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RESEARCH ARTICLE

Discovery of 2′,4′-dimethoxychalcone as a Hsp90 inhibitor and its effect on iressa-resistant non-small cell lung cancer (NSCLC)

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Abstract Heat shock protein 90 (Hsp90) is a ATP dependent molecular chaperone and has emerged as an attractive therapeutic target in the war on cancer due to its role in regulating maturation and stabilization of numerous oncogenic proteins. In this study, we discovered that 2′,4′-dimethoxychalcone (**1b**) disrupted Hsp90 chaperoning function and inhibited the growth of iressa-resistant non-small cell lung cancer (NSCLC, H1975). The result suggested that 2′,4′-dimethoxychalcone (**1b**) could serve as a potential therapeutic lead to circumvent the drug resistance acquired by EGFR mutation and Met amplification.

Keywords 2′,4′-dimethoxychalcone · Hsp90 · Inhibitor · Iressa-resistance · Lung cancer

Introduction

Over the last two decades, numerous cancer drugs designed to hit a single biological target have been discovered as "targeted cancer drugs" to specifically kill cancerous cells while leaving healthy cells unharmed (Aggarwal 2010). Despite the robust efficacy of the targeted drugs, the 'one target, one disease' approach is being challenged by the occurrence of drug resistance. Cancer cells are intrinsically heterogeneous and tend to result from multiple molecular abnormalities (Petrelli and Giordano 2008; Boran and Iyengar 2010). Therefore, pinpointing a single target is unlikely to eradicate cancer cells, which often find ways to compensate for the blockage of the protein by amplifying alternative signals or mutating targeted proteins. Therefore, to elucidate a drug simultaneously to attack multiple targets has been emerged as a new paradigm to overcome the drug resistance in cancer research. Alternatively, to find a single protein that integrates multiple signaling pathways and disable its function might be best suited to overcome the genetic and molecular heterogeneity of cancers.

In this regard, heat shock protein 90 (Hsp90) represents an attractive cancer therapeutic target (Mahalingam et al. 2009; Whitesell and Lindquist 2005). Hsp90 is an abundant and ubiquitous ATP dependent molecular chaperone that plays an important role in regulating maturation and stabilization of numerous oncogenic proteins including EGFR, Met, Her2, Akt, Cdk4, Hif-1, and MMP2 (Mahalingam et al. 2009). When cancer cells are exposed to environmental insults such as poor nutrition, hypoxia and acidosis, the cells dramatically increased the production of chaperone protein Hsp90 that is an adaptive response to various proteotoxic stressors (Whitesell and Lindquist 2005). Accordingly, Hsp90 is constitutively expressed at 2–10 fold higher levels in cancer cells than their normal counterparts and Hsp90 inhibitors display selective anti-proliferative effects on cancer cells as compared to normal cells (Solit and Chiosis 2008; Chiosis and Neckers 2006).

Historically, natural products provided ample source of chemical diversity for drug discovery and to discover new drugs from natural products proved to be the most successful strategy (Li and Vederas 2009). Hence, several natural products, such as geldanamycin (GA, **3**), radicicol (**4**), novobiocin (**5**), and derrubone (**6**) have been reported to target Hsp90 (Fig. 1) (Whitesell et al. 1994; Hadden et al. 2007; Marcu et al. 2000; Sharma et al. 1998).

Flavokawain B is one of chalcones extracted from Kava (*Piper methylsticum*), that is an native plant traditionally

