An Improved Synthesis of Resorcylic Acid Macrolactone Inhibitors of Hsp90

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Abstract: A synthesis of resorcylic acid macrolactone analogues of the natural product radicicol is described in which the key steps are the acylation and ring opening of a homophthalic anhydride to give an isocoumarin, followed by a ring-closing metathesis to form the macrocycle.

Key words: macrocyclic lactones, isocoumarins, ring-closing metathesis reaction

Heat shock protein 90 (Hsp90) is an ATP-dependent chaperone that plays a central role in regulating the stabilization, activation, and degradation of a range of client proteins,[1,2] including a number of known over-expressed or mutant oncogenic proteins such as C-RAF, B-RAF, ERBB2, AKT, telomerase, and p53, many of which are associated with the six hallmarks of cancer.[3,4] It is one of the most abundant proteins in eukaryotic cells, and has emerged as a very attractive target for novel cancer therapeutic agents, with several Hsp90 inhibitors having now entered clinical trial.[5–10] Early pioneering work on Hsp90 inhibition was carried out with two natural products, radicicol (**1**) and geldanamycin (**2**, Figure 1), both of which, in common with most other inhibitors, bind to the ATP-binding pocket in the N-terminal domain of the protein.[11–14]

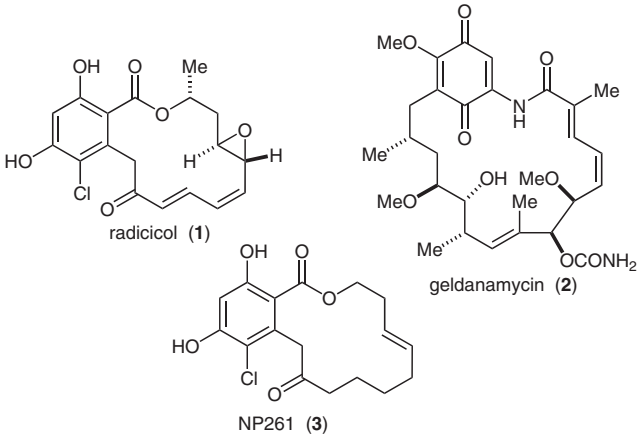

Figure 1 Structures of the naturally occurring Hsp90 inhibitors radicicol (**1**) and geldanamycin (**2**), together with the simpler radicicol analogue NP261 (**3**)

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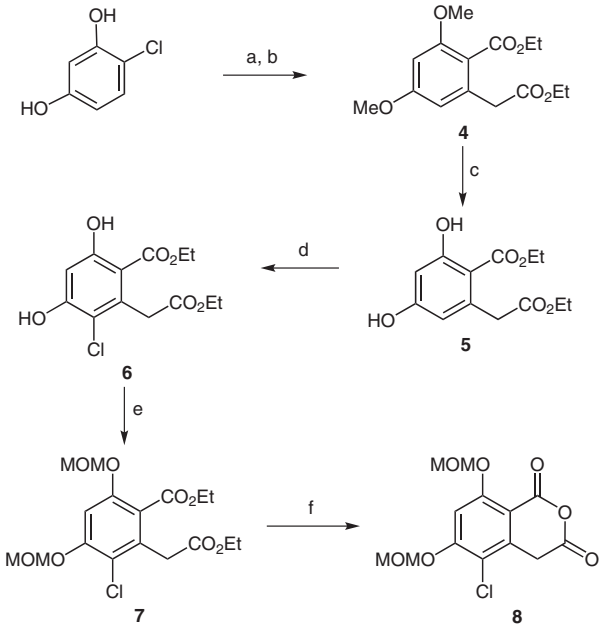
Radicicol (**1**), also known as monorden, was originally isolated from the fungus *Monocillium nordinii* over 50 years ago,[15] is a member of a larger class of natural products known as resorcylic acid lactones, many of which also exhibit potent biological activity.[16–18] The compound has attracted the interest of synthetic chemists and has been the subject of total synthesis by the groups of Lett,[19–22] Danishefky,[23] and Winssinger.[24]

