interactions which lessens the requirement for multiple washing steps.

[0012] Therefore, there is a need for methods for detecting interactions between a protein and ligands that ameliorate the above problems. The present invention seeks to fulfill these needs and provides further related advantages.

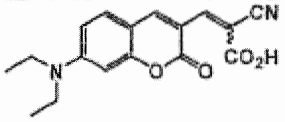
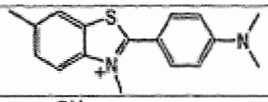
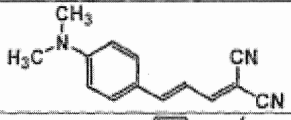
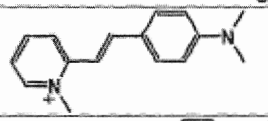
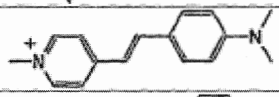
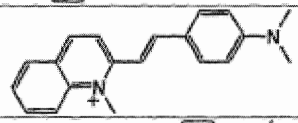
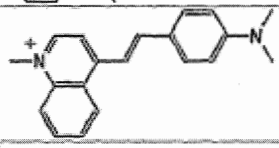
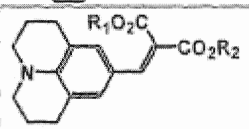
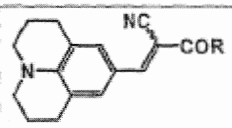
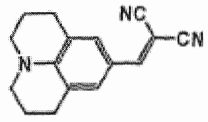
SUMMARY

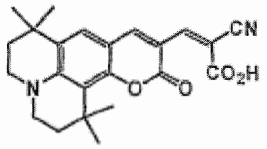
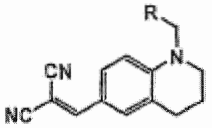
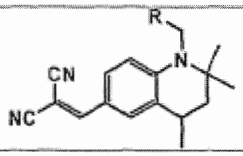
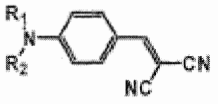
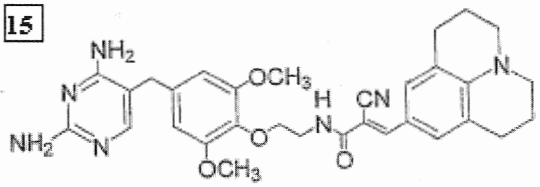
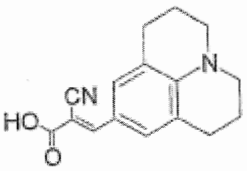
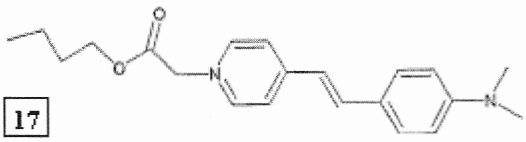
[0013] According to the present disclosure~~a first aspect~~, there is provided a method for detecting an interaction between a protein and a ligand, comprising:

(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.

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		

[0013a] In a first aspect, there is 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 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 ligand or protein 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,

with the proviso that the fluorescent molecular rotor is not a compound selected from the following table:

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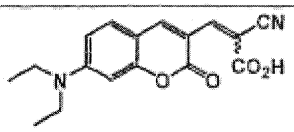
1		
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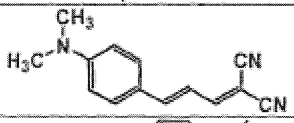
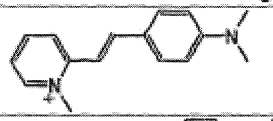
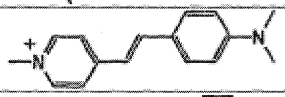
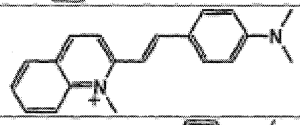
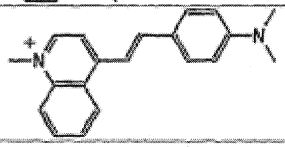
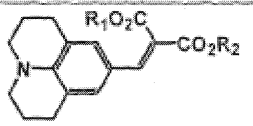
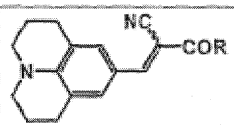
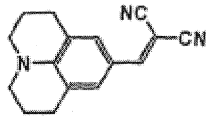
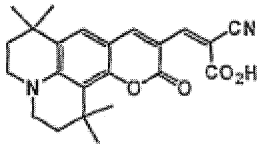
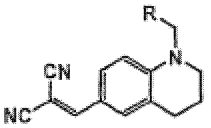
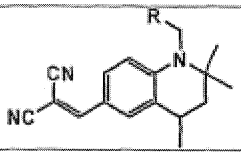
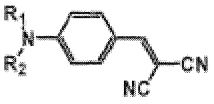
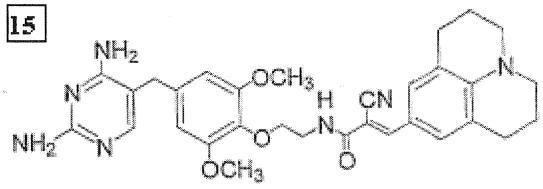
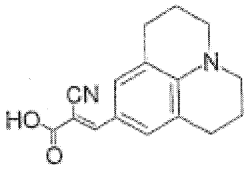
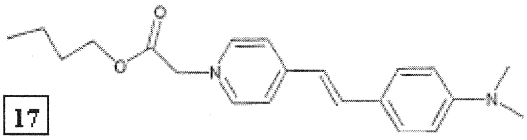
3		
4		
5		
6		
7		
8		
9		R=OH, OEt, NH_2
10		

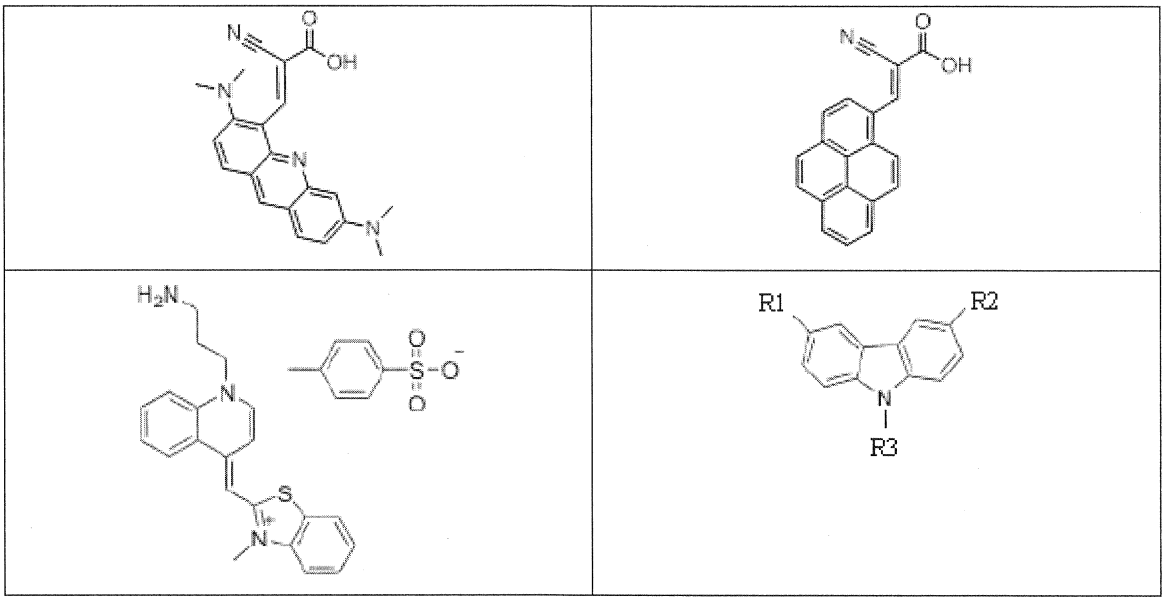
	Structure	R groups
11		
12		R=COOH, CO_2Et, CH_2OH, CH_2OAc, CH_2Cl
13		R=CH_2CO_2Ph
14		R_1, R_2 = CH_3
15		
16		
17		

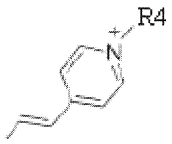
[0014] ~~In a second aspect, t~~There is also provided a method for detecting an interaction between a protein and a ligand, comprising:

(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.

