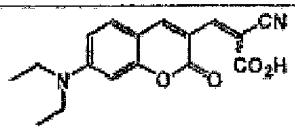
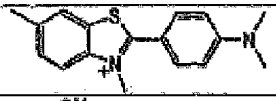
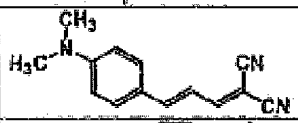
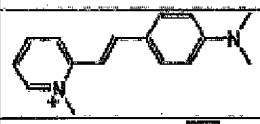
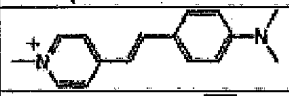
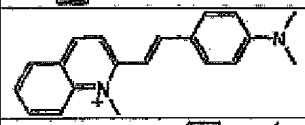
	Structure	R groups
1		
2		
3		
4		
5		
6		
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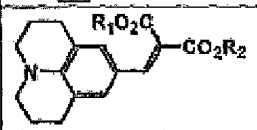
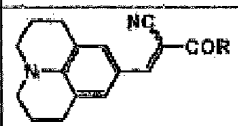
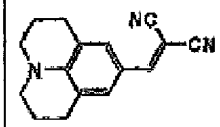
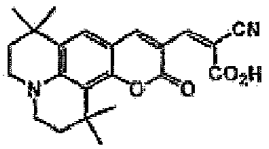
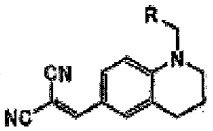
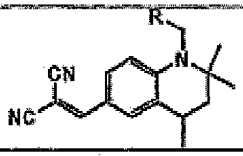
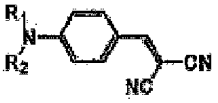
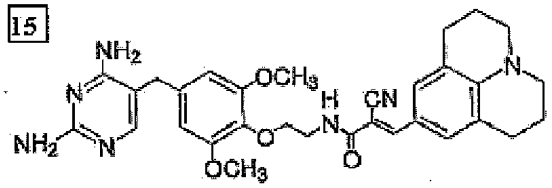
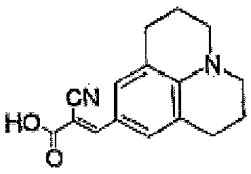
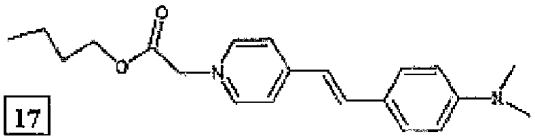
8		
9		R=OH, OEt, NH_2
10		

	Structure	R groups
11		
12		R=COOH, CO_2Et, CH_2OH, CH_2OAc, CH_2Cl
13		R=CH_2CO_2Ph
14		R_1, R_2 = CH_3
15		
16		
17		

[0013a] In an aspect, there is provided a method for detecting an interaction between (a) DNA and p53 protein, (b) MDM2 protein and a peptide, or (c) biotin and streptavidin, the method comprising:

(i) binding at least one fluorescent molecular rotor to DNA or p53 protein of (a), peptide or MDM2 protein of (b), or biotin or streptavidin of (c); and

detecting a change in fluorescence emitted by said fluorescent molecular rotor after contact of the bound fluorescent molecular rotor with the other of said DNA or p53 protein of (a), peptide or MDM2 protein of (b), or biotin or streptavidin of (c), thereby detecting an interaction between DNA and p53 protein of (a), peptide and MDM2 protein of (b), or biotin or streptavidin of (c);

wherein the fluorescent molecular rotor is selected from the group consisting of:

a rotating σ-bond;

an electron-donating moiety;

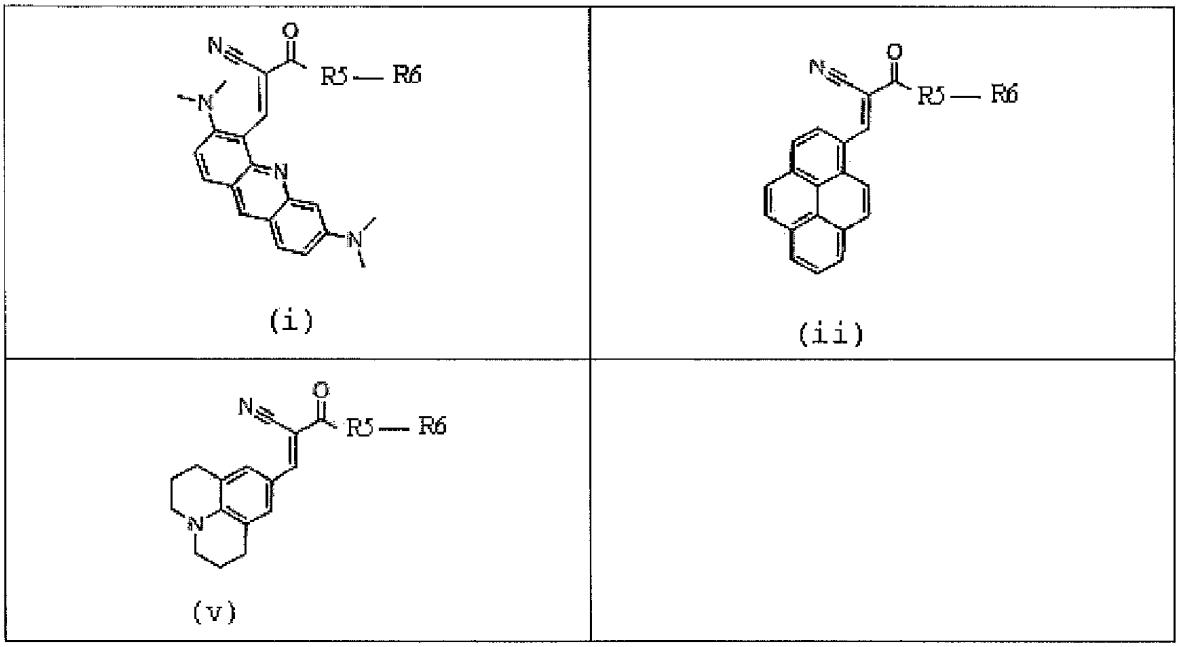
an electron-accepting moiety; and

a π-conjugated linker

wherein the fluorescent molecular rotor(s) is bound to DNA of (a), peptide of (b), or biotin of (c), via a linking moiety, or bound to the p53 protein of (a), the MDM2 protein of (b), or streptavidin of (c) via a linking moiety,

wherein said linking moiety is selected from the group consisting of a single bond; or optionally substituted heteroalkyl, wherein the main chain atoms of said optionally substituted heteroalkyl are optionally interrupted by one or more optionally substituted cyclic groups,

wherein the bound fluorescent molecular rotor is selected from the group consisting of:



R5 is the linking moiety; and

R6 is absent, the DNA, the p53 protein, the peptide, the MDM2 protein, the biotin or the streptavidin.

[0013b] In another aspect, there is provided a composition comprising:

a) fluorescent molecular rotor, DNA and p53 protein;

b) fluorescent molecular rotor, peptide and MDM2 protein; or

c) fluorescent molecular rotor, biotin and streptavidin,

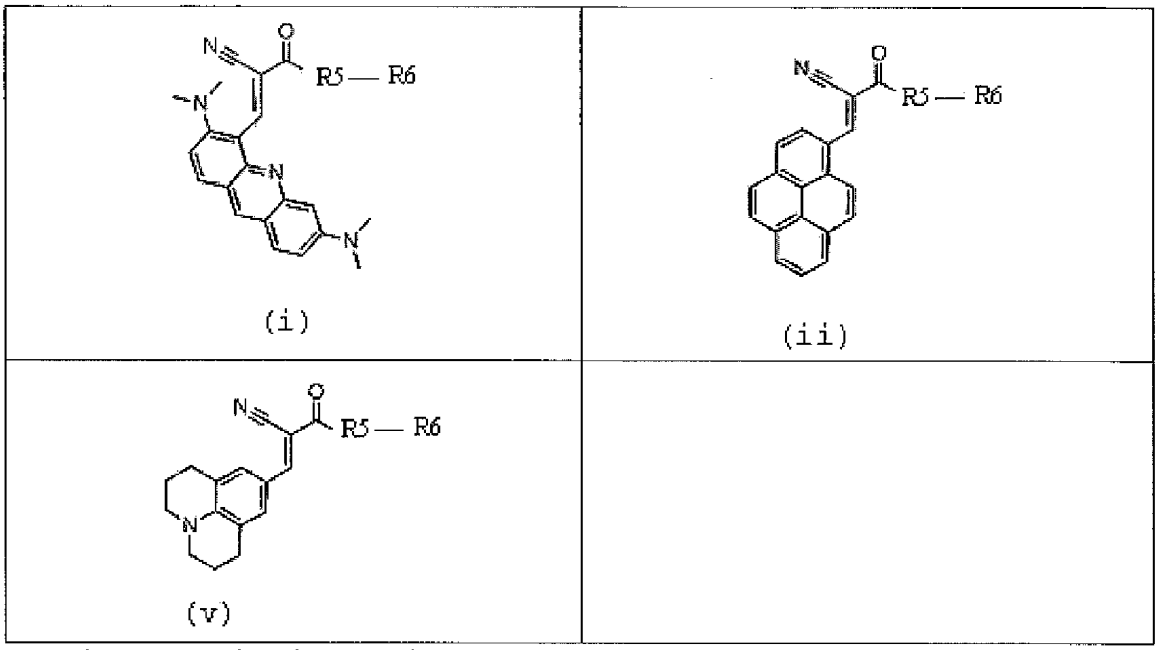
wherein said fluorescent molecular rotor is bound to one of DNA or p53 protein in (a), one of peptide or MDM2 protein in (b), or one of biotin or streptavidin in (c), and wherein the fluorescent molecular rotor(s) comprises:

a rotating σ-bond;

an electron-donating moiety;

an electron-accepting moiety; and

a π-conjugated linker, wherein the fluorescent molecular rotor(s) is bound to DNA of (a), peptide of (b), or biotin of (c), via a linking moiety, or the p53 protein of (a), MDM2 protein of (b), or streptavidin of (c), via a linking moiety, wherein said linking moiety is selected from the group consisting of a single bond; or optionally substituted heteroalkyl, wherein the main chain atoms of said optionally substituted heteroalkyl are optionally interrupted by one or more optionally substituted cyclic groups, wherein the bound fluorescent molecular rotor is selected from the group:



R5 is the linking moiety; and

R6 is absent, the DNA, the p53 protein, the peptide, the MDM2 protein, the biotin or the streptavidin.

[0013c] In another aspect, there is provided a screening assay for identifying an interaction between DNA and p 53 protein, or peptide and MDM2 protein, the assay comprising:

a) providing a p53 protein or MDM2 protein to be tested for binding affinity with candidate DNA or peptide;

b) providing candidate DNA or peptide bound to at least one fluorescent molecular rotor;

c) testing the candidate DNA or peptide for binding affinity by contacting said p53 protein or MDM2 protein with said candidate DNA or peptide bound to at least one fluorescent molecular rotor; and

d) identifying a relevant interactions by measuring a change in fluorescence emitted by the fluorescent molecular rotor(s),

wherein the fluorescent molecular rotor(s) comprises:

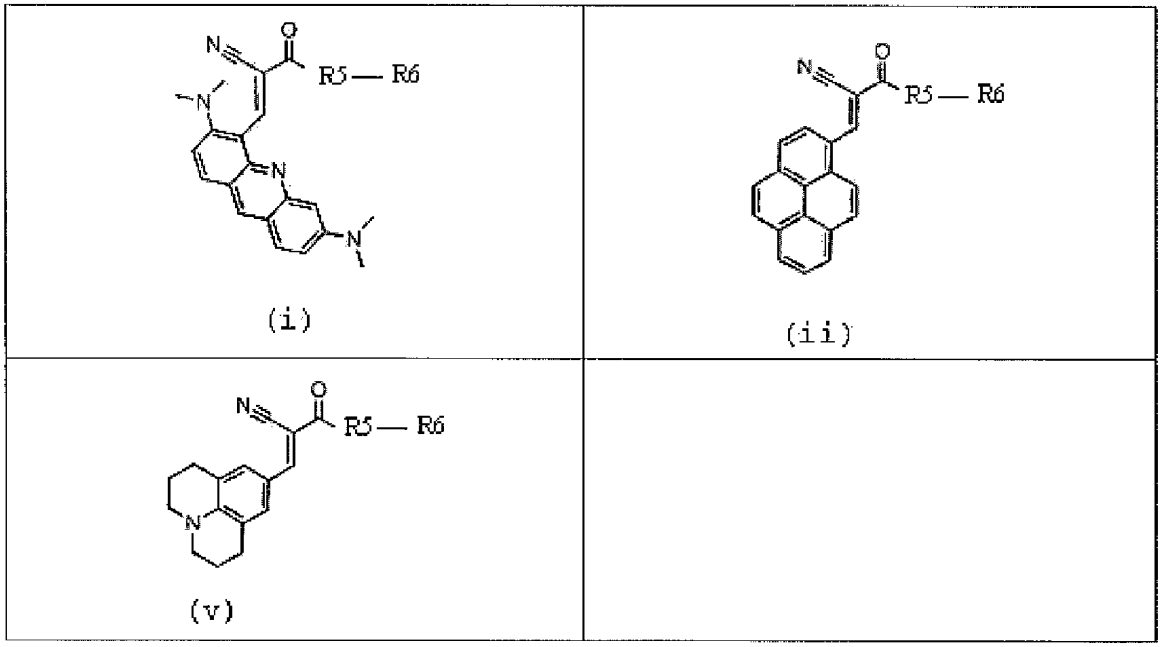
a rotating σ-bond;

an electron-donating moiety;

an electron-accepting moiety; and

a π-conjugated linker, wherein the fluorescent molecular rotor is bound to the candidate DNA or peptide via a linking moiety, wherein said linking moiety is selected from the group consisting of a single bond; or optionally substituted heteroalkyl, wherein the main chain atoms of said optionally substituted heteroalkyl are optionally interrupted by one or more optionally substituted cyclic groups,

wherein the bound fluorescent molecular rotor is selected from the group consisting of:



R5 is the linking moiety; and

R6 is absent, the DNA, the p 53 protein, the peptide or the MDM2 protein.

[0013d] In another aspect, there is provided a screening assay for identifying a candidate compound, comprising:

(a) providing a candidate compound to be tested for binding affinity with MDM2 protein;

(b) providing a complex comprising a probe bound to protein, wherein said probe comprises a peptide selected from the group consisting of JP1: MPRFMDYWEGLSK and JP2: MPRFMDYWEGLNK and at least one fluorescent molecular rotor bound to peptide;

(c) testing the candidate compound for binding affinity with said MDM2 protein by contacting said candidate compound with said complex; and

(d) identifying a relevant candidate compound by measuring the change in fluorescence emitted when said complex is disrupted, wherein the fluorescent molecular rotor(s) comprises:

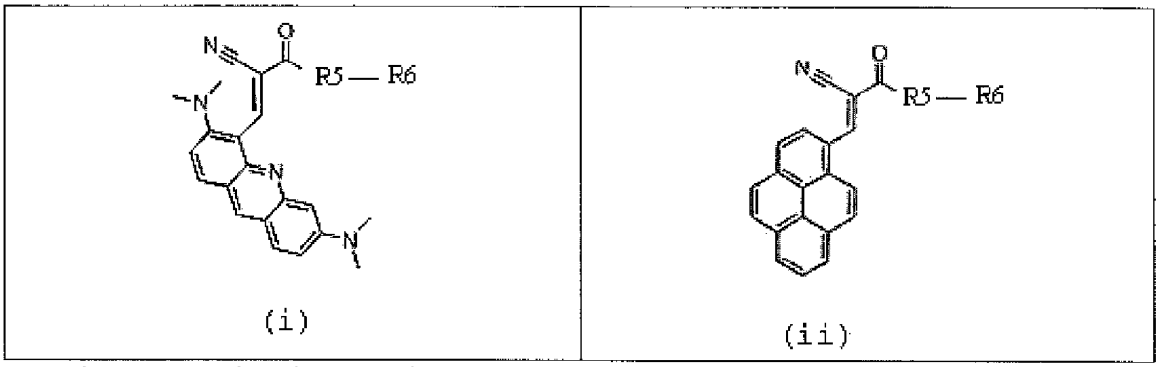
a rotating σ-bond;

an electron-donating moiety;

an electron-accepting moiety; and

a π-conjugated linker, wherein the fluorescent molecular rotor is bound to the peptide via a linking moiety, wherein said linking moiety is selected from the group consisting of a single bond; or optionally substituted heteroalkyl, wherein the main chain atoms of said optionally substituted heteroalkyl are optionally interrupted by one or more optionally substituted cyclic groups,

wherein the bound fluorescent molecular rotor is selected from the group consisting of:



R5 is the linking moiety; and

R6 is absent or the candidate compound or the MDM2 protein.

