Supporting Information

The stepwise evolution of fragment hits against MAPK Interacting Kinases 1 and 2

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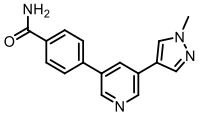
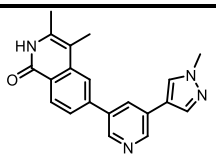
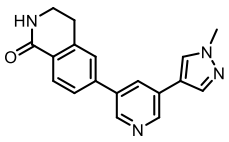
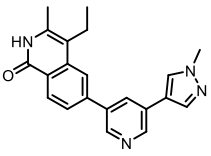
Contents

1. Inhibitory activity of additional compounds

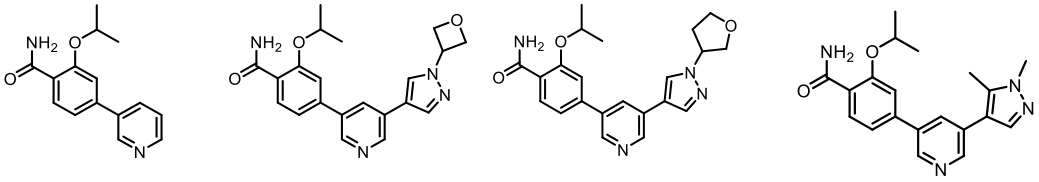
SI Table 1. Biochemical potency of cyclic amide analogues of compound 36.

Cmpd	Structure	IC_{50} [μM] (LE) MNK2	IC_{50} [μM] (LE) MNK1	Cmpd	Structure	IC_{50} [μM] (LE) MNK2	IC_{50} [μM] (LE) MNK1
36		0.35 (0.42)	0.8 (0.40)	**51**		0.23 (0.36)	1.0 (0.33)
50		0.11 (0.41)	0.32 (0.39)	**52**		0.097 (0.37)	0.21 (0.35)

2. *In vitro* and *in vivo* PK compilation and mouse PK for compounds 44 and 48

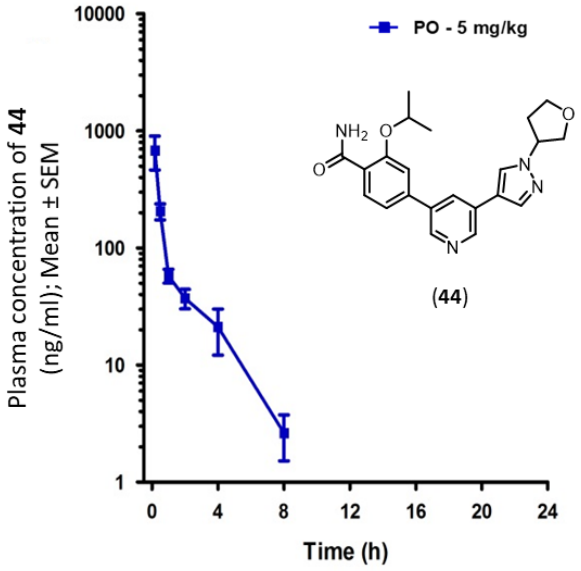
Compounds were shortlisted for *in vivo* PK based on their potency and *in vitro* PK profile; compounds with high clearance in mouse liver microsome assay were not progressed to animal studies. **44** is a close analogue of compound **43**, which in turn is considered the best in the series based on balanced PK and decent potency in biochemical and cellular assay. On the other hand, as shown in **SI Table 2**, clearance of **44** is 10-fold higher reaching 37 $\mu L \cdot min^{-1} \cdot mg^{-1}$. As such it was expected that the *in vivo* exposure would be decreased as compared to **43**. Indeed, significantly lower AUC was observed for **44** ($AUC_{0\text{-}last}$ = 387 $ng \cdot h \cdot ml^{-1}$), while C_{max} for both compounds was similar (**SI Table 2** and **3**). Similarly, compound **48** showed decreased AUC, which corresponded to increased rate of clearance as compared to **43**; however, **48** also had the lowest C_{max} in the series, signifying poorer absorption.

SI Table 2. *In vitro* and *in vivo* PK profiles compared for the 4 key compounds.



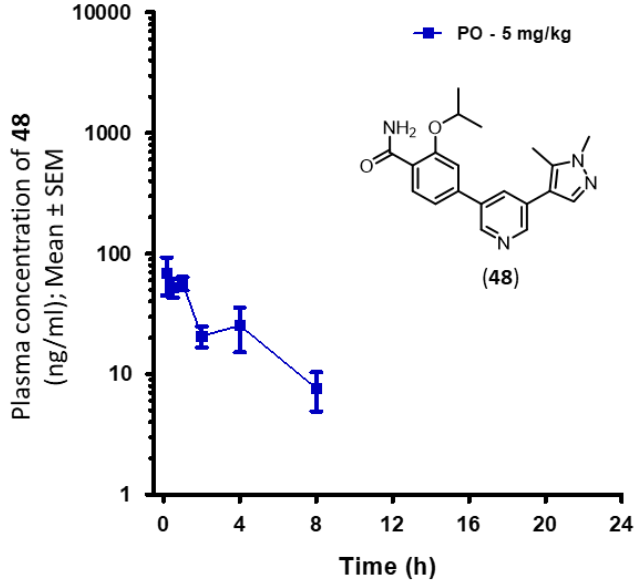
Profile/ cmpd		21	43	44	48
IC_{50} [μM]	MNK2	0.34	0.089	0.044	0.060
	MNK1	0.88	0.20	0.12	0.12
IC_{50} [μM]	eIF4E	0.90	0.2	0.39	2.2
	hERG	--	> 30	--	--
Sol. [7.4] μg/mL		45	28	3.8	7.3
CACO-2 [A-B] / efflux ratio [10^{-6} cm/s]		32 / 0.62	10 / 2.4	14 / 1.5	27 / 0.82
CL MLM/HLM ($\mu L \cdot min^{-1} \cdot mg^{-1}$)		9.4 / 5.8	3.1 / <10	36.8 / 8.2	25 / 7.9
2D6 / 3A4 IC_{50} [μM]		> 30 / >30	>30 / >30	>30 / >30	>30 / >30
C_{max} [ng/mL]		615	658	682	69
$AUC_{0\text{-last}}$ [$ng \cdot h \cdot mL^{-1}$]		494	2079	387	221

SI Table 3. ***In vivo*** **PK of compound 44.** Higher rate of clearance of compound **44**, as compared to **43** translated into lower AUC, despite similar C_{max}.



Parameters	Unit	PO
Dose	mg/kg	5
C_{max}	Ng/ml	682
T_{max}	h	0.17
$T_{1/2}$	h	1.5
CL/F	$L \cdot h^{-1} \cdot kg^{-1}$	12.7
V_z	L/kg	28
$AUC_{0\text{-}t(last)}$	$ng \cdot h \cdot mL^{-1}$	387
$AUC_{0\text{-}inf}$	$ng \cdot h \cdot mL^{-1}$	393

SI Table 4. ***In vivo*** **PK of compound 48.** Decreased C_{max} and AUC were observed for **48** indicating poor absorption.



Parameters	Unit	PO
Dose	mg/kg	5
C_{max}	ng/ml	69
T_{max}	h	0.17
$T_{1/2}$	h	2.7
CL/F	$L \cdot h^{-1} \cdot kg^{-1}$	22
V_z	L/kg^{-1}	88
$AUC_{0\text{-}t(last)}$	$ng \cdot h \cdot mL^{-1}$	221
$AUC_{0\text{-}inf}$	$ng \cdot h \cdot mL^{-1}$	226

3. Molecular Modelling and analysis of MNK isoforms similarity

The X-ray structure of MNK2 complexed with Staurosporine was downloaded from the protein data bank (www.rcsb.org, PDB code 2HW7). The structure was prepared using the protein preparation wizard in Maestro release 2017-3 (www.schrodinger.com) with standard settings. This included the addition of hydrogen atoms, bond assignments, removal of all water molecules, protonation state assignment and optimization of the hydrogen bond network. The missing atoms of residues Ile122 and Gly370 was added using Prime release 2017-3

(www.schrodinger.com). Staurosporine was deleted from the prepared structure and the inhibitors were then manually docked into the ATP-site using Maestro. It was assumed that the amino-pyridine part of our compounds forms the same hydrogen bond with the kinase hinge residue Met162 as the acceptor in the adenine of ATP and that the phenyl interacts with the sidechain of the gatekeeper residue Phe159. The crystal water hydrogen bonding to residues Glu129 and Asp226 was kept as the inhibitor amine may interact with the water. Residues beyond 9 Å of the inhibitor was constrained and the complex was then subjected to 500 steps of TNCG minimization using the OPLS3 force field and GB/SA solvation model implemented in MacroModel release 2017-3 (www.schrodinger.com). A map of the binding site was calculated for the minimized complex with compound **13** using SiteMap release 2017-3 (www.schrodinger.com).

MNK1 & MNK2 alignment

In contrast to the majority of kinases that have a DFG-motif, both MNK kinases have a DFD-motif. Analysis of the published MNK X-ray structures revealed that MNK1 is crystalized in the DFD-out conformation only, while MNK2 is crystalized in the DFD-in conformation as well as a state in-between in and out. Apart from the conformational difference that may be a crystalization artefact, the binding-sites are very similar. There are no obvious residue differences in the binding-sites that may be targeted for achieving MNK subtype selectivity. The homology between MNK1 and MNK2 is 84% and the sequence identity is 73% (**SI Figure 1**).

SI Figure 1. Comparison of MNK1 and MNK2 structures; a) sequences of MNK1 and MNK2 with crystallized residues in bold font; b) alignment of MNK1 and MNK2 with colour-coded secondary structures and binding sites.

a)

Full Protein Sequences from Uniprot
Crystalized residues in black

>Q9BUB5|MNK1
MVSSQKLEKPIEMGSSEPLPIADGDRRRKKKRRGRATDSLPGKFEDMYKLTSELLGEGAY
AKVQGAVSLQNGKEYAVKIIEKQAGHSRSRVFREVETLYQCQGNKNILELIEFFEDDTRF
YLVFEKLQGGSILAHIQKQKHFNEREASRVVRDVAAALDFLHTKDKVSLCHLGWSAMAPS
GLTAAPTSLGSSDPPTSASQVAGTTGIAHRDLKPENILCESPEKVSPVKICDFDLGSGMK
LNNSCTPITTPELTTPCGSAEYMAPEVVEVFTDQATFYDKRCDLWSLGVVLYIMLSGYPP
FVGHCGADCGWDRGEVCRVCQNKLFESIQEGKYEFPDKDWAHISSEAKDLISKLLVRDAK
QRLSAAQVLQHPWVQGQAPEKGLPTPQVLQRNSSTMDLTLFAAEAIALNRQLSQHEENEL
AEEPEALADGLCSMKLSPPCKSRLARRRALAQAGRGEDRSPPTAL

>Q9HBH9|MNK2
MVQKKPAELQGFHRSFKGQNPFELAFSLDQPDHGDSDFGLQCSARPDMPASQPIDIPDAK
KRGKKKKRGRATDSFSGRFEDVYQLQEDVLGEGAHARVQTCINLITSQEYAVKIIEKQPG
HIRSRVFREVEMLYQCQGHRNVLELIEFFEEEDRFYLVFEKMRGGSILSHIHKRRHFNEL
EASVVVQDVASALDFLHNKGIAHRDLKPENILCEHPNQVSPVKICDFDLGSGIKLNGDCS
PISTPELLTPCGSAEYMAPEVVEAFSEEASIYDKRCDLWSLGVILYILLSGYPPFVGRCG
SDCGWDRGEACPACQNMLFESIQEGKYEFPDKDWAHISCAAKDLISKLLVRDAKQRLSAA
QVLQHPWVQGCAPENTLPTPMVLQRNSCAKDLTSFAAEAIAMNRQLAQHDEDLAEEEAAG
QGQPVLVRATSRCLQLSPPSQSKLAQRRQRASLSSAPVVLVGDHA

b)

CLUSTAL W (1.83) multiple sequence alignment of Mnk1 & Mnk2

Structural information from X-ray structures
Mnk1: 2HW6
Mnk2: 2AC3, 2AC5, 2HW7
Crystalized residues in black

Binding Site
Binding Site & Beta Strand
Binding Site & Alpha Helix
Alpha Helix
Beta Strand

```
Q9BUB5|MNK1     1 ------------------------------------MVSSQKLEKPIEMGSSEPLPIADGD  25
Q9HBH9|MNK2     1 MVQKKPAELQGFHRSFKGQNPFELAFSLDQPDHGDSDFGLQCSARPDMPASQPIDIPDAK  60
                  ____________________________________._:_.___:*_:*:*:_*.*..

Q9BUB5|MNK1    26 RRRKKKRRGRATDSLPGKFEDMYKLTSELLGEGAYAKVQGAVSLQNGKEYAVKIIEKQAG  85
Q9HBH9|MNK2    61 KRGKKKKRGRATDSFSGRFEDVYQLQEDVLGEGAHARVQTCINLITSQEYAVKIIEKQPG 120
                  :*_***:*******:.*:***:*:*_.::*****:*:**_.:.*_..:**********.*

Q9BUB5|MNK1    86 HSRSRVFREVETLYQCQGNKNILELIEFFEDDTRFYLVFEKLQGGSILAHIQKQKHFNER 145
Q9HBH9|MNK2   121 HIRSRVFREVEMLYQCQGHRNVLELIEFFEEDDRFYLVFEKMRGGSILSHIHKRRHFNEL 180
                  *_*********_******::*:********::_********::*****:**:*::****_

Q9BUB5|MNK1   146 EASRVVRDVAAALDFLHTKDKVSLCHLGWSAMAPSGLTAAPTSLGSSDPPTSASQVAGTT 205
Q9HBH9|MNK2   181 EASVVVQDVASALDFLHN----------------------------------------K 199
                  ***_**:***:******._____________________________________.

Q9BUB5|MNK1   206 GIAHRDLKPENILCESPEKVSPVKICDFDLGSGMKLNNSCTPITTPELTTPCGSAEYMAP 265
Q9HBH9|MNK2   200 GIAHRDLKPENILCEHPNQVSPVKICDFDLGSGIKLNGDCSPISTPELLTPCGSAEYMAP 259
                  ***************_*::**************:***..*:**:****_**********

Q9BUB5|MNK1   266 EVVEVFTDQATFYDKRCDLWSLGVVLYIMLSGYPPFVGHCGADCGWDRGEVCRVCQNKLF 325
Q9HBH9|MNK2   260 EVVEAFSEEASIYDKRCDLWSLGVILYILLSGYPPFVGRCGSDCGWDRGEACPACQNMLF 319
                  ****.*:::*::************:***:*********:**:********.*_.***_**

Q9BUB5|MNK1   326 ESIQEGKYEFPDKDWAHISSEAKDLISKLLVRDAKQRLSAAQVLQHPWVQGQAPEKGLPT 385
Q9HBH9|MNK2   320 ESIQEGKYEFPDKDWAHISCAAKDLISKLLVRDAKQRLSAAQVLQHPWVQGCAPENTLPT 379
                  ******************._*******************************_***:_***

Q9BUB5|MNK1   386 PQVLQRNSSTMDLTLFAAEAIALNRQLSQHEENELAEEPEALADGLCS------MKLSPP 439
Q9HBH9|MNK2   380 PMVLQRNSCAKDLTSFAAEAIAMNRQLAQHDEDLAEEEAAGQGQPVLVRATSRCLQLSPP 439
                  *_******.:_***_*******:****:**:*:___**._._.:_:_________::****

Q9BUB5|MNK1   440 CKSRLARRRALAQAGRGEDRSPPTAL                                  465
Q9HBH9|MNK2   440 SQSKLAQRRQRASLSSAPVVLVGDHA                                  465
                  .:*:**:**__*._._._________
```

4. X-ray crystal structure details

Protein expression and purification:

MNK2 kinase domain (amino acids 72-385)[1] was cloned into a pGEX-6P-1 vector with a modified thrombin protease site. The GST-thrombin site-Mnk2 kinase construct was transformed into BL21 Star (DE3) *E.Coli* bacterial strain and grown in LB media. The cells were grown to an OD_{600}= 0.7 and induced with 1 mM IPTG and harvested after 6 hours at 25°C.

The cells were lysed using buffer containing 10 mM Na_2HPO_4 pH 7.4, 150 mM NaCl, 1 tablet of protease inhibitor, DNAse (6.25mg/ml), lysozyme (1.25mg/ml), 5 mM β-mercaptoethanol and centrifuged. The GST tagged MNK2 kinase protein was purified from the supernatant using affinity chromatography on a Bio-Scale Mini Profinity GST cartridge using the Bio-Rad Profinia system. The GST tagged protein was incubated with thrombin in a 1:300 ratio (wt:wt, Thrombin:fusion protein) overnight at room temperature. One more GST affinity chromatography was performed to separate the cleaved tags from the protein. The protein was then purified by ion exchange chromatography HiTrapQ HP column using binding buffer (10 mM Tris pH 7.5, 50mM NaCl, 1mM DTT) and elution buffer (10 mM Tris pH 7.5, 1M NaCl, 1mM DTT). Pure fractions of MNK2 protein was concentrated to 12mg/ml and stored at -80°C.

