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# Drug discovery and bio-exploration of nature: toxins, friend or foe?

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## Introduction

Nature has devoted plants, animals and microorganisms with a rich source of biologically active compounds, often for reasons of protection, but also for offensive purposes. Without any doubt, natural compounds always have played a crucial role in the development of interesting pharmaceuticals, and it is expected they still will do so. However, in the era of automated high-throughput screening techniques, the use of large libraries with purely synthetic molecules seems to become a first choice approach, rather than continuing to bio-explore nature's treasures. One of the explanations is the complexity of natural samples, necessitating lots of effort to identify and isolate the hit compound in question, and hence requiring a considerable investment in time and financial cost (Maitra et al., 2022). Indeed, since decades the complexity of natural products has resulted in a wealth of studies that focus only on assessing the activity of the entire extract (e.g. from a plant or a venomous animal), without further identifying the individual compound(s) responsible for the observed biological effects. The pharmaceutical industry has delivered more than 1200 drugs in the past 60 years that have played a key role in increasing life expectancy by an average of two months per year. However, the industry has at the same time reached a state of standstill with the output of new molecular entities (NMEs), remaining approximately constant while the cost of producing NMEs is increasing exponentially (King, 2011).

## Toxins and toxinology

In general, toxins produced by plants, animal and microorganisms, have deleterious effects on a living organism and severe health concerns after being exposed to toxins, cannot be ignored. In this way, toxins are surely a foe. Also taking into account emerging toxins that are becoming increasingly prevalent in contexts of climate change and globalization of food supply chains. On the other hand, the diversity of toxins also has led to a frequent use as experimental tools for studying pharmacological mechanisms, the physiological role of the targets these toxins bind to, as well as the determination of the structure of those targets. Toxinology involves the identification, characterization, production and engineering of toxins, including their application and use as research tools and clinical products with therapeutic potential (Clark et al., 2019). Interest in toxins as tools for scientists goes back a long time, with famous examples like Claude Bernard's experiments in the 1800s with curare to demonstrate the existence of chemical communication between nerves and muscles, and Henry Dale's use of muscarine and nicotine to elucidate different subtypes of receptors for acetylcholine.

Despite the 'competition' with the screening of libraries of synthetic molecules, an increasing number of studies dealing with toxins illustrates today that we have arrived in an era where toxins have made the transition from the laboratory to the clinic. Hence the discipline of toxinology seems to shift from the classical development of anti-venoms towards real drug discovery, opening the door to consider toxins as a friend, not a foe. One justification for this phenomenon can be found in the often unique selectivity and remarkable high potency of toxins in general, which probably is a consequence of evolution during countless numbers of years. Toxic plant alkaloids are examples of the first source of toxin-derived therapeutics with a proof of concept: e.g. digoxin in atrial fibrillation and heart failure, and tubocurarine as a selective

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muscle relaxant for the use as an adjunct to classical anaesthetics in surgery. Snake venoms represent another example of a unique source that provided leads with successful therapeutic application: e.g. captopril, being the first orally active inhibitor of the angiotensin-converting enzyme (ACE), was derived from studies on small peptides known as bradykinin-potentiating peptides, isolated from the venom of the dangerous Brazilian snake *Bothrops jararaca*. It cannot be denied that captopril has led to major advances in the treatment of patients with high blood pressure and heart failure (Fischer and Riedl, 2022). Perhaps the most surprising toxin, both scientifically and economically, that has found therapeutic and cosmetic uses is botulinum toxin, produced by the bacterium *Clostridium botulinum* and related species. It is assumed to be the most potent molecule on planet earth, with applications in various movement disorders and migraine, as well as its familiar cosmetic use.

## Technological developments

Technological developments in several fields of 'omics', have helped to increase our knowledge of toxins. Today we believe to understand how organisms like cone snails, sea anemones, spiders, scorpions and snakes, produce toxins, and how these bio-active substances have evolved. Transcriptomics and proteomics have thus far indeed enabled the identification of many (peptide) toxins, followed by the discovery of their often unique biological properties (Wangchuk, 2018).

Notwithstanding this progress, the limited availability of *in silico* systems that either identify the potential of the toxin for off-target effects, or that predict the presence of a toxin with a completely new mechanism of action, still restrict somehow the efficient valorization of these molecules. With the advent of high resolution analytical techniques, which can couple high-throughput functional screenings to compound-target identification, complex mixtures can be dealt with. The latest NMR and MS instruments provide the potential for a sensitive and accurate detection and identification of toxins, for instance in the foodstuff. The last decade in particular, cryo-EM has significantly advanced our knowledge in the field of structural biology, allowing us now to understand better how toxins interact at the molecular level. Techniques such as voltage-clamp electrophysiology with patch electrodes and high-speed atomic force spectroscopy (HS-AFM) allow the interaction of toxins with respectively targets such as ion channels-receptors, and phospholipids. These approaches allow observations in real time, enabling us to get a better insight into the dynamic nature of interactions of molecules. Thanks to several of these approaches, a significant gain in the reduction of the necessary amount of starting material required for diverse types of analyses, was achieved. The widespread use of toxins as experimental tools has been further facilitated by developments in synthetic chemistry and recombinant expression systems (e.g., bacteria, yeast and insect cells), enabling the production of lab scale quantities of toxins easier. Solid phase synthesis of peptide toxins using orthogonal protection strategies now provides a robust and efficient method to produce toxins with the specific disulfide folding networks that are essential for bioactivity. Nevertheless, solid phase synthesis remains relative expensive, is subject to trial and error and does not always accommodate for post-translational modifications (PTMs). On the other hand, toxin peptides can be easily modified (e.g. radioactive, fluorescent) to provide analogues to facilitate the characterization of cell surface ion channels and receptors. In this way, they can be considered as diagnostics.

## From basic research to commercial applications

Examples of ongoing interest in the transition from basic research to commercial applications, can be found in several areas: e.g. in the treatment of pain, in immunotherapies, and for fighting microbial resistance. Several peptide toxin-derived drugs from the viper family of snakes (i.e. captopril, eptifibatide, tirofiban), one cone snail (i.e. ziconotide), and a lizard (i.e. exenatide) are currently approved by the FDA for clinical use for a diverse range of medical conditions, including blood pressure, pain, and diabetes. Other molecules are at the stage of preclinical development, such as a molecule from a sea anemone intended to use in autoimmune diseases, and snake venom-derived proteins that target clotting and coagulation malfunctions in humans.

Not surprisingly, libraries of diverse toxins for pharmaceutical screening purposes are now also available commercially, illustrating once more the fact that toxins remain interesting targets for therapeutic and diagnostic purposes.

## Conclusion

As highlighted by Clark et al. (2019), technological developments in 'omics-based' approaches are driving the discovery of new toxins from an increasing array of organisms (e.g. from bacteria to animals). Challenges remain in the availability of suitable (*in vitro*, *ex vivo*,or *in vivo*) models for characterizing the biological properties of these novel toxins and enabling their subsequent

development within the biosciences or healthcare sectors. There is also a lack of *in silico* tools that aid the prediction and identification of completely new classes of toxins with unique or distinct mechanisms of action. Similarly, new ways to assess the potential for 'off-target' effects on the body would be of significant value when developing toxins for use in veterinary or clinical applications. Although there are a number of exceptions, the mass production of natural bio-actives (with potential therapeutic benefits) remains largely challenging given the difficulties of producing industrial scale yields of toxins through culture and/or chemical synthesis. Furthermore, work has to be done in terms of necessary regulations in the domains of food safety and environmental toxicology. Such regulations are indeed often lacking nowadays, despite emerging (aquatic) toxins as a consequence of global warming and climatic change conditions on this planet. The future therefore calls for an urgent need to link our understanding of environmental impact on toxin production with the tools and knowledge to combine improved detection and forecasting systems. Finally, it is believed that still more than 80-90 % of the world's biodiversity (including plants, animals and micro-organisms) remain under-explored for medicinal applications and therefore merits our attention in the near future.

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