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Propionate Analogues of Zearalenone Bind to Hsp90

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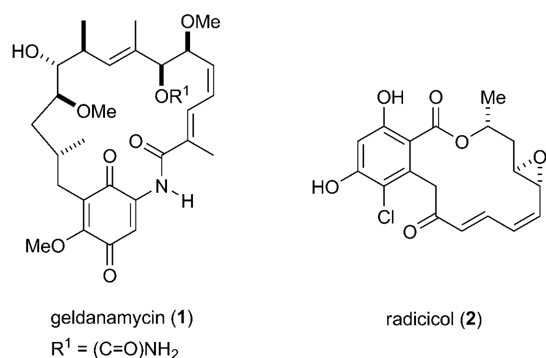
By replacement of an acetate with propionate through organic synthesis a range of zearalenone analogues were prepared. As key steps in the synthesis of the analogues we used the Noyori hydrogenation of methyl acetoacetate followed by Frater alkylation of the enantiomeric 3-hydroxybutyrates. This converted the second acetate to a propionate. Through the derived alkyne, chain extension led to 3-methylundec-10-en-2-ol derivatives. These were condensed with 2,4-dimethoxy-6-vinylbenzoic acid. Ring-closing metathesis of the obtained esters led to macrolactones, which were deproteced to give the zearalenone analogues. Several of the analogues showed cytotoxicity against the L929 mouse fibroblast cell line comparable to zearalenone (9 μM) itself. In the thermal-shift assay, two analogues **35** and *ent*-**35** displayed stronger binding than the natural product geldanamycin to the chaperone Hsp90.

Introduction

Heat shock proteins of the 90 kDa Hsp90 family are promising anticancer targets.[1] As molecular chaperones, these heat shock proteins are responsible for refolding denatured proteins and for the correct folding of newly synthesized nascent polypeptides. The two Hsp90 isoforms, the inducible major form and the constitutive minor form, are found predominantly in the cytosol. The folding or refolding process requires the presence of cochaperones, immunophilins and other partner proteins to produce a functional multiprotein complex. Furthermore, ATP is required during the folding process. The protein complex binds ATP in the N-terminal domain of Hsp90. Disruption of the ATP binding interferes with the function of Hsp90 and causes proteosomal degradation of client proteins. Several natural products bind to the ATP pocket of Hsp90. These compounds include the macrolactam geldanamycin (**1**) and the benzolactone radicicol (**2**; Scheme 1). X-ray structures of these natural products bound to yeast Hsp90 are known.[2–4]



Scheme 1. Structures of natural products that bind to Hsp90.

As is not uncommon for ATP-binding sites, substituted nitrogen heterocycles were also found to bind to Hsp90. An example is the isoxazole **3** published by the company Vernalis, Ltd. (Scheme 2).[5] In addition, Hsp90 function can be disrupted with the antibiotic novobiocin, which binds to the C terminus of the chaperone. While radicicol (**2**) is a high affinity ligand for Hsp90, its in vivo activity is impaired due to chemical reactivity associated with the dienone moiety and the allylic epoxide. Accordingly, studies aimed at the total synthesis[6] of radicicol (**2**) were followed by the synthesis of analogues, for example, cycloproparadicicol (**4**).[7] In addition, a modular synthesis approach was used by Winssinger and colleagues to prepare radicicol-like 14-membered benzolactones.[8] Here, basically the L shape of radicicol was used as a guiding principle. Considering the observation that the aryl groups of radicicol and geldanamycin are located at different positions in the binding site,[9] Blagg et al. designed chimeras containing aryl groups of both natural products. Indeed, radamide (**5**) compared well with geldanamycin (IC_{50} = 5.9 μM vs. 2.5 μM for **1**).[10] A related hybrid connecting the aryl rings via an ester bond was termed radester (**6**).[11] In cell proliferation studies (MCF-7 cells) IC_{50} values in the low μM range were obtained. The group of Moody described a series of benzolactones of varying ring sizes.[12] In enzyme assays, 13–16-membered macrolactones, such as **7**,

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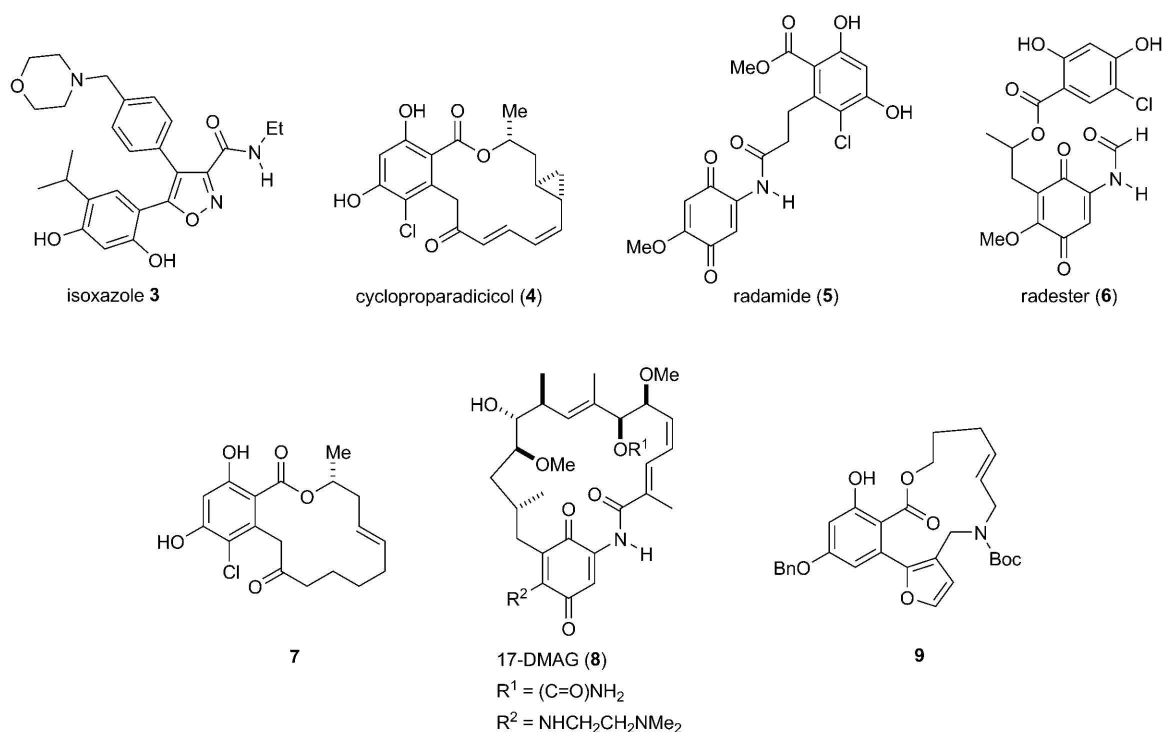


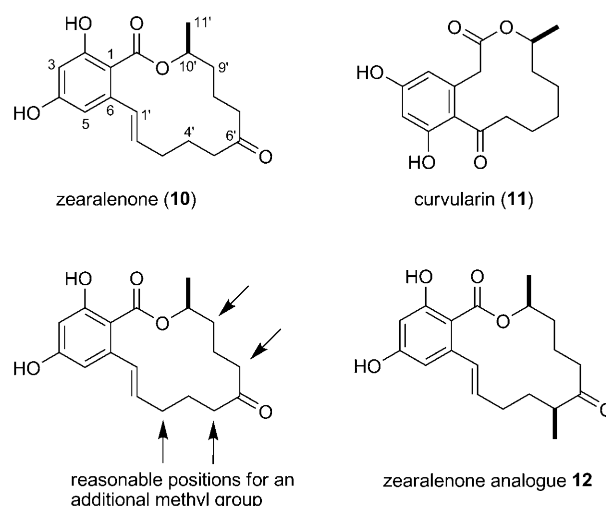
Scheme 2. Structures of various known ligands that bind to Hsp90.

turned out to be good inhibitors. With a view toward improving the solubility of geldanamycin a group at Kosan Biosciences synthesized 17-desmethoxy-17-*N*,*N*-dimethylamino-ethylaminogeldanamycin (**8**, 17-DMAG). This compound turned out to be more potent, even in vivo, than geldanamycin and is currently in clinical trials. Based on radicicol further macrocyclic compounds like **9** were designed and synthesized by McDonald et al.[13] Some of them turned out to be moderate inhibitors of the ATPase activity of Hsp90. Quite recently, the natural product derrubone, an isoflavone, was identified as a Hsp90 inhibitor.[14] However, the exact binding site for this compound is not yet known.

