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Development of Allosteric NIK Ligands from Fragment-Based NMR Screening

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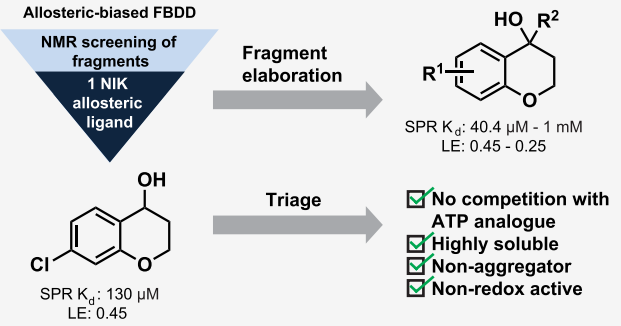
ABSTRACT: NF-κB inducing kinase (NIK) is vital for the induction of many immune responses, and as such, NIK dysregulation has been implicated in various inflammatory diseases and cancers. NIK has been pursued as a potential therapeutic target, and small-molecule inhibitors that bind the orthosteric site on NIK have been reported. However, despite the established chemical matter, NIK inhibitors have not yet reached the clinic. With the goal of developing allosteric NIK ligands using a fragment-based NMR screening approach, we report the identification and development of a series of allosteric, fragment-sized NIK ligands that bind with micromolar potency and good ligand efficiency.



KEYWORDS: *NF-κB inducing kinase, NIK, Allosteric, Fragments, Fragment-based drug discovery, FBDD*

The non-canonical NF-κB signaling pathway is vital for the regulation of several immune responses such as apoptosis and inflammation;[1] however, overactivation of the pathway has been implicated in the pathogenesis and disease progression of various immune disorders and blood cancers.[2] As the central regulatory component of the pathway, NF-κB inducing kinase (NIK) has garnered significant attention as a drug target. Established NIK inhibitors target the ATP-binding site, and they share a propargyl alcohol motif that extends into a small hydrophobic pocket behind the ATP-binding site (Figure 1).[3−6]

The therapeutic potential of inhibiting NIK has been established for a variety of human diseases. For example, the NIK inhibitor NIK SMI1 increased survival in a systemic lupus erythematosus murine model.[4] Inhibition of NIK with B022 has been shown to rescue mice in a toxin-induced liver inflammation model,[6] making NIK a promising target for diet-induced metabolic disorders. Additionally, mangiferin, a natural product NIK inhibitor, slows tumor growth and induces apoptosis in a melanoma mouse model.[7] However, despite these promising experimental data, no NIK inhibitor has received FDA approval. Due to the inability of ATP-competitive NIK inhibitors to confer a clinically viable candidate, we elected to explore alternative strategies for the development of NIK-targeted ligands and inhibitors.

Targeting an allosteric site on NIK, as opposed to the highly conserved ATP-binding site, may provide a viable strategy for targeting this disease-relevant kinase. Fragment-based drug discovery (FBDD) approaches have been used to discover allosteric inhibitors of a variety of proteins,[8−13] including kinases.[14,15] Recently, a FBDD approach was used to discover novel allosteric binders of MEK1 that utilized screening

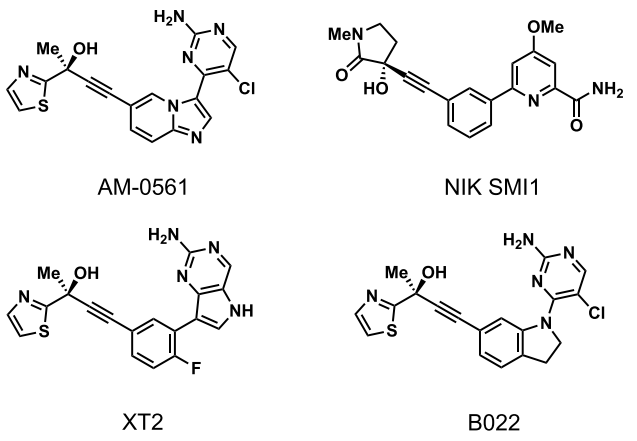


Figure 1. Established active site-directed NIK inhibitors.[3−6]

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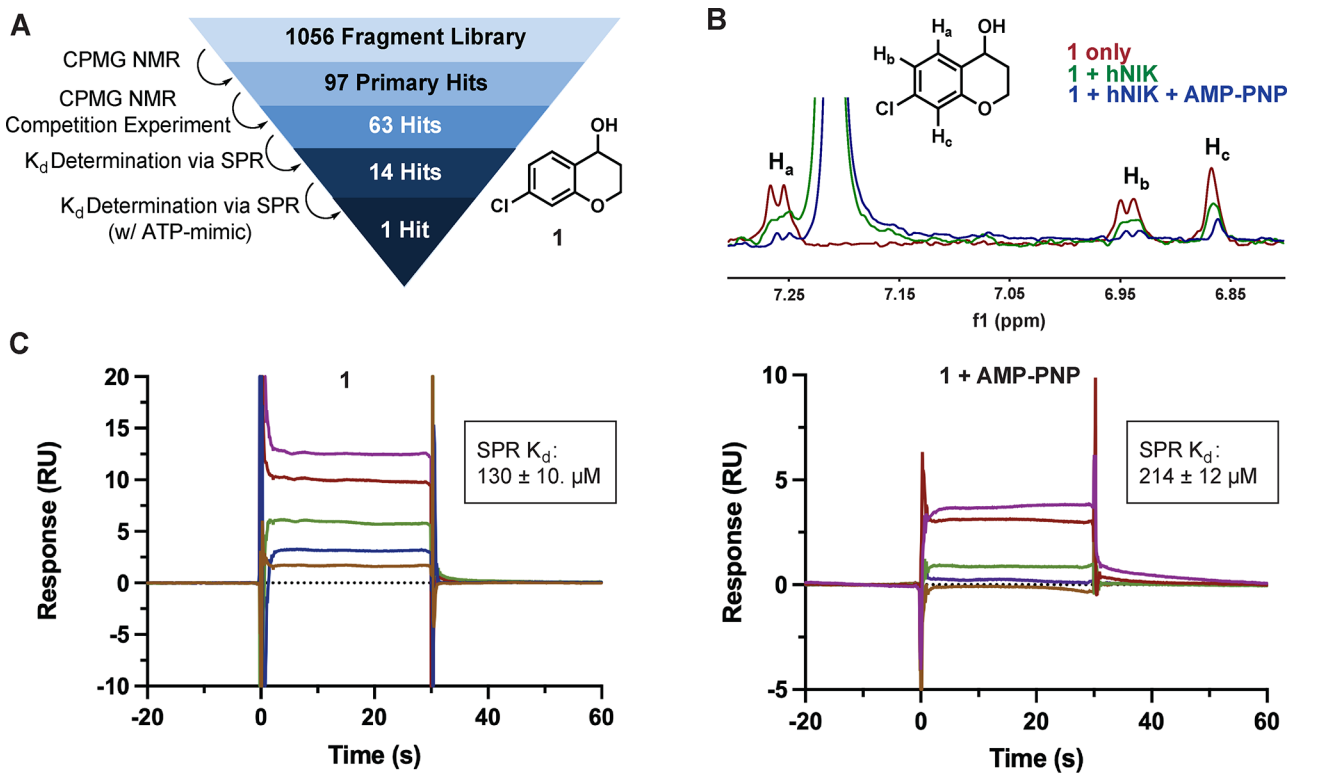

Figure 2. (A) Overview of the allosteric-biased fragment-based screen. (B) CPMG NMR with T_2 filtering competition experiment of **1**. (C) SPR sensorgrams of **1** with and without AMP-PNP. K_d values shown represent the mean ± SEM of two determinations.

conditions to bias for allosteric binding modes.[16] We envisioned using a similar approach in our studies.

We report here the utilization of an in-house fragment library and NMR-based screening to develop allosteric NIK binders. The fragment library consisted of 1056 fragments purchased from the LifeChemicals high-solubility collection. Ligand-observed NMR, using a standard CPMG pulse sequence with T_2 filtering, was employed as the primary screening method.[17] The individual fragments (dissolved in DMSO-d_6) were pooled into cocktails of five compounds and were screened for binding to NIK. To bias for allosteric-binding fragments, a non-hydrolyzable ATP analogue was included in each sample to saturate the NIK orthosteric ATP-binding site. Using this screening protocol, 97 primary hits were identified.

Utilizing the same pulse sequence as before, individual hit fragments were subjected to a competition experiment with AMP-PNP. Three separate NMR spectra were collected for each fragment (fragment alone, fragment with NIK, and fragment with NIK and AMP-PNP). Hits were carried on if the fragment had approximately 50% signal attenuation in the presence of NIK and the signal attenuation was either maintained or increased by adding AMP-PNP. If the signal attenuation was lost upon adding AMP-PNP, then the fragment was determined to be competing with the ATP analogue and rejected. The resulting 63 hits were identified as potential allosteric binders of NIK.

The ligand-observed NMR experiments gave qualitative binding data, but an orthogonal, quantitative experiment was needed to confirm the binding of the hit compounds. Surface plasmon resonance (SPR) with biotinylated NIK protein was chosen to further triage the hit fragments. The 63 hits were tested at five concentrations, and the responses were used to generate a binding curve. After SPR, 14 hits displayed a binding affinity of less than 500 μM. Those 14 hits were then evaluated by SPR in dose−response format in the presence of saturating AMP-PNP, and the response was used to graph a binding curve. After the full screening effort, one hit fragment displayed similar affinity with and without AMP-PNP. Taking all the results together, chromanol **1** was the sole hit from the allosteric bias fragment screening effort (Figure 2). Given the absence of known chemical matter for binding to an allosteric site on NIK, the low hit rate was not unexpected.

The structure of **1** was interesting due to the central chroman ring system. Although there are examples of chromanol structures in biologically active compounds, it is not overly common.[18,19] Therefore, we thought it was necessary to triage hit compound **1** to rule out false positive results.

The solubility of **1** was determined by using a quantitative NMR solubility assay. The hit fragment was determined to have a solubility of at least 5 mM in an aqueous solution. To rule out redox activity contributing to false positive results, a plate-based redox assay was run with **1** and a known redox-active molecule (NSC 663284) as a positive control. Hit fragment **1** showed no redox activity in this assay. Finally, a NMR-based aggregation assay was conducted to ensure that **1** was not aggregating in solution to give false positives during screening. These triage experiments showed that **1** is a highly soluble, non-redox-active and non-aggregating fragment that is suitable to move forward and optimize (Figure 3).

Hit fragment **1** was resynthesized along with a small library of analogues to test the tolerance of substituents on the 4-position of the chromanol structure. The synthesis began by acylating 3-chlorophenol to form **3a** in moderate yield (48%): a phenolic ester was formed *in situ*, and upon adding $AlCl_3$ a

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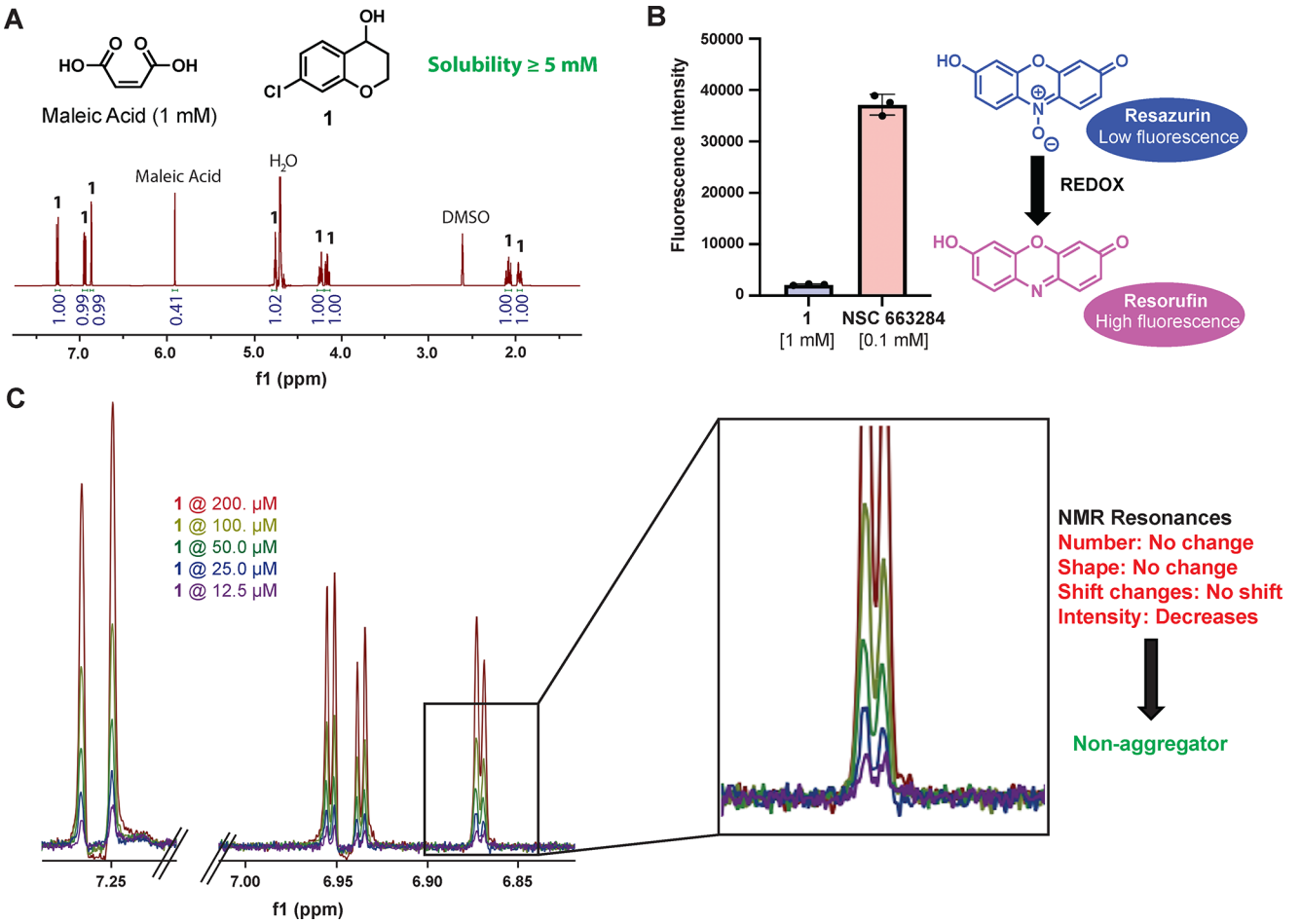


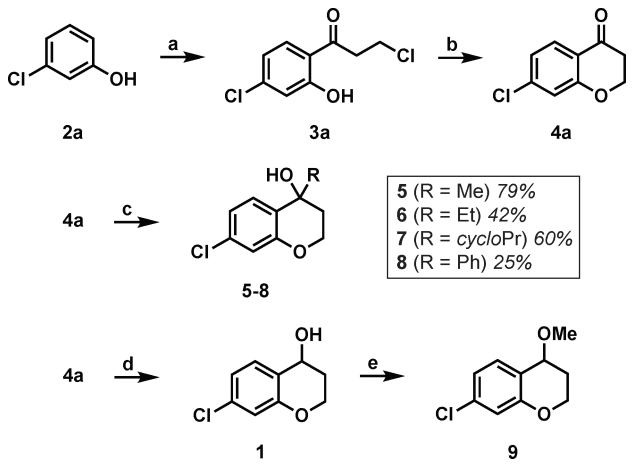
Figure 3. (A) qNMR solubility experiment with **1**. (B) Resazurin redox assay with **1**. (C) NMR aggregation experiment with **1**.

Fries phenolic ester rearrangement gave the desired product.[20,21] Product **4a** was subsequently afforded by an intramolecular cyclization with EtOH and K_2CO_3 in good yield (61%). Grignard addition to the ketone of **4a** yielded tertiary alcohols **5**–**8** in varying yields (25–79%). Reduction of **4a** with $NaBH_4$ in MeOH yielded hit fragment **1** in high yield (75%). Product **1** was then converted to the methoxy derivative with iodomethane and NaH in THF in 68% yield (Scheme 1). Final compounds were tested by SPR for their binding affinity for NIK (Table 1).

Interestingly, all of the tertiary alcohols except for methyl analogue **5** bind NIK with a K_d of less than 1 mM. The cyclopropyl analogue **7** improved upon the binding affinity of the parent hit by approximately 3-fold. The necessity for the hydroxyl was shown by inactive methoxy analogue **9**. These data suggest that fragment elaboration on the 4-position of the chromanol structure is permitted.

To investigate further growth vectors on hit fragment **1**, compounds were synthesized with a methyl walk around the aromatic ring on the chromanol structure. The compounds were synthesized by utilizing chemistry analogous to that used for the synthesis of **1** (Scheme 2). Compounds **10**–**13** were then tested for their affinity for NIK by SPR (Table 2). We were surprised that the only fragment that showed binding was the 5-methyl derivative **10**. Both series of compounds showed that substituents are tolerated if pointing up on the chromanol structure.

Scheme 1. Synthesis of 1, 5–9[a]



[a]Reagents and conditions: (a) 3-chloropropionyl chloride, $AlCl_3$, 90 °C, 2 h, 48%; (b) K_2CO_3, EtOH, rt, 24 h, 61%; (c) RMgBr, THF, 0 → 70 °C, 24 h, 25–79%; (d) $NaBH_4$, MeOH, 0 °C, 2 h, 75%; (e) MeI, NaH, THF, rt, 3 h, 68%.

To test for a synergistic effect of the derivatives displaying micromolar binding affinity for NIK, a series of compounds were synthesized with the 7-chloro and 5-methyl groups, as well as varying substituents at the 4-position (Table S1). We were surprised that none of the analogues displayed enhanced affinity and only phenyl derivative **18** showed a K_d of less than

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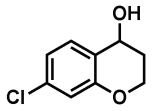
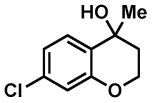
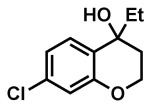
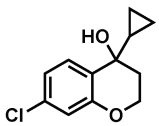
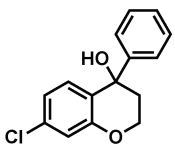
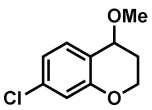
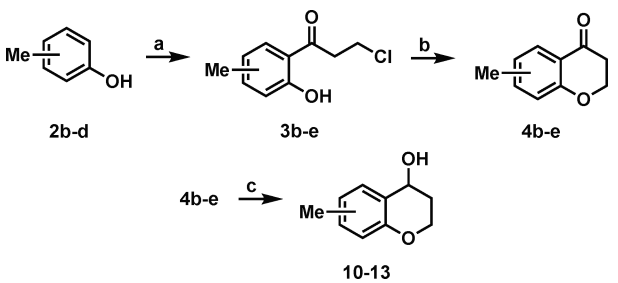
Table 1. NIK Affinity and LE of 1, 5−9

Compound	Structure	SPR K_d(μM) - AMP-PNP[a] SPR K_d(μM) + AMP-PNP	LE[b]
1		130 ± 10. 214 ± 12	0.45
5		> 1 mM > 1 mM	—
6		206 ± 0.50 583 ± 150	0.37
7		40.4 ± 3.1 290 ± 97	0.41
8		572 ± 170 369 ± 110	0.25
9		> 1 mM > 1 mM	—

[a]K_d values shown represent the mean ± SEM of two determinations. [b]LE = 1.37pK_d/HA (heavy atom count).