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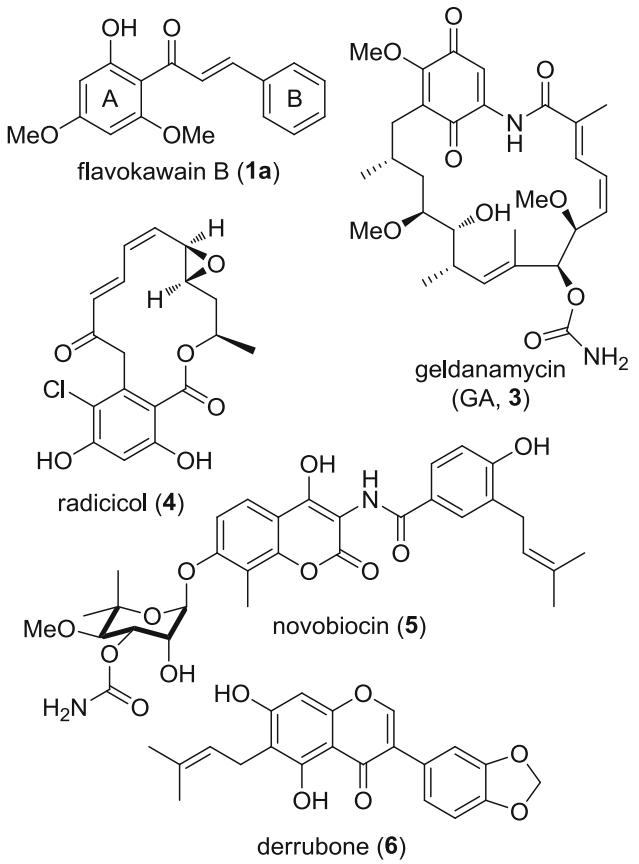


Fig. 1 Structure of natural product Hsp90 inhibitors

used as a medicine and social drink in the South Pacific islands. Interestingly, epidemiological studies illustrated that populations that consume the kava beverage have low incidences of cancer, compared to non-kava-drinking populations (Steiner 2000; Agarwal and Deep 2008). Extracts of kava are classified into two main classes of compounds kavalactone and chalcone, and flavokawain B belongs to chalcone compounds (Warmka et al. 2012; Zhao et al. 2011).

Chalcones are abundant natural products in edible plants such as green tea and display a wide spectrum of biological activities including anti-cancer, anti-inflammation, and anti-microbial activities (Batovska and Todorova 2010; Dimmock et al. 1999). Therefore, chalcones are considered as an important class of molecules and speculated as promising candidates as anticancer agents.

As part of our ongoing efforts to discover Hsp90 inhibitors, we have found that a natural product, flaovokawain B (**1a**) disrupts Hsp90 chaperoning function and impairs the growth of cancer cells (Seo and Oh 2013). These findings prompted us to direct our efforts toward modifying A-ring of flavokawain B in this study, while the modifications of B-ring was not fruitful to improve the potency of flavokawain B in our previous study (Seo and Park 2014). Herein, we describe the modifications of flavokawain B on A-ring and their comparative biological activities against iressa-resistant non-small cell lung cancers (H1975).

Materials and methods

Unless otherwise noted, all reactions were performed under an argon atmosphere in oven-dried glassware. All purchased materials were used without further purification. Thin layer chromatography (TLC) was carried out using Merck silica gel 60 F_{254} plates. TLC plates were visualized using a combination of UV, p-anisaldehyde, ceric ammonium molybdate, ninhydrin, and potassium permanganate staining. NMR spectra were obtained on a Bruker 400 (400 MHz for ^{1}H; 100 MHz for ^{13}C) spectrometer. ^{1}H and ^{13}C NMR chemical shifts are reported in parts per million (ppm) relative to TMS, with the residual solvent peak used as an internal reference. Signals are reported as m (multiplet), s (singlet), d (doublet), t (triplet), q (quartet), bs (broad singlet), bd (broad doublet), dd (doublet of doublets), dt (doublet of triplets), or dq (doublet of quartets); the coupling constants are reported in hertz (Hz). Final products were purified by MPLC (Biotage Isolera One instrument) on a silica column (Biotage SNAP HP-Sil). On the basis of NMR and analytical HPLC data (Shimadzu prominence, VP-ODS C18 column), purity for all the tested compounds was found to be >95 %.

General procedure for preparing compounds (1a-b, and 1d), as exemplified for compound 1a

A mixture of acetophone **2a** (0.30 g, 1.5 mmol), benzaldehyde (0.18 g, 1.7 mmol), KOH (0.19 g, 3.4 mmol) in 5 mL of methanol was stirred at rt for 20 h. The mixture was neutralized with 3 N HCl to pH 6 and then extracted with ethyl acetate. The organic layer was dried over Na_2SO_4, and concentrated under reduced pressure, and purified by column chromatography (10 % ethyl acetate in hexane) to afford compound 1 in 50 % as yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, $J = 15.6$ Hz, 1H), 7.77 (d, $J = 15.6$ Hz, 1H), 7.61–7.57 (m, 2H), 7.42–7.36 (m, 3H), 6.09 (d, $J = 2.4$ Hz, 1H), 5.95 (d, $J = 2.4$ Hz, 1H), 3.90 (s, 3H), 3.82 (s, 3H). ESI MS (m/e) = 285 $[M+1]^+$.

