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

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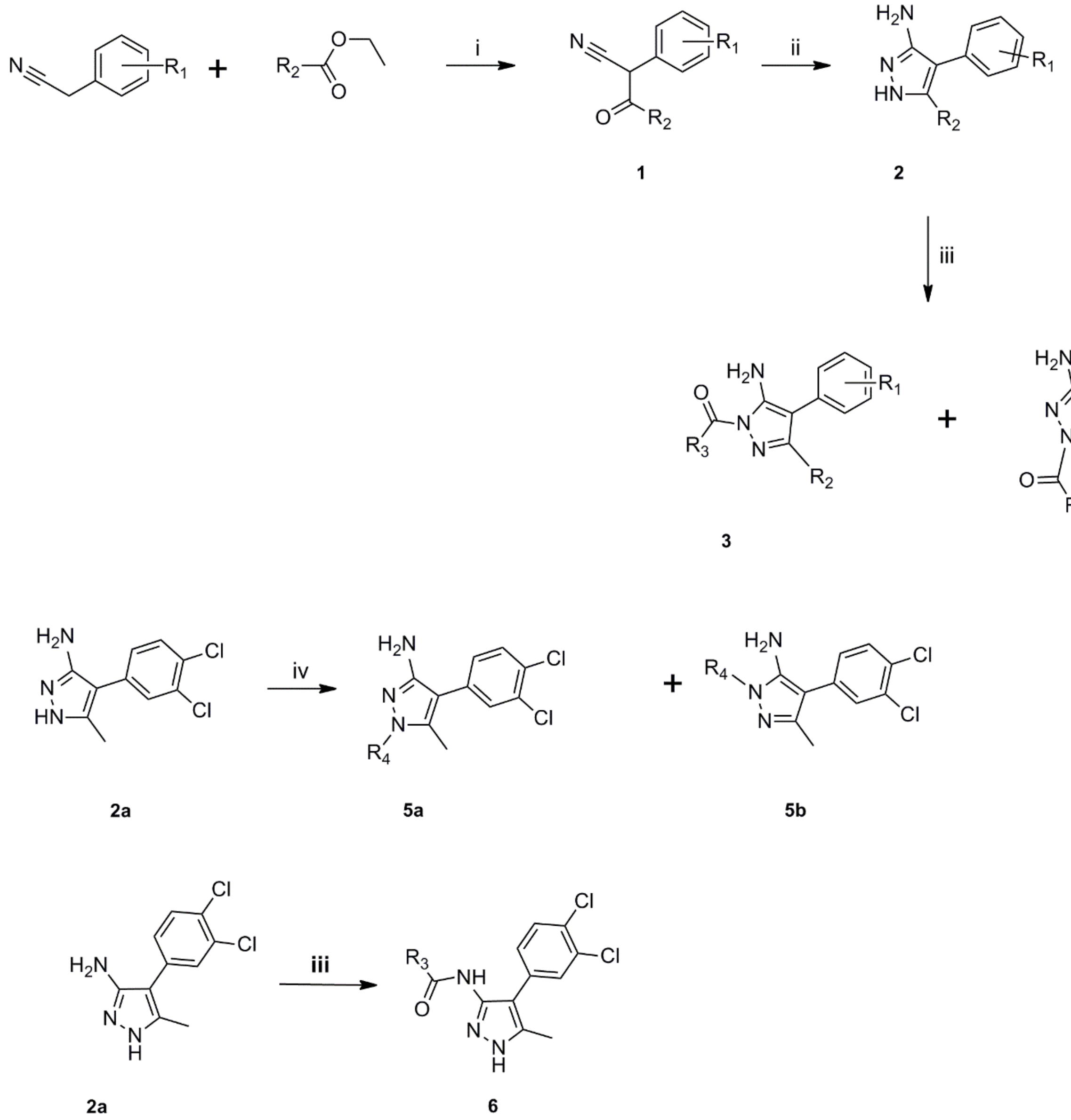
1. *Chemistry*

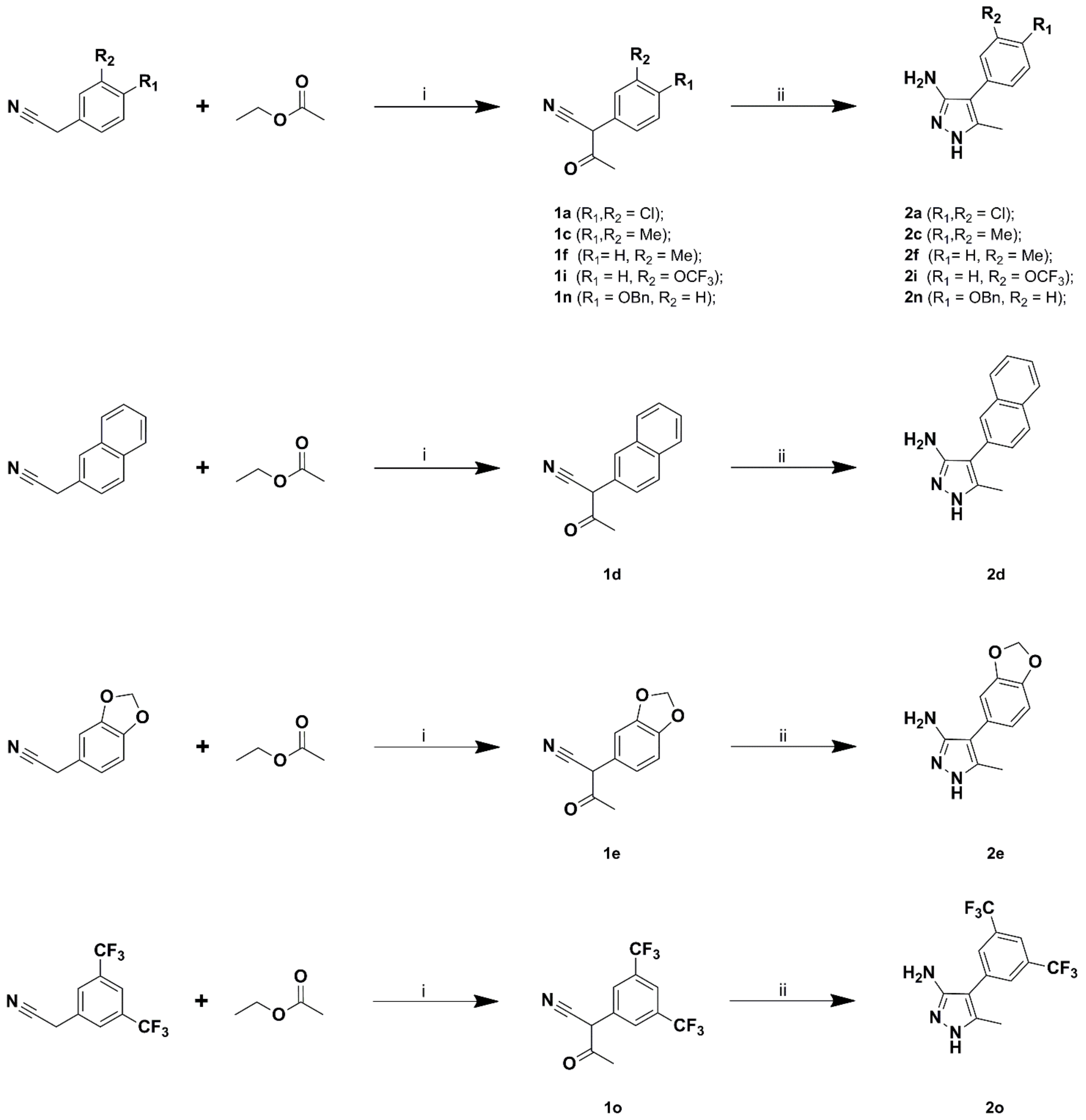
1.1 General methods

All reagents were used as purchased from commercial suppliers without further purification. Analytical grade solvents (acetonitrile, diisopropyl ether, diethyl ether, dichloromethane [DCM], ethyl acetate [EtOAc], hexane, methanol [MeOH], petroleum ether b.p. 40 - 60°C [*petroleum ether*], toluene) were used without further purification. When needed, solvents were dried on 4 Å molecular sieves. Tetrahydrofuran (THF) was distilled immediately prior to use from Na and benzophenone under N_2. Thin layer chromatography (TLC) on silica gel was carried out on 5 x 20 cm plates with 0.25 mm layer thickness to monitor the process of reactions. Anhydrous Na_2SO_4 was used as a drying agent for the organic phases. Purification of compounds was achieved with flash column chromatography on silica gel (Merck Kieselgel 60, 230-400 mesh ASTM) using the eluents indicated. Final compounds were assayed in biological experiments. Their purity was measured by HPLC analyses, showing a chromatogram where the main peak (area at least 95% of all detected peaks) was attributable to the final compound. Some HPLC analyses was performed on a UHPLC chromatographic system (Perkin Elmer, Flexar). The analytical column was an UHPLC Acquity CSH Fluoro-Phenyl (2.1 - 100 mm, 1.7 mm particle size) (Waters). Compounds were dissolved in acetonitrile or methanol, and injected through a 20 ml loop. The mobile phase consisted of acetonitrile/water with 0.1% trifluoroacetic acid; two gradient profiles of mobile phase were used to assay the purity of each compound. UHPLC analysis were run at flow rates of 0.5 mL/min, and the column effluent was monitored at 215 and 254 nm, referenced against a 360 nm wavelength. Other HPLC analyses were performed on a HPLC Agilent 1200 Serie. The analytical column was a Nucleodur C8 ec column, 4.6 x 100 mm from Macherey-Nagel. The mobile phase consisted in an appropriate gradient of water and acetonitrile with 0.1 % formic acid.

All compounds were routinely checked by ^{1}H- and ^{13}C-NMR and mass spectrometry. ^{1}H- and ^{13}C-NMR spectra were performed on a Bruker Avance 300 instrument or Brüker 300 MHz Ultra shield spectrometer. For coupling patterns, the following abbreviations are used: br = broad, s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet. MS spectra were performed on Finnigan-Mat TSQ-700 (70 eV, direct inlet for chemical ionization [CI]) or on Agilent Ion Trap 6340. All chemical yields are unoptimized and generally represent the result of a single experiment. Compounds **2b**, **2d**, **2e**, **2g**, **2h**, **2l** and **2m** are commercial available.



Scheme S1. General Scheme of synthesis of target compounds. Conditions: i) dry THF, NaH, reflux; ii) $NH_2NH_2 \cdot 2HCl$, 3Å sieves, dry EtOH, reflux; iii) R_3COCl, dry pyridine, dry THF, rt, or: appropriate anhydride, dry THF, rt. iv) Cs_2CO_3, CH_3I, dry THF.



Scheme S2: synthesis of compounds **2a - o**: i) dry THF, NaH, reflux; ii) $NH_2NH_2 \cdot 2HCl$, 3Å sieves, dry EtOH, reflux.

*1.2. General method for the preparation of 3-oxo-2-phenylbutanenitrile derivatives **1a – o***

NaH (60% dispersion in oil, 1.0 mmol) was added to a solution of the appropriate phenylacetonitrile (0.5 mmol) in dry THF (5 mL) in 3 portions over 5 minutes. dry EtOAc (10 mmol) was then added to the resulting suspension and the mixture was heated at 60°C overnight. After cooling to room temperature, water was added (10 mL) and the reaction mixture was concentrated to half of its volume under reduced pressure. The mixture was diluted with additional water, adjusted to pH = 5 - 6 with 3N acqueous HCl and extracted with dichloromethane (3 x 15 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford a crude residue that was purified by column chromatography.

*1.3. General method for the preparation of 3-aminopyrazoles **2a** - **o**.*

The appropriate 3-oxo-2-phenylbutanenitrile **1a** - **o** (3.0 mmol) was dissolved in 20 mL of absolute EtOH, and $NH_2NH_2{\cdot}2HCl$ (6.0 mmol) was added. The mixture was heated at reflux overnight on 3Å sieves. After cooling to room temperature, the reaction mixture was concentrated to half of its volume under reduced pressure. The mixture was diluted with additional water, adjusted to pH = 8 with saturated $NaHCO_3$ and extracted with EtOAc (3 x 15 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the desired compound.