[0013e] In another aspect, there is provided a kit for carrying out a method as defined above or the screening assay as defined above comprising:

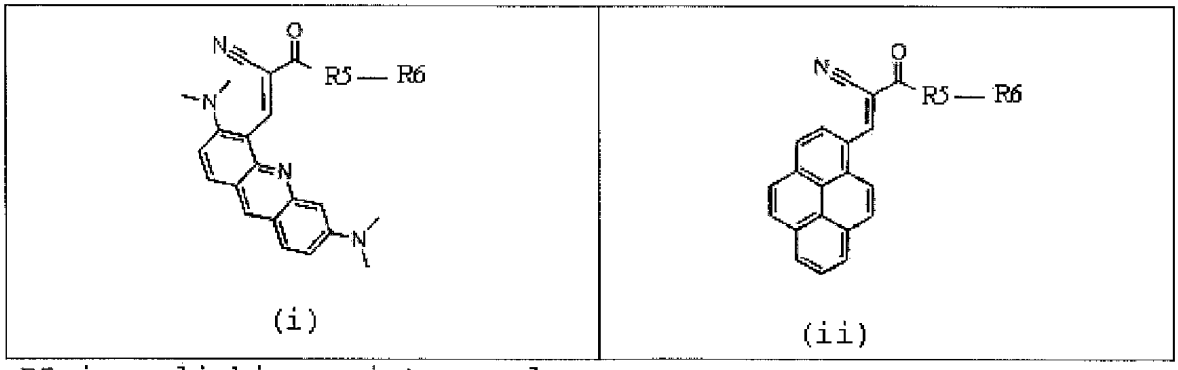
(i) fluorescent molecular rotor, DNA and protein;

(ii) fluorescent molecular rotor, peptide and MDM2 protein; or

(iii) fluorescent molecular rotor, biotin and streptavidin,

as defined above, and a means to detect a change in fluorescence.

[0013f] In another aspect, there is provided a chemical compound selected from the group consisting



R5 is a linking moiety; and

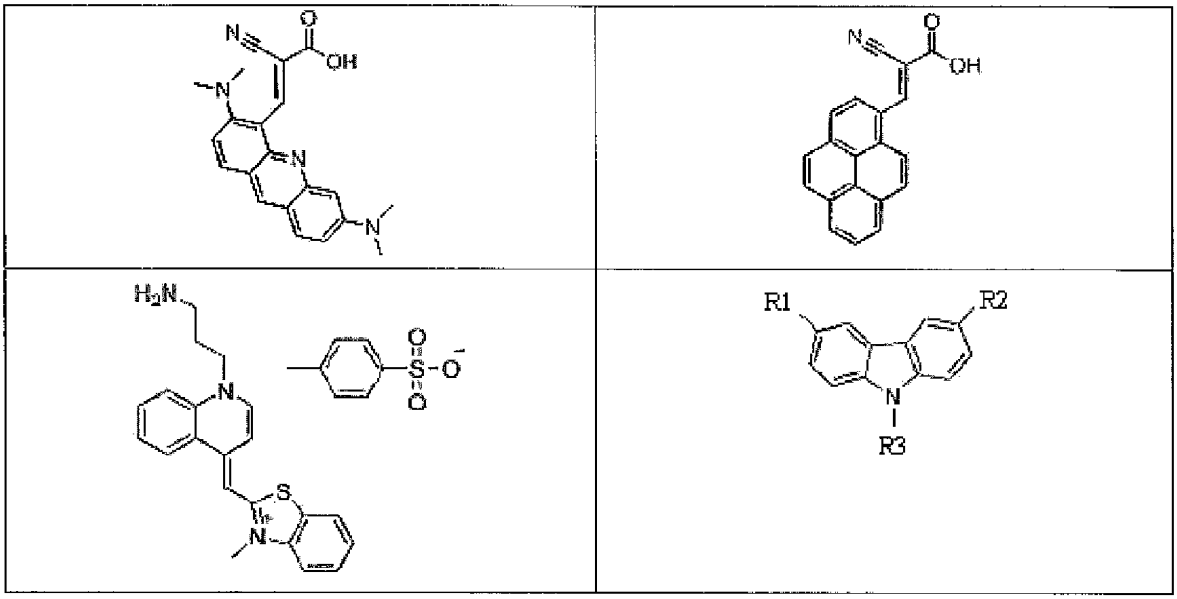
R6 is absent or a ligand.

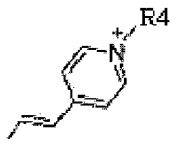
[0014] There is also provided a method for detecting an interaction between a protein and a ligand, comprising:

(i) binding at least one fluorescent molecular rotor to said ligand or protein; and

(ii) detecting a change in fluorescence emitted by said fluorescent molecular rotor after contact of the bound fluorescent molecular rotor with the other of said ligand or protein, thereby detecting an interaction between the ligand and the protein,

wherein the fluorescent molecular rotor is selected from the group consisting of:



wherein at least one of R1, R2 or R3 is  ;

R1, R2, R3 and R4 are independently chosen from the group consisting of: optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted cycloalkyl or optionally substituted heterocyclyl.

[0015] There is further provided a method for detecting an interaction between (a) DNA and protein, (b) MDM2 protein and a peptide, or (c) biotin and streptavidin, the method comprising:

(i) binding at least one fluorescent molecular rotor to said ligand or protein; and

detecting a change in fluorescence emitted by said fluorescent molecular rotor after contact of the bound fluorescent molecular rotor with the other of said ligand or protein, thereby detecting an interaction between the ligand and the protein,

wherein the fluorescent molecular rotor is selected from the group consisting of:

a rotating σ-bond;

an electron-donating moiety;

an electron-accepting moiety; and

a π-conjugated linker.

[0016] Advantageously, the fluorescent molecular rotors may be useful as fluorescent probes for determining protein-ligand interactions. The fluorescent molecular rotors may be useful as a fluorescent probe due to the restriction of intramolecular rotation within the molecular rotor through binding to a ligand.

[0017] The fluorescent molecular rotors may display a change in fluorescence upon being displaced from the ligand, perturbed by protein binding or from greater restriction of their intramolecular rotation.

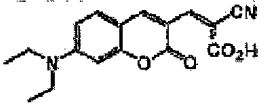
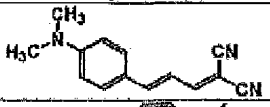
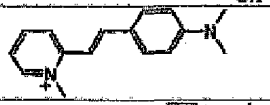
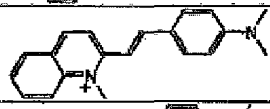
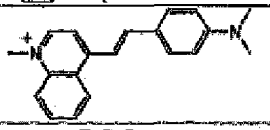
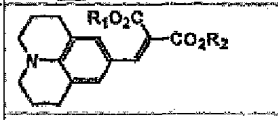
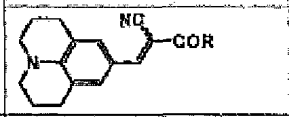
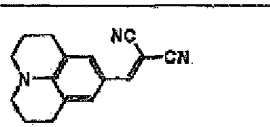
[0018] Advantageously, the fluorescent molecular rotors may be used to investigate protein-DNA interactions. The fluorescent molecular rotors may be engineered such that the intercalating moiety of the rotor encompasses the entire base pair region of DNA, so that the rotating bond will be protruding out of the DNA duplex.

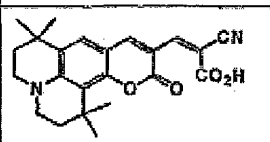
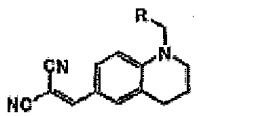
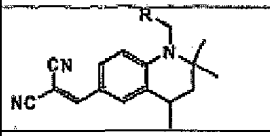
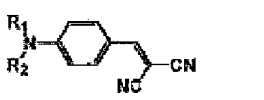
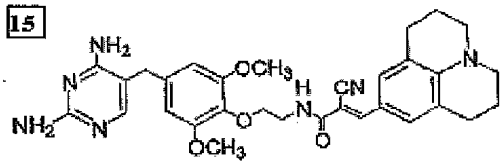
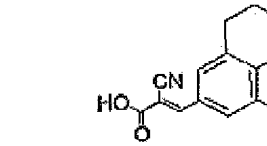
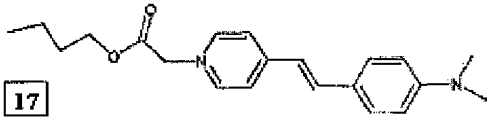
[0019] The fluorescent molecular rotors capable of intercalating DNA can first be bound to DNA then incubated with a protein. Interaction of the protein with the DNA could displace the fluorescent molecular rotor from DNA, or result in a greater restriction of its intramolecular rotation. In both cases, a change in fluorescence may be measured.

