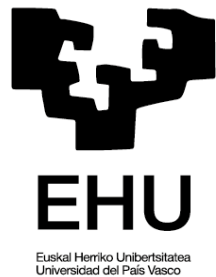


Working in multidisciplinary teams: From Particle physics to oncology

Fernando P. Cossío

The University of the Basque Country (EHU)

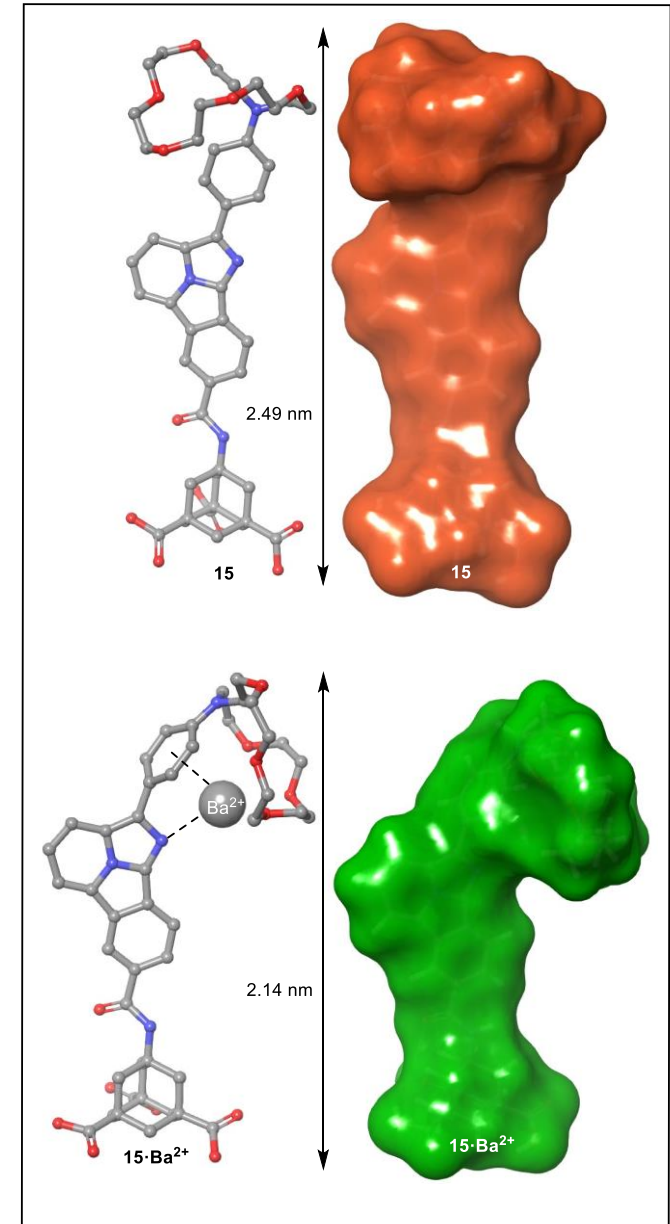
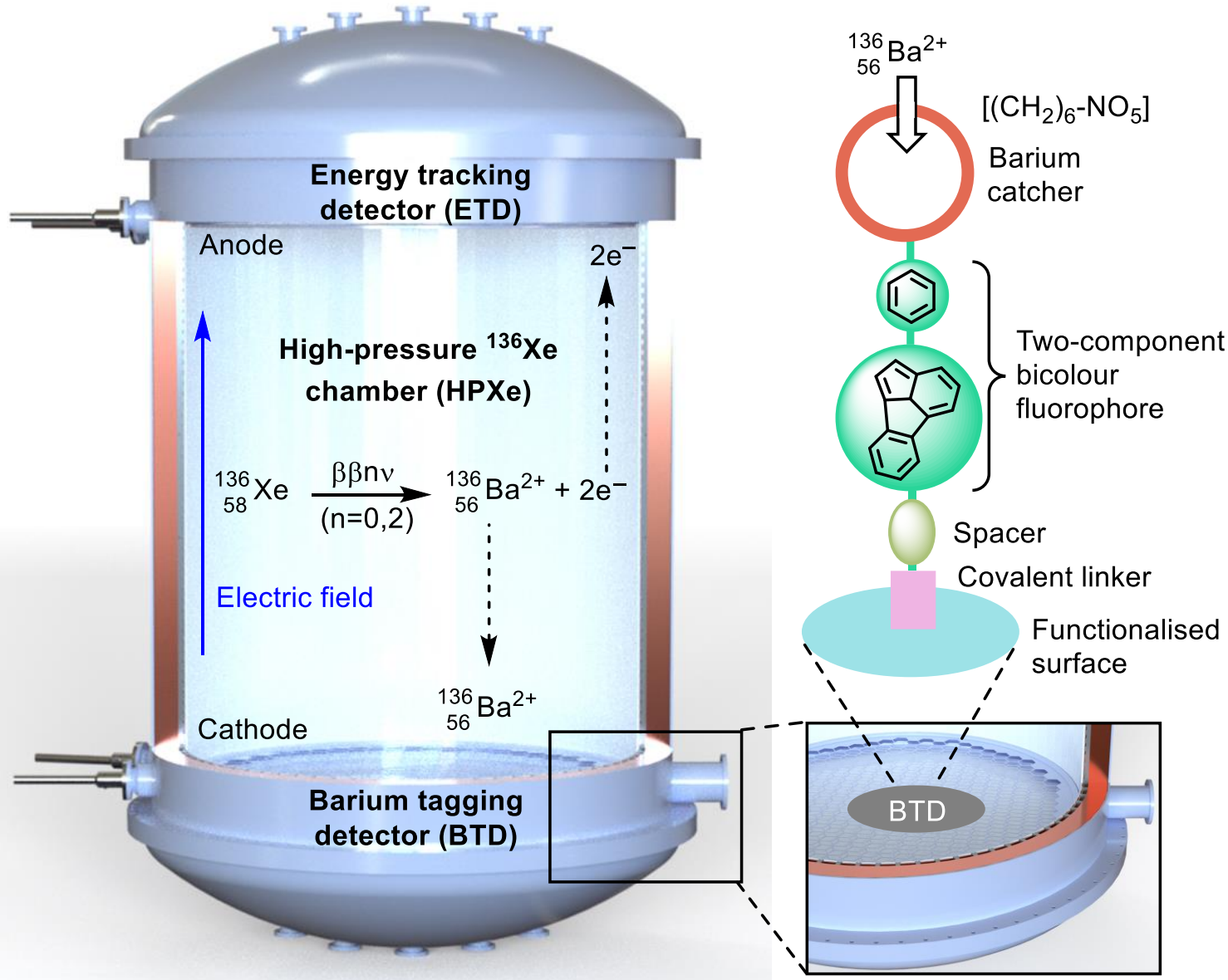


Considerations of use?

Quest for fundamental understanding?

		NO	YES
Quest for fundamental understanding?	NO	Uh... 	Pure applied research (Edison) 
	YES	Pure basic research (Bohr) 	Use-inspired basic research (Pasteur) 

Barium Tagging for $0\nu\beta\beta$: The BOLD Project

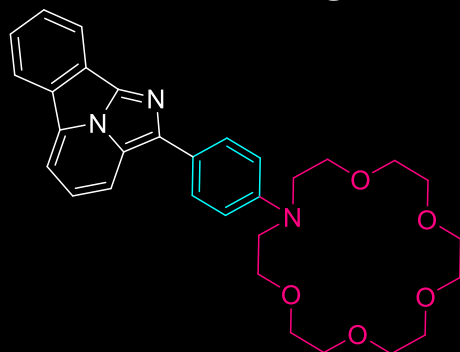


The situation is hopeless, but not serious

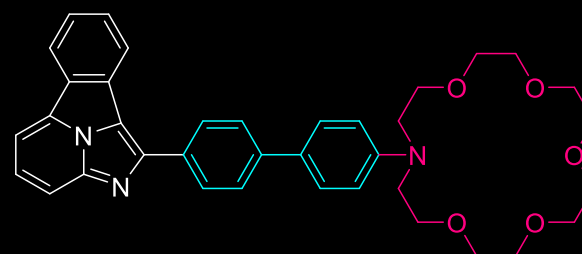
FBI-G1



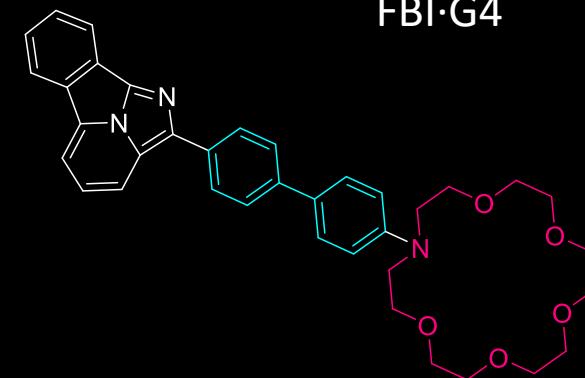
FBI-G2



FBI-G3



FBI-G4



Nature **2020**, 583, 48

Phys. Chem. Chem. Phys. **2021**, 23, 15440

Nat. Commun. **2022**, 13, 7741

Nat. Commun. **2024**, 15, 10595

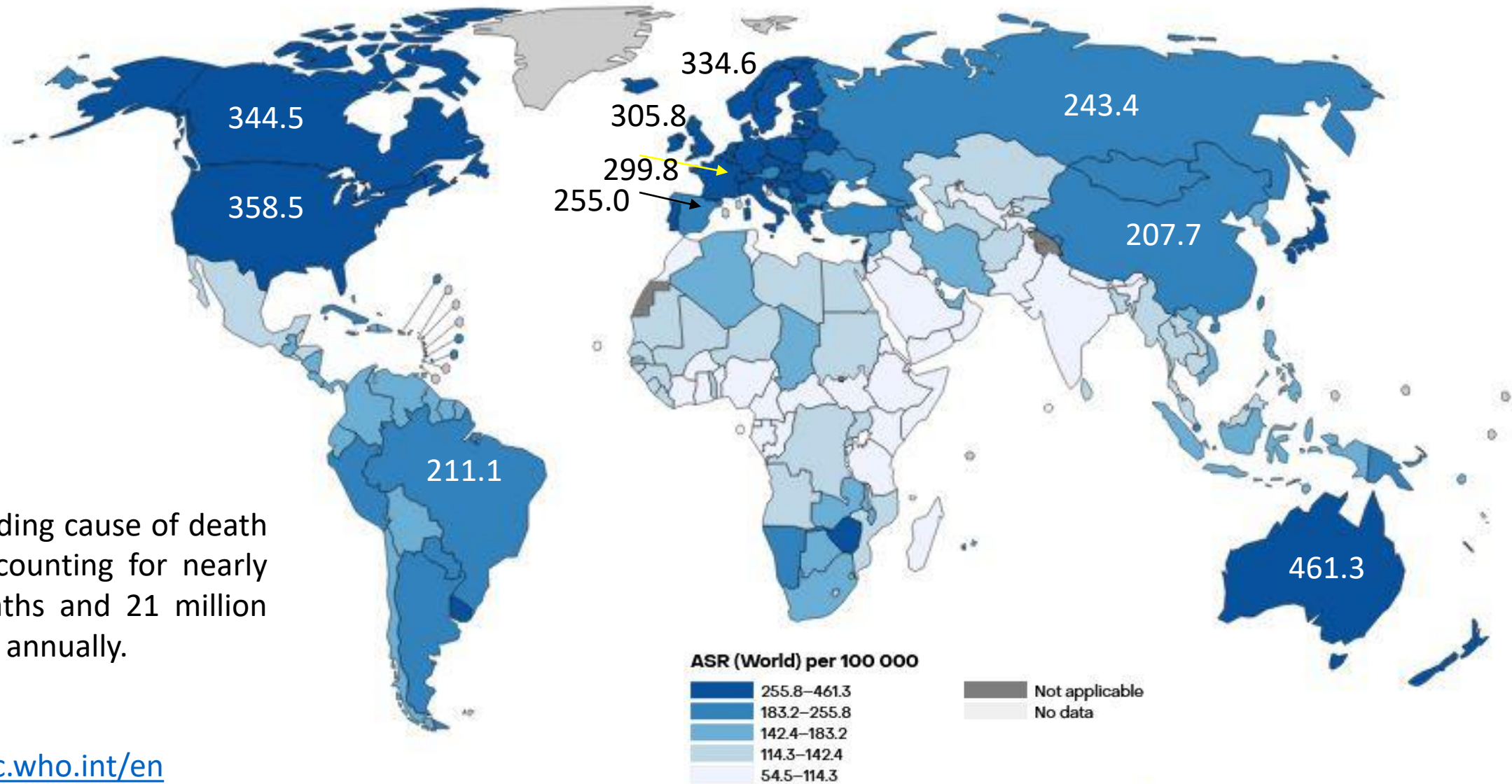
RSC Applied Interfaces **2024**, 2, 185

ACS Sensors **2025**, 10, 2487

Beilstein J. Org. Chem. **2025**, 21, 162

1. The challenge

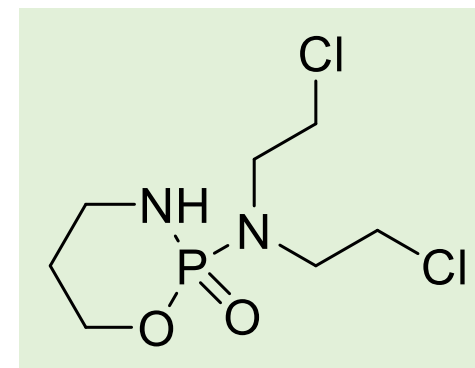
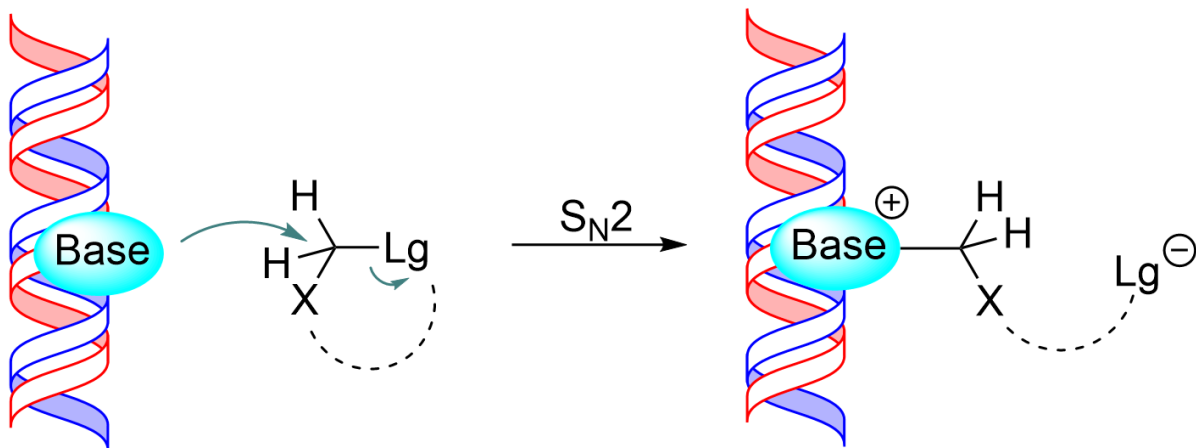
- “The Big C”: Age-Standardized Rate (World) per 100 000, Incidence, both sexes, in 2024



Cancer is a leading cause of death worldwide, accounting for nearly 10 million deaths and 21 million new diagnoses annually.

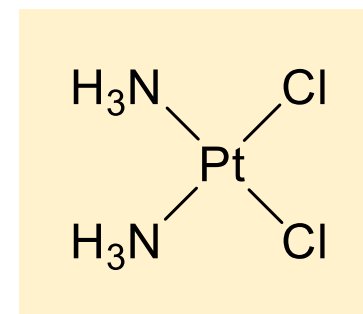
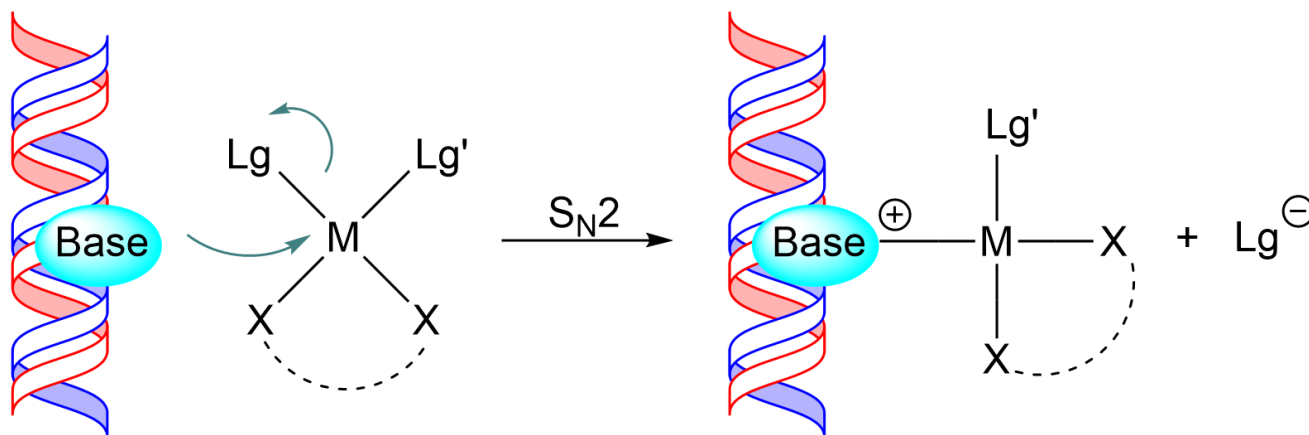
Current chemotherapy: platins and nitrogen mustards

- Nucleophilic substitution reaction (S_N2) of DNA
 - Carbon electrophiles (inversion)



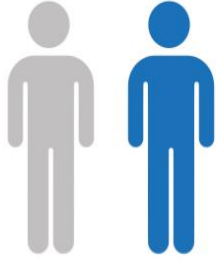
Cyclophosphamide

- Square planar electrophiles (retention)



Cisplatin

Current chemotherapy is not enough



1 in 2

cancer patients receives
platinum-based therapy

However, ...



