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(71) Applicant (for all designated States except US): **MOLECULAR DEVICES CORPORATION** [US/US]; 1311 Orleans Drive, Sunnyvale, CA 94089-1136 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **KLAUBERT,**

**Dieter** [CA/US]; 250 Blue Sky Drive, Arroyo Grande, CA 93420 (US). **DIWU, Zhenjun** [CN/US]; 855 Cascade Drive, Sunnyvale, CA 94087 (US). **YI, Guoliang** [CN/US]; 1007 Valley Forge Drive, Sunnyvale, CA 94087 (US). **KIRK, Martin** [US/US]; 712 Dartmouth Avenue, San Carlos, CA 94070 (US).

(74) Agent: **MCDONNELL, John, J.**; McDonnell Boenhen Hulbert & Berghoff, 300 South Wacker Drive, Suite 3200, Chicago, IL 60606 (US).

(81) Designated States (*national*): CA, US.

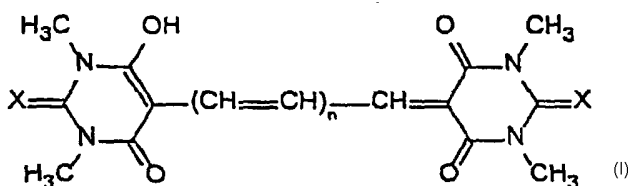
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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: POTENTIOMETRIC PROBE COMPOUNDS



(57) **Abstract:** The invention encompasses an improved method for measuring membrane potential using compounds of the formula I as potentiometric probes. These probes may be used in combination with other fluorescent indicators such as Indo-1, Fura-2, and Fluo-3, such probes may be used in microplate reading devices such as FLIPR<sup>TR</sup>, fluorescent imaging plate reader, sold by Molecular Device Corp., of Sunnyvale, CA; flow cytometers; and fluorometers. Such probes are used to measure membrane potential in live cells. X is O. or S. n is 1 or 2.

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## INTERNATIONAL SEARCH REPORT

International application No:  
PCT/US02/25046

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : C12Q 1/02; G01N 33/53

US CL : 435/29, 968

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)

U.S. : 435/29, 968

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

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

WEST, CHEMICAL ABSTRACTS

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                   | Relevant to claim No. |
|-----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| A         | US 4,370,401 A (WINSLOW et al.) 25 January 1983, see entire document.                                                                                                | 1-6                   |
| T         | US 6,455,271 B1 (LITTLE, II et al.) 24 September 2002, see entire document.                                                                                          | 1-6                   |
| X         | LEE, W. Antifungal Susceptibility Testing of Candida Species by Flow Cytometry. J Korean Med Science. January 1999. Vol. 14, No. 1, pages 21-26, see entire document | 1-6                   |
| X         | BECK, P. Detection of Cell Viability in Cultures of Hyperthermophiles. FEMS Microbiology Letters. 1997. Vol. 147, pages 11-14, see entire document.                  | 1-6                   |



Further documents are listed in the continuation of Box C.



See patent family annex.

|                                                                                                                                                                         |                                                                                                                                                                                                                                                  |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| * Special categories of cited documents:                                                                                                                                | "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                                              |
| "A" document defining the general state of the art which is not considered to be of particular relevance                                                                | "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                                                                     |
| "E" earlier document published on or after the international filing date                                                                                                | "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 |
| "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) | "&" document member of the same patent family                                                                                                                                                                                                    |
| "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                                                                  |                                                                                                                                                                                                                                                  |

Date of the actual completion of the international search

03 JANUARY 2003

Date of mailing of the international search report

23 MAY 2003

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

RALPH GITOMER

Telephone No. (703) 305-1235

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/25046

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                         | Relevant to claim No. |
|-----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X         | dePOORTER, L. et al. Convenient Fluorescence Based Methods to Measure Membrane Potential and Intracellular pH in the Archaeon. J of Microbiological Methods. February 2001. Vol. 47, No. 2, pages 233-241, see entire document.            | 1-6                   |
| X         | LIAO, R. et al. Assessment of the Effect of Amphotericin B on the Vitality of Candida albicans. Antimicrobial Agents and Chemotherapy. May 1999. Vol. 43, No. 5, pages 1034-1041, see entire document.                                     | 1-6                   |
| X         | NAKASHIMA, K. et al. Peroxyoxalate Chemiluminescence Detection of Condensates of Malondialdehyde with Thiobarbituric Acids Using a Flow System. Biomedical Chromatography. February 1992. Vol. 6, No. 2, pages 55-58, see entire document. | 1-6                   |
| A         | SABRY, S. et al. Sensitive Spectrofluorimetric and Spectrophotometric Methods. J. of Pharmaceutical and Biomedical Analysis. February 2000. Vol. 22, No. 2, pages 257-264.                                                                 | 1-6                   |

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US02/25046

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.