Bioorganic & Medicinal Chemistry 20 (2012) 3609–3614

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Novel inhibitors of heat shock protein Hsp70-mediated luciferase refolding that bind to DnaJ

Joel A. Cassel [a,*], Sergey Ilyin [a], Mark E. McDonnell [b], Allen B. Reitz [a]

[a] ALS Biopharma, LLC, Pennsylvania Biotechnology Center, 3805 Old Easton Rd, Doylestown, PA 18902, USA
[b] Fox Chase Chemical Diversity Center, Inc., 3805 Old Easton Rd, Doylestown, PA 18902, USA

ARTICLE INFO

Article history:
Received 3 February 2012
Revised 22 March 2012
Accepted 29 March 2012
Available online 11 April 2012

Keywords:
Hsp70
J-Domain co-chaperones
Luciferase refolding
Thermal shift analysis

ABSTRACT

Inhibitors of both heat shock proteins Hsp90 and Hsp70 have been identified in assays measuring luciferase refolding containing rabbit reticulocyte lysate or purified chaperone components. Here, we report the discovery of a series of phenoxy-*N*-arylacetamides that disrupt Hsp70-mediated luciferase refolding by binding to DnaJ, the bacterial homolog of human Hsp40. Inhibitor characterization experiments demonstrated negative cooperativity with respect to DnaJ and luciferase concentration, but varying the concentration of ATP had no effect on potency. Thermal shift analysis suggested a direct interaction with DnaJ, but not with Hsp70. These compounds may be useful tools for studying DnaJ/Hsp40 in various cellular processes.

1. Introduction

Heat shock proteins (HSPs) are a family of highly conserved, ubiquitously expressed molecular chaperones, some of which are upregulated in response to a variety of cellular stressors.[1–3] Hsp70[†] is one of the best studied and most prevalent HSPs localized in the cytoplasm and the cell surface, and is an inducible form with a corresponding constitutive form, Hsc70, that does not accumulate in response to cellular stress.[4,5] Hsp70 is closely involved in the regulation of proper protein folding and therefore has been linked to neurodegenerative protein misfolding diseases such as amyotrophic lateral sclerosis, Huntington's disease, and Parkinson's disease.[6,7] Hsp70's function involves binding of the hydrophobic C-terminal tail to peptide sequences on a variety of client proteins.[4] This interaction is regulated by the N-terminal ATPase domain of Hsp70, as well as several highly conserved co-chaperones such as Hsp40 and its bacterial homologue DnaJ.[4,8,9] While there is only 35–50% homology between DnaJ and the various Hsp40 proteins that have been identified, they all have a J-domain, which is highly conserved in all isoforms.[10] The J-domain of Hsp40 proteins assists Hsp70 by regulating this ATP-dependent protein binding to Hsp70 leading to the formation of an Hsp70-Hsp40-client protein ternary complex, which is an important intermediate in Hsp70 mediated protein folding.[7–9] In addition, Hsp40 binds to unfolded polypeptide chains and maintains them in a folding competent state, thereby reducing aggregation and resulting cellular stress.[5,8]

High-throughput screening assays measuring protein refolding have been used to identify novel inhibitors of both Hsp70 and Hsp90.[11,12] Both heterogeneous assays using rabbit reticulocyte lysate (RRL) and homogenous assays with purified HSPs and other co-chaperones have been described where either chemically or thermally denatured luciferase is renatured in an ATP-dependent manner yielding a luminescent signal.[13–15] In assays using RRL, luciferase refolding is mediated by the Hsp90 super-chaperone, multi-protein complex composed of heat shock factor 1 (HSF1), Hsp90, Hsp70, Hsp40 and other co-chaperones.[16] While luciferase refolding in RRL is Hsp90-dependent, both Hsp70 and Hsp40 are also critical components.[17] Therefore, in addition to Hsp90 inhibition, compounds which interact with Hsp70, Hsp40, or other co-chaperones would be identified in RRL-mediated luciferase refolding.

We screened a 7200 member diversity library comprised of drug-like compounds in a RRL-mediated luciferase refolding assay and discovered five active compounds that inhibited refolding at micromolar potency These compounds did not behave as typical Hsp90 inhibitors since they did not induce Hsp70 expression in cells. To determine if these compounds were Hsp70 inhibitors, we tested them in a luciferase refolding assay with purified Hsp70 and DnaJ. From these experiments we discovered a class of phenoxy-*N*-arylacetamides that inhibited Hsp70/DnaJ mediated luciferase refolding with sub-micromolar potency. We then characterized the inhibition of Hsp70/DnaJ mediated luciferase

* Corresponding author. Tel.: +1 610 220 4614.
E-mail addresses: jcassel@alsbiopharma.com (J.A. Cassel), silyin@alsbiopharma.com (S. Ilyin), mmcdonnell@fc-cdci.com (M.E. McDonnell), areitz@alsbiopharma.com (A.B. Reitz).

[†] For the purpose of our studies and this discussion, the Hsp70 we are referring to is NCBI Reference Sequence NP_005336 known as 'heat shock 70 kDa protein 1A'.

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.03.067

refolding to be not competitive with denatured luciferase, DnaJ, or ATP. Thermal stability suggested a direct molecular interaction with DnaJ rather than Hsp70. These data highlight the importance of the deconvolution of screening hits from heterogenous assays and represent the first series of small molecules that bind to DnaJ.

2. Materials and methods

2.1. Materials

Purified Hsp70 and DnaJ were produced in *Escherichia Coli* and purchased from Vybion, Inc (Ithaca, NY). RRL was purchased from Green Hectares (Oregon, WI). Phenoxy-*N*-arylacetamides were purchased from Frontier Scientific (Newark, DE). 17-AAG was purchased from Invivogen (San Diego, CA). All other reagents were purchased from Sigma–Aldrich (St. Louis, MO).