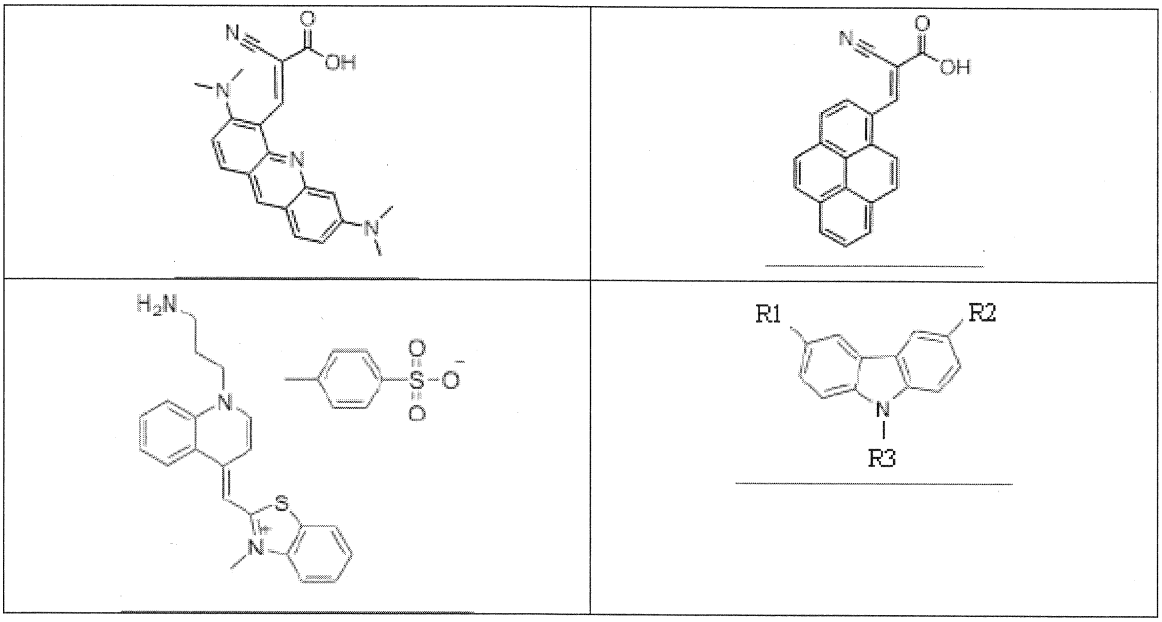
[0014a] In a second aspect, there is provided a method for detecting an interaction between a protein and a ligand, comprising:

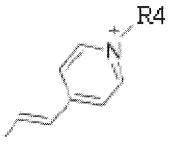
(i) binding at least one fluorescent molecular rotor to said ligand or protein; and

(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 bound to the ligand or protein 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 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; wherein at least one of R1, R2, R3 and R4 are independently chosen 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.

[0015] ~~In a third aspect, t~~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

(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:

a rotating σ-bond;

an electron-donating moiety;

an electron-accepting moiety; and

a π-conjugated linker.

[0015a] In a third 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

(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.

[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.

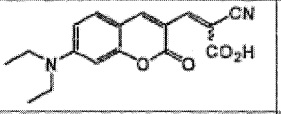
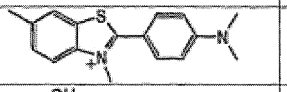
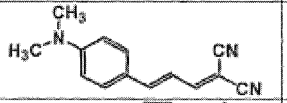
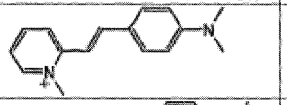
[0018] Advantageously, the fluorescent molecular rotors may be used to investigate protein-DNA interactions. The fluorescent molecular rotors may be engineered such that the intercalating moiety of the rotor encompasses the entire base pair region of DNA, so that the rotating bond will be protruding 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] ~~In a fourth aspect, t~~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		
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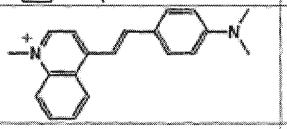
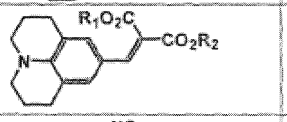
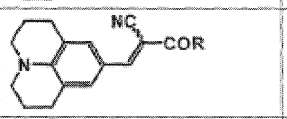
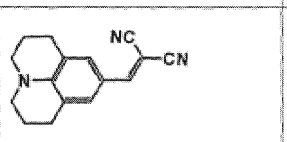
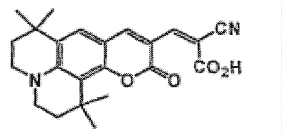
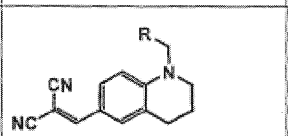
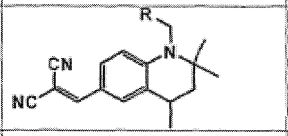
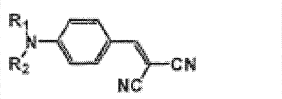
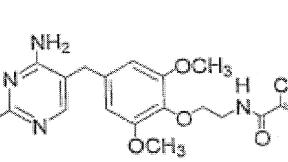
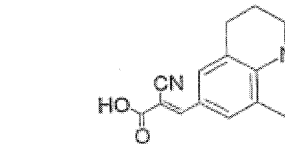
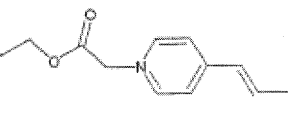
7		
8		
9		R=OH, OEt, NH_2
10		
11		
12		R=COOH, CO_2Et, CH_2OH, CH_2OAc, CH_2Cl
13		R=CH_2CO_2Ph
14		R_1, R_2 = CH_3
15		
16		
17		

[0021a] In a fourth aspect, there is provided a composition comprising a fluorescent molecular rotor, a ligand and a protein, wherein at least one fluorescent

molecular rotor is bound to the ligand or the protein, wherein the fluorescent molecular rotor comprises:

a rotating σ-bond;

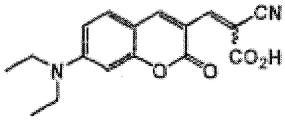
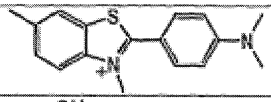
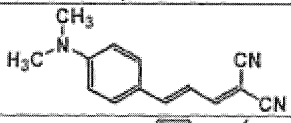
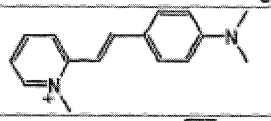
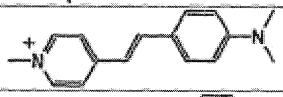
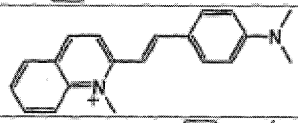
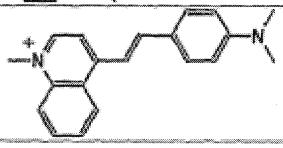
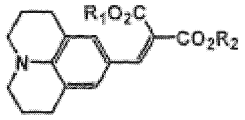
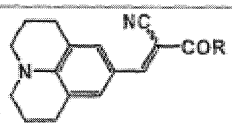
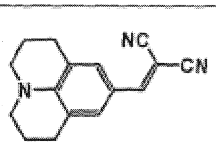
an electron-donating moiety;

