Published in final edited form as:

Chemistry. 2011 January 17; 17(3): 895–904. doi:10.1002/chem.201002402.

Structural Requirements for the Antiproliferative Activity of Pre-mRNA Splicing Inhibitor FR901464

Sami Osman^a, Brian J. Albert^a, Yanping Wang^a, Miaosheng Li^a, Nancy L. Czaicki^a, and Kazunori Koide^{*,a}

^aDepartment of Chemistry, University of Pittsburgh, 219 Parkman Avenue, Pittsburgh, PA, 15260, USA

Abstract

FR901464 is a natural product isolated from a bacterium source that activates a reporter gene, inhibits pre-mRNA splicing, and shows antitumor activity. We previously reported the development of a more potent analogue, meayamycin, through the total synthesis of FR901464. Herein, we report detailed structure-activity relationships of FR901464 that revealed the significance of the epoxide, carbon atoms in the left tetrahydropyran ring, the Z-geometry of the side chain, the 1,3-diene moiety, the C-4 hydroxy group, and the C2"-carbonyl group. Importantly, the methyl group of the acetyl substituent was found to be inessential, leading to a new potent analogue. Additionally, partially based on *in vivo* data, we synthesized and evaluated potentially more metabolically stable analogues for their antiproliferative activity. These structural insights into FR901464 may contribute to the simplification of the natural product for further drug development.

Keywords

carbamate; FR901464; meayamycin; cancer; structure-activity relationship

Introduction

Development of natural product-based anticancer agents

Development of small molecule ligands for specific protein targets is a contemporary approach in drug discovery processes and chemical biology (reverse chemical genetics). The success of imatinib (Gleevec[®]) exemplifies this approach,^[1] but the generality of this rational approach remains to be seen,^[2] as small molecules often bind to off-targets. Historically, especially in cancer medicine, major breakthroughs occurred through the target identifications of previously known anticancer natural products (forward chemical genetics). These targets were usually not obvious prior to these studies. For example, paclitaxel was first identified as an anticancer compound;^[3] however, until Horwitz reported that this natural product bound to microtubule, this protein was presumably not viewed as a viable target.^[4] Similarly, although lactacystin was discovered earlier,^[5] the proteasome was not widely accepted as a viable target to fight cancer until lactacystin's target was found to be a proteasome unit.^[5] Consequently, Bortezomib[®] has been approved by the FDA as the first proteasome inhibitor for cancer treatment.^[6]

FR901464 (Figure 1) may contribute to cancer therapy in a similar fashion. The isolation and antitumor activity of FR901464 was reported in 1996 by Nakajima and his co-

workers.^[7] Although its biological profile was unique and indicative of a new mode of action, the molecular mechanisms of this antitumor agent were not known for a long time. In 2007, the Yoshida-Kitahara group reported that FR901464 inhibited pre-mRNA splicing and that its target was splicing factor 3b (SF3b).^[8] SF3b, consisting of seven proteins,^[9] is required for pre-mRNA splicing and is part of the spliceosome, a complex of approximately 200 proteins and small nuclear RNAs (snRNAs) that catalyzes RNA splicing.^[10] Although the specific molecular target of FR901464 has not yet been determined, it is likely that it is either spliceosomal associated protein (SAP)135 or SAP155.^[8] The same research group also showed that the splicing inhibition of *p27* by FR901464 was linked to cell cycle arrest.^[8] These studies indicate that the anticancer activity of FR901464 is directly linked to pre-mRNA splicing inhibition. This is potentially a breakthrough because splicing processes have never been exploited as therapeutic targets or biomarkers in cancer medicine. Moreover, post-transcriptional RNA modifications are an increasingly important theme in biology,^[11] for which FR901464 or its analogue may be used as a chemical tool. Very recently, the Webb group reported the promising *in vivo* antitumor activity of an FR901464 analogue, which further supports the idea that FR901464 analogues could be antitumor drugs.^[12]

Not surprisingly, several pharmaceutical companies recently used reporter assays related to those that the Nakajima group employed and discovered a series of new natural products with biological profiles similar to that of FR901464.^[13, 14] The most notable natural products are the pladienolides,^[14] a derivative of which is currently in Phase I trials as the first drug candidate with splicing inhibitory activity.^[15]

In addition to the significance of using splicing inhibitors as antitumor agents, there is a great need to develop chemical probes for RNA splicing because the process is not very tractable with currently available biological methods. As the first natural product that inhibits pre-mRNA splicing, FR901464 is now considered a prototype compound for splicing inhibitors. Given the unique mode of action in conjunction with its antitumor activity, we envision that FR901464 or its analogues will be widely used in oncology and RNA biology. Thus, it is important to understand the structure-activity relationships of FR901464, which would enable the rational design of more potent analogues that are compatible with *in vivo* experiments.

Synthetic studies of FR901464

The Jacobsen group accomplished the first total synthesis of FR901464^[16] and systematically studied the structure-activity relationship (SAR) of this natural product.^[17] The results of their SAR studies are described throughout this article where they are directly related to our studies. The second total synthesis was accomplished by the Kitahara group,^[18] who later improved their synthetic scheme.^[19] Our group reported the third total synthesis of FR901464 in 2006,^[20, 21] and later disclosed how the combination of the epoxide at the C3 position and the hydroxy group at the C1 position caused the decomposition of FR901464.^[22]

C1-Hydroxy group of FR901464

Spliceostatin A (Figure 1), the 1-methoxy analogue prepared by the Kitahara group, is more active than FR901464 in enhancing gene expression of a reporter gene.^[23] Unfortunately, their semi-quantitative description of the activity does not allow for complete quantitative assessment. Moreover, the methoxy group at the anomeric center without neighboring electron-withdrawing groups is acid-sensitive,^[24] which raises the question of whether it is simply an FR901464-prodrug with enhanced cell permeability. Alternatively, the improved activity could be a result of the improved stability of spliceostatin A as compared to

FR901464.^[23] 1-Desoxy FR901464, prepared by the Jacobsen group, is slightly more active against Jurkat cells than FR901464.^[17] This analogue shows an important feature about FR901464: its active form contains a cyclic B-ring. It is not clear whether the 1-hydroxy group participates in molecular recognition since the improved stability of 1-desoxy FR901464 and loss of the hydroxy group may compromise each other, resulting in slightly better anticancer activity. We recently substituted the 1-hydroxy group with a methyl group and found that this analog, meayamycin, was 100 times more potent against human breast cancer MCF-7 cells than FR901464.^[22] Moreover, it is more potent than 1-desoxy FR901464 and should be more stable than spliceostatin A. Therefore, in this work, all of the analogues contain the geminal dimethyl group at the C1 position.

