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Structure-based design of 7-carbamate analogs of geldanamycin

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Abstract—The 7-carbamate groups of geldanamycin and its 17-(2-dimethylaminoethyl)amino-17-demethoxy derivative (17-DMAG) bind the N-terminal domain of Hsp90 by establishing a network of hydrogen bonds which involve four buried water molecules. In this study, a structure-based approach was used to investigate the effects of displacing some of these waters by modification of the 7-carbamate. A general loss of binding to human Hsp90 was observed, except for replacement of the carbamate with a hydroxamate group which gave an analog with weak activity. Modeling of Hsp90–ligand interactions suggested that the hydroxamate was not able to displace the buried water molecules, while bulkier substituents able to do so proved inactive.

Geldanamycin, a benzoquinone ansamycin, binds to the N-terminal domain ATP binding site of heat shock protein 90 (Hsp90), inhibiting the chaperone activity of the protein.[1,2] By regulating the function and stability of many key signaling proteins, Hsp90 is of particular importance to the survival of cancer cells. To stabilize its client proteins, the chaperone assembles with other cochaperones and associated proteins to form a 'superchaperone' complex. The disruption of the Hsp90 client protein complexes leads to the ubiquitination and subsequent proteasomal degradation of the client proteins. Because many of the client proteins are oncogenic, Hsp90 is an attractive target for anticancer chemotherapy. Indeed, the geldanamycin analogs 17-allylamino-17-demethoxygeldanamycin (17-AAG) and 17-[2-(dimethylamino)ethyl]amino-17-demethoxygeldanamycin (17-DMAG) have both entered human clinical trials.[3] The crystal structures of human Hsp90–geldanamycin and Hsp90–17-DMAG complexes provided several features of the interaction,[4–6] one of which was that the 7-carbamate of geldanamycin is located deep in the binding pocket and coordinates four buried water molecules through a hydrogen-bonding network with residues D93, G97, T184, S52, I91, V186, L48, and N51 (Fig. 1A). The few structure–activity relationships available suggested that the 7-carbamate substituent of geldanamycin plays an important role in cytotoxic activity. Removal of the carbamate or replacement with urea, thiourea or oxalate reduced cytotoxicity dramatically.[7] Disruption of the carbamate hydrogen bond network by D79N mutation in yeast Hsp90, which corresponds to D93N mutation in the human enzyme, resulted in loss of binding.[8] Notwithstanding, whether or not the carbamate is absolutely required for activity has not been investigated in sufficient detail, as SARs are still limited to a very small number of compounds.

In light of the hydrogen bonding network shown in Figure 1A, we reasoned that binding affinity could be increased by introducing additional hydrogen bonding groups onto the ligand to displace one or more of the water molecules that coordinate the carbamate of geldanamycin, so that they interact directly with the protein. The rationale for this is that displacing water molecules at the protein–ligand interface is, in general, entropically favored. The novobiocin and clorobiocin inhibitors of DNA gyrase B provide a good example of this. Indeed, the crystal structures of human and yeast Hsp90 N-domain revealed structural homology and sequence conservation in the ATP sites of Hsp90s and DNA gyrase B.[9,10] Novobiocin and clorobiocin both are potent DNA gyrase B inhibitors.[11–13] Interestingly, novobiocin has a carbamate functionality which establishes, with gyrase B, a hydrogen-bonding network similar to that of the carbamate of geldanamycin with Hsp90 (Fig. 1B), including the four buried water molecules. In clorobiocin, the 5-methyl-2-pyrrolcarboxylate

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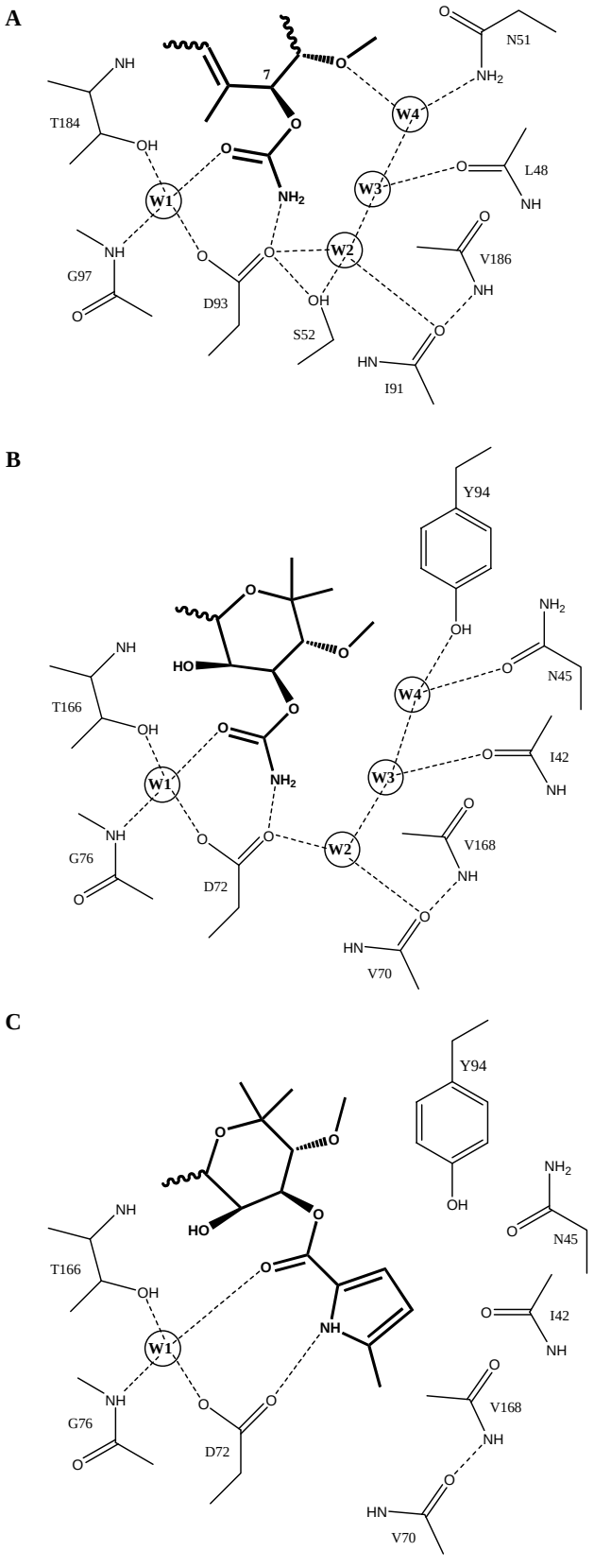


Figure 1. (A) Hydrogen-bonding network established between the 7-carbamate of geldanamycin in complex with human Hsp90; (B) hydrogen bonding network established between the carbamate of novobiocin and DNA gyrase B; (C) hydrogen bonding network established between the 5-methyl-pyrrole ring of clorobiocin and DNA gyrase B.

group, which replaces the carbamate group of novobiocin, binds DNA gyrase B with nearly identical orientation, but three of the four water molecules are displaced (Fig. 1C). Interestingly, isothermal titration calorimetry (ITC) experiments suggested that clorobiocin binds gyrase B with 30-fold better affinity than novobiocin, and that while novobiocin binding is entropically disfavored, that of clorobiocin is both enthalpically and entropically favored.[10]

Because of the structural homology between Hsp90 and gyrase B, we reasoned that carbamate replacements in geldanamycin should also be attempted. By modeling Hsp90–ligand interactions on a set of 17-DMAG derivatives modified at the carbamate using the crystal structure of the human Hsp90–17-DMAG complex[5] as starting point, we found that polar extensions from the carbamate nitrogen could replace one or more bound water molecules without disrupting the rest of the hydrogen bond network. The complexes were modeled with a molecular mechanics and molecular dynamics approach in water using the sander classic module of AMBER6[14] and the Cornell et al.[15] force-field. A carbazide derivative was first proposed. Referring to the compounds reported in Table 1, modeling predicted that the 7-carbazide derivative **8** might displace water molecule w3 from the complex. Compared with the 17-DMAG hydrogen bonding network reported in Figure 2A, the predicted binding mode of this analog has the 7-carbazide carbonyl hydrogen bonded with w1 which, in turn, hydrogen bonds with D93, G97, and T184 (Fig. 2B). Likewise, the adjacent amino function hydrogen bonds with D93 and the terminal amino group of the carbazide is predicted to interact directly with the L48 backbone carbonyl and with w2 and w4. On the contrary, models of the 7-hydroxamate derivative **5** (Table 1) in complex with Hsp90 predicted that the hydroxamate would not be able to displace water molecules (Fig. 2C). A molecular dynamics simulation in which w3 was removed showed that w2 readily moved to occupy the void created by the removal of w3; evidently, the 7-hydroxamate substituent would not be able to reach and effectively replace w3. The hydrogen bonds formed by the hydroxamate are predicted to be similar to those of the 17-DMAG complex, with two additional (long) hydrogen bonds formed by the hydroxyl group of the hydroxamate with w3 and w4. The most significant difference is that the bulkier hydroxamate substituent is predicted to force w3 1.2 Å far from the position occupied in the Hsp90–geldanamycin and Hsp90–17-DMAG complexes; however, this displacement could be tolerated by Hsp90, with a minor shift of the nearby V186 side chain.