Our diversity library was designed by culling from larger commercial libraries purchased from ASDI (Newark, DE) in two successive iterations (ca. ~20% reduction each) to represent a variety of heterocyclic chemotypes compatible with drug suitability and CNS penetration. The molecular weight range was set at 200–400, which allows great flexibility for rapid elaboration from a hit-to-lead perspective and enhanced CNS penetration. Twenty percent of the library comes directly from a lead generation set of compounds. This portion of the library starts by creation of a virtual library, followed by diversity calculations via pairwise Tanamoto coefficients to give the most structurally diverse set of centroids (usual numbering about 500 compounds). Further construction of the library via clustering of most similar compounds around each centroid (5 compounds) provided a library with diversity, but with hit verification properties built in by way of similar compounds around each centroid. The following core heterocycles were included: azaindole, quinolone, pyrimidine, benzopyrimidine, isoxazole, oxadiazole, oxazole, purine, thiazole, and triazole. Filters for removal included two or more Rule of 5 violators, anionically charged compounds such as carboxylates, phosphates, phosphonates, and sulfates, *N*-oxides, nitro compounds, hydrazines, hydrazides, thiocarbonyls, α,β-unsaturated carbonyls, Michael acceptors, and sulfhydryl groups. The average molecular weight of the library was 328, and the mean cLogP value was 3.2.

2.2. Luciferase refolding assay in RRL

The compound library was screened for the inhibition of luciferase refolding in RRL using a procedure that has been previously described.[14] Briefly, luciferase (0.5 mg/mL) was heat denatured for 15 min at 41 °C in a stability buffer (25 mM tricine, pH 7.8, 8 mM $MgSO_4$, 0.1 mM EDTA, 10 mg/mL acetylated BSA, 10% glycerol, 2% Triton X-100). Test compounds were pre-incubated with RRL for 30 min followed by the addition of 3.0 μg/mL denatured luciferase in a final volume of 40 μL assay buffer (20 mM Tris, pH 7.7, 3 mM ATP, 5 mM creatine phosphate, 2.0 mM magnesium acetate, 75 mM KCl, and 0.2 mg/mL creatine phosphokinase). After 1 h incubation at room temperature, 20 μL of detection buffer (75 mM Tricine, pH 7.8, 24 mM $MgSO_4$, 0.3 mM EDTA, 2 mM DTT, 160 μM D-luciferin, 320 μM coenzyme A, 0.66 mM ATP, 150 mM KCl, 20% glycerol, 10% Triton X-100, 3.5% DMSO) was added to each well and luminescence measured using a TopCount scintillation counter (PerkinElmer, Waltham, MA). Active compounds were defined as those having inhibition values >70% at 10 μM relative to 1.0 μM of 17-AAG. Since compounds that inhibit luciferase refolding as well as those that inhibit luciferase activity would be active in the assay, the initial hits were confirmed and tested for direct inhibition of luciferase activity.

2.3. Luciferase refolding assay with purified Hsp70 and DnaJ

Hsp70/DnaJ mediated luciferase refolding was measured using methods previously described by with some minor modifications.[15] Luciferase (0.5 mg/mL) is denatured in buffer A (25 mM Hepes, pH 7.4, 50 mM potassium acetate, and 5 mM DTT) containing 6 M guanidine HCl for 60 min at room temperature and then diluted 1:20 in buffer A and stored at −80 °C until use. Refolding assays contained 0.5 μM Hsp70, 60 nM DnaJ, and 125 ng denatured luciferase in 50 μL of refolding buffer (28 mM Hepes, pH 7.4, 120 mM potassium acetate, 12 mM magnesium acetate, 2.2 mM DTT, 0.1 mM ATP, 8.8 mM creatine phosphate, 35 U/mL creatine phosphokinase). In some experiments, the concentrations of DnaJ or denatured luciferase were varied to characterize the mechanism of inhibition. At various times, 5 μL aliquots were removed from each assay and dispensed into 50 μL of detection buffer (75 mM tricine, pH 7.8, 24 mM $MgSO_4$, 0.3 mM EDTA, 2 mM DTT, 160 mM D-luciferin, 320 mM coenzyme A, 0.66 mM ATP, 150 mM KCl, 20% glycerol, 10% Triton X-100, 3.5% DMSO). After incubation for 10 min, luminescence was measured using a TopCount scintillation counter (PerkinElmer, Waltham, MA).

2.4. Hsp70 ATPase activity assay

Hsp70 ATPase activity was measured using ADP^2 Transcreener assay (BellBrooke Labs, Madison, WI) as described by Rowlands et al.[18] Breifly, Hsp70 (300 nM) was incubated with 3.2 μM ATP at 37 °C for various times in the presence or absence of various concentrations of test compound and/or DnaJ in a final volume of 10 μL of 50 mM HEPES, pH 7.4, 20 mM KCl, 2 mM EGTA, 4 mM $MgCl_2$, and 0.01% Triton X-100 in black opaque 384-well plates. Reactions were stopped by the addition of 5 μL 1.5× stop/detect buffer (30 mM HEPES, 60 mM EDTA, 0.03% Triton X-100) containing ADP tracer and ADP^2 antibody at concentrations recommended by the manufacturer. Fluorescence was measured at excitation λ = 580 nm and emission λ = 620 nm.

