The 7th International Electronic Conference on Medicinal Chemistry (ECMC 2021)
01–30 NOVEMBER 2021 | ONLINE

Identification of Effective Anticancer G-Quadruplex-Targeting Chemotypes through the Exploration of a High Diversity Library of Natural Compounds

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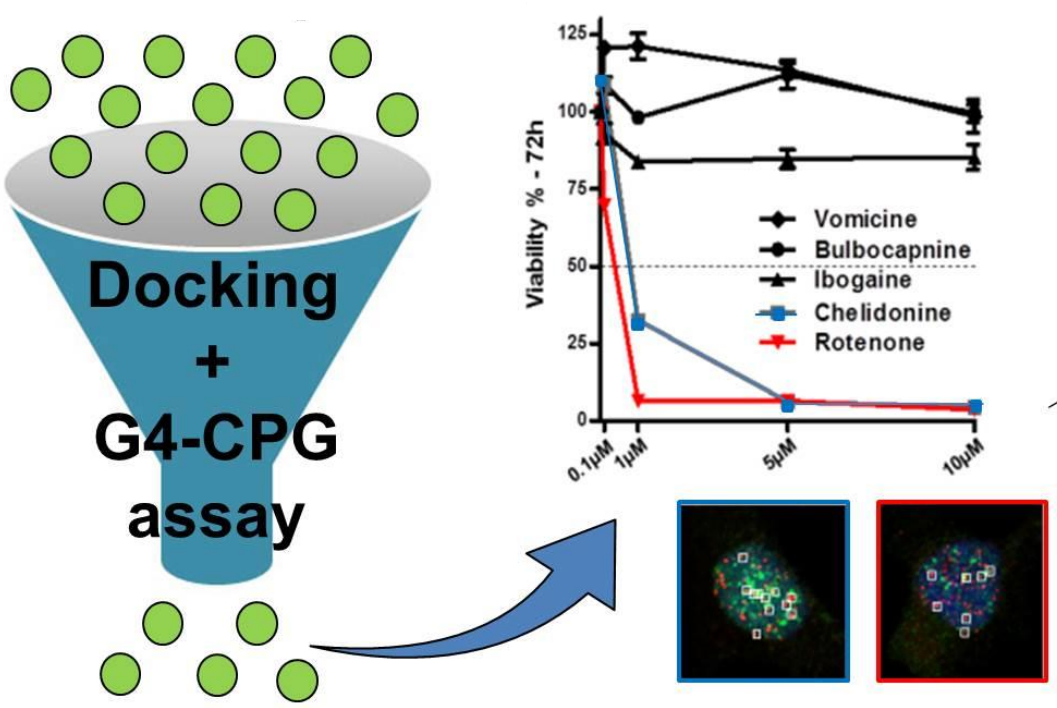
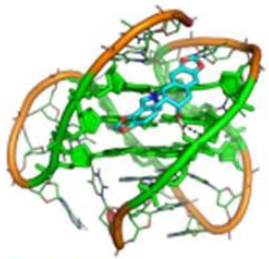
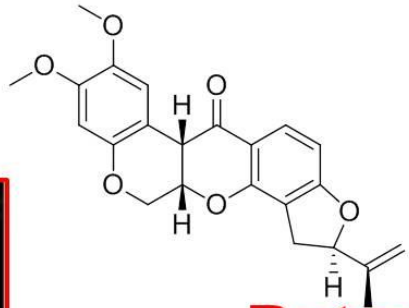
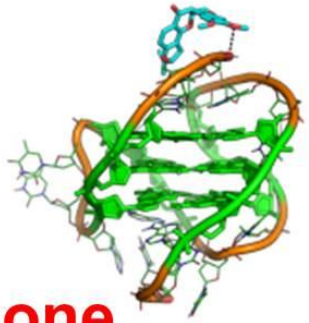