Results and Discussion

Chemistry

Our strategy for finding novel Hsp90 inhibitors was inspired by biosynthetic considerations. In recognizing that benzolactones like zearalenone (**10**) or curvularin (**11**) are only made from acetate building blocks[15,16] we planned to use these naked lactones as scaffolds and to decorate them with typical substituents found in polyketides, like methyl or hydroxyl functions (Scheme 3).[17] For example, we conceived the concept of propionate scanning,[18] which refers to the systematic replacement of an acetate building block by propionate through organic synthesis. An additional methyl group might be able to take advantage of hydrophobic pockets or restrict the conformation of the macrocyclic ring in a positive way. Of course, a methyl group at a certain position might block binding to a receptor or impose selectivity on a promiscuous ligand. The beneficial effect of a methyl group is dramatically illustrated with the epothilone A and B pair of macrolactones.[19] Thus, the additional methyl group of epothilone B makes this compound roughly ten-times more active than epothilone A. Moreover, all epothilone derivatives that are undergoing clinical trials are analogues of epothilone B. Indeed, the monopropionate analogue **12** of zearalenone turned out to be a quite potent (IC_{50} = 210 nM) inhibitor of human carbonyl reductase 1 (CBR1). While the parent compound showed some binding to Hsp90,



Scheme 3. Structures of some acetate-based macrolactones and the concept of propionate scanning.

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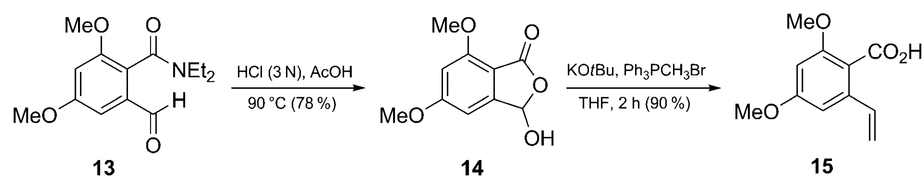
the analogue **12** did not induce any shift in the thermal shift assay.[20–23] In this paper we describe the preparation of zearalenone analogues with a propionate at the second position, that is an additional methyl group at C9′. In the Hsp90 assay two enantiomeric zearalenone analogues surprisingly induced temperature-shift values higher than those of geldanamycin.

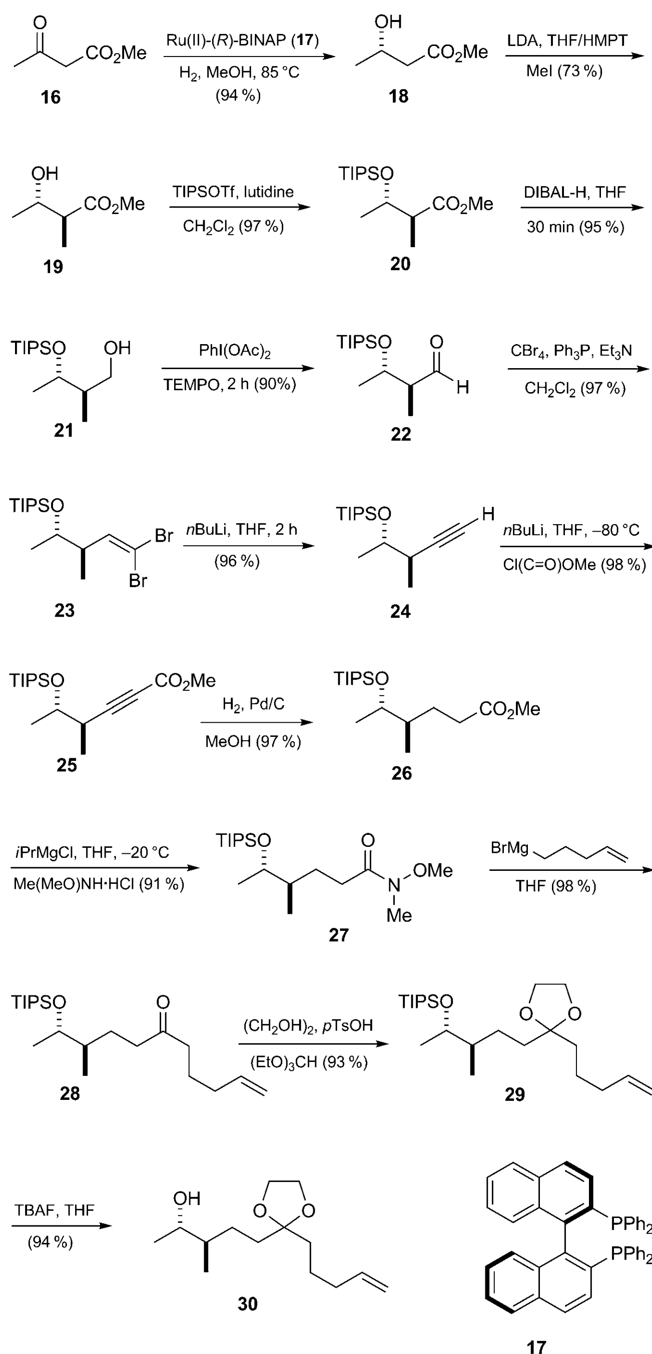


Scheme 4. Synthesis of 6-vinylbenzoic acid (**15**).

For the formation of the macrolactone ring we planned to use a ring-closing methathesis (RCM) strategy related to the work of Fürstner et al.[24,25] Accordingly, the synthetic route was conceived with this in mind. The synthesis of the aromatic fragment, 6-vinylbenzoic acid **15** started with the known *N*,*N*-diethyl-2-formyl-benzamide[26] **13** (Scheme 4). Hydrolysis of the amide function (3 N HCl, 90 °C) provided hemiacetal **14**. It should be noted that refluxing the mixture gave inferior yields. Phthalide **14** was subsequently transformed to styrene **15** by a Wittig reaction.

For the synthesis of the aliphatic fragment **30** we began with the Noyori hydrogenation[27] of acetoacetate **16** using the (*R*)-(+)-BINAP ligand **17** (Scheme 5). The obtained hydroxyester **18** was subjected to Frater alkylation[28] (2.2 equiv LDA, THA/HMPT, MeI); this resulted in the *anti* configured formal aldol product **19**.[29] Routine steps, that is, TIPS protection of the hydroxyl group, DIBAL-H reduction of ester **20** and oxidation of alcohol **21**, led to aldehyde **22**. Chain extension of **22** could be achieved through a Corey–Fuchs–Bestmann olefination[30] and conversion of 1,1-dibromide **23** to pentyne derivative **24**. Deprotonation of alkyne **24** followed by treatment of the intermediate acetylide with methyl chlorocarbonate provided methyl 2-hexynoate **25** in good overall yield. After catalytic hydrogenation of the triple bond, the C6′–C11′ fragment **26** of zearalenone was obtained.[31] The intended RCM strategy required the extension of the carboxyl function of **26** with a pentenyl residue. Thus, ester **26** was converted to the Weinreb amide[32] **27**, which upon treatment with pentenylmagnesium bromide delivered ketone **28**. Acetalization of the keto function and cleavage of the silyl ether produced the key aliphatic building block **30**.

Benzoic acid **15** could now be condensed with secondary alcohol **30** through a Mitsunobu esterification to provide ester **31** in excellent yield (Scheme 6). For the crucial ring-closing metathesis reaction of ester **31** the Grubbs′ 2nd generation catalyst was employed. Within 5 h at 80 °C in toluene the macrolactone **32** was obtained in high yield. Only the *E* isomer was formed. Analogues suitable for biological testing were obtained by first cleaving the acetal under acidic conditions (*p*TsOH, acetone) to provide ketolactone **33**. By using the Lewis acid BCl_3 at low temperature a selective deprotection of the methyl ether *ortho* to the carboxylic group could be achieved to give lactone **34**. Cleavage of both aryl ether functions turned out to be possible with aluminium iodide (AlI_3) in benzene; this led to zearalenone analogue **35**.[33]



Scheme 5. Synthesis of the aliphatic fragment **30** by Frater alkylation of hydroxybutyrate (**18**) and chain extension reactions.

