CHAPTER 14

Case History: Discovery of Eribulin (HALAVEN™), a Halichondrin B Analogue That Prolongs Overall Survival in Patients with Metastatic Breast Cancer

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1. INTRODUCTION

The most recent projections by the National Cancer Institute estimate that one in eight women will be diagnosed with breast cancer at some point in their lifetime [1], making it one of the leading causes of cancer death among women in the United States [2]. Since few therapeutic options exist for patients with advanced or recurrent breast cancer, new and more effective treatment modalities are needed, particularly those that offer improved survival rates over current therapies.

Eribulin mesylate [3,4] (**1**, HALAVEN^{TM}, previously E7389, NSC 707389, and ER-086526) is an antitubulin antimitotic agent with distinct microtubule end-binding properties that result in inhibition of microtubule dynamics in ways that differ from those of vinblastine and paclitaxel [5,6] (Figure 1). This agent was recently approved by the U.S. Food and Drug Administration (FDA) for use in patients with metastatic breast cancer who have previously received at least two chemotherapeutic regimens for the treatment of metastatic disease [7]. Prior therapy should have included an anthracycline and a taxane in either the adjuvant or metastatic setting. HALAVEN therefore represents a new and exciting treatment option that for the first time has been shown to improve overall survival in heavily pretreated women with late stage breast cancer. Building on this success, clinical trials are currently ongoing to evaluate activity in additional cancer indications [8].

Although inspired by the structurally complex marine natural product halichondrin B (**2**, HB), eribulin (**1**) is a totally synthetic macrocyclic ketone analogue. As such, the drug substance can be produced in a controlled and predictable manner with a well-defined and reproducible impurity profile, factors that are essential for all marketed drugs irrespective of source. Since material supply was a critical factor that limited preclinical development of the natural product HB by the U.S. National Cancer Institute (NCI), total synthesis of a structurally simplified and optimized derivative represented the best overall solution to access the needed quantities of material in a sustainable and cost-effective fashion.

In the mid-1980s, the Kishi group was considering a number of chemical targets to showcase the synthetic potential of the Nozaki–Hiyama–Kishi reaction in the construction of structurally complex molecules. From among these, the halichondrins were selected due to their chemical architecture and remarkable biological activity. In 1986, HB was reported by Hirata and Uemura [9] to exhibit subnanomolar cell growth inhibitory potency, good physical properties, and outstanding anticancer activity in animal models [10]. Supported by an NCI grant, the Kishi group initiated a synthetic program, which culminated in the first successful total synthesis of HB in 1992 [11].

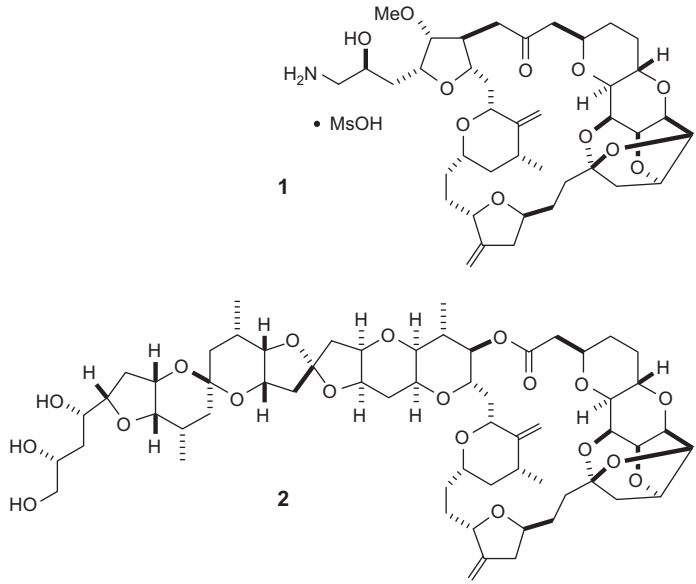

Figure 1 Eribulin mesylate (**1**) and halichondrin B (**2**).