[0020] Advantageously, the fluorescent molecular rotors may be used to investigate protein-peptide interactions. The peptide may be a protein-binding peptide which is conjugated to fluorescent molecular rotor(s). The peptide binding site on the protein may restrict the motion of the

fluorescent molecular rotor(s) attached to the peptide sufficiently to bring about a detectable fluorescence turn-on signal.

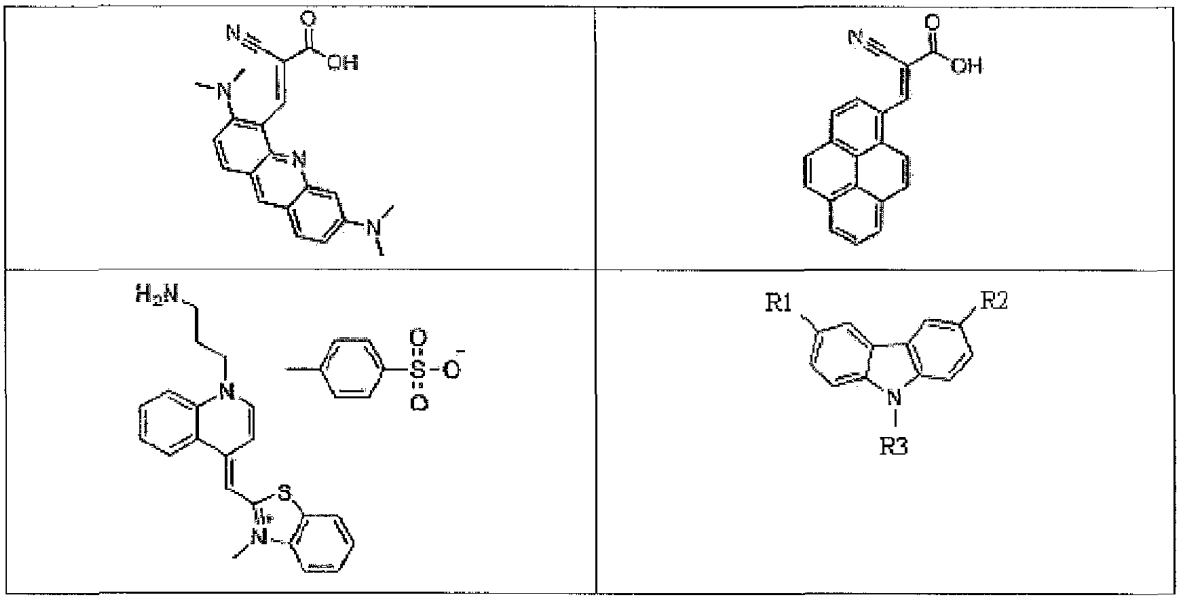
[0021] There is also provided a composition comprising a fluorescent molecular rotor. The composition may further comprise a ligand and a protein, wherein at least one fluorescent molecular rotor is bound to the ligand or the protein, with the proviso that the fluorescent molecular rotor is not a compound selected from the following table:

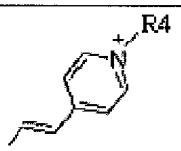
	Structure	R groups
1		
2		
3		
4		
5		
6		
7		
8		
9		R=OH, OEt, NH_2
10		

	Structure	R groups
11		
12		R=COOH, CO_2Et, CH_2OH, CH_2OAc, CH_2Cl
13		R=CH_2CO_2Ph
14		R_1, R_2 = CH_3
15		
16		
17		

[0022] There is also provided a composition comprising a fluorescent molecular rotor,

wherein the fluorescent molecular rotor is selected from the group consisting of:



wherein at least one of R1, R2 or R3 is  ; and R1, R2, R3 and R4 are independently chosen from the group consisting of: optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted cycloalkyl or optionally substituted heterocyclyl.

[0023] There is further provided a composition comprising:

(i) fluorescent molecular rotor, DNA and protein;

(ii) fluorescent molecular rotor, peptide and MDM2; or

(iii) fluorescent molecular rotor, biotin and streptavidin,

wherein said fluorescent molecular rotor is bound to one of DNA or protein in (a), one of peptide or MDM2 in (b), or one of biotin or streptavidin in (c),

and wherein the fluorescent molecular rotor(s) comprises:

a rotating σ-bond;

an electron-donating moiety;

an electron-accepting moiety; and

a π-conjugated linker.

[0024] There is also provided a screening assay for identifying a ligand, the assay comprising:

(i) providing a protein to be tested for binding affinity with a candidate ligand;

[0025] There is further provided a screening assay for identifying an interaction between a protein and ligand, the assay comprising:

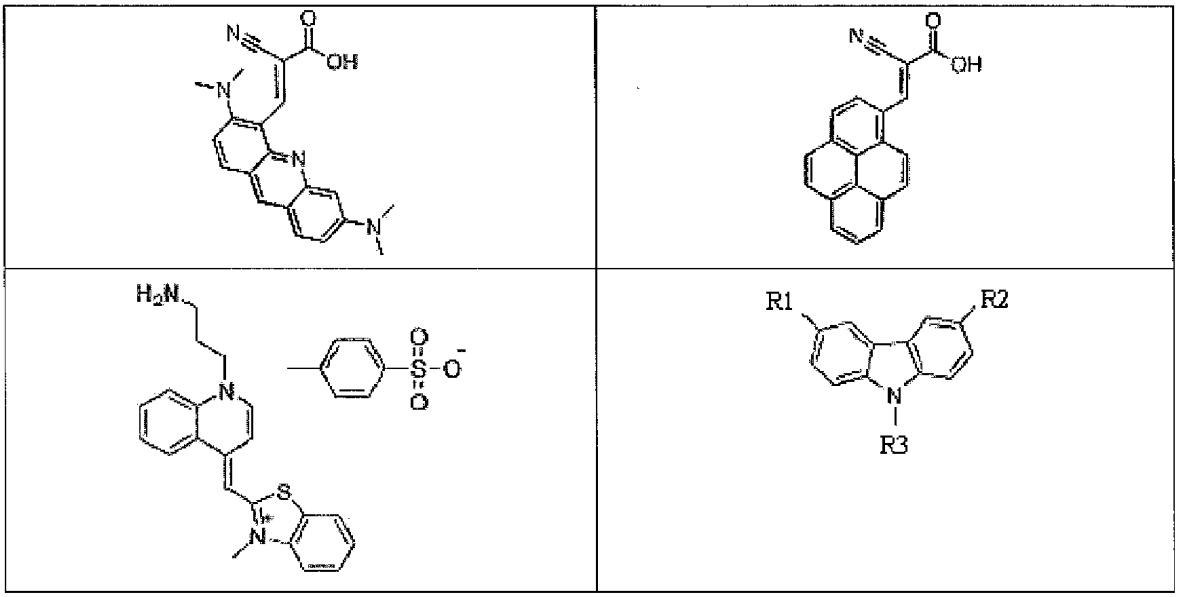
a) providing a protein to be tested for binding affinity with a candidate ligand;

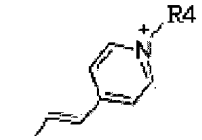
b) providing candidate ligands bound to at least one fluorescent molecular rotor;

c) testing the candidate ligands for binding affinity by contacting said protein with said candidate ligands bound to at least one fluorescent molecular rotor; and

d) identifying a relevant interaction by measuring a change in fluorescence emitted by the fluorescent molecular rotor(s),

wherein the fluorescent molecular rotor is selected from the group consisting of:





wherein at least one of R1, R2 or R3 is ; and R1, R2, R3 and R4 are independently chosen from the group consisting of: optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted cycloalkyl or optionally substituted heterocyclyl.

[0026] There is further provided a screening assay for identifying an interaction between DNA and protein, or peptide and MDM2, the assay comprising:

a) providing a protein to be tested for binding affinity with candidate DNA or peptide;

b) providing candidate DNA or peptide bound to at least one fluorescent molecular rotor;

c) testing the candidate DNA or peptide for binding affinity by contacting said protein with said

candidate DNA or peptide bound to at least one fluorescent molecular rotor; and

d) identifying a relevant interactions by measuring a change in fluorescence emitted by the fluorescent molecular rotor(s),

wherein the fluorescent molecular rotor(s) comprises:

a rotating σ-bond;

an electron-donating moiety;

an electron-accepting moiety; and

a π-conjugated linker.

