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Datasheet for the decision of 5 March 2010

Case Number: T 1197/07 - 3.3.01

Application Number: 04001486.2

Publication Number: 1411047

IPC: C07D 219/14

Language of the proceedings: EN

Title of invention:
Intercalators having affinity for DNA and methods of use

Applicant:
ABBOTT LABORATORIES

Opponent:
-

Headword:
DNA Intercalators/ABBOTT LABORATORIES

Relevant legal provisions:
EPC Art. 123(2), 76, 56, 54
EPC R. 115(2)
RPBA Art. 15(3)

Relevant legal provisions (EPC 1973):
-

Keyword:
"Main request - amendments supported by the application as originally filed (yes)"
"Priority - validly claimed (no)"
"Novelty (yes)"
"Inventive step (no) - obvious alternative DNA intercalators"

Decisions cited:
G 0004/92, T 0181/82

Catchword:
-

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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 1197/07 - 3.3.01

D E C I S I O N
of the Technical Board of Appeal 3.3.01
of 5 March 2010

Appellant: ABBOTT LABORATORIES
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Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 15 February 2007 refusing European application No. 04001486.2 pursuant to Article 97(1) EPC 1973.**

Composition of the Board:

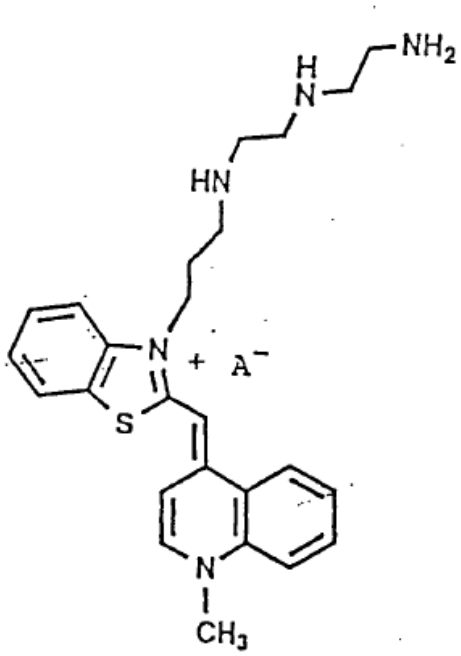
Chairman: P. Ranguis
Members: J.-B. Ousset
C.-P. Brandt

Summary of Facts and Submissions

I. This appeal lies from the decision of the examining division to refuse European patent application No. 04 001 486.2, which is a divisional application from European patent application No. 94 922 010.7. The refusal was for lack of inventive step.

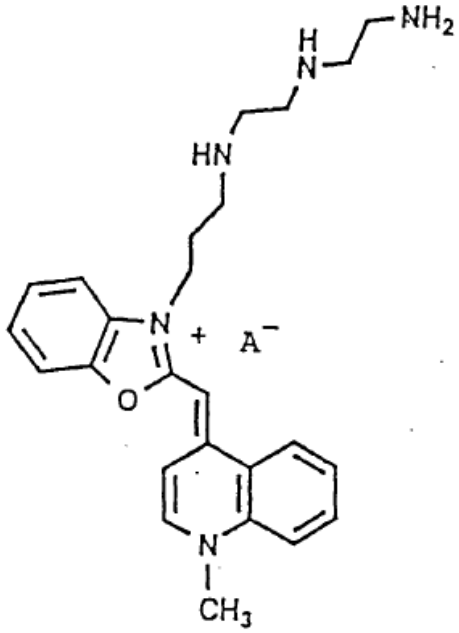
II. Claims 1 to 5 of this application read as follows:

"1. A compound of claim 1 having the formula



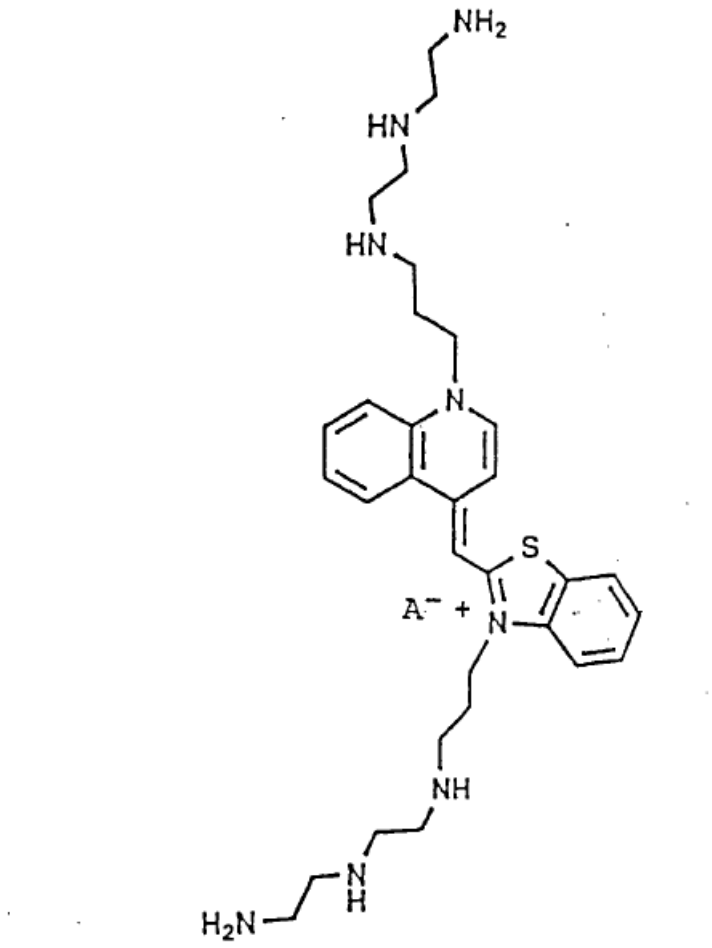
wherein A^- is an acceptable monovalent counter anion.

2. A compound having the formula



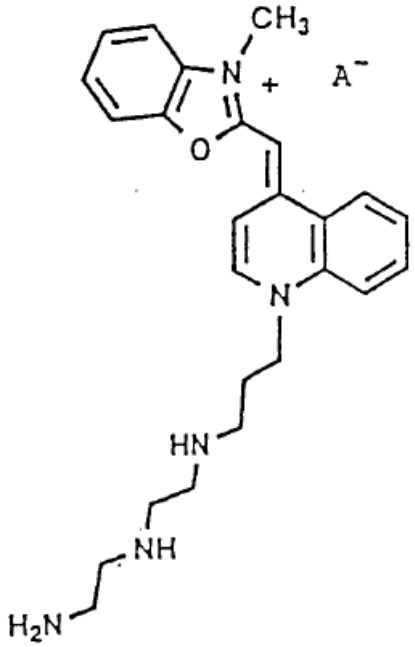
wherein A^- is an acceptable monovalent counter anion.

3. A compound having the formula



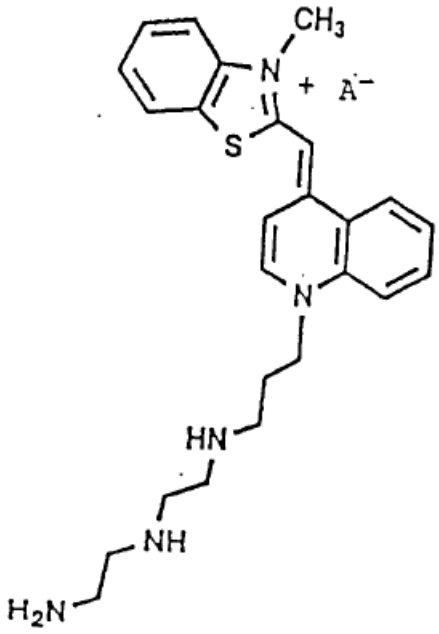
wherein A^- is an acceptable monovalent counter anion.

4. A compound having the formula



wherein A^- is an acceptable monovalent counter anion.

5. A compound having the formula



wherein A^- is an acceptable monovalent counter anion."

III. The examining division considered that the present application did not fulfil the requirements of

Article 56 EPC on the basis of the following cited documents:

(4) US-A-5,312,921 (prior art under Article 54(2) EPC given that priority was not validly claimed)
(5) Nucleic Acids Research, **21**, (1993), pp. 5720-5726

In the absence of any data showing a surprising effect for the claimed compounds and starting from the closest prior art (4), the person skilled in the art would have deduced the structural modifications from (4) to arrive at the claimed compounds without any inventive skills.

IV. In its statements setting out the grounds of appeal, the appellant argued as follows:

- The problem of the invention was to provide further commercially important DNA intercalator and in the absence of any pointer to the specific new structures an inventive step should be acknowledged.

- It was emphasized that "a reasonable expectation of success" was not to be confused with "hope of success".

- The examining division had not shown in its decision that the prior art had given the person skilled in the art a reasonable expectation of success in identifying the claimed DNA intercalators. On the contrary, identifying an important DNA intercalator within acceptable time limits was not a foregone conclusion, because without appropriate guidance the skilled person

might face repeated failure without any guarantee of finding an important DNA intercalator.

- The presently claimed compounds had high affinity for binding to DNA molecules, reduced self-quenching and superior transport kinetics. The claimed intercalators provided also an enhanced fluorescence when bound to DNA molecules. The claimed compounds when bound to DNA molecules are far more sensitive than ethidium homodimer (EthD) in detecting hybridization. The intercalators of the invention provided a thousand-fold improvement on conventional staining methodologies using ethidium homodimer (EthD).

- Document (5) displayed a very short list of fluorescent dyes and attested therefore to the difficulty of finding further commercially useful dyes (see page 5725, paragraph "Discussion", two first sentences). Therefore, neither (4) nor (5) directed the person skilled in the art to use the specific side chain $-(CH_2)_3NH(CH_2)_2NH(CH_2)_2NH_2$ on thiazole orange to obtain intercalators.

- The comparison between the experimental results set out in Figures 5 of document (4) and the ones of page 13 of the present application was used in order to show superior properties for the claimed compounds. In particular, when comparing the fluorescence intensities between the ethidium bromide and the compounds of the present application, that showed the achievement of high fluorescence intensity enhancements.

V. In its communication annexed to the summons to oral proceedings, the board drew the appellant's attention to the following point:

Document (4) discloses compounds used as intercalating fluorescent dyes like the compounds of the present application. Thiazole orange derivatives substituted by an alkyl chain containing a nitrogen atom internal to this chain and a nitrogen atom at the end of this chain are also exemplified in document (4) (see Table 1). Hence, the only structural difference between the claimed compounds and those of document (4) is in the nature of the alkyl chain attached to the thiazolo orange moiety.

The problem to be solved seems to be the provision of intercalator moieties having high affinity to bind to DNA molecules and which show reduced self-quenching and provide superior transport kinetics.

Any alleged advantageous effects should be shown in comparison with the closest prior art. The comparison between Fig. 5 of document (4) and the data of page 13 of the present application cannot meet this requirement, since it is not clear which methodology was used to perform these measurements because it is not described in this document. In view of the content of the description, these data do not show the presence of any technical effect suggesting that the problem underlying the present application was solved.

Therefore, the problem might be reformulated in the provision of alternative compounds able to bind to DNA molecules.