That year proved to be a pivotal one as the NCI accepted the natural product HB for preclinical development. That same year, Eisai researchers received a number of synthetic intermediates from the Kishi laboratory and discovered that the right half (RH) C.1–C.38 fragment **6** exhibited potent cell growth inhibitory activity. The technology to synthesize HB was patented by Harvard University [12] and subsequently licensed to Eisai.

2. HALICHONDRIN B

2.1. Material supply

The amount of HB needed to support clinical development was estimated to be around 10 g [13]. Assuming success in clinical trials and approval by regulatory authorities, the amount of HB projected to meet future commercial demand was predicted to be 1–5 kg/year. However, despite even these modest amounts, collection from the wild was determined to be impractical due to the extremely low yield of HB and the low abundance of known HB-producing sponges in the world. In addition, achieving consistent compound purity represented a significant hurdle since material from natural sources must be separated from closely related family

members as well as structurally unrelated but highly potent co-isolated bioactive metabolites (*e.g.*, okadaic acid).

Supported in part by an NCI grant, preliminary in-sea aquaculture techniques with the HB-producing sponge *Lissodendoryx* sp. were investigated, but significant challenges remained that rendered future scale-up uncertain [13].

2.2. Total synthesis

A number of chemistry research groups initiated programs directed toward the total synthesis of the halichondrins [14]. The first successful total synthesis was reported by the Kishi group at Harvard University in 1992 [11]. Their synthesis is highly convergent and involves a series of Nozaki–Hiyama–Kishi coupling reactions of advanced intermediates **3**–**5** to afford RH intermediate **6** (Figure 2). The latter compound, which represents the macrolactone region of the natural product, was then coupled with left half precursor **7** to provide HB. This total synthesis represents a landmark scientific achievement that highlights the power

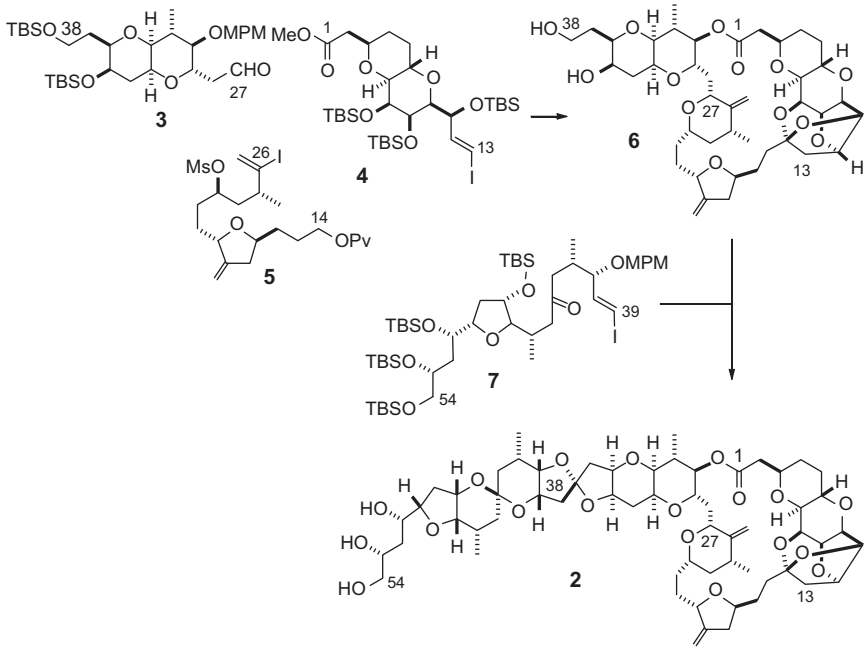

Figure 2 Kishi total synthesis of HB commencing from the C.1–C.13 (**4**), C.14–C.26 (**5**), and C.27–C.38 (**3**) fragments. Coupling of RH (**6**) with the C.39–C.54 left half fragment (**7**) afforded the natural product.

and utility of the Nozaki–Hiyama–Kishi reaction in the construction of structurally complex molecules.

