ORGANIC LETTERS

2004
Vol. 6, No. 24
4459−4462

Design, Synthesis, and Evaluation of a Radicicol and Geldanamycin Chimera, Radamide

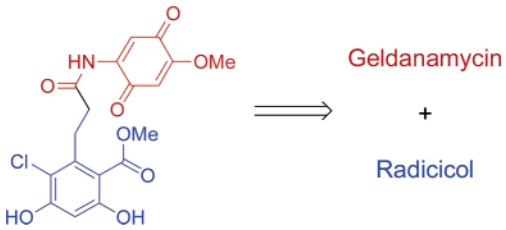
Randell C. Clevenger and Brian S. J. Blagg*

Department of Medicinal Chemistry and The Center for Protein Structure and Function, The University of Kansas, 1251 Wescoe Hall Drive, Malott 4070, Lawrence, Kansas 66045-7562

bblagg@ku.edu

Received August 30, 2004

ABSTRACT



A chimera composed of the natural products radicicol and geldanamycin has been prepared through an amide linkage connecting the resorcinol moiety of radicicol to the quinone ring of geldanamycin. The inhibitory activity of these compounds was determined by their ability to inhibit Hsp90's inherent ATPase activity along with degradation of the Hsp90 client protein, HER-2 in MCF-7 breast cancer cells.

The 90 kDa heat shock proteins (Hsp90) are essential for refolding denatured proteins as well as for the conformational maturation of nascent polypeptides into biologically active three-dimensional structures.[1] Several Hsp90 dependent client proteins have been identified and include Src kinase, Raf, HER-2, p185, mutant p53 (not normal p53), telomerase, steroid hormone receptors, polo 1-kinase (PLK), protein kinase B (AKT), death domain kinase (RIP), MET kinase, focal adhesion kinase (FAK), as well as the aryl hydrocarbon receptor, PKR, nitric oxide synthase, and others.[2] Of these Hsp90 client proteins, HER-2, Raf, PLK, RIP, AKT, FAK, telomerase, and MET are directly associated with all six hallmarks of cancer.[3] Consequently, Hsp90 has emerged as a promising biological target for the development of cancer chemotherapeutics because multiple pathways can be simultaneously disrupted by inhibition of the Hsp90 protein folding machinery.[1]

The Hsp90 protein folding process is ATP dependent. Disruption of ATP binding and hydrolysis results in the degradation of unfolded and partially folded client proteins via the ubiquitin-proteasome pathway.[4] Known inhibitors of the Hsp90 protein folding process include geldanamycin (GDA, Figure 1),[5] herbimycin A (HB),[6] and radicicol (RDC).[7] RDC is the most potent Hsp90 inhibitor in vitro but has no activity in vivo.[8] In contrast, GDA is less potent than RDC in vitro with an IC_{50} of 1−3 μM.[9] However, in cellular studies, a derivative of GDA (17-AAG) was shown to have greater affinity for the Hsp90 multiprotein complex found in malignant cells with an IC_{50} of 5−100 nM.[10]

(1) Walter, S.; Buchner, J. *Angew. Chem., Int. Ed.* **2002**, *41*, 1098.
(2) Adams, J.; Elliot, P. J. *Oncogene* **2000**, *19*, 6687.
(3) Maloney, A.; Workman, P. *Expert Opin. Biol. Ther.* **2002**, *2*, 3.
(4) Binder, R. J.; Blachere, N. E.; Srivastava, P. K. *J. Biol. Chem.* **2001**, *276*, 17163.
(5) Grenert, J. P.; Sullivan, W. P.; Fadden, P.; Haystead, T. A. J.; 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.
(6) Miller, P.; DiOrio, C.; Walter, C.; Moyer, J. D. *Proc. Am. Assoc. Cancer Res.* **1993**, *34*, 2047.
(7) Yang, Z.-Q.; Geng, X.; Solit, D.; Pratilas, C. A.; Rosen, N.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 7881.
(8) Agatsuma, T.; Ogawa, H.; Akasaka, K.; Asai, A.; Yamashita, Y.; Mizukami, T.; Akinaga, S.; Saitoh, Y. *Bioorg. Med. Chem.* **2002**, *10*, 3445.
(9) (a) Panaretou, B.; Prodromou, C.; Roe, S. M.; O'Brien, R.; Ladbury, J. E.; Piper, P. W.; Pearl, L. H. *EMBO J.* **1998**, *17*, 4829. (b) Chiosis, G.; Huezo, H.; Rosen, N.; Mimnaugh, E.; Whitesell, L.; Neckers, L. *Mol. Cancer Ther.* **2003**, *2*, 123.

10.1021/ol048266o CCC: $27.50
Published on Web 10/30/2004

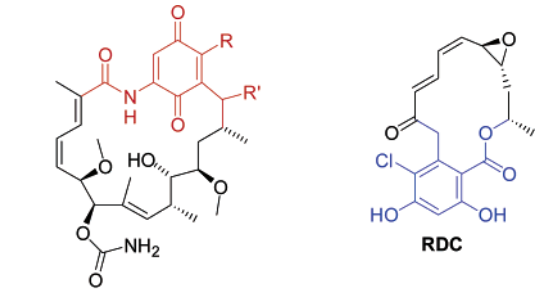


Figure 1. Hsp90 inhibitors.

Although 17-AAG has entered Phase I clinical trials for the treatment of several cancers,[11] 17-AAG still maintains additional toxicity and formulation problems[12] that may prove difficult to overcome. Therefore, the identification of new inhibitors that are more accessible to structural modification is likely to provide clinically useful alternatives to 17-AAG.

Although the entire three-dimensional structure of Hsp90 has not been elucidated, cocrystal structures of the N-terminal region bound to GDA, RDC, and ADP have been solved (Figure 2).[13] The resorcinol moiety of RDC binds in the same

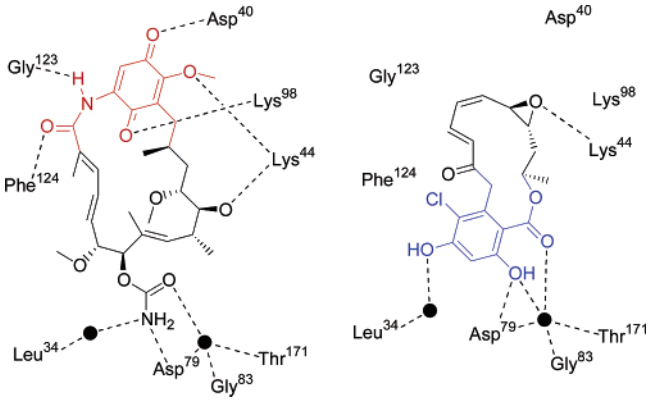


Figure 2. Binding interactions of GDA and RDC with Hsp90. ● = one molecule of H_2O.

location as the adenine ring of ADP and mimics the hydrogen bond donor/acceptor properties of the exo- and N7 endocyclic amine/imine, respectively. In contrast, the quinone ring of GDA binds toward the exterior of the pocket and participates in hydrogen bond interactions with the amino acids that normally bind to the diphosphate region of ADP. Key interactions observed between the quinone and ATP binding pocket suggest that binding to this region is critical to GDA's affinity for Hsp90.

Examination of the cocrystal structures suggested a chimeric molecule composed of RDC's resorcinol ring, and GDA's quinone may provide a molecule with high affinity for Hsp90. Radanamycin amide (radamide, Figure 3) is such

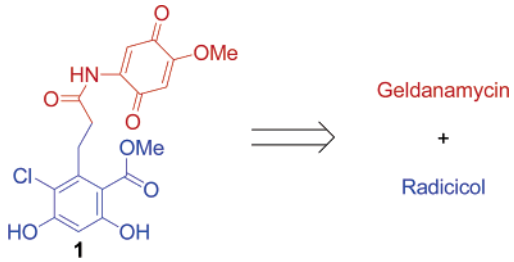


Figure 3. Radanamycin amide, a radicicol and geldanamycin chimera.

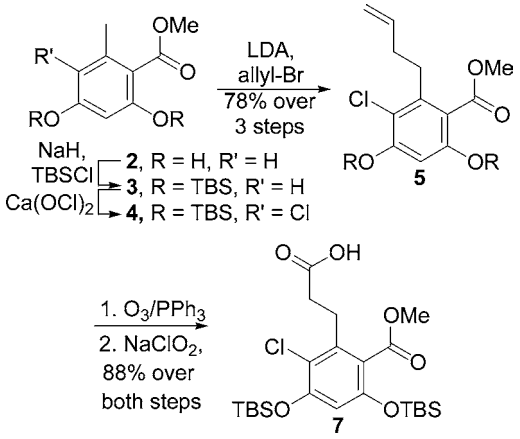
a molecule that connects the resorcinol ring of **rad**icicol to the quinone moiety of geld**anamycin** through an amide linkage. Molecular modeling and docking experiments supported alignment of these two portions into the appropriate locations within the ATP binding site of Hsp90.

We sought to prepare this chimera and to furnish additional analogues that could unveil structure−activity relationships between RDC, GDA, and radamide. Thus, compound **1** was synthesized from methyl 2,4-dihydroxy-5-methyl benzoate[14] (**2**, Scheme 1) by silyl protection of the phenols followed by chlorination of the aromatic ring to provide **4**. Treatment of **4** with lithium diisopropylamide at −78 °C followed by addition of allyl bromide provided the allylated product, **5**. Ozonolysis of the double bond and oxidation of the resultant aldehyde gave the corresponding acid **7**.

The quinone precursor **10** was prepared from 1,4-bis-(methoxymethoxy)-2-methoxybenzene (**8**),[15] by nitration[16] and reduction of the nitro group (Scheme 2). Since the quinone ring is redox-active[17] and an excellent Michael acceptor, we also prepared a trimethoxy phenyl derivative **11**, which is not subject to the same reactivity as a quinone but still maintains hydrogen bond-accepting capabilities. In the event, **7** was coupled with anilines **10** and **11** to provide the corresponding amide products, **12** and **13**. Removal of

(10) Kamal, A.; Thao, L.; Sensintaffar, J.; Zhang, L.; Boehm, M. F.; Fritz, L. C.; Burrows, F. J. *Nature* **2003**, *425*, 407.
(11) Blagosklonny, M. V. *Leukemia* **2002**, *16*, 455.
(12) (a) Sausville, E. A.; Tomaszewski, J. E.; Ivy, P. *Curr. Cancer Drug Targets* **2003**, *3*, 377−383. (b) Neckers, L. M. *Drug Res. Updates* **2000**, *3*, 203−205. (c) Chiosis, G.; Lucas, B.; Shtil, A.; Huezo, H.; Rosen, N. *Bio. Med. Chem.* **2002**, *10*, 3555−3564. (d) Chiosis, G.; Lucas, B.; Huezo, H.; Solit, D.; Bassa, A.; Rosen, N. *Curr. Cancer Drug Targets* **2003**, *3*, 371−376. (e) Neckers, L. *Trends Mol. Med.* **2002**, *8*, S55−S61. (f) Dikalov, S.; Landmesser, U.; Harrison, D. G. *J. Biol. Chem.* **2002**, *277*, 25480−25485. (g) Sreedhar, A. S.; Mihaly, K.; Pato, B.; Schnaider, T.; Stetak, A.; Kis-Petik, K.; Fidy, J.; Simonics, T.; Maraz, A.; Csermely, P. *J. Biol. Chem.* **2003**, *278*, 35231−3523140. (h) Workman, P. *Curr. Cancer Drug Targets* **2003**, *3*, 297−300.
(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) Furstner, A.; Gastner, T. *Org. Lett.* **2000**, *2*, 2467.
(15) Andrus, M. B.; Hicken, E. J.; Meredith, E. L.; Simmons, B. L.; Cannon, J. F. *Org. Lett.* **2003**, *5*, 3859.
(16) Crivello, J. V. *J. Org. Chem.* **1981**, *46*, 3056.
(17) Dikalov, S.; Landmesser, U.; Harrison, D. G. *J. Biol. Chem.* **2002**, *277*, 25480.

Scheme 1. Synthesis of Radicicol's Resorcinol Moiety

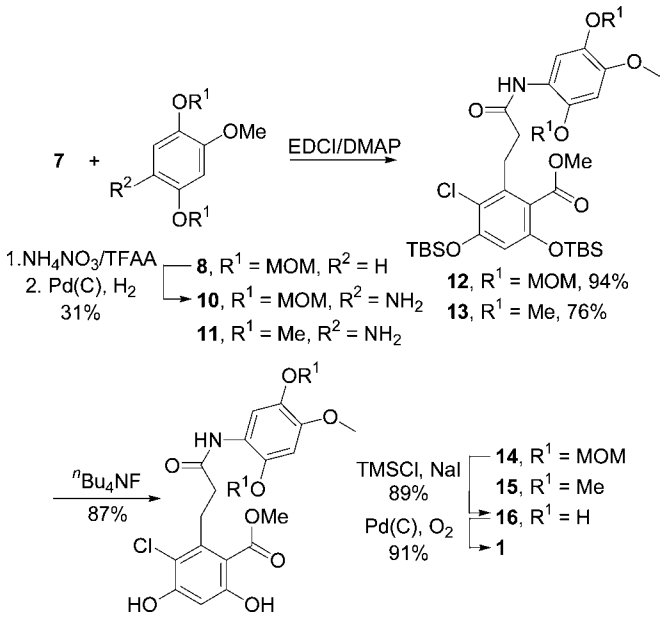


the silyl protecting groups gave **14** and **15**, of which **14** was a direct precursor to **1**. Following the procedure of Andrus and co-workers,[15] the methoxymethyl ethers were cleaved with in situ-derived trimethylsilyl iodide to furnish the hydroquinone product, **16**. Oxidation of the hydroquinone with palladium on carbon[12] provided the paraquinone product **1**.

Upon completion of the synthesis of **1**, our efforts turned toward evaluation of its biological activity. Recombinant yeast Hsp90 was overexpressed and purified according to the procedure of Buchner.[7] The pure protein was incubated with ATP and either **15**, **16**, or **1**, and the production of inorganic phosphate was determined (Figure 4).[8]

The IC_{50} of GDA as determined by this assay was consistent with previously reported values (2.5 μM),[8] and the inhibitory activity of **16** and **1** was 1.8 and 5.9 μM, respectively. The IC_{50} of **15** was higher than 40 μM,

Scheme 2. Preparation of Radamide



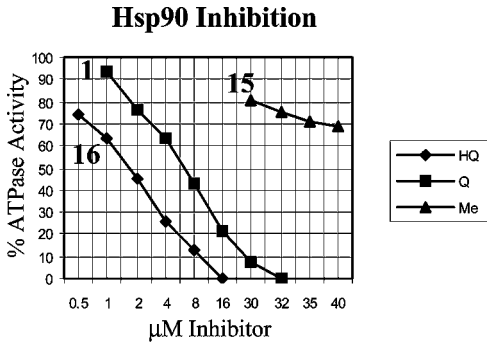


Figure 4. Inhibition of Hsp90's ATPase activity.

suggesting that the incorporation of methyl ethers onto the aromatic ring disrupts a key hydrogen bond network in the ATP binding pocket. Andrus and co-workers recently reported a similar result for the trimethoxy derivative of GDA, which resulted in a substantial loss in cellular efficacy.[18]

As mentioned previously, Hsp90 is responsible for the conformational maturation of several polypeptides into biologically active, three-dimensional structures. When the Hsp90 protein folding process is disrupted, these client proteins are unable to adopt their native structures, which ultimately leads to their degradation. Therefore, one can confirm Hsp90 inhibition by observing whether Hsp90 client proteins are degraded by analysis of cellular lysates in the presence of varying concentrations of Hsp90 inhibitors. When increasing concentrations of **16**, **1**, and **15** were incubated with MCF-7 breast cancer cells, a decrease in the Hsp90-dependent client protein, HER-2 was observed by Western Blot analysis (Figure 5), providing evidence that

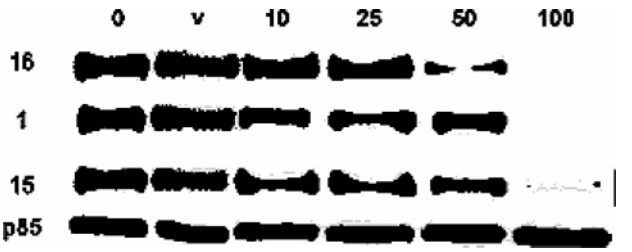


Figure 5. HER-2 degradation assays. Concentrations of drug in μM are listed above each lane. V = vehicle (1% DMSO).

these molecules also inhibit Hsp90 in cells. P85 is not an Hsp90-dependent client protein and was used as a control.

In contrast to GDA, radamide (**1**) is prepared in a minimal number of steps and is amenable to the rapid construction of analogues that will be useful for elucidation of Hsp90

(18) Andrus, M. B.; Meredith, E. L.; Hicken, E. J.; Simmons, B. L.; Glancey, R. R.; Ma, W. *J. Org. Chem.* **2003**, *68*, 8162.

structure−activity relationships. Studies are now underway to prepare more active inhibitors of Hsp90 based upon this chimeric species.

In conclusion, we have designed, synthesized, and provided biological data to support the hypothesis that a chimera comprised of RDC and GDA is a novel lead compound for the future development of Hsp90 inhibitors.

Acknowledgment. The authors gratefully acknowledge support of this project by the NIH COBRE RR017708, The University of Kansas Center for Research, and The University of Kansas New Faculty General Research Fund. Geldanamycin was provided by the Developmental Therapeutics Program at the National Cancer Institute. Recombinant yeast Hsp90 was purified by Boris Kornikaev at The University of Kansas Biochemical Research Service Laboratory. The authors would like to thank Professors Gunda Georg, Robert Hanzlik, and Rick Dobrowski for helpful discussions.

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.

OL048266O