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RESEARCH ARTICLE

N-Acetyl-3-aminopyrazoles block the non canonical NF-kB cascade by selectively inhibiting NIK

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*Agnese C. Pippione,[a] Stefano Sainas,[a] Antonella Federico,[a] Elisa Lupino,[b] Marco Piccinini,[b] Michael Kubbutat,[c] Jean-Marie Contreras,[d] Christophe Morice,[d] Alessandro Barge,[a] Alex Ducime,[a] Donatella Boschi,[a] Salam Al-Karadaghi[e] and Marco L. Lolli.[a]**

Abstract. NF-κB-inducing kinase (NIK), an oncogenic drug target that is associated with various cancers, is a central signalling component of the non-canonical pathway. A blind screening process, which established that amino pyrazole related scaffolds have an effect on IKKbeta, led to a *hit-to-lead* optimization process that identified aminopyrazole **3a** as a low μM selective NIK inhibitor. Compound **3a** effectively inhibited the NIK-dependent activation of the NF-κB pathway in tumour cells, confirming its selective inhibitory profile.

Acting as a key regulator of immune response, cell proliferation, cell death and inflammation, NF-κB is a ubiquitously expressed family of transcription factors known to be constitutively activated in a variety of malignancies, resulting in uncontrolled apoptosis, cell cycle deregulation and metastatic growth.[1] These observations have led to the NF-κB pathway being validated as target, in particular in breast[2] and thyroid cancers.[3] The non-canonical NF-κB pathway is an important component of NF-κB signalling and predominantly targets the activation of the p52/RelB NF-κB complex.[4, 5] Specifically, the non-canonical NF-κB pathway is involved in secondary lymphoid organ development, B cell survival and maturation, dendritic cell activation, lymphocyte recruitment and bone metabolism.[6] NF-κB-inducing kinase (NIK), is a central signalling component of the non-canonical pathway that integrates signals from a subset of TNF receptor family members and activates a downstream kinase, IκB kinase-α (IKKα), triggering p100 phosphorylation and processing. The specific kinase inhibition activity shown by NIK may provide a means to directly inhibit the non-classical NF-κB pathway and thus potentially influence multiple human diseases, including a number of cancers.[7] The crystal structure of the NIK catalytic domain has recently been resolved,[8] allowing potent, conformationally restricted NIK inhibitors to be identified (Figure 1).[6, 9]

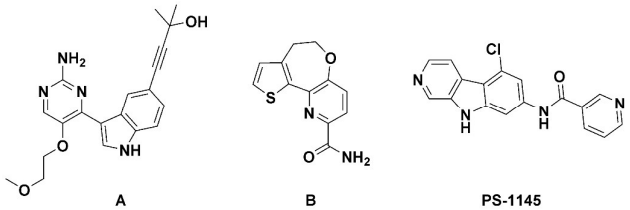

Figure 1: Examples of selective NIK inhibitors A[9] and B.[6] PS-1145 structure of potent IKK*β* inhibitor.[10]

With the aim of identifying new chemical entities[11] that can block the NF-kB cascades, the authors has recently described 4-hydroxy-N-[3,5-bis(trifluoromethyl)phenyl]-1,2,5-thiadiazole-3-carboxamide[12, 13] as a nM-inhibitor of the NF-κB pathway. Following that experience, in this work, a blind screening was conducted on the four kinases, IKKβ, IKKα, IKKε and NIK, that characterise the NF-κB pathway in order to find novel chemotypes. The study involved 2320 compounds from three dedicated Prestwick libraries (*Prestwick Chemical Library*® (1200 cpds), *Prestwick Pyridazine Library* (400 cpds) and *Prestwick Fragment Library* (720 cpds)). An aminopyrazole (**2a**, Figure 2), which is able to weakly (microM range) but selectively inhibit IKKβ, was identified from among the 10 most promising *hits*. Starting from this compound, a *hit-to-lead* optimization process was directed to improve its potency while, simultaneously, retain the observed selectivity. A schematic representation of compound **2a** modulation is presented in Figure 2.

