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Discovery of an in vitro and in vivo potent resorcylic lactone analog of LL-Z1640-2 as anti-inflammatory lead, II

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ABSTRACT

The potent in vitro lead compound, ER-803064 (**2**), a MEK1 and MEKK1 inhibitor inspired from natural product LL-Z1640-2 (f152A1), was further optimized to improve in vitro and in vivo potency. The modifications on C14 position led to discovery of the lead compounds **28** and **29**, which regained full in vitro potency of f152A1 and showed higher in vivo potency by iv administration.

In an earlier Letter,[1] we described the effort in bioassay-guided fractionation through phenotypic screens in identifying a natural product f152A1 (**1**), also known as LL-Z1640-2,[2] from the fermentation broth of a fungus, *Curvularia verruculosa*, in the mid 1990's. f152A1 (**1**) was found to exhibit potent anti-inflammatory activities in vitro and was rapidly inactivated in human or mouse microsomes and plasma.[1] Shown in our last report, chemical modification on C4 position led to ER-803064 (**2**) with increased metabolic stability and reduced potency (Fig. 1).[1] ER-803064 is active in vivo, but the ED_{50} value (13.2 mg/kg, iv) was fairly high in regard to TNFα suppression. Thus, we pursued further optimization on the structure toward a lead compound that would maintain the metabolic stability with improved potency in vitro and in vivo. In this Letter, we described the discovery of lead compounds **28** and **29** through optimization of the C14 position.

Having identified C4-methyl as the critical element for metabolic stability, and explored structure modifications from C3 to C8 positions, we turned our focus to study the SAR in C11–C12 and aromatic positions while keeping a methyl group in the C4 position. The assays used for lead optimization were TNFα-PLAP and Actin-PLAP reporter assays described previously.[1] The C11–C12 analogs and C13-Br analog were not as potent as **2** (IC_{50}s: 136 nM for **2**, 1683 nM for **3**, 293 nM for **4** and 804 nM for **5**) (Fig. 2).[3] The C14-ethyl analog **6** was slightly more potent than **2** (IC_{50}: 100 nM). According to this result, we focused our efforts on C14 modification.

Due to the labile nature of enone functionality, C14 analogs could not be synthesized from ER-803064 (**2**) directly. To facilitate this modification, a practical synthetic route was developed based on these criteria: (1) access to advanced intermediates that could be readily derivatized to a wide range of analogs;

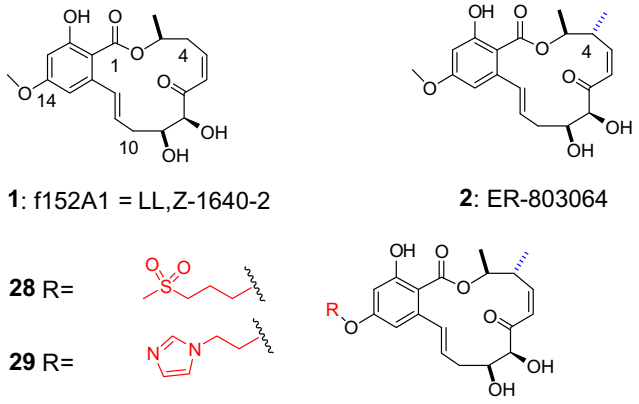

Figure 1. Structures of f152A1 (**1**), ER-803064 (**2**), **28** and **29**.

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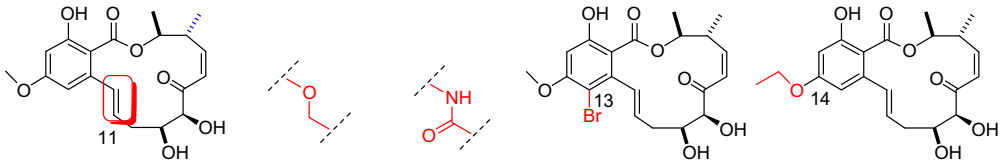
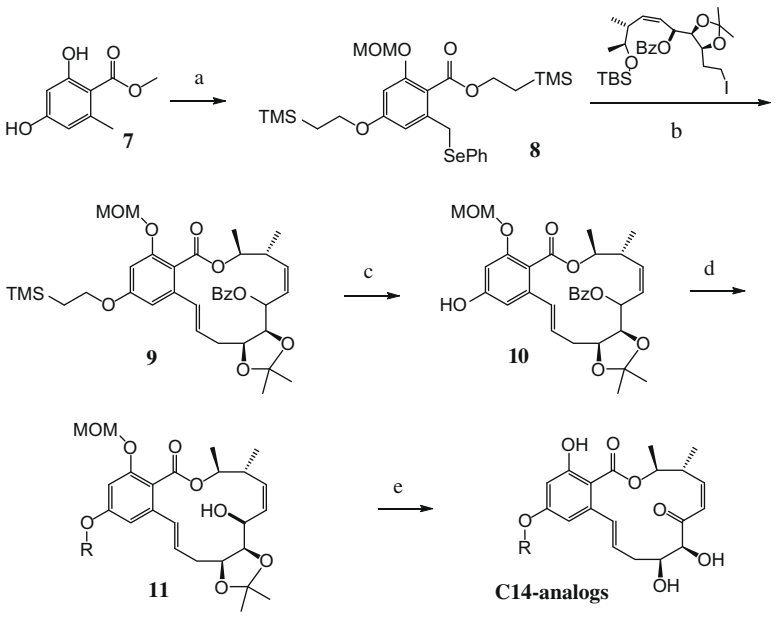


	2	3	4	5	6
TNF-PLAP (nM)	136	1683	293	804	100
ACT-PLAP (nM)	>10000	>10000	>10000	>10000	>10000

Figure 2. Structures and in vitro activities of C11–C14 analogs.



Scheme 1. Synthetic route towards C14 analogs. Reagents and conditions: (a) (i) $HOCH_2CH_2TMS$, DEAD, Ph_3P, 55%; (ii) MOM-Cl, DBU, 74%; (iii) LDA, Ph_2Se_2; (iv) 2.5 N NaOH, EtOH, 92% for two steps; (v) $HOCH_2CH_2TMS$, DEAD, Ph_3P, 77%; (b) (i) LiHMDS, acyclic iodide; (ii) MCPBA, Et_3N, 36% for two steps; (iii) TBAF; (iv) 2-Cl-1-Me pyridinium iodide, 79% for two steps; (c) TBAF, 58%; (d) (i) Mitsunobu; (ii) NaOH, 50% for two steps; (e) PCC OR Swern; (ii) HCl, 60% for two steps.

(2) the enone functionality would be introduced at late stage to avoid the isomerization and decomposition. The synthesis started from commercially available Methyl Orsellinate (**7**) (Scheme 1). Under Mitsunobu condition, *p*-phenol group in **7** was selectively protected as TMS ethylether (55% yield), which further went through a four-step transformation sequence: MOM-Cl protection (74% yield), selenination and saponification (92% yield for two steps), and Mitsunobu esterification (77% yield) to afford aromatic intermediate **8**. Selenide **8** was coupled with acyclic iodide in the presence of LiHMDS (acyclic iodide was synthesized from commercially available 2-deoxy-D-ribose and (*S*)-(+)-3-hydroxy-*N*-butyric acid methyl ester),[3] followed by MCPBA oxidation (36% for two steps), TBAF deprotection and lactone cyclization under the influence of Mukaiyama's reagent (79% yield for two steps) to provide lactone **9**.[4] Fully protected intermediate **9** was treated with TBAF to remove TMS ethyl protection group and afford key advanced intermediate **10** in 58% isolated yield. From phenol **10**, C14 substitutions were introduced through Mitsunobu reaction and benzoyl group was then deprotected under basic condition to produce allylic alcohol **11** in 50% yield over two steps. Alcohol **11** was oxidized (PCC oxidation or Swern oxidation) into labile enone intermediate and MOM group was then removed in the presence of aq HCl to furnish C14 analogs in 60% yield after purification over the final two steps.

Table 1
Structures and activity data for selected C14 analogs

Analog	R	TNFα-PLAP IC_{50} (nM)	Actin-PLAP IC_{50} (nM)
2	MeO–	136	>10,000
6	EtO–	100	>10,000
13	HO–	515	>10,000
14	*n*-PrO–	249	>10,000
15	*n*-BuO–	325	>10,000
16	*i*-PrO–	687	>10,000
17	CF_2HO–	615	>10,000
18	BnO–	319	>10,000
19	3,4-diCl-BnO–	752	>10,000
20	4-MeO–BnO–	168	>10,000

From the advanced intermediate **10**, the C14 phenol group was readily derivatized into analogs with simple alkyl and benzyl groups.[3] Biologically *n*-alkyl groups with 1–4 carbon length was very well tolerated. The two-carbon alkyl substitution (analog **6**) demonstrated the best potency within this group (Table 1).

We explored the size of C14 binding pocket and whether additional interactions with the kinase around C14 position might improve potency.[5] Thus, analogs with C14 extended alkyl side chains bearing *N* or *O*-functional groups were synthesized. Both ethylene and propylene turned out to be good linkers between C14 oxygen

Table 2
Structures and activity data for selected analogs

Analog	C14 R group	TNFa-PLAP IC_{50} (nM)	Actin-PLAP IC_{50} (nM)
21	$Me_2N–CH_2CH_2–O–$	597	>10,000
22	$HO–CH_2–(HO)CH–CH_2–O–$	383	>10,000
23	2-Pyridine-NH–$(CH_2)_2–O–$	113	>10,000
24	Morpholine-$CH_2CH_2–O–$	59	>10,000
25	$CH_3–CO–NHCH_2CH_2–O–$	65	>10,000
26	1-Triazole-$(CH_2)_2–O–$	43	>10,000
27	$Ac–NH–CH_2–CO–CH_2–O–$	31	>10,000
28	$MeSO_2–(CH_2)_3–O–$	32	>10,000
29	1-Imidazole-$(CH_2)_2–O–$	15	>10,000

Table 3
In vivo data for selected analogs

Analog	C14 R group	TNFα-PLAP IC_{50} (nM)	ED_{50}, mg/kg (iv) TNFα
1	–	11	No dose response
2	$CH_3–O–$	136	13.2
6	$CH_3–CH_2–O–$	100	12
28	$MeSO_2–(CH_2)_3–O–$	32	9.8
29	1-Imidazole-$(CH_2)_2–O–$	15	6.5

and the new functional groups. Sulfone analog **28** (R = $MeSO_2–(CH_2)_3–O$) and imidazole analog **29** (R = 1-imidazole-$(CH_2)_2–O$) had significantly improved potency over ER-803064 (**2**) (IC_{50}s: 32 nM for **28**, 15 nM for **29** vs 136 nM for **2**) (Table 2). The in vitro potency of **29** was comparable to f152A1 (IC_{50}: 11 nM). We found that both **28** and **29** were more potent than **2** in vivo (Table 3).[6] In particular, **29** significantly improved in vivo potency over ER-803064 (**2**) (ED_{50}: 6.5 mg/kg for **29** vs 13.2 mg/kg for **2**) (Table 3).

In conclusion, we have studied the SAR of f152A1 analogs with synthetic modifications on C11–C12 and aromatic positions. Modifications on C14 aromatic position resulted in a series of interesting analogs. A practical synthetic route was developed for the synthesis of important C14 analogs. Our medicinal chemistry effort led to the discovery of the analogs **28** and **29**, fully synthetic analogs of f152A1 (**1**). The analog **29** maintained the metabolic stability, regained full in vitro potency similar to **1** and had significant improvement in in vivo potency. These new leads were further evaluated in various animal models to validate the anti-inflammatory application.

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