6th International Electronic Conference on Medicinal Chemistry
1-30 November 2020
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Isoquinolinequinone *N*-oxides as anticancer agents effective against drug resistant cell lines

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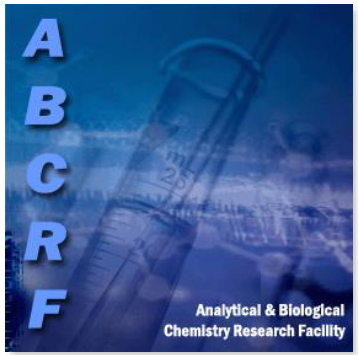

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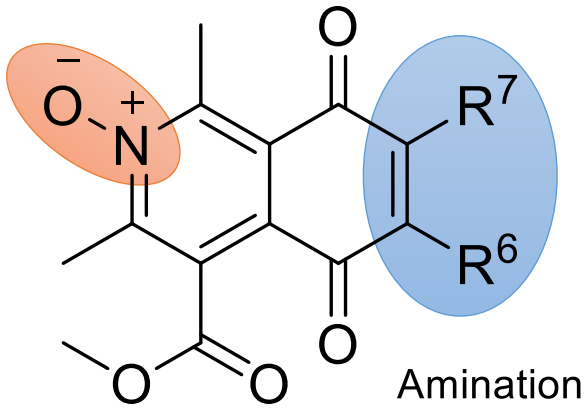
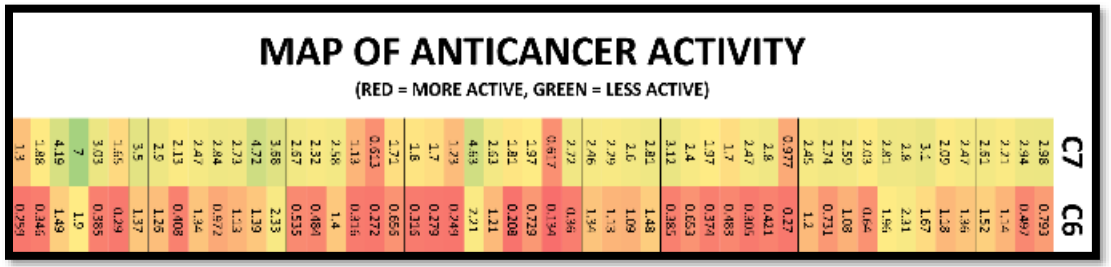
Coláiste na hOllscoile Corcaigh, Éire
University College Cork, Ireland

Isoquinolinequinone *N*-oxides as anticancer agents effective against drug resistant cell lines

Graphical Abstract



IQQ *N*-oxides

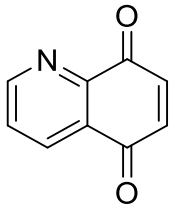


- Nanomolar anticancer activity against ovarian, melanoma and leukaemia tumour cell lines
- Adduct formation *in vitro;* redox cycling; MDR active

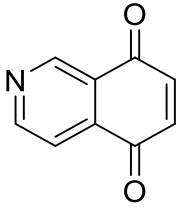
Abstract: The isoquinolinequinone (or isoquinoline-5,8-dione) pharmacophore is a privileged framework in known cytotoxic natural product families, caulibugulones and mansouramycins with notable anticancer properties. Exploiting both families as seeds for drug discovery, we report for the first time on the structured development of an isoquinolinequinone *N*-oxide anticancer framework which exhibits growth inhibition of cancer cells in the nM range across melanoma, ovarian and leukaemia cancer cell lines. A new lead compound (**16**, R6 = benzyl, R7 = H) exhibits nM GI50 values against 31/57 human tumour cell lines screened as part of the NCI60 panel and shows remarkable activity against doxorubicin resistant tumour cell lines. An electrochemical study highlights a correlation between electropositivity of the isoquinolinequinone *N*-oxide framework and cytotoxicity. Preliminary studies were conducted to identify adduct binding to sulfur based biological nucleophiles glutathione and cysteine observed in vitro pointing to a potential mechanism of action. This new framework possesses significant anticancer potential and is the subject of intensive efforts to probe the effect on multidrug resistant cancer cells.

Keywords: bioadduct formation; doxorubicin resistant cancer cells; isoquinolinequinone; multidrug resistant cell lines; *N*-oxide