*1.3.1. 4-(3,4-dichlorophenyl)-5-methyl-1H-pyrazol-3-amine. **(2a)*** This compound is known and commercially available; however, no analytical data were reported. Purified by flash chromatography (eluent dichloromethane / MeOH 95/5 v/v). Yield 73%, white solid. ^{1}H-NMR (300 MHz, DMSO): δ 2.19 (*s*, 3H), 4.66 (*br s*, 2H), 7.33 (*dd*, *J* = 8.5, 1.8 Hz, 1H), 7.51 – 7.65 (*m*, 2H), 11.55 (*br s,* 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 11.2, 102.9, 127.6, 128.6, 129.5, 130.4, 132.1, 134.3, 138.8, 150.7. MS [CI, m/z]: 242 $[M+H]^+$.

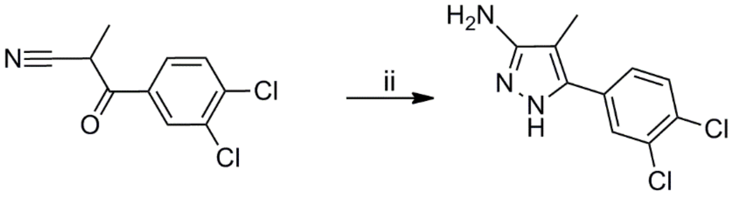
*1.3.2. 4-(3,4-dimethylphenyl)-5-methyl-1H-pyrazol-3-amine **(2c)**.* Purified by crystallization from diisopropyl ether/hexane. Yield 85%, white solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.26 (*s*, 3H), 2.28 (*s*, 6H), 5.92 (*br s*, 3H), 7.06 (*d*, *J* = 7.6 Hz, 1H), 7.10 (*s*, 1H), 7.17 (*d*, *J* = 7.7 Hz, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 10.9, 19.5, 19.9, 106.3, 126.1, 129.8, 130.1, 130.2, 134.7, 137.1, 138.3, 151.5. MS [CI, m/z]: 202 $[M+H]^+$.

*1.3.3. 5-methyl-4-(3-methylphenyl)-1H-pyrazol-3-amine **(2f)**.* This compound is known; however, no analytical data were reported. Purified by crystallization from dichloromethane / *petroleum ether*. Yield 85%, white solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.27 (*s*, 3H), 2.38 (*s*, 3H), 5.43, (*br s*, 3H), 7.04 – 7.18 (*m*, 3H), 7.30 (*t*, *J* = 7.5 Hz, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 10.9, 21.5, 106.4, 125.7, 127.0, 128.8, 129.3, 132.8, 138.3, 138.5, 151.6. MS [CI, m/z]: 188 $[M+H]^+$.

*1.3.4. 5-methyl-4-[3-(trifluoromethoxy)phenyl]-1H-pyrazol-3-amine (**2i**).* The reaction was performed in toluene (instead of MeOH) with AcOH (2.5 eq) at 105°C during 6h. Purified by crystallization from petroleum ether. Yield 80%, white solid. ^{1}H-NMR (300 MHz, DMSO-d6): δ 1.98 (*s*, 3H), 4.56 (*br s*, 2H), 7.12 (*d*, J = 8.0 Hz, 1H), 7.29 (*s*, 1H), 7.38 (*d*, J = 7.8 Hz, 1H), 7.46 (*dd*, J = 8.0 Hz, J = 7.8 Hz, 1H), 11.54 (*br s*, 1H). LC/MS (ESI, m/z) 258.0 $[M + H]^+$.

*1.3.5. 4-(4-benzyloxyphenyl)-5-methyl-1H-pyrazol-3-amine (**2n**).* The reaction was performed in toluene (instead of MeOH) with AcOH (2.5 eq) at 105°C during 5h. Purified by crystallization from diethyl ether. Yield 59%, pale yellow solid. ^{1}H-NMR (300 MHz, DMSO-d6): δ 2.12 (*s*. 3H), 4.32 (*br s*, 2H), 5.10 (*s*, 2H), 7.01 (*d*, J = 8.7 Hz, 2H), 7.24 (*d*, J = 8.7 Hz, 2H), 7.30-7.48 (*m*, 5H), 11.31 (*br s*, 1H). LC/MS (ESI, m/z) 280.1 $[M + H]^+$.

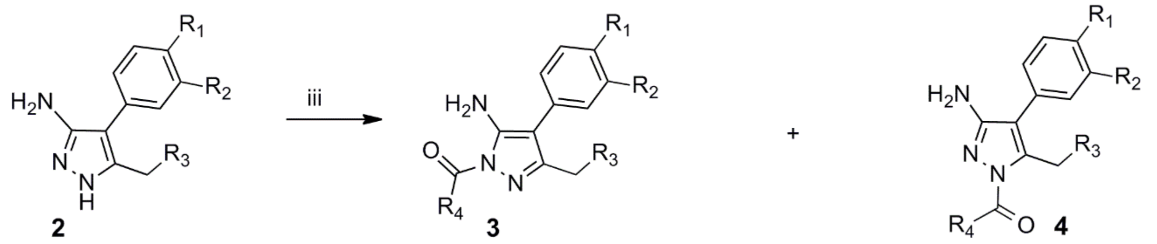
*1.3.6. 4-[3,5-bis(trifluoromethyl)phenyl]-5-methyl-1H-pyrazol-3-amine (**2o**).* Purified by crystallization from diisopropyl ether/hexane. Yield 75%, white solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.34 (*s*, 3H), 5.85 (*br s*, 3H), 7.58 – 7.96 (*m*, 3H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 11.1, 104.2, 119.9 – 120.4 (*m*), 123.4 (*q*, J = 272.8 Hz), 128.2, 128.6 (*m*), 132.4 (*q*, J = 33.2 Hz), 135.2, 139.5, 151.3. MS [CI, m/z]: 310 $[M+H]^+$.



1p **2p**

Scheme S3: synthesis of compound **2p**. ii) $NH_2NH_2 \cdot 2HCl$, 3Å sieves, dry EtOH, reflux.

*1.4. 5-(3,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-amine (**2p**).* This compound was obtained from 2-methyl-3-oxo-3-phenylpropanenitrile (**1*i***) and 2.0 eq. of hydrazine dihydrochloride (Scheme S3) following the same procedure for compounds of structure **2a - h**. Purified by flash chromatography (eluent dichloromethane / MeOH 95/5 v/v). Yield 57%, white solid. ^{1}H-NMR (300 MHz, CD_3OD): δ 2.05 (*s*, 3H), 7.46 (*dd*, J = 8.1, 1.4 Hz, 1H), 7.58 (*d*, J = 8.4 Hz, 1H), 7.68 (*d*, J = 1.0 Hz, 1H). ^{13}C-NMR (75 MHz, DMSO): δ 8.3, 96.3, 126.4, 127.8, 129.3, 130.8, 131.3, 133.7, 140.6, 151.4. MS [CI, m/z]: 242 $[M+H]^+$.



2a (R_1,R_2 = Cl, R_3 = H);
2c (R_1,R_2 = CH_3, R_3 = H);
2g (R_2 = Cl, R_1,R_3 = H);
2j (R_2 = CF_3, R_1,R_3 = H);
2l (R_1 = Cl, R_2,R_3 = H);
2q (R_1,R_2 = Cl, R_3 = CH_3);

3a (R_1,R_2 = Cl, R_3 = H, R_4 = Me);
3b (R_1,R_2 = Cl, R_3 = H, R_4 = Et);
3c (R_1,R_2 = Cl, R_3 = H, R_4 = Prop);
3d (R_1,R_2 = Cl, R_3 = H, R_4 = CycloProp);
3e (R_1,R_2 = Cl, R_3 = H, R_4 = CycloBu);
3f (R_1,R_2 = Cl, R_3 = H, R_4 = CycloPent);
3g (R_1,R_2 = Cl, R_3 = H, R_4 = CycloHex);
3h (R_1,R_2 = Cl, R_3 = H, R_4 = Ph);
3i (R_1,R_2 = Cl, R_3 = H, R_4 = 3-Py);
3j (R_1,R_2 = Cl, R_3 = H, R_4 = 4-Py);
3K (R_1 = Cl, R_2,R_3 = H, R_4 = Me);
3l (R_1 = Cl, R_2,R_3 = H, R_4 = CycloProp);
3m (R_2 = Cl, R_1,R_3 = H, R_4 = Me);
3n (R_2 = CF_3, R_1,R_3 = H, R_4 = Me);
3o (R_1,R_2 = CH_3, R_3 = H, R_4 = Me);
3p (R_1,R_2 = Cl, R_3 = CH_3, R_4 = Me);
3q (R_1,R_2 = Cl, R_3 = H, R_4 = O*t*-Bu);
3r (R_1,R_2 = Cl, R_3 = H, R_4 = HNEt);

4a (R_1,R_2 = Cl, R_3 = H, R_4 = Me);
4e (R_1,R_2 = Cl, R_3 = H, R_4 = CycloBu);
4i (R_1,R_2 = Cl, R_3 = H, R_4 = 3-Py);
4n (R_2 = CF_3, R_1,R_3 = H, R_4 = Me);
4o (R_1,R_2 = CH_3, R_3 = H, R_4 = Me);
4q (R_1,R_2 = Cl, R_3 = H, R_4 = O*t*-Bu);

Scheme S4: synthesis of compounds **3a - q** and **4a - q**: iii) R_4COCl, dry pyridine, dry THF, rt, or: appropriate anhydride, dry THF, rt.

*1.5. 1-[5-amino-4-(3,4-dichlorophenyl)-3-methyl-1H-pyrazol-1-yl]ethan-1-one (**3a**) and 1-[3-amino-4-(3,4-dichlorophenyl)-5-methyl-1H-pyrazol-1-yl]ethan-1-one (**4a**).* Acetic anhydride (0.164 mL, 1.73 mmol) was added to a solution of **2a** (300 mg, 1.24 mmol) in dry dioxane (5 mL). The mixture was stirred for 2 hours at room temperature. The solvent was evaporated under reduced pressure, and the residue was partitioned between water and ethyl acetate. The aqueous layer was extracted twice with ethyl acetate and the combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by flash chromatography (eluent *petroleum ether* / ethyl acetate 90/10 v/v) to obtain two products.

3a) First compound eluted. Yield 69%, white solid. ^{1}H-NMR (300 MHz, DMSO): δ 2.13 (*s*, 3H), 2.55 (*s*, 3H), 6.75 (*br s*, 2H), 7.31 (*dd*, J = 8.3, 2.1 Hz, 1H), 7.54 (*d*, J = 2.0 Hz, 1H), 7.64 (*d*, J = 8.3 Hz, 1H). ^{13}C-NMR (75 MHz, DMSO): δ 13.2, 23.2, 99.3, 128.2, 128.6, 130.0, 130.6, 131.2, 132.6, 147.5, 150.6, 173.2. MS [CI, m/z]: 284 $[M+H]^+$.

4a) Second compound eluted. Yield 15%, white solid. ^{1}H-NMR (300 MHz, DMSO): δ 2.40 (s, 3H), 2.49 (*s*, 3H), 5.47 (*br s*, 2H), 7.31 (*dd*, J = 8.3, 1.5 Hz, 1H), 7.56 (*d*, J = 1.5 Hz, 1H), 7.70 (*d*, J = 8.3 Hz, 1H). ^{13}C-NMR (75 MHz, DMSO): δ 13.2, 23.4, 112.4, 129.7, 129.8, 130.7, 131.1, 131.3, 131.6, 139.9, 154.5, 170.0. MS [CI, m/z]: 284 $[M+H]^+$.

*1.6. General method for the preparation of acylated 3-aminopyrazoles **3b** - **3p** and **4b** - **4p**.*

Pyridine (1.24 mmol) and the appropriate acyl chloride (1.24 mmol) was added to a solution of 3-aminopyrazole (1.24 mmol) in dry THF (10 mL). The reaction mixture was stirred overnight at room temperature. Then the mixture was poured in 0.5 N HCl (20 mL) and reduced to half of its volume by rotary evaporation under reduced pressure. The mixture was extracted twice with ethyl acetate and the combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by flash chromatography, obtaining the 1-acyl-5-amino-pyrazole (general structure **3**) as major product. In some cases, also the 1-acyl-3-aminopirazole (general structure **4**) was isolated as minor product and characterized.

