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Synthesis of novel 4-alkyl-6-(5-aryl-1,2,3-thiadiazol-4-yl) benzene-1,3-diols as potential Hsp90 inhibitors

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The efficient synthesis of 4-alkyl-6-(5-aryl-1,2,3-thiadiazol-4-yl)benzene-1,3-diols as potential isoform-specific Hsp90 inhibitors was developed. The synthetic pathway starts from the commercially available 1,3-benzenediol and employs well-known reactions. The Friedel–Crafts acylation and carbonyl group reduction to the methylene group gave 1-(5-alkyl-2,4-dihydroxyphenyl)-2-arylethan-1-ones. The subsequently carried out condensation with ethyl carbazate and Hurd–Mori cyclisation gave title compounds.

Keywords: potential Hsp90 inhibitors, thiadiazoles, heterocycles, Hurd–Mori cyclisation

INTRODUCTION

Heat shock protein 90 (Hsp90) is a molecular chaperone that is crucial for the correct folding, stabilisation and maturation of its client proteins [1]. The class of heat shock proteins accounts for a total of 1–2% of cellular proteins in non-stress conditions, and the amount is doubled in cell stress situations [2]. Except for archaebacteria, the protein is present in all classes of organisms. In all mammals, Hsp90 has four isoforms: cytosolic Hsp90α and Hsp90β, glucose-regulated protein 94 (Grp94), located in endoplasmic reticulum, and mitochondrial Tumor necrosis factor receptor-associated protein 1 (Trap1) [3]. These major isoforms share over 50% of sequence identity [4] but have different client proteins, as well as slightly varied functions, depending on their location of activity [5]. Hsp90 consists of three regions: the C-domain, responsible for the dimerisation of the protein, middle domain, which interacts with client-proteins, and the N-domain, which has an ATP binding pocket [1]. Due to many of the Hsp90 client-proteins being linked to the development of cancer hallmarks [6], the subject of Hsp90 inhibition has been studied as a potential anticancer treatment since 1994, when the first natural inhibitor geldanamycin was identified [7]. Despite some recent attempts made on C-terminal domain inhibitors [8], the vast majority of research progress has been achieved on N-domain binding, with 18 potential inhibitors recently reported to be in clinical trials [9]. One of the key groups of compounds under investigation is radicicol-based inhibitors, containing 1,3-benzenediol moiety, which is also the focus of our research team. The typical structure of potential Hsp90 inhibitors is comprised of a 5-membered heterocyclic ring, linking two aryl substituents, one of which is a 4-substituted-1,3-benzenediol fragment, connected through the 6th position. Figure depicts several of the compounds in clinical trials, as well

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as our previously published structures of 4-(5-aryl-1,2,3-thiadiazol-4-yl)-6-isopropylbenzene-1,3-diols, that demonstrated a significant binding to Hsp90 [10, 11]. A range of various substituents for the 1,3-benzenediol moiety are featured in biologically active structures. Isopropyl-, ethyl- and chloro- groups are the most prevalent ones. However, none of the drugs undergoing clinical trials show the ability to specifically inhibit just one isoform of Hsp90 [12], which is believed to be causing unwanted side effects [13]. The previously indicated substituents can be characterised by a small sterical hindrance. For this work, more sizeable cyclic alkyl groups, cyclobutyl-, cyclopentyl- and cyclohexyl-, were chosen as R^1. These groups are likely to have more significant interactions with the sidechains of amino acids, positioned in the active centre of Hsp90, therefore demonstrating isoform-specificity. With that goal in mind, an efficient synthetic approach was developed to obtain novel 4-alkyl-6-(5-aryl-1,2,3-thiadiazol-4-yl)benzene-1,3-diols.

EXPERIMENTAL

Reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm Merck silica plates ($60F_{254}$), using UV light as a visualising agent and/or vanillin as a developing agent. Column chromatography was performed with Kieselgel 60 (40–63 μm) silica gel. The measured melting points on a 'Stuart SMP10' were obtained and are uncorrected. The following abbreviations are used for organic solvents: DCM – dichloromethane, EA – ethyl acetate and Tol – toluene. ^{1}H and ^{13}C NMR spectra were recorded in deuterated solvents on a BrukerAscend™ 400 MHz spectrometer. Chemical shifts (δ) are given in ppm with reference to solvent signals ($CDCl_3$: δ = 7.26 ppm for ^{1}H NMR, δ = 77.16 ppm for ^{13}C NMR; DMSO-d_6: δ = 2.50 ppm for ^{1}H NMR, δ = 39.5 ppm for ^{13}C NMR). The following standard abbreviations are used to indicate multiplicity: s – singlet, d – doublet, t – triplet, q – quartet, hept – heptet,