Chelidonine





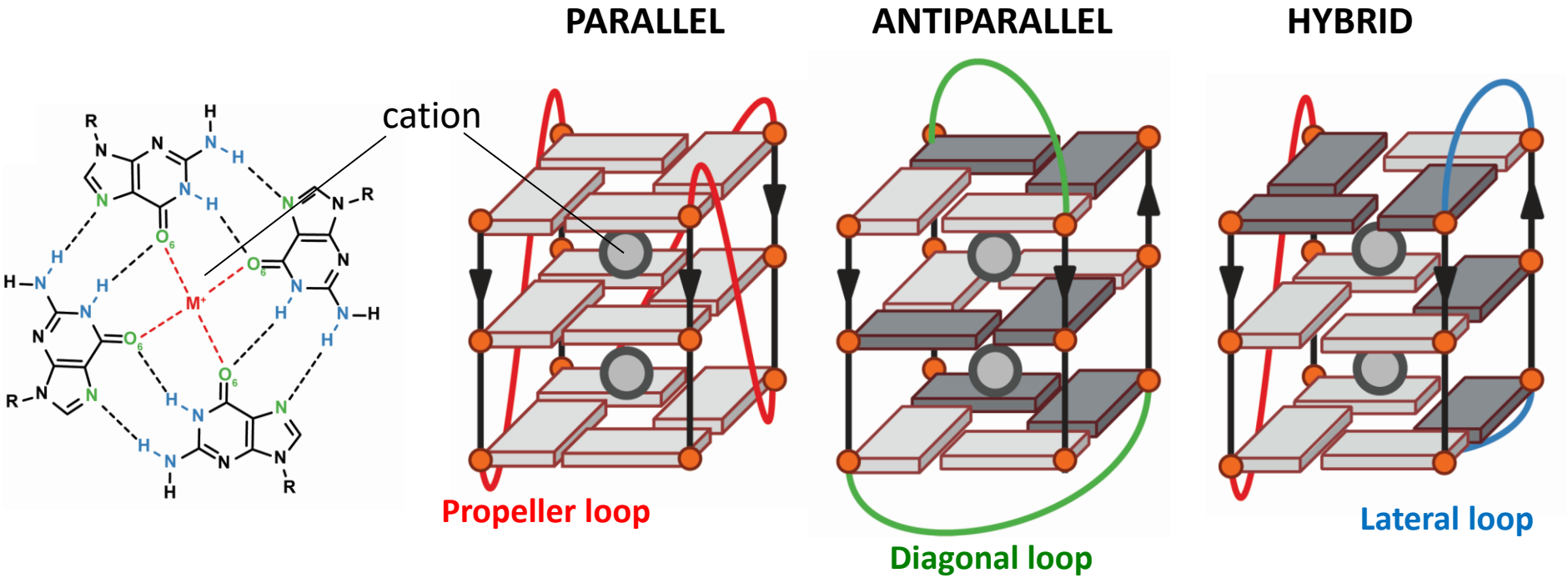
Rotenone

Abstract: In the quest for selective G-quadruplex (G4)-targeting chemotypes, natural compounds have been thus far poorly explored, though representing appealing candidates due to the high structural diversity of their scaffolds. In this regard, a unique high diversity in-house library composed of ca. one thousand individual natural products was investigated. The combination of molecular docking-based virtual screening and the G4-CPG experimental screening assay proved to be useful to quickly and effectively identify—out of many natural compounds—five hit binders of telomeric and oncogenic G4s, i.e., Bulbocapnine, Chelidonine, Ibogaine, Rotenone and Vomicine. Biophysical studies unambiguously demonstrated the selective interaction of these compounds with G4s compared to duplex DNA. The rationale behind the G4 selective recognition was suggested by molecular dynamics simulations. Indeed, the selected ligands proved to specifically interact with G4 structures due to peculiar interaction patterns, while they were unable to firmly bind to a DNA duplex. From biological assays, Chelidonine and Rotenone emerged as the most active compounds of the series against cancer cells, also showing good selectivity over normal cells. Notably, the anticancer activity correlated well with the ability of the two compounds to target telomeric G4s.

Keywords: cancer; G-quadruplex; molecular dynamics; natural compounds.

Introduction – structural features of G4s

G-quadruplexes (G4s) are non-canonical four-stranded structural motifs of nucleic acids formed by guanine-rich sequences

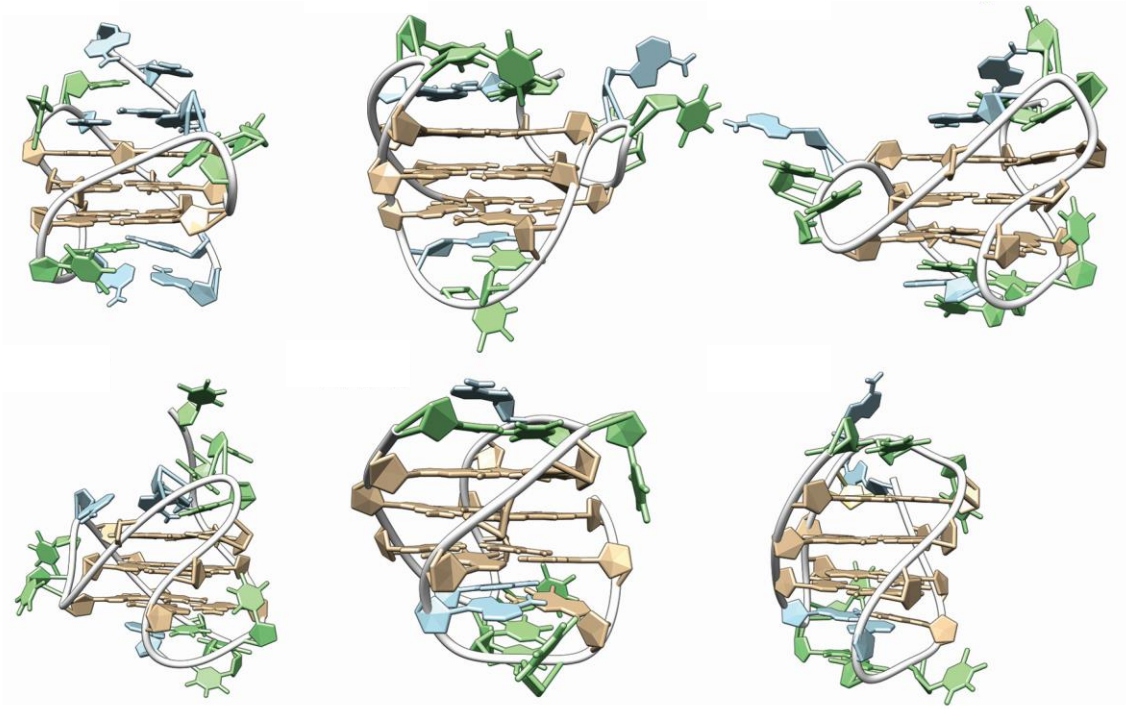


The relative orientation of strands originates different topologies
Loops geometry contribute to shape the 3D architecture of G4s

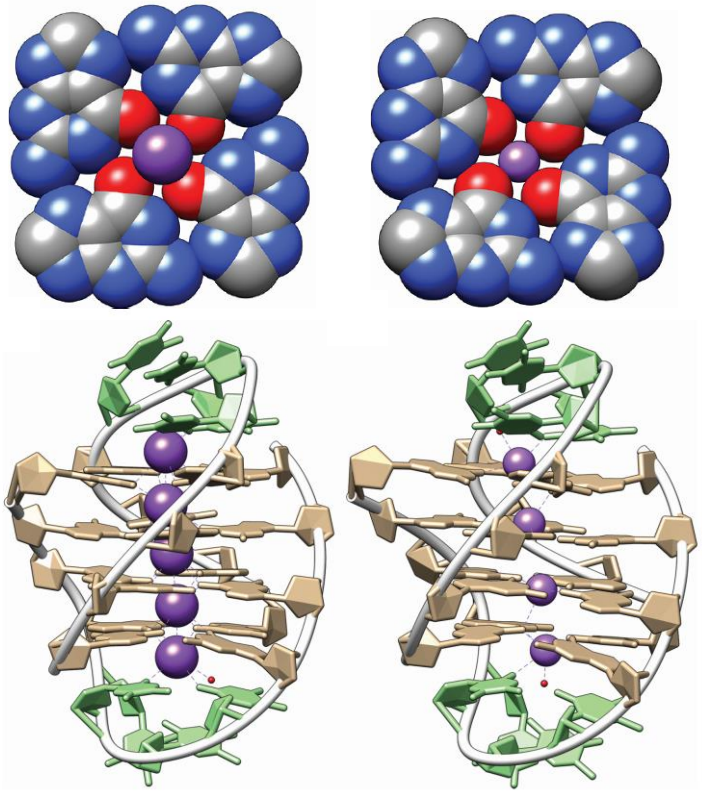
https://doi.org/10.1007/978-3-319-21756-7_7

Introduction – structural features of G4s

Loops length, geometry, and composition have a strong influence on the overall G4 structure.



