Antitumor Agents

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Divergent Synthesis of a Pochonin Library Targeting HSP90 and In Vivo Efficacy of an Identified Inhibitor**

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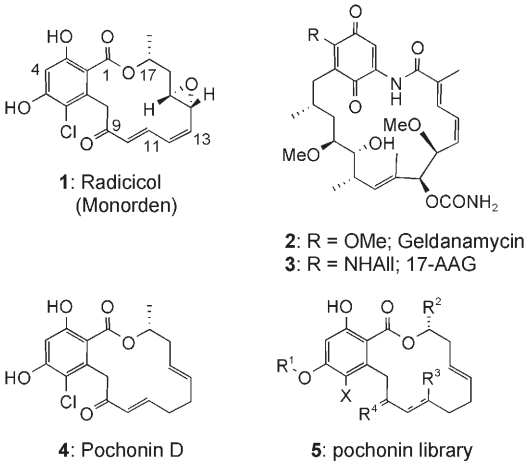
The heat-shock protein 90 (HSP90) has emerged as one of the most exciting therapeutic targets in recent years.[1,2] Despite the seemingly ubiquitous function of this constitutively expressed chaperone, its role in stabilizing conformationally labile proteins has implications in pathologies ranging from oncology to neurodegenerative diseases. Most endogenous clients[3] of HSP90s are key regulators of cell signaling which are destabilized and degraded in the absence of the chaperoning activity of HSP90. 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 counterparts and have an increased dependence on the chaperoning activity of HSP90.[4] A clinically relevant example is the heightened dependence of drug-resistant Bcr-Abl mutants on the activity of HSP90, 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 the treatment of cancer. Furthermore, HSP90 inhibitors can act synergistically with a cytotoxic agent.[7] HSP90 is also implicated in regulat-



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

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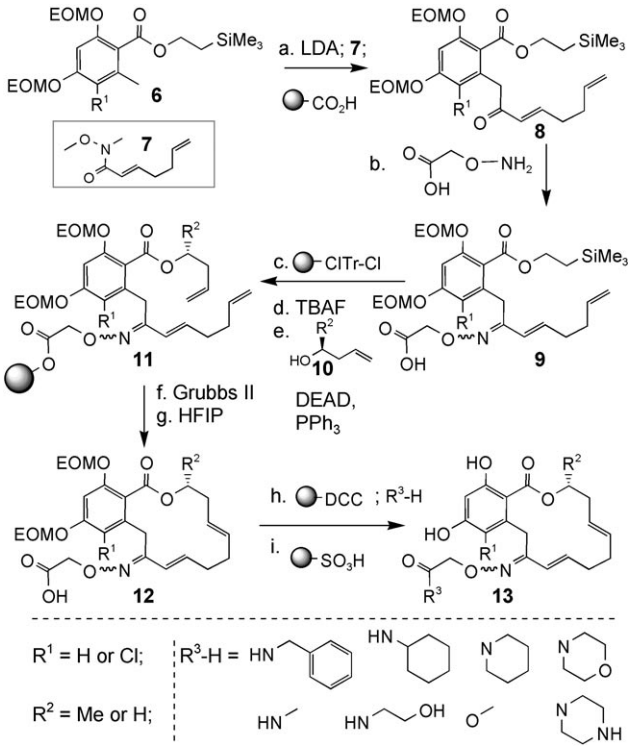
Supporting information for this article (including physical characterization of compounds **13** and **14**) is available on the WWW under http://www.angewandte.org or from the author.



Scheme 2. Reagents and conditions: a) LDA (2.0 equiv), THF, −78 °C, 5 min; **7** (1.0 equiv), 10 min; PS-COOH (5.0 equiv), −78 to 23 °C, 20 min, ca. 50%; b) $NH_2OCH_2CHO_2H$ (5.0 equiv), Py/AcOH 5:1, 40 °C, 24 h, 45–95%; c) PS-ClTr-Cl (3.0 equiv), DIPEA (6.0 equiv), CH_2Cl_2, 23 °C, 24 h; then AcOH (20 equiv), 23 °C, 24 h; d) TBAF (4.0 equiv), 23 °C, 4 h; e) **10** (5.0 equiv), Ph_3P (2.0 equiv), DEAD (2.0 equiv), toluene, 23 °C, 12 h, f) Grubbs II cat. (0.06 equiv), CH_2Cl_2, 120 °C, microwaves, 3×45 min; g) HFIP/CH_2Cl_2 (1:4), 23 °C, 3 h, 20–30% over 5 steps; h) PS-DCC (3.0 equiv), DMAP (cat.), R^3-H (2.0 equiv), 23 °C, 72 h, ca. 75%; i) PS-SO_3H (10 equiv), MeOH, 23 °C, 4 h, ca. 85%. DCC = *N*,*N*′-dicyclohexylcarbodiimide, DEAD = diethylazodicarboxylate, DIPEA = *N*,*N*-diisopropylethylamine, DMAP = 4-dimethylaminopyridine, EOM = ethoxymethyl, HFIP = hexafluoroisopropanol, LDA = lithium diisopropylamide, PS = polystyrene, Py = pyridine, TBAF = tetrabutylammonium fluoride, Tr = trityl.

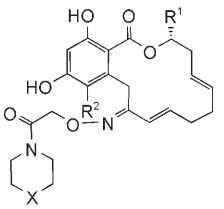
ing the fate of a number of conformationally unstable proteins which are associated with the development of neurodegenerative diseases.[8] It has been shown that HSP90 inhibitors can reduce protein aggregates in cellular and animal models of Huntington disease,[9] spinal and bulbar muscular atrophy,[10] Parkinson disease,[11] and other Tau protein related neurodegenerative diseases.[12]

Two natural products, radicicol and geldanamycin (**1** and **2**, Scheme 1), were instrumental in understanding the role of HSP90 in oncogenic processes as well as 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 the discovery of novel scaffolds 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 17AAG (**3**, Scheme 1), the semisynthetic derivative of geldanamycin, 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, although having a higher affinity than geldanamycin for HSP90, suffers from two limitating features: a strained and highly sensitive epoxide and a conjugate diene which functions as a Michael acceptor. Indeed, the inactivity of radicicol in animal models has been attributed to a conjugate addition of thiol nucleophiles at the C13-position.[21] Akinaga and co-workers overcame this limitation by converting radicicol into an oxime, which showed significant antitumor activity (reduction in tumor growth) in animal models.[21–23] Mindful of the labile epoxide, Danishefsky and co-workers 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 co-workers reported the synthesis of radicicol-related resorcylides, and explored the importance of the size of the macrocyle.[26]

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

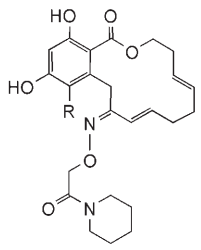
While some of the simpler resorcylides such as pochonin D had good affinity for HSP90, their cellular activity was disappointing in comparison to that of 17AAG. The ambiguous correlation between the affinity of 17AAG for HSP90 and its cellular activity remains a subject of intense investigation,[29–31] 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 resorcylides motif.[26]

Based on the observation that oxime substitutions in the pochonin series did not affect the HSP90 activity, and inspired by previous success with radicicol,[21–23] we developed a divergent synthesis that provided rapid access to this class of compounds. Readily available intermediate **6** was deprotonated with LDA (Scheme 2) and treated with Weinreb

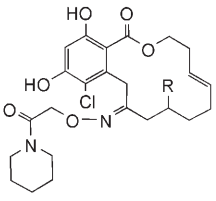


13a: R^1 = H; R^2 = Cl; X = CH_2
HSP90 affinity: 0.021
Client depletion: 0.035
cytotoxicity: 0.125; 0.320
13b: R^1 = H; R^2 = H; X = CH_2
HSP90 affinity: 0.015
Client depletion: 0.050
cytotoxicity: 0.120; 0.220
13c: R^1 = Me; R^2 = H; X = CH_2
HSP90 affinity: 0.018
Client depletion: 0.026
cytotoxicity: 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

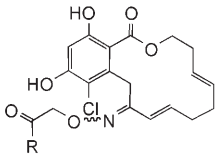
1: radicicol
HSP90 affinity: 0.140
Client depletion: 0.45
4: pochonin D
HSP90 affinity: 0.360
Client depletion: 3.5
3: 17-AAG
HSP90 affinity: 0.032
Client depletion: 0.050



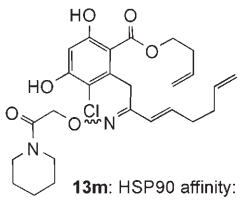
13f: R = Cl
HSP90 affinity: 0.068
Client depletion: 2.4
cytotoxicity: 1.3; 2.8
13g: R = H
HSP90 affinity: 0.081



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:
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 1. Biological activity (μM) of pochonin-oxime derivatives: HSP90α affinity, client depletion (Her-2 from SKBr3 cell line), and cytotoxicity (SKBr3 and HCC1954 respectively). * denotes an approximate 1:1 mixture of *E*/*Z* oximes.

amide **7**. Quenching the reaction with benzoic acid resin sequestered the amine by-products and afforded **8** with sufficient purity to be engaged directly in the formation of an oxime on reaction with aminooxyacetic acid. After evaporation of the solvent, the crude mixture was treated with an acidic resin, which removed the excess hydroxylamine and some by-products stemming from conjugate addition, to afford **9**. Intermediate **9** was then loaded onto 2-chlorotrityl resin and the carboxylate group was deprotected with TBAF to reveal the acid, which was subsequently 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 the formation of coumarin.[32] The resins were then treated with the second-generation Grubbs catalyst under microwave irradiation to obtain the macrocycles **12** in excellent yield and purity after cleavage from the resin with hexafluoroisopropanol (HFIP). In contrast to TFA, these mild cleavage conditions were found to leave the EOM groups intact, thus enabling a subsequent selective esterification or amidation. For this purpose, we used an immobilized carbodiimide reagent followed by treatment with a sulfonic acid resin to obtain a library of pochonin oximes **13**.

The library was then screened for affinity to HSP90α,[33] Her-2 (Hsp90 client) degradation,[34] and cytotoxicity against SKBr3 and HCC1954, two breast cancer cell lines which overexpress Her-2 (Figure 1). The most potent inhibitors were compounds **13a** and **13b**, which contain 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 important for the activity of **13b**. The structure–activity data suggest that the piperidine amide has a relatively good fit in a lipophilic pocket, as the morpholino analogue (**13d**), piperazine analogue (**13e**), and simple methylamide (**13i**) have significantly lower activity. The cyclohexylamide (**13k**) or benzylamide (**13j**) analogues, 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 in activity between the *E* and the *Z* isomers, with the *E* isomer having higher activity (**13a** versus **13f** and **13b** versus **13g**). Compound **13a** was further evaluated in vivo because of its potent activity.

Treatment of CB17/SCID mice with **13a** at 100 mg kg^{-1} for five consecutive days was well tolerated, with minimal weight loss observed. To investigate the in vivo efficacy of **13a**, a xenograft bearing BT-474 (breast-tumor cell line) was used, as this tumorgenic 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) over 28 days were investigated. Gratifyingly, treatment with **13a** resulted in a dose-dependent inhibition of the tumor growth, with an 18% regression in the tumor volume using the q2d schedule ($p = 0.0002$, Figure 2a). In neither schedule was a significant weight loss observed (Figure 2b). Histologic examination of tumors removed from animals receiving either DMSO (as vehicle) or drug for 28 days following the q2d schedule revealed a dramatic loss of cellularity in tumors obtained from drug-treated animals. The nuclei of remaining cells were uniformly condensed, thus suggesting the occurrence of massive apoptosis (Figure 3, top panels). This finding was confirmed by the high degree of nuclear TUNEL staining seen in tumors excised from drug-treated animals (Figure 3, bottom panels). These data suggest that tumor regression in animals treated for 28 days with the q2d schedule may be more dramatic than estimated from measurement of the tumor volume, as depicted in Figure 3, since few to no viable cells could 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 the client proteins of HSP90 than is 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 has facilitated the

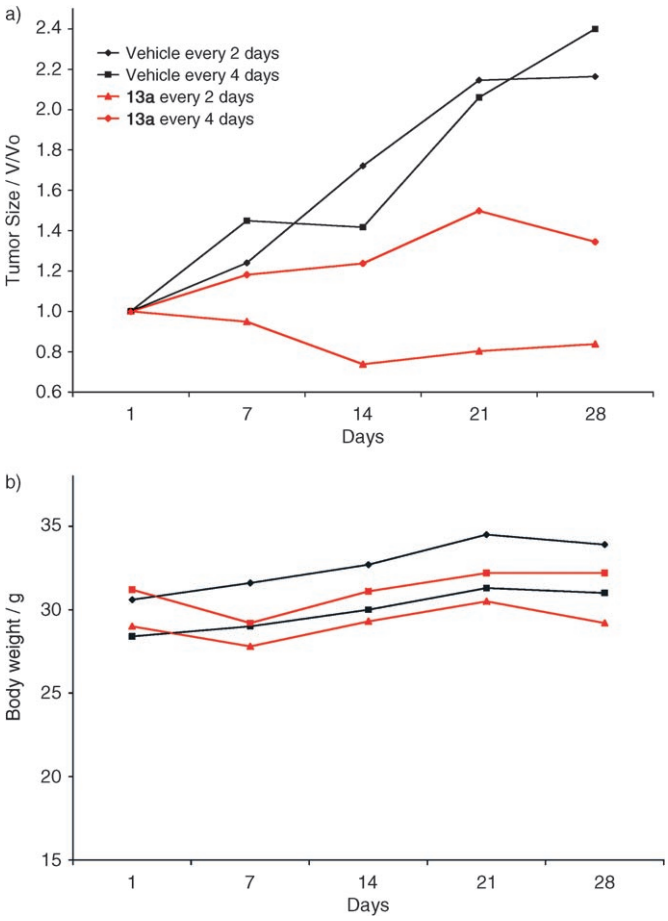


Figure 2. a) Tumor volume (BT474) and b) animal weight following treatment with **13a** or the control vehicle (DMSO). Each point represents the mean of measurements from five (for the vehicle) or six (for **13a**) animals. See the Supporting Information for errors and statistical analysis.

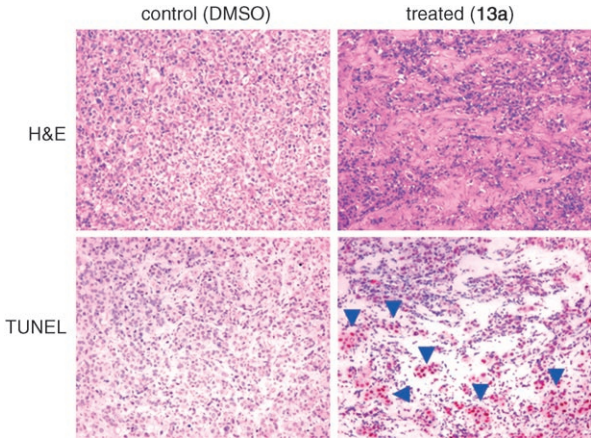


Figure 3. Tumor histology and apoptosis in DMSO- and drug-treated animals. The top panels represent hematoxylin and eosin (H & E) stained paraffin sections. Nuclei appear blue in color. The dark blue condensed nuclei in drug-treated tumors (right) are consistent with apoptotic cells. A dramatic loss 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. The blue arrowheads point to characteristic TUNEL-positive nuclei.

synthesis of new analogues and set a successful precedent for the rapid elaboration of natural product libraries.

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