Introduction: Quinolines and Quinones

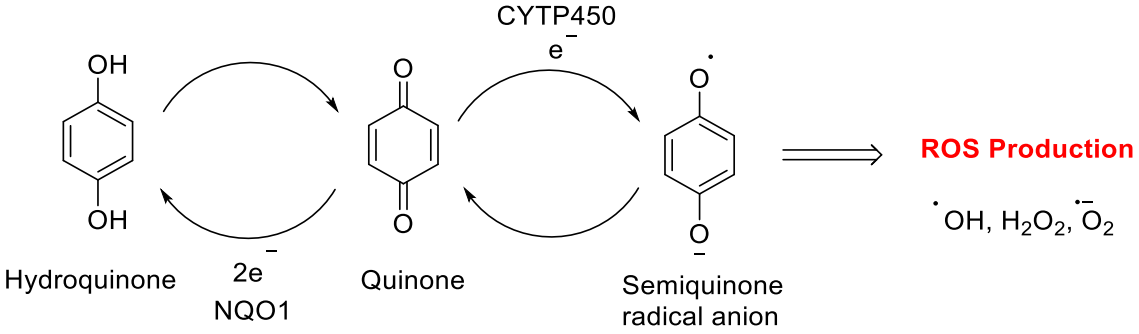


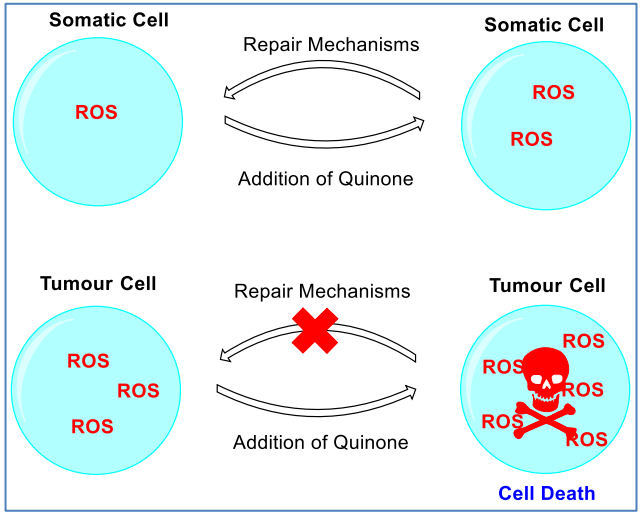
Quinoline-5,8-dione



Isoquinoline-5,8-dione

- Quinolines are natural products used in multiple applications
- Quinones are common substrates in drug design and especially in anticancer compounds
- Quinoline-5,8-dione and isoquinoline-5,8-dione are non-symmetrical substrates which leads to challenging chemistry for drug discovery
- Quinone bioactivity is related to redox cycling and the production of reactive oxygen species (ROS) in addition to electrophilicity and covalent adduct formation
- Due to cancer cell ROS levels interest in multidrug resistant cancers

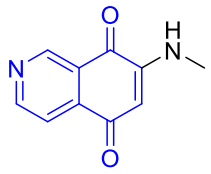




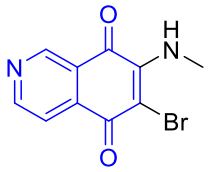
Isoquinolinequinones: Caulibugulones

- Caulibugulones are isolated as an isoquinolinequinone (IQQ) natural product[1]

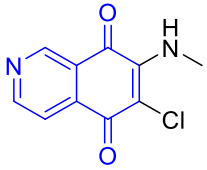
Caulibugulones (A-D)



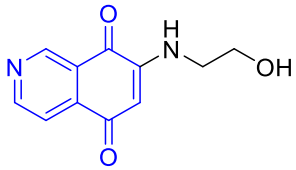
A (1.81 µM)



B (0.82 µM)



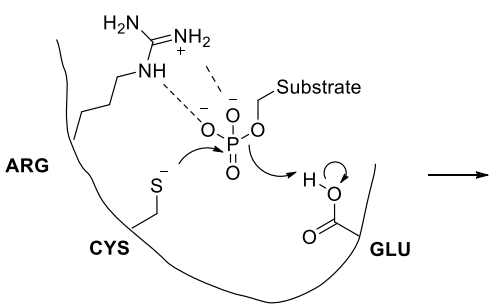
C (1.26 µM)



D (7.65 µM)

Caulibugula intermins[3]
Indo-Pacific off Palau

Caulibugulone	CDC25 inhibition[2] (µM)	
	CDC25A	CDC25B
A	3.38	6.74
B	**1.51**	**2.72**
C	2.57	5.40
D	4.89	32.5

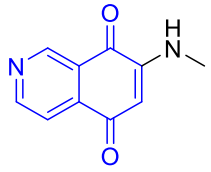


1. IC_{50} of **A-D** against the Murine IC-2WT Cell Line: Milanowski J. D., *et al*, *J. Nat. Prod.*, **2004**, (67), 70-73 2. Brisson M., *et al*, *Mol. Pharmacol.*, **2007**, (71), 184-192 3. http://www.ljaxphotos.com/photoGalleries

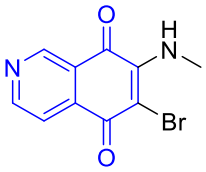
Isoquinolinequinones: Caulibugulones and Mansouramycins

- Caulibugulones are isolated as an isoquinolinequinone (IQQ) natural product[1]

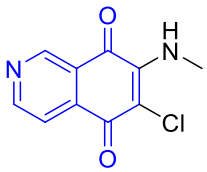
Caulibugulones (A-D)



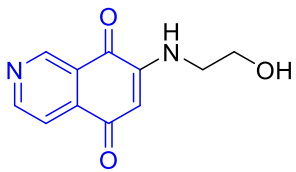
A (1.81 µM)



B (0.82 µM)



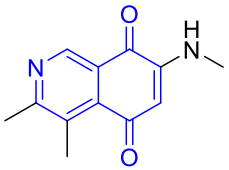
C (1.26 µM)



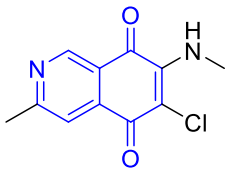
D (7.65 µM)

Caulibugula intermins[3]
Indo-Pacific off Palau

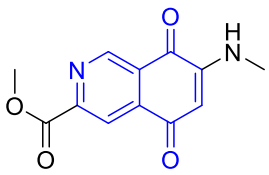
Mansouramycins (A-D)



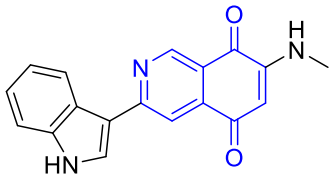
A (13.44 µM)



B (2.70 µM)



C (0.089 µM)



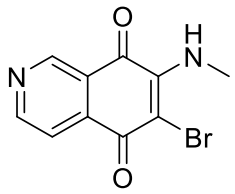
D (N/A)

Petrosia genus[4]
Kalampisauan Island, Philippines

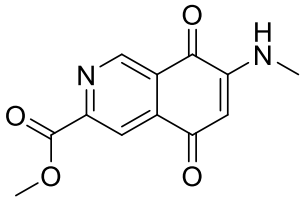
- Redox cycling (Mitochondrial Permeability)[5]; Cancer cell specific (Threshold Theory)

1. IC_{50} of **A-D** against the Murine IC-2WT Cell Line: Milanowski J. D., *et al*, *J. Nat. Prod.*, **2004**, (67), 70-73 2. Brisson M., *et al*, *Mol. Pharmacol.*, **2007**, (71), 184-192 3. http://www.ljaxphotos.com/photoGalleries
3. Average IC_{50}'s against 36 humour tumour cell lines: Hawas W. U., *et al.*, *J. Nat. Prod.*, **2009**, 72, 2120-2124 4. http://coo.fieldofscience.com/2015/09/petrosia-sexual-life-of-sponges.html 5. Kuang S., *et al.*, *Oncotarget*, **2017**, 8, 104057-104071

