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Evaluation of the interactions of selected ligands with (3+1) G-quadruplex sequence within PARP1 gene promoter region

Chaired by **DR. ALFREDO BERZAL-HERRANZ**;
Co-Chaired by **PROF. DR. MARIA EMÍLIA SOUSA**

pharmaceuticals MDPI

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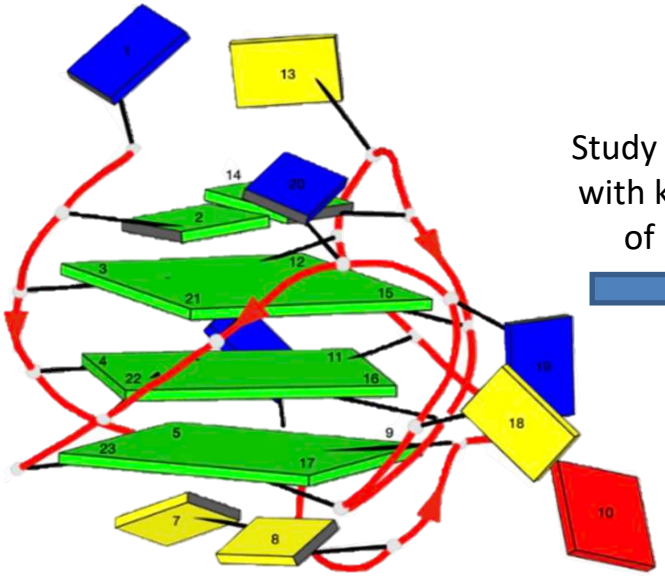
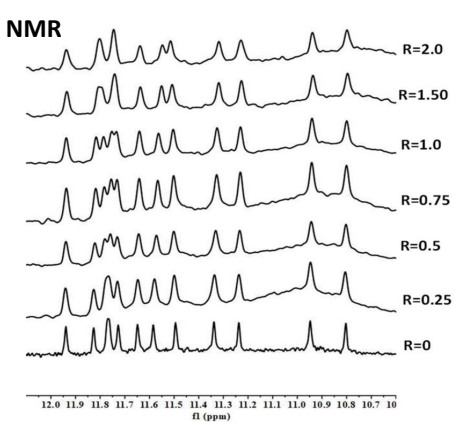
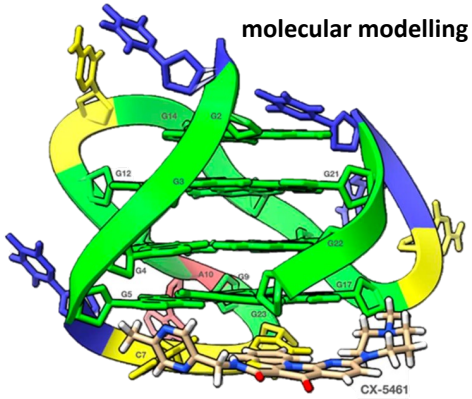
Evaluation of the interactions of selected ligands with (3+1) G-quadruplex sequence within PARP1 gene promoter region

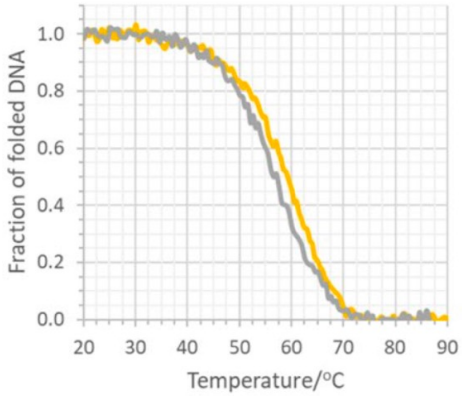


(3+1) GQ from PARP1 promoter region

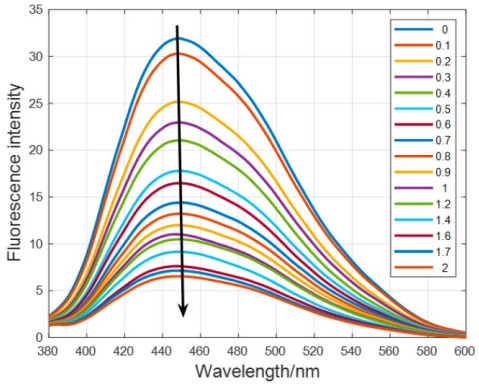
Study of interactions with known binders of parallel GQ