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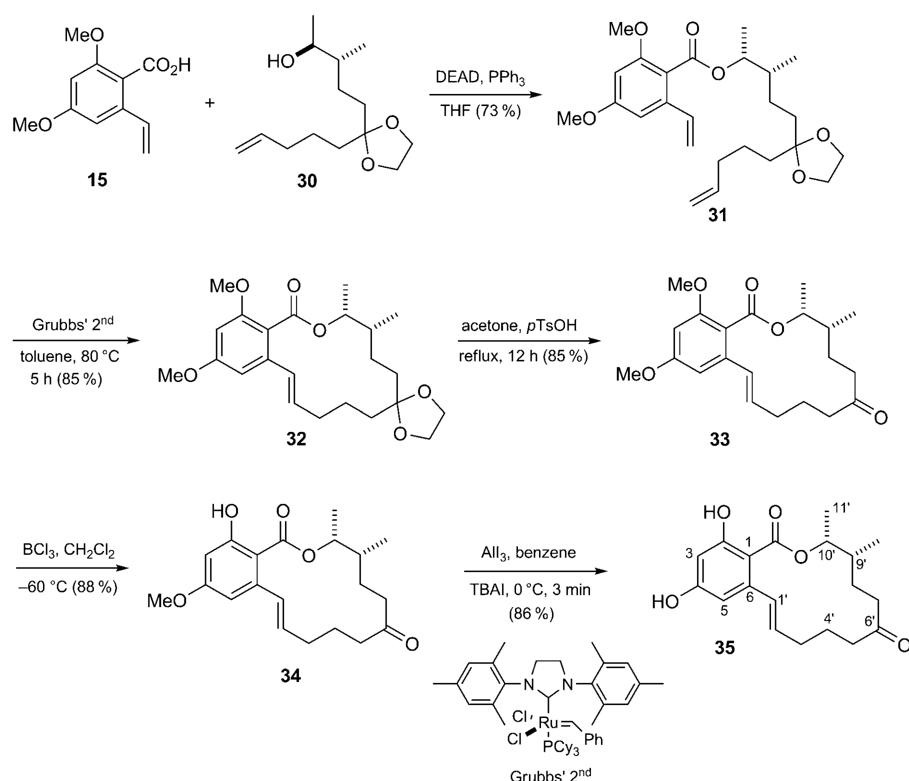

Scheme 6. Ring-closing metathesis of ester **31** to macrolactone **32** and preparation of additional zearalenone analogues **34** and **35** from lactone **33**.

Biology

Zearalenone (**10**) as well as all the analogues **33**–**35**, **40**–**42**, *ent*-**33**–*ent*-**35**, and *ent*-**40**–*ent*-**42** were tested for cytotoxicity against the L929 mouse fibroblast cell line. The obtained IC_{50} values are listed in Table 1. As can be seen zearalenone is cytotoxic in this cell line with an IC_{50} value in the low μM range (9.4 μM; entry 6). The monomethoxy analogues (**41**, **34**, *ent*-**41**, entries 11, 13, 14, respectively) are the least active. The exception is the surprisingly relatively high activity of the monomethoxy compound *ent*-**34** (entry 8); the dimethoxyaryl analogues (**33**, *ent*-**33**, **40**, *ent*-**40**) showed intermediate activity. Furthermore, the compounds in the *cis* series (**33**–**35** and their enantiomers) seem to be more active than the corresponding *trans* isomers. Besides zearalenone itself and the monomethoxy compound *ent*-**34** the two most cytotoxic compounds were the fully deprotected *cis* analogues *ent*-**35** (6.9 μM) and **35** (10.2 μM).

All analogues as well as zearalenone were then evaluated for binding to Hsp90 within the integrated structural and functional genomics platform of the Structural Genomics Consortium (SGC) directed against human medicinal target classes.

Table 1. Cytotoxicity of the tested macrolactones.

Entry[a]	Compound	IC_{50} [μM][b]	T_m shift[c] [°]
1	**2** (RAD)	0.58	5.5
2	**35**	10	3.9
3	*ent*-**35**	6.9	3.6
4	**1** (GEA)	0.0086	3.5
5	*ent*-**42**	15	3.2
6	**10** (ZEA)	9.4	2.9
7	**42**	14	2.5
8	*ent*-**34**	10	2.1
9	**33**	36	0.3
10	*ent*-**33**	44	0.3
11	**41**	46	0.3
12	*ent*-**40**	56	0.3
13	**34**	72	0.3
14	*ent*-**4**	> 120	0.3
15	**40**	33	0.2

[a] The compounds are arranged with decreasing T_m shift values; [b] against the L929 mouse fibroblast cell line; [c] for details see the Experimental Section.

In order to reach the diastereomeric series of analogues (C10′ inverted) a Mitsunobu inversion on the *anti*-configured alcohol **30** was performed (Scheme 7). The obtained *p*-nitrobenzoate **36** was saponified to yield *syn* isomer **37**. This alcohol was condensed with 6-vinylbenzoic acid **15** under Mitsunobu conditions to give ester **38**. Cyclization of **38** by using the Grubbs′ 2nd generation catalyst led to macrolactone **39**. As before, cleavage of the acetal yielded the corresponding ketolactone **40**. This compound served as precursor for the two additional partially and fully deprotected analogues **41** and **42**, respectively.

In this series, recrystallization of lactone **40** yielded crystals suitable for X-ray analysis. A rendering of this structure is shown in Figure 1. The macrolactone is characterized by a typical bend or L shaped conformation. The two methyl groups are in a gauche arrangement.

Access to the enantiomeric series of analogues was initiated by performing the Noyori hydrogenation of methyl 3-oxobutanoate in the presence of (*S*)-BINAP. A Frater alkylation of hydroxyester *ent*-**18** led to (2*R*,3*R*)-methyl 3-hydroxy-2-methylbutanoate (*ent*-**19**). As described in Scheme 5 this ester was converted to the two alcohols *ent*-**30** and *ent*-**37** (Scheme 8, for details see the Supporting Information). Condensation of these alcohols with the vinylbenzoic acid **15** followed by ring-closing metathesis produced the lactones *ent*-**31** and *ent*-**37**. Through acetal and methyl ether cleavage the zearalenone analogues *ent*-**33**–*ent*-**35** and *ent*-**40**–*ent*-**42**, respectively, were obtained.

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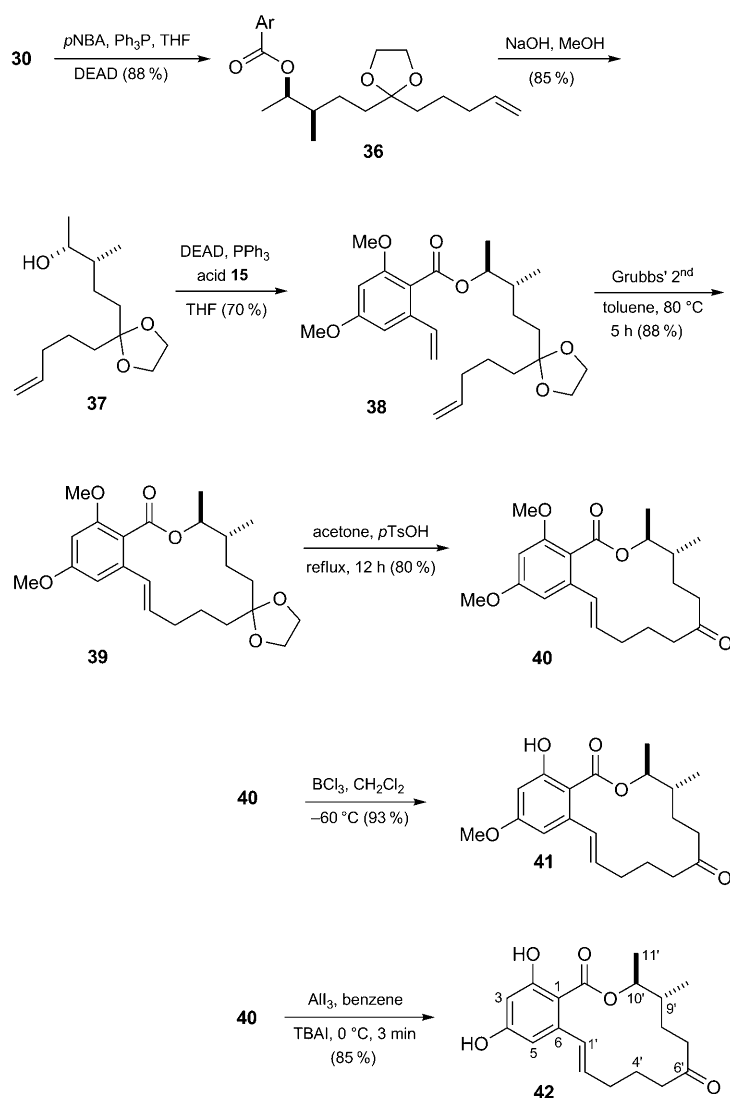

Scheme 7. Synthesis of the *trans* series of lactones **40**–**42** through the inverted alcohol **37**; Ar = pNO_2Ph.

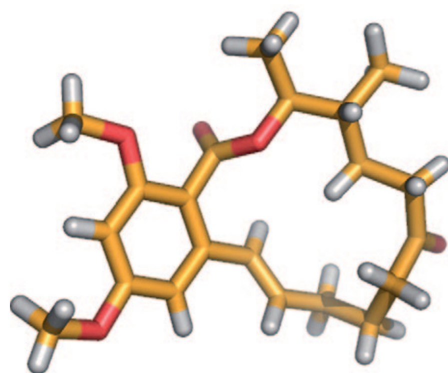

Figure 1. X-ray structure of macrolactone **40**.

Binding of compounds to Hsp90 was detected by using differential scanning fluorimetry (DSF)—a generic thermal-shift (ΔT_m) detection assay—as described.[20–23] For comparison purposes, geldanamycin (**1**, GEA) and radicicol (**2**, RAD) were also included in the assay. As Table 1 indicates several zearalenone analogues showed Hsp90 stabilization. It has been shown that for a series of related compounds T_m values correlate very well with inhibitor binding constants and inhibition of enzyme activity.[34] However, T_m values below 2 °C are usually not reliable and compounds were considered not to interact tightly with the target protein below this threshold (Table 1). As can be seen there is no direct correlation between the T_m shifts and the cytotoxicity data. GEA is clearly unique since it shows a moderate T_m shift, but displays strong growth inhibition (entry 4). Interestingly, the two *cis* compounds **35** and *ent*-**35** surpassed GEA in their T_m shifts. This is an important finding considering the significantly reduced chemical complexity of these two analogues when compared with GEA. Another potent Hsp90 ligand was compound *ent*-**42** (entry 5, 3.2°), which is also fully deprotected but features a *trans* orientation of the methyl groups.

Performing the thermal-shift assay at various concentrations allows for determination of the K_d values. This was measured with the two compounds **35** and *ent*-**35** by using the human Hsp90α N-terminal domain (Hsp90N). Preparation of Hsp90N has been previously described.[35] From these measurements K_d values of 0.25 μM (*ent*-**35**) and 0.33 μM (**35**) were obtained. The corresponding K_d value for radicicol in this assay was 0.001 μM (see the Supporting Information).

Conclusions

Taking the polyacetate-based natural product zearalenone (**10**, ZEA) as a lead compound, the concept of propionate scanning was used in the design of zearalenone analogues with an additional methyl group at C-9′. Thus, by total synthesis we replaced the second acetate by a propionate. Key steps in the syntheses of the analogues were a Noyori hydrogenation of methyl acetoacetate followed by a Frater alkylation of the 3-hydroxy butyrates. Chain extension led to alcohols **30** and *ent*-**31**. Mitsunobu esterification with benzoic acid **15** and ring-closing metathesis provided macrolactones, which could be converted to the *cis* analogues **33**–**35** and their enantiomers. The diasteromeric series of analogues (**40**–**42** and enantiomers) were obtained through a Mitsunobu inversion of alcohols **30** and *ent*-**31** prior to the esterification with the benzoic acid **15**.

Biological testing showed several of the analogues to be comparable in cytotoxicity with zearalenone (**10**). Thus, the five compounds *ent*-**35**, *ent*-**34**, **35**, **42**, and *ent*-**42** displayed IC_{50} values in the low μM range. Screening these compounds in a thermal-shift (ΔT_m) detection assay revealed that they do bind to Hsp90. Two compounds, **35** and *ent*-**35**, were better ligands than GEA based on T_m shift assays. While we were not able to obtain potencies as observed for radicicol we hope

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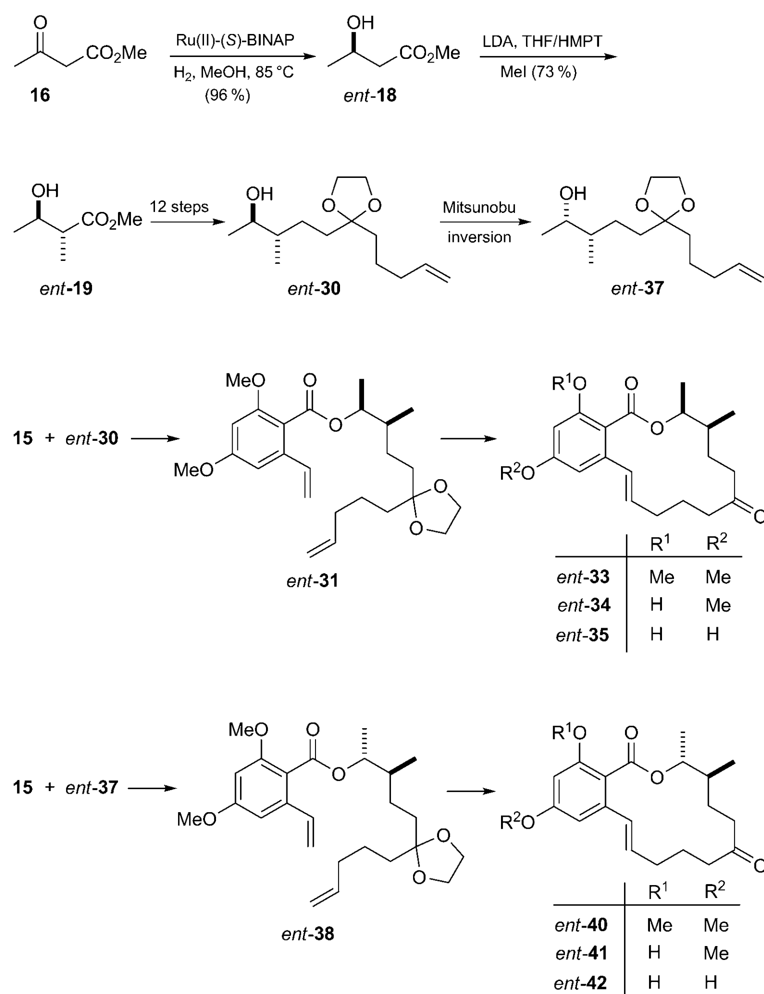

Scheme 8. Summary of the steps leading to the enantiomeric series of analogues *ent*-**33**–*ent*-**35** and *ent*-**40**–*ent*-**42**.

that structure-based design approaches will help to improve these versatile molecules in the future. Further studies will be necessary to measure the degradation of client proteins like Her-2.[36]

Experimental Section

3-Hydroxy-5,7-dimethoxyisobenzofuran-1(3*H*)-one (14): A solution of aldehyde[26] **13** (1.40 g, 5.28 mmol) in a mixture of HCl (1 N)/acetic acid (1:1, 50 mL) was stirred at 90 °C for 22 h. After being cooled, the solvents were removed in vacuo, the resulting solid was taken up in ethyl acetate (50 mL) and was washed with saturated $NaHCO_3$ solution (3×100 mL). Then the aqueous phase was acidified by adding concentrated HCl and the mixture was extracted with ethyl acetate (2×100 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the pure phthalide **14** (862 mg, 78%) as a slightly brown solid; R_f = 0.17 (petroleum ether/EE, 1:1); ^{1}H NMR (400 MHz, acetone): δ = 3.75 (s, 1H; OH), 3.91 (s, 3H; OCH_3), 3.93 (s, 3H; OCH_3), 6.43 (s, 1H; *CH*OH), 6.64 (d, *J* = 1.8 Hz, 1H; 6-H), 6.72 (d, *J* = 1.5 Hz, 1H; 4-H), 10.78 (br s, small); ^{13}C NMR (100 MHz, acetone): δ = 56.9 (OCH_3), 57.1 (OCH_3), 97.1 (CHOH), 97.2 (C-4), 101.0 (C-6), 101.4 (C-1a), 153.7 (C-3a), 160.7 (C-7), 166.7 (C-5), 168.4 (CO_2).

2,4-Dimethoxy-6-vinylbenzoic acid (15): KO*t*Bu (428 mg, 3.81 mmol) was added to a solution of Ph_3PMeBr (1.36 g, 3.81 mmol) in dry THF (20 mL) at 0 °C and the mixture was stirred for 0.5 h at the same temperature. Then phthalide **14** (100 mg, 0.475 mmol) was added to this solution and the mixture was stirred for 1.5 h at room temperature. Subsequently water was added, the mixture was extracted with ethyl acetate (3× 40 mL) and then the combined organic layers were washed with a NaOH solution (1 N; 2×50 mL). The aqueous phase was acidified with concentrated HCl and then extracted with ethyl acetate (3×50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the olefin **15** (89 mg, 90%) as a slightly yellow solid; R_f = 0.47 (petroleum ether/ethyl acetate, 1:1). ^{1}H NMR (400 MHz, acetone): δ = 3.82 (s, 3H; OCH_3), 3.85 (s, 3H; OCH_3), 5.31 (d, *J* = 10.9 Hz, 1H; CH=CH_2), 5.82 (d, *J* = 17.4 Hz, 1H; CH=CH_2), 6.35 (d, *J* = 2.0 Hz, 1H; 3-H), 6.78 (d, *J* = 2.0 Hz, 1H; 5-H), 6.85 (dd, *J* = 17.4, 11.1 Hz, 1H; *CH*=CH_2), 11.22 (br s, 1H; CO_2H); ^{13}C NMR (100 MHz, acetone): δ = 56.3 (OCH_3), 56.8 (OCH_3), 99.5 (C-3), 102.6 (C-5), 117.5 (C-1), 118.3 (CH=CH_2), 135.4 (*CH*=CH_2), 138.4 (C-6), 159.4 (C-4), 162.9 (C-2), 169.0 (CO_2); HRMS (ESI): $[M+Na]^+$ calcd for $C_{11}H_{12}O_4$ 207.06628, found 207.06627.

