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Synthesis and in vitro anti-HSV-1 activity of a novel Hsp90 inhibitor BJ-B11

Huai-Qiang Ju [a,c,†], Yang-Fei Xiang [a,†], Bao-Juan Xin [b], Ying Pei [a], Jia-Xin Lu [a], Qiao-Li Wang [a], Min Xia [a], Chui-Wen Qian [a], Zhe Ren [a], Sha-Yan Wang [c], Yi-Fei Wang [a,*], Guo-Wen Xing [b,*]

[a] Guangzhou Jinan Biomedicine Research and Development Center, National Engineering Research Center of Genetic Medicine, Jinan University, No. 601, West Huangpu Road, Guangzhou 510632, China
[b] Department of Chemistry, Beijing Normal University, Beijing 100875, China
[c] Shenzhen People's Hospital, Clinical Medical College of Jinan University, Shenzhen 518020, China

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ABSTRACT

In this study, a novel Hsp90 inhibitor BJ-B11, was synthesized and evaluated for in vitro antiviral activity against several viruses. Possible anti-HSV-1 mechanisms were also investigated. BJ-B11 displayed no antiviral activity against coxsackievirus B_3 (CVB_3), human respiratory syncytial virus (RSV) and influenza virus (H1N1), but exhibited potent anti-HSV-1 and HSV-2 activity with EC_{50} values of 0.42 ± 0.18 μM and 0.60 ± 0.21 μM, respectively. Additionally, the inhibitory effects of BJ-B11 against HSV-1 were likely to be introduced at early stage of infection. Our results indicate that BJ-B11 with alternative mechanisms of action is potent as an anti-HSV clinical trial candidate.

Herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) are lipid-enveloped DNA viruses that cause various illnesses ranging from asymptomatic infection to fulminant disseminated diseases, such as labials herpes, keratitis, genital herpes, and encephalitis.[1] In taxonomy, both HSV-1 and HSV-2 are members of the genera *Simplexvirus* from the subfamily *Alphaherpesvirinae* in *Herpesviridae* family,[2] which can be distinguished by clinical manifestations and biochemical as well as serological examinations but possess many common characteristics, and that currently available anti-HSV-1 medicines are generally active against HSV-2 with analogical mechanisms.[3,4] The major antiviral therapy for the treatment of HSV infection is to use nucleoside analogues such as acyclovir (ACV). However, the increasing clinical application of this type of antiviral agents has been associated with the emergence of drug-resistant HSV strains.[5] Therefore, the development of new anti-HSV agents with a different mode of action is highly warranted.

Hsp90 is a multifunctional, complex, and highly specialized chaperone machine that is extremely abundant in most organisms and cell types.[6] It can facilitate the assembly of multiprotein complexes and participate in protein trafficking within the cellular milieu in response of various types of stress. It was reported that Hsp90 inhibitors could selectively target and induce apoptosis in tumor tissues but had modest effects in normal cells.[7] Viral infections have also been shown to associate with Hsp90. For instance, Hsp90 is required for full activity of the hepatitis B virus reverse transcriptase. Studies also indicate that HSV-1 employs the Hsp90 chaperone system during infection and the viral polymerase may be a client protein of Hsp90.[8] The possibility that the host–pathogen interaction may represent a novel and specific antiviral target has led us study novel Hsp90 inhibitors as antiviral agents.

In previous reports,[9] we analyzed the molecular mechanism of the apoptosis effect of a selective Hsp90 inhibitor, SNX-2112,[10,11] on human chronic myeloid leukemia (CML) K562 cells, and a sensitive and specific reversed-phase high-performance liquid chromatography method was used for the identification and quantification of SNX-2112 in rat plasma. We also examined the antiviral activities of SNX-2112 and its analogues in order to investigate the correlation between viral infections and Hsp90. Here, we report the synthesis and antiviral activity of 2-(4-acetyloxy-cyclohexylamino)-4-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydro-1*H*-indazol-1-yl)-benzamide (BJ-B11, **1**, Scheme 1), a novel analogue of SNX-2112. Also, the possible anti-HSV-1 mechanisms of BJ-B11 were investigated.