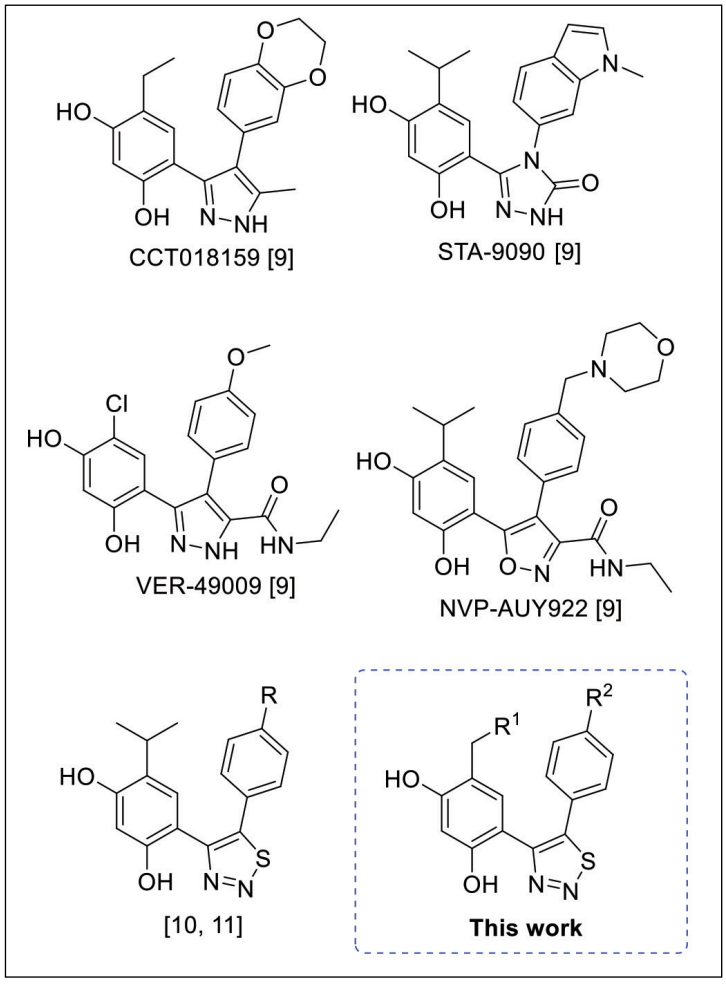


Figure. Potential Hsp90 inhibitors

m – multiplet, dd – doublet of doublets, tt – triplet of triplets and br.s – broad singlet. High resolution mass spectra (HRMS) were recorded on an Agilent LC/MSD TOF mass spectrometer by electrospray ionisation time-of-flight (ESI-TOF) reflection experiments. Infrared spectra were recorded on a PERKIN-ELMER 1000 FT-IR spectrometer with a UATR annex.

General procedure for preparation of alkyl-(2,4-dihydroxyphenyl)methanones (2a–c). A mixture of 1,3-benzenediol (2,2 g, 20 mmol), appropriate carboxylic acid (20 mmol) and $BF_3{\cdot}Et_2O$ (10 mL) was heated at 90°C for 4 h. After cooling to room temperature, 100 mL of 20% NaOAc (aq.) solution was added. The reaction mixture was stirred for 1 h, then extracted with EA (3 × 30 mL), the combined organic phase was washed with sat. $NaHCO_3$ solution (2 × 30 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was used in the next step without further purification.

General procedure for preparation of 4-alkylbenzene-1,3-diols (3a–c). A mixture of previously prepared ketones **2**, $NaBH_3CN$ (3.1 g, 50 mmol) and 20 mg of methyl orange indicator was stirred at room temperature for 24 h. 1M HCl was added as much as required so that the colour of the solution remained red through all reaction time. Next, it was extracted with DCM (3 × 30 mL), the organic layer was washed with brine (30 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography eluting with Tol : EA = 10 : 1.

4-(Cyclobutylmethyl)benzene-1,3-diol (3a). Yellowish crystals, mp 92–94°C. Yield 81%. IR: υ_{max} = 3246 (OH) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 6.90 (d, *J* = 8.1 Hz, 1H), 6.34 (dd, *J* = 8.1, 2.5 Hz, 1H), 6.32 (d, *J* = 2.4 Hz, 1H), 4.90 (s, 1H), 4.85 (s, 1H), 2.63–2.53 (m, 3H), 2.08–1.99 (m, 2H), 1.89–1.78 (m, 2H), 1.75–1.65 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 154.8, 154.5, 130.9, 119.4, 107.7, 102.9, 36.1, 36.0, 28.3, 18.4 ppm. HRMS (ESI): calcd. for $C_{11}H_{15}O_2$ $[M+H]^+$ 179.1067, found 179.1066.

4-(Cyclopentylmethyl)benzene-1,3-diol (3b). Yellowish crystals, mp 103–105°C. Yield 75%. IR: υ_{max} = 3280 (OH) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 6.94 (d, *J* = 8.0 Hz, 1H), 6.34 (dd, *J* = 8.1, 2.4 Hz, 1H), 6.32 (d, *J* = 2.3 Hz, 1H), 4.72 (s, 1H), 4.67 (s, 1H), 2.52 (d, *J* = 7.4 Hz, 2H), 2.09 (hept, *J* = 7.4 Hz, 1H), 1.74–1.60 (m, 5H), 1.55–1.49 (m, 1H), 1.26–1.15 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 154.5, 154.4, 131.4, 120.7, 107.7, 103.0, 40.6, 35.2, 32.6, 25.0 ppm. HRMS (ESI): calcd. for $C_{12}H_{17}O_2$ $[M+H]^+$ 193.1223, found 193.1223.

