UM PEQUENO

PAR *CONTRA UM* GRANDE TRIO

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Resumo

"Um pequeno par contra um grande trio" (Two4Three) é um projeto que se propôs combater Malária, Tuberculose e HIV com Líquidos Iónicos (ILs).

Um dos grandes problemas de saúde do nosso tempo é a resistência dos agentes infeciosos aos fármacos atualmente disponíveis. Este é um problema particularmente dramático no contexto das "3 grandes doenças" - malária, HIV/SIDA e tuberculose, e em que a constante procura pelo próximo fármaco de primeira linha já não parece uma abordagem sustentável. Alternativamente, podemos pensar em olhar para os fármacos "velhos" sob uma perspetiva nova: combinar os fármacos disponíveis de polaridade oposta, no sentido de formar Líquidos Iónicos como uma maneira económica de controlar co-infeções comuns, especialmente em África (HIV/TB, TB/malária, malária/HIV). Estas novas formulações podem exibir não só uma dupla, mas também novas propriedades físico-químicas e biológicas, devido à sua natureza **iónica**, eventualmente contribuindo para melhorar a biodisponibilidade oral, ou até contornar problemas de resistência a cada um dos fármacos individuais.

Com este trabalho demonstramos que a utilização de ILs deve ser incentivada porque pode reciclar muitos dos fármacos que foram colocados na prateleira, não só devido à resistência, mas também devido à sua limitada solubilidade aquosa ou conversão polimórfica.

Abstract

"A small couple for the big-three" (Two4Three) is an innovative and ambitious project where we propose to fight Malaria, Tuberculosis (TB), and HIV-1/AIDS using Ionic Liquids (ILs).

One of the major societal challenges today is the increasing resistance of pathogens against available drugs, a matter of great concern for the Big Three Diseases (BTD). We intend to address this problem from a new perspective: combine known drugs to form IL active against HIV/TB, TB/malaria, malaria/HIV co-infections. Such IL will act as new formulations potentially exhibiting dual action, while possessing physico-chemical properties favoring oral bioavailability, more potent activity, and absence of resistance

This is our working hypothesis which, if proven correct, will open brand new avenues towards cheap and effective dual-action medicines against the BTD. In other words, this is a low-cost innovative approach that may yield a major advance in the control of the most concerning infections of today.

"Two4Three" intends to fight malaria, TB and HIV by using API-derived IL. This project looks at the current group of anti-infective API from a new perspective: recycling ionizable drugs with opposed polarities (basic+acid) to form IL by ion pairing may produce valuable dual-action drug candidates, while returning value to drugs whose clinical use is in decline or put on hold(Cole, Hobden, & Warner, 2015; Prudencio, Vieira, van der Auweraer, & Ferraz, 2020; Teixeira et al., 2014).

The global aim of this project is to bring an innovative concept to the field of co-infections therapy, by taking advantage of both the wide plethora of antimalarial, anti-HIV and anti-mycobacterial drugs available, and the distinct properties of IL.

Assumed that, most drugs are either acidic or basic, their pairing may produce IL with dual anti-infective activity and distinct physic-chemical and biochemical

properties, appropriate for their use as new formulations with enhanced oral bioavailability and limited emergence of resistance. Such innovation will bring safe, effective and affordable medicines against co-infections, given that it will settle on:

a) well-known anti-infectives, previously approved by the relevant agencies (e.g., FDA, EMEA), and whose production pipelines are well-established and optimized to reduce costs;

b) simple ionic combination of two building blocks, one cationic, and one anionic, to form the final IL, hence, no elaborate or expensive chemistry will be required.

There are 5 major experimental goals to attain, which defined the Tasks for this project:

Task 1 - synthesis of novel IL based on acid-base pairing of ionizable anti-malarial, anti-HIV and anti-TB drugs;

Task 2 - biophysical profiling of the API-IL produced;

Tasks 3 and 4 - evaluation in vitro (Task 3) and in vivo (Task 4) of the anti-HIV, anti-TB and anti-malarial properties of the new IL, to both provide proof-of-concept on working hypothesis and propose new co-drug candidates for clinical development against HIV/TB, malaria/TB and HIV/malaria co-infections.;

Task 5 - assessment of structural and functional cellular changes on eukaryote and prokaryote cell models, induced by a subset of IL, chosen according to data from task 3.

Antimalarial activity, as well as toxicity to human host cells, will be evaluated at the Participating Institution "Instituto de Higiene e Medicina Tropical" (IHMT@UNL), in Lisbon, supervised by team member Dr. F. Nogueira. Dr. Nogueira

Anti-TB screenings will be carried out at the Participating Institution "Instituto de Biologia Molecular e Celular" (IBMC@i3S), in Porto, supervised by Prof. M. S. Gomes. Prof. Gomes's team has strong experience in mycobacterial biology and in the screening of anti-mycobacterial compounds. We have been collaborating with this team for some years. Relevantly, some preliminary assays have been recently run that delivered quite promising results regarding the potential of chloroquine-derived IL against TB.

Anti-HIV activity assays will be done on a collaborative basis.

The biological effects of the most promising of the API-IL produced on eukaryote and prokaryote cell models will be evaluated, through assessment of structural and functional changes induced by the IL on those cells. This task will be carried out at "Escola Superior de Saúde do Instituto Politécnico do Porto" (ESS-IPP@I3S), in Porto, supervised by Dr. Cristina Prudêncio.

The first drugs that we used were cationic antimalarials (primaquine (PQ), chloroquine (CQ)). For anionic drugs, we decided to used three types of bioactive compounds: cinnamic acids, fatty acids and fluoroquinolones.

We also produce ionic liquids and covalent compounds from PQ and 7 fatty acids (butyric acid, caprylic acid , lauric acid, myristic acid, palmitic acid, stearic acid and oleic acid) in a total of 10 compounds. We did the same for CQ (A. T. Silva et al., 2020; Ana Teresa Silva et al., 2020).

We synthesized a total of 33 compounds pure and full characterized by NMR and mass spectra with good yields.

About their thermal stability, an important issue for APIs typically employed in the treatment of diseases, such as malaria, that are endemic to tropical and sub-tropical countries, all compounds were analysed by simultaneous thermogravimetric analysis (STA). Herein we demonstrated that the ILs are slightly less thermally stable than the commercial CQ phosphate salt, but still remain unaltered up to about 90 ºC or higher temperatures. As expected, covalent amide analogues displayed higher thermal stability.

Regarding their antimalarial activity, the compounds were submitted to in vitro assays, to assess their activity against a CQ-sensitive (3D7) and a CQ-resistant (Dd2) strain of Plasmodium falciparum. The first remarkable observation was that, while all RTIL displayed adequate solubility in the medium used in the assays, only some amides were sufficiently soluble in these conditions. Besides, RTIL displayed slightly better solubility in aqueous media than covalent analogues. More importantly, all RTIL displayed stronger activity than the parent antimalarial drug, which is classically formulated as a phosphate salt, against both CQ-sensitive and CQ-resistant strains of P. falciparum, the species responsible for the deadliest form of human malaria (A. T. Silva et al., 2020; Ana Teresa Silva et al., 2020).

ILs based on CQ-cinnamic acid conjugates were as effective against M. avium, extracellularly and inside macrophages, as its covalent equivalents. However, they were more soluble and less toxic for the host cells. The conjugation of CQ or primaquine (PQ) with fluoroquinolones, that are sometimes used to treat

mycobacterial infections, resulted in ILs that have the same direct activity as the original antibiotics to M. avium, but are more active against the mycobacteria growing inside macrophages (Bento, Gomes, & Silva, 2020).

The project is in his final months, we now expect to be in shape to deliver novel API-IL as valuable dual-action leads against co-infections arising from host invasion by the most threatening infectious pathogens of today: malaria parasites, TB bacteria, and HIV retro-viruses. This findings will hopefully pave the way towards the next natural step in this context: attracting funds/investors to take forward most promising API-IL into clinical development, towards low-cost and safe medicines, within reach of even the most remote infection sites.

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