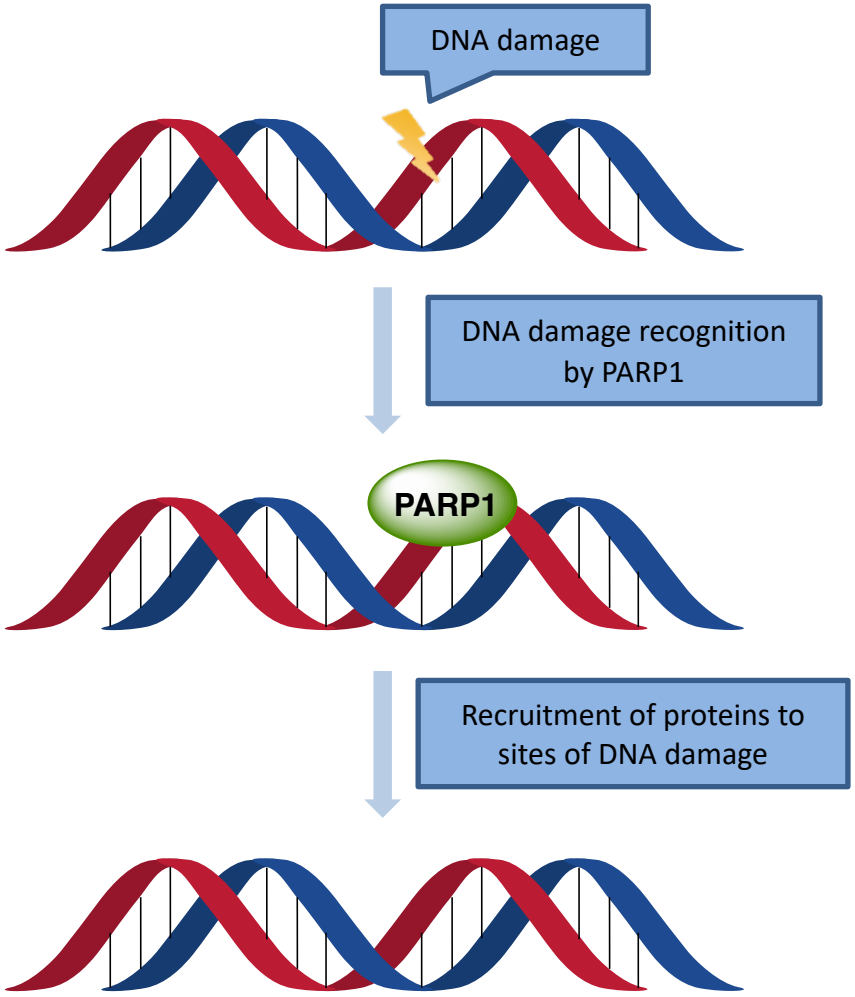
melting point



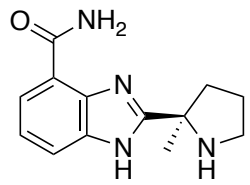
fluorescence titration

Abstract: PARP1 is a nuclear enzyme involved in DNA repair processes. Since its inhibition causes sensitization to DNA damaging chemotherapy (by the so-called “synthetic lethality”), several inhibitors have been recently developed and exploited for clinical use. However, the emergence of resistance to PARP1 inhibitors increased the interest towards alternative approaches able to interfere with PARP1 activity. In particular, within the promoter region of PARP1 a characteristic, non-canonical (3+1) G-quadruplex-forming sequence was identified. A strong correlation between G-quadruplex stabilization in gene promoters and transcriptional regulations has been proposed for several oncogenes. Since no PARP promoter modulators have been described so far, the interaction with a small collection of G-quadruplex binders was investigated, taking into account the particular hybrid topology of PARP1 G-quadruplex. Six structurally diverse compounds, extensively studied and known for showing great affinity towards canonical G-quadruplex, were selected, and NMR, CD, and fluorescence titration studies were carried out. The results from the physico-chemical analyses, confirmed by molecular modelling, showed that only one of the tested compounds showed strong stabilization of the nucleotide, demonstrating that the structural requirements for an optimal interaction between each of the ligands and the peculiar hybrid G-quadruplex region are quite strict. Overall, the studied compounds can be considered as a starting point for the identification of the key features necessary for a selective interaction with the PARP promoter G-quadruplex.

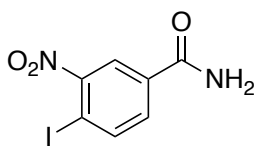
Targeting PARP1: enzyme inhibition



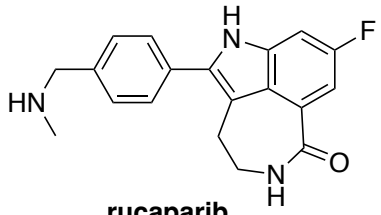
FDA approved PARP1 inhibitors



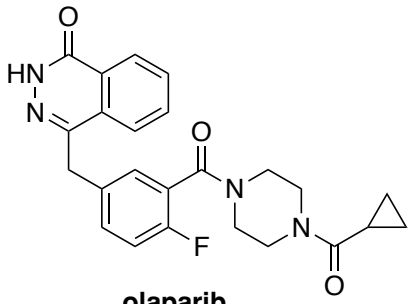
veliparib



iniparib



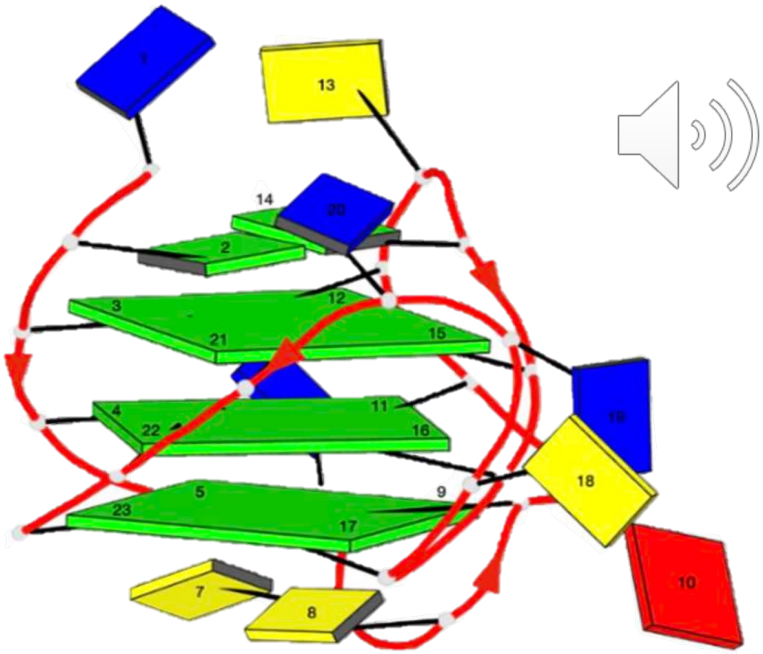
rucaparib



olaparib

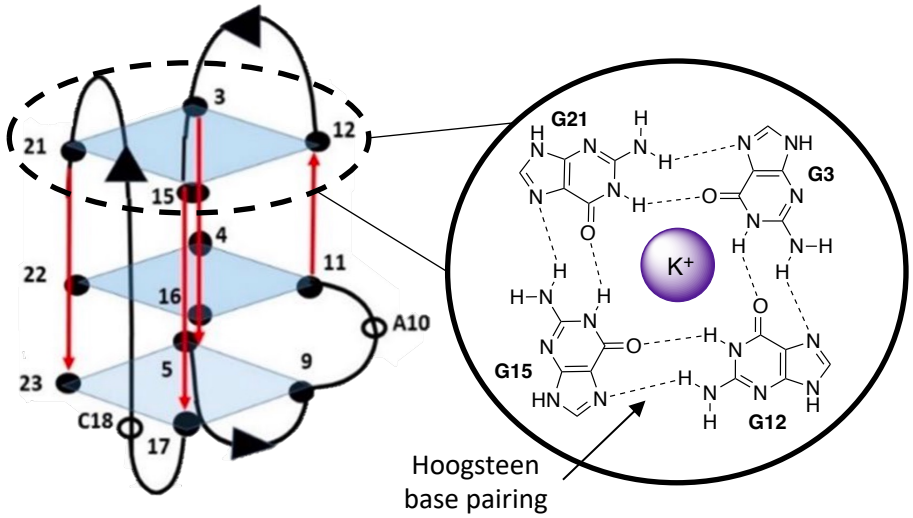
- Involved in DNA repair processes, transcriptional regulation, cell signaling and cell death.
- "synthetic lethality" caused by inhibition, and **sensitization** to DNA damaging radio- and chemotherapies.

Targeting PARP1: promoter stabilization

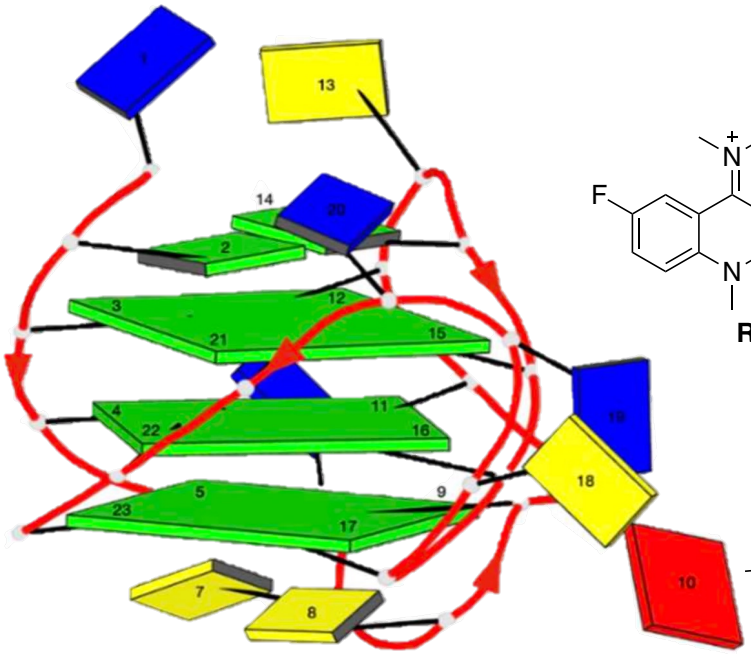


- Emergence of **resistance** to PARP1 inhibitors requires alternative approaches to interfere with PARP1 activity.
- Non-canonical **(3+1)** **G-quadruplex**-forming sequences have been identified in the **promoter region** of the PARP1 gene [TP3T6 sequence].

