Propionate Analogues of Zearalenone Bind to Hsp90


By replacement of an acetate with propionate through organic synthesis a range of zearalenone analogues were prepared. 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] 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 (IC50 = 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) IC50 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, [a] M. Ugele, Prof. Dr. M. E. Maier
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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. 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. 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 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). For example, we conceived the concept of propionate scanning, 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 macro lactones. 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 of zearalenone turned out to be a quite potent (IC₅₀ = 210 nm) inhibitor of human carbonyl reductase 1 (CBR1). While the parent compound showed some binding to Hsp90,
the analogue 12 did not induce any shift in the thermal shift assay.\textsuperscript{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.

For the formation of the macrolactone ring we planned to use a ring-closing metathesis (RCM) strategy related to the work of Fürstner et al.\textsuperscript{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\textsuperscript{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\textsuperscript{27} of acetoacetate 16 using the (R)-(++)-BINAP ligand 17 (Scheme 5). The obtained hydroxyester 18 was subjected to Frater alkylation\textsuperscript{28} (2.2 equiv LDA, THF/HMPT, MeI); this resulted in the anti configured formal aldol product 19.\textsuperscript{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\textsuperscript{30} and conversion of 1,1-dibromide 23 to pentyne derivative 24. De-protonation 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.\textsuperscript{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\textsuperscript{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 (pTsOH, acetone) to provide ketolactone 33. By using the Lewis acid BCl3, 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 (AlI3) in benzene; this led to zearalenone analogue 35.\textsuperscript{33}
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 acetyl 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 (2R,3R)-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.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>IC50 [mM]</th>
<th>Tm shift [°]</th>
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<tbody>
<tr>
<td>1</td>
<td>2 (RAD)</td>
<td>0.58</td>
<td>5.5</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>10</td>
<td>3.9</td>
</tr>
<tr>
<td>3</td>
<td>ent-35</td>
<td>6.9</td>
<td>3.6</td>
</tr>
<tr>
<td>4</td>
<td>1 (GEA)</td>
<td>0.0086</td>
<td>3.5</td>
</tr>
<tr>
<td>5</td>
<td>ent-42</td>
<td>15</td>
<td>3.2</td>
</tr>
<tr>
<td>6</td>
<td>10 (ZEA)</td>
<td>9.4</td>
<td>2.9</td>
</tr>
<tr>
<td>7</td>
<td>42</td>
<td>14</td>
<td>2.5</td>
</tr>
<tr>
<td>8</td>
<td>ent-34</td>
<td>10</td>
<td>2.1</td>
</tr>
<tr>
<td>9</td>
<td>33</td>
<td>36</td>
<td>0.3</td>
</tr>
<tr>
<td>10</td>
<td>ent-33</td>
<td>44</td>
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<td>11</td>
<td>41</td>
<td>46</td>
<td>0.3</td>
</tr>
<tr>
<td>12</td>
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<td>56</td>
<td>0.3</td>
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<td>13</td>
<td>34</td>
<td>72</td>
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<td>14</td>
<td>ent-4</td>
<td>&gt;120</td>
<td>0.3</td>
</tr>
<tr>
<td>15</td>
<td>40</td>
<td>33</td>
<td>0.2</td>
</tr>
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[a] The compounds are arranged with decreasing Tm; [b] against the L929 mouse fibroblast cell line; [c] for details see the Experimental Section.

