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Targeting the Hsp90 Chaperone: Synthesis of Novel Resorcylic Acid Macrolactone Inhibitors of Hsp90

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Abstract: A series of benzo-macrolactones has been prepared by chemical synthesis, and evaluated as inhibitors of heat shock protein 90 (Hsp90), an emerging attractive target for novel cancer therapeutic agents. A new synthesis of these 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. The methodology has been extended to a novel series of macrolactones incorporating a 1,2,3-triazole ring.

Keywords: anticancer agents · cycloaddition · enzyme inhibitors · macrocyclic compounds · metathesis

Introduction

Heat shock protein 90 (Hsp90), one of the most abundant proteins in eukaryotic cells, is an ATP-dependent chaperone that plays a central role in regulating the stabilization, activation and degradation of a range of proteins.[1,2] These "client" proteins include a number of known over-expressed or mutant oncogenic proteins such as C-RAF, B-RAF, ERBB2, AKT, telomerase and p53. Many of these proteins are associated with the six hallmarks of cancer,[3,4] and therefore Hsp90 has emerged as a very attractive target for novel cancer therapeutic agents. Progress in this area has been extensively reviewed in the last five years.[2,3,5–22] Several Hsp90 inhibitors have now entered clinical trial.[22]

The pioneering work on Hsp90 inhibition was done with two natural products, radicicol (**1**) and geldanamycin (**2**) (Figure 1), both of which bind to the ATP-binding pocket in the *N*-terminal domain of the protein.[23,24] The protein-bound structures of both **1** and **2** have been studied by X-ray crystallography[25] and in solution by NMR spectroscopy.[26]

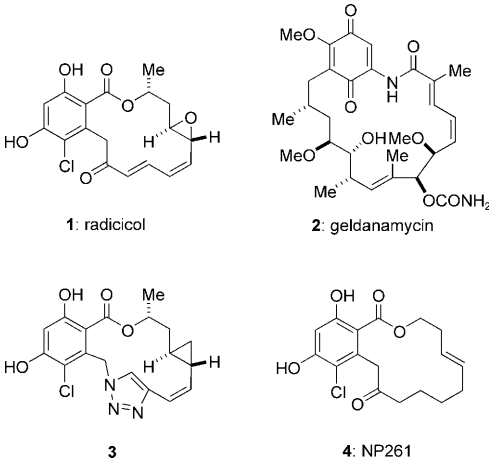

Figure 1. Structures of the naturally occurring Hsp90 inhibitors radicicol **1** and geldanamycin **2**, Danishefsky's cyclopropararadicicol analogue **3**, and our synthetic radicicol analogue **4**.

Radicicol (**1**) was originally isolated from the fungus *Monocillium nordinii* over 50 years ago,[27] and subsequently from both *Nectria radicicola*[28] and from the plant associated fungus *Chaetomium chiversii*,[29] has attracted the interest of synthetic chemists and has been the subject of three total syntheses by the groups of Lett,[30–33] Danishefky,[34] and

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Winssinger.[35] It is one of the most potent Hsp90 inhibitors in vitro,[36] although it has little or no activity in vivo,[37,38] and is a member of a larger class of natural products known as resorcylic acid lactones, many of which also exhibit potent biological activity.[39–41] The lack of in vivo activity of radicicol is presumably a result of its reactive epoxide and dienone moieties, although its oxime derivative did possess some in vivo activity.[42] In a search for further biologically active analogues, Danishefsky and co-workers have developed a novel series of inhibitors based on cyclopropradicicol,[43–45] including the recently reported triazole-cyclopropradicicol **3**.[46]

