Amino Acids (2013) 45:1047–1053
DOI 10.1007/s00726-013-1575-0

MINIREVIEW ARTICLE

Chemical profiling of deoxyhypusine hydroxylase inhibitors for antimalarial therapy

Imke von Koschitzky · Annette Kaiser

Received: 18 June 2013 / Accepted: 2 August 2013 / Published online: 14 August 2013

Abstract A first approach to discover new antimalarials has been recently performed in a combined approach with data from GlaxoSmithKline Tres Cantos Antimalarial Set, Novartis-GNF Malaria Box Data set and St. Jude Children's Research Hospital. These data are assembled in the Malaria Box. In a first phenotypic forward chemical genetic approach, 400 chemicals were employed to eradicate the parasite in the erythrocytic stages. The advantage of phenotypic screens for the identification of novel chemotypes is that no a priori assumptions are made concerning a fixed target and that active compounds inherently have cellular bioavailability. In a first screen 40 mostly heterocyclic, highly active compounds (in nmol range of growth inhibition) were identified with EC_{50} values ≤ 2 μM against chloroquine-resistant *Plasmodium falciparum* strains and a therapeutic window ≥ 10 against two mammalian cell lines. 78 % of the compounds had no violations with the Lipinski Rule of 5 and only 1 % of the compounds showed cytotoxicity when applied at concentrations of 10 μM. This pre-selective step of parasitic eradication will be used further for a test of the Malaria Box with a potential in iron chelating capacity to inhibit deoxyhypusine hydroxylase (DOHH) from *P. falciparum* and *vivax*. DOHH, a metalloprotein which consists of ferrous iron and catalyzes the second step of the posttranslational modification at a specific lysine in eukaryotic initiation factor 5A (EIF-5A) to hypusine. Hypusine is a novel, non-proteinogenic amino acid, which is essential in eukaryotes and for parasitic proliferation. DOHH seems to be a "druggable" target, since it has only 26 % amino acid identity to its human orthologue. For a High-throughput Screening (HTS) of DOOH inhibitors, rapid and robust analytical tools are a prerequisite. A proteomic platform for the detection of hypusine metabolites is currently established. Ultra performance Liquid Chromatography enables the detection of hypusine metabolites with retention times of 7.4 min for deoxyhypusine and 7.3 min for hypusine. Alternatively, the analytes can be detected by their masses with gas chromatography/mass spectrometry or one-dimensional chromatography coupled to mass spectrometry. Moreover, the identified hits will be tracked further to test their efficacy in novel "in vitro assays". Subsequently in vivo inhibition in a humanized mouse model will be tested.

Keywords Deoxyhypusine hydroxylase · Phenotypic screening · DOHH inhibitors · Malaria · Hypusine

I. von Koschitzky · A. Kaiser (✉)
Institute for Pharmacogenetics, University of Duisburg-Essen, Hufelandstrasse 55, 45177 Essen, Germany
e-mail: annette.kaiser@uk-essen.de

Introduction

The Malaria Research and Eradication Agenda (MalERA) (malERA Consultative Group on Drugs et al. 2011) has recently defined the vision of the future antimalarial drug which should eradicate all developmental stages of the six different human malaria pathogenic strains in a single patient encounter independent of the severity and state of infection. During recent years, the spectrum of human pathogenic malaria parasites was extended. *Plasmodium knowlesi* was identified to proliferate in humans as a simian parasite (White 2008) and two sympatric globally spread subspecies of *Plasmodium ovale* occurred (Sutherland et al. 2010) which might further complicate therapy and diagnosis. However, the issues of MalERA cannot be reached in the near future. Moreover, short of this optimal goal, in a

draft research agenda, the consortium emphasizes the necessity to develop gametocytocides and more rapid and robust diagnosis with respect to parasitic liver stages to broaden the knowledge about the biology of *P. vivax,* the benign malaria parasite. In particular, in vitro cultivation methods of the persisting dormant hypnozoites are required. In sum, evaluation of new antimalarials should be enforced in a variety of epidemiological settings.

The genomics revolution has not yet led to new antimalarial medicines and target-based lead discovery has produced disappointing results, generally for lack of whole-cell activity as documented for bacteria (Swinney and Anthony 2011). The lack of new antimalarials and the reduced clinical response to artemisinin-containing drug combinations (Dondorp et al. 2009) led to an accelerated screening for novel antimalarial compounds. These screening approaches had either a virtual, genetic or chemical basis (Penna-Coutinho et al. 2011; Diaz et al. 2006; Rottmann et al. 2010).