[0027] There is also provided a screening assay for identifying a candidate compound, comprising:

(a) providing a candidate compound to be tested for binding affinity with protein;

(b) providing a complex comprising a probe bound to protein, wherein said probe comprises at least one fluorescent molecular rotor bound to peptide;

(c) testing the candidate compound for binding affinity with said protein by contacting said candidate compound with said complex; and

(d) identifying a relevant candidate compound by measuring the change in fluorescence emitted when said complex is disrupted, wherein the fluorescent molecular rotor(s) comprises:

a rotating σ-bond;

an electron-donating moiety;

an electron-accepting moiety; and

a π-conjugated linker.

[0028] Advantageously, the screening assay may be high-throughput, quantitative, label-free, homogenous, non-radioactive and a reproducible method to measure protein-ligand binding.

[0029] Advantageously, the screening assay may be non-laborious and may not require the use of expensive instrumentation.

[0030] Advantageously, the screening method may not require multiple washing steps.

[0031] Advantageously, the assay is single-well, low volume and non-radioactive with minimal pipetting steps and fluorescent readout.

[0032] Advantageously, the screening assay may be a small molecule drug screening assay which may identify small molecules which may be missed in assays, such as fluorescence polarization.

[0033] There is also provided a kit for carrying out the first or sixth aspects of the invention, comprising a ligand, protein and fluorescent molecular rotor(s), and a means to detect a change in fluorescence.

[0034] There is further provided a kit for carrying out the second or seventh aspects of

with the proviso that R5 is not OH in formula (v).

DEFINITIONS

[0037] Unless otherwise defined herein, scientific and technical terms used in this application shall have the meanings that are commonly understood by those of ordinary skill in the art. Generally, nomenclature used in connection with, and techniques of, chemistry, cell and tissue culture, molecular biology, cell and cancer biology, neurobiology, neurochemistry, virology, immunology, microbiology, pharmacology, genetics and protein and nucleic acid chemistry, described herein, are those well-known and commonly used in the art.

[0038] The following are some definitions that may be helpful in understanding the description of the present invention. These are intended as general definitions and should in no way limit the scope of the present invention to those terms alone, but are put forth for a better understanding of the following description.

[0039] Unless the context requires otherwise or specifically stated to the contrary, integers, steps, or elements of the invention recited herein as singular integers, steps or elements clearly encompass both singular and plural forms of the recited integers, steps

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AGENT INPUT CORRESPONDENCE

Agent Input Correspondence: Reporting Office Action

Please complete the relevant fields below and submit by email to your contact.
Please note items marked with a * are mandatory.

Agent Name :	Hechanova & Co., Inc.
Application No. *:	1-2014-500793
S&F Reference No. *:	8767SG29
Your Reference No. *:	P18.0080.SF
Reporting Date (dd/mm/yyyy) *:	19/02/2018

Office Action	
Office Action Date (dd/mm/yyyy) *:	08/02/2018
Office Action Deadline (dd/mm/yyyy) *:	08/04/2018
Extensible *:	(•) Yes If Yes, please provide a due date (dd/mm/yyyy): 08/06/2018 ( ) No

Additional Comments:

☐ See Attached Letter ☑ Other

1. Please provide us with your instructions no later than 2 April 2018, a week before the deadline of 8 April 2018.
2. Kindly note that the Applicant may file a Request for 2nd Extension to File a Response to the outstanding Office Action and shall be given another sixty (60) days which is due on 8

CLAIMS

1. A method for detecting an interaction between (a) DNA and p53 protein, (b) MDM2 protein and a peptide, or (c) biotin and streptavidin, the method comprising:

(i) binding at least one fluorescent molecular rotor to DNA or p53 protein of (a), peptide or MDM2 protein of (b), or biotin or streptavidin of (c); and

(ii) detecting a change in fluorescence emitted by said fluorescent molecular rotor after contact of the bound fluorescent molecular rotor with the other of

said DNA or p53 protein of (a), peptide or MDM2 protein of (b), or biotin or streptavidin of (c), thereby detecting an interaction between DNA and p53 protein of (a), peptide and MDM2 protein of (b), or biotin or streptavidin of (c);

wherein the fluorescent molecular rotor is selected from the group consisting of:

a rotating σ-bond;

an electron-donating moiety;

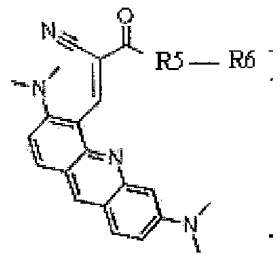
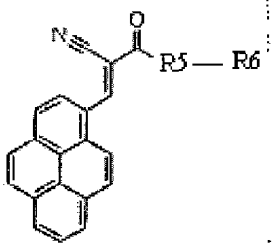
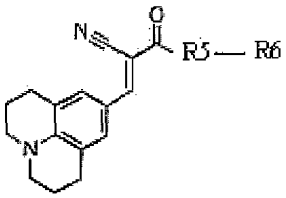
an electron-accepting moiety; and

a π-conjugated linker

wherein the fluorescent molecular rotor(s) is bound to DNA of (a), peptide of (b), or biotin of (c), via a linking moiety, or bound to the p53 protein of (a), the MDM2 protein of (b), or streptavidin of (c) via a linking moiety,

wherein said linking moiety is selected from the group consisting of a single bond; or optionally substituted heteroalkyl, wherein the main chain atoms of said optionally substituted heteroalkyl are optionally interrupted by one or more optionally substituted cyclic groups,

wherein the bound fluorescent molecular rotor is selected from the group consisting of:

 (i)	 (ii)
 (v)	

R5 is the linking moiety; and

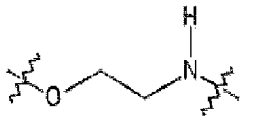
R6 is absent, the DNA, the p53 protein, the peptide, the MDM2 protein, the biotin or the streptavidin.

2. The method of claim 1, wherein the fluorescent molecular rotor(s) is a molecule that exhibits a change in fluorescence when DNA of (a) binds to a p53 protein, peptide of (b) binds to MDM2 protein, or biotin of (c) binds to streptavidin.

3. The method of claim 1 or 2, wherein a change in fluorescence occurs when the binding of p53 protein to DNA of (a), binding of MDM2 protein to peptide of (b), or the binding of streptavidin to biotin of (c) displaces the fluorescent molecular rotor(s) from the DNA or peptide, or perturbs the fluorescent molecular rotor(s).

4. The method of any one of claims 1 to 3, wherein the linking moiety comprises 1 to 20 main chain atoms.

5. The method of any one of claims 1 to 4 wherein the linking moiety is selected from the group consisting of: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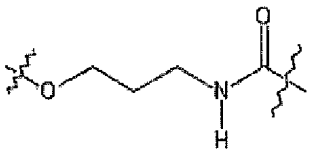
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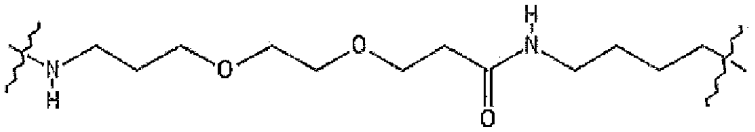
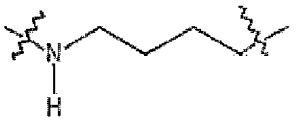

;


and .

6. The method of any one of claims 1 to 5, wherein the protein of (a) is a DNA transacting protein.

7. The method of claim 6, wherein the DNA transacting protein is a transcription factor, polymerase, telomerase or recombinase.

8. The method of any one of claims 1 to 5, wherein the MDM2 protein of (b) is wild-type or mutant MDM2 protein.

9. The method of claim 8, wherein the protein is wild-type or mutant MDM2.

10. The method of any one of claim 1 to 5 and 8 to 9, wherein the peptide of (b) has an amino acid sequence

identical to the sequence of a native peptide that binds to the protein, or a portion thereof.