3. ERIBULIN DRUG DISCOVERY PROGRAM

3.1. Medicinal chemistry strategy

Synthetic intermediates from the Kishi laboratory prepared in connection with the total synthesis of HB and norHB were submitted to both the NCI and Eisai for biological evaluation. Interestingly, *in vitro* cell growth inhibitory activity against DLD-1 cells was associated only with the RH macrolactone fragment **6**. None of the left half intermediates submitted for testing was found to be active under the experimental conditions examined. This finding was significant for a number of reasons. First, it established that structurally simplified derivatives of HB, which exhibit potent anticancer activity *in vitro*, could be identified. Second, it reinforced the belief that the material supply problem associated with the halichondrins could be solved in a practical manner by total synthesis. And third, it clearly demonstrated that significant structural modifications could be made to the left half of the molecule without adversely affecting cell growth inhibitory activity, thereby pointing the direction for future optimization.

As outlined in Figure 2, the synthesis of **6** is highly convergent and remarkably modular, allowing each of the three main fragments to be individually modified in a mix and match approach. By stockpiling key fragments, the preparation of new analogues could be transformed from a scientifically complex challenge to that of a technical and material management problem directed by classical medicinal chemistry conventions.

The axiom that formed the basis for Eisai Research Institute's (ERI) medicinal chemistry strategy was to first identify the pharmacophore and then modify the remainder of the structure to increase synthetic accessibility. However, initially, the drug discovery team at ERI (Andover, MA facility) did not have access to ancillary resources that are now generally assumed in today's drug discovery environment. These include (a) ADME studies, (b) a computational chemistry group, (c) a process chemistry department, (d) kilo lab support, and (e) an analytical chemistry unit. Scale-up work and resupply of key intermediates had to be handled entirely within the discovery chemistry team, and questions regarding pharmacokinetics had to be addressed indirectly through hypothesis-directed establishment of new *in vitro* screens.

Often with chemical leads derived from high-throughput screening of small molecule compound libraries, potency is an issue that may be addressed in an additive fashion, for example, structure optimization

may involve adding functional groups and substituents to improve affinity of the ligand for the target of interest. In the halichondrin program, however, potency was not a critical path issue. Thus, the optimization campaign was envisioned to proceed in a subtractive mode driven entirely by synthetic accessibility and *in vivo* performance concerns. Using this general design principle, we derived the following initial research objectives:

1. Establish proof of concept (*i.e.*, demonstrate *in vivo* activity for RH)
2. Stockpile key fragments to support analogue synthesis
3. Modify the structure of **6** to identify the minimum pharmacophoric substructure and increase synthetic accessibility
4. Optimize for biological activity

To accomplish the first and second objectives, a scale-up synthesis was initiated where the three key fragments **3**–**5** were stockpiled. This initial effort afforded approximately 100 mg of **6**, which was more than sufficient to conduct multiple human tumor xenograft experiments. However, in sharp contrast to the natural product, no *in vivo* activity could be observed under the experimental conditions tested. This was clearly a set-back for the program. If **6** rather than HB represented the starting point and chemical lead for the drug discovery program, then in essence the team lost their advanced starting point. Without access to *in vivo* ADME data, it was simply not possible to explain the difference based on pharmacokinetics. Thus, to provide a rational hypothesis-driven path forward a surrogate quantitative cell-based pharmacodynamic assay was needed to prioritize new analogues for *in vivo* evaluation.

As a result, a secondary, orthogonal *in vitro* evaluation system was developed to complement the primary 3- to 4-day cell growth inhibition assay used to evaluate HB and its synthetic intermediates. In the primary screen, cancer cells were continually exposed to the test substance for the duration of the experiment. However, in the human tumor xenograft models, compounds were dosed intermittently, which presumably gave rise to peaks and troughs in free drug concentration. Thus, the hypothesis was generated that the inability to maintain a complete mitotic block (CMB) under drug washout conditions could explain the observed lack of *in vivo* activity for **6**. A new flow cytometric analysis assay using U937 human histiocytic lymphoma cells was therefore established at ERI [15]. In this system, the reversibility ratio was calculated by dividing the test compound concentration found to give a CMB at the 10-h time point by the minimum compound concentration required to induce a CMB at the 0-h time point.