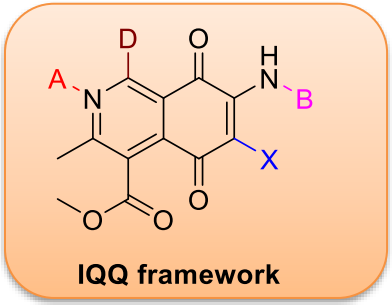
Aims and Objectives



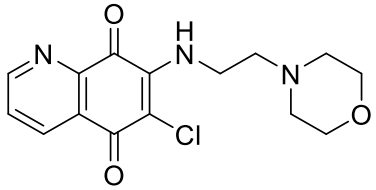
Caulibugulone B



Mansouramycin C

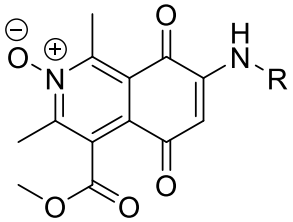


IQQ framework



NSC 663284

NSC 663284, an established Cdc25 inhibitor which shares a similar mechanism of action as caulibugulone family



N-oxide framework

Cytotoxicity Screening
- **NCI60** Cell Screen
- One Dose/Five Dose

***In Silico* Studies**
- COMPARE Analysis

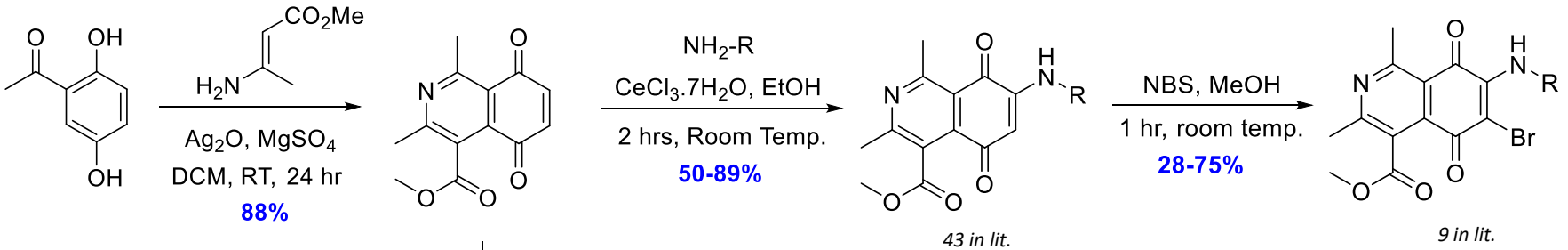
Electrochemical Assays
- Redox cycling
- Mechanism Identification

Clinical Development

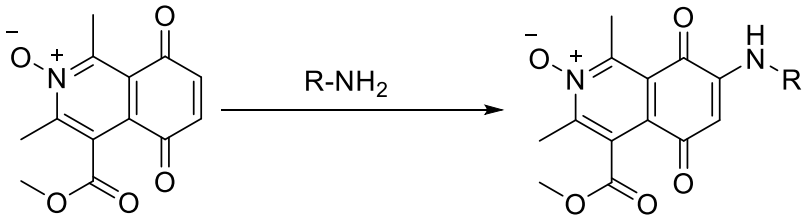
6. Kruschel, R. D., *et al.*, *Org. Biomol. Chem.*, **2020**, 18 (3), 557-568

Isoquinolinequinones: past and future

Previous work



This work

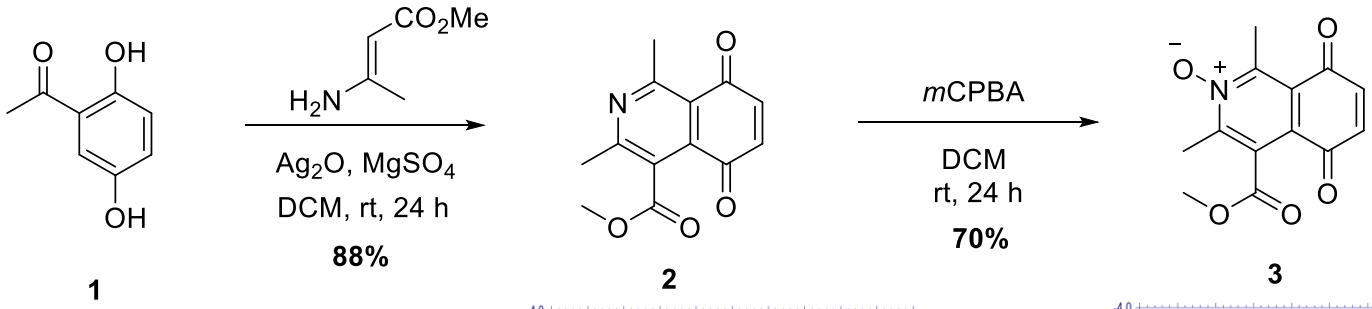


- The IQQ framework **2** was available as a starting point through known methodology
- Increase of electropositivity on brominated IQQ framework[7]
- Effect of *N*-oxide?

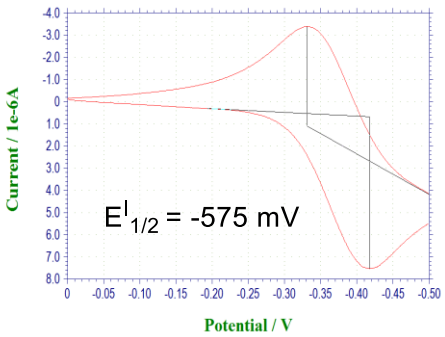
7. Ibacache J. A., *et al.*, *J. Chil. Chem. Soc.*, **2016**, 61, 3191-3194

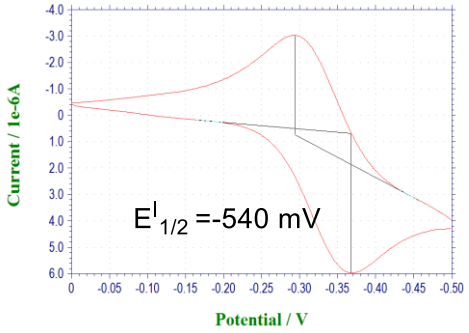
Introduction – Isoquinolines and quinones

- The IQQ framework **2** was synthesised as a starting point through known literature methodology. An IQQ *N*-oxide framework **3** was constructed utilising the *m*-CPBA oxidation of **2.**
- *N*-oxide addition results in a more electropositive IQQ, promoting redox ability and electrophilicity
- IQQ *N*-oxide exhibits a greater positive half wave potential ($E_{1/2}$) than IQQ.

