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Indeno[1,2-*b*]indole Scaffold in Drug Discovery: An Effective Template in Kinase Inhibitor Medicinal Chemistry

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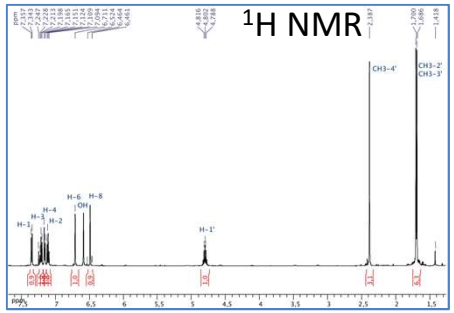
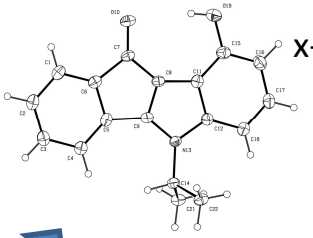
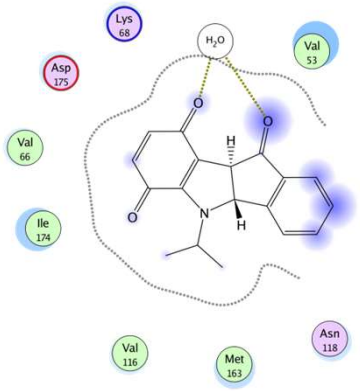
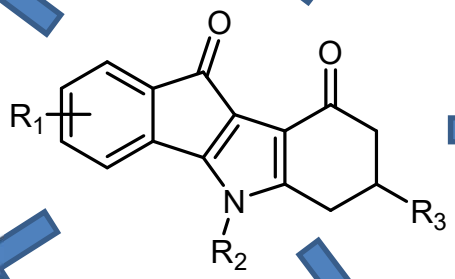
Indeno[1,2-*b*]indole Scaffold in Drug Discovery: An Effective Template in Kinase Inhibitor Medicinal Chemistry

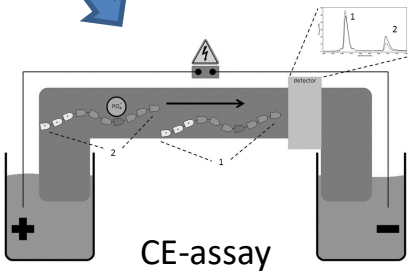
Graphical Abstract

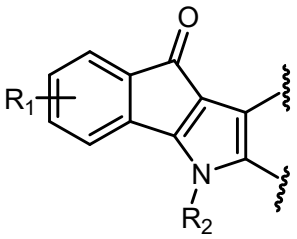




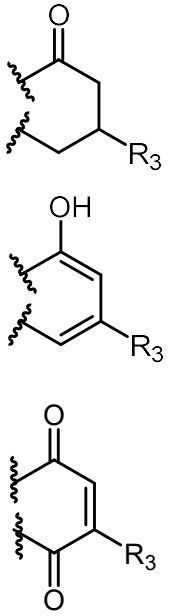








ring D modulation



Abstract:

Casein kinase 2 (CK2) is a highly pleiotropic serine/threonine protein kinase whose list of substrates includes >300 proteins implicated in a wide variety of cell functions. The catalytic subunits of CK2 (alpha and/or alpha') are constitutively active either alone or in combination with the regulatory beta-subunits to give a heterotetrameric protein. High constitutive activity of CK2 is related to contribute to cancer. Based on recent years of effort, a German French collaborative network has developed indeno[1,2-*b*]indole scaffold for designing novel inhibitors of human CK2. **The aim of this study** is to develop functionalized indeno[1,2-*b*]indoles and to investigate their potential inhibitory activity against human CK2. The different aspects of medicinal chemistry will be discussed, namely synthesis, NMR investigations, X-ray crystallography, CK2 inhibition, *in cellulo* activities, molecular modelling and physico-chemical properties.

Keywords: indenoindole synthesis; spectra resources; protein kinase CK2; physico-chemical properties; *in cellulo* permeability

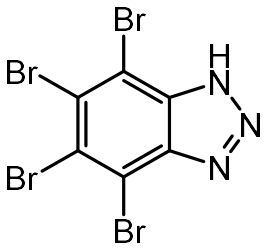
Introduction

Protein kinase CK2

First kinase discovered in 1954

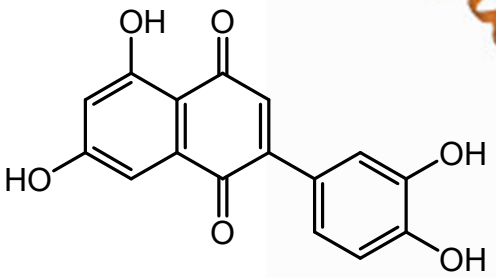
Highly expressed in many cancers

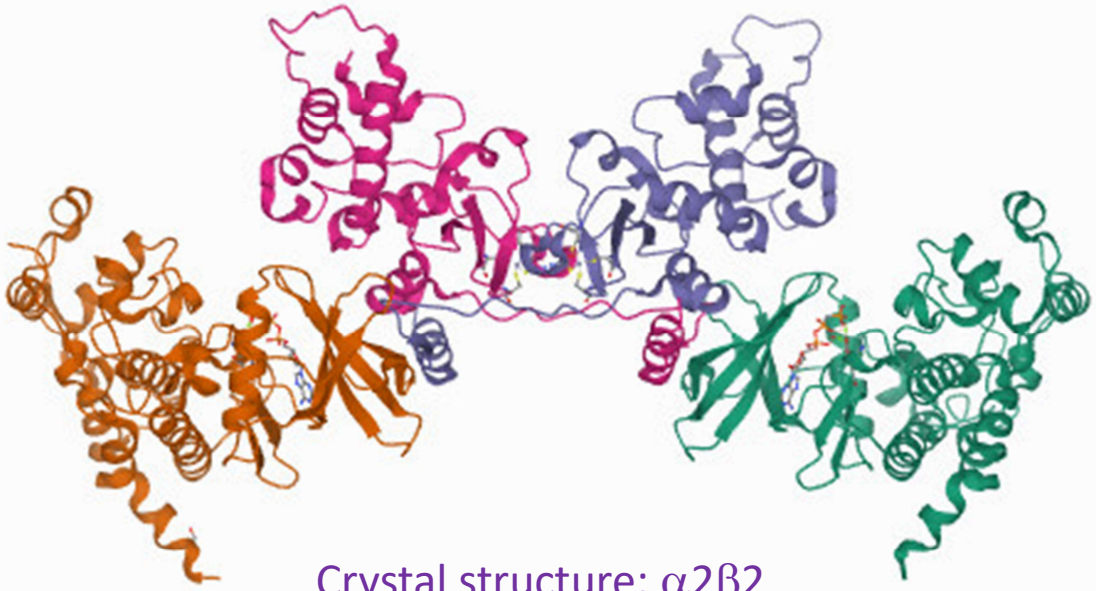
Fused bicyclic molecules as CK2 inhibitors:



TBB

IC_{50}: 0.50 μM



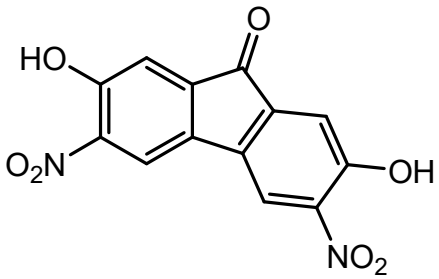


Crystal structure: α2β2
(PDB code 4NH1)

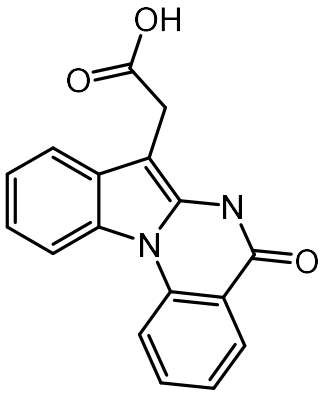
Schnitzler, A. et al. J Mol Biol 2014, **426**:1871-82
Sarno, S. et al. Biochem J 2003, **374**(Pt 3):639-46

Introduction

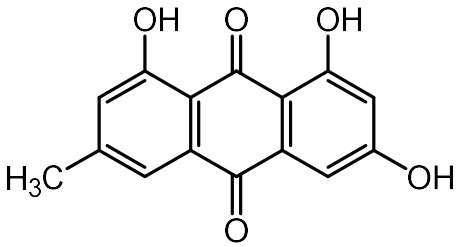
Tricyclic backbones of known ATP-competitive low molecular weight (LMW) inhibitors of CK2



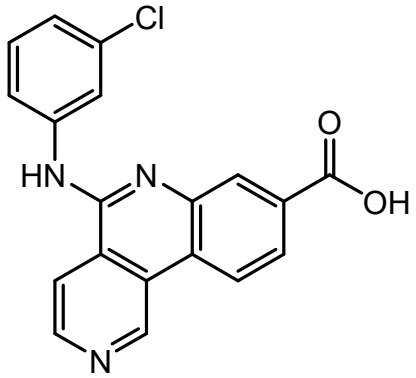
FL12
IC_{50}: 1.00 μM



IQA
IC_{50}: 0.39 μM



Emodin
IC_{50}: 2 μM

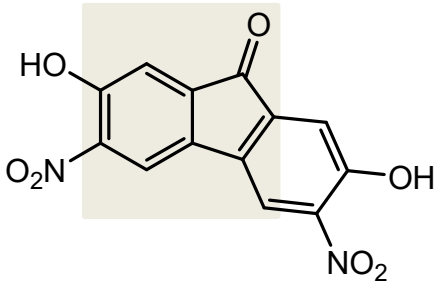


CX-4945
IC_{50}: 1 nM

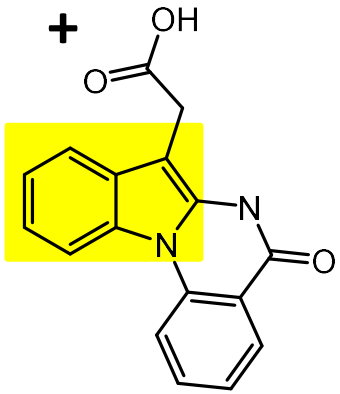
Cozza, G. et al. ChemMedChem 2011, **6**:2273-86
Protopopov, M.V. et al. Bioorg Chem 2020, **102**:104062

