Dual targeting of the thioredoxin and glutathione antioxidant systems in malignant B cells: A novel synergistic therapeutic approach

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B-cell malignancies are a common type of cancer. One approach to cancer therapy is to either increase oxidative stress or inhibit the stress response systems on which cancer cells rely. In this study, we combined nontoxic concentrations of Auranofin (AUR), an inhibitor of the thioredoxin system, with nontoxic concentrations of buthionine-sulfoximine (BSO), a compound that reduces intracellular glutathione levels, and investigated the effect of this drug combination on multiple pathways critical for malignant B-cell survival. Auranofin interacted synergistically with BSO at low concentrations to trigger death in multiple malignant B-cell lines and primary mantle-cell lymphoma cells. Additionally, there was less toxicity toward normal B cells. Low AUR concentrations inhibited thioredoxin reductase (TrxR) activity, an effect significantly increased by BSO cotreatment. Overexpression of TrxR partially reversed AUR+BSO toxicity. Interestingly, the combination of AUR+BSO inhibited nuclear factor κB (NF-κB) signaling. Moreover, synergistic cell death induced by this regimen was attenuated in cells overexpressing NF-κB proteins, arguing for a functional role for NF-κB inhibition in AUR+BSO-mediated cell death. Together, these findings suggest that AUR+BSO synergistically induces malignant B-cell death, a process mediated by dual inhibition of TrxR and NF-κB, and such an approach warrants further investigation in B-cell malignancies. Copyright © 2015 ISEH - International Society for Experimental Hematology. Published by Elsevier Inc.
including breast cancer [10], ovarian cancer [11], multiple myeloma (MM) [12], and chronic lymphocytic leukemia [13].

Homeostasis of the cellular redox state is regulated not only by the TrxR-dependent system but also by the GSH system. Recent studies demonstrate that genetic ablation of TrxR1 results in a compensatory increase in the activity of the GSH system [14], supporting the notion of redundancy in antioxidant pathways. Given this redundancy, it is not surprising that inhibition of the GSH system, in conjunction with genetic inhibition of either TrxR1 or TrxR2, results in the elicitation of pronounced neoplastic cell death [14]. A pharmacologic approach to inhibit GSH is to use buthionine-sulfoximine (BSO), an inhibitor of the rate-limiting enzyme in GSH biosynthesis, γ-glutamylcysteine synthetase [15]. Therefore, combining AUR with agents that inhibit or deplete GSH is a rational therapeutic combination, and in fact, combinations of AUR and BSO have recently been shown to selectively kill human head, neck, and lung cancer cells by inducing oxidative stress [16,17]. Whether this strategy would be effective in malignant hematopoietic cells, specifically malignant B cells, has not yet been investigated, to our knowledge.

Increasing cellular oxidative stress can induce cancer cell death directly as a result of the irreversible damage that reactive oxygen species elicit on cellular lipids, DNA, and/or proteins. Oxidative stress can also induce cancer cell death indirectly by modulating numerous redox-dependent cellular pathways that mediate cell survival. One such redox-dependent regulatory mechanism involves the posttranslational modification of reduced cysteines (or thiols) on proteins involved in diverse cellular functions, a process known as thiol-based redox switching [18]. The nuclear factor κB (NF-κB) signaling pathway, a pivotal cellular prosurvival pathway that is often dysregulated in cancer, is regulated in part by thiol-based redox switching. Notably, AUR inhibits the nuclear translocation, and thus activation, of NF-κB by inhibiting the activation of the inhibitory κB kinase (IKK) complex through its modification of cysteine 779 on IKK [19,20].

In addition to inhibiting NF-κB activation, AUR is thought to inhibit TrxR activation via modification of cysteine and selenocysteine residues (thiols) in the redox centers of TrxR. Thus, given that BSO results in GSH depletion and that GSH reduces free thiols, we hypothesized that BSO would augment the inhibitory effect of AUR on both TrxR activity and NF-κB activation. Further, we hypothesized that the cytotoxicity elicited by the combination of AUR and BSO was due, in part, to inhibition of both TrxR and NF-κB.