*1.6.1. 1-[5-amino-4-(3,4-dichlorophenyl)-3-methyl-1H-pyrazol-1-yl]propan-1-one (**3b**).* Flash chromatography eluent: *petroleum ether* / ethyl acetate 90/10 v/v. Yield 56%, white solid. ^{1}H-NMR (300 MHz, DMSO): δ 1.12 (*t*, J = 7.4 Hz, 1H), 2.12 (*s*, 3H), 1.12 (*t*, J = 7.4 Hz, 1H), 6.77 (*br s*, 2H), 7.31 (*dd*, J = 8.3, 2.0 Hz, 1H), 7.54 (*d*, J = 2.0 Hz, 1H), 7.54 (*d*, J = 2.0 Hz, 1H). ^{13}C-NMR (75 MHz, DMSO): δ 8.2, 13.2, 28.1, 99.2, 128.2, 128.6, 130.0, 130.6, 131.2, 132.7, 147.5, 150.5, 176.3. MS [CI, m/z]: 284 $[M+H]^+$.

*1.6.2. 1-[5-amino-4-(3,4-dichlorophenyl)-3-methyl-pyrazol-1-yl]butan-1-one (**3c**).* Flash chromatography eluent cyclohexane / ethyl acetate 1/1 v/v. Yield 38%, pale yellow oil. ^{1}H-NMR (300 MHz, DMSO): δ 0.95 (*t*, J = 7.4 Hz, 3H), 1.65 (*m*, 2H), 2.12 (*s*, 3H), 2.97 (*t*, J = 7.4 Hz, 2H), 6.74 (*br s*, 2H), 7.30 (*dd*, J = 8.3 Hz, 2.1 Hz, 1H), 7.53 (*d*, J = 2.1 Hz, 1H), 7.64 (*d*, J = 8.3 Hz, 1H). LC/MS (ESI, m/z) 312.1 $[M + H]^+$

*1.6.3. [5-amino-4-(3,4-dichlorophenyl)-3-methyl-1H-pyrazol-1-yl](cyclopropyl)methanone (**3d**).* Flash chromatography eluent: *petroleum ether* / ethyl acetate 90/10 v/v. Yield 56%, white solid. ^{1}H-NMR (300 MHz, DMSO): δ 0.99 – 1.15 (*m*, 4H), 2.15 (*s*, 3H), 2.87 – 3.15 (*m*, 1H), 6.73 (*br s*, 2H), 7.31 (*d*, J = 8.3 Hz, 1H), 7.54 (*s*, 1H), 7.63 (*d*, J = 8.3 Hz, 1H). ^{13}C-NMR (75 MHz, DMSO): δ 10.3, 12.7, 13.3, 99.6, 128.3, 128.8, 130.1, 130.7, 131.4, 132.7, 147.5, 151.0, 176.0. MS [CI, m/z]: 310 [M+H]$^+$.

1.*6.4. [5-amino-4-(3,4-dichlorophenyl)-3-methyl-1H-pyrazol-1-yl](cyclobutyl)methanone* ***(3e)*** *and [3-amino-4-(3,4-dichlorophenyl)-5-methyl-1H-pyrazol-1-yl](cyclobutyl)methanone* ***(4e)***. The two products were separated by flash chromatography (eluent: *petroleum ether* / ethyl acetate 95/5 v/v).

3e) Yield 64%, white solid. ^{1}H-NMR (300 MHz, DMSO): δ 1.71 – 1.85 (*m*, 1H), 1.91 – 2.07 (*m*, 1H), 2.10 (*s*, 3H), 2.14 – 2.38 (*m*, 4H), 3.92 – 4.28 (*m*, 1H), 6.75 (*br s*, 2H), 7.30 (*dd*, J = 8.4, 2.0 Hz, 1H), 7.53 (*d*, J = 1.9 Hz, 1H), 7.63 (*d*, J = 8.3 Hz, 1H). ^{13}C-NMR (75 MHz, DMSO): δ 13.4, 17.8, 24.6, 38.2, 99.4, 128.3, 128.7, 130.1, 130.6, 131.3, 132.8, 147.6, 150.8, 176.7. MS [CI, m/z]: 324 [M+H]$^+$.

4e) Yield 5%, white solid. ^{1}H-NMR (300 MHz, DMSO): δ 1.68 – 1.90 (*m*, 1H), 192 – 2.10 (*m*, 1H), 2.12 – 2.32 (*m*, 4H), 2.41 (*s*, 3H), 3.95 – 4.20 (*m*, 1H), 5.39 (*br s*, 2H), 7.30 (*dd*, J = 8.3 Hz, 1.5 Hz, 1H), 7.56 (*d*, J = 1.2 Hz, 1H), 7.69 (*d*, J = 8.3 Hz, 1H). MS [CI, m/z]: 324 [M+H]$^+$.

1.6.5. [5-amino-4-(3,4-dichlorophenyl)-3-methyl-1H-pyrazol-1-yl] (cyclopentyl)methanone ***(3f)***. Flash chromatography eluent: *petroleum ether* / ethyl acetate 90/10 v/v. Yield 53%, white solid. ^{1}H-NMR (300 MHz, DMSO): δ 1.46 – 1.85 (*m*, 6H), 1.87 – 2.05 (*m*, 2H), 2.13 (*s*, 3H), 3.76 – 4.02 (*m*, 1H), 6.78 (*br s*, 2H), 7.32 (*dd*, J = 8.3, 0.8 Hz, 1H), 7.55 (*d*, J = 0.7 Hz, 1H), 7.64 (*d*, J = 8.3 Hz, 1H). ^{13}C-NMR (75 MHz, DMSO): δ 13.24, 25.58, 29.69, 42.70, 99.31, 128.17, 128.64, 130.01, 130.54, 131.22, 132.65, 147.69, 150.54, 178.53. MS [CI, m/z]: 338 [M+H]$^+$.

1.6.6. [5-amino-4-(3,4-dichlorophenyl)-3-methyl-pyrazol-1-yl]-cyclohexyl-methanone ***(3g)***. Flash chromatography eluent cyclohexane / ethyl acetate 1/1 v/v. Yield 40%, white solid. ^{1}H-NMR (300 MHz, DMSO-d$_6$): δ 1.25-1.47 (*m*, 6H), 1.77 (*m*, 2H), 1.92 (*m*, 2H), 3.53 (*m*, 1H), 6.59 (*br s*, 2H), 7.30 (*d*, J = 8.1 Hz, 1H), 7.52 (*s*, 1H), 7.61 (*d*, J = 8.1 Hz, 1H). LC/MS (ESI, m/z) 352.1 [M + H]$^+$.

*1.6.7. [5-amino-4-(3,4-dichlorophenyl)-3-methyl-1H-pyrazol-1-yl](phenyl)methanone. **(3h)**.* Flash chromatography eluent: *petroleum ether* / ethyl acetate 95/5 v/v. Yield 49 %, white solid. ^{1}H-NMR (300 MHz, DMSO): δ 2.10 (*s*, 3H), 6.86 (*br s*, 2H), 7.36 (*dd*, J = 8.3, 1.8 Hz, 1H), 7.42 – 7.76 (*m,* 5H), 7.94 (*d*, J = 7.3 Hz, 2H). ^{13}C-NMR (75 MHz, DMSO): δ 13.3, 99.6, 127.9, 128.5, 128.91, 130.3, 130.4, 130.7, 131.4, 132.2, 132.6, 133.3, 148.8, 151.4, 169.8. MS [CI, m/z]: 346 $[M+H]^+$.

*1.6.8. [5-amino-4-(3,4-dichlorophenyl)-3-methyl-1H-pyrazol-1-yl](pyridin-3-yl)methanone (**3i**) and [3-amino-4-(3,4-dichlorophenyl)-5-methyl-1H-pyrazol-1-yl](pyridin-3-yl)methanone (**4i**).* In this case, the reaction mixture was filtered. The filtrate was evaporated and the resulting crude material was purified by flash chromatography (eluent: *petroleum ether* / ethyl acetate 60/40 v/v) obtaining two products:

3i) Yield 31%, pale yellow solid. ^{1}H-NMR (300 MHz, DMSO): δ 2.13, (s, 3H), 6.92 (br s, 2H), 7.37 (d, *J* = 8.0 Hz, 1H), 7.51 – 7.76 (m, 4H), 8.33 (d, *J* = 7.7 Hz, 1H), 8.78 (d, *J* = 3.8 Hz, 1H), 9.10 (s, 1H). ^{13}C-NMR (75 MHz, DMSO): δ 13.3, 99.6, 123.0, 128.6, 128.9, 129.5, 130.3, 130.7, 131.4, 132.4, 138.0, 148.7, 150.6, 152.0, 152.3, 168.1. MS [CI, m/z]: 347 $[M+H]^+$.

4i) Yield 10%, white solid. ^{1}H-NMR (300 MHz, DMSO): δ 2.53 (*s*, 3H), 5.58 (*br s*, 2H), 7.37 (*dd*, J = 8.3, 1.9 Hz, 1H), 7.55 (*dd*, J = 7.7, 4.9 Hz, 1H), 7.62 (*d*, J = 1.9 Hz, 1H), 7.37 (*dd*, J = 8.3, 1.9 Hz, 1H), 8.18 – 8.27 (*m*, 1H), 8.73 (*dd*, J = 4.8, 1.5 Hz, 1H), 9.01 (*d*, J = 1.5 Hz, 1H). ^{13}C-NMR (75 MHz, DMSO): δ 13.4, 113.4, 123.0, 129.9, 130.2, 130.5, 130.9, 131.2, 131.3, 131.5, 137.7, 141.4, 150.4, 151.8, 155.4, 165.7. MS [CI, m/z]: 347 $[M+H]^+$.

*1.6.9. [5-amino-4-(3,4-dichlorophenyl)-3-methyl-1H-pyrazol-1-yl](pyridin-4-yl)methanone (**3j**).* In this case, the reaction mixture was filtered. The filtrate was evaporated and the resulting crude material was purified by flash chromatography (eluent: dichloromethane / ethyl acetate 90/10 v/v). Yield 17%, Yellow solid. ^{1}H-NMR (300 MHz, DMSO): δ 2.08 (*s*, 3H), 6.92 (*br s*, 2H), 7.34 (*dd*, J = 8.4, 2.0 Hz, 1H), 7.34 (*dd*, J = 8.4, 2.0 Hz, 1H), 7.66 (*d*, J = 8.3 Hz, 1H), 7.79 (*d*, J = 5.9 Hz, 2H), 8.77 (*d*, J = 5.3 Hz, 2H). ^{13}C-NMR (75 MHz, DMSO): δ 13.3, 99.7, 123.3, 128.7, 128.9, 130.3, 130.7, 131.4, 132.3, 140.9, 148.6, 149.6, 152.4. MS [CI, m/z]: 347 $[M+H]^+$.

1.6.10. 1-[5-amino-4-(4-chlorophenyl)-3-methyl-1H-pyrazol-1-yl]ethan-1-one ***(3k)***. Flash chromatography eluent: *petroleum ether* / ethyl acetate 90/10 v/v. Yield 52 %, white solid. ^{1}H-NMR (300 MHz, DMSO): *δ* 2.20 (*s*, 3H), 2.54 (*s*, 3H), 6.61 (*br s*, 2H), 7.35 (*d*, J = 8.5 Hz, 2H), 7.46 (*d*, J = 8.5 Hz, 2H). ^{13}C-NMR (75 MHz, DMSO):*δ* 13.3, 23.3, 100.4, 128.78, 130.2, 130.5, 130.8, 147.3, 150.8, 173.3. MS [CI, m/z]: 250 $[M+H]^+$.