Our own work in this area started with a consideration of the structure of **1** bound in the ATP pocket in the N-terminal domain of yeast Hsp90 (Figure 2a),[25] together with published biological data on radicicol analogues,[29,45,47–49] and resulted in the synthesis and biological evaluation of a series of novel resorcylic macrolactones of varying ring size and conformation.[50] One of these analogues, NP261 (**4**), not only showed the established molecular signature of Hsp90 inhibitors that bind to the *N*-terminus of the protein, 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 (Figure 2b). Thus both compounds adopt a similar folded conformation with the same key hydrogen-bonding interactions involving the salicylate ester and phenolic groups, the carboxylate side-chain of Asp79, the main-chain amide group of Gly83, the hydroxyl side-chain of Thr171, the main-chain carbonyl of Leu34, and conserved water molecules (Figure 2). Although the radicicol structure clearly shows the involvement of the epoxide oxygen in hydrogen bonding to the ε-amino side-chain of Lys44, the relatively minimal two-fold loss of in vitro activity in the analogue **4** suggests that this interaction, although useful, is not essential.

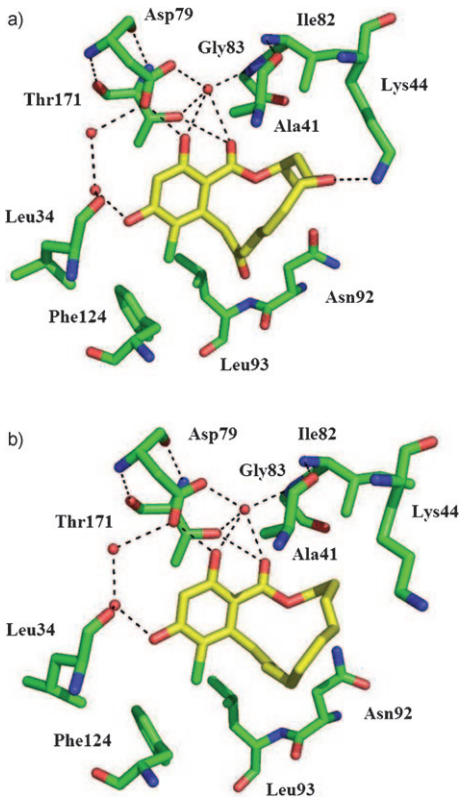


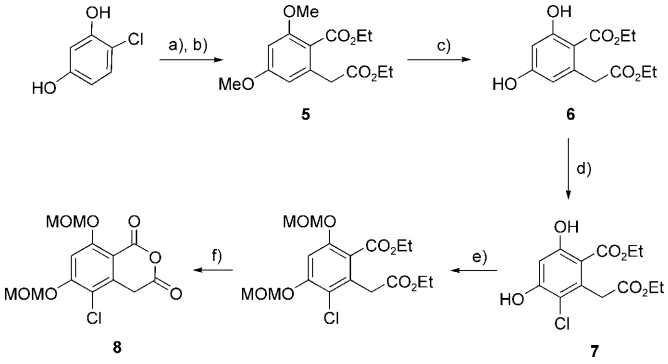
Figure 2. Binding interactions of resorcylic macrolactone inhibitors with yeast Hsp90; in all cases, the hydrogen-bonded interactions in the ATP-binding site are represented as broken lines. a) Radicicol **1** (data taken from Roe et al.[25] and available at PDB ID 1BGQ); b) 14-membered lactone NP261 (**4**) (data taken from Proisy et al.[50] and available at PDB ID 2CGF).

The aim of the present study was two-fold: firstly to develop an improved chemical route to our radicicol analogue **4**,[51] and secondly the application of the new synthetic chemistry to the synthesis of novel resorcylic macrolactones designed to incorporate heteroatoms with the potential to bind to the side chains of Lys44, thereby increasing the potential potency of the inhibitors.