2.5. Protein melting thermal shift assay

Protein melting studies were performed as previously described with some minor modifications.[19] Hsp70 or DnaJ diluted to 0.5 mg/mL in melting buffer (100 mM HEPES, 150 mM NaCl, pH 7.4) and 10 μL (10 μg) added to PCR plate along with test compound and 50× Spyro Orange dye in a final volume of 20 μL melting buffer. The fluorescence was monitored as the assay plate was heated in 1 °C increments up to 95 °C in a AB7500 fast thermocyler (Carlsbad, CA) using settings according to the manufacturer's instructions. Data were plotted as the first derivative of the change in fluorescence versus the change in temperature as described by Crowther et al.[19]

3. Results

3.1. Characterization of screening hits from luciferase refolding in RRL

A drug-like chemical diversity library (~7200 compounds) was screened for inhibition of luciferase refolding in RRL. Active compounds were defined as those having inhibition values greater than 70% at 10 μM relative to treatment with 1 uM of 17-AAG. Z′ values ranged from 0.4 to 0.8. Since compounds that inhibit luciferase refolding as well as those that inhibit luciferase activity would be active in the assay, the initial hits were confirmed and tested for direct inhibition of luciferase activity. Five active compounds were identified which had no effect on luciferase activity at concentrations up to 300 μM (Fig. 1, Table 1).

Hsp90 inhibitors, such as geldanamycin and 17-AAG, inhibit luciferase refolding by binding to Hsp90 leading to dissociation of the heat shock protein complex. In cells, this dissociation then allows heat shock factor 1 (HSF1) to translocate to the nucleus where it upregulates Hsp70. Luciferase refolding in RRL has been used as a surrogate for Hsp90 inhibition,[14] and therefore, we tested these active compounds for Hsp70 induction in both HeLa and A172 cells. Cells were treated with test compound overnight and then Hsp70 levels were measured using a solid state immunoassay, where 1 μM 17-AAG was the positive control. However, none of the actives induced Hsp70 in either cell line with stimulation values relative to 17-AAG less then 10% at 10 μM. Therefore, it is unlikely that these compounds are typical Hsp90 inhibitors.

Another possible mechanism for the inhibition of luciferase refolding is inhibition of Hsp70 directly. As a result, we tested these compounds in Hsp70 mediated luciferase refolding using purified human recombinant Hsp70 as well as the co-chaperone DnaJ. Compound **5** exhibited similar potency for inhibition of Hsp70/DnaJ mediated luciferase refolding compared to inhibition of RRL mediated luciferase refolding (Table 1). These data suggest that **5** inhibits luciferase refolding via an interaction with either Hsp70 or DnaJ, or both of them. We purchased several related phenoxy-*N*-arylacetamides that were close analogs of **5**, and determined their IC_{50} values in Hsp70/DnaJ mediated luciferase refolding (Fig. 2). A range of potencies was observed from 0.13 to 30 μM for these compounds, suggesting a specific biological effect. The length of the ester linkage as well as the position of the chlorine atoms on the phenyl ring were key determinants for the potency of phenoxy-*N*-arylacetamides for inhibition of Hsp70/DnaJ mediated refolding. The combination of the butyl ester linkage together with the 2,4-dichloro substitution resulted in the most potent activity. We then conducted additional experiments to further characterize the mechanism of action.

3.2. Characterization of phenoxy-*N*-arylacetamide inhibition of Hsp70/DnaJ mediated luciferase refolding

Our initial hypothesis was that the phenoxy-*N*-arylacetamides were interacting directly with Hsp70. Therefore, we conducted competition experiments with derivative **6** in Hsp70/DnaJ mediated luciferase refolding by varying the concentrations of DnaJ, denatured luciferase and ATP. Increasing the concentrations of DnaJ from 10 to 1000 nM or denatured luciferase from 0.1 to 10 μg/mL produced a gradual rightward shift in the IC_{50} values for compound **6** (Fig. 3). Plotting the logIC_{50} values for **6** against the log concentration of either DnaJ or luciferase resulted in a curvilinear plot (Fig. 3) which is indicative of an allosteric

Table 1
IC_{50} values of screening hits for inhibition of luciferase refolding mediated by rabbit reticulocyte lysate or purified Hsp70/DnaJ

Compound	IC_{50}[a] (μM), Luciferase refolding	
	RRL	Hsp70/DnaJ
1	4.3 (1.7–10)	>30
2	4.0 (1.3–12)	>100
3	8.8 (4.8–16)	>100
4	1.1 (0.45–2.5)	22 (7.9–80)
5	**0.81** (**0.25–2.6**)	**0.62** (**0.40–1.0**)

[a] IC_{50} values are the geometric mean and 95% confidence intervals of at least 3 determinations.

interaction.[20,21] Linear regression of the linear portion of these plots for both DnaJ and luciferase yielded slope values of ~0.6, whereas a slope value of 1 would be indicative of competitive antagonism. Therefore, based on these data, inhibition of Hsp70/DnaJ mediated luciferase refolding by these phenoxy-*N*-arylacetamides is not competitive with DnaJ or denatured luciferase.

Interestingly, inhibition by **6** was insurmountable with increasing concentrations of ATP, which had no effect on the IC_{50} value at concentrations up to 10 mM (Figure 3). To confirm further investigate this finding, we tested **6** for inhibition of Hsp70 mediated ATPase using the Transcreener FI assay as previously described.[18] However, compound **6** did not inhibit Hsp70 mediated ATPase activity at concentrations up to 10 μM (data not shown). We then tested if compound **6** would inhibit DnaJ stimulated Hsp70 ATPase activity by titrating DnaJ in the presence of increasing concentrations of compound **6**. Compound **6** did modestly induce a rightward shift in the dose-response curve for DnaJ (Fig. 4), which is consistent with the experiments measuring the effect of DnaJ on **6** inhibition of Hsp70 mediated refolding. Similar to the inhibitor characterization experiments in Hsp70 mediated luciferase refolding, plotting the log EC_{50} values against the concentration of **6** yielded a slope value of 0.26 (Fig. 4), which is significantly less than unity, thereby suggesting negative cooperativity. Therefore, the inability of higher ATP concentrations to inhibit the effect of **6** together with specific inhibition of DnaJ stimulated Hsp70 ATPase activity suggests that these compounds may interact with DnaJ rather than Hsp70.

