Gibberellin JRA-003: A Selective Inhibitor of Nuclear Translocation of IKK α

James R. Annand, Andrew R. Henderson, Kyle S. Cole, Aaron J. Maurais, Jorge Becerra, Yejun Liu, Eranthie Weerapana, Angela N. Koehler, Anna K. Mapp, and Corinna S. Schindler*

Cite This: *ACS Med. Chem. Lett.* 2020, 11, 1913–1918

Read Online

ACCESS |

Metrics & More

Article Recommendations

Supporting Information

ABSTRACT: The small molecule gibberellin JRA-003 was identified as an inhibitor of the NF- κ B (nuclear kappa-light-chain-enhancer of activated B cells) pathway. Here we find that JRA-003 binds to and significantly inhibits the nuclear translocation of pathway-activating kinases IKK α (I κ B kinase alpha) and IKK β (I κ B kinase beta). Analogs of JRA-003 were synthesized and NF- κ B-inhibiting gibberellins were found to be cytotoxic in cancer-derived cell lines (HS 578T, HCC 1599, RC-K8, Sud-HL4, CA 46, and NCIH 4466). Not only was JRA-003 identified as the most potent synthetic gibberellin against cancer-derived cell lines, it displayed no cytotoxicity in cells derived from noncancerous sources (HEK 293T, HS 578BST, HS 888Lu, HS 895Sk, HUVEC). This selectivity suggests a promising approach for the development of new therapeutics.

KEYWORDS: Nuclear kappa-light-chain-enhancer of activated B cells (NF- κ B), I κ B kinase (IKK), gibberellins, inhibition of nuclear translocation

Figure 1. NF- κ B pathway is regulated by inhibitory complexes that keep inactive NF- κ B localized in the cytoplasm. Upon phosphorylation by I κ B kinases, and proteolytic degradation of inhibitory subunits, active NF- κ B is imported into the nucleus to bind to DNA and promote gene expression.

Figure 2. Previously reported modulators of the NF- κ B pathway related to the gibberellin family of natural products.^{23, 22}

Chronic inflammation is known to affect all phases of carcinogenesis, and targeting inflammation in the tumor microenvironment has been shown to significantly reduce the development, growth, and spread of malignancies.^{1,2} The NF-

Special Issue: Medicinal Chemistry: From Targets to Therapies

Received: December 16, 2019

Accepted: May 21, 2020

Published: May 21, 2020

Figure 3. Pulldown experiments using molecular probe JRA-031 and inactive analog JRA-032. (A) Pulldowns analyzed by mass spectroscopy in TNFα stimulated HEK 293T cells. *n* = 3 (biological replicates). (B) Pulldowns analyzed by Western blotting in IL-1β stimulated HeLa cells.

Figure 4. Immunohistochemical staining and confocal microscopy of HeLa cells. Cells were pretreated with DMSO or JRA-003 before stimulation by IL-1β or treatment with vehicle. AF488 tagged antibodies were used to image NF-κB pathway members, and DAPI was employed as a nuclear stain.

Figure 5. Western blotting analysis in HEK 293T cellular cytosolic and nuclear fractions. Cells were pretreated with vehicle or JRA-003 before stimulation by IL-1β treatment with vehicles. The cells were then lysed, and the nuclei were separated for independent analysis.

κB (nuclear kappa-light-chain-enhancer of activated B cells) signaling pathway is constitutively active in the majority of cancers and considered a critical link between chronic inflammation and tumorigenesis.^{3–5} Specifically, NF-κB dysregulation has been implicated in all stages of tumorigenesis

Figure 6. Structures of synthetic gibberellins with their EC₅₀ in a commercially available NF-κB driven luciferase assay in TNFα stimulated HEK 293T cells.

