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Referência do projecto
Project reference
PTDC/SAU-FAR/118459/2010 **(Lacrado a 24-02-2011 às 10:47)**

1. Identificação do projecto -

1. Project description

Área científica principal
Main Area
Ciências da Saúde - Ciências Farmacêuticas

Área científica Secundária
Secondary area
Química e Bioquímica - Química

Título do projecto (em português)
Project title (in portuguese)
Contribuição para a erradicação da malária. Uma nova abordagem para atingir multi-alvos no ciclo de vida do parasita

Título do projecto (em inglês)
Project title (in english)
Towards malaria eradication. A novel approach for multi-targeting the parasite´s life cycle

Financiamento solicitado
Requested funding
191.759,00€

Palavra-chave 1
Antimaláricos

Keyword 1
Antimalarials

Palavra-chave 2
Fármacos híbridos

Keyword 2
Hybrid drugs

Palavra-chave 3
Fase sanguínea

Keyword 3
Blood stage

Palavra-chave 4
Fase hepática

Keyword 4
Liver stage

Data de início do projecto
Starting date
01-03-2012

Duração do projecto em meses
Duration in months
36

2. Instituições envolvidas -

2. Institutions and their roles

Instituição Proponente
Principal Contractor
Faculdade de Farmácia da Universidade de Lisboa (FF/UL)
Av. Professor Gama Pinto
1649-003Lisboa

Instituição Participante
Participating Institution
Centro de Neurociências e Biologia Celular (CNBC/UC)
Departamento de Zoologia - Universidade de Coimbra
3004-517Coimbra
Instituto de Medicina Molecular (IMM/FM/UL)
Avenida Professor Egas Moniz
1649-028Lisboa

Unidade de Investigação
Research Unit
Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL)
Av. Prof. Gama Pinto
1649-003Lisboa

Unidade de Investigação Adicional
Additional Research Unit
Centro de Neurociências e Biologia Celular (CNBC/UC)
Departamento de Zoologia - Universidade de Coimbra
3004-517Coimbra
Instituto de Medicina Molecular (IMM/FM/UL)
Avenida Professor Egas Moniz
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Instituição de Acolhimento
Host Institution
Faculdade de Farmácia da Universidade de Lisboa (FF/UL)
Av. Professor Gama Pinto
1649-003Lisboa

3. Componente Científica
3. Scientific Component

3.1. Sumário
3.1 Abstract

3.1.a Em português
3.1.a In Portuguese

A malária continua a ser uma das doenças tropicais mais preocupantes devido ao elevado número de mortes que origina e ao impacto negativo a nível social e económico nos países onde a doença é endémica. O aparecimento e a consequente disseminação de P. falciparum multi-resistente, o agente responsável pela forma mais letal de malária humana, é um dos principais obstáculos ao controlo da doença.
A maior parte dos fármacos em uso foram desenhados como esquizonticidas sanguíneos potentes, para actuarem rapidamente contra as formas eritrocíticas do parasita, responsáveis pelos sintomas, de maneira a curar a doença num tempo razoável (3 dias ou menos) [1]. No entanto, o ciclo de vida do parasita nos humanos inclui também uma fase hepática assintomática anterior à fase sanguínea. Particularmente, as infecções a P. vivax e a P. ovale podem gerar formas latentes, chamadas hipnozoitos, que persistem no fígado por longos períodos de tempo, podendo causar recaídas da doença. Assim, a erradicação da malária, definida como a eliminação completa de todos os parasitas (incluindo os hipnozoítos do P. vivax no fígado) surge agora como um objectivo audacioso (http://malera.tropica.net) [4-6].
A primaquina (PQ) é o único fármaco eficaz contra as infecções a P. vivax e tem sido usado há mais de 60 anos apesar dos seus inconvenientes como por exemplo a anemia hemolítica em doentes deficientes em glucose-6-fosfato desidrogenase [9]. Actualmente, existe um número reduzido de compostos, com pouca diversidade química e com eficácia provada contra os hipnozoítos do P. vivax. Esta lacuna de conhecimento é ainda maior se considerarmos compostos que actuem em simultâneo e com eficácia idêntica em ambas as fases do ciclo de vida do parasita (hepática e sanguínea).
O objectivo geral deste projecto, é pois colmatar esta falha de maneira a obter novas ferramentas para campanhas de erradicação. A ideia central é que combinando 2 estruturas químicas diferentes – cada uma delas direccionada para uma fase específica do ciclo de vida do parasita – numa única molécula, se possam obter antimaláricos efectivos capazes de actuarem nas 2 fases com eficácia semelhante. Esta hipótese tem por base resultados preliminares do nosso grupo em que compostos híbridos baseados na artemisinina (esquizonticida sanguíneo pontente) e primaquina mostraram excelente eficácia in vivo em modelos de roedores contra as fases sanguínea e hepática (Anexo 1). Iremos agora levar esta ideia inovadora mais à frente, (i) diversificando a natureza da unidade estrutural responsável pela actividade sobre a fase sanguínea, (ii) incorporando novas estruturas activas sobre a fase hepática potencialmente menos tóxicas que a PQ e (iii) conferindo aos híbridos melhores propriedades de fármacos por diminuição da massa molecular para aumentar a biodisponibilidade oral. Os objectivos específicos propostos para este projecto são:

1. Desenvolver 3 bibliotecas de compostos híbridos com diversidade química usando diferentes combinações de estruturas

2. Screening in vitro destas bibliotecas para eficácia contra as fases hepática e sanguínea usando estirpes resistentes de P. falciparum e P. berghei
3. Optimizar os 'hits' iniciais em protótipos com base na relação estrutura actividade e nos ensaios in vitro de previsão farmacocinética
4. Seleccionar protótipos usando testes in vivo em modelos de roedor para eficácia contra as fases hepática e sanguínea e modelos de toxicologia

Os métodos para atingir os objectivos específicos envolvem o uso de química terapêutica paralela; avaliação, in vitro e in vivo, da eficácia dos compostos; e avaliação da permeabilidade, metabolismo e toxicidade dos compostos. Estes serão sucessivamente melhorados aplicando ciclos destes métodos e refinando o modelo que define o melhor balanço geral destas características numa molécula entre os vários ciclos (ver diagrama de fluxo no anexo 2 e 'timeline'). Quando completo, este projecto deverá originar protótipos optimizados prontos para serem submetidos a testes pré-clínicos.
Para atingir estes objectivos específicos criaremos uma equipa interdisciplinar altamente experiente com conhecimentos na área da química orgânica e terapêutica (Lopes, Afonso, Moreira, Sá e Melo), na área da biologia da malária e screening de actividades (Mota, Prudêncio, Rosenthal), e na área da modelação farmacocinética (Morais).
Devido ao grande impacto global da malária e à grande necessidade de novos fármacos antimaláricos eficazes contra infecções multi-resistentes, seguros em crianças e grávidas, o sucesso deste projecto representará a melhoria da vida de milhões de pessoas. Também esperamos contribuir para a formação de jovens investigadores nesta área.

3.1.b Em inglês
3.1.b In English

Malaria remains the world's top-priority tropical disease due to its high death burden, as well as to its economic and social impacts on the development of malaria-endemic countries. The emergence and spread of multidrug-resistant Plamodium falciparum, the causative agent of the most lethal form of human malaria, is still the major obstacle in the control of the disease. Current antimalarials have been designed as potent blood-schizontocides, i.e. acting rapidly against the parasitic forms that invade erythrocytes and cause disease symptoms, in order to cure malaria within a reasonable time (ideally 3 days or less) [1]. However, prior tothis erythrocytic stage of infection, malaria parasites in the human host go through an asymptomatic, obligatory developmental phase in the liver, after which they are released into the bloodstream where they infect red blood cells. In particular, P. vivax infections can generate cryptic forms called hypnozoites that persist in the liver for long periods of time, causing relapsing malaria. Thus, malaria eradication, defined as elimination of all parasites (including the long-lived hypnozoites of P. vivax in the liver), has now emerged as an audacious goal (http://malera.tropika.net) [4-6]. Primaquine (PQ), is the only available drug to treat P. vivax infections and has been used for more than 60 years despite its liabilities such as hemolytic anemia in patients with glucose 6-phosphate dehydrogenase deficiency [9]. Currently, there is a reduced number of chemotypes with proofed efficacy against P. vivax hypnozoites. This knowledge gap is even wider when we take into consideration the lack of chemotypes capable of killing both the blood- and liver-stage parasites with identical efficacy. Consequently, the overall goal of this project is to address this gap in order to provide new tools for eradication campaigns. The central hypothesis of the present project application is that combining two chemotypes - each one targeting a specific stage of the parasite's life cycle - in a single chemical entity, can lead to effective antimalarials capable of killing both the blood- and liver-stage parasites. The hypothesis rests on the observation made by our team that hybrid compounds based on the potent blood-schizontocide artemisinin, and the liver-stage compound primaquine have shown excellent efficacy in in vivo rodent models for blood- and liver-stage infections (Annex 1). We will now take this innovative approach one step further, by (i) expanding the scaffolds that are capable of interfering in the blood-stage, (ii) incorporating new chemotypes active against the liver stage that are potentially less toxic than PQ, and (iii) conferring the hybrid molecules drug-like properties such as smaller molecular-weight to improve oral bioavailability. Specifically, the goals proposed for this research are:

1. Development three series of chemically diverse hybrid-based libraries using different combinations of chemotypes
2. Screening of these libraries for efficacy against the blood- and liver-stages in vitro, using drug resistant P. falciparum and P. berghei,
3. Optimization of initial screening hits into lead compounds based on the structure-activity relationships as well as using pharmacokinetic prediction assays in vitro
4. Selection of lead compounds using in vivo rodent efficacy models for blood- and liver-stage infections and toxicology models

The research design and methods for achieving the specific goals involves the use of parallel medicinal chemistry; in vitro and in vivo measures of compound efficacy; and measures of compound permeability, metabolism and toxicity. Compounds will be incrementally improved by applying cycles of these methods and refining the model that defines the overall best balance of these traits in a molecule between cycles (see flowchart in Annex 2 and timeline). When complete, this project will be able to deliver optimized lead compounds ready to be submitted to preclinical trials. To achieve these specific goals, we put together an highly experienced interdisciplinary team comprising expertise in medicinal and organic chemistry (Lopes, Afonso, Moreira, Sa e Melo), malaria biology and activity screenings (Mota, Prudêncio, Rosenthal), and pharmacokinetic modeling (Morais). Given the scope of the global impact of malaria and the great need for new bioavailable and affordable antimalarial drugs that are effective in eliminating all parasites, safe in children and during pregnancy, the significance of success in these efforts would be nothing less than improving the lives of millions of individuals. We also hope to contribute to the training of young researchers in the area of malaria.

3.2. Descrição Técnica
3.2 Technical Description

3.2.1. Revisão da Literatura
3.2.1. Literature Review

The malaria research agenda: changing the paradigm
Malaria research agenda has been focused on developing effective, orally bioavailable, safe, and affordable antimalarials, to improve disease's control and to reduce morbidity and mortality [1]. These efforts resulted in large cell-based high-throughput screens that generated hits with the potential to evolve into drugs acting rapidly against the parasitic forms that invade erythrocytes and cause the usual malaria symptoms [2,3]. However, because of the continuing unacceptable high death

burden and economic and social impacts of malaria, the research agenda has now set up the ultimate goal of global eradication of malaria parasites from the human population [4,5].

The liver stage: a valuable target for malaria eradication
The ideal malaria eradication drug should be suitable for mass administration and lead to the elimination of all parasites in all life cycle stages of the five malaria species infecting humans [6]. In humans, the malaria parasites undergo an asymptomatic, obligatory developmental phase in the liver after which they are released into the bloodstream where they infect red blood cells and cause disease symptoms. Importantly, P. vivax infections can generate cryptic forms called hypnozoites that persist in the liver for long periods of time [7]. The liver stage offers important advantages for prophylactic intervention: (i) the number of parasites present during this stage is relatively low, (ii) intervention at this stage acts before the onset of symptoms, providing a prophylactic strategy and (iii) anti-liver stage drugs would be needed for a malaria eradication campaign, which obviously, includes elimination of the long-lived hypnozoites of P. vivax in the liver [7].

Chemotypes for blocking Plasmodium liver stage
Presently, only the 8-aminoquinolines (8-AQ) are known to be effective against P. vivax hypnozoites [8]. Primaquine (PQ, 1, Fig. 1), an 8-aminoquinoline, has been used for more than 60 years, despite its liabilities (hemolytic anemia in patients with glucose 6-phosphate dehydrogenase deficiency) [9]. Tafenoquine (TFQ, 2, Fig. 1), an 8-AQ discovered in the 70s as a potential successor to PQ [10], still remains in Phase I clinical trials, and only three additional compounds are in pre-clinical or early-stage clinical trials for the radical cure of P. vivax infection [7]. Several groups have been actively involved in filling this knowledge gap of the chemical space active against the liver stage, by rediscovering PQ as a lead compound for further development [9,11]. For example, our team performed structural modifications to solve specific drug metabolism issues that affect pharmacokinetics, e.g. the formation of inactive metabolites such as carboxyprimaquine (CPQ, 3, Fig. 1) resulting from oxidative deamination at the side chain of PQ [12-15]. Formation of CPQ is reflected on the short half-life of PQ (ca. 6 h in humans) and thus, on the 14-day treatment course required to eliminate the latent hypnozoite reservoir of vivax malaria associated with adverse events in G6PD heterozygous patients [9]. Blocking such metabolic pathway, however, has the potential advantage of a shorter treatment course than PQ, and therefore increased patient compliance. Importantly, the strategy adopted by our team resulted in metabolic-stable 8-AQs (4, Fig. 1), without affecting in vitro activity against the liver stage of the rodent P. berghei parasite [15]. Moreover, these novel derivatives displayed potent gametocytocidal activity in a model comprising P. berghei infected-mice and Anopheles stephensi mosquitoes, showing their potential as transmission-blocking agents [12,15], and added-value in eradication campaigns [6].
Other compounds reported to inhibit the development of liver stage parasites, include antibacterial quinolones [16], the antifungal miconazole [17], the flavonoids licochalcone A and ginestein [17,18] or the HIV-1 protease inhibitor saquinavir [17]. However, most of these compounds are not readily accessible from a synthetic point of view - a major requisite to obtain affordable antimalarials - or are not as active as the golden standard PQ. Very recently, 4-pyridonimines and 4-quinolonimines (5 and 6, Fig. 1) were reported as novel chemotypes that are (i) significantly more effective than PQ in inhibiting liver stage P. berghei parasites and (ii) readily accessible from simple starting materials [19]. Thus, they are ideal partners for blood-stage acting antimalarials, to accomplish complete eradication of parasites from infected hosts [6].

Multi-targeting the parasite's life cycle: an unmet need
Unfortunately, a chemotype capable of killing both the blood- and liver-stage parasites has not been discovered yet. An alternative approach to fill this gap is to incorporate two chemotypes - each one targeting a specific stage of the parasite's life cycle - in single chemical entity, called hybrid drug. This concept has been successfully used to develop potent blood-stage-acting antimalarials that interfere with different targets (e.g. 7, Fig. 2) [20] or different sites of the same target (e.g. 8, Fig. 2) [21]. Only recently similar approaches have been reported for hybrids acting on different stages of malaria parasite. For example, our group developed hybrid molecules acting against blood- and liver-stage parasites, encompassing a ferrocene moiety (to generate reactive oxygen species via a Fenton-like redox reaction in the parasite digestive vacuole) and PQ (9, Fig. 2) [22]. The lack of activity against blood-stage parasites displayed by hybrids 9 led to the development of artemisinin-PQ hybrids (10-11, Fig. 2), with excellent activities in vitro and in vivo against both the blood- and liver-stage parasites (Annex 1). These encouraging results strongly suggest that combining peroxides to 8-AQs is a promising strategy to generate drug candidates for eradicating malaria.

3.2.2. Plano e Métodos

3.2.2. Plan and Methods

The central hypothesis to be developed in this project is that hybrid compounds combining two chemotypes - each one targeting a specific stage of the parasite's life cycle - can generate effective antimalarials capable of killing both blood- and liver-stage parasites. The hypothesis rests on the observation made by our team that hybrid compounds based on the potent blood-schizontocide artemisinin and primaquine have shown excellent efficacy in in vivo rodent models for blood- and liver-stage infections (see Annex 1). We will now take this approach one step further, by conferring the hybrid molecules drug-like properties, such as decreased molecular-weight, to improve oral bioavailability. The specific goals to be achieved with this project are: (i) to develop chemically diverse hybrid-based libraries complying with the Lipinski's Rule of Five, to improve activity when given orally, (ii) to screen of these libraries for efficacy against the blood- and liver-stages in vitro, using drug-resistant P. falciparum and P. berghei, (iii) to optimize initial screening hits into lead compounds based on the structure-activity relationships as well as using pharmacokinetic prediction assays in vitro and (iv) to select lead compounds using in vivo rodent efficacy models for blood- and liver-stage infections and toxicology models. Compounds will be incrementally improved by applying cycles of these methods and refining the model that defines the overall best balance of these traits in a molecule between cycles (see flowchart in Annex 2 and timeline).

1. Synthesis of chemical libraries
The first task of the project consists in the synthesis of the hybrid libraries (Fig. 3), based on 1,2,4,5-tetraoxanes and 1,2,4-trioxolanes as substitutes for the artemisinin scaffold. These peroxides were selected on the basis of their (i) high potency against the blood-stage of malaria parasites [23,24] (ii) reduced molecular weight when compared to artemisinins, thus contributing to improved drug-like properties, and (iii) simple and affordable synthetic methodology (Fig. 4A,C). An exploratory approach to the 1,2-dioxolane scaffold (Fig. 4A) as an anchoring point to other chemotypes, will also be pursuit as a backup strategy. 1,2-Dioxolanes have been recently reported as potent antiplasmodials [25] and are completely unexploited

in terms of hybrid drug design. The peroxide scaffolds are designed to provide an anchoring point to attach the second chemotype, e.g. for amide coupling, reductive amination or nucleophilic substitution.
Task 1 also involves the synthesis of the chemotypes targeting the liver-stage and which will be attached to the peroxide anchors. They include the 8-aminoquinoline, 4-quinolonimine and the closely related 4-pyridonimine scaffolds. Regarding the 8-aminoquinoline moiety, the objective is to synthesize compounds containing aryl/heteroaryl groups at position C-5 of the quinoline moiety to avoid P540-induced hemotoxicity [9] The chemistry to be developed is based on the Suzuki, Sonogashira and click reactions (Fig. 5A,B), which are adaptable to a solution-phase parallel synthesis format. The nature of the substituents introduced via these methodologies is expected to block metabolic activation (e.g. polar five-membered heterocycles). The chemical space around 4-quinolonimines and 4-pyridonimines will be also exploited using solution-phase parallel synthesis based on the chemistry already developed at iMed.UL [19,31].
Task 1 covers 36 months and encompasses (i) the synthesis of the three chemical libraries, (ii) optimization of hit compounds generated from in vitro activity and ADME (absorption, distribution, metabolism and elimination) screening of libraries (Tasks 2 to 4), and (iii) scale-up synthesis of lead compounds selected for in vivo activity studies (Task 5). The 1,2,4,5-tetraoxane- and 1,2,4-trioxolane-based libraries are expected to be complete in month 12 (milestone 1), while its 1,2-dioxolane-based counterpart will be complete in month 14. This task joins the Medicinal and Organic Chemistry expertise's of iMed.UL and CNC researchers.

2. Hit discovery. Screening against Plasmodium blood- and liver-stages
In Task 2, all chemical libraries will be screened for (i) activity against cultured P. falciparum (both sensitive and multidrug resistant strains) at Rosenthal's lab (consultant) and (ii) for inhibition of the development of P. berghei schizonts in human hepatoma cells, using the transgenic P. berghei parasite, PbGFP-Luccon, expressing the bioluminescent reporter protein luciferase to quantify parasite development in liver cells, at IMM [26]. From these screens, structure-activity relationships (SAR) will be established for each of the parasite's life-cycle stage studied and hit compounds (i.e. with IC50 values not higher than 100 nM against the blood- and liver-stages, and preferably less than 50 nM against at least against one of the parasite's stages) will be selected for further optimization (milestone 2, month 18). Task 2 also includes the screening of compounds generated from the hit optimization process (see 'Hit-to-lead' section).

3. Hit-to-lead
The next step is to convert these hits into lead compounds. Incorporating predictive ADME assays in earlier stages of drug discovery can help in rejecting candidate molecules that lack necessary pharmacological properties. In this context, the most potent hits emerged from the screens will be subjected to preliminary in vitro metabolism studies (Task 3). These will be performed incubating compounds in human and murine liver microsomes and analyzed for degradation rates and intrinsic clearances (CLint) using HPLC and LC-MS methodologies. Microsomes retain activity of key enzymes involved in drug metabolism that reside in the smooth endoplasmic reticulum, such as cytochrome P450s (CYPs), flavin monooxygenases and glucuronosyltransferases [27]. Since lipophilicity is a key determinant of ADME as well as being an important factor in determining the solubility of a compound, log D7.4 (distribution coefficient octanol-pH 7.4 buffer) will also be determined by HPLC. iMed.UL and CNC facilities are adequately equipped for the analytical measurements required, including analytical- and prep-HPLCs, as well as a LC-MS-MS.
Simultaneously, a human colorectal adenocarcinoma cell line, Caco-2, will be used to assess absorption, permeation and efflux transport properties of compounds [28] (Task 4). Caco-2 cell line forms monolayers of differentiated epithelial cells joined by intercellular tight junctions in polycarbonate membrane inserts, providing a selective barrier for both transcellular (from the apical to the basolateral chamber) and carrier-mediated efflux transport (from the basolateral to the apical chamber) mechanisms, allowing the calculation of apparent permeability (Papp) values as well as net efflux ratios. The use of the P-gp selective inhibitor, GG918, will allow clarification on the physiological role of this transporter. The later capability will be used exclusively for lead candidates, at the final stage of the project.
Once hit compounds have been adequately characterized in terms of intrinsic metabolic stability and permeability, the resulting structure-metabolism-permeability relationships will be analyzed for structural features that affect these properties and to prioritize the structural modifications to be performed in another cycle of Task 1. These structural modifications will be focusing on improving metabolic stability and permeability, which usually requires reduction of lipophilicity or blocking metabolic-labile groups [12,15,27].

4. Lead selection
Compounds displaying adequate intrinsic metabolic stability and cell permeability will progress to in vivo (murine models) activity determination (Task 5) to enable selection of hybrid compounds with suitable profiles for future in vivo ADME studies. The assay for determining activity against the blood-stage uses P. berghei is a four-day suppressive test, in which the efficacy of four daily doses of compounds is measured by comparison of blood parasitaemia and mouse survival in treated and untreated mice [1]. The test to determine the potency against the liver-stage uses a transgenic P. berghei parasite, PbGFP-Luccon, expressing the bioluminescent reporter protein luciferase to visualize and quantify parasite development in liver cells in live mice using real-time luminescence imaging and has been developed by the IMM group [26]. The first cycle of in vivo activity studies is expected to be complete in month 24 (milestone 3), while the second cycle involving the study of compounds generated from hit-optimization will be complete in month 34 (milestone 4). A key issue that will be addressed is the potential haematological toxicity of lead compounds, whether intrinsic or generated following metabolic activation (Task 6). The selected lead hybrid compounds will be tested for metabolism-linked methemoglobin toxicity in vitro (milestone 4) [29]. Briefly, compounds will be simultaneously incubated with human liver microsomes and pooled human erythrocytes followed by methemoglobin determination by a spectrophotometric technique adapted to 96-well plates.

In summary, the main outcomes expected from this project include: (i) a new therapeutic approach to contribute to the malaria eradication agenda and (ii) novel promising validated hybrid lead series based on in vitro and in vivo activity data. The teams involved have internationally recognized merits on this scientific field, including extensive experience in the design, synthesis and evaluation of new antimalarial drug candidates. Thus, we expect to successfully achieve the goals herein proposed. It should be stressed that intellectual property is already settled and that any potential patent regarding a new antimalarial candidate will be dealt through the Knowledge Transfer Office of the University of Lisbon.

3.2.3. Tarefas

3.2.3. Tasks

Lista de tarefas (6)
Task list (6)

Designação da tarefa Task denomination	**Data de início** Start date	**Data de fim** End date	**Duração** Duration	**Pessoas * mês** Person * months
Synthesis of chemical libraries	01-03-2012	28-02-2015	36	80,4

Descrição da tarefa e Resultados Esperados
Task description and Expected results

The primary goal of this task is to synthesise hybrid libraries comprising (i) 1,2,4,5-tetraoxanes, 1,2,4-trioxolanes and 1,2-dioxolanes to target the blood stage, and (ii) 8-aminoquinolines, 4-quinolonimines and 4-pyridonimines to target the liver stage (Fig. 3). This task also includes optimization of hit compounds generated from in vitro activity (Task 2) and ADME screening of libraries (Tasks 3 and 4) as well as the scale-up synthesis of lead compounds selected for in vivo activity studies (Task 5).

Methodologies
(i) Peroxides. 1,2,4,5-Tetraoxanes and 1,2-dioxolane can be prepared from a single common intermediate, the gem-bishydroperoxides (Fig. 4). This can be achieved by a large variety of methods (for a review, see Fig. 4A). iMed.UL team developed an efficient method for the direct synthesis of gem-bishydroperoxides by aqueous H2O2 catalyzed by PTSA at room temperature. Under such conditions, cyclohexane-1,1-dihydroperoxide was obtained from cyclohexanone in 95% yield, in high purity, without the need of further purification (Fig 4B). These preliminary result supports the possibility to create a more efficient synthetic methodology that may allow to prepare a higher range of 1,1-dihydroperoxides (C Afonso/A Rosatella). gem-Bishydroperoxides will be converted to 1,2,4,5-tetraoxanes by reaction with a second ketone using rhenium (VII) oxide chemistry (Fig. 4A) (M Melo/M Silva) [23]. Appropriately functionalized 1,2-dioxolanes will be prepared using silyl peroxyketal (modified gem-bishydroperoxides, Fig. 4A) or 1,2-dioxolan-3-ol intermediates [25] (D Miranda). Access to 1,2,4-trioxolanes involves the preparation of various methyl oximes that can be coupled through ozonolysis with an appropriately functionalised ketone, using the Griesbaum-Vennerstrom chemistry [24] (R Capela) (Fig. 4C). Tetraoxanes and trioxolanes can incorporate structural features that impart chemical stability (e.g. the adamantane moiety) and are designed to provide an anchoring point to attach the second chemotype.

(ii) 8-Aminoquinolines, 4-pyridonimines and 4-quinolinimines (BI Fellow/F Lopes). 8-AQs containing aryl/heteroaryl groups at position C-5 of the quinoline moiety (Fig. 5A) are designed to avoid the cytochrome P540-catalyzed oxidation reaction at this position [9]. Compounds will be prepared using the commercially available starting material 6-methoxy-8-nitroquinoline. Bromination at C-5, followed by coupling with appropriate aryl/hereroaryl boronic acid (Suzuki coupling) to yield the corresponding 5-substituted quinoline (Fig. 5A). This intermediate is reduced and then converted to the 8-AQ via Gabriel synthesis (Fig. 5A). A closely related approach will also be developed to incorporate an electron-rich heterocyclic moiety at C-5. This will be based on the Sonogashira reaction that takes the advantage of using the same intermediate synthesized in the Suzuki approach (Fig. 5B). The Sonogashira reaction generates an alkyne derivative that can react with appropriately substituted azides ('click' reaction) to give the corresponding 1,2,3-triazoles (Fig. 5B). The 5-alkyne intermediate can then evolve to the final 8-AQ derivative using the chemistry described above. 4-Pyridonimines and 4-quinolinimines will be prepared according to the methodologies developed at iMed.UL [19, 31].

(iii) Hybrid compounds (D Miranda/R Capela/BI Fellow/M Melo/Maria Silva/F Lopes). The 8-AQ-peroxide hybrid library (Fig. 6A) will be obtained by coupling both components via amide or reductive amination chemistry, in which the 8-AQ provides de amine moiety (Fig. 6B). For the 4-quinolonimine- and 4-pyridonimine-based libraries, coupling with the peroxide moiety will occur via the N-1 or C-4 positions using linkers of variable length (Fig. 7). This provides a site for modulating pharmacokinetic properties.

This task requires one full-time student (BI) specifically to develop all the parallel chemistry and synthesize lead hybrid compounds for in vivo studies.

Membros da equipa de investigação nesta tarefa
Members of the research team in this task

(BI) Bolseiro de Investigação (Mestre) 1; Andreia de Almeida Rosatella; Carlos Alberto Mateus Afonso; Daniela Filipa Pintassilgo Miranda; Francisca Conceição Lopes; Maria Luisa Campeão Fernandes Vaz Sá Melo; Maria Manuel da Cruz Silva; Rita Sofia Salvador Simões Capela;

Designação da tarefa Task denomination	**Data de início** Start date	**Data de fim** End date	**Duração** Duration	**Pessoas * mês** Person * months
In vitro screening	01-09-2012	30-06-2014	22	23

Descrição da tarefa e Resultados Esperados
Task description and Expected results

Objectives
In this task we will evaluate the ability of compounds synthesized in Task 1 to inhibit both infection of liver cells by Plasmodium in vitro, and the development of cultured sensitive and multidrug resistant strains of P. falciparum. This will establish their efficacy as liver- and blood-stages antimalarials.

Methodologies
(i) Liver-stage assay. An established in vitro infection model using human hepatoma cells Huh7 infected with P. berghei parasites, in 96-well plates, will be employed to assess the effect of the compounds on in vitro infection. Parasites will be obtained from the dissection of the salivary glands of infected female Anopheles mosquitoes, routinely bred in the Malaria Unit of the Instituto de Medicina Molecular (UMA-IMM), our collaborators in this project (MM Mota and M Prudêncio labs).
The effect of compounds on the infection load of Huh7 cells by Plasmodium will be assessed by various methods that have been optimized in the UMA-IMM laboratory. Initially, a newly developed technique based on the measurement of the luminescence of

cells infected with luciferase-expressing P. berghei parasites will be employed [26]. The effect of selected compounds will be re-evaluated by flow cytometry with the use of GFP-expressing P. berghei parasites ([15] and references cited herein). This will not only enable confirmation of the previously observed infection phenotype but will also provide important insights into the mechanism through which such an effect occurs as it will enable distinguishing between effects on invasion and Plasmodium development following drug treatment. Finally, infection will be monitored by automated fluorescence microscopy and image analysis [30]. This will enable the confirmation of the invasion/development phenotypes while providing visual information about the morphology of both the parasite and the host cells after incubation with the compounds. The liver-stage assay involves the collaboration of A Cruz and one BI fellow.

(ii) Blood-stage assay. These assays will be conducted at Phil Rosenthal's lab (consultant; this has been a long-standing collaboration between I.Med.UL and UCSF that have been on the basis of a large number of joint papers), using the methodology reported elsewhere [15]. Briefly, synchronized ring-stage W2 strain P. falciparum parasites are cultured with multiple concentrations of test compounds (added from 1000xstocks in DMSO) in RPMI 1640 medium with 10% human serum. After an 48 h incubation, when control cultures contains new rings, parasites are fixed with 1%formaldehyde in PBS, pH 7.4, for 48 h at room temperature and then labeled with YOYO-1 (1 nM; Molecular Probes) in 0.1% Triton X-100 in PBS. Parasitemias are determined from dot plots (forward scatter vs fluorescence) acquired on a FACSort flow cytometer using CELLQUEST software (Becton Dickinson). IC50s for growth inhibition are determined with GraphPad Prism software from plots of percentages of the level of parasitemia of the control relative to inhibitor concentration. In each case, goodness of curve fit is assessed by R2 values of >0.95.

Expected results
Results obtained in this task will enable (i) assessment of the activities for selected compounds during the liver- and blood-stages of Plasmodium infection, as expressed by their IC50 values (ii) determination of structure-activity relationships (SAR) for each library and for each stage of infection, and (iii) prioritization of compounds to be selected for Task 3 and Task 4. It can be expected that compounds that impair infection will be identified by their IC50s determination.

Taking in account the large number of compounds to be screened, this task requires one full-time student (BI) that will be also involved in the in vivo testing of lead compounds (Task 5).

Membros da equipa de investigação nesta tarefa
Members of the research team in this task
(BI) Bolseiro de Investigação (Lic. ou Bacharel) 1; Ana Filipa Pintéus da Cruz;

Designação da tarefa	**Data de início**	**Data de fim**	**Duração**	**Pessoas * mês**
Task denomination	Start date	End date	Duration	Person * months
Cellular permeability	01-03-2013	28-02-2014	12	2,9

Descrição da tarefa e Resultados Esperados
Task description and Expected results
Objectives
Measurement of the bidirectional transport, apical to basolateral (A to B) and basolateral to apical (B to A), across the Caco-2 cell monolayer allows for the calculation of apparent permeability (Papp) values as well as net efflux ratios (ER = Papp B to A/Papp A to B). The use of the P-gp selective acridone carboxamide inhibitor, GG918 (N-{4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)-ethyl]-phenyl}-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide), will allow clarification on the physiological role of this transporter. This task will be developed at iMed.UL by M Estudante and J Ferreira. Our team has already solid experience in cell transport methodology acquired at the UCSF (Prof. L. Z. Benet's lab). Caco-2 cell lines will be kindly provided by Prof. L. Z. Benet.

Methodology
Caco-2 cells grown (60,000 cells/cm2) as epithelial monolayers on polycarbonate membrane inserts (0.4 micrometers) with 4.2 cm2 surface area will be used in the transport studies 21 days after seeding. Cell media is changed twice a week and the day before the experiment. Transepithelial electrical resistance (TEER) is measured for assessment of monolayer integrity. Caco-2 cells will be used if TEER values are higher than 800–1000 Ω cm2. A paracellular marker, mannitol, will be used complementary to assess monolayer integrity. Cells are incubated with drug solution in the absence or presence of the P-gp inhibitor GG918 (0.5 microM). At selected times (1, 2, and 3h), 200 miliL-samples from the A or B side are taken and replaced with fresh buffer with or without GG918. In order to validate our cellular system regarding the presence and viability of the efflux transporter P-gp, a positive control drug (the well known intestinal Pgp substrate digoxin) will be tested in parallel to study drugs. Drug concentrations in the samples and intracellularly at the end of the experiment will be determined by HPLC or LC-MS/MS. iMed.UL is equipped with state-of-the-art analytical HPLCs and triple-quadrupole LC-MS/MS.

Expected results
To be considered a good drug candidate, the corresponding permeability value in Caco-2 cells should be greater than 10X10-6 cm/s (70-100% absorbed) and the ER should be smaller than 3. When ER is higher than 3, an efflux transporter is considered to be involved. SAR for the tested compounds will elucidate on the structural motifs that enhance cell permeability and thus, this information can be used in the hit-to-lead optimization process and influence the second cycle of Task 1.

Membros da equipa de investigação nesta tarefa
Members of the research team in this task
João Pedro Gomes Roque Borges Ferreira; Maria Margarida André Oliveira Estudante;

Designação da tarefa	**Data de início**	**Data de fim**	**Duração**	**Pessoas * mês**
Task denomination	Start date	End date	Duration	Person * months
Metabolic stability	01-03-2013	30-04-2014	14	15,3

Descrição da tarefa e Resultados Esperados
Task description and Expected results

Objectives
Evaluation of the susceptibility of hit compounds towards degradation in mice and human microsomes. The intrinsic clearance (CLint) is the rate constant of the first-order decay of a given compound, normalized for the protein concentration in the incubation. CLint is calculated after measurement of the first-order rate constant for consumption of substrate in the presence of mice or human liver microsomes. For those compounds displaying higher CLint values, a metabolite identification study by LC-MS-MS will be carried out.

Methodology
For human CLint determination, the first-order rate constant for consumption of substrate at one concentration (1-10mM) in the presence of mice or human liver microsomes (0.5-1.0 mg protein/mL; normally pooled from 10 subjects and kept frozen at – 80 ºC; commercial source: e.g. GENTEST) is measured in the presence of the cofactor NADPH. The reaction is carried out at 37 °C in 0.1 M phosphate buffer pH 7.4 for 30 min while aliquots were taken for at least 5 time points. The samples will be analyzed by HPLC or LC-MS-MS for the loss of substrate and by LC-MS-MS for metabolite identification. The CLint is calculated by the formula CLint = (0.693/in vitro T1/2) x (mL incubation/mg microsomes). This task will be developed jointly by the iMed.UL (R Moreira/A Sitoe/A Santana/J Ferreira) and CNC (M Batista) teams. Both teams have proofed experience in using LC-MS-MS as identification and analytical tool, as well as in the evaluation of metabolic susceptibility of leads towards metabolic stability [12]. Both iMed.UL's triple quad and CNC's ion trap equipments will be used in analyzing the loss of substrate in the incubation mixture. The ion trap MS at CNC will provide additional capabilities in terms of structural identification (LC-MS-MSn) of metabolites. Both equipments are part of National Network of Mass Spectrometry and thus, exchange of samples, methods and personnel are already performed on a regular basis.

Expected results
The results from this task will determine, together with those of Task 4, which hits discovered in Task 2 will be delivered for in vivo studies and those which will be considered for structural modification to impart metabolic administration. For drugs that are foreseen for oral administration, microsomal CLint should be low. A medium clearance (6.5 to 35 mL/min/mg protein) leads to an expected bioavailability higher than 30%, considering the hepatic clearance is the major mechanism of clearance.

Membros da equipa de investigação nesta tarefa
Members of the research team in this task
Ana Bela Fernandes Santana; ANA RAQUEL FERNANDES SITOE; João Pedro Gomes Roque Borges Ferreira; Maria Teresa Pereira Marques Batista; Rui Ferreira Alves Moreira;

Designação da tarefa Task denomination	**Data de início** Start date	**Data de fim** End date	**Duração** Duration	**Pessoas * mês** Person * months
Proof-of-concept: in vivo activity	01-09-2013	31-08-2014	12	11

Descrição da tarefa e Resultados Esperados
Task description and Expected results
Objectives
In this task the ability of the compounds to inhibit infection of liver cells by Plasmodium in vivo will be evaluated. This will establish their efficacy as liver stage antimalarials and will provide important information about their bioavailability on the context of hepatic infection. This task will be performed at Mota's and Prudêncio's labs (consultants from IMM) by A Cruz and BI Fellow

Methodologies
(i) Liver-stage assay. The in vivo effect of the compounds tested will be assessed by measuring liver parasite loads of rodent models of malaria infected with P. berghei. Infection determinations will be carried out by two alternative methods: i) quantitative real-time PCR of liver homogenates employing Plasmodium-specific primers or ii) luminescence measurement following luciferin injection into mice infected with luciferase-expressing P. berghei parasites [26]. In the latter case, the same groups of mice can be used to monitor appearance of parasites in the blood and disease progress. Compounds will be administered to mice either by intra-peritoneal injection or by oral gavage. Intra-peritoneal injection will be used in clearance assays, where a high-dose of compound will be administered 3-6 hours after parasite injection. Oral gavage will be used to assess the prophylactic potential of the compounds, which will be administered at defined intervals prior to infection. Parasite delivery will be carried out either by intravenous injection of isolated Plasmodium sporozoites or by mosquito bite. Liver infection loads will be measured 42-44 hours after infection.

(ii) Blood-stage assay. Selected compounds will be evaluated for their in vivo blood schizonticidal activity. Mice will be infected by intraperitoneal injection of infected red blood cells and blood parasitemia will be allowed to increase to 2-3%, upon which treatment will be initiated by daily subcutaneous or oral administration of the compounds. Different compound doses in the four-day suppressive test, in which the efficacy of four daily doses of compounds is measured by comparison of blood parasitaemia and mouse survival time in treated and untreated mice [1]. Efficacies will also be compared with that of the parent peroxide or equimolar mixtures of the peroxide and liver-stage-acting parent compound. Infection progression will be followed by daily monitoring of parasitemia (flow cytometry) and disease symptoms.

Expected results
Efficacy of lead compounds against both the liver- and blood-stages, following different administration routes, in vivo will be inferred. ED50 values will allow selection of the best candidates.

This task requires one full-time student (BI) that will be also involved in the in vitro testing of the chemical libraries (Task 5).

Membros da equipa de investigação nesta tarefa
Members of the research team in this task
(BI) Bolseiro de Investigação (Lic. ou Bacharel) 1; Ana Filipa Pintéus da Cruz;

Designação da tarefa Task denomination	**Data de início** Start date	**Data de fim** End date	**Duração** Duration	**Pessoas * mês** Person * months
Hematological toxicity	01-03-2014	28-02-2015	12	7,5

Descrição da tarefa e Resultados Esperados
Task description and Expected results
Objectives
In this task we will test selected lead compounds for their direct and metabolism-activated hematological toxicity, and compare with that of primaquine. Selected hybrid compounds will be incubated with human erythrocytes and liver microsomes to generate potential hemotoxic metabolites which might interact with erythrocytes. Methemoglobin and reactive oxygen species (ROS) will be determined as markers of hemotoxicity [29]. This task is performed at iMed.UL by A Sitoe and F Lopes

Methodologies
(i) Methemoglobin formation. A reaction mixture containing pooled erythrocytes (in phosphate buffer saline with glucose, PBSG, for 50% hematocrit), NADPH regeneration cocktail, pooled liver microsomes and the drug will be incubated at 37 ºC in a shaking water bath. Controls without compound and without microsomes will also be used. After 1h, the reaction mixtures will be chilled on ice and centrifuged. The supernatants will be removed, the erythrocyte pellets lysed and the methemoglobin levels measured spectrophotometrically using a potassium ferricyanide/cyanide assay.

(ii) Reactive oxygen species formation. The formation of ROS will be monitored in real-time with a fluorescent probe (2,7-dichlorofluorescein diacetate, DCFDA) using a flat bottom 96 well microplate. A mixture of pooled human erythrocytes (in PBSG, 10% hematocrit) and DCFDA in DMSO will be incubated at 37 ºC for 20 min. and centrifuged. The resulting pellet of DCFDA loaded erythrocytes will be suspended in PBSG to a 50% hematocrit. This suspension will be mixed with NADPH regeneration cocktail and pooled liver microsomes. Controls without drug will be used. The plate will be placed in a microplate reader for kinetic measurement of fluorescence (excitation 488 nm and emission 535 nm).

(iii) Dependence on CYP isoforms. In those cases metabolism-activated hematological toxicity is observed, the contribution of the most usual CYP isoforms to the toxicity will be assessed by using selective CYP inhibitors, such as thiotepa (CYP2B6), fluoxetine (CYP2D6) and troleandomycin (CYP3A4).

Expected results
A safety profile for hybrid candidate molecules will be determined. The results obtained from this task will be fundamental for the decision-making regarding the compound selected for pre-clinical studies following the completion of this project.
Membros da equipa de investigação nesta tarefa
Members of the research team in this task
ANA RAQUEL FERNANDES SITOE; Francisca Conceição Lopes;

3.2.4. Calendarização e Gestão do Projecto
3.2.4. Project Timeline and Management

3.2.4.a Descrição da Estrutura de Gestão
3.2.4.a Description of the Management Structure

Scientific management
The project will be managed via a Scientific Board headed by the RI, comprising all scientists holding a PhD degree, including the consultants (MM Mota, M Prudêncio and P Rosenthal for the screening, and J Morais for the ADME).
Internal collaboration within the project network will be fostered through (i) monthly meetings between chemistry teams from iMed.UL and CNC in the first year to discuss the methodologies being developed for the chemical libraries (ii) annual meetings of Scientific Board to discuss results and planning future work, (iii) meetings between the synthesis and screening teams each 2-3 months; these meetings will be converted into conference calls with P Rosenthal; (iv) exchange of team members associated to Task 4 (metabolic stability) and the National Network of Mass Spectrometry to make use of the different capabilities of the triple quad (iMed.UL) and ion trap (CNC) in the metabolism studies.

Financial management

The project will be run through the accounting department of the Faculty of Pharmacy, which is will prepare all the financial reports. The Faculty of Pharmacy currently runs several national and European research projects.

3.2.4.b Lista de Milestones
3.2.4.b Milestone List

Data Date	**Designação da milestone** Milestone denomination
01-03-2013	Chemical Libraries

Descrição
Description
Synthesis and full characterization, including purity, of the two first chemical libraries

Data Date	**Designação da milestone** Milestone denomination
01-09-2013	Proof-of-concept: in vitro activities and ADME prediction

Descrição
Description
(i) Structure-activity relationships resulting from in vitro screenings and predictive ADME studies; (ii) Selection of hit compounds for further optimization; (iii) Selection of compounds for in vivo studies

Data Date	**Designação da milestone** Milestone denomination

01-03-2014	Proof-of-concept: in vivo activity

Descrição
Description

(i) Efficacy data against the blood- and liver-stages using appropriate animal models; (ii) Selection of lead compounds to enter toxicity studies

Data Date	**Designação da milestone** Milestone denomination
28-02-2015	Selection of lead candidates

Descrição
Description

Selection of one candidate and one backup compound for pre-clinical studies. This selection will be based on in vivo data as well as on the hematological toxicity studies.

3.2.4.c Cronograma
3.2.4.c Timeline

Ficheiro com a designação "timeline.pdf", no 9. Ficheiros Anexos, desta Visão Global (caso exista).
File with the name "timeline.pdf" at 9. Attachments (if exists).

3.3. Referências Bibliográficas
3.3. Bibliographic References

Referência Reference	**Ano** Year	**Publicação** Publication
1	2004	**Fidock DA; Rosenthal PJ; Croft SL; Brun R; Nwaka S Antimalarial drug discovery: efficacy models for compound screening. Nat Rev Drug Discov 2004, 3, 509-520.**
2	2010	**Gamo FJ; Sanz LM; Vidal J; Cozar C; Alvarez E; Lavandera JL; Vanderwall DE; Green DVS; Kumar V; Hasan S; Brown JR; Peishoff CE; Cardon LR; Garcia-Bustos JF Thousands of chemical starting points for antimalarial lead identification. Nature 2010, 465, 305-310**
3	2010	**Guiguemde WA; Shelat AA; Bouck D; Duffy S; Crowther G.J; Davis PH; Smithson DC; Connelly M; Clark J; Zhu F; Jiménez-Díaz MB; Martinez MS; Wilson EB; Tripathi AK; Gut J; Sharlow ER; Bathurst I; El Mazouni F; Fowble JW; Forquer I; McGinley PL; Castro S; Angulo-Barturen I; Ferrer S; Rosenthal PJ; DeRisi JL; Sullivan Jr DJ; Lazo JS; Roos DS; Riscoe MK; Phillips MA; Rathod PK; Van Voorhis WC; Avery VM; Guy RK Chemical genetics of Plasmodium falciparum. Nature 2010, 465, 311-315**
4	2011	**Rodrigues T; Moreira R; Lopes F New hope in the fight against malaria? Future Med. Chem. 2011, 3, 1–3**
5	2011	**Alonso PL; Brown G; Arevalo-Herrera M; Binka F; Chitnis C; Collins F; Doumbo OK; Greenwood B; Hall BF; Levine MM; Mendis K; Newman RD; Plowe CV; Rodriguez MH; Sinden R; Slutsker L; Tanner M A Research Agenda to Underpin Malaria Eradication. PloS Med 2011, 8, e1000406**
6	2011	**The malERA Consultative Group on Drugs. A Research Agenda for Malaria Eradication: Drugs. PloS Med 2011, 8, e1000402**
7	2010	**Wells TNC; Burrows JN; Baird JK Targeting the hypnozoite reservoir of Plasmodium vivax: the hidden obstacle to malaria elimination. Trends Parasitol. 2010, 26, 145-151**
8	2006	**Tekwani BL; Walker LA 8-Aminoquinolines: future role as antiprotozoal drugs. Curr Opin Infect Dis 2006, 19, 623–631**
9	2009	**Vale N; Moreira R; Gomes P Primaquine revisited six decades after its discovery. Eur. J. Med. Chem. 2009, 44, 937-953 and references cited herein**
10	1983	**Schmidt LH Relationships between chemical structures of 8-aminoquinolines and their capacities for radical cure of infections with Plasmodium cynomolgi in Rhesus monkeys. Antimicrob. Agents Ch. 1983, 24, 615-652**
11	2010	**Kaur K; Jain M; Reddy RP; Jain R Quinolines and structurally related heterocycles as antimalarials. Eur. J. Med. Chem. 2010, 45, 3245-3264**
12	2005	**Araújo MJ.; Bom J; Capela R; Casimiro C; Chambel P; Gomes P; Iley J; Lopes F; Morais J; Moreira R; de Oliveira E; do Rosário V; Vale N Imidazolidin-4-one derivatives of primaquine as novel transmission-blocking antimalarials. J. Med. Chem. 2005, 48, 888-892**
13	2008	**Vale N; Matos J; Gut J; Nogueira F; do Rosário V; Rosenthal PJ; Moreira R; Gomes P Imidazolidin-4-one peptidomimetic derivatives of primaquine: synthesis and antimalarial activity. Bioorg. Med. Chem. Lett. 2008, 18, 4150-4153**
14	2009	**Vale N; Nogueira F; do Rosário V; Gomes P; Moreira R Primaquine dipeptide derivatives bearing an imidazolidin-4-one moiety at the N-terminus as potential antimalarial prodrugs. Eur. J. Med. Chem. 2009, 44, 2506-2516**

15	2009	**Vale N; Prudêncio M; Marques CA; Collins MS; Gut J; Nogueira F; Matos J; Rosenthal PJ; Cushion MT; do Rosário V; Mota MM; Moreira R; Gomes P Imidazoquines as antimalarial and anti-pneumocystis agents. J. Med. Chem. 2009, 52, 7800-7807**
16	2003	**Mahmoudi N; Ciceron L; Franetich JF; Farhati K; Silvie O; Eling W; Sauerwein R; Danis M; Mazier D; Derouin F In vitro activities of 25 quinolones and fluoroquinolones against liver and blood stage Plasmodium spp. Antimicrob. Agents Ch. 2003, 47, 2636–2639**
17	2008	**Mahmoudi N; Garcia-Domenech R; Galvez J; Farhati K; Franetich JF; Sauerwein R; Hannoun L; Derouin F; Danis M; Mazier D New active drugs against liver stages of Plasmodium predicted by molecular topology. Antimicrob. Agents Ch. 2008, 52, 1215–1220**
18	2008	**Cunha-Rodrigues M; Portugal S; Prudêncio M; Gonçalves LA; Casalou C; Buger D; Sauerwein R; Haas W; Mota MM Genistein-Supplemented Diet Decreases Malaria Liver Infection in Mice and Constitutes a Potential Prophylactic Strategy. PLoS ONE 2008, 3, e2732**
19	2010	**Rodrigues T Novel mitochondrial electron transport-chain inhibitors as potential antimalarial agents. PhD thesis, University of Lisbon, 2010**
20	2009	**Capela R; Oliveira R; Moreira R; Gonçalves L; Domingos A; Gut J; Rosenthal PJ; Lopes F Artemisinin-dipeptidyl vinylsulfone hybrid molecules: design, synthesis and preliminary SAR for antiplasmodial activity and falcipain-2 inhibition. Bioorg. Med. Chem. Lett. 2009, 19, 3229-3232**
21	2008	**Meunier B Hybrid molecules with a dual mode of action: dream or reality? Acc. Chem.Res. 2008, 41, 69-77**
22	2010	**Matos J; Vale N; Collins MS; Gut J; Rosenthal PJ; Cushion MT; Moreira R; Gomes P Primacenes: novel non-cytotoxic primaquine-ferrocene conjugates with anti-Pneumocystis carinii activity. MedChemComm. 2010, 1, 199-201**
23	2010	**O'Neill P; Amewu R; Nixon G; ElGarah FB; Mungthin M; Chadwick J; Shone A; Vivas L; Lander H; Barton V; Muangnoicharoen S; Bray P; Davies J; Park B; Wittlin S; Brun R; Preschel M; Zhang K; Ward S Identification of a 1,2,4,5-tetraoxane antimalarial drug-development candidate (RKA182) with superior properties to the semisynthetic artemisinins. Angew. Chem. Int. Ed. 2010, 49, 5693-5697**
24	2010	**Verissimo E; Gibbons P; Araujo N; Cristiano MLS; Rosenthal PJ; Gut J; Moreira R; Guedes RC; O'Neill PM; Design, Synthesis, Antimalarial Activity and Modelling Studies of Novel Dipeptidyl Ketone Inhibitors of Falcipain 2/3 J. Med. Chem. 2010, 53, 8202–8206**
25	2011	**Schiaffo CE; Rottman M; Wittlin S; Dussault PH 3-Alkoxy-1,2-dioxolanes: synthesis and evaluation as potential antimalarial agents. ACSMed. Chem. Lett. 2011 doi: 10.1021/ml100308d**
26	2009	**Ploemen IHJ; Prudêncio M; Douradinha BG; Ramesar J; Fonager J; van Gemert GJ; Luty AJF.; Hermsen CC; Sauerwein RW; Baptista FG; Mota MM; Waters AP; Que I; Lowik CWGM; Khan SM; Janse CJ; Franke-Fayard BMD Visualisation and quantitative analysis of the rodent malaria liver stage by real time imaging. PLoS ONE 2009, 4, e7881**
27	2004	**. Nassar AEF; Kamel AM; Caroline C Improving the decision-making process in the structural modification of drug candidates: enhancing metabolic stability. Drug Discov. Today 2004, 9, 1020-1028**
28	2010	**Paixão P; Gouveia LF; Morais JAG Prediction of the in vitro permeability determined in Caco-2 cells by using artificial neural networks. Eur. J. Pharm. Sci. 2010, 41, 107-11**
29	2009	**Ganesan S; Tekwani BL; Sahu R; Tripathi LM; Walker LA Cytochrome P450-dependent toxic effects of primaquine on human erythrocytes Toxicol. Appl. Pharmacol. 2009, 241, 14-22**
30	2008	**Prudêncio M; Rodrigues CD; Hannus M; Martin C; Real E; Gonçalves LA; Carret C; Dorkin R ; Rohl I; Jahn-Hoffmann K; Luty AJF; Sauerwein R; Echeverri CJ; Mota MM Kinome-Wide RNAi Screen Implicates at Least 5 Host Hepatocyte Kinases in Plasmodium Sporozoite Infection. PLoS Pathog 2008, 4, e1000201**

3.4. Publicações Anteriores
3.4. Past Publications

Referência Reference	Ano Year	Publicação Publication
20	2009	**Capela R; Oliveira R; Gonçalves L; Domingos A; Gut J; Rosenthal PJ; Lopes F; Moreira R Artemisinin-dipeptidyl vinylsulfone hybrid molecules: design, synthesis and preliminary SAR for antiplasmodial activity and falcipain-2 inhibition. Bioorg. Med. Chem. Lett. 2009, 19, 3229-3232**

12	2005	**Araújo MJ.; Bom J; Capela R; Casimiro C; Chambel P; Gomes P; Iley J; Lopes F; Morais J; Moreira R; de Oliveira E; do Rosário V; Vale N Imidazolidin-4-one derivatives of primaquine as novel transmission-blocking antimalarials. J. Med. Chem. 2005, 48, 888-892**
24	2010	**Verissimo E; Gibbons P; Araujo N; Cristiano MLS; Rosenthal PJ; Gut J; Moreira R; Guedes RC; O'Neill PM; Design, Synthesis, Antimalarial Activity and Modelling Studies of Novel Dipeptidyl Ketone Inhibitors of Falcipain 2/3 J. Med. Chem. 2010, 53, 8202–8206**
31	2009	**Rodrigues T; Guedes RC; Santos D; Gut J; Rosenthal PJ; Moreira R; Lopes F Design, synthesis and structure activity-relationships of (1H-pyridin-4-ylidene)amines as potential antimalarials. Bioorg. Med. Chem. Lett. 2009, 19, 3476–3480**
15	2009	**Vale N; Prudêncio M; Marques CA; Collins MS; Gut J; Nogueira F; Matos J; Rosenthal PJ; Cushion MT; do Rosário V; Mota MM; Moreira R; Gomes P Imidazoquines as antimalarial and anti-pneumocystis agents. J. Med. Chem. 2009, 52, 7800-7807.**

4. Equipa de investigação
4. Research team

-

4.1 Lista de membros
4.1. Members list

Nome Name	Função Role	Grau académico Academic degree	%tempo %time	CV nuclear Core CV
Francisca Conceição Lopes	Inv. Responsável	DOUTORAMENTO	50	✓
Ana Bela Fernandes Santana	Investigador	DOUTORAMENTO	25	X
Ana Filipa Pintéus da Cruz	Investigador	DOUTORAMENTO	15	X
ANA RAQUEL FERNANDES SITOE	Investigador		50	X
Andreia de Almeida Rosatella	Investigador	DOUTORAMENTO	15	X
Carlos Alberto Mateus Afonso	Investigador	AGREGAÇÃO	15	X
Daniela Filipa Pintassilgo Miranda	Investigador	LICENCIATURA	50	X
João Pedro Gomes Roque Borges Ferreir...	Investigador	MESTRADO	25	X
Maria Luisa Campeão Fernandes Vaz Sá ...	Investigador	AGREGAÇÃO	15	X
Maria Manuel da Cruz Silva	Investigador	DOUTORAMENTO	15	X
Maria Margarida André Oliveira Estuda...	Investigador	LICENCIATURA	15	X
Maria Teresa Pereira Marques Batista	Investigador	DOUTORAMENTO	15	X
Rita Sofia Salvador Simões Capela	Investigador	MESTRADO	15	X
Rui Ferreira Alves Moreira	Investigador	AGREGAÇÃO	15	✓

(O curriculum vitae de cada membro da equipa está disponível clicando no nome correspondente)
(Curriculum vitae for each research team member is available by clicking on the corresponding name)
Total: 14

4.2. Lista de membros a contratar durante a execução do projecto
4.2. Members list to hire during project"s execution

Membro da equipa Team member	Função Role	Duração Duration	%tempo %time
(BI) Bolseiro de Investigação (Lic. ou Bacharel) 1	Bolseiro	30	100
(BI) Bolseiro de Investigação (Mestre) 1	Bolseiro	36	100

Total: 2

5. Outros projectos
5. Other projects

-

5.1. Projectos financiados
5.1. Funded projects

(Sem projectos financiados)
(No funded projects)

5.2. Candidaturas similares
5.2. Similar applications

(Sem Candidaturas Similares)
(No Similar applications)

6. Indicadores previstos
6. Expected indicators

-

Indicadores de realização previstos para o projecto
Expected output indicators

Descrição	2011	2012	2013	2014	2015	Total

Description						
A - Publicações Publications						
Livros Books	0	0	0	0	0	**0**
Artigos em revistas internacionais Papers in international journals	0	2	2	2	0	**6**
Artigos em revistas nacionais Papers in national journals	0	0	0	0	0	**0**
B - Comunicações Communications						
Comunicações em encontros científicos internacionais Communications in international meetings	0	2	2	3	0	**7**
Comunicações em encontros científicos nacionais Communications in national meetings	0	1	1	1	0	**3**
C - Relatórios Reports	0	1	1	1	0	**3**
D - Organização de seminários e conferências Organization of seminars and conferences	0	0	0	0	0	**0**
E - Formação avançada Advanced training						
Teses de Doutoramento PhD theses	0	0	1	1	0	**2**
Teses de Mestrado Master theses	0	0	1	1	0	**2**
Outras Others	0	0	0	0	0	**0**
F - Modelos Models	0	0	0	0	0	**0**
G - Aplicações computacionais Software	0	0	0	0	0	**0**
H - Instalações piloto Pilot plants	0	0	0	0	0	**0**
I - Protótipos laboratoriais Prototypes	0	0	0	0	0	**0**
J - Patentes Patents	0	0	0	0	0	**0**
L - Outros Other						

Acções de divulgação da actividade científica
Scientific activity spreading actions

(i) Communication with wider audiences is also envisioned to introduce the topics related with the scientific area (malaria, chemistry applied to medicine, pharmaceutical sciences). Particular attention will be given to simple workshops targeting students from high schools and undergraduates, organized with different levels of scientific information (from simple information on the disease to more research-oriented workshops). In addition, one-week laboratory courses in the laboratories of iMed-UL will be offered to students from high schools.
(ii) Organization of open one-day conferences on recent advances in drug discovery of antimalarials, opened to scientists and health professionals and involving national and international invited speakers, are planned to take place at iMed.UL or IMM in each year of the project. Both IMM and iMed.UL have experience in organizing meetings and good contacts with several emeritus scientists.
(i) The core teams are members of the Iberian Platform on Malaria, which organizes regular biannual meetings in Portugal and Spain which joins the community involved in malaria research. M Mota is organizing the 2011 meeting.

7. Orçamento
7. Budget

-

Instituição Proponente
Principal Contractor

Faculdade de Farmácia da Universidade de Lisboa

Descrição Description	**2011**	**2012**	**2013**	**2014**	**2015**	**Total**
Recursos Humanos Human resources	0,00	10.890,00	13.150,00	13.224,00	2.208,00	**39.472,00**
Missões Missions	0,00	800,00	1.600,00	2.400,00	800,00	**5.600,00**
Consultores Consultants	0,00	0,00	0,00	0,00	0,00	**0,00**

Aquisição de bens e serviços Service procurement and acquisitions	0,00	12.000,00	20.000,00	21.000,00	3.000,00	**56.000,00**
Registo de patentes Patent registration	0,00	0,00	0,00	0,00	0,00	**0,00**
Adaptação de edifícios e instalações Adaptation of buildings and facilities	0,00	0,00	0,00	0,00	0,00	**0,00**
Gastos gerais Overheads	0,00	4.730,00	6.950,00	7.325,00	1.202,00	**20.207,00**
TOTAL DESPESAS CORRENTES TOTAL CURRENT EXPENSES	**0,00**	**28.420,00**	**41.700,00**	**43.949,00**	**7.210,00**	**121.279,00**
Equipamento Equipment	0,00	20.000,00	0,00	0,00	0,00	**20.000,00**
Total	**0,00**	**48.420,00**	**41.700,00**	**43.949,00**	**7.210,00**	**141.279,00**

Instituições Participantes
Participating Institutions

Centro de Neurociências e Biologia Celular

Descrição Description	**2011**	**2012**	**2013**	**2014**	**2015**	**Total**
Recursos Humanos Human resources	0,00	0,00	0,00	0,00	0,00	**0,00**
Missões Missions	0,00	0,00	0,00	0,00	0,00	**0,00**
Consultores Consultants	0,00	0,00	0,00	0,00	0,00	**0,00**
Aquisição de bens e serviços Service procurement and acquisitions	0,00	2.000,00	2.000,00	1.000,00	0,00	**5.000,00**
Registo de patentes Patent registration	0,00	0,00	0,00	0,00	0,00	**0,00**
Adaptação de edifícios e instalações Adaptation of buildings and facilities	0,00	0,00	0,00	0,00	0,00	**0,00**
Gastos gerais Overheads	0,00	200,00	200,00	100,00	0,00	**500,00**
TOTAL DESPESAS CORRENTES TOTAL CURRENT EXPENSES	**0,00**	**2.200,00**	**2.200,00**	**1.100,00**	**0,00**	**5.500,00**
Equipamento Equipment	0,00	0,00	0,00	0,00	0,00	**0,00**
Total	**0,00**	**2.200,00**	**2.200,00**	**1.100,00**	**0,00**	**5.500,00**

Instituto de Medicina Molecular

Descrição Description	**2011**	**2012**	**2013**	**2014**	**2015**	**Total**
Recursos Humanos Human resources	0,00	3.416,00	10.332,00	10.404,00	1.738,00	**25.890,00**
Missões Missions	0,00	0,00	0,00	0,00	0,00	**0,00**
Consultores Consultants	0,00	0,00	0,00	0,00	0,00	**0,00**
Aquisição de bens e serviços Service procurement and acquisitions	0,00	1.000,00	8.000,00	5.000,00	1.000,00	**15.000,00**
Registo de patentes Patent registration	0,00	0,00	0,00	0,00	0,00	**0,00**
Adaptação de edifícios e instalações Adaptation of buildings and facilities	0,00	0,00	0,00	0,00	0,00	**0,00**
Gastos gerais Overheads	0,00	442,00	1.833,00	1.541,00	274,00	**4.090,00**
TOTAL DESPESAS CORRENTES TOTAL CURRENT EXPENSES	**0,00**	**4.858,00**	**20.165,00**	**16.945,00**	**3.012,00**	**44.980,00**
Equipamento Equipment	0,00	0,00	0,00	0,00	0,00	**0,00**
Total	**0,00**	**4.858,00**	**20.165,00**	**16.945,00**	**3.012,00**	**44.980,00**

Orçamento Global
Global budget

Descrição Description	**2011**	**2012**	**2013**	**2014**	**2015**	**Total**
Recursos Humanos Human resources	0,00	14.306,00	23.482,00	23.628,00	3.946,00	**65.362,00**
Missões Missions	0,00	800,00	1.600,00	2.400,00	800,00	**5.600,00**
Consultores Consultants	0,00	0,00	0,00	0,00	0,00	**0,00**
Aquisição de bens e serviços Service procurement and acquisitions	0,00	15.000,00	30.000,00	27.000,00	4.000,00	**76.000,00**
Registo de patentes Patent registration	0,00	0,00	0,00	0,00	0,00	**0,00**
Adaptação de edifícios e instalações Adaptation of buildings and facilities	0,00	0,00	0,00	0,00	0,00	**0,00**
Gastos gerais Overheads	0,00	5.372,00	8.983,00	8.966,00	1.476,00	**24.797,00**
TOTAL DESPESAS CORRENTES TOTAL CURRENT EXPENSES	**0,00**	**35.478,00**	**64.065,00**	**61.994,00**	**10.222,00**	**171.759,00**
Equipamento Equipment	0,00	20.000,00	0,00	0,00	0,00	**20.000,00**
Total	**0,00**	**55.478,00**	**64.065,00**	**61.994,00**	**10.222,00**	**191.759,00**

Plano de financiamento
Finance plan

Descrição Description	**2011**	**2012**	**2013**	**2014**	**2015**	**Total**
Financiamento solicitado à FCT Requested funding	0,00	55.478,00	64.065,00	61.994,00	10.222,00	**191.759,00**
Financiamento próprio Own funding	0,00	0,00	0,00	0,00	0,00	**0,00**
Outro financiamento público Other public-sector funding	0,00	0,00	0,00	0,00	0,00	**0,00**
Outro financiamento privado Other private funding	0,00	0,00	0,00	0,00	0,00	**0,00**
Total do Projecto Total of the project	**0,00**	**55.478,00**	**64.065,00**	**61.994,00**	**10.222,00**	**191.759,00**

8. Justificação do orçamento
8. Budget rationale

-

8.1. Justificação dos recursos humanos
8.1. Human resources rationale

Tipo
Type
(BI) Bolsa de Investigação (Mestre)

Nº de pessoas
No. of persons
1

Duração (em meses)
Duration (in months)
36

Custo envolvido (€) *(calculado)*
Total cost (€) *(estimated)*
35.280,00

Outros custos (€)
Other costs (€)
4.192,00

Justificação do financiamento solicitado
Rationale for requested funding

A BI Fellowship for one skilled researcher at full time will be required for a 36 month period in order to achieve the proposed goals. Her/he will be fully involved in the synthesis of chemical libraries, hit-to-lead optimization and scale-up synthesis of lead compounds. The funding requested to FCT includes monthly values of the grant,according to FCT rates, plus the social security insurance (€4192).

Tipo
Type
(BI) Bolsa de Investigação (Lic. ou Bacharel)

Nº de pessoas
No. of persons
1

Duração (em meses)
Duration (in months)
30

Custo envolvido (€) *(calculado)*
Total cost (€) *(estimated)*
22.350,00

Outros custos (€)
Other costs (€)
3.540,00

Justificação do financiamento solicitado
Rationale for requested funding

One BI fellowships for one skilled researcher at full time will be required for a 30 month period in order to achieve the proposed
goals for in vitro screening and in vivo assays. The funding requested to FCT includes monthly values of the grant according to FCT rates, plus the social security insurance (€3540).

8.2. Justificação de missões
8.2. Missions rationale

Tipo
Type
Participação em congressos

Nº de deslocações
No. of participations
7

Local
Venue
International Scientific MedChem Meetings

Custo envolvido (€)
Cost (€)
5.600,00

Justificação do financiamento solicitado
Rationale for requested funding
The results of this project will be presented in major international scientific meetings such as the International Symposium in Medicinal Chemistry and the American Chemical Society Meetings. It is envisaged that the members of iMed.UL team will participate in two/three international conferences per year. The requested funding will be used for registration fees, flight tickets
(when necessary) and accommodation (€800/meeting).

8.3. Justificação de consultores
8.3. Consultants rationale

Nome completo
Full name
Maria Manuel Dias da Mota
Instituição
Institution
Malaria Unit of the Instituto de Medicina Molecular (UMA-IMM)
Fase do projecto
Project phase
Tasks 2 and 5
Custo (€)
Cost (€)
0,00
Justificação do financiamento solicitado
Rationale for requested funding
Responsible for the UMA-IMM, with an outstanding experience in the biology of malaria parasites, particularly the liver stage
Página na Internet onde pode ser consultado o CV do consultor
Web page where the consultant's CV can be accessed
(Vazio)
(Void)

Nome completo
Full name
Miguel Prudêncio
Instituição
Institution
Malaria Unit of the Instituto de Medicina Molecular (UMA-IMM)
Fase do projecto
Project phase
Tasks 2 and 5
Custo (€)
Cost (€)
0,00
Justificação do financiamento solicitado
Rationale for requested funding
Responsible for the liver-stage assays at UMA-IMM.
Página na Internet onde pode ser consultado o CV do consultor
Web page where the consultant's CV can be accessed
(Vazio)
(Void)

Nome completo
Full name
José Augusto Guimarães Morais
Instituição
Institution
iMed.UL
Fase do projecto
Project phase
Task 4
Custo (€)
Cost (€)
0,00
Justificação do financiamento solicitado
Rationale for requested funding
Expert in Pharmacokinetics and in silico studies of ADMET
Página na Internet onde pode ser consultado o CV do consultor
Web page where the consultant's CV can be accessed
(Vazio)
(Void)

Nome completo
Full name
Philip J. Rosenthal
Instituição
Institution
Deptartment of Medicine, University of California, San Francisco

Fase do projecto	**Custo (€)**
Project phase	Cost (€)
Task 2	0,00

Justificação do financiamento solicitado
Rationale for requested funding
Philip Rosenthal plays a crucial role in the screening against the blood-stage of malaria parasites. F Lopes and R Moreira have long standing collaboration with P Rosenthal that resulted in a large number of papers in high ranking journals
Página na Internet onde pode ser consultado o CV do consultor
Web page where the consultant's CV can be accessed
http://www.ff.ul.pt/FCT/PTDC/SAU-FAR/118459/2010/NIH_biosketch_Rosenthal.pdf

8.4. Justificação de aquisição de bens e serviços
8.4. Service procurement and acquisitions

Tipo	**Custo (€)**
Type	Cost (€)
Structural characterization of compounds	10.000,00

Justificação do financiamento solicitado
Rationale for requested funding
For the total characterization of compounds prepared (Task 1) it will be necessary to use a 400 MHz NMR equipment that will be available in 2012 at iMed-UL and users will need to contribute to NMR liquid helium and nitrogen consumable (5,000 euros). Elemental analysis, high-resolution mass spectrometry and x-ray crystallography will be done at the unity of mass spectroscopy (Santiago de Compostela, Spain). The total requested for these services is 5,000 euros.

Tipo	**Custo (€)**
Type	Cost (€)
Consumables for synthetic chemistry	28.000,00

Justificação do financiamento solicitado
Rationale for requested funding
The synthetic goals of Task 1 (36 months; 60-80 compounds) have high demands on the quantity and quality of starting materials, solvents (anhydrous, analytical grade or higher) and chromatographic supports for purification of compounds when necessary (bulk silica gel, alumina and RP-C18 adsorbents for column and preparative chromatography). In addition, nitrogen and argon will be used for reactions in dry atmosphere, and oxygen will be needed for the trioxolane synthesis. The total estimated cost is 28,000 euros.

Tipo	**Custo (€)**
Type	Cost (€)
Consumables for LC-MS	10.000,00

Justificação do financiamento solicitado
Rationale for requested funding
Task 3 and Task 4 require heavy use of HPLCs and LC-MS-MS, and thus ultra-high purity solvents and adequate columns will be needed. A total 0f 10,000 euros is requested

Tipo	**Custo (€)**
Type	Cost (€)
In vitro ADME	10.000,00

Justificação do financiamento solicitado
Rationale for requested funding
Both mice and human microsomes will be needed for Tasks 3 and 6. In addition for cell cultures required for Task 4, funds are needed to cover maintenance of mammalian cell cultures, transport substrates, inhibitors, culture media, aditives, sterile material and for the periodic maintenance and control of the laminar flow chamber workstation as well as the refrigerated centrifuge.

Tipo	**Custo (€)**
Type	Cost (€)
Maintenance of equipments	3.000,00

Justificação do financiamento solicitado
Rationale for requested funding
Some equipments, such has HPLCs and the LC-MS-MS will require maintenance during the period of this project. For this, a value of 3000 euros

Tipo	**Custo (€)**
Type	Cost (€)
In vitro screening and in vivo assays	15.000,00

Justificação do financiamento solicitado
Rationale for requested funding

Liver stage assays require sporozoites from mosquitoes. The insectary, based at IMM, is a fundamental piece for the success of the present proposal. This consists of a room as well as 2 independent incubators, all with highly controlled levels of temperature and humidity. their maintenance is crucial. Mice cost approximately 13 euros each (including transport) and each compound should be tested in at least 5-10 mice per group. Expected costs for in vitro and in vivo assays is 15,000 euros

8.6. Justificação do Equipamento
8.6. Equipment rationale

8.6.1. Equipamento já disponível para a execução do projecto
8.6.1 Available equipment

Tipo de equipamento Equipment type	**Fabricante** Manufacturer	**Modelo** Model	**Ano** Year
NMR spectrometer	Bruker	Avance 400	2004
Tipo de equipamento Equipment type	**Fabricante** Manufacturer	**Modelo** Model	**Ano** Year
LC-MS-MS	Waters	Micromass Quattro Micro	2007
Tipo de equipamento Equipment type	**Fabricante** Manufacturer	**Modelo** Model	**Ano** Year
Analytical HPLC system	Merck	Lachrom	2000
Tipo de equipamento Equipment type	**Fabricante** Manufacturer	**Modelo** Model	**Ano** Year
Analytical HPLC system	Merck	Lachrom Elite	2009
Tipo de equipamento Equipment type	**Fabricante** Manufacturer	**Modelo** Model	**Ano** Year
FTIR	Nicolet	Impact 400	1994
Tipo de equipamento Equipment type	**Fabricante** Manufacturer	**Modelo** Model	**Ano** Year
Parallel Synthesis	Heidolph	Synthesis1	2007
Tipo de equipamento Equipment type	**Fabricante** Manufacturer	**Modelo** Model	**Ano** Year
Preparative HPLC	VWR	LaPrep P110	2011
Tipo de equipamento Equipment type	**Fabricante** Manufacturer	**Modelo** Model	**Ano** Year
Microwave reactor	CEM	Labmate	2010
Tipo de equipamento Equipment type	**Fabricante** Manufacturer	**Modelo** Model	**Ano** Year
Voltimeter	Millipore	Millicell	2008
Tipo de equipamento Equipment type	**Fabricante** Manufacturer	**Modelo** Model	**Ano** Year
Laminar low hood	Holten	HVR246	2000
Tipo de equipamento Equipment type	**Fabricante** Manufacturer	**Modelo** Model	**Ano** Year
Inverted microscope	Olympus	CR2-TR	2000
Tipo de equipamento Equipment type	**Fabricante** Manufacturer	**Modelo** Model	**Ano** Year
LC-MS-MSn	Thermo Finnigan	Advantage Ion Max	2007
Tipo de equipamento Equipment type	**Fabricante** Manufacturer	**Modelo** Model	**Ano** Year
Preparative LC	Buchi	C-660 C-615 and C-605	2003
Tipo de equipamento Equipment type	**Fabricante** Manufacturer	**Modelo** Model	**Ano** Year
Incubator Shaker	New Brunswick Scientific	Classic C24	2002
Tipo de equipamento Equipment type	**Fabricante** Manufacturer	**Modelo** Model	**Ano** Year
Orbital incubators	Aralab	Agitorb	2000
Tipo de equipamento Equipment type	**Fabricante** Manufacturer	**Modelo** Model	**Ano** Year
Analytical HPLC system	Shimadzu	LC Solution 2010 CHT	2007
Tipo de equipamento Equipment type	**Fabricante** Manufacturer	**Modelo** Model	**Ano** Year
qRT-PCR	Applied Biosystems	7500 FAST	2005

Tipo de equipamento Equipment type	Fabricante Manufacturer	Modelo Model	Ano Year
qRT-PCR	Corbett Research	Rotorgene 6000	2006

Tipo de equipamento Equipment type	Fabricante Manufacturer	Modelo Model	Ano Year
Nanodrop	Thermo Scientific	ND-1000	2003

Tipo de equipamento Equipment type	Fabricante Manufacturer	Modelo Model	Ano Year
Fluorescence/Bioluminescence Imaging	Caliper LifeSciences	IVIS Lumina	2007

Tipo de equipamento Equipment type	Fabricante Manufacturer	Modelo Model	Ano Year
Widefield Fluorescence Microscope	Carl Zeiss MicroImaging	Axiovert 200M	2003

Tipo de equipamento Equipment type	Fabricante Manufacturer	Modelo Model	Ano Year
96 Well plate reader	Anthos labtec instruments	Zenyth 3100	2005

8.6.2. Discriminação do equipamento a adquirir
8.6.2. New equipment requested

Tipo de equipamento Equipment type	Fabricante Manufacturer	Modelo Model	Custo (€) Cost (€)
Contribution to the 400 NMR accessories 5 mm broad band probe, autosampler and low temperature controller	Bruker or Varian	Various	20.000,00

Justificação do financiamento solicitado
Rationale for requested funding

The NMR facility at University of Lisbon is currently equipped with a single 400 MHz instrument, which is being used by researchers from the Faculties of Pharmacy (iMed.UL) and Sciences. In order to boost the production of new compounds and expand the in-house chemical libraries, as well as to support all the ongoing synthetic chemistry research, iMed.UL is committed to purchase in 2011-12 the core 400 NMR equipment ready to basic work (approx. 215 Keuros) but without 5 mm broad band probe (56 Keuros), autosampler (14 Keuros) and low temperature controller (12 Keuros) (Total = 82 Keuros). The request of 20 Keuros aims the contribution to the acquisition of those accessories (remaining input budget to be supported by iMed.UL own resources during 2012-13).

8.7. Justificação de registo de patentes
8.7. Patent registration

(Vazio)
(Void)

8.8. Justificação de adaptação de edifícios e instalações
8.8. Adaptation of buildings and facilities

(Vazio)
(Void)

9. Ficheiros Anexos
9. Attachments

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Nome Name	Tamanho Size
Annex_1_PTDC_SAU_FAR_118459_2010.pdf	261Kb
Annex_2_PTDC_SAU_FAR_118459_2010.pdf	82Kb
Figures_PTDC_SAU_FAR_118459_2010.pdf	605Kb
timeline.pdf	25Kb

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