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Short communication

Determination of SNX-2112, a selective Hsp90 inhibitor, in plasma samples by high-performance liquid chromatography and its application to pharmacokinetics in rats

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ABSTRACT

A sensitive and specific reversed-phase high-performance liquid chromatography method with ultraviolet detection has been developed and validated for the identification and quantification of SNX-2112 in rat plasma. Following sample preparation using liquid–liquid extraction, the analytes were separated by the mobile phase acetonitrile–water (40:60, v/v) with an Agilent RP-HPLC column (ZORBAX SB-C18, 5 μm, 4.6 mm × 250 mm) at a flow rate of 1 ml/min, column temperature of 30 °C and detection wavelength of 251 nm. The retention time of SNX-2112 was 11.2 min. A good linear relationship was obtained in the concentration range studied (0.07–21 μg/ml, $R^2 > 0.9982$), and the LLOD and LLOQ for SNX-2112 were 0.02 and 0.07 μg/ml, respectively. The mean absolute recovery of SNX-2112 in plasma ranged from 88.58 to 99.61% at the studied concentrations. The intra- and inter-batch relative standard deviations were 1.7–3.5 and 1.9–4.4%, respectively. This method was successfully applied to pharmacokinetic studies in rats after intravenous administration of SNX-2112.

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1. Introduction

Hsp90 is one of the most active cellular chaperones. Its main functions are to maintain protein stability, improve stress tolerance of cells and enhance the anti-oxidation of cells to maintain their normal physiological functions [1]. Hsp90 is mainly in the activated state in tumor cells, and forms a complex with the receptor protein and auxiliary molecular chaperone. It maintains the receptor proteins in a mature and functional conformation to protect the receptor protein from degradation by the proteosome [2,3]. To date, several Hsp90 inhibitors have been reported, and some of them have shown a certain degree of anti-tumor capability, such as radicicol [4,5], geldanamycin (GA) [6] and its derivative 17-allylamino geldanamycin (17-AAG) [7,8] and 17-dimethy laminoethylamino-17-demethoxygeldanamycin (17-DMAG) [9]. In addition to these recognized Hsp90 inhibitors, some other Hsp90 inhibitors have been synthesized or isolated, such as PU3 (purine-scaffold inhibitor), IPI-504 (17-AAG hydroquinone) and NVP-AUY922 (an isoxazole derivative), among others.

SNX-2112, 4-(6,6-dimethyl-4-O-3-trifluoromethyl-4,5,6,7-tetrahydro-1-indoleyl)-2-(1-(4-trans-hydroxy-cyclohexane)amino)benzamide (Fig. 1), is a representative small molecule synthesized by the Serenex company in America; its structural formula was officially announced in 2008 [10]. Experiments have shown that SNX-2112 can degrade Hsp90 receptor protein HER2, arrest the Rb-dependent G0 phase of the cell cycle and differentiate MCF-7 cells in vitro [11]. It can induce solid tumor and leukemia cell apoptosis in vitro, and its effects were significantly higher than 17-AAG. It can also induce apoptosis in multiple myeloma cells and reduce the generation of osteoclasts [12]. SNX-2112 displays a better activity than 17-AAG in the therapy of multiple myeloma. In addition, SNX-2112 can also inhibit the growth of S-180 tumors in Kunming mice through a significant reduction in tumor angiogenesis. At the same time, it has been found that SNX-2112 can inhibit the diffusion of K562 cells in NOD/SCID mice and extend their survival time, and the curative effects are better than 17-AAG [13].

