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Kinase Inhibition by Deoxy Analogues of the Resorcylic Lactone L-783277

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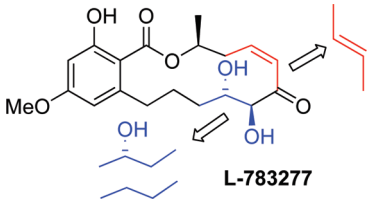
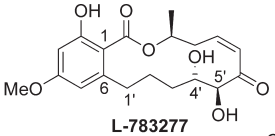
ABSTRACT The natural product L-783277 is a resorcylic lactone type covalent kinase inhibitor. We have prepared the 5′-deoxy analogue of L-783277 (**1**) in a stereoselective fashion. Remarkably, this analogue retains almost the full kinase inhibitory potential of natural L-783277, with low nanomolar IC_{50} values against the most sensitive kinases, and it exhibits essentially the same selectivity profile (within the panel of 39 kinases investigated). In contrast, removal of both the 4′- and the 5′-hydroxyl groups leads to a more significant reduction in kinase inhibitory activity and so does a change in the geometry of the C7′-C8′ double bond in **1** from *Z* to *E*. These findings offer new perspectives for the design of second generation resorcylic lactone-based kinase inhibitors.

KEYWORDS Cancer, irreversible inhibitor, kinase inhibitor, natural product, SAR, stereoselective synthesis, resorcylic lactone (RL)

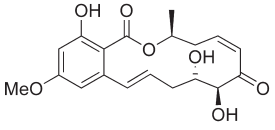


L-783277

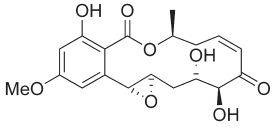
The inhibition of protein kinases has emerged as a powerful mechanistic paradigm for the treatment of diseases that are characterized by dysregulated intracellular signaling or defects in cell cycle regulation.[1−4] A total of 11 kinase inhibitors are currently approved for the treatment of various types of cancers,[1,2] and kinase inhibitor drugs are likely to be of therapeutic value also in other disease indications.[3,4] With the exception of the two rapamycin derivatives temsirolimus and everolimus, which do not inhibit kinase activity directly,[5] all marketed kinase inhibitors are based on different types of (poly)heterocyclic or urea scaffolds.[1−3] These structural features are also shared by the majority of agents in clinical and preclinical development. As an important extension of kinase inhibitor chemical space, the resorcylic lactone (RL)-based fungal metabolites hypothemycin, LL-Z1640-2, and L-783277 have recently been recognized as a new and structurally unique group of kinase inhibitors.[6,7]



L-783277



LL-Z1640-2



Hypothemycin

The suppression of kinase activity by these natural products involves 1,4-addition of a protein thiol to the *cis*-enone moiety in the macrocycle, as has been demonstrated in biochemical[8] as well as structural studies.[9,10] The inhibition of protein kinases by *cis*-enone-containing RL, thus, is largely confined to kinases incorporating a properly located Cys residue in their ATP binding pocket, corresponding to Cys166 in ERK2,[8] which provides a "built-in" selectivity advantage over other inhibitor classes. Importantly, while kinases containing the Cys166 equivalent of ERK2 comprise only ca. 10% of the total kinome, they include a number of important drug targets such as VEGFR2, MEK2, cKIT, or FLT3.[8] At the same time, the use of covalent inhibitors may bear the potential for undesired off-target effects, due to the unspecific modification of protein thiols. However, on the basis of work by Kosan, the second order rate constant for the reaction of hypothemycin with glutathione at pH 7.5 is 6.6 M^{-1} s^{-1} vs an apparent second order rate constant (k_{inact}/K_i) of 1.2 × 10^5 for the reaction with MEK1, thus indicating a high degree of specificity.[8]

Structure−activity relationship (SAR) studies around hypothemycin[11] and LL-Z1640-2 (also called f152A1 or 5*Z*-7-oxozeaenol)[12−19] have included modifications of both the aliphatic and the aromatic portion of the structures, and analogues have been identified with in vivo activity in tumor[20] as well as inflammatory disease[14,18] models; one compound, Eisai's E6201, has been advanced to clinical trials in humans[13] in cancer and plaque type psoriasis

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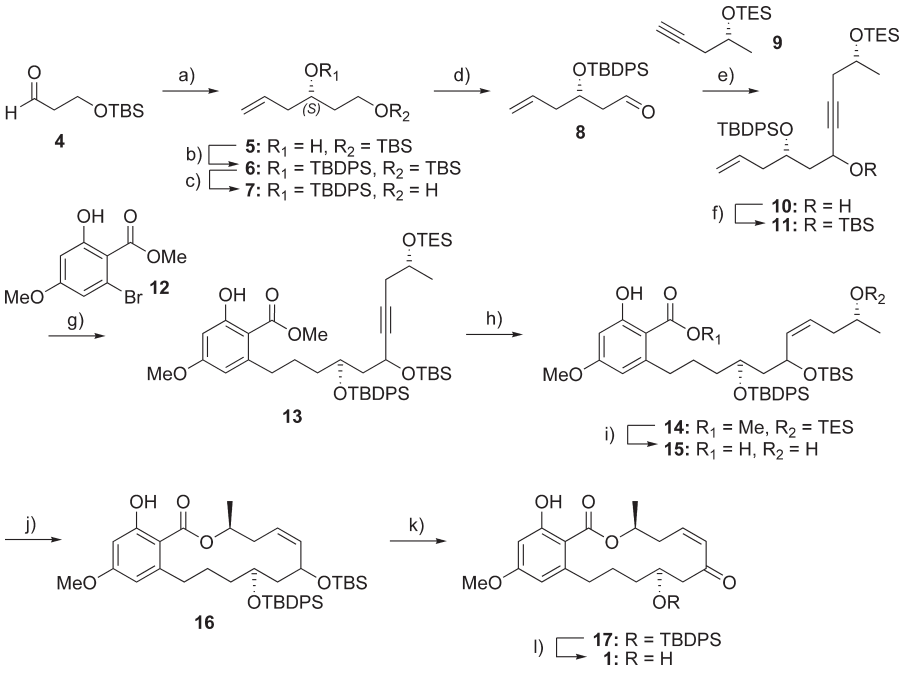
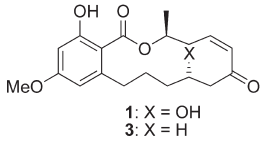
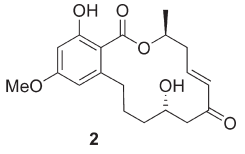
Scheme 1[a]



[a] Reagents and conditions: (a) $Ti(OiPr)_4$ (2.5 mol %), (*S*)-BINOL (5 mol %), $CH_2{=}CHCH_2SnBu_3$, toluene, −20 °C, 139 h, 90%, 98% *ee*. (b) TBDPSCl, imidazole, DMF, room temperature, 21 h, 90%. (c) *p*-TsOH, THF/H_2O (20:1), room temperature, 10 h, 92%. (d) $(COCl)_2$, DMSO, NEt_3, CH_2Cl_2, −78 °C, 1.5 h, 94%. (e) Compound **9**, *n*-BuLi, THF, −78 °C → −10 °C, 40 min; then addition of **8**, −78 °C → room temperature, 2 h, 93%, *dr* 1:1. (f) TBSCl, imidazole, DMF, room temperature, 15 h, 96%. (g) Compound **11**, 9-BBN (1.5 equiv), THF, room temperature, 5 h; then **12**, 2 M K_3PO_4, $Pd(OAc)_2$ (20 mol %), TFP (0.8 equiv), DME, reflux, 19 h, 68%. (h) 7.5 bar H_2, Lindlar catalyst (5 mol %), AcOEt, 50 min, room temperature, 0.1 M, 97%. (i) 1 M NaOH/MeOH (1:3), reflux, 21 h, 60%. (j) DEAD, PPh_3, toluene, 0 °C → room temperature, 2.5 h, 49%. (k) CrO_3, H_2SO_4, KF, H_2O, acetone, −10 °C, 2 h, 64%. (l) HF·Py, THF, room temperature, 9 h, 88%. BBN, 9-borabicyclo[3.3.1]nonane; DEAD, diethyl diazodicarboxylate; TBDPSCl, *tert*-butyldiphenylsilyl chloride; TBSCl, *tert*-butyldimethylsilyl chloride; TFP, tri-2-furanylphosphine; *p*-TsOH, *para*-toluenesulfonic acid.