The synthetic route of compound **1** was shown in Scheme 1. Triketones **3** were conveniently obtained from acetyl chloride and dimedone **2** in the presence of triethylamine and catalytic 4-dimethylaminopyridine (DMAP) at room temperature. 4-Hydrazino-2-(4-hydroxy-cyclohexylamino)-benzonitrile (**5**) was prepared by treatment of the known compound **4**[10] with hydrazine

Abbreviations: Hsp90, heat shock protein 90; MTT, 3-(4,5-diethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromideassay; Vero, African green monkey kidney cell line; MOI, multiplicity of infection; PFU, plaque forming unit.

* Corresponding authors. Tel./fax: +86 20 85223426 (Y.F.W.).
E-mail addresses: twang-yf@163.com (Y.-F. Wang), gwxing@bnu.edu.cn (G.-W. Xing).

† These authors contributed equally to this work.

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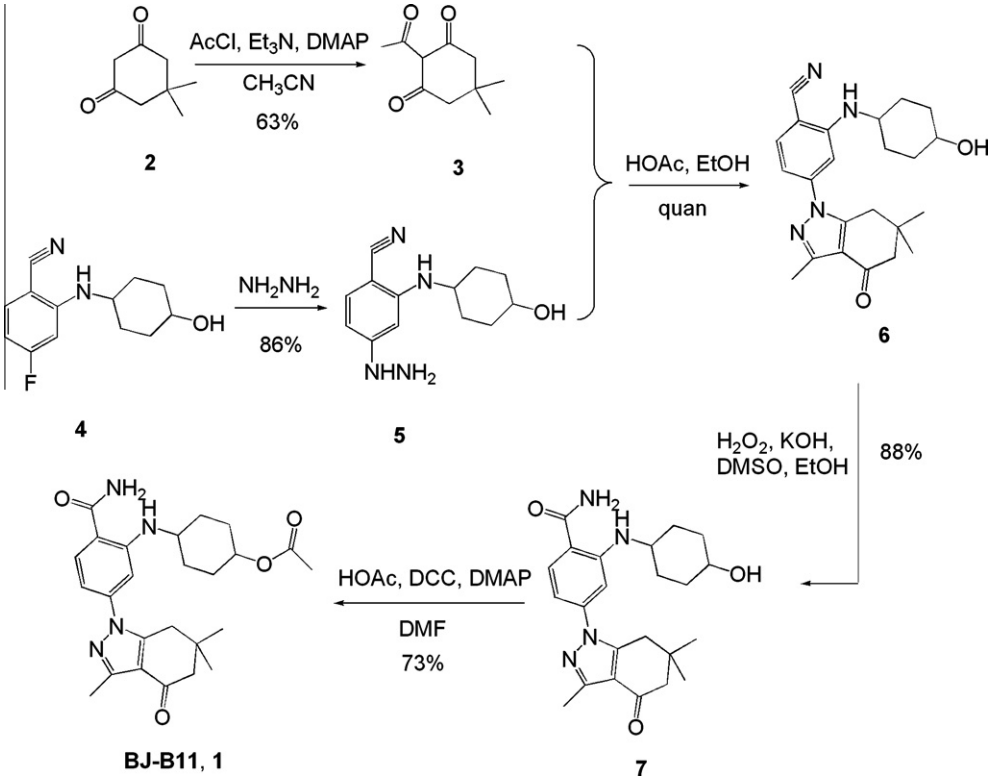

Scheme 1. Chemical structure and general synthesis process of BJ-B11.

at 90 °C for 3 h. Then, indazol-4-one **6** was produced from **4** and **5** in 3:1 ethanol/acetic acid by heated at about 60 °C, followed by hydration of the resulting benzonitriles to afford **7**[10] in high yield. Condensation of **7** and acetic acid under DCC/DMAP coupling conditions provided the expected SNX-2112 analogue BJ-B11.[12] The structure of BJ-B11 is different from SNX-2112 in the cyclohexanol and inazolone moieties. Yields of all compounds are showed in Scheme 1. Both analytical and spectral data of all target compounds were accordant with the proposed structures.