Using this method, an HB concentration of 25 nM (reversibility ratio $= 3$) was found to be sufficient to induce a CMB at the 10-h time point, whereas the concentration of **6** was found to be >880 nM (reversibility

ratio > 30). This result allowed us to reject the null hypothesis and incorporate the reversibility assay into our screening paradigm as a cell-based surrogate for the effects of intermittent dosing.

3.2. Macrolactone

3.2.1. Proof of concept

To demonstrate proof of concept, we needed to identify a RH analogue that exhibited activity in a human tumor xenograft model using a standard intermittent dosing schedule. Since **6** lacks the polyether portion of the natural product and contains a diol, our initial hypothesis was that the C.35–C.38 region needed to more closely resemble that of the natural product. Tetrahydrofuran analogue **8**, which is a direct C.1–C.38 substructure of HB, was therefore evaluated in the mitotic block reversibility assay but found to be highly reversible (Figure 3).

We next hypothesized that the ability of HB to maintain a CMB upon drug washout may be associated with the C.39–C.50 polyether moiety. To test this, a small series of readily accessible ''left half surrogate''

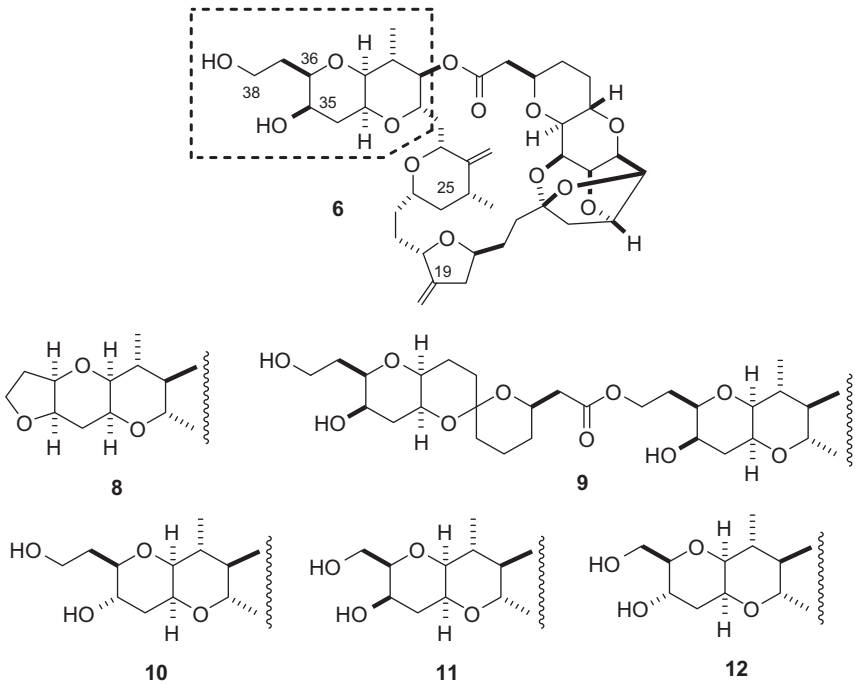

Figure 3 RH macrolactone analogues. Compounds **8–11** were highly reversible in the U937 mitotic block assay. Compound **12** was much less reversible.

derivatives was prepared (*e.g.*, **9**). Unfortunately, these too exhibited unfavorable behavior in the U937 mitotic block reversibility assay, rendering them unsuitable for *in vivo* evaluation.