including initiation,^{6,7} angiogenesis,^{8,9} metastasis,^{10,11} and tumor survival.^{12–14} Though an important target for anticancer therapy, the complex regulation of NF-κB activation currently presents significant challenges for the development of new therapeutics. Consequently, selective inhibitors of the NF-κB pathway hold great potential to improve our current understanding of NF-κB's role in carcinogenesis to ultimately design and advance new cancer therapeutics able to selectively target inflammatory pathways for the prevention and treatment of malignancies.^{15,16}

NF-κB activity is tightly regulated in healthy cells. Transcriptionally active subunits of NF-κB are bound to inhibitory protein subunits which are phosphorylated by activating kinases (IKKα and IKKβ) and subsequently proteolytically degraded by ubiquitin dependent proteases before active NF-κB can be translocated to the nucleus (Figure 1).¹⁷ While inhibition of the activating kinases was shown to decrease NF-κB signaling in cellular models, these strategies were unsuccessful in the production of viable therapeutics.^{18,19} Specifically, complete inhibition of canonical NF-κB signaling via IKKβ inhibition has been associated with systemic toxicity *in vivo*.²⁰

Our laboratories have an active interest in identifying new strategies for NF-κB inhibition relying on small molecule inhibitors. Specifically, recent work by one of our groups

Table 1. High Throughput Evaluation of Synthetic Gibberellin Analogs^a

compound	Cell Titer Glo EC ₅₀ (nm)											
	HEK 293T embryonic kidney	HS 578BST fibroblast	HS 888Lu fibroblast	HS 895Sk fibroblast	PSN-1 pancreatic adenocarcinoma	HS 895.T melanoma	HS 578T breast carcinoma	HCC 1599 breast carcinoma	RC-K8 lymphoma	Sud-HL4 lymphoma	CA 46 Burkett's lymphoma	NCIH 446 small cell lung cancer
JRA-001	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
JRA-002	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
JRA-003	N.S.	N.S.	N.S.	N.S.	N.S.	9200	<40	150	<40	<40	<40	61
JRA-004	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
JRA-005	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
JRA-006	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
JRA-007	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
JRA-008	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
JRA-009	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
JRA-010	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
JRA-011	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
JRA-012	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
JRA-013	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
JRA-014	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
JRA-015	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
JRA-017	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
JRA-018	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
JRA-019	N.S.	N.S.	N.S.	9500	N.S.	N.S.	4800	1100	1900	690	460	2900
JRA-021	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
JRA-022	2900	10,000	N.S.	N.S.	N.S.	N.S.	5200	1400	970	620	2500	2100
JRA-023	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
JRA-026	7100	N.S.	N.S.	N.S.	N.S.	N.S.	5200	830	72	<40	880	<40
JRA-027	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
JRA-028	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
JRA-029	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	5500	4300	3000	3700	5900

^aCell Titer Glo viability assay was conducted by HTE. Data reported as average of $n = 2$. N.S. = Not Significant ($IC_{50} > 10,000$ nM).

showed that allogibberic acid (**2**) and gibberellic acid (**3**) selectively bind NF- κ B, specifically p50, and inhibit the NF- κ B pathway without inhibiting the activation and translocation of NF- κ B (Figure 2).²¹ In related studies aimed toward the synthesis of pharbinilic acid (JRA-008), we identified JRA-003 as an active inhibitor of the NF- κ B pathway.²² Herein we report the identification of IKK α and IKK β as protein targets of JRA-003. Additionally, we show that treatment with JRA-003 significantly inhibits the nuclear translocation of IKK α and IKK β . We also report the synthesis and evaluation of analogs of JRA-003 as inhibitors of the NF- κ B pathway as well as inhibitors of cancer cell viability. Specifically, our studies show that JRA-003 is more than 500-fold selective toward inhibition of lymphoma and breast cancer-derived cell lines than healthy fibroblast derived cell types.