Results and Discussion^[25]

The epoxide moiety

The C3-cyclopropyl analogue **1** (Figure 1) was prepared by the Jacobsen group and shown to be inactive even at 4 μM (i.e., >3 orders of magnitude less active).^[17] This result implies that the epoxide may be crucial for FR901464's biological activity, but it was not clear whether the oxygen atom or the electrophilicity of the epoxide was important. If the electrophilicity is important, such a notion would be contradictory to the Yoshida-Kitahara team's statement that FR901464 does not form a covalent bond with its target protein.^[8] In light of this potential discrepancy, we set out to prepare non-epoxide analogues that still contain the oxygen atom at the C3 position (Scheme 1).

Compound **2**^[22] was reduced with LiAlH_4 to a diol, which was protected as an acetal to give **3** in 67% yield for the two steps (Scheme 1A). This acetal was subjected to olefin cross metathesis conditions with the previously prepared diene **4** and **Ru-1**^[26] to give the cross-coupled compound in 41% yield. The acetal of this compound was hydrolyzed under acidic conditions to give analogue **5** in 66% yield.

To arrive at two similar analogues, alcohol **6**^[22] was used as the starting material. The alcohol was protected as its PMB ether in 74% yield, and its epoxide was subsequently opened with KOH to give diol **7** in 77% yield (Scheme 1B). Diol **7** was cleaved by the action of NaIO_4 , and the resulting ketone was immediately reduced with $\text{L-Selectride}^\circledast$ to give alcohol **8** as a single diastereomer. Alcohol **8** was treated with DDQ to give a mixture of acetals and esters (see Supporting Information), which was treated with acidic methanol and subsequently with KOH to give a diol. This diol was converted to acetal **9** in quantitative yield. Acetal **9** was then subjected to diene **4** and **Ru-1** to give a metathesis adduct in 48% yield, which was deprotected under acidic conditions to give **10** in 68% yield.

Alcohol **8** was also methylated by the action of MeI and NaH to give **11** in 94% yield. The PMB ether **11** was exposed to DDQ to give an alcohol in 89% yield, which was then protected as its THP ether **12** in quantitative yield. Compound **12** was treated with diene **4** and **Ru-1** to give the desired cross-coupled product in 40% yield. Finally, the THP ether of this product was hydrolyzed to give analogue **13** in 63% yield.

None of these non-epoxy analogues (**5**, **10**, **13**) exhibited antiproliferative activity against MCF-7 cells even at 10 μM , meaning that they are at least six orders of magnitude less active than meayamycin (Table 1). These data, together with those by the Jacobsen group,^[17] suggest that the epoxide of FR901464 plays a crucial role as an electrophile and presumably forms a covalent bond. Since non-specific epoxide opening by thiols *in vivo* may not be a major concern,^[27] the potential covalent bond formation may be specific for the putative target(s).

The 12 position

Vasella and co-workers studied the conformations of compound **14** in water and found that a significant population (1/3) of this compound existed as conformer **14_{N-ax}** despite the bulk of the acetamide group (Scheme 2A).^[28] The somewhat surprising stability of the axial conformer prompted us to speculate that the more stable chair conformer of the A-ring of FR901464 might be **FR901464_{N-ax}** (Scheme 2B), provided that the A-ring is indeed in a chair form (Scheme 2B). This preference can be justified by arguing that in this conformer, if the amide NH is hydrogen bonded to the oxygen atom of the A-ring, only the nitrogen lone pair would point toward the methyl group at the C12 position, minimizing the 1,3-diaxial interaction. On the other hand, in conformer **FR901464_{N-eq}**, there is a substantial 1,3-diaxial interaction between the methyl group at the C15 position and the C10-allylic methylene group. Therefore, we hypothesized that FR901464 might bind to its target(s) when it assumed the chair form **FR901464_{N-ax}** in the A-ring.

With this hypothesis, it appeared to us that the 12-methyl group in the axial position destabilized this putative binding conformer. Therefore, removal of this methyl group might indeed increase the population of the bound conformer, thereby enhancing the potency of meayamycin. Moreover, inspired by the work of Wender on a series of bryostatin analogues,^[29] which was followed by the Smith group on phorbosazole analogues,^[30] the substitution of the 12-carbon with an oxygen atom looked very appealing from a synthetic standpoint.

Our synthetic efforts toward analogue **22** began with the one pot DIBALH reduction-Wittig condensation reaction sequence of the L-threonine derivative **15** (Scheme 3A).^[31] The resulting enoate was hydrogenated to form the corresponding saturated compound **16** in 87% yield for the two steps. Treatment of this compound with acetic acid promoted the acetonide cleavage and intramolecular transesterification, giving lactone **17** in 73% yield. The allylation of this lactone followed by the BF₃•OEt₂-Et₃SiH mediated reduction of the resulting hemiketal provided tetrahydropyran **18** in 43% yield for the two steps. The same three-step sequence as our previous synthesis (1. Boc-deprotection–amidation with acid **19**; 2. olefin cross metathesis with methacrolein; 3. Wittig reaction with Ph₃P=CH₂)^[21] afforded the conjugated diene **21** in 43% yield over the three steps. This left fragment was then coupled with the right fragment **6** to give the designed analogue **22** in 50% yield. The total number of steps is 25 for this analogue with 11 steps in the longest linear sequence, which compares favorably to the synthesis of meayamycin (28 total steps and 13 steps in the longest linear sequence).

The acetal analogue synthesis was even more concise; treatment of the commercially available compound **23** with NaBH₄ gave the known diol **24** in 87% yield (Scheme 3B).^[32] This diol was reacted with 1,2-diethoxy-3-butene under acidic conditions to form the cyclic acetal **25** in 67% yield. After the same synthetic procedures as before, we were able to prepare the target analogue **28** in 15% yield for the 4 steps. The total number of steps is 21 for this analogue with only 11 steps in the longest linear sequence.

