks' Balanced Salt Solutions (hereinafter referred to as HBSS), Minimun Essential Medium-Eagle, Earle's Salts Base, with Non-Essential Amino Acid (hereinafter referred to as the MEM-NEAA), or Roswell Park Memorial Institute Medium (hereinafter referred to as RPMI) 1640, a commercially available buffer for FACS analysis, and an infusion solution such as a lactated Ringer's solution. Such a solvent can particularly contain water by 50% or more. Besides, a mixture of two or more of these solvents can be used.

[0229]On the other hand, to such a solvent, a serum such as a fetal bovine serum (hereinafter referred to as the FBS) or a horse serum, or an antibacterial agent such as sodium azide or penicillin-streptomycin (hereinafter referred to as the P/S) can be added before use. In

particular, a physiological saline, a buffer such as a
PBS or a Tris buffer, a cell culture medium such as a
D-MEM, an IMDM, or a HBSS, a commercially available
buffer for FACS analysis, or an infusion solution such
as a lactated Ringer's solution can be suitably used
from the viewpoint of controlling a salt concentration,
pH and the like to be suitable for cells.

[0230] The compound represented by general formula (1) can be
dissolved, before mixing with the aforementioned
solvent, in an organic solvent such as dimethyl
sulfoxide (hereinafter referred to as DMSO) or ethanol
as long as the organic solvent is used at a
concentration not affecting the biological sample.

[0231] Another additive may be added if necessary. The
additive used in the present invention is not
especially limited as long as the staining of
macrophages is not affected, and examples of the
additive include a humectant, a surface tension
adjuster, a thickener, a salt such as sodium chloride,
any of various pH adjusters, a pH buffer solution, an
antiseptic agent, an antibacterial agent, a sweetener
and a perfume. One of these additives may be singly
used, or more of these additives may be used in
combination.

[0232] The concentration of the compound represented by
general formula (1) in the macrophage identification
agent of the present invention is not especially
limited as long as the compound is used in an amount
sufficient for identifying the subtypes, and can be
appropriately decreased/increased according to the
state of a sample in which a target subtype is present.
In general, the compound is used in a concentration of
0.001 nM or more and 1000 µM or less, and more
preferably in a concentration of 0.01 nM or more and
100 µM or less. Particularly when the compound is used
in vivo, the amount of the compound can be as small as

possible.

[0233] One of the compounds represented by general formula (1) for the macrophage identification agent of the present invention can be singly used, or two or more of the compounds may be used in combination. Besides, two or more of the compounds represented by general formula (1) for the macrophage identification agent of the present invention can be used in combination with a known fluorescent dye.

[0234] The staining of cells by exposing the macrophage identification agent of the present invention to the biological sample can be performed, in the case of a use in vitro or ex vivo, by mixing the biological sample with the macrophage identification agent in a suitable vessel, or bringing the macrophage identification agent into contact with the biological sample on a slide glass or the like by dropping, spraying or the like.

[0235] In the case of a use in vivo, the macrophage identification agent may be administered to a living body by orally administering the agent, or by injecting, spraying or applying the agent intravenously or intraarterially intravascularly, orally, sublingually, intrarectally, intraperitoneally, cutaneously, subcutaneously, intravesically, intratracheally (intrabronchially), intraocularly, intranasally or intraaurally. Alternatively, the macrophage identification agent may be used together with a probe of an endoscope such as a catheter.

[0236] The temperature at which the staining is performed is not especially limited, but the macrophage identification agent is exposed to the biological sample at a temperature of preferably 4 to 42°C, more preferably 4 to 38°C, further more preferably 31 to 38°C and most preferably 37°C.

[0237] The time for the staining is not especially limited,

but the macrophage identification agent is exposed to
the biological sample for preferably 1 minute or more
and 24 hours or less, more preferably 1 minute or more
and 4 hours or less, and further more preferably 1
minute or more and 1 hour or less. The staining can be
performed generally in 1 hour.

[0238] <Staining mechanism>

The macrophage identification agent of the present
invention can identify a subtype based on a difference
in the intensity of fluorescence derived from the
macrophage identification agent caused depending on the
subtypes, and this difference is caused, for example,
for the following reason: Depending on the subtypes,
the amount or speed for import of the macrophage
identification agent into cells is different, or the
amount or speed for extracellularly export of the
macrophage identification agent having been imported
into cells is different, the interaction of the
macrophage identification agent, having been imported
into cells, with a component present in the cells, such
as a high molecular weight substance, a low molecular
weight substance, a gaseous molecule, or an ion, is
different depending on the subtypes, or the interaction
of the macrophage identification agent with cell
surfaces is different depending on the subtypes.

[0239] The macrophage identification agent of the present
invention works as a substrate of at least one or more
transporters expressed on cell surfaces of a subtype,
and therefore, the amount or speed for import of the
macrophage identification agent into cells or the
amount or speed for extracellularly export of the
macrophage identification agent having been imported
into cells is different depending on the subtypes.

[0240] Herein, to "work as a substrate of a transporter" means
that the agent can be selectively transported by an
entry transporter in the absence of an influx

transporter inhibitor but cannot be transported in the presence of the influx transporter inhibitor, or that the migration via an influx transporter is changed in the presence of an influx transporter inhibitor. Alternatively, it means that the agent is selectively transported by an efflux transporter, that the agent is transported by an efflux transporter in the absence of an efflux transporter inhibitor but is not transported in the presence of the inhibitor, or that the migration via an efflux transporter is changed in the presence of an efflux transporter inhibitor.

[0241] The transporter is not especially limited, and examples include an ABC transporter, an SLC transporter, a glucose transporter and a dopamine transporter. Among these transporters, an efflux transporter is preferred, an ABC transporter is more preferred, and a transporter working as a substrate of Pgp (P-glycoprotein), BCRP (Breast Cancer Resistance Protein), MRP (Multidrug Resistance-associated Protein) or MDR (Multidrug Resistance) is further more preferred.

[0242] The macrophage identification agent of the present invention is different in the amount or speed of being imported into cells by endocytosis depending on the subtypes. If the macrophage identification agent of the present invention is modified with a liposome, the macrophage identification agent can be easily imported into cells by endocytosis.

[0243] The macrophage identification agent of the present invention shows, depending on the subtypes, a different interaction with a high molecular weight substance such as an enzyme or a protein, a low molecular weight substance such as a lipid, gaseous molecules such as reactive oxygen species or reactive nitrogen species, or ion species such as hydrogen ions present in the cells.

[0244] The macrophage identification agent of the present

invention shows, depending on the subtypes, a different
interaction with cell surfaces. The interaction
between the macrophage identification agent and the
cell surfaces is caused with at least one or more
proteins expressed on the cell surfaces of a subtype,
amino acid or a functional group contained in the
protein, a cell membrane, a lipid contained in the cell
membrane, a charge present on the cell surfaces, a
hydrophilic region present on the cell surfaces, a
hydrophobic region present on the cell surfaces, or the
like.

[0245] <Washing>

After the macrophage identification agent of the
present invention is exposed to the biological sample,
a washing operation may be performed as occasion
demands. The washing operation will now be described.

[0246] If a cell sample obtained by extraction or culture has
been stained, the cell sample is precipitated in a
proper vessel by centrifugation for removing the
staining solution. Next, a solution not containing a
dye compound such as the macrophage identification
agent of the present invention (i.e., a washing
solution) is added to the resultant for washing.

[0247] If a tissue or tissue section has been stained, a
washing operation is performed by exposing, in a proper
vessel or on a slide glass or the like, the resultant
sample to a solution not containing a dye compound such
as the macrophage identification agent of the present
invention (i.e., a washing solution).

[0248] The washed operation may be repeated more than once if
necessary. Besides, the biological sample may be
allowed to stand in a washing solution for a prescribed
period of time. Furthermore, a shaking or heating
operation may be performed if necessary.

[0249] If the washed biological sample is a sample of cells,
in particular, a stirring operation or a filtering

operation may be performed so that the cells cannot be
aggregated.

[0250] Sorting method

A sorting method for a subtype according to Embodiment
6 of the present invention will now be described.

[0251] Through the identification performed by the
aforementioned identification method for a subtype for
selectively sorting, a subtype of interest can be
sorted.

[0252] For sorting cells, a commercially available FACS
apparatus can be suitably used. Besides, if the
identification is performed by using an image, cells
can be sorted by selectively collecting or removing
cells of a subtype of interest or a subtype other than
the subtype of interest. For selectively collecting or
removing, an aspirator or the like can be used.

[0253] It has been revealed that a specific type of
macrophages plays a significant role in healing an
inflammatory disease. For example, there are reports
on research that the M1 macrophage plays a significant
role in removing cancer cells, and that the M2
macrophage plays a significant role in healing
nephritis. Since an arbitrary subtype can be sorted by
the sorting method for a macrophage subtype of the
present invention, the sorting method of the present
invention can be utilized in a treatment for an
inflammatory disease in which an arbitrary subtype is
transplanted in a disease site.

[0254] Evaluation method

An evaluation method for a subtype according to
Embodiment 7 of the present invention includes exposing
the macrophage identification agent of the present
invention to a biological sample.

[0255] Besides, in the evaluation method of the present
invention, after or simultaneously with exposing the
macrophage identification agent to a biological sample,

a test substance may be allowed to work on a part or
the whole of the biological sample.

[0256] The evaluation method of the present invention further
includes detecting the staining properties of a subtype
with the macrophage identification agent. Thus, the
type, number, ratio, optical characteristics or the
like of a subtype contained in the biological sample
can be evaluated.

[0257] If a test substance is allowed to work, an effect of
the test substance on the type, number, ratio, optical
characteristics or the like of the subtype can be
evaluated. At this point, if a biological sample on
which the test substance is allowed work and a
biological sample on which the test substance is not
allowed to work are separately evaluated, the action of
the test substance on the subtype can be evaluated
based on change in the type, number, ratio or the like
of the subtype depending on the presence or absence of
the test substance can be evaluated. Two or more types
of test substances may be used for evaluating a
difference in the action between the test substances.

[0258] Since the type, number, ratio or optical
characteristics of a macrophage subtype can be detected
by the evaluation method of the present invention, the
evaluation method of the present invention can be
utilized for clarifying the relationship between the
pathologic change of an inflammatory disease and a
subtype. Besides, in the research or clinical field,
the evaluation method of the present invention can be
used for determining or diagnosing the pathology of an
inflammatory disease. Furthermore, since there are a
large number of reports that the balance between the
subtypes is correlated with the prognosis or recurrence
of an inflammatory disease, the evaluation method of
the present invention can be used for predicting the
prognosis or recurrence of an inflammatory disease. In

addition, if the change in subtype caused after
administering a drug is examined by the evaluation
method of the present invention, a therapeutic drug can
be developed, an optimal therapeutic drug can be
selected, or the effect of a selected therapeutic drug
can be evaluated.

[0259] Analysis method

In Embodiment 8 of the present invention, an analysis
method in which a macrophage identification agent is
exposed to a biological sample simultaneously with,
before or after exposing a test substance to the
biological sample so as to analyze staining properties
of a subtype is provided. The test substance may be a
substance affecting or not affecting the staining
properties of the subtype with the macrophage
identification agent. Besides, two or more types of
test substances may be used for analyzing a difference
in the action between the test substances.

[0260] In using a test substance affecting the staining
properties of a subtype with the macrophage
identification agent, if a biological sample on which
the test substance is allowed to work and a biological
sample on which the test substance is not allowed to
work are separately evaluated, the influence of the
test substance on the staining properties can be
evaluated. Thus, the correlation between the test
substance and a transporter expressed on surfaces of
cells in which the macrophage identification agent
works as a substrate, or a protein, a functional group
or the like expressed on surfaces of cells with which
the macrophage identification agent interacts can be
analyzed.

[0261] In using a test substance not affecting the staining
properties of a subtype with the macrophage
identification agent, the correlation between the
subtype identified based on the staining properties

with the macrophage identification agent and the test
substance can be analyzed. A test substance not
affecting the staining properties of a subtype with the
macrophage identification agent may be a substance
affecting or not affecting the subtype.

[0262] In using a test substance not affecting the staining
properties of a subtype with the macrophage
identification agent but affecting the subtype, if a
biological sample on which the test substance is
allowed to work and a biological sample on which the
test substance is not allowed to work are separately
evaluated, the action of the test substance on the
subtype can be evaluated based on change in the type,
number, ratio or the like of the subtype depending upon
the presence or absence of the test substance.

[0263] In using a test substance affecting neither the
staining properties of a subtype with the macrophage
identification agent nor the subtype, optical
characteristics derived from the test substance may be
measured for detecting the interaction between the test
substance and the subtype. If a test substance or a
material used for labeling the test substance has
fluorescence, the interaction between the test
substance and the subtype can be detected by
fluorescence measurement. For accurately performing
the analysis, the test substance or the material used
for labeling the test substance can be selected to have
a different excitation wavelength or a different
fluorescence wavelength from that of the macrophage
identification agent of the present invention. If the
excitation wavelength or the fluorescence wavelength is
different, the binding, incorporation or the like of
the test substance to the subtype can be analyzed by
detecting a fluorescent signal derived from the
macrophage identification agent and a fluorescent
signal derived from the test substance or the material

used for labeling the test substance. The test
substance having fluorescence is not especially limited,
and may be a fluorescent surface antigen marker, or an
organic or inorganic molecule having fluorescence.

[0264] If the correlation between a subtype and a drug (i.e.,
change in the subtype caused after administering a
drug) is examined by the analysis method of the present
invention, a therapeutic drug can be developed, an
optimal therapeutic drug can be selected, or the effect
of a selected therapeutic drug can be analyzed.

[0265] Screening method
In Embodiment 9 of the present invention, a screening
method in which a macrophage identification agent is
exposed to a biological sample simultaneously with,
before or after exposing a substance to the biological
sample so as to screen the substance based on the
staining properties of a subtype is provided. In the
screening, the evaluation method or the analysis method
of the present invention described above can be used,
so as to evaluate or analyze the effect or the
interaction of a plurality of test substances on or
with the subtype.

[0266] For example, a biologically active substance working on
the action of influx and efflux transporters of a
compound expressed in a subtype can be evaluated. In
particular, the action of an efflux transporter can be
suitably evaluated. More preferably, an ABC
transporter can be evaluated. In using the macrophage
identification agent of the present invention, if the
action of an efflux transporter is inhibited by the
influence of a biologically active substance and hence
the subtype is poorly separated or satisfactorily
separated, or is not different from other cells, it can
be evaluated that the biologically active substance
acts on the efflux transporter.

[0267] Alternatively, if a fluorescent labeled anti-surface

antigen antibody is used as a test substance in order
to examine a surface antigen expressed in a subtype
identified by the macrophage identification agent, a
type of antibody having a high affinity with the
subtype can be screened.

[0268] Alternatively, if a fluorescent substance unknown in
the incorporation or interaction into or with a subtype
is used as a test substance, the function of the test
substance on a subtype identified by the macrophage
identification agent can be evaluated based on
fluorescence intensity.

[0269] There are a large number of reports stating that a
specific type of macrophage plays a significant role in
healing a disease in addition to reports stating that a
subtype reflects the pathologic change of an
inflammatory disease, and therefore, a therapeutic drug
can be developed, an optimal therapeutic drug can be
selected or the effect of a selected therapeutic drug
can be screened by examining the correlation between a
subtype and a drug (i.e., change in the subtype caused
after administering a drug) by the screening method of
the present invention.

[0270] Kit

An identification kit according to Embodiment 10 of the
present invention contains at least one or more
macrophage identification agents of the present
invention. Besides, although not especially limited,
the identification kit can contain a vessel, a reagent
and the like necessary for exposing the macrophage
identification agent to a biological sample.

[0271] By using the identification kit of the present
invention, an inflammatory disease can be simply
diagnosed in situ at the home, in a place for medical
examination or in an emergency medical scene.

[0272] The present invention will now be described in more
details with reference to examples, and it is noted

that these examples are described merely for making the
present invention more deeply understood and do not
limit the present invention at all.

Examples

[0273] <Synthetic Example 1>

As an example of the macrophage identification agent
represented by general formula (1) of the present
invention, a synthetic example of the compound (1) will
be described.

Aldehyde (1)   Compound (B1)   Compound (1)
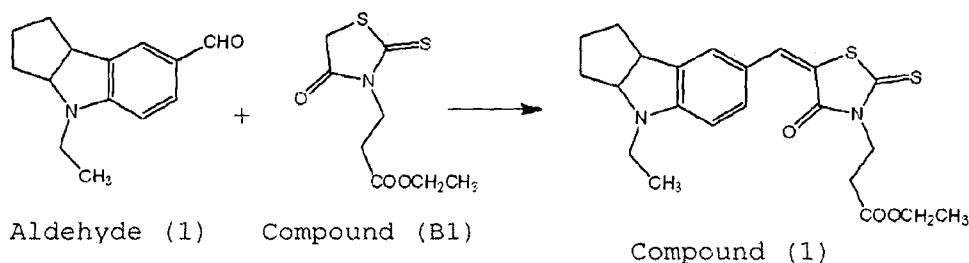
[0274] To a 20 mL acetic acid solution of 2.5 g (11.4 mmol) of
aldehyde (1), 2.7 g (11.5 mmol) of a compound (B1) and
1.6 g of ammonium acetate were added, and the resulting
solution was stirred for 2 hours under reflux. After
completing the reaction, 50 mL of water was slowly
added dropwise thereto while cooling, and the resulting
solution was cooled to room temperature. The thus
deposited solid was filtered out, washed with 100 mL of
water twice, and further washed with 50 mL of 2-
propanol, thereby obtaining 3.0 g (yield: 60.1%) of the
compound (1) of interest.

[0275] The obtained substance was confirmed to be the compound
(1) of interest by ^{1}H nuclear magnetic resonance
spectrometric analysis (ECA-400, manufactured by JEOL
Ltd.) and LC/TOF MS (LC/MSD TOF, manufactured by
Agilent Technologies, Inc.).

[0276] <Other compounds represented by general formula (1)>

In the same manner as in Synthetic Example 1, compounds
shown in Table 4 were synthesized and identified as the
compounds of interest.

[0277] Subtype identification by using macrophage

identification agent represented by general formula (1)

Example 1

[0278]<Culture of subtypes>

The culture of subtypes was performed according to a protocol described in NPL 7. Specifically, bone marrow cells collected from the femur of a Balb/c mouse of 8 to 10 weeks old were dispersed to a concentration of 1 x 10^6/mL in an IMDM containing 20% FBS and 1% P/S, and 1 x 10^7 cells were seeded in a 100 mm dish. After further adding an M-CSF (manufactured by Pepro Tech) thereto to a concentration of 50 ng/mL, the cells were incubated for 3 to 4 days in the presence of 5% CO_2 at 37°C, so as to differentiate macrophage precursor cells to macrophages. After exchanging the medium, the M-CSF was added to a concentration of 50 ng/mL, and the incubation was performed for further 3 to 4 days in the presence of 5% CO_2 at 37°C, and then, the resulting cells were differentiated to macrophage. By adding 200 ng of interferon-γ and 1 μg of polyliposaccharide (both manufactured by Pepro Tech) to the IMDM containing 5% FBS and 1% P/S, the cells were differentiated to the subtype M1. Besides, by adding 100 ng of IL-4 to the IMDM containing 5% FBS and 1% P/S, the cells were differentiated to the subtype M2. After performing the incubation for further 3 days in the presence of 5% CO_2 at 37°C, the resultant was rinsed with PBS, and PBS containing 0.25% trypsin and 1 mM EDTA (ethylenediaminetetraacetic acid) was used for collecting, from the dish, the macrophages having been differentiated to the subtypes M1 and M2.

[0279]<Confirmation of subtypes>

The thus collected M1 and M2 macrophages were subjected to gene expression analysis for confirming that the cells had been differentiated to the subtypes. From a part (approximately 0.5 to 5 x 10^6 macrophages) of each of the M1 macrophages and the M2 macrophages thus

collected, total RNA was extracted according to a
protocol attached to RNeasy mini kit manufactured by
Qiagen. Thereafter, cDNA was synthesized by reverse
transcription performed according to a protocol of
SuperScript (R) VILO (TM) cDNA Synthesis Kit
(manufactured by Invitrogen). Primers for cDNAs of M1
macrophage marker genes (iNOS and TNFα) and cDNA of M2
macrophage marker genes (Arg1 and CD206) were
synthesized. The sequences of the synthesized primers
are shown in Table 2. A polymerase chain reaction was
performed by using Applied Biosystems 7500 Real-Time
PCR system according to a protocol of PowerSYBR (R)
Green PCR Master Mix (manufactured by Applied
Biosystems), so as to quantitatively determine the
expression level of each marker gene. Fig. 1
illustrates a result of comparison in the expression
level among the marker genes obtained from the cells
having been differentiated to the M1 macrophages and
the M2 macrophages. RQ (Relative Quantification) on
the ordinate indicates the expression level of each
marker gene obtained from the cells differentiated to
the M2 macrophages shown as a relative value obtained
by assuming that the expression level of the marker
gene obtained from the cells differentiated to the M1
macrophage is 1. As is obvious from Fig. 1, the
expression levels of the iNOS and the TNFα were higher
in the cells differentiated to the M1 macrophages than
in the cells differentiated to the M2 macrophages. On
the other hand, the expression levels of the Arg1 and
the CD206 were higher in the cells differentiated to
the M2 macrophages than in the cells differentiated to
the M1 macrophages. It was confirmed based on this
result that the M1 macrophages and the M2 macrophages
could be prepared by the differentiation.

[0280]Table 2

| Target gene | Sequence of primer | | Remarks |
	Forward	Reverse	
GAPDH	TGAAGCAGGCATCTGAGGG	CGAAGGTGGAAGAGTGGGAG	Endogenous control gene
iNOS	AGACCTCAACAGAGCCCTCA	TCGAAGGTGAGCTGAACGAG	M1 macrophage marker
TNFα	ACGGCATGGATCTCAAAGAC	AGATAGCAAATCGGCTGACG	M1 macrophage marker
Arg1	CTCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC	M2 macrophage marker
CD206	CGGTGAACCAAATAATTACCAAAAT	GTGGAGCAGGTGTGGGCT	M2 macrophage marker

[0281]<Staining of subtypes>

Two x 10^5 each of the thus collected M1 and M2 subtypes were dispensed into a 1.5 mL tube, the compound (1) was added thereto to a concentration of 1 µM, and the resultant was incubated at 37°C for 30 minutes. Thereafter, the tube was centrifuged at 180 G for 10 minutes to remove the supernatant, a dye for identifying dead cells (aqua fluorescent reactive dye, manufactured by Invitrogen; which is hereinafter sometimes abbreviated as aqua) was added thereto, and the resultant was incubated at 4°C for 10 minutes. Subsequently, the tube was centrifuged at 180 G for 10 minutes to remove the supernatant, cells were suspended in a FACS buffer to be analyzed by using FACSCanto (trademark) II flow cytometry apparatus manufactured by BD.

[0282]<Subtype identification>

The analysis was performed on a cell population showing a low signal for the aqua, so as to analyze a cell population from which dead cells were eliminated.

A fluorescent signal derived from the compound (1) was measured in a FITC channel (excited at 488 nm, 530/30 nm: center wavelength/wavelength width). A histogram was prepared by plotting fluorescence intensity obtained in the FITC channel on the abscissa and plotting the number of cells at each fluorescence

intensity on the ordinate, which is shown in Fig. 2.

[0283] Examples 2 to 54

Fluorescent signals derived from the compounds (3), (4), (6), (7), (9), (11) to (13), (15), (17), (19) to (26), (28), (30) to (33), (35), (36), (38), (42) to (45), (48) to (62), (65), (67), (74) to (76), (82), (83) and (85) were analyzed in the same manner as in Example 1 except that the compound (1) used in Example 1 was replaced with the compounds (3), (4), (6), (7), (9), (11) to (13), (15), (17), (19) to (26), (28), (30) to (33), (35), (36), (38), (42) to (45), (48) to (62), (65), (67), (74) to (76), (82), (83) and (85).

[0284] Comparative Example 1

A fluorescent signal derived from a comparative compound (1) was analyzed in the same manner as in Example 1 except that the compound (1) used in Example 1 was replaced with the comparative compound (1). However, the fluorescent signal derived from the comparative compound (1) was measured in a PE-Cy7 channel (excited at 488 nm, 780/60 nm: center wavelength/wavelength width). A histogram was prepared by plotting fluorescence intensity obtained in the PE-Cy7 channel on the abscissa and plotting the number of cells at each fluorescence intensity on the ordinate, which is shown in Fig. 3.

[0285] Comparative Example 2

A fluorescent signal derived from a comparative compound (2) was analyzed in the same manner as in Example 1 except that the compound (1) used in Example 1 was replaced with the comparative compound (2). A histogram was prepared by plotting fluorescence intensity obtained in the FITC channel on the abscissa and plotting the number of cells at each fluorescence intensity on the ordinate, which is shown in Fig. 4.
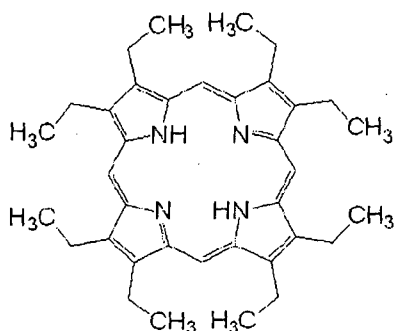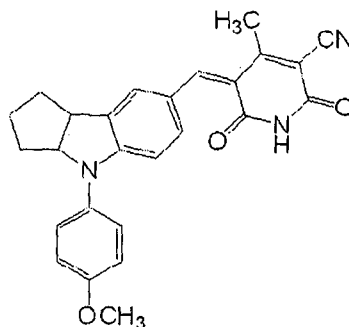
Comparative Compound (1)   Comparative Compound (2)

[0286] The results obtained in Examples 1 to 54 and
        Comparative Examples 1 and 2 described above are shown
        in Table 4.

[0287] <Evaluation method for identification ability of
        macrophage identification agent represented by general
        formula (1)>
        The fluorescent signals derived from the compounds (3),
        (4), (6), (7), (9), (11) to (13), (15), (17), (19) to
        (26), (28), (30) to (33), (35), (36), (38), (42) to
        (45), (48) to (62), (65), (67), (74) to (76), (82),
        (83) and (85) and the comparative compounds (1) and (2)
        were measured in channels suitable to the respective
        compounds. Histograms were prepared by plotting
        fluorescence intensities obtained in the respective
        channels on the abscissa and plotting the numbers of
        cells at each fluorescence intensity on the ordinate.
        A difference between a fluorescence intensity (F_{M1}) at a
        peak top of the histogram of the M1 macrophage and a
        fluorescence intensity (F_{M2}) at a peak top of the
        histogram of the M2 macrophage is divided by a sum of a
        peak width (W_{M1}) of the histogram of the M1 macrophage
        and a peak width (W_{M2}) of the histogram of the M2
        macrophage, so as to obtain a resolution R, which was
        used as an index of the identification ability for a
        subtype (see the following expression (1)).
        Resolution R = $|F_{M1} - F_{M2}| / (W_{M1} + W_{M2})$ ... Expression
        (1)

[0288] Based on the thus obtained values of the resolution R, the identification abilities for the subtypes of the compounds (3), (4), (6), (7), (9), (11) to (13), (15), (17), (19) to (26), (28), (30) to (33), (35), (36), (38), (42) to (45), (48) to (62), (65), (67), (74) to (76), (82), (83) and (85) and the comparative compounds (1) and (2) were evaluated as any of A to C according to the following criteria, and if the resolution R was 0.15 or more, the compound was determined to be good in the subtype identification.