JRA-003 is an effective inhibitor of canonical NF- κ B signaling upon pathway stimulation by either IL-1 β (6.0 μ M) or TNF α (2.6 μ M) (see Supporting Information, Figure S1). To gain more direct insight into the mechanism of action of JRA-003, pull down experiments were performed with the alkyne-tagged analog of JRA-003 (JRA-031) (Figure 3A). The experiments were conducted with pretreatment of cells with either JRA-003 or the negative control JRA-002 followed by treatment with JRA-031; enrichment was important as many components of the NF- κ B pathway are present in low copy numbers.²³ Under these conditions, it is expected that upon pretreatment with JRA-003, but not with JRA-002 or DMSO, specific targets of JRA-003 would be competed away from interacting with the probe molecule, JRA-031. The results obtained are consistent with IKK α and IKK β as specific targets

of JRA-003. Additionally, proteome wide stable isotopic labeling with amino acids in cell culture (SILAC) experiments was performed and only 11 other proteins were found to be targets of JRA-003, none of which are known to be modulators of the NF- κ B pathway nor are they known oncogenes (see Supporting Information, Figure S2). Consistent with these results, direct pulldown of IKK α from IL-1 β stimulated HEK 293T cells was observed upon treatment with JRA-031 but not inactive analog JRA-032 (Figure 3B). Additionally, no loss of binding was observed even with rigorous washing, suggesting that JRA-031 is an irreversible covalent binder of IKK.

Next, we investigated the localization of NF- κ B family members. As IKK kinases are responsible for phosphorylating and degrading I κ B, leading to the nuclear translocation of transcriptionally active NF- κ B subunits, RelA, RelB, and c-Rel, we anticipated that IKK inhibitors would inhibit the nuclear translocation of Rel.¹⁷ First, we analyzed the cellular compartmentalization of NF- κ B family members by immunohistochemical staining and confocal microscopy in HeLa cells that were treated with either DMSO or JRA-003 for 4 h followed by 1 h of pathway stimulation with IL-1 β or treatment with vehicle (Figure 4, see Supporting Information, Figure S1). Additionally, we further corroborated these data by Western blotting in HEK 293T cytosolic and nuclear fractions in cells that had been similarly pretreated with DMSO or JRA-003 prior to stimulation with IL-1 β or treatment with vehicle (Figure 5). Significant reduction of nuclear IKK α and IKK β was observed in these nuclear translocation assays. Nuclear IK kinases are known to have a myriad of targets including cotranscriptional mediators and histones. In particular, the

nuclear activity of IKK α has been connected to an upregulation of NF- κ B signaling²⁴ and has been linked to cell cycle regulation and survival in colorectal,^{25,26} breast,^{27,28} pancreatic,²⁹ gastric,³⁰ osteo-sarcoma,³¹ and prostate cancers.^{32–34}

These results prompted us to synthesize a small library of gibberellin and allogibberic acid analogs based on a synthetic strategy we had previously developed toward the synthesis of pharbinilic acid,²² with the goal of identifying structural features necessary for activity. We initially evaluated this compound library in Luciferase reporter gene assays in commercially available HEK 293T cell lines in order to identify the structural features required to inhibit the NF- κ B pathway (Figure 6). JRA-003 was found to be among the most potent against the NF- κ B pathway both upon pathway stimulation by TNF α as well as IL-1 β (Figure 6, see Supporting Information, Figure S3). Among the active analogs, the stereochemical configuration of H-9, which is historically challenging to control,^{22,35–38} plays a significant role in the activity of NF- κ B inhibiting gibberellins (Figure 6, JRA-022 vs JRA-019). Additionally, only gibberellins bearing electrophilic functionalities were found to be active in the NF- κ B pathway. Experiments to determine the contribution of electrophile reactivity are ongoing (See Supporting Information, Figure S4).

In ensuing efforts, all gibberellin analogs were subjected to high throughput evaluation (HTE) upon their ability to inhibit the growth of 22 different cell lines derived from cancerous and noncancerous sources (Table 1 and Supporting Information Table S1). Interestingly, the gibberellin analogs capable of modulating the NF- κ B pathway were also found to be active in the HTE Cell Titer Glo viability assay while non-NF- κ B inhibiting gibberellins were found to be inactive even at the highest concentrations tested. We found that highly electrophilic gibberellins, namely JRA-019, JRA-022, and JRA-026, were broadly cytotoxic with single digit micromolar EC₅₀s across several cell lines and significant cytotoxicity at the highest concentrations tested. In contrast, JRA-003 showed unique potency, with EC₅₀s < 100 nM against breast cancer derived cell lines (HS 578T, HCC1599), lymphoma derived cell lines (RC-K8, Sud-HL4, CA 46) and a small cell lung cancer derived cell line (NCIH 446). Importantly, JRA-003 also displayed high selectivity with a >10 μ M EC₅₀ against all noncancer derived cell lines as well as several noninflammatory cancer cell types. Together, this demonstrates that JRA-003 provides more than 500-fold selectivity against inflammatory cancer *in vitro*.

In conclusion, these results suggest that JRA-003 directly acts on IKK α and ultimately prevents it from entering the nucleus, representing a new approach toward inhibition of the NF- κ B pathway. Further efforts to identify a specific site and manner of binding are ongoing areas of research within our research programs. Both the SILAC proteomics and the HTE cell viability assay suggest that JRA-003 is selective in its biological activity. Finally, the selectivity observed in the cell viability assay demonstrates that small molecules with the ability to affect the localization of IKK α can provide a promising avenue for the discovery and development of new therapeutics.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsmmedchemlett.9b00613>.

Supplemental figures and tables, experimental conditions, and synthesis and characterization of all compounds (PDF)

Proteins detected by mass spectroscopy after pull down with JRA-031 or negative control and complete readout of the proteome-wide SILAC mass spectrometry screen (XLSX)

■ AUTHOR INFORMATION

Corresponding Author

Corinna S. Schindler – Department of Chemistry, Willard-Henry-Dow Laboratory and Program in Chemical Biology, University of Michigan, Ann Arbor, Michigan 48109, United States; orcid.org/0000-0003-4968-8013; Phone: 764-763-6853; Email: corinnas@umich.edu

Authors

James R. Annand – Department of Chemistry, Willard-Henry-Dow Laboratory and Program in Chemical Biology, University of Michigan, Ann Arbor, Michigan 48109, United States

Andrew R. Henderson – Program in Chemical Biology, University of Michigan, Ann Arbor, Michigan 48109, United States

Kyle S. Cole – Department of Chemistry, Merkert Center, Boston College, Chestnut Hill, Massachusetts 02467, United States

Aaron J. Maurais – Department of Chemistry, Merkert Center, Boston College, Chestnut Hill, Massachusetts 02467, United States

Jorge Becerra – Program in Chemical Biology, University of Michigan, Ann Arbor, Michigan 48109, United States

Yejun Liu – Program in Chemical Biology, University of Michigan, Ann Arbor, Michigan 48109, United States

Eranthie Weerapana – Department of Chemistry, Merkert Center, Boston College, Chestnut Hill, Massachusetts 02467, United States; orcid.org/0000-0002-0835-8301

Angela N. Koehler – David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, United States

Anna K. Mapp – Department of Chemistry, Willard-Henry-Dow Laboratory and Program in Chemical Biology, University of Michigan, Ann Arbor, Michigan 48109, United States; orcid.org/0000-0003-0791-8327

Complete contact information is available at: <https://pubs.acs.org/doi/10.1021/acsmmedchemlett.9b00613>

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding

This work was supported by generous grants from the Alfred P. Sloan Foundation, the David and Lucile Packard Foundation, and the Camille and Henry Dreyfus Foundation. The HTS core facilities were supported by the Koch Institute Cancer Center Support Core Grant (P30-CA14051). J.R.A. was additionally supported by a GAANN fellowship.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

In addition to our generous funding sources we would like to thank Paul Bruno for invaluable advice and conversations. For maintaining and providing access to and training for confocal microscopes we thank the BRCF Microscopy Core at the University of Michigan Medical School. We would like to thank Jaime Cheah and the Swanson Biotechnology Center High Throughput Screening Facilities at the Koch Institute for Integrative Cancer Research at MIT for help designing and carrying out HTE Cell Titer Glo® viability assays.

■ ABBREVIATIONS

NF- κ B, nuclear kappa-light-chain-enhancer of activated B cells; IKK α , nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor kinase, alpha; IKK β , nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor kinase, beta; DMSO, dimethyl sulfoxide

■ REFERENCES

- (1) (a) Coussens, L. M.; Werb, Z. Inflammation and Cancer. *Nature* **2002**, *420* (6917), 860–867.
- (2) Mantovani, A.; Allavena, P.; Sica, A.; Balkwill, F. Cancer-Related Inflammation. *Nature* **2008**, *454* (7203), 436–444.
- (3) Bennett, J.; Capece, D.; Begalli, F.; Verzella, D.; D'Andrea, D.; Tornatore, L.; Franzoso, G. NF- κ B in the Crosshairs: Rethinking an Old Riddle. *Int. J. Biochem. Cell Biol.* **2018**, *95*, 108–112.
- (4) Aggarwal, B. B.; Gehlot, P. Inflammation and Cancer: How Friendly Is the Relationship for Cancer Patients? *Curr. Opin. Pharmacol.* **2009**, *9* (4), 351–369.
- (5) Grivennikov, S. I.; Greten, F. R.; Karin, M. Immunity, Inflammation, and Cancer. *Cell* **2010**, *140* (6), 883–899.
- (6) Habib, A. A.; Chatterjee, S.; Park, S.-K.; Ratan, R. R.; Lefebvre, S.; Vartanian, T. The Epidermal Growth Factor Receptor Engages Receptor Interacting Protein and Nuclear Factor- κ B (NF- κ B)-Inducing Kinase to Activate NF- κ B IDENTIFICATION OF A NOVEL RECEPTOR-TYROSINE KINASE SIGNALOSOME. *J. Biol. Chem.* **2001**, *276* (12), 8865–8874.
- (7) Romashkova, J. A.; Makarov, S. S. NF- κ B Is a Target of AKT in Anti-Apoptotic PDGF Signalling. *Nature* **1999**, *401* (6748), 86–90.
- (8) Aggarwal, B. B.; Shishodia, S.; Sandur, S. K.; Pandey, M. K.; Sethi, G. Inflammation and Cancer: How Hot Is the Link? *Biochem. Pharmacol.* **2006**, *72* (11), 1605–1621.
- (9) Levine, L.; Lucci, J. A.; Pazdrak, B.; Cheng, J.-Z.; Guo, Y.-S.; Townsend, C. M.; Hellmich, M. R. Bombesin Stimulates Nuclear Factor κ B Activation and Expression of Proangiogenic Factors in Prostate Cancer Cells. *Cancer Res.* **2003**, *63* (13), 3495–3502.
- (10) van de Stolpe, A.; Caldenhoven, E.; Stade, B. G.; Koenderman, L.; Raaijmakers, J. A.; Johnson, J. P.; van der Saag, P. T. 12-O-Tetradecanoylphorbol-13-Acetate- and Tumor Necrosis Factor Alpha-Mediated Induction of Intercellular Adhesion Molecule-1 Is Inhibited by Dexamethasone. Functional Analysis of the Human Intercellular Adhesion Molecule-1 Promoter. *J. Biol. Chem.* **1994**, *269* (8), 6185–6192.
- (11) Helbig, G.; Christopherson, K. W.; Bhat-Nakshatri, P.; Kumar, S.; Kishimoto, H.; Miller, K. D.; Broxmeyer, H. E.; Nakshatri, H. NF- κ B Promotes Breast Cancer Cell Migration and Metastasis by Inducing the Expression of the Chemokine Receptor CXCR4. *J. Biol. Chem.* **2003**, *278* (24), 21631–21638.
- (12) Aggarwal, B. B. Nuclear Factor-KappaB: The Enemy Within. *Cancer Cell* **2004**, *6* (3), 203–208.
- (13) Ahn, K. S.; Aggarwal, B. B. Transcription Factor NF-KappaB: A Sensor for Smoke and Stress Signals. *Ann. N. Y. Acad. Sci.* **2005**, *1056*, 218–233.
- (14) Dolcet, X.; Llobet, D.; Pallares, J.; Matias-Guiu, X. NF- κ B in Development and Progression of Human Cancer. *Virchows Arch.* **2005**, *446* (5), 475–482.
- (15) Chaturvedi, M. M.; Sung, B.; Yadav, V. R.; Kannappan, R.; Aggarwal, B. B. NF- κ B Addiction and Its Role in Cancer: “One Size Does Not Fit All. *Oncogene* **2011**, *30* (14), 1615–1630.
- (16) Sethi, G.; Sung, B.; Aggarwal, B. B. Nuclear Factor-KappaB Activation: From Bench to Bedside. *Exp. Biol. Med. (London, U. K.)* **2008**, *233* (1), 21–31.
- (17) Gilmore, T. D. Introduction to NF- κ B: Players, Pathways, Perspectives. *Oncogene* **2006**, *25* (51), 6680–6684.
- (18) Miller, S. C.; Huang, R.; Sakamuru, S.; Shukla, S. J.; Attene-Ramos, M. S.; Shinn, P.; Van Leer, D.; Leister, W.; Austin, C. P.; Xia, M. Identification of Known Drugs That Act as Inhibitors of NF- κ B Signaling and Their Mechanism of Action. *Biochem. Pharmacol.* **2010**, *79* (9), 1272–1280.
- (19) Gamble, C.; McIntosh, K.; Scott, R.; Ho, K. H.; Plevin, R.; Paul, A. Inhibitory Kappa B Kinases as Targets for Pharmacological Regulation. *Br. J. Pharmacol.* **2012**, *165* (4), 802–819.
- (20) Prescott, J. A.; Cook, S. J. Targeting IKK β in Cancer: Challenges and Opportunities for the Therapeutic Utilisation of IKK β Inhibitors. *Cells* **2018**, *7* (9), 115.
- (21) Koehler, A. N. Methods for modulating NF- κ B using gibberellins. *U.S. Pat.* 2009/005938, 2009.
- (22) Annand, J. R.; Bruno, P. A.; Mapp, A. K.; Schindler, C. S. Synthesis and Biological Evaluation of Phorbilic Acid and Derivatives as NF- κ B Pathway Inhibitors. *Chem. Commun.* **2015**, *51* (43), 8990–8993.
- (23) Zhao, Y.; Widen, S. G.; Jamaluddin, M.; Tian, B.; Wood, T. G.; Edeh, C. B.; Brasier, A. R. Quantification of Activated NF- κ B/RelA Complexes Using SsDNA Aptamer Affinity – Stable Isotope Dilution—Selected Reaction Monitoring—Mass Spectrometry. *Mol. Cell. Proteomics* **2011**, *10* (6), M111.008771
- (24) Huang, W.-C.; Hung, M.-C. Beyond NF- κ B Activation: Nuclear Functions of I κ B Kinase α . *J. Biomed. Sci.* **2013**, *20* (1), 3.
- (25) Fernández-Majada, V.; Aguilera, C.; Villanueva, A.; Vilarde, F.; Robert-Moreno, A.; Aytés, A.; Real, F. X.; Capella, G.; Mayo, M. W.; Espinosa, L.; et al. Nuclear IKK Activity Leads to Dysregulated Notch-Dependent Gene Expression in Colorectal Cancer. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104* (1), 276–281.
- (26) Hoberg, J. E.; Yeung, F.; Mayo, M. W. SMRT Derepression by the I κ B Kinase Alpha: A Prerequisite to NF- κ B Transcription and Survival. *Mol. Cell* **2004**, *16* (2), 245–255.
- (27) Park, K.-J.; Krishnan, V.; O'Malley, B. W.; Yamamoto, Y.; Gaynor, R. B. Formation of an IKK α -Dependent Transcription Complex Is Required for Estrogen Receptor-Mediated Gene Activation. *Mol. Cell* **2005**, *18* (1), 71–82.
- (28) Tu, Z.; Prajapati, S.; Park, K.-J.; Kelly, N. J.; Yamamoto, Y.; Gaynor, R. B. IKK α Regulates Estrogen-Induced Cell Cycle Progression by Modulating E2F1 Expression. *J. Biol. Chem.* **2006**, *281* (10), 6699–6706.
- (29) Shiah, H.-S.; Gao, W.; Baker, D. C.; Cheng, Y.-C. Inhibition of Cell Growth and Nuclear Factor-KappaB Activity in Pancreatic Cancer Cell Lines by a Tylophorine Analogue, DCB-3503. *Mol. Cancer Ther.* **2006**, *5* (10), 2484–2493.
- (30) Hirata, Y.; Maeda, S.; Ohmae, T.; Shibata, W.; Yanai, A.; Ogura, K.; Yoshida, H.; Kawabe, T.; Omata, M. Helicobacter Pylori Induces I κ B Kinase Alpha Nuclear Translocation and Chemokine Production in Gastric Epithelial Cells. *Infect. Immun.* **2006**, *74* (3), 1452–1461.
- (31) Furuya, K.; Ozaki, T.; Hanamoto, T.; Hosoda, M.; Hayashi, S.; Barker, P. A.; Takano, K.; Matsumoto, M.; Nakagawara, A. Stabilization of P73 by Nuclear I κ B Kinase-Alpha Mediates Cisplatin-Induced Apoptosis. *J. Biol. Chem.* **2007**, *282* (25), 18365–18378.
- (32) Luo, J.-L.; Tan, W.; Ricono, J. M.; Korchynskyi, O.; Zhang, M.; Gonias, S. L.; Cheres, D. A.; Karin, M. Nuclear Cytokine-Activated IKK α Controls Prostate Cancer Metastasis by Repressing Masp1. *Nature* **2007**, *446* (7136), 690–694.