Introduction

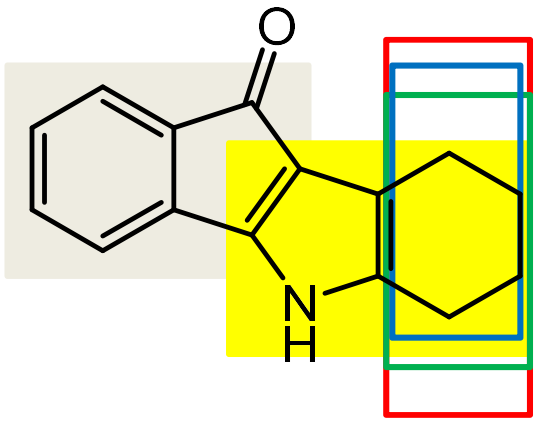
Connecting two bicyclic backbones



+



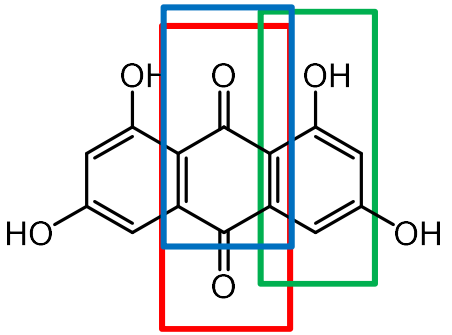
Indeno[1,2-*b*]indoles



ring D

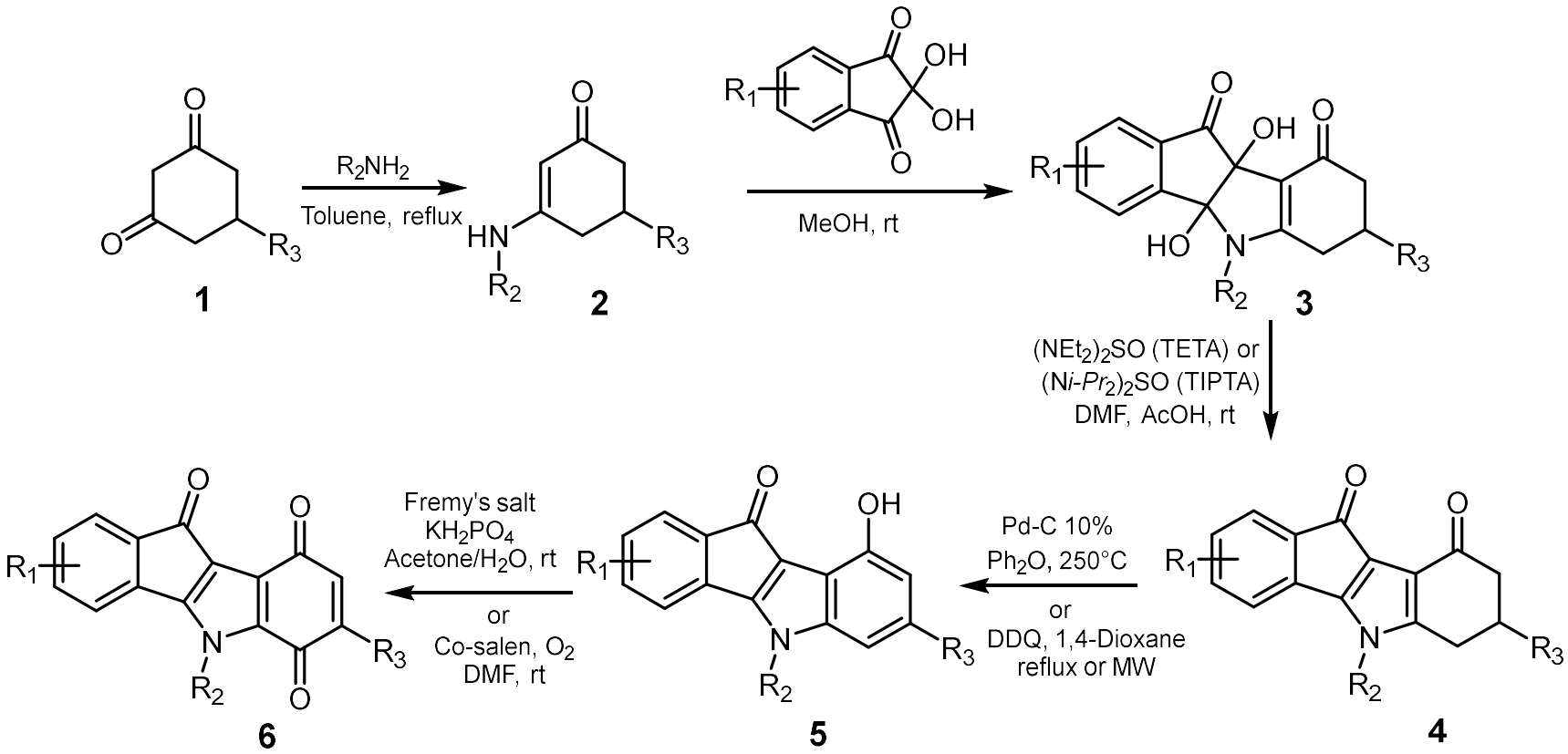
for ring D

keto **phenol**



***para*-quinone**

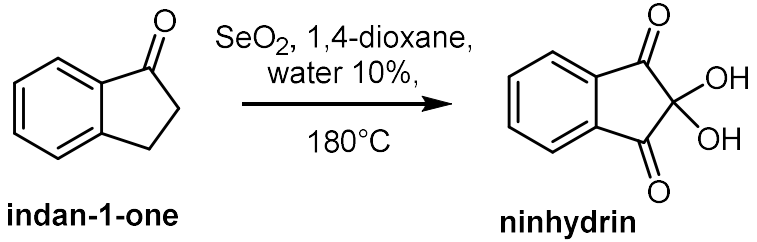
Results and discussion – *Synthesis of indeno[1,2-b]indoles*



Results and discussion – *Synthesis of indeno[1,2-b]indoles*

✓ Microwaves for oxidation of indan-1-one derivatives into ninhydrin derivatives

PROCESS OPTIMIZATION

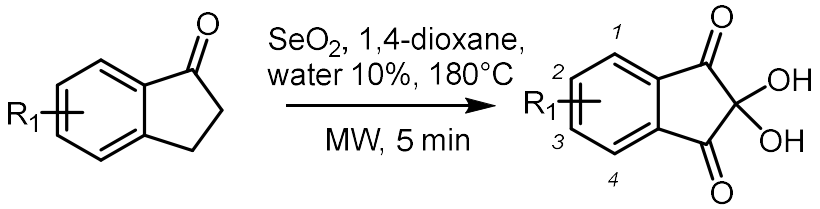


indan-1-one → **ninhydrin**

Sealed vessel

Classic 20 min, **77%**

MW 5 min, **84%**



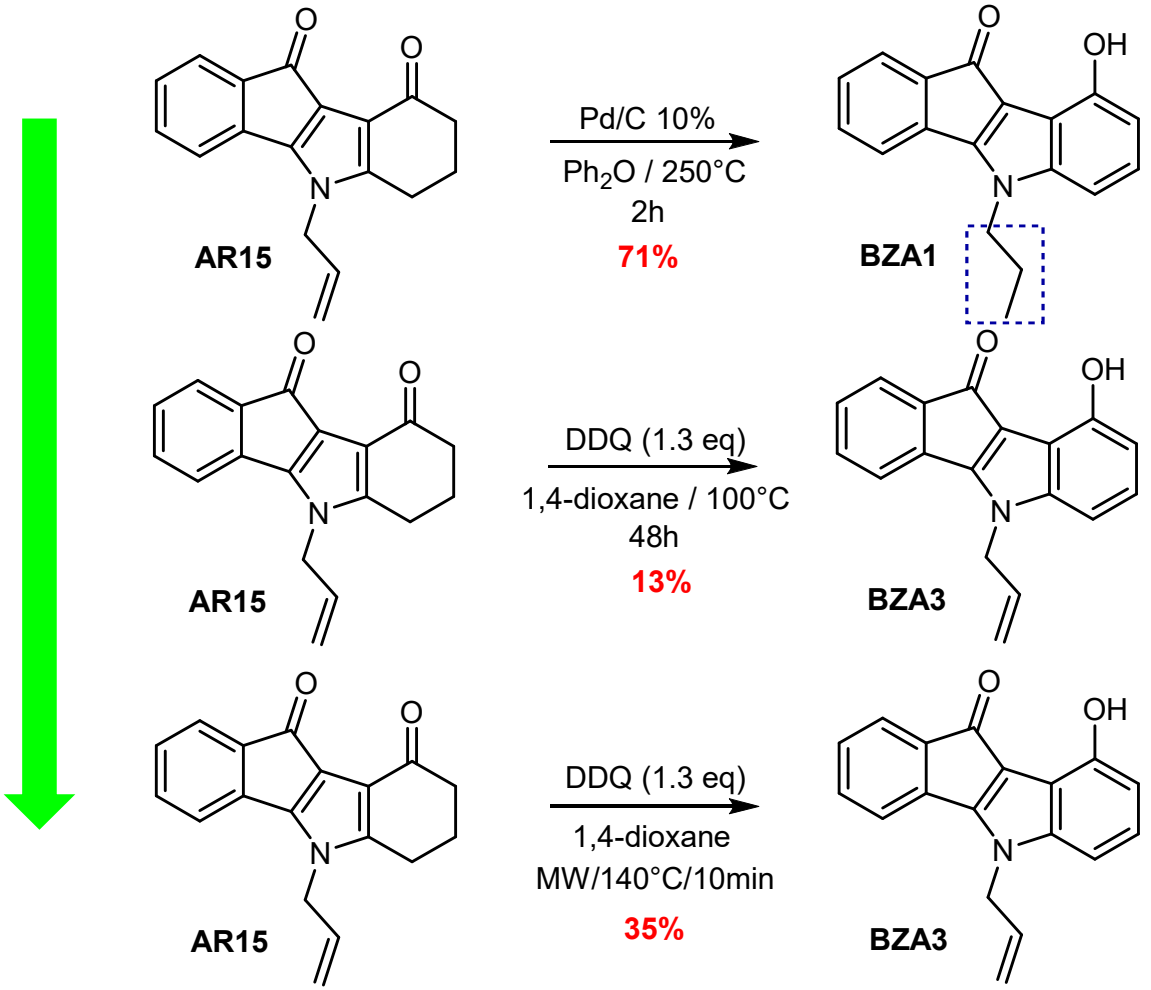
R_1= 1-CH_3, **79%** (litt. 21%)
R_1= 1-Br, **70%**
R_1= 1-OCH_3, **87%**
R_1= 3-OH, **73%**

