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Review

Microbial Steroids: Novel Frameworks, and Bioactivity Profiles

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Abstract

Endophytic and marine-derived fungi represent prolific and structurally innovative sources of steroidal natural products. In recent years, extensive chemical investigations of diverse fungal taxa—including *Aspergillus, Penicillium*, and numerous other genera—have revealed an extraordinary variety of steroids and steroid-like metabolites featuring unprecedented carbon skeletons, unusual ring rearrangements, heterocyclic fusions, and hybrid architectures. These metabolites encompass ergostane-, lanostane-, pregnane-, and abeo-type derivatives; secosteroids; polyoxygenated and polycyclic frameworks; meroterpenoid–steroid hybrids; and rare Diels–Alder adducts. Many of these compounds exhibit significant biological activities, including cytotoxic, anti-inflammatory, antimicrobial, antiviral, immunosuppressive, antioxidant, enzyme-inhibitory, larvicidal, neuroprotective, and herbicidal effects. Several metabolites, such as cordycepsterols, citristerones, rubensteroid A, anicequol, and various hybrid steroidal structures, demonstrate potent inhibition of key molecular targets (e.g., COX-2, NF-κB, PTP1B, AChE, NO production) and show promise as leads for anticancer, anti-infective, anti-neuroinflammatory, and metabolic disease therapeutics. Collectively, the rapidly expanding diversity of fungal steroids underscores the remarkable biosynthetic capabilities of fungi and highlights their continued potential as reservoirs of structurally novel and biologically valuable natural products. This review summarizes recent discoveries and structural classes of fungal-derived steroids, emphasizing their chemical diversity, biosynthetic features, and bioactivity profiles.

Keywords: microorganisms; bacteria; fungal endophytes; steroids; activity

1. Introduction

Endophytic fungi are microorganisms that inhabit plant tissues for all or part of their life cycle without causing apparent disease. This symbiotic association is often mutualistic, conferring a range of physiological and ecological benefits to the host plant. Endophytes are highly diverse and have been documented in nearly all plant species, colonizing roots, stems, leaves, flowers, and seeds [1–3].

The interaction between endophytic fungi and their hosts is particularly compelling due to the multifaceted advantages these microorganisms provide. In exchange for shelter and nutrients, endophytic fungi promote plant growth by enhancing biomass accumulation, height, root development, and the uptake of water and nutrients. These effects are mediated through mechanisms such as the production of phytohormones and the solubilization of essential nutrients, including phosphorus and nitrogen. Endophytes also improve plant tolerance to abiotic stresses—such as drought, salinity, and extreme temperatures—by regulating osmotic balance, modulating hormone levels, and synthesizing antioxidant compounds. Furthermore, several endophytic fungi function as biological control agents, protecting plants against pathogens, insects, and herbivores through the production of antimicrobial and insecticidal secondary metabolites [4–9].

Among endophytic fungi, species belonging to the genera *Aspergillus* [10–12] and *Penicillium* [13–15] are particularly noteworthy. These genera are ubiquitous in nature and are recognized for

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their remarkable capacity to biosynthesize structurally diverse and pharmacologically active secondary metabolites, including steroids, terpenoids, alkaloids, and polyketides. Many of these compounds have demonstrated significant biological activities, such as anti-inflammatory, antibacterial, cytotoxic, and antitumor effects [16–22].

This review summarizes recent advances in the discovery, structural diversity, and biological activity of bioactive and structurally unusual steroids produced by microbial sources.

2. The Genus Aspergillus

Aspergillus (Ascomycota) is a genus of filamentous fungi characterized by predominantly asexual reproduction. Species within this genus are cosmopolitan and ecologically versatile, playing important roles in natural ecosystems as well as in various sectors of the human economy. Owing to their capacity to produce a wide array of extracellular enzymes and organic acids, *Aspergillus* species have long attracted scientific interest for their biotechnological and industrial applications. They also synthesize numerous secondary metabolites of relevance to biotechnology and natural product discovery [23–26].

Despite their beneficial uses, several *Aspergillus* species are pathogenic to plants and animals. In humans, aspergillosis refers collectively to diseases caused by members of this genus. Systemic *Aspergillus* infections generally occur in immunocompromised individuals, and the rising incidence of invasive aspergillosis has placed an increasing burden on clinical healthcare systems [26–28].

1.1. Steroid Production in Aspergillus aculeatus

Recent work by Yue and colleagues [29] reported the isolation of novel steroids from the endophytic fungus *Aspergillus aculeatus*. This strain was isolated from the internal tissues of a surface-sterilized leaf of *Pseudostellaria heterophylla*, collected in Liaoning Province, China. *Ps. heterophylla*, known as “hai er shen” or “tai zi shen” and commonly referred to as false chickweed, is a eudicot species in the family Caryophyllaceae. It is widely used in traditional Chinese medicine as a qi tonic and a yin-nourishing herb [30,31].

Six new steroids (compounds **1–5** and **7**, the structures are shown in Figure 1) were isolated from an ethyl acetate extract obtained after incubating the fungal strain on a rice substrate for 15 days. Structural elucidation was accomplished through comprehensive spectroscopic analyses and comparison with previously reported data. These compounds were identified as camphoratin L (**1**), camphoratin K (**2**), camphoratin M (**3**), camphoratin O (**4**), camphoratin N (**5**), and camphoratin P (**7**). The anti-inflammatory properties of compounds **1, 2, 4, 6**, and **8** were assessed by measuring nitric oxide (NO) production in LPS-stimulated RAW264.7 macrophages. Compounds **1**, **2**, and **6** exhibited moderate inhibitory effects on NO release, indicating potential anti-inflammatory activity [29,32].

Several known steroids were also identified: (22*E*,24*S*)-5α,8α-epidioxy-24-ethylcholesta-6,22-dien-3β-ol (**6**), previously isolated from the mushroom *Lactarium volemus* [33]; ergosta-7,22-dien-6β-methanoxy-3β,5α-diol (**8**), previously reported in *Aspergillus awamori* [34]; and cerevisterol (**9**), known from *Penicillium brasilianum* [35].

Further investigation of other fractions from *A. aculeatus* revealed a broader diversity of steroidal metabolites. HSQC spectral datasets were exported as CSV files and analyzed using the SMART system to predict potential structural types. The computational analysis indicated that the fractions likely contain additional steroids (compounds **10–24**), corresponding to structures present in existing spectral databases. Thus, it is reasonable to infer that *A. aculeatus* also produces steroids (**10–24**) [29,32].

