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Macrolactam analogues of macrolide natural products

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Abstract

The chemical modifications of macrolide natural products into *aza*- or lactam analogues is a strategy employed to improve their metabolic stability and biological activity. The methods for the synthesis of several lactam analogues of macrolide natural products is highlighted and aspects of their biological properties presented.

Keywords: macrolactams, *aza*-epothilone B, 15-*aza*-salicylhalamide A, migrastatin lactam, radicicol macrolactams

Introduction

The utilization of biologically active heterocyclic macrocycles in *Nature* is a reflection of their efficacy and benefit to biological processes. One drug analysis¹ has revealed that 59% of small-molecule drugs contain a ring-nitrogen and most of the approved nitrogen macrocycles are natural products or derivatives of natural products. The major liabilities of macrocyclic natural products² are *in vivo* instability and significant toxic side effects. In this regard nitrogen or *aza*-analogues (lactams) often display advantageous properties such as improved reactivity, aqueous solubility, bioavailability, metabolic stability and often also reduced toxicity.

Figure 1. Selected *aza* analogues of macrolide natural products.

Macrolide natural products^{3,4} with 12, 14, or 16 membered rings and their semisynthetic derivatives are important clinically relevant compounds predominantly used for the treatment of various types of cancer.⁵ Macrolactams are easily synthesized and offer access to multiple analogues with chemical and enzymatic stability⁶ as well as desirable biological properties. In this short review article, the synthesis and biological activity of some key examples of lactam analogues of 12, 14, and 16 membered macrolides in which only the oxygen atom is substituted by nitrogen in the macrocycle are presented. Examples of *aza* modified macrolides include the 16-membered 15-*aza*-epothilone B or ixabepilone (**1**), the most successful lactam modified macrolide which has been approved for the treatment of aggressive metastatic taxol resistant breast cancer,⁷ the 12-membered

aza-salicylilalamide analogue **2**,⁸ the 14-membered *aza*-migrastatin⁹ analogue **3**, and the 14-membered *aza*-radicol analogue **4** (Figure 1).¹⁰

15-Aza-epothilone B [Ixabepilone] (**1**)

The epothilones A (**6**) and B (**5**) (Figure 2) are members of a group of 16-membered bacterial macrolides originally identified¹¹ as secondary metabolites produced by the soil-dwelling myxobacterium *Sorangium cellulosum* (strain So ce90) which possesses the largest bacterial genome sequenced to date¹² (13,033,779 base pairs). Interest in the epothilones increased when it was discovered in 1995 that they possess potent tubulin binding properties, rivaling taxol in killing dividing cells by inducing tubulin polymerization and enhancing microtubule stability.¹³ Epothilone B (**5**) shares the same binding site as taxoids,⁷ however their mode of binding within the binding pocket is distinctive.¹⁴ In addition to epothilone A and B, a diverse number of natural epothilone compounds have been isolated from the same myxobacterium.¹⁵ Epothilone B (**5**) was more potent than paclitaxel in inhibiting cell growth.¹⁶

Figure 2. Selected biological for ixabepilone (**1**), epothilone B (**5**) and epothilone A (**6**) (LCK = log cell kill and IC₅₀ Pat-7 ovarian carcinoma model; PPB = Plasma protein binding)^{13c}

Intensive chemical analogue synthesis unveiled structure activity relationships¹⁷ for epothilone B (**5**). Fortunately, it was quickly recognized that the lactone group is vulnerable to hydrolytic cleavage by esterases which may account for the short half-life of epothilones in the early animal studies.¹⁸ Further studies resulted in several lead-structures for the discovery of clinical candidates for cancer treatment.¹⁷ The lactam 15-*aza*-epothilone B (**1**) (ixabepilone)¹⁹ was identified as a potent analogue of epothilone B (**5**) (Figure 2) and this compound showed high potency against ovarian cancer (IC₅₀ = 2.6 nM) and leukemia cell lines (CCRF-CEM: IC₅₀ = 2.1 nM) comparable to that for epothilone B (**5**) (IC₅₀ = 0.41 nM) but was less toxic. Macrolactam **1** was only 10-fold lower in activity when compared with epothilone B (**5**) but also more importantly displayed a 50-fold decrease in cytotoxicity.^{13a} In 2007, ixabepilone (**1**) by Bristol-Myers Squibb (marketed as Ixempra), was approved by the FDA for the treatment of aggressive metastatic taxol resistant breast cancer.⁷ Key to its success as a cancer therapeutic agent is the fact that the *lactam* had better metabolic stability (less prone to hydrolysis). Ixabepilone (**1**) had a metabolic hydrolysis rate [mouse S9 liver fraction: 0.01 nmol/(min mg)] 100 times slower than epothilone B (**6**) [1.02 nmol/(min mg)].^{13c} In addition, ixabepilone has increased water solubility, reducing the use of formulation agent such as cremophor (polyethoxylated castor oil) which can cause allergic reactions in some patients.

