Resorcylic acid lactones (RALs) and their structural congeners: recent advances in their biosynthesis, chemical synthesis and biology

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Resorcylic acid lactones (RALs) are naturally occurring 14-membered macrolactones that constitute a class of polyketides derived from fungal metabolites and that possess significant and promising biological activity. Their core structural feature consists of a β-resorcylic acid framework (2,4-dihydroxybenzoic acid) fused with an alicyclic side unit decorated with numerous functional groups in a stereodefined fashion. In this review, we focus our attention on the chemistry and biology of this novel class of macrolactones. Only recent developments from the year 2008 to date will be covered, and the core attention will be given to the synthesis and biosynthesis of RALs published during these times. We also delineate the chemistry and biology of several structural congeners of RALs that have also come into existence in recent years.

1. Introduction

Resorcylic acid lactones (RALs) are a class of mycotoxins isolated from various strains of fungi and are defined by the presence of a β-resorcylic acid ring and a 14-membered lactonemacrocycle with a methyl substituent at the C10′-position (Fig. 1) in the core structure.

The first example of a RAL, radicicol, was isolated from Monocillium nordinii in 1953.1 Historically, radicicol (1) was a vanguard of RAL research, generating interest due to its potential antibacterial2–4 and cytotoxic5 properties. Also of early historical importance was zearalenone (2), which was isolated from Gibberella zeae in 1962.6 Initially, radicicol was known to have a mild sedative activity along with moderate antibiotic activity, while zearalenone was known to be a toxic material of extensive concern to livestock and poultry producers. Decades later it was found that zearalenone is an oestrogen agonist and radicicol is a potent HSP90 inhibitor, with news of these two important biological activities placing this class of molecules into the limelight and leading afterwards to numerous studies to find new potent members of this class. Additionally, the majority of work elucidating the mechanism of the biosynthesis of RALs was done on zearalenone, which was shown to be possible to biosynthesize through a polyketide synthase (PKS) pathway, the details of which are provided in Section 4. A few other RALs, such as LL-Z1640-1 to LL-Z1640-4, were isolated in 1978,7 together with hypothemycin (3) in 1980,8 monocillins I–V in 19879 and zeanenol in 1992 (Fig. 2). After that, a series of 14-membered resorcylic acid lactones, such as aigialomycins A–E, pochonins A–P, paecilomycins A–F and cochliomycins, were reported as fungal polyketide metabolites. All of these compounds have received considerable attention, due to their potent biological properties, which include antifungal, cytotoxic, antimalarial, antiviral, antiparasitic, estrogenic, nematicidal,
protein tyrosine kinase and ATPase inhibition activities. We
discuss the biological activities associated with several RALs in
detail in Section 5.

Initially, RALs only garnered limited interest from the
known biological properties of radicicol and it was not until
the late 1990s that RALs became a significant class of com-
 pounds for medicinal research, initiated by the discovery of
radicicol as a potent and selective inhibitor of Hsp90,10,11 as
well as from the discovery of hypothemycin (3) and L-783,277
(8) as kinase inhibitors.12 Interest in research on RALs was
further bolstered by the discovery of additional compounds
displaying interesting biological activity, such as the aigialo-
mycins in 200213 and the pochonins in 2003.14 Further details
of some reported RALs (natural and synthetic) and their known
biological properties are given in Section 5. Some examples of
selected RALs and their subsequent biological profile are
provided in Fig. 2. These examples highlight the potential value
of SAR (structure–activity relationship) studies, with minor
variations to the general RAL skeletal structure resulting in
significant changes to selectivity and activity. In this review, we
intend to focus primarily on the chemistry and biology of RALs
isolated very recently (2008 afterward). Interested readers are
encouraged to go through earlier reviews delineating different
aspects of RALs.15–19

2. Isolation and structural features of RALs

The main structural features of RALs consist of a β-resorcylic acid
unit (2,4-dihydroxy benzoic acid) embedded in a 14-membered
macrolactone core. The C6-position of the aromatic ring is
usually functionalized with an alicyclic side chain and esterified with C1-carboxylic acid with a C10′-Me substitution to close the macrocycle. The C2 and C4 in the aromatic ring usually have hydroxyl substitution but in many cases the C4-contains a methoxy (–OMe) group. The C3-position in the aromatic ring is usually free in most of the RALs, except for the recently isolated 3,5-dibromozeaenol, in which the C3-position is functionalized with a –Br group; whereas at the C5-position, the presence of some halogen-containing functional groups (–Cl and Br) are known in a few instances.

The aliphatic side chain originating from the C6-position of the aromatic ring is usually decorated with several functional groups in a stereochemical fashion. The C-10′ position always has a –Me appendage in all the RAL structures. The C1′ and C2′ positions contain olefinic unsaturation, though C2′ also might contain a carbonyl functionality. There might exist an epoxy functionality between C1′ and C2′ in some RALs. The C4′, C5′ and C6′ positions usually contain stereochemically pure –OH functionality. We discuss the structural features separately in the subsequent section for the individual RALs.

3. Various types of RALs

3.1. RALs isolated earlier (radicicol and other RALs)

Radicicol (1), the first known naturally occurring RAL, was isolated way back in 1953 from a few fungal strains, such as Nectria radicicola20 and the plant-associated fungus Chaetomium chiversii.21 Initially, mild sedative and antibiotic activity was exhibited by radicicol, which was later found to be an excellent inhibitor of HSP90, a molecular chaperone that has a significant effect in cancer biology.22–25

The other known popular RALs, such as zearalenone, zearalanol, LL-Z1640-2, hypothemycin, radicicol A and L-783,277, were isolated between 1978 and 1999 and reviewed nicely by Murphy et al.,26 hence they are not discussed here. However, in a later section, we discuss a few of their latest chemical syntheses reported after 2008.

3.2. Queenslandon and caryospomycins

Queenslandon (10), a relatively new RAL, was isolated in 2002 from the fungal strain Chrysosporium queenslandicum IFM51121 and has exhibited antifungal activity.27 The aromatic ring in queenslandon was almost fully functionalized (except C3) and shown to have a C5-OMe group in its structure, which was relatively new. The side chain functionality in queenslandon was also relatively new as it contained carbonyl functionality (at C5′) flanked by two –OH functionalities at C4′ and C6′. Queenslandon was subsequently assayed for its antifungal activity and showed moderate activities against Alternaria alternata (IFM 41348), Paecilomyces variotii (IFM 40913), Penicillium chrysogenum (IFM 40614), Aspergillus flavus (IFM 41934), Aspergillus fumigatus (IFM 41088), Aspergillus terreus (IFM 40851) and Aspergillus niger (IFM 5368).

A bioassay-guided fractionation from the extracts of C. carllicarpa YMF1.01026 led to the isolation of a few relatively new RAL molecules in this regard. The caryospomycins A–C (11–13), another molecule in the series (Fig. 3), were isolated in 2007 from the fresh-water fungus Caryospora callicarpa YMF1.01026, and has been shown to possess moderate nematicidal activity against the pine wood nematode B. xylophilus with LC50 values around 100 ppm over a 36 h period.28 No research into the cytotoxic or kinase inhibition properties of any caryospomycins has been reported. This finding also demonstrated that fungi inhabiting freshwater environments could produce nematicidal metabolites. The occurrence of a nematicidal substance in freshwater fungi might be linked to their survival strategies. The structural feature of caryospomycin A was unique as it contained rare acetonide functionality in it, whereas all the RALs contain E-olefinic unsaturation at C1′–C2′ and C7′–C8′.

3.3. Hamigeromycins

A group of structurally similar RALs, the hamigeromycins (14–20), was isolated by Isaka et al. (Fig. 4), from the soil fungus Hamigera avellanea BCC 17816 and showed limited biological activity. Tests against three human cancer cell lines (KB, MCF-7 and NCI-H187) at 50 μM and Plasmodium falciparum K1 at 10 μM showed no significant biological activity. Against Vero cells, only hamigeromycin A and C displayed growth inhibition, with respective IC50 values of 42 and 13 μM.29,30 While the structural features of hamigeromycins A and C–E are similar to that of B, F and G are a little different, as shown in Fig. 4. Hamigeromycin B (15) has a unique dihydro-2H-pyran-4(3H)-one core embedded in the 14-membered macrocycle core. In hamigeromycins F and G, the presence of γ-hydroxy keto functionality is the key feature, whereas the other RALs in the same series contain the usual pattern of functional groups. Their structures have been confirmed through extensive 2D-NMR analysis (COSY and HMBC techniques).

3.4. Aigialomycins

Five new resorcylic macrorides named aigialomycins A–E (21–23, 9 and 24) were isolated from a lignicolous mangrove...
ascomycete, *Aigialus parvus* BCC 5311, in 2002 by Isika et al.\textsuperscript{13} There was no early report for the secondary metabolites from the genus *Aigialus*. Hence this finding is extremely important regarding finding structurally novel bioactive components. *In vitro* antimalarial activity was exhibited by aigialomycin D (IC\textsubscript{50} value of 6.6 \(\mu\)g mL\(^{-1}\)), whereas the other compounds seemed not to be active. The structures of all the newly isolated RALs were confirmed by extensive NMR and X-ray crystallography analyses. Aigialomycin A–C (21–23) have similar structural features with the known RAL hypothemycin (3) as all of them contain an epoxy linkage along C1' and C2', though the stereochemistry differs as well as the geometry of the olefinic unsaturation along C7' and C8' (in hypothemycin, it was \(Z\); whereas, in aigialomycin A–C it was \(E\)). Aigialomycin D (9) does not consist of an epoxy functionality, and the geometry of both the olefinic unsaturation along C1'–C2' and C7'–C8' was \(E\). Aigialomycin E (24) featured a rare \(Z\) olefinic double bond geometry along C1'–C2', which was not very common in RALs (Fig. 5).

### 3.5. Pochonins

Pochonins A–F (25–26, 4, 7 and 27–28), six relatively new RALs were isolated from the cultures of the clavicipitaceous hyphomycete *Pochonia chlamydosporia* var. *catenulata* strain P 0297 in 2003 by Hellwig et al. in a mission to find new antiviral compounds for the treatment of infection caused by HCV (Herpes Simplex Virus). A systematic HTS (high throughput screening) was carried out by the researchers to find some new antiviral agents, as acyclovir the known drug at the time had encountered several limitations, such as resistance towards certain strains. Six new RALs named pochonins A–F with other known RALs, such as monodern and tetrahydro-monodern, were isolated and structurally characterized during the study.\textsuperscript{14}

Pochonins A–B (25–26) both have an epoxide appendage along C7'–C8' and an enone moiety (C2'–C4'), which seems to be responsible for its biological profile. Pochonin C (4) has a trans-chlorohydrin moiety at C7'–C8' with the \(E\)-enone moiety, whereas pochonin D/E (7 and 27) is devoid of the chlorohydrin part. Pochonin F (28) differs mostly in the aromatic substitution and in the truest sense it cannot really be regarded as a RAL as the aromatic part is somewhat different to the case for the other known RALs (Fig. 6).

### 3.6. Paecilomycins

Six new \(\beta\)-resorcylic acid lactones, named paecilomycins A–F,\textsuperscript{31} were isolated recently from the mycelial solid culture of
Paecilomyces sp. SC0924 in late 2010 by Chen and Wei et al. along with other known RALs. Paecilomycin A (29) possessed a $1',2'$-epoxy linkage with three hydroxyl groups at the $4',5',6'$-positions, whereas paecilomycins C and D (31 and 32) possessed 5-membered $\gamma$-lactones instead of the 14-membered macro-lactones seen in other RALs (Fig. 7). Paecilomycin B (30) consisted of a unique tetrahydropyran ring connecting C1' and C5'. Paecilomycin E exhibited antiplasmodial activity against the Plasmodium falciparum line 3D7 with IC$_{50}$ values of 20.0 nM. Paecilomycin E and F (33 and 34) showed moderate activity against the P. falciparum line Dd2. Later, a structural revision for paecilomycin E and F was reported.$^{32}$ The stereochemistry of the hydroxyl containing the C-6' carbon is inverted in both the molecules in the corrected form; hence paecilomycin E and F became paecilomycin F and E, respectively.

Three new RALs paecilomycins G–I (Fig. 8) were isolated in 2012 from a MeOH extract of the solid culture of Paecilomyces sp. SC0924 and showed antifungal activity against Peronosphythora litchii, one of the main pathogens causing Lithi (Litchi chinensis Sonn.) fruit rot.$^{13}$ Close structural inspection revealed that paecilomycin H (36) was the acetonide-protected aigialomycin D, whereas paecilomycin I (37) was structurally related to aigialomycin A and contained an extra ethoxy group at the C8' position.

3.7. Cochliomycins

Most recently, three new 14-membered resorcylic acid lactones named cochliomycin A–C (38–40)$^{14}$ were isolated by Wang et al. from the culture broth of Cochliobolus lunatus, a fungus obtained from the gorgonian Dichotella gemmacea collected in the South China Sea together with four known analogues. Zeaenol, a phytotoxic RAL first isolated in 1992$^{35}$ by Sugawara et al., was also found in the same fungus. Two of the newly found RALs (cochliomycin A and B; Fig. 9) included a rare natural acetonide group, while one had a 5-chloro-substituted (cochliomycin C; 40) resorcylic acid lactone. The structures and relative configurations of cochliomycins A–C were investigated through extensive NMR analysis (NOESY). These resorcylic acid
lactones were subsequently evaluated against the larval settlement of the barnacle *Balanus amphitrite*, and cochliomycin A (38) was found to exhibit the best inhibitory effect. Antifouling activity was detected and measured for the first time for this class of secondary metabolites. These compounds were also tested for antibacterial activity and cytotoxicity. Cochliomycin A exhibited better antifouling activity against the larval settlement of barnacle *B. Amphitrite* compared to zeaenol, suggesting that the acetonide moiety might play a major role in the antifouling activity.

### 3.8. Neocosmosins

Three new RALs named neocosmosin A–C (43–45; Fig. 10) were isolated through a bioassay-guided screening approach from a fungus *Neocosmospora* sp. (UM-031509). The isolated RALs were tested for in vitro binding assays using opioid receptors (subtype δ, κ and μ) and cannabinoid receptors (CB1 and CB2). Neocosmosin C was found to have significant inhibitory activity against the specific binding of [3H]-enkephalin to CHO-K1 cell membranes, expressing human δ-opioid receptors at a concentration of 10 μM (IC50 = 14.82 μM). Further study revealed that neocosmosin C (45) acted as a potent and full agonist of the human δ-opioid receptor. An earlier study also indicated that an agonist or antagonist of opioid or cannabinoid receptors (G-coupled protein receptors) have a profound effect on pain modulator activity. The structural features of neocosmosins are simpler compared to other RALs, as the alkylated chain (C10–C100) does not have any oxygenated functionality.

### 3.9. Cryptosporiopsin A

In 2012, Laatsch *et al.* reported the isolation of a new resorcyclic acid lactone, cryptosporiopsin A (46) from *Cryptosporiopsis* sp., an endophytic fungus from the healthy leaves, stems and branches of *Zanthoxylum leprieurii* (Rutaceae). The relative and absolute configuration of the natural product (cryptosporiopsin A) was assigned by extensive NMR analysis. Cryptosporiopsin A (46) showed motility inhibitory and lytic activities against zoospores of the grapevine downy mildew pathogen *Plasmopara viticola* as well as potent inhibitory activity against the mycelial growth of phytopathogens, *Pythium ultimum*, *Aphanomyces cochlioides* and a basidiomycetous fungus *Rhizoctonia solani*. It also exhibited weak cytotoxic activity against brine shrimp larvae. Its structural features were similar to another naturally occurring RAL radicicol (1) as both of them contained a “Cl” group in the aromatic moiety. It also contained an E-enone functionality and carbonyl group at the C7′ position (Fig. 11).

### 3.10. Brominated zeaenols

The chemical epigenetic manipulation approach is a novel and efficient approach for the generation of novel secondary
metabolites through promoting the silent biosynthetic pathway involved in the formation of polyketide, non-ribosomal and hybrid polyketide natural products. Recently such a technique was applied to the marine-derived fungus Cochliobolus lunatus (TA26–46) with histone deacetylase inhibitors, resulting in significant changes in the overall production of secondary metabolites. In 2014, another metabolically stable strain, C. lunatus (TA26–46), isolated from the sea anemone Palythoa haddoni, was found to produce resorcylic acid lactones. In order to achieve hitherto undiscovered lactones, the chemical epigenetic perturbation method was applied to the fermentation of C. Lunatus (TA26–46). Consequently, brominated resorcylic acid lactones were obtained from the culture treated with sodium butyrate. The new compounds 3-bromo-zeaenol (47a) and 3,5-dibromo-zeaenol (47b) were the first examples of isolated brominated naturally occurring RALs. These two compounds, being the bromo derivatives of the known RAL zeaenol, were evaluated for their cytotoxicity, antifouling activity, and zebrafish embryo teratogenicity. Unfortunately, both compounds showed no activity in these bioassays. Their absolute configurations were confirmed by advanced Mosher's methods.

3.11. Hydroxyzearalenone

Very recently, three new β-resorcylic acid lactones were isolated from the seagrass-derived fungus Fusarium sp. PSU-ES123 (Fig. 13). Seagrasses are marine plants and are regarded as a rich source of endophytic fungi with the capability of producing structurally interesting bioactive compounds, such as the antifeedants luteolin, apigenin and luteolin 4′-glucuronide, the antibacterial meroterpenoid nodosol and antimicrobial aspegillumarins A and B. 5’β-hydroxyzearalenone (48), 7’β-hydroxyzearalenone (49) and 9’α-hydroxyzearalenone (50) were isolated as new RALs, and their structures were well characterized by standard spectroscopic techniques and then their absolute configurations were confirmed by advanced Mosher’s method. Among the isolated RALs, compound 48 only exhibited weak antifungal activity against Cryptococcus neoformans with an MIC value of 128 μg mL−1 and no cytotoxic activity against noncancerous Vero cell lines.

3.12. Hyalodendriellins

Six new 14-membered resorcylic acid lactones (RALs), named hyalodendriellins A–F (Fig. 14), were isolated in 2016 from a culture of the endophytic fungus Hyalodendriella sp. All the isolated compounds were evaluated for their antinematodal, larvicidal, cytotoxic, antibacterial and antifungal activities. Hyalodendriellin A (51a) displayed moderate antinematodal activity against Caenorhabditis elegans and Meloidogyne incognita. Hyalodendriellin C (51c) exhibited a larvicidal effect against the fourth-instar larvae of the mosquito Aedes aegypti.

4. Biogenesis of RALs

Iterative polyketide synthases (PKSs) are large, multifunctional modular enzymes that resemble eukaryotic fatty acid synthases, but can be used to produce highly functionalized secondary metabolites using intricate and unresolved programming rules and have been found to be involved in the biosynthesis of RALs. PKSs, which often occur in fungi, have only a single copy of each domain (e.g. keto reduction, dehydration and enoyl reduction) that can be utilized efficiently and repeatedly for multiple cycles of chain elongation and for tailoring of the functionality (Fig. 15). The control of product functionality by an iterative PKS exclusively depends on the structure of the growing chain covalently attached to the enzyme as a thioester as well as the exact protein sequence. Although the understanding of the biosynthesis of polyketides is advancing rapidly, knowledge of the detailed programming by iterative PKSs is still inadequate. A key requirement for understanding the mechanisms of PKS enzymes is the determination of the structures of the intermediates that remain enzyme-bound.
during numerous successive steps of elongation and modification. A putative scheme of RAL biosynthesis is presented below, which highlights the salient features of PKS. RAL biosynthesis (Fig. 15) is normally catalyzed by two iterative polyketide synthase (PKS) proteins: a highly reducing PKS (hrPKS) and a nonreducing PKS (nrPKS). The hrPKS cluster has a full set of reductive domains and generates the alkyl portion of the RALs (C1–C10). The alcohol required for the macrocyclization as well as other oxygen-containing functional groups present in various RALs (such as the epoxy in hypothemycin) are introduced by elegant programming that enables the hrPKS to skip various reductive domains based on the length of the growing chain. The nrPKS, which usually lacks the reductive domains, next takes the hrPKS product and inserts additional malonate units, generating a poly β-keto intermediate, which is then cyclized by a product template domain into the resorcylate group. The completed polyketide chain is then released via late-stage macrocyclization from the nrPKS by a thioesterase (TE) domain.

Research on zearalenone (2) revealed that the biosynthesis occurred through a polyketide synthase (PKS) pathway, involving the condensation and subsequent cyclisation of acetyl-CoA, as shown in Scheme 1. The biosynthesis of zearalenone is initiated with a highly reducing PKS (hrPKS), a large multi-domain enzyme with various domains used for condensing and reducing acyl-CoA units to form a reduced hexaketide-thioester chain. This thioester chain was then transferred to a non-reducing PKS (nrPKS), where further acyl-CoA units were condensed to form a mixed reduced/unreduced nonaketide. The resorcylic moiety was then formed through an aldol condensation of the unreduced portion of the chain, followed by esterification between the reduced chain hydroxyl and thioester functional groups to form the macrolactone ring and to release zearalenone from the enzyme cluster.
The overall pathway revealed that a late stage macrolactonization was involved for the construction of the RAL core.

Hypothemycin (3) biosynthesis has been studied in considerable detail.64,65 Two iterative PKS proteins, namely Hpm8 and Hpm3, were thought to construct the polyketide backbone of hypothemycin. Hpm8, a highly reducing PKS (hrPKS), first assembled a reduced hexaketide intermediate. With the support of a starter unit acyl-carrier protein transacylase (SAT) domain, this newly formed hexaketide was then transferred to Hpm3, a nonreducing PKS (nrPKS), where it was further extended to a nonaketide, which then underwent regioselective cyclization and macro lactonization to afford (6'S,10'S)-7',8'-dehydrozearalenol (DHZ).

Subsequent post-PKS modifications of DHZ by other enzymes afforded hypothemycin. Although the general functions of the two PKSs were assigned, it remains unresolved how Hpm8 controls the tailoring of the intermediates en route to its hexaketide product using its reductive domains (KR, keto reduction; DH, dehydration and ER, enoyl reduction) in a permutative fashion. It has been proposed that the biosynthesis of radicicol (1) also proceeds through a similar pathway, involving a hrPKS and nrPKS, with structural variations arising from differing reduction patterns in the hrPKS step and putative post-PKS enzymatic alterations (e.g. epoxidation, halogenation).

Schemes 2 and 3 represent how various post-PKS enzymes may be employed to afford the rich diversity of several compounds in the RAL family. For example, hypothemycin (3) was proposed to be biosynthesized via aigialomycin C (23) from the initial product 7',8'-dehydrozearalenol (Scheme 2). After the formation of 7',8'-dehydrozearalenol through a PKS pathway, the putative post-PSK enzymatic alterations involved selective 4'-O-methylation by an O-methyltransferase (OMT), 1',2'-epoxidation by a flavin-dependent monoxygenase (FMO) and the 5'-hydroxylation of 7',8'-dehydrozearalenol by a cytochrome P450 (CYP) monoxygenase to afford aigialomycin C (23). Then, a subsequent 6'-oxidation by an alcohol oxidase (AOX), Z/E isomerization of the 7'-alkene by a glutathione S-transferase (GST) and 4'-hydroxylation by another CYP afforded hypothemycin (3).64

The biosyntheses of monocillin I (6) and radicicol (1) were also thought to follow a similar mechanistic pathway, except for a chlorination step by putative fungal flavin-dependent halogenase to initially form pochonin D (7) in the biosynthesis of radicicol. A putative CYP epoxidase was responsible for epoxidation of the 7'-alkene to form monocillin I and the 5-chloro equivalent, pochonin A (25). The origin of the (5'Z)-alkene is uncertain, as PKSs almost exclusively provide (E)-alkenes, and the radicicol gene cluster does not possess a GST, which has been shown to facilitate isomerization of the alkene in hypothemycin analogs. A possible origin of the (Z)-alkene of
radicicol and monocillin I was a 5'-hydroxylation catalyzed by a CYP followed by a dehydration step [Scheme 3].

Recent results on the biogenesis of zearalenone and radicicol revealed that the biosynthesis of those two RAL molecules relied on a stereotolerant late stage macrocyclizing of thioesterase enzymes. Zearalenone and radicicol are structurally similar RALs with the only exception being their opposite stereochemical configurations of the secondary alcohol involved in lactone formation (the C10 stereocenter). The abilities of the thioesterases from the zearalenone and radicicol biosynthetic pathways to macrocyclize both R and S configured synthetic acyclic substrate analogues were biochemically characterized and it was shown that both enzymes were highly stereotolerant and able to close the macrocycles from both substrates with similar kinetic parameters. Characterization of the full-length nrPKS proteins from RAL pathways suggested that the TEs embedded into these proteins were able to macrocyclize both R and S configured substrates. In vivo and in vitro work with Hpm3, the nrPKS from hypothemycin biosynthesis, revealed that both the macrocycles with R and epimeric S configuration at the C-10 could be accomplished. In the same way as in the in vivo characterization of Rdc1, the NPCs from the radicicol biosynthesis gene cluster showed that both the macrolactone core with an R and enantiomeric S configuration at C-10 could be generated. This stereotolerant behaviour would be in stark contrast to their bacterial analogues and represented distinctive activity for TE domains. Hence, it might be expected that one could observe substantial kinetic stereoselectivity for the TE domains from zearalenone biosynthesis (Zea TE) and radicicol biosynthesis (Rad TE).

5. Biological activity of RALs and their structure–activity relationship (SAR)

Investigations into the biological activity of the RALs have focused mainly on two groups of compounds: the first group includes radicicol, the pochonins, and related compounds, which have been the focus of considerable research due to their Hsp90 inhibition properties, and the second group includes hypothemycin (3), LL-Z1640-2 (5) and related compounds, which have gained significant interest due to their selective kinase inhibition properties. Other RALs have been isolated that display less noteworthy biological activity, but are useful when considering SARs within the class of compounds.

5.1. As HSP90 inhibitors

Hsp90 is a molecular chaperone that plays an important role in many biological processes related to the transport, activation, stabilization and degradation of various proteins. The important functions of Hsp90 are in the folding of both nascent and denatured proteins, ensuring they are in an activated or stabilized form, and in preventing aggregation. Hsp90 is present under normal conditions to assist in these tasks. When exposed to cellular stresses (e.g. infection, inflammation, changes to temperature or exposure to toxins), Hsp90 is overexpressed to maximize the number of functional proteins, which are known...
to include various oncogenic proteins, such as Raf, mutant p53, Her2 and telomerase. As a result, Hsp90 has the potential to facilitate the proliferation and survival of various proteins associated with oncological pathways, making Hsp90 inhibition an attractive target for cancer therapy. Preliminary studies also suggested that Hsp90 inhibitors accumulate more efficiently in tumour cells than normal cells. The specific Hsp90 inhibitor used in these studies was a geldanamycin analogue, 17-allylamino-17-demethoxygeldanamycin (17-AAG), currently in phase II clinical trials, which has a similar biological mechanism to radicicol. Studies have shown that, despite the lack of structural similarity between ATP and either radicicol or geldanamycin, both inhibit Hsp90 by competitive binding to the ATP binding site of Hsp90. Importantly, radicicol has been shown to only bind to the unique L-shaped binding pocket (Bergerat fold) present in Hsp90, and consequently does not compete with ATP binding in other biological processes. Radicicol also binds to Hsp90 in its lowest energy conformation, suggesting radicicol compounds must adopt a higher energy conformation, suggesting radicicol or radicicol analogues may be a good alternative or even improvement on 17-AAG.

Radicicol has been shown to bind to Hsp90 non-covalently, suggesting the structural features important for biological activity are those which govern the conformation, and hydrogen bonding of radicicol to the ATP binding site of Hsp90. Two other groups of naturally occurring RALs, the monocillins (isolated in 1980 from M. nordinii) and the pochonins (isolated in 2003 from Pochonia chlamydosporia var. catenulata strain P 0297), have also exhibited Hsp90 inhibition properties. Both groups of RALs are highly structurally similar to radicicol, as characterized by a trans-enone functionality and a (10'R)-methyl group. Fig. 16 highlights the minor structural variations between these compounds and their effect on Hsp90 inhibition. The inhibition of herpes simplex virus 1 (HSV 1) and WNT-5A have also been reported, indicating their anti-viral properties and potential for hair growth treatment respectively. The WNT gene family consists of structurally related genes that encode the secreted signalling pathway in lipid-modified glycoproteins. These proteins have been implicated in oncogenesis and in several developmental processes, such as the regulation of cell fate and patterning during embryogenesis.

Fig. 16 shows the isolated RALs that have been found to possess HSV 1, Hsp90 and WNT-5A inhibition properties. These compounds highlight the effect of various alterations around the C5–C8 portion of the molecule and from chlorination at the C5-position. Similar RALs have also been isolated (monocillin IV, monocillin V, nordinone and nordinonediol, Fig. 17), which do not possess significant biological activity. In contrast to the previously mentioned RAL, these RALs possess no functionality from the C3–C6 portion of the molecule or from chlorination at the C5-position, suggesting a certain functionality is required at those positions for the compound to inhibit the desired biological targets.

Natural RALs provide useful SAR (structural–activity relationship) information but are limited in the type and number of structural features that occur naturally. Analogue synthesis allows for a more extensive and systematic analysis of SARs.
and can provide greater insights about both the importance of the structural features to biological activity and the nature of their role (e.g. Michael acceptor, the effect on conformation, hydrogen bonding). Detailed SAR studies have been conducted around the general structure provided in Fig. 19, and these show the value of analogue synthesis-based SAR studies.82 These studies were done by synthesizing a combination of analogues possessing single or multiple structural variations, then comparing the results of biological testing. Early SAR studies were done by making small variations, such as hydrogernating the alkene moieties and correlating the effect on Hsp90 inhibition. SAR studies also involved a variation of the macrocycle size from 12- to 16-membered, which suggested 13-, 15- and 16-membered macrocycles may retain activity.83 However, these studies were done with 3’,4’-dihydro analogues, which are less active. Later research by Winssinger et al. on the more active pochonin D analogues showed that 13- or 15-membered macrocycles led to a significant reduction in biological activity.84 This highlights a particular flaw of SAR studies, namely that alterations at one position may negate or enhance the effect of alterations at another.

The unpredictability of correlating SARs of varying systems was further highlighted by additional results reported by Winssinger et al.84 It was found that the 5-dechloropochonin D analogue (monocillin II) showed a similar EC50 value as pochonin D, while, in combination with the modification of the 10’-methyl to an ethyl group, chlorination at the C5-position led to a five-fold increase in affinity. However, in combination with methylation at the C6’-position, chlorination was found to lower the affinity to Hsp90. Other results reported by Winssinger et al. suggested that a hydroxyl at the C6’-position and replacement of the C2’-carbonyl with an oxime increased the potency of the compound. Further evidence was also provided for the importance of the enone system for biological activity and stability, showing that analogues lacking an sp2-hybridised centre at the C3’-position exhibited lower biological activity and lower stability under acidic conditions. This research also provided SARs related to the toxicity of analogues, with results suggesting that C5-dechloro analogues are relatively more toxic, while C3’-hydroxyl and C3’-oxime analogues are relatively less toxic.85 A diagram summarizing the SAR information from the studies outlined previously is provided in Fig. 19.

5.2 As kinase inhibitors

Kinases are phosphotransferase enzymes responsible for transferring phosphate groups from a donor molecule (usually ATP) to various substrates. An important class of these enzymes is protein kinases. Through phosphorylation of protein substrates, protein kinases can play a critical role in various cellular processes, including those associated with oncological pathways. One such pathway is the mitogen-activated protein kinase (MAPK) pathway, a three-tiered kinase cascade of signal transducing enzymes that directly influences many processes, such as cell survival, proliferation and adaption. The cascade comprises a MAPK, which is activated via phosphorylation by a MAPK kinase (commonly abbreviated as MAPKK, MKK or MEK), which in turn is activated via phosphorylation by a MEK kinase (commonly abbreviated as MAPKKK, M KK or MEKK), with the entire process upregulated in response to physical and chemical stresses.85 It is the MAPKs involvement in this cascade that is a major target for the RALs being developed for therapeutic cancer treatment.

The first RALs discovered to possess kinase inhibition properties, namely hypothemycin and L-783,277, were found to inhibit MEK1, with IC50 values of 15 nM and 4 nM, respectively.16 Other notable RALs exhibiting kinase inhibition are LL-Z1640-2, an inhibitor of TAK1 and ERK2, with IC50 values of 8.1 nM and 8.0 nM, respectively,16 and radicicol A, an inhibitor of VEGF-R2/R3, FLT3 and PDGFRβ, with IC50 values of 26, 66, 110 and 210 nM, respectively.86 These compounds display high structural similarities, including a (10’S)-methyl group [compared with the (10’R)-methyl seen in Hsp90-inhibiting RALs], a 6’-8’-cis-enone, (4’S,5’S)-diol and 4-O-methyl (Fig. 20).

A more comprehensive study showed that hypothemycin displayed significant inhibition against 21 out of the 123 kinases tested. Twenty of these kinases contained a cysteine (cys) residue, of those, 18 incorporated a cys residue corresponding to the cys-166 in ERK1/2.86 From co-crystallisation with ERK2, LL-Z1640-2 was shown to be covalently bound to the cys-166...
residue. It was proposed that this covalent bonding would occur through a Michael addition of the cysteine thiol to the C8'-position (Scheme 4). This proposed mechanism would indicate that the Michael acceptor properties of the RAL are a crucial factor in determining the kinase-inhibition properties.

The synthesis and subsequent biological testing of a 4-O-desmethyl hypothemycin analogue (54a) was performed and it showed an increased potency against three human cancer lines (COL829, HT29 and SKOV3) compared to hypothemycin. Two radicicol A analogues (Fig. 21) were also synthesized and tested, specifically a 5-desmethoxy analogue (54b) and a 5-chloro analogue (54c), both of which were found to be less potent compared to radicicol A against all targets based on IC50 values. The most comprehensive SAR studies on kinase-inhibiting RALs through analogue synthesis were done based on the development of LL-Z1640 as an anti-inflammatory drug.

6. Chemical synthesis of naturally occurring RALs

The discovery of some interesting biological activities of this natural product family has led to significantly increased

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**Scheme 4** Proposed Michael addition of a cysteine thiol to the enone functionality.
interest in the chemistry of RALs in recent years. Diverse biological functions and curious skeletal features of these lactones have tempted synthetic organic chemists to try to synthesize them. In general, the chemical synthesis of RALs can be categorized into two parts: (a) construction of a fully functionalized aromatic moiety (either from an aromatic precursor or an acyclic precursor) and (b) construction of the alkyl side chains in an enantioselective way. Subsequently, the coupling of both fragments can be strategically performed in numerous ways, as reported in the scientific literature. One of the finest synthetic strategies reported for RALs involves a biomimetic synthesis featuring a late stage aromatization of properly substituted \( b,\delta,\zeta \)-tri-keto esters, as demonstrated by Barrett et al. in their total synthesis of several RALs and also as recently reviewed by them.\(^{94}\) A brief overview of the earlier syntheses of RALs is presented as a schematic in Scheme 5, highlighting the crucial strategic reactions explored for the total synthesis of several RALs.

### 6.1. Synthetic studies towards RALs in 2008

**a) Synthesis of \((S)\)-zeralenone.** A biomimetic synthesis of \((S)\)-zeralenone (2) was reported by Barrett et al. with the help of a late-stage aromatization approach. The synthetic strategy was greatly inspired by the polyketide biosynthesis pathway.\(^{95}\) The synthesis was commenced from the known enantiopure secondary alcohol 55 and the dioxinone 56. Compound 56 on thermolysis at 110 °C in toluene afforded the corresponding triketo ketene, which was immediately trapped in situ with the alcohol 55 to yield the triketo ester 57. The triketo ester was then subjected to treatment under strong basic conditions, facilitating the aromatization as described previously, followed by acidic cleavage of the ketal functionality to furnish the resorcylate core 58 in an 82% yield. Compound 58, upon exposure to HG-II catalyst,\(^{96}\) afforded the ring-closed product \((E:Z)\) ratio = 6:1, from which \((S)\)-zeralenone was isolated in a 71% yield (Scheme 6). Even a one-pot reaction by mixing 55 and 56 followed by thermolysis and subsequent aromatization/acetal deprotection/RCM (G-II catalyst) was also successful, and this four step reaction could even be carried out in a single vessel without isolating any intermediates.\(^{97}\)

**b) Synthesis of \(\text{epi-aigialomycin D and deoxy-aigialomycin C.}\) In the same year, Jennings’ group accomplished the total synthesis of 6’-\(\text{epi-aigialomycin D}\) and \(\text{deoxy-aigialomycin C}\) through a remote stereocentered RCM macrocyclization method.\(^{98}\) The initial target was to synthesize the naturally occurring aigialomycin and aigialomycin C through the successful exploration of RCM-based ring-closing reactions.\(^{99}\) The synthesis was initiated with alkyne 63, which underwent a base-mediated reaction with aldehyde 62 (synthesized from epoxide 59, as depicted in Scheme 7) to furnish the allyl alcohol 64 in a modest 2:1 diastereomeric ratio in favour of the anti-Cram product. The alkyne was selectively reduced in favour of the ‘’\(E\)’’ isomer upon treatment with Red-Al to furnish compound 65.

Oxidation of the alcohol 65 followed by Red-Al reduction in a chelation-controlled way afforded the alcohol 66/67 with good diastereocontrol (6:1). Subsequent removal of the MOM group and acetonide protection afforded compounds 68 and 69 as non-separable diastereomers in a 6:1 ratio. The esterification of 68/69 with a known aromatic precursor (70) furnished compounds 71/72. The RCM reaction was attempted with a G-II catalyst to furnish the 14-membered macrocycle 74 (13%) and acyclic compound 73 (84%). Finally, debenzylation of 74 with BBr\(_3\) afforded the 6’-\(\text{epi-aigialomycin D}\) (75) in a 74% yield.

![Reported analogues of hypothemycin and radicicol A.](image)

**Fig. 21** Reported analogues of hypothemycin and radicicol A.

**Scheme 5** Brief survey of some of the reported synthetic procedures for RALs (1970–2007).\(^{26}\)
It was noteworthy that the stereochemistry at C6’ in the mixture of 68/69 controlled the course of the reaction. One of the diastereomers, namely 68, reacted with G-II catalyst to furnish the six-membered cyclic acetonide, whereas the minor diastereomer 69 afforded the 14-membered ring-closed RAL through a classical resolution kind of reaction. As the formation of the six-membered trans acetonide was thermodynamically unfavourable, compound 69 afforded the 14-membered RAL analogue. By applying the same strategy compound, 68 (with its diastereomer) was synthetically elaborated to afford deoxy-aigialomycin C (77) and another stereoisomer, namely deoxy-C6-epi-aigialomycin C (78) (Scheme 7a and b).

(c) Synthesis of aigialomycin D through ynal macrocyclization. A truly unique synthesis of aigialomycin D (9) was reported by Montgomery et al.106 involving Ni-catalyzed ynal macroclactonization as a key step.107 The synthesis was initiated from the substituted aromatic precursor 79, which upon protection and Mitsunobu esterification furnished the compound 81. The known alkyne diol 82 was converted to the corresponding boronic acid 84 by the conventional protocol. The Suzuki coupling102 of 84 with 81 afforded the coupled product 85 and constructed C6–C1′ connectivity in the target molecule. Selective mono desilylation and DMP oxidation108 afforded the aldehyde 87. Ynal macrocyclization was accomplished by treating the aldehyde 87 with Ni(COD)2, Et3SiH (5 equiv.) and IMes-HCl in THF solvent to afford compound 88 in a 1:1 diastereomeric ratio. The deprotection of MOM, TBS, and TES group was accomplished by treating compound 88 with 0.5 M HCl in MeOH and then separating the diastereomers by preparative HPLC. aigialomycin D (9) to obtain its 6’-epimer (89) (Scheme 8).

(d) Synthesis of L-783,277. In the same year, the synthesis of another RAL, L-783,277 (8) was reported by Altmann et al.,104 as presented in Scheme 9. Initially, the authors had decided to explore the Sharpless asymmetric dihydroxylation method105 to fix C4’ and C5’ stereocenters in the target molecule, but the unusually low enantioselectivity in this method compelled them to adopt a chiral pool approach. The synthesis commenced with isopropylidene-α-erythro-1,4-lactone, which upon functional group manipulation furnished the alcohol 94. The alcohol was then subjected to Grieco elimination106 with β-NO2C6H4SeCN to furnish the olefin, which upon subsequent desilylation and oxidation afforded the corresponding aldehyde 95. The aldehyde 95 was then coupled with a known alkyl in the presence of n-BuLi to furnish the alcohol, which upon subsequent protection afforded compound 96. The Suzuki coupling reaction of organoborane species generated from olefin 96 and aryl bromide 97 proceeded smoothly to furnish compound 98 in a good yield. Partial reduction of the alkene with the H2/Lindlar catalyst afforded Z-olefin in a good yield; subsequent di-desilylation then afforded the seco-acid, which upon Mitsunobu macrolactonization furnished lactone, which then upon acetonide removal and allylic oxidation with polymer-supported IBX afforded the naturally occurring RAL L-783,277 (8), as presented in Scheme 9.

6.2. Synthetic studies towards RALs in 2009

(a) Synthesis of aigialomycin D. The synthesis of aigialomycin D (9) was disclosed by Barrett’s group in 2009.107 The synthesis was unique in the sense that protection in the phenolic group was avoided and subsequently a triple cascade could be performed involving ketene generation, alcohol trapping and aromatization to lead to the resorcylate core structure. Finally, a late-stage RCM reaction enabled the total synthesis of the target molecule concisely. The synthesis commenced from the known alcohol 99 (readily accessed from a commercially available precursor) and involved oxidation with DMP and Wittig olefination to afford the terminal alkenes 100/101 in a good yield. The DIBAL-H reduction of ethyl esters in 100/101 afforded...
the corresponding aldehydes which upon further Wittig olefination with the known ylide 102 furnished the E-\(\alpha,\beta\)-unsaturated Weinreb amides 103/104. A dianion generated from keto dioxinone 106 was then reacted with Weinreb amides 103/104 to furnish the desired diketo-dioxinone 107/108 (Scheme 10). Upon heating, in toluene, the dioxinone furnished ketene 109/110.
through a retro Diels–Alder sequence. The ketenes were then immediately trapped with the known (S)-(+)-4-penten-2-ol (111), then subsequent aromatization in the presence of CsOAc and acetic acid proceeded smoothly to yield the resorcylates 114/115 in good yield. A one-pot mild Claisen condensation pathway was also investigated by the same authors for the synthesis of 114/115 in an alternative approach to increase the reactivity and selectivity of C-acylation only. Finally, RCM with HG-II under microwave irradiation followed by acetonide group deprotection accomplished obtaining the target molecule as a major product (Scheme 10).

(b) Synthesis of aigialomycin D by Harvey et al. A combined RCM and RB reaction (Ramberg–Backlund) was employed for the efficient synthesis of aigialomycin D (9), as shown in Scheme 11. The initial part of the synthesis involved preparation of the benzylic bromide 117 (required for RB reaction) from methyl orsellinate. Next, the base-mediated condensation reaction of ethyl acetoacetate afforded methyl orsellinate, which upon subsequent treatment with Ac2O afforded the di-acetylated product. Benzylic bromination was accomplished by treating the di-acetylated compound with NBS and Bz2O in CCl4 solvent to afford compound 117. The other coupling partner required for the RB reaction was accessed from D-ribose. (D)-Ribose, upon functional group manipulation, afforded the iodide 118, which upon Bernet–Vasella fragmentation afforded the olefinic aldehyde 119. The aldehyde was then subjected to Wittig olefination and subsequent reduction of the α,β-unsaturated double bond with CuCl/NaBH4 system followed by LAH treatment to furnish the alcohol 120. A conventional functional group transformation, as shown in Scheme 11, subsequently afforded the thioacetate 121. The thioacetate 121 and benzyl bromide derivative 117 were then coupled together in the presence of K2CO3 to furnish the sulfide 122 in good yield. The free phenolic hydroxyl group in 122 was protected as a –MOM ether followed by methylester hydrolysis to afford the carboxylic acid 123. The Mitsunobu esterification of the acid 123 with (R)-(+)-4-penten-2-ol yielded the RCM precursor 124. Prior to the RCM reaction, the sulfide 124 was oxidized with mCPBA to give a sulfone in excellent yield. Exposure of the sulfone with G-II catalyst under microwave irradiation afforded the cyclised product (E-geometry was observed in the newly formed olefinic unsaturation at C7=C8). The macrocyclic sulfone was then subjected to the Meyers modified RB reaction in refluxing CCl4 in the presence of KOH to accomplish the core framework of the target molecule with the formation of exclusive E-isomer at C1=C2. Deprotection with methanolic HCl furnished aigialomycin D (Scheme 11).

c) Synthesis of L-783,277, LL-Z1640-2 and hypothemycin. Research from Winssinger's group in 2009 revealed an elegant synthetic strategy for the above RALs employing the alkylation of a suitable electrophile on a sulfide or selenide and a
subsequent reductive elimination.\textsuperscript{111} The synthesis was carried out both in solution and in the solid phase to have a better and wider applicability for the overall strategy. The acyclic core of the molecule was synthesized as shown in Scheme 12. The properly substituted olefin was subjected to a CM reaction with $\text{Z}$-2-butene-1,4-diol in the presence of HG-II catalyst to afford allylic alcohol, which upon subsequent Sharpless asymmetric epoxidation,\textsuperscript{112} Parikh–Doering oxidation \textsuperscript{113} and Wittig olefination\textsuperscript{114} afforded the epoxy olefin 131 in good yield.

Epoxide opening and subsequent protection of the diol furnished the corresponding acetonide 132. The ozonolysis of 132 yielded the aldehyde 133, which could also be synthesized from acetonide protected deoxy-(D)-ribose as shown in Scheme 12. The aldehyde 133 was then coupled with (Z)-vinyllic bromide (127, easily accessible from R-methyl-3-hydroxy butyrate or R-pente-4-en-2-ol, as shown in Scheme 12a) to furnish compound 134. Compound 134, upon benzoylation, desilylation and Appel reaction,\textsuperscript{115} afforded the corresponding iodide 135 required for coupling with the properly functionalized sulfide or selenide. Another coupling partner iodide 136 was also synthesized from compound 134, as shown in Scheme 12a.

The sulfide compound 137 was then coupled with the iodoalcohol 136 under Mitsunobu conditions\textsuperscript{116} to furnish compound 138. The sulphide was oxidized to the corresponding sulfoxide, which upon subsequent treatment with KO\textsubscript{t}Bu, furnished the intramolecular alkylated product, and then further thermolysis afforded the olefin 139 with exclusively the E-geometry with the newly created olefinic (C1–C2') unsaturation. Finally, global deprotection with BCl\textsubscript{3} and allylic oxidation with polymeric-resin-supported IBX furnished LLZ-1640-2 (5) in good yield (Scheme 12b).

The remaining two RALs were also synthesized as shown in Scheme 13. Initially, the sulfide 140 (anchored on a polymeric support) was coupled with the iodo compound 135 in the presence of LDA as a base, followed by removal of the PMB group and desilylation to afford the sec-o-acid 141. Mitsunobu macrocyclization of the acid 141 afforded the macroactone core 142 in good yield. Reductive desulphurization was achieved by treating compound 142 with AIBN and FrOct\textsubscript{3}SnH (F = fluorous phase) to afford compound 143. DMP oxidation and deprotection (acetonide and EOM) furnished a phenolic compound, which upon mono methylation by treatment with diazomethane afforded L-783,277 (8). On the contrary, the sulfide 142 was oxidized to the sulfoxide, followed by thermolysis to afford the elimination product 144 (exclusively the E isomer). The benzoyl group deprotection, DMP oxidation, OEOM group deprotection and selective monomethylation proceeded smoothly to afford compound LLZ-1640-2 (5) in excellent yield. Stereoselective epoxidation with DMDO on compound 5 accomplished obtaining hypothenycin (3), as depicted in Scheme 13.

In a subsequent study by Winssinger’s group, the synthesis of several RAL libraries was accomplished by using a fluorous-mixture synthetic strategy, albeit the essence of the main chemistry was similar to in their earlier report.

6.3. Synthetic studies towards RALs in 2010

(a) Synthesis of L-783,277 by Banwell et al. An asymmetric synthesis of the target molecule L-783,277 (8) was accomplished
by an intramolecular Weinreb ketone synthesis protocol. The Weinreb amide and the alkyne were connected through the resorcylic moiety, as shown in Scheme 14. The synthesis commenced with the aromatic aldehyde, which was reacted with a known Weinreb amide under Heck conditions to furnish the olefin. The aldehyde functionality in compound was oxidized under Pinnick conditions, followed by hydrogenation of the olefinic unsaturation with Pd–C to afford compound . The acid was then esterified with enantiopure alkynol under Mitsunobu conditions to furnish the ester in a 70% yield with a neat inversion at the carbon. Finally, ring closing was accomplished by treating compound with LHMDS as a base to generate the acetylide anion, which subsequently attacked the Weinreb amide part to close the ring. The alkyne functionality in compound was then partially reduced to Z-olefin by hydrogenation with Lindlar catalyst, followed by demethylation and acetone deprotection with BCl₃ to afford the natural product L-783,277.

(b) Biomimetic synthesis of \((-\)\)-zearealenone. A novel biomimetic synthesis of \((S)\)-zearealenone \((2)\) was accomplished by Barrett et al. by employing a transannular aromatization and macrocyclization method. Conceptually the strategy was similar to that reported for other RALs by Barrett’s group involving ketene generation through a retro Diels–Alder reaction, trapping the ketene with a chiral alcohol and finally a transannular aromatization cascade sequence, as shown in Scheme 15. The synthesis was initiated from norbornene-2-carboxylic acid \((151)\), which upon Claisen condensation with EtOAc in the presence of LDA afforded the \(\beta\)-ketoester. Subsequent carbonyl protection and a retro Diels–Alder reaction under FVP (flash vacuum pyrolysis) conditions afforded the \(\beta\)-ketoester. Subsequent carbonyl protection and a retro Diels–Alder reaction under FVP (flash vacuum pyrolysis) conditions afforded the \(\beta\)-ketoester.
the keto-dioxinone 156. Compound 156 was then reacted with the known alcohol 157 under CM conditions with G-II catalyst (10 mol%) to afford compound 158. The thermolysis of compound 158 under a retro Diels–Alder pathway afforded the ketene 159, which was intramolecularly trapped by the alcohol moiety to afford the 18-membered macrolactone 160. Subsequent ketal deprotection provided the triketo ester 161, which on immediate transannular aromatization with Cs₂CO₃ afforded the target RAL as anticipated.

(c) Asymmetric synthesis of LL-Z1640-2. In 2010 asymmetric synthesis of the TAK-kinase inhibitor LL-Z1640-2 (5) was reported by Barrett’s group. Conceptually the synthetic strategy was very much similar to that depicted for (S)-zearealenone (Scheme 15) involving a biomimetic macrocyclization and aromatization strategy. The synthesis began with the commercially available 2-deoxy-D-ribose, which upon Wittig olefination with Ph₃P=CHCO₂Et afforded the unsaturated ester 162. Oxidation of the free alcohol group in 162 under Parrikh–Doering conditions afforded the aldehyde 163. The aldehyde 163 was then coupled with lithiated alkyn, followed by protection with EOM-Cl to furnish the alkyne 164 in good yield. The alkyne 164 was next converted to the corresponding Weinreb amide, followed by partial hydrogenation under Lindlar conditions to yield the Z-alkene. The dianion derived from the keto-dioxinone (165) was then coupled with the alkene, and then a subsequent deprotection of the PMB group with DDQ furnished the precursor 166. The thermolysis of 166 afforded the ketene (167), which was immediately trapped intramolecularly by the alcohol moiety to afford the macrocycle 168. Transannular aromatization was attempted with Cs₂CO₃ to furnish the resorcylic core and subsequent protection of the phenolic –OH at the four position afforded compound 169. Finally, acetonide and EOM deprotection with polymer-supported sulfonic acid and allylic oxidation with DMP furnished the desired target, as shown in Scheme 16.

(d) Synthesis of queenslandon. Synthesis of the proposed structure of queenslandon (10) was accomplished by Maier et al. by using a triol-containing chiral building block prepared from β-ribose, as shown in Scheme 17a. Initially, a RCM reaction was attempted to construct the macrocycle core in queenslandon. Initially, β-ribose was converted to compound 170, as shown in Scheme 17a, with a three-step protocol (acetoniode protection, Wittig reaction and pivolyt protection). Acetoniode cleavage in compound 170 with CuCl₂ and treatment of the generated triol with benzaldehyde dimethyl acetal afforded the 1,3-dione derivative 171, which upon treatment with NaH and PMB-Br afforded compound 172. The cross metathesis of 172 with olefin 121 in the presence of G-II catalyst in refluxing
toluene furnished compound 173. Compound 173 was next converted to 174, as shown in Scheme 15. Here, initially, the hydrogenation of 173 with Pd–C and reductive removal of the –OPiv group with DIBAL-H afforded the primary alcohol, which was immediately converted to the corresponding olefinic compound 174 in a two-step protocol, as shown in Scheme 15. The aromatic fragment (175) was readily prepared from the known phthalide by adopting a Wittig olefination, as presented in Scheme 16. Coupling of the acid and alcohol fragment was smoothly performed under Mitsunobu conditions to afford the RCM precursor 176. A slight functional group adjustment was also performed to generate several other RCM precursors (177–181), but the attempted RCM reaction with all the probable RCM precursors (176–181) did not occur as anticipated under numerous conditions (Scheme 17a). The reason for such unsuccessful RCM reactions to yield the macrocycle core of queenslandon was not provided by the researchers.

Finally, the same authors decided to complete the synthesis by using a macro lactonization method. The macro lactonization precursor was synthesized by alkylation of a fully functionalized...
Scheme 13  Synthesis of LL Z1640-2, L-783,277 and hypothemycin.

Scheme 14  Total synthesis of L-783,277.
Scheme 15  Synthesis of (S)-zearalenone.

Scheme 16  Asymmetric synthesis of LL-Z1640-2.
aromatic selenide precursor (184) with the iodide 183. After the alkylation, the –SePh group was oxidatively eliminated by treatment with H₂O₂ to furnish compound 185. Cleavage of the –TBS group and trimethylsilylthanyl (TMSE) group was performed under TBAF conditions to furnish the macrolactonization precursor 186. The lactonization under Mitsunobu conditions proceeded
smoothly to furnish the core resorcylate structure 187, which on the subsequent removal of the –PMB group and further oxidation yielded compound 189. The benzylidine acetal was then removed under acidic conditions, followed by treatment with BCl₃ to furnish the target demethylated compound (proposed structure of queenslandon) in good yield (Scheme 17b).

(e) Total synthesis of L-783,290. The first asymmetric synthesis of L-783,290, the trans isomer of the known RAL L-783,277, was accomplished by Banwell’s group124 by adopting a chemoenzymatic strategy and a late-stage RCM reaction to construct the E-C7’-C8’ unsaturation. The assembly of the C1’-C6’ fragment was done from enzymatically derived arene-cis-1,2-diol (190) derived from the whole-cell biotransformation of chlorobenzene 125 (Scheme 15). The selective hydrogenation and acetonide protection of the diol functionality of 190 furnished compound 191. The ozonolysis of 191 and reduction with NaBH₄ afforded the alcohol. The ester functionality was then converted to its corresponding Weinreb amide 192 by treatment with iPrMgCl and (MeO)MeNH₂/C₂HCl. The free alcohol was then converted to the terminal olefin by the Grieco elimination protocol to furnish compound 193. The Heck coupling of olefin 193 with its styrene derivative 201 with the G-II catalyst furnished the alcohol 202. Further synthetic elaboration of compound 202, as presented in Scheme 17, afforded 203. Preparation of the alkyne fragment was begun from commercially available (S)-propylene oxide, which upon epoxide opening with lithium trimethylsilylacetylene and further protecting group manipulation, accomplished the RCM reaction with the G-II catalyst followed by deprotection afforded the target molecule, as depicted in Scheme 18.

(f) Enantioselective synthesis of L-783,277. An efficient stereoselective synthesis of naturally occurring L-783,277 (8) was achieved by Sim et al.126 in 2010 by employing the CM reaction, acetylide addition to an aldehyde and the Yamaguchi macrolactonization method. The aldehyde (197) derived from β-mannitol upon enantioselective Brown allylation127 afforded the homoalylalcohol 198. Acetonide group deprotection under acidic conditions then furnished the triol 199. Selective protection of the primary alcohol group with TBDPS-Cl and subsequent treatment with 2,2-DMP then afforded compound 200, as depicted in Scheme 19. The aromatic moiety was accessed from readily available 2,4,6-trihydroxy benzoic acid, which upon treatment with acetone in the presence of TFAA/TFA yielded the corresponding acetonide. Regioselective methylation was performed at the C4-position under Mitsunobu conditions to furnish the corresponding methyl ether. The compound was then readily converted to its triflate, followed by Stille coupling128 to furnish the styrene derivative 201 in good yield. The CM reaction of olefin 200 with its styrene derivative 201 with the G-II catalyst furnished the alcohol 202. Further synthetic elaboration of compound 202, as presented in Scheme 17, afforded 203. Preparation of the alkyne fragment was begun from commercially available (S)-propylene oxide, which upon epoxide opening with lithium trimethylsilylacetylene and further protecting group manipulation, accomplished the
desired compound. The DMP oxidation of alcohol 203 yielded the corresponding aldehyde, which upon subsequent reaction with the alkyne afforded the alkynol 204 as a diastereomeric mixture. Partial reduction of the alkyne under Lindlar conditions, followed by PMB protection of the free hydroxyl group and desilylation with TBAF afforded 205. Basic hydrolysis of 205 with NaOH afforded the seco-acid, which upon macrolactonization under Yamaguchi conditions accomplished obtaining the macrocycle 206. Subsequent removal of the PMB group with DDQ and oxidation under DMP afforded the ketone 207.

Finally, acetonide deprotection and regioselective demethylation were performed to access the natural product, as shown in Scheme 19.

(g) **Total synthesis of LL-Z1640-2.** An efficient total synthesis of the potent kinase-inhibitor LL-Z1640-2 (5) was accomplished by Thomas et al. through utilization of a successful exploration of the late-stage NHK (Nozaki–Hiyama–Kishi) reaction, as shown in Scheme 20. The assembly of the Z-vinylhalogen fragment was initiated with a known alcohol. Protection with TBS-OTf, followed by ozonolysis, afforded the aldehyde 213, which upon Stork–Zhao olefination yielded the corresponding Z-vinyllic iodide 214b. In another attempt, the CM reaction of alkene 211 with vinyl boronic acid, followed by treatment with Br₂/MeOH, selective HBr elimination and subsequent desilylation, furnished the Z-vinyllic bromide 214a. The phenyl selenide coupling reaction developed by Winssinger et al. was used to couple the alkyl iodide (210; easily prepared as shown from compound 208 in three steps) with the aromatic fragment 215, with subsequent selenoxide elimination to afford compound 216. Deprotection of the -TMSethanyl (TMSE) group and Mitsunobu esterification with the Z-vinyllic halides (214a/214b) furnished compounds 217a/217b. Deprotection and oxidation under DMP conditions furnished aldehydes (218a/218b), which smoothly underwent intramolecular NHK reactions to afford ring-closed products as diastereomeric mixtures, as shown in Scheme 20, to afford the resorcylic core,
which upon further oxidation and regioselective demethylation with BCl₃ yielded the natural product in an overall good yield.

6.4. Synthetic studies towards RALs in 2011

(a) Multicomponent coupling approach. An expeditious total synthesis of the RAL framework was reported in 2011 by Takahashi et al.¹³⁴ employing a three-component coupling approach. The key reactions involved were intermolecular coupling of a benzyl iodide with a protected cyanohydrin in an umpolung fashion,¹³⁵ carbonylative esterification of an aryl iodide with an alcohol and a late-stage RCM reaction. The strategy enabled a rapid construction of the RAL framework without extra protection/deprotection and installed carbonyl functionality at the C₂⁰-position (as a protected cyanohydrin), as shown in Scheme 21. The synthesis was initiated from 3,5-dimethoxybenzyl alcohol, which upon electrophilic iodination with NIS afforded the aryl iodide 219. The Appel reaction of compound 219 with TPP/CBr₄ furnished the benzyl bromide derivative 220. The protected cyanohydrin was readily accessed from geraniol, which upon allylic oxidation with MNO₂, yielded geranial. The treatment of geranial with TMS-CN/18-c-6 afforded the corresponding cyanohydrin, which was instantly protected as its –OEE derivative 221. The intermolecular alkylation of bromide 220 with the protected cyanohydrins 221 went smoothly in the presence of LHMD to furnish compound 222 in an 83% yield. Compound 222, upon a carbonylative esterification ¹³⁶ reaction with 4-buten-1-ol in the presence of a Pd(Ph₃P)₂Cl₂ and “CO” atmosphere, afforded the ester 223 in a 61% yield. Finally, the RCM reaction of 223 with the G-II catalyst furnished the macrocycle core with absolute stereocontrol in favour of the “E” geometry in the newly formed olefinic unsaturation (C₇⁻C₈).

(b) Synthesis of the proposed structure of pochonin-J. The synthesis of the proposed structure of the naturally occurring RAL pochonin-J was accomplished by Jennings’ s group in 2011.¹³⁷ The key reaction involved in the synthesis was a chemoselective Wacker oxidation and Evans–Saksena anti-reduction to access an advanced intermediate. Finally, stereoselective allylation of an oxocarbenium precursor and a late-stage RCM reaction was explored to complete the synthesis, as delineated in Scheme 22. Initially, prenyl Grignard addition to TBDPS-protected S-glycidol ether (227) in the presence of Li₂CuCl₄ proceeded smoothly and yielded the alcohol 228 in an almost quantitative yield.
Protection of the free hydroxyl group in 228 was accomplished by treatment with MOM-Cl, followed by desilylation of the corresponding primary TBDPS ether-furnished alcohol 229. Ley–Griffith oxidation of 229 yielded the aldehyde, which upon subsequent asymmetric allylation with Brown’s protocol readily afforded the homoallylic alcohol 230 with excellent diastereoselection. The free hydroxyl group in 230 was then protected as its corresponding –TES ether under normal conditions. Wacker oxidation proceeded smoothly with 10 mol% of PdCl₂ and 0.2 equivalent of Cu(OAc)₂ under an oxygen atmosphere to furnish the methyl ketone in a 77% yield. Removal of the –TES group was achieved with TBAF to afford the β-hydroxy ketone, which upon reduction under Evans–Saksena conditions with Me₄N+B(OAc)₃H furnished the anti 1,3-diol 231. The oxidative cleavage under ozonolysis conditions of compound 231 followed by 6-exo-trig cyclization afforded the hemiacetal, which was immediately acetylated to furnish the bis-acetyl hemiacetal 232. Upon the exposure of compound 232 to BF₃/CO₂Et₂, the generated endocyclic oxocarbenium cation, which was stereoselectively trapped with allyltrimethyl silane, furnished the α-C-glycoside. Apparently, it seems that the allylation of oxocarbenium cations (233) occur through a stabilized chair-like transition state via the axial addition of the allylsilane to afford the α-C-glycoside. Finally, removal of the acetate functionality with K₂CO₃ afforded compound 234 in an almost quantitative yield.

The transesterification of the known styrene derivative 226 with compound 234 proceeded smoothly in the presence of NaH in THF/DMF (1 : 1) to furnish the RCM precursor 235 in a 77% yield. Upon exposure to the G-II catalyst, the RCM reaction underwent smoothly to furnish the core RAL framework in a 97% yield. The free phenolic hydroxyl group was subsequently protected as its MOM ether 236. The stereoselective epoxidation of compound 236 with mCPBA furnished the corresponding β-epoxide as a single diastereomer in an impressive 82% yield. The reductive ring opening of epoxide was accomplished with Pd–C/H₂ to afford the homo-benzylic alcohol (237), which upon subsequent oxidation with DMP and MOM deprotection afforded the proposed structure of ent-pochonin-J. Though the spectroscopic discrepancies between the synthetic and the natural product suggested that a structural revisit was required to assign the correct structure, the synthetic strategy was unique with its own merits.

6.5. Synthetic studies towards RALs in 2012

(a) Asymmetric synthesis of cochliomycin A and zeaenol. In 2012, we first disclosed the asymmetric synthesis of a rare acetone containing RAL, cochliomycin A and another earlier known RAL named zeaenol. The synthetic strategy involved a successful exploration of ME-DKR (metal enzyme combined dynamic kinetic resolution) strategy to access an enantiopure secondary alcohol as an advanced intermediate.
moiety was synthesized from the commercially available cheap starting material 3,5-dihydroxybenzoic acid. Esterification and reduction with LAH afforded the corresponding benzyl alcohol, which upon PCC oxidation,\(^\text{144}\) Wittig reaction and Vilsmeier–Haack formylation\(^\text{145}\) afforded the aldehyde \(239\) as a sole product. Selective demethylation with \(\text{BBr}_3\) and Pinnick oxidation furnished the carboxylic acid \(240\), as shown in Scheme 23. The chiral alcohol \((241)\) obtained through a ME-DKR (metal enzyme dynamic kinetic resolution) pathway was functionally manipulated to the corresponding sulfone \(243\), as shown below. JK-olefination\(^\text{146}\) was explored to construct the \(\text{C}^7'–\text{C}^8'\) olefinic unsaturation in a stereoselective way. The known \(\text{C}_2\)-symmetric diol derived from l-tartaric acid was chosen as a starting material. Selective monoprotection and oxidation under Swern conditions afforded the aldehyde \(244\). The aldehyde \(244\) was then subjected to Keck asymmetric allylation\(^\text{147}\) with allyltributylstannane and \((S)\)-BINOL to furnish homo-allylic alcohol with excellent diastereocontrol. The free alcohol was protected as its –PMB ether by treatment with \(\text{NaH}\) and \(\text{PMB-Br}\) to afford compound \(245\). The subsequent desilylation of \(245\) with \(\text{TBAF}\) afforded the primary alcohol, which upon oxidation with \(\text{DMP}\) furnished the desired aldehyde \(246\) in good yield. The aldehyde \(246\) was immediately subjected to JK-olefination with the sulfone \(243\), as depicted in Scheme 23. Coupling of the alcohol with the acid \(240\) under Mitsunobu conditions, followed by removal of the PMB group with \(\text{DDQ}\), proceeded smoothly to furnish the RCM
precursor 248. RCM with the G-II catalyst in refluxing DCM for 6 h afforded the ring-closed product cochliomycin A (38) with excellent stereocontrol for the newly formed C1'-C2' olefinic unsaturation. Finally, deprotection of the acetonide functionality afforded another natural product zeaenol (41), as shown in Scheme 20.

(b) Total synthesis of 5',6'-epi-paecilomycin F. In the same year, we also disclosed the synthesis of another stereoisomer of naturally occurring RAL paecilomycin F.148 The molecule we synthesized was named as 5'-epi-paecilomycin F in the original report, but as structural reassignment of the paecilomycin was done at a later stage,22 our synthesized molecule should be now regarded as 5',6'-epi-paecilomycin F. The reported synthesis was conceptually similar with our earlier work as successful exploration of the late-stage RCM reaction was employed. The synthetic journey began with 1,5-pentanediol. Functional group manipulation, as shown in Scheme 24, furnished the alcohol 249. ME-DKR of the alcohol 249 was then attempted with a Ru-based racemization catalyst and CAL-B as an enzyme with isopropenyl acetate as an acyl donor. The reaction proceeded smoothly with excellent enantioselection (ee = 98%) in favour of the desired stereoisomer to afford the acetate 250. Removal of the acetate functionality in 250, protection of the free hydroxyl group as its -TBDPS ether, deprotection of PMB ether with DDQ and oxidation under Swern conditions149 furnished the aldehyde 251 in a reasonably good yield. HWE olefination150 of the aldehyde 251 with triethyl phosphonoacetate furnished the E-α,β-unsaturated ester, which was next subjected to dihdroxylation under Sharpless conditions with Admix-β to furnish the diol 252 with excellent stereocontrol. The diol 252 upon subsequent treatment with 2,2-DMP and partial reduction of the ester group with DIBAL-H furnished the aldehyde 253. The aldehyde 253 was next subjected to Keck asymmetric allylation and afforded the homo-allylic alcohol with commendable stereocontrol. The free hydroxyl group was protected as its MOM ether, followed by desilylation with TBAF to furnish compound 254. The alcohol 254 was then coupled with the known acid (240) under Mitsunobu conditions to accomplish the RCM precursor 255. RCM of 255 with G-II catalyst in refluxing DCM furnished the resorcylate core (256), which upon deprotection of acetonide and the MOM group yielded the 5',6'-epi-paecilomycin F (Scheme 24).

(c) Total synthesis of pochonin E and F and structural revision. The asymmetric total syntheses of pochonin E and F and their C6'-epimers were accomplished by Winssinger et al. in 2012.151 The reaction of the vinyluous silyl dienol ether 257 with 2-butenal in the presence of Denmark's catalyst152 afforded the
alcohol 258 in a 60% yield (ee = 94%). The free alcohol was then protected as its -TBS ether, and subsequent treatment with the magnesium salt of Weinreb amine afforded the amide 259, as shown in Scheme 25. The enantiomer Weinreb amide was also synthesized by adopting a similar protocol. The known aromatic precursors (260/261) were deprotonated at the benzylic position with LDA to furnish the corresponding lithiated species, which immediately reacted with the Weinreb amide 259 to furnish the RCM precursors. Finally, RCM reaction with the G-II catalyst (0.5 mol%) and subsequent deprotection of the -EOM and TBS group with polymer-supported sulfonic acid yielded all the stereoisomers of pochonins E and F. Upon close comparison with the spectral data it was revealed that the naturally occurring pochonin E and F both had a 6R configuration, which was later confirmed by X-ray crystallographic analysis.

(d) Total synthesis of paeclimycin F. Srihari et al. in 2012 reported an asymmetric synthesis of paeclimycin F by adopting a late-stage RCM approach. Later on, structural revision revealed that paeclimycin F should now be regarded as paeclimycin F. Their reaction involved the synthesis of the aromatic precursor from 2,4,6-trihydroxybenzoic acid through a known protocol to access the styrene derivative 268, as shown in Scheme 26. The other precursor was accessed from L-DET, as presented in Scheme 26. The known diol was mono protected as its benzyl ether (269), which upon oxidation under Swern conditions furnished the aldehyde 270. The aldehyde was next homologated to the alkyne 271 by an Ohira–Bestman protocol154 and by subsequent reaction of the lithiated alkyne with the enantiopure epoxide (R-propylene oxide) to furnish the homopropargylic alcohol 272. The free alcohol was protected as its -TBS ether and hydrogenated with Pd–C/H2 (debenzylation also occurred) to afford compound 273. The Swern oxidation of alcohol 273, followed by Barbier allylation and subsequent protection with MOM-Cl and DIPEA afforded compound 274 with good stereocontrol. Protection group adjustment by desilylation was performed with TBAF to furnish the corresponding alcohol in a good yield. Esterification under the Mitsunobu protocol with the styrene derivative 268 afforded the RCM precursor 275. RCM with the G-II catalyst in refluxing DCM, followed by deprotection of the acetonide and MOM functionality furnished the natural product, as shown in Scheme 26.

6.6. Synthetic studies towards RALs in 2013

(a) Synthesis of 5’,6’-epi-cochliomycin C. The asymmetric total synthesis of chlorine containing naturally occurring RAL was reported by our group in 2013. Initially the compound was named as 5’-epi-cochliomycin C, but later on structural revision suggested that it should be considered as the 5’,6’-epi stereoisomer of the natural product. The introduction of the “Cl” group at the C5-position of the aromatic ring was accomplished from the known aldehyde under modified Pinnick conditions. Thus, when the aldehyde was subjected to oxidation with NaClO2 and NH2SO3H, the oxidation and the incorporation of the “Cl” group took place to furnish the acid 276. The acid was next coupled with a known alcohol precursor (254) earlier synthesized by our group to afford the ester 277 under Mitsunobu conditions. Attempted RCM reaction of the ester under numerous conditions all failed (Scheme 27a), and instead the dimerized product 278 was always obtained through a CM reaction. Hence, later on, we opted for the macrolactonization
Scheme 25  Total synthesis of pochonin E and F and their structural confirmation.

Scheme 26  Synthesis of paecilomycin F.
method to construct the RAL framework. The acid 276 was protected as its acetonide and then subsequent oxidative cleavage afforded the aldehyde 280. The alcohol (281) was next converted to its corresponding sulfone by the use of conventional methods, as disclosed in Scheme 27b. JK-olefination of the sulfone and aldehyde in the presence of KHMDS afforded the olefin with great stereocontrol at the newly formed olefinic unsaturation (which is in favour of the E-geometry). Cleavage of the cyclic ester moiety and removal of the –TBDPS group furnished the seco-acid 284 in good yield. Finally, macrolactonization under the Mitsunobu protocol afforded the ring-closed product (285), which upon subsequent deprotection furnished 5′,6′-epi-cochliomycin C (Scheme 27b).

6.7. Synthetic studies towards RALs in 2014

(a) Synthesis of paecilomycin E and F. The asymmetric total synthesis of paecilomycin E and F was accomplished by Mahapatra et al.\textsuperscript{157} by employing a protecting-group-directed diastereoselective intermolecular NHK reaction and late-stage macrolactonization under De Brabander conditions.\textsuperscript{158}
The synthesis was started with the known aromatic precursor 286, which upon methylation under Mitsunobu conditions furnished compound 287. Benzyl bromination was performed with compound 287 by treatment with NBS and benzoyl peroxide (BPO, Bz2O) to furnish compound 288. The benzylic bromide 288 upon treatment with 1-phenyl-1H-tetrazole-5-thiol afforded the sulfide 289 in a reasonably good yield. Later on, the sulfide was oxidized in the presence of mCPBA to furnish the sulfone 290, as shown in Scheme 28. The other fragment was accessed from a known precursor 291 (easily prepared from D-mannitol). Protecting group manipulation of compound 291 afforded the diol 292 (i.e. deprotection of the ketal functionality and selective protection at the primary alcohol as its –TBS ether). Acetonide protection of the diol functionality was achieved by treatment with 2,2-DMP and subsequent removal of the TBS group, followed by oxidation of the alcohol under DMP conditions to furnish the aldehyde 293. The aldehyde 293 was next subjected to an intermolecular NHK coupling reaction with the known vinylic iodide 117 to afford the diastereomeric alcohols 294 and 295 in 7:3 ratios. Later on, the authors found that changing the protecting groups had a dramatic effect on the product ratio; for instance, when the protecting groups in aldehdye 293 were changed to –TBS, the diastereomeric ratio was enhanced to an impressive 19:1. However eventually, both compounds 294 and 295 were required for total synthesis of the above RALs. Protection of the free hydroxyl group in 294 as its –MOM ether and oxidative cleavage under Johnson–Lemieux conditions afforded the aldehyde 296, which was then subjected to JK-olefination with the sulfone 290 in the presence of KHMDS to furnish the olefin 297 with overall good stereocontrol. Desilylation under standard conditions and base-induced intramolecular transesterification afforded the macrocyclic core. Finally, deprotection of the acetonide and MOM group with HCl furnished paecilomycin E (33). The alcohol 295 was later on synthetically elaborated by following a same series of transformations to access paecilomycin F (34), as shown in Scheme 28.

Scheme 28  Total synthesis of paecilomycin E and F.
Overall the synthetic pathway delineated in Scheme 28 was shown to be very efficient as both natural products could be accessed in a stereodivergent way.

(b) **Total synthesis of cryptosporiopsin A.** In the same year, Mohapatra et al. investigated the synthesis of another recently isolated RAL: cryptosporiopsin A (46), which seems to exhibit motility inhibitory and lytic activities against zoospores of the grapevine downy mildew pathogen *Plasmopara viticola*. The synthesis was completed by the exploration of Jacobsen's HKR reaction, Stille coupling and late-stage RCM reaction. The enantiopure epoxide 298, obtained by HKR reaction from the corresponding racemic epoxide, was reductively opened with LAH to afford an alcohol. The free alcohol was then protected as its –PMB ether, and a subsequent debenzylation with RANEY-Ni/H₂ afforded the alcohol 299. The oxidation of the alcohol 299 with BAIB and TEMPO, followed by the Grignard addition of 3-butenyl magnesium bromide afforded compound 300 as a mixture of diastereomers. The free alcohol functionality in compound 300 was subsequently protected as its –TBS ether to furnish 301. The removal of the –PMB group in 301 was achieved with DDQ to furnish compound 302. Regioselective protection of the sterically less hindered phenolic –OH group in compound 302 under Mitsunobu conditions with 4-methoxybenzyl alcohol, followed by a subsequent conversion of another free phenolic-OH group to its triflate and then Stille coupling with allyltributyl stannane furnished compound 304. Oxidative cleavage by a modified Jin’s protocol furnished the corresponding aldehyde in good yield. The aldehyde was then immediately reacted with CH₂=CHMgBr with concomitant protection of the free alcohol as its –TBS ether to furnish compound 305. The transesterification of compound 305 by De Brabander’s protocol with the alcohol 302 and its subsequent methylation with Mel afforded the required ester in a good yield. Installation of the required “Cl” group at the C₅-position of the aromatic ring was accomplished by electrophilic chlorination with NCS to afford compound 306 in a 77% yield. The RCM reaction of compound 306 proceeded smoothly with the G-II catalyst in refluxing DCM for 18 h, and yielded the desired RAL framework in a 74% yield. Didesilylation with TBAF and oxidation under DMP afforded the diketone, which upon subsequent removal of the –PMB group with TiCl₄ furnished the natural product (Scheme 29).

(c) **Total synthesis of paecilomycin E and its structural analogues.** Recently in a detailed study, we disclosed the asymmetric total synthesis of naturally occurring paecilomycin E and two of its close structural congeners 10-epi-paecilomycin E and 6-epi-cochliomycin C. The synthetic strategy involved the successful application of late-stage Mitsunobu macrolactonization (through hydroxyl group activation by SN₂ inversion), E-stereoselective JK-olefination, substrate-directed stereoselective dihydroxylation and Z-selective Wittig olefination. The aliphatic fragment was accessed from the known racemic alcohol 307. EKR (enzymatic kinetic resolution) coupled with Mitsunobu inversion furnished the enantiopure (S)-307. The free hydroxy group was protected with TBS-Cl to afford the corresponding –TBS ether 309. Subsequent removal of the –PMB group, 

![Scheme 29](image-url)
conversion of the free –OH to its iodo (by a two-step reaction) and refluxing with Ph₃P furnished the phosphonium salt 310. The salt 310 was then treated with KHMD to generate the ylide, which was subsequently reacted with the aldehyde 313 (synthesized from the known compound in two steps as shown) to afford the Z-olefin 314 as a major diastereomer. The substrate-directed dihydroxylation of olefin 314 furnished the diastereomeric diols 316 and 315 in an 8:1 ratio. The origin of this good diastereoselection can be explained through the Kishi empirical model, which utilizes the $A^{1,3}$ strain as a governing factor. The major diol 316 was protected as its acetone and then subsequent removal of the –PMB group and functional group adjustment afforded the sulfone 320.

Two of the required aromatic fragments were synthesized from 3,5-dimethoxy benzaldehyde. Regioselective bromination furnished the bromo compound, which upon aldehyde protection with ethylene glycol furnished the acetal. The bromo acetal was then treated with n-BuLi, and then subsequent reaction with ethylchloroformate and acetal deprotection with PTSA furnished the corresponding ester 321. The introduction of the desired “Cl” atom in the aromatic ring was done by treating compound 321 with SO₂Cl₂ to furnish compound 322. Both compounds 321 and 322 are required for the overall synthesis. The sulfone 320 was then coupled with the aromatic aldehyde 321 under JK-olefination conditions to furnish the olefin 323 with excellent stereocontrol in favour of the E-olefin (C1’–C2’). The base-mediated hydrolysis of the ester group in 323 met limited success, probably due to the depleted nucleophilicity of the ester carbonyl in the presence of the electron-releasing –OMe group at the C2- and C4-positions of the aromatic ring. Nevertheless, an extra three-step protocol, involving reduction of the alcohol and re oxidation of the alcohol to the acid, furnished carboxylic acid. The –TBS group in the acid was judiciously deprotected in the presence of acetonide by treating the compound with an excess of 2,2-DMP and PPTS in acetonitrile, to furnish smooth removal of TBS group to afford the seco acid 324. Numerous macrolactonization methods involving a carboxylic activation protocol, such as the Yamaguchi, Keck and Shiina methods all failed miserably. On the contrary, the alcohol activation method through the Mitsunobu protocol worked nicely with clean inversion at the C10 stereocenter to furnish compound 325. Regioselective demethylation, acetonide removal and then desilylation with HF-pyridine afforded 10-$\text{epi}$-paecilomycin E. Later on, the same strategy was applied to access the natural product paecilomycin E and its chloro analogue 6-$\text{epi}$-cochliomycin C, as shown in Schemes 30a and b.

(d) Synthesis of zeaenol, 7-$\text{epi}$-zeaenol and a few analogues. Mohapatra et al. reported the asymmetric synthesis of zeaenol (41) and a few of its structural analogues through successful exploration of protecting-group-directed intermolecular NHK coupling as a key reaction, as disclosed in Scheme 31. The synthetic planning was similar to that reported for paecilomycin E and F from the same group early in 2014. The vinylic iodide was accessed from the known epoxide 333, as depicted in Scheme 31. Reductive opening and protecting the free hydroxyl group as its –TBS ether furnished compound 241. Removal of the –PMB group, followed by DMP oxidation and subsequent Takai olefination afforded the E-iodide 334. The benzyl sulfone 335 was also previously prepared by their group;
whereas the aldehyde partner 337, required for the JK-olefination, was synthesized from D-mannitol by an established protocol. The JK-olefination of sulfone 335 went smoothly with aldehyde 337 in the presence of KHMDMS to yield the E-olefin 338. Later on, protecting-group manipulation and oxidation furnished the aldehyde 339, which upon NHK coupling with the iodide 334 furnished the coupled alcohol. The free alcohol was then protected as its –MOM ether, together with removal of the TBS group with TBAF, and then upon subsequent treatment with NaH, the intramolecular transesterification proceeded smoothly, followed by benzyl protection to yield the lactone 340. Finally, the global deprotection of the benzyl and MOM group in compound 340 with TiCl4 in DCM afforded the 6'-epi-zeaenol (341). For the synthesis of the natural product, first the lactone 340 was subjected to –MOM deprotection and oxidation under DMP conditions to afford the ketone. The ketone was then next reduced with (S)-CBS reagent to furnish the alcohol with the required configuration, followed by deprotection of the benzyl group with TiCl4 to afford the zeaenol, as shown in Scheme 31. Zeaenol (41) and 6'-epi-zeaenol both were hydrogenated with Pd–C/H2 to furnish two close structural analogues of related RALs.

**Scheme 31** Total synthesis of zeaenol and a few of its stereoisomers.

(e) **Total synthesis of cochliomycin A.** A convergent and flexible total synthesis of the naturally occurring RAL cochliomycin A (38) was reported by Du’s group by employing a chiral pool approach. The synthesis was initiated from L-arabinose as a chiral pool starting material, which was initially protected as its diacetonide by treatment with 2,2-DMP. Next, Wittig olefination of the aldehyde with Ph3P=CH2 and selective deprotection of the terminal acetonide group with 75% AcOH furnished the diol 343. Regioselective mono-tosylation of the diol was achieved by treating it with dibutyltin oxide and Ts-Cl, and then subsequent treatment with a base (K2CO3) afforded the epoxy olefin 344 in good yield. Cross metathesis (CM) reaction of olefin 344 with the known enantiopure homoallylic alcohol 169 in the presence...
of G-II catalyst yielded the \(E\)-olefin 345 in an 85% yield. The treatment of lithiated TMS-acetylene with the epoxoolefin 345 in the presence of BF\(_3\)/OEt\(_2\), followed by desilylation furnished the corresponding homopropargylic alcohol. The alkynol was later converted to its corresponding iodo compound, while subsequent treatment of lithiated TMS-acetylene with the epoxyolefin in the presence of BF\(_3\) afforded the olefinic alcohol, as shown in Scheme 32. The stannane was then coupled with the known aromatic triflate under Stille coupling conditions to afford the \(E\)-olefin 347 in an excellent yield. Intramolecular transesterification of compound 347 under De Brabander conditions furnished cochliomycin A (38).

(f) **Total synthesis of L-783,290.** An asymmetric synthesis of L-783,290 was disclosed by Subba-Reddy et al. in 2014 through an elegant exploration of the Alder–Rickert reaction for the construction of the aromatic moiety present in L-783,290. The synthesis began with the known tosylate 348, which could be easily accessed from \(\alpha\)-ribose. The tosylate 348 was treated with lithium acetylide ethylenediamine complex to furnish the alkynyl species, which upon subsequent methoxy carbonylation with methyl chloroformate and \(\alpha\)-BuLi afforded the carbohydrate-tethered alkynol 349. The Alder–Rickert reaction was next attempted with the alkynyl ester 349 with 1,3-dimethoxy-cyclohexa-1,4-diene in the presence of \(N,N\)-dimethyl aniline at 180 °C. The role of \(N,N\)-dimethyl aniline was speculated to help in the isomerization process of the diene to convert it to a 1,3-diene to afford \([4+2]\) cycloaddition. The cycloaddition and cycloreversion (expulsion of ethylene) proceeded smoothly to furnish compound 350. Desilylation of the TBS group was achieved by treating compound 350 with TBAF to furnish the corresponding primary alcohol. Appel reaction with I\(_2\) and TPP afforded the corresponding iodo compound, while subsequent Barrett–Varsella fragmentation afforded the olefinic alcohol, as shown in Scheme 33. The free hydroxyl group was next protected as its \(-\)MOM ether with MOM-Cl and DIPEA to furnish compound 351. The basic hydrolysis of the ester 351 afforded the corresponding carboxylic acid, which was next esterified with \((R)\)-pentene-2-ol under Mitsunobu conditions to furnish compound 352. The RCM of compound 352 with the HG-II catalyst in refluxing toluene afforded the ring-closed product in a 78% yield. Deprotection of \(-\)MOM and acetonide and regioselective demethylation with BCl\(_3\) afforded the triol, which upon allylic oxidation with the polymeric-resin-supported IBX afforded the natural product L-783,290, as depicted in Scheme 33.

(g) **Total synthesis of cochliomycin B and zeaenol.** The first asymmetric synthesis of cochliomycin B (39) was reported by Du et al. through an elegant exploration of a Suzuki cross-coupling reaction for the construction of an aromatic fragment with a fully functionalized aliphatic fragment. \(\alpha\)-Arabinose was used as a chiral pool starting material for the synthesis. Acetonide protection and subsequent Wittig olefination afforded the diol 353. The free hydroxyl groups in diol 353 were protected as its \(-\)TBS ether, followed by selective desilylation with PPTS to furnish the alcohol 354. The oxidation of compound 354 under DMP conditions furnished the aldehyde 355 in an 86% yield. Wittig olefination of the aldehyde 355 with Ph\(_3\)P\(\text{CH_2OMeCl}\) in the presence of KO\(_2\)Bu as a base, followed by hydrolysis of the resultant enol–ether afforded the homologated aldehyde 356. The aldehyde 356 was then subjected to Takai olefination to furnish the \(E\)-vinylc iodide 357 \((E:Z = 4.5:1)\). The Suzuki coupling reaction of the fully functionalized aromatic boronic acid 358 with the iodide 357 went smoothly to furnish the olefinic ester 359. Base-induced transesterification with \((S)\)-4-penten-2-ol afforded the ester 360, which upon exposure with G-II catalyst afforded the ring-closed product containing the RAL framework with overall excellent...
stereocontrol in favour of E-olefin. Finally, desilylation afforded cochliomycin B, which upon further acetoneide deprotection yielded zeenaol, as depicted in Scheme 34.

(h) Total synthesis of neocosmocin A. Neocosmocin A (43), having an in vitro binding affinity towards human opioid and cannabinoid receptors, was first isolated in 2012. The first asymmetric synthesis of this RAL molecule was disclosed by Das et al. in 2014. The synthesis was done in a convergent way through the coupling of individual fragments in a sequential manner. The fragments could be accessed separately, as delineated in Scheme 35. The aromatic fragment was first prepared from methyl acetoacetate, which upon base treatment afforded the aromatic compound methyl 2,4-dihydroxy-6-methylbenzoate. Subsequent regioselective methylation under Mitsunobu conditions, followed by MOM protection of the free phenolic –OH and basic hydrolysis then furnished compound 361.

The alcohol part was synthesized from homoallylic alcohol, which upon epoxidation, followed by the Jacobsen HKR method provided the enantiopure epoxide 362. Reductive cleavage with LAH and silylation with TBSCI afforded compound 363, which upon debenzylization with Li-naphthalene afforded the alcohol 364. Finally, Swern oxidation, Wittig olefination and desilylation afforded (R)-4-pentene-2-ol, as shown in Scheme 35. The Weinreb amide fragment 366 was synthesized from cyclohexanone through a conventional pathway, as shown below. Coupling of the aromatic fragment 361 with (R)-4-pentene-2-ol under Mitsunobu conditions afforded the ester 367. The ester 367 then, upon treatment with LDA, furnished the benzylic lithiated species, which upon subsequent treatment with the Weinreb amide 366 provided the keto olefin 368. The –MOM ether in compound 368 was then deprotected by treatment with HCl to furnish the RCM precursor. A late-stage RCM reaction with G-II catalyst in refluxing DCM afforded the target molecule in excellent yield. The synthesis was unique in the sense that all the used starting materials were very cheap and commercially available.

6.8. Synthetic studies towards RALs in 2015

(a) Total synthesis of neocosmocin A and structural reassignment. In 2015, Banwell reported an elegant synthesis of neocosmocin A and ent-neocosmocin A and established the correct absolute configuration in the naturally occurring RAL. The main highlight of the synthesis was the successful exploration of the metathesis reaction (CM and late-stage RCM) and Pd-catalyzed Meinwald type rearrangement. The synthesis was initiated from the known styrene compound 201 and the olefinic acetal 369, following a successful CM reaction in the presence of G-II catalyst to afford compound 370 in favour of the exclusive formation of the E-geometry at the newly formed olefinic bond. The attempted transesterification
of compound 373 with a known alcohol under De Brabander conditions did not work; hence the complete hydrolysis of 373 was accomplished to furnish an acid, which upon subsequent esterification under Mitsunobu conditions with both the enantiomers of 4-pentene-2-ol worked excellently to furnish both the enantiomers of ester 374. Finally, RCM with G-II catalyst in refluxing DCM afforded neocosmocin A and ent-neocosmocin A in good yields.

(b) Total synthesis of cochliomycin A and B and zeaenol. In the same year, investigations performed by Banwell’s group revealed an efficient synthesis of the above RAL molecules through the successful execution of an intramolecular NHK coupling reaction (for the construction of C6–C7). Initially, the E-vinyllic iodide 376 was synthesized from the known alcohol, as shown in Scheme 37. The free alcohol was protected as its –TBS ether, and then a subsequent CM reaction with commercially available pinacol ester of vinylboronic acid in the presence of HG-II catalyst was achieved and furnished compound 380 in an 86% yield. The base-mediated transesterification of 380 with E-iodide 376 proceeded smoothly to provide the corresponding phenolic compound. The free phenolic –OH group was next protected as its –SEM ether by treatment with SEM-Cl and Hunig’s base, followed by selective desilylation with TBAF at 0 °C to furnish the alcohol 381. The oxidation of compound 381 with DMP afforded the corresponding aldehyde. The aldehyde was then immediately subjected to an intramolecular NHK reaction to furnish compound 382 as a single diastereomer. Treatment with TBAF afforded the naturally occurring cochliomycin B in a 73% yield; whereas the treatment of compound 382 with methanolic HCl furnished SEM deprotection and acetonide isomerization to afford cochliomycin A in a good yield. The deprotection of both the SEM and acetonide was achieved under a methanolic HCl/water mixture to furnish zeaenol, as shown in Scheme 37.

(c) Total synthesis of zeaenol. In 2015, Meshram et al. disclosed the synthesis of zeaenol through two alternative and independent pathways for accessing a key intermediate. In the initial strategy, a Stille coupling with the aromatic triflate with E-stannane was the key reaction, whereas in the second pathway, a Sonogashira coupling followed by a Trost
Intramolecular hydrosilylation protocol was the key reaction. The synthesis was initiated from D-xylose, which was first converted to the known compound 383. The functional-group manipulation of 383 (thioketal removal, aldehyde reduction and benzyl protection) furnished compound 384, which upon selective acetonide removal with TFA furnished the diol 385. Selective protection of the primary hydroxyl group in compound 385 as its –TBS ether, followed by treatment with MsCl afforded the mesylate, which upon treatment with TBAF yielded the epoxide 386 in good yield. The epoxide was then opened with lithium acetylide, followed by –MOM protection to generate an alkyne. The alkyne was next converted to its corresponding E-stannane 387 by a conventional method, as shown in Scheme 36. The Stille coupling between the aromatic triflate and E-stannane 387 proceeded smoothly to furnish the coupled product, which upon debenzylation with DDQ afforded the alcohol 388. The oxidation of the alcohol 388 with IBX afforded the aldehyde 389 in good yield. In another attempt, the triflate was coupled directly with the alkyne 390 under Sonogashira conditions to furnish compound 391 in good yield. The homopropargylic compound 391 then underwent hydrosilylation upon treatment with tetramethyldisilazane (TMDS) in the presence of [Cp*Ru(MeCN)3]PF6 to afford the cyclic siloxane product 392. The cyclic siloxane was then immediately treated with a catalytic amount of CuI in the presence of TBAF to afford the protodesilylated product, which upon further protection as its –MOM ether, followed by debenzylation with DDQ and IBX oxidation furnished the aldehyde 389 in an alternative way (Scheme 38a). The aldehyde 389 upon stereoselective JK-olefination with a known sulfone (synthesized from methyl acetoacetate, as shown in Scheme 38b) afforded the olefin 393. Removal of the –TBDPS group, base-induced intramolecular transesterification and subsequent deprotection afforded zeaenol, as shown in Scheme 38b.

(d) Total synthesis of cochliomycin C and paecilomycin E and F. The first asymmetric synthesis of chlorinated RAL cochliomycin C (40) was reported by Srihari's group in 2015. In addition, they also completed the synthesis of paecilomycin E and F and 6'-epi-cochliomycin C. The synthesis was initiated from D-lyxose, as shown in Scheme 39, with acetonide protection performed to afford compound 395. Formation of the alkyne was achieved under Ohira–Bestmann conditions to furnish compound 396 as diastereomeric mixtures. The free dial was then further protected as its di-acetonides 397 (major) and 398. Compound 397 was then reacted with the enantiopure epoxide in the presence of n-BuLi and BF3:OEt2 to furnish the alkylnol 399.
which upon further reduction with RANEY™-Ni/H₂ furnished the alcohol 400 in good yield. The alcohol 400 was then reacted with the known styrene compound 189 to afford the ester 401, as depicted in Scheme 39a. Regioselective terminal acetonide cleavage was achieved by the treatment of compound 401 with methanolic H₂SO₄ at 10 °C to furnish the aldehyde 402. The aldehyde 402 upon Barbier allylation with Zn/allyl bromide furnished the homoallylic alcohol 403 as a single diastereomer. The RCM reaction of 403 with Hg-II catalyst, followed by acetonide deprotection furnished paecilomycin F (34). Electrophilic chlorination of paecilomycin F with SO₂Cl₂ at 0 °C afforded cochliomycin C (40) in a 90% yield.

For the synthesis of paecilomycin E, the same authors changed the strategy a little bit to overcome the unwanted epimerization that occurred during alkyne synthesis under Ohira–Bestman conditions. So the initially obtained acetonide-protected D-lyxose (395) was subjected to Wittig olefination to furnish the olefin, which upon further acetonide protection afforded the diacetonide olefin 404. Oxidative cleavage, followed by treatment with TPP/CBr₄ afforded the dibromide 405, which was immediately treated with n-BuLi to furnish the alkyne, which was reacted with the epoxide in the same flask to furnish the alkyne 406. By adopting a similar sequence of reactions, the total synthesis of paecilomycin E (33) and 6-epi-cochliomycin C was achieved (Scheme 39b).

6.9. Synthetic studies towards RALs in 2016

(a) Asymmetric synthesis of neocosmocin A. An elegant synthesis of neocosmocin A (43) was accomplished by Cho et al. in 2016 through successful exploitation of an intramolecular Diels–Alder addition (IMDA) of a bromopyrone containing a bromo-propiolate as a dienophile, as presented in Scheme 40. The synthesis began with the E-selective JK-olefination of an alkyne 411 with the known sulfone 412 to afford the enyne 413. A Sonogashira cross-coupling reaction between the enyne 413 with the bromo-pyrone afforded compound 414. HgO-Mediated alkyne hydration followed by desilylation furnished the keto alcohol 415 in a 90% yield, which was then immediately esterified with propiolic acid under Mitsunobu conditions to afford the alkyne ester 416. Treatment of the alkyne with NBS–AgNO₃ system afforded the bromo-alkyne as an IMDA precursor in a 76% yield. The IMDA reaction proceeded smoothly to furnish the dibromobenzo macrocyclic lactone 417 in a 65% yield. The dibromo species was converted to a dipinacolboryl derivative by Miyura conditions, which then upon subsequent oxidation yielded the phenolic compound. Finally, regioselective methylation afforded the natural product neocosmocin A, as shown in Scheme 40.

(b) Total synthesis of paecilomycin F and cochliomycin C. Banwell et al. reported the asymmetric total synthesis of paecilomycin F (34) and its chlorinated derivative,
cochliomycin C (40), through successful exploitation of an intramolecular Loh-type allylation as a key step. The synthesis was initiated with the CM reaction of the two known intermediates to furnish the E-olefin, which upon hydrogenation and debenzylation furnished compound 418. An intermolecular transesterification of the alcohol 418 with the known aromatic triflate afforded the ester 419 in a 91% yield. The free phenolic-OH group was protected as its –SEM ether, as shown in Scheme 41. Stille coupling of the triflate with the known stannane afforded the coupled product 420 in a 76% yield. An Appel reaction with NCS-Ph3P afforded the E-allylic chloride (84% yield), which upon further desilylation and oxidation of the resulting alcohol with DMP furnished the aldehyde 421.

The authors then attempted the intramolecular allylation reaction under NHK conditions and furnished the undesired γ-allylated product 422 as a major product. Whereas allylation under Loh conditions (in the presence of In metal) provided the breakthrough and yielded the desired α-allylated product through a Felkin-Anh type transition state to yield macrocycle 423 as a single stereoisomer. Deprotection of the acetonide and –SEM group with methanolic HCl afforded bafilomycin F, which upon subsequent chlorination afforded cochliomycin C (Scheme 41).

(c) Asymmetric synthesis of paecilomycin F, cochliomycin C and zeenol derivatives. The asymmetric total syntheses of five naturally occurring RALs were reported by the author’s group in 2016. The key reactions involved in the syntheses were Heck coupling, Barbier propargylation and a late-stage macrolactonization protocol. Two of the synthesized RALs, 3-bromo-zeenol (47a) and 3,5-dibromo-zeenol (47b), were synthesized for the first time. The synthesis began with the CM reaction of two known olefinic partners in the presence of HG-II catalyst to furnish compound 424 in a 88% yield in favour of exclusive E-geometry for the newly formed olefinic unsaturation. Reductive removal of the –PMB group and subsequent hydrogenation was achieved in a H2

Scheme 37 Total synthesis of cochliomycin A and B and zeenol.
atmosphere to afford the corresponding alcohol, which upon subsequent oxidation with DMP afforded the aldehyde 425. Barbier propargylation of compound 425 with propargyl bromide in the presence of metallic Zn provided the corresponding propargylic alcohol with excellent diastereoselection under Felkin–Anh control. Partial hydrogenation of the alkyne functionality with a Lindlar
catalyst, followed by –MOM protection yielded compound 426. The stereoselective Heck coupling of 426 with the fully functionalized aromatic iodo compound furnished the olefin 427 with absolute stereocontrol at the newly formed olefin unsaturation in favour of E-geometry. Pinnick oxidation and further desilylation afforded the seco-acid, which upon macrolactonization under Mitsunobu
conditions afforded the resorcylate macrocycle 428. The global deprotection of acetonide and MOM and regioselective demethylation were achieved by treating compound 428 with BCl3 at –20 °C to furnish paecilomycin F. The electrophilic chlorination of paecilomycin F with SO2Cl2 furnished cochliomycin C (Scheme 42a). Another target molecule, namely zeaenol, was synthesized as shown in Scheme 42b by following similar strategies as depicted earlier starting from compound 424. Oxidative removal of the –PMB group with DDQ, followed by oxidation under DMP conditions afforded the aldehyde 429. Barbier propargylation of the aldehyde 429, followed by partial hydrogenation of the alkyne group with a Lindlar catalyst and finally –MOM protection of the free hydroxy group furnished compound 430. The stereoselective Heck coupling of compound 430 with 2-iodo-4,6-dimethoxybenzaldehyde furnished compound 431 in excellent yield. Pinnick oxidation, desilylation and macrolactonization under Mitsunobu conditions afforded the lactone 432. The deprotection of –MOM and acetonide and selective demethylation of 432 with BBr3 furnished zeaenol. The bromination of zeaenol with NBS afforded 3-bromoozaenol and 3,5-dibromo-zeaenol in a 1:4 ratio (separated by preparative HPLC), as shown in Scheme 42b.

(d) Asymmetric synthesis of paecilomycin G. A straightforward synthesis of paecilomycin G (35) was reported by Das et al. in 2016 for the first time.192 The main highlight of their synthesis was to use a Sharpless asymmetric dihydroxylation method to fix C50 and C60 stereocenters and a late-stage RCM to construct the macrocycle. The synthesis was started with the known α,β-unsaturated ester 433, which was converted to the corresponding ene–yne 434 by a three-step protocol, as shown in Scheme 43. The alkyne was then reacted with the known enantiopure epoxide to furnish the alkynol 435, which was instantly protected as its –TBS ether by a conventional method. Sharpless asymmetric dihydroxylation with AD-mix α afforded the corresponding diol with excellent diastereoselection to furnish the diol. The diol was then protected as its acetonide to afford compound 436. Compound 436 upon hydrogenation with Raney Ni/H2 furnished the corresponding alcohol (where debenzylation also occurred). The alcohol was then oxidized with IBX, followed by Wittig olefination and a subsequent desilylation reaction to afford the olefin 437. The compound 437 was then esterified with the known aromatic precursor 2-hydroxy-4-methoxy-6-vinylbenzoic acid under Mitsunobu conditions to accomplish compound 438 in an 84% yield. Finally, an RCM reaction with HG-II catalyst in refluxing toluene, followed by removal of the acetonide protecting-group furnished paecilomycin G (35), as shown in Scheme 43.

(e) Synthesis of paecilomycin F. Another total synthesis of paecilomycin F was disclosed by Srihari’s group,194 which represented an extension of their previous work for the synthesis of another RAL paecilomycin E. The (±)-tartrate-derived known compound 440 was coupled with the known alkyne to furnish compound 442. Compound 442 upon hydrogenation with Pd–C/H2 afforded the respective alcohol. A subsequent oxidation under Swern conditions and the Barbier allylation reaction furnished a homo-alcoholic with excellent diastereorecontrol. Protection of the free hydroxyl group as its –MOM ether and subsequent desilylation afforded compound 443. Alcohol 443 was then coupled with a known aromatic acid under DCC/DMAP conditions to furnish the ester 444 in a 67% yield. RCM reaction with G-II catalyst proceeded smoothly to afford the macrocycle core in an 85% yield. Deprotection of the acetonide and the –MOM group was achieved under acidic conditions to furnish the target molecule paecilomycin F (34), as shown in Scheme 44.

Scheme 40  Total synthesis of neocosmocin A.
7. Structural congeners of RALs (synthesis and biology)

7.1. Synthesis of RAL analogues using a fluorous mixture strategy

A library of RAL analogues containing a Z-enone moiety that could possibly target several kinases (bearing a cysteine residue) through a Michael type of attack was synthesized using fluorous mixture methods by Winnsinger et al. in 2009. The general synthetic planning is outlined in Scheme 45 by adopting chemistry that had already previously been established by the same group. In general, the RAL fragment 1 was thought to be accessed from the coupling of the aromatic fragment and the aliphatic fragment bearing the fluorous tag. The aromatic component had an “X” group, which could be a selenide, Me or a phenolic –OH group, all of which could be coupled in the presence of a base with the fluorous tag containing an aliphatic partner bearing a primary iodo group at one end. Later on, the final lactonization was envisioned to proceed through a Mitsunobu method to afford the macrocycles.

Post-synthetic modification of the RAL analogues through epoxidation (at C1–C2), oxime formation or methylation with diazomethane and oxime formation was also carried out to access a series of structurally similar RAL libraries. The diversities originated by changing the nature of R1, R2 and the aromatic ring, as shown in Scheme 45.

Finally, all the synthesized RAL analogues were screened against a panel of kinases, and two of the best inhibitors were chosen for further studies. The two chosen RALs were further screened against a large pool of kinases, consisting of 402 in number. The detailed study was outlined in the article by Winnsigner et al. and they concluded by stating that the synthetic analogues of RALs bearing a cis-enone moiety may have a large and significant potential for use against kinase inhibition and cancer oncology.

7.2. Synthesis of RAL analogues by intramolecular HWE reaction and photoisomerization

A series of RAL analogues were synthesized by Murphy et al. by adopting an intramolecular HWE reaction between an
aldehyde and phosphonate. The synthesized RALs were then post-synthetically modified by other methods. The synthesis was initiated between a Mitsunobu coupling of a known alcohol and aromatic acid to furnish the ester 446 in an 87% yield. Removal of the –PMB group, followed by DMP oxidation furnished the aldehyde. The CM reaction between an aldehyde
and the alkene keto-phosphonate in the presence of HG-II catalyst afforded the compounds 449/450. An attempted HWE reaction (Still–Genari or Ando) proceeded well but the obtained geometry in the newly created olefinic unsaturation was always E rather than the expected Z. Finally, compound 451 was post-synthetically modified by numerous ways, as depicted
in Scheme 46, to generate a few structurally interesting RAL analogues. The compound 451 upon reduction with Stryker’s reagent,199 followed by regioselective demethylation afforded the RAL analogue compound 452. The demethylation of 451 afforded 453, which upon further treatment with BBr₃ or BCl₃ afforded the Michael adducts of the bromo or chloro RALs, as shown above (where installation of the halogen took place at C8). Compound 453 upon photoisomerization furnished a mixture of compounds 454 and 455 in a 50% yield. Whereas the hydrogenation of 456/457 with Pd–C/H₂ afforded the completely saturated chloro/bromo RALs 458/459, which upon elimination afforded compound 460. Compound 460 upon subsequent photoisomerization yielded Z-461 in an 83% yield. Hence, a series of simple RAL analogues were accessed through intramolecular HWE reactions, followed by photoisomerization and a Michael reaction (Scheme 46).

### 7.3. Synthesis of the RAL framework by sequential Pd-catalyzed coupling reactions

In 2010, Takahashi et al. disclosed an elegant and novel piece of work for the construction of several RAL frameworks with the successful exploitation of sequential Pd-catalyzed coupling reactions.200 The detailed strategy used in their investigation is presented in Scheme 47. Aromatic bromo compounds or
boronic acid derivatives were chosen as the initial precursors. Pd-Catalyzed carbonylation with various substituted alkenols afforded the corresponding esters (with the more reactive “I” group reacting). In a later stage, the “Br” group was replaced with the help of Pd-catalyzed carbonylation or amination to furnish aromatic esters. The esters were then subjected to Pd-catalyzed Sonogashira, Stille and Suzuki coupling reactions to furnish bis-olefinic esters, as shown in Scheme 47. The aromatic boronic ester was also coupled with alkenols, as shown below under Pd catalysis, followed by a successful Suzuki coupling with E-vinylic iodides to afford esters. Finally, all the esters were then subjected to RCM reaction with G-II catalyst to furnish the RAL frameworks concisely. Regioselective demethylation then furnished the RAL analogues, as shown in Scheme 47. By adopting this strategy, it was possible to introduce new functionality, such as an alkyne moiety at C1⁻C2⁻, amine at C1’, ester at C1’, no Me substitution at C10’. It was considered that these newly generated RALs might be useful for kinase inhibition studies.

7.4. Synthesis of radicicol analogues and binding studies

Investigations by Moody’s group revealed a series of radicicol analogues that were synthesized and evaluated as potential inhibitors of Hsp90. The synthetic strategy involved the generation of a dianion from properly functionalized toluic acid and then a subsequent reaction with a Weinreb amide derivative, followed by a late-stage RCM. The synthesis started with bisallylic chloride, which upon treatment with PMB-OH furnished the mono protected allylic chloride. The compound was then coupled with organo zinc species derived from iodobutyrate in the presence of CuCN to afford the corresponding ester, which was next converted to the corresponding Weinreb amide, as shown in Scheme 48. The anion generated from the bis-EOM-protected toluic acid was then coupled with the Weinreb amide to furnish the bis-olefinic ketone. Mitsunobu esterification of the acid with but-3-en-1-ol afforded the RCM precursor, which was then immediately cyclised to the macrocycle under RCM conditions. The compound
upon removal of the PMB group afforded the alcohol 467, which was then synthetically manipulated to several radicicol analogues, as shown below.

The synthesized RAL macrocyclic lactones 471–473 were then tested for binding to the ATP site of the N-terminal domain of human Hsp90β in two Hsp90 assays: the fluorescence polarization (FP) assay and the TR-FRET assay. Their growth inhibitory activity against a human colon cancer cell line (HCT116) was also determined specifically by the SRB assay. It was evident from the obtained results that introduction of groups into the C7₀-position of the macrocyclic ring showed a significant reduction in binding to Hsp90 compared to the quite potent (IC₅₀ 40 nm in FP assay) NP261, another RAL analogue synthesized from the same group in 2006. However, closer inspection revealed that compounds 471–473 were only marginally less potent than the previous compound in the SRB growth inhibition assay. The loss of potency in inhibition of Hsp90 was presumably a result of the change in conformation of the macrolactone ring upon introduction of the substituent at C7₀.

7.5. Synthesis of fluorenone RALS

Winssinger et al. reported two novel 7₀-F-cis-enone resorcylides (482–483) for the irreversible inhibition of kinase based on the fact that this compound could react with a cysteine residue of the protein. Their synthesis was started with the known enantiopure ester, which was converted to the corresponding aldehyde in two steps. The aldehyde was then subjected to HWE reaction with (EtO)₂POCHFCO₂Et to afford the α,β unsaturated ester. The ester was then reduced to the aldehyde with DIBAL-H/Swern oxidation to furnish the aldehyde 474. The aldehyde was then coupled with the lithiated alkyne 475 to afford the alcohol 476. Partial hydrogenation of the alkyne functionality with Lindlar catalyst, followed by protection of the free alcohol as its benzoyl ester afforded the olefin 477. The compound 477 was next dihydroxylated with OsO₄, followed by acetonide protection to afford compound 478, which was then converted to iodide 479 in a two-step method, as shown in Scheme 49. With the iodide 479 in hand, it was then coupled with fully functionalized aromatic selenide and phenol separately to furnish the coupled product. A subsequent selenoxide elimination then furnished the corresponding olefin 480. Compounds 480/481 were then next converted to the RAL analogues (482/483) by a series of known reactions, as shown in Scheme 49.

Both the compounds were then tested for kinase inhibition assays, whereby even though their activity was good when compared to the known inhibitor LL-Z-1640-2, they seemed to...
be inferior. The ether-containing RAL (483) analogue seemed to have a more potent inhibitory effect when compared with the other molecule. Thus, the fluorenone-containing RAL analogues were well tolerated in the kinase active site but did not exhibit enhanced activity compared to other known inhibitors.

7.6. Triazole-containing RAL macrolactones

In 2010, Moody et al. synthesized \(^{204}\) a series of novel RAL analogues (488a–488b; 490a–490c) in search of new Hsp90 inhibitors, which was become an emerging target for searching for novel cancer therapeutic agents. The synthetic strategy involved the coupling of an acyl derivative with a substituted homophthalic anhydride to furnish isocoumarins. The isocoumarins were then synthetically manipulated to construct the RAL analogues. The initial phase of synthesis involved formation of the homophthalate ester 485 from 4-chlororesorcinol (484) by adopting a unique protocol developed by Danishefsky. \(^{205}\) Regioselective chlorination and phenolic –OH protection afforded the di MOM ether, which upon hydrolysis and dehydration afforded the homophthalic anhydride 486. Treatment with acylchloride derived from the malonate derivative compound 486 afforded the corresponding acylated product, which then instantaneously underwent cyclization, ring opening and CO\(_2\) elimination to furnish the iso coumarin derivatives 487a–487d, as shown in Scheme 50. \(^{206}\) Hydrolysis of the isocoumarins yielded the resorcylic acids, which upon coupling with properly substituted alcohols furnished the esters 488a–488b. The esters then underwent rapid RCM reaction with G-II catalyst, followed by MOM-group deprotection to furnish the RAL analogues 489a–489b. Terminal-alkyne-based isocoumarins were coupled with azido alcohols to afford the azido esters 490a–490e. A intramolecular click reaction between the azide and alkyne in the presence of CuSO\(_4\) and Na-ascorbate afforded the triazole-containing RALs 491a–491e, as shown in Scheme 50. All the newly synthesized RAL analogues (488a–b; 491a–e) were evaluated for Hsp90 inhibition in two known Hsp90 binding assays: the fluorescence polarization (FP) assay and the TR-Fret assay. Introduction of a –Me group in compound 489b resulted in a loss of potency in the HCT116 human colon cancer cell line compared to the radicicol analogue 489a. Among the synthesized
compounds 491a–e, only 491d showed weak Hsp90 inhibition, suggesting a weak detrimental factor for the triazole ring to bind to Hsp90. In addition, the triazole-containing RAL analogues 491a–e showed no significant growth inhibition of the HCT116 cell line in comparison with the naturally occurring radicicol (1).

7.7. Synthesis and Hsp90 inhibition with resorcylic acid macrolactam analogues

Several new nitrogen analogues (500a–500f) of the RAL were synthesized by Moody et al.207 by applying a similar isocoumarin-based strategy as discussed in the preceding section. A late-stage RCM reaction was used to form the macrocycle, and then subsequent manipulation of the pendant ester group with a range of amides led to a diverse set of macrolactams, as shown in Scheme 51.

To understand the structural details of the binding novel resorcylic acid lactams to Hsp90, the selected representative macrolactams 494 and 500a were successfully co-crystallized with the N-terminal domain of the yeast protein, where molecular replacement solved the structures of the resulting complexes. Comparison with their previously established structure of Hsp90-bound NP261-7 showed that although the macrolactone ring of NP261-7 was superimposed with the macrolactone ring of radicicol, the macrolactam rings of 494 and 500a adopted a slightly different conformation. The replacement of the lactone with a lactam ring produced compounds that were metabolically more stable and that could act as Hsp90 inhibitors with equivalent or even superior biological profiles in some instances. Investigations also demonstrated that the growth-inhibitory activity of the potent compound 500a against human colon cancer cells was due to Hsp90 inhibition, as established by the specific molecular signature of chaperone client protein depletion combined with Hsp72 upregulation. Protein crystallography also showed that the ATP site could readily accommodate analogues of differing substitution patterns and conformations. Interestingly, the macrolactam 500a, containing a benzyl amide group, opened a hydrophobic pocket in the N-terminal ATP site by displacing the loop between Leu93 and Lys98, which may be attributed to its better biological profile. Although this activity was reported for purine-based drugs (PU3 and H71), this was indeed unprecedented for the radicicol-based chemical class of inhibitors. The mobility of this loop raises the possibility of designing and developing new inhibitors that can form favorable interactions with the alternate conformations of this loop.

7.8. Synthesis of deoxy analogues of L-783,277

In a subsequent study reported by Altmann et al.,208 a few deoxy analogues of naturally occurring kinase inhibitor L-783,377 were synthesized and screened for their kinase inhibition activity. Mainly, they synthesized 5′-deoxy L-783,277 (508 and 509),
a dideoxy analogue and a C7′-C8′ E-deoxy analogue for their study. The synthetic strategy is highlighted in Scheme 52. The synthesis of 5′-deoxy L-783,277 was initiated with the Keck alkylation of the known aldehyde, followed by protecting-group readjustment and oxidation under Swern conditions to afford the aldehyde 501, which was then immediately coupled with the TES-protected alkyne 502 to furnish compound 503. A Suzuki coupling reaction with alkyl borane generated from olefin 503 with the fully substituted aromatic bromide afforded compound 504. Partial hydrogenation under Lindlar conditions afforded Z-olefin, which upon subsequent ester hydrolysis and removal of the –TES group with 1 M NaOH in refluxing MeOH afforded compound 505. Lactonization under Mitsunobu conditions afforded the macrocycle core 506. The treatment of compound 506 with Jones reagent and KF afforded a desilylated product first, while the concomitant allylic oxidation furnished compound 507. Removal of the TBDPS group with HF-pyridine furnished 5′-deoxy L-783,277 (508). The synthesis of 4′,5′-dideoxy L-783,277 was achieved as depicted in Scheme 54 by adopting a similar strategy described for the synthesis of 5′-deoxy L-783,277.

Finally, an inhibition study for all the newly synthesized compounds was carried out against a panel of 39 kinases. It was found that 5′-deoxy L-783,277 exhibited a similar kind of activity profile when compared with L-783-277; whereas the other two analogues were significantly less active towards kinase inhibition compared to their natural counterpart.

7.9. Synthesis of RAL analogue containing E-enone and a halogen at the C-8′ position

Murphy et al. synthesized209 a series of RAL analogues (512a–512n) based on their early work, described in Section 7.2. The main chemistry involved in the synthesis was the application of an intramolecular HWE reaction. The photo-isomerization reaction was adapted for the isomerization reaction, with the BX3 (X = Cl, Br)-mediated Michael addition of halides to enones the key reactions employed to access those analogues (Scheme 53).

All the newly synthesized RAL analogues (512a–512n) were next screened against a panel of 13 available kinases. It was speculated that all the kinases were cysteine-containing proteins that are known to exhibit irreversible inhibition by all the synthesized RALs. A known commercially available potent
kinase inhibitor LL-Z1640-2 and staurosporine were also tested together with the synthetic library as positive controls. All of the synthesized compounds were shown to selectively inhibit the kinases through a Michael-type attack of the conserved cysteine residue present in the ATP-binding site (i.e. PDGFRα vs. ERK5) of the proteins. However, they did not inhibit all kinases with the same potency, demonstrating that selectivity could be achieved within this subset of kinomes. The absence of the C4, C5-diol functionality generally resulted in a significant reduction in inhibitory activity compared to the highly potent LL-Z1640-2 (0.24 nM, PDGFRα) and deletion of the ability of the RALs to inhibit a number of the kinases (ERK1, ERK2, GSK3β, GSK3α). However, E-enones 512b (27 nM) and 512c (89 nM) were considerably more active as inhibitors of PDGFRα than Z-enone 512k (440 nM). All the β-haloketones (512a, 512d–512f, 512h, 512l–512m) were found to be less potent than the corresponding E-enones, but the bromide 512e (87 nM, PDGFRα) was also a more potent inhibitor than the Z-enone. The lower activity of the chloro-RAL analogues (512d, 512h and 512m) may be attributed due to the slower rate of reaction of the corresponding chlorides with the cysteine residue in the kinase active site or to the depleted affinity of the chlorides for the kinase compared to the corresponding enone analogues of the RALs.

7.10. Synthesis of aigialomycin D analogues and their kinase inhibition studies

A series of new analogues of the potent kinase inhibitor aigialomycin D was synthesized in 2011 by Chai et al.210 The synthetic strategy was similar to that reported earlier for this kind of compounds. The diversity was created by hydrogenation at C1'–C2' and C7'–C8', by introducing carbonyl/oxime functionality at C2', deoxy analogue at C5'–C6', and by selective methylation at the phenolic group of the aromatic ring (Scheme 54). The protected toluic acid was initially coupled with the alcohols under Mitsunobu conditions to furnish the esters. The esters were then lithiated with LDA and subsequently treated with the Weinreb amides to furnish the keto-olefins. RCM reaction with HG-II catalyst, followed by dehydration and removal
of the MOM groups afforded the aigialomycin D analogues. The obtained compounds were then post-synthetically modified by conventional functional-group manipulation, as shown in Scheme 54.

Initially, the activity of aigialomycin D against a pool of 96 kinases was tested. Having identified MNK2 as a promising new target of aigialomycin D, the inhibitory activities of all the 16 synthesized compounds were determined using a validated in-house IMAP protocol. The results showed that compounds aigialomycin D (9), 514d and 516 had similar activities, with IC₅₀ values in the range of 0.45–1.6 μM, while the rest were much less active, with IC₅₀ values > 10 μM. These results indicated that an unprotected resorcinol unit together with a 1′,2′-double bond and (S)-10′-methyl group were critical for the high activity. Although removal of the 5′,6′-diol (514d) or saturation of the 7′,8′-double bond (516) caused a decrease of 3.0- to 3.5-fold in activity, masking the diol 522 as its acetonide only had a marginal effect. These preliminary results obtained by them should provide helpful guidance for the design of the next generation of RAL analogues and for future SAR studies.

7.11. Synthesis of a RAL analogue containing amide and E-enone functionality

Murphy et al. reported the synthesis of a RAL analogue in which they replaced the hydroxymethylene group (at the C4′ position) with an amide group and that also had an E-enone moiety (C7′–C8′). The synthesis involved intramolecular HWE-type olefination, Suzuki coupling and late-stage intramolecular amide coupling. In the beginning, Suzuki coupling of the aromatic triflate (524) with the boronic acid derivative proceeded smoothly to furnish the E-olefin 525 in a 77% yield. Base-mediated ester hydrolysis and Mitsunobu esterification then furnished the ester compound 526, as shown in Scheme 55. The treatment of compound 526 with DDQ afforded the α,β-unsaturated aldehyde 527 (involving deprotection of both the –MPM groups and oxidation of the allylic alcohol).
A subsequent Pinnick oxidation and regioselective protection of the carboxylic acid group to its –TBS ester afforded compound 528. Compound 528 upon oxidation under DMP conditions furnished the aldehyde 529 in a 76% yield. The reaction of the aldehyde 529 with the anion of Still–Genary phosphonate afforded the E-olefin 530 in place of the expected Z-olefin. The treatment of compound 530 with TFA furnished the acid-amine precursor, which upon coupling with EDCI/HOBt afforded the target amide 531. The compound 531 was then tested for its kinase-inhibition activity, and it was found that it was less potent compared to the known analogue LL-Z-1640-2. It was confirmed that introduction of the amide group had a definite role in lowering the overall inhibitory activity, as the other structural part remained the same in the synthesized analogue.

7.12. Synthesis of pochonin E and F analogues and Hsp90 inhibition study

Winssinger et al. reported\textsuperscript{33} the synthesis of some novel analogues of naturally occurring RAL pochonin E and F. The known ketones (both the epimers 533a–b/536a–b) were condensed with 2-(aminooxy)piperidyl acetamide to yield the corresponding oxime derivatives 534a–b/537a–b. RCM followed by desilylation furnished both the epimers of pochoxime. In efforts to further explore modifications at the C-6' position of the pochoxime scaffold, the fully protected pochoxime obtained from the RCM cyclization was treated with TBAF to selectively remove the silyl group. The free hydroxyl group was then converted to an azide in two steps (Ms-Cl, NaN\textsubscript{3}) to obtain compounds 538a–b, which were instantly reduced to an amine with trimethylphosphine (Me\textsubscript{3}P). Complete deprotection of the EOM groups with a sulfonic acid resin thus afforded the C-6' amino pochoximes 539a–b. Alternative strategies to convert the hydroxyl group using palladium-catalyzed \(\pi\)-ally chemistry through its acetylated or carbonated form were not productive. The key amine 539 could also be derivatized as a chloroacetamide and conjugated to 1-\(\beta\)-thiogluucose to afford compounds 540a–b. Although the chemistry shown in Scheme 56 starts with the (S) isomer of 532a–b, the same reactions were also carried out with the \(R\) isomer, thus affording the related products. All the products were obtained as a mixture of oxime geometries, which were separated by column chromatography.

Conversion of the pochonin to the corresponding pochoxime led to a consistent and significant gain in affinity, with the best ligand (\textit{epi}-pochoxime F) being 80 times more potent than \textit{epi}-pochonin F. A significant difference between the pochonins and their pochoxime derivatives was the fact that the aryl chloride no longer seemed important for Hsp90 binding in the pochoxime. Indeed, the most potent pochoxime did not have an aryl chloride. There was also a significant difference in binding affinity (16-fold) depending on the stereochemistry and
nature of the substituent at C-6. To gain insights into the origin of these differences, the impact on the conformational profile of modifications at C-6 was evaluated for the newly synthesized compounds, as shown in Schemes 56 (6-\(S\) and 6-\(R\) diastereoisomers with and without chlorine and 6-\(S\) and 6-\(R\) diastereoisomers). A detailed analysis of the structure–activity relationship of those compounds was discussed in the original article, and interested readers are advised to follow that.

7.13. Synthesis of aigialomycin D analogues and their kinase inhibition

A series of aigialomycin analogues was synthesized by Harvey et al.\textsuperscript{212} by employing a previously explored strategy involving Ramberg–Backelund and RCM reactions, as shown in Scheme 57. Next, the bioactivities of all the synthesized analogues and the parent aigialomycin D were screened against the human promyelocytic leukemia HL-60 cell line using an MTT cell proliferation assay. It was found that the ring-enlarged 15-membered macrocyclic sulfone (547) compound was a similarly potent inhibitor, which possibly showed that this compound intercepts a different cellular target to the 14-membered RALs since kinases typically have fairly specific substrate requirements. The tetrahydro aigialomycin D analogue (549a–d) obtained after the complete hydrogenation of aigialomycin D led to only an insignificant loss of potency. In comparison, the diastereomer of aigialomycin D differing at both diol centres (548a–c) had no noticeable activity up to 100 mM. Unfortunately, 2,4-dideoxy-AmD (548d) was found to be decomposed before it could be tested.

The development of a reliable synthetic procedure for the preparation of glucoside and sulphate derivatives of a naturally occurring RAL, zearalenone, was investigated by Mikula et al. in 2014. Different protective group strategies were employed to enable the synthesis of glucosides and sulphates at the aromatic positions of the zeralenone that had never been synthesized before. Initial attempts involving acetyl and \( p \)-methoxybenzyl protection led to unsuccessful results and were abandoned. Finally, triisopropylsilyl-protected zearalenone was successfully used as an intermediate for the first synthesis of the corresponding mycotoxin glucoside and sulfate, which are highly valuable as reference materials for further studies in the emerging field of masked mycotoxins. Furthermore, high stability was observed for the aryl sulfates prepared as tetrabutylammonium salts. Overall, these findings should be applicable for the synthesis of similar RAL-type and natural-product conjugates. The basic strategy is outlined in Scheme 58.

7.15. Synthesis and activity of a triazole-containing RAL analogue

In 2014, Chen et al. reported the synthesis of a triazole analogue of naturally occurring RAL LL-Z 1640-2. The synthesis involved a successful click reaction between an azide and alkyne, as shown in Scheme 59. The synthesis was started with the known 2-deoxy-D-ribose acetonide, which upon Colvin rearrangement with \( n \)-BuLi yielded the corresponding alkyne. The alkyne upon hydrostannylation with \( n \)-BuSnH afforded the \( E \)-vinyl stannane. Stille coupling of compound 553 with the aromatic triflate afforded the olefin in a 90% yield. The alcohol functionality in 555 was then converted to the corresponding azide under Mitsunobu conditions with diphenylphosphoryl azide. The azide was then clicked with \( S \)-pent-4-yn-ol under standard conditions to furnish the triazole 557 in a 78% yield. Finally, base-mediated intramolecular transesterification and a subsequent acetonide deprotection afforded the triazole-containing RAL 558 in good yield.

The kinase-inhibitory activity of the newly synthesized triazole RAL analogue 558 was studied against a panel of 96 selected protein kinases at 10 \( \mu \)M by using KINOME scan technology. Disappointingly, the compound exhibited much less activity compared to that of the parent compound 1 for those kinases containing a cysteine residue at the ATP binding site. The significant low activity was apparently due to the absence of a highly active enone system in 558, which is known to bind irreversibly to the cysteine residue in these kinases. These results further bolstered the crucial role of the enone motif in the high activity of the RALs.

7.16. Synthesis of macrolactam analogues of radicicol

A new series of macrolactam analogues of naturally occurring radicicol was synthesized by Moody et al. from methyl orsellinate as a starting material. Initially, chlorination with \( SO_2Cl_2 \) afforded the chloro derivative, which upon protection...
of the phenolic OH group and basic hydrolysis afforded the acid 560, as depicted in Scheme 60. Dianion generation was triggered by treatment of the orsellinate derivative with 2.2 equivalent of s-BuLi and subsequent acylation with olefinic Weinreb amide (561) to afford compounds 562a–d. The acids 562a–d were then coupled with an amine under standard conditions to afford the bis-olefinic precursor containing amide functionality. The macrocycles were then constructed via ring-closing metathesis, employing G-II catalyst, in moderate to good yield and as mixtures of E/Z-isomers (564a–d).

Deprotection of the EOM groups proceeded smoothly with TFA, affording the N-methyl resorcylic acid macrolactams 565a–d in up to 12% yield over four steps from the known starting material.

Next, the thermodynamics of the binding with Hsp90 of the newly synthesized macrocycles was investigated through isothermal titration calorimetry (ITC) measurement. The results show that there was a significant enthalpic penalty compared to radicicol, although the binding of the macro lactams was found to be superior to that with the macro lactone analogues previously reported by the same group 33 (\( K_d = 210-1200 \mu M \)). It should be noted that compounds 565d are mixtures of geometric isomers, albeit with a heavy predominance of one isomer, which makes interpretation of the binding data less clear. The radicicol analogues 565a/565c were then co-crystallized (major E/Z-isomer crystallized in all cases) with yeast Hsp90 to probe the interaction of the compounds with the Hsp90 N-terminal domain. The resorcylic acid macrolactams 565a–d bind to Hsp90 in a similar fashion to radicicol, with the same interactions exhibited by the resorcicol group in all cases. However, there was a clear conformational change in the macrocycle from radicicol to the macrolactams, emphasizing the importance of the interaction between the radicicol epoxide and the Hsp90 backbone.


An efficient synthesis of a 13-membered exo-enone analogue of the naturally occurring RAL molecule LL-Z1640-2 was synthesized by Chen’s group in 2016.217 The synthesis involved an intramolecular reductive macrocyclization coupling reaction of an aldehyde–alkyne by Ni-catalysis, as presented in Scheme 61. The synthesis began with the known compound 566 prepared from 2-deoxy-D-ribose acetonide, as reported earlier. Base-mediated transesterification with (S)-pent-4-yn-ol afforded the alkyne. The free phenolic –OH group was then protected as its –MOM ether to furnish compound 568. The desilylation of compound 568 with TBAF/THF afforded the corresponding alcohol, which was next subjected to DMP oxidation to furnish the alkyne–aldehyde 569.
A Ni-catalyzed, exo-selective reductive coupling macrocyclization of the alkyne–aldehyde (569) based on an intermolecular reaction reported by Takai et al. was then attempted. The reductive macrocyclization coupling reaction proceeded smoothly as anticipated and furnished the exo-methylene allylic alcohol (570) with high regiocontrol. The reaction is known to proceed via an alkenynickel species generated by a regioselective hydronickelation of the substrate alkyne. The presence of water is extremely crucial as it serves as a hydride source for the formation of nickel hydride required for hydronickelation of the alkyne, while the addition of a catalytic amount of Ph₃P accelerates the reaction and stabilizes the catalyst, thereby preventing the formation of inactive nickel metal particles. Finally, oxidation under DMP conditions afforded the exo-enone RAL analogue 571. A few other compounds, namely 572a–572d, were also synthesized by applying this reductive macrocyclization reaction.

The kinase inhibition activity of the synthesized exo-enone RAL analogue was then tested by screening against a panel of 62 selected kinases at 10 μM using DMSO as a negative control. The results indicated a strong inhibition of compound 571 against several kinases, comparable to the natural product LL-Z1640-2. These strongly inhibited kinases mainly fall into two families: TK (tyrosine kinases) and STE (homologues of yeast Sterile 7,
Sterile 11 and Sterile 20 kinases) and all belong to a kinase group that contains a cysteine residue at the ATP binding site. It appeared that the change of the ring size from a 14-membered (\(5\)) to 13-membered (\(571\)) macrocycle did not have any profound effect on the activities. However, further investigation would be required to establish if this new compound indeed covalently binds to the cysteine residue at the ATP binding site.

### 7.18. Synthesis of zearalenone analogues

Very recently a unique catalytic and regioselective method of hydrocarbofunctionalization of unactivated olefins was developed by Engle et al.\(^\text{219}\) The reaction employed a palladium(II) catalyst and utilized an easily removable directing group (8-aminoquinoline, AQ) to control the regioselectivity of carbopalladation and to enable subsequent protodepalladation. A wide range of C–H nucleophiles (1,3-dicarbonyls and electron-rich aromatic systems) was then reacted with this system to afford a Michael type of adduct. By adopting this methodology, the naturally occurring RAL zearalenone was structurally modified to access a novel RAL analogue in a 55% yield (Scheme 62).

**Summary of the strategical blueprints for the synthesis of RALs and their analogues during 2008–2016.** A snapshot of different synthetic strategies (2008–2016) adopted for the total synthesis of naturally occurring RALs and their analogues is provided in Scheme 63. A direct comparison with the previously reported syntheses (Scheme 5) seems to be relevant in this context. Careful analysis revealed that new strategies have evolved with new strategic bond disconnections and new synthetic transformations. Metathesis reactions (RCM and CM) for the construction of C–C bonds in RALs, such as C1’–C2’ and C7’–C8’, have been mostly explored. While the formation of C3’–C4’ and C5’–C6’ with the help of RCM reaction has also been attempted and met with great success. The Loh-type allylation reaction was explored for C3’–C4’ bond construction, which had never been attempted before. For the construction of the fully substituted aromatic ring, the acetoacetate condensation route explored by Barett et al. seems to be novel and unique. Whereas other researchers relied heavily on starting from aromatic precursors and performed functional group manipulation. The construction of C1’–C2’ bonds is always regarded as the most strategic and hence has been widely explored by several workers. New methodologies, such as Heck coupling, Weinreb ketone synthesis, Pd-mediated Meinwald type rearrangement and Julia–Kocienski (JK) olefination, have been mainly adopted to construct C1’–C2’ in RALs. Cross-coupling reactions, such as Suzuki and Stille, have often been used to construct the C6–C1’ connectivity in RALs. Other adventitious reactions, such as intramolecular NHK coupling between aldehyde and vinylic halide...
Scheme 59  Synthesis of triazole-containing RALs.

Scheme 60  Synthesis of macro lactam analogues of radicicol.
species (for making C7′–C6′ bonds) and “Ni”-catalyzed ynal macrocyclization, are worth mentioning. Finally, it was established that macrolactonization from a suitable seco-acid for the construction of a lactone core in RALs (through C1–C10) mimics the biogenesis of such natural products. The macrolactonization method through Mitsunobu inversion (hydroxy group activation protocol) was mainly explored as a carboxylic acid group activation method, such as Yamaguchi, but failed mainly due to the depleted electrophilicity of the aromatic system (mainly due to the presence of OMe groups). De-Brabander lactonization seemed to be very useful and was explored by many researchers for the total synthesis of RALs through a late-stage lactonization method. Whereas the Pd-catalyzed carbonylation method for constructing the ester unit of macrolactone was relatively unexplored, but is potentially a very useful reaction.
8. Conclusions

In general, a fairly large number of naturally occurring RALs have been isolated so far, and the structural variations and diversities of these species are also quite evident from the above discussion. From a structural perspective, they possess a relatively simple architectural pattern consisting of a substituted aromatic part fused with an acyclic side chain bearing certain functionality in a proper stereochemical fashion. Their biogenesis seems to be interesting as it involved an intriguing PKS pathway and a late-stage macroalactonization, which was responsible for the construction of the lactone framework. Recent investigations showing that naturally occurring RALs and their analogues could be potent inhibitors of ATPases, such as HSP90, or kinases have raised significant interest in this family of natural products. From a chemical or biology perspective, kinases are unique proteins involved in various signalling cascades, and the selective inhibitors of such systems are predominantly useful to scrutinize the implication of individual kinases in complex biological networks. From an organic synthesis perspective, several elegant approaches to important RALs have been disclosed herein. The synthetic strategies delineated in this review are unique in their own merit and cover many important organic transformations and strategies. RALs have stimulated the creative impulses of synthetic organic chemists, and many elegant total syntheses have been reported based on the application of contemporary methods based on novel C–C bond-forming reactions. The development of such concise and modular syntheses has enabled researchers to generate a variety of new analogues of RALs as well as the natural compounds themselves in solution and also in the solid phase. The research in some cases has also led to structural revisions of a few naturally occurring RALs. Even conceptually interesting biomimetic strategies have also been investigated to access those molecules in a flexible manner. In the future, efforts to synthesize such compounds through concise and modular routes to extend the diversity of this family by relatively unexplored strategy will be the major challenge.

From a therapeutic perspective, the target proteins of many RALs (HSP90 and kinases) are considered amongst the most promising targets for chemotherapy as well as inflammation treatment. Various RALs have already been proven to be effective in animal models (hypothemycin and radicicol derivatives) for such treatment. The fact that some RALs have been shown to act as irreversible inhibitors of those target proteins may demonstrate them to be an asset as they can be subsequently used to selectively label or as probes for activity-based profiling of those proteins. Various structural congeners of naturally occurring RALs have also been synthesized and found to be as potent as their natural counterpart. To what extent the activity and potency of given inhibitors can be modulated with changes in the functionalities around the macrocycle remains to be defined, but it is clear that small changes of the functional groups around the macrocycle can have a dramatic impact on the overall conformation and hence on the activities of those analogues.

Conflicts of interest

There are no conflicts to declare.

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