NPR

RSC Publishing

REVIEW

Cite this: *Nat. Prod. Rep.*, 2013, **30**, 941

Angular tricyclic benzofurans and related natural products of fungal origin. Isolation, biological activity and synthesis

Sebastián O. Simonetti, Enrique L. Larghi, Andrea B. J. Bracca and Teodoro S. Kaufman*

Covering: 1997 to 2013

Naturally-occurring angular tricyclic benzofuran/isobenzofuran derivatives of fungal origin and related compounds, in which two heterocyclic rings are fused to a central benzenoid moiety, are covered. Emphasis is placed on the structure of the compounds, together with their relevant biological activities, source microorganisms, country or region of origin and environmental conditions. In addition, proposed biosynthetic pathways, as well as the total syntheses of some of the compounds, including those that lead to structural revision or to correct stereochemical assignments, and related synthetic efforts, are discussed in detail.

Received 18th February 2013

DOI: 10.1039/c3np70014c

www.rsc.org/npr

1 Introduction

Microorganisms, particularly fungi, are a constant stimulus for natural product research, and as such they remain the subject of vigorous chemical investigation. Fungi represent the second largest group of organisms, next to insects, and are widely distributed across the planet.[1] They inhabit almost all ecological niches, occurring between the tropics, but also in temperate regions, and even in Artic and Antarctic ice. Fungi have been found in places as different as seawater[2] and dry soils, including the surface of mountain rocks. They parasitize plants, protozoa,

Instituto de Química Rosario (CONICET-UNR), Suipacha 531, S2002LRK, Rosario, SF, Argentina. E-mail: kaufman@iquir-conicet.gov.ar; tkaufman@fbioyf.unr.edu.ar; Fax: +54-341-4370477; Tel: +54-341-4370477, ext. 105

fishes, insects, and mammals, but also display symbiotic lives with algae, sponges and other members of the plant kingdom.

Fungi are completely heterotrophic because of their inability to photosynthesise. Thus, they must acquire their nutrients from the environment, including living, dying, or dead organisms. Therefore, fungi have acquired the ability to survive under extreme environmental conditions, and have evolved to biosynthesize a wide variety of fascinating natural products not for their own growth but for other purposes such as detoxification, cell differentiation, defense, signaling and communication.[3]

Many of the fungi-derived biologically active small molecules range from highly potent toxins to plant growth promoters and life-saving drugs. Thus, fungi have historically been a gold mine of lead compounds for the pharmaceutical industry.[4]

The enormous diversity of fungal-derived natural products is a consequence of the large manifold of fungal species and also a result of their ability to change metabolic pathways when exposed to stress conditions or different environments, where even usually silent metabolic routes are awakened.[5] Recent access to the detailed structure of some fungal genomes has confirmed the large number of secondary metabolite pathways at their disposal, which can be unlocked to serve as potential sources for new and useful natural products.[6]

Most fungal secondary metabolites and their biosynthetic pathways still remain unveiled.[7] However, knowledge of their structure and function may facilitate a better understanding of fungal diversity and environmental adaptation processes. It should also allow the development of bioactive compounds as medicines, flavors, cosmetics, agrochemicals, crop protection agents and others.[8]

Historically, fungal natural product research has been strongly related with specimens isolated from soil. However, in mankind's search of remote regions to find new ways to fight disease, fungi of marine origin are becoming an important source of novel skeletons and bioactive compounds for drug discovery. Likewise, analysis of products from fungi growing in

Sebastián O. Simonetti was born in Chabás (Santa Fe, Argentina) and received his BS degree in Chemistry in 2011 from the National University of Rosario (UNR). He is currently performing research work towards his Ph. D. as a Doctoral fellow of the Argentine National Research Council (CONICET) at the Institute of Chemistry of Rosario (IQUIR, CONICET-UNR), in Dr Kaufman's group. His research interests include the total synthesis of heterocyclic marine natural products with unique structures and interesting biological activity. In 2011, he received the XIX SINAQO award for the best work in synthetic organic chemistry.

Andrea B. J. Bracca was born in Rosario (Santa Fe, Argentina) and graduated in 2001 with a BS in Biotechnology from the National University of Rosario (Argentina). She received her Ph. D. in 2009 under the guidance of Prof. Kaufman. After a two years period of post-doctoral training with Prof. Guillermo R. Labadié, she returned to work in Dr Kaufman's group as an Assistant Research Scientist of the Argentine National Research Council (CONICET) at the Institute of Chemistry of Rosario (IQUIR, CONICET-UNR), where she is currently doing research work related to the synthesis of heterocyclic natural products.

Enrique L. Larghi was born in Rosario. After receiving his B.S. in Chemistry in 1997 from the UNR, he started research work at the Universidade Federal de Santa María (Brazil) where he received his M.Sc. (1999, Professor Claudio C. Silveira) and his Ph.D. in Chemistry (2003, Professor Ademir Farias Morel). After a short experience as Head of Research and Development in the pharmaceutical industry, he joined Dr Kaufman's group at IQUIR as a post-doctoral fellow in 2005. Currently, he is Researcher of the Argentine National research Council (CONICET). His interests cover bioorganic chemistry and the synthesis of heterocyclic natural products.

Teodoro S. Kaufman was born near Moisés Ville (Santa Fe, Argentina). He graduated in biochemistry (1982) and pharmacy (1985) at the National University of Rosario (UNR), and received his Ph.D. (1987) under the guidance of Prof. Edmundo A. Rúveda. After two years of post-doctoral training at The University of Mississippi (USA), he returned to Rosario. Currently, he is full Professor of the UNR, Research Head of the Argentine National Research Council (CONICET) and Head of the Institute of Chemistry of Rosario (IQUIR). His research interests include heterocyclic chemistry and the synthesis and evaluation of bioactive natural products and analogs.

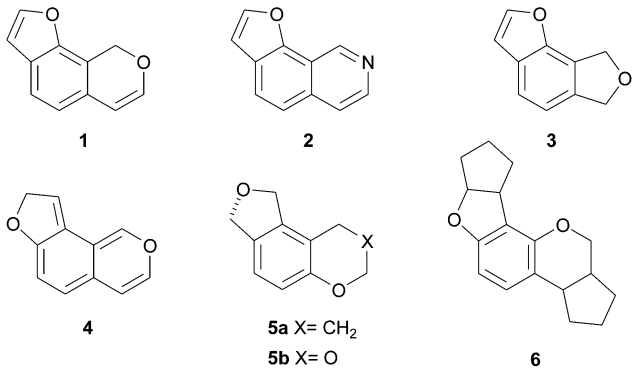


Fig. 1 Naturally-occurring angular tricyclic benzofuran motifs of interest.

environments subjected to decomposition, such as damp buildings, are providing new clues to the pathogenesis of specific conditions and offer an opportunity to discover new drug candidates.[9]

Reviewed here are the isolation, biological activity profiles and reported syntheses of naturally-occurring angular tricyclic benzofuran/isobenzofuran derivatives of fungal origin, and related compounds, in which two heterocyclic rings are fused to a central benzenoid moiety. They comprise furo[3,2-*h*]isochroman (**1**), furo[3,2-*h*]isoquinoline (**2**), furo[4,5-*g*]isobenzofuran (**3**), furo[2,3-*h*]isochroman (**4**) and furo[3,4-*f*]chroman (**5a**) type natural products (Fig. 1), as well as a novel furo[3,4-*f*]benzo[5,6-*e*]-[1,3]dioxine derivative (**5b**).

The isolation of some natural products to which pyrano[3,2-*h*] isochroman and pyrano[3,2-*h*]isoquinoline skeletons have been assigned, and their synthesis which demonstrated them to actually be furo[3,2-*h*]isochroman (**1**) and furo[3,2-*h*]isoquinoline (**2**) derivatives, respectively, are also included. On the other hand, the pentacyclic aflatoxins belonging to series B, M, G and GM, which share the furo[2,3-*h*]chroman skeleton (**6**) and have been extensively studied over the last 50 years, are not covered.[10]

The majority of these compounds have been isolated during the last 15 years, mainly from saprophytic fungi commonly found in soil, decaying vegetation, seeds and grains; others have been obtained from fungi isolated from other environments, including marine and indoor spaces, or have long been known.

2 Furo[3,2-*h*]isochromans

2.1 Pergillin, pseudodeflectusin, penicisochromans, ustusoranes, and related compounds. Isolation, proposed biosynthesis and biological activity

Aspergillus ustus is one of the most prevalent fungi in soil and decaying vegetation. It is a known contaminant of stored foodstuffs such as cereals, pulses and cheese; it has also been found in indoor environments and is known to produce a number of toxic secondary metabolites of varied structure and biosynthetic origin.

The tricyclic hemiacetals pergillin (**7**) and dihydropergillin (**8**) were the first known naturally-occurring furo[3,2-*h*]isochromans (Fig. 2). They were isolated from *Aspergillus ustus* found growing on pea seed of *Pisum sativum* var. *Macrocarpon*[11] and

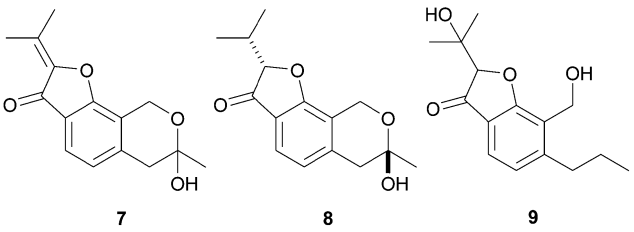


Fig. 2 Chemical structures of pergillin (**7**), dihydropergillin (**8**) and brassicadiol (**9**).

recently reported again in *Aspergillus ustus* 094102.[12] Pergillin is also produced by *A. insuetus.*[13]

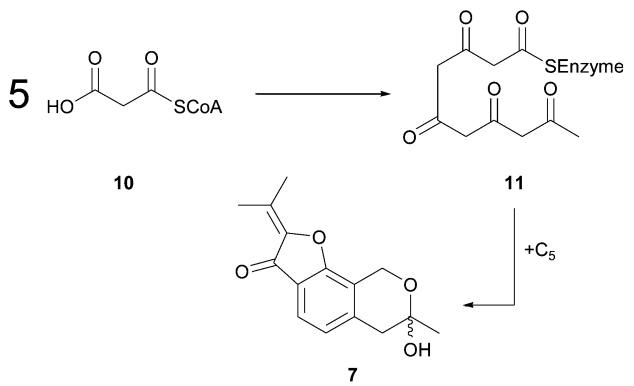
Pergillin and other extrolites have been used as taxonomic markers for *Aspergilli*. In that sense and based on chemical, molecular and morphological data, *A. insuetus* has been recently separated from *A. ustus* and differentiated from *A. keveii*.[14]

It was proposed that pergillin could result from the condensation of a pentaketide and one molecule of mevalonic acid, which can yield γ,γ′-dimethylallyl pyrophosphate, and that its hemiketal feature would result from the union of the two ends of the pentaketide chain (Scheme 1).[15] Even though the hemiketal moiety of pergillin contains a stereogenic center, the natural product has no optical activity.

Pergillin and dihydropergillin displayed moderate plant growth inhibiting properties in the etiolated wheat coleoptile assay, which were significantly ($p < 0.01$) inhibited by 10^{-4} M solutions, with dihydropergillin being the most potent.[16] Interestingly, however, pergillin was nontoxic to chicks at doses up to 250 mg Kg^{-1}.

It was proposed that reduction of the double bond allows the isopropyl group to form a staggered conformation with the substituted furan ring. The resulting added flexibility of this end of the molecule may account for the enhanced biological activity.

Brassicadiol (**9**) contains the carbon skeleton of the furo[3,2-*h*]isochromans. The natural product was isolated by Ayer and Peña Rodríguez in 1987 during their study of the fungal metabolites that produced the damage of the black spot disease of canola,[17] one of the most widespread diseases of rapeseed. The black spot disease of canola is caused by the fungal pathogen *Alternaria brassica* (Berkeley) Saccardo, and results in low



Scheme 1 Proposed biosynthetic pathway of pergillin (**7**).

oilseed yields through reduction in photosynthesis, premature defoliation, and shattering of fruits. However, it was found that brassicadiol is not the responsible agent for the observed effects of the disease.

In 2004, the group of Mizushina isolated pseudodeflectusin (**12**), from the culture broth of *Aspergillus pseudodeflectus* Hiji005, in turn isolated from the sea weed *Sargassum fusiform*, collected in the Miura Peninsula, Kanagawa, Japan. This new tricyclic angular isochroman derivative was found together with the structurally related alkaloid TMC-120B (**23**, *vide infra*).[18]

Pseudodeflectusin features a cyclic hemiacetal motif, which suggests that it may exist as an interconverting mixture of two isomers; however, the natural product was obtained as a single isomer. The absolute configuration of its stereogenic centers at C-7 and C-9 were not determined at that time.

The natural product exhibited cytotoxicity towards several human cancer cell lines, including those from the stomach (NUGC-3), cervix (HeLa-S3), and peripheral blood (HL-60, $LD_{50} = 39$ μM), but did not affect those from the lung (A549) or colon (DLD-1). Since **12** did not display any inhibition of DNA metabolic enzymes, it was considered as a promising candidate of a new type of antitumor agent, acting through an unprecedented mechanism.[18]

The group of Zhu reported in 2009 the isolation of ustusoranes A–F (Fig. 3) from *Aspergillus ustus* 094102 [isolated from the rhizosphere soil of the mangrove *Bruguiera gymnorrhiza*, (Wenchang, Hainan Province, China)],[12] together with daldinin B [**13**, a carbinolic isomer of ustusorane E (**18**) exhibiting the *R* configuration at C2][19] and the known pergillin (**7**). Their structures (**14–19**) were elucidated by NMR and HRMS analyses.

The molecular formula of ustusorane C (**16**) was close to that of pseudodeflectusin,[18] and it was suggested to be an artifact, resulting from sequential dehydration of the hemiacetal of pseudodeflectusin with silica gel (to form an oxonium ion) and quenching of the oxonium ion by MeOH, during the isolation process.

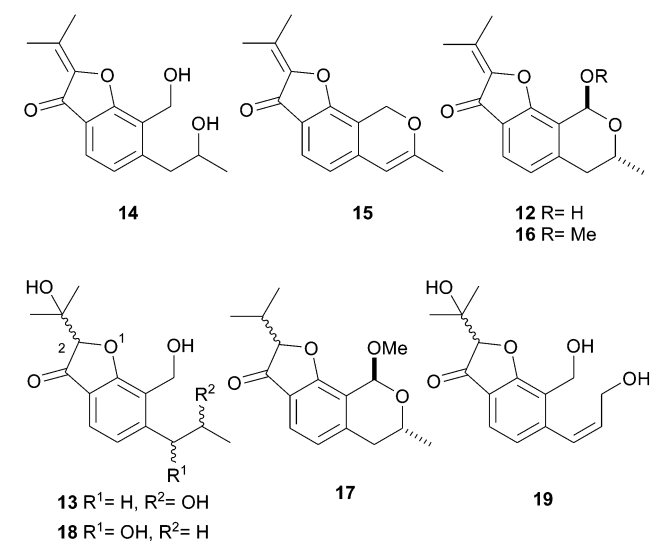


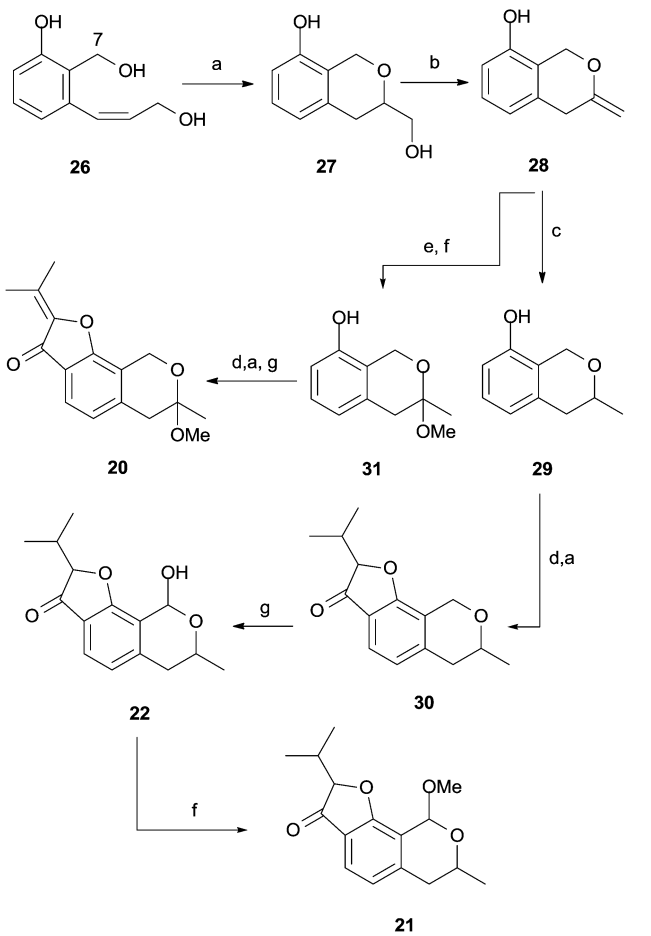
Fig. 3 Chemical structures of pseudodeflectusin (**12**), daldinin B (**13**) and ustusoranes A–F (**14–19**).

The 1,3-*anti*-configuration of **16**, the same of pseudodeflectusin, was inferred considering that the nucleophilic attack of MeOH on the oxonium ion should occur preferably from the less hindered side. The specific rotation of **16** ($[\alpha]_D^{20} = +6$) is of the same sign as that of natural (+)-pseudodeflectusin, whose absolute configuration had been confirmed as 7*R*,9*S* by chemical synthesis,[20] suggesting that **16** also possesses the 7*R*,9*S* configuration.

The cytotoxicity of the isolated compounds was evaluated against A549 and HL-60 cell lines using the sulforhodamine B[21] and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide[22] methods, respectively.

In 2010, the group of Rukachaisirikul isolated the penicisochromans A–C (**20–22**) as colorless gums, together with alkaloids TMC-120B (**23**) and TMC-120C (**24**) (*vide infra*) and the related (−)-penicisoquinoline (**25**), a chiral isoquinoline derivative ($[\alpha]_D^{20}$ −21), from the extract of the mycelia of the marine-derived fungus *Penicillium* PSU-F40 (Scheme 2).[23]

This fungal strain was isolated from the sea fan *Annella sp.* collected near the Similan Islands, in the Phangnga Province of Thailand. Some of the natural compounds were tested for antibacterial activity against *Staphylococcus aureus* and Methycillin-resistant *S. aureus*, but none of them were active.



Scheme 2 Biosynthesis of penicisochromans. Proposed transformations: (a) cyclization; (b) dehydration; (c) hydrogenation; (d); acylation; (e) hydration; (f) methylation; (g) oxidation.

The authors hypothesized that penicisochromans A–E and the structurally related furo[2,3-*h*]isoquinolines **23–25** (*vide infra*) could derive from peniciphenol (**26**), which was concomitantly isolated, through an acid-catalyzed intramolecular cyclization of the (*Z*)-3-hydroxyl-1-propenyl side chain with the benzyl alcohol, to yield isochroman A **27** (Scheme 2).

According to their proposal, dehydration and subsequent hydrogenation of **28** would generate isochroman intermediate **29**. Next, acylation of **29** with a 3-methyl-2-butenoyl unit and subsequent cyclization would yield **30**, the oxidation and *O*-methylation of which would afford penicisochromans C (**22**) and B (**21**) respectively. On the other hand, penicisochroman A (**20**) would result from Markovnikov hydration and subsequent *O*-methylation of **28**, followed by acylation of intermediate **31**, cyclization and final oxidation.

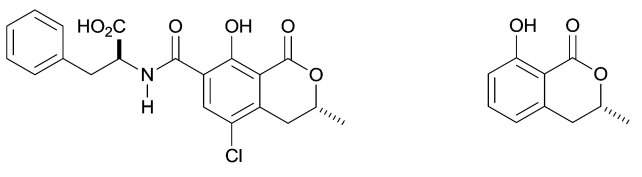
Analogously, it was proposed that the biosynthesis of the related furo[3,2-*h*]isoquinolines (*vide infra*) would proceed along the same lines as for the furo[3,2-*h*]isochromans, from a 7-amino derivative of peniciphenol as the biosynthetic precursor.

2.2 Total syntheses of pseudodeflectusin

The group of Kobayashi[18,24] disclosed in 2006 the first total synthesis of (+)-pseudodeflectusin, which served to assign the absolute configuration of the natural product. The authors observed that ochratoxin A (**32**, Fig. 4), isolated from some strains of *Aspergillus ochraceus*,[25] has a benzoisochroman skeleton similar to that of pseudodeflectusin; therefore, they considered it very likely that the latter has the same absolute configuration. Since the absolute configuration of ochratoxin A was determined by comparison of the optical rotation of the degraded natural product with *R*-(−)-mellein (**33**),[26] a total synthesis of **32** employing *R*-(−)-mellein (**33**) as a key intermediate was proposed.

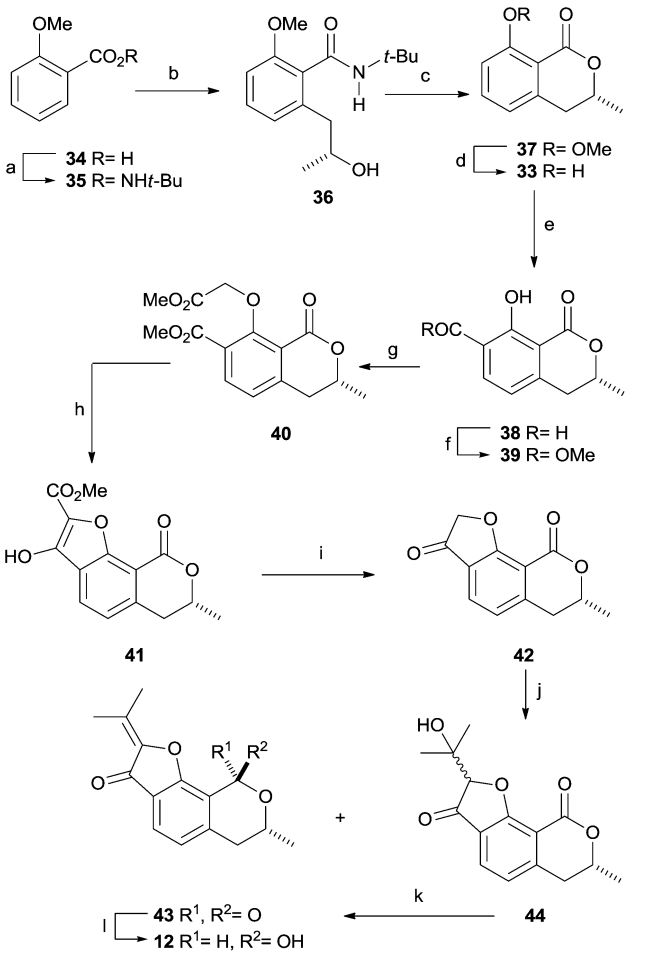
As shown in Scheme 3, condensation of *o*-anisic acid (**34**) with *tert*-butylamine under DCC promotion afforded 75% of benzamide **35**, which once submitted to *ortho*-metalation with *n*-BuLi-TMEDA, followed by quenching of the orange-colored dianion with *R*-(+)-propylene oxide,[27] gave 61% of alcohol (−)-**36**. Next, intramolecular cyclization assisted by *p*-tosic acid furnished δ-lactone (−)-**37** in 72% yield, while final demethylation with BCl_3 in CH_2Cl_2 afforded (−)-mellein [(−)-**33**] as key intermediate in 95% yield.

Formylation of (−)-**33** with Cl_2CHOCH_3 under $TiCl_4$ promotion afforded 88% of aldehyde (−)-**38**, accompanied by 8% of the *para* isomer;[28] the former was transformed into the related methyl ester (−)-**39** in 91% yield with the NaCN/MnO_2 reagent



32 33

Fig. 4 Chemical structures of ochratoxin A (**32**) and *R*-(−)-mellein (**33**).



