Bioorganic & Medicinal Chemistry 18 (2010) 5732–5737

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Efficient synthesis of Hsp90 inhibitor dimers as potential antitumor agents

Hironori Sekiguchi [a], Kazuhiro Muranaka [a], Akiko Osada [b], Satoshi Ichikawa [a,*], Akira Matsuda [a,*]

[a] Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060-0812, Japan
[b] Discovery & Development Laboratory I, Hanno Research Center, Taiho Pharmaceutical Co., Ltd, 1-27 Misugidai, Hanno, Saitama 357, Japan

ARTICLE INFO

Article history:
Received 7 May 2010
Revised 25 May 2010
Accepted 26 May 2010
Available online 9 June 2010

Keywords:
Hsp90
Antitumor agent
Copper-catalyzed alkyne azide coupling

ABSTRACT

The PU-H58-dimers **13a**–**15b** were efficiently synthesized and their biological properties were evaluated. The copper-catalyzed alkyne azide coupling was effective in simultaneously linking three components via a triazole formation to afford the target dimers. These synthesized dimers exhibited binding affinity to the N-terminal domain of Hsp90, cytotoxicity, and client degradation activity although these activities were comparative or weak comparable with that of the parent compound.

1. Introduction

Cancer cells acquire sets of functional capacities known as the six hallmarks of cancer[1] and consequently it is difficult to treat cancer cells by malfunction of a single cancer-causing target in current cancer chemotherapy. The heat shock protein 90 (Hsp90) family is a group of molecular chaperones[2–5] that plays a key role in the folding of polypeptide chains called client proteins.[6] Many of the Hsp90 client proteins, for example, Bcr-abl, Akt, C-Raf, CDK4, Her2, and the estrogen receptor (ER), are oncogenic and are also considered hallmarks of the disease.[7,8] Increased expression of Hsp90 helps the cancer cell manage not only the stress caused by mutation and overexpression of oncogenes but also the stress due to hypoxia, nutrient deprivation and acidosis.[9,10] Inhibition of Hsp90 leads to degradation of these various client proteins by the proteasome. The simultaneous combinatorial depletion of many cancer-causing client proteins and the modulation of all of the hallmarks of cancer are the major advantages of Hsp90 inhibitors. Hsp90 exists predominantly as a dimer in the cell, with each subunit being made up of three functional domains: an N-terminal ATP-binding domain; a middle domain; and a C-terminal dimerization domain. A natural product, geldanamycin,[11] is known to be a strong ATP-competitive inhibitor of the Hsp90 N-terminal domain,[12–15] and the 17-allylamino derivative (17-AAG) and the 17-dimethylaminoethyl derivative (17-DMAG) are currently in phase II clinical studies.[16] The 8,9-disubstituted purine class of molecules, for example, PU3 (Fig. 1) (**1**)[17] and PU-H58 (**2a**),[18] are the first fully synthetic Hsp90 inhibitors with the ability to bind at the ATP-binding site of the N-terminal domain. This class of synthetic inhibitors is also active in cells causing degradation of Erb B2 kinase, ER, and *C*-Raf kinase.

We have recently synthesized PU3-dimers **3** according to a structure-based drug design using the X-ray crystal structure of the full length Hsp90 dimer and found that by increasing the

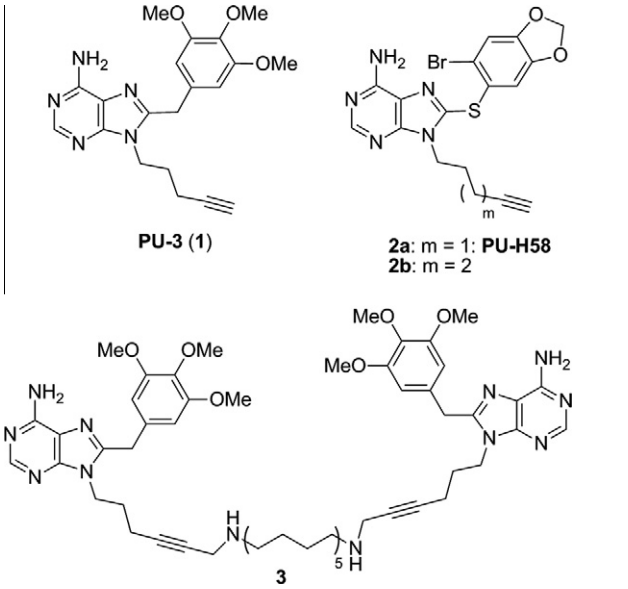


Figure 1. Structure of PU-3, PU-H58, and its dimer.

* Corresponding authors. Tel.: +81 11 706 3763; fax: +81 11 706 4980 (S.I.); tel.: +81 11 706 3228; fax: +81 11 706 4980 (A.M.).
E-mail addresses: ichikawa@pharm.hokudai.ac.jp (S. Ichikawa), matuda@pharm.hokudai.ac.jp (A. Matsuda).

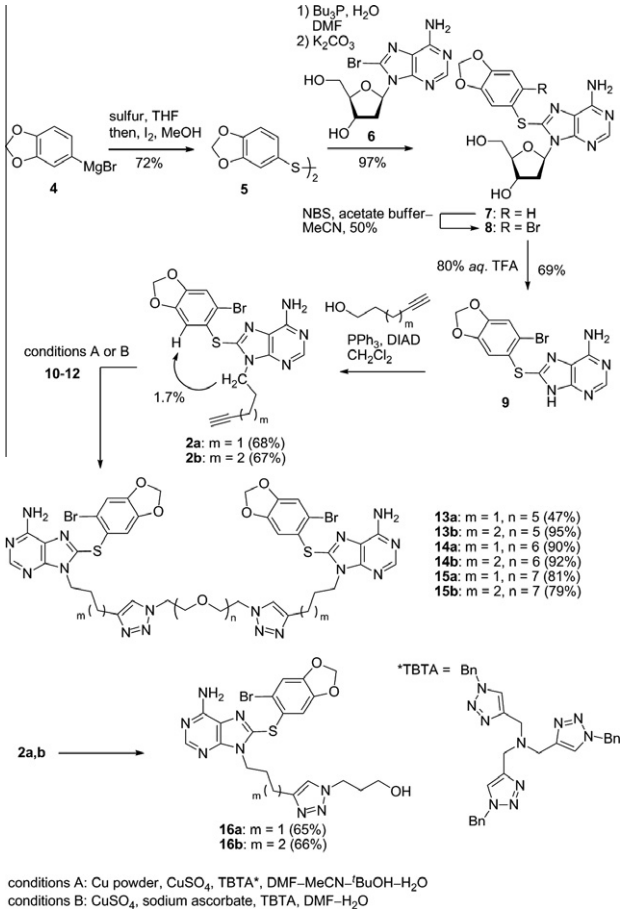
0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.05.075

length of the bridging linker, the PU3 dimers became more cytotoxic against human breast cancer cell lines.[19] Compound **3**, with a C-20 linker, exhibited a 20–30-fold increase in cytotoxicity compared with that of PU3. However the preparation of these analogs was not feasible in terms of the yields and product isolation to pursue further optimization. Herein we describe the efficient synthesis of Hsp90 inhibitor dimers using a copper-catalyzed alkyne azide coupling (CuAAC). Biological evaluations of the synthesized analogs are also described.

