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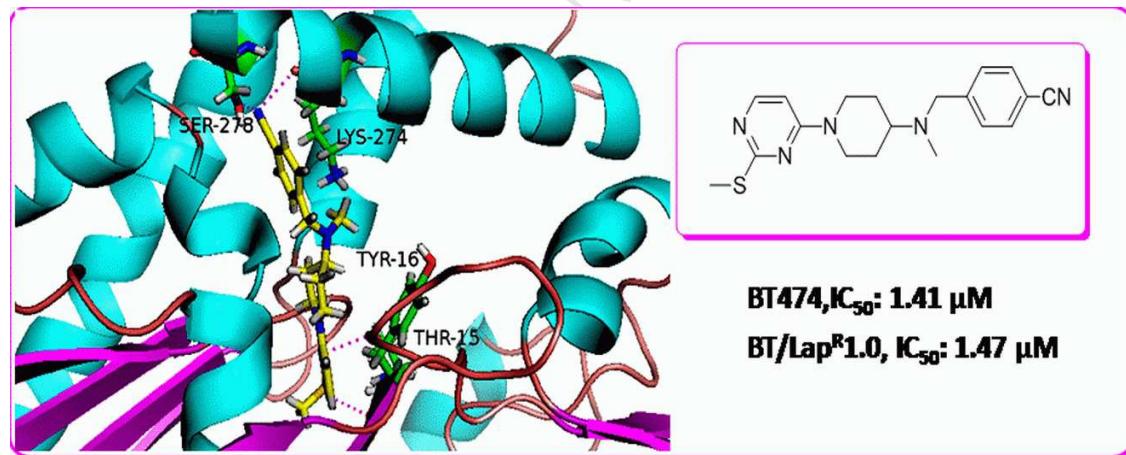
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HSP70 is a potential target for tumor treatments. Herein, novel HSP70 inhibitors, piperidine derivatives, were designed and biologically evaluated. The results show that the HSP70 inhibitors have a good antitumor activity, especially in lapatinib-resistant cancer cells.



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ABSTRACT

HSP70 is a potential target for tumour treatment. HSP70 plays significant roles in several biological processes, including the regulation of apoptosis. In this study, piperidine derivatives were designed as novel HSP70 inhibitors based on virtual fragment screening performed in Dock 4.0, Discovery Studio 2.5 and SYBYL 6.9. A total of 67 novel piperidine derivatives were synthesized. Cell viability assays were performed in 16 cancer cell lines. The emphasis was placed on lapatinib-resistant breast cancer cells (BT/Lap^R1.0, MDA-MB-361, SK/Lap^R1.0, and MDA-MB-453). The compounds HSP70-36/37/40/43/46 significantly inhibited the proliferation of human breast cancer cells. Compound HSP70-36 inhibited the growth of BT474 and BT/Lap^R1.0 cells with IC₅₀ values of 1.41 μM and 1.47 μM, respectively. The binding affinity of HSP70-36 / HSP70 was evaluated by surface plasmon resonance and yielded K_d values of 2.46 μM. The LD₅₀ was 869.0 mgkg⁻¹. These data suggest that HSP70-36 may be a potential candidate compound for tumour treatment.

Keywords: HSP70 inhibitors, Lapatinib-resistant breast cancer cell, Biological evaluation

1. Introduction

Heat Shock Protein 70 (HSP70) maintains protein homeostasis by controlling protein quality and turnover during both normal and stress conditions. [1] Genetic and biochemical studies have implicated HSP70 in a range of diseases and conditions, such as cancer, allograft rejection, neurodegeneration, and infection, and this variety of conditions may be consistent with HSP70's diverse activities. HSP70 plays significant roles in several biological processes, including the regulation of apoptosis, via its roles in intrinsic and extrinsic apoptotic pathways, such as blocking the release of cytochrome c from mitochondria [2] and the binding of death ligands (Tumour necrosis factor (TNF) and TNF-related apoptosis inducing ligand (TRAIL)) to their corresponding receptors. [3]

Multidrug resistance to chemotherapeutic drugs is an important reason underlying clinical chemotherapy failure. Consistent with the hypothesis that HSP70 is involved in cancer resistance during tumour treatment, [4, 5] HSP70 has been found to be abundantly expressed in a variety of human cancers and is often related to metastasis and poor outcome in cancer patients. [6, 7] HSP70 inhibitors can reduce or suppress its physiological function in tumour cells, which may suppress or reduce cancer resistance. [7] Many reports have shown that autophagy plays a protective role in cell death. [8] RNA-seq results indicated that the genes HSPA1A/B, [9] which can promote autophagy, were significantly upregulated in HYF127c/Cu-treated cells (HSPA1A: fold=6.3, p=0.0004). The mitogen-activated protein kinase (MAPK) 11/12/13/14 inhibitor SB203580 downregulated autophagy by inhibiting the transcription of the autophagy genes HSPA1A, promoted HYF127c/Cu-induced cell death. [8] Thus, HSPA1A/B likely contributes to chemotherapy resistance in cancer cells. [8]

Only a small number of HSP70 inhibitors have been identified. [10, 11] In most cases, the active site and mode of binding of these molecules to HSP70 are unknown. [12, 13] Here, we aim to identify new chemical entities based on the HSP70 N-terminal nucleotide binding domain for the potential treatment of clinical tumour resistance. In the next section, we discuss novel small molecules that were designed based on the N-terminal nucleotide binding domain that may reduce the viability of resistant cancer cells. These compounds are novel small molecule drugs that had not been identified to date as

HSP70 inhibitors.

2. Results and Discussion

2.1 Computational studies

The design of small molecule drugs by virtual fragment screening and combination virtual library screening is a new approach. Virtual fragment screening and combination virtual library screening were performed to design novel HSP70 inhibitors. The skeleton structures of these small molecules, which are novel, are shown in Fig. 1.

Fig. 1

The calculated binding mode of the compound HSP70-40 at the active site of the HSP70 ATPase domain is shown in Fig. 2, which was generated with the Discovery Studio 2.5 software. The ligand docking results showed that the binding pocket involves the key amino acid residues Thr14, Thr15, Gly205, Arg274 and Ser278. The N3 atom on the pyridine ring of compound HSP70-40 donates 2 hydrogen bonds from to the backbone of Thr15 and Gly205. Two hydrogen bonds that formed deep in the ATP-binding site seem to act as an anchor to facilitate the binding of compound HSP70-40. Compound HSP70-40 can be further stabilized in the ATP-binding site by the hydrophobic interactions of its nonpolar groups with the side chains of Gly13, Tyr16, Gly204, Gly206, Thr207, Ser343, Gly345 and Asp369. Most small compounds share the key feature of occupying a significant portion of the ATP-binding site.

Fig. 2

2.2. Chemistry

(All the novel compounds were named by “HSP70-number”, and the numbering of the compounds in table was simply represented only by number.)

2.2.1. Synthesis of compounds HSP70-(1–20)

Compounds HSP70-(1–20) were synthesized according to the procedure outlined in Scheme 1. 2-chloro-5-(chloromethyl)thiazole (**1a**) was reacted with tert-butyl methyl (piperidin-4-yl)carbamate (**2**) in the presence of K_2CO_3 to produce tert-butyl 1-((2-chlorothiazol-5-yl)methyl)piperidin-4-yl(methyl)carbamate(**3a**), which was subsequently transformed into **4a** via one successive substitution reaction. Next, **5a** was obtained via the deprotection of amino groups with trifluoroacetic acid in dichloroethane. Amide **5a** was reacted with the corresponding substituted benzyl chloride (**6**) to afford HSP70-(1–20) via a substitution reaction.

Scheme 1

2.2.2. Synthesis of compounds HSP70-(21–31)

Compounds HSP70-(21–31) were synthesized according to the procedure outlined in Scheme 2. **3a** was subsequently transformed into 1-((2-chlorothiazol-5-yl)methyl)-N-methylpiperidin-4-amine(**5b**) via the deprotection of amino groups with trifluoroacetic acid in dichloroethane. Amide **5b** was reacted with the corresponding substituted benzyl chloride (**6**) to afford HSP70-(21–34) via a substitution reaction.

Scheme 2

2.2.3. Synthesis of compounds HSP70-(32–34)

Compounds HSP70-(32–34) were synthesized according to the procedure outlined in Scheme 3. 2-chloro-5-(chloromethyl)thiazole (**1a**) and tert-butyl piperidin-4-ylcarbamate (**2a**) were reacted in the presence of K_2CO_3 to produce tert-butyl 1-((2-chlorothiazol-5-yl)methyl)piperidin-4-ylcarbamate (**3d**). Compound **3d** was treated with trifluoroacetic acid in dichloroethane and refluxed for 5 h to obtain the intermediate product 1-((2-chlorothiazol-5-yl)methyl)piperidin-4-amine (**5c**), followed by an amine substitution reaction of the intermediate **5c** with substituted benzyl chloride (**6**) to yield HSP70-(32–34).

Scheme 3

2.2.4. Synthesis of compounds HSP70-(35–56)

Compounds HSP70-(35–56) were synthesized according to the procedure outlined in Scheme 4. 2,4-dichloropyrimidine(**1b**) and tert-butyl methyl (piperidin-4-yl)carbamate (**2**) were reacted in dimethylformamide at room temperature in the presence of K_2CO_3 to yield tert-butyl 1-(2-chloropyrimidin-4-yl)piperidin-4-yl(methyl)carbamate(**3b**). Subsequently, **3b** was converted to the ether via a treatment with CH_3SNa or CH_3ONa to produce intermediates of type **4d**. Next, a stirred solution of **4d** in dichloroethane was treated with trifluoroacetic acid at room temperature to obtain **5d**. Amide **5d** was reacted with the corresponding substituted benzyl chloride (**6**) to yield HSP70-(35–56) via a substitution reaction.

Scheme 4

2.2.5. Synthesis of compounds HSP70-(57–67)

Compounds HSP70-(57–67) were synthesized according to the procedure outlined in Scheme 5. 4,6-dichloropyrimidine (**1c**) was reacted with tert-butyl methyl (piperidin-4-yl)carbamate (**2**) in the presence of K_2CO_3 to produce tert-butyl 1-(6-chloropyrimidin-4-yl)piperidin-4-yl(methyl)carbamate (**3c**), which was subsequently transformed into tert-butyl 1-(6-methoxypyrimidin-4-yl)piperidin-4-yl(methyl)carbamate (**4e**) via one successive substitution reaction. Subsequently, 1-(6-methoxypyrimidin-4-yl)-N-methylpiperidin-4-amine (**5e**) was obtained via the deprotection of amino groups with trifluoroacetic acid in dichloroethane, followed by the amine substitution reaction of intermediate **5e** with substituted benzyl chloride (**6**) to afford HSP70-(57–67).

Scheme 5

2.3. Cell viability assay

As previously reported, HSP70 over expression has been associated with poor prognosis in multiple forms of cancer and is thought to offer a survival advantage to cancer cells by interacting with multiple components of both caspase-dependent and caspase-independent apoptotic pathways. [14, 15] To identify molecules that induce apoptosis in cells, 67 piperidine derivatives were evaluated in six breast cancer cell lines, including BT474, BT/Lap^R1.0, MDA-MB-361, SK-BR3, SK/Lap^R1.0 and MDA-MB-453. A dose exceeding the IC₅₀ was selected to improve the detection of cell viability. Lapatinib is used in combination with capecitabine to treat advanced or metastatic breast cancers that express high levels of ErbB-2 or are resistant to anthracycline, paclitaxel or trastuzumab (Herceptin). Therefore, lapatinib was selected as a positive control in the cell viability assay. Furthermore, emphasis was placed on lapatinib-resistant breast cancer cells (BT/Lap^R1.0, MDA-MB-361, SK/Lap^R1.0 and MDA-MB-453), whose data may be more clinically applicable. As shown in Fig. 3, inhibitors HSP70-36/37/40/43/46 demonstrated significantly potent anti-cancer activities against the six breast cancer cell lines, especially the lapatinib-resistant breast cancer cells (lapatinib 1 μ M). Therefore, the scaffolds of these piperidine derivatives will be further probed to improve their activity. Specifically, a dose of 5 μ M achieved inhibitory rates of more than 50% of the piperidine derivatives in MDA-MB-453 cells, indicating that most of these compounds were

cytotoxic to MDA-MB-453 cells. IC₅₀ values were also measured in BT474 and BT/Lap^R1.0 human breast cancer cells and are shown in Table 1.

Fig. 3

Table 1

We also assessed the abilities of HSP70-36/37/40/43/46 to induce cell death in several other cancer cell lines, including hepatocellular carcinoma (HepG2 and Huh-7), gastric cancer (N87 and BGC823), pancreatic cancer (AsPC-1 and MIA-PaCa-2), colorectal cancer (COLO205 and HT-29), lung cancer (A549 and H460) and normal cell lines (human embryonic lung fibroblast WI-38 cells and lung epithelial fibroblast MRC5 cells). The test samples were diluted to final concentrations of 4, 1, 0.25, 0.063, and 0.016 μM and added to wells containing 50 μL of medium. Samples were set up in triplicate, and cells were incubated for 72 h at 37°C. As shown in Fig. 4, compounds HSP70-36/43 somewhat inhibited and were cytotoxic to both tumour and normal cells, and these effects did not differ between cell types. Compounds HSP70-37/40/46 exerted a slightly stronger effect on hepatocellular carcinoma cells than other cancer cells. Moreover, the cytotoxicity was lower to normal cells than to other tumour cells, indicating that these compounds may have fewer side effects.

Fig. 4

2.4. SPR studies

To understand the molecular basis by which piperidine derivatives induce apoptosis, surface plasmon resonance (SPR) studies of HSP70 inhibitors were performed using an optical biosensor Biacore T100 (GE), as reported elsewhere. [16, 17] This process was first highlighted in mass-based chemical experiments. The exponential curves produced according to concentration-dependent responses during both the association and dissociation phases indicated efficient interactions with immobilized HSP70 protein. Each compound was examined at different concentrations (0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 μM), T=25°C and 5% dimethyl sulfoxide (assay buffer). A typical analysis cycle consisted of a 90 s sample injection (30 μl/min) and 120 s of buffer flow (dissociation phase). The dissociation constant (K_d value) for Ver-155008/HSP70 was determined to be 0.2060 μM, which is similar to the reported 0.3 μM value. [18] As shown in Table 2, the thermodynamic dissociation constants (K_d values) for HSP70-12/HSP70 and HSP70-36/HSP70 were 0.1999 μM and 0.2463 μM, respectively, indicating they have the same binding affinity for HSP70 as the positive control Ver-155008 at 10 μM. Furthermore,

HSP70-36 inhibited the proliferation of human breast cancer cells BT474 and BT/Lap^R1.0 with IC₅₀ values of 1.41 μM and 1.47 μM, respectively. Both results identified HSP70 as the main HSP70-36 target in cancer cells. Thus, compound HSP70-36 may prolong the survival time of cancer patients by inhibiting HSP70.

Table 2

2.5. Colorimetric determination of HSP70 ATPase activity

To examine the ability of the tested compounds to act on the HSP70 ATPase domain, the HSP70 ATPase activity was evaluated using an assay outlined in previous reports with some modifications. [19] In the preliminary screening system, the compounds that inhibited HSP70 ATPase activity by 20% at a concentration of 200 μM were defined as effective inhibitors. [20, 21] Two positive control compounds were used to verify the accuracy of the screening system. The inhibition rate of the control compounds AZ and VER155008 reached 53.13% and 43.03%, respectively, indicating the high efficacy and accuracy of the experimental system. Dose-dependent effects were observed for various concentrations of the positive controls incubated with HSP70 (Fig. 5). HSP70-37 was identified as a potential inhibitor because its inhibition rate exceeded 20% in multiple experiments. Other compounds could not effectively inhibit the ATPase turnover (Table 3). Therefore, the SPR and ATPase activity results indicate that the piperidine derivatives have a strong binding affinity for HSP70. However, these compounds may not act on the HSP70 ATPase domain. Identifying the specific site on HSP70 which these piperidine derivatives act requires more in-depth studies. Further genetic testing may also be required.

Fig. 5

Table 3

2.6. Acute toxicity test by single oral gavage with HSP70-36 in mice

We tested the acute toxicity of HSP70-36 by treating mice with a single oral gavage. Mice that received HSP70-36 at 1555 mg/kg were dead after 5 min. None of the control mice died from d 1 to d 14, which was the end of the test. We observed 10, 9, 8, 5, 4, 1 and 0 deaths in the groups treated with 1555, 1296, 1080, 900, 750, 625 and 520 mg/kg HSP70-36, respectively (Table 4). No obvious abnormalities were observed from the organ coefficients at the end of the experiment. Using the Bliss method[22, 23], the LD₅₀

was calculated to be $869.0 \text{ mg} \cdot \text{kg}^{-1}$ with 95% a confidence interval of 776.5–969.4 $\text{mg} \cdot \text{kg}^{-1}$.

Table 4

3. Conclusions

Novel piperidine derivatives significantly inhibit the growth of lapatinib-resistant breast cancer cells and other tumour cell lines, indicating that they may be effective clinical therapies for drug-resistant breast cancer patients by targeting HSP70. The R^1 of ring A was substituted from a thiazole to a pyrimidine. Pyrimidine is more active than thiazole. The methylthio group at the position R^1 was well suitable to the function of the compound. These substituents exhibited better anti-tumour activity than substitutions with halogens, such as chlorine and fluorine. However, the position of the substituent is critical because the inhibition rate markedly decreased when 2-substituents were changed to 4-substituents. Placing R^3 of ring C in the ortho and/or para rather than intero position improved the anti-tumour activity.

These piperidine derivatives showed good anticancer bioactivities with a very low inhibiting rate of the HSP70 ATPase. However, these compounds showed moderate binding to HSP70. Therefore, these compounds did not interact with the HSP70 ATPase domain while acting on HSP70. An allosteric site on the HSP70 N-terminal nucleotide binding domain has been reported. [24, 25] We identified this allosteric site as the purple areas shown in Fig.6. In other words, the virtual screening domain included the allosteric site and ATPase domain, which indicates that these compounds may have acted on the allosteric site. Therefore, these compounds affect HSP70 function without affecting ATPase, which allows them to inhibit the growth of tumour cells. Nevertheless, in-depth studies are required to clarify the mechanism of action.

These findings may be relevant to the possible therapeutic applications of piperidine derivatives and design of new therapeutic approaches. Our findings may hold key importance for the development of effective HSP70 inhibitors. Based on the determination of dissociation constants, we can examine how these compounds suppress the viability of cancer cells by inhibiting HSP70, which can provide a favourable clinical profile. In addition, the identification of piperidine derivatives as inhibitors of HSP70 suggests that these compounds may serve as a new tool to further explore the biology of

this chaperone protein and perhaps help unravel the affected complex processes.

4. Experimental Section

4.1 Computational methods

The crystal structures of the nucleotide binding domain (NBD) in complex with ADP (adenosine diphosphate) were recovered from the Brookhaven Protein Data Bank (<http://www.rcsb.org/pdb/home/home.do>) (entry codes 3I33[26]). The docking domain was detected using the MVD4.0 software and divided into red, yellow and purple areas for virtual fragment screening (Fig. 6). The red and yellow areas constituted the domain that the ADP acted on. The fragment libraries were taken from the Cambridge database and our patented compound library containing a total of 243,712 compounds. The receptor files were prepared in Chimera. The grid-based energy scoring function was used to judge the affinity of the fragment for HSP70. The energy-scoring component of Dock 4.0 was based on the implementation of force field scoring. Force field scores are approximate molecular mechanics interaction energies, consisting of van der Waals and electrostatic components: $\Delta G_{\text{binding}} \approx \Delta H_{\text{binding}}$. Anchor-first search was used for small molecule conformation searches. Optimization fragments were selected with critical site point matching, chemical matching and energy minimization. A virtual combination library was established using the module Reaction of Enumerate Library of Discovery Studio 2.5. ADMET screening was used to estimate a range of ADMET-related properties for small molecules, and we excluded compounds with poor druggability. Precise docking was then performed on the Surflex-Dock of SYBYL 6.9. The geometries of these compounds were subsequently optimized using the Tripos force field [27] with Gasteiger-Hückel charges. The Powell method encoded in SYBYL 6.9 was used for energy minimization under an 8 Å nonbonded cutoff and an energy convergence gradient value of 0.005 kcal/(mol Å). The 3D grid was established by the AutoGrid algorithm to evaluate the binding energies between the inhibitors and HSP70 ATPase domain. The number of generations, energy evaluations, and docking runs were set to 380000, 1600000, and 20, respectively. The atomic charges used were Gasteiger-Hückel [27] for the inhibitors and Kollmal-all-atom for HSP70 NBD. Precise docking was then performed on the Surflex-Dock of SYBYL 6.9.

Fig. 6

4.2. General chemistry

¹H NMR spectra were recorded in CDCl₃-d on a Bruker-ARX300 spectrometer with TMS as an internal standard. ¹³C NMR spectra were recorded in DMSO-d6 on a Bruker-axr400 spectrometer. All chemical shifts are quoted in δ values (ppm). MS spectra were recorded using a Micromass ZabSpect. Reagents and solvents were purchased from common commercial suppliers.

4.2.1 General procedure for the synthesis of compounds HSP70-(1-20)

General procedure 1: 8.40 g (50 mmol) of 2-Chloro-5-(chloromethyl)thiazole, 10.70 g (50 mmol) of tert-butyl methyl(piperidin-4-yl)carbamate, and 6.90 g (50 mmol) of anhydrous K₂CO₃ were added to a 1000 ml three-necked flask containing 300 ml of dimethyl formamide (DMF). The mixture was stirred at room temperature for 6 h. A large amount of ice water was added to precipitate a solid. The solid was washed with large amounts of petroleum ether, filtered, and dried in a vacuum. A white powder consisting of N-methyl-N-{1-[2-chloro-5-thiazolyl] methyl}-4-piperidinyl carbamic acid tert-butyl ester (3a) (16.90 g) was obtained (yield 98%).

A solution of 3a (6.92 g, 20 mmol) in anhydrous ethanol (100 ml) was treated with sodium ethoxide or sodium methyl mercaptan (25 mmol) and refluxed for 8 h. The mixture was cooled to room temperature in a water bath. Solvent was removed under a vacuum at 50°C. The residue was dissolved in water, extracted with ethyl acetate, dried over anhydrous MgSO₄, and filtered. The organic phase was removed under a vacuum at 45°C to obtain colourless oily substance 4a or 4b.

Compound 4a (10 mmol) was dissolved in dichloromethane (50 ml), and an excess of trifluoroacetic acid was added in an ice bath. The mixture was stirred at room temperature for 5 h. The solvent was removed in a vacuum at 40°C to obtain yellow oily substance of 5a.

A solution of compound 5a (0.2 mmol) was dissolved in DMF (5 ml). Anhydrous K₂CO₃ (0.2 mmol) and benzyl chloride (0.2 mmol) were added, and the mixture was stirred at room temperature for 4 h. Water (40 ml) was added, and the compound was extracted with dichloromethane, dried with Na₂SO₄, and the solvent was removed to produce a colourless oily substance, HSP70-(1-20).

4.2.1.1. N-(3-Chlorobenzyl)-1-(2-ethoxythiazol-5-yl)methyl)-N-methylpiperidin-4-

amine (HSP70-1). ^1H NMR (300 MHz, CDCl_3 -d): δ 1.46–1.43 (t, $J=7.2$ Hz, 3H); 1.67–1.64 (m, 2H); 1.82–1.79 (m, 2H); 82.03–1.98 (m, 2H); 82.21 (s, 3H); 82.44 (m, 1H); 83.01–2.99 (m, 2H); 83.56 (s, 4H); 84.46–4.41 (q, $J=7.2$ Hz, 2H); 86.90 (s, 1H); 87.35–7.22 (m, 4H). MS(TOF) 379.9 (M^+).

4.2.1.2. 2-(((1-((2-Ethoxythiazol-5-yl)methyl)piperidin-4-yl)(methyl)amino)methyl)benzonitrile (HSP70-2). ^1H NMR (300 MHz, CDCl_3 -d): δ 1.45–1.42 (t, 3H); 1.70–1.69 (m, 2H); 1.82 (m, 2H); 82.02 (m, 2H); 82.22 (s, 3H); 82.51 (m, 1H); 83.02–2.99 (m, 2H); 83.56 (s, 2H); 83.78 (s, 2H); 84.46–4.40 (q, 2H); 86.90 (s, 1H); 87.64–7.28 (m, 4H). MS(TOF) 370.5 (M^+).

4.2.1.3. N-(3,4-dichlorobenzyl)-1-((2-ethoxythiazol-5-yl)methyl)-N-methylpiperidin-4-amine (HSP70-3). ^1H NMR (300 MHz, CDCl_3 -d) δ 1.42–1.46 (t, 3H); 1.63–1.66 (m, 2H); 1.77–1.80 (d, 2H); 82.00 (t, 2H); 82.20 (s, 3H); 82.43 (m, 1H); 82.98–3.01 (d, 2H); 82.53–3.56 (d, 4H); 84.41–4.46 (m, 2H); 86.90 (s, 1H); 87.15–7.18 (dd, 1H); 87.37–7.39 (d, 2H); 87.44–7.75 (d, 1H). MS(TOF) 414 (M^+).

4.2.1.4. 4-(((1-((2-Ethoxythiazol-5-yl)methyl)piperidin-4-yl)(methyl)amino)methyl)benzonitrile (HSP70-4). ^1H NMR (300 MHz, CDCl_3 -d) δ 1.42–1.46 (t, 3H); 1.63–1.67 (m, 2H); 1.77–1.81 (d, 2H); 81.98–2.03 (t, 2H); 82.20 (s, 3H); 82.43 (m, 1H); 82.98–3.01 (d, 2H); 83.56–3.63 (d, 4H); 84.40–4.46 (m, 2H); 86.90 (s, 1H); 87.44–7.46 (d, 2H); 87.60–7.62 (d, 2H). MS(TOF) 370.5 (M^+).

4.2.1.5. N-(2-chloro-6-fluorobenzyl)-1-((2-ethoxythiazol-5-yl)methyl)-N-methylpiperidin-4-amine (HSP70-5). ^1H NMR (300 MHz, CDCl_3 -d) δ 1.43–1.46 (t, 3H); 1.72–1.75 (m, 2H); 81.84–1.87 (d, 2H); 82.00–2.03 (t, 2H); 82.26 (s, 3H); 82.53 (m, 1H); 83.01–3.04 (d, 2H); 83.58 (s, 2H); 83.73 (s, 2H); 84.41–4.46 (m, 2H); 86.91 (s, 1H); 86.98–6.99 (m, 1H); 87.18–7.28 (m, 2H). MS(TOF) 397.9 (M^+).

4.2.1.6. N-(2-chlorobenzyl)-1-((2-ethoxythiazol-5-yl)methyl)-N-methylpiperidin-4-amine (HSP70-6). ^1H NMR (300 MHz, CDCl_3 -d) δ 1.42–1.46 (t, 3H); 1.67–1.71 (m, 2H); 1.82–1.85 (d, 2H); 81.99–2.04 (t, 2H); 82.26 (s, 3H); 82.49 (m, 1H); 82.99–3.02 (d, 2H); 83.56 (s, 2H); 83.70 (s, 2H); 84.41–4.46 (m, 2H); 86.91 (s, 1H); 87.17–7.20 (m, 1H); 87.22–7.24 (m, 1H); 87.22–7.24 (m, 1H); 87.33–7.35 (dd, 1H); 87.49 (dd, 1H). MS(TOF) 379.9 (M^+).

4.2.1.7. 1-((2-Ethoxythiazol-5-yl)methyl)-N-(4-fluorobenzyl)-N-methylpiperidin-4-amine (HSP70-7). ^1H NMR (300 MHz, CDCl_3 -d) δ 1.42–1.46 (t,3H); 1.68–1.69 (m,2H); 1.80 (d,2H); 1.98–2.03 (t,2H); 2.21 (s,3H); 2.45 (m,1H); 2.98–3.01 (d,2H); 3.56 (s,4H); 4.41–4.46 (m,2H); 6.90 (s,1H); 6.98–7.03 (t,2H); 7.28–7.30 (d,2H). MS(TOF) 363.5 (M^+).

4.2.1.8. N-(2,4-dichlorobenzyl)-1-((2-ethoxythiazol-5-yl)methyl)-N-methylpiperidin-4-amine (HSP70-8). ^1H NMR (300 MHz, CDCl_3 -d) δ 1.42–1.46 (t,3H); 1.61–1.69 (m,2H); 1.80–1.83 (d,2H); 1.99–2.04 (t,2H); 2.24 (s,3H); 2.47 (m,1H); 2.99–3.02 (d,2H); 3.57 (s,2H); 3.65 (s,2H); 4.41–4.46 (m,2H); 6.91 (s,1H); 7.21–7.23 (dd,1H); 7.36 (d,1H); 7.45 (d,1H). MS(TOF) 414.4 (M^+).

4.2.1.9. N-(2,6-dichlorobenzyl)-1-((2-ethoxythiazol-5-yl)methyl)-N-methylpiperidin-4-amine (HSP70-9). ^1H NMR (300 MHz, CDCl_3 -d) δ 1.42–1.46 (t,3H); 1.78–1.84 (m,4H); 2.05 (m,2H); 2.26 (s,3H); 2.56 (m,1H); 3.01–3.06 (m,2H); 3.59 (m,2H); 3.85 (s,2H); 4.43–4.45 (m,2H); 6.92 (s,1H); 7.14–7.16 (m,1H); 7.30–7.31 (m,3H). MS(TOF) 414.4 (M^+).

4.2.1.10. N-(2,5-dichlorobenzyl)-1-((2-ethoxythiazol-5-yl)methyl)-N-methylpiperidin-4-amine (HSP70-10). ^1H NMR (300 MHz, CDCl_3 -d) δ 1.42–1.44 (t,3H); 1.66–1.69 (m,2H); 1.81 (m,2H); 2.02 (m,2H); 2.26 (s,3H); 2.48 (m,1H); 3.00–3.03 (m,2H); 3.58 (s,1H); 3.65 (s,1H); 4.09–4.46 (m,2H); 6.92 (s,1H); 7.16–7.17 (m,1H); 7.27–7.28 (m,1H); 7.54 (s,3H). MS(TOF) 414.4 (M^+).

4.2.1.11. N-methyl-N-(2-methylbenzyl)-1-((2-(methylthio)thiazol-5-yl)methyl)piperidin-4-amine (HSP70-11). ^1H NMR (300 MHz, CDCl_3 -d): 1.71–1.72 (m,2H); 1.82 (d,2H); 2.03–2.04 (t,2H); 2.20 (s,3H); 2.37 (s,3H); 2.47 (m,1H); 2.70 (s,3H); 2.98–3.01 (d,2H); 3.57 (s,2H); 3.67 (s,2H); 7.16–7.17 (m,3H); 7.29 (m,1H); 7.42 (s,1H). MS(TOF) 361.6 (M^+).

4.2.1.12. N-methyl-N-(4-methylbenzyl)-1-((2-(methylthio)thiazol-5-yl)methyl)piperidin-4-amine (HSP70-12). ^1H NMR (300 MHz, CDCl_3 -d): 1.67–1.68 (m,2H); 1.80 (d,2H); 1.99–2.01 (t,2H); 2.21 (s,3H); 2.35 (s,3H); 2.45 (m,1H); 2.69 (s,3H); 2.97–2.99 (d,2H); 3.56 (s,2H); 3.66 (s,2H); 7.12–7.14 (d,2H); 7.20–7.28 (d,2H); 7.41 (s,1H). MS(TOF) 361.6 (M^+).

4.2.1.13. N-(3-fluorobenzyl)-N-methyl-1-((2-(methylthio)thiazol-5-yl)methyl)piperidin-4-amine (HSP70-13). ^1H NMR (300 MHz, CDCl_3 -d): δ 1.63–1.67 (m, 2H); δ 1.79–1.82 (d, 2H); δ 1.99–2.05 (t, 2H); δ 2.22 (s, 3H); δ 2.45 (m, 1H); δ 2.69 (s, 3H); δ 2.97–3.00 (d, 2H); δ 3.58 (s, 2H); δ 3.67 (s, 2H); δ 6.93–6.94 (t, 2H); δ 7.06–7.10 (m, 2H); δ 7.26–7.28 (m, 1H); δ 7.42 (s, 1H). MS(ToF) 365.5 (M^+).

