Molecular Mechanisms Mediating Anti-Myeloma Activity of Proteasome Inhibitor PS-341

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Abstract

We have recently shown that proteasome inhibitor PS-341 induces apoptosis in drug resistant multiple myeloma (MM) cells, inhibits binding of MM cells in the bone marrow microenvironment, and inhibits cytokines mediating MM cell growth, survival, drug resistance, and migration in vitro. PS-341 also inhibits human MM cell growth and prolongs survival in a SCID mouse model. Importantly, PS-341 has achieved remarkable clinical responses in patients with refractory relapsed MM. We here demonstrate molecular mechanisms whereby PS-341 mediates anti-MM activity include: induction of p53 and MDM2 proteins; phosphorylation$^{\text{Ser15}}$ of p53; activation of c-Jun NH$_2$-terminal kinase (JNK), caspases-8 and –3; as well as cleavage DNA protein kinase catalytic subunit, ATM, and MDM2. Inhibition of JNK activity abrogates PS-341-induced MM cell death. These studies identify molecular targets of PS-341, and provide the rationale for development of next generation more targeted therapies.
Introduction

The ubiquitin-proteasome pathway (UPP) is a proteolytic system in the cytosol and nucleus which regulates cyclins and cyclin dependent kinase inhibitor cell cycle regulatory proteins, and thereby regulates cell cycle progression. UPP also has a critical role in selective removal of mutant, damaged, and misfolded proteins. Proteasome inhibitors have recently demonstrated promise as potential novel anti-cancer therapies, since they induce apoptosis of tumor cells in vitro, despite the accumulation of p21 and p27 and irrespective of the p53 wild type or mutant status in tumor cells. Specifically, PS-341 (pyrazylcarbonyl-Phe-Leu-boronate) is representative of a class of peptide boronate proteasome inhibitors which inhibit 26S proteasome activity. This agent induces marked in vivo anti-tumor activity against human prostate cancer, Burkitt’s lymphoma in a murine model and adult T cell leukemia; with 5-fluorouracil, cisplatin, taxol and adriamycin produces additive growth delays against Lewis lung carcinoma; and demonstrates anti-angiogenic activity in an orthotopic pancreatic cancer model.

Multiple myeloma (MM) is a hematologic malignancy which affected 14,000 new individuals in the United States in 2000 and remains incurable with conventional therapies; novel biologically-based therapies are therefore urgently needed. We have previously demonstrated that PS-341 directly induces apoptosis, by activation of caspase-3 and without associated alteration of Bax or Bcl-2 protein expression, even in MM cell lines and patients MM cells which are resistant to conventional therapies. It also overcomes the anti-apoptotic effects of IL-6 or adherence to bone marrow stromal cells (BMSCs). In vivo PS-341 inhibits human MM cell growth and associated angiogenesis, as well as prolonging survival, in a murine SCID mouse model. Most importantly, PS-341 has achieved responses, even
complete responses, in a Phase II clinical trial treating patients with relapsed MM refractory to conventional therapies, and has an acceptable toxicity profile. This drug therefore represents a new treatment paradigm targeting not only the tumor cell, but also the MM cell-host interaction and the BM milieu, to overcome drug resistance and improve patient outcome.

Both pro-apoptotic and anti-apoptotic proteins are substrates of UPP, and the molecular mechanisms whereby PS-341 mediates its anti-MM activity are not yet defined. In this study, we demonstrate that PS-341 induces p53 and MDM2 protein expression; induces phosphorylation (Ser15) of p53 protein; and activates JNK, which in turn activates caspases-8 and -3. Importantly, activated caspase-3 in turn cleaves DNA protein kinase catalytic subunit (DNA-PKcs), ATM, and MDM2; conversely, caspase inhibitors Z-VAD-FMK and Z-IETD-FMK abrogate these effects. Furthermore, inhibition of JNK activity by SP600125 reduces PS-341-induced MM cell death. Our results therefore demonstrate that PS-341 induces caspase activation, inhibits DNA repair, and activates p53 by phosphorylation and degradation of MDM2. Given the early clinical promise and favorable toxicity profile of PS-341 in patients with relapsed refractory MM, these studies provide the framework for further clinical evaluation of PS-341, alone and coupled with conventional or other novel therapies, to improve patient outcome in MM.
**Abbreviations used:** Abbreviations: multiple myeloma (MM); bone marrow stromal cell (BMSC); dexamethasone (Dex); c-Jun NH₂-terminal kinase (JNK); ubiquitin-proteasome pathway (UPP); DNA protein kinase catalytic subunit (DNA-PKcs).
Materials and Methods

MM derived cell lines and patient MM cells.