(according to the Thomson Reuters Integrity database). With the exception of their clinical candidate E6201,[17] however, no kinase inhibition data have been reported by the Eisai group on the numerous LL-Z1640-2 analogues that were prepared as part of the work leading to the discovery of E6201. In general, the presence of two oxygen functionalities at C4′ and C5′ and the retention of the natural configuration at these chiral centers have been considered as important determinants for potent kinase inhibition by *cis*-enone-containing RL.[15,19] Thus, all three non-natural epimeric diols of LL-Z1640-2 were found to be > 50-fold less active than the natural product in a cellular reporter assay for TNFα release.[19] At the same time, the structure of hypothemycin bound to ERK2 does not show any significant interactions between the C5′-OH and the protein.[9] This is in line with the observation that methylation of the 5′-OH group in hypothemycin produces no more than a 3−5-fold loss in antiproliferative activity;[11] in contrast, the corresponding C4′-*O*-methyl derivative was found to be significantly less potent, but no in vitro kinase inhibition data have been reported for either of these methylated derivatives.[11] While the presence of a 5′-OH (or -methoxy) group may be important for the stabilization of the bioactive conformation of RL-based kinase inhibitors, even in the absence of discernible hydrogen bonding with the protein, the structural data leave open the possibility for the 5′-OH group to be a functionally less relevant (or even irrelevant) inborn structural feature of the natural products. In light of this and because the presence of a chiral center α to the carbonyl had caused synthetic problems in some of our previous work,[21] we have investigated the activity of 5′-deoxy L-783277 (**1**). For reference purposes, we have also prepared the corresponding C7′-C8′ *E* analogue **2** and dideoxy L-783277 (**3**) and determined their kinase inhibitory properties.





Neither of these three analogues has been investigated previously; 4′,5′-dideoxy LL-Z1640-2 has been described as part of a model study toward the total synthesis of LL-Z1640-2,[22] but the inhibition of kinase activity by this compound was not studied.

The synthesis of **1** was based on the same overall strategy that we had followed in our recent total synthesis of L-783277[23] and involved the use of building blocks **8**, **9**, and **12** for the assembly of the macrolide skeleton (Scheme 1). Thus, the addition of the Li-acetylide derived from protected homopropargylic alcohol **9** to aldehyde **8** followed by TBS protection of the secondary hydroxyl group provided the

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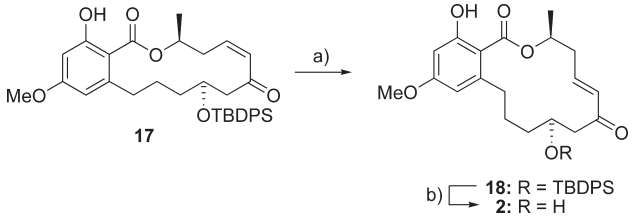
protected C1′-C10′ fragment **11** (as a mixture of diastereoisomers at C6′) in excellent overall yield. Building block **8** (which is the distinguishing element from the synthesis of the natural product L-783277[23]) was prepared from TBS-protected 3-hydroxy propanal (**4**) in a high-yielding four-step sequence (69% overall yield) involving Keck allylation[24] of **4** (which proceeded with 98% ee) followed by protecting group reshuffling and oxidation of the primary hydroxyl group under Swern conditions. Olefin **11** was then connected to aryl bromide **12** by Suzuki−Miyaura coupling to produce the protected *seco* acid **13**; subsequent partial hydrogenation of the triple bond with Lindlar catalyst followed by simultaneous ester saponification and TES cleavage with NaOH/MeOH gave the *seco* acid **15** as the immediate cyclization precursor (38% over three steps). It should be noted here that the clean cleavage of the methyl ester group in **13** proved to be difficult and could be realized in acceptable yield only after extensive optimization.[26] Cyclization of **15** under Mitsunobu conditions gave the protected macrolide **16** in moderate but acceptable yield (49%). Treatment of **16** with Jones reagent complemented with KF resulted in cleavage of the allylic TBS ether and immediate oxidation to ketone **17**. The latter was deprotected with HF·pyridine to furnish the desired 5′-deoxy L-783277 (**1**) in 56% yield from **16**. Upon standing in chloroform solution for 4 days, the protected macrolactone **17** isomerized to its C7′-C8′ *E* analogue **18** in high yield (Scheme 2).[25] Treatment of **18** with HF·pyridine then gave *E*-5′-deoxy-L-783277 (**2**) (81%).