Different antimalarial compound libraries from various pharmaceutical drug companies were released to public and applied in High-throughput Screening assays (HTS). The Open Access Malaria Box (Spangenberg et al. 2013) has different resources i.e., Glaxxo SmithKline (GSK) The Tres Cantos Antimalarial Dataset (TCAMS) (Gamo et al. 2010), Novartis, and St. Jude Children's Research Hospital. The St. Jude's dataset comprised 1,523 unique structures, while the Novartis dataset included 5,661 structures. 1,986,056 compounds of the Tres Cantos Antimalarial dataset (TCAMS) were tested for inhibition of chloroquine-resistant *P. falciparum* 3D7 at 2 μM under in vitro conditions. 13,533 compounds were identified displaying 80 % growth inhibition. Several analogs of 4-aminoquinolines (for example, chloroquine), 8-aminoquinolines (for example, primaquine), methanol-quinolines (for example, quinine), diaminopyrimidines (for example, pyrimethamine), diaminotriazines (for example, cycloguanil) and naphthoquinones (for example, atovaquone) are all present in the hit collection. They do not represent more than 10 % of all the hits by the similarity criteria. Liver cytotoxicity was checked with human hepatoma HepG2 cells. The compound library was checked against multidrug resistant *P. falciparum* strains. However, these strains exhibited sensitivity against more than 60 % of the compounds.

Target-based drug screening has dominated the drug discovery process during the last decades. However, it has been shown in some cases that it was not successful. In this context, screening activities against lactate dehydrogenase (LDH) (Granchi et al. 2010; Cameron et al. 2004) identified azole derivatives responsible for anti-parasitic activity but could not be linked to enzyme inhibition. Thus LDH was not druggable. Meanwhile, phenotype-based screens are applied which have the advantage to act against their antimalarial target in a cellular context (Guiguemde et al. 2012).

Referring to this novel screening approach, a combined screening has been started with the Malaria Box based on two strategies, i.e., a phenotypic and a target-based screening for inhibitors of deoxyhypusine hydroxylase (DOHH). The enzyme catalyzes the second step of a posttranslational modification of lysine 50 in eukaryotic translation initiation factor 5A (EIF-5A). This review summarizes the most important biological and biochemical data obtained for DOHH from *P. falciparum* and *P. vivax* and focuses on the analytical challenge for a HTS screening.

In the phenotypic screening from 400 compounds 10 % exhibited antiplasmodial activity in the erythrocytic stages. These compounds were assembled on their drug-like properties and as molecular probes in the Malaria Box (Spangenberg et al. 2013). Forty active compounds ($IC_{50} < 2$ μM) were selected because of high activity in the blood stages. Applying empirical medicinal chemistry most of the 40 compounds can function as chelators or kinase inhibitors dependent on their scaffolds. These drugs will be selectively applied for a target-based screen to inhibit hypusine biosynthesis.

Hypusine is a non-proteinogenic amino acid present in eukaryotic translation initiation factor 5A (eIF-5A) (Shiba et al. 1971). A unique characteristic of eIF-5A is its activation through hypusination. This step is catalyzed by the sequential action of deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH). The resulting posttranslational modification occurs at lysine 50 in eukaryotic translation initiation factor 5A. Hitherto, a lot of different functions have been attributed to hypusinated EIF-5A. Recent data have shown a putative role of hypusinated EIF-5A in stress-induced signaling which appears in diabetes (Maier et al. 2010), malaria (Schwentke et al. 2012), and HIV (Hauber et al. 2005). In diabetes type 2 pancreatic stressed β-cells (Maier et al. 2010) and in HIV-infected T cells (Hofmann et al. 2001), eIF-$5A^{Hyp}$ is functional as a nucleocytoplasmic shuttle protein for the transport and translation of specific mRNAs (Rosorius et al. 1999). Particularly in HIV, eIF-$5A^{Hyp}$ is essential for the nucleocytoplasmic transport and translation of incompletely spliced mRNAs encoding viral proteins (Mittal et al. 2013). During the infectious process of cerebral malaria proinflammatory cytokines including tumor necrosis factor α (TNF-α), interferon γ (IFN-γ) and interleukin (IL)-1β are produced locally before the "entry of the systemic phase" in which cytokines activate macrophages and $CD8^+$ T cells. It was recently shown that a knock down of the *eIF-5A* or the *dhs* gene in *Plasmodium* prevented iNos induction in the human host, which protects against the infection (Schwentke et al. 2012).

