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# Exploring Aigialomycin D and Its Analogues as Protein Kinase Inhibitors for Cancer Targets

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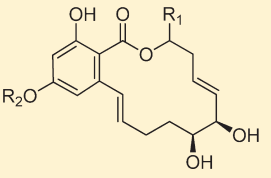
Supporting Information

**ABSTRACT:** The natural product aigialomycin D (**1**) is a member of the resorcylic acid lactone (RAL) family possessing protein kinase inhibitory activities. This paper describes the synthesis of aigialomycin D and a series of its analogues and their activity for the inhibition of protein kinases related to cancer pathways. A preliminary study of these compounds in the inhibition of CDK2/cyclin A kinase has found that aigialomycin D and analogues **11** and **23** are moderate CDK2/cyclin A inhibitors with $IC_{50}$ values of ca. 20 $\mu$M. Kinase profiling of aigialomycin D against a panel of kinases has led to the identification of MNK2 as a promising target ($IC_{50}$ = 0.45 $\mu$M), and preliminary structure−activity relationship studies have been carried out to identify the essential functional groups for activity.




(**1**) $R_1$ = (*S*)-Me, $R_2$ = H ($IC_{50}$ = 0.45 μM for MNK2)
(**11**) $R_1$ = (*R*)-Me, $R_2$ = H
(**23**) $R_1$ = (*S*)-Me, $R_2$ = Me

**KEYWORDS:** Aigialomycin D, CDK2, MNK, kinase inhibitor, resorcylic acid lactone

Kinases are essential components of cellular signaling networks and play key roles in the regulation of many important biological processes relating to cell growth, differentiation, apoptosis, etc. Studies on the inhibition of protein kinase signaling pathways over the last two decades have developed into a major paradigm for the discovery of new drugs, primarily in the area of oncology, and others such as inflammation and cardiovascular disease.[1−4] Currently, there are 11 kinase inhibitors that have been approved as drugs for the treatment of various types of cancers.[5] All of these approved drugs, along with most of the known protein kinase inhibitors, contain at least one *N*-aromatic heterocyclic motif. However, with increasing reports of resistance responses among patients to these drugs[6−8] as well as the need to develop new drugs for different types of cancers, small molecule kinase inhibitors with new scaffolds are actively being sought. One of the more promising groups of natural products that have recently emerged as new lead structures for kinase inhibition is the resorcylic acid lactones (RALs),[9−11] which are $\beta$-resorcylic acid derivatives possessing a 14-membered macrolactone ring. To date, more than 30 naturally occurring RALs have been reported, and these have a broad spectrum of biological activities, particularly as potent and selective inhibitors of protein kinases, such as transforming growth factor-$\beta$-activated kinase 1 (TAK1), mitogen-activated protein (MAP) kinase, and MAP kinase kinase (MEK).[9−11] Among the most extensively investigated RALs are those containing a *cis*-enone moiety (e.g., hypothemycin,[12−14] LL-Z-1640-2,[15−19] and L-783277[20,21]) because of their potent kinase inhibitory properties (Figure 1). The mechanism of inhibition is thought to be due to the Michael acceptor nature of the *cis*-enone system, which enables covalent binding to the cysteine residue in the ATP-binding pocket of a subgroup of kinases.[22,23] In contrast, aigialomycin D (**1**)[24−28] does not possess an enone system and yet is a cyclin-dependent kinase (CDK) 1 and 5 inhibitor albeit with less activity ($IC_{50}$ ∼6 $\mu$M).[26] This suggests that different RAL scaffolds may inhibit different kinases via various mechanisms. To understand this further, a structure−activity relationship (SAR) study of aigialomycin D and analogues is necessary. At the outset of this study, we examined the kinase inhibitory activity of aigialomycin D and analogues against CDK2, a validated target for anticancer drug development[29−31] yet unexplored for RALs.

The target compounds for SAR studies were designed to identify the functionalities that are critical to their kinase inhibition activity. Specifically, compounds **22**−**24** and **27** were synthesized to examine the nature of the substitution on the aromatic ring of the RAL scaffold while other compounds enabled the SAR study of the macrocyclic system. The analogues were synthesized based on the route previously reported by us for aigialomycin D (Scheme 1).[27] The aromatic fragment **2** was coupled with alcohols (**3a**−**c**) via the Mitsunobu reaction, providing the benzoates (**4a**−**c**) in excellent yields (>90%). The benzoates (**4a**−**c**) were then acylated at the benzylic position by deprotonation with lithium diisopropylamide (LDA) followed by condensation with the Weinreb amides (**9** and **10**)[28] to access

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the dienes (**5a**−**d**). The dienes (**5a**−**d**) were cyclized by ring-closing olefin metathesis (RCM) to give the macrocyclic compounds (**6a**−**d**) in high yields. Reduction of the C-2′ carbonyl group followed by mesylation and elimination installed the *trans*-1′,2′-double bond. Finally, global deprotection of the protecting groups afforded aigialomycin D and its analogues (**1** and **11**−**13**).