High toxicity

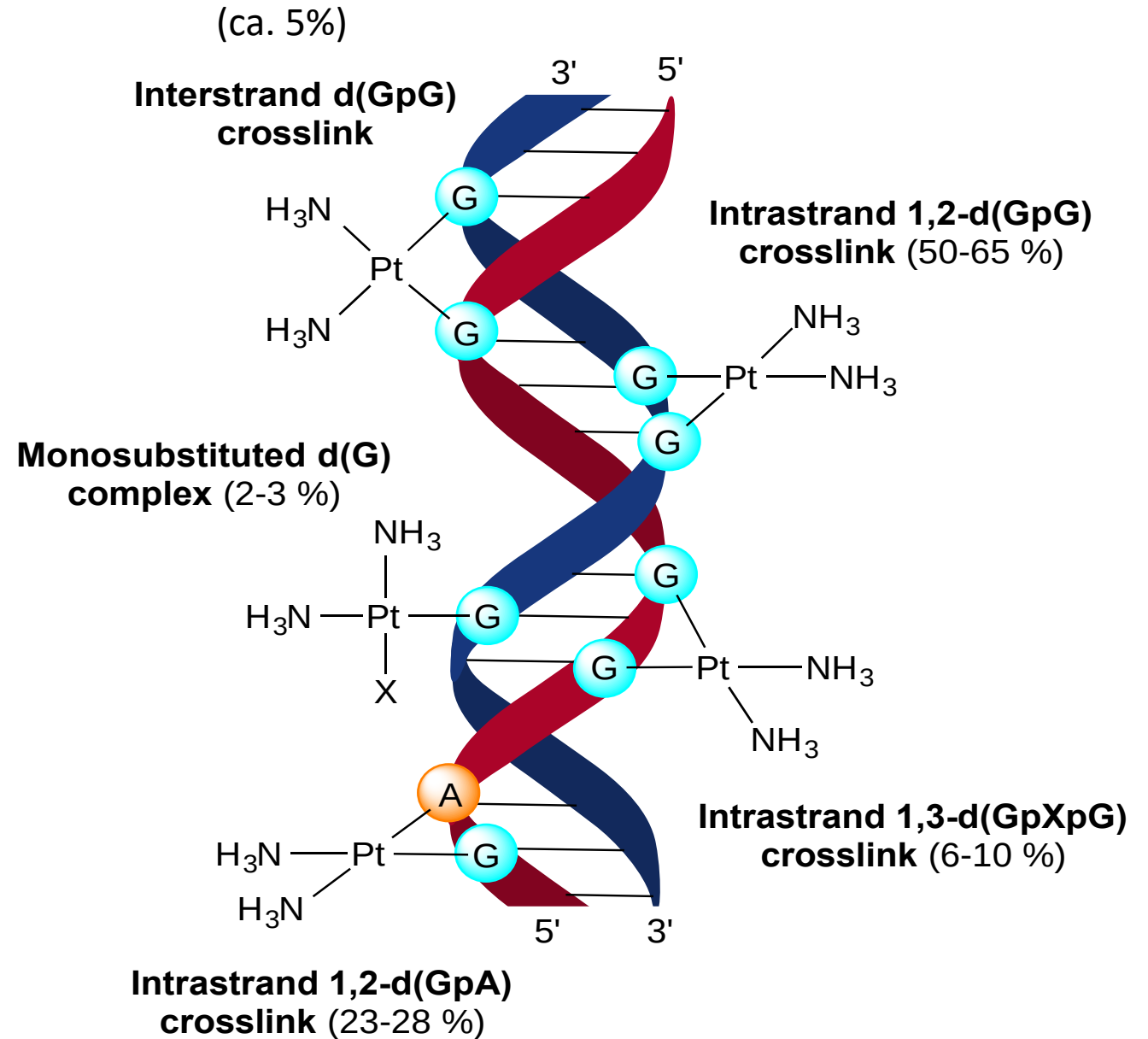
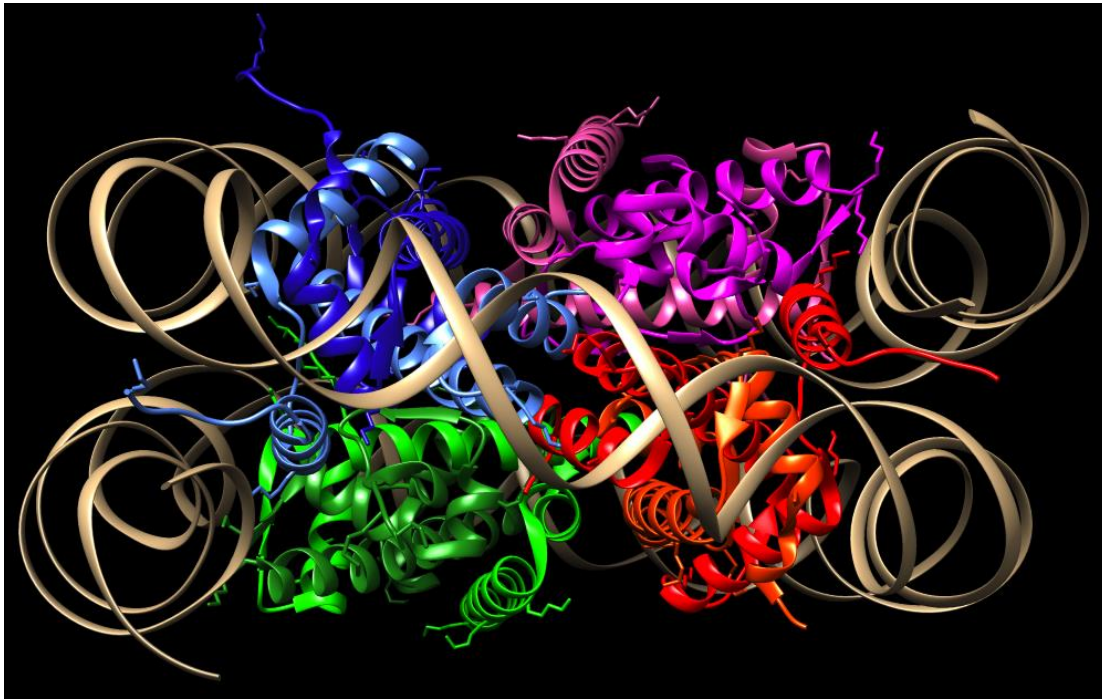


~70%
develop resistance

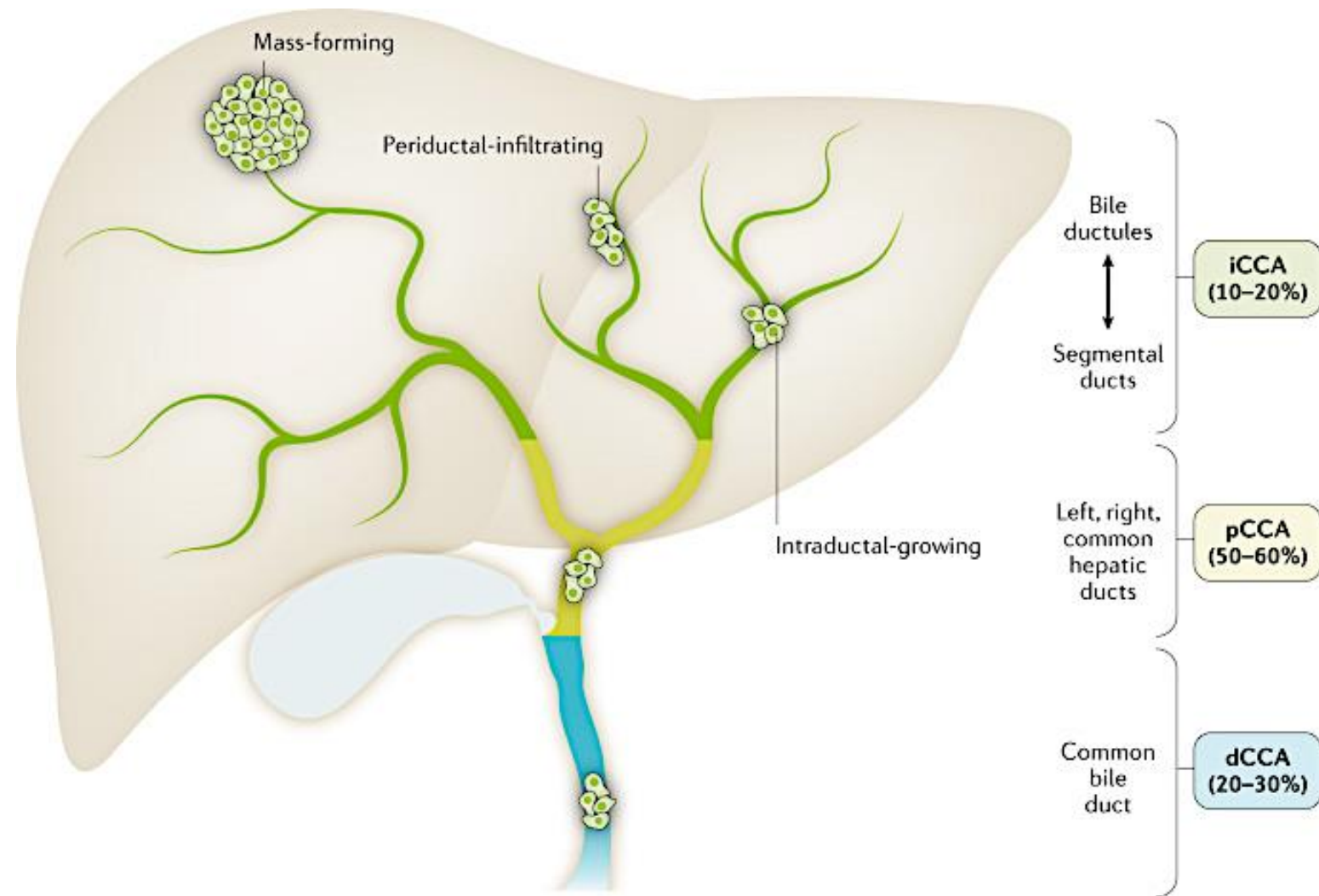
Around 70% of patients either exhibit intrinsic resistance or eventually develop resistance to these drugs, limiting their long-term effectiveness

**URGENT NEED TO DEVELOP NEW CHEMOTHERAPEUTIC AGENTS WITH
MORE ACTIVITY AND ABLE TO OVERCOME CISPLATIN RESISTANCE**

3. On the road: Cisplatin as starting point



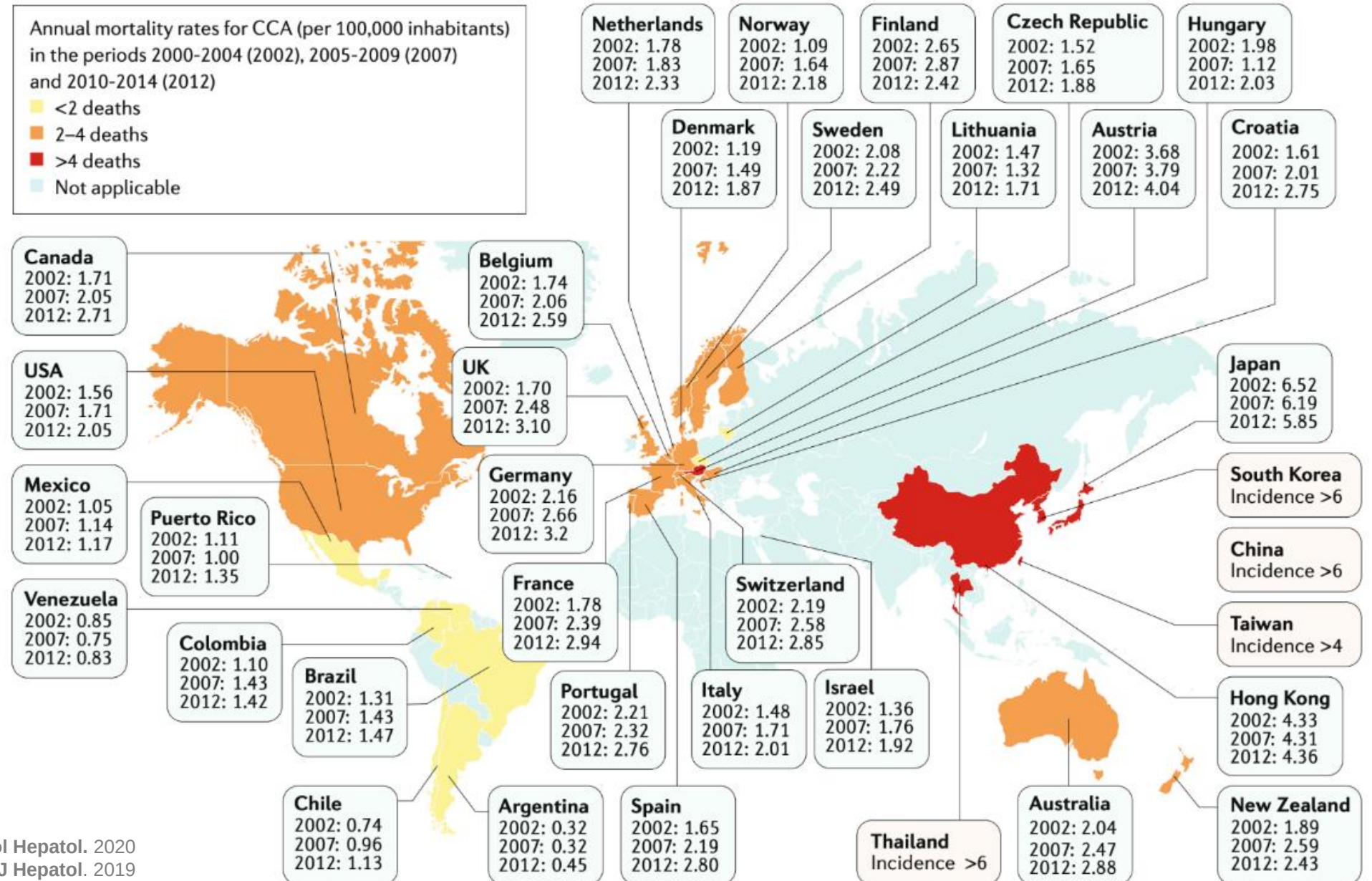
Even worse: cholangiocarcinoma



¹ World Health Organization. International Classification of Diseases 11th Revision. Version 02/2022

² Banales JM, et al. *Nat Rev Gastroenterol Hepatol.* 2020

Annual mortality rate associated with cholangiocarcinoma

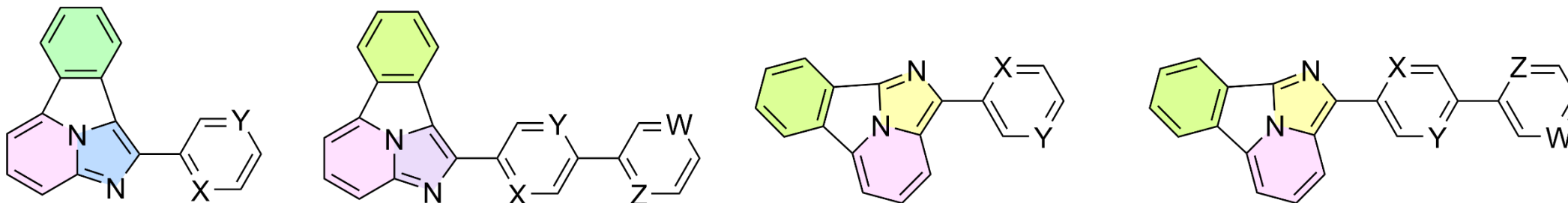


¹ Banales JM, et al. Nat Rev Gastroenterol Hepatol. 2020

² Bertuccio P, et al. J Hepatol. 2019

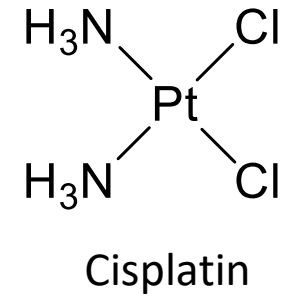
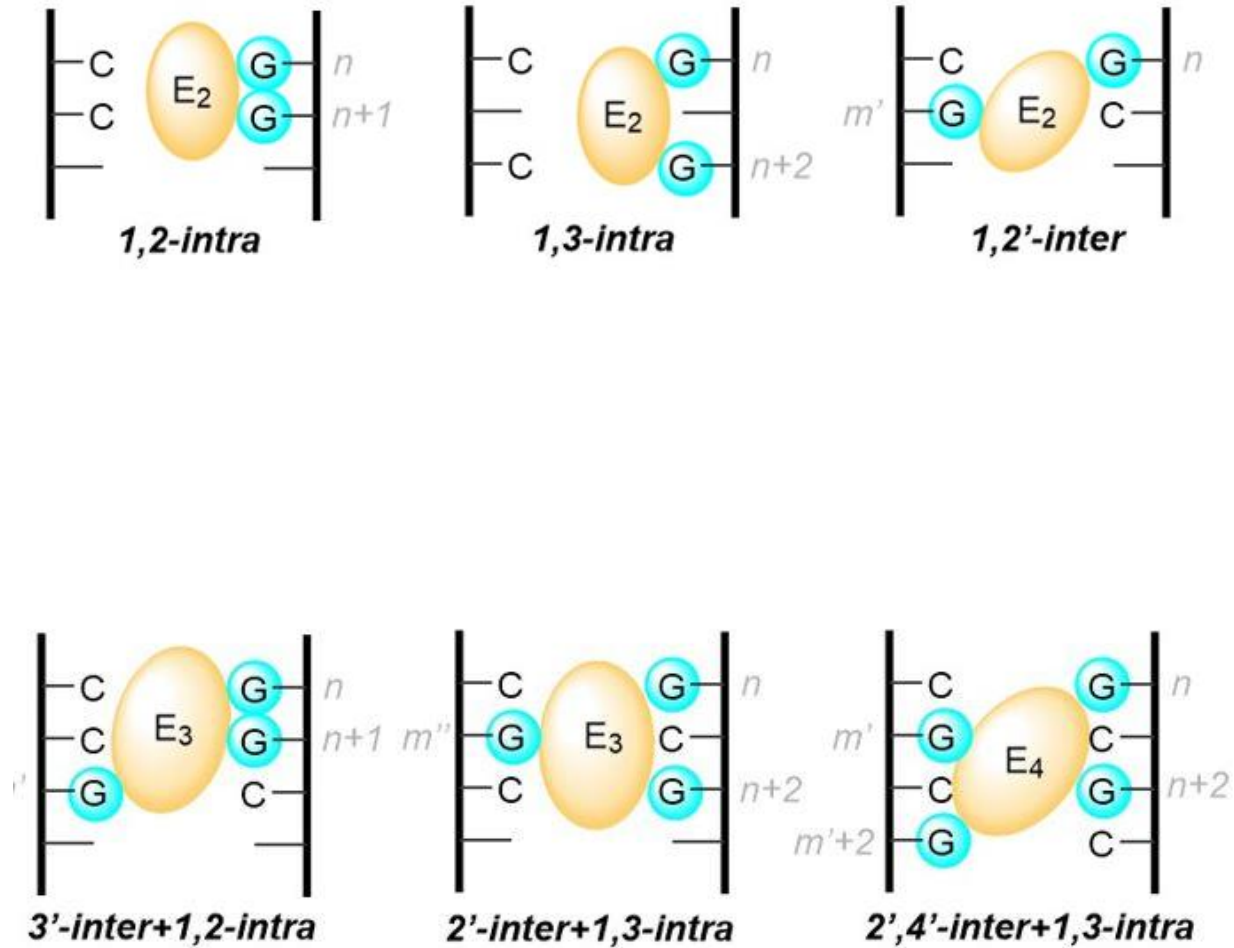
2. The BOLD solution (I)

- We have obtained diverse families of fluorescent polyheterocycles that can interact with metals

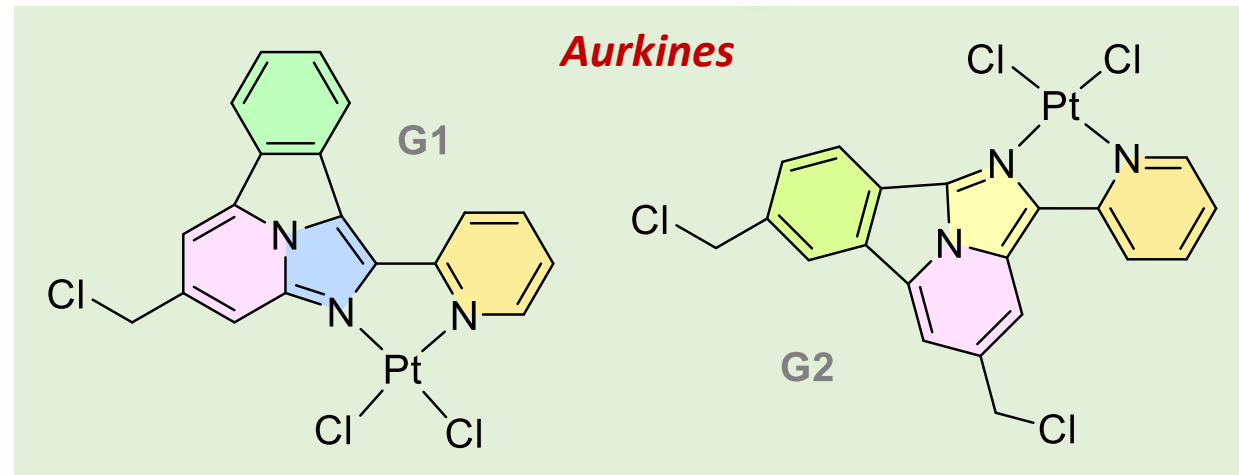


- Can we apply this expertise to the synthesis of novel chemotherapeutic agents?
- Requirements:
 - High potency -binding constants (K_i)
 - Favorable photophysics
 - Biological probes
 - Application to dynamic cancer phototherapy
 - Low toxicity
 - Good ADMETox profile

2. The BOLD solution (II)



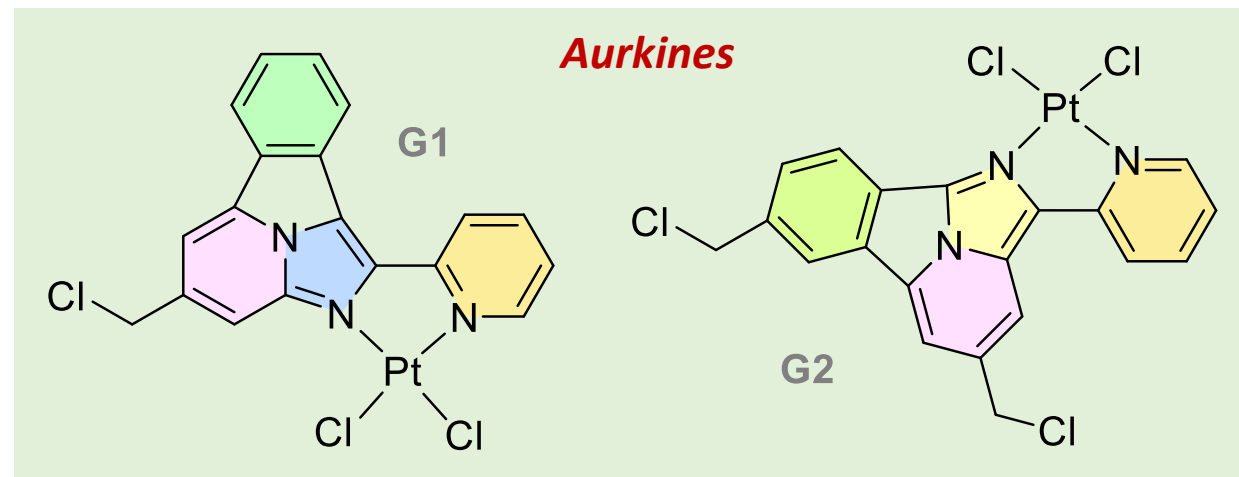
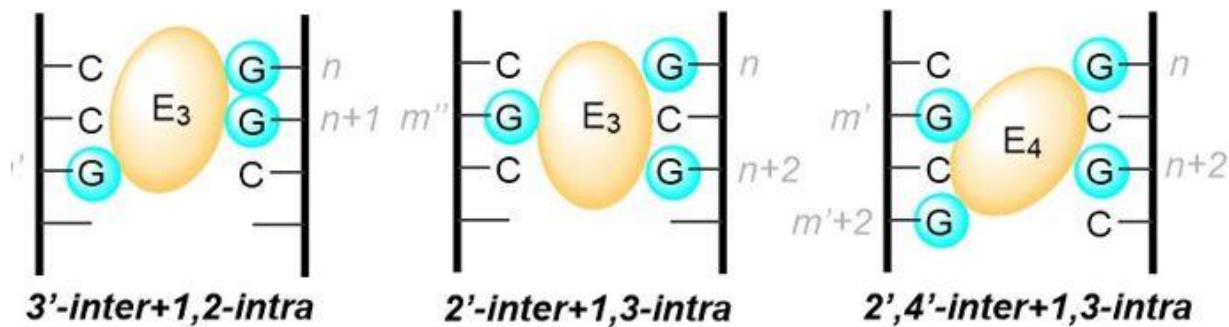
Aurkitu = to find
Aurka = against
Aurki = quickly

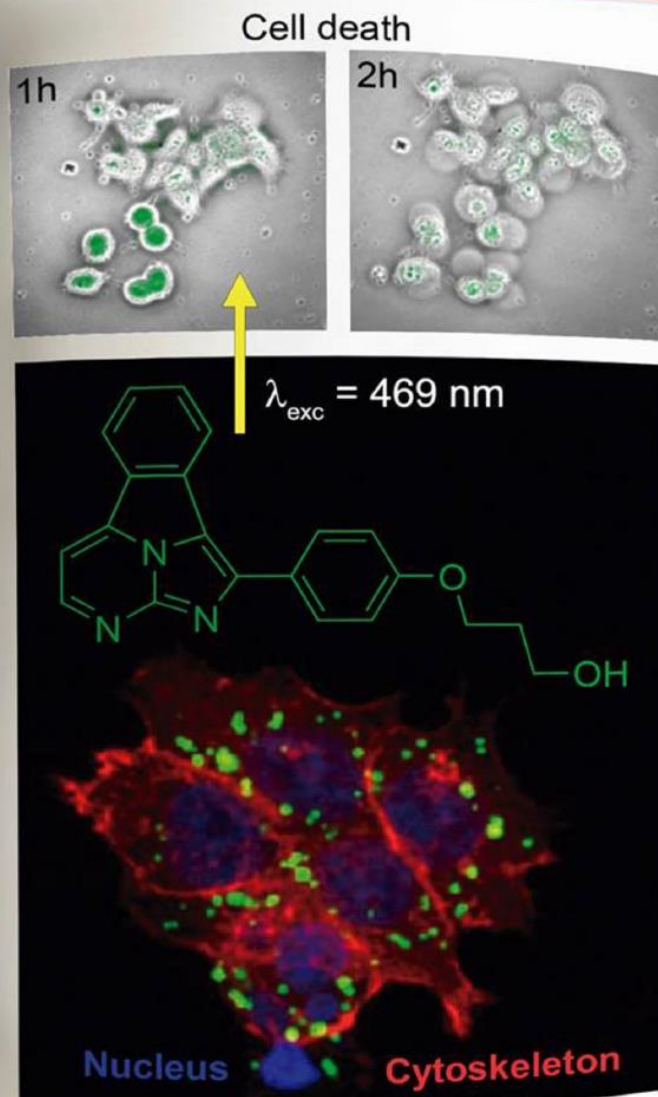
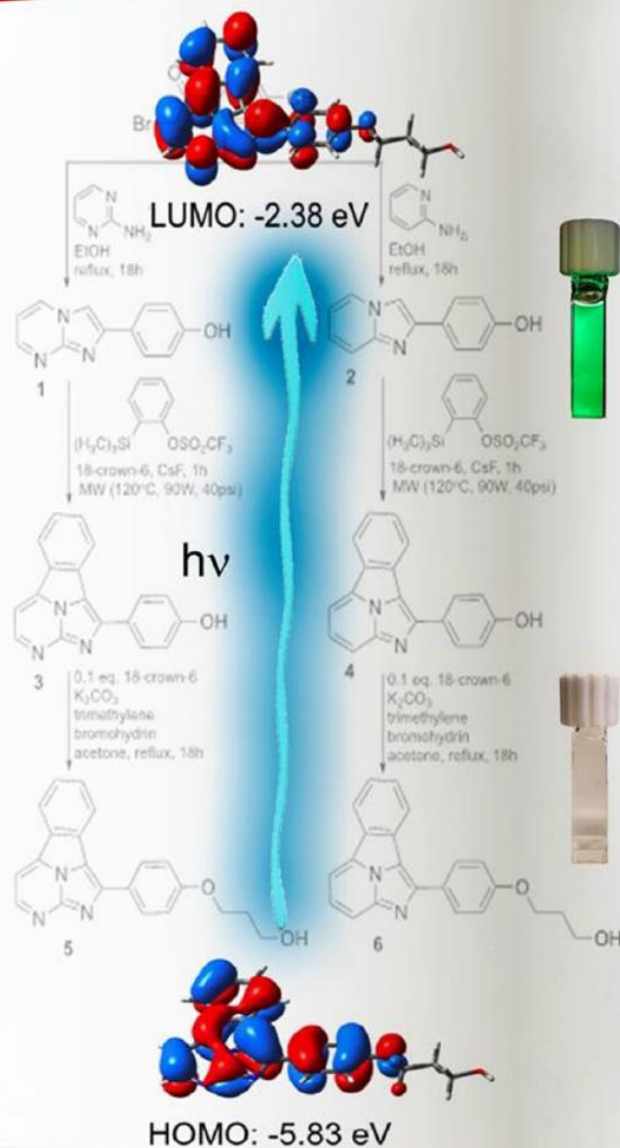


2. The BOLD solution (II)

Design criteria:

- Increasing the number n of electrophilic positions (chlorine atoms) of the reagent by making $n > 2$.
- Inclusion of **intercalating** (π -stacking) **moieties**.
- Inclusion of **basic centres** to make the molecule accessible in vivo to organic cationic transporters.
- Generation of **fluorophores** that could facilitate the monitoring of the distribution of the molecules within cellular organelles, thus combining chemotherapeutic activity of these molecules and their use as **molecular probes**.





(51) International Patent Classification:

C07F 1/09 (2006.01) A61P 35/00 (2006.01)
C07F 15/00 (2006.01) C07D 471/04 (2006.01)
A61K 33/243 (2019.01) C07D 471/16 (2006.01)

(21) International Application Number:

PCT/EP2023/083500

(22) International Filing Date:

29 November 2023 (29.11.2023)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

P202231032 30 November 2022 (30.11.2022) ES

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(74) Agent: ABG INTELLECTUAL PROPERTY LAW, S.L., Avda. de Burgos, 16D, Edificio EUROMOR, E-28036 Madrid (ES).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Emission (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

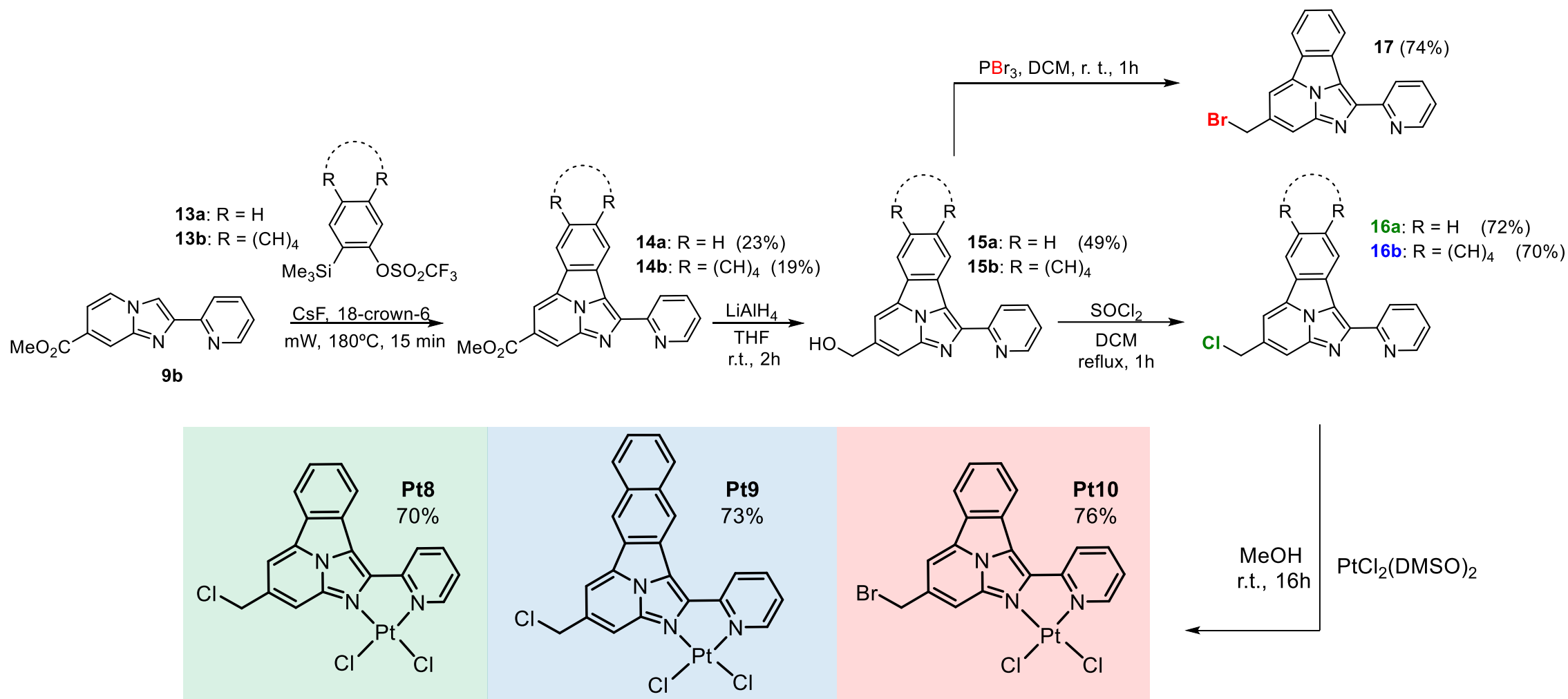
Published:

— with international search report (Art. 21(3))
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(ii))

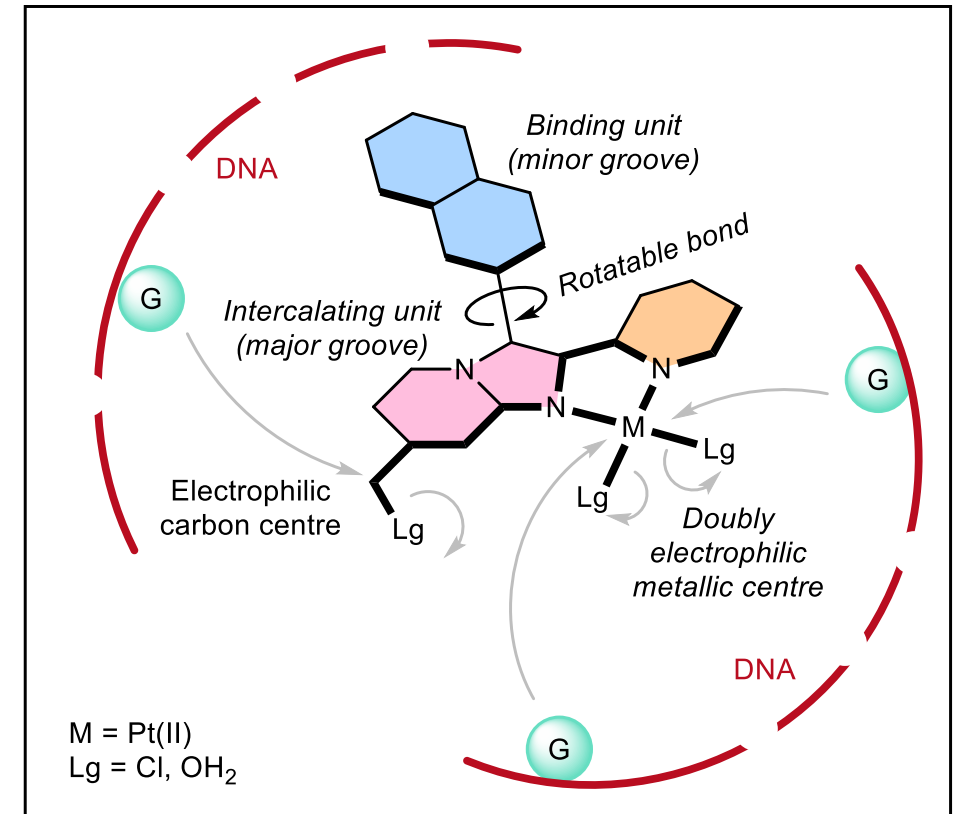
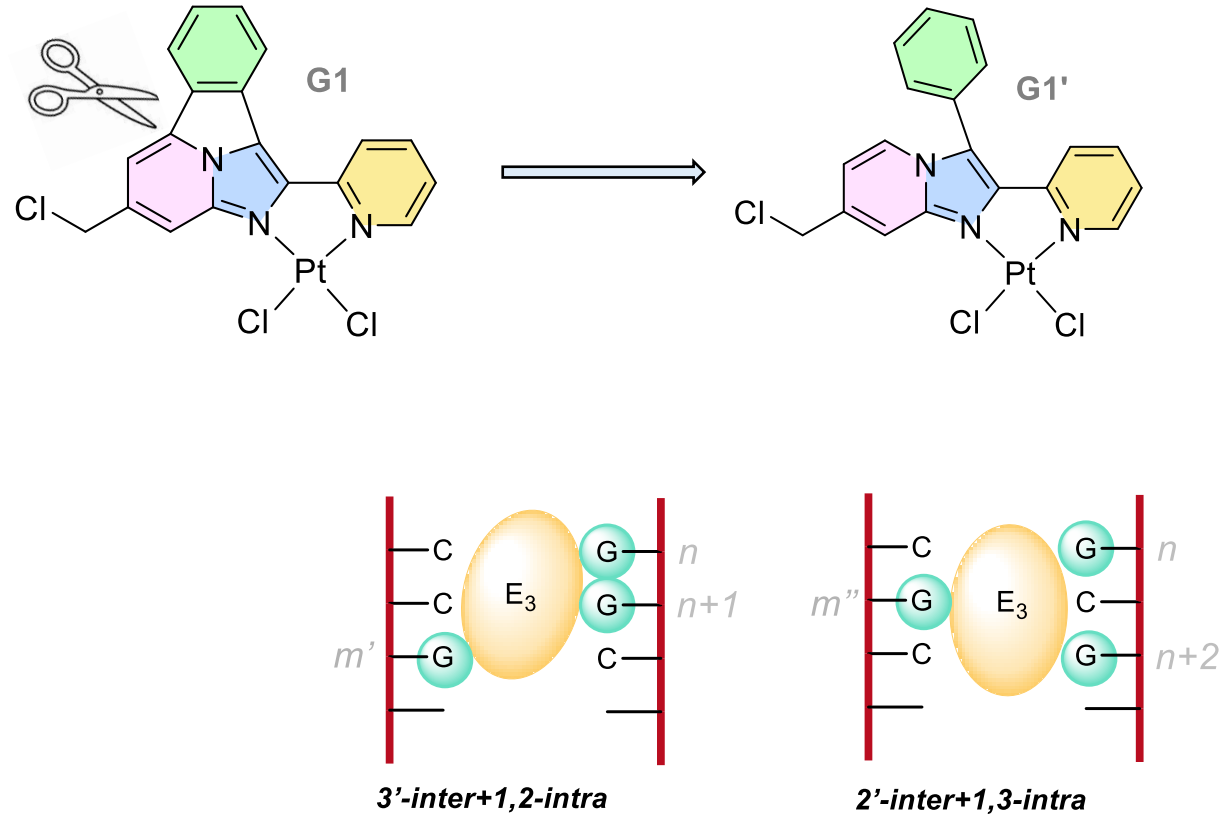
(54) Title: POLYELECTROPHILIC METALATED HETEROCYCLES DERIVED FROM 2-(PYRIDIN-2-YL)IMIDAZO[1,2-A]PYRIDINE AND USE THEREOF AS CHEMOTHERAPEUTIC AGENTS

(57) Abstract: The present invention is directed to new compounds based on polyelectrophilic metalated heterocycles derived from 2-(pyridin-2-yl)imidazo[1,2-a]pyridine, to their obtaining procedure, as well as to their use as chemotherapeutic agents, in particular against tumors sensitive or resistant to other chemotherapeutic agents, such as cisplatin.

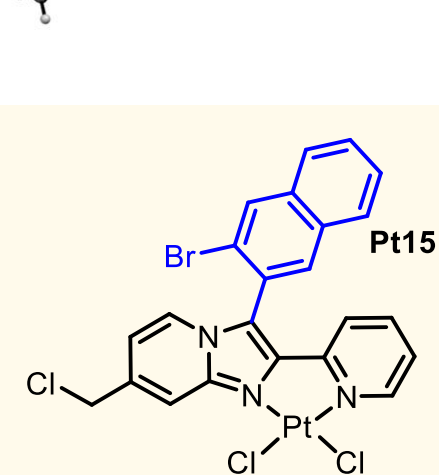
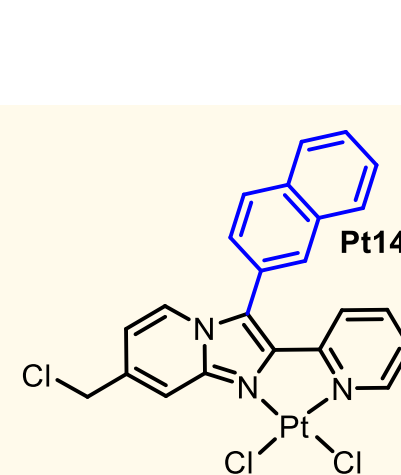
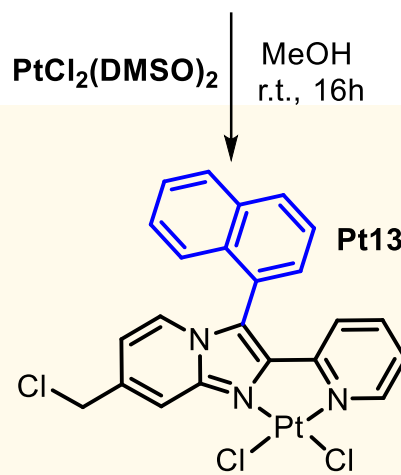
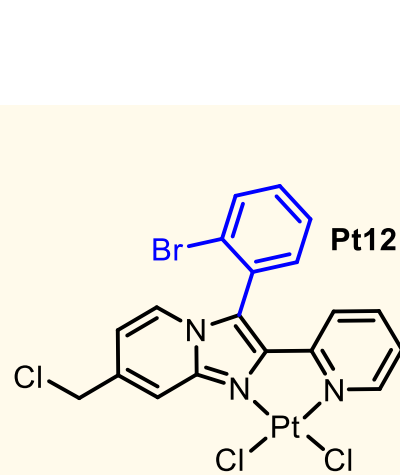
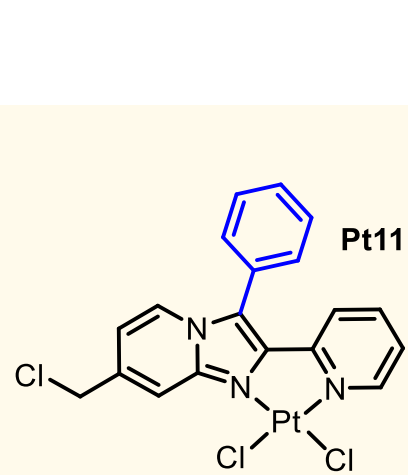
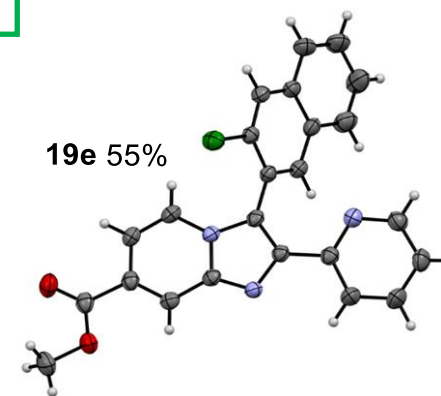
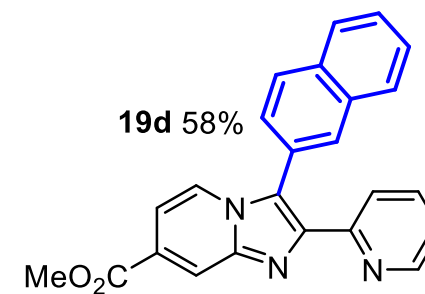
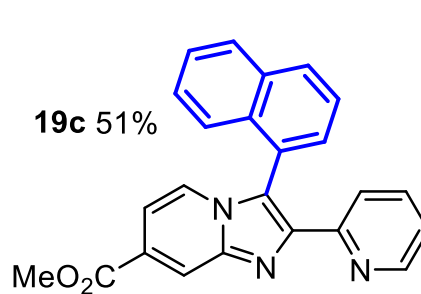
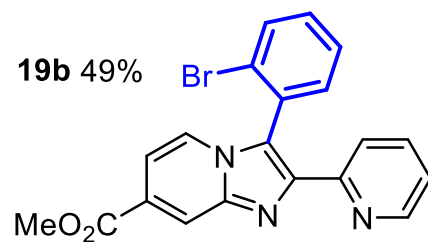
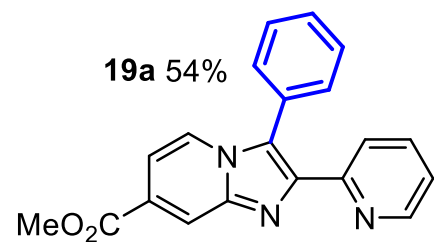
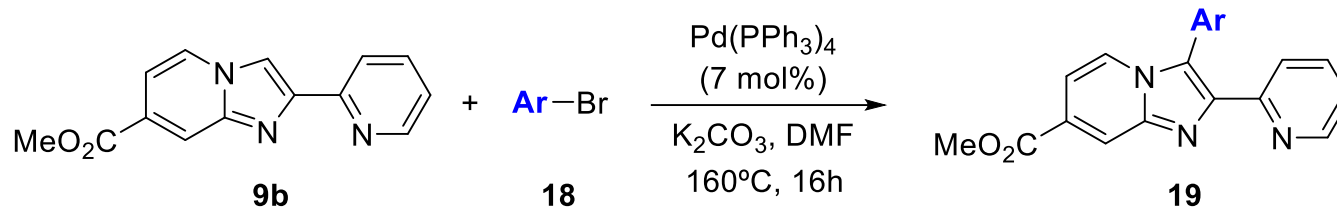
2. The BOLD solution (II): platinum complexes



2. The BOLD solution (III): a new design



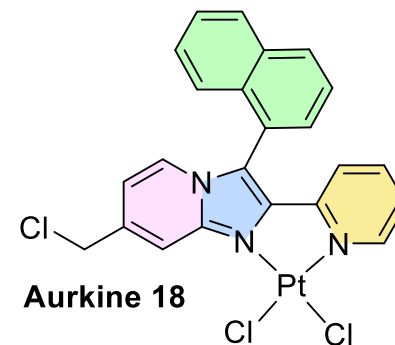
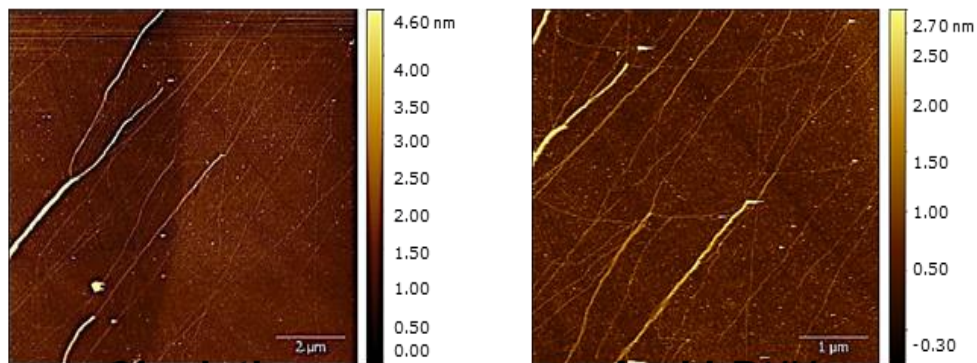
2. The BOLD solution (III): chemical synthesis



The BOLD solution (IV): results in naked DNA

AFM

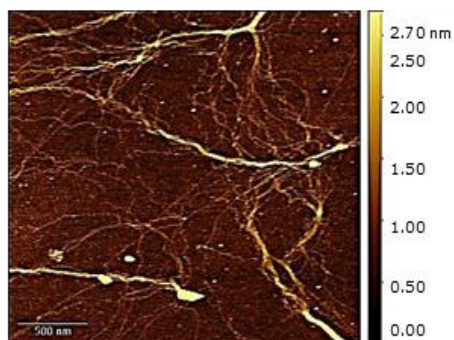
Non-treated
DNA



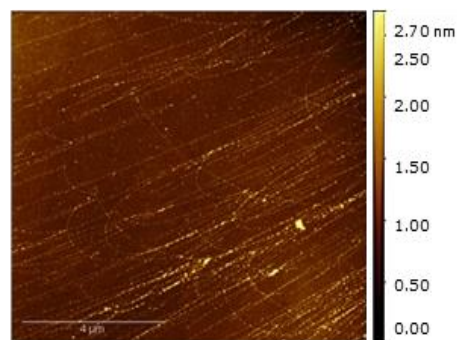
Time
(min)

2

Cisplatin

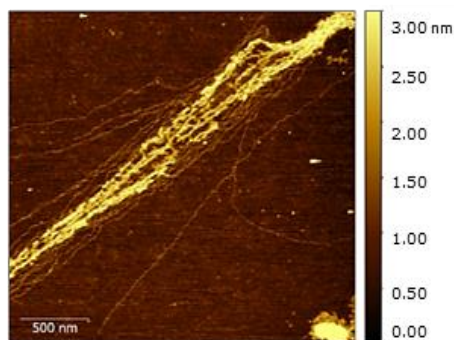


Aurkine 18

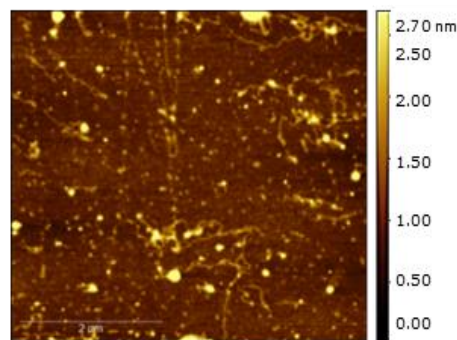


10

Cisplatin

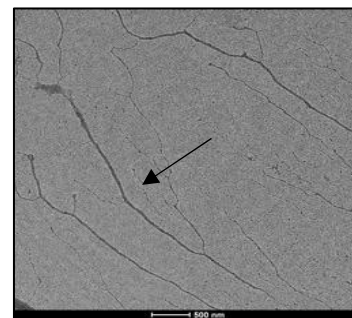


Aurkine 18

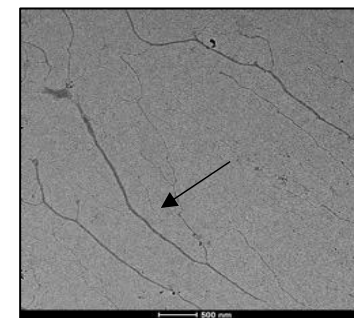


TEM

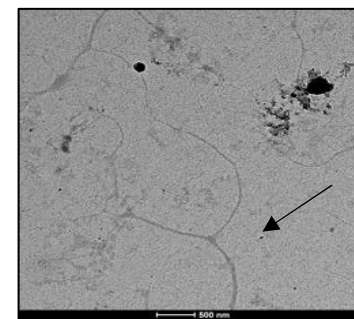
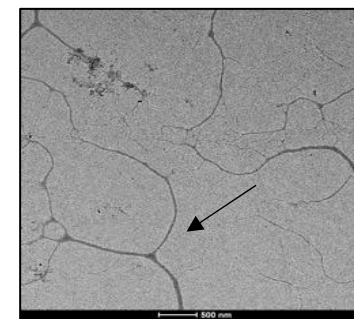
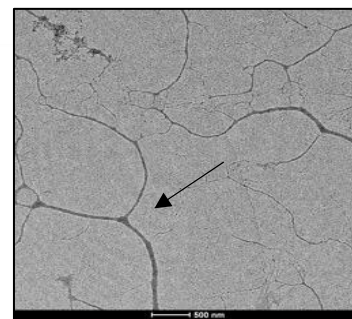
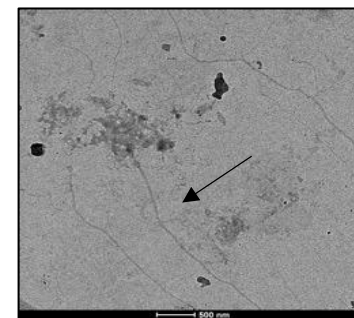
Non-treated
DNA



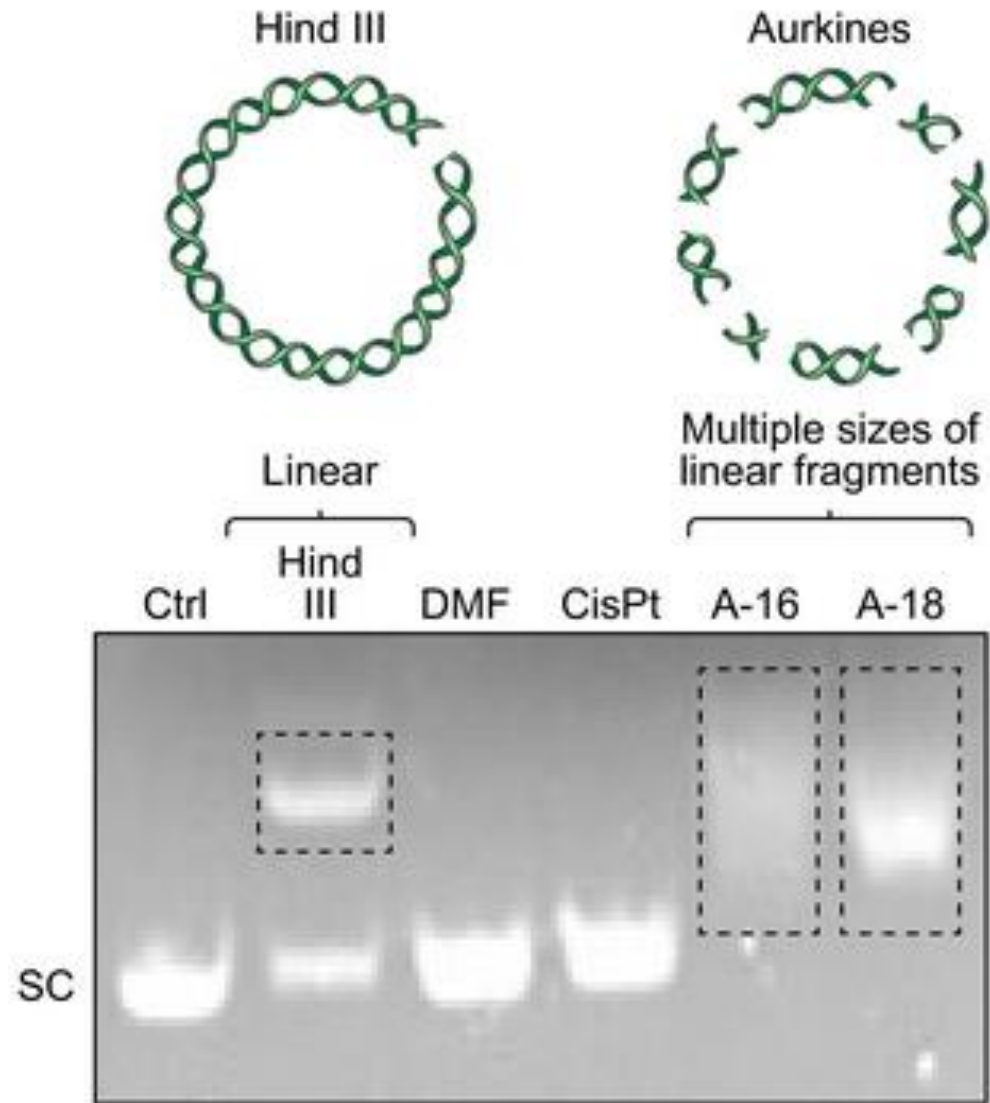
2 min



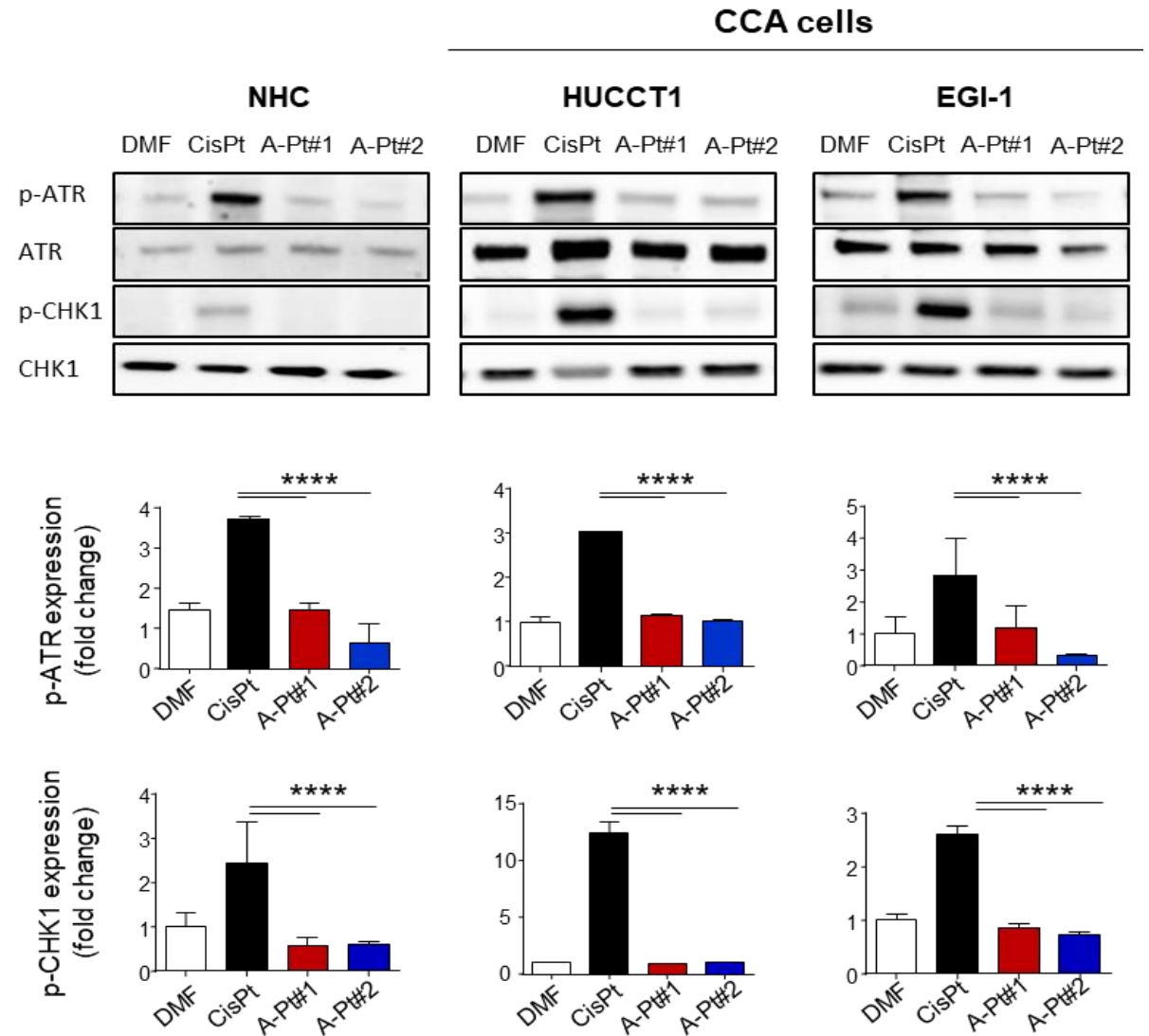
10 min



The BOLD solution (IV): results in plasmids

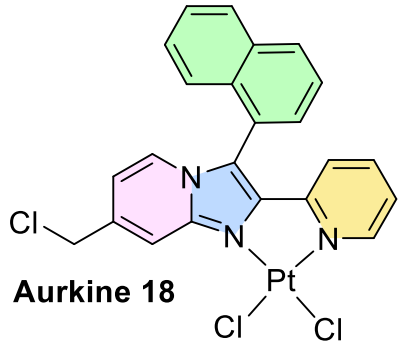
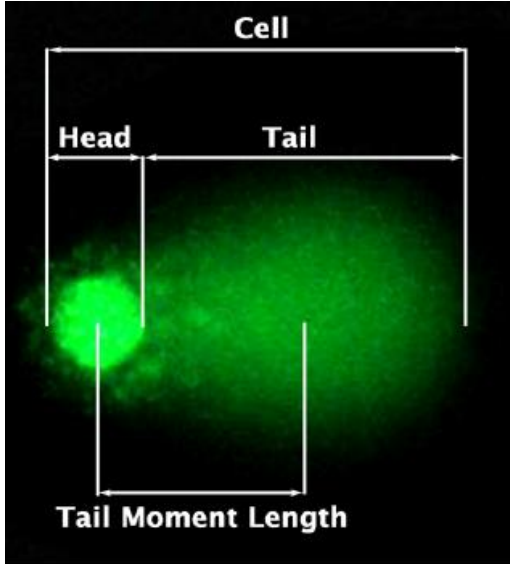


pUC18 plasmid mobility

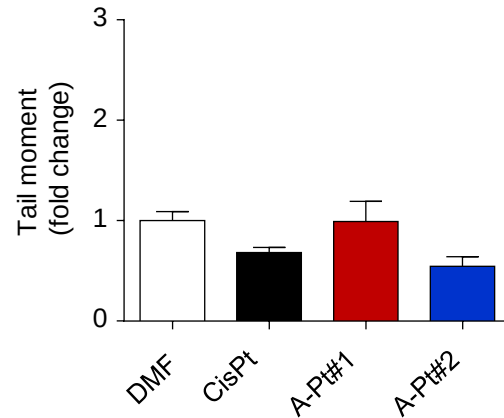
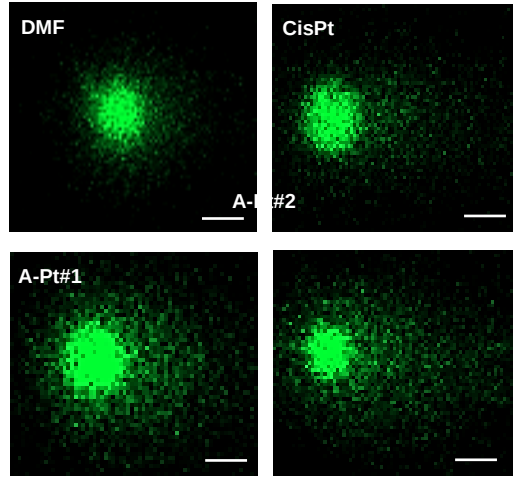


The BOLD solution (IV): results in cells

Comet assay



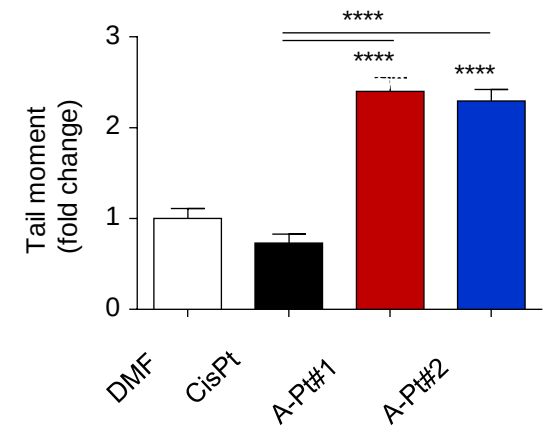
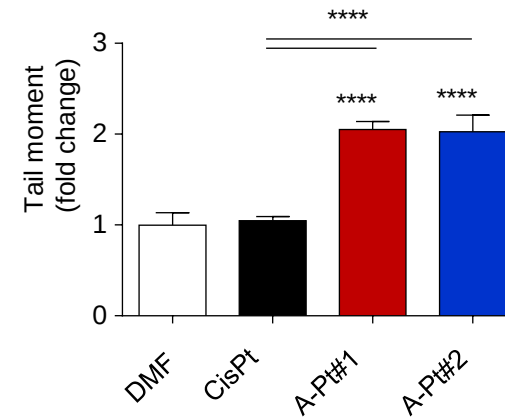
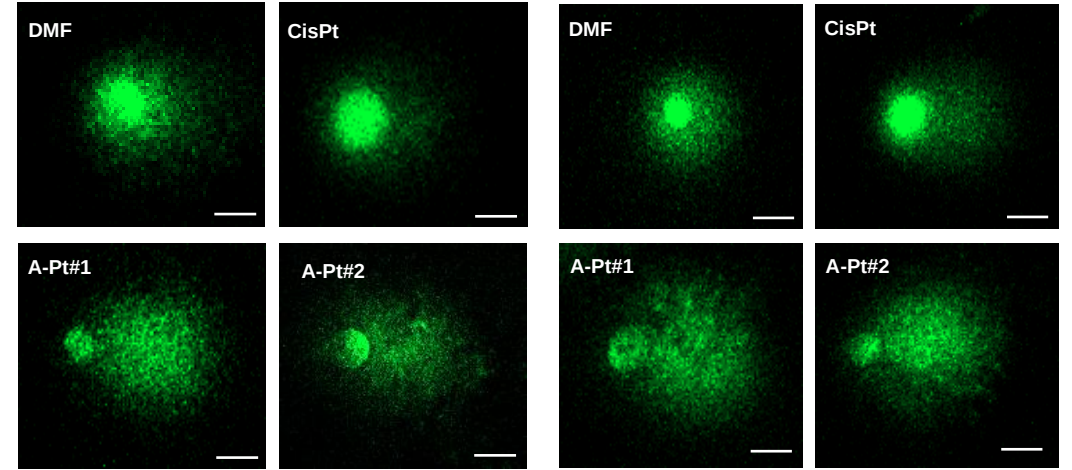
NHC: Normal human cholangiocytes



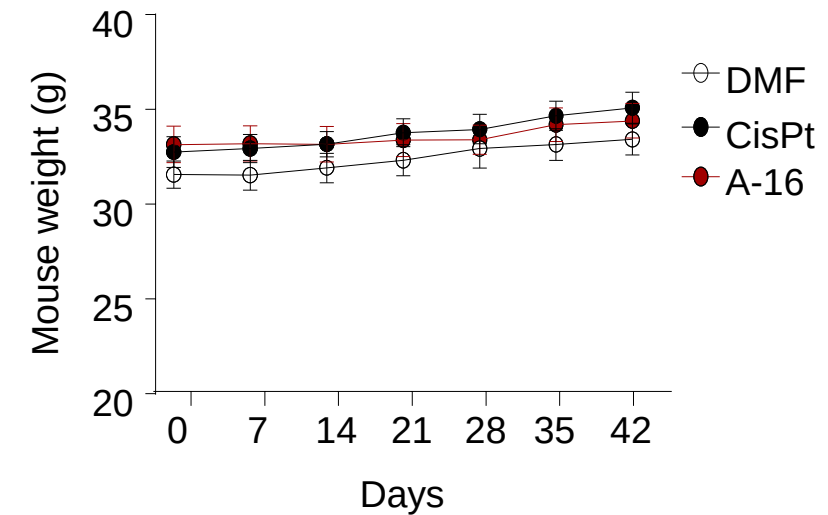
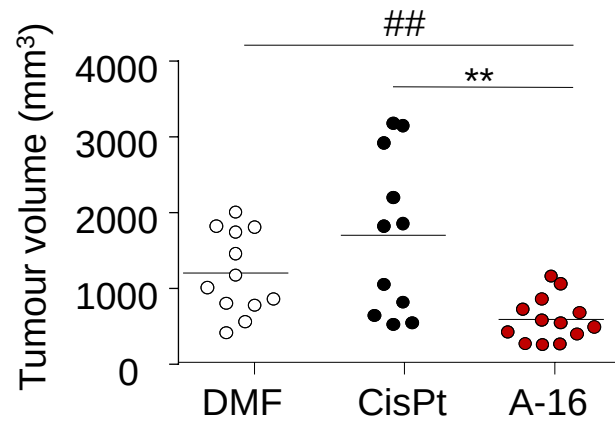
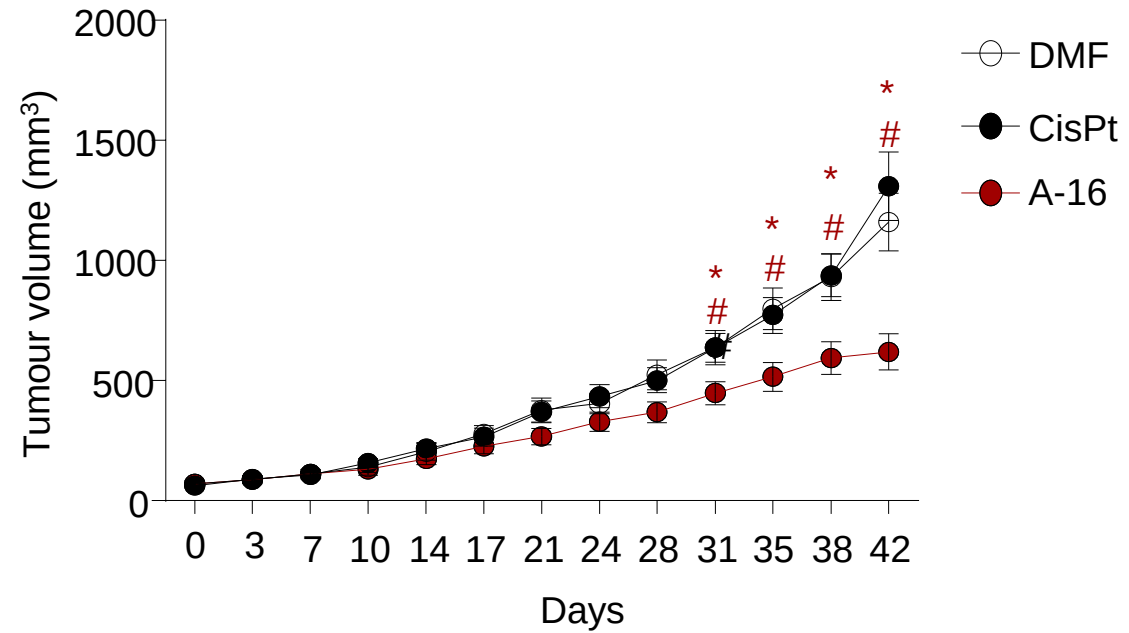
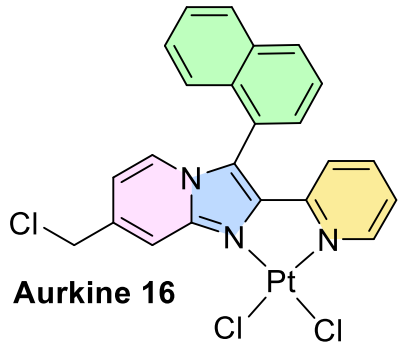
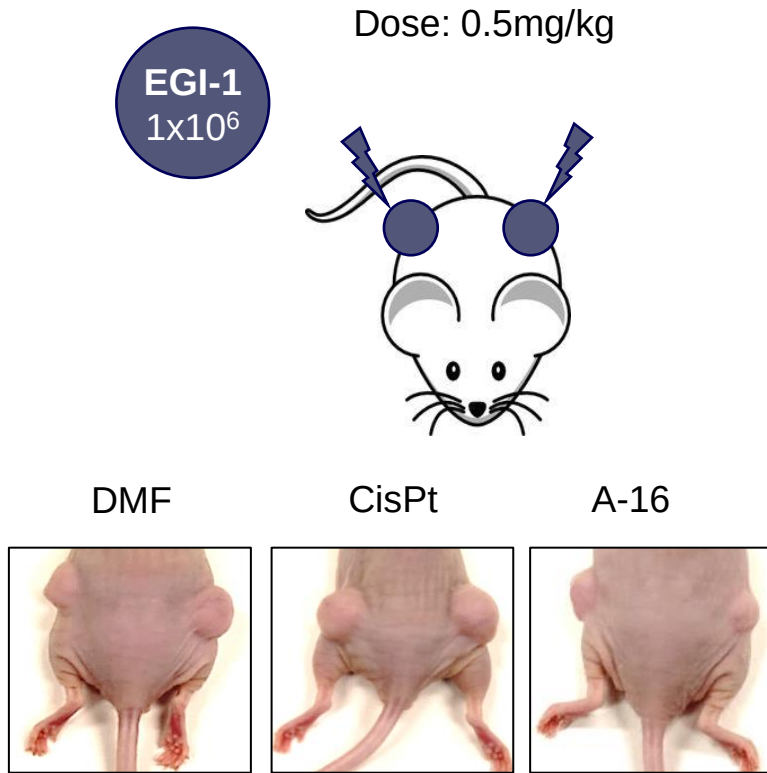
CCA cells

HUCCT1

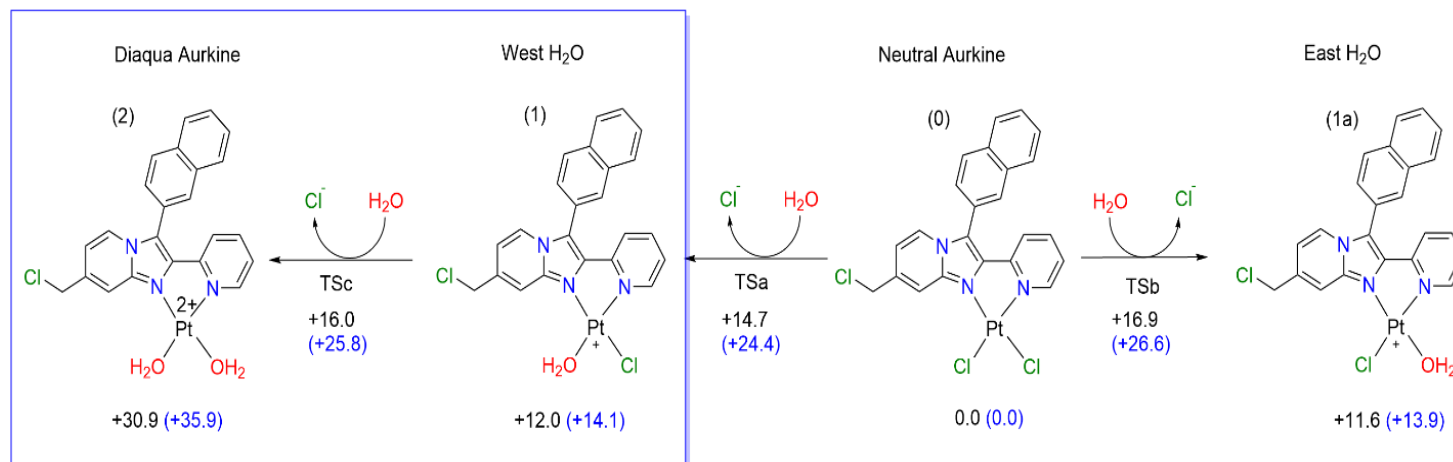
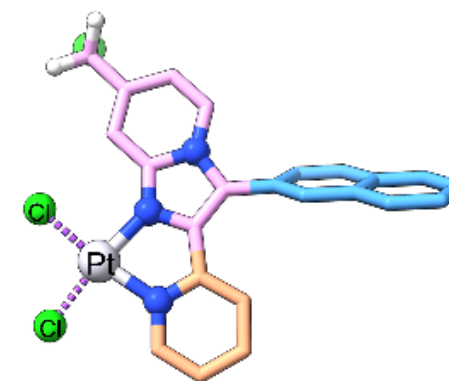
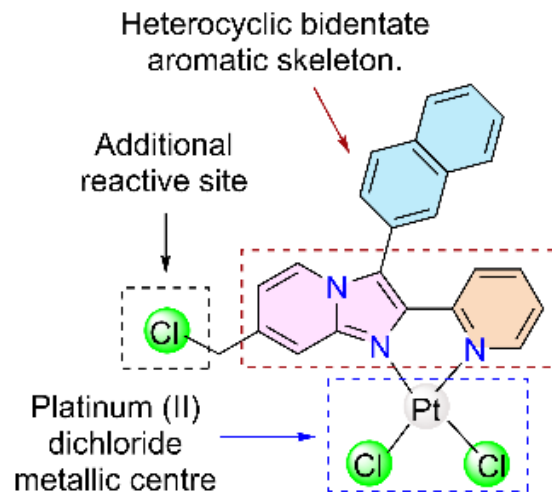
EGI-1



The BOLD solution (IV): results in cells (xenografts)

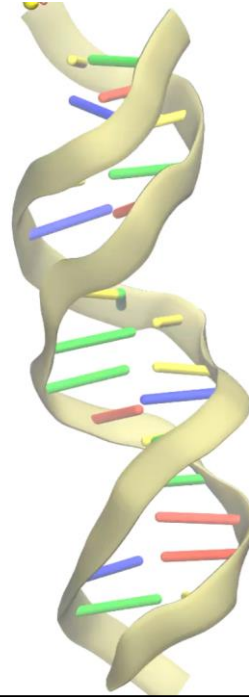
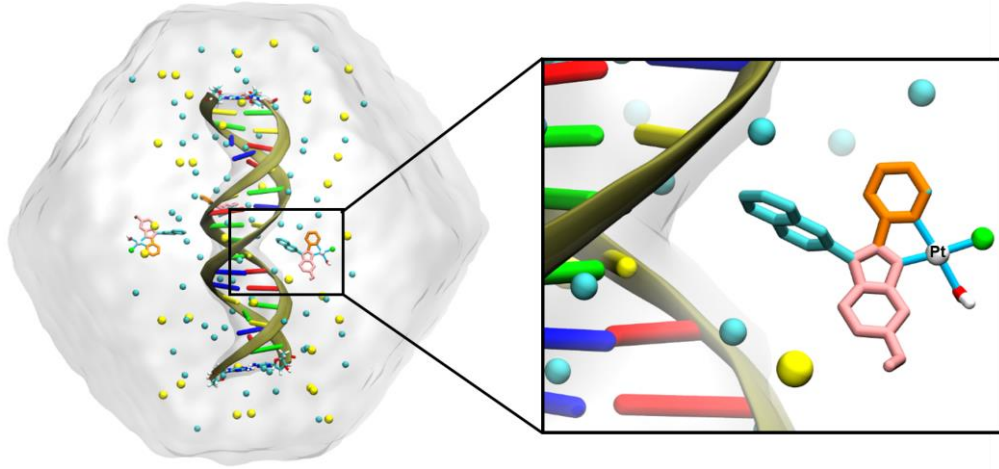


The BOLD solution (V): computational studies

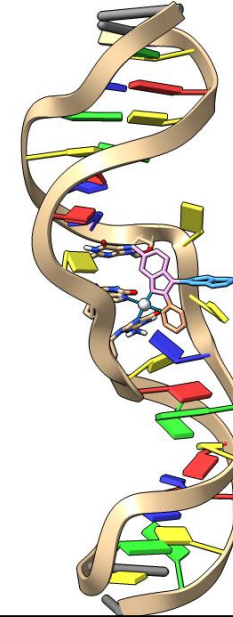


The BOLD solution (V): computational studies

DNA intercalation

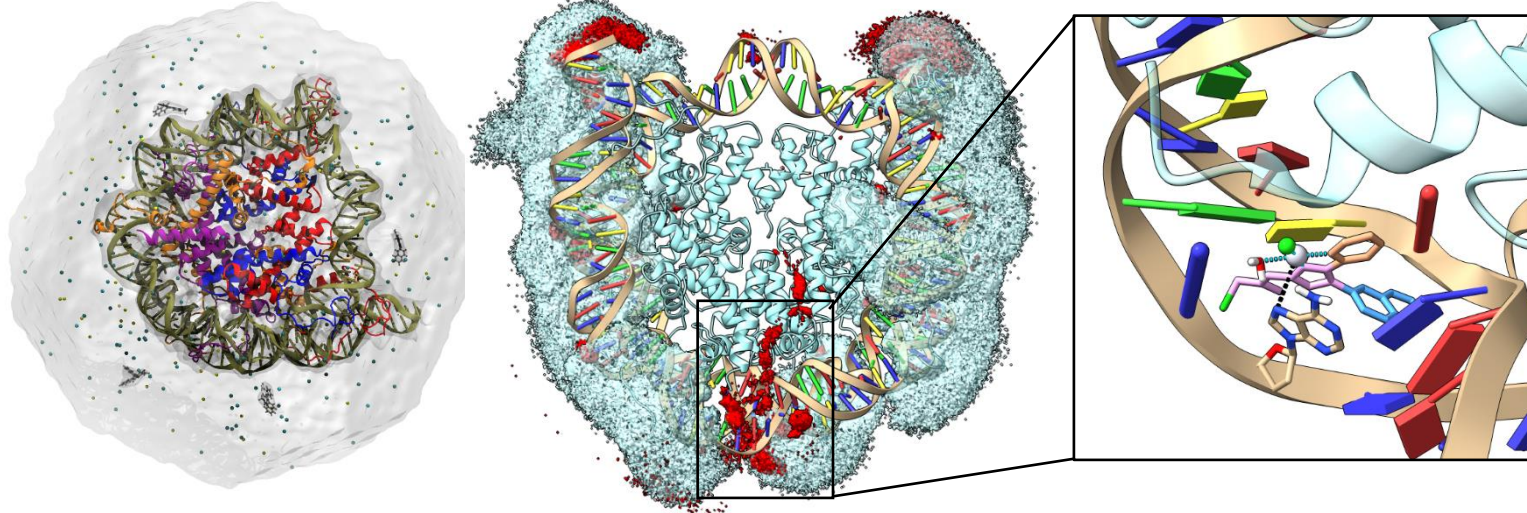


Aurkine16
3-'inter+1,2-intrastrand



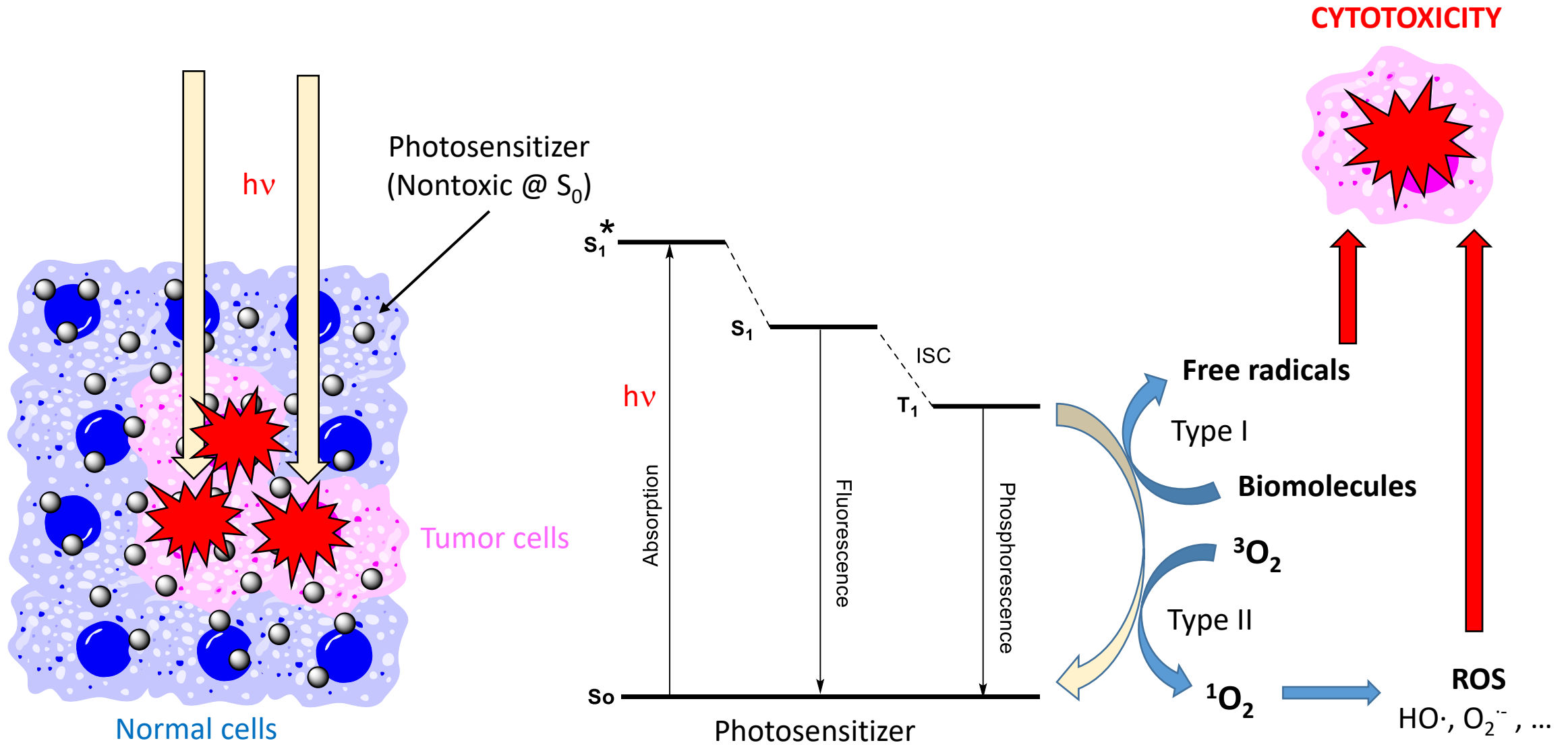
Intercalation or
covalent bond
compromises the
DNA structure

Aurkine16 with the Nucleosome core particle

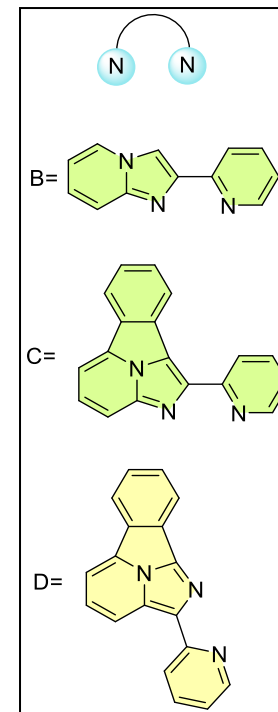
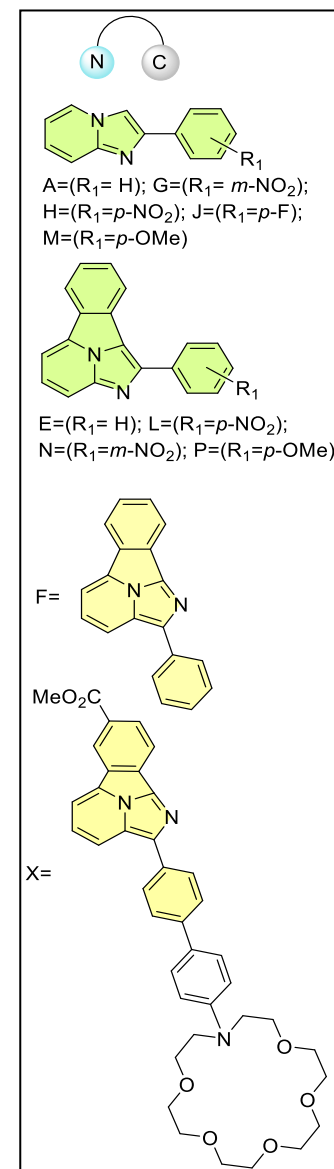
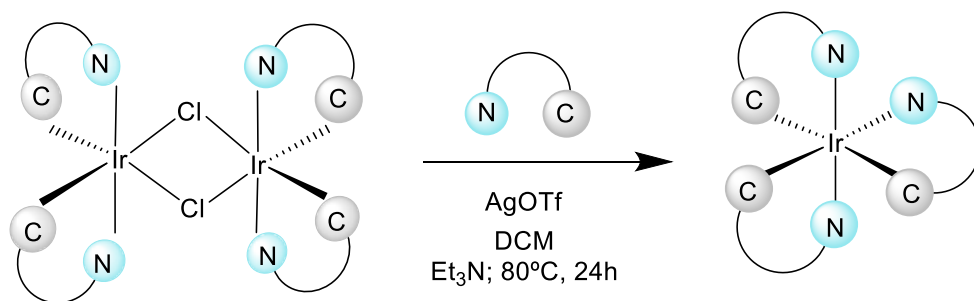
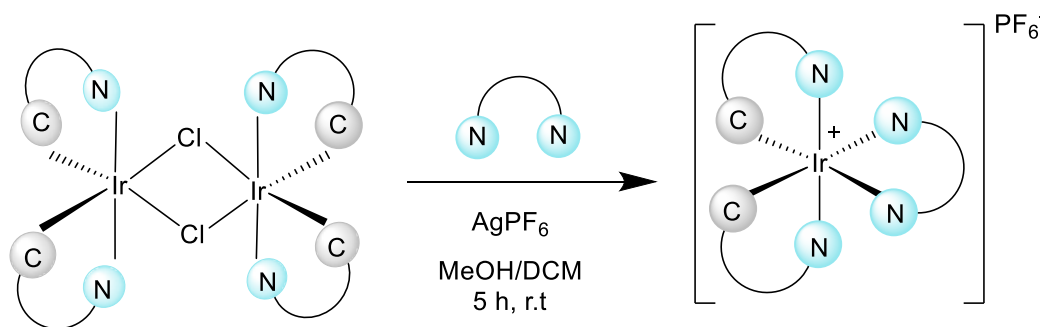
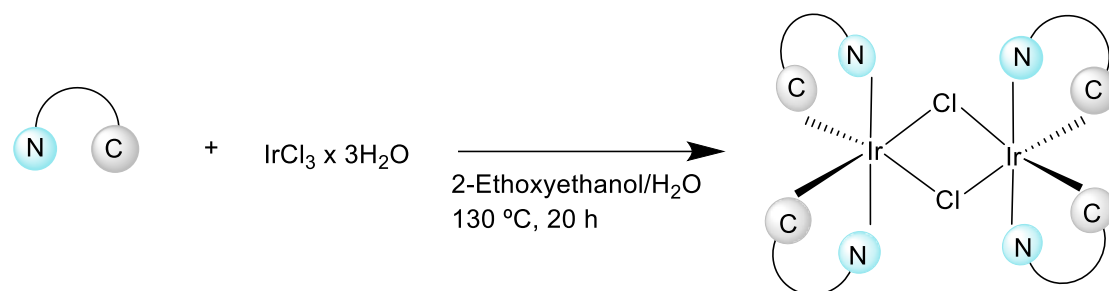


Aurkine16 does not permeate into the
histones and do not compromise the
nucleosome core particle

3. The BOLD solution: dynamic phototherapy



From BOLD to Chemotherapeutic photosensitizers



Cholangiocarcinoma models: intrahepatic CCA + extrahepatic CCA



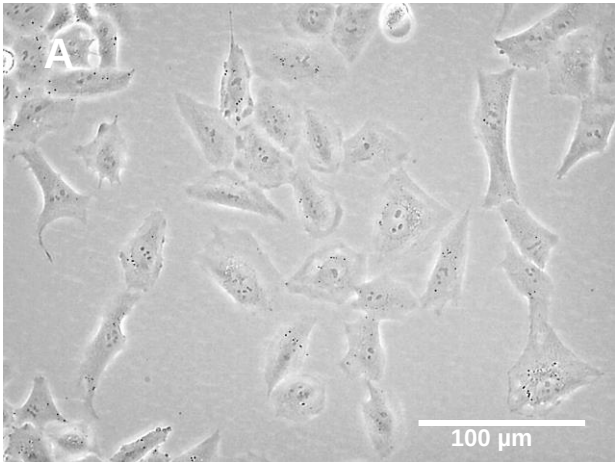
Dr. Pilar Acedo Nuñez



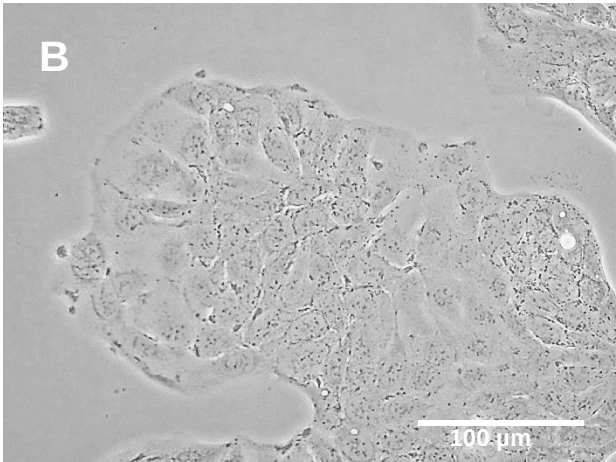
María Rosado Rodríguez

University College London

CCLP-1 (iCCA)



SNU-1196 (eCCA)

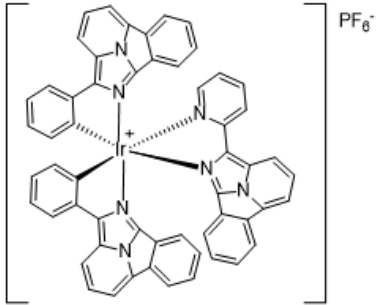


	CCLP-1	SNU-1196
Lineage	Bile duct	Bile duct
Disease	CCA	CCA
CCA type	iCCA	eCCA
Population	Caucasian	East Asian

CCA cells cultured in DMEM media supplemented with 10% FBS and 1% penicillin-streptomycin. Incubated at 37°C in a humidified atmosphere with 5% CO₂



JCP-67



Tiempo $\lambda_{\text{exc max}} 450\text{nm}$ $\lambda_{\text{em max}} 550\text{ nm}$:

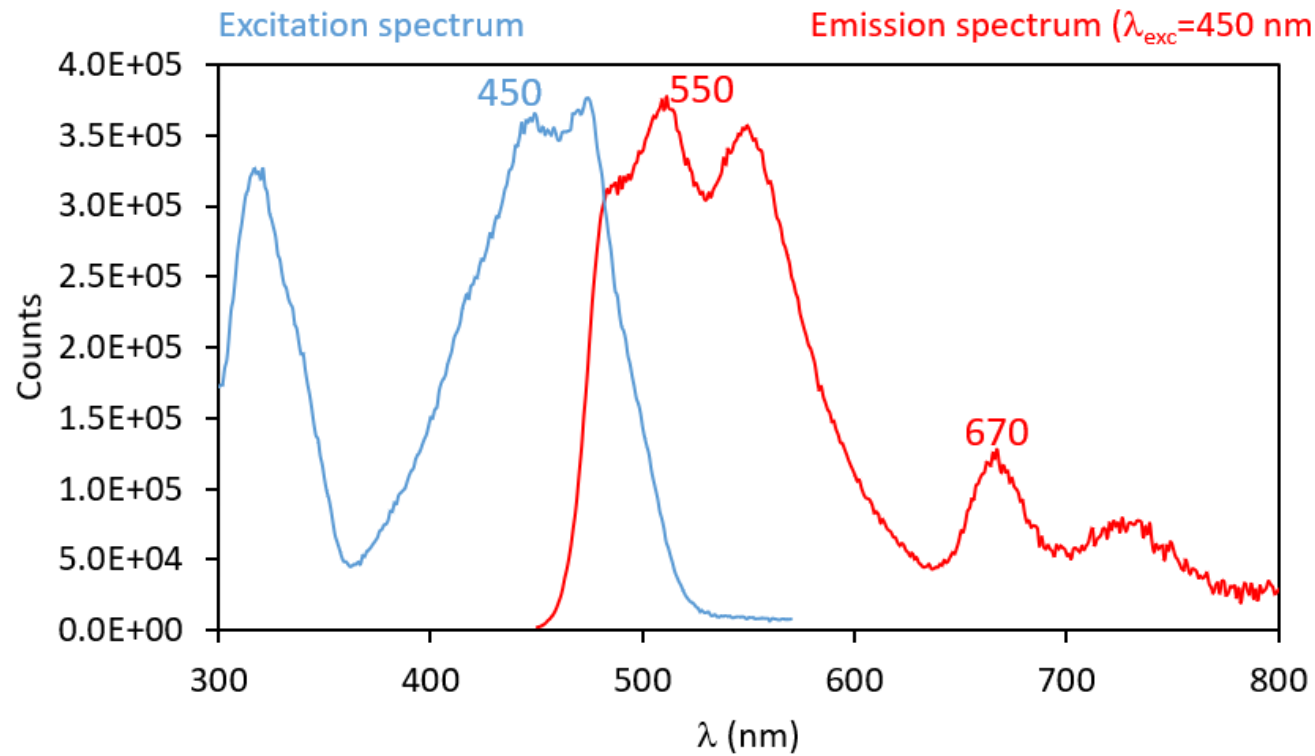
$$\tau_{\text{O}_2} = 9.5\text{ ns}$$

$$\tau_{\text{Ar}} = 13.9\text{ ns}$$

Tiempo $\lambda_{\text{exc max}} 450\text{nm}$ $\lambda_{\text{em}} 670\text{ nm}$ o 730nm :

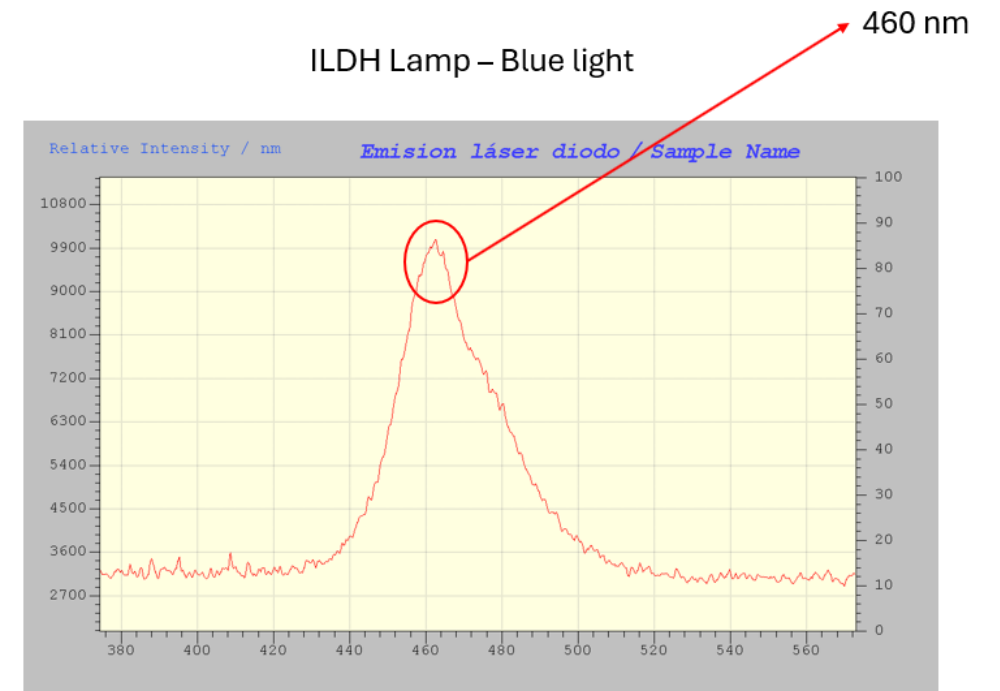
$$\tau_{\text{O}_2} = 525\text{ ns}$$

$$\tau_{\text{Ar}} = 2.75\text{ }\mu\text{s}$$



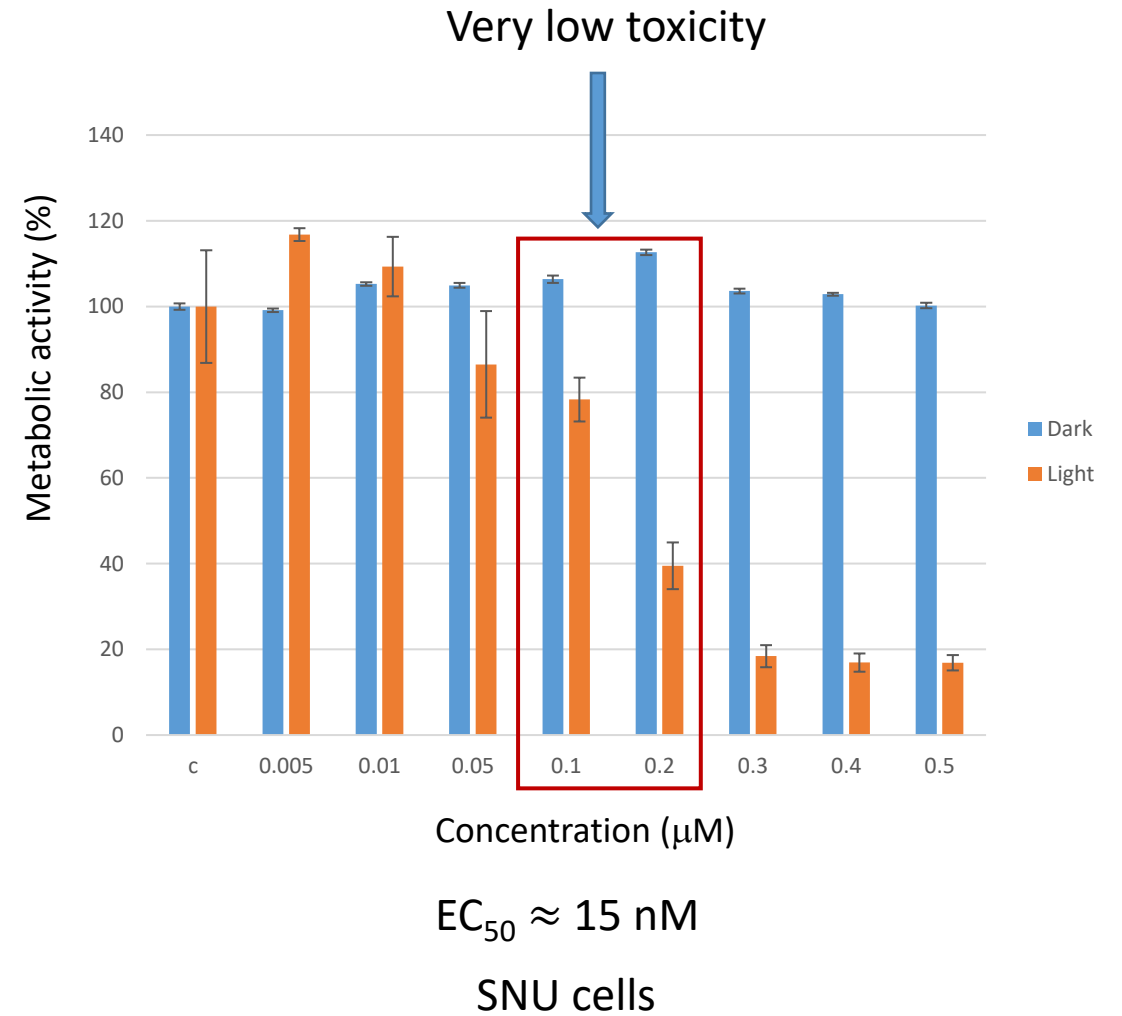
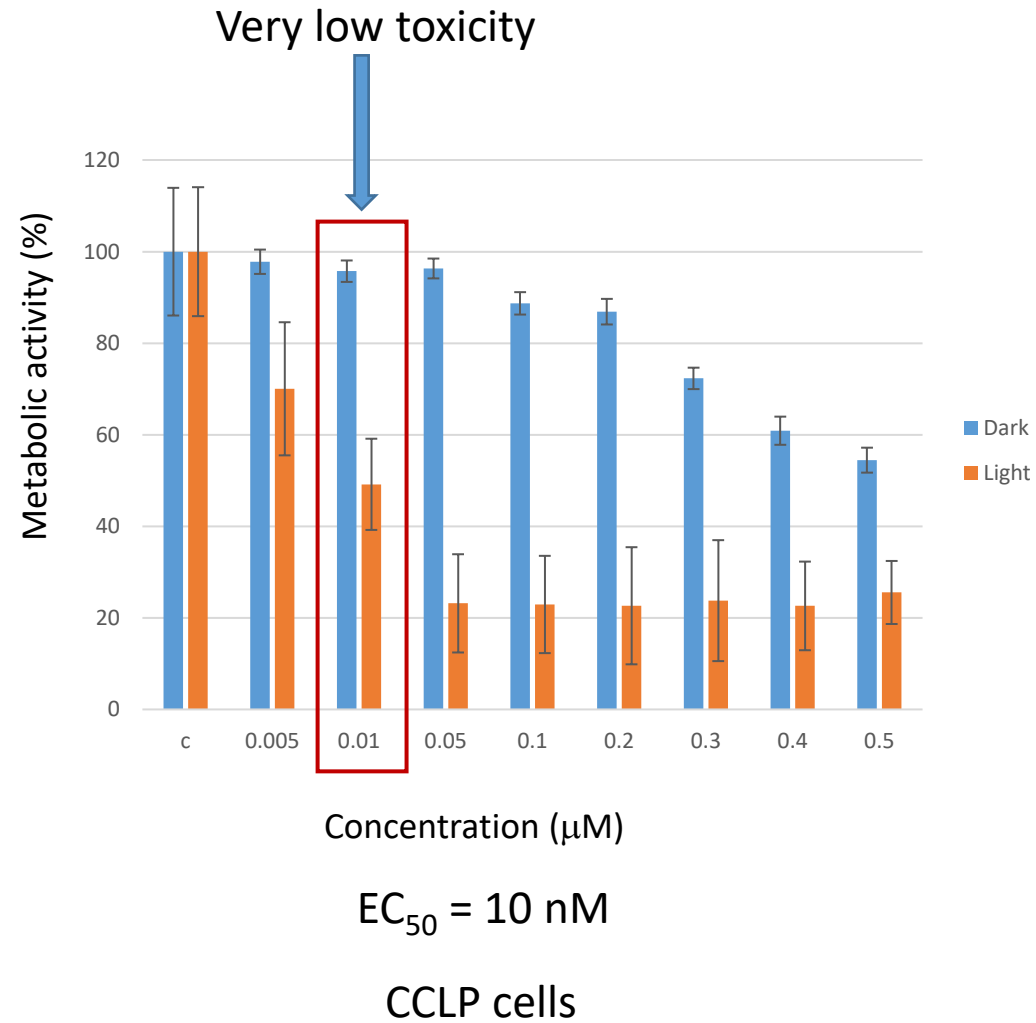
JCP 67

ROS: 75%-80%

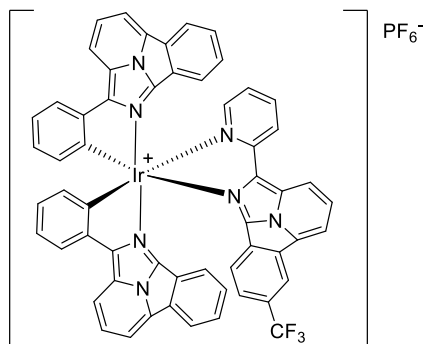


MTT assay

ILDH lamp (blue light):
7min 35s = 5J/cm²



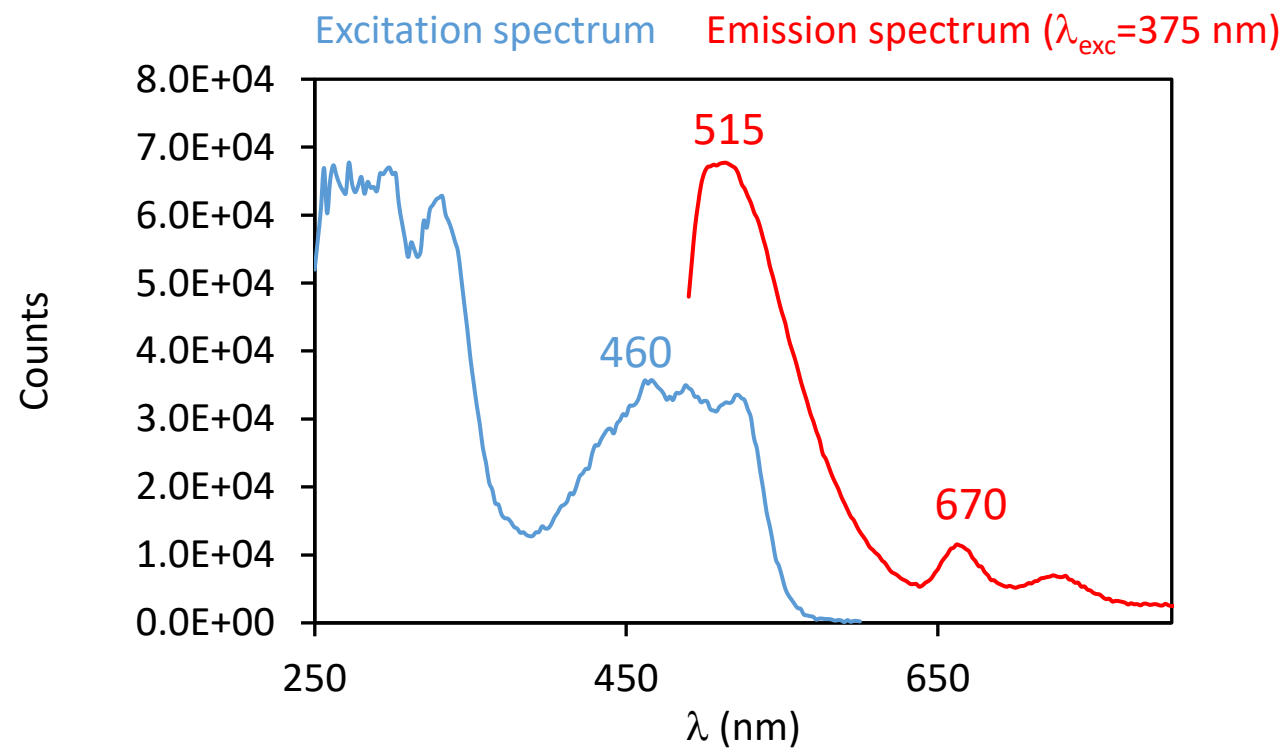
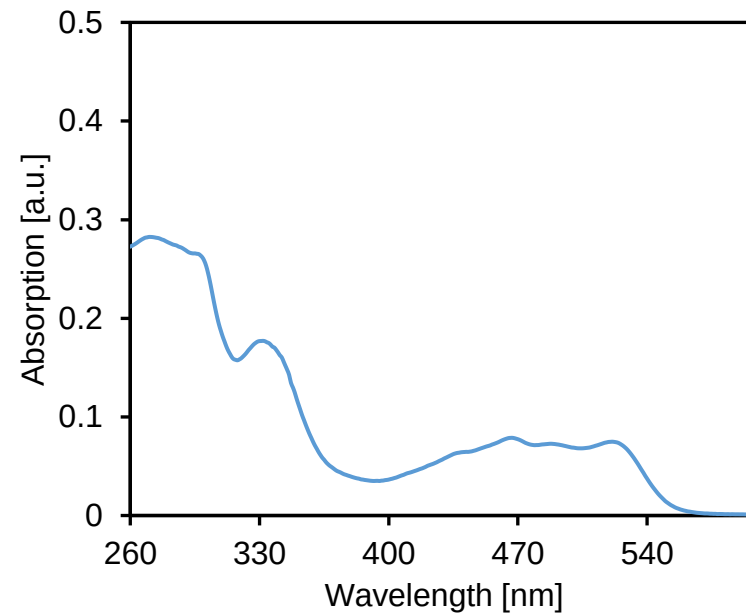
JCP-490



Lifetime $\lambda_{\text{exc}_{\text{max}}}$ 485nm $\lambda_{\text{em}_{\text{max}}}$ 670nm :

$\tau_{\text{O}_2} = 454 \text{ ns}$

$\tau_{\text{Ar}} = 2.97 \mu\text{s}$



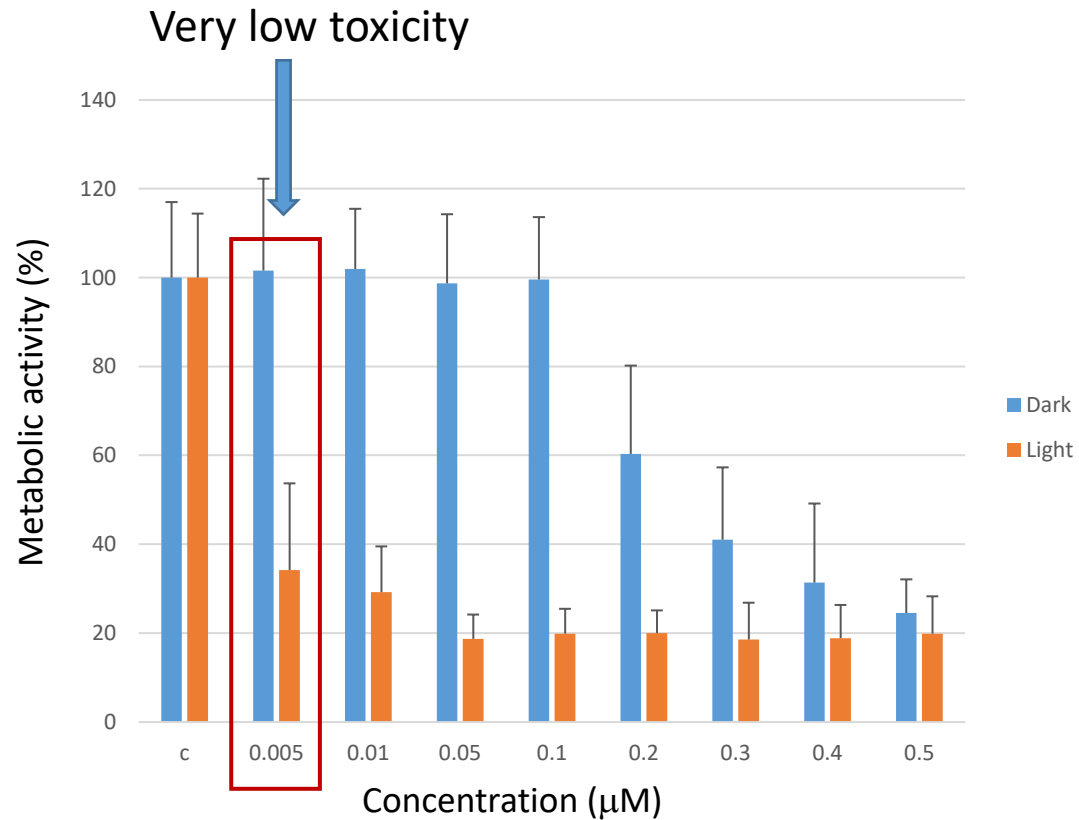
JCP-490

ROS: 56%

MTT assay

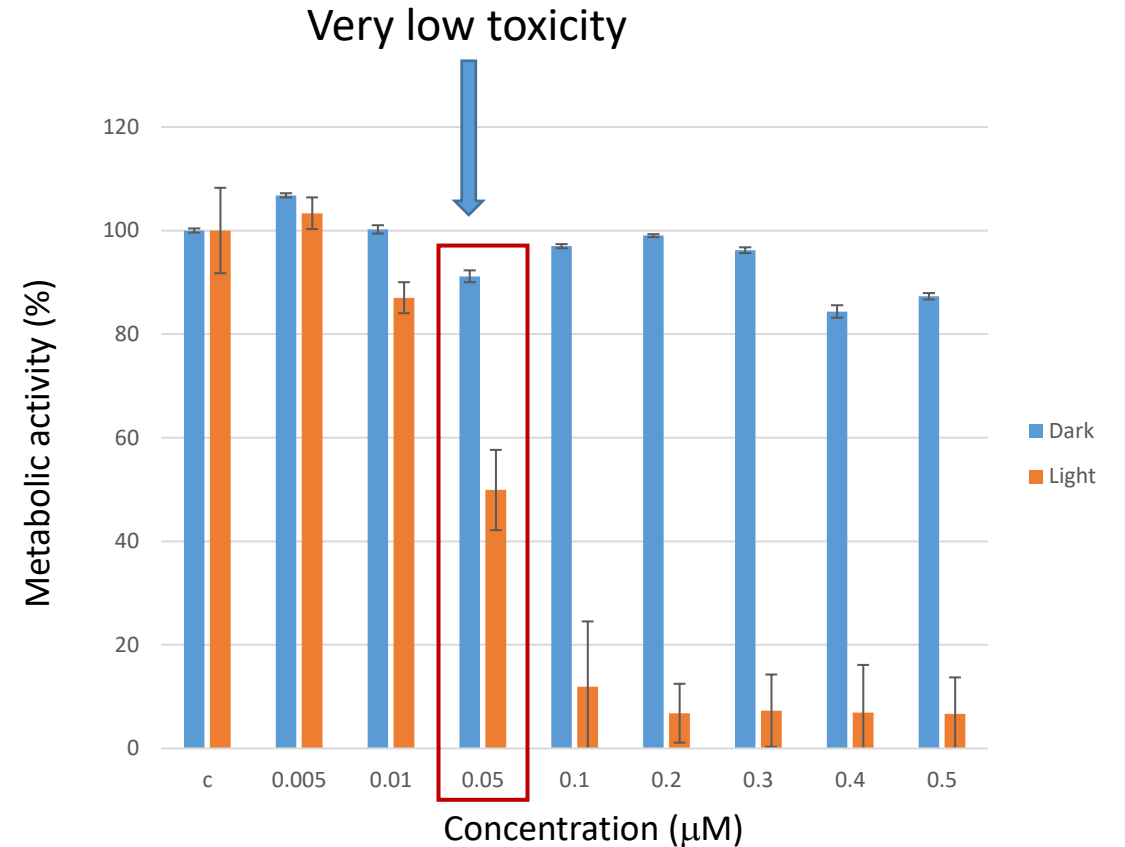
MTT JCP-490 24h + 24h post illumination - High concentrations

ILDH lamp (blue light):
7min 35s = 5J/cm²



$EC_{50} < 5 \text{ nM}$

CCLP cells



$EC_{50} = 5 \text{ nM}$

SNU cells

Conclusions

- BOLD G1-G4 generation-derived from 1-(pyridin-2-yl)benzo[a]imid-azo[5,1,2-cd]indolizine molecules are efficient molecular probes in cancer cells.
- These molecules exhibit promising properties in photodynamic therapy of cancer.
 - Bathochromic absorption shifts are still necessary for the treatment of internal tumors.
- Aurkines are very promising new chemical entities for the treatment of platinum-resistant cholangiocarcinoma.
 - These molecules show very low toxicity in normal cholangiocytes

The situation is serious, but not hopeless



Iván Rivilla
Ikerbaque. R. Assoc. Prof.



Fernando Auria Luna
Postdoc Associate



Jesús (Txus) Bañales, Irene Olaizola



Amanda Ribeiro PhD



Óscar Rodríguez PhD



Mikel Odriozola PhD



Amaia Larumbe PhD



*Virginia
San Nacianceno PhD*

