The 9th International Electronic Conference on Medicinal Chemistry (ECMC 2023)
01–30 November 2023 | Online

Thiosugars as vectors for G-quadruplex ligands: potent anticancer and antiparasitic drugs

Chaired by Dr. Alfredo Berzal-Herranz and Prof. Dr. Maria Emília Sousa

pharmaceuticals MDPI

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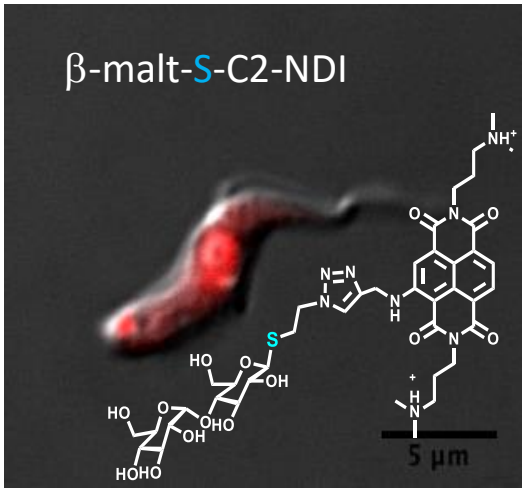
Thiosugars as vectors for G-quadruplex ligands: potent anticancer and antiparasitic drugs

Graphical Abstract



Abstract:

G-quadruplexes (G4) are DNA secondary structures that play important roles in the regulation of gene expression in human cells and have been proposed as therapeutic targets in cancer. At the same time, putative G-quadruplex forming sequences have also been found on the genome of parasites *T. brucei*, *L. major* and *P. falciparum* suggesting they could also be explored as therapeutic targets.[1]

We have designed and prepared thio sugars as vectors for naphthalene diimides, a classical G-quadruplex ligand, aiming to improve binding to G4 and biostability. Recent results on their efficacy against cancer cells and parasitic infections will be presented.[2]

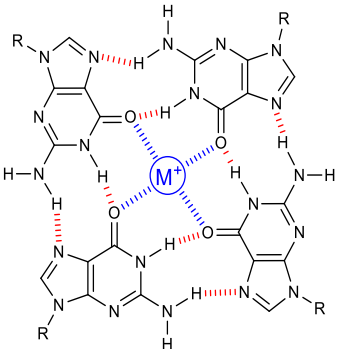
1. Belmonte-Reche et al., *J. Med. Chem.* **2018,** *61* (3), 1231-1240.
2. Belmonte-Reche et al., *Eur J Med Chem* **2022**, 232: 114183.

Keywords: anticancer; antiparasitic; DNA G-quadruplex; Trypanosoma brucei

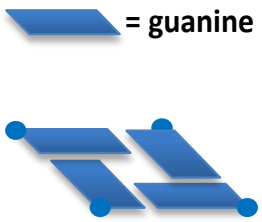
Introduction

What are DNA G-Quadruplexes (G4)?

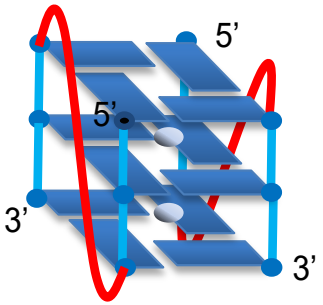
- G-quadruples (G4) form in G-rich tracts: X_n**GGG**X_n**GGG**X_n**GGG**.....



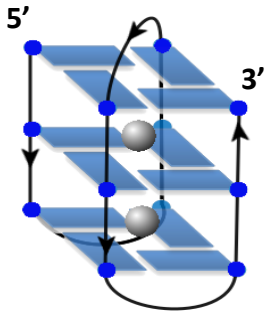
Hoogsteen hydrogen bonding



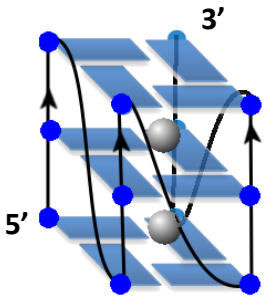
Guanine tetrad



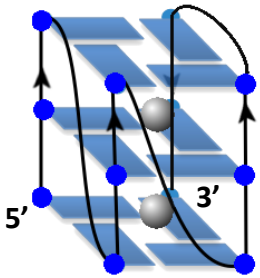
G-quadruplex (G4)



Antiparallel Quadruplex



Parallel Quadruplex



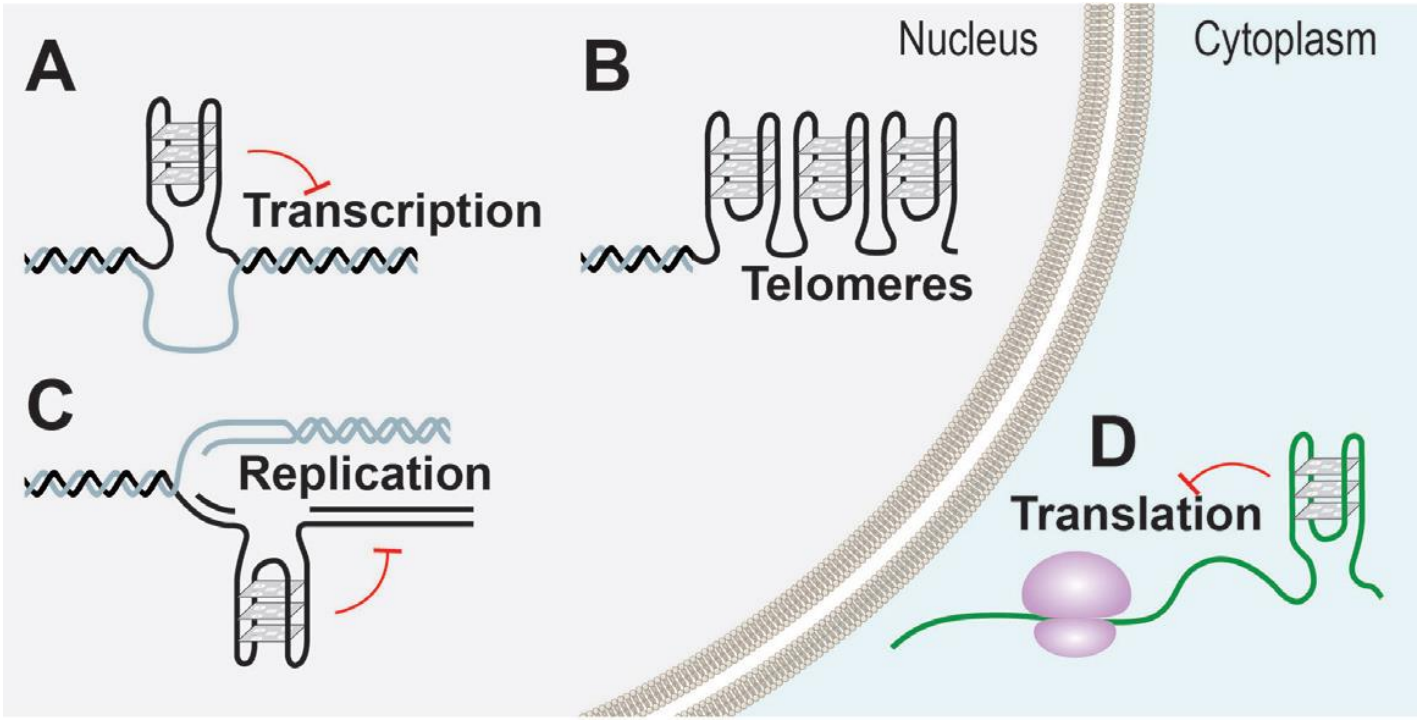
Hybrid Quadruplex

DIFFERENT TOPOLOGIES DEPENDING ON:

- **LINEAL SEQUENCE**
- **CATION**
- **LOOP**
- **pH**
- **TEMPERATURE**

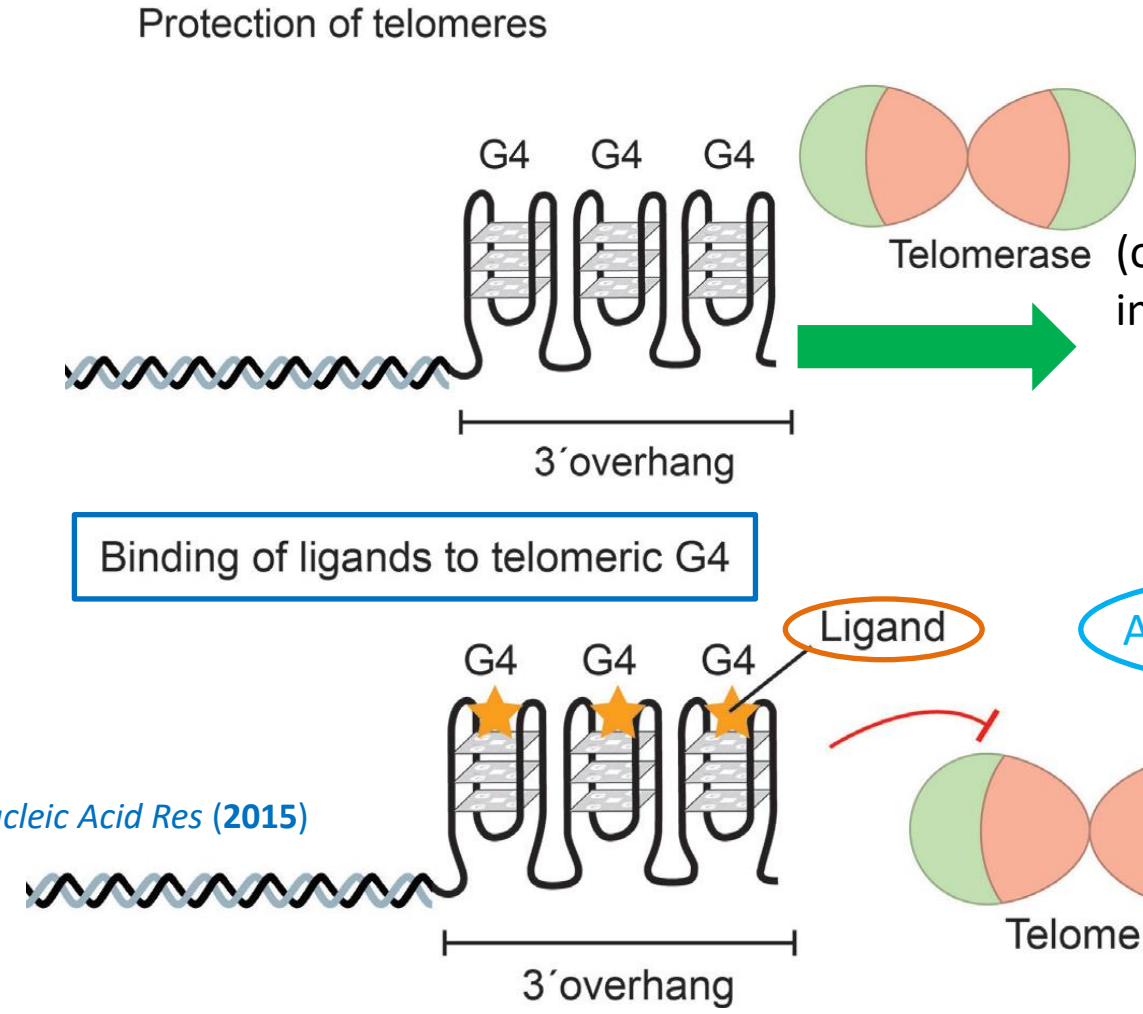
Introduction

Location of G4s in cells



Introduction

Roles of G4s in cells

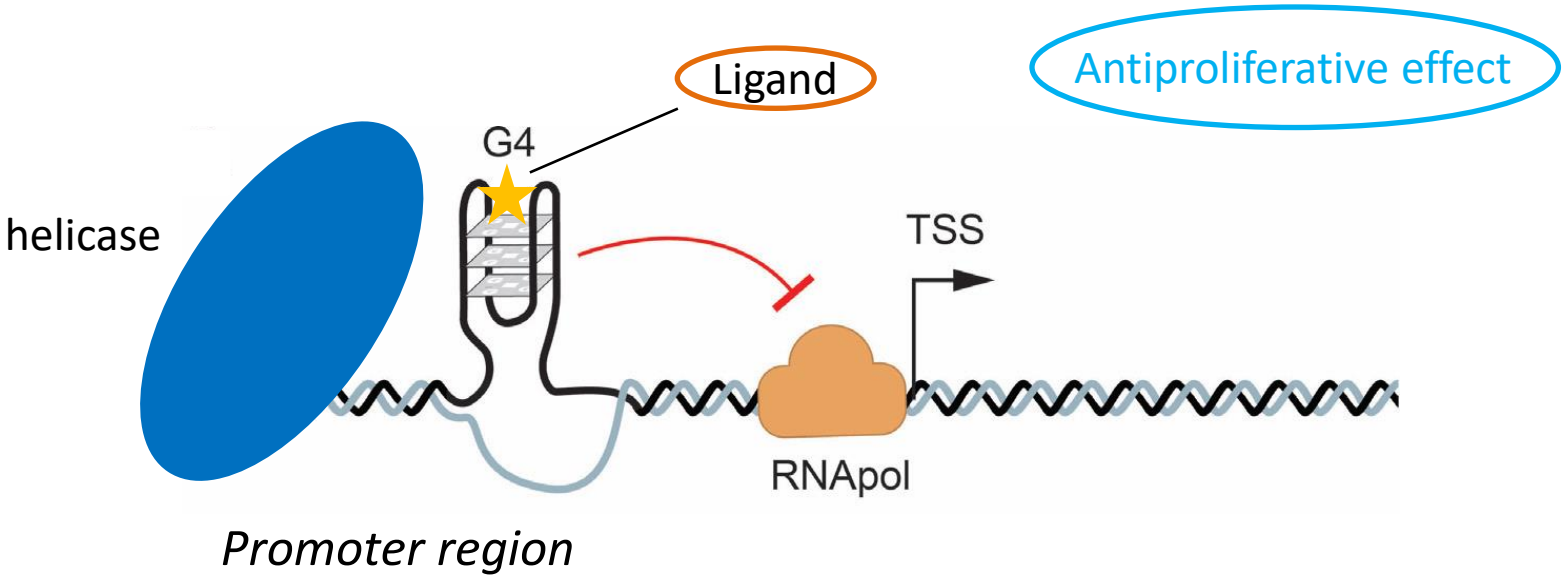


Rhodes et al. *Nucleic Acid Res* (**2015**)

Introduction

Antiproliferative activity of G4 ligands

Regulation of gene expression



Promoter region

ONCOGENE

Introduction

Antiparasitic activity of G4 ligands

Sleeping sickness, is an insect-borne parasitic disease of humans and other animals caused by *Trypanosoma brucei*.

Putative G-quadruplex (PQS) sequences in *T. brucei* genome and Frequency (number of times each sequence occurs in the genome)

PQS	Name	Frequency
GGGCAGGGGGTGATGGGGAGGAGCCAGGG	EBR1	33
GGGTTAGGGTTAGGGTTAGGG	hTel	26
GGGAGAGGGAGAGGGAGAGGG		5

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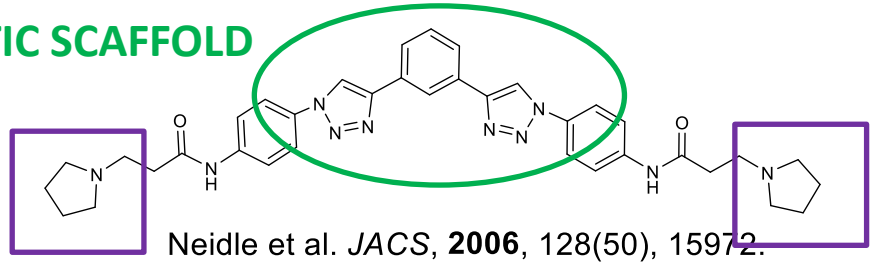
Introduction

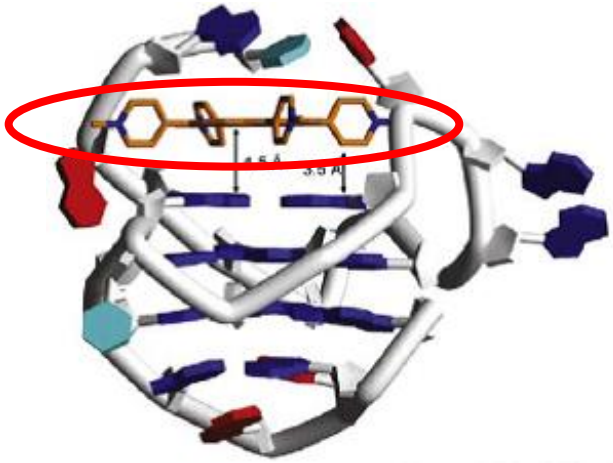
G4 ligands

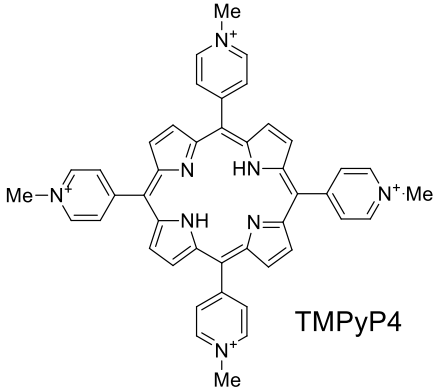
AROMATIC SCAFFOLD

CATIONIC SIDE CHAINS

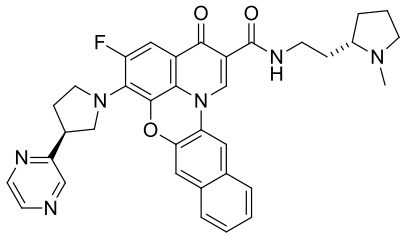


Neidle et al. *JACS*, **2006**, 128(50), 15972.





TMPyP4



Quarfloxin

Clinical trials for leucemia

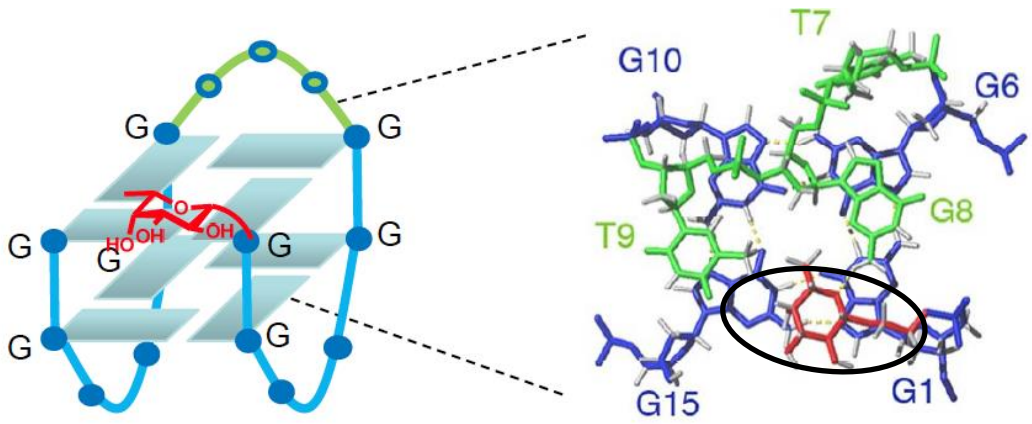
Drawbacks to become anticancer drugs:

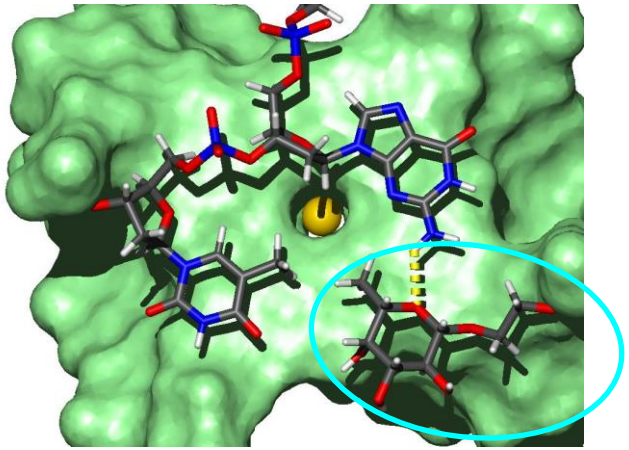
-low selectivity G4 vs dsDNA

-Not drug-like molecules

Introduction

Carbohydrates G-quadruplex conjugates





ΔG (Carb-π) = 0.2-0.6 kcal/mol

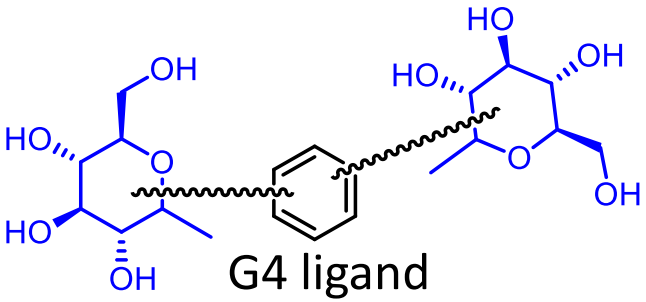
Possible interactions carb-G4:

- by stacking to guanines
- by Hydrogen bonding

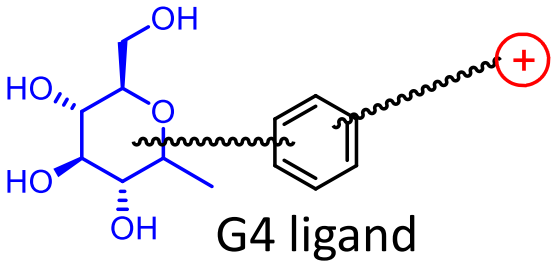
Gómez-Pinto et al. *Chem. Eur. J.* **2013**, *19,* 1920-1927.

Introduction

Sugar modified G4 ligands



G4 ligand



G4 ligand

WHY?

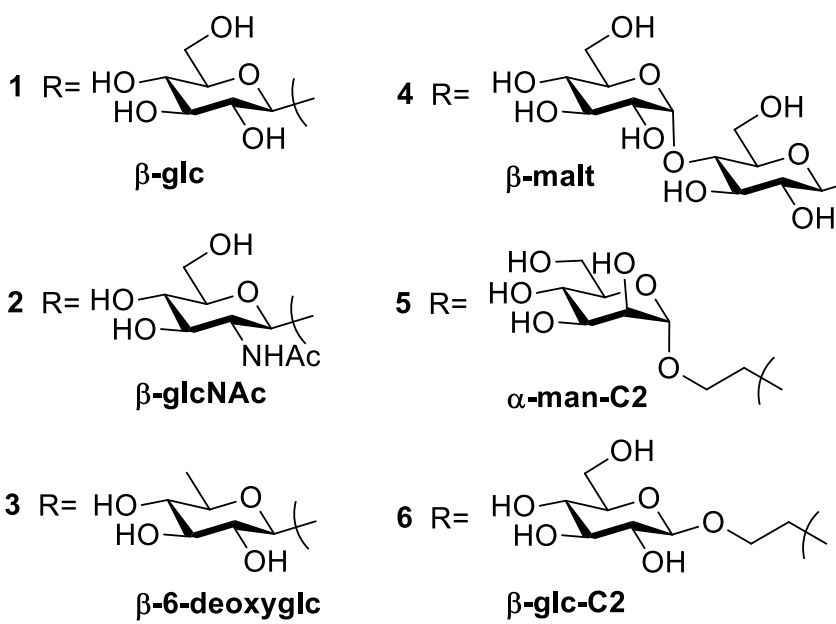
a) To increase binding and selectivity to G4 targets (sugar-mediated interactions to G4's)

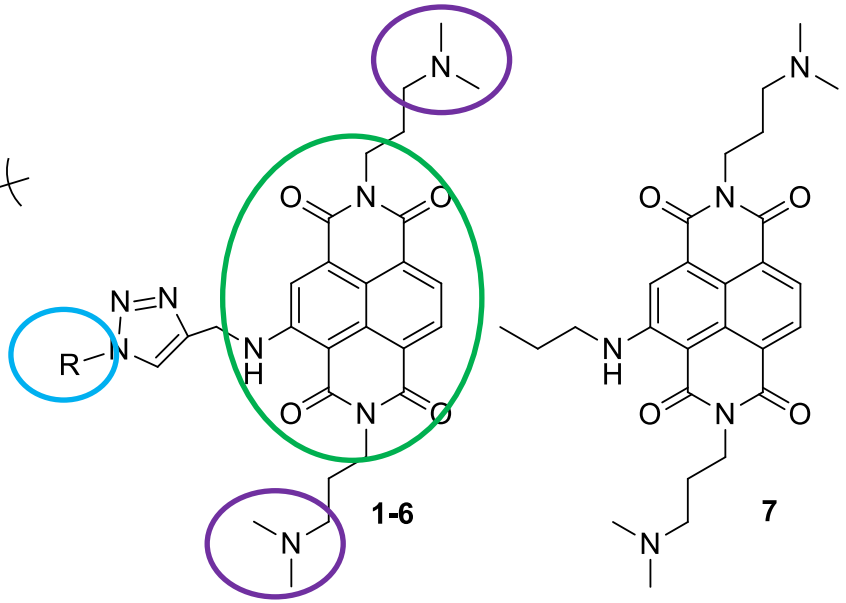
b) To promote selective entrance into tumoral cells through glucose transporters (GLUT)

c) To improve drug-like properties of these molecules

Results and discussion

Carb-NDI ligands



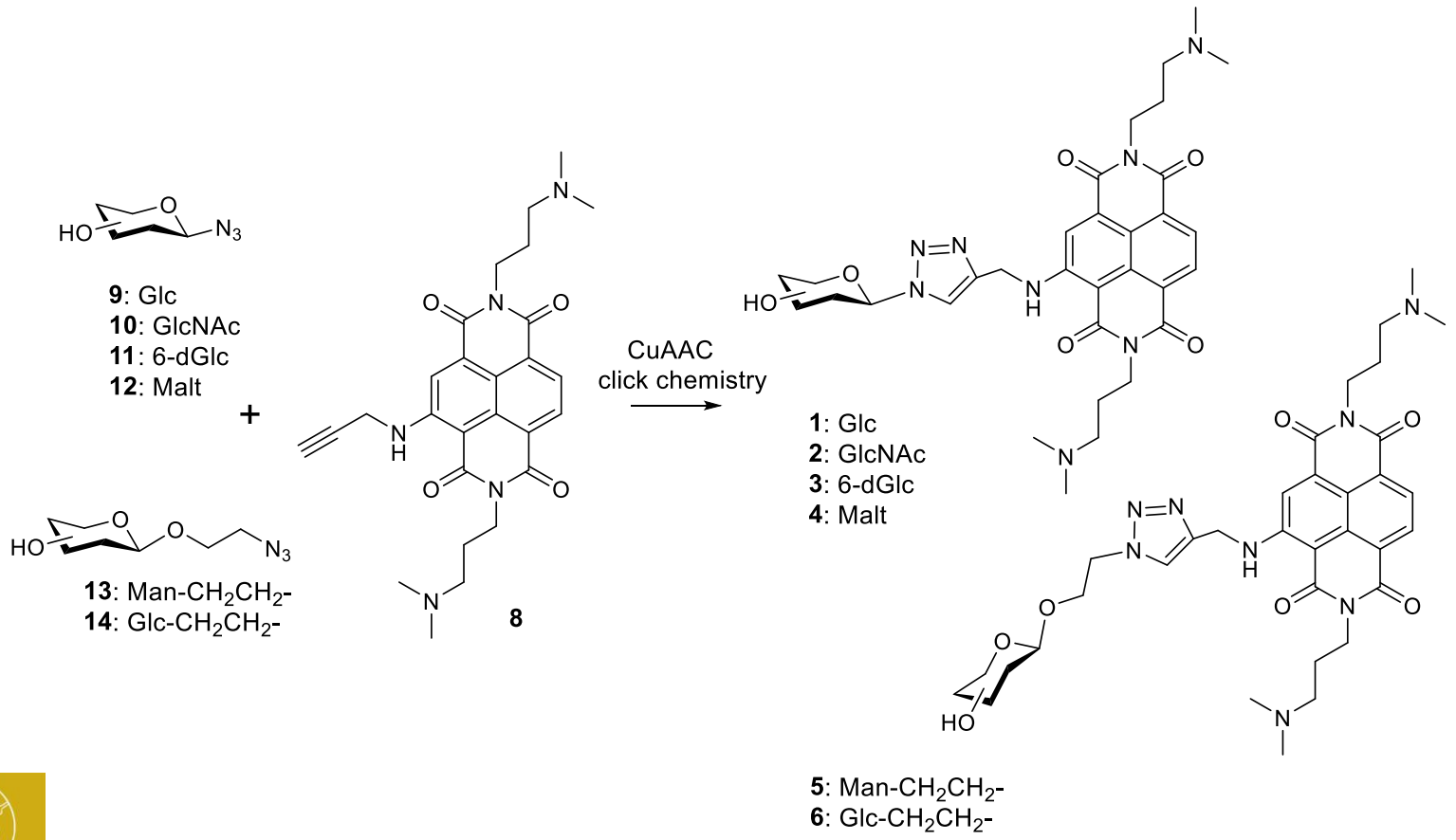


Carb-NDI NDI control

Prof. M. Freccero
UNIVERSITÀ DI PAVIA
CSIC ipbln

Results and discussion

Synthesis



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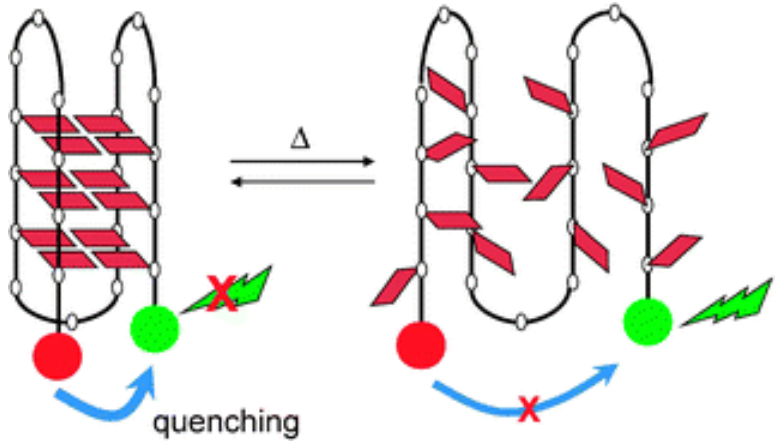
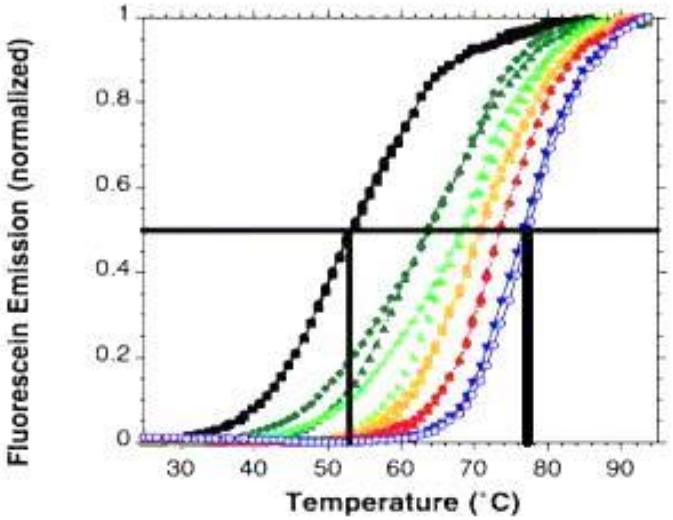
Results and discussion

Binding to G4



FRET assay

ΔT_m



G4 ligand addition

Prof. JL Mergny

Institut Européen de Chimie et Biologie
I E C B

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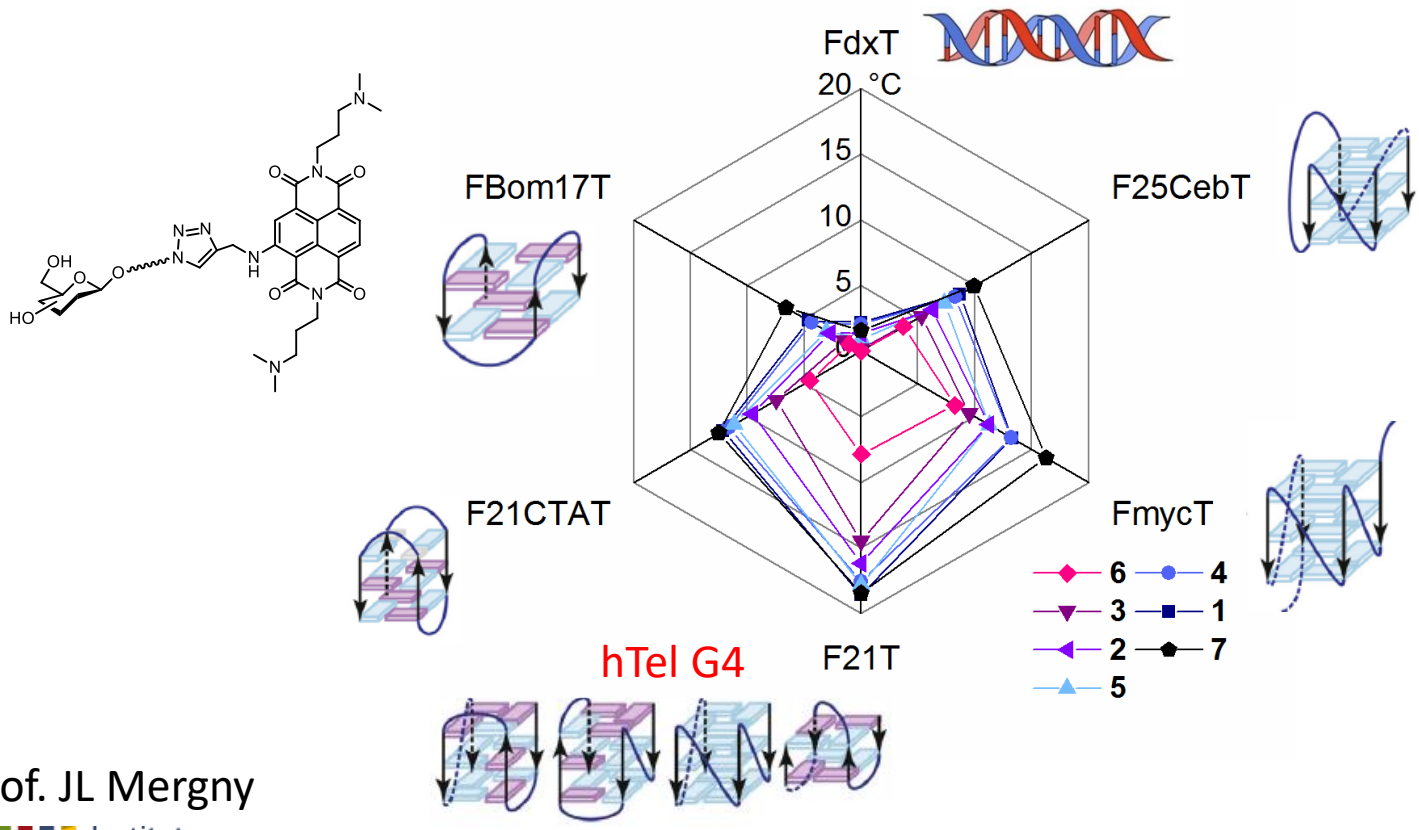
Results and discussion

Binding to G4

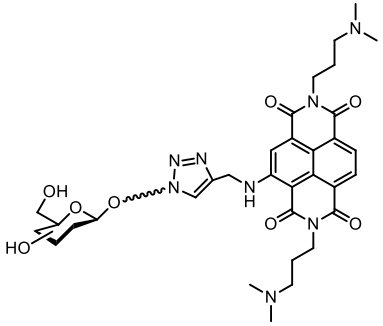


Prof. JL Mergny
Institut Européen de Chimie et Biologie
I E C B

- Selectivity for G4 vs dsDNA
- Carb-NDI show small differences when binding G4

Results and discussion

Cytotoxicity assays



Carb-NDIs

IC50 values (μM) after 72 h incubation

Compound	MRC-5	HT-29	Selectivity
β-glc-NDI	1.2 ± 0.3	2.9 ± 0.5	0.4 x
β-glcNAc-NDI	0.5 ± 0.1	2.3 ± 1.0	0.2 x
β-6deoxyglc-NDI	0.9 ± 0.3	0.4 ± 0.1	2.2 x
β-maltose-NDI	2.0 ± 0.1	1.8 ± 0.2	1.1 x
α-man-C2-NDI	0.8 ± 0.1	**0.4 ± 0.1**	1.9 x
β-glc-C2-NDI	0.7 ± 0.1	**0.4 ± 0.1**	2.0 x
NDI-prop	0.3 ± 0.1	0.1 ± 0.1	3.0 x

Carb-C2-NDIs

No carbohydrate

- IC50 in the micromolar range and low selectivity
- Higher cytotoxicity for aglycone-NDI (prop-NDI)
- Carb-NDIs are less toxic than carb-C2-NDIs

Arévalo-Ruiz et al., ***Chem. Eur. J.*** 2017, 23(29), 2157-2164.

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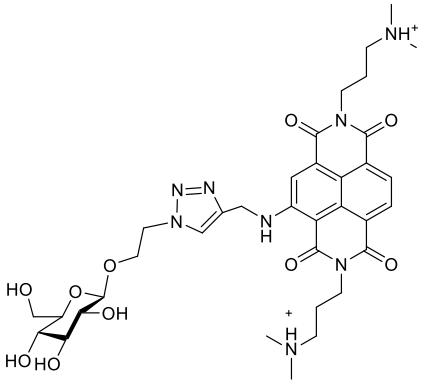
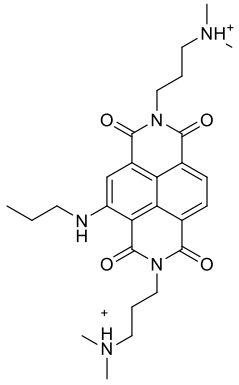
Results and discussion

In vivo activity

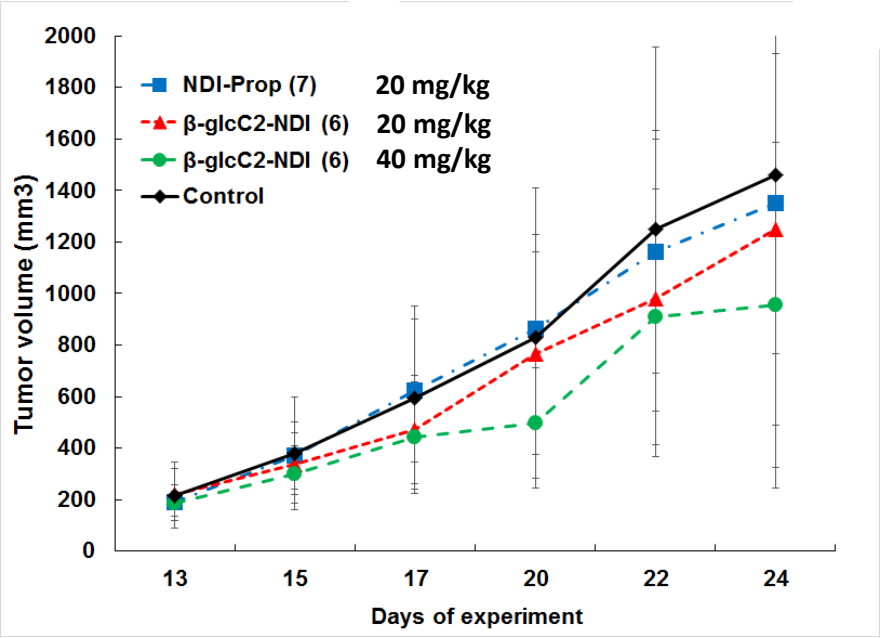
- HT-29 human colon cancer xenograft mice model.
  drug ip administration, 3 doses/wk during two weeks (6 doses)



β-glc-C2-NDI (**6**)



NDI-prop (**7**)

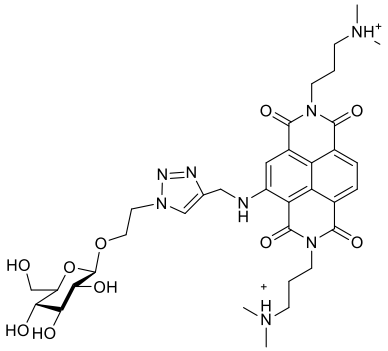


- β-glc-C2-NDI (**6**) shows a dose-dependent anti-tumour response and 35% decrease in tumour size.

Results and discussion

In vivo activity

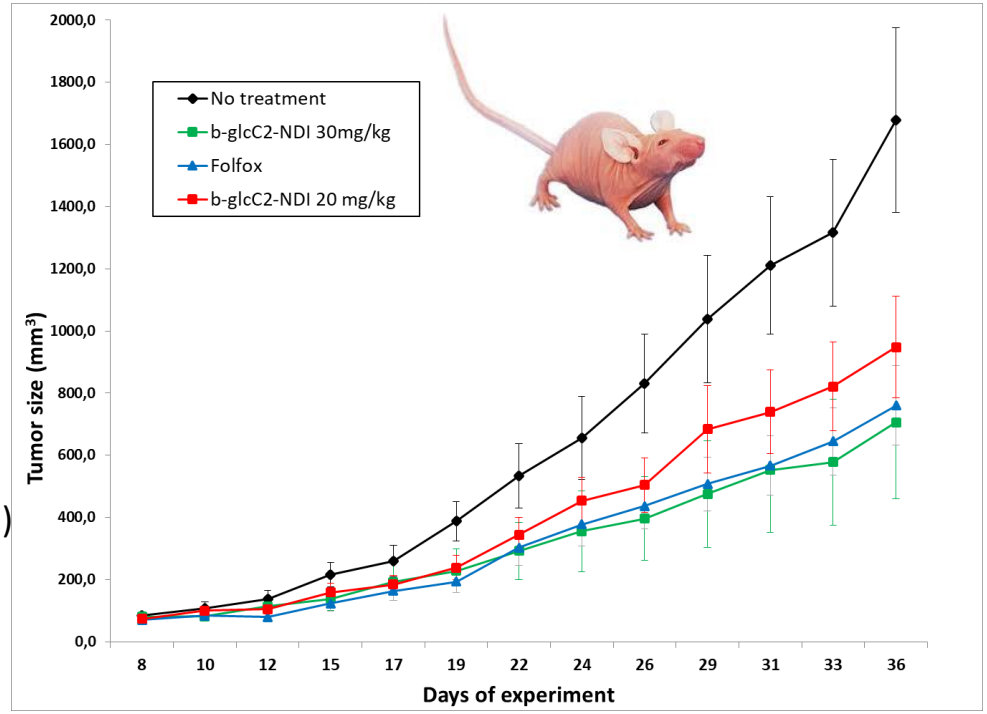
- HT-29 human colon cancer xenograft mice model.
  drug ip administration, 3 doses/wk during four weeks (12 doses)



β-glc-C2-NDI (**6**)

FOLFOX:

FOL– Folinic acid (leucovorin)
F – Fluorouracil (5-FU)
OX – Oxaliplatin (Eloxatin)



- β-glc-C2-NDI (**6**) shows similar antiproliferative effect as FOLFOX.

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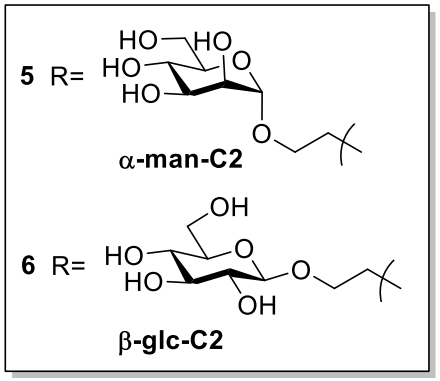
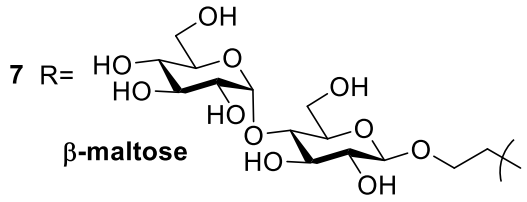
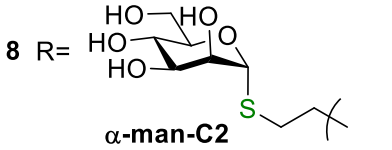
Results and discussion

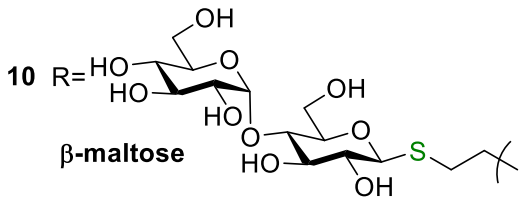
Thiosugars as vectors?



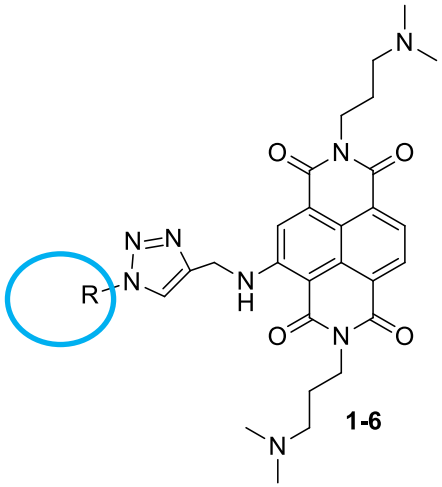


Carb-NDI

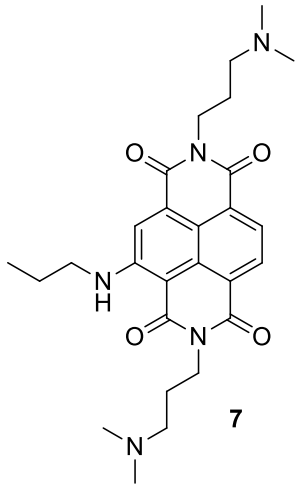




Thiocarb-NDI



Carb-NDI

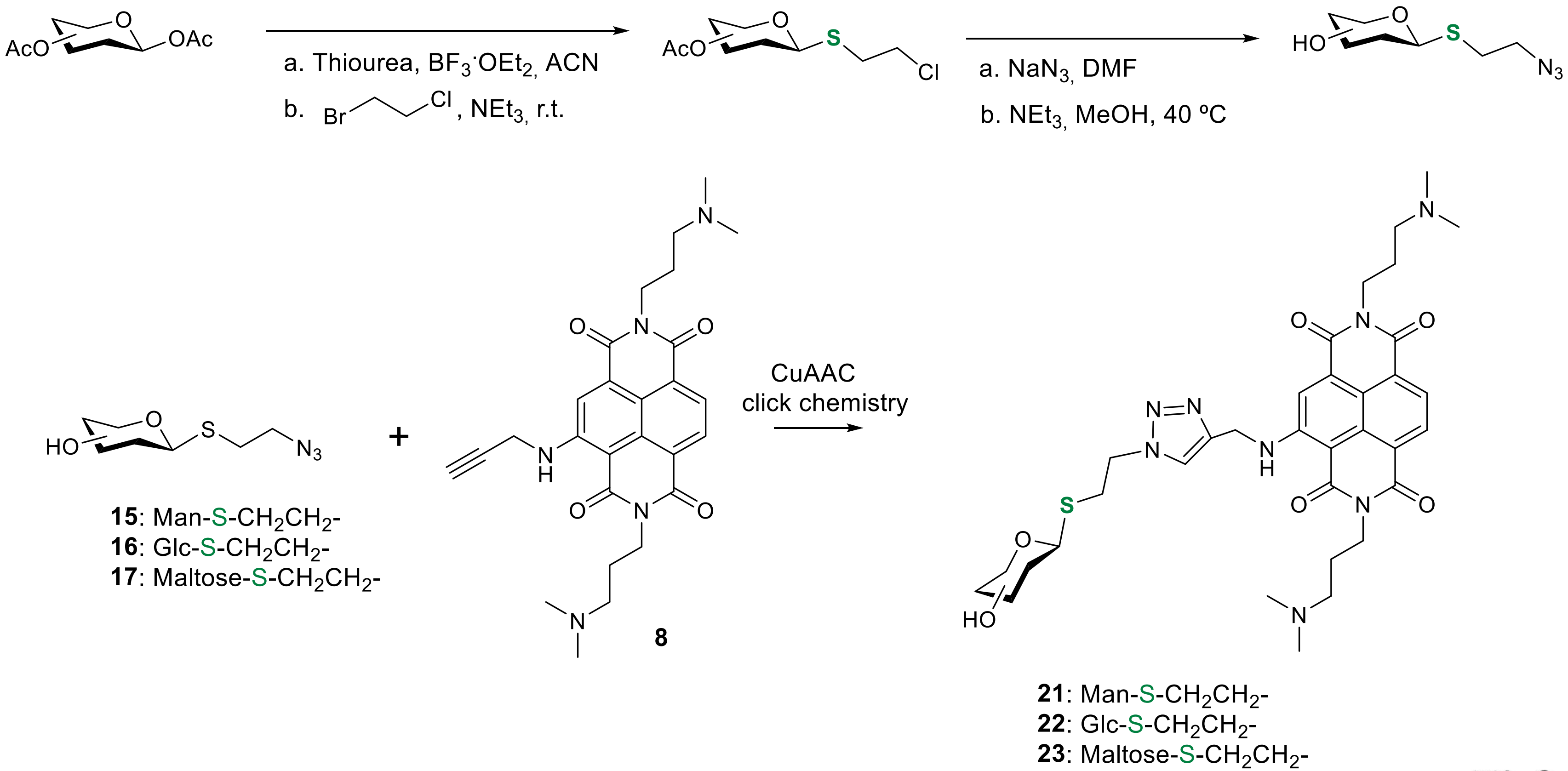


NDI control

a) More stable than carb-NDIs!
b) Further interactions with GLUT and with DNA G-quadruplexes?

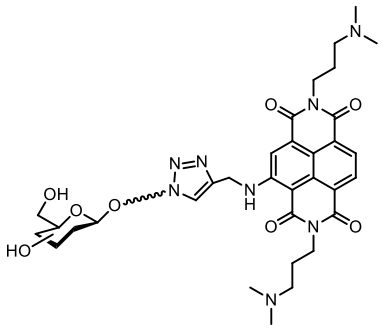
Results and discussion

Synthesis of Thiosugar-NDIs



Results and discussion

Cytotoxicity assays



Carb-C2-NDIs

Carb-C2-S-NDIs

IC50 values (μM) after 72 h incubation

Compound	MRC-5	HT-29	Selectivity
α-man-C2-NDI	0.8 ± 0.1	0.4 ± 0.1	1.9 x
β-glc-C2-NDI	0.7 ± 0.1	0.4 ± 0.1	2.0 x
β-maltose-C2-NDI	1.8 ± 0.5	0.5 ± 0.2	4.0 x
α-man-S-C2-NDI	2.7 ± 2.1	0.9 ± 0.2	3.1 x
β-glc-S-C2-NDI	2.7 ± 1.8	0.7 ± 0.1	3.7 x
β-maltose-S-C2-NDI	**2.9 ± 0.4**	**0.3 ± 0.1**	**9.9 x**

- Thiocarb-NDIs are less toxic than carb-NDIs for healthy cells
- β-maltose-S-C2-NDI is the most selective carb-NDI

Belmonte-Reche et al., ***Eur. J. Med. Chem.*** 2022, *232, 114183*

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Results and discussion

G4 found in *T.brucei* genome

Sleeping sickness, is an insect-borne parasitic disease of humans and other animals caused by *Trypanosoma brucei*.

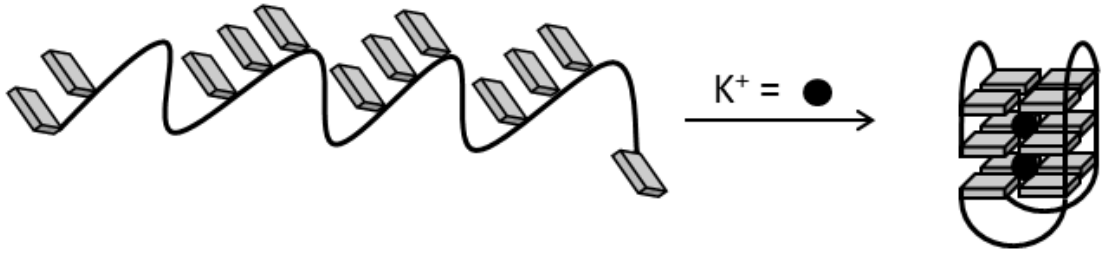
Putative G-quadruplex (PQS) sequences in *T. brucei* genome and Frequency (number of times each sequence occurs in the genome)

PQS	Name	Frequency
GGGCAGGGGGTGATGGGGAGGAGCCAGGG	EBR1	33
GGGTTAGGGTTAGGGTTAGGG	hTel	26
GGGAGAGGGAGAGGGAGAGGG		5

Results and discussion

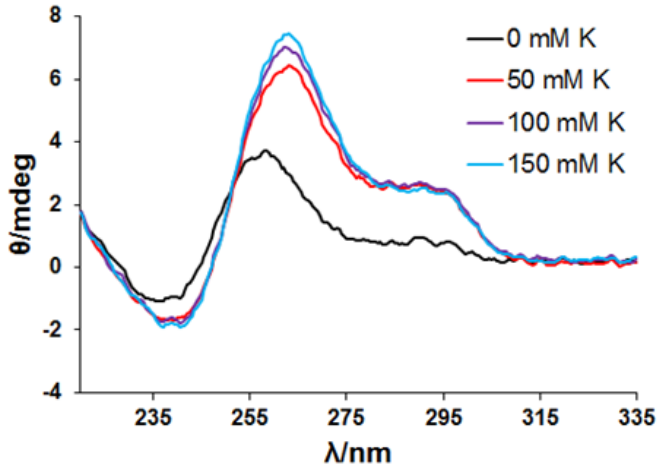
EBR1 forms a G-quadruplex

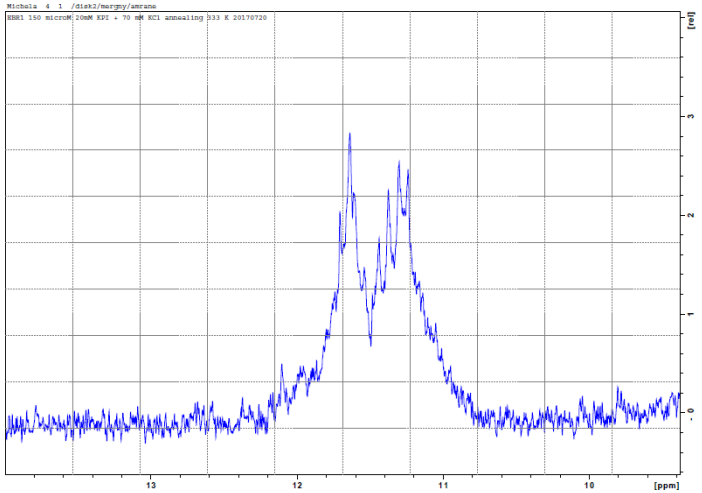
EBR1: GGGCAGGGGGTGATGGGGAGGAGCCAGGG



G-rich DNA single strand

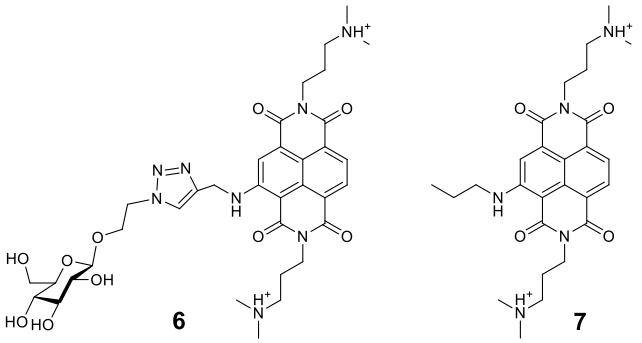
G-quadruplex DNA

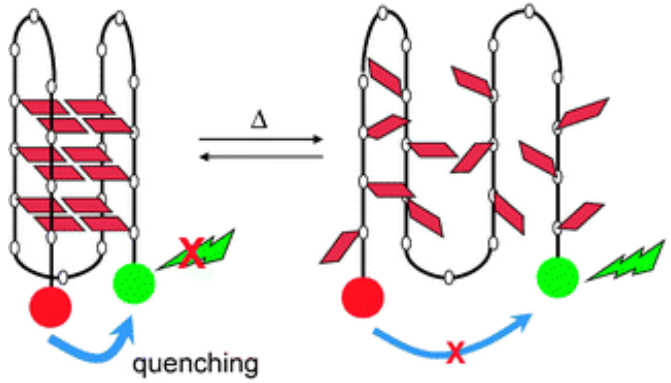


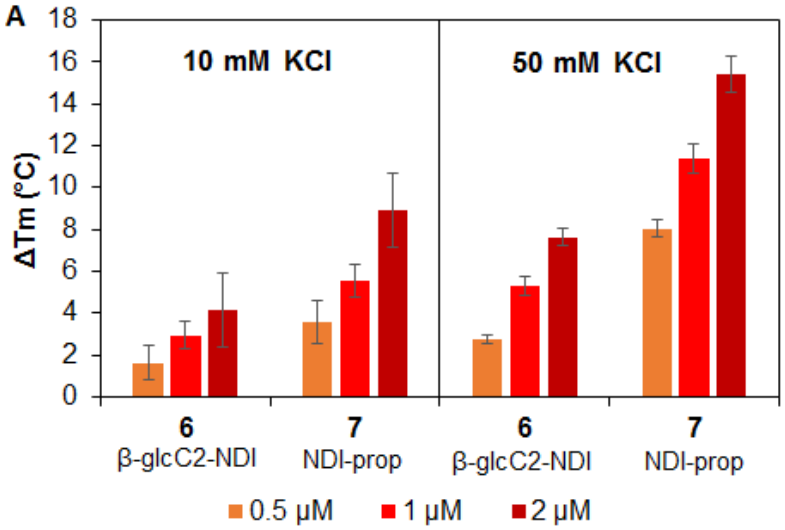


Results and discussion

Carb-NDIs bind to G4 EBR1







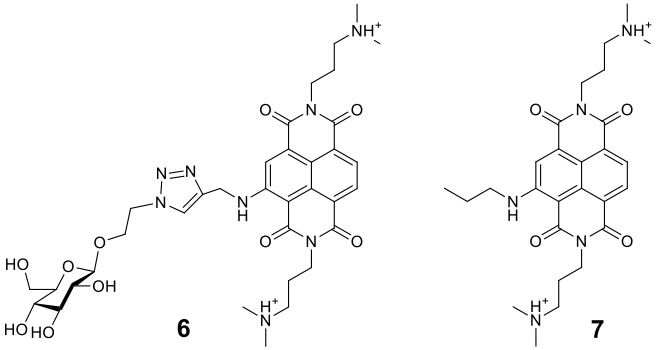
Results and discussion

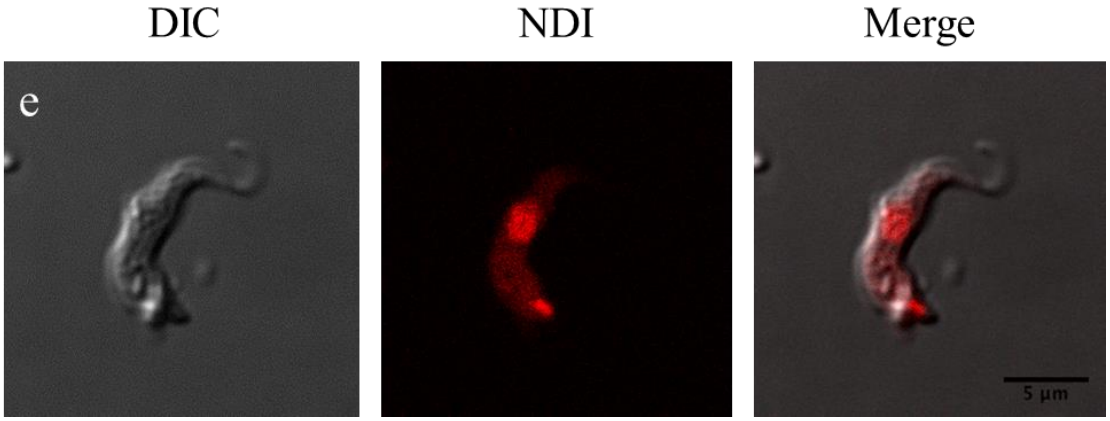
Cytotoxicity assays

	IC_{50} Parasites (uM)				SI		
	L. major	***T. brucei***	*P. falciparum*	MRC-5	*L. major*	***T. brucei***	*P. falciparum*
1 β-glc-NDI	0.244 ± 0.007	0.024 ± 0.001	1.350 ± 0.636	1.15 ± 0.29	4.7	47.9	0.9
2 β-glcNAc-NDI	1.041 ± 0.027	0.089 ± 0.007	0.360 ± 0.071	0.51 ± 0.01	0.5	5.7	1.4
3 β-6dglc-NDI	0.184 ± 0.009	**0.017 ± 0.007**	0.225 ± 0.120	0.91 ± 0.32	4.9	**53.5**	4.0
4 β-malt-NDI	0.921 ± 0.051	0.099 ± 0.010	0.370 ± 0.085	2.04 ± 0.05	2.2	20.6	5.5
5 α-manC2-NDI	0.306 ± 0.019	**0.021 ± 0.003**	0.180 ± 0.099	0.81 ± 0.44	2.6	**38.6**	4.5
6 β-glcC2-NDI	0.537 ± 0.030	**0.017 ± 0.009**	0.275 ± 0.191	0.71 ± 0.25	1.3	**41.8**	2.6
7 NDI-prop	0.034 ± 0.005	0.009 ± 0.001	0.091 ± 0.013	0.36 ± 0.16	10.6	40.0	4.0
BRACO-19		5.51 ± 0.99	9.70 ± 4.67	8.33 ± 2.96		1.5	0.9
suramin		0.038 ± 0.003					

CSIC ipbln

Results and discussion

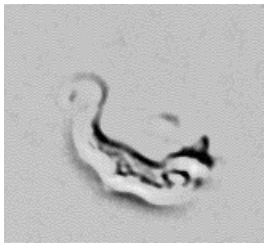


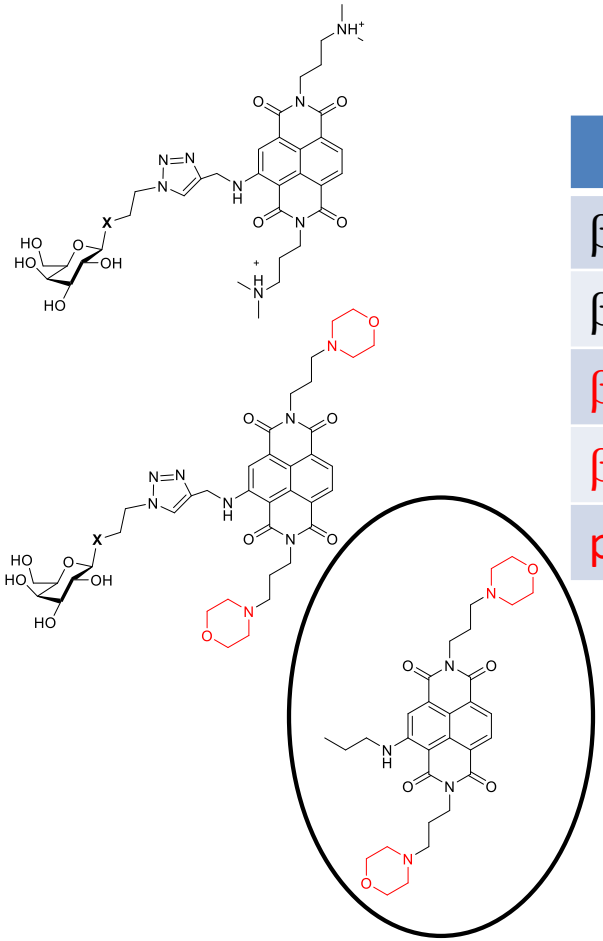


Carb-NDIs locate into the nucleus and kinetoplast of *T. brucei*

E.Belmonte-Reche, *et al.*, ***J. Med. Chem.*** 2018, 61, 1231-1240.

Results and discussion





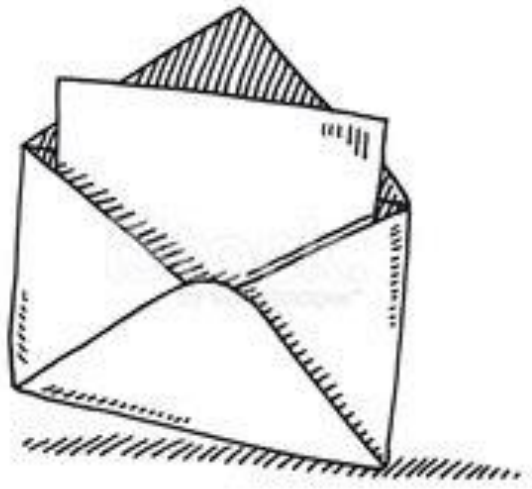
IC50 values (μM)

Compound	MRC-5	*T.brucei*	Selectivity
β-gal-O-C2-NDI	1.9 ± 0.7	0.05 ± 0.01	38.8 x
β-gal-S-C2-NDI	1.5 ± 0.2	0.04 ± 0.01	38.7 x
β-gal-O-C2-NDI-morph	10.4 ± 3.2	0.15 ± 0.02	69.3 x
β-gal-S-C2-NDI-morph	23.5 ± 6.3	0.85 ± 0.29	27.6 x
prop-NDI-morph	47.2 ± 1.6	0.05 ± 0.02	**131.1 x**

- Some carb-NDIs-morph are more selective than carb-NDIs
- **prop-NDI-morph** is the most selective compound for *T.brucei*

Belmonte-Reche et al., ***Eur. J. Med. Chem.*** 2022, *232, 114183*

Conclusions



Take on messages

☺ Carbohydrate conjugated G4 ligands are promising drugs

☺ DNA G-quadruplexes are potential targets for cancer and for parasitic infections

Acknowledgments

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