

Concepts Paper

Small Molecule Therapeutics for the Initial and Adjunctive Treatment of Snakebite

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Abstract: The World Health Organization (WHO) recently added snakebite envenoming to the priority list of Neglected Tropical Diseases (NTD). It is thought that ~75% of mortality following snakebite occurs outside the hospital setting, making the temporal gap between a bite and antivenom administration a major therapeutic challenge. Small molecule therapeutics (SMTs) have been proposed as potential pre-referral treatments for snakebite to help address this gap. Herein, we discuss the characteristics, potential uses and development of SMTs as potential treatments for snakebite envenomation. We focus on SMTs that are secretory phospholipase A2 (sPLA2) inhibitors and metalloprotease (MP) inhibitors.

Keywords: snakebite; antidote; inhibitor; small molecule therapeutics; SMT; secretory phospholipase; sPLA2; Neglected Tropical Disease; NTD

1. Introduction

Snakebite envenomation is a neglected tropical disease that causes more than 100,000 deaths every year [1]. Of the snakebites that are ultimately fatal, it is estimated that more than 75% occur before victims can reach the hospital for antivenom treatment [2–4]. There is an urgent need for novel interventions to address the therapeutic and temporal gap between a bite and hospital-level care. Small molecule therapeutics (SMTs) have been proposed for initiating the treatment of snakebite in the pre-hospital environment and as adjuncts to antivenom therapy [5,6].

2. Small Molecule Therapeutics

SMTs represent a potentially useful adjunctive therapy to antivenoms, the current mainstay of care for symptomatic snakebite. Most SMTs are naturally occurring (e.g., alkaloid) or synthetic molecules that are usually intended to act on specific targets. SMTs could be used in multiple ways to decrease morbidity and mortality caused by snake envenomation (Figure 1). Ideally, an SMT could be given orally in the pre-referral setting to diminish or slow down the effects of envenomation. An SMT could also be used in an in-patient setting, either orally or intravenously, as an adjunct to antivenom and to increase the breadth of treatment efficacy. These uses could potentially reduce the required dosage of antivenom, and improve treatment costs by improving the performance of imperfectly matched antivenoms. Finally, an SMT could be administered post-hospitalization to reduce the chances of rebound effects from venom components not effectively or durably covered by antivenom.

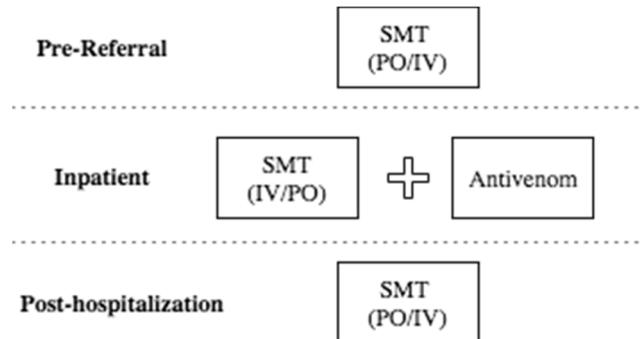


Figure 1. Potential uses of an SMT, via PO (Oral) or IV (Intravenous).

The search for non-serotherapy antidotes to snakebite is not new. Traditional healers have long used poultices and teas derived from plants to attempt treatment. Plants and fungi remain the basis for most active pharmaceutical ingredients used in modern medicine today [7]. The spectrum of small molecule inhibitor compounds for the treatment of snakebite has been reviewed by Carvalho, Soares, Lohse, Laustsen, Bastos and others [5,8–11]. As a field, only a small number of individuals and groups have directly addressed the question of developing small molecules for the treatment of snakebite and it remains largely unexplored [6,12–15]. No SMT for snakebite treatment has ever been approved for use in humans or animals; however, a clinical trial with cepharanthine, a small molecule, was recently conducted for the treatment of a Mamushi bite [16]. The potential benefits of using SMTs as an adjunctive therapy deserve further study.

SMTs have many characteristics that make them potentially useful as an adjunctive therapy for snakebite treatment. If proven effective, SMTs might address some limitations of antivenom and vice-versa (Figure 2). By their nature, SMTs are at low risk for allergenicity or anaphylactic shock as compared to most serum-based therapies [17]. In addition, many venoms have in common active toxic components that could be targeted by SMTs, including the secreted phospholipase A2 (sPLA2), metallo- and serine-proteases (svMPs and SPs, respectively), and the non-enzymatic three-finger toxins (3-FTX). If the inhibitory targets are common among snake species, SMTs could potentially have “venom agnostic” effects. This would potentially decrease the importance of snake identification and would increase the usefulness of SMTs as first-line therapeutics. Venom agnostic SMTs could be used in a broad range of geographical areas and potentially eliminate the need of an expert to confirm snake species prior to initializing treatment, though newly developed rapid diagnostics could rapidly refine the specificity of treatment and improve the clarity and powering of clinical studies where more than one type of venomous snake is prevalent [18,19]. Finally, in theory, multiple SMTs developed against various venom proteins could be combined to inhibit wider varieties of toxins present across snake species.

	Antivenom	Small Molecule Therapeutic (SMT)
Advantages	Long half-life Broad spectrum venom antigen coverage	Heat Stable Low Allergenicity Low COGS Easy route of administration Superior Penetration Compatible with Antivenom
Limitations	Cold chain (Some, not all) Allergenicity Higher COGS Professional administration only Limited tissue penetration Supply/Access	Short Half-Life Spectrum (depends on molecule/target)

Figure 2. Advantages and limitations of antivenom and SMTs. If proven effective, an SMT might address some limitations of antivenom and vice-versa. (COGS = Cost of Goods).

For SMTs to be potentially useful as an adjunctive therapy for snakebite, they should be heat stable and easily administered, allowing point-of-care treatments in the field. Also, the manufacturing cost-of-goods (COGS) of SMT should be comparatively low. Consideration of repositioned (repurposed) compounds with a history of use in humans, a strategy discussed in detail below, could further decrease costs of development [6,20]. Also, venom distributes outside the blood, with an average volume of distribution in animals of 0.054–0.070 L/Kg, where antivenom cannot distribute [21]. Given their small molecular weight and charge, SMTs will generally have much higher volumes of distribution and tissue penetration than antivenom, allowing them to distribute within vulnerable tissues [21–25].

WHO has provided a detailed pre-clinical assessment for antivenom development for snakebite treatment and has recently been reviewed in detail by Gutierrez *et al.* [26,27]. However, none exists for SMT development for snakebite. Based on the WHO guidelines, previous studies on antivenom development, and our experience in the development of SMTs, the preclinical assessment of SMTs for snakebite treatment can be envisioned (Table 1).

Table 1. Assessment of desirable pre-clinical characteristics of an SMT.

Safe	<ul style="list-style-type: none"> Minimize off-target toxicity [28] Without adverse interactions with antivenom Broad therapeutic index
Efficacy (<i>in vitro</i>)	<ul style="list-style-type: none"> Nano- or sub-nanomolar <i>in vitro</i> potency (IC_{50}) for scalability [28–30] Determination of affinity, minimum active concentrations, physical characteristics, stability, mechanisms of action, dose-response, and drug effects [6,26,27,29–39]
Efficacy (<i>in vivo</i>)	<ul style="list-style-type: none"> Tested with both: <ul style="list-style-type: none"> Minimum acceptable: Pre-mixing of venom and antidote prior to injection (ED_{50} determination) [27] Ideal: Venom administration prior to administration of antidote [6,26,40]
Broad Spectrum	<ul style="list-style-type: none"> Depending on target selection (ubiquity and medical importance of inhibitory target among snake species) [41,42]
Heat Stable	<ul style="list-style-type: none"> Real-time stability studies up to 30 °C (± 2 °C) and relative humidity of 75% ($\pm 5\%$) (WHO “Climatic Zone IVb”) [43]
Ease of Administration	<ul style="list-style-type: none"> Oral solution, rectal or nasal formulations Auto-Injectable [34]
Bioavailability	<ul style="list-style-type: none"> For oral formulations, adequate bioavailability in fed state
Half-life	<ul style="list-style-type: none"> For field antidotes, half-life of at least 5 to 7 hours [44–46] Consideration of re-dosing strategies

3. Pathway for Development of an SMT

3.1. Venom Target Selection

As put by Laustsen, snake venom is likely to be the “most complex pharmaceutical target” known, composed of a multitude of toxin components and complex biochemical interactions [42]. Thus, SMT targets for inhibition should be, ideally, abundant across as many of the medically

important snake species as possible. For understanding venom properties and targeting, proteomic analysis of snake venom has been crucial to reveal species variation in venom composition and toxicity [41,47,48]. Proteomic analysis has revealed a wide array of active toxic ingredients from at least 26 protein families, but the most common medically relevant components are found within four families in varying proportions [49–51]. These proteins are the phospholipase A2 (PLA2s), snake venom metallo- and serine-proteases (svMPs and SPs, respectively), and the non-enzymatic three-finger toxins (3-FTX) [41,50–52]. Not all snake venoms, however, have unique toxins that are in this group of four, including mambas with dendrotoxins and some rattlesnakes with low molecular mass cationic myotoxins [53–55]. Continued research into the proteomic and toxicovenomic characterization of the most medically relevant venoms are crucial in order to have a more comprehensive understanding of drug and antivenom targeting in these species, as well as to understand the nature of therapeutic failures when they occur. The use of tools, such as the newly developed Toxicity Score, which combines the medical importance and the relative abundance of a specific toxin, can aid the identification of a target [41,42].

Herein, we focus on sPLA2 as a candidate for inhibition by SMTs because of its ubiquity and clinically significant effects [26,51]. Snake venom sPLA2s play roles in early- and late-onset symptomology, as well as synergistic and regulatory roles for other co-existing snake venom components [56–63]. sPLA2s are also some of the most pharmacologically active, multi-effect (neuro- myo- cyto- hemotoxic) venom components (Table 2) [56–63].

Table 2. Generic Pathogenesis of Major Toxins in Snake Venom.

Family	PLA2	svMP	SP	3-FTX
Neurotoxic	Yes	-	-	Yes
Hemotoxic	Yes	Yes	Yes	-
Myotoxic	Yes	Yes	-	-
Cytotoxic	Yes	Yes	-	Yes

While sPLA2 inhibition might prove sufficient as a “bridge-to-survival” for many types of venoms when administered in a pre-referral setting and, at times, be sufficient for treatment, future SMTs might be mixtures of other SMT (Figure 3a). Some targets could also be inhibited indirectly by SMTs, such as three-finger toxins, whose effects might sometimes be mitigated by acetylcholinesterase inhibitors, though the use of these inhibitors remains controversial despite decades of use for this purpose [52,61,64–66]. In addition, SMTs might be used to slow the spread of venom by paralyzing lymphatic smooth muscles (e.g., with lignocaine) [67]. SMTs could also be paired with antibodies or other biologicals to increase the range of efficacy or extend their paraspécificité (Figure 3b).

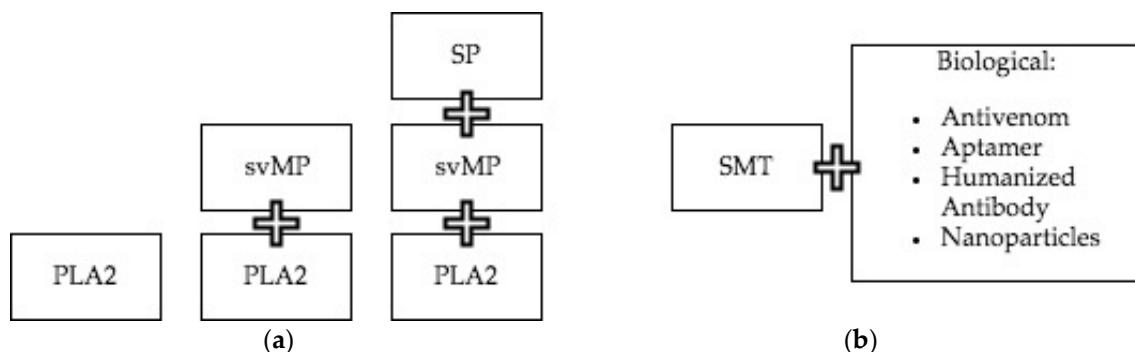


Figure 3. Hypothetical pipeline of SMTs for snakebite treatment. (a) Targeted inhibition of major snake venom enzymatic toxins through a combination of multiple inhibitory small molecules. (b) In combination with biologicals or others as adjuncts to antivenom for hospital administration (e.g., for targeting non-enzymatic toxins, such as 3-FTX).

3.2. Strategies for Discovery of Lead Compounds

There is a variety of strategies to discover new SMTs as listed in Figure 5. Some strategies involve the screening of entire compound libraries against the selected target, such as High Throughput Screening (HTS) (Figure 3). HTS requires no previous knowledge of potentially successful chemotypes. Compounds that show a pre-determined percentage of inhibition, for example more than 50% inhibition at 10 μ M, are advanced to the Confirmation of Hits stage. Confirmation of Hits would test the screened compounds with a dose-response curve, utilizing multiple concentrations, to determine the IC₅₀ (half-maximal Inhibitory Concentration) and, therefore, the effectiveness of the compound at inhibiting the active components of snake venom. Other methods, such as focused screening, are less time consuming but require more knowledge. Focused screening involves screening a small amount of existing developed drugs for potential repurposing for snakebite. The strategy chosen to discover a lead snakebite SMT depends on the resources and knowledge available to the investigator.

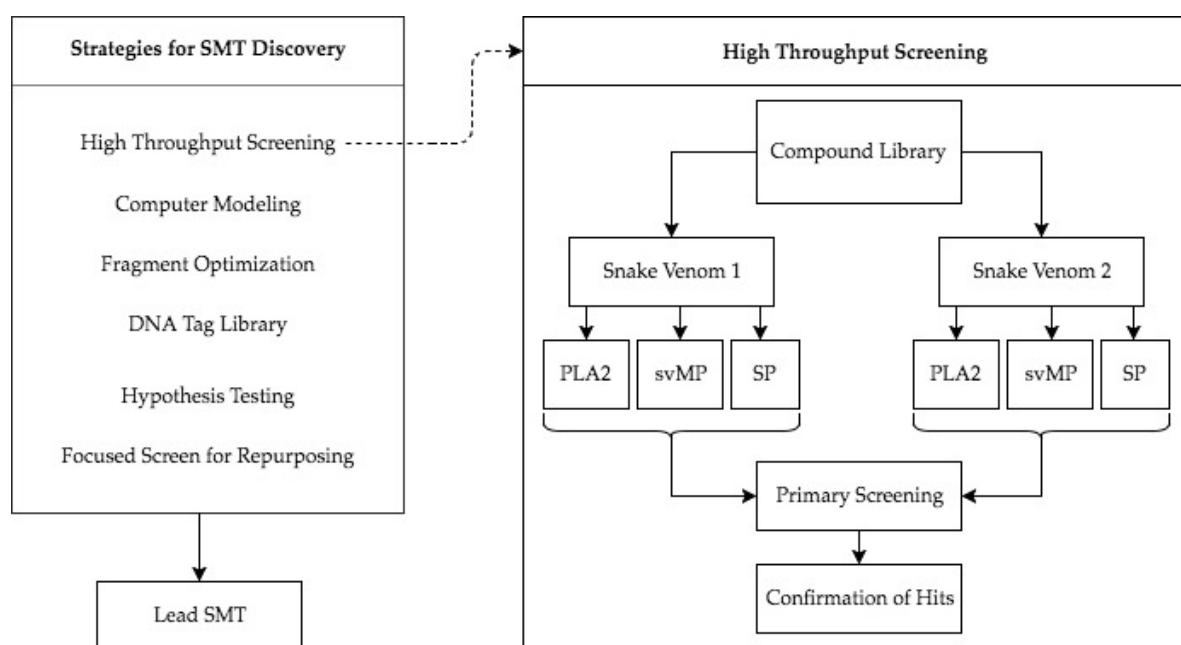


Figure 4. Hit to Lead: a variety of strategies to discover new SMTs and an example of processes and targets for High Throughput Screening (HTS) of candidate snake venom SMTs. There are many potential targets in addition to sPLA₂, and svMP and SP. 3-FTX lack enzymatic activity and present a challenging target for an SMT. Different assay methods are used for each type of enzymatic activity so screens would be run separately even if compound libraries were the same.

3.3. Repurposing as a Strategy for Discovery and Development

To achieve a lower-cost SMT product that can be commercialized and priced sustainably, its development costs need to be lowered. The development of a new drug from lead discovery to launch can take many years and cost more than one billion USD [28]. Repurposing, a strategy for accelerated drug development by reviving or expanding indications of existing drugs, might be beneficial to the development of a potential SMT for snakebite. Repurposing, or repositioning, is a powerful way to reduce the cost of drug development, particularly for NTDs that do not offer sufficiently alluring markets to larger pharmaceutical companies, such as snakebite [20,68]. Repurposing compounds already in development can accelerate entry to clinical trials and result in significant savings. Repurposing can also revive the potential of drugs that never reached commercialization, or expand the purpose of existing drugs by applying them to new indications [69]. Examples of successfully repurposed drugs include Thalomid (Thalidomide) for side effects of

leprosy and Viagra (Sildenafil) for pulmonary hypertension [20,69]. In 1972, Banerjee *et al.* presented an early example of repurposing an SMT for snakebite when neostigmine was used to treat the paralytic effects of an elapid bite [52]. Multiple groups have since investigated the use of this class of acetylcholinesterase inhibitors in the clinical setting with variable results [52,61,65,66]. Development of a hypothetical SMT using a repurposing pathway are shown in Figure 4.

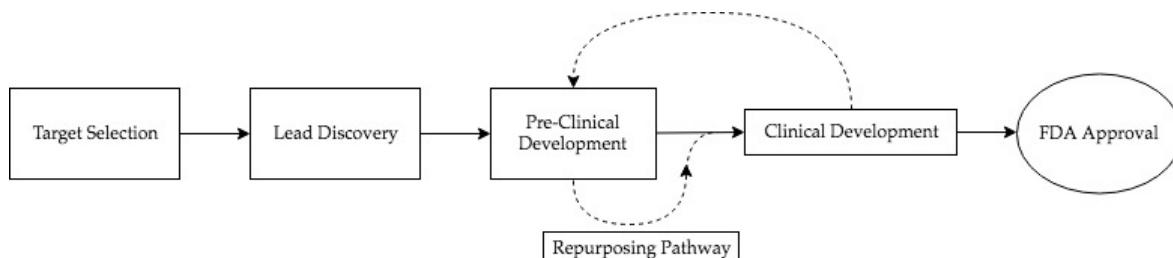


Figure 5. Scheme of a potential SMT development pathway. “Sections” correspond to paragraphs that follow. The repurposing pathway accelerates development and lowers costs by starting at a more advanced stage of development than a new chemical entity

Drug repurposing does not generally require further optimization or structural modification of an FDA-approved drug or lead compound, though formulations may require extra characterization and testing if altered from the original studies in order to establish equivalency [20]. Therefore, data from efficacy, safety, pharmacokinetics/dynamics studies and others conducted for the initial indication of the compound can be re-used for the new indication if the originators donate, license, or sell access to their data [31]. In regards to safety, Klug *et al.* note that “the most common side effects of the repurposed drugs are minor in comparison to those of many existing NTD therapeutics” [20]. Repurposing can be a cost-effective, lower-risk strategy to rapidly develop new SMTs for snakebite treatment.

4. Repurposed Drugs and Model SMT Candidates for Enzymatic Inhibition of Snake Venom

We recently identified a previously studied sPLA2 inhibitor, varespladib (*syn* LY315920, S-920) and its orally bioavailable prodrug, methyl-varespladib (*syn* LY333013, A-002) as a candidate treatment for snake envenomation [6]. Varespladib appears to be a potent sPLA2 inhibitor against a broad spectrum of snake venom sPLA2s. As mentioned above, the ubiquity and clinically significant effects of snake venom sPLA2s across venom types make it a plausible candidate for inhibition by an SMT with potential for broad spectrum of efficacy. Figure 6 shows the structures of the sPLA2 inhibitor, varespladib and the anti-svMP peptidomimetic SMTs, prinomastat and marimastat [12,13,34].

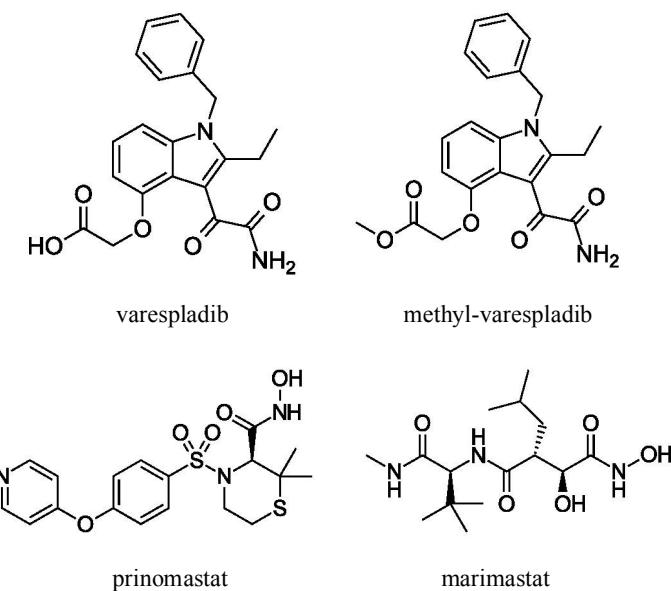


Figure 6. Structure of: varespladib (top left), its orally bioavailable pro-drug, methyl-varespladib (top right), prinomastat (bottom left) and marimastat (bottom right). Marimastat and prinomastat are both orally bioavailable and could be combined (mixed or co-packaged) for more extensive coverage as field antidotes [1,34]

In vitro, varespladib was observed to be a surprisingly potent inhibitor of snake venom sPLA2s. The observed consistent potency (nano- and sub-nanomolar range) against a range of sPLA2s from more than 25 medically important snakes from six continents suggests that these could be scaled for human use at reasonable dose volumes and dosage forms [6]. Varespladib, for the indication snakebite, is an example of a potentially repurposed compound. In recent decades, several large pharmaceutical industry endeavors focused on sPLA2 inhibition for potential anti-inflammatory and cardiovascular drugs, but to date, none came to market [70,71]. sPLA2 enzymes are present throughout the animal kingdom and are involved in multiple key processes, such as synaptic transmission and inflammation. PLA2s are implicated as having roles in various important diseases such as sepsis, cardiovascular disease, neurological disease, rheumatological disease, and cancer [72]. In attempts to treat these diseases, several companies devoted extensive resources to developing sPLA2 inhibitors as targets for drug development. These inhibitors could be considered for repurposing as SMTs for snakebite treatment. Similarly, MP inhibitors that have previously been developed for cancer treatment such as batimastat, marimastat, and prinomastat could also be considered [11,12,34,51]. Varespladib is a sPLA2 inhibitor originally developed by Shionogi, Lilly for treatment of pancreatitis and sepsis, and later, licensed to Anthera, for treatment of acute chest syndrome and heart disease [74,75]. This makes it an inviting candidate for repurposing because of the known safety profile and, thus, potentially reduced development costs [6,76,77].

In vivo, rescue studies using lethal doses of coral snake (*M. fulvius*) and common adder (*V. berus*) venom were performed on mice to whom venom was administered subcutaneously followed by intravenous varespladib in the lateral tail vein, and all survived for at least 24 h, while those receiving only venom died in a matter of minutes or hours. Similarly, mice subjected to intraperitoneal administration of venoms were rescued by intravenous, intramuscular and per-oral routes of drug administration against venoms from snakes such as *D. russelli*, *E. carinatus sochureki*, *O. scutellatus*, *C. scutulatus*, and *C. durissus terrificus* (unpublished data). In addition, recent results by Wang *et al.* (2018) showed the inhibitory effect of varespladib treatment on *D. acutus*, *A. halys*, *N. atra* and *B. multicinctus* *in vitro* and *in vivo* [78]. The results of these experiments have led to several new questions, including the exact mechanism by which survival is enhanced by these experimental drugs.

For a repurposed compound, attention should be given to the safety signals seen in prior trials. Consideration of the difference in use of an SMT between previous indication and snakebite might mitigate safety risks: for example, an SMT for snakebite would be used acutely, with one or few doses, rather than chronically. In terms of efficacy, the risk that inhibition of one toxin (e.g. sPLA2, svMP or 3FTX) might not be sufficient and can be mitigated by addressing additional targets, and having backup molecules potentially more suitable for different geographic regions [34]. Lastly, means for commercialization should be evaluated early on to determine real world feasibility of implementing an SMT.

5. Conclusions

SMTs constitute a potentially useful class of compounds meeting criteria for development as initial field treatment for snakebite and as adjuncts to antivenom with heretofore unrealized potential.

The development of an SMT commences with selecting an inhibitory target and determination of the breadth of efficacy across snake species. The breadth of effect helps determine the applicability to specific geographical regions and snake types. Aiding the discovery step is the availability of SMTs already developed by the pharmaceutical industry for other indications, which, if repurposed, could substantially lower development costs and increase the potential for accelerated approvals. Preclinical studies to evaluate the safety and efficacy of the SMT follow similar assays used in antivenom testing, but other assays to test heat stability, ease of administration, and bioavailability are likely to be additionally performed. Overall, the development of varespladib, can be used as an example for pathways of SMT development. A careful, systematic, and multidisciplinary approach will be required to determine the most appropriate next steps in the development and deployment of new therapeutic classes for the initial treatment and overall management of snakebite.

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Conflicts of Interest: XXX is associated with intellectual property related to the commercial development of XXXX. XXX has no conflicts of interest.

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