3.3. Effects of phenoxy-*N*-arylacetamides on thermal stability of Hsp70 or DnaJ

In order to further investigate whether these compounds bound to Hsp70 or DnaJ, we conducted protein melting experiments using

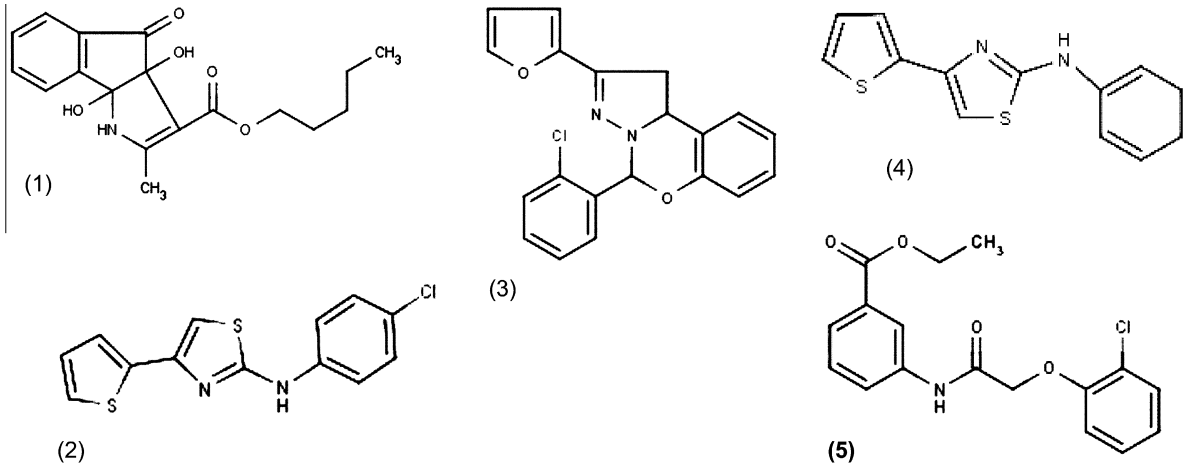

Figure 1. Compounds **1** to **5** identified from the luciferase refolding screen with rabbit reticulocyte lysate.

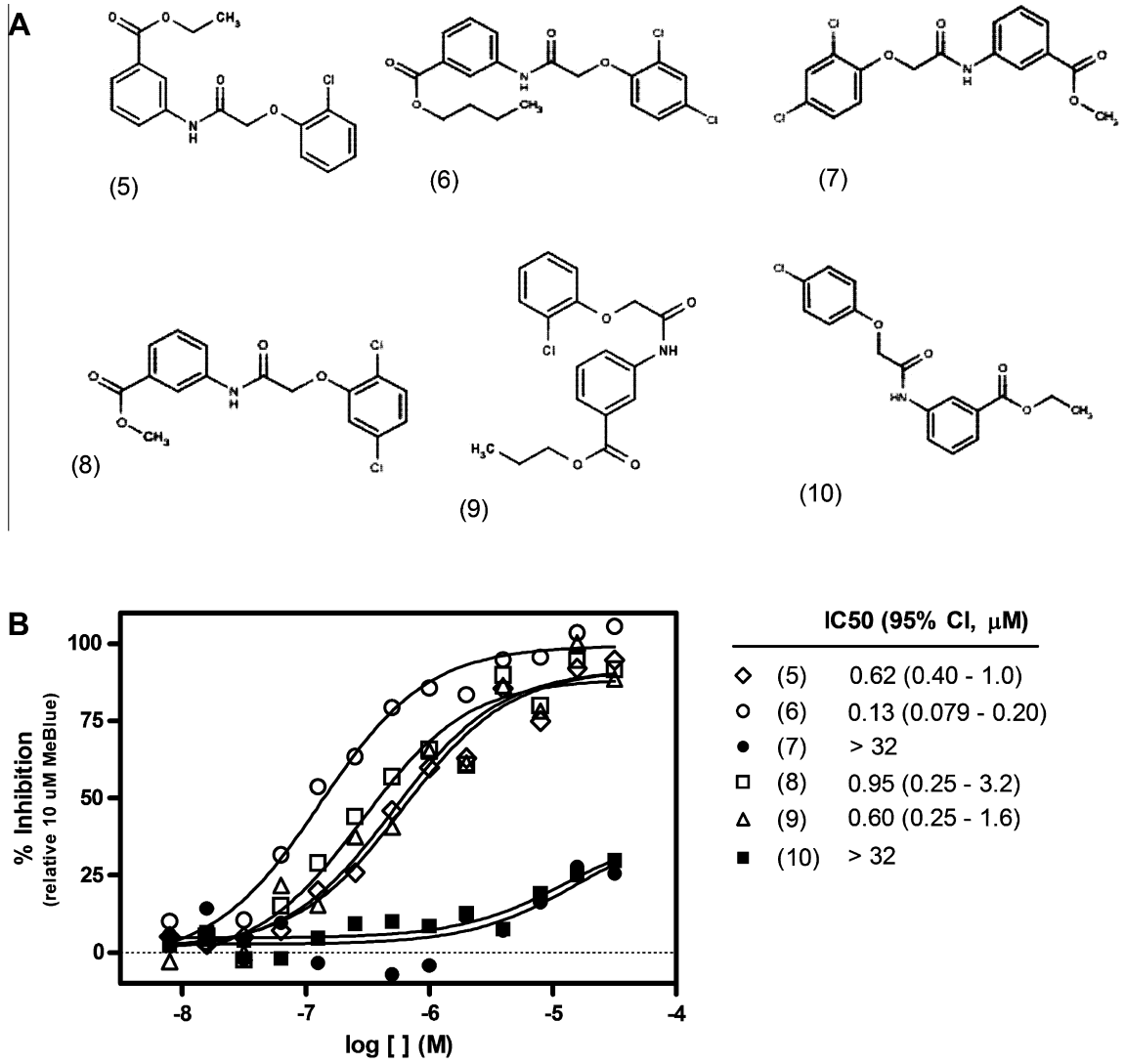

Figure 2. Inhibition of Hsp70/DnaJ mediated luciferase refolding by phenoxy-*N*-arylacetamides. (A) Structures of phenoxy-*N*-arylacetamide compounds **6**–**10**. (B) Dose-response curves and IC_{50} values for inhibition of Hsp70/DnaJ mediated luciferase refolding. Test compound was incubated with Hsp70, DnaJ, and denatured luciferase for 1 h followed by measurement of luciferase activity as described in methods. Dose–response curves are an example of a single experiment that was repeated at least three times with similar results. IC_{50} values are the geometric mean and 95% confidence intervals of at least three determinations.

the Spyro orange dye with DnaJ or Hsp70 in the presence of **6** or **7** as an inactive control. Spyro orange interacts with hydrophobic groups on proteins which become more accessible as a protein is heated and begins to denature. Using a thermocycler, changes in fluorescence can be measured during a constant rise in temperature and obtain a melting curve for a protein. The temperature where the protein is halfway denatured, or the point where the change in fluorescence is the steepest is the called the T_m. Typically, these data are then plotted as the first derivative, where the T_m is the temperature where the first derivative plot is maximal. Compounds which interact with the protein tend to stabilize the protein and produce a rightward shift in the protein's melting curve, resulting in a larger value for T_m.[22] As shown in Figure 5, analog **6** produced a dose-dependent rightward shift in the melting curve for DnaJ, shifting the apparent T_m by ~2 °C at 10 and 30 μM. However, **6** had no effect on the melting curve of Hsp70 (Fig. 5). In addition, **7**, the inactive control compound did not shift the melting curves for either DnaJ or Hsp70 (Fig. 5). These data suggest that the mechanism of inhibition in Hsp70/DnaJ mediated luciferase refolding for these phenoxy-*N*-arylacetamides via a direct molecular interaction with DnaJ.

4. Discussion

The data presented in this report underscores the need for deconvolution when screening using heterogeneous systems, such as RRL. The initial goal of this screening campaign was to identify novel Hsp90 inhibitors that induce Hsp70 in cellular assays; however, we did not discover such compounds. While we were able to identify a mechanism of inhibition for **5** and structurally-related analogs, we were unable to account for the inhibition mediated by **1**–**4**. These compounds may interact with other proteins in the reticulocyte lysate that are necessary for luciferase refolding, or may exhibit poor cellular permeability. It should also be noted that there are some known Hsp90 inhibitors which do not upregulate Hsp70.[23] Thus, **1**–**4** may actually be Hsp90 inhibitors, however, we were not able to obtain sufficient quantities of purified Hsp90 to test this hypothesis.

One of the benefits of screening using a heterogeneous system is the ability to identify compounds with unique and different mechanisms of action. The discovery of the class of phenoxy-*N*-arylacetamides that interact with DnaJ directly that we have described here is an example of such an advantage. To our