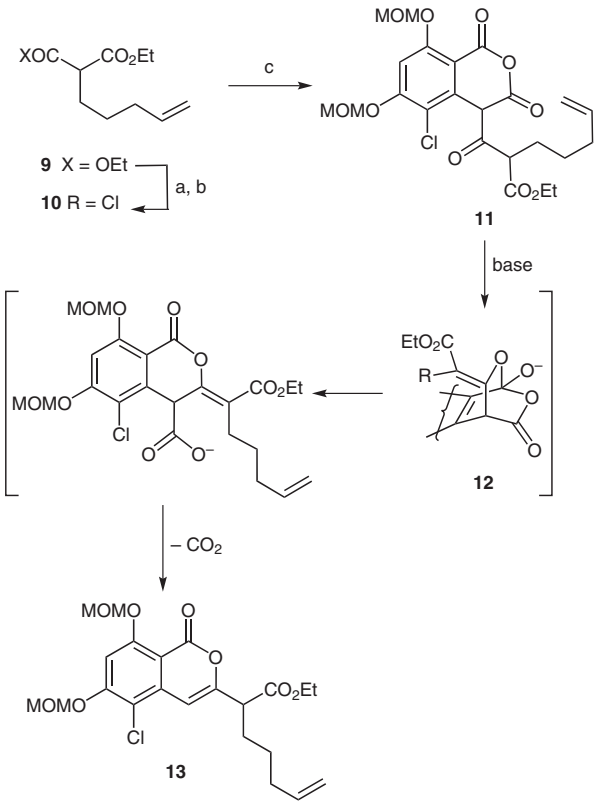
Recently we reported the synthesis and biological evaluation of a simple analogue of radicicol, NP261 (**3**), which not only showed the established molecular signature of Hsp90 inhibition, that is, depletion of client proteins with upregulation of Hsp70, but also bound to the protein in a similar way to the structurally more complex natural product.[25] Our original approach to the synthesis of radicicol analogues, such as NP261 (**3**), was based on Danishefsky and co-workers' first-generation synthesis of radicicol itself,[23] employing a ring-closing metathesis (RCM) reaction to form the macrocycle, a tactic commonly used in related syntheses.[24,26–28] We have now developed a more versatile route to the RCM precursor that is based on isocoumarin chemistry, and incorporates the necessary chlorine from an early stage, thereby avoiding a late stage chlorination on every analogue.

Our pivotal intermediate was the 5-chlorohomophthalic anhydride **8**, available by adaptation of chemistry reported by Bauta et al.[29] The starting point for the synthesis was diester **4**,[29] obtained in two steps from 4-chlororesorcinol, using the addition of malonate anion to 3,5-dimethoxybenzyne and subsequent rearrangement to the homophthalate ester **4**, a reaction originally developed by Danishefsy.[30] The removal of both methoxy groups of diester **4** using aluminium chloride in dichloromethane proved to be problematic when increasing the scale of the reaction (>5.0 g). However, using aluminium bromide (1.2 equiv) to remove the methoxy next to the ester first, followed by a large excess of aluminium chloride (8.0 equiv) to remove the second methyl group, reproducible results and a good yield of the resorcylic compound **5** was achieved (71%). The chlorination of compound **5** was carried out by cooling to low temperature (–30 °C) and using a slight excess of chlorinating agent (1.1 equiv). At higher temperatures (–10 °C and 0 °C), double chlorination was observed. The protection of the chlororesorcinol (**6**) with two methoxymethyl (MOM) groups proceeded in excellent yield. Cleavage of both esters using sodium hydroxide and dehydration with acetic anhydride gave the key homophthalic anhydride intermediate **8** in 59% yield over two steps (Scheme 1).[31]

Our projected conversion of anhydride **8** into an isocoumarin suitable for a subsequent RCM reaction to give the macrolactone **3** (and analogues) required its acylation by the ethyl malonyl chloride **10** bearing a pentenyl side chain. This was obtained by monohydrolysis of the corresponding malonate **9**, itself obtained by alkylation of triethyl methanetricarboxylate, followed by removal of one of the ester groups.[32] The mono ethyl malonate **9** was converted into the acid chloride **10** using thionyl chloride. With the synthesis of both the anhydride **8** and acid chloride **10** complete, both were subjected to the reaction conditions for formation of the isocoumarin developed by Bauta et al. (Scheme 2),[29] using tetramethylguanidine (TMG) to effect the initial acylation. In this process, the initial product **11** of acylation undergoes further base-mediated cyclization to give **12**, ring opening of which followed by loss of carbon dioxide gives the isocoumarin (**13**, Scheme 2). The presence of two MOM-protected phenols and the chlorine adjacent to the reaction center had no adverse effect, and a good yield of the isocoumarin (**13**) was obtained (75%).[33]

