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ANTIBIOTICS FROM *Xenorhabdus* spp. AND *Photorhabdus* spp. (ENTEROBACTERIACEAE) (REVIEW)

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Xenorhabdus spp. and *Photorhabdus* spp. (Enterobacteriaceae), bacterial symbionts of the entomopathogenic nematodes *Steinernema* spp. and *Heterorhabditis* spp., respectively, are a unique, natural source of novel antibiotics. Several groups of antibiotics, such as xenorhabdins, xenorxides, xenocoumacins, indole derivatives including nematophin, genistein, stilbene derivatives, and anthraquinone derivatives in addition to bacteriocins, xenorhabdicin (phage tail-like bacteriocin), phages, and chitinases have been reported since the early 1980s. The antibiotics have not only shown promising activity against a variety of bacterial and fungal pathogens of medicinal and agricultural importance, including that against clinical-resistant strains of *Staphylococcus aureus*, but some also have shown other activities such as insecticidal, nematicidal, antiulcer, and anticancer activity. The suggested mechanisms of action of the antibiotics include inhibition of RNA and protein synthesis. These naturally occurring antibiotics provide useful leads in the research and development of drugs and agrochemicals. This review summarizes the chemistry and biology of these antibiotics with emphasis on the authors' work.

INTRODUCTION

The excess use and the consequent adverse effects of synthetic pesticides in the environment and of synthetic pharmaceutical drugs in humans is reported with increasing frequency [1-4]. Of particular concern is the increasing frequency of reports of the development of multi-drug resistance of humans to many bacterial pathogens, and this is resulting in the lives of many patients being in danger or lost [1, 2, 5, 6]. There is, therefore, an urgent need for new agrochemicals and antimicrobial drugs [4]. *Xenorhabdus* spp. and *Photorhabdus* spp. (Enterobacteriaceae), bacterial symbionts of the entomopathogenic nematodes *Steinernema* spp. and *Heterorhabditis* spp., respectively, are natural sources of novel antibiotics. One major advantage that some of these new antibiotics have over many of those currently in use is that they are not structurally related to current, clinical antibiotics, such as penicillin [7]. Some of the antibiotics derived from *Xenorhabdus* and *Photorhabdus* not only showed excellent activity against some clinical, multi-drug-resistant strains of bacterial pathogens, such as *Staphylococcus aureus* [8], but they also showed a broad spectrum of activities including insecticidal, nematicidal, antiulcer, and anticancer activities [9-13]. This review addresses the antibiotic compounds derived from the bacterial cultures of *Xenorhabdus* spp. and *Photorhabdus* spp. with emphasis on the chemical properties and bioactivities of the antibiotic compounds.

Xenorhabdus spp. and *Photorhabdus* spp.

Xenorhabdus spp. and *Photorhabdus* spp. are unique genera of bacteria that are symbiotically associated with the entomopathogenic nematodes *Steinernema* spp. and *Heterorhabditis* spp., respectively [14-16]. The infective ju-

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veniles of the nematode symbionts living in the soil carry the symbiotic bacteria in their gut [17, 18]. They release the bacterial cells into the insect's haemocoel after entering the insect by way of natural openings (*Steinernema* spp.) or by boring directly through the insect cuticle (*Heterorhabditis* spp.). The bacterial cells multiply in the haemocoel and, with the help of nematode symbiont, overcome the insect's defense system and kill the insect host, usually within 24-48 h. Bacterial breakdown of the insect's tissues provides nutrients and an optimal environment for nematode development. Subsequently, new generations of infective juveniles emerge from the insect cadaver carrying some cells of the bacterial symbiont in their gut. They then seek out another insect host in order to start a new infection cycle.

Xenorhabdus spp. and *Photorhabdus* spp. are Gram-negative, facultatively anaerobic rods, classified within the family Enterobacteriaceae [19, 20]. Five species of *Xenorhabdus* have been described, namely *X. nematophilus*, X. *bovenii*, *X. poinarii*, *X. beddingii*, and *X. japonicus*, and one species of *Photorhabdus* (probably a polyspecies), *P. luminescens* [21, 22]. These bacteria have been isolated only from entomopathogenic nematode-infected insects, infective juveniles of the nematode symbiont, and, a rare record, from a human wound [23]. Most species of *Xenorhabdus* and *Photorhabdus* have been found to have two forms. The primary form cells are natural symbionts of entomopathogenic nematodes which can be isolated from nematode-infected insects and the infective juveniles of the nematode. They produce antibiotics when in aerobic *in vitro* culture [24] and in infected insect larvae [12, 25]. The primary form is unstable in *in vitro* culture where it reverts to the secondary form after about 2 weeks. The secondary form cells differ in many respects from the primary form, and are not common natural symbionts of the nematode. They occur under certain culture conditions, such as prolonged incubation or low-osmolarity [26], and usually do not produce antibiotics [24].

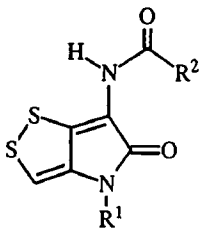
A significant biological phenomenon of this nematode–bacterium–insect complex is that the bacteria produce a variety of antibiotics under both *in vitro* and *in vivo* conditions [12, 25, 27, 28]. The reason for the production of the antibiotics is not clear. However, it is generally believed that the antibiotics help to maintain an optimal environment for the developing nematode in the cadaver relatively free from competition from other bacteria, fungi, and nematode species. Once killed, the infected insect host is a ripe target for fungi and other decay-inducing organisms in the soil or in the insect gut. The bacterial symbionts produce a variety of broad-spectrum antibiotics that prevent putrefaction and enable nematode development relatively free from competition [16, 18, 29].

IDENTIFICATION OF THE ANTIBIOTICS

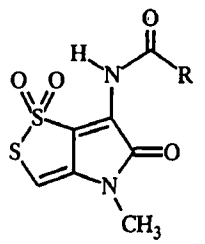
Dutky [30] was the first to note that the bacteria associated with the entomopathogenic nematode, DD-136 strain of *Neoaplectana carpocapsae* (now named *S. carpocapsae*), produces antibiotics that inhibit the putrefaction of insect cadavers 5 infected with *S. carpocapsae*. Paul et al. [31] reported the first two groups of antibiotics, namely stilbene and indole derivatives (Table 1), from bacterial cultures of *Xenorhabdus* and *Photorhabdus*. The commercialization and partial success of the entomopathogenic nematodes as biocontrol agents against a variety of insect pests [32], and the biological complexity of the symbiotic association between the nematode and bacterial symbiont have led to more detailed studies over the past few years. Consequently, several novel antibiotics have been isolated from different species of these bacteria [8-10, 27, 28, 31, 33]; these are summarized in Table 1 and their structures are illustrated below.

Most species of *Xenorhabdus* and *Photorhabdus* produce more than one group of antibiotics, but the antibiotics are less diverse from *Photorhabdus* than those from *Xenorhabdus* (Table 1). Antibiotics from these bacterial symbionts are highly active against Gram-positive bacteria but less active against Gram-negative bacteria, and some of them are also active against fungi (Table 2) [9, 10, 27, 28]. Other antimicrobial agents, such as phages and some proteinaceous components, are selective against closely related *Xenorhabdus* spp., and other bacteria and fungi [29, 34-37].

The indole derivatives are presumably produced via tryptophan and the stilbenes via polyketide pathways [31]. Nematophin is most likely derived from tryptophan and isoleucine, and xenorxides are obviously the oxidized products of xenorhabdins [38]. The production of such a variety of stilbene and anthraquinone derivatives by *P. luminescens* under *in vitro* and *in vivo* conditions [25, 28] is unusual, because they are not common, secondary metabolites of bacteria [39-43]. Some of the pigments have antibiotic activity [28, 44].



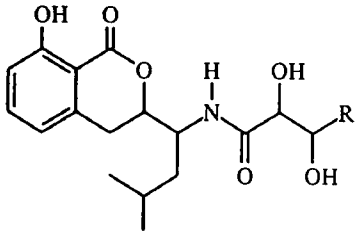
a: Xenorhabdins



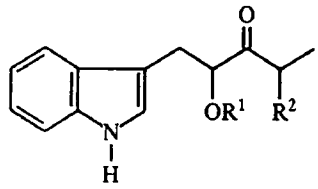
b: Xenorxides

I $R^1 = H$, $R^2 = n\text{-}C_5H_{11}$;
II $R^1 = H$, $R^2 = (CH_2)_3CH(CH_3)_2$;
III $R^1 = H$, $R^2 = (CH_2)_3CH(CH_3)_2$
IV $R^1 = CH_3$, $R^2 = n\text{-}C_5H_{11}$;
V $R^1 = CH_3$, $R^2 = (CH_2)_3CH(CH_3)_2$;
VI $R^1 = CH_3$, $R^2 = CH_2CH(CH_3)_2$
VII $R^1 = CH_3$, $R^2 = CH_2CH_2CH_3$