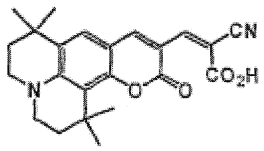
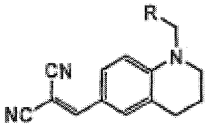
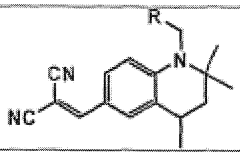
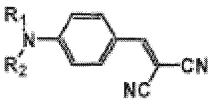
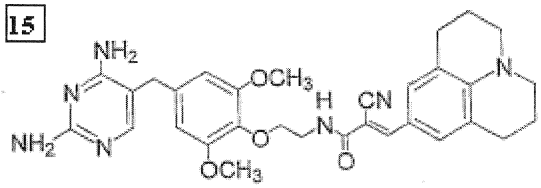
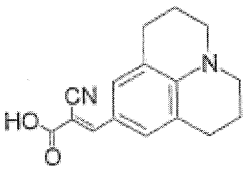
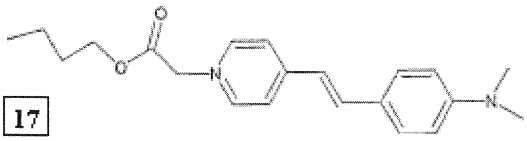
an electron-accepting moiety; and

a π-conjugated linker,

wherein the fluorescent molecular rotor is bound to the ligand or protein 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,

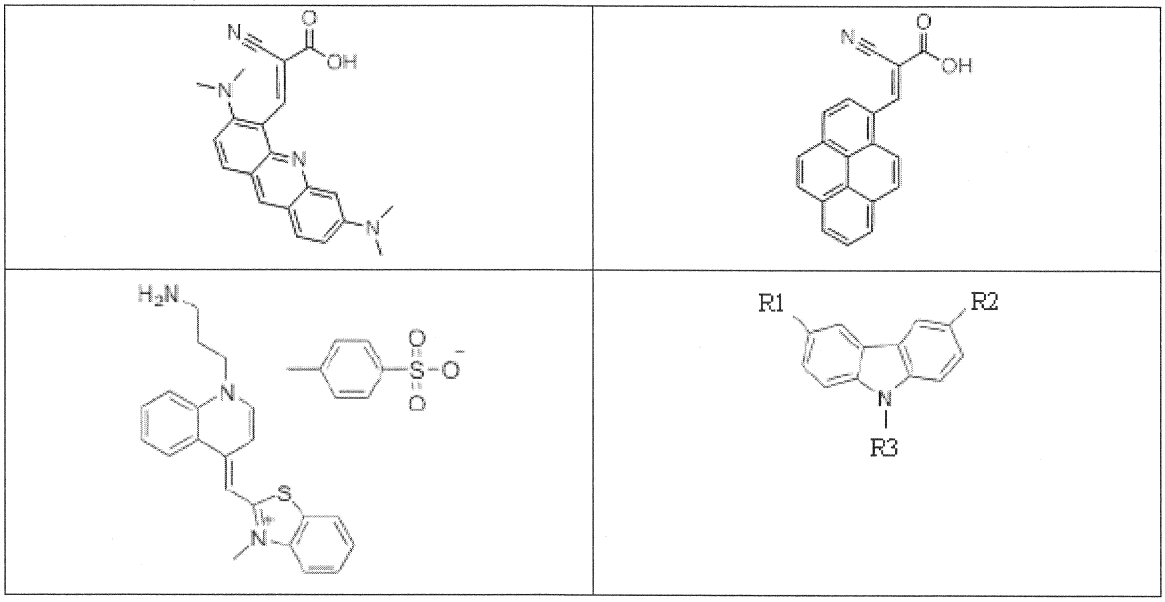
with the proviso that the fluorescent molecular rotor is not a compound selected from the following table:

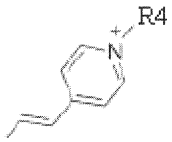
	Structure	R groups
1		
2		
3		
4		
5		
6		
7		
8		
9		R=OH, OEt, NH_2
10		

	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] ~~In a fifth aspect, t~~There is also provided a composition comprising a fluorescent molecular rotor,

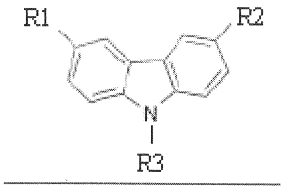
wherein the fluorescent molecular rotor is selected from the group consisting of:

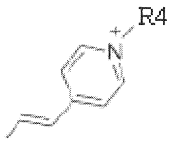


wherein at least one of R1, R2 or R3 is  ; and R1, R2, R3 and R4 are independently chosen from the group consisting of: optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted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

[0022a] In a fifth aspect, there is provided a composition comprising a fluorescent molecular rotor, a ligand and a protein, wherein at least one fluorescent molecular rotor is bound to the ligand or the protein 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 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; wherein at least one of R1, R2, R3 and R4 are independently chosen 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.

[0023] ~~In a sixth aspect, t~~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.

[0023a] In a sixth 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.

[0024] ~~In a seventh aspect, t~~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;

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

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

identifying relevant candidate ligands by measuring a change in fluorescence emitted by the fluorescent molecular rotor(s), with the proviso that the fluorescent molecular rotor is not a compound selected from the following table:

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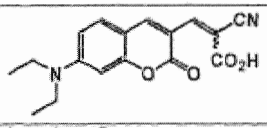
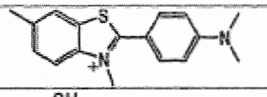
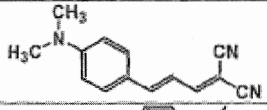
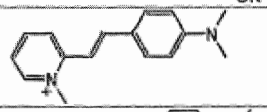
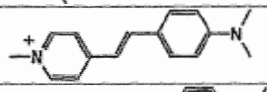
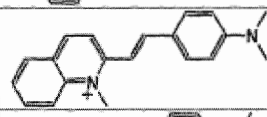
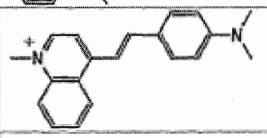
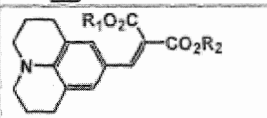
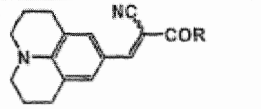
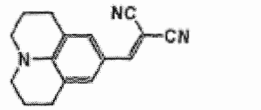
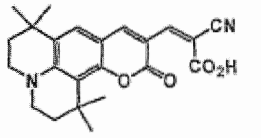
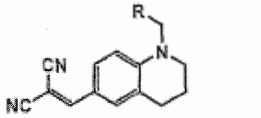
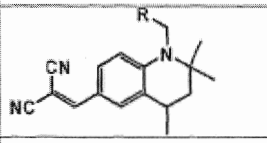
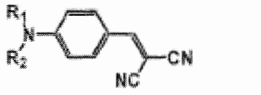
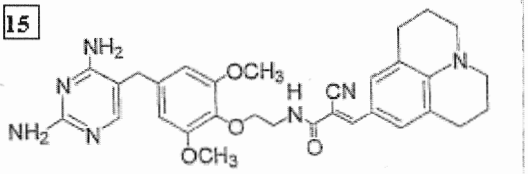
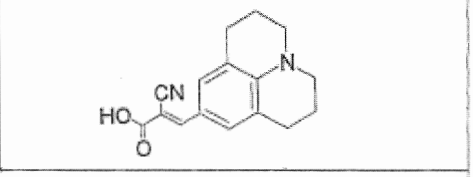
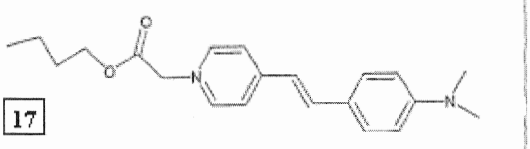
1		
2		
3		
4		
5		
6		
7		
8		
9		R=OH, OEt, NH_2
10		

	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		

[0024a] In a seventh aspect, there is provided a screening assay for identifying an interaction between protein and ligand, the assay comprising:

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

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

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

(iv) 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;

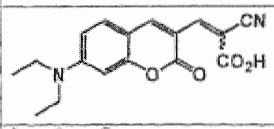
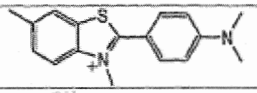
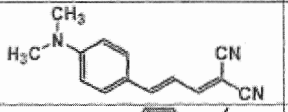
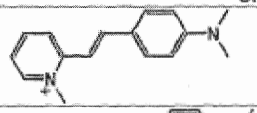
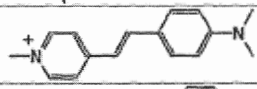
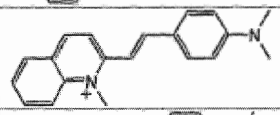
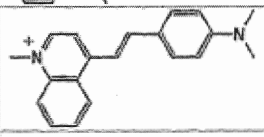
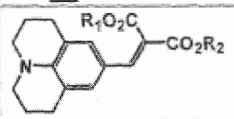
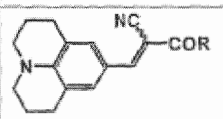
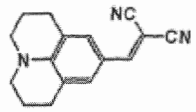
an electron-donating moiety;

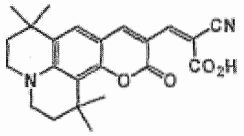
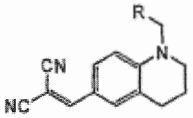
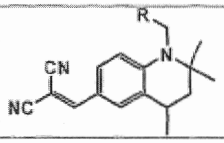
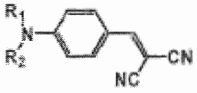
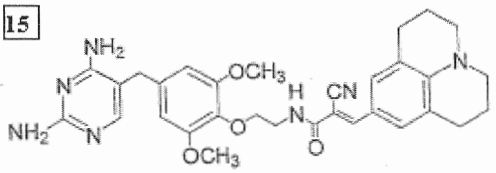
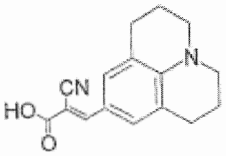
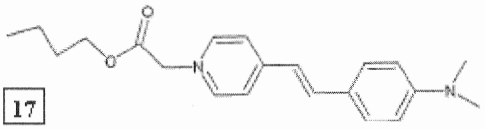
an electron-accepting moiety; and

a π-conjugated linker,

wherein the fluorescent molecular rotor is bound to the ligand or protein 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,

with the proviso that the fluorescent molecular rotor(s) is not a compound selected 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		

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

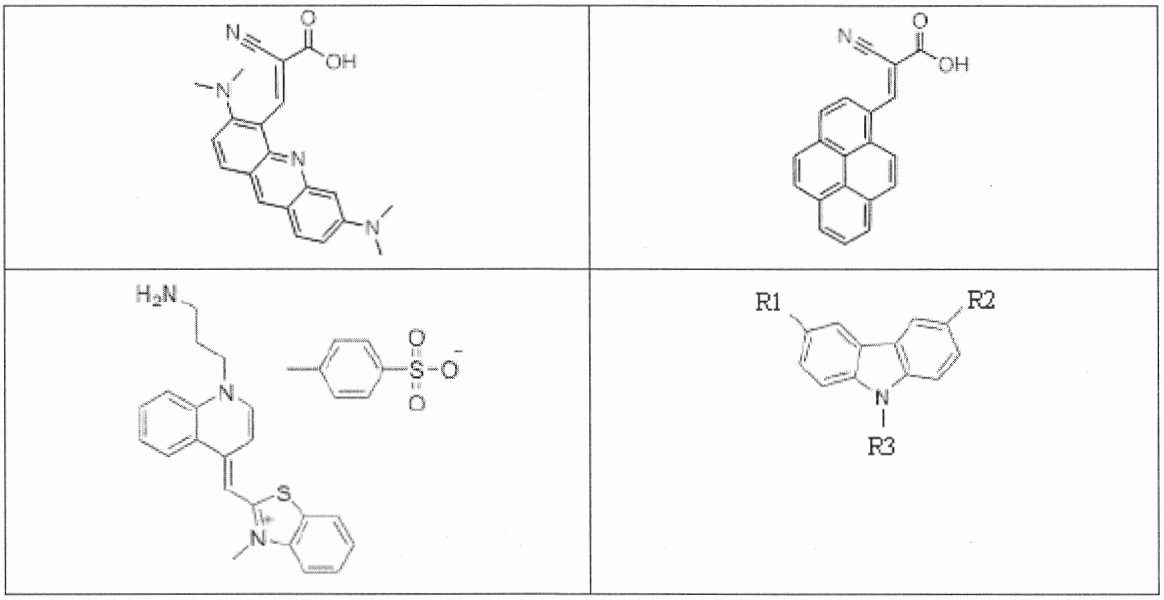
a) providing a protein to be tested for binding affinity with a candidate ligand;

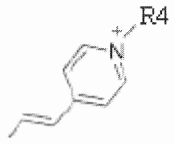
b) providing candidate ligands bound to at least one fluorescent molecular rotor;

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.

[0025a] In an eighth aspect, there is 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;

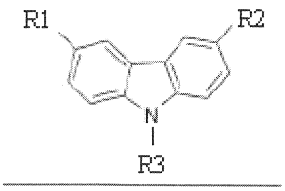
c) testing the candidate ligands for binding affinity by contacting said protein with said

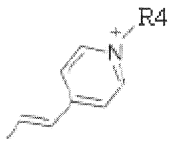
candidate ligands bound to at least one fluorescent molecular rotor; and

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

wherein the fluorescent molecular rotor is bound to the ligand or the protein 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 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; wherein at least one of R1, R2, R3 and R4 are independently chosen 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.

[0026] ~~In a ninth aspect, t~~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.

[0026a] In a ninth aspect, there is provided a screening assay for identifying an interaction between DNA and protein, or peptide and MDM2 protein, 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., 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.

[0027] ~~In a tenth aspect, t~~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.

[0027a] In a tenth 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 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, 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.

[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.

[0032a] In an eleventh aspect, there is provided a kit for carrying out a method disclosed herein, or a screening assay disclosed herein, comprising the ligand, protein and fluorescent molecular rotor(s) as defined herein, and a means to detect a change in fluorescence.

[0033] ~~In an eleventh aspect, t~~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.

[0033a] In a twelvth aspect, there is provided a kit for carrying out a method disclosed herein, or the screening assay disclosed herein, 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 herein, and a means to detect a change in fluorescence.

[0034] ~~In a twelfth aspect, t~~There is further provided a kit for carrying out the second or seventh aspects of

the invention comprising the ligand, protein and fluorescent molecular rotor(s), and a means to detect a change in fluorescence.

[0035] ~~In a thirteenth aspect, t~~There is also provided a kit for carrying out the third or eighth aspects of the invention comprising the ligand, protein and fluorescent molecular rotor(s), and a means to detect a change in fluorescence.

[0036] ~~In a fourteenth aspect, t~~There is also provided a chemical compound selected from the group consisting of:

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

[0036a] In a thirteenth aspect, there is provided a chemical compound selected from the group consisting of:

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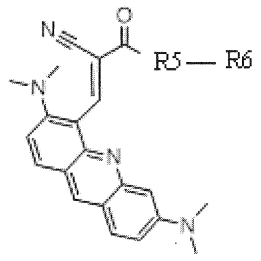
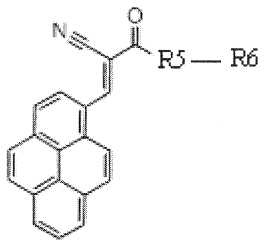
<tr><td>
(i)</td><td>
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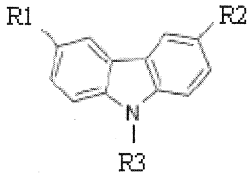
<tr><td></td><td>
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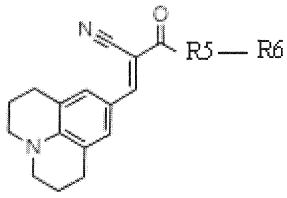
<tr><td>
(v)</td><td></td></tr>
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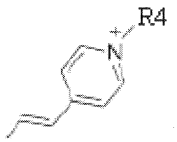
wherein at least one of R1, R2 or R3 is  ;

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; wherein at least one of R1, R2, R3 and R4 are independently chosen 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;

R5 is a OH or a linking moiety 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; and

R6 is absent or a ligand,

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

DEFINITIONS

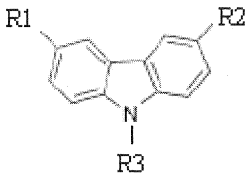
[0037] Unless otherwise defined herein, scientific and technical terms used in this application shall have the meanings that are commonly understood by those of ordinary skill in the art. Generally, nomenclature used in connection with, and techniques of, chemistry, cell and tissue culture, molecular 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.

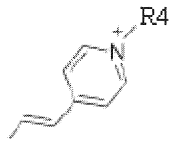
[0038] The following are some definitions that may be helpful in understanding the description of the present invention. These are intended as general definitions and should in no way limit the scope of the present invention to those terms alone, but are put forth for a better understanding of the following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

or elements.

[0040] As used herein, unless otherwise specified, the following terms have the following meanings, and unless otherwise specified, the definitions of each term (i.e. moiety or substitutent) apply when that term is used individually or as a component of another term (e.g., the definition of aryl is the same for aryl and for the aryl portion of arylalkyl, alkylaryl, arylalkynyl, and the like).





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; wherein at least one of R1, R2, R3 and R4 are independently chosen 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.

68. A kit for carrying out a method of any one of claims 37 to 51 or the screening assay claim 67 comprising the ligand, protein and fluorescent molecular rotor(s) as defined in any one of claims 37 to 51, or 67 , and a means to detect a change in fluorescence.

69. 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,

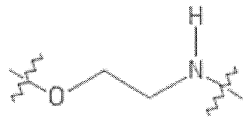
wherein said linking moiety is selected from the group consisting of a single bond; or optionally substituted heteroalkyl, wherein the main chain atoms of said optionally substituted heteroalkyl are optionally interrupted by one or more optionally substituted cyclic groups.

70. The method of claim 69, 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.

71. The method of any one of claims 69 or 70, 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).

72. The method of any one of claims 69 to 71, wherein the linking moiety comprises 1 to 20 main chain atoms.

73. The method of any one of claims 69 to 72 wherein the linking moiety is selected from the group consisting of: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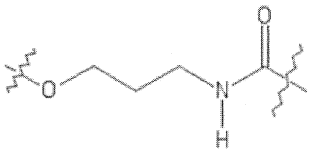
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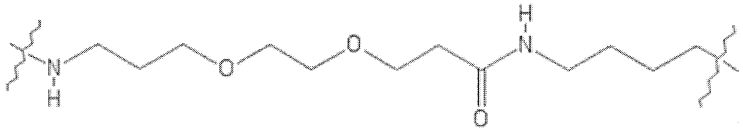
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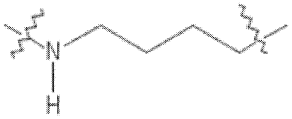

and .

74. The method of any one of claims 69 to 73, wherein the protein of (a) is a DNA transacting protein.

75. The method of claim 74, wherein the DNA transacting protein is a transcription factor, polymerase, telomerase or recombinase.

~~76. The method of claims 69 to 75, wherein the protein is p53 protein.~~

~~77.~~76. The method of any one of claims 69 to 73, wherein the MDM2 protein of (b) is wild-type or mutant MDM2 protein.

~~78.~~77. The method of claim ~~77~~76, wherein the protein is wild-type or mutant MDM2.

~~79.~~78. The method of any one of claim 69 to 73 and ~~77~~76 to ~~78~~77, 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.

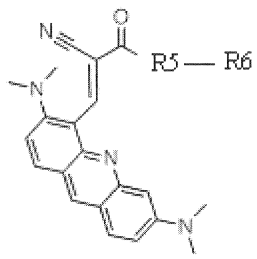
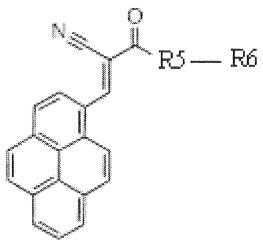
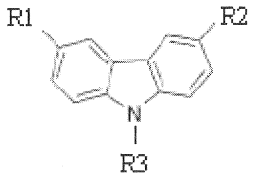
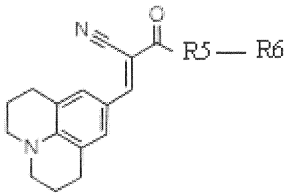
~~80.~~79. The method of claim 69 to 73 and ~~77~~76 to ~~79~~78, wherein the peptide of (b) is derived from p53 protein.

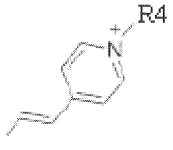
~~81.~~80. The method of any one of claims 69 to 73 and ~~77~~76 to ~~80~~79, wherein the peptide of (b) is selected from the group consisting of:

JP1: MPRFMDYWEGLSK; and

JP2: MPRFMDYWEGLNK.

~~82.~~81. The method of any one of claims 69 to ~~82~~80, wherein the unbound or bound fluorescent molecular rotor is selected from the group consisting of:

 (i)	 (ii)
	 (iv)
 (v)	

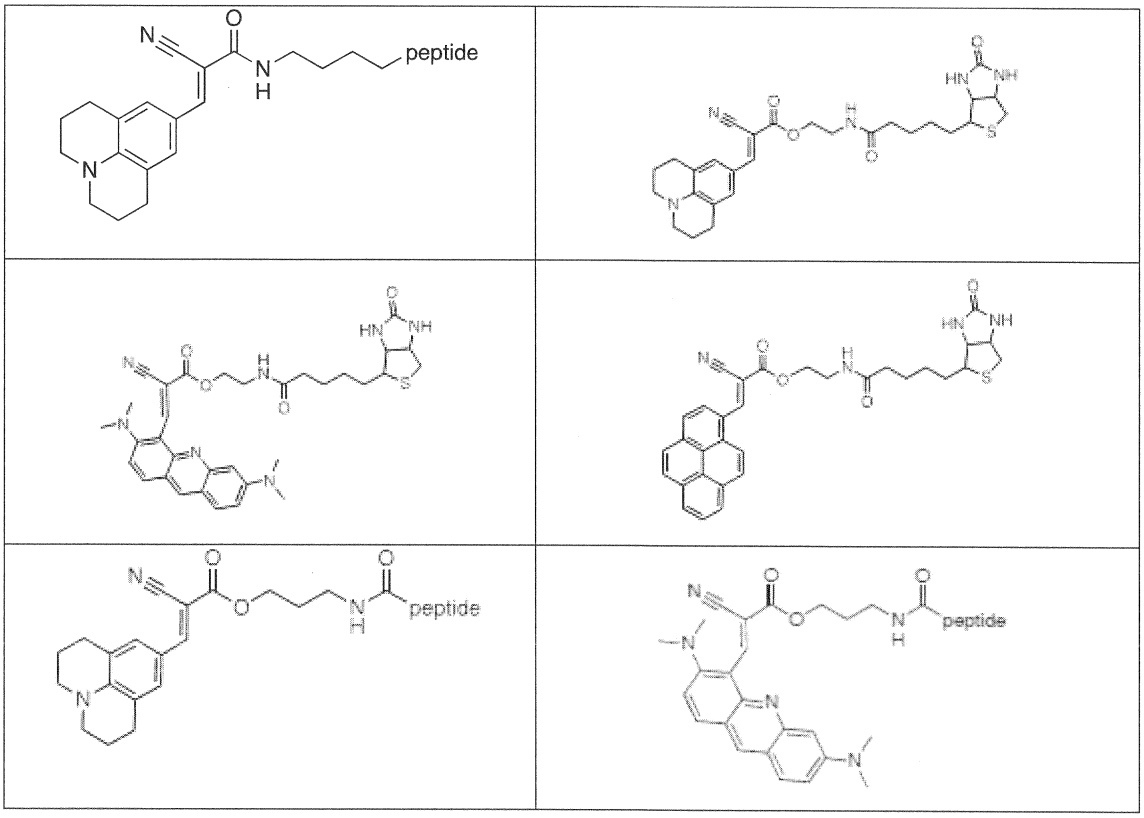
wherein at least one of R1, R2 or R3 is  ;