4.2.1.14. N-(3-chlorobenzyl)-N-methyl-1-((2-(methylthio)thiazol-5-yl)methyl)piperidin-4-amine (HSP70-14). ^1H NMR (300 MHz, CDCl_3 -d): δ 1.63–1.70 (m, 2H); δ 1.79–1.82 (d, 2H); δ 1.99–2.05 (t, 2H); δ 2.22 (s, 3H); δ 2.44 (m, 1H); δ 2.69 (s, 3H); δ 2.97–3.00 (d, 2H); δ 3.56 (s, 2H); δ 3.66 (s, 2H); δ 7.21–7.24 (m, 3H); δ 7.34 (s, 1H); δ 7.42 (s, 1H); δ 7.42 (s, 1H). MS(ToF) 382 (M^+).

4.2.1.15. N-(4-fluorobenzyl)-N-methyl-1-((2-(methylthio)thiazol-5-yl)methyl)piperidin-4-amine (HSP70-15). ^1H NMR (300 MHz, CDCl_3 -d): δ 1.63–1.67 (m, 2H); δ 1.79–1.82 (d, 2H); δ 1.99–2.05 (t, 2H); δ 2.20 (s, 3H); δ 2.44 (m, 1H); δ 2.69 (s, 3H); δ 2.97–3.00 (d, 2H); δ 3.55 (s, 2H); δ 3.66 (s, 2H); δ 6.99–7.02 (t, 2H); δ 7.26–7.30 (t, 2H); δ 7.41 (s, 1H). MS(ToF) 365.5 (M^+).

4.2.1.16. N-(3,4-dichlorobenzyl)-N-methyl-1-((2-(methylthio)thiazol-5-yl)methyl)piperidin-4-amine (HSP70-16). ^1H NMR (300 MHz, CDCl_3 -d): δ 1.62–1.66 (m, 2H); δ 1.79 (d, 2H); δ 2.00 (t, 2H); δ 2.20 (s, 3H); δ 2.37–2.46 (m, 1H); δ 2.69 (s, 3H); δ 2.97–3.00 (d, 2H); δ 3.37 (s, 2H); δ 3.84 (s, 2H); δ 6.02–7.17 (d, 1H); δ 7.37–7.72 (m, 3H). MS(ToF) 416.4 (M^+).

4.2.1.17. N-(2-chloro-6-fluorobenzyl)-N-methyl-1-((2-(methylthio)thiazol-5-yl)methyl)piperidin-4-amine (HSP70-17). ^1H NMR (300 MHz, CDCl_3 -d): δ 1.71–1.75 (m, 2H); δ 1.84–1.87 (m, 2H); δ 2.02–2.05 (m, 2H); δ 2.26 (s, 3H); δ 2.52 (m, 1H); δ 2.69 (s, 3H); δ 3.00–3.03 (d, 2H); δ 3.68 (s, 2H); δ 3.72 (s, 2H); δ 6.98–6.99 (m, 1H); δ 7.17–7.20 (m, 2H); δ 7.42 (s, 1H). MS(ToF) 416.4 (M^+).

4.2.1.18. N-(2,6-dichlorobenzyl)-N-methyl-1-((2-(methylthio)thiazol-5-yl)methyl)piperidin-4-amine (HSP70-18). ^1H NMR (300 MHz, CDCl_3 -d): δ 1.72–1.76 (m, 2H); δ 1.83–1.86 (m, 2H); δ 2.02–2.08 (m, 2H); δ 2.25 (s, 3H); δ 2.55 (m, 1H); δ 2.69 (s, 3H); δ 3.00–3.03 (d, 2H); δ 3.68 (s, 2H); δ 3.85 (s, 2H); δ 7.12–7.14 (m, 1H); δ 7.28–7.30 (m, 2H); δ 7.42 (s, 1H). MS(ToF) 416.4 (M^+).

4.2.1.19. N-(2,4-dichlorobenzyl)-N-methyl-1-((2-(methylthio)thiazol-5-yl)methyl)piperidin-4-amine (HSP70-19).

¹H NMR (300 MHz, CDCl₃-d): δ1.42–1.44 (m,3H); δ1.66–1.69 (m,2H); δ1.81 (m,2H); δ2.02 (m,2H); δ2.26 (s,3H); δ2.48 (m,1H); δ3.00–3.03 (d,2H); δ3.58 (s,1H); δ3.65 (s,1H); δ4.41–4.46 (m,2H); δ6.91 (s,1H); δ7.16–7.17 (m,1H); δ7.25–7.27 (m,1H); δ7.54 (s,1H). MS(TOF) 416.4 (M⁺).

4.2.1.20. N-(2,5-dichlorobenzyl)-N-methyl-1-((2-(methylthio)thiazol-5-yl)methyl)piperidin-4-amine (HSP70-20).

¹H NMR (300 MHz, CDCl₃-d): δ1.68–1.69 (m,2H); δ1.81 (m,2H); δ2.04 (m,2H); δ2.26 (s,3H); δ2.47 (m,1H); δ2.69 (s,1H); δ2.98–3.01 (m,2H); δ3.65–3.68 (m,4H); δ7.14–7.17 (m,1H); δ7.27–7.28 (m,1H); δ7.42 (s,1H); δ7.54 (s,1H). MS(TOF) 416.4 (M⁺).

4.2.2 General procedure for the synthesis of compounds HSP70-(21-31)

General procedure 2: Tert-butyl 1-((2-chlorothiazol-5-yl)methyl)piperidin-4-yl(methyl) carbamate (3a, 3.46 g, 10 mmol) was dissolved in dichloromethane (50 ml), and an excess of trifluoroacetic acid was added in an ice bath. The mixture was stirred at room temperature for 5 h. Solvent was removed under a vacuum to obtain a yellow oily substance consisting of 1-((2-chlorothiazol-5-yl)methyl)-N-methylpiperidin-4-amine. A stirred solution of 5b (0.5 mmol) in DMF (30 ml) at room temperature was treated with substituted benzyl chloride (0.51 mmol) and anhydrous K₂CO₃ (0.51 mmol) for 4 h. The solution was extracted with dichloromethane, dried with anhydrous MgSO₄, filtered, and concentrated to produce a yellow oily residue that was purified by flash chromatography to yield the desired product as a white powder, HSP70-(21-31).

4.2.2.1. 2-(((1-((2-Chlorothiazol-5-yl)methyl)piperidin-4-yl)(methyl)amino)methyl) benzonitrile (HSP70-21).

¹H NMR (300 MHz, CDCl₃-d): δ1.66–1.70 (m,2H); δ1.84–1.87 (d,2H); δ2.03–2.09 (t,2H); δ2.22 (s,3H); δ2.52 (m,1H); δ2.98–3.01 (d,2H); δ3.65 (s,2H); δ3.79 (s,2H); δ7.34–7.37 (m,2H); δ7.55–7.57 (m,2H); δ7.63–7.65 (d,2H). MS(TOF) 360.9 (M⁺).

4.2.2.2. 4-(((1-((2-Chlorothiazol-5-yl)methyl)piperidin-4-yl)(methyl)amino)methyl) benzonitrile (HSP70-22).

¹H NMR (300 MHz, CDCl₃-d): δ1.62–1.66 (m,2H); δ1.78–1.81 (d,2H); δ1.99–2.05 (t,2H); δ2.18 (s,3H); δ2.43 (m,1H); δ2.69 (s,3H); δ2.69–3.00 (d,2H); δ3.62–3.67 (d, 4H); δ7.41 (s,1H); δ7.44–7.46 (d,2H); δ7.60–7.62 (d,2H).

MS(TOF) 360.9 (M^+).

4.2.2.3. 1-((2-Chlorothiazol-5-yl)methyl)-N-(4-fluorobenzyl)-N-methylpiperidin-4-amine (HSP70-23). 1H NMR (300 MHz, CDCl₃-d): δ 1.64–1.71 (m, 2H); 1.80–1.83 (d, 2H); 2.01–2.07 (t, 2H); 2.20 (s, 3H); 2.45 (m, 1H); 2.97–3.00 (d, 2H); 3.56 (s, 2H); 3.65 (s, 2H); 6.98–7.03 (t, 2H); 7.27–7.316 (m, 2H); 7.34 (s, 1H). MS(TOF) 353.9 (M^+).

4.2.2.4. 1-((2-Chlorothiazol-5-yl)methyl)-N-(3,4-dichlorobenzyl)-N-methylpiperidin-4-amine (HSP70-24). 1H NMR (300 MHz, CDCl₃-d): δ 1.62–1.69 (m, 2H); 1.80–1.82 (d, 2H); 2.01–2.07 (t, 2H); 2.20 (s, 3H); 2.44 (m, 1H); 2.97–3.00 (d, 2H); 3.54 (s, 2H); 3.65 (s, 2H); 7.16–7.18 (d, 1H); 7.35–7.39 (m, 2H); 7.45 (d, 1H). MS(TOF) 404.8 (M^+).

4.2.2.5. N-(3-chlorobenzyl)-1-((2-chlorothiazol-5-yl)methyl)-N-methylpiperidin-4-amine (HSP70-25). 1H NMR (300 MHz, CDCl₃-d): δ 1.64–1.71 (m, 2H); 1.82–1.85 (d, 2H); 2.02–2.08 (t, 2H); 2.22 (s, 3H); 2.47 (m, 1H); 2.97–3.00 (d, 2H); 3.60 (s, 2H); 3.65 (s, 2H); 7.23 (m, 3H); 7.35–7.36 (m, 2H). MS(TOF) 370.3 (M^+).

4.2.2.6. N-(2-chloro-6-fluorobenzyl)-1-((2-chlorothiazol-5-yl)methyl)-N-methylpiperidin-4-amine (HSP70-26). 1H NMR (300 MHz, CDCl₃-d): 1.74–1.75 (m, 2H); 1.85 (d, 2H); 2.07 (t, 2H); 2.26 (s, 3H); 2.54 (m, 1H); 3.00–3.03 (d, 2H); 3.67 (s, 2H); 3.74 (s, 2H); 6.99 (m, 1H); 7.19–7.21 (m, 2H); 7.34 (s, 1H). MS(TOF) 388.3 (M^+).

4.2.2.7. 1-((2-Chlorothiazol-5-yl)methyl)-N-(2,4-dichlorobenzyl)-N-methylpiperidin-4-amine (HSP70-27). 1H NMR (300 MHz, CDCl₃-d): 1.45 (m, 2H); 1.68–1.69 (m, 2H); 2.02–2.08 (t, 2H); 2.25 (s, 3H); 2.47 (m, 1H); 2.98–3.01 (d, 2H); 3.65 (s, 4H); 7.22–7.25 (m, 1H); 7.35–7.37 (m, 2H); 7.45 (s, 1H). MS(TOF) 404.8 (M^+).

4.2.2.8. 1-((2-Chlorothiazol-5-yl)methyl)-N-methyl-N-(2-methylbenzyl)piperidin-4-amine (HSP70-28). 1H NMR (300 MHz, CDCl₃-d): 1.71–1.72 (m, 2H); 1.83 (d, 2H); 2.05–2.06 (t, 2H); 2.20 (s, 3H); 2.37 (s, 3H); 2.48 (m, 1H); 2.98–3.01 (d, 2H); 3.58 (s, 2H); 3.66 (s, 2H); 7.16–7.17 (m, 3H); 7.29 (m, 1H); 7.35 (s, 1H). MS(TOF) 349.9 (M^+).

4.2.2.9. 1-((2-Chlorothiazol-5-yl)methyl)-N-methyl-N-(4-methylbenzyl)piperidin-4-

amine (HSP70-29). ^1H NMR (300 MHz, CDCl_3 -d): δ 1.67 (m, 2H); δ 1.82 (d, 2H); δ 2.04 (t, 2H); δ 2.22 (s, 3H); δ 2.35 (s, 3H); δ 2.47 (m, 1H); δ 2.97–3.00 (d, 2H); δ 3.57 (s, 2H); δ 3.64 (s, 2H); δ 7.13–7.15 (d, 2H); δ 7.21–7.23 (d, 2H); δ 7.34 (s, 1H). MS(TOF) 349.9 (M^+).

4.2.2.10. 1-((2-Chlorothiazol-5-yl)methyl)-N-(3-fluorobenzyl)-N-methylpiperidin-4-amine (HSP70-30). ^1H NMR (300 MHz, CDCl_3 -d): δ 1.63–1.67 (m, 2H); δ 1.80–1.83 (d, 2H); δ 2.01–2.07 (t, 2H); δ 2.22 (s, 3H); δ 2.46 (m, 1H); δ 2.97–3.00 (d, 2H); δ 3.59 (s, 2H); δ 3.65 (s, 2H); δ 6.94 (m, 1H); δ 7.07–7.10 (m, 2H); δ 7.24–7.28 (m, 1H); δ 7.34 (s, 1H). MS(TOF) 353.9 (M^+).

4.2.2.11. 1-((2-Chlorothiazol-5-yl)methyl)-N-(2,6-dichlorobenzyl)-N-methylpiperidin-4-amine (HSP70-31). ^1H NMR (300 MHz, CDCl_3 -d): δ 1.76–1.84 (m, 2H); δ 2.08 (m, 2H); δ 2.26 (m, 2H); δ 2.56 (s, 3H); δ 3.01–3.04 (m, 1H); δ 3.67 (m, 2H); δ 3.86 (s, 2H); δ 7.15–1.17 (s, 1H); δ 7.28–7.30 (t, 1H); δ 7.32–7.36 (m, 3H). MS(TOF) 404.8 (M^+).

4.2.3 General procedure for the synthesis of compounds HSP70- (32-34)

General procedure 3: General procedures 1: 2-Chloro-5-(chloromethyl)thiazole (8.40 g, 50 mmol), tert-butyl piperidin-4-ylcarbamate (50 mmol), and anhydrous K_2CO_3 (6.90 g, 50 mmol) were to a 1000 ml three-necked flask containing 300 ml of dimethyl formamide (DMF). The mixture was stirred at room temperature for 6 h. A large amount of ice water was added to precipitate a solid. The solid was washed with large amounts of petroleum ether, filtered, and dried in a vacuum. A white powder consisting of tert-butyl 1-((2-chlorothiazol-5-yl)methyl)piperidin-4-ylcarbamate (3d, 16.90 g) was obtained (yield 98%).

One part F_3CCOOH was added to 10 mmol of tert-butyl 1-((2-chlorothiazol-5-yl)methyl)piperidin-4-ylcarbamate in anhydrous dichloroethane (50 ml). The solution was stirred at room temperature for 5 h. The solution was removed at 35°C under a vacuum and recrystallized from ethanol-ethyl acetate to yield a white powder. A stirred solution of white powder (5 mmol) in DMF (30 ml) at room temperature was treated with substituted benzyl chloride (5.1 mmol) and anhydrous K_2CO_3 (5.1 mmol) for 4 h. The solution was extracted with dichloromethane, dried with anhydrous MgSO_4 , filtered, and concentrated to provide yellow oily residue that was purified by flash chromatography to

yield the desired product as a white powder, HSP70-(32-34).

4.2.3.1. 1-((2-Chlorothiazol-5-yl)methyl)-N-(3,4-dichlorobenzyl)piperidin-4-amine (HSP70-32). ^1H NMR (300 MHz, CDCl_3 -d): δ 1.44–1.45 (m, 2H); 1.871 (m, 2H); 82.09–2.10 (d, 2H); 82.51 (m, 1H); 82.86–2.89 (d, 2H); 83.64–3.65 (d, 2H); 83.78 (s, 2H); 87.17–7.19 (dd, 1H); 87.34 (s, 1H); 87.38–7.41 (d, 1H); 87.46 (d, 1H). MS(ToF) 390.8 (M^+).

4.2.3.2. 1-((2-chlorothiazol-5-yl)methyl)-N-(4-fluorobenzyl)piperidin-4-amine (HSP70-33). ^1H NMR (300 MHz, CDCl_3 -d): δ 1.46–1.51 (m, 2H); 1.89–1.92 (m, 2H); 82.05–2.12 (d, 2H); 82.54–2.56 (m, 1H); 82.87–2.90 (d, 2H); 83.64 (s, 2H); 83.80 (s, 2H); 86.99–7.04 (m, 2H); 87.31–7.34 (d, 3H). MS(ToF) 339.9 (M^+).

4.2.3.3. N-(3-chlorobenzyl)-1-((2-chlorothiazol-5-yl)methyl)piperidin-4-amine (HSP70-34). ^1H NMR (300 MHz, CDCl_3 CDCl_3 -d): δ 1.43–1.46 (m, 2H); 1.88–1.92 (m, 2H); 82.07–2.13 (m, 2H); 82.53 (m, 1H); 82.86–2.90 (d, 2H); 83.64 (s, 2H); 83.81 (s, 2H); 87.21–7.26 (m, 3H); 87.34–7.35 (m, 2H). MS(ToF) 356.3 (M^+).

4.2.4 General procedure for the synthesis of compounds HSP70-(35-56)

General procedure 4: 2, 4-dichloropyrimidine (40 mmol), tert-butyl methyl(piperidin-4-yl)carbamate (41 mmol), and anhydrous K_2CO_3 (41 mmol) were added to a 1000 ml three-necked flask containing 300 ml of DMF. The mixture was stirred at room temperature for 6 h. A large amount of ice water was added to precipitate a solid. The solid was washed with large amounts of petroleum ether, filtered, and dried in a vacuum. Flash chromatography (ethyl acetate-petroleum ether, 1:2) of the residue was performed to obtain white powder 3b (tert-butyl 1-(2-chloropyrimidin-4-yl) piperidin-4-yl (methyl)carbamate).

A solution of 3b (20 mmol) in anhydrous THF (100 ml) was treated with CH_3SNa or CH_3ONa (30 mmol) and KI (20 mmol) and refluxed for 8 h. The mixture was cooled to room temperature in a water bath. Solvent was removed under vacuum at 50°C. The residue was dissolved in water, extracted with ethyl acetate, dried over anhydrous Na_2SO_4 , and filtered. The organic phase was removed under a vacuum at 45°C to obtain colourless oily residue 4 d.

The colourless oily residue (10 mmol) was dissolved in dichloromethane (50 ml). An

excess of trifluoroacetic acid was added in an ice bath. The mixture was stirred at room temperature for 5 h. Solvent was removed under a vacuum to obtain yellow oily residue 5d.

A solution of yellow oily residue 5d (0.3 mmol) was dissolved in DMF (5 ml). Anhydrous K₂CO₃ (0.31 mmol) and substituted benzyl chloride (0.31 mmol) were added, and the solution was stirred at room temperature for 4 h. A total of 40 ml water was added, and the compound was extracted with dichloromethane and dried with Na₂SO₄, and the solvent was removed to produce a colourless oily substance, HSP70-(35-56)

4.2.4.1. N-(4-fluorobenzyl)-N-methyl-1-(2-(methylthio)pyrimidin-4-yl)piperidin-4-amine (HSP70-35). ¹H NMR (300 MHz, CDCl₃-d): δ1.56–1.60 (m,2H); δ1.90–1.93 (d,2H); δ2.20 (s,3H); δ2.53 (s,3H); δ2.73 (m,1H); δ2.85–2.92 (m,2H); δ3.57 (s,2H); δ4.47–4.49 (d,2H); δ6.21–6.23 (d,1H); δ6.99–7.03 (t,2H); δ7.28–7.31 (m,2H); δ8.01–8.03 (d,1H). MS(TOF) 346.5 (M⁺).

4.2.4.2. 4-((Methyl(1-(2-(methylthio)pyrimidin-4-yl)piperidin-4-yl)amino)methyl)benzonitrile (HSP70-36). ¹H NMR (300 MHz, CDCl₃-d): δ1.55–1.59 (m,2H); δ1.91–1.94 (d,2H); δ2.21 (s,3H); δ2.50 (s,3H); δ2.75 (m,1H); δ2.84–2.90 (t,2H); δ3.65 (s,2H); δ4.48 (d,2H); δ6.21–6.22 (d,1H); δ7.46–7.48 (d,3H); δ7.61–7.63 (d,2H); δ8.01–8.02 (d,1H). ¹³C-NMR (DMSO, 100 MHz) δ13.90; 27.80; 37.97; 43.40; 57.29; 60.83; 99.58; 109.95; 119.52; 129.63; 132.63; 146.97; 156.06; 160.77; 170.48. MS(TOF) 353.5 (M⁺).

4.2.4.3. N-(2-chloro-6-fluorobenzyl)-N-methyl-1-(2-(methylthio)pyrimidin-4-yl)piperidin-4-amine (HSP70-37). ¹H NMR (300 MHz, CDCl₃-d): δ1.62–1.69 (m,2H); δ1.97–2.00 (d,2H); δ2.28 (s,3H); δ2.50 (s,3H); δ2.85–2.92 (t,3H); δ3.71 (s,2H); δ4.5 (s,2H); δ6.21–6.23 (d,1H); δ6.99–7.05 (m,1H); δ7.20–7.22 (m,2H); δ8.00–8.02 (d,1H). ¹³C-NMR (DMSO, 100 MHz) δ13.90; 27.56; 37.19; 43.52; 48.32; 61.48; 99.56; 114.72; 125.42; 126.08; 130.40; 135.96; 136.02; 156.06; 160.75; 163.29; 170.48. MS(TOF) 380.9 (M⁺).

4.2.4.4. N-(3,4-dichlorobenzyl)-N-methyl-1-(2-(methylthio)pyrimidin-4-yl)piperidin-4-amine (HSP70-38). ¹H NMR (300 MHz, CDCl₃-d): δ1.55–1.57 (m,2H); δ1.90–1.93 (d,2H); δ2.20 (s,3H); δ2.50 (s,3H); δ2.74–2.90 (m,3H); δ3.55 (s,2H); δ4.48 (s,2H); δ6.20–6.22 (d,1H); δ7.19 (s,1H); δ7.37–7.45 (m,2H); δ8.00–8.02 (d,1H). MS(TOF) 397.4

(M⁺).

4.2.4.5. N-(3-chlorobenzyl)-N-methyl-1-(2-(methylthio)pyrimidin-4-yl)piperidin-4-amine (HSP70-39). ¹H NMR (300 MHz, CDCl₃-d): δ1.57–1.60 (m,2H); δ1.90–1.93 (d,2H); δ2.22 (s,3H); δ2.52 (s,3H); δ2.74 (m,1H); δ2.89–2.90 (m,2H); δ3.58 (s,2H); δ4.47–4.49 (d,2H); δ6.21–6.23 (d,1H); δ7.24–7.28 (m,3H); δ7.36 (s,1H); δ8.02–8.03 (d,1H). MS(TOF) 362.9 (M⁺).

4.2.4.6. N-(2,4-dichlorobenzyl)-N-methyl-1-(2-(methylthio)pyrimidin-4-yl)piperidin-4-amine (HSP70-40). ¹H NMR (300 MHz, CDCl₃-d): δ1.64–1.68 (m,2H); δ1.96 (d,2H); δ2.27 (s,3H); δ2.53 (s,3H); δ2.86–2.89 (t,3H); δ3.89 (s,2H); δ4.54 (m,2H); δ6.23–6.24 (d,1H); δ7.17–7.19 (t,1H); δ7.32–7.34 (d,2H); δ8.02–8.04 (d,1H). ¹³C-NMR (DMSO, 100 MHz) δ13.39; 27.30; 37.21; 38.87; 42.95; 53.97; 60.57; 99.07; 127.18; 128.59; 131.87; 133.91; 136.47; 155.55; 160.25; 169.97. MS(TOF) 397.4 (M⁺).

4.2.4.7. 2-((Methyl(1-(2-(methylthio)pyrimidin-4-yl)piperidin-4-yl)amino)methyl)benzonitrile (HSP70-41). ¹H NMR (300 MHz, CDCl₃-d): δ1.59–1.65 (m,2H); δ1.96–1.99 (d,2H); δ2.21 (s,3H); δ2.51 (s,3H); δ2.82–2.93 (t,3H); δ3.80 (s,2H); δ4.50–4.52 (m,2H); δ6.22–6.23 (d,1H); δ7.35–7.38 (m,1H); δ7.56–7.57 (m,2H); δ7.64–7.66 (d,1H); δ8.01–8.02 (d,1H). MS(TOF) 353.5 (M⁺).

4.2.4.8. N-(2,6-dichlorobenzyl)-N-methyl-1-(2-(methylthio)pyrimidin-4-yl)piperidin-4-amine (HSP70-43). ¹H NMR (300 MHz, CDCl₃-d): δ1.63–1.67 (m,2H); δ1.94–1.97 (d,2H); δ2.24 (s,3H); δ2.86–2.90 (m,3H); δ3.87 (s,2H); δ3.94 (s,3H); δ4.51–4.53 (m,2H); δ6.21–6.22 (d,1H); δ7.13–7.17 (t,1H); δ7.30–7.33 (m,2H); δ8.02–8.03 (d,1H). ¹³C-NMR (DMSO, 100 MHz) δ13.39; 26.93; 36.09; 38.87; 43.14; 52.22; 60.95; 99.05; 128.64; 128.59; 129.72; 134.72; 136.09; 155.57; 160.23; 169.97. MS(TOF) 396.3(M⁺).

4.2.4.9. N-methyl-N-(4-methylbenzyl)-1-(2-(methylthio)pyrimidin-4-yl)piperidin-4-amine (HSP70-42). ¹H NMR (300 MHz, CDCl₃-d): δ1.58–1.62 (m,2H); δ1.92–1.95 (d,2H); δ2.23 (s,3H); δ2.36 (s,3H); δ2.52 (s,3H); δ2.76 (m,1H); δ2.85–2.92 (t,2H); δ3.59 (s,2H); δ4.47 (m,2H); δ6.21–6.23 (d,1H); δ7.14–7.16 (d,2H); δ7.22–7.24 (d,2H); δ8.02–8.03 (d,1H). MS(TOF) 342.5 (M⁺).

4.2.4.10. N-(2,5-dichlorobenzyl)-N-methyl-1-(2-(methylthio)pyrimidin-4-yl)piperidin-4-amine (HSP70-44). ¹H NMR (300 MHz, CDCl₃-d): δ1.58–1.62 (m,2H);

δ 1.92–1.95 (d,2H); δ 2.25 (s,3H); δ 2.52 (s,3H); δ 2.86–2.87 (m,1H); δ 2.89–2.93 (t,2H); δ 3.67 (s,2H); δ 4.49–4.51 (m,2H); δ 6.22–6.23 (d,1H); δ 7.23–7.26 (dd,1H); δ 7.38–7.45 (s,1H); δ 7.45–7.47 (d,1H); δ 8.02–8.04 (d,1H). MS(TOF) 397.4 (M^+).

4.2.4.11. N-(3-fluorobenzyl)-N-methyl-1-(2-(methylthio)pyrimidin-4-yl)piperidin-4-amine (HSP70-45). 1 H NMR (300 MHz, CDCl₃-d): δ 1.57–1.61 (m,2H); δ 1.92–1.95 (d,2H); δ 2.23 (s,3H); δ 2.52 (s,3H); δ 2.86–2.87 (m,1H); δ 2.89–2.93 (t,2H); δ 3.61 (s,2H); δ 4.48–4.50 (m,2H); δ 6.22–6.23 (d,1H); δ 6.95 (t,1H); δ 7.09–7.11 (m,2H); δ 7.29 (s,1H); δ 8.02–8.04 (d,1H). MS(TOF) 346.5 (M^+).

4.2.4.12. N-(2-chlorobenzyl)-N-methyl-1-(2-(methylthio)pyrimidin-4-yl)piperidin-4-amine (HSP70-46). 1 H NMR (300 MHz, CDCl₃-d): δ 1.57–1.67 (m,2H); δ 1.94–1.97 (d,2H); δ 2.27 (s,3H); δ 2.76–2.79 (m,1H); δ 2.88–2.94 (t,2H); δ 3.72 (s,2H); δ 3.95 (s,3H); δ 4.48–4.50 (m,2H); δ 6.21–6.22 (d,1H); δ 7.18–7.26 (m,2H); δ 7.35–7.37 (dd,1H); δ 7.49–7.51 (d,1H); δ 8.02–8.04 (d,1H). 13 C-NMR (DMSO, 100 MHz) δ 13.90; 27.80; 37.70; 43.48; 55.02; 61.10; 99.58; 127.50; 128.93; 129.71; 131.20; 133.63; 137.66; 156.08; 160.77; 170.48. MS(TOF) 361.9 (M^+).

4.2.4.13. N-(2-chlorobenzyl)-1-(2-methoxypyrimidin-4-yl)-N-methylpiperidin-4-amine (HSP70-47). 1 H NMR (300 MHz, CDCl₃-d): δ 1.60–1.64 (m,2H); δ 1.95–1.98 (d,2H); δ 2.27 (s,3H); δ 2.52 (s,3H); δ 2.87–2.88 (m,1H); δ 2.91–2.94 (t,2H); δ 3.72 (s,2H); δ 4.49–4.50 (m,2H); δ 6.21–6.24 (d,1H); δ 7.20–7.26 (m,2H); δ 7.35–7.37 (d,1H); δ 7.49 (d,1H); δ 8.02–8.03 (d,1H). MS(TOF) 362.9 (M^+).

4.2.4.14. 4-(((1-(2-Methoxypyrimidin-4-yl)piperidin-4-yl)(methyl)amino)methyl)benzonitrile (HSP70-48). 1 H NMR (300 MHz, CDCl₃-d): δ 1.54–1.58 (m,2H); δ 1.89–1.92 (d,2H); δ 2.20 (s,3H); δ 2.72 (m,1H); δ 2.85–2.92 (t,2H); δ 3.64 (s,2H); δ 3.92 (s,3H); δ 4.45–4.48 (m,2H); δ 6.19–6.20 (d,1H); δ 7.44–7.46 (d,2H); δ 7.59–7.61 (d,2H); δ 7.49–7.51 (d,1H); δ 8.01–8.02 (d,1H). MS(TOF) 337.4 (M^+).

4.2.4.15. 2-(((1-(2-Methoxypyrimidin-4-yl)piperidin-4-yl)(methyl)amino)methyl)benzonitrile (HSP70-49). 1 H NMR (300 MHz, CDCl₃-d): δ 1.60–1.64 (m,2H); δ 1.97–2.00 (d,2H); δ 2.23 (s,3H); δ 2.84–2.94 (m,3H); δ 3.82 (s,2H); δ 3.95 (s,3H); δ 4.49–4.52 (m,2H); δ 6.21–6.22 (d,1H); δ 7.31–7.39 (m,1H); δ 7.57–7.58 (m,2H); δ 7.65–7.67 (d,1H); δ 8.03–8.04 (d,1H). MS(TOF) 337.4 (M^+).

4.2.4.16. 1-(2-Methoxypyrimidin-4-yl)-N-methyl-N-(4-methylbenzyl)piperidin-4-amine (HSP70-50). ^1H NMR (300 MHz, CDCl_3 -d): δ 1.57–1.61 (m, 2H); 1.91–1.92 (d, 2H); 2.22 (s, 3H); 2.35 (s, 3H); 2.74 (m, 1H); 2.86–2.92 (t, 2H); 3.58 (s, 2H); 3.94 (s, 3H); 4.45–4.47 (m, 2H); 6.20–6.21 (d, 1H); 7.13–7.15 (dd, 2H); 7.21–7.23 (dd, 2H); 8.02–8.03 (d, 1H). MS(TOF) 326.4 (M^+).

4.2.4.17. N-(2,5-dichlorobenzyl)-1-(2-methoxypyrimidin-4-yl)-N-methylpiperidin-4-amine (HSP70-51). ^1H NMR (300 MHz, CDCl_3 -d): δ 1.57–1.61 (m, 2H); 1.92–1.95 (d, 2H); 2.24 (s, 3H); 2.76 (m, 1H); 2.86–2.93 (t, 2H); 3.67 (s, 2H); 3.93 (s, 3H); 4.47–4.50 (m, 2H); 6.20–6.21 (d, 1H); 7.22–7.25 (dd, 2H); 7.37 (d, 1H); 7.45–7.47 (d, 1H); 8.02–8.03 (d, 1H). MS(TOF) 381.3 (M^+).

4.2.4.18. N-(3,4-dichlorobenzyl)-1-(2-methoxypyrimidin-4-yl)-N-methylpiperidin-4-amine (HSP70-52). ^1H NMR (300 MHz, CDCl_3 -d): δ 1.54–1.60 (m, 2H); 1.92–1.95 (d, 2H); 2.24 (s, 3H); 2.76 (m, 1H); 2.90–2.92 (t, 2H); 3.55 (s, 2H); 3.93 (s, 3H); 4.48 (s, 2H); 6.19–6.21 (d, 1H); 7.17–7.19 (d, 2H); 7.30–7.44 (m, 1H); 8.01–8.03 (d, 1H). MS(TOF) 381.3 (M^+).

