Supplementary Information

Synthetic routes for the synthesis of all novel compounds 4-19

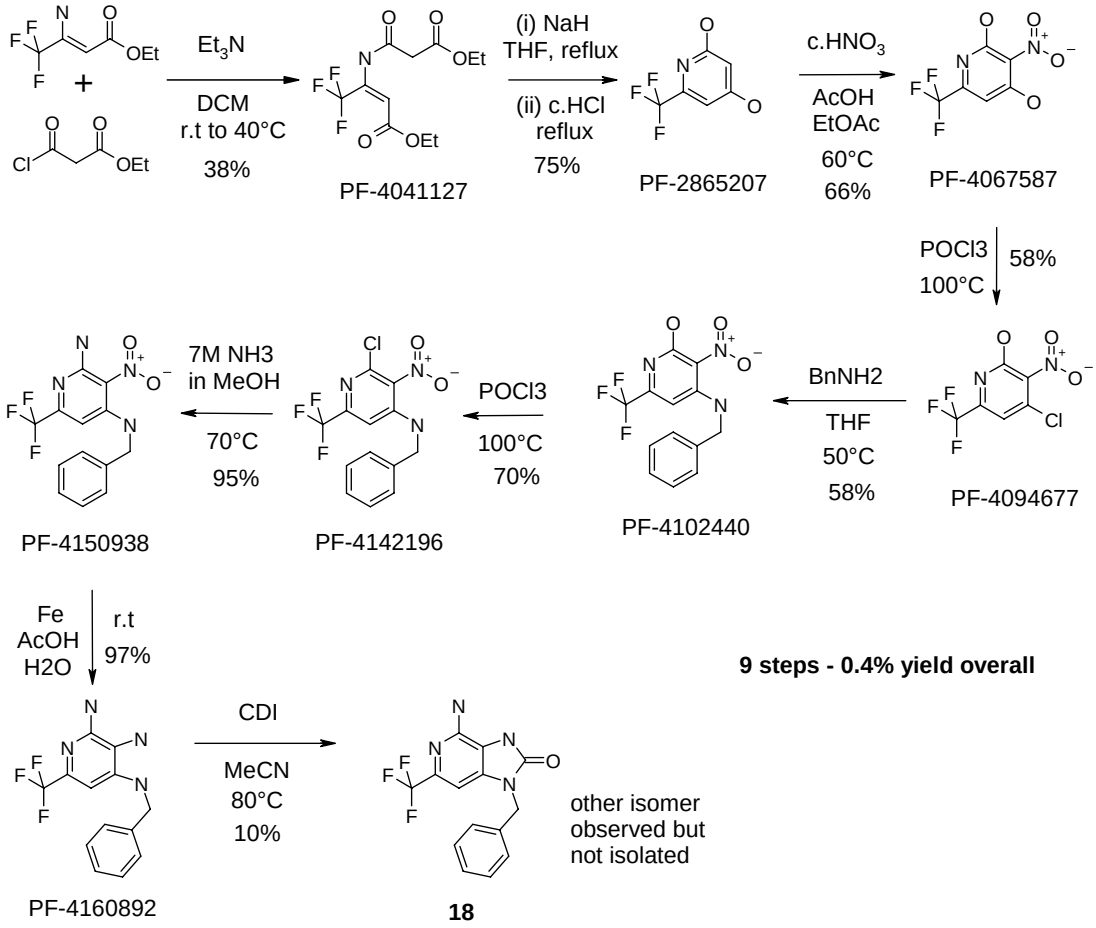
Full synthetic detail for the synthesis of compound 18

Abbreviations used:

CDI – *N*,*N*-Carbonyldiimidazole
THF - Tetrahydrofuran
MeCN - Acetonitrile
AcOH – Acetic acid
DCM - Dichloromethane
MS – Mass spectrometry
AP/ES – Atmospheric pressure chemical ionisation/electrospray ionisation
APCI – Atmospheric pressure chemical ionisation
LC-MS – Liquid chromatography-mass spectrometry
ES – Electrospray ionisation
MeOH - Methanol
SM – Starting material
DMSO - Dimethylsulfoxide
pTSA – 4-Methylbenzenesulfonic acid
IPA – Propan-2-ol
mCPBA – 3-Chloroperoxybenzoic acid
Ra-Ni – Raney Nickel
DME – 1,2-Dimethoxyethane
DIPEA – *N*,*N*-Diisopropylethylamine
EtOAc – Ethyl acetate
TLC – Thin Layer Chromatography
$MgSO_4$ – Magnesium sulphate
K_2CO_3 – Potassium carbonate
Et_3N- Triethylamine
NaH – Sodium hydride
c.HCl – Concentrated hydrochloric acid
c.HNO_3 – Concentrated nitric acid

$POCl_3$ – Phosphorus oxychloride
c.NH_3 – Concentrated ammonia solution
$NaHCO_3$ – Sodium bicarbonate

Compound 18



PF-4041127

Ethyl 3-amino-4,4,4-trifluorocrotonate (5g, 27.3mmol) was dissolved in DCM (25ml) and triethylamine (3.8ml, 27.3mmol) was added and the mixture was cooled in ice/water. The ethyl malonyl chloride (3.5ml, 27.3mmol) was added drop wise. The mixture was allowed to warm to r.t and stirred under N_2 at r.t overnight. The mixture was quenched with saturated $NaHCO_3$ (aq) (25ml) and extracted with DCM (50ml). The extract was washed with brine (25ml), dried over $MgSO_4$ and concentrated in vacuo to give the crude (6g). TLC analysis 90:10 Pentane:EtOAc showed some enamine SM (R_f 0.42) and a major product (R_f 0.11). Column chromatography through silica eluting with 98:2 Pentane:EtOAc gave fractions 8-23 which contained the SM. Increasing the eluent gradually to 80:20 Pentane:EtOAc gave fractions 37-76 which contained the major product (3.08g) as a pale yellow oil. ^1H NMR ($CDCl_3$, 400MHz) δ 10.74 (brs, 1H), 5.95 (s,1H), 4.30-4.25 (q, 4H), 3.48 (s, 2H), 1.34-1.30 (m, 6H). MS (AP/ES) $[M-H]^-$ @ 296m/z and a weak ion $[M+H]^+$ @ 298m/z.

PF-2865207

Sodium hydride (0.81g, 20.2mmol) was suspended in THF (20ml) and cooled in an ice bath. A solution of PF-4041127 (3g, 10.1mmol) in THF (10ml) was added drop

wise and then the mixture was heated to reflux and stirred at reflux under N_2 overnight. TLC analysis 95:5 DCM:MeOH showed no SM (R_f 0.81) and a new product spot (R_f 0.32). LC-MS showed $[M+H]^+$ @ 252m/z for the desired cyclised intermediate. The reaction was quenched with MeOH (15ml) and then concentrated in vacuo to give the crude intermediate. The intermediate was suspended in c.HCl (20ml) and the mixture was refluxed for two days. The reaction solution was adjusted to pH 7-8 with c.NH_3 and the resulting precipitate was collected by filtration, washed with water and MeCN and dried in vacuo at 40°C overnight. The dried sample gave the product (1.99g) as a beige solid which was triturated in MeCN and filtered, washing with fresh MeCN. The solid was dried over the weekend *in vacuo* at 40°C. The dried sample gave the title compound (1.35g) as a pale beige solid. ^{1}H NMR (d6-DMSO, 400MHz) δ 10.91 (brs, 2H), 6.70 (d,1H), 6.17 (s, 1H). MS APCI and ES shows $[M+H]^+$ @ 180m/z and $[M-H]^-$ @ 178m/z .