The antiproliferative activity of these two analogues was evaluated against MCF-7 cells (Table 1). The 12-desmethyl meayamycin **22** was less potent than meayamycin, but the GI₅₀ is still in the respectable sub-nanomolar range (0.3 nM). The acetal analogue **28** was far less active, with the GI₅₀ value at 2.5 nM. These data indicate the significance of hydrophobicity at the 12-position. The replacement of the A-ring with a cyclic acetal provided a means to probe the impact of substituents at positions 13 and 15. Thus, through similar synthetic schemes (Supporting Information), analogues **29** and **30** were prepared and evaluated. The additional methyl group at the 13 position in analogue **29** was detrimental to the biological activity. The replacement of the 15-methyl with a hydrogen atom in analogue **30** also had a

negative impact with respect to the biological activity. All together, the substituents at positions 12, 13, and 15 are intricately responsible for the biological activity of FR901464. Although these analogues are more synthetically accessible, we tentatively decided to exclude these simplifications because a sufficient quantity of meayamycin could be produced for the purpose of this study using tissue-cultured cells.

To understand if the loss in activity of these A-ring analogues was due to a conformational change or a loss of hydrophobicity, NMR experiments were undertaken for dienes **4**, **21**, and **27** in CD₃OD (Figure 2). The J_{14-15} values were 2.1, 1.6, and 1.8 Hz for **4**, **21**, and **27**, respectively, indicating similar chair-like conformers. Therefore, the loss in activities for 12-desmethyl meayamycin, **22**, and **28** is mainly attributed to the loss of favorable hydrophobic interactions, and, to a lesser degree, a conformational change in the A-ring.

Why is compound **28** less potent than compound **22**? The lower potency of **28** can be attributed to the additional conformer **28β** (Figure 3). From ¹H NMR experiments, the secondary N-H signal was very prominent in CDCl₃, which did not diminish upon the addition of CD₃OD. This indicates that there is strong H-bonding between the N-H and the oxygen atom on the A-ring (pyran or acetal). An additional reason for the lower potency of **28** may be because the additional oxygen is bound to a water molecule, which may need to be dissociated to enter the putative hydrophobic binding pocket.

The 4'-position

Due to a 1,3-allylic strain, the 4'-H is nearly in the same plane as the conjugated amide, as suggested by the ¹H NMR spectrum of FR901464 ($J_{3'-4'} = 7.8$ Hz, Figure 4). Inversion of the 4'-stereocenter places its methyl and acetoxy substituents in opposite faces of the plane defined by the conjugated amide. This analogue, 4'-*epi*-FR901464, was synthesized by the Jacobsen group and found to be 15 times less active than FR901464.^[17] The reduced activity could be because either the acetoxy or 5'-methyl group is in the wrong orientation (or both groups). To probe this idea, we prepared 4'-desacetyl meayamycin, which began with the methanolysis of the previously prepared **4**^[21, 22] to give alcohol **31** in 82% yield (Scheme 4A). This alcohol was then subjected to olefin cross metathesis conditions with **6** and **Ru-1** to give 4'-desacetyl meayamycin in 81% yield. The potency of 4'-desacetyl meayamycin dropped by 24-fold (GI₅₀ = 0.48 nM; Table 1) as compared to meayamycin, further indicating the significance of the 4'-position.

From this result, it was not clear whether this reduced activity was due to the loss of the acetyl group itself or possibly placing the methyl group in the α-face by virtue of the intramolecular hydrogen bonding between the 4'-hydroxy group and the amide group. Therefore, we also prepared 4'-*epi*-desacetyl meayamycin according to Scheme 4B. The enantiomer of acid **19** (i.e., *epi*-**19**) was prepared by the following route: *N*-acetyl morpholine was coupled to an in situ prepared lithium acetylide to give **32** in 90% yield.^[33] This ynone was reduced with HCO₂Na and Noyori's catalyst,^[34] **Ru-2**, to give an alcohol, which was acetylated to give **33** in 73% yield for the two steps. This material was then exposed to acidic methanol followed by the Jones' reagent to give a ynoic acid in 72%. The ynoic acid was hydrogenated in the presence of Lindlar's catalyst to give 4'-*epi*-**19** in 68% yield. Acid 4'-*epi*-**19** was then used to prepare 4'-*epi*-desacetyl meayamycin by the same synthetic route previously outlined.^[21, 22] This analogue was found to be one order of magnitude less active (GI₅₀ = 4.7 nM, Table 1) than 4'-desacetyl meayamycin, indicating that the acetyl group is important for the biological activity.

The correlation between the loss of the acetyl group and activity was a concern to us because esterases might hydrolyze the ester group in tissue culture and *in vivo*. Consequently, we measured the half-lives of meayamycin in mouse and human sera at 10

μM . As we expected, meayamycin was hydrolyzed to its desacetyl analogue ($t_{1/2} = 2$ h in mouse serum, 9 h in human serum), which was then converted to uncharacterized products. We hypothesized that if we prolong the half-life of meayamycin in sera, effective concentrations of this compound might be higher during the assays, thus improving the potency. To test this hypothesis, we replaced the acetyl group with a morpholine-based carbamate (meayamycin B) to enhance stability under biological conditions as carbamates are known to be more stable than esters under biological conditions.^[35]

The synthesis of this analogue was accomplished by the reaction of **31** with carbonyldiimidazole (CDI) followed by morpholine to give **36** in 80% yield (Scheme 4C). This carbamate was then coupled to **6** in the presence of **Ru-1** to give meayamycin B in 44% yield. The half-life of this carbamate analogue was 13 h in mouse serum (Figure 5), significantly longer than that of meayamycin, presumably due to the esterase-resistant nature of this compound. Encouraged by this result, the antiproliferative activity of this new analogue was measured; it was gratifying to find that meayamycin B was even more potent than extremely potent meayamycin in MCF-7 cells (Table 2). With this result, we tested meayamycin^[36] and meayamycin B with various cancer cell lines. MCF-7 and MDA-MB-231, both human breast cancer cell lines, were the most responsive. PC3, HCT-116 and H1299 were also very responsive, exhibiting GI_{50} values in a low picomolar range. A549 and DU145 cells were less responsive, but the GI_{50} values were still sub-nanomolar. On the average, meayamycin B is 3.1 ± 1.9 times more potent than meayamycin in these seven cancer cell lines.

After the synthesis and biological evaluation of meayamycin B were complete in our laboratory, the Webb group published similar carbamate analogues with great loss of activity.^[12] In retrospect, we were fortunate that we chose morpholine for water solubility, the size of which appeared to be adequately small to fit in an unknown binding pocket unlike Webb's larger carbamate groups.