3D structure of telomeric G4s.



K^+ and Na^+ bind in a different way to G-tetrads, providing different stabilizing effects on the G4s.

https://doi.org/10.1007/978-3-319-21756-7_7

Introduction – G4s as drug targets

WHERE: G4s form in specific sequences of both DNA and RNA with functional significance, such as telomeres, oncogene-promoter regions, and 5′- and 3′-untranslated region (UTR) of mRNA.

WHAT: G4s are involved in key genome functions, such as transcription, replication, genome stability, and epigenetic regulation.

WHY: Several evidences link G4s to disease onset and progression, and they are considered as profitable targets mainly for the therapy of cancer and infectious diseases.

HOW: Small molecule binders of G4s might be valuable leads for further development.

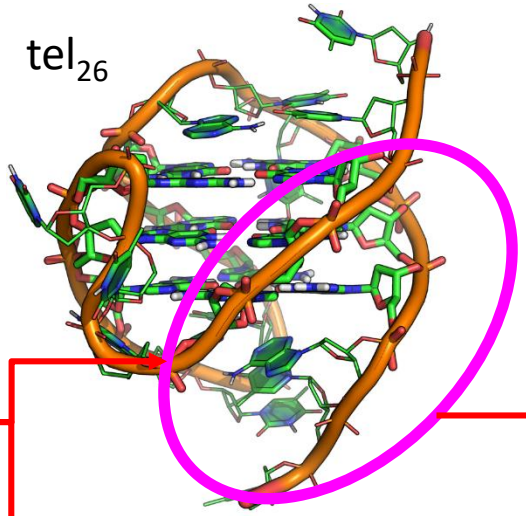
Quarfloxin (CX-3543) first-in class G4 binder in Phase II against various solid tumors → discontinued due to off-target effects.

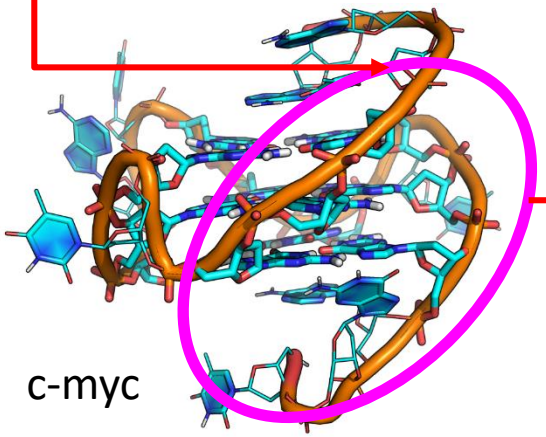
Novel and selective G-quadruplex (G4)-binding chemotypes are highly needed to advance anticancer therapies based on targeting G4s

Introduction – our multidisciplinary approach

In-house library

Virtual screening





Biophysical assays

In vitro tests

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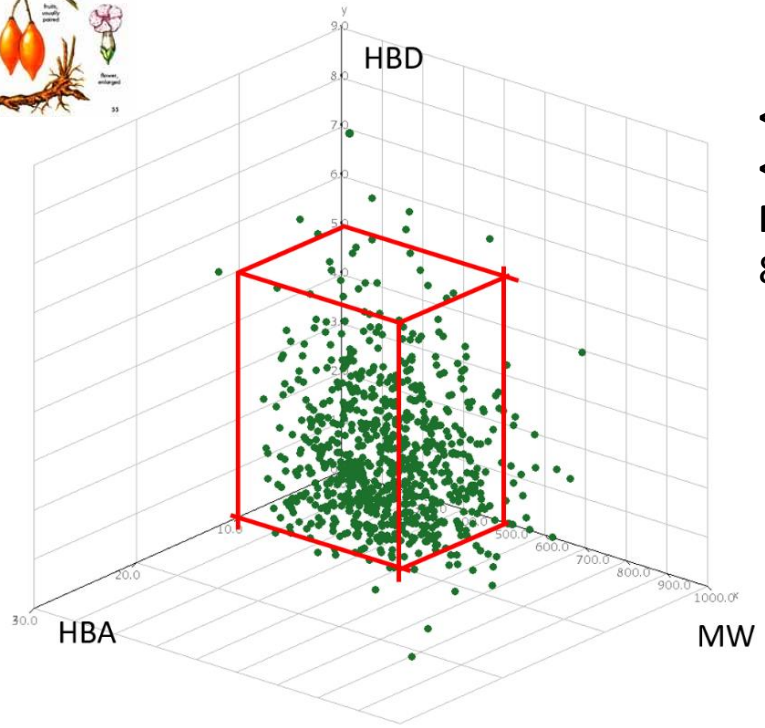
Results and discussion – *in-house* natural products library

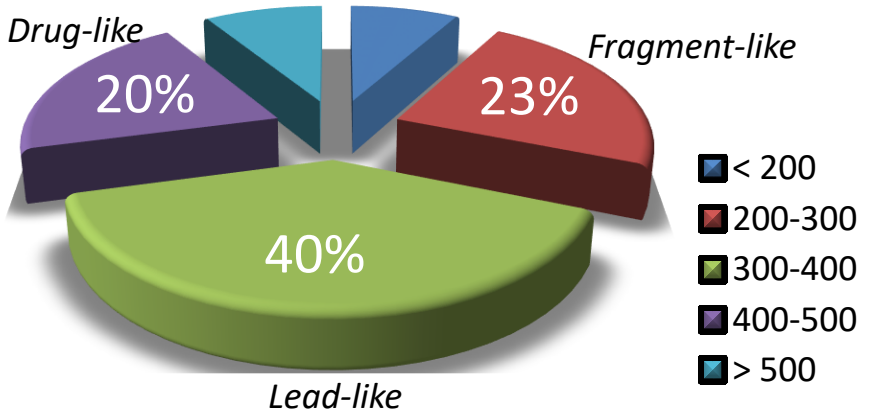
Natural compounds have been poorly studied as G4 ligands compared to synthetic compounds. *In-house* library of around 1,000 natural products and their derivatives from plants collected in biodiversity-rich countries.



