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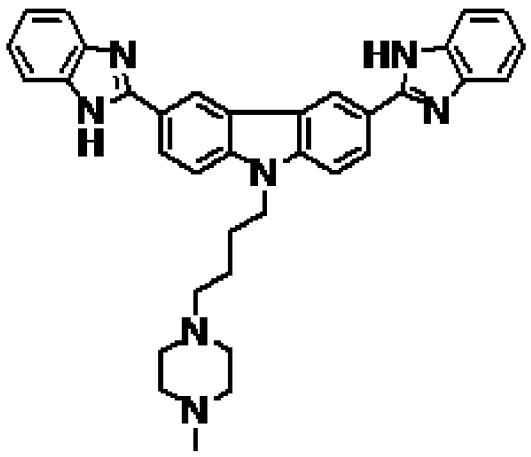

FIG. 1

(57) Abstract: The invention provides a method for obtaining a compound specific for rapid detection of HearNPV. The method includes obtaining a bromide precursor, 9-(4-bromobutyl)-9H-carbazole and constituting the bromide precursor in acetonitrile at a predefined temperature to obtain a piperazine derivative, 9-(4-(4-methylpiperazin-1-yl) butyl)-9H-carbazole. Subsequent to obtaining the piperazine derivative, the piperazine is reacted to obtain an intermediate aldehyde derivative, 9-(4-(4-methylpiperazin-1-yl)butyl)-9H- carbazole-3,6-dicarbaldehyde. The aldehyde derivative is further refluxed to obtain a carbazole derivative 3, 6 -di(1H-benzo [d]imidazol-2-yl)-9-(4-(4- methylpiperazin-1-yl)butyl)-9H-carbazole. Further, the invention provides a method for obtaining a probe for rapid detection for HearNPV. The method includes selecting a carbazole derivative 3, 6 -di(1H-benzo [d]imidazol-2-yl)-9-(4-(4-methylpiperazin-1-yl)butyl)-9H-carbazole, constituting the carbazole derivative in an organic medium and embedding the constituted carbazole derivative onto a substrate to obtain the probe. The invention also provides a device comprising a probe, a detection means and an analyser for rapid detection of HearNPV.

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METHOD AND DEVICE FOR RAPID DETECTION OF HearNPV

FIELD OF INVENTION

The invention generally relates to the field of biopesticide detection and more particularly to a device for rapid detection of HearNPV.

BACKGROUND

Helicoverpa armigera (Hübner), hereinafter referred to as *H.armigera,* is a moth, the larvae of which feed on a wide range of plants and cultivated crops. *H.armigera* is considered to be one of the most serious pests worldwide. *H.armigera* is one of the significant contributors to cause damage to a great number of agricultural crops including but not limited to, cereal, vegetable and garden crops, among them beans, leek, zucchini, lemon, sunflower, artichoke, pigeonpea, sorghum and groundnut, hereinafter collectively referred to as the produce. The worldwide loss due to *H.armigera* pest attack has been estimated to be more than US$78 billion per annum. In India, *H. armigera* caused infestation of the produce has also been observed in different parts from Kashmir to Kanyakumari, throughout the whole year.

There are a number of methods used to control the *H. armigera* caused infestation. Examples of commonly adopted methods include but are not limited to the use of sex pheromone traps, horticultural control, genetically improved plant varieties, biological control, chemical control and/or combinations thereof. One example of biological control method includes the use of *Helicoverpa armigera nucleopolyhedrovirus* biopesticide, hereinafter referred to as HearNPV.

One of the methods of application of HearNPV formulation includes spraying a water-dispersible liquid formulation of HearNPV to the infested produce. A significant advantage of the formulation is that the formulation is a biopesticide and is less hazardous to the environment, where the spraying is done. However, one significant disadvantage of the spray method is that the insecticidal activity of the formulation lowers with time

due to decrease in the number of viable virus. One of the techniques for determining efficacy of the HearNPV formulation is the quantification of the occlusion bodies present in HearNPV formulation.

In general, the known method of counting of virus particles is by using a Hemocytometer with improved Neubauer. However, this presently known light microscopy based method has several disadvantages. The use of poor quality microscopes with non-phase optics can add errors in counting. The virus suspension should be pure or semi-pure for microscopic analysis to avoid confusion with other polyhedra like particles (for example dust or tiny oil droplets). Virus suspension should not have any clumping and aggregation of virus particles. On the other hand, special care must be taken to ensure that the microscopy instrument and its associated devices such as coverslip, slides should be free of any dust and dirt. Further, the material cost for conducting the experiment is high and the experiment requires strict maintenance of several parameters. Also, the method requires intervention by skilled personnel. Hence, there is a need for quantification of HearNPV in commercial formulations which is rapid, cost-effective, easy and does not require any expert knowledge in handling.

BRIEF DESCRIPTION OF DRAWINGS

So that the manner in which the recited features of the invention can be understood in detail, some of the embodiments are illustrated in the appended drawings. It is to be noted, however, that the appended drawings illustrate only typical embodiments of this invention and are therefore not to be considered limiting of its scope, for the invention may admit to other equally effective embodiments.

FIG. 1 shows a representative structure of the compound I, according to an embodiment of the invention.

FIG. 2a shows a plot of emission spectra of the compound I in different solvent systems, according to an embodiment of the invention.

FIG. 2b shows TEM images of the compound I in water at a pH of about 6.5, according to an embodiment of the invention.

FIG. 2c shows AFM images of the compound I in water at a pH of about 6.5, according to an embodiment of the invention.

FIG. 3a shows a plot of emission spectra of the compound I in different pH ranges, according to an embodiment of the invention.

FIG. 3b shows a plot of emission spectra of the compound I in different temperature ranges, according to an embodiment of the invention.

FIG. 4a shows time dependent emission studies of the compound I in different solvent systems, according to an embodiment of the invention.

FIG. 4b shows a plot of emission spectra of the compound I in a viscous medium, according to an example of the invention.

FIG. 5a shows plot of emission spectra of compound I with HearNPV at pH 7.5, according to an embodiment of the invention.

FIG. 5b shows plot for calculating detection limit for HearNPV, according to an embodiment of the invention.

FIG. 6a shows plot of emission spectra of compound I in the presence of HearNPV, according to an embodiment of the invention.

FIG. 6b shows plot of emission spectra of compound I with different analytes, according to an embodiment of the invention.

FIG. 7a shows plot of emission spectra of heat treated HearNPV, according to an embodiment of the invention.

FIG. 7b shows plot of CD spectra of heat treated HearNPV, according to an embodiment of the invention.

FIG. 7c shows plot of emission spectra of compound I with heat treated HearNPV, according to an embodiment of the invention.

FIG. 7d shows the comparison of compound I interaction with normal and heat treated HearNPV, according to an embodiment of the invention.

FIG. 8a shows a plot depicting reversible interaction of HearNPV with the probe, according to an embodiment of the invention.

FIG. 8b shows a plot of emission spectra of the probe with a commercial formulation of HearNPV, according to an embodiment of the invention.

FIG. 9a shows changes in thermodynamic parameters of compound I upon interaction with HearNPV, according to an embodiment of the invention.

FIG. 9b shows changes in zeta potential values of HearNPV upon interaction with compound I, according to an embodiment of the invention.

FIG. 9c shows changes in extent of interaction of compound I with HearNPV in presence of NaCl, according to an embodiment of the invention.

FIG. 9d shows time dependent emission spectra of compound I in presence of HearNPV, according to an embodiment of the invention.

FIG. 10a shows changes in the emission intensity of the probe upon gradual addition of HearNPV, according to an embodiment of the invention.

FIG. 10b shows selective emission response of the probe towards HearNPV in presence of other components, according to an embodiment of the invention.

FIG. 11a shows preferential response of the probe towards freshly obtained HearNPV in comparison to its heat treated analog, according to an embodiment of the invention.

FIG. 11b shows comparable emission response of the probe towards the pure HearNPV and commercially available HearNPV according to an embodiment of the invention.

FIG. 12a shows emission intensity of compound with HearNPV in cereals, according to an embodiment of the invention.