Dr. Steven Rosen (Northwestern University, Chicago, IL) kindly provided MM.1S (wild-type p53) human MM cell line. RPMI8226 and U266 human MM cells were obtained from American Type Culture Collection (Rockville, Maryland). All MM cell lines were cultured in RPMI-1640 containing 10% fetal bovine serum (FBS, Sigma Chemical Co., St. Louis, MO), 2 \(\mu\)M L-glutamine, 100 U/ml penicillin, and 100 \(\mu\)g/ml streptomycin (GIBCO, Grand Island, NY). Patient MM cells were purified from patient bone marrow (BM) aspirates by negative selection using anti-CD2, CD14, CD33, CD41, CD45RA, and CD66b Abs (RosetteSep separation system, StemCell Technologies, Vancouver, Canada). The purity of MM cells is >95%, as confirmed by flow cytometric analysis using anti-CD138 Ab (BD Pharmingen, San Diego, CA).

Reagents

Proteasome inhibitor PS-341 (Millennium Pharmaceuticals, Cambridge, MA) was dissolved in DMSO, stored at \(\sim\)20 \(^\circ\)C, and used at 5-80 nM in the presence of 5% FBS. JNK inhibitor 600215 (Calbiochem, San Diego, CA) was stored at \(\sim\)20 \(^\circ\)C and used at 5-40 \(\mu\)M. Pan caspase inhibitor Z-VAD-FMK and caspase-8 inhibitor Z-IETD-FMK (Calbiochem) were also stored at \(\sim\)20 \(^\circ\)C and used at 100 \(\mu\)M and 25 \(\mu\)M, respectively. For inhibition of caspase cleavage, cells were incubated with caspase inhibitors for 1 h prior to incubation with PS-341 (20 nM) for 8 h. Caffeine (Sigma) (0.5 and 1 mM) was used as an inhibitor of ATM/ATR.

Growth inhibition assay
MM.1S cells were cultured for 24 h with 6-25 nM PS-341 in the presence (5-10 µM) or absence of SP600125. The inhibitory effect of PS-341 on MM cell growth in the presence or absence of SP600125 was assessed by measuring 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasodium bromide (MTT, Chemicon International, Temecula, CA) dye absorbance, as previously described 14,17.

Preparation of biotinylated probes and hybridization on microarrays

MM.1S cells were incubated with 20 nM PS-341 for 0.5, 1, 3 and 6 h in the presence of 5% FBS. mRNA expression profiling was analyzed using Affymetrix huGene FL™ arrays (SantaClara, CA) containing 12626 genes, as previously described 18.

Immunoblotting

MM cell lines and patient MM cells cultured with PS-341 were lysed and protein lysates (50 µg) subjected to Western blotting, as in prior studies 11,17. Nuclear extracts were prepared using the Nuclear Extract Kit (Activemotif, Carsbad, CA), according to the manufacturer’s instructions. The Abs used for immunoblotting included: anti-phospho-p53 (Ser 6, 9, 15, 20, 37, 46, 392), p53, phospho-SEK1, phospho-SAPK, phospho-c-Jun, phospho-ATF-2, phospho-Bcl-2, and caspase-8 (Cell Signaling, Beverly, MA); anti-MDM2, -JNK1, -DNA-PKcs, -Bcl-2, -Bcl-XL, -α-tubulin and -nucleolin (Santa Cruz Biotechnology, Santa Cruz, CA); anti-ATM (Oncogene Research Products, Boston, MA); and anti-caspase-3 (BD Pharmingen, San Diego, CA). For immunoprecipitation, whole cell lysates were incubated with anti-p53 and MDM2 overnight at 4°C, and then incubated with protein A/G PLUS-Agarose (Santa Cruz Biotechnology) for 4 h at 4°C as in our prior study 14,19.
Statistical analysis

Statistical significance of differences observed in drug-treated versus control cultures was determined using the Wilcoxon signed-ranks test. The minimal level of significance was $p < 0.05$. 
Results

PS-341 upregulates expression of p53 and MDM2 proteins, and induces phosphorylation (Ser15) of p53