Compound 1b

$R_f = 0.22$ (1:9 ethyl acetate: hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, $J = 8.8$ Hz, 1H), 7.68 (d, $J = 15.6$ Hz, 1H), 7.58 (dd, $J = 7.6$ Hz, 2.4 Hz, 2H), 7.52 (d, $J = 16.0$ Hz, 1H), 7.40–7.26 (m, 3H), 6.55 (dd,

$J = 8.8$ Hz, 2.4 Hz, 1H), 6.48 (d, $J = 2.4$ Hz, 1H), 3.88 (s, 3H), 3.84 (s, 3H). ESI MS (m/e) = 269 $[M+1]^+$.

Compound 1d

$R_f = 0.28$ (1:9 ethyl acetate:hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, $J = 8.8$ Hz, 1H), 7.69 (d, $J = 15.6$ Hz, 1H), 7.61 (d, $J = 16.0$ Hz, 1H), 7.56–7.54 (m, 2H), 7.34–7.31 (m, 3H), 6.53 (dd, $J = 8.6$ Hz, 2.0 Hz, 1H), 6.47 (d, $J = 2.0$ Hz, 1H), 6.06–5.96 (m, 2H), 5.44–5.37 (m, 2H), 5.29–5.22 (m, 2H), 4.55–4.51 (m, 4H). ESI MS (m/e) = 321 $[M+1]^+$.

Procedure of preparing compound 1c

The resulting compound **1d** was stirred under microwave irradiation (Biotage Initiator) for 30 min at 120 °C in the presence of $PdCl_2(PPh_3)_2$ (13 mg) and ammonium formate (80 mg) in 4 mL of THF. The reaction mixture was diluted with ethyl acetate. The organic layer was washed with water, dried over Na_2SO_4, concentrated under reduced pressure, and purified by MPLC to afford compound **1c** in 35 % yield. $R_f = 0.24$ (1:4 ethyl acetate:hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.41 (s, 1H), 7.88 (d, $J = 15.6$ Hz, 1H), 7.84 (d, $J = 9.2$ Hz, 1H), 7.66–7.63 (m, 2H), 7.57 (d, $J = 15.2$ Hz, 1H), 7.44–7.42 (m, 3H), 6.47 (d, $J = 2.4$ Hz, 1H), 6.45 (s, 1H). ESI MS (m/e) = 241 $[M+1]^+$.

Materials

Antibodies specific for EGFR, Her2, Met, Akt, Cdk4, Hsp90, Hsp70, and β-actin were purchased from Cell Signaling Technology. Goat anti-rabit IgG horseradish peroxidase conjugate was purchased from Santa Cruz Biotechnology. Cell Titer 96 Aqueous One Solution cell proliferation assay kit was purchased from Promega.

Cell culture

H1975 cells were grown in RPMI 1640 with L-glutamine supplemented with streptomycin (500 mg/mL), penicillin (100 units/mL), and 10 % fetal bovine serum (FBS). Cells were grown to confluence in a humidified atmosphere (37 °C, 5 % CO_2).

Cell proliferation assay

Cells were seeded at 3000 cells per well in a clear 96-well plate, the medium volume was brought to 100 μL, and the cells were allowed to attach overnight. The next day, varying concentrations of compounds (**1a–d**), 1 μM geldanamycin (GA) as a positive control or 1 % DMSO vehicle control was added to the wells. Cells were then incubated at 37 °C for 1, 2, and 3 days. Cell viability was determined using the Promega Cell Titer 96 Aqueous One Solution cell proliferation assay. After incubation with compounds, 20 μL of the assay substrate solution was added to the wells, and the plate was incubated at 37 °C for an additional 1 h. Absorbance at 490 nm was then read on Tecan Infinite F200 Pro plate reader, and values were expressed as percent of absorbance from cells incubated in DMSO alone.