The breakthrough came when compound **12** was submitted for biological evaluation. At that time, precursors derived from the gluco rather than the galacto series of starting materials were being used to develop the asymmetric Nozaki–Hiyama–Kishi reaction at Harvard. The resulting ''norgluco'' product **12** contains two structural changes relative to the original RH intermediate **6**—a one carbon truncation at C.37 and inversion of stereochemistry at C.35. Surprisingly, this material exhibited a reversibility ratio of 24, which was the first time that a RH derivative was found to possess an improved reversibility profile. On the basis of the primary and secondary assay results, the synthesis of norgluco RH analogue **12** was scaled up and the material evaluated in the LOX human melanoma xenograft model. Gratifyingly, the compound demonstrated sustained inhibition of *in vivo* tumor growth at 5 mg/kg on a (Q1Dx5) $\times$ 2 dosing schedule, thereby validating the reversibility hypothesis and providing in vivo proof of concept.

Compounds **10** and **11**, which represent single point changes relative to RH, were subsequently prepared at ERI, but both were found to be highly reversible in the secondary *in vitro* screen. Thus, both structural changes present in compound **12** relative to **6** were needed to generate an improved reversibility profile.

The next step was to increase synthetic accessibility by removing or modifying non-pharmacophoric functional groups in a systematic stepwise fashion using the existing stockpile of synthetic intermediates.

3.2.2. C.1–C.13 fragment

Consideration was given to modifying the C.1–C.13 fragment **4**, but there were no clear directions for how that would simplify the chemistry and thus the synthetic accessibility of the compound. Consequently, we decided that this fragment should be left unmodified.

3.2.3. C.14–C.26 fragment

Removal or modification of the C.19 and C.26 exo olefins in fragment **5** was considered next. Since the C.19 center is derived from arabinose, a structure ''simplification'' would be to retain the asymmetric center already present in the starting material and omit the multistep sequence to introduce the exo olefin. In the event, the resulting C.19 methoxy derivative was active in the primary screen, but less potent than **6** and therefore not pursued further. Complete removal of the exo olefins at C.19 and C.26 similarly afforded less potent compounds.

The C.25 methyl group was then queried. This substituent was introduced by a stereoselective alkylation reaction of an early lactone

intermediate. If that could be removed, then the chemistry of the C.14–C.26 fragment would be simplified, albeit incrementally. Disappointingly, however, either deletion or homologation of the methyl group only led to a substantial decrease in activity. Thus, we concluded that the functionality embedded within the C.14–C.26 fragment was critically important and should be retained in the synthesis of future analogues.

3.2.4. C.27–C.38 fragment

Based on the above studies, the decision was made to leave the C.1–C.26 region of the molecule unchanged. Thus, effort focused on the fused C.27–C.38 ring system, which we believed offered the greatest opportunity for structure optimization given the initial observation that HB could be truncated at C.38 and still retain biological activity. Toward that end, formal removal of the C.36 carbon and its attendant hydroxyl group from pivotal compound **12** afforded tetrahydropyran analogue **13** (Figure 4) [16]. Since the C.31 methyl group was introduced *via* a multistep procedure involving a carbohydrate precursor, we envisioned that the existing hydroxyl group in the starting material could instead be converted to a methoxy group in a straightforward manner to ultimately afford **14**. This derivative exhibited good cell growth inhibitory activity and an acceptable level of desired irreversibility in the U937 mitotic block assay [4]. However, despite meeting all of the *in vitro* criteria for compound advancement, it was inactive in the mouse LOX melanoma