A: The compound is very good in the subtype identification (namely, the resolution R is 0.5 or more);

B: the compound is good in the subtype identification (namely, the resolution R is 0.15 or more and less than 0.5); and

C: the compound cannot identify the subtypes (namely, the resolution R is less than 0.15).

[0289] Besides, with respect to each of the compounds evaluated to have the identification ability rated as A or B among the compounds (3), (4), (6), (7), (9), (11) to (13), (15), (17), (19) to (26), (28), (30) to (33), (35), (36), (38), (42) to (45), (48) to (62), (65), (67), (74) to (76), (82), (83) and (85) and the comparative compounds (1) and (2), a subtype strongly stained, out of the M1 macrophage (M1) and the M2 macrophage (M2), is shown in a column of "Selectivity" in Table 4.

[0290] In Table 3, "Channel" refers to the channel used in measuring the fluorescent signal derived from the corresponding compound. The details of the respective channels are shown in Table 3.

[0291]Table 3

Channel	Excitation wavelength (nm)	Center wavelength (nm)	Wavelength width (nm)
FITC	488	530	30
PE	488	585	42
PerCP-Cy5.5	488	670-735[*1]	
PE-Cy7	488	780	60
APC	633	660	20
APC-Cy7	633	780	60
Pacific Blue	405	450	50
AmCyan	405	510	50

[*1] Fluorescence wavelength measurement region

[0292]Table 4

Example	Compound	Channel	Peak position of M1 macrophage	Peak position of M2 macrophage	Resolution ratio R	Evaluation of macrophage subtype identification	Selectivity
Example 1	(1)	FITC	85.8	12.4	1.150	A	M1
Example 2	(3)	PE	11.2	25.0	0.622	A	M2
Example 3	(4)	FITC	158	45.4	1.125	A	M1
Example 4	(6)	PE-Cy7	26.5	50.4	0.504	A	M2
Example 5	(7)	FITC	65.4	23.6	0.567	A	M1
Example 6	(9)	FITC	2.63	6.69	0.828	A	M2
Example 7	(11)	PE	30.5	16.2	0.544	A	M1
Example 8	(12)	PE	50.7	97.7	0.594	A	M2
Example 9	(13)	PE	7.74	20.4	0.714	A	M2
Example 10	(15)	PE	7.52	10.5	0.700	A	M2
Example 11	(17)	FITC	7.74	16.5	0.577	A	M2
Example 12	(19)	Pacific Blue	0.90	0.64	0.319	B	M1
Example 13	(20)	PE-Cy7	4.47	2.10	0.290	B	M1
Example 14	(21)	FITC	40.0	20.3	0.351	B	M1
Example 15	(22)	PE-Cy7	199	313	0.389	B	M2
Example 16	(23)	PE	1.10	1.48	0.185	B	M2
Example 17	(24)	PE	18.1	27.6	0.347	B	M2
Example 18	(25)	PE	27.4	18.2	0.346	B	M1
Example 19	(26)	PE	3.51	4.49	0.202	B	M2
Example 20	(28)	PE	9.54	15.8	0.431	B	M2
Example 21	(30)	PE-Cy7	55.1	73.9	0.257	B	M2
Example 22	(31)	PE-Cy7	65.1	89.0	0.248	B	M2
Example 23	(32)	PE-Cy7	88.7	155	0.476	B	M2
Example 24	(33)	Am Cyan	5.00	9.05	0.522	A	M2
Example 25	(35)	Pacific Blue	1.15	1.29	0.192	B	M2
Example 26	(36)	Pacific Blue	5.70	3.82	0.344	B	M1
Example 27	(38)	FITC	3.74	1.63	0.724	A	M1
Example 28	(42)	FITC	4.19	1.79	0.616	A	M1
Example 29	(43)	PE	7.14	13.0	0.411	B	M2
Example 30	(44)	PE	24.5	12.9	0.330	B	M1

Table 4 (continued)

Example	Compound	Channel	Peak position of M1 macrophage	Peak position of M2 macrophage	Resolution ratio R	Evaluation of macrophage subtype identification	Selectivity
Example 31	(45)	FITC	18.2	4.84	0.822	A	M1
Example 32	(48)	FITC	65.4	9.74	0.621	A	M1
Example 33	(49)	PE	3.26	5.02	0.486	B	M2
Example 34	(50)	FITC	3.24	0.99	1.025	A	M1
Example 35	(51)	PE-Cy7	87.5	23.3	0.684	A	M1
Example 36	(52)	PE	3.90	1.68	0.756	A	M1
Example 37	(53)	PerCP-Cy5.5	128	190	0.650	A	M2
Example 38	(54)	PerCP-Cy5.5	154	211	0.531	A	M2
Example 39	(55)	PE	2.34	1.86	0.206	B	M1
Example 40	(56)	PE	4.90	6.36	0.207	B	M2
Example 41	(57)	PE-Cy7	4.20	3.59	0.167	B	M1
Example 42	(58)	PE	1.67	6.20	0.698	A	M2
Example 43	(59)	APC-Cy7	188	325	0.733	A	M2
Example 44	(60)	PE	4.55	7.65	0.621	A	M2
Example 45	(61)	PE	2.39	3.15	0.219	B	M2
Example 46	(62)	PerCP-Cy5.5	193	333	0.561	A	M2
Example 47	(65)	PE	2.96	4.67	0.236	B	M2
Example 48	(67)	PE	8.28	27.6	0.808	A	M2
Example 49	(74)	Pacific Blue	2.52	1.25	0.538	A	M1
Example 50	(75)	PE-Cy7	96.1	235	0.665	A	M2
Example 51	(76)	PerCP-Cy5.5	7.77	10.5	0.283	B	M2
Example 52	(82)	Pacific Blue	3.18	1.42	0.783	A	M1
Example 53	(83)	PE-Cy7	20.3	10.6	0.516	A	M1
Example 54	(85)	PE-Cy7	225	285	0.179	B	M2
Comparative Example 1	Comparative compound (1)	PE-Cy7	1.70	1.86	0.006	C	not identified
Comparative Example 2	Comparative compound (2)	FITC	0.86	1.05	0.007	C	not identified

[0293] As is obvious from Table 4 and Figs. 2 to 4, although there is no difference in the staining intensity between the M1 macrophages and the M2 macrophages stained by using the comparative compound (1) or (2) and hence these macrophages cannot be identified, if the macrophage identification agent containing the dye compound represented by general formula (1) of the present invention is used, the M1 macrophages and the M2 macrophages can be identified from each other based on a difference in the fluorescence intensity.

[0294] Example 55

Two x 10^5 each of M1 macrophages and M2 macrophages cultured in the same manner as in Example 1 were dispensed into a 1.5 mL tube, and the compound (1) and

the compound (33) were added thereto each to a
concentration of 1 µM.   Dead cells were identified by
using TO-PRO-3 (manufactured by Invitrogen).

[0295] A fluorescent signal derived from the compound (1) was
measured in the FITC channel (excitation wavelength:
488 nm, center wavelength: 530 nm, wavelength width: 30
nm) and a fluorescent signal derived from the compound
(33) was measured in an AmCyan channel (excitation
wavelength: 405 nm, center wavelength: 510 nm,
wavelength width: 50 nm).   A cytogram developed by
plotting the intensity of the fluorescent signal
derived from the compound (1) and obtained in the FITC
channel on the ordinate and plotting the intensity of
the fluorescent signal derived from the compound (33)
and obtained in the AmCyan channel on the abscissa was
prepared.

[0296] The analysis was performed on a cell population showing
a low signal for the TO-PRO-3, so as to analyze a cell
population from which dead cells were eliminated.

[0297] The operation was performed in the same manner as in
Example 1 except for the above.

[0298] As a result, as is obvious from Figs. 5A to 5E, when
the compound (1) is used, the M1 macrophages are more
strongly stained than the M2 macrophages.   On the
contrary, when the compound (33) is used, the M2
macrophages are more strongly stained than the M1
macrophages.   Therefore, the M1 macrophages and the M2
macrophages stained with the compound (1) and the
compound (33) are plotted, on the cytogram developed
with respect to the intensities of the fluorescent
signals derived from these compounds, as cell
populations having different fluorescent
characteristics, and hence, these macrophages can be
easily identified.

[0299] This example reveals that the subtypes can be
identified even when two or more types of compounds for

the macrophage identification agent represented by
general formula (1) of the present invention are used
in combination.

[0300] Sorting of subtype by using macrophage identification
agent represented by general formula (1)
Example 56
One x 10^5 each of M1 macrophages and M2 macrophages
cultured in the same manner as in Example 1 were mixed
in a 1.5 mL tube, and the compound (1) was added
thereto to a concentration of 1 μM.
The operation was performed in the same manner as in
Example 1 except for the above.

[0301] As a result, as is obvious from Figs. 6A to 6C, the M1
macrophages and the M2 macrophages stained with the
compound (1) were plotted, on a cytogram developed with
respect to the intensity of forward scattered light
affected by the size of cells and the intensity of a
fluorescent signal derived from the compound (1), as
cell populations having different fluorescence
intensity distributions.

[0302] Since the compound (1) has a characteristic to stain
the M1 macrophages more strongly than the M2
macrophages, the M1 macrophages can be identified as a
cell population having a high fluorescence intensity
derived from the compound (1), and on the other hand,
the M2 macrophages can be identified as a cell
population having a low fluorescence intensity derived
from the compound (1).

[0303] Besides, the M1 macrophages and the M2 macrophages
identified by using the compound (1) could be each
sorted with an FACS apparatus. The sorted subtypes
were confirmed by a fluorescent antibody method and
gene expression analysis.

[0304] It was found through Examples 1 to 56 described above
that the subtypes can be identified and sorted by using
the macrophage identification agent represented by

general formula (1) of the present invention.

[0305] Analysis and screening of subtype by using macrophage identification agent represented by general formula (1)

Example 57

Two x 10⁵ each of M1 macrophages and M2 macrophages cultured in the same manner as in Example 1 were dispensed into a 1.5 mL tube, and the compound (1) was added thereto to a concentration of 1 μM. Besides, Fumitremorgin C (manufactured by Sigma-Aldrich, hereinafter referred to as FTC) was added thereto, as a substance for inhibiting export of the compound (1) having been imported into cells, to a concentration of 10 μM, and the resultant was incubated at 37°C for 30 minutes. Thereafter, the tube was centrifuged at 180 G for 10 minutes to remove the supernatant, and then, 1 mL of a HBSS buffer containing the FTC in a concentration of 10 μM was added thereto to suspend the cells, and the resultant was further incubated at 37°C for 30 minutes.

[0306] Furthermore, each tube was centrifuged at 180 G for 10 minutes to remove the supernatant, the cells were suspended by adding the dye aqua for identifying dead cells thereto, and the resultant was incubated at 4°C for 10 minutes. Thereafter, the tube was centrifuged at 180 G for 10 minutes to remove the supernatant, and then, the cells were suspended in a FACS buffer and analyzed by using a FACSCanto (trademark) II flow cytometry apparatus manufactured by BD.

The analysis was performed on a cell population showing a low signal for the aqua, so as to analyze a cell population from which dead cells were eliminated.

[0307] Comparative Example 3

The analysis was performed in the same manner as in Example 57 except that the FTC was not added. As a result, as is obvious from Figs. 7A and 7B, in a histogram in which the fluorescence intensity obtained

in the FITC channel used for measuring a fluorescent
signal derived from the compound (1) was plotted on the
abscissa and the number of cells at each fluorescence
intensity was plotted on the ordinate, the enhancement
of the fluorescence intensity caused by the inhibition
by the FTC of the export of the compound (1) from the
cells was observed merely in the M2 macrophages. Thus,
it was found that the influence of the export
inhibition by the FTC is different depending on the
subtypes.

[0308] Example 58

Two x 10^5 each of M1 macrophages and M2 macrophages
cultured in the same manner as in Example 1 were
dispensed into a 1.5 mL tube, the compound (1) was
added thereto to a concentration of 1 μM, and the
resultant was incubated at 37°C for 30 minutes.
Thereafter, the tube was centrifuged at 180 G for 10
minutes to remove the supernatant, and then, a blocking
treatment for cell surfaces was performed by using
2.4G2 (anti-mouse CD16/32 antibody, manufactured by
Biolegend). To the cells having been subjected to the
blocking treatment, a FACS buffer containing 0.2 μg of
Pacific Blue-labeled anti-CD40 antibody and 0.1 μg of
Alexa Fluor647-labeled anti-Dectin-1 antibody was added
for performing immunofluorescent staining. The
fluorescent labeling with these antibodies was selected
so that the fluorescence wavelengths of the antibodies
could overlap neither with each other nor with the
fluorescence wavelengths of the compound (1) and the
aqua.

[0309] Thereafter, each tube was centrifuged at 180 G for 10
minutes to remove the supernatant, the cells were
suspended by adding the dye aqua for identifying dead
cells, and the resultant was incubated at 4°C for 10
minutes. Then, after the tube was centrifuged at 180 G
for 10 minutes to remove the supernatant, the cells

were suspended in a FACS buffer and analyzed by using
the FACSCanto (trademark) II flow cytometry apparatus
manufactured by BD. The analysis was performed on a
cell population showing a low signal for the aqua, so
as to analyze a cell population from which dead cells
were eliminated.

[0310] As a result, in the M1 macrophages identified based on
the strong staining with the compound (1), a
fluorescent signal derived from the Pacific Blue-
labeled anti-CD40 antibody was observed in a Pacific
Blue channel (excited at 405 nm, 450/50 nm: center
wavelength/wavelength width). On the contrary, in the
M2 macrophages identified based on the weak staining
with the compound (1), a fluorescent signal derived
from the Alexa Fluor647-labeled anti-Dectin-1 antibody
was observed in an APC channel (excited at 633 nm,
660/20 nm: center wavelength/wavelength width). It was
found based on this result that the anti-CD40 antibody
and the anti-Dectin-1 antibody are antibodies
specifically binding respectively to the M1 macrophages
and the M2 macrophages.

[0311] Identification of macrophage subtypes differentiated
from mouse cell line by using macrophage identification
agent represented by general formula (1)
Example 59

[0312] The subtypes were identified by using the compound (1)
in the same manner as in Example 1 except that the
macrophage subtypes differentiated from the mouse bone
marrow cells were changed to macrophage subtypes
differentiated from a cell line established from mouse
monocytic leukemia.

[0313] <Culture of subtypes>
The culture of subtypes was performed with reference to
NPL 9. Specifically, RAW264.7 cells purchased from
American Type Culture Collection, that is, a cell line
established from mouse monocytic leukemia, were

subcultured several times, and the resultant cells were dispersed to a concentration of 3.3 x 10^4/mL in a D-MEM containing 10% FBS, 1% P/S and 1% MEM-NEAA, and 3.3 x 10^5 cells were seeded in a 100 mm dish. The cells were grown by incubation performed in the presence of 5% CO_2 at 37°C. After 2 to 4 days, it was confirmed that an area of about to 70% of the bottom of the dish had been covered by the cells, the medium was then exchanged, and by adding 200 ng of interferon-γ and 1 μg of polyliposaccharide (both manufactured by Pepro Tech) to the medium, the cells were differentiated to the subtype M1. Besides, by adding 100 ng of IL-4, the cells were differentiated to the subtype M2. After incubation performed in the presence of 5% CO_2 at 37°C for 24 hours, the resultant was rinsed with PBS, and PBS containing 0.25% trypsin and 1 mM EDTA was used for collecting, from the dish, the macrophages having been differentiated to the subtypes M1 and M2.

[0314]<Confirmation of subtypes>

The thus collected M1 and M2 macrophages were subjected to the gene expression analysis performed in the same manner as in Example 1, resulting in confirming that the cells had been differentiated to the subtypes. The result is illustrated in Fig. 8. As is obvious from Fig. 8, the expression levels of the iNOS and the TNFα were higher in the cells differentiated to the M1 macrophages than in the cells differentiated to the M2 macrophages. On the other hand, the expression levels of the Arg1 and the CD206 were higher in the cells differentiated to the M2 macrophages than in the cells differentiated to the M1 macrophages. It was confirmed based on this result that the M1 macrophages and the M2 macrophages could be prepared by the differentiation.

[0315]After staining the M1 macrophages and the M2 macrophages differented from the RAW264.7 cells with the compound (1) in the same manner as in Example 1, a

fluorescent signal derived from the compound (1) was
analyzed in the same manner as in Example 1 except that
dead cells were identified by using the TO-PRO-3. A
histogram was prepared by plotting fluorescence
intensity obtained in the FITC channel on the abscissa
and plotting the number of cells at each fluorescence
intensity on the ordinate, which is shown in Fig. 9.

[0316] As a result, as is obvious from Fig. 9, the M1
macrophages were stained more strongly than the M2
macrophages by the compound (1), and it was thus found
that these macrophages can be identified based on a
difference in the fluorescence intensity.

[0317] Identification of macrophage subtypes differentiated
from human cell line by using macrophage identification
agent represented by general formula (1)
Examples 60 to 64

[0318] The subtypes were identified by using the compound (1)
in the same manner as in Example 1 except that the
macrophage subtypes differentiated from the mouse bone
marrow cells were changed to macrophage subtypes
differentiated from a cell line established from human
acute monocytic leukemia.

[0319] <Culture of subtypes>
The culture of subtypes was performed with reference to
NPL 10. Specifically, THP-1 cells purchased from JCRB
Cell Bank of National Institute of Biomedical
Innovation, that is, a cell line established from human
acute monocytic leukemia, were subcultured several
times, and the resultant cells were dispersed to a
concentration of 5.0×10^5/mL in an RPMI1640 containing
10% FBS and 1% P/S, and 5.0×10^6 cells were seeded in
a 100 mm dish. The monocytes were differentiated into
macrophages by incubating the resultant in the presence
of 5% CO_2 at 37°C for 2 days with 500 ng of PMA added
thereto. After exchanging the medium, by adding 200 ng
of interferon-γ and 1 μg of polyliposaccharide (both

manufactured by Pepro Tech) to the medium, the cells
were differentiated to the subtype M1. Besides, by
adding 200 ng of IL-4 and IL-13, the cells were
differentiated to the subtype M2. After incubation
performed in the presence of 5% CO_2 at 37°C for 24 hours,
the resultant was rinsed with PBS, and PBS containing
0.25% trypsin and 1 mM EDTA (ethylenediaminetetraacetic
acid) was used for collecting, from the dish, the
macrophages having been differentiated to the subtypes
M1 and M2.

[0320] Through the gene expression analysis performed in the
same manner as in Example 1 by using primers shown in
Table 5, the collected M1 macrophages and M2
macrophages were confirmed to have been differentiated
to the subtypes. The result is illustrated in Fig. 10.
As is obvious from Fig. 10, the expression levels of
the TNFα and IL-6 were higher in the cells
differentiated to the M1 macrophages than in the cells
differentiated to the M2 macrophages. On the other
hand, the expression level of the CD206 was higher in
the cells differentiated to the M2 macrophages than in
the cells differentiated to the M1 macrophages. It was
confirmed based on this result that the M1 macrophages
and the M2 macrophages could be prepared by the
differentiation.

[0321] Table 5

Target gene	Sequence of primer		Remarks
	Forward	Reverse	
GAPDH	CAACAGCGACACCCACTCCT	CACCCTGTTGCTGTAGCCAAA	Endogenous control gene
TNFα	TGCACTTTGGAGTGATCGGC	CTCAGCTTGAGGGTTTGCTAC	M1 macrophage marker
IL-6	AGCCACTCACCTCTTCAGAAC	GCCTCTTTGCTGCTTTCACAC	M1 macrophage marker
CD206	CCCTCAGAAAGTGATGTGCCTA	TACTTGTGAGGTCACCGCCT	M2 macrophage marker

[0322] After staining the M1 macrophages and the M2
macrophages differentiated from the THP-1 cells with

the compounds (1)(3)(4)(12) and (15) in the same manner
as in Example 1 , fluorescent signals derived from the
compounds (1)(3)(4)(12) and (15) were analyzed in the
same manner as in Example 1 except that dead cells were
identified by using the TO-PRO-3. Specifically,
fluorescent signals derived from the compounds
(1)(3)(4)(12) and (15) were measured by using the
channels which are appropriate for each of the
compounds and the identification abilities for the
subtypes of the compounds were evaluated based on the
values of the resolution R.

[0323] Comparative Example 4

After staining the M1 macrophages and the M2
macrophages differentiated from the THP-1 cells with
the compound (1) in the same manner as in Example 1,
dead cells were identified by using the TO-PRO-3, and
fluorescent signal derived from the compound (1) was
analyzed in the same manner as in Example 1 except that
the fluorescent signal derived from the comparative
compound (2) was measured using the PE-Cy7 channel
(excited at 488 nm, 780/60 nm: center
wavelength/wavelength width). The identification
abilities for the subtypes of the compounds were
evaluated based on the values of the resolution R.

[0324] Comparative Example 5

After staining the M1 macrophages and the M2
macrophages differentiated from the THP-1 cells with
the compound (2) in the same manner as in Example 1,
dead cells were identified by using the TO-PRO-3, and
fluorescent signal derived from the compound (2) was
analyzed in the same manner as in Example 1. The
identification abilities for the subtypes of the
compounds were evaluated based on the values of the
resolution R.

[0325] The results obtained in Examples 60 to 64 and
Comparative Examples 4 and 5 described above are shown

in Table 6. The details of channels shown in Table 6 are shown in Table 3.

[0326]Table 6

Example	Compound	Channel	Peak position of M1 macrophage	Peak position of M2 macrophage	Resolution ratio R	Evaluation of macrophage subtype identification	Selectivity
Example 60	(1)	FITC	58.1	33.4	0.409	B	M1
Example 61	(3)	PE	9.63	12.3	0.257	B	M2
Example 62	(4)	FITC	26.5	17.9	0.372	B	M1
Example 63	(12)	PE	37.4	46.5	0.276	B	M2
Example 64	(15)	PE	5.62	7.4	0.216	B	M2
Comparative Example 4	Comparative compound (4)	PE-Cy7	0.24	0.21	0.021	C	No selectivity
Comparative Example 5	Comparative compound (5)	FITC	0.25	0.21	0.061	C	No selectivity

[0327]As is obvious from Table 6, when using the M1 macrophages and the M2 macrophages differentiated from the THP-1 cell, there was no difference in the staining intensity between the M1 macrophages and the M2 macrophages stained by using the comparative compound (1) or (2) and hence these macrophages cannot be identified. On the other hand when the macrophage identification agent containing the dye compound represented by general formula (1) of the present invention is used, the M1 macrophages and the M2 macrophages could be identified from each other based on a difference in the fluorescence intensity.

[0328]It is understood from Examples 57 and 58 that the macrophage identification agent represented by general formula (1) of the present invention can be used for analyzing the correlation between a subtype and a substance.

[0329]It is understood from Examples 1 to 58 and Comparative Examples 1 and 2 described above that the macrophage identification agent represented by general formula (1) of the present invention can be used for identifying, sorting, evaluating and analyzing a subtype.

[0330]Besides, by performing operations similar to those of

Examples 57 and 58 on a plurality of substances, the
correlation between a subtype and the substances can be
screened by using the macrophage identification agent
represented by general formula (1) of the present
invention.

[0331] Besides, it was revealed based on Example 59 that not
only the subtypes differentiated from bone marrow cells
but also the subtypes differentiated from a mouse cell
line can be identified by using the macrophage
identification agent represented by general formula (1)
of the present invention.
This result supports that subtypes can be identified by
using the macrophage identification agent represented
by general formula (1) of the present invention even if
the cell type is different.

[0332] Besides, it was revealed based on Examples 60 to 64 and
comparative examples 4 and 5 that not only the subtypes
differentiated from bone marrow cells but also the
subtypes differentiated from a human cell line can be
identified by using the macrophage identification agent
represented by general formula (1) of the present
invention. This result further supports that subtypes
can be identified by using the macrophage
identification agent represented by general formula (1)
of the present invention even if human-derived cells
are used, and probably suggests that the present
invention is applicable to pathologic tissues like
human tissues or even a human individual.

[0333] <Synthetic Example 2>
As an example of the macrophage identification agent
represented by general formula (6) of the present
invention, a synthetic example of the compound (86)
will be described.

Compound (A1)   Compound (B2)   Compound (86)
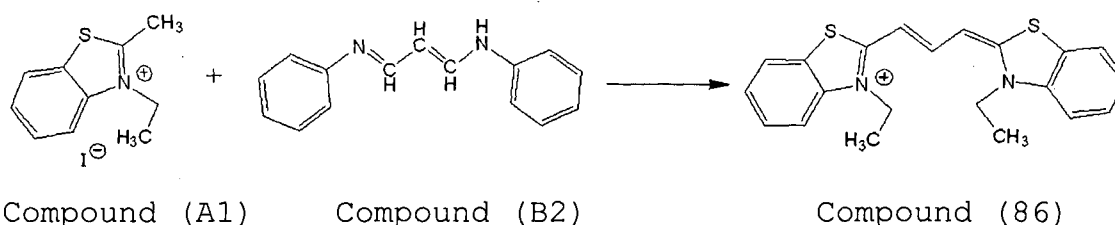
Under a nitrogen atmosphere, 0.22 g (1.0 mmol) of a
compound (B2) and 0.16 g (2.0 mmol) of anhydrous sodium
acetate were added to a 10 mL acetic anhydride solution
of 0.61 g (2.0 mmol) of a compound (A1), and the
resulting solution was stirred at 100°C for 1 hour.
After completing the reaction, 100 mL of a saturated
saline was slowly added dropwise thereto while cooling,
and the resulting solution was cooled to room
temperature. The resultant solution was extracted with
50 mL of dichloromethane twice. After drying the
resultant over anhydrous sodium sulfate, an organic
layer was concentrated under reduced pressure. The
residue was purified by silica gel chromatography and
recrystallized from diethyl ether, thereby obtaining
0.29 g (yield: 59%) of the compound (86). The obtained
substance was confirmed to be the compound of interest
by the ^{1}H nuclear magnetic resonance spectrometric
analysis (ECA-400, manufactured by JEOL Ltd.) and the
LC/TOF MS (LC/MSD TOF, manufactured by Agilent
Technologies, Inc.).

