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Stereocontrolled Synthesis of a Complex Library via Elaboration of Angular Epoxyquinol Scaffolds

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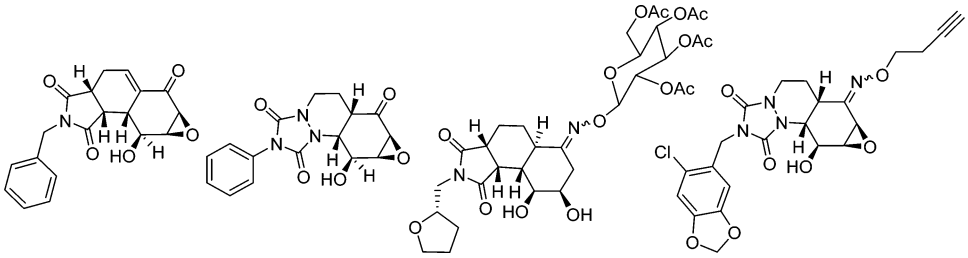

We have accomplished the synthesis of a complex chemical library via elaboration of angular epoxyquinol scaffolds with distinct skeletal frameworks. The key strategy involves highly stereocontrolled [4 + 2] Diels−Alder cycloadditions of chiral, nonracemic epoxyquinol dienes to generate the scaffolds. Further scaffold diversification involves hydrogenation, epimerization, dehydration, and condensation of the carbonyl group with alkoxyamine and carbazate building blocks. Further elaboration of the scaffolds also provided new skeletal frameworks using hydroxyl-directed Diels−Alder cycloaddition and reductive N−N bond cleavage. The overall process afforded 244 highly complex and functionalized compounds. Preliminary biological screening of the library uncovered six compounds which showed significant inhibition of Hsp 72 induction.

Introduction

Due to the growing use of small-molecule libraries for studies of protein function and discovery of novel drug leads, the need for efficient generation of small-molecule libraries with structural features of bioactive natural products has grown dramatically.[1] In this regard, stereocontrolled synthesis of complex chemical libraries with distinct skeletal frameworks via diversity-oriented synthesis (DOS)[2] provides access to such libraries, including those based on natural product-like scaffolds.[3]

As part of our overall interest in complex natural product synthesis, we have accomplished syntheses of a number of epoxyquinol-containing natural products.[4] A recent example is the enantioselective total synthesis of the ubiquitin-activating enzyme inhibitor (+)-panepophenanthrin **1** (Scheme 1).[5] In this study, we success-

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(1) For recent reviews, see: (a) Weber, L. *Curr. Opin. Chem. Biol.* **2000**, *4*, 295. (b) Wessjohann, L. A. *Curr. Opin. Chem. Biol.* **2000**, *4*, 303. (c) Hall, D. G.; Manku, S.; Wang, F. *J. Comb. Chem.* **2001**, *3*, 125. (d) Ganesan, A. *Pure Appl. Chem.* **2001**, *73*, 1033. (e) Breinbauer, R.; Vetter, I. R.; Waldmann, H. *Angew. Chem., Int. Ed.* **2002**, *41*, 2878. For selected examples, see: (f) Lee, D.; Sello, J. K.; Schreiber, S. L. *J. Am. Chem. Soc.* **1999**, *121*, 10648. (g) Nicolaou, K. C.; Pfefferkorn, J. A.; Roecker, A. J.; Cao, G. Q.; Barluenga, S.; Mitchell, H. J. *J. Am. Chem. Soc.* **2000**, *122*, 9939. (h) Pelish, H.; Westwood, N.; Feng, Y.; Kirchhausen, T.; Shair, M. D. *J. Am. Chem. Soc.* **2001**, *123*, 6740. (i) Brohm, D.; Phillippe, N.; Mertzger, S.; Bhargava, A.; Muelle, O.; Lieb, F.; Waldmann, H. *J. Am. Chem. Soc.* **2002**, *124*, 13171. (j) Couladouros, E. A.; Strongilos, A. T. *Angew. Chem., Int. Ed.* **2002**, *41*, 3677. (k) Lo, M. M.-C.; Neumann, C. S.; Nagayama, S.; Perlstein, E. O.; Schreiber, S. L. *J. Am. Soc. Chem.* **2004**, *126*, 16077.

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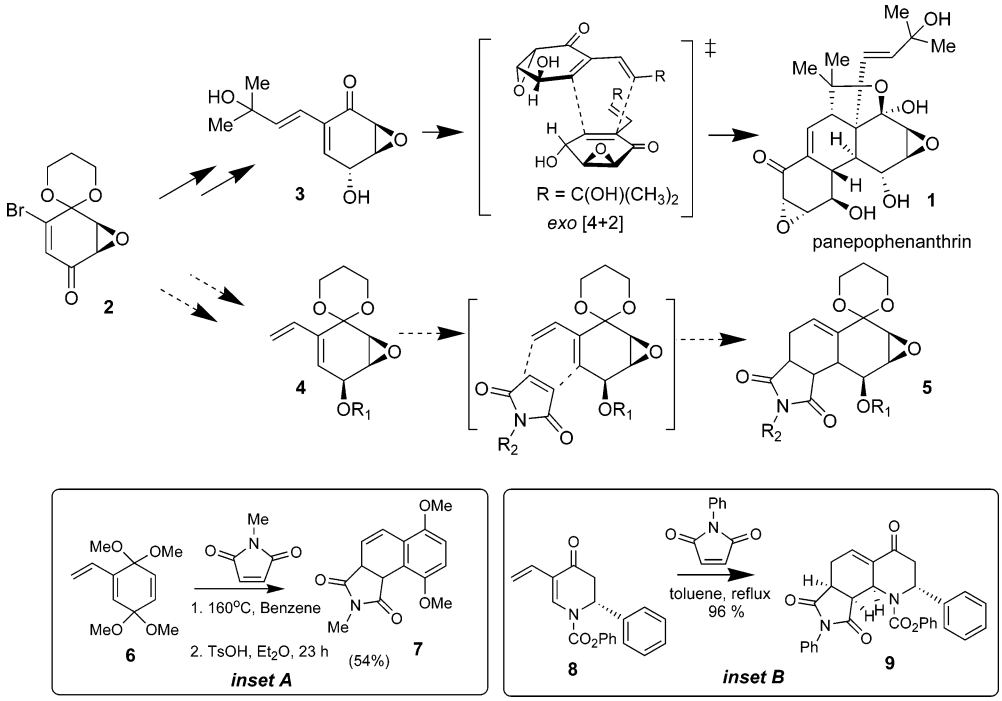
SCHEME 1. Target and Diversity-Oriented Synthesis Using Epoxyketone Scaffolds



fully transformed chiral, nonracemic bromo epoxy ketone **2** to diene monomer **3**, which smoothly underwent highly selective *exo*-Diels–Alder cycloaddition to generate **1**. We also considered use of bromo epoxy ketone **2** for diversity-oriented synthesis[2] of natural product-like molecules. Previous investigations have demonstrated the utility of [4 + 2] cycloadditions in complex chemical library construction.[6] Diels–Alder cycloadditions of derived diene **4** with dienophiles such as maleimides may be used to prepare angular compounds such as **5**. Literature reports have demonstrated the use of related cycloaddtions to produce complex angular skeletons including studies by Parker and co-workers employing an achiral diene **6** and *N*-ethylmaleimide to prepare angular compound **7** (Scheme 1, inset A)[7] and by Comins and co-workers using chiral 5-vinyldihydropyridones **8** and *N*-phenylmaleimide to generate cycloadduct **9** (Scheme 1, inset B).[8] We planned to use solution-phase parallel synthesis methods (extractive workups, polymer reagent, and scavenger techniques) for elaboration of scaffolds to prepare target library structures.[9]

Herein, we report the highly stereocontrolled solution-phase synthesis of a complex chemical library via elaboration of angular epoxyquinol scaffolds. Our approach was initiated by parallel synthesis of angular epoxyquinol scaffolds and further elaboration to afford distinct molecular frameworks. Subsequently, a library of 244 highly functionalized and stereochemically well-defined compounds was completed. Further elaboration of the scaffolds and preliminary biological screening of library members for inhibition of heat shock protein (HSP) induction will also be described.