FIG. 12b shows emission intensity of compound with HearNPV in fruits, according to an example of the invention.

FIG. 12c shows emission intensity of compound with HearNPV in vegetable extracts, according to an example of the invention.

SUMMARY OF THE INVENTION

One aspect of the invention provides a method for obtaining a compound specific for rapid detection of HearNPV. The method includes obtaining a bromide precursor, 9-(4-bromobutyl)-9H-carbazole and constituting the bromide precursor in acetonitrile at a predefined temperature to obtain a piperazine derivative, 9-(4-(4-methylpiperazin-1-yl) butyl)-9H-carbazole. Subsequent to obtaining the piperazine derivative, the piperazine is reacted to obtain an intermediate aldehyde derivative, 9-(4-(4-methylpiperazin-1-yl)butyl)-9H-carbazole-3,6-dicarbaldehyde. The aldehyde derivative is further refluxed to obtain a carbazole derivative 3, 6 -di(1H-benzo [d]imidazol-2-yl)-9-(4-(4-methylpiperazin-1-yl)butyl)-9H-carbazole.

Another aspect of the invention provides a method for obtaining a probe for rapid detection for HearNPV. The method includes selecting a carbazole derivative 3, 6 -di(1H-benzo [d]imidazol-2-yl)-9-(4-(4-methylpiperazin-1-yl)butyl)-9H-carbazole, constituting the the carbazole derivative in an organic medium and embedding the constituted carbazole derivative onto a substrate to obtain the probe.

Yet another aspect of the invention provides a device comprising a probe, a detection means and an analyser for rapid detection of HearNPV.

DETAIL DESCRIPTION OF THE INVENTION

Various embodiments of the invention provide a method of measuring the insecticidal efficacy of HearNPV by quantifying the number of occulsuion bodies present in its commercial formulation. One embodiment of the invention provides a method of obtaining a probe for measuring the insecticidal efficacy of HearNPV by quantifying the number of occulsuion bodies present in its commercial formulation. The method involves synthesizing a carbazole derivative, 3, 6 -di(1H-benzo [d]imidazol-2-yl)-9-(4-(4-methylpiperazin-1-yl)butyl)-9H-carbazole, hereinafter referred to as compound I. The method for synthesis of compound I includes obtaining a bromide precursor, 9-(4-bromobutyl)-9H-carbazole of compound I. The

bromide precursor of compound I is synthesized by stirring the mixture of 9H-carbazole with a long chain alkyl bromide in DMF medium. In one example of the invention the long chain alkyl bromide is 1, 4-dibromobutane. The mixing is carried out in presence of strong hydride base at room temperature in the range of about 20°C to about 37°C to obtain the bromide precursor. In one example of the invention the strong hydride base is 50% aqueous NaOH. The bromide precursor obtained is then constituted in acetonitrile and mixed with N-methyl piperazine and a mild carbonate base to obtain a mixture. In one example of the invention the mild carbonate base is K_2CO_3. The constituted mixture is heated at a temperature in the range of about between 90 ºC - 100 ºC to yield a piperazine derivative. In one example, the piperazine derivative obtained is 9-(4-(4-methylpiperazin-1-yl) butyl)-9H-carbazole.

The piperazine derivative is then taken in a container having dry DMF and $POCl_3$ is added drop wise to obtain a mixture. The mixture is subjected to cooling by placing the container over an ice-bath for about 15 minutes. Subsequent to cooling, the mixture is heated at 100 ºC for a time duration of about 12 hours to about 16 hours. The solution is then evaporated under vacuum to remove the excess DMF and the resultant solution is neutralized with concentrated KOH solution. The solution is extracted using ethyl acetate and the organic layer is passed through Na_2SO_4. The filtrate is then evaporated to obtain a gummy mass. The gummy mass is then purified by silica gel chromatography using $CHCl_3$/MeOH as eluent. The filtrate product obtained is an aldehyde derivative, 9-(4-(4-methylpiperazin-1-yl)butyl)-9H-carbazole-3,6-dicarbaldehyde. The aldehyde derivative obtained is a mass of yellow solid.

The aldehyde derivative obtained is constituted with phenylene diamine in 1:2 ratio and $Na_2S_2O_5$ in EtOH and is refluxed for a time period of 12 hours to about 16 hours. Then the reaction mixture is subjected to cooling and filtered. The supernatant from the filtrate is then evaporated to obtain the

compound I, 3, 6 -di(1H-benzo [d]imidazol-2-yl)-9-(4-(4-methylpiperazin-1-yl)butyl)-9H-carbazole.

FIG.1 shows a representative structure of compound I, according to an embodiment of the invention. The synthesized compound is then characterized to determine structural and emission properties. The compound I thus obtained has a planar structure. The compound I shows strong fluorescence property.

FIG. 2a shows a plot of emission spectra of the compound I in different solvent systems, according to an embodiment of the invention. In non-polar solvent like THF, the compound I exhibits moderate emission with a maximum at about 395 nm. However, in presence of water i.e. Tris-HCl buffer at pH 6.5, a strong red shift, of about 70 nm, in fluorescence maximum is observed. This shift is due to formation the formation of nano-aggregates in the aqueous medium. The aggregate formation is also evidenced by the conventional microscopic TEM analysis FIG. 2b shows TEM images of the compound I in water at a pH of about 6.5, according to an embodiment of the invention. The aggregate formation is also evidenced by AFM analysis. FIG. 2c shows AFM images of the compound I in water at a pH of about 6.5, according to an embodiment of the invention.

FIG. 3a shows a plot of emission spectra of the compound I in different pH ranges, according to an embodiment of the invention. Variable pH studies indicate that the maximum inter-chromophore association occurs at pH range of about 4.5 to about 6.5 due to protonation at the terminal units. FIG. 3a shows a plot of emission spectra of the compound I at different pH ranges, according to an embodiment of the invention. Variation in temperature at pH range of about 6.5 shows that the aggregation phenomenon is highly thermo-reversible in nature. FIG. 3b shows a plot of emission spectra of the compound I at different temperatures, according to an embodiment of the invention. A clear shift from aggregate to monomer emission is observed during temperature scan from 10°C to 90°C. When

the temperature of the same solution is brought back to 10°C, reappearance of the aggregate emission is noticed.

FIG. 4a shows time dependent emission studies of the compound I in different solvent systems, according to an embodiment of the invention. The time dependent emission studies suggests that in pure THF, compound followed single exponential decay due to presence of uniformly dispersed monomer species. On the other hand, in water of pH about 6.5, aggregation could restrict the twisting motion of the flexible terminal groups and stabilized the excited state in higher extent, resulting in the formation of long-lived emissive species with multi-exponential decay pathways. FIG. 4b shows a plot of emission spectra of the compound I in a viscous medium, according to an example of the invention. When emission spectrum of the compound I is recorded in highly viscous medium of 10 mg/ml PEG in water, an enhancement in aggregation is observed, confirming the role of conformational rigidity in the agglomeration process.

The compound I, as synthesized herein and as characterized is then embedded into a substrate to be used as a probe. The method of embedding the compound I onto the substrate includes constituting the synthesized compound I in an organic medium. Examples of the organic medium include but are not limited to MeOH, DMSO, Acetonitrile, and EtOH. Subsequent to the constitution of the compound I in the organic medium, a substrate suitable for embedding of the compound I is selected. Examples of the substrate include but are not limited to a glass, a quartz, a cellulose matrix wherein the cellulose matrix is synthesized or naturally obtained. The selected substrate is then coated with compound I to obtain a probe. The probe thus obtained is used for detection of HearNPV.

Another embodiment of the invention provides a device for rapid detection of HearNPV in biopesticidal formulations. The device includes a probe, a detection means and an analyser. The probe is obtained by embedding compound I onto a substrate. Examples of the substrate include but are not limited to a glass, a quartz, a cellulose matrix wherein the cellulose matrix

is synthesized or naturally obtained. The detection means is a UV source. In one example of the invention the source is a UV torch. The step of detection includes the determination of the insecticidal efficacy of HearNPV by quantifying the number of occlusion bodies present.

In one embodiment of the invention, the quantification of HearNPV in commercial formulation is performed prior to spraying of the HearNPV formulation onto a field having a desired produce. The method involves addition of aqueous dispersion of HearNPV onto the probe. The probe is then placed under UV lamp to observe change in molecular emission of the compound embedded in the substrate. A change in molecular emission from blue to cyan is observed. The emission change is then verified with the help of an analyser. In one embodiment of the invention the analyser is a reference chart of emission intensity for known concentrations of HearNPV formulations. The chart provides reference emission intensities, with respect to a preferred concentration value of the HearNPV formulation which is indicative of a minimum threshold value of the concentration required for spraying.

Example: The method as described above is adopted to obtain a probe for measuring the insecticidal efficacy of HearNPV by quantifying the number of occlusion bodies present in the commercial formulations. The method involves selecting 0.443 µg of compound I. The selected compound I is then constituted in 40µl of MeOH to get a final concentration 0.02 mM. Subsequent to the constitution of the compound I in MeOH, a cellulose based material, having a pore size of 40 microns is selected as the substrate for embedding the compound I. The selected substrate is then coated with the constituted compound I to obtain a probe. The probe thus obtained has strong fluorescence property and is used for the measuring the insecticidal efficacy of the biopesticide, by estimating the concentration of occlusion bodies present in the commercial formulations of HearNPV.

In one embodiment of the invention the compound I is treated with aqueous solution of HearNPV at pH 7.5. FIG. 5a shows plot of emission spectra of

compound I with HearNPV at pH 7.5, according to an embodiment of the invention. At pH 7.5 and at a concentration of 10 µM, compound I showed a concentration dependent quenching of emission with HearNPV at 395 nm with formation of a new emission maxima at 470 nm. FIG. 5b shows plot for calculating detection limit for HearNPV, according to an embodiment of the invention. The minimum detectable concentration for HearNPV at a pH of 7.5 in Tris-HCl buffer is 2.82 x 10^3 PIBs/mL.

FIG. 6a shows plot of emission spectra of compound I in the presence of HearNPV, according to an embodiment of the invention. The change in emission intensity of compound I in the presence of HearNPV is observed at different pH ranges, with emission maxima at 395nm. In all pH ranges, the probe showed quenching in emission intensity at 395 nm. Hence, the probe is suitable for estimation of HearNPV over a wide range of pH, including but not limited to pH 6.5, 7.0 and 7.5.

To quantify the amount of HearNPV present in the commercial formulations, the cross-reactivity of compound I towards the other adjuvants present in the mixture is tested. FIG. 6b shows plot of emission spectra of compound I with different analytes, according to an embodiment of the invention. There is no significant alteration in the emission spectra upon addition of these analytes. Hence, the probe is suitable for estimation of HearNPV even in commercially available bio-pesticide formulations.

In one embodiment of the invention the emission a spectrum of active HearNPV is compared to the ineffective heat treated HearNPV. FIG. 7a shows plot of emission spectra of heat treated HearNPV, according to an embodiment of the invention. The heat treated HearNPV solution exhibited significantly different emission signature in comparison to the active one. This is characteristic of the HearNPV which undergoes denaturation of outer protein layer owing to temperature conditions during peak summer. The change in the emission spectra of the heat treated HearNPV indicates heat mediated denaturation of outer protein layer in HearNPV. FIG. 7b shows plot of CD spectra of heat treated HearNPV, according to an

embodiment of the invention. The heat treated HearNPV solution exhibited significantly different emission signature in comparison to the active one. The change in the emission spectra of the heat treated HearNPV indicates heat mediated denaturation of the outer protein layer in HearNPV.

FIG. 7c shows plot of emission spectra of compound I with heat treated HearNPV, according to an embodiment of the invention. Addition of heat-treated HearNPV in the aqueous solution of compound I significantly decreases the extent of emission quenching. There is no red-shift in emission maximum under this condition. FIG. 7d shows the comparison of the emission response of compound I towards the normal and heat treated HearNPV, according to an embodiment of the invention.

In yet another embodiment of the invention, the probe coated with compound I is tested for its reusability. FIG. 8a shows a plot depicting reversible interaction of HearNPV with the probe, according to an embodiment of the invention. The compound I is allowed to interact with HearNPV at buffered solution of pH 7.5. Then the pH of probe-HearNPV conjugate solution is lowered up to pH 4.5. As it essentially changes the charge distribution on the polyhedral surface of HearNPV, this preformed complex is no longer be stable in solution. Thus formation of white colored precipitate of HearNPV is observed, which is then separated by centrifugation. After that, the pH of the probe solution is adjusted again to 7.5 and the same is utilized again for HearNPV sensing.

FIG. 8b shows a plot of emission spectra of the probe with a commercial formulation of HearNPV, according to an embodiment of the invention. Quantitative estimation of HearNPV is observed even in the commercial formulation without any significant loss. This shows that the present probe acts as a quality marker for commercially available HearNPV based biopesticides. To unveil the mechanism of HearNPV interaction with compound I, series of spectroscopic studies are conducted. FIG. 9a shows changes in the thermodynamic parameters of compound I during interaction with HearNPV, according to an embodiment of the invention. A

decrease in ΔH value with simultaneous increase in ΔS suggests that the interaction is electrostatic in nature. FIG. 9b shows the changes in zeta potential values of HearNPV upon gradual addition of compound I, according to an embodiment of the invention. HearNPV shows highly negative zeta potential value in absence of compound I. Addition of compound I shows lowering of zeta potential value ie. it becomes more positive. This indicates again the formation of electrostatic complex between compound I and HearNPV. Electrostatic interaction is known to be affected by the presence of electrolyte. FIG. 9c shows changes in the extent of interaction of compound I with HearNPV in presence of NaCl, according to an embodiment of the invention. A decrease in the extent of interaction was observed in presence of higher amount of NaCl. FIG. 9d shows time dependent emission studies of the compound I in presence of HearNPV, according to an embodiment of the invention. The time dependent emission studies suggest that in presence of HearNPV, the average lifetime of compound I increases. This suggests restriction in the twisting motion of the flexible terminal groups during electrostatic complex formation.

The probe is developed by drop casting the constitution of compound I in MeOH onto the selected substrate. FIG. 10a shows changes in the emission intensities of the probe in presence of various amounts of HearNPV, according to an embodiment of the invention. FIG. 10b shows selective emission response of the probe towards HearNPV in presence of other components, according to an embodiment of the invention. FIG. 11a shows preferential response of the probe towards freshly obtained HearNPV in comparison to its heat treated analog, according to an embodiment of the invention. A higher emission response of the probe towards is observed in the freshly prepared HearNPV in comparison to the heat treated HearNPV. FIG. 11b shows comparable emission response of the probe towards the pure HearNPV and commercially available HearNPV according to an embodiment of the invention.

INDUSTRIAL APPLICATION

The most common way to introduce HearNPV in agriculture field is to spray the water dispersible formulation of HearNPV using conventional spray equipment. Sixteen different types of crops known to be infected by *H. Armigera* were tested for this study. FIG. 12a shows emission intensity of compound with HearNPV in cereals, according to an embodiment of the invention. The dose-dependent gradual decrease in emission intensity at 395 nm shows that the probe can detect HearNPV even in the complex biological mixtures such as crop extracts.

FIG. 12b shows emission intensity of compound with HearNPV in fruits, according to an example of the invention.

FIG. 12c shows emission intensity of compound with HearNPV in vegetable extracts, according to an example of the invention.

The table below shows minimum detectable concentration of HearNPV in different crop extracts.

Crops	**LOD (10^3PIBs/mL)**	**Crops**	**LOD (10^3PIBs/mL)**
Cabbage (Brassica oleracea)	17.36	Pigeon peas spilt (Cajanus cajan)	20.35
Okra (Abelmoschus esculentus)	21.78	Red Lentils (Lens culinaris)	17.54
Cardamom (Elettaria cardamomum)	17.60	Chickpeas (brown) (Cicer arietinum)	26.20
Cumin (Cuminumcyminum)	23.75	Maize (Zea mays)	25.12

Black pepper (Piper nigrum)	24.82	Oat (Avena sativa)	22.62
Moong Bean (Vigna radiata)	28.61	Mango (Mangifera indica)	20.35
Brinjal (Solanummelongena)	19.60	Banana (Musa paradisiaca)	17.21
Cucumber (Cucumis sativus)	21.96	Apple (Malusdomestica)	24.85

The device allows rapid detection of HearNPV concentration in commercially available biopesticides. The device is easy-to-use, with portable probes that help in measuring insecticidal efficacy of HearNPV by quantifying the number of occlusion bodies present. Additionally, the probes are reusable in nature.

The foregoing description of the invention has been set for merely to illustrate the invention and is not intended to be limiting. Since modifications of the disclosed embodiments incorporating the spirit and substance of the invention may occur to person skilled in the art, the invention should be construed to include everything within the scope of the appended claims and equivalents thereof.

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1) Manual of Techniques in Invertebrate Pathology (Second Edition), edited by Lawrence A Lacey. ACADEMIC PRESS, An imprint of Elsevier Science.

We claim:

1) A method for synthesizing a compound specific for rapid detection of HearNPV, the method comprising of:

obtaining a bromide precursor, 9-(3-bromobutyl)-9H-carbazole;

constituting the bromide precursor in acetonitrile at a predefined temperature to obtain a piperazine derivative, 9-(4-(4-methylpiperazin-1-yl) butyl)-9H-carbazole; and

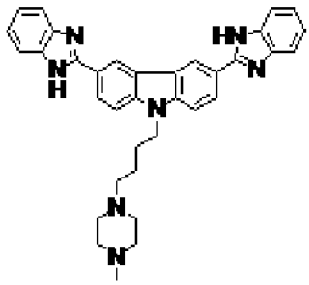
purifying the piperazine derivative to obtain an intermediate, wherein the intermediate is further refluxed to obtain a carbazole derivative.

2. The method of claim 1, wherein the predefined temperature is between 90°C -100°C.

3. The method of claim 1, wherein the intermediate is an aldehyde derivative 9-(4-(4-methylpiperazin-1-yl)butyl)-9H-carbazole-3,6-dicarbaldehyde.

4. The method of claim 1, wherein the intermediate is refluxed for time duration of about 12hours to about 16 hours.

5. The compound of claim 1, wherein the carbazole derivative is represented by a formula (I):



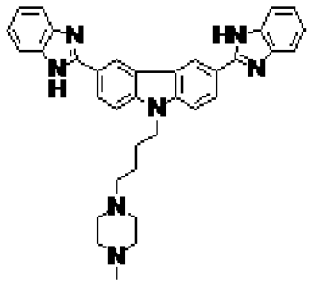
6. A method for obtaining a probe for rapid detection of HearNPV, the method comprising of :

selecting a carbazole derivative of formula (I);

constituting the selected carbazole derivative in an organic medium; and

embedding the constituted carbazole derivative onto a substrate to obtain the probe.

7. The method of claim 6, wherein the carbazole derivative is represented by a formula (I):



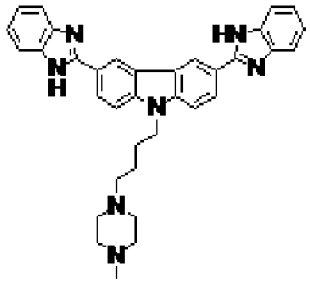
8. The method of claim 6, wherein the organic medium is selected from the group comprising of MeOH, DMSO, Acetonitrile, EtOH and/or combinations thereof.
9. The method of claim 6, wherein the substrate is selected from the group comprising of glass, quartz, cellulose matrix and/or combinations thereof.
10. A device for rapid detection of HearNPV, the device comprising of a:

a probe;

a detection means; and

an analyser.

11. The device of claim 10, wherein the probe comprises of a substrate embedded with a carbazole derivative.
12. The probe of claim 11, wherein the substrate is selected from the group comprising of glass, quartz, cellulose matrix and/or combinations thereof.
13. The probe of claim 11, wherein the carbazole derivative is represented by a formula (I):



14. The device of claim 10, wherein the detection means is a UV source.
15. The device of claim 10, wherein the analyser is a reference chart indicating predefined efficacy concentration of HearNPV.

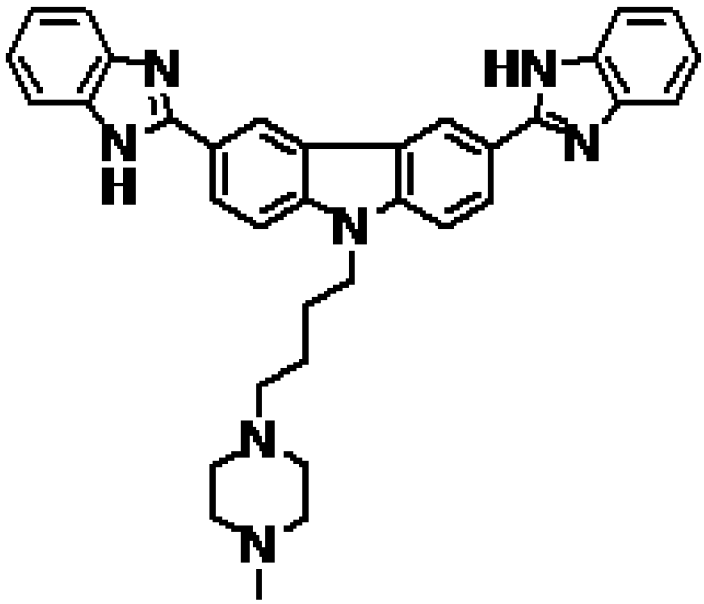


FIG. 1

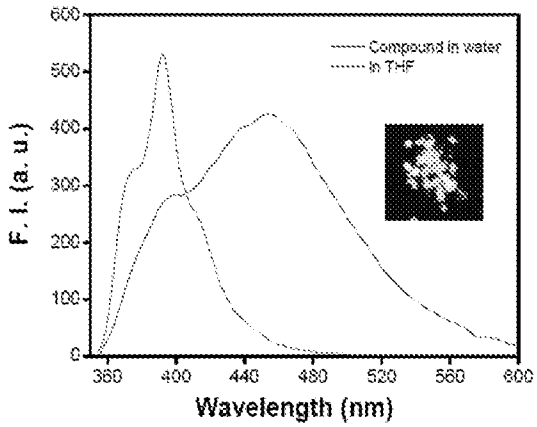


FIG. 2a

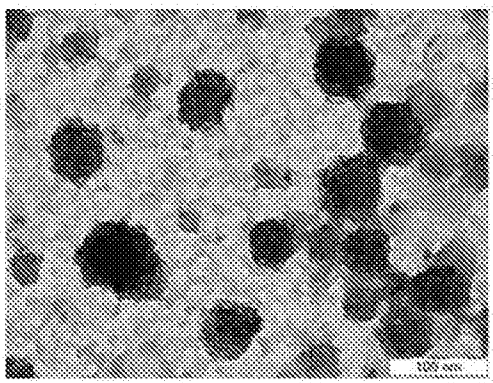


FIG. 2b

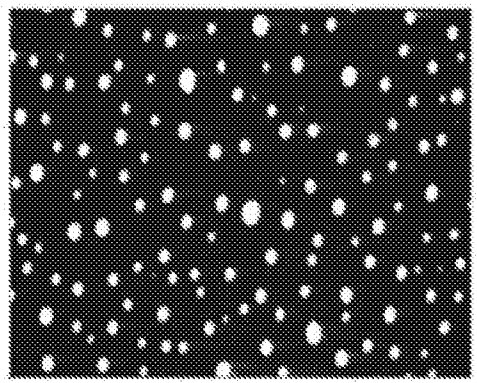


FIG. 2c

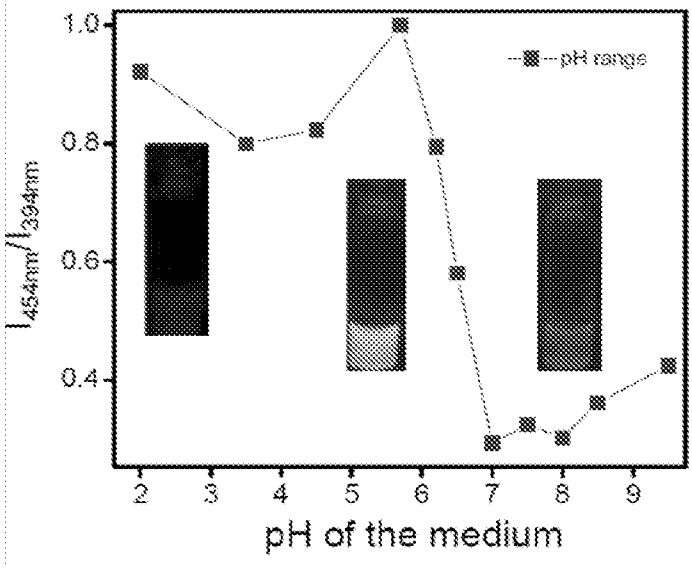

1.0
0.8
0.6
0.4
I_{454nm}/I_{394nm}
pH range
2
3
4
5
6
7
8
9
pH of the medium

FIG. 3a

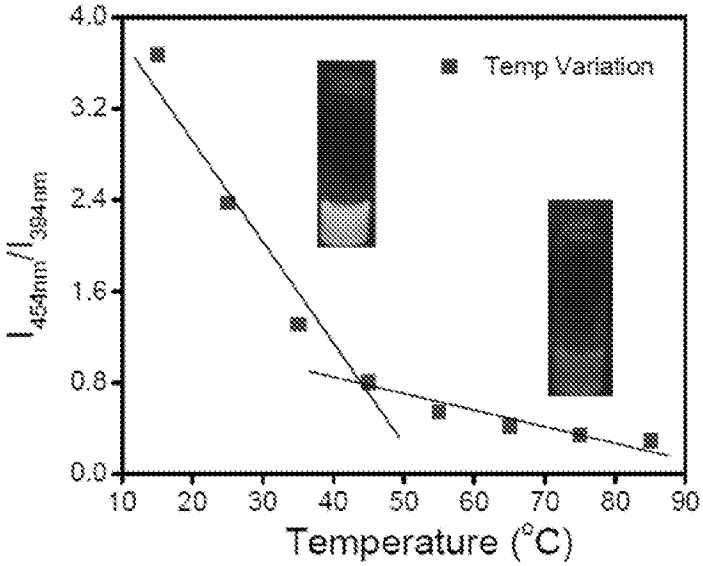

4.0
3.2
2.4
1.6
0.8
0.0
I_{454nm}/I_{394nm}
Temp Variation
10
20
30
40
50
60
70
80
90
Temperature (°C)

FIG. 3b

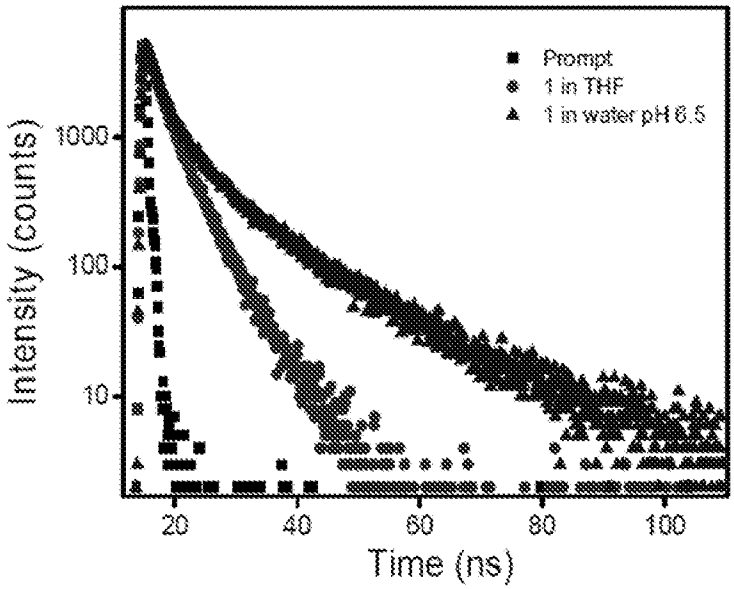

Prompt
1 in THF
1 in water pH 6.5
Intensity (counts)
1000
100
10
20
40
60
80
100
Time (ns)

FIG. 4a

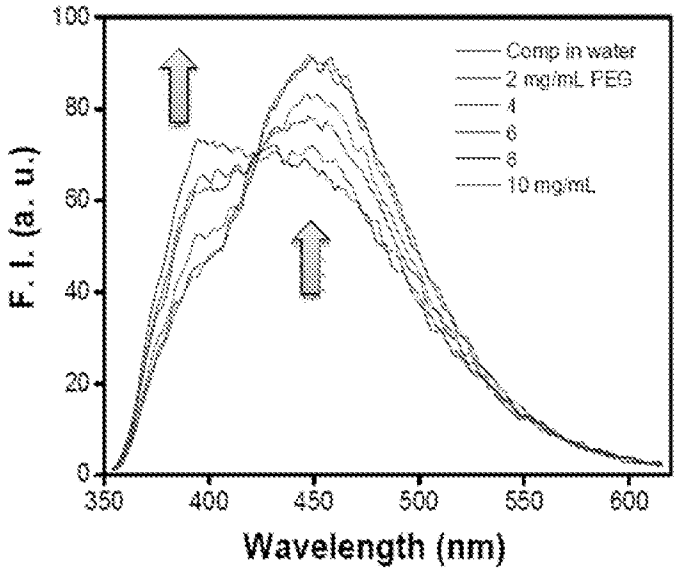

Comp in water
2 mg/mL PEG
4
6
8
10 mg/mL
F. I. (a. u.)
100
80
60
40
20
0
350
400
450
500
550
600
Wavelength (nm)

FIG. 4b

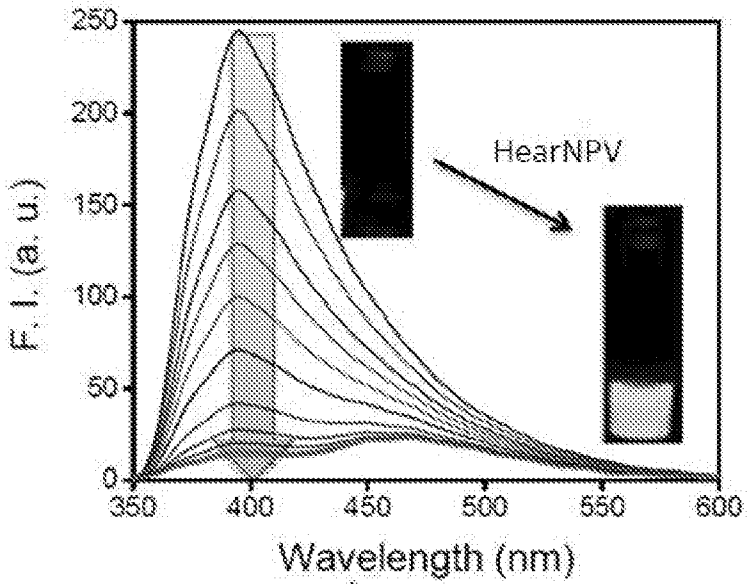

250
200
150
100
50
0
F. I. (a. u.)
HearNPV
350
400
450
500
550
600
Wavelength (nm)

FIG. 5a

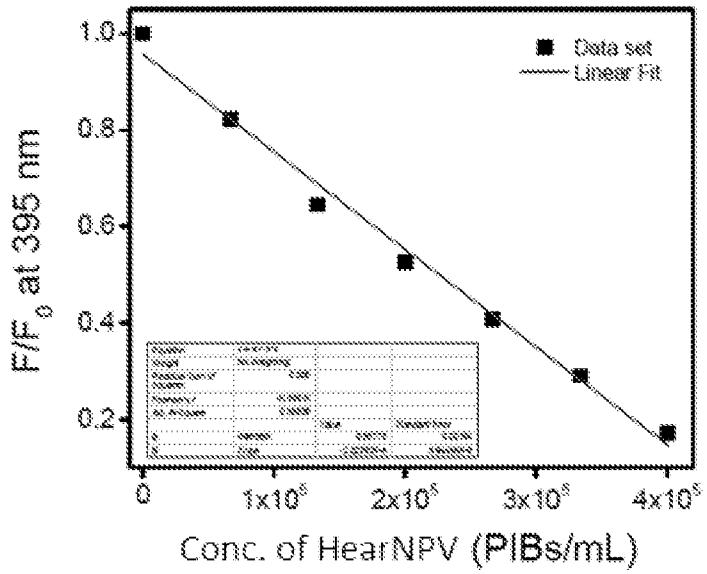

1.0
0.8
0.6
0.4
0.2
F/F0 at 395 nm
Data set
Linear Fit
0
1x10^5
2x10^5
3x10^5
4x10^5
Conc. of HearNPV (PIBs/mL)

FIG. 5b

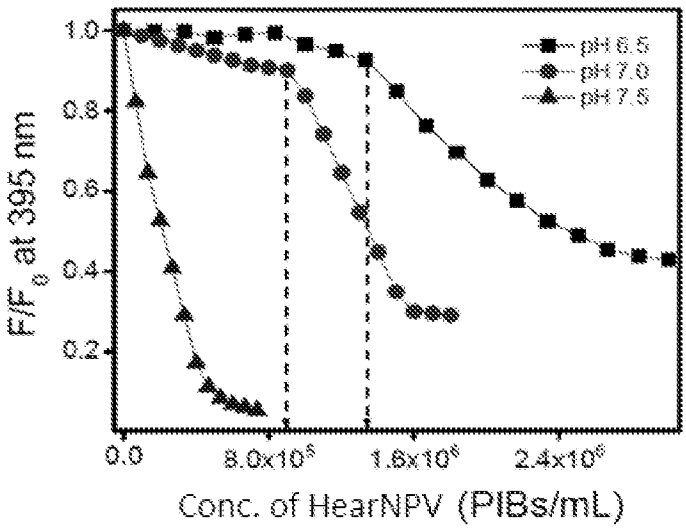

1.0
0.8
0.6
0.4
0.2
F/F$_0$ at 395 nm
pH 6.5
pH 7.0
pH 7.5
0.0
8.0x10^5
1.6x10^6
2.4x10^6
Conc. of HearNPV (PIBs/mL)

FIG. 6a

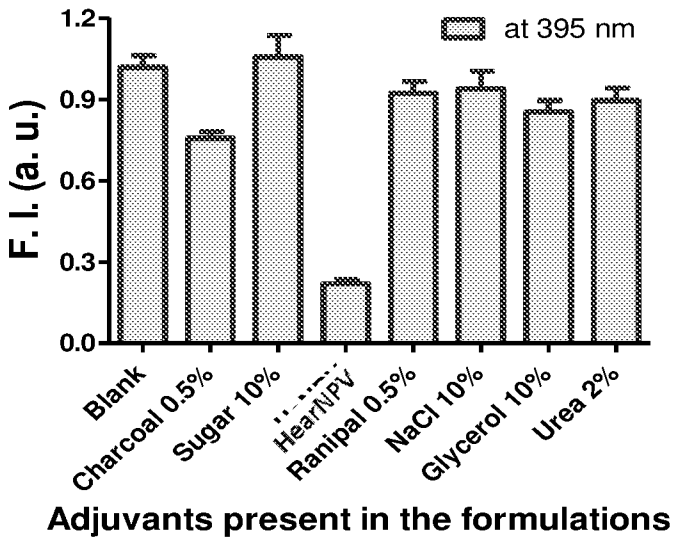

1.2
0.9
0.6
0.3
0.0
F. I. (a. u.)
at 395 nm
Blank
Charcoal 0.5%
Sugar 10%
HearNPV
Ranipal 0.5%
NaCl 10%
Glycerol 10%
Urea 2%
Adjuvants present in the formulations

FIG. 6b

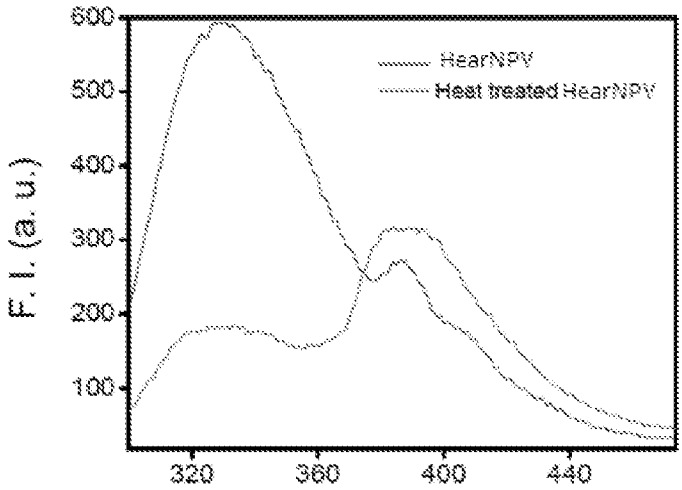

F. I. (a. u.)
600
500
400
300
200
100
320
360
400
440
HearNPV
Heat treated HearNPV

FIG. 7a

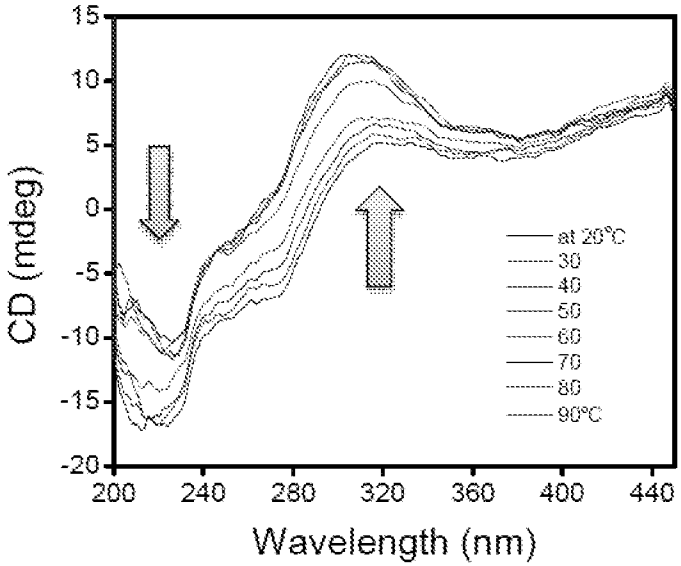

CD (mdeg)
15
10
5
0
-5
-10
-15
-20
200
240
280
320
360
400
440
Wavelength (nm)
at 20°C
30
40
50
60
70
80
90°C

FIG. 7b

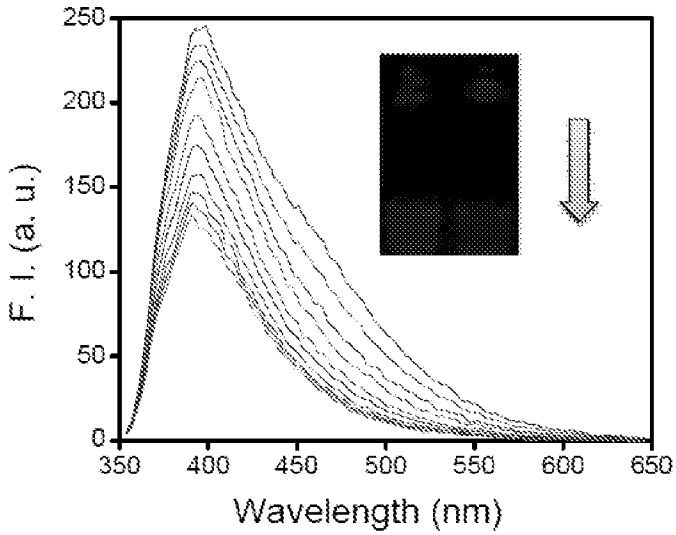

250
200
150
100
50
0
F. I. (a. u.)
350
400
450
500
550
600
650
Wavelength (nm)