The person skilled the art can find the generic information characterizing the compounds which are useful as intercalators to bind to DNA molecules in document (4) (see column 3, line 9 to column 4, line 24 and more particularly, column 3, lines 20 to 22, 24 to 25, 34 to 35, 42 to 48, 51 to 62; column 4, lines 6 to 9, 16 to 17). As already pointed out by the examining division, the specific claimed compounds of the invention are encompassed by this generic disclosure.

The person skilled in the art would thus expect from document (4) that the specific compounds claimed in the present application would exhibit the binding properties. He would thus arrive at the claimed compounds without inventive skills.

In view of the above, the inventive step of the claimed subject-matter is not acknowledged.

VI. With a fax dated 26 February 2010, the appellant informed the board that he would not be attending the oral proceedings scheduled for 5 March 2010 but maintained its request for oral proceedings and requested that a decision be taken on the file as it stands.

VII. Oral proceedings took place on 5 March 2010 before the board.

VIII. The appellant did not appear at the oral proceedings but, according to its written submissions, requested that the decision of the examining division be set

aside and that a patent be granted on the basis of the request refused by the examining division.

IX. At the end of the oral proceedings, the decision of the board was announced.

Reasons for the Decision

1. The appeal is admissible.

Procedural matters

2. The appellant was informed in due time by the communication of the board of the objection based on Article 56 EPC. Since the appellant had an opportunity to present its arguments in respect thereof, the requirements of Article 113(1) EPC are fulfilled. Although the appellant did not appear at the oral proceedings, the board is therefore empowered to decide on these matters (see G 4/92, OJ EPO 1994, 149, Order 1, Rule 115(2) EPC and Article 15(3) of the RPBA).

3. The requirements of Article 76 EPC are fulfilled. The claims of the present application as originally filed are identical to the ones of the parent application as originally filed.

Amendments

4. Claims 1 to 5 of the main request are identical to claims 2 to 6 of the set of claims as originally filed. Claims 7 to 11 are based on the original description as follows:

- claim 7 (see page 4, lines 20 to 27)
- claim 8 (see page 8, line 13 to page 9, line 2)
- claim 9 (see page 88, example 39)
- claim 10 (see page 13, lines 22 to 26)
- claim 11 (see page 81, example 32)

4.1 The requirements of Article 123(2) EPC are met.

Priority

5. Although generically encompassed in the scope described in the US-document 08/086,285, claimed as priority document, none of the five compounds claimed in claims 1 to 5 of the present application was disclosed in the priority document.

Consequently, as already found by the examining division (see point II of the decision), priority is not validly claimed for the subject-matter claimed in the present application. Therefore, document (4) which was published between the priority document not validly claimed and the publication date of the parent application is to be considered as a prior art document within the meaning of Article 54(2) EPC.

6. Novelty

None of the five specific compounds claimed in claims 1 to 5 of the main request is disclosed in the cited prior art. Novelty is thus acknowledged.

7. Inventive step - claim 5

7.1 The first step for assessing inventive step according to the problem-solution approach is to identify the closest state of the art in order to determine in view thereof the technical problem to be solved.

7.1.1 Document (5) discloses that dyes differing widely in structure, such as phenanthridinium derivative, ethidium homodimer, and the asymmetric cyanine dyes thiazole orange and oxazole yellow homodimer bind with high affinity to double-stranded (ds) DNA to form fluorescent complexes stable to gel electrophoresis. Such high affinity complexes of dsDNA with dyes are valuable in the detection of DNA in solution, and in various applications dependent on gel electrophoresis (see page 5720, left-hand column, "Introduction"). Specific dyes forming fluorescent complexes with dsDNA are disclosed, *inter alia* Ethidium Bromide, (1,3-propanediamino)propidium, (diethylenetriamino)propidium, N,N'-tetramethyl-1,2-ethane-diamino)propyl thiazole orange, (tetramethylpropanediamino)propyl thiazole orange, ethidium homodimer, compounds (1), (2), (3), (5), (6), and (9) of Figure 1 respectively.

7.1.2 Document (4), the inventors of which are cited in document (5), also relates to dyes designed for high sensitivity detection of double-stranded DNA and also discloses the compounds mentioned in the previous paragraph, except ethidium dimer. In addition, however, document (4) offers a more general teaching. Whereas document (5) is a scientific publication limited to the results obtained with the compounds actually made and tested, document (4) generalizes the results obtained.

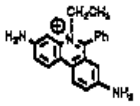
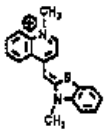
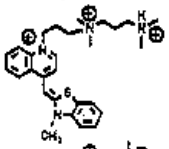
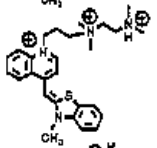
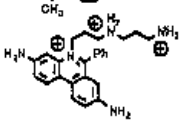
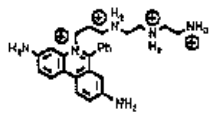
In document (4), the subject dyes are characterized by having at least one polycationic chain (side-chain) linked to an annular member, where the annular member will normally be carbon or nitrogen (see column 3, lines 9 to 12). Fluorophore moieties of particular interest will involve two ring systems, which are joined by a bond or a linking group having one or more ethylenic groups which are in conjugation with the aromatic moieties. Aromatic groups of interest include phenanthridine (benzquinoline), benzimidazole, benzthiazole, benzoxazole, quinoline, acridine and xanthine. Illustrative groups include thiazole orange, thiazole blue, ethidium, fluorescein, acridine, phenanthridine, xanthenes, and fluorones (see column 4, lines 6 to 15). The side-chain will have at least two positive charges, under the conditions in which the dye is used, and usually not more than five positive charges, more usually not more than about four positive charges. For the most part, the positive charges will be based on amino groups (see column 3, lines 12-17). The amino groups which are internal to the chain will be at least disubstituted and may be tri- or tetra-substituted. The terminal amino group may be monosubstituted to tetrasubstituted. Normally, the nitrogen atoms will be separated by at least two carbon atoms. Preferably, alkylene amines will be employed, where the alkylene is two to three carbon atoms, and the nitrogen atoms, if substituted, are substituted with lower alkyl groups of from 1-3 carbon atoms (see column 3, lines 20 to 37).

However, as correctly noted in decision T 181/82 (OJ EPO 1982, 401), when assessing inventive step of

organic compounds a strict distinction must be drawn between the purely intellectual content of the definitions and their information content in the sense of a specific teaching (see point 8). When the teaching from a citation is interpreted, special attention must be paid to the material actually disclosed in the sense of a complete technical rule.

7.1.3 In the present case, the Board finds that the technical state of the art actually disclosed is represented by the compounds of Table 1:

Table 1

Structure	Dye	$t_{1/2}$ (min)	F_{bound} / F_{free}
	Ethidium Bromide	3.6	40
	Thiazole Orange	4.3	2300
	Thiazole Orange Tetramethyl Propanediamine	22	3800
	Thiazole Orange Tetramethyl Ethanediamine	26	3800
	Ethidium Propanediamine	31	40
	Ethidium Diethylenetriamine	75	30

or ethidium dimer.

7.1.4 By arguing that the intercalators of the invention provide enhanced fluorescence when bound to a DNA molecule within a fluorescent flow cytometry environment which is about eight to ten times brighter in fluorescence than ethidium homodimer (EthD) utilized in the same flow cytometry environment, or provide about a thousand-fold improvement over conventional staining methodologies using ethidium bromide, the Appellant relies implicitly on ethidium homodimer or ethidium bromide as the state of the art to which the present invention must be compared to define the technical problem to be solved.

7.1.5 However, this is wrong because the intercalators to which the invention must be compared are the most closely structurally related compounds. In that case the fluorophore moiety is fundamental in order to have the fluorescent effect. In that respect the compounds thiazole orange, thiazole orange tetramethyl propane diamine and thiazole orange tetramethyl ethane diamine are closer than the ethidium derivatives from the compound of Claim 5 and among them the two last are even closer as they have an alkylene diamino side-chain.