The second enzyme of the hypusine pathway, DOHH has recently been assessed to be a druggable target for antimalarial chemotherapy in *P. falciparum* and *Leishmania major* (Mittal et al. 2013). The occurrence of E-Z HEAT-like repeats and ferrous iron in the active site of the DOHH protein might be exploited for the discovery of novel interacting proteins or chelators with new lead structures. In sum, separation of DOHH by native PAGE electrophoresis revealed two bands, i.e., one band referred to the apoprotein, which does not contain ferrous iron while the latter represents the holoenzyme with ferrous iron, which is coordinated to four histidine glutamate residues (Kerscher et al. 2010). The molecular cloning of the DOHH orthologue from the neglected human malaria parasite *P. vivax* (Atemnkeng et al. 2013) has confirmed the previous target assessment for *P. falciparum* DOHH. Purified DOHH protein shows functional activity and has only four E-Z HEAT-like repeats opposed to the five present in its orthologue from *P. falciparum*. Moreover, it was demonstrated that the active catalytic site of DOHH from *P. vivax* can be selectively inhibited by zileuton, (RS)-1-(1-Benzothiophen-2-ylethyl)-1-hydroxyurea (Rossi et al. 2010), an inhibitor of human 5-lipoxygenase (5-LOX) which catalyzes the first two reactions in the production of leukotrienes from arachidonic acid. Moreover, 5-LOX is a validated target for anti-inflammation drug design. All lipoxygenases are homologous in sequence and have the same two domain structure which is an *N*-terminal β-barrel domain and a *C*-terminal catalytic domain (lipoxygenase domain) (Rossi et al. 2010) containing a ferrous iron. Moreover, the iron is ligated in an octahedral arrangement by three conserved histidines, one His/Asn/Ser, and the *C*-terminal isoleucine. By contrast, the ferrous iron in *Plasmodium* DOHH is coordinated by four histidine glutamate residues. The structural similarities between 5-LOX and plasmodial DOHH might reflect a common mechanism resulting from iron complexation of the inhibitor in both enzymes.

One of the main issues of this combined screening approach is to close the major gaps in current chemotherapy of *P. vivax* and *P. falciparum* infections. Primaquine, a 8-aminoquinoline is the only drug to cure the dormant hypnozoites present in the liver which switch to the infectious form due to unknown signals. In case of *P. falciparum*, there is a lack of gametocytocides since most 4-aminoquinolines have only weak or no activity against these developmental stages which would decrease the transmission rate. In this context, DOHH can be used as a common target to screen the 40 hits obtained from a phenotypic screen of the highly active substances of the erythrocytic stages from the Malaria Box. Based on an empirical medicinal chemistry approach different inhibitors with a potential of iron chelating activity will be selected to validate DOHH from two human malaria parasites.

A proteomic platform for hypusine detection

Since a HTS can only be successful with rapid and robust analytical methods, we installed a proteomic analysis platform during recent years which is able to analyze data in a microplate formate. Moreover, non-radioactive assays had to replace the former radioactive filter assay with [^{14}C]- or [^{3}H]-spermidine for DHS and DOHH (Park et al. 2011; Sasaki et al. 1996).

Since hypusine is formed by two subsequent enzymatic reactions, i.e., deoxyhypusine synthase and deoxyhypusine hydroxylase, purification was performed with the *N*-terminal histidine tagged fusion proteins of EIF-5A, DHS or DOHH in recombinant pET-15b/pET-28a. Each protein was expressed separately in *E. coli* BL21 (DE3) or Rosetta cells. Purification of the different proteins was performed by Nickel-chelate affinity chromatography. Enrichment of deoxyhypusinylated eIF-5A (Dhp) and hypusinated eIF-5A (Hyp) was achieved with two steps of size-exclusion chromatography applying an Amicon Ultra 100 and an Amicon Ultra 30 column cutting off DHS and DOHH, respectively. Modified EIF-5A was recovered by size-exclusion chromatography and analysed by subsequent peptide hydrolysis (Fig. 1).