2. Results and discussion

2.1. Chemistry

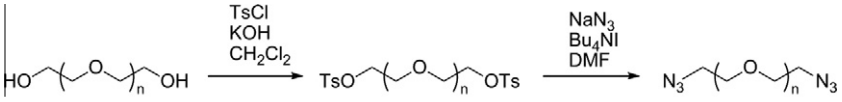
We planned to systematically prepare the dimers of PU-H58 (**2a**), which was reported to be a better ligand of the Hsp90 N-terminal domain than PU-3, and its homolog (**2b**) by varying the length of the linker enough to cover the distance (ca. 25 Å) of the two ATP-binding sites of the N-terminal domains.[20] Considering water solubility, we chose an ethylene glycol repeat as the linker connecting the two N-terminal ligands. The CuAAC[21] was chosen for the reaction linking the linker and the two N-terminal ligands, since a variety of functional groups are well tolerated in the CuAAC, which proceeds even in aqueous media in good yields, thereby making it suitable for linking polar molecules and/or macromolecules. This strategy allowed us to prepare the target molecules without using any protecting groups during the synthesis. The synthesis of the dimers **13a**–**15b** is outlined in Scheme 1. 3,4-Methylenedioxyphenyl magnesium bromide (**4**) was treated with sulfur to give the corresponding thiol, which was difficult to isolate because of its susceptibility to air oxidation. Therefore the thiol was sequentially oxidized by I_2 in MeOH to give the disulfide **5** in 72% over two steps. After **5** was reduced with Bu_3P and H_2O, the resulting thiol was reacted with 8-bromo-2′-deoxyadenosine (**6**) in the presence of K_2CO_3 in DMF to give **7** in 97% yield. Bromination of the methylenedioxyphenyl ring by NBS gave **8** selectively in 50% yield. Acid-catalyzed hydrolysis of the glycosidic bond of **8** afforded **9** in 69% yield. Mitsunobu reaction of **9** with 4-pentyn-1-ol or 5-hexyn-1-ol in CH_2Cl_2 gave the desired N^9-substituted adenine derivatives **2a** or **2b** as the major products along with the corresponding N^3-isomers. This was confirmed by NOE experiments, where the correlation of the H-6′ proton (1.7%) in **2a** was observed upon irradiation at the methylene protons connected to the *N*-9 position and no correlation was observed between amino protons at the *N*-6 position and the methylene protons. With the alkyne units in hand, we next examined the CuAAC linking two molecules of **2** with the bis(azido)ethylene glycol linkers **10**–**12**. The linkers **10**–**12** were prepared from the corresponding diols **17**–**19** by conventional methods as shown in Scheme 2. Two types of conditions for CuAAC were used to prepare the dimmers, namely Cu powder, $CuSO_4$, and tris(benzyltriazolylmethyl)amine[22] (TBTA) ligand in DMF–MeCN–*t*BuOH–H_2O (conditions A); and $CuSO_4$, sodium ascorbate, and TBTA in DMF–H_2O (conditions B). Under both sets of conditions, the reaction proceeded effectively at both termini of the linker, and the desired dimers **13a**–**15b** were obtained in good yields (47–95%).



Scheme 1.

2.2. Biological activity

The biological activity of the synthesized compounds was then evaluated, and the results are summarized in Table 1. The binding affinity of the compounds to the N-terminal domain of human Hsp90α was first evaluated by competitive binding to a biotinylated geldanamycin, which was quantified by Alpha screen. The IC_{50} values of **16a**,**b**, which are reference monomeric analogues with a triazole substituent, were 0.49 μM and 0.51 μM, respectively, and they exhibited a similar potency to **2a**,**b** (0.3 μM for **2a**, 1.4 μM for **2b**). This indicated that introducing the triazole moiety did not influence the binding of the PU-H58 moiety to the N-terminal domain. All the dimers also retained affinity to the N-terminal domain, which was regarded as a monomeric state of Hsp90α. It was observed that homologation reduced the binding affinity in all cases. The cytotoxic activity of these analogues was next evaluated against the SKBr3 human breast cancer cells. All the synthesized dimers showed moderate cytotoxicity with IC_{50}s ranging from 1.4–5.6 μM, and their activity was comparable or reduced in some cases (**15a**) compared to the parent **2a**, which exhibited the previously reported activity (0.33 μM for SKBr3). To determine whether the observed cytotoxicity was related to Hsp90 inhibition, degradation of the Hsp90 client protein Her2 upon treatment with the compounds was evaluated. Thus, compounds were incubated with SKBr-3 breast cancer cells for 24 h, and the protein lysates were analyzed by Western blot with the anti-Her2 antibody. The amount of Her2 was normalized by the expression level of GAPDH, which is not dependent on the Hsp90 protein folding machinery. The dimers **13a**–**15b** did exhibit the Her2 degradation activity, which was well-correlated to the cytotoxicity. These results clearly indicated that the dimers exhibited cytotoxicity against SKBR-3 breast cancer cells through Hsp90 inhibition. Contrary to our previous study,[19] the length of the linker did not correlate to the cytotoxicity or to the client degradation activity, in spite of the length of the linkers (approximate distance between the two triazole rings for **13**, **14**, or **15** is 22, 25, or 29 Å in linear length, respectively) being enough to fit the two ATP-binding sites of the N-terminal domains of the closed form Hsp90 (ca. 25 Å). This may be attributed



17: n = 5
18: n = 6
19: n = 7

20: n = 5 (quant.)
21: n = 6 (quant.)
22: n = 7 (71%)

10: n = 5 (61%)
11: n = 6 (57%)
12: n = 7 (83%)

Scheme 2.

Table 1
Biological evaluation of the dimers

Compound	IC_{50} (μM)		
	Hsp90α binding[a]	Her2 degradation[b]	Cytotoxicity (SKBR-3)[c]
2a	0.30	0.46	0.33
2b	1.4	2.6	1.4
13a	0.65	6.3	3.8
13b	5.1	5.2	3.0
14a	0.79	7.1	5.6
14b	3.4	4.7	5.0
15a	1.1	9.2	4.8
15b	3.1	4.2	4.8
16a	0.49	3.9	1.8
16b	0.51	2.9	1.4

[a] Binding of biotinylated geldanamycin to the N-terminal domain of human Hsp90a was evaluated in the presence of drugs by Alpha screen.
[b] SKBR-3 cells were plated 2×10^5 cells/well and seeded for 24 h. After 24 h incubation in the presence of drugs or DMSO, lysates were prepared using M-PER® reagent. Clarified protein lysates were analyzed by Western blotting with anti-Her2 and anti-GAPDH.
[c] Cells were cultured in the presence of test compounds for 72 h and then fixed with glutaraldehyde. Fixed cells were stained in 0.05% crystal violet solution. The dye was extracted and the absorbance was read at 540 nm on a microplate reader.

to the reduced membrane-permeability of the dimers because of the physicochemical properties of the ethylene glycol linker.

3. Conclusion

The PU-H58-dimers **13a**–**15b** were efficiently synthesized and their biological properties were evaluated. The CuAAC was effective in simultaneously linking three components via triazole formation to afford the target dimers having relatively high molecular weight. The synthesized dimers exhibited binding affinity to the N-terminal domain of Hsp90, cytotoxicity, and client degradation activity although these activities were comparable or weak compared with that of the parent compound. However, the length of the linker did not correlate to cytotoxicity and client degradation activity. This may be attributed to the reduced membrane-permeability of the dimers because of the physicochemical properties of the ethylene glycol linker. Optimization of the linker will be necessary to improve the cytotoxicity to develop potent Hsp90 inhibitors.

4. Experimental

4.1. General experimental methods

NMR spectra were obtained on a JEOL ECX400 or JEOL ECA500, and were reported in parts per million (δ) relative to tetramethylsilane (0.00 ppm) as internal standard otherwise noted. Coupling constant (J) was reported in hertz (Hz). Abbreviations of multiplicity were as follows; s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, br: broad. Data were presented as follows; chemical shift (multiplicity, integration, coupling constant). Assignment was based on ^{1}H–^{1}H COSY, HMBC, and HMQC NMR spectra. FAB-MS was obtained on a JEOL JMS-HX110. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60F254 plates. Normal-phase column chromatography was performed on Merck silica gel 5715. Flash column chromatography was performed on Merck silica gel 60.

4.1.1. Di-(3,4-methylenedioxyphenyl) disulfide (5)

A solution of 3,4-methylenedioxyphenylmagnesium bromide **4** (1.0 M solution in toluene/THF, 1:1, 95 mL, 95 mmol) in THF (480 mL) was treated with sulfur (3.0 g, 95 mmol) at 0 °C for 1 h. The reaction mixture was allowed to room temperature and stirred for additional 4 h. The reaction was quenched with 5 N HCl, and the mixture was extracted three times with AcOEt. The collected organic layers were washed with H_2O and brine, dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (ϕ 7 × 14 cm, 5% AcOEt/hexane) to give the mixture of **5** and 3,4-methylenedioxybenzenethiol. The mixture of **5** and 3,4-methylenedioxybenzenethiol in MeOH (200 mL) was treated with I_2 (5.1 g, 20 mmol) at room temperature for 2 h. The reaction was quenched with saturated aqueous $Na_2S_2O_3$, and the mixture was partitioned between AcOEt and H_2O. The organic layer was washed with brine, dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (ϕ 7 × 14 cm, 5% AcOEt/hexane) to give **5** (11 g, 72%) as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.00 (d, 2H, H-2, J = 1.8 Hz), 6.94 (dd, 2H, H-6, J = 1.8, 7.7 Hz), 6.72 (d, 2H, H-5, J = 7.7 Hz), 5.97 (s, 4H, OCH_2O); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.3, 148.2, 129.9, 124.8, 111.1, 108.7, 101.6; EIMS-LR m/z = 306 $(M)^+$; EIMS-HR calcd for $C_{14}H_{10}O_4S_2$ 306.0021, found 306.0016.

4.1.2. 9-(2-Deoxy-β-D-*ribo*-pentofuranosyl)-8-(3,4-methylenedioxyphenylthio)adenine (7)

A solution of **5** (7.7 g, 25 mmol) in DMF (250 mL) was treated with tributylphosphine (6.2 mL, 25 mmol) and H_2O (0.68 mL, 38 mmol) at room temperature for 1 h. Potassium carbonate (10 g, 75 mmol) and **6**[23] (12 g, 35 mmol) were added to the mixture, which was stirred for additional 2 h. The mixture was concentrated in vacuo, and the residue was triturated from MeOH and $CHCl_3$ to give **7** (14 g, 97%) as a pale yellow solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.08 (s, 1H, H-2), 7.47 (br s, 2H, NH_2), 7.02 (s, 1H, H-2″), 6.94 (s, 2H, H-5″ and H-6″), 6.48 (dd, 1H, H-1′, J = 6.3, 8.3 Hz), 6.04 (s, 2H, OCH_2O), 5.52 (dd, 1H, OH-5′, J = 4.0, 8.3 Hz), 5.29 (d, 1H, OH-3′, J = 4.6 Hz), 4.42 (m, 1H, H-3′), 3.88 (m, 1H, H-4′), 3.66 (dt, 1H, H-5′, J = 4.0, 12.3 Hz), 3.49 (ddd, 1H, H-5′, J = 4.0, 8.3, 12.3 Hz), 3.04 (ddd, 1H, H-2′b, J = 5.8, 8.3, 13.0 Hz), 1.98 (ddd, 1H, H-2′a, J = 2.9, 6.3, 13.0 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 155.5, 152.4, 150.0, 148.2, 148.0, 145.0, 125.7, 122.7, 119.9, 111.8, 109.3, 101.8, 88.5, 85.7, 71.4, 62.3, 37.8; ESIMS-LR m/z = 426 $(M+Na)^+$; ESIMS-HR calcd for $C_{17}H_{17}N_5O_5SNa$ 426.0848, found 426.0864.

4.1.3. 8-(6-Bromo-3,4-methylenedioxyphenylthio)-9-(2-deoxy-β-D-*ribo*-pentofuranosyl)adenine (8)

A suspension of **7** (10 g, 25 mmol) in 1 M acetate buffer (pH 4.0)/CH_3CN (2:1, 750 mL) was treated with NBS (8.9 g, 50 mmol)

at room temperature for 3 h. The mixture was extracted three times with $CHCl_3$. The collected organic layers were washed with saturated aqueous $NaHCO_3$, H_2O and brine, dried (Na_2SO_4), filtered and concentrated in vacuo. The residue was triturated from hexane and AcOEt to give **8** (6.1 g, 50%) as a brown solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H, H-2), 7.50 (br s, 2H, NH_2), 7.37 (s, 1H, H-5″), 6.83 (s, 1H, H-2″), 6.42 (dd, 1H, H-1′, J = 6.3, 8.2 Hz), 6.09 (s, 2H, OCH_2O), 5.47 (m, 1H, OH-5′), 5.29 (d, 1H, OH-3′, J = 3.6 Hz), 4.43 (m, 1H, H-3′), 3.88 (m, 1H, H-4′), 3.66 (m, 1H, H-5′), 3.50 (m, 1H, H-5′), 3.11 (ddd, 1H, H-2′b, J = 5.9, 8.2, 12.7 Hz), 2.05 (ddd, 1H, H-2′a, J = 1.9, 6.3, 12.7 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.5, 152.5, 150.0, 148.6, 147.9, 143.6, 124.3, 120.1, 115.3, 113.1, 111.6, 102.7, 88.4, 85.6, 71.3, 62.2, 37.7; ESIMS-LR m/z = 504 (M+Na)$^+$; ESIMS-HR calcd for $C_{17}H_{16}BrN_5O_5SNa$ 503.9953, found 503.9950.

4.1.4. 8-(6-Bromo-3,4-methylenedioxyphenylthio)adenine (9)

A solution of **8** (24 mg, 0.050 mmol) in 80% aqueous TFA (2 mL) was stirred at room temperature for 1 h. The reaction was quenched with saturated aqueous $NaHCO_3$, and the mixture was extracted five times with $CHCl_3$. The collected organic layers were washed with brine, dried (Na_2SO_4), filtered and concentrated in vacuo to give **9** (13 mg, 69%) as a yellow solid, properties of which were identical in all respects to those for previously reported:[18b] ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.06 (s, 1H, H-2), 7.36 (s, 1H, H-5′), 7.22 (br s, 2H, NH_2), 6.99 (s, 1H, H-2′), 6.10 (s, 2H, OCH_2O).

4.1.5. 8-(6-Bromo-3,4-methylenedioxyphenylthio)-9-(pent-4-ynyl)adenine (2a: PU-H58)

A suspension of **9** (500 mg, 1.4 mmol), 4-pentyn-1-ol (190 μL, 2.1 mmol) and PPh_3 (790 mg, 3.0 mmol) in CH_2Cl_2 (14 mL) was treated with DIAD (1.4 mL, 6.9 mmol) at room temperature for 1 h. The solvent was removed in vacuo, and the residue was purified by flash silica gel column chromatography (ϕ 1 × 22 cm, 1% MeOH/$CHCl_3$) to give **2a** (400 mg, 68%) as a pale yellow foam: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 (s, 1H, H-2), 7.36 (br s, 2H, NH_2), 7.35 (s, 1H, H-5′), 6.83 (s, 1H, H-2′), 6.08 (s, 2H, OCH_2O), 4.21 (t, 2H, CH_2CH$_2$CH$_2$C≡CH, J = 7.4 Hz), 2.78 (t, 1H, CH$_2$CH$_2$CH$_2$C≡CH, J = 2.6 Hz), 2.20 (dt, 2H, CH$_2$CH$_2$CH_2C≡CH, J = 2.6, 6.9 Hz), 1.87 (tt, 2H, CH$_2$CH_2CH$_2$C≡CH, J = 6.9, 7.4 Hz).

4.1.6. 8-(6-Bromo-3,4-methylenedioxyphenylthio)-9-(hex-5-ynyl)adenine (2b)

Compound **2b** (410 mg, 67%) was obtained from **9** (500 mg, 1.4 mmol) and 5-hexyn-1-ol (230 μL, 2.1 mmol) as described for the synthesis of **2a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.32 (s, 1H, H-2), 7.07 (s, 1H, H-5′), 6.83 (s, 1H, H-2′), 5.97 (s, 2H, OCH_2O), 5.88 (br s, 2H, NH_2), 4.23 (t, 2H, CH_2CH$_2$CH$_2$CH$_2$C≡CH, J = 7.4 Hz), 2.22 (dt, 2H, CH$_2$CH$_2$CH$_2$CH_2C≡CH, J = 2.6, 7.4 Hz), 1.93 (t, 1H, CH$_2$CH$_2$-CH$_2$CH$_2$C≡CH, J = 2.6 Hz), 1.90 (tt, 2H, CH$_2$CH_2CH$_2$CH$_2$C≡CH, J = 6.9, 7.4 Hz), 1.55 (tt, 2H, CH$_2$CH$_2$CH_2CH$_2$C≡CH, J = 6.9, 7.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.7, 153.2, 151.7, 149.2, 148.2, 145.7, 123.9, 120.3, 117.2, 113.5, 112.7, 102.5, 83.7, 69.1, 43.5, 29.0, 25.6, 18.2; ESIMS-LR m/z = 446 (M+H)$^+$; ESIMS-HR calcd for $C_{18}H_{17}BrN_5O_2S$ 446.0286, found 446.0280.

4.1.7. 3,6,9,12,15-Pentaoxaheptadecane-1,17-diol ditosylate (20)

A mixture of hexaethyleneglycol **17** (140 mg, 0.50 mmol) and KOH (220 mg, 4.0 mmol) in CH_2Cl_2 (1.0 mL) was treated with *p*-toluenesulfonyl chloride (190 mg, 1.0 mmol) at 0 °C for 1 h. The reaction was quenched with H_2O, and the mixture was extracted three times with CH_2Cl_2. The collected organic layers were washed with brine, dried (Na_2SO_4), filtered and concentrated in vacuo to give **20** (300 mg, quant.) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, 4H, Ts, J = 8.2 Hz), 7.34 (d, 4H, Ts, J = 8.1 Hz), 4.15 (t, 4H, TsOCH_2CH$_2$, J = 5.0 Hz), 3.67 (t, 4H, TsOCH$_2$CH_2, J = 5.0 Hz), 3.64–3.56 (m, 16H, CH_2), 2.43 (s, 6H, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.4, 133.1, 129.9, 128.1, 70.8, 70.7, 70.6, 69.4, 68.8, 21.7; FABMS-LR m/z = 591 (M+H)$^+$; FABMS-HR calcd for $C_{26}H_{39}O_{11}S_2$ 591.1934, found 591.1957.

4.1.8. 3,6,9,12,15,18-Hexaoxaeicosane-1,20-diol ditosylate (21)

Compound **21** (320 mg, quant.) was obtained from heptaethyleneglycol **18** (160 mg, 0.50 mmol) as described for the synthesis of **20**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, 4H, Ts, J = 8.2 Hz), 7.32 (d, 4H, Ts, J = 8.2 Hz), 4.13 (t, 4H, TsOCH_2, J = 4.9 Hz), 3.67–3.56 (m, 24H, CH_2), 2.42 (s, 6H, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.9, 133.1, 129.9, 128.1, 77.5, 77.2, 76.8, 70.9, 70.8, 70.7, 70.6, 69.4, 68.7, 21.7; FABMS-LR m/z = 635 (M+H)$^+$; FABMS-HR calcd for $C_{28}H_{43}O_{12}S_2$ 635.2196, found 635.2186.

4.1.9. 3,6,9,12,15,18,21-Heptaoxatrieicosane-1,23-diol ditosylate (22)

Compound **22** (320 mg, 71%) was obtained from octaethyleneglycol **19** (190 mg, 0.50 mmol) as described for the synthesis of **20**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, 4H, Ts, J = 8.5 Hz), 7.33 (d, 4H, Ts, J = 8.0 Hz), 4.14 (t, 4H, TsOCH_2CH$_2$, J = 4.6 Hz), 3.67 (t, 4H, TsOCH$_2$CH_2, J = 4.6 Hz), 3.62–3.57 (m, 24H, CH_2), 2.43 (s, 6H, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.9, 133.1, 129.9, 128.1, 70.9, 70.7, 70.6, 69.4, 68.8, 21.8; FABMS-LR m/z = 679 (M+H)$^+$; FABMS-HR calcd for $C_{30}H_{47}O_{13}S_2$ 679.2474, found 679.2458.

4.1.10. 1,17-Diazide-3,6,9,12,15-pentaoxaheptadecane (10)

A mixture of **20** (120 mg, 0.20 mmol) and Bu_4NI (5.5 mg, 0.015 mmol) in DMF (5 mL) was treated with NaN_3 (78 mg, 1.2 mmol) at 80 °C for 3 h. After cooling to room temperature, the solvent was removed in vacuo. The residue was diluted with Et_2O (2 mL), and the insoluble was filtered off. The filtrate was concentrated in vacuo, and the residue was purified by silica gel column chromatography (ϕ 1 × 6 cm, 80% AcOEt/hexane) to give **16b** (40 mg, 61%) as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.67–3.64 (m, 20H, CH_2), 3.37 (t, 4H, N$_3$CH_2, J = 5.1 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 70.8, 70.7, 70.1, 50.8; FABMS-LR m/z = 355 (M+Na)$^+$; FABMS-HR calcd for $C_{12}H_{24}N_6O_5Na$ 355.1706, found 355.1712.

4.1.11. 1,20-Diazide-3,6,9,12,15,18-hexaoxaeicosane (11)

Compound **11** (64 mg, 57%) was obtained from **21** (190 mg, 0.30 mmol) as described for the synthesis of **10**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.67–3.63 (m, 24H, CH_2), 3.37 (t, 4H, N$_3$CH_2, J = 5.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 70.8, 70.7, 70.1, 50.8; FABMS-LR m/z = 399 (M+Na)$^+$; FABMS-HR calcd for $C_{14}H_{28}N_6O_6Na$ 399.1968, found 399.1962.

4.1.12. 1,23-Diazide-3,6,9,12,15,18,21-heptaoxatrieicosane (12)

Compound **12** (100 mg, 83%) was obtained from **22** (180 mg, 0.27 mmol) as described for the synthesis of **10**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.65–3.61 (m, 28H, CH_2), 3.35 (t, 4H, N$_3$CH_2, J = 5.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 70.7, 70.6, 70.1, 50.7; FABMS-LR m/z = 443 (M+Na)$^+$; FABMS-HR calcd for $C_{16}H_{32}N_6O_7Na$ 443.2230, found 443.2216.

4.1.13. Dimer (13a)

A solution of **2a** (34 mg, 0.079 mmol), **10** (12 mg, 0.036 mmol), $CuSO_4$ (8.6 mg, 0.054 mmol) and tris(benzyltriazolylmethyl)amine (TBTA, 19 mg, 0.036 mmol) in DMF (1 mL) was treated with copper powder at room temperature, and the mixture was stirred for overnight. The solvent was removed in vacuo. The residue in $CHCl_3$ (1 mL) was treated with diamine silica gel for removal of copper at room temperature for 5 min. Copper powder and diamine silica gel were filtered off, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography

(ϕ 1.1 × (6 + 1) cm, 10% MeOH/$CHCl_3$) to give **13a** (20 mg, 47%) as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.29 (s, 2H, H-2), 7.49 (s, 2H, triazole), 7.04 (s, 2H, H-5′), 6.82 (s, 2H, H-2′), 6.08 (br s, 4H, NH_2), 5.96 (s, 4H, OCH_2O), 4.47 (t, 4H, NCH_2CH_2O, J = 5.1 Hz), 4.29 (t, 4H, $NCH_2CH_2CH_2$, J = 7.4 Hz), 3.81 (t, 4H, NCH_2CH_2O, J = 5.1 Hz), 3.57–3.56 (m 16H, OCH_2CH_2O), 2.74 (t, 4H, $NCH_2CH_2CH_2$, J = 8.0 Hz), 2.19 (tt, 4H, $NCH_2CH_2CH_2$, J = 7.4, 8.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.7, 153.1, 151.7, 149.3, 148.3, 146.4, 146.0, 123.6, 122.2, 120.2, 117.4, 113.5, 112.8, 102.6, 70.6, 70.5, 69.7, 50.2, 43.4, 29.3, 23.0; ESIMS-LR m/z = 1217 $(M+Na)^+$; ESIMS-HR calcd for $C_{46}H_{52}Br_2N_{16}O_9S_2Na$ 1217.1809, found 1217.1795.

4.1.14. Dimer (13b)

Compound **13b** (35 mg, 95%) was obtained from **2b** (29 mg, 0.066 mmol) and **10** (10 mg, 0.030 mmol) as described for the synthesis of **13a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.28 (s, 2H, H-2), 7.41 (s, 2H, triazole), 7.03 (s, 2H, H-5′), 6.81 (s, 2H, H-2′), 6.15 (br s, 4H, NH_2), 5.95 (s, 4H, OCH_2O), 4.46 (t, 4H, NCH_2CH_2O, J = 5.0 Hz) 4.22 (t, 4H, $NCH_2CH_2CH_2CH_2$, J = 7.2 Hz), 3.81 (t, 4H, NCH_2CH_2O, J = 5.0 Hz), 3.60–3.55 (m, 16H, OCH_2CH_2O), 2.71 (t, 4H, NCH_2CH_2-CH_2CH_2, J = 7.2 Hz), 1.84 (tt, 4H, $NCH_2CH_2CH_2CH_2$, J = 7.2, 8.1 Hz), 1.67 (tt, 4H, $NCH_2CH_2CH_2CH_2$, J = 7.2, 8.1 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.8, 153.1, 151.6, 149.2, 148.2, 147.3, 145.6, 123.7, 122.1, 120.2, 117.2, 113.4, 112.7, 102.5, 70.6, 70.5, 69.7, 50.2, 43.6, 29.4, 26.6, 25.3; ESIMS-LR m/z = 1245 $(M+Na)^+$; ESIMS-HR calcd for $C_{48}H_{56}Br_2N_{16}O_9S_2Na$ 1245.2122, found 1245.2143.

4.1.15. Dimer (14a)

Compound **14a** (33 mg, 90%) was obtained from **2a** (29 mg, 0.066 mmol) and **11** (11 mg, 0.030 mmol) as described for the synthesis of **13a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.28 (s, 2H, H-2), 7.51 (s, 2H, triazole), 7.03 (s, 2H, H-5′), 6.81 (s, 2H, H-2′), 6.25 (br s, 4H, NH_2), 5.94 (s, 4H, OCH_2O), 4.47 (t, 4H, NCH_2CH_2O, J = 5.1 Hz), 4.28 (t, 4H, $NCH_2CH_2CH_2$, J = 6.8 Hz), 3.81 (t, 4H, NCH_2CH_2O, J = 5.1 Hz), 3.56–3.55 (m 20H, OCH_2CH_2O), 2.74 (t, 4H, $NCH_2CH_2CH_2$, J = 7.5 Hz), 2.18 (tt, 4H, $NCH_2CH_2CH_2$, J = 6.8, 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.8, 153.1, 151.7, 149.2, 148.2, 146.4, 145.7, 123.5, 122.2, 120.1, 117.4, 113.5, 112.8, 102.6, 70.6, 70.5, 69.6, 50.2, 43.4, 29.3, 23.0; ESIMS-LR m/z = 1261 $(M+Na)^+$; ESIMS-HR calcd for $C_{48}H_{56}Br_2N_{16}O_{10}S_2Na$ 1261.2071, found 1261.2044.

4.1.16. Dimer (14b)

Compound **14b** (35 mg, 92%) was obtained from **2b** (29 mg, 0.066 mmol) and **11** (11 mg, 0.030 mmol) as described for the synthesis of **13a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.28 (s, 2H, H-2), 7.42 (s, 2H, triazole), 7.03 (s, 2H, H-5′), 6.82 (s, 2H, H-2′), 6.17 (br s, 4H, NH_2), 5.95 (s, 4H, OCH_2O), 4.47 (t, 4H, NCH_2CH_2O, J = 5.2 Hz) 4.22 (t, 4H, $NCH_2CH_2CH_2CH_2$, J = 7.5 Hz), 3.81 (t, 4H, NCH_2CH_2O, J = 5.2 Hz), 3.60–3.56 (m, 20H, OCH_2CH_2O), 2.71 (t, 4H, NCH_2CH_2-CH_2CH_2, J = 7.5 Hz), 1.84 (tt, 4H, $NCH_2CH_2CH_2CH_2$, J = 6.9, 7.5 Hz), 1.67 (tt, 4H, $NCH_2CH_2CH_2CH_2$, J = 6.9, 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.8, 153.1, 151.6, 149.2, 148.2, 147.3, 145.6, 123.7, 122.1, 120.2, 117.3, 113.4, 112.8, 102.6, 70.6, 70.5, 69.7, 50.2, 43.6, 29.4, 26.6, 25.3; ESIMS-LR m/z = 1289 $(M+Na)^+$; ESIMS-HR calcd for $C_{50}H_{60}Br_2N_{16}O_{10}S_2Na$ 1289.2384, found 1289.2360.

4.1.17. Dimer (15a)

Compound **15a** (31 mg, 81%) was obtained from **2a** (29 mg, 0.066 mmol) and **12** (13 mg, 0.030 mmol) as described for the synthesis of **13a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (s, 2H, H-2), 7.52 (s, 2H, triazole), 7.03 (s, 2H, H-5′), 6.82 (s, 2H, H-2′), 6.26 (br s, 4H, NH_2), 5.94 (s, 4H, OCH_2O), 4.48 (t, 4H, NCH_2CH_2O, J = 5.2 Hz), 4.27 (t, 4H, $NCH_2CH_2CH_2$, J = 6.9 Hz), 3.81 (t, 4H, NCH_2CH_2O, J = 5.2 Hz), 3.57–3.56 (m, 24H, OCH_2CH_2O), 2.74 (t, 4H, $NCH_2CH_2CH_2$, J = 7.4 Hz), 2.17 (tt, 4H, $NCH_2CH_2CH_2$, J = 6.9, 7.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.8, 153.1, 151.6, 149.3, 148.2, 146.4, 145.7, 123.4, 122.3, 120.1, 117.5, 113.5, 112.9, 102.6, 70.6, 70.5, 69.6, 50.2, 43.4, 29.3, 23.0; ESIMS-LR m/z = 1305 $(M+Na)^+$; ESIMS-HR calcd for $C_{50}H_{60}Br_2N_{16}O_{11}S_2Na$ 1305.2333, found 1305.2307.

4.1.18. Dimer (15b)

Compound **15b** (31 mg, 79%) was obtained from **2b** (29 mg, 0.066 mmol) and **12** (13 mg, 0.030 mmol) as described for the synthesis of **13a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.29 (s, 2H, H-2), 7.43 (s, 2H, triazole), 7.05 (s, 2H, H-5′), 6.82 (s, 2H, H-2′), 6.07 (br s, 4H, NH_2), 5.97 (s, 4H, OCH_2O), 4.47 (t, 4H, NCH_2CH_2O, J = 5.2 Hz) 4.23 (t, 4H, $NCH_2CH_2CH_2CH_2$, J = 6.9 Hz), 3.82 (t, 4H, NCH_2CH_2O, J = 5.2 Hz), 3.60–3.57 (m, 24H, OCH_2CH_2O), 2.72 (t, 4H, NCH_2CH_2-CH_2CH_2, J = 8.0 Hz), 1.85 (tt, 4H, $NCH_2CH_2CH_2CH_2$, J = 6.9, 7.4 Hz), 1.68 (tt, 4H, $NCH_2CH_2CH_2CH_2$, J = 7.4, 8.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.7, 153.1, 151.6, 149.2, 148.2, 147.3, 145.8, 123.7, 122.1, 120.2, 117.4, 113.5, 112.8, 102.6, 70.6, 70.5, 69.7, 50.2, 43.6, 29.4, 26.6, 25.3; ESIMS-LR m/z = 1333 $(M+Na)^+$; ESIMS-HR calcd for $C_{52}H_{64}Br_2N_{16}O_{11}S_2Na$ 1333.2646, found 1333.2611.

4.1.19. 8-(6-Bromo-3,4-methylenedioxyphenylthio)-9-[{1-(3-hydroxypropyl)-1*H*-1,2,3-triazol-4-yl}propyl]adenine (16a)

A solution of **2a** (43 mg, 0.10 mmol), 3-azidopropanol (10 mg, 0.10 mmol), $CuSO_4$ (24 mg, 0.15 mmol) and TBTA (53 mg, 0.10 mmol) in DMF (1 mL) was treated with copper powder at room temperature, and the mixture was stirred for overnight. The solvent was removed in vacuo. The residue in $CHCl_3$ (1 mL) was treated with diamine silica gel for removal of copper at room temperature for 5 min. Copper powder and diamine silica gel were filtered off, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (ϕ 1 × 7 cm, 10% MeOH/$CHCl_3$) to give **16a** (35 mg, 65%) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.30 (s, 1H, H-2), 7.36, (s, 1H, triazole), 7.06 (s, 1H, H-5′), 6.84 (s, 1H, H-2′), 5.98 (s, 2H, OCH_2O), 5.86 (br s, 2H, NH_2), 4.46 (t, 2H, $NCH_2CH_2CH_2O$, J = 6.8 Hz), 4.27 (t, 2H, $NCH_2CH_2CH_2C$, J = 6.8 Hz), 3.59 (t, 2H, $NCH_2CH_2CH_2O$, J = 6.3 Hz), 2.78 (t, 2H, $NCH_2CH_2CH_2C$, J = 7.4 Hz), 2.20 (tt, 2H, $NCH_2CH_2CH_2C$, J = 6.8, 7.4 Hz), 2.08 (tt, 2H, $NCH_2CH_2CH_2O$, J = 6.3, 6.8 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.5, 153.1, 151.7, 149.3, 148.3, 146.5, 146.1, 123.4, 121.6, 120.2, 117.6, 113.6, 113.0, 102.6, 58.7, 46.9, 43.3, 32.6, 29.2, 23.0; ESIMS-LR m/z = 555 $(M+Na)^+$; ESIMS-HR calcd for $C_{20}H_{21}BrN_8O_3SNa$ 555.0538, found 555.052.

4.1.20. 8-(6-Bromo-3,4-methylenedioxyphenylthio)-9-[{1-(3-hydroxypropyl)-1*H*-1,2,3-triazol-4-yl}butyl]adenine (16b)

A solution of **2b** (45 mg, 0.10 mmol), 3-azidopropanol (10 mg, 0.10 mmol), $CuSO_4$ (1.9 mg, 0.012 mol) and TBTA (10 mg, 0.019 mmol) in DMF/^{t}BuOH/H_2O/CH_3CN (4:2:1:1, 2 mL) was treated with sodium L-ascorbate (8.0 mg, 0.040 mmol) at room temperature, and the mixture was stirred for overnight. The solvent was removed in vacuo. The residue in $CHCl_3$ (1 mL) was treated with diamine silica gel for removal of copper at room temperature for 5 min. Diamine silica gel were filtered off, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (ϕ 1.1 × (6 + 2) cm, 12% MeOH/$CHCl_3$) to give **16b** (36 mg, 66%) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.31 (s, 1H, H-2), 7.28 (s, 1H, triazole), 7.07 (s, 1H, H-5′), 6.86 (s, 1H, H-2′), 6.00 (s, 2H, OCH_2O), 5.80 (br s, 2H, NH_2), 4.46 (t, 2H, $NCH_2CH_2CH_2O$, J = 6.9 Hz) 4.24 (t, 2H, $NCH_2CH_2CH_2CH_2C$, J = 7.5 Hz), 3.60 (t, 2H, $NCH_2CH_2CH_2O$, J = 5.7 Hz), 2.74 (t, 2H, $NCH_2CH_2CH_2CH_2C$, J = 7.5 Hz), 2.09 (tt, 2H, $NCH_2CH_2CH_2O$, J = 5.7, 6.9 Hz), 1.85 (tt, 2H, $NCH_2CH_2CH_2CH_2C$, J = 7.5, 8.0 Hz), 1.70 (tt, 2H, $NCH_2CH_2CH_2CH_2C$, J = 7.5, 8.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.5, 153.1, 151.7, 149.2, 148.3, 147.5, 146.0, 123.7, 121.4, 120.3, 117.4, 113.5, 112.8, 102.6, 59.0, 46.8, 43.7, 32.6, 29.3, 26.5,

25.2; ESIMS-LR $m/z = 569$ $(M+Na)^+$; ESIMS-HR calcd for $C_{21}H_{23}BrN_8O_3SNa$ 569.0695, found 569.0669.

4.2. HSP90 competitive binding assay

His-tagged N-terminal fragment of human HSP90α (amino acids 2–236) was expressed in *Escherichia coli* and purified by Ni-NTA column chromatography. Competitive binding assay was done with purified HSP90α and biotin-labeled geldanamycin (InvivoGen) in the presence of different concentrations of test compounds. The binding of bition-labeled geldanamycin to HSP90α was detected using the AlphaScreen Histidine Detection Kit (PerkinElmer).

4.3. HER2 degradation assay

HER2 degradation was determined by cell-based ELISA assay. SK-BR-3 cells were exposed to the compounds for 6 h and then fixed in 4% paraformaldehyde. Fixed cells were permeabilized in 0.1% Triton-X100. HER2 and GAPDH was probed with anti-HER2 antibody (#2165, Cell signaling Technology) and anti-GAPDH antibody (#H86504 M, BioDesign), respectively, and then probed with IRDye-labeled secondary antibodies (LI-COR Biosciences). Both proteins were imaged with the Odyssey IR Imaging System (LI-COR Biosciences).

4.4. Cytotoxicity assay

Antiproliferative activities of the compounds against SK-BR-3 cells were measured using the crystal violet assay.[24] Cells were cultured in the presence of test compounds for 72 h and then fixed with glutaraldehyde. Fixed cells were stained in 0.05% crystal violet solution. The dye was extracted and the absorbance was read at 540 nm on a microplate reader.

Acknowledgments

This work was supported financially by a Grant-in-Aid from the Ministry of Education, Science, Sports, and Culture. We thank Ms. S. Oka (Center for Instrumental Analysis, Hokkaido University) for measurement of mass spectra.

References and notes

1. Hanahan, D.; Weinberg, R. A. *Cell* **2000**, *100*, 57.
2. Bukau, B.; Deuerling, E.; Pfund, C.; Craig, E. A. *Cell* **2000**, *101*, 119.
3. Hartl, F. U.; Hayer-Hartl, M. *Science* **2002**, *295*, 1852.
4. Young, J. C.; Agashe, V. R.; Siegers, K.; Hartl, F. U. *Nat. Rev. Mol. Cell Biol.* **2004**, *5*, 781.
5. Smith, D. F.; Whitesell, L.; Katsanis, E. *Pharmacol. Rev.* **1998**, *50*, 493.
6. See for example: (a) Maloney, A.; Workman, P. *Expert Opin. Biol. Ther.* **2002**, *2*, 3; (b) Wegele, H.; Muller, L.; Buchner, J. *Rev. Physiol. Biochem. Pharmacol.* **2004**, *151*, 1. The updated list is available at http://www.picard.ch/downloads/Hsp90interactors.pdf.
7. Pratt, W. B. *Proc. Soc. Exp. Biol. Med.* **1998**, *217*, 420.
8. Xu, W.; Neckers, L. *Clin. Cancer Res.* **2007**, *13*, 1625.
9. Whitesell, L.; Bagatell, R.; Falsey, R. *Curr. Cancer Drug Targets* **2003**, *3*, 349.
10. Mosser, D. D.; Morimoto, R. I. *Oncogene* **2004**, *23*, 2907.
11. (a) Deboer, C.; Meulman, P. A.; Wnuk, R. J.; Peterson, D. H. *J. Antibiot.* **1970**, *23*, 442; (b) Rinehart, K. L., Jr.; Sasaki, K.; Slomp, G.; Grostic, M. F.; Olson, E. C. *J. Am. Chem. Soc.* **1970**, *92*, 7591.
12. (a) Whitesell, L.; Shifrin, S. D.; Schwab, G.; Neckers, L. M. *Cancer Res.* **1992**, *52*, 1721; (b) Whitesell, L.; Mimnaugh, E. G.; De Costa, B.; Myers, C. E.; Neckers, L. M. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 8324.
13. (a) Grenert, J. P.; Sullivan, W. P.; Fadden, P.; Haystead, T. A.; Clark, J.; Mimnaugh, E.; Krutzsch, H.; Ochel, H. J.; Schulte, T. W.; Sausville, E.; Neckers, L. M.; Toft, D. O. *J. Biol. Chem.* **1997**, *272*, 23843; (b) Stebbins, C. E.; Russo, A. A.; Schneider, C.; Rosen, N.; Hartl, F. U.; Pavletich, N. P. *Cell* **1997**, *89*, 239; (c) Prodromou, C.; Roe, S. M.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. *Cell* **1997**, *90*, 65.
14. (a) Schulte, T. W.; Akinaga, S.; Soga, S.; Sullivan, W.; Stensgard, B.; Toft, D.; Neckers, L. M. *Cell Stress Chaperones* **1998**, *3*, 100; (b) Sharma, S. V.; Agatsuma, T.; Nakano, H. *Oncogene* **1998**, *16*, 2639.
15. Roe, S. M.; Prodromou, C.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. *J. Med. Chem.* **1999**, *42*, 260.
16. (a) Schnur, R. C.; Corman, M. L.; Gallaschun, R. J.; Cooper, B. A.; Dee, M. F.; Doty, J. L.; Muzzi, M. L.; Moyer, J. D.; DiOrio, C. I.; Barbacci, E. G.; Miller, P. E.; O'Brien, A. T.; Morin, M. J.; Foster, B. A.; Pollack, V. A.; Savage, D. M.; Sloan, D. E.; Pustilnik, L. R.; Moyer, M. P. *J. Med. Chem.* **1995**, *38*, 3806; (b) Schulte, T. W.; Neckers, L. M. *Cancer Chemother. Pharmacol.* **1998**, *42*, 273; (c) Banerji, U. *Proc. Am. Assoc. Cancer Res.* **2003**, *44*, 677; (d) Sausville, E. A. *Curr. Cancer Drug Targets* **2003**, *3*, 377; (e) Tian, Z.-Q.; Liu, Y.; Zhang, D.; Wang, Z.; Dong, S. D.; Carreras, C. W.; Zhou, Y.; Rastelli, G.; Santi, D. V.; Myles, D. C. *Bioorg. Med. Chem.* **2004**, *12*, 5317; (f) Jez, J. M.; Chen, J. C.-H.; Rastelli, G.; Stroud, R. M.; Santi, D. V. *Chem. Biol.* **2003**, *10*, 361.
17. Chiosis, G.; Timaul, M. N.; Lucas, B.; Munster, P. N.; Zheng, F. F.; Sepp-Lorenzino, L.; Rosen, N. *Chem. Biol.* **2001**, *8*, 289.
18. (a) He, H.; Zatorska, D.; Kim, J.; Aguirre, J.; Llauger, L.; She, Y.; Wu, N.; Immormino, R. M.; Gewirth, D. T.; Chiosis, G. *J. Med. Chem.* **2006**, *49*, 381; (b) Llauger, L.; He, H.; Kim, J.; Aguirre, J.; Rosen, N.; Peters, U.; Davies, P.; Chiosis, G. *J. Med. Chem.* **2005**, *48*, 2892.
19. Muranaka, K.; Sano, A.; Ichikawa, S.; Matsuda, A. *Bioorg. Med. Chem.* **2008**, *16*, 5862.
20. Ali, M. M.; Roe, S. M.; Vaughan, C. K.; Meyer, P.; Panaretou, B.; Piper, P. W.; Prodromou, C.; Pearl, L. H. *Nature* **2006**, *440*, 1013. PDB ID; 2cge and 2cg9.
21. (a) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057; (b) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596; (c) Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128.
22. Chan, T. R.; Hilgraf, R.; Sharpless, K. B.; Fokin, V. V. *Org. Lett.* **2004**, *6*, 2853.
23. Ettner, R. G.; Haak, U.; Niederweis, M.; Hillen, W. *Nucleosides Nucleotides* **1993**, *12*, 757.
24. Utsugi, T.; Aoyagi, K.; Asao, T.; Okazaki, S.; Aoyagi, Y.; Sano, M.; Wierzba, K.; Yamada, Y. *Jpn. J. Cancer Res.* **1997**, *88*, 992.