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Radester, a Novel Inhibitor of the Hsp90 Protein Folding Machinery

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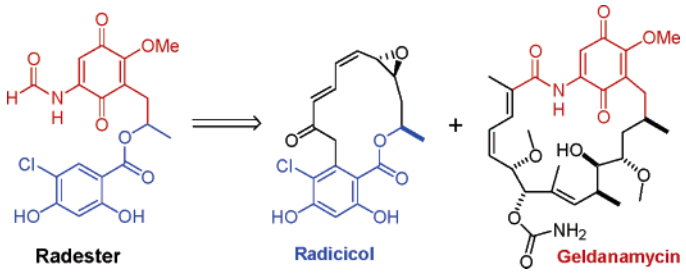
ABSTRACT



The antitumor antibiotics radicicol and geldanamycin are potent inhibitors of the Hsp90 protein folding machinery. Radester is a hybrid composed of radicicol's resorcinol ring and geldanamycin's quinone through an isopropyl ester. Radester was prepared, and the cytotoxicity of it and the corresponding hydroquinone were determined in MCF-7 breast cancer cells to be 13.9 and 7.1 μM, respectively. Protein degradation assays were performed on Hsp90-dependent client proteins, Her-2 and Raf, to correlate Hsp90 inhibition to cytotoxicity.

Hsp90 (90 kDa heat shock protein) is a molecular chaperone responsible for the conformational maturation of numerous oncogenic proteins.[1] Inhibition of Hsp90 turns the protein folding machinery into a catalyst for protein degradation. Recent studies have demonstrated that Hsp90 multiprotein complexes from tumor cells have higher affinity for ligands than Hsp90 in normal cells, because malignant cells are highly dependent upon the Hsp90 protein folding machinery for the maturation of mutated and over expressed client proteins that are vital to cell proliferation and growth.[2] In fact, proteins represented in all six hallmarks of cancer are dependent on the Hsp90 maturation process. Consequently, Hsp90 has emerged as a promising biological target for the treatment of cancer, because inhibition of Hsp90 results in a combinatorial blockade of multiple signaling cascades that are essential to tumor cell survival.[3]

Hsp90 is an ATP-dependent protein with two nucleotide-binding domains, one of which is located in the N-terminus and the other in the C-terminal region.[4] The energy derived from ATP hydrolysis is used to fold the nascent polypeptide into a biologically active protein. Disruption of Hsp90's ATPase activity results in the destabilization of multiprotein complexes and subsequent degradation of the client via the ubiquitin−proteasome pathway.[5]

Known inhibitors of Hsp90 manifest their activity by binding to the N-terminal ATP binding pocket and preventing Hsp90-catalyzed hydrolysis of ATP. Such inhibitors include the antitumor antibiotics geldanamycin (GDA), a 17-allyl-amino derivative of GDA (17-AAG), radicicol (RDC), and the synthetic ATP analogue PU3 (Figure 1).[6] The IC_{50}'s as determined in MCF-7 cells are 49 nM, 23 nM,[7] and 50 μM for GDA, RDC, and PU3, respectively.

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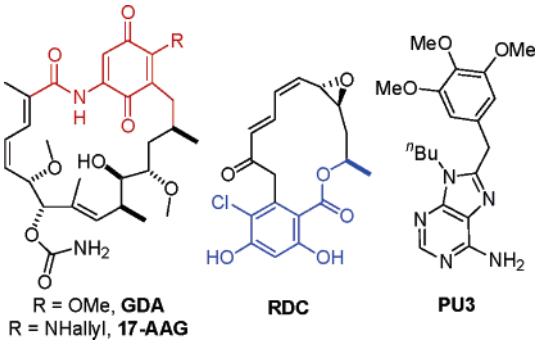


Figure 1. Known inhibitors of Hsp90.

Although 17-AAG has entered phase I clinical trials[8,9] for the treatment of several cancers, the quinone ring is redox-active. GDA has been shown to generate superoxide radicals in cells, which can lead to cell death without interfering directly with Hsp90.[10] In vivo, RDC is rapidly converted to inactive metabolites that have little or no affinity for Hsp90.[11] Consequently, researchers throughout industry and academia have pursued the development of new Hsp90 inhibitors without these detrimental properties.[6]

The previously solved co-crystal structures of RDC and GDA bound to Hsp90 (Figure 2) clearly demonstrated that

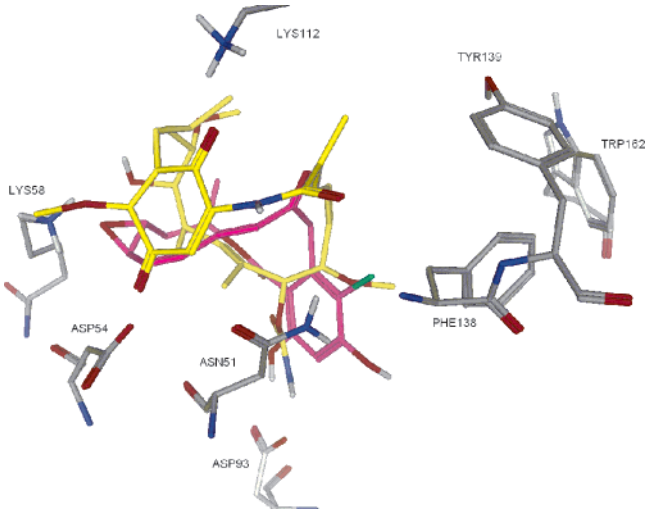


Figure 2. Superimposed co-crystal structures of GDA (yellow) and RDC (magenta) with Hsp90.

the resorcinol ring of RDC and the quinone of GDA bound in opposite orientations.[12] The resorcinol moiety provides a key hydrogen bond network with conserved water molecules coordinated through Asp93 in the region that typically binds the adenine ring of ATP. The carbamate on GDA provides similar interactions with this portion of the binding pocket. The quinone ring of GDA interacts with Asp54, Lys58, Lys112 and other amino acids, while the epoxide on RDC maintains only one hydrogen bond with Lys58. As a consequence of increasing interactions with Hsp90, we proposed that a molecule containing the quinone ring of GDA and the resorcinol ring of RDC would provide a new scaffold for the development of Hsp90 inhibitors. This chimera or hybrid of **rad**icicol and geld**anamycin** we named **radanamycin** ester (Radester, Figure 3).

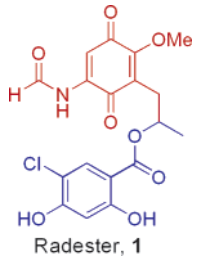
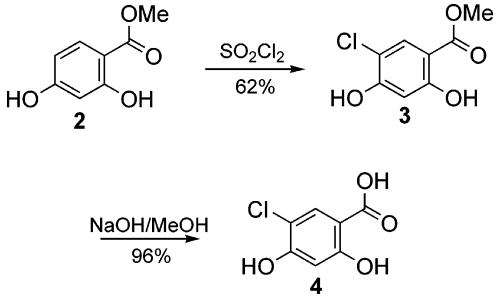


Figure 3. Hsp90 inhibitors.

Radester not only contains the quinone ring of GDA and the resorcinol ring of RDC but also maintains the isopropyl ester that is present on RDC and the same substituent attached to the GDA quinone. Because this molecule can be prepared in a minimal number of steps, we envision radester as a starting point for the construction of Hsp90 inhibitors with tunable properties that can be enhanced by derivatization of either the quinone ring to produce non-redox-active analogues or modification of the resorcinol ring to incorporate additional hydrophobic moieties that can project into the π-rich lipophilic pocket formed by amino acids Phe138, Trp162, and Tyr139.

The synthesis of radester began by preparation of the resorcinolic moiety. Chlorination of methyl 2,4-dihydroxybenzoate (**2**) with sulfuryl chloride furnished **3**, which was hydrolyzed to furnish acid **4** in good yield, Scheme 1.

Scheme 1. Synthesis of 5-Chloro-2,4-dihydroxybenzoic Acid



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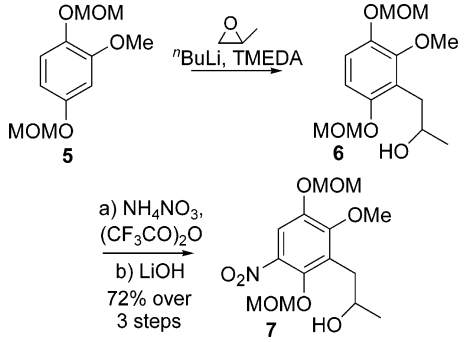
In light of previous work by our laboratory that suggested the hydroquinone was more potent than the corresponding quinone,[13] we designed a synthetic sequence that would allow for the preparation of radester quinone and the corresponding hydroquinone. As such, we chose to mask the hydroquinone as the bis(methoxymethylene)ether, which could be removed to provide the hydroquinone and upon subsequent oxidation afford the corresponding quinone.

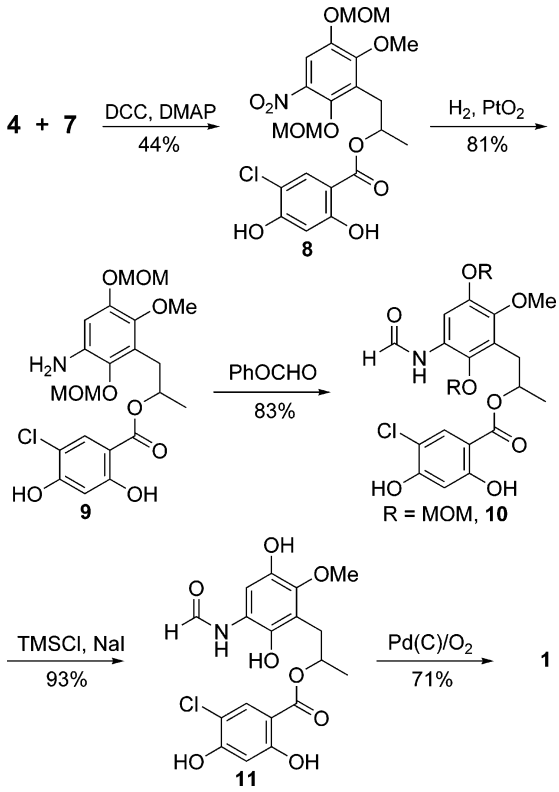
The quinone precursor was prepared from 2-methoxy-1,4-bis(methoxymethyleneoxy)-benzene[14] (**5**, Scheme 2). Treatment of this protected hydroquinone with *n*-butyllithium in the presence of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine afforded the lithium anion, which upon addition of propylene oxide provided the secondary alcohol, **6**, in good yield. Nitration of the aromatic ring containing the acid-labile protecting groups proved to be problematic under normal nitration conditions. However, success was granted by treatment of the aromatic ring with ammonium nitrate and trifluoroacetic anhydride,[15] which afforded the nitrated product in good yield. However, under these conditions the secondary alcohol was converted to the trifluoroacetyl ester, which was hydrolyzed by the addition of lithium hydroxide to furnish **7**.

Scheme 2. Synthesis of MOM-Protected Precursor to the Quinone



Coupling of **4** with **7** proved to be difficult, and after considerable optimization, **8** was best provided by treatment with dicyclohexylcarbodiimide and *N*,*N*-(dimethylamino)-pyridine, Scheme 3. The aromatic nitro substituent was converted to aniline **9** without reduction of the aryl−chloride bond in the presence of platinum (IV) oxide and hydrogen. Addition of phenolformate[16] to **9** gave the N-formylated product, **10**, which mimicked the same amide functionality found in GDA. Following the procedure of Andrus and coworkers,[14] the bis(methoxymethyleneoxy) groups were cleaved upon exposure to in situ-derived trimethylsilyl iodide to provide the hydroquinone precursor (**11**) to radester. Oxidation of the hydroquinone required stoichiometric palladium on carbon and oxygen to give radester **1**.[17]

Scheme 3. Synthesis of Radester



Cell proliferation studies with **11** and **1** were performed in the MCF-7 breast cancer cell line. As demonstrated by Figures 4A and 4B, the IC_{50} for **11** and **1** was 7.1 ± 0.3 μM and 13.9 ± 1.4 μM, respectively.

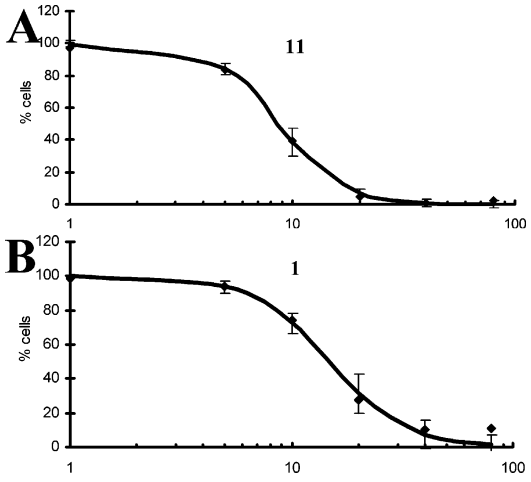


Figure 4. Cytotoxicity profiles for **11** and **1** in MCF-7 breast cancer cells (μM). The data represent the average of three experiments.

To determine whether the observed cytotoxicity was related to Hsp90 inhibition, **11** and **1** were incubated with

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MCF-7 breast cancer cells for 24 h. As can be seen from Western blot analyses of the protein lysates, both **11** and **1** resulted in the concentration-dependent degradation of Hsp90 client proteins, Her-2 and Raf (Figure 5). Hsp70 levels

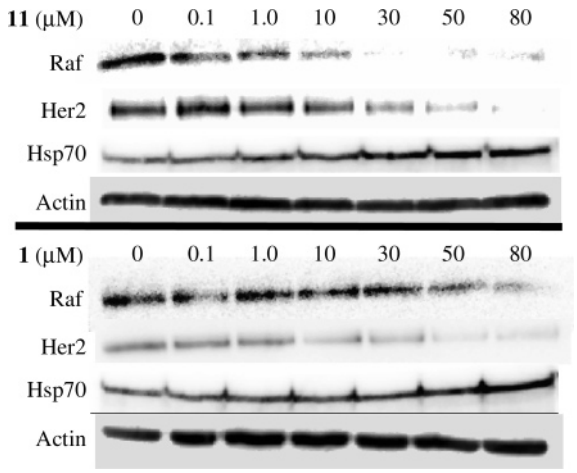

Figure 5. Western blot analyses of Hsp90 client protein degradation assays. Concentration of inhibitors (in μM) are denoted above each lane.

increased in a concentration-dependent manner, which is consistent with other Hsp90 inhibitors.[6] Since actin is not dependent upon the Hsp90 protein folding machinery, actin levels remained unchanged.

These results suggest the importance of a hydrogen bond donor in the region that typically binds the triphosphate moiety of Hsp90's natural substrate, ATP. After our initial publication disclosing radamide,[13] researchers at Kosan Biosciences[18] and Novartis[19] also reported molecules with hydrogen bond donors in the same proximity, KOSN1559 and G3130, respectively (Figure 6). Together, these data support our hypothesis that the quinone-binding region of Hsp90 plays an important role in ligand recognition and binding, despite its close proximity to the aqueous media (vida infra the co-crystal structure of the Hsp90 N-terminal domain bound to inhibitors).

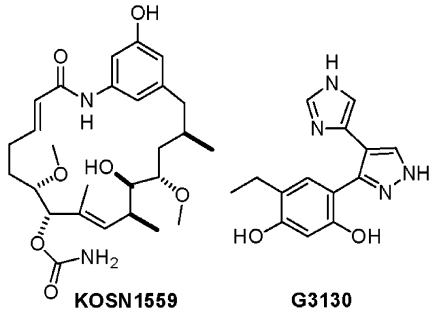

Figure 6. Hsp90 inhibitors with a hydrogen bond donor in the "quinone" binding region of the N-terminus.

These studies support our hypothesis that chimeric molecules derived from the two most potent natural product inhibitors, RDC and GDA, represent a promising new class of Hsp90 inhibitors. Additional structure−activity relationship studies are needed to provide non-redox-active analogues and chimeric molecules with greater inhibitory activity. Such studies are currently underway and will be reported in due course.

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

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