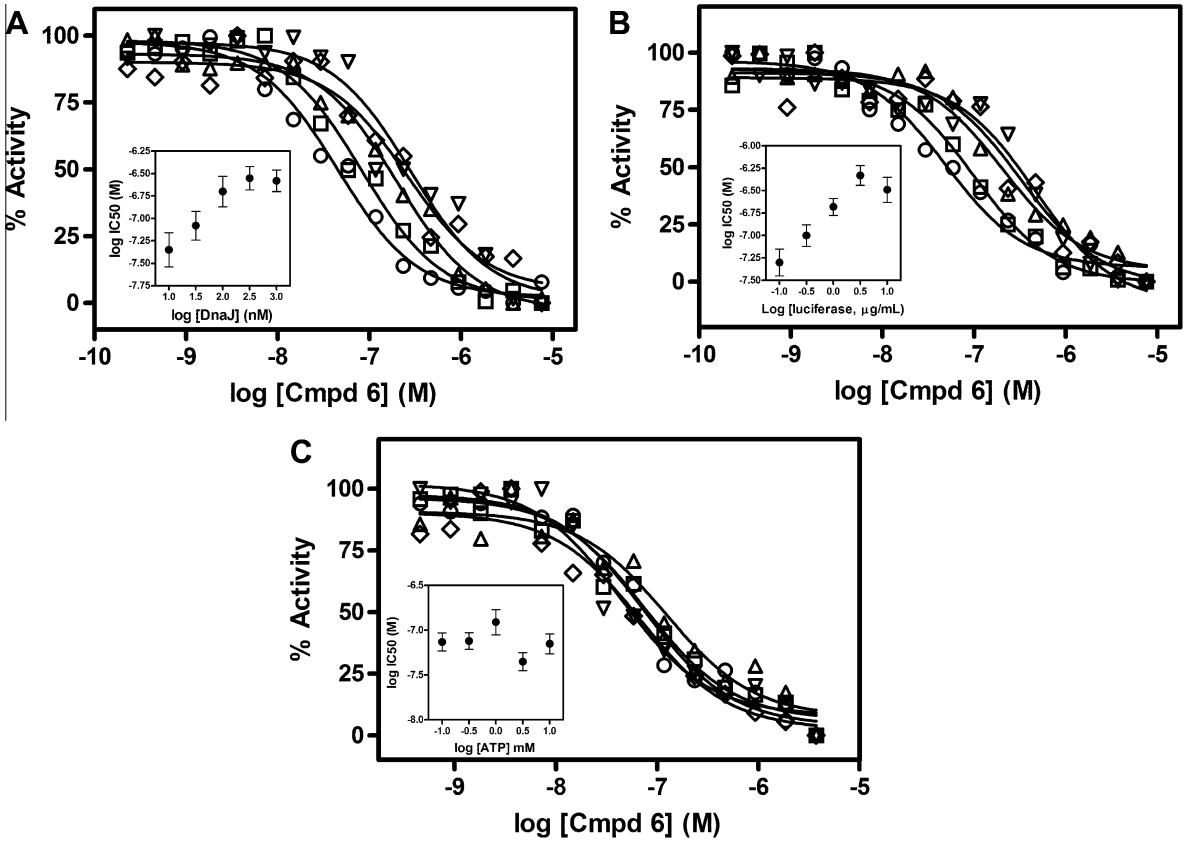

Figure 3. Inhibition of Hsp70/DnaJ mediated luciferase refolding by **6** is not competitive with DnaJ, denatured luciferase, or ATP. (A) Effect of increasing DnaJ concentration on inhibition by compound (**6**). Compound **6** was incubated with Hsp70 and denatured luciferase in the presence of 10 nM (○), 32 nM (□), 100 nM (△), 320 nM (▽) or 1000 nM (◇) DnaJ for 1 h followed by measurement of luciferase activity. (B) Effect of increasing denatured luciferase concentration on inhibition by **6**. Compound **6** was incubated with Hsp70 and DnaJ in the presence of 0.1 μg/mL (○), 0.32 μg/mL (□), 1.0 μg/mL (△), 3.2 μg/mL (▽) or 10 μg/mL (◇) denatured luciferase for 1 h followed by measurement of luciferase activity. (C) Effect of increasing ATP concentration on inhibition by **6**. Compound **6** was incubated with Hsp70, DnaJ, and denatured luciferase in the presence of 0.1 mM (○), 0.32 mM (□), 1 mM (△), 3.2 mM (▽) or 10 mM (◇) ATP for 1hr followed by measurement of luciferase activity. Data are an example of single experiments that were repeated three times with similar results. (Insets) Plot of log IC_{50} values ± S.E.M. of three determinations vs log concentration of DnaJ (A), denatured luciferase (B), or ATP (C).

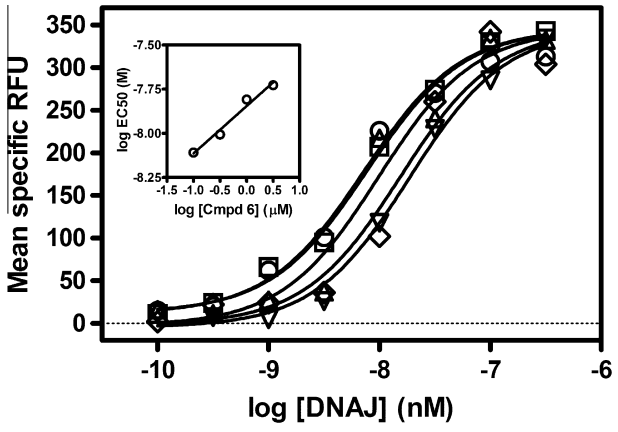

Figure 4. Compound **6** inhibits DnaJ-mediated stimulation of Hsp70 ATPase activity. Hsp70 (300 nM) was incubated for various times from 5 min to 40 min at 37 °C with a series of concentrations of DnaJ (0.1 nM to 320 nM), 3.2 μM ATP, in the absence (○) and presence of 0.1 μM (□), 0.32 μM μM (△), 1 μM (◇) and 3.2 μM (▽) of compound **6**. Nonspecific RFU was determined in the absence of Hsp70 and subtracted from total RFU values. (Inset) Plot of log EC_{50} values obtained for DnaJ stimulation of Hsp70 ATPase activity as a function of log concentration of compound **6**. Data represent a single experiment that was repeated with similar results.

knowledge, these are the first such compounds that have been reported to bind to DnaJ, however, several compounds have been identified which specifically interact with the DnaJ binding site on Hsp70. For example, the flavonoid myricetin, has been reported to specifically inhibit DnaJ stimulated Hsp70 mediated ATPase activity, but the mechanism of inhibition is via binding to a unique site on Hsp70.[24] In addition, a class of dihydropyrimidines have been identified which interact with Hsp70 at the DnaJ interface.[25] Both myricetin and the dihydropyrimidines affect the expression of the microtubule associated protein tau, which is a key target for Alzheimer's disease.[26] As a result, we investigated the effects of the phenoxy-*N*-arylacetamides described here on tau levels in human neuroglioma H4 cells in a solid state immunoassay using a tau primary antibody with methylene blue as the positive control. However, we did not see any change in tau levels after a 24 h exposure to these compounds relative to methylene blue (data not shown).