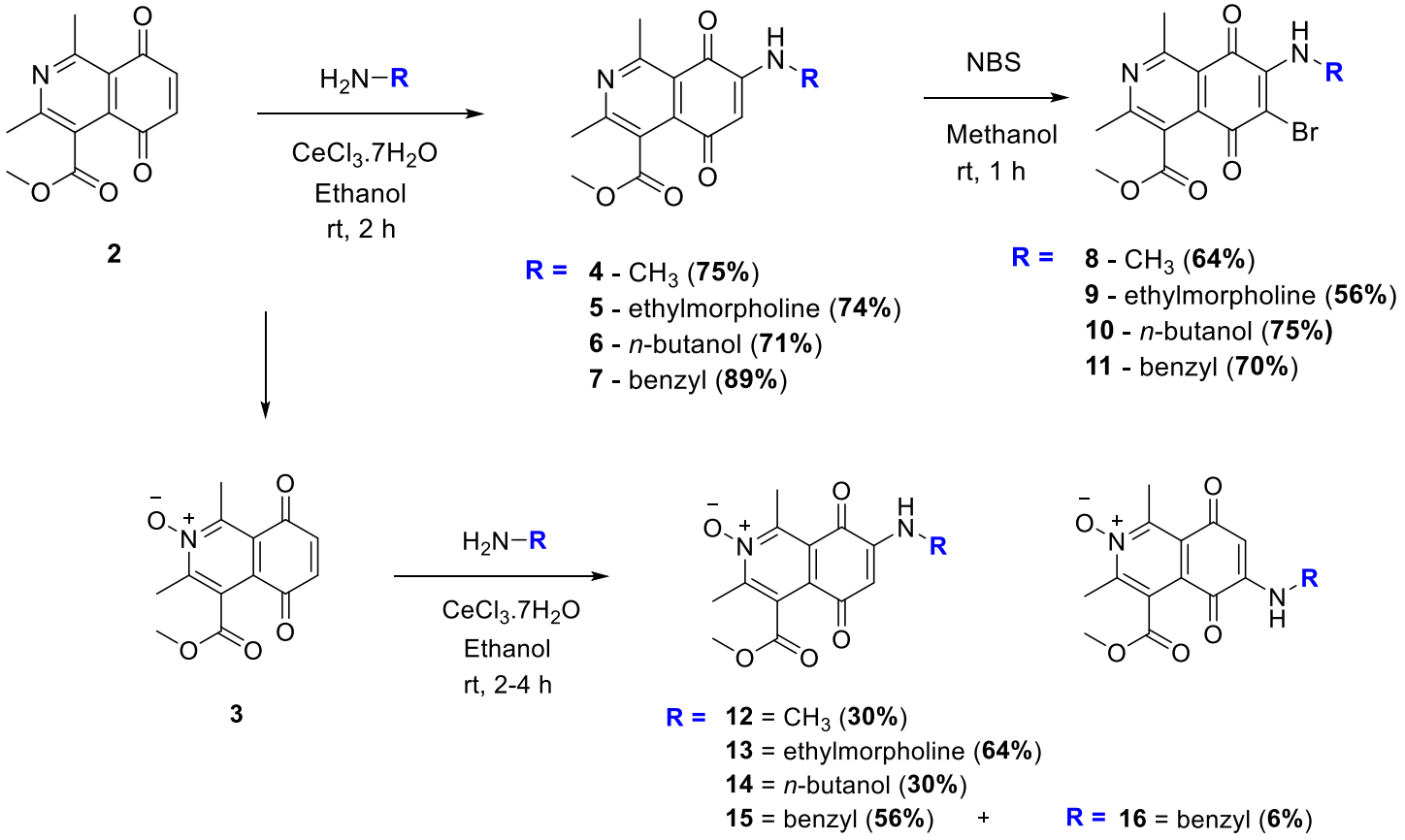


Scheme 1. Synthesis of the IQQ scaffold **2** and its subsequent oxidation with *m*-CPBA resulting in the novel IQQ *N*-oxide framework **3**.





Synthesis of Aminoisoquinolines and *N*-oxides



Scheme 2. Synthesis of novel derivatives of the IQQ scaffold **2** and the novel IQQ *N*-oxide framework **3**.

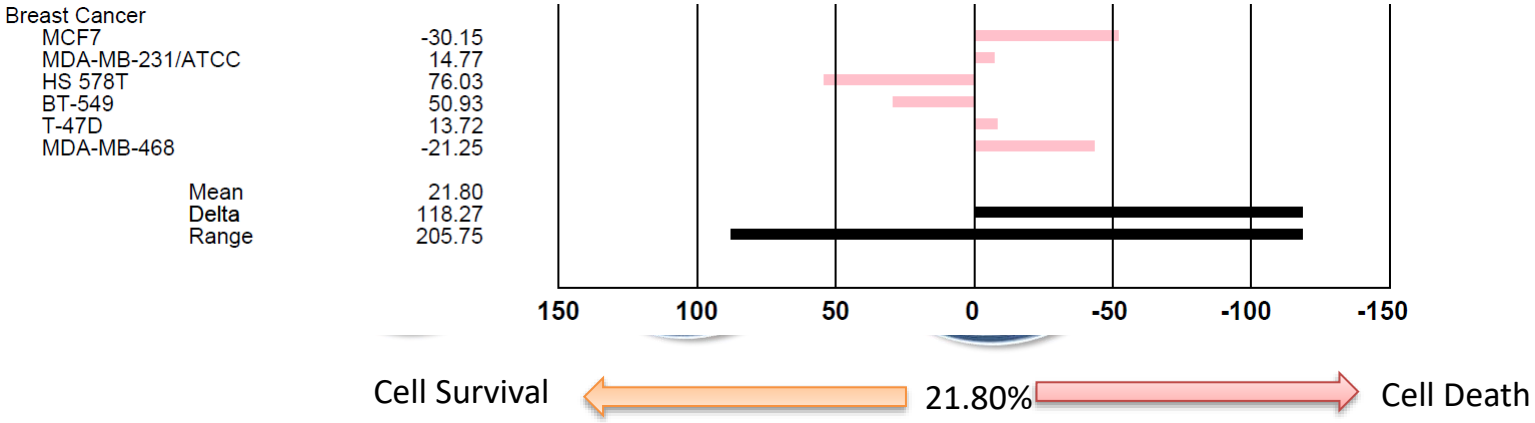
6. Kruschel, R. D., *et al.*, *Org. Biomol. Chem.*, **2020**, 18 (3), 557-568