Scheme 2. Synthesis of 10−13[a]

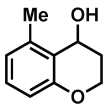
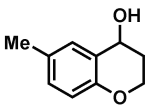
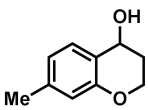
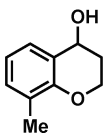


[a]Reagents and conditions: (a) 3-chloropropionyl chloride, $AlCl_3$, 90−120 °C, 2−24 h, 19−72%; (b) K_2CO_3, EtOH, rt, 24 h, 28−73%; (c) $NaBH_4$, MeOH, 0 °C, 2 h, 55−66%.

1 mM (Table 3). We hypothesize that steric bulk on that side of the chromanol structure, along with the rigidity of the ring system, was not optimal for binding.

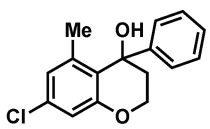
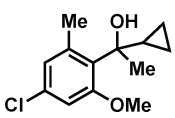
One possible solution is the addition of rotatable bonds on this fragment series, which may permit a more favorable conformation for binding. To test this hypothesis, we synthesized ring-opened analogues where the bond between C-2 and C-3 was disconnected and measured their binding affinity by SPR (Table S2). Only cyclopropyl **21** showed binding of less than 1 mM (Table 3); however, the affinity of **21** for NIK was similar to that of **1**. Currently we are pursuing fragment−NIK co-structure studies to better understand the

Table 2. NIK Affinity and LE of 10−13

Compound	Structure	SPR K_d(μM) - AMP-PNP[a] SPR K_d(μM) + AMP-PNP	LE[b]
10		134 ± 13 131 ± 61	0.45
11		> 1 mM > 1 mM	—
12		> 1 mM > 1 mM	—
13		> 1 mM > 1 mM	—

[a]K_d values shown represent the mean ± SEM of two determinations. [b]LE = 1.37pK_d/HA (heavy atom count).

Table 3. NIK Affinity and LE of 18 and 21

Compound	Structure	SPR K_d(μM) - AMP-PNP[a] SPR K_d(μM) + AMP-PNP	LE[b]
18		367 ± 23 717 ± 46	0.25
21		150 ± 53 143 ± 83	0.33

[a]K_d values shown represent the mean ± SEM of two determinations. [b]LE = 1.37pK_d/HA (heavy atom count).

allosteric binding pocket on NIK and the binding mode of these chromanol-based fragments, which will better inform the rational design of new compounds.

In conclusion, we have reported chromanol analogues that bind to NIK in the micromolar range and do not show competition with the non-hydrolyzable ATP analogue (AMP-PNP). Growth of these chromanol compounds at the 4-position gave promising results, with fragment **7** showing a 3-fold improved binding affinity over parent hit fragment **1**. This class of chromanol analogues provides evidence that NIK possesses allosteric binding sites that are amenable to small-molecule targeting. Ongoing efforts are focused on structurally enabling this project, as well as defining features on the chromanol chemotype that may be modified/elaborated to improve NIK-targeting potency. The development of high-affinity, NIK-targeting ligands even in the absence of enzymatic inhibition would enable therapeutic approaches to regulate this biomedically important kinase by targeted protein degradation, which is currently under investigation by our team.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.3c00429.

Detailed description of synthesis and chemical characterization of compounds; materials and methods for CPMG NMR, SPR, qNMR, aggregation, and redox assays; supplementary SPR sensorgrams and data (PDF)

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Author Contributions

The study was conceptualized and designed by J.J.A., M.J.G., and D.A.H. M.J.G. contributed to curation of the fragment collection and setup of the CPMG NMR assay. All syntheses and collection of experimental data were conducted by J.J.A. The manuscript was written by J.J.A. with editing from M.J.G. and D.A.H.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

NIK, NF-κB inducing kinase; SPR, surface plasmon resonance; NMR, nuclear magnetic resonance; ATP, adenosine triphosphate; LE, ligand efficiency

■ REFERENCES

(1) Cheng, J.; Feng, X.; Li, Z.; Zhou, F.; Yang, J.; Zhao, Y. Pharmacological inhibition of NF-κB-inducing kinase (NIK) with small molecules for the treatment of human diseases. *RSC Med. Chem.* **2021**, *12*, 552.

(2) Zinatizadeh, M. R.; Schock, B.; Chalbatani, G. M.; Zarandi, P. K.; Jalali, S. A.; Miri, S. R. The Nuclear Factor Kappa B (NF-κB) signaling in cancer development and immune diseases. *Genes Dis.* **2021**, *8*, 287.

(3) Demchenko, Y. N.; Brents, L. A.; Li, Z.; Bergsagel, L. P.; McGee, L. R.; Kuehl, M. W. Novel inhibitors are cytotoxic for myeloma cells with NFkb inducing kinase-dependent activation of NFkb. *Oncotarget* **2014**, *5*, 4554.

(4) Brightbill, H. D.; Suto, E.; Blaquiere, N.; Ramamoorthi, N.; Sujatha-Bhaskar, S.; Gogol, E. B.; Castanedo, G. M.; Jackson, B. T.; Kwon, Y. C.; Haller, S.; Lesch, J.; Bents, K.; Everett, C.; Kohli, P. B.; Linge, S.; Christian, L.; Barrett, K.; Jaochico, A.; Berezhkovskiy, L. M.; Fan, P. W.; Modrusan, Z.; Veliz, K.; Townsend, M. J.; DeVoss, J.; Johnson, A. R.; Godemann, R.; Lee, W. P.; Austin, C. D.; McKenzie, B. S.; Hackney, J. A.; Crawford, J. J.; Staben, S. T.; Ismaili, M. H. A.; Wu, L. C.; Ghilardi, N. NF-κB inducing kinase is a therapeutic target for systemic lupus erythematosus. *Nat. Commun.* **2018**, *9*, 179.

(5) Li, Z.; Li, X.; Su, M.; Gao, L.; Zhou, Y.; Yuan, B.; Lyu, X.; Yan, Z.; Hu, C.; Zhang, H.; Luo, C.; Chen, Z.; Li, J.; Zhao, Y. Discovery of a Potent and Selective NF-κB-Inducing Kinase (NIK) Inhibitor That Has Anti-inflammatory Effects in Vitro and in Vivo. *J. Med. Chem.* **2020**, *63*, 4388.

(6) Ren, X.; Li, X.; Jia, L.; Chen, D.; Hou, H.; Rui, L.; Zhao, Y.; Chen, Z. A small-molecule inhibitor of NF-κB-inducing kinase (NIK) protects liver from toxin-induced inflammation, oxidative stress, and injury. *FASEB J.* **2017**, *31*, 711.

(7) Takeda, T.; Tsubaki, M.; Sakamoto, K.; Ichimura, E.; Enomoto, A.; Suzuki, Y.; Itoh, T.; Imano, M.; Tanabe, G.; Muraoka, O.; Matsuda, H.; Satou, T.; Nishida, S. Mangiferin, a novel nuclear factor kappa B-inducing kinase inhibitor, suppresses metastasis and tumor growth in a mouse metastatic melanoma model. *Toxicol. Appl. Pharmacol.* **2016**, *306*, 105.

(8) Christopher, J. A.; Aves, S. J.; Bennett, K. A.; Doré, A. S.; Errey, J. C.; Jazayeri, A.; Marshall, F. H.; Okrasa, K.; Serrano-Vega, M. J.; Tehan, B. G.; Wiggin, G. R.; Congreve, M. Fragment and Structure-Based Drug Discovery for a Class C GPCR: Discovery of the mGlu5 Negative Allosteric Modulator HTL14242 (3-Chloro-5-[6-(5-fluoropyridin-2-yl)pyrimidin-4-yl]benzonitrile. *J. Med. Chem.* **2015**, *58*, 6653−6664.

(9) Coutard, B.; Decroly, E.; Li, C.; Sharff, A.; Lescar, J.; Bricogne, G.; Barral, K. Assessment of Dengue virus helicase and methyltransferase as targets for fragment-based drug discovery. *Antiviral Res.* **2014**, *106*, 61−70.

(10) Orgován, Z.; Ferenczy, G. G.; Keserű, G. M. Fragment-Based Approaches for Allosteric Metabotropic Glutamate Receptor (mGluR) Modulators. *Curr. Top. Med. Chem.* **2019**, *19*, 1768−1781.

(11) Scott, D. E.; Coyne, A. G.; Hudson, S. A.; Abell, C. Fragmentbased approaches in drug discovery and chemical biology. *Biochemistry* **2012**, *51*, 4990−5003.

(12) Tiefenbrunn, T.; Forli, S.; Happer, M.; Gonzalez, A.; Tsai, Y.; Soltis, M.; Elder, J. H.; Olson, A. J.; Stout, C. D. Crystallographic fragment-based drug discovery: use of a brominated fragment library targeting HIV protease. *Chem. Biol. Drug Des.* **2014**, *83*, 141−148.

(13) Vance, N. R.; Gakhar, L.; Spies, M. A. Allosteric Tuning of Caspase-7: A Fragment-Based Drug Discovery Approach. *Angew. Chem., Int. Ed.* **2017**, *56*, 14443−14447.

(14) Li, C.; Zhang, X.; Zhang, N.; Zhou, Y.; Sun, G.; Zhao, L.; Zhong, R. Identification and Biological Evaluation of CK2 Allosteric Fragments through Structure-Based Virtual Screening. *Molecules* **2020**, *25*, 237.

(15) Schoepfer, J.; Jahnke, W.; Berellini, G.; Buonamici, S.; Cotesta, S.; Cowan-Jacob, S. W.; Dodd, S.; Drueckes, P.; Fabbro, D.; Gabriel, T.; Groell, J. M.; Grotzfeld, R. M.; Hassan, A. Q.; Henry, C.; Iyer, V.; Jones, D.; Lombardo, F.; Loo, A.; Manley, P. W.; Pellé, X.; Rummel, G.; Salem, B.; Warmuth, M.; Wylie, A. A.; Zoller, T.; Marzinzik, A. L.; Furet, P. Discovery of Asciminib (ABL001), an Allosteric Inhibitor of

the Tyrosine Kinase Activity of BCR-ABL1. *J. Med. Chem.* **2018**, *61*, 8120−8135.
(16) Di Fruscia, P.; Edfeldt, F.; Shamovsky, I.; Collie, G. W.; Aagaard, A.; Barlind, L.; Börjesson, U.; Hansson, E. L.; Lewis, R. J.; Nilsson, M. K.; Öster, L.; Pemberton, J.; Ripa, L.; Storer, R. I.; Käck, H. Fragment-Based Discovery of Novel Allosteric MEK1 Binders. *ACS Med. Chem. Lett.* **2021**, *12*, 302−308.
(17) Meiboom, S.; Gill, D. Modified Spin-Echo Method for Measuring Nuclear Relaxation Times. *Rev. Sci. Instrum.* **1958**, *29*, 688.
(18) Vakalopoulos, A.; Schmeck, C.; Thutewohl, M.; Li, V.; Bischoff, H.; Lustig, K.; Weber, O.; Paulsen, H.; Elias, H. Chromanol Derivatives − A Novel Class of CETP Inhibitors. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 488.
(19) Liu, L.; Wang, F.; Lu, H.; Ren, X.; Zou, J. Chromanol 293B, an Inhibitor of KCNQ1 Channels, Enhances Glucose-Stimulated Insulin Secretion and Increases Glucagon-Like Peptide-1 Level in Mice. *Islets* **2014**, *6*, No. e962386.
(20) Fries, K.; Finck, G. Über Homologe des Cumaranons und ihre Abkömmlinge. *Chem. Ber.* **1908**, *41*, 4271.
(21) Martin, R. Uses of the Fries Rearrangement for the Preparation of Hydroxyarylketones. A Review. *Org. Prep. Proced. Int.* **1992**, *24*, 369.

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