Scheme 3 *Reagents and conditions*: (a) *t*-$BuNH_2$, DCC, DMAP/CH_2Cl_2 (75%); (b) *n*-BuLi, TMEDA, *R*-(+)-propylene oxide/THF, −78 °C (61%, 32% recovery of **35**); (c) *p*-TsOH·H_2O/toluene, reflux (72%); (d) BCl_3/CH_2Cl_2, −78 °C → 0 °C (95%); (e) Cl_2CHOCH_3, $TiCl_4/CH_2Cl_2$, −10 °C (88%); (f) NaCN, MnO_2/MeOH (91%); (g) K_2CO_3, $BrCH_2CO_2Me$, DMF, 50 °C (98%); (h) NaOMe/MeOH, reflux (93%); (i) LiOH·H_2O, DMSO, H_2O, 75 °C (80%); (j) *p*-TsOH·H_2O/acetone, reflux (**43**, 49%; **44**, 24%, **42**, 9%); (k) MsCl, DMAP, pyridine (72%); (l) DIBAL (2.5 equiv.), THF, −78 °C (**12**, 28%; **43**, 49%).

in MeOH.[29] Interestingly, ester **39** was previously prepared both in racemic and optically active forms.[30]

Alkylation of the phenol moiety of **39** with methyl bromoacetate and potassium carbonate proceeded in 98% yield, while NaOMe-assisted cyclization of the resulting (−)-**40** furnished intermediate β-ketoester (−)-**41**, which upon hydrolysis and decarboxylation with LiOH in aqueous DMSO gave 80% of furanone (−)-**42**.[31]

The methylethylidene moiety was installed by reaction of (−)-**42** with acetone under *p*-TsOH·H_2O promotion, which yielded a 2 : 1 mixture of the expected product **43** and tertiary alcohol **44** in combined 73% yield. Mesylation of alcohol **44** in pyridine was followed by elimination of MsOH from the tertiary mesylate and furnished 72% of (−)-**43**. To complete the synthesis, lactone (−)-**43** was partially and selectively reduced with DIBAL at −78 °C, affording 28% of the expected lactol **12** [(+)-pseudodeflectusin].[32]

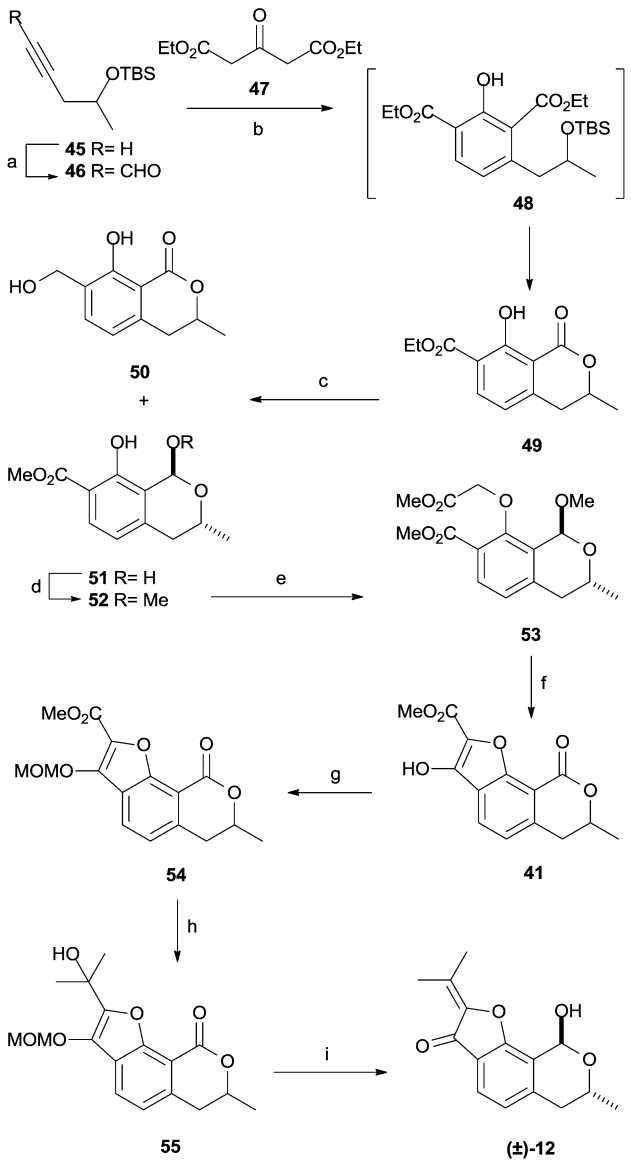
However, it was found that the specific optical rotation of synthetic **12** {$[\alpha]_D^{23} = +63.7$ ($c = 0.08$, MeOH)} was higher than that of natural **12** {$[\alpha]_D^{23} = +11$};[18] this was ascribed to contaminants in the sample of the latter.[20] On the other hand, chiral HPLC comparison between the synthetic compound, its racemate and the natural product confirmed the stereochemistry of the latter as **12**. Furthermore, the authors unambiguously established the 1,3-*anti* configuration of both stereogenic centers and thus the configuration of the C-9 stereocenter by X-ray crystallography.

In order to avoid the inconveniencies associated to the chemoselective reduction of the lactone, and taking into account the relatively moderate performance of the final transformations of Kobayashi's synthesis, Tobe *et al.* developed in 2007 an alternative synthesis of (±)-pseudodeflectusin (Scheme 4), which features the preparation of an acetalic intermediate (**51**) early in the sequence.[33] Thus, formylation of alkyne **45**[34] by lithiation followed by reaction with DMF afforded 91% of aldehyde **46**, which was cyclocondensed with 3-oxopentane dicarboxylate (**47**) to afford 43% of lactone **49**[35] after deprotection of intermediate TBS ester **48** and subsequent cyclization in the presence of HCl.

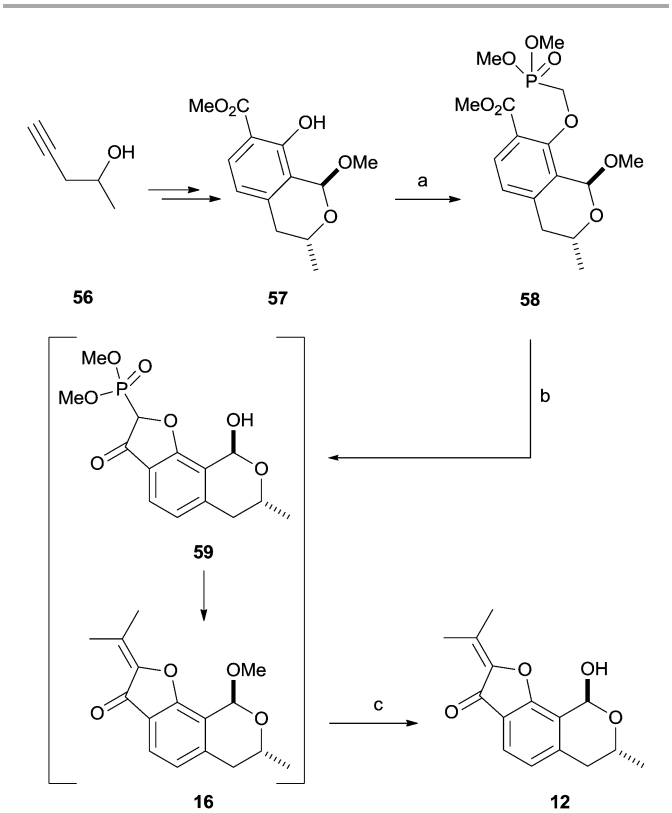
Submission of the lactone moiety to the critical reduction with DIBAL gave lactol **51** in mixture with benzylic alcohol **50** (19%), while acetalization of the lactol furnished acetal **52** in 58% overall yield, when the transformation was carried out as a one-pot process. Remarkably, both the lactol and the acetal were isolated as single 1,3-*trans* diastereomers.

Next, alkylation of the free phenol with ethyl bromoacetate (**55**) paved the way for a Dieckmann condensation,[36] which afforded enolester **41** in 84% overall yield. In turn, the enol was captured as the corresponding MOM ether, which enabled to add MeLi to the ester moiety to access **54**, without risking addition to the carbonylic carbon.

Final treatment with tosic acid in aqueous THF effected the removal of the MOM ether protective group and dehydration of the tertiary alcohol, affording the desired product (**12**) in 43% overall yield for the last three steps. Despite most of the synthetic work being devoted to construction of the properly functionalized furanone motif, this 8-step route proceeded in 8.2% overall yield.



Scheme 4 *Reagents and conditions*: (a) *n*-BuLi, THF, DMF (91%); (b) 1. Cs_2CO_3, THF; 2. HCl_{dil} (43%); (c) DIBAL, PhMe, −78 °C; (d) MeOH, AcOH (58%); (e) $BrCH_2CO_2Et$, K_2CO_3, acetone, reflux (90%); (f) *t*-BuOK, THF (93%); (g) MOMCl, DBU, CH_2Cl_2; (h) MeLi, THF; (i) *p*-TsOH, THF-H_2O (43% overall).



Scheme 5 *Reagents and conditions*: (a) $TfOCH_2P(O)(OMe)_2$, Cs_2CO_3, MeCN, rt, 4h (99%); (b) 1. LDA, THF, 0 °C, 0.5h; 2. Me_2CO, NH_4Cl, rt, 24h; (c) 1M HCl, rt, 32h (82%, overall).

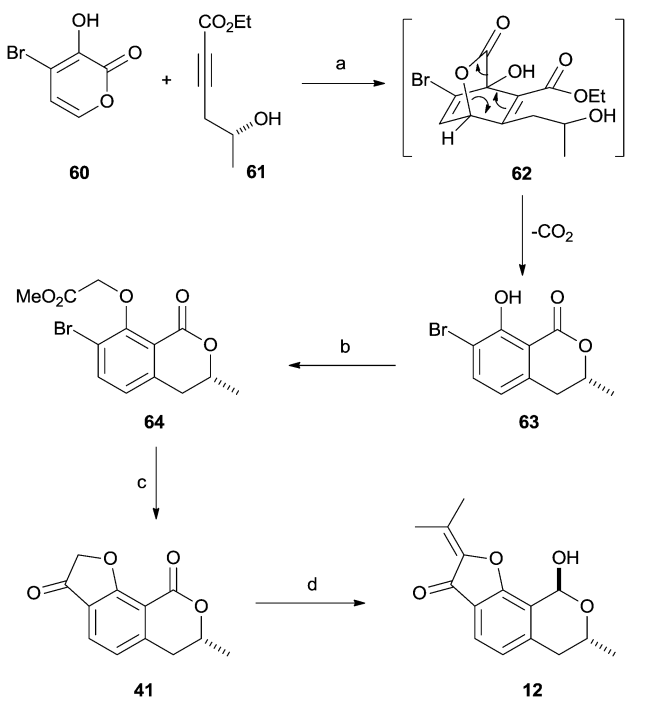
The group of Fujiota disclosed in 2012 a short alternative synthesis of (±)-pseudodeflectusin which hinges on an efficient elaboration of the furanone moiety (Scheme 5).[37] The sequence entailed a four step transformation of alkyne **56** into acetal **57**, reminiscent of Tobe's lactonic intermediate **49**, which was then almost quantitatively converted to the *O*,*P*-acetal **58** by reaction with $TfOCH_2P(O)(OMe)_2$.

This was followed by a one-pot cyclization to **59** under LDA promotion, followed by a Horner–Wadsworth–Emmons reaction with acetone to afford (±)-pseudodeflectusin (**12**) *via* **16** in 82% overall yield. The synthetic sequence involved 6-steps from **56** and proceeded in 16% global yield.

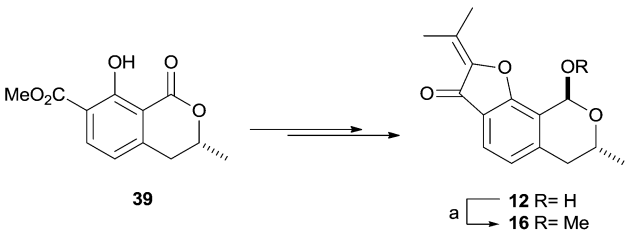
In 2011, the group of Kobayashi reported a more efficient second generation synthesis of (+)-pseudodeflectusin (Scheme 6).[38] To that end, pyrone **60** was subjected to a Diels–Alder reaction with chiral alkyne **61** in the presence of a base. Under these conditions, deprotonation of the hydroxy group of the pyrone favors the reaction.[39]

The reaction was performed at 200 °C, where the pyrone salt is stable, employing dioxane, which cleanly afforded the product **63** in 78% yield, through the intermediacy of **62**, in a cascade process involving a regioselective Diels–Alder reaction, followed by lactonization to form the heterocyclic ring and decarboxylation to yield the aromatic moiety.

Alkylation of the free phenol with methyl bromoacetate to give **64**, followed by treatment with TMSS*n*-Bu_3[40] cleanly afforded moderate yields of the intermediate furanone **64**, which was easily converted into the natural product **12** following known steps.[20]



Scheme 6 *Reagents and conditions*: (a) NaH (1.1 equiv.), dioxane, 200 °C, 5h (78%); (b) $BrCH_2CO_2Me$, K_2CO_3, DMF, rt (93%); (c) TMSSn-Bu_3, CsF, 4 Å MS, THF-DMF, −78 °C → −50 °C (58%); (d) ref. 19.



Scheme 7 *Reagents and conditions*: (a) $TsOH \cdot H_2O$, MeOH (100%).

2.3 Total synthesis of ustusorane C

Ustusorane C (**16**) is the methyl acetal derivative of pseudodeflectusin (**12**). Kuramochi and co-workers reported in 2010 the total synthesis of ustusorane C (**16**) employing an extension of their sequence, concomitantly used for the preparation of pseudodeflectusin (Scheme 7).[41]

3 Furo[3,2-*h*]isoquinolines

3.1 Isolation of TMC-120-type alkaloids, panaefluorolines, and related compounds. biological activity and proposed biosynthesis

Interleukin (IL)-5 is a cytokine that attracts, activates, and prolongs the survival of eosinophils; it is important in causing eosinophilic inflammation in the asthmatic airway and contributing to eosinophil viability in the sputum of asthmatic patients during attacks.[42] Therefore, compounds that inhibit the prolongation of eosinophil survival are useful candidates for the development of drugs to treat bronchial asthma.

In 1999, during the course of a screening for inhibitors of interleukin-5 (IL-5)-mediated prolongation of eosinophil survival, Kohno *et al.* disclosed the isolation of several alkaloids [TMC-120A-C (**65**, **23**, **24**)] from *Aspergillus ustus* TC 1118 (Bain.) Thom & Church (Fig. 5).[43] The fungus was isolated from the rhizosphere of grass collected in Kawaguchi-shi, Saitama, Japan.

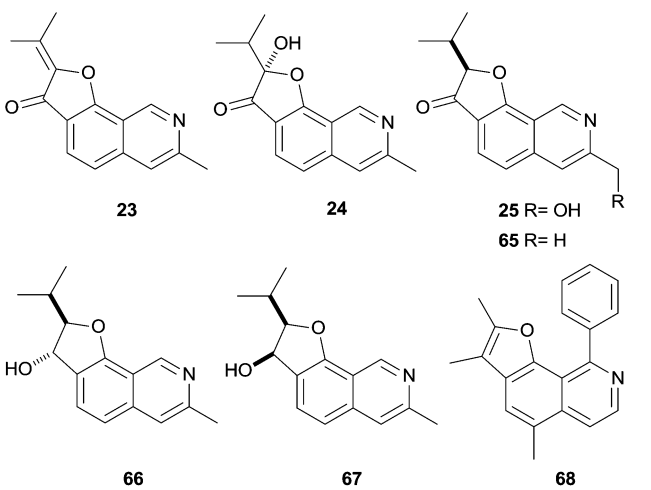


Fig. 5 Chemical structures of TMC-120A-C (**65**, **23**, **24**), $NaBH_4$-mediated reduction products of TMC-120B (**66**, **67**) and **68**.

Their structural elucidation was carried out by a combination of NMR spectroscopy, X-ray crystallographic analysis of TMC-120B and chemical studies on TMC-120B. The latter included its conversion into (±)-TMC-120A by catalytic hydrogenation over 10% Pd/C (36% yield) and also the reduction of its α,β-unsaturated carbonyl system with $NaBH_4$, to a mixture of alcohols (±)-**66** and (±)-**67** (Fig. 5).

TMC-120A is optically active; however, it was suggested that TMC-120C, which has a hemiacetalic structure and displays $[\alpha]_D = 0$, may be a racemate.[44]

Taking into account that the synthetic 5-methyl-2,3-dimethyl-9-phenylfuro[3,2-*h*]isoquinoline **68** can be regarded as the first known furo[3,2-*h*]isoquinoline,[45] alkaloids TMC-120A-C can be considered as the first furo[3,2-*h*]isoquinoline-type alkaloids isolated from natural sources.[44]

In 2004, the group of Mizushina found TMC-120B in the culture broth of *A. pseudodeflectus* Hiji005, isolated from the sea weed *Sargassum fusiform*, collected in the Miura Peninsula, Kanagawa, Japan, together with pseudodeflectusin (**12**).[18]

When TMC-120A-C (**65**, **23** and **24**, respectively) and the reduced derivatives **66** and **67** were subjected to the bioassay, TMC-120B (**23**) exhibited the best performance ($IC_{50} = 2.0$ μM), suggesting that the α,β-unsaturated ketone system of TMC-120B plays an important role for the activity, which probably involves the alkylation of biological nucleophiles through a Michael-type addition. Interestingly enough, TMC-120B was non-toxic against human leukemia HL-60 at a 42 μM level.

More recently, the group of Hibino disclosed the effects of TMC-120A (**65**), TMC-120B (**23**) and synthetic benzylidene derivative **69** in several cellular assay systems.[46] They found that the heterocycles were potent inhibitors of the production of interferon-γ (IFN-γ), induced by stimulating ovalbumin (OVA)–specific murine T cells with an OVA peptide antigen (Table 1).[47]

Stimulation by the peptide also induced production of interleukin-4 (IL-4), but the effects of the tested compounds were relatively weak at the 3 μM level. At this concentration, the heterocycles had no effect on the proliferative response, as assessed by [^{3}H] thymidine uptake. This was interpreted as evidence that suppression of the production of IFN-γ is not a result of toxic effects of the compounds.

Miller *et al.* employed TMC-120A (**65**) as a probe to study the inflammatory potential and molecular mechanisms underscoring inflammatory responses of lung cells to compounds from fungi that grow on damp building materials.

The effect of the pure fungal compounds on potentiating acute inflammatory response in primary mouse alveolar macrophages was evaluated testing the hypothesis that alveolar macrophages' responses to low molecular weight fungal compounds exhibit temporal and compound specificity that mimic that observed in the whole lung.

These authors found that exposure to the natural product produced statistically-significant changes ($p < 0.05$) in gene transcription of interleukins IL1β, IL1r1, IL4 and IL10. The results confirm that the inflammatory nature of this fungal metabolite can contribute to the development of non-allergenic respiratory health effects.[48]

After LC-MS analysis of building-derived isolates, Nielsen and co-workers suggested in 2003 that TMC-120-related compounds were present in *A. ustus* var. *pseudodeflectus* (revised as *A. insuetus*) found in these isolates.[49] However, it was the group of Slack the one that produced the first confirmatory report of the presence of TMC-120A and TMC-120B in indoor isolates.

Aspergillus insuetus and *A. calidoustus* grown in CY medium produced several major metabolites identified from the filtrate extract as almost exclusively TMC-120A-C and novel TMC-120 derivatives **70** and **71** (Fig. 6).

These isoquinoline alkaloids were detected primarily in the *A. calidoustus* strains, and only in one strain of *A. insuetus*.[13] This novel TMC-120 derivative **71** may be a product resulting from ring opening of TMC-120C between C-2 and the oxygen at position 1.

The group of Takahashi isolated the mycobiont *Arthonia cinnabarina* (DC.) Wallr[50] from spores discharged from apothecia of the lichen *Amygdalaria panaeola* (Ach.) Hertel & Brodo, collected in Finland, which is usually found on dead and decomposing twigs. When the mycobiont was cultured on

Table 1 Effects of TMC-120A (**65**), TMC-120B (**23**) and **69** on the production of interferon-γ (IFN-γ) and interleukin-4 (IL-4) from antigen-stimulated T-cells and on cell proliferation

		OVA peptide (μg mL^{-1})			
		0	0.3	3	30
IFN-γ (ng mL^{-1})	Control	0.0	13.7	20.0	39.0
	65 (3 μM)	0.0	8.1	12.5	18.4
	23 (3 μM)	1.6	6.7	8.9	9.1
	69 (3 μM)	0.7	9.6	12.4	18.8
IL-4 (pg mL^{-1})	Control	0.0	81.0	135.0	164.0
	65 (3 μM)	0.0	103.5	152.5	166.0
	23 (3 μM)	0.0	82.5	133.0	168.0
	69 (3 μM)	0.0	88.5	128.5	132.0
Thymidine uptake (fold)	Control	1.0	6.32	5.93	5.92
	65 (3 μM)	1.23	8.35	7.49	7.37
	23 (3 μM)	0.95	8.79	8.02	7.78
	69 (3 μM)	0.83	6.42	5.75	5.69

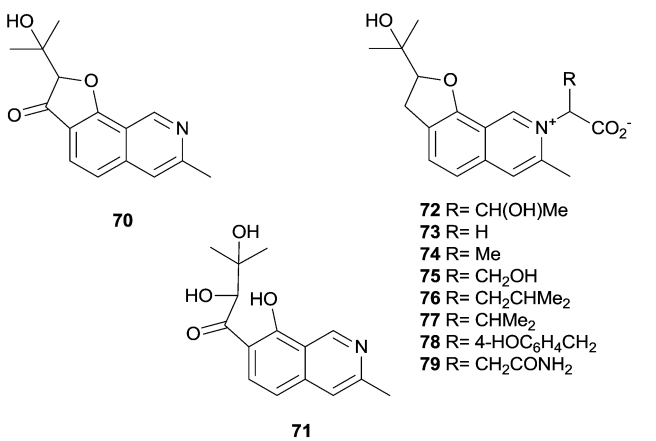


Fig. 6 Chemical structures of **70**, **71** and panaefluorolines A–H (**72–79**).

malt-yeast liquid medium, it became fluorescent and panaefluorolines A–C (**72–79**) were isolated in 2003, as yellowish green amorphous solids (Fig. 6).[51]

Their structures were elucidated by spectroscopic means and by comparison with the published data of TMC-120A. The fluorescent pigments could not be detected in the lichen thallus by HPLC analysis.

The authors conjectured that despite isoquinoline alkaloids in higher plants are biosynthesized from tyrosine, the structures of panaefluorolines provide clues with regards to the source of their nitrogen atom, and this may be amino acids such as threonine, glycine and alanine. The more recent isolation of the panaefluorolines D–H (**75–79**) from the same source[52] reinforces this hypothesis.

On the other hand, the group of Rukachaisirikul[23] proposed that the furo[3,2-*h*]isoquinolines would be biosynthesized by a pathway very similar to that proposed for the related furo[3,2-*h*]-iso-chromans (Scheme 2); however, a 7-amino derivative of peniciphenol (**26**) would be used as the biosynthetic precursor.

The panaefluorolines were separated as single enantiomers, and except for panaefluoroline G (**78**), and all of them were dextrorotatory; the relative configuration of the stereogenic centers of panaefluoroline D (**75**) was determined by X-ray crystallography, resulting 2,2′-*anti*.

3.2 Total syntheses of alkaloid TMC-120B

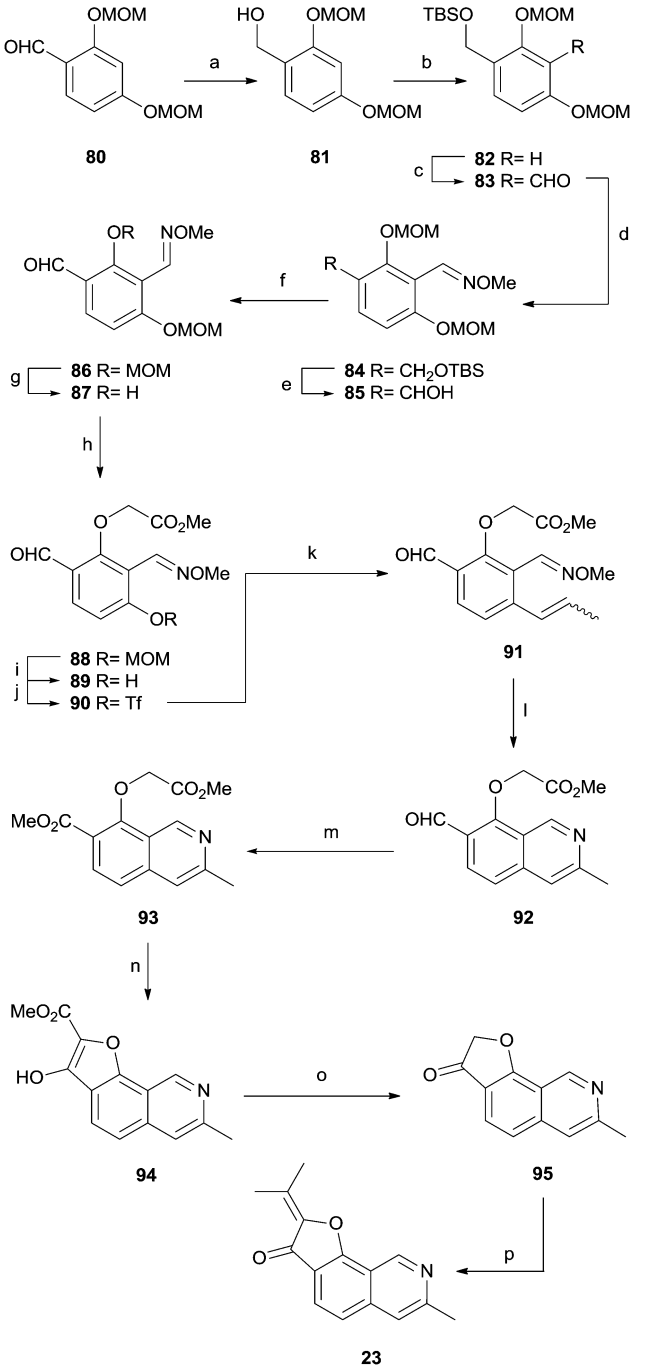
The group of Hibino reported the first total synthesis of TMC-120B in 2003[24,53] from the known aldehyde **80** (Scheme 8).[54] Reduction of the formyl moiety of **80** followed by silylation of the resulting alcohol **81** afforded 77% of intermediate **82**. This was followed by *ortho*-lithiation of **82** and DMF quench of the lithiated species to give 75% of aldehyde **83**.[55] In turn, this was treated with hydroxylamine methyl ether in EtOH to yield 89% of the oxime methyl ether **84**.

Next, desilylation of **84** was carried out with TBAF in 92% yield and oxidation of the resulting benzylic alcohol **85** afforded benzaldehyde **86** in 89% yield. Selective cleavage of the *ortho*-disubstituted MOM-ether group furnished 92% of **87**, which was alkylated with methyl bromoacetate to give 93% of **88**.

The removal of the remaining MOM ether in hot AcOH gave 80% of 4-hydroxy benzaldehyde **89**, which was transformed into 85% of a mixture of propenyl derivatives **91** by Stille cross-coupling of the intermediate triflate **90** with tributyl-1-propenyltin under $PdCl_2(PPh_3)_2$ catalysis. The 6π-electrocyclization of the propenyl methoxime was performed in refluxing *ortho*-dichlorobenzene,[56] furnishing 44% of the isoquinoline **92**.

Continuing with the reaction sequence, the formyl group was oxidized to the related methyl ester **93** in 83% yield employing Corey's protocol[29a] and the resulting product was subjected to an intramolecular Dieckmann condensation with NaMeO to afford β-keto ester **94** in 66% yield.

Hydrolysis and decarboxylation of **94** with LiOH in aqueous DMSO[31] gave 75% of the expected furanone **95**, which upon treatment with LDA followed by acetone quench of the intermediate anion afforded an aldol intermediate.



Scheme 8 *Reagents and conditions*: (a) $NaBH_4$, EtOH, rt, 2h (90%); (b) TBDMSCl, imidazole, DMF, rt, 12h (85%); (c) 1. *n*-BuLi, THF, 40 min; 2. DMF, 0 °C, 20 min (75%); (d) $MeONH_2$.HCl, NaOAc, EtOH, 80 °C, 12h (89%); (e) TBAF, THF, rt, 1.5h (92%); (f) MnO_2, CH_2Cl_2, rt, 24h (89%); (g) $HCl_{conc.}$, MeOH, 0 °C, 3h (92%); (h) NaH, DMF, $BrCH_2COOMe$, rt, 12h (93%); (i) AcOH, 90 °C, 12h (80%); (j) Tf_2O, pyridine, CH_2Cl_2, 0 °C, 4h (85%); (k) $Bu_3SnCH{=}CHMe$, Et_4NCl, $PdCl_2(PPh_3)_2$, DMF, 80 °C, 4h (83%); (l) 1,2-$Cl_2C_6H_4$, 180 °C, 30 min (44%); (m) NaCN, AcOH, MnO_2, MeOH, rt, 4h (83%); (n) NaOEt, MeOH, 80 °C, 12h (66%); (o) LiOH·H_2O, DMSO-H_2O, 70 °C, 2h (75%); (p) 1. LDA, Me_2CO, THF, −78 °C, 4h; 2. $MeSO_2Cl$, DMAP, pyridine, 0 °C, 2h (33%).

This intermediate was transformed to the final product by reaction with mesyl chloride in pyridine, under DMAP promotion in 33% yield.[57] The synthesis proceeded in 16 steps with

2.5% overall yield; 13 of these steps were devoted to build the trisubstituted isoquinoline moiety (7% yield).

Recently, these authors presented an improved access to key intermediate **93** and reported a more efficient synthesis of TMC-120B, together with a total synthesis of TMC-120A (Scheme 9).[58] The starting material was methyl 3-formyl-2,4-dihydroxy benzoate (**96**),[59] which was subjected to reaction with benzyl bromide and NaH in DMF.

This performed the selective benzylation of the less hindered 6-hydroxy group to give 62% of **97**, and was followed by alkylation of the product with $BrCH_2CO_2Me_3$ to afford 78% of the diester **98**. Catalytic hydrogenolysis of the benzyl ether gave the 4-hydroxybenzoate **99** in near quantitative yield, which was transformed into triflate **100** with triflic anhydride and pyridine at 0 °C (80%).

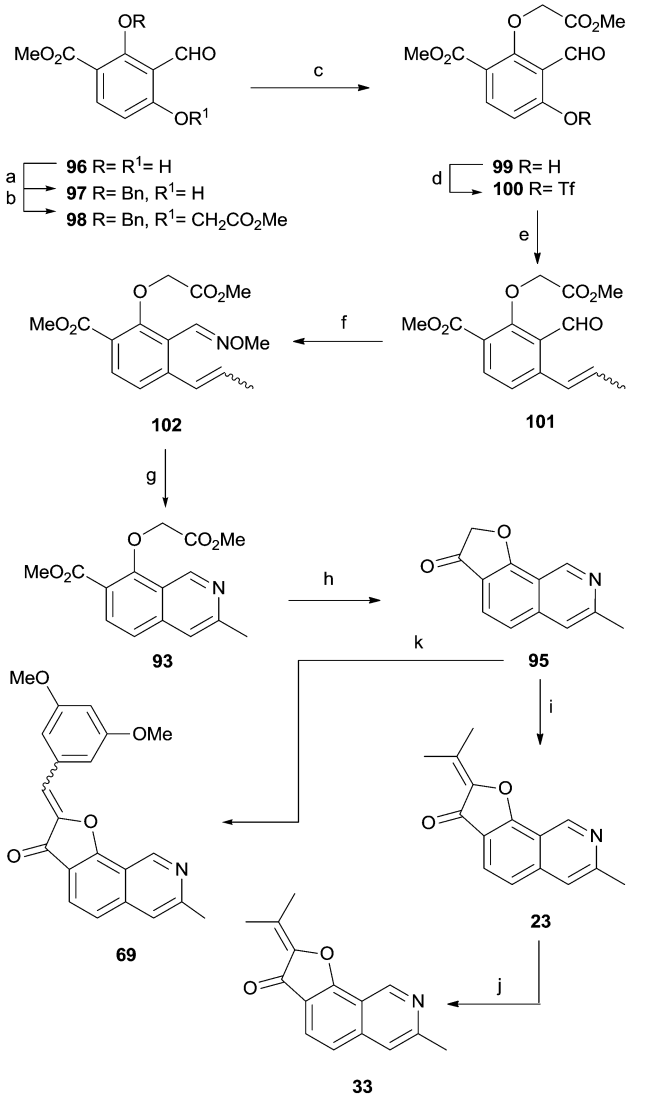
This paved the road to a Stille cross-coupling reaction with tributyl propenylstannane under $PdCl_2(PPh_3)_2$ catalysis, in the presence of Et_4NCl, which afforded 94% of the 2-propenylbenzaldehyde derivative **101**.

Oximation of the latter with hydroxylamine methyl ether in refluxing EtOH gave the corresponding methoxime **102**, which was subjected to a microwave-assisted 6π-electrocyclization reaction in *ortho*-dichlorobenzene. Under these conditions, the expected 3,7,8-trisubstituted isoquinoline **93** was obtained in 47% yield from **101**. In this way, the intermediate isoquinoline **93** was accessed from **96** in seven steps and 16% overall yield.

While catalytic hydrogenation of TMC-120B under the same conditions reported by Kohno (10% Pd/C, H_2, 1 atm, EtOAc)[44] afforded 99% TMC-120A, attempts to asymmetric reduction with several chiral reagents met with failure.

On the other hand, LDA-mediated metallation of the furanone intermediate **95** followed by addition of 3,5-dimethoxy benzaldehyde and treatment with the methanesulfonyl chloride/pyridine reagent afforded 61% of the benzylidene derivative **69**, the activity of which was also tested (*vide supra*).

Interestingly, 4-phenyl tetrahydroisoquinoline derivatives including the furo[3,2-*h*]isoquinoline tricyclic skeleton of panaefluorolines and the TMC-120 alkaloids have been patented as useful for the treatment of various neurological and psychological disorders.[60]



Scheme 9 *Reagents and conditions*: (a) NaH, BnBr, DMF, rt, 12h (62%); (b) $BrCH_2CO_2Me$, K_2CO_3, DMF, 50 °C, 12h (78%); (c) 10% Pd/C, H_2 (1 atm), EtOAc, rt, 2h (98%); (d) Tf_2O, pyridine, CH_2Cl_2, 0 °C, 2.5h (80%); (e) $Bu_3SnCH{=}CHMe$, Et_4NCl, $Pd(PPh_3)_2Cl_2$, DMF, 80 °C, 50 min (94%); (f) $NH_2OMe.HCl$, NaOAc, EtOH, reflux, 1h; (g) 1,2-Cl_2-C_6H_4, MW (180 °C), 15 min (47% overall); (h) 1. NaOEt, MeOH, 80 °C, 12h (66%); 2. $LiOH{\cdot}H_2O$, DMSO-H_2O, 70 °C, 2h (75%); (i) 1. LDA, Me_2CO, THF, −78 °C, 4h; 2. $MeSO_2Cl$, DMAP, pyridine, 0 °C, 2h (33%); (j) 10% Pd/C, H_2 (1 atm), EtOAc, rt, 1h (99%); (k) 1. LDA, 3,5-$(MeO)_2$-C_6H_3CHO, −78 °C → rt, 4h; 2. MsCl, DMAP, pyridine, rt, 1h (61%).

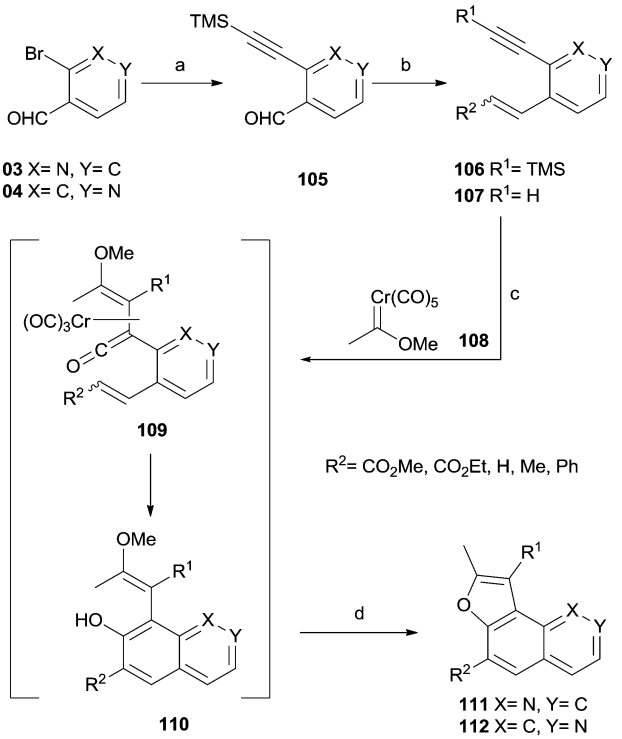
3.3 Synthesis of furo[2,3-h]isoquinoline derivatives employing fischer carbene complexes and the Ninitzescu reaction

Some furo[2,3-*h*]isoquinoline and derivatives have been recently synthesized by coupling appropriate enyne derivatives with Fischer carbene complexes in a reaction involving the simultaneous one-pot formation of three carbon–carbon bonds and one carbon–oxygen bond, leading to the construction of both the furan and benzene rings.[61]

The related furo[2,3-*h*]quinolines have also been prepared employing the same strategy. In these syntheses (Scheme 10), the starting bromoaldehydes **103** and **104**[62] were subjected to a Sonogashira reaction with (trimethylsilyl)acetylene and the resulting alkynyl aldehydes **105**, were treated with appropriate Wittig reagents to afford the enyne derivatives **106** in good yield. Remarkably, however, when reactive or semi-stabilized ylides were employed, desilylated terminal alkynes **107** were obtained exclusively.

Coupling of the enyne derivative **107** ($R^2 = CO_2Me$) with the carbene complex **108** in refluxing THF for 20 h in the presence of PPh_3, followed by treatment with catalytic H_2SO_4 gave furoquinoline **111** in 73% yield, through the intermediacy of chromium complex **109** and enolether **110**. Analogously, isoquinoline derivative **112** was isolated in 71% yield.

The mechanism of this transformation resembles that of Dötz benzannulation, because the chromium carbene-generated ketene complex **109** formed by CO insertion cyclizes to

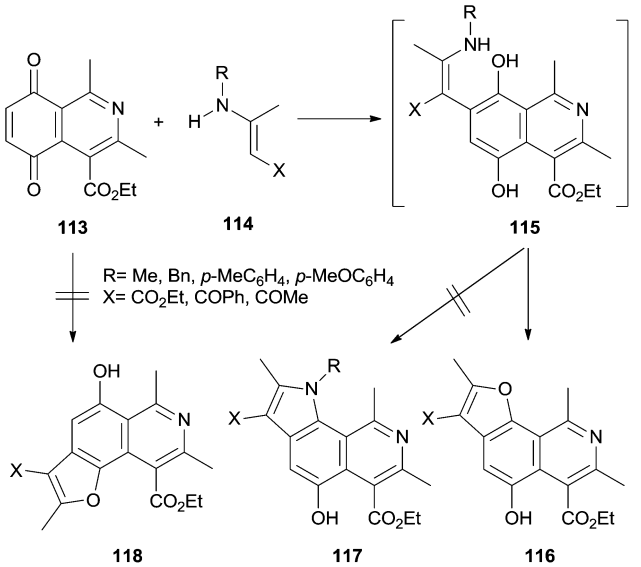


Scheme 10 *Reagents and conditions*: (a) (Trimethylsilyl)acetylene, $(Ph_3P)_2PdCl_2$, CuI, THF, Et_3N, rt; (b) $Ph_3P=CHR_2$, THF, rt; (c) PPh_3, THF, reflux; (d) $H_2SO_{4(cat)}$ (R^1 = H, R^2 = CO_2Me, X = N, Y = C, 73%; R^1 = TMS, R^2 = CO_2Me, X = C, Y = N, 71%).

afford a phenol derivative (**110**), which provides the furoquinoline or furoisoquinoline products (**111**/**112**), upon acid treatment.[63] PPh_3 as a ligand additive greatly enhanced the yields of the cyclized products.[64] Interestingly, furo[2,3-*h*]isoquinoline derivatives and the related thieno heterocycles have been prepared as potential photochemo therapeutic agents with increased antiproliferative activity and decreased toxic side effects.[65]

On the other side, polysubstituted furo[2,3-*h*]isoquinolines have been prepared by use of the Ninitzescu reaction, entailing the condensation of quinones with enamines (Scheme 11).[66]

In view of the results, it seems that reaction of quinone **113** with enamines **114** led to the hydroquinone-adducts **115**. Nucleophilic attack of the phenolic hydroxyl on the α-position of the enamines would yield the observed benzofurans (**116**); this path prevails over that leading to the five-membered nitrogen derivatives **117** or the furans **118**, resulting from formation and subsequent reactions of the alternate quinone adducts.



Scheme 11 Furo[2,3-*h*]isoquinoline derivatives by the Ninitzescu reaction.

4 Pyrano[3,2-*h*]isoquinoline and pyrano [3,2-*h*] isochromans

4.1 Isolation of aspergillitine and the aspergiones

In 2001, Lin *et al.* reported that fungal isolates of *Aspergillus versicolor* (Vuill) Tirab., which were obtained from the marine sponge *Xestospongia exigua* (Pterosiidae) collected along the coast line of Bali, Indonesia, produced a series of angular tricyclic 2,3-dimethylchromones, the aspergiones A–F (**119–124**).

Their structures were proposed as shown in Fig. 7 on the basis of extensive spectroscopic (UV, MS, ^1H and ^{13}C NMR, COSY, HMQC and HMBC) analyses.[67]

In a second communication, the structures of the aspergiones (**125–130**) were reformulated as depicted in Fig. 8, and a new compound was reported (**131**), to which the name aspergillitine was given.[68] Except for aspergillitine and aspergione D, all the isolated compounds were optically active.

The structural proposal was quite surprising, since the 2,3-dimethylchromone motif is rare[69a] and 2,3-dimethylchromones are uncommon among natural products. A few exceptions being chromones **132**, isolated from the mycobiont of the lichen *Graphis* scripta,[69b] compound **133** obtained from *Thitonia diversifolia*[69c] and *Tussilago farfara*,[69d] chromone **134**, isolated from *Ligularia microphylla*,[69e] and chaetochromin D, a bis-(naphtho-γ-pyrone) derivative produced by the fungus *Chaetomium gracile*.[69f] In addition, 2,3-dimethylchromones have been employed as key intermediates for the synthesis of more complex natural and other products.[70]

Interestingly enough, aspergillitine displayed moderate antibacterial activity against *Bacillus subtilis*, being inactive against *Escherichia coli* and *Saccharomyces cerevisiae*. On the other hand, the aspergiones C (**127**) and E (**129**) were inactive.

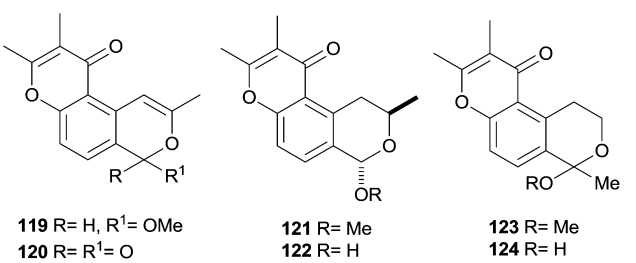


Fig. 7 Proposed chemical structures of the aspergiones A–F (**119–124**).

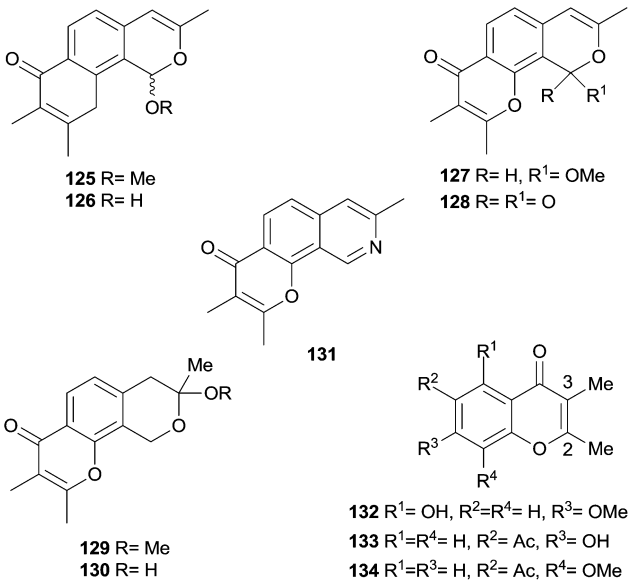
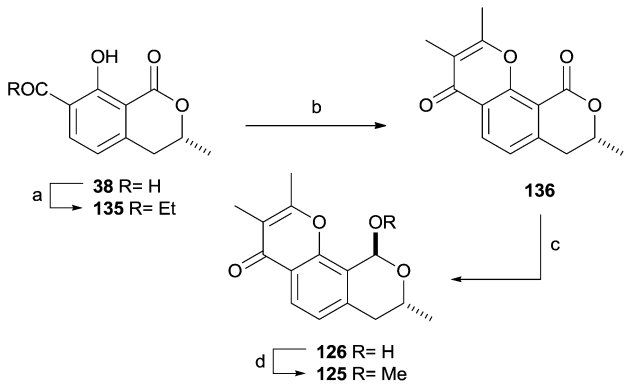


Fig. 8 Chemical structures of aspergillitine (**131**), revised chemical structures proposed for the aspergiones A–F (**125–130**) and some natural 2,3-dimethyl-chromones (**132–134**).

4.2 Synthesis of the structure assigned to aspergione B and relationship with pseudodeflectusin

Because the published NMR data of aspergione B (*vide infra*) were close to those of pseudodeflectusin, in 2006 the group of Kobayashi also synthesized the structure originally assigned to (±)-aspergione B, employing aldehyde (±)-**38** as a key intermediate (Scheme 12).

The addition of EtMgBr to aldehyde (±)-**38** gave a 1 : 1 diastereomeric mixture of the alcohols, which upon MnO_2 oxidation afforded ketone (±)-**135**. Kostanecki-Robinson synthesis of the chromone framework proceeded in 53% yield of **136** by treatment of (±)-**135** with Ac_2O and DBU in pyridine at 60 °C,[71] while final selective reduction of the lactone moiety in (±)-**136** with DIBAL in CH_2Cl_2 afforded (±)-**126.** NMR data comparison confirmed that the compound to which the structure of aspergione B was assigned, is actually pseudodeflectusin.



Scheme 12 *Reagents and conditions*: (a) 1. EtMgBr, THF, −5 °C; 2. MnO_2, CH_2Cl_2 (52% overall); (b) Ac_2O, DBU, pyridine, 60 °C (53%); (c) DIBAL, CH_2Cl_2, −78 °C (99%); (d) *p*-TsOH, MeOH (91%).

After their synthesis of ustusorane C (**16**) through the intermediacy of pseudodeflectusin (**12**), the group of Kuramochi[41] agreed with the above proposal. Moreover, based on an exhaustive analysis of NMR data of synthetic and natural products, these authors also suggested that the compound isolated as aspergione A (**125**) is actually ustusorane C (**16**).

4.3 Synthesis of the structure originally assigned to aspergillitine. relationship with alkaloid TMC-120B

Paralleling the relationship between aspergiones A and B (**119** and **120**) with ustusorane C and pseudodeflectusin (**16** and **12**), respectively, the structure proposed by Proksch *et al.* for aspergillitine (**131**) is identical with that of TMC-120B with regards to the isoquinoline moiety.

However, they differ in that ring *A* of **131** contains a 2,3-dimethyl-pyran-4-one (2,3-dimethyl-γ-pyrone) motif, while ring *A* of **23** is the isomeric 3-isopropylidene-3*H*-furan-2-one. The synthesis of the structure originally assigned to aspergillitine was undertaken with the double aim of accessing a unique and unprecedented polycyclic structure and in order to contribute to reveal structural relationships between the natural product isolated by the group of Proksch and TMC-120B. To that end, the commercially available propiophenone derivative **137** was subjected to a Kostanecki-Robinson synthesis, affording 56% of chromone intermediate **138** (Scheme 13).[72]

O-allylation, followed by Claisen rearrangement and mesylation of the free phenol afforded **141**, which was treated with catalytic OsO_4 and KIO_4, affording 20% of aldehyde **143**, presumably through the intermediacy of the readily enolizable phenylacetaldehyde **142**.[73] However, carrying out the reaction on the free phenol gave 50% of **143**.

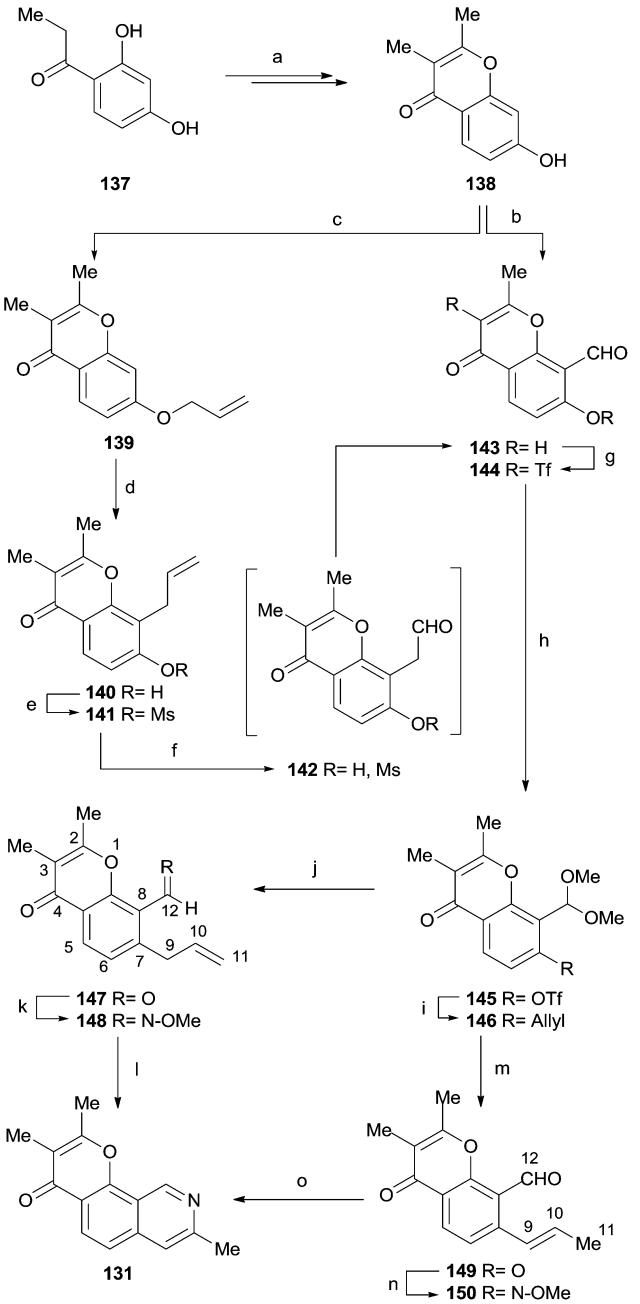
Because the overall sequence for installation of the 8-formyl unit was deemed unsatisfactory, alternative formylation strategies were sought, among them a Duff formylation. In an optimized process, modification of the hydrolysis stage of the iminium intermediates, which included the use of milder conditions (H_2O, 100 °C),[74] afforded 72% of the 8-formyl derivative **143.**

Attempts of triflating the phenol **143** with Tf_2O and a base (*N*,*N*-diisopropylethylamine, 2,4,6-lutidine) were unsuccessful;[75] therefore, the transformation was carried out by treatment of **143** with NaH and *N*-phenyltriflimide in a THF-DMF solvent mixture, furnishing 95% of **145.**[76]

Stille's cross-coupling of aldehyde **147** with *n*-$Bu_3SnCH_2CH{=}CH_2$ met with failure,[77] yielding products resulting from 1,2-addition to the carbonyl or decarbonylation of the formyl moiety.[78]

Therefore, the reaction was performed on the dimethyl acetal (prepared in 97% yield by reaction with $HC(OMe)_3$ in MeOH under CSA catalysis). This gave access to **146** in 80% yield when $Pd(PPh_3)_2Cl_2$ in DMF was employed.[79] The acetal readily hydrolyzed to **147** during acidic work-up and chromatography on silica gel; oximation of the resulting aldehyde **147** with methoxylamine afforded 76% of the *syn*-methoxime **148.**

The direct amino-Heck cyclization of the 1,3,6-azatriene moiety under the conditions of Tsutsui and Narasaka gave 18%



Scheme 13 *Reagents and conditions*: (a) 1. Ac_2O, NaOAc, Δ; 2. Et_3N, Δ; 3. HCl (56% overall); (b) 1. Hexamine, H_2O, AcOH, 100 °C, 2.5h (72%); (c) $BrCH_2CH{=}CH_2$, K_2CO_3, EtOH, reflux, 3h (83%); (d) 1,2-Cl_2-C_6H_4; Δ, 36h (80%); (e) MsCl, pyridine, CH_2Cl_2, 40 °C, 48h (93%); (f) OsO_4, KIO_4, *t*-BuOH:0.1M phosphate buffer, pH 8.0 (1 : 1), rt, overnight (**141** → **143**, 20%; **140** → **143**, 50%); (g) *N*-phenyltriflimide, NaH, THF-DMF, rt, 4h (95%); (h) $HC(OMe)_3$, CSA, MeOH, rt, 24h (97%); (i) *n*-$Bu_3SnCHCH_2{=}CH_2$, $Pd(PPh_3)_2Cl_2$, LiCl, PPh_3, BHT, DMF, Δ, 18h (80%); (j) SiO_2 (100%); (k) $MeONH_2$.HCl, NaOAc, EtOH, 50 °C, 12h (76%); (l) $Pd(PPh_3)_4$, *n*-Bu_4NCl, Et_3N, DMF, 80 °C (18%); (m) 1. $Pd(PPh_3)_2Cl_2$, LiCl, DMF, Δ, 24h; 2. H_2O–THF, 80 °C, 2h (75%, overall); (n) $MeONH_2$.HCl, NaOAc, EtOH, 50 °C, 12h (85%); (o) 1,2-Cl_2–C_6H_4, MW, 180 °C, 30 min (80%).

of the tricyclic product.[80] Therefore, acetal **146** was subjected to isomerization with $Pd(PPh_3)Cl_2$ in DMF, affording 75% of aldehyde **149** after work-up and chromatography.[81]

Finally, oximation of **149** to the *O*-methyl oxime **150** (obtained in 85% yield as a 4 : 1 *syn*/*anti* mixture of isomers), followed by a microwave-assisted 6π-electrocyclization,[82] provided 80% yield of tricyclic compound **131**. The synthesis was completed in 11 steps and 15% overall yield from 2,4-dihydroxypropiophenone (**137**).

The ^1H and ^{13}C NMR spectroscopic data of synthetic and natural TMC-120B, as well as those of "natural" and synthetic aspergillitine were compared. Resonances of the synthetic aspergillitine did not match those reported by Proktsch *et al.* for the natural product; being very close to those recorded for the synthetic and natural alkaloid TMC-120B (**23**).

Taken together, this means that both, the compound isolated by Proksch *et al.* and TMC-120B should be the same compound, and that the tricyclic structure originally assigned to aspergillitine still remains unobserved among natural products.[83]

5 Furo[4,5-*g*]isobenzofurans

5.1 Isolation of daldinins and concentricolide

Qin *et al.* studied a Chinese strain of the Ascomycete *Daldinia concentrica* collected at Lijing, in the Southwestern Chinese province of Yunnan, reporting in 2006 the isolation of concentricolide (**151**). The structure of the natural product (Fig. 9) was established by NMR spectroscopy and single crystal X-ray crystallography, and the 6*R* stereochemistry was proposed for its stereogenic center.[84]

Concentricolide inhibited HIV-1-induced cytopathic effects with an EC_{50} value of 0.31 μg mL^{-1}; it also exhibited the blockage (EC_{50} = 0.83 μg mL^{-1}) on syncytium formation between HIV-1 infected cells and normal cells, and had a therapeutic index of 247, suggesting that it is safe and effective against HIV-1.

The natural product and several related compounds, including 2,5-dinitro, 2,3-dibromo and 3-bromo derivatives were patented claiming their usefulness for the treatment and prevention of the infection caused by the human immunodeficiency virus (HIV).[85]

The OSMAC (one strain-many compounds) approach towards metabolite diversity is based on the observation that individual fungal strains are able to produce more metabolites than normally detected in a routine screening program, and that very small changes in the cultivation parameters (for example, culture

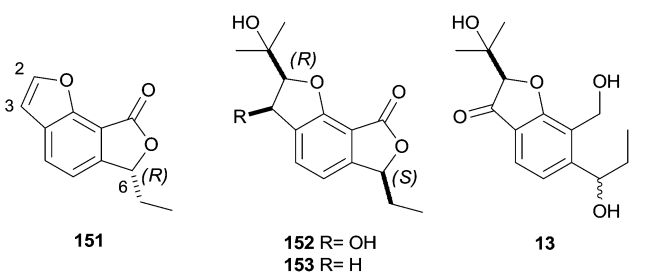


Fig. 9 Chemical structures of concentricolide (**151**, originally proposed configuration) and daldinins A (**152**), B (**13**) and C (**153**).

vessel, media composition, addition of enzyme inhibitors, *etc.*), can completely shift their metabolic profile.[86]

Ascomycetes of the genus *Daldinia* (Xylariaceae) are rich in secondary metabolites. European and American *Daldinia sp.* have been screened for secondary metabolites since the late 1950s. Shao *et al.* studied the ascomycete *Daldinia concentrica* S0318, collected at Laojunshan (Yunnan Province, China). The researchers found that this strain produced concentricolide (**151**, 160 mg Kg^{-1} dried fruiting bodies) when cultivated in brown reagent bottles; however, when cultivated in colorless Erlenmeyer flasks, despite employing the same medium and conditions, they produced the above metabolite in addition to novel benzofuranoid derivatives, the daldinins A–C (**152**, **13** and **153**). Compound **13** has been recently found in *A. ustus* 094102,[12] together with other related compounds.

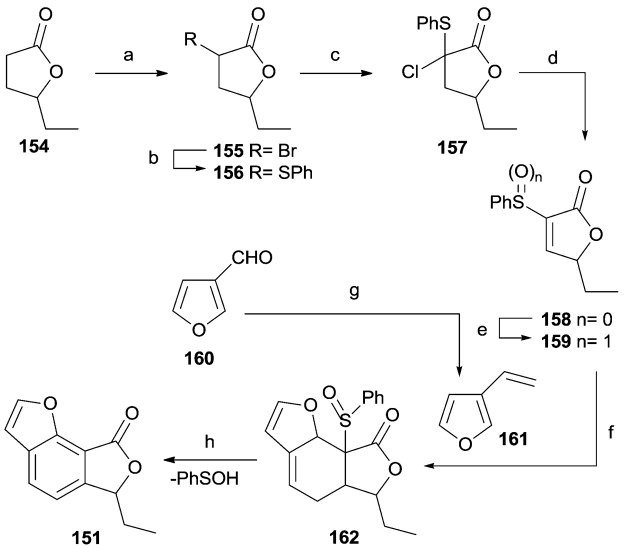
The structures of the natural products were elucidated by NMR spectroscopy and X-ray crystallography of daldinin A, which allowed establishment of the relative configuration of C2 and C6. Taking into account the simultaneous production of daldinins A, B and concentricolide, and that the latter has been unambiguously determined as the *S*-(−) enantiomer, it is not unlikely that the daldinins A and B share the 2*S*,6*S* absolute configuration. It has been suggested that the secondary metabolite constituents of *Daldinia* are of great chemotaxonomic significance for this and other genera of the Xylariaceae.[87]

Interestingly enough, in 1994, the group of Asakawa reported three azaphilone natural products from *D. concentrica* collected in Tokushima, Japan, to which the names daldinin A–C were given.[88] An unsuccessful attempt to synthesize the azaphilone-type daldinin A was performed in 2001.[89]

5.2 Total syntheses of concentricolide. determination of its absolute configuration

The total synthesis of concentricolide (**151**) and further investigations on the requirements of its partial analogs for pharmacological activity are undoubtedly important to find new medicines for treating AIDS. Fang and Liu reported in 2009 the first total synthesis of racemic concentricolide from 5-ethyl-dihydrofuran-2(3*H*)-one (**154**).[90] To that end, the starting furanone was brominated to give 3-bromo-5-ethyl-dihydrofuran-2(3*H*)-one (**155**),[91] which was subjected to nucleophilic attack with thiophenol to afford the 3-phenylthio derivative **156** in 56% yield (Scheme 14). Exposure of **156** to NCS effected an α-chlorination yielding 82% of **157**. Elimination of HCl by treatment with Li_2CO_3 in refluxing THF gave 35% of the key intermediate furan-2(5*H*)-one **158**.

Oxidation of the sulfide with *m*-CPBA afforded sulfoxide **159** in 82% yield. Next, a Diels–Alder reaction was performed between sulfoxide **159** and vinylfuran **161** (prepared by reaction of 3-formylfuran (**160**) with $TMSCH_2Cl$) for 3 days in the presence of hydroquinone to prevent unwanted reactions.[92] This afforded **162**, which without purification was stirred with $CaCO_3$ in anhydrous toluene to give **151** after loss of PhSOH and further oxidation with concomitant aromatization during column chromatography. The yield of product was less than 4%.



Scheme 14 *Reagents and conditions*: (a) PBr_3/Br_2, 70–90 °C (57%); (b) PhSH, Et_3N, Et_2O (98%); (c) NCS, CCl_4, reflux (82%); (d) Li_2CO_3, LiBr, THF, reflux (35%); (e) *m*-CPBA, CH_2Cl_2, 0 °C (82%); (f) hydroquinone (0.1 equiv.), rt, PhMe, 72h; (g) 1. $TMSCH_2Cl$, Mg, Et_2O, reflux, 9h; 2. 3-formylfuran, 0 °C, 4h (50%); (h) 1. $CaCO_3$, PhMe, reflux, 19h; 2. [O]-Column chromatography (3.8%).

When concentricolide was isolated, its absolute configuration was proposed as (6*R*) based on an X-ray crystallographic study.[84] However, Mo radiation is unsuitable to determine absolute configurations in X-ray experiments. This prompted Ren *et al.* to perform a density functional theory (DFT) study of the absolute configuration of concentricolide, which allowed the proposal of its configurational reassignment, employing the B3LYP/aug-cc-pVDZ//B3LYP/6-31G(d) and B3LYP/aug-cc-pVDZ//MP2/6-31+G(d) methods, respectively. Similar studies have been successfully performed for other natural products, helping to establish their absolute configurations.[93]

Analogs **163** (*ee* = 99%) and **164** (*ee* = 63%, *S*-enantiomer purified by chiral HPLC to *ee* = 99%) were obtained as racemates and in optically active forms by catalytic enantioselective addition of Et_2Zn to the corresponding aldehydes (Fig. 10),[94] employing (*S*)-2-[(3,3-dimethylbutyl)(methyl)amino]-3-ethyl-1-(1*H*-indol-3-yl)pentan-3-ol or 3-[(1*S*,3*S*)-2-methyl-1-neopentyl-2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]-indol-3-yl]pentan-3-ol, as chiral auxiliaries.[84]

Compounds (*R*)-**163**, (*S*)-**163**, (*R*)-**164**, (*S*)-**164**, (±)-**163**, (±)-**164** and **165** were submitted to theoretical (DFT) and anti-HIV (experimental) studies.[95]

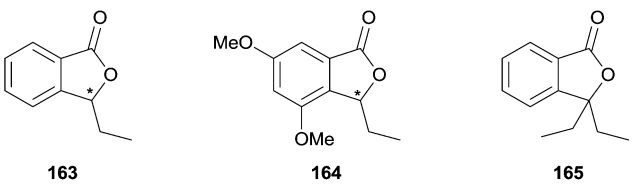


Fig. 10 Test compounds employed for the theoretical study on the absolute stereochemistry of concentricolide.

Table 2 The computed and experimental specific optical rotation values for compounds **151**, **163** and **164** in the gas phase

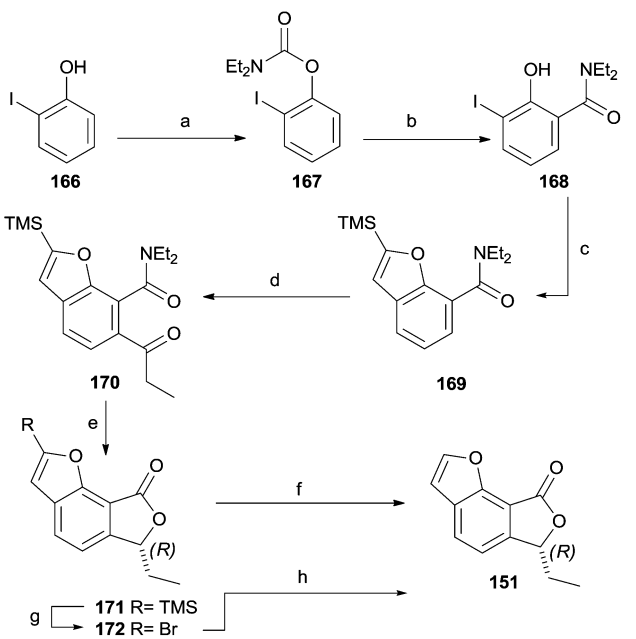
Compound	Purity (%)	$[\alpha]_D$ Calcd.	Method[a]	Proposed Config.	$[\alpha]_D$ Exptl.
151	97	−43.8	A	*S*	−59.2
		−66.1	B		
163	99	−97.3	A	*S*	−81.9
		−102.0	B		−76.0
164	99	−102.8	A	*S*	−98.8
		−83.2	B		
151		Assigned configuration →		*S*	−59.2

[a] Method A: B3LYP/aug-cc-pVDZ//B3LYP/6-31G(d); Method B: B3LYP/aug-cc-pVDZ//MP2/6-31+G(d).

The (*R*) configurations of **163** and **164** exhibited better anti-HIV-1 activity than the corresponding (*S*)-enantiomers, being (*R*)-**164** the most potent (EC_{50} = 28 μM; control of zidovudine EC_{50} = 12 μM) in MT4 + HIV-LLAI tests. The 3,3-diethyl isobenzofuran-1(3*H*)-one displayed very low activity.

The calculated values (Table 2) were close to the experimental or reported ones;[96] therefore, the absolute configuration of concentricolide {$[\alpha]_D^{22}$ −59.2} was re-assigned as (6*S*).

Chang and Chien recently reported the first enantioselective total synthesis of *R*-(+)-concentricolide[97] from 2-iodophenol (**166**) in 7 steps, which served to unambiguously establish the absolute configuration of the natural product (Scheme 15). To that end, the phenol was converted into the carbamate (**167**),[98] which in turn was subjected to an anionic *ortho*-Fries rearrangement to afford **168**.[99]



Scheme 15 *Reagents and conditions*: (a) *N*,*N*-diethyl chloroformamide, K_2CO_3, CH_3CN, reflux, 2h (95%); (b) LDA, THF, −60 °C (72%); (c) trimethylsilylacetylene, Et_3N, $Pd(PPh_3)_2Cl_2$ (1 mol%), CuI (2 mol%), CH_3CN, 65 °C, 96h (88%); (d) TMEDA, *t*-BuLi, THF, −80 °C, 1.5h; *N*-methoxy-*N*-methylpropionamide (76%); (e) (*S*)-B-*n*-Bu-CBS catalyst (0.5 equiv), BH_3·THF, 0 °C (86%); (f) KOAc, 1,2-$Me_2C_6H_4$, 130 °C, 1h; (g) TBAF, AcOH, rt, 10 min (82%); (h) Br_2, CH_2Cl_2, rt, 10 min; (i) Zn, AcOH, H_2O, 100 °C, 1h (95%).

A Sonogashira coupling of **168** with TMS-acetylene under cuprous and palladium catalysis provided 88% of intermediate benzofuran **169** after cyclization of the acetylenic intermediate.[100] *Ortho*-metallation of benzofuran **169** with *t*-BuLi-TMEDA followed by addition of *N*-methoxy-*N*-methylpropionamide afforded 76% of the propanoyl derivative **170**.[101]

Enantioselective reduction of the ketone moiety under CBS conditions with the (*S*)-*n*-Bu-CBS catalyst[102] afforded 86% of **171** as a mixture of rotamers, which upon treatment with KOAc furnished 82% of phthalide **151** (*ee* = 80%). The unexpectedly low enantioselectivity of the product was assigned to the participation of the anilide group in the pre-transition state of the borane-CBS catalyst-ketone assembly. Slow interconversion of the conformers may negatively affect the facial selectivity of the hydride attack. Desilylation of **171** with TBAF proceeded in a medium buffered with acetic acid to prevent loss of chirality,[103] affording 86% of *R*-(+)-concentricolide {$[\alpha]_D^{25}$ = +26.1}.

The configuration of the final product was deduced by application of the CBS rule and also by X-ray analysis of bromide **172**, prepared in 82% yield by treatment of **171** with bromine in CH_2Cl_2. Zinc/AcOH-mediated debromination of **172** also provided *R*-(+)-concentricolide {$[\alpha]_D^{25}$ = +34.8}. Employing the enantiomer of the CBS catalyst for the key reduction step, afforded *S*-(−)-concentricolide {$[\alpha]_D^{25}$ −35.1}.

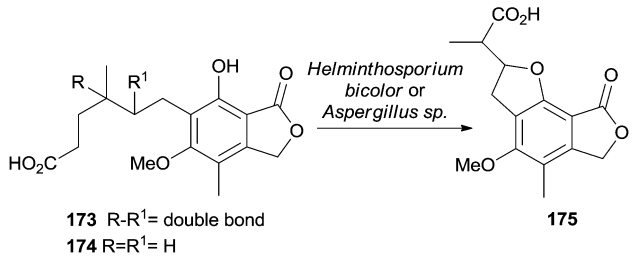
5.3 Syntheses and uses of some tricyclic mycophenolic acid derivatives

Mycophenolic acid (**173**) is an antifungal, antitumor, antiviral, antimitotic, immunosuppressive and antipsoriatic[104] agent isolated from several species of Penicillium, especially *Penicillium brevicompactum*. Fermentation of dihydromycophenolic acid **174** by *Helminthosporium bicolor* and *Aspergillus sp.* yielded tricycle **175**, possessing a furo[4,5-*g*]isobenzofuran core (Scheme 16).

The compound was also found when mycophenolic acid itself was submitted to fermentation by other microorganisms, especially *Penicillium daleae*, *P. raistrickii* and *Aspergillus carbonarius*.[105]

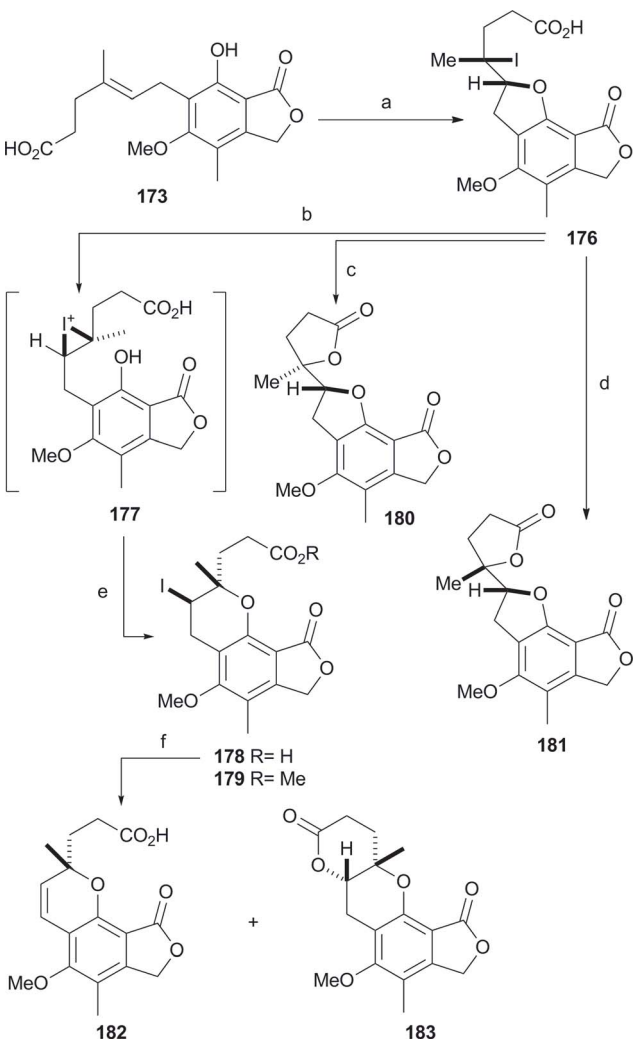
The tricyclic derivative **176** of mycophenolic acid was prepared in roughly 50% yield by shaking mycophenolic acid in bicarbonate solution with iodine in ethyl acetate (Scheme 17).

Its stereochemistry was initially proposed on mechanistic grounds, but it was recently confirmed by single crystal X-ray



Scheme 16 Biotransformation of **173** to furo[4,5-*g*]isobenzofuran **175**.

diffraction studies of its methyl ester.[105c] Precipitation of the sodium salt and warming yielded dilactone **180**, while treatment with MeOH and concentrated H_2SO_4 afforded the rearranged chromane ester **179** in nearly quantitative yield, presumably though the intermediacy of iodonium species **177**. The most stable conformer of **179** displays the methyl group quasi axial to the pyran ring, while the iodine is equatorial.



Scheme 17 *Reagents and conditions*: (a) $NaHCO_3$, I_2, H_2O–EtOAc (50%); (b) H_2SO_4, MeOH; (c) 1. $NaHCO_3$; 2. 50 °C, 5 min; (d) NaOH; (e) H_2SO_4, MeOH; (f) $NaHCO_3$.

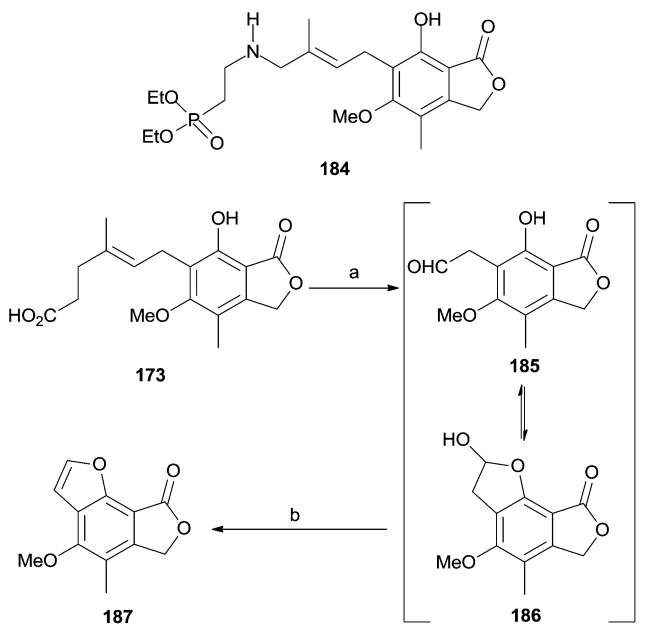
The stereochemistry of the related iodoacid **178** was deduced from formation of mycochromenic acid (**182**) together with tetracyclic dilactone (**183**) upon treatment with bicarbonate. This dilactone showed an equatorial proton geminal to the lactone ether oxygen, which requires the *cis* fused ring system (J_{vic} 2 and 4 Hz). Interestingly, however, exposure of iodide **176** to NaOH afforded lactone **181**, diasteromeric with **180**.[105a,b]

It has been recently discovered that the phosphonate-containing analogue of mycophenolic acid **184** might act as inositol monophosphate dehydrogenase isoforms I and II (IMPDH) inhibitor (IC_{50} = 37 and 13 nM, respectively), while exhibiting more prolonged cellular retention.[106] An improved synthesis of **184** was conceived employing tricyclic derivative **187** as a starting material (Scheme 18). This compound features a furan moiety as a joint protecting group for a salicylaldehyde moiety.[107]

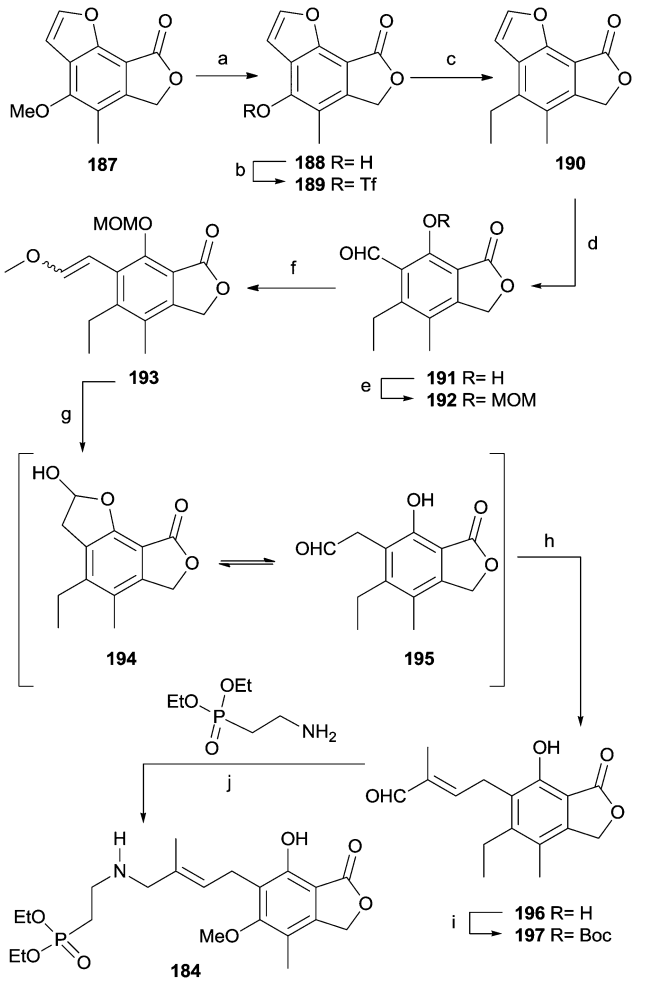
Tricycle **187** was easily made available by oxidation of the double bond of the side chain with peracetic acid and cleavage of the so produced diol[108] with periodate (79% overall yield). This was followed by acetalization (**185**) and quantitative dehydration of the resulting phenylacetic acid derivative **186** under *p*-tosic acid promotion.

The synthesis (Scheme 19) started with the installation of the ethyl side chain.[109] To this end, boron-tribromide-assisted demethylation of **187** was carried out to yield phenol **188** (97%), which was converted into the related triflate (**189**) and subsequently submitted to Suzuki coupling with ethylboronic acid under palladium catalysis to afford 96% of intermediate **190**. Ozonation of **190** proceeded in 61% yield, simultaneously uncovering the neighbor formyl and phenol moieties (**191**).

Temporary protection of the phenol as the MOM ether (**192**) was followed by homologation of the benzaldehyde to the corresponding phenylacetaldehyde by way of a Wittig reaction with



Scheme 18 *Reagents and conditions*: (a) 1. CH_3CO_3H, THF, rt, overnight; 2. NaOH, rt, 30 min; 3. $NaIO_4$, H_2O, 5 °C, 45 min (79%); (b) TsOH, PhMe, reflux, 4h (100%).



Scheme 19 *Reagents and conditions*: (a) BBr_3, CH_2Cl_2, 4h (97%); (b) Tf_2O, pyridine, CH_2Cl_2, rt, 1.5 h (88%); (c) $EtB(OH)_2$, Cs_2CO_3, $PdDppdCl_2$-CH_2Cl_2, THF, 70 °C, overnight (96%); (d) 1. O_3, CH_2Cl_2, −78 °C; 2. SMe_2 (61%); (e) MOMCl, DIPEA, CH_2Cl_2 (89%); (f) $Ph_3PCH_2OCH_3Cl$, KHDMS, THF, −78 °C → rt, 1h (71%); (g) H_2SO_4 (77%); (h) Ph_3P = (Me)CHO, PhMe, 80 °C (75%); (i) $(Boc)_2O$, pyridine, CH_2Cl_2, 30 min (100%); (j) 1. $H_2NCH_2P(O)(OEt)_2$. oxalate, $NaBH(OAc)_3$, AcOH, DMF; 2. 20% TFA, CH_2Cl_2, 0 °C.

methoxymethyl triphenylphosphonium chloride and acid hydrolysis of the methyl vinyl ether intermediate,[110] which concomitantly removed the MOM ether protecting group.

Subjection of aldehyde **195** to a second Wittig reaction towards the unsaturated aldehyde **196** was carried out without need of protecting the phenol, which was quantitatively transformed into Boc derivative **197** before performing the reductive amination required to complete the side chain. Final acidic hydrolysis of the phosphate and Boc moieties furnished the final product.

6 Furo[2,3-*h*]isochromans

6.1 Isolation, bioactivity and biosynthesis of lactonic azaphilones

The azaphilones are a structurally diverse family of fungal secondary metabolites, which have been isolated mainly from perfect and imperfect stages of ascomycetes such as *Aspergillus*, *Penicillium*, *Hypoxylon*, *Chaetomium*, *Hypoxylon*, *Monascus* and others.[111] These secondary metabolites are a relatively small subset of the polyketide class of natural products and feature a pyrone-quinone structure containing a highly oxygenated bicyclic core and a quaternary centre.

The name azaphilone reflects their affinity for nitrogen-containing compounds like ammonia; they react with amines (proteins, amino acids and nucleic acids) to yield red or purple vinylogous γ-pyridones where the pyranic oxygen is replaced with a nitrogen atom.[112] This reaction has been mentioned repeatedly as the ultimate cause behind the wide range and potency of biological activities associated to these compounds.[113]

Currently, the azaphilone family has approximately 180 known members; around 40 naturally-occuring azaphilones contain the furo[2,3-*h*]isochroman motif, and some representative examples are depicted in Fig. 11.

The subjects of isolation and biological activities of the azaphilones have been extensively reviewed in recent times.[111,114] Many azaphilones have long been known. Ochrephilone (**198**), (+)-5-bromoochrephilone (**200**), isochromophilone I (**199**) and tetrahydroisochromophilone (**203**), (+)-isorotiorin (**201**) and 5-chloroisorotiorin (**202**), (Fig. 11) were isolated from *Penicillium multicolor* FO-2338 and *P. sclerotiorum* X11853, and most of them demonstrated to be gp120-CD4 binding inhibitors, albeith with different potencies.[115] Ochrephilone (**73**) and (+)-5-chloroisorotiorin (**202**) also displayed endothelin receptor binding properties,[116] while ochrephilone and isochromophilone I proved to possess antitrypanosomal activity. Ochrephilone was fivefold less active against trypanosomes than isochromophilone I, a result attributed to the presence of a chlorine atom in the latter.[117]

On the other hand, rubrorotiorin (**204**) was isolated from *Penicillium multicolor* FO-2338, *Chaetomium cupreum* CC3003 (isolated from Thai soil) and *P. hirayamae* Udawaga, and was demonstrated to be a cholesteryl ester transfer protein (CETP), gp120-CD4 binding inhibitor, and also to display antifungic activity against *Candida albicans* (IC_{50} = 0.6 μg mL^{-1}).[118]

The related deflectins have been isolated by the group of Anke in 1981 from *Aspergillus deflectus*, a member of the *A. ustus* group,[119] and reisolated in 2010 from cultures of a strain of *Aspergillus deflectus* CBS 109.55 grown on RA medium.[120] Deflectins 1b (**205**) and 2a exhibited antibacterial and weak antifungic properties, with cytotoxic activity against Ehrlich carcinoma cells of mice, being lytic towards bacteria and red blood cells. In this assay, deflectins 1a (**206**), 1c (**207**) and 2b were inactive. This prompted the authors to suggest that in sensitive cells, one of the primary targets of the deflectins might be the cytoplasmic membranes.

The luteusins C (**209**), D (**209a**) and E were isolated in 1996 by the group of Yoshida from *Talaromyces luteus* IFM42239, and their structures were established by spectroscopic means.[121] The compounds were tested as MAO inhibitors, but demonstrated to be inactive. On the other hand, trichoflectin (**215**) was obtained from *Trichopezizella nidulus*.[122] This azaphilone was

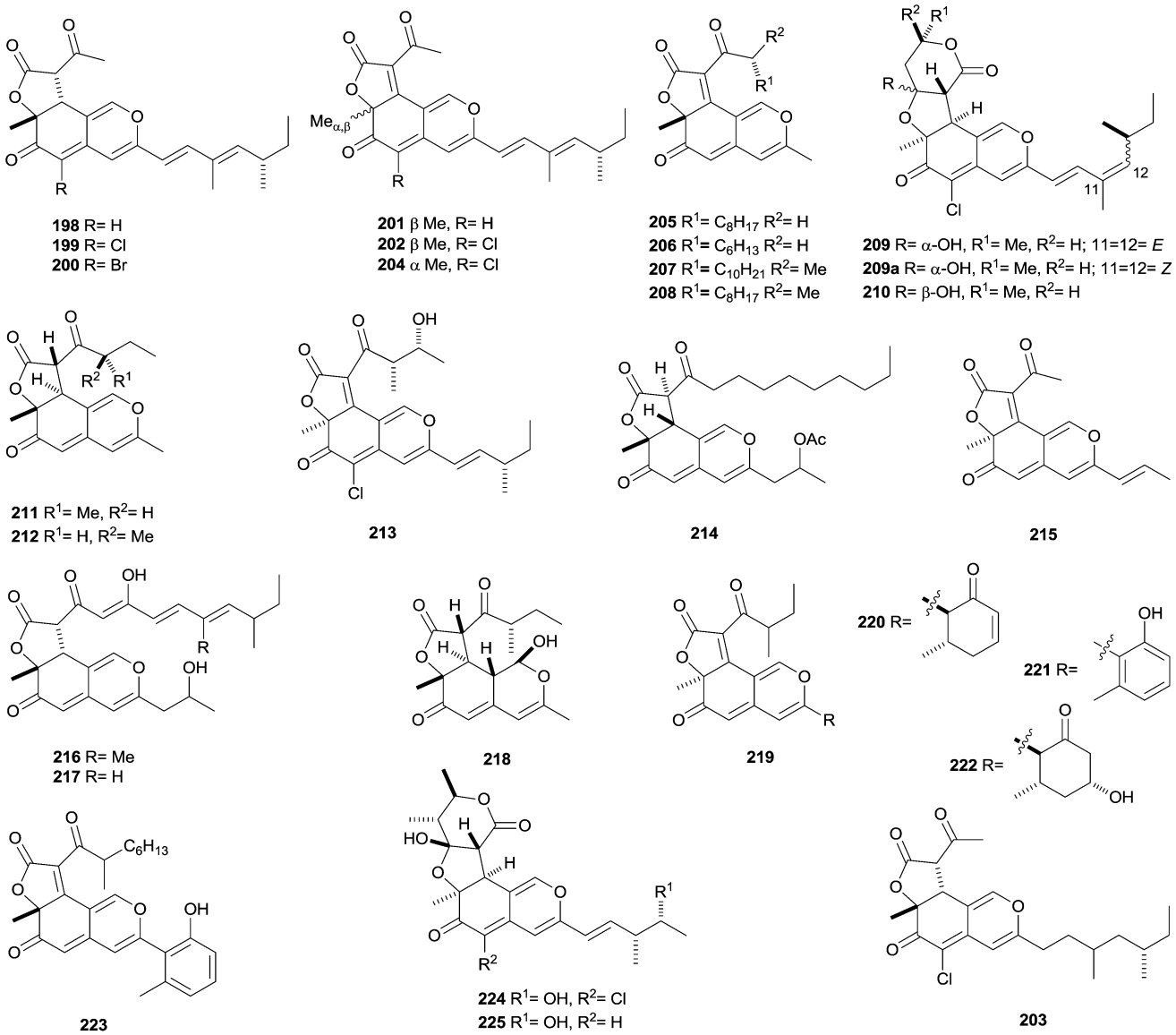


Fig. 11 Structural diversity of the lactonic angular azaphilones. Chemical structures of ochrephilone (**198**), isochromophilone I (**199**), (+)-5-bromoochrephilone (**200**), isorotiorin (**201**), 5-chloroisorotiorin (**202**), rubrorotiorin H (**204**), deflectin 1b (**205**), deflectin 1a (**206**), deflectin 1c (**207**), luteusin C (**209**), luteusin D (**209a**), RP1551-1 (**210**), chermesinone B (**211**), monochaetin (**212**), chaetoviridin A (**213**), monascuskaolin (**214**), trichoflectin (**215**), sassafrin A (**216**), sassafrin B (**217**), chermesinone C (**218**), multiformin B (**220**), multiformin C (**221**), multiformin D (**222**), cohaerin E (**223**), chaetomugilin A (**224**), dechloro-chaetomugilin A (**225**), tetrahydroisochromophilone (**203**).

found to be an antimicrobial agent and an inhibitor of the biosynthesis of dihydroxynaphthalene melanin of *Lachnellula sp.* A32–89 as the test organism. In this test, 50 μg of **215** produced an inhibition halo of 19 mm diameter whereas in the same system 50 μg of 1-methoxy-8-hydroxynaphthalene produced a 13-mm inhibition zone.[122b]

Melanins are not essential for fungal growth and development; however, they enhance their survival and competitive abilities in certain environments. The dark brown-to-black melanins in the cell walls of Ascomycotina and Deuteromycotina are generally synthesized *via* the pentaketide pathway, and the immediate precursor of the polymer is 1,8-dihydroxynaphthalene (DHN).[123]

Azaphilone derivatives RP 1551-1 (**208**), 3, 4, 5, 6, 8 and M1, isolated by the group of Toki in 1999 from *Penicillium sp.* SPC-21609, demonstrated to possess antibiotic activity against *Bacillus subtilis*, *Enterococcus faecium* and *Staphylococcus aureus*.[124] They also proved to inhibit the binding of platelet-derived growth factor (PDGF) AA homodimer to the extracellular domain of the PDGF α-receptor (IC_{50} = 0.1 to 2 μM) without affecting the PDGF BB homodimer binding to the extracellular domain of PDGF α-receptor.

The group of Asakawa disclosed in 2005 the isolation of sassafrins A (**216**), B (**217**) and C. These are the red pigment of *Creospharea sassafras* (Schwein.: Fr.) Y.-M. Ju, F. San Martin & J. D. Rogers (previously classified as *Hypoxylon sassafras*) collected

in Rimont (Ariége, France). *H. sassafras* is a xylariaceous fungus widespread around the world and found in Brazil, Canada, Chile, France, Italy, Taiwan, and North America;[125] the sassafrins were suggested as taxonomic markers of this fungus, placing it as a member of a distinct genus within the Xylariaciae.[126]

The natural products displayed moderate-to-strong antibacterial and antifungal activities against a panel of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella enteritidis*, *Escherichia coli*, *Aspergillus niger* and *Candida albicans*. However, collectively observed, these effects appeared to be non-selective, because all of the azaphilones affected both, fungi and bacteria.[123]

These results confirmed previously reported bioactivities for other azaphilones, pointing towards the role of azaphilones as defense metabolites that may protect the stromata of Xylariaceae against colonizing microbes and feeding enemies.

On the other hand, this may also explain the lack of total syntheses of these compounds and why they have not been pursued intensively for drug development. Sassafrins A–C were tested together with another 12 xylariaceous azaphilones as agents that suppresses nitric oxide (NO) production stimulated by lipopolysaccharide (LPS) in RAW 264.7 cells and as antioxidants.[127] Nitric oxide (NO) is a mediator in the inflammatory response involved in host defense. Although these compounds were not the most potent, they helped to establish structure–activity relationships.

The group of Asakawa also reported in 2005 the isolation of multiformins A–D as novel azaphilones from the xylariaceous inedible mushroom *Hypoxylon* (=*Annulohypoxylon*) *multiforme.*[123,128] These compounds displayed non-selective antibacterial activity, being Multiformins B (**94a**) and C (**94b**) the most potent examples; these were also the most active as antifungic. In addition, multiformin D (**94**) proved to suppress nitric oxide (NO) production stimulated by lipopolysaccharide (LPS) in RAW 264.7 cells and behaves as an antioxidant.[127] Some sassafrins and multiformins also displayed nematicidal activity against *Caenorhabditis elegans* (Table 3).[129]

These pigments are located in extremely high concentrations in granules directly beneath the stromatal surface, particularly in young stromata. This suggests that they may act as an outward directed chemical defense to protect the maturing teleomorphs. Being essential for survival of their producers, they co-evolved with morphological and biological features, and therefore may have taxonomic significance.[130]

The tricyclic cohaerins C–E, isolated in 2006 from *Hypoxylon* (=*Annulohydroxylon*) *cohaerens* (collected from decaying tree trunks of *Fagus sylvatica* near Niš city, Serbia and Montenegro), also proved to inhibit the production of nitric oxide (NO) stimulated by lipopolysaccharide (LPS) in RAW 264.7 cells and to be strong non-selective antimicrobials.[127,131]

On the other hand, chermesinones B (**211**) and C (**218**), were isolated in 2011 from the culture of the mangrove (*Kandelia candel*, collected from the South China Sea in Guangdong Province, China) endophytic fungus *Penicillium chermesinum* (ZH4-E2).[132]

Chermesinone B is the C-12 epimer of monochaetin (**212**), a natural product isolated in 1970 from *Monochaetia compta*, that was reformulated as a tricyclic lactonic azaphilone by Steyn and Vleggaar in 1986.[133]

Monascuskaolin (**214**) was isolated in 2012 from the ethanolic extract of the red yeast rice fermented with the yellow mutant of the fungus *Monascus kaoliang* BCRC 31506. The natural product also exhibited inhibition of NO production in LPS-stimulated RAW 264.7 macrophages *in vitro* (MIC = 7.62 μg mL^{-1}).[134]

Chaetomium sp. is the third most common indoor fungal contaminant of mouldy damp buildings and the largest saprophytic Ascomycetes genera. It is also a common colonizer of soil and cellulose-containing substrates. *Chaetomium* has been the source of various families of azaphilone natural products during the last quarter of century.

The chaetoviridins A–D were first isolated in 1990 from the soil strain *Chaetomium globosum flavo-viridae* by the group of Takahashi. Since then, chaetoviridins A (**213**) and B were also found in *Penicillium multicolor* FO-2338 and demonstrated to be antifungal agents against *Puccinia recondita* (wheat leaf rust). These were also found to be inhibitors of the CETP and of the growth (*in vitro*) of *Magnaporthe grisea* (rice blast) and *Pythium ultimum* mycelia, with MIC values of 1.23 and 33.3 μg mL^{-1}, respectively. Chaetoviridin A (**213**) also displayed inhibition activity in the 12-*O*-tetradecanoylphorbol-13-acetate model of induced inflammation in mice.[115a,135,136]

The chaetomugilins A–F (**224**, **226–230**), seco-chaetomugilins[137] as well as the dechlorinated derivatives dechloro-chaetomugilins[138] have been isolated since 2005 by several research groups, from various strains of *Chaetomium globosum* obtained from different sources.[136] Particularly relevant was the exhaustive study carried out by the group of Yamada, of products from *C. globosum* isolated from the marine fish *Mugil cephalus*, which unveiled most of this family members.[137,139]

Table 3 Activity of multiformins A–D and sassafrins A–C as inhibitors of NO production, antioxidants and nematicides

Compound	Inhibition of NO production (IC_{50}, μM)	Antioxidant activity (IC_{50}, μM)	Nematicidal activity (LD_{50}, μM)
Multiformin B	—	—	10
Multiformin C	—	—	10
Multiformin D	13.8	418.0	100
Sassafrin A	14.7	105.0	50
Sassafrin B	15.7	54.4	50
Sassafrin C	10.0	62.4	25

The seco-chaetomugilin A as well as the chaetomugilins A–F (Table 4) and K–O displayed selective cytotoxicity or growth inhibition activity against several tumor cell lines. In addition, chaetomugilin B (**226**) was also isolated from a strain of *C. globosum* found as an endophyte of *Gingko biloba*.[140]

Other members of this group have been isolated more recently. Collado, Pupo and co-workers greatly expanded in 2011 the amount of known chaetoviridins with their isolation of chaetoviridins G–I and several epimeric compounds from *Chaetomium globosum* isolated, in turn, from the leaves of *Viguiera robusta* (Asteraceae) and cultivated in PDB medium for 21 days.[141]

The structure of chaetoviridin A (**213**) was confirmed by a single-crystal X-ray diffraction analysis[141] and the absolute configurations of chaetoviridin D, 5′-epichaetoviridin A and 4′-epichaetoviridin A were determined employing Mosher's method.

The antibiotic activity of chaetoviridins was evaluated using an *in vivo Caenorhabditis elegans* infection model. Chaetoviridin A showed weak inhibitory activity of monoamine oxidase (IC_{50} = 0.012 μg mL^{-1}); it also proved to be an inductor of chlamidospore-like cells in *Cochliobolus lunatus* (40–50% at 100 μg $disc^{-1}$) as well as an inhibitor of the growth of *Pyricuralia oryzae* (2.5 μg mL^{-1}).[135a]

On the other hand, Chen and co-workers reported in 2012 the isolation of chaetomugilin S (**231**), 7,5′-bis-*epi*-chaetoviridin A (**232**), and 7-*epi*-chaetoviridin E (**233**) from *Chaetomium elatum* (Fig. 12). These compounds exhibited caspase-3 inhibitory activity.[142]

Caspase-3 (cysteine aspartyl-specific protease-3) is one of the executioners in the caspase-dependent apoptosis. The enzyme, which is activated in nearly every model of apoptosis, has been regarded as a promising therapeutic target for excessive apoptosis-related diseases (Alzheimer's, Huntington's, ischemic damage, and autoimmune disorders).

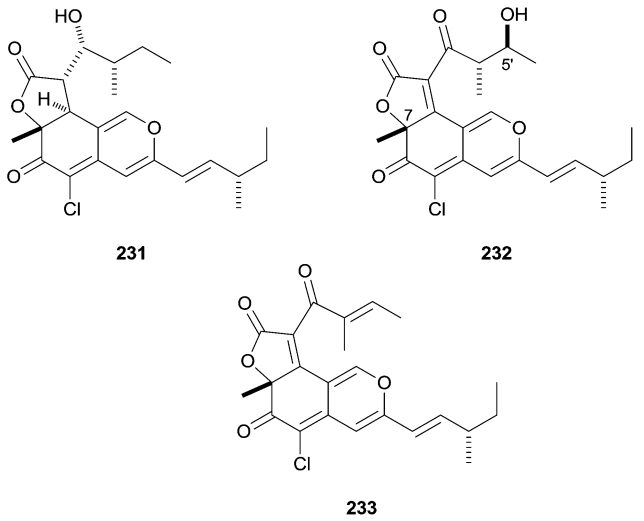


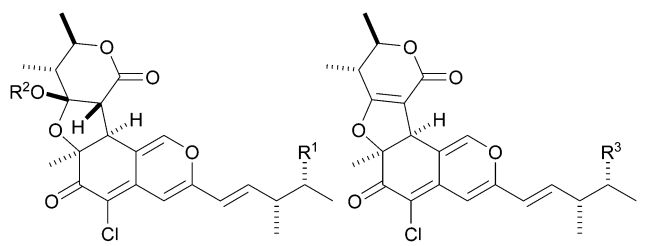
Fig. 12 Azaphilones which inhibit caspase 3.

Interestingly, according to the literature, *Chaetomium spp.* have yielded so far more than 30 azaphilones possessing a five-membered lactone ring C-7/C-8 fused to a chlorinated isochromen displaying a 7*S* absolute configuration.[111a] These are the first 7*R*-configurated azaphilones.

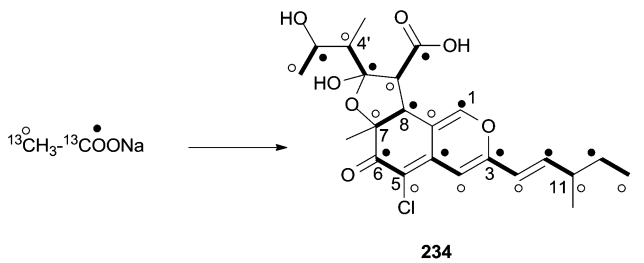
Biosynthetic studies on ochrephilone (**198**)[115e,143] and related compounds unveiled the polyketide origins of the azaphilones. These are essentially composed of two chains; the main chain which starts from the C-3 side chain and ends at C-1, and a subsidiary chain attached at the C-7 oxygen. The labelling pattern from [1,2-$^{13}C_2$]acetate was employed to ascertain the angular nature of this azaphilone. In these derivatives, it was demonstrated that the C-5 atom and the adjacent carbonyl carbon atom at C-6 are derived from the same acetate unit.

When the culture of *Chaetomium flavo-viridae* was exposed to sodium [1,2-$^{13}C_2$]acetate for the incorporation study, chaetoviridin B (**234**) and not chaetoviridin A (**213**), was isolated as the main metabolite.[135a] The ^{13}C-NMR of **234** enriched in ^{13}C exhibited the satellite doublet signals due to ^{13}C–^{13}C couplings at all carbon signals except those for the methyl groups attached at C-7, C-11 and C-4, which originated from C-1 units. The ^{13}C–^{13}C coupling constants indicated that the labelling pattern (Scheme 20) originated from two polyketide chains; the observed coupling of C-5 with the C-6 carbonyl carbon provided

Table 4 Cytotoxicity of the chaetomugillins A–F against P388 and HL-60 cell lines



Chaetomugillin	Compound	R^1	R^2	R^3	P388 IC_{50} (μM)	HL-60 IC_{50} (μM)
A	**224**	OH	H	—	8.7	7.3
B	**226**	OH	Me	—	18.7	16.5
C	**227**	—	—	OH	3.6	2.7
D	**228**	H	H	—	7.5	6.8
E	**229**	H	Me	—	15.7	13.2
F	**230**	—	—	H	3.3	1.3
	5-Fluorouracil	—	—	—	1.7	2.7



Scheme 20 Biosynthesis of chaetoviridin B and ^{13}C label distribution.

the key evidence for the angular nature of the structure of chaetoviridin B.

On the other hand, Tang and co-workers recently disclosed the identification and characterization of the *caz* biosynthetic cluster from *Chaetomium globosum* and the characterization of a highly reducing polyketide synthase (PKS) that acts in both a sequential and convergent manner with a nonreducing PKS to biosynthesize chaetomugilins and chaetoviridins. Their studies, involving genetic inactivation, assessed the involvement of individual *caz* genes in the biosynthesis of the azaphilones. In addition, through *in vitro* reconstitution, the *in vitro* synthesis of chaetoviridin A from cazisochromene, a pyranoquinone intermediate, using the highly reducing PKS and an acyltransferase was demonstrated.[144]

6.2 Synthetic efforts in the area of lactonic azaphilones and synthesis of similar tricycles

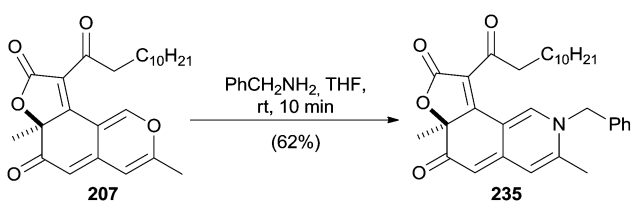
Although different approaches towards the synthesis of azaphilones have been reported in recent times,[145] only scattered efforts have been applied to the elaboration of these tricyclic heterocycles.[146] This may be a result of their high reactivity towards primary amines or because of their structural complexity.

The group of Porco Jr. has reported the preparation of the azaphilone bicyclic core by oxidation of a benzopyrylium salt with IBX[146] or through a buffer-mediated cycloisomerization of a vinylogous acid prepared from oxidative dearomatization of the corresponding o-alkynylbenzaldehyde derivative.[145f] Utilizing these methodologies, the azaphilone core was further employed as a scaffold for the preparation of a chemical library containing orthogonal diversification points.[147]

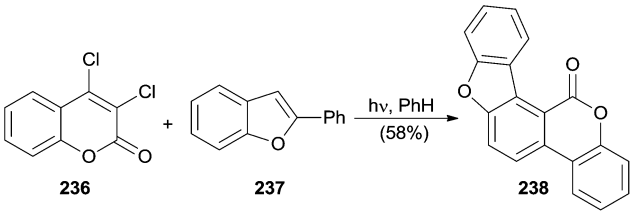
In an attempt to identify the structural requirements involved in the Hsp90 inhibitory activity and exploiting the well-known reactivity of azaphilones with amines, the group of Dallavalle[120] converted deflectin **207** into benzylamine derivative **236** (Scheme 21). The derivative exhibited no appreciable Hsp90 binding, like the parent azaphilones, thus confirming the detrimental effect on bioactivity of an angular structure.

Interestingly, the photoassisted naphthoannulation of 3,4-dichlorocoumarin (**236**) with 2-phenylbenzofurans, which involves radical cyclization and tandem electrocyclic reactions (Scheme 22), afforded 58% of polycyclic lactone **238**,[148] which shares the tricyclic furo[2,3-*h*]isochroman skeleton with the azaphilones.

This compound may be of interest from the point of view of its optoelectric properties; it is bright yellow (UV absorption



Scheme 21 Reactivity of azaphilones with amines.



Scheme 22 The photoassisted naphthoannulation of 3,4-dichloro-coumarin (**236**) with 2-phenylbenzofuran (**237**).

$\lambda_{max} = 403$ nm, $\varepsilon = 2.31 \times 10^4$ L mol^{-1} cm^{-1} in benzene) and strongly fluorescent ($\lambda_{em} = 441$ nm, $\phi_f = 0.21$) in the blue region.

7 Furo[3,4-*f*]chromans

7.1 Isolation of fuscinarin and related tricycles

Fuscinarin (**239**) is a novel pentaketide metabolite, recently isolated from the mitosporic fungus *Oidiodendron griseum*, a widespread fungus that has been isolated from wood pulp and soil,[149] together with the known fuscin (**240**), repeatedly isolated from *Oidiodendron* species[150] and 10-methoxydihydrofuscin (**241**).

These natural products (Fig. 13) effectively competed with the macrophage inflammatory protein 1R (MIP-1*R*) for binding

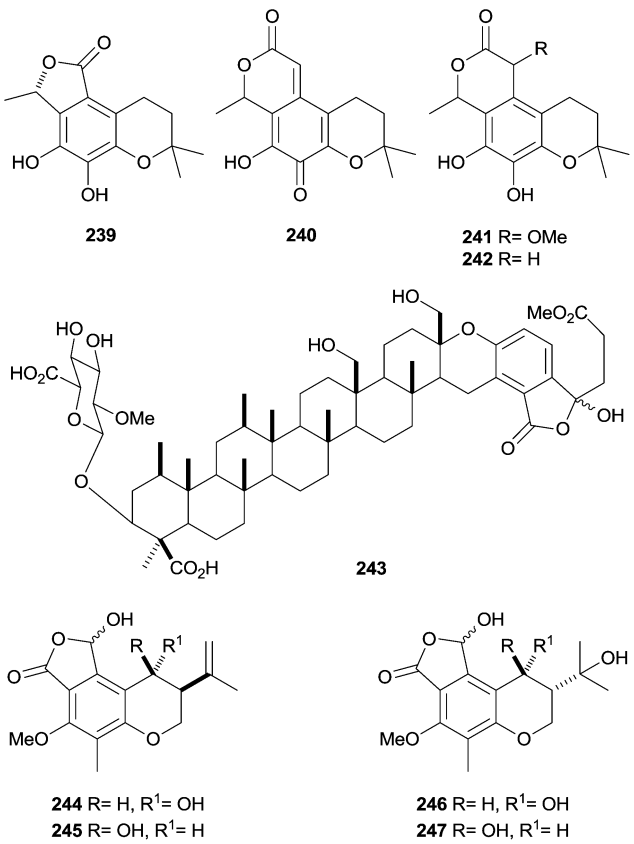


Fig. 13 Chemical structures of fuscinarin (**239**), fuscin (**240**), 10-methoxydihydrofuscin (**241**), dihydrofuscin (**242**), KS-505a (**243**) and tricycles **244–247**, from *A. duracaulis*.

to human chemokine receptor CCR5 in a scintillation proximity binding assay, exhibiting IC_{50} values of 80, 21 and 154 μmol L^{-1}, respectively. Human CCR5 is a G-coupled receptor that binds to the envelope proteins gp120 and CD4, and mediates the HIV-1 viral entry into the cells. The blockade of this binding by a small molecule receptor antagonist could lead to a new mode of action agent for HIV-1 and AIDS. On the other hand, fuscin (**240**), an inhibitor of mitochondrial SH-dependent transport-linked functions, has been repeatedly targeted for synthesis.[151]

Fuscinarin shares its tricyclic motif with the tetraterpenoid lactonic antibiotic KS-505a (**243**, longestin), isolated from *Streptomyces argenteolus* A-2. Compound **243** is an inhibitor of bovine brain Ca^{2+} and calmodulin-dependent cyclic-nucleotide phosphodiesterase and has effect on neurite formation in NG108-15 neuroblastoma × glioma hybrid cells. When administered intraperitoneally, the natural product exhibited *in vivo* anti-amnesia activity in an electroconvulsive shock-induced amnesia model in the rat.[152]

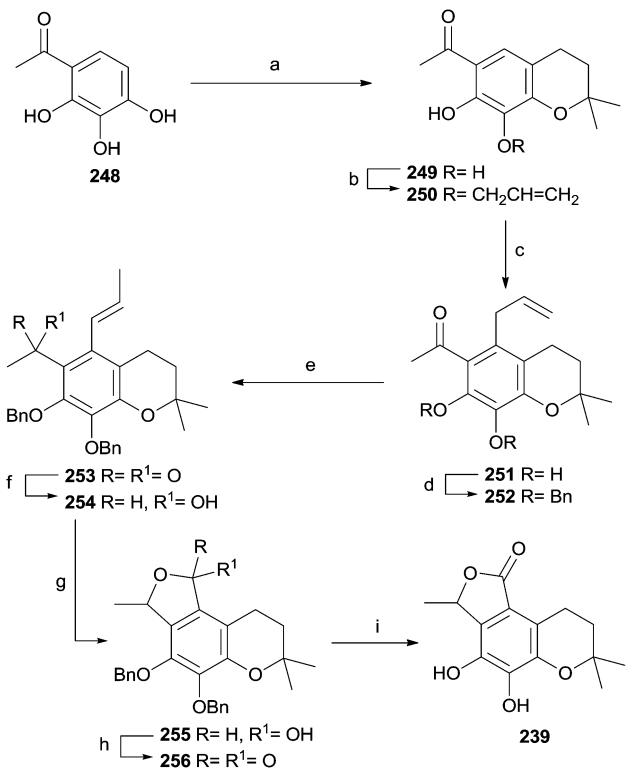
Compound **243** also exhibits antitrypanosomal activity *in vitro* and *in vivo* against *Trypanosoma brucei brucei* strain GUTat 3.1 and *T. brucei rhodesiense* strain STIB900 (IC_{50} = 1.03 and 1.66 μg mL^{-1}, respectively), with selectivity indices of 26.5 and 16.5, respectively, in the acute mouse model. Data suggest that KS-505a is possibly a new candidate compound for discovering more potent new antitrypanosomal drugs.[153]

On the other hand, the four optically inactive diastereomeric chromanols **244–247** were found in *Aspergillus duracaulis*, as probable metabolic derivatives of 5-*O*-isopentenyl cyclopaldic acid.[154] Compound **245** was also isolated from submerged cultures of the Ascomycete *Lachnum papyraceum* (Karst.) Karst (Hyaloscyphaceae) and shown to possess antibiotic activity.[155]

7.2 Total synthesis of fuscinarin

The group of Zi-Xiang recently reported the first total synthesis of fuscinarin (**239**) from gallacetophenone (**248**).[156] The starting ketone (Scheme 23) was reacted with 2-methyl-3-buten-2-ol in refluxing 80% HCOOH to afford 86% of chroman **249**;[157] in turn, this was selectively transformed into the monoallyl ether **250** in 88% yield and further converted into **251** by means of a conventional *para*-Claisen rearrangement (140 °C, 6 days, 97% yield). Conducting the reaction under microwave irradiation for 5 min also furnished the product in 96% yield.[158]

The catechol **251** was protected as the bis-benzyl ether **252** in 93% yield, which was isomerized to the *trans* β-methylstyrene **253** in 96% yield, after treatment with NaOH under phase transfer catalysis.[159] Reduction of the carbonyl moiety with $NaBH_4$ afforded alcohol **252** in 91% yield, the ozonolysis of which gave moderate yield of lactol **255** after quenching with SMe_2.[160] PCC/Al_2O_3 oxidation of the lactol resulted in 90% of lactone **256**, which once subjected to hydrogenolytic debenzylation afforded fuscinarin almost quantitatively.



Scheme 23 *Reagents and conditions*: (a) 2-methyl-3-buten-2-ol; 80% HCO_2H, reflux, 8h (86%); (b) $BrCH_2CH{=}CH_2$, K_2CO_3, acetone, rt, overnight (88%); (c) MW, 5 min, 100 °C (96%); (d) BnBr, acetone, K_2CO_3, reflux, 6h (93%); (e) TBAB, $NaOH_{aq.}$, PhMe, 75 °C (96%); (f) $NaBH_4$, EtOH, 8h (91%); (g) O_3, CH_2Cl_2, −78 °C, Me_2S (65%); (h) PCC/Al_2O_3, CH_2Cl_2, rt (90%); (i) 5% Pd/C, H_2, EtOH, rt (98%).

8 Microsphaerophthalide G, a novel furo [3,4-*f*] benzo[5,6-*e*][1,3]dioxine

Microsphaerophthalide G (**257**) is one of the phthalides recently isolated from the endophytic fungus *Microsphaeropsis arundinis* PSU-G18, in turn isolated from the leaves of *Garcinia hombroniana*.[58] Compound **257** (Fig. 14) was isolated in minor amounts, together with several other phthalide derivatives, among them the related phenols **258** and **259**. The (*S*)-configuration was assigned based on its specific optical rotation ($[\alpha]_D^{28}$ −40.9), compared with other 3-substituted naturally-occurring phthalides. Interestingly, despite more than 180 phthalides having been reported to date,[161] the 3-oxygenated phthalides[162] are rare natural products.[163]

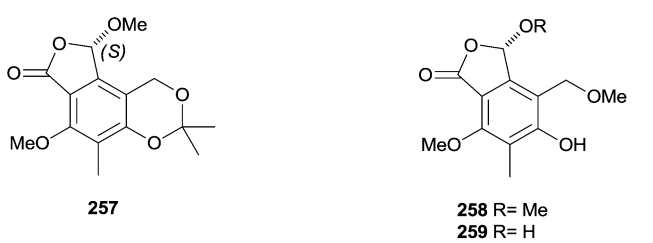


Fig. 14 Chemical structures of microsphaerophthalide G (**257**) and related phtalides (**258** and **259**).

Table 5 Summary of the bioactivity data of the naturally-occurring angular tricyclic benzofuran derivatives of fungal origin of interest

Compound	Activity	Potency	Ref.
Pergillin (**7**)	Plant growth inhibitor (etiolated wheat coleoptile assay)	Significant inhibition ($p < 0.01$) at 100 μM	16
Pseudodeflectusin (**12**)	Cytotoxic towards human cancer cell lines	$LD_{50} = 39$ μM (HL-60 cells)	18
TMC-120A (**65**)	Inhibitor of interleukin-5 mediated prolongation of eosinophil survival	$IC_{50} = 13.7$ μM	43
	Enhancer of the acute inflammatory response in primary mouse alveolar macrophages	Statistically-significant changes in gene transcription of IL1β, IL1r1, IL4 and IL10	48*a*,*b*
TMC-120B (**23**)	Inhibitor of interleukin-5 mediated prolongation of eosinophil survival	$IC_{50} = 2.0$ μM	43
	Inhibitor of the production of interferon-γ induced by stimulating ovalbumin (OVA)–specific murine T cells with an OVA peptide antigen (30 μg mL^{-1})	81% Inhibition at a 3 μM concentration	46
Aspergillitine (**131**) (TMC-120B, **23**)	Antimicrobial	Moderate activity against *Bacillus subtilis*	68*a*
Concentricolide (**151**)	Inhibitor of HIV-1-induced cytopathic effects	$EC_{50} = 0.31$ μg mL^{-1}	84
	Blocker on syncytium formation between HIV-1 infected cells and normal cells	$EC_{50} = 0.83$ μg mL^{-1}	84
Ochrephilone (**198**)	Rabbit endothelin A receptor binding	$IC_{50} = 26$ μM	116
	Antitrypanosomal (*T. Brucei brucei* GUTat 3.1)	$IC_{50} = 1.02$ μg mL^{-1}	117
	Binding inhibitors of gp120-CD4	$IC_{50} = 114$ μM	115*a*
Rubrorotiorin (**204**)	Inhibitor of cholesteryl ester transfer protein	$IC_{50} = 41.1$ μM	115*d*
	Antifungal against *Candida albicans*	$IC_{50} = 0.6$ μg mL^{-1}	118*a*
	Binding inhibitors of gp120-CD4	$IC_{50} = 240$ μM	115*a*
(+)-5-Bromo-ochrephilone (**200**)	Binding inhibitor of gp120-CD4	$IC_{50} = 2.5$ μM	115*a*
(+)-Isorotiorin (**201**)	Binding inhibitor of gp120-CD4	$IC_{50} = >260$ μM	115*a*
Isochromophilone I (**199**)	Binding inhibitor of gp120-CD4	$IC_{50} = 6.6$ μM	115*a*
	Antitrypanosomal (*T. Brucei brucei* GUTat 3.1)	$IC_{50} = 0.21$ μg mL^{-1}	117
Tetrahydro-isochromophilone (**203**)	Binding inhibitor of gp120-CD4	$IC_{50} = >260$ μM	115*a*
5-Chloroisorotiorin (**202**)	Rat endothelin B receptor binding	$IC_{50} = 8$ μM	116
Chaetoviridin A (**213**)	Inhibitor of Monoamine oxidase	$IC_{50} = 0.012$ μg mL^{-1}	135*a*
	Inhibitor of cholesteryl ester transfer protein	$IC_{50} = 31.6$ μM	115*d*
	Antifungic (plant pathogens) – growth inhibitor of the fungi *Puccinia recondita* (leaf rust)	83% Inhibition at 62.5 μg mL^{-1}	136
	Magnaporthe grisea (*Pyricularia oryzae*, rice blast)	MIC = 1.23 μg mL^{-1}	136
	Pythium ultimum mycelia	MIC = 1.23 μg mL^{-1}	136
	Pyricularia oryzae	Active at 2.5 μg mL^{-1}	135*a*
	Anti-inflammatory (TPA-induced inflammation)	$IC_{50} = 0.6$ μM	135*b*
	Inductor of chlamidospore-like cells in *Cochliobolus lunatus*	Weak (40–50% at 100 μg/disc)	135*a*
Deflectin A (1b) (**205**)	Antibacterial	Weak-moderate. Reversed by addition of serum	119
	Antifungal	Weak	120
	Cytotoxic against Ehrlich carcinoma cells of mice	Incorporation of thymidine: 6% at 10 μg mL^{-1}	119
	Lytic towards bacteria and RBC	100% Lysis at 20 μg mL^{-1}	119
RP 1551-1 (**208**)	Antimicrobial against *B. subtilis*, *E. faecium* and *S. aureus*	Weak	124
	Binding inhibitor of platelet-derived growth factor (PDGF) AA to the	$IC_{50} = 1.7$ μM	124

Table 5 (*Contd.*)

Compound	Activity	Potency	Ref.
	extracellular domain of the PDGF α-receptor		
Chaetomugillins A–F (**224, 226–230**)	Cytotoxic against P388 and HL-60 cell lines	For IC_{50} values, see Table 2	139*b*
Chaetomugilin S (**231**)	Inhibitor of caspase 3	$IC_{50} = 20.6$ μM	142
7,5′-*bis*-*epi*-Chaetoviridin A (**232**)	Inhibitor of caspase 3	$IC_{50} = 10.9$ μM	142
7-*epi*-Chaetoviridin E (**233**)	Inhibitor of caspase 3	$IC_{50} = 7.9$ μM	142
Trichoflectin (**215**)	Antimicrobial	Moderate	122*a*
	Inhibitor of fungal DHN-melanin biosynthesis	Moderate	122*a*
Sassafrin A (**216**)	Antioxidant (DPPH)	$IC_{50} = 105$ μM	127
	Nematicidal (against *Caenorhabditis elegans*)	$LD_{50} = 50$ μM	129*a*
	Antimicrobial (against *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. enteritidis*)	Moderate to strong	129*b*
	Inhibitor of NO production in LPS-stimulated RAW 264.7 macrophages	$IC_{50} = 14.7$ μM	126
	Antifungic (against *A. niger* and *C. Albicans*)	Relatively strong	126
Sassafrin B (**217**)	Antioxidant (DPPH)	$IC_{50} = 54.4$ μM	127
	Nematicidal (against *Caenorhabditis elegans*)	$LD_{50} = 50$ μM	129*a*
	Antimicrobial (against *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. enteritidis*)	Moderate to strong	129*b*
	Inhibitor of NO production in LPS-stimulated RAW 264.7 macrophages	$IC_{50} = 15.7$ μM	126
	Antifungal (against *A. niger* and *C. Albicans*)	Relatively strong	126
Multiformin B (**220**)	Antifungal (*A. Niger and C. albicans*)	Comparable to nystatin	128*b*
	Antimicrobial (against *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. enteritidis*)	Moderate	128*b*
Multiformin C (**221**)	Antifungic (*A. Niger and C. albicans*)	Comparable to nystatin	128*b*
	Antimicrobial (against *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. enteritidis*)	Antimicrobial	128*b*
Multiformin D (**222**)	Inhibitor of NO production in LPS-stimulated RAW 264.7 macrophages	$IC_{50} = 13.8$ μM	127
	Antioxidant (DPPH)	$IC_{50} = 418$ μM	127
	Nematicidal (against *Caenorhabditis elegans*)	$LD_{50} = 100$ μM	129*b*
	Antimicrobial (bacteriostatic and fungistatic)	Moderate	128*b*
Monascuskaolin (**214**)	Inhibitor of NO production in LPS-stimulated RAW 264.7 macrophages	$IC_{50} = 7.62$ μg mL^{-1}	134
Fuscinarin (**239**)	Competitor with the macrophage MIP-1R for binding to human CCR5	$IC_{50} = 80$ μM	149, 156
245	Antimicrobial	(inactive after Achenbach and co-workers[154*a*])	155
KS-505a (**243**)	Antitrypanosomal (acute mouse model)		
	T. brucei brucei GUTat 3.1 (*in vitro*)	$IC_{50} = 1.03$ μg mL^{-1}; Selectivity Index = 26.5	153
	T. brucei rhodesiense STIB900 (*in vivo*)	$IC_{50} = 1.66$ μg mL^{-1}; Selectivity Index = 16.5	153
	Inhibitor of bovine brain Ca^{2+} and calmodulin-dependent cyclic-nucleotide phosphodiesterase	$IC_{50} = 0.065$ μM	152*a*
	Effect on neurite formation in NG108-15 neuroblastoma × glioma hybrid cells	Noticeable at 90 μM	152*b*

9 Summary of biological activity

Table 5 summarizes the most relevant findings among the studied families of naturally-occurring tricyclic heterocycles, referring to their bioactivity. From the wide array of activities reported, the magnitude of the current lack of knowledge about this topic can be inferred.

Many members were isolated as a result of the search for some specific bioactivity (bioguided fractionation of extracts), while others were obtained by studying phylogenetically related fungi, which had yielded structurally similar compounds.

As a consequence, different members of the already unveiled set of naturally-occurring angular tricyclic heterocycles of fungal origin, have been shown to possess activity as antioxidants, enzymatic inhibitors of monoamino oxidase (MAO), cholesteryl ester transfer protein (CETP), the bovine brain Ca^{2+} and calmodulin-dependent cyclic-nucleotide phosphodiesterase and the fungal DHN-melanin biosynthesis, as well as of the production of nitric oxide.

They were demonstrated to interact with endothelin A and B, the platelet-derived growth factor (PDGF), gp120-CD4 and chemokine receptor 5 (CCR5), competing with the binding of other substrates. Some were also shown to exhibit activity as antiviral (HIV-1), antimicrobials, antifungals (against plant and human disease-causing species), cytotoxics against various cancer cell lines, bacteriolytics, hemolytics and antiparasitaries (including as nematicidal and antitrypanosomal). Other activities relate to their ability to act as modulators of the inflammatory response, neurite formation and plant growth.

10 Conclusions

In conclusion, fungi are a rich source of chemical diversity, being the source of many drugs and other useful compounds. As only a small part of the mycota is known and most fungi produce several unknown metabolites, fungi are still one of the most promising sources for new lead compounds.

Fungi-derived naturally-occurring angular tricyclic benzofurans in which two heterocyclic rings are fused to a central six-membered motif are a continuously growing set of families of compounds. They were isolated mostly during the last 15 years from a handful of fungal species growing in different environments around the world, and exhibit a wide range of interesting biological activities, many of them potentially useful.

Some of these tricycles have been the subjects of total syntheses for structural or stereochemical confirmation and biological activity examination, while others still await synthetic and bioactivity studies. It is expected that in the near future, more compounds sharing the angular tricyclic benzofuran motif will be isolated and evaluated as candidates for new developments in the areas of chemistry and biology.

11 Acknowledgements

The authors gratefully acknowledge CONICET, ANPCyT and SECyT-UNR for financial support. SOS thanks CONICET for his fellowship.

12 Notes and references

1 E. P. Feofilova, *Appl. Biochem. Microbiol.*, 2001, **37**, 124–137.
2 R. Ebel, Natural Product Diversity from Marine Fungi, *Compr. Nat. Prod. II*, 2010, **2**, 223–262.
3 (*a*) D. L. Hawksworth and A. Y. Rossman, *Phytopathology*, 1997, **87**, 888–891; (*b*) E. M. Fox and B. J. Howlett, *Curr. Opin. Microbiol.*, 2008, **11**, 481–487.
4 (*a*) M. S. Butler, *J. Nat. Prod.*, 2004, **67**, 2141–2153; (*b*) M. S. Butler, *Nat. Prod. Rep.*, 2005, **22**, 162–195; (*c*) M. Misiek and D. Hoffmeister, *Planta Med.*, 2007, **73**, 103–115.
5 (*a*) R. B. Williams, J. C. Henrikson, A. R. Hoover, A. E. Lee and R. H. Cichewicz, *Org. Biomol. Chem.*, 2008, **6**, 1895–1897; (*b*) Fungal Secondary Metabolism. Methods and Protocols In *Methods in Molecular Biology*, Vol. 944; N. P. Keller and G. Turner (ed.), Springer, Heidelberg, Germany, 2013; (*c*) A. A. Brakhage and V. Schroeckh, *Fungal Genet. Biol.*, 2011, **48**, 15–22.
6 N. Khaldi, F. T. Seifuddin, G. Turner, D. Haft, W. C. Nierman, K. H. Wolfe and N. D. Fedorova, *Fungal Genet. Biol.*, 2010, **47**, 736–741.
7 K. Duarte, T. A. P. Rocha-Santos, A. C. Freitas and A. C. Duarte, *TrAC, Trends Anal. Chem.*, 2012, **34**, 97–110.
8 P. Bhadury, B. T. Mohammad and P. C. J. Wright, *J. Ind. Microbiol. Biotechnol.*, 2006, **33**, 325–337.
9 D. J. Newman and G. M. Cragg, *J. Nat. Prod.*, 2007, **70**, 461–477.
10 (*a*) S. Bräse, A. Encinas, J. Keck and C. F. Nising, *Chem. Rev.*, 2009, **109**, 3903–3990; (*b*) S. C. Bassapa, *Aflatoxins: Formation, Analysis and Control*, Alpha Science International Ltd., Oxford, UK, 2009.
11 H. G. Cutler, F. G. Crumley, J. P. Springer, R. H. Cox, R. J. Cole, J. W. Dorner and E. Thean, *J. Agric. Food Chem.*, 1980, **28**, 989–991.
12 Z. Lu, Y. Wang, C. Miao, P. Liu, K. Hong and W. Zhu, *J. Nat. Prod.*, 2009, **72**, 1761–1767.
13 G. J. Slack, E. Puniani, J. C. Frivstad, R. A. Samson and J. D. Miller, *Mycol. Res.*, 2009, **13**, 480–490.
14 (*a*) J. Houbraken, M. Due, J. Varga, M. Meijer, J. C. Frisvad and R. A. Samson, *Studies in Mycology*, 2007, **59**, 107–128; (*b*) R. A. Samson, J. Varga, M. Meijer and J. C. Frisvad, *Studies in Mycology*, 2011, **69**, 81–97.
15 S. A. Ahmed, F. E. Scott, D. J. Stenzel, T. J. Simpson, R. N. Moore, L. A. Trimble, K. Arai and J. C. Vederas, *J. Chem. Soc., Perkin Trans. 1*, 1989, 807–816.
16 H. G. Cutler, F. G. Crumley, J. P. Springer and R. H. Cox, *J. Agric. Food Chem.*, 1981, **29**, 981–983.
17 W. Ayer and L. M. Peña–Rodríguez, *J. Nat. Prod.*, 1987, **50**, 408–417.
18 A. Ogawa, C. Murakami, S. Kamisuki, I. Kuriyama, H. Yoshida, F. Sugawara and Y. Mizushina, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 3539–3543.
19 H. J. Shao, X. D. Qin, Z. J. Dong, H. B. Zhang and J. K. Liu, *J. Antibiot.*, 2008, **61**, 115–119.
20 F. Saito, K. Kuramochi, A. Nakazaki, Y. Mizushina, F. Sugawara and S. Kobayashi, *Eur. J. Org. Chem.*, 2006, 4796–4799.

21 P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney and M. R. Boyd, *J. Natl. Cancer Inst.*, 1990, **82**, 1107–1112.

22 T. Mosmann, *J. Immunol. Methods*, 1983, **65**, 55–63.

23 K. Trisuwan, V. Rukachaisirikul, Y. Sukpondma, S. Phongpaichit, S. Preedanon and J. Sakayaroj, *Tetrahedron*, 2010, **66**, 4484–4489.

24 T. Kumemura, T. Choshi, C. Hirata, M. Sera, Y. Takahashi, J. Nobuhiro and S. Hibino, *Heterocycles*, 2003, **61**, 13–17.

25 (*a*) P. S. Steyn and C. W. Holzapfel, *Tetrahedron*, 1967, **23**, 4449–4461; (*b*) K. J. van der Merwe, P. S. Steyn, L. Fourie, D. B. Scott and J. J. Theron, *Nature*, 1965, **205**, 1112–1113.

26 K. J. Van der Merwe, P. S. Steyn and L. Fourie, *J. Chem. Soc.*, 1965, 7083–7088.

27 B.-S. Kwon and J. Tae, *Heterocycles*, 2004, **62**, 137–141.

28 K. van Laak and H.-D. Scharf, *Tetrahedron*, 1989, **45**, 5511–5516.

29 (*a*) E. J. Corey, N. W. Gilman and B. E. Ganem, *J. Am. Chem. Soc.*, 1968, **90**, 5616–5617; (*b*) G. Lai and W. K. Anderson, *Synth. Commun.*, 1997, **27**, 1281–1287.

30 (*a*) G. A. Kraus, *J. Org. Chem.*, 1981, **46**, 201–202; (*b*) Y. Sasaki, T. Fujita, K. Okazaki, K. Kamata and S. Tobinaga, *Heterocycles*, 1992, **33**, 357–374; (*c*) C. D. Gill and M. Donner, *Aust. J. Chem.*, 2002, **55**, 213–217; (*d*) A. McClay, H. Van Den Berg, P. Johnston, W. Watters, K. McGarell, D. Waugh, P. Armstrong, Z. Delbederi, C. Higgins and T. Mills, WO2006046071; Chem. Abstr., 2006, **144**, 450616 and others cited therein.

31 Y. Takeuchi, I. Watanabe, K. Misumi, M. Irea, Y. Hirose, K. Hirata, M. Yamato and T. Harayama, *Chem. Pharm. Bull.*, 1997, **45**, 2011–2015.

32 T. A. Munro, M. A. Rizzacasa, B. L. Roth, B. A. Toth and F. Yan, *J. Med. Chem.*, 2005, **48**, 345–348.

33 M. Tobe, T. Tashiro, M. Sasaki and H. Takikawa, *Tetrahedron*, 2007, **63**, 9333–9337.

34 C. H. Lin and D. L. Alexander, *J. Org. Chem.*, 1982, **47**, 615–620.

35 A. Covarrubias–Zúñiga and E. Ríos–Barrios, *J. Org. Chem.*, 1997, **62**, 5688–5689.

36 J. P. Schaefer and J. J. Bloomfield, *Org. React.*, 1967, **15**, 1–203.

37 T. Maegawa, K. Otake, K. Hirosawa, A. Goto and H. Fujioka, *Org. Lett.*, 2012, **14**, 4798–48801.

38 Y. Sato, K. Kuramochi, T. Suzuki, A. Nakazaki and S. Kobayashi, *Tetrahedron Lett.*, 2011, **52**, 626–629.

39 H. Okamura, T. Iwagawa and M. J. Nakatani, *J. Synth. Org. Chem. Jpn.*, 1999, **57**, 84–91.

40 M. Mori, N. Kaneta, N. Isono and M. Shibasaki, *J. Organomet. Chem.*, 1993, **455**, 255–260.

41 K. Kuramochi, F. Saito, A. Nakasaki, T. Takeuchi, K. Tsubaki, F. Suagawara and F. Kobayashi, *Biosci., Biotechnol., Biochem.*, 2010, **74**, 1635–1640.

42 T. Adachi, S. Motojima, A. Hirata, T. Fukuda and S. Makino, *Am. J. Respir. Crit. Care Med.*, 1995, **151**, 618–623.

43 J. Kohno, M. Sakurai, N. Kameda, M. Nishio, K. Kawano, N. Kishi, T. Okuda and S. Komatsubara, *J. Antibiot.*, 1999, **52**, 913–916.

44 J. Kohno, H. Hiramatsu, M. Nishio, M. Sakurai, T. Okuda and S. Komatsubara, *Tetrahedron*, 1999, **55**, 11247–11252.

45 R. Royer, J. Guillaumel, P. Demerseman, N. Platzer and J. P. Buisson, *Bull. Soc. Chim. Fr.*, 1972, **11**, 4201–4208.

46 T. Choshi, T. Kumemura, H. Fujioka, Y. Hieda and S. Hibino, *Heterocycles*, 2012, **84**, 587–595.

47 J. M. Robertson, P. E. Jensen and B. D. Evavold, *J. Immunol.*, 2000, **164**, 4706–4712.

48 (*a*) T. G. Randa, J. DiPenta, C. Robbins and J. D. Miller, *Chem.-Biol. Interact.*, 2011, **190**, 139–147; (*b*) J. D. Miller, M. Sun, A. Gilyan, J. Roy and T. G. Rand, *Chem.-Biol. Interact.*, 2010, **183**, 113–124.

49 K. F. Nielsen and J. Smedsgaard, *J. Chromatogr., A*, 2003, **1002**, 111–136.

50 Y. Yamamoto, Y. Kinoshita, G. R. Thor, M. Hasumi, K. Kinoshita, K. Koyama, K. Takahashi and I. Yoshimura, *Phytochemistry*, 2002, **60**, 741–745.

51 K. Kinoshita, Y. Yamamoto, K. Koyama, K. Takahashi and I. Yoshimura, *Tetrahedron Lett.*, 2003, **44**, 8009–8011.

52 K. Kinoshita, Y. Yamamoto, K. Takatori, K. Koyama, K. Takahashi, K.-I. Kawai and I. Yoshimura, *J. Nat. Prod.*, 2005, **68**, 1723–1727.

53 T. Kumemura, T. Choshi, A. Hirata, M. Sera, Y. Takahashi, J. Nobuhiro and S. Hibino, *Chem. Pharm. Bull.*, 2005, **53**, 393–397.

54 (*a*) M. Miyake and Y. Fujimoto, *Chem. Lett.*, 1993, 1683–1686; (*b*) M. Miyake, Y. Hanaoka, Y. Fujimoto, Y. Sato, N. Takemoto, I. Yokota and Y. Yoshiyama, *Heterocycles*, 1996, **43**, 665–674.

55 M. Pocci, V. Bertini, F. Lucchesini, A. De Munno, N. Picci, F. Iemma and S. Alfei, *Tetrahedron Lett.*, 2001, **42**, 1351–1354.

56 (*a*) T. Choshi, T. Kumemura, J. Nobuhiro and S. Hibino, *Tetrahedron Lett.*, 2008, **49**, 3725–3728; (*b*) Y. Ishihara, S. Azuma, T. Choshi, K. Kohno, K. Ono, H. Tsutsumi, T. Ishizu and S. Hibino, *Tetrahedron*, 2011, **67**, 1320–1333.

57 S. Danishefsky and S. J. Etheredge, *J. Org. Chem.*, 1978, **43**, 4604–4605.

58 U. Sommart, V. Rukachaisirikul, K. Tadpetch, Y. Sukpondma, S. Phongpaichit, N. Hutadilok-Towatana and J. Sakayaroj, *Tetrahedron*, 2012, **68**, 10005–10010.

59 R. C. Shah and M. C. Laiwalla, *J. Chem. Soc.*, 1938, 1828–1832.

60 J. P. Beck, A. D. Pechulis and A. E. Harms, US Patent 7,084,152, 2006; Chem. Abstr., **136:**, 118393.

61 P. Roy and B. K. Ghorai, *Tetrahedron Lett.*, 2011, **52**, 251–253.

62 A. Numata, Y. Kondo and T. Sakamoto, *Synthesis*, 1999, 306–311.

63 (*a*) K. H. Dötz and P. Tomuschat, *Chem. Soc. Rev.*, 1999, **28**, 187–198; (*b*) Y. Zhang, D. Candelaria, Herndon, J. W. Y. Zhang, D. Candelaria and J. W. Herndon, *Tetrahedron Lett.*, 2005, **46**, 2211–2214.

64 J. Zhang, Y. Zhang, W. F. Schnatter and J. W. Herndon, *Organometallics*, 2006, **25**, 1279–1284.

65 (*a*) P. Fossa, L. Mosti, G. Menozzi, C. Marzano, F. Baccichetti and F. Bordin, *Bioorg. Med. Chem.*, 2002, **10**, 743–751; (*b*) A. Chilin, C. Marzano, F. Baccichetti, M. Simonato and A. Guiotto, *Bioorg. Med. Chem.*, 2003, **11**, 1311–1318; (*c*) C. Marzano, A. Chilin, F. Baccichetti,

F. Bettio, A. Guiotto, G. Miolo and F. Bordin, *Eur. J. Med. Chem.*, 2004, **39**, 411–419.
66 T. I. Mukhanova, L. M. Alekseeva and V. G. Granik, *Chem. Heterocycl. Compd.*, 2002, **38**, 586–589.
67 W. H. Lin, H. Z. Fu and P. Proksch, *Chin. Chem. Lett.*, 2001, **12**, 235–238.
68 (*a*) W. H. Lin, G. Brauers, R. Ebel, V. Wray, A. Berg, Sudarsono and P. Proksch, *J. Nat. Prod.*, 2003, **66**, 57–61; (*b*) P. Proksch, R. Ebel, R. A. Edrada, P. Schupp, W. H. Lin, Sudarsono, V. Wray and K. Steube, *Pure Appl. Chem.*, 2003, **75**, 343–352.
69 (*a*) S. B. Singh, D. L. Zink, G. F. Bills, A. Teran, K. C. Silverman, R. B. Lingham, P. Felock and D. J. Hazuda, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 713–717; (*b*) Y. Takenaka, Y. Tanahashi, N. Nagakura and N. Hamada, *Heterocycles*, 2000, **53**, 1589–1593; (*c*) Y. H. Kuo and B. Y. Lin, *Chem. Pharm. Bull.*, 1999, **47**, 428–429; (*d*) D. Wu, M. Zhang, C. Zhang and Z. Wang, *Biochem. Syst. Ecol.*, 2008, **36**, 219–222; (*e*) Q. Wang and D. Chen, *Helv. Chim. Acta*, 2007, **90**, 2432–2437; (*f*) K. Koyama and S. Natori, *Chem. Pharm. Bull.*, 1987, **35**, 578–584.
70 (*a*) K. S. Rehder and J. A. Kepler, *Synth. Commun.*, 1996, **26**, 4005–4021; (*b*) F. Zamattio, J. D. Brion, L. Belachmi and G. Le Baut, *J. Heterocycl. Chem.*, 1991, **28**, 2013–2019; (*c*) D. Yu, C.-H. Chen, A. Brossi and K.-H. Lee, *J. Med. Chem.*, 2004, **47**, 4072–4082; (*d*) N. R. Ayyangar, R. A. Khan and V. H. Deahpande, *Tetrahedron Lett.*, 1988, 2347–2348; (*e*) L. W. McGarry and M. R. Detty, *J. Org. Chem.*, 1990, **55**, 4349–4356.
71 C. Riva, C. De Toma, L. Donadel, C. Boi, R. Pennini, G. Motta and A. Leonardi, *Synthesis*, 1997, 195–201.
72 S. Kirkiacharian, A. T. Lormier, H. Chidiack and F. Bouchoux, *Farmaco*, 2004, **59**, 981–986.
73 (*a*) J. R. Keeffe, A. J. Kresge and N. P. Schepp, *J. Am. Chem. Soc.*, 1990, **112**, 4862–4868; (*b*) Y. Chiang, A. J. Kresge, P. A. Walsh and Y. Yin, *J. Chem. Soc., Chem. Commun.*, 1989, 869–871.
74 K. B. Bahnck and S. D. Rychnovsky, *J. Am. Chem. Soc.*, 2008, **130**, 13177–13181.
75 (*a*) C. W. Y. Chung and P. H. Toy, *Tetrahedron*, 2005, **61**, 709–715; (*b*) B. F. Zhong and F. E. McDonald, *Org. Lett.*, 2005, **7**, 3617–3620.
76 Y.-S. Yao and Z.-J. Yao, *J. Org. Chem.*, 2008, **73**, 5221–5225.
77 (*a*) R. Bautista, P. Bernal, L. E. Montiel, F. Delgado and J. Tamariz, *Synthesis*, 2011, 929–933; (*b*) T. Shirasaka, Y. Takuma and N. Imaki, *Synth. Commun.*, 1990, **20**, 1213–1221; (*c*) Q. Yao and M. Sheets, *J. Org. Chem.*, 2006, **71**, 5384–5387.
78 J. M. Saá, G. Martorell and A. García-Raso, *J. Org. Chem.*, 1992, **57**, 678–685.
79 G. Martorell, A. García-Raso and J. M. Saá, *Tetrahedron Lett.*, 1990, **31**, 2357–2358.
80 (*a*) H. Tsutsui and K. Narasaka, *Chem. Lett.*, 2001, 526–527; (*b*) M. Kitamura and K. Narasaka, *Chem. Rec.*, 2002, **2**, 268–277.
81 D. Gauthier, A. T. Lindhart, E. P. Olsen, J. Overgaard and T. Skrydstrup, *J. Am. Chem. Soc.*, 2010, **132**, 7998–8009.
82 C. C. Silveira, E. L. Larghi, S. R. Mendes, A. B. J. Bracca, F. Rinaldi and T. S. Kaufman, *Eur. J. Org. Chem.*, 2009, 4637–4645.
83 A. M. Sarotti and S. C. Pellegrinet, *J. Org. Chem.*, 2012, **77**, 6059–6065.
84 X. D. Qin, Z. J. Dong, J. K. Liu, L. M. Yang, R. R. Wang, Y. T. Zheng, Y. Lu, Y. S. Wu and Q. T. Zheng, *Helv. Chim. Acta*, 2006, **89**, 127–133.
85 (*a*) J.-K. Liu, Y. T. Zheng, X. D. Qin, L. M. Yang, Z. J. Dong, R. R. Wang and J. Tan, J. *US Patent*, 7,659,308, 2010; Chem. Abstr., **142:**, 39220; (*b*) J.-K. Liu, *Drug Discov. Ther.*, 2007, **1**, 94–103.
86 (*a*) H. B. Bode, B. Bethe, R. Hoefs and A. Zeeck, *ChemBioChem*, 2002, **3**, 619–627; (*b*) R. Hoefs, M. Walker and A. Zeeck, *Angew. Chem., Int. Ed.*, 2000, **39**, 3258–3261.
87 (*a*) M. Stadler, M. Baumgartner, T. Grothe, A. Mühlbauer, S. Seip and H. Wollweber, *Phytochemistry*, 2001, **56**, 787–793; (*b*) D. N. Quang, T. Hashimoto, M. Tanaka, M. Baumgartner, M. Stadler and Y. Asakawa, *J. Nat. Prod.*, 2002, **65**, 1869–1874.
88 (*a*) T. Hashimoto, S. Tahara, S. Takaoka, M. Tori and Y. Asakawa, *Chem. Pharm. Bull.*, 1994, **42**, 2397–2399; (*b*) T. Hashimoto and Y. Asakawa, *Heterocycles*, 1998, **47**, 1067–1110.
89 S. Suzuki, K. Tanemura, C. Okada, K. Arai, A. Awaji, T. Shimizu and T. Horaguchi, *J. Heterocycl. Chem.*, 2001, **38**, 1409–1418.
90 L.-Z. Fang and J.-K. Liu, *Heterocycles*, 2009, **78**, 2107–2113.
91 (*a*) G. J. Hollingworth, G. Perkins and J. Sweeney, *J. Chem. Soc., Perkin Trans. 1*, 1996, 1913–1919; (*b*) A. Kaydos and D. L. J. Smith, *J. Org. Chem.*, 1983, **48**, 1096–1099.
92 T. Sasaki, Y. Ishibashi and M. Ohno, *Heterocycles*, 1983, **20**, 1933–1936.
93 (*a*) D. Z. Liu, F. Wang, T. G. Liao, J. G. Tang, W. Steglich, H. J. Zhu and J. K. Liu, *Org. Lett.*, 2006, **8**, 5749–5752; (*b*) J. X. Pu, S. X. Huang, J. Ren, W. L. Xiao, L. M. Li, R. T. Li, L. B. Li, T. G. Liao, L. G. Lou, H. J. Zhu and H. D. Sun, *J. Nat. Prod.*, 2007, **70**, 1706–1711; (*c*) Y. Hua, J. Ren, C. X. Chen and H. J. Zhu, *Chem. Res. Chin. Univ.*, 2007, **23**, 592–596; (*d*) H. X. Liang, F. K. Bao, X. P. Dong, H. J. Zhu, X. J. Lua, M. Shi, Q. Lua and Y. X. Cheng, *Chem. Biodiversity*, 2007, **4**, 2810–2816.
94 (*a*) M. Hatano and K. Ishihara, *Chem. Rec.*, 2008, **8**, 143–155; (*b*) M. Hatano and K. Ishihara, *Synthesis*, 2008, 1647–1675.
95 A. N. Phillips, C. A. Lee, J. Elford, A. Webster, G. Janossy, P. D. Griffiths and P. B. A. Kernoff, *AIDS*, 1991, **5**, 1217–1222.
96 (*a*) P. V. Ramanchandran, G. M. Chen and H. C. Brown, *Tetrahedron Lett.*, 1996, **37**, 2205–2208; (*b*) H. Takahashi, T. Tsubuki and K. Higashiyama, *Chem. Pharm. Bull.*, 1991, **39**, 3136–3139; (*c*) H. Nakano, N. Kumagai, H. Matsuzaki, C. Kabuto and H. Hongo, *Tetrahedron: Asymmetry*, 1997, **8**, 1391–1401; (*d*) M. Watanabe, N. Hashimoto, S. Araki and Y. Butsugan, *J. Org. Chem.*, 1992, **57**, 742–744.
97 C.-W. Chang and R.-J. Chein, *J. Org. Chem.*, 2011, **76**, 4154–4157.

98 F. García, M. McPartlin, J. V. Morey, D. Nobuto, Y. Kondo, H. Naka, M. Uchiyama and A. E. H. Wheatley, *Eur. J. Org. Chem.*, 2008, 644–647.

99 (*a*) M. P. Sibi and V. Snieckus, *J. Org. Chem.*, 1983, **48**, 1935–1937; (*b*) N. Assimomytis, Y. Sariyannis, G. Stavropoulos, P. G. Tsoungas, G. Varvounis and P. Cordopatis, *Synlett*, 2009, 2777–2782; (*c*) R. E. Miller, T. Rantanen, K. A. Ogilvie, U. Groth and V. Snieckus, *Org. Lett.*, 2010, **12**, 2198–2201.

100 D. J. Hart and A. Mannino, *Tetrahedron*, 1996, **52**, 3841–3856.

101 P. Beak and V. Snieckus, *Acc. Chem. Res.*, 1982, **15**, 306–312.

102 (*a*) E. J. Corey and C. J. Helal, *Angew. Chem., Int. Ed.*, 1998, **37**, 1986–2012; (*b*) R.-J. Chein, Y.-Y. Yeung and E. J. Corey, *Org. Lett.*, 2009, **11**, 1611–1614.

103 E. J. Corey and A. Venkateswarlu, *J. Am. Chem. Soc.*, 1972, **94**, 6190–6191.

104 (*a*) H. W. Florey, K. Gilliver, M. A. Jennings and A. G. Sanders, *Lancet*, 1946, 46–49; (*b*) A. Mitsui and S. Suzuki, *J. Antibiot.*, 1969, **22**, 358–363; (*c*) A. Covarrubias-Zúñiga, A. González-Lucas and M. M. Domínguez, *Tetrahedron*, 2003, **59**, 1989–1994.

105 (*a*) D. F. Jones, R. H. Moore and G. C. Crawley, *J. Chem. Soc. (C)*, 1970, 1725–1737; (*b*) R. M. Carman, *Aust. J. Chem.*, 1978, **31**, 353–364; (*c*) P. V. Bernhardt, R. M. Carman and T. T. Le, *Aust. J. Chem.*, 2007, **60**, 354–357.

106 W. J. Watkins, J. Chen, A. Cho, L. Chong, N. Collins, M. Fardis, W. Huang, T. Kirschberg, W. A. Lee, W. Thomas, J. Zhang, J. Xu and A. Zeynalzadegan, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 3479–3483.

107 A. Lewis, I. Stefanuti, S. A. Swain, S. A. Smith and R. J. K. Taylor, *Org. Biomol. Chem.*, 2003, **1**, 104–116.

108 K. W. Pankiewics, K. B. Lesiak-Watanabe, K. A. Watanabe, S. E. Patterson, H. N. Jayaram, J. A. Yalowitz, M. D. Miller, M. Seidman, A. Majumdar, G. Prehna and B. M. Goldstein, *J. Med. Chem.*, 2002, **45**, 703–712.

109 M. Fardis, M. Mertzman, W. Thomas, T. Kirschberg, N. Collins, R. Polniaszek and W. J. Watkins, *J. Org. Chem.*, 2006, **71**, 4835–4839.

110 S. J. Yadav, R. S. Babu and G. Sabitha, *ARKIVOC*, 2003, **iii**, 125–139.

111 (*a*) N. Osmanova, W. Schultze and N. Ayoub, *Phytochem. Rev.*, 2010, **9**, 315–342; (*b*) J.-M. Gao, S.-X. Yang and J.-C. Qin, *Chem. Rev.*, 2013, **113**, DOI: 10.1021/cr300402y.

112 (*a*) W. G. Wei and Z. J. Yao, *J. Org. Chem.*, 2005, **70**, 4585–4590; (*b*) J. Dong, Y. Zhou, R. Li, W. Zhou, L. Li, Y. Zhu, R. Huang and K. Zhang, *FEMS Microbiol. Lett.*, 2006, **264**, 65–69; (*c*) W.-J. Qian, W.-G. Wei, Y.-X. Zhang and Z.-J. Yao, *J. Am. Chem. Soc.*, 2007, **129**, 6400–6401.

113 *Studies in Natural Products Chemistry: Bioactive Natural Products (Part (L)*, A.-U. Rahman (Ed.), Elsevier, 2005, p. 481.

114 T. Rezanka and J. Spizek, Griseofulvin and other biologically active, halogen containing compounds from fungus. In *Studies in Natural Products Chemistry*. Vol. 32, Part L, A.-U. Rahman (Ed.), Elsevier, Amsterdam, Netherlands, 2005.

115 (*a*) K. Matsuzaki, H. Tahara, J. Inokoshi, H. Tanaka, R. Masuma and S. Omura, *J. Antibiot.*, 1998, **51**, 1004–1011; (*b*) K. Matsuzaki, H. Tanaka and S. Omura, *J. Antibiot.*, 1995, **48**, 703–707; (*c*) K. Matsuzaki, H. Tanaka and S. Omura, *J. Antibiot.*, 1995, **48**, 708–713; (*d*) H. Tomoda, C. Matsushima, N. Tabata, I. Namatame, H. Tanaka, M. J. Bamberger, H. Arai, M. Fukazawa, K. Inoue and S. Omura, *J. Antibiot.*, 1999, **52**, 160–170; (*e*) H. Seto and M. Tanabe, *Tetrahedron Lett.*, 1974, **15**, 651–654.

116 L. Pairet, S. K. Wrigley, I. Chetland, E. E. Reynolds, M. A. Hayes, J. Holloway, A. M. Ainsworth, W. Katzer and X. M. Cheng, *J. Antibiot.*, 1995, **48**, 913–923.

117 M. Iwatsuki, K. Otoguro, A. Ishiyama, M. Namatame, A. Nishihara-Tukashima, J. Hashida, T. Nakashima, R. Masuma, Y. Takahashi, H. Yamada and J. Omura, *J. Antibiot.*, 2010, **63**, 619–622.

118 (*a*) S. Kanokmedhakul, K. Kanokmedhakul, P. Nasomjai, S. Louangsysouphanh, K. Soytong, M. Isobe, P. Kongsaeree, S. Prabpai and A. Suksamrarn, *J. Nat. Prod.*, 2006, **69**, 891–895; (*b*) R. W. Gray and W. B. Whalley, *J. Chem. Soc.*, 1971, 3575–3577.

119 H. Anke, T. Kemmer and G. Höfle, *J. Antibiot.*, 1981, **34**, 923–928.

120 L. Musso, S. Dallavalle, L. Merlini, A. Bava, G. Nasini, S. Penco, G. Giannini, C. Giommarelli, A. De Cesare, V. Zuco, L. Vesci, C. Pisano, F. Dal Piaz, N. De Tommasi and F. Zunino, *Bioorg. Med. Chem.*, 2010, **18**, 6031–6043.

121 (*a*) E. Yoshida, H. Fujimoto and M. Yamazaki, *Chem. Pharm. Bull.*, 1996, **44**, 284–287; (*b*) E. Yoshida, H. Fujimoto and M. Yamazaki, *Chem. Pharm. Bull.*, 1996, **44**, 1775.

122 (*a*) E. Thines, H. Anke and O. Sterner, *J. Nat. Prod.*, 1998, **61**, 306–308; (*b*) E. Thines, T. Daussmann, M. Semar, O. Sterner and H. Anke, *Z. Naturforsch.*, 1995, **50C**, 813–819; (*c*) H. Anke, O. Bergendorff and O. Sterner, *Food Chem. Toxicol.*, 1989, **27**, 393–398.

123 A. A. Bell and M. H. Wheeler, *Annu. Rev. Phytopathol.*, 1986, **24**, 411–451.

124 S. Toki, T. Tanaka, Y. Uosaki, M. Yoshida, Y. Suzuki, K. Kita, A. Mihara, K. Ando, N. A. Lokker, N. A. Giese and Y. Matsuda, *J. Antibiot.*, 1999, **52**, 235–244.

125 Y.-M. Ju, F. San Martín González and J. D. Rogers, *Mycotaxon*, 1993, **47**, 219–228.

126 D. N. Quang, T. Hashimoto, N. Radulovíc, J. Fournier, M. Stadler and Y. Asakawa, *Tetrahedron*, 2005, **61**, 1743–1748.

127 D. N. Quang, L. Harinantenaina, A. Nishizawa, T. Hashimoto, C. Kohchi, G.-I. Soma and Y. Asakawa, *Biol. Pharm. Bull.*, 2006, **29**, 34–37.

128 (*a*) D. N. Quang, T. Hashimoto, N. Radulovic, M. Stadler and Y. Asakawa, *Int. J. Med. Mushrooms*, 2005, **7**, 452–455; (*b*) D. N. Quang, T. Hashimoto, M. Stadler, N. Radulovíc and Y. Asakawa, *Planta Med.*, 2005, **71**, 1058–1062.

129 (*a*) M. Stadler, D. N. Quang, A. Tomita, T. Hashimoto and Y. Asakawa, *Mycol. Res.*, 2006, **110**, 811–820; (*b*) D. N. Quang, T. Hashimoto and Y. Asakawa, *Chem. Rec.*, 2006, **6**, 79–99.

130 M. Stadler and J. Fournier, *Rev. Iberoam. Micol.*, 2006, **23**, 160–170.

131 D. N. Quang, M. Stadler, J. Fournier, A. Tomita and T. Hashimoto, *Tetrahedron*, 2006, **62**, 6349–6354.

132 H. Huang, X. Feng, Z. Xiao, L. Liu, H. Li, L. Ma, Y. Lu, J. Ju, Z. She and Y. Lin, *J. Nat. Prod.*, 2011, **74**, 997–1002.

133 P. S. Steyn and R. Vleggaar, *J. Chem. Soc., Perkin Trans. 1*, 1986, 1975–1976.

134 M.-J. Cheng, M.-D. Wua, Y.-S. Su, G.-F. Yuan, Y.-L. Chen and I.-S. Chen, *Phytochem. Lett.*, 2012, **5**, 262–266.

135 (*a*) M. Takahashi, K. Koyama and S. Natori, *Chem. Pharm. Bull.*, 1990, **38**, 625–628; (*b*) K. Yasukawa, M. Takahashi, S. Natori, K. I. Kawai, M. Yamazaki, M. Takeuchi and M. Takido, *Oncology*, 1994, **51**, 108–112; (*c*) N. Phonkerd, S. Kanokmedhakul, K. Kanokmedhakul, K. Soytong, S. Prabpai and P. Kongsearee, *Tetrahedron*, 2008, **64**, 9636–9645.

136 J. H. Park, G. J. Choi, K. S. Jang, H. K. Lim, H. T. Kim, K. Y. Cho and J. C. Kim, *FEMS Microbiol. Lett.*, 2005, **252**, 309–313.

137 T. Yamada, Y. Muroga and R. Tanaka, *Mar. Drugs*, 2009, **7**, 249–257.

138 T. Yamada, M. Jinno, T. Kikuchi, T. Kajimoto, A. Numata and R. Tanaka, *J. Antibiot.*, 2012, **65**, 413–417.

139 (*a*) T. Yamada, M. Doi, H. Shigeta, Y. Muroga, S. Hosoe, A. Numata and R. Tanaka, *Tetrahedron Lett.*, 2008, **49**, 4192–4195; (*b*) Y. Muroga, T. Yamada, A. Numata and R. Tanaka, *J. Antibiot.*, 2008, **61**, 615–622; (*c*) Y. Muroga, T. Yamada, A. Numata and R. Tanaka, *Helv. Chim. Acta*, 2010, **93**, 542–549; (*d*) M. Yasuhide, T. Yamada, A. Numata and R. Tanaka, *Tetrahedron*, 2009, **65**, 7580–7586; (*e*) T. Yamada, Y. Muroga, H. Shigeta, A. Numata and R. Tanaka, *J. Antibiot.*, 2009, **62**, 353–357.

140 J.-C. Qin, Y.-M. Zhang, J.-M. Gao, M.-S. Bai, S.-X. Yang, H. Laatsch and A.-L. Zhang, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 1572–1574.

141 W. S. Borges, G. Mancilla, D. O. Guimaraes, R. D. Patrón, I. G. Collado and M. T. Pupo, *J. Nat. Prod.*, 2011, **74**, 1182–1187.

142 G.-D. Chen, Y.-J. Li, H. Gao, Y. Chen, X.-X. Li, J. Li, L.-D. Guo, Y.-Z. Cen and X.-S. Yao, *Planta Med.*, 2012, **78**, 1683–1689.

143 W. B. Turner and D. C. Aldridge, *Fungal metabolites II*. Academic Press, London, 1983.

144 J. M. Winter, M. Sato, S. Sugimoto, G. Chiou, N. K. Garg, Y. Tang and K. Watanabe, *J. Am. Chem. Soc.*, 2012, **134**, 17900–17903.

145 (*a*) A. R. Germain, D. M. Bruggemeyer, J. Zhu, C. Genet, P. O'Brien and J. A. Porco, Jr., *J. Org. Chem.*, 2011, **76**, 2577–2584; (*b*) R. C. Clark, S. Y. Lee and D. L. Boger, *J. Am. Chem. Soc.*, 2008, **130**, 12355–12369; (*c*) L. Lin, N. Mulholland, Q. Y. Wu, D. Beattie, S. W. Huang, D. Irwin, J. Clough, Y. C. Gu and G. F. Yang, *J. Agric. Food Chem.*, 2012, **60**, 4480–4491; (*d*) J. Zhu and J. A. Porco, Jr., *Org. Lett.*, 2006, **8**, 5169–5171; (*e*) M. A. Marsini, K. M. Gowin and T. R. Pettus, *Org. Lett.*, 2006, **8**, 3481–3483; (*f*) J. Zhu, N. P. Grigoriadis, J. P. Lee and J. A. Porco, Jr., *J. Am. Chem. Soc.*, 2005, **127**, 9342–9343; (*g*) T. Takeuchi, Y. Mizushina, S. Takaichi, N. Inoue, K. Kuramochi, S. Shimura, Y. Myobatake, Y. Katayama, K. Takemoto, S. Endo, S. Kamisuki and F. Sugawara, *Org. Lett.*, 2012, **14**, 4303–4305.

146 J. Zhu, A. R. Germain and J. A. Porco, Jr., *Angew. Chem., Int. Ed.*, 2004, **43**, 1239–1243.

147 M. Achard, A. B. Beeler and J. A. Porco, Jr., *ACS Comb. Sci.*, 2012, **14**, 236–244.

148 M. Zhang, H.-Y. An, B.-G. Zhao and J.-H. Xu, *Org. Biomol. Chem.*, 2006, **4**, 33–35.

149 K. Yoganathan, C. Rossant, S. Ng, Y. Huang, M. S. Butler and A. D. Buss, *J. Nat. Prod.*, 2003, **66**, 1116–1117.

150 (*a*) S. E. Michael, *Biochem. J.*, 1948, **43**, 528–533; (*b*) D. H. R. Barton and J. B. Hendrickson, *J. Chem. Soc.*, 1956, 1028–1034; (*c*) G. C. Crawley, *J. Chem. Soc., Perkin Trans. 1*, 1981, 221–223; (*d*) C. A. Boulet and G. A. Poulton, *Can. J. Chem.*, 1983, **61**, 2285–2286.

151 (*a*) J. H. Birkinshaw, A. Bracken, J. E. Michael and H. Raistrick, *Biochem. J.*, 1951, **48**, 67–74; (*b*) D. H. R. Barton and J. B. Hendrickson, *Chem. Ind.*, 1955, 682; (*c*) A. J. Birch, *Chem. Ind.*, 1955, 682–683; (*d*) A. J. Birch, A. J. Ryan, J. Schofield and H. Smith, *J. Chem. Soc.*, 1965, 1231–1234; (*e*) B. Pyuskyulev, B. Rindone and C. Scolastico, *Tetrahedron*, 1973, **29**, 2849–2850.

152 (*a*) S. Nakanishi, K. Osawa, Y. Saito, I. Kawamoto, K. Kuroda and H. Kase, *J. Antibiot.*, 1992, **45**, 341–347; (*b*) K. Nagashima, S. Toki, S. Nakanishi, H. Kase and Y. Matsuda, *J. Antibiot.*, 1993, **46**, 1481–1483; (*c*) M. Ichimura, R. Eiki, K. Osawa, S. Nakanishi and H. Kase, *Biochem J.*, 1996, **316**, 311–316.

153 A. Ishiyama, K. Otoguro, M. Namatame, A. Nishihara, T. Furusawa, R. Masuma, K. Shiomi, Y. Takahashi, M. Ichimura, H. Yamada and S. Omura, *J. Antibiot.*, 2008, **61**, 627–632.

154 (*a*) H. Achenbach, A. Miihlenfeld, B. Weber and G. U. Brillinger, *Tetrahedron Lett.*, 1982, **23**, 4659–4660; (*b*) H. Achenbach, A. Muhlenfeld and G. U. Brillinger, *Liebigs Ann. Chem.*, 1985, 1596–1628.

155 R. Shan, M. Stadler, H. Anke and O. Sterner, *J. Nat. Prod.*, 1997, **60**, 804–805.

156 C. Di, L.-Q. Li, S.-J. Da, Y. Li and Z.-X. Xie, *Chin. J. Chem.*, 2008, **26**, 693–698.

157 Q. L. Wang, X. G. She, X. F. Ren, J. Y. Ma and X. F. Pan, *Tetrahedron: Asymmetry*, 2004, **15**, 29–34.

158 A. M. Martín Castro, *Chem. Rev.*, 2004, **104**, 2939–3002.

159 M. Halpern, Y. Sasso and M. Rabinovitz, *J. Org. Chem.*, 1983, **48**, 1022–1025.

160 W. H. Bunnelle, M. A. Rafferty and S. L. Hodges, *J. Org. Chem.*, 1987, **52**, 1603–1605.

161 G. Lin, S.-K. Chan, H.-S. Chung and S.-L. Li, In *Studies in Natural Products Chemistry*, A.-U. Rahman, Ed.; Elsevier: Amsterdam, Netherlands, 2005, p. 611–669.

162 B. J. Bradbury, P. Bartyzel, T. S. Kaufman, M. J. Nieto, R. D. Sindelar, S. M. Scesney, B. R. Gaumond and H. C. Marsh Jr., *J. Med. Chem.*, 2003, **46**, 2697–2705.

163 (*a*) H. Achenbach, A. Muehlenfeld and D. Hunkler, *Z. Naturforsch. B*, 1985, **40B**, 426–428; (*b*) Y. S. Tsantrizos, K. K. Ogilvie and A. K. Watson, *Can. J. Chem.*, 1992, **70**, 2276–2284.