Since p53 is a substrate of UPP, we first examined the effect of PS-341 on p53 protein expression in MM.1S cells. MM.1S cells were treated with PS-341 (20 nM) for up to 16 h in the presence of 5% FBS. As expected, p53 protein expression was transiently upregulated after PS-341 treatment in MM.1S cells, with peak expression at 8 h (Figure 1A). Importantly, PS-341 also induces transient p53 phosphorylation on serine 15 (Ser15) without phosphorylation of Ser 6, 9, 20, 37, 46, or 392 residues (data not shown), in the same pattern as p53 protein expression. MDM2 protein is also transiently upregulated after PS-341 treatment, with peak protein expression observed at 6 h. We next examined the dose dependent effect of PS-341 on both phosphorylation and protein expression of these proteins. Phosphorylation (Ser15) and protein expression of p53 are upregulated in a dose-dependent fashion; in contrast, MDM2 expression was decreased only after high dose PS-341 treatment (Figure 1B). To assess the effect of PS-341 in patient MM cells, we next purified the MM cells from patients' bone marrow aspirates. As can be seen in Figure 1C, PS-341 treatment (20 nM, 8 h) induces phosphorylation of p53 (Ser15), and also upregulates p53 and MDM2 protein expression in patient MM cells. Since DNA damage induces p53 phosphorylation, these results suggest that PS-341 induces DNA damage in both MM cell lines and patient MM cells. We further examined the distribution and interaction of p-p53, p53 and MDM2 proteins. As expected, most p-p53 is present in the nucleus, and PS-341 triggered increased protein expression associated with nuclear translocation of p-p53. In contrast, cytoplasmic p53 is in its non-phosphorylated form (Figure 1D). We also examined the
association of p53 and MDM2 protein using co-immunoprecipitation with anti-p53 and MDM2 Abs, and demonstrate that PS-341 induces co-immunoprecipitation of p53 with MDM2 protein in a time-dependent fashion (Figure 1E). PS-341-induced interaction of p53 and MDM2 occurs earlier than induction of p53 protein.
PS-341 induces c-Jun NH₂-terminal kinase (JNK) activation

We next examined whether PS-341 induces stress response in MM.1S cells. PS-341 induces phosphorylation of JNK, as well as both upstream (SEK-1) and down stream (c-Jun and ATF-2) proteins, in a time- (Figure 2A) and dose-(Figure 2B) dependent fashion. PS-341 similarly induces phosphorylation of JNK in U266 and RPMI8226 MM cell lines (Figure 2C). As we have done in dexamethasone-treated in MM.1S cells ¹⁸, we next performed gene microarray profile analysis in MM.1S cells before and after PS-341 treatment. As seen in Figure 2D, PS-341 induces a significant (2.5 - 72 fold) increase in heat shock protein (HSP) transcription in a time-dependent fashion. These results indicate that PS-341 induces a stress response in MM cell lines.

Inhibition of JNK activity reduces PS-341-induced cell death by inhibition of caspase-3 activation

To examine whether activation of JNK plays a role in mediating PS-341-induced apoptosis, we used JNK specific inhibitor SP600125 ¹⁵,¹⁶. In the presence of SP600125, phosphorylation of JNK triggered by PS-341 is completely abrogated in a dose-dependent fashion (Figure 3A). Phosphorylation of c-Jun or ATF-2 is also blocked by SP600125 (data not shown). Importantly, SP600125 does not block phosphorylation of p53 or induction of MDM2 by PS-341. We further examined whether inhibition of JNK activity also inhibits caspase-3 cleavage. As seen in Figure 3B, SP600125 inhibits PS-341-induced caspase-3 cleavage, consistent with its inhibition of JNK phosphorylation. SP600125 in a dose-dependent fashion also significantly ($P < 0.01$) inhibits PS-341-induced cytotoxicity in
MM.1S cells, assessed by MTT assay (Figure 3C). These results suggest that JNK plays a critical role in mediating PS-341-induced apoptosis via activation of caspase-3.
PS-341 induces DNA-PKcs and ATM cleavage in MM