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.
Binding of compounds to Hsp90 was detected by using differential scanning fluorimetry (DSF)—a generic thermal-shift ($\Delta T_m$) detection assay—as described.\textsuperscript{[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.\textsuperscript{[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$\mu$m), 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 Hsp90a N-terminal domain (Hsp90N). Preparation of Hsp90N has been previously described.\textsuperscript{[35]} From these measurements $K_d$ values of 0.25$\mu$m (ent-35) and 0.33$\mu$m (35) were obtained. The corresponding $K_d$ value for radicicol in this assay was 0.001$\mu$m (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 diastereomeric 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 $\mu$m range. Screening these compounds in a thermal-shift ($\Delta T_m$) 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
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(3H)-one (14): A solution of aldehyde[26] 13 (1.40 g, 5.28 mmol) in a mixture of HCl (11 V)/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 NaHCO₃ solution (3×100 mL). Then the aqueous phase was acidified with concentrated HCl and then extracted with ethyl acetate (3×40 mL) and 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 MgSO₄, filtered, and concentrated under reduced pressure to give the olefin 15 (89 mg, 90 %) as a slightly yellow solid; Rf=0.47 (petroleum ether/ethyl acetate, 1:1). ¹H NMR (400 MHz, acetone): δ=3.82 (s, 3 H; OCH₃), 3.85 (s, 3 H; OCH₃), 5.31 (d, J= 10.9 Hz, 1 H; CH≡CH₂), 5.82 (d, J= 17.4 Hz, 1 H; CH≡CH₂), 6.35 (d, J= 2.0 Hz, 1 H; 3-H), 6.78 (d, J= 2.0 Hz, 1 H; 5-H), 6.85 (dd, J= 17.4, 11.1 Hz, 1 H; CH≡CH₂), 11.22 (brs, 1 H; CO₂H); ¹³C NMR (100 MHz, acetone): δ=56.9 (OCH₃), 57.1 (OCH₃), 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 (CO₂).

2,4-Dimethoxy-6-vinylbenzoic acid (15): KOBu (428 mg, 3.81 mmol) was added to a solution of Ph₃PMeBr (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 MgSO₄, filtered, and concentrated under reduced pressure to give the olefin 15 (89 mg, 90 %) as a slightly yellow solid; Rf=0.47 (petroleum ether/ethyl acetate, 1:1). ¹H NMR (400 MHz, acetone): δ=3.82 (s, 3 H; OCH₃), 3.85 (s, 3 H; OCH₃), 5.31 (d, J=10.9 Hz, 1 H; CH≡CH₂), 5.82 (d, J=17.4 Hz, 1 H; CH≡CH₂), 6.35 (d, J=2.0 Hz, 1 H; 3-H), 6.78 (d, J=2.0 Hz, 1 H; 5-H), 6.85 (dd, J=17.4, 11.1 Hz, 1 H; CH≡CH₂), 11.22 (brs, 1 H; CO₂H); ¹³C NMR (100 MHz, acetone): δ=56.9 (OCH₃), 57.1 (OCH₃), 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 (CO₂).

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

NH₄Cl solution was added and the mixture was stirred for 45 min at room temperature. Then phthalide 14 (0.20 g, 0.47 mmol) was added dropwise to the LDA solution and the mixture was stirred for 45 min at −60 °C. Then a solution of Mel (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 NH₄Cl solution was added and the mixture was extracted with EtO (3×20 mL). The combined organic layers were dried over MgSO₄, filtered, and concentrated in vacuo. Purification of the residue by flash chromatography (petroleum ether/ethyl acetate, 35:1) provided 4.23 g (97 %) of the ester 20; Rf=0.24 (petroleum ether/ethyl acetate, 35:1); ¹H NMR (400 MHz, CDCl₃); δ=1.04 (s, 21 H; [(CH₃)₂CH]S), 1.10 (d, J=7.1 Hz, 3 H; 2-
CH3), 1.12 (d, J = 6.1 Hz, 3H; 4-H), 2.57–2.64 (m, 1H; 2-H), 3.65 (s, 3H; OCH3), 4.22–4.28 (m, 1H; 3-H); 1H NMR (100 MHz, CDCl3): δ = 11.2 (CHS & B, 12.5 (2-Ch), 18.0, 18.1 (OCH3)2CHSi), 19.9 (C-4), 47.7 (C-2), 51.4 (OCH3), 69.6 (C-3), 175.3 (C-1); HRMS (ESI): [M+Na]+ calcld for C14H32O2Si 283.20638, found 283.20643.