Here, we show that mantle cell lymphoma (MCL) cells exhibit dramatically reduced viability following combined exposure to AUR and BSO, even at low concentrations of both agents. Similar effects were observed for both diffuse large B-cell lymphoma (DLBCL) and MM cells; however, normal B-cell viability was less affected. Notably, BSO augmented AUR-induced inhibition of both TrxR and NF-κB signaling. Finally, the cytotoxic effects of AUR and BSO were attenuated by overexpressing either TrxR or NF-κB proteins, supporting a significant functional role for inhibition of these signaling pathways in the AUR+BSO synergistic toxicity. Collectively, these findings support the notion that dual targeting of cellular antioxidant systems may exert selective toxicity toward malignant B cells.

Materials and methods

Cell culture and primary cells
The human DLBCL cell lines SUD-HL6 and OCI-LY10, the MCL cell lines Rec-1 and Granta, and the MM cell lines U266 and KMS-12-PE were cultured as previously described [21–24].

B cells were isolated from fresh peripheral blood mononuclear cells from one healthy donor by negative magnetic bead selection of non–B cells, according to the manufacturer’s protocol (Miltenyi Biotec, Auburn, CA). The donor provided signed, written, informed consent. All procedures and methods were approved by the Research Subjects Review Board at the University of Rochester Medical Center.

Primary patient-derived MCL cells were obtained from the University of Rochester Medical Center Tissue Core under an Institutional Review Board–approved protocol. These tissue specimens were fully anonymous; hence patient demographics are not available.

Chemical reagents
All chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise stated.

Cell viability assays
Cells were treated with varying concentrations of AUR or BSO, alone or together, for 24 hours, and a MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay was performed as described previously [23]. The alamarBlue cell viability assay (Life Technologies, Grand Island, NY) was used according to the manufacturer’s protocol to assess the viability of primary cells. The Annexin V-FITC Apoptosis Detection kit (BD Biosciences, San Jose, CA) was also used according to the manufacturer’s instructions.

Thioredoxin reductase activity
To measure TrxR activity, Granta cells were incubated with 100 nmol/L AUR or 5 μmol/L BSO, alone or in combination, for 18 hours. Subsequently, TrxR activity was measured spectrophotometrically using a kit, according to the manufacturer’s protocol (Sigma-Aldrich).

Electrophoretic mobility shift assay
Granta cells were treated with the indicated concentrations of AUR and BSO, alone or in combination, for 6 hours. Following these treatments, nuclear extracts were prepared as described previously [25–27]. Electrophoretic mobility shift assays were performed by incubating nuclear extracts with IR-Dye-700-conjugated, double-stranded DNA probe at room temperature for
10 min, or an additional 10 min incubation with NF-kB-specific antibodies for supershift analysis, followed by resolution of the complexes on native 4% polyacrylamide gels. The bands were then visualized using images acquired on an Odyssey infrared imager (LI-COR Biosciences, Lincoln, NE). The double-stranded oligonucleotide probe is as follows (upper strand): 5'/ SIRD7000/CAACGCCAGGGGAATTCCTTCTCCTT-3'.

Luciferase assay
Luciferase reporter plasmids containing either the NF-kB- or OCT-1-responsive elements upstream of a firefly luciferase gene were transfected into Granta cells, together with a vector expressing IκBzs1/2 or an empty vector, using Nucleofector (Amaxa/ Lonza, Basel, Switzerland). The total amount of plasmid DNA was kept constant at 5 μg for each transfection. Thirty minutes after the transfection, cells were plated into single wells and were left untreated or incubated for 6 hours with the indicated doses of AUR or BSO, alone or in combination. Cell lysates were prepared using reporter lysis buffer, and luciferase activity was measured with a SpectraMax M3 plate reader (Molecular Devices, Sunnyvale, CA). In these assays, total protein amount as determined by Bradford assay was used to normalize the samples, as a single transfection was divided into multiple wells for subsequent treatments.

Immunoblot analyses
Following the indicated manipulations, whole-cell lysates were prepared in E lysis buffer[28]. Lysates were fractionated on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels, and protein was electrophoretically transferred to Hybond enhanced chemiluminescence (ECL) nitrocellulose membrane (GE Healthcare Bio-Sciences Corporation, Piscataway, NJ). The membranes were analyzed for immunoreactivity with primary antibodies raised against RelA (1:1000), RelB (1:1000), IκBzs (1:1000), or α-Tubulin (1:1000; all from Santa Cruz Biotechnologies, Santa Cruz, CA), TrxR, p100/p52, or actin (1:1000; from Cell Signaling Technology, Danvers, MA). Bound antibodies were detected by species-specific, IRDye-conjugated secondary antibodies for supershift analysis, followed by resolution of the complexes on native 4% polyacrylamide gels. The bands were then subjected to Odyssey infrared imaging (LI-COR Biosciences, Lincoln, NE).

Quantitative real-time polymerase chain reaction analysis
B-cell lymphoma-2-like protein 1 (Bcl-xL) mRNA levels were determined via quantitative real-time polymerase chain reaction (qRT-PCR) using the following primers: Bcl-xL F 5'-GATCCCCCATTCAGTTAAC-3', R 5'-CATTCAGGTCAGCCTGGAAGGT-3'. Levels of mRNA were compared using the ΔΔCt method.

Adenovirus transduction
We transduced U266 cells with recombinant adenovirus vectors that express green fluorescent protein (GFP) and RelA [26], RelB (Cell Biolabs, San Diego, CA), or an empty control vector (Cell Biolabs; biosafety level 2). Vectors were constructed and propagated using previously described methods [29], and they were handled following standard biosafety level 2 procedures. The viral vectors were used to transduce U266 cells at a multiplicity of infection of 100. Between 18 and 24 hours posttransduction, cells were treated with AUR or BSO, alone or in combination, for an additional 24 hours.

Statistical analyses
Synergy was analyzed at each testable dose combination using Laska et al.'s model-free test of synergy [30] with unequal-variance t tests (in R software, http://www.r-project.org) as the building blocks. Adjustment for multiple comparisons over dose combinations via control of the false discovery rate was considered but deemed unnecessary, given that the majority of the synergy p values were significant and that they always clustered in contiguous synergistic dose combination regions.

Mean data values and the standard error of the mean were calculated for each variable. One-way analysis of variance, followed by Bonferroni’s test for multiple comparisons, was used to analyze data involving the analysis of multiple sample groups. A value of p < 0.05 was designated as statistically significant (Prism 4.0 software; GraphPad Software, La Jolla, CA).

Results
Combined toxicity of Auranofin and buthionine-sulfoximine
The effect of AUR in combination with BSO on the viability of various malignant B-cell lines was determined using an MTT assay following 24-hour exposure to these drugs, either alone or together. At low nanomolar concentrations, AUR alone caused minimal levels of cell death, and BSO alone was nontoxic in the MCL cell lines Granta and Rec-1. However, simultaneous exposure of the cells to both drugs dramatically enhanced the cytotoxicity, even at the lowest tested AUR concentration of 100 nmol/L (Fig. 1A). Similarly, BSO enhanced AUR-mediated toxicity in the DLBCL cell lines OCI-LY10 and SUDHL6 (Fig. 1B) and in the MM cell lines U266 and KMS-12-PE (Fig. 1C). Laska et al.'s model-free test of synergy [30] confirmed the synergistic action of these two drugs together on cancer cell death at the multiple-dose combinations that were tested. This method has previously been used to test the effects of drug combinations on cancer cell growth [31-33]. The most profound combined toxicity of these drugs occurred in Granta cells and U266 cells; hence, these two cell lines were used in subsequent experiments investigating potential mechanisms underlying this synergism.