4.2.4.19. N-(2-chloro-6-fluorobenzyl)-1-(2-methoxypyrimidin-4-yl)-N-methylpiperidin-4-amine (HSP70-53). ^1H NMR (300 MHz, CDCl_3 -d): δ 1.63–1.66 (m, 2H); 1.95–1.98 (d, 2H); 2.26 (s, 3H); 2.78 (m, 1H); 2.86–2.92 (t, 2H); 3.74 (s, 2H); 3.93 (s, 3H); 4.51 (s, 2H); 6.20–6.22 (d, 1H); 6.99–7.01 (m, 2H); 7.19–7.21 (m, 1H); 8.01–8.03 (d, 1H). MS(TOF) 364.8 (M^+).

4.2.4.20. N-(3-chlorobenzyl)-1-(2-methoxypyrimidin-4-yl)-N-methylpiperidin-4-amine (HSP70-54). ^1H NMR (300 MHz, CDCl_3 -d): δ 1.55–1.62 (m, 2H); 1.91–1.94 (d, 2H); 2.21 (s, 3H); 2.74 (s, 1H); 2.85–2.90 (t, 2H); 3.58 (s, 2H); 3.93 (s, 3H); 4.48 (m, 2H); 6.19–6.21 (d, 1H); 7.23 (m, 2H); 7.34 (s, 2H); 8.00–8.02 (d, 1H). MS(TOF) 346.9 (M^+).

4.2.4.21. N-(3-fluorobenzyl)-1-(2-methoxypyrimidin-4-yl)-N-methylpiperidin-4-amine (HSP70-55). ^1H NMR (300 MHz, CDCl_3 -d): δ 1.56–1.64 (m, 2H); 1.91–1.94 (d, 2H); 2.22 (s, 3H); 2.74–2.75 (m, 1H); 2.86–2.93 (t, 2H); 3.60 (s, 2H); 3.94 (s, 3H); 4.46–4.49 (m, 2H); 6.20–6.21 (d, 1H); 6.93–6.97 (t, 2H); 7.08–7.11 (m, 2H); 7.25–7.30 (m, 1H); 8.02–8.04 (d, 1H). MS(TOF) 330.4 (M^+).

4.2.4.22. N-(4-fluorobenzyl)-1-(2-methoxypyrimidin-4-yl)-N-methylpiperidin-4-amine (HSP70-56). ^1H NMR (300 MHz, CDCl_3 -d): δ 1.56–1.60 (m, 2H); 1.91–1.94 (d, 2H); 2.20 (s, 3H); 2.74 (s, 1H); 2.84–2.90 (t, 2H); 3.58 (s, 2H); 3.93 (s, 3H); 4.47 (s, 2H); 6.18–6.20 (d, 1H); 6.98–7.02 (t, 2H); 7.30 (m, 2H); 7.25–7.30 (m, 1H); 8.00–8.02 (d, 1H). MS(TOF) 330.4 (M^+).

4.2.5 General procedure for the synthesis of compounds HSP70-(57-67)

General procedure 5: 4, 6-dichloropyrimidine was processed instead of 2, 4-dichloropyrimidine as described in General procedure 4 to provide the product HSP70-(57-67).

4.2.5.1. N-(2-chlorobenzyl)-1-(6-methoxypyrimidin-4-yl)-N-methylpiperidin-4-amine (HSP70-57). ^1H NMR (300 MHz, CDCl_3 -d): 1.60–1.67 (m, 2H); 1.93–1.96 (d, 2H); 2.27 (s, 3H); 2.77 (m, 1H); 2.85–2.92 (t, 2H); 3.72 (s, 2H); 3.93 (s, 3H); 4.40–4.44 (m, 2H); 5.86 (s, 1H); 7.20–7.26 (m, 2H); 7.35–7.37 (dd, 1H); 7.49–7.51 (d, 1H); 8.34 (s, 1H). MS(TOF) 346.9 (M^+).

4.2.5.2. 1-(6-Methoxypyrimidin-4-yl)-N-methyl-N-(4-methylbenzyl)piperidin-4-amine (HSP70-58). ^1H NMR (300 MHz, CDCl_3 -d): 1.58–1.62 (m, 2H); 1.91–1.94 (d, 2H); 2.22 (s, 3H); 2.35 (s, 3H); 2.74 (m, 2H); 2.83–2.90 (t, 2H); 3.58 (s, 2H); 3.93 (s, 3H); 4.38–4.42 (m, 2H); 5.85 (s, 1H); 7.13–7.15 (d, 2H); 7.22–7.24 (d, 2H); 8.34 (s, 1H). MS(TOF) 326.4 (M^+).

4.2.5.3. N-(2,6-dichlorobenzyl)-1-(6-methoxypyrimidin-4-yl)-N-methylpiperidin-4-amine (HSP70-59). ^1H NMR (300 MHz, CDCl_3 -d): 1.63–1.67 (m, 2H); 1.93–1.96 (d, 2H); 2.24 (s, 3H); 2.83–2.90 (m, 3H); 3.87 (s, 2H); 3.93 (s, 3H); 4.43–4.46 (m, 2H); 5.86 (s, 1H); 7.15–7.17 (t, 1H); 7.29–7.32 (m, 2H); 8.34 (s, 1H). MS(TOF) 381.3 (M^+).

4.2.5.4. N-(3,4-dichlorobenzyl)-1-(6-methoxypyrimidin-4-yl)-N-methylpiperidin-4-amine (HSP70-60). ^1H NMR (300 MHz, CDCl_3 -d): 1.43–1.58 (m, 2H); 1.88–1.90 (m, 2H); 2.19 (s, 3H); 2.72 (m, 1H); 2.82–2.89 (m, 2H); 3.53 (s, 2H); 3.91 (s, 3H); 4.38–4.42 (m, 2H); 5.84 (s, 1H); 7.16 (s, 1H); 7.37–7.44 (m, 2H); 8.32 (s, 1H). MS(TOF) 381.3 (M^+).

4.2.5.5. N-(4-fluorobenzyl)-1-(6-methoxypyrimidin-4-yl)-N-methylpiperidin-4-amine

(HSP70-61). ^1H NMR (300 MHz, CDCl_3 -d): δ 1.56–1.62 (m, 2H); 1.89–1.92 (m, 2H); 32.19 (s, 3H); 32.72 (s, 1H); 32.82–2.89 (m, 2H); 33.56 (s, 2H); 33.88 (s, 3H); 34.38–4.41 (m, 2H); 35.84 (s, 1H); 36.98–7.02 (t, 1H); 37.27–7.29 (m, 2H); 38.32 (s, 1H). MS(TOF) 330.4 (M^+).

4.2.5.6. N-(3-chlorobenzyl)-1-(6-methoxypyrimidin-4-yl)-N-methylpiperidin-4-amine

(HSP70-62). ^1H NMR (300 MHz, CDCl_3): δ 1.55–1.62 (m, 2H); 1.89–1.92 (m, 2H); 32.20 (s, 3H); 32.72 (m, 1H); 32.83–2.89 (m, 2H); 33.57 (m, 2H); 33.91 (s, 3H); 34.38–4.41 (m, 2H); 35.83 (s, 1H); 37.23 (m, 3H); 37.34 (s, 1H); 38.32 (s, 1H). MS(TOF) 346.9 (M^+).

4.2.5.7. N-(2-chloro-6-fluorobenzyl)-1-(6-methoxypyrimidin-4-yl)-N-

methylpiperidin-4-amine (HSP70-63). ^1H NMR (300 MHz, CDCl_3 -d): δ 1.59–1.68 (m, 2H); 1.94–1.96 (m, 2H); 32.25 (s, 3H); 32.75 (m, 1H); 32.84–2.87 (m, 2H); 33.73 (s, 2H); 33.91 (s, 3H); 34.42–4.45 (m, 2H); 35.84 (s, 1H); 36.99 (t, 1H); 37.19 (d, 2H); 38.32 (s, 1H). MS(TOF) 364.8 (M^+).

4.2.5.8. 2-(((1-(6-Methoxypyrimidin-4-yl)piperidin-4-yl)(methyl)amino)methyl)benzonitrile (HSP70-64). ^1H NMR (300 MHz, CDCl_3): δ 1.58–1.62 (m, 2H); 1.93–1.96 (m, 2H); 32.20 (s, 3H); 32.72 (m, 1H); 32.83–2.87 (m, 2H); 33.78 (s, 2H); 33.91 (s, 3H); 34.41–4.44 (m, 2H); 35.84 (s, 1H); 37.35 (s, 3H); 37.54 (s, 1H); 37.63–7.65 (d, 1H); 38.32 (s, 1H). MS(TOF) 337.4 (M^+).

4.2.5.9. 4-(((1-(6-Methoxypyrimidin-4-yl)piperidin-4-yl)(methyl)amino)methyl)benzonitrile (HSP70-65). ^1H NMR (300 MHz, CDCl_3): δ 1.55–1.61 (m, 2H); 1.88–1.91 (m, 2H); 32.22 (s, 3H); 32.71 (m, 1H); 32.80–2.89 (m, 2H); 33.63 (s, 2H); 33.93 (s, 3H); 34.39–4.41 (d, 2H); 35.8 (s, 1H); 37.26 (s, 3H); 37.44–7.46 (d, 1H); 37.60–7.62 (d, 1H); 38.32 (s, 1H). MS(TOF) 337.4 (M^+).

4.2.5.10. N-(2,5-dichlorobenzyl)-1-(6-methoxypyrimidin-4-yl)-N-methylpiperidin-4-amine (HSP70-66). ^1H NMR (300 MHz, CDCl_3): δ 1.59–1.62 (m, 2H); 1.91–1.94 (m, 2H); 32.25 (s, 3H); 32.75 (m, 1H); 32.85–2.92 (m, 2H); 33.66 (s, 2H); 33.93 (s, 3H); 34.41–4.44 (m, 2H); 35.86 (s, 1H); 37.23–7.25 (t, 1H); 37.38 (m, 1H); 37.45–7.47 (m, 1H); 38.34 (s, 1H). MS(TOF) 381.3 (M^+).

4.2.5.11. N-(2,5-dichlorobenzyl)-1-(6-methoxypyrimidin-4-yl)-N-methylpiperidin-4-amine (HSP70-67). ^1H NMR (300 MHz, CDCl_3 -d): δ 1.58–1.64 (m, 2H); 1.91–1.94

(m,2H); δ2.21 (s,3H); δ2.74 (m,1H); δ2.84–2.91 (m,2H); δ3.61 (m,2H); δ3.93 (s,3H); δ4.40–4.43 (m,2H); δ5.86 (s,1H); δ6.95 (t,1H); δ7.11 (m,2H); δ7.28–7.30 (s,2H); δ8.34 (s,1H). MS(TOF) 330.4 (M^+).

4.3 Biological evaluation

4.3.1 Cell viability assay

A luminescent signal indicating the ATP levels was used as a marker of cell viability. The breast cancer cell lines BT474, BT/Lap^R1.0, MDA-MB-361, SK-BR3, SK/Lap^R1.0 and MDA-MB-453 were selected for the cell viability assays. The cell lines were obtained from the American Type Culture Collection (ATCC). The epidermal growth factor (EGFR: ErbB-1, ErbB-2) tyrosine kinase inhibitor lapatinib (10 mM in DMSO, BioVision, Cat: 1624-100, Lot: 50324) was used as the positive control. The amount of ATP was estimated with the use of an ATPlite kit (CellTiter-Glo Substrate, Promega, Part: G755B, Lot: 32513501, EXP: 2014-05) according to the manufacturer's protocol. Briefly, the cells were trypsinized in 1 mL of 0.25% trypsin (Gibco) supplemented with 2 mL of medium (containing 10% FBS, Gibco) and seeded (1×10^5 cells/mL, 50 µL/well, 5000 cells/well) into 96-well plates. The plates were incubated for 24 h at 37°C. The compounds were diluted to 10, 5, 2.5, 1.25, 0.625, 0.31, 0.16, 0.08, 0.04 and 0 µM. The serially diluted compounds or control (50 µL) were added to each well in triplicate. The cells were incubated for 72 h at 37°C. A volume of the CellTiter-Glo Reagent (50 µL) equal to the volume of the cell culture medium present in each well was added. The plates were shaken for 3 min on an orbital shaker to induce cell lysis. To stabilize the luminescent signal, the plate was incubated at room temperature for 10 min. The liquid supernatant (100µL/well) was aspirated, and the luminescent signal was measured. The cell viability was determined based on the luminescence: cell viability (%) = $\frac{RLU_{\text{experimental group}}}{RLU_{\text{blank group}}} \times 100\%$.[28]

4.3.2 Surface plasmon resonance (SPR) analyses

SPR studies of HSP70 inhibitors were performed using optical biosensor Biacore T100 (GE Company) as reported elsewhere. [16, 17] Briefly, human HSP70 (ADI-ESP-550-D, Enzo Life Sciences) was immobilized on a CM5 sensor chip (GE) using 10 mmol/L phosphate-buffered saline (PBS), pH 5.0, RU=11209. Binding experiments were performed at 25°C using a flow rate of 30 µl ml⁻¹, with 90 s of monitoring for association

and 120 s of monitoring for dissociation. The complete binding study was performed using an eight-point concentration series (0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 μM), which were prepared with 5% DMSO in PBS. Different concentrations of compounds were typically injected over the sensor chip, and 5% DMSO in PBS was used as a running buffer and blank control. Ver-155008 was used as a positive control. The data were processed using an equilibrium dissociation constant (K_d value), which was calculated with the steady state model: Response= Conc * Rmax / [conc + K_d] + offset.

4.3.3 Colorimetric determination of ATPase activity

The assay procedure was adopted from previous reports [19] with some modifications where indicated. Stock solutions of malachite green (0.1% w/v), polyvinyl alcohol (2.3% w/v), and ammonium molybdate tetrahydrate (1% w/v in 1 M HCl) were prepared and mixed with water at a ratio of 2:1:1:2 to prepare the malachite green reagent, which is stable at room temperature for at least 2 h. The reagent should be filtered through a 0.22- μM membrane prior to use when its colour changes from dark brown to yellow. ATP, malachite green, polyvinyl alcohol and ammonium molybdate tetrahydrate were purchased from Sigma and used without further purification.

For compound screening, an aliquot of a master mixture of DnaK:DnaJ (2.0 μM :3.5 μM) prepared in assay buffer (0.017% Triton X-100, 100 mM Tris-HCl, 20 mM KCl, and 6 mM MgCl₂, pH 7.4) was added to each well of a 96-well plate (14 μL per well). To this solution, 1 μL of either one of the test compounds (5 mM) or DMSO was added, and the plate was shaken for 10 min and incubated for 30 min at 37°C. Ten microliters of 2.5 mM ATP was added to start the reaction. The final reaction volume was 25 μL , and the concentration of the tested compounds was 200 μM . After 3 h of incubation at 37°C, 80 μL of the malachite green reagent was added to each well, and the plates were gently shaken. Immediately following this step, 10 μL of 34% sodium citrate was added to terminate the nonenzymatic hydrolysis of ATP. After the samples were thoroughly mixed and incubated at 37°C for 15 min, the OD₆₂₀ was measured using a SpectraMax M5 (Molecular Devices, Sunnyvale, CA, USA).

4.3.4 Acute toxicity test by single oral gavage with HSP70-36 in mice

Seventy mice (KM) were randomly divided into seven groups, with 10 mice per group (5 males and 5 females). HSP70-36 was diluted to concentrations of 155.5, 129.6, 108.0, 90.0, 75.0, 62.5, and 52.0 mg*ml⁻¹ with 0.5% sodium carboxymethyl cellulose. The fixed-dose procedure was used for this test. According to the acute toxicity test results, the mice received 1555, 1296, 1080, 900, 750, 625 and 520 mg*kg⁻¹ HSP70-36 via oral gavage and were continuously observed for 14 days. Death and toxicity were recorded after 5 min, 10 min, 20 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h, 18 h, 1 d, 2 d, 3 d, 7 d and 14 d. The LD₅₀ was calculated based on a statistical analysis with SAS.

5. Acknowledgments

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Fig. 1. Skeleton structures containing piperidine.

Fig. 2. Binding modes of HSP70-40 at the active site of HSP70 ATPase. The Figure was generated by the Discovery Studio 2.5 software. The compound HSP70-40 is represented in stick form, and the blue dotted lines are hydrogen-binding interactions and H- π interactions.

Scheme 1. Synthetic pathway of derivatives HSP70-(1–20). Reagents and conditions: (a) DMF, K_2CO_3 , rt., 6 h; (b) $R_1=CH_3CH_2O$: CH_3CH_2ONa , MeOH, reflux, 8 h; $R_1=CH_3S$: CH_3SNa , KI, THF, reflux, 10 h (d) CF_3CCOOH , dichloroethane, rt., 5 h; (e) DMF, K_2CO_3 , rt., 4 h.

Scheme 2. Synthetic pathway of derivatives HSP70-(21–31). Reagents and conditions: (d) CF_3CCOOH , dichloroethane, rt., 5 h; (e) DMF, K_2CO_3 , rt., 4 h.

Scheme 3. Synthetic pathway of derivatives HSP70-(32–34). Reagents and conditions: (a) DMF, K_2CO_3 , rt., 6 h; (d) CF_3CCOOH , dichloroethane, rt., 5 h; (e) DMF, K_2CO_3 , rt., 4 h.

Scheme 4. Synthetic pathway of derivatives HSP70-(35–56). Reagents and conditions: (a) DMF, K_2CO_3 , rt., 6 h; (b) $R_1=CH_3CH_2O$: CH_3ONa , MeOH, reflux, 8 h; $R_1=CH_3S$: CH_3SNa , KI, THF, reflux, 10 h (d) CF_3CCOOH , dichloroethane, rt., 5 h; (e) DMF, K_2CO_3 , rt., 4 h.

Scheme 5. Synthetic pathway of derivatives HSP70-(57–67). Reagents and conditions: (a) DMF, K_2CO_3 , rt., 6 h; (b) CH_3ONa , MeOH, reflux, 8 h; (d) CF_3CCOOH , dichloroethane, rt., 5 h; (e) DMF, K_2CO_3 , rt., 4 h.

Fig. 3. Inhibitory rates of piperidine derivatives in BT474, BT/LapR1.0, MDA-MB-361, SK-BR3, SK/LapR1.0 and MDA-MB-453 cells at 5 μ M.

Table 1. IC₅₀ values of compounds in BT474 and BT/Lap^R1.0 human breast cancer cells.

Fig. 4. Survival rates of HepG2, Huh-7, N87, BGC823, AsPC-1, MIA-PaCa-2, COLO205, HT-29, A549, H460 and normal cell lines (WI-38 and MRC5) exposed to different concentrations of compounds HSP70-36/37/40/43/46.

Table 2. Kinetic characterization of inhibitor binding to HSP70^a

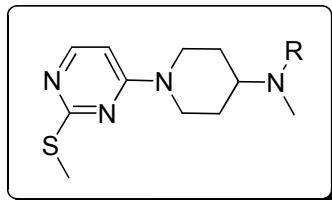
Fig. 5. Percentage of inhibition of HSP70 ATPase activity achieved by different concentrations of the positive controls VER 155008 and AZ.

Table 3. Inhibition rate of compounds (200 μ M) on HSP70 ATPase.

Table 4. Number of surviving and deceased mice in each dose group.

Fig. 6. Active pocket of HSP70 ATPase domain with ADP. The nucleoside binding domain is divided into red, yellow and purple areas for virtual screening using Dock 4.0.

Table 1. IC₅₀ values of compounds in BT474 and BT/Lap^R1.0 human breast cancer cells.



NAME	R	BT474 (μ M)	BT/Lap ^R 1.0 (μ M)
36	P-cyanobenzyl	1.41	1.47
37	2-chloro-6-fluoro-benzyl	1.04	0.94

40	2,4-dichlorobenzyl	1.62	1.68
43	2,6-dichlorobenzyl	0.78	0.70
46	2-chlorobenzyl	1.99	1.70

Table 2. Kinetic characterization of inhibitor binding to HSP70^a

NAME	K _d (molL ⁻¹)
VER-155008	2.060×10 ⁻⁷
12	1.999×10 ⁻⁷
14	2.454×10 ⁻⁶
35	2.045×10 ⁻⁶
36	2.463×10 ⁻⁷
56	2.811×10 ⁻⁶

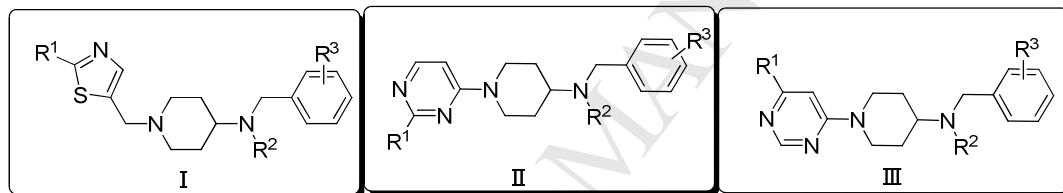
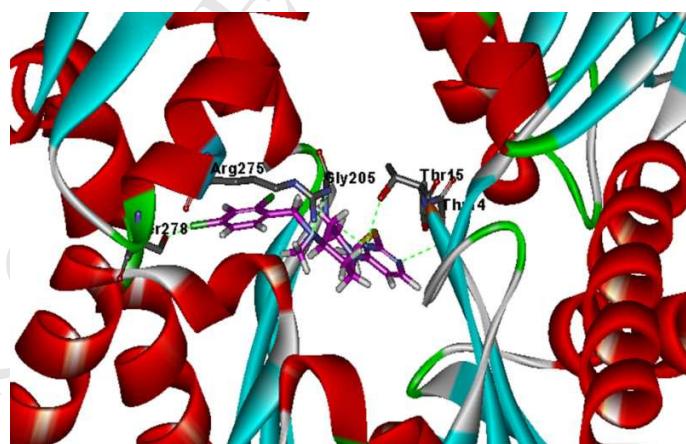
^aTo determine the affinities and kinetic rate constants, each compound was examined at different concentrations (0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 µM), T=25°C and 5% dimethyl sulfoxide (assay buffer). A typical analysis cycle consisted of a 90 s sample injection (30 µlmin⁻¹) and 120 s of buffer flow (dissociation phase).

Table 3. Inhibition rate of compounds (200 µM) on HSP70 ATPase.

NAME	Inhibition rate (%)	SD
4	5.49	8.04
8	4.5	15.32
12	10.59	8.52
16	10.74	11.51
19	6.21	9.32
22	11.28	1.34
35	7.73	2.52
36	8.14	2.72
37	20.39	3.31
40	8.28	10.58
41	16.67	1.6
43	12.81	4.41
46	11.63	4.64
47	6.11	5.05

Table 4. Number of surviving and deceased mice in each dose group.

ID	Dose mgkg ⁻¹	Number of animals (survived/dead)	
		Male	Female
1	1555	0/5	0/5
2	1296	1/4	0/5
3	1080	0/5	2/3
4	900	3/2	2/3
5	750	4/1	2/3
6	625	4/1	5/0
7	520	5/0	5/0

**Fig. 1.** Skeleton structures containing piperidine.**Fig. 2.** Binding modes of HSP70-40 at the active site of HSP70 ATPase. The Figure was generated by the Discovery Studio 2.5 software. The compound HSP70-40 is represented in stick form, and the blue dotted lines are hydrogen-binding interactions and H-π interactions.

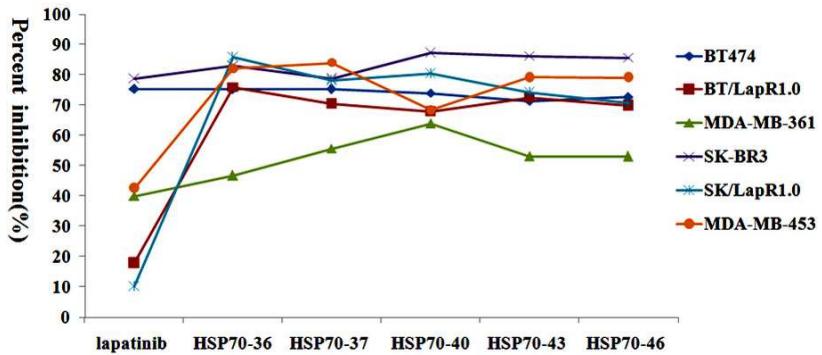
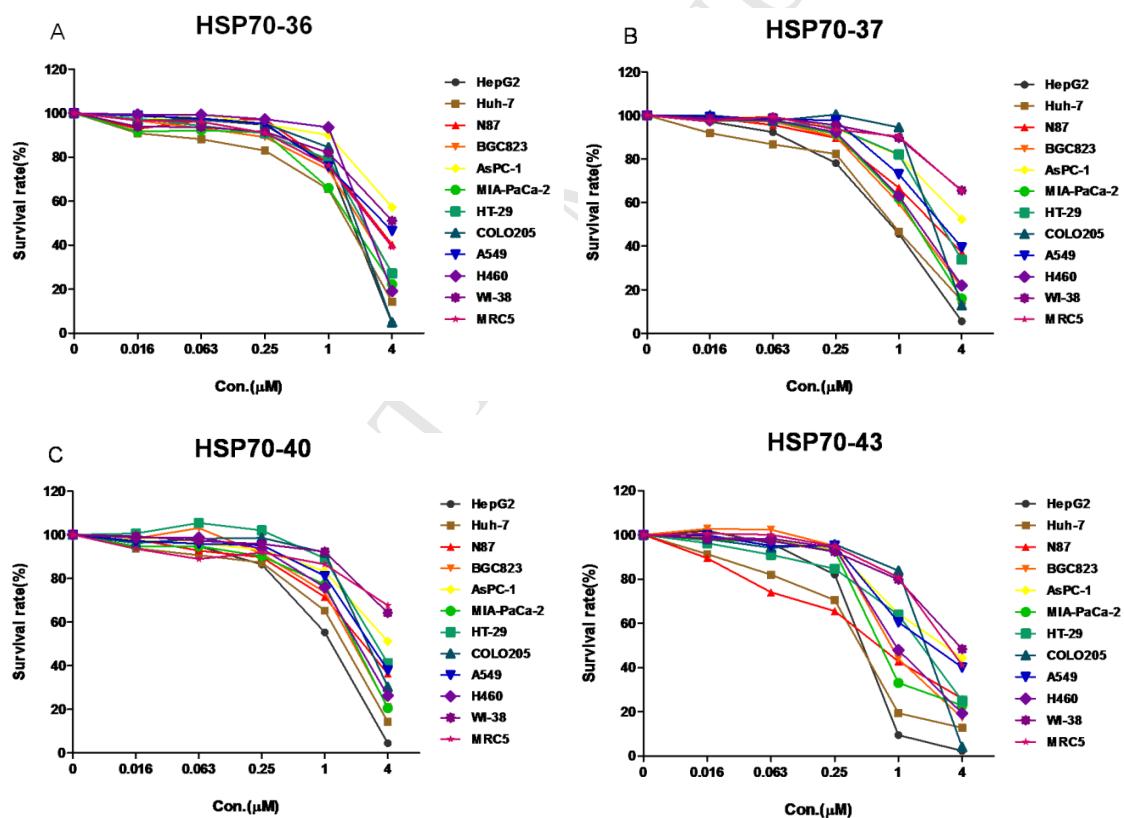


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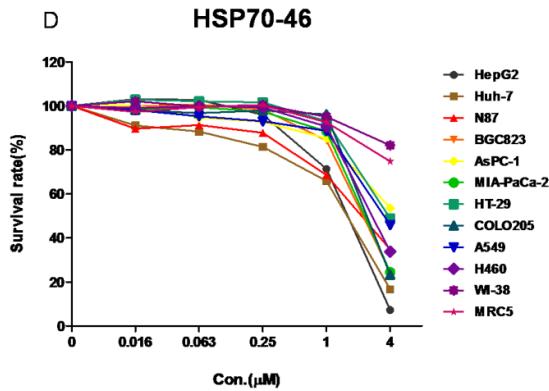


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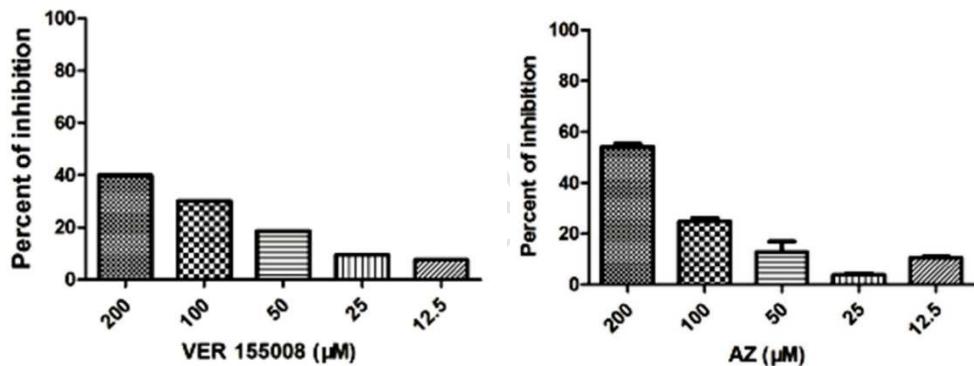


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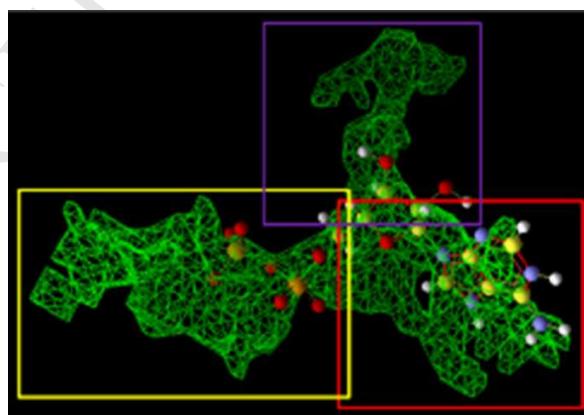
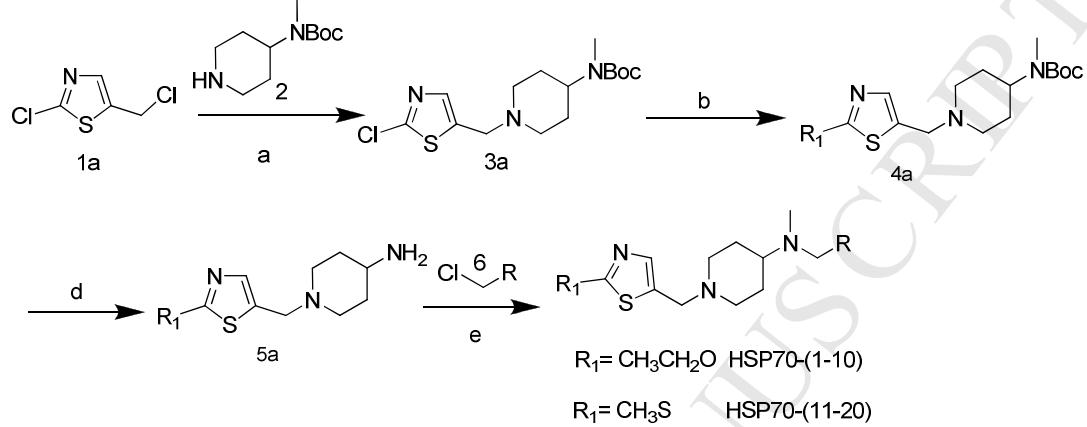
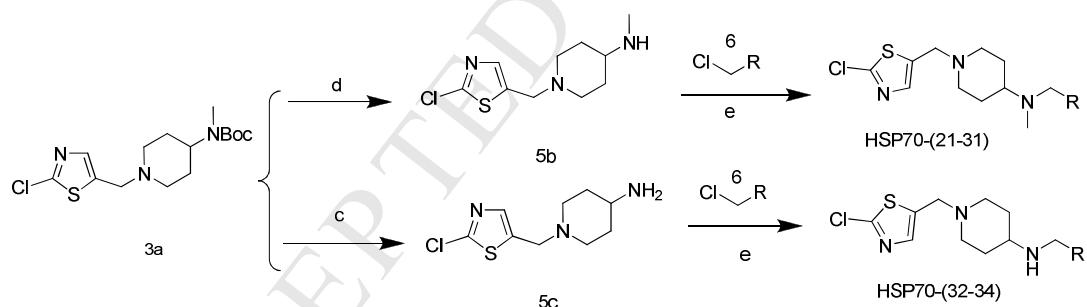


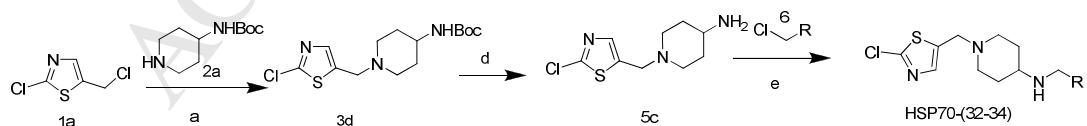
Fig. 6. Active pocket of HSP70 ATPase domain with ADP. The nucleoside binding domain is divided into red, yellow and purple areas for virtual screening using Dock 4.0.



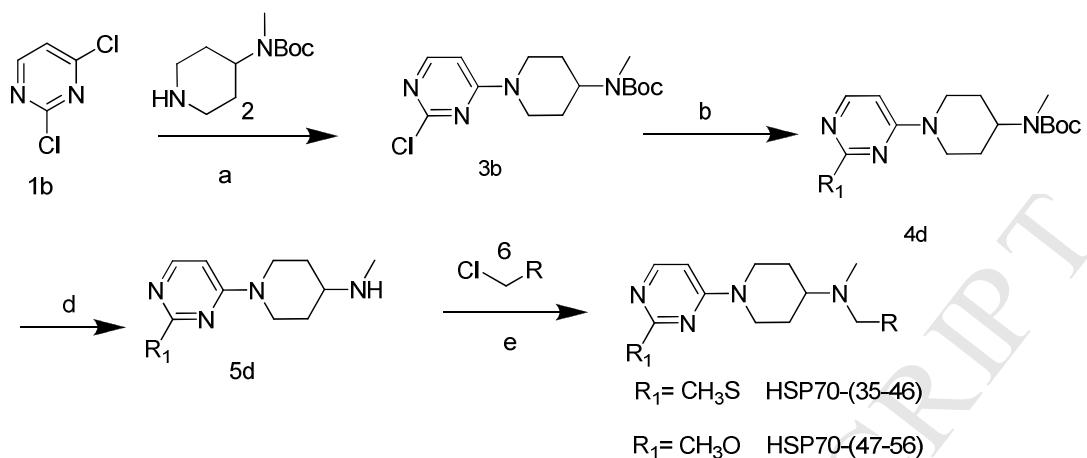
Scheme 1. Synthetic pathway of derivatives HSP70-(1-20). Reagents and conditions:
 (a) DMF, K_2CO_3 , rt., 6 h; (b) $R_1=CH_3CH_2O$: CH_3CH_2ONa , MeOH, reflux, 8 h;
 $R_1=CH_3S$: CH_3SNa , KI, THF, reflux, 10 h (d) CF_3CCOOH , dichloroethane, rt., 5 h;
 (e) DMF, K_2CO_3 , rt., 4 h.



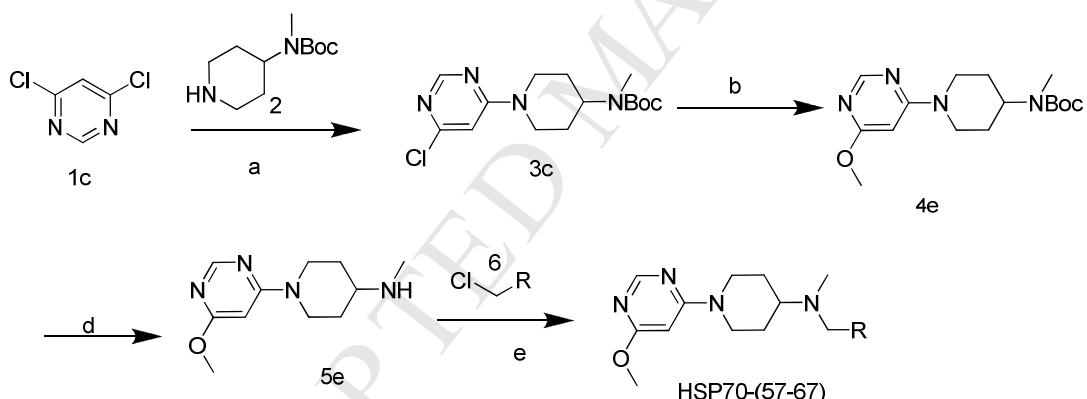
Scheme 2. Synthetic pathway of derivatives HSP70-(21–31). Reagents and conditions: (d) CF_3CCOOH , dichloroethane, rt., 5 h; (e) DMF, K_2CO_3 , rt., 4 h.



Scheme 3. Synthetic pathway of derivatives HSP70-(32–34). Reagents and conditions: (a) DMF, K₂CO₃, rt., 6 h; (d) CF₃CCOOH, dichloroethane, rt., 5 h; (e) DMF, K₂CO₃, rt., 4 h.



Scheme 4. Synthetic pathway of derivatives HSP70-(35-56). Reagents and conditions: (a) DMF, K_2CO_3 , rt., 6 h; (b) $\text{R}_1=\text{CH}_3\text{O}$: CH_3ONa , MeOH , reflux, 8 h; $\text{R}_1=\text{CH}_3\text{S}$: CH_3SNa , KI , THF , reflux, 10 h (d) CF_3CCOOH , dichloroethane, rt., 5 h; (e) DMF, K_2CO_3 , rt., 4 h.



Scheme 5. Synthetic pathway of derivatives HSP70-(57-67). Reagents and conditions: (a) DMF, K_2CO_3 , rt., 6 h; (b) CH_3ONa , MeOH , reflux, 8 h; (d) CF_3CCOOH , dichloroethane, rt., 5 h; (e) DMF, K_2CO_3 , rt., 4 h.

Highlights

1. A novel HSP70 inhibitors, piperidine derivatives were designed and synthesized.
2. The virtual fragment screening was performed.
3. The surface plasmon resonance and HSP70 ATPase activity were evaluated.
4. Cell viability assay were evaluated in 16 cancer cell lines and 2 normal cell lines.

Supporting Information

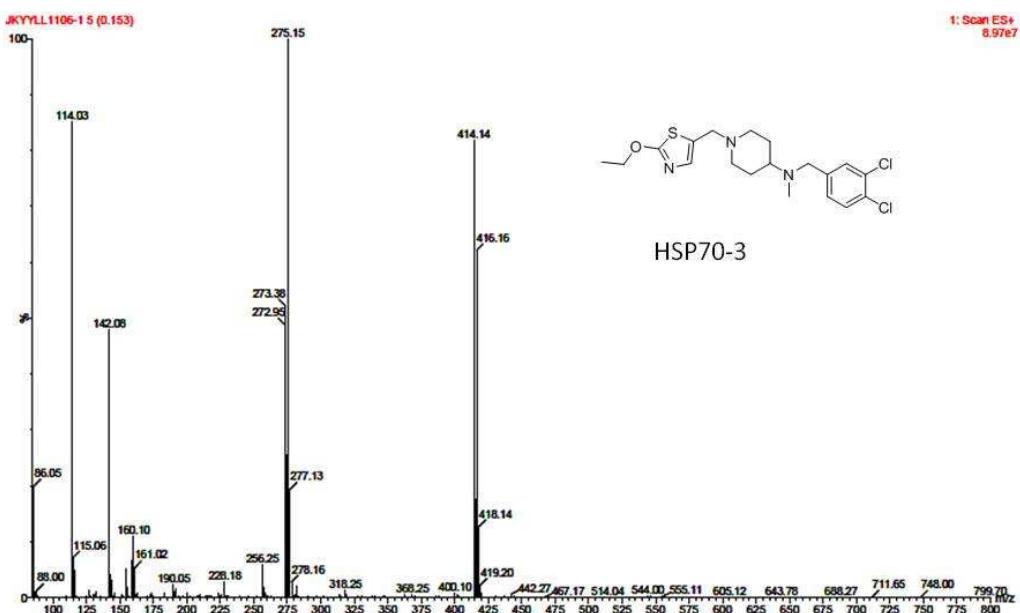
Table 1. Cell inhibitory rates of piperidine derivatives HSP70-(1-67) (5 uM) in BT474, BT/Lap^R1.0, MDA-MB-361, SK-BR3, SK/Lap^R1.0 and MDA-MB-453 cells.

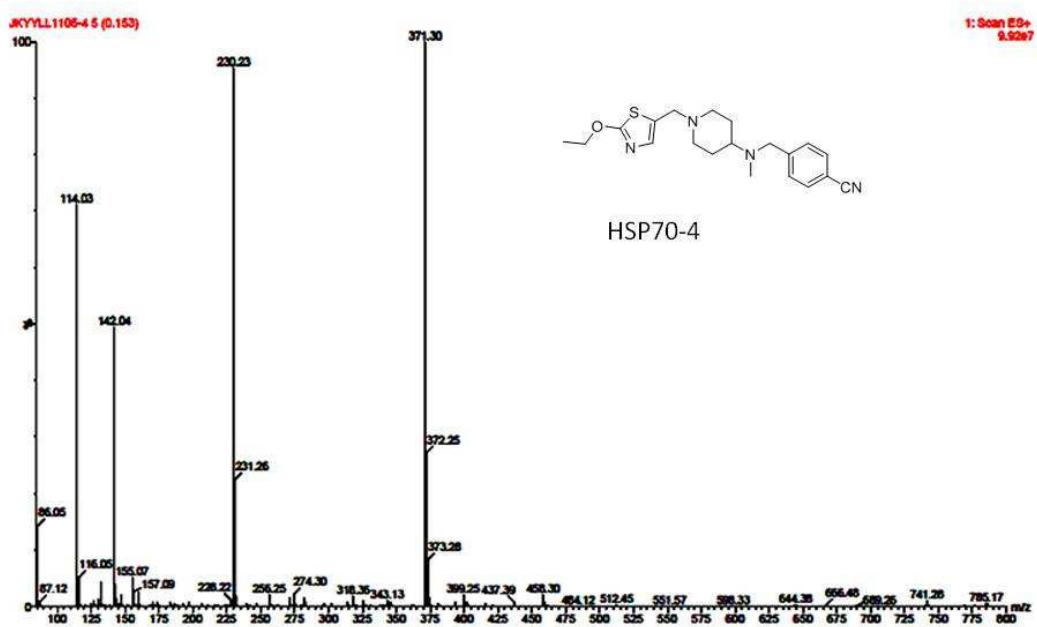
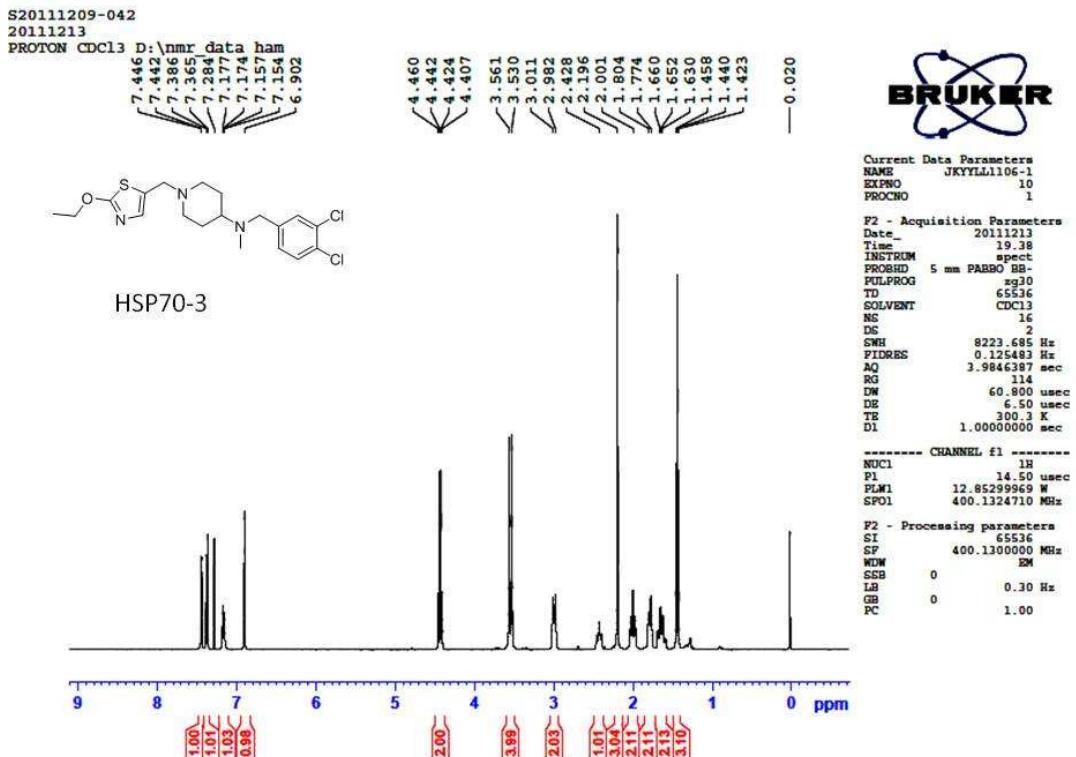
NAME μM)	Inhibitory rate % (5					
	BT474	BT/ Lap ^R 1.0	MDA- MB-361	SK-BR3	SK/ Lap ^R 1.0	MDA- MB-453
Lapatinib	75.6	18	40.2	79	10.4	42.6
1	14.4	12.5	14.2	16.7	5.2	41.2
2	17.6	19.4	12.3	13.6	7.2	43.8
3	62.9	67.4	35.8	41.2	13.1	71.6
4	61.7	66.1	51.3	45.5	6.7	70.5
5	54.4	50.5	32.8	40.9	11.7	67.4
6	10.1	13.3	33.3	12.6	0.6	36.4
7	36.4	20.7	24.2	26	25.1	52.9
8	70.3	65.9	49.2	41.6	12	71.3
9	38.2	25.6	24.5	17.9	5.1	51.5
10	5.6	9.6	25.3	9.4	5.2	23.9
11	15.5	1.4	31.7	21.9	6.9	35.1
12	68.3	66.7	38.3	49.6	12.1	67.1
13	26.3	19.8	31.7	19.5	2.1	44.2
14	44.9	36.1	31.1	26.7	7.3	62.3
15	54.4	50.5	32.8	40.9	11.7	67.4
16	62.6	67.1	42.1	42.4	10.1	69.9
17	49.5	59.9	47.2	9.2	4.5	65.3
18	54.9	54.9	45.2	24.3	26.8	63.7
19	70	66.3	36.3	37.8	40.4	72.4
20	42.3	34.4	25.4	23.3	11.4	59.4
21	-4.1	4	-8.5	7.3	4.2	8.2
22	69.3	68.7	35.6	41.5	7.5	68.5
23	7.1	9	-1.3	18.6	-0.1	11
24	45.4	53.4	42.9	34.3	5.3	63.8
25	7.1	8.4	9.7	15.5	8.5	12.6
26	10.6	3.2	10.4	9.8	1.5	22.4
27	64.1	61.8	30.1	47.1	11.4	60.8
28	1.5	4	4.4	9.7	5.1	3.5

29	14.2	16	10.9	26.2	8.4	29.6
30	2.4	5.7	3.2	4.2	1.3	4.4
31	70	66.3	36.3	37.8	40.4	72.4
32	11	10.6	13.9	26.3	25.1	32.9
33	70	66.3	36.3	37.8	40.4	72.4
34	3.8	7.2	3.2	17.9	4.1	2.4
35	40.5	44.7	46	31.5	30.9	82.3
36	75.5	76.2	46.9	83.4	86.1	82.4
37	75.5	70.7	55.9	78.9	78.3	84.2
38	39.5	29.9	29.5	30.1	12.9	60
39	35.1	25.1	39.1	26.4	24.4	65.7
40	74.2	68.1	64.1	87.6	80.7	68.7
41	74.2	68.1	64.1	87.6	80.7	68.7
42	69.2	65.8	55.7	38.9	42	66.6
47	36.7	28.6	45.1	41.4	35.8	60.8
44	61	56.9	45.7	19.3	29.4	69.3
45	67.1	63.9	50.4	32.6	35.2	66.2
46	21.3	17.5	36.2	22.2	34.2	45.3
43	73.1	70	53.2	85.9	71	79.2
48	60.4	58.1	27.7	31.7	16.6	66.2
49	71.6	72.7	53.3	86.5	74.3	79.5
50	61.5	58.8	30.1	19.7	13.5	68.9
51	27.5	22.7	22	24.7	11.7	50.6
52	52.9	35.8	41.7	27.7	16.8	60.2
53	65.4	62.4	48.3	27.7	17.2	64.7
54	36.2	26.2	23.2	18.5	15.1	56.7
55	9.9	10.5	8.5	9.2	3.9	27.9
56	12.5	14.6	12.8	14.4	9	40.4
57	27.7	18.9	20.3	13.5	29	46.7
58	5	8	10.6	20.6	22	13.4
59	53.6	52.5	30.8	22.8	19.2	65.1
60	9.8	9.2	6.5	16.1	28	12.7
61	7.8	8.1	4.9	7.6	5.7	5
62	11.9	9.4	25.6	12.6	24.8	23.8
63	45.9	48.5	39.6	15.5	16.6	59.4
64	65.8	62.2	42.7	23.3	16.8	66.1
65	25.1	16.9	20.3	17.3	8.8	46.1
66	45.9	32.1	27.6	29.2	9.4	61.1
67	5.4	5.6	8.4	5.2	30.1	9.4

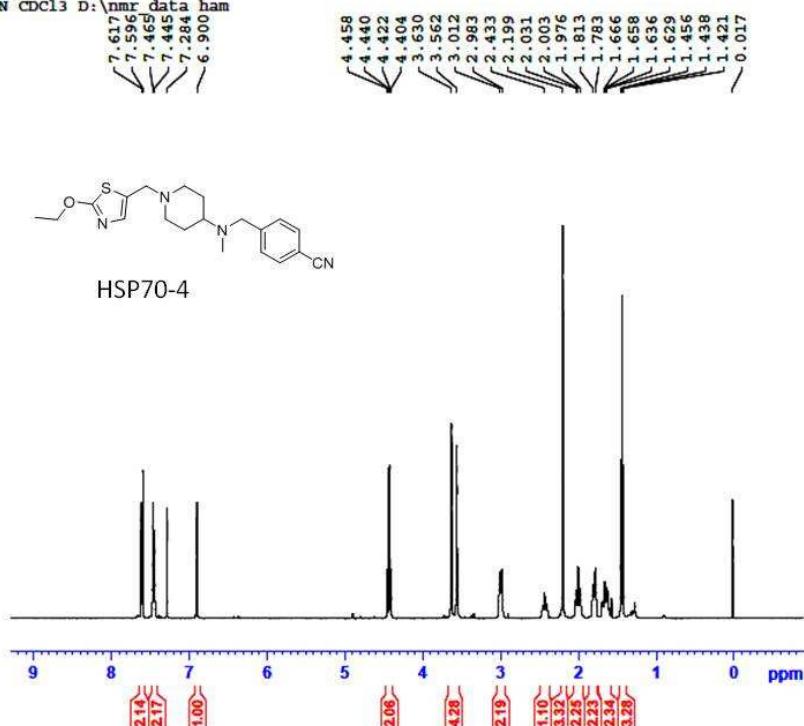
Table 2. Preparation of HSP70-36 for acute toxicity studies

Dose (mgkg ⁻¹)	HSP70-36 (mg)	0.5%CMC-Na (ml)	HSP70-36 (mgml ⁻¹)
1555	466.5	3	155.5
1296	388.8	3	129.6
1080	324.0	3	108.0
900	270.0	3	90.0
750	225.0	3	75.0
625	187.5	3	62.5
520	156.0	3	52.0

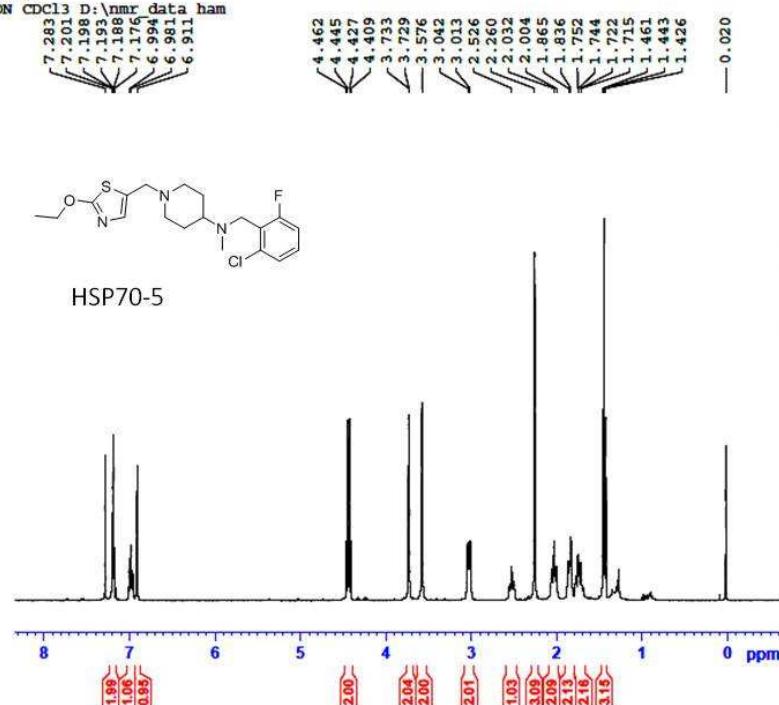
¹H NMR Spectra, MS Spectra and ¹³C NMR Spectra

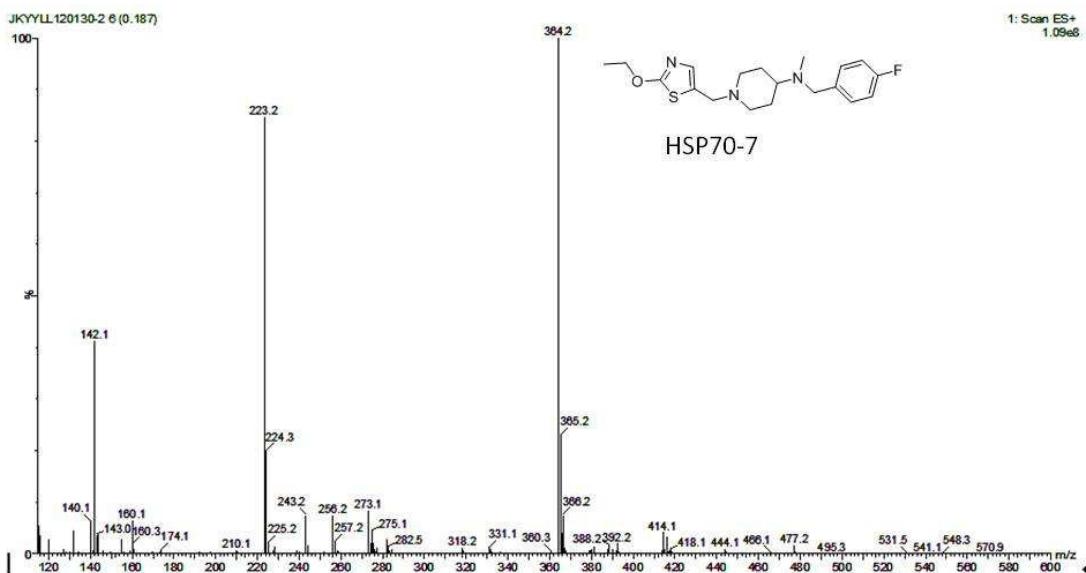
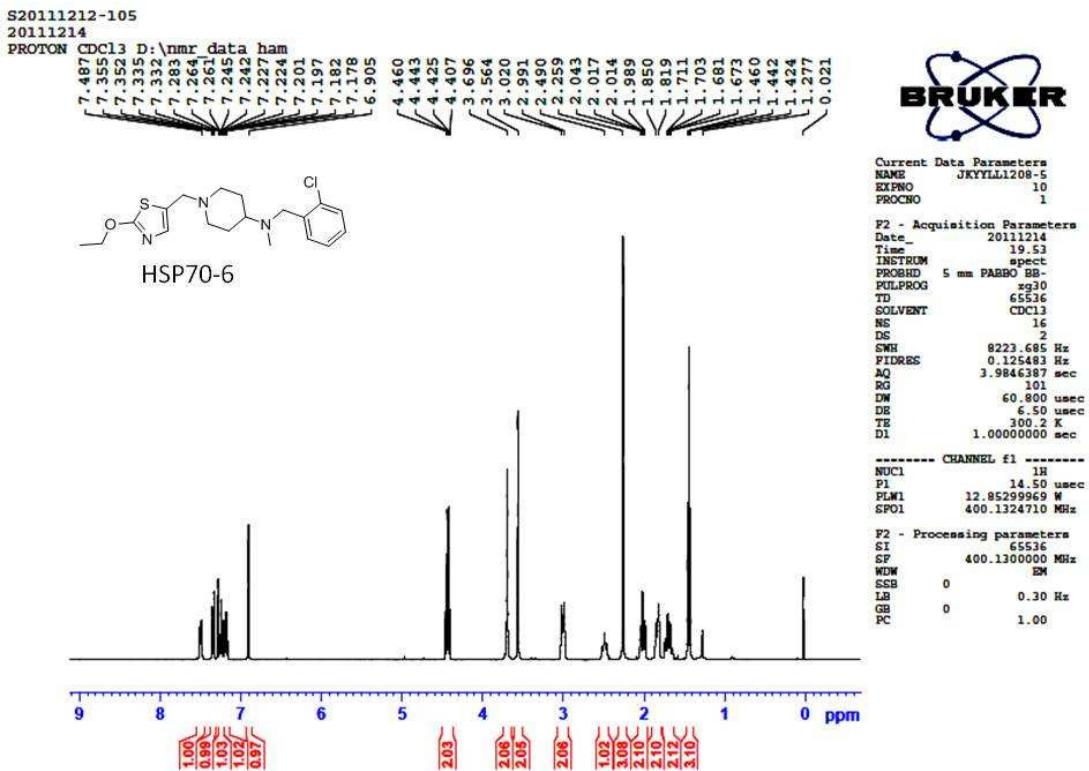


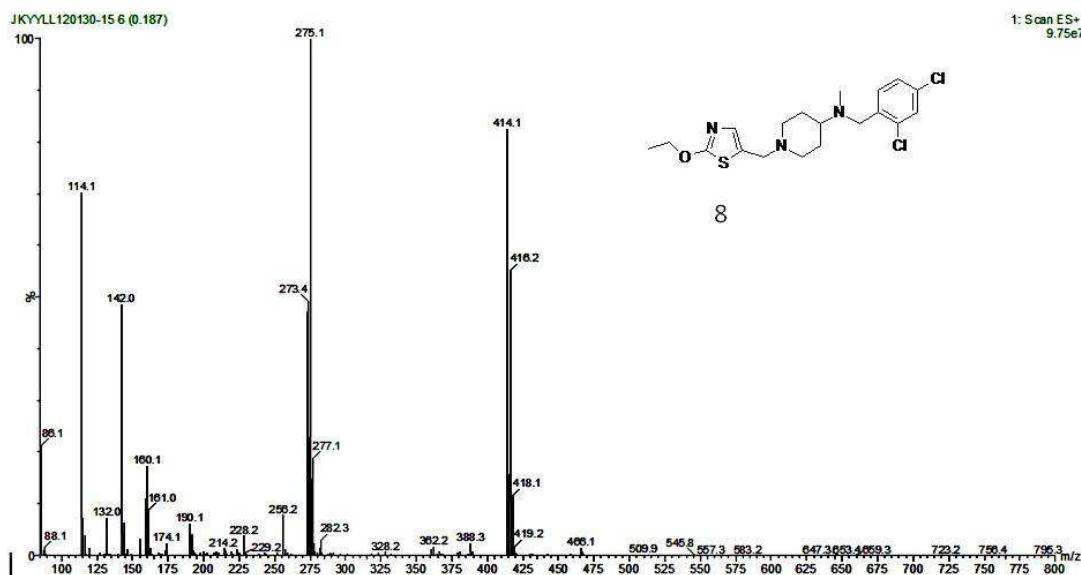
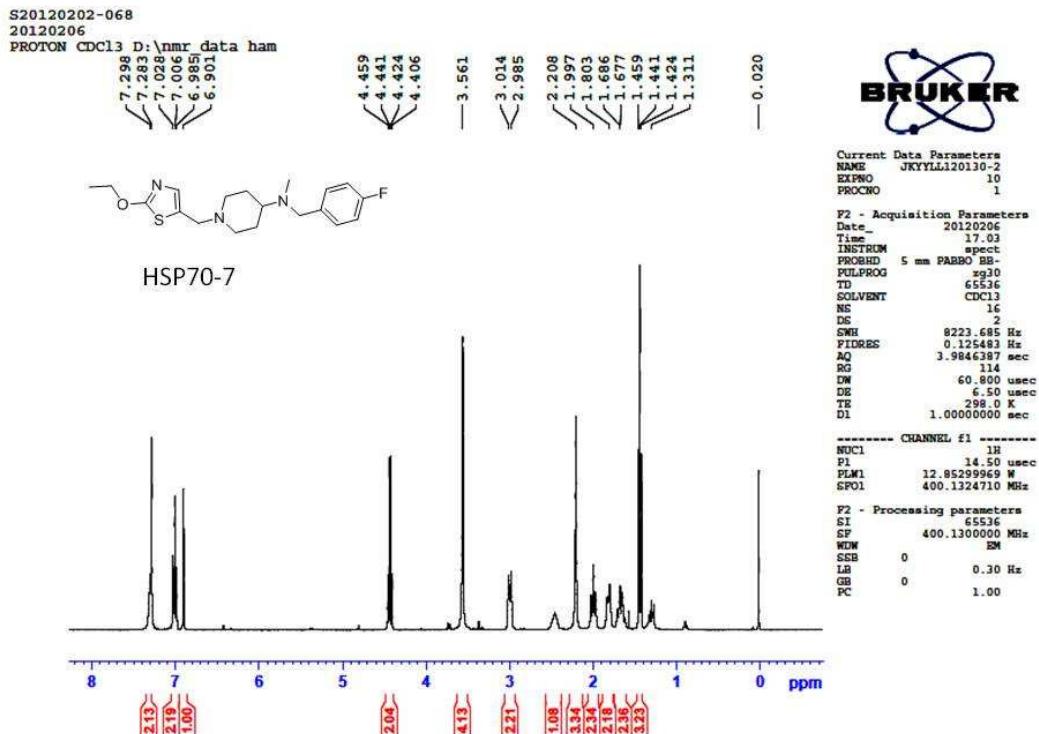
S20111209-045
20111213
PROTON CDCl₃ D:\nmr data ham

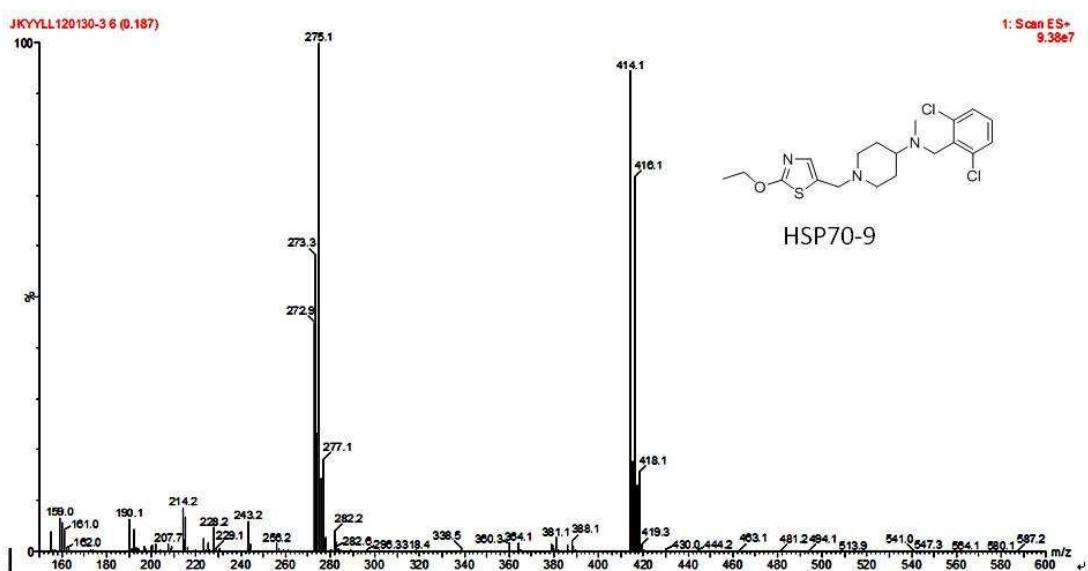
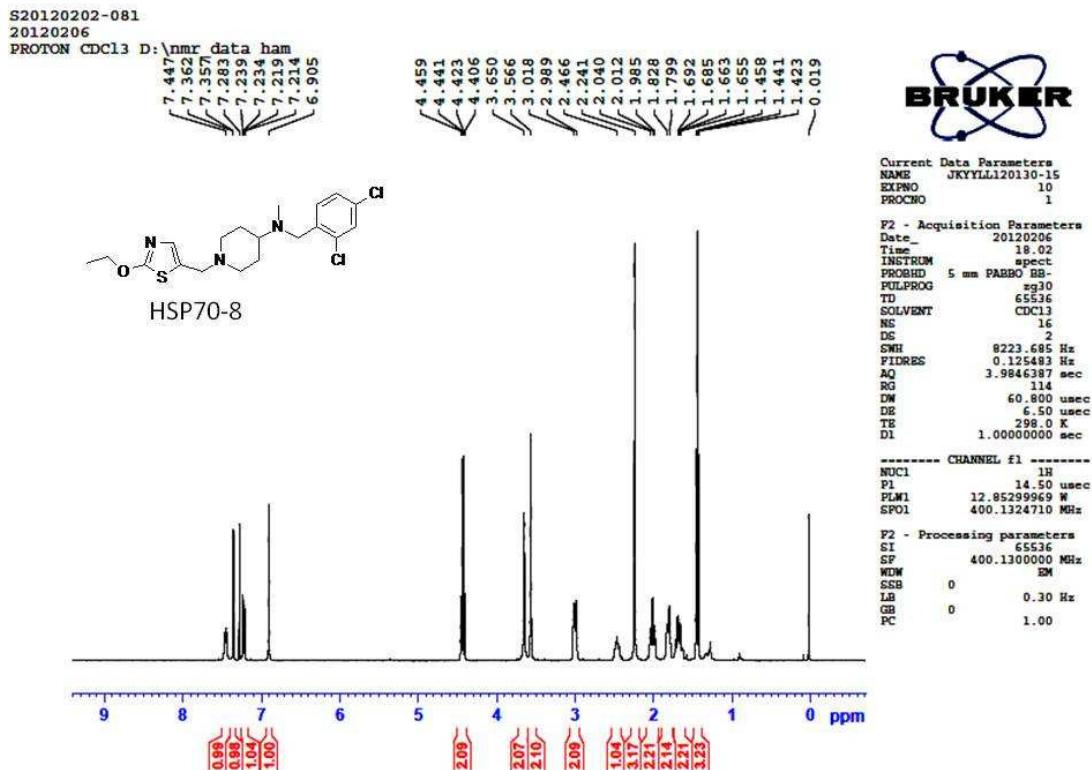


S20111212-101
20111214
PROTON CDCl₃ D:\nmr data ham

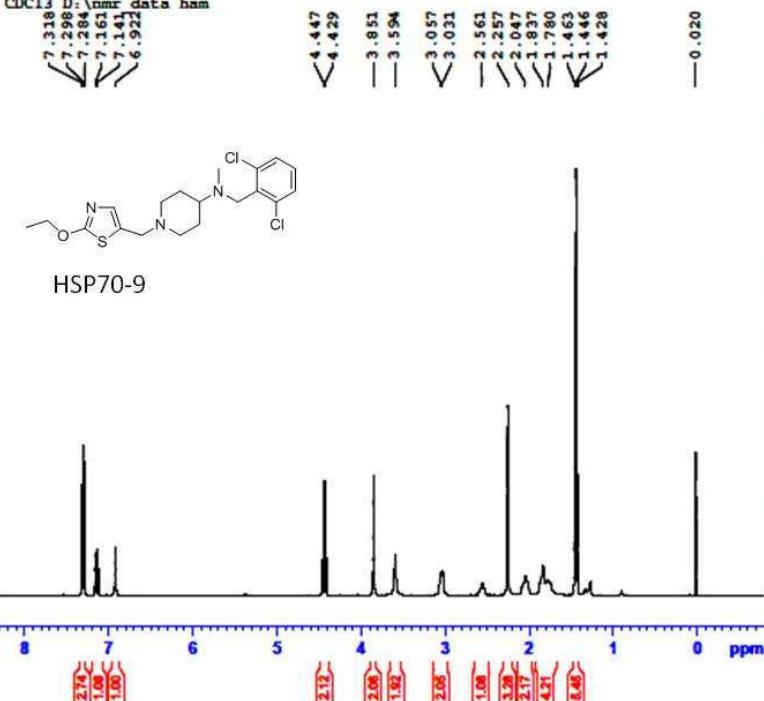








S20120202-069
20120206
PROTON CDCl₃ D:₁nmr data.hdm

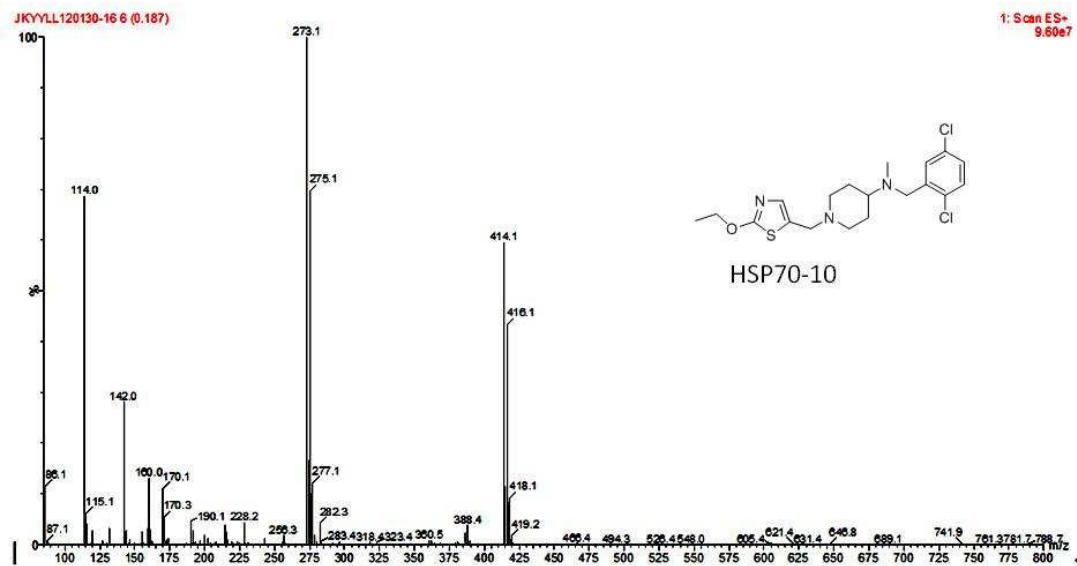


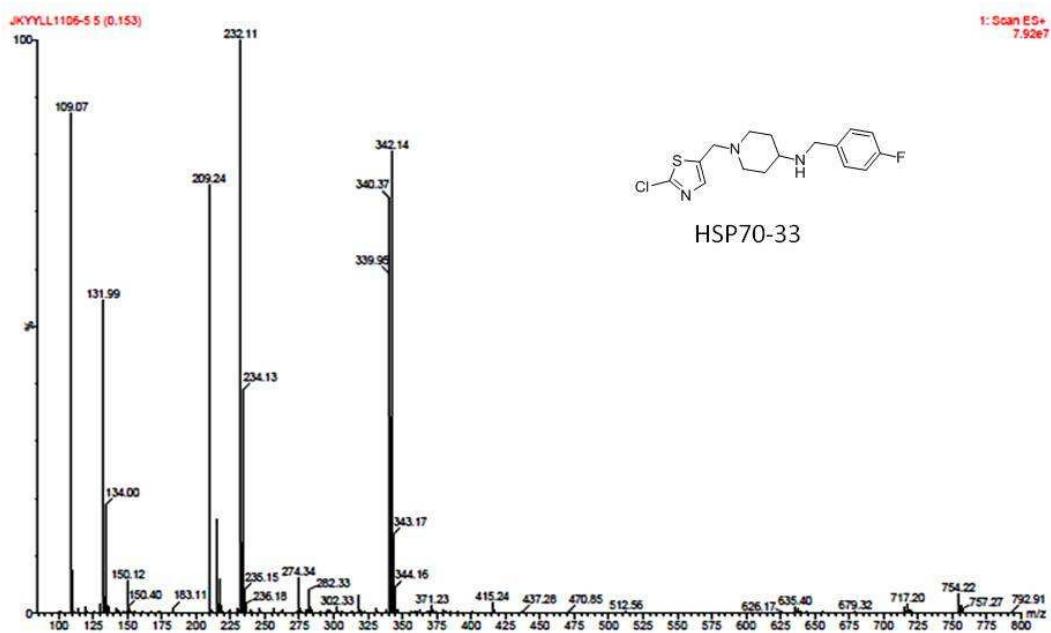
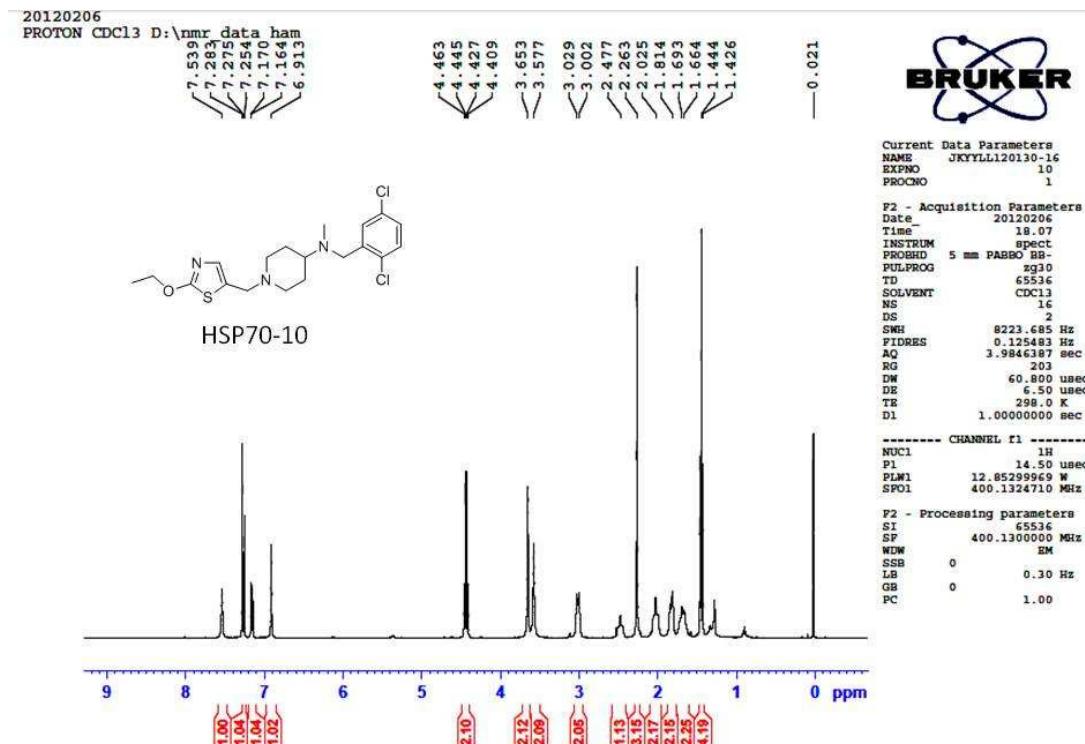
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NAME JKYYLL120130-3
EXPNO 10
PROCNO 1

P2 - Acquisition Parameters
D1NUC 1H
Time 201.170 sec
INSTRUM spect
PROGPRG 5 mm PARBO SS-
PULPROG zg30
TD 45536
SOLVENT CDCl₃
NS 16
DS 2
SW0 8323.485 Hz
FIDRES 0.125483 Hz
AQ 2.9846287 sec
RG 203
DW 60.800 usec
DR 6.50 usec
TE 298.0 K
D1 1.0000000 sec

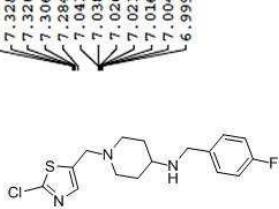
***** CHANNEL f1 *****
NUC1 1H
P1 14.50 usec
PLW1 12.85299969 M
SFO1 400.1324710 MHz

P2 - Processing parameters
SI 65536
SF 400.1300000 MHz
WDW 0
SSB 0
LB 0.30 Hz
TC 1.00

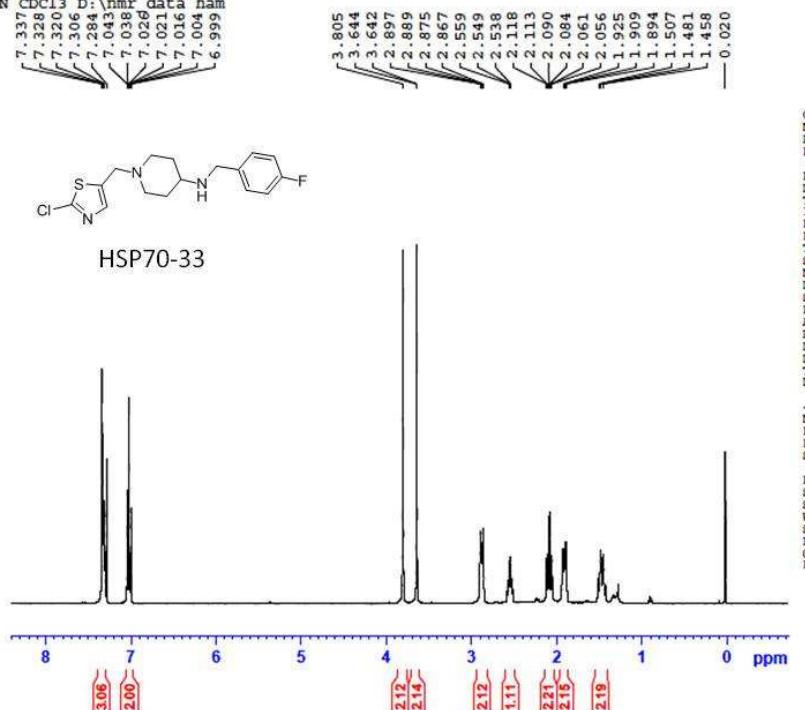




S20111209-046
20111213
PROTON CDCl₃ D:\nmr data ham



HSP70-33



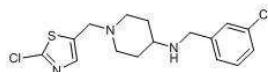
Current Data Parameters
NAME JKYYLL1106-5
EXPNO 10
PROCNO 1

P2 - Acquisition Parameters
Date_ 2011213
Time_ 19.58
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDCl₃
NS 16
DS 2
SWH 8223.685 Hz
PIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 128
DW 60.000 usec
DE 6.50 usec
TE 300.3 K
D1 1.0000000 sec

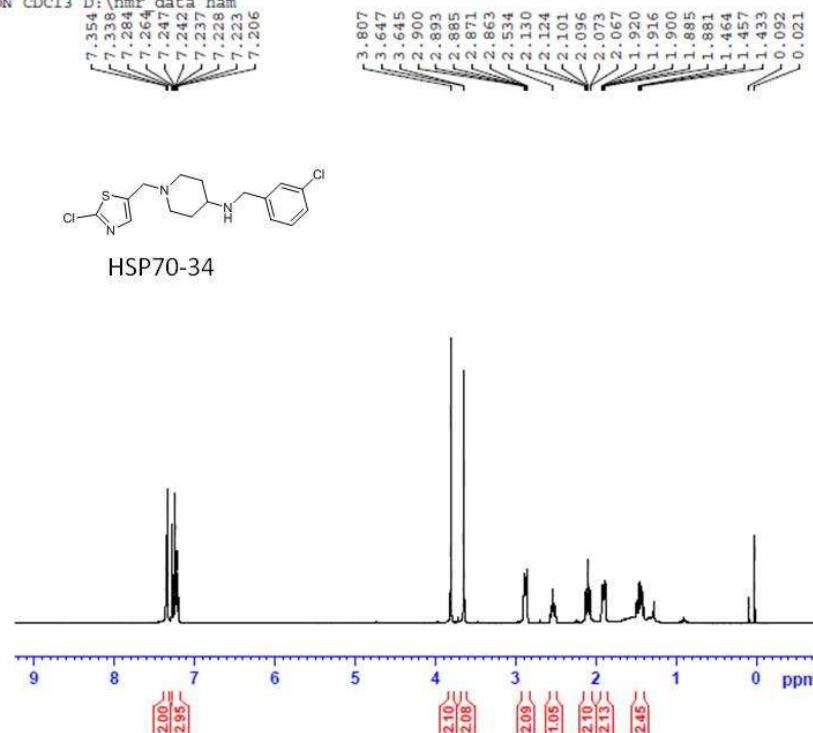
----- CHANNEL f1 -----
NUC1 IH
P1 14.50 usec
PLM1 12.85299969 W
SPO1 400.1324710 MHz

P2 - Processing parameters
SI 65536
SF 400.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

S20111212-064
20111213
PROTON CDCl₃ D:\nmr data ham



HSP70-34

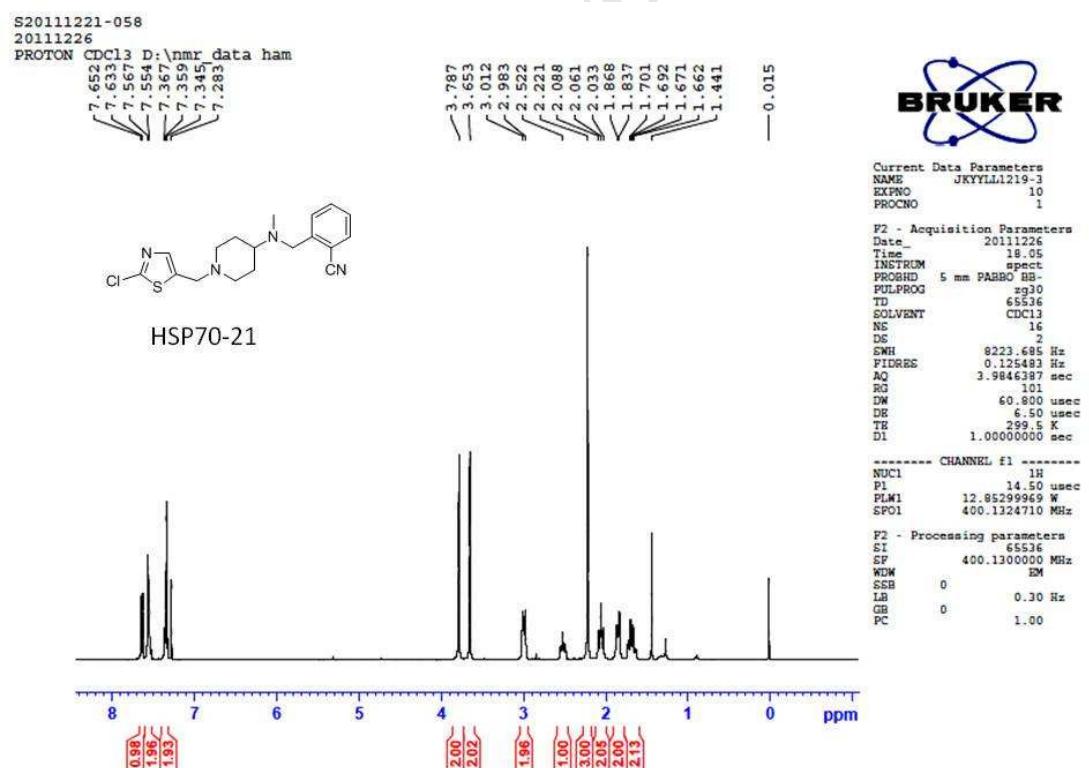
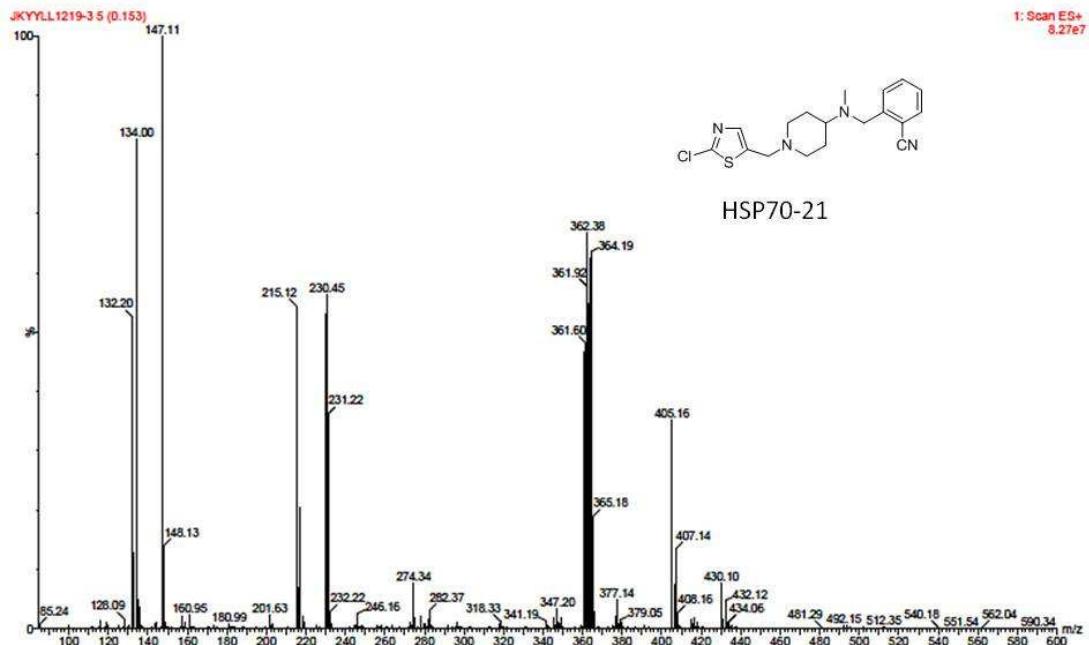


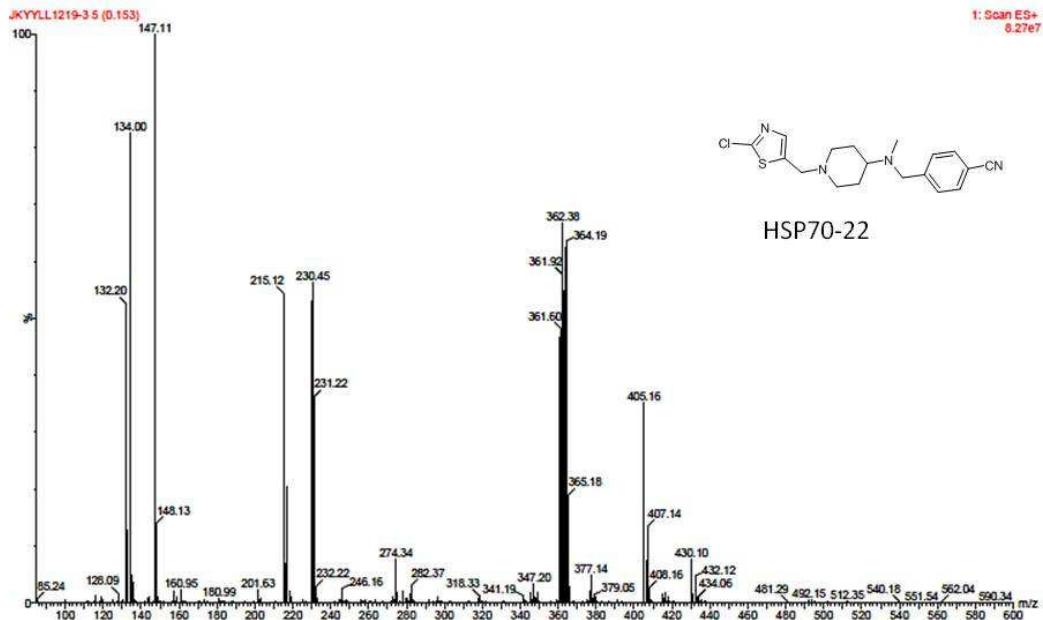
Current Data Parameters
NAME JKYYLL1107-6
EXPNO 10
PROCNO 1

P2 - Acquisition Parameters
Date_ 20111213
Time_ 18.08
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDCl₃
NS 16
DS 2
SWH 8223.685 Hz
PIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 114
DW 60.000 usec
DE 6.50 usec
TE 300.3 K
D1 1.0000000 sec

----- CHANNEL f1 -----
NUC1 IH
P1 14.50 usec
PLM1 12.85299969 W
SPO1 400.1324710 MHz

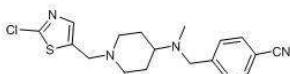
P2 - Processing parameters
SI 65536
SF 400.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00





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20111226
PROTON CDCl₃ D:\nmr_data\ham

7.6159
7.4433
7.4411
7.2333
7.2333



HSP70-22

0.015

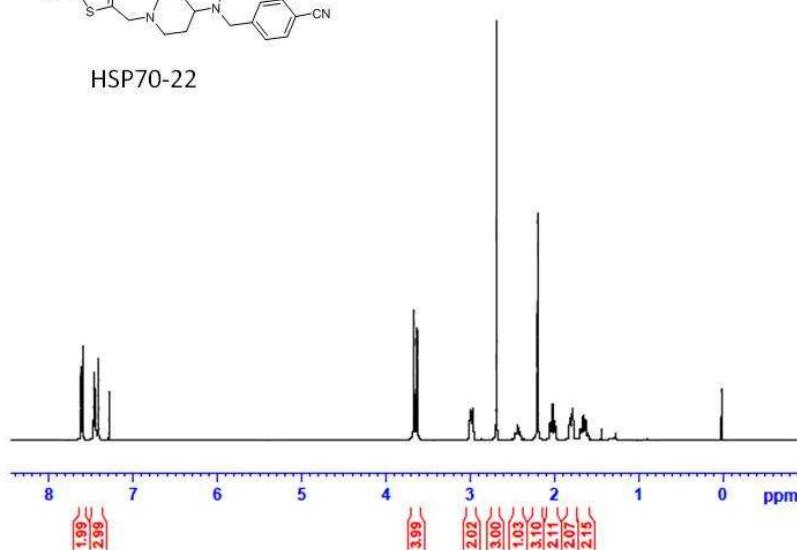


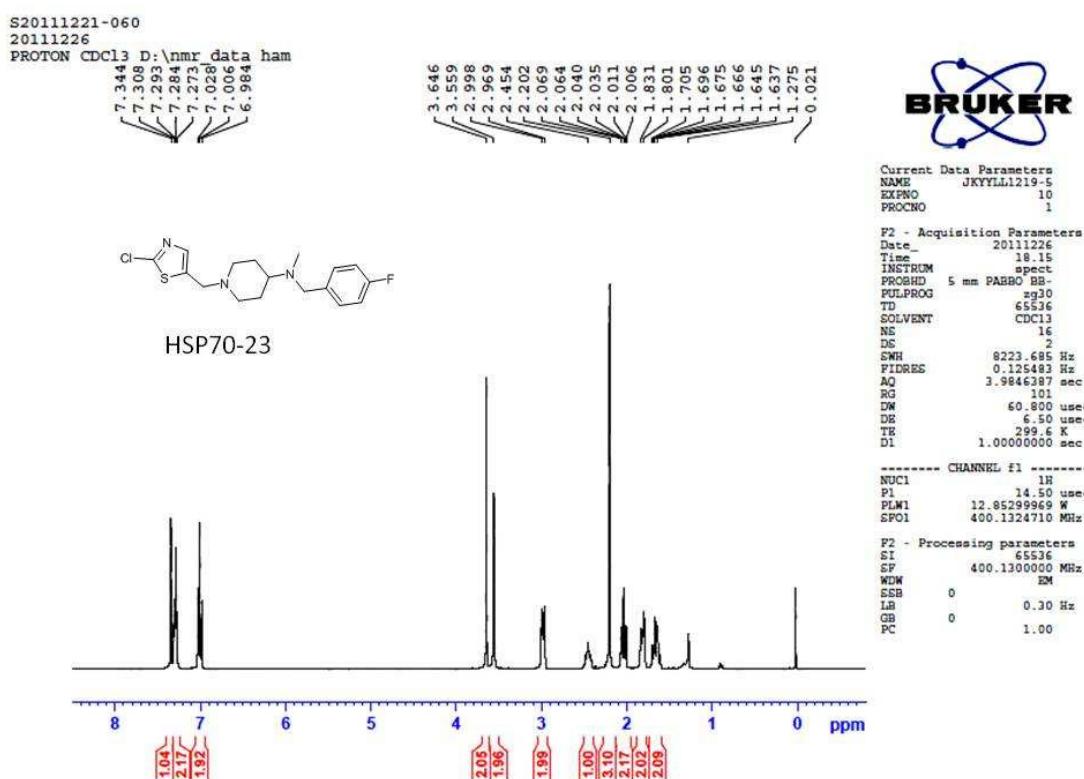
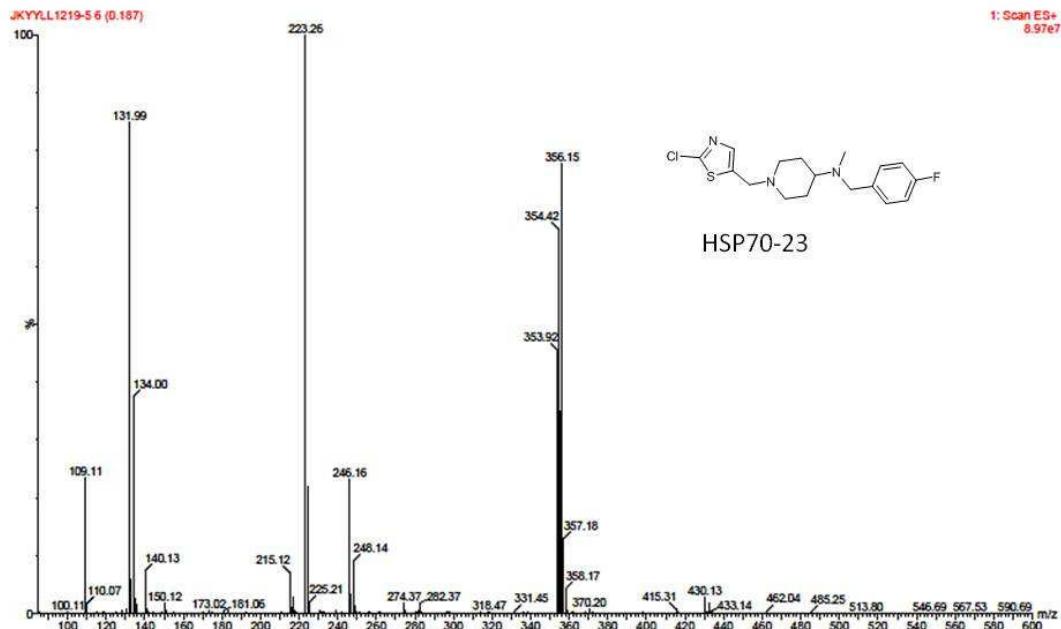
Current Data Parameters
NAME JKYLL1219-4
EXPNO 10
PROCNO 1

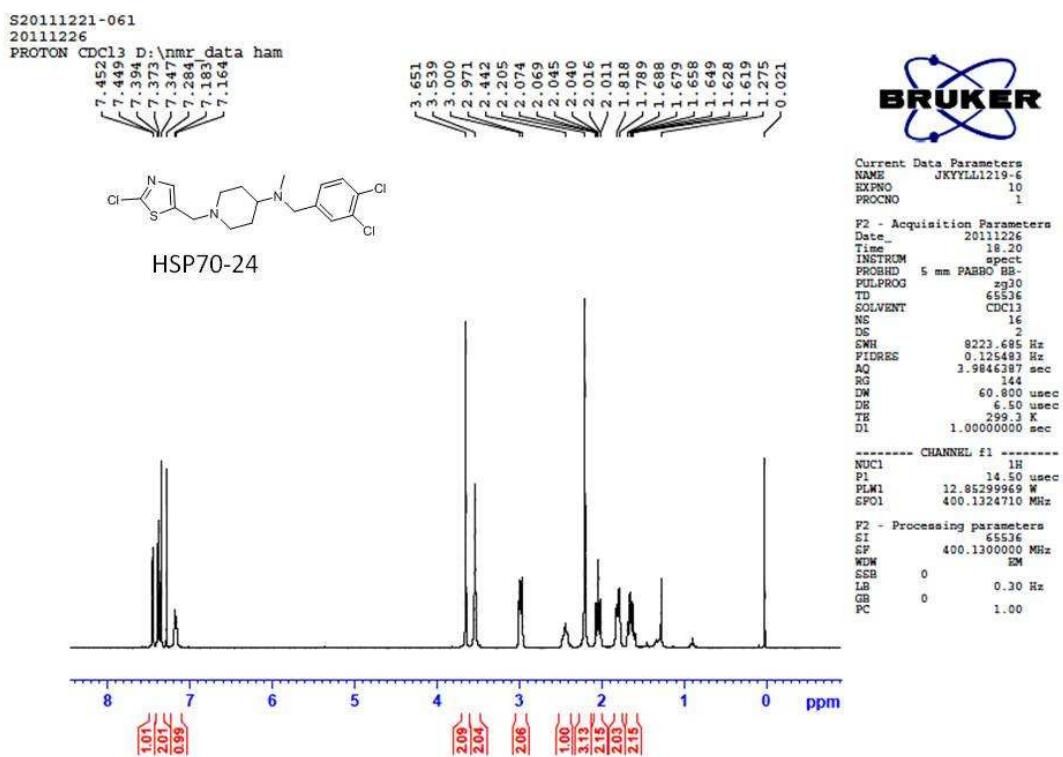
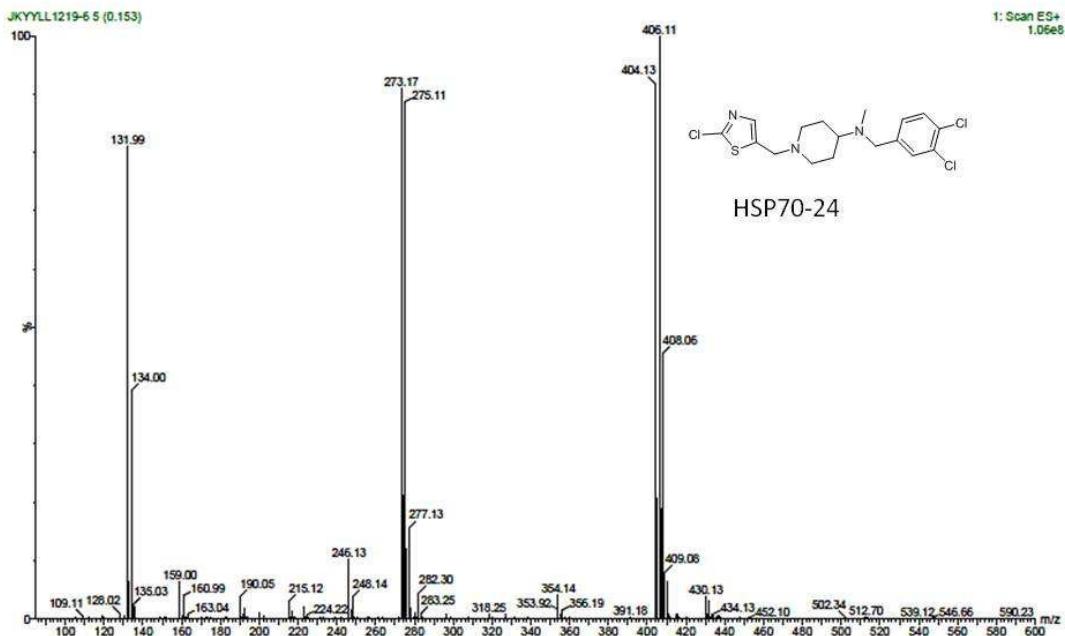
P2 - Acquisition Parameters
Date 20111226
Time 18.09
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 6556
SOLVENT CDCl₃
NS 16
DS 2
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 101
DM 60.00 usec
TE 299.9 K
TR 1.0000000 sec

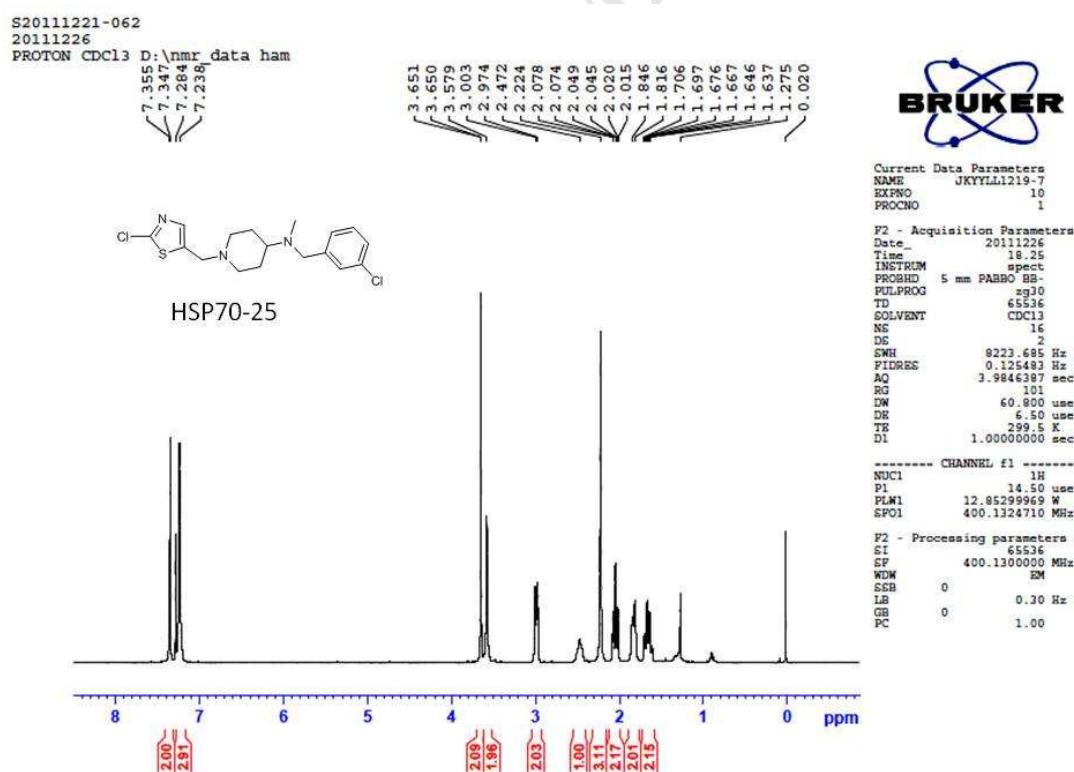
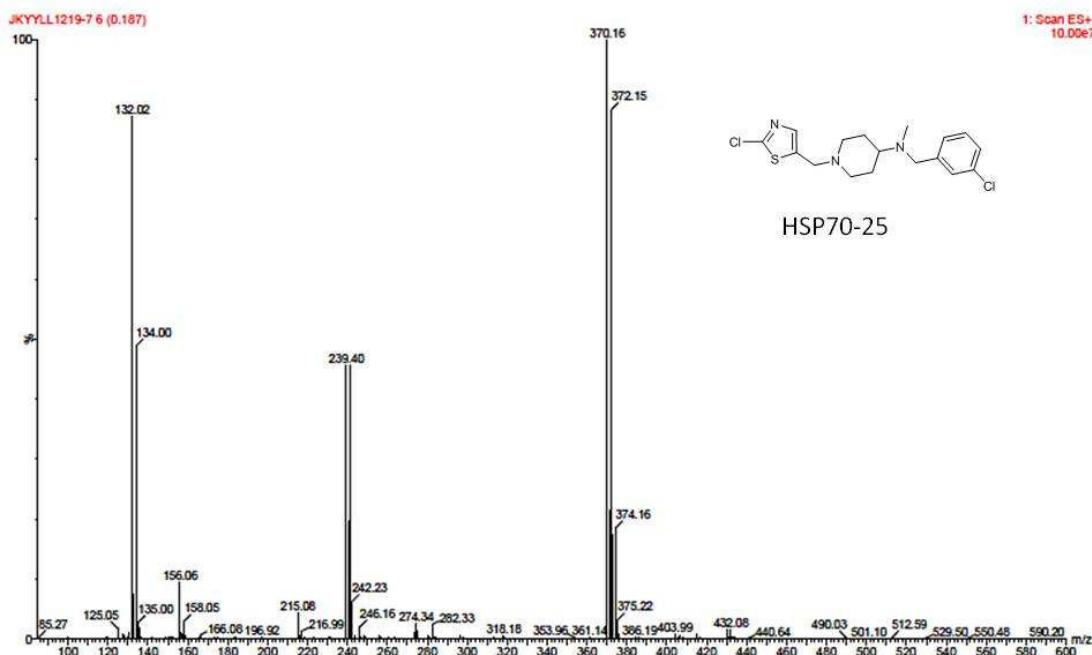
----- CHANNEL f1 -----
NUC1 1H
PI 14.50 usec
PLW1 12.85299969 W
SF01 400.1324710 MHz

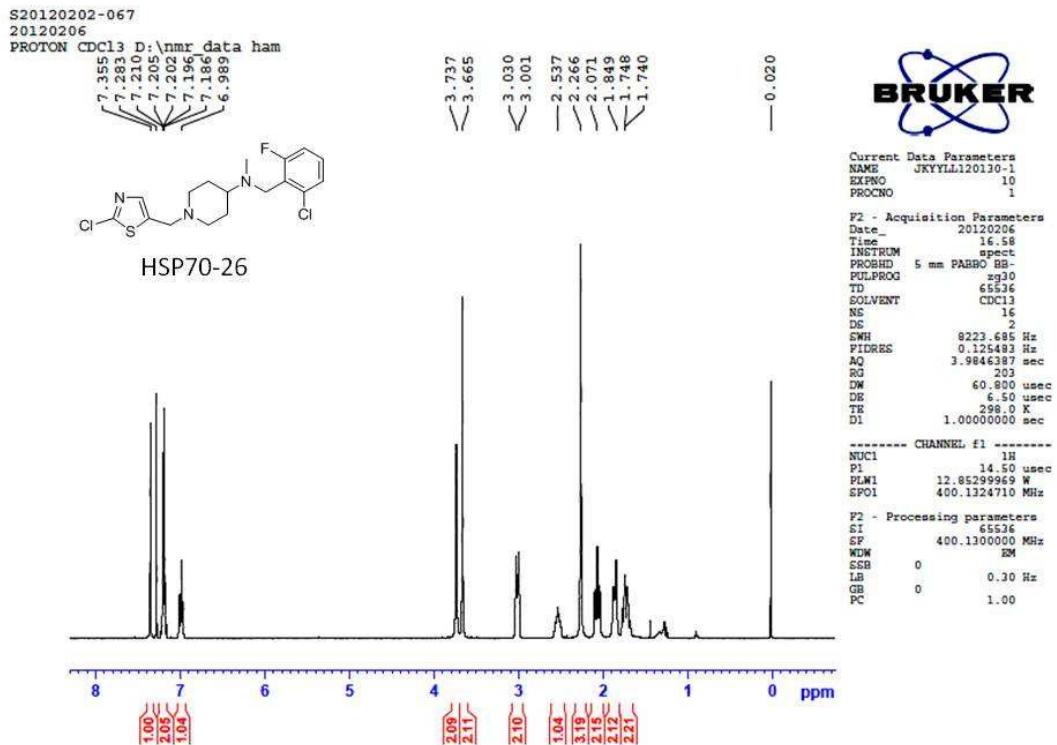
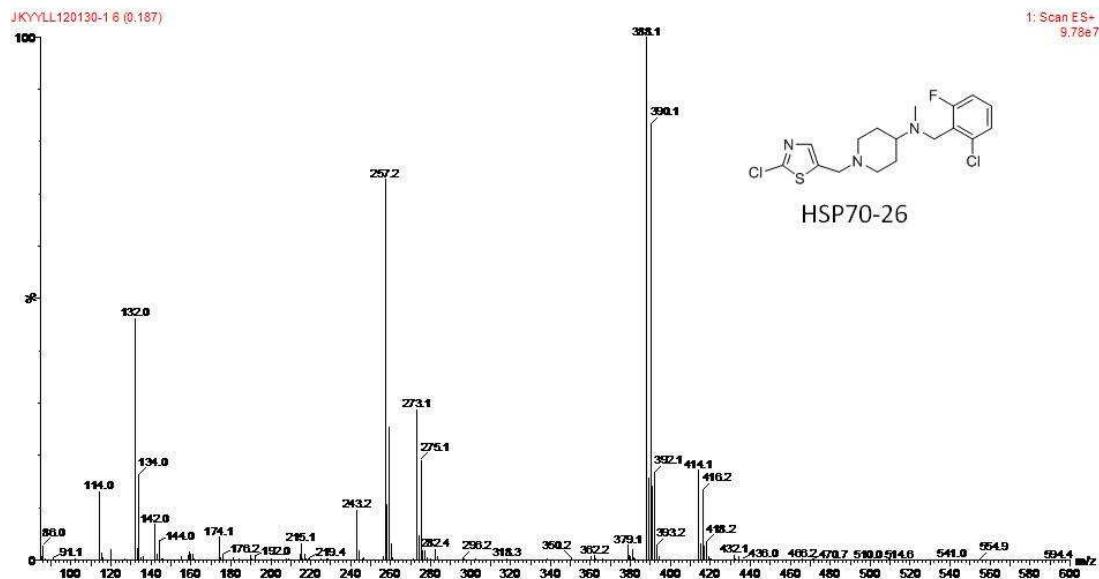
P2 - Processing parameters
SI 65536
SF 400.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

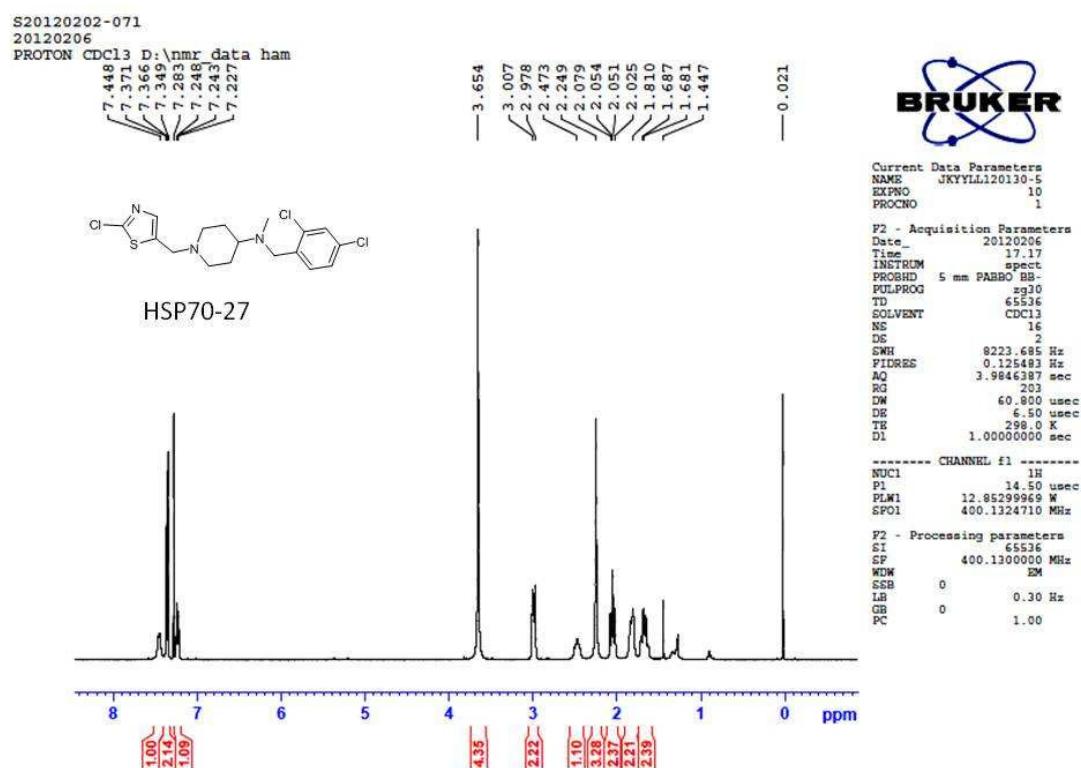
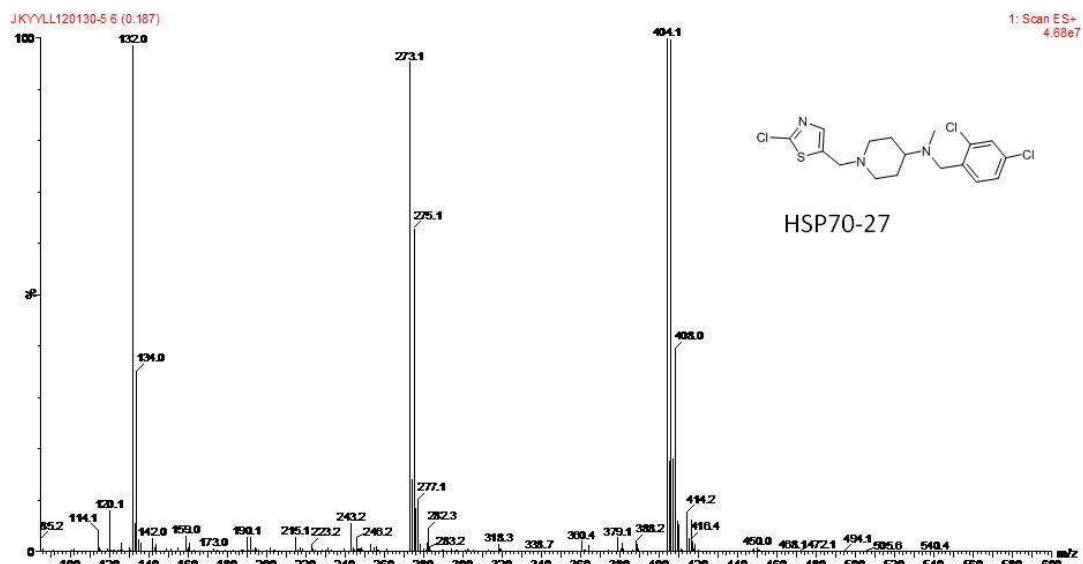


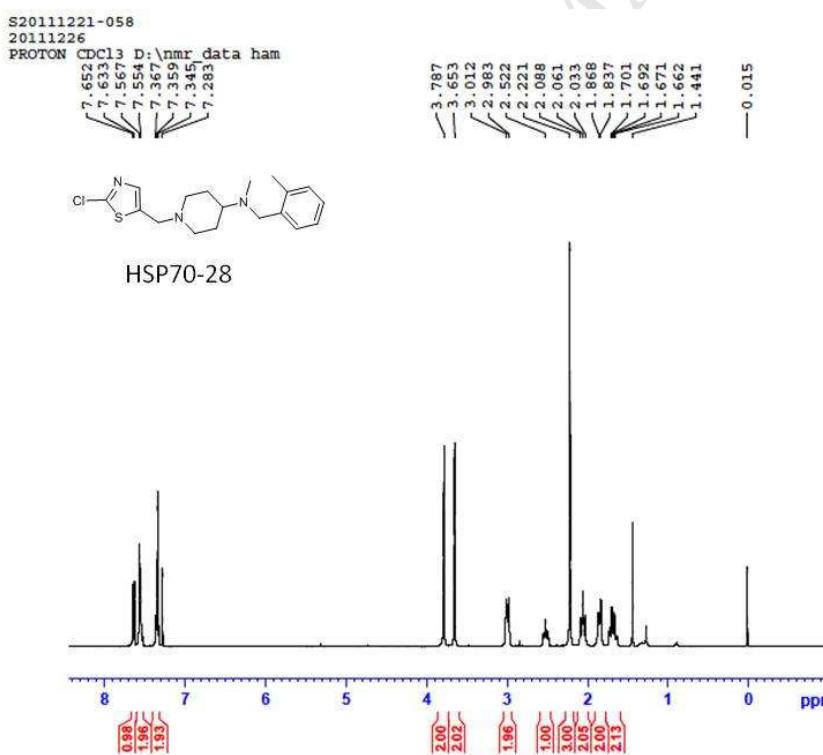
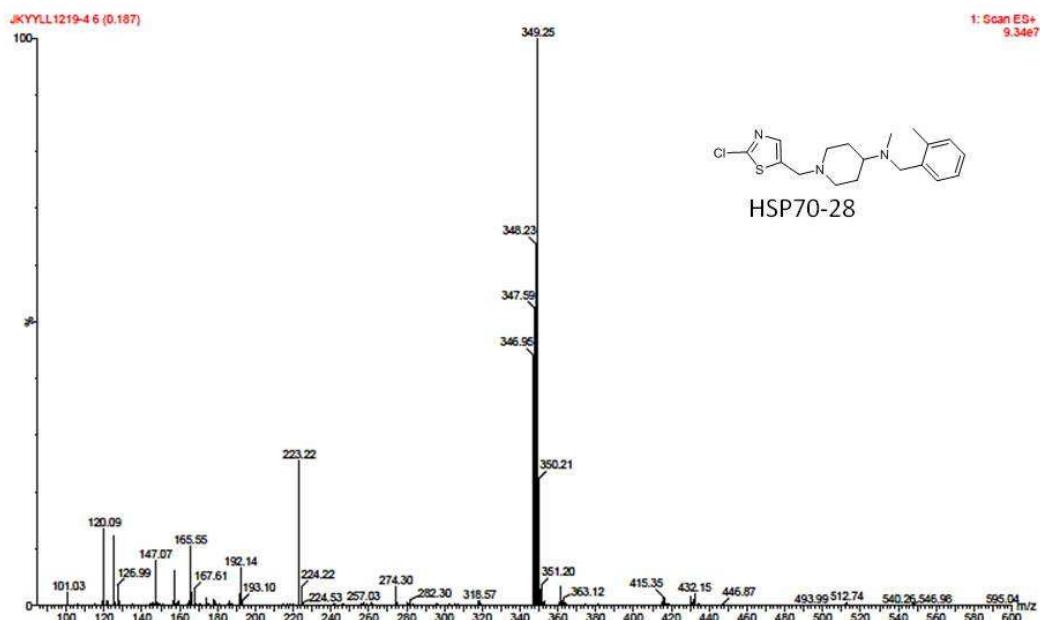


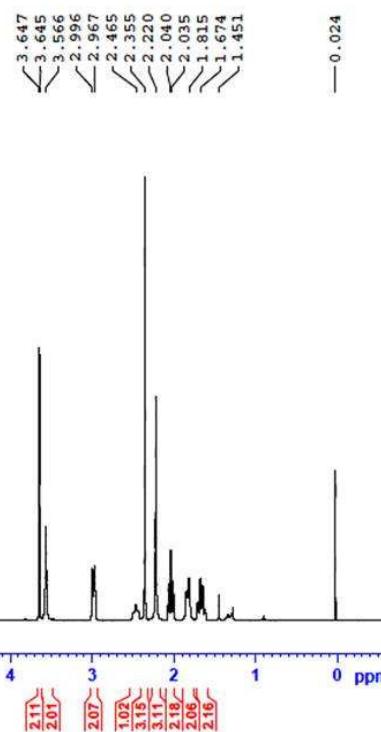
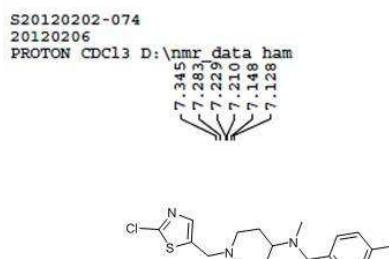
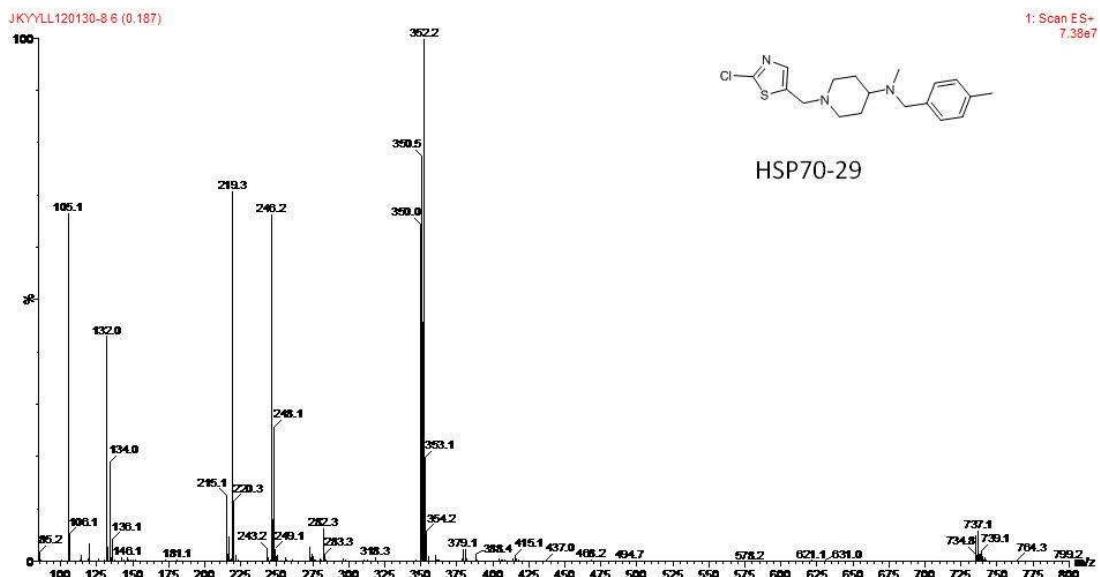


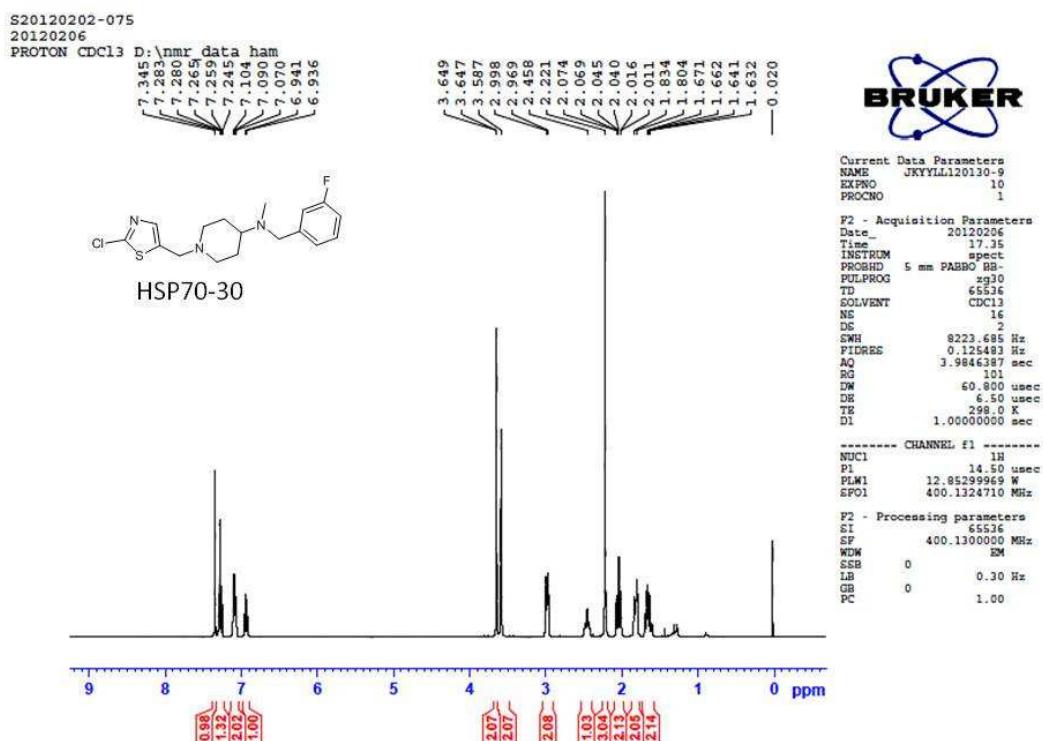
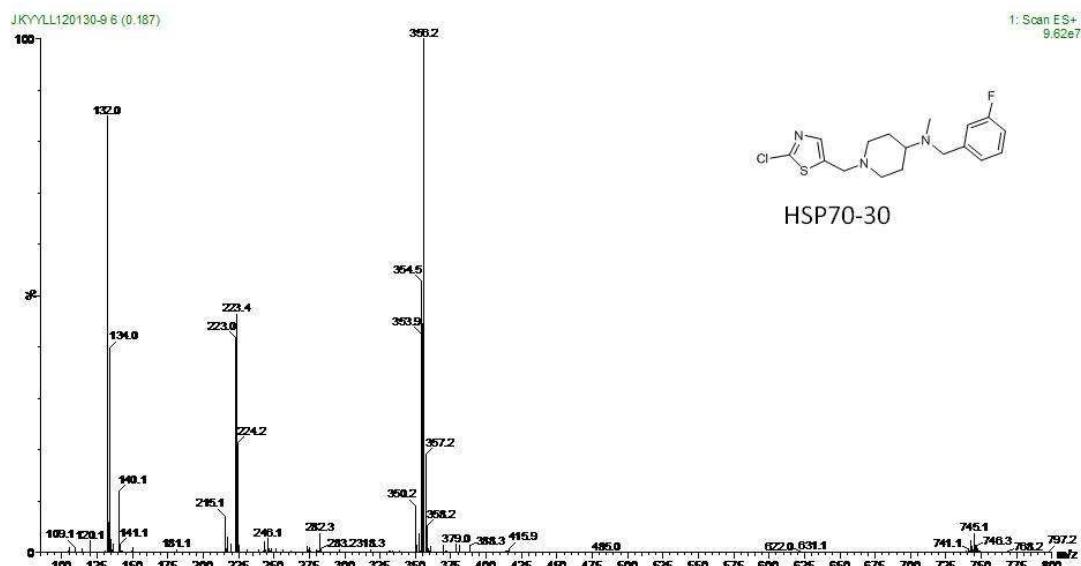


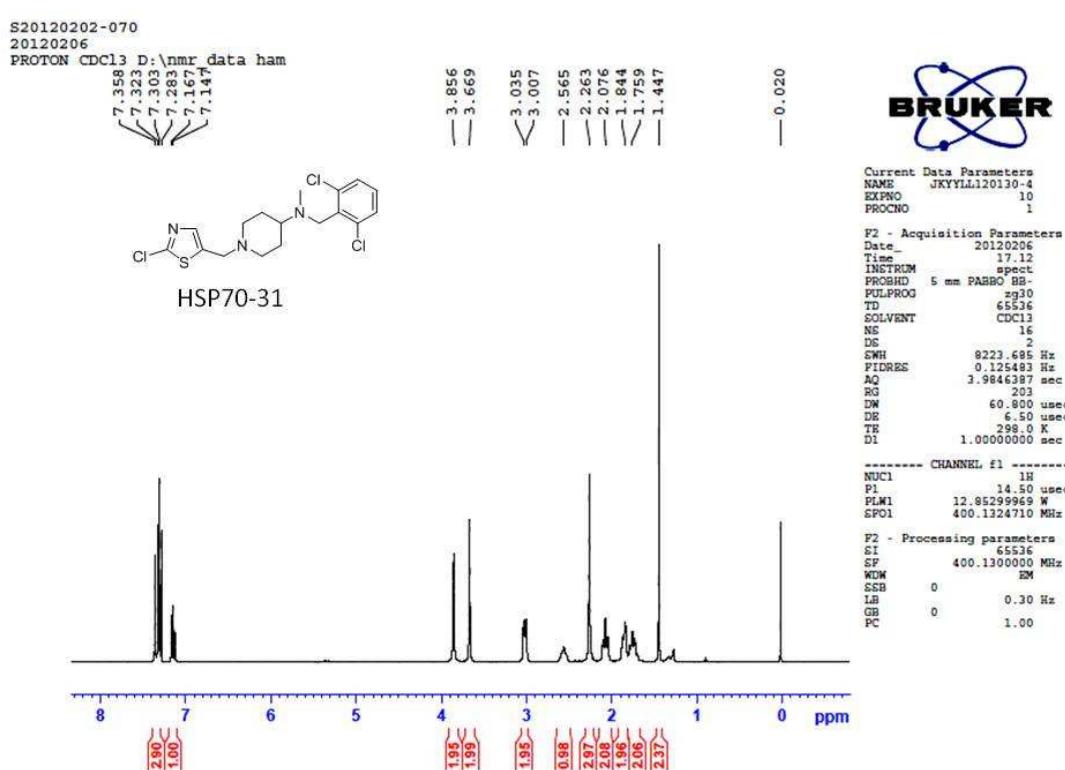
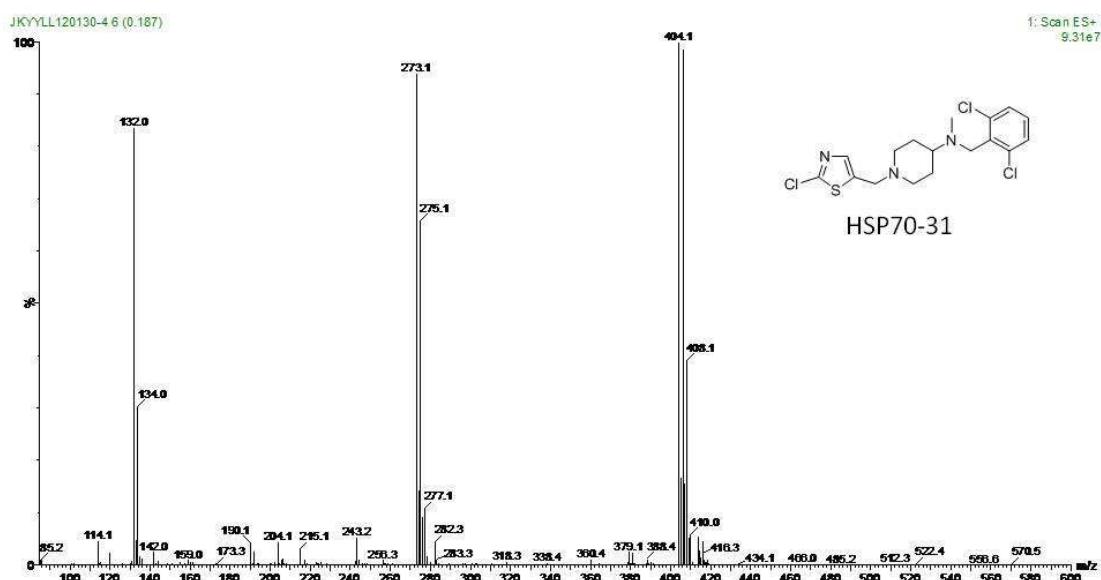