<5 HBD
<10 HBA
MW < 500
84% of the library

HIGHLY SUITABLE FOR EARLY-STAGE DRUG DISCOVERY PROJECTS

Min	118.2
Max	982.1
Average	353.5



Org. Chem. Front. 2021, 8, 996–1025

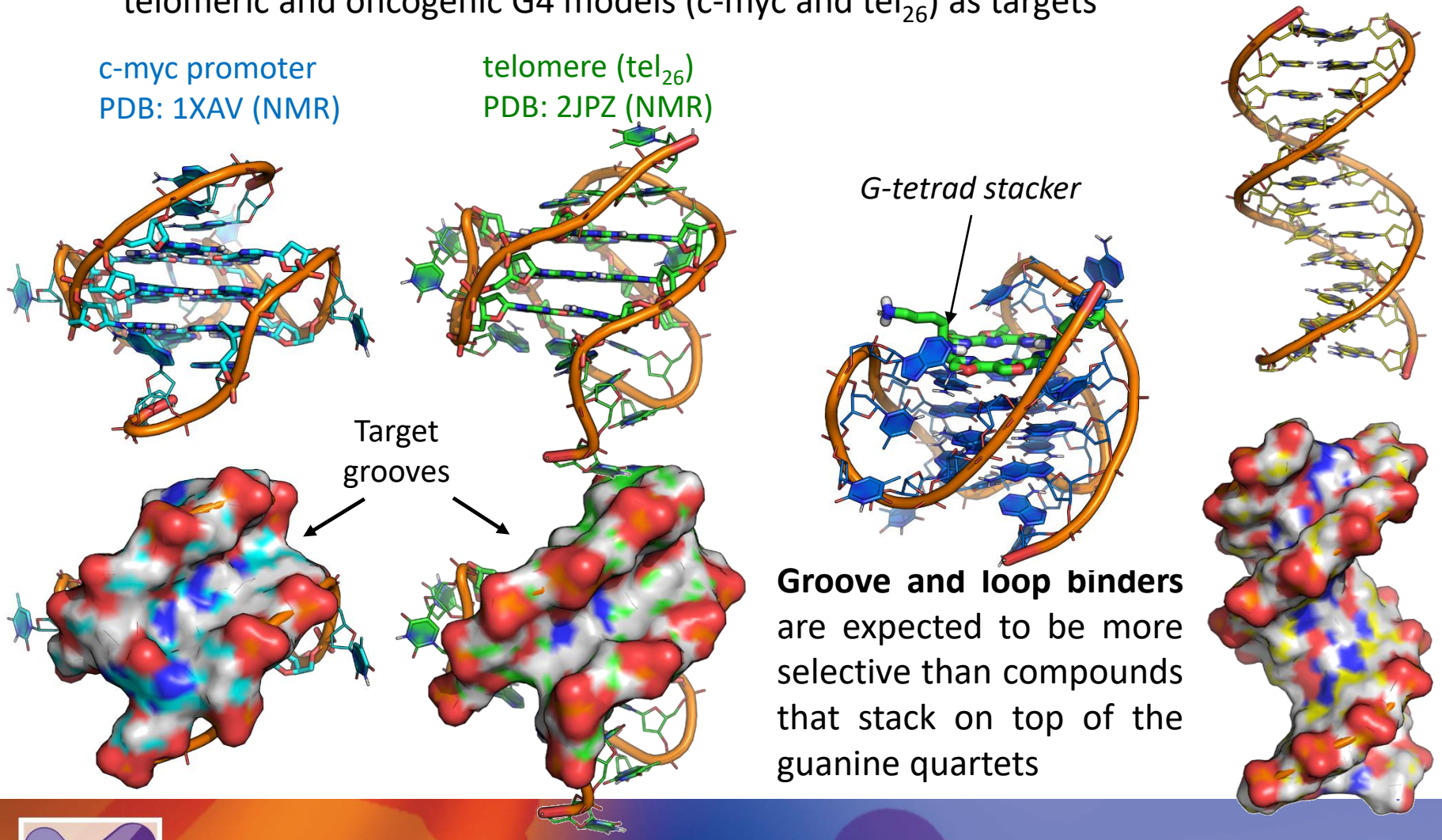
Results and discussion – virtual screening

telomeric and oncogenic G4 models (c-myc and tel_{26}) as targets

c-myc promoter
PDB: 1XAV (NMR)

telomere (tel_{26})
PDB: 2JPZ (NMR)

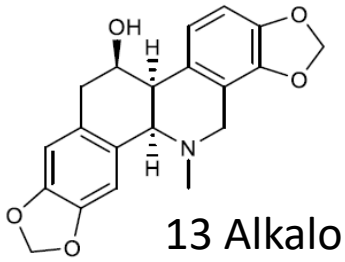
unspecific DNA duplex
PDB: 1NAJ (NMR)



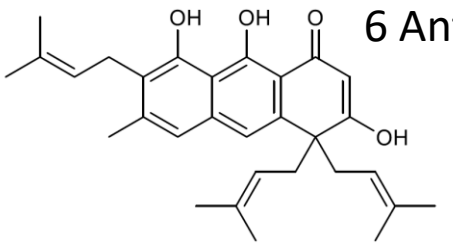
Groove and loop binders are expected to be more selective than compounds that stack on top of the guanine quartets

Results and discussion – virtual screening

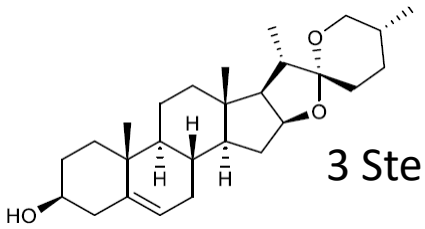
Score → G4s/duplex selectivity *in silico* → visual inspection → **28 compounds for testing**



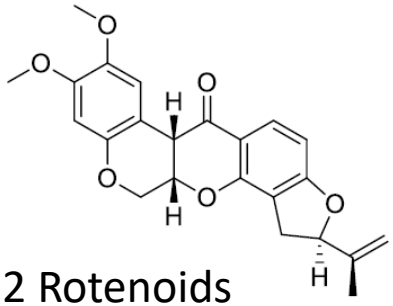
13 Alkaloids



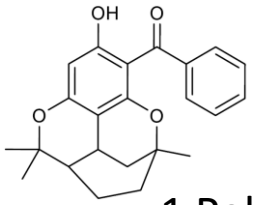
6 Anthranoids



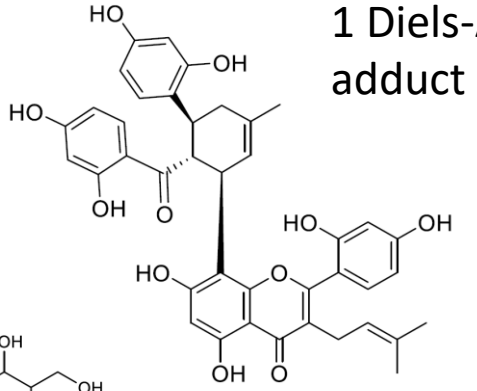
3 Steroids



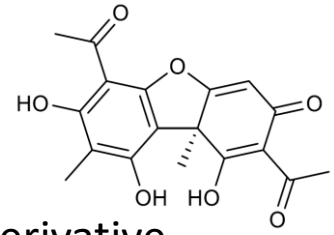
2 Rotenoids



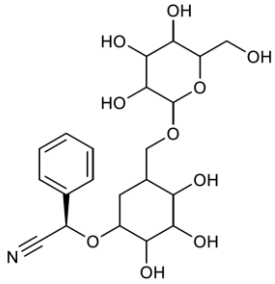
1 Polyphenol



1 Diels-Alder type adduct



1 Dibenzofuran derivative



1 Cyanogenic glycoside

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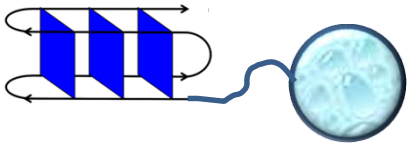
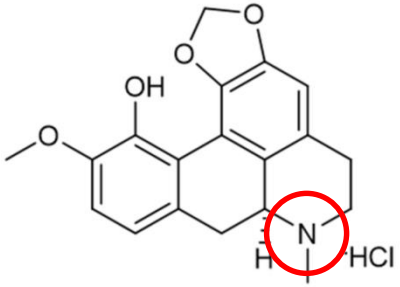
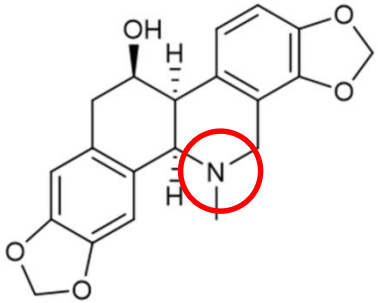
Results and discussion – G4-CPG assay

1) Controlled Pore Glass-based oligonucleotide affinity support (G4-CPG) assay, an affinity chromatography-based method for the screening of putatively selective G4 ligands (c-myc, tel_{26}, duplex) → **5 compounds**