Marminon, C. et al. Tetrahedron Lett 2015, **56**:1840-42

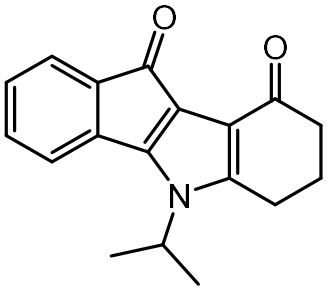
Results and discussion – *Synthesis of indeno[1,2-b]indoles*

- ✓ Microwaves for oxidation of ketonic derivatives into phenols

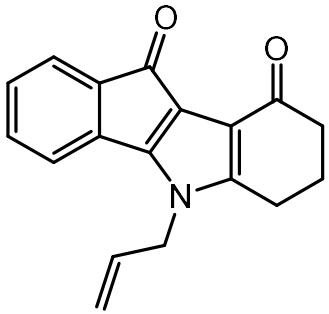
PROCESS OPTIMIZATION



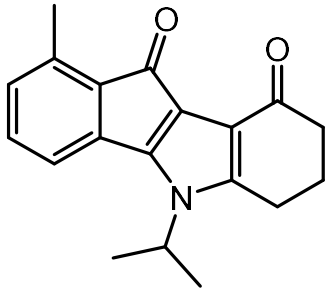
Results and discussion – *24 indeno[1,2-b]indoles synthesized*



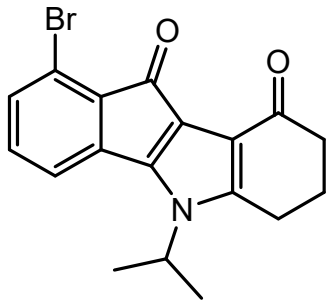
4b



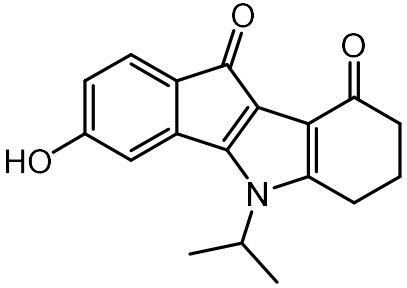
AR15



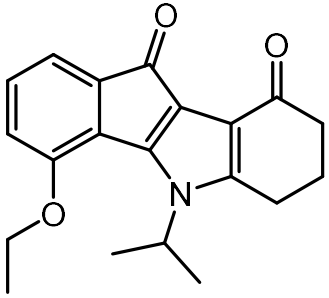
CM3146B



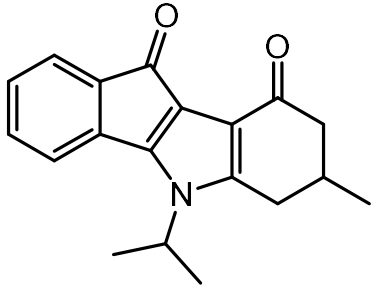
4p



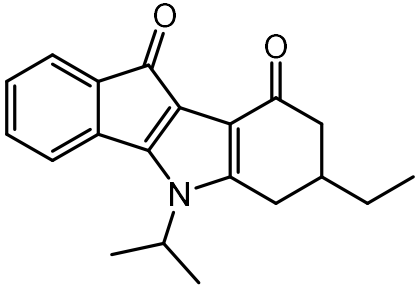
NA8b



NA28-1

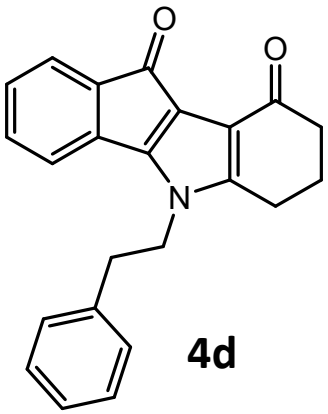


AF3

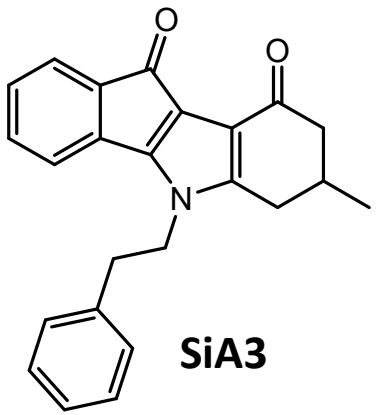


4v

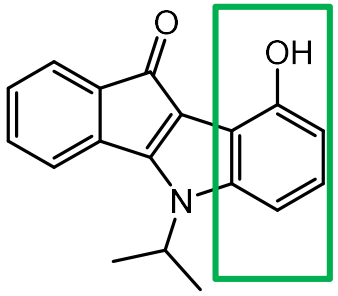
Results and discussion – *24 indeno[1,2-b]indoles synthesized*



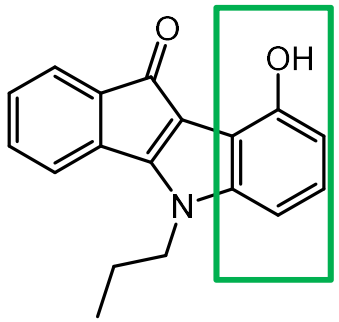
4d



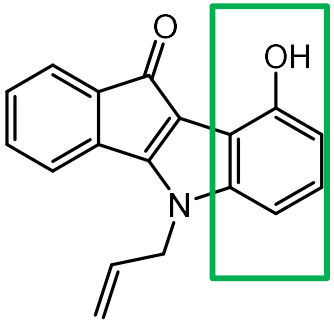
SiA3



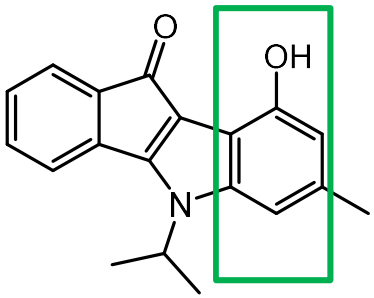
5b



BZA1



BZA3



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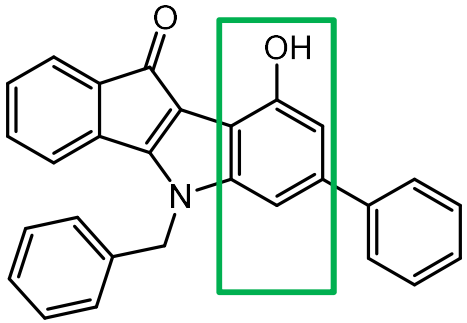
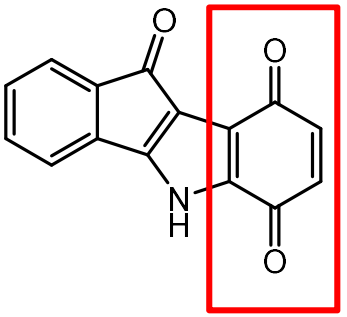
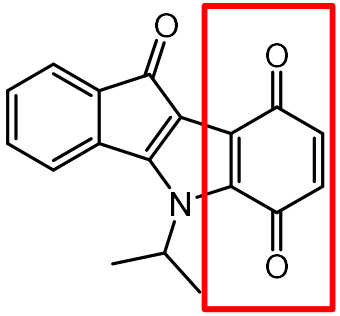