[a] *Department of Science and Drug Technology, University of Torino, via Pietro Giuria 9, 10125 Torino (Italy).*
[b] *Department of Oncology, University of Torino, via Michelangelo 27/B, 10126 Torino (Italy).*
[c] *ProQinase GmbH, Breisacher Str. 117, 79106 Freiburg, Germany.*
[d] *Prestwick Chemical, 220 Boulevard Gonthier d'Andernach 67400 Illkirch (France).*
[e] *SARomics Biostructures, Lund (Sweden).*

† Electronic Supplementary Information (ESI) available: additional biochemical data, chemistry, NMR characterization of final compounds, biochemical protocols. See DOI: XXXXXX

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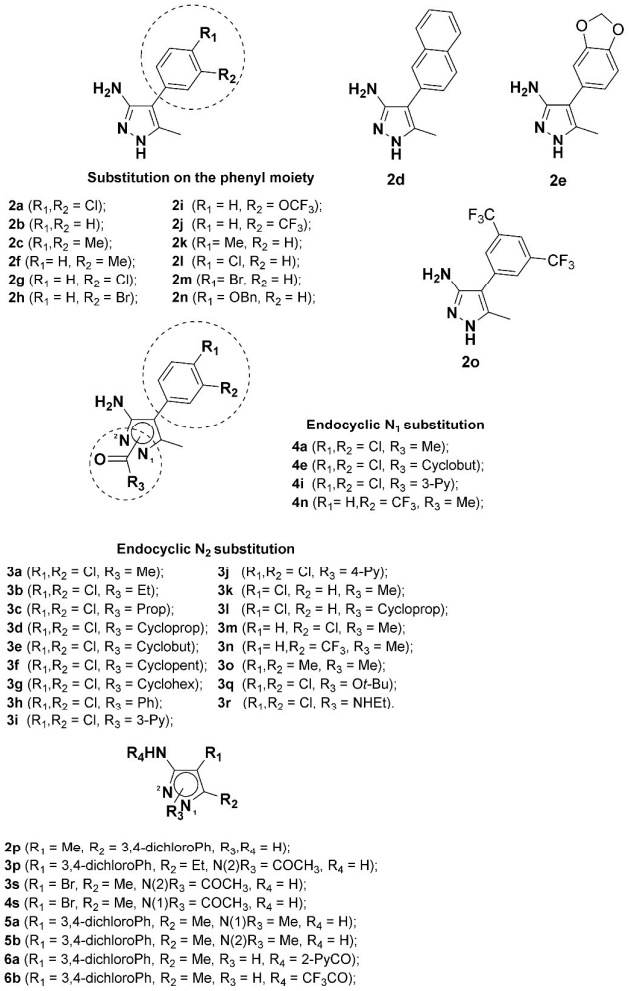
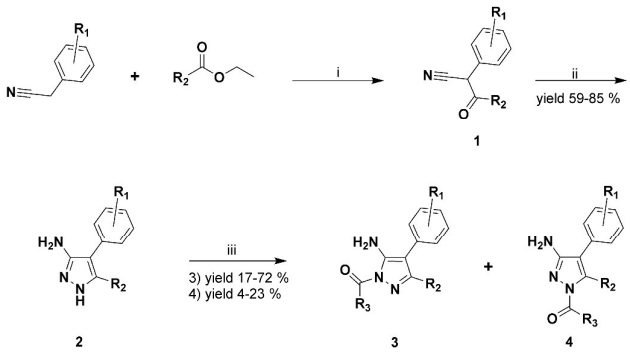


Figure 2: A schematic description of the compounds investigated in the *hit-to-lead* process applied to **2a**.

Of the 44 aminopyrazole derivatives involved in this study, 39 were synthesised using the general procedures described in Scheme 1, in which a Claisen condensation between the appropriate ethyl ester and a benzyl cyanide gave the 3-oxo-2-phenylpropionitriles, structure type **1**. These systems, had been allowed to react, according to a previously-reported procedure,[14] with an excess of hydrazine dihydrochloride to yield the desired 3-aminopyrazoles (structure type **2**). Acetylated product types **3** and **4** were prepared from these compounds. The 3-aminopyrazoles, type **2**, can be acetylated/alkylated in three positions; the primary exocyclic NH_2 group and the two annular nitrogen atoms. The variations in the regiochemistry of the acylation reactions of 3-aminopyrazoles have been described in several studies,[15-18] although 5-methyl-1*H*-pyrazol-3-amines, substituted with a phenyl-substituted group in the 4 position, have never been used as a substrate, to the best of our knowledge. Nevertheless, it appears that regioselective substitution is heavily dependent on the nature of the electrophilic reagent,[15] as well as the reaction conditions and substrate.[19-22]



Scheme 1. General synthesis scheme of the lead compounds with general structure formula **2**, **3** and **4**. Reaction conditions: i) dry THF, NaH, reflux, overnight; ii) $NH_2NH_2 \cdot 2HCl$, 3Å sieves, dry EtOH, reflux, overnight; iii) R_3COCl, dry pyridine, dry THF, rt, or appropriate anhydride, dry THF, rt, from 2h to overnight.

The treatment of compound **2a** with acetic anhydride gave two mono-acetylated products; **3a** (main product), and **4a**. The functionalization of the two annular nitrogen atoms was established using the ^{1}H NMR spectra of the two products obtained; broad singlet signals with δ values of 5.47 ppm and 6.75 ppm, which correspond to the NH_2 protons, were observed in both spectra (Figure 3).

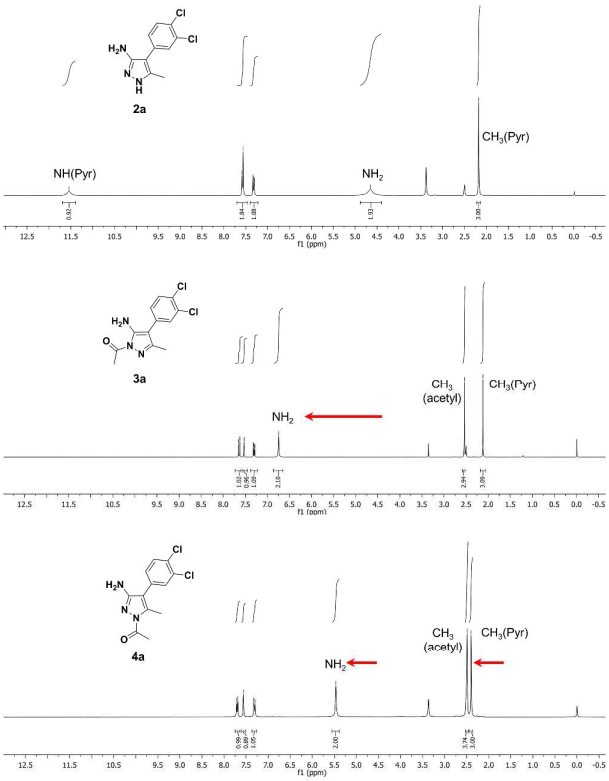


Figure 3: ^{1}H-NMR spectra of starting material **2a** and acylated **3a** - **4a**.

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The two regioisomers were distinguished by comparing their NH_2 proton signals with a similar isomeric couple described in the literature (compounds **C** and **D**, Figure 4).[17] The authors observed the presence of two intramolecular N-H---O hydrogen bonds between the 5-exoamino group and the carbonyl of the N1-acetyl group in compound **C**, resulting in its NH_2 signal being downfield compared to compound **D**. Notably, a downfield chemical shift in the pyrazolic CH_3 signal, which was probably due to the shielding effect of the vicinal CH_3CO group, can also be seen in compound **4a**.

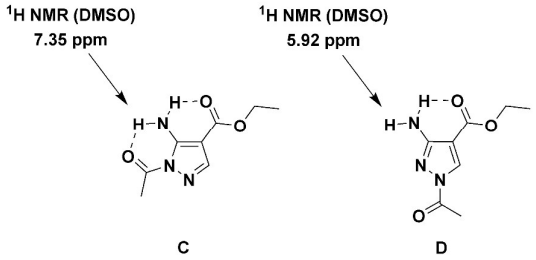
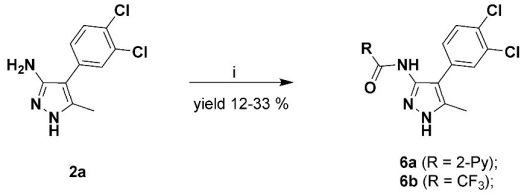


Figure 4: chemical shift of compounds described by Kusakiewicz-Dawid *et al.*[17]

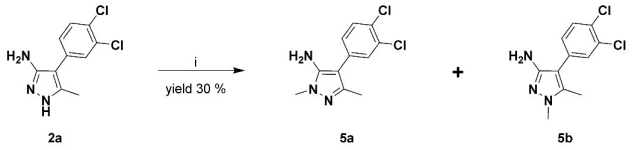
In order to modulate the active isomer **3a**, other 1-acyl-5-aminopirazoles (see Figure 2, Scheme 1), were synthesised under Scheme 1 conditions. If the appropriate anhydrides were not commercially available, the corresponding and more accessible acyl chlorides were involved. As for **2a**, acylation generally gave type **3** as the main product. In some cases, traces of compound type **4** were isolated and characterized (see Supplementary). In only two cases (compounds **6a,b**), did the acylation of the primary exocyclic NH_2 group occur (Scheme 2).



Scheme 2: Synthesis of compounds **6a** and **6b**. Reaction conditions: i) pyridine-2-carbonyl chloride or trifluoroacetic anhydride, dry pyridine, dry THF, rt, overnight.

Surprisingly, when compound **2a** was treated with pyridine-2-carbonyl chloride, in an attempt to obtain the corresponding 2-pyridinyl regioisomer **3**, only carboxamide **6a** was produced in modest yield. On the other hand, the synthesis of **6b** should be carried out using trifluoroacetic anhydride instead of acetic anhydride. Pierce *et al.* have observed the same acylation trend in a number of 3,4-diaryl-5-aminopyrazoles.[19]

In an attempt to modulate **2a** via the insertion of an alkyl group at endocyclic N positions of the pyrazole ring, it was reacted with 1.0 equivalent of methyl iodide, producing a mixture of compounds **5a** and **5b** (Scheme 3).



Scheme 3: Methylation of **2a** to give compounds **5a** and **5b**. Reaction conditions: i) Cs_2CO_3, CH_3I, dry THF, rt, overnight.

An effort to increase conversion yields, which were low for both regioisomers (19 and 11 %), by adding more than 1 equivalent of methyl iodide resulted in dimethylated / polymethylated mixtures that were difficult to resolve. The two regioisomers (**5a** and **5b**), were easily resolved by flash chromatography and distinguished using 2D-NMR (see Supplementary).

The designed compounds (Figure 2), were biologically evaluated both at enzymatic and cellular levels and compared with Amgen compound **A** (Figure 1),[9] and PS-1145,[23, 24], which were used as NIK and IKKβ reference inhibitors respectively (Table 1).

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CPD	IKKβ IC_{50} (μM)	IKKα IC_{50} (μM)	IKKε IC_{50} (μM)	NIK IC_{50} (μM)
PS-1145	0.09±0.01	>100	>100	>100
Cpd A	>100	>100	>100	0.07±0.01
2a	50.9±3.2	>100	>100	>100
2b	>100	>100	>100	>100
2c	>100	>100	>100	>100
2d	42.8±1.3	>100	>100	>100
2e	>100	>100	>100	>100
2f	>100	>100	>100	>100
2g	>100	>100	93.4	>100
2h	88.0±2.4	>100	>100	>100
2i	>100	>100	>100	>100
2j	22.4±1.8	>100	>100	>100
2k	>100	>100	>100	>100
2l	37.9±3.3	>100	>100	61.1
2m	60.1	89.7	>100	>100
2n	>100	>100	15.4	>100
2o	22.9±3.5	>100	>100	>100
2p	75.7±2.7	>100	>100	>100
3a	>100	>100	>100	8.4±1.3
3b	>100	>100	>100	27.4±3.2
3c	>100	>100	>100	>100
3d	>100	>100	>100	24.3±4.3
3e	>100	>100	>100	2.9±1.1
3f	>100	>100	>100	41.0±1.2
3g	>100	>100	>100	3.3±0.4
3h	>100	>100	>100	23.0±1.4
3i	>100	>100	>100	>100
3j	>100	>100	>100	58.2±4.1
3l	>100	>100	>100	>100
3k	>100	>100	>100	>100
3m	>100	>100	>100	>100
3n	>100	>100	>100	>100
3o	>100	>100	>100	39.6±0.9
3p	>100	>100	>100	>100
3q	>100	>100	>100	>100
3r	>100	>100	>100	>100
3s	13.6±1.6	>100	22.9±3.0	>100
4a	>100	>100	>100	>100
4e	>100	>100	>100	>100
4i	>100	>100	>100	>100
4s	47.7±4.0	>100	>100	12.5±2.1
5a	>100	>100	>100	>100
5b	>100	>100	>100	>100
6a	>100	>100	>100	>100
6b	>100	34.9±2.3	>100	

Table 1. The effects of PS-1145, Amgen compound **A** and the designed compounds on: ATP-based kinase assays for IKKβ, IKKα, IKKε and NIK (expressed as IC_{50} value, μM). All experiments were performed in triplicate and data represent means ± SD

In order to complete the biological investigation, we assayed all the compounds against the other three kinases that are involved in the canonical and non-canonical NF-κB activation pathways (IKKα, IKKε and NIK). The first group contains compounds (**2b - 2o**), that were derived from **2a** via modulation of the phenyl moiety. In **2b**, the attempt to remove both the chlorine atoms present in **2a** led to inactivity towards all four kinases. The same behaviour was observed when both the chlorine atoms present in **2a** are replaced with lipophilic/bulky substituents (cpds **2c - 2e**). The meta substitution of compounds **2f - 2j** was investigated and it was observed that the presence of a trifluoromethyl group on **2i** was beneficial for its IKKβ activity. **2j** was two times more active against IKKβ (IC_{50} = 22.4 μM), than **2a**, but retains the same selectivity profile. Similar behaviour was observed in **2o** (IC_{50} = 22.9 μM), which bears two meta trifluoromethyl groups. The presence of a single meta substitution (cpds **2k - 2n**), does not generally appear to be beneficial for activity. The only interesting behaviour here was observed in **2l**, which showed NIK, as well as IKKβ, activity. Inverting the phenyl and methyl moieties in compound **2p**, which is an isomer of **2a**, did not improve IKKβ activity, although selectivity was retained. The major breakthrough in the series was obtained when 3(5)-aminopyrazoles were acetylated/alkylated in one of the three nitrogen positions. While the substitution of the exocyclic NH_2 (cpds **6a** and **6b**), and N1 acylation (cpds **4a**, **4e** and **4i**), led to a complete loss of activity, it became evident that the N(2) acetyl analogue **3a** was the most interesting compound in the entire series due to its selective NIK profile in the low μM range (IC_{50} = 8.4 μM). As the presence of the acetyl moiety was required for activity (the N(2) methyl analogue **5b** was found to be inactive), the acyl substituent was investigated further (cpds **3b - 3r**), leading to the cyclobutyl analogue **3e** (IC_{50} = 2.9 μM), and the cyclohexyl analogue **3g** (IC_{50} = 3.32 μM), being observed as the most potent NIK inhibitors.

The cellular activity of the compounds with a NIK selective profile was evaluated using a gene reporter assay to measure NF-kB activation in EJM cells (ACC-560, DSMZ); a multiple myeloma cell line characterized by constitutively high levels of nuclear NF-κB as a consequence of NIK gene amplification.[25] The inhibitory activity of each compound was determined at the highest concentration resulting in ≥ 80 % cell viability on the Dual Luciferase reporter assay (Table 2). Cell viability was determined using a Promega CellTiter Glo luminescent assay. In order to check the selectivity of the compounds, gene reporter assays were carried out in SKBr3 (HTB-30, ATCC) and MDA-MB-231 (ACC-732, DSMZ), two cell lines in which constitutive activation of nuclear NF-κB is unrelated to NIK.[26, 27] All the compounds were found to inhibit the NF-κB activity in EJM cells, but not in MDA-MB-231 and SKBr3 cells, thus indicating their potential for NIK-selective inhibitory activity in cells. Compound **3a** was also tested against two purified NIK kinase domains. The first was mouse NIK kinase domain (mNIK) comprising amino acids 345-675 of in which V480 was mutated to L, originating the so called humanized KD-mNIK. The second one was human NIK kinase domain (hNIK) comprising amino acids 330-680 in which S549 was mutated to

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D, originating the constitutively active form. Compound **3a** did not show appreciable inhibitory activity against either domain (data not shown). Further investigations are in progress to identify the mode of action of compound **3a**.

NIK in a panel of 44 kinases. It also displayed acceptable log D and solubility parameters. Further experiments will be performed to elucidate the binding mode of **3a** and improve its metabolic stability.

Cpd	NF-kB reporter assay - % of inhibition (concentration)		
	MDA[a]	EJM[b]	SKBr3[c]
PS-1145	87.9±3.3 (50 μM)	89.3±4.3 (20 μM)	3.6±0.2 (50 μM)
Cpd A	7.1±0.4 (1 μM)	93.5±2.4 (1 μM)	2.3±0.1 (1 μM)
2l	29.9±3.4 (50 μM)	10.4±1.1 (50 μM)	4.2±0.7 (100 μM)
3a	-0.77±0.1 (100 μM)	83.4±3.6 (25 μM)	3.9±0.2 (100 μM)
3b	10.3±1.3 (100 μM)	66.8±2.2 (50 μM)	-3.14±0.1 (100 μM)
3d	5.8±0.3 (50 μM)	68.9±3.8 (100 μM)	3.66±0.1 (50μM)
3e	2.36±0.2 (100 μM)	96.2±4.1 (25 μM)	5.2±0.2 (100 μM)
3g	2.36±0.6 (100 μM)	94.7±3.4 (25 μM)	3.7±1.3 (100 μM)
3h	13.8±1.1 (100 μM)	75.2±2.8 (50 μM)	0.31±0.1 (100μM)

Table 2. The effects of PS-1145, Amgen compound **A** and the designed compounds on the NF-κB signalling pathway. NF-κB activation was assessed using a Dual Luciferase reporter assay in [a] MDA-MB-231, [b] EJM and [c] SKBr3 cells. Data are expressed as % of inhibition at the highest concentration tested, indicated in brackets. All experiments were performed in triplicate and data represent means ± SD

Given **3a** was found to be selective towards the other kinases involved in NF-κB activation, we investigated its activity towards a larger kinase panel (Table 4). The determination of IC_{50} values for 44 representative kinases showed that **3a** had a very good selectivity profile versus NIK (IC_{50} kinases > 100 μM).

The preliminary ADME profile of **3a** was evaluated as the final step in this work. Whereas **3a** showed acceptable log D and solubility parameters at this stage, the relative metabolic instability of the compound was highlighted and must be considered in any future exploration.

	Cpd 3a
Metabolic stability [a]	1% [b]
log D [c]	4.09
Kinetic solubility [d]	12 μM

Table 3. Early ADME profiling for **3a**. [a] Human liver microsome (obtained by Xenotech: Xtreme 200 Human Liver Microsomes) 1 μM, 37 °C, NADPH cofactor; [b] cpd found instable also in absence of NADPH, indicating chemical instability or a non-NADPH dependent enzymatic degradation; [c] PBS pH 7.4 / octanol; [d] PBS pH 7.4, 60 min, room temperature.

In conclusion, we have herein identified N-acetyl-3-aminopyrazoles as a new chemotype that can block NF-kB cascade by selectively inhibiting NIK. Compound **3a** is the most representative of the series, which exhibited selectivity for

Kinase	IC_{50} (μM)	Kinase	IC_{50} (μM)
AMPK-alpha1 aa1-550	> 100	JAK1	> 100
AXL	> 100	MAP3K11	> 100
B-RAF wt	> 100	MAP4K2	> 100
CAMKK1	> 100	MEK1 wt	> 100
CDK1/CycB1	> 100	MELK	> 100
CHK2	> 100	MET wt	> 100
CK1-alpha1	> 100	MST1	> 100
CK2-alpha1	> 100	mTOR	> 100
CLK1	> 100	NEK6	> 100
COT	> 100	p38-alpha	> 100
CSK	> 100	PAK1	> 100
DAPK1	> 100	PCTAIRE1/CycY	> 100
DYRK1A	> 100	PDGFR-alpha wt	> 100
EGF-R wt	> 100	PDK1	> 100
EPHA6	> 100	PIM1	> 100
ERK2	> 100	PKC-alpha	> 100
FGF-R1 wt	> 100	PLK1	> 100
FLT3 wt	> 100	SRC (GST-HIS-tag)	> 100
GSK3-beta	> 100	SRPK2	> 100
HIPK1	> 100	SYK	> 100
INS-R	> 100	TAOK2	> 100
IRAK4 (untagged)	> 100	TLK1	> 100

Table 4. IC_{50} profiling for **3a** against 44 protein kinases

Conflict of interest

The authors declare no competing interest.

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Notes and references

1. H. Park, Y. Shin, H. Choe and S. Hong, *J Am Chem Soc*, 2015, **137**, 337-348.
2. W. Wang, S. A. Nag and R. Zhang, *Current Medicinal Chemistry*, 2015, **22**, 264-289.
3. N. Pozdeyev, A. Berlinberg, K. Wuensch, W. M. Wood, Q. Zhou, H. Shibata and B. R. Haugen, *PLoS One*, 2015, **10**, e0134901.
4. S.-C. Sun, *Cell Research*, 2011, **21**, 71-85.

View Article Online
DOI: 10.1039/C8MD00068A

5. S.-C. Sun, *Nat. Rev. Immunol.*, 2017, **17**, 545-558.
6. G. M. Castanedo, N. Blaquiere, M. Beresini, B. Bravo, H. Brightbill, J. Chen, H.-F. Cui, C. Eigenbrot, C. Everett, J. Feng, R. Godemann, E. Gogol, S. Hymowitz, A. Johnson, N. Kayagaki, P. B. Kohli, K. Knuppel, J. Kraemer, S. Kruger, P. Loke, P. McEwan, C. Montalbetti, D. A. Roberts, M. Smith, S. Steinbacher, S. Sujatha-Bhaskar, R. Takahashi, X. Wang, L. C. Wu, Y. Zhang and S. T. Staben, *J. Med. Chem.*, 2017, **60**, 627-640.
7. K. Vazquez-Santillan, J. Melendez-Zajgla, L. E. Jimenez-Hernandez, J. Gaytan-Cervantes, L. Munoz-Galindo, P. Pina-Sanchez, G. Martinez-Ruiz, J. Torres, P. Garcia-Lopez, C. Gonzalez-Torres, V. Ruiz, F. Avila-Moreno, M. Velasco-Velazquez, M. Perez-Tapia and V. Maldonado, *Sci. Rep.*, 2016, **6**, 37340.
8. G. de Leon-Boenig, K. K. Bowman, J. A. Feng, T. Crawford, C. Everett, Y. Franke, A. Oh, M. Stanley, S. T. Staben, M. A. Starovasnik, H. J. A. Wallweber, J. Wu, L. C. Wu, A. R. Johnson and S. G. Hymowitz, *Structure (Oxford, U. K.)*, 2012, **20**, 1704-1714.
9. K. Li, L. R. McGee, B. Fisher, A. Sudom, J. Liu, S. M. Rubenstein, M. K. Anwer, T. D. Cushing, Y. Shin, M. Ayres, F. Lee, J. Eksterowicz, P. Faulder, B. Waszkowycz, O. Plotnikova, E. Farrelly, S.-H. Xiao, G. Chen and Z. Wang, *Bioorg. Med. Chem. Lett.*, 2013, **23**, 1238-1244.
10. T. Hideshima, D. Chauhan, P. Richardson, C. Mitsiades, N. Mitsiades, T. Hayashi, N. Munshi, L. Dang, A. Castro, V. Palombella, J. Adams and K. C. Anderson, *J Biol Chem*, 2002, **277**, 16639-16647.
11. M. Lolli, S. Narramore, C. W. G. Fishwick and K. Pors, *Drug discovery today*, 2015, **20**, 1018-1026.
12. S. Sainas, A. C. Pippione, M. Giorgis, E. Lupino, P. Goyal, C. Ramondetti, B. Buccinna, M. Piccinini, R. C. Braga, C. H. Andrade, M. Andersson, A. C. Moritzer, R. Friemann, S. Mensa, S. Al-Kadaraghi, D. Boschi and M. L. Lolli, *Eur J Med Chem*, 2017, **129**, 287-302.
13. A. C. Pippione, A. Federico, A. Ducime, S. Sainas, D. Boschi, A. Barge, E. Lupino, M. Piccinini, M. Kubbutat, J. M. Contreras, C. Morice, S. Al-Karadaghi and M. L. Lolli, *MedChemComm*, 2017, **8**, 1850-1855.
14. D. R. Compton, S. Sheng, K. E. Carlson, N. A. Rebacz, I. Y. Lee, B. S. Katzenellenbogen and J. A. Katzenellenbogen, *Journal of Medicinal Chemistry*, 2004, **47**, 5872-5893.
15. H. F. Anwar and M. H. Elnagdi, *Arkivoc*, 2009, 198-250.
16. S. Plescia, G. Daidone and M. L. Bajardi, *Journal of Heterocyclic Chemistry*, 1982, **19**, 685-687.
17. A. Kusakiewicz-Dawid, E. Masiukiewicz, B. Rzeszotarska, I. Dybala, A. E. Koziol and M. A. Broda, *Chem Pharm Bull (Tokyo)*, 2007, **55**, 747-752.
18. D. Clarke, R. W. Mares, H. McNab and F. G. Riddell, *Magnetic Resonance in Chemistry*, 1994, **32**, 255-257.
19. L. T. Pierce, M. M. Cahill and F. O. McCarthy, *Tetrahedron*, 2011, **67**, 4601-4611.
20. C. M. Pask, K. D. Camm, C. A. Kilner and M. A. Halcrow, *Tetrahedron Lett*, 2006, **47**, 2531-2534.
21. R. Deprez-Poulain, N. Cousaert, P. Toto, N. Willand and B. Deprez, *European Journal of Medicinal Chemistry*, 2011, **46**, 3867-3876.
22. W. M. Al-Adiwish, M. I. Tahir, A. Siti-Noor-Adnalizawati, S. F. Hashim, N. Ibrahim and W. A. Yaacob, *Eur J Med Chem*, 2013, **64**, 464-476.
23. t. H. Obtained from Sigma (code: P6624, ,).
24. A. C. Castro, L. C. Dang, F. Soucy, L. Grenier, H. Mazdiyasni, M. Hottelet, L. Parent, C. Pien, V. Palombella and J. Adams, *Bioorg Med Chem Lett*, 2003, **13**, 2419-2422.
25. C. M. Annunziata, R. E. Davis, Y. Demchenko, W. Bellamy, A. Gabrea, F. Zhan, G. Lenz, I. Hanamura, G. Wright, W. Xiao, S. Dave, E. M. Hurt, B. Tan, H. Zhao, O. Stephens, M. Santra, D. R. Williams, L. Dang, B. Barlogie, J. D. Shaughnessy, W. M. Kuehl and L. M. Staudt, *Cancer Cell*, 2007, **12**, 115-130.
26. E. C. Merkhofer, P. Cogswell and A. S. Baldwin, *Oncogene*, 2009, **29**, 1238.
27. N. Yamaguchi, T. Ito, S. Azuma, E. Ito, R. Honma, Y. Yanagisawa, A. Nishikawa, M. Kawamura, J.-i. Imai, S. Watanabe, K. Semba and J.-i. Inoue, *Cancer Science*, 2009, **100**, 1668-1674.

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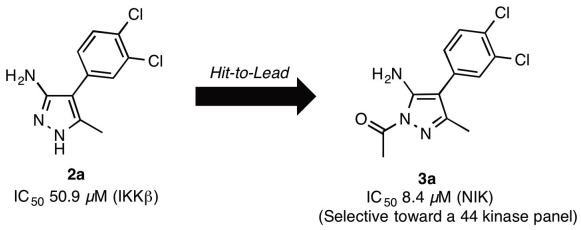
Resulted from a hit-to-lead optimization, the aminopyrazole **3a** is a NIK inhibitor selective over 44 kinases.

Figure (8 cm x 4 cm)



2a
IC_{50} 50.9 μM (IKKβ)

3a
IC_{50} 8.4 μM (NIK)
(Selective toward a 44 kinase panel)