For the detection of hypusine mainly three different analytical tools were applied. GC/MS analysis is currently the most suitable technique for a large and rapid analysis of compounds like the Malaria Box. The main advantages are the easy derivatization with methyl chloroformate according to a method described by (Husek 1991) leading either to *N*-methylated derivatives of the amino groups or to the esterification of the free carboxylic groups. A typical ion mass spectrum of hypusine isolated from a DOHH activity assay in a peptide hydrolysate after MCF derivatization identified the molecular ion [M] + at 347 and a molecular fragment of [M-OCH3] with [M-31]+. This fragment is absent in deoxyhypusine and thus enables a differentiation between both metabolites.

Recently, cellular polyamines have been separated by HPLC after 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AccQ-Tag, Waters Germany) derivatization (Kaiser et al. 2012). This prompted us to develop an alternative assay to quantify the intermediate deoxyhypusine and the final product hypusine by Ultra performance Liquid Chromatography (UPLC). Derivatization with AccQ-Tag occurred at all three amino groups in hypusine, while derivatives with one or two derivatized amino groups resulted in different retention times.

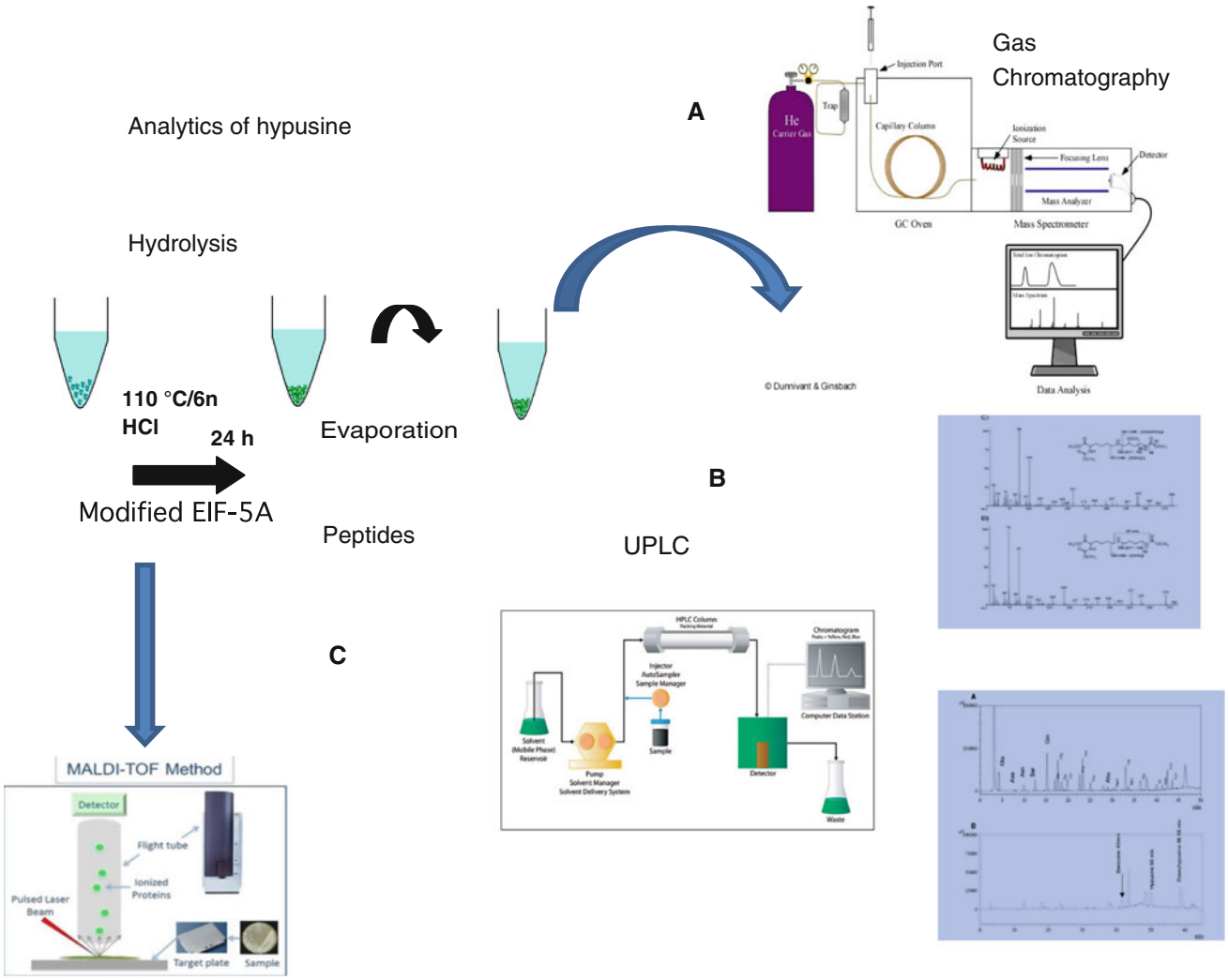

Fig. 1 Demonstrates the whole capacity of the proteomics working platform. The first step to establish a non-radioactive assay was to develop a rapid and robust, but sensitive procedure to evaluate the efficacy of the inhibitors. This can be achieved by three different bioanalytical techniques. **a** Gas chromatography/Mass Spectrometry (GC/MS): GC/MS was originally used in the field of metabolomics to determine the active metabolites in tissues or body fluid. For the detection of hypusine in a peptide hydrolysate methyl chloroformate derivatives with esterified reactive side chains or carboxylic groups are formed. **b** Ultra performance Liquid Chromatography (UPLC) coupled to mass spectrometry is a rapid assay to detect hypusine metabolites after derivatization with 6-aminoquinolyl-*N*-hydroxy-succinimidyl carbamate (AccQ-Tag, Waters Germany) at the primary amino groups. Separation was performed on an Acquity system (Waters) equipped with a binary solvent manager, a sample manager with column heater and a photodiode-array detector (PDA). **c** For Matrix-Assisted Laser Desorption Ionization time-of-flight spectrometry (MALDI-TOF/TOF), the DOHH protein is only purified and embedded in a matrix. There is laser ionization of a molecular beam, laser desorption of solid samples from an alternative dual-stage reflectron. The kinetic energy distribution in the direction of ion flight can be corrected using a reflectron. Time-of-flight is a method of mass spectrometry in which an ion's mass-to-charge ratio is determined via a time measurement