For *in vivo* studies, the most ideal vehicle is saline; therefore, the solubility of drugs in saline is a crucial parameter. Although meayamycin was not entirely soluble in saline at 0.1 mg mL^{-1} , meayamycin B was easily soluble at this concentration (presumably soluble at higher concentrations), showing an additional benefit for meayamycin B.^[37]

C2'–C3' Olefin on the side chain

Next, we asked whether the C2'–C3' trans isomer was the actual active species after the potential isomerization of the cis enamide to its trans form. To address this question, we synthesized analogue **41** according to Scheme 5. A key step is the deliberate isomerization of the cis acid **19** to the activated trans acid derivative during the amide-bond-forming reaction. Specifically, we exploited the facile cis-trans isomerization of the HATU-activated acid **19** prior to the addition of amine **34** to yield a $\approx 1:1$ cis:trans mixture as illustrated in Scheme 5A. Analogue **41** was assembled in three additional steps as illustrated in the scheme. The GI_{50} of this analogue was 3 orders of magnitude higher than that of the corresponding cis compound, indicating that the cis enamide is the active species.

Considering that an α,β -unsaturated amide could serve as an electrophile,^[38] we asked whether the side chain was an electrophile. To answer this question, analogue **48** was designed and synthesized according to Scheme 5B. The GI_{50} of this side chain analogue was found to be 19 nM with MCF-7 cells (Table 1) (93 nM with HCT-116). The antiproliferative activity of two analogues **41** and **48** implies that the cis C2'–C3' enamide is not an electrophile but rather a structural requirement.

4-Hydroxy group

4-*O*-Alkyl FR901464 analogues were prepared and evaluated by others.^[39] However, it was not clear to us whether the loss of activity was due to the large alkyl groups or the loss of a hydrogen bond donor. To minimize the additional sterics, we prepared the 4-*O*-methyl analogue **50** (Scheme 6). This analogue, meayamycin C, showed respectable potency with the GI₅₀ of 0.38 nM. The diminished activity may be explained by the loss of a hydrogen bond donor or the additional steric bulk. Nonetheless, this analogue may prove to be an important one because in a separate study we observed glucuronidation of meayamycin B as a major metabolite in mice, for which this hydroxy group is the only site. Detailed pharmacokinetic studies of meayamycin B and meayamycin C will be reported by our laboratory in due course. It is noteworthy that the olefin cross metathesis of **36** with **49** was reproducibly higher yielding than that with **6** (Scheme 4C). This difference proved to be crucial in the preparation of cyclopropyl analogues (see below).

1,3-Diene moiety

We previously observed relatively facile isomerization of the 1,3-diene moiety under acidic conditions.^[22] The same might occur in live cancer cells, raising a question of whether the trans, trans-diene moiety is actually the biologically active species. To examine the significance of the diene moiety, we synthesized cyclopropyl analogues **59** and **60** (Scheme 7).

Compound **34** was first cross-coupled with methacrolein in the presence of **Ru-1** in 84% yield. This aldehyde was converted to alcohol **52**, which was stereoselectively cyclopropanated by Charett's method^[40] to form **53** in 70% yield with a diastereoselective ratio of 89:11. We also prepared the epimer at the C8 and C9 position in 88% with a diastereoselective ratio 87:13 (Supporting Information). The allylic cyclopropyl group, after the conversion of the hydroxy group of **53** to an olefin, was not compatible with acidic conditions for the Boc removal (data not shown). These failures prompted us to remove the Boc group from **53** with TFA before the derivatization of the hydroxy group. The resulting amine **54** was condensed with carboxylic acid **19** to form **55** in 48% yield for the two steps. The oxidation-olefination sequence gave alkene **57** in 56% yield for the two steps. Further transformations yielded alkene **58**. Interestingly, this alkene did not undergo cross olefin metathesis with alkene **6** but did so with alkene **49** to form **59** in 30% yield. By a similar sequence, diastereomer **60** was prepared (Supporting Information).

The GI₅₀ values of analogues **59** and **60** with MCF-7 cells were found to be 15 and 10 nM, respectively (Table 1). The potencies of these analogues were approximately one order of magnitude lower than meayamycin C but were still respectable. These results imply that the C8–C9 olefin of FR901464 remains trans as an active species in live cells. In order to fully understand the significance of the C8–C9 olefin, cyclopropyl analogues that would mimic the C8–C9 cis geometry must be prepared and evaluated. Nonetheless, the similar activity of **59** and **60** compared to meayamycin C implies that the C8–C9 olefin would remain trans as the active FR901464 species in cells.

Summary

The C3-spiroepoxide (Figure 6) was shown to be crucial for the retention of the antiproliferative activity, indicating that a covalent bond is presumably formed between target protein(s) and FR901464. The C12 position prefers to be hydrophobic, and C4'-acetate is important but the ester is labile, forming a less potent alcohol (4'-desacetyl meayamycin). The C2'–C3' side chain olefin needs to be cis to be active. The replacement of one of the 13-H with a methyl group diminished biological activity. Replacement of the

15-methyl group with a hydrogen atom was also somewhat detrimental. The C8–C9 olefin remains trans as the active species of FR901464. Masking the 4-hydroxy group with a methyl group reduced the biological activity. Finally, the development of the more biologically stable and more saline-soluble C4'-carbamate analogue, meayamycin B, was shown to be significantly more potent than meayamycin in seven cancer cell lines. FR901464 binds SF3b and inhibits pre-mRNA splicing, which could be very exciting because the meayamycins should also inhibit the same process and exhibit anticancer activity. Since there is no approved antitumor drug that acts by inhibiting pre-mRNA splicing, the mode of action, potency, and water solubility of meayamycin B might further trigger interest in both oncology and RNA biology.

Experimental Section

Materials and Methods for the Growth Inhibition Assays

Materials

FR901464 and its analogues were dissolved in dimethyl sulfoxide (DMSO) as 10 mM stocks and stored at –20 °C. For the experiments, aliquots were thawed at room temperature and dilutions were prepared in RPMI 1640 medium containing 2% DMSO at 2× the desired concentration prior to addition to the cells.

Cell Culture

MCF-7, A549, DU145, HCT116, MDA-MB-231, H1299, and PC3 cells were grown at 37 °C in an atmosphere containing 5% carbon dioxide in Corning cell culture dishes (150 mm) in RPMI-1640 cell culture medium containing 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin-streptomycin solution (Invitrogen).