Others have demonstrated that DNA-PKcs is a target for an ICE-like and CPP32-like
apoptotic protease protease. Since we have previously reported that PS-341 induces caspase-3 cleavage in MM cells, we next examined whether DNA-PKcs and/or ATM is cleaved by caspase activation triggered by PS-341. As expected, PS-341 induces cleavage of DNA-PKcs (180 kD protein) in a time- (Figure 4A, upper panel), and dose- (Figure 4A, lower panel) dependent fashion in MM.1S cells. PS-341-induced DNA-PKcs cleavage also occurs in RPMI8226 (Figure 4B, upper panel) and U266 (Figure 4B, lower panel) MM cell lines. Although there is constitutive DNA-PKcs cleaved form in patient's MM cells, PS-341 also induces DNA-PKcs cleavage in these cells (Figure 4C). Importantly, PS-341 similarly cleaves ATM in a time- (Figure 4D, upper panel), and dose- (Figure 4D, lower panel) dependent fashion in MM.1S cells. These results suggest that PS-341 may inhibit DNA repair by cleavage of DNA-PKcs and/or ATM in both MM cell lines and patient MM cells.
Mechanism whereby PS-341 cleaves DNA-PKcs and ATM

Having shown that PS-341 induces DNA-PKcs and ATM cleavage, we next examined the
mechanism whereby PS-341 triggers cleavage of these protein kinases. As in our prior study \(^4\), PS-341 induces caspase-3 cleavage (Figure 5A, upper panel) and caspase-8 cleavage (Figure 5A, lower panel) in a time-dependent fashion. In contrast, there is no caspase-9 cleavage (data not shown), and no changes in phosphorylation of Bcl-2 or in protein expression of Bcl-2 and Bcl-XL (Figure 5B). To determine whether caspase-3 activation mediates DNA-PKcs and/or ATM cleavage induced by PS-341, we next examined the effect of caspase inhibitors on DNA-PK and ATM cleavage (Figure 5C). As expected, pan-caspase inhibitor Z-VAD-FMK (4th lane) and caspase-8 inhibitor Z-IETD-FMK (3rd lane) completely abrogate DNA-PKcs and ATM cleavage induced by PS-341. Importantly, these caspase inhibitors also abrogate PS-341-induced phosphorylation, but not protein expression, of p53 (Figure 5C). Since MDM2 is cleaved during apoptosis \(^24\), we next examined whether PS-341 also cleaves MDM2. As seen in Figure 5D, MDM2 is completely cleaved after treatment with PS-341 for 10 h. To define the role of ATM and/or ATR in phosphorylation of p53 induced by PS-341 treatment, we used caffeine as an inhibitor of both ATM \(^25\) and ATR \(^26\). Importantly, caffeine inhibits PS-341 induced p53 phosphorylation, but not p53 protein expression, in MM.1S cells in a time- (Figure 5E, upper panel) and dose- (Figure 5E, lower panel) dependent fashion. These results suggest that PS-341 induces: DNA-PKcs and ATM cleavage; phosphorylation of p53 and MDM2 cleavage via caspase-3 activation; and activation of ATM or ATR activation by activation of caspase-3 via caspase-8.
**Fig 5**

A) ps-341 (20 nM)

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B) ps-341 (20 nM)

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C) ps-341 (20 nM, 8h)

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D) ps-341 (20 nM)

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E) Caffeine (0.5 mM)

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Fig 5
Discussion

UPP is a major proteolytic system which selectively removes mutant, damaged, or misfolded proteins. It also regulates expression of proteins mediating cell cycle progression (p21\textsuperscript{Cip1}, p27\textsuperscript{Kip1}, cyclins), oncogenesis (p53, I\textsubscript{kB}), and apoptosis (Bcl, cIAP, XIAP, Bax).\textsuperscript{1} Proteasome inhibitors block UPP and induce caspases despite expression of anti-apoptotic proteins (Bcl, cIAP, XIAP) and independent of p53 status. The proteasome inhibitor PS-341 demonstrates remarkable anti-MM activity both \textit{in vitro}\textsuperscript{11} and in murine MM models\textsuperscript{27}, and most recently has achieved stabilization or response in 77% of patients with refractory relapsed MM\textsuperscript{13}. The \textit{in vivo} targets whereby PS-341 mediates anti-MM activity, and conversely, mechanisms of PS-341 resistance, are at present undefined. In this study we define the molecular mechanisms of anti-MM activity of this agent, in order to provide the framework for its optimal clinical use and to form the basis for the development of second generation more targeted potent and less toxic proteasome inhibitors.