Employment of UPLC facilitated the separation of the hypusine metabolites in a peptide hydrolysate within approximately 7 min of detection (Kaiser et al. 2012). Retention times for deoxyhypusine were 7.44 min and for hypusine 7.30 min, respectively. The limit of detection for both compounds was 0.144 ng/l. However, although this method can be applied for HTS it has to be coupled to a mass spectrometer. Different fragmentation patterns of hypusine have been obtained with characteristic molecular masses [see "Introduction" part within].

A third analytical alternative for hypusine determination is one-dimensional chromatography (SDS-PAGE) coupled to mass spectrometry. In this case, deoxyhypusinated EIF-5A protein is extracted after separation from the SDS-PAGE gel and analysed by MALFI-TOF (time-of-flight) or MALDI-TOF–TOF (time-of-flight) (time-of-flight) mass spectrometry after digestion with either trypsin or the endoproteinase *LysC* which cleaves proteins on the *C*-terminal side of lysine (Dyshlovoy et al. 2012). MALDI-TOF is a laser-based soft ionization method producing

single-charged ions that can be routinely used for peptide mass fingerprints (typically 1–5 proteins). Tandem high-resolution mass spectrometry will even lead to a higher resolution (Fig. 1).

Blocking hypusine biosynthesis in different stages of parasitic development and subsequent assay in a humanized mouse model

Hits deriving from the proteomic platform after DOHH target evaluation will be further analysed for their activity against different developmental stages.

Hitherto, there is no in vitro *culture* system for *P. vivax* although it remains the most widely distributed human parasite. One of the main reasons is that *P. vivax* prefers reticulocytes, which are non-mature erythrocytes for its growth. The only alternative is a short-term in vitro culture of *P. vivax* and *P. ovale* for drug-susceptibility testing. An in vitro maturation of the parasites to schizonts in a single asexual life cycle is the minimal requirement for the drug-susceptibility assay which has been shown for the short-term in vitro culture (Basco and Le Bras 1994). The advantages of using hematopoietic stem cells are that the asynchronous maturation of the erythroid cells enables continuous production of fresh reticulocytes and that the cells contain hemoglobin A. This facilitates a simpler cultivation procedure, and weekly addition of the growing erythrocytes is sufficient to maintain the parasites.