Scheme 2[a]



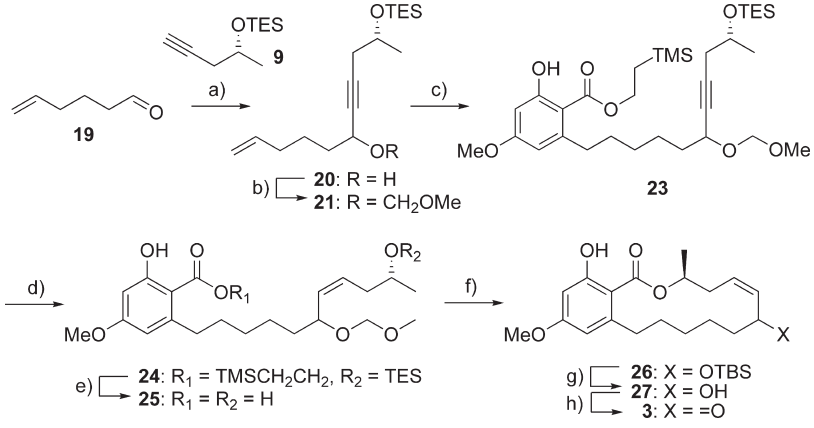
[a] Reagents and conditions: (a) $CDCl_3$, room temperature, 4 days, 96% conversion, 83%. (b) HF·Py, THF, room temperature, 10 h, 81%.

The synthesis of dideoxy-L-783277 (**3**) is summarized in Scheme 3. Conceptually, the synthesis parallels that of **1**, except for the use of a different protecting group strategy [i.e., MOM protection for the secondary hydroxyl group formed in the acetylide addition step and TMS-ethyl ester protection for the carboxyl group in the aromatic building block **22**[23] (Scheme 3)]. Analogue **3** was ultimately obtained from aldehyde **19** in eight steps and ca. 10% overall yield.

The in vitro kinase inhibitory activity of L-783277 and compounds **1**−**3** were assessed against a panel of 39 kinases. Table 1 provides a listing of those kinases that were inhibited with an IC_{50} value $< 10\ \mu M$ by at least one of the compounds; in all other cases, IC_{50} values were $> 10\ \mu M$.

As expected, L-783277 is highly selective for the inhibition of kinases with an appropriately located Cys residue in the ATP binding site, with nanomolar activity against VEGFR2 (IC_{50} = 2 nM) and PDGFRα (V651E)[28] (IC_{50} = 2.4 nM). cKIT, MK5, ERK2, and TYK2, although they do contain the requisite Cys residue, are inhibited with much lower potency, thus indicating that L-783277 (as other RL-based kinase inhibitors)[8,15] does not indiscriminately inhibit all potentially susceptible kinases with the same potency. IC_{50} values of ca. 800 nM and 7 μM were observed against ALK and LCK, respectively, which do not contain an ERK Cys166 equivalent; inhibition of LCK by L-783277 has been reported previously by the Merck group with an IC_{50} value of 750 nM.[29] The IC_{50} value of L-783277 for VEGFR2 is one order of magnitude lower than has been reported by others;[15] the reasons for this discrepancy are unknown, but it may (at least in part) reflect differences in assay format.[30] L-783277 was found to inhibit MEK2 with an IC_{50} of 15 nM, which is in

Scheme 3[a]



[a] Reagents and conditions: (a) Compound **9**, *n*-BuLi, THF, −10 °C, 20 min; then **19**, −78 °C → −18 °C, 1.5 h, 51%, *dr* 1:1.1. (b) MOM-Cl, $(i\text{-Pr})_2NEt$, Bu_4NI, DMF, 19 h, 91%. (c) Compound **21**, 9-BBN, THF, room temperature, 2 h; then 2-(trimethylsilyl)ethyl 2-bromo-6-hydroxy-4-methoxybenzoate (**22**), 2 M K_3PO_4, $Pd(OAc)_2$ (15 mol %), TFP (0.6 equiv), DME, reflux, 1.5 h, 83%. (d) H_2, Lindlar catalyst, AcOEt, 3 h, 92%. (e) TBAF, THF, room temperature, 2 h, 90%. (f) DEAD, Ph_3P, toluene/THF, 1 h, 64%. (g) Sulfonic acid resin, MeOH, reflux, 3 h, 73%. (h) DMP, CH_2Cl_2, 4.5 h, 72%. DMP, Dess−Martin periodinane (1,1,1-tris(acetyloxy)-1,1-dihydro-1,2-benziodoxol-3(1*H*)-one); TBAF, *tetra*-*n*-butylammonium fluoride.

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Table 1. Inhibition of Protein Kinases by RL L-783277, **1**, **2**, and **3**[a]

	IC_{50} (nM)			
Kinase[b]	L-783277	**1**	**2**	**3**
ALK	763 ± 40	763 ± 75	> 10000	3500/4400
ERK2	950/1300	6600/6400	> 10000	> 10000
EPHB4	> 10000	7400/7500	> 10000	> 10000
cKIT	303 ± 134	673 ± 119	7600/8500	> 10000
LCK	6200/7000	4800/4700	> 10000	> 10000
MEK2[c]	15[d]	ND	ND	6840[d]
MK5	640[d]	ND	ND	> 10000
MNK2	ND	150[d]	ND	> 10000
PDGFRα[e]	2.38 ± 1.90	7.23 ± 1.15	180/220	95/100
RET	> 10000	9900/7200	> 10000	> 10000
TYK2	4100/5600	4400/5700	> 10000	> 10000
VEGFR2	2.0 ± 1.4	5.80 ± 1.01	230/190	170/190

[a] IC_{50} values from two independent experiments; for L-783277 and **1**, values against ALK, cKIT, PDGFRα, and VEGFR2 are averages ± SDs from three independent experiments. Kinase assays were essentially carried out as described in ref 27. IC_{50} values for AUR A, CDK2A, CDK4D1, COT1, ERK2, JAK1-3, PDK1, PKCα, PKN1-2, ROCK2, ABL, and p38α were determined in a Caliper mobility shift assay; TR-FRET-based LanthaScreen assay technology was used for all other kinases. ND, not determined. No significant inhibition was observed at concentrations < 10 μM for the following kinases: AUR A (aurora kinase A), AXL, BTK (Bruton's tyrosine kinase), CDK2A/4D1 (cyclin-dependent kinases 2A and 4D1), ZAP70 (ζ-chain-associated protein kinase), COT1 (colonial temperature-sensitive gene-encoded kinase 1), EPHA4 (ephrin A4), FGFR3 (fibroblast growth factor receptor 3, K651E mutant), GSK3β (glycogen synthase kinase 3β), HER2 (human epidermal growth factor receptor 2), HGFR (hepatocyte growth factor receptor; cMet), IGF1R (insulin-like growth factor 1 receptor), INSR (insulin receptor), JAK1/2/3 (Janus kinases 1, 2, and 3), PDK1 (phosphoinositide-dependent protein kinase 1), PKA/α/τ (protein kinases A, α, and τ), PKN1/2 (protein kinases N 1 and 2), ROCK2 (rho-associated protein kinase 2), cABLT315I (Abelson kinase, T315I mutant), and p38α (mitogen-activated protein kinase 14). [b] ALK, anaplastic lymphoma receptor tyrosine kinase; ERK2, extracellular-signal-regulated kinase 2; EPHB4, ephrin B4; LCK, lymphocyte-specific protein tyrosine kinase; MEK2, mitogen-activated protein kinase kinase 2; MK5, mitogen-activated kinase-activated protein kinase 5; MNK2, mitogen-activated protein kinases-interacting kinase 2; PDGFRα, platelet-derived growth factor receptor α; RET, "rearranged during transfection" gene-derived kinase; TYK2, nonreceptor tyrosine kinase 2; and VEGFR2, vascular endothelial growth factor receptor 2. [c] MEK2 testing was performed at Millipore/Upstate (Charlottesville, VA). [d] Single measurement. [e] V561D mutant.[28]

good agreement with the activity originally reported by the Merck group.[29] Activity was also observed at the cellular level, where L-783277 inhibited VEGFR2 autophosphorylation[27] with an IC_{50} of 57 nM.

Strikingly, the potency of L-783277 against VEGFR2 and PDGFRα (V561D), the most sensitive kinases in our panel, is only slightly reduced (2−3-fold) upon removal of the 5′-OH group (Table 1). Thus, 5′-deoxy L-783277 (**1**) inhibits VEGFR2 and PDGFRα (V561E) with IC_{50} values of 5.8 and 7.2 nM, respectively. Other kinases that are inhibited by **1** with submicromolar potency are ALK, MNK2 (ERK Cys166 equivalent present), and cKIT; against the latter, **1** is ca. 2-fold less potent than L-783277, in accordance with the results obtained for VEGFR2 and PDGFRα (V561D). Collectively, the data indicate that **1** is almost equipotent with L-783277 and exhibits a very similar selectivity profile. Further modification of **1** by a change in double bond geometry from *Z* to *E*, to produce **2**, or the removal of the OH group on C4′, to give **3**, lead to a significant loss in potency, although both compounds retain clearly submicromolar activity against VEGFR2 and PDGFRα (V561D). The data for *E* analogue **2** provide broad confirmation for the substantially reduced activity of C7′-C8′ *E*-configured RL-based kinase inhibitors, which had been reported previously for the C7′-C8′ *E* isomers of L-783277 and LL-Z1640-2 against MEK2[29] and TAK1,[31] respectively, and also for *E*-LL-Z1640-2 in a cellular reporter assay for TNFα signaling.[18] There appears to be a trend for **3** to be more potent than **2**, but additional data are required to consolidate this conclusion. It should also be noted that the significant difference in activity between **3** and **1** does not necessarily imply the 4′-(mono)-deoxy analogue of L-783277 to be less active than **1**. Unfortunately, efforts to prepare 4′-deoxy L-783277 by a route analogous to that employed for **1** (Scheme 1) have failed so far, due to problems with protecting group migration and isomerization in the final oxidation and deprotection steps (Neuhaus, C.; Altmann, K.-H. Unpublished data). An alternative synthesis of this compound is currently under investigation.

LL-Z1640-2 has been reported to be converted to its less active C7′-C8′ *E* isomer in mouse blood or plasma with a half-life of roughly 20 min.[18] We have independently investigated the stability of L-783277 in human plasma and, in agreement with the work of Du et al. on LL-Z1640-2,[18] found the compound to be transformed into its C7′-C8′ *E* isomer as the only detectable metabolite (ca. 50% conversion after 20 min at 37 °C and 55 μM compound concentration; data not shown). The compound is fully stable in pH 7.0−7.5 HEPES buffer for 24 h at 37 °C (< 1% isomerization), which implicates the involvement of plasma components in the isomerization process, most likely reduced glutathione and glutathione-*S*-transferase.[18] Unsurprisingly, the stability of 5′-deoxy L-783277 (**1**) in human plasma was unchanged as compared to L-783277.

In summary, we have shown that 5′-deoxy L-783277 (**1**) retains almost the full kinase inhibitory potential of the parent natural product L-783277. In contrast, C7′-C8′ *E* derivative **2** and 4′,5′-dideoxy analogue **3** are significantly less active. Our remarkable finding on the activity of **1** broadens the perspectives for the design of new RL-based kinase inhibitors, which may be based on the monohydroxylated scaffold of **1**. Studies to determine the effects of the removal of the 5′-hydroxyl group in combination with other modifications are ongoing in our laboratories.

SUPPORTING INFORMATION AVAILABLE

Synthetic procedures and analytical data for all new compounds (**1**−**3**, **5**−**8**, **10**, **11**, **13**, and **20**−**27**). Individual IC_{50} values for inhibition of ALK, cKIT, PDGFRα, and VEGFR2 by L-783277 and **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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