FIG. 7c

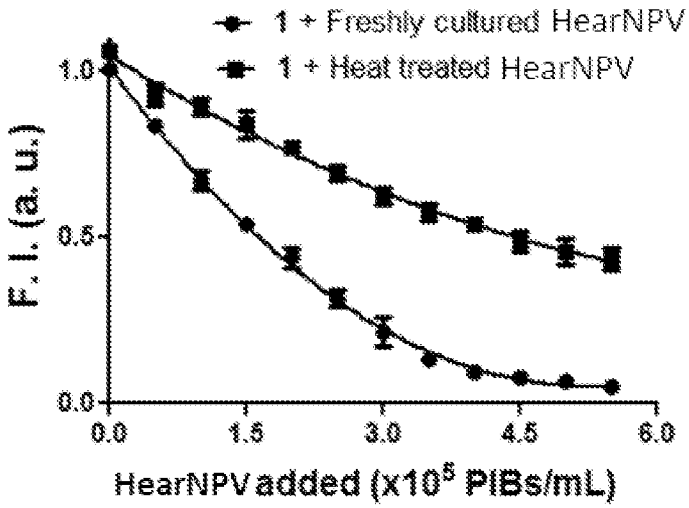

1 + Freshly cultured HearNPV
1 + Heat treated HearNPV
1.0
0.5
0.0
F. I. (a. u.)
0.0
1.5
3.0
4.5
6.0
HearNPV added (x10^5 PIBs/mL)

FIG. 7d

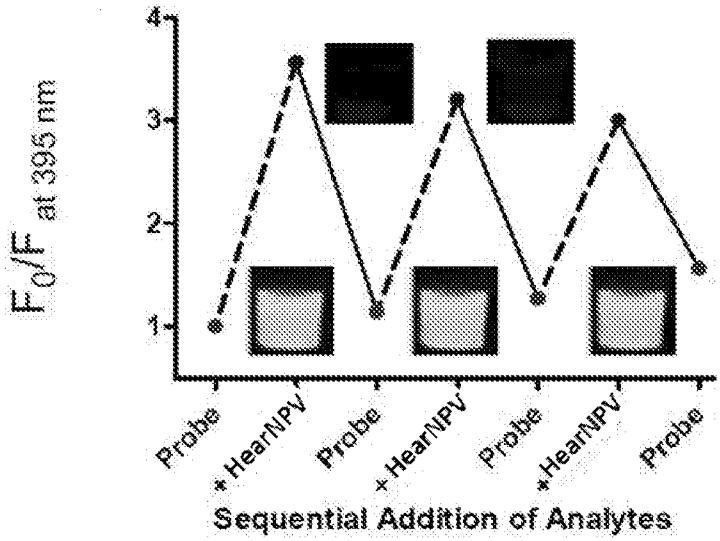

F_0/F at 395 nm
4
3
2
1
Probe
+ HearNPV
Probe
+ HearNPV
Probe
+ HearNPV
Probe
Sequential Addition of Analytes

FIG. 8a

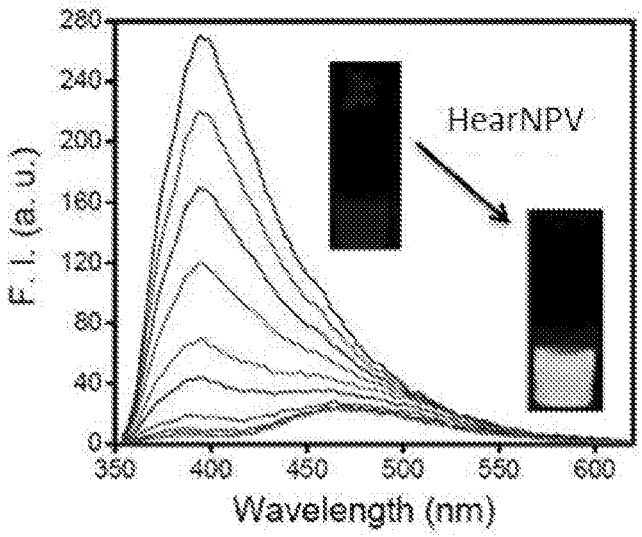

F. I. (a. u.)
280
240
200
160
120
80
40
0
350
400
450
500
550
600
Wavelength (nm)
HearNPV

FIG. 8b

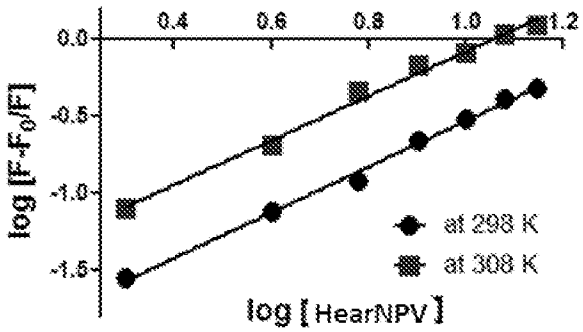


FIG. 9a

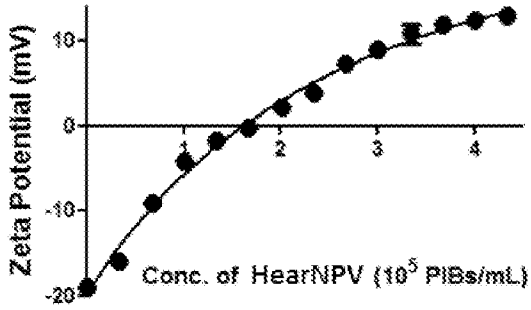


FIG. 9b

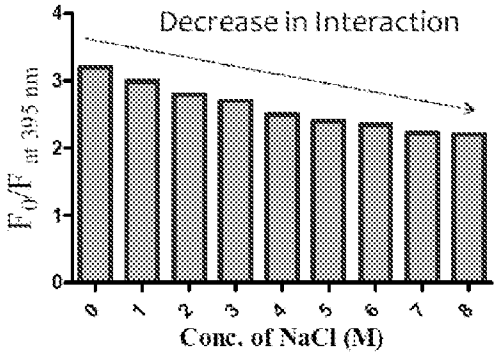


FIG. 9c

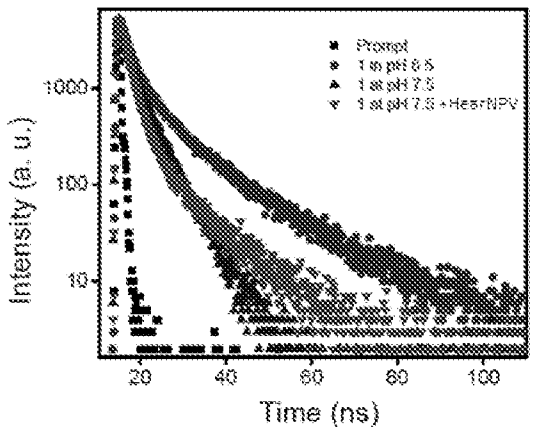


FIG. 9d

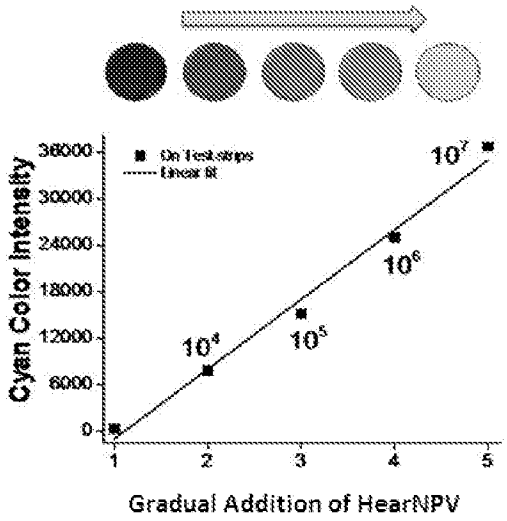


FIG. 10a

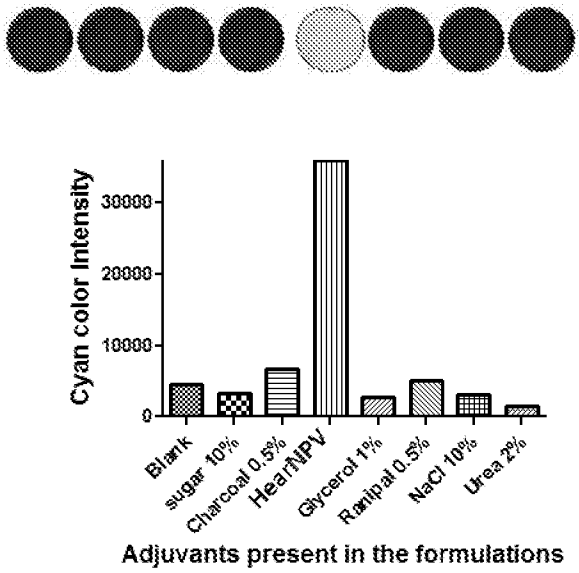


FIG. 10b

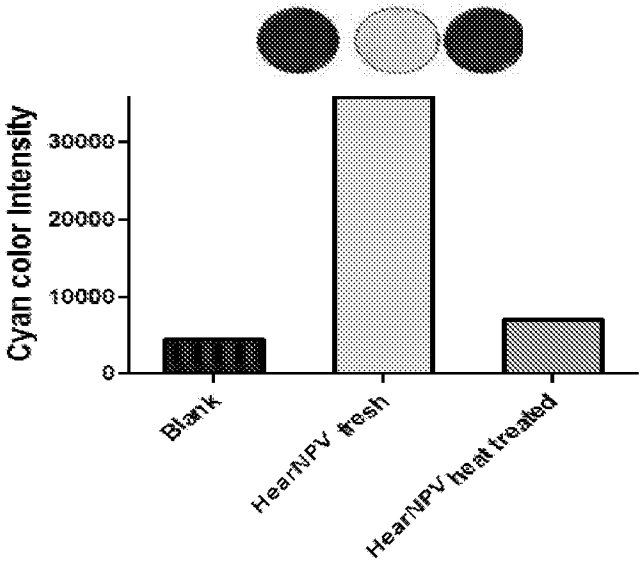


FIG. 11a

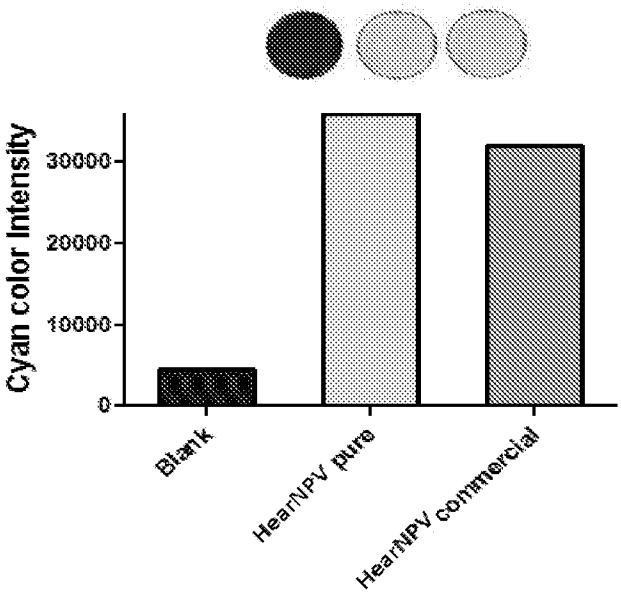


FIG. 11b

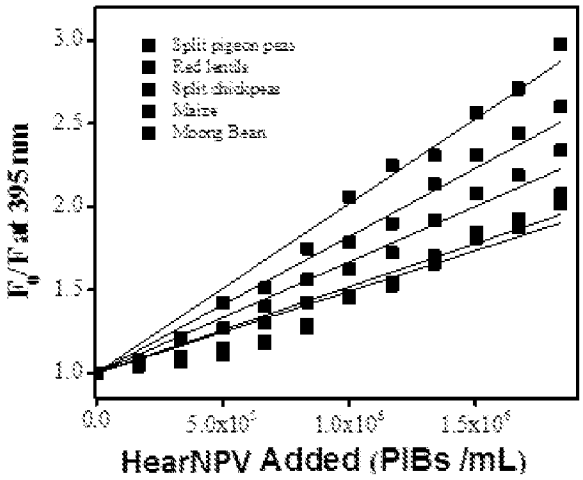


FIG. 12a

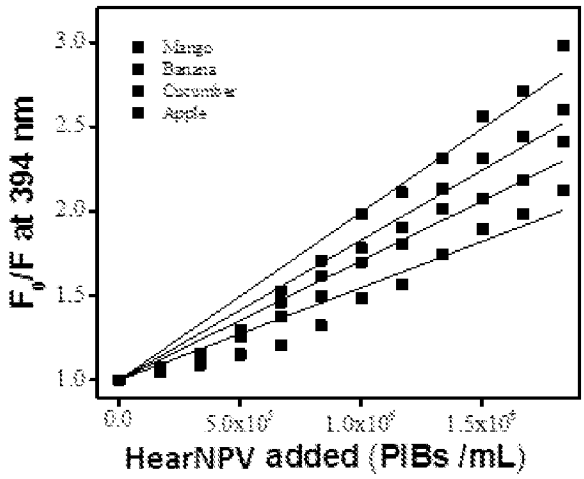


FIG. 12b

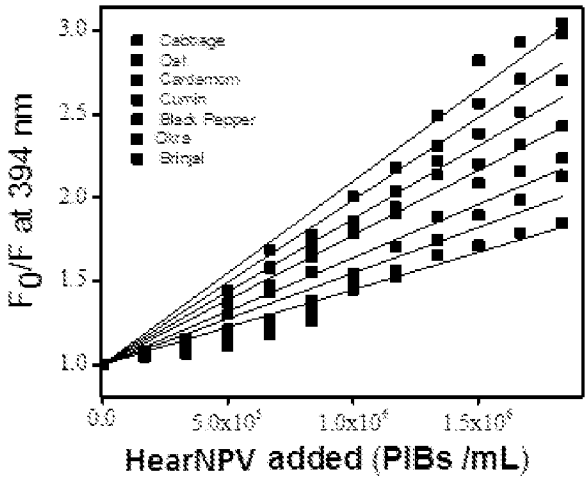


FIG. 12c

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2018/050365

A. CLASSIFICATION OF SUBJECT MATTER
C12Q1/68,C12Q1/70,C07D209/82 Version=2018.01

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)

C12Q, C07D

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)

TotalPatent One, IPO Internal Database

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SANTANU BHATTACHARYA ET AL: "Design and Synthesis of New Benzimidazole-Carbazole Conjugates for the Stabilization of Human Telomeric DNA, Telomerase Inhibition, and Their Selective Action on Cancer Cells", Journal of Medicinal Chemistry, 25 July 2014, Vol. 57, No. 16, pages 6973-6988. *Scheme 1; synthesis of 9, 10, 11, 1-3 under the heading Materials and Methods*	1-5
A	*The whole document*	6-15
X	CN105950782 A (HENAN JIYUAN BAIYUN IND CO LTD) 20 September 2016 (2016-09-21) (Family: None) *The whole document especially Abstract*	10
A	*The whole document*	1-9, 11-15
A	SMITH T.F. "Rapid Methods for the Diagnosis of Viral Infections", LABORATORY MEDICINE, Vol. 18, No. 1, January 1987, pages 16-20.	1-15

☒ 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
04-10-2018	04-10-2018
Name and mailing address of the ISA/ Indian Patent Office Plot No.32, Sector 14,Dwarka,New Delhi-110075 Facsimile No.	Authorized officer Lokesh Kumar Rajwanshi Telephone No. +91-1125300200

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	*The whole document* --- MOORE ET AL: "Evaluation of Helicoverpa armigera Nucleopolyhedrovirus (HearNPV) for Control of Helicoverpa armigera (Lepidoptera: Noctuidae) on Citrus in South Africa", Biocontrol Science and Technology, May 2004, Vol. 14, No. 3, pages 239-250. *The whole document*	1-15