4-(Cyclohexylmethyl)benzene-1,3-diol (3c). Yellowish crystals, mp 113–114°C. Yield 86%. IR: υ_{max} = 3287 (OH) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 6.90 (d, *J* = 8.0 Hz, 1H), 6.38–6.31 (m, 2H), 4.83–4.72 (m, 2H), 2.40 (d, *J* = 7.1 Hz, 2H), 1.73–1.59 (m, 5H), 1.56–1.43 (m, 1H), 1.26–1.09 (m, 3H), 1.01–0.88 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 154.7, 154.5, 132.0, 119.4, 107.6, 102.9, 38.7, 37.3, 33.4, 26.6, 26.4 ppm. HRMS (ESI): calcd. for $C_{13}H_{19}O_2$ $[M+H]^+$ 207.1380, found 207.1377.

General procedure for preparation of 1-(5-alkyl-2,4-dihydroxyphenyl)-2-arylethan-1-ones (4a–d). A mixture of 4-alkylbenzene-1,3-diol **3** (5 mmol), appropriate 2-arylacetic acid (5 mmol) and $BF_3{\cdot}Et_2O$ (5 mL) was heated at 90°C for 4 h. After cooling to room temperature, 25 mL of 20% NaOAc (aq.) solution was added. The reaction mixture was stirred for 1 h, then extracted with ethyl acetate (3 × 15 mL), the combined ethyl acetate washed with sat. $NaHCO_3$ solution (2 × 15 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography eluting with Tol : EA = 20 : 1.

1-(5-(Cyclobutylmethyl)-2,4-dihydroxyphenyl)-2-(4-methoxyphenyl)ethan-1-one (4a). Yellowish crystals, mp 139–141°C. Yield 43%. IR: υ_{max} = 3249 (OH), 1604 (C=O) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 12.52 (s, 1H), 7.52 (s, 1H), 7.19 (d, *J* = 8.2 Hz, 2H), 6.88 (d, *J* = 8.3 Hz, 2H), 6.30 (s, 1H), 5.61 (s, 1H), 4.15 (s, 2H), 3.79 (s, 3H), 2.64–2.52 (m, 3H), 2.08–1.98 (m, 2H), 1.92 – 1.81 (m, 2H), 1.74–1.63 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 202.5, 163.9, 161.0, 158.8, 132.3, 130.5, 126.7, 119.4, 114.4, 113.4, 103.5, 55.4, 44.3, 36.0, 35.6, 28.2, 18.4 ppm. HRMS (ESI): calcd. for $C_{20}H_{23}O_4$ $[M+H]^+$ 327.1591, found 327.1589.

1-(5-(Cyclopentylmethyl)-2,4-dihydroxyphenyl)-2-(4-methoxyphenyl)ethan-1-one (4b). Yellowish crystals, mp 118–120°C. Yield 45%. IR: υ_{max} = 3265 (OH), 1604 (C=O) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ: 12.50 (s, 1H), 7.56 (s, 1H), 7.18 (d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 8.6 Hz, 2H), 6.31 (s, 1H), 4.15 (s, 2H), 3.79 (s, 3H), 2.53 (d,

J = 7.4 Hz, 2H), 2.08 (hept, *J* = 7.6 Hz, 1H), 1.71–1.59 (m, 4H), 1.55–1.48 (m, 2H), 1.21–1.10 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 202.5, 163.9, 161.0, 158.8, 132.9, 130.5, 126.7, 120.5, 114.4, 113.3, 103.5, 55.4, 44.4, 40.3, 35.3, 32.6, 25.1 ppm. HRMS (ESI): calcd. for $C_{21}H_{25}O_4$ $[M+H]^+$ 341.1747, found 341.1745.

1-(5-(Cyclohexylmethyl)-2,4-dihydroxyphenyl)-2-(4-methoxyphenyl)ethan-1-one (4c). Yellowish crystals, mp 134–136°C. Yield 63%. IR: υ_{max} = 3262 (OH), 1600 (C=O) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 12.51 (s, 1H), 7.51 (s, 1H), 7.18 (d, *J* = 8.6 Hz, 2H), 6.87 (d, *J* = 8.6 Hz, 2H), 6.31 (s, 1H), 5.77 (br.s, 1H), 4.15 (s, 2H), 3.78 (s, 3H), 2.41 (d, *J* = 7.1 Hz, 2H), 1.74–1.59 (m, 5 H), 1.52–1.42 (m, 1H), 1.22–1.12 (m, 3H), 0.97–0.86 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 202.6, 163.8, 161.1, 158.7, 133.5, 131.0, 130.5, 126.7, 119.3, 114.3, 113.2, 103.5, 55.4, 44.3, 38.3, 37.3, 33.3, 26.6, 26.4 ppm. HRMS (ESI): calcd. for $C_{22}H_{27}O_4$ $[M+H]^+$ 355.1904, found 355.1902.

2-(4-Chlorophenyl)-1-(5-(cyclohexylmethyl)-2,4-dihydroxyphenyl)ethan-1-one (4d). Yellowish crystals, mp 178–180°C. Yield 29%. IR: υ_{max} = 3275 (OH), 1628 (C=O) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 12.37 (s, 1H), 7.46 (s, 1H), 7.31 (d, *J* = 8.4 Hz, 2H), 7.20 (d, *J* = 8.5 Hz, 2H), 6.32 (s, 1H), 5.52 (s, 1H), 4.18 (s, 2H), 2.41 (d, *J* = 7.1 Hz, 2H), 1.75 – 1.66 (m, 5H), 1.52 – 1.41 (m, 1H), 1.21 – 1.11 (m, 3H), 0.94–0.88 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 201.5, 163.9, 161.1, 133.3, 133.2, 133.1, 130.8, 129.0, 119.4, 113.2, 103.6, 44.4, 38.3, 37.4, 33.3, 26.6, 26.3 ppm. HRMS (ESI): calcd. for $C_{21}H_{24}ClO_3$ $[M+H]^+$ 359.1408, found 359.1392.

General procedure for preparation of ethyl 2-(1-(5-alkyl-2,4-dihydroxyphenyl)-2-arylethylidene)hydrazine-1-carboxylates (5a–d). A mixture of ketone **4** (2 mmol), ethyl hydrazinecarboxylate (1.25 eq., 2.5 mmol, 0.26 g) and acetic acid (0.5 mL) in ethanol (25 mL) was refluxed for 40 h. The precipitate was filtered and recrystallised from 2-propanol.

Ethyl 2-(1-(5-(cyclobutylmethyl)-2,4-dihydroxyphenyl)-2-(4-methoxyphenyl)ethylidene) hydrazine-1-carboxylate (5a). White crystals, mp 187–189°C. Yield 75%. IR: υ_{max} = 3378, 3257 (OH, NH), 1711 (C=O) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.86 (s, 1H), 10.93 (s, 1H), 9.64 (s, 1H), 7.11 (s, 1H), 7.09 (d, *J* = 8.7 Hz, 2H), 6.86 (d, *J* = 8.5 Hz, 2H), 6.27 (s, 1H), 4.19 (q, *J* = 7.1 Hz, 2H), 4.16 (s, 2H), 3.68 (s, 3H), 2.47–2.36 (m, 3H), 1.84–1.68 (m, 4H), 1.57–1.46 (m, 2H), 1.26 (t, *J* = 7.0 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ: 158.4, 157.8, 157.3, 154.6, 154.1, 129.6, 129.1, 128.2, 117.6, 114.0, 110.2, 102.9, 61.1, 55.0, 35.7, 35.0, 30.5, 27.3, 17.7, 14.5 ppm. HRMS (ESI): calcd. for $C_{23}H_{29}N_2O_5$ $[M+H]^+$ 413.2071, found 413.2068.

Ethyl 2-(1-(5-(cyclopentylmethyl)-2,4-dihydroxyphenyl)-2-(4-methoxyphenyl)ethylidene) hydrazine-1-carboxylate (5b). White crystals, mp 168–170°C. Yield 76%. IR: υ_{max} = 3329, 3216 (OH, NH), 1702 (C=O) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.69 (s, 1H), 10.93 (s, 1H), 9.64 (s, 1H), 7.11 (s, 1H), 7.06 (d, *J* = 8.3 Hz, 2H), 6.83 (d, *J* = 8.4 Hz, 2H), 6.27 (s, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 4.15 (s, 2H), 3.67 (s, 3H), 2.32 (d, *J* = 7.3 Hz, 2H), 2.00–1.90 (m, 1H), 1.56–1.47 (m, 2H), 1.44–1.34 (m, 4H), 1.25 (t, *J* = 7.1 Hz, 3H), 1.04–0.94 (m, 2H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ: 158.3, 157.7, 157.3, 156.7, 154.2, 130.2, 129.1, 128.3, 118.7, 114.0, 110.1, 102.9, 61.1, 55.0, 34.8, 31.7, 30.6, 24.4, 14.5, 14.4 ppm. HRMS (ESI): calcd. for $C_{24}H_{31}N_2O_5$ $[M+H]^+$ 427.2227, found 427.2223.

Ethyl 2-(1-(5-(cyclohexylmethyl)-2,4-dihydroxyphenyl)-2-(4-methoxyphenyl)ethylidene) hydrazine-1-carboxylate (5c). White crystals, mp 175–176°C. Yield 58%. IR: υ_{max} = 3556, 3218 (OH, NH), 1700 (C=O) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.77 (s, 1H), 10.88 (s, 1H), 9.53 (s, 1H), 7.04–7.00 (m, 3H), 6.79 (d, *J* = 8.5 Hz, 2H), 6.21 (s, 1H), 4.15 (q, *J* = 7.1 Hz, 2H), 4.10 (s, 2H), 3.63 (s, 3H), 2.18 (d, *J* = 6.9 Hz, 2H), 1.54–1.47 (m, 3H), 1.40–1.31 (m, 2H), 1.25–1.19 (m, 3H), 1.05–0.66 (m, 4H), 0.75–0.64 (m, 2H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ: 158.3, 157.7, 157.3, 154.1, 135.3, 130.7, 129.1, 128.2, 117.5, 113.9, 109.9, 102.9, 62.0, 61.1, 54.9, 37.4, 36.7, 32.6, 26.2, 25.7, 14.5 ppm. HRMS (ESI): calcd. for $C_{25}H_{33}N_2O_5$ $[M+H]^+$ 441.2384, found 441.2384.

Ethyl 2-(2-(4-chlorophenyl)-1-(5-(cyclohexylmethyl)-2,4-dihydroxyphenyl)ethylidene) hydrazine-1-carboxylate (5d). White crystals, mp 119–120°C. Yield 46%. IR: υ_{max} = 3520, 3224 (OH, NH), 1710 (C=O) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.70 (s, 1H), 10.94 (s, 1H), 9.56 (s, 1H), 7.29 (d, *J* = 8.1 Hz, 2H), 7.11 (d, *J* = 8.1 Hz, 2H), 6.94 (s, 1H), 6.22 (s, 1H), 4.15 (q, *J* = 7.1 Hz, 2H), 2.16 (d, *J* = 6.6 Hz, 2H), 1.58–1.42 (m, 4H), 1.32–1.27 (m, 2H), 1.22 (t, *J* = 7.0 Hz, 3H), 1.03 – 0.95 (m,

3H), 0.71–0.63 (m, 2H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ: 158.2, 157.4, 154.1, 143.4, 135.6, 130.9, 130.7, 129.9, 128.4, 117.5, 109.7, 102.9, 61.1, 60.1, 37.3, 36.6, 32.5, 26.1, 25.7, 14.4 ppm. HRMS (ESI): calcd. for $C_{24}H_{30}ClN_2O_4$ $[M+H]^+$ 445.1889, found 445.1888.

General procedure for preparation of 4-alkyl-6-(5-aryl-1,2,3-thiadiazol-4-yl)benzene-1,3-diols (6a–d). A mixture of compound **5** (1 mmol) and thionyl chloride (5 mL) was stirred for 4 h at 60°C temperature. The solvent was evaporated under reduced pressure, then the oily substance was redissolved in ethanol (10 mL), conc. HCl (0.5 mL) was added and the mixture was refluxed for 1 h. Ethanol was evaporated under reduced pressure and the residue was purified by column chromatography eluting with Tol : EA = 10 : 1.

4-(Cyclobutylmethyl)-6-(5-(4-methoxyphenyl)-1,2,3-thiadiazol-4-yl)benzene-1,3-diol (6a). White crystals, mp 163–164°C. Yield 52%. IR: υ_{max} = 3286 (OH) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.36 (d, *J* = 8.6 Hz, 2H), 7.01 (d, *J* = 8.7 H, 2H), 6.88 (s, 1H), 6.53 (s, 1H), 3.88 (s, 3H), 2.39 (d, *J* = 7.4 Hz, 2H), 2.32–2.23 (m, 1H), 1.84–1.68 (m, 4H), 1.52–1.42 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 161.2, 156.0, 155.8, 155.7, 149.7, 130.9, 129.6, 119.8, 118.9, 115.1, 108.5, 104.4, 55.7, 35.8, 35.4, 28.3, 18.4 ppm. HRMS (ESI): calcd. for $C_{20}H_{21}N_2O_3S$ $[M+H]^+$ 369.1267, found 369.1267.

4-(Cyclopentylmethyl)-6-(5-(4-methoxyphenyl)-1,2,3-thiadiazol-4-yl)benzene-1,3-diol (6b). White crystals, mp 179–180°C. Yield 56%. IR: υ_{max} = 3292 (OH) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.36 (d, *J* = 8.8 Hz, 2H), 6.98 (d, *J* = 8.7 Hz, 2H), 6.91 (s, 1H), 6.53 (s, 1H), 3.87 (s, 3H), 2.29 (d, *J* = 7.4 Hz, 2H), 1.89–1.79 (m, 1H), 1.56–1.40 (m, 6H), 1.01–0.93 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 161.1, 155.9, 155.8, 155.8, 149.7, 130.9, 130.2, 120.0, 119.7, 115.1, 108.5, 104.4, 55.6, 39.9, 35.1, 32.6, 25.0 ppm. HRMS (ESI): calcd. for $C_{21}H_{22}N_2O_3S$ $[M+H]^+$ 383.1424, found 383.1429.

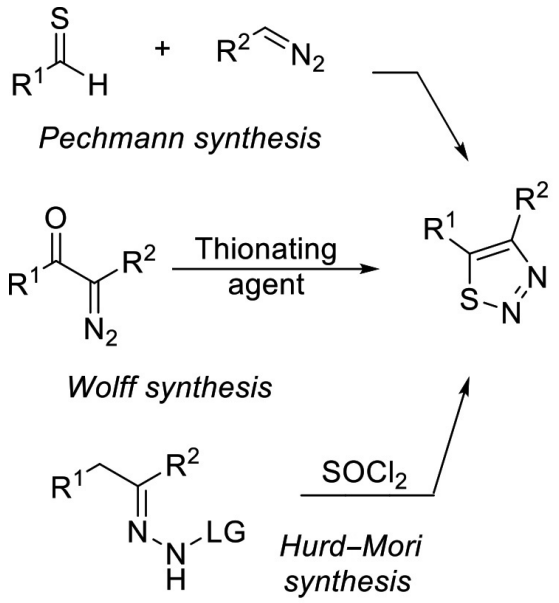
4-(Cyclohexylmethyl)-6-(5-(4-methoxyphenyl)-1,2,3-thiadiazol-4-yl)benzene-1,3-diol (6c). White crystals, mp 180–182°C. Yield 46%. IR: υ_{max} = 3292 (OH) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.36 (d, *J* = 8.2 Hz, 2H), 6.98 (d, *J* = 8.3 Hz, 2H), 6.85 (s, 1H), 6.54 (s, 1H), 5.10 (s, 1H), 3.87 (s, 3H), 2.16 (d, *J* = 7.1 Hz, 2H), 1.69–1.58 (m, 3H), 1.57–1.48 (m, 2H), 1.32–1.21 (m, 1H), 1.13–1.06 (m, 3H), 0.80–0.64 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 160.9, 155.8, 155.7, 155.5, 149.7, 130.8, 130.7, 119.5, 118.8, 114.9, 108.2, 104.2, 55.4, 38.0, 37.0, 33.2, 26.5, 26.2 ppm. HRMS (ESI): calcd. for $C_{22}H_{25}N_2O_3S$ $[M+H]^+$ 397.1580, found 397.1580.

4-(5-(4-Chlorophenyl)-1,2,3-thiadiazol-4-yl)-6-(cyclohexylmethyl)benzene-1,3-diol (6d). Yellowish crystals, mp 203–204°C. Yield 72%. IR: υ_{max} = 3288 (OH) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.66 (s, 1H), 7.46 (d, *J* = 8.2 Hz, 2H), 7.37 (d, *J* = 8.2 Hz, 2H), 6.71 (s, 1H), 6.54 (s, 1H), 4.90 (s, 1H), 2.16 (d, *J* = 7.0 Hz, 2H), 1.72–1.57 (m, 3H), 1.54–1.46 (m, 2H), 1.23–1.19 (m, 1H), 1.13–1.06 (m, 3H), 0.80–0.64 (m, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.2, 156.0, 155.7, 147.9, 136.2, 130.8, 130.7, 129.7, 126.4, 119.0, 107.8, 104.4, 38.0, 37.0, 33.2, 26.4, 26.2 ppm. HRMS (ESI): calcd. for $C_{21}H_{22}ClN_2O_2S$ $[M+H]^+$ 401.1085, found 401.1085.

RESULTS AND DISCUSSION

The 1,2,3-thiadiazole ring has recently gained increasing popularity in the search for new medicinal substances. A recent review highlights antiviral, anticancer and antifungal activities, exhibited by compounds containing this fragment [14]. There are three main synthetic approaches to 1,2,3-thiadiazole formation (Scheme 1). The Pechmann synthesis [15], first described in 1896, involves 1,3-dipolar cycloaddition of diazoalkanes onto a C=S bond. The Wolff synthesis [16] requires the generation of α-diazo thiocarbonyl compounds and their cyclisation, or using thionating compounds (i.e. Lawesson's reagent, P_4S_{10}, arylsulfonyl azides) on diazocarbonyl compounds. The Hurd–Mori reaction, first described in 1955 [17], involves hydrazone formation from ketone and its subsequent cyclisation using thionylchloride. Several other approaches can be found in literature, such as oxidative heterocyclisation and certain rearrangements [18]. The most recently used methods for the synthesis and chemical properties of 1,2,3-thiadiazoles were covered in an entire chapter in Volume IV of Comprehensive Heterocyclic Chemistry, published in 2022 [19].

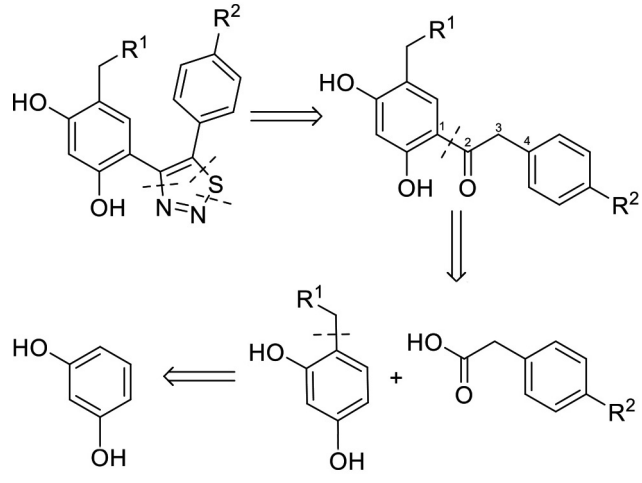
Currently, the Hurd–Mori reaction is the most prevalent method for thiadiazole preparation, and despite being readily used



Scheme 1. Main synthetic pathways to thiadiazole formation

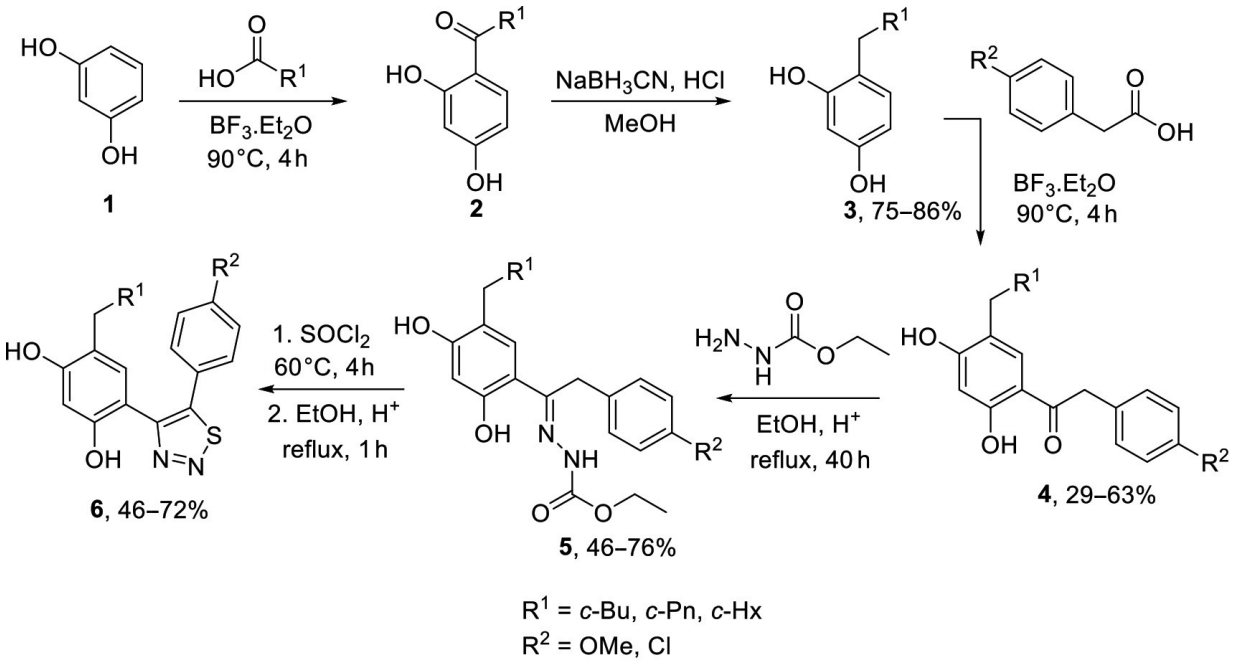
numerous times in the last 15 years, improvements are constantly being made. One research group demonstrated the production of ionic liquid-supported sulphonyl hydrazines, whose condensation with acetyl derivatives and subsequent cyclisation results in thiadiazole formation [20]. It has the benefit of the convenient product separation from the ionic liquid. Another group showed the solid-phase organic synthesis technique [21], the advantages of which are target molecule isolation by simple washing of the solid phase, and its sustainability via the potential regeneration and reuse of resin-bound reagents. The 2016 study [22] focused on improving Hurd–Mori synthesis by substituting the commonly used thionylchloride, which is sensitive to water and humid conditions. Attempts were made to replace it with sulphur-catalyst-oxidating agent system. Viable reaction conditions were determined to be heating of *N*-tosylhydrazones at 100°C in DMA with sulfur and $K_2S_2O_8$. In another recent publication [23], the modification with 1,10-bridged alloxazinium salt and ammonium iodide catalytic system was used. The Hurd–Mori reaction was chosen for the synthetic purposes of 1,2,3-thiadiazole formation in this work.

The retrosynthetic analysis of 4-alkyl-6-(5-aryl-1,2,3-thiadiazol-4-yl)benzene-1,3-diols was started from the ring cleft and hydrazone disconnection, since the 1,2,3-thiadiazole fragment is susceptible to side reactions, certain rearrangements or even cleavage of the ring (Scheme 2) [19]. The synthesis of ketone was considered by disconnecting the two fragments in-between 1st and 2nd marked carbon atoms, 2nd – 3rd and 3rd – 4th. Both last options would result in the need of C–C bond formation requiring difficult procedures and expensive palladium catalysts, as described in literature for the cases of bond formation at C^2–C^3 [24] and C^3–C^4 [25]. On the other hand, the detachment at C^1–C^2 leads to synthons such as 2-arylacetic acid and 4-alkyl-1,3-benzenediol. The former is readily commercially available, and the latter can be prepared by aromatic ring substitution reactions from 1,3-benzenediol.