We performed Annexin V/propidium iodide (PI) staining in Granta cells at multiple time points following treatment to determine the mechanism of AUR+BSO-induced cell death. At 6 hours posttreatment, there were no changes in levels of Annexin V single-positive (early apoptotic) or Annexin V/PI double-positive (late apoptotic/dead) cell populations. However, at both 18 hours (data not shown) and 24 hours (Fig. 1D) posttreatment, there was a large increase in the Annexin V/PI double-positive (late apoptotic/dead) cell population, thus suggesting that AUR+BSO may be directly inducing necrotic cell death in Granta cells. This
Figure 1. Toxicity of Auranofin and BSO in malignant B-cell lines. (A) Granta cells and Rec-1 cells (human mantle cell lymphoma cell lines) were treated with Auranofin or BSO, alone or together, at the indicated concentrations for 24 hours. Cell viability was measured using the MTT assay. Percent survival was calculated as compared with dimethyl sulfoxide (DMSO)-treated control cells. (B) LY10 and SUDHL6 (human diffuse large B-cell lymphoma cell lines) were treated as indicated for 24 hours, and cell viability was measured as described above. (C) U266 and KMS-12-PE cells (human multiple myeloma cell lines) were treated as indicated for 24 hours, and cell viability was measured as described above. (D) Granta cells were treated as indicated for 24 hours, and cell death was analyzed using Annexin V/PI staining followed by flow cytometry. Annexin V−/PI− = viable cells; Annexin V+/PI− = early apoptotic cells; Annexin V+/PI+ = late apoptotic/dead cells.
is consistent with the report by Sobhakumari et al. [16], where the researchers suggest that AUR + BSO induces necrosis in human head and neck cancer cells.

Enhanced lethality involves inhibition of TrxR activity
Auranofin is a well-known inhibitor of the Trx antioxidant system through inhibition of mitochondrial TrxR [34–38]. As expected, we observed a significant reduction in TrxR activity after 18 hours of treatment with AUR in Granta cells. Interestingly, TrxR activity was further reduced following exposure to AUR and BSO in combination (Fig. 2A). The effect of TrxR overexpression on AUR + BSO-mediated cell death was next determined using an MTT assay in U266 cells stably overexpressing either

![Graph showing thioredoxin reductase activity](image)

**Figure 2.** Effect of AUR and BSO on thioredoxin reductase activity. (A) Granta cells were treated with AUR or BSO, alone or together, at the indicated concentrations for 18 hours. Thioredoxin reductase activity was measured using the Sigma Thioredoxin Reductase assay kit. Results are shown as percent activity compared with DMSO-treated control cells. (B,C) U266 cells either untransfected (control), or stably overexpressing mutant thioredoxin reductase (mutTrxR) or wild-type thioredoxin reductase (TrxR) were treated with the indicated concentrations of AUR, either (B) alone or (C) together with 5 μmol/L BSO for 24 hours. Cell viability was measured using the MTT assay. Percent survival was calculated as compared with DMSO-treated control cells. Immunoblot analysis was performed on whole-cell lysates from U266 cells, either untransfected (control) or stably overexpressing mutTrxR or wild-type TrxR. Overexpression of TrxR was confirmed using an anti-TrxR antibody. Actin was used as a loading control. Statistical significance is indicated as compared with untransfected control cells (**p < 0.01, *p < 0.05) or as compared with mutTrxR expressing cells (**p < 0.01, *p < 0.05).
wild-type TrxR or mutated TrxR (mutTrxR), which was unable to reduce oxidized thioredoxin. The untransfected parent cell line U266 was used as a control. A higher level of TrxR expression in both the TrxR and mutTrxR stable cell lines was confirmed by immunoblot analysis (Fig. 2B). Auranofin alone caused minimal cell death, and this was not significantly altered with TrxR overexpression (Fig. 2B). When combined with 5 μmol/L BSO, AUR drastically decreased cell viability. Interestingly, at the two lowest AUR concentrations, TrxR overexpression significantly protected against AUR+BSO-mediated toxicity (Fig. 2C), thus confirming that AUR’s inhibition of TrxR contributed to the cell death induced by this drug combination.