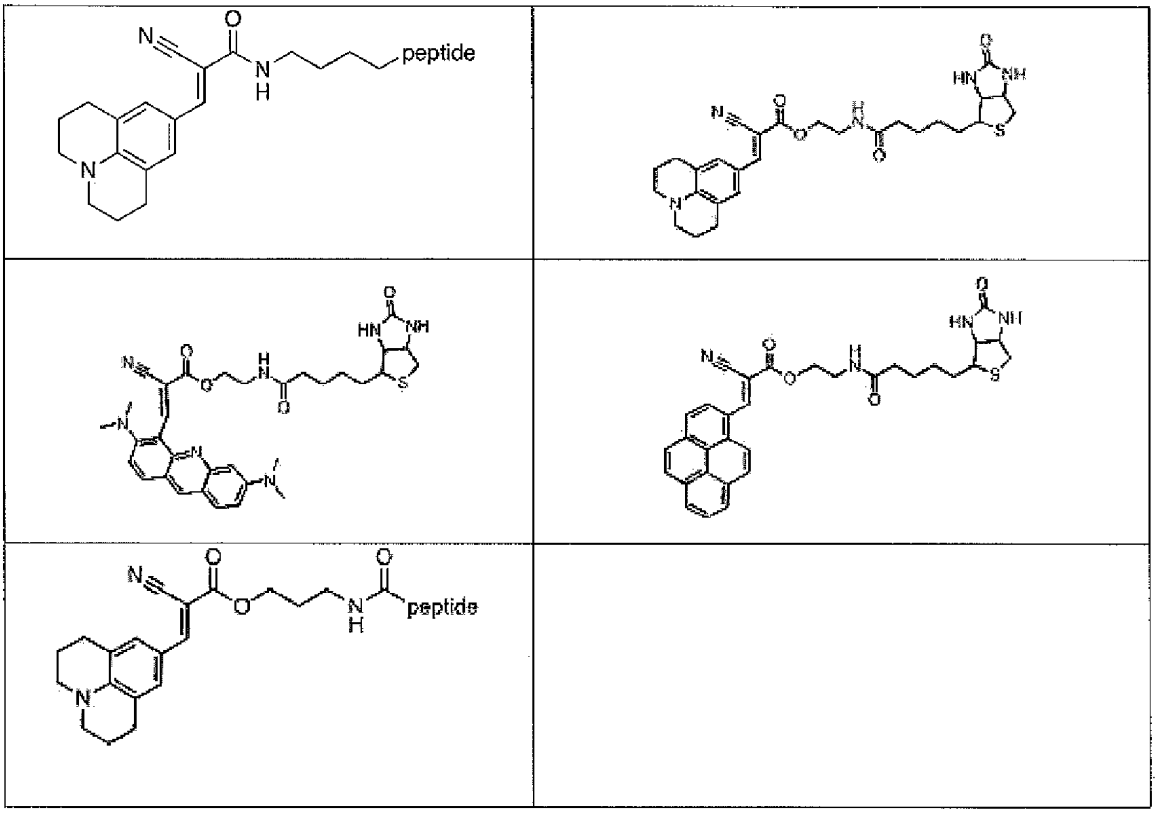
11. The method of claim 1 to 5 and 8 to 10, wherein the peptide of (b) is derived from p53 protein.

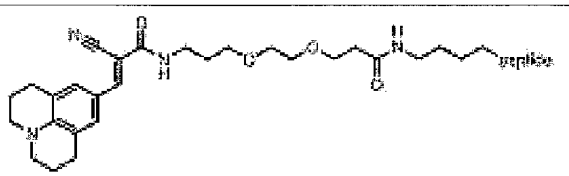
12. The method of any one of claims 1 to 11 and 8 to 11, wherein the peptide of (b) is selected from the group consisting of:

JP1: MPRFMDYWEGLSK; and

JP2: MPRFMDYWEGLNK.

13. The method of any one of claims 4 to 12, wherein the fluorescent molecular rotor bound to the ligand is selected from the group consisting of:



14. A composition comprising:

a) fluorescent molecular rotor, DNA and p53 protein;

b) fluorescent molecular rotor, peptide and MDM2 protein; or

c) fluorescent molecular rotor, biotin and streptavidin,

wherein said fluorescent molecular rotor is bound to one of DNA or p53 protein in (a), one of peptide or MDM2 protein in (b), or one of biotin or streptavidin in (c),

and wherein the fluorescent molecular rotor(s) comprises:

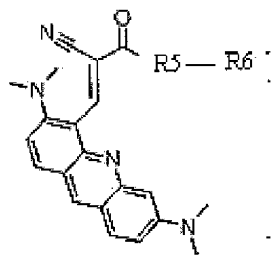
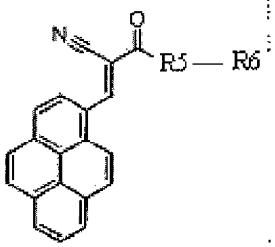
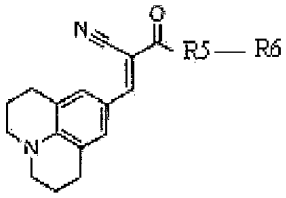
a rotating σ-bond;

an electron-donating moiety;

an electron-accepting moiety; and

a π-conjugated linker, wherein the fluorescent molecular rotor(s) is bound to DNA of (a), peptide of (b), or biotin of (c), via a linking moiety, or the p53 protein of (a), MDM2 protein of (b), or streptavidin of (c), via a linking moiety,

wherein said linking moiety is selected from the group consisting of a single bond; or optionally substituted heteroalkyl, wherein the main chain atoms of said optionally substituted heteroalkyl are optionally interrupted by one or more optionally substituted cyclic groups, wherein the bound fluorescent molecular rotor is selected from the group:

 (i)	 (ii)
 (v)	

R5 is the linking moiety; and

R6 is absent, the DNA, the p53 protein, the peptide, the MDM2 protein, the biotin or the streptavidin.

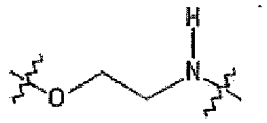
15. The composition of claim 14, wherein the fluorescent molecular rotor(s) exhibits a change in fluorescence when the DNA of (a), peptide of (b), or biotin of (c), binds to the p53

protein of (a), MDM2 protein of (b), or streptavidin of (c), respectively.

16. The composition of any one of claims 14 or 15, wherein a change in fluorescence occurs when the binding of protein to DNA, MDM2 protein to peptide, or biotin to streptavidin, displaces the fluorescent molecular rotor(s) from DNA or perturbs the fluorescent molecular rotor(s).

17. The composition of any one of claims 14 to 16, wherein the linking moiety comprises 1 to 20 main chain atoms.

18. The composition of any one of claims 14 to 17, wherein the linking moiety is selected from the group consisting of: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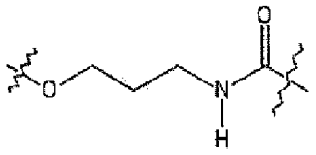
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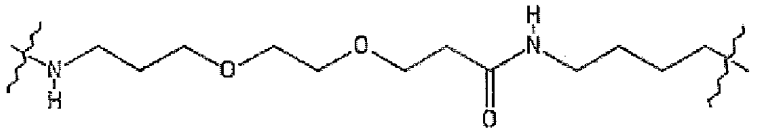
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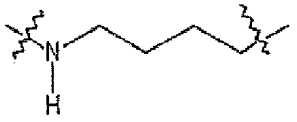

and .

19. The composition of any one of claims 14 to 18, wherein the protein of (a) is a DNA transacting protein.

20. The composition of claim 19, wherein the DNA transacting protein is a transcription factor, polymerase, telomerase or recombinase.

21. The composition of any one of claims 14 to 18, wherein the MDM2 protein of (b) is a wild-type or mutant MDM2 protein.

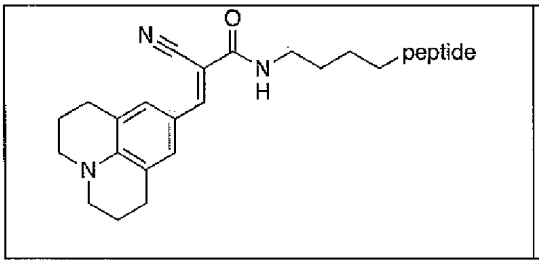
22. The composition of any one of claims 14 to 18 and 21, wherein the MDM2 protein is wild-type or mutant MDM2 protein.

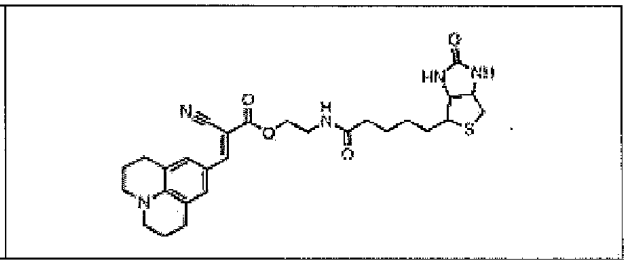
23. The composition of any one of claims 14 to 18 and 21 to 22, wherein the peptide of (b) has an amino acid sequence identical to the sequence of a native peptide that binds to the protein, or a portion thereof.