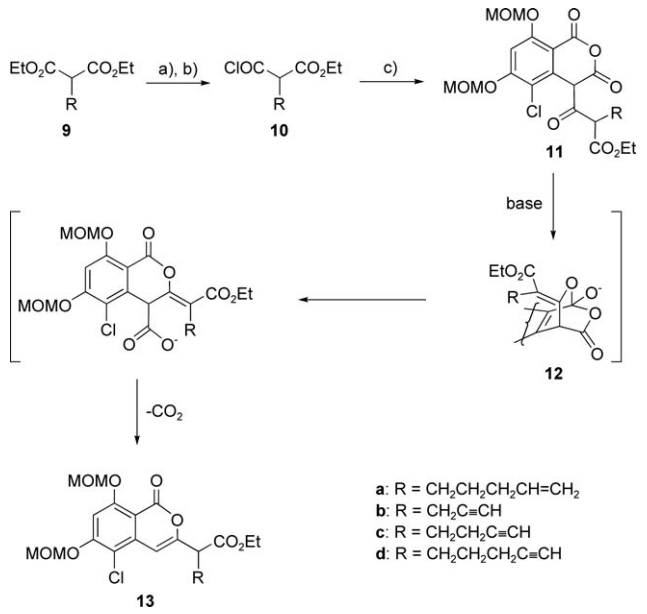
Results and Discussion

Our original approach to the synthesis of radicicol analogues such as **4** was based on Danishefsky and co-workers' first-generation synthesis of radicicol itself,[34] employing a ring-closing metathesis (RCM) reaction to form the macrocycle, a tactic commonly used in related syntheses.[35,44,49,52] We have now developed a more versatile route to the RCM precursors that is based on isocoumarin chemistry and incorporates the necessary chlorine from an early stage, thereby avoiding a late stage chlorination on every analogue. Interestingly, isocoumarins have been postulated as biosynthetic precursors to resorcylic lacones, a view supported by the recent isolation of isocoumarins from the radicicol producing fungal strain *Chaetomium chiversii*,[53] and were also used as intermediates in Lett's first synthesis of radicicol.[30,31] The starting point for the synthesis was the homophthalate diester **5**,[54] obtained in two steps from 4-chlororesorcinol, using the addition of malonate anion to 3,5-dimethoxybenzyne and subsequent rearrangement to the homophthalate ester **5**, an elegant reaction used by Danishefsky during a synthesis of dynemicin A.[55] Although both methoxy groups of diester **5** could be removed using aluminium chloride in dichloromethane on a small scale, this proved difficult when increasing the scale of the reaction (>5.0 g). However, using aluminium bromide (1.2 equiv) that initially removes the methoxy adjacent to the ester, followed by a large excess of aluminium chloride (8.0 equiv) to remove the second methyl group, resulted in reproducible results and a good yield of the resorcylic compound **6** (71%). The chlorination of compound **6** was carried out at −30°C with a slight excess of sulfuryl chloride (1. equiv). At higher temperatures (−10 and 0°C), double chlorination was observed. The protection of the chlororesorcinol (**7**) with two methoxymethyl (MOM) groups proceeded in excellent yield, and this was followed by cleavage of both esters using sodium hydroxide and dehydration with acetic anhydride to give the

pivotal homophthalic anhydride intermediate **8** in 59% yield over the last two steps (Scheme 1).



Scheme 1. Synthesis of the key homophthalic anhydride intermediate **8** from 4-chlororesorcinol. a) Me_2SO_4, aq NaOH, heptane (88%); b) diethyl malonate, LDA, THF, −78°C (57%); c) i) $AlBr_3$, CH_2Cl_2, RT to 45°C, ii) $AlCl_3$, RT (71%); d) SO_2Cl_2, THF, −30°C (81%); e) MOMCl, iPr_2NEt, DMF, 0°C (85%); f) i) NaOH, THF/MeOH/H_2O, 70°C, ii) Ac_2O, PhMe, 125°C (59%).