[0334] <Synthetic Example 3>
As an example of the macrophage identification agent
represented by general formula (6) of the present
invention, a synthetic example of the compound (110)
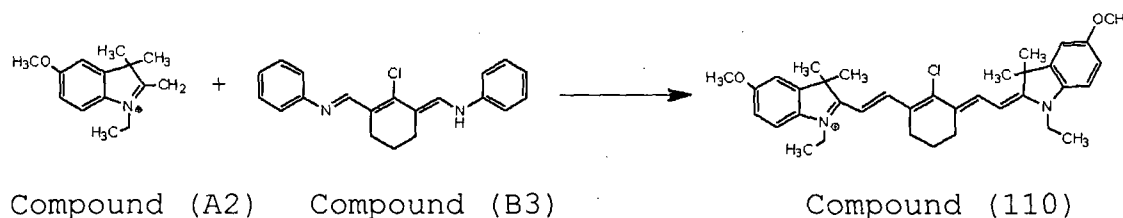will be described.

Compound (A2)   Compound (B3)   Compound (110)

Under a nitrogen atmosphere, 0.32 g (1.0 mmol) of a compound (B3) and 0.25 g (3.0 mmol) of anhydrous sodium acetate were added to a 10 mL acetic anhydride solution of 0.48 g (2.2 mmol) of a compound (A2), and the resulting solution was stirred at 100°C for 1 hour. After completing the reaction, 100 mL of a saturated saline was slowly added dropwise thereto while cooling, and the resulting solution was cooled to room temperature. The resultant solution was extracted with 50 mL of dichloromethane twice. After drying the resultant over anhydrous sodium sulfate, an organic layer was concentrated under reduced pressure. The residue was purified by the silica gel chromatography and recrystallized from diethyl ether, thereby obtaining 0.38 g (yield: 54%) of the compound (110). The obtained substance was confirmed to be the compound (110) of interest by the ^{1}H nuclear magnetic resonance spectrometric analysis (ECA-400, manufactured by JEOL Ltd.) and the LC/TOF MS (LC/MSD TOF, manufactured by Agilent Technologies, Inc.).

[0335]<Other compounds represented by general formula (6)>
In the same manner as in Synthetic Examples 2 and 3, compounds shown in Table 7 were synthesized and identified as the compounds of interest.

[0336]Subtype identification by using macrophage identification agent represented by general formula (6)
Example 65
A fluorescent signal derived from the compound (86) was analyzed in the same manner as in Example 1 except that the compound (1) used in Example 1 was replaced with the compound (86). However, the signal of the compound (86) was measured in an APC-Cy7 channel (excited at 633 nm, in a fluorescence wavelength measurement region of 780/60 nm of center wavelength/wavelength width). A histogram was prepared by plotting fluorescence intensity obtained in the APC-Cy7 channel on the

abscissa and plotting the number of cells at each
fluorescence intensity on the ordinate, which is shown
in Fig. 11.

[0337] Examples 66 to 76

Fluorescent signals derived from the compounds (88),
(90), (91), (92), (95), (98), (99), (100), (103), (104)
and (113) were analyzed in the same manner as in
Example 1 except that the compound (1) used in Example
1 was replaced with the compounds (88), (90), (91),
(92), (95), (98), (99), (100), (103), (104) and (113).

[0338] Comparative Example 6

A fluorescent signal derived from a comparative
compound (3) was analyzed in the same manner as in
Example 1 except that the compound (1) used in Example
1 was replaced with the comparative compound (3).
However, the fluorescent signal of the comparative
compound (3) was measured in the FITC channel (excited
at 488 nm, 530/30 nm: center wavelength/wavelength
width). A histogram was prepared by plotting
fluorescence intensity obtained in the FITC channel on
the abscissa and plotting the number of cells at each
fluorescence intensity on the ordinate, which is shown
in Fig. 12.
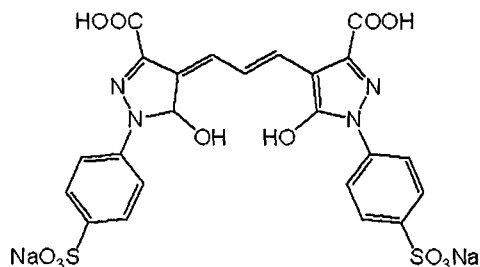
Comparative Compound (3)

[0339] The results obtained in Examples 65 to 76 and
Comparative Examples 1 and 6 described above are shown
in Table 7.

[0340] <Evaluation method for identification ability of
macrophage identification agent represented by general
formula (6)>

Fluorescent signals derived from the compounds (88),
(90), (91), (92), (95), (98), (99), (100), (103), (104)
and (113) and the comparative compound (3) were
measured in channels suitable to the respective
compounds in the same manner as in the evaluation
method for the identification ability of the macrophage
identification agent represented by general formula (1)
except that the compounds (3), (4), (6), (7), (9), (11)
to (13), (15), (17), (19) to (26), (28), (30) to (33),
(35), (36), (38), (42) to (45), (48) to (62), (65),
(67), (74) to (76), (82), (83) and (85) and the
comparative compounds (1) and (2) used in the
evaluation method for the identification ability of the
macrophage identification agent represented by general
formula (1) were replaced with the compounds (88), (90),
(91), (92), (95), (98), (99), (100), (103), (104) and
(113). By using a resolution R thus obtained as an
index, the identification ability for a subtype of each
compound was evaluated.

[0341] The evaluation results are shown in Table 7. The
details of channels shown in Table 7 are shown in Table
3.

[0342]Table 7

Example	Compound	Channel	Peak position of M1 macrophage	Peak position of M2 macrophage	Resolution ratio R	Evaluation of macrophage subtype identification	Selectivity
Example 65	(86)	APC-Cy7	2.27	6.37	0.602	A	M2
Example 66	(88)	APC-Cy7	0.95	1.48	0.241	B	M2
Example 67	(90)	APC-Cy7	0.86	1.31	0.321	B	M2
Example 68	(91)	APC-Cy7	2.96	13.89	0.476	B	M2
Example 69	(92)	PE-Cy7	2.27	3.9	0.534	A	M2
Example 70	(95)	PE	71.57	133.45	0.421	B	M2
Example 71	(98)	FITC	21.95	61.59	0.507	A	M2
Example 72	(99)	PE	191.14	331.51	0.673	A	M2
Example 73	(100)	PE	16.41	32.87	0.372	B	M2
Example 74	(103)	PE-Cy7	2.97	8.23	0.597	A	M2
Example 75	(104)	PE-Cy7	95.26	197.19	0.698	A	M2
Example 76	(113)	APC-Cy-7	9.05	21.39	0.857	A	M2
Comparative Example 1	Comparative compound (1)	PE-Cy7	1.70	1.86	0.006	C	No selectivity
Comparative Example 6	Comparative compound (3)	FITC	0.86	0.97	0.079	C	No selectivity

[0343]As is obvious from Table 7, Fig. 1 and Figs. 11 and 12,
although there is no difference in the staining
intensity between the M1 macrophages and the M2
macrophages stained by using the comparative compound
(1) or (3) and hence these macrophages cannot be
identified, if the macrophage identification agent
containing the dye compound represented by general
formula (6) of the present invention is used, the M1
macrophages and the M2 macrophages can be identified
from each other based on a difference in the
fluorescence intensity.

[0344]Example 77
Two x 10^5 each of M1 macrophages and M2 macrophages
cultured in the same manner as in Example 1 were
dispensed into a 1.5 mL tube, and the compound (103)
and the compound (113) were added thereto each to a
concentration of 1 μM.

[0345]A fluorescent signal derived from the compound (103)

was measured in the PE-Cy7 channel (excited at 488 nm,
780/60 nm: center wavelength/wavelength width) and a
fluorescent signal derived from the compound (113) was
measured in the APC-Cy7 channel (excited at 633 nm,
780/60 nm: center wavelength/wavelength width). A
cytogram developed by plotting the intensity of the
fluorescent signal derived from the compound (103) and
obtained in the PE-Cy7 channel on the ordinate and
plotting the intensity of the fluorescent signal
derived from the compound (113) and obtained in the
APC-Cy7 channel on the abscissa was prepared.

[0346] The operation was performed in the same manner as in
Example 1 except for the above.

[0347] As a result, when the compound (103) is used for
staining, the M2 macrophage shows a higher fluorescence
intensity in the PE-Cy7 channel than the M1 macrophage,
and on the contrary, when the compound (113) is used
for staining, the M2 macrophage shows a higher
fluorescence intensity in the APC-Cy7 channel than the
M1 macrophage. Therefore, on the cytogram developed
with respect to the intensity of the fluorescent signal
obtained in the PE-Cy7 channel and the intensity of the
fluorescent signal obtained in the APC-Cy7 channel, the
M1 macrophages and the M2 macrophages stained with the
compound (103) and the compound (113) are plotted as
cell populations having different fluorescent
characteristics, and hence, these macrophages can be
identified.

[0348] This example reveals that a subtype can be identified
even when two or more types of compounds for the
macrophage identification agent represented by general
formula (6) of the present invention are used in
combination.

[0349] Examples 65 to 77 and Comparative Examples 1 and 6
described above reveal that a subtype contained in a
biological sample can be identified by using one or

more macrophage identification agents represented by
general formula (6) of the present invention.

[0350] Sorting of subtype by using macrophage identification
agent represented by general formula (6)

Example 78

One x 10^5 each of M1 macrophages and M2 macrophages
cultured in the same manner as in Example 1 were mixed
in a 1.5 mL tube, and the compound (86) was added
thereto to a concentration of 1 µM.

The operation was performed in the same manner as in
Example 1 except for the above.

[0351] As a result, as illustrated in Figs. 13A to 13C, the M1
macrophages and the M2 macrophages stained with the
compound (86) were plotted, on a cytogram developed
with respect to the intensity of forward scattered
light affected by the size of cells and the intensity
of a fluorescent signal derived from the compound (86),
as cell populations having different fluorescence
intensity distributions. Since the compound (86)
having a characteristic to stain the M2 macrophages
more strongly than the M1 macrophages was used for
staining, the M2 macrophages can be identified as a
cell population having a high fluorescence intensity
derived from the compound (86), and on the other hand,
the M1 macrophages can be identified as a cell
population having a low fluorescence intensity derived
from the compound (86). Besides, the M1 macrophages
and the M2 macrophages identified by using the compound
(86) could be each sorted with an FACS apparatus. The
sorted subtypes were confirmed by the fluorescent
antibody method and the gene expression analysis.

[0352] It was found through Examples 65 to 78 described above
that a subtype can be identified and sorted by using
the macrophage identification agent represented by
general formula (6) of the present invention.

[0353] Analysis and screening of subtype by using macrophage

identification agent represented by general formula (6)

Example 79

Two x 10⁵ each of M1 macrophages and M2 macrophages
cultured in the same manner as in Example 1 were
dispensed into a 1.5 mL tube, and the compound (113)
was added thereto to a concentration of 1 µM, and
furthermore, MK-571 (manufactured by Sigma-Aldrich) was
added thereto, as a substance for inhibiting export of
the compound (113) having been imporated into cells, to
a concentration of 10 uM, and the resultant was
incubated at 37°C for 30 minutes. Thereafter, the tube
was centrifuged at 180 G for 10 minutes to remove the
supernatant, and then, 1 mL of a HBSS buffer containing
the MK-571 in a concentration of 10 µM was added
thereto to suspend the cells, and the resultant was
further incubated at 37°C for 30 minutes.

The operation was performed in the same manner as in
Example 57 except for the above.

[0354] Comparative Example 7

The analysis was performed in the same manner as in
Example 79 except that the MK-571 was not added.

As a result, as is obvious from Figs. 14A and 14B, in a
histogram in which the fluorescence intensity obtained
in the FITC channel for measuring a fluorescent signal
derived from the compound (113) was plotted on the
abscissa and the number of cells at each fluorescence
intensity was plotted on the ordinate, the enhancement
of the fluorescence intensity caused by the inhibition
by the MK-571 of the export of the compound (113) from
the cells was observed in both the M1 macrophages and
the M2 macrophages. The degree of the enhancement of
the peak intensity caused by adding the MK-571 was
evaluated by calculating the resolution R described
above by using peak intensities in histograms prepared
with and without adding the MK-571. As a result, the
degree was 0.406 in the M1 macrophages and 0.438 in the

M2 macrophages. Thus, it was found that the influence
on the export inhibition by the MK-571 is slightly
different depending on the subtypes.

[0355] Example 80

Two x 10^5 each of M1 macrophages and M2 macrophages
cultured in the same manner as in Example 1 were
dispensed into a 1.5 mL tube, the compound (86) was
added thereto to a concentration of 1 uM, and the
resultant was incubated at 37°C for 30 minutes.
Thereafter, the tube was centrifuged at 180 G for 10
minutes to remove the supernatant, and then, a blocking
treatment for cell surfaces was performed by using
2.4G2 (anti-mouse CD16/32 antibody, manufactured by
Biolegend). To the cells having been subjected to the
blocking treatment, a FACS buffer containing 0.2 µg of
Alexa Fluor647-labeled anti-CD40 antibody and 0.2 µg of
PE-labeled anti-Dectin-1 antibody was added for
performing the immunofluorescent staining. The
fluorescent labeling with these antibodies was selected
so that the fluorescence wavelengths of the antibodies
could overlap neither with each other nor with the
fluorescence wavelengths of the compound (86) and the
aqua.

The operation was performed in the same manner as in
Example 58 except for the above.

[0356] As a result, in the M2 macrophages identified based on
the strong staining with the compound (86), a
fluorescent signal derived from the PE-labeled anti-
Dectin-1 antibody was observed in a PE channel (excited
at 488 nm, 585/42 nm: center wavelength/wavelength
width). On the contrary, in the M1 macrophages
identified based on the weak staining with the compound
(86), a fluorescent signal derived from the Alexa
Fluor647-labeled anti-CD40 antibody was observed in the
APC channel (excited at 633 nm, 660/20nm: center
wavelength/wavelength width). It was found based on

this result that the anti-Dectin-1 antibody and the
anti-CD40 antibody are antibodies respectively
specifically binding to the M2 macrophages and the M1
macrophages.

[0357] It is understood from Examples 79 and 80 that the
macrophage identification agent represented by general
formula (6) of the present invention can be used for
analyzing the correlation between a subtype and a
substance.

[0358] It is understood from Examples 65 to 80 and Comparative
Examples 1 and 6 described above that the macrophage
identification agent of the present invention can be
used for identifying, sorting, evaluating and analyzing
a subtype.

[0359] Besides, by performing operations similar to those of
Examples 79 and 80 on a plurality of substances, the
correlation between a subtype and the substances can be
screened by using the macrophage identification agent
of the present invention.

[0360] <Synthetic Example 4>

As an example of the macrophage identification agent
represented by general formula (10) of the present
invention, a synthetic example of the compound (116)
will be described.

Aldehyde (2)   Ethyl   Amine (1)   Compound (116)
   cyanoacetate
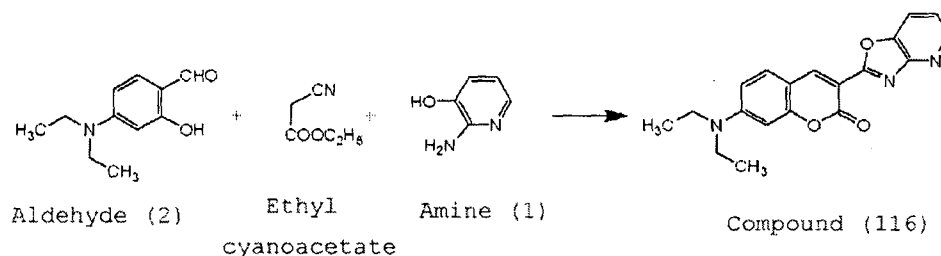
To a 200 mL n-butyl alcohol solution of 5.5 g (50 mmol)
of amine (1), 12.1 g (62.5 mmol) of aldehyde (2), 6.7
mL (62.5 mmol) of ethyl cyanoacetate and 1.4 g (12.5
mmol) of benzoic acid were added, and the resulting
solution was heated to reflux at 150°C for 11 hours.
After completing the reaction, the resulting solution

was cooled to room temperature, and the solvent was distilled off under reduced pressure. To the residue, 100 mL of isopropyl alcohol and 15 mL of a 1 mol/L sodium hydroxide aqueous solution were added, followed by stirring. The thus obtained solid was filtered out and washed with 100 mL of isopropyl alcohol and 66 mL of DMSO, thereby obtaining 7.8 g (23 mmol, yield: 47%) of the compound (116) of interest.

[0361]<Synthetic Example 5>

As an example of the macrophage identification agent represented by general formula (10) of the present invention, a synthetic example of the compound (135) will be described.

Aldehyde (2)   Ethyl   Amine (2)   Compound (135)
                cyanoacetate
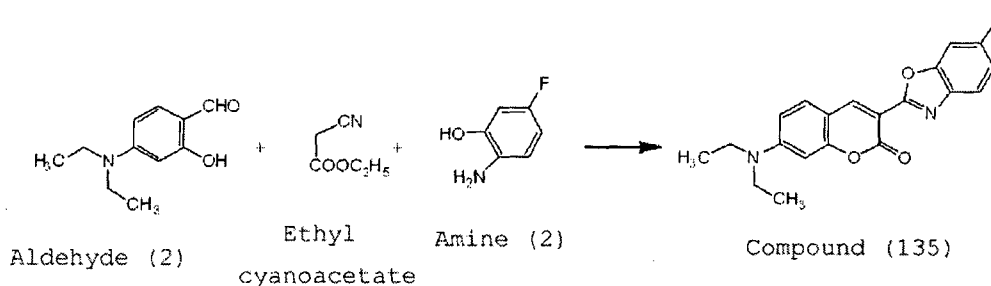
The operation was performed in the same manner as in Synthetic Example 4 except that amine (2) was used in Synthetic Example 5 instead of the amine (1), thereby obtaining 8.46 g (24 mmol, yield: 48%) of the compound (135) of interest.

[0362]Subtype identification by using macrophage identification agent represented by general formula (10)

Example 81

A fluorescent signal derived from the compound (116) was analyzed in the same manner as in Example 1 except that the compound (1) used in Example 1 was replaced with the compound (116) and the TO-PRO-3 was used for identifying dead cells. A histogram was prepared by plotting fluorescence intensity obtained in the FITC channel on the abscissa and plotting the number of

cells at each fluorescence intensity on the ordinate, which is shown in Fig. 15.

[0363] Examples 82 to 90

Fluorescent signals derived from the compounds (117), (119), (120), (122), (127), (129), (131), (132) and (138) were analyzed in the same manner as in Example 1 except that the compound (1) used in Example 1 was replaced with the compounds (117), (119), (120), (122), (127), (129), (131), (132) and (138).

[0364] Comparative Example 8

A fluorescent signal derived from a comparative compound (4) was analyzed in the same manner as in Example 1 except that the compound (1) used in Example 1 was replaced with the comparative compound (4). A histogram was prepared by plotting fluorescence intensity obtained in the Pacific Blue channel on the abscissa and plotting the number of cells at each fluorescence intensity on the ordinate, which is shown in Fig. 16.
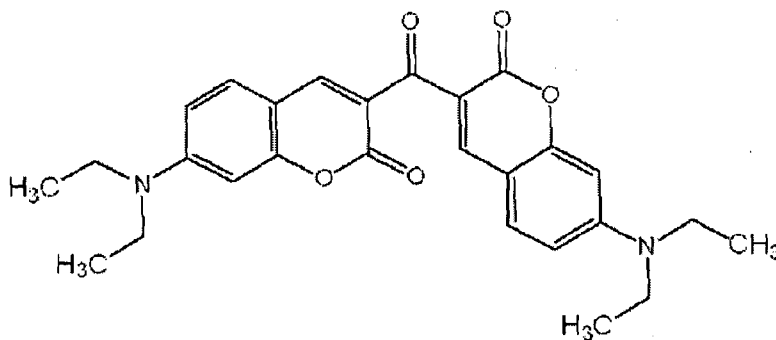
Comparative Compound (4)

[0365] The results obtained in Examples 81 to 90 and Comparative Examples 1 and 8 described above are shown in Table 8.

[0366] <Evaluation method for identification ability of macrophage identification agent represented by general formula (10)>

Fluorescent signals derived from the compounds (117),

(119), (120), (122), (127), (129), (131), (132) and (138) and the comparative compound (4) were measured in channels suitable to the respective compounds in the same manner as in the evaluation method for the identification ability of the macrophage identification agent represented by general formula (1) except that the compounds (3), (4), (6), (7), (9), (11) to (13), (15), (17), (19) to (26), (28), (30) to (33), (35), (36), (38), (42) to (45), (48) to (62), (65), (67), (74) to (76), (82), (83) and (85) and the comparative compounds (1) and (2) used in the evaluation method for the identification ability of the macrophage identification agent represented by general formula (1) were replaced with the compounds (117), (119), (120), (122), (127), (129), (131), (132) and (138) and the comparative compound (4). By using a resolution R thus obtained as an index, the identification ability for a subtype of each compound was evaluated.

The evaluation results are shown in Table 8. The details of channels shown in Table 8 are shown in Table 3.

[0367] Table 8

Example	Compound	Channel	Peak position of M1 macrophage	Peak position of M2 macrophage	Resolution ratio R	Evaluation of macrophage subtype identification	Selectivity
Example 81	(116)	FITC	4.91	12.1	0.772	A	M2
Example 82	(117)	FITC	7.35	9.73	0.318	B	M2
Example 83	(119)	AmCyan	2.60	3.94	0.273	B	M2
Example 84	(120)	Pacific Blue	10.1	5.60	0.676	A	M1
Example 85	(122)	FITC	142	211	0.377	B	M2
Example 86	(127)	FITC	3.99	6.00	0.381	B	M2
Example 87	(129)	Pacific Blue	31.0	13.0	0.709	A	M1
Example 88	(131)	FITC	8.06	13.5	0.480	B	M2
Example 89	(132)	FITC	30.3	53.8	0.624	A	M2
Example 90	(138)	AmCyan	20.6	28.0	0.312	B	M2
Comparative Example 1	Comparative compound (1)	PE-Cy7	1.70	1.86	0.006	C	No selectivity
Comparative Example 8	Comparative compound (4)	Pacific Blue	0.88	1.05	0.053	C	No selectivity

[0368]As is obvious from Table 8 and Fig. 1 and Figs. 15 and
        16, although there is no difference in the staining
        intensity between the M1 macrophages and the M2
        macrophages stained by using the comparative compound
        (1) or (4) and hence these macrophages cannot be
        identified, if the macrophage identification agent
        containing the dye compound represented by general
        formula (10) of the present invention is used, the M1
        macrophages and the M2 macrophages can be identified
        from each other based on a difference in the
        fluorescence intensity.

[0369]Example 91

        Two x 10^5 each of M1 macrophages and M2 macrophages
        cultured in the same manner as in Example 1 were
        dispensed into a 1.5 mL tube, and the compound (116)
        and the compound (120) were added thereto each to a
        concentration of 1 μM.

[0370]A fluorescent signal derived from the compound (116)
        was measured in the FITC channel (excited at 488 nm,
        530/30 nm: center wavelength/wavelength width) and a
        fluorescent signal derived from the compound (120) was
        measured in the Pacific Blue channel (excited at 405 nm,
        450/50 nm: center wavelength/wavelength width). A
        cytogram developed by plotting the intensity of the
        fluorescent signal derived from the compound (1) and
        obtained in the FITC channel on the ordinate and
        plotting the intensity of the fluorescent signal
        derived from the compound (5) and obtained in the
        Pacific Blue channel on the abscissa was prepared.
        The operation was performed in the same manner as in
        Example 1 except for the above.

[0371]As a result, as is obvious from Figs. 17A to 17E, the
        compound (116) has a characteristic to more strongly
        stain the M2 macrophages than the M1 macrophages, and
        on the contrary, the compound (120) has a
        characteristic to more strongly stain the M1

macrophages than the M2 macrophages. Therefore, on the
cytogram developed with respect to the intensity of the
fluorescent signal derived from the compound (116) and
the intensity of the fluorescent signal derived from
the compound (120), the M1 macrophages and the M2
macrophages stained with the compound (116) and the
compound (120) are plotted as cell populations having
different fluorescent characteristics, and hence, these
macrophages can be identified.

[0372] This example reveals that a subtype can be identified
even when two or more types of compounds for the
macrophage identification agent represented by general
formula (10) of the present invention are used in
combination.

[0373] Examples 81 to 91 and Comparative Examples 1 and 8
described above reveal that a subtype contained in a
biological sample can be identified by using one or
more macrophage identification agents represented by
general formula (10) of the present invention.

[0374] Sorting of subtype by using macrophage identification
agent represented by general formula (10)
Example 92
One x 10^5 each of M1 macrophages and M2 macrophages
cultured in the same manner as in Example 1 were mixed
in a 1.5 mL tube, and the compound (120) was added
thereto to a concentration of 1 uM.
The operation was performed in the same manner as in
Example 1 except for the above.

[0375] As a result, as is obvious from Figs. 18A to 18C, the
M1 macrophages and the M2 macrophages stained with the
compound (120) were plotted, on a cytogram developed
with respect to the intensity of forward scattered
light affected by the size of cells and the intensity
of a fluorescent signal derived from the compound (120),
as cell populations having different fluorescence
intensity distributions. Since the compound (120)

having the characteristic to stain the M1 macrophages
more strongly than the M2 macrophages was used for
staining, the M1 macrophages can be identified as a
cell population having a high fluorescence intensity
derived from the compound (120), and on the other hand,
the M2 macrophages can be identified as a cell
population having a low fluorescence intensity derived
from the compound (120). Besides, the M1 macrophages
and the M2 macrophages identified by using the compound
(120) could be each sorted with an FACS apparatus. The
sorted subtypes were confirmed by the fluorescent
antibody method and the gene expression analysis.

[0376] It was found through Examples 81 to 92 described above
that a subtype can be identified and sorted by using
the macrophage identification agent represented by
general formula (10) of the present invention.

[0377] Analysis and screening of subtype by using macrophage
identification agent

[0378] Example 93

Two x 10^5 each of M1 macrophages and M2 macrophages
cultured in the same manner as in Example 1 were
dispensed into a 1.5 mL tube, and the compound (120)
was added thereto to a concentration of 1 µM, and
furthermore, MK-571 (manufactured by Sigma-Aldrich) was
added thereto, as a substance for inhibiting export of
the compound (120) having been imported into cells, to
a concentration of 10 µM, and the resultant was
incubated at 37°C for 30 minutes. Thereafter, the tube
was centrifuged at 180 G for 10 minutes to remove the
supernatant, and then, 1 mL of a HBSS buffer containing
the MK-571 in a concentration of 10 µM was added
thereto to suspend the cells, and the resultant was
further incubated at 37°C for 30 minutes.
The operation was performed in the same manner as in
Example 58 except for the above.

[0379] Comparative Example 9

The analysis was performed in the same manner as in
Example 93 except that the MK-571 was not added.
As a result, as is obvious from Figs. 19A and 19B, in a
histogram in which the fluorescence intensity obtained
in the FITC channel for measuring a fluorescent signal
derived from the compound (120) was plotted on the
abscissa and the number of cells at each fluorescence
intensity was plotted on the ordinate, the enhancement
of the fluorescence intensity caused by the inhibition
by the MK-571 of the export of the compound (120) from
the cells was observed in both the M1 macrophages and
the M2 macrophages. The degree of the enhancement of
the peak intensity caused by adding the MK-571 was
evaluated by calculating the resolution R described
above by using peak intensities in histograms prepared
with and without adding the MK-571. As a result, the
degrees were substantially the same, and were 0.739 in
the M1 macrophages and 0.730 in the M2 macrophages.
Thus, it was found that the influence on the export
inhibition by the MK-571 does not depend on the
subtypes.

[0380] Example 94

Two x 10^5 each of M1 macrophages and M2 macrophages
cultured in the same manner as in Example 1 were
dispensed into a 1.5 mL tube, the compound (116) was
added thereto to a concentration of 1 uM, and the
resultant was incubated at 37°C for 30 minutes.
Thereafter, the tube was centrifuged at 180 G for 10
minutes to remove the supernatant, and then, a blocking
treatment for cell surfaces was performed by using
2.4G2 (anti-mouse CD16/32 antibody, manufactured by
Biolegend). To the cells having been subjected to the
blocking treatment, a FACS buffer containing 0.2 µg of
Pacific Blue-labeled anti-CD40 antibody and 0.05 µg of
PerCP-labeled anti-Dectin-1 antibody was added for
performing the immunofluorescent staining. The

fluorescent labeling with these antibodies was selected
so that the fluorescence wavelengths of the antibodies
could overlap neither with each other nor with the
fluorescence wavelengths of the compound (116) and the
TO-PRO-3.

The operation was performed in the same manner as in
Example 58 except for the above.

[0381] As a result, in the M1 macrophages identified based on
the strong staining with the compound (116), a
fluorescent signal derived from the Pacific Blue-
labeled anti-CD40 antibody was observed in the Pacific
Blue channel (excited at 405 nm, 450/50 nm: center
wavelength/wavelength width). On the contrary, in the
M2 macrophages identified based on the weak staining
with the compound (116), a fluorescent signal derived
from the PerCP-labeled anti-Dectin-1 antibody was
observed in a PerCP channel (excited at 488 nm, in a
fluorescence wavelength measurement region of 670 to
735 nm). It was found based on this result that the
anti-CD40 antibody and the anti-Dectin-1 antibody are
antibodies respectively specifically binding to the M1
macrophages and the M2 macrophages.

[0382] Identification of macrophage subtypes differentiated
from mouse cell line by using macrophage identification
agent represented by general formula (10)
Example 95

[0383] A fluorescent signal derived from the compound (129)
was measured in the Pacific Blue channel (excited at
405 nm, 450/50 nm: center wavelength/wavelength width)
in the same manner as in Example 1 except that the
compound (1) used in Example 59 was replaced with the
compound (129). A histogram was prepared by plotting
the fluorescence intensity obtained in the FITC channel
on the abscissa and plotting the number of cells at
each fluorescence intensity on the ordinate, which is
shown in Fig. 20.

[0384] As a result, as is obvious from Fig. 20, the M1 macrophages were stained more strongly by the compound (129) than the M2 macrophages, and hence, it was found that these macrophages can be identified based on a difference in the fluorescence intensity.

[0385] Identification of macrophage subtypes differentiated from human cell line by using macrophage identification agent represented by general formula (10)

Example 96

[0386] A fluorescent signal derived from the compound (120) was measured in the Pacific Blue channel (excited at 405 nm, 450/50 nm: center wavelength/wavelength width) in the same manner as in Example 1 except that the compound (1) used in Example 60 was replaced with the compound (120). A histogram was prepared by plotting the fluorescence intensity obtained in the Pacific Blue channel on the abscissa and plotting the number of cells at each fluorescence intensity on the ordinate, which is shown in Fig. 21.

[0387] As a result, as is obvious from Fig. 21, the M1 macrophages were stained more strongly by the compound (120) than the M2 macrophages, and hence, it was found that these macrophages can be identified based on a difference in the fluorescence intensity.

[0388] Example 97

A fluorescent signal derived from the compound (129) was measured in the Pacific Blue channel (excited at 405 nm, 450/50 nm: center wavelength/wavelength width) in the same manner as in Example 1 except that the compound (1) used in Example 60 was replaced with the compound (129). A histogram was prepared by plotting the fluorescence intensity obtained in the Pacific Blue channel on the abscissa and plotting the number of cells at each fluorescence intensity on the ordinate, which is shown in Fig. 22.

[0389] As a result, as is obvious from Fig. 22, the M1

macrophages were stained more strongly by the compound
(129) than the M2 macrophages, and hence, it was found
that these macrophages can be identified based on a
difference in the fluorescence intensity.

[0390] It is understood from Examples 93 and 94 that the
macrophage identification agent represented by general
formula (10) of the present invention can be used for
analyzing the correlation between a subtype and a
substance.

[0391] It is understood from Examples 81 to 94 and Comparative
Examples 1 and 8 described above that the macrophage
identification agent of the present invention can be
used for identifying, sorting, evaluating and analyzing
a subtype.

[0392] Besides, by performing operations similar to those of
Examples 93 and 94 on a plurality of substances, the
correlation between a subtype and the substances can be
screened by using the macrophage identification agent
of the present invention.

[0393] Besides, it was revealed based on Example 95 that not
only the subtypes differentiated from bone marrow cells
but also the subtypes differentiated from a mouse cell
line can be identified by using the macrophage
identification agent represented by general formula
(10) of the present invention.
This result supports that subtypes can be identified by
using the macrophage identification agent represented
by general formula (10) of the present invention even
if the cell type is different.

[0394] Besides, it was revealed based on Examples 96 and 97
that not only the subtypes differentiated from bone
marrow cells but also the subtypes differentiated from
a human cell line can be identified by using the
macrophage identification agent represented by general
formula (10) of the present invention.
This result further supports that subtypes can be

identified by using the macrophage identification agent
represented by general formula (10) of the present
invention even if human-derived cells are used, and
probably suggests that the present invention is
applicable to pathologic tissues like human tissues or
even a human individual.

[0395]<Synthetic Example 6>

As an example of the macrophage identification agent
represented by general formula (11) of the present
invention, a synthetic example of the compound (139)
will be described.

Intermediate   Compound (139)
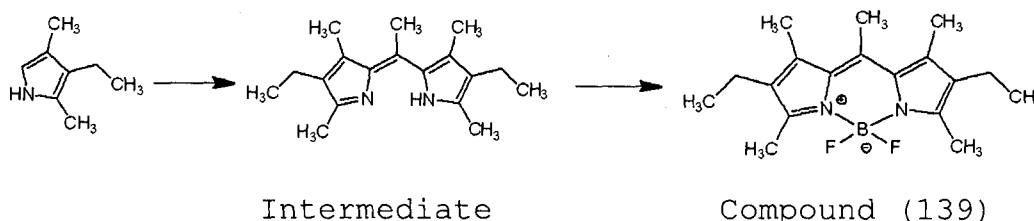
Under a nitrogen atmosphere, a 50 mL dichloromethane
solution of 10 g (81 mmol) of 3-ethyl-2,4-dimethyl
pyrrole was cooled to 0°C, and 13.3 mL of acetyl
chloride was slowly added dropwise thereto. After
completing the dropwise addition, the resulting
solution was slowly heated to room temperature and
diluted with 200 ml of petroleum ether, and the solvent
was distilled off under reduced pressure. To the
residue, 5 mL of ethyl acetate and 70 mL of hexane were
added, and the thus obtained solid was filtered out.
Furthermore, the solid was washed with 28 mL of hexane
and 2 mL of ethyl acetate, thereby obtaining 11.1 g of
an intermediate.

[0396] Under a nitrogen atmosphere, to a 500 mL
dichloromethane suspension of the intermediate, 45 mL
of triethyl amine was slowly added dropwise, and
subsequently, 61 mL of a boron trifluoride ethyl ether
complex was added dropwise thereto over 30 minutes or
more. After stirring the resulting solution at 25°C for
2 hours, the solution was cooled to 5°C or lower and

then extracted by adding 300 mL of water and 1000 mL of
diethyl ether. After the extraction, an organic layer
was washed with 300 mL of water twice and further with
300 mL of a saturated sodium hydrogencarbonate aqueous
solution. After drying over anhydrous sodium sulfate,
the resultant was filtered out, and the solvent was
distilled off under reduced pressure. The resultant
was purified by the silica gel column chromatography
and then washed with methanol, thereby obtaining 9.3 g
(29.2 mmol, yield: 36%) of the compound (139) of
interest.

[0397] The obtained substance was confirmed to be the compound
(139) of interest by the ^{1}H nuclear magnetic resonance
spectrometric analysis (ECA-400, manufactured by JEOL
Ltd.) and the LC/TOF MS (LC/MSD TOF, manufactured by
Agilent Technologies, Inc.).

[0398] <Synthetic Example 7>

As an example of the macrophage identification agent
represented by general formula (11) of the present
invention, a synthetic example of the compound (154)
will be described.

Compound (139)   Compound (154)
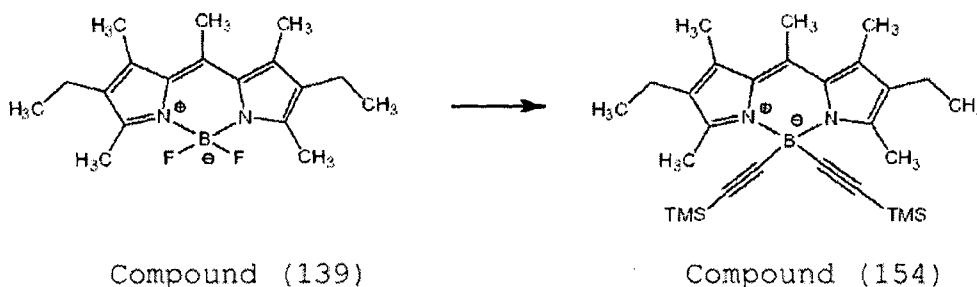
Under a nitrogen atmosphere, a 250 mL anhydrous
tetrahydrofuran solution of 6.9 mL (50 mmol) of
trimethylsilyl acetylene was cooled to -78°C, and 30 mL
(50 mmol) of a 1.65 mol/L n-butyllithium hexane
solution was added dropwise thereto over 8 minutes.
After stirring the resulting solution at -78°C for 1
hour, the solution was slowly heated to room
temperature and stirred for 30 minutes to prepare

trimethylsilyl acetylene lithium.

[0399] Under a nitrogen atmosphere, a 750 mL anhydrous
tetrahydrofuran solution of 6.4 g (20 mmol) of the
compound (139) was cooled to 0°C or lower, and the
trimethylsilyl acetylene lithium prepared as described
above was added dropwise thereto over 20 minutes or
more. After stirring the resulting solution at 0°C for
20 minutes, the solution was diluted with 500 mL of
water to stop the reaction. The resultant was
extracted with 250 mL of ethyl acetate three times, and
the combined organic layers were washed with 250 mL of
a saturated saline. After drying over anhydrous sodium
sulfate, the resultant was filtered out, and the
solvent was distilled off under reduced pressure. The
resultant was purified by the silica gel column
chromatography and then washed with hexane, thereby
obtaining 6.6 g (13.9 mmol, yield: 70%) of the compound
(154) of interest.

[0400] The obtained substance was confirmed to be the compound
(154) of interest by the ^{1}H nuclear magnetic resonance
spectrometric analysis and the LC/TOF MS.

[0401] <Synthetic Examples 8 and 9>
The compounds (141) and (148) were obtained in the same
manner as in Synthetic Example 6 except that the 3-
ethyl-2,4-dimethyl pyrrole used in Synthetic Example 6
was replaced with a corresponding pyrrole derivative.
The obtained substances were confirmed to be the
compounds (141) and (148) of interest by the ^{1}H nuclear
magnetic resonance spectrometric analysis and the
LC/TOF MS.
Besides, commercially available products were used as
the compounds (157), (161), (165), (166), (167) and
(169).

[0402] Subtype identification by using macrophage
identification agent represented by general formula
(11)

Example 98

A fluorescent signal derived from the compound (139)
was analyzed in the same manner as in Example 1 except
that the compound (1) used in Example 1 was replaced
with the compound (139) and the TO-PRO-3 was used for
identifying dead cells. A histogram was prepared by
plotting fluorescence intensity obtained in the FITC
channel on the abscissa and plotting the number of
cells at each fluorescence intensity on the ordinate,
which is shown in Fig. 23.

[0403] Examples 99 to 107

Fluorescent signals derived from the compounds (141),
(148), (154), (157), (161), (165), (166), (167) and
(169) were analyzed in the same manner as in Example 1
except that the compound (1) used in Example 1 was
replaced with the compounds (141), (148), (154), (157),
(161), (165), (166), (167) and (169).

[0404] The results obtained in Examples 98 to 107 described
above are shown in Table 9.

[0405] <Evaluation method for identification ability of
macrophage identification agent represented by general
formula (11)>

Fluorescent signals derived from the compounds (141),
(148), (154), (157), (161), (165), (166), (167) and
(169) were measured in channels suitable to the
respective compounds in the same manner as in the
evaluation method for the identification ability of the
macrophage identification agent represented by general
formula (1) except that the compounds (3), (4), (6),
(7), (9), (11) to (13), (15), (17), (19) to (26), (28),
(30) to (33), (35), (36), (38), (42) to (45), (48) to
(62), (65), (67), (74) to (76), (82), (83) and (85) and
the comparative compounds (1) and (2) used in the
evaluation method for the identification ability of the
macrophage identification agent represented by general
formula (1) were replaced with the compounds (141),

(148), (154), (157), (161), (165), (166), (167) and
(169). By using a resolution R thus obtained as an
index, the identification ability for a subtype of each
compound was evaluated.

The evaluation results are shown in Table 9. The
details of channels shown in Table 9 are shown in Table
3.

[0406] Table 9

Example	Compound	Channel	Peak position of M1 macrophage	Peak position of M2 macrophage	Resolution ratio R	Evaluation of macrophage subtype identification	Selectivity
Example 98	(139)	FITC	220	47.8	1.023	A	M1
Example 99	(141)	FITC	16.6	9.77	0.650	A	M1
Example 100	(148)	FITC	109	151	0.193	B	M2
Example 101	(154)	FITC	1.25	1.63	0.160	B	M2
Example 102	(157)	FITC	2.57	5.62	0.637	A	M2
Example 103	(161)	APC	38.4	9.02	0.582	A	M1
Example 104	(165)	FITC	1.25	2.00	0.183	B	M2
Example 105	(166)	FITC	127	56.3	0.722	A	M1
Example 106	(167)	FITC	14.5	7.82	0.525	A	M1
Example 107	(169)	FITC	10.1	8.35	0.247	B	M1
Comparative Example 1	Comparative compound (1)	PE-Cy7	1.70	1.86	0.006	C	-

[0407] As is obvious from Table 9 and Figs. 1 and 23, although
there is no difference in the staining intensity
between the M1 macrophages and the M2 macrophages
stained by using the comparative compound (1) and hence
these macrophages cannot be identified, if the
macrophage identification agent containing the dye
compound represented by general formula (11) of the
present invention is used, the M1 macrophages and the
M2 macrophages can be identified from each other based
on a difference in the fluorescence intensity.

[0408] Example 108

Two x 10^5 each of M1 macrophages and M2 macrophages
cultured in the same manner as in Example 1 were
dispensed into a 1.5 mL tube, and the compound (139)
and the compound (161) were added thereto each to a
concentration of 1 uM.

[0409]A fluorescent signal derived from the compound (139) was measured in the FITC channel (excitation wavelength: 488 nm, center wavelength: 530 nm, wavelength width: 30 nm) and a fluorescent signal derived from the compound (161) was measured in the APC channel (excitation wavelength: 633 nm, center wavelength: 660 nm, wavelength width: 20 nm). A cytogram developed by plotting the intensity of the fluorescent signal derived from the compound (139) and obtained in the FITC channel on the abscissa and plotting the intensity of the fluorescent signal derived from the compound (161) and obtained in the APC channel on the ordinate was prepared.
The operation was performed in the same manner as in Example 1 except for the above.

[0410]As a result, if the compound (139) is used for staining, the M1 macrophages show higher fluorescence intensity in the FITC channel than the M2 macrophages, and on the contrary, if the compound (161) is used for staining, the M1 macrophages show higher fluorescence intensity in the APC channel than the M2 macrophages. Therefore, as is obvious from Figs. 24A to 24E, on the cytogram developed with respect to the intensity of the fluorescent signal obtained in the FITC channel and the intensity of the fluorescent signal obtained in the APC channel, the M1 macrophages and the M2 macrophages stained with the compound (139) and the compound (161) are plotted as cell populations having different fluorescent characteristics, and hence, these macrophages can be identified.

[0411]This example reveals that a subtype can be identified even when two or more types of compounds for the macrophage identification agent represented by general formula (11) of the present invention are used in combination.

[0412]Examples 98 to 108 and Comparative Example 1 described

above reveal that a subtype contained in a biological
sample can be identified by using one or more
macrophage identification agents represented by general
formula (11) of the present invention.

[0413] Sorting of subtype by using macrophage identification
agent represented by general formula (11)
Example 109
One x 10^5 each of M1 macrophages and M2 macrophages
cultured in the same manner as in Example 1 were mixed
in a 1.5 mL tube, and the compound (139) was added
thereto to a concentration of 1 μM.
The operation was performed in the same manner as in
Example 1 except for the above.

[0414] As a result, as is obvious from Figs. 25A to 25C, the
M1 macrophages and the M2 macrophages stained with the
compound (139) were plotted, on a cytogram developed
with respect to the intensity of forward scattered
light affected by the size of cells and the intensity
of a fluorescent signal derived from the compound (139),
as cell populations having different fluorescence
intensity distributions. Since the compound (139)
staining the M1 macrophages more strongly than the M2
macrophages was used for staining, the M1 macrophages
can be identified as a cell population having a high
fluorescence intensity derived from the compound (139),
and on the other hand, the M2 macrophages can be
identified as a cell population having a low
fluorescence intensity derived from the compound (139).
Besides, the M1 macrophages and the M2 macrophages
identified by using the compound (139) could be each
sorted with an FACS apparatus. The sorted subtypes
were confirmed by the fluorescent antibody method and
the gene expression analysis.

[0415] It was found through Examples 98 to 109 described above
that a subtype can be identified and sorted by using
the macrophage identification agent represented by

general formula (11) of the present invention.

[0416] Analysis and screening of subtype by using macrophage
identification agent represented by general formula
(11)

Example 110

Two x 10^5 each of M1 macrophages and M2 macrophages
cultured in the same manner as in Example 1 were
dispensed into a 1.5 mL tube, and the compound (139)
was added thereto to a concentration of 1 µM, and
furthermore, 4,4'-diisothiocyanatostilbene-2,2'-
disulfonic acid (manufactured by Sigma-Aldrich,
hereinafter referred to as DIDS) was added thereto, as
a substance for inhibiting export of the compound (139)
having been imported into cells, to a concentration of
10 µM, and the resultant was incubated at 37°C for 30
minutes. Thereafter, the tube was centrifuged at 180 G
for 10 minutes to remove the supernatant, and then, 1
mL of a HBSS buffer containing the DIDS in a
concentration of 10 µM was added thereto to suspend the
cells, and the resultant was further incubated at 37°C
for 30 minutes.

The operation was performed in the same manner as in
Example 57 except for the above.

[0417] Comparative Example 10

The analysis was performed in the same manner as in
Example 110 except that the DIDS used in Example 110
was not added.

As a result, as is illustrated in Figs. 26A and 26B, in
a histogram in which the fluorescence intensity
obtained in the FITC channel for measuring a
fluorescent signal derived from the compound (139) was
plotted on the abscissa and the number of cells at each
fluorescence intensity was plotted on the ordinate, the
enhancement of the fluorescence intensity caused by the
inhibition by the DIDS of the export of the compound
(139) from the cells was observed merely in the M1

macrophages, and thus, it was found that the influence
on the export inhibition by the DIDS is different
depending on the subtypes.

[0418]Example 111

Two x 10^5 each of M1 macrophages and M2 macrophages
cultured in the same manner as in Example 1 were
dispensed into a 1.5 mL tube, the compound (161) was
added thereto to a concentration of 1 μM, and the
resultant was incubated at 37°C for 30 minutes.
Thereafter, the tube was centrifuged at 180 G for 10
minutes to remove the supernatant, and then, a blocking
treatment for cell surfaces was performed by using
2.4G2 (anti-mouse CD16/32 antibody, manufactured by
Biolegend). To the cells having been subjected to the
blocking treatment, a FACS buffer containing 0.2 μg of
Pacific Blue-labeled anti-CD40 antibody and 0.1 μg of
Alexa Fluor647-labeled anti-Dectin-1 antibody was added
for performing the immunofluorescent staining. The
fluorescent labeling with these antibodies was selected
so that the fluorescence wavelengths of the antibodies
could overlap neither with each other nor with the
fluorescence wavelengths of the compound (161) and the
aqua.

The operation was performed in the same manner as in
Example 59 except for the above.

[0419]As a result, in the M1 macrophages identified based on
the strong staining with the compound (161), a
fluorescent signal derived from the Pacific Blue-
labeled anti-CD40 antibody was observed in the Pacific
Blue channel (excited at 405 nm, 450/50 nm: center
wavelength/wavelength width). On the contrary, in the
M2 macrophages identified based on the weak staining
with the compound (161), a fluorescent signal derived
from the Alexa Fluor647-labeled anti-Dectin-1 antibody
was observed in the APC channel (excited at 633 nm,
660/20 nm: center wavelength/wavelength width). It was

found based on this result that the anti-CD40 antibody
and the anti-Dectin-1 antibody are antibodies
respectively specifically binding to the M1 macrophages
and the M2 macrophages.

[0420]Identification of macrophage subtypes differentiated
from mouse cell line by using macrophage identification
agent represented by general formula (11)
Example 112

[0421]A fluorescent signal derived from the compound (141)
was measured in the FITC channel (excited at 488 nm,
530/30 nm: center wavelength/wavelength width) in the
same manner as in Example 1 except that the compound
(1) used in Example 59 was replaced with the compound
(141). A histogram was prepared by plotting the
fluorescence intensity obtained in the FITC channel on
the abscissa and plotting the number of cells at each
fluorescence intensity on the ordinate, which is shown
in Fig. 27.

[0422]As a result, as is obvious from Fig. 27, the M1
macrophages were stained more strongly by the compound
(141) than the M2 macrophages, and hence, it was found
that these macrophages can be identified based on a
difference in the fluorescence intensity.

[0423]Identification of macrophage subtypes differentiated
from human cell line by using macrophage identification
agent represented by general formula (11)
Example 113

[0424]A fluorescent signal derived from the compound (139)
was measured in the FITC channel (excited at 488 nm,
530/30 nm: center wavelength/wavelength width) in the
same manner as in Example 1 except that the compound
(1) used in Example 60 was replaced with the compound
(139). A histogram was prepared by plotting the
fluorescence intensity obtained in the FITC channel on
the abscissa and plotting the number of cells at each
fluorescence intensity on the ordinate, which is shown

in Fig. 28.

[0425]As a result, as is obvious from Fig. 28, the M1
macrophages were stained more strongly by the compound
(139) than the M2 macrophages, and hence, it was found
that these macrophages can be identified based on a
difference in the fluorescence intensity.

[0426]Example 114

A fluorescent signal derived from the compound (141)
was measured in the FITC channel (excited at 488 nm,
530/30 nm: center wavelength/wavelength width) in the
same manner as in Example 1 except that the compound
(1) used in Example 60 was replaced with the compound
(141). A histogram was prepared by plotting the
fluorescence intensity obtained in the FITC channel on
the abscissa and plotting the number of cells at each
fluorescence intensity on the ordinate, which is shown
in Fig. 29.

[0427]As a result, as is obvious from Fig. 29, the M1
macrophages were stained more strongly by the compound
(141) than the M2 macrophages, and hence, it was found
that these macrophages can be identified based on a
difference in the fluorescence intensity.

[0428]It is understood from Examples 110 and 111 that the
macrophage identification agent represented by general
formula (11) of the present invention can be used for
analyzing the correlation between a subtype and a
substance.

[0429]It is understood from Examples 98 to 111 and
Comparative Example 1 described above that the
macrophage identification agent represented by general
formula (11) of the present invention can be used for
identifying, sorting, evaluating and analyzing a
subtype.

[0430]Besides, by performing operations similar to those of
Examples 110 and 111 on a plurality of substances, the
correlation between a subtype and the substances can be

screened by using the macrophage identification agent
represented by general formula (11) of the present
invention.

[0431] Besides, it was revealed based on Example 112 that not
only the subtypes differentiated from bone marrow cells
but also the subtypes differentiated from a mouse cell
line can be identified by using the macrophage
identification agent represented by general formula
(11) of the present invention.
This result supports that subtypes can be identified by
using the macrophage identification agent represented
by general formula (11) of the present invention even
if the cell type is different.

[0432] Besides, it was revealed based on Examples 113 and 114
that not only the subtypes differentiated from bone
marrow cells but also the subtypes differentiated from
a human cell line can be identified by using the
macrophage identification agent represented by general
formula (11) of the present invention.
This result further supports that subtypes can be
identified by using the macrophage identification agent
represented by general formula (11) of the present
invention even if human-derived cells are used, and
probably suggests that the present invention is
applicable to pathologic tissues like human tissues or
even a human individual.

[0433] Example 115
Two x 10^5 each of M1 macrophages and M2 macrophages
cultured in the same manner as in Example 1 were
dispensed into a 1.5 mL tube, and the compound (1) and
the compound (113) were added thereto each to a
concentration of 1 μM. Dead cells were identified by
using the TO-PRO-3.

[0434] A fluorescent signal derived from the compound (1) was
measured in the FITC channel (excitation wavelength:
488 nm, center wavelength: 530 nm, wavelength width: 30

nm) and a fluorescent signal derived from the compound (113) was measured in the APC-Cy7 channel (excitation wavelength: 633 nm, center wavelength: 780 nm, wavelength width: 60 nm). A cytogram developed by plotting the intensity of the fluorescent signal derived from the compound (1) and obtained in the FITC channel on the ordinate and plotting the intensity of the fluorescent signal derived from the compound (113) and obtained in the APC-Cy7 channel on the abscissa was prepared.

[0435] The analysis was performed on a cell population showing a low signal for the TO-PRO-3, so as to analyze a cell population from which dead cells were eliminated. The operation was performed in the same manner as in Example 1 except for the above.

[0436] As a result, as is obvious from Figs. 30A to 30E, when the compound (1) is used, the M1 macrophages are more strongly stained than the M2 macrophages. On the contrary, when the compound (113) is used, the M2 macrophages are more strongly stained than the M1 macrophages. Therefore, the M1 macrophages and the M2 macrophages stained with the compound (1) and the compound (113) are plotted, on the cytogram developed with respect to the intensities of the fluorescent signals derived from these compounds, as cell populations having different fluorescent characteristics, and hence, these macrophages can be easily identified.

[0437] This example reveals that a subtype can be identified even when two or more types of compounds for the macrophage identification agent represented by general formulas (1), (6), (10), and (11) are used in combination.

INDUSTRIAL APPLICABILITY

[0438] The macrophage identification agent containing any of the organic compounds represented by general formulas

(1), (6), (10) and (11) provided by the present
invention is a useful material for realizing subtype
identification simply, highly safely and inexpensively.
Besides, also in evaluation of a substance affecting a
subtype, the macrophage identification agent of the
present invention can be used for performing high
throughput screening at low cost. Use of the
macrophage identification agent of the present
invention can remarkably develop the research for
clarifying the role of a subtype in an inflammatory
disease, and can contribute to development of an
effective diagnosis or treatment method for an
inflammatory disease, and thus, an extremely useful
basic technology industrially and practically can be
provided.

[0439] While the present invention has been described with
reference to exemplary embodiments, it is to be
understood that the invention is not limited to the
disclosed exemplary embodiments. The scope of the
following claims is to be accorded the broadest
interpretation so as to encompass all such
modifications and equivalent structures and functions.

[0440] This application claims the benefit of Japanese Patent
Application No. 2013-114578, filed May 30, 2013, which
is hereby incorporated by reference herein in its
entirety.

CLAIMS

[Claim 1] A method for identifying macrophage subtype
M1 and macrophage subtype M2, wherein the
subtype M1 and the subtype M2 are identified
based on a spectral characteristic obtained
with an organic compound added.

[Claim 2] The method according to claim 1, wherein the
organic compound is a dye compound having a
molecular weight of 2000 or less.

[Claim 3] The method according to claim 2, wherein the
organic compound comprises one or more
compounds represented by the following
general formula (1):

General formula (1)
wherein, in general formula (1), R_1
represents a hydrogen atom, an alkyl group,
an aralkyl group, an alkenyl group, or an
aryl group; R_2 to R_5 each independently
represent a hydrogen atom, an alkyl group, an
aryl group, a carboxyl group, a carboxylalkyl
group, or an alkylcarbonyl group, wherein R_2
and R_4 may be bound to each other to form an
aliphatic ring; R_6 represents a hydrogen atom,
an alkyl group, an alkoxy group, or a halogen
atom; R_7 and R_8 each independently represent a
hydrogen atom, an alkyl group, or an aryl
group; R_9 represents a dicyanomethylene group,
a cyanocarboxymethylene group, or any one of
the following general formulas (2) to (4); Y_1

represents a carbon atom having aryl group, a cyclopentene ring, a cyclohexene ring or a carbon atom; and n represents 0 or 1,

General formula (2)   General formula (3)   General formula (4)

in general formula (2), R_{10} represents an alkyl group, a carboxylalkyl group, an alkoxycarbonylalkyl group, or an alkylcarbonyloxyalkyl group; R_{11} represents a sulfur atom, a 2-thioxothiazolidin-4-one group, a thiazoline-2,4-one group, or the following general formula (5); and a sign "*" represents a binding site,

General formula (5)

in general formula (5), R_{12} represents an alkyl group, a carboxylalkyl group, an alkoxycarbonylalkyl group, or an alkylcarbonyloxyalkyl group; R_{13} and R_{14} each represent a hydrogen atom and may be bound to each other to form a benzene ring; Q_1 represents an oxygen atom, a sulfur atom, a N-alkyl nitrogen atom, or $-C(R_{15})(R_{16})-$,

wherein R_{15} and R_{16} each independently represent an alkyl group and may be bound to each other to form a ring; X_1^- represents an anionic group; and a sign "*" represents a binding site, in general formula (3), R_{17} represents an alkyl group, a carboxylalkyl group, an alkoxycarbonylalkyl group, or an alkylcarbonyloxyalkyl group; R_{18} and R_{19} each represent a hydrogen atom and may be bound to each other to form a benzene ring; Q_2 represents an oxygen atom, a sulfur atom, a N-alkyl nitrogen atom, or $-C(R_{20})(R_{21})-$, wherein R_{20} and R_{21} each independently represent an alkyl group and may be bound to each other to form a ring; X_2^- represents an anionic group; and a sign "*" represents a binding site, and in general formula (4), R_{22} represents an alkyl group or an aryl group; R_{23} represents an alkyl group or a carboxyl group; and a sign "*" represents a binding site.

[Claim 4] The method according to claim 3, wherein the ring formed when R_2 and R_4 of general formula (1) are bound to each other is a cyclopentane ring.

[Claim 5] The method according to claim 3 or 4, wherein R_9 of the compounds represented by general formula (1) is a compound represented by general formula (2).

[Claim 6] The method according to claim 3 or 4, wherein R_9 of the compounds represented by general formula (1) is a compound represented by general formula (3).

[Claim 7] The method according to claim 3 or 4, wherein R_9 of the compounds represented by general formula (1) is a compound represented by

general formula (4).
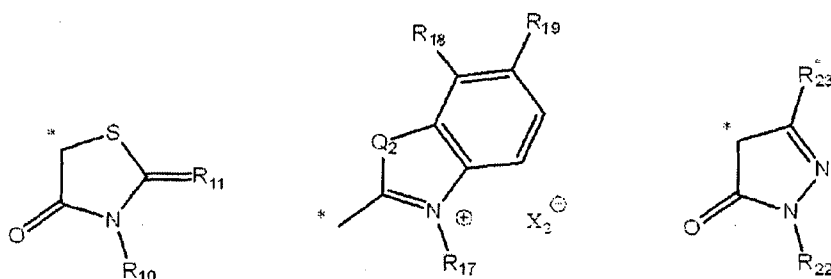
[Claim 8] The method according to claim 2, wherein the organic compound contains one or more compounds represented by the following general formula (6):
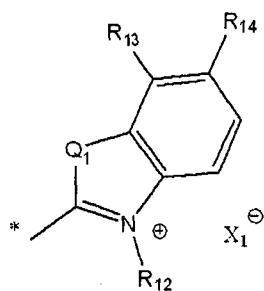

General formula (6)

wherein, in general formula (6), R_{24} and R_{25} each independently represent an alkyl group, a carboxylalkyl group, an alkoxycarbonylalkyl group, or an alkylcarbonyloxyalkyl group; R_{26} to R_{33} each independently represent a hydrogen atom, an alkyl group, an aryl group, an alkoxy group, a halogen atom, an alkoxysulfonyl group, a N-alkylsulfamoyl group, an alkyloxycarbonyl group, a carbamoyl group, or a N-alkylcarbamoyl group, wherein R_{26} and R_{27}, R_{28} and R_{29}, R_{30} and R_{31}, and R_{32} and R_{33} may be independently cyclized to form a benzene ring; X_1^- represents an anionic group; Y_2 represents a group containing $*1$, $*2$ and $*5$ and containing an oxygen atom, a sulfur atom, a N-alkyl nitrogen atom or $-C(R_{34})(R_{35})-$, or a group containing $*1$, $*2$ and $*5$ and represented by $*1-C*5-CH=CH-*2$ or $*1-CH=CH-*5-*2$; Y_3 represents a group containing $*3$, $*4$ and $*6$ and containing an oxygen atom, a sulfur atom, a N-alkyl nitrogen atom or $-C(R_{34})(R_{35})-$, or a group containing $*3$, $*4$ and $*6$ and represented by

*3-Y$_3$-*6-*4, *3-C*6-CH=CH-*4, or
*3-CH=CH-*6-*4,
wherein R$_{34}$ and R$_{35}$ each independently
represent an alkyl group and may be bound to
each other to form a ring; and
A represents a 3-oxocyclobutenolate ring or
is represented by the following general
formula (7) or (8):

General formula (7)

General formula (8)
in general formula (7), R$_{36}$ to R$_{38}$ each
independently represent a hydrogen atom, an
alkyl group or an aryl group, and n
represents a value of 0 to 2,
in general formula (8), R$_{39}$ represents a
hydrogen atom, a phenyl group, a thiol group,
an alkoxy group, an aryloxy group, or a
halogen atom; and
R$_{40}$ and R$_{41}$ each independently represent a
hydrogen atom, an alkyl group or an
alkyloxycarbonyl group.
[Claim 9] The method according to claim 8, wherein the

compound represented by general formula (6)
is represented by the following general
formula (9):
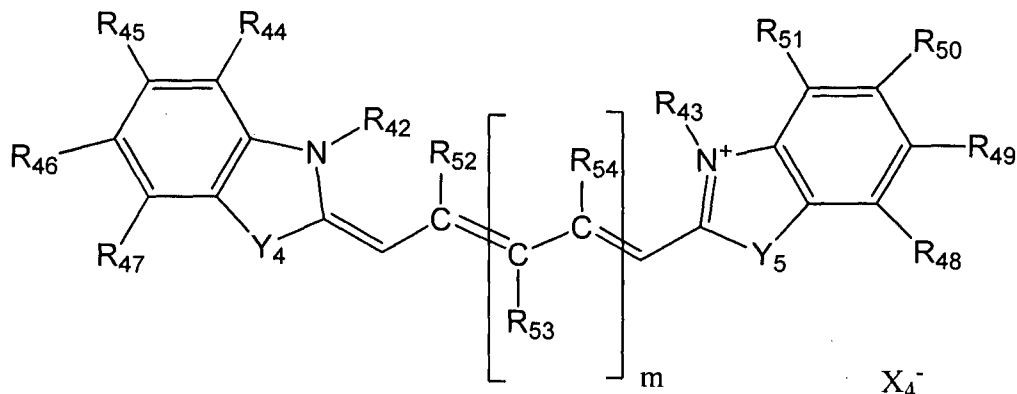
General formula (9)

wherein, in general formula (9), R_{42} and R_{43}
each independently represent an alkyl group,
a carboxylalkyl group, an
alkylcarbonyloxyalkyl group, or an
alkoxycarbonyl alkyl group; and R_{44} to R_{51}
each independently represent a hydrogen atom,
an alkyl group, an aryl group, an alkoxy
group, a halogen atom, an alkoxysulfonyl
group, a N-alkylsulfamoyl group, an
alkyloxycarbonyl group, or a N-alkylcarbamoyl
group, wherein R_{44} and R_{45}, R_{46} and R_{47}, R_{48} and
R_{49}, and R_{50} and R_{51} may be independently
cyclized to form a benzene ring;
R_{49} to R_{51} each independently represent a
hydrogen atom, an alkyl group or an aryl
group; m represents a value of 0 to 2; X_2^-
represents an anionic group;
Y_4 and Y_5 each represent an oxygen atom, a
sulfur atom or an alkylene group, wherein the
alkylene group may have a substituent, the
substituent used in this case is an alkyl
group, and the substituents may be bound to

each other to form an aliphatic ring.

[Claim 10] The method according to claim 8, wherein A of
general formula (6) is a 3-oxocyclobutenolate
ring.
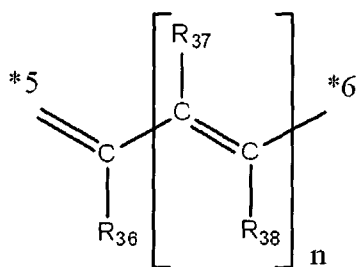
[Claim 11] The method according to claim 2, wherein the
organic compound contains one or more
compounds represented by the following
general formula (10):

General formula (10)

wherein, in general formula (10), R_{55} and R_{56}
each independently represent an alkyl group;
R_{57} and R_{58} each independently represent a
hydrogen atom or an alkyl group, wherein R_{55}
and R_{57}, and R_{56} and R_{58} may be independently
bound to each other to form a ring; R_{59}
represents a hydrogen atom, an alkyl group,
an aryl group, an alkoxy group or a halogen
atom; X_5 represents a sulfur atom, an oxygen
atom or $-NR_6-$; and Y_6 represents a carbon atom
or a nitrogen atom.

[Claim 12] The method according to claim 2, wherein the
organic compound contains one or more
compounds represented by the following
general formula (11):

General formula (11)
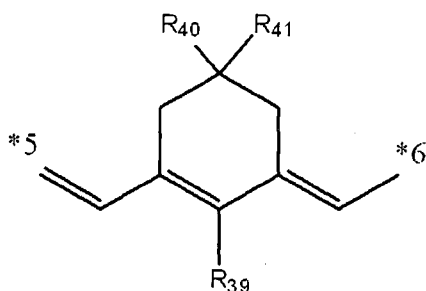
wherein, in general formula (11), R_{60}
represents a hydrogen atom, an alkyl group,
an aryl group, a thioalkyl group, an amino
group, a hetero ring group, an alkenyl group,
a hydroxyl group, a halogen atom, or an
alkoxy group; R_{61} to R_{66} each independently
represent a hydrogen atom, an alkyl group, an
aryl group, a hetero ring group, an aralkyl
group, or a sulfonyl group, wherein R_{62} and
R_{63}, and R_{65} and R_{66} may be bound to each other
to form a hetero ring; and R_{67} and R_{68} each
independently represent a fluorine atom or an
alkynyl group.

[Claim 13] The method according to any one of claims 1
to 12, wherein the M1 macrophage contained in
a biological sample is identified by
utilizing that the M1 macrophage has
different staining properties from the M2
macrophage.

[Claim 14] The method according to any one of claims 1
to 12, wherein the M2 macrophage contained in
a biological sample is identified by
utilizing that the M1 macrophage has
different staining properties from the M2
macrophage.

[Claim 15] The method according to any one of claims 1
to 12, wherein the M1 macrophage and the M2

macrophage contained in a biological sample
are identified by utilizing that the M1
macrophage has different staining properties
from the M2 macrophage.

[Claim 16] A macrophage identification agent for
identifying macrophage subtypes M1 and M2,
comprising one or more organic compounds,
wherein a spectral characteristic obtained
when the organic compound is added is
different between the subtype M1 and the
subtype M2.

[Claim 17] The macrophage identification agent according
to claim 16, wherein the organic compound is
an organic compound having a molecular weight
of 2000 or less.

[Claim 18] The macrophage identification agent according
to claim 17, wherein the organic compound
contains one or more compounds represented by
the following general formula (1):

General formula (1)
wherein, in general formula (1), R_1
represents a hydrogen atom, an alkyl group,
an aralkyl group, an alkenyl group, or an
aryl group; R_2 to R_5 each independently
represent a hydrogen atom, an alkyl group, an
aryl group, a carboxyl group, a carboxylalkyl
group, or an alkylcarbonyl group, wherein R_2
and R_4 may be bound to each other to form an
aliphatic ring; R_6 represents a hydrogen atom,

an alkyl group, an alkoxy group, or a halogen
atom; R_7 and R_8 each independently represent a
hydrogen atom, an alkyl group, or an aryl
group; R_9 represents a dicyanomethylene group,
a cyanocarboxymethylene group, or any one of
the following general formulas (2) to (4); Y_1
represents a carbon atom having aryl group, a
cyclopentene ring, a cyclohexene ring or a
carbon atom; and n represents 0 or 1,

    General formula (2)   General formula (3)   General formula (4)

in general formula (2), R_{10} represents an
alkyl group, a carboxylalkyl group, an
alkoxycarbonylalkyl group, or an
alkylcarbonyloxyalkyl group; R_{11} represents a
sulfur atom, a 2-thioxothiazolidin-4-one
group, a thiazoline-2,4-one group, or the
following general formula (5); and a sign "*"
represents a binding site,

General formula (5)
in general formula (5), R_{12} represents an
alkyl group, a carboxylalkyl group, an

alkoxycarbonylalkyl group, or an alkylcarbonyloxyalkyl group; R_{13} and R_{14} each represent a hydrogen atom and may be bound to each other to form a benzene ring; Q_1 represents an oxygen atom, a sulfur atom, a N-alkyl nitrogen atom, or $-C(R_{15})(R_{16})-$, wherein R_{15} and R_{16} each independently represent an alkyl group and may be bound to each other to form a ring; X_1^- represents an anionic group; and a sign "*" represents a binding site, in general formula (3), R_{17} represents an alkyl group, a carboxylalkyl group, an alkoxycarbonylalkyl group, or an alkylcarbonyloxyalkyl group; R_{18} and R_{19} each represent a hydrogen atom and may be bound to each other to form a benzene ring; Q_2 represents an oxygen atom, a sulfur atom, a N-alkyl nitrogen atom, or $-C(R_{20})(R_{21})-$, wherein R_{20} and R_{21} each independently represent an alkyl group and may be bound to each other to form a ring; X_2^- represents an anionic group; and a sign "*" represents a binding site, and in general formula (4), R_{22} represents an alkyl group or an aryl group; R_{23} represents an alkyl group or a carboxyl group; and a sign "*" represents a binding site.

[Claim 19] The macrophage identification agent according to claim 18, wherein the ring formed when R_2 and R_4 of general formula (1) are bound to each other is a cyclopentane ring.

[Claim 20] The macrophage identification agent according to claim 18 or 19, wherein R_9 of the compounds represented by general formula (1) is a compound represented by general formula (2).

[Claim 21] The macrophage identification agent according
to claim 18 or 19, wherein R_9 of the
compounds represented by general formula (1)
is a compound represented by general formula
(3).

[Claim 22] The macrophage identification agent according
to claim 18 or 19, wherein R_9 of the
compounds represented by general formula (1)
is a compound represented by general formula
(4).

[Claim 23] The macrophage identification agent according
to claim 17, wherein the organic compound
contains one or more compounds represented by
the following general formula (6):

General formula (6)

wherein, in general formula (6), R_{24} and R_{25}
each independently represent an alkyl group,
a carboxylalkyl group, an alkoxycarbonylalkyl
group, or an alkylcarbonyloxyalkyl group; R_{26}
to R_{33} each independently represent a hydrogen
atom, an alkyl group, an aryl group, an
alkoxy group, a halogen atom, an
alkoxysulfonyl group, a N-alkylsulfamoyl
group, an alkyloxycarbonyl group, a carbamoyl
group, or a N-alkylcarbamoyl group, wherein
R_{26} and R_{27}, R_{28} and R_{29}, R_{30} and R_{31}, and R_{32} and
R_{33} may be independently cyclized to form a
benzene ring; X_1^- represents an anionic group;
Y_2 represents a group containing *1, *2 and

*5 and containing an oxygen atom, a sulfur
atom, a N-alkyl nitrogen atom or —C(R$_{34}$)(R$_{35}$)—,
or a group containing *1, *2 and *5 and
represented by *1—C*5—CH=CH—*2 or
*1—CH=CH—*5—*2; Y$_3$ represents a group
containing *3, *4 and *6 and containing an
oxygen atom, a sulfur atom, a N-alkyl
nitrogen atom or —C(R$_{34}$)(R$_{35}$)—, or a group
containing *3, *4 and *6 and represented by
*3—Y$_3$—*6—*4, *3—C*6—CH=CH—*4, or
*3—CH=CH—*6—*4,
wherein R$_{34}$ and R$_{35}$ each independently
represent an alkyl group, and may be bound to
each other to form a ring; and
A represents a 3-oxocyclobutenolate ring or
is represented by the following general
formula (7) or (8):

General formula (7)

General formula (8)
in general formula (7), R$_{36}$ to R$_{38}$ each
independently represent a hydrogen atom, an
alkyl group or an aryl group, and n

represents a value of 0 to 2,

in general formula (8), R_{39} represents a
hydrogen atom, a phenyl group, a thiol group,
an alkoxy group, an aryloxy group, or a
halogen atom; and

R_{40} and R_{41} each independently represent a
hydrogen atom, an alkyl group or an
alkyloxycarbonyl group.

[Claim 24] The macrophage identification agent according
to claim 23, wherein the organic compound is
a compound represented by the following
general formula (9):

General formula (9)

wherein, in general formula (9), R_{42} and R_{43}
each independently represent an alkyl group,
a carboxylalkyl group, an
alkylcarbonyloxyalkyl group, or an
alkoxycarbonyl alkyl group; and R_{44} to R_{51}
each independently represent a hydrogen atom,
an alkyl group, an aryl group, an alkoxy
group, a halogen atom, an alkoxysulfonyl
group, a N-alkylsulfamoyl group, an
alkyloxycarbonyl group, or a N-alkylcarbamoyl
group, wherein R_{44} and R_{45}, R_{46} and R_{47}, R_{48} and
R_{49}, and R_{50} and R_{51} may be independently

cyclized to form a benzene ring;

R_{52} to R_{54} each independently represent a
hydrogen atom, an alkyl group or an aryl
group; m represents a value of 0 to 2; X_2^-
represents an anionic group;

Y_4 and Y_5 each represent an oxygen atom, a
sulfur atom or an alkylene group, wherein the
alkylene group may have a substituent, the
substituent used in this case is an alkyl
group, and the substituents may be bound to
each other to form an aliphatic ring.

[Claim 25] The macrophage identification agent according
to claim 23, wherein A of general formula (6)
is a 3-oxocyclobutenolate ring.

[Claim 26] The macrophage identification agent according
to claim 17, wherein the organic compound
contains one or more compounds represented by
the following general formula (10):

General formula (10)

wherein, in general formula (10), R_{55} and R_{56}
each independently represent an alkyl group;
R_{57} and R_{58} each independently represent a
hydrogen atom or an alkyl group, wherein R_{55}
and R_{57}, and R_{56} and R_{58} may be independently
bound to each other to form a ring; R_{59}

represents a hydrogen atom, an alkyl group,
an aryl group, an alkoxy group or a halogen
atom; X_5 represents a sulfur atom, an oxygen
atom or $-NR_6-$; and Y_6 represents a carbon atom
or a nitrogen atom.

[Claim 27] The macrophage identification agent according
to claim 17, wherein the organic compound
contains one or more compounds represented by
the following general formula (11):

General formula (11)
wherein, in general formula (11), R_{60}
represents a hydrogen atom, an alkyl group,
an aryl group, a thioalkyl group, an amino
group, a hetero ring group, an alkenyl group,
a hydroxyl group, a halogen atom, or an
alkoxy group; R_{61} to R_{66} each independently
represent a hydrogen atom, an alkyl group, an
aryl group, a hetero ring group, an aralkyl
group, or a sulfonyl group, wherein R_{62} and
R_{63}, and R_{65} and R_{66} may be bound to each other
to form a hetero ring; and R_{67} and R_{68} each
independently represent a fluorine atom or an
alkynyl group.

[Claim 28] The method according to any one of claims 1
to 15, wherein the organic compound is
exposed to a biological sample.

[Claim 29] A method for sorting a macrophage subtype
that is contained in the biological sample

and is identified by the identifying method according to any one of claims 1 to 15.

[Claim 30] An evaluation method for a macrophage subtype, at least comprising detecting a type, the number, a ratio or an optical characteristic of a macrophage subtype that is contained in the biological sample and is identified by the identifying method according to any one of claims 1 to 15.

[Claim 31] An evaluation method for a macrophage subtype, comprising allowing the macrophage identification agent according to any one of claims 16 to 30 and a test substance to work on a part or whole of the biological sample.

[Claim 32] An analysis method for analyzing correlation between a macrophage subtype and a test substance based on the evaluation method for a macrophage subtype according to claim 31.

[Claim 33] An analysis method for analyzing correlation between a macrophage subtype and a test substance based on the evaluation method for a macrophage subtype according to claim 30, comprising detecting an optical characteristic derived from the test substance.

[Claim 34] A screening method for examining correlation between a macrophage subtype and a test substance by using the analysis method according to claim 32 or 33.

[Claim 35] An identification kit for a macrophage subtype, comprising at least the macrophage identification agent according to any one of claims 16 to 30.

[Claim 36] A sorting method for macrophages, wherein two or more types of the macrophage identification agent according to any one of

claims 16 to 27 are used.

[Claim 37] An evaluation method for macrophages, wherein
two or more types of the macrophage
identification agent according to any one of
claims 16 to 27 are used.

[Claim 38] An analysis method for macrophages, wherein
two or more types of the macrophage
identification agent according to any one of
claims 16 to 27 are used.

[Claim 39] A screening method for macrophages, wherein
two or more types of the macrophage
identification agent according to any one of
claims 16 to 27 are used.

[Claim 40] An identification kit for a macrophage
subtype, comprising two or more types of the
macrophage identification agent according to
any one of claims 16 to 27.