In Vitro Anticancer Screening

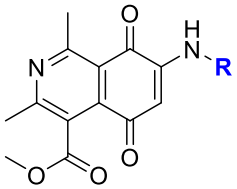
US National Cancer Institute 60 human tumour cell line panel (NCI60)

- Drug discovery tool with aim of identifying compounds which exhibit cytotoxic effects on particular tumours
- 60 tumour cell lines utilised from cancers such as renal, breast, CNS, melanoma, leukaemia, colon, lung, prostate and ovary

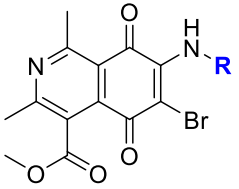
Screening Methodology



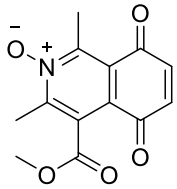
NCI Cancer Cell Growth
- One dose screen at 10μM



R = **4** - CH_3
5 - ethylmorpholine
6 - *n*-butanol
7 - benzyl



R = **8** - CH_3
9 - ethylmorpholine
10 - *n*-butanol
11 - benzyl



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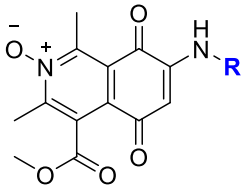
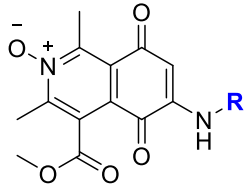


R = **12** = CH_3
13 = ethylmorpholine
14 = *n*-butanol
15 = benzyl



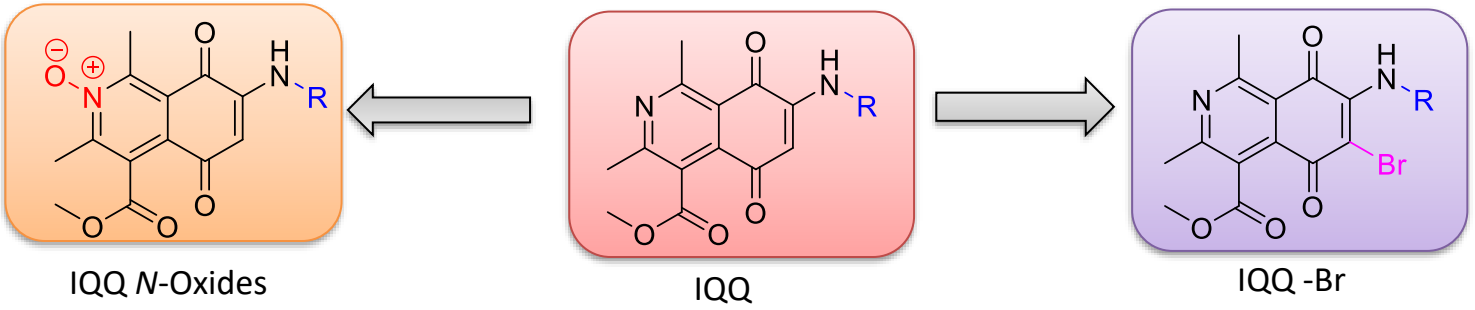
R = **16** = benzyl

Series	Compound	Mean (%) Growth (%)	Growth of selected cell lines (%)					
			MCF-7	COLO-205	MALME-3M	SK MEL-5	OVCAR-3	OVCAR-4
IQQ 6H	**4**	102	95	106	59	84	113	88
	5	97	81	96	65	86	95	81
	6	91	90	76	67	88	89	86
	7	83	76	79	-2	51	44	65
IQQ 6Br	**8**	59	45	31	-27	-91	2	35
	9	93	82	56	37	71	13	74
	10	64	38	17	-3	-53	2	38
	11	22	-30	-12	-75	-96	-13	-57
IQQ NOX 6H	**3**	-29	-59	-78	-88	-99	-9	-17
	12	61	57	8	-46	-48	7	26
	13	73	56	7	-13	49	5	35
	14	94	82	60	33	66	71	76
	15	20	-34	-9	-78	-85	1	-79
7H	**16**	-35	-62	-37	-97	-98	-35	-95

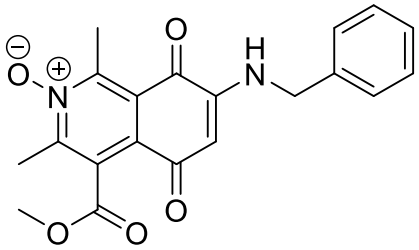
Table 1. Preliminary cytotoxic NCI60 one-dose mean % cell growth

6. Kruschel, R. D., *et al.*, *Org. Biomol. Chem.*, **2020**, 18 (3), 557-568

Isoquinolines and quinones: Anticancer screen



Isoquinolines and quinones: Anticancer screen



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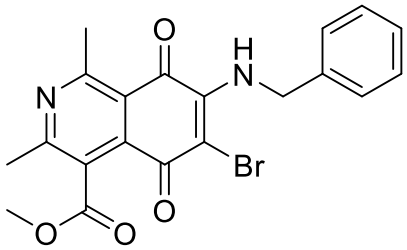


11

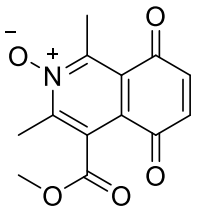
Entry	Mean	Breast	Leukaemia	Colon	Melanoma		Ovarian		NSCLC
	(56 cell)	MDA-MB*	CCRF-CEM	COLO-205	MAL**	MDA#	OVCAR-3	OVCAR-4	HOP-62
15	2.52	1.30	2.98	0.977	0.617	1.81	0.613	1.13	3.10
11	2.93	1.10	3.11	1.70	1.47	1.86	2.77	1.55	3.12

Entry	Imatinib	Lapatinib	Bleomycin	Mitomycin C	Doxorubicin	Etoposide	Cisplatin
GI_{50} (Mean)[8]	15	2.9	1.3	0.71	0.097	6.6	1.4

8. Holbeck S. L., *et al*., *Mol. Cancer. Ther*., **2010**, 9(5), 1451-1460

All figures are of µM concentration. Abbreviation: *MDA-MB-231/ATCC, **MALME-3M, #MDA-MB-435

Comparison of 5 Dose anticancer activity



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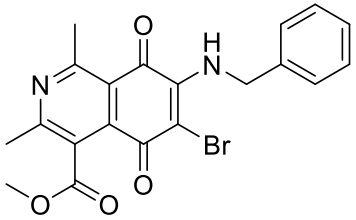

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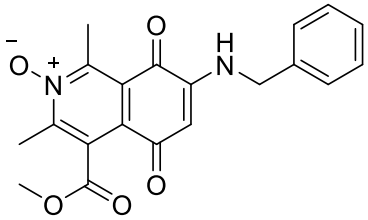

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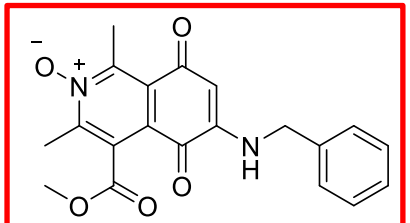


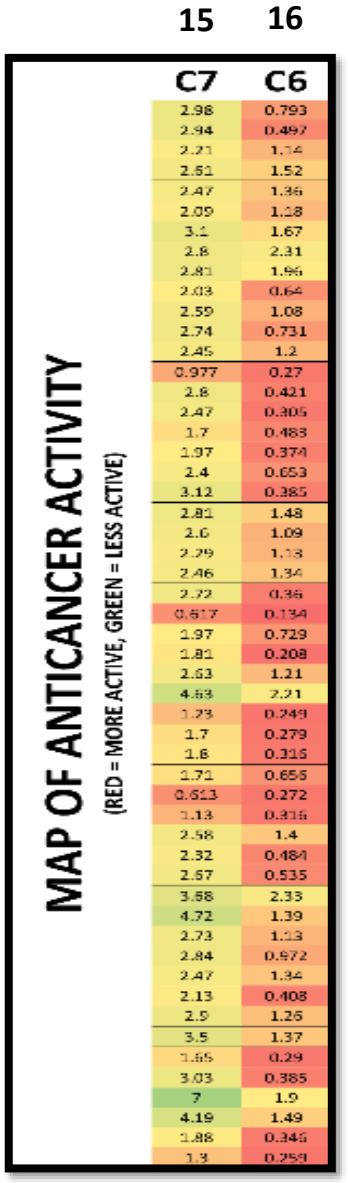
16

Entry	3	11	15	16
Mean GI_{50}	1.75	2.93	2.52	0.91
Mean LC_{50}	28.30	42.14	52.99	23.86

Table 2. GI_{50}'s of active IQQ benzyl amine analogues across multiple cancer cell lines

All figures are of μM concentration. Mean GI_{50} was calculated for 53 common human tumour cell lines. Mean LC_{50} was calculated for 50 common cancer cell lines.

- 7-Benzylamine isomer **16** most potent and identified with excellent MDR potential
- NCI/ADR-RES expresses a high level of multidrug resistance and *P*-glycoprotein
- Addition of benzylamine to the 6-position overcomes doxorubicin resistance resulting in a GI_{50} for **16** (0.54 μM) less than 40 times than that of doxorubicin



Heat map of potency

- Mean GI_{50} (left) and LC_{50} (right)was calculated from 53 human cancer cell lines common across the four compounds **3**, **11**, **15** and **16**.
- Conditional formatting was applied to the GI_{50} values per compound row with the colour green representing more un-responsive cell lines and red representing more-responsive cell lines.
- Remarkable potency seen for **16.**

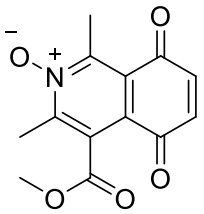
	3	11	15	18
CCRF-CEM	0.0559	3.11	2.98	0.793
K-562	3.04	3.36	2.94	0.497
MOLT-4	1.69	3.09	2.21	1.14
RPMI-8226	2.07	3.24	2.61	1.52
A549/ATCC	3.12	2.56	2.47	1.36
EKVX	2.02	2.12	2.09	1.18
HOP-62	0.744	3.12	3.1	1.67
HOP-92	2.12	2.46	2.8	2.31
NCI-H226	1.42	4.01	2.81	1.96
NCI-H23	1.63	1.89	2.03	0.64
NCI-H322M	1.68	3.45	2.59	1.08
NCI-H460	2.72	2.94	2.74	0.731
NCI-H522	1.66	2.25	2.45	1.2
COLO-205	1.64	1.7	0.977	0.27
HCC-2998	1.64	3.4	2.8	0.421
HCT-116	0.945	2.5	2.47	0.305
HCT-15	1.43	2.76	1.7	0.483
HT-29	2.41	2.97	1.97	0.374
KM12	1.99	2.93	2.4	0.653
SW-620	1.54	3.39	3.12	0.385
SF-268	2.05	3.46	2.81	1.48
SF-295	1.78	3.04	2.6	1.09
SF-539	1.8	2.85	2.29	1.13
U251	1.48	2.74	2.46	1.34
LOX IMVI	1.44	2.45	2.72	0.36
MALME-3M	1.53	1.47	0.617	0.134
M14	1.78	2.06	1.97	0.729
MDA-MB-435	1.79	1.86	1.81	0.208
SK-MEL-2	1.89	2.52	2.63	1.21
SK-MEL-28	1.96	5.79	4.63	2.21
SK-MEL-5	1.14	1.47	1.23	0.249
UACC-257	1.57	1.62	1.7	0.279
UACC-62	1.63	1.41	1.8	0.316
IGROV1	1.74	2.05	1.71	0.656
OVCAR-3	0.314	2.27	0.613	0.272
OVCAR-4	1.2	1.55	1.13	0.316
OVCAR-5	1.76	3.11	2.58	1.4
OVCAR-8	0.339	2.51	2.32	0.484
NCI-ADR/RES	2.33	2.83	2.67	0.535
786-0	2.07	4.65	3.68	2.33
A498	3.58	4.48	4.72	1.39
ACHN	1.9	3.08	2.73	1.13
CAKI-1	1.95	5.66	2.84	0.972
RXF 393	1.42	3.08	2.47	1.34
SN12C	1.62	1.49	2.13	0.408
UO-31	1.69	2.41	2.9	1.26
DU-145	1.82	4.26	3.5	1.37
MCF7	1.55	1.3	1.65	0.29
MDA-MB-231/ATCC	2.68	2.11	3.03	0.385
HS 578T	2.84	10.8	7	1.9
BT-549	1.72	4.08	4.19	1.49
T-47D	1.97	2.26	1.88	0.346
MDA-MB-468	0.946	1.1	1.3	0.259
Mean GI_{50}	1.75	2.93	2.52	0.91