Scheme 1. Retrosynthetic analysis and key bond connections of ixabepilone (**1**)

The convergent retrosynthetic strategy for the first total synthesis of ixabepilone (**1**) is depicted in Scheme 1 and this was utilised by Danishefsky and coworkers.^{20,21} The key transformations involved a late stage epoxidation and a Suzuki coupling and subsequent macrolactamisation to form

the macrolactam 12,13-desoxy-15-*aza*-epothilone B (*aza*-dEpoB) (**7**). This led to the two precursors vinyl iodide **8** and β,δ -diketoester **9**.

The triethylsilyl protected vinyl iodide **12** was formed in 98% yield *via* a Horner-Emmons type condensation with the readily prepared phosphine oxide **10** and ketone **11** (Scheme 2). Deprotection of the TES ether gave an alcohol that was converted into the azide **8** by a Mitsunobu reaction. Acylation of β -ketoester **13** followed by methylation gave enol ether **14**. Aldol coupling of the enolate derived from **14** with *S*-2-methyl-4-pentenal **15** and protection as the trichloroethyl carbonate ester (Troc) then afforded adduct **9**. The Pd-catalyzed Suzuki cross-coupling reaction of *in situ* prepared B-alkyl-9-BBN derivative of **9** with the azido vinyl iodide **8** delivered the corresponding *cis*-olefin **16** in 78 % yield. A Staudinger reduction of azide **16** to the amine, primary amine protection and enol hydrolysis gave carbamate **17**. The subsequent asymmetric reduction of C3 carbonyl group was optimized with a modified Noyori catalyst²² and yielded a single C3-hydroxy diastereoisomer in 78% yield. Exposure to trifluoroacetic acid deprotected both Boc group and tert-butyl ester and macrolactamisation of the resultant amino acid with HATU in dichloromethane gave macrolactam **18** in 90% yield. The O7 Troc protecting group was removed in 88% yield by sonication of **18** in acetic acid and zinc dust to afford *aza*-dEpoB (**7**), and epoxidation of the C12-13 (*Z*)-alkene with 2,2-dimethyldioxirane at -50°C provided a 70% yield of 15-*aza*-epothilone B (**1**) as a single diastereoisomer. Both ixabepilone (**1**) and *Aza*-dEpoB (**7**) showed potent activity against human leukemia cells lines (CCRF-CEM) with **1** slightly being more active ($\text{IC}_{50} = 2.1$ and 9.5 nM, respectively).

Ixabepilone (**1**) can also be semi-synthesized from epothilone B (**5**) and this is close to how the material is produced on a large scale for clinical use (Scheme 3).¹⁹ Remarkably, macrolide ring opening of epothilone B (**5**) mediated with $\text{Pd}(\text{PPh}_3)_4$ gave the π -allylpalladium complex **19** that could be intercepted by an azide nucleophile. The resultant allylic azide **20** was then reduced to the amine **21** and cyclized to yield the lactam macrolide **1**. Since the natural product epothilone B (**5**) can be produced in quantity through optimized bacterial fermentation,²³ this one-pot three-step transformation [20-25% overall yield] enables ready access to ixabepilone (**1**).

Scheme 3. Semisynthesis of ixabepilone (1) from epothilone B (5).¹⁹

Molecular energy calculations conducted on epothilone B and ixabepilone²⁴ using the MP2//B3LYP method found that the C10-C11-C12-O12 torsion angle directs the *exo* (epoxide out of macrocycle) and *endo* (epoxide into macrocycle) conformations of the macrocycle. Importantly, the macrocycles include either two intramolecular hydrogen bonds for epothilone B (5) or three for ixabepilone (1) that accounts for the ring conformations for both lactone/lactam structures. Epothilone B (5) prefers to adopt the compact *exo* conformation **5ex** (Figure 3), as a consequence of the short, near-linear hydrogen bonding between the 3-hydroxyl group and the side-chain thiazole N atom, and from the 7-hydroxyl to the C5-ketone that contribute to the total macrolide conformation energy. In contrast, the macrocyclic hydrogen bonding involving the amide group facilitates ixabepilone (1) to exist as competitive mixtures of *exo* (**1ex**) and *endo* (**1en**) conformers enabling the slightly less stable *endo* conformer to energetically compete for the taxol binding site. The *endo*-ixabepilone (**1en**) conformer thus has cooperative hydrogen bonding between the amide NH, the 3-hydroxyl, and the epoxide. This illustrates that the lactone to lactam change induces significant conformational flexibility within the macrocyclic structure offering more hydrogen bonding patterns and conformational diversity relative to the natural macrolide. This conformational mobility may also contribute to low plasma protein binding observed (see Figure 2) for ixabepilone (1) which could result in better *in vivo* efficacy.

