Modular Synthesis of Radicicol A and Related Resorcylic Acid Lactones, Potent Kinase Inhibitors**

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Radicicol A (F87-2509.04, **1**, Scheme 1) belongs to the family of resorcylic acid lactones^[1] (RAL) and was first reported by



Scheme 1. Structure of radicicol A and related resorcylides.

researchers from Sandoz who identified this fungal metabolite from a screen for IL1 β inhibition.^[2] While it was observed that **1** accelerated the degradation of specific mRNA sequences including those of IL1 β , its precise molecular

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- Supporting information for this article, including experimental procedures, physical characterization for the synthesis of 1, 3, and 31 as well as selectivity profile of compound 31 against a panel of 127 kinases, is available on the WWW under http://www. angewandte.org or from the author.

target was not identified.^[2,3] Subsequently, two other related resorcylic acid lactones containing a *cis*-enone (**3** and **5**, Scheme 1) were reported to be potent irreversible yet selective kinase inhibitors.^[4,5] More recently, Santi and co-workers showed that hypothemycin, which also bears the *cis*-enone moiety, covalently inactivates ERK2 by reacting with a cysteine residue positioned in the active site (Cys166).^[6]

Our interest in RALs stems from the observation that a significant fraction of this family of natural products has been shown to inhibit kinases and ATPases (ATP = adenosine 5'-triphosphate). Despite the lack of obvious similarities between the RALs and ATP, these compounds have been shown to bind to the ATP-binding pocket of ATPases. We initiated a program to extend the diversity of RALs and to capitalize on this privileged structure.^[1,7-9] A cornerstone of these efforts is the development of chemistry that is compatible with high-throughput synthesis. The disconnections that were envisioned for radicicol A and related *cis*-enone RALs are summarized in Scheme 2. A significant challenge is to



 $\textit{Scheme 2.}\ Retrosynthetic disconnection of radicicol A. PG = protecting group.$

control the *cis* geometry of the enone as it can isomerizes to the thermodynamically more favorable *trans* isomer as in compound **4**, which is known to be significantly less active.^[5] It was thus anticipated that the enone should be revealed only at a late stage through a selective allylic oxidation and that the *cis*-alkene would originate from a vinyl lithium addition onto an aldehyde. As shown in Scheme 2, the molecule could thus be disconnected into three fragments (**7**, **8**, and **9**) of even complexity, in which the aldehyde or iodide of fragment **8** 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 **8**+**9** would allow the use of a fluorinated protecting group for the alcohol **9**, thus enabling the use of fluorous isolation technology^[10,11]

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until the penultimate cyclization. Two features make this strategy particularly attractive. First and foremost, it has been shown that multiple components tagged with fluorinated tags of different lengths can be carried through a synthesis as a mixture and ultimately resolved using fluorous chromatog-raphy.^[12,13] Second, this technology has now been automated, making it amenable to high-throughput synthesis.^[14]

The aromatic fragment **7** was prepared with a silyl-based protecting group (TMSE, 2-(trimethylsilyl)ethyl) for the carboxylic acid. As shown in Scheme 3, this synthesis was





Scheme 3. Synthesis of aromatic moieties **13–15**. a) $(COCI)_2$, (1.0 equiv), DMF (cat.), CH_2Cl_2 , 0°C, 1 h, then 2-(trimethylsilyl)ethanol (1.0 equiv), Et₃N (2.6 equiv), 4-DMAP (cat.), 23 °C, 1 h, 96–98%; b) LDA (2.0 equiv), (PhSe)₂ (1.0 equiv), THF, -78 °C, 1 h, 89–91%, 4-DMAP = 4-dimethylamino pyridine, LDA = lithium diisopropylamide, TMS = trimethylsilyl.

achieved in two steps from the different acids 10-12 by esterification with 2-(trimethylsilyl)ethanol and subsequent formation of the selenide via LDA deprotonation of the benzylic position and reaction with diphenyldiselenide. This procedure was found to be effective for the trimethoxysubstituted aryl ring corresponding to radicicol A (10) as well as for the dimethoxy aryl ring corresponding to 5-(Z)-7oxozeaenol (11) and the chlorodimethoxy substitution present in radicicol (12). While several protecting groups for the ortho-phenol (MOM (methoxymethyl), EOM (ethoxymethyl), PMB (para-methoxybenzyl)) were found to be suitable in the case of the 2,4-dimethoxy substitution, these protecting groups were not stable for the 2,4,5-trimethoxysubtituted aryl ring. The presence of the 5-methoxy substituent renders any acid-labile protecting group at the 2-position particularly susceptible to acids and, more generally, makes this aryl moiety prone to oxidation. Nevertheless, it was found that a methyl ether could be cleaved very selectively in the presence of the other methyl ethers using boron trichloride for all three aromatic systems.

Intermediate **8**, with the iodide masked in the form of a silyl-protected hydroxyl group, was conveniently obtained from acetal-protected deoxyribose^[15] in three steps (Scheme 4). Protected deoxyribose (obtained in one step from 2-deoxy-D-ribose) was reduced using LiAlH₄, and the crude reaction product was selectively silylated on the sterically less encumbered alcohol using TBDPSCI (greater than 15:1 selectivity) in the presence of imidazole in DMF. The free alcohol was then oxidized using an immobilized version of IBX,^[16] thus affording the aldehyde, which could be used directly in the subsequent reaction without workup or further purification.

The synthesis of fragment 9 began with the protection of (R)-2-hydroxypentene (18, Scheme 5) with a fluorous version



Scheme 4. Synthesis of key intermediate **17**. a) LiAlH₄ (1.4 equiv), THF, from 0°C to 23 °C, 2 h, 95%; b) TBDPSCl (1.0 equiv), imid. (1.5 equiv), DMF, 23 °C, 2 h, 66%; c) PS-IBX (3.0 equiv), CH_2Cl_2 , 23 °C, 2 h, 100%, DMF = dimethyl formamide, IBX = 2-iodoxybenzoic acid, TBDPS = *tert*-butyldiphenylsilyl.

of PMB trichloroacetimidate 19.^[12,17] Cross-metathesis with the vinyl borolane 20 afforded the *trans*-vinyl borolane^[18] 21 in excellent yield and stereoselectivity (greater than 20:1 E/Z) using the second-generation Grubbs catalyst or even faster and with equally good stereoselectivity using the Grela modified catalyst.^[19] Importantly, the *trans*-vinylborolane 21 could be stereospecifically converted to the cis-vinyl bromide 22 in excellent yield using the protocol of Brown et al.^[20] Transmetalation of 22 with tBuLi and addition onto the crude aldehyde 17 afforded, after EOM protection, product 23 as a mixture of diastereoisomers (3:1). This lack of selectivity is inconsequential, as the stereogenic center in question will ultimately be oxidized to the ketone. Conversion of the silylprotected hydroxyl group to the iodide (TBAF; Ph_3P , I_2) afforded compound 24, which was alkylated in excellent yield with the three different aromatic fragments 13-15 previously deprotonated by LDA. The selenide was then oxidized and eliminated to afford compounds 25-27. This reaction sequence did not require a single traditional workup. The crude reaction mixtures were simply loaded on fluorous-silica columns and eluted first with 75 % MeOH in water to remove all non-fluorous-tagged components and then washed with MeOH to recover the desired compounds. All the compounds (21-27) came at the solvent front with the MeOH wash and could be recovered in excellent yields. Reactions that were performed under basic conditions (22 to 23 and alkylation with 24) were quenched with benzoic acid resin prior to loading on the column. 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 TMSE ester was remove with TBAF. The hydroxyacids thus obtained were engaged in Mitsunobu macrocyclisations using fluorous-tagged triphenyl phosphine and diazo dicaboxylate, thus yielding the macrocycles 28-30 after a fluorous solid-phase extraction. The EOM and acetonide groups could be quantitatively deprotected using sulfonic acid resin in MeOH, while the ortho-phenol could be selectively cleaved in the presence of the other methyl ethers using BCl₃, or more conveniently, all operations could be achieved in one step with BCl₃. The allylic alcohols were then selectively oxidized with the polymer-bound version of IBX to afford radicicol A (1),^[21] 5-(Z)-7-oxozeaenol (3),^[22] and radicicol analogue 31 in nearly quantitative yield following a simple filtration.

Radicicol A and the analogues bearing different substituents on the aromatic ring as well as their corresponding allylic alcohols were tested in vitro against a panel of 24 kinases to assess their activity and specificity. In agreement with the



Scheme 5. Synthesis of radicicol A (1), 5-(Z)-7-oxozeaenol (3), and radicicol A analogue 31. a) 19 (1.0 equiv), CSA (cat.), CH₂Cl₂, 23 °C, 12 h, 92 %; b) 20 (2.0 equiv), Grubbs II (2.5 mol%); toluene, 80°С, 12 h,92%; c) Br₂ (1.0 equiv, 1 м in CH₂Cl₂), Et₂O, -20°С 10 min, then NaOMe (2.2 equiv, 1 м in MeOH), -20°С, 30 min, 89%; d) tBuLi (2.0 equiv), THF/Et₂O, -100°C, 15 min, then 17 (1.0 equiv), -100°C, 15 min, 88%; e) EOMCI (8.0 equiv), iPr2EtN (8.0 equiv), TBAI (cat.), DMF 23 °C, 12 h, 96 %; f) TBAF (2.5 equiv), THF, 23 °C, 12 h, 92 %; g) PPh₃ (1.5 equiv), I₂ (1.5 equiv), imidazole (2.5 equiv), THF, 0 °C, 1 h, 91%; h) 13-15 (1.0 equiv), LDA (2.0 equiv), THF/HMPA 10:1, -78°C, 20 min, 88-91%, i) H2O2 (2.0 equiv), THF, 23°C, 2 h, 79-82%, j) DDQ (1.2 equiv), CH2Cl2/H2O 2:1, 23 °C, 2 h, 70-80%; k) TBAF (3.0 equiv), THF, 23 °C, 2 h, 87%; l) R^FPh₃P (2.0 equiv), R^FDEAD (2.0 equiv), toluene (10 mM), 23 °C, 2 h, 81 %; m) BCl₃ (3.0 equiv), CH₂Cl₂, 0 °C, 15 min, 86%; PS-IBX (3.0 equiv), CH₂Cl₂, 23 °C, 1 h, greater than 90%. CSA = camphor-10sulfonic acid, DEAD = ethoxycarbonylazocarboxymethyl, DDQ = dichlorodicyanoquinone, Grubbs II = ruthenium[1,3-bis (2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(phenylmethylene) (tricyclohexylphosphine), HMPA = hexamethylphosphoramide, LDA = lithium diisopropylamide, $R^F = C_3 H_6 C_6 F_{13}$, TBAF = tetrabutylammonium fluoride, TBAI = tetrabutylammonium iodide, TBDPS = tert-butyldiphenylsilyl.

proposal that the enone moiety acts as a Michael acceptor, none of the products lacking the *cis*-enone showed significant activity (data not shown). Radicicol A, on the other hand, exhibited potent inhibitory activity (low nanomolar range) against VEGF-R2, VEGF-R3, FLT3, and PDGFR- β (Table 1). From a therapeutic perspective, radicicol A's 2hydroxy-5-methoxy aryl substitution may present a liability, as such electron-rich aryl moieties may be oxidized to the

corresponding quinones. We have also noted that compounds related to 3 could be oxidized to the quinone, albeit with stronger oxidizing agents, such as mCPBA (m-chloroperbenzoic acid). A number of RALs, such as pochonins C and D, are substituted with a chlorine atom at the 5position rather than a methoxy group. Inspired by these analogues, we tested compound 31 in the same panel of kinases. While it was overall slightly less active than 1 and 3, it remained a potent inhibitor of therapeutically important kinases (Table 1). The activity of this compound was further evaluated in a panel of 127 kinases, which showed that the only kinases inhibited at nanomolar concentrations were VEGF-R2 and 3, PDGFR- α and β , as well as MEK1 (see the Supporting Information), all of which have a cysteine residue in the active site. Kinases inhibited at low micromolar concentrations included FLT3, KIT, and VEGF-R1, which also bear a cysteine residue in the active site. The discrimination by 31 among the subset of kinases bearing an appropriately positioned cysteine residue 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 in vivo was then assessed by measuring the level of VEGF-R2 autophosphorylation in the presence of its ligand (VEGF₁₆₅). Immortalized human umbilical vein endothelial cells (HUVECs) known to express high levels of VEGF-R2 were incubated with inhibitor 31 for 90 min and then stimulated with $VEGF_{165}$ for 7 min.

The level of autophosphorylation was measured by ELISA using anti-VEGF-R2 as capture antibody and anti-phosphotyrosine as detection antibody. Results are expressed as a percentage of maximal autophosphorylation in the absence of inhibitor (Figure 1). Compound **31** was found to have a cellular IC₅₀ of 440 nm, which is consistent with its inhibition at the enzymatic level (90 nm). Consistently, PDGFR- β autophosphorylation was mildly inhibited by **31** (IC₅₀ =



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radicicol A (1)									9 100			9 600	26	66	110			210		
oxozeaenol (3)					6 300							6 600	52	110	170			340		
CI-radicicol A (31)													90	210	1 800			370		

[[]a] Empty fields: IC₅₀ > 100 000 пм.

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Figure 1. Cellular inhibition of VEGF-R2 (left) and PDGFR- β (right) autophosphorylation upon stimulation with their cognate ligand after preincubation with compound **31** (the y axis represents the percentage of phosphorylation compared to maximal phosphorylation).

13 μ M), whereas autophosphorylation of TIE2 was unaffected. The demonstration that radicicol A inhibits key kinases involved in MAPK cascades can rationalize the original observation that it accelerates the degradation of Il1 β '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.^[23,24]

In conclusion, we have developed a concise synthesis of radicicol A and related resorcylic acid lactones using a combination of fluorous tag-isolation and polymer-bound reagents, thus making the chemistry amenable to high-throughput synthesis. 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 design of selective inhibitors towards engineered kinases^[25,26] as well as for chemical proteomics.^[27]

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