(2*S*,3*S*)-Methyl 3-hydroxy-2-methylbutanoate (19): A solution of LDA was prepared by adding *n*BuLi (2.77 mL, 6.93 mmol) at −30 °C to a solution of *i*Pr_2NH (701 mg, 0.98 mL) in dry THF (9 mL). After being stirred for 30 min the solution was cooled to −60 °C and the hydroxyester **18** (409 mg, 3.46 mmol) was added dropwise to the LDA solution and the mixture was stirred for 45 min at −60 °C. Then a solution of MeI (491 mg, 0.215 mL, 3.46 mmol) in HMPT (1 mL) was added dropwise to the cooled solution. After complete addition, the solution was allowed to reach room temperature. Then saturated NH_4Cl solution was added and the mixture was extracted with Et_2O (3×20 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification of the residue by flash chromatography (petroleum ether/ethyl acetate, 2:1) gave hydroxy ester **19** (334 mg, 73%) as a colorless oil; R_f = 0.47 (petroleum ether/ethyl acetate, 1:1); $[\alpha]_D^{20}$ = +26.9 (*c* = 1.0, CH_2Cl_2); in ref. [29a] *ent*-**19**: $[\alpha]_D^{20}$ = −32.9 (*c* = 1.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.15 (d, *J* = 7.1 Hz, 3H; 2-CH_3), 1.18 (d, *J* = 6.4 Hz, 3H; 4-H), 2.37–2.50 (m, 2H; 2-H), 2.71 (d, *J* = 4.3 Hz, 1H; OH), 3.68 (s, 3H; OCH_3), 3.82–3.90 (m, 1H; 3-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.0 (2-CH_3), 20.7 (C-4), 46.9 (C-2), 51.7 (OCH_3), 69.4 (C-3), 176.3 (C-1).

(2*S*,3*S*)-Methyl 2-methyl-3-(triisopropylsilyloxy)butanoate (20): 2,6-Lutidine (2.65 mL, 22.7 mmol) was added to a solution of hydroxyester **19** (2.0 g, 15.13 mmol) in CH_2Cl_2 (40 mL) at 0 °C followed by the addition of TIPSOTf (4.88 mL, 18.16 mmol). The mixture was stirred for 8 h at room temperature. Then saturated NH_4Cl solution was added and the mixture was extracted with CH_2Cl_2 (3×60 mL). The combined CH_2Cl_2 layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification of the residue by flash chromatography (petroleum ether/ethyl acetate, 35:1) provided 4.23 g (97%) of the ester **20**; R_f = 0.24 (petroleum ether/ethyl acetate, 35:1); $[\alpha]_D^{20}$ = +28.5 (*c* = 1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.04 (s, 21H; $((CH_3)_2CH)_3Si$), 1.10 (d, *J* = 7.1 Hz, 3H; 2-

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CH_3), 1.12 (d, $J=6.1$ Hz, 3H; 4-H), 2.57–2.64 (m, 1H; 2-H), 3.65 (s, 3H; OCH_3), 4.22–4.28 (m, 1H; 3-H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=$ 11.2 (CHSi), 12.5 (2-CH_3), 18.0, 18.1 ($((CH_3)_2CH)_3Si$), 19.9 (C-4), 47.7 (C-2), 51.4 (OCH_3), 69.6 (C-3), 175.3 (C-1); HRMS (ESI): $[M+Na]^+$ calcd for $C_{15}H_{32}O_3Si$ 311.20125, found 311.20135.

(2*R*,3*S*)-2-Methyl-3-(triisopropylsilyloxy)butan-1-ol (21): DIBAL-H (16.5 mL, 16.5 mmol, 1 M in hexane) was added in a dropwise fashion to a solution of ester **20** (2.16 g, 7.48 mmol) in dry CH_2Cl_2 (60 mL) that had been cooled to −80 °C. After complete addition, the mixture was stirred for 30 min at −80 °C and then treated with saturated NH_4Cl solution. The mixture was extracted with CH_2Cl_2 (3×40 mL), dried over $MgSO_4$, filtered, and concentrated, in vacuo. The residue was purified by flash chromatography (petroleum ether/ethyl acetate, 9:1) to give 1.85 g (95%) of the pure alcohol **21** as a colorless oil; $R_f=0.33$ (petroleum ether/ethyl acetate, 9:1); $[\alpha]_D^{20}=+9.0$ ($c=1.0$, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): $\delta=0.98$ (d, $J=7.1$ Hz, 1H; 2-CH_3), 1.07 (s, 21H; $((CH_3)_2CH)_3Si$), 1.22 (d, $J=$ 6.4 Hz, 3H; 4-H), 1.62–1.70 (m, 1H; 2-H), 2.68 (br, 1H; OH), 3.53–3.58 (m, 1H; 1-H), 3.72–3.74 (m, 1H; 1-H), 3.97–4.03 (m, 1H; 3-H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=12.7$ (CHSi), 13.9 (2-CH_3), 18.1, 18.2 ($((CH_3)_2CH)_3Si$), 21.6 (C-4), 42.2 (C-2), 66.0 (C-1), 73.4 (C-3); HRMS (ESI): $[M+Na]^+$ calcd for $C_{14}H_{32}O_2Si$ 283.20638, found 283.20643.

(2*S*,3*S*)-2-Methyl-3-(triisopropylsilyloxy)butanal (22): $PhI(OAc)_2$ (4.27 g, 13.28 mmol) and TEMPO (260 mg, 1.66 mmol) were added to a solution of alcohol **21** (2.16 g, 8.29 mmol) in dry CH_2Cl_2 (40 mL) and the mixture was stirred for 2 h at room temperature. Then a solution of $Na_2S_2O_3$ (10%; 10 mL) was added, the mixture was stirred for 10 min before it was extracted with CH_2Cl_2 (3× 40 mL). The combined CH_2Cl_2 layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (petroleum ether/ethyl acetate, 50:1) to give 1.93 g (90%) of pure aldehyde **22**; $R_f=0.25$ (petroleum ether/ethyl acetate, 50:1); $[\alpha]_D^{20}=+32.8$ ($c=1.0$, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): $\delta=0.99$ (s, 21H; $((CH_3)_2CH)_2Si$), 1.05 (d, $J=7.1$ Hz, 3H; 2-CH_3), 1.16 (d, $J=6.1$ Hz, 3H; 4-H), 2.42–2.50 (m, 1H; 2-H), 4.24–4.30 (m, 1H; 3-H), 9.71 (s, 1H; CHO); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=9.7$ (2-CH_3), 12.5 (CHSi), 18.08, 18.13 ($((CH_3)_2CH)_3Si$), 21.3 (C-4), 54.0 (C-2), 69.4 (C-3), 204.9 (C-1); HRMS (ESI): $[M+Na]^+$ calcd for $C_{14}H_{30}NaO_2Si$ 281.46207, found 281.46219.

Dibromoalkene 23: Triethylamine (0.2 mL, 1.44 mmol) was added to a solution of aldehyde **22** (100 mg, 0.464 mmol) in CH_2Cl_2 (3 mL) at 0 °C. Then a solution (prepared at 0 °C) containing PPh_3 (593 mg, 2.26 mmol) and CBr_4 (365 mg, 1.10 mmol) in CH_2Cl_2 (3 mL) was added via canula to the aldehyde/amine solution at 0 °C. The mixture was stirred for 45 min at the same temperature. After that silica gel was added, the mixture was evaporated and applied to a column. The crude product was subjected to flash chromatography (petroleum ether/ethyl acetate, 50:1) to give 183 mg (97%) of the dibromide **23**; $R_f=0.83$ (petroleum ether/ethyl acetate, 25:1). The dibromide was used directly in the subsequent alkyne formation reaction.

(3*R*,4*S*)-4-Triisopropylsilyloxy-3-methylpent-1-yne (24): *n*BuLi (2.35 mL, 5.89 mmol) was added to a solution of dibromide **23** (610 mg, 1.47 mmol) in THF (16 mL) at −80 °C. This mixture was stirred for 2 h at −80 °C, and then quenched by the addition of water (5 mL). Then the mixture was extracted with Et_2O (3×20 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (petroleum ether/ethyl acetate, 50:1) to give 335 mg (96%) of pure alkyne **24**; $R_f=0.4$ (petroleum ether); $[\alpha]_D^{20}=+3.4$ ($c=0.45$, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): $\delta=1.06$ (s, 21H; $((CH_3)_2CH)_3Si$), 1.16 (d, $J=7.1$ Hz, 3H; 3-CH_3), 1.20 (d, $J=6.1$ Hz, 3H; 5-H), 2.04 (d, $J=$ 2.3 Hz, 1H; 1-H), 2.58–2.65 (m, 1H; 3-H), 4.05–4.11 (m, 1H; 4-H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=12.4$ (CHSi), 13.7 (3-CH_3), 18.08, 18.11 ($((CH_3)_2CH)_3Si$), 18.7 (C-5), 33.4 (C-3), 69.5 (C-4), 70.1 (C-1), 86.9 (C-2); HRMS (ESI): $[M+Na]^+$ calcd for $C_{15}H_{30}OSi$ 277.19581, found 277.19586.

(4*R*,5*S*)-Methyl 4-methyl-5-(triisopropylsilyloxy)hex-2-ynoate (25): *n*BuLi (0.59 mL, 1.48 mmol, 2.5 M in hexane) was added to a solution of alkyne **24** (327 mg, 1.284 mmol) in dry THF (2 mL) at −80 °C and the mixture was stirred for 2 h at −80 °C. After that methylchloroformate (0.15 mL, 1.93 mmol) was added, the mixture was stirred for 1 h at −80 °C and then warmed to 0 °C. Thereafter, saturated NH_4Cl solution was added and the mixture was extracted with Et_2O (3×30 mL). The combined organic layers were washed with saturated NaCl solution, dried over $MgSO_4$, filtered and concentrated, in vacuo. The residue was purified by flash chromatography (petroleum ether/ethylacetate, 30:1) to provide ester **25** as a colorless oil (393 mg, 98%); $R_f=0.41$ (petroleum ether/ethyl acetate, 25:1); $[\alpha]_D^{20}=+3.3$ ($c=1.0$, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): $\delta=1.04$ (s, 21H; $Si(CH(CH_3)_2)_3$), 1.21 (dd, $J=6.6$, 3.3 Hz, 6H; 6-H, 4-CH_3), 2.70–2.76 (m, 1H; 4-H), 3.74 (s, 3H; OCH_3), 4.08–4.14 (m, 1H; 5-H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=12.4$ (CHSi), 13.2 (4-CH_3), 18.0, 18.1 ($((CH_3)_2CH)_3Si$), 19.4 (C-6), 33.8 (C-4), 52.5 (OCH_3), 69.9 (C-5), 74.1 (C-2), 91.4 (C-3), 154.2 (C-1); HRMS (ESI): $[M+Na]^+$ calcd for $C_{17}H_{32}O_2Si$ 335.20129, found 355.20128.

(4*R*,5*S*)-Methyl 4-methyl-5-(triisopropylsilyloxy)hexanoate (26): The alkyne **25** (410 mg, 1.31 mmol) was dissolved in MeOH (7 mL), Pd/C (10 mg) was added and the suspension was stirred for 3 h under a H_2 atmosphere. After that the suspension was filtered through a short pad of celite and washed with Et_2O. The filtrate was concentrated under reduced pressure to give **26** as a colorless oil (404 mg, 97%); $R_f=0.33$ (petroleum ether/ethyl acetate, 25:1); $[\alpha]_D^{20}=+4.6$ ($c=1.0$, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): $\delta=0.88$ (d, $J=6.8$ Hz, 3H; 6-H), 1.05 (s, 24H; 4-CH_3, $((CH_3)_2CH)_3Si$), 1.34–1.42 (m, 1H; 4-H), 1.55–1.73 (m, 2H; 3-H), 2.32 (m, 2H; 2-H), 3.65 (s, 3H; OCH_3), 3.84–3.89 (m, 1H; 5-H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=12.9$ (CHSi), 13.6 (4-CH_3), 18.6 ($((CH_3)_2CH)_3Si$), 18.8 (C-6), 28.6 (C-3), 32.8 (C-2), 40.3 (C-4), 51.9 (OCH_3), 71.8 (C-5), 174.7 (C-1); HRMS (ESI): $[M+Na]^+$ calcd for $C_{17}H_{36}O_3Si$ 339.23259, found 339.23263.

(4*R*,5*S*)-*N*-Methoxy-*N*,4-dimethyl-5-(triisopropylsilyloxy)hexanamide (27): *N*,*O*-Dimethylhydroxylamine hydrochloride (14 mg, 0.145 mmol) was added in one portion to a solution of ester **26** (27 mg, 0.085 mmol) in THF (4 mL) at −20 °C followed by the dropwise addition of *i*PrMgCl (0.145 mL, 0.29 mmol). Then the solution was allowed to warm to −10 °C and was stirred for 30 min. Next, saturated NH_4Cl solution was added and the mixture was extracted with Et_2O (3×30 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (petroleum ether/ethyl acetate, 5:1) to yield pure amide **27** as a colorless oil (27 mg, 91%); $R_f=0.27$ (petroleum ether/ethyl acetate, 5:1); $[\alpha]_D^{20}=+3.8$ ($c=1.0$, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): $\delta=0.89$ (d, $J=6.6$ Hz, 3H; 6-H), 1.03–1.05 (m, 24H; 4-CH_3, $((CH_3)_2CH)_3Si$), 1.33–1.41 (m, 1H; 4-H), 1.57–1.71 (m, 2H; 3-H), 2.31–2.52 (m, 2H; 2-H), 3.16 (s, 3H; NCH_3), 3.67 (OCH_3), 3.86–3.92 (m, 1H; 5-H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=11.9$ (CH-Si), 12.7 (C-6), 17.6 ($((CH_3)_2CH)Si$), 17.7 (4-CH_3), 27.4 (C-3), 29.7 (C-2), 31.6 (NCH_3), 39.6 (C-4), 60.6 (OCH_3), 70.9 (C-5), 174.3 (C-1); HRMS (ESI): $[M+Na]^+$ calcd for $C_{18}H_{39}NO_3Si$ 368.25914, found 368.25920.

(9*R*,10*S*)-9-Methyl-10-(triisopropylsilyloxy)undec-1-en-6-one (28): Mg turnings (67 mg, 2.73 mmol) were placed in a flask with a

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reflux condenser and a septum. Then they were covered with dry Et_2O (1 mL) and a few drops of 1-bromopentene were added to start the reaction. After the reaction has started the remaining 1-bromopentene (406 mg, 2.73 mmol), which was dissolved in Et_2O (2 mL), was added slowly. After complete addition, the mixture was stirred for 45 min at room temperature. In a separate flask a solution of amide **27** (314 mg, 0.91 mmol) in dry THF (15 mL) was cooled to −80 °C. To this solution the prepared Grignard solution was added dropwise and the mixture was stirred for 15 min at −80 °C before it was slowly warmed to room temperature. Next, HCl (1 N; 1 mL) was added until the formed precipitate disappeared. The mixture was extracted with Et_2O (3×30 mL) and the combined organic layers were washed with saturated NaCl, dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification of the crude ketone by flash chromatography (petroleum ether/ethyl acetate, 40:1) gave ketone **28** as a colorless oil 315 mg (98%); R_f=0.43 (petroleum ether/ethyl acetate, 25:1); $[\alpha]_D^{20}=+5.0$ (c=1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.86 (d, J=6.6 Hz, 3H; 11-H), 1.04–1.05 (m, 24H; 9-CH_3, $((CH_3)_2CH)_3Si$), 1.24–1.34 (m, 2H; 8-H), 1.49–1.56 (m, 1H; 9-H), 1.63–1.70 (m, 4H; 8-H, 4-H), 2.04 (q, J=7.2 Hz, 2H; 3-H), 2.33–2.45 (m, 4H; 5-H, 7-H), 3.82–3.88 (m, 1H; 10-H), 4.96 (d, J=11.2 Hz, 1H; 1-H), 5.00 (d, J=17.0 Hz, 1H; 1-H), 5.71–5.81 (m, 1H; 2-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=12.5 (10-CH_3), 13.4 (9-CH_3), 18.1, 18.2 ($(CH_3)_2CH)_3Si$, 18.4 (C-5), 22.8 (C-11), 27.0 (C-8), 33.1 (C-3), 40.0 (C-7), 41.1 (C-9), 41.8 (C-5), 71.5 (C-10), 115.2 (C-1), 138.0 (C-2), 211.1 (C-6); HRMS (ESI) $[M+Na]^+$ calcd for $C_{21}H_{42}O_2Si$ 377.28463, found 377.28459.