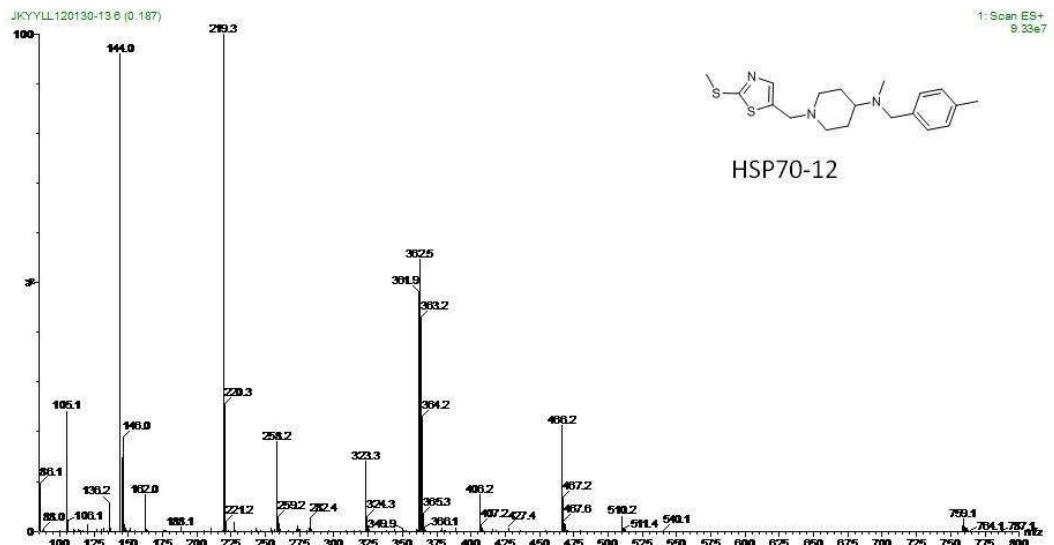






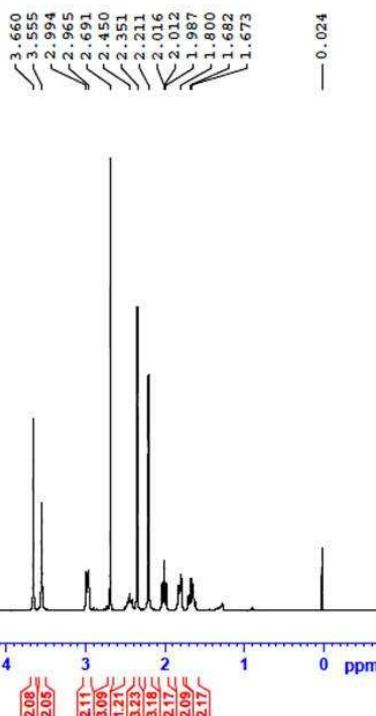


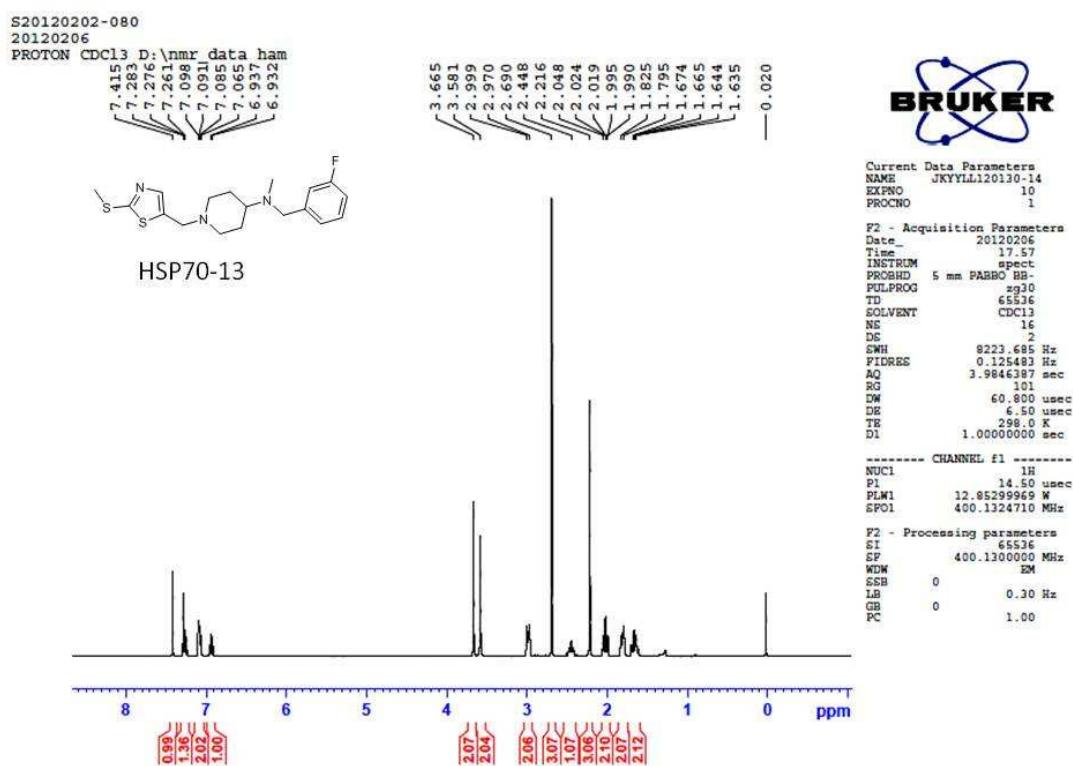
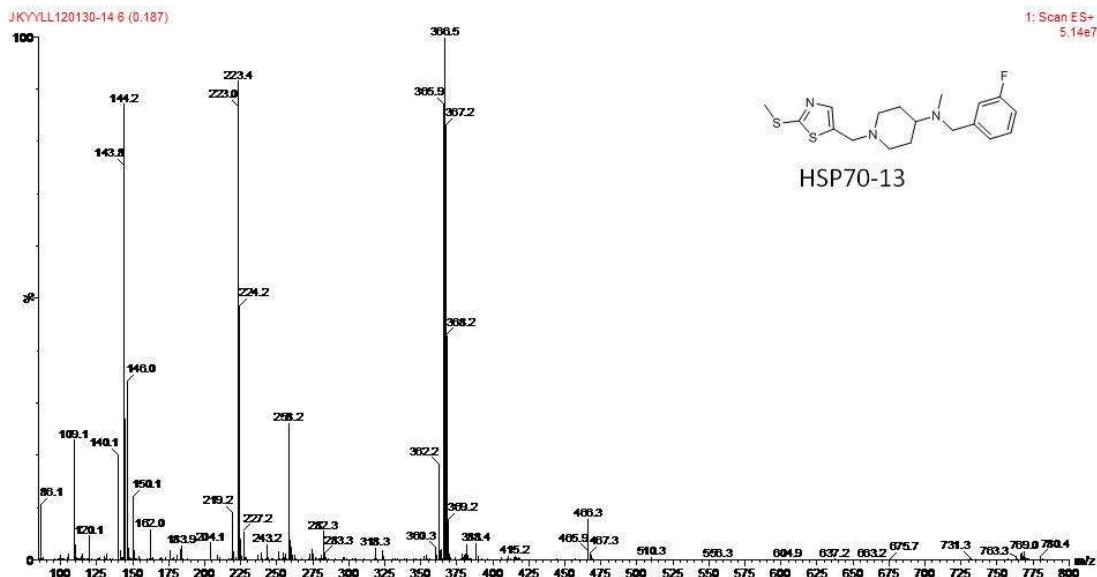


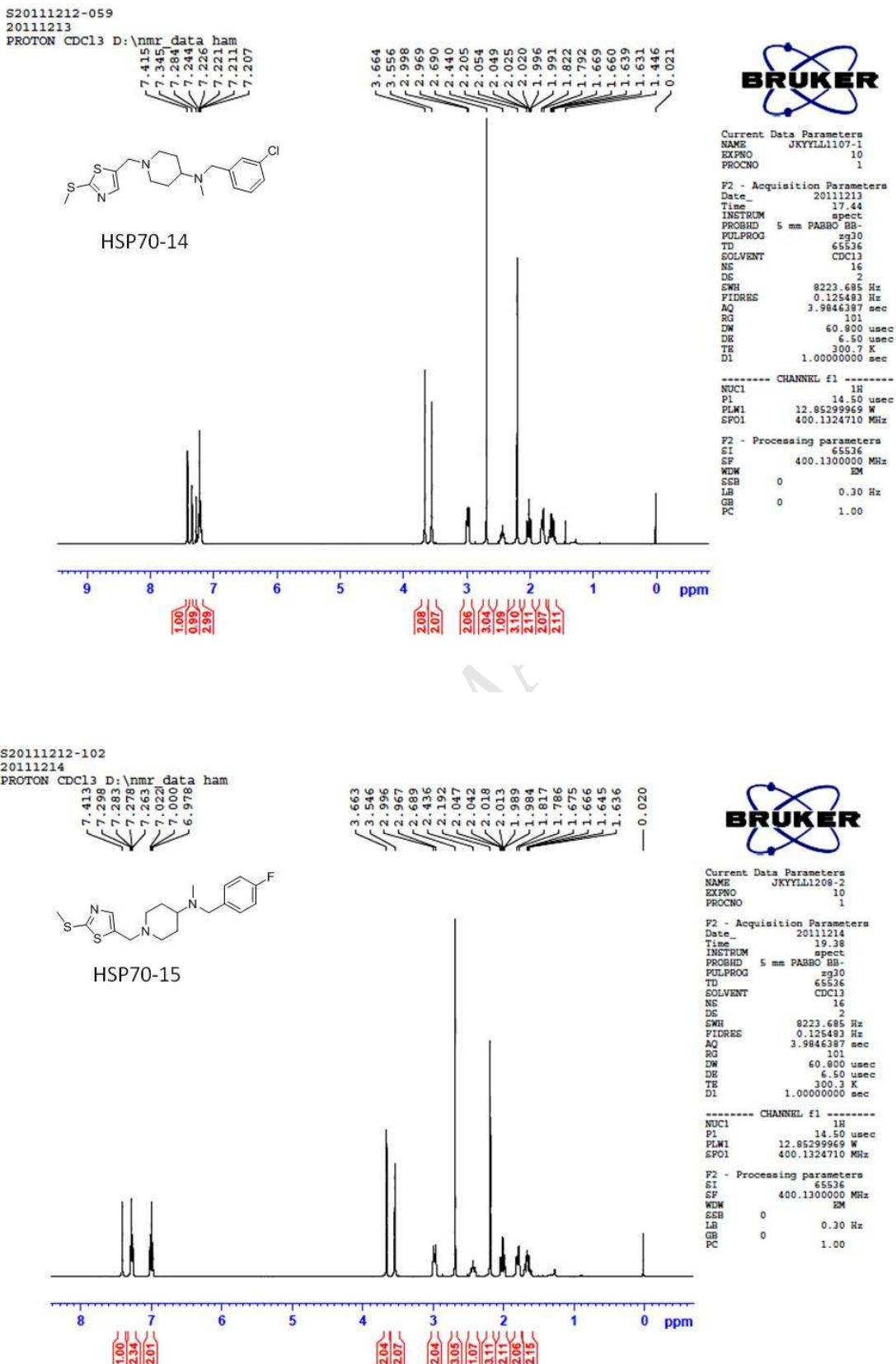


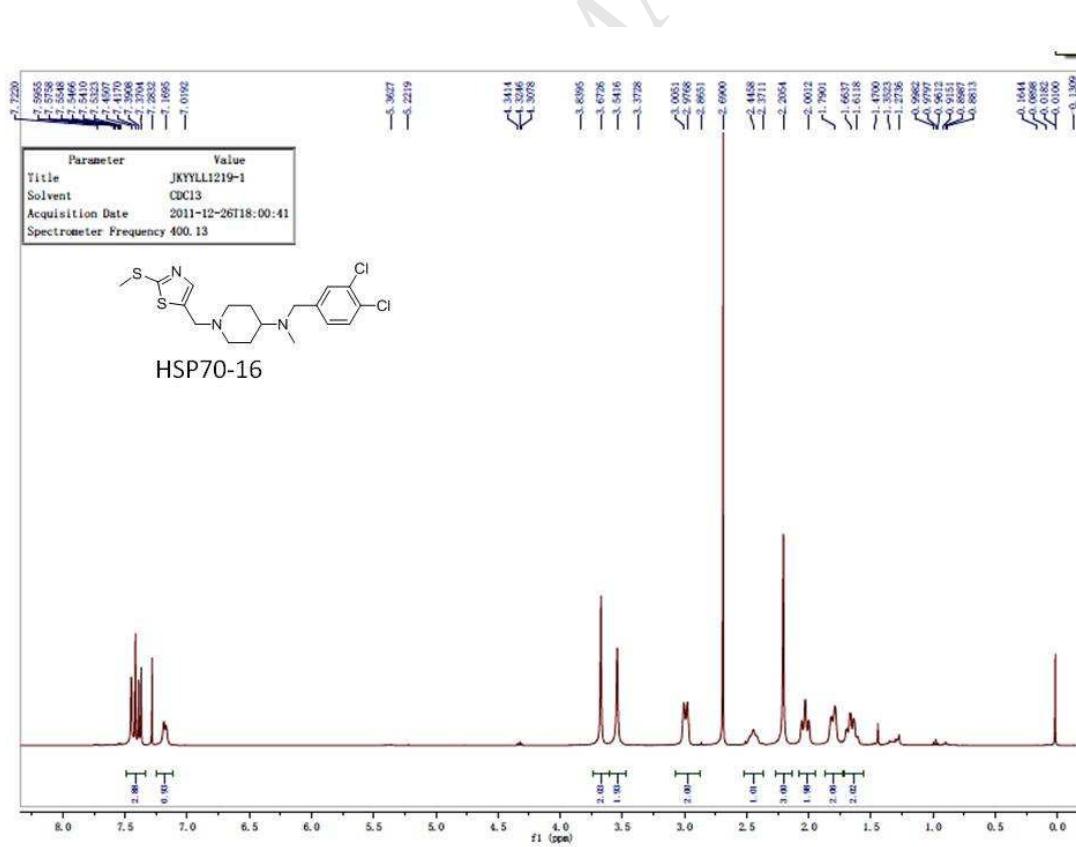
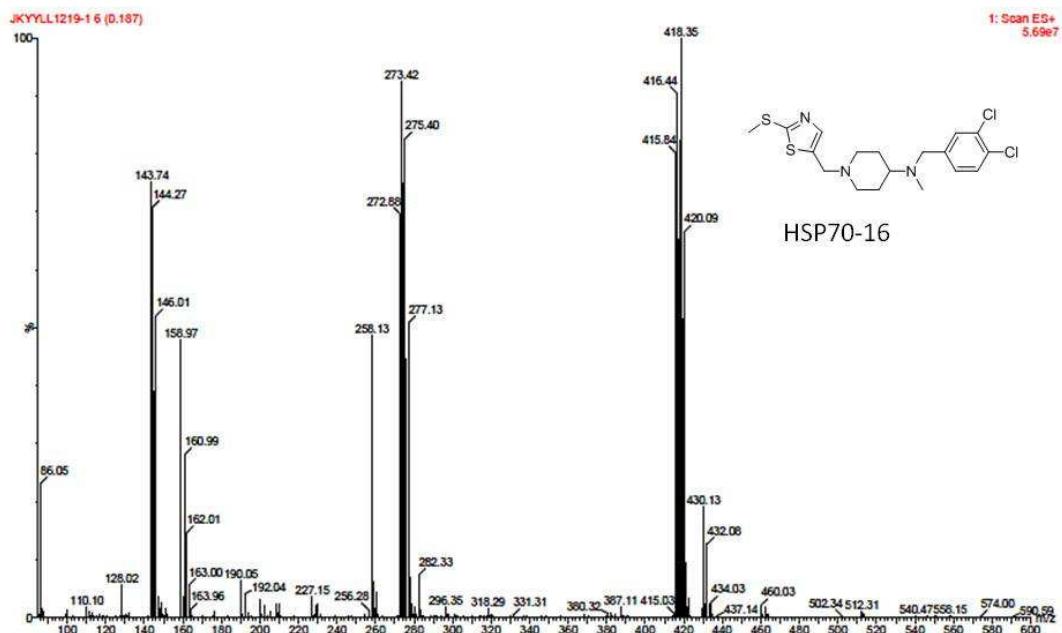
S20120202-079
20120206
PROTON CDCl₃ D:\nmr data\ham
7.413 7.284 7.221 7.202 7.141 7.122
7.122

HSP70-12

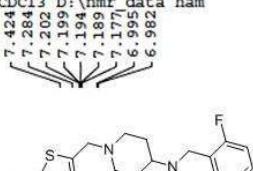




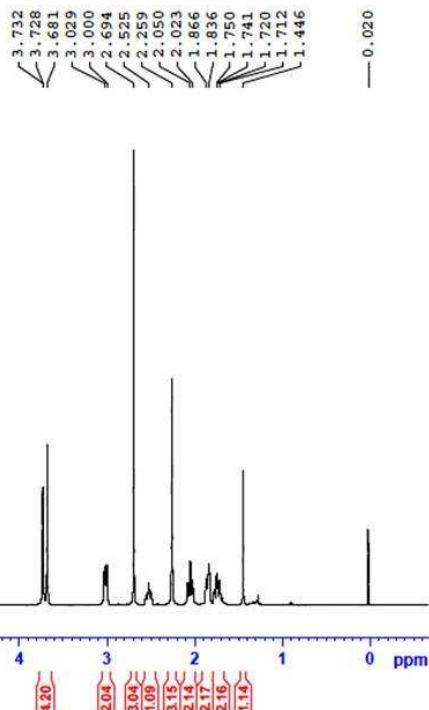




S20111212-103
20111214
PROTON CDCl₃ D:\nmr data ham



HSP70-17



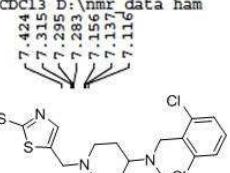
Current Data Parameters
 NAME JKYYLL1208-3
 EXPNO 10
 PROCNO 1
 P2 - Acquisition Parameters
 Date_ 20111214
 Time 19:57:43
 INSTRUM spect
 PROBHD 5 mm PARBO BB-
 PULPROG zg30
 TD 65536
 SOLVENT CDCl₃
 NS 16
 SWH 8223.685 Hz
 FIDRES 0.125483 Hz
 AQ 3.9846387 sec
 RG 128
 DW 60.800 usec
 DE 6.50 usec
 TB 300.1 K
 DI 1.0000000 sec

----- CHANNEL f1 -----

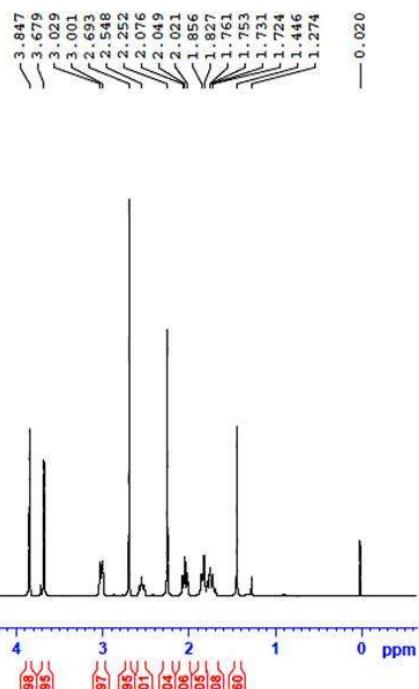
NUC1 1H
 PI 14.50 usec
 PLW1 12.8529969 W
 SPO1 400.1324710 MHz

P2 - Processing parameters
 SI 65536
 SF 400.1300000 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00

S20111212-106
20111214
PROTON CDCl₃ D:\nmr data ham



HSP70-18

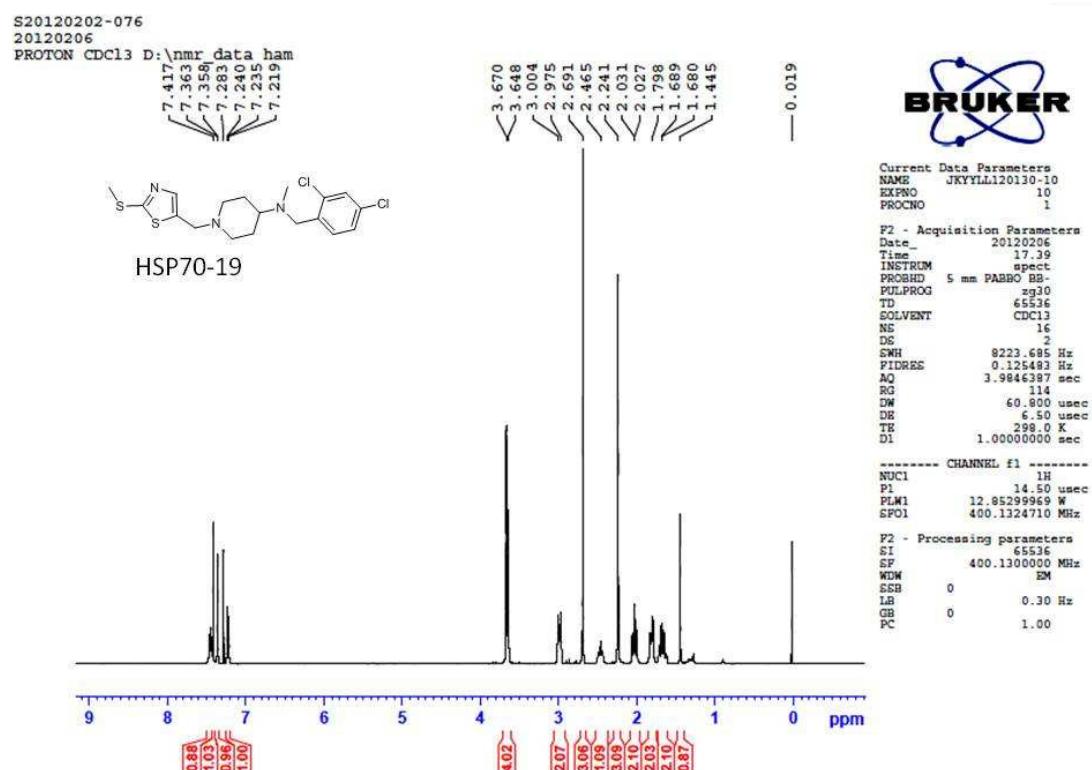
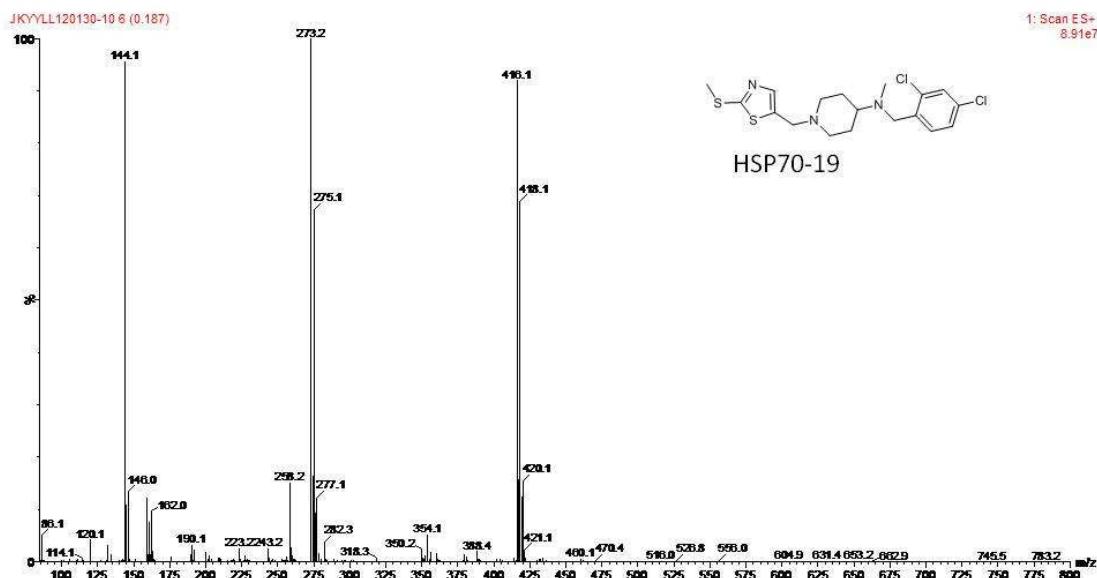


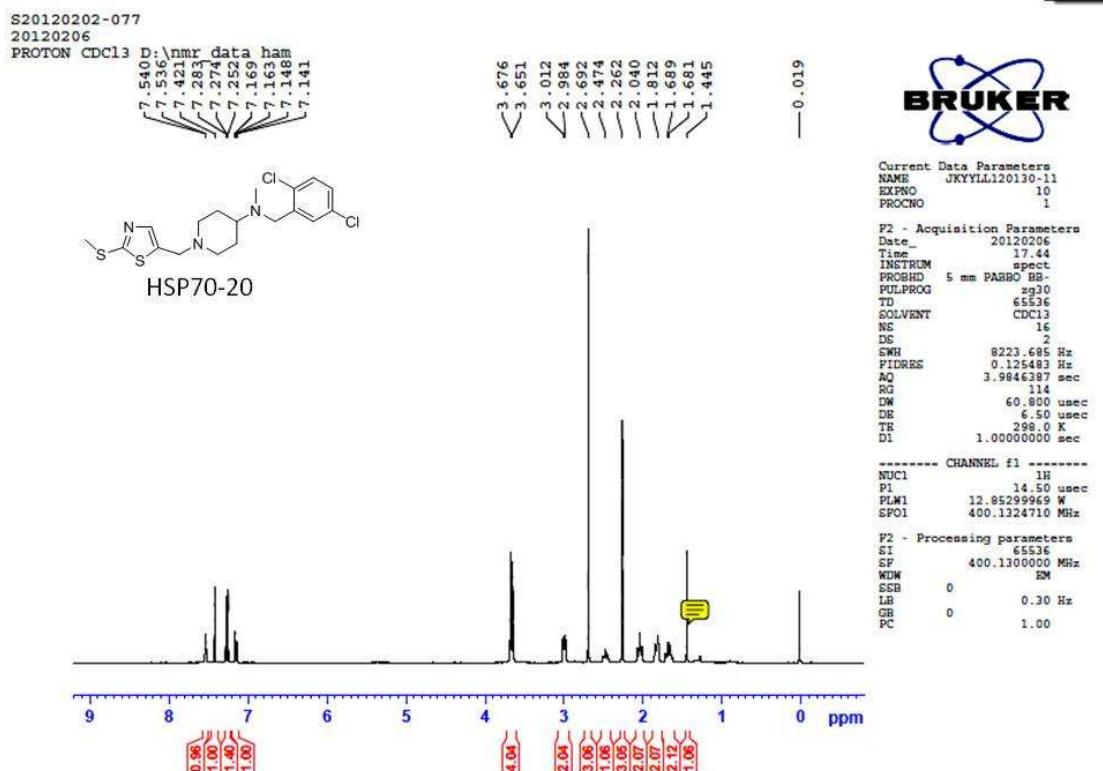
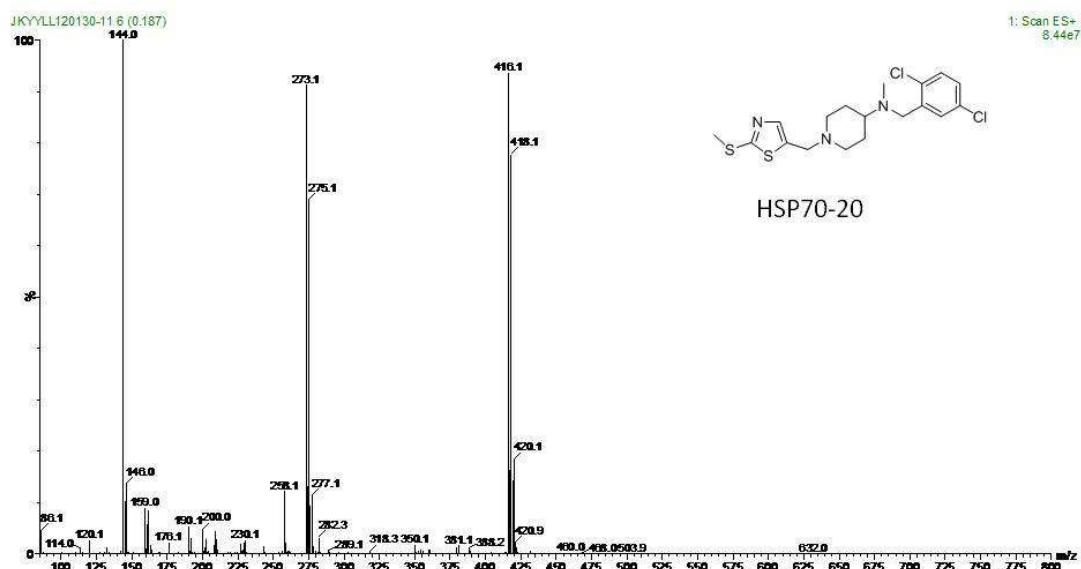
Current Data Parameters
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 EXPNO 10
 PROCNO 1
 P2 - Acquisition Parameters
 Date_ 20111214
 Time 19:57:43
 INSTRUM spect
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 PULPROG zg30
 TD 65536
 SOLVENT CDCl₃
 NS 16
 SWH 8223.685 Hz
 FIDRES 0.125483 Hz
 AQ 3.9846387 sec
 RG 101
 DW 60.800 usec
 DE 6.50 usec
 TB 300.1 K
 DI 1.0000000 sec