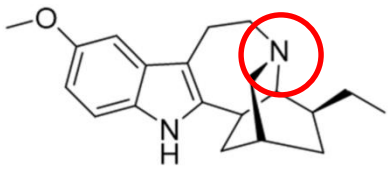




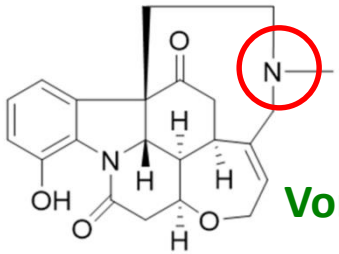
Bulbocapnine



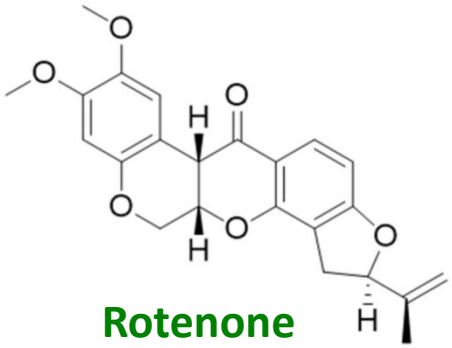
Chelidonine



Ibogaine



Vomicine



Rotenone

Anal. Chim. Acta **2018**, 1030, 133–141

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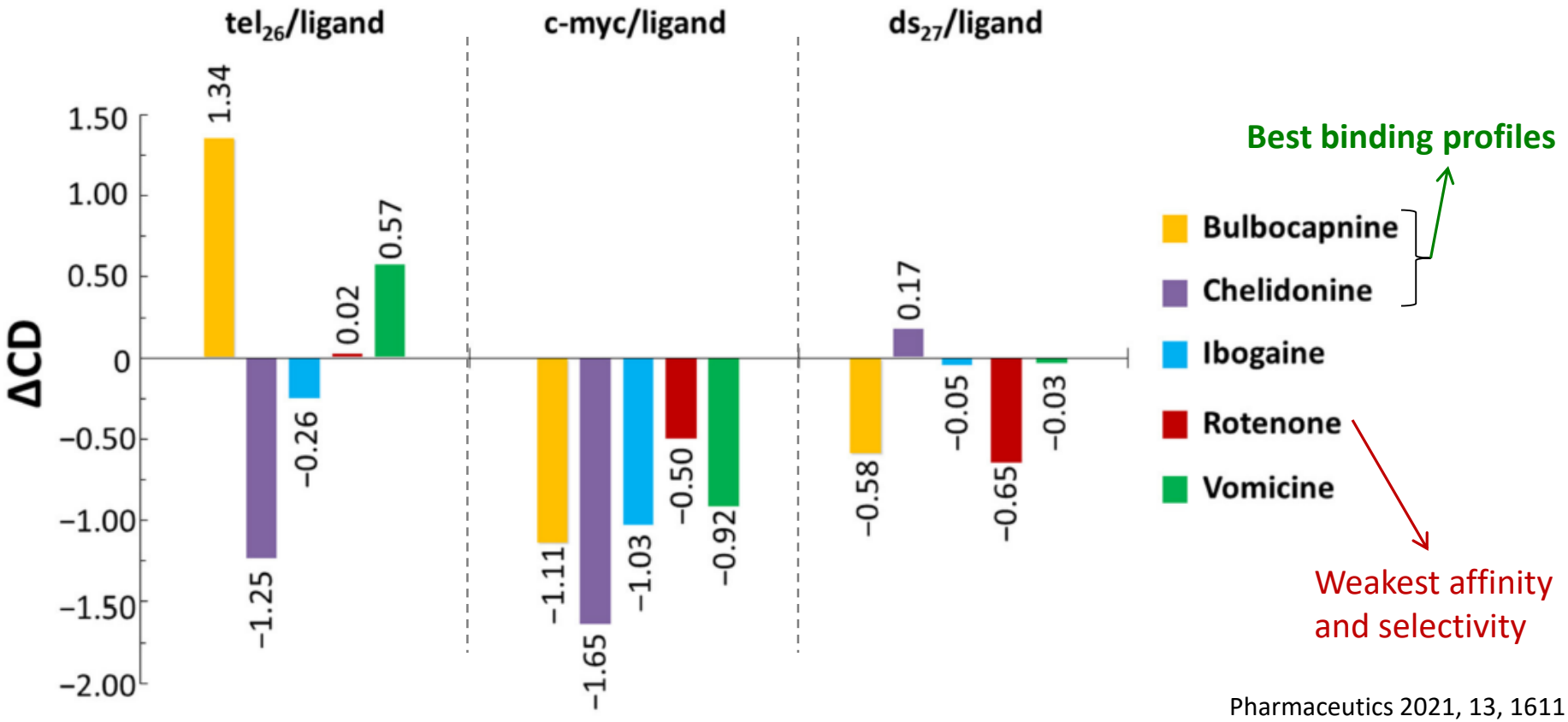
Results and discussion – Circular Dichroism

<u>2) CD titration up to 1:10 molar ratio</u>, to confirm binding in solution and to estimate binding parameters



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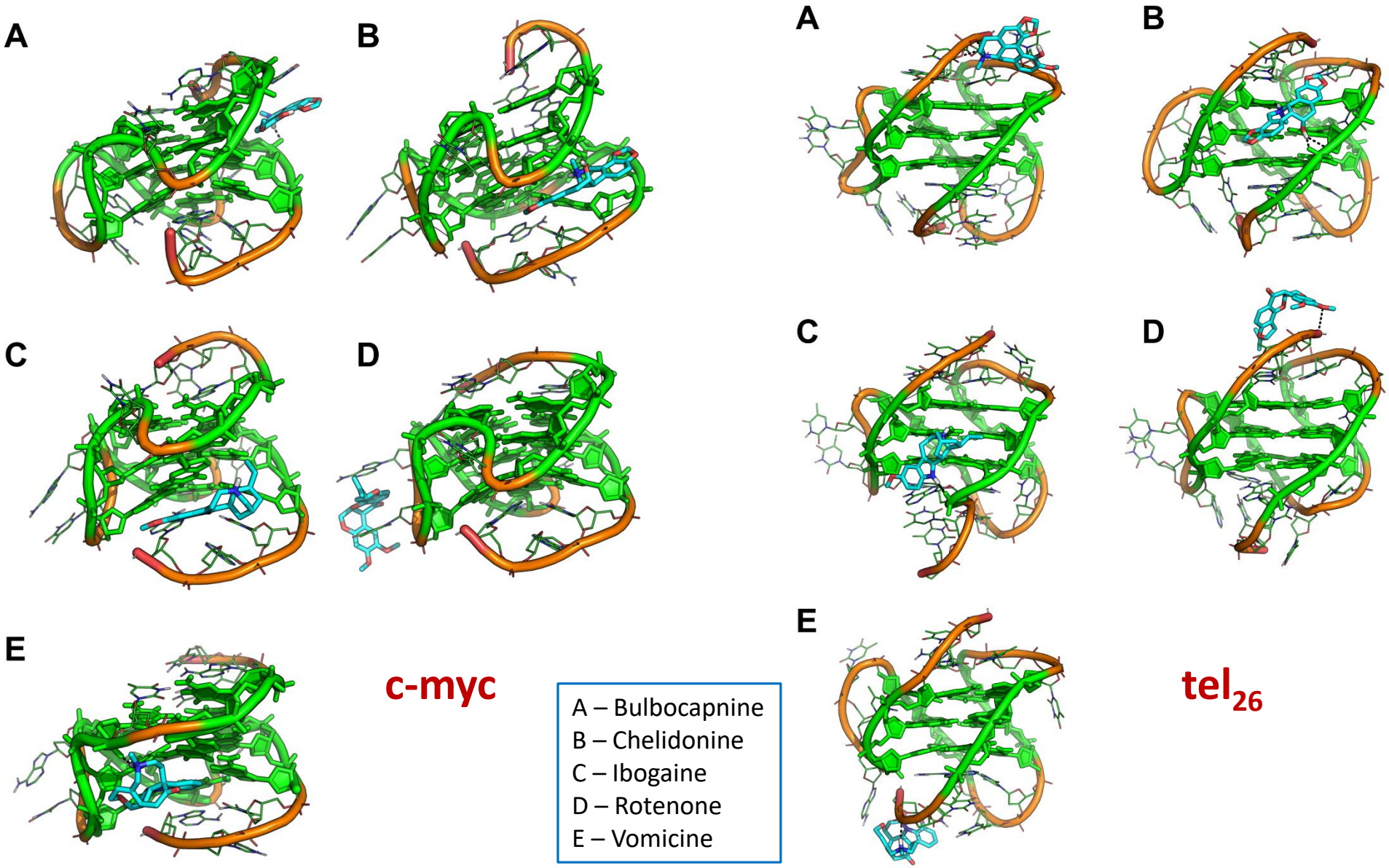
Results and discussion – MD simulations