2-((3*R*,4*S*)-4-Triisopropylsilyloxy-3-methylpentyl)-2-(pent-4-enyl)-1,3-dioxolane (29): Ketone **28** (615 mg, 1.73 mmol) was dissolved in ethane-1,2-diol (1.93 mL, 34.68 mmol), then triethylorthoformate (1.15 mL, 6.94 mmol) and *p*TsOH (29 mg, 0.173 mmol) were added and the mixture was stirred for 8 h at room temperature. After this, saturated $NaHCO_3$ solution was added and the mixture was extracted with Et_2O (3×40 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. Purification of the residue by flash chromatography (petroleum ether/ethyl acetate, 25:1) afforded the pure ketal **29** (650 mg, 93%) as a colorless oil; R_f=0.29 (petroleum ether/ethyl acetate, 25:1). $[\alpha]_D^{20}=+4.1$ (c=1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.86 (d, J=6.9 Hz, 3H; 5′-H), 1.03 (d, J=6.9 Hz, 3H; 3′-CH_3), 1.04 (s, 21H; $((CH_3)_2CH)_3Si$), 1.35–1.71 (m, 8H; 2-H, 1-H, 1′-H, 2′-H), 2.04 (q, J=7.0 Hz, 2H; 3-H), 3.83–3.88 (m, 1H; 4′-H), 3.91 (s, 4H; OCH_2CH_2O), 4.93–5.02 (m, 2H; 5-H), 5.73–5.84 (m, 1H; 4-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=12.5 (C-5′), 13.4 (3′-CH_3), 18.16, 18.19 ($((CH_3)_2CH)_3Si$), 23.1 (C-2), 27.3 (C-2′), 33.9 (C-1′), 35.2 (C-1), 36.5 (C-3), 40.6 (C-3′), 64.9 (OCH_2CH_2O), 71.6 (C-4′), 111.8 ($C(OR)_2$), 114.6 (C-5), 138.7 (C-4); HRMS (ESI): $[M+Na]^+$ calcd for $C_{23}H_{46}O_3Si$ 421.31084, found 421.31093.

(2*S*,3*R*)-3-Methyl-5-(2-(pent-4-enyl)-1,3-dioxolan-2-yl)pentan-2-ol (30): TBAF (778 mg, 2.47 mmol) was added to a solution of silyl ether **29** (655 mg, 1.64 mmol) in THF (10 mL) at room temperature. The mixture was stirred for 6 h at room temperature before it was treated with saturated NH_4Cl solution (20 mL) and extracted with Et_2O (3×50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (petroleum ether/ethyl acetate, 2:1) to provide alcohol **30** (383 mg, 94%) as a colorless oil; R_f=0.2 (petroleum ether/ethyl acetate, 3:1); $[\alpha]_D^{20}=+12.9$ (c=1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.85 (d, J=6.9 Hz, 3H; 1-H), 1.11 (d, J=6.4 Hz, 3H; 3-CH_3), 1.39–1.61 (m, 8H; 4-H, 5-H, 1′-H, 2′-H), 2.03 (q, J=7.1 Hz, 2H; 3′-H), 3.59–3.66 (m, 1H; 3-H), 3.91 (s, 4H; OCH_2CH_2O), 4.93 (d, J=10.1 Hz, 1H; 5′-H), 4.98 (dd, J=17.0, 1.5 Hz, 1H; 5′-H), 5.72–5.83 (m, 1H; 4′-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=14.7 (3-CH_3), 19.5 (C-1), 23.1 (C-2′), 26.4 (C-4), 33.8 (C-5), 34.5 (C-1′), 36.5 (C-3′), 40.2 (C-3), 64.9 (OCH_2CH_2O), 71.5 (C-2), 111.8 ($C(OR)_2$), 114.6 (C-5′), 138.6 (C-4′); HRMS (ESI): $[M+Na]^+$ calcd for $C_{14}H_{26}O_3$ 265.17742, found 265.17739.

(2*R*,3*R*)-3-Methyl-5-(2-(pent-4-enyl)-1,3-dioxolan-2-yl)pentan-2-yl 2,4-dimethoxy-6-vinylbenzoate (31): DEAD (342 mg, 1.96 mmol, 40% solution in toluene) was added dropwise to a solution of alcohol **30** (190 mg, 0.748 mmol), acid **15** (245 mg, 1.176 mmol) and PPh_3 (525 mg, 2.0 mmol) in dry THF (6 mL). After complete addition, the mixture was stirred for 3 h at room temperature before the solvent was removed under reduced pressure. The resulting oil was purified by flash chromatography (petroleum ether/ethyl acetate, 4:1) to give the pure ester **31** (260 mg, 73%) as a colorless oil; R_f=0.34 (petroleum ether/ethyl acetate, 4:1); $[\alpha]_D^{20}=-0.4$ (c=1.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.94 (d, J=6.9 Hz, 3H; 3′-CH_3), 1.19–1.30 (m, 1H; 4′-H), 1.28 (d, J=6.4 Hz, 3H; 1′-H), 1.40–1.47 (m, 2H; 2″-H), 1.56–1.67 (m, 6H; 3′-H, 4′-H, 5′-H, 1″-H), 2.03 (q, J=7.1 Hz, 2H; 3″-H), 3.78 (s, 3H; OCH_3), 3.82 (s, 3H; OCH_3), 3.90 (s, 4H; OCH_2CH_2O), 4.93 (d, J=10.2 Hz, 1H; 5″-H), 4.98 (dd, J=17.2, 1.7 Hz, 1H; 5″-H), 5.07–5.13 (m, 1H; 2′-H), 5.30 (d, J=10.9 Hz, 1H; 8-H), 5.69 (d, J=17.6 Hz, 1H; 8-H), 5.74–5.82 (m, 1H; 4″-H), 6.38 (d, J=2.0 Hz, 1H; 3-H), 6.63 (d, J=2.0 Hz, 1H; 5-H), 6.72 (dd, J=17.3, 10.9 Hz, 1H; 7-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=14.8 (3′-CH_3), 17.0 (C-1′), 23.0 (C-2″), 26.4 (C-4′), 33.9 (C-5′), 34.6 (C-1″), 36.6 (C-3″), 37.9 (C-3′), 55.4 (OCH_3), 55.8 (OCH_3), 64.9 (OCH_2CH_2O), 74.9 (C-2′), 98.2 (C-3), 101.2 (C-5), 111.7 (C-1), 114.6 ($C(OR)_2$), 116.8 (C-8), 116.9 (C-5″), 133.9 (C-7), 137.4 (C-4″), 138.6 (C-6), 157.9 (CO_2), 161.2 (C-2), 167.6 (C-4); HRMS (ESI): $[M+Na]^+$ calcd for $C_{25}H_{36}O_6$ 455.24041, found 455.24038.

Macrolactone 32: Diene **31** (205 mg, 0.472 mmol) was dissolved in dry toluene (120 mL), then Grubbs' 2nd generation catalyst (20.0 mg, 0.024 mmol) was added and the mixture was heated for 4 h at 80 °C. After being cooled, the reaction mixture was concentrated under reduced pressure and the resulting residue was purified by flash chromatography (petroleum ether/ethyl acetate, 3:1). The pure lactone **32** (163 mg, 85%) was obtained as a slightly brown oil; R_f=0.3 (petroleum ether/ethyl acetate, 3:1); $[\alpha]_D^{20}=-67.6$ (c=2.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.93 (d, J=6.9 Hz, 3H; 9′-CH_3), 1.21 (d, J=6.6 Hz, 3H; 10′-CH_3), 1.24–1.32 (m, 1H; 3′-H), 1.40–1.48 (m, 1H; 5′-H), 1.55–1.72 (m, 5H; 3′-H, 4′-H, 8′-H), 1.85–1.92 (m, 1H; 5′-H), 1.95–2.00 (m, 1H; 7′-H), 2.10–2.17 (m, 1H; 7′-H), 2.30–2.39 (m, 1H; 9′-H), 3.77 (s, 3H; OCH_3), 3.80 (s, 3H; OCH_3), 3.90 (dd, J=3.5, 1.3 Hz, 4H; OCH_2CH_2O), 5.14–5.20 (m, 1H; 10′-H), 6.22–6.29 (m, 1H; 2′-H), 6.32 (d, J=2.0 Hz, 1H; 3-H), 6.37 (d, J=16.1 Hz, 1H; 1′-H), 6.59 (d, J=2.0 Hz, 1H; 5-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=14.8 (9′-CH_3), 15.2 (10′-CH_3), 21.1 (C-4′), 25.1 (C-8′), 30.2 (C-3′), 32.7 (C-9′), 33.6 (C-7′), 37.1 (C-5′), 55.4 (OCH_3), 56.0 (OCH_3), 64.3 (OCH_2CH_2O), 64.4 (OCH_2CH_2O), 74.3 (C-10′), 97.5 (C-3), 100.7 (C-5), 112.1 (C-6′), 117.1 (C-1), 126.0 (C-1′), 132.8 (C-2′), 136.6 (C-6), 157.5 (C-2), 161.0 (C-4), 168.0 (CO_2); HRMS (ESI) $[M+Na]^+$ calcd for $C_{23}H_{32}O_6$ 427.20911, found 427.20915.