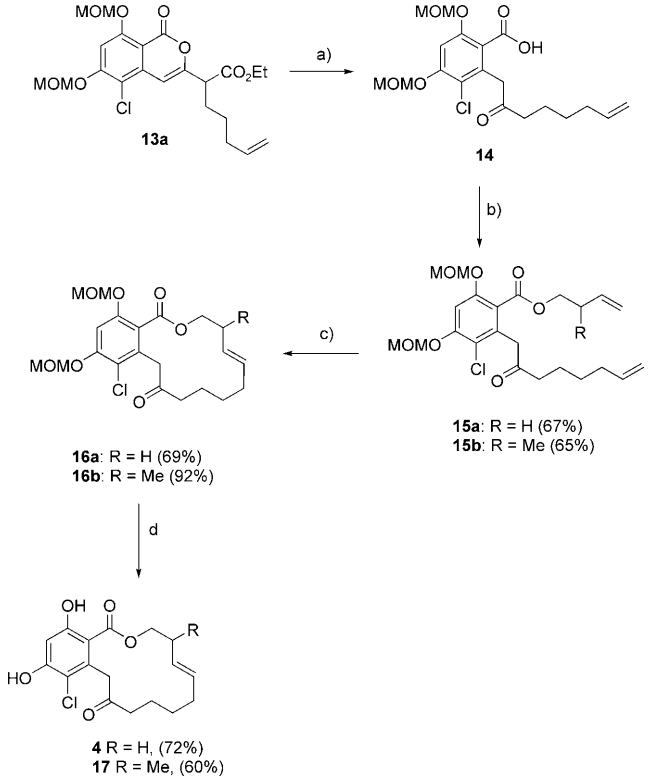


Scheme 2. Synthesis of isocoumarins **13**. a) KOH, EtOH, 0°C to RT; b) $(COCl)_2$, DMF, CH_2Cl_2, 0°C to RT; c) **8**, TMG, MeCN, 0°C, then Et_3N, 0°C to RT.

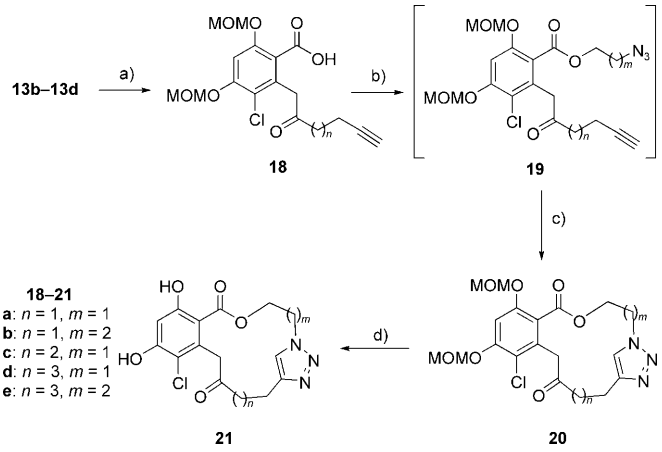
The crucial component of our strategy involved the conversion of anhydride **8** into an isocoumarin suitable for a subsequent RCM reaction to give the macrolactone **4** (and analogues thereof). This necessitated its acylation by an ethyl malonyl chloride **10** bearing an appropriate alkenyl side chain. The pentenyl derivative **10a** was obtained by mono-hydrolysis of the corresponding malonate **9a**, itself obtained by alkylation of triethyl methanetricarboxylate followed by removal of one of the ester groups,[56] and conversion into the acid chloride **10a** using thionyl chloride. The stage was now set for the coupling of anhydride **8** and acid chloride **10a** to give the isocoumarin **13a** under the reaction conditions for developed by Bauta et al. (Scheme 2),[54] using tetramethylguanidine (TMG) to effect the initial acylation. In this cascade process, the initial acylation product **11a** undergoes further base-mediated cyclization to give **12a**, ring opening of which followed by loss of carbon dioxide gives the desired isocoumarin **13a** (Scheme 2). The process is tolerant of the two MOM-protected phenols and the chlorine adjacent to the reaction center, and a good yield of the isocoumarin **13a** was obtained (75%).

