



ELSEVIER

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Future directions for the discovery of natural product-derived immunomodulating drugs: an IUPHAR positional review

Cherry L. Wainwright^{a,*}, Mauro M. Teixeira^b, David L. Adelson^c, Fernão C. Braga^d, Eric J. Buenz^e, Priscilla R.V. Campana^d, Bruno David^f, Keith B. Glaser^g, Yuka Harata-Lee^c, Melanie-Jayne R. Howes^{h,i}, Angelo A. Izzo^j, Pasquale Maffia^k, Alejandro M.S. Mayer^l, Claire Mazars^f, David J. Newman^m, Eimear Nic Lughadha^h, Rodrigo M. Pádua^d, Adriano M. C. Pimentaⁿ, John A.A. Parraⁿ, Zhipeng Qu^c, Hanyuan Shen^c, Michael Spedding^o, Jean-Luc Wolfender^p

^a Centre for Natural Products in Health, Robert Gordon University, Aberdeen, UK

^b Department of Biochemistry and Immunology, Universidade Federal de Minas Gerais, Brazil

^c Molecular & Biomedical Science, University of Adelaide, Australia

^d Department of Pharmaceutical Products, Faculty of Pharmacy, Universidade Federal de Minas Gerais, Brazil

^e Nelson Marlborough Institute of Technology, New Zealand

^f Green Mission Pierre Fabre, Pierre Fabre Laboratories, Toulouse, France

^g AbbVie Inc., Integrated Discovery Operations, North Chicago, USA

^h Royal Botanic Gardens Kew, Richmond, Surrey, UK

ⁱ Institute of Pharmaceutical Science, Faculty of Life Sciences & Medicine, King's College London, UK

^j Department of Pharmacy, School of Medicine, University of Naples Federico II, Italy

^k Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, UK

^l Department of Pharmacology, College of Graduate Studies, Midwestern University, Illinois, USA

^m NIH Special Volunteer, Wayne, Pennsylvania USA

ⁿ Laboratory of Animal Venoms and Toxins, Department of Biochemistry and Immunology, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

^o Spedding Research Solutions, Paris, France

^p School of Pharmaceutical Sciences, University of Geneva, Switzerland

ARTICLE INFO

Keywords:

Natural products
Immunomodulators
Marine
Venoms and toxins
Metabolomics
Bioinformatics

ABSTRACT

Drug discovery from natural sources is going through a renaissance, having spent many decades in the shadow of synthetic molecule drug discovery, despite the fact that natural product-derived compounds occupy a much greater chemical space than those created through synthetic chemistry methods. With this new era comes new possibilities, not least the novel targets that have emerged in recent times and the development of state-of-the-art technologies that can be applied to drug discovery from natural sources. Although progress has been made with some immunomodulating drugs, there remains a pressing need for new agents that can be used to treat the wide variety of conditions that arise from disruption, or over-activation, of the immune system; natural products may therefore be key in filling this gap. Recognising that, at present, there is no authoritative article that details the current state-of-the-art of the immunomodulatory activity of natural products, this in-depth review has arisen from a joint effort between the International Union of Basic and Clinical Pharmacology (IUPHAR) Natural Products and Immunopharmacology Sections, with contributions from a number of world-leading researchers in the field of natural product drug discovery, to provide a "position statement" on what natural products has to offer in the search for new immunomodulatory agents. To this end, we provide a historical look at previous discoveries of

* Corresponding author.

E-mail addresses: c.wainwright@rgu.ac.uk (C.L. Wainwright), mmtex@icb.ufmg.br (M.M. Teixeira), david.adelson@adelaide.edu.au (D.L. Adelson), Eric.Buenz@nmit.ac.nz (E.J. Buenz), brunoxdavid@gmail.com (B. David), keith.glaser@abbvie.com (K.B. Glaser), m.howes@kew.org, melanie-jayne.howes@kcl.ac.uk (M.-J.R. Howes), aaizzo@unina.it (A.A. Izzo), pasquale.maffia@glasgow.ac.uk (P. Maffia), amayer@midwestern.edu (A.M.S. Mayer), claire.mazars@pierre-fabre.com (C. Mazars), djnewman664@verizon.net (D.J. Newman), apimenta@gmail.com (A.M.C. Pimenta), michael@speddingresearchsolutions.fr (M. Spedding), jean-luc.wolfender@unige.ch (J.-L. Wolfender).

<https://doi.org/10.1016/j.phrs.2022.106076>

Received 21 December 2021; Accepted 7 January 2022

Available online 21 January 2022

1043-6618/© 2022 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

naturally occurring immunomodulators, present a picture of the current status of the field and provide insight into the future opportunities and challenges for the discovery of new drugs to treat immune-related diseases.

1. Introduction

Drug discovery from natural sources is going through a renaissance, having spent many decades in the shadow of synthetic molecule drug discovery, despite the fact that natural product-derived compounds occupy a much greater chemical space than those created through synthetic chemistry methods. With this new era comes new possibilities, not least the novel targets that have emerged in recent times and the development of state-of-the-art technologies that can be applied to drug discovery from natural sources. Although progress has been made with some immunomodulating drugs, there remains a pressing need for new agents that can be used to treat the wide variety of conditions that arise from disruption, or over-activation, of the immune system; natural products may therefore be key in filling this gap. Recognising that, at present, there is no authoritative article that details the current state-of-the-art of the immunomodulatory activity of natural products, this in-depth review has arisen from a joint effort between the International Union of Basic and Clinical Pharmacology (IUPHAR) Natural Products and Immunopharmacology Sections, with contributions from a number of world-leading researchers in the field of natural product drug discovery, to provide a “position statement” on what natural products has

to offer in the search for new immunomodulatory agents. To this end, we provide a historical look at previous discoveries of naturally occurring immunomodulators, present a picture of the current status of the field and provide insight into the future opportunities and challenges for the discovery of new drugs to treat immune-related diseases.

2. Immunomodulatory drugs from natural sources – a historical perspective

2.1. Traditional medicines – the origin of natural product -derived drug discovery

A majority of the world’s population relies on traditional medicine for their healthcare [1] and at least 130 countries have formal programs to engage traditional medicines at the national level [2]. Thus, the scientific study of substances used medicinally, especially folk remedies, by different ethnic or cultural groups (ethnopharmacology) has made a substantial contribution to the discovery and development of modern therapeutics [3]. Recent studies suggest that the historical ethnopharmacological uses of natural products as a preliminary screening tactic are beneficial in the ultimate identification of bioactive



Fig. 1. The intergenerational transfer of traditional medicine knowledge is the foundation of the ethnopharmacological approach to bioprospecting natural products. There is a transfer of knowledge from individuals which is applied by the traditional healer and refined based on the results achieved through administration of the therapy across generations. Ultimately, this iterative process results in a refined identification of the most effective natural product to treat a specific disorder. The collection of ethnopharmacological data requires working with individuals possessing the knowledge of the use of the natural products as therapeutics. Individuals with knowledge of the medicinal properties will often have cultivated specimens of the materials frequently used (A) and share standard documentation describing the activity and goals of the work with all participants involved in the activity (B). The individuals with the medical knowledge are enthusiastic in sharing their ethnopharmacological knowledge (C).

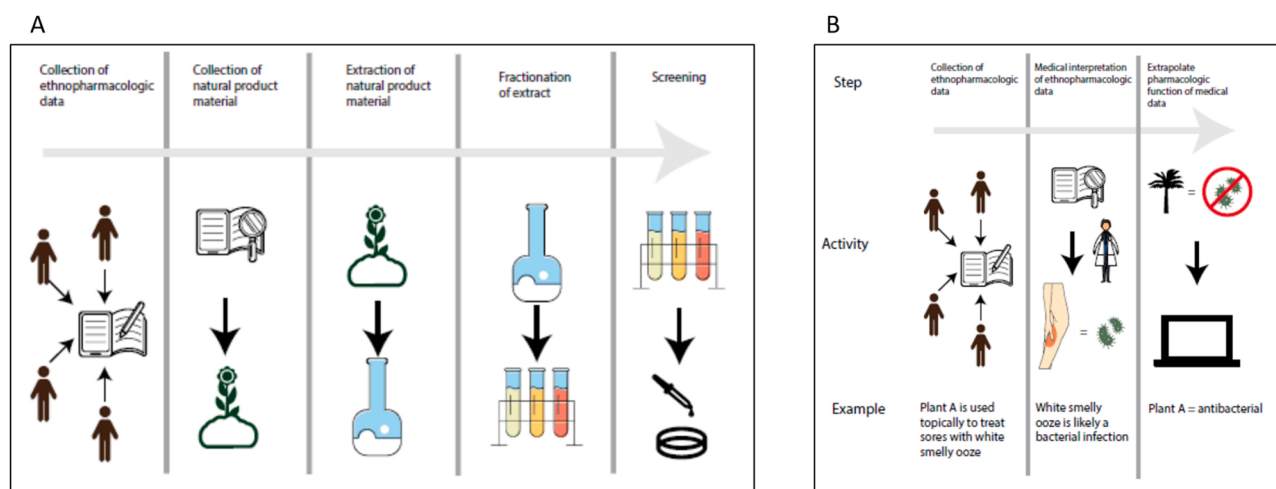


Fig. 2. A. The ethnopharmacological approach to drug discovery. Ethnopharmacological data based on traditional use of a natural product is collected and compiled into a database. The plants described with potential medicinal use are collected and extracts of the plants created. Those crude extracts are fractionated to remove the common potential confounders and improve hit rate in standard screening assays. B. The requirement for a standardized resource to allow the broad and regular application of ethnopharmacological data in the drug discovery process. Currently, the application of ethnopharmacological data is irregular and the data disparately available except for a few notable resources. In order for ethnopharmacological data to be used regularly in the drug discovery process a standardized resource containing the extrapolated pharmacologic function is necessary. In this example, there is documentation that the specific natural product is used topically to treat sores with a white smelly ooze. That documented treatment paradigm is extrapolated to be treating a topical bacterial infection. Creation of a standardized database with these extrapolated pharmacologic functions would allow efficient screening natural products with a historic use.

compounds [4–7] and there may be advantages further down the drug development pipeline. However, these ethnopharmacological data have yet to be broadly integrated and employed in drug discovery and development. There is, however, an exciting resurgence of natural products in drug discovery [8] and the emerging use of ethnopharmacological data [9,10] has the potential to further accelerate the search for new therapeutics [3].

2.1.1. The opportunity to use ethnopharmacological data as a tool in drug discovery

Ethnopharmacology is based on the recognition that natural products have been used as therapeutics throughout history [11]. This theory is centred on the hypothesis that an iterative trial-and-error approach was used to develop a collective understanding of the medicinal properties of natural products in the immediate environment (Fig. 1). This collective medicinal understanding can be used as a tool to extract information regarding the therapeutic properties of the natural product [12,13]. Employing ethnopharmacology in the process of drug discovery and development is grounded in the information regarding how natural products are used as medicines. A collection of these data requires working directly with the individuals who are knowledgeable regarding the uses of the natural products (Fig. 1), or using resources such as historic texts describing how medicinal natural products have been used [13]. The value of using these historic resources is that it is possible to resurrect traditional medicine knowledge that may be lost through time [13–15], and these efforts have been successful [12,16] and in one situation resulted in a clinical investigation [17]. However, work focusing on using documented historical knowledge is obviously predicated on the accurate documentation of the historic use of the natural products in the first instance [18], and the heterogeneity of approaches to the documentation of the uses of medicinal natural products [19] and the varying quality of the ethnopharmacological assessment [20] are particular challenges. Fortunately, guidelines for rigorous standards have been developed [13] which allow statistical methods to be applied [21,22] as the basis for a bioinformatics analysis assessing how well the ethnopharmacological data fit expected models [23].

Screening of natural products is a resurging drug discovery paradigm grounded in a range of advantages over combinatorial chemistry libraries [8]. Introducing ethnopharmacology into this

natural-product-based investigation allows further, more informed, refinement of the starting compounds for the screening effort (Fig. 2A). Thus, as tools to take advantage of these benefits, and in some situations secure intellectual property, there are multiple databases of natural products such as the Universal Natural Product Database [24], Drug Discovery Portal [25] and NAPRALERT [26] that provide information for virtual screening programs. Furthermore, other databases such as the Chinese Natural Product Database [27], Database from Historic Medicine Plants [28], AfroDb [29], and NuBBE [30] provide information on natural products used in the traditional medicines. While these natural products based in traditional medicine use have been used to conduct virtual screening programs [8,12,16] the traditional uses of these natural product resources are not always clearly included in the databases and the diversity of approaches to documentation can be problematic [19].

Beyond these databases, there are countless isolated studies conducted by various groups throughout the world, documenting the historic use of natural products as medicines. A sorely needed resource in the field of ethnopharmacology is a comprehensive database that allows mining of the extrapolated pharmacologic function of a natural product from the historic uses of the natural product material (Fig. 2B). Although there are standardized resources to categorize diseases [31] which could provide the basis for this extrapolation, and some databases, such as NAPRALERT™ provide pharmacologic information [26], there is no systematic resource for extrapolation of pharmacologic function from the reported uses of medical plants. This proposed database resource would facilitate prospective evaluation to determine the value of including the ethnopharmacological data in the natural product drug discovery process.

2.1.2. The risks of using ethnopharmacological data in the drug discovery process

While the theory underpinning the use of ethnopharmacology as a tool in drug discovery is uncomplicated, and the promise of using ethnopharmacological data in the process is exciting, there are risks to foundations of the field and the opportunity is irrefutable:

- The two greatest risks are the generational loss of this medicinal knowledge [32,33] and the loss of the biodiversity of natural product

materials used in traditional medicines [34,35]. These two extinction processes are particularly problematic because the loss of a species used for medicinal purposes makes the generational passing of knowledge difficult, and potentially impossible. There are multiple studies showing that the loss of species, or reduction in prevalence, requires healers to seek substitute species [36]. Thus, efforts to document the ethnopharmacological use of natural products are critical and simultaneously demonstrate the value of these data through incorporating this information in the drug discovery and development processes.

- Detractors from ethnopharmacology as a tool in drug discovery posit that the drug discovery process is too complex to randomly get correct [37] based upon the fact that the robust design and detailed interpretation of randomized double-blind placebo-controlled studies are complex, requiring rigorous training and methodology that was unavailable until relatively recently. Nevertheless there are numerous examples of ethnopharmacological data leading to new therapeutics [38–40].
- Early efforts to employ ethnopharmacology as a tool in bioprospecting were confounded by issues around intellectual property and equity sharing; however now there are established frameworks and templates to navigate this potential challenge (see Section 5.5).

2.1.3. The future value of ethnopharmacology in drug discovery

Clearly screening both combinatorial chemistry and natural product libraries is an effective approach to developing new drugs. However, the discovery of artemisinin [39] and other ethnopharmacological successes [40] shows that there is value in leveraging historic medicinal knowledge of natural products. While the contemporary contribution of ethnopharmacology to drug discovery has generally been intermittent, the goal moving forward, particularly in the context of the resources available today, is to institutionalize the use of ethnopharmacology to augment the drug discovery process and thereby accelerate the identification of new therapeutics. While it would be unwise to exclude combinatorial chemistry libraries in screening efforts, it has been suggested that frontloading the screening process with natural products could potentially be beneficial [8]. We agree with that suggestion and extend it further: frontloading the screening process with ethnopharmacological data has the potential to raise the hit rate even further and accelerate the discovery of new therapeutics.

There has never been a more appropriate time to integrate ethnopharmacological data into the drug discovery and development processes, and a concerted effort is required as these ethnopharmacological

resources, and therefore their use as tools to facilitate the discovery of new therapeutics, gradually disappear. One good example of how ethnopharmacological data can support drug discovery comes from the historical treatment of malaria with natural products such as quinine (from the cinchona tree; 45), only to be supplanted by the synthetic drug chloroquine starting in the 1960s [41]. Unfortunately, resistance to these compounds was soon established and continues to spread [42]. In 1960, Youyou Tu initiated a screening program of Chinese Traditional Medicines for antimalarials to support the communist allies of the Vietnam War [43–45]. Extracts of *Artemisia annua* appeared promising, but inconsistent, until the first-in-class compound, artemisinin, was isolated and identified. Importantly, artemisinin affects the parasite earlier in the life cycle compared to other available therapeutics, and in 2015 the Nobel Prize in Medicine was awarded for its discovery. However, the exact mechanism of action remains to be identified and is believed to be promiscuous [46]. Unfortunately, resistance to Artemisinin is also established [47] and new therapeutics continue to be needed.

With respect to bioprospecting, India is unique in the efforts it makes to preserve [48] and protect the intellectual property [49] surrounding the uses of their traditional medicines [50] and maintain the desire to keep ties with the historic use of the traditional medicine material [51]. Specifically, the Council of Scientific & Industrial Research has created the Traditional Knowledge Digital Library (<http://www.tkdil.res.in/>) with details of ~250,000 formulations [52] for global patent offices to use as a reference tool for determining prior art. This resource has become particularly important because of the resurgence of natural products and traditional medicines as a tool for drug discovery [8]. Furthermore, the Indian Council of Medical Research (ICMR) has worked to validate the efficacy of many traditional Indian therapies and curate resources of historic use of medicinal plants [53,54]. This effort is globally important as ~80% of the world's population rely on traditional medicines for their primary healthcare tool [55] and Indian traditional medicines are used globally [56,57]. There is a call for more structured and rigorous evaluations of Indian traditional medicines [55, 58,59] and the Council of Scientific & Industrial Research has built a robust foundation to facilitate these necessary investigations.

A popular biopiracy legend employed to illustrate the purported evils of bioprospecting natural products surrounds the identification of vincristine and vinblastine from *Catharanthus roseus* [60]. While the legend varies, the general story is that scientists at Eli Lilly & Company collected *C. roseus* in collaboration with individuals in Madagascar and identified the plant as a potential chemotherapeutic. From this

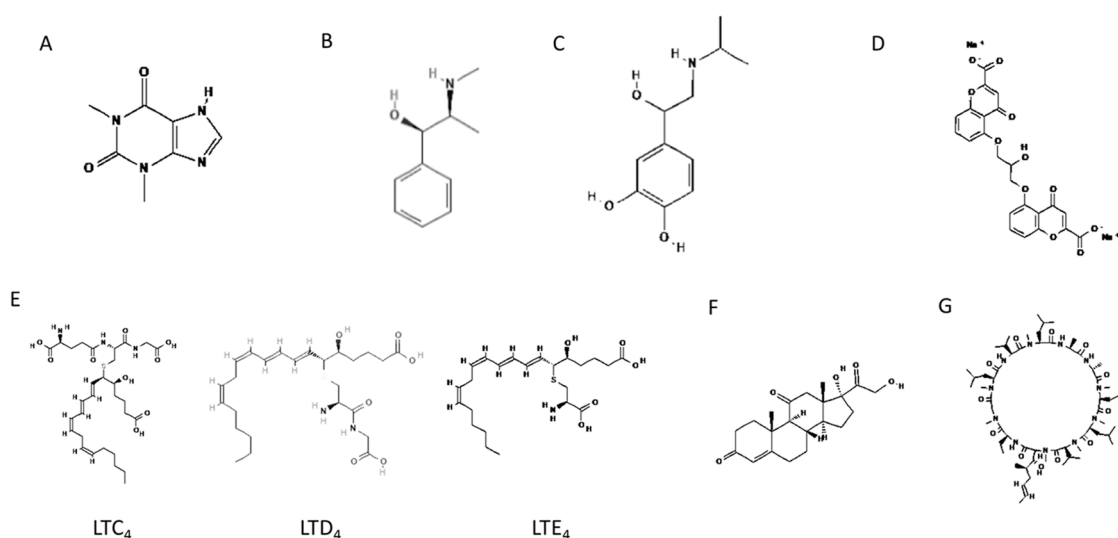


Fig. 3. Chemical Structures of early natural product-derived drugs. A – Aminophylline (theophylline derivative); B – Ephedrine; C – Isoproterenol; D - Sodium cromoglycate; E - LTC₄, LTD₄ and LTE₄; F – Cortisone; G- Cyclosporin.

information, Eli Lilly & Company developed Velban® and Oncovin® which generated a fortune for the organization, none of which went back to individuals in Madagascar. The truth is different; since the mid-1700s *C. roseus* has certainly been cultivated globally allowing it to be integrated into traditional medicine practices in numerous countries as a treatment for diabetes, not cancer [61]. The chemotherapeutic potential of *C. roseus* was identified by a chance observation in 1958 by a Canadian investigator evaluating samples of *C. roseus* collected in Jamaica [62]. The United Nations Convention on Biological Diversity assigns the sovereign right to the providing country; however, as this plant is spread throughout the world and was not previously used to treat cancer, there was no biopiracy [63]. The important message contained in this story is that bioprospecting is not inherently evil, but that assigning intellectual property rights is challenging [64] and has the risk of becoming emotionally charged.

2.2. Natural product and natural product-derived NME's - early immunosuppressive drugs (Before the Late 1970s)

Although today, (06/2021) there are many drug entities that will suppress (or if required, enhance) human immune processes, until the advent of cyclosporin and a few other agents with similar activities (all isolated from microbes in the later part of the 20th century) only a few drug classes were available to aid in treatment of immunological diseases of man, almost all of which required suppression of overactive immune systems. The range of materials available for such use in 1970 and earlier was well covered in a review by Aisenberg in 1971 [65]. Suffice to say, none of the agent classes listed, with the exception of the corticosteroids (covered later), were used to any extent after the identification/use of the microbial products (cyclosporin, rapamycin, the FK series etc.) in the early to late 1970s. Those readers who are old enough will remember the failures of attempts at long-term organ replacements, unless from very closely matched donor/recipient pairs from an HLA perspective. Even then, the majority of transplanted organs were rejected by the recipient(s) in relatively short time frames (frequently less than a year).

This section will consider the early/historical treatment of asthma with natural products and their derivatives, as an optimal example of both an allergic and an immune related disease and briefly discuss the revolution caused by the discovery of cyclosporins and similar drugs as immunosuppressants in the 1970s

2.2.1. Natural product-derived immunosuppressants and asthma

2.2.1.1. Theophylline and derivatives. One "relative success" in the early days, before the full recognition that asthma had an IgE component in many cases of this disease, was the use of the purine derivative theophylline and simple derivatives (Fig. 3A) before the advent of bronchodilators (vide infra). Theophylline was first isolated from tea leaves in the middle 1880s, with the first reports by Kossel in 1888 [66] and 1889 [67] followed relatively soon afterwards by total syntheses by Fischer and Ach [68] in 1895 and a much fuller report covering syntheses of xanthines, including theophylline, by Traube [69] in 1900. Since there are no useful natural sources of theophylline that can be "extracted to give large quantities" today (despite reports in the late 1990s that there were elevated levels in some cocoa beans; *T. cacao*, strain Criollo), then either total synthesis or simple demethylation of caffeine is used. Granted these are not immunomodulators, but in the early 1900s the basic treatment for asthma could be best described as "opening the passageways", as the only (partially) effective technique. A 1947 paper by Prigal et al. [70] gives some of the history of the usage of theophylline and its simple derivatives in the USA, beginning with the report by Herrmann and Aynesworth [71] of the IV use of the theophylline derivative aminophylline (theophylline ethylene diamine; Fig. 3A) in 1937. In 1941, the subsequent review by Young and Gilbert

covered the use of the same theophylline derivative [72]. Even today, some variants of theophylline remain part of the physician's armamentarium in specific cases.

2.2.1.2. Adrenergic agents and derivatives. Initially these agents, which were either products isolated from the adrenal gland(s) of mammals or synthetic chemical analogues that bound to the same receptor site(s), were based upon adrenaline (ephedrine; Fig. 3B). Adrenaline itself was first isolated from adrenal glands in 1901 by Takamine [73]. Although not related to asthma or immuno-modifying agents, a 2016 review by Newman¹⁰ covered the history of these agents from a chemical/biochemical aspect and pointed out that the later concept of different types of β -adrenoceptors enabled the pharmacology of these agents/derivatives to be understood at the molecular level as time and usage evolved. As treatments evolved pre and during WWII, the use of isoproterenol (Fig. 3C), a non-specific β -adrenoceptor agonist first synthesized in the late 1930s¹¹, took over from adrenaline and, as shown in Newman, [74] many more specific agents directed at adrenoceptors have now been discovered and remain in use.

2.2.1.3. Mixed treatments including sodium cromoglycate. Interestingly, there are numerous publications (even into the 1970s) that continued to advocate the use of bronchodilators plus theophylline, and even suggested the use of sodium cromoglycate (Fig. 3D), in the management of asthma. Although a synthetic compound, the base structure of sodium cromoglycate was related to was related to khellin, a plant chromone, and was used for some years as a direct treatment for asthma. Sodium cromoglycate acts on mast cells, to inhibit the release of histamine and "slow reacting substance of anaphylaxis" (SRS-A) when challenged by an appropriate antigen [75]. In 1980, SRS-A was identified as a mixture of leukotrienes (Fig. 3E) LTC₄, LTD₄ and LTE₄ which, following secretion from mast cells, induces inflammation and is a major bronchoconstrictor in asthma [76]. Thus another immunoactive secretion was identified that had significant effects on the etiology of asthma. A review by Lai and Manley [77] in 1984 explains in more detail the interplay in the arachidonic acid cascades and has extensive citations to prior chemical, biochemical and pharmacological reports. A more recent review by Di Gennaro and Haeggstrom in 2011 on these agents provides information on later developments on their involvement in immune-related diseases [78].

2.2.1.4. Corticosteroids. Concomitant with the use of bronchodilators was the recognition that administration of corticosteroids could help reduce the severity of asthmatic attacks, since it was assumed at the time that this disease was always an inflammatory response to allergens and steroids were a primary treatment for inflammation. The compounds used ranged from "natural steroids" (though usually made synthetically) to "molecules related to the basic steroid chemical nucleus" but modified to give better pharmacological properties. (Fig. 3F). Until the advent of synthetically modified agents, cortisone or its hydroxy equivalent cortisol were the major steroids used. In the review by Aisenberg [65], there is one reference to the "extensive use" of prednisone as an immunosuppressant in man [79]. In the 7th (1985) edition of Goodman and Gilman [80] a table of relative anti-inflammatory potencies of the corticosteroids demonstrates that, although the natural agents had up to a four-fold increase in efficacy when compared to cortisol (hydrocortisone), fluorine substituted derivatives were up to 25 times more effective as anti-inflammatory agents. Likewise, in the 2nd (1980) edition of Bowman and Rand [81], there is a comparable table that also shows the greater efficacy of the fluorinated corticosteroids such as triamcinolone and beclomethasone.

2.2.2. Microbial-derived immunosuppressants

2.2.2.1. Cyclosporin. Although microbial products as antibiotics were

HEALTHCARE : Two Worlds

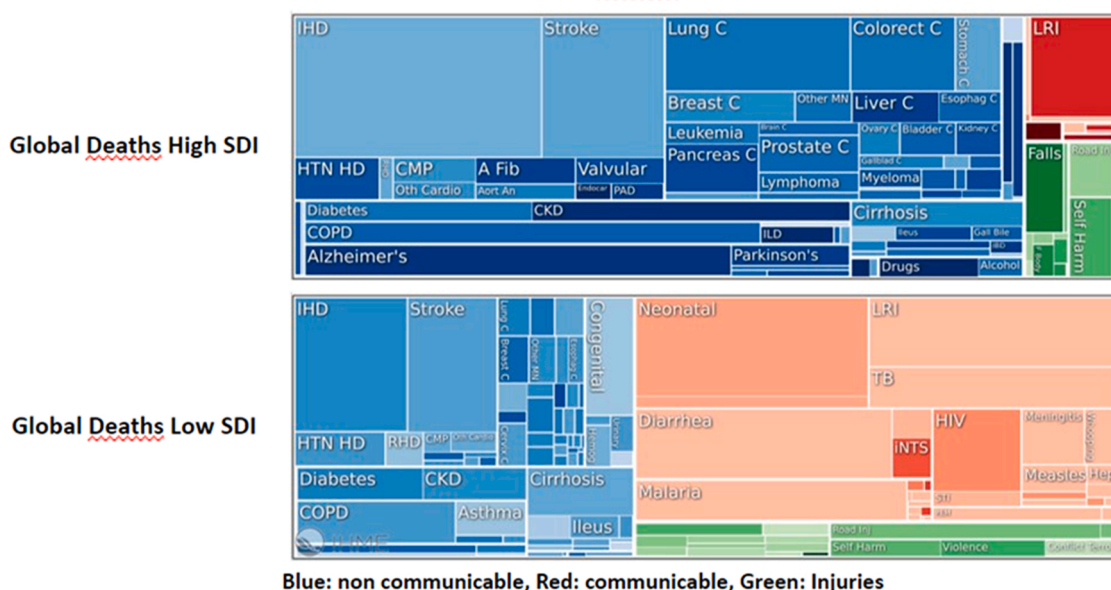


Fig. 4. Causes of mortality differ markedly between rich and poor countries (social development index, SDI, average of the rankings of the incomes per capita, average educational attainment, and fertility rates) worldwide. Data are from Institute of Health Metrics and Evaluation, University of Washington (<https://vizhub.healthdata.org/gbd-compare/> organized by MS), 2019, pre-COVID.

well established by the middle 1950s, it was not until the report in 1976 by the Sandoz group, led by Stahelin, on the discovery of cyclosporin (Fig. 3G) that opened up the potential for their use in organ transplantation from non-matched donors [82]. Cyclosporine, isolated from the fungus *Tolypocladium inflatum*, was initially discovered in 1971 using a screening test for immunosuppression, and was further confirmed the same year. That it had potential immunosuppressive activities, and indeed was the first immunosuppressive drug that allowed selective immunoregulation of T cells without excessive toxicity, is covered in an exhaustive report published by Rügger et al. [83]. The first report of the use of cyclosporin in human transplantation was the paper in the Lancet by Calne et al. [84], and since then it has been one of the mainstays of immunosuppressive therapy for transplantation. A second microbial-derived compound, rapamycin (also known as sirolimus), was first isolated from the bacterium *Streptomyces hygroscopicus* in 1972 and was initially developed as an antifungal agent [85] until its immunosuppressant properties (through inhibition of mTor) were identified. Rapamycin is now often used alongside a further microbial-derived immunosuppressant tacrolimus (FK506), which was first isolated from the soil bacterium *Streptomyces tsukubaensis* in 1987 [86], that binds to the protein immunophilin (FKBP12) to prevent activation of nuclear factor of activated T-cells (NF-AT). Historically speaking, these agents and derivatives effectively revolutionized the management of rejection following organ transplantation.

3. Sources of natural products as novel immunomodulating drugs

3.1. The importance of natural products immunopharmacology

Noncommunicable diseases (NCDs) include all the diseases that are not transmissible directly from one person to another. They comprise cardiovascular disease, cancer, chronic respiratory diseases, diabetes, neurological disorders and most of the chronic inflammatory pathologies. It is now established that the immune system and inflammatory processes are involved in most NCDs that represent the most common cause of mortality and morbidity worldwide [87]. Furthermore, communicable diseases (CDs) are pivotally dependent on immune

system responses (see below) where the effects of natural products have not been fully evaluated. The difference between rich and poor in their susceptibility to CDs and NCDs is still highly apparent (Fig. 4), so the contribution of immunopharmacology in the developing world is crucial to healthcare. Nevertheless, the Global Burden of Disease (GBD) report published in 2019 [88] has clearly shown that most of the sub-Saharan African countries are undergoing a demographic transition leading to increasing prevalence of NCDs, as is already occurring in countries such as India. This is posing an increasing challenge for the fragile and overburdened health systems which are the norm in many low- and middle-income countries (LMICs), and which have to date largely focused on tackling infectious diseases and maternal/child mortality. In this context, the access to affordable and reliable immunomodulatory and anti-inflammatory drugs will be key for the future of LMICs and will help to achieve the United Nations' Sustainable Development Goal 3.4 of "a one-third reduction in premature deaths between 30 and 70 years of age from the four major NCDs".

To date, we have access to broad spectrum of immunomodulators, from the conventional glucocorticoids that are still widely used, and indeed one of the very few drugs able to significantly reduce mortality in hospitalised severe COVID-19 patients [89], to the broad range of monoclonal antibodies widely used in chronic inflammatory disorders [90]. More recently, checkpoint inhibitors have represented a major breakthrough in cancer immunotherapy, being able to boost the immune system curing patients with end-stage cancer, a discovery that led to the award of the 2018 Nobel Prize in Physiology or Medicine to Tasuku Honjo and James Allison [91]. However, the major factor limiting the widespread use of biologics in LMICs is their prohibitive cost. The potential market for novel and established NP immunotherapies is huge worldwide and will allow greater patient access to treatment through cost savings. The following sections will give some insight as to the existing range of immunomodulating agents available from a variety of natural sources, to provide a starting point for further exploitation of these natural resources for the development of novel drugs acting on the immune system.

3.2. Terrestrial plants as sources of Immunomodulators

3.2.1. Vaccine adjuvants

The role of terrestrial plants as sources of medicines is evolving; plants are not just confined to providing or inspiring pharmaceutical drugs as 'single active ingredients', or to their value as traditional medicines globally. Indeed, the soapbark tree (*Quillaja saponaria*), native to Central Chile [92], provides vaccine adjuvant chemicals (QS-21) for our armory against diseases including COVID-19, the coronavirus that caused a global pandemic in 2020 (Supplementary Table 1). QS-21 is a mixture of two isomeric triterpene saponins that have the same adjuvanticity and only differ in the terminal sugar unit (either β -D-apiofuranosyl or β -D-xylopyranosyl) of the tetrasaccharide connected to the C28 carboxyl group of the quillaic acid aglycone [93]. There are some limitations for the use of QS-21 as a vaccine adjuvant, including chemical instability, dose-limiting toxicity, and supply and purification challenges, therefore QS-21 is typically formulated as a matrix (e.g. ISCOMATRIX™, Matrix-M™) with other agents to retain stability, adjuvant activity and to reduce toxicity [94]. Alternative strategies to overcome these limitations are also being evaluated, which include investigation of other *Q. saponaria* saponins, and the development of synthetic analogues and conjugates [95–97]. These alternatives could offer hope for more sustainable sources of effective vaccine adjuvants for the future. For example, QS-7, also from *Q. saponaria*, has similar adjuvanticity to QS-21 (induces a balanced Th1/Th2 response and potent CD8 + CTL production) but is less haemolytic, so has been used as a template to design synthetic analogues, including those with different immunomodulatory profiles [95]. A range of other terrestrial plants have been the subject of scientific research to identify saponins with immunomodulatory effects that have potential for development as future vaccine adjuvants; these include *Q. lancifolia*, a tree native to Peru, Brazil, Uruguay and Argentina, in addition to plants in various other genera [98] (Supplementary Table 1).

To meet global demands for vaccine manufacture, there has also been interest in the development of carbohydrate-based adjuvants, including those derived from plants, as a number of these activate both humoral and cellular immune responses against pathogens. Plant-derived polysaccharides, including fructans (e.g. inulin), arabinans (e.g. from almond [*Prunus amygdalus*] seeds), mannans and glucans, have shown adjuvant effects, as reviewed [99,100]. Polysaccharides from *Angelica sinensis*, *Alhagi pseudoalhagi*, flaxseed (*Linum usitatissimum*) and *Actinidia eriantha* mediate adjuvant effects, thus are under investigation for this purpose [100,101]; while ginsan, a polysaccharide from ginseng (*Panax ginseng*) shows immunomodulatory and adjuvant effects, the latter when combined with an H5N1 influenza vaccine [102]. Ginseng saponins also show promise as potential vaccine adjuvant candidates (Supplementary Table 1).

A number of carbohydrate-based vaccine adjuvants are under investigation in clinical trials and have been the subject of patents; these include a novel plant saccharide based on β -glucopyranosiduronic acid, a trehalose glycolipid derivative, and glucans [100]. α -Glucans with adjuvant activity include those from the roots of certain *Astragalus* species (also a source of adjuvant saponins; Supplementary Table 1), *Tinospora cordifolia*, *Lonicera japonica* and *Actinidia chinensis*; this class of adjuvants (including glucan derivatives) has been of particular interest for application as mucosal adjuvants due to their biocompatible and biodegradable nature, although their isolation and purification poses technical challenges [99], and if not adequately purified, may be toxic [100]. The β -glucan curdlan (occurs in higher plants and also fungi) and its derivatives have also been investigated as potential nasal mucosal adjuvants [100]. Carbohydrates derived from traditional Chinese medicinal plants are also under evaluation as potential vaccine adjuvants [103]. More research is needed to further assess the potential of carbohydrate-based adjuvants for vaccine development, particularly their potential as mucosal adjuvants, and their efficacy and safety. If the technical challenges of the chemical complexity and purification of

carbohydrate-derived adjuvants can be overcome, there is much potential to develop these as biocompatible vaccine adjuvants, derived sustainably from plant sources, to expand the currently limited repertoire of available vaccine adjuvants.

With the global demand for vaccines against current and emerging infectious diseases, the search for suitable vaccine adjuvants may accelerate. Yet it has been proposed that the future of vaccine adjuvants will involve advances in synthetic components, rather than natural products [104]. However, if effective natural product analogues can be synthesised, the role of natural products as lead scaffolds for adjuvant use would remain important in future vaccine development. Furthermore, the untapped potential of terrestrial plants as potential sources of useful vaccine adjuvants remains largely unexplored; indeed, there are an estimated 343,000 vascular plant species known to science [105] with the majority not explored for their potential to yield next generation adjuvants or indeed, new pharmaceuticals for other medicinal applications. A recent review highlights the unmet need for new candidate adjuvants, emphasising that their discovery needs to be more systematic, combining high-throughput screening, computational virtual screening and QSAR optimisation [106]. Here we propose that a systematic, more strategic, focused and efficient approach for vaccine discovery from plants could also be underpinned by applying phylogenetic approaches for drug discovery (see 5.1).

Another consideration is that plant-derived vaccine adjuvants should be sustainably sourced, so that obtaining the required plant material does not threaten the survival of species. For example, harvesting certain plant parts, particularly bulbs, bark and roots, can be destructive and often leads to the death of the plants [107]. Therefore, of the potential vaccine adjuvants from plant sources already identified (including those in Supplementary Table 1), or to be identified in future, those sourced from species in a way that is not destructive to the plant, and sourced sustainably from cultivated instead of wild-harvested material, or sourced through other technologies, such as through plant cell cultures or total synthesis, would be preferred so that strategies for sourcing them are harmonised with those for biodiversity conservation. Other scientific advances may also support more sustainable sourcing of vaccine adjuvants originally from plants. Understanding the genes and enzymes involved in the biosynthetic pathways for synthesis of natural product vaccine adjuvants, mean these pathways can be expressed in other organisms (fungal, plant or bacterial) such that the resulting engineered 'cell factories' offer an alternative way to synthesise plant natural products for medicinal applications [107]. This approach has already been shown to be successful for the synthesis of certain pharmaceutical compounds originally produced by plants [107] and could enable the more sustainable production of natural product-derived adjuvants for the future to meet global demands for vaccine production.

3.2.2. Immunomodulators for cancer treatment

In addition to infectious diseases, modulation of the immune system is also a target in current and emerging cancer therapies [108]. In addition to plant-derived vaccine adjuvants (discussed above; Supplementary Table 1) for use in cancer immunotherapy, certain plant compounds can modulate the immunogenicity of cancer cells [108]. For example, the naphthoquinone shikonin from *Lithospermum erythrorhizon* root (a traditional Chinese medicine) and cardenolides and their glycosides from foxgloves (*Digitalis* species) induce the hallmarks of immunogenic cell death; for example, shikonin induces immunogenic apoptosis, while cardenolide-based compounds stimulate anticancer immune responses in vivo [108]. Other plant-derived compounds can also modulate immunogenic cell death, including those already in clinical use for cancer, such as paclitaxel (originally from the Pacific yew tree, *Taxus brevifolia*), which although has the principal mode of action of stabilising microtubule assembly [109], new insights into the immunogenic effects could refresh current approaches for its clinical use. Colchicine, an alkaloid from the autumn crocus (*Colchicum autumnale*) is used for gout [110], but its ability to modulate immunogenic cell

Table 1
Marine natural products with anti-inflammatory activity and affecting the immune system in 2016–2017.

Drug Class	Natural product/ marine source ^a	Chemistry	Pharmacological activity	IC ₅₀ ^b	MMOA ^c	Country ^d	References
Anti-inflammatory	AMT-E/alga	Terpenoid ^e	Murine colitis inhibition model	10 mg/kg* *	Inhibition of TNF- α , IL-6	ESP, MAR	[131]
Anti-inflammatory	<i>Bacillus</i> sp. diketopiperazines /bacterium	Peptide ^f	Murine sepsis model	10 μ M* *	TGF β 1p inhibition in vivo	S. KOR	[132]
Anti-inflammatory	6-bromoisatin/mollusk	Alkaloid	Murine lung inflammation model	0.05 mg/g* *	Inhibition of TNF- α , IL-6	AUS	[133]
Anti-inflammatory	excavatulide B/soft coral	Terpenoid ^e	Rat arthritis model	2.5,5 mg/kg* *	Decreased MMP-2, MMP-9, CD11b in tissues	CHN, TWN	[134]
Anti-inflammatory	fucoxanthin/alga	Terpenoid ^e	Murine paw edema and ear inflammation model	4 mg/kg* *	Modulation of iNOS, PLA ₂ , COX-2, ACC, IL-6 and Nrf2 expression	JPN, S. KOR, MEX	[135]
Immune system	LPS/cyanobacterium	Polysaccharide/ Polyketide ^g	Rat brain microglia model	10 μ g/mL*	TNF- α , IL-6, MMP-9, MIP-2, IP-10, MIP-1 α , MCP-1	USA	[139]
Immune system	cucumarioside A ₂ -2/sea cucumber	Terpenoid ^e	Murine spleen and macrophage activation model	3 mg/kg* *	Increased B cell PCNA and M1 macrophages	RUS, TWN	[138]
Anti-inflammatory	cucumarioside A ₂ -2/sea cucumber	Terpenoid ^e	Murine macrophage P2X purinergic receptors binding	0.02 μ M*	Induction Ca ²⁺ oscillations	RUS	[137]
Anti-inflammatory	curvularin derivative/fungus	Polyketide ^g	Murine macrophage PGE ₂ and NO release inhibition	1.9–2.7 μ M	NF- κ B signaling inhibition	S. KOR	[138]
Anti-inflammatory	9,11-dihydrogracilin A /sponge	Terpenoid ^e	PBMC proliferation inhibition	3 μ M*	IL-6 and IL-10 inhibition	ITA	[140]
Anti-inflammatory	ogipeptins A-D /bacterium	Peptide ^f	Human macrophage TNF- α release inhibition	1 μ M *	Block LPS binding to CD14	JPN	[141]
Immune system	gracilins A, H and L/sponge	Terpenoid ^e	Human peripheral lymphocytes IL-2 release inhibition	1 μ M *	CD147 receptor modulation	ESP, GBR	[142]

Abbreviations: ACC: acetyl-CoA carboxylase; CD: cluster of differentiation; COX: cyclooxygenase; IL: interleukin; iNOS: inducible nitric oxide synthase; IP-10: interferon gamma-induced protein 10 kDa; MCP-1: monocyte chemotactic protein-1; MIP-1 α : macrophage inflammatory protein-1; MIP-2: macrophage inflammatory protein-2; MMP-9: matrix metalloproteinase-9; NF- κ B: nuclear factor kappa-light-chain-enhancer of activated B cells; NO: nitric oxide; Nrf2-ARE: nuclear transcription factor E2-related factor antioxidant response element; PBMC: human peripheral blood mononuclear cells; PCNA: proliferating cell nuclear antigen; PLA₂: phospholipase A₂; TGF β 1p: transforming growth factor β - induced protein; TNF- α : tumor necrosis factor- α .

^a Organism: *Kingdom Animalia*: coral (Phylum Cnidaria); sea cucumber (Phylum Echinodermata); turril snail (Phylum Mollusca); sponge (Phylum Porifera); *Kingdom Fungi*: fungus; *Kingdom Plantae*: alga; *Kingdom Monera*: bacterium.

^b IC₅₀: concentration of a compound required for 50% inhibition, * : apparent IC₅₀, ** in vivo study.

^c MMOA: molecular mechanism of action.

^d Country: AUS: Australia; CHN: China; ESP: Spain; GBR: United Kingdom; JPN: Japan; MAR: Morocco; MEX: Mexico; RUS: Russian Federation; S. KOR: South Korea; TWN: Taiwan.

^e Terpene.

^f Nitrogen-containing compound.

^g Chemistry: Polyketide.

death has stimulated interest in its potential use in cancer immunotherapy [108].

3.2.3. Other plant-derived compounds as immunomodulators

Plant-derived steroidal compounds such as diosgenin from yams (*Dioscorea* species) and hecogenin from *Agave sisalana* have been used in the manufacture of pharmaceutical corticosteroids to provide drugs for immune-mediated disorders [107,110]; although other than this role in drug manufacture, plant compounds have had a limited role in immunomodulatory drug development. Greater scrutiny of plant compounds is therefore needed if plants are to be a future source of new immunomodulatory drugs. Since numerous plant-derived alkaloids have been instrumental in drug discovery for various diseases [110,111], this compound class could be prioritised for future immunomodulatory drug discovery. Certain plant alkaloids with immunomodulatory activity have been investigated in this respect, including those with potential applications in autoimmune disorders (e.g. alkaloids from *Thalictrum cirrhosum*) [112], and in type I diabetes (e.g. tetrandrine from *Stephania tetrandra*) [113].

3.2.4. Drug repurposing opportunities

The future for natural product immunomodulators derived from, or inspired by, plant compounds could also be in drug repurposing. For example, one compound class of current and future interest to develop as potential immunomodulatory drugs are the cannabinoids, which occur in *Cannabis sativa*. Natural cannabinoids or synthetic derivatives have pharmaceutical applications for anti-emesis following chemotherapy, managing seizures and for multiple sclerosis (MS). Cannabinoids have been shown to target cannabinoid 2 receptors expressed on immune cells, including CNS microglia, to modulate immune responses during inflammatory processes [114]. Although nabiximols (includes cannabidiol and dronabinol) are currently used for symptomatic relief of spasticity in MS [115], the immunomodulatory effects of cannabinoids have opened up other opportunities for their therapeutic use in MS beyond reducing spasticity, and for use in other diseases. Cannabinoids, cannabidiol in particular, have been evaluated in MS models and they improve disease outcomes and mediate immunomodulatory effects via a range of mechanisms, including suppression of T-cell proliferation, as reviewed previously [114,116]. In animal models of arthritis and systemic sclerosis, cannabinoids show immunomodulatory effects; however, when they have been evaluated for efficacy in RA, osteoarthritis and fibromyalgia in clinical trials, results have been inconclusive; therefore, robust controlled trials are needed to establish whether cannabinoids could modulate disease progression and symptoms, not only in rheumatic diseases [117], but other diseases involving immune function. Another pharmaceutical drug example for potential drug repurposing is the hypoglycaemic drug metformin, a compound with a chemical structure that has its origins in an alkaloid that occurs in the plant goat's rue (*Galega officinalis*) [110]. Although a drug licensed for use in diabetes, metformin's immunomodulatory effects have sparked interest in repurposing this drug for treating COVID-19 [118] and tuberculosis [119].

3.3. Marine organisms as sources of immunomodulators

3.3.1. Marine-derived anti-inflammatory and immunomodulating natural products

The anti-inflammatory and immune system pharmacology of marine natural products (MNPs) has been consolidated in a series of comprehensive and systematic reviews since 1998 [120–130]. This brief overview is limited to immunomodulating MNPs that have been studied mechanistically in either *in vivo* or *in vitro* experimental models during 2016–2017. The MNPs shown in Table 1 were sourced from a diversity of marine organisms: brown algae, mollusks, sponges, soft corals, sea cucumbers, bacteria and fungi.

In vitro preclinical pharmacology research of MNP was completed

using several *in vitro* experimental inflammation paradigms: murine dextran sodium sulphate (DSS)-induced colitis, murine sepsis, murine lung inflammation, rat arthritis, murine paw edema and ear inflammation, and murine spleen and macrophage activation (Table 1). The meroterpene 11-hydroxy-1'-O-methylamentadione (AMT-E), isolated from the marine brown alga *Cystoseira usneoides*, prevented body weight loss and reduced colonic damage in a murine (DSS)-induced colitis model through a mechanism that reduced myeloperoxidase activity, the release of cytokines tumour necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), and IL-10, and both nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression [131]. Three cyclic peptide diketopiperazines, isolated from Korean marine sediment-derived bacteria *Bacillus* sp. HC001 and *Piscicoccus* sp. 12L081, were found to inhibit lipopolysaccharide (LPS)-induced and transforming growth factor beta-induced protein (TGF β 1p)-mediated septic responses in a murine cecal sepsis and pulmonary injury model [132]. Orally administered brominated indol 6-bromoisatin, isolated from the Australian marine gastropod mollusk *Dicathais orbita*, inhibited inflammation in a murine LPS-induced acute lung injury model by reducing pro-inflammatory TNF- α and IL-1 β production in bronchoalveolar lavage [133]. Lin and colleagues evaluated the terpenoid excavatolide, isolated from the Taiwanese gorgonian coral *Briareum excavatum*, reporting rat paw oedema reduction in two rat models of experimental arthritis by attenuating expression of cathepsin K, matrix metalloproteinase-2 (MMP-2), MMP-9 and IL-17A [134], while the carotenoid fucoxanthin, isolated from the marine edible brown alga *Undaria pinnatifida* was shown to inhibit murine paw edema and ear inflammation by reducing activation of iNOS, COX-2 and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) [135]. Studies on the triterpene glycoside cucumarioside A₂-2, isolated from the Russian sea cucumber *Cucumaria japonica*, demonstrated changes in mouse spleen morphology, B-cell proliferation and macrophage activation, as well as concomitant increase of IL-1 β , iNOS, reactive oxygen species (ROS) and nitric oxide (NO) [136].

In vitro preclinical pharmacology of MNPs was investigated in several *in vitro* inflammation paradigms: murine peritoneal macrophages, murine macrophage/monocyte cell line RAW264.7, rat microglia, human peripheral blood mononuclear cells, human monocyte cell line U937 and human T lymphocytes (Table 1). From these studies evidence has emerged that the triterpene glycoside cucumarioside A₂-2, isolated from the Russian edible sea cucumber *Cucumaria japonica*, potentially interacted with the P2X₄ purinergic receptor on murine macrophage membranes and enhanced reversible ATP-dependent Ca²⁺ signalling, thus leading to the activation of cellular immunity [137]. Moreover, the anti-inflammatory effects of a known curvularin-type metabolite, derived from an Antarctic Ross Sea sponge-derived fungus *Penicillium* sp. SF-5850, potentially inhibited LPS-induced RAW264.7 macrophages production of pro-inflammatory NO and PGE₂, as well as the expression of iNOS and COX-2 by inhibiting NF- κ B signalling [138]. Mayer and colleagues demonstrated that LPS, isolated from the cyanobacterium *Oscillatoria* sp., induced classical and alternative activation of rat brain microglia as well as release of pro-inflammatory ROS, MMP-9, TNF- α and IL-6, and the chemokines macrophage inflammatory protein-1 α and 2, interferon gamma-induced protein 10 kDa monocyte chemoattractant protein-1, and alternative activation cytokine IL-10 [139]. The terpenoid 9,11-dihydrogracilin A (DGA), isolated from the Antarctic marine sponge *Dendrilla membranosa*, was shown to downregulate NF- κ B and inhibit IL-10 in human peripheral blood mononuclear cells, while significantly attenuating mouse ear edema and inflammation [140]. The novel cyclic peptides ogipeptins A-D from the culture broth of the Japanese marine Gram-negative bacterium *Pseudoalteromonas* sp. SANK 71903 were shown to block the binding of LPS to cluster of differentiation (CD) 14 receptors on human U937 monocytic cells *in vitro* and decreased TNF- α release [141] and three terpenoids (gracillin A, H and L) isolated from the marine sponge *Spongionella gracillis*, inhibited human T lymphocyte IL-2 production, as well as CD147 expression by

Table 2
Current marine natural product pipeline of ADCs.

	Targeting Antigen	Payload	Indications	Trade Name (Year approved)	References
APPROVED					
Brentuximab vedotin	CD30	MMAE	Relapsed Hodgkin's Lymphoma (HL) and relapsed anaplastic large cell lymphoma (ALCL)	Adcetris (2011)	[152]
Enfortumab vedotin-ejfv	Nectin4	MMAE	Locally advanced or metastatic urothelial cancer	Padcev (2019)	[415]
Polatuzumab vedotin-piiq	CD79	MMAE	R/R diffuse large B-cell lymphoma (DLBCL)	Polivy (2019)	[416]
Belantamab mafodotin-blmf	BCMA	MMAF	R/R multiple myeloma	Blenrep (2020)	[417]
Disitamab vedotin	HER2	MMAE	Locally advanced or metastatic gastric cancer	Aidixi (China only 2021)	
Phase II/III					
AGS-16C3F (AGS-16M8F)	ENPP3	MMAF	Renal Cell Cancer (discontinued)		[418]
Tisotumab vedotin	Tissue Factor	MMAE	Previously treated recurrent or metastatic cervical cancer		[420]
Ladiratumab vedotin	LIV1	MMAE	advanced gastric and gastroesophageal junction adenocarcinoma		[424]
Telisotuzumab vedotin	cMET	MMAE	c-MET-positive Stage IV or recurrent squamous cell lung cancer		[426]
Enapotamab vedotin	AXL	MMAE	Solid Tumors		Discontinued on Nov 24, 2020 (https://ir.genmab.com/news-releases/news-release-details/genmab-announces-enapotamab-vedotin-update)
Disitamab vedotin	HER2	MMAE	Urothelial cancer	Phase II US/ Approved China	[150]
CX-2029	CD71	MMAE	Advanced solid tumors or DLBCL		[421]
W0101	IGF-1R	Aur0101; PF-06380101	Advanced or metastatic solid tumors		[419]
Phase I					
Farletuzumab-eribulin (MORAb-202)	FR α	Eribulin	Solid tumors		[160]
ARX-788	HER2	Amberstatin 269	Resistant breast and gastric cancers		[162]
Uiftamab rilsodotin (XMT-1536)	NaPi2b	AF-HPA	Solid tumors		[163,428]
ALT-P7	HER2	MMAE	Breast cancer		[164]
PF-06804103	HER2	Aur0101	Breast cancer		[164]
ZW-49	HER2xHER2	N-acyl sulfonamide auristatin	HER2 expressing tumors		[166]
MRG-003	EGFR	MMAE	Relapsed/refractory tumors		[427]
RC-88	Mesothelin	MMAE	Advance malignant solid tumors		[143]
SGN-B6A	ITGB6	MMAE	Advanced solid tumors		[422]
SGN-CD228A	CD288	MMAE	Advanced solid tumors		[423]
FOR-46	CD46	MMAF	Multiple Myeloma (MM)		[425]
A166	HER2	Duostatin 5	Breast cancer		[164]
Cofetuzumab pelidotin	PTK7	Aur0101	Solid tumors		[161]
STI-6129	CD38	Duostatin 5.2	Multiple Myeloma (MM)		[165]

Abbreviations: 1 MOA: molecular mechanism of action; MMAE: Monomethyl-auristatin E, vedotin; MMAF: Monomethyl-auristatin F, mafodotin; AF-HPA: Auristatin F – hydroxypropylamide, rilsodotin. Aur0101: auristatin 0101, pelidotin, DLBCL: Diffuse Large B Cell Lymphoma, MM: Multiple Myeloma, HL: Hodgkin's Lymphoma, ALCL: Anaplastic Large Cell Lymphoma

reducing calcineurin phosphatase activity [142].

3.3.2. Antibody drug conjugates: the role of marine natural products

The concept of targeted therapy (magic bullet) has been around for many years and with the advent of therapeutic use of monoclonal antibodies (mAb) this concept came to fruition in the form of antibody drug conjugates (ADCs) [143]. ADCs are targeted therapies consisting of a targeting antibody (mAb) connected by a linker to a small molecule payload (warhead). The original ADC concept was the targeted delivery of the warhead, typically a highly potent molecule that as monotherapy had an insufficient therapeutic index to be independently approved, thus avoiding, or minimizing systemic toxicities. The development of ADCs has been more complex than originally expected as each of the three components of an ADC requires optimization considering each

individual component and how they interact to provide efficacy [144, 145]. As the technology to develop clinically viable ADCs developed, marine natural products (MNPs) became a dominant source of highly potent payloads, particularly the auristatins and derivatives thereof [143,146,147].

Currently, there are 12 approved ADCs, which includes subsequent re-approval of Mylotarg® at a lower dose and modified dosing schedule [148], and the auristatin containing disitamab vedotin (Aidixi/RC48) that was recently conditionally approved by the Chinese NMPA (June 2021) [149]. Disitamab vedotin had already been granted Breakthrough Therapy Designation by the FDA in September of 2020 and is currently in Phase II clinical trials in the US. (Table 3) [150]. Five out of the twelve approved ADCs contain MNPs or derivatives thereof, as well as a considerable portion of ADCs currently in clinical trials [143,151]. After

Table 3
Marine natural products currently used in ADCs – approved and in clinical trials.

MNP	Source	Description	MMOA ^a	Reference
Dolastatin 10	Mollusc/ Cyanobacterium	Original cytotoxic isolated from <i>Dolabella auricularia</i>	Microtubule polymerization inhibitor	[154, 155]
MMAE	Mollusc/ Cyanobacterium	Permeable derivative of Dolastatin 10	Microtubule polymerization inhibitor	[153]
MMAF	Mollusc/ Cyanobacterium	Impermeable derivative of Dolastatin 10	Microtubule polymerization inhibitor	[153]
Eribulin	Sponge	E7389	Microtubule polymerization inhibitor	[429]
Dolaflexin	Mollusc/ Cyanobacterium	AF-HPA	Microtubule polymerization inhibitor	[163, 428]
Duostatin 5	Mollusc/ Cyanobacterium	MMAF derivative	Microtubule polymerization inhibitor	[164]
Ambrestatin 269	Mollusc/ Cyanobacterium	MMAF analog	Microtubule polymerization inhibitor	[162]
Auristatin 0101 (pelidotin)	Mollusc/ Cyanobacterium	PF-06380101; Aur0101	Microtubule polymerization inhibitor	[159]

Abbreviations: 1 MOA: molecular mechanism of action; MMAE: Monomethylauristatin E, vedotin; MMAF: Monomethylauristatin F, mafodotin; AF-HPA: Auristatin F – hydroxypropylamide, rilsodotin. Aur0101: auristatin 0101, pelidotin.

the first ADC (Mylotarg®; original approval in 2000) was approved, it took 11 years to approve the first MNP containing ADC, brentuximab vedotin (Adcetris) [152]. This new generation of ADCs was ushered in by the utilization of highly potent microtubule inhibitors, the auristatins [153], derived from dolastatin 10 [154,155]. Dolastatin 10, a MNP originally isolated from the gastropod mollusc *Dolabella auricularia* by the Pettit group [154] and subsequently shown to be derived from a marine cyanobacterium *Symploca sp.* VP642 [156]; several of its derivatives have been clinically evaluated as monotherapy but did not achieve a suitable therapeutic index and/or efficacy to gain approval to treat cancer patients [157,158]. Brentuximab vedotin, FDA approved in 2011, is a CD30 targeting mAb attached by a maleimide-based cleavable linker to monomethylauristatin E (MMAE) – CD30 mAb-Vit-Cit-PABC-MMAE [152]. Subsequently, 4 additional ADCs, all containing either MMAE or monomethylauristatin F (MMAF) have been approved by medical authorities (Table 2). Polatumumab vedotin-piiq, FDA approved in 2019, is a CD79 mAb linked to MMAE. Enfortumab vedotin, also FDA approved in 2019, is a Nectin-4 mAb linked to MMAE. Belantamab mafodotin-blmf, FDA approved in 2020, is a BCMA mAb linked to MMAF. Disitamab vedotin, conditionally approved by the Chinese NMPA in 2021, is a HER2 mAb linked to MMAE and is currently in Phase II studies in the US and given Breakthrough Designation by the FDA for treatment of urothelial cancer. The role of MNP toxins is clear in the development of ADCs, with 5 of the 12 approved ADC derived from the auristatins and the number of MNP containing ADCs currently in clinical trials [151]. The variety of warheads derived from dolastatin 10 used in clinical trials continues to develop beyond MMAE and MMAF [143] and includes other microtubule inhibitors being studied as payloads in ADCs (Table 3).

As of June 2021, in Phase II/III, the auristatin analogue Aur0101 [159] has been conjugated to the IGF-R mAb in W0101 (Table 2). The majority of ADC containing MNPs in Phase II/III contain either MMAE or MMAF and consist of agents targeting ENPP3 (AGS-16C3F), Tissue Factor (tisotumab vedotin), LIV1 (adiratuzumab vedotin), cMET (telisotuzumab vedotin), AXL (enapotamab vedotin), and CD71 (CX-2029)

(Table 2). In Phase I there is a greater variety of dolastatin 10 analogues being developed for ADCs (Table 2) [157]. Eribulin is one such MNP and possesses the same mechanism of action (inhibition of microtubule polymerization) as the auristatins, (Table 3). In Phase I development, eribulin (Halaven, E7389) has been conjugated to a FR α mAb in farletuzumab-eribulin (MORAb-202) [160], Aur0101 has been conjugated to a PTK7 mAb (cofetuzumab pelidotin) [161] and a HER2 mAb (PF-06804103). The novel auristatin derivative ambrastatin 269, has also been conjugated to a HER2 mAb (ARX-788) [162]. In a new ADC format, the Dolaflexins, auristatin F-hydroxypropylamide (AF-HPA) has been conjugated to a NaPi2b mAb (Upifitamab rilsodotin; XMT-1536) [163], while duostatin 5 and 5.2 have been conjugated to a HER2 mAb (A166) [164] and a CD38 mAb (STI-6129) [165], respectively. An interesting HER2xHER2 bispecific (biparatopic) antibody has been conjugated to a novel N-acyl sulfonamide auristatin in ZW-49 [166]. The remainder of ADCs in Phase I containing MNPs are conjugated to MMAE or MMAF [151,157] (Table 2).

The prospect of other MNPs being used as ADC payloads has the potential to increase substantially as the field moves away from highly toxic payloads and focuses more on novel mechanism of action. Also included are those mechanisms that are known to have clinical efficacy, e.g. SN-38 analogs and therapeutic steroids [157] that have the potential to move ADCs beyond a cancer centric approach. This avenue is essential to the continued development of ADCs [145] as based on the clinical data on currently available ADCs, a general observation suggests that toxicities (Adverse Events) are primarily associated with off-target, off-tumour effects of the drug payloads [167] and independent of the target antigen [145] demonstrating the complexity necessary to achieve clinical efficacy with our current approach to ADCs.

3.4. Venoms and toxins as sources of immunomodulators

Animal venoms are complex mixture of proteins, enzymes, peptides, small organic and inorganic molecules synthesized by specialized glands and introduced into their prey or predators by sting or bite [168]. Once injected, they interact with their targets, such as membrane receptors or voltage-gated ion channels from nervous system, causing envenomation symptoms that varies from harmless to lethal, which might also involve the participation of the immune system [169]. Venoms and venom-based compounds trigger, directly or indirectly, immunological responses such as inflammation, complement activation, immune cell recruitment, immune cell activation and differentiation, cytokine secretion amongst others [169,170]. Similarly, several isolated venom-derived molecules down-regulates the immune response [169, 171], showing the immunomodulatory properties that such molecules may have (Fig. 5).

To date, eleven venom-based drugs have been approved by FDA, and several others are in preclinical stage or clinical trials [172]. This section emphasizes the potential that venoms and venom-derived molecules may have, as drugs or scaffolds for novel therapeutics as immunomodulators in the treatment of several diseases.

3.4.1. Snake venom

Snake venoms are complex mixtures of proteins, enzymes, carbohydrates, amino acids, lipids, peptides, which are synthesized and stored in modified salivary glands, located below their eyes. Although snake envenomation represents a significant public health problem, mainly due to their myotoxic, cardiotoxic and neurotoxic effects [173], some snake venom compounds have shown potential immunomodulatory properties for treatment of several diseases and, therefore, could be envisaged as novel drug leads [171].

Crototoxin (CTX) is the main component of *Crotalus durissus terrificus* (Cdt) venom. CTX was able to shift the pro-inflammatory status to anti-inflammatory environment in a colitis model, induced by chemical agent in mice. CTX reduced the secretion of IL-1 β , TNF- α , and IL-6, accompanied by a major secretion of TGF- β , PGE₂ and LXA₄, showing

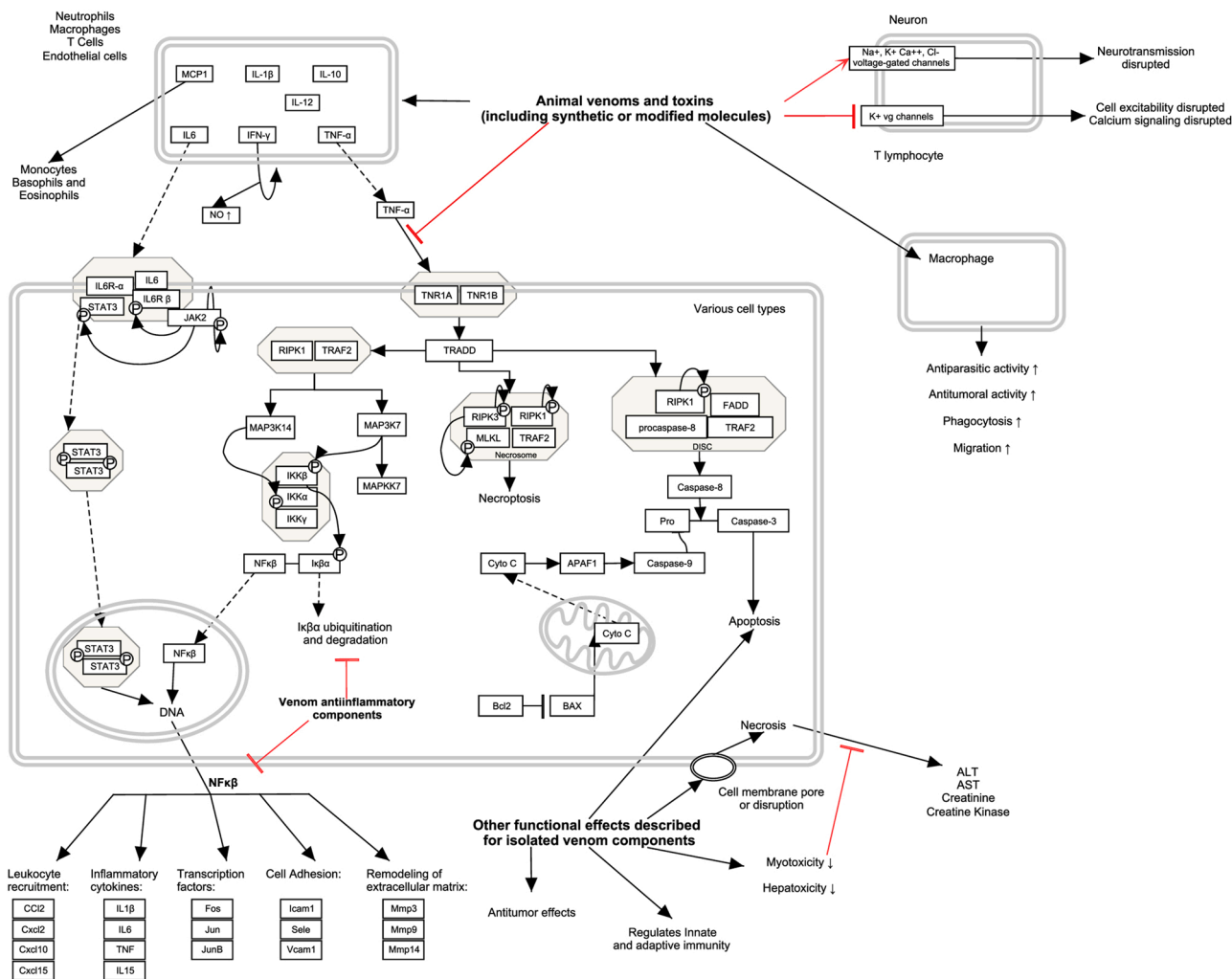


Fig. 5. Immunomodulatory activities of venoms and toxins molecules. The scheme shows some potential therapeutic uses of whole venoms or isolated molecules by acting on key points of signaling pathways and their effects. For more information concerning the venoms or molecules and the related pathways as well as activities regulated, please review the content on the papers cited in each topic. Created with PathVisio (doi.org/10.1371/journal.pcbi.1004085).

a potent *in vivo* immunomodulatory activity that could point to a potential application of CTX to ameliorate ulcerative colitis [174]. CTX also modulates the activity of neutrophils by inhibiting the SyK-GTPase signalling pathway, involved in actin polymerization necessary for the adhesion and migration of neutrophils [175].

Anti-inflammatory activity of whole venom was demonstrated in the Indian monocellate cobra *Naja kaouthia*, which suppress the immune system, exerting an anti-arthritic activity against adjuvant induced arthritis in rats [176]. Cobrotoxin and Neurotoxin-Nna, isolated from *Naja Naja atra* venom, significantly inhibited the NF-κB pathway and production of IL-1β, TNF-α and iNOS, in animal models of arthritis and inflammatory pain, respectively [177,178]. Hidrostattin-TL1 from *Hydrophis cyanocinctus*, which inhibits the interaction between TNF-α R1 and TNF-α, is a potential peptide for the design of agents to treat TNF-α inflammatory diseases, such as sepsis and inflammatory bowel disease [179].

3.4.2. Bee venom

Peptides melittin and apamin are among the main components of *Apis mellifera* (European honeybee) venom. Other constituents comprised adolopin and mast cell degranulating (MCD) peptides and enzymes such as Phospholipase A₂ (bvPLA₂) and hyaluronidase [180], among others. Bee venom (BV) has been used to treat inflammatory disorders like rheumatoid arthritis since antiquity. BV therapy – under

the commercial name of Apitox® – is approved human use to treat multiple sclerosis, rheumatoid arthritis and other inflammatory-related diseases [172].

BV melittin presents a great bioactive potential with antineoplastic, anti-inflammatory and antioxidant activities [181,182]. BV is a promise anti-inflammatory therapy for periodontitis, suppressing NF-κB and AP-1 signalling pathway, thus reducing pro-inflammatory cytokines levels induced by LPS of *Porphyromonas gingivalis* [183]. BV exhibits anti-neuroinflammatory activity in microglial cells, via MyD88-dependent NF-κB signalling pathway [184]. BV also controls the allergic-inflammatory response, by reducing inflammatory mediators via attenuation of STAT1/IRF3 signalling pathway [185]. BvPLA₂ can ameliorate airway inflammation in an ovalbumin-induced asthma model by the induction of regulatory T cells, reducing the levels of Th2 cytokines and inflammatory cells [186]. BvPLA₂ is responsible for the activation of human T cells via CD1 antigen-presenting proteins, displaying self-phospholipids, sphingolipids, acyl-glycerides, and other lipid antigens, stimulators of T cells. BV is a feasible approach to modulate the inflammatory response in non-hypersensitive individuals. Also, BV immunotherapy may create a lifesaving tolerance for some allergens, in individuals with IgE-mediated allergy [169,185].

3.4.3. Scorpion venom

Scorpion venoms are composed of enzymes, proteins, peptides,

carbohydrates, amino acids, inorganic salts, amines and lipids. Scorpion venoms are well characterized for being neurotoxin-rich compounds that interact mainly with Na^+ , K^+ , Ca^{2+} and Cl^- voltage-gated channels [187]. Among these, potassium channels (Kv) play a key role in immune cell excitability and calcium signalling and are chief targets for T cell regulation [188]. The peptide HsTX1 and its analog HsTX1[R14A] from *Heterometrus spinnifer*, inhibits the activation of effector memory T lymphocytes (T_{EM}), drivers of inflammation in autoimmune disease such as multiple sclerosis and rheumatoid arthritis, by blocking Kv1.3 channels [189]. Anti-inflammatory and analgesic activity from *Heterometrus laoticus* venom, is also based on the affinity of Hetlaxin toxin for Kv1.3 channels [190].

Ts14 is an anti-hypertensive peptide that belongs to *Tityus serralatus* Hypotensins (TsHpT) [191], a group of bradykinin potentiating peptides isolated from *Tityus serralatus* venom (TsV) that act as bradykinin 2 receptor agonists. Ts14-derived tripeptide (KPP) was able to mimic the hypotensive activity of Ts14 [192]. Ts14 also evokes anti-inflammatory, proangiogenic and anti-fibrogenic activities, as verified by means of a murine sponge model of angiogenesis. In such conditions, these processes are associated to ischemic condition, wound healing and inflammatory disease, which reflects the therapeutic potential of Ts14 [193]. Aiming to identify chemotherapeutic-compounds for the treatment of Toxoplasmosis and Chagas Disease, TsV-active molecules were found. An active molecule similar to TsV 2 toxin (Ts2) and mimetic peptides (Pep1, Pep2a and Pep2b), reduced the replication of *Toxoplasma gondii* (Tg) tachyzoites in macrophages without host cell toxicity. Similarly, TsV, Ts14 and KPP were able to attenuate *Trypanosoma cruzi* (Tc) infection in macrophages *in-vitro*. In both cases, nitric oxide (NO) and pro-inflammatory mediators (IL-12/TNF/IL-6) production were induced. Ts2-mimetic peptides were able to decrease the number of cerebral cysts in Tg-infected mice and KPP treatment decreased parasitaemia in Tc-infected mice. Authors observed that anti-Tc activity of TsV-peptides triggers MAPKs (ERK1/2, JNK1/2, p38 α) pathways [194, 195].

3.4.4. Immunomodulatory activities of other venoms

A variety of molecules with immunomodulatory activity have been isolated from other sources like amphibian secretions and wasps [182]. Venom from the *Nasonia vitripennis* wasp inhibited the NF- κ B signalling pathway, dampening the expression of IL-6 in LPS-treated macrophages, activity that demands the activation of the JNK pathway [196]. Although ticks are not considered venomous animals, its saliva contain protein families that are present in venomous taxa. The tick salivary protein (Salp15) from *Ixodes scapularis*, blocks CD4^+ T cell activation by binding specifically to the CD4 coreceptor. Upon binding, Salp15 inhibits TCR ligation-induced T cell signalling, showing a potential immunosuppressive activity [197].

The venom of the spider *Phoneutria nigriventer* (PnV) is rich in toxins that affect Na^+ , Ca^{2+} and K^+ voltage-gated channels and glutamate transporters. PnV and PnV-peptides have shown analgesic, anti-hypertensive, neuroprotective, antimicrobial activities, as well as modulation of erectile function [198]. PnV have shown antineoplastic activity *in-vitro* and *in-vivo* for glioblastoma cells (GB), the most common and aggressive malignant brain tumour in adults [199]. PnV impaired GB tumour development in a murine xenogeneic model. Unlike classical chemotherapeutic drugs, PnV did not induce immunosuppression. Furthermore, PnV-activated macrophages become more phagocytic without inducing a pro or anti-inflammatory cytokines profile [199]. Further studies have confirmed the immunomodulatory activities of PnV-derived molecules. PnV subfraction LW-9 displays an interesting immunomodulatory effect. Bone marrow-derived macrophages treated with LW-9 showed an activated morphological profile, increased phagocytosis and migration and increased cytotoxic effect on tumour cells. Immunomodulation is a relevant approach in the treatment of malignant tumours, and useful immunoadjuvants to cancer treatment might be found in LW-9-molecules [200].

Immunomodulatory molecules have also been found in venoms and toxins of sea animals including jellyfish, corals, sea anemones, cone snails, fish and others [171]. Dalazatide, a synthetic-designed peptide based on Shk-186 (a peptide isolated from *Stoichactyla helianthus* sun sea anemone) has shown potential for the treatment of autoimmune diseases such as psoriasis, arthritis, lupus, multiple sclerosis, acting as a Kv1.3 channel inhibitor. Preclinical trial and phase trials I for Dalazatide were completed in 2015. Since then, no phase II study has been started [172].

In the last years, improvements in isolation, analyses, and chemical syntheses techniques together with advance in genomic, transcriptomic and proteomic platforms, have shortened the way for venom-based novel therapeutics. Besides the therapeutic application, venoms and venoms-based compounds have proved be important diagnostic tools and their cosmeceutical applications have become more profitable [168, 172].

The identification of compounds that downregulate the immune system during inflammation, autoimmunity and organ transplant and/or upregulate the immune response through pathological conditions like cancer and infection diseases, will pave the way forward for novel drug therapeutics. Research in some immunology and venomics areas are still in its infancy, however the coming years could bring novel therapeutic avenues for disease treatment and envenomation (Fig. 5), together with discoveries of new immune-biochemical pathways.

4. Bringing natural products drug discovery into the 21st century

4.1. New molecules versus phytomedicines

As can be seen from the previous section, the use of natural products and/or natural product structures from a broad range of sources (including microbes, marine organisms, animals, fungi and higher plants) continue to play a pivotal role in the drug discovery and development process [201–204]. Plant products have been predominant in books containing recipes for various medicines and, historically, have been probably the most important source of therapeutic agents [205]. Plant-derived therapeutics may reach the pharmaceutical market as pure compounds or as complex mixtures containing thousands of different molecules, the latter referred as “botanical drugs” by the USA FDA and as “phytomedicines” in Europe [206]. It is noteworthy that many botanical mixtures are marketed not in the form of medicine, but as less strictly regulated product groups, such as dietary supplements [206, 207]. The marketing authorization of dietary supplements is very simplified, with the need for the producer to prove efficacy, safety, and quality of the marketed product less enforced than in the pharmaceutical sector [206,208].

Before the 19th century, plant-derived medicines consisted mainly of impure preparations with a substantial variability in the phytochemical profile and pharmacological response [209]. The isolation of morphine crystals from the tarry poppy seed juice by the German pharmacist Friedrich Wilhelm Adam Serturner, at the beginning of the 19th century, started the modern process of drug discovery [210]. In the following years, phytochemicals isolated in pure form (e.g., colchicine, atropine, quinine) were increasingly supplanting the plant impure preparations from which they had been isolated [209]. Despite the advent of combinatorial chemistry and high-throughput screening campaigns in the last decades, the search for drugs from plants has continued over the years, with several new drugs approved in the 21st century (e.g. galantamine, from *Galanthus woronowii* for Alzheimer’s disease dementia, ingenol mebutate, from *Euphorbia peplus* for actinic keratosis, homoharringtonine, from *Cephalotaxus harringtonia*, indicated for treatment of chronic myeloid leukemia) [201,211].

The identification of single bioactive constituent represents the common procedure for scientists involved in drug discovery from plants. When the most important constituent is known, it can be further evaluated in pharmacokinetic and pharmacodynamic studies, being the

evaluation simpler with pure isolated phytochemicals than with the entire herbal mixtures. Furthermore, plant-derived pure constituents may be chemically tailored and structurally modified as a strategy to increase potency and selectivity over a given target, to improve physicochemical, biochemical, and pharmacokinetic properties and to eliminate or reduce unwanted side effects [212]. Challenges associated with the discovery and the pharmaceutical development of botanical mixtures include solubility problems (not all the components of the mixture are equally soluble), interference with assay by mixture components in pharmacodynamic evaluations, and impossibility to follow the fate of each component in pharmacokinetic studies [207,213,214]. The complexity of the plant extracts, their viscosity, their tendency to aggregate or precipitate, the presence of components that non-specifically bind proteins or that may give false positive or false negative results make quite problematic high-throughput screening [215].

Despite these challenges, the interest in the therapeutic use of mixtures is still thriving. Examples of FDA-approved “botanical drugs” include sinecatechins (Veregen®, approved in the 2006), a mixture of catechins and other green tea components (indicated for the topical treatment of external genital and perianal warts in immunocompetent patients) and crofelemer (MytesiMT, approved in 2012), extracted from the latex of the *Croton lechleri* tree (dragon’s blood), indicated for the symptomatic relief of non-infectious diarrhoea in adult patients with HIV/AIDS on anti-retroviral therapy. A recent review article covering drugs from 1981 to 2019 retrieved fourteen “botanical drugs” approved as therapeutic agents [201]. One of the rationales for using mixtures of molecules rather than single compounds rely on the observation that many disorders have a multifactorial aetiology, with different targets involved in disease pathophysiology [216]. For example, several chemically unrelated compounds, acting on different targets, are believed to be responsible for the antidepressant effect of St John’s wort (*Hypericum perforatum*) extracts [217]. Also, supporters of the therapeutic use of plant-derived mixtures claim that additive or synergistic effects may occur among the components of the mixture, making it more effective than the isolated pure compounds [206,216]. Even though synergistic effects are many often advocated without a clear pharmacological demonstration, many examples have been reported [218–220].