Figure 3. Low energy conformers of *exo*-epothilone B (**5ex**) and *exo* and *endo*-ixabepilone (1) with critical H-bonds indicated (reproduced from SI data: see Lozynski²⁴)

The electron crystallography structure of epothilone A (6) bound to zinc-stabilized α,β -tubulin was determined¹⁴ to a resolution of 2.89 Å and this, along with NMR based conformational analysis, revealed that the bound conformation of 6 has the epoxide in the *endo* orientation (Figure

4A). Key interactions with Arg276, Thr274 and Arg282 were present along with a H-bond between the thiazole nitrogen atom and His227. As shown in the overlay (Figure 4B), the conformation of bound epothilone A (**6**) is very close to that the calculated conformer of ixabepilone (**1en**) above and this may provide an explanation as to why that the macrolactam analogue **1** can effectively bind.

Figure 4. (A) Surface map of epothilone A (**6**) (yellow C atoms) bound to zinc-stabilized two-dimensional crystals of tubulin showing H-bonds as dashed black lines (produced from electron crystallography structure from PDB code 1tvk see Nettles *et. al.*¹⁴).

(B) Overlay of bound **6** (yellow C atoms) and calculated *endo* conformer of ixabepilone (**1en**)²⁴ (blue C atoms)

12,13-desoxy-15-Aza-epothilone A (**22**)

A total synthesis of the 12,13-desoxy-*aza*-epothilone A analogue **22** has been reported by Schinzer and co-workers²⁵ as shown in Scheme 4. Wittig coupling of ylide **23** and ketone **24** gave the diene **25** in high yield after removal of the Boc group. The acid coupling partner **28** was synthesized from ketone **26** and aldehyde **27** *via* aldol condensation followed by acetal hydrolysis, protection and oxidation. Coupling of amine **25** and acid **28** proceeded in good yields using benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (pyBOP) to afford diene **29**. RCM using Grubbs I catalyst gave the macrocycle **30** along with the *E*-isomer as a 1:1 mixture and separation followed by deprotection of the TBS ethers afforded 12,13-desoxy-*aza*-epothilone A **22** which was active against human epidermoid cancer cell lines KB-31 and KB-8511 (IC₅₀ = 464 and 285 nM respectively) but was less active than ixabepilone (**1**) indicating the importance of the epoxide. Compound **22** also induced tubulin polymerization at 26% that measured for epothilone B (**5**).²⁵

Scheme 4. Synthesis of 12,13-desoxy-*aza*-epothilone A analogue (**22**).²⁵

15-Aza-salicylihalamide analogue 2

The salicylate enamide macrolide natural products shown in Figure 5 are potent antitumor compounds and mammalian vacuolar type proton ATPase (V-ATPase) inhibitors.²⁶ Inhibition of these proton pumps eventually leads to cell death via apoptosis. Salicylihalamide A (**31**) was isolated²⁷ from a sponge of the genus *Haliclona* and is a novel highly cytotoxic macrolide. The myxobacteria metabolite apicularen A²⁸ (**32**) is a potent cytotoxin against several human tumor cell lines (cervical IC₅₀ 0.4 ngmL⁻¹; kidney IC₅₀ 0.3 ngmL⁻¹; lung IC₅₀ 0.1 ngmL⁻¹; prostate IC₅₀ 0.5 ngmL⁻¹) and contains the same novel secondary enamide functionality²⁹ as found in salicylihalamide A (**31**).²⁷ It also possesses a similar benzolactone with an internal 9,13-*trans*-tetrahydropyran ring and shows highly effective growth inhibition of several human tumor cell lines.

Figure 5. Salicylihalamide A (**31**) and analogues **33** and **2** and apicularen A (**32**).

The simplified salicylihalamide analogue **33** also showed potent activity against human tumor cells lines [SF268 (CNS glioma), GI₅₀ 0.27 μM; DU-145 (prostate cancer) 2.12 μM].³⁰ The discovery and effectiveness of the potent ixabepilone (**1**) that was found to exhibit a better therapeutic range than epothilone B inspired the research into modification of the salicylihalamide (**31**) 12-membered macrolactone into *aza*-salicylihalamide A analogue **2**.

Scheme 5. Synthesis of 15-*aza*-salicylihalamide A analogue (**2**).⁸

The synthesis of the simplified 15-*aza*-salicylihalamide A analogue **2** shown in Scheme 5 involved the following key synthesis steps that are all scalable.⁸ To form the amide, a photochemical acylation reaction³¹ was utilised in which the quinoketene intermediate **35**, formed from photolysis of 1,3-dioxanolone **34**, was captured by the optically pure amine **36** to yield the diene **37** in 65% yield. This method of amide formation was far superior to alternatives involving traditional benzoic acid-amine cross couplings. The highest *E* selectivity in the ring closing

metathesis was observed using Grubbs 1st generation catalyst on the acetate protected diene **38** which gave the lactam **39** in 75% yield. Other protecting groups, such as methyl and silyl based ones gave inferior *E:Z* selectivities in the RCM reaction. PMB group removal gave alcohol **40** and oxidation provided aldehyde **41**, the structure of which was confirmed by X-ray crystallography. A Takai homologation gave the vinyl iodide **42**. The final step involved the copper mediated cross coupling³² of iodide **42** with dienamide **43** which resulted in the CuI catalyzed attachment of enamide side chain giving the aza-salicylihamide analogue **2** in 64% yield.