Our previous study demonstrates that PS-341-induced apoptosis in MM cells with both wild type and mutant p53\textsuperscript{4}, consistent with previous reports that proteasome inhibitor-induced apoptosis occurs dependent\textsuperscript{5} or independent\textsuperscript{28} of p53 status. In this study, we hypothesized that DNA damage triggered by PS-341 treatment in MM cells is associated with activation of DNA-PKcs and/or ATM/ATR and activation of p53. We first report that PS-341 specifically induces phosphorylation of p53 (Ser15). This induction of p53 phosphorylation is associated with increased p53 protein expression, as previously reported\textsuperscript{29}. PS-341 induces MDM2 protein and association of p53 and MDM2, earlier than phosphorylation of p53. Previous studies have demonstrated that phosphorylation of p53 (Ser15) dissociates p53 from p53/MDM2 complex; however, our results demonstrate p53 is
still associated with MDM2 even after phosphorylation. Since previous reports demonstrated that DNA damage induced phosphorylation of p53 via activation of DNA-PKcs\(^{20,30}\), our results therefore strongly suggest that PS-341 induces DNA damage, activates DNA-PKcs and/or ATM/ATR, and phosphorylates p53 (Ser15) in both MM cell lines and primary patients' MM cells.

We have studied mechanisms whereby conventional and novel therapies trigger MM cell apoptosis. For example, Dex triggers caspase-9 mediated MM cell death\(^{19}\) whereas immunomodulatory derivatives of thalidomide\(^{31}\) and TRAIL\(^{32}\) induce caspase-8-mediated apoptosis. Our recent gene microarray data of MM.1S cells treated with PS-341 demonstrates transcriptional triggering of apoptotic cascades, downregulation of growth/survival kinases, upregulation of UPP, and induction of stress kinases, including heat shock proteins (HSPs). C-Jun kinase (JNK), one of these stress-response proteins, mediates apoptosis triggered by unfolded proteins\(^{33}\). Importantly, JNK inhibitor SP600125 blocks PS-341-induced cell death by abrogation of caspase-3 cleavage, but does not affect phosphorylation of p53. These data suggest that activation of JNK modulates PS-341-induced caspase activation and apoptosis. Previous reports demonstrate that JNK increases phosphorylation of anti-apoptotic proteins Bcl-2 and Bcl-x, thereby reducing their antiapoptotic activity\(^{34,35}\); however, our data demonstrate that PS-341 does not alter protein expression of Bcl-2 and Bcl-XL, or phosphorylation of Bcl-2. This result is consistent with our data that PS-341 does not induce activation of caspase-9, but rather induces caspase-8 apoptotic signaling.

DNA-PKcs is a phosphatidylinositol (PI) kinases which has a crucial role in repair of damaged DNA and phosphorylation of selective serine residues (Ser15) on p53\(^{20,30}\), and is a
possible target for an ICE-like protease or CPP32-like apoptotic protease. We therefore next determined whether activation of caspases by PS-341 induces cleavage of DNA-PKcs and/or ATM. We demonstrate that PS-341 cleaves DNA-PKcs and ATM in both MM cell lines and primary patient's MM cells. Importantly, PS-341 activates caspase-3 via caspase-8 activation, whereas pan-caspase and caspase 8 inhibitors completely abrogate PS-341 induced caspase-8/caspase-3 activation and DNA-PKcs and ATM cleavage. These data suggest that cleavage of DNA-PKcs and ATM triggered by PS-341 is dependent on caspase8/caspase3 signaling. Our results further demonstrate that inhibition of caspase activation also inhibits p53 phosphorylation, but not protein expression of p53, suggesting that phosphorylation of p53 is a secondary event following DNA damage induced by caspase-3. Taken together, our findings indicate that PS-341 activates caspase-3 via caspase-8 activation; impairs DNA repair by cleavage of DNA-PKcs and/or ATM; and activates p53 through both phosphorylation of p53 and degradation of MDM2 (Figure 6). Importantly, these effects all occur with PS-341 (20 nM), serum levels which are achieved in clinical trials; moreover, PS-341 irreversibly binds to proteasomes and accumulates in target cells. Further delineation of the molecular mechanisms mediating anti-tumor activity of these agents will provide the framework for their use, alone or coupled with other novel agents, to improve outcome. These studies will also establish the molecular basis for the development of more targeted, potent, and less toxic next generation proteasome inhibitors.
Fig 6
Acknowledgements

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mitochondria and interaction with Bcl-x(L) in response to DNA damage. J Biol Chem. 1999;275:322-327
Figure legends.