One of the advantages of using the botanical extracts is their peculiarity to be chemically manipulated without losing the distinctiveness of being entirely natural products. Indeed, the process of extraction may be optimized to obtain extracts enriched of a specific component. Similarly, the cultivation of specific plant chemical genotypes, with high yields of a specific compound, may lead to botanical extracts with high content of the desired phytochemical (for example, large-scale cannabinoid chemotypes *Cannabis sativa* are cultivated for the therapeutic use of extracts with high content – about 65% - of cannabidiol, [221]). Conversely, during the production it is possible to remove undesirable phytochemicals from a herbal mixture, to produce safe botanicals retaining the pharmacological activity [222]. Elimination of unwanted constituents lead to the so-called refined extracts [222]. Theoretically, if extracts can be manipulated, by selective removal or enhancement of a constituent, the same plant may yield medicines tailored for different indications [206].

The choice to move towards mixtures rather than to pure compounds for pharmaceutical development is, in some instances, a mandatory step. It is not uncommon that the pharmacological activity of a specific extract is lost upon bioassay-guided fractionation, making it impossible to isolate the active ingredient of the mixture [216,222]. Bioassay-guided fractionation is a technique whereby the plant extract is chromatographically fractionated and re-fractionated until a pure biologically active molecule is isolated. Each fraction produced during the fractionation process is screened for biological activity and only active (or most active) fractions are subsequently fractionated. The process is repeated until the pharmacologically active compound has been isolated

[223].

The use of mixtures rather than pure compounds raises the issue of the reproducible pharmacological activity. While for extracted pure compounds only minimal standards are required (phytochemical characterization, purity, methods used to determine compound identity and major impurities, stability) [224], the phytochemical complexity of the mixture requires that a number of important pieces of information must be disclosed. Botanical mixtures must be clearly described with respect to the part plant used (the part being indicated with the binomial Latin name), the drug extract ratio, solvent type and its concentration, method of extraction [224,225]. To ensure reproducible pharmacological activity, the extract must be chemically characterized (e.g., by HPLC fingerprint and metabolomics) and/or the content of marker compound (s) must be determined with validated analytical methods [224,226]. The selection of the marker compound(s) is based on several aspects, including claimed pharmacological relevance, accessibility of analytical methods, chemical standards, and previous use of a marker compound [227]. According to EMA guidelines on herbal medicinal products/traditional herbal medicinal products, standardization means “adjusting the herbal substance/preparation to a defined content of a constituent or a group of constituents with known therapeutic activity respectively either by adding excipients or by blending batches of the herbal substance and/or herbal preparation (e.g. standardized extracts)”.

From a clinical point of view, there is no conceptual difference between botanical mixtures and pure compounds. Botanical mixtures must be subjected to the same phase 1 to phase 3 efficacy/safety trials as pure compounds before they can be approved by the regulatory authorities for medicinal use in the general population. As it is the case for pure compounds, the evidence obtained from high-quality randomized controlled trials (RCTs) represents the gold standard for assessing clinical efficacy of botanical mixtures [208]. During the past years, an impressive number of randomized clinical trials in which herbal extracts have been compared, in terms of efficacy and safety, to standard medicines (pure synthetic or plant-derived compounds) have been published. Surprisingly, in several trials, therapeutic equivalence and even superiority of the botanical extracts over the standard treatment (pure synthetic or natural compounds) have been reported. For example, a randomised, multicentre, double blind, parallel group trial, published by the British Medical Journal, performed on 324 patients with mild to moderate depression, found that an *Hypericum perforatum* (St John’s wort) extract was therapeutically equivalent to imipramine, but patients tolerated the extract better than imipramine [228]. In general, the reported efficacy of herbal mixture must be interpreted cautiously. Although the reporting quality of clinical trials related to botanical mixture has improved over the last several decades, the poor methodological quality of the available trials and the possible presence of publication bias must be considered [207,208]. Weaknesses include small sample size, short duration and non-appropriate information on the mixture under investigation (e.g. the part of the plant used, the Latin name of the plant, solvent/type of extraction and standardization of the mixture) ([207,226]. Reporting recommendations have been developed in which several CONSORT items were elaborated to become relevant and complete for randomized controlled trials of herbal medicines [229].

In conclusion, the current method of drug discovery from plants is not easily adaptable to mixtures, being it typically devoted to reducing complexity and identifying single active plant constituents for drug development. Pharmacokinetics and pharmacodynamic studies are simpler to perform with pure compounds than with mixtures. Also, isolated compounds may be lead compounds for further pharmaceutical development. Conversely, strengths for the possible development and therapeutic use of mixtures are the possibility of synergy among the components, the presence of multiple compounds which may theoretically act on different targets involved in diseases pathophysiology, and the possibility that extracts can be chemically refined, through selective removal or enhancement of a constituent. The discovery of pure

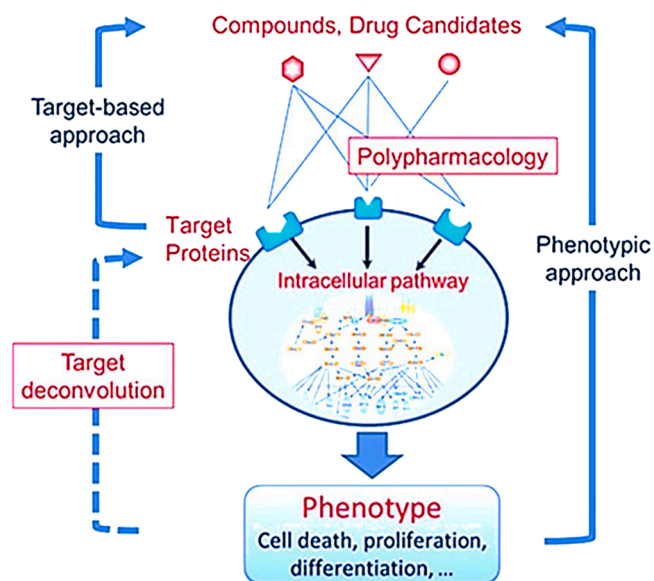


Fig. 6. Combining phenotypic and target-based screening. Phenotypic screening data that yields “hit” compounds can be used to retrospectively determine the biological targets through target deconvolution; once a target is identified, compound libraries can then be screened against that target to find a drug that binds to it with high affinity and elicits the desired effect.

compounds rather than mixtures, however, must be not seen in contraposition, as exemplified by the cannabis research, which has generated both pure compounds (for example, cannabidiol for treatment for refractory paediatric epilepsies) and botanical extracts (i.e. Sativex®, a mixture of two different cannabis extracts indicated for the treatment of spasticity due to multiple sclerosis) for therapeutic use. From a clinical point of view, to achieve market authorization as a medicine, the efficacy must be documented in high-quality randomized clinical trials, both for pure compounds and for mixtures.

4.2. Harnessing small molecule drug discovery technology for natural product screening

The “traditional” phenotypic screening approach using cell, tissue or animal models of disease for bioassay-guided fractionation for natural product drug discovery is extremely laborious and expensive in terms of time and animal use and has a number of limitations, including the relevance of the model, the limited knowledge generated of the biological target and lack of insight into the mechanism of action of the compounds. Small molecule drug discovery revolutionised the drug discovery process by adopting a combined phenotypic and target-based approach that could be performed using high throughput screening (HTS). The target-based approach, based upon the concept that “if you want a new drug you must find a new target”, involves the identification of a target protein associated with a specific disease as a starting point, followed by screening of large libraries of compounds to determine binding affinity and the ability to elicit the desired effect. This is in contrast to the phenotypic screening approach (i.e. “if you want to treat a disease you must find a cause”), which identifies compounds that cause a desirable change in phenotype in disease models. “Forward Pharmacology” uses these two approaches together, whereby phenotypic screening data that yields “hit” compounds can then be used to retrospectively determine the biological targets through target deconvolution; once a target is identified, compound libraries can then be screened against the target to find a drug that binds to it with high affinity and elicits the desired effect (Fig. 6). The HTS technologies that were developed and used extensively by the pharmaceutical industry in the small molecule discovery sphere are now being adopted in the

natural product drug discovery domain. It is beyond the scope of this section to go into detail around the need for assay optimisation and the post-screening journey, but two excellent reviews contain some excellent recommendations, and highlight the advantages, limitations and pitfalls for the successful development of high throughput target-based and phenotypic screens for the discovery of both natural product-based single compounds [230] and combination drugs [231]. What this section will focus on is some examples of how screening platforms developed for small molecule drug discovery have been utilised for natural product drug discovery, with an emphasis on discovery of novel immunomodulators.

4.2.1. High throughput phenotypic cell-based screening

In high throughput (HT) screening, thousands of compounds are tested in parallel for their activity in one or more biological assays. There are many examples of HT phenotypic screening of small molecule compound libraries (see [232,233] for two reviews), and there are increasing reports of natural product libraries being screened by this method. One recent search for compounds to prevent cardiac fibrosis screened a library of 480 chemically diverse natural product compounds against human cardiac fibroblast proliferation using an ELISA-based assay, which yielded 15 active compounds. Further screening using an *in vitro* and *in vivo* pipeline of analysis identified bufalin (from Chinese toad venom) and lycorine (from Amaryllidaceae plant species) as the lead compounds, and subsequent miRNA deep sequencing identified the pro-fibrotic microRNA 671-5p as a common effector [234]. In terms of immunomodulating natural products, a high throughput method to measure nitrite levels in murine macrophages (RAW264.7 cells) identified 79 samples from a subset of 5976 extracts (1.32% hits) from the MEDINA microbial extracts collection as immunomodulators, and, following deconvolution, one extract was eventually selected for large scale-up growth [235]. Florian et al. developed a 96-well plate fluorescence end-point assay for cytotoxic T lymphocyte lytic granule exocytosis, which they initially used to screen a synthetic compound library [236] and subsequently enhanced and validated to be suitable for use with natural product collections [237]. Using a 384-well format, HTS has also been applied to screen microbial extracts from the MEDINA Natural Products library for agents effective against some neglected tropical diseases (Human African trypanosomiasis, leishmaniasis and Chagas disease [238]).

4.2.2. High content screening

In contrast to HT screening assays, which focus upon determination of a single cellular response, high content screening (HCS) involves assays of more complex cellular phenotypes as outputs, such as increases or decreases in the production of cellular products and/or changes in cellular morphology. Thus, HCS typically involves automated digital microscopy and image analysis and flow cytometry, in combination with software systems for the analysis and storage of the data. The purpose of HCS is two-fold; first to acquire spatially or temporally resolved information on an event and second to automatically quantify it. It is beyond the scope of this review to describe the detailed theoretical and practical aspects of this technique, but interested readers are referred to an excellent book edited by Haney [239] for an in-depth description. Like HT screening, HCS is increasingly finding its place within the natural product drug discovery field. One of the first reports of its use was in a screening programme for nuclear export inhibitors using the MEDINA library of microbial extracts [240]. Using a green fluorescent protein reporter assay (U2nesRELOC; [241]) and a human osteosarcoma (U2OS) cell line, 14,000 microbial extracts were screened, of which 151 demonstrated activity and following bioassay-guided fractionation several fungal-derived molecules were identified as being in the same chemical class as known nuclear export inhibitors [240]. HCS is also being used extensively in drug discovery from TCM (recently reviewed in [242]) and natural product libraries for a range of therapeutic applications. Some examples using this technique include the discovery of

two novel inhibitors of mTOR (1,4-O-diferuloylsecoisolariciresinol and pterreione B) [243] and the alkaloid sanguinarine as a novel inhibitor of mitogen-activated protein kinase phosphatase-1 (MPK-1; [244]). HCS has also been used to confirm mechanism and/or site of action, as exemplified by the demonstration that pseudolaric acid B, a diterpene acid isolated from *Pseudolaria kaempferi* inhibits cancer cell growth through inhibition of COX-2 by interrupting cytokine-induced translocation of NF- κ B to the nucleus and inhibiting constitutive STAT3 activation [245]. Likewise, panduratin A, isolated from *Boesenbergia rotunda*, was shown to inhibit NF- κ B translocation in a human lung cancer cell line [246]. In a search for novel drugs for neurodegenerative diseases a synthesized curcumin derivative (C1) was shown through HCS to be a novel activator of transcription factor EB (TFEB), independently of mechanistic target of rapamycin (serine/threonine kinase) (mTOR) activity [247], unlike existing TFEB inhibitors which act through inhibition of mTOR. Finally, HCS was utilised to screen for cytotoxicity in a study designed to determine the antifibrotic potential of the components and metabolites of Fuzheng Huayu recipe (FZHY) [248].

4.2.3. Label-free screening assays

Many HTS and HCS methods require the use of fluorescent or radio-labelled tags, which has raised the question around the ability of these probes to change the properties of the proteins under study and thus result in experimental bias. The advent of label-free technologies, which are based on measuring changes in electrical impedance or the refractive index of cells, is a rapidly growing area of drug discovery [249]. One such technique that has been used for screening natural products is dynamic mass redistribution (DMR), which uses light to measure ligand-induced cytoskeletal re-arrangement of cells adjacent to the biosensor and provides an integrated cellular response comprising multiple pathways and cellular events. This can be applied to any cell type, including inflammatory cells [250], as either a phenotypic screen (i.e. does a particular type of cell respond to a ligand?) or a target-based screen (i.e. do libraries of compounds cause a response in cells over-expressing a protein of interest?). Several studies have successfully used DMR to identify novel compounds or to determine their toxicity. For example, a study of 82 compounds from fungi of the *Ganoderma* genus identified the site of action of four novel phytocannabinoids using DMR [251]. A similar approach was taken in screening a plant-based compound library for novel mu-opioid ligands [252], while DMR confirmed biased β_2 -adrenoceptor signalling as the mechanism underlying the anti-secretory and anti-bronchospasmodic effects of an ivy leaves extract (EA 575®) used clinically for the treatment of respiratory disorders [253]. DMR can also be used to screen for activity against multiple targets, as recently demonstrated by screening of a TCM formula (Qing Fei Pai Du Decoction) that was widely used in China for treatment of COVID-19 infection; using data from DMR screening of 144 compounds present in the preparation against six GPCR's linked to inflammatory, immunity and respiratory actions, alongside pathway de-convolution and construction of a herb-compound-effect network, twenty active compounds and their effective concentrations were identified for each target, along with the pathways underlying their mechanism(s) of action [254]. Integration of DMR alongside micro-fractionation and high-resolution MS was also highly useful in assisting in the isolation of bioactive alkaloids from different plant species (*Corydalis yanhusuo*, *Dactylicapnos scandens*, *Chelidonium majus* and *Corydalis decumbens*) [255]. In a proof of concept study to characterise toxic herbs contained within TCMs, DMR was an integral part of a biosensor-based two-phase pharmacological profiling (BTTP) method which, combined with HPLC, identified 25 naturally-occurring and potentially toxic muscarinic M₂-receptor antagonists in *Datura* species [256], a herbal medicine used in the treatment of asthma; since TCM mixtures could contain incorrect plant species due to mis-authentication DMR could be used to help confirm or detect the presence of toxic contaminants within preparations.

4.2.4. Non-mammalian in vivo models

As mentioned earlier, traditional approaches to natural product drug discovery often required whole animal or animal tissue experiments to perform dose response and toxicity studies, usually in rodent species. This approach is not only expensive in financial terms, but is also ethically questionable in light of the very large numbers of animals required to yield meaningful results. However, as valuable as cell-based screening programmes are, they do not give a full picture of how a compound may work in the whole organism. Thus, non-mammalian models as *in vivo* screens are becoming increasingly important in the natural products field. The two most commonly used models are zebrafish (*Danio rerio*) and the nematode worm *Caenorhabditis elegans* (*C. elegans*); an added benefit of these two species is that they can be genetically modified to generate models that mimic human disease.

The zebrafish was initially developed as a model for the study of developmental biology and embryology, however the production of gene targeting for the generation of transgenic fish has resulted in this becoming a popular model for studying human disease. Although zebrafish were first used to screen for biological activity of naturally-occurring compounds over 60 years ago [257] it is only very recently that they have been adopted as a model for routine drug screening against various diseases such as diabetes [258], neurological diseases [259,260], and immunological disorders [261]. The zebrafish model offers significant versatility in that it can be used as straightforward phenotypic or toxicity screens, or utilised to determine mechanism of action by taking advantage of the wide variety of genetic strains available. Examples of the former include studies on the acute toxic, anti-oxidant and anti-inflammatory actions of *Hypericum hookerianum* extract [262] and the immunomodulatory effects of a polysaccharide isolated from *Rosa laevigata* [263] and an arabinofuranan from *Garcinia mangostana* [264], assessed by NO and ROS production in zebrafish embryos. The latter approach helped to identify how pectin nanoparticles derived from *Spirulina maxima* improved wound healing through upregulation of a range of innate immune-related genes [265]. Finally, zebrafish have also been utilised in bioassay-guided fractionation to identify anti-angiogenic bioactives from East African medicinal plants [266].

The potential value of *C. elegans* as a suitable screening model for bioactivity screening of natural compounds against a range of conditions including oxidative stress, ageing, metabolic and neurodegenerative disorders is now well recognised [267,268], although challenges remain, particularly in terms of throughput. Consequently, most *C. elegans* studies on natural products are performed relatively late-on in the drug discovery cycle as a means of confirming bioavailability and bio-efficacy of an identified compound or characterized extract in an *in vivo* model. Examples include confirmation of the anti-oxidant activity of methyl cinnamoyl catalpol (DAM-1, isolated from *Dolichandrone atrovirens*) [269], a neuroprotective and anti-oxidant effect of an extract from *Vitis vinifera* leaves [270] and suppression of degeneration by an extract from rapeseed pomace [271]. The potential value of using genetic models of *C. elegans* for studying innate immunity has been recognised for some time [272], and in terms of screening for immunomodulating activity of natural products, a high-throughput (96-well plate) liquid culture-based screening method in the IG692 strain of *C. elegans* has been recently developed and validated for rapid screening of libraries, including libraries of natural compounds [273].

4.3. "Synergism" between compounds in complex mixtures

As is becoming increasingly clear from the previous sections, natural products continue to be a very important resource for the modern pharmaceutical industry and, with the development of chemistry and pharmacology technology, more and more bioactive compounds from plant extracts are being identified and shown to have medicinal properties. Most bioactive compounds in herbal medicines are plant secondary metabolites, many of which are individually present at very low

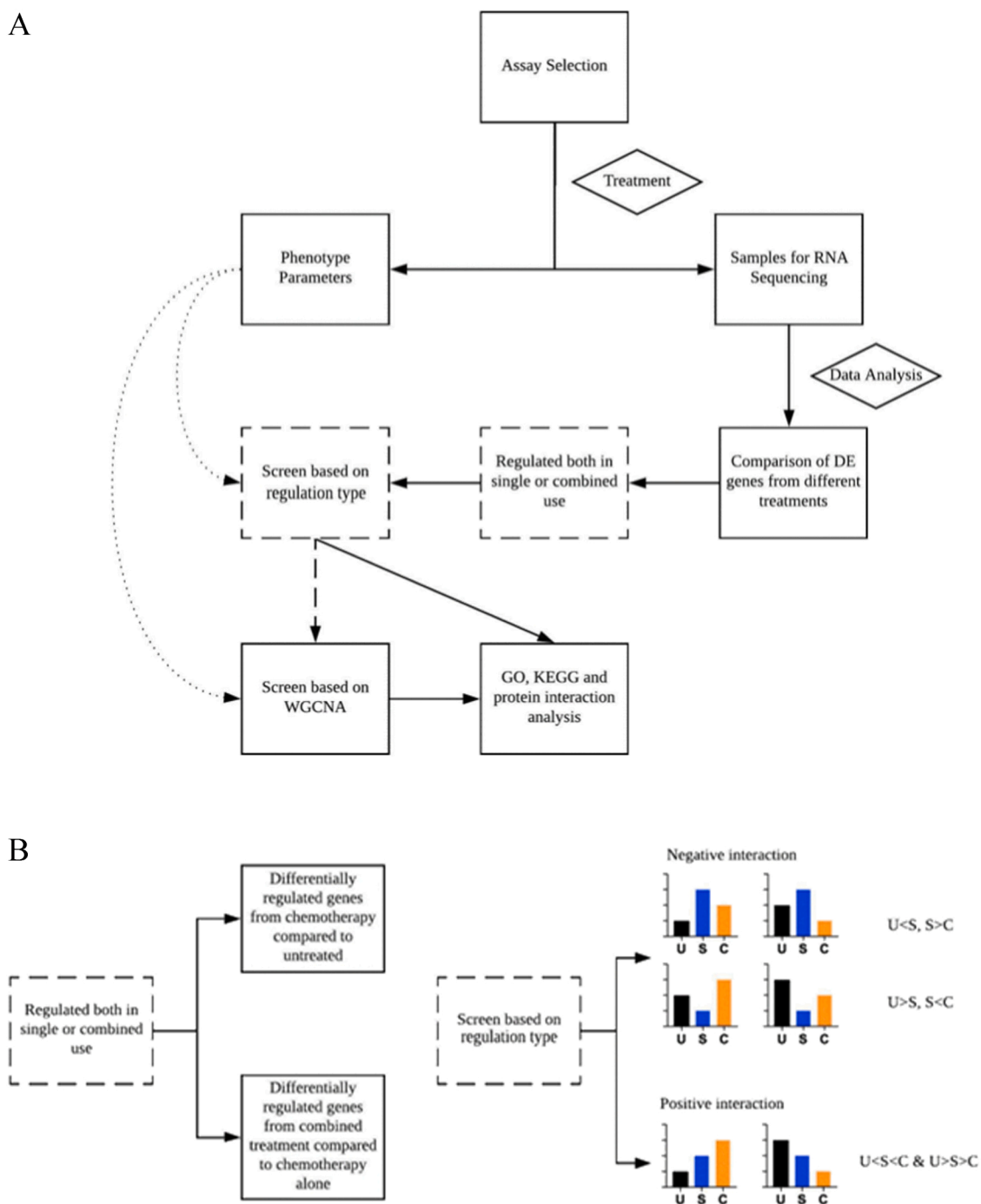


Fig. 7. Experimental and data analysis workflow for applying omics to drug-drug interactions. A. The overall design of the study. B. Further details of 2 specific procedures indicated with dashed-line boxes in A. The black, blue and orange bars represent untreated, single treatment and combined treatment, respectively.

levels in extracts [274]. However, purified compounds usually cannot achieve similar therapeutic effects from equivalent amounts. In addition, increases in dosage of these compounds often result in more side effects [275,276]. The hidden principle here is the synergism between different ingredients in herbal medicines/plant extracts. One of the ways by which synergism occurs in herbal medicine is the presence of active compound analogues in these mixtures that often affect similar drug-targets. However, limited by the analytical techniques and complexity of secondary metabolites, in most cases only a few compounds with relatively high concentration can be fully investigated and selected as bioactive products to represent a medical herb [277,278]. The other cause for synergism is that herbs usually contain more than

one series of active products, such as alkaloids and flavones in *Sophora flavescens*, which often work on different targets to achieve synergistic effects [279]. This is further exacerbated as herb prescriptions can contain several, or even over a dozen, medicinal plants; thus, herbal medicines may work in such a way that multiple compound groups perturb multiple targets to achieve a combined effect on disease. This makes synergistic mechanisms an important, but also challenging, area in natural product/herbal medicine research.

Natural compounds are the pharmacodynamic material basis of herbal medicines, and ultimately, the synergism of natural medicine depends on the interactions among compounds during production and resulting from in vivo processes. Although it may not be comprehensive,

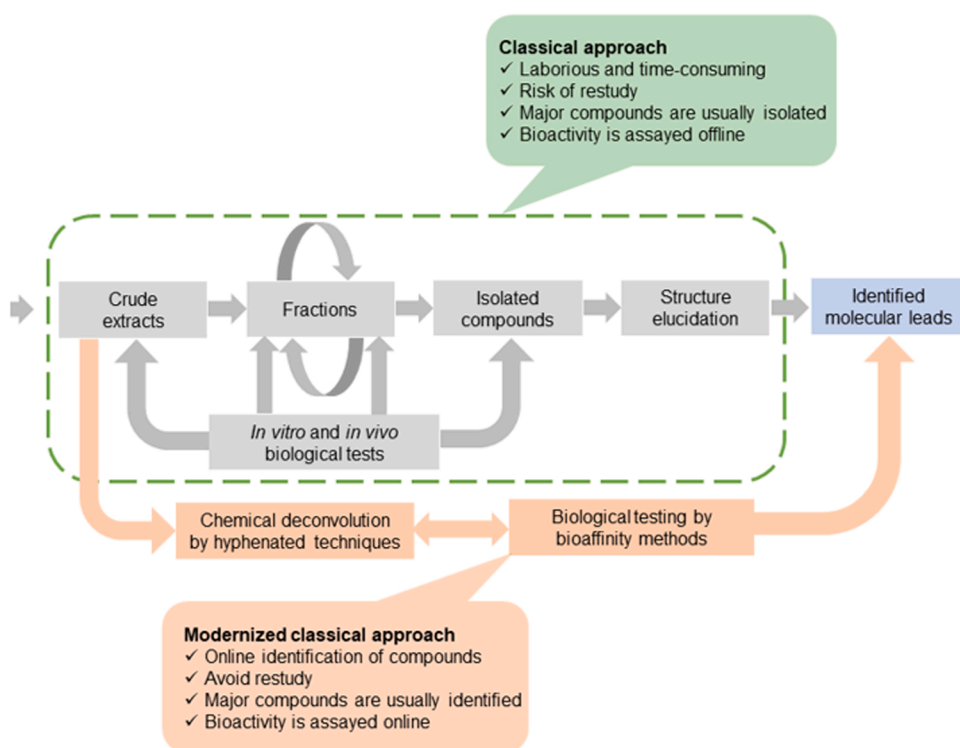


Fig. 8. The classical workflow for research and development of NP-based drugs starts with the selection of living organisms for study using different strategies. The extracts are prepared and empirically tested for bioactivity employing *in vitro* and/or *in vivo* tests. The active extracts are then submitted to recurring steps of fractionation and biological activity testing until the single bioactive compounds are isolated, which have their chemical structures confirmed or assigned by spectroscopic analyses. This classical approach was accelerated by incorporating dereplication tools based on hyphenated techniques, consisting of online coupling of chromatographic separation devices to spectrometers like UV, MS, and NMR. *In vitro* bioaffinity assays were also coupled with classical chemical deconvolution methods to accelerate the identification of bioactive constituents.

selecting representative compounds to conduct research on drug-interactions is a compromise. For most herbs, the main secondary metabolites have been identified and pharmacological activities of those compounds have been studied. These results, combined with known drug-targets and molecular docking methods, can provide useful information to predict and explain synergistic effects among bioactive compounds in complex mixtures [280,281]. In addition, *in vivo* disposal processes for active compound groups, such as absorption, distribution, metabolism and excretion (ADME), can also influence how different ingredients in a complex mixture can interact to show synergistic or antagonistic effects [282]. Many studies have been done to determine prescription compatibility between herbal medicines based on pharmacokinetics analysis. These studies have mainly focused on the inhibition or activation of different drug transporters or metabolic enzymes induced by ingredients in adjuvant herbs, which can change the pharmacokinetic parameters of other important compounds to achieve better treatment effects or reduce toxicity. For example, components in *Gastrodia Rhizome* (dried tuber of *Gastrodia elata*) can inhibit the p-glycoprotein and multidrug resistance associated protein 2, improving the absorption of bioactive compounds in *Puerariae radix* [283]. It has also been shown that *Herba ephedra* can reduce the toxicity of *Semen armeniacae amarum* by inducing stereoselective metabolism of amygdalin [283,284]. Through these traditional pharmacologic methods, some mechanisms of synergistic effects between major compounds can be specifically explained. However, complex mixtures like herb prescriptions can contain several active compound groups, which may interact with each other in different pharmacokinetic processes and on different drug-targets. This complexity makes it almost impossible to comprehensively explain synergism in a complex mixture using typical pharmacologic research tools. However, the introduction of network pharmacology or systems biology methods have been useful in this context. Using Compound Kushen Injection (CKI), which is a mixture of extracts from Kushen (*Sophora flavescens*) and Baituling (*Heterosmilax chinensis*) as a model, deletion of 9 main compounds one by one, or in different combinations, followed by apoptosis assays and transcriptome analysis of cancer cell lines treated with CKI showed that removal of no

single compound could alter the effects of CKI on cancer cells [285]. Although oxymatrine and oxysophocarpine together seem important to the cytotoxicity effects of CKI, transcriptome results showed that many compounds are required for CKI's effect, and many targets/pathways are involved [285]. These results support the concept of multi-compound/multi-target interactions for complex mixtures based on plant extracts and illustrate the complexity of synergism in herbal medicines.

Although many studies show the feasibility of applying network pharmacology methods to the research of complex mixtures, there are still gaps in understanding the specific mechanisms of how herbal prescriptions exert their effects [286]. Investigation and development of modern medicine use network pharmacology methods based on molecular structures, pharmacokinetic characteristics and target information for drugs and diseases [287]. However, the data from clinical and basic experimental studies for herbal prescriptions are still insufficient. Furthermore, as most active compounds have a wide target spectrum but relatively low affinities, along with limited information on the quantity of each ingredient, it is difficult to describe the interactions between compounds within a mixture. These challenges make it impractical to clearly elucidate the mechanism of herbal prescriptions. As a compromise, herbal medicines or prescriptions could be initially characterized using network pharmacology followed by application of the network to validate synergism or interaction. Based on this concept the analysis process for complex mixtures of natural products and determining their effects on multiple targets/pathways can be simplified. This idea has been applied to the study of CKI and were able to demonstrate that the main active compounds and cytotoxic effects of CKI derive from Kushen [288]. However, transcriptome analysis showed that Baituling can activate immune-related pathways and therefore enhance the overall effects of CKI [288]. In addition to understanding interactions of compounds within herbal extracts it is also important to understand how herbal prescriptions may interact with pharmaceuticals, as herbal extracts are often used with them [289,290]. For this purpose, Adelson's group introduced a workflow to apply transcriptome analysis to drug-drug interaction research (Fig. 7). Taking CKI as an example, they

found that CKI can counteract the cytotoxic effects of fluorouracil and doxorubicin. The transcriptome analysis results demonstrated that this difference is primarily the result of opposing effects on DNA synthesis and metabolism. Subsequently they showed that MYD88 was required for these interactions [288].

When investigating synergism within complex mixtures, a network approach and a traditional compound-target-effect route all have their own merits, and are complementary, and we have proposed several ideas for both approaches.

1. Introduce the concept of an active compound group as a unit, to represent a series of compounds with similar structures and pharmacologic effects in complex mixtures. Major compounds can be representative of active groups but would be weighted to show the effects of compound groups in studies.
2. The effects of complex mixtures and interactions between bioactive compounds are related to their in vivo processes. Therefore, a pharmacokinetic-pharmacodynamic model could be very useful to reveal the mechanisms of complex mixtures. The critical part of such a model would be how to weight different compound groups and their separate effects compared to the overall mixture and its therapeutic effects.
3. Many drug targets are located in cells, including DNA, nuclear receptors, intracellular kinases, etc. Therefore, intracellular processing of the compounds by target cells are directly related to the therapeutic effects. Research on interactions between compounds at the cellular level may be able to connect pharmacokinetic and pharmacodynamic results [291,292].
4. Although the accuracy and completeness of existing databases are currently lacking, network pharmacology could be a very promising approach to reveal the mechanisms of natural herbal mixtures in the future.
5. Disease progression and treatment effects can be expressed at different biological levels; genome, epigenome, transcriptome, proteome and metabolome. Integrating these large-scale data to create pathological and therapeutic models could be very useful for studying natural compound synergism [293,294].

Progress in these areas is gradually revealing the extent and degree of synergism in complex mixtures. Once we understand the underlying mechanisms for synergism, we will be in a position rationally apply these principles to systems biology and pharmacology.

5. Chemical deconvolution of immunomodulatory natural products

As highlighted in the previous sections, NP-based drug discovery follows a workflow (Fig. 8) that encompasses analytical technologies, biological methods and biotechnological approaches that have improved significantly over the last two decades. Extracts of living organisms are initially selected for biological testing based on different strategies [295] including ethnopharmacological, ecological and metabolomics approaches, each one of them presenting benefits and weaknesses. Progress in gene sequencing and molecular biology has shifted the screening process towards a combination of target-based and phenotypic screening, which bears a better correlation with disease states, especially those with highly complex biology [296]. Following screening for bioactivity, active extracts are then submitted to recurring steps of fractionation and biological activity testing until the single bioactive compounds are isolated and their chemical structures confirmed or assigned by spectroscopic analyses (Fig. 8). This stage has been significantly improved by new chromatographic and analytical technologies, especially mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy methods [297–299].

NP-based drugs display greater chemical diversity and occupy larger regions of chemical space than drugs from completely synthetic origins

[300]. They present superior druggability due to intrinsic features like larger numbers of sp^3 carbon and oxygen atoms (but fewer nitrogen and halogen atoms), higher hydrophilicity, and greater molecular rigidity that favors the interaction with proteins [202]. Moreover, they usually possess higher numbers of asymmetric centers, which might be implied in higher selectivity to the biological targets [301]. While the chemical variability of natural products offers unique attributes for drug development, such diversity constitutes a challenge for the time-consuming processes of isolation and structure elucidation. Moreover, bioactive NPs frequently occur as complex mixtures in the living organisms, mostly at low concentrations. Thus, rapid deconvolution strategies that scrutinize the chemical composition of the active extracts and identify the major compounds in the active extract (dereplication) is of special relevance in accelerating the subsequent bioactivity-guided fractionation procedures [302]. Here we describe some classical methods of extract deconvolution and highlight some innovations accelerate the process, still following the established workflow of NP-based drug development.

5.1. Classical methods with new flavors – advantages and drawbacks

5.1.1. Isolation of constituents by chromatographic methods, offline structure elucidation and bioassays

The isolation and purification of compounds from crude extracts often requires the combination of several separation techniques. Open column chromatography (CC) is widely employed for initial fractionation due to its low cost and high sample capacity. However, due to the chemical complexity of plant extracts, it is frequently necessary to employ chromatographic techniques with higher resolution power, such as medium pressure liquid chromatography (MPLC) or semi-preparative high-performance liquid chromatography (HPLC), and multiple steps may be required to achieve the final purification [211,303,304], the selection of which will depend on the chemical composition of the sample. Complete structure elucidation of the isolated compounds can be achieved by using spectroscopic techniques such as ultraviolet-visible (UV-Vis), Infrared (IR), MS and NMR, while the absolute configuration of stereogenic centers can be determined by X-ray crystallography or by electronic circular dichroism (ECD) [304,305].

There are several recent reports on the isolation and identification of new immunomodulatory natural products employing this classical strategy. Six new lathyane diterpenoids from defatted ethanolic extract of seeds from *Euphorbia lathyris* have been isolated using liquid-liquid partition followed by silica gel CC and semi-preparative HPLC [306]. Structural elucidation was performed by the analyses of UV, IR, ESIHRMS, and NMR data and the absolute configuration of one compound was assigned by X-ray crystallography, while the C-2' configuration of another compound was determined by theoretical and experimental ECD. Three compounds inhibited LPS-stimulated NO production in RAW264.7 cells, while one of these also inhibited the production of pro-inflammatory cytokines IL-6 and IL-1 β , reduced the expression of iNOS and NF- κ B, inhibited I κ B α phosphorylation, and blocked LPS-induced nuclear translocation of NF- κ B, indicating its anti-inflammatory potential [306]. In another study [307], three new sesquiterpenoids, (1Z,4E)-lepidoza-1(10),4-dien-14-ol, rel-(1(10) Z,4S,5E,7R)-germacra-1(10),6 diene-11,14-diol, and rel-(1(10) Z,4S,5E,7R)-humula-1(10),5-diene-7,14-diol, were isolated from an ether ethyl extract of *Conocephalum conicum*. The crude extract was subjected to silica gel dry-flash CC in sequential steps and the chemical structure of the new compounds was assigned by complete spectroscopic analysis. Two of these were found to significantly decrease lymphocyte proliferative response to concanavalin A, a T cell mitogen. In addition, fourteen new eudesmane sesquiterpenoids, namely artemihedinic acids A-M and artemihedinin A, including three pairs of epimers, were isolated from the defatted ethanolic extract of *Artemisia hedini* (whole plant) using liquid-liquid partitioning followed by multiple steps CC fractionation using MCI gel CHP20P, ODS, Sephadex LH-20 or silica gel as stationary

analysis are the main advantages of this technique: the extraction process and analysis of volatile compounds can be achieved within 24 h. The main classes of natural products analyzed by this technique include terpenes (mono- and sesquiterpenes), phenylpropanoids, lipides (fatty acids) and sugars (after derivatization) [311]. For example, CG-MS data on the chemical structure assignment of the polysaccharide MP1, isolated from the mesocarp of *Orbignya phalerata* fruits, indicated that MP1 (shown to enhance phagocytosis in mice and exhibit anti-inflammatory activity) has a highly branched glucan type structure composed of alpha-(1→4) linked D-glucopyranose residues with (3→4), (4→6), and with (3→6) branching points [312].

HPLC/UHPLC-DAD is routinely employed to obtain the chemical fingerprint of extracts and to collect preliminary information on their chemical composition. Dereplication by this technique relies on the availability of commercial standards, whose retention times and UV spectra can be compared with those of the extract's constituents. Using a DAD detector also requires a suitable chromophore group in the chemical structure of the analyzed compound. HPLC/UHPLC-DAD is broadly employed for the identification of several classes of plant secondary metabolites, including phenylpropanoids, flavonoids, anthraquinones, coumarins, aromatic alkaloids and terpene derivatives with detectable chromophore groups (e.g. *Digitalis* cardenolides and sesquiterpene lactones). As an example, the use of HPLC-DAD disclosed a high concentration of catechin in a *n*-butanol fraction derived from the ethanol extract of *Acacia catechu* heartwood. This fraction induced immunomodulatory effects on non-specific, humoral, and cell-mediated immune functions, as demonstrated by different assays [313].

The use of HPLC/UHPLC-ELSD for dereplication purposes is also dependent on commercially available standards. The evaporative light scattering detector (ELSD) is based on turbidimetric measurements: the column effluent is nebulized into droplets, which are carried by a gas and directed towards a light beam; the light is scattered by residual particles of non-volatile material and measured by a photomultiplier or a photodiode [314]. Therefore, the technique is mainly used for the analysis of compounds without chromophore groups that absorb in the near UV region, like sugars, terpenes, and lipids. HPLC-DAD-ELSD-ESI-MS has been used to investigate the chemical composition of extracts from *Acanthosicyos naudinianus*, *Gomphocarpus fruticosus*, and *Cryptolepis decidua* [315], which were shown to inhibit T-lymphocyte proliferation (at concentrations that did not cause apoptosis or necrosis) through inhibition of lymphocyte activation by suppression of CD25 and CD69 surface receptor expression and also to reduce production of IFN- γ and IL-2. In this case the ELSD detection was useful for monitoring peaks that lack UV absorbance and MS ionization and, based on the HPLC profile, the possible classes of active compounds were identified as cucurbitacins for *A. naudinianus* and indole alkaloids for *C. decidua*, whereas for *G. fruticosus* they were not disclosed [315]. In another study, the phenolic profile of leaves from *Beta vulgaris* (subspecies *vulgaris*; variety *rubra*) was acquired by HPLC-ESI-HRMS-MS [316]. Twelve known compounds (ferulic acid hexoside, vitexin, (iso) vitexin pentoside, (iso)vitexin hexoside, (iso)vitexin pentoside, apigenin-C-pentoside-C-hexoside, isovitexin, acetyl (iso)vitexin, acacetin-C-glucoside, apigenin-C-pentoside-C-hexoside, acetyl (iso)vitexin, acetyl O-methyl (iso)vitexin, and (iso)swertisin 2''acetate) were putatively identified, seven of which had not been previously described for the species. The extract presented immunomodulatory effects (reduction of TNF- α , IL-1 β , and IL-6; down-regulation of the expression of hepatic NF- κ B) *in vitro* and *in vivo*, demonstrating its potential to reduce the progression of diabetic complications [316].

UHPLC-MS is the hyphenated technique that exhibits higher sensitivity and specificity to deconvolute constituents of complex matrices, allowing the identification of several compounds in a single run. Additionally, the use of MS/MS techniques with high resolution mass spectrometry increases the applicability of this method in the structural elucidation of new chemical entities. The generated spectrum furnishes information on the molecular weight, along with typical structural

fragmentations patterns, revealing the loss of fragments like hydroxyl, carboxyl, and esters groups, in addition to sugar residues, among others, however an important limitation of the technique is the impossibility of differentiating isomers [316]. Fig. 9 depicts the workflow of a dereplication procedure based on UHPLC-MS.

HPLC NMR is the most powerful hyphenated technique for deconvolution that allows the unequivocal structure assignment of compounds in complex mixtures. The main limitation of the technique is obtaining satisfactory ^{13}C NMR spectra due to ^{13}C lower frequency and low natural abundance in comparison to ^1H . The use of solid phase extraction (SPE) and multiple injections (sometimes over 10) can be adopted as a strategy to overcome this restriction, thus affording sufficient sample quantities for mono- and bidimensional NMR experiments. To accomplish this, after multi-injections the SPE-bound compounds are eluted with deuterated acetonitrile and the NMR analyses are undertaken [317]. Semi-hyphenated LC-MS-SPE NMR has been employed to elucidate the chemical structures of three eremophilane sesquiterpenes produced by cultures of *Penicillium roqueforti* [318]. One of these was found to be a new compound, namely (3S)-3-acetoxyeremophil-1(2),7(11),9(10)-trien-8-one, a probable biosynthetic precursor of PR toxin. A further compound, structurally related to the immunosuppressant cuspidatol, exhibited immunosuppressive activity in the Mixed Lymphocyte Reaction assay [319].

5.1.3. Adding new flavors into the classical deconvolution methods

While hyphenated techniques are undoubtedly a valuable tool for the deconvolution of crude extracts, permitting the identification of common compounds at an early stage of drug development, the procedures currently adopted for chemical deconvolution do not disclose the bioactive constituents of extracts, which must be isolated and further tested for bioactivity. There are strategies available to speed up the classical workflow process of NP-based drug research and development that combine simple *in vitro* bioaffinity assays with classical methods of chemical deconvolution. Although there are inherent limitations for testing immunomodulating compounds by simple *in vitro* assays, their use as tandem methods for dereplication can be employed as a screening strategy for selecting compounds for further *in vivo* assays. Here we describe three different approaches - ultrafiltration coupled to LC-MS, cell membrane chromatography (CMC) coupled to LC-MS, and Ligand fishing.

Ultrafiltration-based affinity selection combined with liquid chromatography-mass spectrometry (LC-MS) was originally developed for screening synthetic or combinatorial libraries [320] and it is currently employed to screen extracts and traditional medicines [321]. The extract is incubated with the target protein for a defined time under established conditions and non-specific binding can be addressed by incubating the sample with or without the denatured protein. Ultrafiltration (UF) is performed after incubation using a regenerated cellulose ultrafiltration membrane with a 10,000 molecular weight cutoff, to accomplish the separation of bound from unbound compounds and the UF cell containing the ligand-protein complex is washed to remove any unbound compounds. The aqueous-organic buffer is then run through the chamber to dissociate the ligand-protein interactions. The bound compounds are either analyzed directly by the mass spectrometer or trapped on a column and analyzed by LC-MS [317]. This approach has been used in several studies for the rapid deconvolution of bioactive constituents from plant extracts; for example, bio-affinity UF-LC-MS was used to fish out the potential cyclooxygenase-2 (COX-2) ligands of a flavonoid-rich fraction from the Chinese herb *lotus plumule*. A total of 12 flavonoids showing specific binding to COX-2 had their chemical structure identified [322]. The antioxidant and anti-inflammatory constituents of *Gynura procumbens* was investigated, targeting DPPH and COX-2 by this approach, and disclosed caffeic acid, kynurenic acid, and chlorogenic acids as compounds with highly binding affinity to both targets [323]. A multitarget approach can also be employed, as analysis of *Warburgia ugandensis*, subjected to UF-LC-MS analysis using COX-2,

5-lipoxygenase, and topoisomerases I and II as targets elicited two isomeric neolignanamides as the active compounds [324]. A different investigation of *W. ugandensis* using UF-LC-MS revealed 9 and 12 potential superoxide dismutase (SOD) and xanthine oxidase (XOD) ligands [325]. A search for inhibitors of protein tyrosine phosphatase 1B (PTP1B) a regulator of multiple signalling pathways, including the Janus kinase and signal transducer and activator of transcription (JAK-STAT) signalling, in Chinese red yeast rice by UF-LC-MS resulted in the identification of the selective ligand monascorubramine [321]. Limitations of the UF-LC-MS approach include the requirement for high amounts of purified proteins and its restriction to cytosolic proteins, since transmembrane proteins are prone to non-specific interactions that may result in false positives [317].

Cell membrane chromatography (CMC) employs the integrated cell membrane to investigate the interaction between ligands and transmembrane receptors. The cell membranes containing specific receptors are immobilized on silica and packed into a column [326]. The extract is eluted through the CMC column and those ligands with high affinity are retained, whereas nonspecific unretained analytes are quickly eliminated. The combination of CMC with LC-MS is a valid strategy for the synchronized bioactivity screening and chemical deconvolution of complex matrices like crude extracts. This approach has been used to identify bioactive natural products directed towards different biological activities [327,328], including potential immunomodulating compounds. For example, *Saposhnikovia divaricate* extract, a plant species used in traditional Chinese anti-allergic preparations subjected to a high-expression Mas-related G protein-coupled receptor X2 (MRGPRX2) cell membrane chromatography coupled with LC-MS and the process yielded three constituents, prim-O-glucosylcimifugin, cimifugin, and 4'-O-β-D-glucosyl-5-O-methylvisaminol, whose antiallergic activity was further confirmed *in vivo* [329]. Another Chinese herbal drug (*Radix Salviae Miltiorrhiae*) subjected to dual CMC containing the epidermal growth factor receptor (EGFR) and fibroblast growth factor receptors 4 (FGFR4) as immobilized targets, coupled to LC-MS identified salvianolic acid C, tanshinone I, tanshinone IIA and cryptotanshinone as ligands, with EGFR and FGFR4 activities [330]. A CMC prepared with murine macrophage cell line RAW 264.7 and coupled to LC-MS to screen three Chinese herbs, *Rheum officinale*, *Angelica dahurica* and *Radix bupleuri* disclosed emodin, dehydrocostus lactone, scopoletin, isomeratorin and phellopterin as anti-inflammatory ligands [331]. The stability of CMC columns is expected to be improved in the future and a larger number of suitable cells or enzymes shall be available to be used as screening tools Hou et al. [327]. A limitation of CMC is the requirement of using a predominantly aqueous mobile phase (typically >95%) to ensure proper ligand-protein interactions, which may result in increased non-specific binding [317].

The ligand fishing technique, which can handle either large or micro liter scale of biological samples [327] involves immobilizing a targeted protein onto the surface of magnetic beads and the active ligands are subsequently fished out by suspending the protein-coated beads directly in a crude plant extract. Combining this approach with chromatographic and spectroscopic methods permits rapid identification of bioactive compounds from a complex matrix and has been applied to the screening of a variety of enzyme inhibitors [332,333], in addition to fishing out immunomodulating compounds from plant extracts. An aqueous extract of *Saussurea laniceps*, a plant species traditionally used in China to treat arthritis, was incubated with COX-2-functionalized magnetic nanoparticles, resulting in the identification of scopoletin, syringin, chlorogenic acid and umbelliferone as ligands [334]. The anti-arthritic activity of scopoletin and syringin was further confirmed in rat models of rheumatoid arthritis and osteoarthritis, thus demonstrating the validity of the approach. Ligand fishing was also employed to identify cyclooxygenase-1 (COX-1) inhibitors from a turmeric extract [335], disclosing four curcuminoids (curcumin, demethoxycurcumin, bisdemethoxycurcumin, and 1-(4-hydroxy-3,5-dimethoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-(1E,6E)-1,6-heptadiene-3,5-dione)

whose chemical structures assignments in the original MS spectrum was not straightforward as COX-1 inhibitors. Although ligand fishing in tandem with MS is a valid strategy, the relationship between immobilized enzyme activity and fishing results (accuracy and sensitivity) are still poorly understood. In a study utilising immobilized COX-2 magnetic microspheres with different activities, fished inhibitors from a green tea extract reduced significantly in line with decreased activity of immobilized COX-2, and revealed 3-galloylquinic acid, 5-caffeoylquinic acid, and (-)-epicatechin 3-O-gallate as the active compounds [336]. In addition to its dependence on immobilized enzyme activity, ligand fishing has been limited to cytosolic proteins, since using transmembrane protein coated beads may promote the retention of non-specific ligands due to boundary lipids [317].

In summary, the workflow of research into NP-based drugs has been modernized by the incorporation of new analytical and biological strategies in the last decades. The online identification of the chemical structure of compounds in crude extracts has accelerated the process, mainly resulting from new tandem chromatographic methods, especially LC-MS. In spite of that, new flavors are being incorporated more and more to the old formula by using *in vitro* bio-affinity assays combined (either online or offline) with spectroscopic methods for structure elucidation of the bioactive compounds. There are currently several bio-affinity methods, other than ultrafiltration, cell membrane chromatography and ligand fishing briefly reviewed here, that have been established for this purpose. The common limitation to all of them seems to be the use of simple *in vitro* assays, which have a poor predictive value for complex disease states, as those targeted for immunomodulating drugs. The development of more robust bio-affinity phenotypic models to be employed in the screening of extracts in combination with LC-MS is a challenge that has yet to be overcome to foster NP-based drug development. It is also worthy to note that the isolation of bulk amounts of active compounds will be necessary for further preclinical development, including ADME studies.

5.2. Metabolomic approaches – advantages and limitations

As mentioned in other sections, natural products have played an important role in the development of drugs with immunomodulatory properties. Potent agents (purified natural products) have been identified for their immunosuppressive properties, notably ciclosporin and then tacrolimus, which act as anti-calcineurins. Other NPs acting on the mTOR protein (e.g. sirolimus, everolimus) are also known to have similar clinical effects.

Often phytopreparations (e.g. *Echinacea*), plants used in traditional medicine (e.g. Siberian Ginseng; *Eleutherococcus senticosus*) or mushrooms (*Ganoderma lucidum*) have been used for their immunomodulatory, and particularly immunostimulant, properties. For NPs used in the form of mono-substances as immunosuppressants, while the *in vitro* pharmacological effects are well documented, a complete understanding of the *in vivo* mechanism(s) still requires advanced studies. For phytopreparations, of the many studies that have been conducted to investigate the active ingredients, the vast majority have not led to clear conclusions for pure compounds that can explain the recorded clinical effects and therefore more holistic studies to explain the immunomodulatory mode of action *in vivo* are needed. For many of these phytopreparations, the actions of polysaccharides or various glycosides in particular have been demonstrated, although extensive research to model the pharmacokinetic/pharmacodynamic (PK/PD) properties of the various components of natural extracts that are commonly taken as medicines is lacking. Thus, in order to try to understand, at the molecular level, how these NPs act and what their mode of action may be, modern holistic approaches are necessary. In this context, metabolomics, which has been used since the early 2000 s in parallel with all the omics approaches of systems biology, has become an essential tool [337].

The aim of metabolomics is to perform a comprehensive and

quantitative analysis of the largest possible number of low molecular weight (<1000 Da) metabolites in biological samples and it plays an increasingly important role in various aspects of the life sciences, and in particular in natural product research. Metabolomics is indeed a component of systems biology, which includes a number of other "omics" technologies such as transcriptomics (gene expression) and proteomics (protein expression). Among the "omics" technologies, metabolomics provides the most "functional" information by offering a comprehensive view of the biochemical state of an organism [338,339] and can be used to monitor significant changes in metabolite levels. Indeed, as metabolites are the end products of cellular regulatory processes, their levels can be considered as the ultimate response of biological systems to genetic or environmental changes. This information can be used with other systems biology approaches to assess gene function (functional genomics) and provide a holistic view of a living system for further study.

In the context of NP research, metabolomics-type approaches can take different forms: 1) those linked to the search for biomarkers as described above, which are based on the comparison of metabolic fingerprints with only identification of significant biomarkers; 2) others which are not comparative in scope, which allow the complete characterisation of the metabolome by metabolite profiling (deep metabolome) with annotation/identification of a large number of metabolites. Detailed metabolite profiling of natural extracts extends the chemical compositional coverage of what is more conventionally done by dereplication (see above) in NP-based drug discovery [340].

Currently, immense compositional data obtained at the scale of a large number of extracts opens up new possibilities for investigation in bioactivity screening campaigns, as this potentially allows trends in composition to be linked to the response to biological assays and thus to predict which compound (or class of compound) may be active for targeted isolation without having to go through classical and time-consuming activity-guided isolation. This is becoming especially possible with the recent development of chemometric methods and artificial intelligence algorithms [341]. From a practical point of view, a metabolomics approach is generally divided as follows: sample collection, extraction (as exhaustive as possible; different solvents depending on the polarity), metabolite profiling of the extracts by different spectroscopic methods (coupled or not to chromatographic methods), multivariate analysis of the data, highlighting of differences among the samples, identification of associated biomarkers, interpretation of the data at the pathway biosynthetic level and integration with other omics data types. Such an approach can have several iteration cycles and is mainly used to generate hypotheses in an unbiased, data dependent, manner [337].

The profiling of the extracts is done either by high resolution MS (large number of compounds, sensitive detection) or by NMR (major compounds only, quantitative response). For metabolite profiling in the vast majority of cases, coupled methods linked to ultra-high pressure chromatography are used (UHPLC-HR-MS) [340]. LC-MS is indeed very versatile and well adapted for profiling secondary metabolites, while GC-MS after derivations is also often used for the study of metabolites from primary metabolism [342]. For these different profiling approaches, however, the accurate identification of NPs remains a major challenge. Today, there are more than 300,000 described NPs, and there is no comprehensive database that allows easy comparison of spectral fingerprints for unambiguous dereplication of known compounds, and the structural predication for unknowns is even more challenging. In order to overcome this deficiency, *in silico* structure-based spectral simulation approaches have recently been developed [343]. These approaches allow the calculation of MS/MS fragmentation spectra that can be compared with data recorded on natural extracts. In the context of extract metabolite profiling, current approaches allow fragmentation spectra to be recorded automatically for the largest number of compounds detected. In addition, so-called 'molecular network' (MN) approaches have also emerged and are increasingly used in NP research

[344]. A molecular network is used to group together all the molecular mass detected in a given extract or set of extracts [345] and taxonomic or bioactivity metadata can be added to this information. It is thus possible to obtain clusters that often correspond to the same type in a graphical way. This way of organising the data allows to efficiently identify the different classes of structure that can be found in an extract, and to see if they are common between extracts [346]. An application of this approach has, for example, enabled the identification of compounds with anticancer activities in a set of more than 300 *Euphorbiaceae* extracts [347].

A large number of efficient structural annotation tools are open to the research community and have been developed very recently in this field [348]. The extensive annotation data generated are thus complementary to bioinformatics and artificial intelligence approaches that are increasingly becoming prevalent in drug discovery [349]. Thus, metabolomics play an important and increasingly prominent role in NP research to provide detailed information, notably on the complex plant metabolome. This, in turn, can serve as a functional complement to other omics approaches, support the prioritisation of NPs of interest, and help to understand the complex *in vivo* effects associated with the intake of phytopreparations.

In the context of the search for novel immunomodulating drugs, metabolomics has been conducted both to characterise the composition of phytopreparations of interest and to try to gain an understanding of their effects *in vivo*. Furthermore, this type of information potentially allows to get an insight into possible synergistic effects likely to occur when phytopreparations are taken as medication. Thus, for example, Ginseng-based phytopreparations are used as immune-modulating dietary supplements in various diseases [350] or as potential vaccine adjuvants. Ginseng has been extensively investigated and is well documented in the literature, and metabolomics approaches have been developed to document its complex phytochemical composition and to differentiate the quality of extracts found on the market [351]. Members of the *Araliaceae* family, *Panax ginseng* (Asian/Korean ginseng) and *Panax quinquefolius* (American ginseng) have been extensively studied and the composition of their roots is well established, consisting of saponins, ginsenosides, phenolic compounds, including carbohydrates and carotenoids. The pharmacokinetic profiles of ginsenosides have also been investigated [352] and the immune potential of ginseng has been investigated both *in vitro* and *in vivo*, including clinical data in humans [350]. The phytochemical composition of ginseng products has been characterized in depth [353]; notably a metabolomics study based on ultra-performance liquid chromatography coupled to quadrupole time of flight mass spectrometry (UHPLC-QTOF/MS) was applied for the quality evaluation of white ginseng, tae-geuk ginseng, red ginseng, and black ginseng. The generated LC-MS data were analysed and the four processed ginseng products were well differentiated, allowing the identification of several ginsenosides as major components of each product. This study underlined the importance of this type of approach for the quality control of such a complex herbal preparation [351].

To our knowledge, despite the large number of studies on the composition and pharmacology of ginseng, very few studies have been carried out using *in vivo* metabolomics. A recent study revealed that serum and faeces metabolomics revealed a significant metabolic distinction between American and Asian ginseng in diet-induced obese (DIO) mice. The results indicated that both ginsengs attenuate glucose and lipid metabolism disorders in DIO mice, and metabolic pathway analysis shows that they both dynamically correct metabolic disorders mainly by regulating linoleic acid metabolism, cysteine and methionine metabolism and unsaturated fatty acid biosynthesis [354]. In another study, the anti-stress effects and corresponding mechanisms of ginseng total saponins (GTS) on hindlimb-unloaded rats were assessed by an LC-MS metabolomics study. Three groups of rats were compared, one control group, one groups of rats submitted to stress caused by microgravity and a group of rat treated by GTS. Levels of plasma corticosterone (CORT) and weights of immune organs including the thymuses,

spleens, and adrenal glands were determined. Urinary metabolic profiles of the rats under the simulated microgravity condition, with and without GTS intervention, were compared by LC-MS based metabolomics. Multivariate statistical analysis revealed that compared with control, the plasma CORT level of the SM rats was significantly elevated, and GTS could restore this elevation to a lower level. GTS could also significantly alleviate the atrophy of the thymuses and the spleens, as well as the hypertrophy of the adrenal glands of the SM rats. Urinary metabolite profiling indicated that a series of metabolic pathways including taurine and hypotaurine, purine and pyridine, and amino acid were affected and eleven potential biomarkers were identified. The findings of this study revealed a molecular basis for the anti-stress benefits of GTS in the management of microgravity-related disorders [355].

At the level of pure constituents another study was performed on *Eleutherococcus senticosus* (Siberian ginseng), a medicinal plant containing apoptogenic substances believed to regulate immune responses [356]. In this study the effect of Eleutheroside E (EE), a lignan glycoside known to have protective effects in ischemic tissue and anti-inflammatory actions, were assessed by metabolomics using GC-MS, which is known to be particularly suited for the detection of primary metabolites after derivatisation [342], to quantitatively measure the abundance of known primary metabolites in H9c2 cells. Pre-treatment with EE dramatically inhibited mitochondrial oxidative stress and various markers were assessed and 68 metabolites with reproducible signals were monitored in each sample and visualized in a heat map. Pathway analysis highlighted the inhibition of fatty acid biosynthesis and alternation of arginine and proline metabolism as two potential links to the favourable effect of EE on hypoxia-reoxygenation-injured cardiomyocytes. This provided evidence that EE may be a potential drug for myocardial ischemia-reperfusion injury by reducing oxidative stress, NF-kappa B activation, and metabolic reprogramming [357].

As discussed here, metabolomics has great potential for understanding, at the molecular level, how NPs act on the immune system, whether as a single substance or a complex phytopreparation. Although introduced almost 20 years ago in NP research, this approach should be more systematically used to understand how NPs act in order to discover new active ingredients and new targets. Metabolite profiling methods have evolved considerably and now allow the detection of a very large number of metabolites and provide information rich high-quality spectroscopic data. However, the unambiguous structural identification of biomarkers or NPs in extracts remains a major challenge. Recent methods relying on spectral matching against large *in silico* databases as well as data analysis in the form of molecular networks have allowed substantial progress. These methods are beginning to have the ability to predict even unknown metabolites [348]. However, major efforts are still needed in this direction to achieve confident structural annotation. This is important because only accurate structural knowledge can enable correct interpretation and thus pave the way for important changes in Pharmacognosy in the digital era [358]. We believe that the contextualization of the mass of information obtained by metabolomics with the establishment of an integrated and open databases ecosystem will nurture the discipline.

There is thus great potential to link the pharmacological data reported for many NPs to their effects reported at the clinical level, and this will become possible through the rapid advances in artificial intelligent methods, provided that data are correctly reported in searchable format. To explain the clinical effects observed with the use of specific plants, reverse pharmacology approaches will be important and, in this context, metabolomics should allow full characterisation of the composition of the used phytopreparation on the one hand, but also ideally to monitor the metabolism of the compounds in biological fluids, which should make it possible to obtain PK/PD data ideally on all the constituents. Studies in this direction have already been conducted *in vivo* in animals for Traditional Chinese Medicine preparations [359] and advanced ex-vivo models of intestinal permeation also show good

potential in this context [360]. A better knowledge of the permeation and metabolism of NPs should then allow the testing of compounds that have a real role *in vivo* on relevant pharmacological targets to explain a given immunomodulatory effect. In this context, metabolomics and other omics approaches used in systems biology will undoubtedly allow deciphering at the molecular level how various NPs modulate the immune system. For phytopreparation, investigations of the gut microbiota must also be carried out because ingested NPs certainly have an important influence at the intestinal level and probably can thus indirectly explain the clinical effects observed [361]. Although very complex, research is beginning to be carried out in this field [362] and metabolomics also has an important role to play.

6. Future perspectives, opportunities and challenges

6.1. Phylogenetic approach to identifying sources of NP-derived immunomodulating drugs

The fact that plants in certain families are more likely to be known for medicinal use has long been reported by ethnobotanists [363,364]. Families reported as being particularly rich in medicinal species differ between temperate and tropical regions, and between New World and Old-World flora, but often include Apocynaceae, Fabaceae and Lamiaceae [363–366]. At finer taxonomic scales, plants from the same genus are often used more or less interchangeably for a particular disease; for example, several different species of the genus *Aspidosperma* are used as antimalarials in Latin America [367]. These patterns in the taxonomic distribution of plants traditionally used as medicines are also apparent in plants from which clinically approved drugs have been derived, and there is increasing evidence that they are attributable to shared chemical pathways in closely related plants [368].

Over the past decade, growth in the availability of genetic data for plants coupled with development of software for comparative phylogenetic analysis has facilitated a more comprehensive and detailed understanding of how medicinal plant species are distributed across the plant Tree of Life (Reviewed in [369]). Numerous studies confirm the earlier inferences of ethnobotanists that, far from being randomly distributed, plants used for medicine are strongly clustered on the Tree of Life [9]. Furthermore, clusters of medicinal plants are not confined to genus or family level, toward the tips of the Tree of Life, where they can be readily detected using taxonomic classifications; such clustering can be traced to deep lineages in the Tree of Life (as indicated in [367]) and these same deep lineages rich in medicinal plants are reported from the floras of geographically and culturally distant areas of the world [369].

Phylogenetic approaches are powerful not only for detecting, but also for quantifying how traits of interest are clustered across the Tree of Life. A wide range of metrics is now available for this purpose [370] but the most fundamental metrics measure phylogenetic signal, i.e. the extent to which related species resemble each other more with respect to the trait(s) of interest than species drawn randomly from the same phylogenetic tree [371]. Correlations between trait (phenotypic) similarity and phylogenetic relatedness may be attributable to closely related species retaining a trait shared by a common ancestor, or to them responding in similar ways to similar ecological pressures. While distinguishing between these two situations is of great interest for ecologists [372], such fine distinctions are not necessary to recognise and realise the value of phylogenetic signal for bioprospecting.

Combining well-supported plant phylogenies, enhanced knowledge of plant chemistry and one of the phylogenetic metrics mentioned above, a recent comprehensive analysis of phylogenetic patterns in the distribution of plant secondary metabolites (PSM) across the plant Tree of Life showed significant but weak phylogenetic signal for all eight classes of plant secondary metabolites studied, highlighting the potential to use such phylogenetic signal for bioprospecting [373]. Here we explore the extent to which these new findings are directly applicable to the urgent search for new immunomodulators.

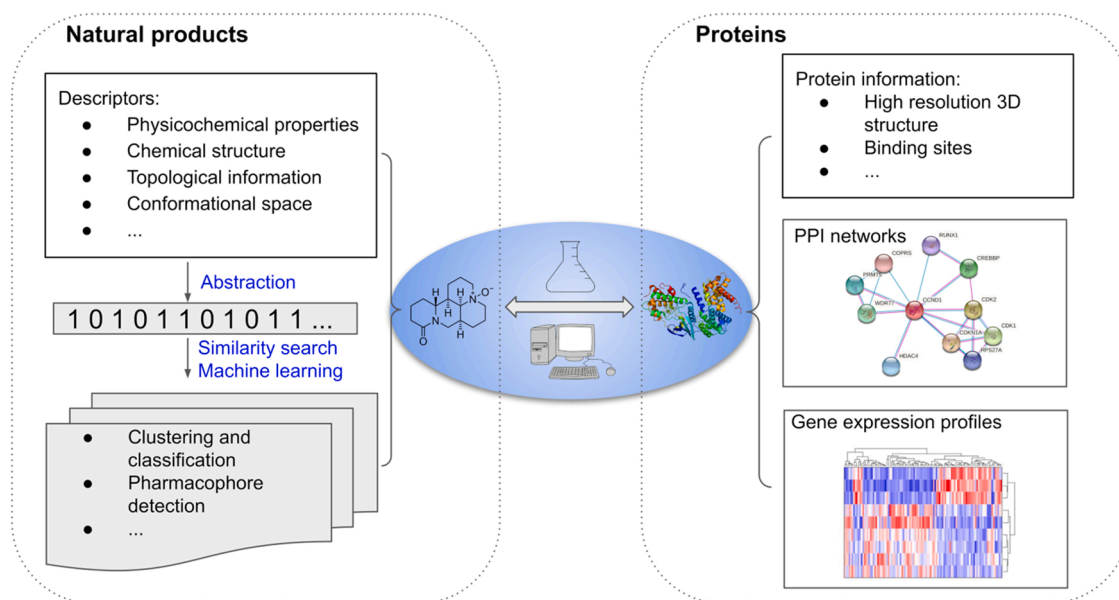


Fig. 10. Overview of cheminformatics in natural product discovery. Different descriptors of natural products are collected and abstracted. These can subsequently be clustered and classified or pharmacophore structures can be detected with similarity searches or machine learning approaches. Basic information about proteins, such as high-resolution 3D structure and binding sites, as well as additional information, such as PPI networks and gene expression profiles are collected. Links between natural products and proteins can be built based on either experimental assays or computational approaches, such as virtual screening. Based on the collected information from natural products and proteins and the links constructed between them, druggable natural products could be screened based on common targets of well characterised drugs, or the mode of action of newly discovered drugs could be identified by target prediction and network analysis.

Immunomodulatory activity has been reported from a wide range of phytochemical classes [374,375], sourced from many different plant families and genera across the Tree of Life. Many of these plants and their constituents are associated with nutraceutical or traditional medicinal uses, rather than for pharmaceutical drug discovery or development. Clear taxonomic patterns in the source plants are not evident from the lists and tabulations published to date [376]. Furthermore, any search for such patterns must be undertaken in the knowledge that most of the available data is ‘presence only’: the absence of a report of immunomodulatory activity from a particular plant does not imply that it will not be found in the future (by a different method, from a different plant part, in a different season), merely that it has not been reported to date. Moreover, since scientists have learned to look for similar pharmacological activity in closely related species, some clusters of closely related species with immunomodulatory activity may represent evidence of targeted sampling as much as phylogenetic signal. For example, paclitaxel, originally reported from the Pacific yew tree (*Taxus brevifolia*), prompted a successful search for molecules with similar activity in other species of *Taxus* [107,109]. Notwithstanding all these caveats, we consider a phylogenetically informed search for potential sources of immunomodulators a worthwhile endeavour, not least because significant phylogenetic signal for medicinally relevant activity can often be detected in large datasets, even if incompletely sampled (as indicated in [367]).

Although many compound classes have immunomodulatory activity, only certain phytochemical classes have been developed, or are under evaluation, for drug or vaccine adjuvant discovery (see Section 2.2.). For example, a phylogenetically-informed search for triterpene saponins, a compound class of high interest for vaccine adjuvant development, could take into account the recent findings that, across seed plants, terpenoids are the PSM class to show strongest phylogenetic signal, and that they tend to show phylogenetic signal across different taxonomic scales (from genera and families to deep nodes in the Tree of Life), in sharp contrast to steroids which tend to be randomly distributed across most taxonomic scales [373].

Importantly, detecting strong phylogenetic signal for a particular PSM class in a particular clade does not necessarily reflect strong

conservation of PSM compounds; it could equally be attributable to bursts of diversification, which are also of interest from a bioprospecting perspective. Across all seed plants, [373] interpreted the observed phylogenetic signal as consistent with strong diversifying selection and/or relatively weak evolutionary constraints on PSMs. They suggest that the patterns of PSMs across major clades might be attributable in large part to multiple origins of PSM biosynthesis due to external selective forces for different genetic pathways. This interpretation of frequent multiple origins of major classes of secondary metabolites across the seed plant Tree of Life is consistent with studies at finer taxonomic scales (on smaller species groups) of smaller groupings of PSMs. For example, the low degree of relatedness of enzymes recruited for triterpene saponin biosynthesis, which appears to have occurred multiple times during evolution, suggests that triterpene saponin biosynthesis in plants is not restricted to a specific phylogenetic origin [377]. Indeed, a separate study revealed that triterpenes with an oleanane aglycone are the most common triterpene class and are present in most orders of the plant kingdom [378]. Since the majority of triterpene saponins evaluated for potential vaccine adjuvant activity are derived from oleanane (Supplementary Table 1), their phylogenetic distribution in plants, and particularly any specific glycoside substitution patterns that correlate with structure-adjuvant activity, merit more extensive scrutiny to establish if a phylogenetic signal can be detected. This approach could facilitate prediction of plants as potential new sources of triterpene saponin-derived vaccine adjuvants. An initial focus for this approach could concentrate on plant species in the Malvid clade, where terpenoids show strong phylogenetic signal, perhaps contrasting them with species of the closely related Fabid clade in which terpenoid distribution appears random [373].

Although our exploration of opportunities for phylogenetic prospecting for natural product derived immunomodulators focused primarily on seed plants, many of the principles discussed are applicable to the fungi. In fact, opportunities for phylogenetic prospecting in the fungal kingdom are arguably more exciting than in plants. As discussed for plants, most reports of immunomodulatory activity in fungi likely represent ‘presence only’ data, however this limitation may be offset by the fact that the decades-long reliance of fungal taxonomists on DNA

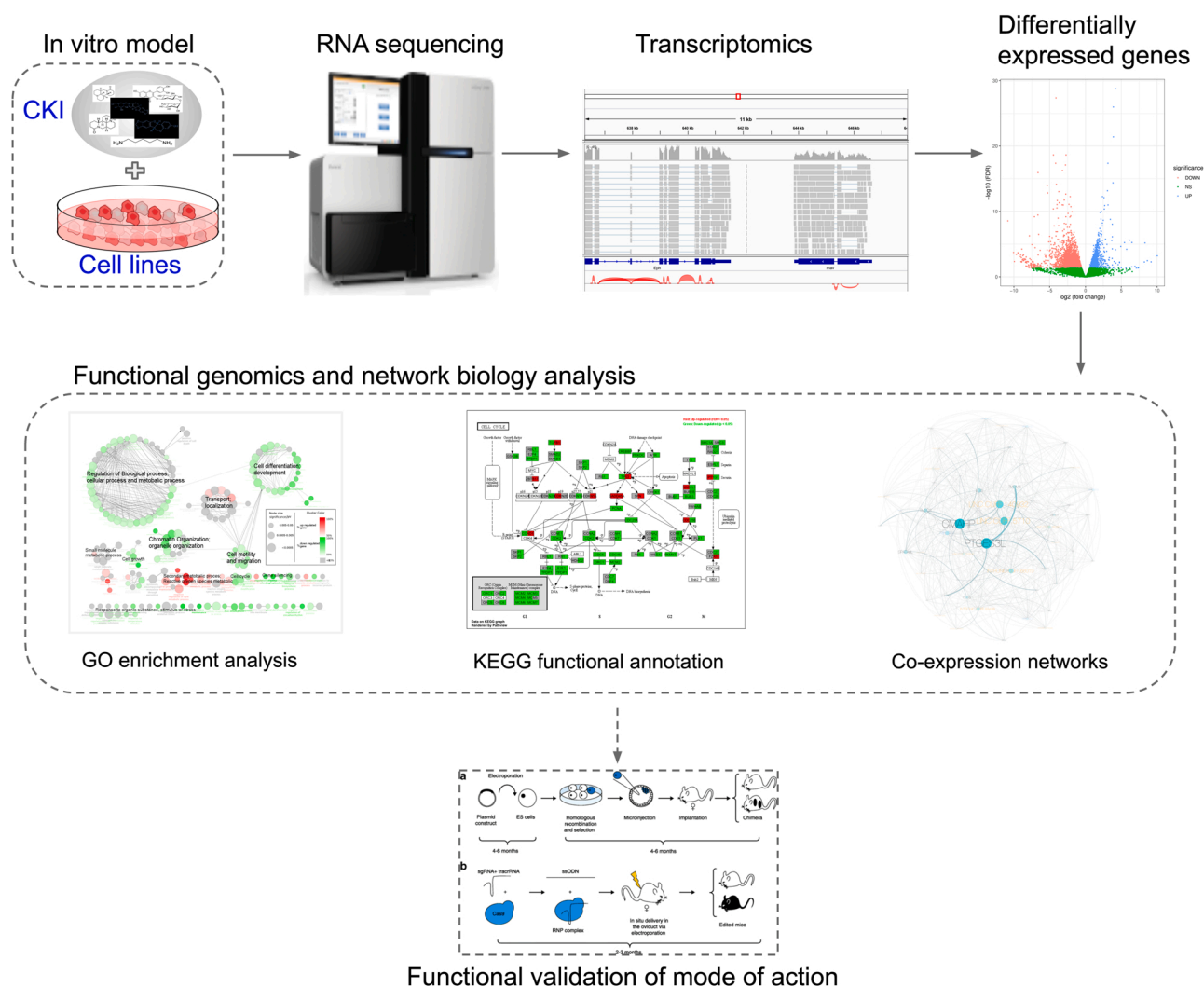


Fig. 11. Framework for transcriptomics analysis of the mode of action of CKI. An in vitro model using CKI in cancer cell lines was used. Transcriptomes were obtained using RNA-Seq and differentially expressed genes representing the perturbation caused by CKI were identified. Genes and pathways perturbed by CKI were identified based on differentially expressed genes using functional genomics and network biology approaches. These genes and pathways were used as candidates for further validation to confirm the mode of action of CKI.

sequence data for classification has resulted in a relatively large proportion of the fungal species known to science being represented by sequence data, providing an exceptionally well-sampled phylogeny in terms of the known diversity. From a sustainability perspective, collecting fungi does not cause detriment to species or ecosystems as only minuscule quantities of mycelium are sampled. In fact, such samples, if cultured in vitro, can be conserved in biobanks, safeguarding genetic diversity which might otherwise become extinct [107]. Moreover, the fungal diversity yet to be scientifically described is estimated to greatly exceed plant diversity in terms of species numbers [379] offering vast potential for discovery of uncharacterised biosynthetic pathways and novel molecules of interest for drug discovery.

6.2. Bioinformatics approach to identifying sources of NP-derived immunomodulating drugs

The advances in sequencing technologies and computational methods in the post-genomic era have propelled bioinformatics into significant roles in almost every aspect of biological research. In pharmacology, computational approaches have been broadly used, from characterising and identifying novel drugs, to exploring the molecular mechanisms of existing drugs. This is particularly true for natural

products or natural product-derived drugs, since most natural products are compounds that are biosynthesized by primary or secondary metabolic pathways. Genomic approaches, such as whole genome sequencing, will be useful tools to understand the biosynthesis of natural products. More importantly, in contrast to synthetic drugs which are identified with clear and specific target(s), natural products are likely to have multiple gene or pathway targets, many of which are still uncharacterised. Systems biology, network biology and functional genomics are well suited to this task.

Cheminformatics is the most widely used bioinformatics area in the study of natural products. Cheminformatics compiles computational approaches to build and extend the links between two core elements, natural products and proteins, based on the molecular similarity principle of “similar molecules have similar biological effects” [380] (Fig. 10). As the first core element, descriptors representing various properties of natural products are collected and prepared. These descriptors might be molecular fingerprints abstracted from 2-dimensional chemical structure, physicochemical properties, and topological information. In addition, 3-dimensional conformational space and molecular space can also be translated as descriptors to reconstruct the profile arrays of natural products for computational methods. Once the profile arrays of natural products have been reconstructed, molecular similarity

analysis can be performed to group natural products based on their pharmacophore structures, or to infer the potential mode of action of novel identified natural products if they share a similar profile array with well characterised drugs. Protein structures are the other core element for cheminformatics, as high-resolution protein structures are essential for virtual screening of potential binding ligands. PPI (Protein-protein interaction) networks could greatly extend our understanding in polypharmacology after building the bridge between natural products and their target proteins. Links between natural products and proteins could be bi-directional. Novel natural products and their potential clinical applications could be screened and inferred based on molecular similarity searches against well-characterised drugs. On the other hand, the polypharmacology of natural products means that they may have multiple targets. By building the links between natural products and proteins with additional information, such as PPI, new targets of natural products could be predicted for repurposing their clinical applications. In addition, unwanted adverse or side effects of natural products could also be identified. Furthermore, many natural products are used as complementary medicines in clinical settings and precision administration of natural products could be based on their targets of proteins and pathways. Although traditional experimental assays, such as affinity chromatography, three-hybrid systems, genetic screening, functional interference approaches, are still fundamental tools for building the links between natural products and proteins [381], novel computational approaches, such as machine learning and deep neural networks, have been developed and implemented in natural product discovery and target deconvolution. For example, a method with trained multi-task neural networks based on large-scale medical indication data was developed to find the privileged scaffolds of natural products, which could serve as an improved source of leading structures for the purpose of natural product abstraction [382]. Another target prediction model leveraging the transfer learning method was built and fine-tuned using a natural product dataset to improve the prediction performance of the targets of natural products [383]. Although the development of cheminformatics has dramatically enriched our “toolkit” for discovery of hidden “treasures” in natural products, new challenges are also posed. First, available knowledge bases, including both natural product and protein databases, and biological data linking these two core elements, are still limited. The limited size of knowledge bases will lead to false negative discoveries, while the poor quality of these knowledge bases due to lack of validation will lead to false positive predictions. Second, many approaches in cheminformatics were initially designed from synthetic compounds/drugs, therefore, the results should be interpreted with caution when they are applied to natural products. Third, computational cheminformatics approaches are powerful tools for simplifying the screening of bioactive natural products, however additional validations are still needed to confirm those predictions.

In addition to well-characterised cheminformatics approaches used in the study of natural products, other genomic and transcriptomic based methods, including functional genomics, network biology and systems biology can also be applied to study the mode of action of natural products. This is particularly useful for many natural products that include multiple compounds, such as herbal supplements or many Traditional Chinese Medicines (TCM), which might target multiple genes and pathways. Instead of fractionating the mixtures into individual ingredients, and studying their mode of action individually, Adelson's group have investigated CKI (Compound Kushen injection), a TCM from extracts of two medicinal plants (Fig. 11). Transcriptomes of *in vitro* models including multiple cancer cell lines treated with different doses of CKI, were obtained using RNA-Seq. Differentially expressed genes in CKI-treated cells compared to untreated cells were identified, and functional enrichment methods together with network biology were employed to identify the potential gene targets of CKI, along with potential CKI-perturbed molecular pathways [384,385]. With this transcriptomics-based framework, they were able to identify cell cycle, energy metabolism and DNA repair as potential target molecular

pathways for CKI inhibition of cell proliferation and induction of apoptosis in cancer cells [386]. In addition, combined with a fractionation, deletion, and reconstitution method, they found that many compounds in CKI interact with each other and function together. This approach allowed identification of innate immune system cytokine signalling pathways as critical targets for CKI driven perturbation of the cell cycle in cancer cells [285]. As sequencing costs keep dropping, more genomic resources are becoming available for the characterisation of novel bioactive natural products using genome mining methods. The biosynthetic gene clusters (BGC) in bacterial and fungal genomes are central targets in genome mining. Recently, Robey et al. used comparative genomics to explore the BGCs from more than 1000 fungal genomes and constructed a comprehensive repertoire for natural products in fungi [387]. Moreover, third generation sequencing makes the correct assembly of large medicinal plant genomes possible, allowing more genes and pathways involved in the biosynthesis of bioactive compounds in medicinal plants to be identified and characterised [388,389].

Compared to traditional experimental approaches, bioinformatics is still a young but fast-growing research area. Novel methods have emerged at a remarkable rate thanks to increased high performance computing and sequencing techniques. Except for cheminformatics, many bioinformatics approaches are still to be adapted to study natural products. Single cell DNA sequencing-based metagenomics has been used to investigate BGCs of natural products from uncultured microorganisms in environmental samples [390]. In addition, single cell RNA sequencing (scRNA-Seq) has the potential to revolutionize our understanding of immunology as our immune response requires a complex variety of immune cell types working together spatially and temporally. For example, the composition and heterogeneity of immune cells in the liver have been profiled using scRNA-Seq. scRNA-Seq analysis was also used to reconstruct the trajectory of immune cell populations, such as Th17 (T helper 17) cells, in pathogenic tissues [391,392]. Given that many natural products target multiple pathways, scRNA-Seq analysis has exciting potential to help us understand the process of differentiation, maturation and activation of immune cells triggered by natural products *in vivo* during homeostasis as well as pathological conditions. Another RNA-Seq approach is expression perturbation analysis at network or pathway level. The traditional approach in transcriptomics-based gene expression analysis is to identify differentially regulated genes first, and then identify perturbed networks or pathways based on the overrepresentation analysis of differentially expressed genes. However, genes and their protein products in living organisms interact with each other as regulatory networks and pathways. Therefore, we can quantify the perturbation status of interacting regulatory networks or pathways by integrating the expression profiles of genes with their hierarchical organisation in the network or pathway. This approach will enable us to better understand the mode of action of natural products at network or pathway scale, based on the polypharmacological nature of natural products.

6.3. Nagoya protocol – the importance of the Convention on Biological Diversity (CBD) and benefit sharing

Since the Rio Convention (1992), the Nagoya Protocol (2010) and the progressive implementation of national legislations, access to Genetic Resources (GR), extracts, natural molecules and traditional knowledge associated to GR (TK) is being regulated in most countries of the world. Therefore, it is now necessary to pay particular attention in order to ensure the legal security essential to the work of researchers in the public or industrial sectors. Access to natural resources from biodiversity that are of interest to pharmacological researchers, is nowadays within the scope of new regulations implemented in most countries of the world. Therefore, ensuring the legal security is a key step before the conduct of laboratory research.

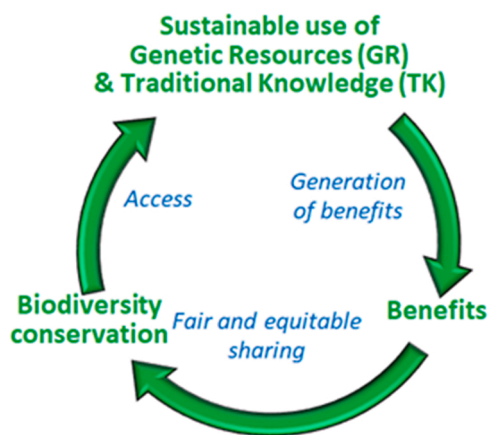


Fig. 12. The virtuous circle that connects the three objectives of the Convention on Biological Diversity and the Nagoya Protocol.

6.3.1. Findings and considerations that led to the CBD and the adoption of the Nagoya Protocol (NP)

The duty to preserve our environment has developed at least since ancient Greek times and really became an environmental conscience in the 1970s with the creation of the United Nations Environment Program and the first Earth Summit in Stockholm (1972). During the third Earth Summit (Rio de Janeiro, 1992), it appeared necessary for the sake of our biosphere to combine the objectives of preservation, sustainable development and sharing the wealth resulting from biodiversity within a virtuous circle (Fig. 12).

A major advance of the Convention on Biological Diversity (CBD)

[393] was the recognition of the sovereignty of states over their biodiversity (plants, animals, fungi and microorganisms) and associated traditional knowledge. Previously these resources were considered as common heritage of humanity. Initially, CBD only concerned genetic resources in the strict sense, i.e. the functional units of heredity. But since neither the pharmaceutical, cosmetic nor perfumery industries use genetic resources the system set up by the CBD did not work. The Nagoya Protocol (NP) [394] brought clarity in 2010 by integrating extracts, materials themselves and naturally occurring molecules. However, shortly after the CBD some nations, such as the Andean nations, Costa Rica, Brazil, India and South Africa, had already taken such regulatory measures (Fig. 13).

6.3.2. Scope of the Nagoya Protocol

Which materials and resources are in the scope of Nagoya Protocol? The Nagoya Protocol applies to the utilization and the valorisation of genetic resources e.g. whole or parts of plants and animals, fungi and microorganisms (virus, bacteria etc.). It also concerns the use of traditional knowledge associated with genetic resources, which means ancestral knowledge and practices held by local communities for years. This knowledge plays an important role in the discovery of new activities of molecules contained in genetic resources, particularly for pharmaceuticals applications. It does not apply to human genetic resources, genetic resources located outside of national jurisdictions, genetic resources for food consumption, such as those listed in the annex 1 of the International Treaty on Plant Genetic Resources for Food and Agriculture [395] and commodities, except otherwise specified in national regulations. Special provisions are provided in Article 8 of the NP [394] for emergency situations such as pandemic.

What activities are covered by the Nagoya Protocol? Utilization means any activity of research and/or development with commercial

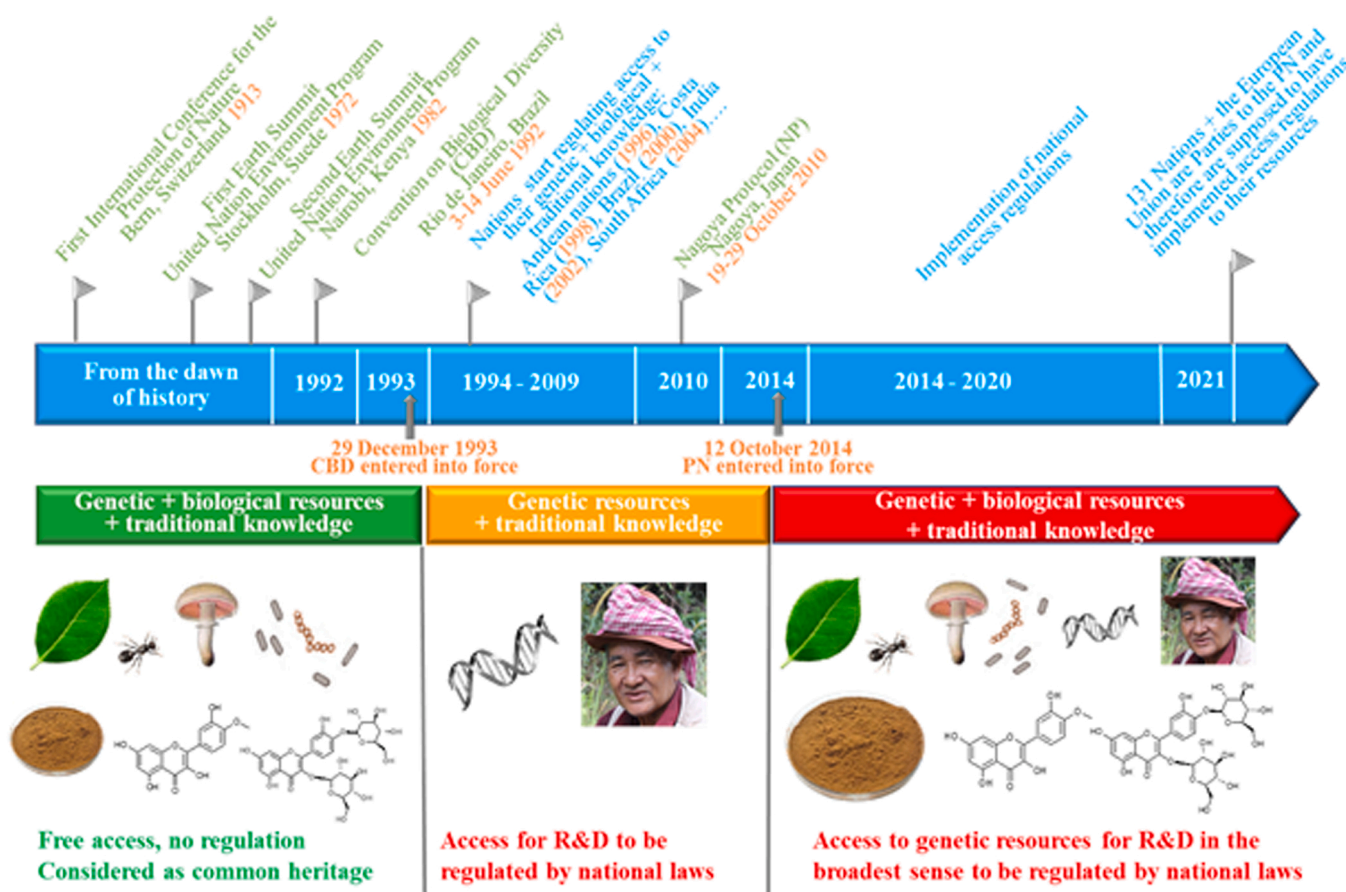


Fig. 13. Evolution of access to genetic resources following the Convention on Biodiversity (CBD) and the Nagoya Protocol (NP).

Table 4

Under the Convention on Biodiversity, each source country has a unique combination of access parameters based on its own legislation. This does not simplify the understanding of potential GR users.

Wild native resources	Cultivated/breeding resources	Introduced resources	Ex situ resources	Wild resources related species
Natural Products	Natural products derivatives	Genetic sequences	Traditional knowledge	Nature of R&D or of industrial sectors
National Actors	Non-national actors	Industry actors	Academic actors	Public lands Private lands
Commodity formulation	Extracts valorisation	Research & Development	Intellectual protection	Possible retroactivity
Who should share, benefits in value chain? Intermediates, final user....		Implications of traditional communities by source country		Evolution of regulation
				Mandatory local collaboration ...

Table 5

Risks and opportunities for users of genetic resources and associated traditional knowledge.

Opportunities	Risks
Securing the sourcing and supply of elements issued from biodiversity	Misunderstanding between parties and difficulties in determining the legal and practical meaning of provisions defined in ABS framework
Sharing practices, developing transparency and trust all along the value chain (from suppliers to customers)	Legal uncertainty all along the value chain actors due to unawareness of small actors regarding the provisions of ABS
Supporting partners and developing strong partnerships and implementing collaborative projects	Granting access permit often requires time; timelines for entering into PIC/MAT may not be compatible with R&D schedules and objectives
Developing climate of trust with National Competent Authorities of the supplier countries development and concretisation of the projects' meaning and purpose for organisations and their employees	Possibility of failure of the ABS negotiation and therefore impossibility of starting the research project
Developing awareness/consciousness within companies towards more "fair" use of natural resources, and the need for biodiversity protection	Discouragement of GR/biodiversity conservation studies
Implementing concrete actions for preserving or restoring biodiversity, creating jobs, sustainable valorisation and improvement of local livelihoods	
Supporting innovation	

intent or not, performed on natural resources. Thus, any researcher or company working with materials obtained from biodiversity, performing research about the properties of biochemical compounds to acquire

new knowledge and/or to develop new ingredients/products would be considered falling within the definition "utilization of genetic resources" as described in the NP, and subsequent national laws; for example, research on plant extracts to develop new pharmaceutical or cosmetic ingredients, analysis and research of plant samples to develop new fragrances or new colorants. The valorisation of any of the above-mentioned activities is concerned by the scope of the NP. The definition of valorisation relates not only to the commercial exploitation of products issued from R&D activities, but also to scientific publications, technological transfer, patents etc. While access to genetic resources not intended to be used for research and development is not in the scope of NP, measures applicable to those accesses may have been adopted by national competent authorities. Indeed, as specified in article 15 of the CBD [393], natural resources are placed under the sovereignty of states. Each state is thus responsible for determining the measures applicable for access occurring in its territory; for example, to regulate only the access to wild genetic resource, to include or not cultivated plants, to include introduced resources etc. Non-parties to the NP can also implement Access and Benefit Sharing (ABS) laws, independently of the Protocol ratification, resulting in specific-country requirements and heterogeneity in the rules to be applied around the world and amounts/nature of benefit to be shared (Table 4). All these different factors have a direct impact on potential users regarding the freedom/possibility of access, duration of negotiation, administrative costs, amount of benefit to be shared and finally on the economic feasibility of their project. Thus, it is important for users to refer to the national regulation in place in the country concerned with the sourcing of the materials, and not only to the principles of the NP or the state of ratification (Fig. 14) to know their obligations.

6.3.3. Obligations of states/providers/users

It is important to note that all too often GR users are convinced that

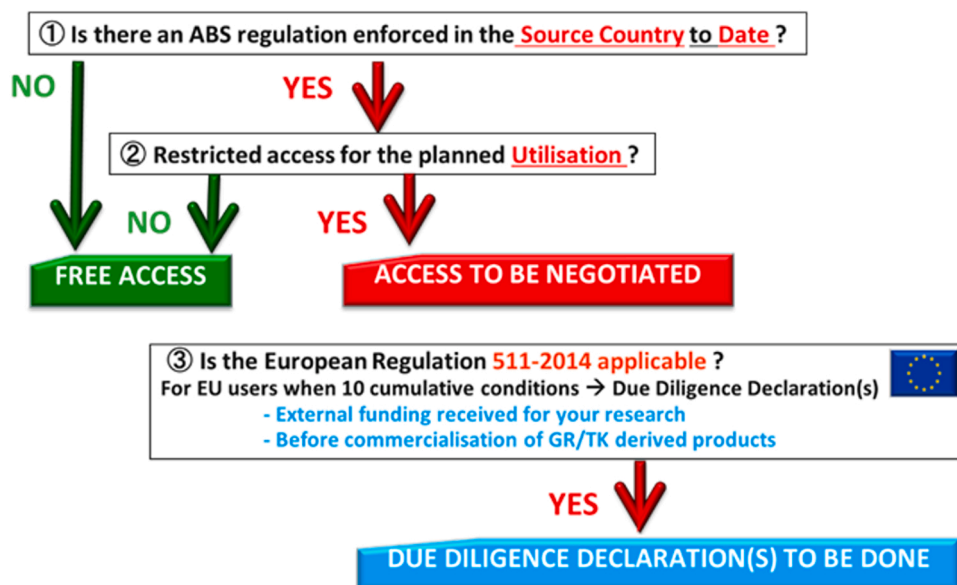


Fig. 14. Key questions to be answered before accessing Genetic Resources (GR) or Traditional Knowledge (TK).

Table 6
Structural and functional differences between providers and users of genetic resources.

Providers	Users
“Poor” countries of the South Biodiversity & TK (traditional knowledge) rich	“Rich” countries in the North Technology & Industry rich
Technology & Industry poor Governments, local communities, NGOs Control over their GR & TK	Biodiversity poor Academic or Industry researchers Fair & Transparent access
Certainty of benefits sharing after access negotiation	Realistic timelines, clear and fair negotiation
Great expectation of funding Interested in economic development, preservation	Limited funding opportunities Interested in innovation
(Biodiversity) legislation often poorly developed	Need of legal security, clarity and transparency

the Nagoya Protocol or the Convention on Biological Diversity applies to them. In fact, GR users are only concerned by the national biodiversity access laws of the source country (Supplementary Fig. 3). In contrast, nations that are signatories to the CBD [393] or the NP [394] must respect their international commitments by transcribing the rules of access to their biodiversity into national law and implementing measures to check users' compliance. Information regarding regulations in place are available on the website of the ABS Clearing House [396]. In European Union member states, users have due diligence and compliance obligations imposed by European Regulation 511/2014 [397–399]. When some conditions are cumulated, they need to declare via an electronic portal external funding received for their research and to testify before commercialization of a GR/TK derived product.

6.3.4. Risks and opportunities related to ABS regulations

ABS regulations are fully enforced within about 50 countries and are currently under development in numerous countries parties to the Nagoya Protocol (e.g. China, Morocco). To date (December 2021), 131 nations are parties to the NP out of a total of 198 countries. Some parties (e.g. India) are currently revisiting their ABS rules, thus users are working in a constantly evolving legal environment. If ABS regulations are often considered as a constraint by users, they also represent opportunities (Table 5). Among them, the development of awareness and consciousness within universities/ companies and researchers towards a more sustainable and fair use of GR. ABS can support the sense to belong to a community acting for protection of biodiversity and allowing all stakeholders to identify themselves to the purpose of their organization. It's important to notice that stakeholders involved around ABS within private companies are belonging to various departments (R&D, intellectual property, legacy, purchases, sourcing, marketing, regulatory...): dealing with ABS requires various experts and team cooperation in order to comply with ABS laws. ABS regulations are also an opportunity to create value and develop strong partnerships between organizations, local communities, and competent authorities.

6.3.5. Assessment of the NP 10 years after its adoption vs. its objectives

Due to different states of maturity of the ABS framework in the world, and to the absence of international harmonization, users face many questions and many challenges when they intend to access genetic resources or natural extracts. Ten years after the adoption of the Nagoya Protocol, grey zones for users remain. Among them, we can find definition of traded commodities exempted from ABS rules, what activities are involved under the definition of Research and Development, and utilization of a genetic resource accessed prior to the implementation of the National ABS law. Moreover, some of the expectations of the Nagoya Protocol have not yet been met, since the conditions to access to genetic resources and natural extracts are country dependent. The combination of opposite identities and expectations between providers countries and users may therefore lead to some difficulties in the implementation of

national regulations (Table 6) and to legal uncertainty. Moreover, strong divergences between expectations and reality resulting from the first decade of Nagoya protocol, have generated frustration for all stakeholders involved in the process [400]. Although the ABS process can contribute to reaching some of the objectives defined in the CBD and the NP, it is insufficient alone in view of the importance of the environmental issues [401].

The urge of biodiversity conservation/restoration is recognized worldwide, but there is a need for a clear, comprehensive legal framework and international harmonization. Locally, several or different laws may regulate wild plant collection and/or, agriculture; fragmented provisions providing different levels of protection for biological resources, with different administrative authorities, may be complex for users to handle [402] when instead they need both a significant level of legal certainty and a comprehensive and coherent framework. Flexibility in the applicable laws is also expected, for example, simplified access measures for academic research or at early stages of a company project. The imposition of long, difficult and costly access procedures may discourage users to operate in some countries, as they have no guarantee in the success of their project or a return on investment. Clear dispositions are also expected from users regarding benefit sharing itself, including precise and understandable calculation methods, a reasonable level of monetary benefits to be shared, or a preference for non-monetary benefit sharing which directly benefits both biodiversity and local people.

Processes to obtain access permits and to enter in ABS agreement are time consuming for stakeholders and require substantial human and financial resources from both governments and private/public sectors [403]. Simplified procedures are expected from all stakeholders, to make the ABS process easier to understand and to apply, and to be more efficient. Enhancing awareness among stakeholders, concretizing coordination among different agencies are continuing challenges in the implementation of the ABS [404].

6.3.6. Current and future perspectives

The complexity of national ABS measures, costs and long delays associated with finalising ABS agreements are well known within the scientific community and there are emerging legal issues pertaining to these aspects of ABS around the world, to ensure that clear national access measures and legal frameworks are decisive for the future. Over the next decade, the international community will adopt the global biodiversity framework during the upcoming 15th Convention of Parties (COP15) which should be held in China in 2022, where many questions about enlargement of the scope of ABS, such as “Digital Sequence Information on Genetic Resources”, [405] and GR beyond national jurisdiction [406], are to be discussed. If Access and Benefit Sharing in the scope of Research and Development, as agreed by the international community can play an important role in the protection of biodiversity, not all the biodiversity issues can be solved through ABS. Other actors and industries that use elements of biodiversity should also contribute to the international effort of biodiversity preservation and restoration. The size of challenge to counter the loss of biodiversity in the next decades is important, and will probably need major reforms in public policies, regulations and economic models.

7. Concluding comments

From a general point of view, insufficient access to quality, safe and affordable drugs in LMICs has represented a significant challenge to public health for decades. Importantly, for example in Africa, the recent creation of the African Medicines Agency will help the establishment of a global, harmonized regulatory framework for medicines [407]. In this context, the potential market for novel and established NP immunotherapies is huge worldwide and will allow greater patient access to treatment through cost savings.

A larger and stronger market for NP therapies in LMICs would also

Table 7

Natural Products and Immunology Biomarkers. Numbers represent the number of publications in Pubmed associated with the indicated keywords shown in the row and column headings.

		Natural Products	Curcumin	Resveratrol	Polyphenol	Flavonoid	Adenosine
PD-1	19,250	615	12	7	13	30	130
CTLA-4	10,851	589	3	3	5	6	63
FoxP3	18,789	782	33	19	32	66	168
CD40L (CD154)	9434	526	6	2	10	38	143
CD25	19,319	989	24	12	14	61	171
CD28H	13	0	0	0	0	0	0
CD28	12,173	471	17	11	14	50	73
ICOS	2193	58	2	0	0	1	4

help closing a virtuous economic circle. First of all, several natural product sources typically come from emerging economies. Secondly, moving toward a more sustainable development, industrial wastes from plants offer a wide spectrum of possibilities for their valorisation, still being enriched in high added-value molecules such as secondary metabolites, that can be used in various fields of pharmacotherapy [408]. Local production and access to medicines in LMICs will result in lower prices, greater availability, local capacity building in manufacturing, supply chain management, and ultimately health system strengthening [409].

Thus, LMICs need to have drugs available, of good quality (a major problem in some LGICs) and also to have the capacity to develop their own drugs, with appropriate control of quality and safety, which necessitates having good immunological research. As an example, Iran has been able to produce its own vaccine against SARS-CoV-2, thanks to an ongoing collaboration with Institute Pasteur-Iran. However, it is important that some capacity for molecular immunological research is present in LMICs. It is a waste of scarce resources just to show reduction of paw oedema, following Freund's adjuvant, by herbal mixtures, or even purified natural products, without a molecular mechanism of action, even though there are hundreds of such papers. Education for immunopharmacology, with freely available websites, has therefore been initiated by a collaboration between the International Union of Immunological Sciences (IUIS) immunopaedia [410] and the International Union of Basic and Clinical Pharmacology (IUPHAR) IUPHAR/BPS Guide to Pharmacology [411] leading to the IUPHAR Guide to Immunopharmacology [412] and the IUPHAR Pharmacology Education Project [413] which links to immunopaedia. Best practice is discussed in Section 5, as is quality of natural products, return of benefits to the country of origin (Nagoya) and deconvolution of activity from herbal mixtures using metabolomics. Safety is a critical issue, which requires quality control of the substances, and also specific exclusion of substances known to cause toxicities, such as drug-induced liver injury [414]. Metabolomics can play a critical role in the safety of mixtures by ensuring exclusion of such substances. It is therefore essential that LMICs have access to centralised facilities such as metabolomics, which could be set-up on a national or continental scale. Centralised immunological facilities in LMICs can also be critical resources for the development of natural products, particularly as research on the interactions of natural products with check point inhibitors (Table 7) is growing rapidly.

As a final word, the principal aim of this review was to provide a position statement on the natural product drug discovery process in the search of novel immunomodulators, to serve as a guide to researchers who are active (or planning to be) in this field. While the review contains a number of examples of studies demonstrating clinical effectiveness of natural product-derived immunomodulators, it was beyond the scope to include detailed descriptions of the large amount of clinical data that has been collected. Therefore, a position statement on the clinical status of natural product immunomodulators will follow.

Declaration of Competing Interest

I confirm that that there is no financial or personal interest or belief that could affect the objectivity of the authors who have contributed to this article.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.phrs.2022.106076.

References

- [1] R.R. Alves, L.L. Rosa, Why study the use of animal products in traditional medicines? *J. Ethnobiol. Ethnomed.* 1 (5) (2005) 4269–1–5.
- [2] World Health Organization, WHO Global Report on Traditional and Complementary Medicine 2019, World Health Organisation, Geneva, 2019.
- [3] E.J. Buenz, R. Verpoorte, B.A. Bauer, The ethnopharmacologic contribution to bioprospecting natural products, *Annu. Rev. Pharmacol. Toxicol.* 58 (2018) 509–530.
- [4] C. Gyllenhaal, M.R. Kadushin, B. Southavong, K. Sydara, S. Bouamanivong, M. Xaiveu, L.T. Xuan, N.T. Hiep, N.V. Hung, P.K. Loc, L.X. Dac, T.Q. Bich, N. M. Cuong, H.M. Ly, H.J. Zhang, S.G. Franzblau, H. Xie, M.C. Riley, B. G. Elkington, H.T. Nguyen, D.P. Waller, C.Y. Ma, P. Tamez, G.T. Tan, J. M. Pezzuto, D.D. Soejarto, Ethnobotanical approach versus random approach in the search for new bioactive compounds: support of a hypothesis, *Pharm. Biol.* 50 (2012) 30–41.
- [5] F. Ntie-Kang, L.L. Lifongo, L.M. Mbaze, N. Ekwelle, L.C. Owono Owono, E. Megnassan, P.N. Judson, W. Sippl, S.M. Efange, Cameroonian medicinal plants: a bioactivity versus ethnobotanical survey and chemotaxonomic classification, *BMC Complement. Alter. Med.* 13 (147) (2013) 6882–13–147.
- [6] F. de Almeida Cde, E.L. de Amorim, U.P. de Albuquerque, Insights into the search for new drugs from traditional knowledge: an ethnobotanical and chemical-ecological perspective, *Pharm. Biol.* 49 (2011) 864–873.
- [7] L. Svetaz, F. Zuljan, M. Derita, E. Petenatti, G. Tamayo, A. Cáceres, V. Cechinel Filho, A. Giménez, R. Pinzón, S.A. Zaccino, M. Gupta, Value of the ethnomedicinal information for the discovery of plants with antifungal properties. A survey among seven Latin American countries, *J. Ethnopharmacol.* 127 (2010) 137–158.
- [8] A.L. Harvey, R. Edrada-Ebel, R.J. Quinn, The re-emergence of natural products for drug discovery in the genomics era, *Nat. Rev. Drug Discov.* 14 (2015) 111–129.
- [9] C.H. Saslis-Lagoudakis, V. Savolainen, E.M. Williamson, F. Forest, S.J. Wagstaff, S.R. Baral, M.F. Watson, C.A. Pendry, J.A. Hawkins, Phylogenies reveal predictive power of traditional medicine in bioprospecting, *Proc. Natl. Acad. Sci. U.S.A.* 109 (2012) 15835–15840.
- [10] C.H. Saslis-Lagoudakis, B.B. Klitgaard, F. Forest, L. Francis, V. Savolainen, E. M. Williamson, J.A. Hawkins, The use of phylogeny to interpret cross-cultural patterns in plant use and guide medicinal plant discovery: an example from Pterocarpus (Leguminosae), *PLoS One* 6 (2011), e22275.
- [11] B. Patwardhan, Ethnopharmacology and drug discovery, *J. Ethnopharmacol.* 100 (2005) 50–52.
- [12] E.J. Buenz, B.A. Bauer, H.E. Johnson, G. Tavana, E.M. Beekman, K.L. Frank, C. L. Howe, Searching historical herbal texts for potential new drugs, *BMJ* 333 (2006) 1314–1315.
- [13] E.J. Buenz, D.J. Schneppe, B.A. Bauer, P.L. Elkin, J.M. Riddle, T.J. Motley, Techniques: bioprospecting historical herbal texts by hunting for new leads in old tomes, *Trends Pharmacol. Sci.* 25 (2004) 494–498.
- [14] V. Martinez-Frances, D. Rivera, M. Heinrich, C. Obon, S. Rios, An ethnopharmacological and historical analysis of "Dictamnus", a European traditional herbal medicine, *J. Ethnopharmacol.* 175 (2015) 390–406.
- [15] G. Scott, M.L. Hewett, Pioneers in ethnopharmacology: the Dutch East India Company (VOC) at the Cape from 1650 to 1800, *J. Ethnopharmacol.* 115 (2008) 339–360.
- [16] E.J. Buenz, H.E. Johnson, E.M. Beekman, T.J. Motley, B.A. Bauer, Bioprospecting Rumphius's ambonese herbal: volume I, *J. Ethnopharmacol.* 96 (2005) 57–70.

- [17] E.J. Buenz, B.A. Bauer, D.J. Schnepfle, D.L. Wahner-Roedler, A.G. Vandell, C. L. Howe, A randomized phase I study of *Atuna racemosa*: a potential new anti-MRSA natural product extract, *J. Ethnopharmacol.* 114 (2007) 371–376.
- [18] P. Barrett, A. Flower, V. Lo, What's past is prologue: Chinese medicine and the treatment of recurrent urinary tract infections, *J. Ethnopharmacol.* 167 (2015) 86–96.
- [19] S.S. Ningthoujam, A.D. Talukdar, K.S. Potsangbam, M.D. Choudhury, Challenges in developing medicinal plant databases for sharing ethnopharmacological knowledge, *J. Ethnopharmacol.* 141 (2012) 9–32.
- [20] J. Bajorath, Compound data mining for drug discovery, *Methods Mol. Biol.* 1526 (2017) 247–256.
- [21] C.S. Weckerle, S. Cabras, M.E. Castellanos, M. Leonti, Quantitative methods in ethnobotany and ethnopharmacology: considering the overall flora–hypothesis testing for over- and underused plant families with the Bayesian approach, *J. Ethnopharmacol.* 137 (2011) 837–843.
- [22] K. Kindscher, S. Corbett, K. McClure, A statistical analysis of medicinal plants: a case study of plant families in Kansas and the Great Plains, *Trans. Kans. Acad. Sci.* 116 (2013) 149–155.
- [23] V. Sharma, I.N. Sarkar, Bioinformatics opportunities for identification and study of medicinal plants, *Brief. Bioinform.* 14 (2013) 238–250.
- [24] J. Gu, Y. Gui, L. Chen, G. Yuan, H.Z. Lu, X. Xu, Use of natural products as chemical library for drug discovery and network pharmacology, *PLoS One* 8 (2013), e62839.
- [25] R.L. Clark, B.F. Johnston, S.P. Mackay, C.J. Breslin, M.N. Robertson, A.L. Harvey, The drug discovery portal: a resource to enhance drug discovery from academia, *Drug Discov. Today* 15 (2010) 679–683.
- [26] W.D. Loub, N.R. Farnsworth, D.D. Soejarto, M.L. Quinn, NAPRALERT: computer handling of natural product research data, *J. Chem. Inf. Comput. Sci.* 25 (1985) 99–103.
- [27] R.K. Petersen, K.B. Christensen, A.N. Assimopoulou, X. Fretté, V.P. Papageorgiou, K. Kristiansen, I. Kouskoumvekaki, Pharmacophore-driven identification of PPAR γ agonists from natural sources, *J. Comput. Aided Mol. Des.* 25 (2011) 107–116.
- [28] D. Schuster, L. Kern, D.P. Hristozov, L. Terfloth, B. Bienfait, C. Laggner, J. Kirchmair, U. Grienke, G. Wolber, T. Langer, H. Stuppner, J. Gasteiger, J. M. Rollinger, Applications of integrated data mining methods to exploring natural product space for acetylcholinesterase inhibitors, *Comb. Chem. High. Throughput Screen* 13 (2010) 54–66.
- [29] F. Ntie-Kang, D. Zofou, S.B. Babiake, R. Meudom, M. Scharfe, L.L. Lifongo, J. A. Mbah, L.M. Mbaze, W. Sippl, S.M. Efang, AfroDb: a select highly potent and diverse natural product library from African medicinal plants, *PLoS One* 8 (2013), e78085.
- [30] M. Valli, R.N. dos Santos, L.D. Figueira, C.H. Nakajima, I. Castro-Gamboa, A. D. Andricopulo, V.S. Bolzani, Development of a natural products database from the biodiversity of Brazil, *J. Nat. Prod.* 76 (2013) 439–444.
- [31] P.O. Staub, M.S. Geck, C.S. Weckerle, L. Casu, M. Leonti, Classifying diseases and remedies in ethnomedicine and ethnopharmacology, *J. Ethnopharmacol.* 174 (2015) 514–519.
- [32] P.A. Cox, Will tribal knowledge survive the millennium? *Science* 287 (2000) 44–45.
- [33] K. Srithi, H. Balslev, P. Wangpakapattanawong, P. Srisanga, C. Trisonthi, Medicinal plant knowledge and its erosion among the Mien (Yao) in northern Thailand, *J. Ethnopharmacol.* 123 (2009) 335–342.
- [34] E.J. Buenz, Country development does not presuppose the loss of forest resources for traditional medicine use, *J. Ethnopharmacol.* 100 (2005) 118–123.
- [35] I. Hedberg, Botanical methods in ethnopharmacology and the need for conservation of medicinal plants, *J. Ethnopharmacol.* 38 (1993) 121–128.
- [36] M. Balick, P. Cox, *Plants, People, and Culture: The Science of Ethnobotany*, first ed., Scientific American Library, 1996.
- [37] E. Elisabetsky, N. Etkin, *Ethnopharmacology: an overview*, *Ethnopharmacology* (2009).
- [38] M. Heinrich, *Ethnopharmacology and drug discovery*, in: L. Mander, H. Liu (Eds.), *Comprehensive Natural Products II: Chemistry and Biology, Development & Modification of Bioactivity*, Elsevier, 2013, pp. 351–381.
- [39] L. Cui, X.Z. Su, Discovery, mechanisms of action and combination therapy of artemisinin, *Expert Rev. Anti Infect. Ther.* 7 (2009) 999–1013.
- [40] M. Heinrich, *Ethnopharmacology in the 21st century - grand challenges*, *Front Pharmacol.* 1 (2010) 8.
- [41] G.R. Coatney, O. Mickelsen, R.W. Burgess, M.D. Young, C.I. Pirkle, Chloroquine or pyrimethamine in salt as a suppressive against sporozoite-induced vivax malaria (Chesson strain), *Bull. World Health Organ.* 19 (1958) 53–67.
- [42] S. Vandekerckhove, M. D'hooghe, Quinoline-based antimalarial hybrid compounds, *Bioorg. Med. Chem.* 23 (2015) 5098–6119.
- [43] C.J. Paddon, P.J. Westfall, D.J. Pitera, K. Benjamin, K. Fisher, D. McPhee, M. D. Leavell, A. Tai, A. Main, D. Eng, D.R. Polichuk, K.H. Teoh, D.W. Reed, T. Treynor, J. Lenihan, M. Fleck, S. Bajad, G. Dang, D. Dengrove, D. Diola, G. Dorin, K.W. Ellens, S. Fickes, J. Galazzo, S.P. Gaucher, T. Geistlinger, R. Henry, M. Hepp, T. Horning, T. Iqbal, H. Jiang, L. Kizer, B. Lieu, D. Melis, N. Moss, R. Regentin, S. Secrest, H. Tsuruta, R. Vazquez, L.F. Westblade, L. Xu, M. Yu, Y. Zhang, L. Zhao, J. Lievense, P.S. Covello, J.D. Keasling, K.K. Reiling, N. S. Renninger, J.D. Newman, High-level semi-synthetic production of the potent antimalarial artemisinin, *Nature* 496 (2013) 528–532.
- [44] D.A. Fidock, Drug discovery: priming the antimalarial pipeline, *Nature* 465 (2010) 297–298.
- [45] T.N. Wells, R. Hoof van Huijsdijnen, Van, W.C. Voorhis, Malaria medicines: a glass half full? *Nat. Rev. Drug Discov.* 14 (2015) 424–442.
- [46] J. Wang, C.J. Zhang, W.N. Chia, C.C. Loh, Z. Li, Y.M. Lee, Y. He, L.X. Yuan, T. K. Lim, M. Liu, C.X. Liew, Y.Q. Lee, J. Zhang, N. Lu, C.T. Lim, Z.C. Hua, B. Liu, H. M. Shen, K.S. Tan, Q. Lin, Haem-activated promiscuous targeting of artemisinin in *Plasmodium falciparum*, *Nat. Commun.* 6 (2015) 10111.
- [47] E.A. Ashley, M. Dhorda, R.M. Fairhurst, C. Amaratunga, P. Lim, S. Suon, S. Sreng, J.M. Anderson, S. Mao, B. Sam, C. Sopha, C.M. Chhur, C. Nguon, S. Sovannaroth, S. Pukrittayakamee, P. Jittamala, K. Chotivanich, K. Chutasmit, C. Suchatsoonthorn, R. Runcharoen, T.T. Hien, N.T. Thuy-Nhien, N.V. Thanh, N. H. Phu, Y. Htut, K.T. Han, K.H. Aye, O.A. Mokuolu, R.R. Olaosebikan, O. O. Folaranmi, M. Mayxay, M. Khanthavong, B. Hongvanthong, P.N. Newton, M. A. Onyamboko, C.I. Fanello, A.K. Tshetu, N. Mishra, N. Valecha, A.P. Phyto, F. Nosten, P. Yi, R. Tripura, S. Borrmann, M. Bashraheil, J. Peshu, M.A. Faiz, A. Ghose, M.A. Hossain, R. Samad, M.R. Rahman, M.M. Hasan, A. Islam, O. Miotto, R. Amato, B. MacLinnis, J. Stalker, D.P. Kwiatkowski, Z. Bozdech, A. Jeeyapant, P.Y. Cheah, T. Sakulthaew, J. Chalk, B. Intharabut, K. Silamut, S. J. Lee, B. Vihokhern, C. Kunasol, M. Imwong, J. Tarning, W.J. Taylor, S. Yeung, C. J. Woodrow, J.A. Flegg, D. Das, J. Smith, M. Venkatesan, C.V. Plowe, K. Stepniewska, P.J. Guerin, A.M. Dondorp, N.P. Day, N.J. White, Tracking Resistance to Artemisinin Collaboration, Spread of artemisinin resistance in *Plasmodium falciparum* malaria, *New Engl. J. Med.* 371 (2014) 411–423.
- [48] R. Chakravarty, P. Mahajan, Preserving traditional knowledge: initiatives in India, *IFLA J.* 36 (2010) 294–299.
- [49] K. Jayaraman, India protects traditional medicines from piracy, *Nature* 457 (2009) 933.
- [50] V.K.J. Jeevan, Digitallibrary development: identifying sources of content for developing countries with special reference to India, *Int. Inf. Libr. Rev.* 36 (2004) 185–197.
- [51] A. Mukherjee, M. Banerjee, V. Mandal, A.C. Shukla, S.C. Mandal, Modernization of Ayurveda: a brief overview of Indian initiatives, *Nat. Prod. Commun.* 9 (2014) 287–290.
- [52] B. Patwardhan, R.A. Mashelkar, Traditional medicine-inspired approaches to drug discovery: can Ayurveda show the way forward? *Drug Discov. Today* 14 (2009) 804–811.
- [53] N. Tandon, S.S. Yadav, Contributions of Indian Council of Medical Research (ICMR) in the area of Medicinal plants/Traditional medicine, *J. Ethnopharmacol.* 197 (2017) 39–45.
- [54] M.P. Chander, C. Kartick, J. Gangadhar, P. Vijayachari, Ethno medicine and healthcare practices among Nicobarese of Car Nicobar - an indigenous tribe of Andaman and Nicobar Islands, *J. Ethnopharmacol.* 158 (Pt A) (2014) 18–24.
- [55] A. Parveen, B. Parveen, R. Parveen, S. Ahmad, Challenges and guidelines for clinical trial of herbal drugs, *J. Pharm. Bioallied Sci.* 7 (2015) 329–333.
- [56] L. Qu, W. Zou, Z. Zhou, T. Zhang, J. Greef, M. Wang, Non-European traditional herbal medicines in Europe: a community herbal monograph perspective, *J. Ethnopharmacol.* 156 (2014) 107–114.
- [57] Y. Jaiswal, Z. Liang, Z. Zhao, Botanical drugs in Ayurveda and Traditional Chinese medicine, *J. Ethnopharmacol.* 194 (2016) 245–259.
- [58] P.D. Gupta, P.G. Daswani, T.J. Birdi, Approaches in fostering quality parameters for medicinal botanicals in the Indian context, *Indian J. Pharmacol.* 46 (2014) 363–371.
- [59] N.J. Gogtay, H.A. Bhatt, S.S. Dalvi, N.A. Kshirsagar, The use and safety of non-allopathic Indian medicines, *Drug Saf.* 25 (2002) 1005–1019.
- [60] I. Mgbeoji, Global Biopiracy: Patents, Plants and Indigenous Knowledge, In: UBC Press, Vancouver, BC, 2006.
- [61] R.L. Noble, The discovery of the vinca alkaloids—chemotherapeutic agents against cancer, *Biochem. Cell Biol.* 68 (1990) 1344–1351.
- [62] R.L. Noble, C.T. Beer, J.H. Cutts, Role of chance observations in chemotherapy: Vinca rosea, *Ann. N.Y. Acad. Sci.* 76 (1958) 882–894.
- [63] K. Saraviita, Indigenous heritage and intellectual property. Genetic resources traditional knowledge and folklore, *Int. J. Minor. Group Rights* 17 (2010) 383–386.
- [64] T. Efferth, M. Banerjee, N.W. Paul, S. Abdelfatah, J. Arend, G. Elhassan, S. Hamdoun, R. Hamm, C. Hong, O. Kadioglu, J. Naß, D. Ochwangi, E. Ooko, N. Onzver, M.E. Saeed, M. Schneider, E.J. Seo, C.F. Wu, G. Yan, M. Zeino, Q. Zhao, M.S. Abu-Darwish, K. Andersch, G. Alexie, D. Bessarab, D. Bhakta-Guha, V. Bolzani, E. Dapat, F.V. Donenko, M. Efferth, H.J. Greten, L. Gunatilaka, A. A. Hussein, A. Karadeniz, H.E. Khalid, V. Kuetze, I.S. Lee, L. Liu, J. Midiwo, R. Mora, H. Nakagawa, O. Ngassapa, C. Noysang, L.K. Omosa, F.H. Roland, A. A. Shahat, A. Saab, E.M. Saeed, L. Shan, S.J. Titinchi, Biopiracy of natural products and good bioprospecting practice, *Phytomedicine* 23 (2016) 166–173.
- [65] A.C. Aisenberg, An introduction to immunosuppressants, *Adv. Pharmacol. Chemother.* 8 (1970) 31–55.
- [66] A. Kossel, Über eine neue Base aus dem Pflanzenreich, *Ber. Dtsch Chem. Ges.* 21 (1888) 2164–2167.
- [67] A. Kossel, Über das Theophyllin, einen neuen Bestandtheil des Thees, *Hoppe Seyler's Z. Physiol. Chem.* 13 (1889) 298–308.
- [68] E. Fischer, L. Ach, Synthese des Caffeins, *Ber. Dtsch Chem. Ges.* 28 (1895) 3139–3143.
- [69] W. Traube, Der synthetisch Aufbau der Harnsäure, des Xanthins, Theobromins, Theophyllins und Caffeins aus der Cyanessigsäure, *Chem. Ber.* 33 (1900) 3035–3056.
- [70] S.J. Prigal, A.M. Brooks, R. Harris, The treatment of asthma by inhalation of aerosol of aminophyllin, *J. Allergy* 18 (1947) 16–28.
- [71] G. Herrmann, M.B. Aynesworth, Successful treatment of persistent extreme dyspnea "status asthmaticus". Use of theophylline ethylene diamine (aminophylline, U. S. P.) intravenously. 1938, *J. Lab Clin. Med.* 115 (1990) 512–525.

- [72] R. Young, R. Gilbert, The use of theophylline with ethylene diamine (aminophylline) for the control of bronchial spasm: a pharmacologic study, *J. Allergy* 12 (1941) 235–241.
- [73] J. Takamine, Adrenalin the active principle of the suprarenal glands and its mode of preparation, *Am. J. Pharm.* 73 (1901) 523–531.
- [74] D. Newman, Natural product-derived drugs based on β -adrenergic agents and nucleosides, *J. Braz. Chem. Soc.* 27 (2016) 1320–1333.
- [75] R.N. Brogden, T.M. Speight, G.S. Avery, Sodium cromoglycate (cromolyn sodium): a review of its mode of action, pharmacology, therapeutic efficacy and use, *Drugs* 7 (1974) 164–282.
- [76] H.R. Morris, G.W. Taylor, P.J. Piper, J.R. Tippins, Structure of slow-reacting substance of anaphylaxis from guinea-pig lung, *Nature* 285 (1980) 104–106.
- [77] S.M. Lai, P.W. Manley, Prostaglandins, thromboxanes, leukotrienes, and related arachidonic acid metabolites, *Nat. Prod. Rep.* 1 (1984) 409–441.
- [78] A. Di Gennaro, J.Z. Haeggström, The leukotrienes: immune-modulating lipid mediators of disease, *Adv. Immunol.* 116 (2012) 51–92.
- [79] J. Mannick, R. Egdahl, in: F. Rapaport, J. Dausset (Eds.), *Human Transplantation*, Grune & Stratton, New York, 1968, pp. 501–509.
- [80] A. Gilman, L. Goodman, T. Rall, F. Murad, *The Pharmacological Basis of Therapeutics*, seventh ed., Macmillan, USA, 1985.
- [81] W. Bowman, M. Rand (Eds.), *Textbook of Pharmacology*, second ed., Wiley Blackwell, UK, 1980.
- [82] J.F. Borel, C. Feurer, H.U. Gubler, H. Stähelin, Biological effects of cyclosporin A: a new antilymphocytic agent, *Agents Actions* 6 (1976) 468–475.
- [83] A. Rüegger, M. Kuhn, H. Lichti, H.R. Loosli, R. Huguenin, C. Quiquerez, A. von Wartburg, Cyclosporin A, a peptide metabolite from *Trichoderma polysporum* (Link ex Pers.) Rifai, with a remarkable immunosuppressive activity, *Helv. Chim. Acta* 59 (1976) 1075–1092.
- [84] R.Y. Calne, D.J. White, S. Thiru, D.B. Evans, P. McMaster, D.C. Dunn, G. N. Craddock, B.D. Pentlow, K. Rolles, Cyclosporin A in patients receiving renal allografts from cadaver donors, *Lancet* 2 (1978) 1323–1327.
- [85] C. Vézina, A. Kudelski, S.N. Sehgal, Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle, *J. Antibiot.* 28 (1975) 721–726.
- [86] T. Kino, H. Hatanaka, M. Hashimoto, M. Nishiyama, T. Goto, M. Okuhara, M. KOHSAKA, H. AOKI, H. IMANAKA, FK-506, a novel immunosuppressant isolated from a Streptomyces. I. Fermentation, isolation, and physico-chemical and biological characteristics, *J. Antibiot.* 40 (1987) 1249–1255.
- [87] D. Furman, J. Campisi, E. Verdin, P. Carrera-Bastos, S. Targ, C. Franceschi, L. Ferrucci, D.W. Gilroy, A. Fasano, G.W. Miller, A.H. Miller, A. Mantovani, C. M. Weyand, N. Barzilai, J.J. Goronzy, T.A. Rando, R.B. Effros, A. Lucia, N. Kleinstreuer, G.M. Slavich, Chronic inflammation in the etiology of disease across the life span, *Nat. Med.* 25 (2019) 1822–1832.
- [88] H.N. Gouda, F. Charlson, K. Sorsdahl, S. Ahmadzada, A.J. Ferrari, H. Erskine, J. Leung, D. Santamauro, C. Lund, L.N. Aminde, B.M. Mayosi, A.P. Kengne, M. Harris, T. Achoki, C.S. Wiysonge, D.J. Stein, H. Whiteford, Burden of non-communicable diseases in sub-Saharan Africa, 1990–2017: results from the Global Burden of Disease Study 2017, *Lancet Glob. Health* 7 (2019) e1375–e1387.
- [89] Recovery Collaborative Group, P. Horby, W.S. Lim, J.R. Emberson, M. Mafham, J. L. Bell, et al., Dexamethasone in Hospitalized Patients with Covid-19, *New Engl. J. Med.* 384 (2021) 693–704.
- [90] G. Schett, I.B. McInnes, M.F. Neurath, Reframing immune-mediated inflammatory diseases through signature cytokine hubs, *New Engl. J. Med.* 385 (2021) 628–639.
- [91] G. Kroemer, L. Zitvogel, Immune checkpoint inhibitors, *J. Exp. Med.* 218 (2021), e20201979, <https://doi.org/10.1084/jem.20201979>.
- [92] G.C. Kite, M.J. Howes, M.S. Simmonds, Metabolomic analysis of saponins in crude extracts of *Quillaja saponaria* by liquid chromatography/mass spectrometry for product authentication, *Rapid Commun. Mass Spectrom.* 18 (2004) 2859–2870.
- [93] C. Pifferi, R. Fuentes, A. Fernández-Tejada, Natural and synthetic carbohydrate-based vaccine adjuvants and their mechanisms of action, *Nat. Rev. Chem.* (2021) 1–20.
- [94] E. Bigaeva, E. Doorn, H. Liu, E. Hak, Meta-analysis on randomized controlled trials of vaccines with QS-21 or ISCOMATRIX adjuvant: safety and tolerability, *PLoS One* 11 (2016), e0154757.
- [95] P. Wang, D. Skalamera, X. Sui, P. Zhang, S.M. Michalek, Synthesis and evaluation of QS-7-based vaccine adjuvants, *ACS Infect. Dis.* 5 (2019) 974–981.
- [96] A. Fernández-Tejada, E.K. Chea, C. George, J.R. Gardner, P.O. Livingston, G. Ragupathi, D.S. Tan, D.Y. Gin, Design, synthesis, and immunologic evaluation of vaccine adjuvant conjugates based on QS-21 and tucaresol, *Bioorg. Med. Chem.* 22 (2014) 5917–5923.
- [97] W.E. Walkowicz, A. Fernández-Tejada, C. George, F. Corzana, J. Jiménez-Barbero, G. Ragupathi, D.S. Tan, D.Y. Gin, Quillaja saponin variants with central glycosidic linkage modifications exhibit distinct conformations and adjuvant activities, *Chem. Sci.* 7 (2016) 2371–2380.
- [98] S. Cibulski, M. Rivera-Patron, N. Suárez, M. Pirez, S. Rossi, A.C. Yendo, F. de Costa, G. Gosmann, A. Fett-Neto, P.M. Roehf, F. Silveira, Leaf saponins of *Quillaja brasiliensis* enhance long-term specific immune responses and promote dose-sparing effect in BVDV experimental vaccines, *Vaccine* 36 (2018) 55–65.
- [99] S. Moreno-Mendieta, D. Guillén, R. Hernández-Pando, S. Sánchez, R. Rodríguez-Sanoja, Potential of glucans as vaccine adjuvants: a review of the α -glucans case, *Carbohydr. Polym.* 165 (2017) 103–114.
- [100] P. García-Vello, I. Speciale, F. Chiodo, A. Molinaro, C. De Castro, Carbohydrate-based adjuvants, *Drug Discov. Today Technol.* 35–36 (2020) 57–68.
- [101] C. Gamazo, J.M. Irache, New adjuvants: from empiricism to science, *Expert Rev. Vaccines* 8 (2009) 1333–1337.
- [102] B. Zhao, C. Lv, J. Lu, Natural occurring polysaccharides from *Panax ginseng* C. A. Meyer: a review of isolation, structures, and bioactivities, *Int. J. Biol. Macromol.* 133 (2019) 324–336.
- [103] B. Sun, S. Yu, D. Zhao, S. Guo, X. Wang, K. Zhao, Polysaccharides as vaccine adjuvants, *Vaccine* 36 (2018) 5226–5234.
- [104] D.T. O'Hagan, L.R. Friedland, E. Hanon, A.M. Didierlaurent, Towards an evidence based approach for the development of adjuvanted vaccines, *Curr. Opin. Immunol.* 47 (2017) 93–102.
- [105] R. Govaerts, E. Nic Lughadha, N. Black, R. Turner, A.J. Paton, The World Checklist of Vascular Plants, a continuously updated resource for exploring global plant diversity, *Sci. Data* (2021), <https://doi.org/10.1038/s41597-021-00997-6>.
- [106] D.R. Flower, Systematic identification of small molecule adjuvants, *Expert Opin. Drug Discov.* 7 (2012) 807–817.
- [107] M.J.R. Howes, C.L. Quave, J. Collemare, E.C. Tatsis, D. Twilley, E. Lulekal, A. Farlow, L. Li, M.E. Cazar, D.J. Leaman, T.A.K. Prescott, W. Milliken, C. Martin, M.N. De Canha, N. Lall, H. Qin, B.E. Walker, C. Vásquez-Londoño, B. Allkin, M. Rivers, M.S.J. Simmonds, E. Bell, A. Battison, J. Felix, F. Forest, C. Leon, C. Williams, E. Nic Lughadha, Molecules from Nature: reconciling biodiversity conservation and global healthcare imperatives for sustainable use of medicinal plants and fungi, *Plants People Planet* 2 (2020) 463–481.
- [108] F. Radogna, M. Dicato, M. Diederich, Natural modulators of the hallmarks of immunogenic cell death, *Biochem. Pharmacol.* 162 (2019) 55–70.
- [109] M.R. Howes, The evolution of anticancer drug discovery from plants, *Lancet Oncol.* 19 (2018) 293–294.
- [110] E. Dauncey, M. Howes, Plants that Cure. Plants as a Source of Medicines - from Pharmaceuticals to Herbal Remedies, UK: Kew Publishing, Royal Botanic Gardens Kew; Quarto Publishing, London, 2020.
- [111] G.A. Cordell, M.L. Quinn-Beattie, N.R. Farnsworth, The potential of alkaloids in drug discovery, *Phytother. Res.* 15 (2001) 183–205.
- [112] H. Khan, H. Ullah, S. Khattak, M. Aschner, C.N. Aguilar, S.M.A. Halimi, O. Cauli, S.M.M. Shah, Therapeutic potential of alkaloids in autoimmune diseases: promising candidates for clinical trials, *Phytother. Res.* 35 (2021) 50–62.
- [113] M.K. Apaya, T.F. Kuo, M.T. Yang, G. Yang, C.L. Hsiao, S.B. Chang, Y. Lin, W. C. Yang, Phytochemicals as modulators of β -cells and immunity for the therapy of type 1 diabetes: recent discoveries in pharmacological mechanisms and clinical potential, *Pharmacol. Res.* 156 (2020), 104754.
- [114] M. Mecha, F.J. Carrillo-Salinas, A. Feliú, L. Mestre, C. Guaza, Perspectives on Cannabis-based therapy of multiple sclerosis: a mini-review, *Front. Cell Neurosci.* 14 (2020) 34.
- [115] **The Complete Drug Reference 2021 Royal Pharmaceutical Society Martindale.**
- [116] A. Furguiele, M. Cosentino, M. Ferrari, F. Marino, Immunomodulatory potential of Cannabidiol in multiple Sclerosis: a systematic review, *J. Neuroimmune Pharmacol.* 16 (2021) 251–269.
- [117] D. Katz-Talmor, I. Katz, B.S. Porat-Katz, Y. Shoenfeld, Cannabinoids for the treatment of rheumatic diseases - where do we stand? *Nat. Rev. Rheumatol.* 14 (2018) 488–498.
- [118] X. Chen, H. Guo, L. Qiu, C. Zhang, Q. Deng, Q. Leng, Immunomodulatory and antiviral activity of Metformin and its potential implications in treating coronavirus disease 2019 and lung injury, *Front. Immunol.* 11 (2020) 2056.
- [119] S. Fatima, A. Bhaskar, V.P. Dwivedi, Repurposing immunomodulatory drugs to combat tuberculosis, *Front. Immunol.* 12 (2021), 645485.
- [120] A.M.S. Mayer, V. Lehmann, Marine pharmacology in 1998: marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, anthelmintic, antiplatelet, antiprotozoal, and antiviral activities; with actions on the cardiovascular, endocrine, immune, and nervous systems; and other miscellaneous mechanisms of action, *Pharmacologist* 42 (2000) 62–69.
- [121] A.M.S. Mayer, M.T. Hamann, Marine pharmacology in 1999: compounds with antibacterial, anticoagulant, antifungal, anthelmintic, anti-inflammatory, antiplatelet, antiprotozoal and antiviral activities affecting the cardiovascular, endocrine, immune and nervous systems, and other miscellaneous mechanisms of action, *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 132 (2002) 315–339.
- [122] A.M.S. Mayer, M.T. Hamann, Marine pharmacology in 2000: marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiplatelet, antituberculosis, and antiviral activities; affecting the cardiovascular, immune, and nervous systems and other miscellaneous mechanisms of action, *Mar. Biotechnol.* 6 (2004) 37–52.
- [123] A.M.S. Mayer, M.T. Hamann, Marine pharmacology in 2001–2002: marine compounds with anthelmintic, antibacterial, anticoagulant, antidiabetic, antifungal, anti-inflammatory, antimalarial, antiplatelet, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems and other miscellaneous mechanisms of action, *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 140 (2005) 265–286.
- [124] A.M.S. Mayer, A.D. Rodríguez, R.G. Berlink, M.T. Hamann, Marine pharmacology in 2003–4: marine compounds with anthelmintic antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiplatelet, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems, and other miscellaneous mechanisms of action, *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 145 (2007) 553–581.
- [125] A.M.S. Mayer, A.D. Rodríguez, R.G. Berlink, M.T. Hamann, Marine pharmacology in 2005–6: Marine compounds with anthelmintic, antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems, and other miscellaneous mechanisms of action, *Biochim. Biophys. Acta* 1790 (2009) 283–308.

- [126] A.M.S. Mayer, A.D. Rodríguez, R.G. Berlinck, N. Fusetani, Marine pharmacology in 2007-8: Marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, antimalarial, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous system, and other miscellaneous mechanisms of action, *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 153 (2011) 191–222.
- [127] A.M.S. Mayer, A.D. Rodríguez, O. Tagliatalata-Scafati, N. Fusetani, Marine pharmacology in 2009-2011: marine compounds with antibacterial, antidiabetic, antifungal, anti-inflammatory, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous systems, and other miscellaneous mechanisms of action, *Mar. Drugs* 11 (2013) 2510–2573.
- [128] A.M.S. Mayer, A.D. Rodríguez, O. Tagliatalata-Scafati, N. Fusetani, Marine pharmacology in 2012-2013: marine compounds with antibacterial, antidiabetic, antifungal, anti-inflammatory, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous systems, and other miscellaneous mechanisms of action, *Mar. Drugs* 15 (2017) 273, <https://doi.org/10.3390/md15090273>.
- [129] A.M.S. Mayer, A.J. Guerrero, A.D. Rodríguez, O. Tagliatalata-Scafati, F. Nakamura, N. Fusetani, Marine pharmacology in 2014-2015: marine compounds with antibacterial, antidiabetic, antifungal, anti-inflammatory, antiprotozoal, antituberculosis, antiviral, and anthelmintic activities; affecting the immune and nervous systems, and other miscellaneous mechanisms of action, *Mar. Drugs* 18 (2019) 5, <https://doi.org/10.3390/md18010005>.
- [130] A.M.S. Mayer, A.J. Guerrero, A.D. Rodríguez, O. Tagliatalata-Scafati, F. Nakamura, N. Fusetani, Marine pharmacology in 2016-2017: marine compounds with antibacterial, antidiabetic, antifungal, anti-inflammatory, antiprotozoal, antituberculosis and antiviral activities; affecting the immune and nervous systems, and other miscellaneous mechanisms of action, *Mar. Drugs* 19 (2021) 49, <https://doi.org/10.3390/md19020049>.
- [131] H. Zbakh, E. Talero, J. Avila, A. Alcáide, C. de Los Reyes, E. Zubía, V. Motilva, The algal meroterpene 11-hydroxy-1'-o-methylamentadiene ameliorates dextran sulfate sodium-induced colitis in mice, *Mar. Drugs* 14 (2016) 149, <https://doi.org/10.3390/md14080149>.
- [132] B. Jung, S.K. Ku, M. Gao, K.M. Kim, M.S. Han, H. Choi, J.S. Bae, Suppressive effects of three diketopiperazines from marine-derived bacteria on TGFβ1-mediated septic responses in human endothelial cells and mice, *Arch. Pharm. Res.* 39 (2016) 843–854.
- [133] T.B. Ahmad, D. Rudd, K. Benkendorff, L.K. Mahdi, K.A. Pratt, L. Dooley, C. Wei, M. Kotiw, Brominated indoles from a marine mollusc inhibit inflammation in a murine model of acute lung injury, *PLoS One* 12 (2017), e0186904.
- [134] Y.Y. Lin, Y.H. Jean, H.P. Lee, S.C. Lin, C.Y. Pan, W.F. Chen, S.F. Wu, J.H. Su, K. H. Tsui, J.H. Sheu, P.J. Sung, Z.H. Wen, Excavatolide B attenuates rheumatoid arthritis through the inhibition of osteoclastogenesis, *Mar. Drugs* 15 (2017) 9, <https://doi.org/10.3390/md15010009>.
- [135] J.H. Choi, N.H. Kim, S.J. Kim, H.J. Lee, S. Kim, Fucoxanthin inhibits the inflammation response in paw edema model through suppressing MAPKs, Akt, and NFκB, *J. Biochem. Mol. Toxicol.* 30 (2016) 111–119.
- [136] E.A. Pisylyagin, I.V. Manzhulo, T.Y. Gorpenchenko, P.S. Dmitrenko, S.A. Avilov, A. S. Silchenko, Y.M. Wang, D.L. Aminin, Cucumariocide A₂-2 causes macrophage activation in mouse spleen, *Mar. Drugs* 15 (2017) 341, <https://doi.org/10.3390/md15110341>.
- [137] D. Aminin, E. Pisylyagin, M. Astashev, A. Es'kov, V. Kozhemyako, S. Avilov, E. Zelepuga, E. Yurchenko, L. Kaluzhskiy, E. Kozlovskaya, A. Ivanov, V. Stonik, Glycosides from edible sea cucumbers stimulate macrophages via purinergic receptors, *Sci. Rep.* 6 (2016) 39683.
- [138] T.M. Ha, W. Ko, S.J. Lee, Y.C. Kim, J.Y. Son, J.H. Sohn, J.H. Yim, H. Oh, Anti-inflammatory effects of curvularin-type metabolites from a marine-derived fungal strain *penicillium* sp. SF-5859 in lipopolysaccharide-induced RAW264.7 macrophages, *Mar. Drugs* 15 (2017) 282, <https://doi.org/10.3390/md15090282>.
- [139] A.M.S. Mayer, J. Murphy, D. MacAdam, C. Osterbauer, I. Baseer, M.L. Hall, D. Feher, P. Williams, Classical and alternative activation of cyanobacterium *Oscillatoria* sp. lipopolysaccharide-treated rat microglia in vitro, *Toxicol. Sci.* 149 (2016) 484–495.
- [140] E. Ciaglia, A.M. Malfitano, C. Laezza, A. Fontana, G. Nuzzo, A. Cutignano, M. Abate, M. Pelin, S. Sosa, M. Bifulco, P. Gazzo, Immuno-modulatory and anti-inflammatory effects of Dihydrograicilin A, a terpene derived from the marine sponge *Dendrilla membranosa*, *Int. J. Mol. Sci.* 18 (2017) 1643, <https://doi.org/10.3390/ijms18081643>.
- [141] S. Kozuma, Y. Hirota-Takahata, D. Fukuda, N. Kuraya, M. Nakajima, O. Ando, Identification and biological activity of ogipeptins, novel LPS inhibitors produced by marine bacterium, *J. Antibiot.* 70 (2017) 79–83.
- [142] J.A. Sánchez, A. Alfonso, I. Rodríguez, E. Alonso, J.M. Cifuentes, R. Bermudez, M. E. Rateb, M. Jaspars, W.E. Housen, R. Ebel, J. Tabudravu, L.M. Botana, Spongionella secondary metabolites, promising modulators of immune response through CD147 receptor modulation, *Front. Immunol.* 7 (2016) 452.
- [143] D.J. Newman, Natural product based antibody drug conjugates: clinical status as of november 9, 2020, *J. Nat. Prod.* 84 (2021) 917–931.
- [144] V. Boni, M.R. Sharma, A. Patnaik, The resurgence of antibody drug conjugates in cancer therapeutics: novel targets and payloads, *Am. Soc. Clin. Oncol. Educ. Book* 40 (2020) 1–17.
- [145] J.Z. Drago, S. Modi, S. Chandralapaty, Unlocking the potential of antibody-drug conjugates for cancer therapy, *Nat. Rev. Clin. Oncol.* 18 (2021) 327–344.
- [146] D.J. Newman, The “Utility” of highly toxic marine-sourced compounds, *Mar. Drugs* 17 (2019) 324, <https://doi.org/10.3390/md17060324>.
- [147] D.J. Newman, G.M. Cragg, Current status of marine-derived compounds as warheads in anti-tumor drug candidates, *Mar. Drugs* 15 (2017) 99, <https://doi.org/10.3390/md15040099>.
- [148] FDA. FDA Approves Gemtuzumab Ozogamicin for CD33-positive AML. (<https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-gemtuzumab-ozogamicin-cd33-positive-aml>), 2017 (Accessed June 2021).
- [149] ADC Review. Chinese Regulator Conditionally Approves Disitamab Vedotin in Advanced or Metastatic Gastric Cancer. (<https://www.adcreview.com/clinical-trials-update/chinese-regulator-conditionally-approves-disitamab-vedotin-in-advanced-or-metastatic-gastric-cancer/>), 2021 (Accessed September 2021).
- [150] Newswire CP. RemeGen Announces US FDA Has Granted Breakthrough Therapy Designation for Disitamab Vedotin (RC48) in Urothelial Cancer. (<https://www.prnewswire.com/news-releases/remegen-announces-us-fda-has-granted-breakthrough-therapy-designation-for-disitamab-vedotin-rc48-in-urothelial-cancer-301138315.html>), 2020 (Accessed September 2021).
- [151] Marine Pharmacology. The Global Marine Pharmaceuticals Pipeline. (<https://www.marinepharmacology.org/>) (Accessed September 2021).
- [152] P.D. Senter, E.L. Sievers, The discovery and development of brentuximab vedotin for use in relapsed Hodgkin lymphoma and systemic anaplastic large cell lymphoma, *Nat. Biotechnol.* 30 (2012) 631–637.
- [153] S.O. Doronina, B.E. Toki, M.Y. Torgov, B.A. Mendelsohn, C.G. Cervený, D. F. Chace, R.L. DeBlanc, R.P. Gearing, T.D. Bovee, C.B. Siegall, J.A. Francisco, A. F. Wahl, D.L. Meyer, P.D. Senter, Development of potent monoclonal antibody auristatin conjugates for cancer therapy, *Nat. Biotechnol.* 21 (2003) 778–784.
- [154] G.R. Pettit, Y. Kamano, C.L. Herald, A.A. Tuinman, F.E. Boettner, H. Kizu, J. M. Schmidt, L. Baczynskyj, K.B. Tomer, R.J. Bontems, The isolation and structure of a remarkable marine animal antineoplastic constituent: dolastatin 10, *J. Am. Chem. Soc.* 109 (1987) 6883–6885.
- [155] G.R. Pettit, The dolastatins, *Fortschr. Chem. Org. Nat.* 70 (1997) 1–79.
- [156] H. Luesch, R.E. Moore, V.J. Paul, S.L. Mooberry, T.H. Corbett, Isolation of dolastatin 10 from the marine cyanobacterium *Symplocos* species VP642 and total stereochemistry and biological evaluation of its analogue symplostatins 1, *J. Nat. Prod.* 64 (2001) 907–910.
- [157] Newman D. The Evolution of Dolastatin 10 Derivative as Sources of ADC Warheads. *Hot Topics in Pharmacognosy* 2021; 56 (4). American Society of Pharmacognosy (<https://www.pharmacognosy.us/hot-topics-in-pharmacognosy-the-evolution-of-dolastatin-10-derivatives-as-sources-of-adc-warheads/>) (Accessed September 2021).
- [158] U. Vaishampayan, M. Glode, W. Du, A. Kraft, G. Hudes, J. Wright, et al., Phase II study of dolastatin-10 in patients with hormone-refractory metastatic prostate adenocarcinoma, *Clin. Cancer Res.* 6 (2000) 4205–4208.
- [159] A. Maderna, M. Doroski, C. Subramanyam, A. Porte, C.A. Leverett, B.C. Vetelino, Z. Chen, H. Risley, K. Parris, J. Pandit, A.H. Varghese, S. Shanker, C. Song, S. C. Sukuru, K.A. Farley, M.M. Wagenaar, M.J. Shapiro, S. Musto, M.H. Lam, F. Loganzo, C.J. O'Donnell, Discovery of cytotoxic dolastatin 10 analogues with N-terminal modifications, *J. Med. Chem.* 57 (2014) 10527–10543.
- [160] T. Shimizu, Y. Fujiwara, K. Yonemori, T. Koyama, J. Sato, K. Tamura, A. Shimomura, H. Ikezawa, M. Nomoto, K. Furuuchi, R. Nakajima, T. Miura, N. Yamamoto, First-in-human phase 1 study of MORAb-202, an antibody-drug conjugate comprising farletuzumab linked to eribulin mesylate, in patients with folate receptor-α-positive advanced solid tumors, *Clin. Cancer Res.* 27 (2021) 3905–3915.
- [161] M.L. Maitland, J.C. Sachdev, M.R. Sharma, V. Moreno, V. Boni, S. Kummer, E. Stringer-Reasor, N. Lakhani, A.R. Moreau, D. Xuan, R. Li, E.L. Powell, A. Jackson-Fisher, M. Bowers, S. Alekar, X. Xin, A.W. Tolcher, E. Calvo, First-in-human study of PF-06647020 (Cofetuzumab Pelidotin), an antibody-drug conjugate targeting protein tyrosine kinase 7, in advanced solid tumors, *Clin. Cancer Res.* 27 (2021) 4511–4520.
- [162] L. Skidmore, S. Sakamuri, N.A. Knudsen, A.G. Hewet, S. Milutinovic, W. Barkho, S.L. Biroc, J. Kirtley, R. Marsden, K. Storey, I. Lopez, W. Yu, S.Y. Fang, S. Yao, Y. Gu, F. Tian, ARX788, a site-specific Anti-HER2 antibody-drug conjugate, demonstrates potent and selective activity in HER2-low and T-DM1-resistant breast and gastric cancers, *Mol. Cancer Ther.* 19 (2020) 1833–1843.
- [163] N.D. Bodyak, R. Mosher, A.V. Yurkovetskiy, M. Yin, C. Bu, P.R. Conlon, D. R. Demady, M.J. DeVit, D.R. Gumerov, V.R. Gurijala, W. Lee, D. McGillicuddy, P. U. Park, L.L. Poling, M. Protopova, L. Qin, C.A. Stevenson, E. Ter-Ovanesyan, A. Uttard, D. Xiao, J. Xu, L. Xu, D.A. Bergstrom, T.B. Lowinger, The dolaflexin-based antibody-drug conjugate XMT-1536 targets the solid tumor lineage antigen SLC34A2/NaPi2b, *Mol. Cancer Ther.* 20 (2021) 896–905.
- [164] G. Rinnerthaler, S.P. Gampenrieder, R. Greil, HER2 directed antibody-drug-conjugates beyond T-DM1 in breast cancer, *Int. J. Mol. Sci.* 20 (2019) 1115, <https://doi.org/10.3390/ijms20051115>.
- [165] L. Li, A. Hau, W. Tong, M. Lau, T. Zhu, K. Fells, et al., Abstract LB-227: preclinical development and characterization of STI-6129, an anti-CD38 antibody-drug conjugate, as a new therapeutic agent for multiple myeloma, *Cancer Res.* 80 (2020) LB-227. LB-227.
- [166] K.J. Hamblett, P.W. Hammond, S.D. Barnscher, V.K. Fung, R.H. Davies, G. R. Wickman, A. Hernandez, T. Ding, A.S. Galey, G.C. Winters, J.R. Rich, J. S. Babcook, Abstract 3914: ZW49, a HER2-targeted biparatopic antibody-drug conjugate for the treatment of HER2-expressing cancers, *Cancer Res.* 78 (2018) 3914.
- [167] F.A. Lievano, L.J. Scarazzini, J.E. Tyczynski, C.M. Schubert, C.L. Renz, Risk minimization of antibody-drug conjugates in oncology: a review, *Drug Saf.* 44 (2021) 733–742.
- [168] G.F. King, Venoms as a platform for human drugs: translating toxins into therapeutics, *Expert Opin. Biol. Ther.* 11 (2011) 1469–1484.

- [169] R.Y.M. Ryan, J. Seymour, A. Loukas, J.A. Lopez, M.P. Ikonomopoulou, J.J. Miles, Immunological responses to envenomation, *Front. Immunol.* 12 (2021), 661082.
- [170] R. Jimenez, M.P. Ikonomopoulou, J.A. Lopez, J.J. Miles, Immune drug discovery from venoms, *Toxicon* 141 (2018) 18–24.
- [171] C. Minutti-Zanella, E.J. Gil-Leyva, I. Vergara, Immunomodulatory properties of molecules from animal venoms, *Toxicon* 191 (2021) 54–68.
- [172] K.C.F. Bordon, C.T. Cologna, E.C. Fornari-Baldo, E.L. Pinheiro-Júnior, F.A. Cerni, F.G. Amorim, F.A.P. Anjolette, F.A. Cordeiro, G.A. Wiesel, I.A. Cardoso, I. G. Ferreira, I.S. Oliveira, J. Boldrini-França, M.B. Pucca, M.A. Baldo, E.C. Arantes, From animal poisons and venoms to medicines: achievements, challenges and perspectives in drug discovery, *Front. Pharmacol.* 11 (2020) 1132.
- [173] Y.S. Chan, R.C.F. Cheung, L. Xia, J.H. Wong, T.B. Ng, W.Y. Chan, Snake venom toxins: toxicity and medicinal applications, *Appl. Microbiol. Biotechnol.* 100 (2016) 6165–6181.
- [174] S. Almeida Cde, V. Andrade-Oliveira, N.O. Câmara, J.F. Jacysyn, E.L. Faquim-Mauro, Crotoxin from *Crotalus durissus terrificus* is able to down-modulate the acute intestinal inflammation in mice, *PLoS One* 10 (2015), e0121427.
- [175] T.S. Lima, C.L. Neves, V.O. Zambelli, F.S.R. Lopes, S.C. Sampaio, M.C. Cirillo, Crotoxin, a rattlesnake toxin, down-modulates functions of bone marrow neutrophils and impairs the Syk-GTPase pathway, *Toxicon* 136 (2017) 44–55.
- [176] A. Gomes, S. Bhattacharya, M. Chakraborty, P. Bhattacharjee, R. Mishra, A. Gomes, Anti-arthritis activity of Indian monocellate cobra (*Naja kaouthia*) venom on adjuvant induced arthritis, *Toxicon* 55 (2010) 670–673.
- [177] Q. Zhu, J. Huang, S.Z. Wang, Z.H. Qin, F. Lin, Cobrotoxin extracted from *Naja atra* venom relieves arthritis symptoms through anti-inflammation and immunosuppression effects in rat arthritis model, *J. Ethnopharmacol.* 194 (2016) 1087–1095.
- [178] Y. Ruan, L. Yao, B. Zhang, S. Zhang, J. Guo, Anti-inflammatory effects of Neurotoxin-Nna, a peptide separated from the venom of *Naja Naja atra*, *BMC Complement. Altern. Med.* 13 (2013) 86.
- [179] N. Wang, Y. Huang, A. Li, H. Jiang, J. Wang, J. Li, L. Qiu, K. Li, Y. Lu, Hydrostatin-TL1, an anti-inflammatory active peptide from the venom gland of hydrophis cyanocinctus in the South China Sea, *Int. J. Mol. Sci.* 17 (2016) 17.
- [180] H.J. An, J.Y. Kim, W.H. Kim, M.G. Gwon, H.M. Gu, M.J. Jeon, S.M. Han, S.C. Pak, C.K. Lee, I.S. Park, K.K. Park, Therapeutic effects of bee venom and its major component, melittin, on atopic dermatitis in vivo and in vitro, *Br. J. Pharmacol.* 175 (2018) 4310–4324.
- [181] F. Sobral, A. Sampaio, S. Falcão, M.J. Queiroz, R.C. Calhelha, M. Vilas-Boas, I. C. Ferreira, Chemical characterization, antioxidant, anti-inflammatory and cytotoxic properties of bee venom collected in Northeast Portugal, *Food Chem. Toxicol.* 94 (2016) 172–177.
- [182] M. Dadar, Y. Shahali, S. Chakraborty, M. Prasad, F. Tahoori, R. Tiwari, K. Dhama, Antiinflammatory peptides: current knowledge and promising prospects, *Inflamm. Res.* 68 (2019) 125–145.
- [183] W.H. Kim, H.J. An, J.Y. Kim, M.G. Gwon, H. Gu, J.B. Park, W.J. Sung, Y.C. Kwon, K.D. Park, S.M. Han, K.K. Park, Bee venom inhibits porphyromonas gingivalis lipopolysaccharides-induced pro-inflammatory cytokines through suppression of NF- κ B and AP-1 signaling pathways, *Molecules* 21 (2016) 21.
- [184] E.J. Im, S.J. Kim, S.B. Hong, J.K. Park, M.H. Rhee, Anti-inflammatory activity of bee venom in BV2 microglial cells: mediation of MyD88-dependent NF- κ B signaling pathway, *Evid. Based Complement Altern. Med.* 2016 (2016), 3704764.
- [185] H.J. Chung, J. Lee, J.S. Shin, M.R. Kim, W. Koh, M.J. Kim, J.W. Lee, E.J. Kim, I. H. Lee, W.K. Kim, Y.J. Lee, S.K. Lee, I.H. Ha, In vitro and in vivo anti-allergic and anti-inflammatory effects of eBV, a newly developed derivative of bee venom, through modulation of IRF3 signaling pathway in a Carrageenan-induced edema model, *PLoS One* 11 (2016), e0168120.
- [186] S. Park, H. Bae, K.H. Jung, G. Lee, H. Lee, G.H. Kang, G. Lee, H. Bae, Bee venom phospholipase A2 suppresses allergic airway inflammation in an ovalbumin-induced asthma model through the induction of regulatory T cells, *Immun. Inflamm. Dis.* 3 (2015) 386–397.
- [187] V. Quintero-Hernández, J.M. Jiménez-Vargas, G.B. Gurrola, H.H. Valdivia, L. D. Possani, Scorpion venom components that affect ion-channels function, *Toxicon* 76 (2013) 328–342.
- [188] Y. Zhao, J. Huang, X. Yuan, B. Peng, W. Liu, S. Han, X. He, Toxins targeting the Kv1.3 channel: potential immunomodulators for autoimmune diseases, *Toxins* 7 (2015) 1749–1764.
- [189] M.R. Tanner, R.B. Tajhya, R. Huq, E.J. Gehrman, K.E. Rodarte, M.A. Atik, R. S. Norton, M.W. Pennington, C. Beeton, Prolonged immunomodulation in inflammatory arthritis using the selective Kv1.3 channel blocker HsTX1[R14A] and its PEGylated analog, *Clin. Immunol.* 180 (2017) 45–57.
- [190] A.N. Hoang, H.D. Vo, N.P. Vo, K.S. Kudryashova, O.V. Nekrasova, A.V. Feofanov, M.P. Kirpichnikov, T.V. Andreeva, M.V. Serebryakova, V.I. Tsetlin, Y.N. Utkin, Vietnamese *Heterometrus laoticus* scorpion venom: evidence for analgesic and anti-inflammatory activity and isolation of new polypeptide toxin acting on Kv1.3 potassium channel, *Toxicon* 77 (2014) 40–48.
- [191] T. Verano-Braga, C. Rocha-Resende, D.M. Silva, D. Ianzer, M.F. Martin-Eauclaire, P.E. Bougis, M.E. de Lima, R.A. Santos, A.M. Pimenta, Tityus serrulatus Hypotensins: a new family of peptides from scorpion venom, *Biochem. Biophys. Res. Commun.* 371 (2008) 515–520.
- [192] T. Verano-Braga, F. Figueiredo-Rezende, M.N. Melo, R.Q. Lautner, E.R. Gomes, L. T. Mata-Machado, A. Murari, C. Rocha-Resende, M. Elena de Lima, S. Guatimosim, R.A. Santos, A.M. Pimenta, Structure–function studies of Tityus serrulatus Hypotensin-I (TsHpt-I): a new agonist of B2 kinin receptor, *Toxicon* 56 (2010) 1162–1171.
- [193] P. Cassini-Vieira, M. Felipetto, L.B. Prado, T. Verano-Braga, S.P. Andrade, R.A. S. Santos, M.M. Teixeira, M.E. de Lima, A. Pimenta, L.S. Barcelos, Ts14 from Tityus serrulatus boosts angiogenesis and attenuates inflammation and collagen deposition in sponge-induced granulation tissue in mice, *Peptides* 98 (2017) 63–69.
- [194] P. Pimentel, D. de Assis, M. Gualdrón-Lopez, A. Barroso, F. Brant, P.G. Leite, B. C. de Lima Oliveira, L. Esper, S. McKinnie, J.C. Vederas, M. do Nascimento Cordeiro, P. Dos Reis, M.M. Teixeira, A.M. de Castro Pimenta, M.H. Borges, M. E. de Lima, F.S. Machado, Tityus serrulatus scorpion venom as a potential drug source for Chagas' disease: trypanocidal and immunomodulatory activity, *Clin. Immunol.* 226 (2021), 108713.
- [195] D.R.R. de Assis, P.M.O. Pimentel, P.V.M. Dos Reis, R.A.N. Rabelo, R.W.A. Vitor, M.D.N. Cordeiro, L.F. Felicori, C. Olórtegui, J.M. Resende, M.M. Teixeira, M. H. Borges, M.E. de Lima, A. Pimenta, F.S. Machado, Tityus serrulatus (Scorpion): from the crude venom to the construction of synthetic peptides and their possible therapeutic application against toxoplasma gondii infection, *Front. Cell Infect. Microbiol.* 11 (2021), 706618.
- [196] E.L. Danneels, S. Gerlo, K. Heyninc, K. Van Craenenbroeck, K. De Bosscher, G. Haegeman, D.C. de Graaf, How the venom from the ectoparasitoid Wasp nasonia vitripennis exhibits anti-inflammatory properties on mammalian cell lines, *PLoS One* 9 (2014), e96825.
- [197] R. Garg, I.J. Juncadella, N. Ramamoorthi, Ashish, S.K. Ananthanarayanan, V. Thomas, M. Rincón, J.K. Krueger, E. Fikrig, C.M. Yengo, J. Anguita, Cutting edge: CD4 is the receptor for the tick saliva immunosuppressor, Salp15, *J. Immunol.* 177 (2006) 6579–6583.
- [198] F. De Marco Almeida, A.M. de Castro Pimenta, M.C. Oliveira, M.E. De Lima, Venoms, toxins and derivatives from the Brazilian fauna: valuable sources for drug discovery, *Sheng Li Xue Bao* 67 (2015) 261–270.
- [199] A.P. Bonfanti, N. Barreto, J. Munhoz, M. Caballero, G. Cordeiro, T. Rocha-E-Silva, R. Sutti, F. Moura, S. Brunetto, C.D. Ramos, R. Thomé, L. Verinaud, C. Raposo, Spider venom administration impairs glioblastoma growth and modulates immune response in a non-clinical model, *Sci. Rep.* 10 (5876) (2020) 020–62620–62629.
- [200] J. Munhoz, G. Peron, A.P. Bonfanti, J. Oliveira, T. da Rocha-E-Silva, R. Sutti, R. Thomé, A.L. Bombeiro, N. Barreto, G.M. Chalbatani, E. Garagouzloo, J. L. Vitorino-Araujo, L. Verinaud, C. Raposo, Components from spider venom activate macrophages against glioblastoma cells: new potential adjuvants for anticancer immunotherapy, *J. Biochem.* 170 (2021) 51–68.
- [201] D.J. Newman, G.M. Cragg, Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019, *J. Nat. Prod.* 83 (2020) 770–803.
- [202] A.G. Atanasov, S.B. Zotchev, V.M. Dirsch, International natural product sciences taskforce, *Supuran CT. Natural products in drug discovery: advances and opportunities*, *Nat. Rev. Drug Discov.* 20 (2021) 200–216.
- [203] L. Zhang, J. Song, L. Kong, T. Yuan, W. Li, W. Zhang, B. Hou, Y. Lu, G. Du, The strategies and techniques of drug discovery from natural products, *Pharmacol. Ther.* 216 (2020), 107686.
- [204] E. Lautié, O. Russo, P. Ducrot, J.A. Boutin, Unraveling plant natural chemical diversity for drug discovery purposes, *Front. Pharmacol.* 11 (2020) 397.
- [205] F. Carmona, Soares, A.M. Pereira, Herbal medicines: old and new concepts, truths and misunderstandings, *Rev. Bras. Farmacogn.* 23 (2013) 379–385.
- [206] E.M. Williamson, X. Liu, A.A. Izzo, Trends in use, pharmacology, and clinical applications of emerging herbal nutraceuticals, *Br. J. Pharmacol.* 177 (2020) 1227–1240.
- [207] E.M. Pferschy-Wenzig, R. Bauer, The relevance of pharmacognosy in pharmacological research on herbal medicinal products, *Epilepsy Behav.* 52 (2015) 344–362.
- [208] R. Andrew, A.A. Izzo, Principles of pharmacological research of nutraceuticals, *Br. J. Pharmacol.* 174 (2017) 1177–1194.
- [209] B.B. Petrovska, Historical review of medicinal plants' usage, *Pharmacogn. Rev.* 6 (2012) 1–5.
- [210] C. Krishnamurti, S.C. Rao, The isolation of morphine by Serturmer, *Indian J. Anaesth.* 60 (2016) 861–862.
- [211] A.G. Atanasov, B. Waltenberger, E.M. Pferschy-Wenzig, T. Linder, C. Wawrosch, P. Uhrin, V. Temml, L. Wang, S. Schwaiger, E.H. Heiss, J.M. Rollinger, D. Schuster, J.M. Breuss, V. Bochkov, M.D. Mihovilovic, B. Kopp, R. Bauer, V. M. Dirsch, H. Stuppner, Discovery and resupply of pharmacologically active plant-derived natural products: a review, *Biotechnol. Adv.* 33 (2015) 1582–1614.
- [212] Z. Guo, The modification of natural products for medical use, *Acta Pharm. Sin. B* 7 (2017) 119–136.
- [213] V. Butterweck, A. Nahrstedt, What is the best strategy for preclinical testing of botanicals? A critical perspective, *Planta Med.* 78 (2012) 747–754.
- [214] G.P. Dominguez More, P.A. Cardenas, G.M. Costa, C.M.O. Simoes, D.M. Aragon, Pharmacokinetics of botanical drugs and plant extracts, *Mini Rev. Med. Chem.* 17 (2017) 1646–1664.
- [215] C.J. Henrich, J.A. Beutler, Matching the power of high throughput screening to the chemical diversity of natural products, *Nat. Prod. Rep.* 30 (2013) 1284–1298.
- [216] L.K. Caesar, N.B. Cech, Synergy and antagonism in natural product extracts: when 1 + 1 does not equal 2, *Nat. Prod. Rep.* 36 (2019) 869–888.
- [217] S. Nicolussi, J. Drewe, V. Butterweck, H.E. Meyer Zu Schwabedissen, Clinical relevance of St. John's wort drug interactions revisited, *Br. J. Pharmacol.* 177 (2020) 1212–1226.
- [218] P. Rasoanaivo, C.W. Wright, M.L. Willcox, B. Gilbert, Whole plant extracts versus single compounds for the treatment of malaria: synergy and positive interactions, *Malar. J.* 10 (Suppl 1) (2011) 4. S4,2875-10-S1-S4.
- [219] Y. Yang, Z. Zhang, S. Li, X. Ye, X. Li, K. He, Synergy effects of herb extracts: pharmacokinetics and pharmacodynamic basis, *Fitoterapia* 92 (2014) 133–147.

- [220] U. Anand, B. Pacchetti, P. Anand, M.H. Sodergren, Cannabis-based medicines and pain: a review of potential synergistic and entourage effects, *Pain. Manag.* 11 (2021) 395–403.
- [221] S. Chandra, H. Lata, M.A. ElSohly, L.A. Walker, D. Potter, Cannabis cultivation: methodological issues for obtaining medical-grade product, *Epilepsy Behav.* 70 (2017) 302–312.
- [222] A. Nahrstedt, V. Butterweck, Lessons learned from herbal medicinal products: the example of St. John's Wort (perpendicular), *J. Nat. Prod.* 73 (2010) 1015–1021.
- [223] N. Malviya, S. Malviya, Bioassay guided fractionation-an emerging technique influence the isolation, identification and characterization of lead phytomolecules, *Int. J. Hosp. Pharm.* 2 (2017) 5.
- [224] A.A. Izzo, M. Teixeira, S.P.H. Alexander, G. Cirino, J.R. Docherty, C.H. George, P. A. Insel, Y. Ji, D.A. Kendall, R.A. Panatier, C.G. Sobey, S.C. Stanford, B. Stefanska, G. Stephens, A. Ahluwalia, A practical guide for transparent reporting of research on natural products in the British Journal of Pharmacology: Reproducibility of natural product research, *Br. J. Pharmacol.* 177 (2020) 2169–2178.
- [225] M. Heinrich, G. Appendino, T. Efferth, R. Fürst, A.A. Izzo, O. Kayser, J.M. Pezzuto, A. Viljoen, Best practice in research - overcoming common challenges in phytopharmacological research, *J. Ethnopharmacol.* 246 (2020), 112230.
- [226] A.A. Izzo, S. Hoon-Kim, R. Radhakrishnan, E.M. Williamson, A critical approach to evaluating clinical efficacy, adverse events and drug interactions of herbal remedies, *Phytother. Res.* 30 (2016) 691–700.
- [227] S. Li, Q. Han, C. Qiao, J. Song, C. Lung Cheng, H. Xu, Chemical markers for the quality control of herbal medicines: an overview, *Chin. Med.* 3 (7) (2008) 8546–3–7.
- [228] H. Woelk, Comparison of St John's wort and imipramine for treating depression: randomised controlled trial, *BMJ* 321 (2000) 536–539.
- [229] J.J. Gagnier, H. Boon, P. Rochon, D. Moher, J. Barnes, C. Bombardier, G. Consort, Recommendations for reporting randomized controlled trials of herbal interventions: explanation and elaboration, *J. Clin. Epidemiol.* 59 (2006) 1134–1149.
- [230] B.A.P. Wilson, C.C. Thornburg, C.J. Henrich, T. Grkovic, B.R. O'Keefe, Creating and screening natural product libraries, *Nat. Prod. Rep.* 37 (2020) 893–918.
- [231] M. Isgut, M. Rao, C. Yang, V. Subrahmanyam, P.C.G. Rida, R. Aneja, Application of combination high-throughput phenotypic screening and target identification methods for the discovery of natural product-based combination drugs, *Med. Res. Rev.* 38 (2018) 504–524.
- [232] H. Aldewachi, R.N. Al-Zidan, M.T. Conner, M.M. Salman, High-throughput screening platforms in the discovery of novel drugs for neurodegenerative diseases, *Bioengineering* 8 (2021) 30, <https://doi.org/10.3390/bioengineering8020030>.
- [233] D.W. McMillin, C.S. Mitsiades, High-throughput approaches to discover novel immunomodulatory agents for cancer, *Oncoimmunology* 1 (2012) 1406–1408.
- [234] K. Schimmel, M. Jung, A. Foinquinos, G.S. José, J. Beaumont, K. Bock, L. Grote-Levi, K. Xiao, C. Bär, A. Pflanze, A. Just, K. Zimmer, S. Ngoy, B. López, S. Ravassa, S. Samolovac, H. Janssen-Peters, J. Remke, K. Scherf, S. Dangwal, M.T. Piccoli, F. Kleemiss, F.P. Kreutzer, F. Kenneweg, J. Leonardy, L. Hobuß, L. Santer, Q.T. Do, R. Feffers, J.H. Braesen, J. Schmitz, C. Brandenberger, D.N. Müller, N. Wilck, V. Kaefer, H. Bähre, S. Batkai, J. Fiedler, K.M. Alexander, B.M. Wertheim, S. Fisch, R. Liao, J. Diez, A. González, T. Thum, Natural compound library screening identifies new molecules for the treatment of cardiac fibrosis and diastolic dysfunction, *Circulation* 141 (2020) 751–767.
- [235] J. Pérez Del Palacio, C. Díaz, M. de la Cruz, F. Annang, J. Martín, I. Pérez-Victoria, V. González-Menéndez, N. de Pedro, J.R. Tormo, F. Algieri, A. Rodríguez-Nogales, M.E. Rodríguez-Cabezas, F. Reyes, O. Genilloud, F. Vicente, J. Gálvez, High-throughput screening platform for the discovery of new immunomodulatory molecules from natural product extract libraries, *J. Biomol. Screen.* 21 (2016) 567–578.
- [236] A.E. Florian, C.K. Lepensky, O. Kwon, M.K. Haynes, L.A. Sklar, A. Zweifach, Flow cytometry enables a high-throughput homogeneous fluorescent antibody-binding assay for cytotoxic T cell lytic granule exocytosis, *J. Biomol. Screen.* 18 (2013) 420–429.
- [237] Z. Zhao, J.A. deMayo, A.M. West, M.J. Balunas, A. Zweifach, Development of an enhanced phenotypic screen of cytotoxic T-lymphocyte lytic granule exocytosis suitable for use with synthetic compound and natural product collections, *J. Biomol. Screen.* 21 (2016) 556–566.
- [238] F. Annang, G. Pérez-Moreno, R. García-Hernández, C. Cordon-Obras, J. Martín, J. R. Tormo, L. Rodríguez, N. de Pedro, V. Gómez-Pérez, M. Valente, F. Reyes, O. Genilloud, F. Vicente, S. Castanys, L.M. Ruiz-Pérez, M. Navarro, F. Gamarro, D. González-Pacanowska, High-throughput screening platform for natural product-based drug discovery against 3 neglected tropical diseases: human African trypanosomiasis, leishmaniasis, and Chagas disease, *J. Biomol. Screen.* 20 (2015) 82–91.
- [239] S.A. Haney (Ed.), *High Content Screening: Science, Techniques and Applications*, Wiley-Interscience, New York, 2008.
- [240] B. Cautain, N. de Pedro, V. Murillo Garzón, M. Muñoz de Escalona, V. González Menéndez, J.R. Tormo, J. Martín, N. El Aouad, F. Reyes, F. Asensio, O. Genilloud, F. Vicente, W. Link, High-content screening of natural products reveals novel nuclear export inhibitors, *J. Biomol. Screen.* 19 (2014) 57–65.
- [241] F. Zanella, A. Rosado, F. Blanco, B.R. Henderson, A. Carnero, W. Link, H.T.S. An, approach to screen for antagonists of the nuclear export machinery using high content cell-based assays, *Assay Drug Dev. Technol.* 5 (2007) 333–341.
- [242] J. Wang, M.Y. Wu, J.Q. Tan, M. Li, J.H. Lu, High content screening for drug discovery from traditional Chinese medicine, *Chin. Med.* 14 (5) (2019), 019-0228-y. eCollection.
- [243] J. Yan, H. Zhou, L. Kong, J. Zhang, Q. Zhao, Y. Li, Identification of two novel inhibitors of mTOR signaling pathway based on high content screening, *Cancer Chemother. Pharmacol.* 72 (2013) 799–808.
- [244] A. Vogt, A. Tamewitz, J. Skoko, R.P. Sikorski, K.A. Giuliano, J.S. Lazo, The benzo [c]phenanthridine alkaloid, sanguinarine, is a selective, cell-active inhibitor of mitogen-activated protein kinase phosphatase-1, *J. Biol. Chem.* 280 (2005) 19078–19086.
- [245] L. Hou, B. Xu, W. Guo, F.X. Ran, J.T. Liu, X. Yuan, H.Z. Fu, J.R. Cui, Pseudolaric acid B inhibits inducible cyclooxygenase-2 expression via downregulation of the NF- κ B pathway in HT-29 cells, *J. Cancer Res. Clin. Oncol.* 138 (2012) 885–896.
- [246] S.C. Cheah, D.R. Appleton, S.T. Lee, M.L. Lam, A.H. Hadi, M.R. Mustafa, Panduratin A inhibits the growth of A549 cells through induction of apoptosis and inhibition of NF- κ B translocation, *Molecules* 16 (2011) 2583–2598.
- [247] J.X. Song, Y.R. Sun, I. Peluso, Y. Zeng, X. Yu, J.H. Lu, Z. Xu, M.Z. Wang, L.F. Liu, Y.Y. Huang, L.L. Chen, S.S. Durairajan, H.J. Zhang, B. Zhou, H.Q. Zhang, A. Lu, A. Ballabio, D.L. Medina, Z. Guo, M. Li, A novel curcumin analog binds to and activates TFEB in vitro and in vivo independent of MTOR inhibition, *Autophagy* 12 (2016) 1372–1389.
- [248] T. Yang, D.P. Shen, n Wang, Y.Y. Tao, C.H. Liu, Investigation of the absorbed and metabolized components of Danshen from Fuzheng Huayu recipe and study on the anti-hepatic fibrosis effects of these components, *J. Ethnopharmacol.* 148 (2013) 691–700.
- [249] L.K. Minor, Label-free cell-based functional assays, *Comb. Chem. High. Throughput Screen.* 11 (2008) 573–580.
- [250] H.B. Christensen, D.E. Gloriam, D.S. Pedersen, J.B. Cowland, N. Borregaard, H. Bräuner-Osborne, Applying label-free dynamic mass redistribution assay for studying endogenous FPR1 receptor signalling in human neutrophils, *J. Pharmacol. Toxicol. Methods* 88 (2017) 72–78.
- [251] H. Zhou, X. Peng, T. Hou, N. Zhao, M. Qiu, X. Zhang, X. Liang, Identification of novel phytocannabinoids from Ganoderma by label-free dynamic mass redistribution assay, *J. Ethnopharmacol.* 246 (2020), 112218.
- [252] T. Hou, F. Xu, X. Peng, H. Zhou, X. Zhang, M. Qiu, J. Wang, Y. Liu, X. Liang, Label-free cell phenotypic study of opioid receptors and discovery of novel mu opioid ligands from natural products, *J. Ethnopharmacol.* 270 (2021), 113872.
- [253] F. Meurer, J. Schulte-Michels, H. Häberlein, S. Franken, Ivy leaves dry extract EA 575® mediates biased $\beta(2)$ -adrenergic receptor signaling, *Phytomedicine* 90 (2021), 153645.
- [254] F. Xu, T. Hou, A. Shen, H. Jin, Y. Xiao, W. Yu, X. Li, J. Wang, Y. Liu, X. Liang, Mechanism deconvolution of Qing Fei Pai Du decoction for treatment of Coronavirus Disease 2019 (COVID-19) by label-free integrative pharmacology assays, *J. Ethnopharmacol.* 280 (2021), 114488.
- [255] R. Wang, Y. Liu, H. Zhou, Y. Chen, J. Wang, X. Zhang, R. Yu, X. Liang, Integration of micro-fractionation, high-performance liquid chromatography-ultraviolet detector-charged aerosol detector-mass spectrometry analysis and cellular dynamic mass redistribution assay to accelerate alkaloid drug discovery, *J. Chromatogr. A* 1616 (2020), 460779.
- [256] H.P. Song, H. Wang, X. Zhao, L. He, H. Zhong, S.Q. Wu, P. Li, H. Yang, Label-free pharmacological profiling based on dynamic mass redistribution for characterization and authentication of hazardous natural products, *J. Hazard. Mater.* 333 (2017) 265–274.
- [257] R.W. Jones, M.N. Huffman, Fish embryos as bio-assay material in testing chemicals for effects on cell division and differentiation, *Trans. Am. Microsc. Soc.* 76 (1957) 177–183.
- [258] N. Tabassum, H. Tai, D.W. Jung, D.R. Williams, Fishing for nature's hits: establishment of the zebrafish as a model for screening antidiabetic natural products, *Evid. Based Complement Altern. Med.* 2015 (2015), 287847.
- [259] A. Pitchai, R.K. Rajaretinam, J.L. Freeman, Zebrafish as an emerging model for bioassay-guided natural product drug discovery for neurological disorders, *Medicines* 6 (2019) 61, <https://doi.org/10.3390/medicines6020061>.
- [260] D. Wang, G. Hu, J. Wang, D. Yan, M. Wang, L. Yang, N. Serikuly, E. Alpyshov, K. A. Demin, D.S. Galstyan, T.G. Amstislavskaya, M.S. de Abreu, A.V. Kalueff, Studying CNS effects of Traditional Chinese Medicine using zebrafish models, *J. Ethnopharmacol.* 267 (2021), 113383.
- [261] P.R. Rauta, B. Nayak, S. Das, Immune system and immune responses in fish and their role in comparative immunity study: a model for higher organisms, *Immunol. Lett.* 148 (2012) 23–33.
- [262] P.S. Pradeep, S. Manisha, J. Monica Amala Nayaki, D. Sivaraman, R. Selvaraj, S. Seeni, Potential antioxidant and anti-inflammatory action of *Hypericum hookerianum* extracts in a liposome system evaluated with zebrafish embryos, *J. Microencapsul.* 36 (2019) 513–522.
- [263] J. Zhang, Z. Song, Y. Li, S. Zhang, J. Bao, H. Wang, C. Dong, Y. Ohizumi, J. Xu, Y. Guo, Structural analysis and biological effects of a neutral polysaccharide from the fruits of *Rosa laevigata*, *Carbohydr. Polym.* 265 (2021), 111808.
- [264] S. Zhang, Z. Li, X. Wang, L. An, J. Bao, J. Zhang, J. Cui, Y. Li, D.Q. Jin, M. Tuerhong, M. Abudukeremu, Y. Ohizumi, J. Xu, Y. Guo, Isolation, structural elucidation, and immunoregulation properties of an arabinofuranan from the rinds of *Garcinia mangostana*, *Carbohydr. Polym.* 246 (2020), 116567.
- [265] D.C. Rajapaksha, S.L. Edirisinghe, C. Nikapitiya, S. Dananjaya, H.J. Kwun, C. H. Kim, C. Oh, D.H. Kang, M. De Zoysa, *Spirulina maxima* derived pectin nanoparticles enhance the immunomodulation, stress tolerance, and wound healing in zebrafish, *Mar. Drugs* 18 (2020) 556, <https://doi.org/10.3390/md18110556>.
- [266] A.D. Crawford, S. Liekens, A.R. Kamuhabwa, J. Maes, S. Munck, R. Busson, J. Rozenski, C.V. Esguerra, P.A. de Witte, Zebrafish bioassay-guided natural product discovery: isolation of angiogenesis inhibitors from East African medicinal plants, *PLoS One* 6 (2011), e14694.

- [267] V.H. Liao, Use of *Caenorhabditis elegans* to study the potential bioactivity of natural compounds, *J. Agric. Food Chem.* 66 (2018) 1737–1742.
- [268] S. Bulterijs, B.P. Braeckman, Phenotypic screening in *C. elegans* as a tool for the discovery of new geroprotective drugs, *Pharmaceuticals* 13 (2020) 164, <https://doi.org/10.3390/ph13080164>.
- [269] M.L. Yellurkar, V. Singh, V. Sai Prasanna, P. Das, S. Nanjappan, R. Velayutham, S. Arumugam, Evaluation of a natural compound extracted from *Dolichandrone atrovirens* as a novel antioxidant agent using *Caenorhabditis elegans*, *PLoS One* 16 (2021), e02577702.
- [270] C. Duangjan, P. Rangsinth, S. Zhang, X. Gu, M. Wink, T. Tencomnao, Vitis vinifera leaf extract protects against glutamate-induced oxidative toxicity in HT22 hippocampal neuronal cells and increases stress resistance properties in *Caenorhabditis elegans*, *Front. Nutr.* 8 (2021), 634100.
- [271] F. Pohl, A. Teixeira-Castro, M.D. Costa, V. Lindsay, J. Fiúza-Fernandes, M. Goua, G. Bermanno, W. Russell, P. Maciél, P. Kong Thoo Lin, GST-4-dependent suppression of neurodegeneration in *C. elegans* models of Parkinson's and Machado-Joseph disease by Rapeseed pomace extract supplementation, *Front. Neurosci.* 13 (2019) 1091.
- [272] C.L. Kurz, J.J. Ewbank, *Caenorhabditis elegans*: an emerging genetic model for the study of innate immunity, *Nat. Rev. Genet.* 4 (2003) 380–390.
- [273] V.T.T. Nguyen, S.C. Meyer, H. Rieger, K. Endres, A liquid-culture-based screening approach to study compounds affecting inflammatory processes in *Caenorhabditis elegans*, *Biol. Chem.* 403 (2021) 123–129.
- [274] Y. Li, D. Kong, Y. Fu, M.R. Sussman, H. Wu, The effect of developmental and environmental factors on secondary metabolites in medicinal plants, *Plant Physiol. Biochem.* 148 (2020) 80–89.
- [275] E. Ernst, Herbal medicines: balancing benefits and risks, *Novartis Found. Symp.* 282 (154) (2007) 67, discussion 167–72, 212–8.
- [276] X. Lu, L. Zhang, P. Li, J. Wang, R. Li, Y. Huang, M. Wu, H. Zhou, Y. Li, S. Wei, K. Li, H. Li, X. Zhou, Y. Zhao, X. Xiao, The protective effects of compatibility of *Aconitii Lateralis Radix Praeparata* and *Zingiberis Rhizoma* on rats with heart failure by enhancing mitochondrial biogenesis via Sirt1/PGC-1 α pathway, *Biomed. Pharmacother.* 92 (2017) 651–660.
- [277] M. Zhao, W.F. Xu, H.Y. Shen, P.Q. Shen, J. Zhang, D.D. Wang, H. Xu, H. Wang, T. T. Yan, L. Wang, H.P. Hao, G.J. Wang, L.J. Cao, Comparison of bioactive components and pharmacological activities of ophiopogon japonicus extracts from different geographical origins, *J. Pharm. Biomed. Anal.* 138 (2017) 134–141.
- [278] Y.J. Zhou, Y.J. Guo, X.L. Yang, Z.L. Ou, Anti-cervical cancer role of Matrine, Oxymatrine and *Sophora flavescens* alkaloid gels and its mechanism, *J. Cancer* 9 (2018) 1357–1364.
- [279] X. Cao, Q. He, Anti-tumor activities of bioactive phytochemicals in *Sophora flavescens* for breast cancer, *Cancer Manag. Res.* 12 (2020) 1457–1467.
- [280] F. Fontana, M. Raimondi, M. Marzagalli, A. Di Domizio, P. Limonta, Natural compounds in prostate cancer prevention and treatment: mechanisms of action and molecular targets, *Cells* 9 (2020) 460, <https://doi.org/10.3390/cells9020460>.
- [281] T. Joshi, T. Joshi, P. Sharma, S. Mathpal, H. Pundir, V. Bhatt, S. Chandra, In silico screening of natural compounds against COVID-19 by targeting Mpro and ACE2 using molecular docking, *Eur. Rev. Med. Pharmacol. Sci.* 24 (2020) 4529–4536.
- [282] Y. Li, Y. Wang, W. Tai, L. Yang, Y. Chen, C. Chen, C. Liu, Challenges and solutions of pharmacokinetics for efficacy and safety of Traditional Chinese Medicine, *Curr. Drug Metab.* 16 (2015) 765–776.
- [283] C.S. Liu, X. Liang, X.H. Wei, F.L. Chen, Q.F. Tang, X.M. Tan, Comparative pharmacokinetics of major bioactive components from *Puerariae Radix-Gastrodiae Rhizome* extracts and their intestinal absorption in rats, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 1105 (2019) 38–46.
- [284] S. Song, Q. Ma, Q. Tang, F. Chen, X. Xing, Y. Guo, S. Guo, X. Tan, J. Luo, Stereoselective metabolism of amygdalin-based study of detoxification of *Semen Armeniacae Amarum* in the *Herba Ephedrae-Semen Armeniacae Amarum* herb pair, *J. Ethnopharmacol.* 179 (2016) 356–366.
- [285] T.N. Aung, S. Nourmohammadi, Z. Qu, Y. Harata-Lee, J. Cui, H.Y. Shen, A.J. Yool, T. Pukala, H. Du, R.D. Kortschak, W. Wei, D.L. Adelson, Fractional deletion of compound kushen injection indicates cytokine signaling pathways are critical for its perturbation of the cell cycle, *Sci. Rep.* 9 (14200) (2019) 019–50271–50274.
- [286] R. Zhang, X. Zhu, H. Bai, K. Ning, Network pharmacology databases for Traditional Chinese Medicine: review and assessment, *Front. Pharmacol.* 10 (2019) 123.
- [287] A.S. Azmi, Adopting network pharmacology for cancer drug discovery, *Curr. Drug Discov. Technol.* 10 (2013) 95–105.
- [288] H. Shen, Z. Qu, Y. Harata-Lee, T.N. Aung, J. Cui, W. Wang, R.D. Kortschak, D. L. Adelson, Understanding the mechanistic contribution of herbal extracts in compound kushen injection with transcriptome analysis, *Front. Oncol.* 9 (2019) 632.
- [289] L. Teng, K. Jin, K. He, C. Bian, W. Chen, K. Fu, T. Zhu, Z. Jin, Use of complementary and alternative medicine by cancer patients at Zhejiang University Teaching Hospital Zhujui Hospital, China, *Afr. J. Tradit. Complement. Alter. Med.* 7 (2010) 322–330.
- [290] R.W. van Leeuwen, T. van Gelder, R.H. Mathijssen, F.G. Jansman, Drug-drug interactions with tyrosine-kinase inhibitors: a clinical perspective, *Lancet Oncol.* 15 (2014) e315–e326.
- [291] J. Zhang, F. Zhou, F. Niu, M. Lu, X. Wu, J. Sun, G. Wang, Stereoselective regulations of P-glycoprotein by ginsenoside Rh2 epimers and the potential mechanisms from the view of pharmacokinetics, *PLoS One* 7 (2012), e35768.
- [292] F.Y. Li, H.P. Hao, K. Hao, T.T. Yan, G.J. Wang, Effect of diammonium glycyrrhizinate on entecavir pharmacokinetics in rats, *Chin. J. Nat. Med.* 11 (2013) 309–313.
- [293] O.A. Balbin, J.R. Prensner, A. Sahu, A. Yocum, S. Shankar, R. Malik, D. Fermin, S. M. Dhanasekaran, B. Chandler, D. Thomas, D.G. Beer, X. Cao, A.I. Nesvizhskii, A. M. Chinnaiyan, Reconstructing targetable pathways in lung cancer by integrating diverse omics data, *Nat. Commun.* 4 (2013) 2617.
- [294] K. Cisek, M. Krochmal, J. Klein, H. Mischak, The application of multi-omics and systems biology to identify therapeutic targets in chronic kidney disease, *Nephrol. Dial. Transpl.* 31 (2016) 2003–2011.
- [295] S.L. Schwikkard, D.A. Mulholland, Useful methods for targeted plant selection in the discovery of potential new drug candidates, *Planta Med.* 80 (2014) 1154–1160.
- [296] M. Jörg, K.S. Madden, The right tools for the job: the central role for next generation chemical probes and chemistry-based target deconvolution methods in phenotypic drug discovery, *RSC Med. Chem.* 12 (2021) 646–665.
- [297] T.F. Molinski, Nanomole-scale natural products discovery, *Curr. Opin. Drug Discov. Devel* 12 (2009) 197–206.
- [298] A. Bouslimani, L.M. Sanchez, N. Garg, P.C. Dorrestein, Mass spectrometry of natural products: current, emerging and future technologies, *Nat. Prod. Rep.* 31 (2014) 718–729.
- [299] A. Zhang, H. Sun, X. Wang, Mass spectrometry-driven drug discovery for development of herbal medicine, *Mass Spectrom. Rev.* 37 (2018) 307–320.
- [300] C.F. Stratton, D.J. Newman, D.S. Tan, Cheminformatic comparison of approved drugs from natural product versus synthetic origins, *Bioorg. Med. Chem. Lett.* 25 (2015) 4802–4807.
- [301] W.H. Brooks, W.C. Guida, K.G. Daniel, The significance of chirality in drug design and development, *Curr. Top. Med. Chem.* 11 (2011) 760–770.
- [302] J. Hubert, J. Nuzillard, J. Renault, Dereplication strategies in natural product research: how many tools and methodologies behind the same concept? *Phytochem. Rev.* 16 (2017) 55–95.
- [303] F. Bucar, A. Wube, M. Schmid, Natural product isolation—how to get from biological material to pure compounds, *Nat. Prod. Rep.* 30 (2013) 525–545.
- [304] Q.W. Zhang, L.G. Lin, W.C. Ye, Techniques for extraction and isolation of natural products: a comprehensive review, *Chin. Med.* 13 (20) (2018) 018–0177-x, eCollection 2018.
- [305] A. Altemimi, N. Lakhssassi, A. Baharlouei, D.G. Watson, D.A. Lightfoot, Phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts, *Plants* 6 (2017) 42, <https://doi.org/10.3390/plants6040042>.
- [306] C.Y. Zhang, Y.L. Wu, P. Zhang, Z.Z. Chen, H. Li, L.X. Chen, Anti-inflammatory lathyrane diterpenoids from *Euphorbia lathyris*, *J. Nat. Prod.* 82 (2019) 756–764.
- [307] N.S. Radulović, S.I. Filipović, M.S. Nešić, N.M. Stojanović, K.V. Mitić, M. Z. Mladenović, V.N. Radelović, Immunomodulatory constituents of *Conocephalum conicum* (Snake Liverwort) and the relationship of Isolepidozenes to Germacrane and Humulane, *J. Nat. Prod.* 83 (2020) 3554–3563.
- [308] X. Wang, X. Peng, C. Tang, S. Zhou, C.Q. Ke, Y. Liu, S. Yao, J. Ai, Y. Ye, Anti-inflammatory eudesmane sesquiterpenoids from *Artemisia hedinii*, *J. Nat. Prod.* 84 (2021) 1626–1637.
- [309] C.R. Pye, M.J. Bertin, R.S. Lokey, W.H. Gerwick, R.G. Lington, Retrospective analysis of natural products provides insights for future discovery trends, *Proc. Natl. Acad. Sci. U.S.A.* 114 (2017) 5601–5606.
- [310] N.J. Chear, F. León, A. Sharma, S.R.R. Kanumuri, G. Zwolinski, K.A. Abboud, D. Singh, L.F. Restrepo, A. Patel, T. Hiranita, S. Ramanathan, A.J. Hampson, L. R. McMahon, C.R. McCurdy, Exploring the chemistry of alkaloids from Malaysian *Mitragyna speciosa* (Kratom) and the role of oxindoles on human opioid receptors, *J. Nat. Prod.* 84 (2021) 1034–1043.
- [311] O.S. Olayeriju, A. Papetti, R. Colombo, B. Mannucci, M.T. Olaleye, A. A. Akindahumsi, Phytochemical profiling of aqueous methanolic leaf extract of *Triclisia gillettii* by gas chromatography (GC/MS) and liquid chromatography (HPLC-PDA-ESI/MS(n)) tandem mass spectrometry (MS): a pointer to its nephroprotection, *Nat. Prod. Res.* (2020) 1–6.
- [312] B.P. da Silva, J.P. Parente, An anti-inflammatory and immunomodulatory polysaccharide from *Orbignya phalerata*, *Fitoterapia* 72 (2001) 887–893.
- [313] M.A. Sunil, V.S. Sunitha, A. Ashitha, S. Neethu, S.J. Midhun, E.K. Radhakrishnan, M. Jyothis, Catechin rich butanol fraction extracted from *Acacia catechu* L. (a thirst quencher) exhibits immunostimulatory potential, *J. Food Drug Anal.* 27 (2019) 195–207.
- [314] K. Ali, A. Ali, M.N. Khan, S. Rahman, S. Faizi, M.S. Ali, S. Khalifa, H.R. El-Seedi, S. G. Musharraf, Rapid identification of common secondary metabolites of medicinal herbs using high-performance liquid chromatography with evaporative light scattering detector in extracts, *Metabolites* 11 (2021) 489, <https://doi.org/10.3390/metabo11080489>.
- [315] C.I. Du Preez, C. Gründemann, J.K. Reinhardt, D.R. Mumbengegwi, R. Huber, Immunomodulatory effects of some Namibian plants traditionally used for treating inflammatory diseases, *J. Ethnopharmacol.* 254 (2020), 112683.
- [316] E.A. Abd El-Ghffar, N.M. Hegazi, H.H. Saad, M.M. Soliman, M.A. El-Raey, S. M. Shehata, A. Barakat, A. Yasri, M. Sobeh, HPLC-ESI-MS/MS analysis of beet (*Beta vulgaris*) leaves and its beneficial properties in type 1 diabetic rats, *Biomed. Pharmacother.* 120 (2019), 109541.
- [317] L. Cieřla, R. Moaddel, Comparison of analytical techniques for the identification of bioactive compounds from natural products, *Nat. Prod. Rep.* 33 (2016) 1131–1145.
- [318] D. Sorensen, A. Raditsis, L.A. Trimble, B.A. Blackwell, M.W. Sumarah, J.D. Miller, Isolation and structure elucidation by LC-MS-SPE/NMR: PR toxin- and cuspidatol-related eremophilane sesquiterpenes from *Penicillium roqueforti*, *J. Nat. Prod.* 70 (2007) 121–123.

- [319] Hiroyukikawamura, M. Pulici, H. Koshino, Y. Esumi, J. Uzawa, H. Kumagai, F. Sugawara, New potent immunosuppressive eremophilane isolated from *Trichothecium roseum*, *Nat. Prod. Lett.* 14 (2000) 299–304.
- [320] R.B. van Breemen, C.R. Huang, D. Nikolic, C.P. Woodbury, Y.Z. Zhao, D. L. Venton, Pulsed ultrafiltration mass spectrometry: a new method for screening combinatorial libraries, *Anal. Chem.* 69 (1997) 2159–2164.
- [321] Y. Jin, X. Cheng, F. Jiang, Z. Guo, J. Xie, L. Fu, Application of the ultrafiltration-based LC-MS approach for screening PTP1B inhibitors from Chinese red yeast rice, *Anal. Methods* 8 (2016) 353–361.
- [322] G.L. Chen, M.X. Fan, J.L. Wu, N. Li, M.Q. Guo, Antioxidant and anti-inflammatory properties of flavonoids from lotus plumule, *Food Chem.* 277 (2019) 706–712.
- [323] H.H. Kim, S.E. Ha, P. Vetrivel, P.B. Bhosale, S.M. Kim, G.S. Kim, Potential antioxidant and anti-inflammatory function of *Gynura procumbens* polyphenols ligand, *Int. J. Mol. Sci.* 22 (2021) 8716, <https://doi.org/10.3390/ijms22168716>.
- [324] X.C. Zhuang, Y.L. Zhang, G.L. Chen, Y. Liu, X.L. Hu, N. Li, J.L. Wu, M.Q. Guo, Identification of anti-inflammatory and anti-proliferative neolignanamides from *Warburgia ugandensis* employing multi-target affinity ultrafiltration and LC-MS, *Pharmaceuticals* 14 (2021) 313, <https://doi.org/10.3390/ph14040313>.
- [325] X.C. Zhuang, G.L. Chen, Y. Liu, Y.L. Zhang, M.Q. Guo, New lignanamide with antioxidant and anti-inflammatory activities screened out and identified from *Warburgia ugandensis* combining affinity ultrafiltration LC-MS with SOD and XOD enzymes, *Antioxidants* 10 (2021) 370, <https://doi.org/10.3390/antiox10030370>.
- [326] L. He, G. Yang, X. Geng, Enzymatic activity and chromatographic characteristics of the cell membrane immobilized on silica surface, *Chin. Sci. Bull.* 44 (1999) 826e31–831.
- [327] X. Hou, M. Sun, T. Bao, X. Xie, F. Wei, S. Wang, Recent advances in screening active components from natural products based on bioaffinity techniques, *Acta Pharm. Sin.* B 10 (2020) 1800–1813.
- [328] W. Ma, C. Wang, R. Liu, N. Wang, Y. Lv, B. Dai, L. He, Advances in cell membrane chromatography, *J. Chromatogr. A* 1639 (2021), 461916.
- [329] Q. Jia, W. Sun, L. Zhang, J. Fu, Y. Lv, Y. Lin, S. Han, Screening the anti-allergic components in *Saposhnikovia Radix* using high-expression Mas-related G protein-coupled receptor X2 cell membrane chromatography online coupled with liquid chromatography and mass spectrometry, *J. Sep. Sci.* 42 (2019) 2351–2359.
- [330] J. Fu, Y. Lv, Q. Jia, Y. Lin, S. Han, Dual-mixed/CMC model for screening target components from traditional Chinese medicines simultaneously acting on EGFR & FGFR4 receptors, *Talanta* 192 (2019) 248–254.
- [331] Y. Cao, S. Wang, Y. Li, X. Chen, L. Chen, D. Wang, Z. Zhu, Y. Yuan, D. Lv, A method for screening active components from Chinese herbs by cell membrane chromatography-offline-high performance liquid chromatography/mass spectrometry and an online statistical tool for data processing, *J. Chromatogr. A* 1540 (2018) 68–76.
- [332] R. Zhuo, H. Liu, N. Liu, Y. Wang, Ligand fishing: a remarkable strategy for discovering bioactive compounds from complex mixture of natural products, *Molecules* 21 (2016) 1516, <https://doi.org/10.3390/molecules21111516>.
- [333] I.A. Trindade Ximenes, P.C.O. de Oliveira, C.A. Wegermann, M.C. de Moraes, Magnetic particles for enzyme immobilization: a versatile support for ligand screening, *J. Pharm. Biomed. Anal.* 204 (2021), 114286.
- [334] Q. Chen, L. Zhu, K.M. Yip, Y. Tang, Y. Liu, T. Jiang, J. Zhang, Z. Zhao, T. Yi, H. Chen, A hybrid platform featuring nanomagnetic ligand fishing for discovering COX-2 selective inhibitors from aerial part of *Saussurea laniceps* Hand.-Mazz, *J. Ethnopharmacol.* 271 (2021), 113849.
- [335] Y. Zhang, S. Shi, X. Chen, M. Peng, Functionalized magnetic nanoparticles coupled with mass spectrometry for screening and identification of cyclooxygenase-1 inhibitors from natural products, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 960 (2014) 126–132.
- [336] D. Li, L. Xu, J. Qi, B. Yu, Screening and analysis of cyclooxygenase-2 inhibitors from the complex matrix: a case study to illustrate the important effect of immobilized enzyme activity in magnetic ligand fishing, *J. Pharm. Biomed. Anal.* 175 (2019), 112795.
- [337] J.L. Wolfender, S. Rudaz, Y.H. Choi, H.K. Kim, Plant metabolomics: from holistic data to relevant biomarkers, *Curr. Med. Chem.* 20 (2013) 1056–1090.
- [338] G.J. Patti, O. Yanes, G. Siuzdak, Innovation: Metabolomics: the apogee of the omics trilogy, *Nat. Rev. Mol. Cell Biol.* 13 (2012) 263–269.
- [339] F.R. Pinu, D.J. Beale, A.M. Paten, K. Kouremenos, S. Swarup, H.J. Schirra, D. Wishart, Systems biology and multi-omics integration: viewpoints from the metabolomics research community, *Metabolites* 9 (2019) 76, <https://doi.org/10.3390/metabo9040076>.
- [340] J.L. Wolfender, J.M. Nuzillard, J.J.J. van der Hooft, J.H. Renault, S. Bertrand, Accelerating metabolite identification in natural product research: toward an ideal combination of liquid chromatography-high-resolution tandem mass spectrometry and nmr profiling, in silico databases, and chemometrics, *Anal. Chem.* 91 (2019) 704–742.
- [341] J. Wolfender, E.F. Queiroz, P. Allard, Massive metabolite profiling of natural extracts for a rational prioritization of bioactive natural products: a paradigm shift in pharmacognosy, *Food Front* 1 (2020) 105–106.
- [342] D.J. Beale, F.R. Pinu, K.A. Kouremenos, M.M. Poojary, V.K. Narayana, B. A. Boughton, K. Kanojia, S. Dayalan, O. Jones, D.A. Dias, Review of recent developments in GC-MS approaches to metabolomics-based research, *Metabolomics* 14 (152) (2018) 018–1449-2.
- [343] P.M. Allard, T. Pèresse, J. Bisson, K. Gindro, L. Marcourt, V.C. Pham, F. Roussi, M. Litaudon, J.L. Wolfender, Integration of molecular networking and in-silico MS/MS fragmentation for natural products dereplication, *Anal. Chem.* 88 (2016) 3317–3323.
- [344] R.A. Quinn, L.F. Nothias, O. Vining, M. Meehan, E. Esquenazi, P.C. Dorrestein, Molecular networking as a drug discovery, drug metabolism, and precision medicine strategy, *Trends Pharmacol. Sci.* 38 (2017) 143–154.
- [345] L.F. Nothias, D. Petras, R. Schmid, K. Dührkop, J. Rainer, A. Sarvepalli, I. Protsyuk, M. Ernst, H. Tsugawa, M. Fleischauer, F. Aicheler, A.A. Aksenov, O. Alka, P.M. Allard, A. Barsch, X. Cachat, A.M. Caraballo-Rodríguez, R.R. Da Silva, T. Dang, N. Garg, J.M. Gauglitz, A. Gurevich, G. Isaac, A.K. Jarmusch, Z. Kamenik, K.B. Kang, N. Kessler, I. Koester, A. Korf, A. Le Gouellec, M. Ludwig, C. Martin, H. L. McCall, J. McSayles, S.W. Meyer, H. Mohimani, M. Morsy, O. Moyné, S. Neumann, H. Neuweger, N.H. Nguyen, M. Nothias-Esposito, J. Paoletti, V.V. Phelan, T. Pluskal, R.A. Quinn, S. Rogers, B. Shrestha, A. Tripathi, J. van der Hooft, F. Vargas, K.C. Weldon, M. Witting, H. Yang, Z. Zhang, F. Zubeil, O. Kohlbacher, S. Böcker, T. Alexandrov, N. Bandeira, M. Wang, P.C. Dorrestein, Feature-based molecular networking in the GNPS analysis environment, *Nat. Methods* 17 (2020) 905–908.
- [346] J.L. Wolfender, M. Litaudon, D. Touboul, E.F. Queiroz, Innovative omics-based approaches for prioritisation and targeted isolation of natural products - new strategies for drug discovery, *Nat. Prod. Rep.* 36 (2019) 855–868.
- [347] F. Olivon, P.M. Allard, A. Koval, D. Righi, G. Genta-Jouve, J. Neyts, C. Apel, C. Pannecoque, L.F. Nothias, X. Cachat, L. Marcourt, F. Roussi, V.L. Katanaev, D. Touboul, J.L. Wolfender, M. Litaudon, Bioactive natural products prioritization using massive multi-informational molecular networks, *ACS Chem. Biol.* 12 (2017) 2644–2651.
- [348] M.A. Hoffmann, L.F. Nothias, M. Ludwig, M. Fleischauer, E.C. Gentry, M. Witting, P.C. Dorrestein, K. Dührkop, S. Böcker, High-confidence structural annotation of metabolites absent from spectral libraries, *Nat. Biotechnol.* (2021).
- [349] M.A. Schorn, S. Verhoeven, L. Ridder, F. Huber, D.D. Acharya, A.A. Aksenov, G. Alefi, J.A. Moghaddam, A.T. Aron, S. Aziz, A. Bauermeister, K.D. Bauman, M. Baunach, C. Beemelmans, J.M. Beman, M.V. Berlanga-Clavero, A.A. Blacutt, H.B. Bode, A. Boullie, A. Brejnrod, T.S. Bugni, A. Calteau, L. Cao, V.J. Carrión, R. Castelo-Branco, S. Chanana, A.B. Chasne, M.G. Chevrete, L.V. Costa-Lotufo, J. M. Crawford, C.R. Currie, B. Cuypers, T. Dang, T. de Rond, A.M. Demko, E. Dittmann, C. Du, C. Drozd, J.C. Dujardin, R.J. Dutton, A. Edlund, D.P. Fewer, N. Garg, J.M. Gauglitz, E.C. Gentry, L. Gerwick, E. Glukhov, H. Gross, M. Gugger, D.G. Guillén Matus, E. Helfrich, B.F. Hempel, J.S. Hur, M. Iorio, P.R. Jensen, K. B. Kang, L. Kayser, N.L. Kelleher, C.S. Kim, K.H. Kim, I. Koester, G.M. König, T. Leao, S.R. Lee, Y.Y. Lee, X. Li, J.C. Little, K.N. Maloney, D. Männle, C. Martín H, A.C. McAvoy, W.W. Metcalf, H. Mohimani, C. Molina-Santiago, B.S. Moore, M. W. Mallowney, M. Muskat, L.F. Nothias, E.C. O'Neill, E.I. Parkinson, D. Petras, J. Piel, E.C. Pierce, K. Pires, R. Reher, D. Romero, M.C. Roper, M. Rust, H. Saad, C. Saenz, L.M. Sanchez, S.J. Sørensen, M. Sosio, R.D. Süsmuth, D. Sweeney, K. Tahlan, R.J. Thomson, N.J. Tobias, A.E. Trindade-Silva, G.P. van Wezel, M. Wang, K.C. Weldon, F. Zhang, N. Ziemert, K.R. Duncan, M. Crüsemann, S. Rogers, P.C. Dorrestein, M.H. Medema, J. van der Hooft, A community resource for paired genomic and metabolomic data mining, *Nat. Chem. Biol.* 17 (2021) 363–368.
- [350] M. Riaz, N.U. Rahman, M. Zia-Ul-Haq, H.Z.E. Jaffar, R. Manea, Ginseng: a dietary supplement as immune-modulator in various diseases, *Trends Food Sci. Technol.* 83 (2019) 12–30.
- [351] J.W. Lee, S.H. Ji, B.R. Choi, D.J. Choi, Y.G. Lee, H.G. Kim, G.S. Kim, K. Kim, Y. H. Lee, N.I. Baek, D.Y. Lee, UPLC-QTOF/MS-based metabolomics applied for the quality evaluation of four processed *Panax ginseng* products, *Molecules* 23 (2018) 2062, <https://doi.org/10.3390/molecules23082062>.
- [352] Y. Liu, H. Zhang, X. Dai, R. Zhu, B. Chen, B. Xia, Z. Ye, D. Zhao, S. Gao, A. N. Orekhov, D. Zhang, L. Wang, S. Guo, A comprehensive review on the phytochemistry, pharmacokinetics, and antidiabetic effect of Ginseng, *Phytomedicine* 92 (2021), 153717.
- [353] Y. Yang, Z. Ju, Y. Yang, Y. Zhang, L. Yang, Z. Wang, Phytochemical analysis of *Panax species*: a review, *J. Ginseng Res.* 45 (2021) 1–21.
- [354] W.S. Zhang, A. Pan, L. Yang, Y.Y. Cai, B.L. Liu, P. Li, L.W. Qi, J. Li, Q. Liu, American Ginseng and Asian Ginseng intervention in diet-induced obese mice: metabolomics reveals distinct metabolic profiles, *Am. J. Chin. Med.* 47 (2019) 787–801.
- [355] L. Feng, X.M. Liu, F.R. Cao, L.S. Wang, Y.X. Chen, R.L. Pan, Y.H. Liao, Q. Wang, Q. Chang, Anti-stress effects of ginseng total saponins on hindlimb-unloaded rats assessed by a metabolomics study, *J. Ethnopharmacol.* 188 (2016) 39–47.
- [356] L. Jin, M. Schmich, M. El Gaafary, X. Zhang, T. Syrovets, T. Simmet, A comparative study on root and bark extracts of *Eleutherococcus senticosus* and their effects on human macrophages, *Phytomedicine* 68 (2020), 153181.
- [357] S. Wang, X. Yang, Eleutheroside E decreases oxidative stress and NF- κ B activation and reprograms the metabolic response against hypoxia-reoxygenation injury in H9c2 cells, *Int. Immunopharmacol.* 84 (2020), 106513.
- [358] P.M. Allard, J. Bisson, A. Azzollini, G.F. Pauli, G.A. Cordell, J.L. Wolfender, Pharmacognosy in the digital era: shifting to contextualized metabolomics, *Curr. Opin. Biotechnol.* 54 (2018) 57–64.
- [359] Q. Chen, S. Xiao, Z. Li, N. Ai, X. Fan, Chemical and metabolic profiling of Si-Ni decoction analogous formulae by high performance liquid chromatography-mass spectrometry, *Sci. Rep.* 5 (2015) 11638.
- [360] J. Houriet, Y.E. Arnold, L. Pellissier, Y.N. Kalia, J.L. Wolfender, Using porcine jejunum ex vivo to study absorption and biotransformation of natural products in plant extracts: *pueraria lobata* as a case study, *Metabolites* 11 (2021) 541, <https://doi.org/10.3390/metabo11080541>.
- [361] X. An, Q. Bao, S. Di, Y. Zhao, S. Zhao, H. Zhang, F. Lian, X. Tong, The interaction between the gut Microbiota and herbal medicines, *Biomed. Pharmacother.* 118 (2019), 109252.

- [362] E.M. Pferschy-Wenzig, K. Koskinen, C. Moissl-Eichinger, R. Bauer, A combined LC-MS metabolomics- and 16S rRNA sequencing platform to assess interactions between herbal medicinal products and human gut bacteria in vitro: a pilot study on willow bark extract, *Front. Pharmacol.* 8 (2017) 893.
- [363] D.E. Moerman, Symbols and selectivity: a statistical analysis of native American medical ethnobotany, *J. Ethnopharmacol.* 1 (1979) 111–119.
- [364] D.E. Moerman, The medicinal flora of Native North America: an analysis, *J. Ethnopharmacol.* 31 (1991) 1–42.
- [365] R. Allkin, A. Booker, C. Canteiro, E. Dauncey, S. Edwards, F. Forest, et al., Useful plants – medicines: current resource and future potential, in: K. Willis (Ed.), *State of the World's Plants*, UK: Royal Botanic Gardens, Kew, 2017, pp. 22–29.
- [366] M. Leonti, O. Sticher, M. Heinrich, Antiquity of medicinal plant usage in two Macro-Mayan ethnic groups (Mexico), *J. Ethnopharmacol.* 88 (2003) 119–124.
- [367] W. Milliken, B.E. Walker, M.R. Howes, F. Forest, E. Nic Lughadha, Plants used traditionally as antimalarials in Latin America: mining the tree of life for potential new medicines, *J. Ethnopharmacol.* 279 (2021), 114221.
- [368] F. Zhu, C. Qin, L. Tao, X. Liu, Z. Shi, X. Ma, J. Jia, Y. Tan, C. Cui, J. Lin, C. Tan, Y. Jiang, Y. Chen, Clustered patterns of species origins of nature-derived drugs and clues for future bioprospecting, *Proc. Natl. Acad. Sci. U.S.A.* 108 (2011) 12943–12948.
- [369] D. Lei, T. Al Jabri, I. Teixidor-Toneu, C. Sasilis-Lagoudakis, S. Ghazanfar, J. Hawkins, Comparative analysis of four medicinal floras: phylogenetic methods to identify cross-cultural patterns, *Plants People Planet* 2 (2020) 614–626.
- [370] C.M. Tucker, M.W. Cadotte, S.B. Carvalho, T.J. Davies, S. Ferrier, S.A. Fritz, R. Grenyer, M.R. Helmus, L.S. Jin, A.O. Mooers, S. Pavoine, O. Purschke, D. W. Redding, D.F. Rosauer, M. Winter, F. Mazel, A guide to phylogenetic metrics for conservation, community ecology and macroecology, *Biol. Rev. Camb. Philos. Soc.* 92 (2017) 698–715.
- [371] T. Münkemüller, S. Lavergne, B. Bzeznik, S. Dray, T. Jombart, K. Schiffers, W. Thuiller, How to measure and test phylogenetic signal, *Methods Ecol. Evol.* 3 (2012) 743–756.
- [372] M. Cadotte, T. Davies, *Phylogenies in Ecology*, Princeton University Press, USA, 2016.
- [373] Y. Zhang, T. Deng, L. Sun, J.B. Landis, M.J. Moore, H. Wang, Y. Wang, X. Hao, J. Chen, S. Li, M. Xu, P.T. Puno, P.H. Raven, H. Sun, Phylogenetic patterns suggest frequent multiple origins of secondary metabolites across the seed-plant 'tree of life', *Natl. Sci. Rev.* 8 (2020) 8.
- [374] L. Masuelli, M. Benvenuto, C. Focaccetti, S. Ciuffa, S. Fazi, A. Bei, M.T. Miele, L. Piredda, V. Manzari, A. Modesti, R. Bei, Targeting the tumor immune microenvironment with "nutraceuticals": from bench to clinical trials, *Pharmacol. Ther.* 219 (2021), 107700.
- [375] J.E. Talmadge, Natural product derived immune-regulatory agents, *Int. Immunopharmacol.* 37 (2016) 5–15.
- [376] J. Martel, Y.F. Ko, D.M. Ojcius, C.C. Lu, C.J. Chang, C.S. Lin, H.C. Lai, J.D. Young, Immunomodulatory properties of plants and mushrooms, *Trends Pharmacol. Sci.* 38 (2017) 967–981.
- [377] J.M. Augustin, V. Kuzina, S.B. Andersen, S. Bak, Molecular activities, biosynthesis and evolution of triterpenoid saponins, *Phytochemistry* 72 (2011) 435–457.
- [378] J.P. Vincken, L. Heng, A. de Groot, H. Gruppen, Saponins, classification and occurrence in the plant kingdom, *Phytochemistry* 68 (2007) 275–297.
- [379] M. Cheek, E. Nic Lughadha, P. Kirk, H. Lindon, J. Carretero, B. Looney, B. Douglas, D. Haelewaters, E. Gaya, T. Llewellyn, A.M. Ainsworth, Y. Gafforov, K. Hyde, P. Crous, M. Hughes, B.E. Walker, R. Campostrini Forzza, K.M. Wong, T. Niskanen, New scientific discoveries: plants and fungi, *Plants People Planet* 2 (2020) 371–388.
- [380] A. Bender, R.C. Glen, Molecular similarity: a key technique in molecular informatics, *Org. Biomol. Chem.* 2 (2004) 3204–3218.
- [381] G.C. Terstappen, C. Schlüpen, R. Raggiacchi, G. Gaviraghi, Target deconvolution strategies in drug discovery, *Nat. Rev. Drug Discov.* 6 (2007) 891–903.
- [382] J. Lai, J. Hu, Y. Wang, X. Zhou, Y. Li, L. Zhang, Z. Liu, Privileged scaffold analysis of natural products with deep learning-based indication prediction model, *Mol. Inform.* 39 (2020), e2000057.
- [383] B. Qiang, J. Lai, H. Jin, L. Zhang, Z. Liu, Target prediction model for natural products using transfer learning, *Int. J. Mol. Sci.* 22 (2021) 22.
- [384] Z. Qu, J. Cui, Y. Harata-Lee, T.N. Aung, Q. Feng, J.M. Raison, R.D. Kortschak, D. L. Adelson, Identification of candidate anti-cancer molecular mechanisms of compound Kushen Injection using functional genomics, *Oncotarget* 7 (2016) 66003–66019.
- [385] J. Cui, Z. Qu, Y. Harata-Lee, H. Shen, T.N. Aung, W. Wang, R.D. Kortschak, D. L. Adelson, The effect of compound kushen injection on cancer cells: integrated identification of candidate molecular mechanisms, *PLoS One* 15 (2020), e0236395.
- [386] J. Cui, Z. Qu, Y. Harata-Lee, T. Nwe Aung, H. Shen, W. Wang, D.L. Adelson, Cell cycle, energy metabolism and DNA repair pathways in cancer cells are suppressed by compound Kushen Injection, *BMC Cancer* 19 (103) (2019) 018–5230–5238.
- [387] M.T. Robey, L.K. Caesar, M.T. Drott, N.P. Keller, N.L. Kelleher, An interpreted atlas of biosynthetic gene clusters from 1,000 fungal genomes, *Proc. Natl. Acad. Sci. U.S.A.* 118 (2021), e2020230118, <https://doi.org/10.1073/pnas.2020230118>.
- [388] Y. Liu, B. Wang, S. Shu, Z. Li, C. Song, D. Liu, Y. Niu, J. Liu, J. Zhang, H. Liu, Z. Hu, B. Huang, X. Liu, W. Liu, L. Jiang, M.M. Alami, Y. Zhou, Y. Ma, X. He, Y. Yang, T. Zhang, H. Hu, M.S. Barker, S. Chen, X. Wang, J. Nie, Analysis of the *Coptis chinensis* genome reveals the diversification of protoberberine-type alkaloids, *Nat. Commun.* 12 (3276) (2021) 021–23611–0.
- [389] L. Ma, C. Dong, C. Song, X. Wang, X. Zheng, Y. Niu, S. Chen, W. Feng, De novo genome assembly of the potent medicinal plant *Rehmannia glutinosa* using nanopore technology, *Comput. Struct. Biotechnol. J.* 19 (2021) 3954–3963.
- [390] T. Mori, J.K.B. Cahn, M.C. Wilson, R.A. Meoded, V. Wiebach, A.F.C. Martinez, E. Helfrich, A. Albersmeier, D. Wibberg, S. Dätwyler, R. Keren, A. Lavy, C. Rückert, M. Ilan, J. Kalinowski, S. Matsunaga, H. Takeyama, J. Piel, Single-bacterial genomics validates rich and varied specialized metabolism of uncultivated *Entotheonella* sponge symbionts, *Proc. Natl. Acad. Sci. U.S.A.* 115 (2018) 1718–1723.
- [391] E. Azizi, A.J. Carr, G. Plitas, A.E. Cornish, C. Konopacki, S. Prabhakaran, J. Nainys, K. Wu, V. Kiseliovas, M. Setty, K. Choi, R.M. Fromme, P. Dao, P. T. McKenney, R.C. Wasti, K. Kadaveru, L. Mazutis, A.Y. Rudensky, D. Pe'er, Single-cell map of diverse immune phenotypes in the breast tumor microenvironment, *Cell* 174 (1293) (2018), 1308–1308.e36.
- [392] P. Savas, B. Virassamy, C. Ye, A. Salim, C.P. Mintoff, F. Caramia, R. Salgado, D. J. Byrne, Z.L. Teo, S. Dushyanthen, A. Byrne, L. Wein, S.J. Luen, C. Poliness, S. S. Nightingale, A.S. Skandarajah, D.E. Gyorki, C.M. Thornton, P.A. Beavis, S. B. Fox, P.K. Darcy, T.P. Speed, L.K. Mackay, P.J. Neeson, S. Loi, Kathleen Cuninghame Foundation Consortium for Research into Familial Breast Cancer, Single-cell profiling of breast cancer T cells reveals a tissue-resident memory subset associated with improved prognosis, *Nat. Med.* 24 (2018) 986–993.
- [393] United Nations, Convention on Biological Diversity. (<https://www.cbd.int/doc/legal/cbd-en.pdf>), 1992 (Accessed July 2021).
- [394] United Nations, Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity. (<https://www.cbd.int/abs/doc/protocol/nagoya-protocol-en.pdf>), 2010 (Accessed July 2021).
- [395] United Nations, Food and Agriculture Organization, International Treaty on Plant Genetic Resources for Food and Agriculture. (<https://www.fao.org/plant-treaty/en/>) 2001 (Accessed July 2021).
- [396] United Nations, A. Access and Benefit Sharing Clearing House. (<https://absch.cbd.int/>), (Accessed July 2021).
- [397] European Union, Regulation (EU) No 511/2014 of the European Parliament and of the Council of 16 April 2014 on compliance measures for users from the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization in the Union. (<https://op.europa.eu/en/publication-detail/-/publication/6b16d48a-dff0-11e3-8cd4-01aa75ed71a1/language-en>), 2014 (Accessed July 2021).
- [398] European Commission. Commission implementing Regulation (EU) 2015/1866 of 13 October 2015 laying down detailed rules for the implementation of Regulation (EU) No 511/2014 of the European Parliament and of the Council as regards the register of collections, monitoring user compliance and best practices. (<http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32015R1866&from=EN>), 2015 (Accessed July 2021).
- [399] European Commission. Guidance document on the scope of application and core obligations of Regulation (EU) No 511/2014 of the European Parliament and of the Council on the compliance measures for users from the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization in the Union. (<https://ec.europa.eu/transparency/regdoc/rep/3/2020/EN/C-2020-8759-F1-EN-MAIN-PART-1.PDF>), 2020, (Accessed July 2021).
- [400] F. Michiels, U. Feiter, S. Paquin-Jaloux, D. Jungmann, A. Braun, M.A.P. Sayoc, et al., Facing the harsh reality of access and benefit sharing (ABS) legislation. An industry perspective, *Sustainability* 14 (2022) 277.
- [401] International Chamber of Commerce. Towards a new implementation strategy for access and benefit sharing. (<https://icwbo.org/publication/towards-a-new-implementation-strategy-for-access-and-benefit-sharing/>), 2020, (Accessed July 2021).
- [402] X. Zheng, Key legal challenges and opportunities in the implementation of the Nagoya Protocol: the case of China, *RECIEL* 28 (2019) 175–184.
- [403] S. Laird, R. Wynberg, M. Rourke, F. Humphries, M.R. Muller, C. Lawson, Rethink the expansion of access and benefit sharing, *Science* 367 (2020) 1200–1202.
- [404] M. Sangeetha, G. Gangappa, M. Bhatta, Access and benefit sharing (ABS) of bio-resources extent of awareness among the stakeholders in India, *Asian J. Microbiol. Biotechnol. Environ. Sci.* 23 (2021) 96–98.
- [405] F. Rohden, A.H. Scholz, The international political process around Digital Sequence Information under the Convention on Biological Diversity and the 2018–2020 interessional period, *Plants People Planet* (2021) (n/a).
- [406] Heinrich K. Biological Prospecting in Antarctica – A Solution-Based Approach to Regulating the Collection and Use of Antarctic Marine Biodiversity by Taking the BBNJ Process into Account. The Yearbook of Polar Law Online 2020, 12: 41–60.
- [407] B.M. Ncube, A. Dube, K. Ward, Establishment of the African Medicines Agency: progress, challenges and regulatory readiness, *J. Pharm. Policy Pract.* 14 (29) (2021) 020–00281–9.
- [408] I. Chiochio, M. Mandrone, P. Tomasi, L. Marinich, F. Poli, Plant secondary metabolites: an opportunity for circular economy, *Molecules* 26 (2021) 495, <https://doi.org/10.3390/molecules26020495>.
- [409] Access to Medicines Foundation. (<http://www.accessmedicineindex.org/>), (Accessed November 2021).
- [410] International Union of Immunological Sciences. (<https://www.immunopaedia.org.za/>) (Accessed November 2021).
- [411] J.F. Armstrong, E. Faccenda, S.D. Harding, A.J. Pawson, C. Southan, J. L. Sharman, B. Campo, D.R. Cavanagh, S. Alexander, A.P. Davenport, M. Spedding, J.A. Davies, NC-IUPHAR, The IUPHAR/BPS guide to pharmacology in 2020: extending immunopharmacology content and introducing the IUPHAR/

- MMV Guide to malaria pharmacology, *Nucleic Acids Res.* 48 (2020) D1006–D1021.
- [412] S. Milling, M. Spedding, P. Maffia, Guide to Immunopharmacology: a database to boost immunology education, research and therapy, *Immunology* 160 (2020) 1–2.
- [413] E. Faccenda, S. Maxwell, J.L. Szarek, The IUPHAR pharmacology education project, *Clin. Pharmacol. Ther.* 105 (2019) 45–48.
- [414] CIOMS Working Group. Drug-Induced Liver Injury (DILI): Current Status and Future Directions for Drug Development and the Post-Market Setting: Council for International Organizations of Medical Sciences; 2020. ISBN: 978–929036099-5.
- [415] T. Powles, J.E. Rosenberg, G.P. Sonpavde, Y. Loriot, I. Durán, J.L. Lee, N. Matsubara, C. Vulsteke, D. Castellano, C. Wu, M. Campbell, M. Matsangou, D. P. Petrylak, Enfortumab vedotin in previously treated advanced urothelial carcinoma, *New Engl. J. Med.* 384 (2021) 1125–1135.
- [416] L.H. Sehn, A.F. Herrera, C.R. Flowers, M.K. Kamdar, A. Mcmillan, M. Hertzberg, S. Assouline, T.M. Kim, W.S. Kim, M. Ozcan, J. Hirata, E. Penuel, J.N. Paulson, J. Cheng, G. Ku, M.J. Matasar, Polatuzumab vedotin in relapsed or refractory diffuse large b-cell lymphoma, *J. Clin. Oncol.* 38 (2020) 155–165.
- [417] G. Lassiter, C. Bergeron, R. Guedry, J. Cucarola, A.M. Kaye, E.M. Cornett, A. D. Kaye, G. Varrassi, O. Viswanath, I. Urits, Belantamab mafodotin to treat multiple myeloma: a comprehensive review of disease, drug efficacy and side effects, *Curr. Oncol.* 28 (2021) 640–660.
- [418] C. Kollmannsberger, T.K. Choueiri, D.Y.C. Heng, S. George, F. Jie, R. Croitoru, S. Poondru, J.A. Thompson, A randomized phase ii study of ags-16c3f versus axitinib in previously treated patients with metastatic renal cell carcinoma, *Oncologist* 26 (2021) 182.
- [419] B. Akla, M. Broussas, N. Loukili, A. Robert, C. Beau-Larvor, M. Malissard, N. Boute, T. Champion, J.F. Haeuw, A. Beck, M. Perez, C. Dreyfus, M. Pavlyuk, E. Chetaille, N. Corvaia, Efficacy of the antibody-drug conjugate w0101 in preclinical models of igf-1 receptor overexpressing solid tumors, *Mol. Cancer Ther.* 19 (2020) 168–177.
- [420] R.L. Coleman, D. Lorusso, C. Gennigens, A. González-Martín, L. Randall, D. Cibula, B. Lund, L. Woelber, S. Pignata, F. Forget, A. Redondo, S.D. Vindeløv, M. Chen, J.R. Harris, M. Smith, L.V. Nicacio, M. Teng, A. Laenen, R. Rangwala, L. Manso, M. Mirza, B.J. Monk, I. Vergote, C. innovaTV /GOG-/ENGOT-, Efficacy and safety of tisotumab vedotin in previously treated recurrent or metastatic cervical cancer (innovaTV 204/GOG-3023/ENGOT-cx6): a multicentre, open-label, single-arm, phase 2 study, *Lancet Oncol.* 22 (2021) 609–619.
- [421] M. Johnson, J. Rodon Ahnert, N. Lakhani, R.E. Sanborn, A. El-Khoueiry, N. Hafez, H. Mamdani, V. Boni, H. Castro, A.L. Hannah, A. Spira, P01.06 CX-2029, a proboddy drug conjugate targeting CD71 in patients with selected tumor types: PROCLAIM-CX-2029 dose expansion phase, *J. Thorac. Oncol.* 16 (S238) (2021) S238.
- [422] A. Patnaik, E. Calvo, S.A.A. Piha-Paul, A. Hollebecque, V. Galvao, J.S. Lopez, F. S. Braiteh, R.E. Sanborn, P. Zhou, N.N. Nazarenko, A. Dowlati, A phase 1 study of SGN-B6A, an antibody-drug conjugate targeting integrin beta-6, in patients with advanced solid tumors (SGNB6A-001, Trial in Progress), *JCO* 39 (2021) TPS3144.
- [423] A. Patnaik, F. Meric-Bernstam, F. Robert, A. Dowlati, H.L. Kindler, C.M.S.P. R. Lima, et al., SGN228-001: a phase I open-label dose-escalation, and expansion study of SGN-CD228A in select advanced solid tumors, *JCO* 38 (2020).
- [424] A.F. Sher, J.Y. Bruce, S.Y. Oh, I.C. Anderson, D.Y. Oh, L.M. Nott, J.S. Lee, C.C. Lin, R. Mehra, B.Y. Shim, W.C. Su, T.H. Guthrie, N.Y. Gabrail, R.E. Sanborn, A.S. Lee, D.L. Cortinovis, P. Swiecicki, Y. Wang, Z. Wang, J.Y. Cho, Open-label, phase II study of ladiratuzumab vedotin (LV) for advanced gastric and gastroesophageal junction adenocarcinoma (SGNLVA-005, Trial-in-Progress), *JCO* 39 (2021). TPS256-TPS256.
- [425] D.W. Sherbenou, B.T. Aftab, Y. Su, C.R. Behrens, A. Wiita, A.C. Logan, D. Acosta-Alvear, B.C. Hann, P. Walter, M.A. Shuman, X. Wu, J.P. Atkinson, J.L. Wolf, T. G. Martin, B. Liu, Antibody-drug conjugate targeting CD46 eliminates multiple myeloma cells, *J. Clin. Investig.* 126 (2016) 4640–4653.
- [426] S.N. Waqar, M.W. Redman, S.M. Arnold, F.R. Hirsch, P.C. Mack, L.H. Schwartz, D. R. Gandara, T.E. Stinchcombe, N.B. Leighl, S.S. Ramalingam, S.H. Tanna, R. S. Raddin, K. Minichiello, J.D. Bradley, K. Kelly, R.S. Herbst, V. A. Papadimitrakopoulou, A phase II study of telisotuzumab vedotin in patients with c-met-positive stage iv or recurrent squamous cell lung cancer (LUNG-MAP Sub-study S1400K, NCT03574753), *Clin. Lung Cancer* 22 (2021) 170–177.
- [427] R. Xu, M. Qiu, Y. Zhang, X. Wei, C. Hu, First-in-human dose-escalation study of anti-EGFR ADC MRG003 in patients with relapsed/refractory solid tumors, *JCO* 38 (2020) 3550–3550.
- [428] A.V. Yurkovetskiy, N.D. Bodyak, M. Yin, J.D. Thomas, S.M. Clardy, P.R. Conlon, C.A. Stevenson, A. Uttard, L. Qin, D.R. Gumerov, E. Ter-Ovanesyan, C. Bu, A. J. Johnson, V.R. Gurijala, D. McGillicuddy, M.J. DeVit, L.L. Poling, M. Protopopova, L. Xu, Q. Zhang, P.U. Park, D.A. Bergstrom, T.B. Lowinger, Dolaflexin: a novel antibody-drug conjugate platform featuring high drug loading and a controlled bystander effect, *Mol. Cancer Ther.* 20 (2021) 885–895.
- [429] M.J. Towle, K.A. Salvato, J. Budrow, B.F. Wels, G. Kuznetsov, K.K. Aalfs, S. Welsh, W. Zheng, B.M. Seletsky, M.H. Palme, G.J. Habgood, L.A. Singer, L.V. Dipietro, Y. Wang, J.J. Chen, D.A. Quincy, A. Davis, K. Yoshimatsu, Y. Kishi, M.J. Yu, B. A. Littlefield, In vitro and in vivo anticancer activities of synthetic macrocyclic ketone analogues of halichondrin B, *Cancer Res.* 61 (2001) 1013–1021.

Update

Pharmacological Research

Volume 180, Issue , June 2022, Page

DOI: <https://doi.org/10.1016/j.phrs.2022.106207>



Corrigendum

Corrigendum to “Future directions for the discovery of natural product-derived immunomodulating drugs: An IUPHAR positional review” [Pharmacol. Res. 177 (2022) 106076]



Cherry L. Wainwright^{a,*}, Mauro M. Teixeira^b, David L. Adelson^c, Eric J. Buenz^d, Bruno David^e, Keith B. Glaser^f, Yuka Harata-Lee^c, Melanie-Jayne R. Howes^{g,h}, Angelo A. Izzoⁱ, Pasquale Maffia^{i,j}, Alejandro M.S. Mayer^k, Claire Mazars^e, David J. Newman^l, Eimear Nic Lughadha^g, Adriano M.C. Pimenta^m, John A.A. Parra^m, Zhipeng Qu^c, Hanyuan Shen^c, Michael Speddingⁿ, Jean-Luc Wolfender^{o,p}

^a Centre for Natural Products in Health, Robert Gordon University, Aberdeen, UK

^b Department of Biochemistry and Immunology, Universidade Federal de Minas Gerais, Brazil

^c Molecular & Biomedical Science, University of Adelaide, Australia

^d Nelson Marlborough Institute of Technology, New Zealand

^e Green Mission Pierre Fabre, Pierre Fabre Laboratories, Toulouse, France

^f AbbVie Inc., Integrated Discovery Operations, North Chicago, USA

^g Royal Botanic Gardens Kew, Richmond, Surrey, UK

^h Institute of Pharmaceutical Science, Faculty of Life Sciences & Medicine, King's College London, UK

ⁱ Department of Pharmacy, School of Medicine, University of Naples Federico II, Italy

^j Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, UK

^k Department of Pharmacology, College of Graduate Studies, Midwestern University, IL, USA

^l NIH Special Volunteer, Wayne, PA, USA

^m Laboratory of Animal Venoms and Toxins, Department of Biochemistry and Immunology, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

ⁿ Spedding Research Solutions, Paris, France

^o School of Pharmaceutical Sciences, University of Geneva, Switzerland

^p Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva, Switzerland

1. The authors regret that due to a mistake during the manuscript editing, the name of three co-authors are missing.

The missing names are:

Fernão C. Braga, Priscilla R.V. Campana and Rodrigo M. Pádua

Affiliation: Department of Pharmaceutical Products, Faculty of Pharmacy, Universidade Federal de Minas Gerais, Brazil.

Email addressees: fernaobraga@farmacia.ufmg.br; prvcampana@gmail.com; mpadua@hotmail.com.

2. The chemical structure is missing in Fig. 9. The following figure is the correct Fig. 9:

The authors apologize to the journal and the readers for any inconvenience this mistake may have caused.

DOI of original article: <https://doi.org/10.1016/j.phrs.2022.106076>.

* Corresponding author.

E-mail address: c.wainwright@rgu.ac.uk (C.L. Wainwright).

<https://doi.org/10.1016/j.phrs.2022.106207>

Available online 12 April 2022

1043-6618/© 2022 Published by Elsevier Ltd.

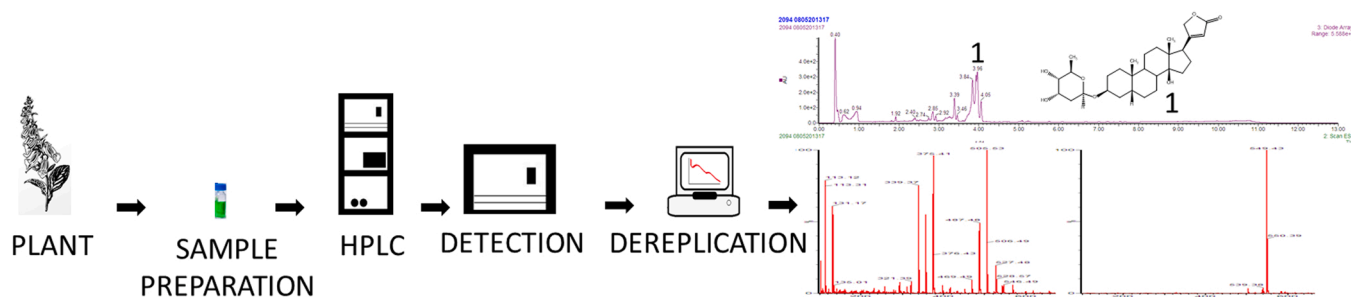


Fig. 9. Workflow of a dereplication procedure based on UPLC-MS.