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Original article

# 4,5,6,7-Tetrahydro-[1,2,3]triazolo[1,5-a]pyrazine as a new scaffold for heat shock protein 90 inhibitors

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ABSTRACT

Heat shock protein 90 (hsp90) is a promising anticancer drug target. A library of 2,4-dihydroxyphenyl (resorcinol) substituted 4,5,6,7-tetrahydro-[1,2,3]triazolo[1,5-a]pyrazine compounds that target this protein were designed and prepared based on our earlier study. The compounds were tested in five cancer cell lines and seven of them showed notable anticancer activity ($IC_{50}$ 2–10 μmol/L). The active subset compounds were further subjected to a polarized fluorescent assay and exhibited high binding affinity toward purified hsp90 ($IC_{50}$ 60–100 nmol/L). These results indicated that the tetrahydro-triazolopyrazine motif of the molecules may represent a novel scaffold for the development of hsp90 inhibitors.



## 1. Introduction

Heat shock protein 90 (Hsp90) is an ATP-dependent 90 kD molecular chaperone ubiquitously expressed in eukaryotes [1]. An array of downstream proteins, which are termed as Hsp90 clients, are dependent on the chaperon function of Hsp90 during the late stage of their synthesis and structure maturation. Under stressed conditions, Hsp90 is also responsible for the structural maintenance and repairing of the clients, to prevent them from aggregation, dysfunction and degradation.

In cancer cells, Hsp90 is 2–10 fold overexpressed and plays critical role in cell survival, growth and tumor progression [2]. A number of well-known oncogenic proteins, for example AKT, Eerb-2, Hif-1α and telomerase, are now known to be Hsp90 clients. As a consequence, the maintenance of these malignant pathways could be highly dependent on the chaperone function of Hsp90. It was hypothesized that inhibition of Hsp90 in cancer cells could result in the spontaneous depression of its oncogenic clients and thereby disruption of the related signaling pathways, making the molecular chaperone a "one stone two birds" anticancer drug target. The hypothesis, however, was not widely accepted until the seminal study published by Neckers' team in 1994 [3]. It was demonstrated that benzoquinone ansamycin geldanamycin (**1**, Fig. 1) was able to bind to the ATP-binding cite of Hsp90 located in its N-terminal domain, and significantly lowered the cellular level of c-Raf, a well-defined oncogenic Hsp90 client. Since then, research attentions devoted to this subject has become intense.

A large number of Hsp90 inhibitors based on varied scaffolds, mostly targeting the N-terminal ATP-binding pocket of Hsp90, were reported, and nearly twenty of them received clinical studies for treatment of cancer [4,5]. Among these molecules, resorcinol containing compounds, *e.g.* AUY-922 (**2**) [6], STA-9090 (**3**) [7] and AT13387 (**4**) [8], made a major class. LD-053(**5**) is a new resorcinol-type Hsp90 inhibitor identified in our laboratory [9]. It showed notable anticancer activities in several cellular models and was proved to be effective *in vivo*. We report herein an extended study based on these preliminary results, to demonstrate that the tetrahydro[1,2,3]triazolopyrazin central segment presented in LD-053 actually represents a new scaffold for further discovery of novel Hsp90 inhibitors.

## 2. Experimental

### 2.1. Chemicals and instruments

Starting materials, reagents were purchased from commercial suppliers (Acros, Alfa Aesar) and used without further purification

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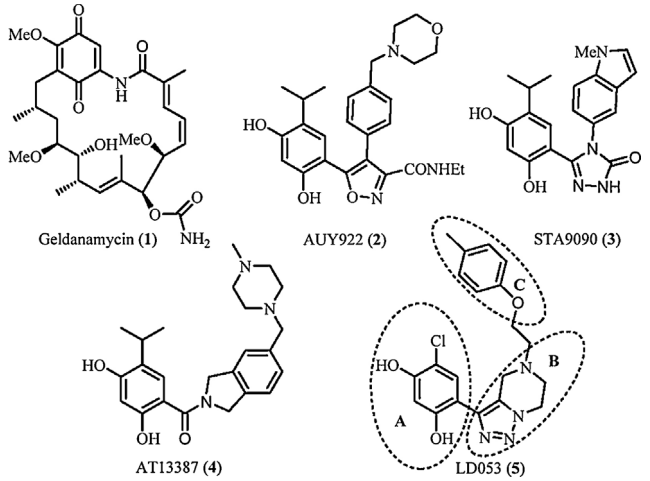



**Fig. 1.** Selected examples of Hsp90 inhibitors.

unless otherwise stated. Pyridine (Py.) was dried over solid potassium hydroxide and distilled. Tetrahydrofuran (THF) and toluene were distilled over sodium. Dry dichloromethane (DCM) was distilled over $P_2O_5$ before use. Proton nuclear magnetic resonance ($^1$H NMR) spectra and carbon-13 ($^{13}$C NMR) spectra were recorded on a Varian Mercury-300, Varian Mercury-400, Varian Avance-500 or Varian System-600 spectrometer. $^1$H spectra was referenced to the residual solvent ($\delta$ 7.26 ppm for $CDCl_3$, $\delta$ 2.04 ppm for $CD_3COCD_3$) or tetramethylsilane ($\delta$ 0 ppm, $CDCl_3$) as an internal reference. For $^{13}$C spectra, chemical shifts are reported relative to the $\delta$ 77.0 ppm resonance of $CDCl_3$ or the $\delta$ 28.9 ppm resonance of $CD_3COCD_3$. Coupling constants are reported in Hz. Infrared (IR) spectra were recorded with Microscope Transmission on a Nicolet 5700 FT-IR. Optical rotations were measured on a Perkin-Elmer 240 using a quartz cell with 1 mL capacity and a 10 cm path length at 20 °C. Mass spectra were recorded on a Thermo Finnigan LTQ FT mass spectrometry manufactured by Thermo Fisher Scientific (San Jose, CA, USA). Column chromatography was generally performed using HaiyangZCX. II (200–300 mesh) silica gel. Unless noted otherwise, all compounds isolated by chromatography were sufficiently pure by $^1$H NMR analysis for use in subsequent reactions.

### 2.2. Synthesis of compounds ***8–13a***

*N*-(2-(*tert*-Butyldimethylsilyloxy)ethyl)-*N*-(3-(5-isopropyl-2,4-bis(methoxymethoxy)phenyl)prop-2-ynyl)benzamide (**8**): 1-Iodo-5-isopropyl-2,4-bis(methoxymethoxy)benzene (**6**) (2.8 g, 7.65 mmol), *N*-(2-(*tert*-butyldimethylsilyloxy)ethyl)-*N*-(prop-2-ynyl)benzamide (**7**) (2.67 g, 8.4 mmol) and CuI (0.29 g, 1.53 mmol) were mixed in $Et_3N$ (35 mL) (See Supporting information for preparation of **6** and **7**). The mixture was ultrasonically deoxygenated under an argon atmosphere and then $PdCl_2(PPh_3)_2$ (540 mg, 0.77 mmol) was added. The reaction mixture was heated at 60 °C for 2 h and filtered. The filtrate was concentrated and the residue was stirred in hexanes (30 mL). The resulting mixture was filtered again. The filtrate was concentrated and the residue purified by chromatography on silica gel (petroleum ether/ethyl acetate, 8/1) to give compound **8** (3.34 g, 95%) as yellow oil. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.66–7.36 (m, 5*H*), 7.22 (s, 1*H*), 6.86 (s, 1*H*), 5.21 (s, 2*H*), 5.20 (s, 2*H*), 4.39 (m, 2*H*), 4.00–3.58 (m, 4*H*), 3.51 (s, 3*H*), 3.48 (s, 3*H*), 3.32–3.18 (m, 1*H*), 1.22 (d, 6*H*, *J* = 12.3 Hz), 0.90 (s, 9*H*), 0.08 (s, 6*H*). $^{13}$C NMR (151 MHz, DMSO-$d_6$): $\delta$ 170.91, 157.19, 155.40, 136.41, 131.15, 130.58, 130.00, 128.87, 127.31, 106.10, 102.73, 95.35, 94.59, 87.77, 60.63, 56.41, 56.29, 50.26, 47.57, 41.64, 26.37, 26.20, 23.01, 18.32, −5.02. IR ($cm^{-1}$): 2228, 1614, 1256, 1219, 1082. HRMS ($ESI^+$) calcd. for $C_{31}H_{46}O_6NSi$ $(M + H)^+$ 556.3089, found 556.3077.

*N*-(2-Hydroxyethyl)-*N*-(3-(5-isopropyl-2,4-bis(methoxymethoxy)phenyl)prop-2-ynyl)benzamide (**9**): Tetrabutylammonium fluoride (3.49 g, 8.4 mmol) was added to a solution of compound **8** (3.33 g, 6.0 mmol) in THF (30 mL). The mixture was stirred at 40 °C for 6 h and diluted with ethyl acetate (20 mL). The resulting solution was successively washed with water (20 mL × 2) and brine (20 mL). The organic layer was dried over anhydrous $Na_2SO_4$ and filtered. The filtrate was concentrated and the residue purified by chromatography on silica gel (petroleum ether/ethyl acetate, 5/1) to give alcohol **9** (2.12 g, 85%) as light yellow oil. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.73–7.36 (m, 5*H*), 7.21 (s, 1*H*), 6.88 (s, 1*H*), 5.22 (s, 2*H*), 5.20 (s, 2*H*), 4.29 (m, 2*H*), 3.98 (m, 2*H*), 3.91 (m, 2*H*), 3.51 (s, 3*H*), 3.48 (s, 3*H*), 3.23 (m, 1*H*), 1.20 (d, 6*H*, *J* = 6.9 Hz). $^{13}$C NMR (151 MHz, $CDCl_3$): $\delta$ 173.14, 157.23, 155.79, 135.19, 131.43, 130.54, 130.37, 128.48, 127.34, 105.24, 101.84, 95.20, 94.48, 86.41, 81.90, 61.72, 56.39, 56.25, 49.44, 41.92, 26.46, 22.71. IR ($cm^{-1}$): 2228, 2101, 1643, 1502, 1262, 1081. HRMS ($ESI^+$) calcd. for $C_{25}H_{32}O_6N$ $(M + H)^+$442.2224, found 442.2205.

*N*-(2-Azidoethyl)-*N*-(3-(5-isopropyl-2,4-bis(methoxymethoxy)phenyl)prop-2-ynyl)benzamide (**10**): $CBr_4$ (1.08 g, 3.2 mmol) was added portion-wide to a solution of compound **9** (1.20 g, 2.7 mmol) and $PPh_3$ (0.85 g, 3.2 mmol) in dry DMF (15 mL) at 0 °C under argon atmosphere. Upon completion of addition, the mixture was stirred for additional 1 h. $NaN_3$ (0.23 g, 3.5 mmol) was added in one portion and the reaction mixture was stirred for 48 h at room temperature before it was diluted with ethyl acetate (30 mL). The resulting mixture was successively washed with water (10 mL × 3) and brine (10 mL). The organic layer was dried over anhydrous $Na_2SO_4$ and filtered. The filtrate was concentrated and the residue purified by chromatography on silica gel (petroleum ether/ethyl acetate, 8/1) to give compound **10** (0.84 g, 66%) as light yellow oil. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.68–7.39 (m, 5*H*), 7.22 (s, 1*H*), 6.87 (s, 1*H*), 5.22 (s, 2*H*), 5.20 (s, 2*H*), 4.33 (m, 2*H*), 3.87 (m, 2*H*), 3.73 (m, 2*H*), 3.51 (s, 3*H*), 3.49 (s, 3*H*), 3.33–3.16 (m, 1*H*), 1.20 (d, *J* = 6.9, 6*H*). $^{13}$C NMR (151 MHz, $CDCl_3$): $\delta$ 171.60, 157.21, 155.71, 135.41, 131.40, 130.64, 130.08, 128.50, 127.01, 105.38, 102.00, 95.25, 94.46, 86.01, 81.96, 56.30, 56.22, 49.15, 45.27, 42.01, 26.44, 22.68. IR ($cm^{-1}$): 2960.7, 2905.8, 2102.2, 1642.5, 1501.4, 1151.3. HRMS ($ESI^+$) calcd. for $C_{25}H_{31}O_5N_4$ $(M + H)^+$467.2289, found 467.2272.

(3-(5-Isopropyl-2,4-bis(methoxymethoxy)phenyl)-6,7-dihydro-[1,2,3]triazolo[1,5-a]pyrazin-5(4*H*)-yl)(phenyl)methanone (**11**): Compound **10** (520 mg, 1.11 mmol) was dissolved in toluene (10 mL) and heated at reflux for 10 h. The reaction mixture was the concentrated and the residue was purified by chromatography on silica gel (petroleum ether/ethyl acetate, 2/1) to give **11** (470 mg, 91%) as white solid. $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 7.72–7.33 (m, 6*H*), 7.06–6.71 (m, 1*H*), 5.24 (s, 2*H*), 5.04 (s, 2*H*), 4.52 (s, 2*H*), 4.34–3.61 (m, 2*H*), 3.55–2.91 (m, 9*H*), 1.36 (m, 6*H*). $^{13}$C NMR (151 MHz, DMSO-$d_6$): $\delta$ 170.31, 155.38, 152.53, 138.96, 138.79, 135.46, 131.01, 130.73, 129.20, 128.47, 127.66, 113.94, 102.10, 95.41, 94.74, 56.34, 46.34, 45.85, 44.86, 44.13, 26.42, 23.23.IR ($cm^{-1}$): 1641.8, 1506.4, 1150.8, 1079.6. HRMS ($ESI^+$) calcd. for $C_{25}H_{31}O_5N_4$ $(M + H)^+$467.2289, found 467.2271.

3-(5-Isopropyl-2,4-bis(methoxymethoxy)phenyl)-4,5,6,7-tetrahydro-[1,2,3]triazolo[1,5-a]pyrazine (**12**): Benzamide **11** (4.40 g, 9.43 mmol) was heated at reflux with KOH (1.59 g, 28.4 mmol) in a mixture of MeOH/$H_2O$ (50 mL, v/v, 1/1) for 10 h. After the mixture was cooled to room temperature, water was added and then extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over anhydrous $Na_2SO_4$, filtered and evaporated to dryness to give **12** (3.0 g, 88%) as white solid. This material is pure enough for the next reaction. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.55 (s, 1*H*), 6.96 (s, 1*H*), 5.23 (s, 2*H*), 5.11 (s, 2*H*),

4.42 (t, 2*H*, *J* = 5.6 Hz), 4.14 (s, 2*H*), 3.50 (d, 3*H*, *J* = 5.5 Hz), 3.41 (s, 3*H*), 3.35 (t, 2*H*, *J* = 5.6 Hz), 3.33–3.24 (m, 1*H*), 1.24 (d, 6*H*, *J* = 6.9 Hz).$^{13}$C NMR (151 MHz, $CDCl_3$): $\delta$ 155.34, 152.74, 139.27, 131.70, 129.66, 128.14, 114.40, 101.98, 95.54, 94.66, 56.32, 56.22, 46.69, 42.79, 42.52, 26.73, 22.82. IR ($cm^{-1}$): 1658.2, 1112.7, 1053.0. HRMS ($ESI^+$) calcd. for $C_{18}H_{27}O_4N_4$ $(M + H)^+$ 363.2027, found 363.2014.

2-(3-(5-Isopropyl-2,4-bis(methoxymethoxy)phenyl)-6,7-dihydro-[1,2,3]triazolo[1,5-a]pyrazin-5(4*H*)-yl)ethanol (**13**): $K_2CO_3$ (571 mg, 4.14 mmol) and 2-bromoethanol (1.04 g, 8.29 mmol) was added to a solution of compound **12** (1.00 g, 2.76 mmol) in dry acetone (10 mL) in a seal tube. The reaction was kept sealed and stirred at 50 °C for 48 h. Water was added and the mixture was extracted with ethyl acetate. The organic layers were combined, washed with brine, dried over anhydrous $Na_2SO_4$ and filtered. The filtrate was concentrated and residue purified using silica gel chromatography (petroleum ether/ethyl acetate, 1/1) to give compound **13** (1.6 g, 95%) as yellow oil. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.54 (s, 1*H*), 6.94 (s, 1*H*), 5.23 (s, 2*H*), 5.10 (s, 2*H*), 4.50 (t, 2*H*, *J* = 5.6 Hz), 3.84 (s, 2*H*), 3.70 (s, 2*H*), 3.51 (s, 3*H*), 3.41 (s, 3*H*), 3.30 (m, 1*H*), 3.11 (t, 2*H*, *J* = 5.6 Hz), 2.83–2.75 (m, 2*H*), 1.24 (d, 6*H*, *J* = 6.9 Hz). $^{13}$C NMR (151 MHz, $CDCl_3$): $\delta$ 155.43, 152.66, 139.65, 131.84, 129.14, 128.23, 114.22, 102.06, 95.67, 94.63, 58.44, 58.34, 56.37, 56.20, 53.44, 49.44, 45.59, 26.74, 22.81. IR ($cm^{-1}$): 3338.6, 2952.0, 1510.6, 1268.9, 1211.0, 1144.3, 988.1. HRMS ($ESI^+$) calcd. for $C_{20}H_{31}O_5N_4$ $(M + H)^+$407.2289, found 407.2274.

2-(3-(5-Isopropyl-2,4-bis(methoxymethoxy)phenyl)-6,7-dihydro-[1,2,3]triazolo[1,5-a]pyrazin-5(4*H*)-yl)ethyl methanesulfonate (**13a**): Compound **13** (200 mg, 0.49 mmol) and $Et_3N$ (60 mg, 0.59 mmol) were dissolved in dry dichloromethane (2.5 mL). Methanesulfonyl chloride (62.4 mg, 0.54 mmol) was added dropwise with stirring at 0 °C. The mixture was kept stirred for 3 h and quenched by addition of water. The mixture was extracted with dichloromethane (10 mL × 3). The organic layers were combined, washed with brine (20 mL), dried over anhydrous $Na_2SO_4$. After filtration, the filtrate was concentrated under reduced pressure to yield intermediate **13a**. This crude material was used directly in the next step.

### 2.3. General method for synthesis of compound *15*

Substituted phenol (1.0 equiv) was dissolved in dry DMF. NaH (3.0 equiv) was added portion-wise at 0 °C under stirring. Upon completion of addition, stirring was continued for another 0.5 h. A solution of crude **13a** (1 equiv) in dry DMF (1 mL) was injected to the reaction mixture through a syringe. The ice bath removed and the mixture was stirred for 8 h at room temperature before water was added dropwise at 0 °C to quench the reaction. The mixture was then extracted with ethyl acetate. The organic layers were combined, washed with brine and then concentrated. The residue was purified by chromatography on silica gel (petroleum ether/ethyl acetate, 1/1) to give **14**. **14** was dissolved in HCl-methanol, and stirred at room temperature for 12 h. Then the reaction mixture was evaporated under reduced pressure at 35 °C to give the crude product. The crude product was purified by chromatography on silica gel (petroleum ether/ethyl acetate, 1/1) to give **15** as white solid.

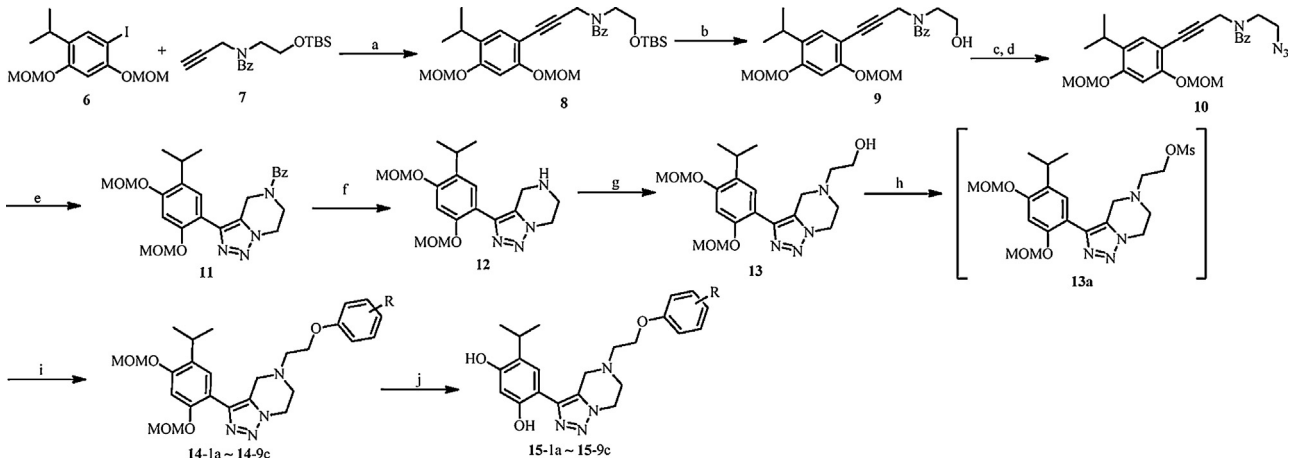
Characterization data from compound **15-1** to **15-9** can be found in Supporting information.

## 3. Results and discussion

The validated druggability of Hsp90 has provoked numerous attempts aiming at novel Hsp90 inhibitors, leading to the discovery of stockpiles of small molecules targeting Hsp90. Despite that the structures of these agents ranging from semisynthetic natural product derivatives to target based rational-designed compounds, chemical space within this scope still remains under-explored. Novel structures possessing elevated potency, reduced off-target toxicity and more favorable pharmacokinetic profiles are still of considerable interest. In this context, randomly discovered LD053 positioned us at a good start point for further investigations, and hence an extended series of compounds were designed out of this prototype molecule to tackle a general view of structure activity relationships.

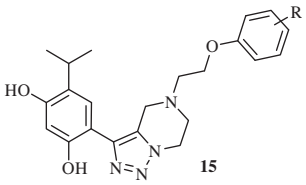
The molecule of LD053 (**5**, Fig. 1) virtually comprises three sections. We intended to retain the framework except that a more commonly adopted iso-propyl was used in place of the chlorine in section "A". The major variations, on the other hand, were made to section "C", into which a series of mono-substituted phenoxyls were to be introduced with the substituent varying from simple alkyls, halogens to more polar sulfonylsand the site of substitution including *othro-*, *meta-* and *para-*.

The designed molecules were synthesized as depicted in Scheme 1. Thus, Sonogashira coupling between 1-iodo-5-isopropyl-2,4-bis(methoxymethoxy)benzene **6** and *N*-propargyl benzamide **7** to give compound **8** [10]. The product was desilylated (**9**) and brominated. Upon treatment of the bromo intermediate with $NaN_3$ in DMF, the resultant azide **10** was heated at reflux in toluene to effect the intramolecular [3 + 2]




**Scheme 1.** The synthesis of LD053 analogues. Reagent and conditions: (a) $PdCl_2(PPh_3)_2$ (0.1 equiv.), CuI (0.2 equiv), $Et_3N$, 60 °C, 95%; (b) TBAF, THF, 40 °C, 85%; (c) $CBr_4$, $PPh_3$, DMF, 0 °C; (d) $NaN_3$, DMF, r.t., 66% for 2 steps; (e) toluene, reflux, 91%; (f) KOH (3 equiv.), MeOH/$H_2O$ (v/v 1/1), 88%; (g) $BrCH_2CH_2OH$, $K_2CO_3$, dry acetone, 50 °C in seal tube, 95%; (h) MsCl, $Et_3N$, 0 °C; (i) Selected phenols, NaH, DMF, 0 °C to r.t.; (j) HCl–MeOH (1 M), r.t., 65%–88% for 3 steps.

**Table 1**
Cancer cell growth inhibitory effects of compound **15**




**15**

| Entry | Compound | R | $IC_{50}$ (μmol/L) | | | | |
|---|---|---|---|---|---|---|---|
| | | | HCT116 | BGC823 | A375 | MCF7 | Mia-paca2 |
| 1 | **15-1a** | *o*-Me | >100 | >100 | >100 | >100 | >100 |
| 2 | **15-1b** | *m*-Me | >100 | >100 | 9.57 | >100 | >100 |
| 3 | **15-1c** | p-Me | 60.95 | >100 | 91.33 | >100 | >100 |
| 4 | **15-2a** | o-Et | >100 | >100 | >100 | >100 | >100 |
| 5 | **15-2b** | *m*-Et | 15.32 | >100 | 31.85 | 72.63 | 9.66 |
| 6 | **15-2c** | *p*-Et | >100 | >100 | >100 | >100 | >100 |
| 7 | **15-3a** | *o*-i-Pr | 26.53 | 13.99 | >100 | >100 | 88.22 |
| 8 | **15-3b** | *m*-i-Pr | 52.38 | >100 | >100 | >100 | >100 |
| 9 | **15-3c** | *p*-i-Pr | >100 | 47.05 | >100 | 7.37 | >100 |
| 10 | **15-4a** | *o*-Cl | >100 | >100 | >100 | >100 | >100 |
| 11 | **15-4b** | *m*-Cl | >100 | 4.66 | >100 | >100 | >100 |
| 12 | **15-4c** | *p*-Cl | >100 | >100 | >100 | >100 | >100 |
| 13 | **15-5a** | *o*-CN | >100 | >100 | >100 | >100 | >100 |
| 14 | **15-5b** | *m*-CN | **3.99** | **12.33** | **2.65** | **6.02** | **6.02** |
| 15 | **15-5c** | *p*-CN | **6.65** | **6.90** | **2.24** | **5.83** | **6.15** |
| 16 | **15-6a** | *o*-OMe | >100 | 8.63 | >100 | >100 | >100 |
| 17 | **15-6b** | *m*-OMe | >100 | >100 | >100 | >100 | >100 |
| 18 | **15-6c** | *p*-OMe | **10.22** | **4.15** | **2.29** | **5.58** | **6.74** |
| 19 | **15-7a** | *o*-SMe | 96.62 | >100 | >100 | >100 | >100 |
| 20 | **15-7b** | *m*-SMe | **7.24** | **28.71** | **4.15** | **6.60** | **3.47** |
| 21 | **15-7c** | p-SMe | >100 | >100 | >100 | >100 | 74.13 |
| 22 | **15-8a** | *o*-SOMe | 11.45 | >100 | 4.97 | 18.76 | 7.23 |
| 23 | **15-8b** | *m*-SOMe | **5.18** | **10.35** | **2.78** | **4.23** | **2.69** |
| 24 | **15-8c** | *p*-SOMe | **8.46** | **18.37** | **3.83** | **7.41** | **4.56** |
| 25 | **15-9a** | *o*-$SO_2$Me | >100 | 8.33 | 13.15 | >100 | >100 |
| 26 | **15-9b** | *m*-$SO_2$Me | >100 | >100 | >100 | >100 | >100 |
| 27 | **15-9c** | *p*-$SO_2$Me | **7.57** | **12.19** | **3.80** | **6.22** | **3.21** |
| 28 | AUY-922 | – | <0.1 | 0.57 | <0.1 | <0.1 | <0.1 |

cycloaddition, giving tetrahydro-[1,2,3]-triazolopyrazin adduct **11** in 91% yield. Removal of the *N*-benzoyl by using regular basic hydrolysis condition led to the isolation of **12**, which was heated with 2-bromoethanol and $K_2CO_3$ in acetone at 50 °C in a seal tube to give compound **12**. Alcohol **12** was mesylated and coupled with the selected phenols in the presence of sodium hydride in DMF to deliver compound **14-1a**–**14-9c**. These precursors were then stirred in methanolic hydrochloride to remove the MOM protection, giving the final products (**15-1a**–**15-9c**) mostly in forms of precipitated solids. Structures of these compounds were summarized in Table 1.

Compounds synthesized as described above were submitted to MTT assays [9] in five different cancer cell lines to determine their *in vitro* effects on cell growth (Table 1), and seven were found active (entries 14, 15, 18, 20, 23, 24 and 27) with the average $IC_{50}$s lower than 10 μmol/L.

It is noteworthy that simple aliphatic (chlorine as well) substituted compounds in this series generally exhibited low activities (entries 1–12, Table 1). This is also the case for the molecules carrying *othro*-substitution in section C (entries 13, 16, 19, 22 and 25), indicating substitution at this position is detrimental to the compounds' anticancer activity. For the active compounds, on the other hand, a polar substituent, either at *meta*- or *para*-position, seemed necessary; yet more interesting is that both electron donating and withdrawing groups contributed positively.

These selected compounds were further subjected to a polarized fluorescent assay using the known protocol [9] to determine their affinities toward hsp90. As it is showed in Table 2, all compounds appeared highly potent upon binding to purified hsp90, indicating that the anti-cancer activity of these molecules is associated with their capability of hsp90 inhibition. It is unfortunate that the high target affinity of our compounds was not fully reflected in the cellular level examinations, but the results still evidenced that the 4,5,6,7-tetrahydro-[1,2,3]triazolo[1,5-a]pyrazine substructure, or section B of the target molecules, may represent a new scaffold for the discovery of novel hsp90 inhibitors.

**Table 2**
Hsp90 affinity of the selected compounds.

| Compound | **15-5b** | **15-5c** | **15-6c** | **15-7b** | **15-8b** | **15-8c** | **15-9c** |
|---|---|---|---|---|---|---|---|
| R | *m*-CN | *p*-CN | *p*-OMe | *m*-SMe | *m*-SOMe | *p*-SOMe | *p*-$SO_2$Me |
| $IC_{50}$ (μmol/L) | 0.090 | 0.082 | 0.102 | 0.060 | 0.060 | 0.090 | 0.064 |

## 4. Conclusion

In summary, a library of analogues was designed and synthesized based on our randomly discovered prototype hsp90 inhibitor, LD053. *In vitro* anticancer activities were tested in a panel of cellular tumor models, and seven of the compounds were found active ($IC_{50}$ 2–10 μmol/L). The selected active compounds also exhibited notable binding affinities toward purified hsp90 ($IC_{50}$ 60–100 nmol/L). These results, in combination to our earlier findings, helped the recognition of the 4,5,6,7-tetrahydro-[1,2,3]triazolo[1,5-a]pyrazine motif as a new scaffold for hsp90 inhibitors. With the aid of the preliminary structure activity relationships illustrated in this study, further optimization of the molecule is ongoing.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2015.09.024.

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