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Bioorganic & Medicinal Chemistry Letters 16 (2006) 2459–2462
Bioorganic & Medicinal Chemistry Letters

Radanamycin, a macrocyclic chimera of radicicol and geldanamycin

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Received 17 January 2006; revised 20 January 2006; accepted 23 January 2006
Available online 7 February 2006

Abstract—Radicicol and geldanamycin are potent inhibitors of the Hsp90 protein folding machinery, which is an emerging target for the development of cancer chemotherapeutics. However, radicicol is inactive in vivo and geldanamycin suffers from its redox-active behavior that produces toxicity unrelated to Hsp90 inhibition. It was proposed that a chimeric molecule containing the resorcinol ring of radicicol and the quinone of geldanamycin could provide an opportunity to elucidate structure–activity relationships for both natural products and serve as a starting point for the development of more potent inhibitors. Synthesis of the macrocyclic chimera named radanamycin is reported along with the biological activity exhibited by this compound in MCF-7 breast cancer cells.

The 90 kDa heat shock proteins (Hsp90) represent a promising target for the development of cancer chemotherapeutics as a result of their ability to fold nascent oncogenic polypeptides into biologically active proteins.[1–3] Not surprisingly, the Hsp90 protein folding process is dependent upon ATP-hydrolysis to provide energy for the conformational maturation of nascent polypeptides.[4] However, unlike most ATP-binding proteins, Hsp90 binds ATP in a bent conformation that is very unique and only eukaryotic enzymes MutL and histidine kinase have been shown to bind nucleotides in a similar manner.[5] Moreover, two natural products have been identified as potent inhibitors of the Hsp90-mediated protein folding process and there are currently more than 20 clinical trials in progress based on Hsp90-targeted drugs. All of these trials utilize derivatives of geldanamycin (GDA, $IC_{50} = 49$ nM, Fig. 1), which has been shown to produce toxicity unrelated to Hsp90 inhibition.[6] Another natural product inhibitor of Hsp90 is radicicol (RDC, $IC_{50} = 23$ nM), which produces the most potent inhibitory activity yet identified, however, it has no activity in vivo because it is rapidly metabolized into inactive compounds that have little or no affinity for Hsp90.[7]

To circumvent problems associated with the natural product inhibitors of Hsp90, several institutions have developed small molecule antagonists of the Hsp90 molecular chaperone that are either based upon the purine skeleton (PU24FCl, $IC_{50} = 2$ μM),[8] dihydroxyphenyl pyrazoles (CCT018159, $IC_{50} = 4.1$ μM),[9] or more recently novobiocin ($IC_{50} \sim 700$ μM).[10] In an effort to develop new Hsp90 inhibitors and to understand structure–activity relationships for the natural products, we superimposed the co-crystal structures of GDA and RDC bound to Hsp90.[5] As can be seen in Figure 2, the resorcinol moiety of RDC occupies the same binding region as the carbamate of GDA. Both of these moieties provide similar interactions as those observed between the adenine ring of ADP and Hsp90. In contrast, the quinone ring of GDA extends toward the protein surface in analogy to the macrocyclic ring and the epoxide of RDC. Although the entire three-dimensional crystal structure of Hsp90 has not been determined, experimental data and individual structures of the N- and middle domains have supported contact between these two regions, suggesting the region that binds to the quinone and epoxide may not be solvent exposed.[11–13] To increase interactions of each inhibitor with Hsp90, we proposed that a molecule containing portions of each natural product should be capable of producing effective Hsp90 inhibitors that could be modified for increased potency.

Originally, our proposed inhibitor was designed by comparing the co-crystal structure of each natural product bound to Hsp90. As can be seen in Figure 2, both molecules adopt a bent conformation upon Hsp90 binding, which is similar to that of the natural substrate, ADP.[5]

Keywords: Hsp90; Inhibitors; Geldanamycin; Radicicol; Cancer.
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doi:10.1016/j.bmcl.2006.01.086

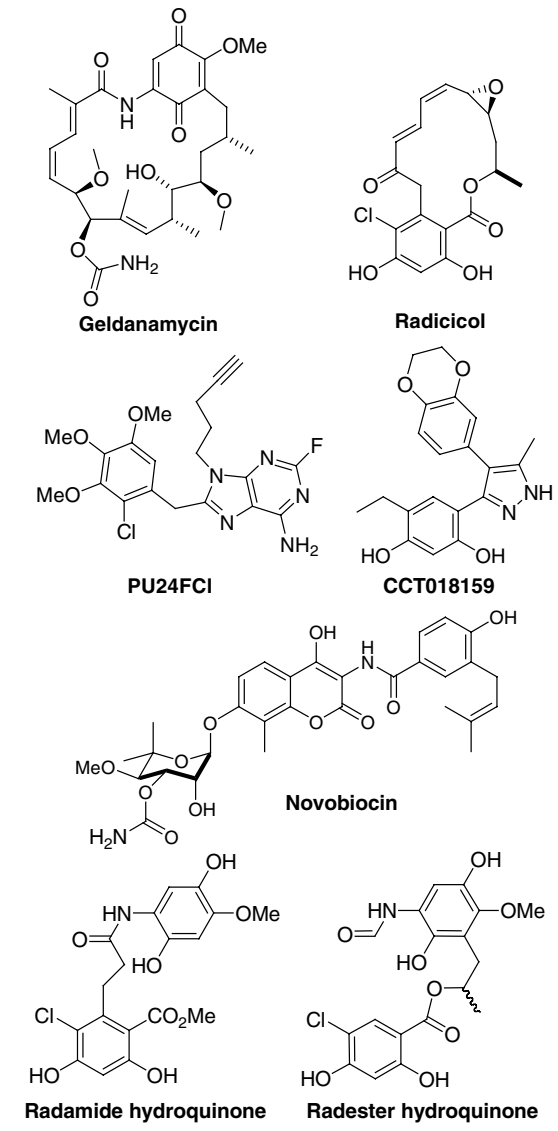


Figure 1. Hsp90 inhibitors.

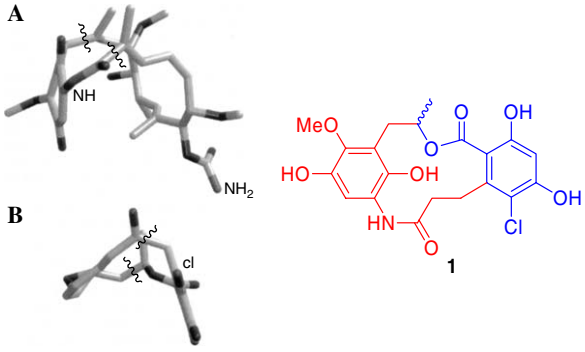


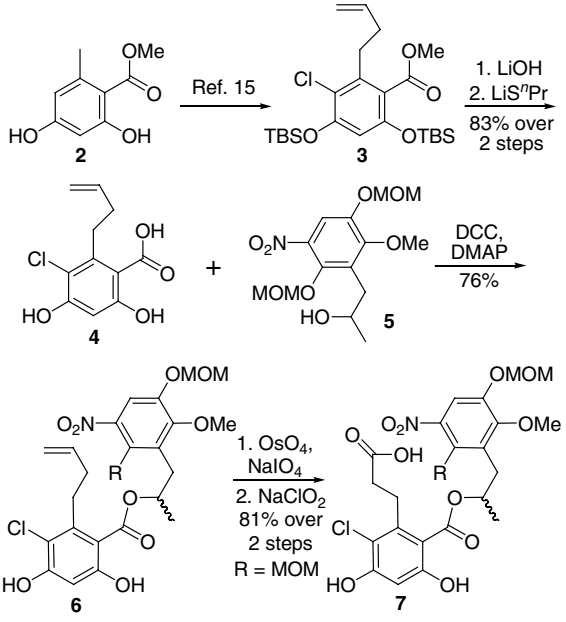
Figure 2. Conformations of geldanamycin and radicicol bound to yeast Hsp90 as well as the chimeric compound, radanamycin (**1**).

A simple disconnection across the apex of each natural product, followed by hybridization of the RDC resorcinol moiety with the GDA quinone, afforded the macrocyclic compound. Previously, we reported the syntheses and inhibitory activities of the open chain chimeras of RDC and GDA (radester and radamide)[14,15] and based on their activities proposed that the conformationally constrained macrocyclic analogue would be more effective as entropic penalties would be minimized. In this letter, we report the synthesis and evaluation of radanamycin (**1**), a macrocyclic chimera of radicicol and geldanamycin.

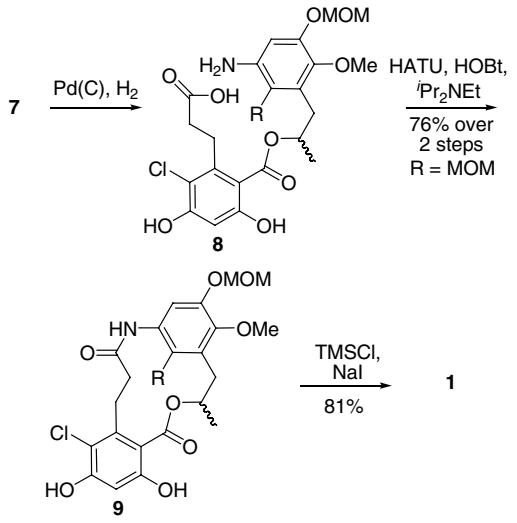
Synthesis of radanamycin began by modification of the procedure used to prepare the seco-derivatives. The TBS-protected resorcinolic methyl ester **3** was synthesized from ester **2** by previously reported methods (Scheme 1).[14] The silyl groups were removed and the ester dealkylated with the lithium anion of *n*-propylthiol to furnish the corresponding acid, **4**. DCC-mediated coupling of the free acid with the MOM-protected precursor to the quinone ring (**5**)[15] provided the ester product **6**, in good yield. Subsequent oxidation of the olefin and conversion to the acid, gave **7** in high yield.

The nitro substituent was reduced without competitive removal of the protecting groups by brief exposure to palladium on carbon under hydrogen gas for a limited time (Scheme 2).[16] Prolonged exposure to these conditions resulted in the accumulation of several undesired products.

Macrolactamization was problematic at the onset, but was finally achieved by treatment of the crude amino acid **8**, with HATU and HOBt to furnish the desired macrocycle **9**, in excellent yield. ^{1}H NMR of **9** provided evidence for a bent conformation similar to that observed for the natural products bound to Hsp90 by virtue of the upshield shift and anisotropy of several aliphatic protons. In analogy to the method used for the construction of the seco-agents, radester and radamide,[14,15] the MOM-protecting groups were removed



Scheme 1. Synthesis of **7**.



Scheme 2. Synthesis of radanamycin, **1**.

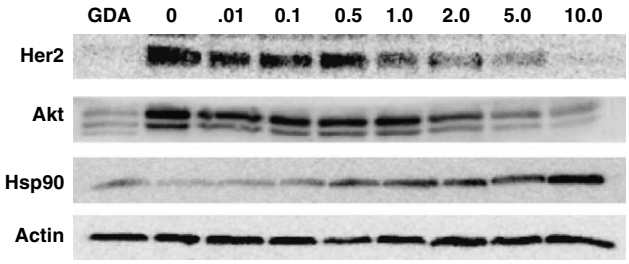


Figure 3. Western blot analyses of Her2, Akt, Hsp90, and actin. Concentrations of radanamycin are listed above each lane in μM. GDA was used as a positive control at 0.5 μM.

with in situ generated trimethylsilyl iodide to give hydroquinone **1**.[17]

With radanamycin in hand, we sought to determine its ability to inhibit Hsp90 as determined by the degradation of Hsp90-dependent client proteins. When **1** was incubated with MCF-7 breast cancer cells for 24 h, a dramatic effect on Hsp90-dependent client proteins (Her2 and Akt) was observed (Fig. 3). These Hsp90-dependent substrates were readily degraded in the presence of 1–5 μM of radanamycin, which correlated inversely with Hsp90 levels. Actin is not an Hsp90-dependent substrate and remains unaffected by Hsp90 inhibition, thereby allowing a suitable control for comparing relative protein concentrations.

Consistent with the degradation of Hsp90-dependent substrates, radanamycin exhibited anti-proliferative activity with an $IC_{50} = 1.2 \pm 0.1$ μM (Fig. 4), thereby linking Hsp90 inhibition directly to the inhibition of cell growth. The values obtained from these studies compare well to those of previously identified inhibitors of the Hsp90 protein folding machinery as noted earlier in this letter.[8–10,13,14]

Results from these studies indicate that chimeric compounds composed of RDC's resorcinol ring and GDA's quinone ring produce potent Hsp90 inhibitors

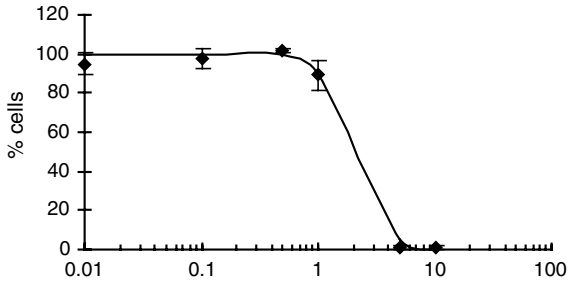


Figure 4. Anti-proliferative activity of radanamycin in MCF-7 breast cancer cells. Concentrations are reported on the *x* axis in μM.

and further derivatization of these molecules is likely to afford analogues with increased activity and perhaps, useful alternatives to the geldanamycin derivatives in clinical trials. Additional studies with these chimeric species are in progress and structure–activity relationships for these agents will be reported in due course.

Acknowledgments

The authors gratefully acknowledge support of this project by the NIH COBRE RR017708 and The University of Kansas Center for Research. G.S. is the recipient of a Susan G. Komen Dissertation Award.

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16. Intermediate **7** (69 mg, 0.12 mmol) was dissolved in EtOH (2.0 mL) before 10% palladium on carbon (15 mg) was

added. The mixture was purged with argon before H_2 gas was added. The heterogeneous mixture was stirred for 3 h and then filtered through a plug of Celite. The eluent was concentrated and the residue was redissolved in DMF (10 mL) before slow addition to a mixture of HATU (137 mg, 0.36 mmol), HOBt (49 mg, 0.36 mmol), and diisopropyl ethyl amine (0.189 mL, 1.08 mmol) in DMF (120 mL) over 4 h. Upon addition, the solution was heated at 70 °C for 15 h. The solvent was removed by distillation at reduced pressure. EtOAC (20 mL) was added to the residue and the resulting solution was washed with saturated aqueous NH_4Cl (3× 5 mL), H_2O (5 mL), and saturated aqueous NaCl (5 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was purified via chromatography (SiO_2, 20% hexanes in EtOAc) to afford **9** (48 mg, 76% yield, 2 steps) as a white solid: ^{1}H NMR (acetone-d_6, 400 MHz) δ 9.73 (s, 1H, –OH), 9.15 (s, 1H, –OH), 8.22 (s, 1H), 6.97 (s, 1H), 5.52 (m, 1H), 5.18 (dd, J = 6.6, 24.1 Hz, 2H), 4.90 (dd, J = 6.6, 24.1 Hz, 2H), 4.06 (s, 3H), 3.53 (s, 3H), 3.48 (s, 3H), 3.19 (dt, J = 4.8,13.3 Hz, 1H,), 3.19 (dd, J = 11.5, 13.2 Hz, 1H,), 3.06 (dd, J = 3.1, 13.2 Hz, 1H), 2.68 (dt, J = 4.0,13.3 Hz, 1H), 2.40 (dt, J = 3.9,13.1 Hz, 1H), 2.04 (m, 1H), 1.45 (d, J = 6.3 Hz, 2H); ^{13}C NMR (acetone-d_6, 125 MHz) δ 174.3, 168.7, 157.3, 156.0, 148.9, 147.6, 147.1, 139.6, 126.9, 126.8, 115.6, 113.4, 112.7, 102.4, 100.2, 95.8, 73.0, 60.8, 57.4, 56.0, 36.2, 31.8, 27.3, 20.9; IR (film) ν_{max} 3301, 3119, 2929, 1651, 1597, 1487, 1235, 1129, 1070 cm^{-1}; HRMS (TOF-ES+) found 626.1475 (M+H$^+$), calcd 626.1480 for $C_{24}H_{29}Cl\ NO_{10}$.

17. MOM-protected radanamycin **9** (76 mg, 0.144 mmol) was dissolved in CH_3CN (1.2 mL) and CH_2Cl_2 (1.2 mL) before NaI (216 mg, 1.44 mmol) and TMSCl (0.182 mL, 1.44 mmol) were added at rt. Upon addition, the solution turned cloudy and yellow. After 20 min, a saturated aqueous solution of $Na_2S_2O_4$ (2 mL) was added to the mixture and stirred for 10 min. The aqueous phase was extracted with EtOAc (3× 5 mL) and the combined organic layers were washed with H_2O (2× 5 mL), saturated aqueous NaCl (5 mL), dried (Na_2SO_4), filtered, and concentrated. The residue was purified via chromatography (SiO_2, 10% MeOH in CH_2Cl_2) to afford **1** (53 mg, 84% yield) as a white solid: ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.82 (br s, 2H), 6.59 (s, 1H), 6.41 (s, 1H), 5.55 (m, 1H), 3.97 (s, 3H), 3.54 (dt, J = 5.4, 13.3 Hz, 1H), 3.27 (dd, J = 11.6, 13.5 Hz, 1H), 2.96 (dd, J = 3.2, 13.5 Hz, 1H), 2.77 (dt, J = 3.6, 13.2 Hz, 1H), 2.18 (m, 1H), 1.97 (dt, J = 5.4, 11.8 Hz, 1H), 1.41 (d, J = 6.3 Hz, 3H); ^{3}C NMR (acetone-d_6, 125 MHz) δ 174.1, 168.8, 158.0, 156.0, 146.8, 145.2, 143.0, 139.8, 120.4, 120.0, 114.5, 112.7, 111.9, 102.0, 73.2, 59.9, 35.7, 29.3, 27.1, 20.5; IR (film) ν_{max} 3323, 3021, 1643, 1603, 1445, 1380, 1299, 1236, 1170 cm^{-1}; HRMS (TOF-ES+) found 438.0949 (M+H$^+$), calcd 438.0956 for $C_{20}H_{21}Cl\ NO_8$.