Inspiralis

15 Years Supporting Drug Discovery

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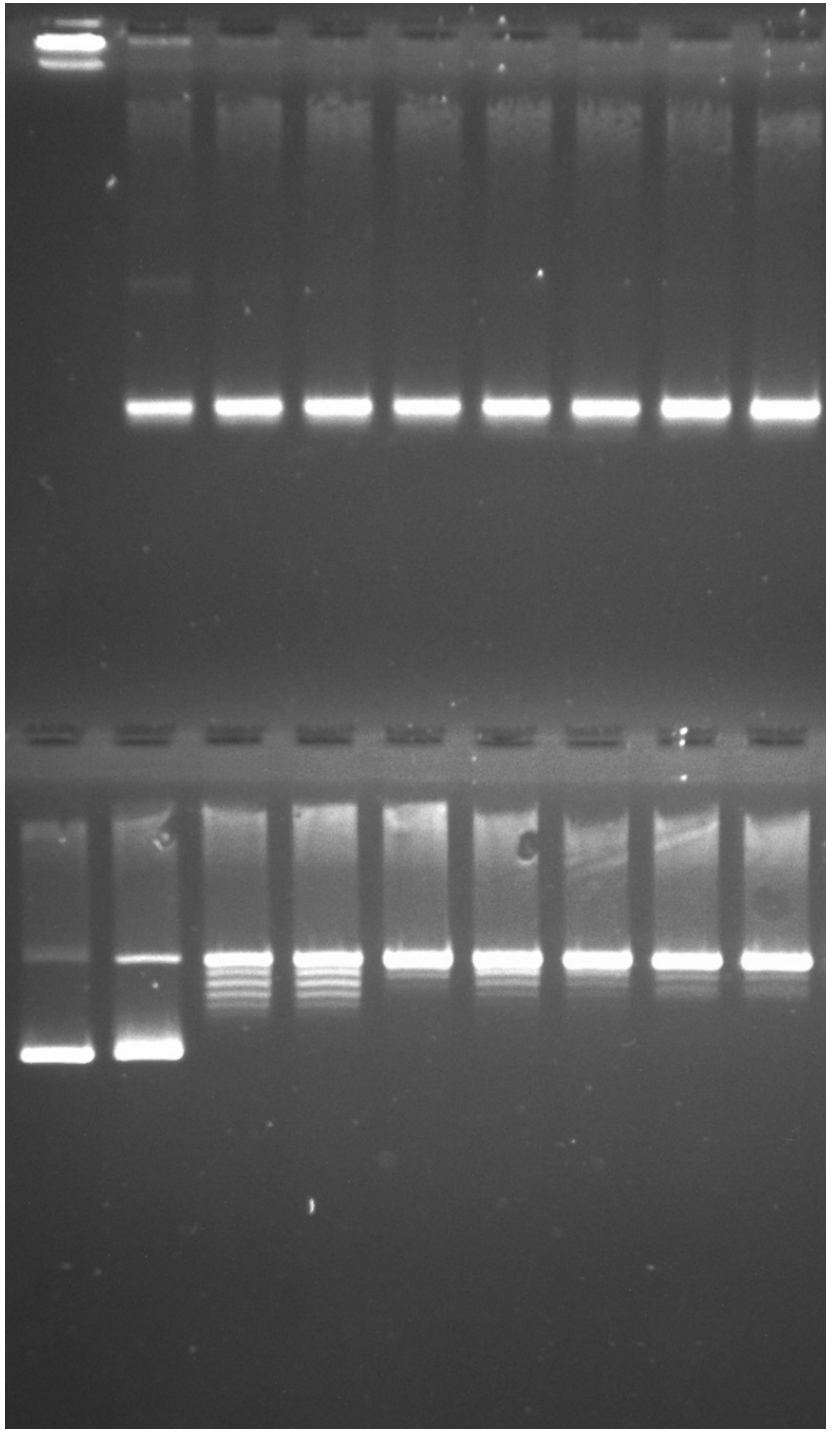
Inaphaea Lab Launch

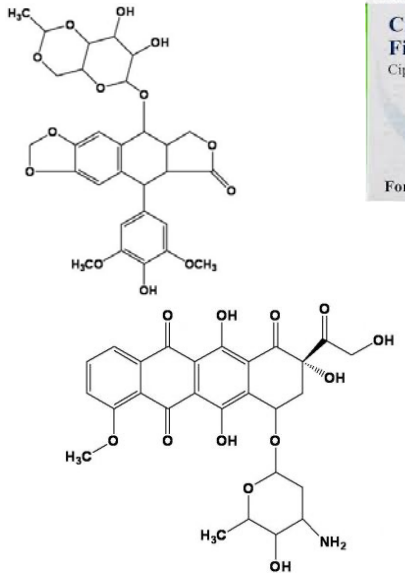
26 September 2023



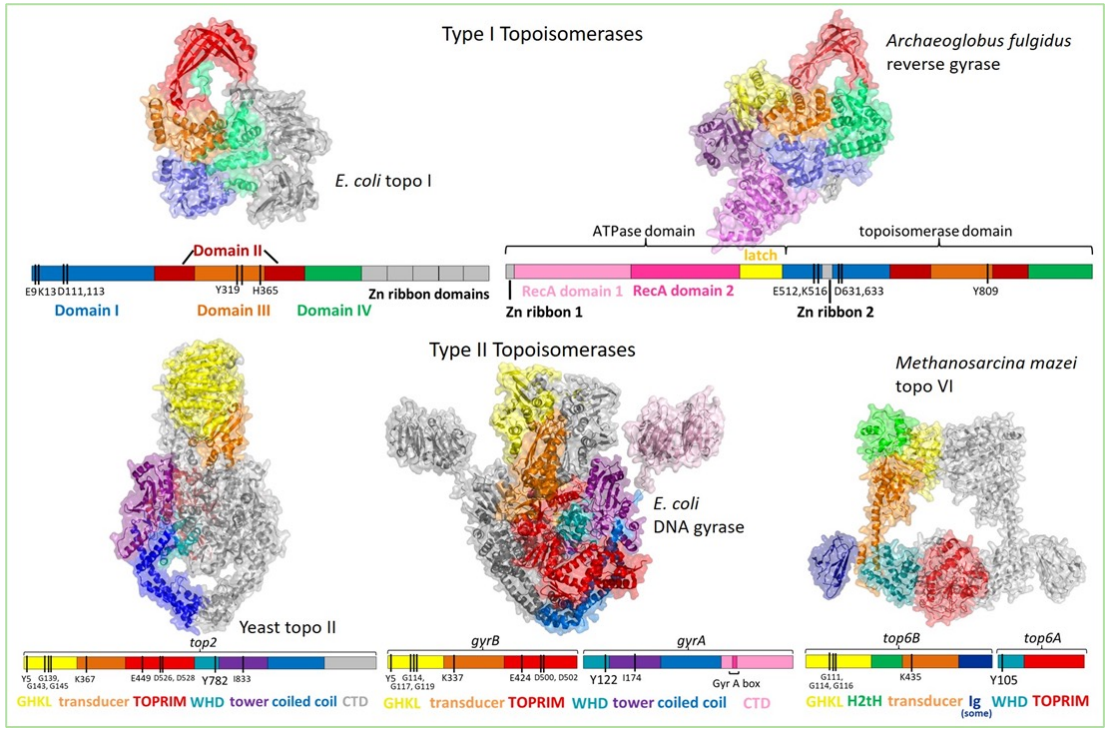
Who are we?

- Global suppliers of DNA modifying enzymes and DNA substrates
- Supplied alone or in easy-to-use kits
- DNA topoisomerases
  - essential enzymes
  - control DNA topology
  - validated drug targets

Cancer drugs



Antibiotics



Bush *et al*. 2018

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How we support drug discovery:

- Contract research or collaborations/consortiums
- Preclinical development
  - Inhibitor screening
  - Hit to lead/lead optimisation – IC_{50}s
  - Mode of Action studies
    - Complex biochemical assays
    - Crystallography
    - Biophysical analysis
  - Custom protein production
    - Mutant protein production
    - Alternative species/other proteins

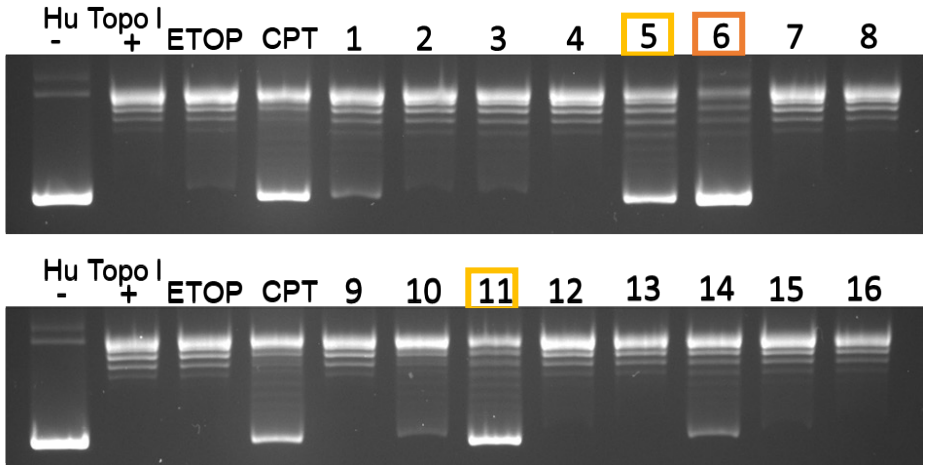
Case Study 1 – Hit identification

- Pharmaceutical company – looking for novel human topoisomerase inhibitors

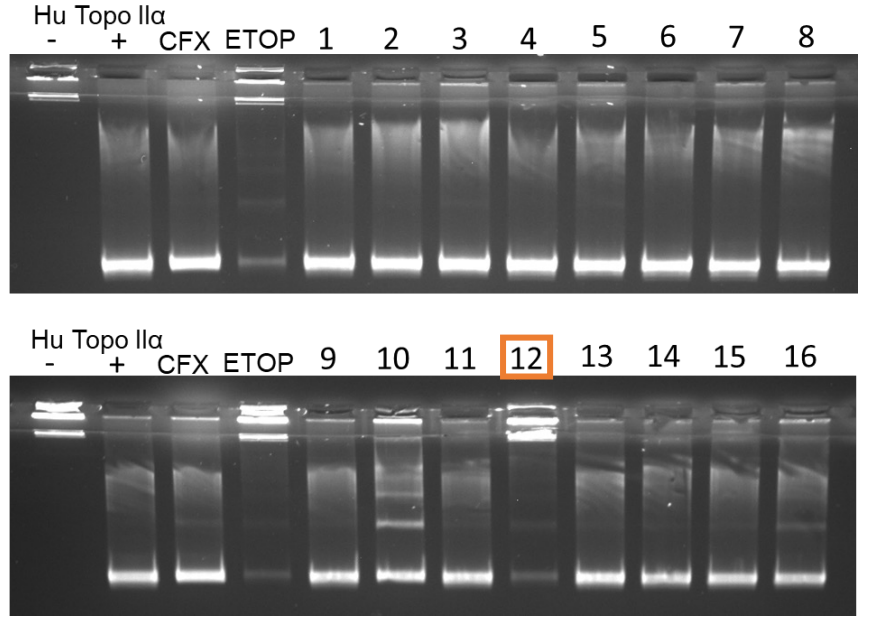
Compound	Human topo I relaxation % Inhibition at 100 µM		
	Assay 1	Assay 2	Avg
Etoposide (ETOP)	0.81	2.33	1.57
Camptothecin (CPT)	59.07	59.55	59.31
1	14.02	10.28	12.15
2	0.29	2.39	1.34
3	1.48	5.76	3.62
4	0.00	4.47	2.23
5	55.40	49.63	52.52
6	90.33	91.42	90.88
7	0.00	0.47	0.24
8	0.00	2.43	2.00
9	0.00	2.66	1.33
10	3.86	5.42	4.64
11	62.93	56.69	59.81
12	0.00	0.97	0.48
13	0.00	1.46	0.73
14	16.47	12.41	14.44
15	0.12	1.93	1.03
16	0.00	0.25	0.13

Compound	Human topo IIα decatenation % Inhibition at 100 µM		
	Assay 1	Assay 2	Avg
Ciprofloxacin (CFX)	6.77	0.00	3.39
Etoposide (ETOP)	84.27	90.77	87.52
1	4.53	1.53	3.03
2	6.20	0.00	3.10
3	0.24	5.98	3.11
4	0.00	0.01	0.00
5	2.34	1.58	1.96
6	1.02	0.00	0.51
7	4.58	6.53	5.56
8	6.48	0.22	3.35
9	2.19	0.00	1.09
10	14.38	33.90	24.14
11	3.71	3.86	3.78
12	84.86	86.68	85.77
13	0.00	8.02	4.01
14	0.00	0.00	0
15	0.00	0.00	0
16	0.00	5.79	2.6

Human topo I relaxation assays - Test Compounds 1-16



Human topo IIα relaxation assays - Test Compounds 1-16



Case Study 2 – Lead Optimisation

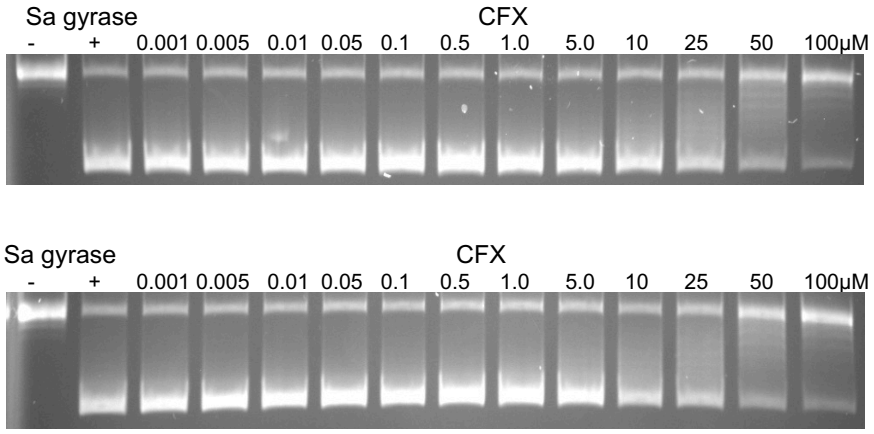
- Pharmaceutical company – looking for novel antibiotics
- Medicinal chemistry effort to improve lead compound

Compound	*S.aureus* gyrase supercoiling IC_{50} (µM)			*S.aureus* topo IV decatenation IC_{50} (µM)			*S.aureus* S84L gyrase supercoiling IC_{50} (µM)		
	Assay 1	Assay 2	**Avg**	Assay 1	Assay 2	**Avg**	Assay 1	Assay 2	**Avg**
CFX	14.20	16.15	**15.18**	1.15	0.81	**0.98**	>500	>500	**>500**
Lead compound	1.60	1.73	**1.67**	2.94	2.34	**2.64**	21.45	17.78	**19.61**
Compound 1	3.00	3.70	**3.35**	2.13	2.09	**2.11**	-	-	**-**
Compound 2	0.56	0.73	**0.65**	0.94	0.87	**0.91**	2.33	1.90	**2.11**

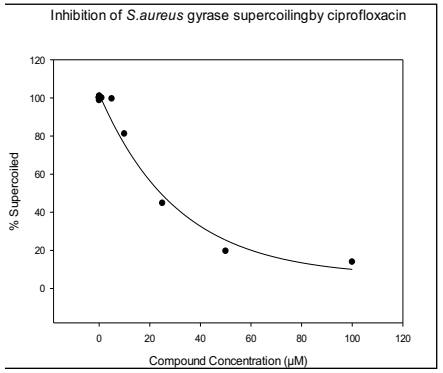
Compound	Human Topo II decatenation IC_{50} (µM)		
	Assay 1	Assay 2	**Avg**
Etoposide	52.3	37.8	**45.05**
Lead compound	~250	100-250	**250**

Gel Images: *S. aureus* gyrase supercoiling assays

Ciprofloxacin

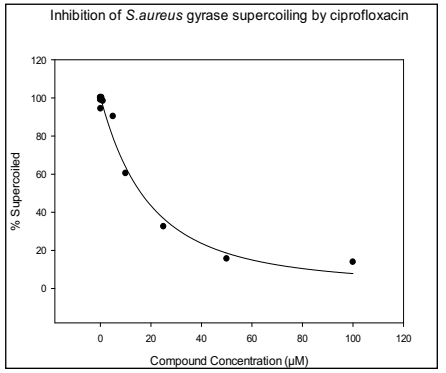


Assay 1



Calculated IC_{50} 14.20 µM

Assay 2



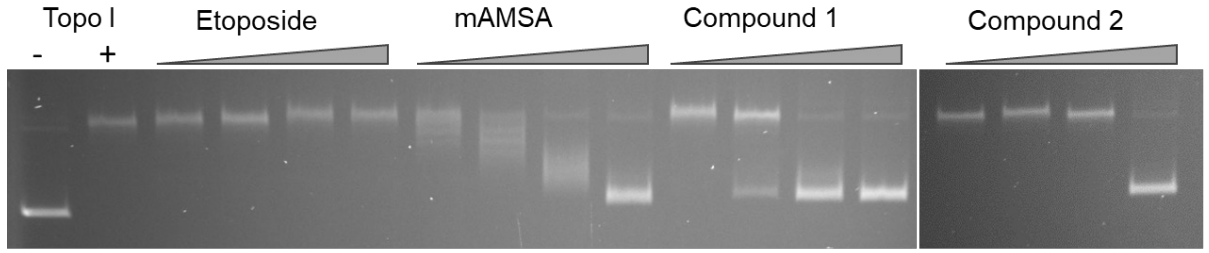
Calculated IC_{50} 16.15 µM

Case Study 3 – Mode of Action: Biochemical Assays

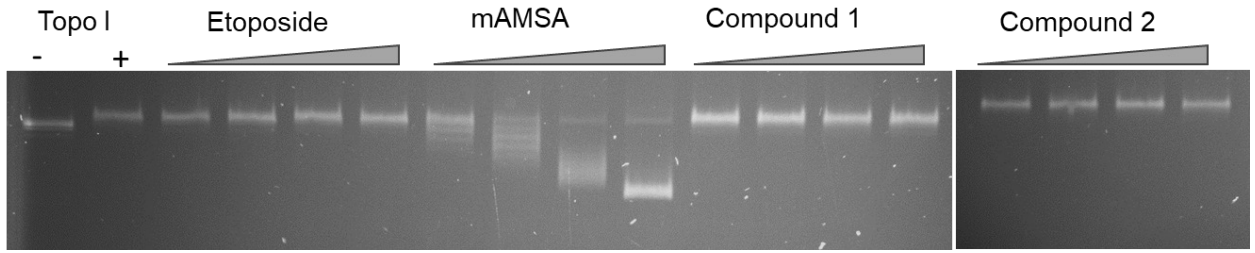
- Academic group – looking at novel Human topoisomerase inhibitors

Assay to check for Intercalation

Wheatgerm Topo I Unwinding Assay with Supercoiled pBR322 Substrate

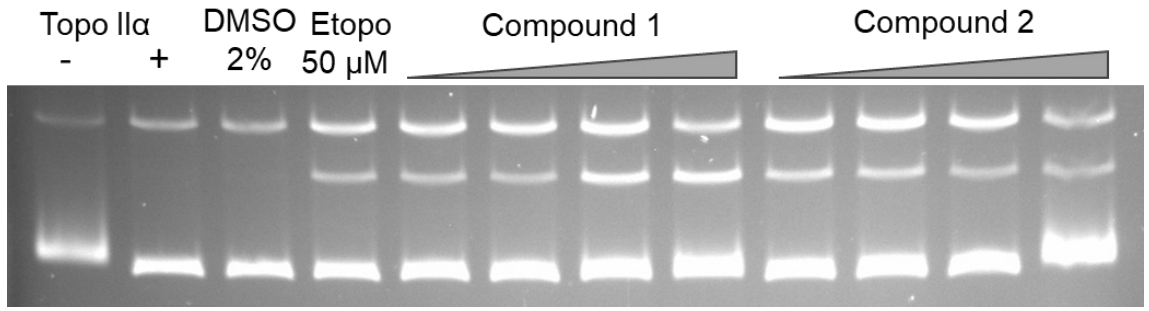


Wheatgerm Topo I Unwinding Assay with Relaxed pBR322 Substrate



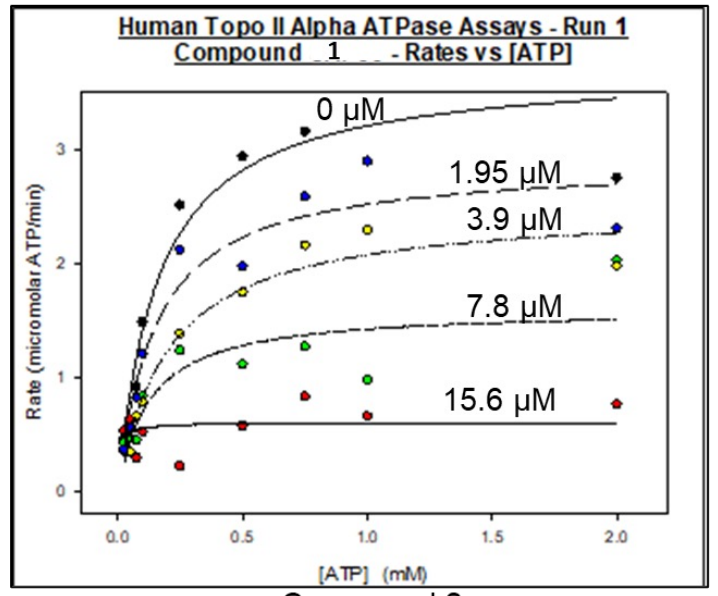
		% Linear
DNA alone		0
DNA + topo IIα		0
DNA + topo II +DMSO		0
Etoposide	50 µM	13.70
Compound 1	7.8 µM	12.14
Compound 1	15.6 µM	10.44
Compound 1	31.25 µM	22.80
Compound 1	125 µM	21.84
Compound 2	7.8 µM	16.87
Compound 2	15.6 µM	14.86
Compound 2	1.25 µM	10.47
Compound 2	125 µM	10.12