Western blot

Cells were seeded in 60 mm culture dishes (5×10^5/dish), and allowed to attach overnight. Compounds (**1a–d**) were

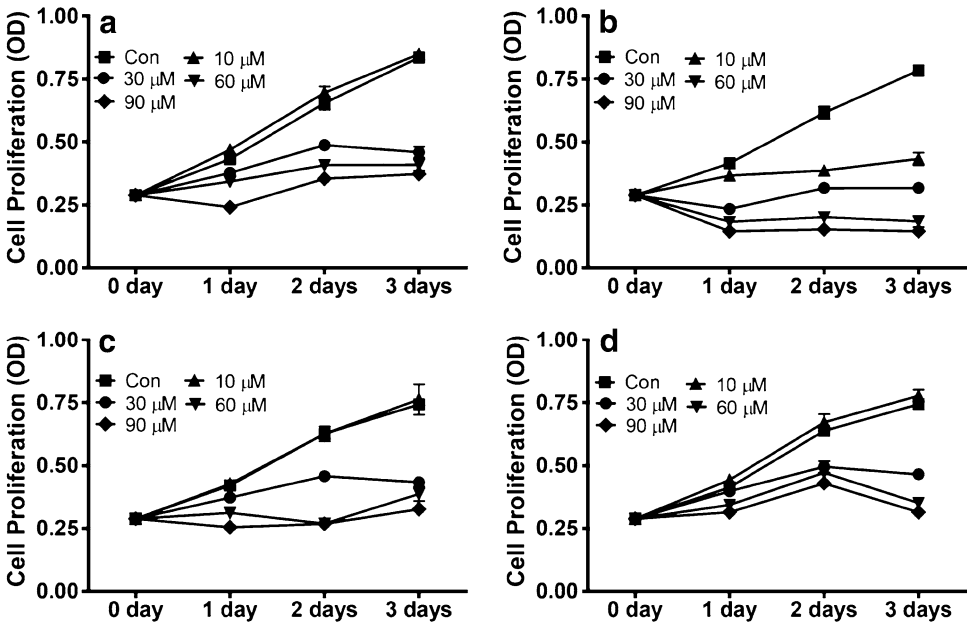


Fig. 2 Anti-proliferative effect of compound **1a–d** on H1975 cells. Cell proliferation was measured at 1, 2, and 3 days using MTS assay at the indicated concentrations of each compound. *Data* are presented as mean ± SD (n = 4)

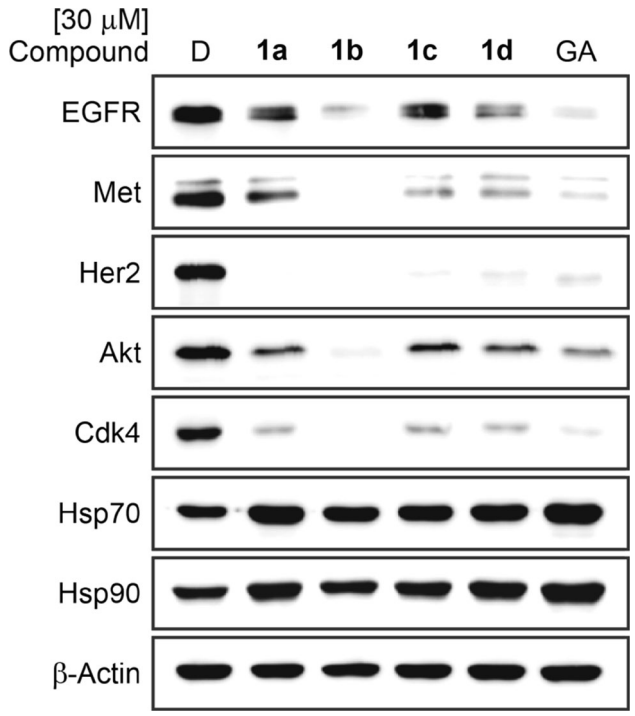


Fig. 3 Effect of compounds (**1a–d**) on cellular biomarkers of Hsp90 inhibition. H1975 cells were treated with 30 μM of each compound for 24 h and the expression level of EGFR, Met, Her2, Akt, Cdk4, Hsp70, Hsp90 and β-actin were analyzed by western blot. Geldanamycin (GA, 1 μM) and DMSO (D) were employed as positive and negative controls, respectively