Results and Discussion

Synthesis of Epoxyquinol Scaffolds. Scaffold synthesis was initiated by selective reduction of epoxyketone **2**[5] with Super-Hydride to afford a *syn*-epoxyquinol as a single diastereomer[5,10] followed by silyl protection of the secondary alcohol to provide compound **10** (Scheme 2). Stille cross-coupling[11] of **10** afforded chiral diene **11** in good yield. Diels–Alder cycloaddition of **11** with *N*-benzylmaleimide smoothly generated angular derivative **12** as a single diastereomer in quantitative yield. The *endo* stereoselectivity of the cycloaddition was confirmed by NOE analysis.[12] Global deprotection of both the cyclic ketal and the TBS ether with HF–CH_3CN, followed by quenching with methoxytrimethysilane (HF scavenger),[13] afforded angular epoxyquinol **13** (95%). [4 + 2] cyclo-

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(12) See the Supporting Information for further details.

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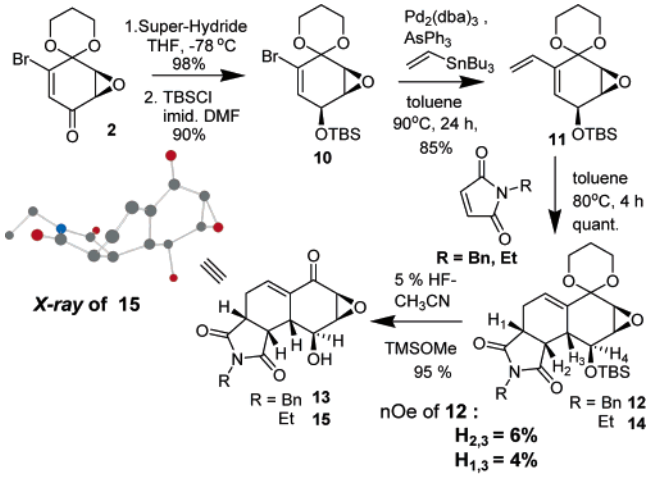
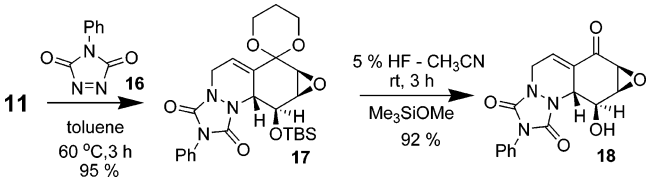
SCHEME 2. Synthesis of Maleimide-Derived Angular Epoxyquinol Scaffolds



SCHEME 3. Synthesis of a Urazole-Containing, Angular Epoxyquinol Scaffold

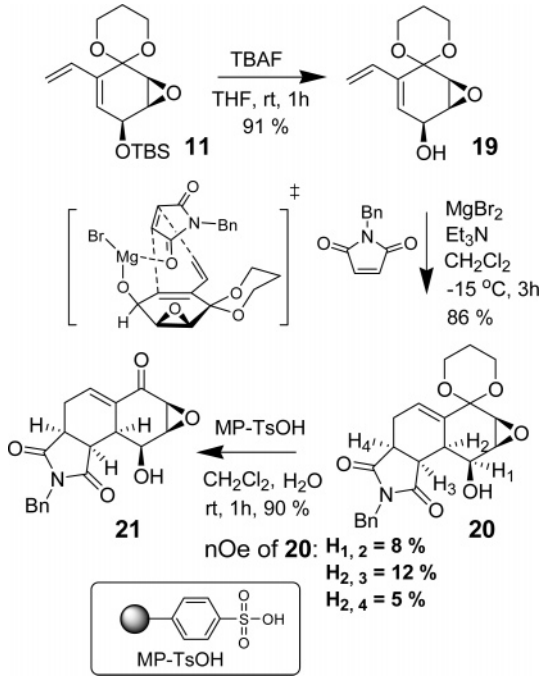


addition of **11** with *N*-ethylmaleimide also produced cycloadducts **14** and the derived epoxyquinols **15** in an analogous manner. X-ray crystallographic analysis of angular epoxyketone **15** unambiguously confirmed that **14** was an *endo*-selective cycloadduct in which the addition of the maleimide occurred *anti* to both the epoxide and silyl ether moieties.[8]

To alter the skeleton of angular [4 + 2] cycloadducts such as **13**, *N*-phenyltriazolinedione **16** was selected as an alternative dienophile (Scheme 3). Cycloaddition of diene **11** and **16** (toluene, 60 °C) afforded cycloadduct **17** as a single diastereomer in good yield. The stereochemistry of **17** was confirmed by X-ray crystallographic analysis of a crystalline derivative (cf. Scheme 7). Global deprotection (HF−CH_3CN), followed by HF neutralization with Me_3SiOMe, afforded epoxyquinol **18**, a scaffold with a slightly altered framework relative to the maleimide cycloadducts **13** and **15**.

Hydroxyl-Directed Diels−Alder Cycloaddition. Recently, Barriault and co-workers reported the highly stereoselective hydroxyl-directed Diels−Alder reaction of secondary and tertiary dienols with activated dienophiles.[14] This interesting precedent prompted us to evaluate use of a free secondary alcohol to direct Diels−Alder cycloaddition to the proximal face of diene **19** (Scheme 4). Desilylation of **11** with TBAF (THF, rt) afforded dienol **19** in good yield. Treatment of **19** with *N*-benzylmaleimide under conditions described by Barriault[14a] ($MgBr_2$, Et_3N, CH_2Cl_2, −15 °C) afforded

SCHEME 4. Hydroxyl-Directed Diels−Alder Cycloaddition



cycloadduct **20** as a single diastereomer. NOE analysis confirmed that **20** was an *endo* cycloadduct in which the facial selectivity of the maleimide cycloaddition occurred *syn* to the secondary alcohol.[12] The stereochemistry of **20** is consistent with a cycloaddition proceeding through the chelated transition state shown in Scheme 4. In contrast to previous results by Barriault and co-workers involving use of a chiral diene bearing a tertiary alcohol, in the present case we did not observe further ring closure of **20** to an amido lactone as evidenced by IR analysis.[12] A control experiment of **19** with *N*-benzylmaleimide under thermal conditions (80 °C, toluene) afforded a 2:1 mixture of diastereomers in which the apparent hydroxyl-directed isomer **20** was the minor product. Deprotection of **20** using polymer-supported arylsulfonic acid (MP-TsOH) resin (1.45 mmol/g)[15] afforded the angular epoxyquinol **21**, which has a distinct skeleton in comparison to cycloadducts such as **13**.

Further Elaboration of Angular Scaffolds. With a number of angular epoxyquinol scaffolds in hand, we proceeded to explore further transformations to achieve skeletal modifications. Initial efforts to develop selective Michael addition or epoxide opening of substrates including **13** and **18** using amines and thiols as nucleophiles typically generated mixtures of products. Thus, we chose to hydrogenate the enone of scaffolds such as **13**. Comparison of nickel-,[16] palladium-,[17] rhodium-,[18] and copper-based[19] catalysts showed no reactivity for reduction of substrate **13**. After considerable experimentation, we found that Adams' catalyst (PtO_2) gave promising re-

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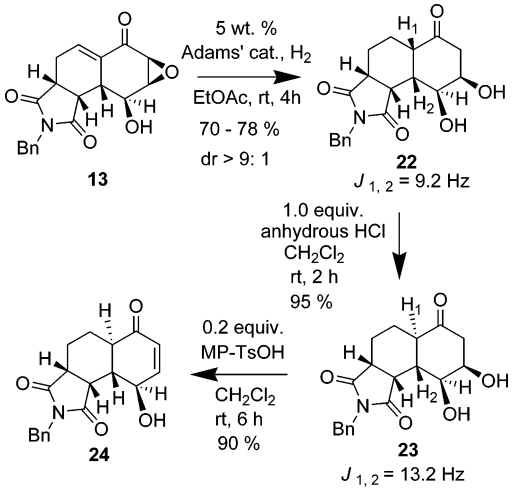
SCHEME 5. Elaboration of an Angular Epoxyquinol Scaffold

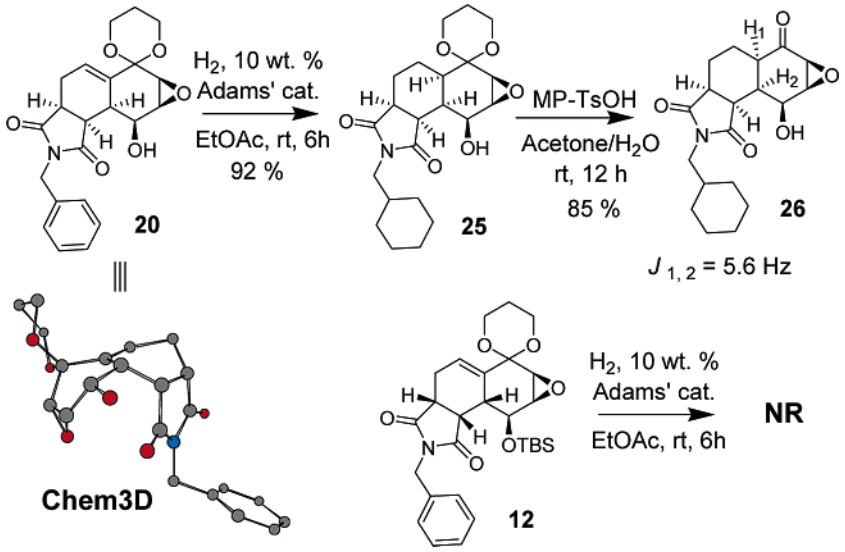


sults.[20] Treatment of **13** with 5 wt % PtO_2 under a hydrogen atmosphere in EtOAc afforded the hydrogenated *cis* diol **22** as a major product (Scheme 5). The epoxyketone moiety also underwent hydrogenolysis under the reaction conditions.[21] The ratio of *cis* and *trans* isomers **22** and **23** was determined to be 9:1 by ^{1}H NMR analysis. The relative stereochemistries of **22** and **23** were determined by evaluation of ^{1}H NMR coupling constants. In decalin ring systems, the *trans* protons generally have larger coupling constants than the corresponding *cis* protons.[22] Interestingly, we found that by treatment with 1.0 equiv of anhydrous HCl, *cis* isomer **22** could be cleanly transformed to the thermodynamically favored *trans* isomer **23** in good yield.[23] Further evaluation of acid catalysts led to the serendipitous finding that *trans* isomer **23** could be cleanly dehydrated to enone **24** using 0.2 equiv of a macroporous *p*-toluenesulfonic acid resin (MP-TsOH). Thus, hydrogenation, epimerization, and dehydration processes may be employed to alter the angular epoxyquinol scaffold to generate a number of advanced scaffolds with distinct molecular frameworks bearing stereochemical and functional group diversity.[2]

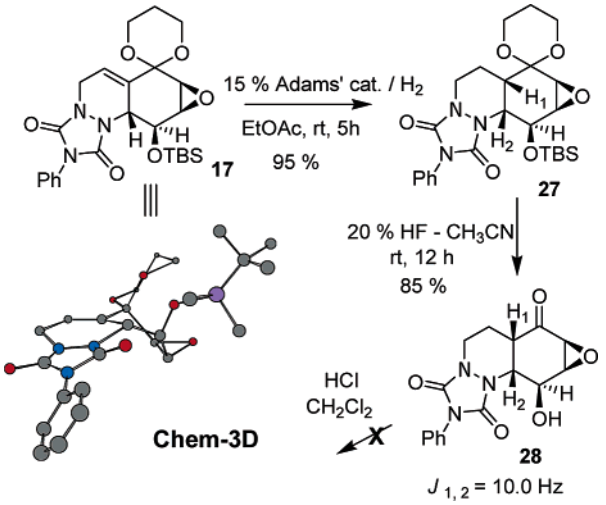
To expand the skeletal diversity of angular scaffolds, hydrogenation using Adams' catalyst (PtO_2) was conducted on substrate **20**. In the event, hydrogenation of **20** cleanly afforded a single *cis* isomer ($J_{1,2}$ = 6.8 Hz) possessing an intact epoxide, identified as the dearomatized compound **25** by both ^{1}H NMR and HRMS analysis (Scheme 6).[12] Literature reports have demonstrated examples of catalytic hydrogenation of the benzene nucleus using Adam's catalyst.[24] The high selectivity observed in the hydrogenation of **20** may be explained by the cup-shaped conformation of **20** (see Scheme 6, ChemBats3D structure) in which case hydrogenation of **20** occurs from the less hindered, convex face. In contrast to this result, attempted hydrogenation of the TBS-protected [4 + 2] cycloadduct **12** under the same conditions led to no reaction. The low reactivity of **12** toward hydrogenation is likely due to strong steric hindrance, in which the bulky TBS group blocks the β face and the benzyl group blocks the α face. Deprotection of **25** using MP-TsOH resin in acetone/water provided epoxyquinol **26** in good yield. ^{1}H NMR analysis revealed that **26** was a decalin-like *cis* isomer ($J_{1,2}$ = 5.6 Hz).