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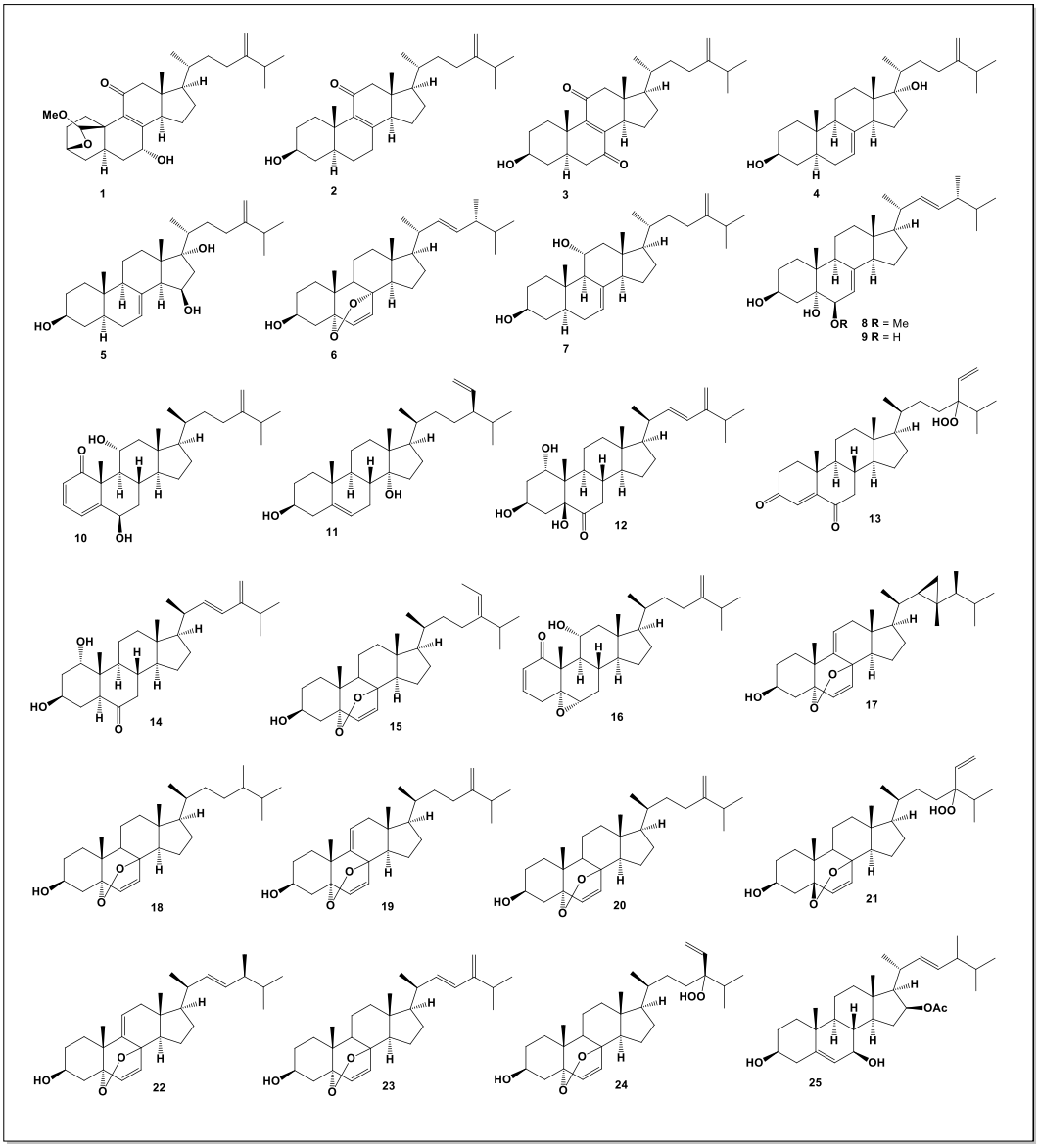

Figure 1. New steroids produced by Ascomycetes of the genus Aspergillus.

1.2. Steroids from other Aspergillus Species

Penicysteroid C (**25**), a polyoxygenated steroid, was isolated from a co-culture of *Aspergillus niger* and *Streptomyces pyomogenus* AS63D grown on rice medium under solid-state fermentation. This compound demonstrated antimicrobial and cytotoxic activities, highlighting the potential of interspecies co-cultivation strategies for activating otherwise silent biosynthetic pathways [36].

Aspersteroid A (**26**, Figure 2), a highly rearranged 1(10→6)-abeo-18,22-cyclosterol, together with two additional 18,22-cyclosterols (**27** and **28**), was isolated from the culture extract of *Aspergillus ustus* NRRL 275. Sterol **26** features a highly unusual carbon framework, comprising a 6/6/6/5/5 fused-ring system. Its proposed biosynthetic pathway involves A-ring scission, sequential 1,2-shifts, and subsequent C-18/C-22 cyclization. Notably, aspersteroid A exhibited potent immunosuppressive and antimicrobial activities [37].

An endophytic strain *Aspergillus* sp. 1022LEF, inhabiting the internal tissues of a red marine alga, produced a novel polyketide–terpene hybrid metabolite, tennessenoid A (**29**). This compound represents an unprecedented steroid–sorbicillinoid adduct linked through a C–C bond, a rare structural motif in natural products. Tennessenoid A showed broad-spectrum antifungal activity against *Sclerotium rolfsii, Fusarium oxysporum, Fusarium* sp., *Coniella diplodiella, Physalospora piricola, Fusarium graminearum, Alternaria mali, Colletotrichum orbiculare*, and *Alternaria porri* [38].

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Spectasterols A–E (**30**–**34**), aromatic ergosterols with distinctive ring architectures, were isolated from *Aspergillus spectabilis*. Sterols **30** and **31** contain a 6/6/6/5/5 ring system with an additional cyclopentene moiety, whereas compounds 32 and 33 possess an unusual 6/6/6/6 system generated through D-ring expansion mediated by 1,2-alkyl shifts. Compound **33** demonstrated cytotoxic activity (IC_{50} = 6.9 μM) and induced cell cycle arrest and apoptosis in HL-60 cells. It also exhibited anti-inflammatory effects by reducing COX-2 expression at both transcriptional and protein levels and by inhibiting the nuclear translocation of NF-κB p65 [39].

Two structurally unusual naturally occurring Diels–Alder adduct steroids, ergosterdiacids A and B (**35** and **36**), featuring a 6/6/6/6/5 pentacyclic system, were isolated from a mangrove-derived *Aspergillus* sp. Both compounds inhibited *Mycobacterium tuberculosis* protein tyrosine phosphatase B (MptpB), with IC_{50} values of 15.1 and 30.1 μM, respectively, acting through a noncompetitive mechanism. Additionally, both sterols displayed potent anti-inflammatory activity, suppressing nitric oxide production at 4.5 and 3.6 μM, respectively [40].

From *Aspergillus nidulans*, two previously undescribed 30-norlanostane triterpenoids, nidulanoids A and B, were isolated along with an ergostane-type steroid featuring an unusual Δ17,20 double bond, designated as (17*E*,22*E*,24*R*)-3β,5α-dihydroxyergosta-7,17,22-trien-6,16-dione (**37**), and a pregnane derivative, (7*Z*,9*Z*,17*Z*)-2α,3β-dihydroxypregna-7,9,17(20)-trien-18-al (**38**).

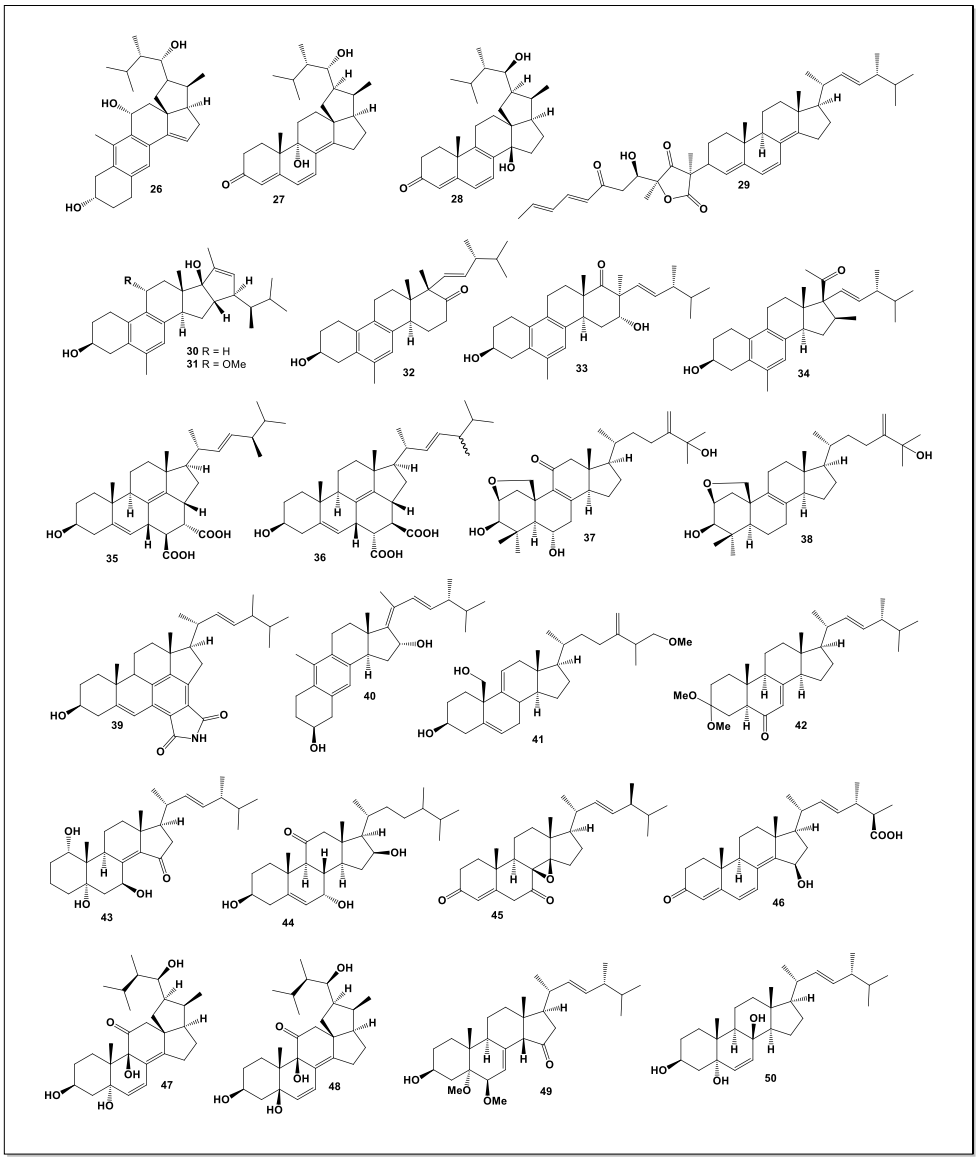

Figure 2. Bioactive rare and uncommon steroids derived from fungal endophytes.

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Nidulanoids A and B represent the first natural examples of 30-norlanostane triterpenoids bearing a C9 side-chain at C-17 and a hemiacetal moiety linking C-3 and C-19, suggesting their role as intermediates between lanostanes and conventional steroids. Compound **37** is notable for its atypical Δ17,20 unsaturation, while **38** is the first reported C-21 steroid containing an aldehyde group at C-13. Among these metabolites, compound 38 displayed moderate cytotoxicity against PC12 rat neuronal cancer cells (IC_{50} = 7.34 μM). Collectively, these findings expand the structural diversity of triterpenoid and steroidal metabolites produced by *A. nidulans*, and highlight compound **38** as a promising anticancer lead [41].

A sterol derivative, ergosterimide B (**39**), was isolated from the rice fermentation broth of *Aspergillus tubingensis* YP-2. This newly characterized compound exhibited weak cytotoxicity, with IC_{50} = 11.05 μM against tested cell lines [42].

The deep-sea fungus *Aspergillus unguis* IV17-109 yielded an ergostane-type sterol, aspersterol A (**40**), distinguished by a rare side chain featuring conjugated Δ17 and Δ22 double bonds. Aspersterol A is an uncommon anthra-steroid bearing a hydroxyl group at C-2 and demonstrated broad cytotoxicity across six cancer cell lines, with a GI_{50} value of 3.4 μM [43].

A fungal strain, 1901NT-1.40.2, isolated from a *Cliona* sp. sponge collected at 8–10 m depth in Nha Trang Bay, Vietnam, was identified as *Aspergillus subramanianii*. This strain produced an undescribed ergostane-type triterpenoid, aspersubrin A (**41**), representing a new addition to the structural repertoire of *Aspergillus* metabolites [44].

From the marine-derived endophytic fungus *Aspergillus ochraceus* MCCC 3A00521, a new ergostane-type sterol, ochrasterone (**42**), was isolated. This species, an established sterol producer, is preserved in the Marine Culture Collection of China [45].

Finally, a new sterol, aspersterol E (**43**), was obtained from an endophytic *Aspergillus* species associated with *Hibiscus tiliaceus* (Malvaceae, see Figure 3). Compound **43** exhibited cytotoxicity toward MFC mouse pre-gastric cancer cells (IC_{50} = 153.43 μM) [46]. In addition, a newly isolated *Aspergillus* species from soil in Qalubiya Governorate, Egypt, displayed strong antimicrobial activity. Chemical analysis revealed the presence of a polyhydroxysterol (**44**), which demonstrated potent antimicrobial effects [47].

An endophytic fungal strain was isolated from the fruit of the mangrove tree *Avicennia marina*, collected at Kilo 17, Safaga, Red Sea, Egypt. From this isolate, identified as *Aspergillus versicolor*, a new epoxy-ergostane sterol named versicolor (**45**) was obtained as a minor metabolite from fermented rice cultures. Versicolor exhibited inhibitory activity against the SARS-CoV-2 3CL protease (3CLpro), with an IC_{50} value of 2.168 ± 0.09 μM, indicating its potential as a candidate inhibitor of SARS-CoV-2 3CLpro [48].

A second marine-derived fungus, *Aspergillus* sp. ZJUT223, was isolated from seawater collected in the Grant Trough near the Marceau Trough and identified through ITS sequencing. Extraction was performed using ethanol followed by purification with ethyl acetate. From this strain, a new steroid, ganodermanic acid (**46**), was isolated [49].

Additionally, a new 18,22-cyclosterol, aspersteroline A (**47**), together with a structurally related analogue (**48**), was obtained from the soil-derived *Aspergillus versicolor* QC812. Both compounds showed moderate cytotoxic activity against the HL-60 human leukemia cell line [50].

Two oxygenated ergostane-type steroids—one new compound, 3β-hydroxy-5α,6β-methoxyergosta-7,22-dien-15-one (**49**), and one known analogue, ergosta-6,22-dien-3β,5α,8α-triol (**50**)—were isolated from crude extracts of a marine sponge-derived *Aspergillus* sp. Notably, compound **49** represents a marine ergostane-type steroid featuring two methoxy groups at C-5 and C-6 and demonstrated antibacterial activity against *Staphylococcus aureus* [51].

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Figure 3. Fungal endophytes related to the genus *Aspergillus*, which are producers of active steroids, are found in a variety of organisms, which are presented below. **a**) *Pseudostellaria heterophylla* (Tai Zi Shen or False Starwort) is a vital Chinese medicinal herb, known as "lung ginseng," prized for its mild, ginseng-like properties, strengthening spleen, boosting qi, moistening lungs, and supporting immunity, often used for fatigue, poor appetite, and post-illness weakness, with its roots containing bioactive compounds like steroids and saponins, cultivated in China and used in health foods. **b**) *Cliona* is a genus of boring sponges, known for their ability to excavate tunnels and chambers within calcium carbonate substrates, such as limestone, coral, and mollusk shells. They are found worldwide and play a significant role in marine ecosystems by recycling calcium carbonate and shaping benthic habitats. **c**) *Hibiscus tiliaceus*, or Beach Hibiscus, is a versatile plant used in traditional medicine for fevers, coughs, chest congestion, diarrhea, and skin issues like abscesses, with leaves, flowers, bark, and sap all utilized for ailments like infections, inflammation, and as laxatives, possessing antioxidant and antimicrobial properties. **d**) The fruits of *Avicennia marina* (Grey Mangrove) are green, oval capsules, about 20-25 mm in diameter, with a short beak, developing from creamy-yellow flowers and often germinating on the tree before falling to be dispersed by water. They are rich in nutrients, offering high caloric value and essential proteins, fats, and carbs, and are used traditionally for food and medicine due to their strong antioxidant properties and bioactive compounds, though high doses might have mild effects on liver/kidneys.

3. The Genus Penicillium

Penicillium is a globally distributed genus of blue–green molds belonging to the kingdom Fungi. Species within this genus reproduce asexually (anamorphic or deuteromycetous forms) and play major ecological roles as decomposers of organic matter. Many species cause destructive spoilage in the food industry and produce a broad range of mycotoxins, while others function as prolific producers of industrial enzymes or are recognized as common indoor allergens. Although DNA sequencing is essential for accurate species identification, the lack of a comprehensive and validated reference database continues to challenge *Penicillium* taxonomy [52–54].

With the adoption of the "one fungus, one name" principle under the International Code of Nomenclature for algae, fungi, and plants, the generic boundaries of *Penicillium* have been revised,

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incorporating species previously assigned to genera such as *Chromocleista*, *Eladia*, *Eupenicillium*, *Torulomyces*, and *Thysanophora*. Together, these taxa form a broader monophyletic clade. Reflecting the numerous new species described in recent years, the list of accepted *Penicillium* species has been updated and currently includes 354 recognized species. This revision also includes new combinations involving *Aspergillus crystallinus*, *A. malodoratus*, and *A. paradoxus*, all of which have been reassigned to *Penicillium* section *Paradoxa* [55–57].

To increase the taxonomic utility of the updated species list, information is provided on MycoBank numbers, live ex-type cultures, and GenBank accession numbers for ITS, β-tubulin, calmodulin, and RPB2 sequences, thereby supplying a verified reference set for researchers working with this genus. Standardized protocols for species description and identification are also recommended to improve reliability and reproducibility across laboratories [58–63].

3.1. Steroidal Metabolites from Penicillium

A new steroid, persteroid (**51**, see Figure 4), was isolated from the marine-derived *Penicillium* sp. ZYX-Z-143. Persteroid exhibited inhibitory activity against protein tyrosine phosphatase 1B (PTP1B), with an IC_{50} value of 46 μM, and strongly suppressed nitric oxide (NO) production in LPS-stimulated RAW264.7 macrophages, suggesting potential anti-inflammatory properties [64].

Another new steroid, penivariod A (**52**), was isolated from *Penicillium variabile* EN-394, an endophytic strain obtained from the marine red alga *Rhodomela confervoides*. Penivariod A demonstrated potent antimicrobial activity, particularly against *Escherichia coli* and *Pseudomonas aeruginosa*, with MIC values of 1.0 and 2.0 μg/mL, respectively [65].

A series of unusual C_{25} steroids (**53**–**66**), characterized by a distinctive bicyclo[4.4.1] A/B ring system, were isolated from an antitumor mutant strain of *Penicillium purpurogenum* G59 AD-1-2. The isolated metabolites included antineocyclocitrinols A (**53**) and B (**54**), and 23-O-methylantineocyclocitrinol (**55**), all featuring a bicyclo[4.4.1] A/B framework with a Z-configured $\Delta^{20,22}$ double bond. Additional C_{25} steroids—neocyclocitrinols A (**57**), B (**56**), C (**59**), and D (**58**), *threo*-23-O-methylneocyclocitrinol (**60**), *erythro*-23-O-methyl-neo-cyclocitrinol (**61**), 24-epi-cyclocitrinol (**62**), cyclocitrinol (**63**), 20-O-methyl-24-epi-cyclocitrinol (**64**), 20-O-methylcyclocitrinol (**65**), and isocyclocitrinol B (**66**)—were also identified. All compounds displayed varying degrees of cytotoxicity against multiple human cancer cell lines, highlighting the pharmacological potential of this structurally unique class of C_{25} steroids [66].

Two new C_{23}-steroid derivatives, cyclocitrinic acid A (**67**) and cyclocitrinic acid B (**68**), were isolated from the mangrove-derived fungus *Penicillium* sp. SCSIO 41429. Cyclocitrinic acid B demonstrated moderate pancreatic lipase inhibition, with an IC_{50} value of 32 μM, and exhibited both pancreatic lipase inhibitory and antioxidant properties [67].

From the plant-associated fungus *Penicillium fellutanum*, an unusual clathrate-type meroterpenoid, isoatlantinone A (**69**), along with two new steroids, acrocalysterols E (**70**) and F (**71**), was isolated. Isoatlantinone A is notable for its highly oxygenated meroterpenoid structure featuring a unique caged bioxatetracyclo-[6.3.2.$0^{1,6}$.$0^{1,12}$]-tridecane ring system. All isolates were screened for antifungal and cytotoxic activities, among which compound **71** exhibited potent cytotoxicity toward HCC-1806 human breast cancer cells (IC_{50} = 18.15 ± 1.05 μM). These findings highlight *P. fellutanum* as a promising source of structurally novel and bioactive metabolites [68].

Chemical investigation of *Penicillium oxalicum* 2021CDF-3, an endophytic fungus associated with marine red algae, led to the discovery of a new polyoxygenated ergostane steroid, peniciloxatone A (**72**). This compound showed cytotoxic activity against FADU and HepG2 cell lines, with IC_{50} values of 9.5 and 18.1 μM, respectively [69].

A new steroid with strong antibacterial activity, rubensteroid A (**73**), along with its decarboxylated analogue, solitumergosterol A (**74**), was isolated from the Magellan Seamount-derived fungus *Penicillium rubens* AS-130. Rubensteroid A features a rare 6/6/6/6/5 pentacyclic ring system, proposed to originate from a [4+2] Diels–Alder cycloaddition between 14,15-didehydroergosterol (14-DHE) and maleic acid or maleimide, followed by decarboxylation.

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Compound **73** demonstrated potent antibacterial activity against *Escherichia coli* and *Vibrio parahaemolyticus*, both with MIC values of 0.5 μg/mL [70].

From the lichen-associated fungus *Penicillium aurantiacobrunneum*, two new sterols—(20*S*)-hydroxy-24(28)-dehydrocampesterol (**75**) and 7α-methoxy-8β-hydroxy-paxisterol (**76**)—were obtained. Sterol **75** showed cytotoxicity against the HPAC pancreatic adenocarcinoma epithelial cell line (IC_{50} = 17.76 ± 5.35 μM) [71].

A new cytotoxic steroid, 16α-methylpregna-17α,19-dihydroxy-(9,11)-epoxy-4-ene-3,18-dione-20-acetoxy (**77**), was isolated from *Penicillium citrinum* SCSIO 41017, associated with the sponge *Callyspongia* sp. This compound displayed moderate cytotoxicity against MCF-7 human breast cancer cells, with IC_{50} values of 13.5–18.0 μM [72].

From mangrove sediments collected in the Dongzhaigang Mangrove Reserve (Hainan, China), strain ABC190807 of *Penicillium brefeldianum* was isolated. Its EtOAc extract exhibited potent larvicidal activity against *Aedes aegypti* third-instar larvae (LC_{50} = 0.089 mg/mL). A novel purinyl steroid, ergosta-4,6,8(14),22-tetraen-3-(6-amino-9H-purin-9-yl) (**78**), was isolated from this extract [73].

Sterolic acid (**79**, see Figure 5), an unusual sterol, was isolated from a deep-sea sediment-derived *Penicillium* sp. This metabolite features a diepoxy moiety within its A-ring and an oxabicyclo[2.2.2]octane system—structural motifs previously known only from plant-derived natural products. Sterolic acid additionally contains a carboxylic acid group at C-27, further distinguishing it from typical fungal sterols [74].

Penicillitone (**80**), a sterol with a rare tetracyclic skeleton, was obtained from *Penicillium purpurogenum*. Penicillitone exhibited notable cytotoxicity toward multiple cancer cell lines, including A549 (IC_{50} = 5.57 μM), HepG2 (IC_{50} = 4.44 μM), and MCF-7 (IC_{50} = 5.98 μM), with adriamycin serving as a positive control [75].

A novel inhibitor of anchorage-independent tumor cell growth was isolated from the culture broth of *Penicillium aurantiogriseum* TP-F0213. The compound, identified as 16-acetoxy-3,7,11-trihydroxyergost-22-en-6-one (**81**), known as anicequol, possesses an ergostane-type carbon skeleton with substituent configurations 3β, 5α, 7β, 11β, 16β, and 24S. Anicequol inhibited anchorage-independent proliferation of DLD-1 human colon cancer cells with an IC_{50} of 1.2 μM, while showing substantially lower activity against anchorage-dependent growth (IC_{50} = 40 μM) [76].

A new polyoxygenated steroid, penicisteroid A (**82**), was isolated from the culture extract of *Penicillium chrysogenum* QEN-24S, an endophytic strain from an unidentified *Laurencia* species (see Figure 6) of marine red algae [77].

The steroid 8(14),22E-dien-3β,5α,6β,7α-tetraol (**83**) was isolated from a *Penicillium* sp. associated with South Pole sea moss. This compound showed anticancer activity toward HepG2 liver cancer cells, with an IC_{50} of 10.4 μg/mL [78].

Two novel naturally occurring [4+2] Diels–Alder cycloaddition ergosteroids (**84** and **85**) were isolated from *Penicillium herquei*. These compounds represent the first known steroidal cycloadducts formed with 1,4,6-trimethyl-1,6-dihydropyridine-2,5-dione or 4,6-dimethyl-1,6-dihydropyridine-2,5-dione [79].

Several novel steroids—citristerones A (**86**), B (**87**), D (**88**), E (**89**), and a new series of 23,24-diol-containing ergosterols (**90**)—along with three known analogues, were isolated from *Penicillium citrinum* TJ507, an endophytic strain from *Hypericum wilsonii*. Citristerone B exhibited exceptional anti-neuroinflammatory activity (IC_{50} = 0.60 μM) in LPS-stimulated BV-2 microglial cells. Further mechanistic studies revealed that citristerone B markedly reduced NO and cytokine release, inhibited TNF-α, iNOS, and NF-κB expression, and suppressed ROS accumulation [80].

Three andrastin-type meroterpenoids, hemiacetalmeroterpenoids A–C (**91–93**), were isolated from the mangrove-soil-derived *Penicillium* sp. N-5. Hemiacetalmeroterpenoid A (**91**) possesses a unique, highly congested 6,6,6,6,5,5 hexacyclic skeleton and exhibited strong antimicrobial activity against *Penicillium italicum* and *Colletotrichum gloeosporioides* (MIC = 6.25 μg/mL) [81].

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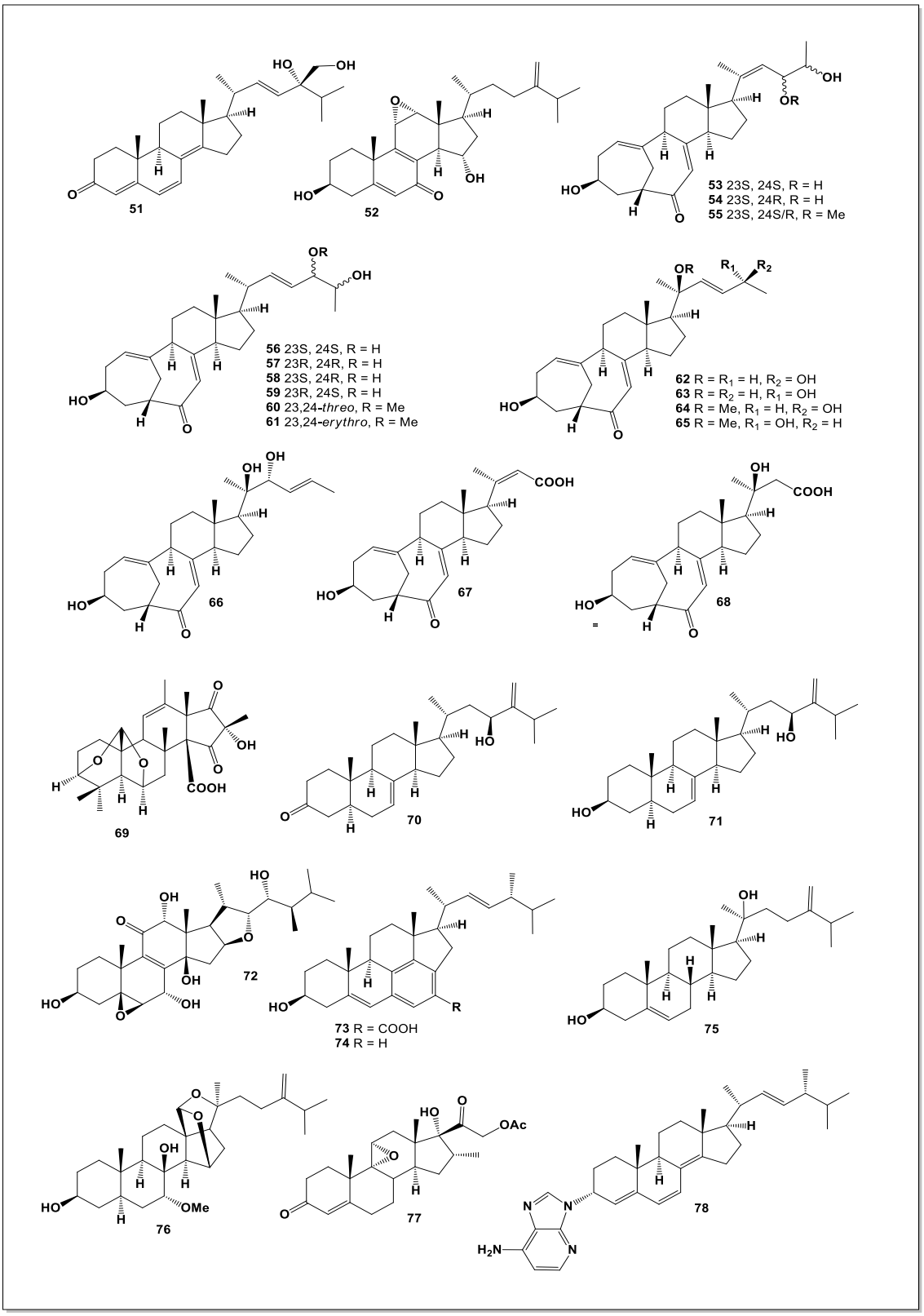

Figure 4. Steroidal hormones derived from Penicillium species.

Two novel nortriterpenoids (**94** and **95**) were isolated from the endophytic fungal strain *Penicillium ochrochloron* SWUKD4.1850, collected from healthy *Kalmia angustifolia* in Yunnan Province, China. Compound **95** represents the first naturally occurring 27-nor-3,4-secocycloartane shinortriterpenoid and exhibited moderate cytotoxicity toward HL-60, SMMC-7721, and MCF-7 cell lines (IC_{50} = 6.5–17.8 μM) [82].

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From *Penicillium expansum* WTJP1, isolated from *Aconitum carmichaelii*, a previously undescribed compound named expansinin (**96**) was discovered. Expansinin represents the first naturally occurring conjugate of an indole alkaloid and an ergosteroid, and was evaluated for cytotoxicity against five human cancer cell lines [83].

Scabrosteroid A (**97**), a novel steroidal heterodimer, was isolated from *Penicillium scabrosum* FXI744. This compound represents the first example of a naturally occurring pyrrolidinone–ergosterol hybrid, linked *via* a C-3/C-3′ bond. Scabrosteroid A inhibited NO production (IC_{50} = 9.5 μM) and showed moderate immunosuppressive activity [84].

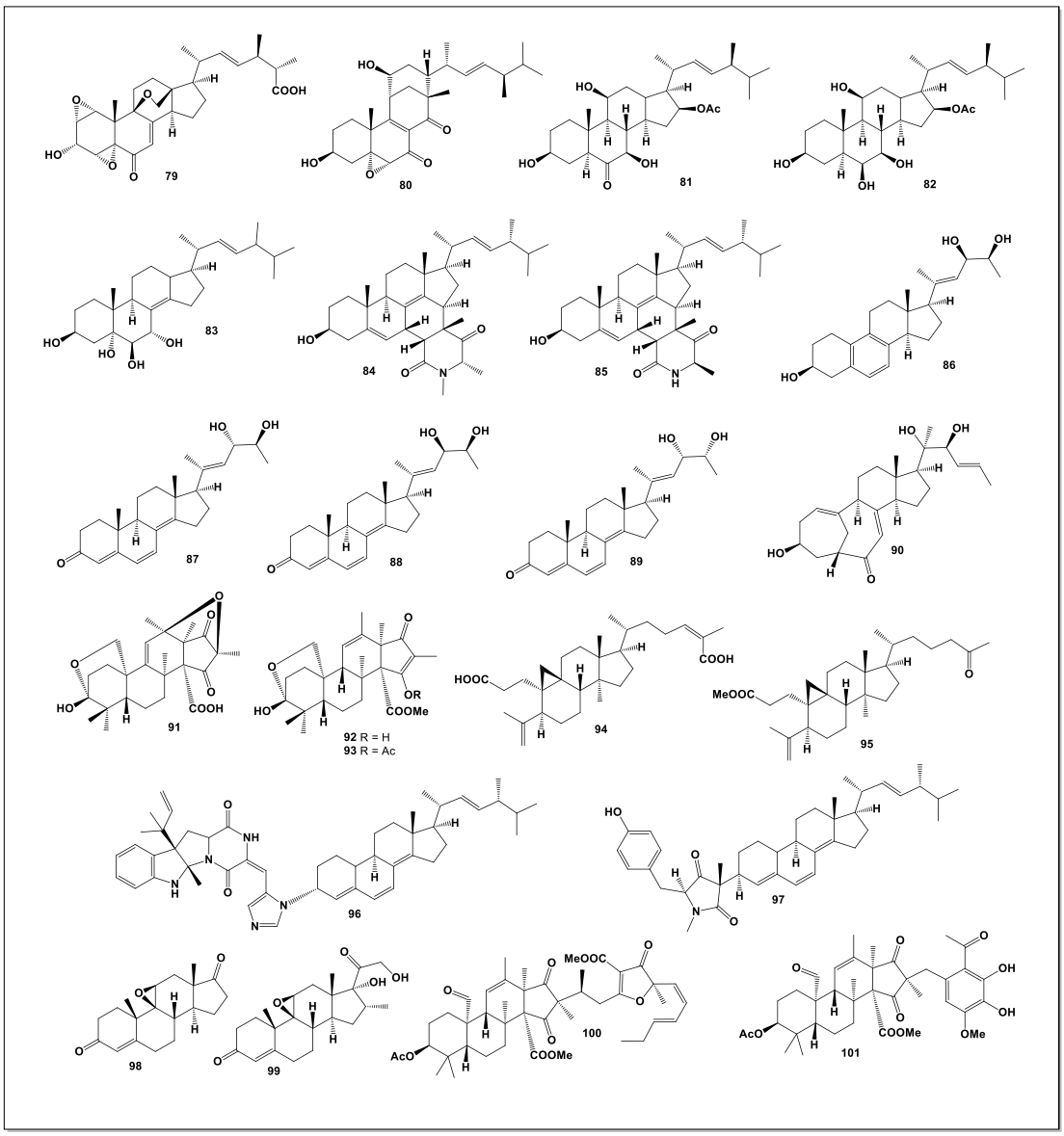

Figure 5. Steroid and their unusual derivatives derived from Penicillium species.

Penicildiones A (**98**) and B (**99**), two new steroids, were isolated from the soft-coral-derived fungus *Penicillium* sp. SCSIO 41201 cultured in 1% NaCl potato dextrose broth [85].

Finally, isopenicins A (**100**) and C (**101**)—novel meroterpenoids with unprecedented terpenoid–polyketide hybrid skeletons—were isolated from *Penicillium* sp. sh18. Compound **102** was identified as a potent inhibitor of the Wnt/β-catenin signaling pathway [86].

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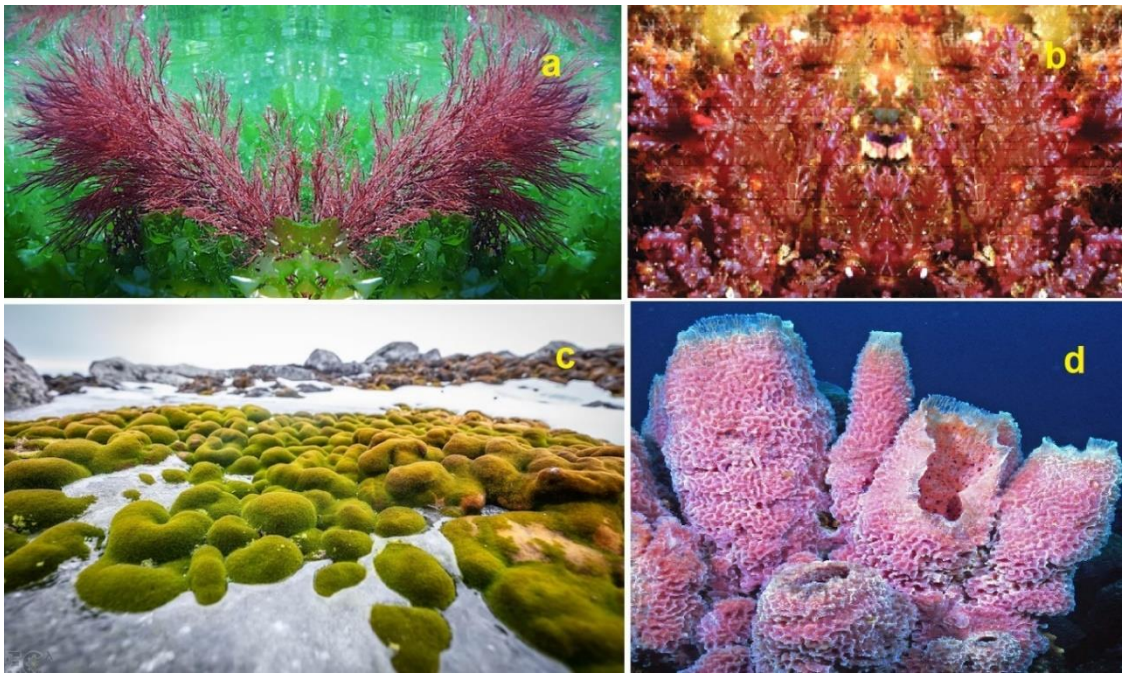

Figure 6. Fungal endophytes related to the genus *Penicillium*, which are producers of active steroids, are found primarily in marine red algae, sea mosses, and sea sponges, some of which are presented below. **a**) *Rhodomela confervoides* is a common species of brownish-red, bushy marine red algae (seaweed) that is widely distributed in intertidal pools. It belongs to the family Rhodomelaceae. **b**) The genus *Laurencia* comprises approximately 130 to 137 taxonomically accepted species of marine red algae worldwide. These species are widely distributed in tropical, subtropical, and temperate waters and are known for producing a large number of secondary metabolites with various biological activities. **c**) South Pole (Antarctic) sea moss refers to the unique, hardy mosses that thrive in Antarctica's extreme coastal environments, acting as vital mini-ecosystems in the icy desert, growing slowly in green carpets or banks, absorbing sun for warmth, drying out to survive winter, and providing habitat for tiny creatures like tardigrades, with their growth patterns also serving as key indicators of climate change. **d**) The genus *Callyspongia* encompasses a wide variety of marine sponges found in tropical coral reef ecosystems worldwide, known for their diverse forms and colors. They are filter feeders and play an important ecological role in reef communities.

4. Steroids Produced by Miscellaneous Microorganisms

A structurally unique C_{25} steroid, phomarol (**103**, see Figure 7), was isolated from a cultured *Phoma* sp. derived from the giant jellyfish *Nemopilema nomurai*. Phomarol is distinguished by a seven-membered carbocyclic A ring [1(10→19)abeo], an aromatic B ring, and a cyclized side chain forming a fused pentacyclic skeleton [87].

From the mangrove endophytic fungus *Talaromyces* sp. SCNU-F0041, a rare 9,11-secosteroid—cyclosecosteroid A (**104**)—was isolated. This compound exhibited moderate acetylcholinesterase (AChE) inhibitory activity, with an IC_{50} value of 46 μM [88].

Four new 9,11-secosteroid-derived γ-lactones—altersteroids A (**105**), B (**106**), C (**107**), and D (**108**)—were obtained from cultures of the ascomycete *Alternaria* sp. These metabolites contain a γ-lactone fused to the steroid D ring at C-13/C-14. Among them, compound **107** demonstrated moderate cytotoxicity against several tumor cell lines and induced apoptosis in A549 cells; notably, it displayed equal potency against both cisplatin-sensitive MB49 and cisplatin-resistant MB49 CisR cells (IC_{50} = 12.7 μM) [89].

Two new steroids, acrocalysterols A (**109**) and B (**110**), were isolated from *Acrocalymma* sp., an endophytic fungus obtained from the tender stems of *Sinomenium acutum*. Compound **110** displayed potent cytotoxicity toward HeLa, HCC-1806, and RKO cancer cell lines, with IC_{50} values ranging from 18.37 to 19.64 μM [90].

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A new ergostane-type steroid, (22E)-3α,6α,9α-ergosta-7,22-diene-3,6,9-triol (**111**), was isolated from *Periconia pseudobyssoides* K5 grown on fermented unpolished rice. This endophytic fungus was isolated from a deciduous tree *Toona sureni*. Compound **111** inhibited heme polymerization with an IC_{50} of 8.24 ± 0.03 mg/mL [91].

Investigation of the endophyte *Phaeosphaeria spartinae*, associated with the marine red alga *Ceramium* sp., yielded spartopregnenolone (112), a metabolite combining features of triterpenes and steroids. Its structure—including a $\Delta^{8,9}$ double bond, a C-4 carboxyl group, and an acetylated side chain—identifies it as a 4α-carboxy-8,9-pregnene derivative representing a transitional form in triterpene-to-steroid biosynthesis [92].

Two new functionalized ergostane-type steroids, phomopsterones A (**113**) and B (**114**), were isolated from *Phomopsis* sp. TJ507A. Phomopsterone A features an unprecedented rearranged bicyclo[3.3.1]nonane motif formed through B-ring scission and subsequent 180° rotation of the A ring. Phomopsterone B exhibited anti-inflammatory activity [93].

Phytochemical analysis of *Stereum hirsutum* fermentation broth led to the identification of ten steroids, including two previously unreported ones—steresterones A (**115**) and B (**116**)—and compound **117**. Compound **117** displayed significant anti-proliferative activity, with IC_{50} values as low as 2.3 μM [94].

Two unusual steroid-like metabolites, asterogynin A (**118**) and asterogynin B (**119**), were obtained from an endophytic fungus isolated from the small palm *Asterogyne martiana* [95].

From *Trichoderma koningiopsis*, an endophytic fungus isolated from the gut of a centipede collected on *Tongji campus*, a steroidal derivative (**120**) was isolated. Named trichosterol A, it represents the first naturally occurring steroid–alkaloid hybrid containing a rare 6/6/6/5/6 pentacyclic skeleton incorporating an unusual 1,2-oxazine moiety. Compound 120 exhibited notable herbicidal activity against *Medicago sativa*, highlighting its potential as a natural bioherbicide lead molecule [96].

Two novel steroids—microascusteroids A and B (**121** and **122**)—were isolated from the marine-derived fungus *Microascus* sp. SCSIO 41821. These metabolites are unusual 5,6-seco-9,10-seco ergostane derivatives featuring a rearranged C ring. Both compounds moderately inhibited NO production in LPS-stimulated RAW 264.7 cells and showed mild inhibitory effects on PTP1B as well as cytotoxicity against H1975 and HepG2 cancer cells [97].

Chaeglobol A (**123**), obtained from *Chaetomium globosum* HBU-45, possesses a highly unusual octacyclic 6/6/6/5/6/5/6/5 skeleton. Its biosynthesis is proposed to involve a [4+2] cycloaddition followed by enzymatic cyclization. Chaeglobol A inhibited Botryosphaeria dothidea by disrupting cell membrane integrity and inducing oxidative damage [98].

Three unprecedented hybrid steroids—striasteroids A–C (**124**–**126**)—were isolated from *Striaticonidium cinctum* SCSIO 41432. Striasteroids A and B feature a new 6/6/6/6/6/5 hexacyclic framework forming polyketide–steroid hybrids bridged by an oxabicyclo[3.3.1]nonane moiety. Striasteroid C (**126**), the first naturally occurring adenine–steroid hybrid, showed strong neuraminidase inhibition (IC_{50} = 6.15 ± 1.15 μM) [99].

The sponge-derived fungus *Gymnacella dankaliensis* produced two exceptionally unusual steroids, dankasterones A (**127**) and B (**128**), when cultured in a modified malt extract medium containing soluble starch instead of glucose. Gymnasterone A (**129**) was obtained from standard malt extract medium. All steroids except **129** inhibited growth of murine P388 cancer cells, and dankasterone A also showed potent activity against human cancer cell lines [100].

Two undescribed steroids, bipolarsterols A (**130**) and B (**131**), were isolated from pathogenic fungus *Bipolaris oryzae*, along with nine known congeners. Bipolarsterol A represents the first example of a 19(10→5)-abeo-7(8→9)-abeo-ergostane featuring a spiro[4.5]decan-6-one system, while bipolarsterol B is a new member of the rare steroid–phenylpropanoid hybrid class [101].

Matsutakone (**132**), a novel sterol featuring an unprecedented polycyclic ring system, along with a new norsteroid, matsutoic acid (**133**), was isolated from the fruiting bodies of *Tricholoma matsutake*. Bioassay results demonstrated that both compounds exhibited inhibitory activity against acetylcholinesterase, with compound **132** displaying an IC_{50} value of 20.9 μM [102].

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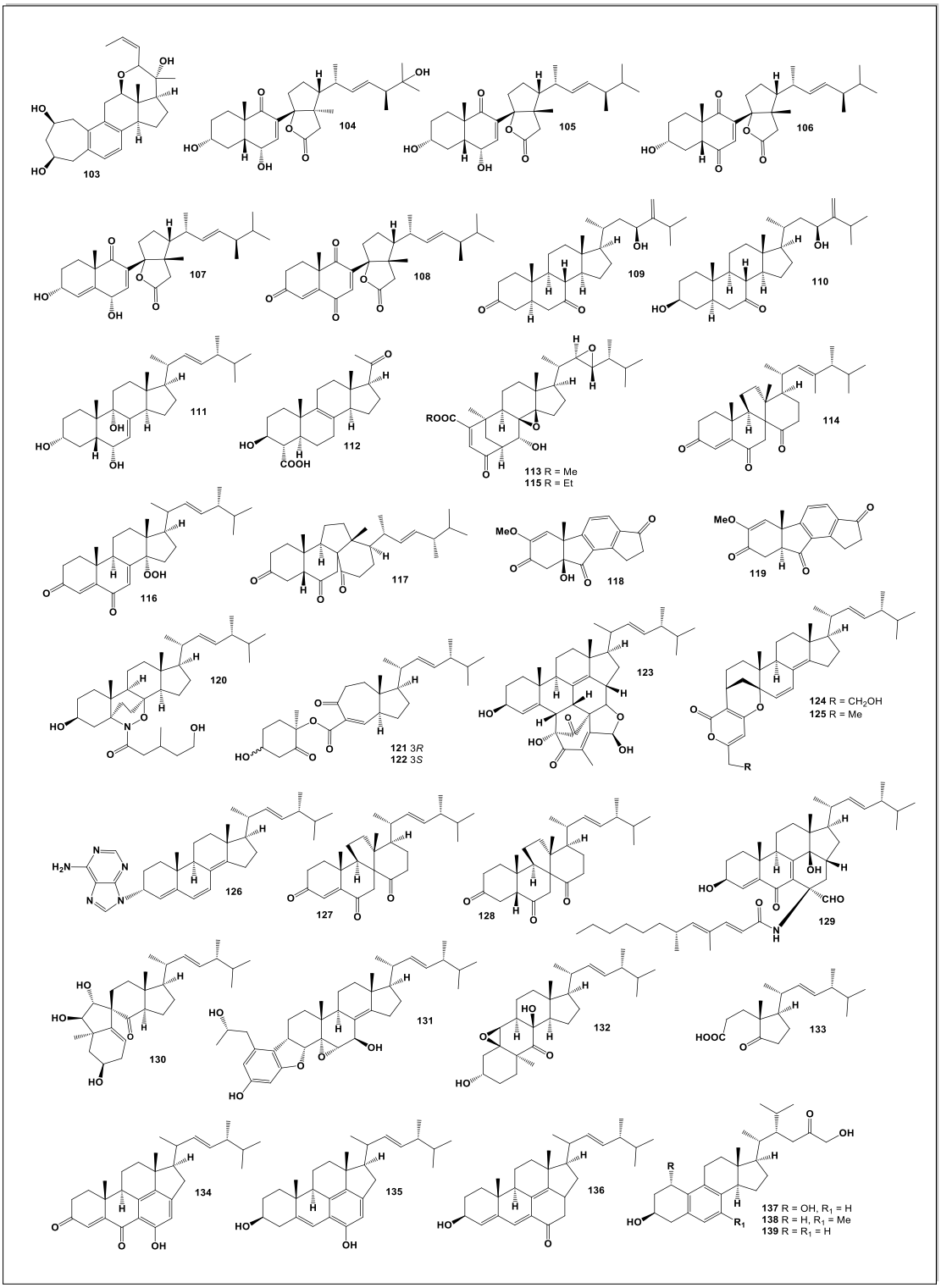

Figure 7. Steroids and their unusual derivatives derived from miscellaneous microorganisms.

Three unusual C_{30} ergosterols—cordycepsterols A–C (**134–136**)—were isolated from the medicinal fungus *Cordyceps militaris*. These sterols possess an uncommon 6/6/6/5/6 pentacyclic skeleton that is hypothesized to arise from a canonical ergosterol framework through extension by two additional skeletal carbons. All three metabolites showed potent inhibition of nitric oxide production, with IC_{50} values of 3.0, 0.9, and 2.3 μM, respectively. At 5 μM, they significantly suppressed the secretion of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), underscoring their promising anti-inflammatory potential and highlighting *C. militaris* as a valuable source of bioactive sterols [103].

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Mangrove endophytic fungi, as extremophilic microorganisms, are known to produce diverse and biologically active secondary metabolites. A strain of *Dothiorella* sp. ZJQQYZ-1, isolated from the mangrove plant *Kandelia candel*, yielded six metabolites, including three new benzofuran derivatives and three new steroids: phomosterol C (**137**), phomosterol B (**138**), and phomosterol A (**139**). Among these, phomosterol A exhibited significant anti-inflammatory activity with an IC_{50} value of 4.6 μM. Mechanistic studies further revealed that compound **139** effectively suppressed the protein expression of inducible nitric oxide synthase (iNOS) in LPS-stimulated RAW264.7 macrophages [104].

5. Conclusion

Fungal organisms—particularly endophytic, marine-derived, and extremophilic species—continue to demonstrate exceptional biosynthetic richness, yielding steroids with unprecedented structural diversity. The discoveries summarized in this review highlight an expanding repertoire of ergostane, lanostane, pregnane, abeo-rearranged, secosteroid, and hybrid scaffolds, many of which feature rare ring systems, extensive oxygenation, or heterocyclic fusions not previously observed in nature. These structural innovations frequently translate into notable biological activities, including cytotoxic, anti-inflammatory, antimicrobial, neuroactive, and enzyme-inhibitory effects, positioning fungal steroids as promising leads for drug discovery and agricultural applications. As advances in isolation techniques, spectroscopy, genome mining, and coculture strategies continue to evolve, the rate of discovering novel steroidal metabolites is likely to accelerate. Overall, fungi represent a largely untapped reservoir of chemically and biologically compelling steroids, underscoring their value in natural product research and their potential contribution to future therapeutic development.

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