Significant differences were noted for the pyrrole derivative **17** (Table 1), which is predicted to bind Hsp90 by displacing water molecules w3 and w4 (Fig. 2D). Except for the 5-methyl group, this compound mimics the novobiocin/clorobiocin substitution reported for gyrase B. Hydrogen bonds with D93, either direct via the amino of the pyrrole or w1-mediated via the carbonyl, were still predicted. The pyrrole ring was still compatible with the presence of w2, as molecular dynamics simulations

Table 1. Structure and biological activities of the 7-carbamate analogs

Compound	R^2 or R^3	R^1	K_d (μM) FL.Hsp90	IC_{50} (nM) $SKBr_3$
1	$-NH_2$	$-NHCH_2CH_2N(CH_3)_2$	0.5	24
2	$-NH_2$	$-NHCH_2CH_2F$	0.8	17
3	$-NH_2$	–Azetidinyl	1.4	24
4	$-NH_2$	$-NH_2$	0.1	33
5	–NHOH	$-NHCH_2CH_2N(CH_3)_2$	18	220
6	–NHOH	$-NHCH_2CH_2F$	>100	1500
7	–NHOH	–Azetidinyl	>100	640
8	$-NHNH_2$	$-NHCH_2CH_2N(CH_3)_2$	>100	140
9	$-NHNH_2$	$-NHCH_2CH_2F$	>100	2100
10	$-NHNH_2$	–Azetidinyl	>100	530
11	$-NHCH_3$	$-NHCH_2CH_2N(CH_3)_2$	>100	420
12	$-N(CH_3)NH_2$	$-NHCH_2CH_2N(CH_3)_2$	>100	2500
13	$-NHCH_2CONH_2$	$-NHCH_2CH_2N(CH_3)_2$	>100	1400
14	$-NHCH_2CH_2OH$	$-NHCH_2CH_2N(CH_3)_2$	>100	390
15	–NHOCH3	$-NHCH_2CH_2N(CH_3)_2$	>100	490
16	$-NHCH_2CH_2N(CH_3)_2$	$-NHCH_2CH_2N(CH_3)_2$	>100	460
17	2-Pyrrole	$-NHCH_2CH_2N(CH_3)_2$	>100	410
18	2-Pyrrole	$-NH_2$	>100	1900
19	4-Imidazole	$-NHCH_2CH_2N(CH_3)_2$	>100	1200
20	Cyclic carbamate	$-NHCH_2\ CH_2N(CH_3)_2$	>100	4500

in which w2 was removed showed that the pyrrole did not reach this position.

To examine these possibilities, these and other derivatives, collected in Table 1, were synthesized and tested for binding affinity to full length human Hsp90 as well as cytotoxicity in SKBr3 mammalian cells. Since substitution at position 17 of geldanamycin largely affects the cytotoxicity of the analogs with little effect on the in vitro binding to purified Hsp90,[16] the 7-carbamate modifications were coupled with the four most potent 17-substituents: 17-[2-(dimethylamino)ethyl]amino-, 17-(2-fluoroethyl)amino-, 17-azetidin-1-yl-, and 17-amino-17-demethoxygeldanamycins. As shown in Scheme 1, analogs **1**–**4** in Table 1 were readily synthesized in good yields by treating geldanamycin (GDM) with the corresponding amines in 1,2-dichloroethane.[16] The 7-carbamate group was removed by treating the 17-alkylamino-17-demethoxygeldanamycins with 2.5 equiv of potassium *t*-butoxide in DMSO at room temperature.[7] Decarbamoylation of 17-DMAG gave the expected 7-hydroxy product along with a minor product **20**, presumably formed via Michael addition of the carbamate nitrogen to C-5. However, decarbamoylation of 17-(2-fluoroethyl)amino-17-demethoxylgeldanamycin under the same condition resulted in decomposition of the material. The desired 7-hydroxyl intermediate was synthesized from 7-*O*-decarbamoylgeldanamycin and 2-fluoroethylamine. 7-Carbazides and 7-hydroxamates **5**–**10** were prepared by reacting the 7-*O*-decarbamoyl intermediate with 1,1′-carbonyldiimidazole (CDI) and subsequently trapping the CDI-adduct with hydrazine or hydroxylamine, respectively. Treatment of the CDI adduct of 7-*O*-decarbamoyl-17-DMAG with alkylamines yielded a series of N-substituted carbamate analogs **11**–**16**. The pyrrole-2-carboxylate and imidazole-4-carboxylate esters **17**–**19** were prepared by dicyclohexylcarbodiimide (DCC) coupling of the 7-hydroxy intermediates with the appropriate carboxylic acids (Scheme 1).

The 11-hydroxyl group is more sterically hindered than the 7-hydroxyl group, resulting in the observed selectivity for the 7-hydroxyl. Given the diminished activity observed for the pyrrole-2-carboxylate analog and other carbamate analogs, the 5-methylpyrrole-2-carboxylate ester[17] derivative of geldanamycin was not pursued further.

The biological activities (Table 1) of the analogs synthesized were evaluated using the Hsp90-binding assay and tumor cell growth inhibition assay reported in a previous study.[16] The binding affinity of these analogs for purified Hsp90 was measured using a scintillation proximity assay previously described.[16] Analogs of 17-DMAG and other 17-aminogeldanamycins with modifications at the 7-positions ranging from simple substitution at the carbamate nitrogen to replacing the carbamoyl group with a pyrrole-2-carboxylate group showed diminished binding affinity to purified human Hsp90. The only exception was the 7-hydroxamate derivative **5**, which retains binding to Hsp90 ($K_d \sim 18\ \mu M$), though weaker than 17-DMAG ($K_d \sim 0.5\ \mu M$), and has an IC_{50} of 220 nM. However, the 7-hydroxamate substituent is predicted to be unable to displace water molecules from the binding site. All other analogs were practically inactive ($K_d > 100\ \mu M$) in the binding assay. The cyclic carbamate analog **20** was also inactive. Several compounds (**5**, **8**, **11**, and **14**–**17**), however, retained cytotoxicity to a certain extent, with an IC_{50} less than 500 nM in SKBr3 cells.

In light of the poor biological results obtained by molecules designed to displace water molecules in the 17-DMAG series, the hydrogen-bonding network between the carbamate and Hsp90 appears crucial, if not essential. Despite the positive result obtained with clorobiocin binding to gyrase B, in Hsp90 the only example of water displacement remains radicicol, another Hsp90 inhibitor, which binds Hsp90 by displacing one of the

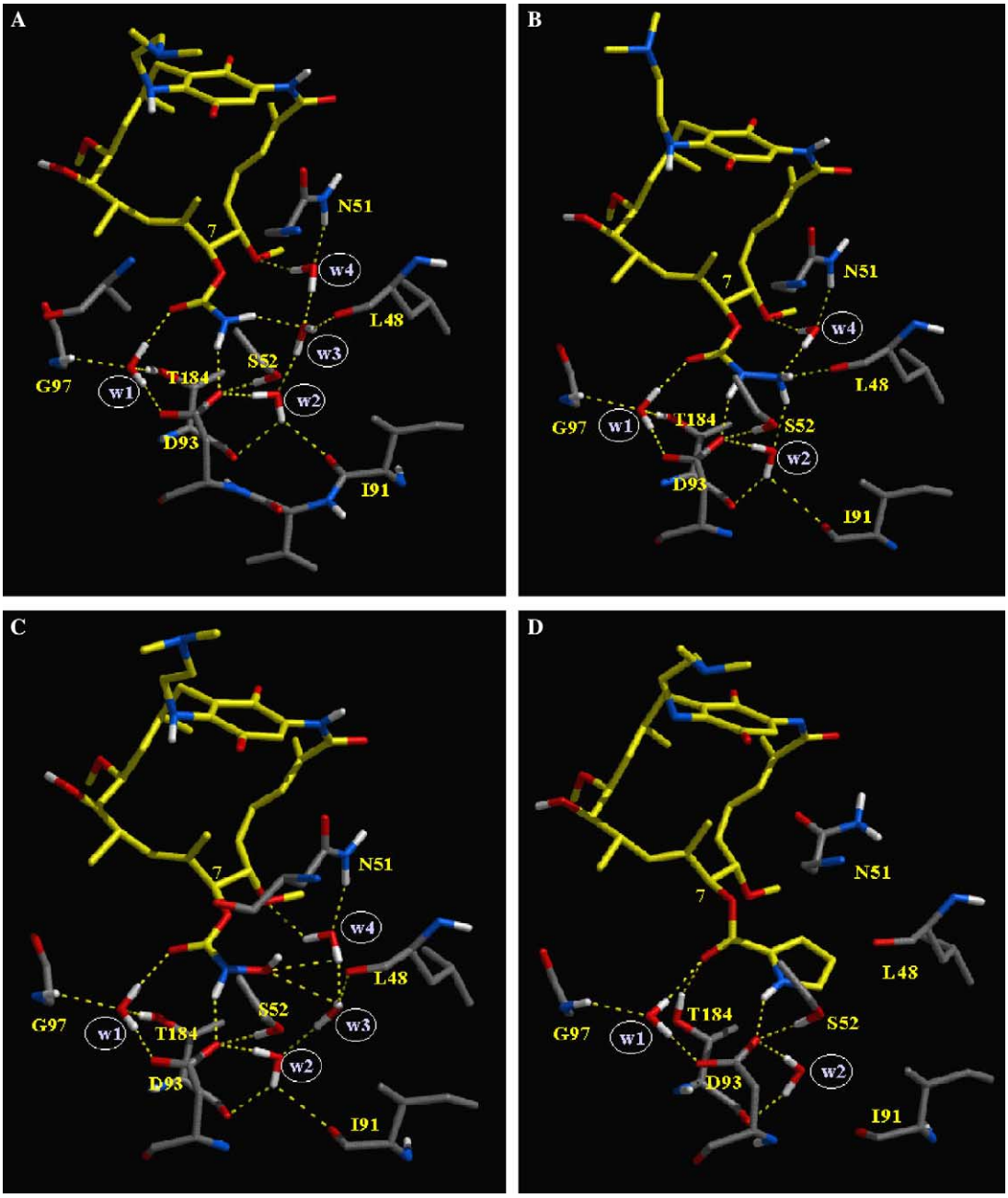
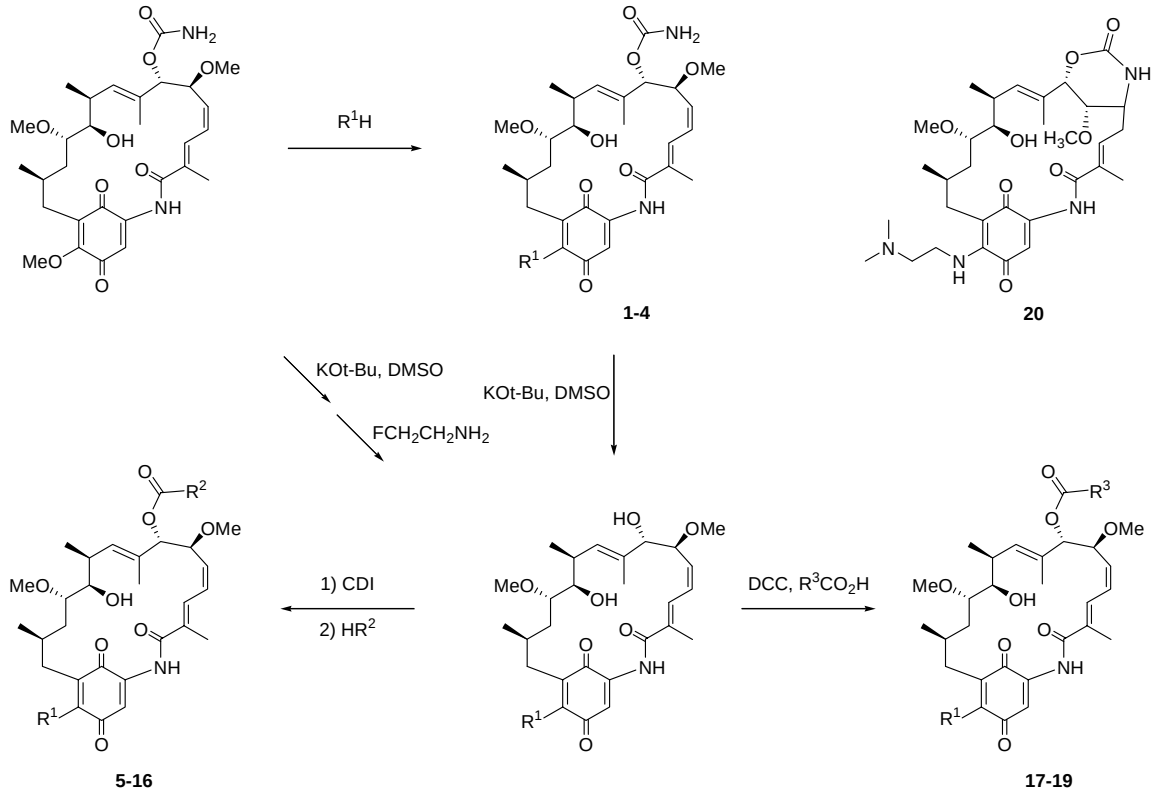


Figure 2. Structures of Hsp90–ligand complexes at the bottom of the active site. The four buried water molecules are noted as w1–w4; for clarity, only polar hydrogens are shown. (A) Hsp90–17-DMAG complex; (B) Hsp90–**8** complex; (C) Hsp90–**5** complex; (D) Hsp90–**17** complex.

four water molecules (w4).[6] Of course, we cannot exclude that substituents different from the ones presented here would be able to do so, and further work will be needed to investigate this possibility.

Many issues are still open: first of all, whether it is best to treat water molecules at the interface between protein and ligand as part of the structure of the protein with a ligand designed to hydrogen bond to the water, or whether it is best to gain entropy from displacement of water by introducing a hydrogen bonding group onto the ligand. This is a choice that is influenced by many factors.[18] For example, it is possible that the 7-carbazide of compound **8**, which was predicted to displace one water molecule, does not make stronger hydrogen bonds than the interfacial water w3 because it is more constrained by attachment to the rest of the ligand. Since the macrocycle of geldanamycin binds Hsp90 by almost entirely filling the binding pocket and establishing a number of hydrophobic contacts and hydrogen bonds, there is little room for conformational adjustment when additional hydrogen bonding groups are introduced. Novobiocin and clorobiocin, in this respect, are significantly more flexible than geldanamycins and might be better able to do so. Second, it is possible that the large conformational changes required to bring the macrocycle of geldanamycin derivatives in a conformation suited



Scheme 1. Synthesis of 7-carbamate derivatives.

for binding[5] might be perturbed by modifying the carbamate. Finally, another issue is the cross-talk between the carbamate derivatives and the 17-substituents. Unexpectedly, while the hydroxamate **5** retained some binding for Hsp90, hydroxamates with different substituents at position 17 (**6**, **7**) are inactive. There is no clear explanation for this, because the 7- and 17-positions are distant and the 17-substituents are solvent exposed. However, manual docking of geldanamycin 17-derivatives into the newly released crystal structure of *E. coli* Hsp90 containing both the N-terminal and the middle domains[19] suggests that the 17-substituents would not be as solvent exposed as originally inferred from the structure of the N-terminal domain alone, rather they significantly interact with residues of the middle domain. Therefore, the binding process might be more complicated than expected.

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2005.08.013

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