Growth Inhibition Assay

Cells were plated in 96 well plates at an initial density of 2,000 or 4,000 cells per well in 100 µL of medium and were incubated for 24–48 h (depending on cell line) prior to compound addition (plating either 2,000 or 4,000 cells/well led to similar GI₅₀ values). Serial two-fold dilutions were used in this experiment for the indicated ranges. The compounds were added to the cells at 2× the desired concentration in 100-µL cell culture medium (containing 2% DMSO). The cells were then incubated for an additional 5 to 10 days. Cell proliferation was measured using the commercial 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) dye reduction assay with phenazine methosulfate (PMS) as the electron acceptor (20 µL per well). The absorbance (at 490 nm minus that at 630 nm) was measured by a Spectromax M5 plate reader (Molecular Devices) or Modulus II Microplate Multimode Reader (Turner BioSystem). Evaluation of FR901464 analogues was performed in quadruplicate at each concentration, and the final numbers were averaged. Microsoft Excel and GraphPad Prism 5 were used to construct dose-response curves and calculating GI₅₀. Growth inhibition is calculated as defined by the National Cancer Institute [GI₅₀ = 100 × (T – T₀)/(C – T₀); T₀ = cell density at time zero; T = cell density of the test well after period of exposure to test compound; C = cell density of the vehicle treated].⁴¹ Vincristine or cisplatin were used as controls.

Procedure for the half-life determination for the decomposition of meayamycin and B in mouse serum.

To a 1 dram glass vial was added mouse serum (1.0 mL), rhodamine B (10 mM in DMSO, 2.0 µL), and DMSO (7.0 µL) under an open atmosphere and warmed to 37 °C. To the

mixture was added meayamycin or meayamycin B (10 mM in DMSO, 1.0 μ L), and the resulting solution was sealed with a cap, vortexed for 15 s, and then placed in a 37 °C incubator. The decomposition was monitored by HPLC at the indicated times. The resulting data were normalized by dividing the ratios of meayamycin or meayamycin B/rhodamine B by the ratio of the first data point. HPLC monitoring was performed on a Varian Pursuit XRs 5 C₁₈ column, 250 $\times$ 10.0 cm, 2.5 mL/min, 30% MeOH in H₂O (containing 1% HCO₂H) to 100% MeOH linear gradient elution from 0.5 to 22 min, monitored at 230 nm (for meayamycins) and 550 nm (for rhodamine B).

Acknowledgments

We thank Professors Billy W. Day and Andreas Vogt (University of Pittsburgh) for helpful discussions. We thank Professor Stephen G. Weber (University of Pittsburgh) for allowing us to use his Spectromax M5 plate reader. B.J.A. and S.O. are recipients of a Graduate Excellence Fellowship from the University of Pittsburgh. N.L.C. is thankful for the Arnold and Mabel Beckman Scholar Award and the Averill Scholarship. We thank Dr. Damodaran Krishnan and Dr. John Williams for assisting with NMR and mass spectroscopic (NIH grant S10RR017977-01) analyses, respectively. This work was supported by the US National Cancer Institute (R01 CA120792).

References

1. Capdeville R, Buchdunger E, Zimmermann J, Matter A. *Nat. Rev. Drug Disc.* 2002; 1:493–502.
2. Kamb A, Wee S, Lengauer C. *Nat. Rev. Drug Disc.* 2007; 6:115–120.
3. Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT. *J. Am. Chem. Soc.* 1971; 93:2325–2327. [PubMed: 5553076]
4. a) Schiff PB, Fant J, Horwitz SB. *Nature.* 1979; 277:665–667. [PubMed: 423966] b) Schiff PB, Horwitz SB. *Proc. Natl. Acad. Sci. U.S.A.* 1980; 77:1561–1565. [PubMed: 6103535]
5. Fenteany G, Standaert RF, Lane WS, Choi S, Corey EJ, Schreiber SL. *Science.* 1995; 268:726–731. [PubMed: 7732382]
6. a) Adams J. *Cancer Cell.* 2004; 5:417–421. [PubMed: 15144949] b) Rajkumar SV, Richardson PG, Hideshima T, Anderson KC. *J. Clin. Oncol.* 2005; 23:630–639. [PubMed: 15659509]
7. a) Nakajima H, Hori Y, Terano H, Okuhara M, Manda T, Matsumoto S, Shimomura K. *J. Antibiot.* 1996; 49:1204–1211. [PubMed: 9031665] b) Nakajima H, Sato B, Fujita T, Takase S, Terano H, Okuhara M. *J. Antibiot.* 1996; 49:1196–1203. [PubMed: 9031664] c) Nakajima H, Takase S, Terano H, Tanaka H. *J. Antibiot.* 1997; 50:96–99. [PubMed: 9066774]
8. Kaida D, Motoyoshi H, Tashiro E, Nojima T, Hagiwara M, Ishigami K, Watanabe H, Kitahara T, Yoshida T, Nakajima H, Tani T, Horinouchi S, Yoshida M. *Nat. Chem. Biol.* 2007; 3:576–583. [PubMed: 17643111]
9. a) Das BK, Xia L, Palandjian L, Gozani O, Chyung Y, Reed R. *Mol. Cell. Biol.* 1999; 19:6796–6802. [PubMed: 10490618] b) Krämer A, Grüter P, Gröning K, Kastner B. *J. Cell Biol.* 1999; 145:1355–1368. [PubMed: 10385517] c) Will CL, Schneider C, MacMillan AM, Katopodis NF, Neubauer G, Wilm M, Lührmann R, Query CC. *EMBO J.* 2001; 20:4536–4546. [PubMed: 11500380] d) Will CL, Urlaub H, Achsel T, Gentzel M, Wilm M, Lührmann R. *EMBO J.* 2002; 21:4978–4988. [PubMed: 12234937]
10. Zhou ZL, Licklider LJ, Gygi SP, Reed R. *Nature.* 2002; 419:182–185. [PubMed: 12226669]
11. Licatalosi DD, Darnell RB. *Nat. Rev. Genet.* 2010; 11:75–87. [PubMed: 20019688]
12. Lagisetty C, Pourpak A, Goronga T, Jiang Q, Cui X, Hyle J, Lahti JM, Morris SW, Webb TR. *J. Med. Chem.* 2009; 52:6979–6990. [PubMed: 19877647]
13. a) Sakurai M, Kohno J, Nishio M, Yamamoto K, Okuda T, Kawano K, Nakanishi N. *J. Antibiot.* 2001; 54:628–634. [PubMed: 11592499] b) Sakai Y, Tsujita T, Akiyama T, Yoshida T, Mizukami T, Akinaga S, Horinouchi S, Yoshida M, Yoshida T. *J. Antibiot.* 2002; 55:863–872. [PubMed: 12523819] c) Sakai Y, Yoshida T, Ochiai K, Uosaki Y, Saitoh Y, Tanaka F, Akiyama T, Akinaga S, Mizukami T. *J. Antibiot.* 2002; 55:855–860. [PubMed: 12523818]
14. a) Sakai T, Asai N, Okuda A, Kawamura N, Mizui Y. *J. Antibiot.* 2004; 57:180–187. [PubMed: 15152803] b) Sakai T, Sameshima T, Matsufuji M, Kawamura N, Dobashi K, Mizui Y. *J. Antibiot.* 2004; 57:173–179. [PubMed: 15152802]

