Doctoral Thesis

Resorcylic lactone L-783277 as a new lead structure for kinase inhibition
total synthesis and SAR studies

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Resorcylic Lactone L-783277 as a New Lead Structure for Kinase Inhibition –
Total Synthesis and SAR Studies

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Abstract

The inhibition of disease-relevant kinases leads to interference with cellular signaling pathways or cell cycle progression and represents a new paradigm in modern drug discovery. In particular, several kinase inhibitors have been successfully developed in recent years for the clinical treatment of different types of cancers. While most of these agents are low-molecular-weight synthetic molecules based on different types of heteroaromatic or urea scaffolds, a number of naturally occurring resorcylic acid lactones (RALs) have recently emerged as alternative new lead structures for kinase inhibition. Most of the members of this family of natural products exhibit a cis-enone moiety as part of their macrolactone ring. A 1,4-addition of an active site cysteine residue to the β-carbon of the α,β-unsaturated carbonyl system is responsible for the high potency kinase inhibition of these compounds. One of the most potent representatives is L-783277 (6) which is depicted in Fig. 1.

Figure 1. Resorcylic lactone L-783277 (6).

Total syntheses have been successfully achieved for several RALs. However, no efforts on the total synthesis of 6 had been reported before our own work, although 6 is a highly potent inhibitor of the Ser/Thr kinase Mek1 (IC$_{50}$ = 4 nM).

The goal of this research project was the development of an efficient enantioselective synthesis of 6 and the characterization of its biological activity with respect to the selectivity of kinase inhibition and its effects on human cancer cells. In a second step, the chemistry developed for the preparation of 6 was planned to be used for the synthesis of a limited number of analogs for SAR and biophysical studies.

The initial approach to 6 had to be abandoned due to instability of advanced intermediates. Thus, it was impossible to cleave the ester group in 173 or 177 (Fig. 2) under conditions that did not lead to the destruction of the molecule. The investigation of different ester groups or different protecting groups for the C4’/C5’ hydroxyl groups did not allow to overcome the lack of chemical stability of these advanced intermediates.
As illustrated in Fig. 3, a new strategy was developed, which successfully led to the natural product L-783277 (6). The design of the second generation approach was based on the convergent assembly of three key intermediates 129R, 191 and 176, which were assembled through addition of the lithiated alkyne 129R to aldehyde 191, followed by Suzuki coupling of 176 with the MOM-protected addition product 192. The resulting protected linear precursor for the macrolactonization, 194, was partially hydrogenated, transformed into the respective seco-acid and cyclized under Mitsunobu conditions. One of the key features of this strategy towards 6 was the late introduction of the ketone moiety at C6’ through selective allylic oxidation of the deprotected intermediate 198.

Only one of the diastereomers 198 (which arise as a consequence of the non-selective addition of the lithiated alkyne 129R to aldehyde 191) was cleanly converted into the final product by selective allylic oxidation. As a consequence the enantioselective coupling of key intermediates 129R and 191 was developed, leading to an enantioselective and highly efficient total synthesis of the natural product 6. In addition, a third approach that aims at ring closure based on the Still-Gennari reaction has been investigated.

Based on the synthetic approach developed for 6 the total syntheses of two selected analogs of 6 have been successfully accomplished (Fig. 4). The dideoxy analog D6
lacks the C4’ and C5’ hydroxyl groups, while the phenyl analog P6 exhibits a phenylene moiety instead of a Z-configured double bond between C7’ and C8’.

6 and its dideoxy analog D6 were tested against a panel of 34 kinases (collaboration with Dr. Doriano Fabbro, Novartis Institute for Biomedical Research, Basel). L-783277 (6) effectively inhibits VEGFR-2, Mek2, PDGFRα and MK5, with IC₅₀ values of 8 nM, 15 nM, 87 nM and 640 nM, respectively. These kinases are known to be involved in cancer relevant and inflammatory signaling pathways of cells. Moreover, 6 inhibited Erk2, Tyk2 and cKit with μM IC₅₀ values, while IC₅₀ values for all other kinases tested were above 10 μM. Interestingly, D6 also inhibited VEGFR-2, but with approx. 60-fold lower potency than 6.

Additionally, 6 was tested in proliferation experiments using primary lymphatic endothelial cells, (collaboration with Prof. Michael Detmar and Benjamin Vigl, ETH Zurich), whose proliferation was induced by VEGF-A. In these experiments the proliferation of cells could be effectively inhibited by 6.

In in vitro capillary-tube formation studies (collaboration with Prof. James Lorens and Dr. Lasse Evensen, University of Bergen) 6 showed an inhibitory effect on tube formation of human umbilical vein endothelial cells.

In conclusion, we have accomplished the first total synthesis of the resorcylic lactone kinase inhibitor L-783277 (6) in an enantioselective and highly efficient manner. Based on the chemistry developed for the preparation of 6, we have completed the total syntheses of two selected analogs. First results of the biological activity of 6 and analog D6 show selective inhibition of a subset of kinases, which are involved in inflammation and cancer-relevant signaling pathways. The applicability of our approach to analog synthesis has been proven. Our strategy thus grants access to novel analog structures for SAR and biophysical studies around this potent lead structure for anticancer and anti-inflammatory drug discovery.
Zusammenfassung


Abbildung 1: Struktur von L-783277 (6), ein Vertreter der resorzyklischen Laktone.

Für einige Vertreter der resorzyklischen Laktone wurden erfolgreich Totalsynthesen entwickelt. Obwohl 6 ein sehr potenter Kinasehemmer der Ser/Thr-Kinase Mek1 (IC_{50} = 4 nM) ist, wurden zu diesem Vertreter der RALs noch keine synthetischen Arbeiten veröffentlicht.


Die zunächst verfolgte Synthesestrategie für die Totalsynthese von 6 musste aufgrund unzureichender Stabilität fortgeschrittener Zwischenprodukte aufgegeben werden. So war die Spaltung der Estergruppe in den Verbindung 173 und 177 (Abbildung 2) nicht möglich und entsprechende Versuche führten unter verschiedenen Reaktionsbedingungen immer wieder zur Zersetzung der Moleküle. Trotz der Verwendung anderer Estergruppen und unterschiedlicher Schutzgruppenstrategien für die C4’- und C5’-Hydroxylgruppen, konnte die chemische Labilität dieser fortgeschrittenen Zwischenprodukte nicht überwunden werden.

Basierend auf der entwickelten Totalsynthese von 6 wurden zwei seiner Analoga erfolgreich synthetisiert (Abbildung 4). Das Dideoxyanalogon D6 besitzt keine Hydroxylgruppen in der C4’ und C5’ Position, während das Phenylanalogon P6 anstatt der Z konfigurierten Doppelbindung einen mit der C7’-C8’ Bindung annelierten Phenylrest aufweist.

6 und sein Dideoxyanalogon D6 wurden gegen eine Reihe von 34 Kinasen getestet, (in Zusammenarbeit mit Dr. Doriano Fabbro, Novartis Institut für Biomedizinische Forschung, Basel). 6 hemmt VEGFR-2, Mek2, PDGFRα und MK5 mit IC_{50} Werten von 8 nM, 15 nM, 87 nM bzw. 640 nM. Es ist bekannt, dass diese Kinasen in bestimmten Signalwegen von Zellen bei Krebs und auch bei entzündlichen Erkrankungen eine wichtige Rolle spielen. Darüber hinaus weist 6 gegen Erk2, Tyk2 und cKit IC_{50} Werte im unteren mikromolaren Bereich auf. Für alle anderen getesteten Kinasen wurden IC_{50} Werte von über 10 μM ermittelt. Interessanterweise inhibiert ebenfalls D6 VEGFR-2, wenn auch mit einer 60-fach geringeren Aktivität als 6.

Zusätzlich wurde 6 in Zellproliferationsexperimenten mit primären lymphatischen Endothelzellen untersucht (in Zusammenarbeit mit Prof. Michael Detmar und Benjamin Vigl, ETH Zürich), wobei das Wachstum der Zellen durch VEGF-A induziert wurde. In diesen Experimenten konnte gezeigt werden, dass das Zellwachstum durch 6 wirksam reduziert werden konnte.

In sogenannten in vitro “capillary tube formation” Studien (in Zusammenarbeit mit Prof. James Lorens und Dr. Lasse Evensen, Universität Bergen) zeigte 6 eine inhibierende Wirkung auf die Gefässbildung von menschlichen Nabelschnurn-endothelzellen.