The aim of this study was to develop and validate a simple, rapid, sensitive and reproducible HPLC method for the determination of

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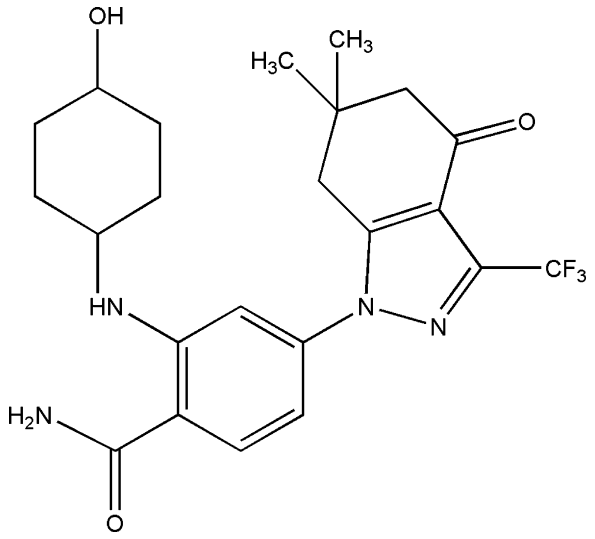

Fig. 1. Formal chemical structure of SNX-2112 (from ChemOffice 2004 software).

SNX-2112 in plasma suitable for pharmacokinetic studies after i.v. administration to rats. To our knowledge, this is the first report on the pharmacokinetics of SNX-2112.

2. Experimental

2.1. Chemicals and reagents

SNX-2112 injections (10 mg/ml) were made at the Biomedicine Research & Development Center of Jinan University. Methanol and acetonitrile were of chromatographic grade (Tedia Company Inc., USA). All other reagents were of analytical grade. Ultrapure water (ELGA Lab Water, VEOLIA) was used throughout the study.

2.2. Animals

Thirty male Sprague–Dawley rats, weighing 180–220 g, were supplied by the Medical Laboratory Animal Center of Guangdong Province (Guangdong, China). The rats were housed under controlled environmental conditions (temperature, $23 \pm 3\,^{\circ}C$; humidity, 55–75%) with a commercial food diet and water freely available. Animal experiments were carried out according to institutional guidelines for the care and use of laboratory animals and were approved by the Animal Ethics Committee of the Chinese Academy of Medical Sciences.

2.3. Preparation of stock solutions, standards and quality control samples

The stock solution of SNX-2112 (2.1 mg/ml) was prepared in methanol. Working standard solutions of SNX-2112 were obtained at concentrations of 7, 42, 210 and 1050 μg/ml by further dilutions of the stock solution with methanol. All of the solutions were stored at 4 °C. The stock solutions and standard solutions used were prepared freshly every time.

Calibration standards were prepared by spiking blank rat plasma with working standard solutions of SNX-2112. The effective concentrations in the standard plasma samples were 0.07, 0.14, 0.42, 1.05, 2.1, 5.25, 10.5 and 21 μg/ml. One calibration curve was constructed on each analysis day using freshly prepared calibration standards. The quality control samples (QCs) were prepared with blank plasma at low, middle and high concentrations of 0.2, 10 and 20 μg/ml and were stored at −20 °C after preparation. The standards and quality controls were extracted on each analysis day with the same procedure for the plasma samples as described below.

2.4. Liquid chromatography conditions

Analysis of the calibration, quality control and experimental samples was performed on a Waters LC system (Waters company, USA) equipped with a Waters 600E pump, 2487 UV detector, on-line degasser, 717 plus autosampler, column compartment (30 °C) and Empower software. The analyte was eluted with a mobile phase consisting of acetonitrile–water (40:60, v/v) at a flow rate of 1.0 ml/min on a reversed-phase column (ZORBAX SB-C18, 5 μm, 4.6 mm × 250 mm; USA) with a C18 guard column (5 μm, 4.6 mm × 8 mm; USA), followed by specific measurement at 251 nm.

2.5. Sample preparation

Each collected blood sample was immediately centrifuged at 3000 rpm for 15 min and then transferred into a 1.5 ml eppendorf tube. A 200 μl volume of blank plasma, calibration standards, QC samples and plasma samples were prepared using liquid–liquid extraction, where 200 μl of plasma was added to 400 μl of ethyl acetate, which was then vortexed for 2 min, centrifuged at 8000 rpm for 10 min, and then the separated supernatant was set in an eppendorf tube. The residue was then extracted one more time with 200 μl of ethyl acetate, and then the extracted supernatants were combined. These sample extracts were evaporated at 50 °C under a stream of nitrogen, reconstituted with 200 μl of mobile phase, vortexed for 1 min, filtered through a 0.45 μm pore size filter (Millipore), and then 20 μl of the solution was injected into the HPLC system.