The 15-*Aza*-salicylihamide A analogue **2** showed potent activity⁸ against several leukemia cell lines and this was compared to the readily available V-ATP-ase inhibitor bafilomycin A1 (**44**)³³ since salicylihamide (**31**) was not available (Figure 6). Analogue **2** was 10 times less active on average than bafilomycin A1 (**44**) but in a linear relationship, suggesting analogue **2** has the same mode of action.

Cell line	<i>Aza</i> -analogue 2 IC ₅₀ (nM)	Bafilomycin A1 (44) (nM)
CCRF-CEM	371	30
HL-60	116	17
K562	506	43
MOLT-4	277	23
RPMI-8226	117	13

Bafilomycin A1 **44**

Figure 6. Sensitivity of various leukemia cell lines to 15-*aza*-salicylihamide analogue **2** versus bafilomycin A1 (**44**).⁸

Migrastatin macrolactam (**3**)

Migrastatin (**45**) is a novel 14-membered macrolide from *Streptomyces* sp. MK929-43F and is a potent inhibitor of tumor cell migration, invasion and metastasis.⁷ A series of analogs with enhanced tumor cell migration inhibitory properties have been synthesized and their biological activities investigated by *in vitro* and *in vivo* studies. Danishefsky and co-workers^{9, 34} synthesized the aza migrastatin lactam analogue (**3**) according to the retrosynthesis in Scheme 6 based on their total synthesis of migrastatin.³⁵ The key intermediates amine **47** and acid **46** were utilized to generate the macrolactam analogue **3** (Scheme 6) by a diverted total synthesis (DTS) approach. Murphy and co-workers also reported the synthesis of a related aza migrastatin analogue **48** using a similar synthetic approach.³⁶ A 1 μ M dose of analogue **48** inhibited migration of gastric cancer cells by up to 50% in a trans-well cellular locomotion assay.

Scheme 6. Strategy for the synthesis of *aza*-migrastatin analogue **3**.

A stereoselective hetero-Diels-Alder reaction³⁷ between aldehyde **49** and diene **50** followed by hydrolysis with TFA gave enone **51** in good yield as a single diastereoisomer. Reduction and hydrolysis followed by further reduction gave the primary alcohol **52**, which was a key intermediate in the total synthesis of migrastatin (**45**). Selective protection provided an alcohol that was converted into the amine **47**. Coupling with acid **46** provided diene **53**. The synthetic macrolide was prepared by ring closing metathesis of the diene **53** using Grubbs-II catalyst and deprotection gave the *aza*-migrastatin analogue **3** which showed *better activity* against the 4T1 breast cancer cell line than migrastatin (**45**) (IC₅₀ = 0.255 μ M for **3** vs. 29 μ M for **45**). Mice injected with 4T1 cells showed that lactam **3** was also active against human colon tumor LOVO cells (IC₅₀ = 0.174 μ M)

and prostate tumor PC-3 cells ($IC_{50} = 1.285 \mu M$).^{34b} In addition, mice implanted with 4T1 cells that were injected with 10 mg kg^{-1} of lactam **3** daily had a 91-99% reduction in the numbers of metastasized 4T1 cells in the lung after 20 days when compared to control mice.

Scheme 7. The synthesis of migrastatin analogue **3** from the migrastatin by a DTS approach.⁹

Radicalol macrolactam analogues

The essential chaperone heat shock protein 90 (Hsp90) is one of the most common of its type found in eukaryotic cells and captures the energy from ATP-hydrolysis to promote the folding or stabilization of client proteins. Its function and importance has been implicated in diverse diseases including processes in oncogenesis such as self-sufficiency in growth signals.¹⁰ Hsp90 is an important host factor, promoting several steps of the HIV-1 life cycle,³⁸ malaria³⁹ and Alzheimer's disease.^{40, 42} The overexpression of Hsp90 in tumors by some 2~10-fold and its ability to control the stability and activity of oncogenic proteins, makes targeting HSP90 an attractive anticancer strategy. Consequently Hsp90 inhibitors have emerged as a promising therapeutic intervention for a wide variety of human cancers in the past two decades.

