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The mycotoxin definition reconsidered towards fungal cyclic depsipeptides

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Abstract

Currently, next to the major classes, cyclic depsipeptides beauvericin and enniatins are also positioned as mycotoxins. However, as there are hundreds more fungal cyclic depsipeptides already identified, should these not be considered as mycotoxins as well? The current status of the mycotoxin definition revealed a lack of consistency, leading to confusion about what compounds should be called mycotoxins. Because this is of pivotal importance in risk assessment prioritization, a clear and quantitatively expressed mycotoxin definition is proposed, based upon data of widely accepted mycotoxins. Finally, this definition is applied to a set of fungal cyclic depsipeptides, revealing that some of these should indeed be considered as mycotoxins.

Keywords

Toxicity, cyclic depsipeptides, mycotoxins, definition, hazard, risk

1. Introduction

The cyclic depsipeptides beauvericin (BEA) and enniatins (ENNs) have recently been positioned as mycotoxins, i.e. metabolites that are at least carcinogenic or toxic in experimental systems (1-3). However, as there are hundreds more fungal cyclic depsipeptides already identified, an important question arises: should these not be considered as mycotoxins as well? An answer to this question is urgently required, due to its impact on the priority status in risk assessment. To determine whether or not they should be considered as mycotoxins, these compounds should meet the criteria posed by the mycotoxin definition. However, since the term was first introduced in the mid 1950s, when it was discovered that aflatoxins, which are secondary metabolites from the fungus *Aspergillus*, had caused the death of more than 100,000 turkeys in the England's poultry industry (4-6), a lack of consistency in the definition and use of the word "mycotoxin" has arisen. Today a huge amount of information about mycotoxins has been already available, but in scientific literature, the authors do not all share the same vision about what should be called a mycotoxin. A re-evaluation of the traditional concepts is thus most certainly required. Therefore, by means of a literature review, we propose here a clear, unambiguous and quantitatively expressed definition, based upon data of some already well-known and widely accepted mycotoxins, allowing more awareness of the now underestimated potential hazard of some of these metabolites. Moreover, we apply our definition to a set of fungal cyclic depsipeptides to determine whether or not these metabolites should be classified as mycotoxins.

2. The mycotoxin definition: current status

In Table 1, an overview of the different mycotoxin definitions found in the literature is presented. Although it is clear that the used definitions differ greatly, by the term 'mycotoxins'

they all mean compounds produced by fungi which are potentially toxic to a certain degree. So all definitions have similarities, however, some of them include more details concerning certain aspects, whereas others are more detailed about other aspects.

Additionally, Berthriller and co-workers defined a mycotoxin subgroup, termed ‘masked’ mycotoxins, as mycotoxin derivatives that are undetectable by conventional analytical techniques because their structure has been changed in the contaminated plant/crop (8). Others have introduced another terminology, namely ‘emerging’ mycotoxins, as mycotoxins represent an emerging food safety (26,27). According to the European Food and Safety Authority (EFSA) (28), an emerging risk can be defined as a risk to human, animal and/or plant health resulting from a newly identified hazard to which a significant exposure may occur, or from an unexpected new increased significant exposure and/or susceptibility to a known hazard.

Already several hundreds of mycotoxins have been identified, although only a few of them have been thoroughly investigated (14), with or without maximum acceptable levels regulated by law (29), and there are even indications that thousands of mycotoxins exist (30). The latter was based on a study comprising a database of 474 mycotoxins and fungal metabolites (31). However, it is not explicitly stated which of the investigated fungal metabolites were considered as mycotoxins, nor does it say that all investigated fungal metabolites are considered as mycotoxic. Finally, the European Mycotoxin Awareness Network (EMAN) (19) has raised the question of whether further important mycotoxins remain to be discovered.

Overall, the confusion concerning the ‘mycotoxin claim’ is understandable and a few major shortcomings cannot be overlooked. It is obvious that there is definite disagreement about the mycotoxin definition, e.g. should mycotoxin identification and classification be viewed from a

hazard or risk point of view? Furthermore, do we consider a precautionary approach or adapt a rather wait-and-see policy? What is considered to be toxic, i.e. how should this toxicity be tested and what quantitative specification thresholds should be applied? Should structurally related compounds automatically be considered as mycotoxins, although they are not produced by the commonly referred fungi, such as *Aspergillus*, *Penicillium* and *Fusarium*, or although their toxicity has not yet been (fully) evaluated/elucidated, i.e. to what extent do we allow false negatives and positives?

3. Proposed mycotoxin definition

Something is a **mycotoxin** if and only if it is a **secondary metabolite** produced by micro**fungi**, posing a **health hazard** to human and vertebrate animal species by exerting a **toxic activity** on human or vertebrate animal cells ***in vitro*** **with 50% effectiveness levels < 1000 μM.**

3.1. Secondary microfungal metabolites

It is generally accepted that mycotoxins are indeed secondary fungal metabolites (7,8,11,12,14-18,21,23-25). The term ‘mycotoxin’ refers only to metabolites produced by microfungi and by convention thus excluding mushroom and yeast toxins. However, opinions remain unclear and divided about the type of microfungi and its occurrence, responsible for the production of mycotoxins. International organizations, i.e. EFSA (28), FAO (7) and FDA (15), as well as scientific researchers (9,23), explicitly state that it especially involves common fungi such as *Aspergillus*, *Penicillium* and *Fusarium*. However, these definitions are all agricultural/food oriented, focusing only on fungi that contaminate feed and food, thereby completely neglecting other possible sources of mycotoxin production. Therefore, this angle towards a proper and unambiguous mycotoxin definition seems insufficient, as traditional mycotoxins are also found

in air particles or on walls of badly maintained, unventilated, humid houses (18,25). Furthermore, marine derived fungi are not mentioned once, although these can also produce toxic secondary metabolites, some of which are structurally very similar to known mycotoxins such as beauvericin and enniatins, e.g. zygosporamide (32,33), causing thus a potential hazard not only to marine ecology, but also to humans through the fishing industry (34).

Thus, restricting the mycotoxin definition to common agricultural fungal origin is inadequate, because it underestimates the impact on the public health. Therefore, we suggest only including the term 'microfungal secondary metabolites' as such into the definition, without further confining the fungal origin.

3.2. Hazard

The International Programme on Chemical Safety (IPCS) has harmonised the context of hazard and risk assessments. Some authors consider introduction via a natural route an important factor in the determination of mycotoxins (20) and therefore attach little importance to cytotoxicity tests or studies evaluating toxicity based on intraperitoneal injection (35). However, based on the information available, the whole mycotoxin definition, discussion should, in our opinion, be best placed within the context of hazard assessment, an important and first part of risk assessment, since the intrinsic toxicity of a compound cannot be altered, meaning that it will always remain a hazard, because it possesses the potential to cause an adverse/toxic effect. Risk, on the other hand, through the process of exposure assessment to the hazard concerned, can be reduced by preventive actions and is very hard to unambiguously determine. Moreover, especially early on, the routes of exposure are usually not clarified. Therefore, while awaiting more toxicity exposure data concerning hazardous compounds, which can take up to several years, a precautionary

approach is preferred above a long during risk policy. In this respect, cytotoxicity studies performed on human or vertebrate animal cells are a cost-effective and animal-friendly way to determine potential toxicity already at an early stage in the hazard assessment. The mycotoxins identified by this approach could first carefully be called 'pseudomycotoxins', while awaiting formal mycotoxin nomination, which involves exposure and risk assessment.

Also to be included in this discussion is the 'low molecular weight' condition for defining a mycotoxin as proposed by Bennet and Klich (18), which is essentially directed towards exposure/risk and not a hazard, since high molecular weight compounds are considered not to be bioavailable due to unfavorable pharmacokinetic properties, i.a. not absorbed through cell membranes. In fact, it is generally accepted that small or low molecular weight molecules have a molecular weight (MW) cut-off of 600 -- 700 Da (36,37). This limit, however, based upon the Lipinski's rule of five, is an artificial limit associated with the observation that the attrition rates of oral drug compounds in the clinical development are significantly reduced if the MW is kept below this 500 Da limit. Molecules with a MW below this cut-off are believed to rapidly diffuse across cell membranes so that they can reach intracellular sites of action, the molecular size thus reflects bioavailability, i.e. exposure and risk (38-40). Recently, it has been shown by our group that also larger cyclic depsipeptide mycotoxins such as beauvericin and enniatins, with MW's up to 783.96 Da, are capable of crossing the human skin barrier and reaching the viable epidermis and dermis (41). Furthermore, a recent *in vivo* study in pigs demonstrated a high oral bioavailability (91%) for enniatin B1 (3), confirming also an earlier *in vitro* study, which assessed the biovailability of ENNs with Caco-2 cells to be 55-66% (42). Moreover, the mycotoxin bassianolide, which has been shown to be cytotoxic to human cell lines in the low µM

range, has a MW of 909.36 Da and thus already exceeding this low molecular weight cut-off (43,44). Also, toxic metabolites of high MW compounds, such as glycopeptides, may not be overlooked, as these can also pose an important health hazard. Therefore, a quantitative molecular weight restriction should not be included in the mycotoxin definition.

To further illustrate this, we would like to refer to the fumonisin paradox. Fumonisin B1 was confirmed to cause i.a. carcinoma, cirrhosis and nephrosis, but was shown to have a very poor oral bioavailability. These conflicting results are called the fumonisin paradox: "How can the toxin cause agriculturally significant diseases and possibly human cancer if it is not effectively absorbed after oral administration?" Shier explained that a higher bioavailability at lower doses, bioaccumulation and/or effective uptake of derivatives that are readily converted back in the body are plausible explanations. Also other important routes of exposure (e.g. dermal or respiratory) should be considered. As a consequence, the complete impact of a compounds threat will thus not be identified until all elements affecting oral bioavailability are understood (45).

3.3 Toxicity

Following the hazard approach, one could argue "*dosis sola facit venenum"*, meaning everything can be considered toxic as long as the dose is high enough: the amount of a substance is what makes it harmful, not the substance itself (Paracelsus, 16th century). Therefore, a quantitative toxicity limit is required as a condition for defining compounds as mycotoxins.

In a first approach, the most objective/standardised, economic and ethically acceptable way for measuring a compound's toxicity is screening it's *in vitro* cytotoxic capacity on various cell lines, preferably of human origin. *In vitro* tests in general have proven their value, i.a. in order to reduce animal/human toxicological studies and/or full-scale trials. Moreover, *in vitro-in vivo*

correlation mathematical models, which describe the relationship between an *in vitro* property and an *in vivo* response, are widely acknowledged. A direct cytotoxic effect is quantitatively expressed as IC_{50}, the concentration required for 50% inhibition of cell viability, a value generally agreed upon. The cell lines and concentration ranges used, together with the demonstrated IC_{50} of some already accepted mycotoxins are given in Supplementary Information S1. As we want to propose a quantitatively expressed definition, a toxicity IC_{50} threshold as a condition for claiming compounds as mycotoxins was set: $IC_{50} < 1000$ µM. This value can be justified by the data from Supplementary Information S1, which show that of all studies the maximum IC_{50} values quantitatively reported for well-established mycotoxins are within the 100-1000 µM class and by the fact that other low-molecular molecules toxic to humans, like ethanol, are not considered mycotoxins because these are only toxic in high concentrations (46). Moreover, it is also important to note that multiple mycotoxins, such as ENNs, T-2, deoxynivalenol (DON), nivalenol (NIV), BEA and fusarenon X (FUS-X), can influence each other's toxicity, showing synergistic, additive and antagonistic effects (27,47-49), considering the well-documented co-occurrence of mycotoxins in real-life (50). As the majority of the IC_{50} values are located between 1 and 100 µM, the chosen upper limit of 1000 µM can therefore also be considered as a 'worst case safety margin' for toxicity.

Moreover, it is recognised that toxic effects are not only concentration- but also time-dependent; therefore, both acute and chronic effects are of interest and are understood to be included in the mycotoxin definition. Furthermore, besides such direct cytotoxic effects, compounds can also exert indirect toxicity due to the formation of metabolites. It should thus be mentioned that a demonstrated *in vitro* toxicity does not per se implicate an *in vivo* effect and vice versa, e.g. *N*-

acyl metabolites are more cytotoxic than the parent fumonisin B1 (51). Therefore, we strongly encourage the inclusion of metabolites (from liver extracts, for example) in these *in vitro* cell tests, to lower the probability of false negatives. And what about newly discovered compounds, of which the toxicity has not yet been thoroughly investigated? Since a lack of toxicity data does not mean that the compound itself is not toxic or has no toxic potential, within the context of risk assessment prioritization and precautionary approach (see also section 3.2.), we suggest terming these compounds appropriately 'potential mycotoxins', defined as: secondary metabolites produced by microfungi, posing a health hazard to human and animal species, but for which the toxic activity is not yet investigated, i.e. no IC_{50} values are available. It goes without saying that these compounds should be further investigated with a prioritization according to their exposure level to humans.

Lastly, it should also mentioned that endocrine disrupting properties, which could occur at lower non-cytotoxic doses, might also suggest an important potential hazard and such effects have already been reported for the mycotoxins DON, ENN B, T-2 and HT-2 toxins (52,53). Screening potential mycotoxins for their endocrine disrupting properties in cell based assays (e.g. using U2OS and H295R cells) should therefore also be considered during hazard assessment.

4. Fungal cyclic depsipeptides

Lastly, we have applied our definition to a set of fungal cyclic depsipeptides (CDPs), in order to determine whether or not these should also be considered as (potential) mycotoxins.

4.1. Data handling

Structural and functional information, as well as the origin of the producing species of nearly 800 naturally occurring cyclic depsipeptides were gathered, of which 194 individual compounds were

found to be produced by fungi. Since these compounds have currently not yet been properly classified, although this information can be of great biological importance, i.a. understanding their structure-property relationships, these fungal cyclic depsipeptides were also subjected to a clustering analysis based upon their chemical properties. Considering that the majority of the compounds have already been categorised in small existing groups (e.g. enniatin A, B, A1, E, etc. all belong to the 'enniatins group'), only a limited number of model compounds was selected, representative for the whole fungal CDP population. Therefore, sampling was done in a randomised way: first, all cyclic depsipeptides were stratified according to their already known existing groups, from which then randomly at least one representative cyclic depsipeptide compound per group was included. However, not every structure reported in literature has a completely clarified stereochemistry, which is of huge importance for many 3D descriptors, so wherever possible, another member of a group was taken. This strategy ultimately lead to 32 CDPs retained for further analysis. Consequently, more than 3000 molecular descriptors were calculated, using Dragon 5.5 (Talete, Milan, Italy), HyperChem 8.0 (Hypercube, Gainesville, FL, USA) and MarvinSketch 5.10.3 (ChemAxon, Budapest, Hungary), for which the MM+ in vacuo structure was optimized, using HyperChem 8.0. The non-discriminating descriptors were eliminated, resulting in a 32×1363 data-matrix. All descriptors were transformed by z-scaling, ensuring an equal contribution of each descriptor to the resulting model (54). Multivariate data-analysis was performed using both principal component analysis (PCA) and hierarchical cluster analysis (HCA) with SIMCA-P+12.0.1.0 (Umetrics AB, Umea, Sweden) and SPSS Statistics 22.0.0 (IBM Corp., Armonk, NY, USA) software programs, respectively. Average-linkage HCA

clustering was performed using the Euclidean distance as the dissimilarity criterion. PCA resulted in an explained variance of PC1 = 0.51 and PC2 = 0.16 (cumulative R^2 of 0.67).

4.2. Chemical clustering classification

Based on the score plot of the first two principal components of the PCA, the 32 cyclic depsipeptides could be categorized into four main clusters with six subclusters, which is confirmed by the dendrogram of the HCA (Figure 1). The corresponding loading plot of the PCA allowed for further interpretation of these groups, by means of the most discriminating molecular descriptors displayed on both principal components. From this, it was deduced that components situated towards the right side of the space are larger, have larger ring sizes, have higher molecular weights and are folded and less flexible, i.e. have less conformational variability, compared to the compounds on the left. Cyclic depsipeptides located in the lower part of the space are more symmetrical than compounds located in the upper part. Other discriminating descriptors at the second axis are the number of terminal primary and tertiary carbons, which may indicate that CDPs located at the upper right part of the space most likely contain more valine, leucine, isoleucine amino acids and/or long branched alkyl chain(s). Moreover, a number of molecular descriptors indicate a higher presence of aromatic/benzene-like rings in CDPs at the lower side of the y-axis. More detailed information can be found in Supplementary Information S2.

4.3. Mycotoxin claim

For these compounds, classified according to the clustering analysis, (i) available toxicity data, (ii) their fungal origin and (iii) mycotoxin claim from literature as well as (iv) their mycotoxin claim resulting from our definition, are all gathered in Table 2.

Beside beauvericin and enniatins, only three of these (groups of) compounds have been previously called mycotoxins in scientific literature: bassianolide (44), beauverolides (71) and destruxins (76). *In vitro* cytotoxicity of these compounds has indeed been studied and was found significant (< 100 µM). Therefore, based upon our proposed definition, these compounds should indeed be defined as mycotoxins. However, according to our definition, seven other fungal metabolites should also be considered as mycotoxins, namely 1962A, emericellamides, guangomides, PF1022A, sansalvamides, scopularamides and zygosporamide, of which sansalvamides and zygosporamide currently seem the most investigated and also the most toxic, based on the available data. So, for these identified hazards, further investigations, i.a. exposure and risk assessment, are strongly recommended. For the other cyclic depsipeptides mentioned in Table 2 no quantitative toxicity data are currently available and hence at this point, these peptides are considered as 'potential mycotoxins' for which further toxicity testing is required.

5. Conclusions

Evaluation of the current status of the mycotoxin definition revealed a lack of consistency, confounding approaches and definite disagreement. We propose here a clear, unambiguous and quantitatively expressed mycotoxin definition, by means of explication and based upon hazard data of some already well-known and widely accepted "traditional" mycotoxins. This definition was then applied to a set of fungal cyclic depsipeptides concluding that some of these compounds should also be considered as mycotoxins, for which exposure and risk assessment investigations are to be considered.

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Table 1: Overview and current status of applied mycotoxin definitions.

Reference	Definition	Status discussion (level of detail)
FAO (7)	Mycotoxins are toxic secondary metabolites of fungi belonging, essentially, to the *Aspergillus*, *Penicillium* and *Fusarium* genera.	– Secondary metabolites of specific fungi – Definitely toxic
Berthiller et al. (8)	Mycotoxins are secondary metabolites of fungi toxic to animals and humans and have been reviewed.	– Secondary metabolites of fungi – Extensively investigated: definitely toxic
Pitt (9)	Mycotoxins are insidious poisons, produced when, and only when, common fungi grow in foods and feeds.	– Metabolites of different fungi – Agricultural/food and feed industry – Definitely toxic (poison)
Frisvad (10)	Mycotoxins are fungal specific (secondary) metabolites that are toxic to vertebrates when introduced in small amounts via a natural route.	– Secondary metabolites of fungi – Exposure via a natural route – Definitely toxic: in low amounts
Bhatnagar et al. (11)	Mycotoxins are secondary metabolites produced by filamentous fungi that cause a toxic response (mycotoxicosis) when ingested by higher animals.	– Secondary metabolites of filamentous fungi – Agricultural/food and feed industry – Definitely toxic when ingested
Raghavender and Reddy (12)	Mycotoxins are toxic secondary metabolites produced by certain fungi or moulds in agricultural products that are susceptible to mould infestation.	– Secondary metabolites of certain fungi/molds – Agricultural/food and feed industry – Definitely toxic
EFSA (13)	Mycotoxins are toxic compounds produced by different types of fungi, belonging mainly to the *Aspergillus*, *Penicillium* and *Fusarium* genera. They commonly enter the food chain through contaminated food and feed crops, mainly cereals.	– Metabolites of different fungi – Agricultural/food and feed industry – Definitely toxic
Devreese et al.	Mycotoxins are secondary metabolites produced by different fungal species contaminating several	– Secondary metabolites of different fungi

(14)	food and feed commodities. Over 100 mycotoxins have been identified, although only a few of them have been thoroughly investigated because of their distinct toxic effects.	– Agricultural/food and feed industry – No thorough investigation: potentially toxic
FDA (15)	Mycotoxins are natural poisons produced by fungi as secondary metabolites. Foods may become contaminated with mycotoxins as a result of mold growth during harvest, or storage. Three genera are responsible for the majority of the mycotoxins with which FDA is concerned: the *Aspergillus*, *Penicillium* and *Fusarium sp*.	– Secondary metabolites of fungi – Agricultural/food and feed industry – Definitely toxic (natural poison)
Yazar and Omurtag (16)	Mycotoxins are secondary metabolites produced by a wide variety of fungal species that cause nutritional losses and represent a significant hazard to the food chain. The exposure risk to human is either directly through foods of plant origin (cereal grains) or indirectly through foods of animal origin (kidney, liver, milk and eggs).	– Secondary metabolites of different fungi – Agricultural/food and feed industry – Hazard/risk identification
Varga et al. (17)	Mycotoxins are secondary fungal metabolites, which are found in a broad range of food and feed, such as cereals, spices, coffee, nuts or dried fruits. They have the capability of causing acute toxic, carcinogenic, mutagenic, teratogenic, immunotoxic or oestrogenic effects in animals and humans and they show a huge structural diversity resulting in a variety of chemical and physicochemical properties.	– Secondary metabolites of fungi – Agricultural/food and feed industry – Potentially toxic
Bennet and Klich (18)	All mycotoxins are low-molecular-weight natural products produced as secondary metabolites by filamentous fungi. These metabolites constitute a toxigenically and chemically heterogeneous assemblage that are grouped together only because the members can cause disease and death in human beings and other vertebrates. They are toxic to vertebrates and other animal groups in low concentrations… Skin contact with mold infested substrates and inhalation of spore-borne toxins are also important sources of exposure.	– Secondary metabolites of filamentous fungi – Other routes of exposure: dermal/respiratory – Definitely toxic: in low concentrations

Table 2: Fungal cyclic depsipeptides as mycotoxin test group.

No°	Cyclic depsipeptide	Fungal origin	Available toxicity data	References	Mycotoxin claim	
					Lit.[a]	Definition
Group 1: small cyclic depsipeptides						
3	Angolide	*Pithomyces* sp.	n.a.	Russell and Ward (55)	no	Potential mycotoxin
Group 2: medium cyclic depsipeptides						
12 → 17[c]	Enniatins[b]	*Alternaria* sp. *Fusarium* sp. *Halosarpheia* sp. *Verticillium* sp.	IC_{50} = 1.6 -- 6.8 µM (Caco-2 cells) IC_{50} = 13 -- 14.8 µM (Caco-2 cells) IC_{50} = 11.7 µM (Caco-2 cells) IC_{50} = 2.8 -- 11.3 µM (Caco-2 cells) IC_{50} = 15.80 µM (IPEC-1 cells)	Herrmann et al. (56) Ivanova et al. (57) Kolf-Clauw et al. (27) Prosperini et al. (49)	yes	Mycotoxins
9	Conoideocrellide A	Scale insect pathogenic fungus *Conoideocrella tenuis* (BCC 18627)	n.a.	Isaka et al. (58)	no	Potential mycotoxin
20	Gliotide	Marine alga-derived fungus *Gliocladium* sp.	n.a.	Lang et al. (59)	no	Potential mycotoxin
6	Beauvericin[b]	*Beauveria* sp. *Paecilomyces fumosoroseus* *Fusarium* sp.	IC_{50} = 12.08 -- 17.22 µM (CHO-K1 cells) IC_{50} = 6.25 -- 11.08 µM (Vero cells) IC_{50} = 12.75 -- 20.62 µM (Caco-2 cells) IC_{50} = 9.75 -- 15.00 µM (HT-29	Ferrer et al. (60) Fukuda et al. (61) Prosperini et al. (62) Ruiz et al. (47) Sifou et al. (63) Song et al.	yes	Mycotoxin

			cells)	(64) Wang et al. (65)		
19	Exumolides	Marine-derived *Scytalidium* sp.	Demonstrated antimicroalgal activity	Jenkins et al. (66)	no	Potential mycotoxins
1	1962A	Mangrove endophytic fungus (no. 1962)	IC_{50} = 165.64 μM (MCF-7 cells)	Huang et al. (67)	no	Mycotoxin
2	Alternaramide	Marine-derived *Alternaria* sp. (SF-5016)	n.a.	Kim et al. (68)	no	Potential mycotoxin
7	Beauverolides	*Beauveria bassiana* *Paecilomyces fumosoroseus*	IC_{50} = 76.20 μM (CEM-DNR cells) IC_{50} = 32.16 μM (K562 cells) IC_{50} = 53.63 μM (K562-Tax cells) IC_{50} = 15.64 μM (B2.4 cells) IC_{50} = 22.77 μM (A549 cells) IC_{50} = 60.31 μM (HT-29 cells) IC_{50} = 52.41 μM (MCF-7 cells) IC_{50} = 82.63 μM (PC-3 cells) IC_{50} = 100.00 μM (U87MG cells) IC_{50} = 45.63 μM (lymphocytes)	Jegorov et al. (69) Kuzma et al. (70) Onstad et al. (71) Sarek et al. (72)	yes	Mycotoxins
10	Destruxins[d]	Imperfect fungus (D1084) Marine-derived fungus *Beauveria f. Metarhizium anisopliae* *Nigrosabulum globosum*	IC_{50} = 0.05 -- >10 μM (KB-3-1 cells) IC_{50} = 0.05 -- >10 μM (Caco-2 cells) IC_{50} = 0.04 -- >10 μM (HCT116 cells)	Boot et al. (73) Che et al (74) Dornetshuber-Fleiss et al. (75) Dumas et al. (76)	yes	Mycotoxins

		Trichothecium roseum	IC_{50} = 0.22 -- >10 μM (A549 cells)	Engstrom et al. (77) Kawazu et al. (78) Lira et al. (79) Springer et al. (80) Sree et al. (81)		
11	Emericellamides	*Aspergillus nidulans* Marine-derived *Emericella* sp.	IC_{50} = 23 μM (HCT116 cells)	Chiang et al. (82) Ghosh and Pradhan (83) Oh et al. (84)	no	Mycotoxins
21 - 22	Guangomides[e]	Terrestrial filamentous fungus, *Trichothecium* sp. Unidentified marine-derived fungus	IC_{50} = ± 15 μM (MCF-7 cells) IC_{50} = ± 15 μM (H460 cells) IC_{50} = ± 15 μM (SF268 cells)	Amagata et al. (85) Sy-Cordero et al. (86)	no	Mycotoxins
23	Hirsutatins	Insect pathogenic fungus *Hirsutella nivea* (BCC 2594)	Noncytotoxic at 73.9 μM (Vero cells)	Isaka et al. (87)	no	Potential mycotoxins
24	Hirsutellides	Entomopathogenic fungus *Hirsutella kobayasii (*BCC 1660)	Noncytotoxic at 75.2 μM (Vero cells)	Vongvanich et al. (88)	no	Potential mycotoxins
25	Leualacin	*Hapsidospora irregularis* (SANK17182)	Demonstrated calcium blocker	Hamano et al. (89)	no	Potential mycotoxin
26	Petrosifungins	Marine-derived *Penicillium brevicompactum*	n.a.	Bringmann et al. (90)	no	Potential mycotoxins
28	Pithomycolide	*Pithomyces chartarum*	n.a.	Moussa and Le Quesne (91)	no	Potential mycotoxin

29	Sansalvamides	Marine *Fusarium* sp.	IC_{50} = 5.96 μM (Colo 205 cells) IC_{50} = 10.06 μM (SK-MEL-2 cells) IC_{50} = 46.70 μM (mean of 60 cancer cell lines) Demonstrated inhibition of a poxvirus topoisomerase (IC50 for inhibition of DNA binding = ± 80 μM) IC_{50} = 3.6 -- 8.3 μM (mean NCI human tumor cell line screen)	Belofsky et al. (92) Cueto et al. (93) Hwang et al. (94)	no	Mycotoxins
30	Trichodepsipetides	*Trichothecium* sp.	IC_{50} = >10 μM (noncytotoxic) (various cell lines)	Sy-Cordero et al. (86)	no	Potential mycotoxins
32	Zygosporamide	Marine-derived fungus *Zygosporium masonii*	IC_{50} = 0.0065 -- 4.6 μM (SF268 cells) IC_{50} = 4.25 -- 15 μM (SF295 cells) IC_{50} = 2.5-- 7.4 μM (A549 cells) IC_{50} = 537 -- 8.5 μM (MDA-MB-231) IC_{50} = < 5.0 nM (RXF 393 cells)	Oh et al. (32) Wang et al. (33)	no	Mycotoxin
Group 3: medium to large cyclic depsipeptides						
5	Bassianolide	*Beauveria bassiana* *Verticillium lecanii* Wood-decaying fungus *Xylaria*	IC_{50} = 4.00 μM (KB cells) IC_{50} = 2.74 μM (BC-1 cells) IC_{50} = 1.21 μM (NCI-H187 cells)	Jirakkakul et al. (43) Suzuki et al. (95) Yun et al. (96)	yes	Mycotoxin

		sp.	IC_{50} = 5.28 µM (Vero cells)			
27	PF1022A	Imperfect fungus *Mycelia sterilia* (*Rosellinia* sp.)	IC_{50} = 4.6 -- 9.6 µM (HCT116 cells) IC_{50} = 4.3 -- 7.5 µM (A549 cells)	Dornetshuber et al. (97) Sasaki et al. (98)	no	Mycotoxin
31	W493s	*Fusarium* sp.	Demonstrated antifungal activity	Nihei et al. (99)	no	Potential mycotoxins
Group 4: large cyclic depsipeptides						
4	Aureobasidins	Marine-derived *Aureobasidin* sp.	Demonstrated antibiotic effects	Abdel-Lateff et al. (100) Detwiller and Lubell (101) Sonda et al. (102) Tan and Tay (103) Tanaka et al. (104)	no	Potential mycotoxins
8	Clavariopsins	*Clavariopsis aquatica*	Demonstrated antifungal activity	Kaida et al. (105)	no	Potential mycotoxins
18	Eujavanicins	*Eupenicillium javanicum*	Demonstrated antifungal activity	Nakadate et al. (106)	no	Potential mycotoxins
Cyclic depsipeptides with no assigned group[f]						
-	Acremolides	Marine-derived *Acremonium* sp.	"Noncytotoxic"	Ratnayake et al. (107)	no	Potential mycotoxins
-	Brevigellin	*Penicillium brevicompactum*	n.a.	McCorkindale and Baxter (108)	no	Potential mycotoxin
-	Glomosporin	*Glomospora* sp. (BAUA 2825)	Demonstrated antifungal activity	Sato et al. (109)	no	Potential mycotoxin
-	Isarfelin	*Isaria felina*	Demonstrated antifungal and insecticidal activity	Guo et al. (110)	no	Potential mycotoxin

-	Isaridins	Entomopathogenic fungus *Beauveria felina* *Isaria* sp.	Demonstrated insecticidal activity	Langenfeld et al. (111) Ravindra et al. (112)	no	Potential mycotoxins
Cyclic depsipeptides with no assigned group[f]						
-	Isariins	Entomopathogenic fungus *Beauveria feline* *Isaria* sp.	Demonstrated insecticidal activity	Langenfeld et al. (111) Sabareesh et al. (113)	no	Potential mycotoxins
-	Scopularides	Marine-derived *Scopulariopsis brevicaulis*	IC_{50} = <15.51 μM (Colo357, Panc89, HT29 cells)	Yu et al. (114)	no	Mycotoxins
-	Sporidesmolides	Pasture fungus *Sporidesmium bakeri* *Pithomyces chartarum*	n.a.	Gillis et al. (115) Russell, 1960 (116) Russell, 1962 (117)	no	Potential mycotoxins
-	Stevastelins	*Penicillium* sp.	Demonstrated immunosuppressive effects	Morino et al. (118)	no	Potential mycotoxins

n.a. Not available.

a Has literature ever referred (in any way) to this compound as being a mycotoxin?

b Already accepted mycotoxins, see also Table 2.

c No° 12, 13, 14, 15, 16, 17 = enniatin A, A1, B, B1, C, D.

d And its analogues (*i.a.* bursaphelocides, pseudodestruxins, roseotoxin, roseocardin).

e No° 21, 22 = guangomide A, B.

f These compounds' structures have not yet been fully elucidated. Therefore these were excluded from the clustering analysis.

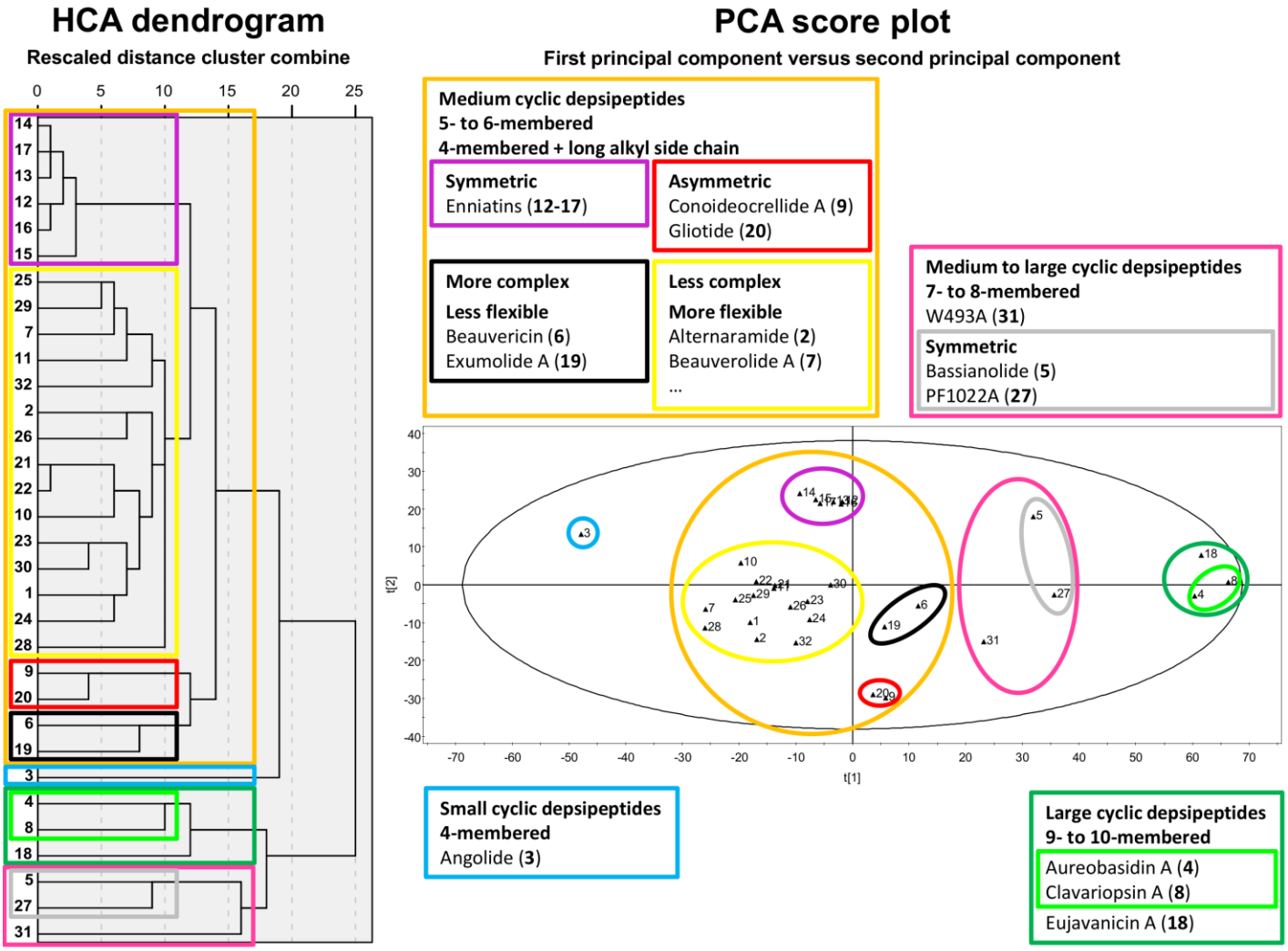

Figure 1. Clustering of 32 fungal cyclic depsipeptides. The four main clusters (blue, orange, pink and green clusters) and six subclusters (purple, yellow, black, red and grey clusters) are indicated by a bold coloured line. Number identifications can be found in Table 2. Left: HCA dendrogram. Right: PCA score plot of the first versus the second principal component.