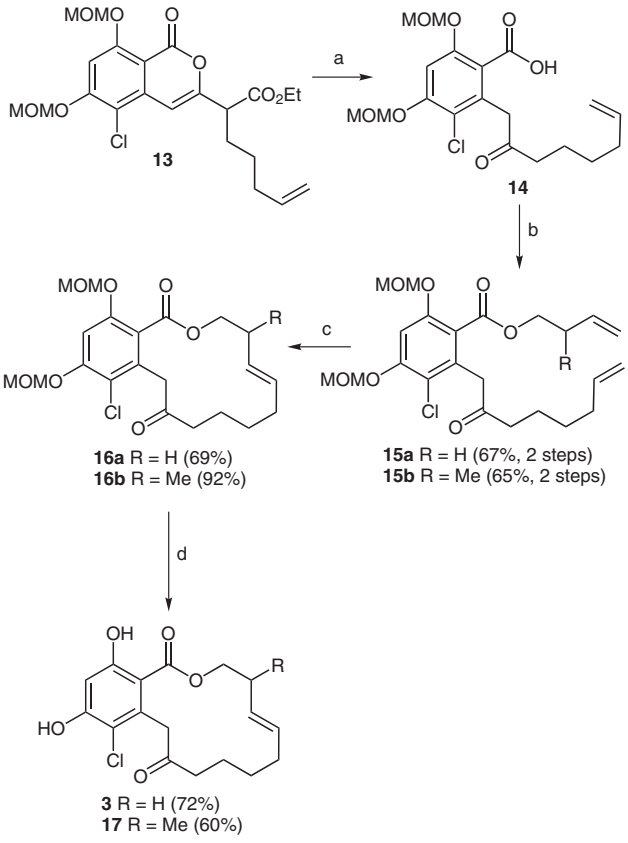


Scheme 1 *Reagents and conditions*: (a) Me_2SO_4, aq NaOH, heptane (88%); (b) diethyl malonate, LDA, THF, –78 °C (57%); (c) (i) $AlBr_3$, CH_2Cl_2, r.t. to 45 °C, (ii) $AlCl_3$, r.t. (71%); (d) SO_2Cl_2, THF, –30 °C (81%); (e) MOMCl, *i*-Pr_2NEt, DMF, 0 °C (85%); (f) (i) NaOH, THF–MeOH–H_2O, 70 °C, (ii) Ac_2O, PhMe, 125 °C (59% over two steps).



Scheme 2 *Reagents and conditions*: (a) KOH, EtOH, 0 °C to r.t. (98%); (b) $SOCl_2$, 70 °C (not purified); (c) **8**, TMG, MeCN, 0 °C, then Et_3N, 0 °C to r.t. (75%).

Next, efforts turned to the conversion of isocoumarin (**13**) into radicicol analogue NP261 (**3**), and started with the hydrolysis of the ester – lactone. It was found that this could be achieved using an excess of lithium hydroxide (20 equiv, Scheme 3), and resulted in hydrolysis of the lactone and ester moieties with concomitant decarboxylation of the resulting β-keto acid fragment to give the desired keto acid **14** directly without the need for a separate decarboxylation step. The keto acid **14** was used directly for the Mitsunobu reaction [diisopropyl azodicarboxylate



Scheme 3 *Reagents and conditions*: (a) LiOH, THF–MeOH–H_2O, r.t.; (b) H_2C=CHCHRCH_2OH, Ph_3P, DIAD, PhMe, r.t.; (c) Grubbs II catalyst (5 mol%), CH_2Cl_2, 45 °C; (d) HCl, dioxane, r.t.