1.6.11. [5-amino-4-(4-chlorophenyl)-3-methyl-1H-pyrazol-1-yl](cyclopropyl)methanone. ***(3l)***. Flash chromatography eluent: *petroleum ether* / ethyl acetate 90/10 v/v. Yield 72 %, white solid. ^{1}H-NMR (300 MHz, DMSO): *δ* 0.89 – 1.40 (*m*, 4H), 2.15 (*s*, 3H), 2.87, 3.18 (*m*, 1H), 6.60 (*br s*, 2H), 7.36 (*d*, J = 7.9 Hz, 2H), 7.46 (*d*, J = 8.2 Hz, 2H). ^{13}C-NMR (75 MHz, DMSO):*δ* 10.3, 12.7, 13.4, 100.6, 128.7, 130.2, 130.5, 130.8, 147.2, 151.1, 175.0. MS [CI, m/z]: 275 $[M+H]^+$.

1.6.12. 1-[5-amino-4-(3-chlorophenyl)-3-methyl-1H-pyrazol-1-yl]ethan-1-one ***(3m)***. Flash chromatography eluent: *petroleum ether* / ethyl acetate 85/15 v/v. Yield 40 %, white solid. ^{1}H-NMR (300 MHz, DMSO): *δ* 2.13 (*s*, 3H), 2.55 (*s*, 3H), 6.67 (*br s*, 2H), 7.24 – 7.34 (*m*, 2H), 7.36 (*t*, J = 1.6 Hz, 1H), 7.44 (*t*, J = 7.8 Hz, 1H). ^{13}C-NMR (75 MHz, DMSO): *δ* 13.3, 23.2, 100.4, 125.9, 127.1, 127.9, 130.4, 133.4, 134.1, 147.4, 150.85, 173.3. MS [CI, m/z]: 250 $[M+H]^+$.

1.6.12. 1-{5-amino-3-methyl-4-[3-(trifluoromethyl)phenyl]-1H-pyrazol-1-yl}ethan-1-one ***(3n)*** *and 1-{3-amino-5-methyl-4-[3-(trifluoromethyl)phenyl]-1H-pyrazol-1-yl}ethan-1-one* ***(4n)***. The two products were separated by flash chromatography (eluent: *petroleum ether* / ethyl acetate 80/20 v/v).

3n) Yield 23 %, white solid. ^{1}H-NMR (300 MHz, DMSO): *δ* 2.13 (*s*, 3H), 2.55 (*s*, 3H), 6.72 (*br s*, 2H), 7.60 – 7.65 (*m*, 4H). ^{13}C-NMR (75 MHz, DMSO): *δ* 13.3, 23.3, 100.3, 122.6 (*q*, J = 3.5 Hz), 124.3 (*q*, J = 272.4 Hz), 124.7 (*q*, J = 3.7 Hz), 129.5 (*q*, J = 31.5 Hz), 129.8, 132.5, 133.0, 147.6, 150.8, 173.4. MS [CI, m/z]: 284 $[M+H]^+$.

4n Yield 7 %, white solid. ^{1}H-NMR (300 MHz, DMSO): *δ* 2.40 (*s*, 3H), 2.50 (*s*, 3H), 5.44 (*br s*, 2H), 7.64 – 7.71 (*m*, 4H). MS [CI, m/z]: 284 $[M+H]^+$.

*1.6.13. 1-[5-amino-4-(3,4-dimethylphenyl)-3-methyl-1H-pyrazol-1-yl]ethan-1-one **(3m)** and 1-[3-amino-4-(3,4-dimethylphenyl)-5-methyl-1H-pyrazol-1-yl]ethan-1-one **(4m)**.* The two products were separated by flash chromatography (eluent: petroleum ether/ ethyl acetate 80/20 v/v).

3o) Yield 53 %, white solid. ^{1}H-NMR (300 MHz, DMSO): δ 2.09 (*s*, 3H), 2.22 (*s*, 3H), 2.24 (*s*, 3H), 2.53 (*s*, 3H), 6.46 (*br s*, 2H), 7.02 (*d*, J = 7.6 Hz, 1H), 7.09 (*s*, 1H), 7.17 (*d*, J = 7.7 Hz, 1H). ^{13}C-NMR (75 MHz, DMSO): δ 13.4, 19.1, 19.5, 23.3, 101.7, 125.9, 129.1, 129.6, 129.84, 134.0, 136.5, 146.9, 151.1, 173.2. MS [CI, m/z]: 244 $[M+H]^+$.

4o) Yield 4 %, white solid. ^{1}H-NMR (300 MHz, DMSO): δ 2.25 (*s*, 6H), 2.38 (*s*, 3H), 2.48 (*s*, 3H), 5.25 (*br s*, 2H), 7.02 (*d*, J = 7.7 Hz, 1H), 7.09 (*s*, 1H), 7.21 (*d*, J = 7.6 Hz, 1H). MS [CI, m/z]: 244 $[M+H]^+$.

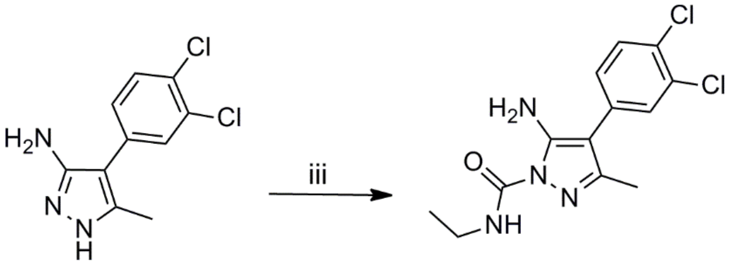
*1.6.14. 1-[5-amino-4-(3,4-dichlorophenyl)-3-ethyl-1H-pyrazol-1-yl]ethan-1-one **(3p)**.* Yield 39 %, white solid. ^{1}H-NMR (300 MHz, DMSO): δ 1.05 (*t*, J = 7.5 Hz, 1H), 2.50 – 2.57 (*m*, 5H), 6.68 (*br s*, 2H), 7.30 (*dd*, J = 8.3, 2.0 Hz, 1H), 7.52 (*d*, J = 2.0 Hz, 1H), 7.65 (*d*, J = 8.3 Hz, 1H). ^{13}C-NMR (75 MHz, DMSO): δ 11.9, 20.3, 23.2, 98.8, 128.5, 129.0, 130.3, 130.7, 131.3, 132.8, 147.6, 155.3, 173.3. MS [CI, m/z]: 298 $[M+H]^+$.

*1.7. Tert-butyl 5-amino-4-(3,4-dichlorophenyl)-3-methyl-1H-pyrazole-1-carboxylate **(3q)** and tert-butyl 3-amino-4-(3,4-dichlorophenyl)-5-methyl-1H-pyrazole-1-carboxylate **(4q)**.* BOC-anhydride (270 mg, 1.24 mmol), bismuth(III) chloride (13.0 mg, 0.0413 mmol) and **2a** (200 mg, 0.826 mmol) were stirred under solvent-free conditions at 30°C for 2 hours. Then the mixture was partitioned between saturated $NaHCO_3$ and ethyl acetate. The aqueous layer was extracted twice with ethyl acetate and the combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by flash chromatography (eluent: from *petroleum ether* / ethyl acetate 85/15 to 70/30 v/v) to obtain two products.

3q) Yield 65 %, white solid. ^{11}H-NMR (300 MHz, DMSO): δ 1.54 (*s*, 9H), 2.34 (*s*, 3H), 5.32 (*br s*, 2H), 7.26 (*d*, J = 8.2 Hz, 1H), 7.49 (*s*, 1H), 7.60 (*d*, J = 7.7 Hz, 1H). ^{13}C-NMR (75 MHz, DMSO): δ

13.0, 28.5, 85.1, 100.0, 129.0, 129.6, 130.9, 131.5, 132.1, 133.9, 148.6, 150.6, 151.0. MS [CI, m/z]: 342 $[M+H]^+$.

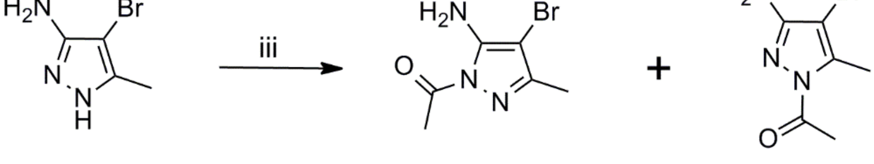
4q) Yield 23 %, white solid. ^{1}H-NMR (300 MHz, DMSO): δ 1.54 (*s*, 9H), 2.06 (*s*, 3H), 6.42 (*br s*, 2H), 7.29 (*d*, J = 8.1 Hz, 1H), 7.54 (*s*, 1H), 7.69 (*d*, J = 8.1 Hz, 1H). ^{13}C-NMR (75 MHz, DMSO): δ 13.1, 27.6, 82.9, 111.2, 129.5, 129.7, 130.6, 131.1, 131.2, 132.0, 140.4, 148.5, 154.3. MS [CI, m/z]: 342 $[M+H]^+$.



3r

Scheme S5: synthesis of compound **3r**: iii) ethyl isocyanate (1.0 eq), acetone, rt, 18h.

*1.8. 5-amino-4-(3,4-dichlorophenyl)-N-ethyl-3-methyl-pyrazole-1-carboxamide (**3r**).* Conditions indicated in scheme S5 were followed. Flash chromatography eluent dichloromethane / ethyl acetate 1/1 v/v. Yield 82%, beige gum. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.26 (*t*, J = 7.2 Hz, 3H), 2.19 (*s*, 3H), 3.42 (*m*, 2H), 5.63 (*br s*, 2H), 7.14 (*dd*, J = 8.2 Hz, 2.0 Hz, 1H), 7.20 (*br s*, 1H), 7.23 (*d*, J = 2.0 Hz, 1H), 7.48 (*d*, J = 8.2 Hz, 1H). LC/MS (ESI, m/z) 313.0 $[M + H]^+$.



3s **4s**

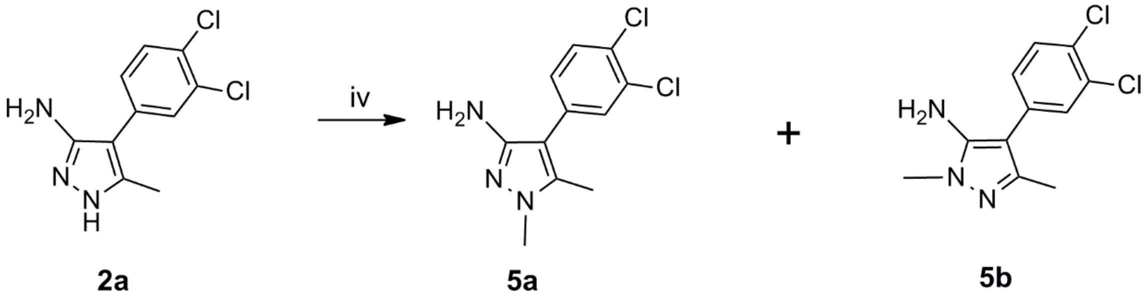
Scheme S6: synthesis of compounds **3s** and **4s**: iii) acetyl chloride, dry pyridine, dry THF, rt.

*1.9. 1-(5-amino-4-bromo-3-methyl-1H-pyrazol-1-yl)ethan-1-one (**3s**) and 1-(3-amino-4-bromo-5-methyl-1H-pyrazol-1-yl)ethan-1-one (**4s**).* The "general method for the preparation of acylated 3-aminopyrazoles 3 and 4" was applied to commercial available 4-bromo-5-methyl-1*H*-pyrazol-3-

amine, obtaining compounds **3s** and **4s** (Scheme S6). The two compounds were separated by flash chromatography (eluent: from *petroleum ether* / ethyl acetate 85/15 to 60/40 v/v).

3s) Yield 57 %, white solid. ^{1}H-NMR (300 MHz, DMSO): δ 2.07 (*s*, 3H), 2.50 (overlapped with DMSO signal), 6.64 (*br s*, 2H).

4s) Yield 10 %, white solid. ^{1}H-NMR (300 MHz, DMSO): δ 2.41 (*s*, 3H), 2.43 (*s*, 3H), 5.76 (*br s*, 2H). MS [CI, m/z]: 218 [M+H]$^+$.

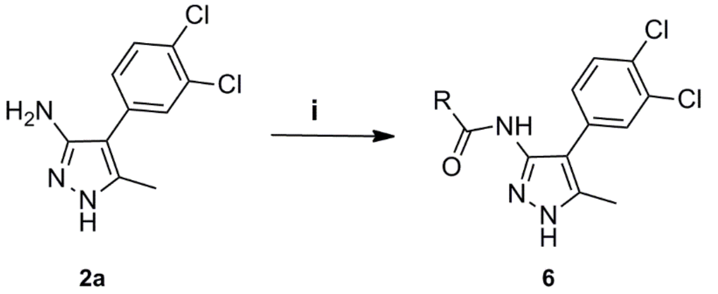


Scheme S7: synthesis of compounds **5a** and **5b**. iv) Cs_2CO_3, CH_3I, dry THF.

*1.10. 4-(3,4-dichlorophenyl)-1,5-dimethyl-1H-pyrazol-3-amine (**5a**) and 4-(3,4-dichlorophenyl)-1,3-dimethyl-1H-pyrazol-5-amine (**5b**).* Cs_2CO_3 (269 mg, 0.826 mmol) and CH_3I (117 mg, 0.826 mmol) was added to a solution of **2a** (200 mg, 0.826 mmol) or an appropriate 3-aminopyrazole (structure **2**, 0.826 mmol) in dry THF (30 mL). The mixture was stirred at room temperature overnight under inert atmosphere. The mixture was quenched in water (50 mL), reduced to half of its volume and extracted with diethyl ether (3 x 30 mL). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by flash chromatography (eluent: dichloromethane / MeOH 95/5 v/v) to obtain two products.

5a) First compound eluted. Yield 19%, white solid. The structure was determined unequivocally using heteronuclear 2D-NMR (HSQC and HMBC). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.21 (*s*, 3H), 3.46 (*br s*, 2H), 3.66 (*s*, 3H), 7.15 (*dd*, J = 8.3, 2.0 Hz, 1H), 7.40 (*d*, J = 2.0 Hz, 1H), 7.45 (*d*, J = 8.3 Hz, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 10.5, 35.7, 105.0, 128.1, 130.0, 130.4, 130.8, 132.9, 133.7, 137.1, 150.6. MS [CI, m/z]: 256 [M+H]$^+$.

5b) Second compound eluted. Yield 11%, white solid. The structure was determined unequivocally using heteronuclear 2D-NMR (HSQC and HMBC). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.19 (*s*, 3H), 3.65 (*br s*, 5H), 7.11 (*dd*, J = 8.2, 1.8 Hz, 1H), 7.11 (*dd*, J = 8.2, 1.8 Hz, 1H), 7.45 (*d*, J = 8.3 Hz, 1H). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 13.0, 34.3, 103.0, 127.8, 129.8, 130.1, 130.9, 133.0, 134.0, 142.5, 144.8. MS [CI, m/z]: 256 $[M+H]^+$.



2a

6

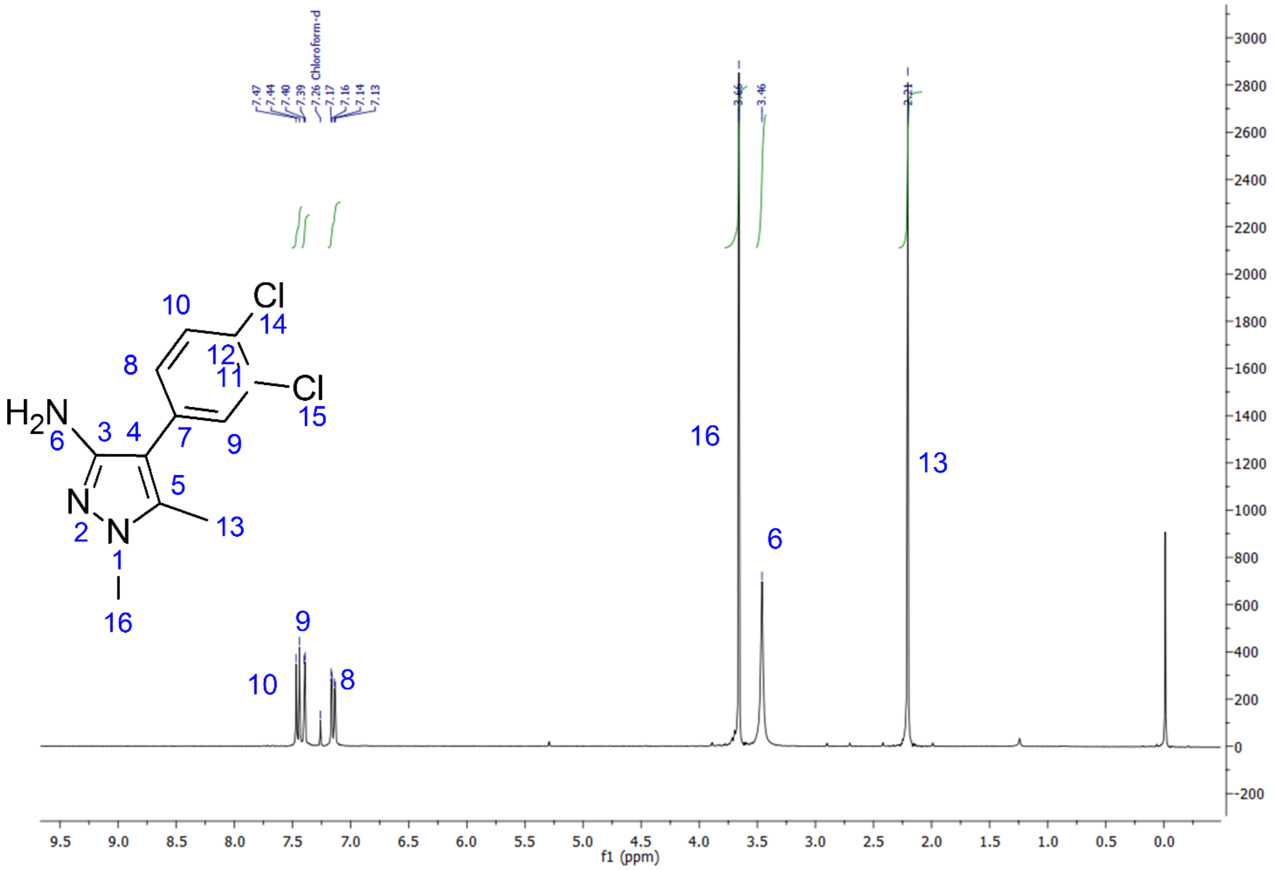
6a R = 2-Py
6b R = CF_3

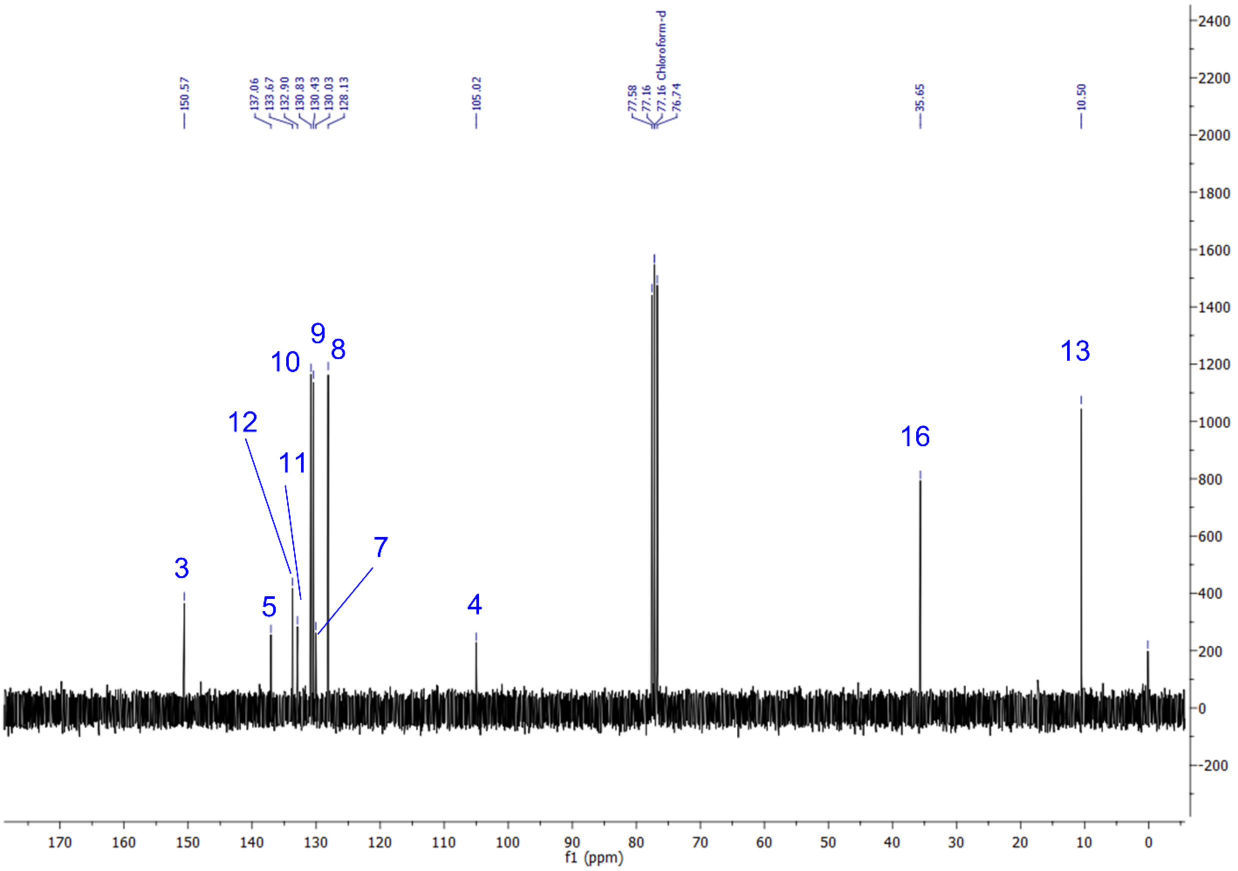
Scheme S8: Synthesis of compounds **6a** and **6b**. i) pyridine-2-carbonyl chloride, dry pyridine, dry THF, rt, or: trifluoroacetic anhydride, dry THF, rt.

*1.11. N-[4-(3,4-dichlorophenyl)-5-methyl-1H-pyrazol-3-yl]pyridine-2-carboxamide (**6a**).* Pyridine (1.24 mmol) and pyridine-2-carbonyl chloride (1.24 mmol) was added to a solution of **2a** (1.24 mmol) in dry THF (10 mL). The reaction mixture was stirred overnight at room temperature, then it was filtered. The filtrate was evaporated and the resulting crude material was purified by flash chromatography (eluent: dichloromethane / ethyl acetate 70/30 v/v) obtaining the desired product as a white solid. Yield 12 %. ^{1}H-NMR (300 MHz, DMSO): δ 2.32 (*s*, 3H), 7.34 (*dd*, J = 8.3, 1.3 Hz, 1H), 7.57 (*d*, J = 8.3 Hz, 1H), 7.60 – 7.71 (*m*, 2H), 7.93 – 8.09 (*m*, 2H), 8.70 (*d*, J = 4.7 Hz, 1H), 10.39 (*s*, 1H), 12.98 (*s*, 1H). ^{13}C-NMR (75 MHz, DMSO): δ 10.5, 112.1, 122.3, 126.0, 128.3, 128.4, 129.6, 130.5, 131.0, 133.9, 137.2, 138.0, 143.0, 148.7, 149.5, 164.2. MS [CI, m/z]: 347 $[M+H]^+$.

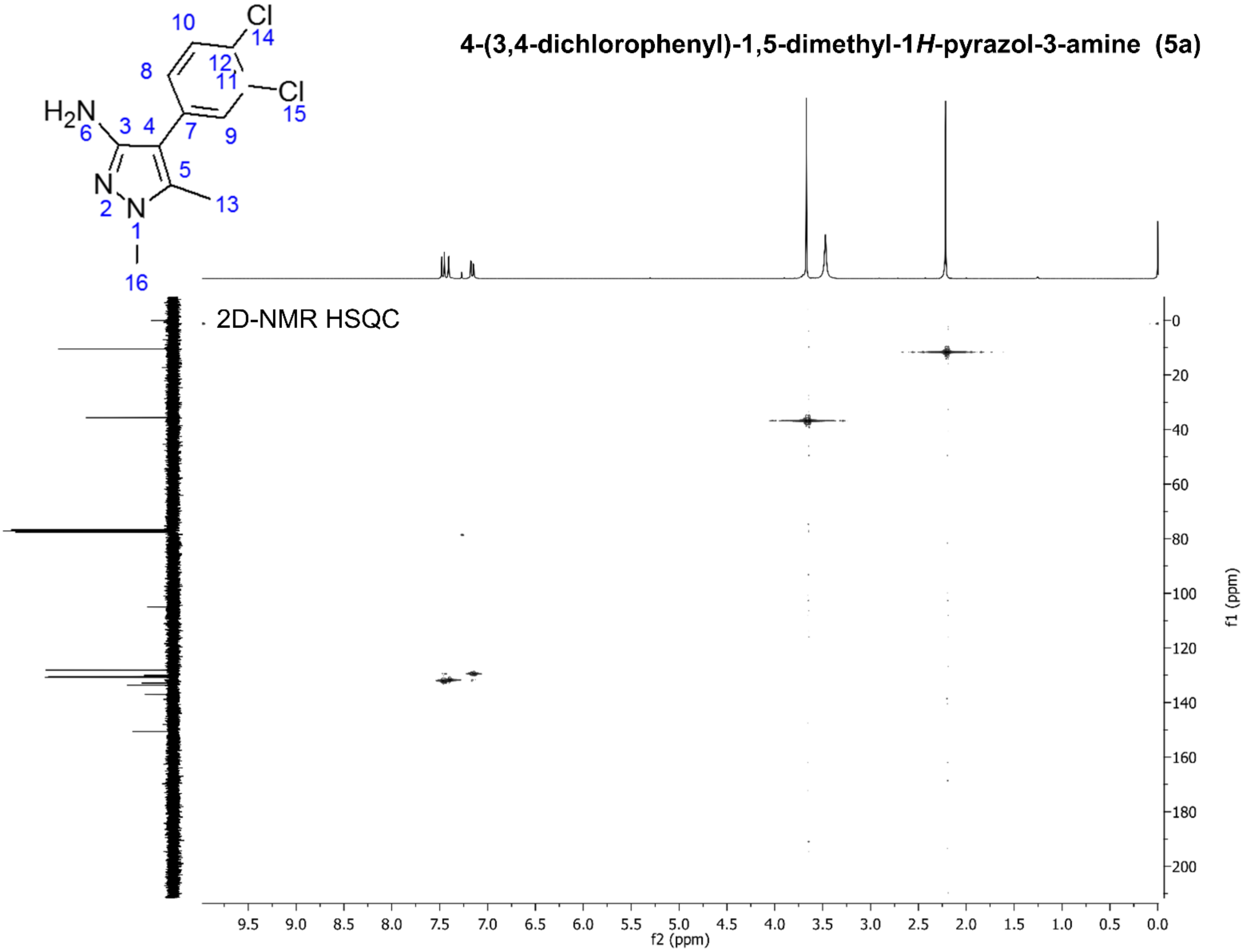
*1.12. N-[4-(3,4-dichlorophenyl)-5-methyl-1H-pyrazol-3-yl]-2,2,2-trifluoroacetamide (**6b**).* Trifluoroacetic anhydride (0.241 mL, 1.73 mmol) was added to a solution of **2a** (300 mg, 1.24 mmol) in dry THF (5 mL). The mixture was stirred overnight at room temperature. The solvent was evaporated under reduced pressure, and the residue was partitioned between water and ethyl acetate. The aqueous layer was extracted twice with ethyl acetate and the combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude material was purified by flash chromatography (eluent dichloromethane / ethyl acetate 80/20 v/v) to obtain the desired product as a white solid. Yield 33 %. ^{1}H-NMR (300 MHz, DMSO): δ 2.33 (*s*, 3H), 7.28 (*dd*, J = 8.3, 1.8 Hz, 1H), 7.51 (*d*, J = 1.7 Hz, 1H), 7.28 (*dd*, J = 8.3, 1.8 Hz, 1H), 11.30 (*s*, 1H), 12.98 (*br s*, 1H). ^{13}C-NMR (75 MHz, DMSO): δ 10.4, 111.7, 115.8 (*q*, J = 288.6 Hz), 128.1, 129.0, 129.3, 130.8, 131.3, 132.9, 138.1, 140.3, 156.2 (*q*, J = 36.7 Hz). MS [CI, m/z]: 347 $[M+H]^+$.

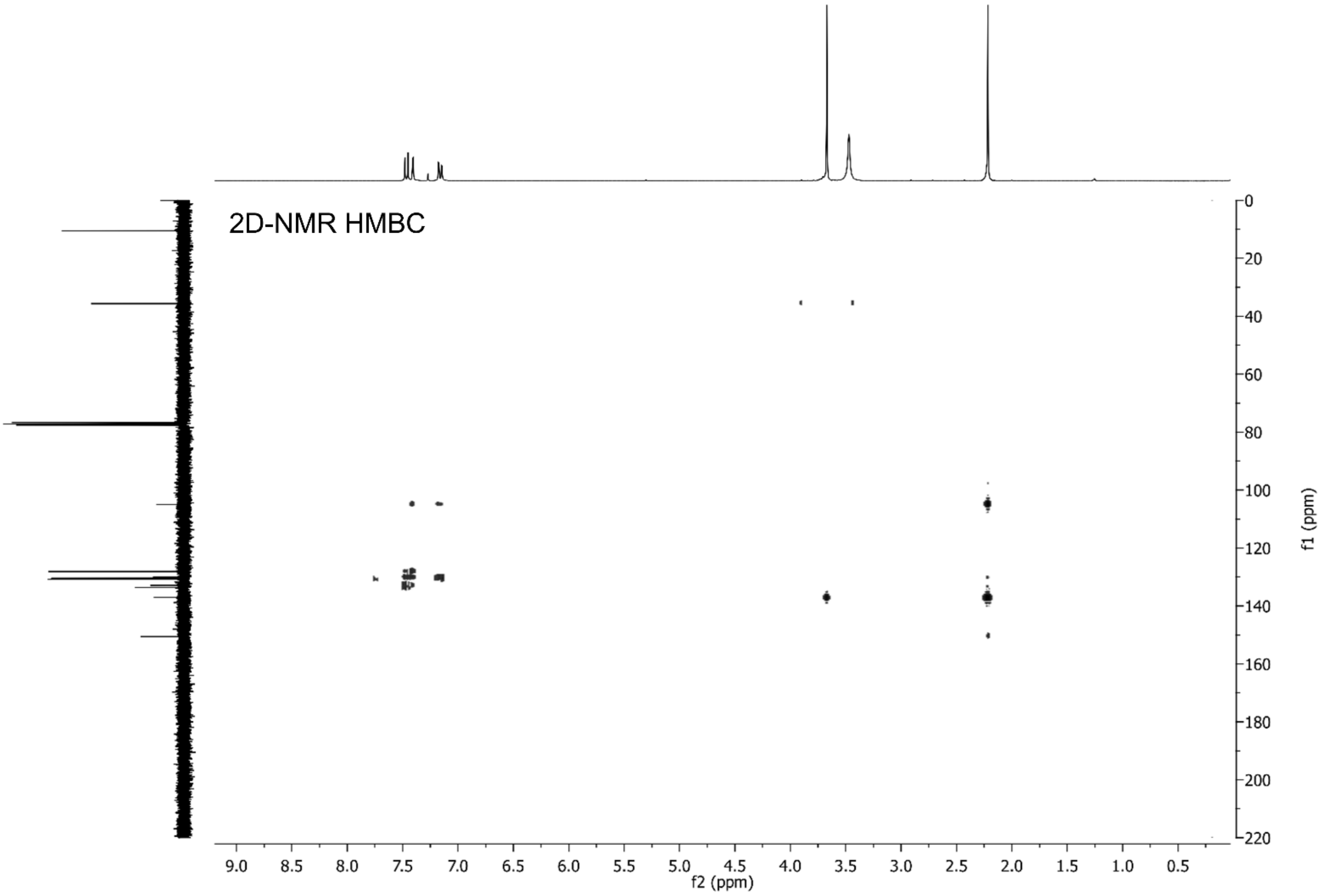
4-(3,4-dichlorophenyl)-1,5-dimethyl-1*H*-pyrazol-3-amine (5a)



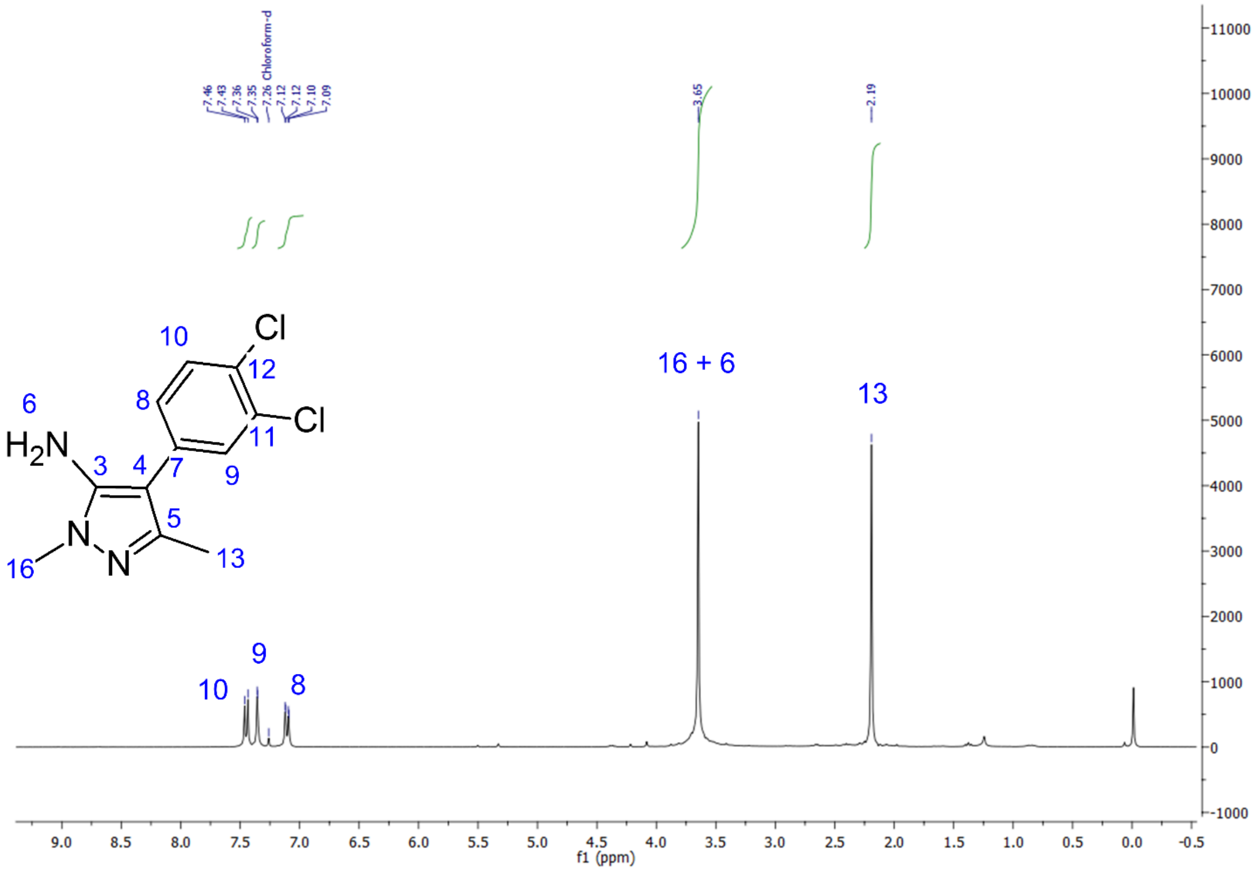


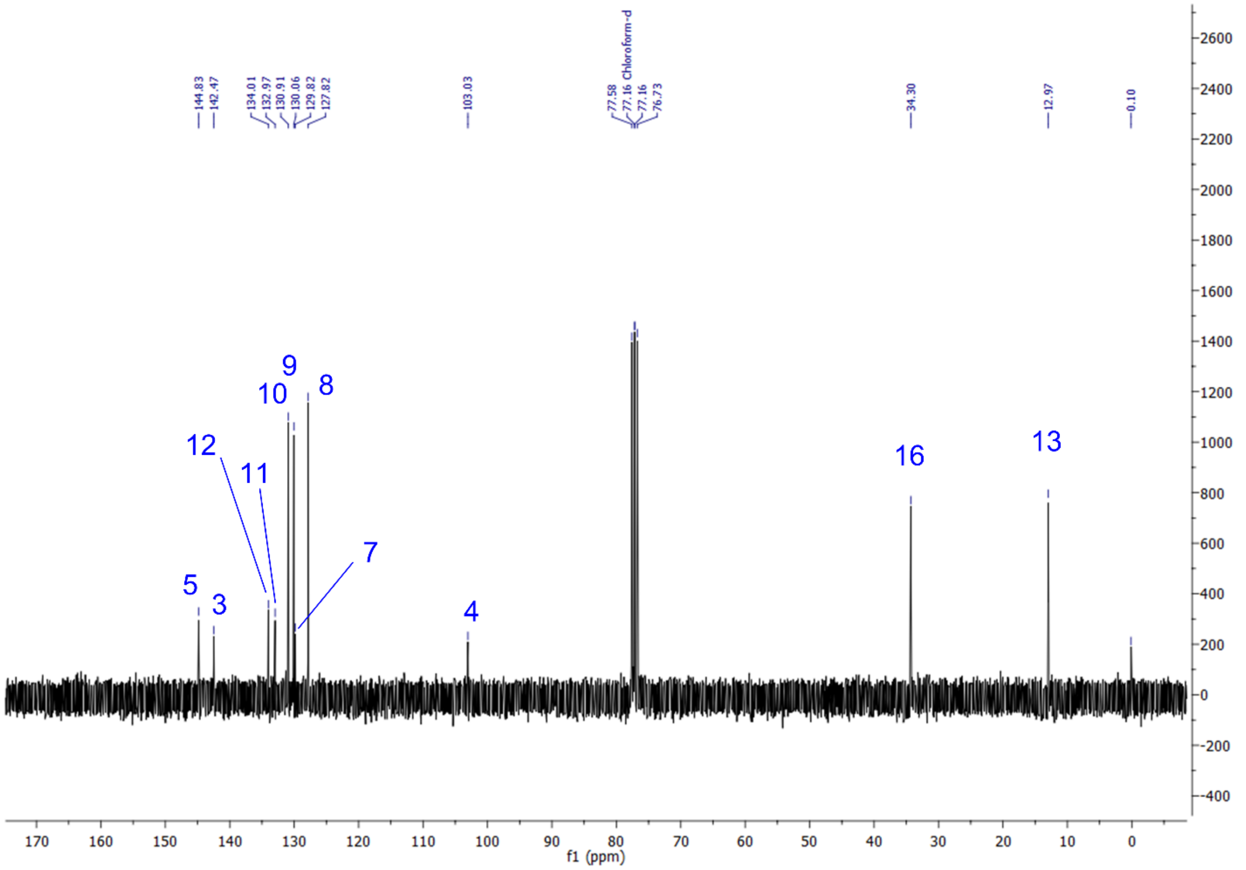
4-(3,4-dichlorophenyl)-1,5-dimethyl-1*H*-pyrazol-3-amine (5a)



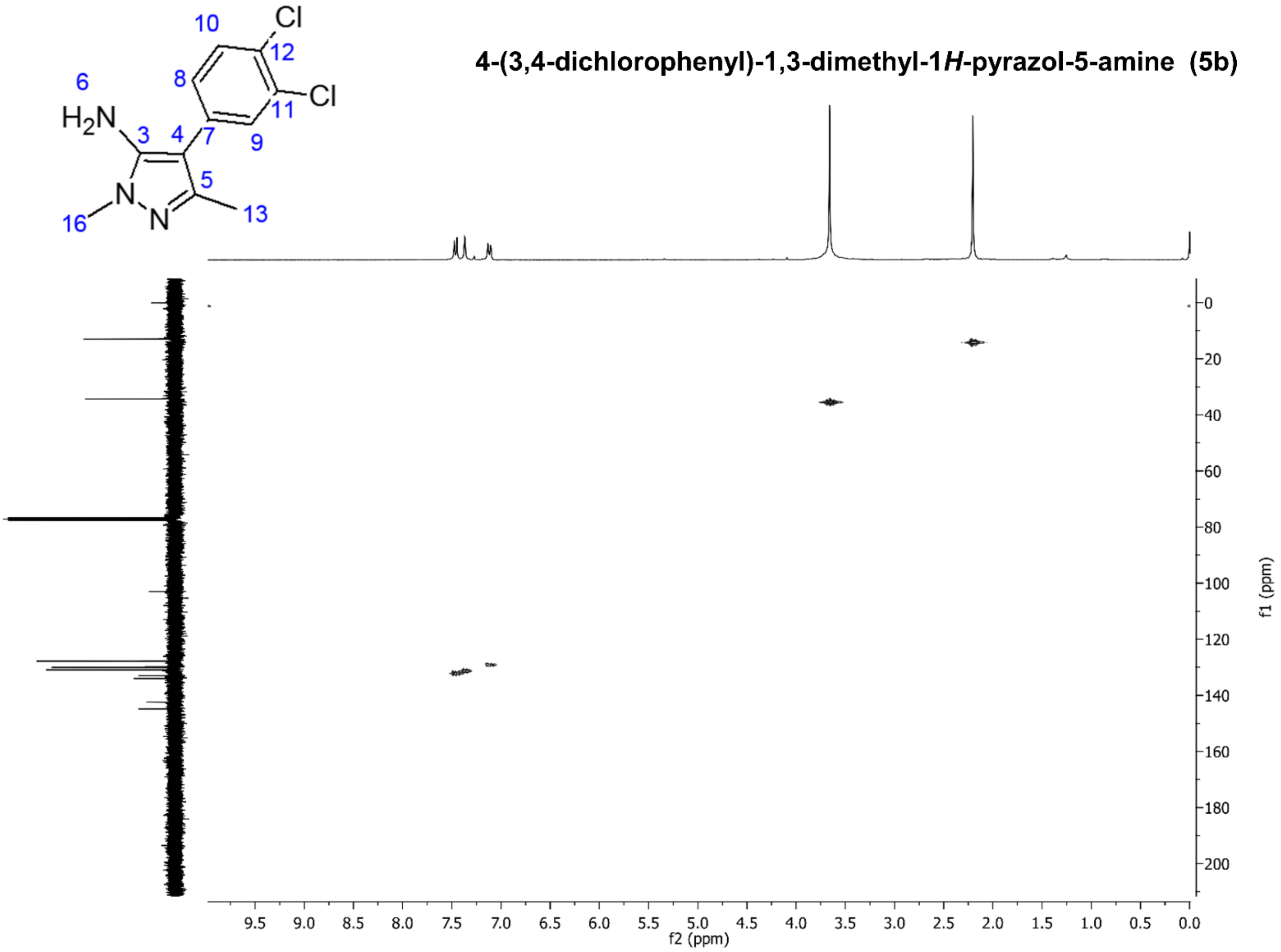


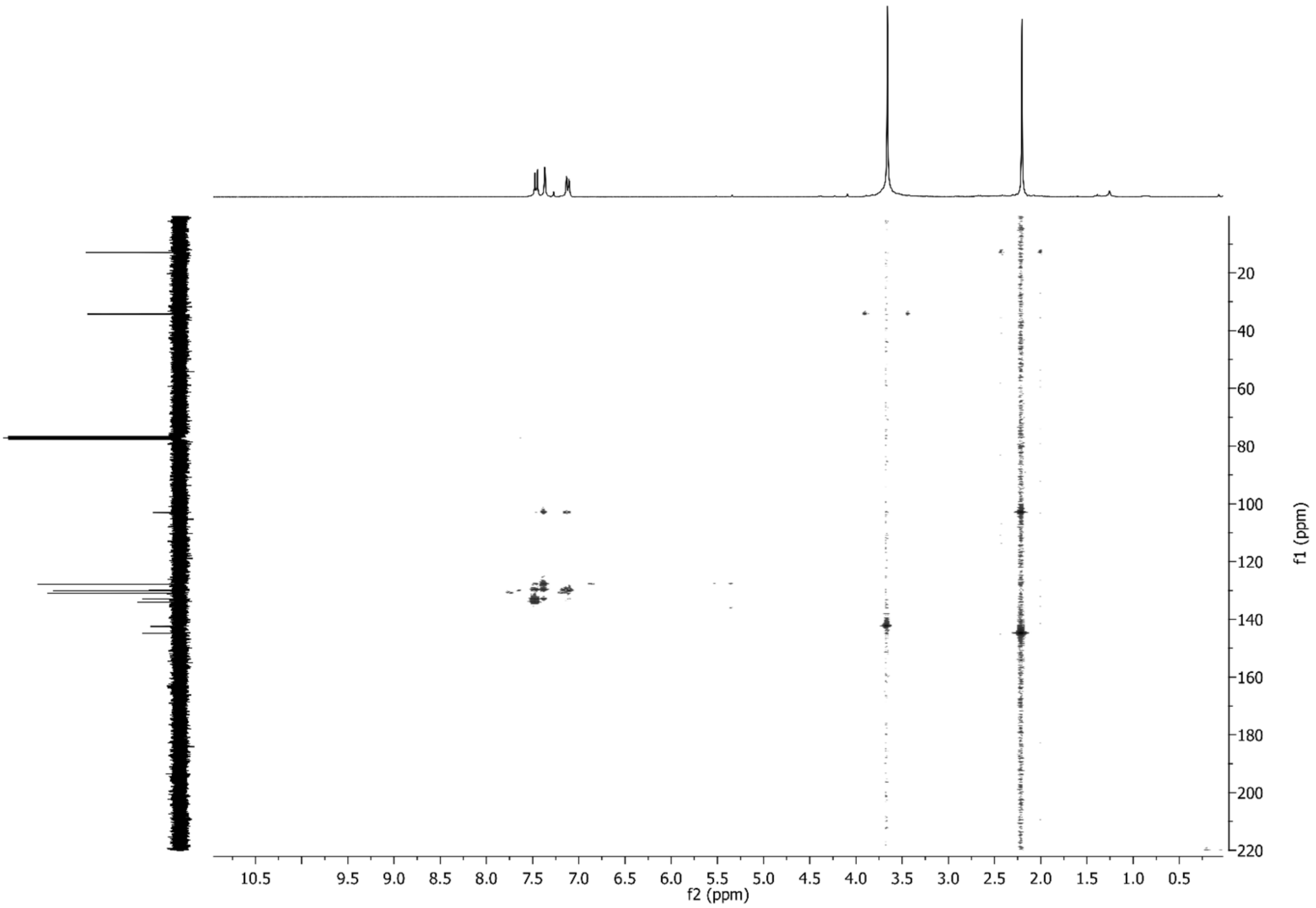
4-(3,4-dichlorophenyl)-1,3-dimethyl-1*H*-pyrazol-5-amine (5b)





4-(3,4-dichlorophenyl)-1,3-dimethyl-1*H*-pyrazol-5-amine (5b)





Biological Assays

Kinase assay. Reactions were carried out in 96 well microtiter plates in the presence of : 1 µM ATP, enzyme and substrate peptide at concentration reported in Table S1, reaction buffer (40 mM Tris-HCl, pH 7.5, 20 mM $MgCl_2$, 2 mM $MnCl_2$, 2 mM DTT, 100 µM Na_3VO_4, 0.1 mg/mL BSA), inhibitory compound or vehicle (DMSO), in a final volume of 25 µL. Temperature 25°C. Incubation length 60 min. Enzyme activity was evaluated using ADP-Glo kinase assay (Promega). Luminescence was measured using GloMax detection system (Promega). Peptide substrates were synthesized by Caslo. Recombinant human IKKβ, IKKε and NIK were provided by ProQinase. Recombinant human IKKα was from Life Technologies. IC_{50} values were obtained by exposing enzymes to 10 concentrations 100-50-10-5-1-0.5-0.1-0.05-0.01-0.005 µM of each compound in single assay. IC_{50} values were determined with nonlinear regression plots using GraphPad Prism6. Values are means ± SD of three independent experiments. During the setup, different amounts of substrate peptides and enzymes were tested to determine the linear range of the assay (Figure S1 and S2). The assays were validated by calculating the IC_{50} values of PS-1145 for IKKβ and staurosporine for IKKα, IKKε and NIK as reference inhibitors. (Figure S3).

	Peptide sequence	Substrate concentration	Enzyme concentration
IKKα assay	ERLLDDRHDSGLDSMKDEE	100 µM	8 ng/µL
IKKβ assay	ERLLDDRHDSGLDSMKDEE	200 µM	3.2 ng/µL
IKKε assay	ERLLDDRHDSGLDSMKDEE	200 µM	4 ng/µL
NIK assay	AKDVDQGSLCTSFVGTLQY	200 µM	5.2 ng/µL

Sequence of peptide substrate used for each kinase assay. Final concentration of peptide substrate and enzyme in each kinase reaction.

NF-κB gene reporter assay. Effects of Cpd A, PS-1145 and selected compounds on NF-κB gene reporter assay in EJM, SKBr3 and MDA-MB-231 cells. Cells were co-transfected with 3 µg of plasmids pGL4.32[*luc2P*/NF-κB-RE/Hygro] and pGL4.74[*hRluc*/TK] in the ratio 10:1 using Amaxa nucleofector II and cultured for 24 h at 37 °C in humidified CO_2 incubator. Compounds were added at increasing concentrations 10 µM, 25 µM, 50 µM, 100 µM and cells incubated for a further 6 h. The inhibitory activity of each compound was determined at the highest concentration resulting in ≥80% cell viability using Dual Luciferase reporter assay. At the end of incubation, the activities of firefly (*Photinus pyralis*) and Renilla (*Renilla reniformis*) luciferases were measured sequentially from a single sample. Luminescence of Photinus was measured and normalized to the luminescence of Renilla using GloMax detection system (Promega). Normalized signals were expressed as percentage of control (cells exposed to vehicle). Values are means ± SD of three independent experiments.

Cell viability assay. Cells were exposed to compounds at increasing concentrations. Cell viability was determined using Promega CellTiter Glo luminescent assay based on quantitation of the intracellular ATP. Cells (5 x 10^3 / well) were seeded in a white - opaque 96-well plate and exposed to increasing concentrations 10 µM, 25 µM, 50 µM,100 µM of each compound or vehicle (DMSO) for 6 h. Luminescence was measured using GloMax detection system (Promega).

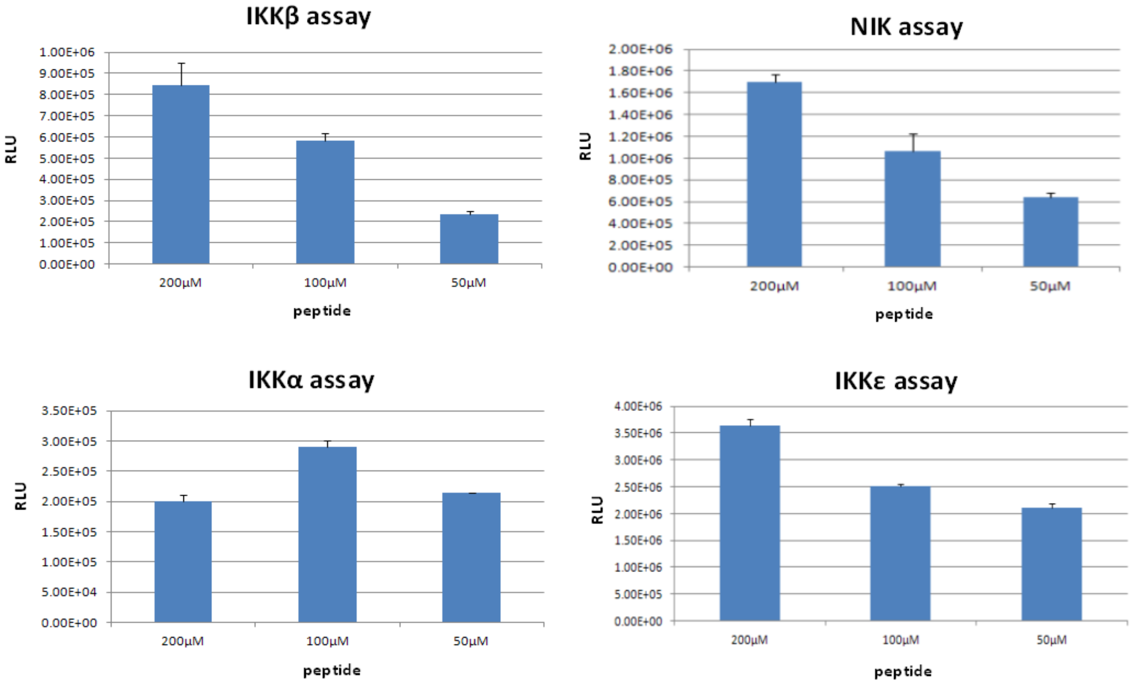


Figure S1. Increasing amounts of substrate peptides were tested to determine the linear range of the kinase assay.

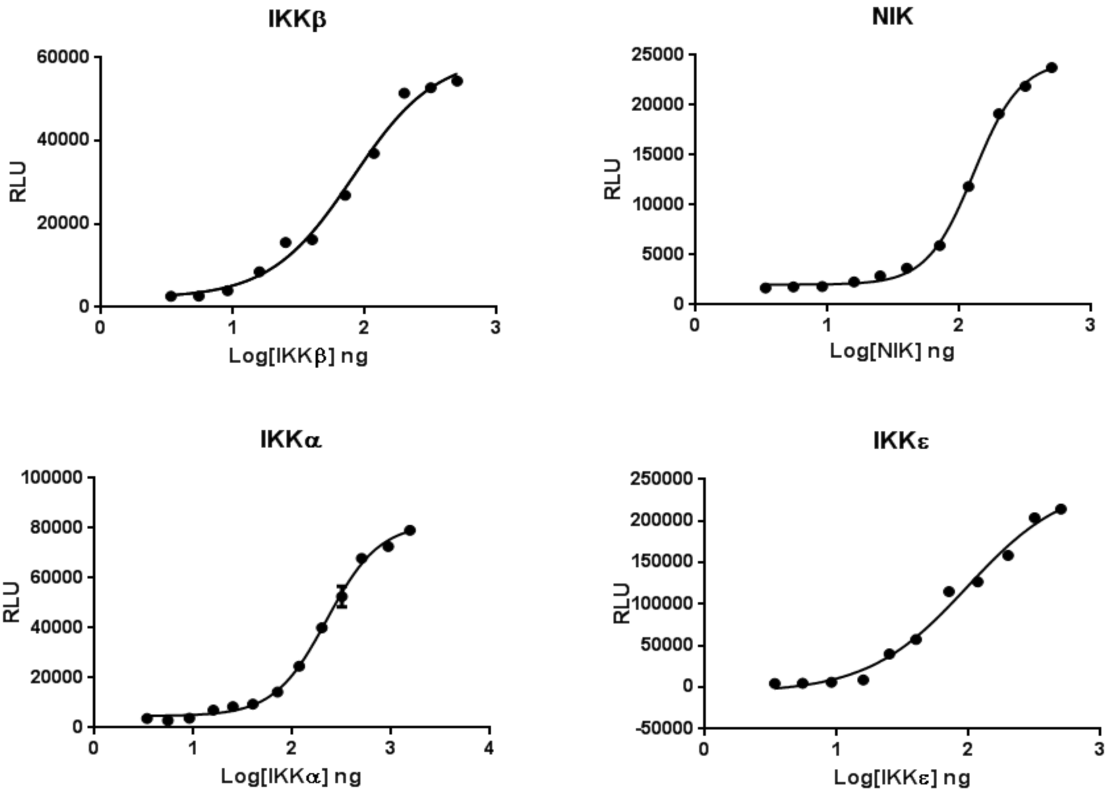


Figure S2. Increasing amounts of enzymes were tested to determine the linear range of the kinase assay.

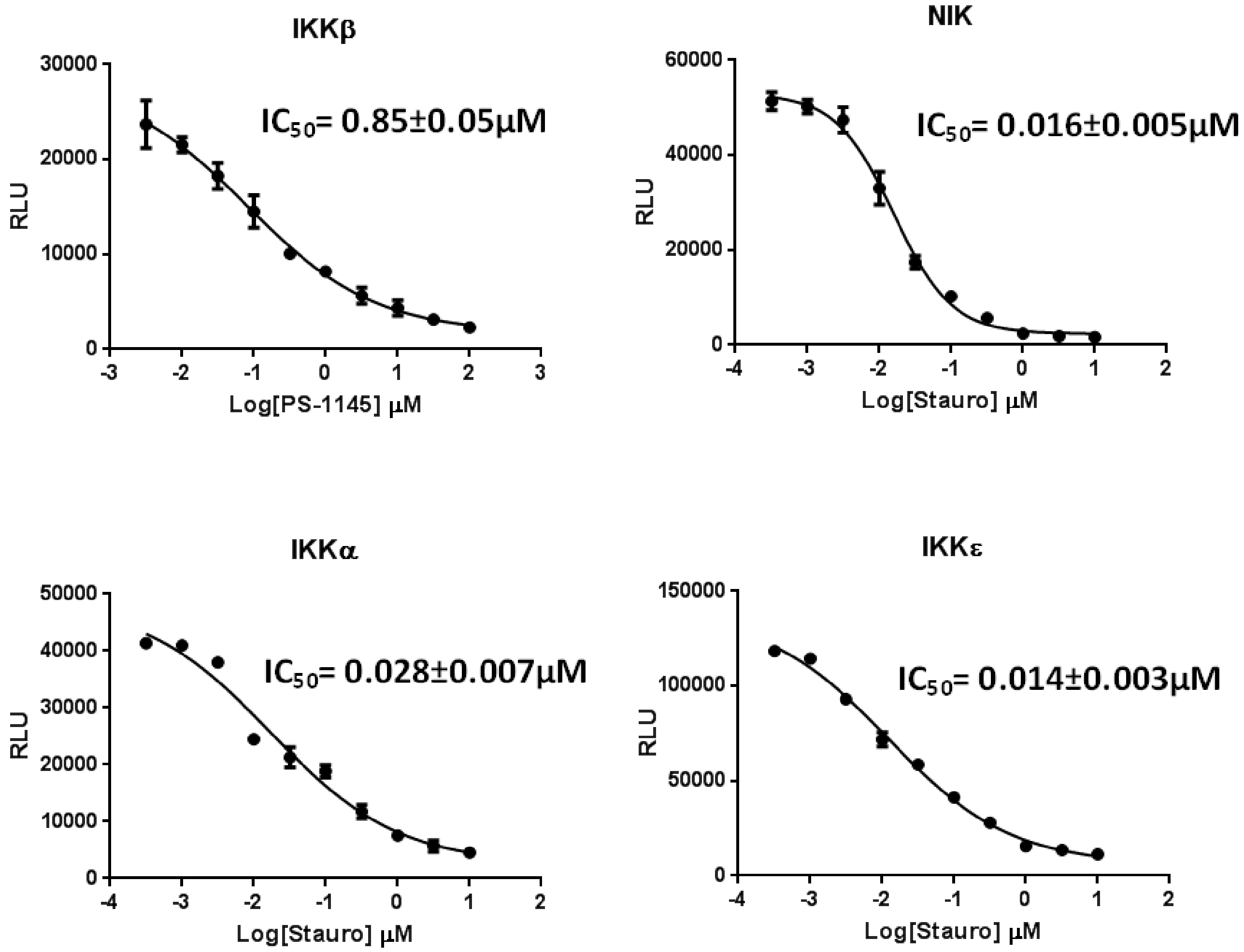


Figure S3. IC_{50} calculation of reference compounds: PS-1145 for IKKβ, Staurosporine for IKKα, IKKε and NIK