Figure 7. Radicalol (**54**) the macrolactone analogue **55** and aza analogues **56** and **4** (TR-FRET and FP assays measure binding of compounds to human Hsp90 β)¹⁰ (GI_{50} against a human colon cancer cell line HCT116)¹⁰

Radicalol (**54**) is a 14-membered resorcylic acid lactone macrolide isolated from various fungi⁴¹ and is a potent *in vitro* Hsp90 inhibitor ($IC_{50} = 20\text{-}23 \text{ nM}$) in cell lines (Figure 7).⁴² However it exhibits no *in vivo* activity and this may be attributed to its highly reactive epoxide ring, the conjugated dienone system Michael acceptor and the macrolactone, all of which are easily metabolised. Consequently, the challenge has focused on the chemical synthesis of analogues with high activity and metabolic stability and a number of groups have reported the studies towards the synthesis of radicalol lactam analogues.^{10, 43} Whilst 12, 13 or 16 membered ring radicalol lactone analogues showed reduced activities,⁴⁴ analogues with 14 or 15-membered rings, including the removal of the diene system, and replacement of the epoxide by a double bond were the most potent Hsp90 inhibitors¹⁰ (Figure 7). Moody and co-workers reported that the novel macrolactone **55** and macrolactam analogue **56** show comparable activity against human colon cancer cell lines.^{10, 43a} The benzylamide analogue **4**¹⁰ exhibited at least a 10-fold higher antiproliferative activity than the

analogues **55** and **56** against human colon cancer and was a 4 fold better binder according to TR-FRET assays ($IC_{50} = 80$ nM vs $IC_{50} = 350$ nM for **55** and $IC_{50} = 220$ nM for **56**).

Scheme 8. The synthesis of radicicol lactam analogues **4**, **56** and **61**.

The synthesis of lactam analogues **4** and **56** is outlined in Scheme 8 and begins with the coupling of **57** and pentenyl malonyl chloride to afford ester **58**. Ring-opening with amino-3-butene gave amide **59** which underwent RCM using Grubbs II catalysts to give the *E*-macrolactam **60** as the major geometric isomer. Hydrolysis followed by decarboxylation and MOM group removal gave the *aza*-radicicol analogue **56**. Alternatively, removal of the MOM ethers from **60** afforded ester analogue **61** which was a strong binder to Hsp90 and had potent activity against HCT116 comparable to analogue **4**. Activation of the ester as the benzotriazole **62** and displacement with benzyl amine followed by MOM ether removal afforded the *aza*-radicicol analogue **4**. Gratifyingly, macrolactam **56** was metabolically more stable than the lactones with 43% metabolized by human liver microsomes (after 30 mins) compared to 83% for radicicol **54**, and 90% for the macrolactone analogue **55**.^{43a}

Scheme 9. The synthetic route to *N*-methyl radicicol macrolactam analogues

A modified synthetic route to generate 14, 15 and 16-membered *N*-methyl radicicol macrolactam analogues is outlined in Scheme 9. Benzylic acylation of **63** with Weinreb amide **64** gave ketone **65** but satisfactory reaction yields were achieved only when the acylation reaction was performed at a

minimum scale of 0.5 mmol of acid **63** at concentration 0.2M in THF at -78. Amide coupling with secondary amine **66** using HATU and Hünig's base provided the diene **67** in 33% yield. As expected, a mixture of *E/Z*-isomers favoring *E* isomer **68** was obtained for the ring closing metathesis of **67** with Grubbs-II. Trifluoroacetic acid deprotection of **68** provided the *N*-methyl macrolactam **69** with overall yields of up to 12% from **63**. Interestingly, removal of the ethoxymethyl groups from the two phenol groups, then the subsequent RCM was found to appreciably change the *E/Z*-isomer ratios of the macrolactam products. This synthetic strategy was utilized to generate *N*-methyl radicicol 15 and 16-membered ring macrolactam analogues **70** and **71**. Surprisingly, *N*-methyl lactams **69-71** are much weaker binders to Hsp90 and exhibit greatly reduced potency against HCT116 (Scheme 9).^{10, 43a}

Radicicol Lactam Analogues: Binding Studies

The thermodynamics of binding to Hsp90 *N*-terminal domain showed that although the macrolactams were often better binders than the macrolactone analogues,^{43a, 44} the natural product radicicol (**54**) was the best (see Figure 6) implying that the epoxide ring exerts a unique binding and conformational interaction with the Hsp90 backbone that is not replicated by the macrolactams. Protein X-ray crystallography showed that radicicol **54** binds to the *N*-terminal domain ATP site of the Hsp90 molecular chaperone with the macrolactone ring in a puckered conformation locked by a key interaction between Lys44 and the epoxide (Figure 8A).⁴⁵ The macrolactone analogue **55** binds to Hsp90 in a very similar manner however without the epoxide there is no interaction with Lys44.⁴⁴ This contributes to the fact that lactone **55** is at least an order of magnitude less effective at binding to Hsp90.

