

Exploring the global animal biodiversity in the search for new drugs – insects

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Abstract

Substances from terrestrial plants and marine organisms have since long been recognized as important sources of bioactive substances. This has led to the development of a large variety of drugs to treat human diseases such as, among others, a number of antihypertensive, hypoglycemic, cardiovascular, antibiotic, and antineoplastic agents. More recently, the amazing biodiversity represented by the myriad of insect species has been realized to produce an equally exceptional source of bioactive chemicals with therapeutic potential. Many of these compounds serve as highly effective defensive and predatory chemicals and have enabled insects to survive for hundreds of millions of years and to diversify to the countless different species known today. As some of these chemicals possess meaningful pharmacological properties, they represent interesting candidates for new drug discovery and development programs. A few examples are substances with microcidal, cytotoxic, cytolytic, apoptotic, anti-angiogenic, or anticoagulant qualities. This paper addresses the significance of bioactive compounds from insects as lead compounds for producing new therapeutics.

Introduction

Today, approximately 80% of the human population living in developing countries still relies for an important part on naturally-derived substances, particularly plants with medicinal properties, for its day-to-day health care [1,2]. The remaining 20% of individuals in the industrialized parts of the world uses in 25% of cases drugs that have been directly derived from natural products [1,2]. This underscores the importance of the biodiversity to both traditional medicinal systems and modern, allopathic medicinal practices.

A myriad of breakthrough therapeutics has been developed by following up naturally-derived leads with medicinal properties, not only from plants but also from microorganisms and marine species [3,4]. A few examples are the cardiac glycoside digoxin from the foxglove *Digitalis lantana* L. (Scrophulariaceae) for treating certain heart conditions [5]; the muscle relaxant D-tubocurarine derived from the Amazon plants *Strychnos toxifera* L. (Loganiaceae) and *Chondrodendron tomentosum* L. (Menispermaceae) [6]; the hypoglycemic agent metformin synthesized on the basis of the chemical structure of galegine in the French lilac *Galega officinalis* L. (Fabaceae) [7]; the antibiotic penicillin from the fungus *Penicillium chrysogenum* Thom, 1910 (Trichocomaceae) [8]; the powerful pain killer ziconotide molded on the basis of conotoxin in the venom of the cone snails *Conus geographicus* Linnaeus, 1758, and *Conus magus* Linnaeus, 1758 (Conidae) [9]; and the novel antineoplastic agent trabectedin (Yondelis®) originally isolated from the Caribbean sea squirt *Ecteinascidia turbinata* Herdman, 1880 (Perophoridae) [10].

However, when compared to plants and marine species, the animal kingdom, particularly the order of the insects (Insecta Linnaeus, 1758), represents at least a similar abundance of bioactive compounds with potentially useful therapeutic properties. The insects are the most varied group of animals on our planet, comprising a biomass of a minimum of 2.6 million and a maximum of 7.8 million individuals [11,12] and have colonized a wide variety of terrestrial, freshwater, and even a few

marine habitats [11,12]. Thus, when taking an average of 5.5 million insect individuals, this group of animals encompasses over 70% of the estimated 7.77 million extant animals on Earth [11,12].

Taxonomically, the Insecta represent a class of invertebrates within the Hexapoda, a subphylum of the Arthropoda ([11,12]; Box 1). The extinct Trilobitomorpha as well as the extant Chelicerata (e.g.,

Kingdom Animalia – animals
Phylum Arthropoda – arthropods
Subphylum Trilobitomorpha
Class Trilobita – trilobites (extinct)
Subphylum Chelicerata
Class Arachnida – spiders, scorpions, etc.
Class Merostomata – horseshoe crabs, eurypterids
Class Pycnogonida – sea spiders
Subphylum Myriapoda
Class Chilopoda – centipedes
Class Diplopoda – millipedes
Class Pauropoda – sister group to millipedes
Class Symphyla – resemble centipedes
Subphylum Crustacea
Class Branchiopoda – brine shrimp etc.
Class Remipedia – blind crustaceans
Class Cephalocarida – horseshoe shrimp
Class Maxillopoda – barnacles, copepods, fish lice, etc.
Class Ostracoda – seed shrimp
Class Malacostraca – lobsters, crabs, shrimp, etc.
Subphylum Hexapoda
Class Insecta – insects
Class Entognatha – springtails, etc.

Box 1. Taxonomic position of the insects in the animal kingdom (Based on Gullan PJ, Cranston PS, 2014).

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spiders and scorpions), Myriapoda (e.g., centipedes and millipedes), and Crustacea (e.g., lobsters, crabs, and shrimps) also belong to the Hexapoda ([11,12]; Box 1). The insects comprise an estimated 950,000 different species classified in approximately thirty orders [11,12]. The orders of the Mantophasmatodea (gladiators), Grylloblattodea (icecrawlers), and Zoraptera (angel insects) only include twenty-four to thirty-two species [11,12]. On the other hand, highly species-rich orders such as the Orthoptera (e.g., grasshoppers, locusts, katydids, and crickets), Diptera (flies), Hemiptera (bugs), Hymenoptera (e.g., wasps, bees, ants, and sawflies), Lepidoptera (e.g., butterflies and moths), and Coleoptera (beetles) roughly consist of 25,000 to 400,000 different species [11,12].

Insects are characterized by a hard, jointed chitinous exoskeleton that encloses a body consisting in adults of three fused segments, *viz.* the head with a series of complex mouthparts and one pair of antennae, the thorax which carries three pairs of legs and usually two pairs of wings, and the abdomen which contains the digestive and reproductive organs ([11,12]; Figure 1). Both the antennae and mouthparts have probably evolved from modified walking legs, and the wings may be reduced or absent in a few very primitive species, becoming non-functional or adapted for a different purpose [11,12]. Notably, a number of other arthropods such as centipedes, millipedes, scorpions, and spiders also have a jointed skeleton (as holds true for all arthropods) but substantially differing features, most noticeably the absence of six legs in the adult stages [11,12].

Insects presumably evolved from a group of crustaceans [13] and first appeared in the Ordovician, some 480 million years ago [14]. The currently existing species can be classified into the subclasses Apterygota and Pterygota. The Apterygota are considered the most primitive insects alive today, and consist of at least four orders characterized by wingless adults (like the immature stages) which have not undergone a metamorphosis [11-14]. The Pterygota comprise winged and secondarily wingless insects, and can further be divided into at least sixteen orders within the Exopterygota and at least nine orders belonging to the Endopterygota [11-14]. The Exopterygota produce larvae that do not enter a pupal stage in their transition to adults, have nymphs that gradually develop into adults through moulting and already resemble adults, and develop wings outside their body [11-14]. The Endopterygota produce larvae that do not resemble adults, undergo an elaborate metamorphosis involving a pupal stage, and develop wings inside the body during pupation [11-14].

Background

Insect species communicate with each other and with their environment using a variety of tactile, visual, auditory, and chemical signals [15,16]. The chemical signals are referred to as semiochemicals or infochemicals and pass on information on, for example, impending

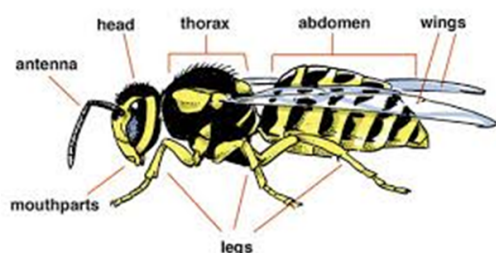


Figure 1. Basic body plan of the insects (from: <http://www.earthlife.net/insects/anatomy.html>).

threats and new food sources [15,16]. Important groups of insect chemicals are pheromones and allelochemicals [15,16]. Pheromones carry information from one individual to (an)other member(s) of the same species and include, among others, sex attractants, intraspecific aggregation signals, alarm substances, and trail-marking compounds [15,16]. Allelochemicals travel from one insect to (a) member(s) of (a) different species and include, for instance, defensive signals such as repellents and toxins as well as compounds to locate suitable host plants [15,16].

Many species of insects synthesize their own chemicals, but others derive these substances from the plants they feed on [15,16]. Some of these chemicals - such as alkaloids, bufadienolides, cantharidins, cyanides, and cardenolides - act as powerful defensive poisons that restrict movement of attackers [15,16]. Such compounds can irritate, injure, induce vomiting, cause blistering, or even kill the enemy [15,16]. Others function as repellents that stimulate scent and taste receptors of predators, discouraging them from feeding [15,16]. Such substances comprise in general volatile and reactive chemicals like acids, aldehydes, aromatic ketones, quinones, and terpenes [15,16]. Some insect species use combinations of defensive chemicals and repellents, and some chemicals can exert multiple effects [15,16].

For instance, when threatened, the soldiers of the pantropical termite subfamily Nasutitermitinae (Blattodea: Termitidae) exude noxious liquids containing several monoterpene hydrocarbons through a horn-like frontal projection [17]. The red spot assassin bug *Platymeris rhadamanthus* Gerstaecker, 1873 (Hemiptera: Reduviidae) immobilizes its prey by using venomous saliva that contains potent lytic enzymes which cause intense local pain, vasodilation, and edema, and help in the external digestion of prey [18]. Soldiers of the Malaysian carpenter ant *Camponotus saundersi* Emery, 1889 (Hymenoptera: Formicidae) violently release a sticky corrosive and irritant substance from their large mandibular glands running through their entire body, causing the body to explode, thereby engulfing the attacker with the poison [19]. And bombardier beetles in the tribes Brachinini Bonelli, 1810; Paussini Latreille, 1807; Ozaenini Hope, 1838; and Metriini LeConte, 1853 (Coleoptera: Carabidae) spray boiling hot, toxic, and foul-smelling quinonoids from their abdomen in the direction of a predator [20].

As well, larvae from the three-lined potato beetle *Lema daturaphila* Kogan & Goeden, 1970 (Coleoptera: Chrysomelidae) use poisonous fecal shields produced from their diet of toxic alkaloid-containing plants such as nightshades to deter predators [21]. The North American eastern lubber grasshopper *Romalea microptera* Houttuyn, 1813 (Orthoptera: Romaleidae) emits a foul-smelling and foul-tasting foamy mixture of phenols, terpenes, and benzoquinones from its thorax when disturbed [22]. And when brought into an anthill, caterpillars from the Japanese oakblue *Arhopala japonica* Murray, 1875 (Lepidoptera: Lycaenidae) secrete a sticky, sweet substance that presumably modifies brain dopamine signaling of the ants, manipulating the ants to protect and even pamper the caterpillars instead of eating them [23].

In light of their effectiveness as warning signals and anti-predator defenses, the meaningful pharmacological properties of certain insect chemicals should be beneficial against human diseases. The above-mentioned reduviid venom, for instance, may treat bacterial infections in humans [24]. Apitoxins such as melittin cause inflammation after bee stings, but these compounds and some of their analogues have been reported to induce immunomodulatory, cytotoxic, and apoptotic effects in various preclinical models [25]. And cantharidin, the defensive chemical from blister beetles, may represent the lead compound of a

promising new class of antineoplastic agents [26].

These and many more examples underscore the pharmacological diversity of insect-derived bioactive substances and their potential therapeutic usefulness. This paper addresses a number of unique bioactive compounds from this group of animals, their (putative) mechanism(s) of action, as well as their potential clinical applicability, and closes with the provisions of new drug development programs exploring these substances. The compounds have been grouped on the basis of the insect order in which they have been encountered (Table 1). The insect orders have been arranged according to their approximate evolutionary complexity with the oldest and most primitive groups addressed first [13,14].

Bioactive compounds from Orthoptera

The order Orthoptera includes, among others, grasshoppers, crickets, and locusts, and represents, with approximately 27,000 species in 235 subfamilies and families worldwide, one of the largest groups of insects [11,12]. Their name derives from the Greek words 'ortho' meaning 'straight' and 'pteron' meaning 'wing' [11,12]. These insects have a characteristically cylindrical body and strong, enlarged legs adapted for jumping, and many species communicate with one another by sounds produced by rubbing their wings against each other or against their legs [11,12].

Representing valuable sources of proteins, certain species of Orthoptera are part of the diets of various societies throughout the world [27]. Others are incorporated in the traditional medicinal practices of, for instance, indigenous communities in India and Brazil [27,28]. Notably, the native peoples of Mexico already used the femurs of grasshoppers for treating liver ailments, presumably because of their resemblance to human liver [29].

Orthoptera feed on a large variety of plants including economically valuable agricultural crops [30]. Particularly grasshoppers and locusts have been regarded as major pests since biblical times, regularly attacking fields of crops in swarms of millions of individuals which wipe out entire areas in a few hours [30]. On the other hand, many Orthoptera are preyed on by various microbial pathogens as well as a number of reptiles, birds, and small mammals, while certain parasitic dipteran and hymenopteran species lay their eggs into the eggs or cuticles of orthopterans [30].

As a result, the Orthoptera have developed a number of remarkable chemical defense mechanisms for their protection. For example, many grasshoppers repel predators by 'tobacco spitting', i.e., by regurgitating their irritant or toxic digestive fluids on the predator [31]. Katydidids from the genus *Eugaster* Serville, 1838 (Tettigoniidae) autohemorrhage

in response to visual stimuli and can accurately eject blood up to forty centimeters in the direction of an attacker [31,32]. Other orthopteran species fortify their blood cells or internal organs with distasteful or toxic allomones [33,34]. And some lubber grasshoppers (family Romaleidae) discharge a defensive secretion from paired metathoracic spiracles to their enemy [31,32]. Studies exploring the chemicals produced by Orthoptera have led to the identification of, among others, a number of isocarbostyryl alkaloids with interesting bioactivity.

Isocarbostyryl alkaloids

Isocarbostyryls are alkaloids that do not contain basic nitrogen atoms and are represented by hydroxylated benzophenanthridone or isoquinolinone types of structure [35,36]. They were first isolated from plants of the Amaryllidaceae family which have long been used for their medicinal and toxic properties [37]. Notably, already in ancient Greece, the oil from Amaryllidaceae members such as *Narcissus* species was used for treating cancer [37]. Particularly the isocarbostyryl alkaloids narciclasine, lycoricidine, and pancratistatin isolated from various Amaryllidaceae members [35] displayed intriguing antitumor activity in various *in vitro* and *in vivo* models [38-42].

These cytotoxic effects occurred at concentrations that did not affect the growth of normal human lung fibroblasts [43]. Narciclasine, for instance, accomplished a 50% reduction in the number of viable tumor cells after three days of treatment at mean concentrations of 0.03 μ M compared to 7.5 μ M for the fibroblasts [43]. Subsequent mechanistic studies indicated that these effects were attributable to the selective induction of internucleosomal DNA fragmentation and the activation of the initiator caspases -8 and -10 of the death receptor pathway in tumor cells but not in normal human fibroblasts [43-47]. These observations suggest that isocarbostyryl alkaloids may represent a promising class of novel apoptosis-inducing anticancer agents.

As part of an effort to explore terrestrial arthropods as potential sources of novel and structurally unique anticancer drugs, the Texas grasshopper *Brachystola magna* Girard, 1854 (Romaleidae) (Figure 2) was also demonstrated to contain isocarbostyryl alkaloids, albeit at levels lower than those found in the Amaryllidaceae [48]. The compounds isolated were pancratistatin, narciclasine, and umgeremine, and presumably act as defensive chemicals for the grasshopper [48]. Particularly the two former compounds displayed meaningful *in vitro* and *in vivo* antitumor effects, comparable to those previously noted for the plant-derived compounds [48], emphasizing their potential usefulness as novel anticancer agents.

There are no records to indicate that *B. magna* specifically or preferentially feeds on plants of the Amaryllidaceae family [49]. This

Table 1. Bioactive compounds with potential clinical applicability derived from insect species. The insect species have been grouped according to the taxonomic order to which they are assigned, and the insect orders have been arranged according to their approximate evolutionary complexity with the oldest and most primitive groups addressed first

(Infra)Order	Species (Family)	Bioactive compound	(Potential) clinical application(s)
Orthoptera	<i>Brachystola magna</i> Girard, 1854 (Romaleidae)	Isocarbostyryl alkaloids	Anticancer agents
Isoptera	<i>Pseudocanthotermes spiniger</i> Sjöstedt, 1900 and <i>Nasutitermis corniger</i> Motschulsky, 1855 (Termitidae)	Termicin and spinigerin	Antimicrobial and antiparasitic agents
Coleoptera	<i>Lytta vesicatoria</i> Linnaeus, 1758 (Meloidae)	Cantharidin and derivatives	Anticancer agents
Diptera	<i>Simulium vittatum</i> Latreille, 1802 (Simuliidae)	Simukunin and analogues	Anticoagulants
Lepidoptera	<i>Pieris rapae</i> Linnaeus, 1758 (Pieridea)	Pierisins	Anticancer agents
Hymenoptera	<i>Apis mellifera</i> Linnaeus 1758 (Apidae) <i>Lasioglossum laticeps</i> Schenck, 1870 (Halictidae) <i>Vespula</i> spp., <i>Vespa</i> spp., and <i>Provespa</i> spp. (Vespidae) <i>Solenopsis invicta</i> Buren 1972 (Formicidae) <i>Pseudomyrmex triplarinus</i> Weddell, 1850 (Formicidae)	Melittin Lassioglossins Mastoparans Solenopsin Myrmexins	Antimicrobial, anti-inflammatory, and anticancer agents Antimicrobial and anticancer agents Antimicrobial and anticancer agents Anticancer agents Rheumatoid arthritis



Figure 2. The Texas grasshopper *Brachystola magna* Girard, 1854 (Orthoptera: Romaleidae). From: <http://www.naturalista.mx/observations/1921224>.

suggests that this insect species produces isocarboxystyris through its own metabolism and, given its biomass, may represent a feasible alternative source of these promising substances. This is all the more important because so far these compounds cannot be synthesized by cost-effective processes [35]. At the same time, several research groups are directing their efforts to acquire new isocarboxystyris derivatives or analogues for further preclinical anticancer testing [35]. Such compounds may not only emerge by modulating the structures of the currently available compounds, but also by further exploring orthopteran species for their ability to synthesize such compounds through modified or alternative biochemical pathways.

Bioactive compounds from Blattodea

The insect order Blattodea (Greek for 'an insect that shuns the light') includes approximately 3,000 species of termites in roughly 300 genera of nine families, besides an estimated 4,600 species of cockroaches in about 500 genera of seven families [11,12]. Previously, the termites were considered a separate order (Isoptera), but relatively recent genetic and molecular evidence strongly suggests a close relationship with the cockroaches, both cockroaches and termites having evolved from a common ancestor [11,12]. For this reason, the termites have been classified as the infraorder Isoptera residing under the order of the Blattodea [11,12].

The Isoptera comprises approximately 3,000 termite species in roughly 300 genera of nine families [11,12]. The name of this infraorder is derived from the Greek words 'iso' and 'pteron' meaning 'equal' and 'wings', respectively, in reference to the similar size, shape, and venation of the four wings [11,12]. Termites are sometimes referred to as white ants but they are not related to this insect family, even though their colonies, like those of ants (as well as those of bees and wasps), comprise several castes including queens, workers, and soldiers [11,12]. They are detritivores, feeding on dead plant material and cellulose, and are encountered on all continents except Antarctica [11,12]. Their colonies can consist of several millions of individuals, and their queens may live up to fifty years [11,12].

Several species of termites - for instance, the Termitidae members *Globitermes sulphureus* Haviland and *Neocapriterme taracua*, as well as members of the family Serritermitidae - have developed dramatic ways to protect their colonies from intruders: they engage in self-destruction while releasing incapacitating and/or toxic compounds on their attackers [50-53]. In addition, presumably as a result of their feeding habits, many termite species produce substances with potent and broad antimicrobial activity that they store in various parts of their gastrointestinal tract [54]. Some of these compounds may represent interesting candidates for developing novel drugs against microbial and parasitic infections.

Termicin and spinigerin

Termite products also have a long use in several traditional medicinal systems, for instance, as a paste consisting of a mixture of macerated termites and parts of the mound [27,55]. In several traditional African medicinal systems, the paste is boiled and topically applied to prevent infection of external wounds, and ingested to treat internal hemorrhages [27]. And in Indian Ayurveda, the boiled paste is used as a remedy for ulcers, rheumatic diseases, anemia, pain, and a declining health [27,55]. Interestingly, termites are also used as a medical device in certain African countries. In order to insert a drug substance subcutaneously, a termite is allowed to bite into the area of the patient's skin coated with the substance, thus effectively injecting it under the skin [55].

Relatively recent insights have made clear that the therapeutic efficacy of at least some of these traditional uses may be attributed to the antibiotics the insects produce [56,57]. Among these are the antimicrobial peptides termicin and spinigerin in the salivary glands of the Termitidae *Pseudocanthotermes spiniger* Sjöstedt, 1900 [58] and *Nasutitermes corniger* Motschulsky, 1855 (Figure 3) [59]. Their natural function is probably to kill a host of microorganisms and parasites [54,60], among others, by disrupting their cell membrane [60].

Not surprisingly, termicin displayed notable *in vitro* activity against several species of fungi as well as various Gram-positive and Gram-negative bacteria [58]. Notably, a decoction of the termite species *N. corniger* acted synergistically with the antibiotics neomycin, gentamicin, and erythromycin against cultured *Escherichia coli* and *Staphylococcus aureus* bacteria including multidrug resistant strains [58,61,62], presumably by inhibiting the drug efflux pump in the plasma membrane of the cells [59]. Moreover, spinigerin reportedly induced apoptotic-like cell death via a caspase-independent pathway in *Leishmania (Leishmania) donovani* parasites without affecting the host macrophages [63].

Together, these observations suggest that termite products - including termicin and spinigerin - may represent unprecedented modalities to overcome bacterial resistance to antibiotics and to fight parasitic infections such as visceral leishmaniasis. Thus, detailed evaluation of these compounds for their potential use against these conditions is warranted.

Bioactive compounds from Coleoptera

The insect order Coleoptera encompasses roughly 400,000 different species of beetles divided in four suborders and about 500 families and subfamilies [11,12]. The name of this group of insects is based on their sheathed wings, deriving from the Greek words 'koleos' for 'sheath' and 'pteron' for 'wing' [11,12]. The Coleoptera are the largest order



Figure 3. The conehead termite *Nasutitermes corniger* Motschulsky, 1855 (Blattodea: Termitidae). From: http://toutunmondedansmonjardin.perso.neuf.fr/EN/pages_EN/nasutitermes_sp_EN.htm



Figure 4. The blister beetle *Lyttavesicatoria* Linnaeus, 1758 (Coleoptera: Meloidae). From: <http://www.terrarium.com.pl/326-zwierzeta-jadowite-i-trujace-w-otoczeniu-czlowieka/>.

in the animal kingdom, comprising approximately 25% of all known life forms and about 40% of all described insect species [11,12]. They characteristically exhibit a particularly hard exoskeleton and hard forewings [11,12]. The exoskeleton is made up of many plates joined by thin sutures, thus providing flexibility to the animal [11,12].

With the exception of oceans and arctic regions, the Coleoptera have colonized all types of habitats on Earth [11,12]. They often feed on fungi, organic debris, and other invertebrates, but also on plants including agricultural crops [11,12]. The Colorado potato beetle *Leptinotarsa decemlineata* Say, 1824 (Chrysomelidae) and the boll weevil *Anthonomus grandis* Boherman, 1843 (Curculionidae), for instance, are infamous as major agricultural pests in the Americas [64]. On the other hand, various ladybird beetles (family Coccinellidae) prey on agricultural pests such as aphids, scale insects, and thrips and are considered useful [11,12].

In their turn, many Coleoptera are prey for various birds and mammals [11,12]. As a result, they have developed a broad range of defensive chemicals to make themselves unpalatable or even toxic to predators [15,16]. Some of these substances are products from their own metabolism while others are secondary metabolites of the plants they feed on [15,16]. The toxic substances may be released by remarkable techniques, such as the jets of formic acid and the earlier mentioned boiling hot toxic quinonoids which are sprayed on potential predators from far distances [20].

Less spectacular but equally effective are the irritating and/or blistering chemical defenses of many Coleoptera such as cantharidin [66]. This substance displayed unique pharmacological properties, making it a subject of various new drug discovery and development programs [67]. The same holds true for some of its naturally occurring derivatives and chemically modified analogues like cantharimides [67-69], cantharidinimide [70,71], palasonin [71], and the demethylated cantharidin analogue norcantharidin [72].

Cantharidin and derivatives

Cantharidin is a terpenoid present in the dried and ground bodies of several species of blister beetles of the genera *Mylabris* and *Lytta* (Meloidae), especially the emerald-green *L. vesicatoria* Linnaeus, 1758 (Figure 4) [67,73]. It is produced by the male who gives it to the female during mating to cover the eggs as a defense against predators [67]. Also known as Spanish fly, cantharidin has a long use as an aphrodisiac in many societies [67,73]. However, the topical use or ingestion of this compound carries a substantial risk of causing toxicity including priapism and even death [67,73].

Topical cantharidin has also been used for treating various skin disease such as furuncles and warts in particularly traditional Chinese and Vietnamese medicine [74], and since the 1950s in the USA for treating warts and molluscum contagiosum, a common viral disease of childhood caused by a poxvirus that presents with small, firm, dome-

shaped, umbilicated papules [75]. This has led to its current approval of the US Food and Drug Administration (FDA) for "topical use in the professional office setting only" against warts and other skin problems [76].

More recent cell culture and animal studies indicated that cantharidin effectively inhibited the growth of various human cancer cells *in vitro* and *in vivo* [77-79]. These findings might be attributable, at least in part, to the induction of apoptosis through the p53-pathway, causing oxidative stress and DNA damage [80-83]. This is followed by activation of the caspase-9 and caspase-3 pathways involving the mitochondrial intrinsic route [80-83], and activation of the JNK pathway subsequent to G_2/M cell cycle arrest [84], respectively.

A number of studies suggest that the tumor growth inhibitory properties of cantharidins could be attributed, at least in part, to anti-angiogenic activity. Cantharidin and some of its derivatives inhibited the proliferation, migration, and invasion of, as well as capillary-like structure formation by cultured endothelial cells [85-88], as well as tumor growth and angiogenesis in various xenograft-mouse models [85-89]. Furthermore, norcantharidin treatment interfered with signaling for vascular development, presumably by inhibiting phosphorylation of critical elements of these pathways [86,91,92]. This led, among others, to a decreased microvessel density and a decreased expression of pro-angiogenic factors such as angiopoietin-2 [88] and upregulation of anti-angiogenic factors such as thrombospondin and tissue inhibitor of metalloproteinase [89]. Notably, norcantharidin also inhibited vasculogenic mimicry [89,90], an important cause of failure of treatment with anti-angiogenic anticancer agents.

The mechanisms underlying these observations probably include, at least in part, potent and selective inhibition of serine/threonine protein phosphatase activities, in particular those of type 1 protein phosphatase and type 2a protein phosphatase [67,93,94]. Together with protein kinases, protein phosphatases act as modulators of many intracellular signaling pathways including those involved in cell cycle progression, apoptosis, cell growth, and vascular development, operating as major 'on' and 'off' switches, respectively, in these pathways [77,84,85]. Clearly, either directly or indirectly manipulating the 'off' switches represented by protein phosphatases may represent an attractive strategy for controlling cancer. In this respect, particularly protein phosphatase 2a has been identified as a tumor suppressor for mainly blood cancers [95]. Thus, cantharidin as well as some of its derivatives may represent very promising lead compounds for a novel class of antineoplastic agents.

Clinical evaluation of cantharidin as an anti-cancer agent was hampered by its relatively short half-life and high toxicity - mostly to kidneys and bone marrow [67] - as well as its poor water-solubility and low oral bioavailability [67]. The problem of the poor water-solubility has been solved by formulating cantharidin as a sodium salt (commercially known as *Qinin*) that has mainly been evaluated in China for its efficacy against various disease conditions including solid tumors [96]. In one such a study, the addition of *Qinin* to chemotherapy did not produce more responses in a cohort of seventy gastric cancer patients, but caused significantly less chemotherapy-related side-effects [97]. The problems of low bioavailability and substantial toxicity may be tackled by delivering cantharidin encapsulated by solid lipid nanoparticles [98] which would carry the drug close to the target, improving its antitumor efficacy and causing less toxicity to the normal tissues.

At the same time, other bioactive substances from blister beetles are

being evaluated in a search for antitumor cantharidins with improved pharmacological properties over those of the parent compound. This has resulted in the isolation and identification of the cantharimides from the Chinese blister beetle *M. phalerata* Pallas (Meloidae) which exhibited protein phosphatase 1 and protein phosphatase 2a inhibitory activities similarly to those of cantharidin [67,68,78,99]. Also, structural modifications of cantharidin have led to several cantharidinimides with retained anticancer activity but less toxicity to non-cancer cells [99].

Particularly norcantharidin displayed interesting features besides countering angiogenesis [87,91,92]: it targeted cancer cells selectively while evoking less toxicity than cantharidin [100]; it overcame multidrug resistance in cultured breast cancer cells by inhibiting sonic hedgehog signaling and P-glycoprotein expression [101-103]; it modulated cancer stem cell self-renewal pathways [104,105]; it acted chemopreventive [106,107]; and it reduced the side-effects of radiation [108,109]. These encouraging results continue to position the Coleoptera and their bioactive products high on the radar of pharmacologists, clinical chemists, molecular biologists, and chemical ecologists who are active in the area of new drug discovery and development.

Bioactive compounds from Diptera

The insect order of the Diptera comprises the so-called true flies and includes an estimated 120,000 described species divided over two suborders [11,12]. The Diptera include many familiar insects such as mosquitoes, midges, house flies, sand flies, gnats, and blow flies which are found in all terrestrial niches with the exception of the polar zones [11,12]. Their name derives from the Greek words '*di*' for 'two', and '*pteron*' for 'wing', as they only use the forewings to fly, the hindwings acting as balancing organs known as halteres. Other distinguishing features of this insect order are their mobile head with a pair of large compound eyes, and mouthparts designed for piercing and sucking or for lapping and sucking [11,12].

Certain dipteran species such as leaf miner flies (family Agromyzidae), fruit flies (families Tephritidae Newman, 1834 and Drosophilidae Rondani, 1856), and gall midges (family Cecidomyiidae) are important agricultural pests, while others such as tsetse flies (family Glossinidae Theobald, 1903) attack livestock causing substantial economic harm [11,12]. Still others such as mosquitos (family Culicidae Meigen, 1818) act as vectors for many infectious diseases like malaria, dengue, chikungunya, zika, West Nile virus, yellow fever, and encephalitis [110]. On the other hand, the Diptera probably are the most important pollinators after the Hymenoptera, particularly in wet and cold environments [11,12].

Many adult dipteran females feed on vertebrate blood which they use as a source of protein for the production of their eggs [11,12]. For this purpose, they pierce the skin of their victim with their sharp stylet and insert a complex cocktail of salivary components into the wound including a host of antihemostatic factors such as anticoagulants, inhibitors of platelet aggregation, anti-inflammatory substances, immunomodulatory components, and vasodilators [111-115]. These substances facilitate blood feeding by helping to overcome host defenses against blood loss, inflammatory reactions, disruption of blood flow, and blood clotting at the feeding site [111-115].

As a result, Diptera have been used for centuries by practitioners of Eastern Medicine to prevent thrombosis [116]. Subsequent studies on these substances led to the identification of, among others, simukunin that may represent the lead compound of a new class of potent anticoagulants.

Simukunin and analogues

Blood coagulation is a complex process that occurs almost immediately following tissue injury, and that involves the aggregation of platelets to form a plug at the site of injury [117]. This is accomplished by the simultaneous activation of an enzymatic cascade primarily consisting of serine proteases that ultimately leads to the formation of fibrin strands which strengthen the platelet plug [117]. Briefly, tissue factor (TF), a protein present in sub-endothelial tissue and leukocytes, binds to the activated blood coagulation factor VII (FVIIa), after which the TF/FVIIa complex activates factor X (FX) to produce factor Xa (FXa) [117]. FXa then combines with activated factor V in the presence of calcium and phospholipids to produce thrombin from prothrombin [117]. Thrombin acts as a serine protease that, among others, converts soluble fibrinogen into insoluble strands of fibrin [117]. Negative regulators of coagulation are primarily protease inhibitors including tissue factor pathway inhibitor (TFPI) [118] that inhibits formation of FXa [118,119].

As mentioned above, blood-feeding arthropods also produce anti-hemostatic factors in their saliva which facilitate blood feeding by interfering with host hemostatic responses [111-115]. A variety of anti-coagulation factors has been isolated from arthropods including the 140- and 308-amino acid proteins ixolaris and penthalaris, respectively, identified in the salivary glands of the deer tick *Ixodes scapularis* Say, 1821 (Ixodida: Ixodidae) [119,120], a vector of Lyme disease [120,121]. These proteins tightly bind to both FX and FXa, strongly inhibiting the TF/FVIIa complex and thus blood clotting [119,120].

Potent anticoagulation factors with activity against thrombin, FXa, and FV were also encountered in the salivary glands of females of the blood-feeding black fly *Simulium vittatum* Latreille, 1802 (Simuliidae) (Figure 5) [122-125]. *S. vittatum* is an important vector of the nematode *Onchocerca volvulus* Bickel, 1982 (Onchocercidae), the causative agent of onchocerciasis or river blindness [126]. One of the anticoagulants identified in the fly's saliva was the polypeptide simukunin [127] that effectively blocked the blood coagulation cascade by inhibiting FXa [128]. Simukunin seems as effective as heparin in preventing the formation of blood clots, but unlike heparin, probably does not cause allergic reactions or thrombocytopenia triggered by immunological attack on platelets [128]. Importantly, this substance also inhibited the serine proteases elastase and cathepsin G and displayed encouraging anti-inflammatory properties [128], providing additional benefits over heparin with respect to antagonizing clot formation and limiting tissue damage caused by inflammatory responses.

Other anticoagulants from the salivary glands of hematophagous insects with potential therapeutic value are tablysin-15, anophelin, and nitrophorin-2. Tablysin-15 is produced by the horsefly *Tabanus yao* Macquart, 1855 (Diptera: Tabanidae) - a vector for filariasis [129]



Figure 5. The black fly *Simulium vittatum* Latreille, 1802 (Diptera: Simuliidae). From: <http://bugguide.net/node/view/624421/bgpage>.

- and inhibits platelet aggregation by interfering with the function of integrins on the platelet membrane [130]. Anophelin is a 6342.4-Da peptide identified in the Central American and African dipterans *Anopheles albimanus* C.R.G. Wiedemann, 1820 (Culicidae) [131] and *A. gambiae* Giles, 1902 [132], respectively, that tightly binds thrombin [131,132]. Nitrophorin-2 (or prolixin S) is a 20-kDa lipocalin in the kissing bug *Rhodnius prolixus* Stål, 1859 (Hemiptera: Reduviidae) [133] that associates with high affinity to either FIX or FIXa [134,135] and generates nitric oxide that causes vasodilatation and inhibition of platelet aggregation at the feeding site [136,137].

Clearly, advances in our understanding of the chemical structures and mechanisms of the components in the salivary glands of arthropods including dipterans, may help in developing novel forms of treatment of conditions requiring the use of anticoagulants such as strokes, cardiovascular blockages, deep vein thrombosis, and pulmonary embolism [117]. In the meantime, ixolaris was found to block intracellular signaling pathways for pro-angiogenic substances in several human carcinoma cell lines while inhibiting the proliferation and metastasis of, and the vascularization of tumor cell grafts in nude mice [138-140]. Apparently, ixolaris also possesses meaningful anti-angiogenic qualities besides useful anticoagulant properties. Whether this also holds true for the substances in the salivary glands of the dipteran species mentioned above - including those of *S. vittatum* - must be verified in future studies.

Bioactive compounds from Lepidoptera

The insect order Lepidoptera consists of about 180,000 different species in 126 families and forty-six superfamilies, and includes moths and butterflies [11,12]. This group of insects comprises roughly 10% of the total number of described species on Earth [11,12] and is, together with the Diptera, Coleoptera, and Hymenoptera, among the largest orders of organisms on our planet [11,12]. The name 'Lepidoptera' is derived from the Greek words '*lepidos*' for 'scale' and '*pteron*' for 'wing', and refers to the scales covering particularly the wings of these insects, giving them their extraordinary variety of colors and patterns [11,12]. The colors and patterns help the animals with, among others, camouflage and mimicry, and act as signals to rivals and potential mates [11,12].

The Lepidoptera are found on all continents except Antarctica, mostly inhabiting terrestrial habitats associated with flowering plants [11,12]. In these ecosystems, many lepidopteran species play vital roles as pollinators [11,12]. This has led to the practice of butterfly rearing, the commercial breeding of butterflies to pollinate crops in greenhouses [141]. On the other hand, moths and butterflies are preyed on by, among others, birds, small mammals, and reptiles [11,12]. Particularly the larvae - caterpillars hatching from eggs laid near or on host plants,

often causing considerable damage to agricultural crops [142] - are easy prey to assailants [11,12]. They defend themselves by camouflage or mimicry, but also by producing noxious and toxic substances, sometimes from secondary metabolites from the plants they feed on [15,16]. Some of these chemicals possess interesting pharmacological properties.

Pierisins

Similarly to many other insect species, lepidopterans are since long used in various traditional medicinal systems. The indigenous Central American peoples, for instance, boiled pupae of the silkworm - the larvae of the silk moth *Bombyx mori* Linnaeus, 1758 (Bombycidae) - to treat apoplexy, aphasy, bronchitis, pneumonia, convulsions, hemorrhages, and polyuria [29]. And in Indian Ayurveda, the larvae from the Jatropha leaf miner *Stomphastis thraustica* Meyrick, 1908 (Gracillariidae) - a major pest to plant species belonging to the genus *Jatropha* L. (Euphorbiaceae) [143] - are boiled and ground to give a paste that is topically applied to induce lactation, reduce fever, and relax an upset gastrointestinal tract [55].

More recently, a series of proteins designated pierisin-1a and -1b as well as pierisin-2 through -5 has been identified in both adults and pupae of butterfly species of the genus *Pieris* (Pieridae) including the cabbage butterfly *P. rapae* Linnaeus, 1758 (Figure 6) [144-147]. The detrimental effects of pierisin-1 on eggs and larvae of endoparasitic wasps that insert their eggs inside *P. rapae* pupae [148] suggest that these compounds function as chemical defenses against microbes and/or parasitoids.

Subsequent *in vitro* studies indicated that pierisins -1 and -2 elicited meaningful cytotoxic and apoptotic effects in various human carcinoma cell lines [144,147,149-152] and suppressed the growth of HeLa cells engrafted in BALB/c nude mice [153]. The induction of apoptosis occurred through a mitochondrial pathway involving Bcl-2 and caspases [152,153].

Notably, pierisin-1 mRNA expression increased during maturation of the pupae and was highly expressed in late-stage pupae, and its protein product accumulated in fat bodies where it persisted during pupation [154,155]. These observations suggest that pierisin-1 plays a critical role during metamorphosis of the pupae by removing unnecessary cells. Thus transforming *P. rapae* pupae into adults, providing support for its presumed ability to cause apoptosis.

Other studies indicated that each pierisin isoform contains a mono(ADP-ribosyl)transferase enzyme domain and receptor-binding domains [146] and that these compounds were able to catalyze the covalent transfer of ADP-ribose moieties from NAD to the N-2 position of guanine bases in DNA [151]. This resulted in the generation of more than 106 ADP-ribosylated DNA adducts per molecule of pierisin-1 [154]. These observations suggest that the cytotoxic and apoptotic actions of pierisins are mediated by the formation of guanine-specific ADP-ribosylated DNA adducts.

Taken together, pierisins and their derivatives, first described as novel DNA-modifying toxins, may play important roles in the treatment of cancer through the activation of apoptosis following the formation of adducts with DNA.

Bioactive compounds from Hymenoptera

The order Hymenoptera comprises the third largest group of insects, consisting of over 150,000 described species including ants,



Figure 6. The cabbage butterfly *Pieris rapae* Linnaeus, 1758 (Lepidoptera:Pieridae). From: <http://www.amateuranthecologist.com/2015/12/a-year-of-pollinators-moths-and.html>.



Figure 7. The honey bee *Apis mellifera* Linnaeus, 1758 (Hymenoptera:Apidae). From: <http://www.insectimages.org/browse/detail.cfm?imgnum=2116051>.

bees, wasps (including hornets), and sawflies [11,12]. The name of the order derives from the Greek words 'hymen' and 'pteron', signifying a key characteristic of this group of insects, viz. the connected hind and fore membranous wings [11,12]. Another morphological feature typical for Hymenoptera - at least in females - is the presence of an ovipositor for inserting eggs into hosts or inaccessible places [11,12]. In many species, the ovipositor is modified to a stinger, and the eggs are laid from the base rather than from the tip which is only used to inject venom to immobilize prey or in defense [11,12].

With the exception of extreme cold, remote, and inhospitable locations, hymenopterans have colonized all terrestrial habitats on Earth [11,12]. As a group, these insects have a wide range of feeding habits. Some are herbivorous, mainly feeding on leaves; bees feed on nectar and pollen; stinging wasps provide their larvae immobilized prey; and the larvae of certain species are parasitoids, feeding on a paralyzed prey after the eggs have hatched inside the victim [11,12].

The venoms from many hymenopteran species comprise a source of numerous bioactive compounds which evolved for prey capture and defense against microorganisms, rival colonies, and predators [157]. The venoms often consist of complex mixtures of bioactive amines including serotonin, histamine, tyramine, dopamine, noradrenaline, and adrenaline; pain-producing peptides such as kinins; and many types of hydrolases such as proteases, hyaluronidases, phosphatases, nucleotidases, and phospholipase A; as well as allergens and neurotoxins [156].

Many ant species produce these compounds in their metapleural glands on their thorax and use them as broad-spectral antimicrobial agents to prevent infections within the colonies [157]. The venoms of most honey bees and wasps (including hornets) also contain antimicrobial peptides [158-160]. Another chemical defense of Hymenoptera includes the venom of the honey bee *Apis mellifera* Linnaeus, 1758 (Apidae) (Figure 7) [161] which contains melittin, a powerful detergent that provokes hemolysis of red blood cells [162]. And wasps (subfamily Vespinae) use their venoms to paralyze and kill prey or defend the nest [163].

When considering the potency of hymenopteran venoms and their relatively high selectivity to their molecular target, it is understandable that these substances have been used medicinally for centuries in various cultures. For instance, traditional Chinese medicine recommends the consumption of the edible black ant *Polyrhachis vicina* Roger, 1863 (Formicidae) to increase virility and fertility, prolong life, delay aging, and enhance mental performance [164]. More recent studies showed encouraging analgesic and anti-inflammatory activities by extracts and fractions from this hymenopteran [165]. Furthermore, the indigenous Central American peoples induced an immunological reaction

via direct stings by the red harvester ant *Pogonomyrmex barbatus* Smith, 1858 (Formicidae) - that causes local inflammation and acts as an anticoagulant [29] - to fight arthritis, rheumatism, polyneuritis, poliomyelitis, and asthma [29]. They also used army ant soldiers (family Formicidae) as stitches to hold together the edges of a wound [29]. For this purpose, the ants were allowed to bite into the wound edges after which their thorax and abdomen were removed, leaving the heads holding the edges together [29]. Later, the antibiotics present in the ants' salivary gland secretions were found to aid in the healing process [29].

Thus, bioactive compounds from the venoms of bees, wasps and ants represent promising lead compounds for developing novel therapeutics against human diseases. Some of these compounds include melittin and lasioglossins from bee species; mastoparan from wasp species; and solenopsin A and analogues as well as myrmexins from ant species.

Melittin

Products from *A. mellifera* such as honey, propolis, royal jelly, and bee bread have been used for centuries in apitherapy on every continent of the world [29,166]. The same holds true for apitoxin (bee venom), a complex mixture of several biologically active proteins and neurotransmitters such as phospholipases A2 and B, hyaluronidase, serotonin, histamine, dopamine, noradrenaline, and adrenaline, some of which can contribute to the clinical signs and symptoms of envenomation [167]. The beneficial properties of apitoxin and several of its components are since long known and have been widely used in Oriental traditional medicine to relieve pain, and to treat inflammatory diseases such as rheumatoid arthritis, multiple sclerosis, and tendonitis [168]. For these purposes, the bee venom was administered by live stings, injection of venom, and venom acupuncture [168].

Apitoxin has also been used for treating cancer [169], skin conditions [170] and even Parkinson's disease [171]. In addition, a purified bee venom preparation called Apitox® has been approved by the FDA as a subcutaneously injectable product for relieving pain and swelling associated with rheumatoid arthritis, tendinitis, bursitis, and multiple sclerosis [172]. The results from a number of clinical studies provided some support for this decision [173,174].

One of the principal components of bee venom is melittin, an amphiphilic linear peptide of twenty-six amino acids with an amino-terminal region that is predominantly hydrophobic and a carboxyl-terminal region that is hydrophilic [175]. As a result, it is water-soluble but yet can spontaneously associate with natural and artificial membranes [176,178]. This feature enables melittin to accumulate in cell membranes, disrupting their phospholipid backbones and causing cell lysis [162,175,177]. Of note, melittin not only induces the lysis of a wide range of plasmatic membranes but also of intracellular



Figure 8. The wild bee *Lasioglossum laticeps* Schenck, 1870 (Hymenoptera:Halictidae). From: <http://www.naturspaziergang.de/Bienen-2.htm>.

membranes such as those of mitochondria [176-178]. Melittin is a powerful stimulator of phospholipase A2 that is also present in bee venom, and the synergistic action of these two substances enforces the cytolytic and cytotoxic effects started by the former compound [189].

These events may lead to the release of other harmful compounds from the damaged cells such as lysosomal enzymes from leukocytes, serotonin from thrombocytes, and histamine from mast cells [167]. However, a large body of preclinical studies also suggest that melittin may elicit multiple pharmacological effects, including antibacterial actions [180,181], antiviral properties [182,183], inhibition of cancer cell growth [184,185], inhibition of cancer cell metastasis [186], interference with angiogenesis [184,187,188], apoptosis-inducing effects [189], radiosensitization [190], immunosuppression [191], anti-inflammatory effects [192,193], as well as anti-atherosclerotic effects [194,195].

The precise mechanisms underlying all these actions remain to be elucidated. However, the antibacterial effects and potential anticancer efficacy of this membrane-active peptide may be related to cytolysis following the activation of phospholipase A2, the induction of pores or perturbations in plasma and subcellular membranes, and the activation of apoptotic pathways such as those mediated by caspases and metalloproteinases [25,162,196]. On the other hand, its presumed ability to inhibit phospholipase A2 [192] suggests that melittin may be useful against inflammatory, rheumatic, and atherosclerotic conditions.

Despite encouraging laboratory data, so far no officially approved medical products for human use that are based on melittin are on the market. Various studies have focused on applications in the field of bacterial and viral infections, inflammatory conditions such as rheumatoid arthritis and arteriosclerosis, and cancer [162,196]. Although the conversion of all these possibilities into therapeutic applications is still far away, these observations underscore the therapeutic perspectives of melittin.

Lasioglossins

Lasioglossins represent a series of three structurally related pentadecapeptides in the venom of the eusocial bee *Lasioglossum laticeps* Schenck, 1870 (Halictidae) (Figure 8) [197]. Lasioglossin I adopted an α -helical structure in a membrane-mimetic environment of dodecylphosphocholine micelles, the polar side chains aligning along one side and the hydrophobic residues along the opposite side of the helical coat [198]. Thus, lasioglossins are membrane-active amphiphilic structures which, comparably to melittin [162,175-178], have the capacity to adsorb to, insert into, readily cross cell membrane bilayers to interact with cytoplasmic constituents [198].

The result is perturbation of biological membranes in a way

comparable to melittin [198,199]. In addition to causing membrane destabilization, lasioglossins may tightly bind to DNA [198]. These actions may be responsible for their strong antimicrobial activity against both Gram-positive and Gram-negative bacteria without causing substantial hemolysis and mast cell degranulation, in addition to cytotoxic activity against various cancer cell lines [197]. Subsequent studies showed that the lasioglossins also displayed encouraging and prompt *in vitro* activity against several *Candida* species [199].

These observations suggest that lasioglossins may represent promising leads for the development of new, effective drugs against bacterial and fungal infections and possibly also against cancer. Their uncommon mechanism of action - perturbation of the structure of membranes and perhaps also that of DNA - may prevent the emergence of resistant microbial strains (and perhaps also that of resistant cancer cells).

Mastoparans

Mastoparans are tetradecapeptides in the venoms of vespid wasps (family Vespidae) [200] and several species of hornets (insects in the genera *Vespa* and *Provespa* residing under the Vespidae) (Figure 9) [201]. Like melittin and lasioglossins, mastoparans are characterized by a hydrophobic and a hydrophilic domain [202] which provides them, similarly to the former compounds, amphiphathic features and the ability to disrupt or cause pores in biological membranes [203,204].

Extensive research over recent decades has shown that mastoparans possess meaningful pharmacological properties [163] including cytolysis following destabilizing of plasma membranes [205]; perturbation of transmembrane signaling by directly interacting with G proteins [203,206-208]; the induction of apoptosis subsequent to the stimulation of phospholipases and mobilization of Ca^{2+} from mitochondria and sarcoplasmic reticulum [209-212].

These properties are probably involved in the antimicrobial studies and antiviral effects of mastoparans seen in preclinical [213-216]. Mastoparans displayed, in addition, noteworthy antitumor activity *in vitro* [203,218,219] and *in vivo* [220]. The main mechanism presumed to underlie these cytotoxic effects is mitochondrial permeability transition initiated by opening of high conductance permeability transition pores in mitochondria, leading to necrosis and apoptosis [209-212]. As a result, however, the toxicities caused by these compounds when used against cancer, may particularly involve mitochondrial permeability transition in normal tissues [221]. Should this holds true, these side-effects may be mimimized by more specifically delivering mastoparans to tumor cell mitochondria encapsulated in a liposomal preparation [221].

Meanwhile, several mastoparan analogues with retained potency but improved pharmacological features have been produced.



Figure 9. The wasp *Vespula* spp. (Hymenoptera: Vespidae). From: <http://www.capetowninvasives.org.za/project/animals/species/vespula-germanica>.



Figure 10. Group of tropical devil-tree ants *Pseudomyrmex triplarinus* Weddell, 1850 (Hymenoptera: Formicidae). From: <https://goo.gl/images/sViBCy>

Examples are the mitoparans which possess greater amphiphilicity when compared to the mastoparans [222], as well as the capacity to selectively bind tumor cell adhesion molecules [223]. Furthermore, a number of other mastoparan peptides called polybia-MPI, polybia-MPII, and polybia-MPIII has been identified in the venom of the Brazilian wasp *Polybia paulista* Ihering, 1896 (Vespidae) [224,225]. Polybia-MPI suppressed the growth of cultured prostate and bladder cancer cells as well as that of multidrug resistant leukemia cells by producing pores in the plasma membranes following interaction with membrane phospholipids [226]. Importantly, this compound induced substantially less cytotoxicity to normal murine fibroblasts [226], suggesting that it had some selectivity for malignant cells. Polybia-MPII and polybia-MPIII caused pronounced lysis of rat mast cells and erythrocytes and displayed antimicrobial activity against both Gram-positive and Gram-negative bacteria [228].

These findings are encouraging and suggest that the mechanistically unique mastoparans will one day be incorporated into every-day clinical practice for treating, among others, microbial infections and cancer.

Myrmexins

The tropical devil-tree ant *Pseudomyrmex triplarinus* Weddell, 1850 (Formicidae) (Figure 10) lives in symbiosis with trees of the South American genus *Triplaris* and protects them from predation by other insects and animals [228]. The ants are very territorial and their sting is extremely painful [229]. The venom has intense phospholipase activity and intermediate hemolytic activity [229]. According to folklore, certain Amazonian tribes punished members who had broken the rules by tying them to a *Triplaris* tree, exposing them to hundreds of ants' stings until they begged for forgiveness for their mistakes [28]. As well, the indigenous people of the Peruvian Amazon treated patients suffering from rheumatoid arthritis by having the afflicted individuals bitten by the ants [28,29].

A relatively recent analysis of the venom of *P. triplarinus* showed that it contained at least twelve proteins including phospholipase, hemolysins, as well as a multi-protein complex designated myrmexins I through VI [230]. Each of the six protein isoforms has a molecular mass around 7,000 Da, and each comprises a heterodimer consisting of a small subunit connected by a disulfide linkage to a larger, structurally unrelated subunit [230]. So far, the biological function(s) of the myrmexins remain(s) unknown.

However, both preclinical [231,232] and a double-blind, controlled clinical study [232,233] have shown that a partially purified extract of the venom from *Pseudomyrmex* spp. decreases pain and inflammation in patients with rheumatoid arthritis and reduces swelling in animal models of inflammation. Further investigations should clarify whether these compounds are indeed useful for treating rheumatoid arthritis

and which myrmexin isoform is best suited as a remedy for this condition.

Solenopsins

Solenopsin A is a piperidine alkaloid in the venom of the so-called red imported fire ant *Solenopsis invicta* Buren, 1972 (Formicidae) (Figure 11). *S. invicta* presumably originates from Brazil and is a major threat to agricultural crops and livestock in various parts of the world [234]. This compound was identified in the 1930s in south-eastern USA during a project aimed at finding ways to exterminate this invasive ant species [234]. Analyses of its venom led in 1979 to the isolation of a number of toxic proteins and piperidines including trans-2-methyl-6-n-undecylpiperidine or solenopsin A [235]. At least some of these substances could be related to the various pharmacological properties of *Solenopsis* venom, including cytotoxic, hemolytic, necrotic, insecticidal, antibacterial, antifungal, anti-HIV, cardiodepressant, and neurologic actions [236].

More recent studies [237] showed that solenopsin A potently inhibited the growth of SVR cells (a transformed murine endothelial cell line) by inhibiting endothelial-specific signaling. This suggested that it has anti-angiogenic activity, which was corroborated by the delayed sprouting of intersomitic vessels from the dorsal aorta it caused in zebrafish [237]. Comparable results were found with solenopsin A and/or its analogues in the p53-deficient renal cell carcinoma cell line 786-O [237] and in various human melanoma cell lines, primary human melanocytes, as well as primary and immortalized human keratinocytes [238].

Studies with mouse embryonic fibroblast cell lines suggested that these effects occurred through the selective inhibition of a series of kinases involved in angiogenesis, most notably phosphatidylinositol-3-kinase (PI3K) and its downstream effector Akt [237,239,240]. These signaling elements play major regulatory roles in the control of apoptosis, proliferation, and angiogenesis [240-242], and are amplified or overexpressed in a number of malignancies [240,241, 243]. The PI3K/Akt pathway regulates the production of the potent angiogenic factor VEGF [240,242], and protects tumor cells against both chemotherapy and reactive oxygen-induced apoptosis [239,240,244].

When considering the selective targeting of Akt by solenopsin and its analogues, these compounds may have potential utility as anti-angiogenic antineoplastic agents. In fact, various inhibitors of PI3K/Akt such as the miltefosin analogue perifosine have undergone clinical testing in patients with advanced forms of cancer [245]. However, unsatisfactory response rates in several phase III studies led to the discontinuation of perifosine as an antineoplastic agent [246]. These disappointing results with perifosine emphasize the importance of solenopsin and its analogues as potentially novel Akt inhibitors for anti-angiogenic anticancer therapy.

Concluding remarks

This paper has reviewed the literature on the potential clinical applicability of a number of bioactive compounds from medicinal insects. Although only very few of these products have reached the clinic today, the results obtained so far are encouraging. Isocarbostryl alkaloids from grasshoppers, cantharidin and its derivatives from blister beetles, pierisins from butterfly larvae, as well as solenopsin A and its analogues from ant species may represent lead compounds of entirely new classes of anticancer agents [48,78,79,67,99-101,144,149,237,238]. Termicin and spinigerin from termite species,



Figure 11. The red imported fire ant *Solenopsis invicta* Buren, 1972 (Hymenoptera: Formicidae). From: <https://goo.gl/images/stX3bM>.

melittin and lasioglossins from bee species, as well as mastoparans from wasp species may help fight microbial and parasitic infections more effectively [58,59,62,64,180-183,197,199,213,216]. Simukunin and its analogues from certain blood-feeding insect species show us a way to develop unprecedented anticoagulants [127,128]. And myrmexins from ant species possess encouraging anti-inflammatory properties that are much needed in the clinic [231-233].

Admittedly, these findings are rather modest when viewed against the background of the massive array of extant insect species on Earth. Nevertheless, they underscore the significance of insect-derived substances for new drug discovery and development programs. Other examples besides those extensively addressed in this paper are the readily available and cost-effective anti-inflammatory glycosaminoglycans from the cricket *Gryllus bimaculatus* De Geer, 1773 (Orthoptera: Gryllidae) [247] and the powerful proteolytic enzyme mantis egg fibrolase (MEF) from the egg cases of the Chinese mantis *Tenodera sinensis* (Saussure, 1871) (Mantodea: Mantidae) [248]. Furthermore, products from the American cockroach *Periplaneta americana* Linnaeus, 1758 (Blattodea: Blattellidae) [249], certain dipteran species [250], and the larvae of the dobsonfly *Protohermes grandis* Thunberg, 1781 (Megaloptera: Corydalidae) [251] displayed broad-spectrum *in vitro* antibiotic activity, including activity against methicillin-resistant *Staphylococcus aureus*. Notably, the insect antimicrobial peptide cecropin from the housefly *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae) elicited interesting anticancer effects in a cell culture model [252]. And the development of bandages, gels, or lotions laced with purified debriding enzymes from the green bottle fly *Lucilia sericata* Meigen, 1826 (Calliphoridae) is anticipated to improve maggot debridement therapy [253].

However, drug discovery and development programs based on medicinal insects face a number of unprecedented obstacles. Firstly, the huge diversity of insect species makes it difficult to distinguish between species having potential therapeutic applicability and those with no apparent medicinal value. Indeed, with so many species altogether, it will take much time and many resources to separate the wheat from the chaff. Incidentally, this is probably an important reason for the failure of several bioprospecting endeavors such as the 1991-multimillion-dollar venture of the Costa Rican National Biodiversity Institute and the pharmaceutical giant Merck & Co. committed to the exploration of bioactive compounds from, among others, insects in the Costa Rican rainforests [254].

Secondly, insects are relatively small and, accordingly, produce only minute amounts of bioactive compounds. For instance, certain blister beetles have cantharidin loads of up to 35 µg per individual [26], and a honey bee holds 0.15 to 0.3 mg of venom in its venom sac, about half of it representing mellitin [255]. This necessitates the collection of vast numbers of animals to obtain sufficient material for mechanistic evaluations, animal pharmacological studies, and if warranted, clinical trials. Obviously, such an endeavor is highly problematic when the bioactive compound of interest is produced by species living solitary or in small groups. As well, many natural products including those from insect have very complex structures, making total synthesis not always feasible or cost-effective [256], while culturing of the desired insects is in many cases not a valid option due to the lack of information about the living conditions and the feeding habits of most species [11,12].

Furthermore, climate change, particularly global warming phenomena caused by certain human activities, may destroy the habitats of many insect species, threatening their continued existence

[257]. And although the medicinal use of insects has been well-documented in, among others, India, China, and Korea [258-260], the preservation of traditional knowledge about medicinal insects is in many cultures far from guaranteed.

Clearly, conversion of the therapeutic potential of medicinal insects into contemporary efficacious medicinal applications will require a collaborative effort of governments, holders of traditional knowledge, scientists, and pharmaceutical companies. Hopefully, such enterprises will eventually overcome the tremendous obstacles mentioned above, and lead to the full exploration of the valuable compounds represented by this largely unexplored source of bioactive substances and the development of structurally novel and mechanistically unique drugs to more effectively manage human diseases.

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