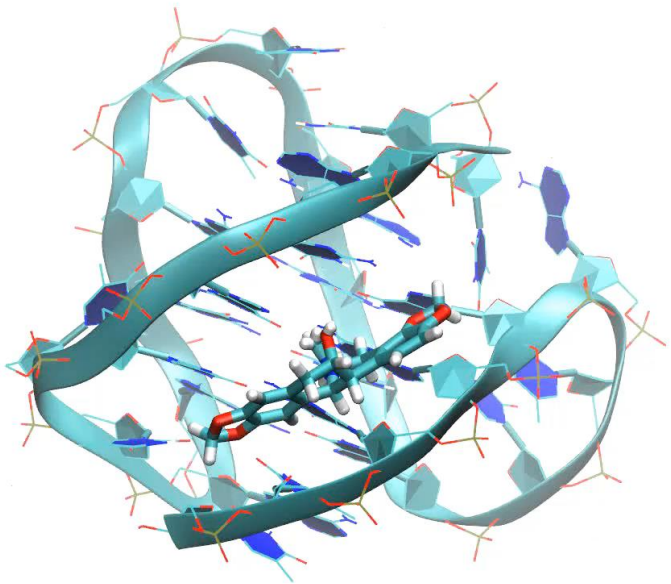
Docking complexes were relaxed through 500 ns of MD simulations in explicit solvent

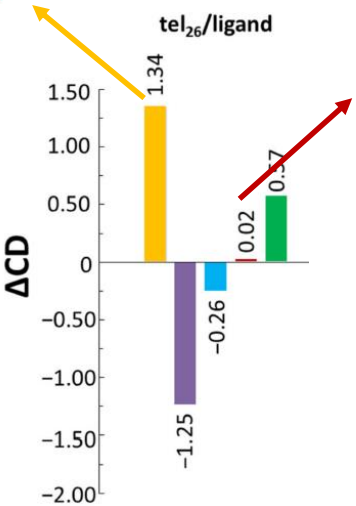


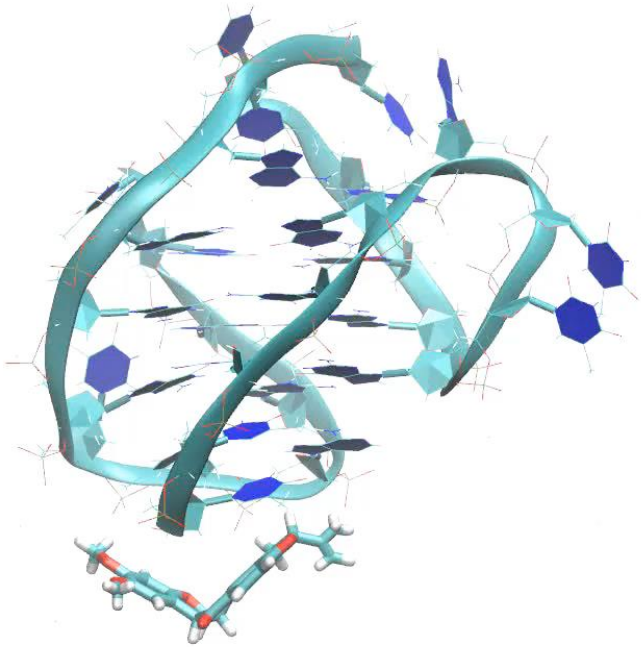
Results and discussion – MD simulations

CHELIDONINE/tel_{26}

ROTENONE/tel_{26}







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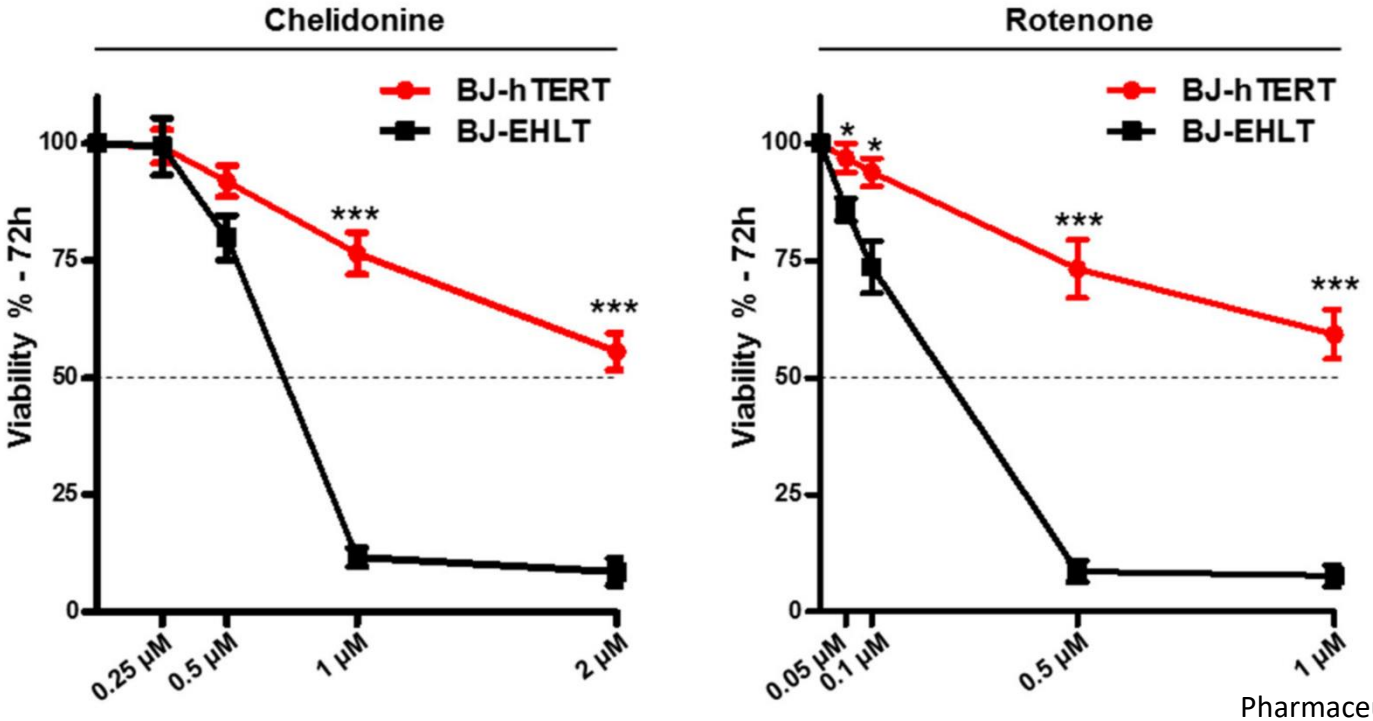
Results and discussion – Biological activity evaluation