(2R,3S)-2-Methyl-3-(triisopropylsilyloxy)butan-1-ol (21): Dibal-H (16.5 mL, 16.5 mmol, 1 mL in hexane) was added in a dry flask to a solution of dibromide (2.35 mL, 5.89 mmol) in dry CH2Cl2 (40 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 NH4Cl solution. The mixture was extracted with CH2Cl2 (327 mg, 1.284 mmol) 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 NH4Cl solution was added and the mixture was extracted with Et2O (3 × 30 mL). The combined organic layers were washed with saturated NaCl solution, dried over MgSO4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (petroleum ether/ethyl acetate, 25:1) to give 183 mg (97%) of the aldehyde.

3-(2-Methyl-3-(triisopropylsilyloxy)propyl)-2-methyl-2-propylpent-1-yne (24): nBuLi (0.59 mL, 1.48 mmol, 2.5 mL in hexane) was added to a solution of alkyn (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 NH4Cl solution was added and the mixture was extracted with Et2O (3 × 30 mL). The combined organic layers were washed with saturated NaCl solution, dried over MgSO4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (petroleum ether/ethyl acetate, 25:1) to give 183 mg (97%) of the aldehyde.

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3-(2-Methyl-3-(triisopropylsilyloxy)propyl)-2-methyl-2-propylpent-1-yne (24): nBuLi (0.59 mL, 1.48 mmol, 2.5 mL in hexane) was added to a solution of alkyn (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 NH4Cl solution was added and the mixture was extracted with Et2O (3 × 30 mL). The combined organic layers were washed with saturated NaCl solution, dried over MgSO4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (petroleum ether/ethyl acetate, 25:1) to give 183 mg (97%) of the aldehyde.
reflux condenser and a septum. Then they were covered with dry Et2O (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 Et2O (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 Et2O (3 × 30 mL) and the combined organic layers were washed with saturated NaCl, dried over MgSO4, 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%); Rf = 0.43 (petroleum ether/ethyl acetate, 25:1); [α]D20 = +5.0 (c = 1.0, CHCl3); 1H NMR (400 MHz, CDCl3); δ = 0.86 (d, J = 6.6 Hz, 3H; 11-H), 1.04–1.05 (m, 24 H; 9-CH3, ((CH3)2CH)3Si), 1.35–1.70 (m, 4H; 8-H, 4-H), 2.04 (q, J = 7.2 Hz, 2H; 3-H), 3.35–3.45 (m, 4H; 4-H, 8-H), 4.54–4.57 (m, 2H; 2-H, 6-H), 5.72–5.83 (m, 3 H; 10-H, 5-H, 6-H); 13C NMR (100 MHz, CDCl3); δ = 20.6 (C-4), 26.4 (C-5), 34.5 (C-3), 34.7 (C-3'), 40.2 (C-3), 64.9 (OCH2CH2O), 71.5 (C-1) 111.2 (C-9), 111.7 (C-10), 132.6 (C-20), 159.3 (C-8); HRMS (ESI): [M+Na]+ calcd for C16H27NO4 Na 277.18863, found 277.18864.

2-(3R,4S,4′R)-4-Trisopropylsilyl-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) and triethylorthoformate (314 mg, 0.472 mmol) was dissolved in acetone/H2O (7 mL, 10:1) containing K2CO3 before it was slowly warmed to room temperature. Next, the mixture was stirred for 4 h at room temperature before it was slowly warmed to room temperature. 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; Rf = 0.34 (petroleum ether/ethyl acetate, 4:1); [α]D20 = −0.4 (c = 1.0, CHCl3); 1H NMR (400 MHz, CDCl3); δ = −0.94 (d, J = 6.9 Hz, 3H; 3'-CH), 1.19–1.30 (m, 3H; 10-H, 1-H, 1'-H), 1.0–1.05 (m, 24 H; 9-CH3, ((CH3)2CH)3Si), 1.35–1.71 (m, 8 H; 2-H, 1-H, 1'-H, 2'-H), 5.71–5.81 (m, 1H; 2-H); 13C NMR (100 MHz, CDCl3); δ = 12.5 (10-CH3), 13.4 (9-CH3), 18.1, 18.2 ((CH3)2CH), 18.4 (C-5), 22.8 (C-11), 27.0 (C-8), 33.1 (40:0.3), 41.1 (C-9), 41.8 (C-7), 71.5 (C-10), 115.2 (C-1), 138.0 (C-2), 211.1 (C-6); HRMS (ESI): [M+Na]+ calcd for C17H29O4Na 377.28463, found 377.28459.

Macrolactone 32: Dien 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, 1:1). The pure lactone 32 (163 mg, 85%) was obtained as a slightly brown oil; Rf = 0.3 (petroleum ether/ethyl acetate, 3:1); [α]D20 = −67.6 (c = 2.0, CHCl3); 1H NMR (400 MHz, CDCl3); δ = 0.93 (d, J = 6.6 Hz, 3H; 9-H), 1.21–1.23 (m, 21 H; 3'-CH3), 1.74–1.82 (m, 1H; 11-H), 1.40–1.48 (m, 1H; 1-H), 1.55–1.72 (m, 5H; 3'-H, 8'-H, 8-H), 1.85–1.92 (m, 2H; 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; OCH3), 3.80 (s, 3H; OCH3), 3.90 (dd, J = 3.5, 13.4 Hz, 4H; OCH2CH2O), 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 = 1.6 Hz, 1H; 1'-H), 6.59 (d, J = 2.0 Hz, 1H; 5'-H); 13C NMR (100 MHz, CDCl3); δ = 14.8 (3'-CH3), 17.0 (C-1), 23.0 (C-2), 26.4 (C-4), 33.9 (C-5), 34.6 (C-1'), 36.3 (C-3), 37.9 (C-3'), 55.4 (OCH3), 55.8 (OCH3), 64.9 (OCH2CH2O), 74.9 (C-2), 98.2 (C-3), 101.2 (C-5), 111.7 (C-1), 114.6 (C-6), 116.8 (C-8), 116.9 (C-10), 133.9 (C-7), 137.4 (C-4), 138.6 (C-6), 157.9 (CO), 161.2 (C-2), 167.6 (C-4); HRMS (ESI): [M+Na]+ calcd for C24H36O5Na 455.24041, found 455.24038.

Macrolactone 33: A solution of lactone 32 (172 mg, 0.424 mmol) in acetone/H2O (7 mL, 10:1) containing pTsOH (4 mg, 0.021 mmol) was refluxed for 12 h. After being cooled, saturated NaHCO3 solution was added and the mixture was extracted with CHCl3 (3 × 30 mL). The combined organic layers were dried over MgSO4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (petroleum ether/ethyl acetate, 1:1) to afford the pure ketone 33 (130 mg, 85%) as a colorless oil; Rf = 0.33 (petroleum ether/ethyl acetate, 3:1); [α]D20 = −29.3 (c = 2.0, CHCl3); 1H NMR (400 MHz, CDCl3); δ = 0.95 (d, J = 6.9 Hz, 3H; 9'-H, 10'-H, 2203 – 2212
Macrolactone 34: 1H NMR (500 MHz, CDCl3): \(\delta = 1.20 (s, 3 H; 10-C), 1.36 (s, 3 H; 9-C), 1.59 (s, 3 H; 8-C), 1.92 (s, 3 H; 7-C), 3.81 (s, 3 H; 5-CH3), 3.83 (s, 3 H; 3-CH3), 5.24 (ddd, J = 13.2, 6.0, 3.0 Hz, 1 H; 10-H), 4.95–6.02 (m, 1 H; 2-H), 2.68 (dd, d = 15.8, 1.0 Hz, 1 H; 1-H), 6.38 (d, d = 2.15, 1 H; 3-H), 6.58 (d, d = 2.10, 1 H; 5-H); \(\delta_{13}C\) NMR (100 MHz, CDCl3): \(\delta = 43.3 (\text{C}-5, \text{CH}_3), 56.7 (\text{OCH}_3), 76.8 (\text{C}-10), 97.7 (\text{C}-3), 102.9 (\text{C}-5), 106.0 (\text{C}-3), 132.0 (\text{C}-2), 135.8 (\text{C}-9), 147.5 (\text{C}-10), 157.2 (\text{C}-8), 167.2 (\text{CO}_2), 211.3 (\text{C}-6); \) HRMS (ESI): [M+Na]+ calc for \(\text{C}_{20}\text{H}_{26}\text{O}_5\) 338.1829, found 338.1827.

Macrolactone 35: A flask was charged with aluminum powder (58 mg, 2.15 mmol) and iodine (85 mg, 0.33 mmol). Then benzene (3 mL) was added and the mixture was heated to reflux. After removal of benzene under reduced pressure the residue was purified by flash chromatography (petroleum ether/ethyl acetate, 3:1) to give lactone 35 (17.5 mg, 0.049 mmol), which were dissolved in benzene (1 mL), were added. After complete addition, the mixture was stirred for 3 hours, and the residue was purified by flash chromatography (petroleum ether/ethyl acetate, 3:1) to give zearelenone analogue 35 (14 mg, 86%) as a colorless oil; \(R_t = 0.28\) (petroleum ether/ethyl acetate, 3:1); \(\delta_{13}C = 22.0 (c = 0.5, \text{THF}); \) \(\delta_{13}NMR (400 MHz, CDCl3); \delta = 0.98 (d, J = 6.4 Hz, 3 H; \text{9-CH}_3), 1.27 (d, J = 6.4 Hz, 3 H; \text{10-CH}_3), 1.40–1.80 (m, 1 H; \text{4-CH}_3), 1.74–1.95 (m, 3 H; \text{8-CH}_3, \text{9-CH}_3), 2.00–2.17 (m, 2 H; \text{4-CH}_3, \text{5-CH}_3), 2.28–2.36 (m, 2 H; \text{3-CH}_3, \text{4-CH}_3), 2.42–2.56 (m, 3 H; \text{8-CH}_3, \text{7-CH}_3), 5.12–5.14 (m, 1 H; \text{10-CH}), 5.81–5.88 (m, 1 H; \text{2-CH}), 6.32 (s, 1 H; \text{3-CH}), 6.45 (s, 1 H; \text{5-CH}), 6.63 (d, J = 15.5 Hz, 1 H; \text{1-CH}), 11.42 (s, 1 H; \text{OH}); \) \(\delta_{13}C\) NMR (100 MHz, CDCl3); \delta = 13.4 (9-CH3), 15.8 (10-CH3), 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 (OCH3), 76.9 (C-10), 104.7 (C-3), 107.5 (C-1), 120.2 (C-1), 123.5 (C-2), 140.2 (C-6), 161.8 (C-4), 170.8 (C-8), 211.6 (C-6); HRMS (ESI): [M+Na]+ calc for \(\text{C}_{19}\text{H}_{24}\text{O}_5\) 369.16725, found 369.16702.

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.[27,30] 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 His6 fusion protein to aid purification. Expression was performed in BL21 (DE3) cells in LB media containing ampicillin (100 µg mL\(^{-1}\)). After induction with IPTG (0.5 mm) the growth temperature was lowered to 18°C and incubation was continued for 24 h. The cells were centrifuged, re-suspended 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, 16,500 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 75 g gel filtration column equilibrated in HEPES buffer (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 85,428 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]