The conversion of isocoumarin **13a** into radicicol analogue **4** started with the hydrolysis of the ester-lactone **13a** using an excess of lithium hydroxide (20 equiv) (Scheme 3). This resulted in hydrolysis of the lactone and ester moieties with concomitant decarboxylation of the resulting β-ketoacid fragment and delivered the desired ketoacid **14** directly without the need for a separate decarboxylation step. The ketoacid **14** was coupled directly with 3-butenol and its 2-methyl derivative under the Mitsunobu protocol (diisopropyl azodicarboxylate (DIAD), Ph_3P and toluene) that gave the esters **15a** and **15b** (65–67%). The crucial ring-closing metathesis using Grubbs' second-generation catalyst (benzylidine[1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene]dichloro(tricyclohexylphosphine)ruthenium) proceeded in good yield to give the macrolactones **16** isolated as the *E* isomer after chromatography. Cleavage of the MOM groups was achieved in good yield under acidic conditions to give the previously synthesized **4** and its novel methyl analogue, the resorcylic macrolactone **17** (Scheme 3), the structure of which was confirmed by X-ray crystallography.[51] Thus, we have established a new route to the Hsp90 inhibitor **4** that proceeds in a 11-step linear sequence in an overall yield of 4%.

In line with our desire to use the above chemistry to access novel resorcylic macrolactones with improved potency, we elected to incorporate a 1,2,3-triazole ring into the macrocycle (cf. recent work by Lei and Danishefsky).[46] The choice of the triazole heterocycle was determined by the fact that it could be suitably positioned as to allow the additional heteroatoms the potential to bind to the side chains of Lys44 and possibly Asn92, and also by its ready accessibility using the copper-catalyzed Huisgen 1,3-dipolar cycloaddition between a terminal alkyne and an alkyl azide (click chemistry).[57] Therefore, as described in Scheme 2, the ethyl malonyl chlorides **10b–c**, containing propargyl, 3-butynyl and 4-pentynyl side chains, were reacted with the homophthallic anhydride **8** to give the isocoumarins **13b–d** bearing terminal alkynyl side chains in good yield. The isocoumarins **13b–d** were ring opened under the conditions previously described, and the resulting ketoacids **18** were reacted directly with either 2-azidoethanol or 3-azidopropanol under Mitsunobu conditions (DIAD, Ph_3P, toluene) to give a range of five azides **19**. The Mitsunobu products **19** could only be partially purified due to the difficulty in removing all the by-products—attempts to use alternative Mitsunobu protocols resulted in poorer yields—and therefore they were directly subjected to the cycloaddition reaction. Initial at-



Scheme 3. Conversion of isocoumarin **13a** into resorcylic macrolactones **4** and **17**. a) LiOH, THF/MeOH/H_2O, RT; b) $H_2C{=}CHCHRCH_2OH$, Ph_3P, DIAD, PhMe, RT; c) Grubbs II catalyst (5 mol%), CH_2Cl_2, 45°C; d) HCl, dioxane, RT.



Scheme 4. Conversion of isocoumarins **13** into 1,2,3-triazole containing resorcylic macrolactones **21** using click chemistry. a) LiOH, THF/MeOH/H_2O, RT; b) $N_3CH_2CH_2OH$ or $N_3CH_2CH_2CH_2OH$, Ph_3P, DIAD, PhMe, RT; c) $CuSO_4$, sodium ascorbate, *t*BuOH/H_2O, RT (8–33% over three steps); d) TFA, CH_2Cl_2, RT (21–84%).

Table 1. Biological activity of the macrocyclic lactones **17** and **21a–e** compared to **1** and **4**.