BJ-B11 was first tested in vitro for antiviral activity against herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), coxsackievirus B_3 (CVB_3), human respiratory syncytial virus (RSV) and influenza virus (H1N1) by cytopathic effect (CPE). Results suggested that BJ-B11 only had antiviral activity against HSV-1 and HSV-2. Possible antiviral mechanisms against HSV-1 were investigated as follows. The cytotoxicity of BJ-B11 and ACV (used as the positive agent) on Vero cells was determined by MTT assay.[13] Based on the results of MTT assay, non-cytotoxic concentrations of compounds were used for determination of in vitro anti-HSV activity in Vero cells by plaque reduction assay.[14] Results are shown in Table 1.

HSV entry involves complex ligand–receptor interactions and has been shown to be an ideal target for antiviral compounds.[15] Effects of BJ-B11 on viral attachment and penetration were individually investigated based on plaque reduction assay. Results suggested that the anti-HSV-1 activity of BJ-B11, like that of ACV, was not attributed to blockage of virus entry (Fig. S1). As shown in Figure 1, to determine at which stage of HSV-1 (MOI = 1) lifecycle BJ-B11 (1 μM) exerts antiviral activity, time-of-addition (filled triangle) and time-of-removal (filled square) experiments were performed.[15] For both assays, at 24 hpi (hours post-infection), the infected cultures were harvested and the virus yield was determined by plaque assay. As shown in Figure 1, the inhibitory effect of BJ-B11 declined insignificantly when added at 9 hpi as compared to that when added at 24 hpi in time-of-addition assay (6.67 vs 7.09 log PFU/mL). Concordantly, virus yield was profoundly affected when BJ-B11 was removed at 6 hpi compared with removal at 0 hpi in time-of-removal experiment (7.13 vs 6.14 log PFU/mL). These data suggested that BJ-B11 mainly inhibited HSV-1 replication before 6 hpi.

Further, real-time PCR was used to explore the effects of BJ-B11 on HSV-1 DNA synthesis and gene expression.[16] Vero cell

Table 1
Cytotoxicity, anti-HSV activity and therapeutic index of BJ-B11

Compound	Cytotoxicity[a] CC_{50} (μM)	Antiviral activity[b]			
		HSV-1		HSV-2	
		EC_{50} (μM)	TI[c]	EC_{50} (μM)	TI[c]
BJ-B11	35.50 ± 2.15	0.42 ± 0.18	84.52	0.60 ± 0.21	59.16
ACV	>200.00	1.16 ± 1.02	>172.42	1.72 ± 0.44	>116.27

Values are mean ± SD of three independent experiments.
[a] Cytotoxic effect was determined by MTT assay. CC_{50} was defined as the concentration reducing 50% cell viability.
[b] Antiviral activity was determined by plaque reduction assay. EC_{50} was the concentration that inhibited 50% of HSV replication in Vero cells.
[c] TI (therapeutic index) was defined as the ratio of CC_{50} to EC_{50}.

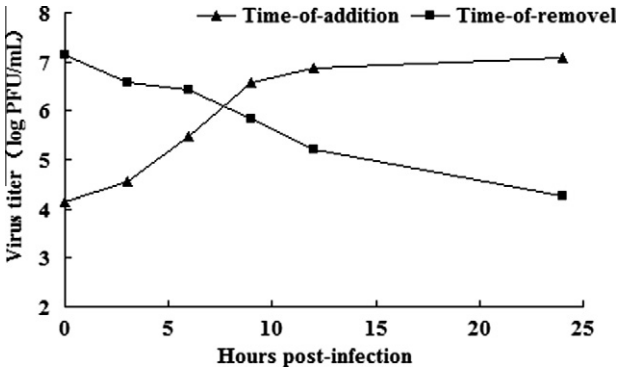

Figure 1. Effects of delayed addition and early removal of BJ-B11 on virus yields. (The data shown here represent the mean of three independent experiments.)

