DOI: 10.1002/cmdc.200700128

Semisynthesis and Cytotoxicity of Hypothemycin Analogues

Brian R. Hearn,* Kurt Sundermann, Jonah Cannoy, and Daniel V. Santi[a]

Hypothemycin (**1**, Figure 1), a naturally occurring 14-membered resorcylic acid lactone (RAL),[1] selectively and covalently inhibits protein kinases that contain a conserved cysteine residue (Cys 166 of human ERK2) within the ATP-binding domain.[2]

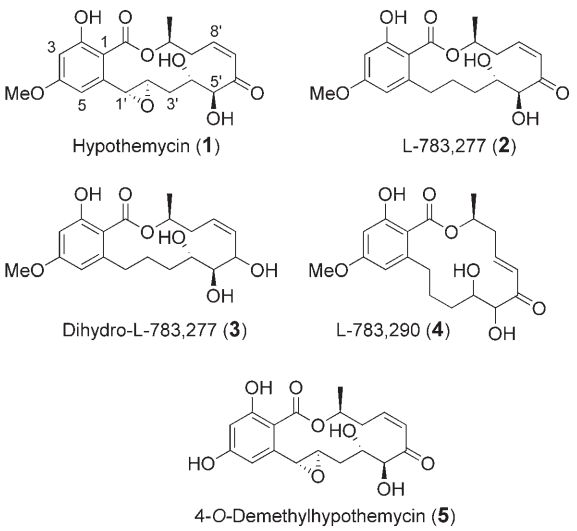


Figure 1. Selected naturally occurring and semisynthetic resorcylic acid lactones.

This subset of the kinome accounts for less than 10% of all identified kinases but includes several targets implicated in aberrant cellular proliferation such as ERK, MEK, FLT, and PDGFR. In cell culture, hypothemycin displays potent cytotoxicity against cancer lines that are dependent on certain activating kinase mutations, particularly the BRAF V600E mutation found in approximately half of all melanomas.[2–4] Additionally, hypothemycin demonstrates significant tumor growth inhibition in at least three separate murine xenograft models.[5] Given these promising in vitro and in vivo results, hypothemycin is an attractive lead compound in the search for new targeted anticancer therapeutics.

The goal of the research described herein involves the evaluation of the hypothemycin cytotoxicity structure–activity relationship (SAR) to ultimately facilitate the identification of potent analogues with optimized pharmacological properties, such as increased aqueous solubility and improved bioavailability. A number of RALs have previously been identified as kinase inhibitors;[6–9] however, very little is known about the structure–cytotoxicity relationship for these compounds.[4,10] Early SAR studies indicate that hypothemycin and related RALs require a C6′–C8′ (*Z*)-enone for both potency and selectivity of kinase inhibition. The basis for this assertion is the finding that L-783,277 (**2**) is approximately 400-fold more potent in MEK inhibition assays than its semisynthetic dihydro derivative (**3**).[8] Furthermore, olefin geometry at C7′–C8′ is of critical importance as RALs with (*E*)-enones, such as **4**, are substantially less potent kinase inhibitors than their (*Z*)-enone analogues.[8,9] Structural diversity is better tolerated at C1′–C2′, as RALs containing either a *trans*-epoxide (**1**), an (*E*)-olefin, or a fully saturated C1′–C2′ hydrocarbon (**2**) inhibit kinases with IC_{50} values in the low nanomolar range.[8,9] To identify the structural requirements for cytotoxicity, we focused on semisynthetic modifications of two RALs that are readily available through fermentation, hypothemycin (**1**) and 4-*O*-demethylhypothemycin (**5**).[4]

Hypothemycin (**1**) was subjected to a variety of reaction conditions targeting the manipulation of its C4′,C5′ diol (Scheme 1). Methylation, using excess (trimethylsilyl)diazomethane and tetrafluoroboric acid, provided the three *O*-methyl analogues **6**, **7**, and **8**. The C4′ hydroxyl group appeared more reactive than its C5′ counterpart based on relative yields of the monomethyl products **6** and **7** (3:1 respectively). Hypothemycin was also treated with 4-toluenesulfonic acid (pTsOH) in dimethoxymethane in an attempt to tether the diol as a cyclic methylidene acetal, but this reaction failed to generate the desired product. Instead acyclic methoxymethyl (MOM) ethers **9**, **10**, and **11** were isolated. Again, the C4′ hydroxyl appeared more reactive than the C5′ hydroxyl as the mono-MOM ethers **9** and **10** were recovered in 4:1 relative yield.

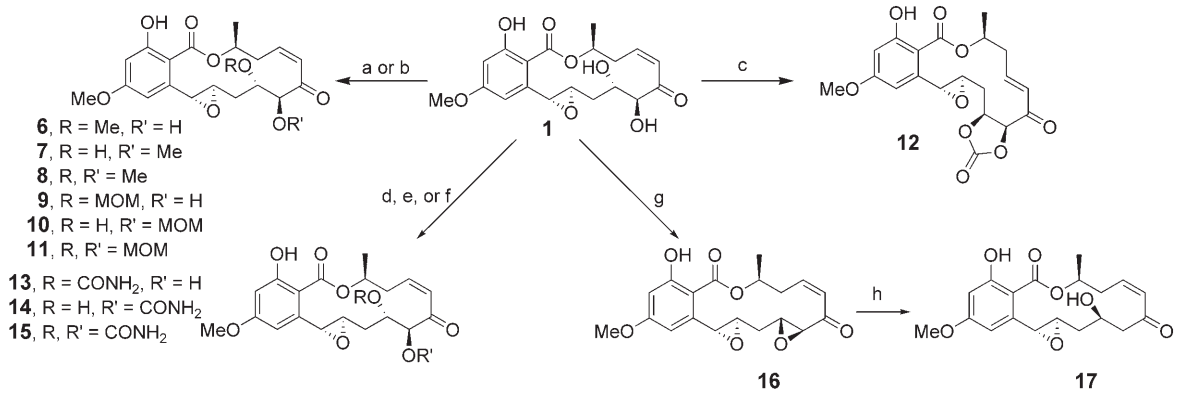
Although cyclic acetal formation was unsuccessful, the C4′,C5′ diol could be tethered as a cyclic carbonate. Treatment of hypothemycin (**1**) with triphosgene and pyridine did generate a C4′,C5′ carbonate. This reaction, unfortunately, also resulted in undesired isomerization of the C7′–C8′ olefin to provide **12** (Scheme 1). Efforts to prevent olefin isomerization to the (*E*)-configuration included the use of alternate bases (NaH or di-*tert*-butyl pyridine) and various carbonylating reagents (phosgene or carbonyl diimidazole), but each failed to generate the desired (*Z*)-C7′–C8′ isomer of **12**.

The C4′,C5′ diol of hypothemycin was also reactive toward isocyanates (Scheme 1). The C4′ monocarbamate **13** and dicarbamate **15** were recovered upon treatment of **1** with trichloroacetyl isocyanate followed by hydrolysis. To selectively produce sufficient quantities for characterization and biological evaluation of the C5′ monocarbamate **14**, a three-step procedure was employed. Selective 4′-OH silyl protection was followed by carbamoylation of the 5′-OH. Acidic desilylation with concomitant hydrolysis of the intermediate trichloroacetyl group yielded the desired monocarbamate **14**.

Finally, hypothemycin was selectively activated with trifluoromethanesulfonic anhydride (Tf_2O) then treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to provide the C4′–C5′

[a] *Dr. B. R. Hearn, Dr. K. Sundermann, J. Cannoy, Dr. D. V. Santi*
Department of Chemistry
Kosan Biosciences, Inc.
3832 Bay Center Place, Hayward, California 94545 (USA)
Fax: (+1) 510-732-8401
E-mail: hearn@kosan.com

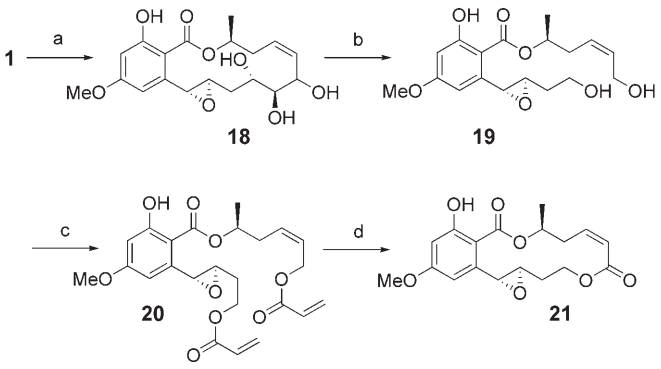
Supporting information for this article is available on the WWW under http://www.chemmedchem.org or from the author.



Scheme 1. Reactivity of the hypothemycin C4′,C5′ diol. Reagents and conditions: a) $TMSCHN_2$, HBF_4, CH_2Cl_2, **6**:**7**:**8**, 3:1:2; b) $(MeO)_2CH_2$, pTsOH, **9**:**10**:**11**, 4:1:2; c) triphosgene, pyridine, CH_2Cl_2; d) For **13**: $Cl_3CCONCO$, CH_2Cl_2, −78 °C, then SiO_2; e) For **14**: 1) TMSCl, Et_3N, THF, 2) $Cl_3CCONCO$, CH_2Cl_2, 3) $NaHSO_4$ (aq), MeOH; f) For **15**: $Cl_3CCONCO$, CH_2Cl_2, rt, then basic Al_2O_3; g) Tf_2O, 2,6-lutidine, CH_2Cl_2, −78 °C to 0 °C, then DBU, −78 °C to rt; h) SmI_2, THF, MeOH, −78 °C.

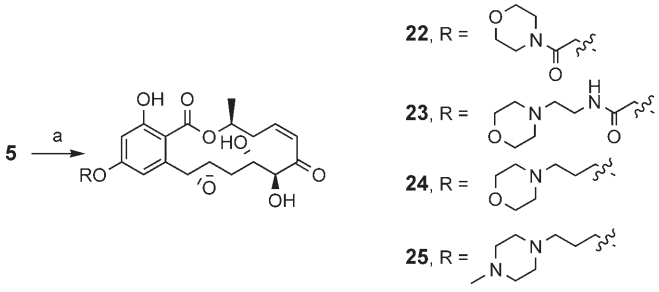
epoxide containing compound **16** as a single isomer (Scheme 1). The chemoselectivity of the sulfonylation reaction was not determined; however, this reaction was predicted to occur at the 4′-OH, given preferential reactivity at this position in previously described transformations (**6** versus **7**, **9** versus **10**, and **13** versus **14**). On the basis of this rationale, intramolecular substitution of the intermediate C4′ triflate would result in the formation of **16** as depicted in Scheme 1. The C4′–C5′ epoxide of **16** was reductively fragmented with samarium(II) iodide to provide **17**. Interestingly, no olefin isomerization was associated with this reaction.

The reactivity of the hypothemycin diol may also provide access to analogues other than simple *O*-functionalized compounds. For example, early degradation studies on hypothemycin indicated that the macrocyclic lactone could be fragmented through a periodate diol cleavage.[1b] A similar approach to degradation of the macrocycle is detailed in Scheme 2. Luche reduction of hypothemycin provided compound **18** as a single, unassigned diastereomer. Periodate cleavage of the triol, followed by sodium borohydride quench, yielded acyclic diol **19**, a versatile intermediate that allows access to a variety of RAL analogues with altered steric and conformational properties. Reconstitution of the macrocycle proceeded in two steps beginning with acylation of **19** using excess acryloyl chloride. The resulting acyclic diacrylate intermediate (**20**) underwent ring-closing metathesis with Grubbs second-generation catalyst to provide the 14-membered dilactone **21**.[11] Remarkably, the ring closure exclusively provided the enoate of (*Z*)-configuration as determined by ^{1}H NMR ($J_{H\text{-}7'\text{–}H\text{-}8'} = 12$ Hz).



Scheme 2. Degradation of the hypothemycin macrocycle. Reagents and conditions: a) $NaBH_4$, $CeCl_3$, MeOH, H_2O; b) $NaIO_4$, THF, H_2O then $NaBH_4$; c) acryloyl chloride, pyridine, CH_2Cl_2; d) Grubbs second-generation ruthenium metathesis catalyst, CH_2Cl_2, 35 °C.

The isolation of 4-*O*-demethylhypothemycin (**5**) from fermentation extracts of *Hypomyces subiculosus* provided a new opportunity to target previously inaccessible semisynthetic analogues.[4] For example, the C4 phenol of **5** selectively undergoes Mitsunobu alkylations with a variety of alcohols, as indicated in Scheme 3. By utilizing the exposed C4 phenol as a handle for structural diversification, a number of polar side chains, both neutral (**22**) and basic (**23**, **24**, and **25**), were appended to the hypothemycin scaffold under standard Mitsunobu conditions using triphenylphosphine and diethyl azodicarboxylate (DEAD).[4]



Scheme 3. Mitsunobu alkylation of the 4-*O*-demethylhypothemycin (**5**) C4 phenol. Reagents and conditions: a) ROH, PPh_3, DEAD, THF.

A summary of the cytotoxicity data for selected hypothemycin analogues is provided in Table 1.[12] Based on the data for analogues **1** and **5**–**16**, it is apparent that the presence of the C4′,C5′ diol is critical for cytotoxic potency. Each modification of the diol results in a significant reduction in cytotoxicity relative to both **1** and **5**. Among these compounds, only **7** and **10** have submicromolar IC_{50} values, indicating that monoalkylation

Table 1. Cytotoxicity IC_{50} values in μM.

Compd	Cell Line		
	COLO829 (BRAF V600E)	HT29 (BRAF V600E)	SKOV3 (BRAF wt)
1	0.082	0.24	5.9
5	0.043	0.18	1.9
6	8.0	7.7	34
7	0.37	0.74	27
8	2.7	2.7	2.5
9	6.1	5.4	3.8
10	0.26	0.99	2.1
11	6.5	8.0	3.2
12	5.6	18	>10
13	6.1	6.4	31
14	4.4	4.6	15
15	>100	>100	>100
16	3.7	3.4	-
18	32	>100	>100
21	>100	>100	>100
22	0.27	0.64	>29
23	0.18	0.24	25
24	0.047	0.29	0.86
25	0.042	0.21	-

of the C5′ alcohol is the best tolerated modification in this region. Compound **18**, the only analogue reported herein that lacks the C6′ carbonyl, is dramatically less potent than hypothemycin. This observation may be attributed in large part to the inability of **18** to form a covalent Michael adduct at C8′ with Cys 166 of the targeted kinase.[13] In contrast to modifications of the macrocyclic lactone, derivatizations of the C4 phenol of **5** are well tolerated. Glycolamides **22** and **23** are cytotoxic with submicromolar IC_{50} values, although the potency of each is somewhat diminished relative to hypothemycin. Flexibility of the C4 side chain may be important, as analogues **24** and **25** are virtually equipotent to hypothemycin.

In summary, initial evaluation of the hypothemycin SAR has identified the C4′–C8′ region as a critical structural component for cytotoxicity. Each modification within this portion of the macrocyclic lactone results in analogues with substantially diminished cytotoxic potency. Alternatively, the C4 position of the resorcylic acid tolerates significant structural diversity, as analogues with polar side chains, **24** and **25**, are as cytotoxic as hypothemycin itself. Modification of the C4 phenol represents the most promising opportunity to improve the solubility and pharmacokinetic properties of the hypothemycins.

Acknowledgements

We thank Dr. J. R. Carney and C. Tran for analytical support of this work and Dr. S. Murli and Dr. J. Kennedy for performing cell viability assays.

Keywords: biologically active compounds · hypothemycin · kinase inhibitors · resorcylic acid lactones · semisynthesis

[1] a) M. S. R. Nair, S. T. Carey, *Tetrahedron Lett.* **1980**, *21*, 2011; b) M. S. R. Nair, S. T. Carey, J. C. James, *Tetrahedron* **1981**, *37*, 2445; c) Revised structure: T. Agatsuma, A. Takahashi, C. Kabuto, S. Nozoe, *Chem. Pharm. Bull.* **1993**, *41*, 373.

[2] A. Schirmer, J. Kennedy, S. Murli, R. Reid, D. V. Santi, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 4234.

[3] D. B. Solit, L. A. Garraway, C. A. Pratilas, A. Sawai, G. Getz, A. Basso, Q. Ye, J. M. Lobo, Y. She, I. Osman, T. R. Golub, J. Sebolt-Leopold, W. R. Sellers, N. Rosen, *Nature* **2006**, *439*, 358.

[4] J. L. Wee, K. Sundermann, P. Licari, J. Galazzo, *J. Nat. Prod.* **2006**, *69*, 1456.

[5] H. Tanaka, K. Nishida, K. Sugita, T. Yoshioka, *Jpn. J. Cancer Res.* **1999**, *90*, 1139.

[6] D. H. Williams, S. E. Wilkinson, T. Purton, A. Lamont, H. Flotow, E. J. Murray, *Biochemistry* **1998**, *37*, 9579.

[7] R. Camacho, M. J. Staruch, C. DaSilva, S. Koprak, T. Sewell, G. Salituro, F. J. Dumont, *Immunopharmacology* **1999**, *44*, 255.

[8] A. Zhao, S. H. Lee, M. Mojena, R. G. Jenkins, D. R. Patrick, H. E. Huber, M. A. Goetz, O. D. Hensens, D. L. Zink, D. Vilella, A. W. Dombrowski, R. B. Lingham, L. Huang, *J. Antibiot.* **1999**, *52*, 1086.

[9] J. Ninomiya-Tsuji, T. Kajino, K. Ono, T. Ohtomo, M. Matsumoto, M. Shiina, M. Mihara, M. Tsuchiya, K. Matsumoto, *J. Biol. Chem.* **2003**, *278*, 18485.

[10] Cytotoxicity data have been reported for hypothemycin and related RALs but only in relatively insensitive cell lines: M. Isaka, C. Suyarnsestakorn, M. Tanticharoen, *J. Org. Chem.* **2002**, *67*, 1561.

[11] a) M. Scholl, S. Ding, C. W. Lee, R. H. Grubbs, *Org. Lett.* **1999**, *1*, 953; b) S. B. Garber, J. S. Kingsbury, B. L. Gray, A. H. Hoveyda, *J. Am. Chem. Soc.* **2000**, *122*, 8168.

[12] Cells were incubated with compound for 72 h, and viability was assessed using CellTiter-Glo Luminescent Cell Viability Assay kit (Promega).

[13] X-ray crystal structure of the hypothemycin-ERK2 covalent complex will be reported elsewhere: G. Rastelli, D. McRee, R. Reid, D. V. Santi.

Received: May 29, 2007
Revised: June 26, 2007
Published online on August 9, 2007