(33) Jackson, P. A.; Widen, J. C.; Harki, D. A.; Brummond, K. M. Covalent Modifiers: A Chemical Perspective on the Reactivity of α,β -Unsaturated Carbonyls with Thiols via Hetero-Michael Addition Reactions. *J. Med. Chem.* **2017**, *60* (3), 839–885.

(34) Kupchan, S. M.; Fessler, D. C.; Eakin, M. A.; Giacobbe, T. J. Reactions of Alpha Methylene Lactone Tumor Inhibitors with Model Biological Nucleophiles. *Science* **1970**, *168* (3929), 376–378.

(35) *Strategies and Tactics in Organic Synthesis*; T. Lindenberg Academic Press Inc., 1994; pp 21–70.

(36) Salman, S. R.; Derwish, G. A. W.; Al-Salih, S. S. Intramolecular Interaction in Gibberic Acid and Its Derivatives. *Spectrochimica Acta Part A: Molecular Spectroscopy* **1986**, *42* (4), 405–408.

(37) Pryce, R. J. New Intermediates in the Aqueous Decomposition of Gibberellic Acid. *J. Chem. Soc., Perkin Trans. 1* **1974**, No. 0, 1179–1184.

(38) Al-Ekabi, H. K.; Derwish, G. A. W. Photochemical and Mass Spectrometric Transformation of Gibberellic Acid to 9-Epiallogibberic Acid. *Can. J. Chem.* **1984**, *62* (10), 1996–1998.