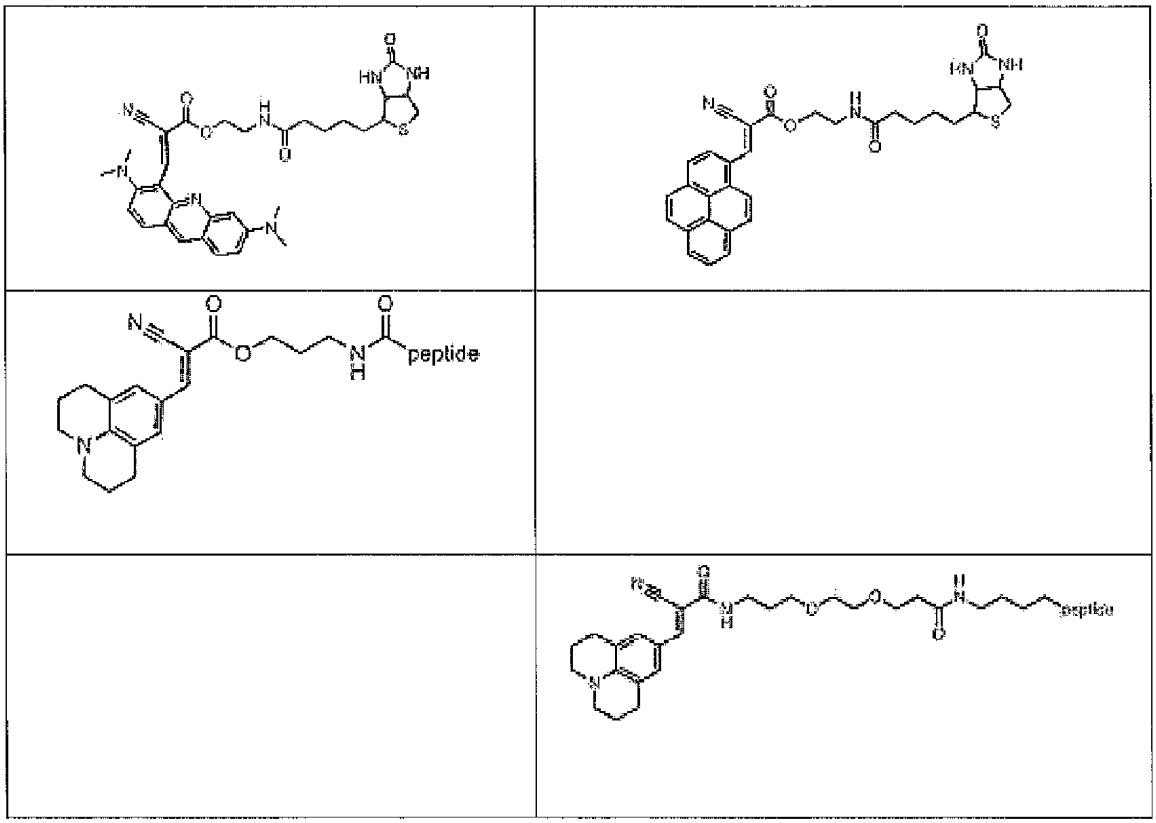
24. The composition of any one of claims 14 to 18 and 21 to 23, wherein the peptide of (b) is derived from p53 protein.

25. The composition of any one of claims 14 to 18 or 21 to 24, wherein the peptide of (b) is selected from the group consisting of:
JP1: MPRFMDYWEGLSK; and
JP2: MPRFMDYWEGLNK.

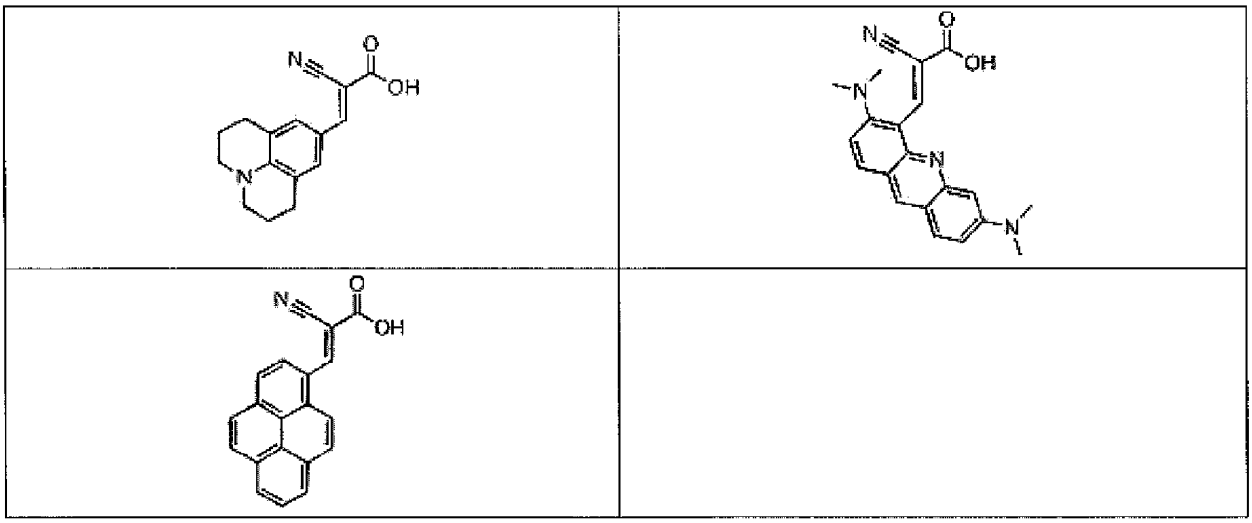
26. The composition of any one of claims 14 to 25, wherein the fluorescent molecular rotor bound to the ligand is selected from the group consisting of:







27. The composition of claim 14 comprising a fluorescent molecular rotor, DNA and protein of (a), said fluorescent molecular rotor bound to DNA, wherein said protein is p53 protein and said fluorescent molecular rotor is selected from the group consisting of:



28. A screening assay for identifying an interaction between DNA and p 53 protein, or peptide and MDM2 protein, the assay comprising:

a) providing a p53 protein or MDM2 protein to be tested for binding affinity with candidate DNA or peptide;

b) providing candidate DNA or peptide bound to at least one fluorescent molecular rotor;

c) testing the candidate DNA or peptide for binding affinity by contacting said p53 protein or MDM2 protein with said candidate DNA or peptide bound to at least one fluorescent molecular rotor; and

d) identifying a relevant interactions by measuring a change in fluorescence emitted by the fluorescent molecular rotor(s),

wherein the fluorescent molecular rotor(s) comprises:

a rotating σ-bond;

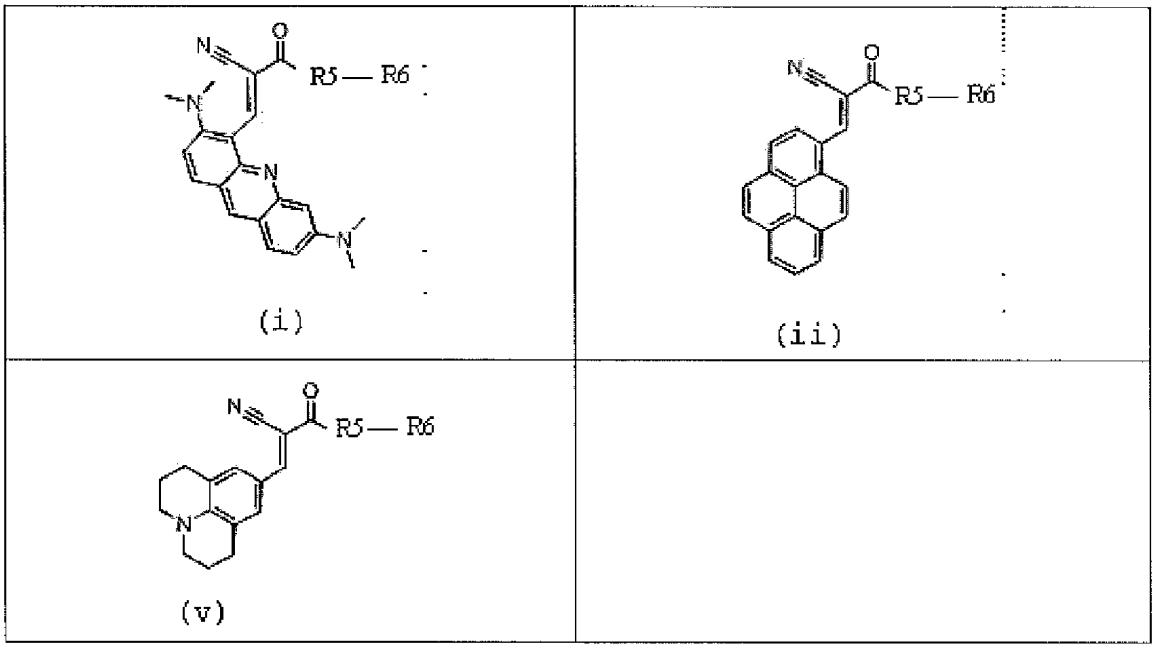
an electron-donating moiety;

an electron-accepting moiety; and

a π-conjugated linker, wherein the fluorescent molecular rotor is bound to the candidate DNA or peptide via a linking moiety, wherein said linking moiety is selected from the group consisting of a single bond; or optionally substituted

heteroalkyl, wherein the main chain atoms of said optionally substituted heteroalkyl are optionally interrupted by one or more optionally substituted cyclic groups,