Angular urazole cycloadduct **18** was also subjected to hydrogenation using Adams's catalyst. This reaction was not clean and showed low selectivity for *cis* versus *trans* isomers. Interestingly we found that the ketal-protected compound **17** could be hydrogenated using 15 wt. % of Adams' catalyst to generate a single diastereomer **27** in which case the epoxide moiety was intact (Scheme 7). Analysis of a molecular model of **17** shows that the hydrogenation has proceeded from the convex (β) face of angular scaffold **17**. Further deprotection of **27** afforded the angular epoxyquinol **28**. X-ray (Figure 1) and ^{1}H NMR analysis unambiguously revealed that hydrogenated product **28** was a *cis* decalin-like isomer. In an effort to epimerize *cis* isomer **28** to a *trans* ring system, the compound was treated with 1.0 equiv of anhydrous HCl which afforded only starting material. Analysis of the X-ray structure of **28** also shows that this *cis* isomer has a $O_1-C_1-C_2-H_2$ dihedral angle of −47°, which is not suitable for σ-C−H/π^*-C=O overlap to facilitate enoliza-

SCHEME 6. Hydrogenation of Cycloadduct 20



SCHEME 7. Hydrogenation and Attempted Epimerization of the Angular Urazole Scaffold



tion/deprotonation to the corresponding *trans* isomer of **28** (Figure 1).[25]

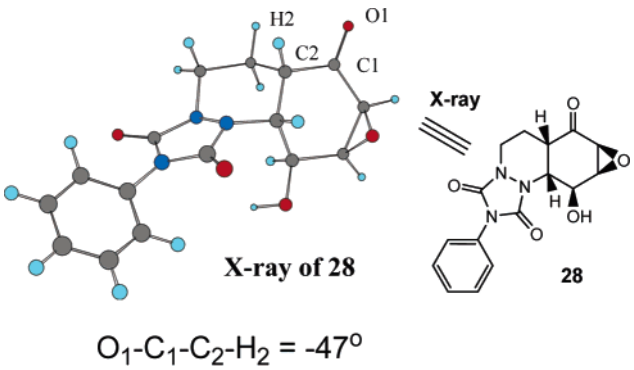
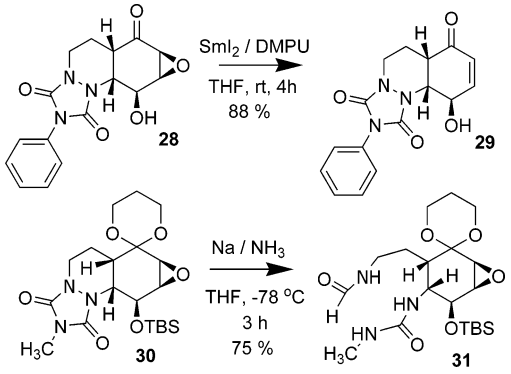


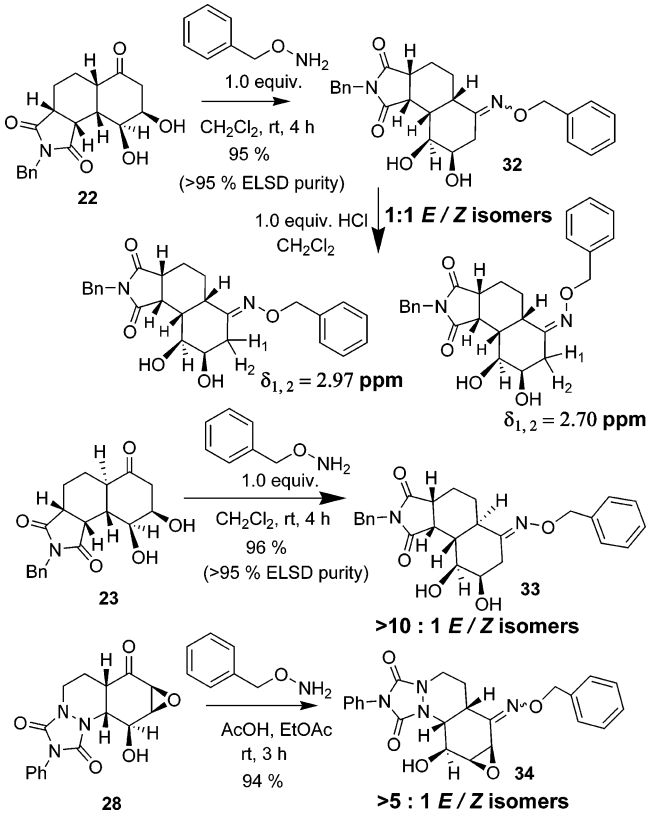
FIGURE 1. Proton alignment for enolization.

Attempted Reductive N−N Bond Cleavage. We were also interested to further modify urazole cycloadducts such as **28**. Recently, Lee and co-workers reported a new approach to macrocyclic amides via ring expansion induced by reductive N−N bond cleavage of a bicyclic hydrazine derivative using Na/NH_3.[26] We applied this protocol to the elaboration of the urazole scaffold with the hope to generate a nine membered-ring dilactam. Use of milder reductive conditions (e.g., SmI_2, DMPU)[27] led to epoxide opening and dehydration to afford enone **29** in 88% yield (Scheme 8). In an effort to selectively reduce the N−N bond, we employed protected compound **30**, prepared in analogy to **27**, in a dissolving metal (Na/NH_3) reduction. In the event, ring-opened compound **31** was isolated in 75% yield. NMR studies, including HMBC analysis[12] revealed that **31** was a primary formamide produced via N−N bond reduction and selective reductive ring opening.[28]

SCHEME 8. Attempted Reductive N−N Bond Cleavage



SCHEME 9. Scaffold Modification Using Oxime Formation



Further Scaffold Modification Using Oxime Formation. Scaffolds such as **22**, **23**, and **28** each contain carbonyl groups which may be subjected to further diversification. We first chose alkoxyamines for diversification of the ketone moiety.[29] Oxime formation with

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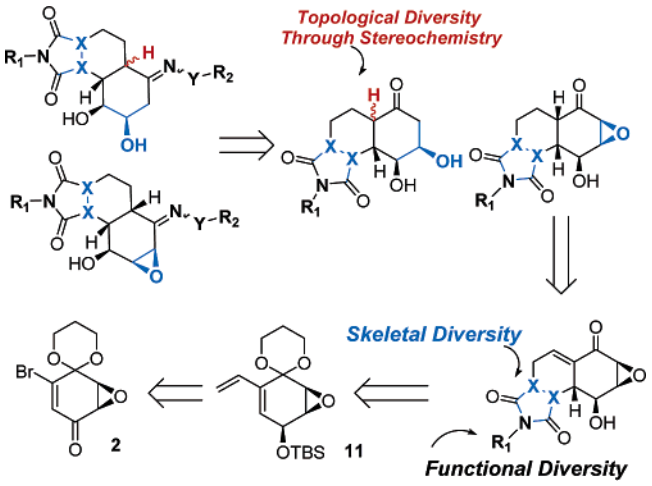
SCHEME 10. Library Design