Figure 8. (A) Surface map of radicicol (yellow C atoms) **54** bound to *N*-terminal domain of yeast Hsp90 showing key H-bond interactions with water molecules represented by red spheres and H-bonds as black dashed lines (produced from X-ray structure from PDB code 1bgq see Pearl *et. al.*⁴⁵)

(B) Surface map of lactone **55** bound to Hsp90 showing key interactions (produced from X-ray structure from PDB code 2cgf see Moody *et. al.*⁴⁴)

Moody and co-workers also demonstrated that macrolactams **56** and **4** are strong binders to Hsp90 and this was also studied by X-ray crystallographic analysis.¹⁰ The macrocyclic lactam **56** binds to Hsp90 with the aromatic ring in the same binding pocket as radicicol (**54**) and lactone **55** but with the macrolactam ring more flattened (Figure 9A). Lactam **4** also bound to Hsp90 in a similar manner but with an additional unusual binding of the benzyl amide group *via* a hydrophobic pocket (lined by residues Met84, Asn92, Leu93, Phe124, Val136, Trp148 and Leu173) and displacing the loop between Leu93 and Lys98 (Figure 9B).¹⁰ Analogue **4** depletes the representative client Hsp90 proteins C-RAF, ERBB2, and CDK4 and upregulates Hsp72 demonstrating that it acts as a Hsp90 inhibitor in the colon cancer cells assays. Thus, the unusual binding of the *N*-benzylamide in **4** (Figure 8B) to the Hsp90 protein may explain its enhanced biological effects (Figure 7).

Figure 9. (A) Surface map of lactam **56** bound to Hsp90 showing key interactions (produced from X-ray structure from PDB code 2xx2 see Moody *et. al.*¹⁰)
(B) Surface map of *aza*-radiccol analogue **4** bound to Hsp90 showing selected H-bonds and the *N*-benzyl group in the key hydrophobic pocket (produced from X-ray structure from PDB code 2xx5 see Moody *et. al.*¹⁰)

The X-ray structure of lactams **61** and **70** bound to Hsp90 (Figure 10) revealed that the key H-bond interactions were similar to those observed for bound lactams **4** and **56** and the macrocyclic ring is again flattened and the ketone carbonyl oriented *endo*. For lactam **61**, the ester group makes additional contacts not observed in lactam **56** (Figure 10A). There is extra H-bonding of the ester via a water molecule to the carbonyl group Phe124 and Asp37. The lactam nitrogen has a contact via water molecule to the ketone carbonyl. For lactam **70**, the *N*-methyl group of the lactam nitrogen points out of the binding pocket and the ketone carbonyl is pushed further away, thus explaining the more flattened macrocycle in the bound conformer (Figure 10B). In addition, the lactam N contacts are not present and this could explain the poor binding of this analogue compared to lactams **4**, **56** and **61**.

Figure 10. (A) Surface map of lactam **61** bound to *N*-terminal domain of yeast Hsp90 showing key interactions. (produced from X-ray structure from PDB code 2xx4 see Moody *et. al.*¹⁰)
(B) Surface map of lactam **70** bound to *N*-terminal domain of yeast Hsp90 showing key interactions. (produced from X-ray structure from PDB code 4ce2 see Moody *et. al.*^{43a})

As shown in Figure 11A, a comparison of the bound conformations of radicicol (**54**) and lactone analogue **55** shows that they are very similar with both in a puckered or 'L-shaped'⁴⁶ conformation with the ketone oriented *exo*. Winssinger and co-workers used molecular dynamics calculations to show that the L-shaped bound conformer of **54** is the lowest in energy with the flattened or P-shaped conformer higher in energy and an alternative L'-shaped conformer (macrocycle on the opposite side with respect to the benzene ring) the most energetic conformer.⁴⁶ The bound conformer of lactam **56** (Figure 11B) is more P-shaped (planar), with the orientation of the ketone *endo*. This suggests that the stronger binding of radicicol **54** can be attributed with the

macrocycle in a more compact L-shaped conformation. Lactam **4** (Figure 11C) also binds in a similar manner to analogue **56** with the ketone *endo* but the extra hydrophobic binding of the benzyl amide increases the binding affinity with the macrolactam ring P-shaped conformation having less effect in this novel binding mode. The last example is lactam **70** compared to **54** (Figure 11D) in which the macrolactam ring is the most P-shaped conformation of all the examples presented. This may explain why the *N*-methyl lactam analogues **69–71** are poor binders to Hsp90 compared to the other macrolactams without *N* methylation. All the bound conformers of the lactam analogues **4**, **56**, **61** and **70** have the ketone oriented *endo* and therefore *cis* to the amide NH or *N*-methyl group. There are extra H-bonds present for the amide NH and ketone in binding to Hsp90 which may contribute to their overall better affinity than the lactone **55**, even though this is in a similar conformation as for radicicol (**54**). Thus, the H-bond to the epoxide in bound radicicol which is not present for **55** is compensated somewhat by the lactam analogues by interactions involving the lactam nitrogen atom and the ketone carbonyl group.

Figure 11. Superimposed bound conformations of radicicol **54** (green) (A) with lactone analogue **55** (red), (B) lactam **56** (blue), (C) lactam **4** (purple) and (D) lactam **70** (orange).