Bulbocapnine, Ibogaine and Vomicine are almost ineffective in **BJ-EHLT** transformed fibroblasts. Chelidonine and Rotenone produce a dose-dependent effect on cell viability (IC_{50} of 0.64 µM and 0.15 µM, respectively) with modest impact on non-transformed fibroblasts **BJ-hTERT** (SELECTIVITY).



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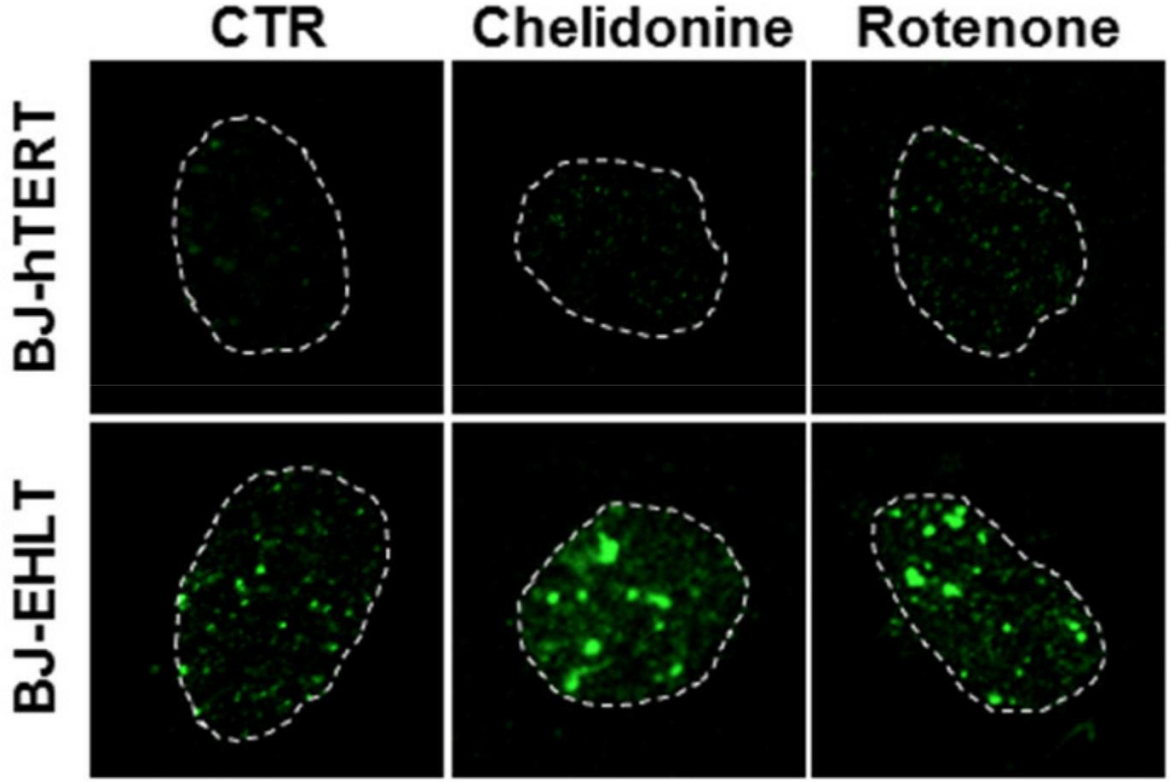
Results and discussion – Biological activity evaluation

The effect of the G4 ligands on cell viability could be due to the capability of these compounds to induce selective DNA damage in transformed cells.



Fluorescent signal due to phosphorylated histone H2Aχ (ϒH2Aχ), a typical hallmark of DNA double-strand breaks.

DNA damage was telomere-located.

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Conclusions

- ✓ By combining virtual and experimental screening of an *in-house* natural products library, Bulbocapnine, Chelidonine, Ibogaine, Rotenone and Vomicine were found to interact with G4s, also selectively stabilizing the G4 vs. duplex structures.

- ✓ Chelidonine has the highest stabilizing effects and affinity on G4 over duplex structures; MD simulations suggest a stable binding in the G4 groove of both c-myc and tel_{26}.

- ✓ Rotenone has the weakest affinity for G4s, also binding the unspecific duplex.

- ✓ Both compounds exhibit a potent anticancer activity at sub-µM concentrations, mediated by their capability to bind and stabilize telomeric G4 structures.

Profitable starting points for further lead optimization studies… ongoing…

Acknowledgments

IG2020 project n. 25046
IG2018 project n. 21579