**Buthionine-sulfoximine augments AUR inhibition of nuclear factor-κB signaling**

The survival of malignant cells is often linked to constitutive activation of the NF-κB signaling pathway [39]. Since AUR has been previously shown to inhibit NF-κB signaling
[19,20], we sought to determine the effect of AUR+BSO on NF-κB activation in Granta cells, having hypothesized that BSO would augment the AUR-induced inhibition of NF-κB. As shown in Figure 3A, following 6-hour treatment with AUR and BSO, alone or together, levels of NF-κB DNA binding activity were dramatically reduced, particularly with the drug combination. To analyze the NF-κB DNA-binding activity further, we performed supershift analyses. As shown in Figure 3B, the antibodies against RelA and RelB altered the mobility of bands, suggesting the presence of these two molecules in NF-κB/DNA complexes. Together, our data suggest that combined treatment of Granta cells with AUR and BSO was able to block RelA- and RelB-specific activities.

We also used luciferase assays as an independent measure of NF-κB activity. Alternatively, Bcl-xL mRNA levels were significantly reduced following 24-hour treatment with AUR+BSO (Fig. 3E). This was expected, considering that Bcl-xL is a prosurvival Bcl-2 homologue and an NF-κB target gene [40].

Genetic evidence for the role of nuclear factor-κB inhibition in toxicity mediated by AUR and buthionine-sulfoximine in combination

To further investigate the involvement of NF-κB signaling in AUR+BSO-induced cell death, we overexpressed the NF-κB proteins RelA and RelB using adenoviral vectors and assessed cell viability via MTT assay following AUR+BSO treatment for 24 hours. Under these conditions, the overexpressed NF-κB molecules are expected to overcome the inhibitory actions of IκBα via stoichiometric changes, thereby assuming a functional presence in the nucleus [41]. Transduction with an empty adenoviral vector, followed by AUR+BSO treatment for 24 hours, was used as a control. U266 cells are highly amenable to adenovirus transduction, as shown by fluorescent microscopy and flow cytometry to measure GFP expression (Fig. 4A); hence, we used this cell type for these analyses. Overexpression of both RelA and RelB was confirmed by immunoblot analysis (Fig. 4B). A longer exposure of the blot containing RelB bands suggests the constitutive presence of this molecule in the cells (data not shown). Enforced RelA and RelB expression completely protected against the cell death caused by AUR alone. Additionally, overexpression of both of these NF-κB proteins significantly protected cells
Figure 4. Overexpression of NF-κB molecules rescues U266 cells from AUR+BSO-induced toxicity. (A) U266 cells were either left untransduced or were transduced with an adenoviral vector expressing RelA and green fluorescent protein (GFP) at a multiplicity of infection of 100 for the indicated time. We captured GFP expression by fluorescence microscopy. Cells were then fixed with 4% paraformaldehyde and were subjected to flow cytometric analysis. The percentage of GFP-positive cells is indicated. (B) U266 cells were transduced with a control adenoviral vector or with an adenoviral vector expressing RelA (first panel) or RelB (second panel) for 24 hours. Cells were then treated with the indicated concentrations of AUR and/or BSO for an additional 24 hours. Cell viability was measured using the MTT assay. Percent survival was calculated as compared with DMSO-treated control cells. Data are presented as mean ± standard error of the mean of values derived from a single experiment that was performed in triplicate. *p < 0.05; **p < 0.01; ***p < 0.001. MOI = Multiplicity of infection.
from AUR+BSO-induced toxicity, an effect particularly apparent at the lowest AUR concentrations (Fig. 4B). Collectively, these data suggest that the combined effect of these two drugs on NF-κB signaling plays an important role in AUR+BSO-mediated cell death.

