ACS Medicinal Chemistry Letters

pubs.acs.org/acsmedchemlett Letter

Kinases Controlling Stability of the Oncogenic MYCN Protein

Nailah Smith,[⊥] Eduard Reznik,[⊥] Brygida Bisikirska, Vasiliki Polychronidou, Qian Wang, Arie Zask, Farhad Forouhar, Andrea Califano, and Brent R. Stockwell*

Cite This: https://doi.org/10.1021/acsmedchemlett.3c00274 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: We previously identified the natural products isopomiferin and pomiferin as powerful, indirect MYCN-ablating agents. In this work, we expand on their mechanism of action and find that casein kinase 2 (CK2), phosphoinositide 3-kinase (PI3K), checkpoint kinase 1 (CHK1) and serine/threonine protein kinase 38-like (STK38L), as well as STK38, work synchronously to create a field effect that maintains MYCN stability. By systematically inhibiting these kinases, we degraded MYCN and induced cell death. Additionally, we synthesized and tested several simpler and more cost-effective pomiferin analogues, which successfully emulated the compound's MYCN ablating activity. Our work identified and characterized key kinases that can be targeted to interfere with the stability of the MYCN protein in NBL cells, demonstrating the efficacy of an indirect approach to targeting "undruggable" cancer drivers.

KEYWORDS: *MYCN, STK38L, STK38, pomiferin, kinase, degradation*

MYCN is an oncogene that codes for a driver protein often found to be aberrantly activated or amplified in the cells of tumors with poor prognoses. Neuroblastoma (NBL) represents the hallmark of MYCN-driven tumors, common brain tumors affecting children. NBL is a cancer derived from undifferentiated cells in the neural crests of embryos.[1] It is the most common cancer plaguing young children under 1, and 9 out of 10 cases are diagnosed by age 5.[2] *MYCN* is amplified in about 25% of NBL cases, generally those that are aggressive and high-risk with poor outcomes.[3] *MYCN* is localized to the tissue of the brain and kidney and present solely during developmental stages.[2] The correlation between *MYCN* expression and NBL prognosis makes it a promising target for therapy. As a transcription factor, *MYCN* plays a part in the activation of thousands of genes that take part in complex pathways participating in processes such as metastasis, proliferation, differentiation, angiogenesis, and cell cycle arrest.[2,4,5]

The MYCN protein has been previously characterized as undruggable due to the absence of adequate direct binding sites for small molecules. However, to circumvent this issue, we hypothesized that a method of intervention would be to identify inhibitors of targets that play a role in the proteasome-mediated regulation of MYCN turnover.

In a previous study, isopomiferin, a naturally occurring isoflavone found in Osage oranges, was found to be highly effective at abrogating MYCN's transcriptional activity and its close analogue pomiferin was even more effective. The study revealed that pomiferin inhibits casein kinase 2 (CK2) and phosphoinositide 3-kinase (PI3K), as well as other kinases, suggesting that isopomiferin may also represent a multi-kinase inhibitor, including CK2, cyclin dependent kinases (CDK2 and CDK4), checkpoint kinase 1 (CHK1), and aurora 48 kinase A (AURKA), all contributing to MYCN's turnover. These results are consistent with the established role of these kinases in regulating MYCN stability. Isopomiferin, a naturally occurring isoflavone found in Osage oranges, was found to be the most effective at reversing the transcriptional activity of MYCN in our prior study. Isopomiferin's close analogue, pomiferin, was found to be even more effective at inducing MYCN degradation. Our previous study revealed that pomiferin inhibits casein kinase 2 (CK2) and phosphoinositide 3-kinase (PI3K), as well as other kinases.[5] These results suggested that isopomiferin inhibits a number of kinases, including CK2, cyclin dependent kinases (CDK2 and CDK4), checkpoint kinase 1 (CHK1), and aurora kinase A (AURKA), as part of its ability to degrade MYCN. There are also data pertaining to individual kinases regulating MYCN stability. For example, CHK1 protein levels have been shown to be higher in *MYCN*-amplified cells and correlated to MYCN expression levels.[6] NBL cells treated with PI3K inhibitors exhibit a decrease in MYCN protein levels as well as decreased tumor mass.[7] We hypothesized that by interfering with these kinases, some compounds may lead to proteasome-mediated MYCN degradation (Figure 1).

Received: June 24, 2023
Accepted: September 28, 2023

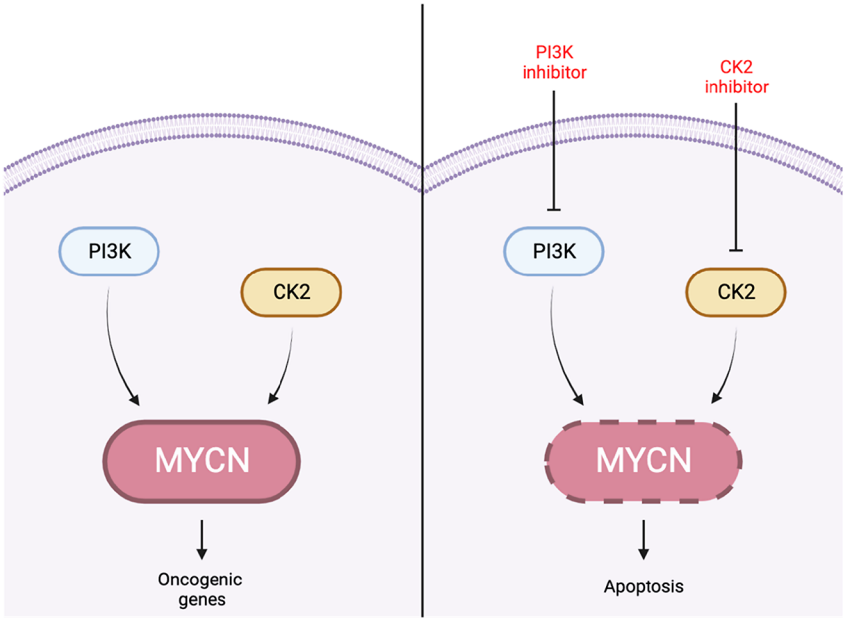

Figure 1. Schematic of the proposed CK2/PI3K dual mechanism that participates in the depletion of MYCN.

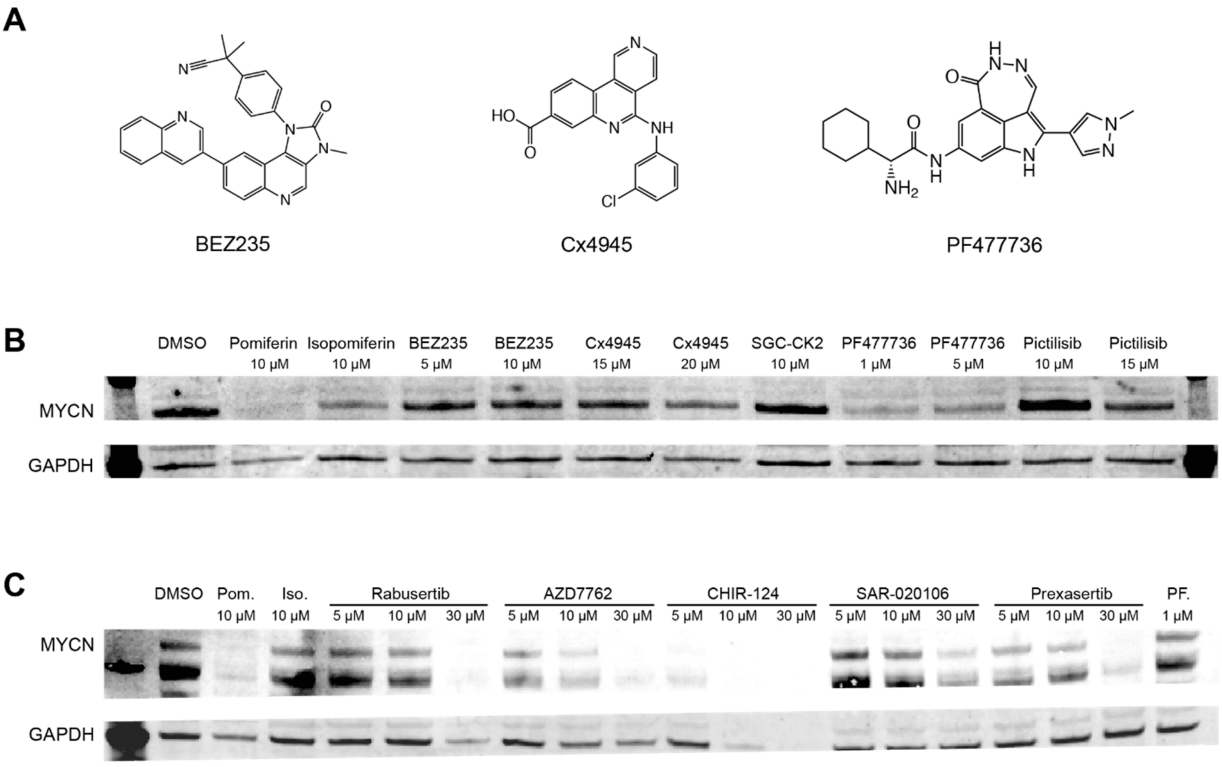

Figure 2. (A) Structures of PI3K inhibitor BEZ235, CK2 inhibitor Cx945, and ChK1 inhibitor PF477736. (B, C) Screening for MYCN degradation activity in SKN-Be2 cells with pomiferin, isopomiferin, and (B) pPI3K and CK2 small-molecule kinase inhibitors and (C) Chk1 inhibtors by Western blot analysis.

In this study, we confirmed that the PI3K and CK2 kinases play a role in MYCN stability; we also discovered three additional kinases that control MYCN stability: CHK1, serine/threonine protein kinase 38-like (STK38L), and another NDR/LATS family kinase member, STK38. We determined that when inhibited in combination, there is an additive field effect on MYCN stability, and the absence of the activity of these kinases decreases MYCN accumulation in a manner comparable to the potent degrader pomiferin. In addition, we designed and synthesized simpler synthetic compounds, derived from the structures of the complex natural products isopomiferin and pomiferin, that still harbor MYCN-degrading activities. These simpler compounds will be more viable starting points for medicinal chemistry and drug development.

Our previous study found that the prenylated isoflavonoid isopomiferin and its structural analogs depleted MYCN protein and reverted MYCN's transcriptional signature through what was postulated to be a dual CK2/PI3K inhibition mechanism.[5]

https://doi.org/10.1021/acsmedchemlett.3c00274
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

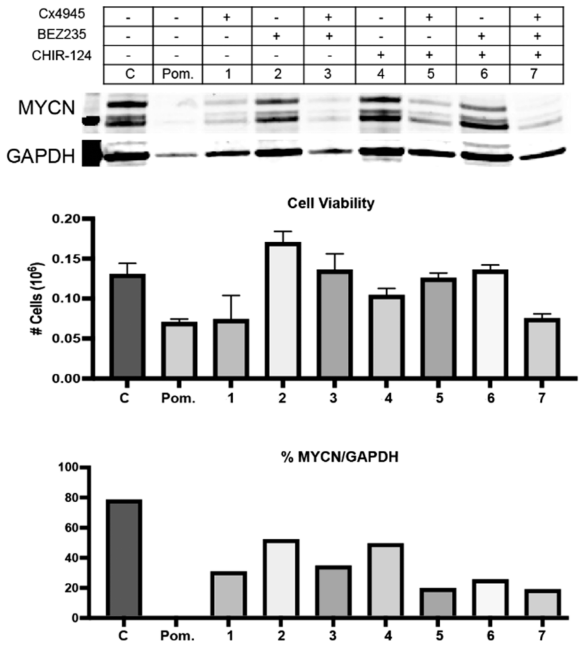

Figure 3. MYCN levels in SKN-Be2 cells following 24 h treatment with DMSO, pomiferin (10 μM), and Cx945 (20 μM). BEZ235 (5 μM) and CHIR-124 (1 μM) determined by Western blot were quantified based on the GAPDH control band to determine % MYCN/GAPDH.

To further assess the MYCN-degrading potential of PI3K/CK2 inhibition, we tested additional PI3K and CK2 small-molecule inhibitors with diverse structures in the MYCN-amplified neuroblastoma cell line SKN-Be2 (Figure 2A,B). Treatment with the most potent PI3K inhibitors, BEZ235 and pictilisib, individually did not result in greater MYCN protein depletion than treatment with pomiferin and isopomiferin, nor did individual treatments with the CK2 inhibitors Cx4945 and SGC-CK2. We also evaluated other potential targets implicated in MYCN transcriptional signature reversion for their MYCN-ablating ability. Among these, the DNA-damage response and cell cycle regulator checkpoint kinase 1 (CHK1) was evaluated; inhibition of CHK1 by PF47776 resulted in significant MYCN protein depletion at concentrations above 1 μM (Figure 2B). Five additional CHK1 inhibitors were tested at three concentrations each, and all depleted MYCN protein (Figure 2C). Of these, CHIR-124 and AZD7762 proved to be the most potent, showing depletion comparable to or better than isopomiferin and pomiferin (Figure 2C).

As noted above, we hypothesized that MYCN depletion occurred as a result of a dual CK2/PI3K dual mechanism. To investigate the role that the CHK1 kinase may play in this mechanism, we explored the combination of CK2/PI3K/CHK1 kinase inhibition. SKN-BE2 cells were treated with CK2, PI3K, and/or CHK1 inhibitors either on their own, in pairs, or with inhibitors of all three kinases together. Cx4945, BEZ235, and CHIR124 were the most potent inhibitors for their specific kinases based on previous assays; concentrations used for combination treatments were slightly lowered to reduce individual effects. MYCN abundance decreased as the number of inhibited kinases increased. By inhibiting all three kinases simultaneously, the strongest depleting effect was observed (Figure 3). In addition, the MYCN/GAPDH ratio

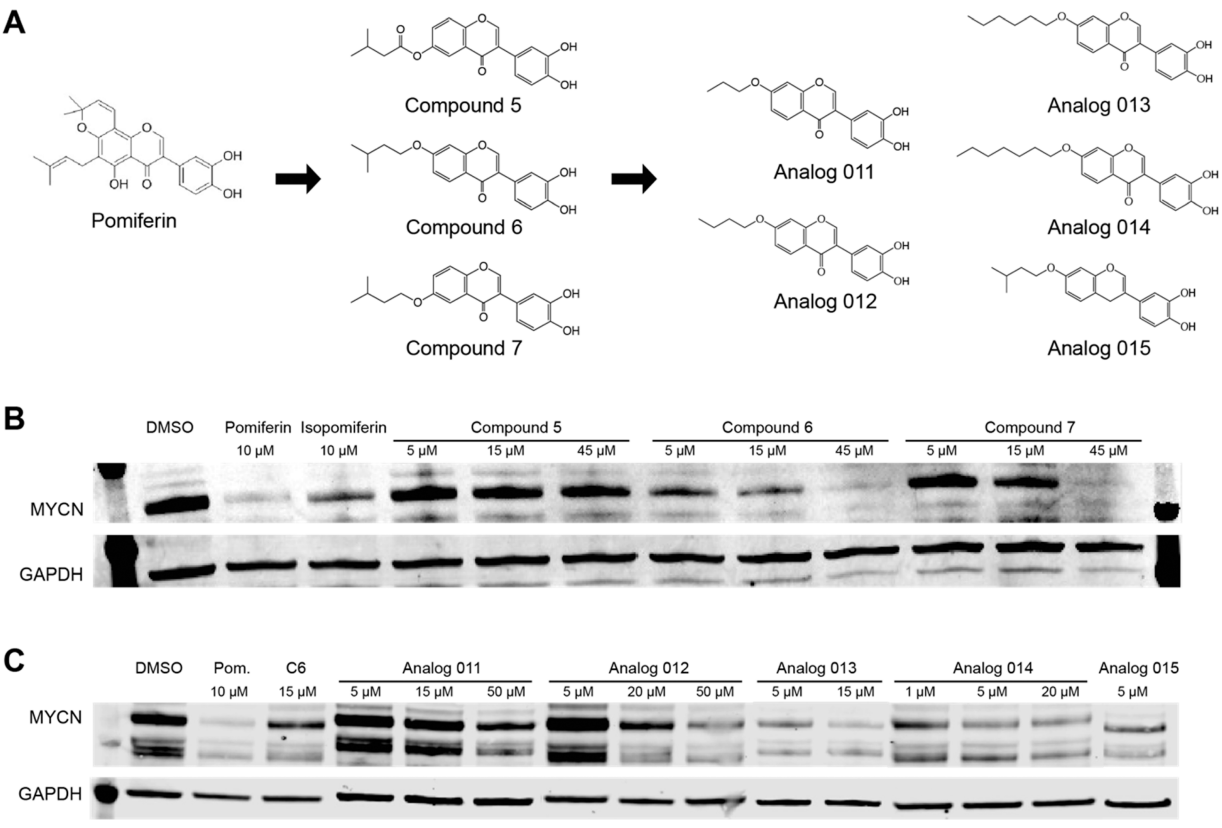

Figure 4. (A) Structures of pomiferin, synthetic analogs **5**, **6**, and **7**, and further-derived analogs **11**, **12**, **13**, **14**, and **15**. (B, C) Western blot analysis of MYCN degrading activity of (B) pomiferin analogs and (C) compound **6** analogs after 24 h treatment at the indicated concentrations.

https://doi.org/10.1021/acsmedchemlett.3c00274
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

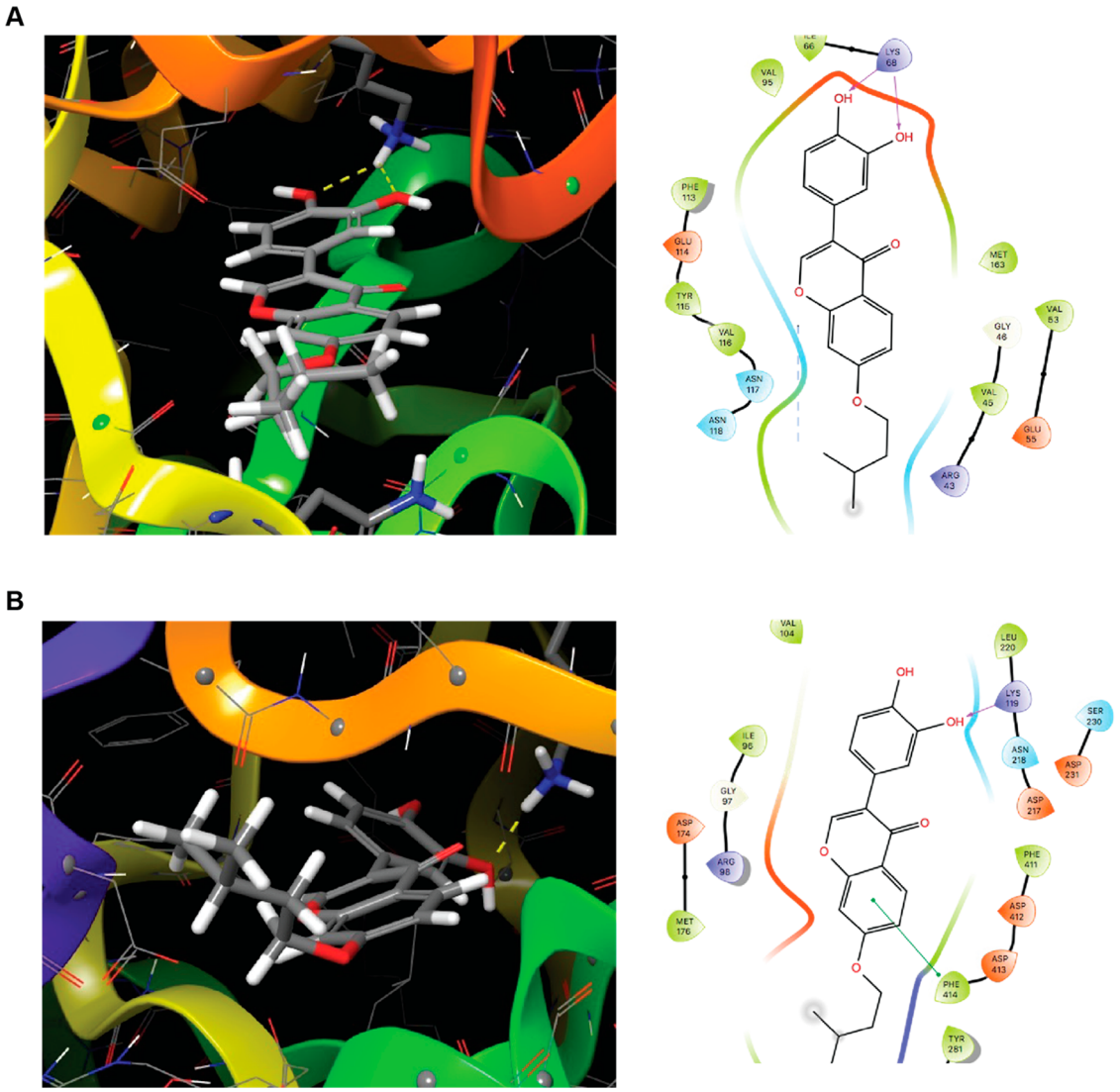

Figure 5. (A) Compound **6** docked in CK2 and (B) the active site of STK38L. The models and ligand interaction diagrams show hydrogen bonding of the OH groups with catalytic Lys68 of CK2 and suggest similar interactions with Lys119 of STK38L.

was the lowest in those cells, indicating the greatest effect on MYCN depletion.

Pomiferin and isopomiferin are complex natural products that are difficult to synthesize and optimize. To create more potent, cheaper, more stable scaffolds, we sought to synthesize simplified analogs of pomiferin. We designed, synthesized, and tested compounds **5**, **6**, and **7**, all of which contained the catechol moiety, but simplified the complex isoflavonoid scaffold of pomiferin (Figure 4A). Of the three compounds, compound **6** showed potency as low as 5 μM, comparable to isopomiferin-treated cells (Figure 4B). Compound **7**'s activity at 45 μM was eliminated by changing the alkoxy side chain to an acyloxy group, as in compound **5**, indicating the significance of this moiety in the compound's activity. By modulation of the length of the hydrophobic side chain of compound **6**, there is an apparent effect on the MYCN-ablating ability. The propoxy tail of compound **11** was the least effective; however, there was an overall increase in potency and lethality as the hydrophobic tail length increased, with peak potency occurring with six carbons (compound **13**) and peak lethality occurring when the carbonyl was removed from the isoflavonoid backbone (compound **15**) (Figure 4A,C). Binding interactions of these simplified pomiferin analogs with the ATP binding site residues of CK2 were similar to those reported previously for pomiferin.[5] Compound **6** was docked into a crystal structure of CK2 (PDB ID 4DGN) using Schrödinger Maestro software. Figure 5A illustrates the phenolic groups of compound **6** making hydrogen-bonding interactions with catalytic Lys68 and the ether oxygen making a hydrogen-bonding interaction with Asn118 of CK2. The ring ether oxygen is in close proximity to the hinge region Val116 and would likely hydrogen-bond to the NH group of the valine. The alkyl group of the ether moiety points out toward the solvent. Docking of compound **6** in a homology model of STK38L showed a similar alignment of the ligand in the ATP binding site with hydrogen bonding of the hydroxy group to the catalytic Lys119 (Figure 5B).

Based on the successful MYCN-depleting abilities of AZD7762, CHIR124, and compound **6**, we sought to compare the kinase targets of these compounds to pomiferin. To do this, we evaluated these small molecules in a kinome-wide binding screen (Table 1). Among the 468 kinases tested, pomiferin only had significant binding with one kinase *in vitro*, NDR2 (also referred to as STK38L). It was the only kinase

https://doi.org/10.1021/acsmedchemlett.3c00274
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

Table 1. Kinome Screen Data of MYCN-Depleting Compounds (% Ctrl)

Table 1 Kinome screen data of MYCN depleting compounds (% Ctrl)					
Target	Pomiferin	Compound 6	Compound 5	CHIR-124	AZD7762
CSNK2A1	45	44	14	1.6	0.8
CSNK2A2	100	100	52	0.35	22
CHEK1	76	71	88	0	0
CHEK2	70	75	76	2.9	0
PIK3C2B	91	100	91	71	37
PIK3C2G	72	75	57	74	98
PIK3CA	96.	100	91	88	86
PIK3CA (C420R)	80	94	87	80	97
PIK3CA (E542K)	92	98	93	93	90
PIK3CA (E545A)	96	100	100	100	100
PIK3CA (E545K)	80	79	66	85	67
PIK3CA (H1047L)	74	80	69	65	49
PIK3CA (H1047Y)	68	98	98	71	74
PIK3CA (I800L)	100	91	98	100	100
PIK3CA (M1043I)	86	77	85	85	79
PIK3CA (Q546K)	100	96	84	62	60
PIK3CB	92	100	92	83	87
PIK3CD	97	97	93	57	81
PIK3CG	93	95	86	21	77
NDR2	28	17	48	0	0

% Ctrl Legend

0 ≤ x < 0.1	0.1 ≤ x < 1	1 ≤ x < 10	10 ≤ x < 35	x ≥ 35

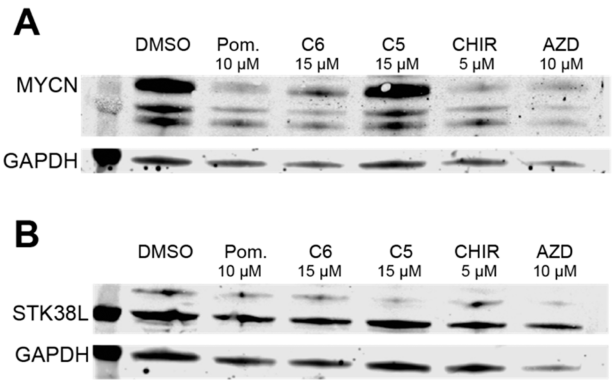

Figure 6. Western blots of SKN Be2 lysates showing (A) MYCN accumulation and correlating (B) STK38L modification after treatment with compounds at concentrations submitted to the kinome screen.

that showed strong binding with CHK1 inhibitors, significant binding with compound **6** and pomiferin, and nonsignificant binding with compound **5**, the negative-control inactive compound. Figure 6 shows MYCN accumulation as a result of treatment with these compounds as well as a visual of corresponding levels of NDR2.

To investigate the role that STK38L may play in MYCN depletion, we tested four STK38L inhibitors, all of which have different targets, with STK38L inhibition noted as an off-target effect. All compounds were tested at multiple concentrations at a single time point and caused a decrease in MYCN accumulation with increasing concentration (Figure 7A). The samples were run in duplicate to visualize the effects of these compounds on MYCN as well as on STK38L (Figure 7B). We noted a band at a higher molecular weight that increased in intensity as MYCN decreased in abundance, indicating that some post-translational modification of STK38L may play a role in the depletion of MYCN. Because the kinome screen was an *in vitro* assay, to determine whether the compounds bound to STK38L in cells, we explored a cellular thermal shift assay (CETSA) (Figure 7C). When a protein is bound to a ligand, it will alter the temperature at which it precipitates; therefore, by treating cells with compound and incubating at a range of temperatures, we can determine whether these compounds that effectively deplete MYCN bind to the STK38L kinase in a cellular context. However, we could not detect that pomiferin, compound **6**, or even the known

https://doi.org/10.1021/acsmedchemlett.3c00274
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

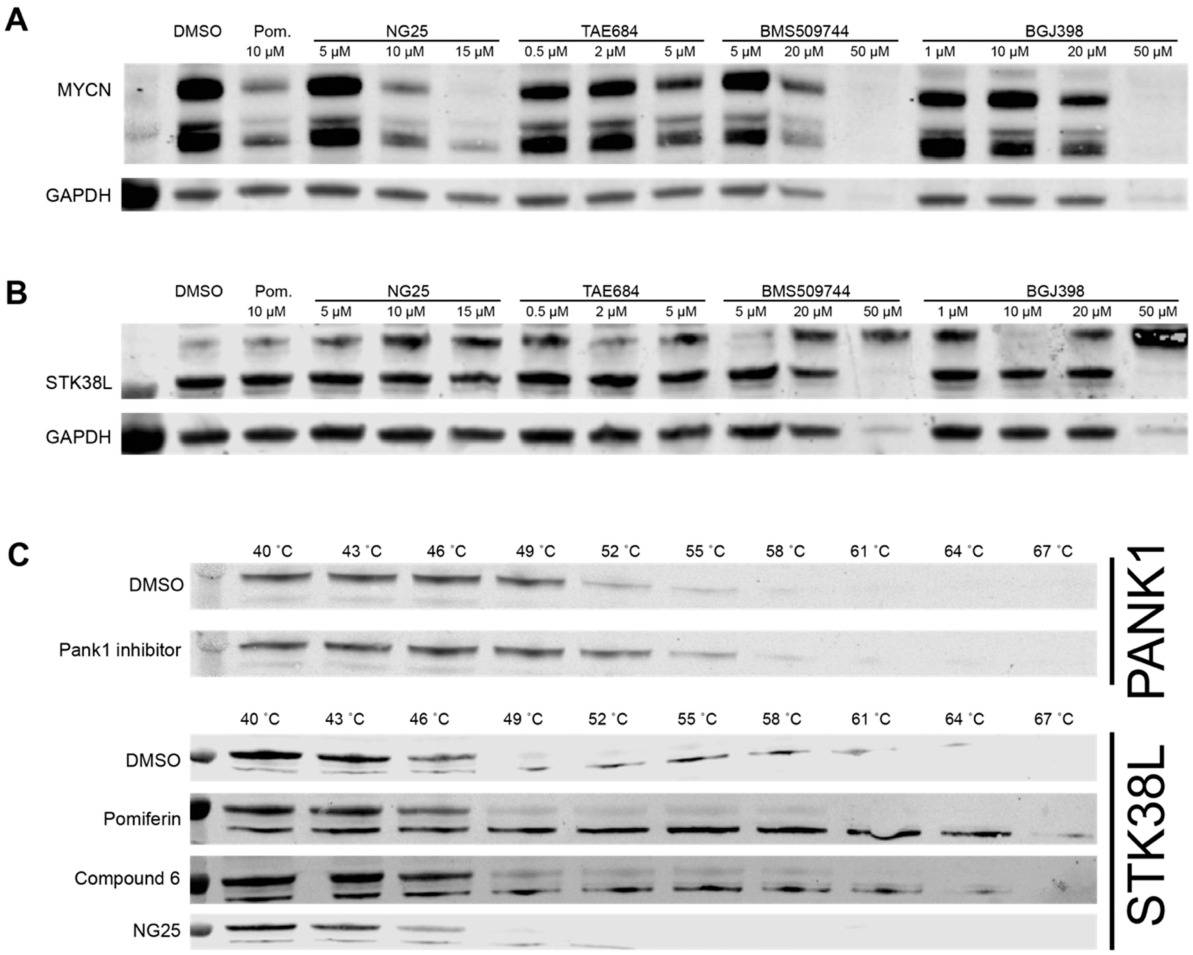

Figure 7. Effects of kinase inhibitors on MYCN and STK38L. (A) Depletion of MYCN protein in SKN Be2 cells after 24 h treatment with NG25 (TAK1 inhibitor), TAE684 (ALK inhibitor), BCJ398 (FGFR inhibitor), and BMS509744, at indicated concentrations. (B) The same samples incubated with STK38L (NDR2) antibody. (C) Western blot of CETSA assay for PANK1 control and STK3L. Pomiferin, compound **6**, and NG25 all bind to the STK38L kinase.

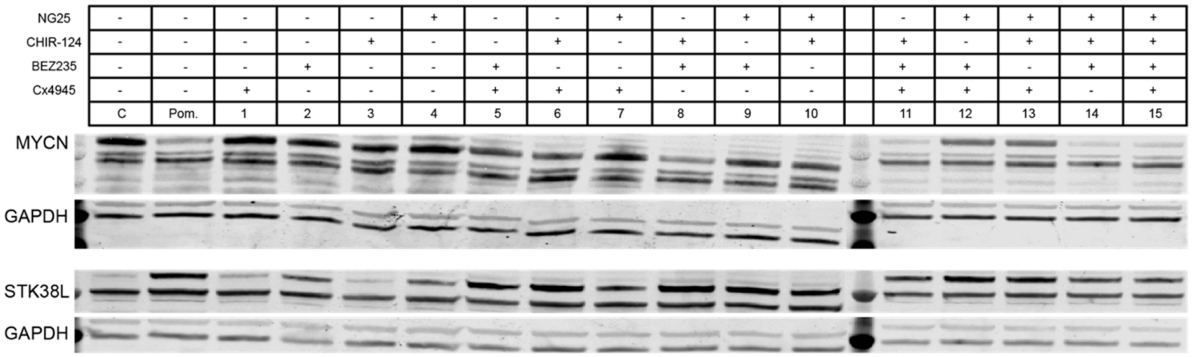

Figure 8. Accumulation of MYCN and modification of STK38L in SKNBe2 cells treated with DMSO (C), pomiferin (10 μM), Cx4945 (20 μM), BEZ235 (5 μM), CHIR-124 (1 μM), and NG25 (10 μM) at the indicated concentrations and combinations after 24 h.

STK38L inhibitor binds directly to the STK38L kinase in cells. STK38L precipitates out by 49 °C in all three treatments, which is consistent with the DMSO control, indicating no detectable change in stability. This may be due to a need to optimize the CETSA assay or may reflect more complex binding dynamics in cells.

However, since the data suggested that NDR2 may play a direct role in MYCN's stability, we performed an additional combination treatment to determine how the inhibition or modification of NDR2 combined with inhibition of CK2, PI3K, and CHK1. Similar to the prior experiment, the SKN-Be2 cells were treated with the compounds alone, as pairs, in a three-way combination, or with all four together. As the number of inhibited kinases increased, so did the degree of MYCN depletion, except in samples that were treated with both NG25, an NDR2 inhibitor, and Cx4945, the CK2 inhibitor (Figure 8). Consistent with previous experiments, the more intense the modified NDR2 band, the more MYCN was

https://doi.org/10.1021/acsmedchemlett.3c00274
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

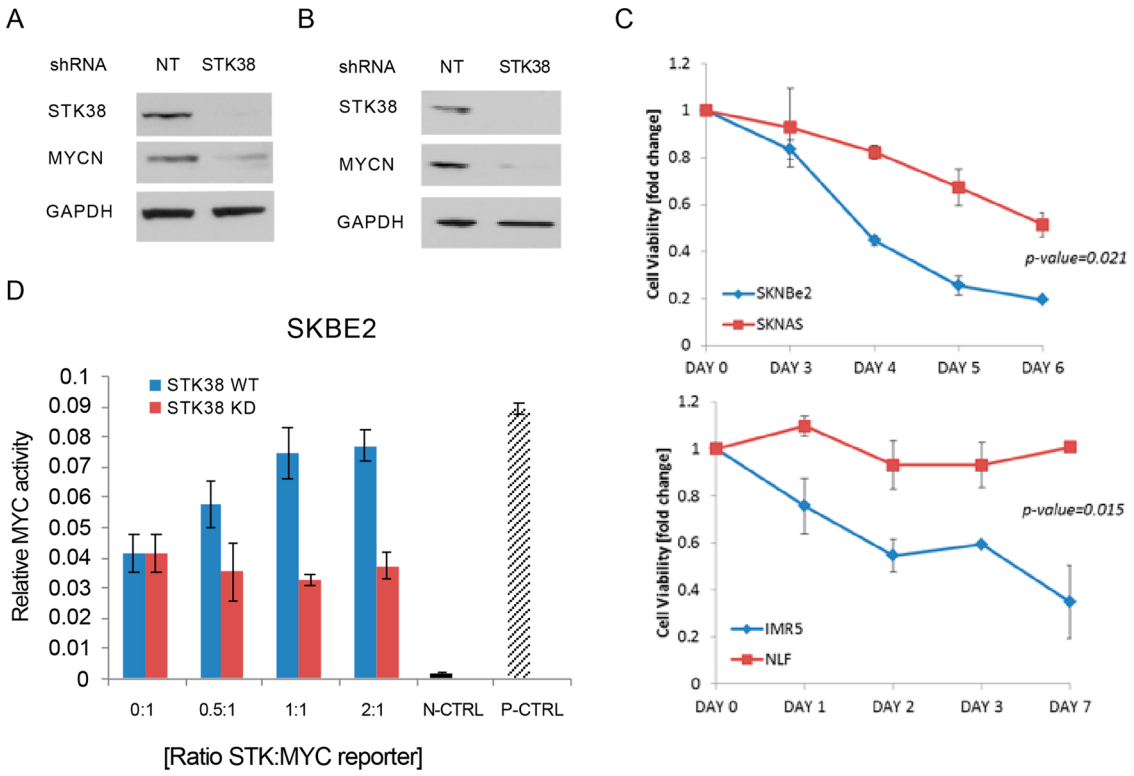

Figure 9. STK38-mediated regulation of MYCN protein in neuroblastoma. Silencing of STK38 affects (A) MYCN expression in SKNBE2MYCN amplified and (B) MYC expression in SKNAS MYCN nonamplified lines. (C) Silencing STK38 inhibits viability in MYCN-amplified lines SKNBE2 and IMR5 cells relative to SKNAS and MYCN-nonexpressing NLF cells. (D) STK38 regulates transcriptional activity of MYCN in SKNBE2; wild-type (WT) STK38 and mutated inactive kinase STK38 (KD) were coexpressed with MYC reporter, and the luciferase activity was measured (N-CTRL, negative control; P-CTRL, positive control).

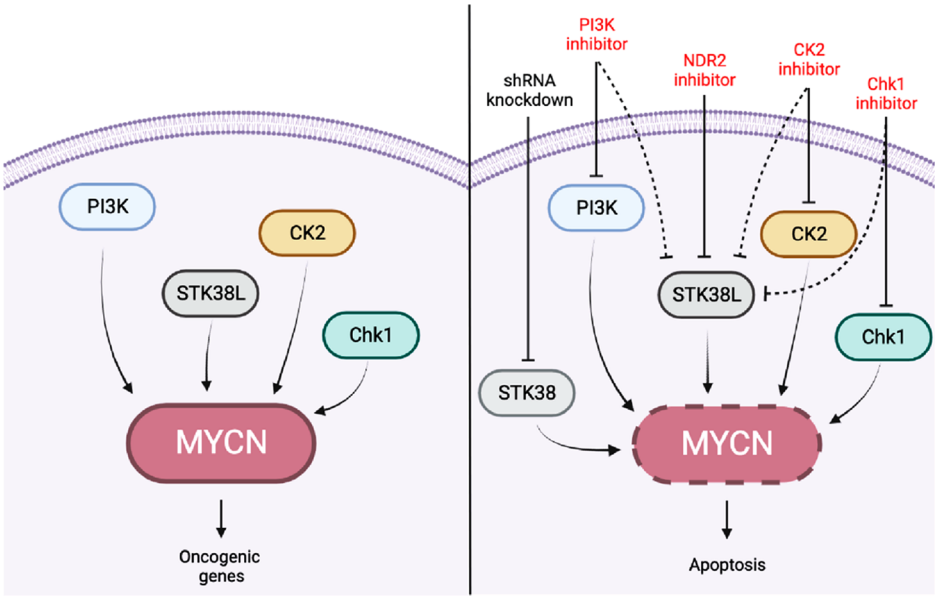

Figure 10. Updated schematic of proposed roles that CK2, PI3K, CHK1, and ST38L kinases play in the depletion of MYCN.

depleted. However, in samples treated with both the STK38L and CK2 inhibitors, the trend was reversed, with MYCN depletion despite less STK38L being lesss affected.

STK38L is a member of the NDR/LATS kinase family that primarily functions in the cytoplasm,[9] and previous work demonstrated that the complementary nuclear NDR/LATS family member NDR1 (STK38), a structural homologue of STK38L, is a critical c-MYC regulator.[10] Although transcribed from distinct genes, MYC and MYCN proteins are both members of the same family of transcription factors, show prominent redundancy in both structure and function,[2] and can regulate some of the same target genes.[11] We therefore investigated the possible effects of STK38 modulation on MYCN protein expression and activity in MYCN-amplified

https://doi.org/10.1021/acsmedchemlett.3c00274
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

cells. No compound in our kinome screen proved to be both a selective and a potent inhibitor of STK38. We therefore used a lentiviral-mediated shRNA silencing approach and observed reduction of MYCN protein expression in MYCN-amplified cells as well as MYC protein depletion in cells lacking expression of MYCN (Figure 9A,B) following STK38 KD, indicating that both MYCN and MYC proteins are stabilized by STK38 independent of cellular context. We examined the effect of STK38 KD on cellular viability in MYCN-amplified and -nonamplified neuroblastoma cell lines. We found that STK38 KD reduced viability in MYCN-amplified cells relative to non-MYCN-amplified cells over a 6 day time course (Figure 9C). Additionally, we tested whether STK38 KD could ablate MYCN protein activity as well as expression level and found that in STK38 KD cells, MYCN activity was indeed suppressed relative to WT (Figure 9D). Taken together, our results indicate that STK38 modulation of MYCN protein activity and abundance results in loss of viability of MYCN-amplified NBL cells.

An undruggable target is challenging to target directly, whether it be due to the nature of its protein−protein interactions or lack of an adequate small-molecule binding site.[8] MYCN is one of many such proteins lacking a sufficient hydrophobic pocket for the binding of a small molecule. One method of overcoming this drawback is to intervene in the pathways regulating such oncoproteins. We sought to investigate the potential of PI3K and CK2 as key kinases that control MYCN stability and by which pomiferin and isopomiferin may exert their MYCN-ablating activity, making them potential targets for intervention.[3]

Pomiferin is a natural product found in the Osage orange. Pomiferin and its analogue isopomiferin are not readily available commercially and must be purified from the fruit, which is a tedious process with low yield. These compounds currently have the greatest MYCN-ablating activity among the screened compounds previously evaluated; therefore, we attempted to create a compound that emulated the activity of the natural products but was more synthetically accessible. We found that the catechol moiety was necessary for pomiferin's activity (Figure S1); therefore, efforts focused on simplifying the isoflavonoid backbone and prenyl groups. While it was unclear how these compounds deplete MYCN mechanistically, we were able to successfully synthesize smaller, simplified compounds whose potencies are comparable to those of pomiferin/isopomiferin and are overall superior candidates for further optimization. Based on the SAR of these analogs and the key protein interactions identified by the docking study, modifications that would increase the drug-like properties of this series of analogs could be designed. For example, phenols are often metabolically labile and are typically replaced by bioisosteres that retain their hydrogen-bonding capabilities but are resistant to metabolism (*e.g.*, indole, 2-aminopyridine). The solvent-exposed regions of kinase inhibitors are frequently modified to increase the water solubility. The aliphatic ether portion of compound **6** could similarly be modified to include groups such as tertiary amines that would increase solubility without affecting the key protein binding interactions.

In addition, we identified three kinases that appear to play a role in MYCN stability: CHK1, NDR1, and NDR2. When cultured neuroblastoma cells were treated with inhibitors of all four kinases simultaneously, the effect on MYCN was similar to the effect of combined inhibition of PI3K/CHK1/NDR2 and closely resembled that of pomiferin. It should be noted, however, that in treatments where both CK2 and STK38L kinases were inhibited, there appeared to be a slight reversal of the MYCN-depleting effect. Additionally, each individual kinase inhibitor appeared to alter STK38L through a higher-molecular-weight band in comparison to the DMSO control. The degree of modification increased as the number of kinases inhibited increased, except in treatments which included both CK2 and STK38L inhibition (Figure 8). It has been reported that for proteins with strong protein−protein interactions, when one pathway is disrupted, it may promote the activity of another pathway, causing resistance to treatment.[8] Individual kinase inhibitors have inherent limitations; *e.g.*, Cx4945 has limited cell penetrance, necessitating increased dosage for MYCN degradation, and PF47776, despite its potency, has multiple off-target effects. However, by utilizing kinase inhibitor combinations to probe the mechanism of MYCN-protein depletion, we were able to simultaneously yield a wider set of targets yet have a more specific area of effect than any small-molecule inhibitor individually can. Based on these data and our STK38 KD findings, we modify our proposed mechanism of MYCN regulation via a multi-kinase field effect that integrates individually weak contributions by PI3K, CK2, CHK1, and STK38L/STK38 in producing a strong effect on regulating MYCN stability in NBL cells, thus abrogating their viability. (Figure 10).

Our finding that silencing of STK38 expression results in loss of MYCN activity and inhibition of cellular viability in MYCN-amplified relative to non-MYCN-amplified cells is particularly intriguing given that MYC and MYCN expression are often inversely corelated in neuroblastoma.[11,12] Taken together with the STK38L data, these results suggest that the NDR/LATS kinase family, a subfamily of the protein kinase A/G/C PKA/PKG/PKC-like group of serine/threonine kinases,[9] could be key druggable upstream modulators of MYCN protein that potentially open up previously inaccessible therapeutic options for elimination of MYCN-addicted tumors. This may provide a new therapeutic approach to targeting the MYC family of oncogenic transcription factors.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.3c00274.

Synthetic and experimental procedures, structures of other analogs previously treated, full results of kinome screen, and NMR spectra and UPLC data (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Brent R. Stockwell − *Department of Biological Sciences and Department of Chemistry, Columbia University, New York City, New York 10027, United States;* orcid.org/0000-0002-3532-3868; Email: bs2198@columbia.edu

Authors

Nailah Smith − *Department of Biological Sciences, Columbia University, New York City, New York 10027, United States*

Eduard Reznik − *Department of Biological Sciences, Columbia University, New York City, New York 10027, United States*

https://doi.org/10.1021/acsmedchemlett.3c00274
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

Brygida Bisikirska − *Department of Physiology and Cellular Biophysics, Columbia University Medical Center, New York City, New York 10032, United States*
Vasiliki Polychronidou − *Department of Biological Sciences, Columbia University, New York City, New York 10027, United States*
Qian Wang − *Department of Biological Sciences, Columbia University, New York City, New York 10027, United States*
Arie Zask − *Department of Biological Sciences, Columbia University, New York City, New York 10027, United States*
Farhad Forouhar − *Department of Biological Sciences, Columbia University, New York City, New York 10027, United States*
Andrea Califano − *Department of Systems Biology, Columbia University Medical Center, New York City, New York 10032, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsmedchemlett.3c00274

Author Contributions

[⊥]N.S. and E.R. contributed equally to this work.

Notes

The authors declare the following competing financial interest(s): B.R.S. is an inventor on patents and patent applications involving ferroptosis, co-founded and serves as a consultant to ProJenX, Inc. and Exarta Therapeutics, holds equity in Sonata Therapeutics, and serves as a consultant to Weatherwax Biotechnologies Corporation and Akin Gump Strauss Hauer & Feld LLP. A.C. is founder, equity holder, and consultant of DarwinHealth Inc., a company that has licensed some of the algorithms used in this work from Columbia University. Columbia University is also an equity holder in DarwinHealth Inc.

■ ACKNOWLEDGMENTS

This work was supported by the National Cancer Institute (NCI) Cancer Target Discovery and Development Program (U01CA217858 and U01CA272610 to A.C. and B.R.S.), NCI Outstanding Investigator Awards (R35CA197745 to A.C. and R35CA209896 to B.R.S.), and Shared Instrumentation Grants (S10OD012351 and S10OD021764 to A.C.). We support inclusive, diverse, and equitable conduct of research.

■ ABBREVIATIONS

CK2, casein kinase 2; PI3K, phosphoinositide 3-kinase; CHK1, checkpoint kinase 1; STK38, serine/threonine protein kinase 38; STK38L, serine/threonine protein kinase 38-like; NBL, neuroblastoma; CDK1, CDK2, CDK4, cyclin dependent kinases 1, 2, and 4; AURKA, aurora kinase A; NDR2, nuclear dbf-related kinase 2; CETSA, cellular thermal shift assay; KD, knockdown; SAR, structure−activity relationship

■ REFERENCES

(1) Ponzoni, M.; Bachetti, T.; Corrias, M. V.; Brignole, C.; Pastorino, F.; Calarco, E.; Bensa, V.; Giusto, E.; Ceccherini, I.; Perri, P. Recent Advances in the Developmental Origin of Neuroblastoma: An Overview. *J. Exp. Clin. Cancer Res.* **2022**, *41* (1), 92.
(2) Huang, M.; Weiss, W. A. Neuroblastoma and MYCN. *Cold Spring Harbor Perspect. Med.* **2013**, *3* (10), No. a014415.
(3) Bartolucci, D.; Montemurro, L.; Raieli, S.; Lampis, S.; Pession, A.; Hrelia, P.; Tonelli, R. MYCN Impact on High-Risk Neuroblastoma: From Diagnosis and Prognosis to Targeted Treatment. *Cancers (Basel)* **2022**, *14* (18), 4421.
(4) Durbin, A. D.; Zimmerman, M. W.; Dharia, N. V.; Abraham, B. J.; Iniguez, A. B.; Weichert-Leahey, N.; He, S.; Krill-Burger, J. M.; Root, D. E.; Vazquez, F.; Tsherniak, A.; Hahn, W. C.; Golub, T. R.; Young, R. A.; Look, A. T.; Stegmaier, K. Selective Gene Dependencies in MYCN-Amplified Neuroblastoma Include the Core Transcriptional Regulatory Circuitry. *Nat. Genet.* **2018**, *50* (9), 1240−1246.
(5) Stokes, M. E.; Vasciaveo, A.; Small, J. C.; Zask, A.; Reznik, E.; Smith, N.; Wang, Q.; Daniels, J.; Forouhar, F.; Rajbhandari, P.; Califano, A.; Stockwell, B. Subtype-selective prenylated isoflavonoids disrupt regulatory drivers of MYCN-amplified Canceras, *Cell Chem. Biol.*, **2023**, in press.
(6) Gu, L.; Chu, P.; Lingeman, R.; McDaniel, H.; Kechichian, S.; Hickey, R. J.; Liu, Z.; Yuan, Y.-C.; Sandoval, J. A.; Fields, G. B.; Malkas, L. H. The Mechanism by Which MYCN Amplification Confers an Enhanced Sensitivity to a PCNA-Derived Cell Permeable Peptide in Neuroblastoma Cells. *eBioMedicine* **2015**, *2* (12), 1923−1931.
(7) Chesler, L.; Schlieve, C.; Goldenberg, D. D.; Kenney, A.; Kim, G.; McMillan, A.; Matthay, K. K.; Rowitch, D.; Weiss, W. A. Inhibition of Phosphatidylinositol 3-Kinase Destabilizes Mycn Protein and Blocks Malignant Progression in Neuroblastoma. *Cancer Res.* **2006**, *66* (16), 8139−8146.
(8) Dang, C. V.; Reddy, E. P.; Shokat, K. M.; Soucek, L. Drugging the "undruggable" Cancer Targets. *Nat. Rev. Cancer* **2017**, *17* (8), 502−508.
(9) Ye, X.; Ong, N.; An, H.; Zheng, Y. (2020) The Emerging Roles of NDR1/2 in Infection and Inflammation. *Front. Immunol.* **2020**, *11*, 534.
(10) Bisikirska, B. C.; Adam, S. J.; Alvarez, M. J.; Rajbhandari, P.; Cox, R.; Lefebvre, C.; Wang, K.; Rieckhof, G. E.; Felsher, D. W.; Califano, A. STK38 is a critical upstream regulator of MYC's oncogenic activity in human B-cell lymphoma. *Oncogene.* **2013**, *32* (45), 5283−5291.
(11) Westermann, F.; Muth, D.; Benner, A.; Bauer, T.; Henrich, K. O.; Oberthuer, Q.; Brors, B.; Beissbarth, T.; Vandesompele, J.; Pattyn, F.; Hero, B.; König, R.; Fischer, M.; Schwab, M. Distinct transcriptional MYCN/c-MYC activities are associated with spontaneous regression or malignant progression in neuroblastomas. *Genome Biol.* **2008**, *9* (10), R150.
(12) Breit, S.; Schwab, M. Suppression of MYC by high expression of NMYC in human neuroblastoma cells. *J. Neurosci. Res.* **1989**, *24*, 21−28.

https://doi.org/10.1021/acsmedchemlett.3c00274
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX