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ortho-Carboranylphenoxyacetanilides as inhibitors of hypoxia-inducible factor (HIF)-1 transcriptional activity and heat shock protein (HSP) 60 chaperon activity

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ABSTRACT

ortho-Carboranylphenoxy derivatives were synthesized and evaluated for their ability to inhibit hypoxia-induced HIF-1 transcriptional activity using a cell-based reporter gene assay. Among the compounds synthesized, compound **1d** showed the most significant inhibition of hypoxia-induced HIF-1 transcriptional activity with the IC_{50} of 0.53 μM. Furthermore, compound **1h** was found to possess the most significant inhibition of heat shock protein (HSP) 60 chaperon activity among the reported inhibitors: the IC_{50} toward the porcine heart malate dehydrogenase (MDH) refolding assay was 0.35 μM.

Heat shock proteins (HSPs), ubiquitous and evolutionary conserved proteins in both prokaryotic and eukaryotic cells, constitute a heterogeneous group of molecules with a wide variety of functions, including assisting the correct folding of nascent polypeptides and the refolding of proteins that were partially denatured as a result of cell stress.[1] HSPs are classified into six families according to their molecular weights: HSP100, HSP90, HSP70, HSP60, HSP40, and small HSPs (15–30 kDa), with several members in each family.[2] HSP60 is a family of sequence-related proteins of ~60 kDa and typically reside in the mitochondria.[3] Hsp60 assembles into a tetradecamer that interacts with the co-chaperonin Hsp10 to assist client polypeptides to fold.[4] Although the primary function of HSP60 is assistance in mitochondrial protein folding, unfolding, and degradation,[5] the expression of HSP60 gene and HSP60 protein has been investigated in relation to disease progression, particularly tumor progression.[6–8] Therefore, HSP60 is used as a biomarker for the diagnosis and prognosis of preneoplastic and neoplastic lesions.[9–11]

Hypoxia-inducible factor (HIF)-1α is a key transcription factor that regulates cellular responses to physiological and pathological hypoxia.[12,13] Under the normoxic conditions, HIF-1α is rapidly degraded via a proteasome-dependent pathway; whereas in hypoxic environment, HIF-1α accumulates in the cytosol and gradually translocate into the nucleus to form a basic helix–loop–helix heterodimeric complex with a constitutively expressed HIF-1β. This HIF-1α/β dimer binds to the DNA of hypoxia response element (HRE) to activate hypoxia-sensitive genes, that encode proteins including cell immortalization, glucose and energy metabolism, vascularization, invasion and metastasis, and so on.[14] Therefore, HIF-1 signal targeting is one of the attractive approaches for development of antitumor agents.[15–17] We have developed various HIF-1 inhibitors, including YC-1 derivatives,[18] *ortho*-carboranylphenoxyacetanilides,[19,20] diaryl-*ortho*-carboranes,[21,22] and indenopyrazoles.[23] We recently identified HSP60 as a primarily target of *ortho*-carboranylphenoxyacetanilide (GN26361), a HIF-1α inhibitor (Fig. 1).[24] We clarified that HSP60 plays an important role for stabilization of HIF-1α protein accumulation under hypoxia and that inhibition of the HSP60 chaperon activity by GN26361 induces the degradation of HIF-1α protein through oxygen-independent pathway. In this Letter, we studied the further structure–activity relationship based on the *ortho*-carboranylphenoxyacetanilides to develop potent HIF-1α and HSP60 inhibitors.

Chemistry: We first synthesized 3-(*ortho*-carboranyl)phenols from 3-iodoanisoles **2** through decaborane coupling with terminal alkynes as shown in Scheme 1. Sonogashira coupling of 3-iodoanisole **2** with ethynyltrimethylsilane proceeded in the presence of $PdCl_2(PPh_3)_2$ catalysts in THF and the resulting alkyne **3** were treated with tetrabutylammonium fluoride (TBAF) to remove trimethylsilyl (TMS) group, giving 3-ethynylanisole **4**. Decaborane coupling of **4**

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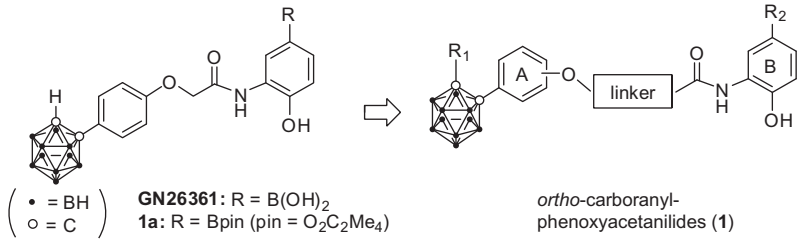
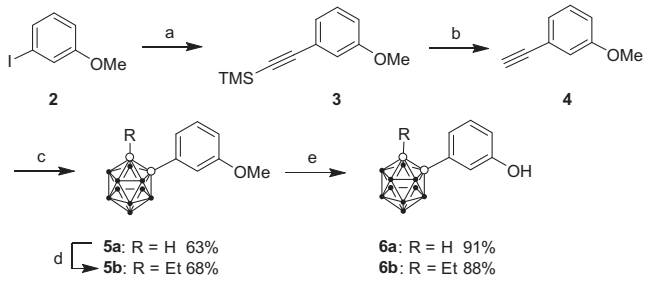
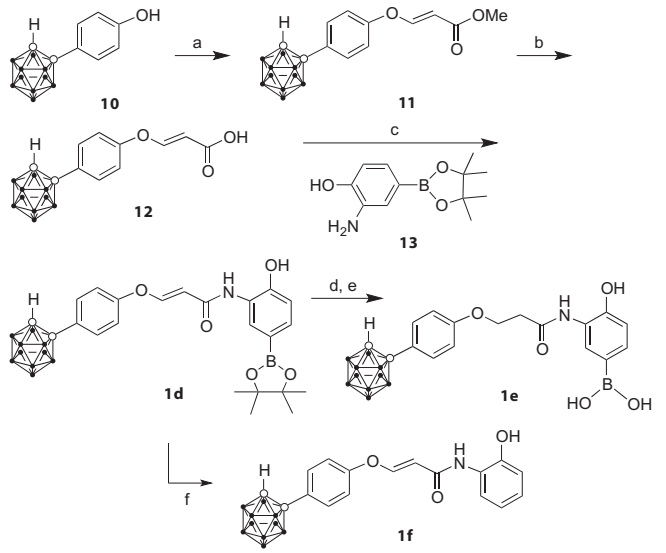


Figure 1. Structure–activity relationship study based on GN26361.



Scheme 1. Reagents and conditions: (a) ethynyltrimethylsilane, $PdCl_2(PPh_3)_2$, PPh_3, CuI, diethylamine, THF, reflux, 5 h; (b) TBAF, THF, rt, 30 min; (c) $B_{10}H_{14}$, *N*,*N*-dimethylaniline, chlorobenzene, microwave, 130 °C, 10 min; (d) (i) *n*-BuLi, THF, −10 °C, 30 min, (ii) EtI, 1.5 h; (e) BBr_3, CH_2Cl_2, rt, overnight.

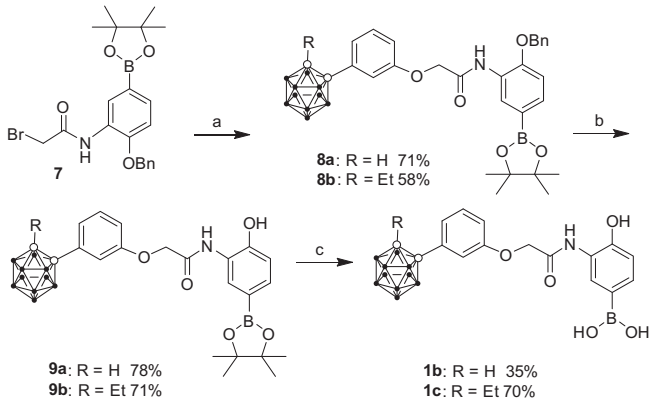


Scheme 3. Reagents and conditions: (a) methyl propiolate, DABCO, CH_2Cl_2, rt, overnight, 80%; (b) LiOH, THF–H_2O, rt, overnight, 60%; (c) (i) oxalyl chloride, CH_2Cl_2, 0 °C, 2 h, (ii) **13**, triisobutylamine, THF, rt, 2 h, 28%; (d) (i) KHF_2 (4 M), MeOH, rt, 30 min, (ii) HCl (1 N), 1 h, overnight; (e) H_2, Pd/C, MeOH–THF, rt, overnight; (f) (i) KHF_2 (4 M), MeOH, rt, 2 h, (ii) HCl (1 N), rt, overnight, 64%.

was carried out in the presence of *N*,*N*-dimethylaniline in chlorobenzene under microwave conditions to give the 3-(*ortho*-carboranyl)anisole **5a** in 63% yield. The treatment of **5a** with *n*-butyllithium generated lithiated *ortho*-carboranyl intermediate, which reacted with ethyl iodide to give **5b** in 68% yield. The methoxy groups of **5a** and **5b** were deprotected by using BBr_3 to afford 3-(*ortho*-carboranyl)phenols **6a** and **6b** in 91% and 88% yield, respectively.

Bromoacetylanilide **7** reacted with 3-(*ortho*-carboranyl)phenols **6a** and **6b** under the basic condition and the resulting 3-(*ortho*-carboranyl)phenoxyacetanilides **8a** and **8b** were hydrogenated using Pd/C under hydrogen atmosphere. Finally the pinacol ester of **9a** and **9b** were hydrolyzed to give **1b** and **1c** as *meta*-derivatives of GN26361 (see scheme 2).

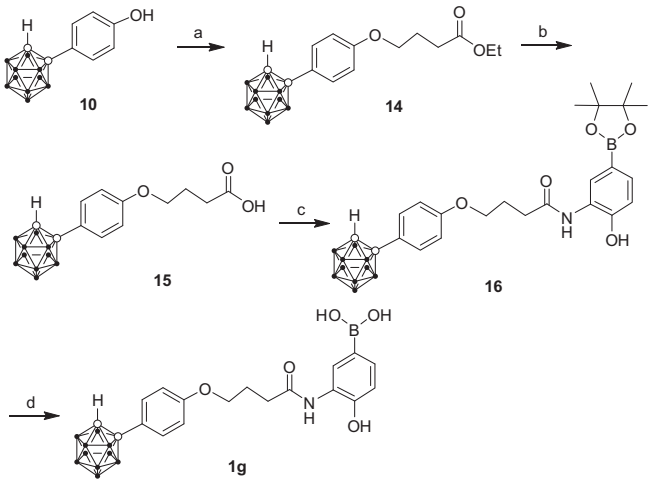
We next synthesized *ortho*-carboranylphenoxy derivatives with an ethylene linker (Scheme 3).[25] 4-(*ortho*-Carboranyl)phenol **10** was treated with methyl propiolate using 1,4-diazabicyclo[2.2.2]octane (DABCO) as a base to give the conjugated methyl ester **11**,[26] which was hydrolyzed with LiOH in aqueous THF solution. The resulting carboxylic acid **12** was converted to the acid chloride, which reacted with **13** to give the corresponding pinacolatoboron ester **1d**. Deprotection of pinacolatoboron ester followed by hydrogenesis gave 3-(*ortho*-carboranyl)phenoxypropananilide **1e**. Interestingly, deboronated product **1f** was obtained when pinacolatoboron ester **1d** treated with KHF_2 was reacted with HCl for overnight.



Scheme 2. Reagents and conditions: (a) **6a** or **6b**, K_2CO_3, DMF, rt, overnight; (b) H_2, Pd/C, MeOH–THF, rt, overnight; (c) (i) KHF_2 (4 M), MeOH, rt, 2 h, (ii) HCl (1 N), rt, overnight.

ortho-Carboranylphenoxy derivative with an propylene linker was also synthesized as shown in Scheme 4. 4-(*ortho*-Carboranyl)phenol **10** was treated with ethyl 4-bromobutyrate using K_2CO_3 as a base and the resulting ethyl ester **14** was hydrolyzed to afford the corresponding carboxylic acid **15**. The amide bond formation of **15** with aniline **13** was carried out using 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium-3-oxidhexafluorophosphate (HATU) as a condensation reagent and the resulting pinacolatoboron ester **16** was treated with KHF_2 followed by HCl to give 3-(*ortho*-carboranyl)phenoxybutananilide **1g**.

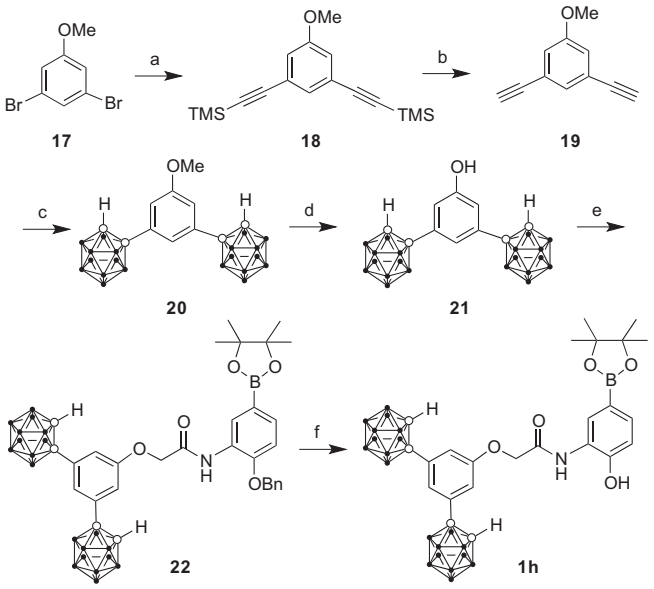
In our previous observation, carborane framework was essential for inhibition of HSP60 chaperon activity,[20] therefore, we synthesized 3,5-(di-*ortho*-carboranyl)phenoxyacetanilide derivative as shown in Scheme 5. Sonogashira coupling of 3,5-dibromoanisole **17** with ethynyltrimethylsilane afforded **18**, which was treated under basic conditions to remove trimethylsilyl (TMS) group, giving 3,5-diethynylanisole **19**. Decaborane coupling of **19** proceeded under the microwave conditions at 130 °C and the resulting anisole



Scheme 4. Reagents and conditions: (a) ethyl 4-bromobutyrate, K_2CO_3, DMF, rt, overnight, 57%; (b) LiOH, THF–H_2O, rt, overnight, 93%; (c) **13**, HATU, DIPEA, DMF, rt, overnight, 48%; (d) (i) KHF_2 (4 M), MeOH, rt, 2 h, (ii) HCl (1 N), rt, overnight, 58%.

20 was converted to the corresponding phenol **21** by treated with BBr_3. Alkylation of **21** with **7** was carried out using NaH as a base and hydrogenesis of the resulting **22** afforded 3,5-(di-*ortho*-carboranyl)phenoxyacetanilide derivative **1h** in 54% yield.

Biological activity: Synthesized substituted *ortho*-carboranylphenoxyacetanilide derivatives **1a**–**h** were evaluated for their ability to inhibit hypoxia-induced HIF-1 transcriptional activity using a cell-based reporter assay in HeLa cells expressing HRE-dependent firefly luciferase reporter construct (HRE-Luc) and constitutively expressing CMV-driven Renilla luciferase reporter. Cell growth inhibition (GI_{50}) by the synthesized compounds was also determined by MTT assay. GN26361 was used as a positive control for comparison. The results are summarized in Table 1. The IC_{50} of GN26361 toward HIF-1 transcriptional activity was 2.2 ± 0.2 μM, whereas the pinacol ester **1a** was more potent than GN26361 (IC_{50} = 1.3 ± 0.1 μM). The higher inhibitory activity of **1a** toward HIF-1 transcription



Scheme 5. Reagents and conditions: (a) ethynyltrimethylsilane, $PdCl_2(PPh_3)_2$, PPh_3, CuI, diethylamine, THF, reflux, 5 h, quant.; (b) KOH, MeOH, rt, 6 h, 78%; (c) $B_{10}H_{14}$, *N*,*N*-dimethylaniline, chlorobenzene, microwave, 130 °C, 37%; (d) BBr_3, CH_2Cl_2, rt, overnight, 100%; (e) **7**, NaH, THF, rt, 2 h, 72%; (f) H_2, Pd/C, MeOH–THF, rt, overnight, 54%.

Table 1
Inhibition of HIF-1 transcriptional activity in HeLa cell-based HRE and CMV dual luciferase assay and cell growth inhibition

Compound	IC_{50}[a] (μM)	GI_{50}[b] (μM)
GN26361	2.2 ± 0.2	13.5 ± 0.6
1a	1.3 ± 0.1	6.9 ± 0.5
1b	4.2 ± 0.8	30
1c	1.1 ± 0.3	5.2 ± 0.8
1d	0.53 ± 0.07	5.3 ± 5.2
1e	2.4 ± 0.6	8.7 ± 0.7
1f	1.3 ± 0.2	7.7 ± 2.3
1g	14.6 ± 2.7	23.6 ± 0.6
1h	16.4 ± 0.6	15.6 ± 1.3

[a] HeLa cells expressing HRE-dependent firefly luciferase reporter construct (HRE-Luc) and constitutively expressing CMV-driven Renilla luciferase reporter with SureFECT Transfection Reagent were established with Cignal™ Lenti Reporter (SABiosciences, Frederick, MD) according to the manufacturer's instructions. The consensus sequence of HRE was 5′-TACGTGCT-3′ from the erythropoietin gene. Cells stably expressing the HRE-reporter gene were selected with puromycin. The cells were incubated for 12 h with or without drugs under the normoxic or hypoxic condition. After removal of the supernatant, the luciferase assay was performed using a Luciferase Assay System (Promega, Madison, WI) according to the manufacturer's instructions. The drug concentration required to inhibit the relative light units by 50% (IC_{50}) was determined from semi-logarithmic dose–response plots, and the results represent means ± SD of triplicate samples.

[b] HeLa cells were incubated for 72 h with various concentrations (100 nM–100 μM) of compounds under the normoxic condition, and viable cells were tested by the MTT assay. The drug concentration required to inhibit cell growth by 50% (GI_{50}) was determined from semi-logarithmic dose–response plots, and results represent means ± SD of triplicate samples.

compared with GN26361 is probably due to the lipophilicity of the pinacol ester functional group, which would affect the transmembrane property. The *meta* derivative **1b** of GN26361 decreased the inhibition of HIF-1 transcriptional activity with IC_{50} of 4.2 ± 0.8 μM. Previously, we demonstrated the effects of substituents at *ortho*-carborane (R^1) in GN26361 on HIF-1 transcriptional activity and found that ethyl group was the most suitable among other substituted derivatives (H, Me, and *i*-Bu).[20] In the case of the *meta* derivatives, the ethyl substituent (**1c**) increased the inhibition of HIF-1 transcriptional activity with IC_{50} of 1.1 ± 0.3 μM. The IC_{50} of compound **1e** having an ethylene linker was slightly decreased to 2.4 ± 0.6 μM, whereas that of compound **1g** having a propylene linker dropped to 14.6 ± 2.7 μM, indicating that the length such as methylene and ethylene linkers is appropriate for the potency of HIF-1 inhibition. The highest inhibition was achieved by compound **1d** with the IC_{50} of 0.53 ± 0.07 μM, suggesting that the rigid conformation by introducing an unsaturated ethyne linker in the molecule is attributed to the potency of HIF-1 inhibition. Interestingly, deboronated derivative **1f** also inhibited HIF-1 transcriptional activity with IC_{50} of 1.3 ± 0.2 μM. However, 3,5-(di-*ortho*-carboranyl) substituent was not effective; the IC_{50} of compounds **1h** dropped to 16.4 ± 0.6 μM. Although the cytotoxicity of the compounds was excluded from the HIF-1 transcriptional activity by correcting the measurement of constitutively expressing CMV-driven Renilla luciferase activity, cell growth inhibition by the compounds (GI_{50}) was related to the inhibition of the hypoxia-induced HIF-1 transcriptional activity except compound **1b**.

We next examined the effects of compounds **1c**, **1d**, **1f**, which showed significant inhibition of HIF-1 transcriptional activity, and di-carboranyl compound **1h**, on human HSP60 chaperone activity using the porcine heart malate dehydrogenase (MDH) refolding assay.[27,28] GN26361 was used as a positive control for comparison. The results are summarized in Figure 2. Inhibition ratio of each compound at 2 μM concentration against HSP60 chaperon activity is indicated by vertical axis. Although epolactaene *tert*-butyl ester (ETB)[27] exhibited significant inhibition (~50%) at 6 μM, a weak inhibition was observed at 2 μM. Moderate inhibition was observed in the cases of compounds **1c** and **1d**, whereas inhibition ratio of

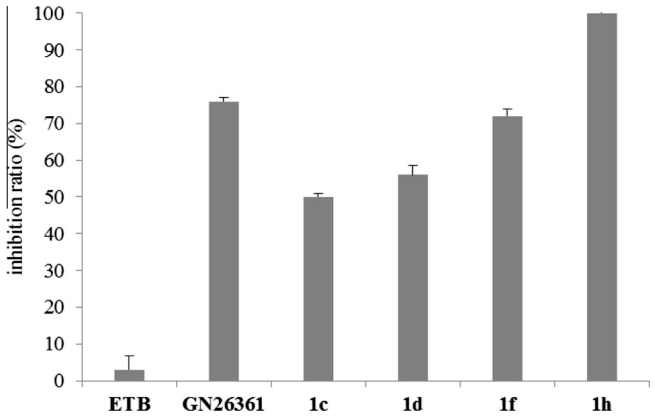


Figure 2. Hsp60 chaperone activity was analyzed by using MDH as substrate. Porcine MDH was denatured in 10 mM HCl at rt for 2 h. HSP60 (4 μM) and human HSP10 (8 μM) were pre-incubated for 90 min at 30 °C in a buffer (50 mM Tris/HCl, pH 7.6, 300 mM NaCl, 20 mM KCl, 20 mM $Mg(OAc)_2$, and 4 mM ATP). Denatured MDH was diluted to a concentration of 0.15 μM with a buffer (0.1 M Tris/HCl, pH 7.6, 7 mM KCl, 7 mM $MgCl_2$, 1 mM DTT, and 2 mM ATP) containing HSP60/10 and each concentrated compound at 30 °C (final concentration of HSP60: 0.2 μM, HSP10: 0.4 μM, compound: 2 μM). After the reaction mixture was treated for 30 min, the reactivation of MDH by the HSP60/10 chaperon activity was determined by measuring the absorbance of NADH at 340 nm, which was converted into NAD^+ by the refolded MDH with absorption reduction. All compounds were confirmed to have no inhibitory activity of MDH at 2 μM.[25]

compound **1f** was similar to that of GN26361. The best result was observed in the case of compound **1h**; 100% inhibition was observed at 2 μM, indicating that the sterically bulky bis-*ortho*-carborane moiety is more effective on inhibition of the HSP60 chaperon activity than the mono-*ortho*-carborane moiety. Therefore the dose-dependent inhibition of HSP60 chaperon activity by compound **1h** was next examined. As shown in Figure 3, compound **1h** inhibited ~70% of HSP60 chaperon activity at 0.6 μM concentration and the IC_{50} was calculated to be 0.35 ± 0.08 μM. So far, compound **1h** is the highest inhibitor of HSP60 chaperon activity among the reported four compounds, ETB,[28] mizoribine,[29] EC3016,[30] and GN26361.[24]

In conclusion, we developed inhibitors[31] of HIF transcriptional activity and HSP60 chaperon activity based on GN26361 as a lead compound. Among the compounds synthesized, 3-(*ortho*-carboranyl)phenoxypropenanilide **1d** suppressed HIF-1 transcriptional activity with the IC_{50} of 0.53 μM, although the inhibition of HSP60 chaperone activity was not as high as that of GN26361. In contrast, the inhibition of HIF-1 transcriptional activity of 3,5-(di-*ortho*-carboranyl)phenoxyacetanilide **1h** with IC_{50} of 16.4 ± 0.6 μM;

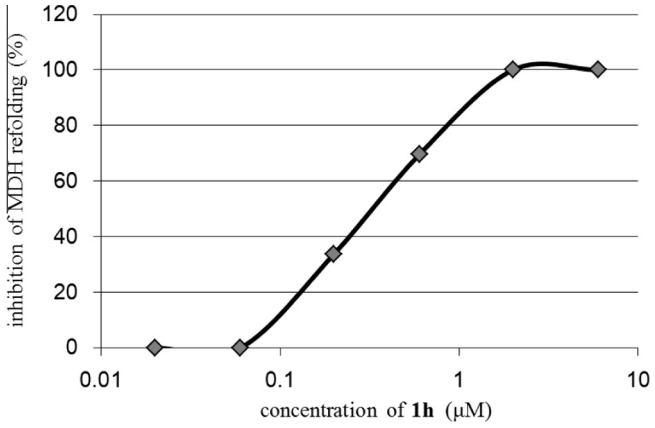


Figure 3. Dose-dependent inhibition of human Hsp60 chaperone activity by compound **1h**. IC_{50} value was calculated to be 0.35 ± 0.08 μM.

however that inhibited HSP60 chaperone activity with the IC_{50} of 0.35 μM. It is known that the stability and activity of HIF-1α are regulated not only by HSP60 but also by HSP90,[32] which is main chaperon of HIF-1α. Therefore HIF-1 inhibition may be involved to some degree in HSP60 inhibition and each inhibitory effect of the compounds might be attributed to different mechanism respectively. So far, compound **1h** possesses the highest inhibitory activity among compounds reported. Since the expression of HSP60 in relation to disease progression, particularly tumor progression, has been investigated, we believe that the current findings of HSP60-binding small molecules would contribute as a tool for investigation of further unknown HSP60 functions and HSP60-related diseases.

Acknowledgments

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References and notes

1. Heilman-Miller, S. L.; Pan, J.; Thirumalai, D.; Woodson, S. A. *J. Mol. Biol.* **2001**, *309*, 57.
2. Schlesinger, M. J. *J. Biol. Chem.* **1990**, *265*, 12111.
3. Jindal, S.; Dudani, A. K.; Singh, B.; Harley, C. B.; Gupta, R. S. *Mol. Cell. Biol.* **1989**, *9*, 2279.
4. Bukau, B.; Horwich, A. L. *Cell* **1998**, *92*, 351.
5. Hansen, J. J.; Bross, P.; Westergaard, M.; Nielsen, M. N.; Eiberg, H.; Borglum, A. D.; Mogensen, J.; Kristiansen, K.; Bolund, L.; Gregersen, N. *Hum. Genet.* **2003**, *112*, 71.
6. Cappello, F. J. *Cancer Biol. Ther.* **2008**, *7*, 801.
7. Bajramovic, J. J.; Geutskens, S. B.; Bsibsi, M.; Boot, M.; Hassankhan, R.; Verhulst, K. C.; van Noort, J. M. *Cell Stress Chaperones* **2000**, *5*, 30.
8. Khalil, A. A. *Cancer Sci.* **2007**, *98*, 201.
9. Ciocca, D. R.; Calderwood, S. K. *Cell Stress Chaperones* **2005**, *10*, 86.
10. Nakamura, H.; Minegishi, H. *Curr. Pharm. Des.* **2013**, *19*, 441.
11. Cappello, F. J. *Expert Opin. Ther. Targets* **2014**, *18*, 185.
12. Semenza, G. L. *Nat. Rev. Cancer* **2003**, *3*, 721.
13. Ke, Q.; Costa, M. *Mol. Pharmacol.* **2006**, *70*, 1469.
14. Semenza, G. L. *Curr. Opin. Genet. Dev.* **2010**, *20*, 51.
15. Semenza, G. L. *Oncogene* **2010**, *29*, 625.
16. Wu, D.; Zhang, R.; Zhao, R.; Chen, G.; Cai, Y.; Jin, J. *PLoS One* **2013**, *8*, e62014.
17. Ban, H. S.; Uto, Y.; Nakamura, H. *Expert Opin. Ther. Pat.* **2011**, *21*, 131.
18. Takeuchi, A.; Hori, M.; Sato, S.; Ban, H. S.; Kuchimaru, T.; Kizaka-Kondoh, S.; Yamori, T.; Nakamura, H. *MedChemComm* **2012**, *3*, 1455.
19. Shimizu, K.; Maruyama, M.; Yasui, Y.; Minegishi, H.; Ban, H. S.; Nakamura, H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1453.
20. Nakamura, H.; Yasui, Y.; Maruyama, M.; Minegishi, H.; Ban, H. S.; Sato, S. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 806.
21. Minegishi, H.; Matsukawa, T.; Nakamura, H. *ChemMedChem* **2013**, *8*, 265.
22. Nakamura, H.; Tasaki, L.; Kanoh, D.; Sato, S.; Ban, H. S. *Dalton Trans.* **2014**, *43*, 4941.
23. Minegishi, H.; Fukashiro, S.; Ban, H. S.; Nakamura, H. *ACS Med. Chem. Lett.* **2013**, *4*, 297.
24. Ban, H. S.; Shimizu, K.; Minegishi, H.; Nakamura, H. *J. Am. Chem. Soc.* **2010**, *132*, 11870.
25. Nail, R.; Won, M.; Kim, B. K.; Xia, Y.; Choi, H. K.; Jin, G.; Jung, Y.; Kim, H. M.; Lee, K. *J. Med. Chem.* **2012**, *55*, 10564.
26. Cong, Z.-Q.; Nishino, H. *Synthesis* **2008**, 2686.
27. Nielsen, K. L.; Cowan, N. J. *Mol. Cell* **1998**, *2*, 93.
28. Nagumo, Y.; Kakeya, H.; Shoji, M.; Hayashi, Y.; Dohmae, N.; Osada, H. *Biochem. J.* **2005**, *387*, 835.
29. Itoh, H.; Komatsuda, A.; Wakui, H.; Miura, A. B.; Tashima, Y. *J. Biol. Chem.* **1999**, *274*, 35147.
30. Chapman, E.; Farr, G. W.; Furtak, K.; Horwich, A. L. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 811.
31. *Spectral data for representative compounds:* 3-[(3-(1-Ethyl-1,2-dicarba-*closo*-carboranyl)phenoxyacetylamino)]-4-hydroxy benzene boronic acid (**1c**): ^1H NMR (500 MHz; CD_3OD): δ 8.23–8.30 (m, 1H), 7.31–7.43 (m, 4H), 7.20 (dd, *J* = 7.0 Hz, 1.5 Hz, 1H), 6.81–6.87 (m, 1H), 4.85 (s, 2H), 1.87 (q, *J* = 7.5 Hz, 2H), 1.00 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 168.6, 159.1, 150.6, 133.5, 132.9, 131.5, 128.5, 125.9, 125.8, 119.3, 118.2, 115.6, 85.0, 84.6, 68.8, 29.7, 14.1. IR (NaCl) 3382, 3285, 2925, 2856, 2588, 2359, 1779, 1604, 1431, 1339, 1200, 757 cm^{-1}; HRMS (ESI) *m/z* Calcd for $C_{18}H_{28}B_{11}NO_5Na$ $[M+Na]^+$: 480.2966. Found: 480.2965. HPLC purity (CH_2Cl_2/MeOH = 20:1): 98.2% (254 nm), retention time: 7.56 min. (*E*)-3-[(4-(1,2-Dicarba-*closo*-carboranyl)-phenoxyacryloylamino]-4-hydroxybenzene boronic acid pinacol ester (**1d**): ^1H NMR (500 MHz; $CDCl_3$): δ 9.92 (s, 1H), 7.87 (d, *J* = 9.0 Hz, 2H), 7.57 (dd,

J = 8.0 Hz, 1.5 Hz, 1H), 7.53 (d, *J* = 9.0 Hz, 2H), 7.39 (s, 1H), 7.52 (d, *J* = 1.5 Hz, 2H), 7.05 (d, *J* = 9.0 Hz, 2H), 7.02 (d, *J* = 8.0 Hz, 2H), 5.80 (d, *J* = 11.5 Hz, 2H), 3.93 (s, 1H), 1.32 (s, 12H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 165.7, 157.5, 156.8, 152.0, 134.2, 130.0, 129.7, 129.0, 125.0, 119.8, 117.8, 104.0, 83.8, 75.6, 60.5, 24.8. IR (NaCl) 2978, 2924, 2852, 2598, 2359, 2345, 1681, 1544 cm^{-1}; HRMS (ESI) *m*/*z* Calcd for $C_{23}H_{34}B_{11}NO_5Na$ $[M+Na]^+$: 546.3435. Found: 546.3438. HPLC purity (CH_2Cl_2/MeOH = 20:1): 100.0% (254 nm), retention time: 7.10 min. 3-[(4-(1,2-Dicarba-closo-carboranyl)phenoxypropanamido)]-4-hydroxybenzene boronic acid (**1e**): ^{1}H NMR (500 MHz; CD_3OD): δ 7.99 (s, 1H), 7.46 (d, *J* = 9.0 Hz, 2H), 7.31 (dd, *J* = 8.0 Hz, 1.5 Hz, 1H), 6.85 (d, *J* = 9.0 Hz,1H), 4.95 (s, 1H), 4.33 (t, *J* = 6.0 Hz, 2H), 2.89 (t, *J* = 6.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 171.9, 161.2, 151.6, 133.0, 130.1, 129.9, 127.5, 126.3, 116.4, 115.7, 115.6, 78.4, 65.5, 62.5, 62.4, 37.6. IR (NaCl) 3309, 2930, 2594, 1540, 1512, 1338, 1254, 1186, 755 cm^{-1}; HRMS (ESI) *m*/*z* Calcd for $C_{17}H_{27}B_{11}NO_5$ $[M+H]^+$: 444.2990. Found: 444.2992. HPLC purity (AcOEt): 95.6% (254 nm), retention time: 4.80 min. 3-[(4-(1,2-Dicarba-closo-carboranyl)phenoxybutanamido)]-4-hydroxybenzene boronic acid (**1g**): ^{1}H NMR (500 MHz; CD_3OD): δ 7.88 (s, 1H), 7.31–7.45 (m, 3H), 6.81–6.88 (m, 3H), 4.92 (s, 1H), 4.07 (t, *J* = 6.0 Hz, 2H), 2.61 (t, *J* = 7.0 Hz, 2H), 2.13–2.18 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 174.4, 161.5, 151.9, 133.2, 130.5, 130.1, 127.1, 126.3, 116.8, 115.6. IR (NaCl) 3296, 3065, 2935, 2596, 1513, 1338, 1256 cm^{-1}; HRMS (ESI) *m*/*z* Calcd for $C_{18}H_{28}B_{11}NO_5Na$ $[M+Na]^+$: 480.2966. Found: 480.2964. HPLC purity (AcOEt): 95.5% (254 nm), retention time: 4.83 min. 3-[(3,5-Di(1,2-dicarba-*closo*-carboranyl) phenoxyacetylamino]-4-hydroxy benzene boronic acid pinacol ester (**1h**): ^{1}H NMR (500 MHz; $CDCl_3$): δ 9.12 (s, 1H), 8.38 (s, 1H), 7.66 (dd, *J* = 10.0 Hz, 1.5 Hz, 1H), 7.46 (d, *J* = 1.5 Hz, 1H), 7.35 (t, *J* = 2.0 Hz, 1H), 7.17 (d, *J* = 2.5 Hz, 2H), 7.08 (d, *J* = 10.0 Hz, 1H), 4.69 (s, 2H), 3.96 (s, 2H), 1.36 (s, 12H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 166.1, 156.6, 151.8, 136.3, 135.0, 128.9, 123.7, 121.5, 120.0, 115.8, 84.0, 74.4, 67.1, 60.0, 24.9. IR (NaCl) 2979, 2924, 2596, 2359, 2341, 1670, 1594, 1506, 1360, 1241, 1141, 1116 cm^{-1}; HRMS (ESI) *m*/*z* Calcd for $C_{24}H_{45}B_{21}NO_5$ $[M+H]^+$: 654.5407. Found: 654.5409. HPLC purity (CH_2Cl_2/MeOH = 20:1): 99.7% (254 nm), retention time: 5.28 min.

32. Liu, Y. V.; Baek, J. H.; Zhang, H.; Diez, R.; Cole, R. N.; Semenza, G. L. *Mol. Cell* **2007**, *25*, 207.