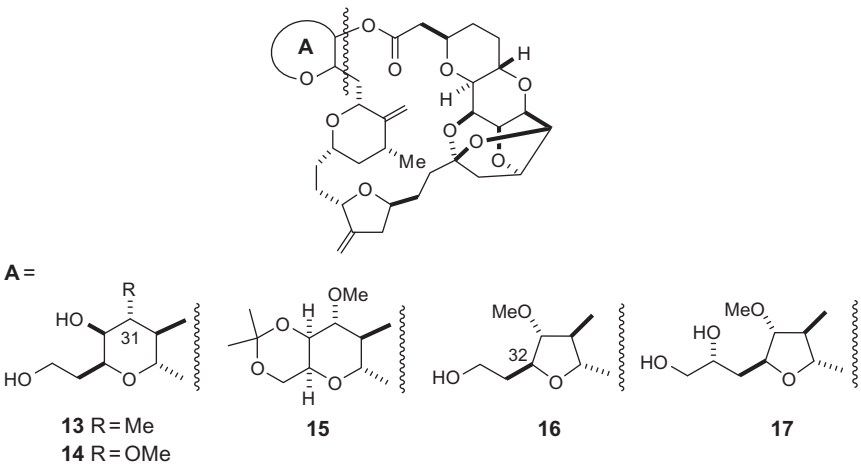

Figure 4 Simplified macrolactone analogues. *In vitro* profile for compounds **13** and **14** was similar. *In vitro* potency for tetrahydrofuran analogues **16** and **17** was superior to that of tetrahydropyran derivative **14**. Compound **17** exhibited superior reversibility characteristics relative to **16**.

xenograft model. Although somewhat puzzling at first, investigation of biological stability *in vitro* revealed that the compound was not stable in the presence of mouse serum [17]. This is in sharp contrast to compound **12**, which was completely stable under the experimental conditions. We hypothesized that the tetrahydropyran ring of **14** was more conformationally flexible than the fused octahydropyrano[3,2-*b*]pyran ring system of **12**, thereby possibly rendering the lactone moiety more susceptible to cleavage by nonspecific mouse serum esterases. As a result, we considered ways to help rigidify the tetrahydropyran ring and indirectly stabilize the macrolactone ring conformation.

One option we pursued was to tie the diol groups into a ketal structure to mimic the fused ring system of **12**. Although **15** was active in the cell growth inhibition assay, it was also highly reversible. As a result, work on this particular approach was abandoned.

The other option that we considered was to reduce the size of the C.29–C.33 six-membered ring to a tetrahydrofuran ring. From earlier studies, we knew that the presence of a C.31 substituent and presumably its conformation relative to the macrocyclic ring were important for cell growth inhibitory activity. Conformational analysis of the norhalichondrin A X-ray crystal structure suggested that the C.31 group in a tetrahydrofuran ring would occupy the appropriate position. Compound **16** (DLD-1 cell growth inhibition $IC_{50} = 0.97$ nM, reversibility ratio = 14) was subsequently prepared and found to be both more potent and more irreversible than **14** ($IC_{50} = 2.1$ nM, reversibility ratio >100) [4], making the tetrahydrofuran series considerably more attractive than the tetrahydropyrans. Structure–activity relationship (SAR) studies of *in vitro* cell growth inhibition potency and reversibility relative to C.32-side chain modifications subsequently identified diol **17** as the most interesting. Unfortunately, this compound and all other tetrahydrofuran lactone analogues tested were unstable in the presence of mouse serum. It was not clear why the fused octahydropyrano[3,2-*b*]pyran analogues exemplified by compound **12** were stable in the presence of mouse serum, but the truncated ring derivatives exemplified by **14** and **16** were not. Thus, in the absence of a specific working hypothesis to guide further structure design efforts, hydrolytically stable C.1 lactone bioisosteres appeared to represent the most expedient path forward.

3.3. Macrocyclic ether

One method to stabilize the lactone moiety was to replace it with a simple ether group. A significant stockpile of the C.27–C.35 tetrahydropyran fragment was available at the time and was therefore used for synthesis. Unfortunately, analogue **18** was found to be much less potent than the macrolactone derivatives (Figure 5). Thus, no further work on the macrocyclic ether series was pursued.

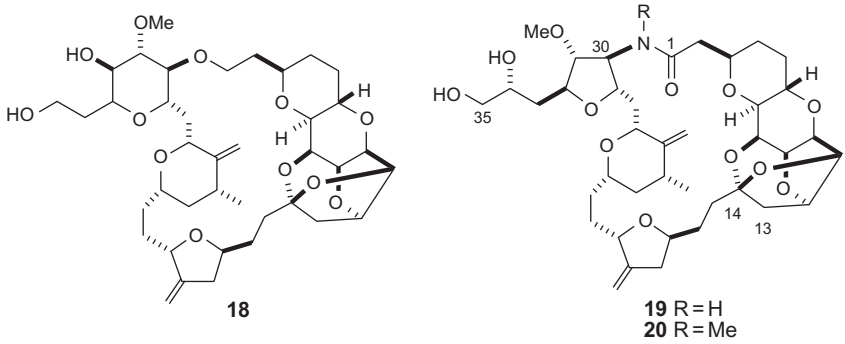

Figure 5 Macrocyclic ether and lactam analogues. All three analogues were much less potent than members of the macrolactone series.

3.4. Macrolactam

Although the lactone to lactam replacement was originally conceived to be a relatively straightforward modification, the existing method to close the macrocyclic ring could not be employed. After considering several possibilities, the macrocyclization precursor was prepared by amide formation between the C.1–C.13 carboxylic acid and an amino derivative of the C.14–C.35 fragment. A Nozaki–Hiyama–Kishi coupling reaction was then used to close the macrocyclic ring at the C.13–C.14 position followed by formation of the ketal ''cage'' structure. Surprisingly, however, the resulting macrolactam analogues **19** and **20** were approximately two orders of magnitude less potent than the corresponding macrolactone derivatives, suggesting that although a lactam linkage was tolerated, activity was compromised [17].

These results were rationalized on the basis of gas phase molecular dynamics simulations. Over a 600-ps time frame, the lactam dihedral angle exhibited two maxima centered around 0° and 180°, representing the s-*cis* and s-*trans* conformations, respectively. A similar gas phase molecular dynamics simulation placed the lactone dihedral angle maximum at 165° with a population distribution that significantly overlapped that associated with the s-*trans* lactam conformation. The X-ray crystal structure of a norhalichondrin A derivative exhibited a lactone dihedral angle of 163° [9], in good agreement with the gas phase molecular dynamics calculation. If we assume that the solid-state conformation of the norhalichondrin A macrolactone represents the bioactive conformation of HB, then the macrolactam analogues should have been more active than observed. Since this was not the case, we concluded that the bioactive conformation must be one where the dihedral angle lies somewhere in-between 90° and 163°. Molecular dynamics calculations placed the

ketone dihedral angle around 90° with a distribution which overlapped that calculated for the lactone, suggesting both the ketone and lactone derivatives could access a common low-energy conformation that would be energetically less accessible by the lactam. On the basis of these modeling studies, we redirected our efforts toward synthesizing a series of C.1 ketone bioisosteric derivatives.

3.5. Macrocyclic ketone

Synthesis of the macrocyclic ketone series proceeded in an analogous manner from the existing stockpile of synthetic intermediates. After modifying the C.27–C.35 tetrahydrofuran fragment to include an appropriately functionalized carbon substituent at C.30, coupling with the C.1–C.13 fragment proceeded smoothly and in high yield. Functional group manipulation and macrocyclic ring formation using the conditions developed for the macrolactam series afforded the desired intermediate that was transformed to the final macrocyclic ketone analogue [18].

In this manner, compound **21** was prepared and found to exhibit excellent potency, good reversibility characteristics, stability in the presence of mouse serum and most importantly, potent inhibition of tumor growth in human cancer xenograft models *in vivo* (Figure 6). Further functional group exploration at C.1 (*e.g.*, exo olefin, alcohol, oxime), C.34 and C.35 (*e.g.*, amides, carbamates, ureas, etc.) led to a series of derivatives, from which eribulin and diol **21** emerged as the most promising [19]. In particular, eribulin exhibited a reversibility ratio of one, indicating irreversible behavior in the mitotic block assay. On the basis of its remarkable biological activity profile, eribulin was nominated and accepted for preclinical development at Eisai. Both eribulin and diol **21** were then submitted to the NCI for further evaluation. Based on a review of all