	3	11	15	16
CCRF-CEM	100	100	100	100
K-562	100	100	100	100
RPMI-8226	100	100	100	100
A549/ATCC	55.3	43.5	66.1	75.4
EKVX	22.2	24.4	28.7	5.55
HOP-62	5.79	33.5	100	6.67
HOP-92	23.8	22.7	56.5	100
NCI-H226	6.26	74.9	77.8	8.87
NCI-H23	100	100	100	5.52
NCI-H322M	5.62	33.4	100	26
NCI-H460	49.9	63.7	100	9.16
NCI-H522	8.11	29.8	52.9	7.68
COLO-205	6.85	7.73	7.19	4.17
HCC-2998	5.95	40	32.8	4.55
HCT-116	46	27.5	34.2	5.22
HCT-15	7.69	30.1	15.7	5.84
HT-29	100	100	100	100
KM12	15.1	62.2	73.1	30.5
SW-620	9.09	42.8	67	6
SF-268	27.6	54.1	100	27.2
SF-295	6.28	34.3	67.4	5.1
SF-539	5.68	28.4	15.8	4.9
U251	5.4	31.9	46	7.15
LOX IMVI	6.42	79.1	66.2	4.45
MALME-3M	5.57	5.55	4.43	0.789
M14	6.69	13.8	9.58	6.19
MDA-MB-435	6.13	6.15	6.01	0.798
SK-MEL-2	8.41	29.5	46.9	6.12
SK-MEL-28	6.62	44.2	100	10.2
SK-MEL-5	5.07	5.54	5.1	2.6
UACC-257	7.11	6.3	9.49	4.33
UACC-62	6.3	6.12	7.05	4.82
IGROV1	11.8	29.2	35.2	6.19
OVCAR-3	22.2	24.6	29.3	3.28
OVCAR-4	5.08	5.8	5.07	3.83
OVCAR-5	5.91	33.4	24.1	5.58
OVCAR-8	4.54	76.8	31	15.3
NCI-ADR/RES	100	100	100	73.8
786-0	9.84	41.1	100	36.1
A498	38.1	42.2	40.9	5.4
CAKI-1	9.05	44	50.4	17.7
RXF 393	5.84	35.7	41.2	6.43
SN12C	5.93	8	20.6	4.21
UO-31	6.8	33	32.7	5.13
DU-145	5.69	38.5	33.9	5.31
MDA-MB-231/ATCC	100	33.3	100	100
HS 578T	100	100	100	100
BT-549	5.73	40.1	41.5	6.5
T-47D	100	33.7	59.9	8.21
MDA-MB-468	7.48	6.87	7.61	4.28
Mean LC_{50}	28.3	42.14	52.99	23.86

Table 3. Five-Dose GI_{50} trend analysis of **3**, **11**, **15** and **16** against the full panel of human tumour cell lines. Red = most responsive cell lines, Green = least responsive cell line. Values are shown in µM.

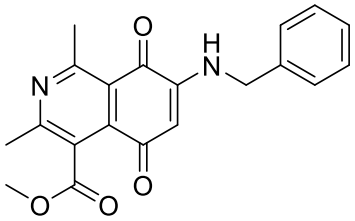
6. Kruschel, R. D., *et al.*, *Org. Biomol. Chem.*, **2020**, 18 (3), 557-568

Probing the mechanism of action – biophysical



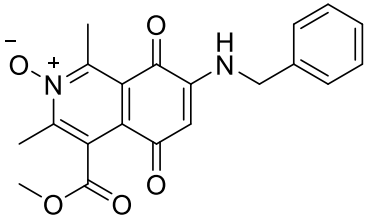
3

$E^{I}_{1/2}$ =-540 mV



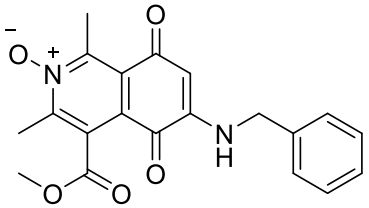
7

$E^{I}_{1/2}$ (mV) = -427



15

$E^{I}_{1/2}$ (mV) = -408



16

$E^{I}_{1/2}$ (mV) = -384

Figure 6. The increasing half-wave potential ($E^{I}_{1/2}$) correlates to the increase in anticancer activity for **7**,**15** and **16**