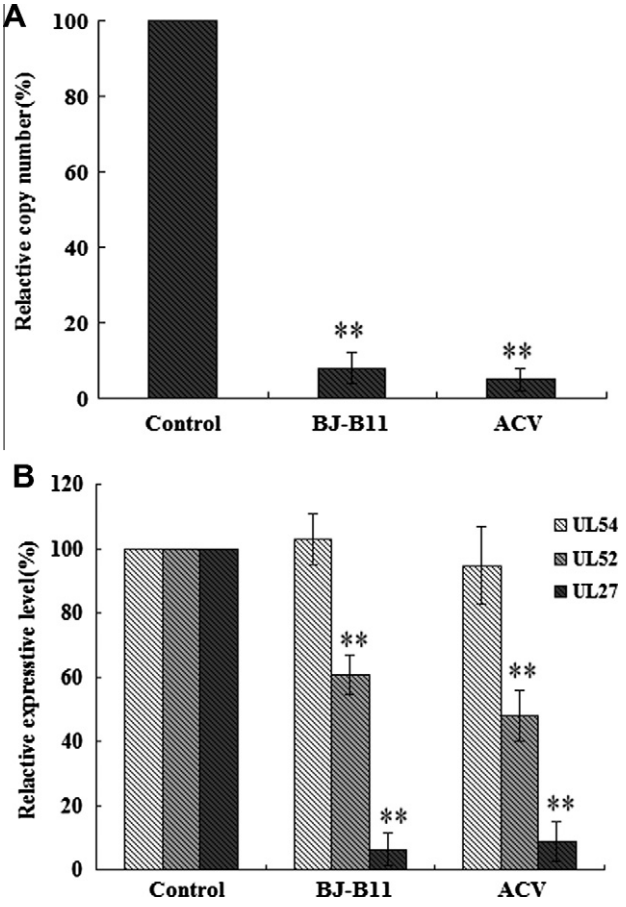

Figure 2. Effects of BJ-B11 on HSV-1 DNA synthesis and HSV-1 gene expression. Each bar represents mean ± SD. The significance was determined by Student's *t*-test (**P <0.01 vs untreated controls) (n = 3).

monolayer in 24-well plate was infected with HSV-1 (MOI = 1) in the presence or absence of 1 μM BJ-B11. The DNA of infected cells was extracted at 24 hpi and subjected to real-time PCR for UL52 (Early gene) detection. The RNA of infected cells was extracted at 3, 6 and 12 hpi, reverse transcribed to cDNA and the serially diluted cDNA was used for UL54 (immediate early gene), UL52 (early gene) and UL27 (late gene) detection by real-time PCR, respectively. As shown in Figure 2A, BJ-B11 could fundamentally reduce HSV-1 DNA synthesis in Vero cells (P <0.01). It did not affect the expression of UL54, but significantly reduced the expression of both UL52 and UL27 (P <0.01) (Fig. 2B). ACV was used as a reference compound here and it showed similar effects. The observation that E gene expression was reduced correlates with the results in Figure 1, and suggested that BJ-B11 mainly exerted anti-HSV-1 effects at the early stage of infection.

In this study, a novel Hsp90 inhibitor BJ-B11 was designed, synthesized and evaluated for in vitro antiviral activity. Results suggested that BJ-B11 had antiviral activity against HSV (HSV-1 and HSV-2). We have focused our study on the anti-HSV-1 mechanisms, which could represent anti-HSV mechanisms of BJ-B11. This study not only demonstrates that the host-pathogen interface may represent a novel and specific antiviral target but also provides information about the basic biology of chaperone-dependent viral processes and cellular responses to stress. In conclusion, the present study describes that BJ-B11 possesses anti-HSV activity, probably by inhibiting early stage of HSV replication. Further studies will be required to explore the detailed antiviral mechanism of BJ-B11, as a possible antiviral therapy.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.01.098.

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12. *Spectral data for BJ-B11* (**1**): White solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.11 (s, 6H), 1.41–1.58 (m, 4H), 2.05 (s, 3H), 2.05–2.16 (m, 4H), 2.41 (s, 2H), 2.55 (s, 3H), 2.82 (s, 2H), 3.41 (br s, 1H), 4.78 (m, 1H), 6.61 (d, J = 8.3 Hz, 1H), 6.78 (s, 1H), 7.48 (d, J = 8.4 Hz, 1H), 8.15 (d, J = 7.1 Hz, 1H); ^{13}H NMR ($CDCl_3$, 100 MHz) δ (ppm): 13.39, 13.43, 21.36, 28.39, 29.54, 29.75, 35.80, 37.67, 49.83, 52.33, 71.75, 106.52, 108.83, 112.13, 117.23, 129.74, 142.67, 149.01, 149.99, 150.10, 170.57, 171.43, 193.41; HRMS (ESI) m/z $[M+H]^+$ calcd for $C_{25}H_{33}N_4O_4$: 453.2502. Found: 453.2512.
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