BZA32

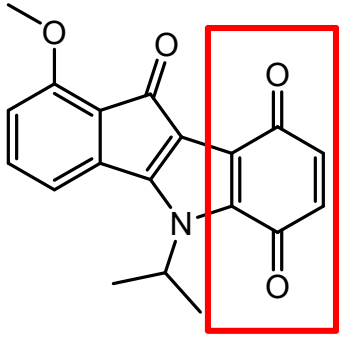


ZW18

Results and discussion – *24 indeno[1,2-b]indoles synthesized*



6b



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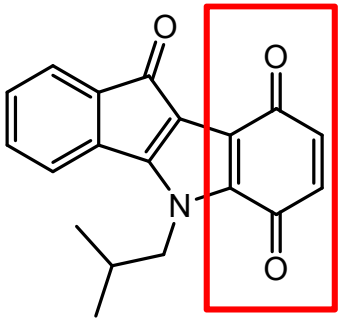
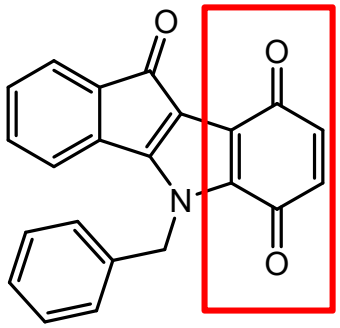
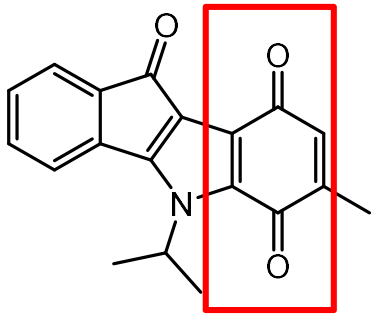


AP05



6c



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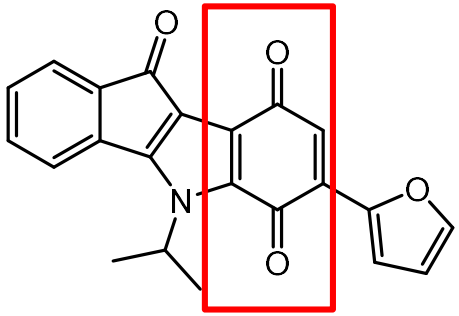

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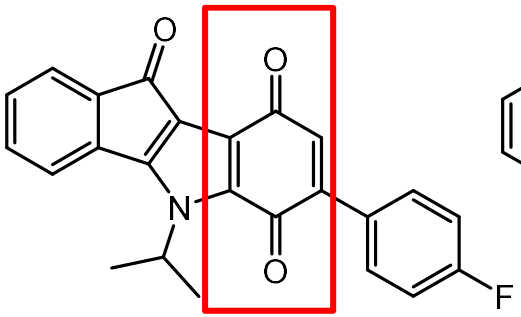

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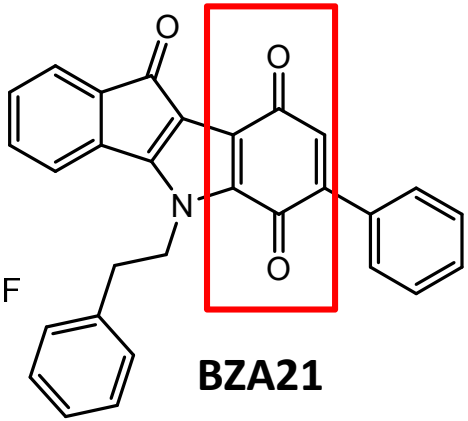
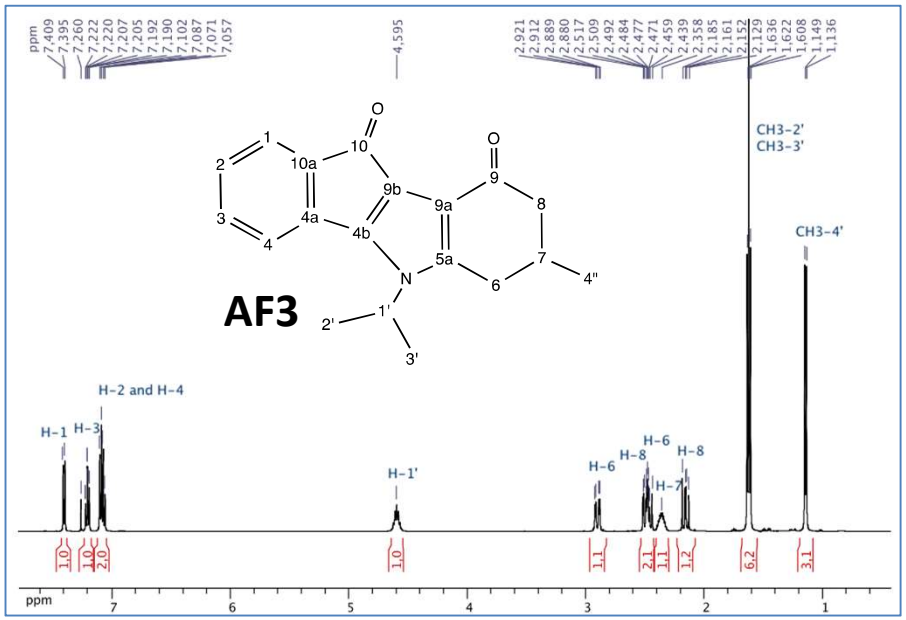
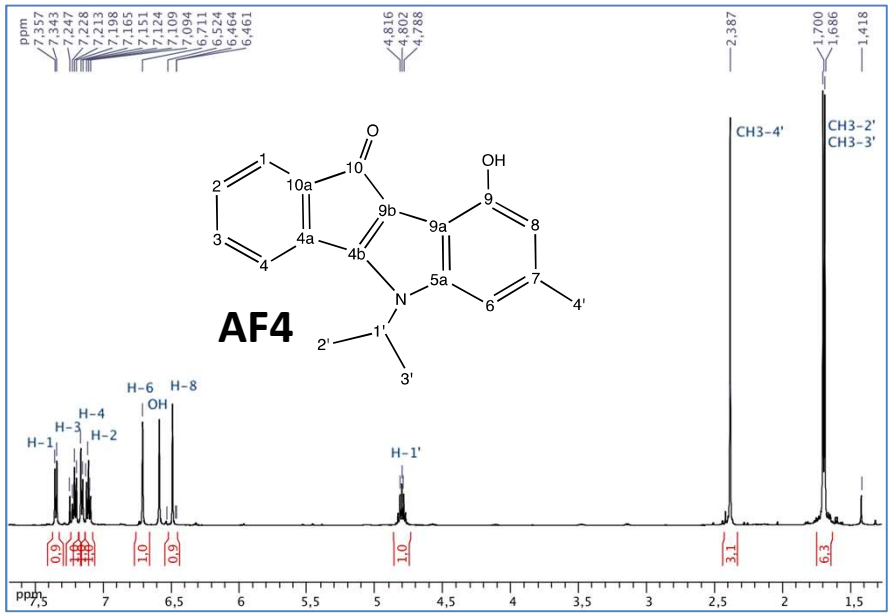
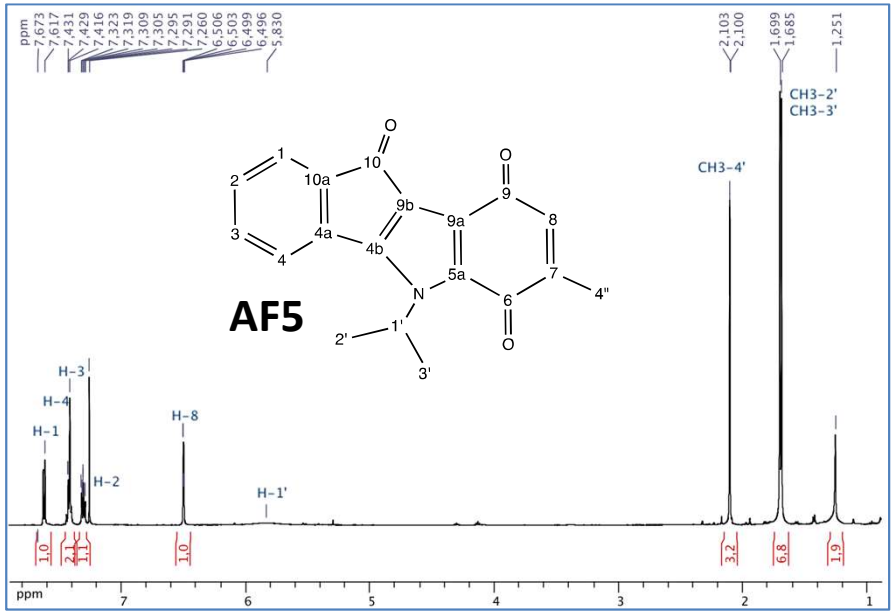