FIG. 1

FIG. 2

FIG. 3

FIG. 4

FIG. 5A

M2 MACROPHAGES   M1 MACROPHAGES

FITC; 530/30 nm
(FLUORESCENT SIGNAL INTENSITY DERIVED
FROM COMPOUND (1))

FIG. 5B

M1 MACROPHAGES   M2 MACROPHAGES

AmCyan; 510/50 nm
(FLUORESCENT SIGNAL INTENSITY DERIVED
FROM COMPOUND (33))

FIG. 5C

FIG. 5D

FIG. 5E

FIG. 6A

FIG. 6B

FIG. 6C

FIG. 7A

FIG. 7B

FIG. 8

FIG. 9

FIG. 10

FIG. 11

FIG. 12

FIG. 13A

FIG. 13B

FIG. 13C

FIG. 14A

FIG. 14B

FIG. 15

M2 MACROPHAGES   M1 MACROPHAGES

THE NUMBER OF CELLS

FITC; 530/30 nm

FIG. 16

M1 MACROPHAGES   M2 MACROPHAGES

THE NUMBER OF CELLS

PACIFIC BLUE; 450/50 nm

FIG. 17A

M1 MACROPHAGES   M2 MACROPHAGES

FITC; 530/30 nm
(FLUORESCENT SIGNAL INTENSITY DERIVED
FROM COMPOUND (116))

FIG. 17B

M2 MACROPHAGES   M1 MACROPHAGES

PACIFIC BLUE; 450/50 nm
(FLUORESCENT SIGNAL INTENSITY DERIVED
FROM COMPOUND (120))
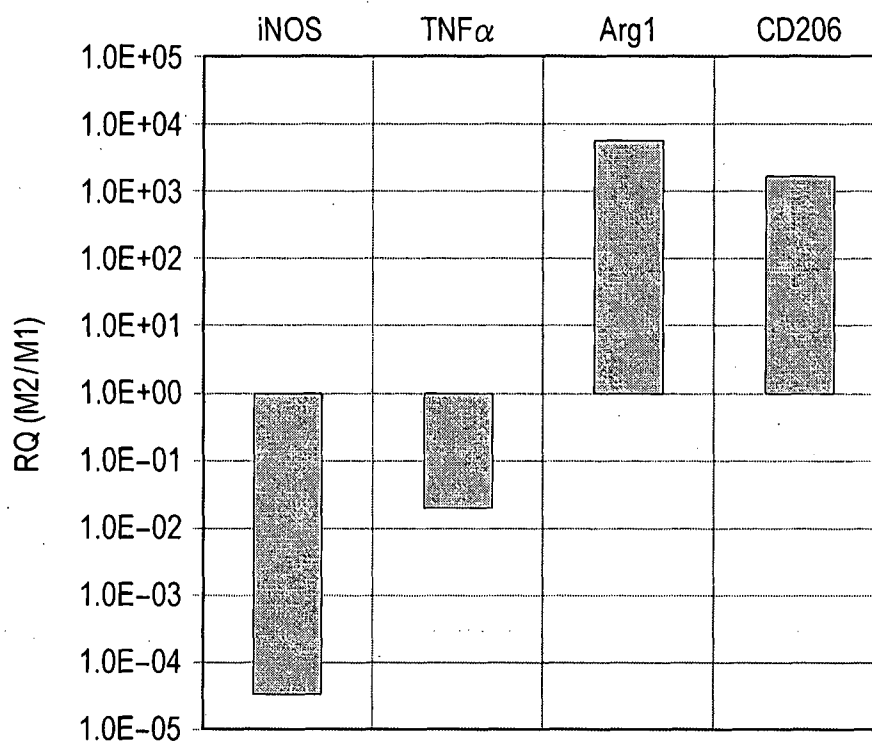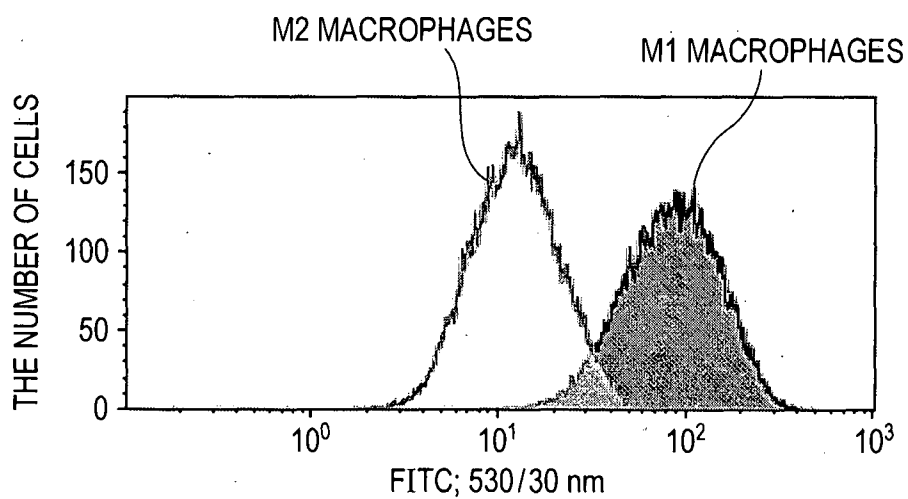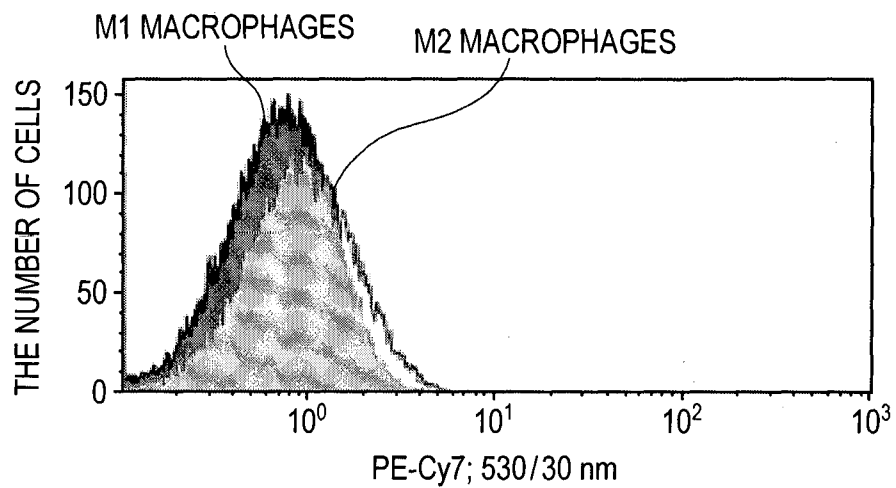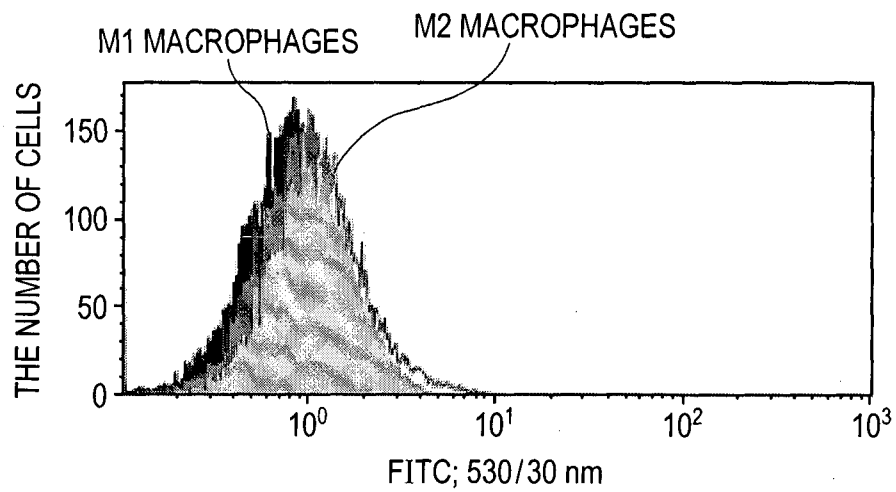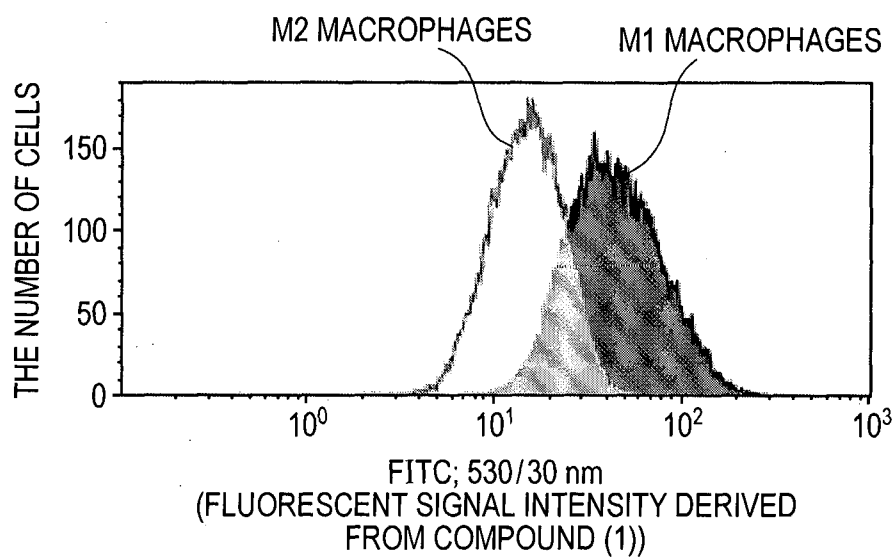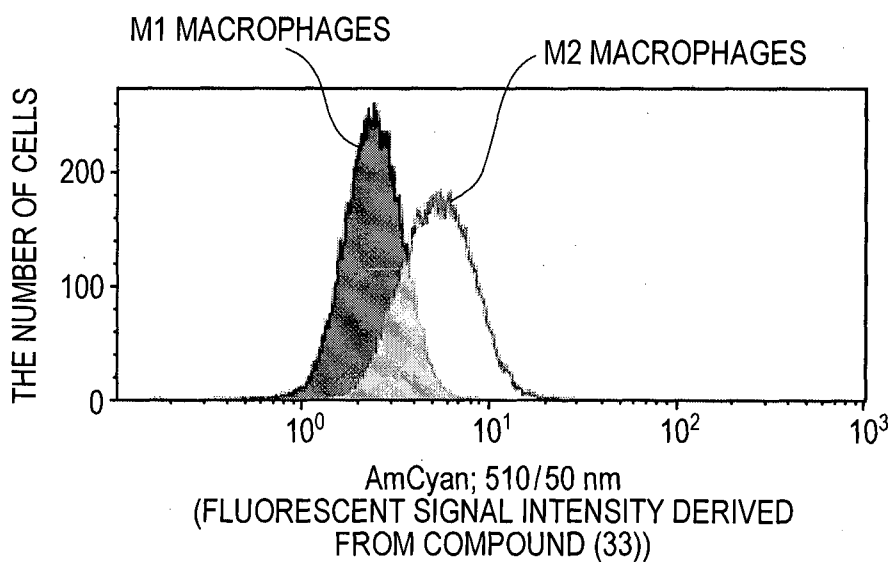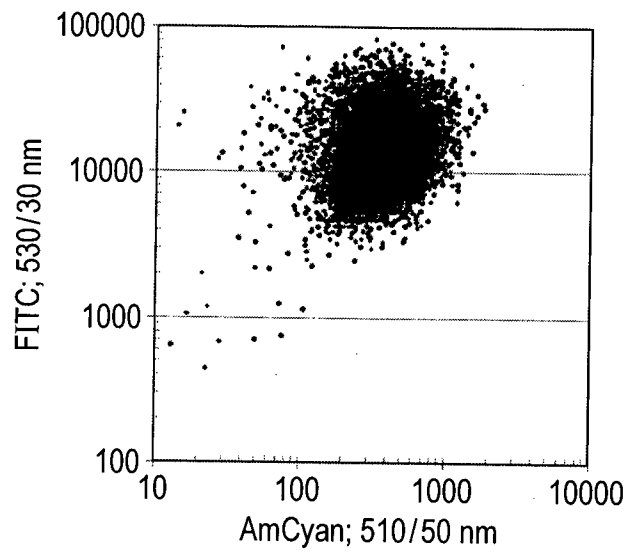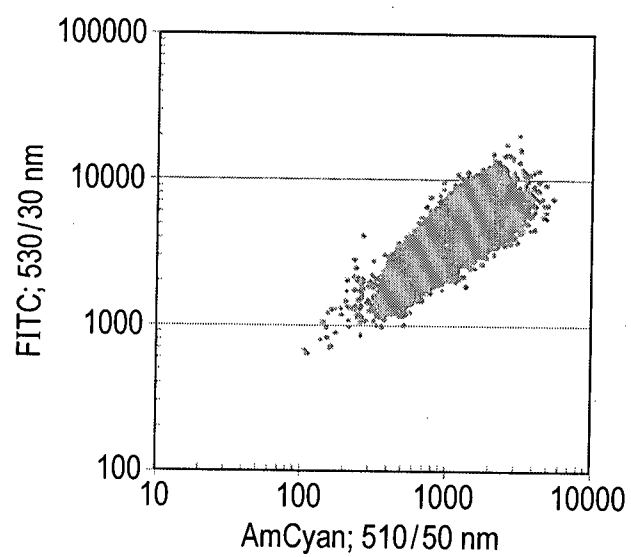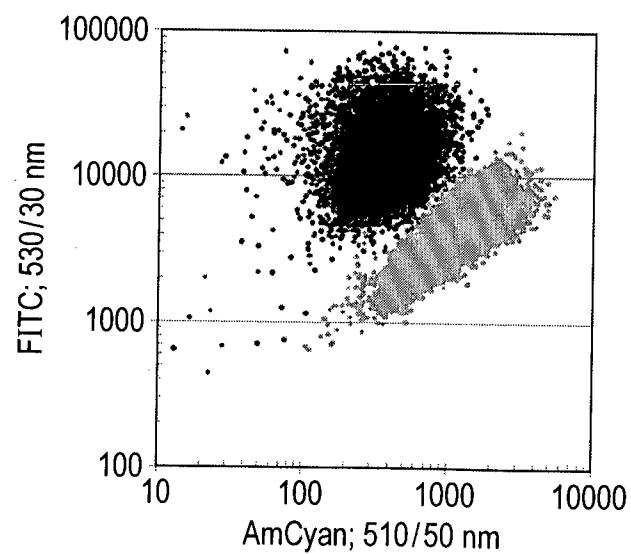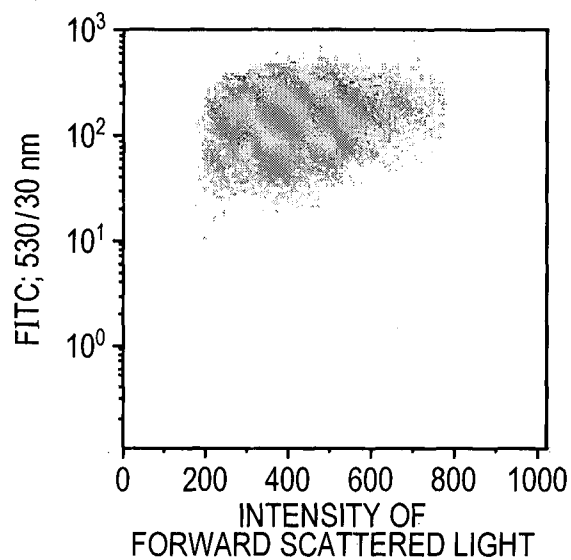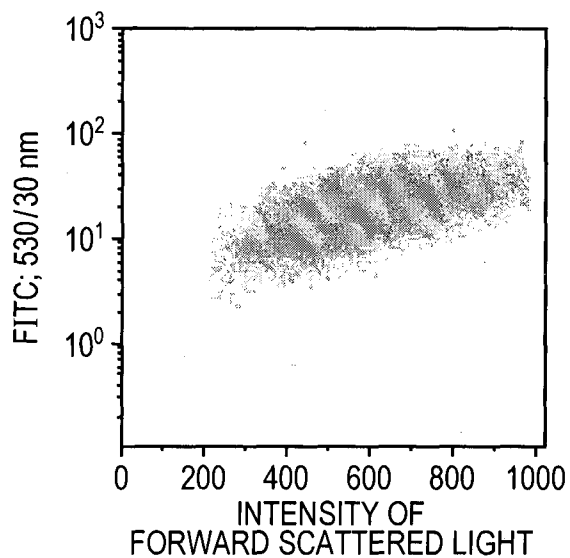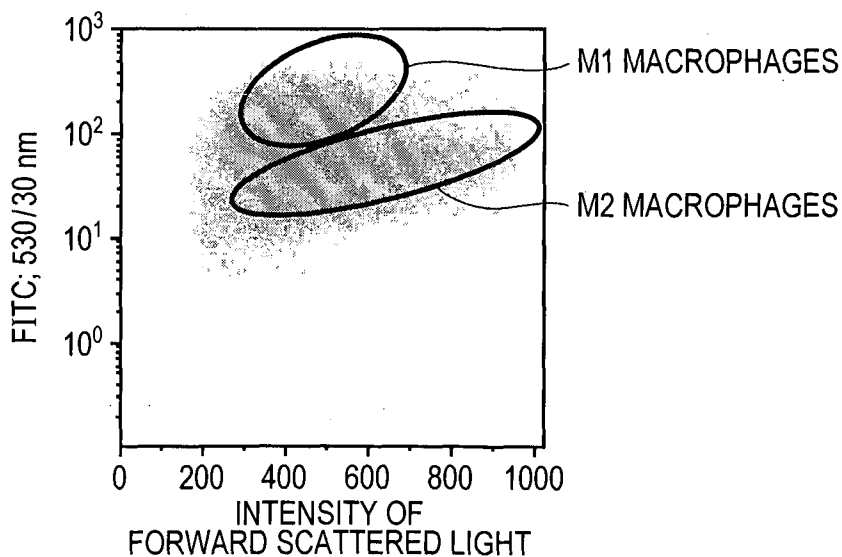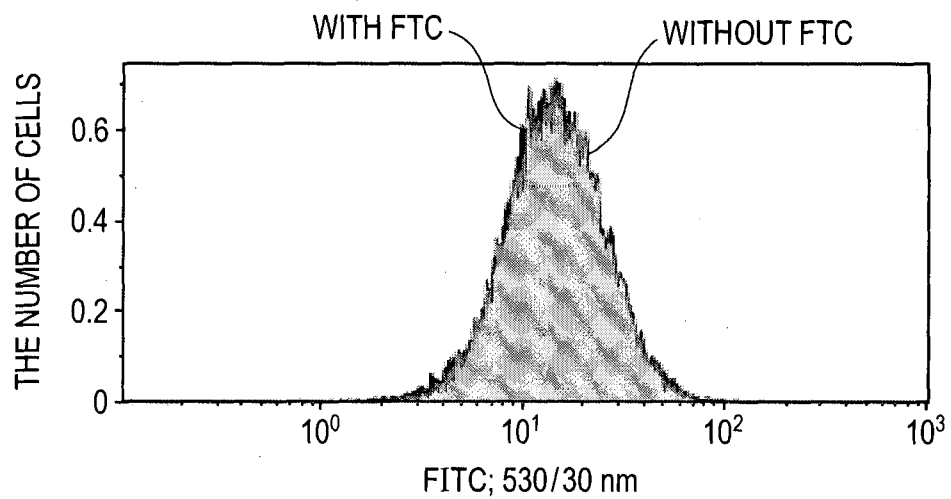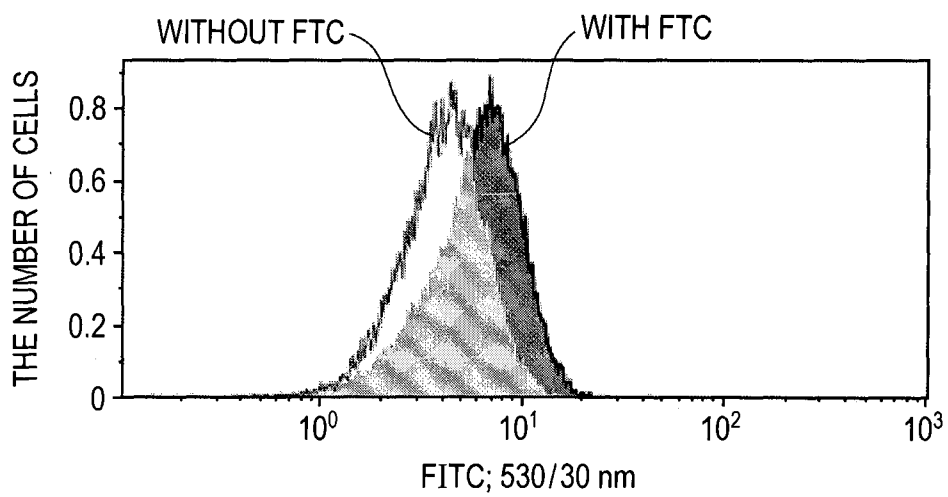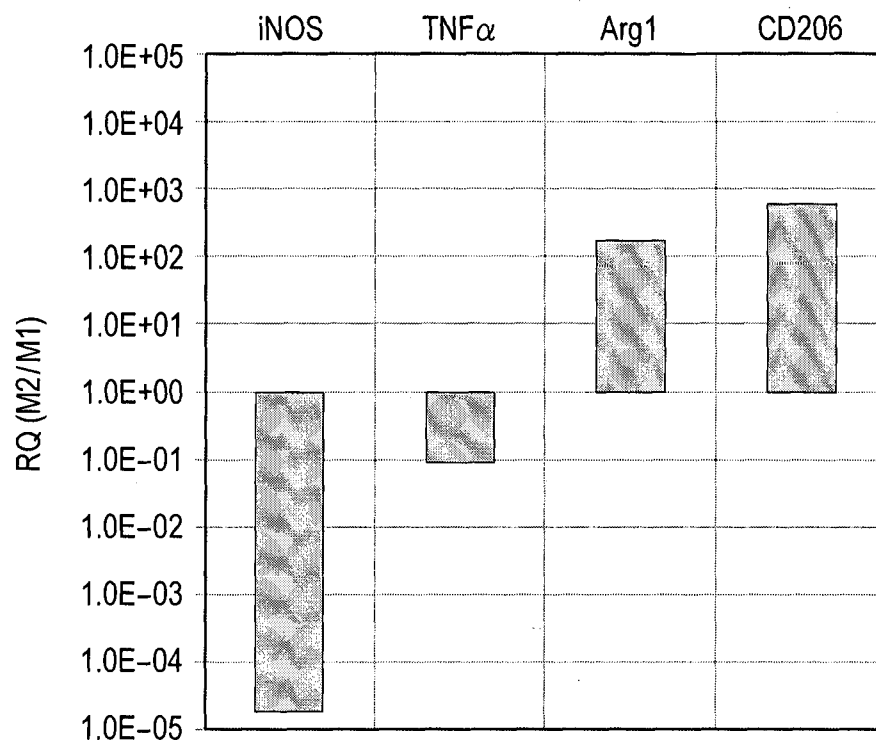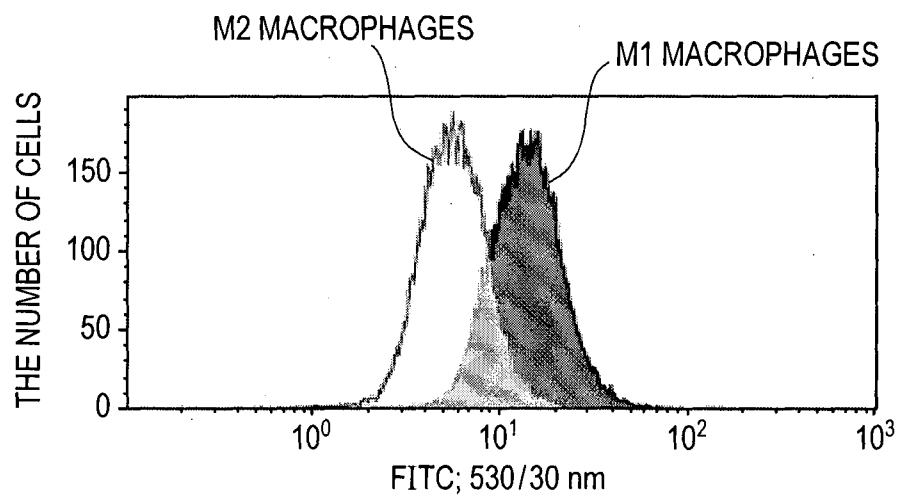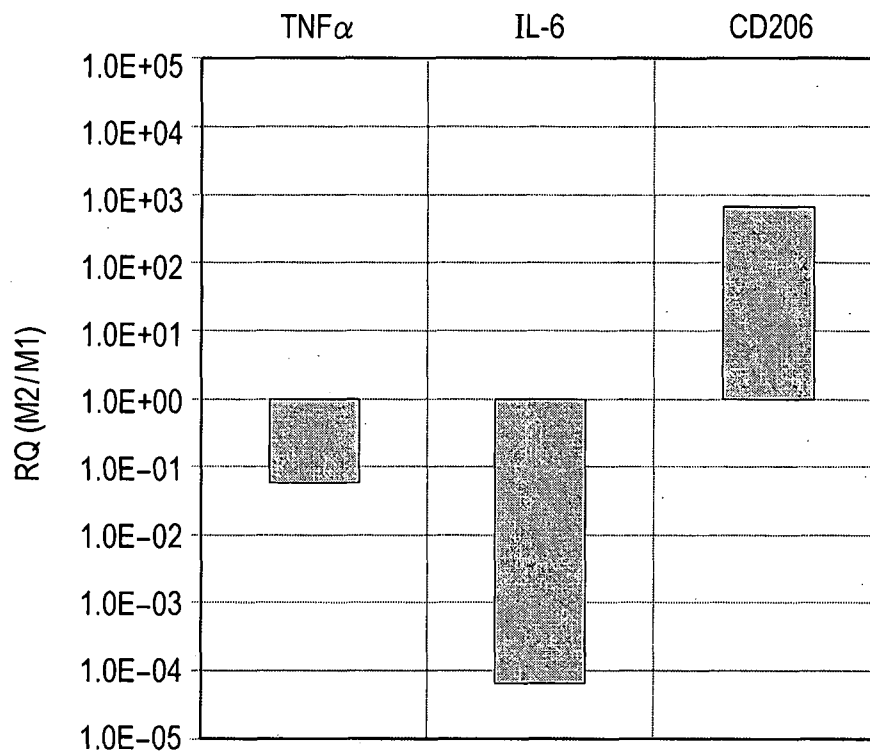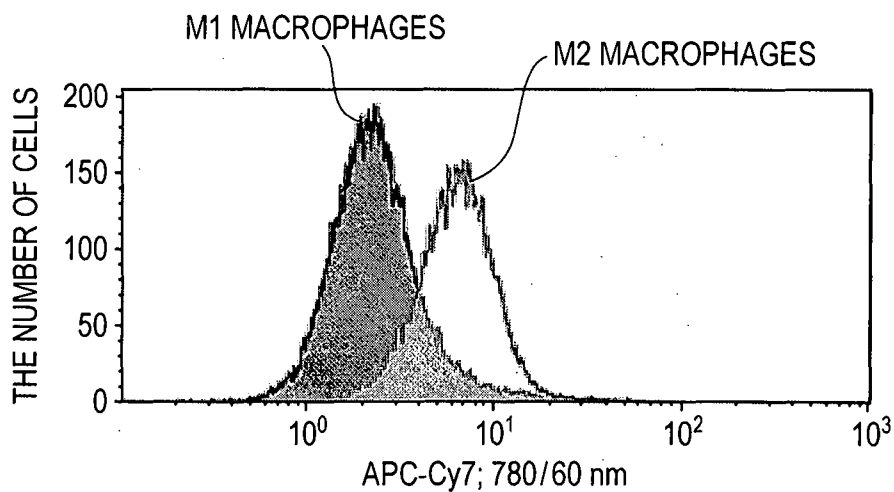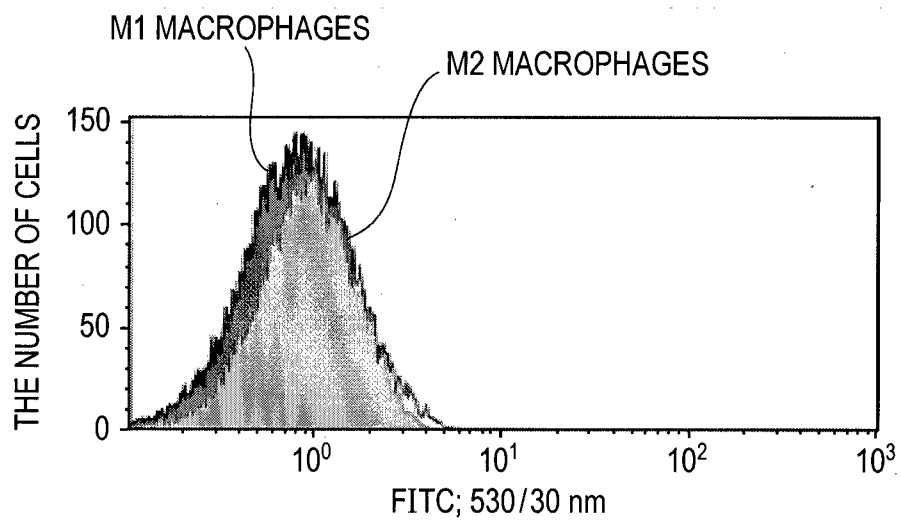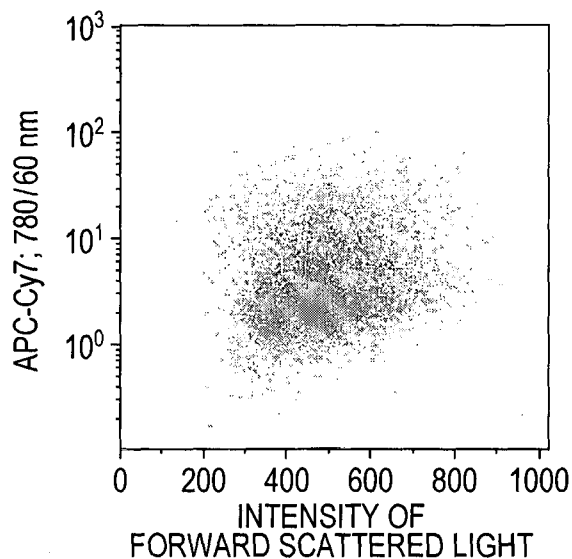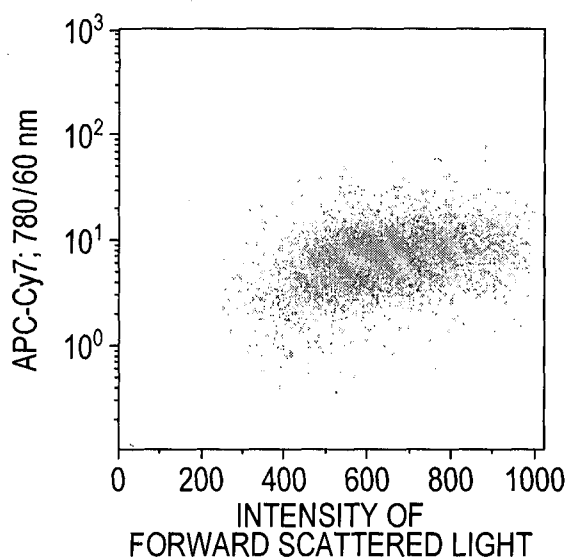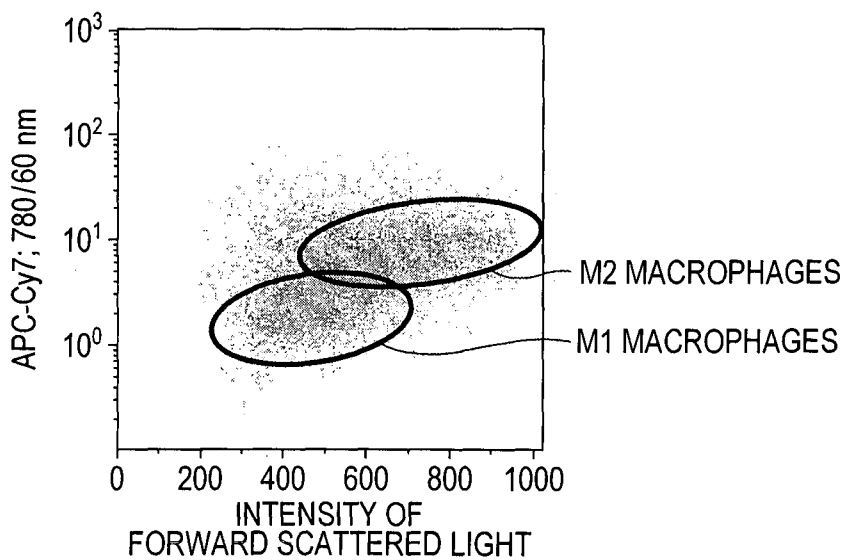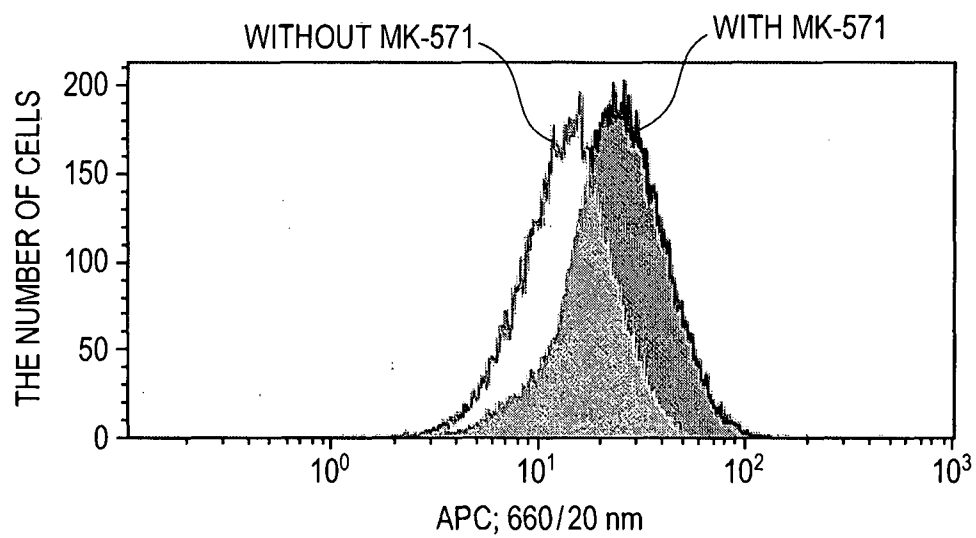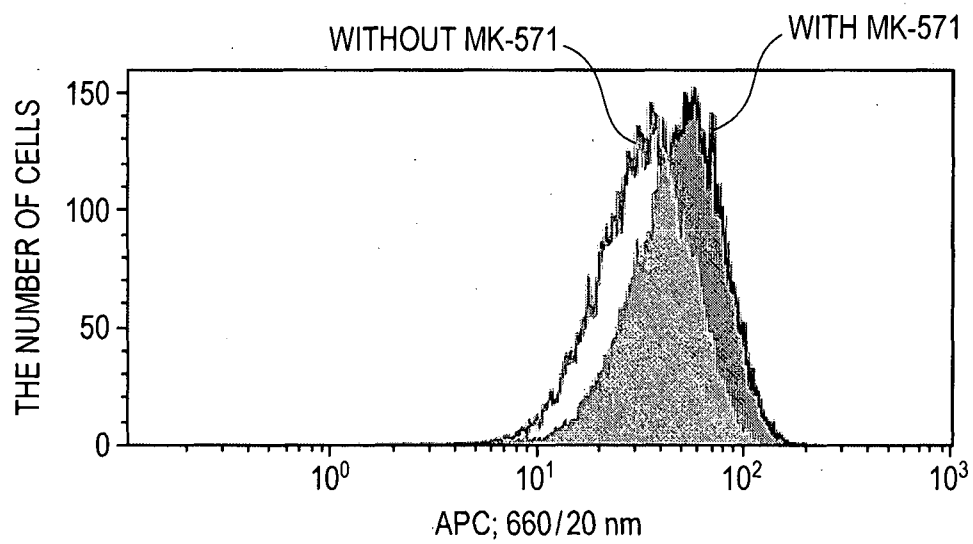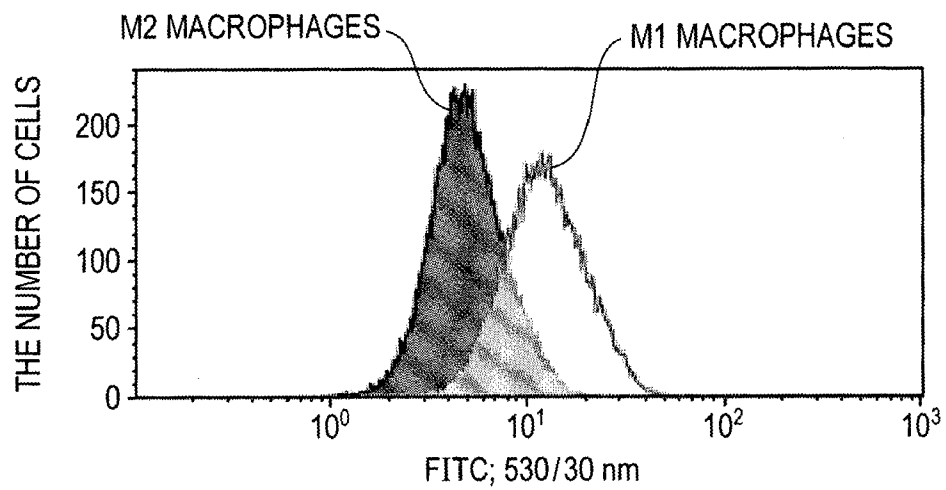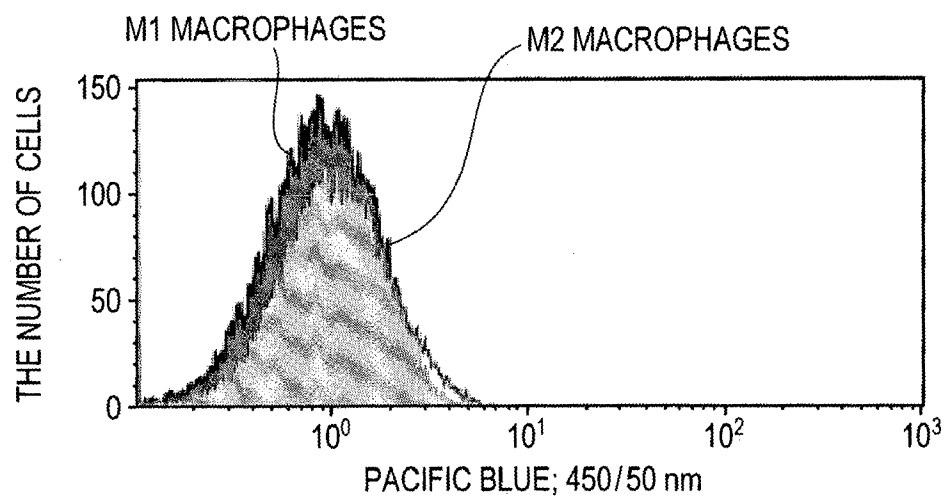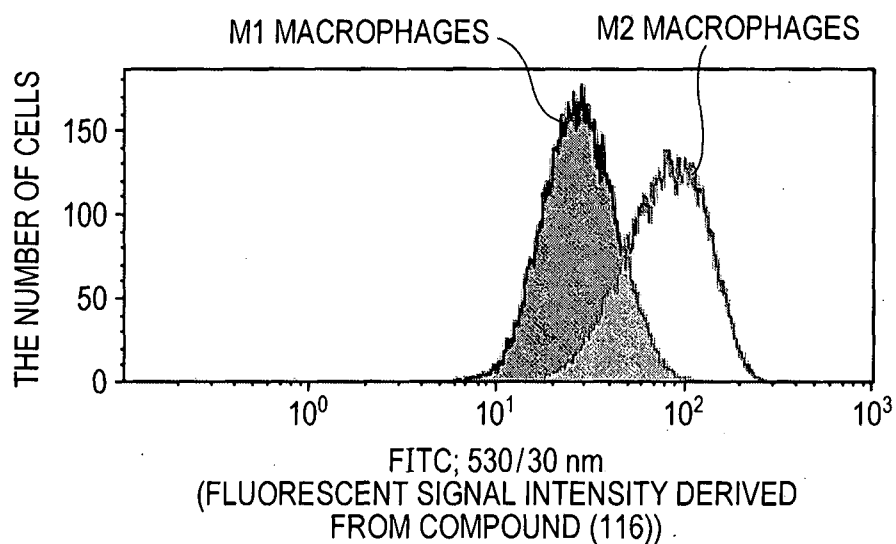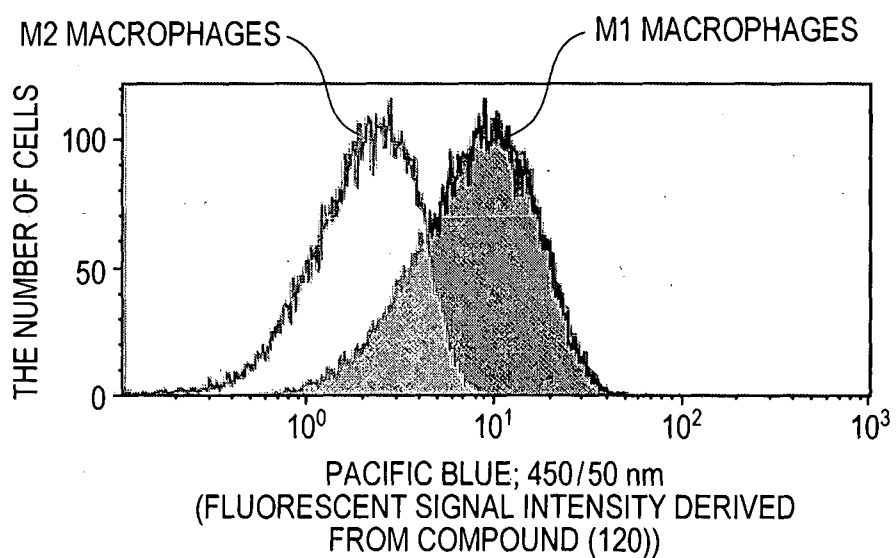
FIG. 17C

