Molecular & Biochemical Parasitology 192 (2013) 1–9

Contents lists available at ScienceDirect

Molecular & Biochemical Parasitology

Review

Chemical biology approaches for the study of apicomplexan parasites

Matthew A. Child*

Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305, USA

ARTICLE INFO

Article history:
Received 27 October 2013
Received in revised form
28 November 2013
Accepted 28 November 2013
Available online 11 December 2013

Keywords:
Apicomplexan
Chemical biology
Small molecule
Bioorthogonal

ABSTRACT

Chemical biology and the techniques the field encompasses provide scientists with the means to address biological questions in ever-evolving and technically sophisticated ways. They facilitate the dissection of molecular mechanisms of cell phenomena on timescales not achievable by other means. Libraries of small molecules, bioorthogonal chemistries and technical advances in mass-spectrometry techniques enable the modern chemical biologist to tackle even the most difficult of biological questions. It is because of their broad applicability that these approaches are well suited to systems less tractable to more classical genetic methods. As such, the parasite community has embraced them with great success. Some of these successes and the continuing evolution of chemical biology applied to apicomplexans will be discussed.

Contents

1. Introduction

The field of chemical biology evolved from the overlap between the disciplines of chemistry and pharmacology, and as such can be considered as an extension of those ideas and principles. Broadly, chemical biology can be defined as the application of chemical techniques, and small molecules synthesized using these techniques, to biological systems in order to manipulate them. Through careful observation of these perturbations, it is possible to gain unique insights into the system being manipulated. The speed with which these chemical tools can be applied and a biological effect manifest makes chemical biological approaches ideally suited to interrogate systems refractory to more traditional approaches. Apicomplexan parasites are one such example, where despite recent advances the continuing lack of robust, rapid and broadly applicable techniques for the conditional regulation of genes highlights the power of studying these parasites through the lens of chemical biology.

This review aims to provide a brief overview of the application of chemical biology tools and techniques to the study of apicomplexan parasites, focusing on *Plasmodium falciparum* and

* Tel.: +1 650 725 4573.
E-mail address: machild@stanford.edu

0166-6851/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.molbiopara.2013.11.003

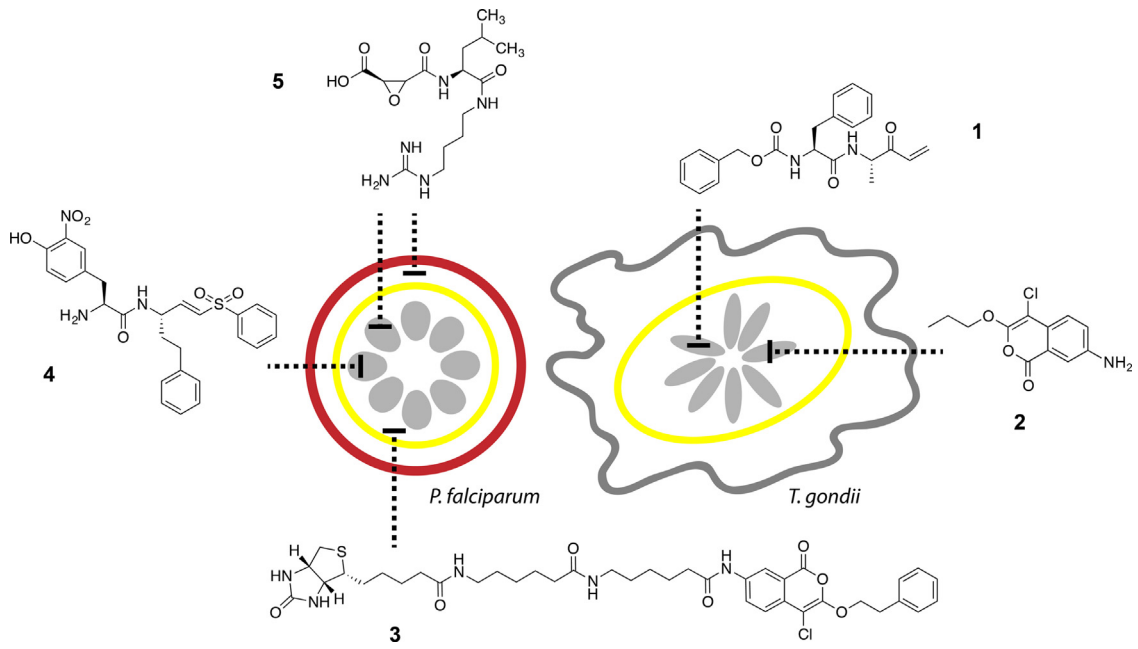

Fig. 1. Examples of small molecules used to study apicomplexan biology. Schematic depicting some successful uses of small molecules to target specific proteins and cellular events in *P. falciparum* and *T. gondii*. (1) WRR-086, a peptidic alpha–beta unsaturated ketone that interacts with TgDJ-1 and blocks microneme secretion in *T. gondii* tachyzoites [33]. (2) JCP174, a substituted chloroisocoumarin that inhibits the depalmitoylating activity of TgPPT1 and enhances invasive capacity of *T. gondii* tachyzoites [36]. (3) JCP104, a biotinylated chloroisocoumarin that binds to PfSUB1 and blocks egress of blood-stage *P. falciparum* merozoites [34]. (4) SAK1, a vinyl sulfone that inhibits PfDPAP3 and blocks *P. falciparum* merozoite egress [34]. (5) E64, an epoxy succinate that inhibits host calpain to block *P. falciparum* merozoite egress [15] and inhibits the activity of parasite digestive food vacuole falcipains [12].

Toxoplasma gondii. Due to space limits, this review will highlight specific examples rather than provide a comprehensive overview of all available literature. Additional reviews are available that more broadly describe the application of small molecules in cellular microbiology [1]. As a conclusion, a view of how this field will continue to evolve with regard to the study of these parasites and other organisms will be presented.

2. Small-molecule tools

Many of the successes of chemical biology in *P. falciparum* and *T. gondii* have been achieved through the use of small molecules to study basic biological processes. In many respects, therapeutic drug treatment of parasitic infections represent the first forays of the parasite field into chemical biology approaches, some of which pre-date the birth of chemical biology as a recognized discipline. In the 17th century Cinchona bark was used for the treatment of malarial fevers, and later chemical extractions characterized the active anti-malarial component as quinine [2]. Further efforts identified a structurally related molecule, chloroquine, as a cheap and efficacious anti-malarial, and it is arguably the most successful anti-malarial drug identified to date [3]. Despite its success and global application, its precise mode of action remains elusive, with best evidence supporting a molecular mechanism whereby chloroquine interacts with heme in the digestive food vacuole of the parasite and blocks its detoxification [4]. Similarly, artemisinin extracted from Chinese wormwood has rapidly emerged from local use in China as a potent antimalarial, and is now a global front-line drug for the treatment of the disease. However, once again, the mechanism of action remains a matter of some debate [5]. Although these are examples of classical pharmacology, they can now be recognized as also being chemical biology studies containing many unanswered questions. Application of the chemical biology approaches outlined below may provide alternative ways to dissect the molecular mechanisms of these drugs, which in turn could indicate novel avenues for therapeutic intervention.

In terms of modern chemical biology, the use of small molecules falls into two distinct types of approach: (1) the application of a small molecule with a previously described target/mechanism of action and (2) phenotypic screening of small-molecule libraries to identify compounds of previously unknown mechanism. Fig. 1 provides an overview of where such approaches have provided unique insights into parasite biology, some of which will be addressed in greater detail below.

2.1. Small molecules with known targets/mechanism of action

Protease inhibitors have been used with great success to probe parasite-specific processes such as egress (host-cell escape) and host-cell invasion [6,7]. Typically, in the case of covalent inhibitors, these compounds contain electrophilic traps that specifically modify reactive nucleophiles such as those found in the active sites of enzymes. These covalent modifications can be both reversible and irreversible, depending upon the electrophilic trap present on the inhibitor. The amino acid environment surrounding the catalytic residue in the active site of the enzyme contributes to both the nucleophilicity of the catalytic residue and the recognition of substrates. As a result, some protease inhibitors are built around a peptidic scaffold that serves to provide a specificity component to the inhibitor. The additional benefit of covalent inhibitors is that they facilitate downstream identification of targets, a point that will be returned to later. The mechanism of protease inhibitors can also be non-covalent, where they often function as competitive inhibitors of an enzyme/substrate interaction. Also, the interaction between the inhibitor and the enzyme need not occur at the active site as exemplified by non-competitive inhibitors. The potential to interact with and block exosites required for substrate recognition is under active investigation [8].

Broad-spectrum inhibitors have been developed for most enzyme classes, though inhibitors specific for a single target remain a largely theoretical ideal. Starting with a compound with a known mechanism of action provides important insight into possible