**Combined toxicity in primary mantle cell lymphoma-derived cells**

Finally, we determined the effect of AUR+BSO on primary human cells derived from MCL patients and one healthy volunteer. Following AUR+BSO treatment for 24 hours, alamarBlue reagent was used to assess cell viability. We chose this assay owing to the small number of cells available in our MCL patient samples, since alamarBlue reagent is sensitive to as few as 50 cells. In two out of the three primary MCL cell samples that were tested, there was decreased cell viability with AUR+BSO, compared with AUR alone (Fig. 5A–5C). With the normal B cells, there was a 40% reduction in cell viability; however, there was no combined effect of AUR+BSO in these cells (Fig. 5D). Together, these data suggest that this drug combination, particularly at low concentrations, may be preferentially toxic to malignant cells as compared with healthy, normal B cells.

**Discussion**

There has been great interest in repurposing existing and approved drugs for the treatment of cancer [42], as this would shorten the time and cost required to provide new and effective cancer therapeutics to patients in need. These agents are already approved for human use, and therefore, ample information is available regarding formulation, dosing, pharmacology, and toxicity. Consequently, their use as anticancer agents can be more rapidly advanced to patients, in contrast to traditional drug discovery pathways. Indeed, AUR represents a prototype of a repurposed agent, as it is currently in clinical trials for patients with chronic lymphocytic leukemia [42].

Auranofin is a gold-based drug approved by the United States Food and Drug Administration for the treatment of patients with rheumatoid arthritis. The pharmacologic activity of AUR is due primarily to its ability to react with physiologic thiols and form stable thiol gold adducts. As described, post-translational modification of protein thiols is a major mechanism for the regulation of multiple cellular pathways. One important target of AUR is TrxR, a component of the cellular thioredoxin system and a major regulator of the cellular thiol state. Additionally, through its regulation of ribonucleotide reductase, TrxR regulates deoxyribonucleotide production and thus regulates DNA synthesis and cellular proliferation [43]. That inhibiting TrxR is an attractive target for cancer therapeutics is supported by the finding that TrxR is overexpressed in a number of cancers compared with its normal cellular counterpart.

Another proposed mechanism of action of AUR is inhibition of NF-κB. Nuclear factor-κB represents a family of
dimeric transcription factors including five members: cRel, RelB, RelA (p65), p50/p105, and p52/p100. Nuclear factor-kB target genes play central roles in a multitude of cellular processes, including immune responses, inflammation, proliferation, and survival [44]. In addition, inhibition of NF-kB has been shown to be an effective therapeutic strategy for B-cell lymphoproliferative disorders such as DLBCL, MCL, and MM [45–48]. Redox regulation of NF-kB activity has been shown to occur both in the nucleus and in the cytoplasm [49,50]. In this regard, AUR inhibits nuclear translocation of NF-kB by inhibiting IKK activation through its modification of cysteine 779 on IKK [19,20].

Overexpression of both RelA and RelB attenuated the cytotoxicity both of AUR alone and of the AUR+BSO combination. The RelA/p50 combination is most commonly involved with activation of the canonical NF-kB pathway, whereas the RelB/p52 combination is mainly involved in the noncanonical NF-kB pathway [51]. Although there is a significant amount of overlap between these two pathways, the different heterodimers differentially regulate gene expression [51,52]. Whereas the canonical NF-kB pathway has been extensively studied in the context of cancer, the same is not true of the noncanonical NF-kB pathway. Therefore, our data showing that overexpression of RelB protects cells from AUR+BSO-induced toxicity in the MM cell line, U266, are interesting, especially considering the results of a very recent study suggesting that the NF-kB-inducing kinase, an important regulator of the noncanonical NF-kB pathway, is a potential therapeutic target for MCL treatment [53].