Macrolactone 33: A solution of lactone **32** (172 mg, 0.424 mmol) in acetone/H_2O (7 mL, 10:1) containing *p*TsOH (4 mg, 0.021 mmol) was refluxed for 12 h. After being cooled, saturated $NaHCO_3$ solution was added and the mixture was extracted with CH_2Cl_2 (3×30 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (petroleum ether/ethyl acetate, 3:1) to afford the pure ketone **33** (130 mg, 85%) as a colorless oil; R_f=0.33 (petroleum ether/ethyl acetate, 3:1); $[\alpha]_D^{20}=-29.3$ (c=2.0, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ=0.95 (d, J=6.9 Hz, 3H; 9′-

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CH_3), 1.21 (d, J=6.6 Hz, 3H; 10′-CH_3), 1.46–1.55 (m, 2H; 4′-H, 8′-H), 1.73–1.87 (m, 2H; 9′-H, 8′-H), 2.02–2.18 (m, 3H; 3′-H, 4′-H, 7′-H), 2.28–2.33 (m, 2H; 3′-H, 5′-H), 2.47–2.53 (m, 1H; 5′-H), 2.67–2.75 (m, 1H; 7′-H), 3.78 (s, 3H; OCH_3), 3.81 (s, 3H; OCH_3), 5.24 (ddd, J=13.2, 6.6, 3.0 Hz, 1H; 10′-H), 5.94–6.02 (m, 1H; 2′-H), 6.28 (dd, J=15.8, 1.0 Hz, 1H; 1′-H), 6.38 (d, J=2.0 Hz, 1H; 3-H), 6.58 (d, J=2.0, 1H; 5-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=14.5 (9′-CH_3), 14.7 (10′-CH_3), 21.1 (C-4′), 26.5 (C-8′), 31.0 (C-3′), 35.4 (C-9′), 37.0 (C-7′), 40.9 (C-5′), 55.4 (OCH_3), 55.9 (OCH_3), 74.3 (C-10′), 97.7 (C-3), 100.9 (C-5), 116.2 (C-1), 128.9 (C-1′), 132.7 (C-2′), 136.8 (C-6), 157.8 (C-3), 161.3 (C-4), 167.2 (CO_2), 211.3 (C-6′); HRMS (ESI): $[M+Na]^+$ calcd for $C_{21}H_{28}O_5$ 383.18290, found 383.18287.

Macrolactone 34: BCl_3 (0.24 mL, 1 M in CH_2Cl_2, 0.24 mmol) was added dropwise to a solution of lactone **33** (11.0 mg, 0.031 mmol) in dry CH_2Cl_2 (2.5 mL) at −60 °C. The mixture was allowed to warm to −20 °C and stirred for 30 min. Then the mixture was cooled to −50 °C before MeOH (1 mL) was added and the mixture was allowed to reach room temperature. After removal of the solvents under reduced pressure the residue was purified by flash chromatography (petroleum ether/ethyl acetate, 3:1) to give lactone **34** (9 mg, 88%) as a colorless oil; R_f=0.45 (petroleum ether/ethyl acetate, 3:1); $[\alpha]_D^{20}$=+43.3 (c=0.5, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ=0.98 (d, J=6.9 Hz, 3H; 9′-CH_3), 1.27 (d, J=6.4 Hz, 3H; 10′-CH_3), 1.42–1.48 (m, 1H 4′-H), 1.75–1.86 (m, 1H; 8′-H), 1.87–1.94 (m, 2H; 8′-H, 9′-H), 2.02–2.07 (m, 1H; 5′-H), 2.10–2.15 (m, 1H; 5′-H), 2.30–2.36 (m, 2H; 3′-H, 4′-H), 2.43–2.56 (m, 3H; 3′-H, 7′-H), 3.81 (s, 3H; OCH_3), 5.11–5.16 (m, 1H; 10′-H), 5.81–5.88 (m, 1H; 2′-H), 6.38 (s, 1H; 3-H), 6.50 (s, 1H; 5-H), 6.84 (d, J=15.5 Hz, 1H; 1′-H), 11.48 (s, 1H; OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ=13.4 (9′-CH_3), 15.8 (10′-CH_3), 22.2 (C-4′), 24.9 (C-8′), 31.3 (C-3′), 37.0 (C-9′), 38.0 (C-7′), 41.8 (C-5′), 55.4 (OCH_3), 76.9 (C-10′), 100.0 (C-5), 104.7 (C-3), 107.5 (C-1), 132.0 (C-1′), 132.5 (C-2′), 142.0 (C-6), 163.8 (C-2), 164.7 (C-4), 170.8 (CO_2), 211.6 (C-6′); HRMS (ESI): $[M+Na]^+$ calcd for $C_{20}H_{26}O_5$ 369.16725, found 369.16702.

Macrolactone 35: A flask was charged with aluminium powder (58 mg, 2.15 mmol) and iodine (203 mg, 0.80 mmol). Then benzene (3 mL) was added and the mixture was heated to reflux until the purple color disappeared. Subsequently, the mixture was cooled to 0 °C and then TBAI (1 mg) and the lactone **33** (17.5 mg, 0.049 mmol), which were dissolved in benzene (1 mL), were added. After complete addition, the mixture was stirred for 3 min, and HCl (2N; 2 mL) and water (10 mL) were added. The mixture was extracted with ethyl acetate (3×25 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (petroleum ether/ethyl acetate, 3:1) to provide zearlenone analogue **35** (14 mg, 86%) as a colorless oil; R_f=0.28 (petroleum ether/ethyl acetate, 3:1); $[\alpha]_D^{20}$=+22.0 (c=0.5, THF); ^1H NMR (400 MHz, $CDCl_3$): δ=0.98 (d, J=6.4 Hz, 3H; 9′-CH_3), 1.27 (d, J=6.4 Hz, 3H; 10′-CH_3), 1.40–1.80 (m, 1H; 4′-H), 1.74–1.95 (m, 3H; 8′-H, 9′-H), 2.00–2.17 (m, 2H; 4′-H, 5′-H), 2.28–2.36 (m, 2H; 3′-H, 5′-H), 2.42–2.56 (m, 3H; 3′-H, 7′-H), 5.12–5.14 (m, 1H; 10′-H), 5.81–5.88 (m, 1H; 2′-H), 6.32 (s, 1H; 3-H), 6.45 (s, 1H; 5-H), 6.83 (d, J=15.5 Hz, 1H; 1′-H), 11.42 (s, 1H; OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ=13.4 (9′-CH_3), 15.8 (10′-CH_3), 22.1 (C-4′), 24.9 (C-8′), 31.3 (C-3′), 36.9 (C-9′), 38.0 (C-7′), 41.8 (C-5′), 77.2 (C-10′), 102.4 (C-3), 105.2 (C-1), 107.7 (C-5), 132.2 (C-2′), 132.3 (C-1′), 142.7 (C-6), 160.1 (C-4), 164.5 (C-2), 170.7 (CO_2), 211.7 (C-6′); HRMS (ESI): $[M+Na]^+$ calcd for $C_{19}H_{24}O_5$ 355.15159, found 355.15170.

Cytotoxicity assay: The viability/toxicity of the compounds with the mouse fibroblast cell line, L929, were tested with an MTT assay after five days of incubation with serial dilutions of the samples.[37,38] The cell line was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany) and cultured in DME medium as reported. Radicicol was purchased from Sigma and geldanamycin was from Serva.

Protein expression and purification: Full-length human Hsp90 was expressed in *E. coli* by using a N-terminal His_6 fusion protein to aid purification. Expression was performed in BL21 (DE3) cells in LB media containing ampicilline (100 $\mu g\,mL^{-1}$). After induction with IPTG (0.5 mM) the growth temperature was lowered to 18 °C and incubation was continued for 12 h. The cells were centrifuged, resuspended in binding buffer (500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 5 mM imidazole) and lysed by sonication. The lysate was centrifuged for 1 h at 4 °C, 16500 rpm. The lysate was applied to a column containing a suspension of Ni-NTA (5 mL) equilibrated in binding buffer. The column was washed with wash buffer (30 mL; 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 30 mM imidazole) and recombinant Hsp90 was eluted by step gradients of 50, 100, 150 and 250 mM imidazole in NaCl (500 mM), glycerol (5%), HEPES (50 mM), pH 7.5. The fractions containing Hsp90 were pooled, concentrated to about 3 mL and applied onto a S75 gel filtration column equilibrated in HEPES (50 mM), pH 7.5, NaCl (500 mM), glycerol (5%), TCEP (0.5 mM). The protein was 95% clean after that purification step. An experimental mass of 85428 Da was measured by using ESI-ToF MS spectroscopy corresponding to the expected molecular weight.

Thermal stability measurements: Thermal melting experiments were carried out by using real time PCR (Mx3005p; Stratagene, La Jolla, CA, USA) according to the protocol described by Vedadi et al.[20]

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