From the key intermediate **6a**, a number of analogues were also accessed (Scheme 2). Deprotection of **6a** provided **14**, which was further hydrogenated to give **15**. Reduction of the carbonyl group in **6a** using $NaBH_4$ followed by deprotection yielded **16** as a C-2′ epimeric mixture, which was further hydrogenated to give **17**. The *N*-oxime analogue (**18**) was obtained by treating **14** with hydroxylamine hydrochloride to give **18** as a *cis*- and *trans*-mixture. 7′,8′-Dihydro aigialomycin D (**19**) was prepared from **6a** by reduction of the carbonyl group followed by saturation of 7′,8′-double bond, elimination and deprotection. Taking advantage of the pivotal intermediate **6a**, two C2-phenol methylated analogues **24** and **27** were also prepared. Because C2-phenol is less reactive than C4-phenol due to intramolecular hydrogen bonding, the synthesis of **24** and **27** require a sequence of multistep transformations. In the sequence of transformations to **24**, the more reactive C2-methoxymethyl (MOM) group and the acetonide group in **25** were removed using 0.2 M TFA in methanol to give intermediate **26**. The C2-phenol of **26** was then methylated followed by the deprotection of the C4-MOM group to provide analogue **24**. Compound **27** was obtained through a sequence of similar but slightly different order of transformations from the ketone **6a**.

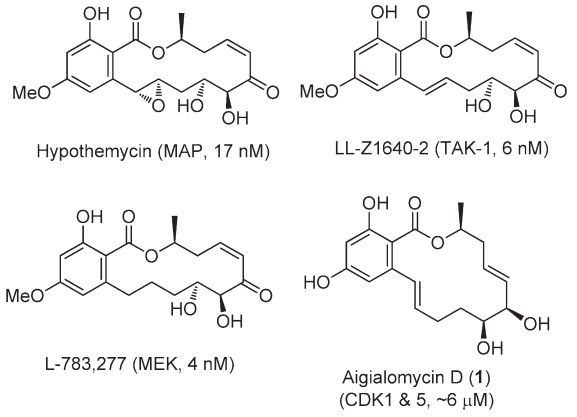
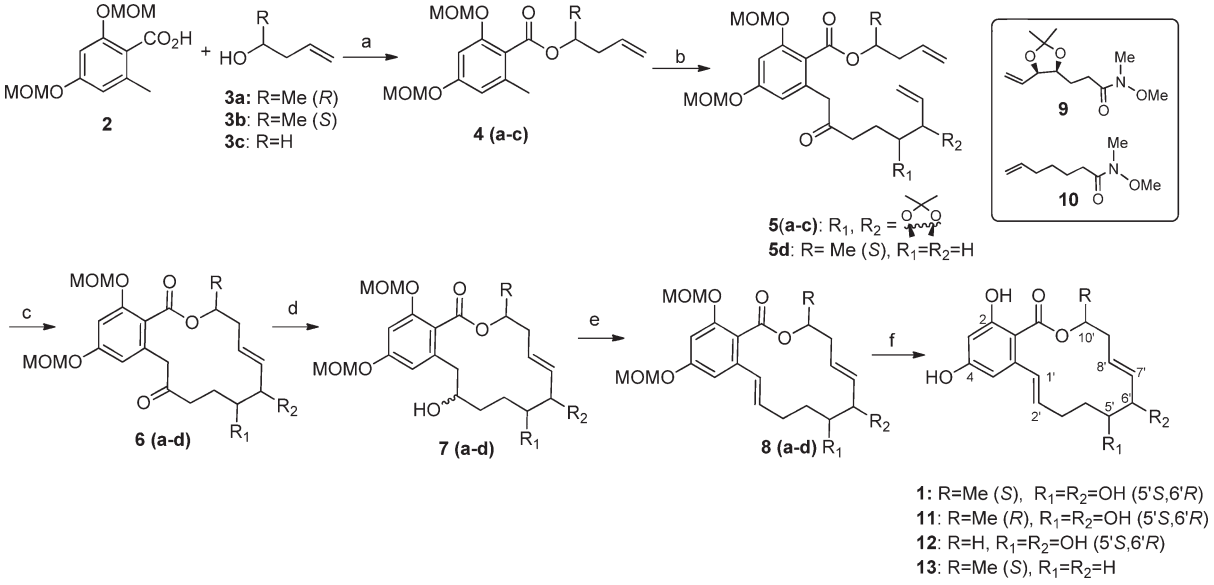



**Figure 1.** Representative RALs and their biological activities.

More analogues were accessed from aigialomycin D (**1**) (Scheme 3). Full saturation of the two double bonds in aigialomycin D (**1**) afforded tetrahydro-agialomycin D (**20**). Attempted methylation of the 2,4-phenolic groups of **1** using excess $Me_2SO_4$ in acetone did not provide either the mono- or the dimethylated product. Unexpectedly, the acetonide **21** was obtained as the sole product in high yield (94%). Alternative methylation of the phenolic groups in compound **1** using trimethylsilyldiazomethane in the presence of methanol and *N*,*N*-diisopropylethylamine[32] provided a mixture of mono- (at C-4) and dimethylated products (**22** and **23**) in a ratio of 1:1 (by $^1$H NMR spectroscopy). The two compounds **22** and **23** were separable by HPLC.

With aigialomycin D and its analogues in hand, the competitive inhibition of these compounds to the ATP-binding site of CDK2/cyclin A was initially screened using an immobilized, metal ion, affinity-based fluorescence polarization (IMAP) enzyme assay.[33] In this assay, purvalanol A (a highly potent CDK inhibitor) was used as the positive control, and percentage inhibitions by aigialomycin D and analogues were measured. From this screen, compounds **1**, **11**, and **23** were identified as the three most active ones at a 10 $\mu$M concentration (Table 1). The $IC_{50}$ values of these three compounds were determined and found to be at ca. 20 $\mu$M (Table 1), indicating that they were only moderate CDK2/cyclin A inhibitors. These results, however,

**Scheme 1. Synthesis of Aigialomycin D 1 and Its Analogues 11−13**[a]




[a] Reagents and conditions: (a) Diisopropylazodicarboxylate (DIAD), $PPh_3$, THF, room temperature, 3 days, >90%. (b) LDA, THF, −78 °C, then **9** or **10**, 15 min, 19−65%. (c) Hoveyda−Grubbs II (5−10 mol %), 1,2-dichloroethane, reflux, 1 h, 76−99%. (d) $NaBH_4$/MeOH, 0 °C, 30 min, 77−99%. (e) (i) MsCl, $Et_3N$, 4-dimethylaminopyridine (DMAP), $CH_2Cl_2$, 0 °C to room temperature, 3 h; (ii) diaza(1,3)bicyclo[5.4.0]undecane (DBU), toluene, reflux, 16 h, 32−65%. (f) 2 N HCl, MeOH, 40 °C, 6 h, quantitative.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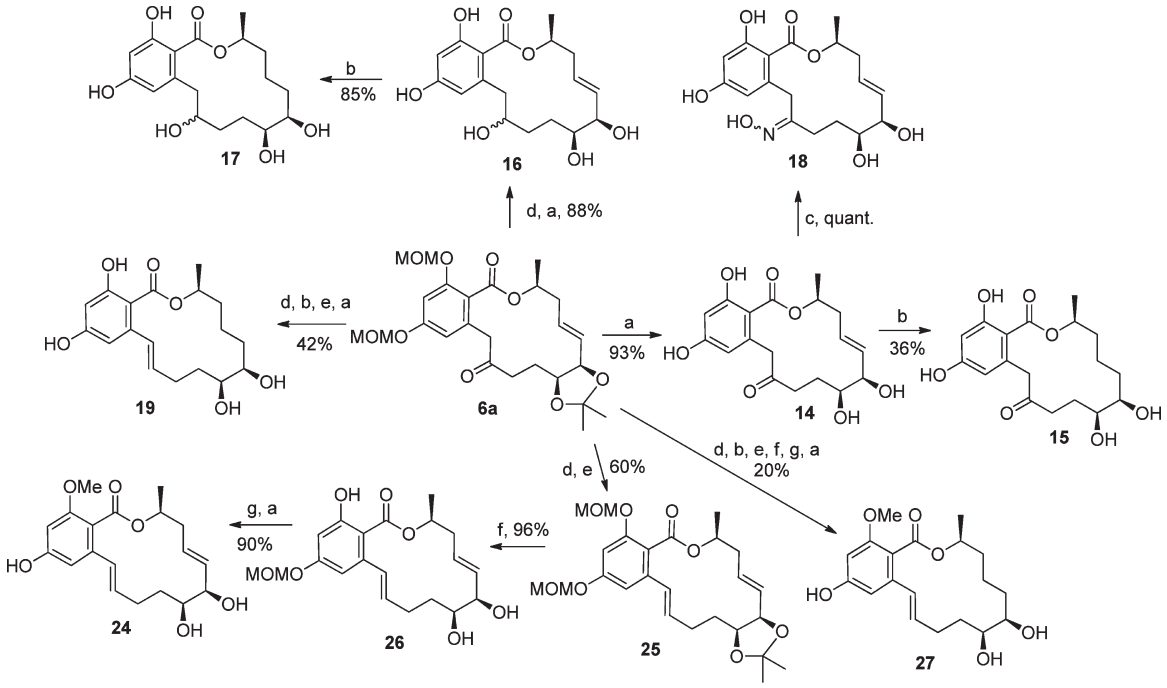
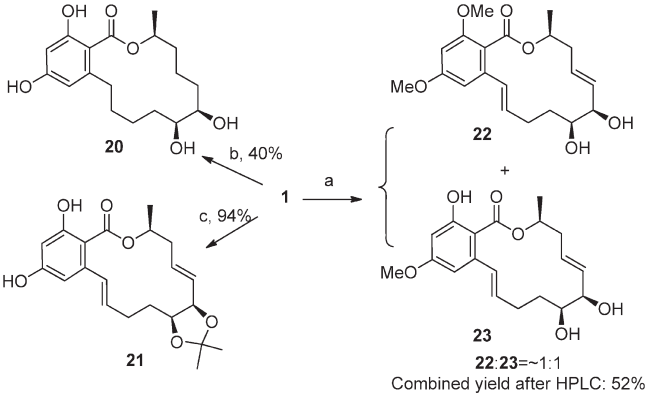
**Scheme 2. Synthesis of Aigialomycin D Analogues 14−19, 24, and 27**[a]




[a] Reagents and conditions: (a) 2 N HCl, MeOH, 40 °C, 6 h. (b) $H_2$, 10%Pd/C, EtOH, room temperature, 20 h. (c) $NH_2OH \cdot HCl$, NaOAc, dioxane, 100 °C, 3 h, quantitative. (d) $NaBH_4$/MeOH, 0 °C, 30 min. (e) (i) MsCl, $Et_3N$, DMAP, $CH_2Cl_2$, 0 °C to room temperature, 3 h; (ii) DBU, toluene, refluxing, 16 h. (f) 0.2 M trifluoroacetic acid, MeOH, room temperature, 3 h. (g) $Me_3SiCHN_2$ (2 equiv), $^iPr_2NEt$, MeOH/MeCN (2:3), room temperature, 18 h.

**Scheme 3. Synthesis of Aigialomycin D Analogues 20−23**[a]




[a] Reagents and conditions: (a) $Me_3SiCHN_2$ (3 equiv), $^iPr_2NEt$, MeOH/MeCN (2:3), room temperature, 18 h. (b) $H_2$, 10%Pd/C, EtOH, room temperature, 20 h. (c) $Me_2SO_4$, $K_2CO_3$, acetone, room temperature, 1 h.

bridged the gap of aigialomycin D against CDK2, which has not been disclosed previously.

To identify better kinase targets, aigialomycin D (**1**) was screened against a panel of 96 kinases at 10 $\mu$M (see the Supporting Information). Five most inhibited kinases with an inhibition of <10% of negative control are listed in Table 2. Among these, UFO/ARK/Tyro7 (AXL),[34] FMS-like tyrosine kinase 3 (FLT3),[35,36] mitogen-activated protein kinases interacting kinases (MKNK, or MNK),[37−39] and multifaceted polo-like kinase 4 (PLK4)[40−42] are validated targets for oncology drug development. The $K_d$ values of these kinases against **1** showed

**Table 1. Percentage Inhibition of CDK2/Cyclin A by 1 and Its Analogues at 10 $\mu$M and $IC_{50}$ Values of Selected Compounds**

| compound | % inhibition[a] | $IC_{50}$ ($\mu$M)[b] | compound | % inhibition[a] | $IC_{50}$ ($\mu$M)[b] |
|---|---|---|---|---|---|
| **1** | 55 ± 8 | 21 ± 5 | **19** | 25 ± 3 | ND |
| **11** | 64 ± 5 | 19 ± 2 | **20** | 16 ± 1 | ND |
| **12** | 38 ± 2 | ND | **21** | 19 ± 5 | ND |
| **13** | 17 ± 11 | ND | **22** | 13 ± 7 | ND |
| **14** | 21 ± 4 | ND | **23** | 49 ± 12 | 19 |
| **15** | 17 ± 4 | ND | **24** | 17 ± 8 | ND |
| **16** | 8 ± 15 | ND | **27** | 10 ± 3 | ND |
| **17** | 6 ± 0 | ND | purvalanol A | 94 ± 1 | ND |
| **18** | 19 ± 5 | ND | | | |

[a] Experiments were carried out at 10 $\mu$M with purvalanol A (1 $\mu$M) as a reference compound. Percentage inhibition values were the averages ± SDs of three independent experiments except for **17**, **19**, **20**, **21**, and **27**, which were given as the means of two experiments. [b] Data are expressed as averages ± SDs from dose−response curves of three independent experiments, except for **23**, which is the mean value of two experiments. ND = not determined.

that MKNK2 (also named MNK2 and referred to hereafter) was the most inhibited with a $K_d$ value of 0.16 $\mu$M. This high activity is very attractive as MNKs are known to phosphorylate and regulate oncogene eIF4E,[43,44] which is an emerging target for cancer drug development.[37−39] In addition, the biological implication of the great selectivity of MNK2 over MNK1 ($K_d$ = 40 $\mu$M, not shown in Table 2) could be worth exploring.


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**Table 2. Kinases with Inhibition <10% Control by 1 and Their $K_d$ Values**

| kinase | AXL | FLT3 | MNK2 | PLK4 | PRKCE |
|---|---|---|---|---|---|
| % control[a] | 4.7 | 7.1 | 0.15 | 9.6 | 7.2 |
| $K_d$ ($\mu$M) | 1.0 | 0.30 | 0.16 | 0.26 | 12 |

[a] Determined at 10 $\mu$M with DMSO as a negative control. A lower value indicates a stronger inhibition.

**Table 3. $IC_{50}$ Values of Compounds against MNK2**

| compound | $IC_{50}$ ($\mu$M)[a] | compound | $IC_{50}$ ($\mu$M)[a] |
|---|---|---|---|
| **1** | 0.45 ± 0.2 | **18** | >10 |
| **11** | >10 | **19** | 1.35 ± 0.07 |
| **12** | >10 | **20** | >10 |
| **13** | 1.61 ± 0.81 | **21** | 0.58 ± 0.36 |
| **14** | >10 | **22** | >10 |
| **15** | >10 | **23** | >10 |
| **16** | >10 | **24** | >10 |
| **17** | >10 | **27** | >10 |

[a] Data are expressed as averages ± SD from dose response curves of 2 independent experiments.

Having identified MNK2 as a promising new target of aigialomycin D, the $IC_{50}$ values of the 16 compounds prepared early on were determined using a validated in-house IMAP protocol (see the Supporting Information). The results showed that compounds **1**, **13**, **19**, and **21** have similar activities with $IC_{50}$ values at the range of 0.45−1.6 $\mu$M, while the rest were much less active with $IC_{50}$ values >10 $\mu$M (Table 3). These results indicated that an unprotected resorcinol unit together with 1′,2′-double bond and (*S*)-10′-methyl group (**1**, **13**, **19**, and **21**) are critical for the high activity. Although removal of 5′,6′-diol (**13**) or saturation of 7′,8′-double bond (**19**) caused a decrease of ca. 3−3.5-fold in activity, masking the diol as an acetonide (**21**) surprisingly only had marginal effects. These preliminary results should provide useful guidance for the design of next generation of compounds and SAR studies.

In summary, we have synthesized aigialomycin D and a series of its analogues based on a convergent and efficient synthetic route developed previously. Three compounds (**1**, **11**, and **23**) have been found to be moderate inhibitors of CDK2/cyclin A complex with $IC_{50}$ values at ca. 20 $\mu$M. These results bridged the gap of previously unknown CDK2 activity of aigialomycin D. Kinase profiling of aigialomycin D (**1**) led to the discovery of MNK2 as a promising new target with an $IC_{50}$ value of 0.45 $\mu$M. Preliminary SAR studies of the synthesized compounds against this kinase revealed that an unprotected resorcinol motif and 1′,2′-double bond are crucial for the activity. These results should provide useful guidance for the design of more potent compounds for MNK2, which is currently underway in our laboratories.

## ■ ASSOCIATED CONTENT

**Supporting Information.** Experimental procedures and characterization data for all new compounds and protocol for IMAP assays for CDK2 and MNK2. This material is available free of charge via the Internet at http://pubs.acs.org.

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