Figure 1
Induction of phosphorylation and protein expression of p53 and MDM2 by PS-341.  (A) PS-341 induces p53 phosphorylation (Ser15), as well as increased protein expression of p53 and MDM2, in MM.1S cells in a time-dependent fashion.  (B) PS-341 induces p53 phosphorylation (Ser15), as well as increased protein expression of p53 and MDM2, in MM.1S cells in a dose-dependent fashion. Anti-α-tubulin Ab is used to confirm equal loading of proteins.  (C) PS-341 induces p53 phosphorylation (Ser15), as well as increased protein expression of p53 and MDM2, in purified patient MM cells.  (D) Localization of p-p53, p53, and MDM2 proteins in cytoplasmic and nuclear fractions of MM.1S cells treated with PS-341. Anti-α-tubulin and anti-nucleolin Abs are used to confirm equal loading of cytoplasmic and nuclear proteins, respectively.  (E) Co-immunoprecipitation of p53 and MDM2 in MM.1S cells treated with PS-341.

Figure 2
PS-341 induces JNK activation and HSP gene expression.  (A) PS-341 induces phosphorylation of JNK, upstream molecule SEK1, as well as downstream targets c-Jun and ATF-2, in MM.1S cells in a time-dependent fashion. Immunoblotting with anti-α-tubulin Ab confirms equal protein loading.  (B) PS-341 induces phosphorylation of JNK and c-Jun in a dose-dependent fashion.  (C) PS-341 induces phosphorylation of JNK in MM.1S, U266, and RPMI8226 MM cell lines.  (D) Gene microarray analysis demonstrates upregulation of HSP27 (●), HSP40 (■), HSP70 (▲) and HSP90 (▲), and HSP110 (○) mRNA in MM.1S cells treated with PS-341.
Figure 3

JNK inhibitor blocks caspase-3 cleavage and abrogates PS-341-induced apoptosis in MM.1S cells.  (A) Inhibition of PS-341-induced JNK phosphorylation, but not of MDM2 expression or p53 phosphorylation, by JNK inhibitor SP600125.  (B) Caspase-3 cleavage triggered by PS-341 is dependent on JNK activity.  SP600125 inhibits phosphorylation of JNK and caspase-3 cleavage triggered by PS-341.  (C) Cell death triggered by PS-341 is partially dependent on JNK activation. MM.1S cell death triggered by PS-341 was measured by MTT assay, in the absence (□) or presence of 5 µM (●) and 10 µM (■) SP600125. *, p<0.01 (versus 0 µM).

Figure 4

Cleavage of DNA-PKcs and ATM induced by PS-341.  (A) PS-341 -induces time (upper panel) and dose (lower panel)-dependent DNA-PKcs cleavage in MM.1S cells.  (B) Time dependent DNA-PKcs cleavage is induced by PS-341 treatment in RPMI8226 (upper panel), and U266 (lower panel) MM cell lines.  (C) DNA-PKcs cleavage is also triggered by PS-341 treatment in purified patient MM cells.  (D) PS-341-induces time (upper panel) and dose (lower panel)-dependent ATM cleavage in MM.1S cells.

Figure 5

Caspase inhibitors block PS-341-induced DNA-PKcs or ATM cleavage.  (A) PS-341 induces time-dependent caspase-3 and -8 cleavage in MM.1S cells.  (B) PS-341 does not affect phosphorylation of Bcl-2 or protein expression of Bcl-2 and Bcl-xL.  Immunoblotting with
anti-α-tubulin Ab confirms equal protein loading. (C) Caspase-8 inhibitor Z-IETD-FMK and pan-caspase inhibitor Z-VDD-FMK protect against PS-341-induced cleavage of DNA-PKcs and ATM, as well as phosphorylation of p53, through inhibition of caspase-3 cleavage. (D) PS-341-induces time-dependent MDM2 protein cleavage in MM.1S cells. (E) Caffeine inhibits PS-341-triggered phosphorylation of p53 in a time-(upper panel) and dose-(lower panel) dependent fashion in MM.1S cells.

Figure 6
Molecular mechanisms mediating anti-myeloma activity of proteasome inhibitor PS-341

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