2.6. Method validation

2.6.1. Sensitivity and specificity

The lowest limit of detection (LLOD) was defined as the lowest concentration of drug in spiked plasma resulting in a signal-to-noise ratio of 3:1; for the lowest limit of quantification (LLOQ), a signal-to-noise ratio of 10:1 was used. The specificity of the method was evaluated by analyzing blank plasma samples from five rats. Each blank sample was tested for interference using the previously described liquid–liquid extraction procedure and HPLC conditions.

2.6.2. Linearity

The linearity of the HPLC method for the determination of SNX-2112 was evaluated by a calibration curve in the range of 0.07–21 μg/ml (weighting factor = $1/x^2$). The calibration curve was obtained by plotting the peak area of each analyte versus SNX-2112 concentration.

2.6.3. Precision and accuracy

The accuracy and precision of the method were assessed by assaying five replicate QC samples. The QC samples (0.2, 10 and 20 μg/ml) were prepared in the same way as the calibration samples. Intra-batch precision and accuracy were determined by repeated analyses of the group of standards in one batch ($n = 5$). To evaluate the intra- and inter-day precision, five replicates of calibration standards were analyzed on the same day and on 5 different days. This allowed the percentage relative standard deviations (RSD, %) of the data to be calculated.

2.6.4. Extraction recovery

The recoveries of SNX-2112 from plasma were determined at different standard concentrations by spiking the drug into the corresponding blank plasma. The percentage of recovery was calculated by comparing the analyte peak area obtained from plasma

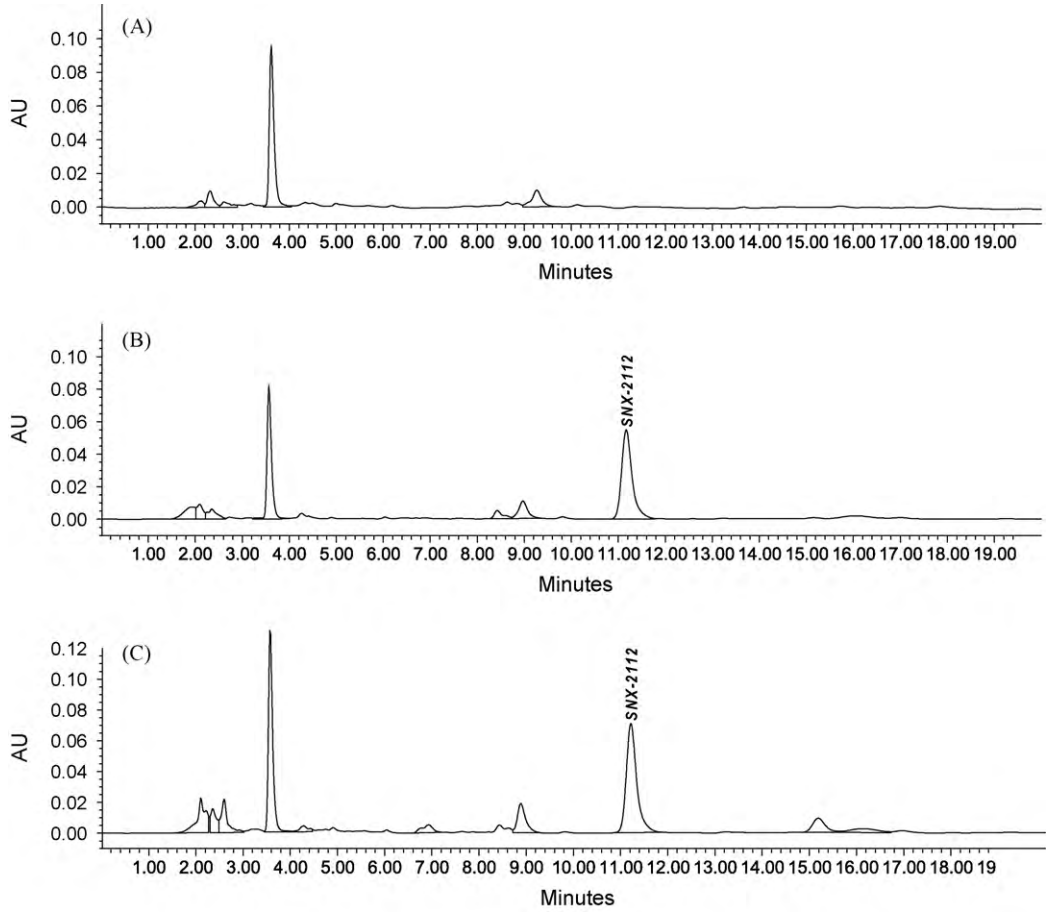

Fig. 2. HPLC chromatograms of (A) blank plasma; (B) blank plasma spiked with SNX-2112 (15 μg/ml); (C) a rat plasma sample 40 min after i.v. administration of SNX-2112 (10 mg/kg) (from Empower software).

samples with those from the standard solutions at the same concentration. The recoveries at three QC concentration levels of SNX-2112 in plasma were examined at least five times.

2.6.5. Stability

To ensure the reliability of the results in relation to handling and storing of the plasma samples and stock standard solutions, stability studies were carried out at three different concentration levels (0.2, 10 and 20 μg/ml). The long-term stability of SNX-2112 in rat plasma was assessed by carrying out the experiment after 15 days of storage at −20 °C. Freeze and thaw stability for three cycles was determined by thawing at room temperature and then refreezing at −20 °C for 24 h. The short-term room temperature stability was examined by keeping the QC plasma samples at room temperature for 10 h. The post-preparative stability of the plasma samples was tested after keeping the samples at room temperature for 24 h.

2.7. Application to pharmacokinetic studies

The rats were acclimatized to the facilities for 7 days, and the six groups (five rats per group, three groups per dose) were randomly assigned and fasted with free access to water for 12 h prior to experiment. The developed HPLC assay method was used in the pharmacokinetic study after intravenous (i.v.) 5 and 10 mg/kg administration of SNX-2112 to the rats. The dosing solution for intravenous administration used for animal studies was prepared by diluting the required amount of concentrated SNX-2112 solution in isotonic sodium chloride solution before administration. Each dosage used three groups of rats to collect blood in turns.

SNX-2112 injection of concentrated liquid diluted in isotonic saline was delivered using a 5 ml syringe into the rat tail veins. The injection volume was 1 ml/100 g body weight. The preparations were made immediately before drug administration. Blood samples of 0.4 ml were taken via the post-orbital venous plexus vein at 0.083, 0.167, 0.333, 0.667, 1, 1.5, 3, 6, 12, 18 and 24 h after intravenous administration. The blood sample was transferred into a heparinized Eppendorf tube, mixed gently, and then centrifuged (3000 rpm, 15 min) to obtain 200 μl of plasma, which was kept at −20 °C until analysis. Pharmacokinetic analysis of SNX-2112 concentrations in plasma was performed using compartmental methods via the proprietary 3P97 computer software package (Chinese Pharmacology Society).

3. Results and discussions

3.1. Liquid chromatography

Representative chromatograms of blank plasma, a spiked plasma standard and a real plasma sample are shown in Fig. 2, showing no co-eluting interference peaks of endogenous compounds in the vicinity of the SNX-2112 peaks. The retention time of SNX-2112 was about 11.2 min.

3.2. Method validation

3.2.1. Linearity and limit of quantification

The calibration model was selected based on the data obtained by linear regression with a $1/x^2$ weighting factor. The calibration curves were all linear with regression correlation coefficients

Table 1
Precision and accuracy for the determination of SNX-2112 in rat plasma (intra-day: $n = 5$; inter-day: $n = 5$ series per day for 5 days).

Concentration added (μg/ml)	Intra-day variability			Inter-day variability		
	Concentration found (mean ± SD) (μg/ml)	Precision (% RSD)	Accuracy (%)	Concentration found (mean ± SD) (μg/ml)	Precision (% RSD)	Accuracy (%)
0.2	0.20 ± 0.01	3.5	106.73	0.20 ± 0.01	4.4	102.00
10	10.08 ± 0.18	1.8	103.32	10.08 ± 0.23	2.0	100.78
20	19.70 ± 0.32	1.7	100.09	19.69 ± 0.37	1.9	98.44

Table 2
Stability of SNX-2112 in rat plasma ($n = 5$; mean ± SD).

Nominal concentration (μg/ml)	Recovered (%)			
	Freeze–thaw (three cycles)	Short-term (room temperature, 10 h)	Long-term (−20 °C, 15 days)	Post-preparative (24 h)
0.2	96.14 ± 2.86	97.04 ± 4.02	96.78 ± 2.11	100.56 ± 5.90
10	95.81 ± 2.38	98.18 ± 3.87	97.06 ± 3.89	96.27 ± 3.23
20	97.05 ± 3.53	99.57 ± 3.84	99.47 ± 3.95	97.20 ± 4.20

Table 3
Pharmacokinetic parameters of SNX-2112 after i.v. administration of 5 and 10 mg/kg; each value represents the mean ± SD ($n = 5$).

Pharmacokinetic parameters	Unit	5 mg/kg	10 mg/kg
A	μg/ml	7.65 ± 0.94	13.86 ± 1.14
α	1/h	1.83 ± 0.14	1.36 ± 0.15
B	μg/ml	0.29 ± 0.08	0.45 ± 0.25
β	1/h	0.07 ± 0.03	0.08 ± 0.04
$V_{(c)}$	(mg/kg)/(mg/ml)	0.64 ± 0.09	0.70 ± 0.05
$T_{1/2\alpha}$	h	0.38 ± 0.03	0.51 ± 0.06
$T_{1/2\beta}$	h	10.43 ± 4.06	10.41 ± 4.38
K21	1/h	0.14 ± 0.04	0.12 ± 0.06
K10	2/h	0.98 ± 0.10	0.91 ± 0.06
K12	3/h	0.79 ± 0.10	0.42 ± 0.12
AUC	(μg/ml)h	8.10 ± 0.77	15.80 ± 1.00
$CL_{(s)}$	ml/h/kg	0.62 ± 0.06	0.63 ± 0.04

$T_{1/2\alpha}$: distribution half-life; $T_{1/2\beta}$: elimination half-life; $CL_{(s)}$: clearance; AUC: the area under the concentration–time curve; $V_{(c)}$: apparent volume of distribution of the central compartment; data are expressed as mean ± SD.

(R^2) > 0.9982 over the concentration ranges tested. The LLOD and the LLOQ for SNX-2112 were 0.02 and 0.07 μg/ml, respectively. They also showed good linear relationships between the peak areas and concentrations.

3.2.2. Accuracy and precision

The intra-batch precision is shown in Table 1. The precision for concentrations of 0.2, 10 and 20 μg/ml SNX-2112 were 3.5, 1.8 and 1.7%, respectively. The accuracy ranged from 100.09 to 106.73% throughout the three concentrations examined. The inter-batch precision was studied over 5 days, and the results are also given in Table 1. The precision ranged from 1.9 to 4.4%, and the inter-day accuracies reached throughout the three concentrations examined were 102.00, 100.78 and 98.44%.

3.2.3. Recovery

The mean extraction recoveries of SNX-2112 from spiked rat plasma under the liquid extraction conditions were 99.61 ± 2.13, 93.34 ± 2.76 and 88.58 ± 2.72% ($n = 5$) at concentrations of 0.2, 10 and 20 μg/ml, respectively. The results showed that there were no significant differences in the signals of analytes extracted from rat plasma and from the mobile phase.

3.2.4. Stability

The stability of SNX-2112 during sample handling (freeze–thaw, short-term temperature, long-term and post-preparative) is shown in Table 2. SNX-2112 was stable for at least 10 h at room temperature in plasma samples, and mean recoveries from the nominal concentrations were more than 97.04% at 0.2, 10 and 20 μg/ml. The mean recoveries from nominal concentrations after three freeze and thaw cycles were more than 95.81%. Additionally, SNX-2112 was stable in plasma samples when stored at −20 °C for a 15-day period, and mean recoveries from the nominal concentrations were all more than 96.78%. Finally, the mean recoveries of post-preparative samples after keeping the samples at room temperature for 24 h were more than 96.27%.

3.2.5. Pharmacokinetic study of SNX-2112 in rats

Plasma concentrations of SNX-2112 in rats after i.v. administration of 5 and 10 mg/kg are shown in Fig. 3, and the corresponding mean pharmacokinetic parameters are listed in Table 3. The

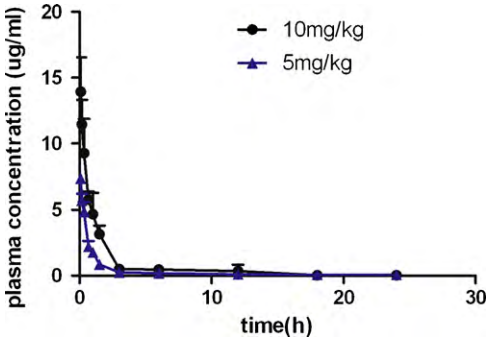

Fig. 3. Mean plasma concentration–time profile after i.v. administration of SNX-2112 (5 and 10 mg/kg). Each point and bar represents the mean ± SD ($n = 5$) (from GraphPad Prism 5.0 software).

area under the plasma concentration (AUC) of SNX-2112 after i.v. administration of 5 and 10 mg/kg were 8.10 ± 0.77 and 15.80 ± 1.00 (μg/ml) h, respectively. The $T_{1/2\beta}$ were 10.43 ± 4.06 and 10.41 ± 4.38 h, respectively. After i.v. injection of SNX-2112 at 5 and 10 mg/kg, we obtained clearances ($CL_{(s)}$) of 0.62 ± 0.06 and 0.63 ± 0.04 ml/h/kg, respectively.

Compartmental model analysis indicated that the SNX-2112 pharmacokinetic profile after i.v. administration fitted well with a two-compartment model. Dose and the area under the plasma concentration curve were positively correlated. The plasma concentration–time curves of SNX-2112 in rats demonstrated that SNX-2112 was eliminated rapidly from the plasma in the first 3 h. The plasma concentration of SNX-2112 was detectable only up to 24 h in rats using the analytical method described above. The previously reported pharmacokinetic studies showed SNX-5422, an oral pro-drug of SNX-2112, to have a half-life of 8–10 h [14]. This is similar to SNX-2112. In addition, there was no significant difference in systemic clearance at the two dose levels, suggesting that SNX-2112 might have linear pharmacokinetic characteristics in rats within the dose ranges tested.

4. Conclusion

A reliable and sensitive HPLC method for the analysis of SNX-2112 in rat plasma has been successfully developed and validated. The method was successfully applied to the pharmacokinetic study of SNX-2112 in rats. The peaks obtained using the HPLC approach enabled the detection of analytes at very low concentrations and with high resolution. A sample preparation method employing the liquid–liquid extraction of SNX-2112 was successfully developed. To our knowledge, this is the first report of an HPLC method on the determination of free SNX-2112 in rats that has a low detection limit. The pharmacokinetic parameters obtained from this study can give some useful information for further research of SNX-2112.

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References

[1] K. Stangl, C. Günther, T. Frank, M. Silke Meiners, T. Röpke, L. Stelter, M. Moobed, G. Baumann, P.M. Kloetzel, V. Stangl, Inhibition of the ubiquitin–proteosome path way induces differential heat shock protein response in cardiomyocytes and renders early cardiac protection, Biochem. Biophys. Res. Commun. 291 (2002) 542–549.
[2] L.H. Pearl, C. Prodromou, Structure, function, and mechanism of the Hsp90 molecular chaperone, Adv. Protein Chem. 59 (2001) 157–186.
[3] W.B. Pratt, The Hsp902 based chaperone system: involvement in signal transduction from a variety of hormone and growth factor receptors, Proc. Soc. Exp. Biol. Med. 217 (1998) 420–434.
[4] H.J. Kwon, M. Yoshida, Y. Fukui, S. Horinouchi, T. Beppu, Potent and specific inhibition of p60v-src protein kinase both in vivo and in vitro by radicicol, Cancer Res. 52 (1992) 6926–6930.
[5] T. Agatsuma, H. Ogawa, K. Akasaka, A. Asai, Y. Yamashita, T. Mizukami, S. Akinaga, Y. Saitoh, Halohydrin and oxime derivatives of radicicol: synthesis and antitumor activities, Bioorg. Med. Chem. 10 (2002) 3445–3454.
[6] W.G. An, T.W. Schulte, L.M. Neckers, The heat shock protein 90 antagonist geldanamycin alters chaperone association with p210bcr-abl and v-src proteins before their degradation by the proteasome, Cell Growth Differ. 11 (2000) 355–360.
[7] G. Chiosis, H. Huezo, N. Rosen, E. Mimnaugh, L. Whitesell, L. Neckers, 17-AAG: low target binding affinity and potent cell activity-finding an explanation, Mol. Cancer Ther. 2 (2003) 123–129.
[8] M.J. Egorin, D.M. Rosen, J.H. Wolff, P.S. Callery, S.M. Musser, J.L. Eiseman, Metabolism of 17-(allylamino)-17-demethoxygeldanamycin (NSC330507) bymurine and human hepatic preparations, Cancer Res. 58 (1998) 2385–2396.
[9] T.W. Schulte, L.M. Neckers, The benzoquinone ansamycin 17-allylamino-17-demethoxygeldanamycin binds to HSP90 and shares important biologic activities with geldanamycin, Cancer Chemother. Pharmacol. 42 (1998) 273–279.
[10] T.E. Barta, J.M. Veal, J.W. Rice, J.M. Partridge, R.P. Fadden, W. Ma, M. Jenks, L. Geng, G.J. Hanson, K.H. Huang, A.F. Barabasz, B.E. Foley, J. Otto, S.E. Hall, Discovery of benzamide tetrahydro-4H-carbazol-4-ones as novel small molecule inhibitors of Hsp90, Bioorg. Med. Chem. Lett. 18 (2008) 3517–3521.
[11] S. Chandarlapaty, A. Sawai, Q. Ye, A. Scott, M. Silinski, K. Huang, P. Fadden, J. Partdrige, S. Hall, P. Steed, L. Norton, N. Rosen, D.B. Solit1, SNX2112, a synthetic heat shock protein 90 inhibitor, has potent antitumor activity against HER kinase-dependent cancers, Clin. Cancer Res. 14 (2008) 240–248.
[12] Y. Okawa, T. Hideshima, P. Steed, S. Vallet, S. Hall, K. Huang, J. Rice, A. Barabasz, B. Foley, H. Ikeda, N. Raje, T. Kiziltepe, H. Yasui, S. Enatsu, K.C. Anderson, SNX-2112, a selective Hsp90 inhibitor, potently inhibits tumor cell growth, angiogenesis, and osteoclastogenesis in multiple myeloma and other hematological tumors by abrogating signaling via Akt and ERK, Blood 113 (2009) 846–855.
[13] L. Jin, C.L. Xiao, C.H. Lu, M. Xia, G.W. Xing, S. Xiong, Q.Y. Liu, H. Liu, Y.C. Li, F. Ge, Q.D. Wang, Q.Y. He, Y.F. Wang, Transcriptomic and proteomic approach to studying SNX-2112-induced K562 cells apoptosis and anti-leukemia activity in K562-NOD/SCID mice, FEBS Lett. 583 (2009) 1859–1866.
[14] J.C. Bryson, J.R. Infante, R.K. Ramanathan, S.F. Jones, D.D. Von Hoff, H.A. Burris, A phase 1 dose-escalation study of the safety and pharmacokinetics (PK) of the oral Hsp90 inhibitor SNX-5422, J. Clin. Oncol. 26 (2008) 14613.