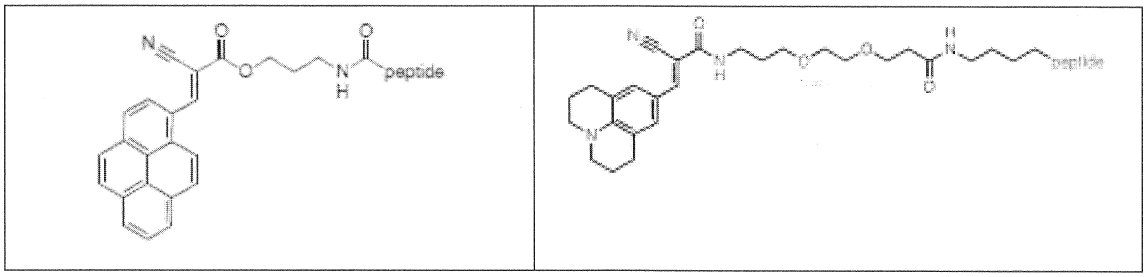
R1, R2, R3 and R4 are independently chosen from the group consisting of: optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted cycloalkyl or optionally substituted heterocyclyl;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

R5 is a OH or a linking moiety as defined in any one of claims 69, 72 or 73; and

R6 is absent or a peptide.

~~83.~~82. The method of claim ~~82~~81, wherein the unbound or bound fluorescent molecular rotor is selected from the group consisting of:





~~84.~~83. 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.

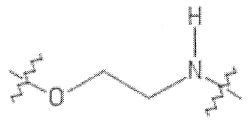
~~85.~~84. The composition of claim ~~84~~83, wherein the fluorescent molecular rotor(s) exhibits a change in fluorescence when the DNA of (a), peptide of (b), or biotin of (c), binds to the p53

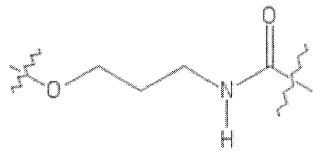
protein of (a), MDM2 protein of (b), or streptavidin of (c), respectively.

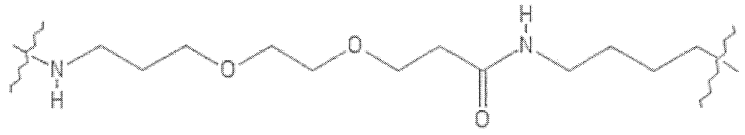
~~86.~~85. The composition of any one of claims ~~84~~—83 or ~~85~~84, wherein a change in fluorescence occurs when the binding of protein to DNA, MDM2 protein to peptide, or biotin to streptavidin, displaces the fluorescent molecular rotor(s) from DNA or perturbs the fluorescent molecular rotor(s).

~~87.~~86. The composition of any one of claims ~~84~~—83 to ~~86~~85, wherein the linking moiety comprises 1 to 20 main chain atoms.

~~88.~~87. The composition of any one of claims ~~84~~—83 to ~~87~~86, wherein the linking moiety is selected from the group consisting of:


;


;


;


and .

~~89.~~88. The composition of any one of claims ~~84~~—83 to ~~88~~87, wherein the protein of (a) is a DNA transacting protein.

~~90.~~89. The composition of claim ~~89~~88, wherein the DNA transacting protein is a transcription factor, polymerase, telomerase or recombinase.

~~91.~~90. The composition of any one of claims ~~84~~—83 to ~~88~~87, wherein the MDM2 protein of (b) is a wild-type or mutant MDM2 protein.

~~92.~~91. The composition of any one of claims ~~84~~83 to ~~88~~87 and ~~91~~90, wherein the MDM2 protein is wild-type or mutant MDM2 protein.

~~93.~~92. The composition of any one of claims ~~84~~83 to ~~88~~87 and ~~91~~90 to ~~92~~91, 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.

~~94.~~93. The composition of any one of claims ~~84~~83 to ~~88~~87 and ~~91~~90 to ~~93~~92, wherein the peptide of (b) is derived from p53 protein.

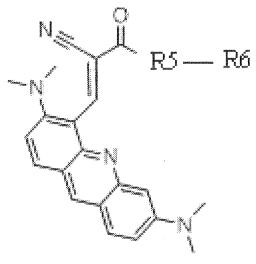
~~95.~~94. The composition of any one of claims ~~84~~83 to ~~88~~87 or ~~91~~90 to ~~94~~93, wherein the peptide of (b) is derived from p53 protein.

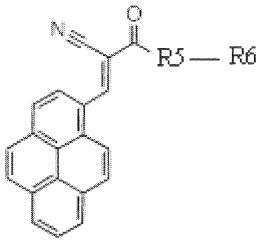
~~96.~~95. The composition of any one of claims ~~84~~83 to ~~88~~87 or ~~91~~90 to ~~95~~94, wherein the peptide of (b) is selected from the group consisting of:

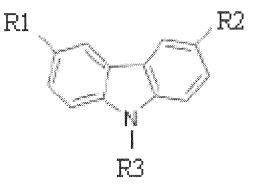
JP1: MPRFMDYWEGLSK; and

JP2: MPRFMDYWEGLNK.

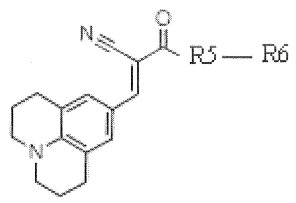
~~97.~~96. A composition of any one of claims ~~84~~83 to ~~96~~95, wherein the unbound or bound fluorescent molecular rotor is selected from the group:

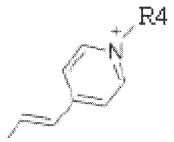
<table>
<tr>
<td>

(i)</td>
<td>

(ii)</td>
</tr>
<tr>
<td></td>
<td>

(iv)</td>
</tr>
</table>

 (v)	



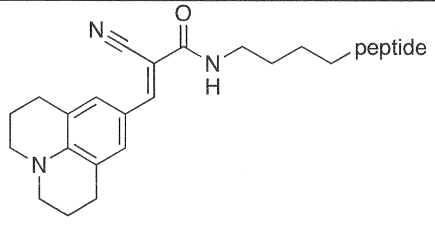
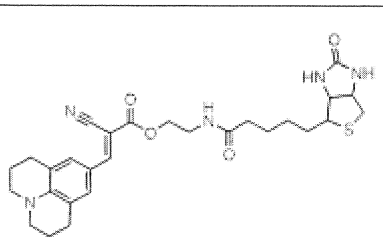
wherein at least one of R1, R2 or R3 is ;