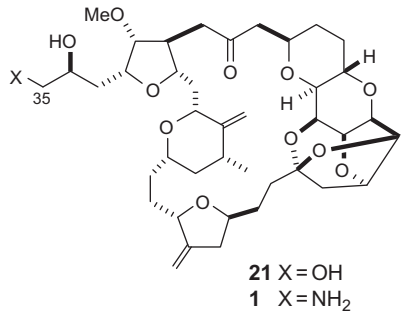

Figure 6 Example macrocyclic ketone analogues. Eribulin (**1**) exhibited a reversibility ratio of one, whereas the diol (**21**) exhibited a reversibility ratio of 13.

available data, including cell-based antiproliferative potency, *in vivo* antitumor activity, pharmacokinetic, and stability parameters, eribulin was ultimately selected as the front running candidate [4].

In addition to its remarkable biological and safety profiles, eribulin mesylate exhibited excellent physicochemical properties, which allowed the compound to be formulated in a simple and straightforward manner. Finally, the stage was set for solving the material supply problem with a structurally simplified, optimized, and totally synthetic derivative that not only retained the remarkable biological activity of the natural product that inspired it, but actually surpassed it.

4. FROM DISCOVERY TO DEVELOPMENT

Despite significant simplification relative to HB, the chemical structure of eribulin remains a synthetically challenging target with 19 stereogenic centers. To the best of our knowledge, this molecule exceeds by far the structural complexity of any drug prepared by total synthesis that is either in development or on the market. Not surprisingly, this presented certain difficulties as the program moved from discovery to development. Serious objections were raised regarding the synthetic feasibility and cost of goods for a totally synthetic compound of such unprecedented structural complexity, concerns that nearly terminated the program as it moved through early clinical development. Notwithstanding, the issues were successfully resolved through continued basic scientific contributions from the Kishi group at Harvard [20] and advances from the Eisai chemical development team [21–23]. At 62 steps, manufacturing eribulin in a cost-effective manner on a scale sufficient to meet commercial demand represents a major leap in demonstrating the power of contemporary organic synthesis to solve problems of this magnitude, and one that effectively resets the bar for what is possible.

At the time of candidate nomination, limitations at Eisai precluded moving eribulin into early clinical development. Thus, the best path forward was through a Cooperative Research and Development Agreement (CRADA) in the form of an NCI-sponsored Phase I clinical trial. The early results were promising, eribulin entered full clinical development and Eisai-sponsored trials were initiated. Details of the clinical trial results have recently been published [24]. In particular, the pivotal EMBRACE phase III trial demonstrated an overall survival benefit for eribulin versus single agent therapy of physician's choice in women who were heavily pretreated for metastatic or locally recurrent breast cancer. Several major advantages offered by HALAVEN over TAXOL®, include the option to formulate HALAVEN using ethanol/water without the need for Cremophor® EL, and the ability for the drug to be administered

as a 2- to 5-min infusion [25] as compared to several hours for Taxol®. Another advantage suggested by preclinical data is a potentially lower incidence of peripheral neuropathy [26]. On November 15, 2010, the FDA announced the approval of HALAVEN for use in patients with metastatic breast cancer who meet certain criteria.

5. CONCLUSION

The path leading to the discovery and development of eribulin included a close three-way collaboration between the Kishi group at Harvard University, the NCI, and Eisai. At many points along the drug discovery path, setbacks and roadblocks arose that threatened to derail the program. Nevertheless, each of the problems was solved in turn thereby demonstrating that a structurally complex molecule could be optimized through total synthesis to successfully deliver a marketed drug that meets all pharmacological, toxicological, and physicochemical requirements.

Although HALAVEN is currently approved for use in patients with metastatic breast cancer who have previously received at least two chemotherapeutic regimens, clinical trials are ongoing to support additional indications [7]. The journey through the discovery and development pipeline was not a smooth or easy one, but one that was ultimately successful. In the end, the project goals were realized and HALAVEN emerged, bringing with it new hope for cancer patients and their families.

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