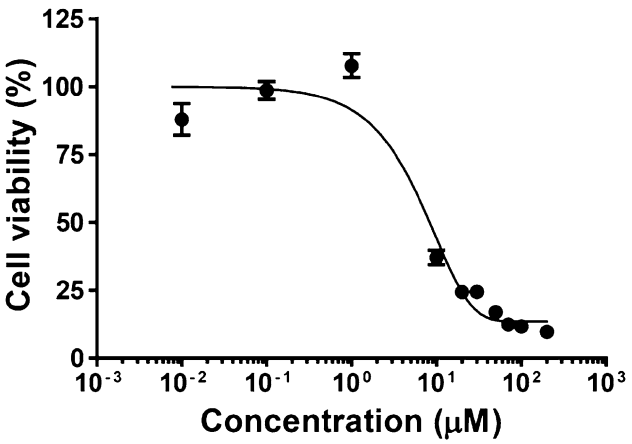


Fig. 4 Effect of compound **1b** on H1975 cell viability. Cells were treated with compound **1b** (0.01, 0.1, 1, 10, 20, 30, 50, 70, 100, and 200 μM) for 72 h and cell viability was measured by MTS assay. *Data* are presented as mean ± SD (n = 4)

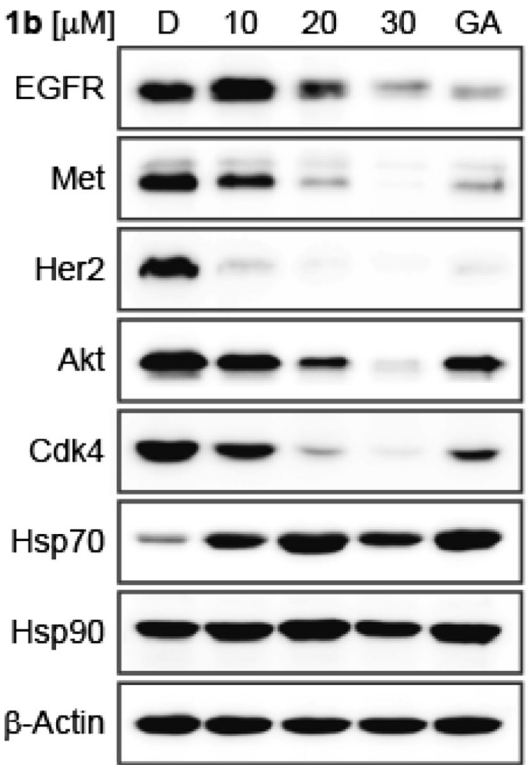


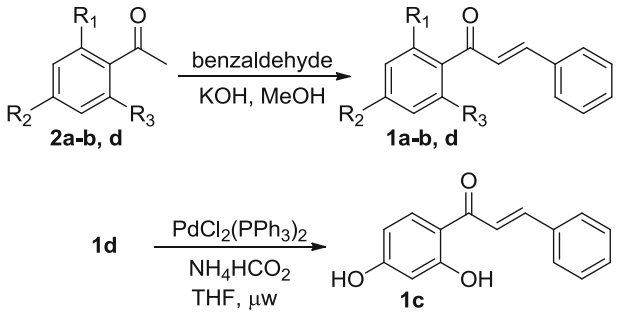
Fig. 5 Effects of compound **1b** on the expression of EGFR, Met, Her2, Akt, Cdk4, Hsp70, Hsp90, and β-actin. Compound **1b** induced proteasomal degradation of Hsp90 client proteins (EGFR, Met, Her2, Akt, and Cdk4) and upregulated Hsp70. Geldanamycin (GA, 1 μM) and DMSO (D) were employed as positive and negative controls, respectively

added at the concentrations indicated in Figs. 3 and 5, and the cells were incubated for an additional 24 h. For comparison, cells were also incubated with DMSO (1 %) or geldanamycin (1 μM) for 24 h. Cells were harvested in ice-cold lysis buffer (23 mM Tris-HCl pH 7.6, 130 mM NaCl, 1 % NP40, 1 % sodium deoxycholate, 0.1 % SDS), and 20 μg of lysate per lane was separated by SDS-PAGE and followed by transferring to a PVDF membrane (Bio-Rad). The membrane was blocked with 5 % skim milk in TBST, and then incubated with the corresponding antibody (EGFR, Her2, Met, Akt, Cdk4, Hsp90, Hsp70, or β-Actin). After binding of an appropriate secondary antibody coupled to horseradish peroxidase, proteins were visualized by ECL chemiluminescence according to the instructions of the manufacturer (Thermo Scientific, USA).