Crystallization and structure solution:

Pure wild type MNK2 protein was crystallized using hanging drop vapor diffusion technique. 1μl of the protein was mixed with 1 μl of the crystallization solution containing 23% polyacrylic acid 5100, 2% 2-methyl-2,4-pentane diol, 100mM HEPES pH 7.7 at 20°C. The apo-crystal obtained was soaked for 30 minutes in a reservoir solution containing 1 mM of the ligand. The ligand soaked crystals were flash frozen in liquid nitrogen after cryo protecting them with 30% glycerol.
The dataset was collected using Rigaku MicroMaxTM 007 HF X-ray diffractometer. The dataset was indexed, integrated and scaled using D*Trek software.[2] The structure of MNK2

[1] Jauch, R.; Jäkel, S.; Netter, C.; Schreiter, K.; Aicher, B.; Jäckle, H.; Wahl, M. C. Crystal Structures of the Mnk2 Kinase Domain Reveal an Inhibitory Conformation and a Zinc Binding Site. *Structure* **2005**, *13*, 1559–1568.

[2] Pflugrath J.W. The finer things in X-ray diffraction data collection. Acta Crystallogr. D Biol. Crystallogr. **1999**, 55, 1718-1725.

(PDB ID: 2AC3) was used as the search model for molecular replacement using PHENIX suite of programs.[3] Refinement of the structure was done using Phenix.refine.[4,5]

SI Table 5. Data collection and refinement statistics*

Resolution range	28.28 - 2.90 (3.00 - 2.90)
Space group	P 32 2 1
Unit cell	105.11 105.11 72.19 90 90 120
Unique reflections	10359 (1002)
Multiplicity	2.0
Completeness (%)	98.52 (99.21)
Mean I/sigma(I)	6.5 (1.5)
Wilson B-factor	86.63
R-meas	0.1 (0.785)
Reflections used in refinement	10333 (1004)
Reflections used for R-free	483 (28)
R-work	0.29 (0.37)
R-free	0.35 (0.50)
Number of non-hydrogen atoms	2136
Macromolecules	2115
Ligands	21
Protein residues	268
RMS(bonds)	0.007

[3] McCoy A.J.; Grosse-Kunstleve, R.W.; Adams, P.D.; Winn, M. D.; Storoni, L. C.; Read, R. J. Phaser Crystallographic Software. *J. Appl. Cryst*. **2007**, *40*, 658–674.

[4] Adams, P. D.; Gopal, K.; Grosse-Kunstleve, R. W.; Hung, L. W.; Ioerger, T. R.; McCoy, A. J.; Moriarty, N. W.; Pai, R. K.; Read, R. J.; Romo, T. D.; Sacchettini, J. C.; Sauter, N. K.; Storoni, L. C.; Terwilliger, T. C. Recent Developments in the PHENIX Software for Automated Crystallographic Structure Determination. *J. Synchrotron Radiat.* **2004,** *11*, 53-55.

[5] Adams, P. D.; Afonine, P. V.; Bunkóczi, G.; Chen, V. B.; Davis, I. W.; Echols, N.; Headd, J. J.; Hung, L.W.; Kapral, G. J.; Grosse-Kunstleve, R. W.; McCoy, A. J.; Moriarty, N. W.; Oeffner, R.; Read, R. J.; Richardson, D. C.; Richardson, J. S.; Terwilliger, T. C.; Zwart, P. H. PHENIX: a Comprehensive Python-based System for Macromolecular Structure Solution. *Acta Crystallogr. D Biol. Crystallogr.* **2010**, *66*, 213-221.

RMS(angles)	1.17
Ramachandran favoured (%)	96.18
Ramachandran allowed (%)	3.82
Ramachandran outliers (%)	0.00
Rotamer outliers (%)	0.00
Clash score	15.15
Average B-factor	86.40
Macromolecules	86.45
Ligands	81.67

* Statistics for the highest-resolution shell are shown in parentheses.

5. Chemistry

General information

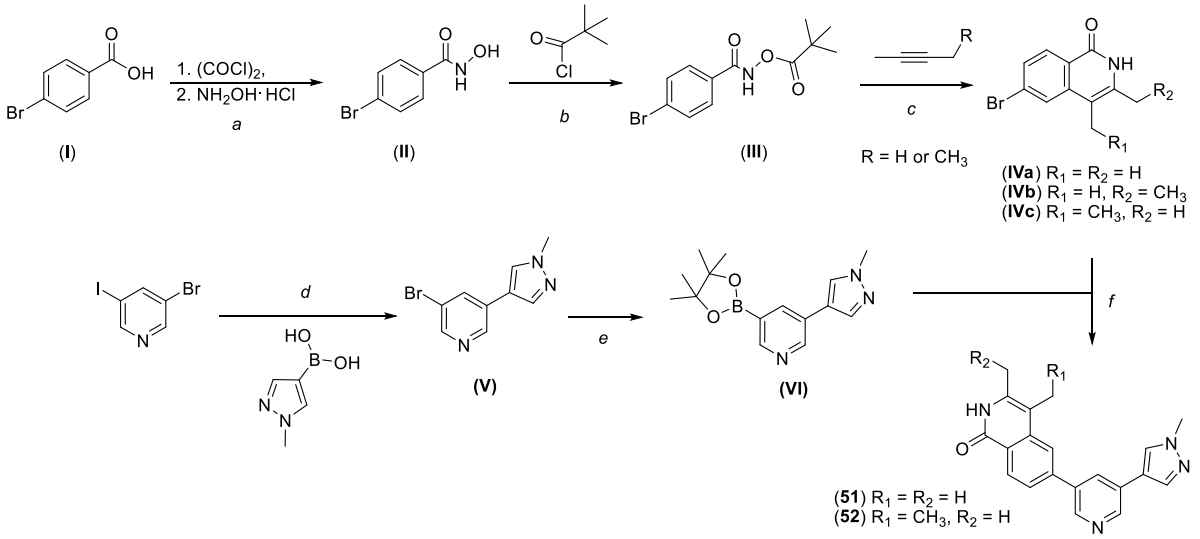
All solvents and reagents were purchased from commercial source and used without further purification. ^{1}H NMR spectra were obtained using a Bruker Ultrashield 400 PLUS/R system, operating at 400 MHz. ^{13}C NMR spectra were acquired on Bruker Cryoprobe 400 MHz. The compounds' purities were ≥ 95% determined according to method A or B. Method A: VARIAN ProStar HPLC instrument using Acetonitrile/water (with 0.1% formic acid) as eluent, Phenomenex, Luna 5u, C18A (2) 100A, 150 x 4.60mm reverse phase column and mobile phase A: 0.1% Formic acid in water and mobile phase B: 0.1% Formic acid in Acetonitrile; run time: 10.5 mins; T/%B: 0.0/3, 3.0/3, 7.50/50, 8.0/95, 10/95, 10.5/3; flow rate: 2.0 mL/min; wavelength: 254 nm. Method B: Agilent UPLC using Acetonitrile/water (with 0.1% formic acid) as eluent; column: Phenomenex, Luna 5u, C18A (2) 100A, 50 x 4.60mm, reverse phase column; mobile phase A: 0.1% Formic acid in water; mobile phase B: 0.1% Formic acid in Acetonitrile; run time: 2.0 min; T/%B: 0.0/5, 1.8/95, 2.0/5; flow rate: 2.0 mL/min; wavelength: 254 nm. HRMS spectra were acquired on Thermo Scientific Orbitrap Fusion mass spectrometer coupled with Advion TriVersa NanoMate (ESI); mass spectrometer setting: detector Orbitrap (high resolution), resolution 60000, mass accuracy <3 ppm.