7.2 In the absence of a comparison between the compound Claim 5 and the closest state of the art (see point 7.1.5 above), the technical problem to be solved can be seen in the provision of thiazole orange derivatives other than thiazole orange tetramethyl propane diamine or thiazole orange tetramethyl ethane diamine which could exhibit a similar high affinity for binding to the DNA molecule and would provide

fluorescence when bound to a DNA molecule within a fluorescent flow cytometry environment.

7.3 From the application as filed, in particular Examples 27, 28, 30, 32, the technical problem is considered as solved.

7.4 It remains to assess whether the claimed solution was obvious in view of the technical problem set out above.

7.4.1 Having in mind the formulas of the compounds thiazole orange tetramethyl propanediamine and thiazole orange tetramethyl ethanediamine, the person skilled in the art knows that the fluorescence effect is due to the thiazole moiety (see column 4, lines 6 to 15 of document (4)). From documents (4) and (5) it is known that dyes having a fluorophore joined to a polycationic chain of at least two positive charges have been found to provide high fluorescence enhancement upon binding to (ds) nucleic acids and have strong binding affinities to the nucleic acid, as compared to the fluorophore without the polycationic chain (see "Summary of the invention" of document (4) and page 5726, left-hand column, lines 15 to 22 of document (5)). Thus, the person skilled in the art identifies the chains tetramethyl propane diamine or tetramethyl ethane diamine linked through a propanylene linker to the nitrogen atom of the fluorophore moiety as the polycationic chain liable to enhance the affinity vis-à-vis DNA.

7.4.2 Furthermore, the person skilled in the art knows that:

The side-chain will have at least two positive charges, under the conditions in which the dye is used, and usually not more than five positive charges, more usually not more than about four positive charges. For the most part, the positive charges will be based on amino groups (see column 3, lines 12-17). The amino groups which are internal to the chain will be at least disubstituted and may be tri- or tetrasubstituted. The terminal amino group may be monosubstituted to tetrasubstituted. Normally, the nitrogen atoms will be separated by at least two carbon atoms. Preferably, alkylene amines will be employed, where the alkylene is two to three carbon atoms, and the nitrogen atoms, if substituted, are substituted with lower alkyl groups of from 1-3 carbon atoms (see column 3, lines 2 to 37).

7.4.3 Thus, the chain can contain two, three or four amino groups. The alkylene can contain two or three carbon atoms and the amino groups can be unsubstituted (apart of course from the links necessary for the construction of the chain). From that information, the person skilled in the art can expect without inventive ingenuity that a compound having a fluorophoric moiety thiazole orange and having a side-chain having three amino groups (the internal nitrogen being disubstituted and the terminal nitrogen being monosubstituted) separated by an alkylene chain having two or three carbon atoms will solve the technical problem defined above. Among the small number of variations (which it is not necessary to detail here), one is - $(CH_2)_3NH(CH_2)_2NH(CH_2)_2NH_2$. Such a designed compound corresponds to the compound of Claim 5, which,

therefore, is obvious in view of the state of the art. Contrary to the Appellant's view, documents (4) and (5) give the person skilled in the art clear guidance towards the compound of Claim 5 to solve the technical problem defined above.

7.4.4 The person skilled in the art seeking to solve the problem set out in point 7.2 would thus further modify the structures of the thiazole orange tetramethyl propane diamine or thiazole orange tetramethyl ethane diamine disclosed in documents (4) or (5) to arrive without inventive skills at the claimed compound of Claim 5 of the present application.

7.4.5 For this reason, Claim 5 does not involve an inventive step within the meaning of Article 56 EPC.

7.5 Since the Board can only decide on a request as a whole, the present request is to be dismissed.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar The Chairman

C. Eickhoff P. Ranguis