In summary, we show that the combination of AUR and BSO elicits significant cell death of MCL, DLBCL, and MM cells through the potent inhibition of both TrxR and NF-kB. Indeed, the synergistic effect of BSO on AUR-induced cell death may be due, in part, to our novel findings that BSO augments AUR-induced inhibition of TrxR and NF-kB activity. Importantly, BSO does not seem to increase the cytotoxicity of AUR in normal peripheral-blood B cells. This selective tumor toxicity speaks to the potential safety of this therapeutic strategy. Auranofin has an established history as a well-tolerated oral agent, and BSO has been studied in phase I trials, where it was found to be safe and well tolerated. This was demonstrated at doses that resulted in serum levels of 50–100 mmol/L and that inhibited cellular glutathione levels to <10% of pretreatment levels [54,55]. Notably, we observed synergy with AUR in vitro at BSO concentrations of 5–15 μmol/L. Taken together, combining AUR and BSO may prove to be a safe, effective treatment strategy against B-cell malignancies and warrants further investigation in prospective clinical trials.

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Conflict of interest disclosure
No financial interest/relationships with financial interest relating to the topic of this article have been declared.

References
15. Griffith OW. Mechanism of action, metabolism, and toxicity of bi- 
thionine sulfoximine and its higher homologs, potent inhibitors of 

human head and neck cancer cells to combined inhibition of glu- 

17. Fath MA, Ahmad IM, Smith CI, Spence J, Spitz DR. Enhancement of 
carboplatin-mediated lung cancer cell killing by simultaneous 
disruption of glutathione and thioredoxin metabolism. Clin Cancer 

18. Antelmann H, Helmann JD. Thiol-based redox switches and gene 

19. Jeon KI, Jeong JY, Yue DM. Thiol-reactive metal compounds inhibit 

20. Jeon KI, Byun MS, Yue DM. Gold compound auranofin inhibits Ikap-
naB kinase (IKK) by modifying Cys-179 of IKKbeta subunit. Exp Mol 


22. Sniderhan LF, Garcia-Bates TM, Burgart M, Bernstein SH, Phripps 
RP, Maggirwar SB. Neurotrophin signaling through tropomyosin re-
ceptor kinases contributes to survival and proliferation of nonHodg-

face protein free thiols: A potential novel mechanism of action of the 

with the point mutation abrogator UCN-01 and MEK1/2 inhibitors poten-
tially induces apoptosis in drug-sensitive and -resistant myeloma cells 
through an IL-6-independent mechanism. Blood. 2002;100:3333–3343.

25. Schreiber E, Matthias P, Muller MM, Schaffner W. Rapid detection 
of octamer binding proteins with ‘mini-extracts’, prepared from a 

26. Ramirez SH, Sanchez JF, Dimitri CA, Gelbard HA, Dewhurst S, 
Maggirwar SB. Neurotrophins prevent HIV Tat-induced neuronal 

27. Maggirwar SB, Ramirez S, Tong N, Gelbard HA, Dewhurst S. Func-
tional interplay between nuclear factor-kappaB and c-Jun integrated 
by coactivator p300 determines the survival of nerve growth factor-

factor-kappaB family member RelB inhibits human immunodefi-
cency virus-1 Tat-induced tumor necrosis factor-alpha production. 

29. He TC, Zhou S, da Costa LT, Yu J, Kinzler KW, Vogelstein B. A 

30. Laska EM, Meisner M, Siegel C. Simple designs and model-free tests 

with the thioredoxin reductase inhibitor auranofin triggers apoptosis through a Bax/Bak-
dependent process that involves peroxiredoxin 3 oxidation. Biochem 

32. Rigobello MP, Bindoli A. Mitochondrial thioredoxin reductase puri-
fication, inhibitor studies, and role in cell signaling. Methods Enzy-

33. Rigobello MP, Callegaro MT, Barzon E, Benetti M, Bindoli A. Puri-
fication of mitochondrial thioredoxin reductase and its involvement in 
the redox regulation of membrane permeability. Free Radic Biol 

34. Rigobello MP, Folda A, Baldwin MC, Scutari G, Bindoli A. Effect of 
auranofin on the mitochondrial generation of hydrogen peroxide. 

35. Liu JJ, Liu Q, Wei HL, Yi J, Zhao HS, Gao LP. Inhibition of thio-
redoxin reductase by auranofin induces apoptosis in adriamycin-
resistant human K562 chronic myeloid leukemia cells. Pharmacie. 

36. Ben-Neriah Y, Karin M. Inflammation meets cancer, with NF-

37. Hythes S. The NF-kappaB family of transcription factors and its 

38. Adams J. The development of proteasome inhibitors as anticancer 

39. O’Connor OA, Wright J, Moskowitz C, et al. Phase II clinical expe-
rience with the novel proteasome inhibitor bortezomib in patients 
with indolent nonHodgkin’s lymphoma and mantle cell lymphoma. 

in previously untreated multiple myeloma: Efficacy, characterization 
of peripheral neuropathy, and molecular correlations with response 

and noncanonical NF-kappaB pathways demonstrates significant anti-
tumor activities in multiple myeloma. Clin Cancer Res. 2012;18: 
4669–4681.

42. Gloire G, Piette J. Redox regulation of nuclear post-translational 
modification, inhibitor studies, and role in cell signaling. Methods Enzy-

and noncanonical NF-kappaB pathways demonstrates significant anti-
tumor activities in multiple myeloma. Clin Cancer Res. 2012;18: 
4669–4681.

and noncanonical NF-kappaB pathways demonstrates significant anti-
tumor activities in multiple myeloma. Clin Cancer Res. 2012;18: 
4669–4681.

45. Rigobello MP, Bindoli A. Mitochondrial thioredoxin reductase puri-
fication, inhibitor studies, and role in cell signaling. Methods Enzy-

46. Rigobello MP, Callegaro MT, Barzon E, Benetti M, Bindoli A. Puri-
fication of mitochondrial thioredoxin reductase and its involvement in 
the redox regulation of membrane permeability. Free Radic Biol 

47. Rigobello MP, Folda A, Baldwin MC, Scutari G, Bindoli A. Effect of 
auranofin on the mitochondrial generation of hydrogen peroxide. 

48. Liu JJ, Liu Q, Wei HL, Yi J, Zhao HS, Gao LP. Inhibition of thio-
redoxin reductase by auranofin induces apoptosis in adriamycin-
resistant human K562 chronic myeloid leukemia cells. Pharmacie. 

49. Ben-Neriah Y, Karin M. Inflammation meets cancer, with NF-

50. Chen C, Edelstein LC, Gelinus C. The Rel/NF-kappaB family 
directly activates expression of the apoptosis inhibitor Bcl-x(L). 

51. Harhaj EW, Sun SC. Regulation of RelA subcellular localization by a 
7095.

52. Skalska J, Brookes PS, Nadtochiy SM, et al. Modulation of cell sur-
face protein free thiols: A potential novel mechanism of action of the 

53. Rigobello MP, Bindoli A. Mitochondrial thioredoxin reductase puri-
fication, inhibitor studies, and role in cell signaling. Methods Enzy-

54. Rigobello MP, Callegaro MT, Barzon E, Benetti M, Bindoli A. Puri-
fication of mitochondrial thioredoxin reductase and its involvement in 
the redox regulation of membrane permeability. Free Radic Biol 

55. Rigobello MP, Callegaro MT, Barzon E, Benetti M, Bindoli A. Puri-
fication of mitochondrial thioredoxin reductase and its involvement in 
the redox regulation of membrane permeability. Free Radic Biol 