Preparation of compounds 51 – 52

Compounds **51** – **52** were prepared as shown on **SI Scheme 1**. Commercially available 4-Bromobenzoic acid was converted into N-hydroxybenzamide **II** which was then esterified with

2,2-Dimethylpropionic acid chloride leading to **III**. Condensation of **III** with 2 butyne or 2-pentyne furnished the key intermediates **IVa-c**. Notably, the mixture of regioisomers **IVb** & **IVc** was difficult to resolve and was carried forward to the next step. The following Suzuki coupling lead to the final compounds **51** and **52**. Compound **52** was separated from regioisomer obtained from intermediate **IVb** – which in turn was not isolated.

SI Scheme 1. Synthesis of compounds 51-52.



[a] Reagents and conditions: (a) 1. Oxalyl chloride (2 equiv.), CH_2Cl_2, rt , 1.5 h; 2. NH_2OH*HCl (4 equiv.), NaOH (6 equiv.), THF / H_2O (1/1), 3 h, rt; (b) Pivaloyl chloride (1.1 equiv.), TEA (5.0 eq), THF, rt, 2 h; (c) 2-Butyne (5 equiv.), carbonyl(η-5-cyclopentadienyl)diiodocobalt (III) (0.1 equiv.), Sodium acetate (1.0 eq), CF_3CH_2OH, rt, 2h; (d) N-methyl-pyrazole-4-boronic acid (1.1 equiv.), Cs_2CO_3 (3 equiv.), $Pd(PPh_3)_4$ (0.05 equiv.), 1, 4-dioxane, H_2O, 100^0C, 4 h; (e) Pinacolatodiboron (1.5 equiv.), NaOAc (3 equiv.), Pd_2Cl_2(dppf).DCM (0.05 equiv.), 1, 4-dioxane, 140 ^{0}C, 2 h, μw; (f) **VI** (2 equiv.), Cs_2CO_3 (2 equiv.), H_2O, 1,4-Dioxane, $Pd(PPh_3)_2$ (0.1 equiv.), 16 h, 80^0C.

Representative procedures – synthesis of compound 51.

Step-1: Preparation of 4-bromo-N-hydroxybenzamide

4-Bromobenzoic acid (2.6 g, 1 equiv.) was treated with Oxalyl chloride (2.22mL, 26 mmol, 2 equiv.) in CH_2Cl_2 (10 ml) at room temperature for 1.5 h. Volatiles were evaporated, residue diluted with 1:1 mixture of THF and water and Hydroxyloamine hydrochloride (3.34 g, 52.3 mmol, 4 equiv.) was added, followed by NaOH (3.12 g, 78.4 mmol, 6 equiv.) and the reaction mixture was stirred for 3 h at room temperature. The reaction mixture was then cooled to room temperature, poured onto water (20 mL) and extracted with Ethyl Acetate (3 x 50 mL). The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by flash chromatography on silica gel eluting with a gradient of Ethyl Acetate in Hexane to yield intermediate **II** (2.3 g). (LCMS: 99%)

Step-2: Preparation of 4-bromo-N-(pivaloyloxy)benzamide

Intermediate **II** (500 mg, 2.3 mmol, 1.0 equiv.) was treated with Pivaloyl chloride (0.3 mL, 2.6 mmol, 1.1 equiv.), TEA (1.6 mL, 11.6 mmol, 5 equiv.) in THF (10 mL) at room temperature

for 2 h. The reaction mixture was poured onto water (10 mL) and extracted using Ethyl Acetate (2 x 20mL). The organic layer was separated, washed with brine, dried over Na_2SO_4 and concentrated in vacuo to obtain crude compound **III** (550mg). (LCMS: 66%)

Step-3: Preparation of 6-bromo-3,4-dimethylisoquinolin-1(2H)-one

Solution of intermediate **III** (550 mg, 1.8 mmol, 1 equiv.) in CF_3CH_2OH (10 mL) was treated with 2-Butyne (109 mg, 89.2 mmol, 5 equiv.), carbonyl(η-5-cyclopentadienyl)diiodocobalt (III) (113 mg, 0.18 mmol, 0.1 equiv.) and Sodium acetate (150 mg, 1.8 mmol, 1.0 equiv.) at room temperature. The reaction was stirred for 2 h at room temperature and monitored by TLC. Upon completion, the reaction mixture was cooled and poured onto water (5 mL) and extracted with Ethyl Acetate (2 x 10mL). Organic layer was separated and washed with brine (5 mL), dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified *via* flash chromatography on silica gel eluting with a gradient of Ethyl Acetate in Hexane to yield intermediate **IV a** (270 mg; LCMS: 84%)

Step-4: Preparation of 3-bromo-5-(1-methyl-1H-pyrazol-4-yl)pyridine

To the solution of 3-Bromo-5-iodopyridine (1.2 g, 1 equiv.) in 1, 4-dioxane (20 mL) and water (5 mL) was added Cs_2CO_3 (3.4 g, 23 equiv.), N-methyl-pyrazole-4-boronic acid (0.488 g, 1.1 equiv.) and the mixture was degassed with Nitrogen gas for 5 mins. Then $Pd(PPh_3)_4$ (0.244 g, 0.05 equiv.) was added and the reaction mixture was heated to 100^0C for 4 h. On completion, the cooled reaction mixture and was poured onto water (50 mL) and extracted with Ethyl acetate (30 mL x 3). Organic layer was washed with brine, dried over Na_2SO_4 and evaporated in vacuo. The crude product was purified by chromatography on silica gel (60-120 mesh) eluting with 10% Ethyl Acetate in Hexane as eluent to yield intermediate **V** as a yellow semi-solid (0.950 g; LCMS: 71 %; M/z=238).

Step-5: Preparation of 3-(1-methyl-1H-pyrazol-4-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine

To the solution of intermediate **V** (1.0 g, 1.0 equiv.) in 1, 4-dioxane (20 mL) was added NaOAc (1.04 g, 3.0 equiv.), Pinacolatodiboron (1.60 g, 1.5 equiv.) and the mixture was degassed with Nitrogen gas for 5 mins. Then Pd_2Cl_2(dppf).DCM (0.172 g, 0.049 equiv.) was added and the reaction mixture was heated at 140^0C for 2 h in microwave reactor. The reaction mixture was then cooled, poured onto water (50 mL) and extracted with Ethyl Acetate (30 mL x 3). The organic layer was dried over Na_2SO_4, evaporated in vacuo to get intermediate **VI** as a brown solid (0.80 g).

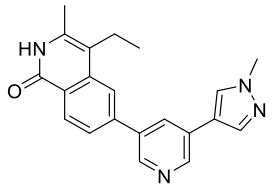
Step-6: Preparation of 3,4-dimethyl-6-(5-(1-methyl-1H-pyrazol-4-yl)pyridin-3-yl)isoquinolin-1(2H)-one

To the solution of intermediate **IVa** (50 mg, 0.20 mmol, 1.0 equiv.), intermediate **VI** (120 mg, 0.40 mmol, 2.0 equiv.) and Cs_2CO_3 (128 mg, 0.4 mmol, 2.0 equiv.) in water (0.7 mL) was added solution of $Pd(PPh_3)_2$ (12 mg, 0.01mmol, 0.1 equiv.) in dioxane (2.8 mL) and the mixture

was degassed by bubbling Nitrogen through for 5 minutes. Reaction vial was then capped and the mixture stirred for 16 h at 80 ^{0}C. The reaction mixture was then cooled to room temperature, poured onto water (10 mL) and extracted with ethyl acetate (2x20mL). The organic layer was separated and washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by flash chromatography on silica gel eluting with 50 % Ethyl Acetate in Hexane to yield compound **51** (5 mg) 3,4-dimethyl-7-[5-(1-methyl-1H-pyrazol-4-yl)pyridin-3-yl]-1,2,3,4-tetrahydroisoquinolin-1-one.

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): δ 11.23 (s, 1H), 8.91 (s, 1H), 8.85 (s, 1H), 8.40 (d, *J* = 16.8 Hz, 2H), 8.31 (d, *J* = 8.2 Hz, 1H), 8.14 (s, 1H), 7.99 (s, 1H), 7.85 (d, *J* = 8.4 Hz, 1H), 3.92 (s, 3H), 3.52 (s, 1H), 2.30 (s, 6H), 1.24 (s, 1H); LCMS (ESI) m/z calcd for $C_{20}H_{20}N_4O_2$ $[M+H]^+$ = 330.4, found 330.3; off-white solid; purity 96 %.

4-Ethyl-3-methyl-7-[5-(1-methyl-1H-pyrazol-4-yl)pyridin-3-yl]-1,2,3,4-etrahydroisoquinolin-1-one (**52**)

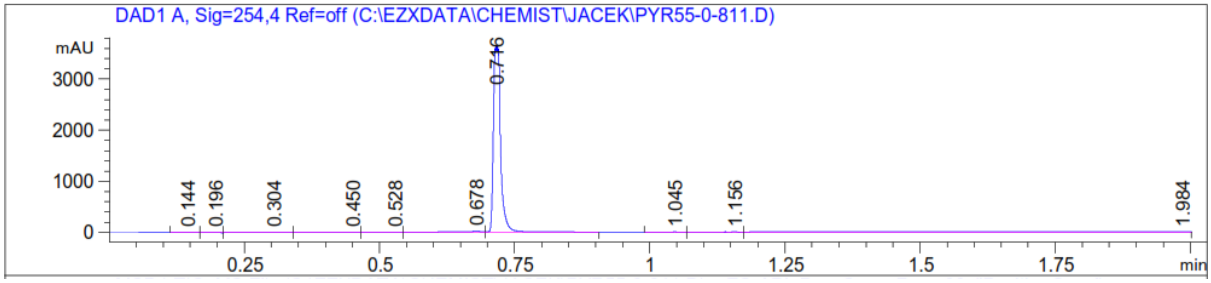


The title compound was obtained analogically to cmpd **51** using 2-pentyne instead on 2-butyne in step 3. The regioisomer of **52** was separated through flash chromatography on silica gel (not recovered).

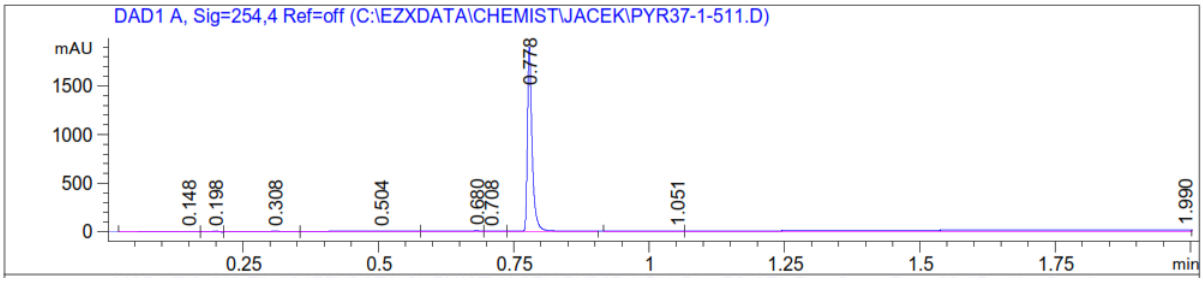
^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.25 (s, 1H), 8.91 (d, *J* = 2.0 Hz, 1H), 8.83 (d, *J* = 2.2 Hz, 1H), 8.42 (s, 1H), 8.38 – 8.29 (m, 2H), 8.14 (s, 1H), 8.00 (d, *J* = 1.6 Hz, 1H), 7.84 (dd, *J* = 8.3, 1.6 Hz, 1H), 3.93 (s, 3H), 2.83 (d, *J* = 7.5 Hz, 1H), 2.30 (s, 3H), 1.15 (t, *J* = 7.4 Hz, 3H); LCMS (ESI) m/z calcd for $C_{21}H_{21}N_4O$ $[M+H]^+$ = 345.42, found 345.2; off-white solid; purity 96 %.

6. HPLC spectrograms of advanced compounds

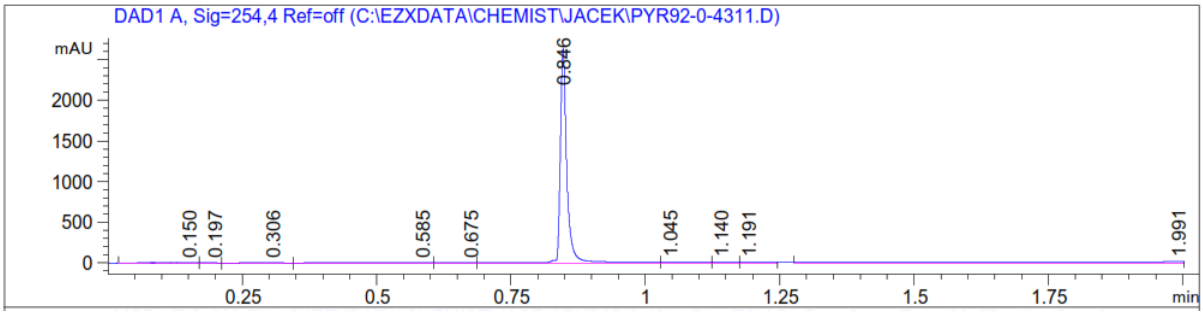
Compound 37



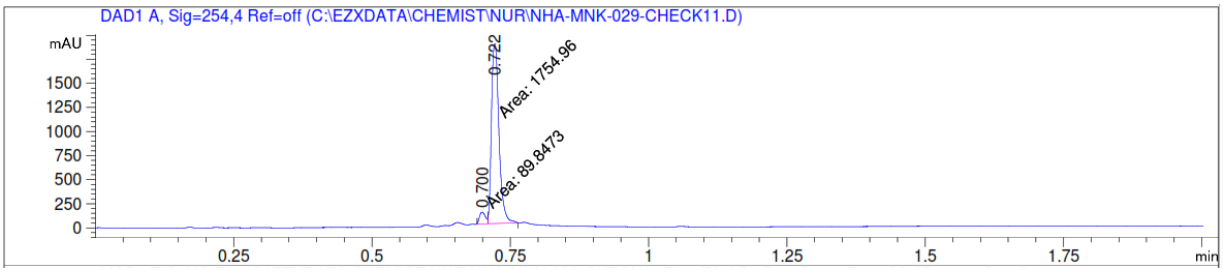
Compound 38

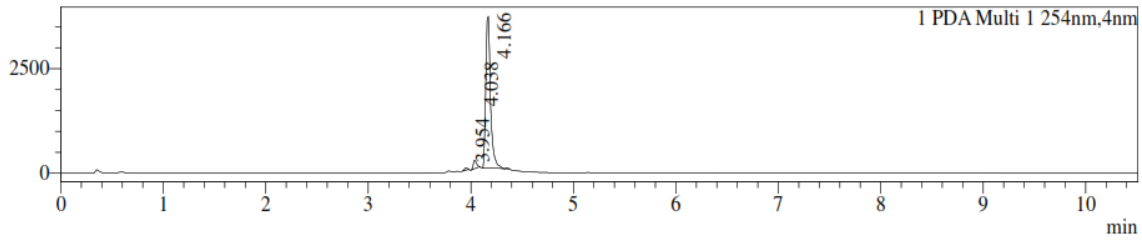


Compound 42



Compound 43



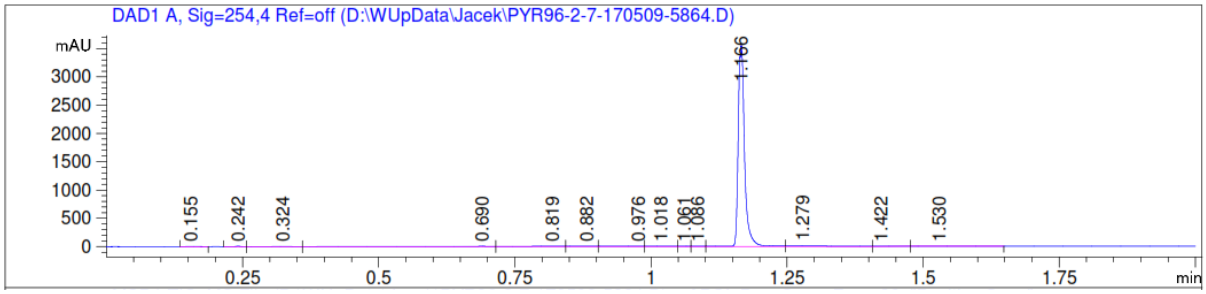


Peak Table

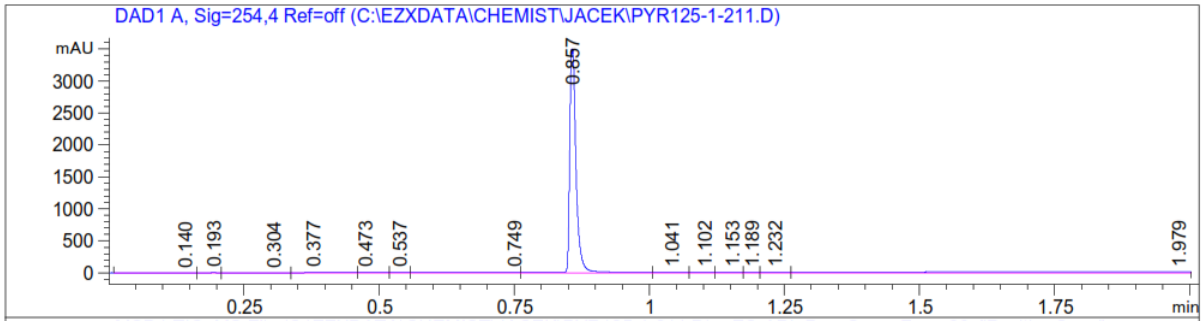
PDA Ch1 254nm

Index#	Name	Ret. Time	Area%	Peak Start	Peak End
1		3.954	0.94	3.925	3.979
2		4.038	3.82	4.011	4.085
3		4.166	95.24	4.117	4.373
Total			100.00		

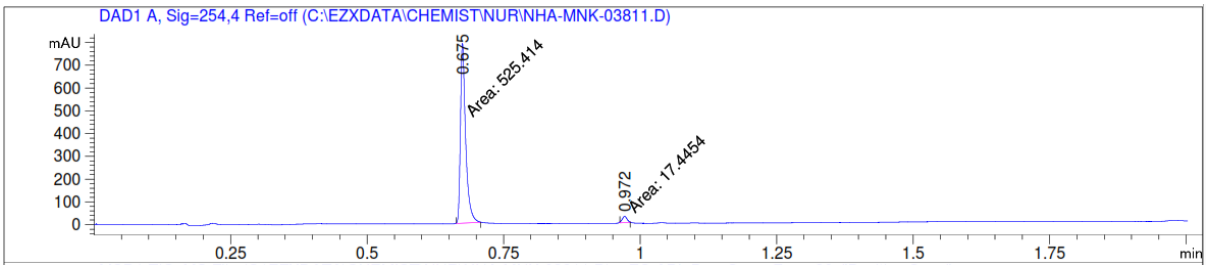
Compound 44



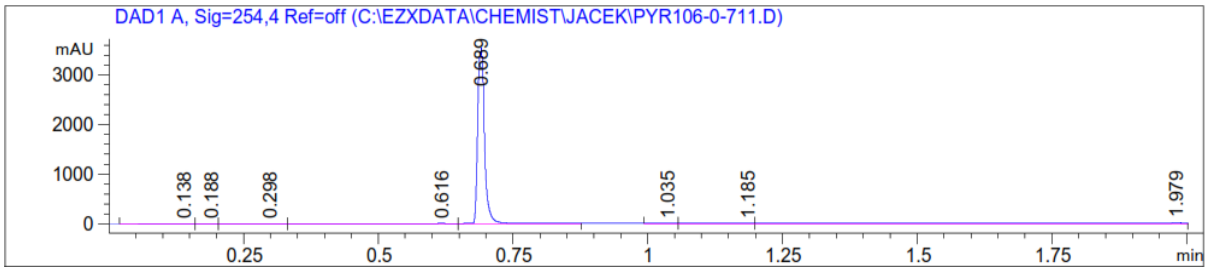
Compound 45



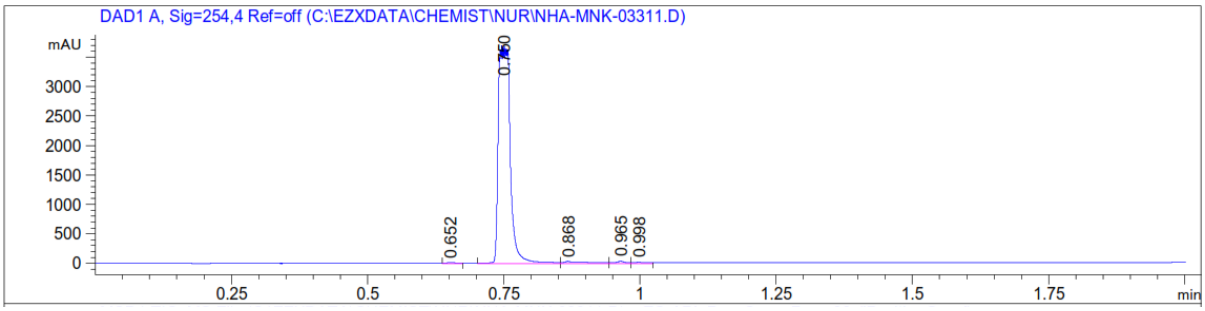
Compound 46



Compound 47



Compound 48



Compound 49