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Divergent Synthesis of a Pochonin Library Targeting HSP90 and in vivo Efficacy of Identified Inhibitor**

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Abstract

The heat shock protein 90 (HSP90) has emerged as one of the most exciting therapeutic target in recent years.[1,2] Despite the seemingly ubiquitous function of this constitutively expressed chaperone, its role in stabilizing conformationally labile proteins has implications in pathologies ranging from oncology to neurodegenerative diseases. Most of HSP90's endogenous clients[3] are key regulators of cell signaling which are destabilized and degraded in the absence of HSP90's chaperoning activity. The dependence of transformed cells on HSP90 is further heightened by the fact that many oncogenic mutations, while increasing the activity of pro-growth signaling pathways, are less stable than their wild type counterpart and have an increased dependence on HSP90's chaperoning activity.[4] A clinically relevant example is the heightened dependence of drug resistant Bcr-Abl mutants on HSP90's activity and the fact that HSP90 inhibitors in combination with Abl inhibitors remain effective against such mutants.[5,6] Accordingly, HSP90 inhibition provides a broad and effective target for anticancer treatment. Furthermore, HSP90 inhibitors can act synergistically with a cytotoxic agent.[7] HSP90 is also implicated in regulating the fate of a number of conformationally unstable proteins which underlie the development of neurodegenerative diseases.[8] It has been shown that HSP90 inhibitors can reduce protein aggregates in cellular and animal models of Huntington disease,[9] spinal and bulbar muscular atrophy,[10] Parkinson disease,[11] and other Tau protein-related neurodegenerative diseases.[12]

Keywords

resorcylic acid lactone; HSP90; 17AAG; pochonin; radicicol

Two natural products, radicicol and geldanamycin (**1** and **2**, Figure 1), were instrumental in understanding the role of HSP90 in oncogenic processes and its therapeutic potential.[13–15] However, neither natural product has acceptable pharmacological properties for clinical application. Structure-based design and high throughput screening have led to discovery of novel scaffold such as purines[16,17] and pyrazoles[18] however, improving the pharmacological properties and potency of the natural pharmacophores remains important. Indeed, the most advanced clinical candidate is a semisynthetic derivative of geldanamycin,

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17AAG (**3**, Figure 1), which is currently in multiple phase II studies.[19] Another semisynthetic derivative with a dimethoxyhydroquinone functionality has recently been reported to have better pharmacological properties than 17AAG while acting as a prodrug.[20] Radicicol, while having a higher affinity than does geldanamycin for HSP90, suffers from two limitations: a strained and highly sensitive epoxide and a conjugate diene which functions as a Michael acceptor. Indeed, the inactivity of radicicol in animals models has been attributed to a conjugate addition of thiol nucleophiles at the C13 position.[21] Akinaga and coworkers overcame this limitation by converting radicicol to an oxime which showed significant anti-tumor activity (reduction in tumor growth) in animal models.[21–23] Mindful of the labile epoxide, Danishefsky and coworkers reported a cyclopropyl analogue of radicicol which was nearly as effective in cellular assays; however its efficacy in animals has not been reported.[24,25] More recently, Moody and coworkers reported the synthesis of radicicol related resorcylics, exploring the importance of the macrocycle ring size.[26]

We have previously suggested that radicicol's epoxide moiety is important as a conformational bias which favors the bioactive conformation of the macrocycle and shown that another natural product, pochonin D (**4**, Figure 1), was also a good ligand for HSP90.[27] Furthermore, we have reported the use of polymer-supported reagents to synthesize a library extending the diversity of the pochonins (**5**, Figure 1).[28] Screening this library for HSP90 affinity and down-regulation of HSP90's client proteins revealed important structure-activity relationships and pointed to the ketone moiety as the most favorable position for improving activity. Herein, we report the structure activity relationship of a focused library of this important pharmacophore, an analogue of pochonin D having 100 fold improvements in its cellular activity as well as its efficacy in a breast tumor xenograft (BT-474).

While some of the simpler resorcylics such as pochonin D had good affinity for HSP90, their cellular activity was disappointing in comparison to 17AAG. The ambiguous correlation between 17AAG's HSP90 affinity and cellular activity remains a subject of intense investigation[29–31] but can be rationalized by the kinetics of binding.[31] Similar discrepancies between HSP90 affinity measured by a fluorescence polarization assay and ATPase inhibition have been noted for inhibitors based on the resorcylics motif.[26]

Based on the observation that oxime substitutions were tolerated for HSP90 activity in the pochonin series and inspired by previous success with radicicol, [21–23] we develop a divergent synthesis providing rapid access to this class of compounds. Readily available intermediate **6** was deprotonated with LDA (Scheme 1) and reacted with Weinreb amide **7**. Quenching the reaction with benzoic acid resin sequestered the amine byproducts and afford **8** with sufficient purity to be engaged directly in the oxime formation with aminooxyacetic acid. After evaporation of the solvent, the crude mixture was treated with an acidic resin which removed the excess hydroxyl amine and some byproducts stemming from conjugate addition to afford **9**. Intermediate **9** was then loaded on 2-chlorotrityl resin and the carboxylate group was deprotected under the action of TBAF thus revealing the acid which was engaged in a Mitsunobu esterification with homoallylic alcohols **10** to obtain polymer-bound intermediates **11**. It is important to note that this reaction sequence is not possible in the absence of the oxime functionality as it leads to coumarin byproducts.[32] The resins were then treated to the second generation Grubbs' catalyst under microwave irradiation to obtain the macrocycles **12** in excellent yield and purity after cleavage from the resin with hexafluoropropanol (HFIP). Contrarily to TFA, these mild cleavage conditions were found to leave the EOMs intact thus enabling a selective esterification or amidation. For this purpose, we used an immobilized carbodiimide reagent followed by a sulfonic acid resin treatment to obtain a library of pochonin oximes **13**.

The library was then screened for affinity to HSP90 α ,[33] Her-2 (Hsp90 client) degradation [34] and cytotoxicity against SKBrS and HCC1954, two breast cancer cell lines which overexpress Her-2 (Figure 2). The most potent inhibitors were compound **13a** and **b** containing the piperidine amide moiety. It is interesting to note that the simplified analogue lacking the chiral methyl group is as active as the parent compound **13c** and that while the chlorine atom is important for the activity of both radicicol and pochonin D, it is not for the activity of compound **13b**. The structure activity data suggests that the piperidine amide has a relatively good fit in a lipophilic pocket as the morpholino analogue (**13d**), piperazine analogues (**13e**) or simple methyl amide (**13i**) lose significant activity. A cyclohexyl amide (**13k**) or benzyl amide (**13j**) on the other hand were also good ligands. Consistent with the previous radicicol oxime,[22] it is interesting to note that there is a significant difference of activity between the E and the Z isomer, with the E isomer having higher activity (**13a** vs **13f** and **13b** vs **13g**). Based on the potent activity of **13a**, this compound was further evaluated *in vivo*.

Treatment of CB17/SCID mice with **13a** at 100mg/kg for five consecutive days was well tolerated with minimal weight loss. To investigate the *in vivo* efficacy of **13a**, a xenograft bearing BT-474 (breast tumor cell line) was used as this tumorigenic cell line has been shown to respond to HSP90 inhibitors[35] in an animal model. Based on the cellular potency of **13a**, two schedules of 100 mg every other day (q2d) or every four days (q4d) during 28 days were investigated. Gratifyingly, the treatment with **13a** resulted in a dose-dependent inhibition of the tumor growth with an 18% regression in tumor volume using the q2d schedule (Figure 3a). In neither schedule was a significant weight loss observed (Figure 3b). Histologic examination of tumors removed from animals receiving either vehicle (DMSO) or drug for 28 days following the q2d schedule revealed a dramatic loss of cellularity in tumors obtained from drug-treated animals. Nuclei of remaining cells were uniformly condensed, suggested the occurrence of massive apoptosis (Figure 4, top panels). This was confirmed by the high degree of nuclear TUNEL staining seen in tumors excised from drug-treated animals (Figure 4, bottom panels). These data suggest that tumor regression in animals treated for 28 days with the q2d schedule may be more dramatic than estimated with tumor volume measurements as depicted in Figure 3, since few to no viable cells can be identified at the end of the treatment period.

In conclusion, pochoximes **13a** and **13b** have a higher affinity for HSP90 and are more active in reducing HSP90's client proteins than radicicol. This is the first report of an HSP90 inhibitor based on the resorcylic macrolide scaffold to show a regression in tumor size and their effectiveness at doses below the maximum tolerated dose suggest a meaningful therapeutic window. The use of polymer bound reagents[36] and solid phase chemistry facilitates the synthesis of new analogues and set a successful precedent for the rapid elaboration of natural product libraries.

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Figure 1. Structure of radicicol, geldanamycin, 17AAG, pochonin D and general structure of the pochonin library (5).

13a: $R^1 = H$; $R^2 = Cl$; $X = CH_2$

HSP90 affinity: 0.021

Client depletion: 0.035

cytotoxicity: 0.125; 0.320

13b: $R^1 = H$; $R^2 = H$; $X = CH_2$

HSP90 affinity: 0.015

Client depletion: 0.050

cytotoxicity: 0.120; 0.220

13c: $R^1 = Me$; $R^2 = H$; $X = CH_2$

HSP90 affinity: 0.018

Client depletion: 0.026

cytotoxicity: 0.450; 0.630

13d: $R^1 = H$; $R^2 = Cl$; $X = O^*$

HSP90 affinity: 0.220

Client depletion: >10

cytotoxicity: >10; >10

13e: $R^1 = H$; $R^2 = Cl$; $X = NH^*$

HSP90 affinity: >10

Client depletion: >10

cytotoxicity: >10; >10

1: radicicol

HSP90 affinity: 0.140

Client depletion: 0.45

4: pochonin D

HSP90 affinity: 0.360

Client depletion: 3.5

3: 17-AAG

HSP90 affinity: 0.032

Client depletion: 0.050

13f: $R = Cl$

HSP90 affinity: 0.068

Client depletion: 2.4

cytotoxicity: 1.3; 2.8

13g: $R = H$

HSP90 affinity: 0.081

14a: $R = MeO$

HSP90 affinity: 1.8

Client depletion: >10

cytotoxicity: >10; 5.2

14b: $R = H^*$

HSP90 affinity: 0.110

Client depletion: 5.0

cytotoxicity: >10; >10

13h: $R = -NHCH_2CH_2OH^*$

HSP90 affinity: 0.390

Client depletion: 7.7

cytotoxicity: 7.5; >10

13i: $R = -NHMe^*$

HSP90 affinity: 1.20

Client depletion: >10

cytotoxicity: >10; >10

13j: $R = -NHBn^*$

HSP90 affinity: 0.11

Client depletion: 5.5

cytotoxicity: 3.5; 8.5

13k: $R = -NHCy^*$

HSP90 affinity: 0.090

Client depletion: 0.25

cytotoxicity: 0.55; 0.45

13l: $R = -OMe^*$

HSP90 affinity: 0.190

Client depletion: 6.5

cytotoxicity: >10; >10

13m: HSP90 affinity: >10

Client depletion: >10

cytotoxicity: >10; >10

Figure 2.

Biological activity (μM) of pochonin-oxime derivatives (* 1:1 mixture of E:Z oximes).

Figure 3.
Tumor volume (BT474) and animal weight following treatment with **13a** or control vehicle.

Figure 4.

Tumor histology and apoptosis in mock- and drug-treated animals. Top panels represent hematoxylin & eosin (H & E) stained paraffin sections. Nuclei appear blue in color. The dark blue condensed nuclei in drug-treated tumors (right) are consistent with apoptotic cells. A dramatic loss of cellularity in drug-treated tumors can also be clearly seen. Bottom panels represent TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling)-stained paraffin sections. The high preponderance of reddish-pink nuclei (positive for TUNEL staining) in the drug-treated tumors reflects DNA fragmentation which is characteristic of apoptosis.

Scheme 1.

Reagents and conditions: a) LDA (2.0 equiv), THF, $-78\text{ }^{\circ}\text{C}$, 5 min; **7** (1.0 equiv), 10 min; PS-COOH (5.0 equiv), -78 to $23\text{ }^{\circ}\text{C}$, 20 min, ~50%; b) $\text{NH}_2\text{OCH}_2\text{CHO}_2\text{H}$ (5.0 equiv), Py/AcOH 5:1, $40\text{ }^{\circ}\text{C}$, 24 h, 45–95%; c) PS-ClTr-Cl (3.0 equiv), DIPEA (6.0 equiv), CH_2Cl_2 , $23\text{ }^{\circ}\text{C}$, 24 h; then AcOH (20 equiv), $23\text{ }^{\circ}\text{C}$, 24 h; d) TBAF (4.0 equiv), $23\text{ }^{\circ}\text{C}$, 4 h; e) **10** (5.0 equiv), Ph_3P (2.0 equiv), DIAD (2.0 equiv), toluene, $23\text{ }^{\circ}\text{C}$, 12 h; f) Grubbs II (0.06 equiv), CH_2Cl_2 , $120\text{ }^{\circ}\text{C}$, MW, 3×45 min; HFIP/ CH_2Cl_2 1/4, $23\text{ }^{\circ}\text{C}$, 3 h, 20–30% over 5 steps; g) HFIP; h) PS-DCC (3.0 equiv), DMAP (cat), $\text{R}^3\text{-H}$ (2.0 equiv), $23\text{ }^{\circ}\text{C}$, 72 h, ~75 %; i) PS-SO₃H (10 equiv), MeOH, $23\text{ }^{\circ}\text{C}$, 4 h, ~85%.