15. Kotake Y, Nijima J, Nagai M, Okano K, Sakai T, Yoshida M, Tsuchida T, Nakashima T, Dobashi K, Mizui Y, Shimizu H, Uenaka T, Iwata M, Asada M, Yoshimatsu K. Clin. Cancer Res. 2003; 9:6208s.
16. Thompson CF, Jamison TF, Jacobsen EN. J. Am. Chem. Soc. 2000; 122:10482–10483.
17. Thompson CF, Jamison TF, Jacobsen EN. J. Am. Chem. Soc. 2001; 123:9974–9983. [PubMed: 11592876]
18. Horigome M, Motoyoshi H, Watanabe H, Kitahara T. Tetrahedron Lett. 2001; 42:8207–8210.
19. Motoyoshi H, Horigome M, Watanabe H, Kitahara T. Tetrahedron. 2006; 62:1378–1389.
20. a) Albert BJ, Koide K. Org Lett. 2004; 6:3655–3658. [PubMed: 15469316] b) Koide K, Albert BJ. J. Synth. Org. Chem., Jpn. 2007; 65:119–126.
21. Albert BJ, Sivaramakrishnan A, Naka T, Koide K. J. Am. Chem. Soc. 2006; 128:2792–2793. [PubMed: 16506745]
22. Albert BJ, Sivaramakrishnan A, Naka T, Czaicki NL, Koide K. J. Am. Chem. Soc. 2007; 129:2648–2659. [PubMed: 17279752]
23. Motoyoshi H, Horigome M, Ishigami K, Yoshida T, Horinouchi S, Yoshida M, Watanabe H, Kitahara T. Biosci. Biotech. Bioch. 2004; 68:2178–2182.
24. Furukawa H, Koide K, Takao K, Kobayashi S. Chem. Pharm. Bull. 1998; 46:1244–1247.
25. A part of these results was also included in the patent; See Koide K, Albert BJ, Sivaramakrishnan A. US 2008096879 A1 20080424 and WO 2009031999 A1 20090312. .
26. Michrowska A, Bujok R, Harutyunyan S, Sashuk V, Dolgonos G, Grela K. J. Am. Chem. Soc. 2004; 126:9318–9325. [PubMed: 15281822]
27. a) Albert BJ, Koide K. ChemBioChem. 2007; 8:1912–1915. [PubMed: 17907119] b) Weerapana E, Simon GM, Cravatt BF. Nat. Chem. Biol. 2008; 4:405–407. [PubMed: 18488014]
28. Bernet B, Piantini U, Vasella A. Carbohydr. Res. 1990; 204:11–25.
29. a) Wender PA, De Brabander J, Harran PG, Jimenez JM, Koehler MFT, Lippa B, Park CM, Shiozaki M. J. Am. Chem. Soc. 1998; 120:4534–4535. b) Wender PA, Baryza JL. Org. Lett. 2005; 7:1177–1180. [PubMed: 15760168] c) Wender PA, Horan JC. Org. Lett. 2006; 8:4581–4584. [PubMed: 16986955] d) Smith AB, Razler TM, Meis RM, Pettit GR. Org. Lett. 2006; 8:797–799. [PubMed: 16468770]
30. Smith AB, Liu ZQ, Hogan AML, Dalisay DS, Molinski TF. Org. Lett. 2009; 11:3766–3769. [PubMed: 19637861]
31. Garner P, Park JM. J. Org. Chem. 1987; 52:2361–2364.
32. Saito K, Saijo S, Kotera K, Date T. Chem. Pharm. Bull. 1985; 33:1342. Nakamura Y, Hirai M, Tamotsu K, Yonezawa Y, Shin CG. Bull. Chem. Soc. Jpn. 1995; 68:1369–1376.
33. Jackson MM, Leverett C, Toczko JF, Roberts JC. J. Org. Chem. 2002; 67:5032–5035. [PubMed: 12098333]
34. Matsumura K, Hashiguchi S, Ikariya T, Noyori R. J. Am. Chem. Soc. 1997; 119:8738–8739.
35. a) Huang TL, Székács A, Uematsu T, Kuwano E, Parkinson A, Hammock BD. Pharm. Res. 1993; 10:639–648. [PubMed: 8321828] b) Ghosh AK, Kincaid JF, Cho WH, Walters DE, Krishnan K, Hussain KA, Koo Y, Cho H, Rudall C, Holland L, Buthod J. Bioorg. Med. Chem. Lett. 1998; 8:687–690. [PubMed: 9871583]
36. Albert BJ, McPherson PA, O'Brien K, Czaicki NL, DeStefino V, Osman S, Li MS, Day BW, Grabowski PJ, Moore MJ, Vogt A, Koide K. Mol. Cancer Ther. 2009; 8:2308–2318. [PubMed: 19671752]
37. With this solubility at 0.1 mgmL⁻¹ in saline, it is possible to reach the maximum tolerated dosage of FR901464 (1 mgkg⁻¹).
38. a) Groll M, Schellenberg B, Bachmann AS, Archer CR, Huber R, Powell TK, Lindow S, Kaiser M, Dudler R. Nature. 2008; 452:755. [PubMed: 18401409] b) Clerc J, Groll M, Illich DJ, Bachmann AS, Huber R, Schellenberg B, Dudler R, Kaiser M. Proc. Natl. Acad. Sci. U. S. A. 2009; 106:6507–6512.
39. Imamura Y, Ohtsu Y, Tanaka H, Hatakeyama M, Manabe T, Kawaguchi H, Handa H, Takahashi T. Heterocycles. 2004; 64:51–56.
40. Charette AB, Marcoux JF. Synlett. 1995:1197–1207.