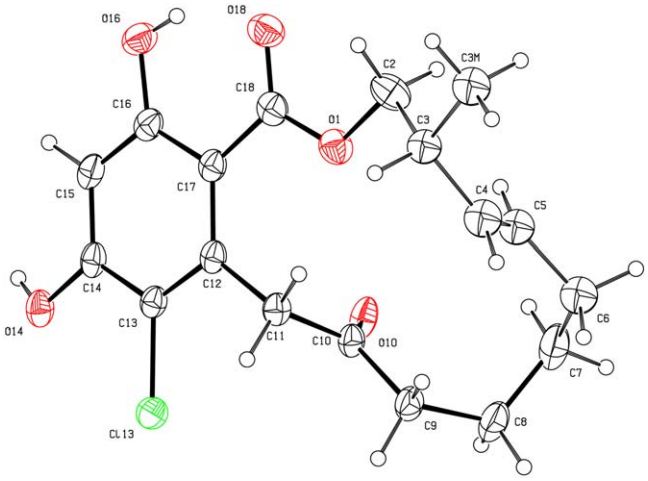


Figure 2 X-ray crystal structure of the (±)-resorcylic macrolactone **17**[35]

(DIAD), Ph_3P and toluene] with 3-butenol and its 2-methyl derivative, which gave the esters **15a** and **15b** in reasonable yield (65–67%). The critical ring-closing metathesis using Grubbs second-generation catalyst {benzylidene[1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(trichlorocyclohexylphosphine)ruthenium} proceeded in good yield to give the macrolactones **16** isolated as the *E*-isomer after chromatography. Cleavage of the MOM groups could be achieved in good yield under acidic conditions to give the previously synthesized NP261 (**3**) and its novel methyl analogue, the resorcylic macrolactone **17** (Scheme 3), the structure of which was confirmed by X-ray crystallography (Figure 2).

Thus, we have established a new route to the Hsp90 inhibitor **3** and its new analogue **17**,[34] that proceeds in an eleven-step linear sequence in an overall yield of 4%.

Acknowledgment

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(31) **5-Chloro-6,8-bis(methoxymethoxy)isochroman-1,3-dione (8)**
Mp 96–98 °C. MS: *m/z* calcd for $C_{13}H_{13}{}^{35}ClNaO_7$: 339.0242; found: 339.0229 [M + Na]. IR (CH_2Cl_2): ν_{max} = 1796, 1756, 1593 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.18 (1 H, s, ArH), 5.48 (2 H, s, OCH_2O), 5.39 (2 H, s, OCH_2O), 4.20 (2 H, s, CH_2), 3.53 (3 H, s, OCH_3), 3.52 (3 H, s, OCH_3). ^{13}C NMR (100 MHz, acetone-d_6): δ = 165.3 (C), 160.6 (C), 159.2 (C), 157.1 (C), 137.7 (C), 114.6 (C), 107.1 (C), 103.4 (CH), 96.3 (CH_2), 96.1 (C), 57.0 (CH_3), 56.9 (CH_3), 34.2 (CH_2). ESI-MS: *m/z* (%) = 341/339 (23/69) [M + Na], 327 (14), 309 (15).
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(33) **Ethyl 2-[5-Chloro-6,8-bis(methoxymethoxy)-1-oxo-1*H*-isochromen-3-yl]hept-6-enoate (13)**
Mp 50–52 °C. MS: *m/z* calcd for $C_{22}H_{27}{}^{35}ClNaO_8$: 477.1292; found: 477.1326 [M + Na]. IR (CH_2Cl_2): ν_{max} = 2987, 1736, 1661, 1585 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6): δ = 7.15 (1 H, s, ArH), 6.85 (1 H, s, ArH), 5.89–5.77 (1 H, m, $CH{=}CH_2$), 5.45 (2 H, s, OCH_2O), 5.34 (2 H, s, OCH_2O), 5.05–4.93 (2 H, m, $CH{=}CH_2$), 4.18 (2 H, q, *J* = 7.1 Hz, $CO_2CH_2CH_3$), 3.66 (1 H, t, *J* = 7.5 Hz, $CHCH_2$), 3.51 (3 H, s, OCH_3), 3.51 (3 H, s, OCH_3), 2.13 (2 H, dt, *J* = 7.1, 7.5 Hz, CH_2), 2.00–1.89 (2 H, m, CH_2), 1.49 (2 H, quin, *J* = 7.1, CH_2), 1.22 (2 H, q, *J* = 7.1 Hz, $CO_2CH_2CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.1 (C), 160.5 (C), 159.2 (C), 157.2 (C), 158.15 (C), 139.1 (CH), 138.6 (C), 115.3 (CH_2), 110.9 (C), 105.9 (C), 103.7 (CH), 101.2 (CH), 96.4 (CH_2), 96.0 (CH_2), 61.8 (CH_2), 56.9 (CH_3), 56.8 (CH_3), 50.5 (CH), 34.0 (CH_2), 27.2 (CH_2), 14.5 (CH_3), 1 × CH_2 unobserved. ESI-MS: *m/z* (%) = 475/477 (36/100) [M + Na].

(34) **(*E*)-1-Chloro-2,4-dihydroxy-7,8,11,12,13,14-hexahydro-6-oxa-16*H*-benzocyclotetradecene-5,15-dione (3)**
Mp 174–176 °C (lit.[25] mp 168–169 °C). MS: *m/z* calcd for $C_{17}H_{19}{}^{35}ClNaO_5$: 361.0813; found: 361.0817 [M + Na]. IR (CH_2Cl_2): ν_{max} = 3722, 3156, 1712, 1659, 1603 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 11.80 (1 H, s, OH), 6.61 (1 H, s, ArH), 6.17 (1 H, s, OH), 5.56–5.39 (2 H, m, CH=CH), 4.44 (2 H, t, *J* = 5.3 Hz, CO_2CH_2), 4.29 (2 H, s, CH_2), 2.58 (2 H, t, *J* = 6.5 Hz, CH_2), 2.44 (2 H, dt, *J* = 5.3, 5.3 Hz, CH_2), 2.10 (2 H, dt, *J* = 6.2, 4.8 Hz, CH_2), 1.71 (2 H, quin, *J* = 6.2 Hz, CH_2), 1.58 (2 H, m, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 206.2 (C), 170.7 (C), 163.0 (C), 156.4 (C), 136.2 (CH), 134.1 (C), 126.8 (C), 114.7 (C), 107.3 (CH), 65.8 (CH_2), 46.8 (CH_2), 41.0 (CH_2), 32.2 (CH_2), 31.54 (CH_2), 25.5 (CH_2), 22.1 (CH_2). ESI-MS: *m/z* (%) = 363/361 (31/100) [M + Na], 341/339 (4/12).

(*E*)-1-Chloro-2,4-dihydroxy-7,8,11,12,13,14-hexahydro-8-methyl-6-oxa-16*H*-benzocyclotetradecene-5,15-dione (17)
Mp 163–164 °C. MS: *m/z* calcd for $C_{18}H_{21}{}^{35}ClNaO_5$: 375.0970; found: 375.0959 [M + Na]. IR (CH_2Cl_2): ν_{max} = 3686, 3512, 1720, 1658, 1604 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 11.78 (1 H, s, OH), 6.63 (1 H, s, ArH), 6.00 (1 H, br s, OH), 5.46 (1 H, dt, *J* = 15.6, 7.5 Hz, =CH), 5.34 (1 H, dt, *J* = 15.6, 7.3 Hz, =CH), 4.27 (1 H, dd, *J* = 10.7, 3.0 Hz, CO_2CH), 4.28 (2 H, s, CH_2), 3.99 (1 H, t, *J* = 10.7 Hz, CO_2CH), 2.58 (3 H, m, CH_2, CH), 2.13 (3 H, m, CH_2, CH), 1.72 (3 H, m, CH_2, CH), 1.04 (3 H, d, *J* = 7.0 Hz, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 205.7 (C), 171.8 (C), 163.8 (C), 158.9 (C), 138.3 (C), 134.3 (CH), 132.5 (CH), 115.9 (C), 107.8 (C), 103.6 (CH), 71.1 (CH_2), 47.1 (CH_2), 41.2 (CH_2), 36.7 (CH), 32.7 (CH_2), 26.6 (CH_2), 23.0 (CH_2), 17.4 (CH_3). ESI-MS: *m/z* (%) = 377/375 (33/100) [M + Na], 355/353 (8/23), 335/337 (31/10).

(35) Crystallographic data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB21EZ, UK; fax: +44 (1223)336033; or deposit@ccdc.cam.ac.uk].