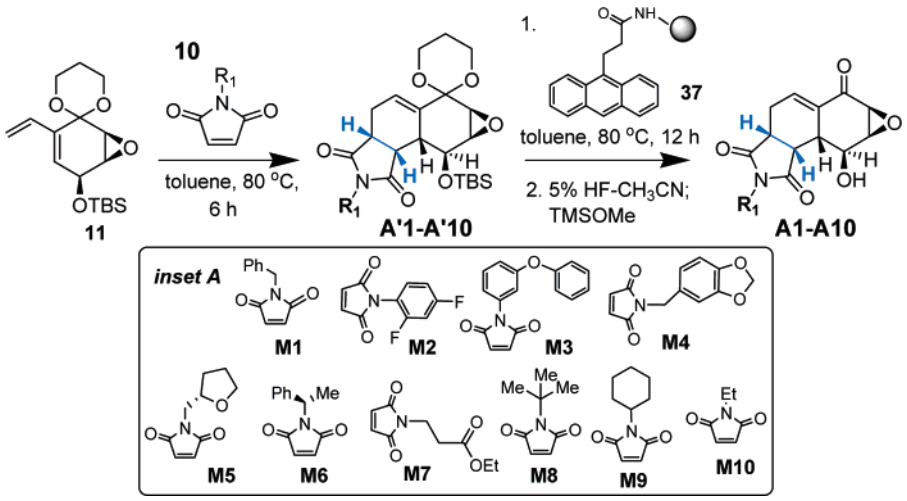


ketones **22** and **23** was conducted in CH_2Cl_2 using 1.0 equiv of *o*-benzylhydroxyamine (Scheme 9). *Cis* compound **22** was found to afford a 1:1 mixture of *E*/*Z* isomers **32**. The *E*/*Z* isomers were not separable by reversed-phase HPLC. Treatment of the *E*/*Z* mixture with 1.0 equivalent of anhydrous HCl afforded a 5:1 mixture of *E*/*Z* isomers. The two isomers were assigned in the ^{1}H NMR based on the difference of chemical shifts of the methylene protons.[30] Due to deshielding effects, the methylene protons of *E* oxime isomer experience a greater downfield ($\delta_{1,2}$ = 2.97 ppm) than the corresponding *Z* isomer protons ($\delta_{1,2}$ = 2.70 ppm). In this acid-catalyzed oxime isomerization process, epimerization of *cis* decalin-like ring to the *trans* structure was not observed. Interestingly, treatment of *trans* compound **23** with *o*-benzylhydroxyamine produced the *E* isomer **33** as the major product. For the relatively labile epoxyketone scaffold **28**, 1.0 equiv of acetic acid was needed to catalyze the oxime formation, and ethyl acetate was found to be an optimal solvent. A mixture of *E*/*Z* isomers **34** (>5:1, based on ^{1}H NMR analysis) was isolated in good yield. In contrast, attempted oxime formation of epoxyketone **13** and enone **24** did not afford clean reactions.

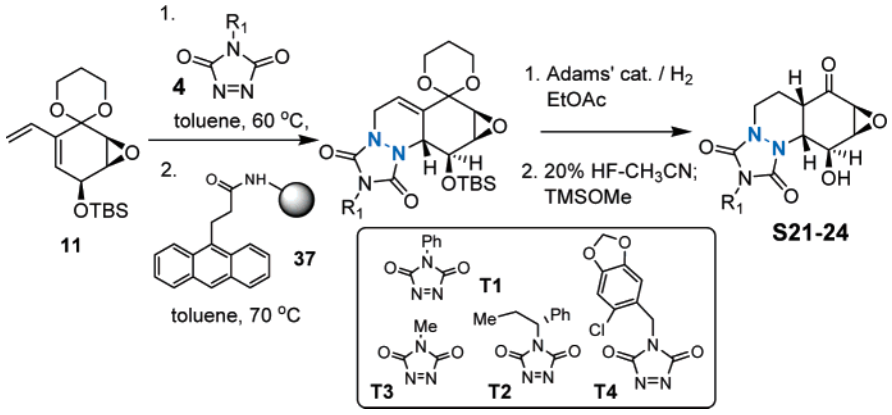
Library Design. After successful methodology development including elaboration of angular epoxyquinol scaffolds, we initiated efforts toward library synthesis. Our library synthesis strategy is depicted in Scheme 10. Condensation of the angular ketone-bearing scaffolds with different nucleophiles at a late stage may be used to incorporate a final diversification point. Three structurally distinct ketone scaffolds prepared by hydrogenation and epimerization of angular epoxyquinol scaffolds were selected for library synthesis (vide infra), in which case topological diversity was achieved through stereochemistry. A key step in our library synthesis was the highly diastereoselective [4 + 2] Diels–Alder cycloaddition of epoxyquinol diene **11** with maleimides and triazolinediones to generate highly funtionalized and complex angular polycyclic angular scaffolds.[6a] Due to difficulty in using $MgBr_2$ in a parallel synthesis setting, we did not include the hydroxyl-directed Diels–Alder cycloadducts (cf. **21**, Scheme 4) in our final library synthesis. Both skeletal and functional diversity are thus achieved by choice of the dienophile component. Diene **11** was prepared rapidly from the chiral nonracemic bromo-epoxyketone **2** in good yield (Scheme 2).

Parallel Synthesis of Angular Epoxyquinol Scaffolds. We employed parallel solution-phase synthesis[9] approaches to produce angular epoxyquinol scaffolds using [4 + 2] cycloaddition with maleimide and triazolinedione dienophiles. Treatment of diene **11** with 10 diverse maleimides[31] (1.2 equiv, inset A, Scheme 11) in toluene at 80 °C for 6 h afforded the Diels–Alder cycloadducts **A′1**–**A′10**. Subsequently, polymer-supported anthracene dienophile scavenger resin **37**[32] (1.12 mmol/gram, 2.0 equivalents) was added to the reaction mixture to sequester excess maleimide. After 12 h, the reaction mixtures were filtered, concentrated, and treated with 5% HF/CH_3CN, followed by HF scavenging using the liquid scavenger TMSOMe[13] to afford 10 angular epoxyquinol scaffolds **A1**–**10** in good yield and purity. For urazole-containing scaffolds (Scheme 12), a hydrogenation step was incorporated after the [4 + 2] cycloadditions and scavenging. Final deprotection led to four additional epoxyquinol scaffolds **S21**–**24**. Figure 2 shows the angular epoxyquinol scaffolds prepared using the Diels–Alder cycloaddition, dienophile scavenging, hydrogenation, and silyl deprotection sequence.

SCHEME 11. Parallel Synthesis of Maleimide-Derived, Angular Epoxyquinol Scaffolds



SCHEME 12. Synthesis of Urazole-Containing Scaffolds



Synthesis of Advanced Scaffolds. Hydrogenation of the angular epoxyquinol scaffolds **A1**−**10** with 5 wt % Adams' catalyst (PtO_2) generated the *cis* isomers **S1**−**10** as major products (Scheme 13). Purification of the *cis* isomers by silica gel chromatography provided the pure *cis* scaffolds **S1**−**10** in 35−50% yields. As previously described (cf. Scheme 5), the mixtures of *cis* and *trans* isomers were subjected to epimerization using a slight excess of anhydrous HCl to generate the pure *trans* scaffolds **S11**−**20** in 30−44% yields based on **A1**−**10**. Dehydration of **S11**−**20** was further conducted using MP-TsOH resin to afford enones **D1**−**10**.

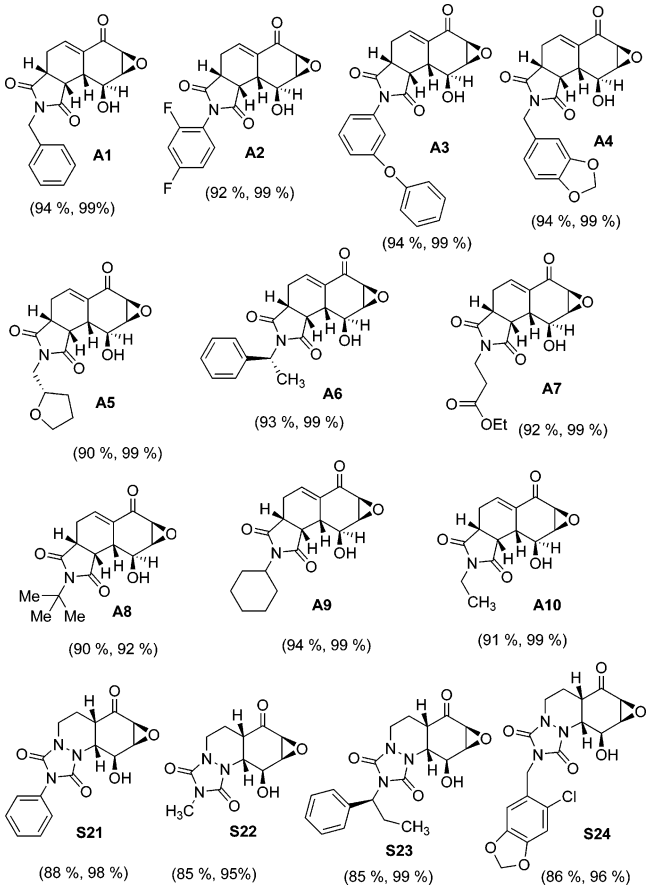
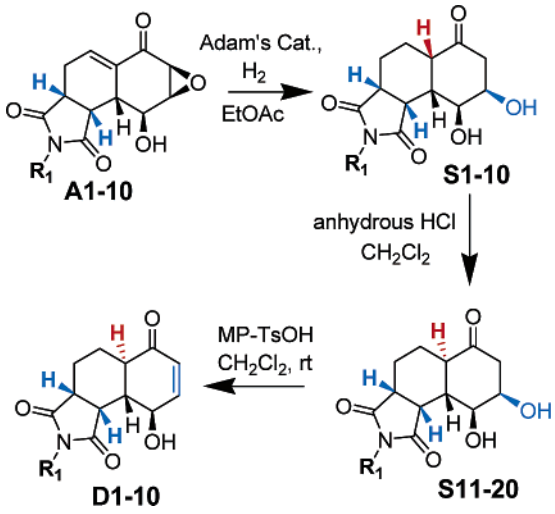


FIGURE 2. Angular epoxyquinol scaffolds (yield (%) and purity (%) shown in parentheses). Purity was measured by HPLC/ELSD.

SCHEME 13. Synthesis of Advanced Scaffolds



Library Synthesis. Final carbonyl diversification reactions of advanced scaffolds were conducted in the solution phase using a resin-scavenging protocol (Scheme 14). Twenty angular maleimide scaffolds **S1**−**20** were incubated with nine different nucleophiles including six alkoxyamines[33] and three carbazates (1.05 equiv, rt, 30 h) in anhydrous CH_2Cl_2; four angular urazole scaffolds **S21**−**24** were treated with five alkoxyamines (1.00 equiv, rt, 12 h) and acetic acid (1.0 equiv) in anhydrous EtOAc. After condensation, reaction mixtures were treated with polymer-supported methylisatoic anhydride resin[34] (2.65

(29) For a review of oxime formation, see: (a) Ābele, E.; Lukevics, E. *Org. Prep. Proced. Int.* **2000**, *32*, 235−264. For recent examples of library syntheses employing alkoxyamines as diversity reagents, see: (b) Maly, D. J.; Choong, I. C.; Ellman, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 2419. (c) Ishikawa, T.; Kamiyama, K.; Matsunaga, N.; Tawada, H.; Iizawa, Y.; Okonogi, K.; Miyake, A. *J. Antibiot.* **2000**, *53*, 1071. (d) Pelish, H. E.; Westwood, N. J.; Feng, Y.; Kirchhausen, T.; Shair, M. D. *J. Am. Chem. Soc.* **2001**, *123*, 6740. (e) Armstrong, J. I.; Ge, X.; Verdugo, D. E.; Winans, K. A.; Leary, J. A.; Bertozzi, C. R. *Org. Lett.* **2001**, *3*, 2657. (f) Wipf, P.; Reeves, J. T.; Balachandran, R.; Day, B. W. *J. Med. Chem.* **2002**, *45*, 1901.

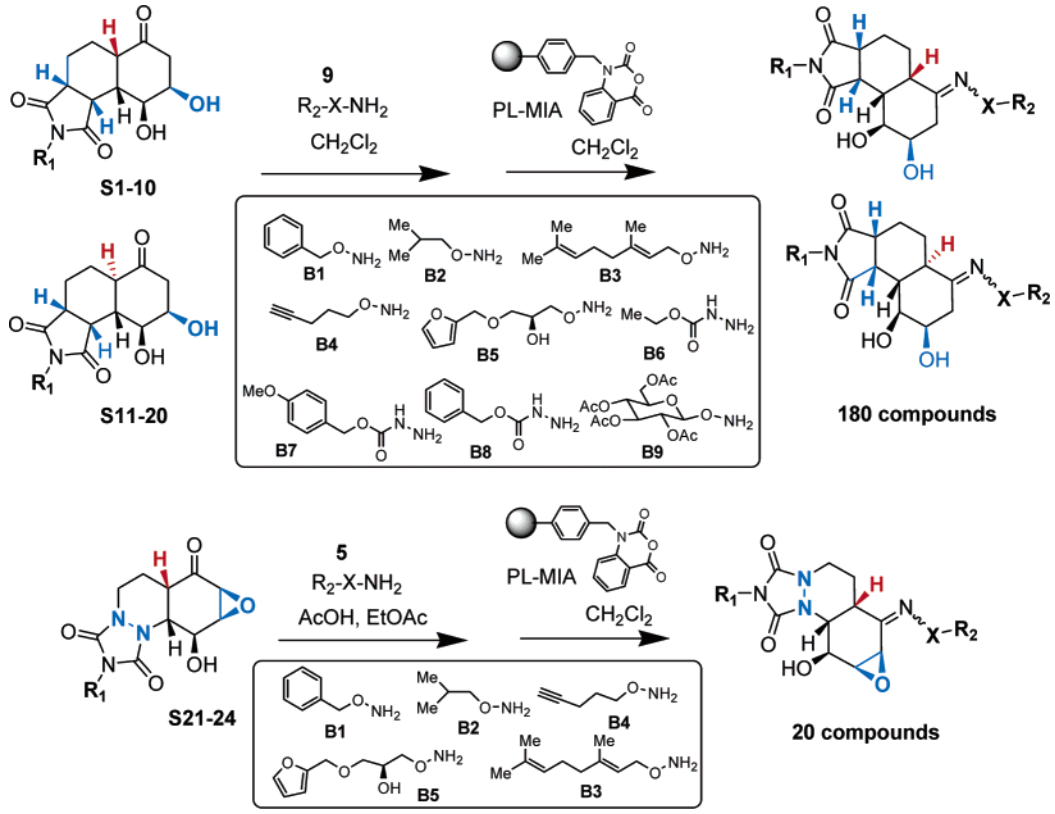
(30) (a) Karabatsos, G. J.; Taller, R. A. *J. Am. Chem. Soc.* **1964**, *86*, 4373. (b) Karabatsos, G. J.; Taller, R. A. *Tetrahedron* **1968**, *24*, 3347.

(31) For details on the syntheses of dienophiles **M5** and **T4**, see the Supporting Information.

(32) For the synthesis and application of dienophile scavenger resin **37**, see: Lei, X.; Porco, J. A., Jr. *Org. Lett.* **2004**, *6*, 795.

(33) (a) **B1**, **B2**, **B6**, **B7**, and **B8** are commercially available. (b) For the syntheses of **B3**, **B4**, and **B5**, see: Supporting Information. (c) For synthesis of **B9**, see: Renaudet, O.; Dumy, P. *Tetrahedron Lett.* **2001**, *42*, 7575.

SCHEME 14. Library Synthesis



mmol/g, 2.0 equiv) in CH_2Cl_2 to afford the desired condensation products after filtration of the resin and concentration in vacuo in good yield and purity. Application of this general protocol led to the preparation of 200 highly functionalized and complex compounds. The library was fully characterized by LC/MS.[12] The yield range was 73−95%, and the purity range was 85−99% (ELSD).[35]

Analysis of Library Members. We included all scaffolds, enones, oximes and alkylidenecarbazates in the final library (total = 244 compounds). As shown in Figure 3, analysis of library members reveals that there are 10 distinct frameworks possessing different stereochemistry and functional groups. Three (6−6−5) fused rings comprise the basic skeleton with five to seven continuous stereogenic centers. Representative natural products with 6−6−5 ring systems are shown in Figure 4.[36] Interestingly, these steroid architectures display functional group (e.g., enone, diol, and glycoside) and stereochemical diversity which is analogous to that found in library members (Figure 3). Evaluation of two important chemical properties (molecular weight and cLogP) of our final library members indicates that the molecular weight range is 263−766 (average molecular weight = 467) and the cLogP range is −0.73−5.83 (average cLogP = 2.21) (Figure 3).[37]

Preliminary Biological Evaluation. The 244-membered library was screened to search for inhibitors of induction of heat shock proteins (HSPs). HSPs are stress-inducible proteins that serve as molecular chaperones in protein folding and degradation. HSPs also protect cells from a variety of stressful treatments, including anticancer drugs.[38] Targeting activity of a major heat shock protein Hsp90 has been suggested as a novel approach to cancer therapy.[39] In fact, specific inhibitors of Hsp90, including radicicol and geldanamycin and its derivatives, have been developed and tested in clinical trials.[40] In the treatment of prostate and some other cancers, radiation and other therapies can be combined with hyperthermia in order to facilitate killing of cancer cells, especially under hypoxic conditions of solid tumors. Hyperthermia can cause rapid and strong induction of a cohort of HSPs,[41] which may counteract its antitumor activity. Furthermore, development of resistance to hyperthermia was associated with overproduction of certain HSPs, including Hsp72 and Hsp27.[42] Inhibitors of the heat shock response have been identified, including querce-

(34) Coppola, G. M. *Tetrahedron Lett*. **1998**, *39*, 8233.
(35) HPLC purity values refer to the sum of the HPLC (ELSD) area % for oxime *E*/*Z* isomers.
(36) Amurensoside D: (a) Riccio, R.; Iorizzi, M.; Minale, L.; Oshima, Y.; Yasumoto, T.. Starfish saponins. *J. Chem. Soc., Perkin Trans. 1* **1988**, *6*, 1337. Withaferin A: (b) Kupchan, S. M.; Anderson, W. K.; Bollinger, P.; Doskotch, R. W.; Smith, R. M.; Renauld, J. A.; Schnoes, H. K.; Burlingame, A. L.; Smith, D. H. *J. Org. Chem.* **1969**, *34*, 3858. Hyocholsaeure: (c) Lehmann, G.; Tepper, H. J.; Hilgetag, G. *Biochem. Z.* **1964**, *340*, 75. Haplosamate A: (d) Qureshi, A.; Faulkner, D. John *Tetrahedron* **1999**, *55*, 8323.
(37) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev*. **1997**, *23*, 3.
(38) For reviews of HSPs, see: (a) Benjamin, I. J.; McMillan, D. R. *Circ. Res*. **1998**, *83*, 117. (b) Gabai, V. L.; Sherman, M. Y. *J. Appl. Physiol*. **2002**, *92*, 1743.
(39) Goetz, M. P.; Toft, D. O.; Ames, M. M.; Erlichman, C. *Ann. Oncol*. **2003**, *14*, 1169.
(40) Banerji, U.; Judson, I.; Workman, P. *Curr. Cancer Drug Targets* **2003**, *3*, 385.
(41) (a) Roti, J. L. *Int. J. Hyperthermia* **2004**, *20*, 109. (b) Jolly, C.; Morimoto, R. I. *J. Natl. Cancer Inst*. **2000**, *92*, 1564.

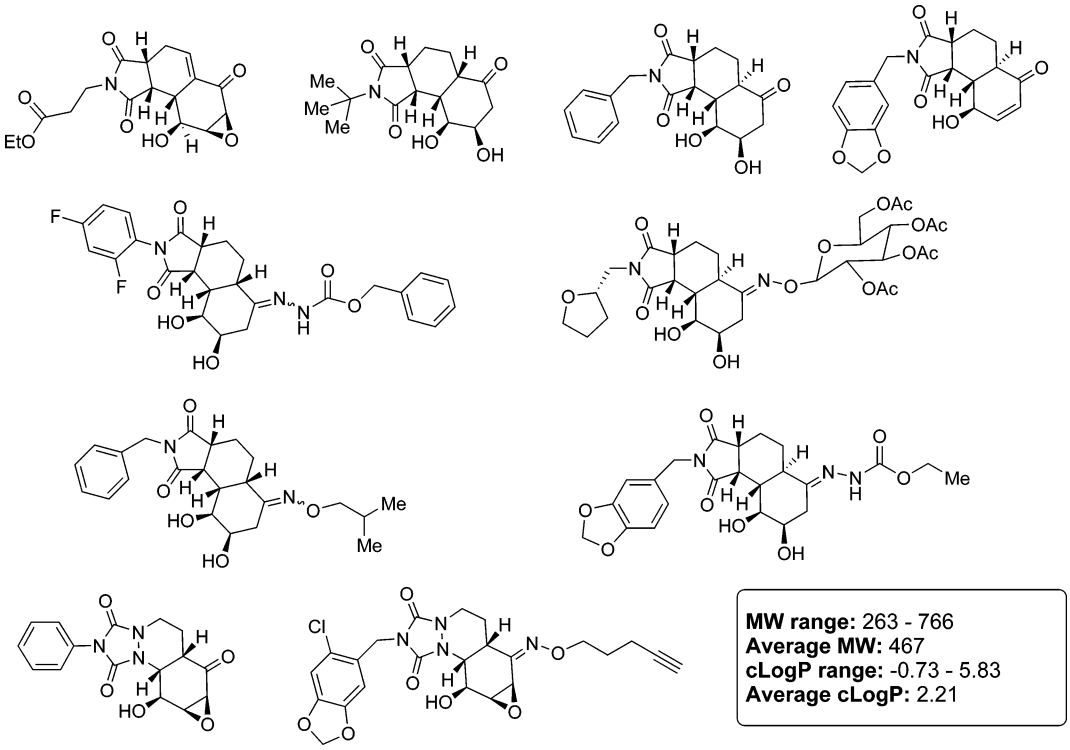


FIGURE 3. Representative library members.

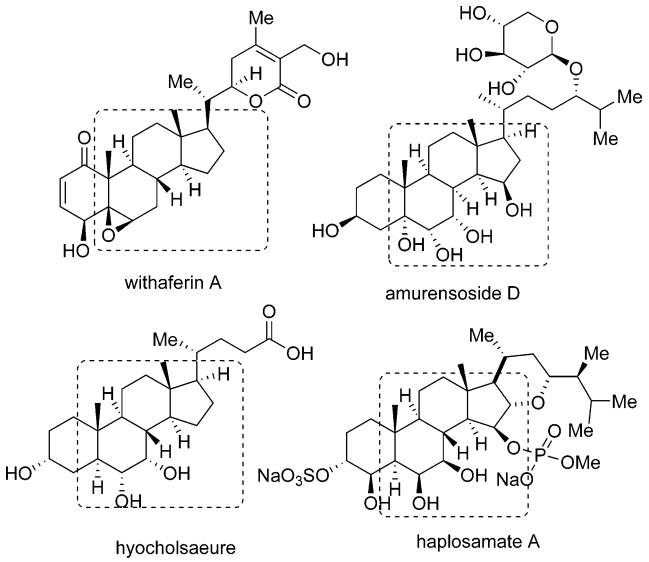


FIGURE 4. Representative natural products with 6−6−5 ring systems.

tin,[43] KNK437,[44] and stresgenin B.[45] These compounds have been shown to repress development of thermotolerance and sensitized cells to heat shock. Furthermore, quercetin was demonstrated to enhance cancer regression after hyperthermia treatments in mice xenografts.[45] All of these compounds, however, have relatively low potency and low specificity and exhibit significant toxicity that is unrelated to their activity as inhibitors of the heat shock response. Therefore, we seek to identify small molecules that will potently inhibit induction of HSPs to overcome resistance and promote antitumor activity of hyperthermia.

To identify inhibitors of induction of HSPs, we developed a two-stage screen of chemical libraries. The first stage involved a cell-based screen for general HSP-mediated refolding of heat-denatured luciferase. In a pilot screen, we evaluated 244 compounds from the chemical library; 22 compounds showed significant inhibitory effects. The hits obtained at the first stage of the screen were further evaluated in the second stage involving a direct testing for inhibition of induction of HSPs by immunoblotting. As a readout, we used induction of Hsp72 in cells exposed to mild heat shock. In this assay, Chinese hamster ovary CHO cells were incubated with various concentrations of the chemical compounds for 16 h at 37 °C before treatment. Control cells were kept without compounds. To induce HSPs, cells were exposed to mild heat shock by immersing plates in a 45 °C water bath for 10 min. Cells were kept for 6 h at 37 °C to allow for induction of HSPs. Finally, cells were lysed and the levels of Hsp72 were monitored by immunoblotting with anti-Hsp72 antibody. Six compounds (scaffolds **A1**, **A3**, **A6**, **A7**, **A8**, and **A9**) showed significant activities in this assay (Figure 5). IC_{50}'s for inhibition of Hsp 72 induction for **A1**, **A3**, **A6**, **A7**, **A8**, and **A9** were found to be 0.75, 1.5, 0.75, 3.0, 1.5, and 1.5 μM, respectively.[12] These initial results suggest that the epoxyketone and enone moieties

(42) (a) Calderwood, S. K.; Asea, A. *Int. J. Hyperthermia* **2002**, *18*, 597. (b) Khoei, S.; Goliaei, B.; Neshasteh, R. A.; Deizadji, A. *FEBS Lett*. **2004**, *561*, 144. (c) Coss, R. A.; Storck, C. W.; Wachsberger, P. R.; Reilly, J.; Leeper, D. B.; Berd, D.; Wahl, M. L. *Int. J. Hypertherm.* **2004**, *20*, 93.

(43) Asea, A.; Ara, G.; Teicher, B. A.; Stevenson, M. A.; Calderwood, S. K. *Int. J. Hypertherm.* **2001**, *17*, 347.

(44) (a) Yokota, S.; Kitahara, M.; Nagata, K. *Cancer Res*. **2000**, *60*, 2942. (b) Ohnishi, K.; Takahashi, A.; Yokota, s.; Ohnishi, T. *Int. J. Radiat. Biol*. **2004**, *80*, 607.

(45) Akagawa, H.; Takano, Y.; Ishii, A.; Mizuno, S.; Izui, R.; Sameshima, T.; Kawamura, N.; Dobashi, K.; Yoshioka, T. *J. Antibiot*. **1999**, *52*, 960.

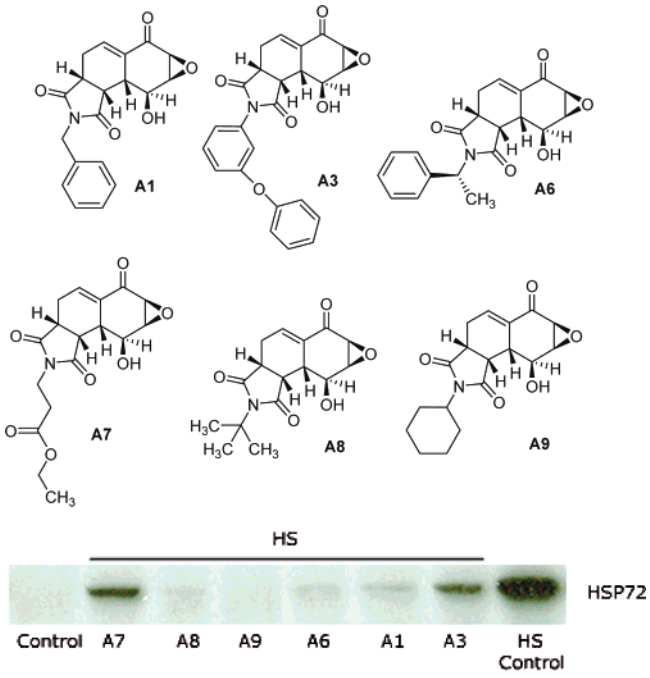


FIGURE 5. Inhibition of induction of Hsp72 by angular compounds **A1**, **A3**, **A6**, **A7**, **A8**, and **A9** (3.0 μM concentration).

may be required for the inhibition of HSPs. Therefore, angular, urazole-containing compound **18** (Scheme 3) was also tested for the inhibition of Hsp72 induction in the direct assay and was found to inhibit induction (IC_{50} = 1.5 μM).[12] To test for nonspecific effects of the selected compounds, we studied whether they inhibit induction of a reporter luciferase under the control of tetracycline-regulated promoter. No inhibition of the induction of luciferase by tetracycline was found for any of the compounds. These data indicate that the compounds do not have general effects on inhibition of transcription or translation or enhancement of protein degradation. Compounds **18** and **A1** were evaluated in an additional control experiment. Cells were infected with a retrovirus encoding the green fluorescent protein (GFP), and after 16 h the compounds were added to the cells. The level of the GFP was measured by immunoblotting. After 16 h of infection, the cells did not express detectable levels of GFP; after 40 h, similar levels of the GFP were found in control and with cells incubated with the compounds. This result reinforces that compounds **18** and **A1** do not have general inhibitory effects on protein synthesis, but rather their effects on induction of HSPs are specific.[12] Compounds identified in this pilot screen will be further developed to obtain a potent, nontoxic inhibitor of induction of HSPs. These preliminary biological screening results indicate that the angular epoxyquinol frameworks described herein should provide access to novel probe molecules for biological research.

Conclusion

We have developed an efficient approach to a highly functionalized, complex and natural product-like chemical library. The key strategies involve highly stereocontrolled [4 + 2] Diels−Alder cycloaddition of a chiral nonracemic epoxyquinol diene with reactive dienophiles to generate angular epoxyquinol scaffolds and their further diversification using hydrogenation, epimerization, and carbonyl condensation with different building blocks. Related studies toward the generation of different skeletal epoxyquinol scaffolds using hydroxyl-directed Diels−Alder cycloadditions, and reductive N−N bond cleavage have also been addressed. As a result, a 244-membered library with 10 distinct skeletons was efficiently synthesized. Preliminary biological screening of this chemical library revealed that six compounds showed significant inhibition of the induction of Hsp 72 with IC_{50}'s in the low micromolar range. Further biological evaluation of the epoxyquinol-derived library is in progress and will be reported in due course.

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

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