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Synthesis of a Resorcylic Acid Lactone (RAL) Library Using Fluorous-Mixture Synthesis and Profile of its Selectivity Against a Panel of Kinases

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Dedicated to Professor Jean-Marie Lehn on the occasion of his 70th birthday

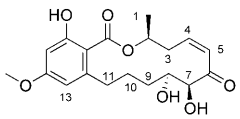
Abstract: A library of resorcylic acid lactones (RAL) containing a *cis*-enone moiety targeting kinases bearing a cysteine residue within the ATP-binding pocket was prepared using a fluorous-mixture synthesis and evaluated against a panel of 19 kinases thus providing important structure–activity trends. Two new analogues were then profiled for their selectivity against a panel of 402 kinases providing the broadest evaluation of this pharmacophores' selectivity.

Keywords: combinatorial chemistry · fluorous chemistry · kinases · lactones · resorcylic acid

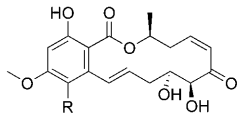
Introduction

Phosphorylation of proteins is one of the most prevalent cellular mechanisms for regulating protein function in a rapid and reversible fashion. There are approximately 518 protein kinases encoded in the human genome[1] as well as a smaller set of protein phosphatases[2] that can work synergistically or concurrently at various levels in cellular pathways, thus resulting in a tremendous combinatorial output. Virtually every signal transduction pathway implicates kinases and as much as 30% of all human proteins may be modified by them. A number of pathologies ranging from oncology to inflammation and neurodegenerative diseases can be attributed to a dysfunctional kinase.[3] Accordingly, kinases have become one of the most intensively pursued classes of proteins for drug discovery, the vast majority being currently investigated for the treatment of cancer. To date, 11 kinase inhibitors have received FDA approval and there are approximately 30 distinct kinase targets being developed at the level of phase I clinical trials.

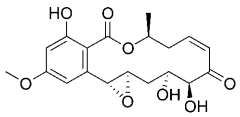
Four natural resorcylic acid lactones (RAL) containing a *cis*-enone moiety have been reported to be potent and irreversible kinase inhibitors[4] (L-783277[5] also named FR265082;[6] LL-Z1640-2[7] also named 5Z-7-oxozeaenol[8] and FR148083;[6] radicicol A[9,10] and hypothemycin[11,12]). Two of these compounds (5Z-7-oxozeaenol and hypothemycin) have already been shown to be effective in animal models.[8,11] As such, these *cis*-enone RAL represent a pharmacologically validated starting point for kinase inhibition. More recently, it has been shown by Santi and co-workers that hypothemycin irreversibly inactivates ERK2 by forming a covalent Michael adduct with the cys166 positioned in the ATP-binding pocket of this kinase.[12] A structure–bioinformatics analysis of the kinome revealed that 46 out of the 518 putative kinases contained a cysteine residue adequately positioned to participate in the Michael addition onto the *cis*-enone of hypothemycin. Nevertheless, it was clearly shown that a significant difference in the kinetics of inactivation existed amongst 16 out of the 46 tested kinases. Our profile of the related chloro-radicicol A against 127 kinases showed that this compound inhibited exclusively kinases from this subset of 46; however, not all were inhibited (GSK3β, for example, was not inhibited).[10]



L-783277 (FR265082)



R = H: LL-Z1640-2
(5Z-7-oxozeaenol or FR148083)
R = OMe: Radicicol A



hypothemycin

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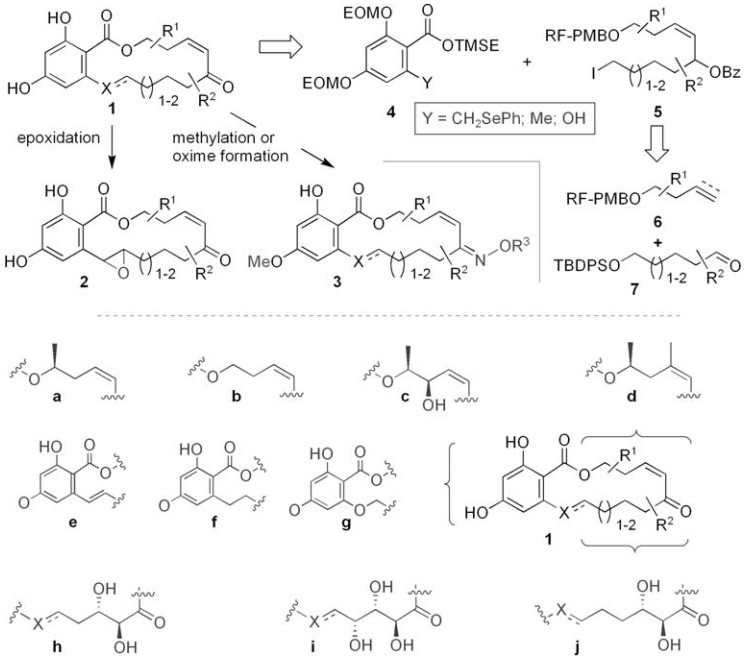
Furthermore it was shown by Nakajima and Miyake that there was a significant difference in activity (5-fold) between LL-Z1640-2 (FR148083) and L-783277 (FR265082) in their inhibition of ERK2, suggesting that a good level of selectivity may be achieved within this subset of the kinome as L-783277 was reported to be a potent MEK inhibitor (4 nM)[5] compared to LL-Z1640-2 (411 nM).[8]

Herein we report a library of over 50 analogues based on this resorcylic acid scaffold. The activity of a representative subset of 31 members was evaluated against a panel of 19 kinases. Two analogues were further evaluated against a panel of 402 kinases which includes 359 distinct kinases and 43 mutants.

Results and Discussion

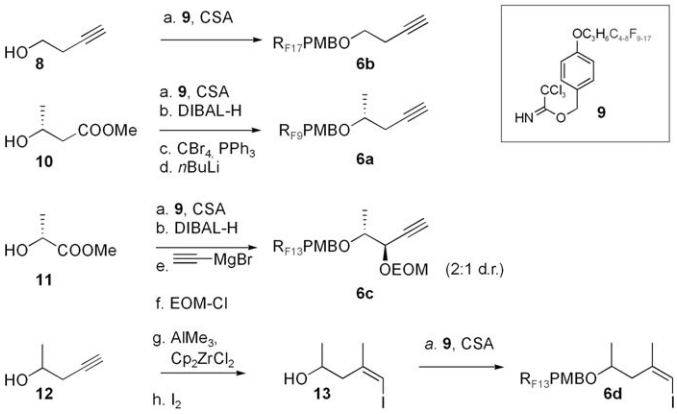
The synthetic planning of the library was based on the previously developed chemistry[10,13] which in turn was based on the use of fluorous tags to facilitate isolation of reaction product and to carry mixtures of products through a common synthetic pathway.[14–16] Thus a library of the general structure **1** (Scheme 1) was envisioned to emanate from the coupling of the key fragment **5** bearing a fluorous tag encoding its structure and the different aromatic moieties **4** bearing either the selenide or methyl or hydroxyl at position Y to obtain respectively an alkene, an alkane or phenolic ether at position X in the library **1**. While we have shown that this coupling chemistry could be carried out on solid phase using a sulfide linker at Y-position of intermediate **4** to access the alkane and alkene functionality,[13] this strategy precluded the formation of phenolic ethers, which were deemed interesting and thus motivated the exclusive use of the fluorous isolation technology for the purpose of the present library. Fragment **5** was foreseen to come from the coupling of fragment **6** and **7**, with fragment **6** being either an alkyne, which would be reduced to the *cis*-alkene with Lindlar catalyst, or a *cis*-vinyl iodide. Of course, the general library **1** could be further elaborated by epoxidation of the benzylic alkene to obtain hypothemycin analogues **2** or by methylation of the more acidic phenol and/or oxime formation on the ketone (**3**). Our choice of the fragments R^1, R^2 and aryl substitutions was based on our preliminary structure activity data coupled to reported structure activity obtained by semi-synthesis of hypothemycin[17] and available structural information.[6,18] Clearly, the nature of the functionality at the benzylic position appeared to be important in dictating the selectivity based on the afore-mentioned difference in activity between LL-Z1640-2 bearing an alkene and L-783277 bearing an alkane for ERK2[6] and MEK1.[5,8] While this modification at the benzylic position may seem rather modest, preliminary modeling experiments have suggested fairly different conformational landscape for both compounds.[19] We thus felt it important to include both of these functionalities in the library. While the epoxide at the benzylic position imparts a similar conformation to an alkene, difference in dipoles may be significant. Alternatively, it appeared that a phenolic ether at that position should also provide significant difference providing a conformational profile more similar to the alkane, but with different dipole moments. While structural information could rule out certain modifications, it should be noted that the crystal structures reported[6,18] are for the Michael adduct and the initial recognition event between the protein and the *cis*-enone may involve different macrocyclic and/or kinase conformations. The modifications which were envisioned for the library are shown in Scheme 1 with four different fragments for the ester moiety R^1 (**a**–**d**), three different functionalities at position X (**e**–**g**) and three different fragments for the lower part of the macrocycle R^2 (**h**–**j**). The chiral methyl substituent on the ester points towards the surface of β-pleaded sheets. Our prior investigation had shown that the *R* chirality at that center dramatically attenuated activity; nevertheless, compounds lacking that methyl group (fragment **b**) may broaden specificity. An extra hydroxyl group at the adjacent position (fragment **c**) was deemed interesting as it would probe whether addition-



Scheme 1. General structure and synthetic planning of a library of *cis*-enone RAL.

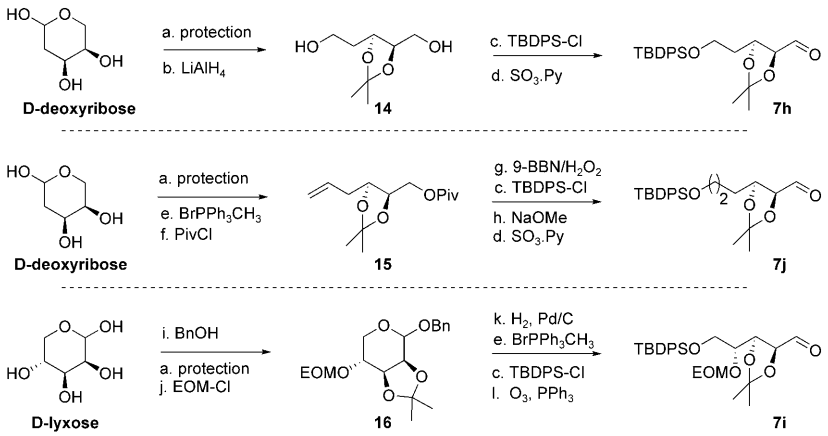
al interactions may be achieved and whether the chiral substituent imparts an important conformational bias on the macrocycle. The same reasoning was applied to the addition of a hydroxyl group on the lower part of the macrocycle (R^2, fragment **i**). Modification of the alkene (fragment **d**) was seen as crucial in modulating the rate of conjugate addition (if the conjugate addition is catalyzed by a hydrogen bond to the carbonyl, the greater stability of a tertiary cation vs. a secondary cation may favor the reaction). Furthermore, having a methyl substituent at that position could alleviate the issues related to *cis*/*trans* isomerization. It is known that the *trans*-enones are significantly less active than the *cis*-enone.[5] The homoallylic diol appeared to be important for biological activity and it was already known that methylation of either led to significant reduction in activity.[17] This functionality was thus kept constant in fragments **h**–**j**. Our preliminary investigations had shown that compounds lacking the diol were indeed inactive as well as acyclic analogues. However, a larger macrocycle appeared interesting in providing subtle changes in conformation profiles and could be probed with fragment **j**. While each of these modifications can be rationalized as providing a potential benefit, the combination of several modifications may provide unanticipated synergistic benefits.

As shown in Scheme 2, fragments **6a–d** were obtained in one to five steps through well-established chemistry. The first step to obtain **6a–c** involved a protection of the alcohols **8**, **10**, and **11** with the trichloroacetimidate of the fluorous *p*-methoxybenzyl (PMB) **9**, bearing different length of the fluorous tag encoding the structure of the starting alcohol. For **10** and **11**, the alcohol protection was followed by a diisobutylaluminium hydride (DIBAL-H) reduction and a Corey–Fuchs reaction or a Grignard addition of acetylene (2:1 d.r., inseparable mixture) followed by ethoxymethyl (EOM) protection to afford **6a** and **6c** respectively. Compound **6d** was prepared from the racemic 4-hydroxylpentyne using a known procedure[20] to access the (*Z*)-vinyl iodide **13**, which was protected with the fluorous tagged PMB. The product of each reaction was isolated by flash chromatography on fluorous silica gel. In general, a 10- to 20-fold ratio of silica to crude product weight was used for the isolation. The elutions were carried out systematically using a three step gradient (7:3 MeOH/H_2O; 8:2 MeOH/H_2O, and pure MeOH) and the product was collected from the MeOH fraction without further attempt to optimize individual isolation or recovered product from mixed fractions.



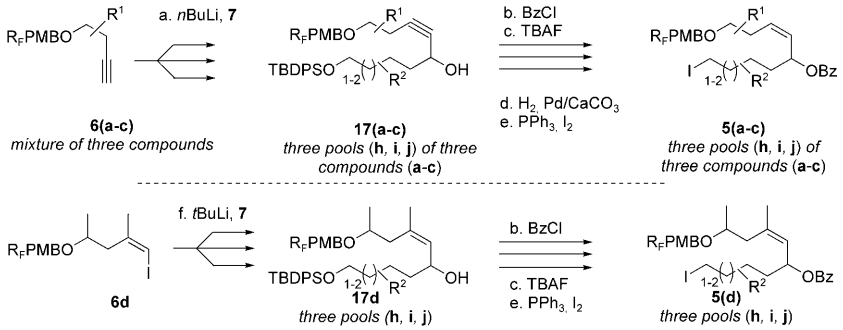
Scheme 2. Synthesis of R_F tagged fragments **6a–d**. a) PMBOC=NHCCl$_3$ (1.0 equiv) , CSA (0.14 equiv), CH_2Cl_2, 23 °C, 12 h, 72–86 %; b) DIBAL-H (1.1 equiv), PhMe, −78 °C, 1 h, 45–52 %; c) CBr_4 (4.0 equiv), PPh_3 (8.0 equiv), CH_2Cl_2, 0 °C, 45 min, 63 %; d) *n*BuLi (2.0 equiv), THF, −78 °C, 1 h, and 23 °C, 1.5 h, 85 %; e) HC_2MgBr (1.5 equiv), THF, −78 °C to 23 °C, 12 h, 88 %; f) EOM-Cl (3.0 equiv), *i*Pr$_2$NEt (3.0 equiv), *n*Bu$_4$N$^+$I (cat), CH_2Cl_2, 23 °C, 12 h, 89 %; g) Cp_2ZrCl_2 (0.25 equiv), $AlMe_3$ (3.0 equiv), CH_2Cl_2, 23 °C, 19 h and reflux 5 days; h) I_2 (1.5 equiv), THF, −30 °C, 15 min, 62 %. CSA = camphorsulfonic acid, Cp = cyclopentadienyl, DIBAL-H = diisobutylaluminium hydride, EOM = ethoxymethyl, PMB = *p*-methoxybenzyl, THF = tetrahydrofuran.

The synthesis of fragments **7h–j** (Scheme 3) was based on the naturally abundant chirality of deoxyribose and lyxose by using well-established methodology. Thus, D-deoxyribose

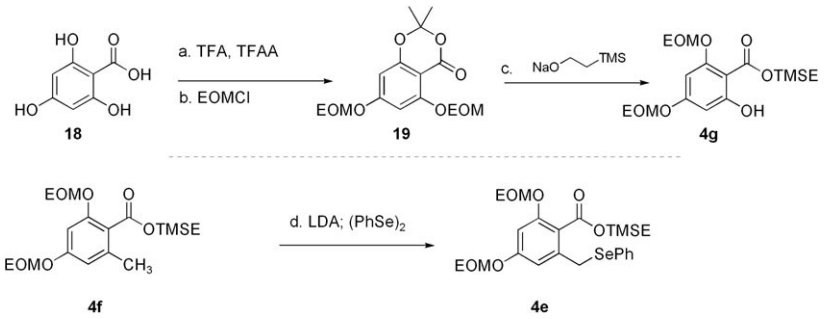


Scheme 3. Synthesis of fragments **7**. a) 2-Methoxypropene (2.0 equiv), pTsA (0.04 equiv), $CaSO_4$ (0.25 equiv), DMF, 0 °C, 3 h, 60 % or 2,2-dimethoxypropane (3.5 equiv), *p*TsA (0.02 equiv), acetone, 23 °C, 12 h, 90 %; b) $LiAlH_4$ (1.4 equiv), THF, 0 °C to 23 °C, 2 h, 95 %; c) TBDPS-Cl (0.9 equiv), imidazole (1.5 equiv), DMF, 23 °C, 2-12 h, 66–99 %; d) SO_3.Py complex (3.5 equiv), Et_3N (4.9 equiv), CH_2Cl_2/DMSO (4:1), 0 °C to 23 °C, 0.5–1 h, 91–94 %; e) $BrPPh_3CH_3$ (3.0 equiv), NaHDMS (2.8 equiv), THF, −78 °C to 23 °C, 1–12 h, 72–86 %; f) PivCl (2.0 equiv), Et_3N (4.0 equiv), DMAP (0.2 equiv), CH_2Cl_2, 0 °C to 23 °C, 12 h, 93 %; g) 9-BBN (2.2 equiv), THF, 0 °C to 23 °C, 3.5 h, then 3 N NaOH/H_2O_2, 0 °C to 23 °C, 1.5 h, 94 %; h) NaOMe (3.0 equiv), MeOH, 23 °C, 16 h, 83 %; i) BnOH (7.2 equiv), 23 °C, 10 h, 96 %; j) EOM-Cl (8.0 equiv), *i*Pr$_2$NEt (8.0 equiv), *n*Bu$_4$NI (cat), CH_2Cl_2, 23 °C, 12 h, 95 %; k) H_2, Pd/C (5 mol %), MeOH, 23 °C, 5 h, 90 %; l) O_3, PPh_3 (2.0 equiv), CH_2Cl_2, −78 °C, 1 h, 90 %. Bn = benzyl, 9-BBN = 9-borabicylo[3.3.1]nonane, DMAP = 4-dimethylaminopyridine, DMF = *N*,*N*-dimethylformamide, DMSO = dimethylsulfoxide, EOM = ethoxymethyl, NaHDMS = sodium bis(trimethylsilyl)amide, Piv = pivaloyl, *p*TsA = *p*-toluene sulfonic acid, py = pyridine, TBAI = tetrabutylammonium iodide, TBDPS = *tert*-butyldiphenylsilyl, THF = tetrahydrofuran.

was selectively protected with an acetonide[21] and reduced with $LiAlH_4$ to obtain diol **14**, which was selectively protected on the less hindered alcohol with TBDPS-Cl (TBDPS = *tert*-butyldiphenylsilyl) and oxidized to aldehyde **7h**. Starting with the same selective protection of ribose but engaging the resulting lactol in a Wittig olefination[22] rather than a reduction afforded alkene **15** after pivaloylation of the primary alcohol. A sequence involving a hydroboration, silyl protection, pivaloyl deprotection, and oxidation afforded fragment **7j**, which has an additional carbon relative to **7h**. The third fragment emanated from D-lyxose by protection of the anomeric position followed by selective acetonide protection of the *cis*-diol, and EOM protection of the remaining hydroxyl group. Deprotection of the anomeric position and conversion of the lactol to an alkene allowed protection of the unique hydroxyl group with TBDPS-Cl followed by ozonolysis of the alkene to obtain fragment **7i**. While a number of alternative strategies can be envisioned for each of these fragments, these procedures were found to be inexpensive, reliable and scalable.



Scheme 4. Synthesis of compounds **5** (12 permutations) by means of fluorous mixture synthesis using three pools. a) *n*BuLi (1.1 equiv), THF, −78 °C, 10 min, then **7** (1.2 equiv), −78 °C, 30 to 60 min, 80 %; b) BzCl (2.5 equiv), pyridine (2.5 equiv), CH_2Cl_2, 0 °C to 23 °C, 4 h, 90 %; c) TBAF (1.5 equiv), THF, 23 °C, 3 h, 86–94 %; d) H_2, $Pd/CaCO_3$ (1.72 equiv), MeOH, 23 °C, 45 min, 90–96 %; e) PPh_3 (1.5 equiv), imidazole (2.5 equiv), I_2 (1.5 equiv), THF, 0 °C°C, 30 min, 70–93 %; f) **6d** (1.0 equiv), *t*BuLi (2.0 equiv), Et_2O, −78 °C, 20 min, then **7** (1.2 equiv), pentane, −78 °C to 0 °C, 7 h, > 80 %. Bz = benzoyl, TBAF = tetrabutylammonium fluoride, THF = tetrahydrofuran.

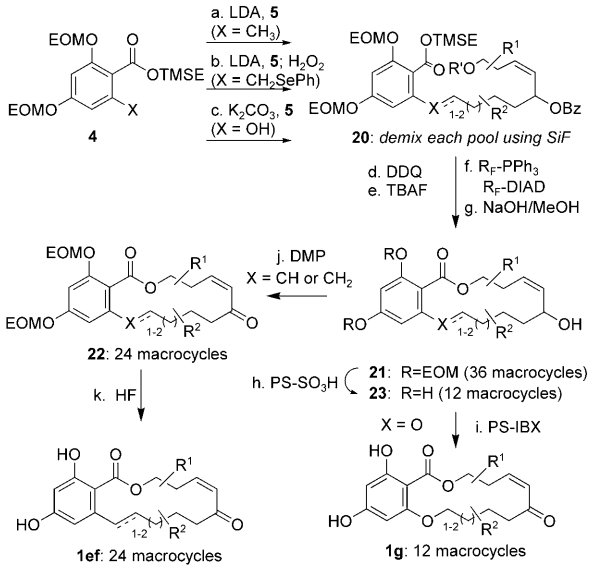


Scheme 5. Synthesis of fragments **4**. a) TFA (20.0 equiv), TFAA (7.6 equiv), acetone, 23 °C, 12 h, 54 %; b) EOM-Cl (4.0 equiv), iPr_2NEt (4.0 equiv), nBu_4N^+I (cat), CH_2Cl_2, 23 °C, 12 h, quant.; c) $TMSCH_2CH_2OH$ (4.0 equiv), NaHDMS (4.4 equiv), THF, 0 °C to 23 °C, 12 h, 69 %; d) LDA (2.0 equiv), THF, −78 °C, 30 min, $(PhSe)_2$ (0.9 equiv), THF, −78 °C, 2 h, 75 %. EOM = ethoxymethyl, LDA = lithium diisopropylamide, NaHDMS = sodium bis(trimethylsilyl)amide, TFA = trifluoro acetic acid, TFAA = trifluoro acetic anhydride, THF = tetrahydrofuran, TMSE = 2-trimethylsilylethyl.

The synthesis of key fragments **5** is shown in Scheme 4. Starting with a mixture of three fluorous-tagged alkynes **6**, which were deprotonated with *n*BuLi and added to three vessels containing the different aldehydes **7** afforded three pools of **17**, each containing three compounds labeled with a unique fluorous tag. Benzoyl protection of the alcohol followed by desilylation, hydrogenation over Lindlar catalyst and iodination afforded the three pools of three compounds **5a–c**. All reactions were monitored by LC-MS and showed greater than 90 % conversion. While the isolated yield of the product following fluorous isolation was not optimized, acceptable yields were generally obtained. The vinyl iodide **6d** was transmetalated with *t*BuLi and similarly added to three different pools of aldehydes **7**. A similar sequence of benzoylation, desilylation, and iodination afforded three pools of **5d**.

The aromatic fragments were obtained as shown in Scheme 5. The fragment **4g** was obtained starting from 2,4,6-trihydroxybenzoic acid (**18**) by protection of the acid and *ortho*-phenol with an acetonide[23] followed by protection of the two remaining phenols with EOM-Cl to obtain **19**, which was treated with the alkoxide of trimethylsilyl ethanol to yield **4g**. Compound **4f** was converted to **4e** by deprotonation with LDA and reaction with diphenyl diselenide.

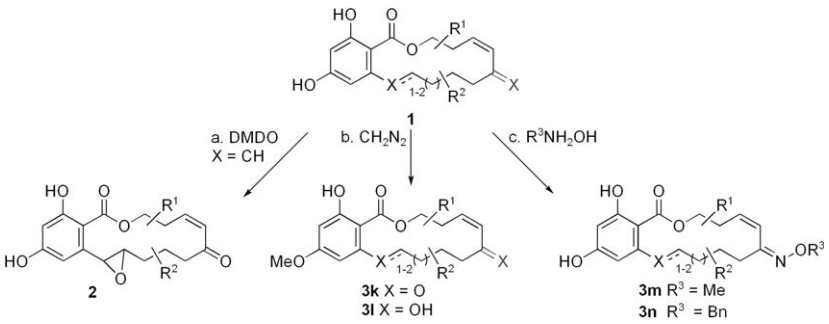
The library synthesis is shown in Scheme 6. Thus each aromatic fragment **4** was coupled to four pools of fragment **5**. Analysis of the reaction mixtures by LC-MS indicated good to excellent conversion for all reactions, with the phenol couplings being systematically the highest yielding followed by the selenide and alkyl couplings. Amongst the different pools of electrophiles, the pool containing fragment **i**, with an additional EOM-protected hydroxyl adjacent to the iodide being displaced, gave the lowest coupling efficiency. However, the desired coupled product was obtained in all cases. Each pool was demixed to resolve the individual components, which were detagged (2,3-dichloro-5,6-dicyanobenzoquinone (DDQ)) and deprotected (tetrabutylammonium



Scheme 6. Library synthesis. a) LDA (2.0 equiv), THF, −78 °C, 10 min, then **5** (0.9 equiv), THF, −78 °C, 2.0 h, 60 %; b) LDA (2.0 equiv), THF/HMPA (10:1), −78 °C, 10 min, then **5** (0.9 equiv), THF, −78 °C, 30 min and then H_2O_2 (2.0 equiv), THF, 23 °C, 2 h, 64–85 %; c) K_2CO_3 (2.0 equiv), **5** (0.9 equiv), DMF, 100 °C, 12 h, 94–100 %; d) DDQ (1.2 equiv), CH_2Cl_2/H_2O (2:1), 23 °C, 2 h, 85–96 %; e) TBAF (3.0 equiv), THF, 23 °C, 2 h, quant.; f) R_F-PPh_3 (2.0 equiv), R_F-DIAD (2.0 equiv), PhMe, 23 °C, 2 h, 50–85 %; g) 1 % NaOH in MeOH, reflux, 12 h, 80–90 %; h) PS-SO_3H (5.0 equiv), MeOH, 50 °C, 2 h, >90 %; i) PS-IBX (3.0 equiv), CH_2Cl_2/few drops of DMSO, 23 °C, 1–3 h (monitored by TLC), 50 %; j) DMP (1.5 equiv), CH_2Cl_2, reflux, 4 h, 80–90 %; k) 40 % aq. HF in CH_3CN (1:10), 23 °C, 3-6 h, 50–70 %. DDQ = 2,3-dichloro-5,6-dicyanobenzoquinone, DIAD = diisopropyl azodicarboxylate, DMF = *N*,*N*-dimethylformamide, DMP = Dess–Martin periodinane, DMSO = dimethylsulfoxide, EOM = ethoxymethyl,, HMPA = hexamethylphophoramide, IBX = 2-iodoxybenzoic acid, LDA = lithium diisopropylamide, PS = polystyrene, TBAF = tetrabutylammonium fluoride, THF = tetrahydrofuran.

fluoride (TBAF)) to be engaged in a macrolactonization. We had previously observed that these reactions could be performed at fairly high concentration (0.1 M) without dimerization or oligomerization. Treatment of each compound with fluorous-tagged PPh_3 and fluorous-tagged diisopropyl azodicarboxylate (DIAD) afforded high yield in the lactonization (>85 %) except for compounds containing the fragment **c**, bearing a sterically more demanding alcohol for which 50 % conversion was achieved. The allylic benzoate was removed in excellent yield for all macrocycles. We had previously noted that a selective allylic oxidation could be achieved in some cases, but was unfortunately not general and depended on the relative geometry of the allylic alcohol.[13] In the cases of the macrocycles bearing an ether (X = O), the selective oxidation of the fully deprotected macrocycle was reliably achieved with polymer-bound 2-iodoxybenzoic acid (IBX). On the other hand, for the oxidation of the macrocycles containing an alkane or alkene at the benzylic position, the oxidation was performed prior to deprotection using Dess–Martin periodinane in refluxing CH_2Cl_2 (the oxidation of one of the diastereoisomer is slower than the deprotected macrocycle and requires more stringent conditions). Removal of the acetonide and EOM by using aqueous HF afforded the desired macrocycles in moderate to good yields. It should be noted that the reaction was never allowed to reach completion as prolonged reaction time led to product decomposition. Macrocycles **1ef** bearing an EOM group on the *para*-phenol were also isolated from most reactions. All compounds were purified by preparative TLC. The macrocycles **1e** could be further elaborated by epoxidation of the benzylic position using DMDO.[13] However, the lability of the benzylic epoxide makes isolation of the product challenging and pharmacologically undesirable. Macrocycles could be also further derivatized by selective methylation of the *para*-phenol with diazomethane. While the reaction was quite selective for the methylation of the *para*-phenol (**3k** and **3l**; Scheme 7), to ensure completion of the reaction, these were performed with a large excess of diazomethane leading to the isolation of 10–40 % of the bis-methylated product (not shown). The ketone could be converted to a methyl or benzyl oxime in high yield under standard conditions. In total, 51 macrocycles were isolated from these efforts.

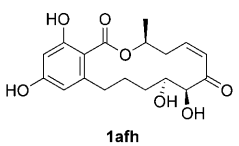
The IC_{50} of a subset of the library (28, containing at least one example of each modification) was assayed against a panel of kinases (19) as representative of kinases bearing the adequately positioned cysteine residue (VGFR-R1-3, PDGF-Rα, FLT3, MEK1, KIT, GSKα, MAP, KAPK5), kinases bearing a cysteine residue at a different position within the ATP binding pocket (EGFR-3, JNK3, NEK2, NIK, SRC, ZAP70), and kinases that do not bear a cysteine residue (PKCα, CK2α, INS-R). It should be noted that as these compounds are expected to be irreversible inhibitors, the measured IC_{50} will depend on the procedure used and caution should be applied in comparing data from experiments performed under different conditions. To obtain a value which best reflects the rate of inactivation, the substrate and inhibitor were added simultaneously to the kinase solution.



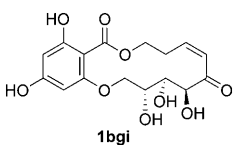
Scheme 7. a) DMDO (4.0 equiv), CH_3CN, 0 °C, 1–2 h, 60–70 %; b) CH_2N_2 (5-10 equiv), Et_2O, 23 °C, 3–12 h, 50–60 %; c) $RNH_2OH.HCl$ (10.0 equiv), pyridine, 40 °C, 12 h, >50 %. DMDO = dimethyldioxirane.

The data is shown in Table 1 (kinases have been ranked from the highest to least inhibited). As evident from Table 1, there is very little difference in relative selectivity of kinase inhibition throughout the library under these conditions. VEGF-R2 is the most highly inhibited kinase in this panel for all library members followed by PDGFR-α, VEGR-R3, Flt3, VEGF-R1, MEK1 SESE (which is a constitutively active form of MEK) and KIT. Overall, the trend of structure activity relationship which emerged from this screen is that the chiral methyl group at the ester position, while not essential, does provide higher activity (entry 6 vs. 10, 7 vs. 11, and 8 vs. 12). Substitution at the adjacent position with a hydroxyl group (entry 14 vs. 7) or at the β-position of the enone with a methyl abrogated activity (15–19). Position X of library **1** (Scheme 1), as suggested from the diverse natural products, is tolerant of modification and, an oxygen is also tolerated at that position. The trend of activity for the three natural products can be ranked as follows: alkene = epoxide > alkane. The ether in the macrocycle is generally comparable to the alkane. The two modifications of R^2 tested were beneficial in certain combinations. For example, the extra hydroxyl group present in fragment **i** does afford a significant (tenfold) gain of activity (8 vs. 7) in combination with fragment **a**. The same beneficial effect was not observed in the presence of fragment **b** (entry 12 vs. 11). The larger macrocycle (fragment **j**) was particularly beneficial in conjunction with the alkane moiety at the benzylic position (**f**). For example, entries 6, 10, and 25 are amongst the most potent compounds tested, whereas a similar gain is not observed for X = CH or oxygen (entries 4, 9, and 13). Generally, methylation of the phenol *para* to the ester had marginal changes in activity, while oxime formation abolished activity. Interestingly, none of the alcohols **23** lacking the ketone showed significant inhibition attesting to the fairly low affinity of the macrocycle for the ATP-binding pocket and the importance of the Michael acceptor.

Two representative library members (**1afh** and **1bgi**) were then evaluated in a larger panel of kinases (402) using the technology described by Lockhart et al.[24,25] In this panel, 31 kinases out of the 46 kinases bioinformatically identified by Santi and co-workers are present along with numerous other kinases bearing a cysteine residue at other positions within the ATP-binding pocket.[3] The two compounds were screened at 1 μM (i.e. well above their IC_{50} concentration for the most potently inhibited kinases) to have a realistic perspective of their selectivity. Interestingly, only two kinases outside of the set of 46 kinases predicted by Santi et al. showed significant inhibition (STK36 and PRKD2) attesting to the potential of exploiting cysteine residues within the ATP-binding pocket as selectivity filters.[26] As shown in Table 2, 11 kinases out of 31 had more than 50% residual activity given this high concentration of **1afh** (MEK4 had 0% residual activity at this concentration). The kinases which showed less than 10% residual activity relatively to control were: Flt1&3; GAK; KIT, MEK1,2,4; MKNK 1&2, PDGFRα&β, TAK1, and TGFR2. Interestingly, some kinases in this group (CDKL5, ERK1, GSK3β, NIK, NLK, PRP4) were not inhibited. For **1bgi**, 19 out of the 31 kinases had over 50% residual activity and MEK4 was also the most highly inhibited kinases, with 1% residual activity. While **1bgi** was overall less potent, it does appear to show subtle differences in reactivity compared to **1afh**. For example, while both compounds have similar activity against GAK, **1bgi** is ten times less active against Flt1 and PDGFRβ.



1afh



1bgi

The two compounds were also evaluated against a series of mutations of Flt3 and KIT. It is known that several mutations in Flt3 lead to a gain of function or constitutive activity. An internal tandem duplication (ITD) in Flt3[27] is found in 20% of patients with acute myeloid leukemia (AML), while the most abundant mutation leading to a gain of function was found to be D835Y for which both compounds (**1afh** and **1bgi**, Table 3) are more active than against the wild type.[28] Other mutants (K663Q[29] and N841 L[30]) leading to a gain of function remained equally sensitive to the inhibitors. Likewise, the compound retained their activity against KIT mutants which results in a gain of function (D816 V[31] L576P[32]and V559D[33]); however, these compounds are not active against the dual mutations that confer resistance to imatinib (V559D, T670I; V559D, V654 A)[34]

From a therapeutic perspective, the kinases inhibited by the *cis*-enone resorcylic acids contribute to the development, progression, and aggressiveness of cancer. More recent in depth characterization of the specificity of kinase inhibitors have shown that all small molecule kinase inhibitors approved for therapeutic intervention or in clinical development do inhibit multiple targets.[25] The pallet of kinases inhibited by the *cis*-enone resorcylides all drive tumor growth. The fact that all compounds lacking the enone moiety failed to show significant inhibition testifies to the importance of the Michael acceptor and its potential as a selectivity filter. An important question is whether a mutation in the cysteine residue, which is so important for the activity, would be viable. However, based on the number of kinases inhibited along the MAP kinase cascade, a concerted evolution of several kinases would be necessary to evade the growth inhibition of these resorcylic acid lactones. The irreversible nature of the inhibition may prove to be important in achieving long-lasting inactivation of specific pathways. The EGFR receptor has also been the target of irreversible inhibitors exploiting a cysteine residue. Results from phase I clinical trials have shown that EKB-569 is safe.[35] From a chemical biology standpoint, compounds that form covalent bonds to their target are particularly useful as they can be used to label the targeted kinases for activity-based[36] profiling and imaging. It is interesting to note how frequently evolution has resorted to irreversible inhibition in the selection

Table 1. IC_{50} of selected library members against a panel of 19 kinases (inhibition reported in μM).

		VEGF-R2[a]	PDGFR-α[a]	VEGF-R3[a]	FLT3[a]	VEGF-R1[a]	MEK1 (SESE)[a]	KIT[a]	NLK[a]	GSK-α[a]	MAP KAPK5[a]	EGFR-3[b]	JNK3[b]	NEK2[b]	NIK[b]	SRC[b]	ZAP70[b]	CK2-α	PKC-α	INS-R
1	hypothemycin	0.009	0.012	0.018	0.033	0.053	0.089	0.189	1.212	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
2	LL-Z1640	0.010	0.015	0.021	0.024	0.042	0.110	0.150	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
3	LL-783277	0.169	0.227	0.253	0.369	2.113	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
4	1aej	0.016	0.022	0.035	0.026	0.058	>3	0.185	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
5	1afh	0.030	0.031	0.069	0.102	0.132	0.152	0.922	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
6	1afj	0.009	0.011	0.017	0.018	0.052	0.142	0.214	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
7	1agh	0.468	0.568	0.974	1.182	1.678	1.749	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
8	1agi	0.028	0.055	0.047	0.102	0.17	0.069	0.702	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
9	1agj	0.229	0.23	0.47	0.379	1.261	0.373	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
10	1bfj	0.044	0.152	0.11	1.271	0.198	1.511	0.676	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
11	1bgh	0.176	0.437	0.386	1.137	1.507	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
12	1bgi	0.208	0.647	0.379	2.213	1.279	0.75	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
13	1bgj	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
14	1cgh	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
15	1deh	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
16	1dfh	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
17	1dfj	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
18	1dgh	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
19	1dgj	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
20	2aj	1.142	1.565	1.66	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
21	3aejk	0.919	1.259	1.482	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
22	3aejkl	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
23	3aeim	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
24	3afjk	0.013	0.020	0.022	0.033	0.061	0.749	0.192	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
25	3aghl	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
26	3agil	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
27	3agjl	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
28	3bfjk	0.040	0.074	0.072	0.939	0.195	2.34	0.701	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
29	3dejk	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
30	23agh	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3
31	23dgh	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3	>3

[a] Part of the group of kinase described by Santi et al containing a cysteine in the same position as ERK2. [b] Kinases containing a cysteine in the ATP-binding pocket but at a different site than ERK2.

Table 2. Residual activity as a % of control for 31 of the 46 putative kinase bearing the suitably positioned cysteine inhibited by two resorcylide containing an alkane (**1afh**) or ether (**1bgi**) at the benzylic position.

Kinase	**1afh**	**1bgi**	Kinase	**1afh**	**1bgi**
AAK1	17	91	MEK2	0.7	11
BIKE	26	76	MEK3	49	73
CDKL2	83	87	MEK4	0	1
CDKL3	69	52	MEK6	28	19
CDKL5	100	100	MKNK1	4.4	23
ERK1	94	95	MKNK2	0	4.4
ERK2	85	100	NIK	100	100
FLT1	5.8	57	NLK	87	100
FLT3	7	40	PDGFRA	4.7	78
FLT4	12	30	PDGFRB	0.65	37
GAK	2.8	3.8	PRP4	100	100
GSK^3A	70	100	TAK1	3.4	27
GSK3B	100	100	TGFBR2	10	18
KIT	8.8	62	VEGFR2	20	77
MAPKAPK5	63	61	ZAK	15	65
MEK1	0.35	5.6			

Table 3. Residual activity as a % of control for kinases bearing mutations.

Kinase	**1afh**	**1bgi**	Kinase	**1afh**	**1bgi**
FLT3	7	40	KIT	8.8	62
FLT3(D835H)	9	12	KIT(D816V)	0.9	13
FLT3(D835Y)	0.4	2.8	KIT(L576P)	5.2	57
FLT3(ITD)	3	26	KIT(V559D)	7.8	38
FLT3(K663Q)	6	19	KIT(V559D,T670I)	68	84
FLT3(N841I)	0	4.8	KIT(V559D,V654A)	66	100

of bioactive secondary metabolites.[37] It should also be noted that irreversible inhibition of kinases leveraged on a conjugate addition is an endogenous mechanism as has been demonstrated for the inhibition of IKB by cyclopentenone prostaglandins.[38]

In conclusion, this library offers important structural activity relationship in the *cis*-enone resorcylic acids. Two modifications were found to independently and synergistically improve the activity of this series of compounds while a modification which dramatically simplifies the synthetic accessibility of these compounds was established. The fact that several members of this family are potent inhibitors of multiple notorious oncogenic kinases should heighten their therapeutic interest.

Experimental Section

Physical characterization for all final library members, selected synthetic intermediates, and the full profile of kinase inhibition are given in the Supporting Information.

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