Pochoxime D lactam analogue **74**

The natural product pochonin D (**72**) is structurally similar to radicicol but does not possess the labile epoxide and was also identified as a good Hsp90 binder by Winssinger *et. al* (Figure 12).⁴⁶ The oxime analogue **73** was found to possess better binding affinity for Hsp90 than the natural product **72**, possibly due to the fact that the oxime has reduced electrophilicity compared to the ketone, making the oxime analogue a less efficient Michael acceptor and therefore less prone to degradation.⁴⁶

Figure 12. Pochonin D (**72**) and the macrolactone analogue **73** and *aza* analogue **74**

Using a solid phase high throughput approach, the macrolactam pochoxime D analogue **74** was synthesised as outlined in Scheme 10.^{43b} Addition of the benzylic anion derived from **75** to the Weinreb amide **76** followed by oxime formation gave acid **77**. This was attached to solid phase via a chlorotrityl ether linkage and subjected to Tms group removal with TBAF to afford **78**. Coupling of acid **78** with the amine **79** and RCM using Grubbs II catalyst gave macrolactam **80** and cleavage from the linker, amide coupling and ethoxyethyl group removal with solid phase sulfonic acid gave analogue **74**. Lactam analogue **74** was a stronger binder to Hsp90 than pochonin D (**72**) but a weaker binder (5 times less active) compared to the pochonin D analogue **73**.⁴⁷

Scheme 10. The synthetic route to macrolactam pochoxime analogue 74.

Aza derivatives of macrolide antibiotics**Antibiotic macrolide A26771B lactam analogues 76 and 77.** 75 (–)-A26771B
S. aureus MIC 1.56 $\mu\text{g mL}^{-1}$

Figure 13. Antibiotic A26771B (75) and the lactam analogues 76 and 77.

The macrolide (–)-A26771B (75) was isolated from *Penicillium turbatum* and showed promise as a new class of antibiotic (Figure 13).⁴⁸ Although compound 75 exhibited moderate *in vivo* activity it was inactive against *Staphylococcus aureus* and *Streptococcus pyogenes* infections in mice. This inactivity was attributed to the instability of the macrolactone and succinate half ester and a number of derivatives were synthesized.⁴⁹ The lactam analogues 76 and 77 were identified as promising antibiotics and the synthesis of these stereoisomers is shown in Scheme 11.

Scheme 11. Synthesis of the A26771B (75) macrolactam analogues 76 and 77.

Treatment of the mesylate derived from serine derivative **78** with Grignard reagent **79** afforded the alkene **80**. Addition of the same Grignard reagent to aldehyde **81** and methylation gave the racemic ether **82**. A cross metathesis between **80** and **82** mediated by Grubbs I catalysts gave alkene **83** as a mixture of four stereoisomers. Hydrogenation followed by oxidative furan ring opening gave aldehyde **84** which was oxidized to acid **85**. Boc group removal and macrolactamisation gave the lactam diastereoisomers **76** and **77** in low yield which were separated by flash chromatography but not fully assigned.

Evaluation of analogues **76** and **77** against a panel of bacterial strains showed that they were both essentially identical in their activity and almost always more active than the natural product **75**. Both **76** and **77** had MIC values of 2 μgml^{-1} against *Enterococcus faecalis* whilst A26771B (**75**) displayed an MIC value of 16 μgml^{-1} . Analogues **76** and **77** were also more active than **75** against strains of *Staphylococcus aureus* (MIC 1 vs 4 μgml^{-1}), *Streptococcus pneumonia* (MIC 2 μM vs 16 μgml^{-1}) and *Enterococcus faecium* (MIC 0.5 vs 4 μgml^{-1}). More interestingly, **76** and **77** were active against *Haemophilus influenzae* ATCC 31517 (MIC 2 μgml^{-1}) and *H. influenza* LS2 Efflux Knock-out (MIC 0.25 μgml^{-1}) whilst the natural product **75** was far less active (MIC >32 μgml^{-1}). Importantly, The macrolactam analogues remained active in the presence of 10% human serum in the *Staphylococcus aureus* assay (MIC 8 μgml^{-1}) while the macrolactone natural product **75** was inactive clearly demonstrating the stability of the macrolactam in human serum. Unfortunately, macrolactams **76** and **77** were orally inactive in a mouse lung infection model at a dose of 50 mgkg^{-1} .

Glycosylated macrolactam analogues of the macrolide antibiotic YC-17 (**87**)

The 14-membered macrolide erythromycin (**86**)⁵⁰ has long been an effective orally active treatment for bacterial infections and many derivatives have also been successful as antibiotics however, the complex nature of these compounds make them difficult to functionalise as macrolactam derivatives (Figure 14). The less complex 12-membered macrolide antibiotic YC-17 (**87**), isolated from *Streptomyces venezuelae* ATCC 15439,⁵¹ was therefore targeted for aza-analogue synthesis and the macrolactam aglycone aza-10-deoxymethynolide (AZDM) (**88**) was synthesized along with the glycosylated derivatives L-rhamnosyl-AZDM (**89**) and D-quinovosyl-AZDM (**90**) (both produced from **88** by chemoezymatic synthesis).⁵²

Figure 14. Antibiotics erythromycin (**86**) and YC-17 (**87**) and the AZDM (**88**) glycoside analogues **89** and **90**.

