

Account / Revue

# Resorcylic acid lactones: A pluripotent scaffold with therapeutic potential

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## Abstract

The recent discoveries of potent HSP90 and MAP kinase inhibitors amongst the resorcylic acid lactones (RALs) have revived interest in this family of natural products. Both HSP90 and MAP kinase inhibition hold tremendous therapeutic potential, particularly in the treatment of cancer. Our synthetic efforts towards the RALs and, in particular, selective inhibitors of HSP90 and kinases are reviewed. *To cite this article: S. Barluenga et al., C. R. Chimie 11 (2008).*

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## Résumé

Les découvertes récentes d'inhibiteurs potentiels de la protéine HSP90 et des kinases MAP parmi les lactones dérivées de l'acide résorcylique (Resorcylic Acid Lactones, RALs) ont suscité un regain d'intérêt pour cette classe de substances naturelles. En effet, l'inhibition de ces protéines présente un potentiel considérable particulièrement pour les thérapies anticancéreuses. Cet article présente nos approches synthétiques vers des inhibiteurs sélectifs de l'HSP90 et de kinases appartenant à la famille des RALs. *Pour citer cet article : S. Barluenga et al., C. R. Chimie 11 (2008).*

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**Keywords:** Resorcylic acid lactones (RALs); Kinase inhibitors; HSP90; Total synthesis; Combinatorial synthesis; Solid-phase synthesis; Polymer supported reagents

**Mots-clés :** Lactone de l'acide résorcylique ; Inhibiteurs de kinases ; HSP90 ; Synthèse total ; Synthèse combinatoire ; Phase solide ; Réactifs supportés

## 1. Introduction

The use of small molecules to perturb biological processes has proven to be an attractive approach to

investigate biological mechanisms and lies at the core of pharmacological intervention [1]. There is a tremendous demand for small molecules which can selectively inhibit pathways with temporal control. It can be argued that biologically active natural products are a privileged starting point in this endeavor based on the fact that they have been selected by evolution to

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bind to given protein motifs [2,3]. Given the prevalence of domain shuffling in protein evolution, it can be anticipated that the privileged scaffolds that have evolved to inhibit a given protein may be suitable for the inhibition of functionally distinct proteins that have a homologous domain. With these considerations, we have been particularly attracted to the resorcylic acid lactones (RALs – Fig. 1). Zearalenone was the first member of this family to attract the attention of both chemists and biologists for its potent agonism of the estrogen receptor which is a transcription factor. It was shown to adopt a conformation which mimics the steroid and competes with estradiol for the estrogen receptor. More recently, radicicol and the *cis*-enone resorcylic acid lactones such as hypothemycin or LL-Z1640-2 have taken the center stage for their potent inhibition of HSP90 and MAP kinases, respectively. Despite the lack of structural similarity between radicicol or LL-Z1640-2 and ATP, a crystal structure of each compound in complex with their respective target has shown them to be bound in the ATP-binding pocket (Fig. 2) [4,5]. Pochonin C which is structurally closely related to radicicol was discovered from a screen for herpes simplex virus (HSV) replication, while aigialomycin D was reported to be an inhibitor of the malaria parasite. However, the precise targets of these compounds have not been reported. Thus, while

zearalenone targets a hormone transcription factor, radicicol targets an ATPase and the *cis*-enone resorcylic acid lactones target kinases. Based on the breadth of biological activity, we have been particularly interested in developing diversity-oriented syntheses which would enable us to broaden the chemical space surrounding this pluripotent natural product motif. We have recently reviewed the chemistry and biology of RALs [6]; herein we review our own synthetic efforts towards the resorcylic acid lactones and discuss their potential therapeutic application.

## 2. Radicicol – a potent HSP90 inhibitor

The heat shock protein 90 (HSP90) is a chaperone responsible for the folding and maturation of its client proteins and is one of the most abundant protein in cells (1–2% of proteomic mass). Despite the seemingly ubiquitous function of this constitutively expressed chaperone, its role in stabilizing conformationally labile proteins has implications in pathologies ranging from oncology to neurodegenerative diseases and has emerged as one of the most exciting therapeutic targets in recent years [7,8]. Most of HSP90's endogenous client proteins are key regulators of cell signaling which are destabilized and degraded in the absence of HSP90's chaperoning activity.<sup>1</sup> The dependence of transformed cells on HSP90 is further accrued by the fact that many oncogenic mutations, while increasing the activity of pro-growth signaling pathways, are less stable than their wild-type counterpart and have an increased dependence on HSP90's chaperoning activity [9]. A clinically relevant example is the heightened dependence on HSP90 of drug resistant mutants of Bcr-Abl. Indeed, HSP90 inhibitors in combination with Abl inhibitors remain effective against cell lines bearing that mutation [10,11]. Accordingly, HSP90 inhibition might provide a broader, more effective target for anticancer treatment than more focused therapies targeting single oncogenic proteins, and can work synergistically with cytotoxics or focused inhibitors. Aside from its involvement in oncogenic processes and potentiating the evolution of drug resistance, HSP90 is also implicated in regulating the fate of conformationally unstable proteins leading to neurodegenerative diseases. Indeed, HSP90 inhibitors have been shown to reduce protein aggregates in cellular and animal models of Huntington [12], spinal and bulbar muscular atrophy [13], Parkinson [14], and other

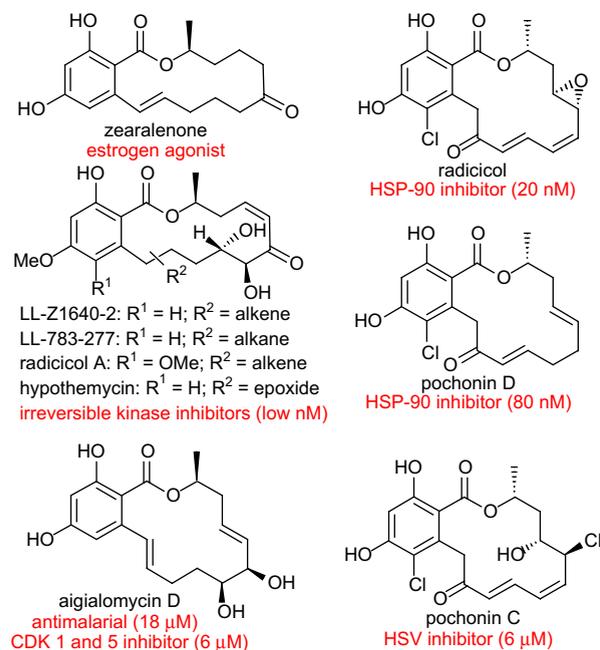


Fig. 1. Selected members of the resorcylic acid lactone family of natural products.

<sup>1</sup> For a complete list of HSP90's clients, see: <<http://www.picard.ch/downloads/Hsp90interactors.pdf>>.

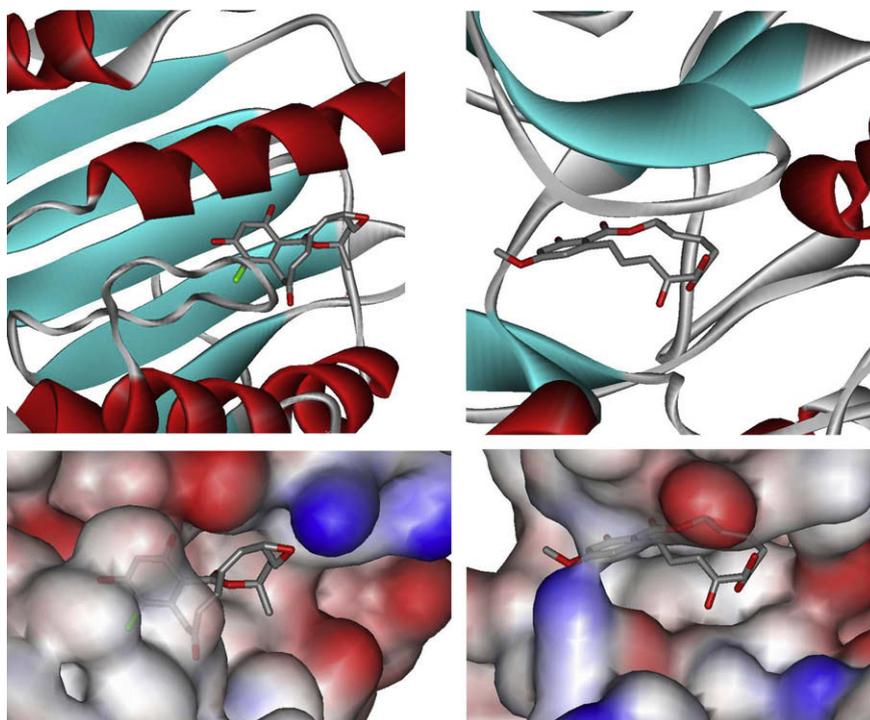


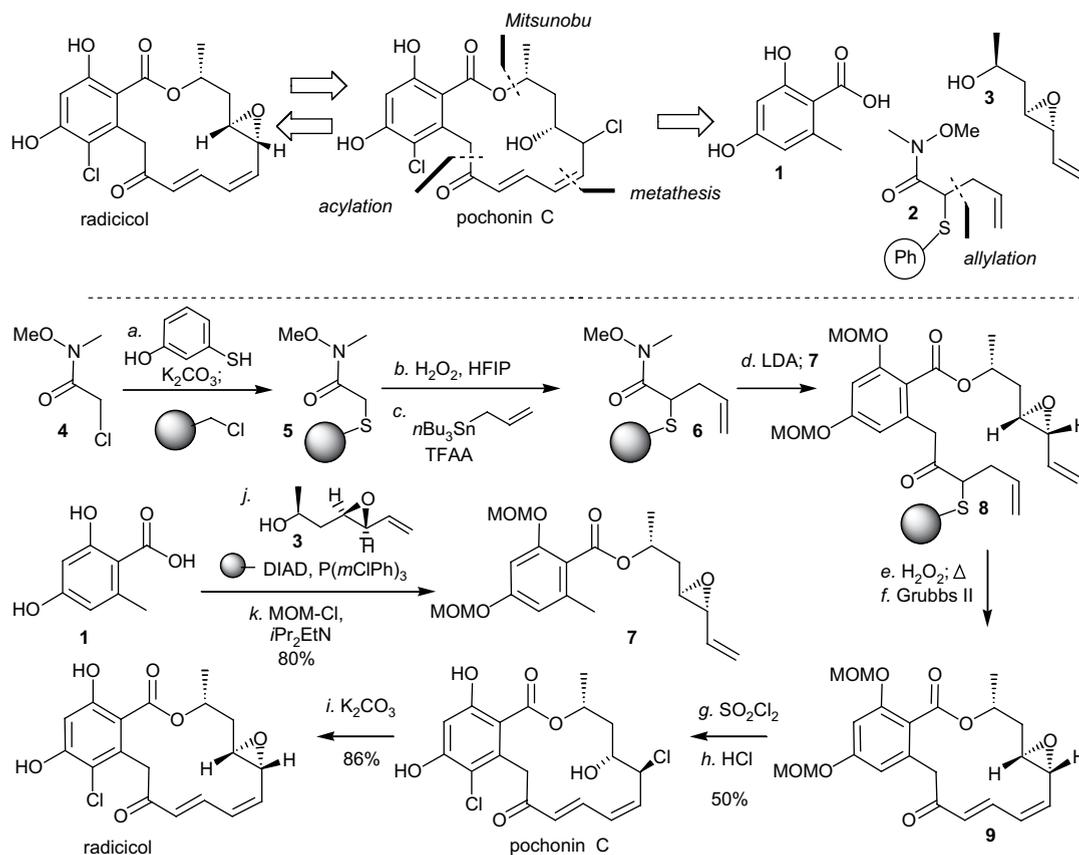
Fig. 2. Co-crystal structure of radicicol bound to HSP90 (pdb ID: 1bjq) and LL-Z1640-2 bound to ERK2 (pdb ID: 2e14).

Tau protein related neurodegenerative diseases [15]. These facts have stimulated tremendous research to obtain inhibitors with good pharmacological properties for clinical application. The most advanced candidate is 17AAG, a semisynthetic analogue of geldanamycin, which is currently in multiple phase II studies, however its hepatotoxicity is limiting its therapeutic potential [16]. Rational design and high throughput screening have led to the discovery of several heterocyclic motifs [17–19] with good pharmacological profile.

Radicicol remains amongst the highest affinity ligand for HSP90 (20 nM) [5]. However, radicicol suffers from two pharmacological liabilities: a conjugated dienone which acts as a Michael acceptor and a strained allylic epoxide. Indeed, while radicicol is effective at the cellular level, it is not in animal models due to metabolic instabilities. It has been shown that thiols such as DTT readily react in a conjugate addition to yield a compound which is inactive [20]. This limitation was partly overcome by converting radicicol's ketone functionality to an oxime which attenuated its Michael acceptor properties and was shown to be active in mouse xenografts [21,22]; however clinical development for this compound series was interrupted due to ocular toxicity. Based on the fact that other HSP90 inhibitors do not engender this toxicity, it can be

ascribed to an off-target activity which may be related to the epoxide serving as an alkylating agent. Mindful of the problematic issues surrounding the labile epoxide of radicicol, Danishefsky and coworkers reported an analogue wherein the epoxide was replaced by a cyclopropyl group and showed this compound to be nearly as active as radicicol [23].

Based on the therapeutic potential of HSP90 inhibition and the different biological activity of structurally related pochonin C, we developed a synthesis to access both of the natural products [24,25]. An important criterion in our planning was the possibility to use high throughput chemical synthesis techniques. It was clear at the onset of this program that the halohydrin of pochonin C could originate from a stereospecific opening of radicicol epoxide. As shown in Scheme 1, a key feature of the synthesis was to mask the conjugate alkene in the form of a thioether which could be converted to the  $\alpha,\beta$ -conjugate system upon oxidation followed by elimination. The remaining disconnections involved a Mitsunobu reaction to form the ester and a ring-closing metathesis. Starting from the commercially available Weinreb amide **4**, which was engaged in a chemoselective *S*-alkylation of 3-hydroxythiophenol followed by an *O*-alkylation to Merrifield resin, polymer-bound Weinreb amide **5** was obtained in one



Scheme 1. Polymer-assisted synthesis of pochonin C and radicicol.

pot. While the selective oxidation to the sulfoxide proved difficult with traditional oxidants such as *m*CPBA and DMDO, it was found to proceed smoothly with  $\text{H}_2\text{O}_2$  in the presence of HFIP [26] without any overoxidation to the sulfone. These conditions were compatible with solid-phase chemistry. Activation of the sulfoxide with trifluoroacetic anhydride in the presence of tributyl(allyl)tin afforded the Pummerer allylation product **6** in excellent yield. Intermediate **7** was obtained in two steps from **1** via Mitsunobu esterification/phenol protection. The use of a polymer-bound version of DIAD precluded the use of traditional purifications. Deprotonation of **7** with LDA and reaction with the polymer-bound Weinreb amide **6** afforded the cyclisation precursor **8** which was released from the resin prior to the metathesis. While the metathesis reaction could be achieved on the resin, carrying out the cyclisation on the triene rather than on the polymer-bound diene was preferable as it afforded exclusively compound **9** with the desired olefin geometry. Chlorination of the aromatic moiety with  $\text{SO}_2\text{Cl}_2$  led to concomitant opening of the epoxide, thus affording

pochonin C after deprotection of the two MOMs. Treatment of pochonin C with potassium carbonate led to radicicol in good yield.

Despite the structural similarity between pochonin C and radicicol, the NMR patterns of these two compounds were dramatically different. As shown in Fig. 3, radicicol exhibited strong NOE between the protons of methyl group (C1) and the protons of C8 and C11 in accordance with its conformation bound in the active site of HSP90 [5]. In comparison, pochonin C showed strong NOE between the protons of C5 and C9 while no NOE between the protons at C1 and C8/C11 suggesting a more planar and less organized conformation. The difference in biological activity reported by Hellwig and coworkers [27] for pochonin C and radicicol may be in part rationalized by their different conformational landscape rather than the difference in functionalities (epoxide vs halohydrin). In collaboration with Karplus and coworkers, the conformational landscape of radicicol and other resorcylics was calculated and it was found that indeed the lowest energy conformer of radicicol had a very similar

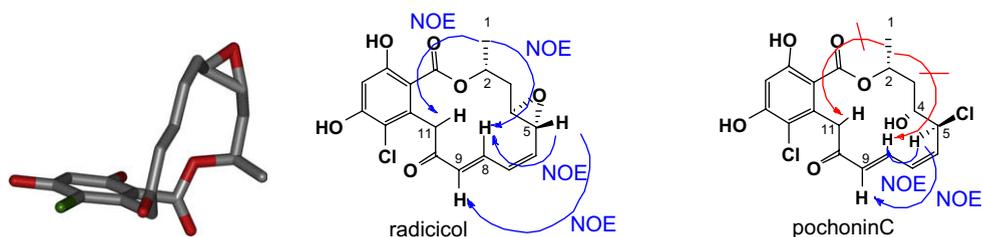
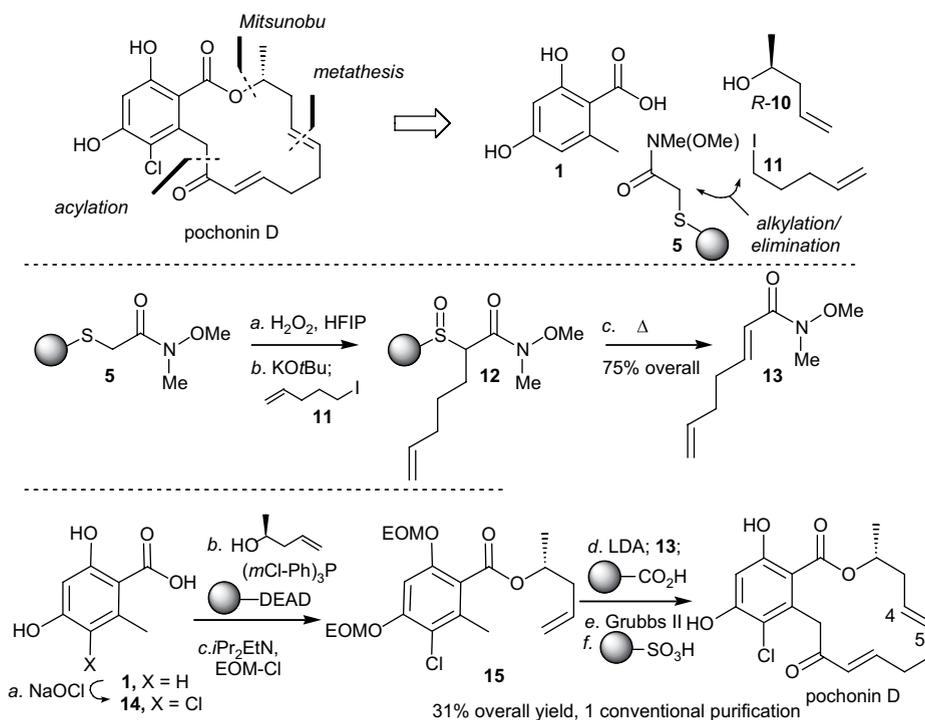


Fig. 3. Conformational aspects of radicicol and pochonin C. Conformation of radicicol bound to HSP90 (left, pdb structure ID 1bgq), NOESY measured for radicicol (middle) and pochonin C (right) [numbering according to pochonin C definition].

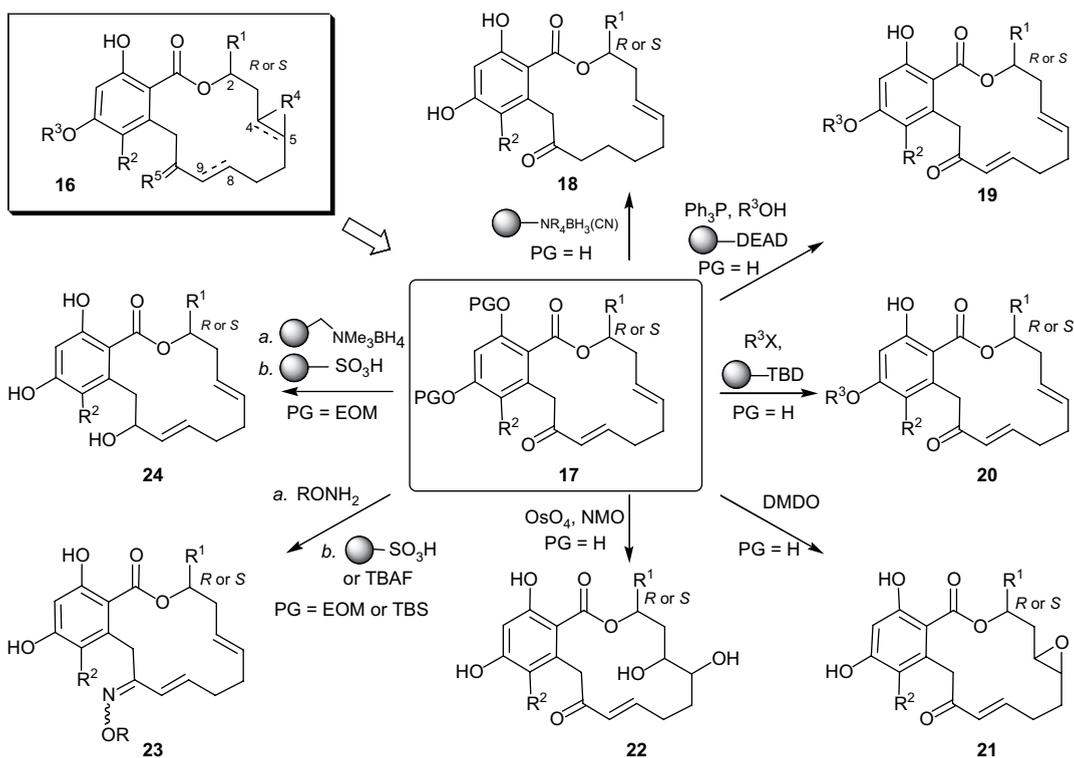
conformation to the bioactive one while the corresponding compound having a diol instead of an epoxide had a minimum energy barrier of 2.7 kcal to adopt the bioactive conformation [28]. Further analysis of a series of resorcylics led to the identification of pochonin D as having a low energy barrier to the bioactive conformation, thus motivating us to pursue its synthesis. The synthesis of pochonin D followed the same logic as the one of pochonin C, namely a disconnection into three fragments (Scheme 2). The Weinreb amide fragment **13** was prepared in three steps using the polymer-bound thioether **5**. The use of polymer-bound reagents [29] obviated the need for regular chromatography and afforded pochonin D in six steps from acid **1** and in 31% overall yield with only one

traditional purification. Importantly, despite its simpler structure, pochonin D was indeed a good ligand for HSP90 with an affinity of 80 nM (compared to 20 nM for radicicol). The difference in affinity is consistent with the calculated 1.2 kcal change in internal energy of pochonin D upon binding. The C4–C5 olefin of pochonin D could be regioselectively epoxidized using dimethyldioxirane to obtain pochonin A which was also found to be an HSP90 ligand; however, it was not superior to pochonin D and presents a labile epoxide [30].

The fact that the synthesis of pochonin D required only six steps and one conventional purification rendered it amenable to combinatorial synthesis. As shown in Scheme 3, a library represented by the



Scheme 2. Polymer-assisted synthesis of pochonin D.



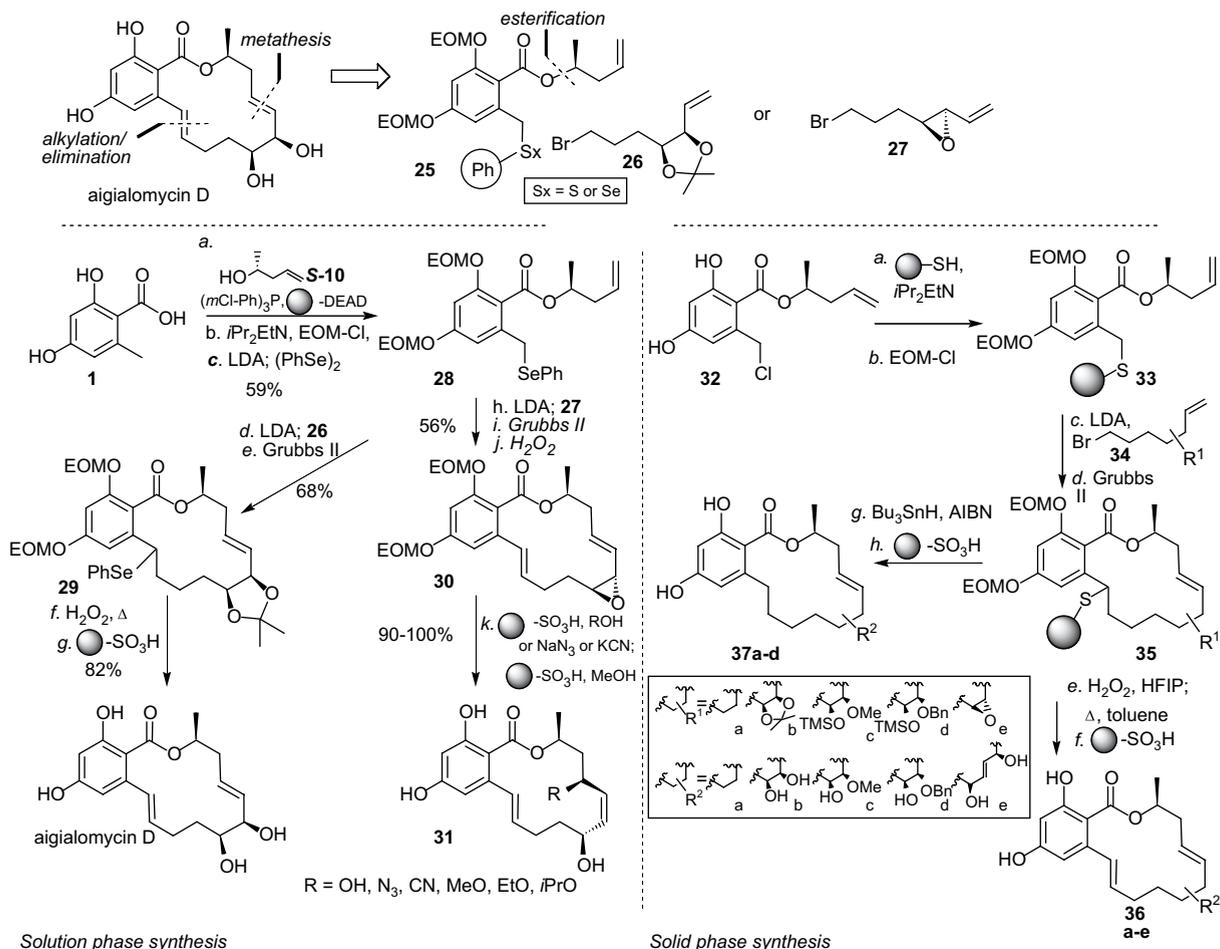
Scheme 3. Diversity-oriented synthesis of a library of pochonins.

general structure **16** which bears five points of diversity was prepared [31] from intermediates **17**, obtained through the aforementioned chemistry using different alcohols. The diversity of the library included the following modifications: the group on C2 ( $R^1$ , both stereochemistries are present in natural resorcylics, however only with a methyl substituent); the *meta* position on the aryl ring ( $R^2$ , a number of natural resorcylics bear a chlorine at that position); the substitution of the *para* phenol ( $R^3$ , a number of natural resorcylics bear a methyl group at that position); the C4–C5 olefin ( $R^4$ ) which was converted to a diol or an epoxide; the C10 carbonyl ( $R^5$ ) which was reduced or converted to oximes and the olefin C8–C9 which was reduced. While not all permutations of the library were pursued, over 100 compounds were prepared using combinations of the chemistry shown in Scheme 3. Screening of this library against HSP90 revealed important structure–activity relationships which led us to the discovery of a compound with 10 0-fold higher activity than pochonin D in cellular assays and which was found to lead to tumor regression in a mouse xenograft bearing a breast tumor cell line [32]. With the goal of exploring the suitability of these compounds for kinase inhibition, a representative

subset of the library (84 compounds) was tested for its inhibition in a panel of 24 kinases which led to the identification of 12 compounds with greater than 50% inhibition for one kinase at 10  $\mu\text{M}$ . The high hit rate in this resorcylic library (14%) testifies to the potential of the RAL scaffold for kinase inhibition. Importantly, the promising kinase leads that were identified were not HSP90 inhibitors and showed diverse selectivity profiles of enzyme inhibition amongst the 24 tested kinases.

### 3. Aigialomycin D – an inhibitor of the malaria parasite (*Plasmodium falciparum*)

As part of our program to further the diversity of RALs beyond the naturally available analogues in the hope of finding new ATPase and kinase inhibitors, we became interested in the aigialomycins [33]. As for the pochonins, our goal was to develop synthetic protocols which were sufficiently flexible as to be carried out in a combinatorial fashion. As shown in Scheme 4, the molecule was disconnected in two fragments using a metathesis to close the macrocycle [34], and a seleno or thioether at the benzylic position to facilitate alkylation chemistry at that position and



Scheme 4. Solution- and solid-phase diverted syntheses of aigialomycin D and analogues.

provide an attachment point to a resin for solid phase synthesis. The synthesis in solution started from benzoic acid **1** which was converted to the selenoether **28** in three steps. Deprotonation of the benzylic position with LDA followed by addition of bromide **26** afforded the alkylated product which was cyclized using Grubbs' second generation catalyst to obtain macrocycle **29** in excellent yield. Oxidation/elimination of the selenide followed by deprotection afforded aigialomycin D in seven steps from acid **1**. The same chemistry was found equally productive with alkyl bromide **27** containing the allylic epoxide, however opening of the epoxide in macrocycle **30** under a variety of conditions failed to give the 1,2-*cis* diol and led in all cases to S<sub>N</sub>2' reactions. While this route cannot be used to access aigialomycin D, this diversion proved quite general for different nucleophiles such as alcohols, cyanide or azide thus obtaining new RAL analogues **31**. Importantly, as in the case of the pochonins, it

was shown that the chemistry could be carried out on solid phase by replacing the phenyl selenide with a polymer-bound thioether. Thus benzyl chloride **32** was loaded on a thiol resin and both phenols were protected to obtain polymer-bound intermediate **33**. Alkylation of the benzylic position with five different alkyl bromides (**34**) followed by RCM afforded macrocycles **35** which could be released from the resin either *via* oxidation/elimination or under reductive conditions to obtain aigialomycin analogues **36** and **37**, respectively, after deprotection with sulfonic acid resin. It is interesting to note that the metathesis conditions that were successfully used with the selenoether in solution (toluene, 80 °C) proved to be ineffective on solid phase with the thioether. Excellent yields were nevertheless obtained by carrying out the RCM in CH<sub>2</sub>Cl<sub>2</sub> at 120 °C using microwave irradiation. This pilot library was screened against a panel of kinases revealing that aigialomycin D is a moderate inhibitor of

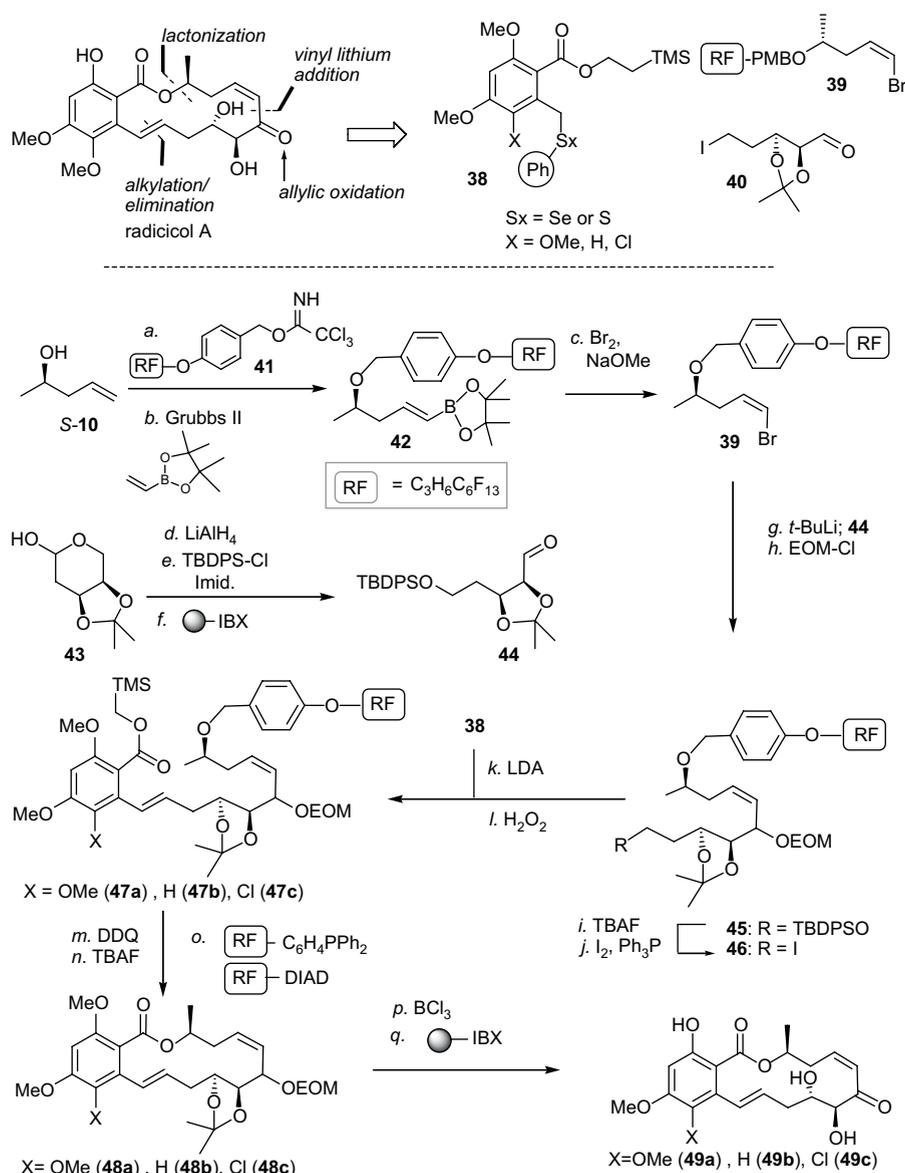
CDK1/cyclin B and CDK5/p25 at 5.7 and 5.8  $\mu\text{M}$ , respectively, as well as GSK-3 at 14  $\mu\text{M}$  but much less of PfGSK-3, the *Plasmodium* homologue of GSK-3. Closely related analogues were not inhibitors of these kinases suggesting that the particular functionalities present on aigialomycin D are important for its activity. It was further confirmed that aigialomycin D is not an inhibitor of HSP90 [23]. HSP90's ATP-binding pocket targeted by radicicol and pochonin D has a specific fold (Bergerat fold) [35] that is present in a superfamily which includes functionally diverse proteins such as DNA topoisomerase II, helicase, MultL and histidine kinase and is characterized by an L-shaped binding pocket [36]. The ATP-binding pocket of kinases on the other hand lies between the two lobes of the kinase fold which are connected by a so-called hinge region and is characterized by a more linear groove than the Bergerat fold. The selectivity of aigialomycin D for kinases rather than HSP90 or other ATPases bearing a Bergerat fold can be rationalized by the fact that this macrocycle's ground state conformation is planar and would need to overcome a minimum energy barrier of 14.7 kcal to adopt the L-shape conformation which fits in the Bergerat fold. Whether the antimalarial activity ascribed to aigialomycin D [33] can be attributed to kinase inhibition remains to be proven but its activity for selected kinases further supports the privileged nature of the RAL scaffold.

#### 4. Radicicol A: a potent MAP kinase inhibitor

Radicicol A (Fig. 1) belongs to the subset of RALs bearing a *cis*-enone and was first reported by researchers from Sandoz who identified this fungal metabolite from a screen for IL1 $\beta$  inhibition [37]. While it was observed that this compound accelerated the degradation of specific mRNA sequences including IL1 $\beta$ 's and inhibited tyrosine phosphorylation, its precise molecular target was not identified [37,38]. Subsequently, two other related resorcylic acid lactones containing a *cis*-enone were reported to be potent irreversible yet selective kinase inhibitors [39,40]. LL-Z1640-2 (Fig. 1) was identified in a screen for TAK1 inhibition [39]. Importantly, it was shown that radicicol and zearalenone had no appreciable activity in this assay while LL-Z1640-2 had an IC<sub>50</sub> of 8.1 nM. The authors further showed this compound to be competitive with ATP and to irreversibly inhibit TAK1. TAK1 is an MAPKKK involved in the p38 signaling cascade for proinflammation signals such as cytokines. In evaluating the selectivity of this compound for TAK1, the authors showed this compound to be

50-fold less active against MEK1 (411 nM), another MAP kinase and having no inhibitory effect on other MAP kinases such as MEKK1, ASKN and MKK4. The authors also demonstrated LL-Z1640-2 to effectively prevent inflammation in an animal model (topical application). LL-783-277 (Fig. 1) was found by researchers at Merck to be a potent and irreversible inhibitor of MEK1 (4 nM) [40]. The authors reported that hypothemycin (another *cis*-enone RAL, Fig. 1) was also an inhibitor of MEK (15 nM) and showed that the *cis*-enone functionality was essential for their activities. It was later shown by Santi and coworkers that this irreversible inhibition observed can be attributed to a Michael addition onto the *cis*-enone of a cysteine residue present in the ATP-binding pocket of a subset of kinases [41]. A structure-bioinformatics analysis of the kinome revealed that 46 out of the 510 identified kinases contain a cysteine residue adequately positioned for reaction. Nevertheless, amongst 16 tested kinases containing this conserved cysteine, hypothemycin had a  $K_i$  in the low nanomolar range for five kinases (MEK1, MEK2, FLT1, FLT3 and KDR), a high nanomolar  $K_i$  for one kinase (TRKB), low micromolar  $K_i$  for seven of them (ERK1, ERK2, PDGFR $\alpha$  and  $\beta$ , PKD1, MAPKAP5, TRKA and SRC) while it inhibited the remaining two kinases only weakly (GSK2 $\alpha$  and  $\beta$ ). The difference in  $K_i$  clearly reflects the fact that, while the ATP-binding pockets are highly conserved and contain the required cysteine, hypothemycin is able to discriminate with some efficiency amongst the 16 tested kinases. These results provide a mechanism for earlier observations that hypothemycin is an inhibitor of the *ras*-signaling pathway [42] and inhibits the production of several cytokines (IL2, IL6, IL10, IFN $\gamma$  and TNF $\alpha$ ) [43]. Hypothemycin was found to inhibit several oncogenic cell lines dependent on kinase activation [41] as well as tumor growth in animal models [42]. As both MEK and TAK kinases are part of the 46 kinases containing the suitably positioned cysteine, the irreversible inhibition previously observed for LL-783-277 and LL-Z1640-2 can be rationalized by the same mechanism. More recently, Santi and coworkers reported semisynthetic derivatives of hypothemycin leading to some preliminary structure–activity relationship [44]. The co-crystal structure of LL-Z1640-2 with ERK2 (Fig. 2) provided the first structural information for this class of compounds and a rationale for the importance of the alcohol stereochemistry [4].

The disconnections that were utilized for the synthesis of radicicol A and related *cis*-enone RALs are summarized in Scheme 5 [45]. A significant challenge



Scheme 5. Fluorous synthesis of radicicol A, LL-Z1640-2 and chlorinated analogue.

is to control the *cis*-geometry of the enone as it can isomerize to the thermodynamically more favorable *trans*-isomer which is known to be significantly less active [40]. We thus planned to reveal the enone at the latest stage through a selective allylic oxidation and to form the *cis*-alkene using a vinyl lithium addition and to form the *cis*-alkene using a vinyl lithium addition onto an aldehyde. The molecule was disconnected into three fragments of even complexity: **38**, **39** and **40** where either the aldehyde or the iodide of fragment **40** would need to be masked depending on the order of assembly. While the order of coupling of these three fragments is conceptually possible in all permutations,

we reasoned that starting the sequence with the coupling of **39** and **40** would allow the use of a fluorinated protecting group for the alcohol in **39** enabling the use of the fluorous isolation technology [46,47] until the penultimate cyclisation. Two features made this strategy particularly attractive, first and foremost, it has been shown that multiple components tagged with different lengths of fluorinated tags can be carried through a synthesis as a mixture and ultimately resolved using fluorous chromatography [48,49]. Second, this technology has now been automated making it amenable to high throughput synthesis [50]. The *cis*-vinyl bromide

functionality was obtained from terminal alkene **S-10** via cross metathesis with vinyl boronate and treatment with bromine under basic conditions thus affording **39** in good yield. Fragment **44** was prepared in three steps from the acetonide-protected 2-deoxy-D-ribose **43** and engaged directly in the coupling with **39**. Protecting group manipulations afforded intermediate **46**, which was then used to alkylate the different benzylic thioethers **38**. Oxidation/elimination of the selenoether afforded compounds **47a–c**. It is noteworthy that this reaction sequence starting from **S-10** did not require a single traditional workup and that the products were isolated by “catch and release” extractions on fluorinated silica. The PMB group was removed under the action of DDQ and again, the byproducts of these reactions were easily sequestered using fluorous solid-phase extraction while the 2-(trimethylsilyl)ethyl ester was removed with TBAF. The hydroxyacids thus obtained were engaged in Mitsunobu macrocyclizations using fluorous-tagged triphenyl phosphine and diisopropyl diazodicarboxylate yielding the macrocycles **48a–c** after a fluorous solid-phase extraction. All protecting groups were removed with  $\text{BCl}_3$  and the allylic alcohols were then selectively oxidized with the polymer-bound version of IBX affording radicicol A, LL-Z1640-2 and radicicol A analogue **49c** in nearly quantitative yield following a simple filtration.

Radicicol A was found to exhibit potent inhibitory activity (low nM) against VEGF-R2, and MEK and moderate activity against VEGF-R3, FLT3 and PDGFR- $\beta$  when tested in a panel of 24 kinases. The demonstration that radicicol A inhibits key kinases involved in MAPK cascades can rationalize the original observation that it accelerates the degradation of  $\text{H1}\beta$ 's RNA as there is mounting evidence that MAP kinases are involved in the regulation of protein translation by controlling the stability of specific mRNAs [51,52]. However, from a therapeutic

perspective, radicicol A may not be suitable as we found it quite prone to form quinone under mild oxidative conditions. On the other hand, analogue **49c** was not prone to quinone formation due to the chlorine substitution on the aryl ring. We found this compound to be nearly as potent as radicicol A. Evaluation of this inhibitor in a larger panel of kinases (127) showed it to be quite selective for therapeutically important kinases (MEK1, VEGFRs, and to a lesser extent PDGFR- $\alpha$  and  $\beta$ , Fig. 4). All inhibited kinases bear the suitably positioned cysteine residue, yet the discrimination among the subset of potential kinases indicates that unique interactions with its target are necessary for the Michael addition to proceed and that its selectivity could be honed or tuned to other kinases. The potential efficacy of this compound was also demonstrated in cells by measuring the level of VEGF-R2 autophosphorylation in the presence of its ligand ( $\text{VEGF}_{165}$ ). From a chemotherapeutic perspective, the fact that the multiple kinases inhibited by the *cis*-enone containing RAL are involved in pro-growth signaling pathways and angiogenesis endow these compounds with potential to have a broader therapeutic application than selective kinase inhibitors. The first broad spectrum kinase inhibitor (sunitinib – SU11248) targeting VEGFR1-3, PDGFRs, KIT, FLT3, RET and CSF1R has been approved in 2006 for the treatment of renal and gastrointestinal cancers [53]. An irreversible kinase inhibitor targeting the EGFRs is currently in phase II clinical trials. This inhibitor targets the same kinase as two approved reversible inhibitors (Iressa and Tarceva). Interestingly, it has been shown that this inhibitor remains effective against mutants that have evolved a resistance to the reversible inhibitor [54]. In addition to the therapeutic potential of this family of natural products, the irreversible nature of these inhibitors also offers a new lead structure for the

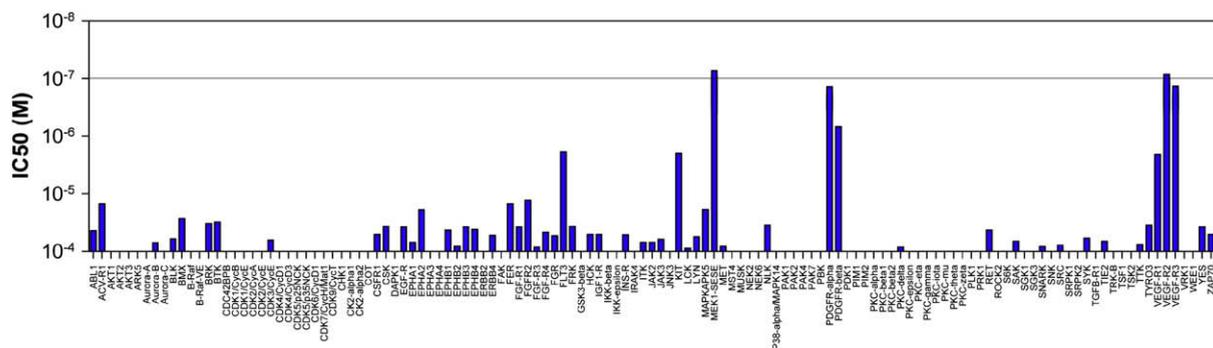
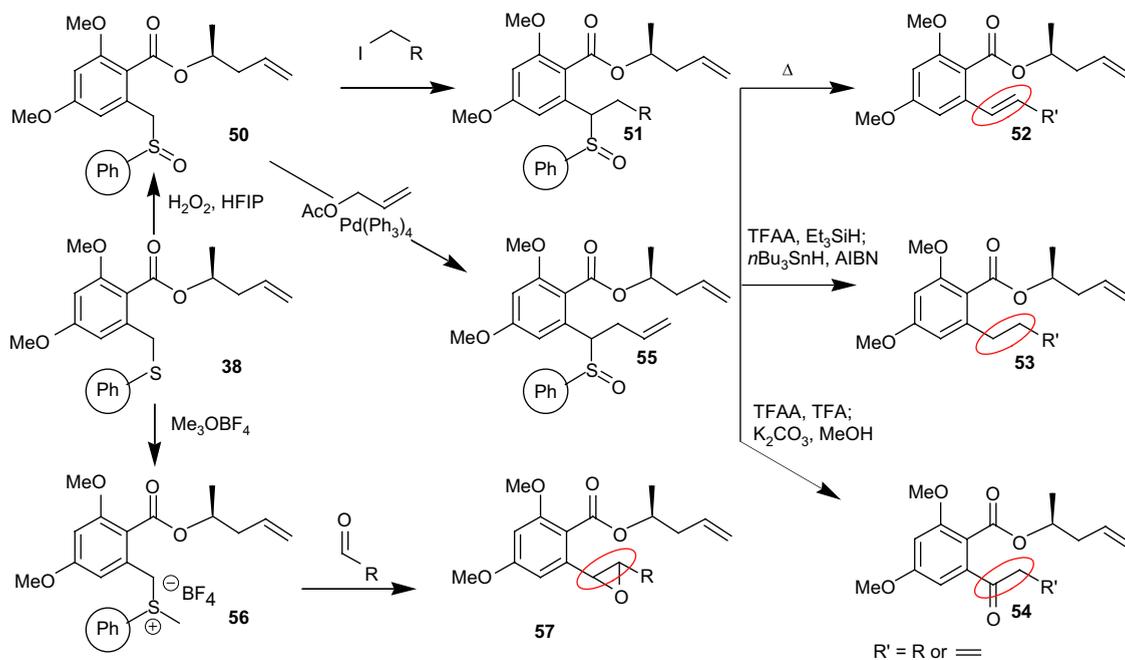


Fig. 4. Profile of compound **49c** against a panel of 127 kinases. In this assay, only VEGF-R2 and MEK are inhibited below 100 nM.



Scheme 6. Divergent chemistry of the thioether linker.

design of selective inhibitors towards engineered kinases [55,56] as well as for chemical proteomics [57].

### 5. Chemical synthesis methodology to access all the functionalities present in the RALs

Beyond the aforementioned syntheses, part of our goal was to develop a general methodology suitable to access the diversity of functionalities present in the RALs which is compatible with high throughput synthesis techniques. In that regard, the thioether functionality is particularly attractive as it offers interesting reactivity but also provides an immobilization strategy. This was exploited in the synthesis of radicicol and aigialomycin D. We have further explored the potential of this linker as shown in Scheme 6. Thus thioether **38** can be oxidized to the sulfoxide **50** which participates in alkylation chemistry under milder conditions than the thioether **38**. Conversely, sulfoxide **50** can also participate in allylation chemistry under palladium catalyzed  $\pi$ -allyl transfer conditions thus affording **55**. Product **51** or **55** can be converted to the styrenyl functionality (**52**) present in a number of RALs. Alternatively, they can also be converted to alkane **53** or ketone **54** under Pummerer condition in the presence of a reducing agent or an acid, respectively. Last but not least, *S*-alkylation of thioether **38** affords the sulfur

ylide **56** which rapidly reacts with aldehydes to afford the benzylic epoxide functionality (**57**) present in hypothemycin and aigialomycins.

### 6. Conclusion and future perspective

It is clear from the diverse biological activity of natural and synthetic resorcylics that this scaffold is a privileged starting point for the discovery of new inhibitors. The thioether methodology developed in our laboratory provides access to all the functionalities present in natural RALs and is compatible with high throughput synthesis. From a therapeutic perspective, HSP90 and kinase inhibition are on the center stage of chemotherapeutic development and the demonstration that several RALs are effective in animal model raises the hope that a natural member, semisynthetic derivative or synthetic analogue may enter clinical development.

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