However, recent advances in establishing a transfection system have paved the way (Sanchez et al. 2013) for episomal expression of indicator genes to study mechanisms of gene regulation, gene replacement and knock down strategies. In sum, episomal expression of indicator genes in *P. vivax* might be a valuable tool to further define the quality of *P. vivax* DOHH inhibitors.

Mostly important is the investigation of the DOHH inhibitors in in vivo assays. Over recent years, several rodent models have been established to study in vivo efficacy of antimalarial compounds. The rodent model with the malaria parasite *P. berghei* is the best available mouse model for human *P. falciparum*-mediated cerebral malaria. Regulatory T cells, in particular CD4 + cells and TH1 cytokines, such as gamma interferon (IFN-γ), which exacerbate the inflammatory cascade responsible for local and systemic inflammation are involved in the induction of the disease. Consistent with this view, C57BL/6 mice, usually prone to developing TH1-dominated responses (Scott et al. 1989), are susceptible to murine cerebral malaria syndrome, whereas BALB/c mice, with a TH2-biased response, are resistant (Kossodo and Grau 1993). While infections with C57BL/6 mice lead to cerebral malaria within 6 days post infection, BALB/c mice do not succumb to cerebral malaria but hyperparasitemia and die due to hemolytic anemia. However, the relevance of rodent models for the study of human cerebral malaria (HCM) is still a controversy since experimental cerebral malaria (ECM) associated with marked accumulation of leukocytes, but not with prominent sequestration of cytoadherent mature trophozoite/schizont iRBC in brain vessels as in HCM (Nie et al. 2007). A clarification is necessary of how inhibitor or intervention studies of adjuvant treatments in mice should be interpreted. In most studies, the inhibitors/drugs are given before the development of neurological symptoms thereby providing information on disease processes in ECM, but not necessarily on the identification of viable therapies. The validity of carrying out human clinical trials, often in underpowered studies, purely on the basis of this type of rodent malaria data is questionable (Craig et al. 2012).

Moreover in the past, much effort has been made by many researches to extrapolate differences in in vivo pharmacokinetics of drugs in human from animal models. A severe combined immunodeficient transgenic mouse line was established recently with replacement in the human liver by more than 80 % human hepatocytes (Katoh and Yokoi 2007). These chimeric mice exhibited human phase I and phase II enzymes in a capacity which was comparable to the human donor. The patterns of the metabolizing enzymes, i.e., CYP2D6, an isoform responsible for metabolism of many drugs, could be induced and inhibited in a similar way as for human orthologues. Drug metabolism consists of oxidation, reduction, hydrolysis, and conjugation. The former three reactions are designated as phase I reactions and the latter as a phase II reaction. Although a transgenic mouse line with human P450s has been established (Zhang et al. 2003), the use of such transgenic mice is limited because only one human P450 can be expressed. This chimeric mouse line should be a very promising model for examining ADME in humans and might be a better model than any other experimental animal to evaluate the in vivo pharmacokinetics in human.

Future perspectives

Two different strategies are pursued to validate DOHH. One strategy is based on iron complexation of the purified target protein, the latter will be started on a combined basis using a phenotypic screening with the Malaria Box for selection of activity in the erythrocytic stages and a subsequent chemical profiling. A bioanalytic, proteomic platform will be applied for quantification of the analytes of the hypusine pathway. The identified hits need further development in the preclinical phase using suitable rodent models. In particular, compounds should be considered

which fill the gaps in antimalarial chemotherapy, i.e., eradication of hypnozoites and gametocytes. As there is no crystal structure for parasite or human DOHH, 3D models of both proteins could be approached exploiting protein homology. A homology modeling or "ab initio" structure prediction to obtain at least the structure of the drug-binding domain will be performed (Sánchez-Jiménez et al. 2013).

Conflict of interest The authors declare that they have no conflict of interest.