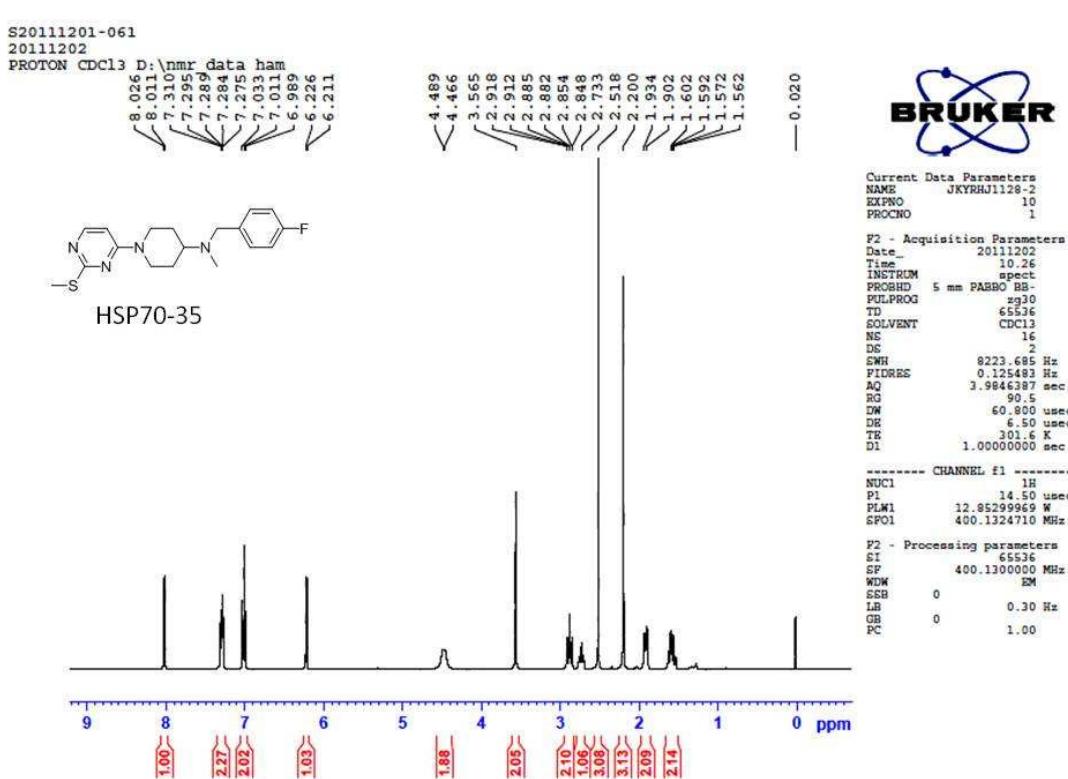
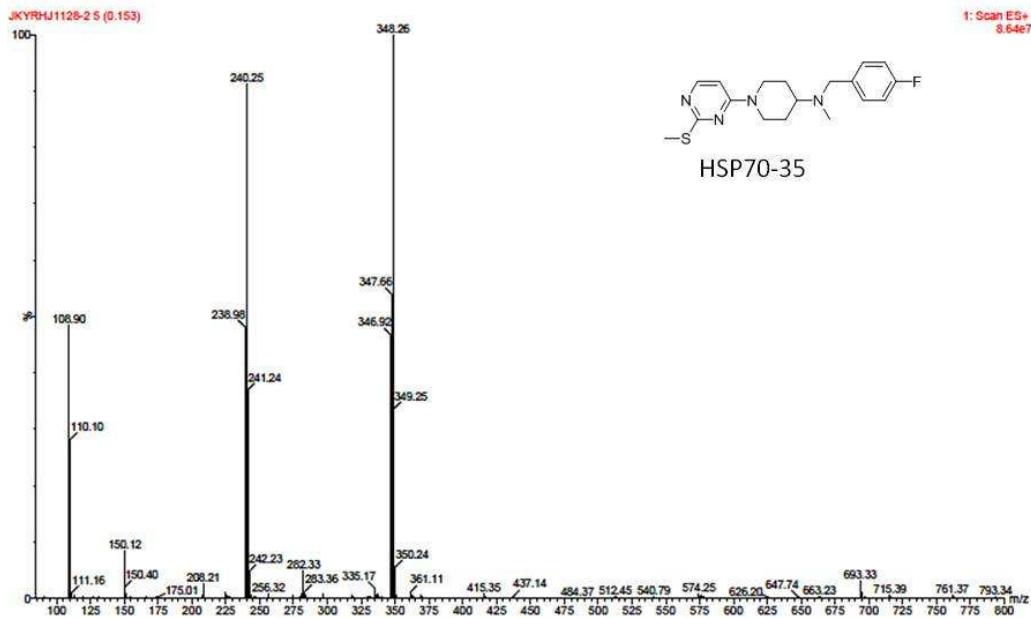
----- CHANNEL f1 -----

NUC1 1H
 PI 14.50 usec
 PLW1 12.8529969 W
 SPO1 400.1324710 MHz

P2 - Processing parameters
 SI 65536
 SF 400.1300000 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00

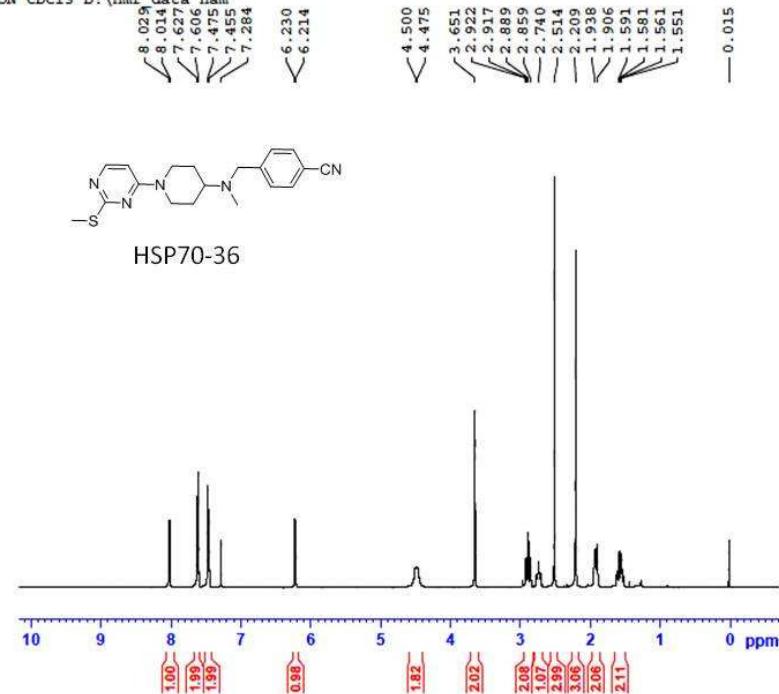




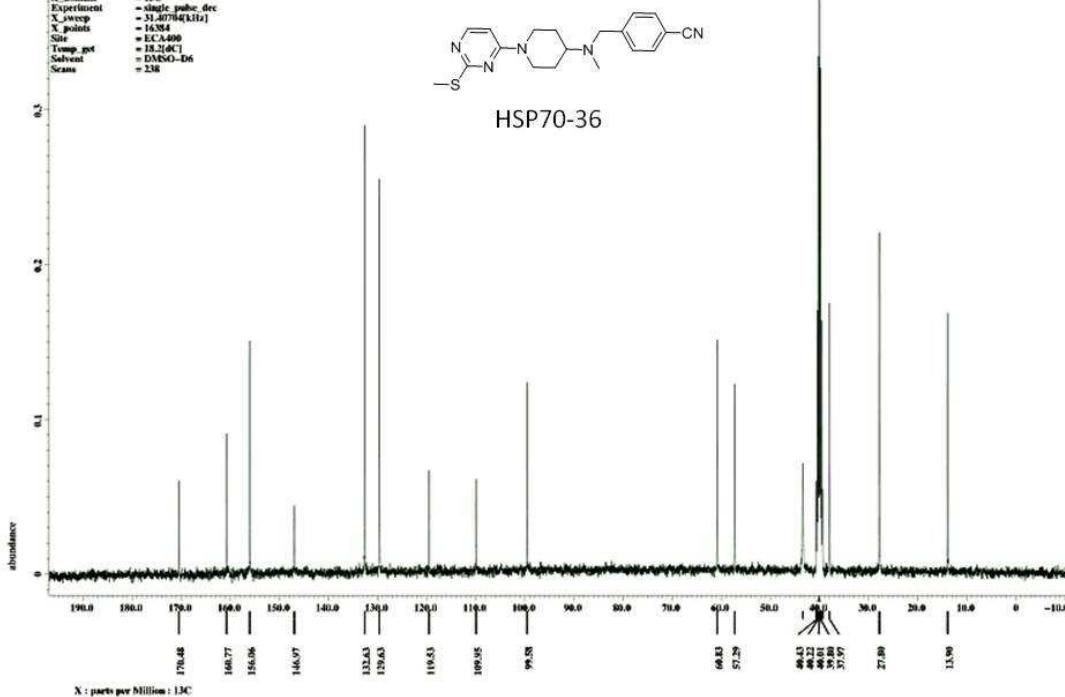


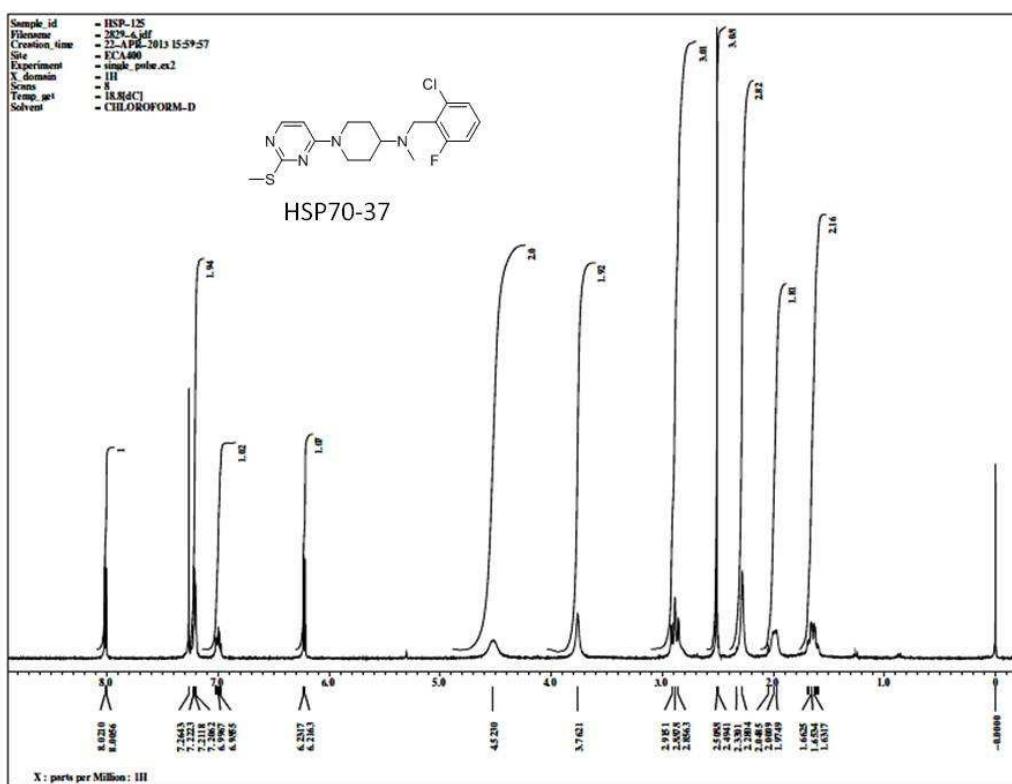
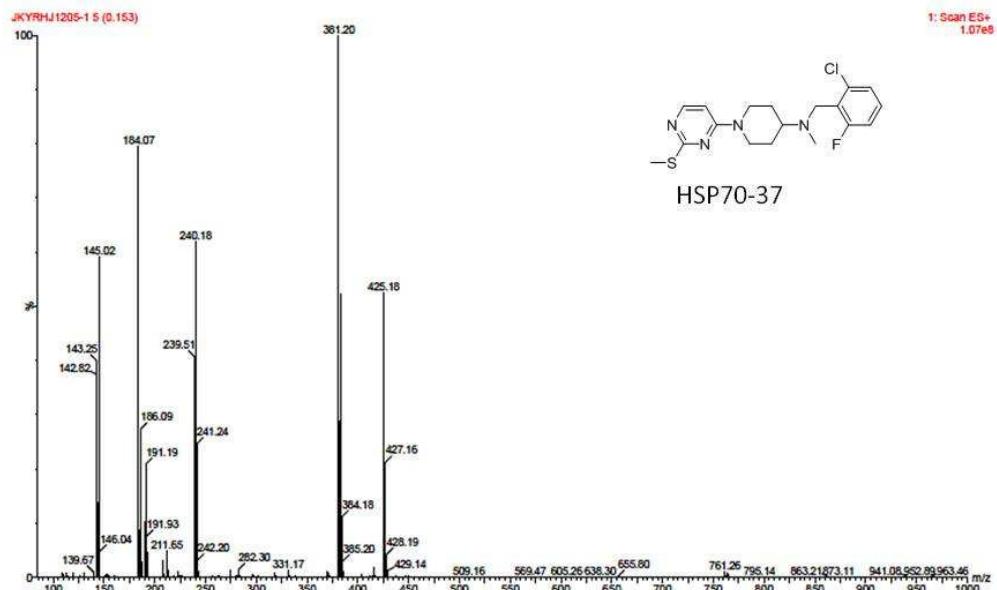
S20111212-093

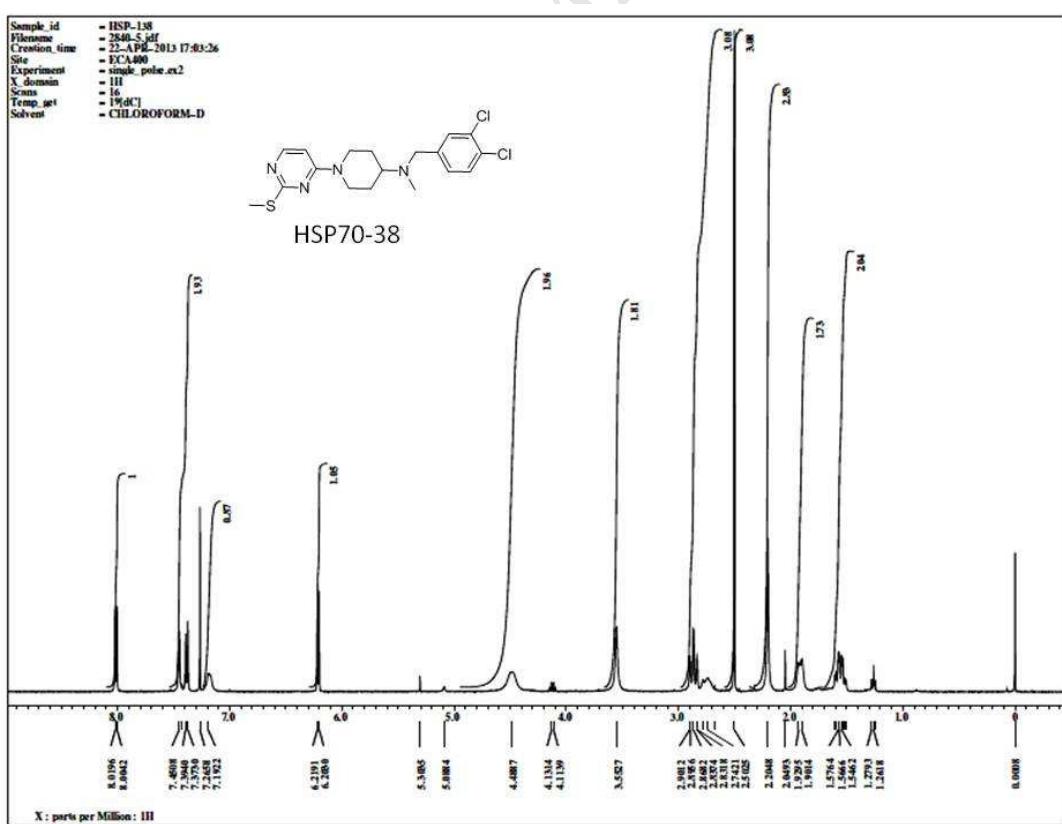
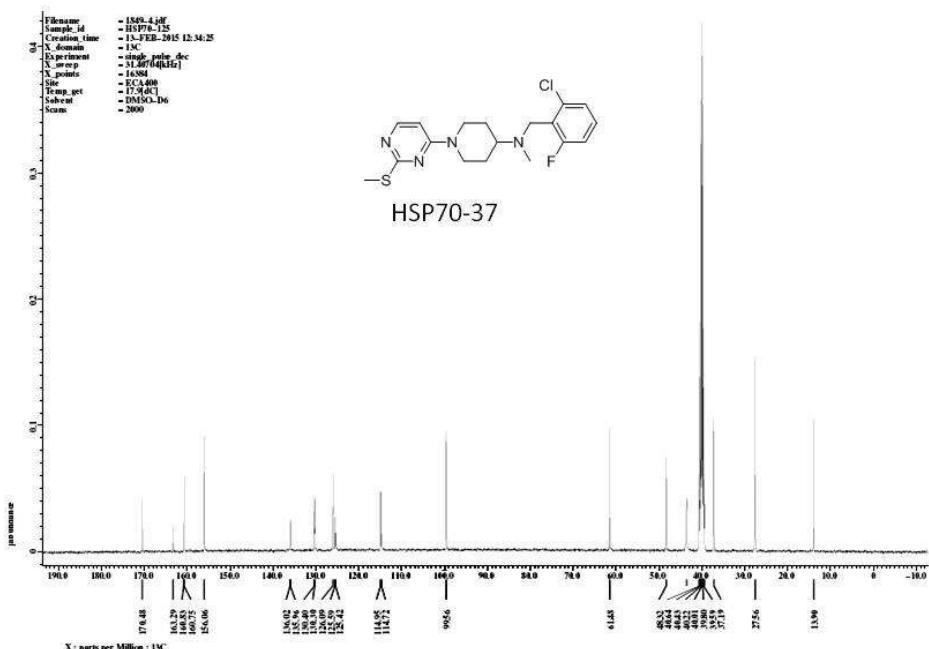
20111214

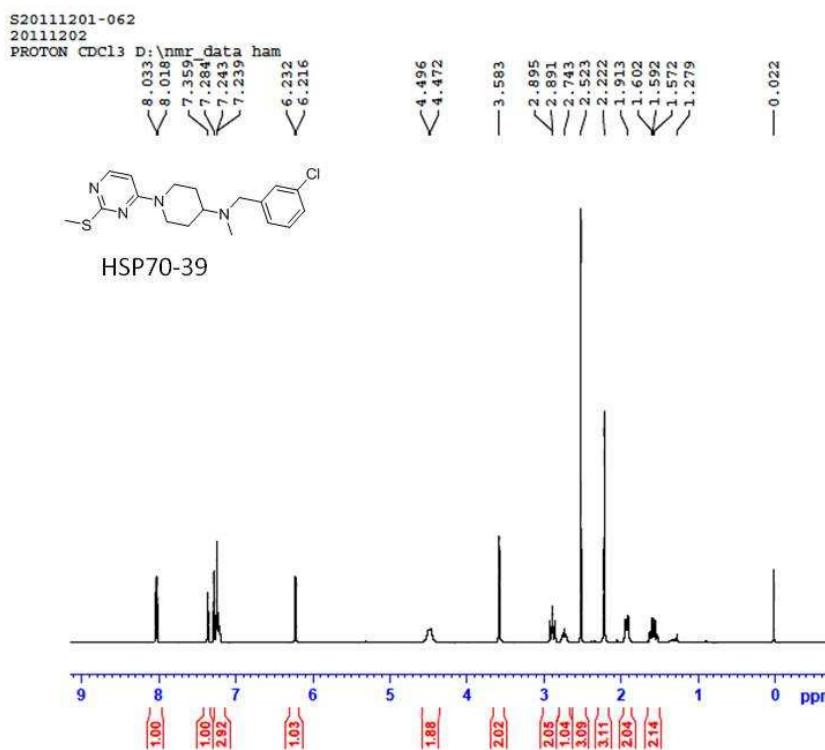
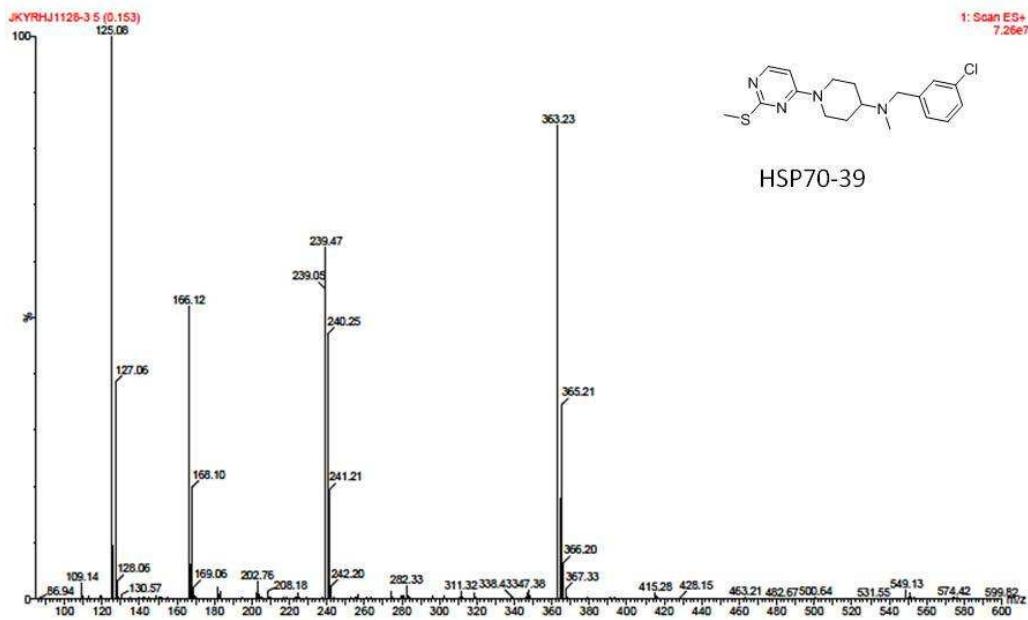
PROTON CDCl₃ D:\nmar data ham

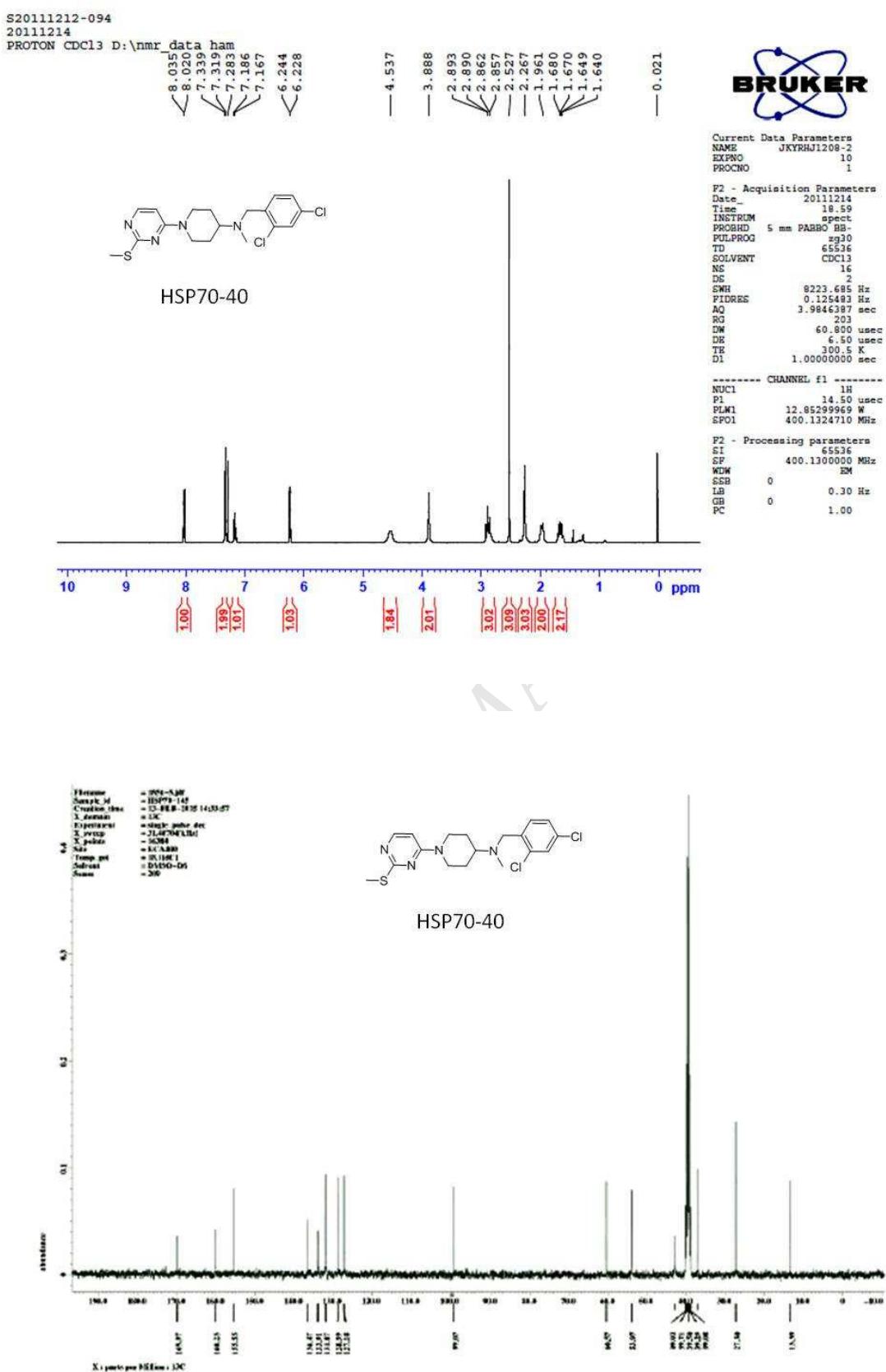
Filename = 1853-3.mf
 Sample_id = HSP70-144
 Creation_time = 13-DEC-2015 15:19:20
 X_start = 190.0
 Experiment = single_pulse dec
 X_sweep = 31.40784(kHz)
 X_points = 16384
 Site = ECA400
 Temp_set = 18.2(6C)
 Solvent = DMSO-D6
 Scale = 1.00





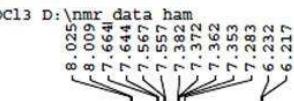




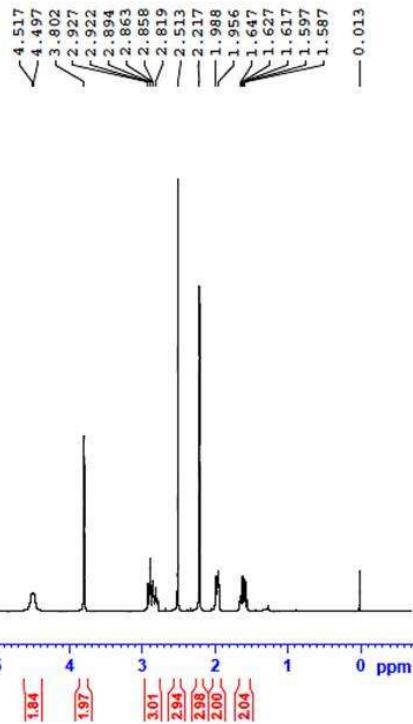


S20111212-095

20111214

PROTON CDCl₃ D:\nmr\data\ham

HSP70-41



Current Data Parameters
NAME JKYRHJ1208-3
EXPNO 10
PROCNO 1

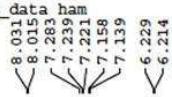
P2 - Acquisition Parameters
Date 20111214
Time 19.04
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDCl₃
NS 16
DS 2
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 90.5
DW 60.800 usec
DE 6.50 usec
TB 300.5 K
D1 1.0000000 sec

----- CHANNEL f1 -----
NUC1 1H
PI 14.50 usec
PLW1 12.85299969 MHz
SPO1 400.1324710 MHz

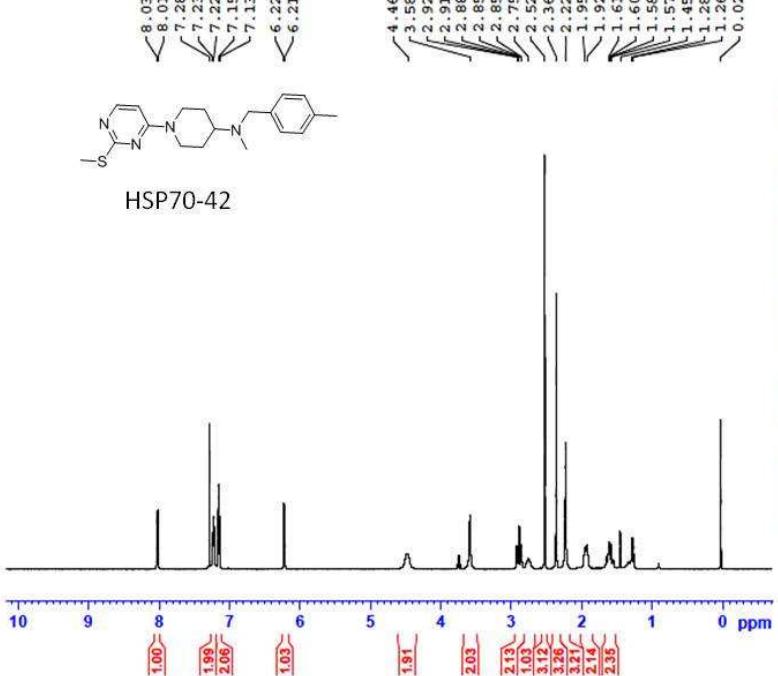
P2 - Processing parameters
SI 65536
SF 400.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

S20111212-096

20111214

PROTON CDCl₃ D:\nmr\data\ham

HSP70-42



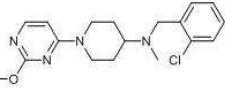
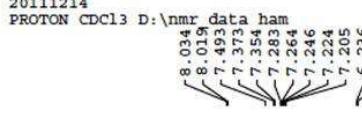
Current Data Parameters
NAME JKYRHJ1209-4
EXPNO 10
PROCNO 1

P2 - Acquisition Parameters
Date 20111214
Time 19.09
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDCl₃
NS 16
DS 2
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 203
DW 60.800 usec
DE 6.50 usec
TB 300.5 K
D1 1.0000000 sec

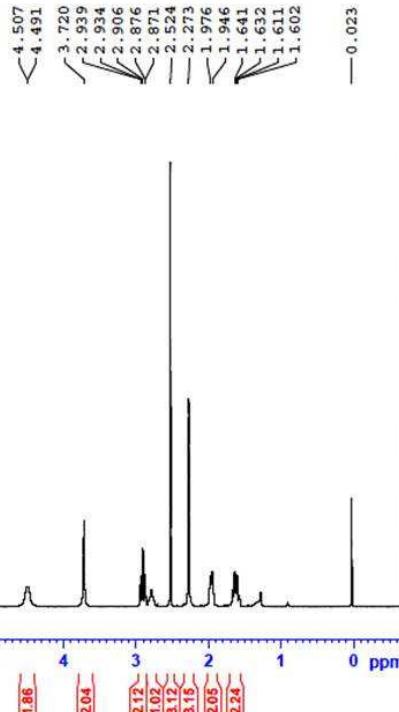
----- CHANNEL f1 -----
NUC1 1H
PI 14.50 usec
PLW1 12.85299969 MHz
SPO1 400.1324710 MHz

P2 - Processing parameters
SI 65536
SF 400.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

S20111212-097
20111214



HSP70-47



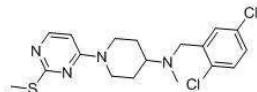
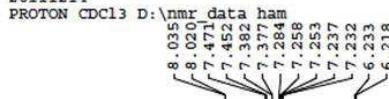
Current Data Parameters
NAME JKYRHJ1208-5
EXPNO 10
PROCNO 1

P2 - Acquisition Parameters
Date_ 20111214
Time_ 19.13
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDCl₃
NS 16
DS 2
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 203
DW 60.800 usec
DE 6.50 usec
TR 300.4 K
DI 1.0000000 sec

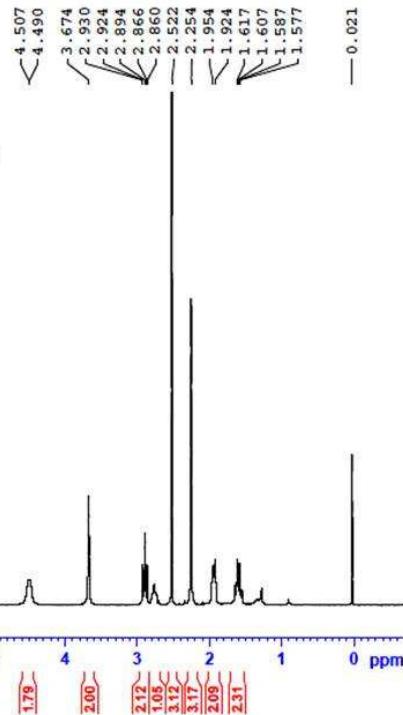
----- CHANNEL f1 -----
NUC1 1H
PL 14.50 usec
PLM1 12.85299969 W
SPO1 400.1324710 MHz

P2 - Processing parameters
SI 65536
SF 400.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

S20111212-098
20111214



HSP70-44



Current Data Parameters
NAME JKYRHJ1208-6
EXPNO 10
PROCNO 1

P2 - Acquisition Parameters
Date_ 20111214
Time_ 19.18
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT CDCl₃
NS 16
DS 2
SWH 8223.685 Hz
FIDRES 0.125483 Hz
AQ 3.9846387 sec
RG 203
DW 60.800 usec
DE 6.50 usec
TR 300.4 K
DI 1.0000000 sec

----- CHANNEL f1 -----
NUC1 1H
PL 14.50 usec
PLM1 12.85299969 W
SPO1 400.1324710 MHz

P2 - Processing parameters
SI 65536
SF 400.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

