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Insights in Fungal Bioprospecting in Mexico*

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ABSTRACT

Fungi have consistently been one of the richest sources of natural products, with unprecedented chemical scaffolds and potent biological activities. During the last 20 years, pharmacognosy researchers in Mexico, in collaboration with mycologists, have discovered many novel bioactive fungi natural products and new fungal species. To date, more than 100 bioactive secondary metabolites from 20 fungi from different ecosystems throughout Mexico have been documented in peer-reviewed literature according to Scopus and SciFinder databases. These include compounds from different biosynthetic origins and structural cores with the potential for the development of anticancer, antidiabetic, and/or pesticide agents.

Introduction

Fungi represent a prized source of natural product bioactive compounds with an enormous and relevant impact in human medicine and crop protection. Some important drugs and products for agriculture have been derived from or inspired by fungal natural products; some important examples include β-lactam antibiotics, griseofulvin, cyclosporine, statins, echinocandins, PF1022A, nodulisporic acid, scyphostatin, and strobilurins. According to new evidence, the number of fungal species might be between 2.2 and 3.8 million, with 120 000 currently accepted [1]. Since the estimations of fungal biodiversity exceed, by far, the number of already identified species, chances of finding unidentified fungal species and novel bioactive products are still high [2]. Furthermore, the advances in genomic analysis techniques have permitted the discovery of innumerable putative gene clusters for potentially bioactive compounds.

The knowledge of fungal biodiversity in Mexico is still poor. A recent analysis proposed that there are 200 000 fungal species, but only 5% of these have been studied [3]. Consequently, the potential of fungal biodiversity from Mexico as a source of bioactive compounds remains largely unexplored. Thus, the aim of this paper is to provide an overview on the status of fungal bioprospecting in Mexico in the last two decades, considering only the work pursued in the authors laboratories at the National Autonomous University of Mexico. Twenty fungi have been investigated from the biological and chemical point of view, leading to the isolation of over 100 bioactive secondary metabolites of medicinal and/or agrochemical relevance. This information has been reported in over 50 peer-reviewed publications according to Scopus and SciFinder databases. In the medicinal area, the studies were focused on the discovery of new leads for the development of antitumoral, Ca^{2+}-calmodulin (CaM) antagonists, and antidiabetic drugs with α-glucosidase inhibitory activity. In the field of agrochemicals, the studies emphasized the discovery of potential herbicidal, antifungal, and anti-oomycete agents.

The fungi discussed in this review were isolated from diverse substrates, mainly from plants (endophytes), dung, soil, insects, and marine environments belonging to different ecosystems in Mexico. Bioprospecting of endophytes from wild medicinal plants is a successful strategy for the discovery of novel and known bioactive compounds [4]. Accordingly, we analyzed endophytes from

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selected medicinal plants, including *Hintonia latiflora* (Sessé & Moc. ex DC.) Bullock (Rubiaceae), *Bursera simaruba* (L.) Sarg. (Burseraceae), *Callicarpa acuminata* Kunth. (Verbenaceae), *Haematoxylon brasiletto* H. Karst. (Fabaceae), *Sapium macrocarpum* Muell. Arg. (Euphorbiaceae), and *Gliricidia sepium* (Jacq.) Kunth ex Walp. (Fabaceae). The endophytic fungi reviewed includes strain MEXU 27095 (Chaetomiaceae), *Preussia minimoides* (S. I. Ahmed & Cain) Valldos. & Guarro (Sporormiaceae), *Xylaria feejeensis* (Berk.) Fr. (Xylariaceae), *Edenia gomezpompae* M. C. González, Anaya, Glenn, Saucedo & Hanlin (Pleosporaceae), *Muscodor yucatanensis* M. C. González, Anaya, Glenn & Hanlin (Xylariaceae), *Acremonium camptosporum* W. Gams (Clavicipitaceae), *X. feejeensis* SM3e-1b, and *Hypoxylon anthochroum* Berk. & Broome strains Blaci and GS4d2II (Xylariaceae).

Coprophilous fungi are a large group of saprotrophic fungi, which represent an important reservoir of useful novel bioactive secondary metabolites. The number of dung fungi undergoing investigation is continuously increasing with new species and genera constantly described [5, 6]. On these grounds, a few coprophilous fungi isolated from bat dung in different regions of Mexico have been investigated in our laboratories. Some of these fungi include *Penicillium spathulatum* B35 Frisvad & Samson (Trichocomaceae), *Penicillium* sp. G1-a14, *Guanomyces polythrix* M. C. González, Hanlin & Ulloa (Chaetomiaceae), and *Malbranchea aurantiaca* Sigler and Carmich (Myxotrichaceae).

Marine fungi (*sensu stricto*) or those isolated from a marine substratum are a rich source of new natural products with interesting chemical scaffolds and potent biological activities [7], therefore *Aspergillus stromatoides* Raper & Fennell (Trichocomaceae), *Aspergillus* sp. MEXU 27854, and *Emericella* sp. MEXU 25379 (Trichocomaceae) were analyzed. Among the entomophatogenic fungi, we have only investigated *Isaria fumosorosea* Wize (Clavicipitaceae), a fungus currently used for the biological control of whiteflies (*Bemisia tabaci* Gennadius). Phytopathogenic fungi are known to be involved in several plant diseases that damage agrarian and forest crops, besides having general adverse effects on wild plant species. These damages are produced by toxins which, in many cases, have been used to obtain products for plant protection. Based on this consideration, we investigated *Phoma herbarum* Westend. (Pleosporales). Finally, two saprophytes were investigated, *Malbranchea flavorosea* Sigler & J. W. Carmich. (Myxotrichaceae), isolated from soil, and *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samso (Ophiocordycipitaceae).

Alpha-Glucosidase Inhibitors

Type II diabetes mellitus is a disease with skyrocketing prevalence. The number of patients today, ~ 380 million, will increase to ~ 450 million in 2030. In this scenario, extraordinary efforts have been made to identify lead compounds for the development of new drugs for treating this disease. Metformin remains the first-line pharmacotherapy for patients with type II diabetes mellitus, whereas the use of other well-established agents, such as sulfonylureas, meglitinides, thiazolidinediones, and α-glucosidase inhibitors, varies in different regions [8]. In areas with a large intake of complex carbohydrates, α-glucosidase inhibitors are widely used. These agents competitively inhibit α-glucosidase enzymes in the brush border of enterocytes lining the intestinal villi. Inhibition of intestinal α-glucosidases delays the digestion of starch and sucrose, flattens the postprandial blood glucose excursions, and thus mimics the effects of dieting on hyperglycemia, hyperinsulinemia, and hypertriglyceridemia [9, 10]. They minimize hypoglycemia and weight gain, increase postprandial glucagon-like peptide-1 secretion, decrease both postprandial hyperglycemia and hyperinsulinemia, and thereby may improve sensitivity to insulin. The α-glucosidase inhibitors class currently comprises three compounds, namely, acarbose, miglitol, and voglibose. Gastrointestinal adverse effects and a rapid absorption rate of some α-glucosidase inhibitors are commonly encountered and can lead to treatment withdrawal.

Based on the above considerations, we have explored a few fungi to discover new α-glucosidase inhibitors. To detect fungi extracts suitable for α-glucosidase inhibitor isolation, first an *in vitro* enzymatic test is carried out using a well-known spectrophotocolorimetric procedure [11]. Those extracts with evident activity in the assay were subjected to activity-guided fractionation. Once the active compounds are obtained and characterized, if the quantity of the active compounds is sufficient (over 100 mg), animal *in vivo* studies using an oral sucrose tolerance test in normal and hyperglycemic mice were performed. In most cases, docking studies are performed using protein models of human *N*-terminal and *C*-terminal subunits of maltase glucoamylase (*h*Nt-MGAM, PDB code 2QMJ; and *h*Ct-MGAM, PDB code 3TOP; respectively), human *N*-terminal sucrose isomaltase (*h*Nt-SI, PDB code 3LPP), and yeast isomaltase (*y*-IM, PDB code 3A4A). Recent work in this area is summarized in the following paragraphs.

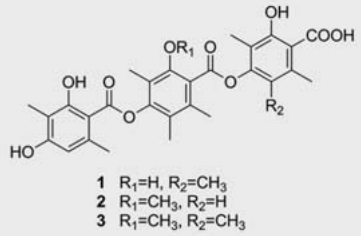
Fungal endophytes from *Hintonia latiflora*

H. latiflora is a Mexican medicinal plant with a long history of use for treating diabetes. Owing to its antidiabetic effect, Mexican and several European drug companies have commercialized herbal products containing the stem bark of this species for decades (e.g., Sucontral D, Copalchi-Bellsolá, Copalchi, Copangel, among others). The plant has been the subject of many chemical and pharmacological studies that have established the hypoglycemic and antihyperglycemic actions. The active principles are a few cucurbitacins and 4-phenylcoumarins type of compounds [12]. With the increasing demand for herbal drugs and natural health products, wild *H. latiflora* and related species are facing accelerated loss and are now threatened with extinction from overexploitation. Therefore, it is important to find alternative approaches to meet the medical demand. This can be achieved using different strategies, including the assessment of the ability of the host microorganisms to biosynthesize pharmacologically active secondary metabolites differently, similarly, or identically to those produced by their host medicinal plant [13]. Therefore, a few endophytes from *H. latiflora* were isolated and investigated.

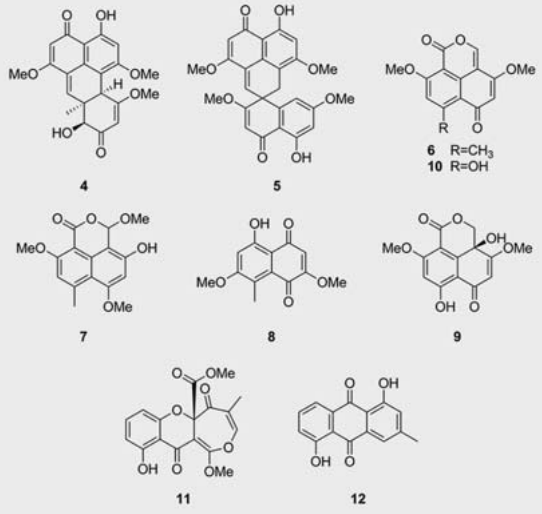
The first endophyte analyzed was MEXU 27095. Bioassay-guided fractionation of the active organic extract obtained from solid media culture led to the separation of three tridepsides, which were identified as thielavins A, J, and K (**1**–**3**) [14] (▶ **Fig. 1**). All three compounds inhibited baker's yeast α-glucosidase (αGHY) in a concentration-dependent manner with IC_{50} val-

ues of 23.8, 15.8, and 22.1 µM, respectively. Their inhibitory action was higher than that of acarbose (IC_{50} = 545 µM), used as a positive control. Kinetic analysis established that the three compounds acted as noncompetitive inhibitors with K_i values of 27.8, 66.2 and 55.4 µM, respectively. Docking analysis predicted that **1**–**3** and acarbose bind to αGHY in a pocket close to the catalytic site for isomaltose. The α-glucosidase inhibitory properties of thielavin K (**3**) were corroborated *in vivo* since it induced noted antihyperglycemic action during an oral sucrose tolerance test (3.1, 10.0, and 31.6 mg/kg) in normal and nicotinamide-streptozotocin (NA-STZ) hyperglycemic mice. Thielavin K (**3**) also showed a hypoglycemic effect *in vivo* at the doses of 3.1 and 10 mg/kg when tested in normal and hyperglycemic mice. In both cases the effect was attained after 5 h and maintained throughout the experiment, and was comparable to that of glibenclamide [14]. Sakemi and coworkers previously demonstrated that compounds **1**–**3** were glucose-6-phosphatase inhibitors *in vitro* [15]. Altogether, these results revealed that thielavin-type tridepsides represent a new lead for the development of α-glucosidase inhibitors; specifically, compound **3** shows potential as an antidiabetic agent acting at different targets, namely, inhibiting the α-glucosidases at the intestinal levels or decreasing hepatic glucose output from glyconeogenesis and glycogenolysis. Unfortunately, compounds **1**–**3** showed poor solubility, which caused problems for further *in vitro* and *in vivo* assays.

P. minimoides was also isolated as an endophyte from *H. latiflora*, although it has been previously obtained from different substrates including dung, *Pinus tabulaeformis* Carr., and *Trametes hirsutum* (Will. Fr.) S. F. Gray, a medicinal fungus collected from a dead hardwood branch in a dry forest in Hawaii [16]. A previous chemical investigation performed by other authors resulted in the isolation of a depsipeptide and two polyketides, sporminarins A and B [16], with antifungal properties against *Aspergillus flavus* Link [17]. In our exploration, extensive fractionation of an extract from the grain-based culture of *P. minimoides* led to the isolation of five new polyketides, two of them with novel skeletons, minimoidiones A (**4**) and B (**5**), corymbiferone C (**6**), corymbiferan lactone E (**7**), and 5-hydroxy-2,7-dimethoxy-8-methylnaphthoquinone (**8**), along with the known compounds preussochromone C (**9**), corymbiferone (**10**), brocaenol B (**11**), and ziganein (**12**) [18, 19] (▶ **Fig. 2**). The structures of the new compounds **4**–**8** were elucidated using 1D and 2D NMR data analysis, along with density functional theory (DFT) calculations of NMR chemical shifts. The absolute configuration of **4** was established by a single-crystal X-ray diffraction analysis and time-dependent DFT electronic circular dichroism (TDDFT-ECD) calculations. Polyketides **4, 5, 9**, and **10** significantly inhibited yeast α-glucosidase with IC_{50}'s ranging from 2.9 to 155 µM. Among them, compound **5** showed the strongest effect (IC_{50} = 2.9 µM). In order to envisage the putative binding mode of compounds **4** and **5** with αGHY, docking analyses were carried out using the crystallized structure of αGHY. Minimoidione A (**4**) and the *R* and *S* enantiomers of **5** were docked into the validated α-glucosidase model. The results predicted that the *R* enantiomer of compound **5** binds in a site different from the catalytic domain. On the other hand, docking of compound **4** and the *S* stereoisomer of **5** suggested that they bind to the catalytic



▶ **Fig. 1** Secondary metabolites isolated from endophyte MEXU 27095.



▶ **Fig. 2** Secondary metabolites isolated from endophyte *P. minimoides*.

site of αGHY with higher affinities. Further work is in progress to test these polyketides *in vivo*.

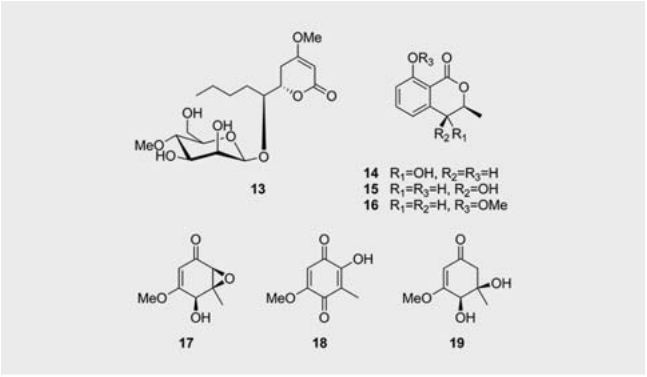
The last endophyte from *H. latiflora* with α-glucosidase inhibitory activity was characterized as *X. feejeensis*. The genus *Xylaria* Hill ex Schrank comprises more than 300 species, some of which have received special attention due to their potential as sources of novel secondary metabolites. A recent review showed that more than 180 compounds, including sesquiterpenoids, diterpenoids, diterpene glycosides, triterpene glycosides, steroids, *N*-containing compounds, pyrone derivatives, and polyketides, have been isolated from this genus [20, 21]. Specifically, from *X. feejeensis*, integric acid, xylaropyrone, and the nonenolide xyolide have been isolated. During our investigations, we isolated two new compounds, pestalotin 4′-*O*-methyl-β-mannopyranoside (**13**) and 3*S*,4*R*-(+)-4-hydroxymellein (**14**), from an organic extract of a solid medium culture (rice). In addition, the known compounds 3*S*,4*S*-(+)-4-hydroxymellein (**15**), 3*S*-(+)-8-methoxymellein (**16**), coriloxine (**17**), and the quinone derivatives **18** and **19** were obtained [22] (▶ **Fig. 3**). The absolute configuration of the stereogenic centers of **13** and **14** was determined using TDDFT-ECD cal-

culations. Compounds **14** and **15** inhibited αGHY with IC_{50} values of 441 and 549 µM, respectively. Their activity was comparable to that of acarbose. Molecular docking predicted that both compounds bind to αGHY in a site different from the catalytic domain, which could imply an allosteric type of inhibition.

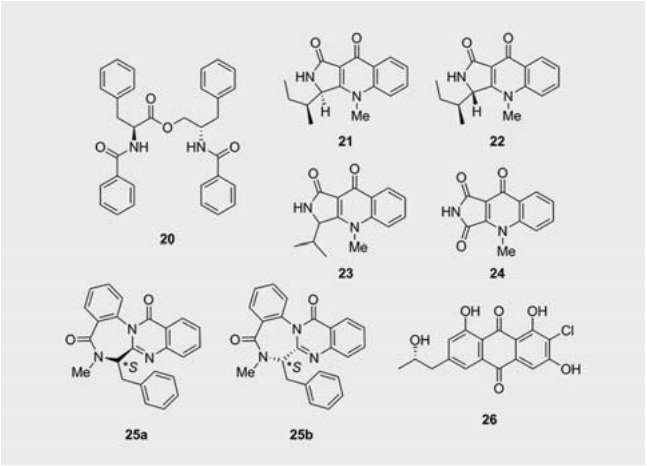
Coprophilous *Penicillium spathulatum*

P. spathulatum was collected aseptically from bat dung obtained in the Chontalcoatlán cave in the State of Guerrero. This fungus has a worldwide distribution and is found commonly in soil, air, water, ice, and food products, and is associated with different plants. HPLC analysis of the extrolites of some strains of *P. spathulatum* revealed that all of the isolates produced asperphenamate (**20**) and perinadine, some benzomalvins, breviones, cyclopenols, and related benzodiazepine alkaloids, and the least quinolactacins [23]. The defatted extract from *P. spathulatum* B35 grown in rice showed inhibitory activity against yeast α-glucosidase (IC_{50} = 56.5 µg/mL). Extensive chromatography of this active fraction led to the isolation of **20**, the epimeric mixture (7 : 3) of quinolactacins A1 (**21**) and A2 (**22**), quinolactacin B as a racemate (**23**), quinolonimide (**24**), benzomalvin A (**25**), and 2-chloro-6-[2′(*S*)-hydroxypropyl]-1,3,8-trihydroxy-anthraquinone (**26**), a new chemical entity [24] (▶ **Fig. 4**). Compound **20**, the mixture of **21** and **22**, racemate **23**, and compound **25** inhibited yeast α-glucosidase in a concentration-dependent fashion with IC_{50} values of 8.3, 273.3, 57.3, and 383.2 µM, respectively. The α-glucosidase inhibitory properties of **25** were confirmed *in vivo* with an oral sucrose tolerance test in normal and hyperglycemic mice. Docking studies performed with conformers **25a** and **25b** indicated that they bind to yeast isomaltase (3A4A) in a different location than acarbose, whereas for human enzymes 3LPP, 2QMJ, and 3TOP, the binding occurred at the catalytic domain [24]. Compound **25** also showed antihyperalgesic activity in NA-STZ hyperglycemic mice, reducing formalin-induced hyperalgesic behavior during phase I at the dose of 31.6 µg/paw, and during phase II at 10 and 31.6 µg/paw. The benzodiazepine-$GABA_A$ receptor was ruled out as the molecular target of **25** on the basis of classical agonist-antagonist experiments.

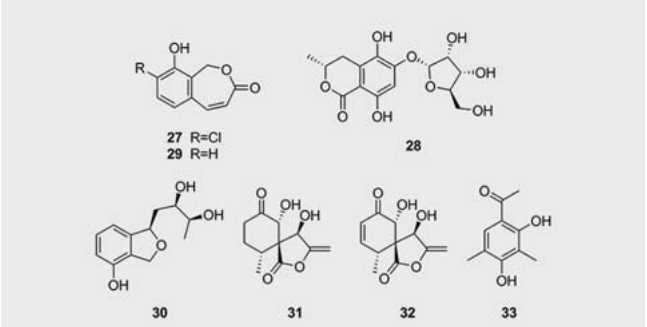
The last fungus selected for the search of α-glucosidase inhibitors was *M. flavorosea*. From the active extract prepared from the grain-based culture, two new polyketides, namely, 8-chloroxylarinol A (**27**) and flavoroseoside (**28**), along with the known compounds xylarinol A (**29**), xylarinol B (**30**), massarigenins B and C (**31** and **32**), and clavatol (**33**), were isolated [25] (▶ **Fig. 5**). The structures of **27, 28**, and **32** were corroborated by single-crystal X-ray diffraction analysis. Compounds **29, 30**, and **32** significantly inhibited αGHY. Oral administration of **32** significantly reduced the postprandial peak in a non-dose-dependent manner during an oral sucrose tolerance test using normal and NA-STZ-induced (50–130 mg/kg) hyperglycemic mice. In all cases, the effect was comparable to that of the positive control acarbose, thus revealing the antihyperglycemic potential of compound **32**. Docking of **32** to *h*Nt-MGAM and *h*Ct-MGAM as well as to *h*Nt-SI and *y*-IM predicted that it would bind to them far from the catalytic site; the interacting forces were mainly by hydrophobic contacts and hydrogen bonding (HB). The overall docking results were consistent with the *in vitro* α-glucosidase inhibitory properties of compound **32** and predicted that its inhibitory action could be exerted throughout an allosteric interaction with the enzymes [25]. Further research is in progress to assess how it is absorbed, distributed, metabolized, and excreted.



▶ **Fig. 3** Secondary metabolites isolated from endophyte *X. feejeensis*.



▶ **Fig. 4** Secondary metabolites isolated from coprophilous *P. spathulatum*.



▶ **Fig. 5** Secondary metabolites isolated from *M. flavorosea*.

CaM Inhibitors

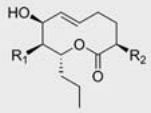
Calmodulin is the major ubiquitous Ca^{2+}-binding protein of all eukaryotes involved in a variety of physiological and pathophysiological roles related to smooth muscle contraction/relaxation, cell motility, cytoskeleton architecture and function, cell proliferation, apoptosis, autophagy, metabolic homeostasis, ion channel function, phosphorylation/dephosphorylation of proteins, plant growth, reproductive processes, and gene expression, to mention a few. CaM controls these processes through the modulation of over 100 different proteins including enzymes such as calmodulin-dependent phosphodiesterase (PDE1), nitric oxide synthases (NOS), several kinases (NADK), ion channels, phosphatases, calcium-ATPase pumps, and ion channels [26].

Many natural products, including drugs, pesticides, and research tools, interact with CaM at different binding sites, provoking a series of conformational changes in the protein. This, in turn, modifies the activity of the enzymes regulated by this important protein. For the discovery of CaM inhibitors, several methods have been used, which were described in a recent review [26]. In our investigations, we have employed functional enzymatic assays with bovine brain CaM-sensitive cAMP phosphodiesterase (PDE1) and/or peas CaM-dependent nicotinamide adenine dinucleotide kinase (NADK) as reporter enzymes, SDS-PAGE, and/or fluorescent biological sensors (FBS) built with human CaM (*h*CaM) developed in recent years [27–33]. In some cases, *in silico* analyses were used to explore the potential binding site of the inhibitors. According to literature data, the Fungi Kingdom has yielded many important CaM inhibitors. Perhaps the ophiobolins isolated from several species of the genus *Bipolaris* has been the most thoroughly investigated [34]. In the course of our investigations, we have also discovered new CaM inhibitors from selected fungi from Mexico, which are summarized in the following sections.

Plant pathogen *Phoma herbarum*

The genus *Phoma* Saccardo consists of over 2000 species found all over the world. Numerous species of this genus are plant pathogens causing root infections and different spot/blotch diseases in several economically important crops [35]. Among the most relevant pathogens of an important variety of corn (*Zea mays* L.) in Mexico (Cacahuacintle corn) is *P. herbarum*. This species is distributed worldwide and found in a variety of substrates, including herbaceous and woody plants, soil, and water, and has also been reported as a pathogen of wild oats and dandelions [36, 37]. Chemical studies of organic extracts prepared from the culture media of *P. herbarum* TOX-01020 led to the isolation of three new nonenolides, namely, herbarumins I–III (**34**–**36**) (▶ **Fig. 6**). The structure elucidation of the herbarumins was accomplished by chemical, spectroscopic, and computational methods [38, 39]. These compounds possess a unique 10-membered macrolide core, which, according to a recent review, has inspired many synthetic works all around the world [40].

The interaction of the nonenolides with CaM was assessed using an SDS-PAGE and the functional enzymatic assay with PDE1. In all cases, CaM treated with the lactones had lower electrophoretic mobility than untreated CaM, and the effect was comparable to that of chlorpromazine (CPZ), used as a positive control. The enzyme inhibition studies were consistent with these results. As in the case of ophiobolin A, the CaM inhibitor properties of herbarumins I–III (**34**–**36**) correlated well with a phytotoxic action because when tested against seedlings of *Amaranthus hypochondriacus* L. using the petri dish bioassay, a significant phytogrowth inhibitory activity was observed. This action was similar or higher than that of 2,2-dichlorophenoxyacetic acid (2,4-D) used as a positive control. Altogether, our results could be related with the observations of Schnick and Boland [41], who demonstrated that a combination of 2,4-D and *P. herbarum* produced enhanced control of dandelion. More recently, a few investigations on this fungus isolated from different substrates allowed for the isolation of several new bioactive compounds. Thus, a marine isolate of *P. herbarum* produced the polyketides arthropsadiol C and massarilactone H, which possessed moderate neuraminidase inhibitory activity [42]. From a *P. herbarum* endophyte of *Aegle marmelos* (L.) Correa, a few new antibacterial naphthalenes were isolated [43], whereas from *P. herbarum* PSU-H256 from *Hevea brasiliensis* (Willd. ex A. Juss.) Müll.Arg. some terezine, tyrosine, and hydantoin derivatives were characterized [44, 45].

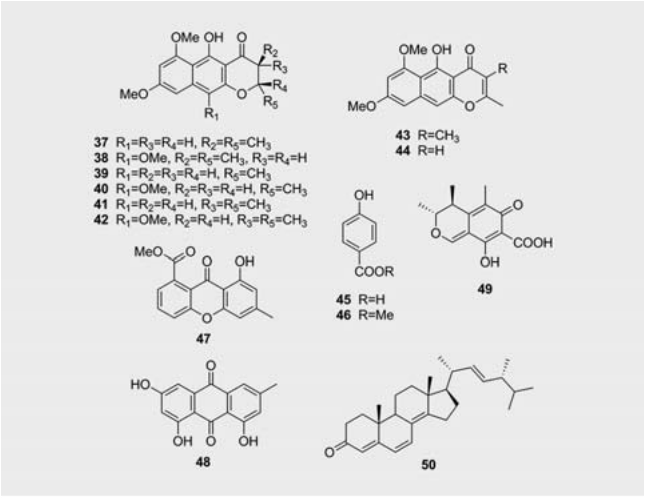


34 R_1=OH, R_2=H
35 R_1=R_2=OH
36 R_1=R_2=H

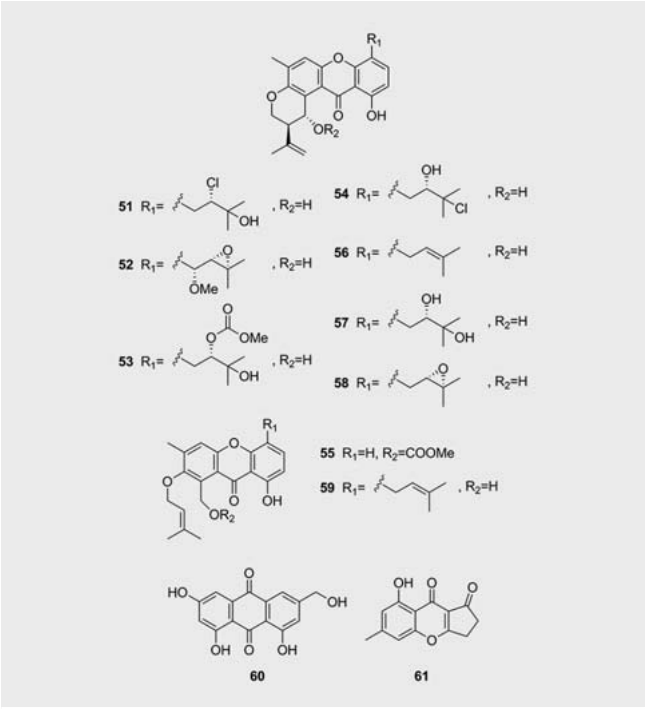
▶ **Fig. 6** Secondary metabolites isolated from plant pathogen *P. herbarum*.

Coprophilous *Guanomyces polythrix*

G. polythrix was isolated from bat guano in the Cueva del Diablo (Devil's cave) in Tepozotlán, Morelos, Mexico. Since the morphological and molecular characteristics of this isolate were unique, the new genus *Guanomyces* M.C. González, Hanlin & Ulloa was proposed to put up the new species [46, 47]. Bioassay-guided fractionation of an organic extract from the fermentation broth and mycelium of *G. polythrix* led to the isolation of several compounds, including seven new naphthopyranone derivatives (**37**–**43**) and the known compounds rubrofusarin B (**44**), *p*-hydroxybenzoic acid (**45**) and its methyl ester (**46**), a xanthene carboxylic acid methyl ester (**47**), emodin (**48**), citrinin (**49**), and the ergostatetraen-3-one (**50**) [47–49] (▶ **Fig. 7**). According to an SDS-PAGE, compounds **37**–**50** interacted with both spinach and bovine brain CaMs, but **37**–**44** and **50** inhibited the activity of PDE1 in a concentration-dependent manner. The inhibitory activity was higher or comparable to that of CPZ (IC_{50} = 10.6 µM), a well-known CaM inhibitor. Natural compounds **38** and **42**–**44** were also potent inhibitors of the complex spinach-CaM-NADK, with IC_{50} values of 22.0, 24.3, 13.3, and 17.1 µM, respectively. Phytotoxins **43** and **44** that possess a double bond between C-2 and C-3 were the most active CaM inhibitors. Once more, all compounds significantly inhibited the radicle growth of *A. hypochondriacus* and *Echi-*



▶ **Fig. 7** Secondary metabolites isolated from coprophilous *G. polythrix*.



▶ **Fig. 8** Secondary metabolites isolated from marine-derived *Emericella* sp. MEXU 25379 and *A. stromatoides*.

nochloa crus-galli (L.) P. Beauv. in a concentration-dependent manner, and were more potent than 2,4-D ($IC_{50} = 1.8 \times 10^{-4}$ and 8.8×10^{-4} M for *A. hypochondriacus* and *E. crus-galli*, respectively). Overall, the effect of the phytotoxins of *G. polythrix* with both enzyme complexes suggested that they may act as CaM antagonists *in vivo*, inhibiting the CaM-dependent phenomena during plant growth, although they could also interfere with other cellular metabolic processes [47–49].

Marine-derived *Emericella* sp. and *Aspergillus stromatoides*

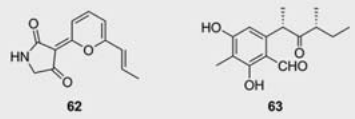
As of now, less than 80 marine fungi have been taxonomically described from the Pacific Coast, Gulf of Mexico Coast, and Caribbean Coast, with just a small percentage of them subjected to chemical studies [50]. Among them, the marine fungus *Emericella* sp. MEXU 25379 and *A. stromatoides* were investigated as a source of new CaM inhibitors.

The genus *Emericella* Berk (*Aspergillus* anamorph) produces a variety of secondary metabolites with important biological properties like antitumor, antioxidant, antimicrobial, immunostimulant, and antiallergic, among others [51]. *Emericella* sp. MEXU 25379 was isolated from the surface of the soft coral *Pacifigorgia rutilia* collected at Marietas Islands in Nayarit, Mexico. Chemical analyses of an organic extract of the broth culture (Czapeck) afforded five new (**51**–**55**) and four known (**56**–**59**) prenylated xanthones (▶ **Fig. 8**). Their structures were elucidated by spectroscopic, chemical (Mosher ester analysis), and molecular modeling methods. Their anti-CaM properties were assessed with the PDE1 assay, SDS-PAGE, and/or FBS [31,51]. Compounds **52** and **57** (IC_{50} = 5.54 and 5.62 µM, respectively) inhibited the activation of PDE1 by CaM in a concentration-dependent manner and retarded the electrophoretic mobility of CaM. In both cases, the effects were comparable to those of CPZ. Furthermore, kinetic analyses revealed that **52** and **57** acted as competitive antagonists of CaM, interfering with the formation of the CaM-PDE1 active complex [51]. The affinity values of compounds **52**–**59** were measured using the fluorescent biosensor *h*CaM L39C-*mBBr*/V91C-*mBBr* (K_d values between 3.7 and 498.4 nM) [52]. Finally, all compounds docked into CaM at site I, except for compounds **54** and **59**, which bound to sites IV and III, respectively. These binding sites have been identified as the most common binding regions for most CaM ligands, and all the predicted binding affinities were in excellent agreement with the experimental values [31,51].

The fungal strain *A. stromatoides* was isolated from the sandy soil of Sánchez Magallanes beach, State of Tabasco, Mexico [52]. Bioassay-guided fractionation of the organic extract from the mycelium and culture medium of *A. stromatoides* led to the isolation of a series of polyketides identified as emodin (**48**), ω-hydroxyemodin (**60**), citrinin (**49**), **47**, and coniochaetone A (**61**) (▶ **Fig. 8**). The latter was unambiguously established by X-ray analysis. The affinity of compounds **48** and **60** with *h*CaM was determined using the fluorescent biosensor *h*CaM M124C-*mBBr* (K_d = 0.33 and 0.76 µM, respectively) and was slightly better than that of CPZ (K_d = 1.25 µM). These results were in agreement with those of the theoretical inhibition constants obtained from docking analysis. The interactions observed in the docking results for **48** and **60** showed that they bind in the same hydrophobic pocket as CPZ and some antitumoral drugs. In turn, our finding might account for the cytotoxic effect of these well-known anthraquinones whose antitumoral properties have been the subject of a few patents [52].

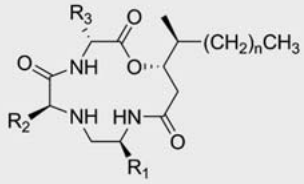
Fungal endophyte MEXU 26343

MEXU 26343 was isolated from selected adult and healthy leaves of *H. latiflora* and tested for its CaM inhibitor properties [53]. The



62 63

▶ **Fig. 9** Secondary metabolites isolated from endophyte MEXU 26343.



64 R_1=R_2=Phe, R_3=Ile; n= 5
65 R_1=R_2=Phe, R_3=Ile; n= 3
66 R_1=Phe, R_2=Ala, R_3=Leu; n= 3
67 R_1=Trp, R_2=Phe, R_3=*allo*-Ile; n= 3
68 R_1=Phe, R_2=Ala, R_3=Leu; n= 5
69 R_1=Val, R_2=Ala, R_3=Leu; n= 3
70 R_1=Tyr, R_2=Ala, R_3=Leu; n= 3

▶ **Fig. 10** Secondary metabolites isolated from entomopathogen *I. fumosorosea*.

endophytic fungus was cultured in moist rice, and its organic extract was subjected to chemical investigation, yielding the mixture *E* and *Z* of the known polyketide vermelhotin (**62**) and a new salicylic aldehyde derivative, namely, 9*S*,11*R*-(+)-ascosalitoxin (**63**) (▶ **Fig. 9**). The mixture *E* and *Z* of **62** was not separable, but in the presence of TFA, the generation of a pyrylium trifluoroacetate was favored and explained the water solubility of **62**. On the other hand, the structure and absolute configuration of the new compound **63** were established through extensive NMR spectroscopy and molecular modeling calculations at the DFT B3LYP/DGDZVP level, which included the comparison between theoretical and experimental optical rotation values. In addition, chemical transformations of **63** yielded suitable derivatives for NOESY and ^{1}H-^{1}H NMR coupling constant analyses, which reinforces the stereochemical assignment [53].

The potential affinity of **62** and **63** with CaM was established using the fluorescent biosensor *h*CaM M124C-*mBBr*, but only compound **62** bound to the protein with a K_d value similar to that of the classical CaM inhibitor CPZ (K_d = 0.25 µM for **62**, and 0.64 µM for CPZ). Docking analysis of the four possible conformers of **62** showed that all bound to CaM at site I, as did trifluoperazine, with affinities consistent with the results from the fluorescence quenching experiments using the *h*CaM M124C-*mBBr* biosensor [53]. These findings suggested that compound **62** might be useful as a tool for studying the role of CaM in several physiological and pathophysiological processes.

Entomopathogen *Isaria fumosorosea*

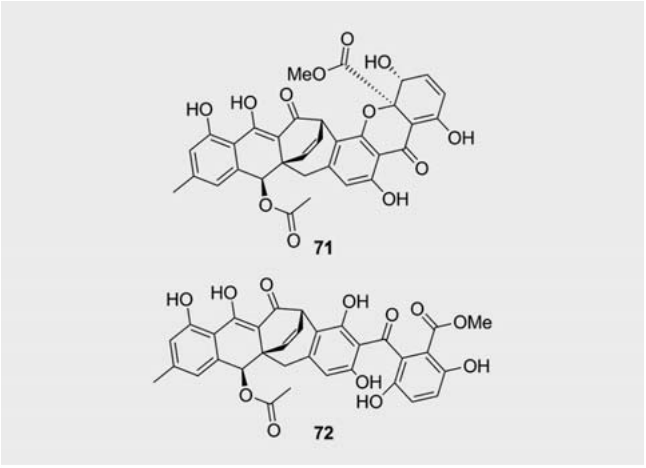
The entomopathogen *I. fumosorosea* was isolated from the whitefly *B. tabaci* Gennadius. Because of its wide arthropod host range, this species has been used for the biological control of several economically important insect pests of agricultural crops [54]. The total extract of *I. fumosorosea* showed significant anti-CaM activity using the CaM biosensor *h*CaM M124C-AF_{350}. Beauverolides C (**64**), F (**65**), I (**66**), Ja (**67**), L (**68**), M (**69**), and N (**70**) were identified as the active constituents of the extract (▶ **Fig. 10**). This group of lipophilic and neutral cyclotetradepsipeptides, containing linear and branched C_9- or C_{11}-β-hydroxy acid residues, has been previously reported in the genera *Beauveria* and *Paecilomyces* [55]. All isolated cyclotetradepsipeptides interacted with CaM with affinities ranging from 0.078 to 3.44 µM. The most active compound, beauverolide Ja (**67**), was almost tenfold more active than CPZ and contains a tryptophan moiety in its structure that could be related to its highest affinity. Docking analyses of compounds **64**–**70** into CaM revealed that, in all cases, they bind in the same pocket of CPZ and showed mainly hydrophobic interactions to the protein [55].

Saprophyte *Purpureocillium lilacinum*

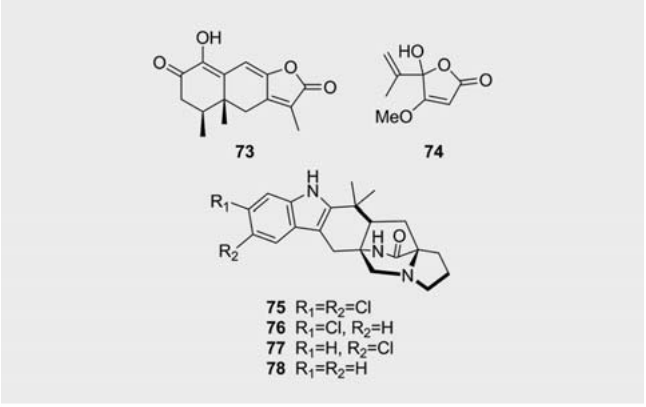
P. lilacinum was isolated from soil collected in a cave in Juxtlahuaca, State of Guerrero, Mexico, in 1998. This species is an emergent pathogen that causes severe human infections, but is also used as an important biological control agent against nematodes [56]. Bioassay-guided fractionation of an extract prepared from the culture medium and mycelium of *P. lilacinum* yielded two CaM inhibitors, namely, acremoxanthone C (**71**) and acremonidin A (**72**) (▶ **Fig. 11**). Both compounds showed an important affinity (70-fold higher than CPZ) for CaM using the fluorescent biosensor *h*CaM M124C-*mBBr*. Interestingly, this level of affinity to CaM is unusual, since most CaM antagonists bind to the protein with much lower affinity, usually in the micromolar range [56]. Based on the observed extraordinary affinity to CaM, we completed the structural characterization of **71** using molecular modeling calculations, which included the comparison between theoretical and experimental specific rotation, $^{3}J_{C,H}$, and $^{3}J_{H,H}$ values. Docking analysis predicted that compounds **71** and **72** bind to CaM at a similar site to KAR-2, which is unusual. This prediction was experimentally supported since **71** quenched the fluorescence of *h*CaM T110C-*mBBr*, a biosensor specially designed to target nonclassical inhibitors of CaM [56].

Coprophilous *Malbranchea aurantiaca*

The genus *Malbranchea* Saccardo comprises around 30 species distributed worldwide and mainly isolated from decaying vegetation, soil, and animal dung (http://www.indexfungorum.org). *M. aurantiaca* was obtained from bat guano collected at the Juxtlahuaca cave located in Ramal del Infierno, State of Guerrero, Mexico. The mycelial and culture broth organic extract of this fungus yielded an eremophilane derivative (**73**), penicillic acid (**74**), and four rare indole alkaloids possessing an unusual bicyclo [2.2.2] diazaoctane ring system, namely, malbrancheamide (**75**) and malbrancheamide B (**76**), isomalbrancheamide B (**77**), and premalbrancheamide (**78**) [57–59] (▶ **Fig. 12**).



▶ **Fig. 11** Secondary metabolites isolated from saprophyte *P. lilacinum*.



▶ **Fig. 12** Secondary metabolites isolated from coprophilous *M. aurantiaca*.

Compounds **75**–**78** have been fully characterized as CaM ligands by NMR, fluorescence, circular dichroism, computational, and enzymatic functional methods. Of the alkaloids with an *h*CaM M124C-*mBBr* biosensor, only **75** quenched significantly (K_d = 1.1 µM). The monochlorinated derivatives (**76** and **77**) provoked only limited decreases in fluorescence quenching, and **78** provoked none. Thus, the presence of two chlorine atoms confers **75** to have the best affinity to *h*CaM. Docking analysis predicted that **75** binds in the hydrophobic pocket of CaM through HB and hydrophobic interactions with a few methionine (Met) residues of the protein. Indeed, NMR experiments confirmed these results, since in solution, **75** induced a diamagnetic shift of most of the Met-methyl resonances of the protein, in particular, to those of Met residues 36, 51, 71, 72, 76, 109, 124, 144, and 145 [27].

As for other CaM inhibitors, **75**–**77** induced significant vasorelaxant activity in an endothelium intact model in rat aorta rings, and a lesser effect in an endothelium denuded model. Compound **75** was the most active (EC_{50} = 2.7 µM) with a maximum effect of almost 100 %, which is unusual for a natural product; the involvement of the nitric oxide-cGMP pathway was experimentally demonstrated. Furthermore, docking studies carried out with two crystallized *e*NOS domains, namely, oxygenase (PDB code 3NOS) and a CaM-binding peptide bound to CaM (PDB code 1NIW), predicted that the endothelium-independent relaxation exerted by **75** could be mediated by its CaM inhibitory properties throughout a positive modulation of *e*NOS. In addition, in a fluorescent-based experiment using the *h*CaM M124C-*mBBr* biosensor, it was demonstrated that **75** (K_d = 0.55 µM) induced a perturbation of the Ca^{2+}-*h*CaM-MLCK complex, which could also account for its vasorelaxant effect [60].

Other Activities

Marine-facultative *Aspergillus* sp.

The marine-facultative *Aspergillus* sp. MEXU 27854 was isolated from sandy soil collected in the intertidal zone located in Caleta Bay, Acapulco, Guerrero, Mexico [61]. The intertidal regions are dynamic environments that support an immense biodiversity. In these ecosystems, fungi play important roles in the process of decomposition and mineralization of organic matter [62]. From the organic extract of a rice-based culture of *Aspergillus* sp. MEXU 27854, a series of dioxomorpholine derivatives (**79**–**84**) were isolated (▶ **Fig. 13**). An extensive literature search revealed that the morpholine-2,5-dione ring system is rare in nature and mainly biosynthesized by fungi. Ergosecalinine, the first reported dioxomorpholine, was isolated in 1959 from *Claviceps purpurea* (Fr.) Tul. [63], the immunomodulator metacytofilin was obtained from the fungus *Metarhizium* sp. TA2759 [64], the compound lateritin, isolated from *Gibberella lateritium* W. C. Snyder & H. N. Hansen in 1993, was recently revised to beauvericin by extensive NMR and MS analyses [65], the cyclodidepsipeptides isolated as a mixture from the fungus *Fusarium sporotrichioides* Sherb., were identified by comparison with the synthetic series, and their stereostructure was studied by quantum chemical calculations [66], and, finally, PF1233 B was isolated from *Aspergillus niveus* Blochwitz in 2003 as a novel inhibitor of voltage-dependent sodium channels, and reported again as a new natural product with P-glycoprotein (Pgp) inhibitor properties in 2014 [67–69].

The structures of **79**–**84** were established by 1D and 2D NMR and HRESIMS data analysis, and the absolute configuration of **79** and **80** was elucidated by comparison of experimental and DFT calculated vibrational circular dichroism spectra. Finally, the cytotoxic properties of derivatives **81, 83**, and **84** were determined using a panel of human cancer cell lines with different functional status for p53 and against a cell line (A549/doxorubicin) that overexpresses Pgp and presents developed resistance to doxorubicin [61].

Agrochemicals

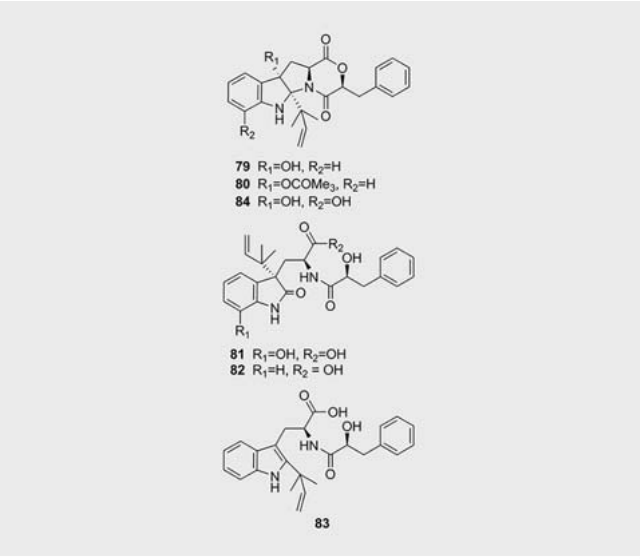
Like pharmaceuticals, many pesticides are based on fungal natural products; these could belong to the category of biopesticides or biochemical pesticides. These agents have become attractive in recent years because the novelty of their structures offers the possibility of finding compounds with new modes of action, thus diminishing the induction of resistance [70]. Among the most rel-

evant pesticides developed from fungal natural products are the strobilurins derivatives. The natural strobilurins were originally found in several fungi, including *Strobilurus tenacellus* (Pers.) Singer, *Xerula* spp., and *Cyphellopsis anomala* (Pers.) Donk. These types of compounds inhibit the electron transfer in mitochondrial respiration by binding to the ubiquinol site of cytochrome *b*. In the case of biopesticides, a few formulations with *Colletotrichum gloeosporioides* f.sp. (Penz.) Penz. & Sacc. (BioMal and Collego), *Phoma macrostoma* Montagne (Ecosense), which has been developed and applied successfully for weed control, and *Myrothecium verrucaria* (Alb. & Schwein.) Ditmar (DiTera) have been made against plant parasitic nematodes. Recently, a few reviews have been published emphasizing the urgent need for new pesticides with safer toxicological and environmental profiles and new modes of action [70, 71]. With these considerations, we have investigated several fungi from Mexico in order to discover new leads for the development of biochemical pesticides, in particular, for weed control affecting different molecular targets.

Endophytes from Plants Collected in El Eden Ecological Reserve, Quintana Roo, Mexico

El Eden Ecological Reserve is a natural protected area situated in the State of Quintana Roo, Mexico, and is part of the Mesoamerican tropical rain forest. Nowadays, many multidisciplinary research projects take place in the region, with fungal biodiversity monitoring and bioprospection projects being two of them [72]. In this context, *E. gomezpompae* was isolated from surface sterilized leaves of the medicinal plant *C. acuminata* [73]. A chemical investigation of the mycelium of *E. gomezpompae* resulted in the isolation of eight naphthoquinone-spiroketals, namely, preussomerins EG_1–EG_4 (**85–88**), palmarumycins EG_1 (**89**), CP_2 (**90**), CP_{17} (**91**), and CP_{19} (**92**) as well as **50** [74, 75] (▶ **Fig. 14**).

Compounds **85–87** and the acetyl derivatives **85a** and **85b** showed significant antifungal and anti-oomycete activities against four economically important phytopathogens, *Phythophtora capsici* Leonian, *Phythophtora parasitica* Dastur, *Fusarium oxysporum* Schltdl., and *Alternaria solani* (Cooke) Wint. Their IC_{50} values ranged between 47.8 and 313.0 µg/mL. Preussomerin EG_1 (**85**) caused complete growth inhibition of *P. parasitica, F. oxysporum*, and *P. capsici*. In the case of *P. capsici*, **85** ($IC_{50} = 5.61 \times 10^{-5}$ M) was more active than the commercial fungicide Captan ($IC_{50} = 1.53 \times 10^{-4}$ M). *A. solani* was only affected by **86** and **87**. Compounds **85–88** inhibited the germination of *A. hypochondriacus, Solanum lycopersicum* L., and *E. crus-galli*, being more potent than the positive control Rival. Compound **86** significantly inhibited (> 50%) root elongation of the three species tested, but **87** and **92** affected (> 50%) both *A. hypochondriacus* and *E. crus-galli*. Compound **86** inhibited respiration of all seedlings tested, but **88** and **89** of *S. lycopersicum* and *E. crus-galli*. Furthermore, oxygen consumption in intact mitochondria was significantly inhibited by compounds **86–88** and **90–93**, but the effect was lower than that of 2,4-dinitrophenol [74]. The study of compounds **85, 88, 90**, and **91** in a series of photosynthesis light reactions in freshly lysed spinach thylakoids (ATP synthesis, electron transport rate basal, phosphorylating, uncoupled and partial reactions of the photosystems I and II, and chlorophyll *a* fluorescence of the photosystem II) revealed that they are Hill's reaction inhibitors [76]. They

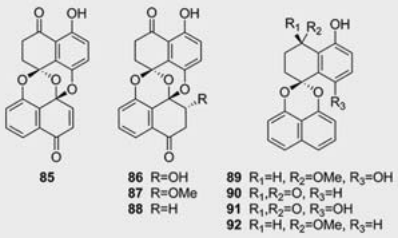


▶ **Fig. 13** Secondary metabolites isolated from marine-facultative *Aspergillus* sp. MEXU 27854.

also interacted at the acceptor site of the photosystem II in a similar way as the commercial herbicide Diuron, as demonstrated by chlorophyll *a* fluorescence experiments. Consequently, these compounds could be considered promising leads for developing new herbicides for utilization in modern agriculture.

Muscodor yucatanenis MEXU 25511 [77], a new fungal species, and *A. camptosporum* MEXU 26354 [78] were isolated from the leaves of *B. simaruba*, which is a frequent codominant medicinal tree of the Yucatan Peninsula; the Mayan communities use this plant as an analgesic, antimycotic, and anti-inflammatory agent. So far, all *Muscodor* species are endophytes that produce mixtures of volatile organic compounds (VOCs). These mixtures are useful as pesticides in living environments since they are readily converted to a gaseous phase at room temperature due to their low vapor pressure [79]. The composition and relative abundance of VOCs produced by microorganisms can be established using headspace solid-phase microextraction.

The VOCs of *M. yucatanensis* were lethal to the endophytes *Colletotrichum* sp., *Phomopsis* sp., and *Guignardia mangiferae*, A. J. Roy, and to the phytopathogens *P. capsici, P. parasitica, Rhizoctonia* sp., and *A. solani.* Root elongation of *A. hypochondriacus, S. lycopersicum*, and *E. crus-galli* was also inhibited by the volatile mixture. *M. yucatanensis*' VOCs included 38 compounds that were identified by GC-MS, mainly, alcohols, acids, esters, ketones, naphthalene derivatives, benzene derivatives, terpenoids, and aliphatic hydrocarbons. The organic extracts from the culture medium and mycelium of *M. yucatanensis* were also active against the same endophytes, phytopathogens, and plants tested. In the case of *A. hypochondriacus*, the culture medium extract (IC_{50} = 194.3 µg/mL) was more active than the commercial herbicide Rival (IC_{50} = 234.42 µg/mL). According to GC-MS, the extracts and VOCs shared 12 compounds, including benzene derivatives, phenolic compounds, cyclopentadienes, esters, lactones, alkanes, aldehydes, and carboxylic acids [80]. Recently, Qadri and



▶ **Fig. 14** Secondary metabolites isolated from endophyte *E. gomezpompae*.

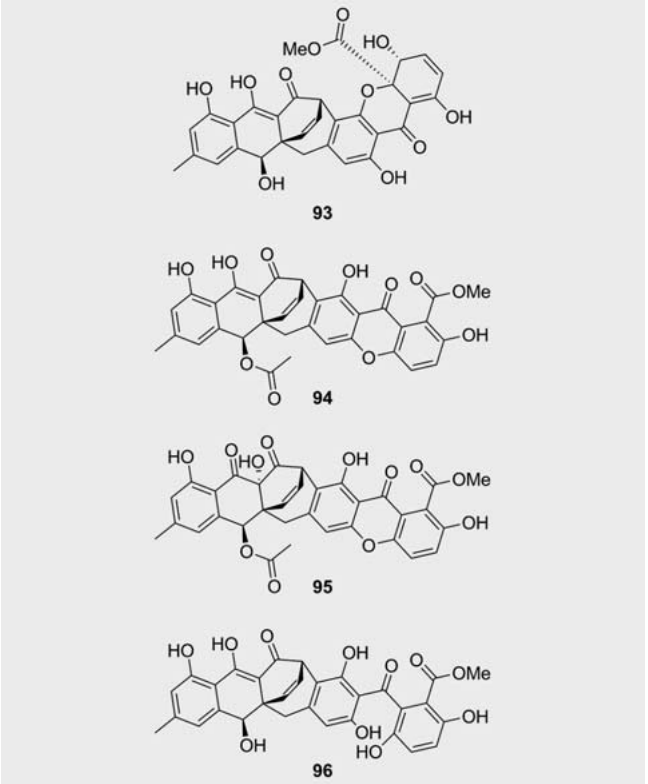
coworkers [81] isolated a new strain of this fungus (Ni30) from a leaf of *Elleanthus* sp. at the Reserva Natural Punta Gorda, Nicaragua. The chemical composition of the volatile mixture of Ni30 was similar to those described by Macías-Rubalcava and coworkers [80]. The Ni30 strain also biosynthesized the macrolactone brefeldin A.

The genus *Acremonium* Link contains more than 200 species, which could be saprophytic, pathogenic, or endophytic. This genus is known for producing many interesting bioactive secondary metabolites, like the *β*-lactam antibiotics cephalosporins, the tremorgenic indole-diterpene alkaloids lolitrems, sesquiterpenoids, diterpenoids and triterpenoids with different skeletons, ophiobiolin D, a few meroterpenoids, a variety of polyketides, and peptides [81]. Bioassay-guided fractionation of the mycelial extract from *A. camptosporum* MEXU 26354 led to the isolation of six heterodimeric polyketides, acremoxanthone E (**93**), acremoxanthone A–C (**94, 95**, and **71**), and acremonidins A (**72**) and B (**96**) [82] (▶ **Fig. 15**). All compounds showed anti-oomycete activity against *Pythium aphanidermatum* Edson, *Phytophthora cinnamomi* Rands, *P. capsici*, and *P. parasitica*, reducing the diameter growth of most target oomycetes in a concentration-dependent manner, with IC_{50} values ranging between 6–38 µM, and the activity was comparable to the commercial fungicide Ridomil Gold 4E. However, in the case of *P. cinnamomi*, polyketides **93, 94**, and **71** were more active than the commercial fungicide. Unusually, this fungus biosynthesizes three different types of heterodimeric polyketides linked by a bicyclo[3.2.2]nonene: xanthoquinodins, acremonidins, and acremoxanthones.

Endophytes from Plants Collected in Sierra de Huautla Biosphere Reserve

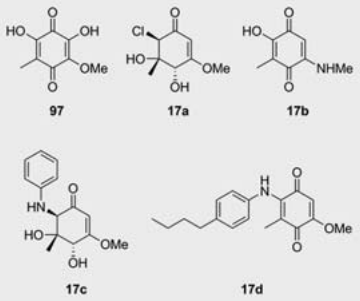
Xylaria feejeensis SM3e-1b

The Sierra de Huautla Biosphere Reserve (REBIOSH), recognized by the MAB-UNESCO program and included in the world net of biosphere reserves, is located in Quilamula at Tlalquitenango, Morelos, Mexico. This area represents the largest natural protected area devoted to the conservation of a tropical dry forest in Central Mexico, and is an important reservoir of endemic species, including fungal endophytes (www.conanp.gob.mx).



▶ **Fig. 15** Secondary metabolites isolated from endophyte *A. camptosporum* MEXU 26354.

From symptomless healthy leaves of the tree *S. macrocarpum*, the strain *X. feejeensis* SM3e-1b was isolated [83]. Bioassay-guided fractionation of the extracts from the culture medium and mycelium led to the isolation of coriloxine (**17**) and two quinone derivatives, **18** and fumiquinone B (**97**). In addition, four semisynthetic compounds from **17** were prepared (**17a–17d**) (▶ **Fig. 16**). All compounds showed a phytotoxic effect in a concentration-dependent manner on germination, elongation of the root, and oxygen uptake (respiration) of seedlings from *Trifolium pratense* L., *Medicago sativa* L., *A. hypochondriacus*, and *Panicum miliaceum* L. In general, the IC_{50} values for all these activities ranged between 0.1–1.1 mM, which were comparable to the values obtained for the commercial herbicides Rival and ExterPro [83]. Of all the compounds, only **17a** and **17d** inhibited ATP synthesis, basal and uncoupled electron transport, and enhanced the phosphorylating electron transport and Mg^{2+}-ATPase enzymatic activity when tested in a series of photosynthetic light reactions in freshly lysed spinach chloroplasts. Overall, these results indicated that **17a** acted as an uncoupler and a weak Hill's reaction inhibitor, whereas **17d** acted as a Hill's reaction inhibitor at the photosystem II [84]. Thus, coriloxine derivatives represented new leads for the development of novel herbicides.



▶ **Fig. 16** Secondary metabolites isolated from endophyte *X. feejeensis* SM3e-1b.

Xylaria sp. PB3f3

The endophyte *Xylaria* sp. PB3f3 was isolated from leaves of *H. brasiletto* collected at the REBIOSH. Based on phylogenetic studies, the genera *Muscodor* and *Xylaria* are anamorphs. In a multiple antagonism bioassay during 40 days, the VOCs produced by *Xylaria* sp. PB3f3 significantly inhibited the radial growth of two oomycetes, *P. aphanidermatum* (78.3%) and *P. capsici* (48.3%), and two fungi, *A. solani* (24.5%), and *F. oxysporum* (24.2%) [85]. Furthermore, *Xylaria* sp. PB3f3, in simple direct antagonism bioassays, produced a significant inhibitory effect at distance of 90% over the growing of the four plant pathogens. On the other hand, the VOCs from 30- and 20-day cultures significantly inhibited the root growth of *A. hypochondriacus* (27.6%) and *S. lycopersicum* (53.2%), respectively. From the mixtures obtained at days 10 and 30 of culturing, 2-methyl-1-butanol and 2-methyl-1-propanol were identified as the most abundant components. Both compounds strongly inhibited root growth and respiration in the seedlings of *A. hypochondriacus* and *S. lycopersicum*, with IC_{50} values ranging between 4.6–48.2 µg/mL and 23.2–130.0 µM, respectively [85].

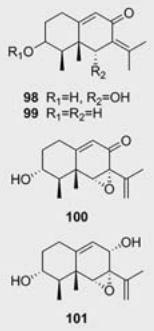
Nodulisporium and *Hypoxylon*

The genus *Hypoxylon* and its anamorph *Nodulisporium* are well-known producers of antimicrobial VOCs [86]. From healthy leaves of *G. sepium* collected in the REBIOSH, the endophyte *Nodulisporium* sp. GS4d2II1a was isolated. VOCs and diffusible metabolites of *Nodulisporium* sp. showed anti-oomycete (*P. aphanidermatum, P. capsici, Phythophtora palmivora, P. cinnamomi, P. parasitica, Pythium ultimum*, and *Pythium polytylum*) and antifungal (*A. solani* and *F. oxysporum*) activities when evaluated using three types of antagonism bioassays, and monitored at different ages of the fungus (3 to 6 days of growth culture) and interaction conditions. The growth of the oomycetes and fungi was inhibited in all cases, and, as visually observed in the petri dish, their mycelia were partially or completely replaced. In addition, VOCs inhibited the growth of three oomycetes (*P. parasitica,* 29.7%; *P. cinnamomi,* 18.1%; and *P. capsici,* 25.7%) and one fungus (*F. oxysporum,* 21.6%) after 3 days of antagonism using divided plates. VOCs were not active after 5 and 7 days of the fungal culture. Finally, the VOCs resulting from *Nodulisporium* sp. grown for 3 and 5 days in PDA at 28 °C, and from the interaction of *Nodulisporium* sp. with *P. aphanidermatum* and *Nodulisporium* sp. with *Nodulisporium* sp., were analyzed by GC-MS (70 VOCs detected). The results revealed four different volatile profiles, however, the main metabolites were terpenes, which represent over 60% of the total, with eucalyptol, limonene, and ocimene being among the most predominant. The remaining compounds were benzene derivatives, esters, ketones, alcohols, and carboxylic acids, including 2-methyl-1-butanol, and 3-methyl-1-butanol as the most relevant [86].

Hypoxylon anthochroum

The endophyte *H. anthochroum* Blaci was isolated from leaves of *Bursera lancifolia* collected at REBIOSH [87]. The VOCs produced by this endophyte exhibited a significant phytotoxic effect on seed germination, root elongation, and seedling respiration of *A. hypochondriacus, P. miliaceum, T. pratense*, and *M. sativa*, the majority with inhibition values over 40%. Furthermore, the VOCs slightly affected the growth of the plant pathogenic fungi *A. solani* and *F. oxysporum*, and the oomycetes *P. ultimum* and *P. capsici*. Once more, eucalyptol was the most predominant compound in the VOCs analyzed. On the other hand, the culture medium and mycelium extracts of *H. anthochroum* showed a high phytotoxic activity on the four test plants. From these, phenylethyl alcohol, 2-methyl-1-butanol, eucalyptol, and terpinolene were the most predominant metabolites. Pure phenylethyl alcohol and eucalyptol were also evaluated in the same vegetal processes, displaying IC_{50} values between 21.4 µg/mL and > 500.0 µg/mL, and 11.6 µg/mL and > 500.0 µg/mL, respectively. The IC_{50} values of the positive control Rival were found between 14.8 µg/mL and > 500.0 µg/mL [87].

Recently, six pure compounds (phenylethyl alcohol, 2-methyl-1-butanol, 3-methyl-1-butanol, eucalyptol, ocimene, and terpinolene) present in the VOCs of *Nodulisporium* sp. GS4d2II1 and *H. anthochroum* were evaluated *in vivo* and *in vitro* against *F. oxysporum* [88]. *In vivo* studies were performed inoculating the pathogen into cherry tomatoes. All compounds, and particularly the mixture of the six, significantly inhibited the growth of the mycelium on the wounds of infected tomatoes in a concentration-dependent manner. The *in vitro* assay, using agar dilution and gas phase methods, revealed that the VOCs mixture significantly affected the respiration and cell membrane permeability of the target fungus. The damage rate caused by individual compounds and the VOCs' mixtures on the cell membrane of *F. oxysporum* was evaluated through the measurement of electrolytes leak (% relative conductivity). After 8 h of treatment, the best effect was observed with 2-methyl-1-butanol (45.4%), phenylethyl alcohol (43.1%), and the VOCs' mixture (42.9%). According to a microscopic analysis of mycelia fresh preparations, the pure compounds and the six compounds' mixture at 1000 µg/mL induced important ultrastructure morphological changes in *F. oxysporum* hyphae. Ocimene and the mixture induced a vacuolization process and thickening and thinning on the middle and the tips, respectively. Finally, an inhibitory effect on the respiration were observed with all treatments, which could be attributed to the proliferation of vacuoles and the severe vacuolization process provoked by the rupture of hyphae [88]. VOCs' mixture showed the greatest oxygen consumption inhibition (97.1%) after 8 h incubation (IC_{50} = 183.9 µg/mL). These results revealed that VOCs are promising candidates for the development of biopesticides, having great po-



▶ **Fig. 17** Secondary metabolites isolated from coprophilous *Penicillium* sp. G1-a14.

tential for tomatoes postharvest treatment against diseases caused by *F. oxysporum*.

Coprophilous *Penicillium* sp. G1-a14

Penicillium sp. G1-a14 was also isolated from bat dung obtained in the Chontalcoatlán cave in the State of Guerrero [89]. This fungus was selected for bioassay-directed fractionation on the basis of its phytogrowth inhibitory activity against seedlings of *A. hypochondriacus* and *E. crus-galli* (IC_{50} = 46.2 and 184.7 µg/mL, respectively). Extensive chromatography of the active extract led to the isolation of a new eremophilane sesquiterpene (**98**), along with three known analogues, namely, isopetasol (**99**), sporogen AO-1 (**100**), and dihydrosporogen AO-1 (**101**) (▶ **Fig. 17**). Only compounds **100** and **101** revealed a concentration-dependent inhibition on radicle elongation against *A. hypochondriacus* (IC_{50} = 0.17 mM for both compounds) and *E. crus-galli* (IC_{50} = 0.17 and 0.30 mM, respectively) with IC_{50} values similar to tricolorin A, used as a positive control [89]. The eremophilane-type sesquiterpenoid is common in *Penicillium*, *Aspergillus*, and *Xylaria* species, and a few of them have also shown important phytotoxic activity.

Concluding Remarks and Outlook

In the last 20 years, more than 100 secondary metabolites from various classes have been identified from fungi from different substrates throughout Mexico. Some of these natural products possessed new scaffolds and have attracted the attention of synthetic chemists, such as in the case of herbarumins I–III (**34**–**36**) and malbrancheamides (**75**–**78**). Most of these fungal compounds displayed diverse bioactivities of medicinal and/or agrochemical interest. Thus, a few showed potential as antidiabetic drugs with α-glucosidase inhibitory activity. Preclinical pharmacological evidence indicated that most of them were more active than acarbose, and some, like benzomalvin A (**25**), also possess antihyperalgesic properties of utility for treating diabetic neuropathies. Another, malbrancheamide (**75**), is a vasorelaxant compound with a maximum effect of almost 100%, which is unusual for a natural product. The effect is mediated by CaM inhibitory properties throughout a positive modulation of *e*NOS and a perturbation of the Ca^{2+}-*h*CaM-MLCK complex. A series of dioxomorpholine derivatives (**81**, **83**, and **84**) were cytotoxic in a panel of human cancer cell lines, with a different functional status for p53, and against a cell line (A549/doxorubicin) that overexpressed Pgp and developed resistance to doxorubicin. A few were CaM inhibitors, valuable for the study of CaM-mediated physiological processes or pharmacological properties in mammals and plants. Among the isolates, the prenylated xanthones **52** and **57** and acremoxanthone C (**71**) were the best CaM inhibitors. In the agrochemical area, many active antifungal, anti-oomycetes, and phytotoxic agents more active than commercial products were discovered. The VOCs of several species, including those from the new species *M. yucatanensis*, showed promise as pest control agents. In some cases, their mode of action was also established. A few new fungal species were discovered, and a new genus, *Guanomyces*, was created to accommodate *G. polythrix*. The fact that Mexican fungi are still understudied shows that many more new scaffolds and several drug and agrochemical leads are likely to be discovered in the future. Thus, bioprospecting for structurally unique and bioactive metabolites from Mexican fungi has great potential for the discovery of new drug candidates in various applications in clinics and the agrochemical area.

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Conflict of Interest

The authors declare no conflict of interest.

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