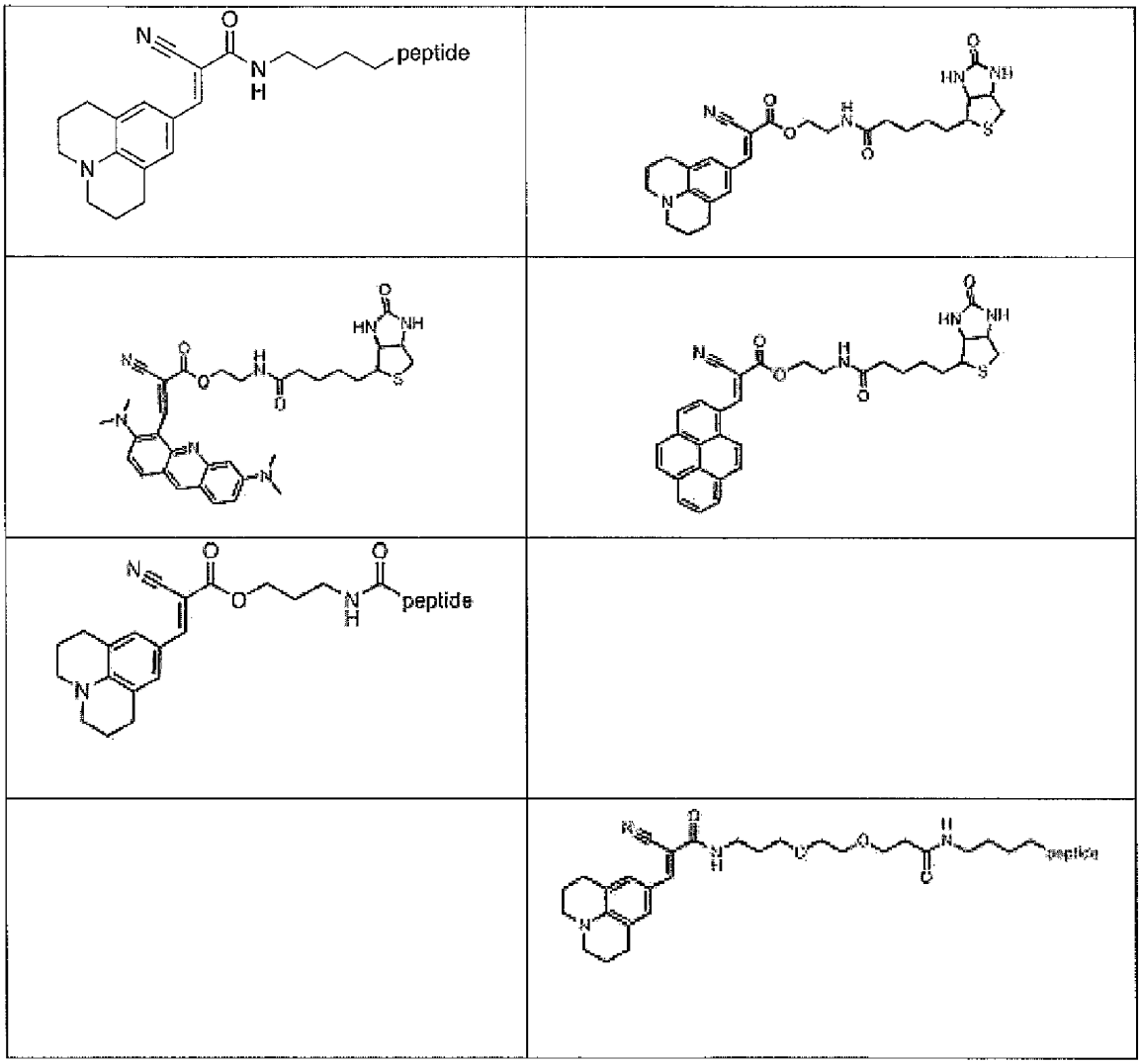
wherein the bound fluorescent molecular rotor is selected from the group consisting of:



R5 is the linking moiety; and

R6 is absent, the DNA, the p 53 protein, the peptide or the MDM2 protein.

29. The screening assay of claim 28, wherein the fluorescent molecular rotor bound to the ligand is selected from the group consisting of:



30. A screening assay for identifying a candidate compound, comprising:

(a) providing a candidate compound to be tested for binding affinity with MDM2 protein;

(b) providing a complex comprising a probe bound to protein, wherein said probe comprises a peptide selected from the group consisting of JP1: MPRFMDYWEGLSK and JP2: MPRFMDYWEGLNK and at least one fluorescent molecular rotor bound to peptide;

(c) testing the candidate compound for binding affinity with said MDM2 protein by contacting said candidate compound with said complex; and

(d) identifying a relevant candidate compound by measuring the change in fluorescence emitted when said complex is disrupted, wherein the fluorescent molecular rotor(s) comprises:

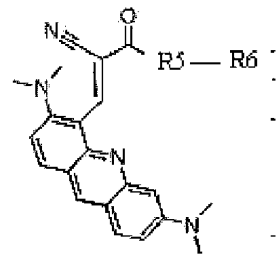
a rotating σ-bond;

an electron-donating moiety;

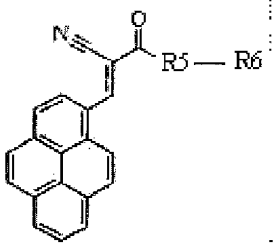
an electron-accepting moiety; and

a π-conjugated linker, wherein the fluorescent molecular rotor is bound to the peptide via a linking moiety, wherein said linking moiety is selected from the group consisting of a single bond; or optionally substituted heteroalkyl, wherein the main chain atoms of said optionally substituted heteroalkyl are optionally interrupted by one or more optionally substituted cyclic groups,

wherein the bound fluorescent molecular rotor is selected from the group consisting of:



(i)



(ii)

R5 is the linking moiety; and

R6 is absent or the candidate compound or the MDM2 protein.

31. The screening assay of claim 30, wherein a change in fluorescence occurs when the probe is displaced from the complex.

32. A kit for carrying out a method of any one of claims 1 to 13 or the screening assay of claims 38 to 31 comprising:

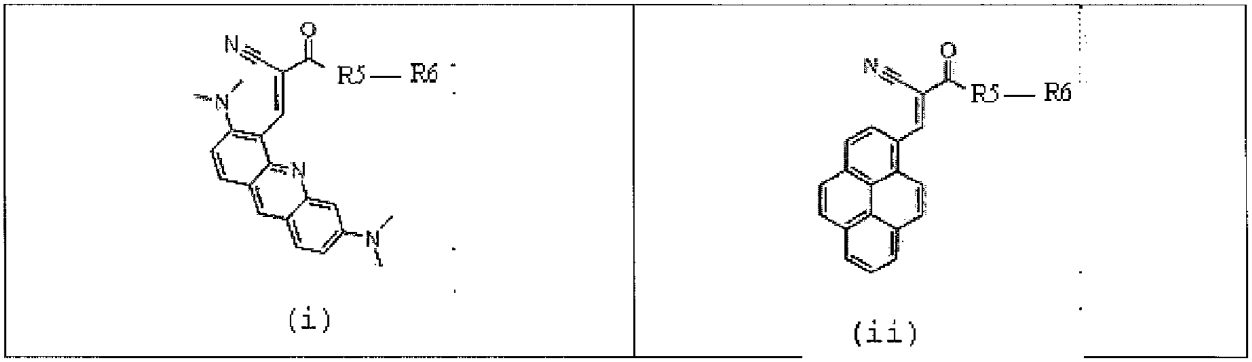
(i) fluorescent molecular rotor, DNA and protein;

(ii) fluorescent molecular rotor, peptide and MDM2 protein; or

(iii) fluorescent molecular rotor, biotin and streptavidin,

as defined in any one of claims 1 to 13 or 28 to 31, and a means to detect a change in fluorescence.

33. A chemical compound selected from the group consisting of:


N
O
R5 — R6
N
N
(i)
N
O
R5 — R6
(ii)

R5 is a linking moiety; and

R6 is absent or a ligand.

34. A chemical compound of claim 33, selected from the group consisting of: