Frontiers in Anti-Infective Drug Discovery

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Frontiers in Anti-Infective Drug Discovery
(Volume 9)

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PREFACE

Infections caused by viruses, bacteria, fungi, and parasites have caused death and suffering to humans and other life forms since their existence. Discoveries of antibiotics in 1928 and the development of vaccines were two important landmarks in our fight against harm caused by these unseen enemies. However, various infection-causing agents soon developed resistance against almost all available antibiotics, making the treatment an ever-growing challenge. Similarly, many fast mutating viruses have rendered many vaccines ineffective. The COVID-19 pandemic has posed an extensional threat to the human race, and it is a stark reminder of our vulnerabilities against infections. To meet these re-emerging challenges, it is imperative to constantly understand the molecular basis of infections and drug resistance, identify new drug targets and develop new chemotherapeutic agents. Unfortunately, till recently, pharmaceutical research for anti-infective agents has been given a low priority due to "economic feasibility" considerations. Only in the last decade, the topic has received global attention and vigorous research pursued in academic and pharmaceutical laboratories.

The book series "*Frontiers in Anti-infective Drug Discovery*" has been publishing review articles on key aspects of this field. Volume 9 is not different from the previous well-received volumes. It contains 5 carefully selected reviews on various key stages of drug development and approaches against infections caused by bacteria and parasites. The Review by Samatani *et al* is focused on a critically important aspect of drug development *i.e*. optimal choice of dose regimen. They explain the use of *in vitro* data against pathogens, animal PK/PD, clinical pharmacokinetics, and Monte Carlo simulations in this process. The chapter by Fadauloglou *et al* is focused on the role of post-translational modifications (PTMs) of microbial and host proteins during a successful bacterial and viral invasion in host cells. As a result, PTMs have recently emerged as novel and promising targets for the discovery of new anti-infective therapies.

Jean Michael Brunel has contributed an article on the potential of hydrazine-based agents as novel drug candidates against resistant bacteria and fungi, as well as their structure-activity relationships. The development of drugs against the second most important neglected parasitic disease, leishmaniasis, is the focus of the chapter by Roy and Mazire. They have reviewed the literature on various new therapeutic options and their current stages of development against this debilitating poor man's disease. Dengue viral hemorrhagic fever is also an important health challenge for the developing world. Leowattana *et al*. have commented on various re-purposed drugs currently in various stages of development against dengue virus infection and its various forms.

The 9th volume of this important book series comprises scholarly contributions from several leading experts to whom we are indebted. "Team Bentham" also deserves our appreciation for a job very well done. Among them, Ms. Asma Ahmed (Senior Manager Publications), and Mr. Mahmood Alam (Editorial Director) of Bentham Science Publishers have played a key role in the timely completion of the volume in hand. We sincerely hope that the efforts of authors and the production team will help readers to better understand and appreciate the importance of vigorous research and development activities currently underway against infections that cause tremendous suffering to humanity.

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CHAPTER 1

Use of Preclinical and Early Clinical Data for Accelerating Antimicrobial Drug Development

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Abstract: Antimicrobial drug development over the last two decades suggests that the choice of dose and dosing regimen can be selected at a very early stage. This is achieved by optimizing several key factors that are properties of the drug, the bug, and the host species. Drug exposure metrics, relative to the potency of the drug, are computed during the early stages of anti-infective drug development. These metrics serve as predictors of efficacy in the animal models of infection. Drug exposure relative to its potency can be expressed using a few metrics such as AUC/MIC, T>MIC, or C_{max}/MIC. The class of drugs that the anti-infective belongs to often determines the optimal choice of the metric for a given anti-microbial (and is empirically chosen based on pre-clinical data). There are various anti-microbial drug classes available on the market. Despite a large number of drug classes, there is reasonable consensus that the PK/PD target, *i.e*. metric of relative drug exposure described above, obtained from *in vitro* and animal experiments can predict the efficacy of specific drugs in humans. The steps involved in the derivation of this crucial PK/PD metric and dosing regimen in humans are as follows: (a) First, the metric is chosen and then the magnitude of the metric is computed using *in vitro* and animal PK/PD experiments; (b) Next, drug properties such as plasma protein binding are included as correction factors for the PK/PD target; (c) Finally, the non-clinical information is combined with early clinical pharmacokinetic data to estimate which dosing regimen has the greatest probability of attaining the PK/PD metric. This methodology of computing the dosing regimen and estimating the probability of successful target attainment accounts for two key sources of variability. These are between-patient variation in clinical pharmacokinetics and the gamut of MIC values that reflect the susceptibility of pathogens to the anti-microbial drug. These sources of variability are incorporated by running Monte Carlo simulations that are population-based in nature *i.e*. they account for variability in both the pathogen and the host. These sophisticated simulations answer the critical question around the rate of target attainment for dosing regimens of the new antibiotic drug. In summary, combining *in-vitro* data, animal PK/PD, early clinical pharmacokinetics, and Monte Carlo simulations expedites decision making in antimicrobial drug development. These efficiencies

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can lead to earlier and faster entry into full development for anti-microbials and aid optimal choice of dose regimen for phase 2/3 studies.

Keywords: Antimicrobial, Drug-development, MIC, Modeling, Monte-Carlo, PK/PD, Probability, Protein-binding, Simulation, Target-attainment.

INTRODUCTION

Drug development of antimicrobials over the last 2 decades has been revolutionized by the pragmatic selection of dose and dosing regimens driven by limited but well defined and validated factors that are characteristics of the drug, the pathogen and the host [1]. A robust predictor of anti-microbial efficacy is achieving the pharmacokinetic/pharmacodynamic (PK/PD) target *i.e*. a drug exposure metric such as area under the curve (AUC) or % time above minimum inhibitory concentration (%T>MIC) or peak concentration (C_{max}) relative to the susceptibility of the organism. Despite a large number of classes of antimicrobial agents, there is increasing consensus that PK/PD targets from *in-vitro* and *in vivo*. preclinical studies are predictive of efficacy in humans [1].

One way of utilizing the PK/PD target is to examine whether the free plasma drug concentrations required for anti-microbial efficacy based on preclinical data, can be safely achieved in early human trials. The technique of examining the adequacy of different regimens to treat a myriad of pathogens is based on Monte Carlo simulation methods that allow assessment of how frequently specific doses of the new drug are expected to achieve therapeutic targets. This methodology has the potential to help with study design for subsequent phases of drug development whereby only those doses with a high probability of success are selected. The antimicrobial development process starts off with assessing antimicrobial activity of an agent *in vitro* against several different laboratory strains of microbes, followed by *in vivo* studies in appropriate animal models with microbes of interest where the right PK/PD target is established. The pathogens causing the infection stay the same across species and this allows translation of efficacy from animals to humans. The PK/PD target is also species independent because the pathogen is susceptible in any species as long as the PK exposure is achieved. The PK/PD target is both a drug and bug property since it allows tailoring the exposure relative to the pathogen's susceptibility *e.g*. exposure should increase with decreasing susceptibility [2]. This is followed by assessing pharmacokinetic characteristics of the drug in healthy human volunteers. Utilizing the totality of such information and reinforcing the knowledge surrounding susceptibility and prevalence of antibacterial strains of interest in the community, extensive Monte-Carlo simulations are undertaken to ascertain the right dose and dosing regimen for a given indication. The objectives of the Monte-Carlo analysis are to (i)

describe the population pharmacokinetic (PK) behavior of a novel anti-microbial in development by capturing the absorption and disposition properties using plasma concentrations collected during Phase 1 studies; (ii) to assess the expected performance of various doses and dosing regimens in clinically attaining PK/PD target measures associated with *in-vivo* efficacy in animal models over a range of pathogen susceptibilities using Monte Carlo simulations; and (iii) utilize the results of the Monte Carlo simulations to identify the optimal dose and dosing regimen for subsequent stages of drug development. The magnitude of the PK/PD target is generally obtained from the murine thigh infection model (but the animal model can vary depending on the infection being treated) and correction factors such as plasma protein binding are incorporated to adjust for species differences. Human PK data are usually obtained from early Phase 1 clinical studies. The pre-clinical efficacy information is then combined with the human PK data to determine which clinical dose has the highest probability of achieving the desired PK/PD target. These dosing computations and the calculation of the probability of successful target attainment explicitly account for inter-subject variability in human PK parameters during simulations, the relative natural prevalence of pathogens for target attainment, and the variability in pathogen susceptibility to allow dual individualization of pathogen and humans to the drug. The results from such exercise aids decision making for the development of novel antimicrobials. These decisions encompass the transition of a novel drug entity into full clinical development and the selection of dosing regimens for future phase II/III trials or making a “no-go” decision if PK/PD target attainment is lower than 90%.

Drug, Bug and Host Interactions: Five Critical Factors

Infections caused by multidrug-resistant bacteria are a serious threat to the general population and continue to cause significant morbidity and mortality worldwide. Application of bio-simulations that allow integration of prior information about the variability in human PK and pathogen susceptibility for assessing the likelihood of success for clinically chosen dose and dosing regimens has increased tremendously in the last 2 decades. The utility of Monte Carlo simulations for dose optimization of anti-microbials was first illustrated in 1998 to the FDA anti-infective drug products advisory committee for the antibiotic evernimicin [3]. Monte Carlo simulation allows integration of the knowledge about the PK profile of the drug and the differences in pathogen susceptibility to the drug to evaluate the expected likelihood of success of a given treatment in a particular disease during future clinical trials.

These bio-simulations are driven by five critical factors that describe the interaction between the drug, pathogen, and host [1]. These five factors include (i) the PK/PD target; (ii) distribution of pathogen susceptibility to the drug; (iii)

CHAPTER 2

Post-Translational Modifications: Host Defence Mechanism, Pathogenic Weapon, and Emerged Target of Anti-Infective Drugs

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Abstract: Post-translational modifications are changes introduced to proteins after their translation. They are the means to generate molecular diversity, expand protein function, control catalytic activity and trigger quick responses to a wide range of stimuli. Moreover, they regulate numerous biological processes, including pathogen invasion and host defence mechanisms. It is well established that bacteria and viruses utilize post-translational modifications on their own or their host's proteins to advance their pathogenicity. Doing so, they evade immune responses, target signaling pathways and manipulate host cytoskeleton to achieve survival, replication and propagation. Many bacterial species secrete virulence factors into the host and mediate host-pathogen interactions by inducing post-translational modifications that subvert fundamental cellular processes. Viral pathogens also utilize post translational modifications in order to overcome the host defence mechanisms and hijack its cellular machinery for their replication and propagation. For example, many coronavirus proteins are modified to achieve host invasion, evasion of immune responses and utilization of the host translational machinery. PTMs are also considered potential targets for the development of novel therapeutics from natural products with antibiotic properties, like lasso peptides and lantibiotics. The last decade, significant progress was made in understanding the mechanisms that govern PTMs and mediate regulation of

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protein structure and function. This urges the identification of relevant molecular targets, the design of specific drugs and the discovery of PTM-based medicine. Therefore, PTMs emerge as a highly promising field for the investigation and discovery of new therapeutics for many infectious diseases.

Keywords: ADP-ribosylation, Antimicrobial drugs, Antiviral drugs, Bacterial effector proteins, Bioengineering, Biopharmaceuticals, Coronavirus, Deamidation, Drug target, Elimination, Glycosylation, Host invasion, Innate immunity, Lantibiotics, Lipidation, Natural antimicrobial peptides, Palmitoylation, Pathogenic bacteria, RiPPs, Secretion systems, Spike protein, Thiopeptides, Ubiquitination, Vaccine design.

INTRODUCTION

After translation at the ribosome, proteins may undergo chemical changes on their amino acids. These changes are called post-translational modifications (PTMs); they display a high diversity and enumerate several hundreds of different kinds [1]. It is expected that their number will be substantially increased in the future along with the advances in technology such as the development of mass spectrometry and proteomics. PTMs can be reversible or irreversible, enzymatically or non-enzymatically catalyzed additions of diverse chemical groups ranging from small moieties to full proteins. These groups are usually added to the side chains, even though they can be linked to main chain atoms as well. Moreover, PTMs include the formation or cleavage of chemical bonds. Again, the new bonds may link side-chain or side-chain to main-chain atoms. Proteolysis is the most trivial example of chemical bond cleavage and disulfide bonds the most common, post-translational, side-chain cross links.

On the molecular level, PTMs may have multiple roles influencing the protein function, the structural stability, or the interaction network of the modified molecule. It has been shown that the addition of small, polar moieties such as hydroxyl-groups in the active site or around the active site of enzymes may increase their catalytic activity by, for example, stabilizing the transition state [2 - 4]. On the contrary, such an addition can cause the opposite effect and inactivate an enzyme if the new group of atoms is located in a place that blocks accessibility of the active site. Likewise, modification of a residue, crucial for the catalysis, will probably result in attenuation or full inactivation of the enzymatic catalysis. The formation of cross-links between different parts of a molecule or alterations to the backbone stereochemistry may increase the molecular rigidity and consequently affect structural stability and flexibility. Often, PTMs result in changes in physicochemical properties [5]. For instance, phosphorylations and deamidations usually add negative charges, Lys acylations eliminate positive

charges, hydroxylations increase the hydrophilicity, while lipidations increase the hydrophobicity of the modification site. These alterations may affect the interaction network of the protein either by introducing constraints that block existent interaction interfaces or by creating novel sites for interactions.

On the cellular level, PTMs are widely used as regulatory factors during multiple physiological processes [6]. Phosphorylation/dephosphorylation is a text-book paradigm of how a PTM may undertake a major regulatory role in signaling pathways. Likewise, histone modifications, primarily through Lys methylation and acetylation, is an essential regulatory mechanism, which controls gene-transcription activation and inactivation. Glycosylation is another well-known PTM which plays a fundamental role in molecular recognition and activation of immune response. In line with well-established knowledge, research in the last decade has discovered novel aspects of PTM biology and has demonstrated its significance and implication in more processes than it was originally expected. There are three properties that render PTMs an ideal control-switch for biological regulation. First, PTMs are usually reversible and therefore highly dynamic. Second, they do not necessarily affect the whole molecular population but just a part of it. Last, they are produced directly on site, where they are needed. This enables the cell to respond quickly and on-demand to many stimuli. We know today that this mechanism is involved in cell homeostasis, adaptation to environmental or intracellular changes including cell stress, as well as cell defence, beyond the typical glycosylation.

Not surprisingly, many pathogens, including bacteria and viruses, use PTM-based strategies to subvert eukaryotic defense and dominate their hosts [7 - 10]. Several recent studies, based on proteomics and mass spectrometry assays, indicate substantial differences in post-translationally modified proteome upon pathogen-host interactions and during infection. Pathogens can post-translationally modify their and their host's proteomes. It has been shown for example, that *Leptospira* is adapted to the host during infection by modulating both protein expression and protein PTMs, including methylation and acetylation [11]. On the other hand, the *Listeria* toxin Listeriolysin induces changes to the host proteome *via* PTMs [12]. In particular, extensive alterations of components of the host ubiquitin machinery were observed in response to the toxin. Last, a proteome comparison of virulent and attenuated *Ehrlichia* demonstrates important differences in phosphorylation and glycosylation [13]. These differences are indicative of dependence on the host interactions.

During invasion, pathogens translocate an arsenal of effectors inside the host cytosol [14 - 16]. A subset of them, in some organisms a noteworthy subset, is related with PTMs. Once within the host, these proteins can either cause

CHAPTER 3

Scope and Limitations on the Potent Antimicrobial Activities of Hydrazone Derivatives

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Abstract: Antimicrobial resistance of Gram-negative bacteria is a major concern, and no new classes of antibiotics that are effective against this type of bacteria have been discovered since the 1960s. During the last decades, multiple approaches have been developed to combat such bacterial resistance. However, the combination of antibiotic resistance mechanisms by bacteria and the limited number of effective antibiotics available, decreases the number of interventions for the treatment of current bacterial infections. The solution to emerging antibiotic resistance will likely involve new therapies or new classes of antibacterial agents. For a few years now, there was a real interest in the design and synthesis of hydrazones possessing an azometine -NHN=CH- proton and constituting an important class of compounds for new drugs development as anticonvulsants, antidepressants, antitumoral agents. In this context, the design and antimicrobial evaluation of hydrazone derivatives have constituted one of the new strategies developed to fight bacterial resistance. As pointed out, the range of biological activities is very broad, and this review will deal exclusively with the synthesis and use of hydrazones as antimicrobial agents and will not cover the other biological properties already well depicted in literature. Thus, we will report herein the scope and limitation of such an approach providing numerous examples demonstrating structure-activity relationships and potent interesting antimicrobial activities against both fungi, Gram-positive and/or Gram-negative bacteria.

Keywords: Aldehydes, Antimicrobial agents, Antifungal activity, Antimicrobial resistance, Azomethine, Gram-positive bacteria, Gram-negative bacteria, Heterocycles, Hydrazones, Hydrazine, Ketones.

INTRODUCTION

An important interest in the design and synthesis of hydrazones possessing an azometine -NHN=CH- proton and constituting an important class of compounds for new drugs development has recently emerged [1, 2]. Since the range of biological activities is very broad, this review will deal exclusively with the synt-

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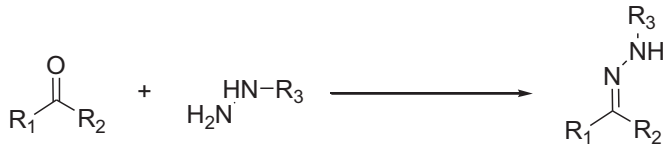
hesis and use of hydrazones as antimicrobial agents, excluding the other well depicted biological properties [1 - 7].

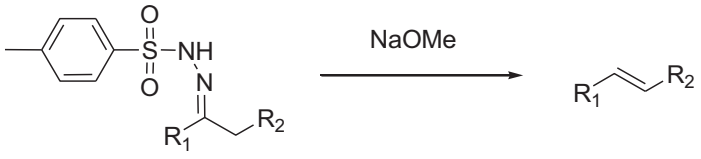
Hydrazones, which general formula is R-NH-N=C, have been widely used and studied in organic chemistry. There are easily produced by condensation of a hydrazine (NH_2-NHR) with a ketone or an aldehyde (Scheme **1**).



Scheme 1.

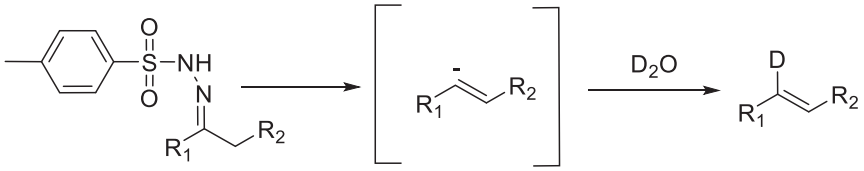
During numerous decades, this condensation has been the only way of detecting the presence of a ketone or an aldehyde group in a molecule. Moreover, hydrazones have also been involved in many reactions such as:

• The Bamford-Stevens reaction allowing the formation of a dual-link Z (Scheme **2**) [8].



Scheme 2.

• The reaction of Shapiro [9] involves the elimination of a tosylhydrazone group and the formation of a double bond. It is noteworthy that during this reaction, the transitional carbanion can be used to graft different functional groups (Scheme **3**).



Scheme 3.

Nevertheless, although these hydrazones are well known in organic chemistry, it is only recently that researchers became interested in their biological potentialities, particularly for the treatment of infectious diseases [10].

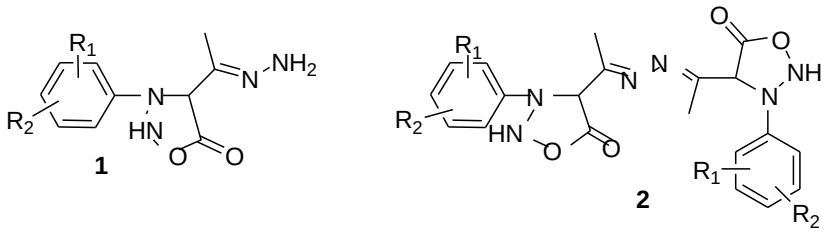
Hydrazones as Potent Antimicrobial Agents

As already mentioned, hydrazones possess a general formula R-NH-N=C. In the following part, we will discuss on the antimicrobial activity of different classes of hydrazones depending on the nature of the R group attached to the nitrogen atom.

R is a Hydrogen

In 2005, Shinge *et al.* reported the reaction of 4-acetyl-3-arylsydnone derivatives [11] with hydrazine leading to the formation of hydrazones **1** and dimers **2** in good yields and the evaluation of their antimicrobial activities against various Gram-negative bacteria and fungi (Table **1**).

Table 1. Structure and antimicrobial activities of derivatives 1a-2f.



			Diameter of Inhibition (mm)			
Compound	**R_1**	**R_2**	***E. coli***	***P. pyocyanous***	***R. bataticola***	***A. niger***
1a	H	4-Cl	29	24	13	18
1b	H	4-Br	30	22	14	18
1c	3-CH_3	4-CH_3	19	18	18	21
1d	4-CH_3	3-Cl	29	21	12	13
1e	2-OCH_3	4-Cl	31	26	11	14
1f	4-Cl	3-F	29	23	13	16
2e	2-OCH_3	4-Cl	18	16	11	14
2f	4-Cl	3-F	21	15	13	16

Determination of the antifungal activity of derivatives **2** led to similar results that their parent monomers **1** (Table **1**, derivatives **1e-1f** and **2e-2f**) whereas weaker activities were encountered against bacteria. Moreover, compounds possessing a chloro, bromo or fluoro substituent group exhibited antibacterial activities greater than norfloxacin reference drug. Derivative **1c** with a methyl group substitution led to an increase of the antifungal activity compared to griseofulvin. Additionally, compound **1d** possessing a methyl and halogen substituent is

CHAPTER 4

Current Scenario of Anti-Leishmanial Drugs and Treatment

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Abstract: Leishmaniasis is a neglected tropical disease caused by a protozoan parasite of the genus *Leishmania*, mainly associated with the lack of community hygiene and poverty in the developing countries. Leishmaniasis can be cured but the emergence of drug resistance makes it difficult to completely eradicate the disease. Even after so many years, there is still no vaccine available against leishmaniasis. Therefore, treatment of the disease is mainly dependent on the available therapeutic drugs. However, the current chemotherapeutic drugs have several drawbacks such as high toxicity, less efficacy, high cost and emergence of drug resistance, *etc*. So, to boost the elimination of disease, development of newer therapeutic agents is imperative. As all this is very well-known, including the current anti-leishmanial drugs with their adverse effects, the authors state that the main objective of this book chapter is to present an overview of the disease, its different clinical forms and the diagnostic tools available for the detection of the disease. Natural sources such as plants and microorganisms have shown great results against *Leishmania* species over the years, indicating that they may be considered as therapeutic agents. Hereafter, potent investigational drugs obtained from the natural sources such as medicinal plants and microorganisms are also discussed in this book chapter.

Keywords: Amastigotes, Amphotericin B, Cutaneous leishmaniasis, Endophytes, Immunological tests, Kinetoplast, Leishmaniasis, Macroalgae, Miltefosine, Molecular diagnostic methods, Mucocutaneous leishmaniasis, Paromomycin, Pentamidine, Pentavalent antimonials, Post Kala-azar Dermal Leishmaniasis (PKDL), Promastigotes, Secondary metabolites, Serological diagnosis, Visceral leishmaniasis or Kala-azar.

INTRODUCTION

Leishmaniasis, a vector-borne disease caused by Trypanosomatid protozoans of the genus *Leishmania,* is classified as a neglected tropical disease having high

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epidemiological and clinical diversity [1, 2]. The disease is endemic and more prevalent in developing countries. The disease is widely spread in about 98 countries located in South and the Central America; Southern Europe, Africa, Middle East, Central Asia and Indian subcontinent. However, more than 90% of the new cases are mainly reported in 13 countries; namely, Afghanistan, Algeria, Bangladesh, Bolivia, Brazil, Columbia, Ethiopia, India, Iran, Perú, South Sudan, Sudan and Syria [1, 3]. Approximately 700,000 to 1 million new cases occur annually. In accordance with WHO (2018), both visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL) are endemic in 68 countries. VL is found to be endemic in 9 countries and CL is endemic in 21 countries. The disease is primarily associated with developing countries that have poor socio-economic status and where problems such as malnutrition, lack of resources, poor housing and sanitary conditions, *etc*. are a major concern. Environmental changes and population mobility are also considered as major risk factors (WHO, 2020) [4].

More than 20 species of *Leishmania* are known to be responsible for the disease in humans [5]. Leishmaniasis is reported to be endemic in Asia, Africa, the Mediterranean, Europe and Middle East. The five-common species of *Leishmania*, responsible for leishmaniasis are *Leishmania tropica*, *Leishmania majo*r, *Leishmania aethiopica*, *Leishmania infantum* and *Leishmania donovani* in Asia, Africa, Middle East and Southern Europe. While, more than six species are responsible for leishmaniasis including *Leishmania mexicana, Leishmania braziliensis*, *Leishmania amazonensis, Leishmania infantum (Leishmania chagasi), Leishmania panamensis*, *Leishmania guyanensis, etc.* in South and the Central America [5]. The three known clinical forms of the disease are i) cutaneous leishmaniasis (CL); ii) mucocutaneous leishmaniasis (MCL) and iii) visceral leishmaniasis (VL) [4]. In accordance with WHO, more than 95% of global VL cases were reported in 10 countries: Brazil, Ethiopia, China, India, Iraq, Nepal, Kenya, Somalia, Sudan and South Sudan in the year 2018. While, 11 countries such as Afghanistan, Algeria, Bolivia, Brazil, Colombia, the Islamic Republic of Iran, Iraq, Pakistan, Perú, the Syrian Arab Republic and Tunisia reported more than 5000 cases of CL, this together accounts for 85% of global reported CL incidence [4, 6]. India, Bangladesh and Nepal have established a collaborative association for elimination of this disease in 2005. It was renewed in 2017 and as the goal was not fulfilled, the program has been extended. The disease is still persistent with high endemicity, mainly in certain regions of India. Moreover, the incidence of VL has increased in Latin America; migration has been one of the sources for increase in number of cases. In accordance to the recent report by the Pan American Health Organization (PAHO) and the World Health Organization (WHO), incidence of VL is expanding geographically. Therefore, the VL elimination program has been extended up to 2020 to facilitate the complete eradication of the disease from the South-Asia region. In addition to

the three mentioned clinical forms of the disease, two major complications are associated with this disease. Post Kala-azar Dermal Leishmaniasis (PKDL) is a complication of VL that mainly occurs in East Africa and India. It is reported that about 5-10% of VL patients develop PKDL [6]. Moreover, HIV-VL co-infection is another complication associated with this disease. In the last few years, reported cases of *Leishmania*-HIV co-infection in endemic areas have increased. VL is considered as an opportunistic infection associated with HIV. Individuals with HIV are particularly more prone to VL infection due to suppressed immune response, leading to higher relapse and mortality rates. HIV-VL co-infection rates are high in Brazil, Ethiopia and Bihar in India. Thus, PKDL and HIV-VL co-infection are major complications of VL that affect the control of leishmaniasis (WHO, 2020).

At present, there is no effective vaccine against leishmaniasis. Therefore, treatment of leishmaniasis is entirely dependent on chemotherapeutic drugs. Pentavalent antimonials, miltefosine, paromomycin, pentamidine and liposomal amphotericin B are widely used drugs for treatment of leishmaniasis [2, 7]. However, the available drugs are toxic; have severe adverse effects and drug resistance is another problem which limits the use of these drugs [2, 7]. Therefore, the development of novel, effective, and less toxic anti-leishmanial agents having reduced side effects is the major priority. Thus, in this chapter an overview of the disease, existing treatment options, diagnostic assays currently available for leishmaniasis and the status of present anti-leishmanial drugs are highlighted.

History of Leishmaniasis

The history of leishmaniasis dates to 2,500 B.C. based on the primitive evidences reported in the ancient writings and recent molecular findings from ancient archaeological material. These reports suggest that the origin of genus *Leishmania* can be traced back in the Mesozoic era. Moreover, subsequent geographical distribution and initial evidence of the disease were reported in ancient times. First account of the infection was identified in the Middle Age, and the discovery of *Leishmania* parasites as causative agents of leishmaniasis was reported in modern times. After observing *Leishmania* organism for the first time in 1885, Russian military surgeon Peter Borovsky found out that the organism was a protozoan which was also confirmed by Wright in 1903. During this time, William Leishman and Charles Donovan described the agent responsible for VL. Leishman observed that VL-infected patients had fever and an enlarged spleen. Further, he also observed the samples from the patients under the microscope using Romanowsky method for staining and he stated that it was something he had never seen before [8]. *L. donovani* was the first identified *Leishmania* species taking its name from William Leishman (genera) and Charles Donovan (species),

CHAPTER 5

Dengue Hemorrhagic Fever: The Potential Repurposing Drugs

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Abstract: Dengue is the most significant arthropod-borne viral infection of humans. More than 3.8 billion people live in endemic areas. Dengue virus infection (DVI) results in more than 500,000 hospitalizations every year, with increased threats of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) during secondary infections. In spite of the high disease burden of the dengue virus, there are no specific antiviral drugs available, and the approved vaccine is harmful in the naïve population with respect to the initiation of primary dengue infection. Several clinically approved drugs have entered human clinical trials. This review addresses the repurposing drug targets that have been investigated in DHF and DSS patients. Furthermore, their essential antiviral action and specific classes of clinically approved drugs have been clarified. These clinical trials' outcomes can enhance our understanding of the antiviral activities of these repurposing drugs to alleviate the clinical severity of dengue viral infection.

Keywords: Antiviral treatment, Balapiravir, Celgosivir, Chloroquine, Clinically approved drugs, Cromolyn, Dengue hemorrhagic fever antibiotics, Doxycycline, Ivermectin, Ketotifen, Montelukast, Repurposing drugs, Ribavirin, Rupatadine, Sofosbuvir, UV-4B.

INTRODUCTION

The Flaviviridae is a family of viruses that use humans and other mammals as natural hosts. This family is primarily spread through arthropod vectors. The common viruses of this family are yellow fever virus (YFV), West Nile virus (WNV), Japanese encephalitis virus (JEV), and dengue virus (DENV) [1]. DENV

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is becoming one of the worst mosquito-borne human pathogens globally. Approximately 400 million people in more than 100 countries are infected each year, leading to 25,000 deaths [2 - 4]. *Aedes aegypti*, which is usually found in tropical areas, and *Aedes albopictus*, commonly found in subtropical areas, are the important vectors of DENV [5]. These mosquitoes are the reservoirs of DENV that could transmit it after infection. Due to the expansion of these vector species, global warming, failure to control the vectors, and urbanization mostly stimulate the drastic increase of DVI worldwide. During 2010-2020, the virus has dramatically re-emerged with significant outbreaks in Africa, South-East Asia, South America, Australia, North America, and Europe [6 - 10]. In endemic areas, most DHF patients are in the younger population, causing a high disease severity. Although DVI is almost asymptomatic and self-limiting, dengue fever (DF) could progress to severe dengue diseases [dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS)]. They usually manifest as retro-orbital pain, severe headaches, muscle pain, bone pain, joint pain, nausea, vomiting, and rash. In the absence of effective prevention by a reliable safe and efficacious vaccine and specific treatment against DVI, patient management is involved in symptomatic treatment and the limitation of transmission progression. Regarding the geographical region, symptomatic patients' mortality rate varies from 1.2% to 3.5%. Hence, effective antiviral drugs to treat DVI are urgently needed [11, 12].

DENV REPLICATION

DENV is a positive-sense, single-stranded RNA virus within an envelope. There are 4 antigenically definite serotypes (DENV 1-4). The first step in infection is the virus particle interaction with the host cell receptors with envelope (E) glycoprotein. There are several host cell receptors such as glycosaminoglycans (GAGs), dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN), and T-cell immunoglobulin-mucin domain (TIM). After that, the virus penetrates the host cells by endocytosis [13]. The conformational modifications in the E glycoprotein are induced by low pH within the endosomal vesicle causing the fusion of membrane, and the nucleocapsid is released into the cytoplasm. Moreover, the viral RNA is translated into 1 polyprotein and then is spliced into three structural proteins; capsid (C), membrane (M), envelop (E), and seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). The NS proteins utilize lipid metabolism in that cell and produce a re-organization of the endoplasmic reticulum (ER) membrane where viral RNA is replicated inside. Many enzymes in cells, such as kinases and α-glucosidases, serve to replicate RNA, translation, and folding of that protein. Several copies of the C protein package newly initiated genomic RNA and the nucleocapsid and then bud into the lumen of ER to create an enveloped immature virion. Hence, virions are transported *via* the secretory pathway where the E and M proteins

encounter post-translational adaptations and conformational changes, comprising the breakdown of precursor M to its mature form by protease furin of the host cell. Progeny virus release occurs through exocytosis and can infect another cell [14]. Molecular study of DENV life cycle and the interaction between virus and host have warranted finding out the small-molecule and peptide antagonists against the interaction of virus and the host cell, the processing of RNA, the genome replication, and the assembly and budding of the mature virus [15 - 17].

The key challenge to dengue therapeutics' success is the rapid decrease in viremia of the patient during a febrile phase, especially in secondary DVI. Hence, the ideal drug that could inhibit DENV should be rapid, active against the four DENV serotypes, possess a good safety window and minimal drug interaction. Many drug candidates have been developed which inhibit either a host or viral protein essential for entry, translation of polyprotein by a proteolytic process, RNA replication, the packaging of the viral genome, and the release of the virion from affected cells. However, all of the candidates have not proved to be better than the placebo in a clinical trial. The discrepancy between pre-clinical and clinical outcomes is the fundamental factor of the study designed. The most common factors included the indefinite and broad spectrum clinical manifestations, the lack of animal models, the limitation of a biological parameter that correlates with clinical outcomes, and the limited financial support for the clinical trials.

Drug repositioning or repurposing can be taken into account as a primary objective and is urgently needed. The repurposing drugs may be authorized or currently used against several clinical situations, or they can be alternatives that have not been successful in any stage of the clinical studies for different purposes. Notably, repurposing drugs are the revelation of new investigations for authorized or unsuccessful agents. The drug repurposing strategy's significance is a quicker and more secure pathway to create new drugs for DHF and DSS management for which a specific treatment is still unavailable. Drug repurposing provides the potentiality to decrease duration and hazards intrinsic to the process of discovery of any drug and urgently advance a candidate drug to the final stage of development [18 - 20]. Moreover, drug repurposing provides an opportunity to develop multi-target drugs able to interfere with several pathways concerned in the pathophysiology of the specific diseases. Multi-target drugs have many advantages such as a synergistic effect, acting on different targets, simplified pharmacokinetics and pharmacodynamics profile, good compliance, and a lower opportunity of drug interferences [21, 22].

SUBJECT INDEX

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PROF. DR. ATTA-UR-RAHMAN, *FRS*

Prof. Atta-ur-Rahman, Ph.D. in Organic Chemistry from Cambridge University (1968) has 1,232 international publications (45 international patents and 341 books). He received the following awards: Fellow Royal Society (FRS) London (2006), UNESCO Science Prize (1999), Honorary Life Fellow Kings College, Cambridge University (2007), Academician (Foreign Member) Chinese Academy of Sciences (2015), Highest Civil Award for Foreigners of China (Friendship Award, 2014), High Civil Award Austria ("Grosse Goldene Ehrenzeischen am Bande") (2007), Foreign Fellow Chinese Chemical Society (2013), Sc.D. Cambridge University (UK) (1987), TWAS (Italy) Prize (2009). He was the President of Network of Academies of Sciences of Islamic Countries (NASIC), Vice President TWAS (Italy), Foreign Fellow Korean Academy of Science & Technology, President Pakistan Academy of Sciences (2003-2006) and (2011 – 2014). He was the Federal Minister for Science and Technology of Pakistan (2000 – 2002), Federal Minister of Education (2002) and Chairman Higher Education Commission/Federal Minister (2002-2008), Coordinator General of COMSTECH (OIC Ministerial Committee) (1996-2012), and the Editor-in-Chief of Current Medicinal Chemistry.

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