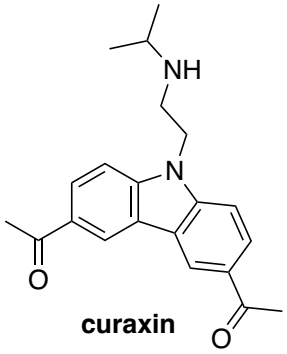
- G-quadruplexes (GQs) are secondary nucleotidic structures.
- The formation of Hoogsteen pairs in guanine-rich regions allows the stack of multiple G-tetrads.
- GQ cover an important role in telomere maintenance and in controlling the **expression** of several **oncogenes** and tumor suppressors.



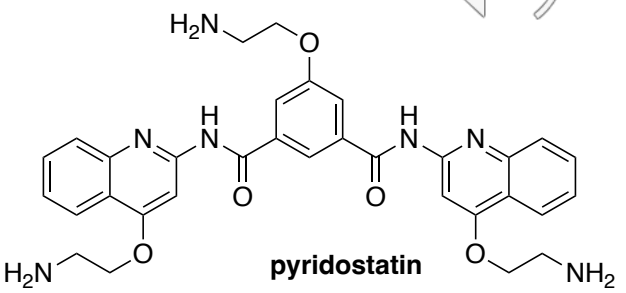
Targeting PARP1: promoter stabilization



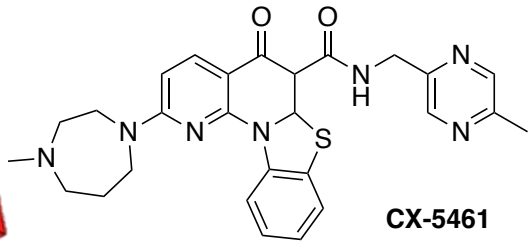
RHPS4



curaxin



pyridostatin



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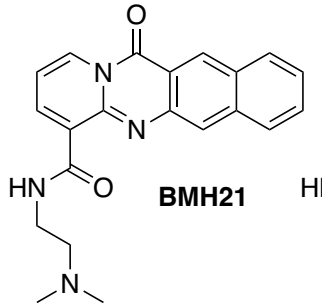
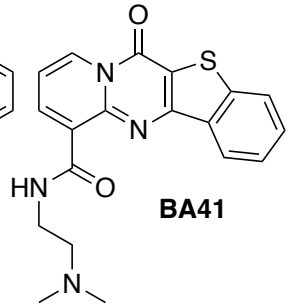


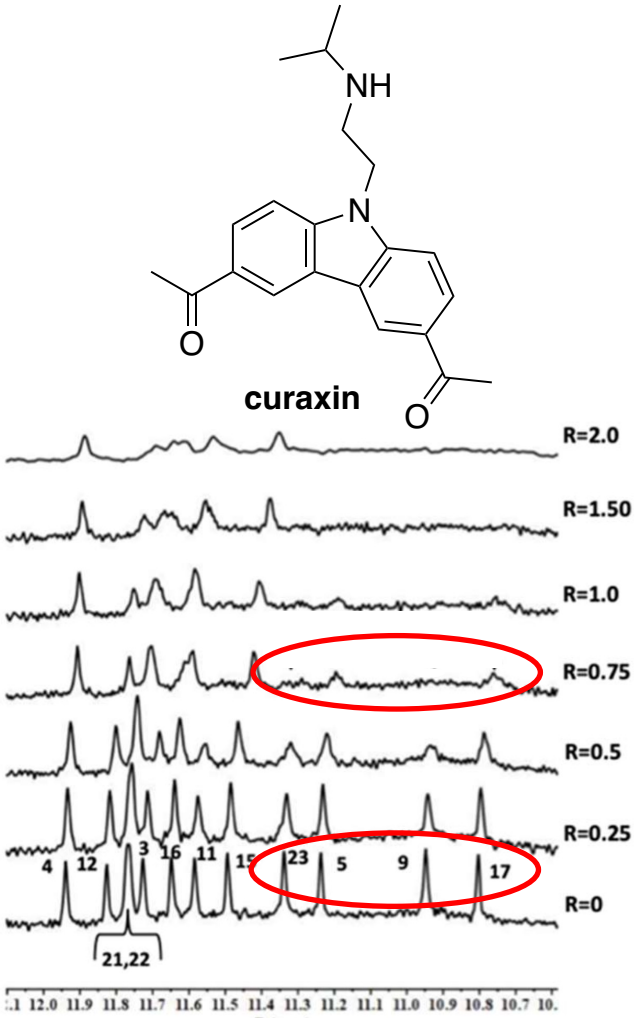
BMH21

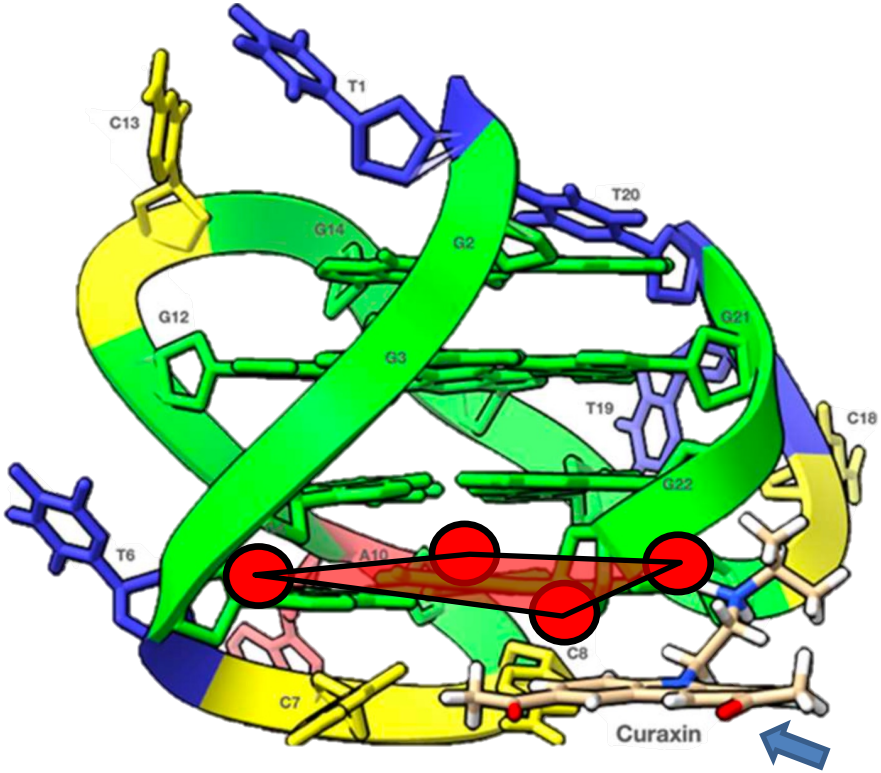


BA41

- To investigate the interaction between **(3+1) hybrid G-quadruplex** with **well-known G-quadruplex binders**.
- NMR, CD, fluorescence and molecular modeling analyses could allow to define **key molecular features** for an effective binding.