References

Atemnkeng VA, Pink M, Schmitz-Spanke S, Wu XJ, Dong LL, Zhao KH, May C, Laufer S, Langer B, Kaiser A (2013) Deoxyhypusine hydroxylase from plasmodium vivax, the neglected human malaria parasite: molecular cloning, expression and specific inhibition by the 5-LOX inhibitor zileuton. PLoS One 8(3):e58318. doi:10.1371

Basco LK, Le Bras J (1994) Short-term in vitro culture of plasmodium vivax and P. ovale for drug-susceptibility testing. Parasitol Res 80(3):262–264

Cameron A, Read J, Tranter R, Winter VJ, Sessions RB, Brady RL, Vivas L, Easton A, Kendrick H, Croft SL, Barros D, Lavandera JL, Martin JJ, Risco F, García-Ochoa S, Gamo FJ, Sanz L, Leon L, Ruiz JR, Gabarró R, Mallo A, Gómez de las Heras F (2004) Identification and activity of a series of azole-based compounds with lactate dehydrogenase-directed antimalarial activity. J Biol Chem 23, 279(30):31429–31439

Craig AG, Grau GE, Janse C (2012) The role of animal models for research on severe malaria. PLoS Pathog 8(2):e1002401. doi:10.1371/journal.ppat.1002401

Diaz CA, Allocco J, Powles MA, Yeung L, Donald RG, Anderson JW, Liberator PA (2006) Characterization of Plasmodium falciparum cGMP-dependent protein kinase (PfPKG): antiparasitic activity of a PKG inhibitor. Mol Biochem Parasitol 146(1):78–88

Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, Lwin KM, Ariey F, Hanpithakpong W, Lee SJ, Ringwald P, Silamut K, Imwong M, Chotivanich K, Lim P, Herdman T, An SS, Yeung S, Singhasivanon P, Day NP, Lindegardh N, Socheat D, White NJ (2009) Artemisinin resistance in plasmodium falciparum malaria. N Engl J Med 30, 361(5):455–467

Dyshlovoy SA, Naeth I, Venz S et al (2012) Proteomic profiling of germ cell cancer cells treated with aaptamine, a marine alkaloid with anti-proliferative activity. J Proteome Res 11(4):2316–2330

Gamo FJ, Sanz LM, Vidal J, de Cozar C (2010) Thousands of chemical starting points for antimalarial lead identification. Nature 465:305–310

Granchi C, Bertini S, Macchia M, Minutolo F (2010) Inhibitors of lactate dehydrogenase isoforms and their therapeutic potentials. Curr Med Chem 17(7):672–697

Guiguemde WA, Shelat AA, Garcia-Bustos JF, Diagana TT, Gamo FJ, Guy RK (2012) Global phenotypic screening for antimalarials. Chem Biol 19(1):116–129. doi:10.1016/j.chembiol.2012.01.004

Hauber I, Bevec D, Heukeshoven J, Krätzer F, Horn F, Choidas A, Harrer T, Hauber J (2005) Identification of cellular deoxyhypusine synthase as a novel target for antiretroviral therapy. J Clin Invest 115(1):76–85

Hofmann W, Reichart B, Ewald A, Müller E, Schmitt I, Stauber RH, Lottspeich F, Jockusch BM, Scheer U, Hauber J, Dabauvalle MC (2001) Cofactor requirements for nuclear export of Rev response element (RRE)- and constitutive transport element (CTE)-containing retroviral RNAs. An unexpected role for actin. J Cell Biol 152(5):895–910. doi:10.1083/jcb.152.5.895

Husek P (1991) Rapid derivatization and gas chromatographic determination of amino acids. J Chromatogr 552:289–299

Kaiser A, Khomutov AR, Simonian A, Agostinelli E (2012) A rapid and robust assay for the determination of the amino acid hypusine as a possible biomarker for a high-throughput screening of antimalarials and for the diagnosis and therapy of different diseases. Amino Acids 42(5):1651–1659

Katoh M, Yokoi T (2007) Application of chimeric mice with humanized liver for predictive ADME. Drug Metab Rev 39(1):145–157

Kerscher B, Nzukou E, Kaiser A (2010) Assessment of deoxyhypusine hydroxylase as a putative, novel drug target. Amino Acids 38(2):471–477

Kossodo S, Grau GE (1993) Profiles of cytokine production in relation with susceptibility to cerebral malaria. J Immunol 151:4811–4820

Maier B, Tersey SA, Mirmira RG (2010) Hypusine: a new target for therapeutic intervention in diabetic inflammation. Discov Med 10(50):18–23

malERA Consultative Group on Drugs et al. (2011) A research agenda for malaria eradication: drugs. PLoS Med 8(1):1–9

Mittal N, Subramanian G, Bütikofer P, Madhubala R (2013) Unique posttranslational modifications in eukaryotic translation factors and their roles in protozoan parasite viability and pathogenesis. Mol Biochem Parasitol 187(1):21–31

