

Design, Synthesis, and Biological Evaluation of Conformationally Constrained *cis*-Amide Hsp90 Inhibitors

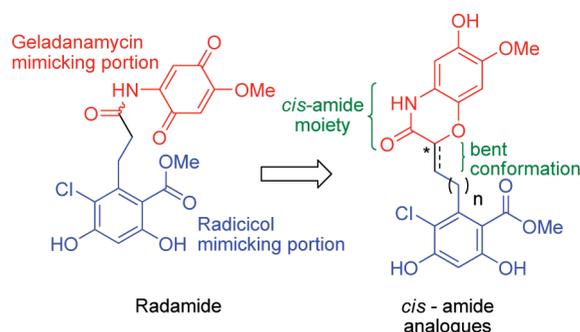
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ABSTRACT



Conformationally constrained *cis*-amide chimeric inhibitors of Hsp90 have been synthesized and evaluated for their Hsp90 inhibitory activity. These new compounds exhibited Hsp90 ATPase inhibition and induced Hsp90-dependent client protein degradation in a dose-dependent manner. Biological data reported herein suggests that amide bond isomerization of geldanamycin derivatives plays an important role in affinity for the heteroprotein complex present in cancer cells.

The 90 kDa heat shock proteins (Hsp90) are ATP-dependent molecular chaperones overexpressed in response to cellular stress and necessary for the folding and rematuration of nascent and denatured polypeptides, respectively.^{1–4} Two natural products, geldanamycin⁵ (GDA, Figure 1) and radicicol⁶ (RDC), bind the Hsp90 N-terminal binding pocket and competitively inhibit ATP binding resulting in degradation of Hsp90-dependent client proteins via the ubiquitin–

proteasome pathway.⁷ Hsp90 clientele play key roles in all six hallmarks of cancer;⁸ therefore, inhibition of the Hsp90 protein folding machinery results in simultaneous disruption of all six mechanisms of oncogenesis.^{9,10} Consequently, not only has Hsp90 emerged as a promising anticancer target,¹¹ but GDA and RDC have proven to represent excellent models for which the development of new Hsp90 inhibitors can be pursued for drug development and mechanistic studies.¹²

Although RDC and GDA bind Hsp90 with high affinity, their modes of binding and inhibitory activities are different.

(1) Freeman, B. C.; Morimoto, R. I. *EMBO J.* **1996**, *15*, 2969.
(2) Walter, S.; Buchner, J. *Angew. Chem., Int. Ed.* **2002**, *41*, 1098.
(3) Schneider, C.; Sepp, L. L.; Nimmesgern, E.; Ouerfelli, O.; Danishefsky, S.; Rosen, N.; Hartl, F. U. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14536.
(4) Schumacher, R. J.; Hansen, W. J.; Freeman, B. C.; Alnemri, E.; Litwack, G.; Toft, D. O. *Biochemistry* **1996**, *35*, 14889.
(5) Whitesell, L.; Mimnaugh, E. G.; De Costa, B.; Myers, C. E.; Neckers, L. M. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 8324.
(6) Schulte, T. W.; Akinaga, S.; Soga, S.; Sullivan, W.; Stensgard, B.; Toft, D.; Neckers, L. M. *Cell Stress Chaperones* **1998**, *3*, 100.

(7) Binder, R. J.; Blachere, N. E.; Srivastava, P. K. *J. Biol. Chem.* **2001**, *276*, 17163.
(8) Hanahan, D.; Weinberg, R. A. *Cell* **2000**, *100*, 57.
(9) Maloney, A.; Workman, P. *Expert Opin. Biol. Ther.* **2002**, *2*, 3.
(10) Neckers, L. *Curr. Top. Med. Chem.* **2006**, *6*, 1163.
(11) Chaudhury, S.; Welch, T. R.; Blagg, B. S. J. *ChemMedChem* **2006**, *1*, 1331.
(12) Hadden, M. K.; Lubbers, D. J.; Blagg, B. S. J. *Curr. Top. Med. Chem.* **2006**, *6*, 1173.

Radicicol exists in the same bent conformation when bound and unbound to Hsp90 and produces a favorable entropy of 8.3 cal/mol upon binding. Not surprisingly, the predisposition of RDC to the bent conformation is believed to be responsible for its similar activity in both cellular and recombinant assays.¹³

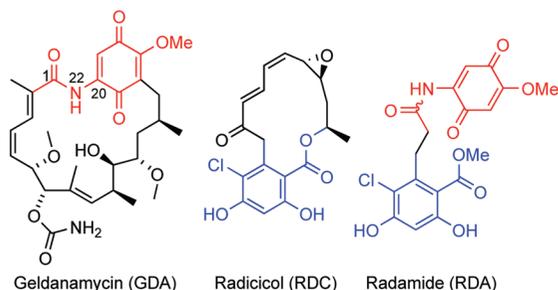


Figure 1. Inhibitors of Hsp90.

In contrast to the bent, *cis*-amide conformation of GDA when bound to Hsp90, both solution and crystal structures have demonstrated that this natural product exists in an extended, *trans*-amide conformation when unbound to Hsp90 (Figure 2).¹⁴ Multiple studies have demonstrated that prior to binding Hsp90, GDA must undergo two conformational changes; the ansa ring must rotate over the benzoquinone moiety and the amide bond must isomerize from *trans* to *cis* by rotation about C₁–N₂₂ and C₂₀–N₂₂ (Figure 1). The first event is reported to occur spontaneously; however, isomerization of the amide bond is estimated to exceed 20 kcal/mol.¹⁵ Accordingly, isothermal titration calorimetry (ITC) experiments have shown that GDA exhibits an entropic penalty of –6.4 cal/mol upon binding Hsp90.^{16,17}

As a consequence of these thermodynamic data, Santi and co-workers hypothesized that GDA analogues containing a predisposed *cis*-amide bond will result in ~1000-fold increase in Hsp90 affinity through reduction of entropic penalties. Such postulations have inspired subsequent studies aimed at determining the effect of *trans*/*cis* isomerization of the GDA-amide moiety.^{18–20} How-

ever, to the best of our knowledge, no analogues have been synthesized that exhibit a predisposed *cis*-amide functionality.

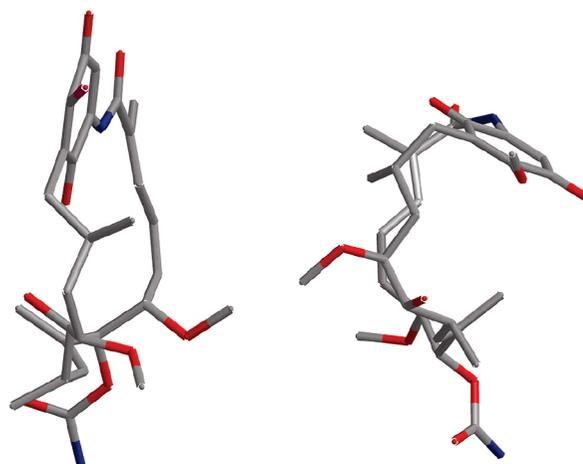


Figure 2. GDA conformations in solution (left) and bound to Hsp90 (right).

Recently, chimeric inhibitors of Hsp90 were disclosed that contained both the quinone ring from GDA and the resorcinol moiety of RDC in an attempt to mimic the hydrogen bonding interactions exemplified by the two natural products when bound to the Hsp90 N-terminal nucleotide-binding pocket. This approach has produced novel scaffolds for Hsp90 inhibition; however, none of the reported analogues exhibit conformational characteristics exhibited by either natural product when bound to Hsp90.^{21–23} Analysis of the *seco* derivative, radamide (RDA, Figure 1), revealed the potential to introduce conformational aspects of both natural products when bound to Hsp90. Herein we present the first series of conformationally constrained chimeric Hsp90 inhibitors exhibiting both a *cis*-amide moiety and a bent conformation.

Retrosynthetically, we envisioned the desired analogues could be obtained via a Horner–Wadsworth–Emmons (HWE) olefination reaction between cyclic phosphonate **1** and homologated aldehydes, **2** and **3** (Scheme 1). Compound **1** was proposed to result from tandem reduction/intramolecular cyclization of compound **4**, which could be obtained from commercially available 4-benzyloxy-3-methoxybenzaldehyde in four steps. Aldehydes **2** and **3** could be prepared directly from **5**²¹ and **6**, respectively.

Commencing with commercially available 4-benzyloxy-3-methoxybenzaldehyde (**7**, Scheme 2), phenol **8** was formed *via* Dakin oxidation.²⁴ Treatment of **8** with diazophosphonate **9** resulted in the phenolic ether **10** upon a rhodium carbenoid

(13) Roe, S. M.; Prodromou, C.; O' Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. *J. Med. Chem.* **1999**, *42*, 260.

(14) Thepchatri, P.; Eliseo, T.; Cicero, D. O.; Myles, D.; Snyder, J. P. *J. Am. Chem. Soc.* **2007**, *129*, 3127.

(15) Lee, Y.; Marcu, M. G.; Neckers, L. *Chem. Biol.* **2004**, *11*, 991.

(16) Chiosis, G.; Huezio, H.; Rosen, N.; Mimnaugh, E.; Whitesell, L.; Neckers, L. *Mol. Cancer Ther.* **2003**, *2*, 123.

(17) Schulte, T. W.; Neckers, L. M. *Cancer Chemother. Pharmacol.* **1998**, *42*, 273.

(18) Onuoha, S. C.; Mukund, S. R.; Coulstock, E. T.; Sengerova, B.; Shaw, J.; McLaughlin, S. H.; Jackson, S. E. *J. Mol. Biol.* **2007**, *372*, 287.

(19) Onodera, H.; Kaneko, M.; Takahashi, Y.; Uochi, Y.; Funahashi, J.; Nakashima, T.; Soga, S.; Suzuki, M.; Ikeda, S.; Yamashita, Y.; Rahayu, E. S.; Kanda, Y.; Ichimura, M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1577.

(20) Zhang, M. Q.; Gaisser, S.; Nur-E-Alam, M.; Sheehan, L. S.; Vousden, W. A.; Gaitatzis, N.; Peck, G.; Coates, N. J.; Moss, S. J.; Radzom, M.; Foster, T. A.; Sheridan, R. M.; Gregory, M. A.; Roe, S. M.; Prodromou, C.; Pearl, L.; Boyd, S. M.; Wilkinson, B.; Martin, C. J. *J. Med. Chem.* **2008**, *51*, 5494.

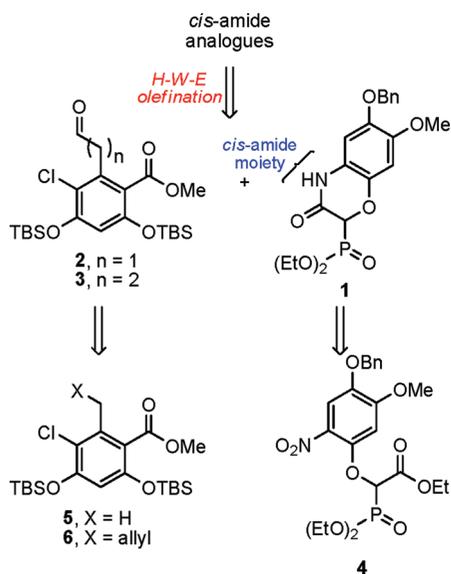
(21) Clevenger, R. C.; Blagg, B. S. J. *Org. Lett.* **2004**, *6*, 4459–4462.

(22) Shen, G.; Blagg, B. S. J. *Org. Lett.* **2005**, *7*, 2157.

(23) Shen, G.; Wang, M.; Welch, T. R.; Blagg, B. S. J. *J. Org. Chem.* **2006**, *71*, 7618.

(24) Li, S.; Chen, L.; Tsai, J.; Tzeng, J.; Tsai, I.; Chi, E. *Tetrahedron Lett.* **2007**, *48*, 2139.

Scheme 1. Retrosynthetic Analysis for the Synthesis of *cis*-Amide Chimeric Analogues



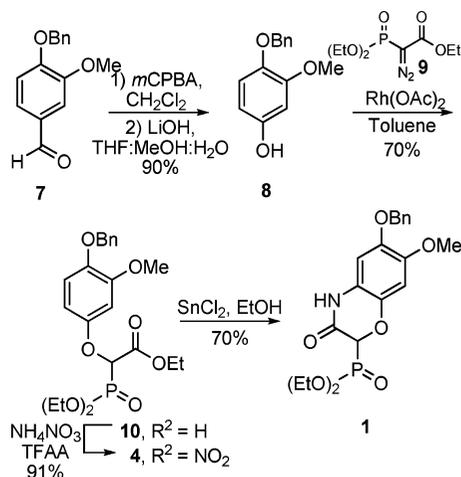
mediated O–H insertion.²⁵ Regioselective nitration of **10** was accomplished under mild conditions enlisting ammonium nitrate and trifluoroacetic anhydride. Treatment of **4** with tin(II) chloride under refluxing conditions resulted in not only reduction of the nitro group to the corresponding aniline but also cyclization to give the desired lactam, **1**. The protected resorcinol precursors were prepared by treatment of **5** with lithium diisopropylamide at $-78\text{ }^{\circ}\text{C}$ to generate the benzylic anion, which was quenched upon addition of dimethylformamide to afford the aldehyde product, **2**, or with allyl bromide to give **6** (Scheme 3). Oxidation of **6** with osmium tetroxide gave the corresponding diol, which was cleaved in situ with sodium periodate to yield homologated aldehyde **3**.

With the synthons in hand, the fragments were joined *via* a Horner–Wadsworth–Emmons olefination reaction (3:1, *E/Z*) and subsequent removal of the protecting groups with tetrabutylammonium fluoride gave compounds **11–14** (Scheme 4).

Originally, we were interested in the saturated analogues, but realizing the olefinated intermediates exhibited higher conformational rigidity than the saturated analogues, we attempted to selectively remove the benzyl ether in the presence of the unsaturated amide with FeCl_3 , $\text{Pd}(\text{OAc})_2/\text{Et}_3\text{SiH}/\text{Et}_3\text{N}$, and NaI/TMSCl . However, none of these conditions afforded the desired products. Finally, conditions were employed utilizing aluminum(III) chloride in anisole that effectively cleaved the benzyl ether without alteration of the α,β -unsaturated amide to afford compounds **15–18** (Scheme 4).

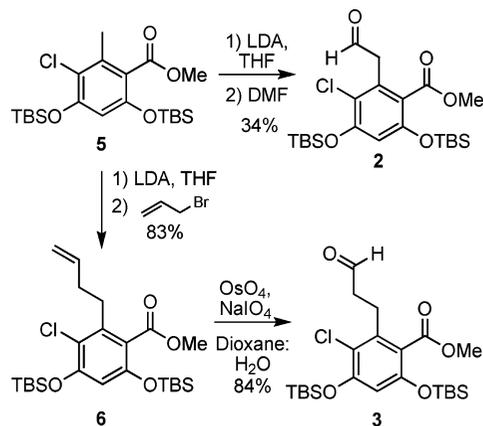
Standard hydrogenation conditions with palladium on carbon under a hydrogen atmosphere yielded racemic

Scheme 2. Synthesis of *cis*-Amide Cyclic Phosphonate



products **19** and **20**, which were subjected to chiral HPLC to afford the enantiopure analogues **21–24** (Scheme 4).

Scheme 3. Synthesis of Homologated Aldehydes



After completing the synthesis of 10 analogues, biological investigation was undertaken. Antiproliferation studies with compounds **15–24** were conducted with MCF7 and SKBr3 breast cancer cell lines. As shown in Table 1, the *E*-olefin is more active than the *Z*-olefin for both linker lengths and the (+)-enantiomer is more active than the (–)-enantiomer.

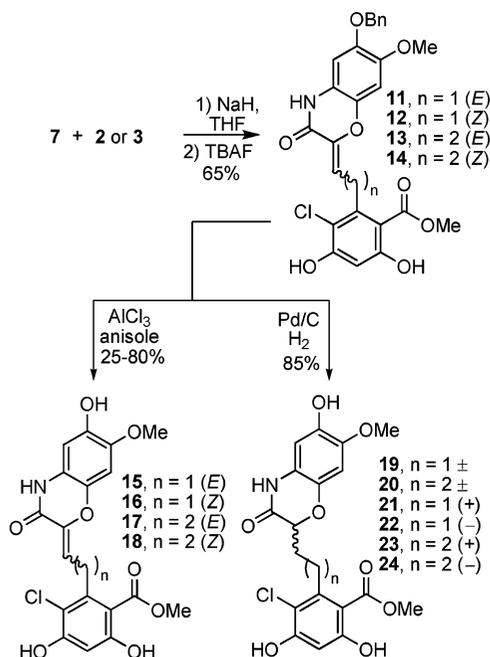
To evaluate this series for inhibition of ATPase activity,²⁶ recombinant yeast Hsp90 was overexpressed and purified.²⁷ The purified protein was incubated with ATP in the presence of **15**, **17**, **21**, and **23**. As shown in Table 1, the IC_{50} values were similar to those found in the antiproliferation studies, suggesting equipotency of these analogues for the hetero-protein complex present in cancer cells versus the purified homodimer representative of normal cells.

(26) Avila, C.; Kornilayev, B. A.; Blagg, B. S. J. *Bioorg. Med. Chem.* **2006**, *14*, 1134.

(27) Richeter, K.; Muschler, P.; Hainzl, O.; Buchner, J. *J. Biol. Chem.* **2001**, *276*, 33689.

(25) Haigh, D. *Tetrahedron* **1994**, *50*, 3177.

Scheme 4. Fragment Coupling and Deprotection



To confirm the cytotoxic nature of these compounds resulted from Hsp90 inhibition, compounds **17** and **23** were incubated with MCF7 cells for 24 h, and Western blot analyses were performed with the cell lysates (see Supporting Information). As expected, the immunoblots confirmed concentration-dependent degradation of Hsp90-dependent client proteins, Her-2 and Raf.

In conclusion, we have designed, synthesized, and provided biological data for a series of analogues that not only contain hydrogen-bonding moieties exhibited by GDA and RDC, but also exhibit the conformational biases adopted by the natural products when bound to Hsp90 *via* inclusion of a bent conformation and a *cis*-amide. In accord with Santi and co-workers, introduction of a *cis*-amide moiety increased Hsp90 inhibitory activity (~10-fold). As indicated by the biological results, introduction of both the *cis*-amide and the bent conformation to the chimeric inhibitors does not influence activity against purified recombinant Hsp90; however, the activity against cancer cells increased ~10-fold with respect to the *seco* derivative, radamide.

These results coincide with the ideas posed by multiple groups, whom suggest a mechanistic difference between the

heteroprotein complex present in cancer cells versus purified recombinant Hsp90, which is reminiscent of the form found in normal cells.^{15,28,29} In addition, the data suggest the heteroprotein complex is more sensitive to amide isomerization and conformational predisposition. Further SAR studies and cocrystallization experiments are ongoing in an effort to delineate the interactions of this class of compounds with Hsp90 and the results from such studies will be reported in due course.

Table 1. Anti-Proliferation and ATPase Activity of *cis*-Amide Analogues

compd	IC ₅₀ ^a (μM)		
	MCF7	SKBr3	ATPase
15	3.2 ± 0.1	1.8 ± 0.1	2.4 ± 0.1
16	78.9 ± 11.3	57.1 ± 8.4	
17	1.5 ± 0.1	1.2 ± 0.1	1.6 ± 0.0
18	9.8 ± 1.9	15.9 ± 4.1	
19	4.8 ± 1.1	7.3 ± 0.2	
20	6.3 ± 0.9	7.8 ± 0.2	
21	3.6 ± 0.1	2.1 ± 0.2	1.5 ± 0.1
22	14.9 ± 0.2	15.4 ± 0.8	
23	2.7 ± 0.5	2.1 ± 0.7	1.2 ± 0.0
24	78.0 ± 4.4	15.4 ± 0.8	
radamide	18.6 ± 0.9 ^b	23.7 ± 1.7 ^b	5.9 ^c
GDA	9.8 ± 0.1 nM	8.5 ± 1.1 nM	2.5 ± 0.4
RDC	47.7 ± 2.6 nM	37.5 ± 4.0 nM	0.4 ± 0.0

^a IC₅₀ = concentration needed to produce 50% inhibition. ^b Hadden, M. K.; Blagg, B. S. J. *J. Org. Chem.* **2009**, in press. ^c Obtained from ref 21.

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Supporting Information Available: Experimental procedures and characterization for all compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(28) Chiosis, G.; Neckers, L. *ACS Chem. Biol.* **2006**, *1*, 279.

(29) Kamal, A.; Thao, L.; Sensintaffar, J.; Zhang, L.; Boehm, M. F.; Fritz, L. C.; Burrows, F. J. *Nature* **2003**, *425*, 407.