The synthesis of AZDM (**88**) is depicted in Scheme 12 and begins with the coupling of amine **91** with acid **92**⁵³ mediated by EDC•HCl and HOBt to afford amide **93**. Selective primary TBS group removal and oxidation with Dess-Martin periodinane gave the aldehyde **94** which upon treatment with vinyl magnesium bromide and oxidation yielded enone **95**. RCM using Grubbs II catalyst gave the macrolactam **96** in good yield as the E-isomer and deprotection gave AZDM (**88**). Chemoezymatic glycosylation of synthetic **88** incubation with a mutant strain of *S. venezuelae* YJ028 in which the gene cluster for encoding the pikromycin PKS and desosamine biosynthetic enzymes were deleted (pLRHM2 expressing thymidine diphosphate (TDP) L-rhamnose biosynthetic genes with glycosyltransferase DesVII/DesVIII) afforded low yields of both L-rhamnosyl-AZDM (**89**) (1.4 mg from 2.4 L culture) and D-quinovosyl-AZDM (**90**).⁵² The production of **90** by this process was attributed to the fact that TDP-4-keto-6-deoxy-D-glucose, which is a biosynthetic intermediate of TDP-L-rhamnose, can be reduced with *S. venezuelae* 4-

ketoreductase to produce TDP-D-quinovose than then glycosylates AZDM (**88**). D-Quinovosyl-AZDM (**90**) (0.3 mg from 1.8 L culture) could also be produced by incubation of **88** with mutant YJ028/pDQNV harboring the D-quinovose biosynthetic genes.

Both lactam glycoside analogues **89** and **90** were at least 4 times more active against several bacterial strains *in vitro* than erythromycin (**86**) (Table 1).⁵² In addition, the macrolactam **89** was much more stable to the macrolide esterase EreB (isolated from the erythromycin-resistant *Escherichia coli*) than erythromycin (**86**). After 1h incubation with EreB, erythromycin (**86**) was completely converted to the linear erythromycin inactivation product while L-rhamnosyl-AZDM (**89**) remained intact, even after 24h incubation. This clearly shows that the macrolactam analogues of AZDM glycosides are stable to bacterial esterases and are active against erythromycin-resistant pathogens. In addition, macrolactam **89** was shown to be stable on exposure to simulated gastric fluid (SGF). Incubation of erythromycin (**86**) with SGF resulted in complete degradation to 5-desosaminylerythronolide A in 2 h but macrolactam **89** remained intact after incubation with SGF over the same time period. Incubation of erythromycin (**86**) or macrolactam **89** with rat liver microsomes and NADPH for 2 h resulted in degradation of the former compound whilst **89** remained unchanged. Finally, both compounds **89** and **90** showed low cytotoxicity against human cell line HEK293 (IC₅₀ > 50 μmol) making them good candidates for further clinical development.

Compound	<i>E. faecium</i> ATCC19434	<i>E. faecium</i> P00558	<i>S. aureus</i> ATCC2592	<i>S. aureus</i> P00740
Erythromycin (86)	30.0	>120.0	60.0	120.0
L-Rhamnosyl-AZDM (89)	7.5	30.0	15.0	30.0
D-Quinovosyl-AZDM (90)	7.5	30.0	15.0	30.0

Table 1. Antibacterial activities [MIC (μM)] of erythromycin (**86**), L-rhamnosyl-AZDM (**89**) and D-quinovosyl-AZDM (**90**).

Conclusion

The lactam analogue of the myxobacteria metabolite epothilone B (**5**), namely ixabepilone (**1**), is the benchmark for *aza* modified macrolides. This great success story, starting with the isolation of the natural product, biological assay, SAR and analogue preparation and final clinical application is a key example of how this simple *aza* modification of macrolides can provide useful active analogues. Inspired by this success, the synthesis of other examples of *aza* analogues of macrolide natural products has been pursued by a number of research groups. Macrolactam analogues based on the marine macrolide salicylhalamide (**31**), bacterial metabolite migrastatin (**45**), fungal metabolite radicicol (**54**) and antibiotic macrolides A26771B (**75**) and YC-17 (**87**) presented in this review show that significant biological and chemical properties such as metabolic stability, reduced toxicity, protein binding and desirable macrocyclic conformations can be achieved. Thus, the

modification of the macrolactone to a macrolactam imparts a number of desirable properties apart from derivatives simply being less susceptible to hydrolysis. With the further development of efficient synthetic technologies for macrolactam formation, natural product *aza* analogues will be even more readily accessible and destined to result in improved therapeutics.

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