Entry	Compound	TR-Fret IC_{50} [μM]	FP IC_{50} [μM]	HCT116 SRB GI_{50} [μM]
1	radicicol (**1**)	0.014	0.0043	0.00061
2	NP261 (**4**)	0.350	0.0060	6.5
3	**17**	5.8	0.425	72.4
4	**21a**	20%@10 μM	15, 21	>100
5	**21b**	40%@10 μM	8.6, 7.0	>100
6	**21c**	20%@10 μM	13, 11	>100
7	**21d**	8.3	2.7, 4.0	>100
8	**21e**	20%@10 μM	10, 13	>100

tempts to effect the macrocyclization using various copper-catalyzed reaction conditions[58,59] only resulted in trace amounts of triazoles being detected. However the optimal conditions were found to be a large excess of both copper(II) sulfate (4.4 equiv) and sodium ascorbate (6.6 equiv) in *tert*-butyl alcohol/water (0.002 M),[60] which gave the desired macrocyclic triazoles **20a–e** in 8-33% yield over three steps. Cleavage of the MOM protecting groups was achieved with TFA in dichloromethane to give the novel triazole–macrocyclic lactones **21a–e** in 21–84% yield (Scheme 4).

The NP261 analogue **17** and the five novel triazole-macrocyclic lactones **21a–e** were evaluated for Hsp90 inhibition in two Hsp90 binding assays: the fluorescence polarization (FP) assay,[61,62] and the TR-Fret assay.[63] Their growth inhibitory potency in HCT116 human colon cancer cell line, as measured by the SRB assay, was also determined (Table 1). As can be seen in Table 1, introduction of a methyl group (analogue **17**) results in a loss of potency compared to **4** itself. With the exception of compound **21d** that is about 20 times less potent than NP261 (**4**), the triazole–macrocyclic lactones **21** only showed weak Hsp90 inhibition, suggesting that introduction of the triazole in the macrocyclic lactone ring is detrimental to the binding of these compounds to Hsp90. None of the compounds show significant growth inhibition of the HCT116 cell line in comparison with radicicol.

Conclusion

In summary, Hsp90 is an important drug target for cancer therapeutics, and we have previously synthesized and evaluated a simple analogue of the natural product radicicol known as NP261 (**4**). We have now developed an improved route to the resorcylic macrolactone **4** and its analogue **17**, and extended it to the synthesis of a series of novel 1,2,3-triazole-containing macrocyclic lactones, although, the introduction of a triazole ring into the macrocyclic lactone appears to be detrimental to Hsp90 inhibition. Further work is ongoing to investigate the effects of ring substituents on conformation, and therefore to optimize Hsp90 inhibition and antitumor activity.

Experimental Section

Full experimental procedures and spectroscopic characterization data are given in the Supporting Information. Data for the compounds that were evaluated in biological assays are given below.

(*E*)-1-Chloro-2,4-dihydroxy-7,8,11,12,13,14-hexahydro-8-methyl-6-oxa-16*H*-benzocyclotetradecene-5,15-dione (17): colorless solid; m.p. 163–164°C; ^{1}H NMR (400 MHz; $CDCl_3$): $\delta = 11.78$ (s, 1H, OH), 6.63 (s, 1H, ArH), 6.00 (br s, 1H, OH), 5.46 (dt, 1H, J = 15.6, 7.5 Hz, =CH), 5.34 (dt,

1H, J = 15.6, 7.3 Hz, =CH), 4.27 (dd, 1H, J = 10.7, 3.0 Hz, CO_2CH), 4.28 (s, 2H, CH_2), 3.99 (t, 1H, J = 10.7 Hz, CO_2CH), 2.58 (m, 3H, CH_2, CH), 2.13 (m, 3H, CH_2, CH), 1.72 (m, 3H, CH_2, CH), 1.04 ppm (d, 3H, 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 ppm (CH_3); IR (dichloromethane): ν_{max} = 3686, 3512, 1720, 1658, 1604 cm^{-1}; MS (ESI): m/z (%): 377/375 (33/100) $[M+Na]^+$, 355/353 (8/23), 335/337 (31/10); HRMS (ESI): m/z: calcd for $C_{18}H_{21}{}^{35}ClNaO_5$: 375.0970; found: 375.0959 $[M+Na]^+$.

Macrocyclic triazole 21a: colorless solid; m.p. >230 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 10.6 (brs, 1H, OH), 10.0 (brs, 1H, OH), 7.26 (s, 1H, triazole-H), 6.54 (brs, 1H, ArH), 4.63 (t, 2H, J = 4.7 Hz, CH_2), 4.53 (brs, 2H, CH_2), 3.48 (brs, 2H, CH_2), 2.87 (t, 2H, J = 6.6 Hz, CH_2), 2.62 ppm (t, 2H, J = 6.6 Hz, CH_2); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 206.7 (C), 167.0 (C), 155.5 (C), 155.0 (C), 144.6 (C), 132.8 (C), 125.0 (CH), 114.0 (C), 113.0 (C), 102.7 (CH), 63.0 (CH_2), 48.6 (CH_2), 47.9 (CH_2), 41.6 (CH_2), 22.0 ppm (CH_2); IR (solid): ν_{max} = 1715, 1604, 1581 cm^{-1}; MS (ESI): m/z (%): 376/374 (37/100) $[M+Na]^+$, 354/352 (17/50) $[M+H]^+$; HRMS (ESI): m/z: calcd for $C_{15}H_{14}{}^{35}ClNaN_3O_5$: 374.0514; found: 374.0516 $[M+Na]^+$; HPLC: t_R = 6.32 min, purity (AUC) 95%.

Macrocyclic triazole 21b: colorless solid; m.p. 207–209 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 10.39 (brs, 1H, OH), 7.66 (s, 1H, triazole-H), 6.27 (brs, 1H, ArH), 4.42 (t, 2H, J = 5.9 Hz, CH_2), 4.11 (t, 2H, J = 4.9 Hz, CH_2), 3.67 (s, 2H, CH_2), 2.86–2.83 (m, 2H, CH_2), 2.64–2.61 (m, 2H, CH_2), 2.18 ppm (quin, 2H, J = 5.9 Hz, CH_2); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 206.7 (C), 168.6 (C), 158.2 (C), 158.0 (C), 146.1 (C), 132.8 (C), 123.2 (CH), 119.1 (C), 116.7 (C), 102.9 (CH), 63.9 (CH_2), 48.5 (CH_2), 47.3 (CH_2), 40.8 (CH_2), 28.6 (CH_2), 21.0 ppm (CH_2); MS (ESI): m/z (%): 390/388 (31/100) $[M+Na]^+$, 366 (42) $[M+H]^+$; HRMS (ESI): m/z: calcd for $C_{16}H_{15}{}^{35}ClNaN_3O_5$: 388.0676; found: 388.0674 $[M+Na]^+$; HPLC: t_R = 5.9 min, purity (AUC) >95%.

Macrocyclic triazole 21c: colorless solid; m.p. 226–227 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 10.42 (s, 1H, OH), 10.07 (s, 1H, OH), 7.69 (s, 1H, triazole-H), 6.47 (s, 1H, ArH), 4.63 (brs, 4H, 2×CH_2), 2.65 (brs, 2H, CH_2), 1.92 ppm (brs, 2H, CH_2); 2×CH_2 hidden under DMSO and water peaks; ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 204.6 (C), 167.34 (C), 155.4 (C), 155.0 (C), 145.2 (C), 133.3 (C), 123.4 (CH), 114.8 (C), 112.1 (C), 102.5 (CH), 63.1 (CH_2), 48.2 (CH_2), 45.3 (CH_2), 25.3 (CH_2), 21.0 ppm (CH_2); CH_2 hidden under solvent peak; IR (solid): ν_{max} = 1708, 1656, 1610, 1579 cm^{-1}; MS (ESI): m/z (%): 390/388 (13/38) $[M+Na]^+$, 368/366 (34/100) $[M+H]^+$; HRMS (ESI): m/z: calcd for $C_{16}H_{17}{}^{35}ClN_3O_5$: 366.0851; found: 366.0844 [M+H]; HPLC: t_R = 6.4 min, purity (AUC) >95%.

Macrocyclic triazole 21d: colorless solid; m.p. 228–230 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 10.36 (brs, 2H, 2×OH), 7.87 (s, 1H, triazole-H), 6.44 (s, 1H, ArH), 4.67 (s, 4H, 2×CH_2), 3.31 (s, 2H, CH_2), 2.67 (m, 2H, CH_2), 2.15 (t, 2H, J = 6.4 Hz, CH_2), 1.68–1.66 (m, 2H, CH_2), 1.55 ppm (quin, 2H, J = 6.1 Hz, CH_2); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 205.5 (C), 167.4 (C), 156.4 (C), 156.0 (C), 147.7 (C), 133.6 (C), 123.0 (CH), 113.2 (C), 112.9 (C), 102.7 (CH), 63.6 (CH_2), 48.8 (CH_2), 44.9 (CH_2), 26.7 (CH_2), 24.9 (CH_2), 22.4 ppm (CH_2); CH_2 hidden under solvent peak; IR (solid): ν_{max} = 1708, 1679, 1609, 1582 cm^{-1}; MS (ESI): m/z (%): 404/402 (32/100) $[M+Na]^+$, 382/380 (28/90) $[M+H]^+$; HRMS (ESI): m/z: calcd for $C_{17}H_{19}{}^{35}ClNaN_3O_5$: 402.0827; found: 402.0836 $[M+H]^+$; HPLC: t_R = 6.9 min, purity (AUC) >95%.

Macrocyclic triazole 21e: colorless solid; m.p. 200–202 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 10.6 (brs, 1H, OH), 10.1 (brs, 1H, OH), 7.94 (s, 1H, triazole-H), 6.53 (brs, 1H, ArH), 4.42 (t, 2H, J = 5.5 Hz, CH_2), 3.81 (s, 2H, CH_2), 3.76 (t, 2H, J = 6.4 Hz, CH_2), 2.70 (t, 2H, J = 5.8 Hz, CH_2), 2.28–2.21 (m, 4H, 2×CH_2), 1.66 (quin, 2H, J = 6.1 Hz, CH_2), 1.27 ppm (quin, 2H, J = 7.5 Hz, CH_2); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 206.2 (C), 167.7 (C), 155.7 (C), 155.3 (C), 146.3 (C), 133.6 (C), 124.1 (CH), 113.8 (C), 112.9 (C), 102.72 (CH), 61.0 (CH_2), 45.9 (CH_2), 45.0 (CH_2), 40.8 (CH_2), 28.5 (CH_2), 27.3 (CH_2), 23.8 (CH_2), 22.7 ppm (CH_2); IR (solid): ν_{max} = 1706, 1655, 1609, 1576 cm^{-1}; MS (ESI): m/z (%): 418/416 (33/100) $[M+Na]^+$, 394/396 (33/100) $[M+H]^+$; HRMS (ESI): m/z: calcd for $C_{18}H_{20}{}^{35}ClN_3O_5$: 416.0989; found: 416.0991 $[M+Na]^+$; HPLC: t_R = 5.6 min, purity (AUC) 92%.

Biology

Compounds were assayed for their ability to inhibit Hsp90 using two assays:

FP assay: This is a measurement of binding competition with a fluorescent probe as described previously.[61,62]

TR-Fret assay: A highly robust time-resolved fluorescence energy transfer (TR-FRET) assay was used to measure the binding of biotinylated geldanamycin (600 nM) to the full length human Hsp90 His-tagged protein (40 nM), as described previously.[63] Briefly, the europium labelled anti-His-tagged protein antibody (Perkin–Elmer Prod.No.AD0110) was added at 1 nM and the streptavidin Surelight APC (Perkin–Elmer Prod. No.AD0201) at 90 nM. Compounds were tested across a ten point concentration range up to 10 μM and the IC_{50} determined.

Growth inhibition assay: The colorimetric sulforhodamine B assay (SRB) was used to measure growth inhibition studies as described previously.[64] The IC_{50} was calculated as the drug concentration that inhibits cell growth by 50% compared with control growth.

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