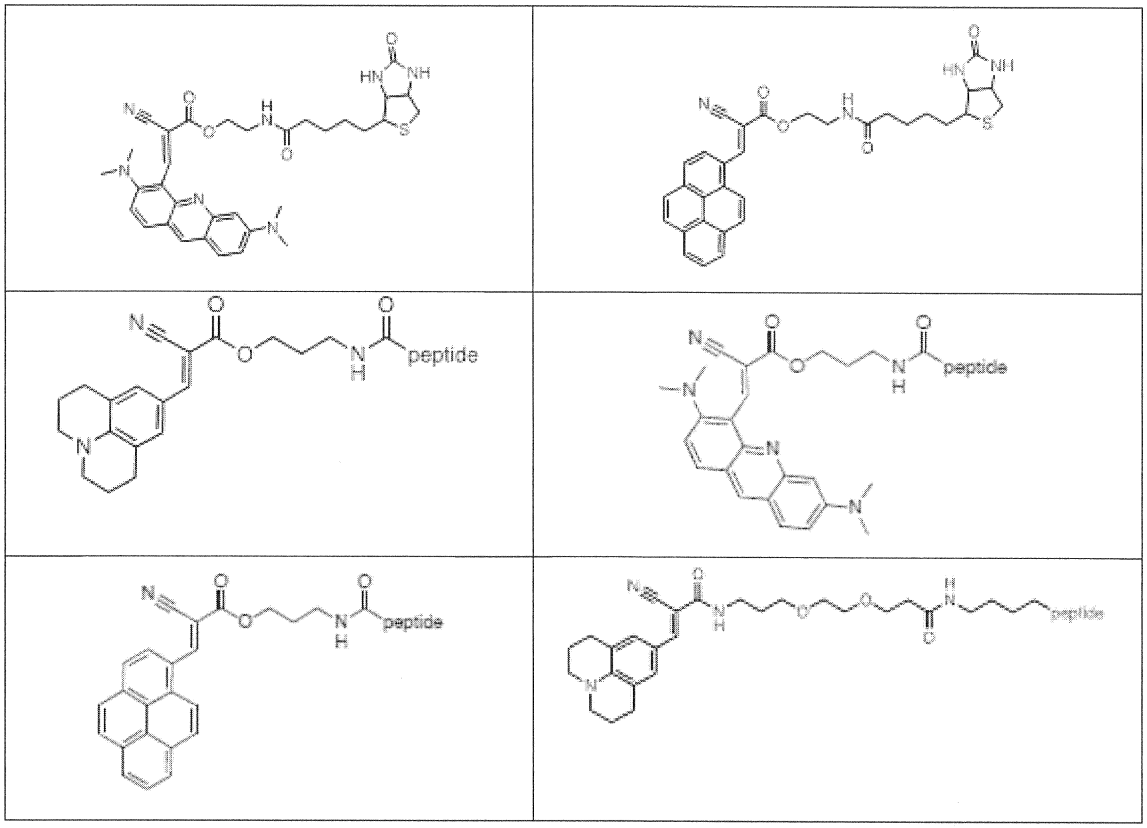
R1, R2, R3 and R4 are independently chosen from the group consisting of: optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted cycloalkyl or optionally substituted heterocyclyl;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

R5 is a OH or a linking moiety as defined in any one of claims 69, 72 or 73; and

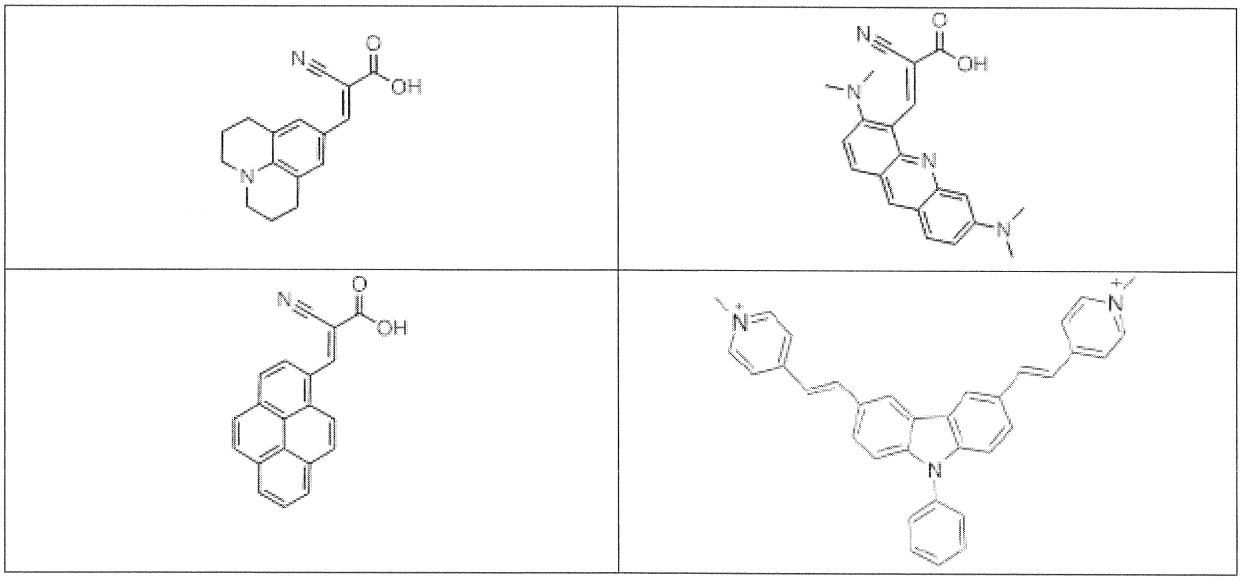
R6 is absent or a peptide.

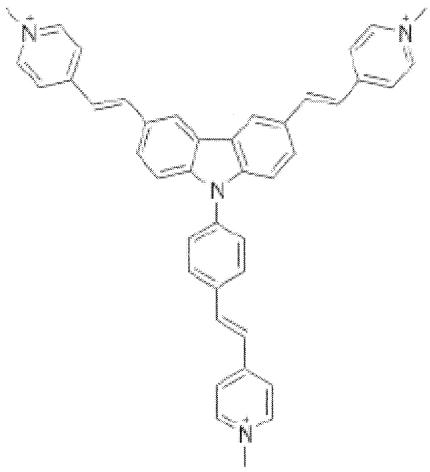
~~98.~~97. The composition of any one of claims ~~84~~83 to ~~97~~96, wherein the unbound or bound fluorescent molecular rotor is selected from the group consisting of:



~~99.~~98. The composition of claim ~~84~~83 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:



~~100.~~99. A screening assay for identifying an interaction between DNA and protein, or peptide and MDM2 protein, 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;

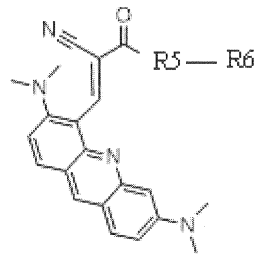
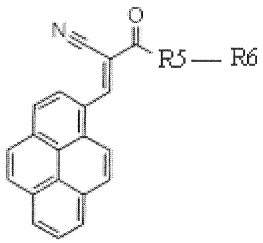
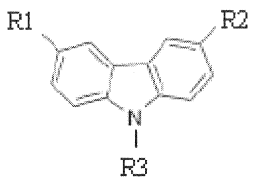
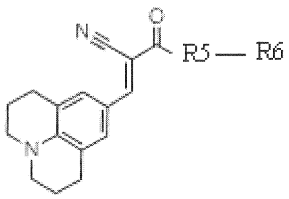
an electron-donating moiety;

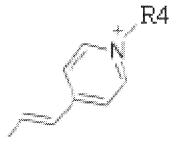
an electron-accepting moiety; and

a π-conjugated linker, 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.

