Development of new antiparasitic drugs targeting DNA duplexes and quadruplexes
(PID2022-136438OB-I00)

Summary of the project

Current therapies to treat diseases caused by kinetoplastid parasites such as *Leishmania* (leishmaniasis), *Trypanosoma cruzi* (Chagas disease or American trypanosomiasis) or *T. brucei* (sleeping sickness or human African trypanosomiasis, HAT) are inappropriate due to several factors, among others, the toxicity of the available drugs and the rapid emergence of resistant strains. These limitations with the lack of vaccines constitute a serious limitation to the control of these diseases. In this sense, the availability of new effective and safe drugs, preferably for oral administration that ensure access to treatment for all patients, is a challenge that we intend to respond to in this project.

To achieve this goal, we will **develop several series of compounds that can exert their antiparasitic activity specifically on guanine (G4) quadruplexes of these parasites**. This will be followed by a comprehensive evaluation of drug formulations and studies of toxicity, bioavailability, and efficacy both *in vitro* and *in vivo*. We will prepare unique fluorescent probes that target G4s as diagnostic and therapeutic ("theranostic") tools to visualize and characterize G4s at the molecular and cellular level, unraveling their role in these parasitic diseases, and potentially in other diseases such as cancer. The detailed study of the drug-target interactions, resistance profile and enzymatic transformations involved in its activation/deactivation, will allow us to learn about its mechanism of action.

1. Background

G-quadruplexes as target for antiprotozoal drugs

G-quadruplexes (**G4s**) are unusual nucleic acid structures formed by guanine-rich DNA (and RNA) sequences that fold into non-canonical secondary structures and can be recognized by specific G4 ligands. G4s exist in two or more G-tetrads formed by hydrogen bonding among four G connected and are held in a planar arrangement with additional stabilization provided by a monovalent cation coordinated to the O^6 atom of each G (Fig. 1).

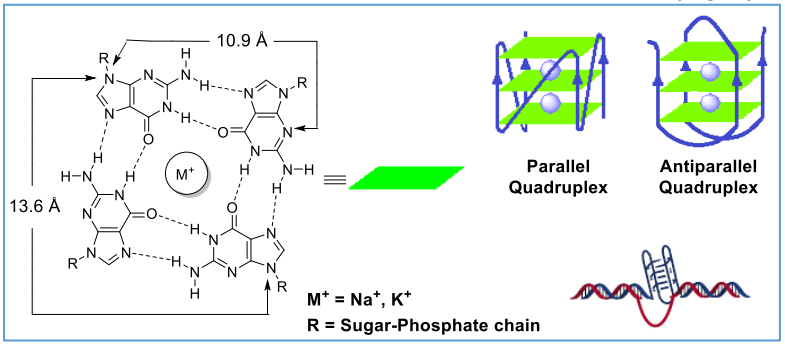

Fig. 1- Structure of a guanine quartet stabilised by a mono-cation, indicating its dimensions (left); G-quadruplex parallel and antiparallel conformations (upper right); sketch of a G4 within double stranded DNA (lower right)

G4s are present in biologically important regions, such as the end of telomeres (h-Tel)[1] as well as in the regulatory regions of oncogenes (*e.g.* c-MYC, c-KIT, B-Raf, K-Ras),[2] and for that reason are considered emerging oncology targets. In the past 2 decades, many examples of small molecule G4 ligands were reported as antitumor drugs.[3] In particular, it has been proven that G4 has a regulatory role in cancer cell growth[28] and it is clear that G4 ligands act selectively on cancer cells and induce focused DNA damage different from that of classical double strand (ds) DNA binders. Moreover, **putative G-quadruplex sequences (PQS) have been found in organisms other than humans, such as** other mammals, yeast, bacteria, viruses, and **parasites**.[4-7] Hence, G4 is an attractive alternative target in these organisms[6] where they play a strong role in immune evasion and virulence.[8]

For instance, DNA G-quadruplexes can be detected in the nuclei of the malaria-causing parasite *Plasmodium falciparum*, which has an extremely AT-rich DNA (>80%) and therefore possesses few guanine-rich sequences with the potential to form G4s.[9] Notwithstanding, *P. falciparum* is sensitive to several G-quadruplex-stabilizing drugs including quarfloxin, a G4 drug that previously reached Phase-2 clinical trials as antitumoral agent.[10] Recently, Cantara *et al.* experimentally confirmed G4 formation for sequences found in four different parasitic helminths. Small molecules able to selectively recognize G4 were found to bind to *Schistosoma mansoni* G4 motifs and two

of these ligands demonstrated potent activity against both larval and adult stages of this parasite.[4, 5]

As regards to diseases caused by trypanosomatids, **PQSs were identified in the pre-edited mRNA of *T. brucei*,[11] and the genomes of *Leishmania* and *T. brucei***.[6] Overall, G4 motifs have been shown to play several different roles in the biology of protozoa. They either organise the genome or regulate both gene expression and transcript editing as in trypanosomatids. Regarding the pathogenic species, most of these mechanisms are essential for parasite survival and this makes G4s potential targets for anti-parasitic drugs.[12] Recent studies reported the potential of G4 ligands as drugs to treat HAT and leishmaniasis by targeting PQS in the *T. brucei* and *Leishmania* genomes.[6, 7, 13-15] Despite the significant progress made in the field, the study of G4s as target in trypanosomatids is still in its infancy and remains a hot, yet understudied, subject.[16]

2. Training program

The PhD student will engage in the Doctoral program in Medicinal Chemistry from the Faculty of Pharmacy at UCM. The PhD project performed at the Medicinal Chemistry Institute (IQM-CSIC) will be related to the "**Synthesis and biophysical study of G-quadruplex portmanteau conjugates as antiparasitic drugs**". To complete his/her formation, the PhD student will attend the "Medicinal chemistry specialization course" taught at IQM every 2 years (next call in 2024). He/She will have the opportunity to make short stays (1-2 months each) in the labs of Prof. Gómez Barrio (UCM, Madrid) and Prof. de Koning (Glasgow, UK) to learn parasitology techniques, and/or Prof. Mergny (INSERM, Paris) to learn fluorescence resonance energy transfer (FRET) melting competition assays. This training abroad will give him/her the opportunity to apply for the "International Doctorate" mention.

3. Scientific and training resources of the group and institution

The multidisciplinary and international nature of our project guarantees the **training of the PhD student in diverse areas of science**. We have **organic chemistry** facilities at IQM-CSIC for the synthesis and optimization of the compounds, and for the study of its physicochemical properties. The PhD candidate will be trained to use different **biophysical techniques** available at IQM-CSIC (i.e., SPR, CD, ITC, UV-titration) for the determination of **drug-DNA interactions**. We have analytical and biochemical facilities for the study of **metabolism and mode of action** of compounds. We have **galenic development** facilities at UCM for the development of formulations and for the study of physicochemical properties.

The mobility plan of the fellow is highly determined by the collaboration with national and international researchers from this project. The fellow will have the opportunity to broaden its knowledge by spending a few months in the laboratories of Prof. Gómez Barrio (UCM, Madrid) and Prof. de Koning (University of Glasgow, UK) to **learn parasitology techniques** for studying the biological activity of his/her compounds against several parasites. A stay in the laboratory of Prof. Mergny (INSERM, France) is also planned to **learn FRET** (fluorescence resonance energy transfer)-**based melting assays** that are used to measure conjugates' binding to several G4 systems. The participation of Prof. González (IQFR-CSIC) in the NMR studies will allow the fellow to interact with another renown expert in G4 research. This collaboration shall be smooth due to the close proximity of IQM and IQFR on the same Campus.

The PhD student will finish his training with a high degree of expertise in organic/medicinal chemistry, biophysics, and parasitology.

The Department for Postgraduate and Specialisation (DPE) contributes to define and implement the CSIC policy regarding the training of research personnel. The PhD student will attend the annual Conference for Doctoral Students of the CSIC which objective is to provide transversal and complementary training, as well as guidance about their future professional career once they have defended their thesis. The CSIC "*WELCOME MANUAL*" is an online document prepared by the Monitoring Group of the Human Resources Strategy for Researchers (HRS4R) which contains very useful general information to facilitate correct and rapid integration of new staff into the CSIC.

4. References

(1) Neidle, S. Human telomeric G-quadruplex: The current status of telomeric G-quadruplexes as therapeutic targets in human cancer. *FEBS J.* **2010**, *277* (5), 1118-1125,

https://doi.org/10.1111/j.1742-4658.2009.07463.x. DOI: https://doi.org/10.1111/j.1742-4658.2009.07463.x (acccessed 2022/10/20).
(2) Balasubramanian, S.; Hurley, L. H.; Neidle, S. Targeting G-quadruplexes in gene promoters: a novel anticancer strategy? *Nature Rev. Drug Discov.* **2011**, *10* (4), 261-275. DOI: 10.1038/nrd3428.
(3) Müller, S.; Rodriguez, R. G-quadruplex interacting small molecules and drugs: from bench toward bedside. *Exp. Rev. Clin. Pharmacol.* **2014**, *7* (5), 663-679. DOI: 10.1586/17512433.2014.945909.
(4) Cantara, A.; Luo, Y.; Dobrovolná, M.; Bohalova, N.; Fojta, M.; Verga, D.; Guittat, L.; Cucchiarini, A.; Savrimoutou, S.; Häberli, C.; et al. G-quadruplexes in helminth parasites. *Nucleic Acids Res.* **2022**, *50* (5), 2719-2735. DOI: 10.1093/nar/gkac129 (acccessed 10/4/2022).
(5) Craven, H. M.; Bonsignore, R.; Lenis, V.; Santi, N.; Berrar, D.; Swain, M.; Whiteland, H.; Casini, A.; Hoffmann, K. F. Identifying and validating the presence of Guanine-Quadruplexes (G4) within the blood fluke parasite Schistosoma mansoni. *PLOS Negl. Trop. Dis.* **2021**, *15* (2), e0008770. DOI: 10.1371/journal.pntd.0008770.
(6) Belmonte-Reche, E.; Martínez-García, M.; Guédin, A.; Zuffo, M.; Arévalo-Ruiz, M.; Doria, F.; Campos-Salinas, J.; Maynadier, M.; López-Rubio, J. J.; Freccero, M.; et al. G-Quadruplex Identification in the Genome of Protozoan Parasites Points to Naphthalene Diimide Ligands as New Antiparasitic Agents. *J. Med. Chem.* **2018**, *61* (3), 1231-1240. DOI: 10.1021/acs.jmedchem.7b01672.
(7) Zuffo, M.; Stucchi, A.; Campos-Salinas, J.; Cabello-Donayre, M.; Martínez-García, M.; Belmonte-Reche, E.; Pérez-Victoria, J. M.; Mergny, J. L.; Freccero, M.; Morales, J. C.; et al. Carbohydrate-naphthalene diimide conjugates as potential antiparasitic drugs: Synthesis, evaluation and structure-activity studies. *Eur. J. Med. Chem.* **2019**, *163*, 54-66. DOI: https://doi.org/10.1016/j.ejmech.2018.11.043.
(8) Harris, L. M.; Merrick, C. J. G-Quadruplexes in Pathogens: A Common Route to Virulence Control? *PLOS Pathogens* **2015**, *11* (2), e1004562. DOI: 10.1371/journal.ppat.1004562.
(9) Gardner, M. J.; Hall, N.; Fung, E.; White, O.; Berriman, M.; Hyman, R. W.; Carlton, J. M.; Pain, A.; Nelson, K. E.; Bowman, S.; et al. Genome sequence of the human malaria parasite Plasmodium falciparum. *Nature* **2002**, *419* (6906), 498-511. DOI: 10.1038/nature01097.
(10) Harris, L. M.; Monsell, K. R.; Noulin, F.; Toyin Famodimu, M.; Smargiasso, N.; Damblon, C.; Horrocks, P.; Merrick, C. J. G-quadruplex DNA motifs in the malaria parasite plasmodium falciparum and their potential as novel antimalarial drug targets. *Antimicrob. Agents Chemother.* **2018**, *62* (3), Article. DOI: 10.1128/AAC.01828-17 Scopus.
(11) Leeder, W. M.; Hummel, N. F. C.; Göringer, H. U. Multiple G-quartet structures in pre-edited mRNAs suggest evolutionary driving force for RNA editing in trypanosomes. *Sci. Rep.* **2016**, *6*, Article. DOI: 10.1038/srep29810 Scopus.
(12) Mendes, E.; Aljnadi, I. M.; Bahls, B.; Victor, B. L.; Paulo, A. Major Achievements in the Design of Quadruplex-Interactive Small Molecules. *Pharmaceuticals* **2022**, *15* (3), 300.
(13) Belmonte-Reche, E.; Benassi, A.; Peñalver, P.; Cucchiarini, A.; Guédin, A.; Mergny, J. L.; Rosu, F.; Gabelica, V.; Freccero, M.; Doria, F.; et al. Thiosugar naphthalene diimide conjugates: G-quadruplex ligands with antiparasitic and anticancer activity. *Eur. J. Med. Chem.* **2022**, *232*, 114183. DOI: https://doi.org/10.1016/j.ejmech.2022.114183.
(14) Street, S. T. G.; Peñalver, P.; O'Hagan, M. P.; Hollingworth, G. J.; Morales, J. C.; Galan, M. C. Imide Condensation as a Strategy for the Synthesis of Core-Diversified G-Quadruplex Ligands with Anticancer and Antiparasitic Activity**. *Chem. Eur. J.* **2021**, *27* (28), 7712-7721. DOI: https://doi.org/10.1002/chem.202100040.
(15) O'Hagan, M. P.; Peñalver, P.; Gibson, R. S. L.; Morales, J. C.; Galan, M. C. Stiff-Stilbene Ligands Target G-Quadruplex DNA and Exhibit Selective Anticancer and Antiparasitic Activity. *Chem. Eur. J.* **2020**, *26* (28), 6224-6233. DOI: https://doi.org/10.1002/chem.201905753.
(16) Dumetz, F.; Merrick, C. J. Parasitic Protozoa: Unusual Roles for G-Quadruplexes in Early-Diverging Eukaryotes. *Molecules* **2019**, *24* (7), 1339.