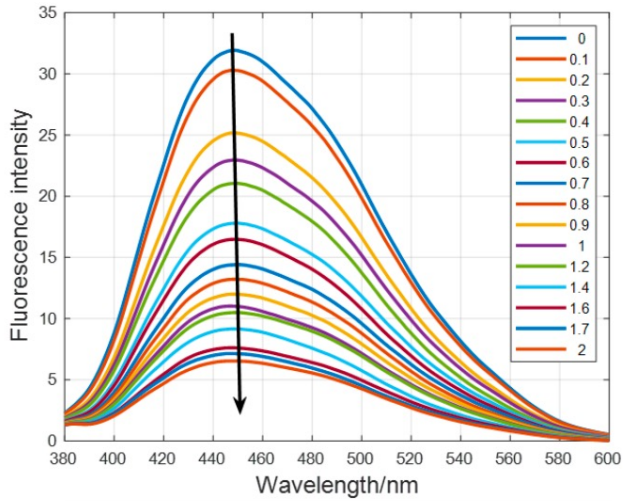
Study of the interactions TP3T6-curaxin

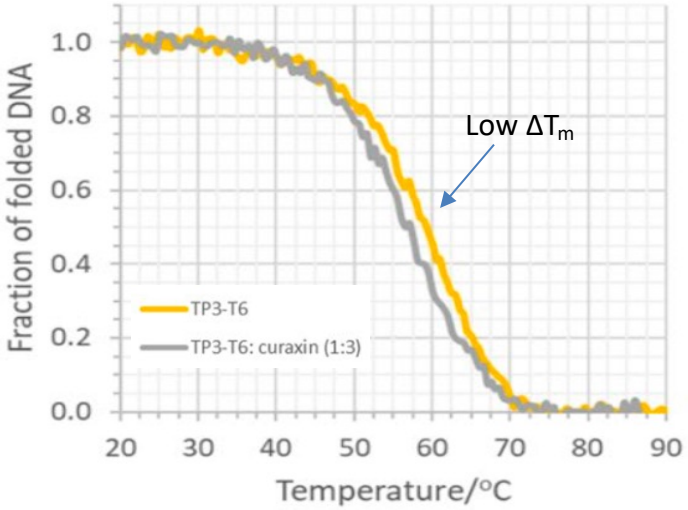


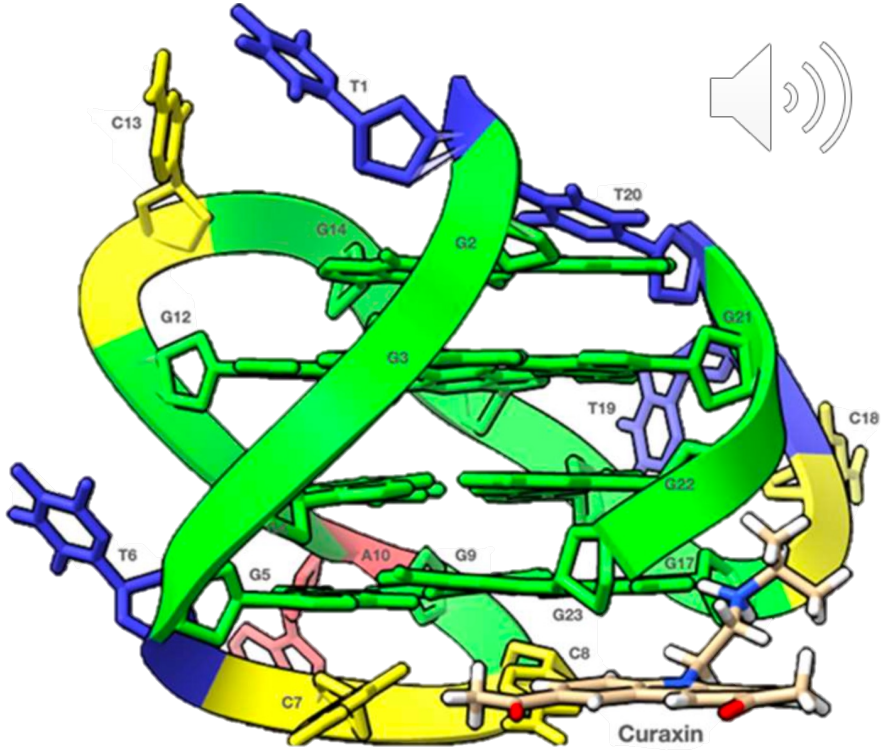


- The side chain arranged along the groove by ionic bonds.
- The rest of the molecule lies below the G5-G9-G17-G23, at the 3'-tetrad.

Study of the interactions TP3T6-curaxin

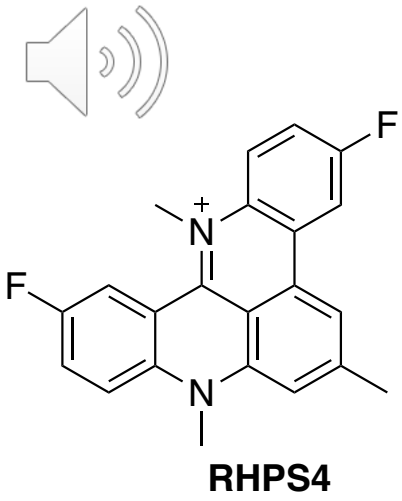


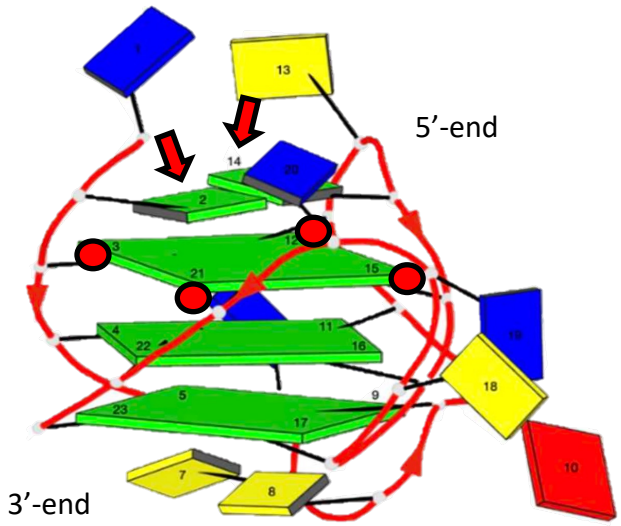


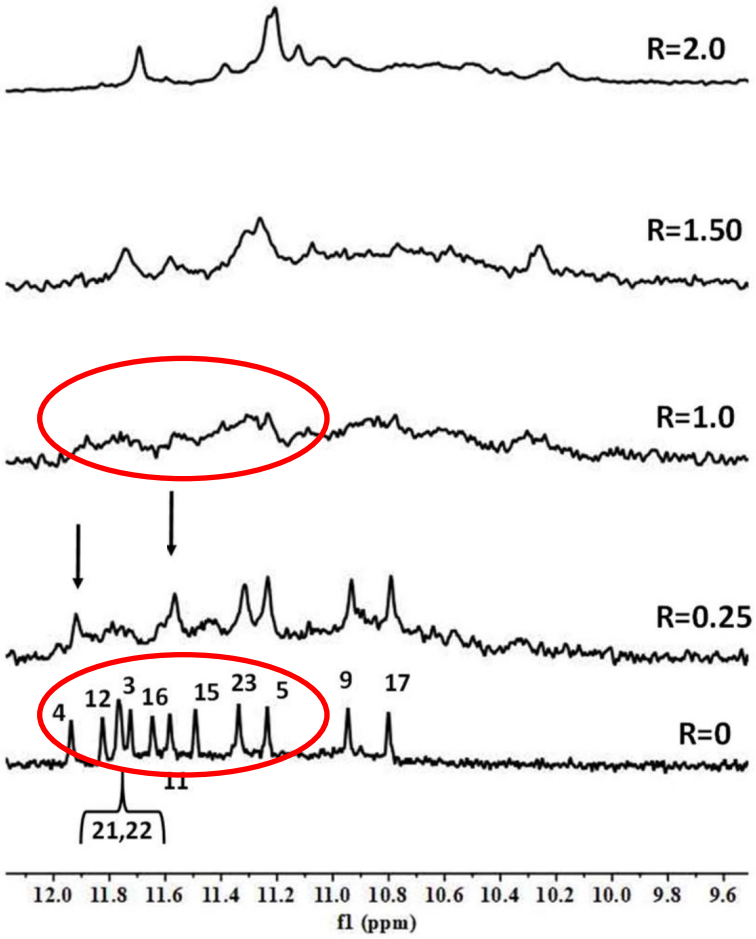


- Fluorescence titration and melting point with nucleotide/ligand in 1:3 ratio.
- No evidence of stabilization.

Study of the interactions TP3T6-RHPS4

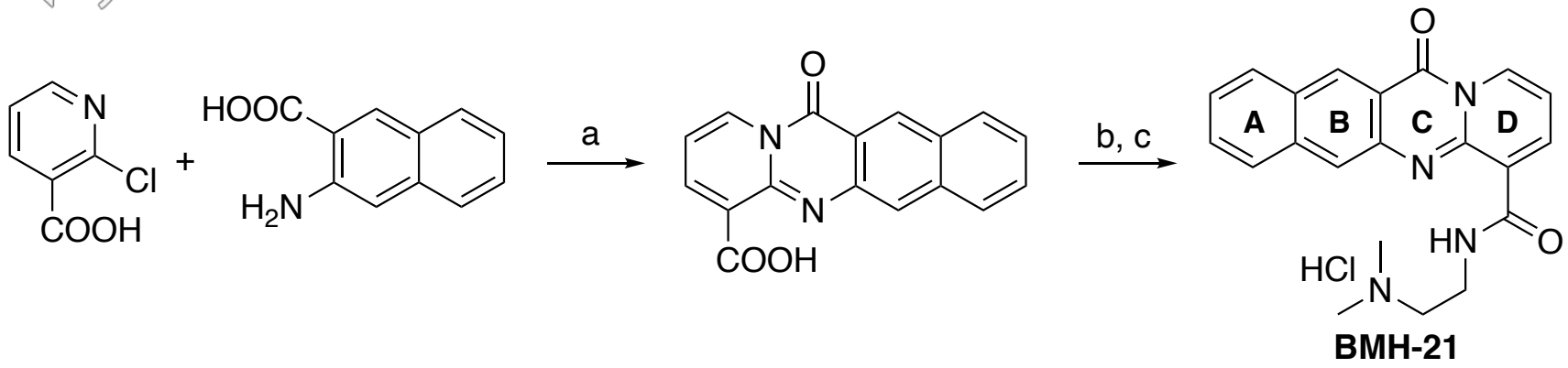






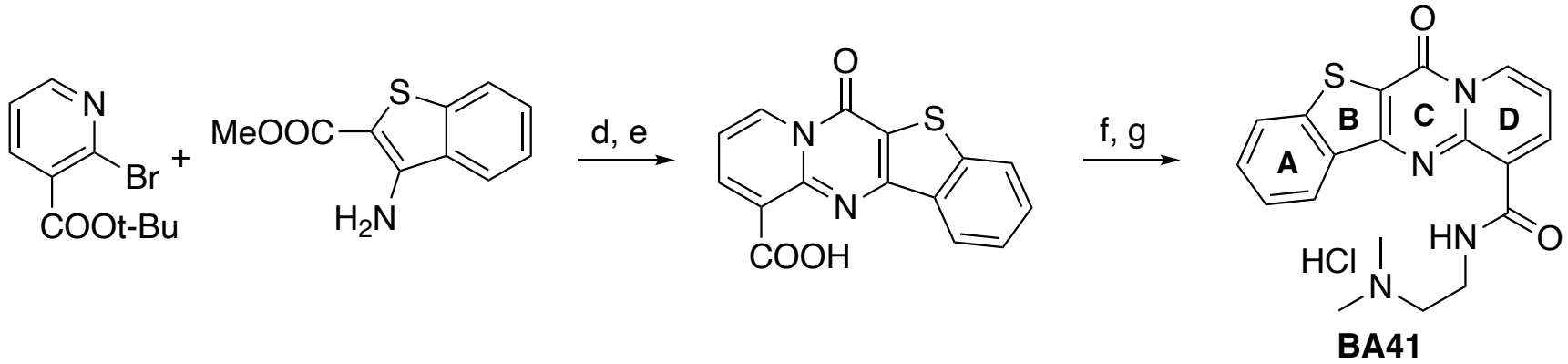
- Broadening of selected ^{1}H imino proton signals at very low ratio (R = 0.25).
- G3, G12, G15 and G21 of the 5'-end tetrad, very broad at R = 1.0
- not well-defined signals detected again at R = 2.0.
- molecular docking investigation did not allow to obtain an unambiguous model of interaction, but hindrance by G2 and G14

Synthesis of BMH-21 and BA41



BMH-21

a) reflux, HCl, EtOH; b) *N*,*N*-dimethyl ethylene diamine, TBTU, DIPEA, DMF, **58%**; c) HCl in MeOH, quantit.



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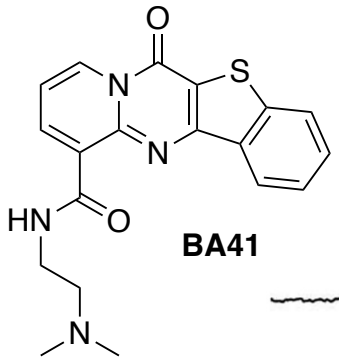
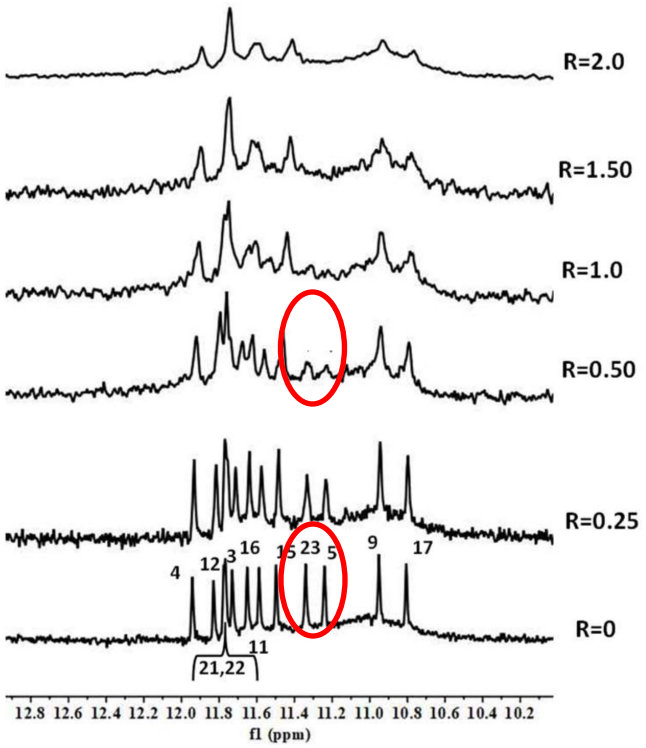
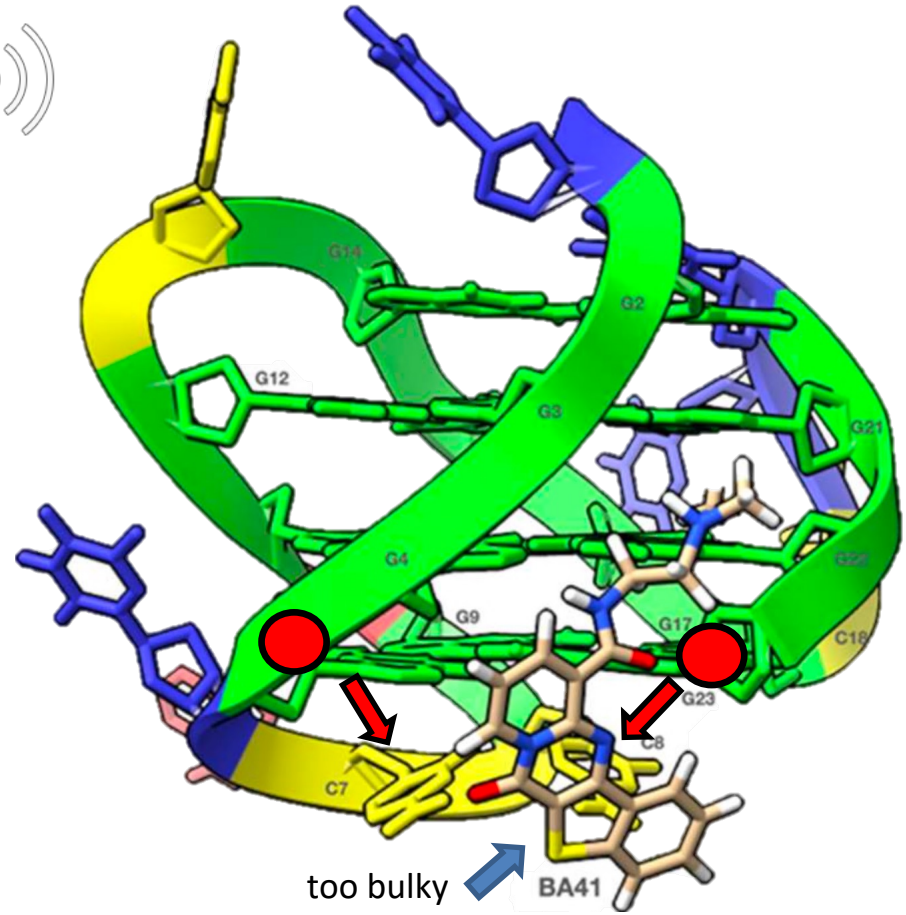
d) $Pd(OAc)_2$, Xantphos, Cs_2CO_3, 120 °C, dioxane, **65%**; e) TFA, CH_2Cl_2, **94%**; f) *N*,*N*-dimethyl ethylene diamine, BOP, DIPEA, CH_2Cl_2, **53%**; g) HCl in MeOH, quantit.

Study of the interactions TP3T6-BA41

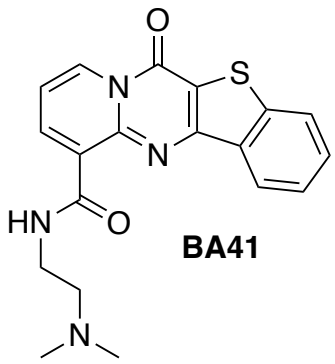


BA41





Study of the interactions TP3T6-BA41



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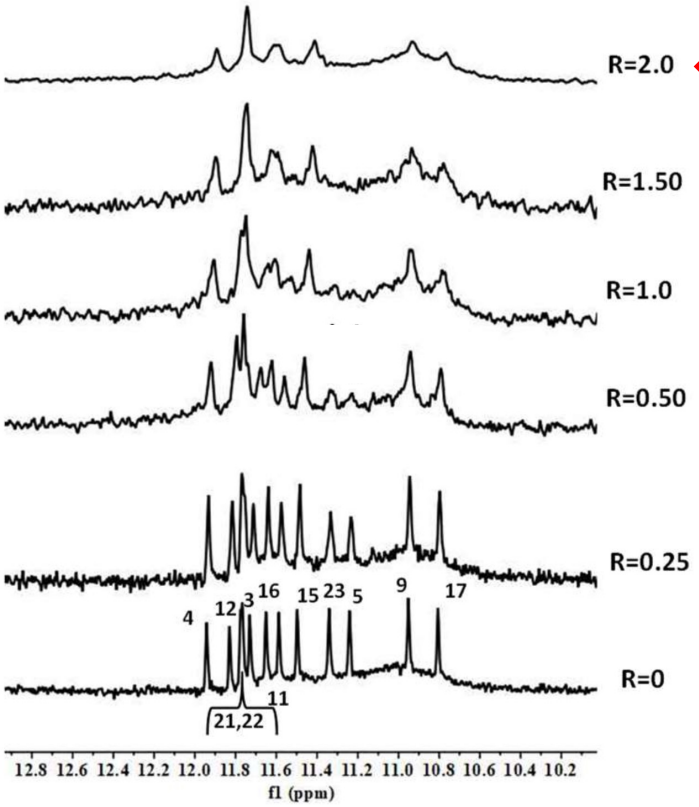
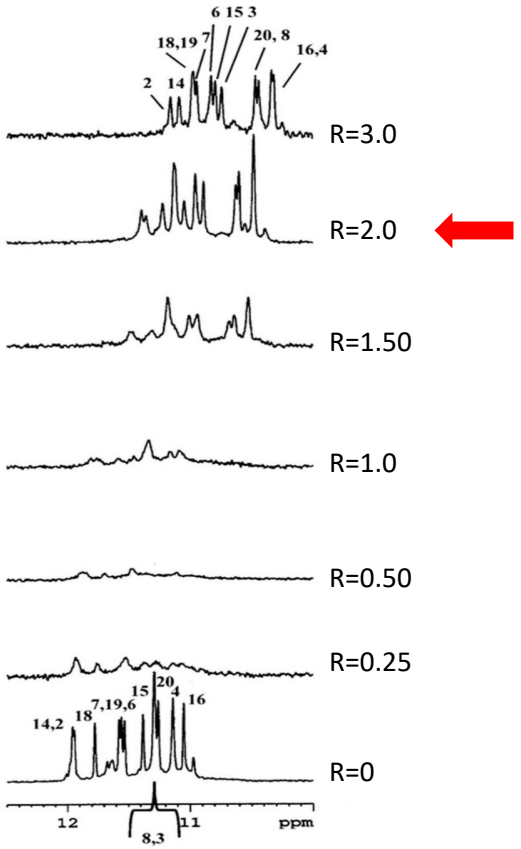


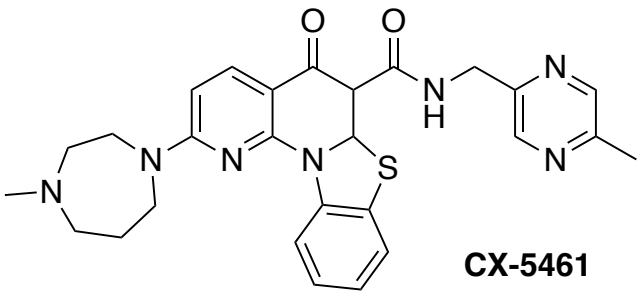
(3+1) HYBRID
[*TP3T6*]

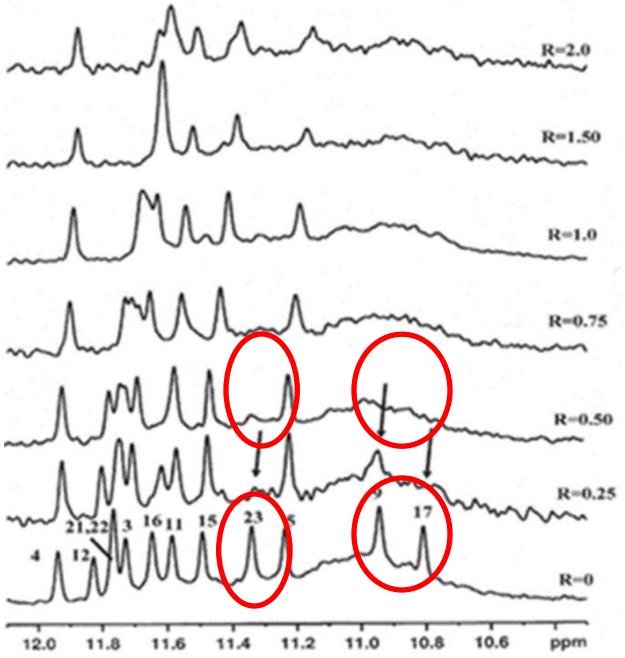


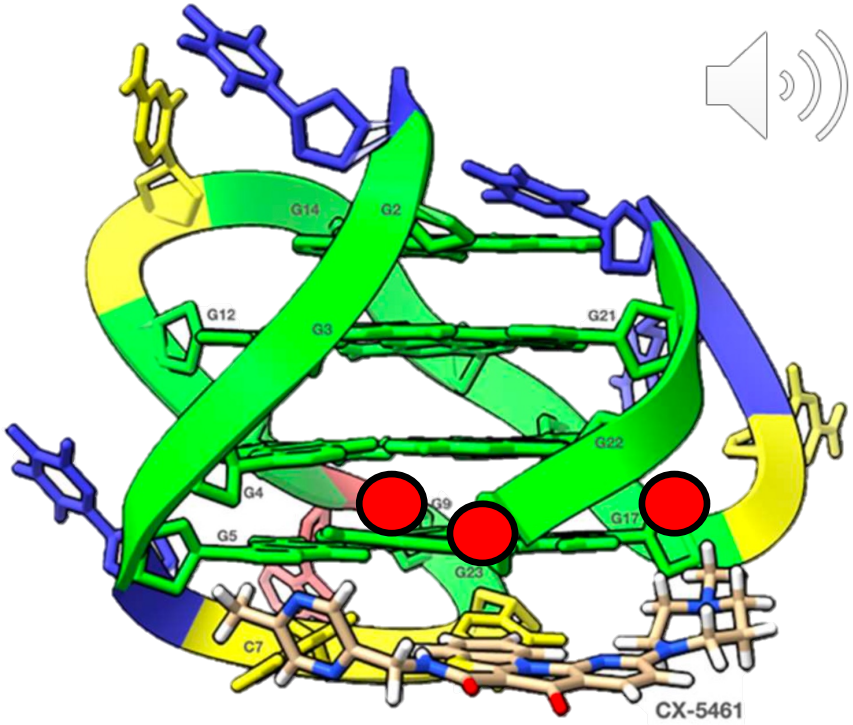
PARALLEL G-QUADRUPLEX
[*c-kit21T12T21*]

Mazzini, S.; Gargallo, R.; Musso, L.; De Santis, F.; Aviñó, A.; Scaglioni, L.; Eritja, R.; Di Nicola, M.; Zunino, F.; Amatulli, A.; Dallavalle, S. Stabilization of c-KIT G-Quadruplex DNA Structures by the RNA Polymerase I Inhibitors BMH-21 and BA-41. *Int. J. Mol. Sci.* **2019**, *20*, 4927. https://doi.org/10.3390/ijms20194927

Study of the interactions TP3T6-CX5461

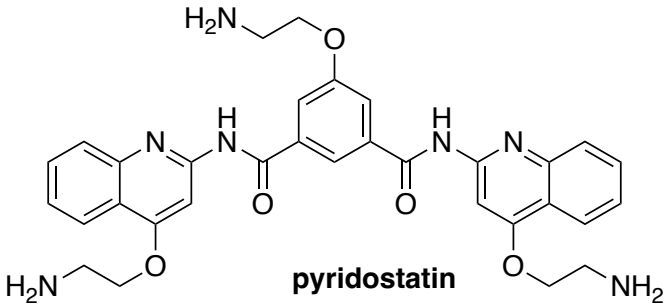


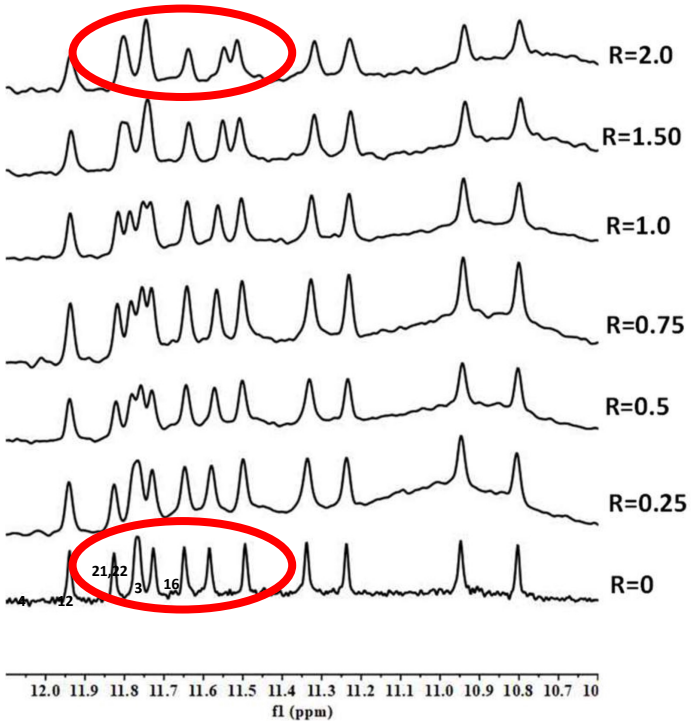




- At R = 0.25, decrease in intensity and/or a disappearance of G17, G9, G23.
- At ratio R > 1.0, general signal broadening.
- Molecular docking confirmed the preferential interaction of the ligand with the 3'-end tetrad, with the side chain arranged along the groove.

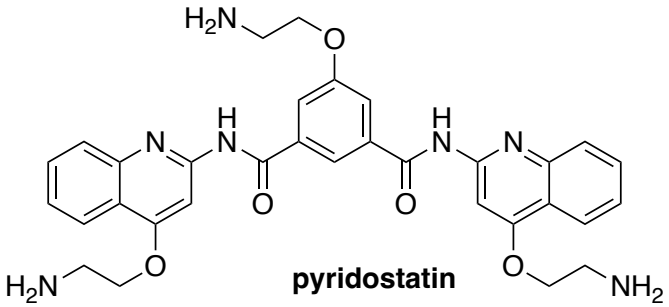
Study of the interactions TP3T6-pyridostatin

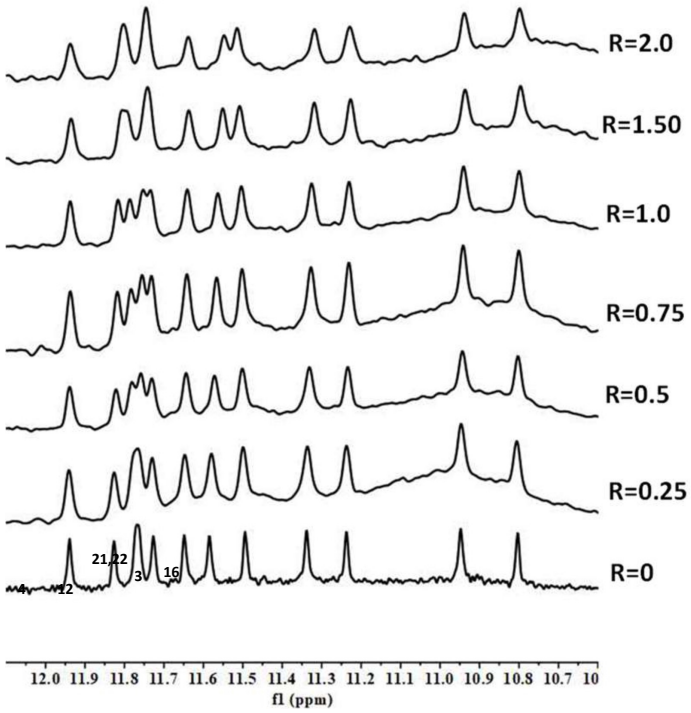




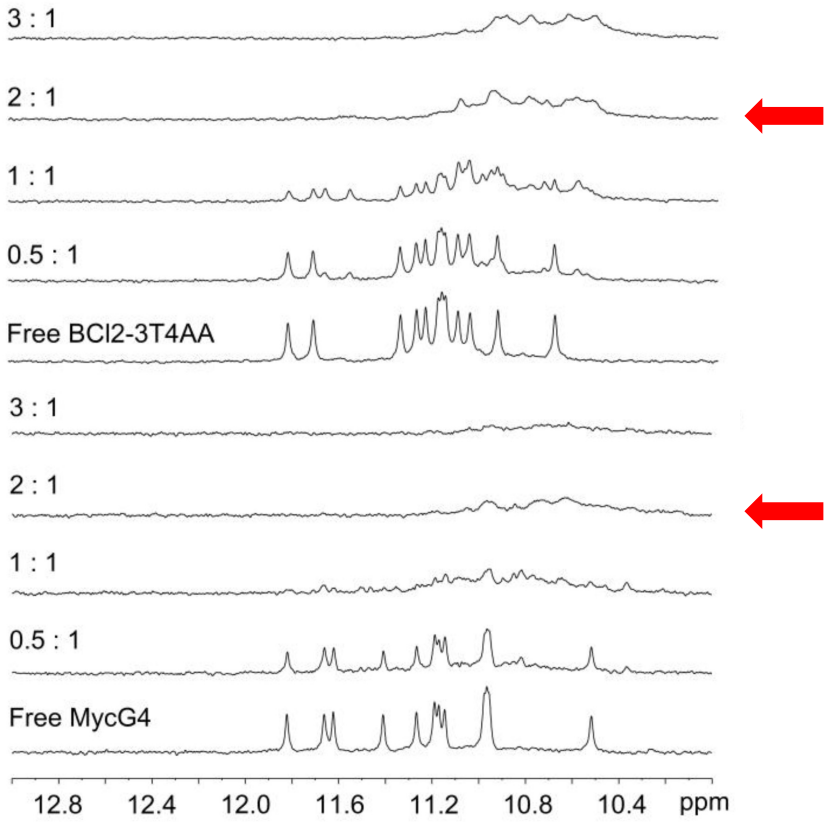
- Only moderate line broadening observed in the ^1H imino protons region of NMR spectra;
- Signals of the imino protons of G4, G12, G21, G22 and G3 tend to clump together, but it seems to have a minimal perturbation on the system.
- ΔT_m > 30 °C, clearly indicating stabilization of the complex

Study of the interactions TP3T6-pyridostatin





With parallel G-quadruplex Bcl2 and Myc



CONCLUSIONS

- NMR, CD, fluorescence and molecular modeling studies suggested that binders that efficiently stabilize parallel G-quadruplex structures showed only a low affinity to (3+1) PARP1 promoter, except for pyridostatin.
- Major limitations for an effective stabilization were:
  - the presence of C7 and C8, preventing efficient π-π stacking, at the 3'-end;
  - the steric hindrance deriving from G2 and G14, at the 5'-end.
- The investigated molecules can serve **as molecular tools** toward the identification of reliable selective PARP G-quadruplex modulators, useful for the regulation of PARP1 activity.

ACKNOWLEDGEMENTS

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