Results and discussion

Compounds (**1a–d**) were synthesized following the literature procedures with slight modifications (Gaur et al. 2014; Boeck et al. 2006). Briefly, Claisen-Schmidt aldol condensations of the corresponding acetophenones (**2a–b** and **2d**) with benzaldehyde were carried out in the presence of KOH in MeOH and the reaction provided the desired compounds (**1a–b** and **1d**) in 50–73 % yields (Scheme 1). To further synthesize compound **1c**, palladium-catalyzed allyl-deprotection reaction of compound **1d** was performed

Scheme 1 Synthesis of flavokawain B and its analogues



entry	R_1	R_2	R_3	yield[c]	product	% inhibition[a]	CLogP
1	OMe	OMe	OH	50	**1a**[b]	62	4.00
2	OMe	OMe	H	59	**1b**	94	3.87
3	OH	OH	H	35[d]	**1c**	68	3.45
4	OAllyl	OAllyl	H	73	**1d**	61	5.42

[a] The percent inhibition of H1975 cell proliferation at 30 μM [b] flavokawain B
[c] % yield of aldol condensation [d] % yield of allyl-deprotection

using $PdCl_2(PPh_3)_2$ and ammonium formate under microwave irradiation to produce the resulting compound **1c** in 35 % yield.

With compounds (**1a–d**) in hands, we decided to investigate the comparative biological effects of the synthesized compounds (**1a–d**). We first examined antiproliferative effects of compounds (**1a–d**) on H1975 cells. H1975 is a gefitinib (iressa)-resistant non-small cell lung cancer cell and its resistance is acquired by T790M-EGFR mutation and Met amplification (Sordella et al. 2004; Paez et al. 2004; Kobayashi et al. 2005). H1975 cells were incubated with varying concentrations (0, 10, 30, 60, and 90 μM) of compounds (**1a–d**) for 0, 1, 2, and 3 days and measured the cell proliferation of H1975 using MTS colorimetric assay (Fig. 2). Among compounds (**1a–d**), compound **1b** displayed the most potent inhibitory activity and efficiently impaired the growth of H1975 cells in a time and concentration dependent manner.

We next investigated whether the observed cytotoxicity was associated to the inhibition of Hsp90 chaperoning function. Accordingly, we treated H1975 cells with 30 μM of each compound (**1a–d**) and examined the expression level of EGFR, Met, Her2, Akt, Cdk4, Hsp70, Hsp90, and β-actin by western blot. The proteasomal degradation of Hsp90 client proteins including EGFR, Met, Her2, Akt, and Cdk4, and the transcriptional induction of cochaperone Hsp70 are considered as cellular hallmarks of Hsp90 inhibition (Wright et al. 2004). As expected, compound **1b** more effectively downregulated Hsp90 client proteins such as EGFR, Met, Her2, Akt, and Cdk4 than other compounds (**1a**, **c–d**) and also induced the upregulation of cochaperone Hsp70 (Fig. 3). The western blot result is consistent with the earlier cell proliferation assay (Fig. 2), in that compound **1b** displayed the highest antiproliferative activity.

To precisely determine half-maximal effective concentration (EC_{50}) of compound **1b** against H1975, cells were incubated with compound **1b** (0.01, 0.1, 1, 10, 20, 30, 50, 70, 100, and 200 μM) for 72 h (Fig. 4). Cell viability was measured by MTS assay and EC_{50} value was calculated using nonlinear least-square curve-fitting. The assay revealed that compound **1b** almost completely inhibited the growth of H1975 cells at 10 μM and provided EC_{50} value of 6.6 μM.

We next evaluated the concentration-dependent effect on cellular hallmarks of Hsp90 inhibition (Fig. 5). H1975 cells were incubated with the indicated concentration of compound **1b** or geldanamycin (GA, 1 μM) as a positive control for 24 h and the expression levels of Hsp90 client proteins including EGFR, Met, Her2, Akt, and Cdk4, along with Hsp70, Hsp90 and β-actin were assessed by western blot. Western blot analysis demonstrated that compound **1b** effectively degraded Hsp90 client proteins. Intriguingly, Her2 responded most sensitively to the administration of compound **1b**. 10 μM of **1b** completely depleted the expression of Her2. The expression levels of EGFR, Met, Akt, and Cdk4 were significantly downregulated when exposed to 20 μM of compound **1b** and completely disappeared at 30 μM. In contrast, the protein level of cochaperone Hsp70 increased upon the administration of 10 μM compound **1b**, and compound **1b** maximized the expression level of Hsp70 at 20 μM concentration. Internal standard, β-actin remained unchanged since β-actin was not a client protein of Hsp90.

Conclusion

In summary, we synthesized compounds (**1a–d**) by modifying A-ring of flavokawain B and evaluated the comparative biological activities of compounds (**1a–d**). Among compounds (**1a–d**), Compound **1b** most effectively impaired the growth of iressa-resistant non-small cell lung cancer cells (H1975) with EC_{50} value of 6.6 μM. Compound **1b** also caused a significant degradation of Hsp90 client proteins, including EGFR, Met, Her2, Akt, and Cdk4 and upregulation of cochaperone Hsp70 in a concentration dependent manner, indicating that compound **1b** disrupted the Hsp90 chaperone machinery. Our finding suggested that compound **1b** could serve as a potential therapeutic lead to circumvent the drug resistance acquired by EGFR mutation and Met amplification. Further structure–activity relationship (SAR) studies to improve the efficacy and the pharmacokinetic properties of the compound are currently underway and will be reported in due course.

References

Agarwal, R., and G. Deep. 2008. Kava, a tonic for relieving the irrational development of natural preventive agents. *Cancer Prevention Research (Phila)* 1(6): 409–412. doi:10.1158/1940-6207.CAPR-08-0172.

Aggarwal, S. 2010. Targeted cancer therapies. *Nature Reviews Drug Discovery* 9(6): 427–428. doi:10.1038/nrd3186.

Batovska, D.I., and I.T. Todorova. 2010. Trends in utilization of the pharmacological potential of chalcones. *Current Clinical Pharmacology* 5(1): 1–29.

Boeck, P., C.A. Bandeira Falcao, P.C. Leal, R.A. Yunes, V.C. Filho, E.C. Torres-Santos, and B. Rossi-Bergmann. 2006. Synthesis of chalcone analogues with increased antileishmanial activity. *Bioorganic & Medicinal Chemistry* 14(5): 1538–1545. doi:10.1016/j.bmc.2005.10.005.

Boran, A.D., and R. Iyengar. 2010. Systems approaches to polypharmacology and drug discovery. *Current Opinion in Drug Discovery & Development* 13(3): 297–309.

Chiosis, G., and L. Neckers. 2006. Tumor selectivity of Hsp90 inhibitors: the explanation remains elusive. *ACS Chemical Biology* 1(5): 279–284. doi:10.1021/cb600224w.

Dimmock, J.R., D.W. Elias, M.A. Beazely, and N.M. Kandepu. 1999. Bioactivities of chalcones. *Current Medicinal Chemistry* 6(12): 1125–1149.

Gaur, R., K.S. Yadav, R.K. Verma, N.P. Yadav, and R.S. Bhakuni. 2014. In vivo anti-diabetic activity of derivatives of isoliquiritigenin and liquiritigenin. *Phytomedicine* 21(4): 415–422. doi:10.1016/j.phymed.2013.10.015.

Hadden, M.K., L. Galam, J.E. Gestwicki, R.L. Matts, and B.S. Blagg. 2007. Derrubone, an inhibitor of the Hsp90 protein folding machinery. *Journal of Natural Products* 70(12): 2014–2018. doi:10.1021/np070190s.

Kobayashi, S., T.J. Boggon, T. Dayaram, P.A. Janne, O. Kocher, M. Meyerson, B.E. Johnson, M.J. Eck, D.G. Tenen, and B. Halmos. 2005. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *New England Journal of Medicine* 352(8): 786–792. doi:10.1056/NEJMoa044238.

Li, J.W., and J.C. Vederas. 2009. Drug discovery and natural products: End of an era or an endless frontier? *Science* 325(5937): 161–165. doi:10.1126/science.1168243.

Mahalingam, D., R. Swords, J.S. Carew, S.T. Nawrocki, K. Bhalla, and F.J. Giles. 2009. Targeting HSP90 for cancer therapy. *British Journal of Cancer* 100(10): 1523–1529. doi:10.1038/sj.bjc.6605066.

Marcu, M.G., A. Chadli, I. Bouhouche, M. Catelli, and L.M. Neckers. 2000. The heat shock protein 90 antagonist novobiocin interacts with a previously unrecognized ATP-binding domain in the carboxyl terminus of the chaperone. *Journal of Biological Chemistry* 275(47): 37181–37186. doi:10.1074/jbc.M003701200.

Paez, J.G., P.A. Janne, J.C. Lee, S. Tracy, H. Greulich, S. Gabriel, P. Herman, et al. 2004. EGFR mutations in lung cancer: Correlation with clinical response to gefitinib therapy. *Science* 304(5676): 1497–1500. doi:10.1126/science.1099314.

Petrelli, A., and S. Giordano. 2008. From single- to multi-target drugs in cancer therapy: When aspecificity becomes an advantage. *Current Medicinal Chemistry* 15(5): 422–432.

Seo, Y.H., and Y.J. Oh. 2013. Synthesis of flavokawain B and its anti-proliferative activity against gefitinib-resistant non-small cell lung cancer (NSCLC). *Bulletin of Korean Chemical Society* 34(12): 3782–3786.

Seo, Y.H., and S.Y. Park. 2014. Synthesis of flavokawain analogues and their anti-neoplastic effects on drug-resistant cancer cells through Hsp90 inhibition. *Bulletin of Korean Chemical Society* 35(4): 1154–1158.

Sharma, S.V., T. Agatsuma, and H. Nakano. 1998. Targeting of the protein chaperone, HSP90, by the transformation suppressing agent, radicicol. *Oncogene* 16(20): 2639–2645. doi:10.1038/sj.onc.1201790.

Solit, D.B., and G. Chiosis. 2008. Development and application of Hsp90 inhibitors. *Drug Discovery Today* 13(1–2): 38–43. doi:10.1016/j.drudis.2007.10.007.

Sordella, R., D.W. Bell, D.A. Haber, and J. Settleman. 2004. Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. *Science* 305(5687): 1163–1167. doi:10.1126/science.1101637.

Steiner, G.G. 2000. The correlation between cancer incidence and kava consumption. *Hawaii Medical Journal* 59(11): 420–422.

Warmka, J.K., E.L. Solberg, N.A. Zeliadt, B. Srinivasan, A.T. Charlson, C. Xing, and E.V. Wattenberg. 2012. Inhibition of mitogen activated protein kinases increases the sensitivity of A549 lung cancer cells to the cytotoxicity induced by a kava chalcone analog. *Biochemical Biophysical Research Communications* 424(3): 488–492. doi:10.1016/j.bbrc.2012.06.140.

Whitesell, L., and S.L. Lindquist. 2005. HSP90 and the chaperoning of cancer. *Nature Reviews Cancer* 5(10): 761–772. doi:10.1038/nrc1716.

Whitesell, L., E.G. Mimnaugh, B. De Costa, C.E. Myers, and L.M. Neckers. 1994. Inhibition of heat shock protein HSP90-pp60v-src heteroprotein complex formation by benzoquinone ansamycins: Essential role for stress proteins in oncogenic transformation. *Proceedings of the National Academy of Sciences United States of America* 91(18): 8324–8328.

Wright, L., X. Barril, B. Dymock, L. Sheridan, A. Surgenor, M. Beswick, M. Drysdale, et al. 2004. Structure-activity relationships in purine-based inhibitor binding to HSP90 isoforms. *Chemistry & Biology* 11(6): 775–785. doi:10.1016/j.chembiol.2004.03.033.

Zhao, X., Y.L. Chao, Q.B. Wan, X.M. Chen, P. Su, J. Sun, and Y. Tang. 2011. Flavokawain B induces apoptosis of human oral adenoid cystic cancer ACC-2 cells via up-regulation of Bim and down-regulation of Bcl-2 expression. *Canadian Journal of Physiology and Pharmacology*. doi:10.1139/y11-088.