While these phenoxy-*N*-arylacetamides do not lower tau levels, they could conceivably influence other cellular processes where J-domain chaperones like Hsp40/DnaJ are involved. Hsp40 chaperones play a role in regulating levels of the hERG potassium channel as well as in glucocorticoid signaling and in α-synuclein aggregation.[27–29] Hsp40/DnaJ has been identified as a key regulator of cancer cell and stem cell function.[30] For example, down-regulation of Hsp40 via siRNA has been shown to potentiate 5-fluorouracil and carboplatin cytotoxicity in hepatoma cell lines.[31] Therefore, Hsp40/DnaJ inhibitors may be useful adjunct chemotherapy agents. In addition to cancer cell function, Hsp40/DnaJ plays an important role in viral pathogenesis.[32] In particular, Hsp40 is

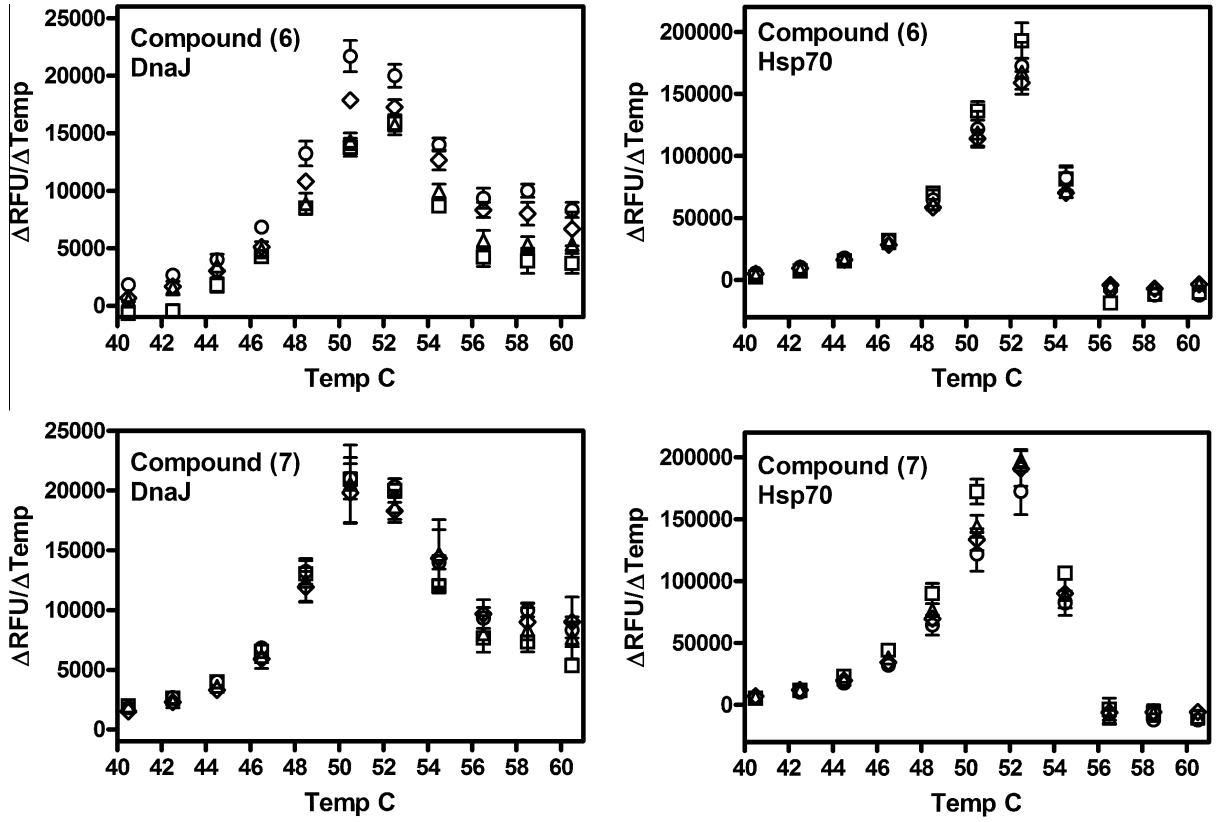

Figure 5. Compound **6**, but not its inactive analog **7**, stabilizes DnaJ from thermal denaturation with no effect on Hsp70. DnaJ or Hsp70 (5 μg) was incubated with Spyro orange dye in the absence (○) or presence of 32 μM (□), 10 μM (△), or 3.2 μM (◇) of either **6** or **7** as indicated in the figure. Fluoresence was monitored in an Applied Biosystems FAST 7500 thermocycler as the temperature was increased from 25 to 95 °C in one degree increments as described in methods. Data points are the mean ± S.E.M. of triplicate determinations obtained between 40 and 60 °C.

critical to both gene expression and viral replication of HIV.[33] As a result, the compounds described here may be useful biochemical probes to further the understanding of the role of Hsp40/DnaJ in a variety of biological processes, possibly leading to the development of novel therapeutics in the areas of oncology and anti-viral therapy.

Acknowledgement

We thank the Alzheimer's Drug Discovery Foundation for providing the funding necessary to perform the work described herein.