Nie CQ, Bernard NJ, Schofield L, Hansen DS (2007) CD4+ CD25+ regulatory T cells suppress CD4+ T-cell function and inhibit the development of Plasmodium berghei-specific TH1 responses involved in cerebral malaria pathogenesis. Infect Immun 75(5):2275–2282

Park JH, Wolff EC, Park MH (2011) Assay of deoxyhypusine hydroxylase activity. Meth Mol Biol 720:207–216

Penna-Coutinho J, Cortopassi WA, Oliveira AA, França TC, Krettli AU (2011) Antimalarial activity of potential inhibitors of Plasmodium falciparum lactate dehydrogenase enzyme selected by docking studies. PLoS One 6(7):e21237

Rosorius O, Reichart B, Krätzer F, Heger P, Dabauvalle MC, Hauber J (1999) Nuclear pore localization and nucleocytoplasmic transport of eIF-5A: evidence for direct interaction with the export receptor CRM1. J Cell Sci 112(Pt 14):2369–2380

Rossi A, Pergola C, Koeberle A, Hoffmann M, Dehm F (2010) The 5-lipoxygenase inhibitor, zileuton, suppresses prostaglandin biosynthesis by inhibition of arachidonic acid release in macrophages. Br J Pharmacol 161:555–570

Rottmann M, McNamara C, Yeung BK, Lee MC, Zou B, Russell B, Seitz P, Plouffe DM, Dharia NV, Tan J, Cohen SB, Spencer KR, González-Páez GE, Lakshminarayana SB, Goh A, Suwanarusk R, Jegla T, Schmitt EK, Beck HP, Brun R, Nosten F, Renia L, Dartois V, Keller TH, Fidock DA, Winzeler EA, Diagana TT (2010) Spiroindolones, a potent compound class for the treatment of malaria. Science 3, 329(5996):1175–1180

Sanchez CP, Pfahler J, Del Portillo HA, Lanzer M (2013) Transient transfection of plasmodium vivax blood-stage parasites. Meth Mol Biol 923:151–159

Sánchez-Jiménez F, Ruiz-Pérez MV, Urdiales JL, Medina MA (2013) Pharmacological potential of biogenic amine-polyamine interplay beyond neurotransmission. Br J Pharmacol. doi:10.1111/bph.12109

Sasaki K, Abid MR, Miyazaki M (1996) Deoxyhypusine synthase gene is essential for cell viability in the yeast *Saccharomyces cerevisiae*. FEBS Lett 384(2):151–154

Schwentke A, Krepstakies M, Mueller AK, Hammerschmidt-Kamper C, Motaal BA, Bernhard T, Hauber J, Kaiser A (2012) In vitro

and in vivo silencing of plasmodial dhs and eIf-5a genes in a putative, non-canonical RNAi-related pathway. BMC Microbiol 12:107. doi:10.1186/1471-2180-12-107

Scott P, Pearce E, Cheever AW, Coffman RL, Sher A (1989) Role of cytokines and $CD4^+$ T-cell subsets in the regulation of parasite immunity and disease. Immunol Rev 1989(112):161–182

Shiba T, Mizote H, Kaneko T, Nakajima T, Kakimoto Y (1971) Hypusine, a new amino acid occurring in bovine brain. Isolation and structural determination. Biochim Biophys Acta 21, 244(3):523–531

Spangenberg T, Burrows JN, Kowalczyk P, McDonald S, Wells TN, Willis P (2013) The open access malaria box: a drug discovery catalyst for neglected diseases. Plos One 8(6):e62906

Sutherland CJ, Tanomsing N, Nolder D et al (2010) Two non-recombining sympatric forms of the human malaria parasite plasmodium ovale occur globally. J Infect Dis 15, 201(10):1544–1550. doi:10.1086/652240

Swinney DC, Anthony J (2011) How were new medicines discovered? Nat Rev Drug Discov 10:507–519

White NJ (2008) *Plasmodium knowlesi*: the fifth human malaria parasite. Clin Infect Dis 46:172–173

Zhang W, Purchio AF, Chen K et al (2003) A transgenic mouse model with a luciferase reporter for studying in vivo transcriptional regulation of the human CYP3A4 gene. Drug Metab Dispos 31:1054–1064