VIII $R = n\text{-}C_5H_{11}$
IX $R = (CH_2)_3CH(CH_3)_2$



c: Xenocoumacins:



d: Indole derivatives

X $R = CH_3(CH_2)_3—NH—C(NH_2)NH$

XI R = 

XII $R^1 = H$, $R^2 = CH_3$;
XIII $R^1 = H$, $R^2 = CH_2CH_3$;
XIV $R^1 = Ac$, $R^2 = CH_3$;
XV $R^1 = Ac$, $R^2 = CH_2CH_3$

BIOACTIVITY OF THE ANTIBIOTICS

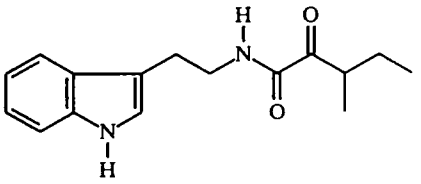
Antimicrobial Activity

As more strains and species of *Xenorhabdus* and *Photorhabdus* were studied, the known spectrum of antimicrobial activity of the metabolites has increased [9, 10, 24, 27, 31, 45]. *In vitro* tests on microorganisms using either cell-free culture broth or solutions of pure compounds derived from these bacteria showed that the growth of many yeasts, fungi, and bacteria, including many of medicinal and agricultural importance, was inhibited [8-10, 24, 27, 28, 31, 45, 46]. The activity of some of these antibiotics is listed in Table 2. The strong antibiotic activity of xenorxides and nematophin, with minimum inhibition concentrations (MICs) of less than 2.0 μg/ml against drug-resistant bacteria, and their relative ease of production by biological or chemical means could make these two groups of chemicals excellent lead compounds for the development of pharmaceutical drugs [7, 8, 38].

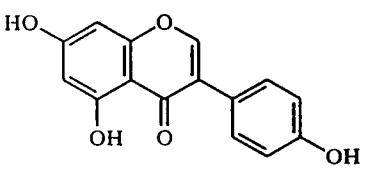
As expected, antibiotics differ in their antimicrobial spectra (Table 2) [10, 27, 28]. Nematophin (XVI), for example, is active against clinical, multidrug-resistant strains of *S. aureus* but not against the plant pathogenic fungus, *Aspergillus fumigatus*. However, xenorxides VIII and IX are active against both *S. aureus* and *A. fumigatus* (Table 2). The insect pathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* have been reported to be resistant to the cell-free culture filtrate of *Xenorhabdus* spp. and *Photorhabdus* spp. [45], although this was contrary to the observations of Barbercheck and Kaya [47]. Olthof et al. [48] noted that an inundative application of *S. feltiae* and *H. bacteriophora* to control a sciarid fly infection on mushrooms in glasshouses led to a decline in growth of the mushroom mycelium. This may have been due to the antimycotic activity of the secondary metabolites of their

respective bacterial symbionts. Also, Maxwell et al. [46] noted that xenocoumacins X and XI which leaked from infected insect cadavers into the soil temporarily diminished the population of soil bacteria. Consequently, further study on the impact of these secondary metabolites on different microorganisms, including beneficial ones in the soil, is needed prior to their development by the agrochemical industry.

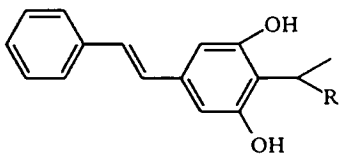
In greenhouse tests in which an organic extract of the culture broth of *X. bovienii* was applied as a spray (10 mg/ml) to the foliage of 4- to 5-week-old potted potato plants (cv. Norchip) infected with *Phytophthora infestans* (potato blight), significant reduction of the symptoms was achieved. The 10 mg/ml treatment inhibited ($P \geq 0.05$) blight 7 days after inoculation with only 4% leaflets showing symptoms of fungal infection, compared with 100% in the controls [49].



e: Nematophin XVI

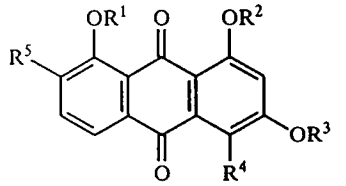


f: Genistein XVII



g: Stilbene derivatives

XVIII R = H; XIX R = CH_3



h: Anthraquinone derivatives

XX	R^1 = H,	R^2 = H,	R^3 = CH_3,	R^4 = H,	R^5 = H
XXI	R^1 = H,	R^2 = CH_3,	R^3 = H,	R^4 = H,	R^5 = H
XXII	R^1 = H,	R^2 = H,	R^3 = H,	R^4 = H,	R^5 = H
XXIII	R^1 = H,	R^2 = CH_3,	R^3 = CH_3,	R^4 = H,	R^5 = H
XXIV	R^1 = CH_3,	R^2 = H,	R^3 = CH_3,	R^4 = H,	R^5 = H
XXV	R^1 = H,	R^2 = CH_3,	R^3 = CH_3,	R^4 = H,	R^5 = OCH_3
XXVI	R^1 = CH_3,	R^2 = H,	R^3 = CH_3,	R^4 = OH,	R^5 = H

Other Bioactivities

These antibiotics are unusual in the breadth of the spectrum of their bioactivity, namely insecticidal, nematicidal, antiulcer, and anticancer activity (Table 1).

The insecticidal activity of xenorhabdin II was demonstrated in larval feeding assays against *Heliothis punctigera* where 100% mortality was achieved at 150 $\mu g/cm^2$ (LC_{50} was 59.5 $\mu g/cm^2$) [9]. Xenorhabdin II at lower concentrations considerably reduced the weight of the surviving larvae. Dudney [50] found that 24 h-old culture broth of *X. nematophilus* killed the fire ant, *Solenopsis invicta*, when applied as a spray, or by pouring directly onto the ant mounds. Such results suggest that *Xenorhabdus* and *Photorhabdus* have the potential to be used directly for insect pest control. An extracellular proteinaceous toxin has been isolated from *P. luminescens* which when ingested by insects in very small amounts (ng) can be fatal to a variety of insects, including ants [51]. With reports in recent years of emerging pest resistance to Bt, a bacterial product of *Bacillus thuringiensis* that is well known for its insecticidal activity, such a toxin from *Photorhabdus* could have the potential to be the next generation of microbial insecticides especially if the genes could be engineered into crop plants [13].

Hu et al. [11, 52, unpubl.] discovered the nematicidal property of a stilbene derivative XIX, and other compounds from cultures of *Xenorhabdus* spp. and/or *Photorhabdus* spp. The stilbene derivative is active against bacterial- (eg. *Caenorhabditis elegans*) and fungal-feeding nematodes (eg. *Aphelenchoides rhyntium*, *Bursaphelenchus* spp.) and some other compounds are also active against the economically important root-knot nematode, *Meloidogyne incognita* (K. Hu, unpubl.).

TABLE 1. Bioactive Agents Associated with or Derived from the Symbiotic Bacteria *Xenorhabdus* spp. and *Photorhabdus* supp.*

Bioactive agents	Bacteria	Activity*	References
Xenorhabdins			
I	*X. nematophilus* *X. bovienii*	1, 2	9
II	*X. nematophilus* *X. bovienii*	1, 4	9
III, IV	*X. nematophilus* *X. bovienii*	1	9, 27
V	*X. bovienii*	1, 2	9, 27
VI, VII	*X. bovienii*	1	27
Xenorxides			
VIII, IX	*X. bovienii*	1, 2	38, 53, 62
Xenocoumacins			
X, XI	*X. nematophilus*	1,2, 3	10
Indole derivatives			
XII—XV	*Xenorhabdus* sp. *X. bovienii*	1, 2	27, 31
Nematophin			
XVI	*X. nematophilus*	1, 2	8, 13, 54, 62
Genistein			
XVII	*P.luminescens*	1	44
Stilbene derivatives			
XVIII	*P. luminescens*	1	31
XIX	*P. luminescens*	1, 2, 5	11, 28, 31, 33
Anthraquinone derivatives			
XX—XXIV	*P. luminescens*	1	28, 33, 44
XXV, XXVI	*P. luminescens*	unknown	25
Phages			
	X. nematophilus *X. bovienii* *X. beddingii* *P. luminescens*	1	17, 35, 36
Bacteriocins			
	X. beddingii *X. bovienii* *X. nematophilus* *Photorhabdus* spp.	1	35, 36
Xenorhabdicin			
	X. nematophilus	1	61
Chitinases			
	X. bovienii *X. nematophilus* *P. luminescens*	2	37
Protein toxin	*P. luminescens*	4	51

* 1 — antibiotic,
2 — antimycotic,
3 — antiulcer,
4 — insecticidal,
5 — nematicidal.

TABLE 2. Minimum Inhibitory Concentrations (MICs) of Some Antibiotics from *Xenorhabdus* spp. and *Photorhabdus* spp. Against Selected Microorganisms of Medicinal and Agricultural Importance

Microorganisms	MIC (μg/ml)						Reference
	II	VIII	IX	X	XVI	XIX	
Aspergillus fumigatus ATCC 13073		0.75	1.5		>100	12	8, 28, 62
Bacillus cereus BTA 432	3.13						9
Corynebacterium xerosis NCTC 9755				1			10
C. neoformans				0.125			10
Escherichia coli ESS				0.5			10
E. coli 10418				2.5			10
Micrococcus luteus BAT 433	0.156						9
Sarcina lutea ATCC8740				1			10
Staphylococcus aureus ATCC 29213		3	3		0.75		8, 62
S. aureus 0012*		0.75	0.75		1.5		8, 62
S. aureus 0017*		0.75	1.5		1.5		8, 62
Streptococcus pyogenes IMR-RNSH	1.25						9
S. pyogenes CN 10				1			10

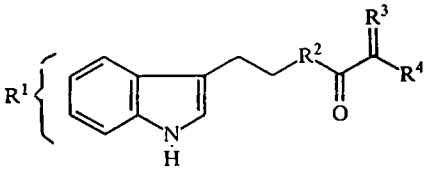
* Clinical, multi-resistant strains.

Antiulcer activity by xenorcoumacins X and XI [10], and anticancer activity [53] by some of the metabolites of *Xenorhabdus* spp. suggest significant pharmaceutical potential. Very recently, a series of new heterocyclic metabolites from selected strains of *Xenorhabdus* spp. have been isolated [38, Li and Hu, unpubl.], and they too may well add to the potential useful array of pharmacological products.

STRUCTURE-ACTIVITY RELATIONSHIP

Several derivatives have been reported of the some of these metabolites from *Xenorhabdus* spp. and *Photorhabdus* spp. (Table 1) [9, 10, 27]. However, there has been little work reported on the structure-bioactivity relationship of the derivatives. Li *et al.* [54] demonstrated that several analogues of nematophin XVI have strong antistaphylococcal activity. Of the 11 analogues tested, nematophin XVI and compounds XXX and XXXIII had MICs of 0.7 μg/ml against *S. aureus* ATCC 29213, compounds XXIX, XXXI, and XXXII had MICs of 3 μg/ml, and the rest were less active or inactive. All the analogues with an α-carbonyl acyl group, such as nematophin, and compounds XXVII—XXXIV, exhibited antibiotic activity. When the α-carbonyl acyl group was transferred to the corresponding α-hydroxy acyl group as in compound XXXV, or was reduced to the corresponding α-hydroxy acyl group as in compound XXXVI, the bioactivity disappeared or was dramatically decreased. This result demonstrated clearly that the conjugated carbonyl acyl group in nematophin and its analogues was essential for their antistaphylococcal activity. The substitutes on the indole ring systems, as shown in nematophin and compounds XXI—XXXIII, have some limited effect on the bioactivity. The result suggests that the ring system could be further modified to improve its solubility and/or bioavailability without losing its bioactivity. Such changes in the side chain have a significant effect on the molecule's bioactivity. The compounds with a branched chain, namely nematophin, and compounds XXIX and XXX, were more active than those with a straight chain, namely XXVII and XXVIII. Also, the change of an amide group, as in nematophin, to the corresponding ester group, as in compound XXXIV, somewhat decreased the bioactivity. Ongoing study of the structure-activity relationship of nematophin and of other antibiotics from *Xenorhabdus* and *Photorhabdus* spp. may lead to clinically important antibiotics being developed.

Structures of nematophin analogues used in the study of structure-activity relationships



	R^1	R^2	R^3	R^4
XXVII	H	NH	O	CH_3
XXVIII	H	NH	O	CH_2CH_3
XXIX	H	NH	O	$CH(CH_3)_2$
XXX	H	NH	O	$CH_2CH(CH_3)CH_3$
XXXI	5-CH_3	NH	O	$CH(CH_3)CH_2CH_3$
XXXII	5-CH_3O	NH	O	$CH(CH_3)CH_2CH_3$
XXXIII	6-F	NH	O	$CH(CH_3)CH_2CH_3$
XXXIV		O	O	$CH(CH_3)CH_2CH_3$
XXXV		NH	$NOCH_3$	$CH(CH_3)CH_2CH_3$
XXXVI		NH	OH+H	$CH(CH_3)CH_2CH_3$

MECHANISM OF ACTION OF THE ANTIBIOTICS

Sundar and Chang [55, 56] investigated the activity and mechanism of action of indole and stilbene derivatives from *Xenorhabdus* and *Photorhabdus*. The derivatives were effective against both Gram-positive and Gram-negative bacteria, causing a severe inhibition of RNA synthesis by inducing an accumulation of the regulatory nucleotide, guanosine-3′,5′-bis-pyrophosphate in susceptible bacteria. Though the mechanism of action of xenorhabdins, dithiolopyrrolone derivatives, from *Xenorhabdus* has not been studied, similar dithiopyrrolones identified from *Streptomyces* spp. have been shown to cause membrane stabilization and platelet aggregation in animals [57], and inhibition of RNA and protein synthesis in yeast [58, 59]. The apparent specificity of nematophin for *S. aureus*, but not *Micrococcus leteus*, under laboratory culture conditions is unusual, especially as it also has antimycotic activity. This in itself may reflect a novel mode of action [7].

PRODUCTION OF THE ANTIBIOTICS

Xenorhabdus spp. and *Photorhabdus* spp. have been cultured successfully on many microbiological media, nutrient broth [24], Luria-Bertani broth [60], and tryptic soy broth [28]. The production of antibiotics by these bacteria changes qualitatively and quantitatively depending on the composition of the medium and the culture conditions. Amounts of nematophin XVI as high as 600 μg/ml of broth could be obtained from *X. nematophilus* cultured in tryptic soy broth [8]. However, little is know about the chemical nature of these antibiotic metabolites which are produced by these bacteria in infected insect hosts. Xenocoumacins X and XI were shown to be produced in a 1:1 ratio in larval cadavers of *G. mellonella* infected with *X. nematophilus* at a total concentration X, XI of 800 ng/ 200 mg (wet weight) of insect tissue [46]. Hu et al. [12] reported that the concentration of the stilbene derivative XIX in nematode-infected *G. mellonella* larvae could be as high as 4,000 μg/g wet insect tissue. No antibiotics were produced in infected *G. mellonella* larvae within the first 24 h of infection (25°C), but the antibiotic concentration increased gradually thereafter, and maintained a relatively stable level after the nematode symbiont had ceased reproducing (K. Hu, unpub). Also, Hu et al. [25] reported that when comparing the *in vitro* and *in vivo* production of antibiotic metabolites by *P. luminescens* they found that an additional stilbene XVIII, more anthraquinone derivatives were present in infected insects but absent from *in vitro* culture broth. The difference of *in vitro* and *in vivo* antibiotic production is most likely due to the difference in nutrient or growth conditions for the bacterium. These results may explain why broader

spectrum and stronger antimicrobial activity was observed for crude extract from bacteria-infected insect than that from *in vitro* culture [46].

CONCLUSION

Xenorhabdus spp. and *Photorhabdus* spp. represent a natural and unique source of novel antibiotics with a broad spectrum of bioactivity including antimicrobial, insecticidal, nematicidal, antiulcer, and anticancer activities. It is remarkable that such a variety of antibiotic products have been detected in only a few strains and species of bacteria. Not only do these bacterial metabolites show excellent antimicrobial activity but so do their analogues. Their promising potential as lead compounds for development of medicinal and agrochemical products needs to be fully explored. It is expected that ongoing research in this field will help mankind in the battle against disease and pestilence.

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