References and notes

1. Lindquist, S.; Craig, E. A. *Annu. Rev. Genet.* **1988**, *22*, 631.
2. Kregel, K. C. *J. Appl. Physiol.* **2002**, *92*, 2177.
3. Feder, M. E.; Hofmann, G. E. *Annu. Rev. Physiol.* **1999**, *61*, 243.
4. Mayer, M. P.; Bukau, B. *Cell. Mol. Life Sci.* **2005**, *62*, 670.
5. Pawlyk, A. C.; Cassel, J. A.; Reitz, A. B. *Curr. Pharm. Des.* **2010**, *16*, 2053.
6. Asea, A. A. A.; Brown, Ian R. In *Heat Shock Proteins and the Brain: Implications for Neurodegenerative Diseases and Neuroprotection*; Springer Science+Business Media B.V.: Dordrecht, Netherlands, 2008.
7. Muchowski, P. J.; Wacker, J. L. *Nat. Rev. Neurosci.* **2005**, *6*, 11.
8. Li, J.; Qian, X.; Sha, B. *Protein Pept. Lett.* **2009**, *16*, 606.
9. Cheetham, M. E.; Caplan, A. J. *Cell Stress Chaperones* **1998**, *3*, 28.
10. Gething, M.-J. *Guidebook to the Molecular Chaperones and Protein-folding Catalysts*; Oxford University Press: Oxford, New York, 1997.
11. Boschelli, F.; Golas, J. M.; Petersen, R.; Lau, V.; Chen, L.; Tkach, D.; Zhao, Q.; Fruhling, D. S.; Liu, H.; Nam, C.; Arndt, K. T. *Cell Stress Chaperones* **2010**, *15*, 913.
12. Matts, R. L.; Manjarrez, J. R. *Curr. Top. Med. Chem.* **2009**, *9*, 1462.
13. Schumacher, R. J.; Hansen, W. J.; Freeman, B. C.; Alnemri, E.; Litwack, G.; Toft, D. O. *Biochemistry* **1996**, *35*, 14889.
14. Galam, L.; Hadden, M. K.; Ma, Z.; Ye, Q. Z.; Yun, B. G.; Blagg, B. S.; Matts, R. L. *Bioorg. Med. Chem.* **2007**, *15*, 1939.
15. Wisen, S.; Gestwicki, J. E. *Anal. Biochem.* **2008**, *374*, 371.
16. Pearl, L. H.; Prodromou, C. *Annu. Rev. Biochem.* **2006**, *75*, 271.
17. Minami, Y.; Minami, M. *Genes Cells* **1999**, *4*, 721.
18. Rowlands, M.; McAndrew, C.; Prodromou, C.; Pearl, L.; Kalusa, A.; Jones, K.; Workman, P.; Aherne, W. *J. Biomol. Screen.* **2010**, *15*, 279.
19. Crowther, G. J.; Napuli, A. J.; Thomas, A. P.; Chung, D. J.; Kovzun, K. V.; Leibly, D. J.; Castaneda, L. J.; Bhandari, J.; Damman, C. J.; Hui, R.; Hol, W. G.; Buckner, F. S.; Verlinde, C. L.; Zhang, Z.; Fan, E.; van Voorhis, W. C. *J. Biomol. Screen.* **2009**, *14*, 700.
20. Arunlakshana, O.; Schild, H. O. *Br. J. Pharmacol. Chemother.* **1959**, *14*, 48.
21. Lanzafame, A.; Christopoulos, A.; Mitchelson, F. *Eur. J. Pharmacol.* **1996**, *316*, 27.
22. Cummings, M. D.; Farnum, M. A.; Nelen, M. I. *J. Biomol. Screen.* **2006**, *11*, 854.
23. Eskew, J. D.; Sadikot, T.; Morales, P.; Duren, A.; Dunwiddie, I.; Swink, M.; Zhang, X.; Hembruff, S.; Donnelly, A.; Rajewski, R. A.; Blagg, B. S.; Manjarrez, J. R.; Matts, R. L.; Holzbeierlein, J. M.; Vielhauer, G. A. *BMC Cancer* **2011**, *11*, 468.
24. Chang, L.; Miyata, Y.; Ung, P. M.; Bertelsen, E. B.; McQuade, T. J.; Carlson, H. A.; Zuiderweg, E. R.; Gestwicki, J. E. *Chem. Biol.* **2011**, *18*, 210.
25. Wisen, S.; Bertelsen, E. B.; Thompson, A. D.; Patury, S.; Ung, P.; Chang, L.; Evans, C. G.; Walter, G. M.; Wipf, P.; Carlson, H. A.; Brodsky, J. L.; Zuiderweg, E. R.; Gestwicki, J. E. *ACS Chem. Biol.* **2010**, *5*, 611.
26. Jinwal, U. K.; Koren, J.; O'Leary, J. C.; Jones, J. R.; Abisambra, J. F.; Dickey, C. A. *Mol. Cell Pharmacol.* **2010**, *2*, 43.
27. Walker, V. E.; Wong, M. J.; Atanasiu, R.; Hantouche, C.; Young, J. C.; Shrier, A. *J. Biol. Chem.* **2010**, *285*, 3319.
28. Murphy, P. J.; Morishima, Y.; Chen, H.; Galigniana, M. D.; Mansfield, J. F.; Simons, S. S., Jr.; Pratt, W. B. *J. Biol. Chem.* **2003**, *278*, 34764.
29. Hinault, M. P.; Cuendet, A. F.; Mattoo, R. U.; Mensi, M.; Dietler, G.; Lashuel, H. A.; Goloubinoff, P. *J. Biol. Chem.* **2010**, *285*, 38173.
30. Sterrenberg, J. N.; Blatch, G. L.; Edkins, A. L. *Cancer Lett.* **2011**, *312*, 129.
31. Sharma, A.; Upadhyay, A. K.; Bhat, M. K. *Cancer Biol. Ther.* **2009**, *8*, 2106.
32. Knox, C.; Luke, G. A.; Blatch, G. L.; Pesce, E. R. *Virus Res.* **2011**, *160*, 15.
33. Kumar, M.; Mitra, D. *J. Biol. Chem.* **2005**, *280*, 40041.