Scheme 2. Retrosynthetic approach to 4-alkyl-6-(5-aryl-1,2,3-thiadiazol-4-yl)benzene-1,3-diols

With all the aforementioned considerations in mind, a synthetic pathway starting from the commercially available 1,3-benzenediol was developed (Scheme 3). The Friedel–Crafts acylation reaction was used to make the first modifications on the resorcinol ring. Compound **1**, an appropriate carboxylic acid, and boron trifluoride etherate, used in excess as Lewis acid and a solvent, gave ketones **2a–c**, which were used without further purification. Next, carbonyl groups were reduced to methylene groups utilising $NaBH_3CN$ in acidic conditions to give **3a–c** in 75–86% yields. Due to the generation of basic side products, a small amount of a methyl orange indicator was used to



Scheme 3. Synthesis of 4-alkyl-6-(5-aryl-1,2,3-thiadiazol-4-yl)benzene-1,3-diols

cleverly determine the exact amount of 1M HCl needed to maintain the required pH. Compounds **3a–c** were modified by the Friedel–Crafts acylation again, utilising the same reaction conditions provided above. 2-(4-Methoxyphenyl)- and 2-(4-chlorophenyl)acetic acids were used to give ketones **4a–d** in moderate yields. The latter was then refluxed in ethanol with ethylcarbazate and an acid catalyst to give hydrazones **5a–d** in moderate to good yields. The Hurd–Mori cyclisation was carried out in two stages. First, compounds **5a–d** were treated with $SOCl_2$ and the reaction mixture was heated at 60°C. As per usual, TLC plates were used to monitor the progress of the reaction; however, formation of two products was observed. As described in literature, the Hurd–Mori cyclization proceeds through intermediate compounds Δ^3-1,2,3-thiadiazolin-1-ones, the formation of which is particularly favoured when ethyl carbazate or tosylhydrazine are employed to generate hydrazones [26]. Therefore, to convert intermediate compounds to desired structures, increase yield and facilitate purification after the reaction, $SOCl_2$ was evaporated from the reaction mixture, the residue was redissolved in EtOH, a catalytic amount of HCl was added, and the solution was refluxed for 1 h. Final products **6a–d** were obtained in 46–72% yields.

CONCLUSIONS

In summary, the efficient synthesis of 4-alkyl-6-(5-aryl-1,2,3-thiadiazol-4-yl)benzene-1,3-diols as potential isoform-specific Hsp90 inhibitors was developed. The synthetic pathway was divided into two parts: preparation of starting materials and Hurd–Mori cyclisation. The process to starting materials employs the Friedel–Crafts acylation, which is a simple way to attach different substituents to a benzene ring from available carboxylic acids, as well as the cyanoborohydride reduction of the carbonyl to methylene group resulting in very good two-step yields. For the 1,2,3-thiadiazole formation, hydrazones, produced by condensation reaction of ketones and ethyl carbazate, underwent the Hurd–Mori cyclisation. The observed intermediate products were converted to target molecules by refluxing a reaction mixture in ethanol with an acid catalyst, which resulted in easier purification and improved yields. The overall yields of 5-step synthesis were sufficient to acquire the quantities necessary for testing of the inhibitory properties of target compound, which is underway and will be disclosed in future reports.

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NAUJŲ 4-ALKIL-6-(5-ARIL-1,2,3-TIADIAZOL-4-IL)BENZEN-1,3-DIOLIŲ, POTENCIALIŲ HSP90 SLOPIKLIŲ, SINTEZĖ

S a n t r a u k a

Hsp90 (šiluminio šoko baltymo 90, angl. *Heat-shock protein* 90) aktyvumo slopinimo tyrimams buvo susintetinta nemažai 4-alkil-6-(5-aril-1,2,3-tiadiazol-4-il)benzen-1,3-diolių. Sintezės kelias buvo padalytas į dvi dalis – pradinių junginių sintezę bei Hurd-Mori ciklizaciją. Pradiniai junginiai buvo paruošti modifikuojant komerciškai prieinamą 1,3-benzendiolį. Pirmiausia, klasikiniu Friedel-Craftso acilinimo metodu į 1,3-benzendiolio žiedo 4-padėtį buvo įvestos ciklobutil-, ciklopentil- ir cikloheksilkarbonilgrupės, kurios vėliau buvo suredukuotos cianoborhidridu rūgštinėje terpėje iki 4-cikloalkilpakaitų. Taip į 1,3-benzendiolio žiedą buvo įvestos ciklinės alkilo grupės, reikalingos, norint gauti Hsp90 slopiklius, kaip tikimasi, atrankius baltymo izoformoms. Pakartotinai modifikuojant gautą 4-cikloalkil-1,3-benzendiolio žiedą Friedel-Craftso acilinimo metodu, susidarę ketonai buvo veikiami etilkarbazatu. Iš susidariusių hidrazonų Hurd-Mori ciklizacijos metodu buvo sintetinami tiksliniai 1,2,3-tiadiazolai. Šios 5 stadijų sintezės metu buvo susintetinti junginiai, kurių Hsp90 slopinimo veiksmingumas šiuo metu yra tiriamas.