41. <http://www.dtp.nci.nih.gov/branches/btb/ivclsp.html>

\$watermark-text

\$watermark-text

\$watermark-text

Figure 1.
Structures of FR901464 and Previously Prepared Analogues.

Figure 2.
Coupling constants for A-ring analogues.

Figure 3.
Rotamers of **28**.

7.8 Hz for FR901464

FR901464: R¹ = CH₃, R² = OAc

4'-*epi*-FR901464: R¹ = OAc, R² = CH₃

Figure 4.
FR901464 and Its 4'-*epi*-Analogue.

Figure 5.

Degradation of meayamycin (triangles) and meayamycin B (squares) in mouse serum at 37 °C. y-axis: The relative quantity of meayamycin (or meayamycin B) compared to the initial meayamycin (or meayamycin B) quantity. The initial meayamycin concentration was 10 μM . Meayamycin: $y = e^{-0.310x}$ ($R^2 = 0.997$). Meayamycin B: $y = e^{-0.053x}$ ($R^2 = 0.954$). MAM: meayamycin or meayamycin B.

Figure 6.
Summary of this work.

Scheme 1.

Preparation of C3 analogues **5**, **10**, and **13**. ^a *Conditions*: (a) LiAlH₄ (5.0 equiv), Et₂O, reflux, 80%. (b) PMPCH(OMe)₂ (2.9 equiv), CSA (0.10 equiv), CH₂Cl₂, 23 °C, 84%. (c) **3** (1.3 equiv), **4** (1.0 equiv), **Ru-1** (0.10 equiv), benzoquinone (0.22 equiv), DCE, 42 °C, 41%. (d) AcOH/THF/H₂O (3:1:1), 23 °C, 66%. (e) PMBCl (2.9 equiv), Ag₂O (1.5 equiv), DMF, 60 °C, 74%. (f) KOH (5.0 equiv), DMSO/H₂O (1.5:1), 55 °C, 77%. (g) NaIO₄ (1.2 equiv), H₂O/THF (2:1), 23 °C; LiSBu₃BH (3.1 equiv), THF, -78 °C, 69% (2 steps). (h) DDQ (3.0 equiv), CH₂Cl₂/sat. aq. NaHCO₃ (9:1), 23 °C; CSA (0.05 equiv), MeOH, 23 °C; 5% KOH/H₂O, 23 °C, 79% (3 steps). (i) PMPCH(OMe)₂ (2.9 equiv), CSA (0.10 equiv), CH₂Cl₂, 23 °C, quant. (j) **4** (1.0 equiv), **9** (1.3 equiv), **Ru-1** (0.10 equiv), benzoquinone (0.22 equiv), DCE, 40 °C, 48%. (k) AcOH/THF/H₂O (3:1:1), 23 °C, 68%. (l) MeI (2.0 equiv), NaH (2.0 equiv), THF, 23 °C, 94%. (m) DDQ (2.0 equiv), CH₂Cl₂/sat. aq. NaHCO₃ (9:1), 23 °C, 89%. (n) 3,4-dihydro-2*H*-pyran (1.5 equiv), CSA (0.05 equiv), CH₂Cl₂, 23 °C, quant. (o) **4** (1.2 equiv), **Ru-1** (0.10 equiv), benzoquinone (0.20 equiv), DCE, 45 °C, 40%. (p) AcOH/THF/H₂O (3:1:1), 23 °C, 63%. CSA: camphorsulfonic acid. PMB: para-methoxybenzyl. DDQ: 2,3-dichloro-5,6-dicyano-1,4-benzoquinone. PMP: para-methoxyphenyl. DCE: 1,2-dichloroethane.

Scheme 2.
Chair Conformers.

Scheme 3.

Preparation of C12 analogues. ^a Conditions: (a) DIBALH (1.8 equiv), CH₂Cl₂, -78 °C; Ph₃P=CHCO₂Me (1.5 equiv), 45 °C, 87%. (b) H₂, Pd/C (1 mol %), EtOAc, 23 °C, quant. (c) AcOH, 80 °C, 73%. (d) CH₂=CHCH₂MgCl (1.9 equiv), THF, -78 °C. (e) Et₃SiH (10 equiv), BF₃•OEt₂ (4.0 equiv), CF₃CH₂OH (8.0 equiv), -78 °C, 43% (2 steps). (f) CH₂Cl₂/TFA (9:1), 23 °C; **19** (1.2 equiv), HATU (1.2 equiv), *i*Pr₂NEt (4.0 equiv), 23 °C, 84%. (g) methacrolein (10 equiv), **Ru-1** (2 mol %), 23 °C, 60%. (h) Ph₃PCH₃Br (2.2 equiv), KO^tBu (2.2 equiv), THF, 0 °C, 86%. (i) **6** (1.6 equiv), **Ru-1** (0.10 equiv), benzoquinone (0.21 equiv), DCE, 35 °C, 50%. (j) NaBH₄ (2.0 equiv), EtOH, 23 °C, 87%. (k) 1,1-diethoxy-3-butene (1.0 equiv), CSA (1 mol %), CH₂Cl₂, 0 °C, 67%. (l) CH₂Cl₂/TFA (9:1), 23 °C; **19** (1.2 equiv), HATU (1.2 equiv), *i*Pr₂NEt (4.0 equiv), 23 °C, 51%. (m) methacrolein (10 equiv), **Ru-1** (2 mol %), 23 °C, 62%. (n) Ph₃PCH₃Br (2.2 equiv), KO^tBu (2.2 equiv), THF, 0 °C, 87%. (o) **6** (1.6 equiv), **Ru-1** (0.10 equiv), benzoquinone (0.22 equiv), DCE, 35 °C, 53%. DIBALH: diisobutylaluminum hydride. TFA: trifluoroacetic acid. HATU: O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate.

Scheme 4.

Preparation of 4'-analogues. ^a Conditions: (a) K₂CO₃ (5.0 equiv), MeOH, 0 °C, 82%. (b) **6** (1.6 equiv), **Ru-1** (0.10 equiv), benzoquinone (0.17 equiv), DCE, 32 °C, 81%. (c) *n*BuLi (2.8 equiv), HC≡CCH₂OTHP (3.0 equiv), THF, -78 to 0 °C, 90%. (d) **Ru-2** (2 mol %), HCO₂Na (10 equiv), CTAB (0.2 equiv), H₂O, 23 °C, 78%. (e) Ac₂O (5.0 equiv), pyridine, 23 °C, 93%. (f) PPTS (0.2 equiv), MeOH, 23 °C, 84%; Na₂Cr₂O₇ (1.5 equiv), acetone, H₂SO₄, 0 to 23 °C, 86%. (g) H₂, Lindlar's catalyst (1 mol %), quinoline (0.10 equiv) EtOH, 23 °C, 68%. (h) **34** (1 equiv), CH₂Cl₂/TFA (9:1), 23 °C; then 4'-*epi*-**19** (1.2 equiv), HATU (1.2 equiv), *i*Pr₂NEt (4.0 equiv), MeCN, 23 °C, 71%. (i) methacrolein (10 equiv), **Ru-1** (2 mol %), 23 °C, 62%. (j) Ph₃PCH₃Br (2.2 equiv), KO^{*t*}Bu (2.2 equiv), THF, 0 °C, 84%. (k) K₂CO₃ (2.0 equiv), MeOH, 0 °C, 86%. (l) **6** (1.5 equiv), **Ru-1** (0.10 equiv), DCE, 35 °C, 34%. (m) CDI (1.5 equiv), CH₂Cl₂, 23 °C; morpholine (4.1 equiv), 23 °C, 80%. (n) **6** (1.6 equiv), **Ru-1** (0.07 equiv), DCE, 42 °C, 44%. CDI: 1,1'-carbonyldiimidazole.

Scheme 5.

Preparation of C2'-C3' analogues **41** and **48**. ^a *Conditions:* (a) CH₂Cl₂/TFA (9:1), 23 °C; **19** (1.7 equiv), HATU (1.3 equiv), *i*Pr₂NEt (5.0 equiv), MeCN, 23 °C, 43%. (b) K₂CO₃ (2.5 equiv), MeOH, 0 °C to 23 °C, 75%. (c) CDI (1.2 equiv), CH₂Cl₂, 23 °C; morpholine (2–5 equiv), 23 °C, 97% (**38**) or 94% (**43**). (d) methacrolein (13–20 equiv), **Ru-1** (2 mol%), CH₂Cl₂ (**39**) or PhH (**46**), 23 °C or 50 °C, 74% (**39**) or 63% (**46**). (e) Ph₃PCH₃Br (2.4 equiv), KO^tBu (2.3 equiv), THF, 0 °C, 50% (**40**) or 76% (**47**). (f) **6** (1.3 equiv), **Ru-1** (0.07–0.2 equiv), benzoquinone (0.2–0.5 equiv), DCE, 42–50 °C, 46% (**41**) or 36% (**48**). (g) DIBALH (1.1 equiv), CH₂Cl₂, –78 °C; Ph₃P=CHCO₂Me (1.5 equiv), 23 °C. (h) H₂, Pd/C (2 mol%), EtOAc, 23 °C. (i) NaOH (2.1 equiv), MeOH/H₂O (1:1.1), 23 °C, 46% (over 3 steps). (j) **34**, CH₂Cl₂/TFA (9:1), 23 °C; **44** (1.3 equiv), HATU (1.2 equiv), *i*Pr₂NEt (3.4 equiv), 23 °C, 66%.

Scheme 6.

Preparation of C4-OMe analogue **50**. ^a *Conditions:* (a) MeI (5.0 equiv), Ag₂O (2.0 equiv), DMF, 23 °C, 87%. (b) **36** (1.0 equiv), **49** (1.5 equiv), **Ru-1** (7 mol%), benzoquinone (0.2 equiv), DCE, 42 °C, 57%.

Scheme 7.

Preparation of cyclopropyl analogues. ^a *Conditions:* (a) methacrolein (20 equiv), **Ru-1** (3 mol%), 23 °C, 84%. (b) DIBALH (2.0 equiv), CH₂Cl₂, -78 °C, 98%. (c) (*S,S*)-dioxaborolane (2.0 equiv), ZnEt₂ (5.0 equiv), CH₂I₂ (7.0 equiv), CH₂Cl₂, 23 °C, 70%, d.r. = 89:11. (d) CH₂Cl₂/TFA (8:2), 0 to 23 °C, quant. (e) **19** (1.2 equiv), HATU (1.0 equiv), *i*-Pr₂NEt (4.0 equiv), MeCN, 23 °C, 48%. (f) Dess-Martin periodinane (2.0 equiv), CH₂Cl₂, 0 °C, 70%. (g) Ph₃PCH₃Br (6.0 equiv), KO^{*t*}Bu (5.0 equiv), THF, 0 °C, 80%. (h) 1. K₂CO₃ (2.5 equiv), THF, 0 °C. (i) CDI (2.5 equiv), CH₂Cl₂, 23 °C; morpholine (5.0 equiv), 23 °C, 94% (over 2 steps). (j) **49** (1.7 equiv), **Ru-1** (0.1 equiv), benzoquinone (0.2 equiv), toluene, 70 °C, 30%.

Table 1Antiproliferative Activities against MCF-7 Cells^a

FR901464 analogue	GI ₅₀ (nM)
meayamycin	0.02
5	>10,000
10	>10,000
13	>10,000
22	0.30
28	2.5
29	85
30	19
meayamycin B	0.008
4'-desacetyl meayamycin	0.48
4'- <i>epi</i> -desacetyl meayamycin	4.7
41	5
48	19
50	0.40
59	15
60	10

^aEach experiment consists of at least twelve data points (twelve different concentrations).

Table 2GI₅₀ Values of Meayamycin and Meayamycin B in pM^a

Cell line	Meayamycin	Meayamycin B
MCF-7	20 ± 8.9	8 ± 2
MDA-MB-231	71 ± 55	15 ± 1.4
A549	258 ± 162	175 ± 77
DU145	1230 ± 697	211 ± 23
HCT116	157 ± 33	4 ± 2
H1299	841 ± 271	146 ± 30
PC3	196 ± 42	101 ± 4.0

^aEach experiment consists of at least twelve data points (twelve different concentrations).