FIG. 17D

FIG. 17E

FIG. 18A

FIG. 18B

FIG. 18C

FIG. 19A

FIG. 19B

FIG. 20

FIG. 21

FIG. 22

FIG. 23

FIG. 24A

FITC; 530/30 nm
(FLUORESCENT SIGNAL INTENSITY DERIVED
FROM COMPOUND (139))

FIG. 24B

APC; 660/20 nm
(FLUORESCENT SIGNAL INTENSITY DERIVED
FROM COMPOUND (161))
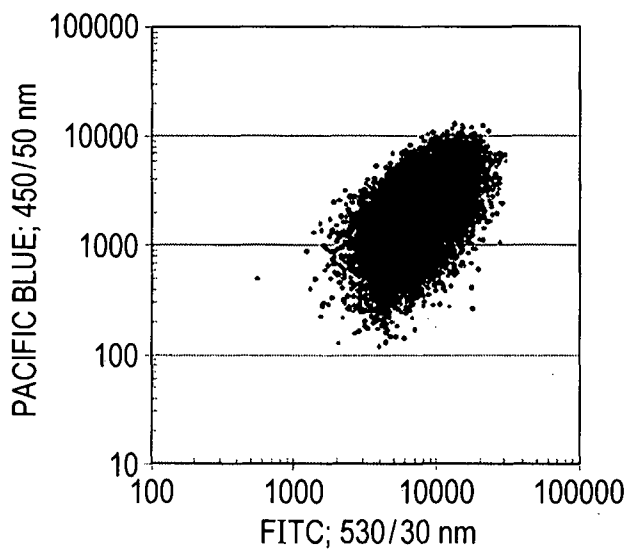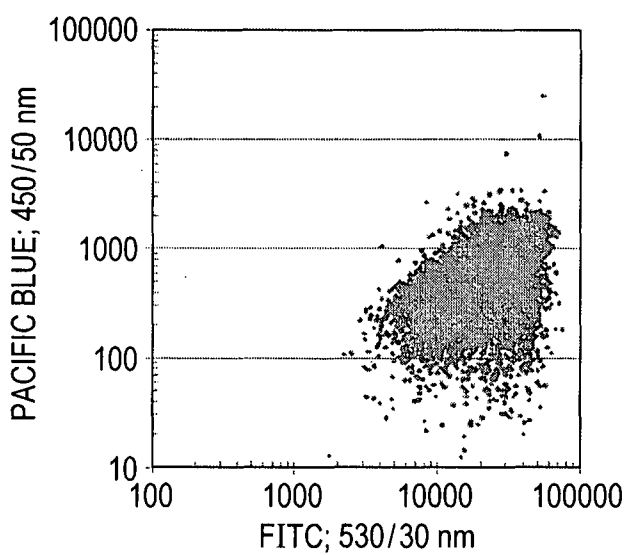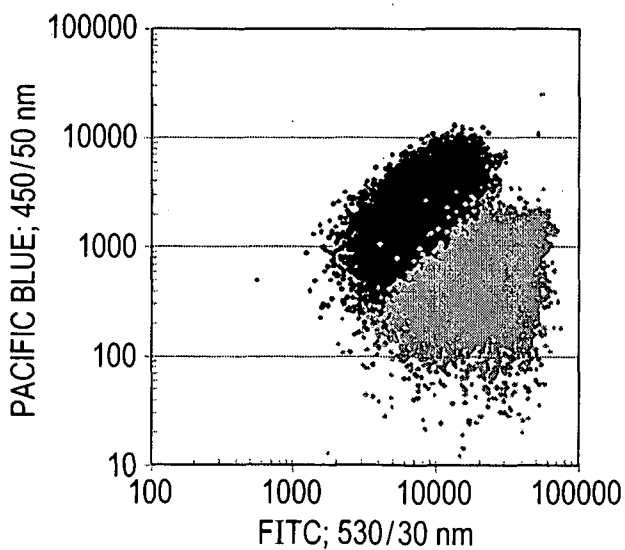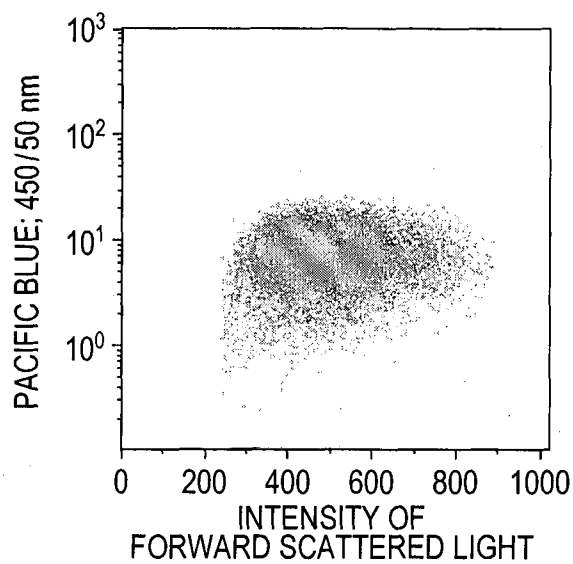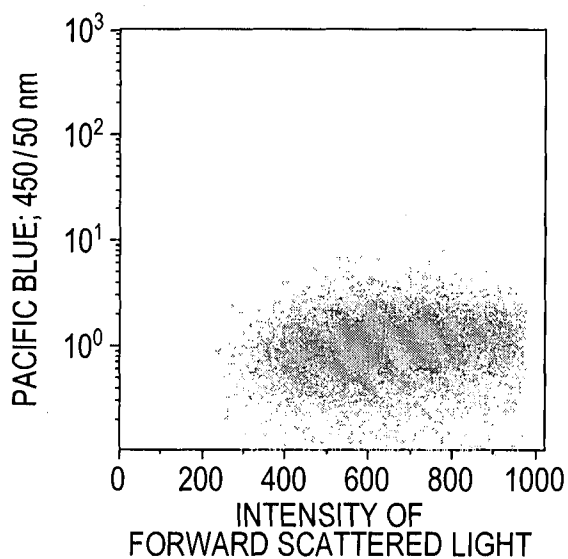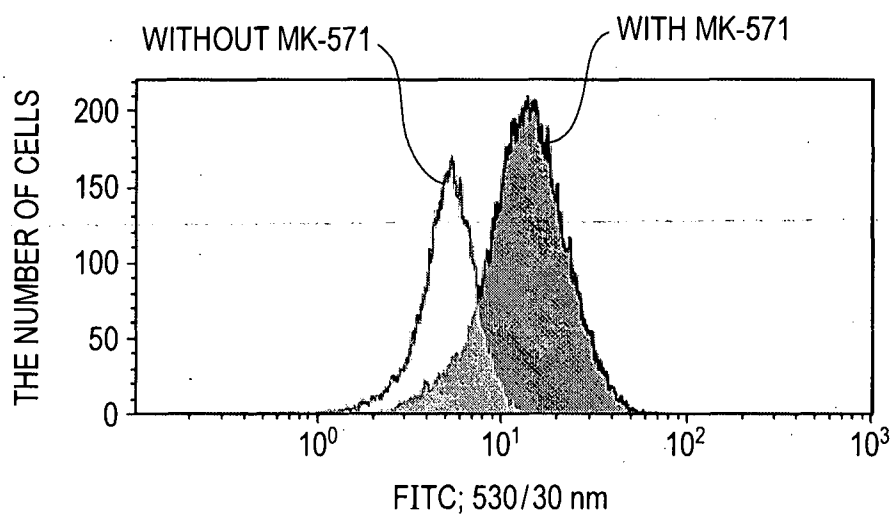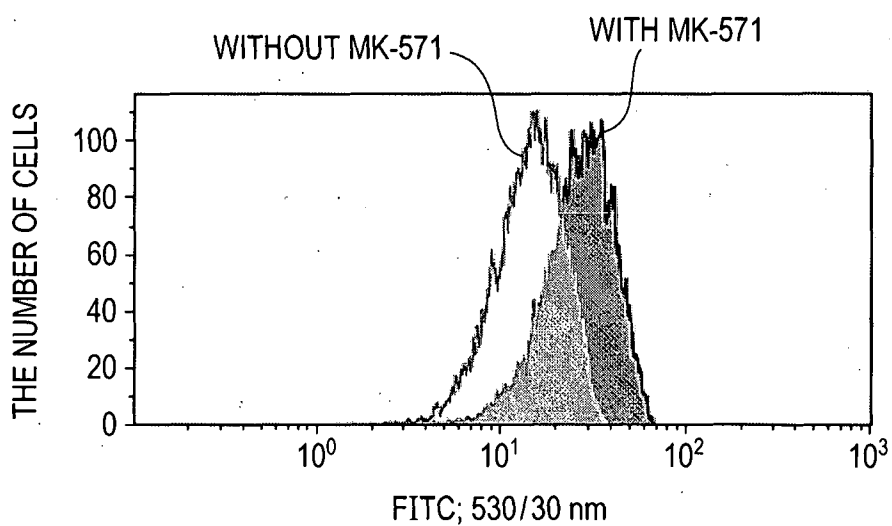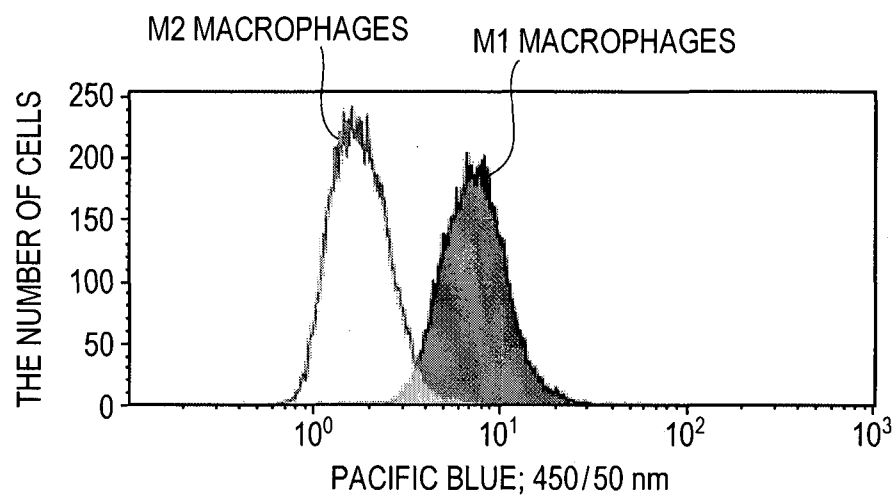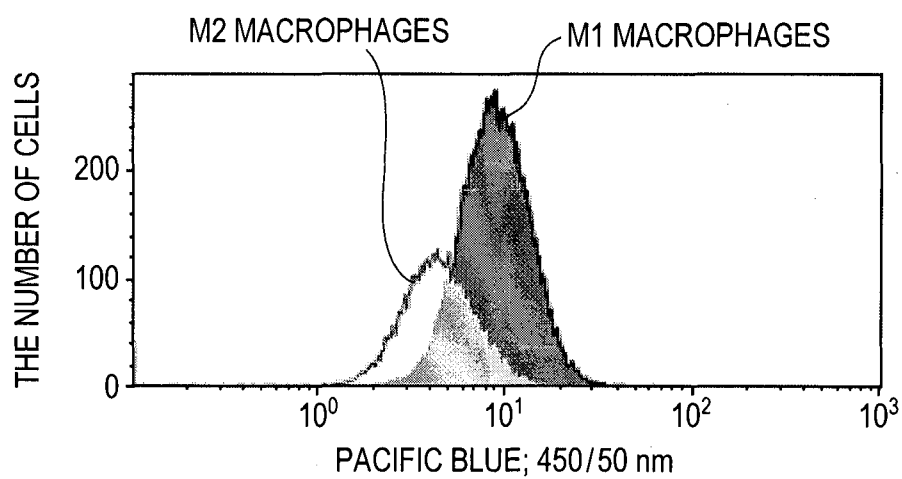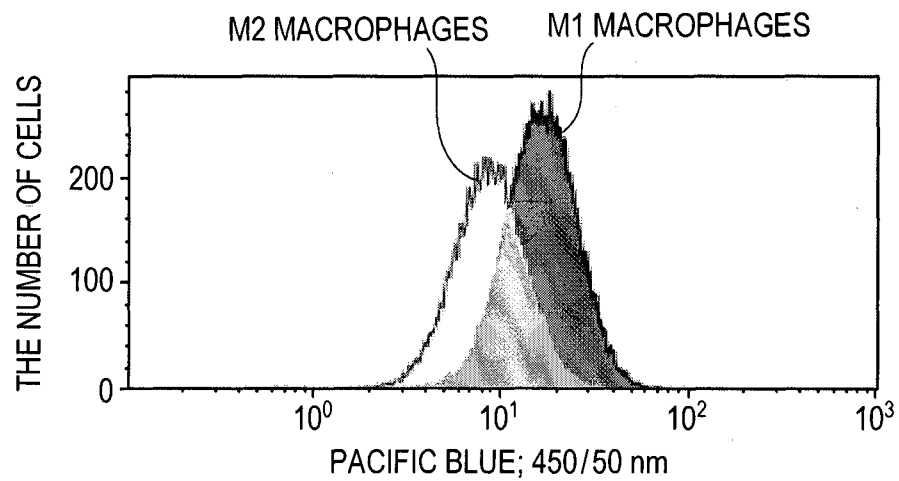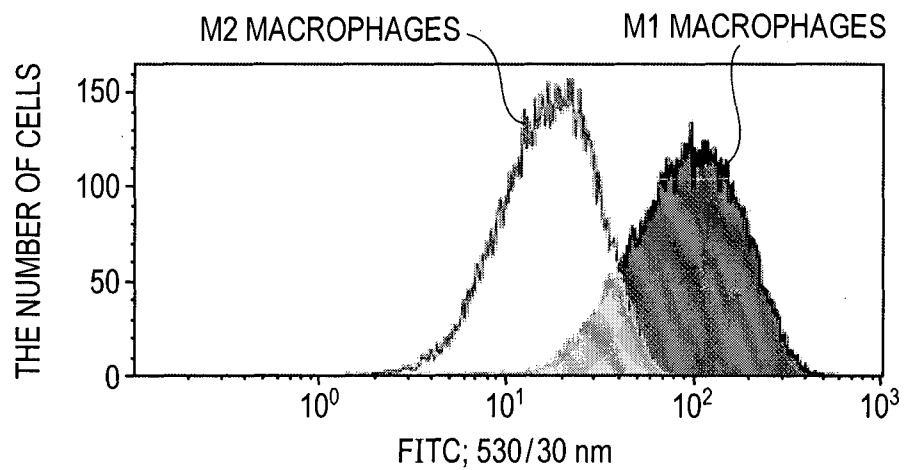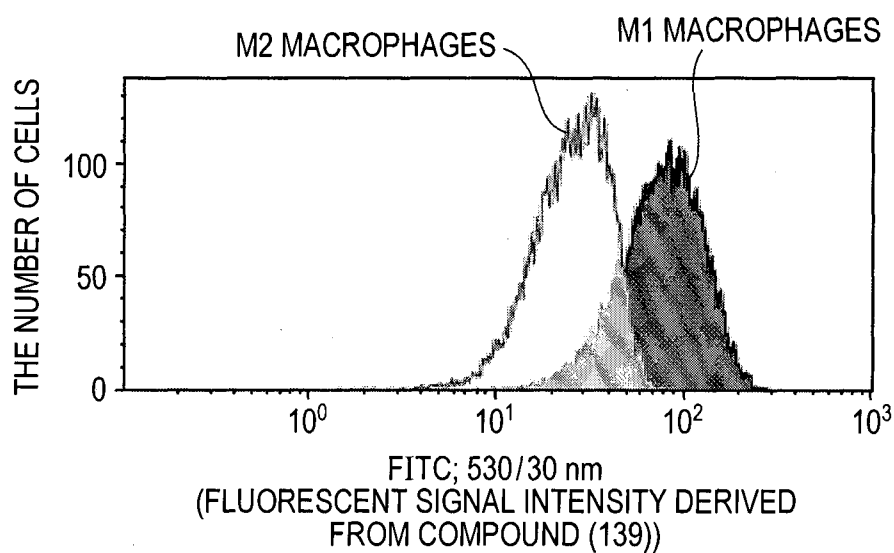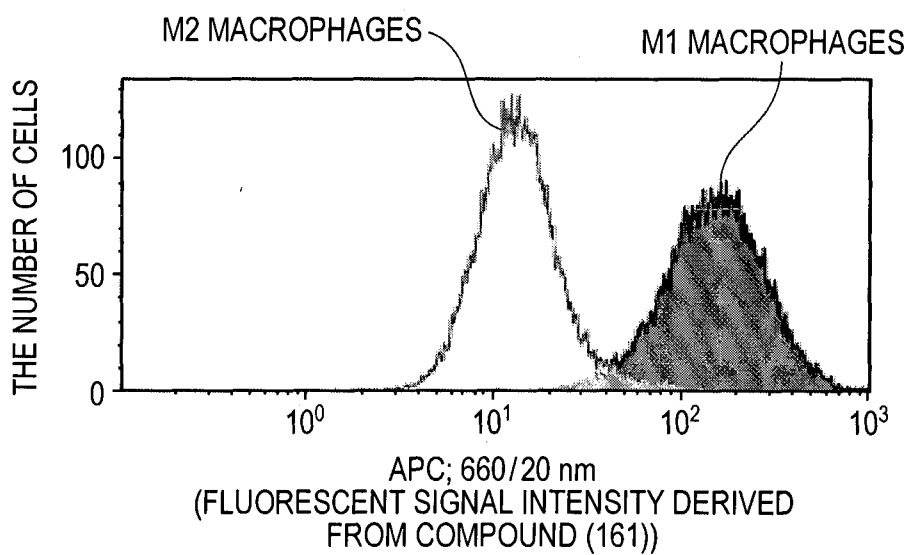
FIG. 24C

FIG. 24D

FIG. 24E

FIG. 25A

FIG. 25B

FIG. 25C

FIG. 26A

FIG. 26B

FIG. 27

FIG. 28

FIG. 29

FIG. 30A

M2 MACROPHAGES   M1 MACROPHAGES

FITC; 530/30 nm
(FLUORESCENT SIGNAL INTENSITY DERIVED
FROM COMPOUND (1))

FIG. 30B

M1 MACROPHAGES   M2 MACROPHAGES

APC-Cy7; 780/60 nm
(FLUORESCENT SIGNAL INTENSITY DERIVED
FROM COMPOUND (113))
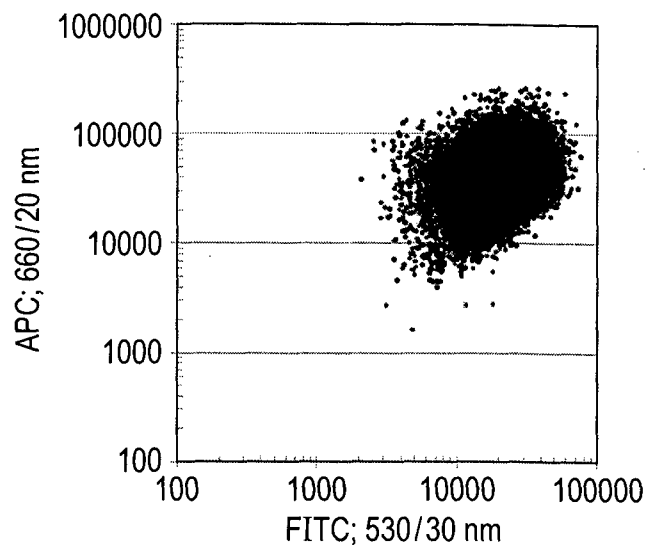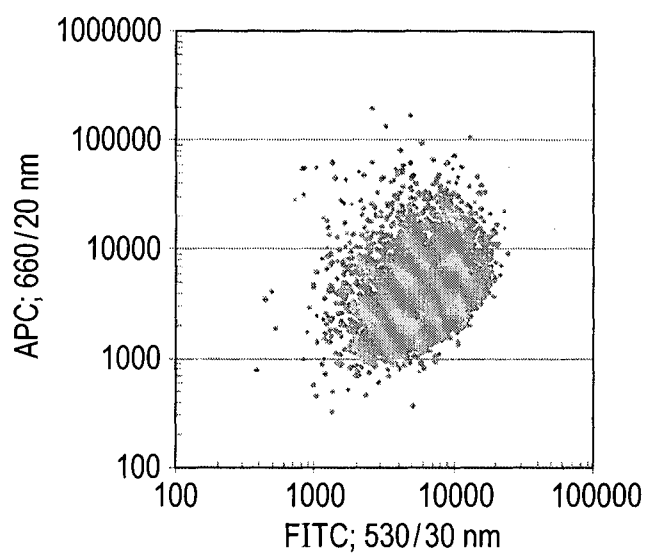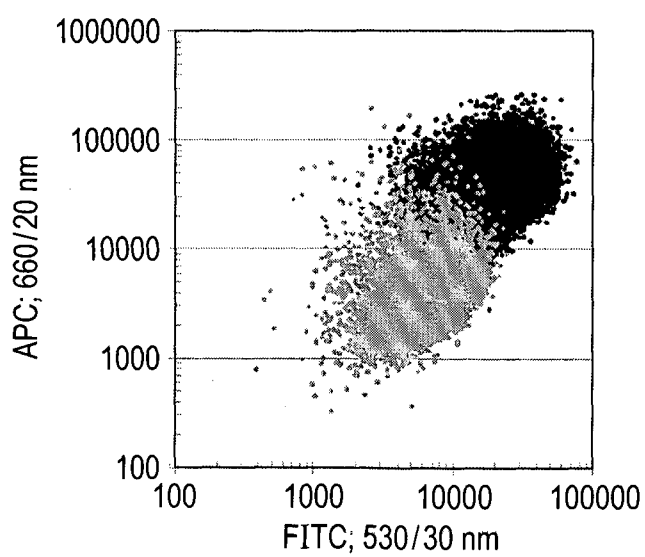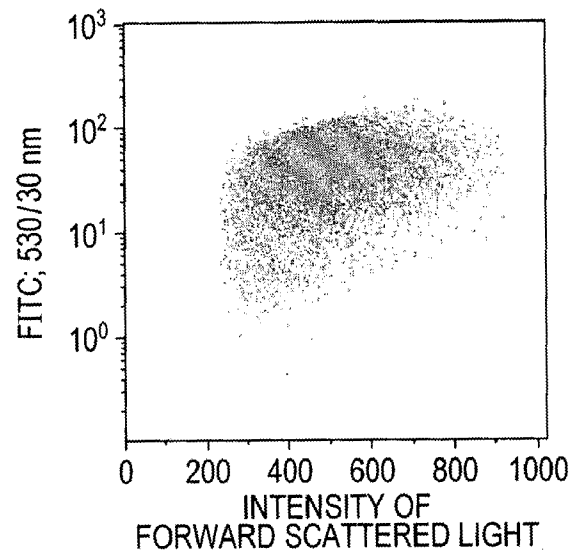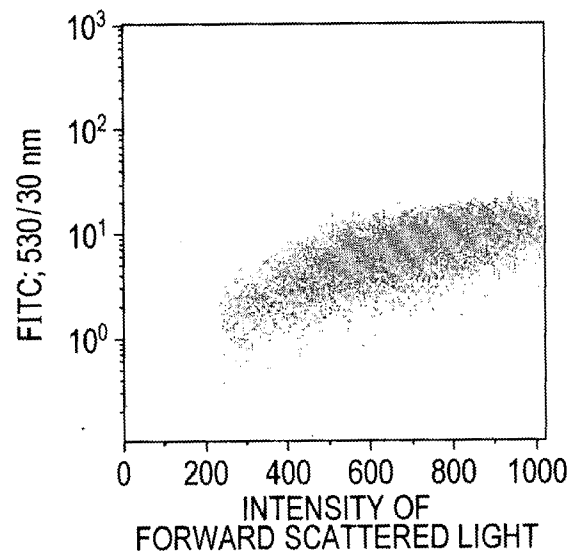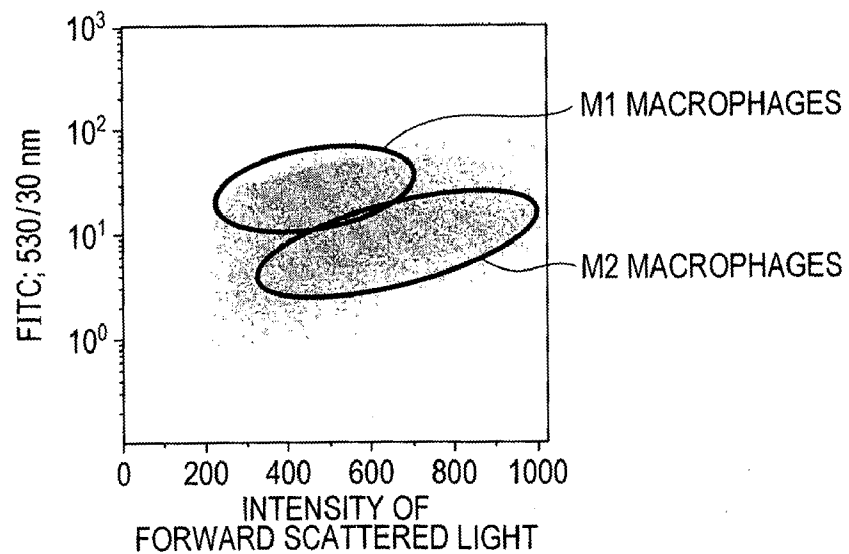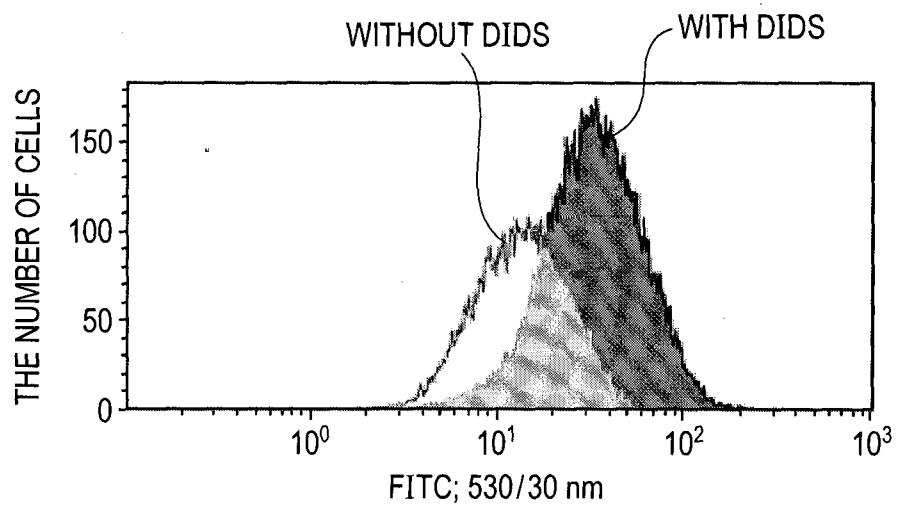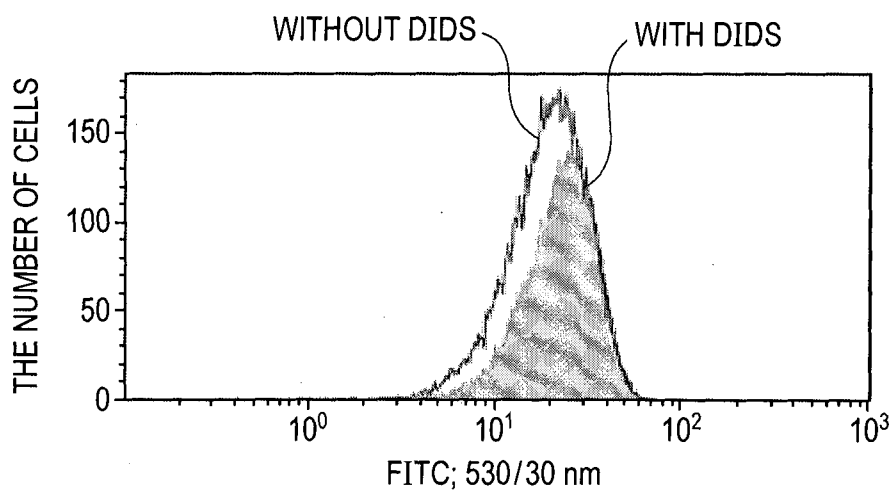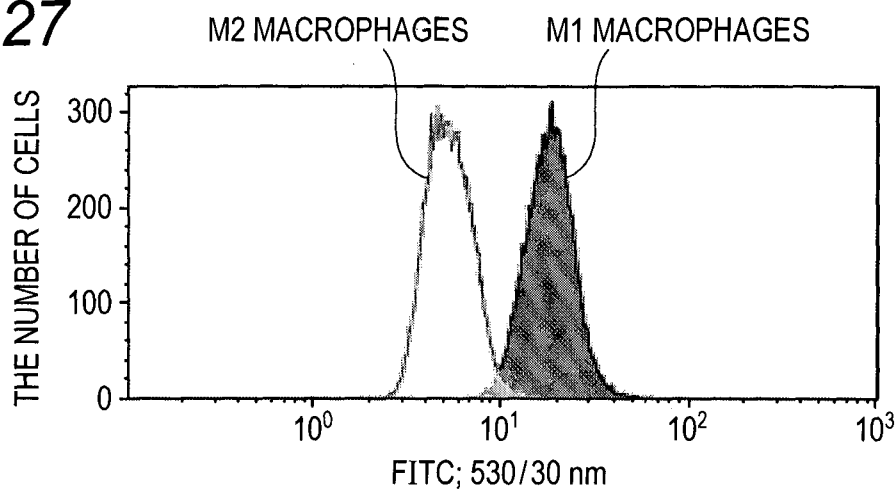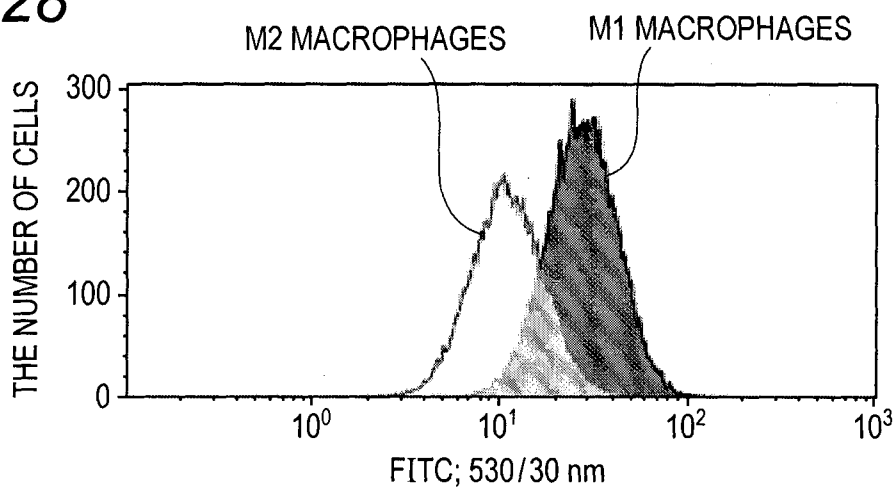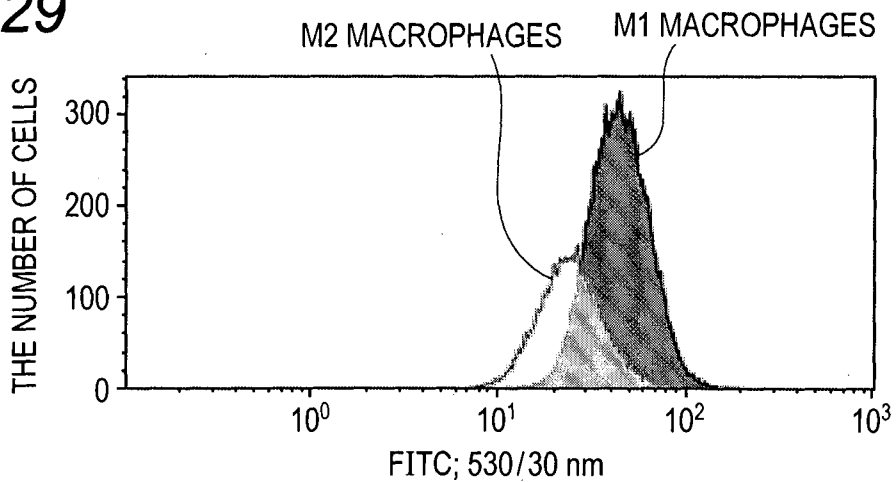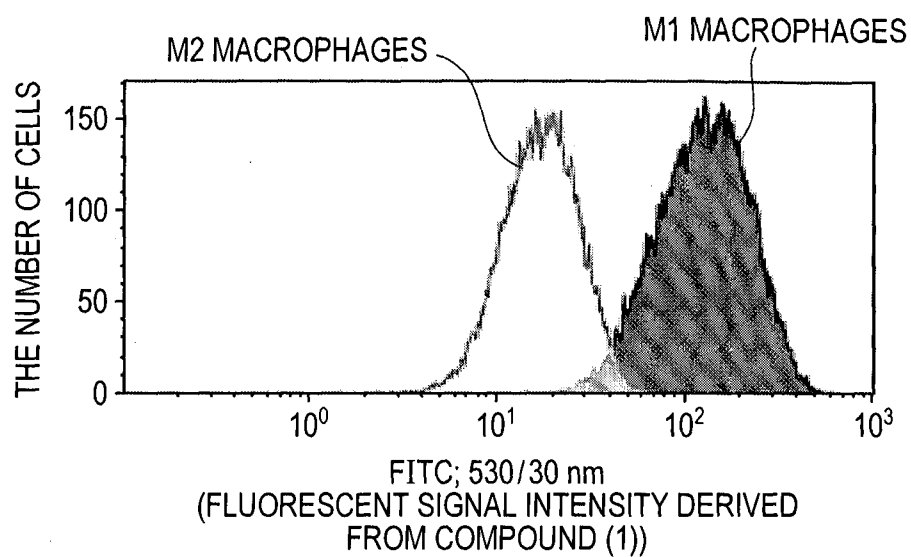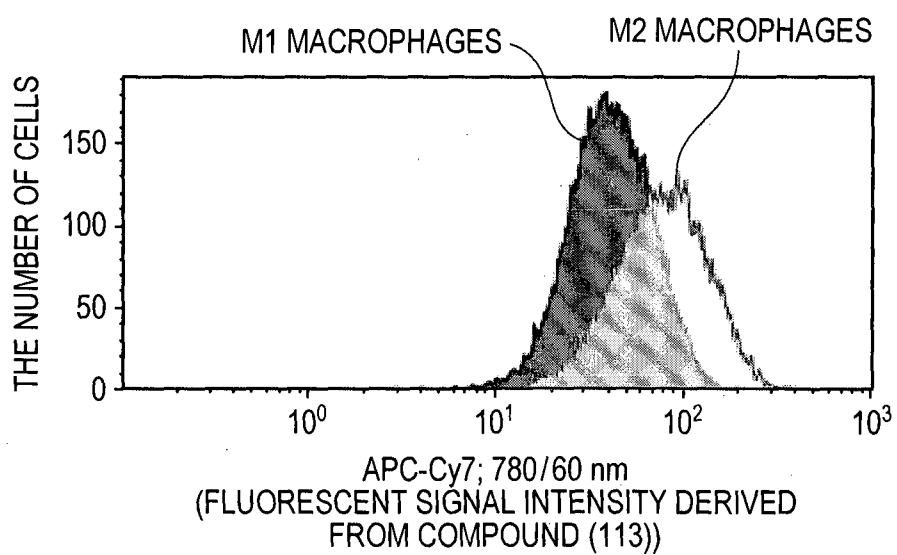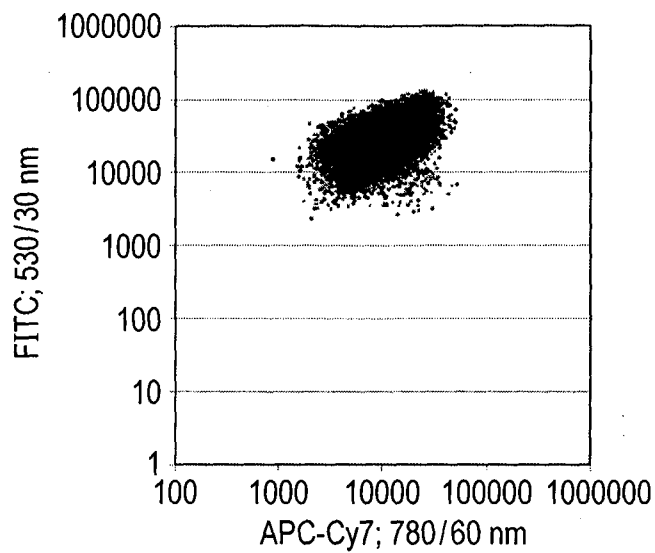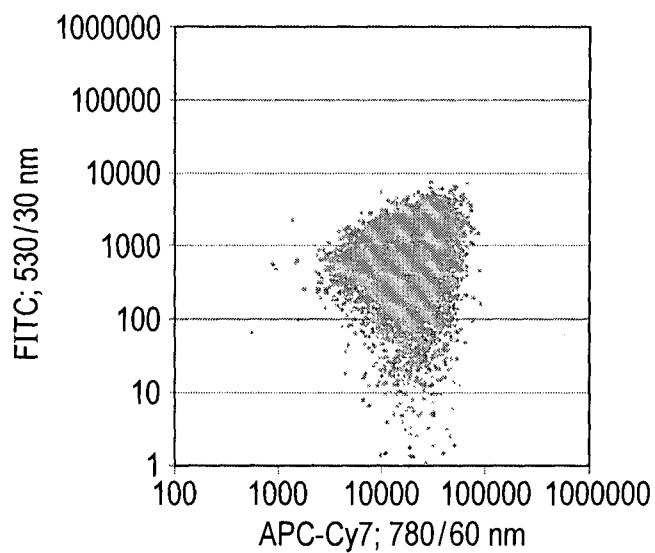
FIG. 30C

FIG. 30D

FIG. 30E
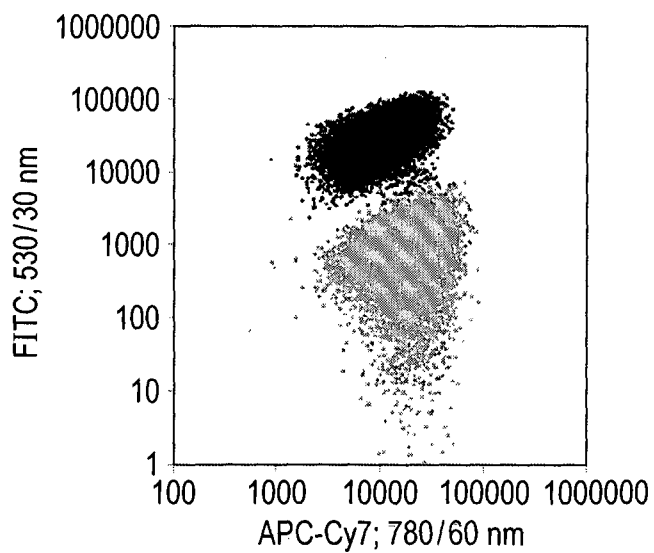
INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2014/064900

A. CLASSIFICATION OF SUBJECT MATTER

Int.Cl. C12Q1/04(2006.01)i, C12M1/00(2006.01)i, C12M1/34(2006.01)i,
C12N5/0786(2010.01)i, C12Q1/02(2006.01)i, G01N33/48(2006.01)i

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)

Int.Cl. C12Q1/04, C12M1/00, C12M1/34, C12N5/0786, C12Q1/02, G01N33/48

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

Published examined utility model applications of Japan 1922-1996
Published unexamined utility model applications of Japan 1971-2014
Registered utility model specifications of Japan 1996-2014
Published registered utility model applications of Japan 1994-2014

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

JSTPlus/JMEDPlus/JST7580(JDreamⅢ)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/012156 A2 (GENENTECH, INC.) 2009.01.22,	1,16
Y	Full text & JP 2010-533866 A & EP 2173438 A2 & CN 101801462 A	1-40
Y	US 4803161 A (BABB, B.E. et al.) 1989.02.07, Full text & JP 1-246240 A & EP 232130 A2	1-40
Y	WO 2003/104210 A1 (CHALMERS TECHNOLOGY LICENSING AB) 2003.12.18, Full text & JP 2006-503119 A & US 2006/0286626 A1 & EP 1509508 A1	1-40
Y	JP 2010-148447 A (CANON K.K.) 2010.07.08, Full text (Family: none)	1-40

☑ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01.09.2014	09.09.2014

Name and mailing address of the ISA/JP	Authorized officer	4B 4155
Japan Patent Office	NOMURA, Hideo	
3-4-3, Kasumigaseki, Chiyoda-ku, Tokyo 100-8915, Japan	Telephone No. +81-3-3581-1101 Ext. 3448	

Form PCT/ISA/210 (second sheet) (July 2009)

C (Continuation).	DOCUMENTS CONSIDERED TO BE RELEVANT	
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2007/102146 A2 (ZETIQ TECHNOLOGIES LTD.) 2007.09.13, Full text & JP 2009-528835 A & US 2009/0117610 A1 & EP 1991852 A2	1-40
A	WO 1985/005179 A1 (MERCK PATENT G.M.B.H.) 1985.11.21, Full text & JP 56-140253 A & US 4615878 A & EP 179107 A1	1-40
P,A	EP 2685261 A1 (CANON K.K.) 2014.01.15, Full text & JP 2014-14344 A & US 2014/0017722 A1	1-40