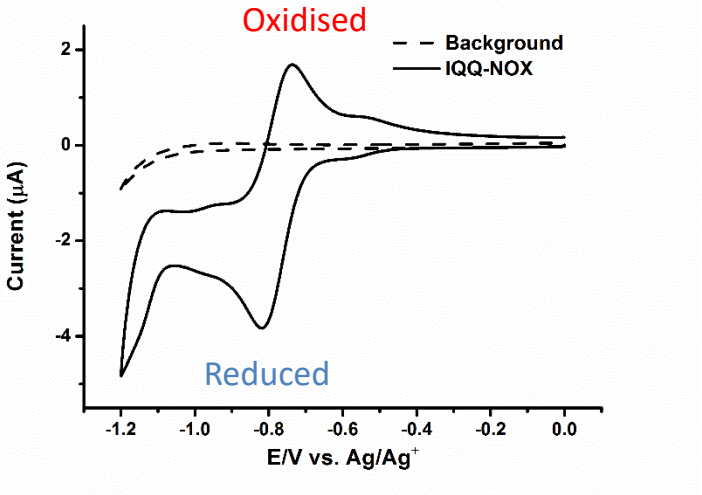


Table 5. Binding IQQ analogues to biological nucleophiles at 37°C

Entry	Adduct Formation		
	Cysteine	Glutathione	Serine
2	✓	✓	✓
3	✓	✓	✓
7	✓	✓	×
11	✓	✓	×
15	✓	✓	×
16	✓	✓	×

✓ = forms adduct, × = no adduct observed. Assay conditions: IQQ (2 mM) incubated with nucleophile (3 mM) in water/methanol at 37°C for 12 hours. The resulting mixture was qualitatively screened using LC/MS for the identification of the adducts. In all cases mono-adduct formation was observed.

Probing the mechanism of action – COMPARE Analysis

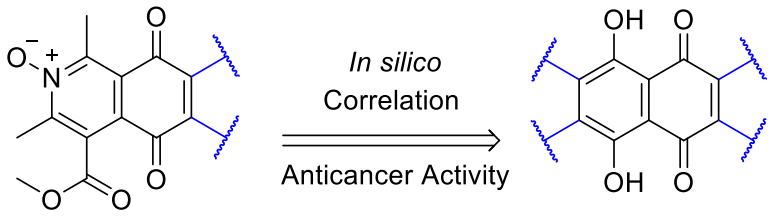
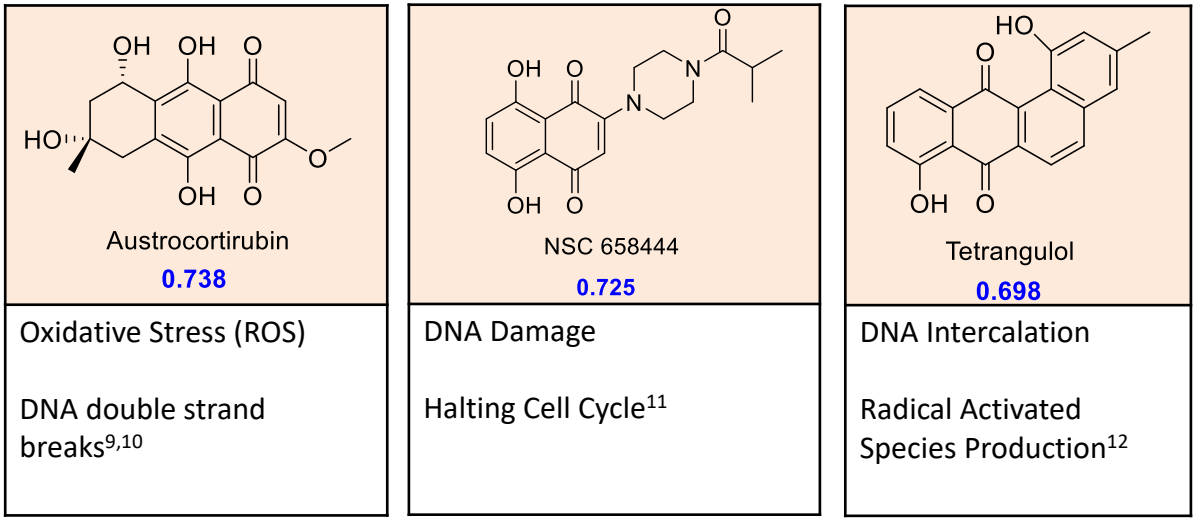


Figure 7. The highest correlations identified by COMPARE analysis were tetrangulol, (1*S*,3*S*)-austrocortirubin and **NSC 65844** which all harbour the naphthazarin framework.



9. Kim J.A., *et al.*, *Int. J. Oncol.*, **2012**, 40, 157-162
10. Zhang J., *et al.*, *Eur. J. Med. Chem.*, **2017**, 10, 435-447
11. Wang Y., *et al.*, *Bioorg. Med. Chem. Lett.*, **2015**, 25, 249-253
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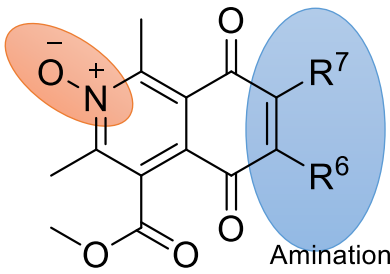
Conclusions



- New IQQ *N*-oxide framework **3** with outstanding activity in the NCI60 screen.
- First single crystal X-ray structure confirmation of the C(7) amination of the IQQ pharmacophore **2**.
- Anticancer activity of three series screened at 10 µM; 6-Bromo substitution leads to a significant increase in cytotoxicity by 32% on average; *N*-oxide moiety equipotent with activity of 6-Br
- IQQ *N*-Oxide **3** and benzylamines **11**, **15** and **16** were selected for five-dose screening.
- C(6) Benzylamine IQQ *N*-oxide **16** exhibited nM GI50 values against 31/57 human tumour cell lines
    - Most responsive cancers identified being ovarian, melanoma, breast and colon
    - Exhibits a GI50 of 535 nM against the doxorubicin resistant tumour cell line NCI/ADR-RES.
- Using CV, identified IQQ *N*-oxide **16** as the most electropositive species in the benzylamine series
    - Suggests correlation between the nature of quinone, redox potential and anticancer activity.
- Adduct formation studies revealed that IQQ frameworks **2** and **3** formed oxygen and sulfur based biological adducts *in vitro*, however, for benzylamine only sulfur based adducts were isolated

Acknowledgments

- Funding
  - Higher Education Authority
  - Irish Research Council
- Quinoline team
  - Ryan Kruschel PhD
  - Kieran Greaney PhD
  - National Cancer Institute
  - Dr Udaya Khandavilli and Dr Simon Lawrence – Single crystal X-ray diffraction
  - Dr Alyah Buzid and Dr Jeremy Glennon - Electrochemistry