~~101.~~100. The screening assay of claim ~~100~~99, wherein the unbound or bound fluorescent molecular rotor is selected from the group consisting of:

<table>
<tr><td>
(i)</td><td>
(ii)</td></tr>
<tr><td></td><td>
(iv)</td></tr>
<tr><td>
(v)</td><td></td></tr>
</table>

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

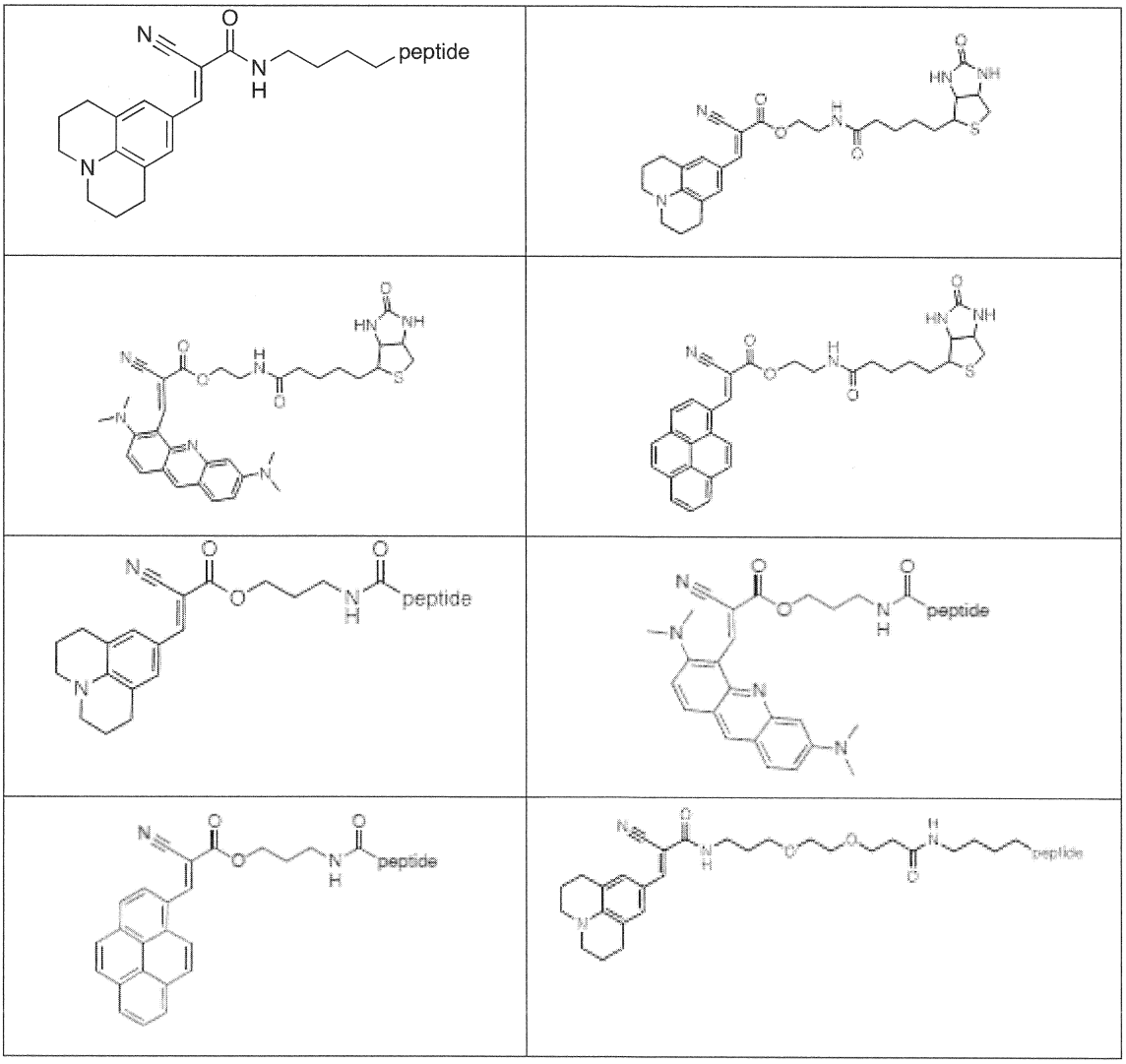
R1, R2, R3 and R4 are independently chosen from the group consisting of: optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted cycloalkyl or optionally substituted heterocyclyl;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

R5 is a OH or a linking moiety as defined in any one of claims 69, 72 or 73; and

R6 is absent or a peptide.

~~102.~~101. The screening assay of claim ~~100~~ 99 or ~~101~~100, wherein the unbound or bound fluorescent molecular rotor is selected from the group consisting of:



~~103.~~102. 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, 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.

~~104.~~103. The screening assay of claim ~~103~~102, wherein a change in fluorescence occurs when the probe is displaced from the complex.

~~105.~~104. The screening assay of any one of claims ~~103~~ 102 or ~~104~~103, wherein the probe comprises peptide selected from the group consisting of JP1: MPRFMDYWEGLSK and JP2: MPRFMDYWEGLNK; and the protein is MDM2 protein.

~~106.~~105. A kit for carrying out a method of any one of claims 69 to ~~83~~82 or the screening assay claims ~~100~~99 to ~~105~~104 comprising:

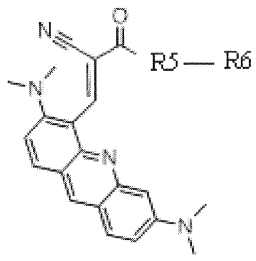
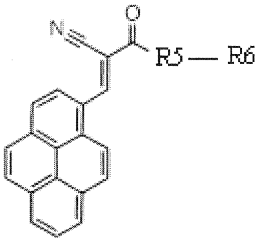
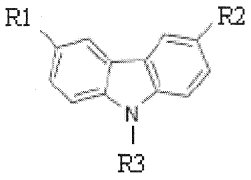
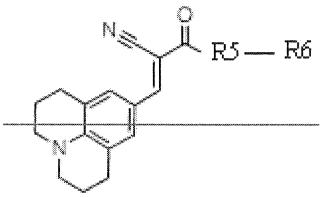
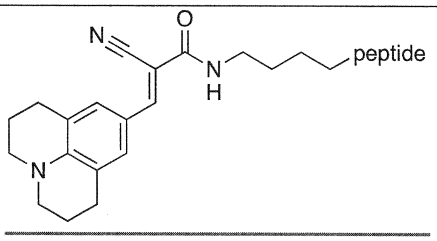
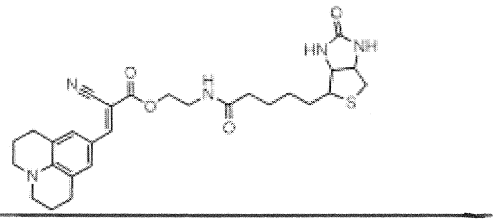
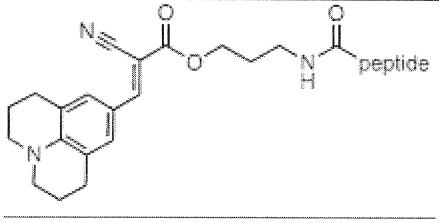
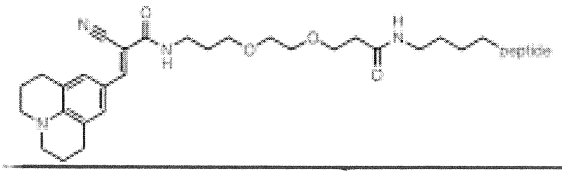
(i) fluorescent molecular rotor, DNA and protein;

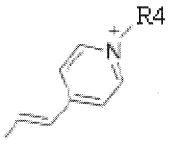
(ii) fluorescent molecular rotor, peptide and MDM2 protein; or

(iii) fluorescent molecular rotor, biotin and streptavidin,,

as defined in any one of claims 69 to 83 or 100 to 105, and a means to detect a change in fluorescence.

~~107.~~106. A chemical compound selected from the group consisting of:

 (i)	 (ii)
	 (iv)
 (v)	
	
	

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; wherein at least one of R1, R2, R3 and R4 are independently chosen 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;

R5 is a OH or a linking moiety 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; and

R6 is absent or a ligand,

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

~~108.~~107. A chemical compound of claim ~~107~~106, selected from the group consisting of:

