Efficient Synthesis of a Novel Resorcyclide as Anticancer Agent Based on Hsp90 Inhibition

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This manuscript is dedicated in honor of the 60th birthday of Professor Chi-Huey Wong for his historic contributions at the intersection of chemistry and biology.

Supporting information for this article is available on the WWW under http://asc.wiley-vch.de/home/.

Abstract: The highly efficient synthesis of a novel heat shock protein 90 (Hsp90)-based anticancer agent, triazole-cycloproparadicicol (**5**), is described. The key step involves a fragment coupling using "click chemistry." The preliminary biological evaluation of triazole-cycloproparadicicol is also reported.

Keywords: antitumor agents; click chemistry; heat shock protein 90; total synthesis; triazole-cycloproparadicicol

Heat shock protein 90 (Hsp90), a molecular chaperone that mediates the folding of a number of oncogenic proteins (cf. *Raf-1* and *Her-2*),[1] is considered to be an attractive target for inhibition by anticancer agents.[2] Recent investigations have argued that, compared to normal cell Hsp90s, the tumor cell variations exist in activated, high-affinity, multi-chaperone form, with particularly heightened affinities for their inhibitors. These increased inhibitor affinities – up to one hundred-fold higher than those of normal cell Hsp90s – provide a basis for the selective targeting of tumor cells.[3] Importantly, it has been suggested that drug-resistant proteins are particularly dependent on association with Hsp90 for proper folding and function. Thus, the selective inhibition of tumor Hsp90 could ultimately provide a means by which to counter the growing problem of drug resistance in cancer treatment.[4]

The interest in the development of selective Hsp90 inhibitors is evidenced by a growing menu of candidates which have been targeted for development directed to this proposition. In particular, the natural product Hsp90 inhibitors, radicicol (**1**)[5] and geldanamycin (**2**)[6] have attracted a great deal of attention (Figure 1).[7] Currently, the geldanamycin derivative,
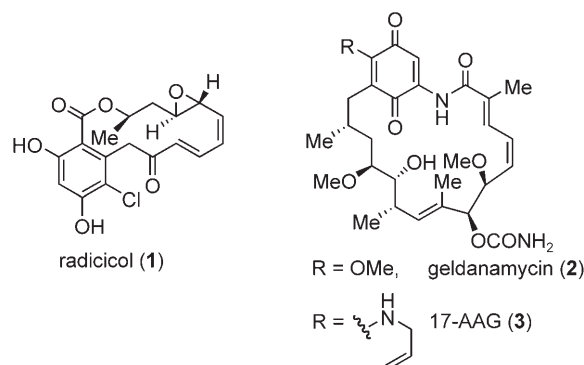
Figure 1. Hsp90 inhibitors.

17-AAG (**3**), is in Phase II clinical trials for breast cancer.[7]

In 1999, our laboratory initiated a program directed toward the diverted total synthesis-based discovery of novel Hsp90-based anticancer agents. We were particularly attracted to radicicol (**1**) as an initial target. The absence of a quinine moiety in **1** seems to be advantageous relative to compounds in the geldanamycin series, such as 17-AAG (**3**). Radicicol (**1**), a metabolite of *Monocillium nordinii*,[5] exhibits potent *in vitro* inhibitory acitivity against Hsp90 (IC$_{50}$ = 20 nM)[8] and has been shown to be significantly less hepatotoxic than 17-AAG (**3**).[9] Even more interestingly, radicicol (**1**) and its analogs demonstrate potent cytotoxicity against Rb (retinoblastoma)-negative cells which are resistant to 17-AAG.[10]
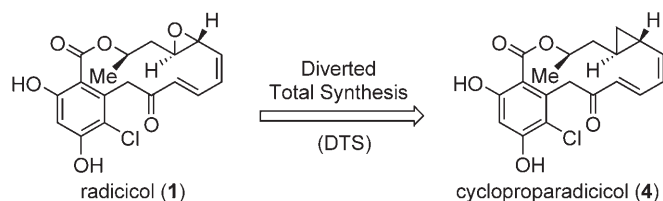
Figure 2. Diverted total synthesis of cycloproparadicicol.

Upon completion of the first total synthesis of radicicol, we were able to confirm its reported *in vitro* activity, but found the compound to be inactive in *in vivo* settings. We postulated that the disappointing lack of *in vivo* activity may be attributable to the presence of the chemically vulnerable epoxide functionality of radicicol.[11] Through application of a diverted total synthesis (DTS)[12] approach, we successfully prepared the congener, cycloproparadicicol (**4**, Figure 2), in which the epoxide was replaced with a more robust cyclopropyl functionality.[13]

Preliminary investigations revealed that the epoxide functionality of radicicol was not crucial for inhibitory activity, as cycloproparadicicol (**4**) inhibited Hsp90 at levels as low as 160 nM. Furthermore, cycloproparadicicol exhibited significant inhibitory activity against MCF-7 breast cancer cells (IC$_{50}$ = 49 nM).[13] In a preliminary *in vivo* study against mice implanted with human colon carcinoma (HCT-116), cycloproparadicicol (75 mg kg^{-1}, QD×7) achieved 68% tumor cell growth suppression. Its stereoisomer SAR profile tracked very closely with that of the corresponding epoxide, suggesting a common recognition pattern with Hsp90.

Our synthesis of cycloproparadicicol suffered from several low-yielding and difficultly scalable steps that significantly limit comfortable access for further pre-clinical studies.[13] Furthermore, we suspected that the dienone moiety of cycloproparadicicol may be responsible for toxicity and loss of *in vivo* activity.[14] We thus sought to design novel analogs based on the cycloproparadicicol framework, which might exhibit a more exploitable therapeutic index than the parent compound and which might be accessible through an efficient and scalable synthetic route.

Given these considerations, we chose as our target compound a novel triazole-containing analog of cycloproparadicicol (**5**). As outlined in Figure 3, we hoped to execute a highly convergent and scalable route to **5**, which would rely on a Cu(I)-mediated triazole formation – "click chemistry"[15] – to achieve the rapid buildup of the backbone (cf. **7**+**8**→**6**). We also anticipated that replacement of the original dienone moiety with a triazole functionality might offer a means by which to overcome toxicity and erosion of *in vivo* activity.[16] The 14-membered macrolactone would be constructed through intramolecular transesterification of alcohol **6**. We proposed accessing fragment A (**7**) from the commercially available orcinol **9**. Fragment B (**8**) would be prepared from the commercially available "chiral non-racemic" β-hydroxy ester **10**. As a consequence of its convergence and scalability, we anticipated that this proposed route would be highly amenable to the preparation of analogs through diverted total synthesis.

The synthesis of fragment A (**7**) began with commercially available orcinol **9**. (Scheme 1). Phenol **11** was efficiently prepared in accordance with literature reported methods[17] in 70% overall yield. TBS-protection of **11** afforded compound **12**. Selective chlorination of the aromatic ring, followed by selective bromination of the methyl group under radical conditions
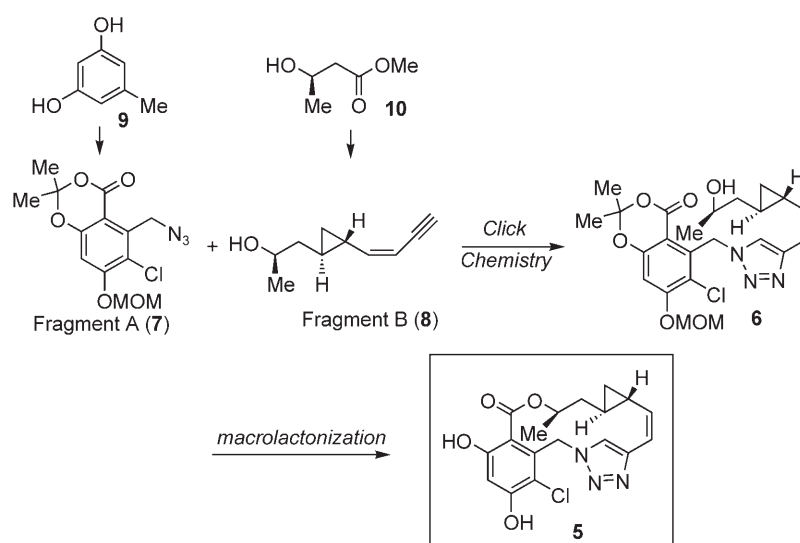
Figure 3. Proposed route to triazole-cycloproparadicicol (**5**).
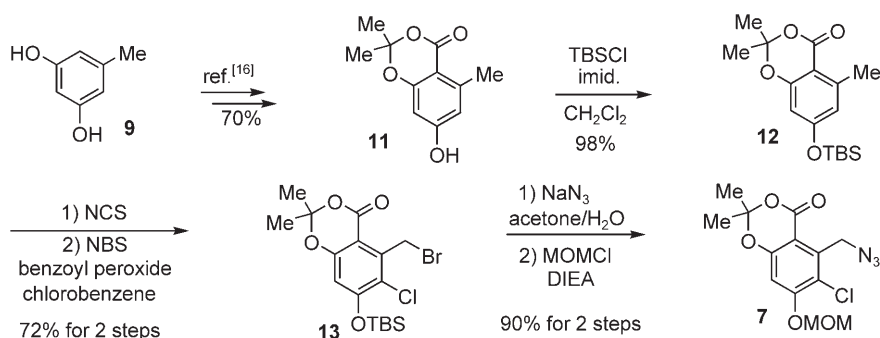
Scheme 1. Synthesis of fragment A (**7**).
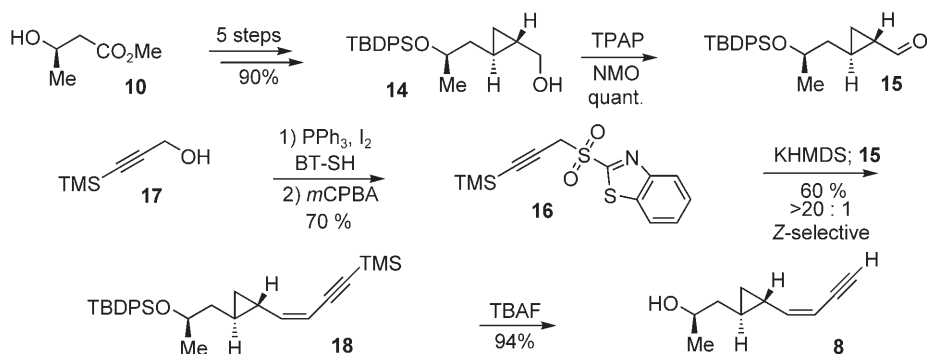
Scheme 2. Synthesis of fragment B (**8**).

smoothly generated benzylbromide **13** in one pot (72% yield). Treatment of the latter with sodium azide at room temperature, followed by phenol protection with MOMCl afforded desired fragment A (**7**) in 90% yield for 2 steps.

Our synthesis of fragment B (**8**) commenced with the readily available alcohol **14** which was itself prepared from the commercially available, chiral non-racemic compound **10** in 90% overall yield (Scheme 2).[13] Oxidation of **14** cleanly afforded aldehyde **15**.[18] Next, sulfone **16** was provided from **17** according to the literature reported method.[19] Julia olefination of aldehyde **15** with sulfone **16**, using KHMDS as base, smoothly afforded the desired enyne compound **18** in 60% yield with excellent Z-selectivity (>20:1).[19] Global desilylation of **18** cleanly provided the desired fragment B (**8**) in 94% yield.

With fragments A (**7**) and B (**8**) in hand, we were able to assemble the triazole **6** through application of the highly efficient Cu(I)-mediated "click chemistry" protocol[20] (Scheme 3). As hoped, the intramolecular macrolactonization of **6** proceeded readily under basic conditions to provide the macrolactone **19** in 70% yield. Finally, HCl-mediated MOM deprotection served to complete the total synthesis of triazole-cyclo-proparadicicol **5**. The structure of **5** was unambiguously confirmed by X-ray crystal structure analysis.[21]

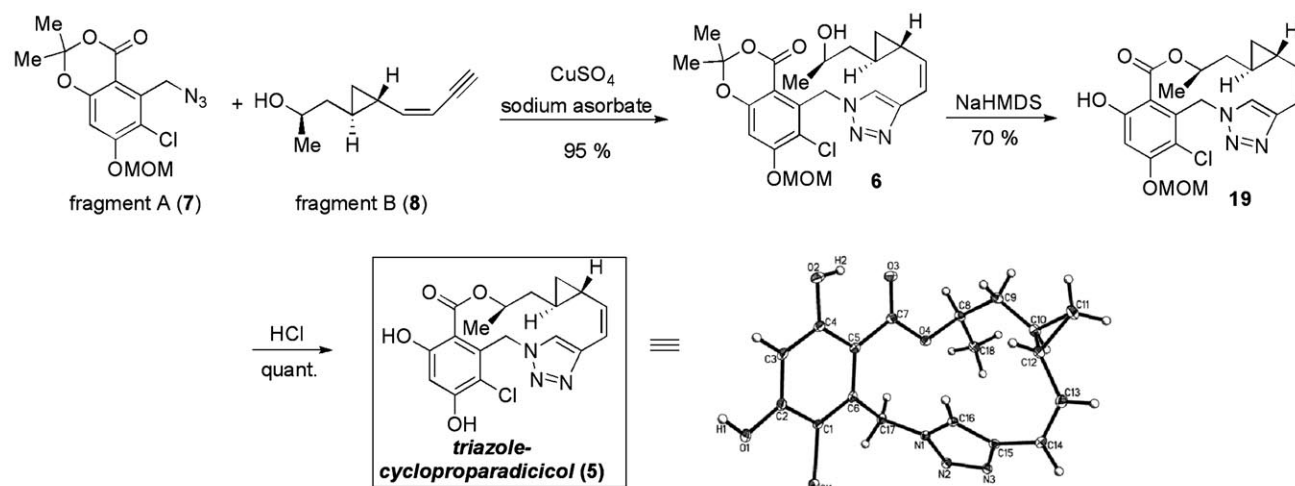Preliminary biological evaluations reveal triazole-cycloproparadicicol **5** to be a reasonably potent Hsp90 inhibitor, with $IC_{50} = 400$ nM. (Table 1). Tri-azole-cycloproparadicicol **5** also displayed significant *in vitro* inhibitory activity, comparable to that of 17-AAG, against the leukemia cell line Kasumi-1 ($IC_{50} = 650$ nM).[22] These preliminary results show that tri-azole-cycloproparadicicol is indeed a promising candidate worthy of further *in vitro* and *in vivo* preclinical studies.

In conclusion, we have developed a highly convergent and robust synthetic route (*only 11 steps in total based on the longest linear sequence, with 20% overall yield*) for the facile synthesis of a novel analog of cycloproparadicicol. The key step involved an efficient cross-coupling reaction using a "click" type construction. The current synthesis should produce substantial amounts of triazole-cycloproparadicicol for further *in vivo* studies and could also provide an excellent platform for further analog synthesis and evaluation.[23]

Experimental Section

General Methods

All non-aqueous reactions were carried out in oven-dried glassware under a slight positive pressure of argon unless otherwise noted. All reagents were commercially available from Sigma–Aldrich and TCI America and used without further purification, unless indicated otherwise. Solvents were reagent grade and purified by standard techniques: THF

Scheme 3. Synthesis of triazole-cycloproparadicicol (**5**).

Table 1. *In vitro* activity of triazole-cycloproparadicicol (preliminary biological evaluations were performed by Dr. Gabriela Chiosis and co-workers at MSKCC).
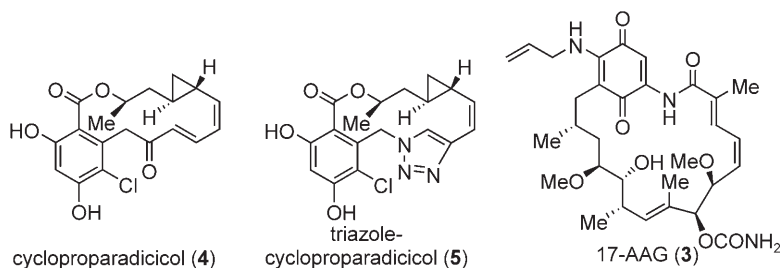
Compound	Hsp90 inhibition (IC$_{50}$)	Inhibition of Kasumi-1 leukemia cell line (IC$_{50}$)
cycloproparadicicol (**4**)	160 nM	–
triazole-cycloproparadicicol (**5**)	400 nM	650 nM
17-AAG (**3**)	600 nM	620 nM

was distilled from Na-benzophenone or filtered through a dry-solvent system; CH$_2$Cl$_2$ was distilled from CaH$_2$ or filtered through a dry-solvent system; all other solvents were Aldrich "anhydrous" grade solvents, unless indicated otherwise. Reactions were magnetically stirred and monitored by thin layer chromatography on Merck silica gel 60-F254 coated 0.25 mm plates. Preparative thin layer chromatography was performed with Merck silica gel 60-F254 coated 0.50 mm plates. Flash chromatography was performed with Sorbent Technology silica gel 60 (particle size 32–63 μm), unless indicated otherwise. Yields reported are for isolated, spectroscopically pure compounds. Melting points are uncorrected. NMR spectra were recorded on Bruker DRX 300 or 400 MHz and DMX 500 MHz spectrometers. Proton and carbon chemical shifts were referenced to residual solvent peaks. Abbreviations for ^{1}H NMR: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, or br = broad. High resolution mass spectra were acquired in the Columbia University Mass Spectral Core facility on a JEOL HX110 spectrometer. Optical rotations were measured on a JASCO DIP-1000 spectrometer.

Procedure for the Synthesis of Representative Compounds

Compound 13: To a solution of compound **12** (2.0 g, 6.2 mmol, 1.0 equiv.) in anhydrous chlorobenzene (10.0 mL) was added *N*-chlorosuccinimide (915 mg, 6.8 mmol, 1.1 equiv.). The reaction mixture was stirred at 100 °C for 12 h. After the reaction mixture had been cooled to room temperature, freshly purified *N*-bromosuccinimide (1.65 g, 9.3 mmol, 1.5 equiv.) and benzoyl peroxide (750 mg, 3.1 mmol, 0.5 equiv.) were added. The reaction mixture was stirred at 100 °C for 16 h. Then the reaction mixture was cooled to room temperature, quenched with saturated aqueous NaHSO$_3$ (10 mL), and extracted with ether (3 × 20 mL). The combined organic phase was washed with brine, dried over MgSO$_4$, concentrated and purified by flash chromatography (hexane/EtOAc: 10/1) to afford product **13** as a white solid; yield: 1.94 g (4.4 mmol, 72%); mp 102–103 °C

Compound 18: To a solution of aldehyde **15** (2.3 g, 6.3 mmol, 1.0 equiv.) and sulfone **16** (2.5 g, 8.2 mmol, 1.3 equiv.) in anhydrous THF (30 mL) was slowly added

KHMDS (0.5 M in toluene, 16.4 mL, 8.2 mmol, 1.3 equiv.) at −78 °C under argon during 1 hour. The reaction mixture was stirred at −50 °C for 6 h, before it was quenched by saturated aqueous NH$_4$Cl. The mixture was diluted with Et$_2$O, washed with water and brine, dried over MgSO$_4$, concentrated and purified *via* flash chromatography (hexane/EtOAc: 20/1) to afford enyne **18** as a yellow oil; yield: 1.7 g (3.8 mmol, 60%).

Compound 6: To a solution of benzyl azide **7** (730 mg, 2.2 mmol, 1.0 equiv.) and alkyne **8** (370 mg, 2.4 mmol, 1.1 equiv.) in *t*-BuOH (2.0 mL) and H$_2$O (2.0 mL) was added CuSO$_4$ (8.0 mg, 0.05 mmol, 0.02 equiv.) followed by sodium asorbate (90 mg, 0.5 mmol, 0.5 equiv.) at room temperature. The reaction mixture was vigorously stirred at room temperature for 12 h. The resulting mixture was diluted with EtOAc (50 mL), washed with water and brine, dried over MgSO$_4$, concentrated and purified *via* flash chromatography (hexane/EtOAc: 1/1) to afford triazole **6** as a colorless oil; yield: 1.01 g (2.1 mmol, 95%).

Compound 5: To the solution of phenol **19** (460 mg, 1.1 mmol) in MeOH (3 mL) was added 2 M HCl (1 mL) at room temperature. The reaction mixture was stirred at 50 °C for 8 h. The solution was concentrated under vacuum and the residue was dissolved with EtOAc (50 mL). The solution was washed with water and brine, dried over anhydrous MgSO$_4$, and filtered. After evaporation of the solvent, the product was collected and further recrystallized by CHCl$_3$/EtOH (10:1) to afford the pure triazole-cycloproparadicicol **5** as a white solid; yield: 410 mg (1.1 mmol, quant.); mp 240–242 °C.

Supporting Information

Additional experimental procedures and spectra data for new compounds and intermediates are available as Supporting Information.

Acknowledgements

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[21] CCDC 682086 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (+44)-1223–336033.

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[23] Limited *in vivo* studies have been performed with triazole-cycloproparadicicol. As yet, it has not demonstrated appreciable *in vivo* activity in mouse xenograft systems, presumably due to solubility issues. We are currently evaluating related analogs in an effort to improve the pharmacokinetic properties of the constructs.