Competitive Cleavage Assay

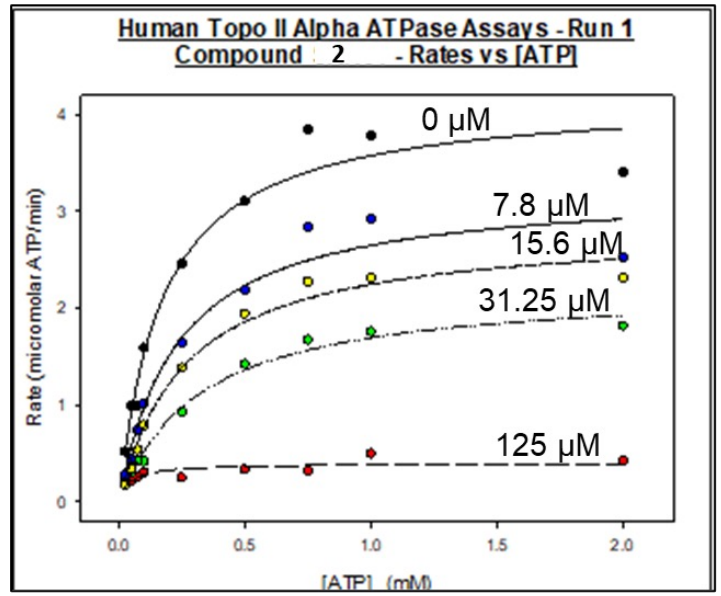


ATPase inhibition

Compound 1

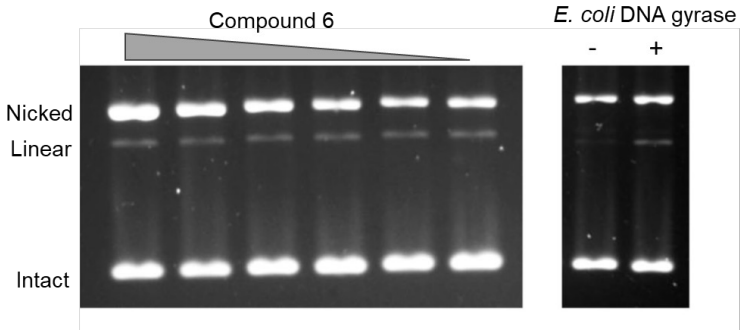


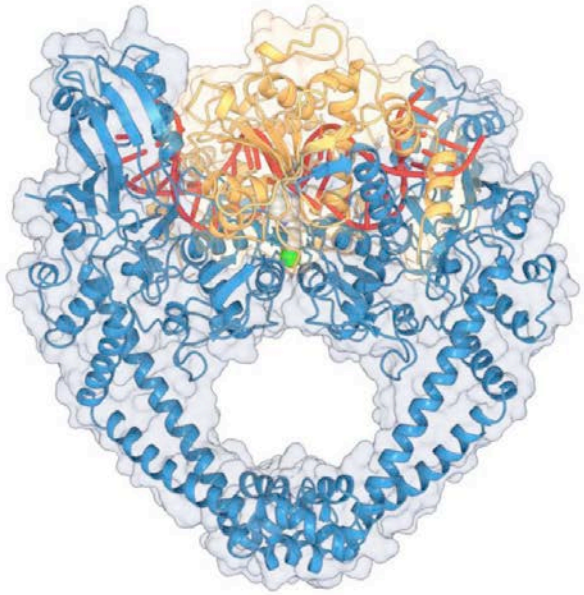
Compound 2

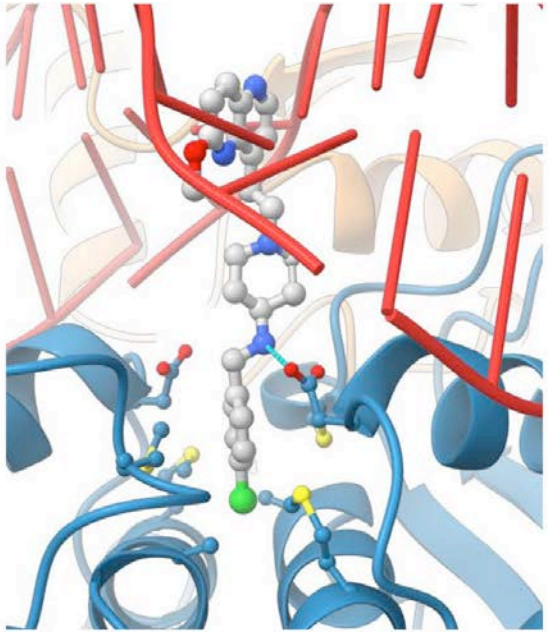


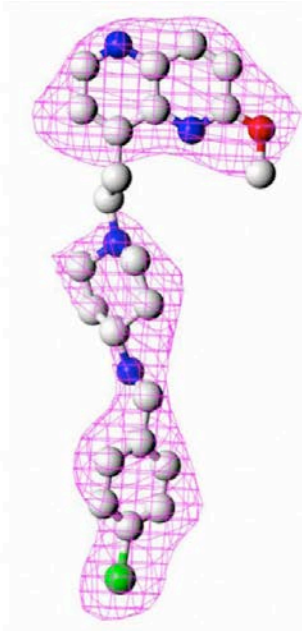
Case Study 4 – Mode of Action: Crystallography

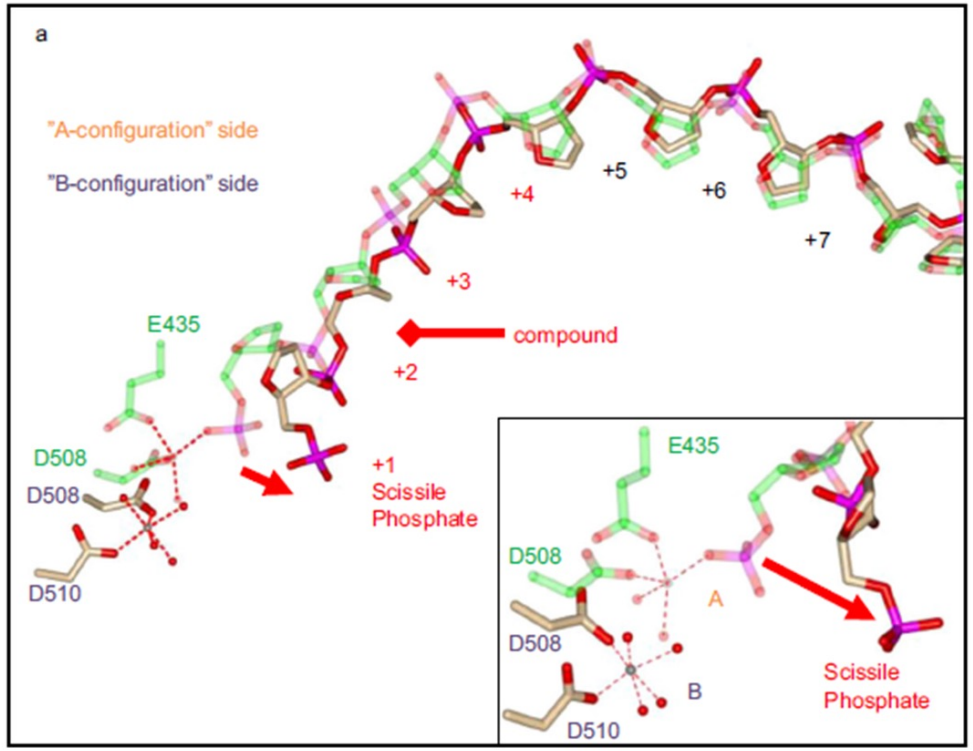
- Academic groups working on novel bacterial type II topoisomerase inhibitors (NBTI's)











Other projects of interest

- 10 000 compound screen in HTS assay – 22 hit compounds
- ENABLE (European Gram Negative AntiBacterial Engine) consortium
- Pharmaceutical company looking at Cardioprotective compounds against Doxorubicin
- Virtual Biotechs - working with in silico screening company

What we have learned as a CRO

- Drug discovery is challenging
- Projects can be pulled at the last minute
- Expectations need to be managed from the outset

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Thank You

Inspiralis values: Quality, consistency and collaboration