BZA21

Results and discussion – *NMR data*







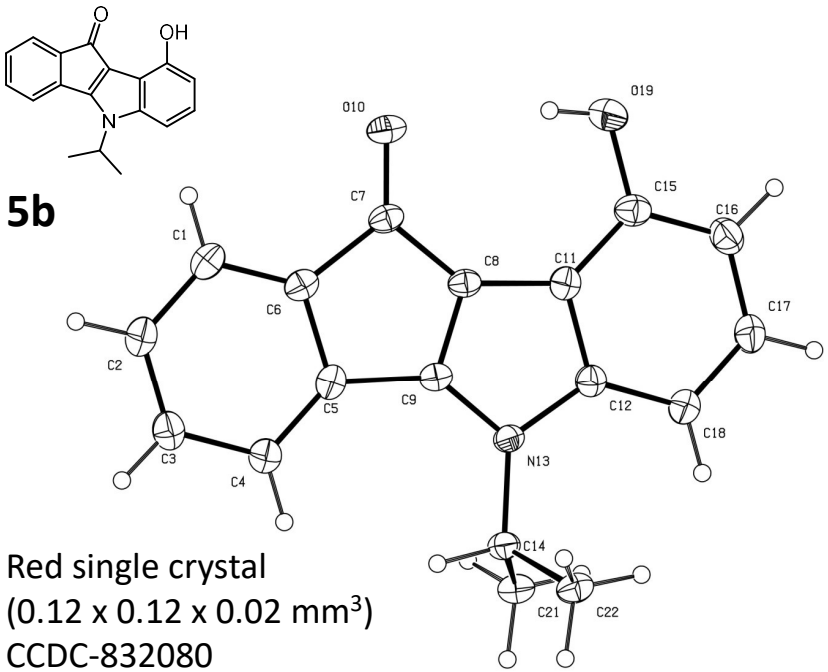
Results and discussion – *NMR data*

No.	AF3			AF4			AF5		
	δH (mult, *J* in Hz)	δC	HMBC	δH (mult, *J* in Hz)	δC	HMBC	δH (mult, *J* in Hz)	δC	HMBC
1	7.44 (d, 7.1)	123.8	C-2,3,4a,10	7,35 (d, 6.9)	123.3	C-2,4a,10	7,62 (d, 7.2)	124.7	C-4,4a,10
2	7.11 m	128.1	C-4a,10a	7,11 (t, 7.3)	129.1	C-4,10a	7,31 (dt, 3;7.1)	130.0	C-1,3,4a,10a
3	7.23 m	132.2	C-1,4a	7,22 (t, 7.5)	132.2	C-1,4a	7,42 m	121.2	C-1,2,4a,10a
4	7.11 m	118.8	C-2,4b,10a	7,16 (d, 7.2)	119.8	C-4b,10a	7,43 (dt, 0.9;7.5)	133.3	C-1,2,4a,4b
4a	—	135.4	—	—	136.5	—	—	134.4	—
4b	—	151.8	—	—	155.3	—	—	154.8	—
5a	—	148.7	—	—	142.9	—	—	146.7	—
6	2.92 (dd, 4.3 and 16.2) 2.50 m	31.7	C-4',5a,6,8,,9a	6,72 s	104.9	C-4',9,9a	—	178.6	—
7	2.39 m	31.1	C-4',7,8	—	135.7	—	6,50 (q, 1.5)	121.6	—
8	2.54 (dd, 3.6 and 16.2) 2.20 (dd, 11.8 and 16.2)	46.1	C-4',7.9,9a,9b	6,49 s	109.2	C-4',6,9,9a	—	132.7	C-4', 5a,6,7,9a,9b
9	—	191.9	—	—	149.7	—	—	181.8	—
9a	—	117.4	—	—	111.4	—	—	123.3	—
9b	—	120.8	—	—	115.7	—	—	134.1	—
10	—	184.2	—	—	186.1	—	—	183.6	—
10a	—	138.9	—	—	140.6	—		140.2	—
1'	4.62 (sept, 7.1)	49.4	C-3'.4b,5a	4,81 (sept 6.9)	49.5	C-2',3',4b,5a	5,83 br s	50.6	—
2'	1.65 (d, 7.3)	22.0	C-1',3'	1,70 (d 7)	22.2	C-1',3'	1,69 (d, 7.1)	21.1	C-1',3'
3'	1.64 (d, 7)	21.9	C-1',2'	1,70 (d 7)	21.8	C-1',2'	1,69 (d, 7.1)	21.1	C-1',2'
4'	1.17 (d, 6.5)	21.3	C-6,8	2,39 s	21.8	C-6,7,8	2,10 (d, 1.5)	16.4	C-5a,6,8,9
OH	—	—	—	6.58 s	—	C-7,9,9a	—	—	—

Al Chab, F. et al. Magn Reson Chem 2013, **51**:837-41

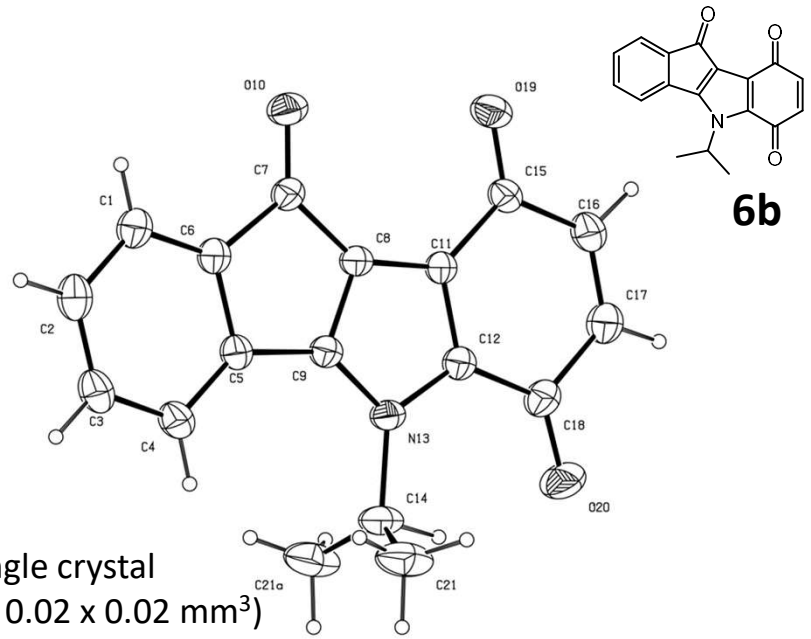
Results and discussion – *X-ray crystallography*

View of the crystal structures of **5b** and **6b** with our numbering scheme, displacement ellipsoids are drawn at the 20% probability level.



Red single crystal
(0.12 x 0.12 x 0.02 mm^3)
CCDC-832080

nearly planar with a mean out-of-plane deviation of 0.0271 Å with the largest deviation of 0.0542 (14) Å for atom C14

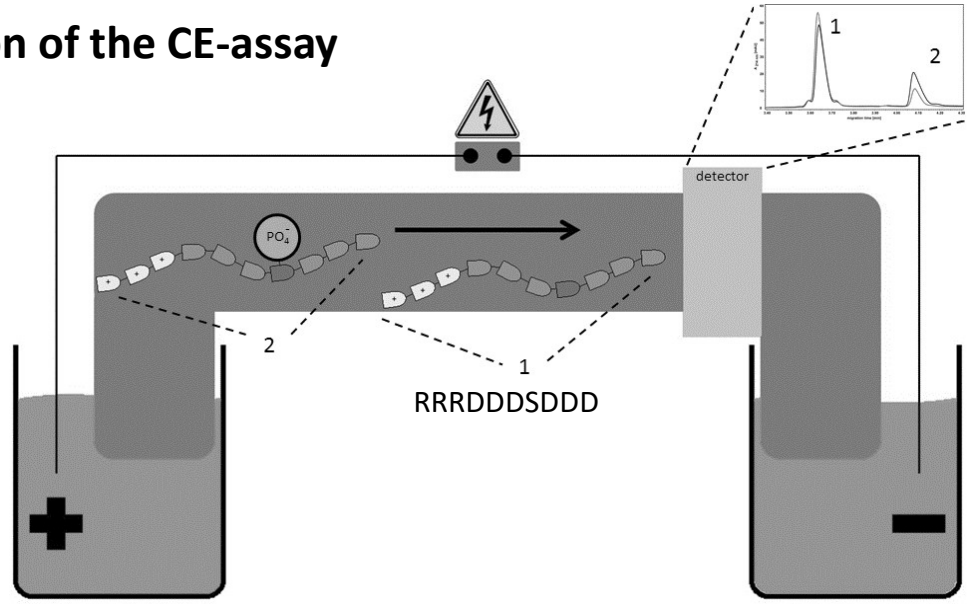


Red single crystal
(0.09 x 0.02 x 0.02 mm^3)
CCDC-832081

almost planar with a maximum deviation from planarity of 0.2430 Å, and the maximum deviation from planarity is found for C(14) lying 0.3379 (9) Å from the plane defined by the hetero-tetracyclic system

Results and discussion – *CK2 inhibitory activities*

Schematic illustration of the CE-assay



CE-electropherograms of CK2 reaction with and without inhibitor

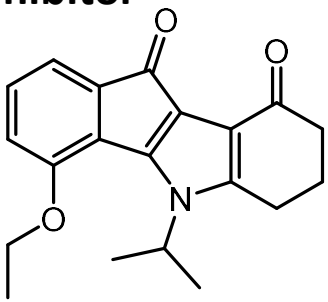
A sample 20 nl of a CK2 reaction is injected into the capillary which is subsequently dipped into buffer vials containing the background electrolyte. The applied electronic field of 30 µA current with flexible voltage separates the CK2 substrate **1** and its phosphorylated analog **2** while they migrate towards the cathode. An UV detector set to 214 nm at the outlet side of the capillary (20 cm effective length) quantitatively detects both peptides **1** and **2**.

Gratz, A. et al. Electrophoresis 2010, **31**:634-40

Results and discussion – *CK2 inhibitory activities*

Best CK2 inhibitor

NA28-1



[a] The percent inhibition of CK2 activity was determined for each compound at a fixed concentration of 10 µM.

[b] Determinations were performed in triplicate in independent experiments.

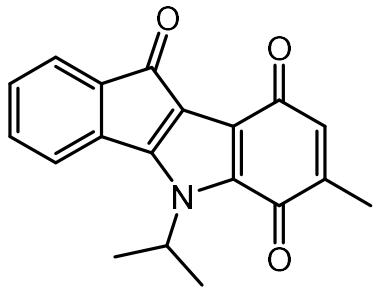
[c] For the best compounds producing at least 50% inhibition at 10 µM, the concentration was varied to precisely determine the IC^{50} values.

No compound	% inhibition (10 µM)[a]	IC_{50} (µM)[b]
4b	99	0.36[c]
AR15	85	0.89
CM3146B	100	0.11
4p	99	0.14
NA8B	100	0.14
NA28-1	100	**0.045**
AF3	94	0.17
4v	94	0.43
4d	59	7.0
SiA3	66	2.50
5b	72	2.0
BZA1	77	2.04

Results and discussion – *CK2 inhibitory activities*

Best CK2 inhibitor

AF5



[a] The percent inhibition of CK2 activity was determined for each compound at a fixed concentration of 10 µM.

[b] Determinations were performed in triplicate in independent experiments.

[c] For the best compounds producing at least 50% inhibition at 10 µM, the concentration was varied to precisely determine the IC^{50} values.

[d]measured by the radiometric assay.

No compound	% inhibition (10 µM)[a]	IC_{50} (µM)[b]
BZA3	81	3.44[c]
AF4	64	1.27
BZA32	48	n.d.
ZW18	91	1.76
6b	60	5.55
CM3079B	40	n.d.
AP05	63	5.93
6c	-	1.49[d]
AF5	87	**0.43**
AF20	82	1.65
AF15	42	n.d.
BZA21	44	n.d.

Results and discussion – *In cellulo activities of 16 indenoindoles*

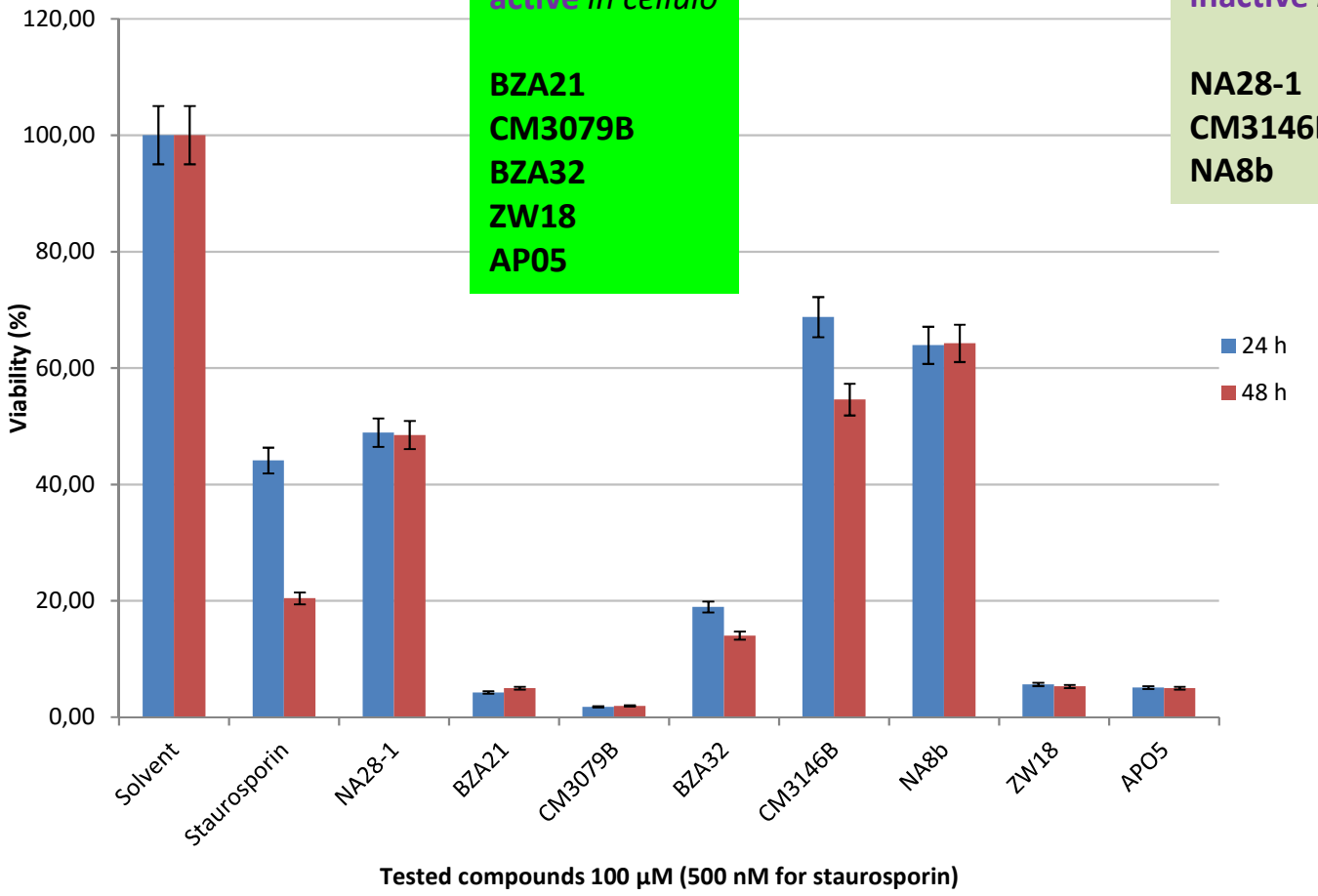
MTT test

5 compounds **active** *in cellulo*

BZA21
CM3079B
BZA32
ZW18
AP05

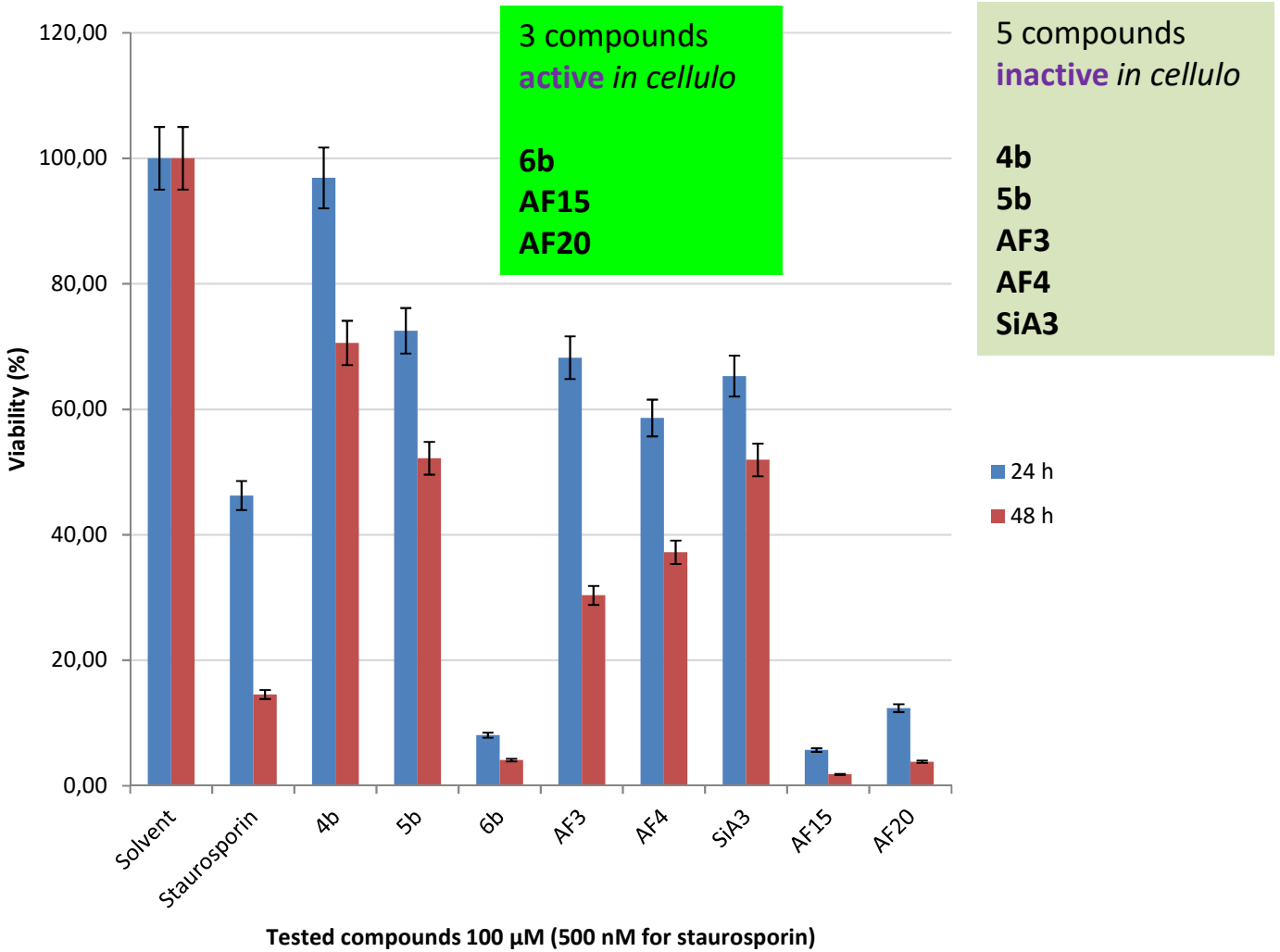
3 compounds **inactive** *in cellulo*

NA28-1
CM3146B
NA8b

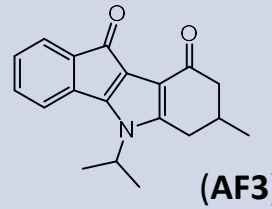
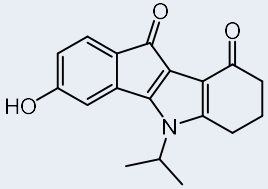
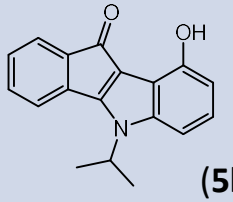


Results and discussion – *In cellulo activities of 16 indenoindoles*

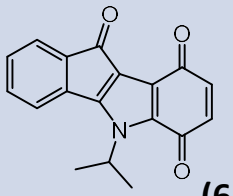
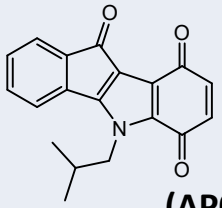
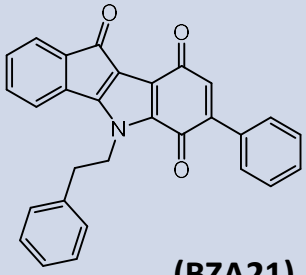
MTT test



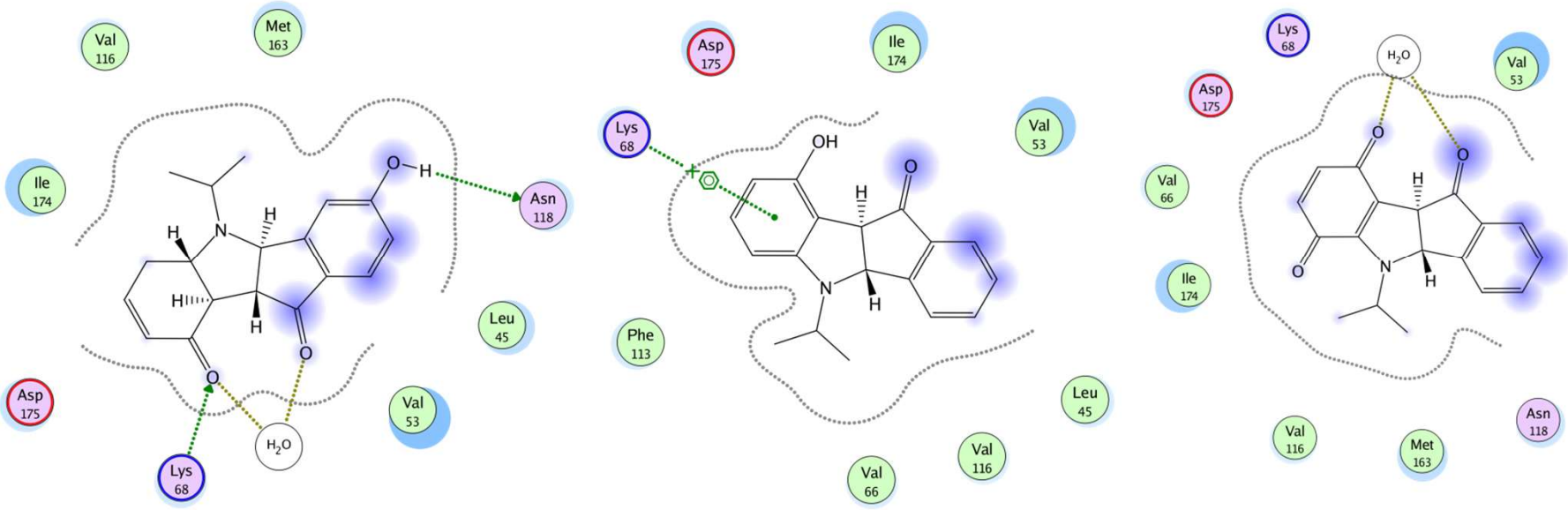
Results and discussion – *Physico-chemical properties and Bio*

Structure (code)	MarvinSketch 20.19.0	Molinspiration v 2018.10	ACD/ChemSketch 2020.1.2	CK2 IC_{50} (μM)	MCF-7 % viability
 (AF3)	3.76	3.79	4.34 +/- 1.27	0.17	24h = 68% 48h = 30%
 (NA8b)	2.87	2.81	3.53 +/- 1.27	0.14	24 h = 64% 48 h = 63%
 (5b)	3.82	4.08	4.04 +/- 1.25	2.0	24 h = 72% 48 h = 52%

Results and discussion – *Physico-chemical properties and Bio*

Structure (code)	MarvinSketch 20.19.0	Molinspiration v 2018.10	ACD/ChemSketch 2020.1.2	CK2 IC_{50} (μM)	MCF-7 % viability
 (6b)	2.54	2.88	3.02 /- 1.50	5.55	24 h = 8% 48 h = 4%
 (AP05)	3.02	3.26	3.55 +/- 1.50	5.93	24 h = 5% 48 h = 4%
 (BZA21)	5.45	5.66	5.97 +/- 1.51	44 (% inh. at 10 μM)	24 h = 6% 48 h = 4%

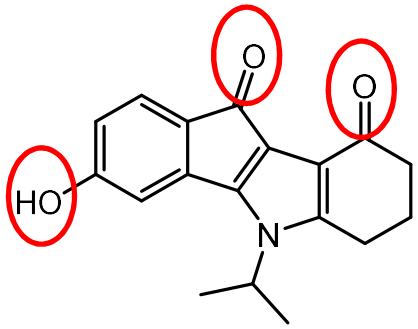
Results and discussion – Computer-assisted docking of indenoindoles



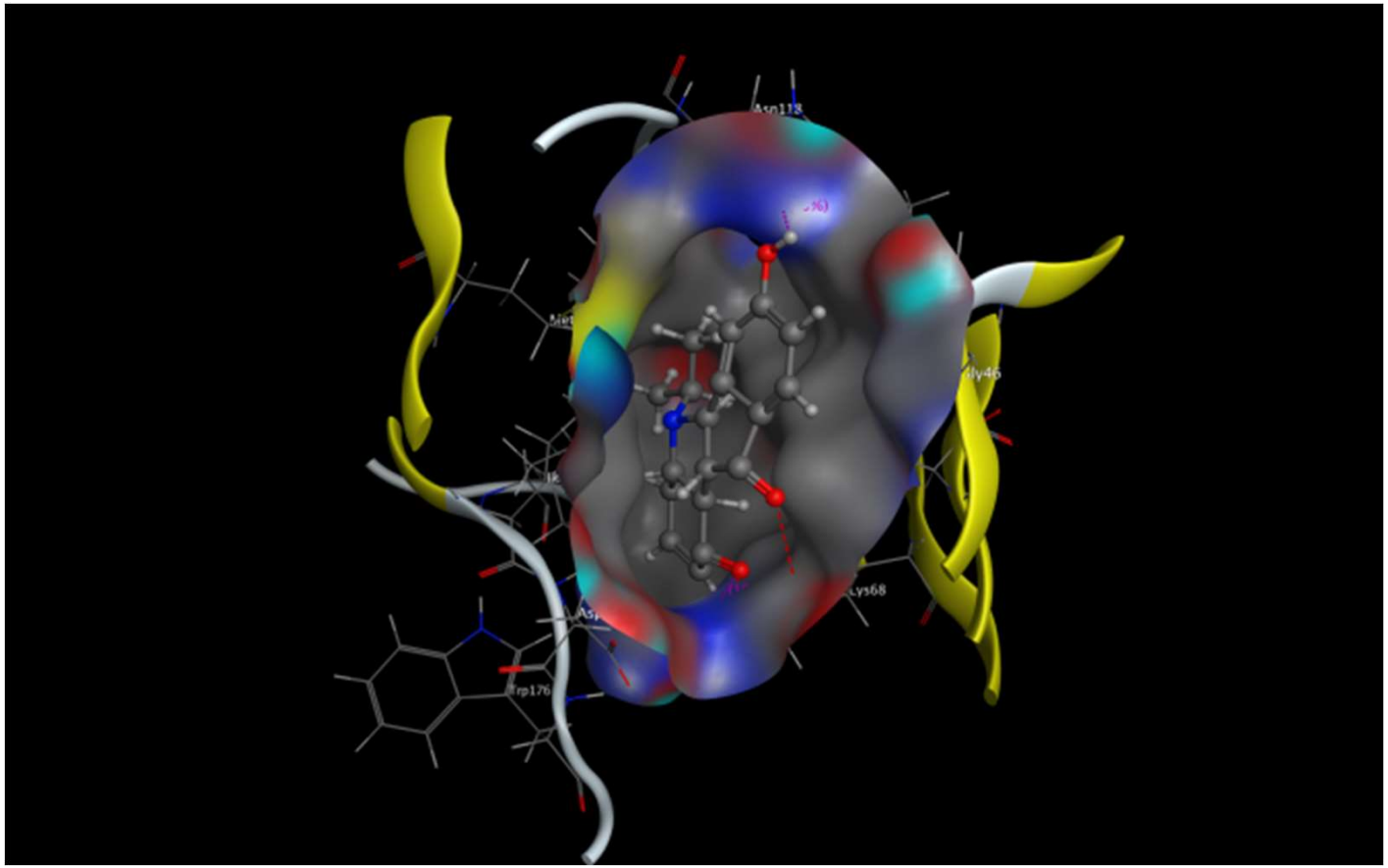
NA-8b **5b** **6b**

2D Interrelation of **NA-8b**, **5b** and **6b** with the amino acid residues of 3C13 crystal structure of CK2 enzyme (in the ATP binding pocket).

Results and discussion – Computer-assisted docking of indenoindoles

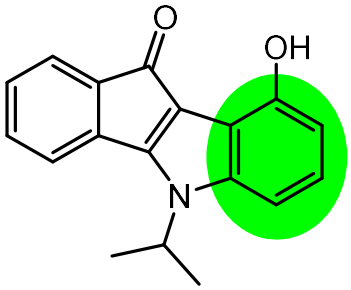


NA-8b

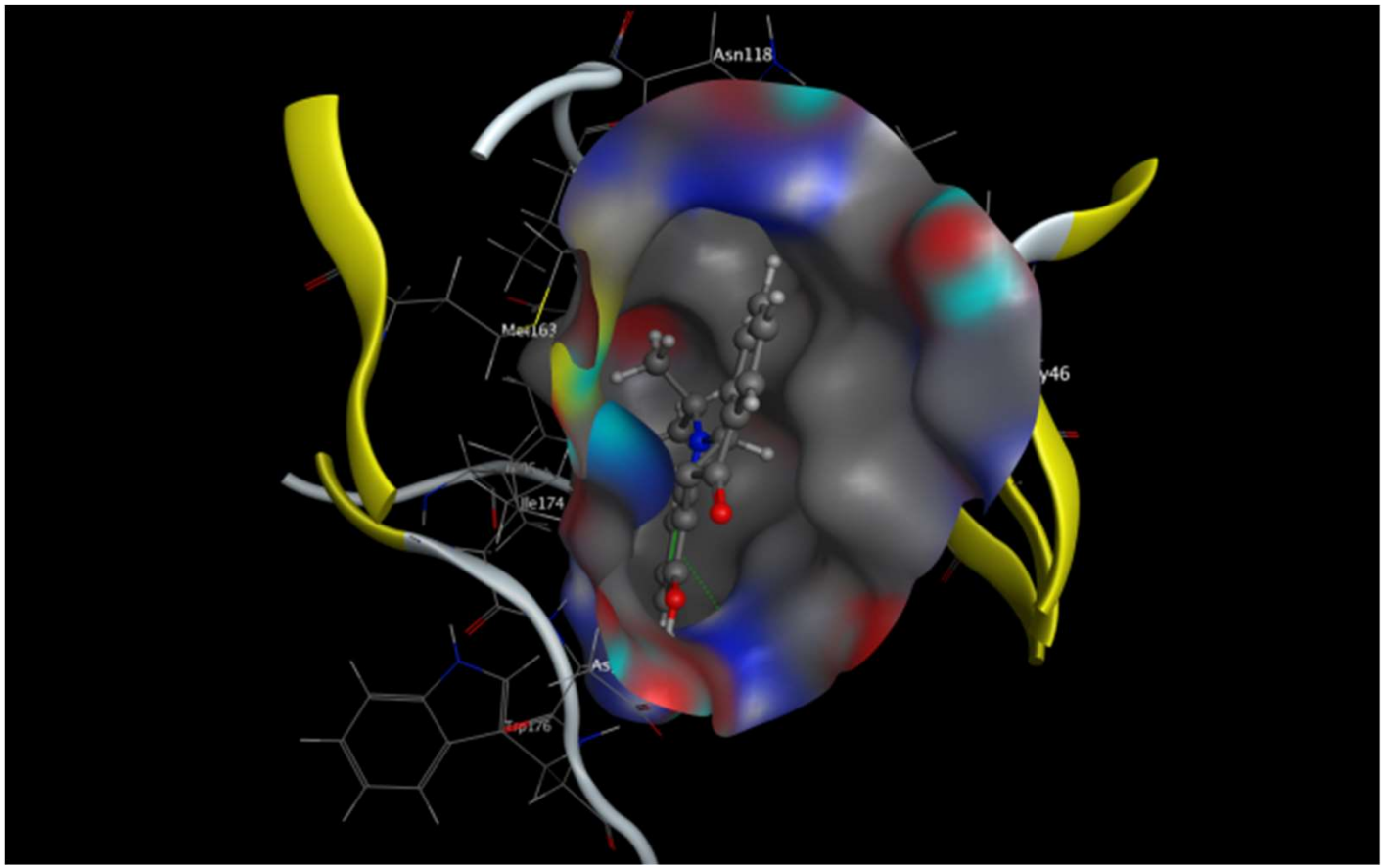


3D Interrelation of **NA-8b** with the amino acid residues of 3C13 crystal structure of CK2 (in the ATP binding pocket).

Results and discussion – Computer-assisted docking of indenoindoles

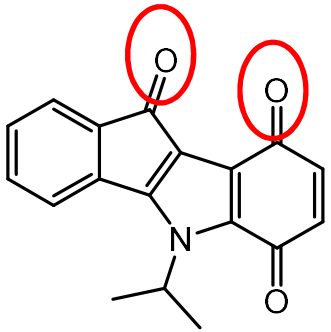


5b

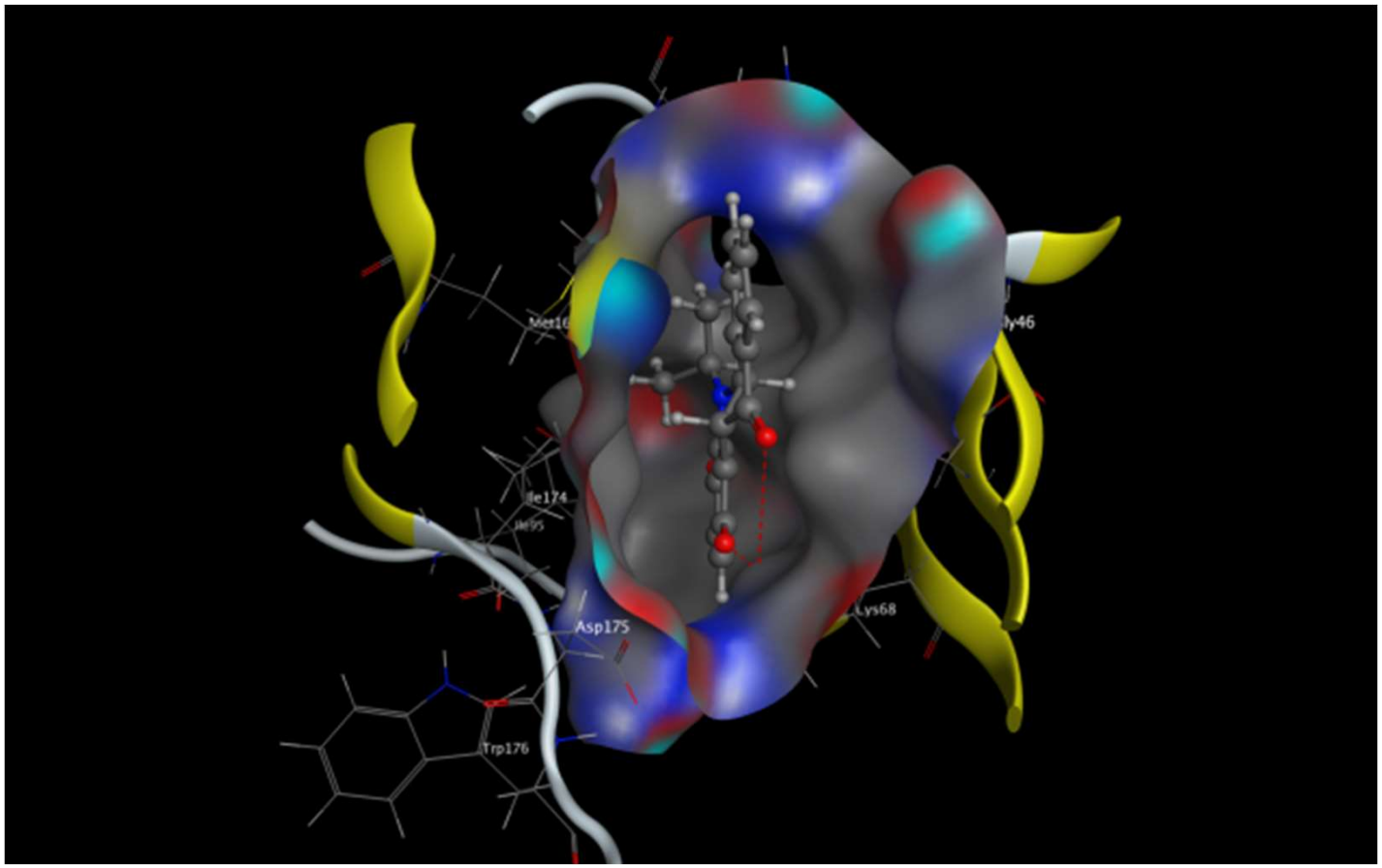


3D Interrelation of **5b** with the amino acid residues of 3C13 crystal structure of CK2 (in the ATP binding pocket).

Results and discussion – Computer-assisted docking of indenoindoles



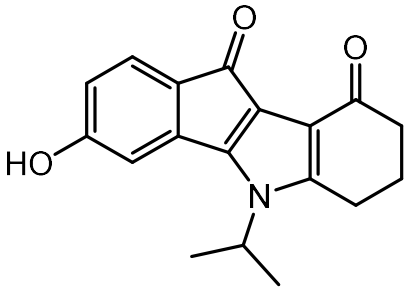
6b



3D Interrelation of **6b** with the amino acid residues of 3C13 crystal structure of CK2 (in the ATP binding pocket).

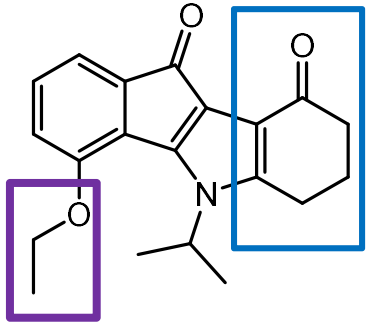
Conclusions

✓ **cLogP and Bio data on CK2: Alkoxy group +++ on keto sub-series**



NA8b

Already best length?



NA28-1

cLogP = 3.53 ± 1.27
[ACD/ChemSketch 2019.2.1]

CK2, **IC_{50}** = 0.14 μM

MCF-7, **% viability** (after 48 h): ≈ 65%

cLogP = 4.29 ± 1.42
[ACD/ChemSketch 2019.2.1]

CK2, **IC_{50}** = 45 nM

MCF-7, **% viability** (after 48 h): < 50%

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