PF-4067587

PF-2865207 (1g, 5.6mmol) was dissolved in AcOH:EtOAc (8:2ml) at r.t. The mixture was heated at 60°C and a small portion of fuming nitric acid (0.05ml, 1.2mmol) was added drop wise. LC-MS showed formation of desired product $[M+H]^+$ @ 225m/z. The remainder of the fuming nitric acid (0.20ml, 4.9mmol) was added dropwise to the reaction mixture at 60°C. The mixture was stirred at 60°C for 1 hour. LC-MS showed no SM remaining $[M+H]^+$ @ 180m/z and the presence of the desired product $[M+H]^+$ @ 225m/z. The mixture was cooled to r.t. The bulk of the solvent was removed in vacuo and the residue was blown dry with nitrogen overnight. The residue was treated with water (10ml). The aqueous was extracted with EtOAc (30ml). The extract was washed with water (10ml) and brine (10ml), dried over $MgSO_4$ and concentrated *in vacuo*. The crude was triturated in pentane to remove the aliphatic impurity observed in the precursor. The solid was collected by filtration, washed with fresh pentane and dried in vacuo at r.t overnight to give the product (827mg) as a pale yellow solid. ^{1}H NMR (d6-DMSO, 400MHz) δ 13.16 (brs, 2H), 6.81 (s,1H). MS APCI and ES show $[M+H]^+$ @ 225m/z, $[M+Na]^+$ @ 247m/z and $[M-H]^-$ @ 223m/z.

PF-4094677

PF-4067587 (720mg, 3.2mmol) was dissolved in $POCl_3$ (5ml) and the mixture was refluxed under a caustic scrubber for 8 days. The mixture was cooled and the $POCl_3$ was removed in vacuo. The residue was dissolved in EtOAc (20ml) and added drop wise to stirred warm water (20ml) using ice to control the temperature. The aqueous was extracted with EtOAc (50ml). The extract was washed with brine (20ml), dried over $MgSO_4$, concentrated and dried *in vacuo* at r.t overnight to give the crude (1g). Column chromatography through silica eluting with 95:5 DCM:MeOH gave fractions 2-4 which contained the product (454mg) as a light brown solid. 1H NMR ($CDCl_3$,400MHz) δ 7.01 (s,1H), exchangeable proton not observed. MS APCI and ES show $[M-H]^-$ @ 241m/z.

PF-4102440

PF-4094677 (450mg, 1.9mmol) was dissolved in THF (5ml). Benzylamine (400ul, 3.7mmol) was added and the mixture was warmed to 50°C and stirred under N_2 for 5 days. TLC analysis 90:10 DCM:MeOH showed trace SM (Rf 0.14), benzylamine (Rf

0.31) and the desired product (Rf 0.48). The THF was removed in vacuo and the residue was treated with water (5ml). The aqueous was extracted with EtOAc (15ml) and the extract was washed with brine (5ml), dried over $MgSO_4$ and concentrated in vacuo to give the crude (710mg). Column chromatography through silica eluting with 99:1 DCM:MeOH removed higher running impurities. Increasing the eluent to 98:2 DCM:MeOH gave fractions 10-24 which contained the major product (335mg) as a pale yellow solid. 1H NMR ($CDCl_3$,400MHz) δ 9.44 (brs, 1H), 7.46-7.38 (m, 3H), 7.35-7.33 (m, 2H), 6.62 (s,1H), 4.63 (d, 2H). MS APCI and ES shows $[M+H]^+$ @ 314m/z, $[2M+Na]^+$ @ 649m/z and $[M-H]^-$ @ 312m/z.

PF-4142196

PF-4102440 (100mg, 0.3mmol) was dissolved in $POCl_3$ (2ml) and the mixture was refluxed under a caustic scrubber for 3 days The mixture was cooled and the $POCl_3$ was removed in vacuo. The residue was dissolved in EtOAc (10ml) and added dropwise to stirred warm water (10ml) using ice to control the temperature. The aqueous was extracted with EtOAc (20ml). The extract was washed with brine (10ml), dried over $MgSO_4$,concentrated and dried in vacuo at r.t overnight to give the crude (190mg). TLC analysis showed trace SM (Rf 0.18) and a product spot (Rf 0.82). Column chromatography through silica eluting with 99:1 DCM:MeOH gave fractions 2-3 which contained the product (74mg) as a yellow solid. 1H NMR ($CDCl_3$,400MHz) δ 7.45-7.37 (m, 3H), 7.32-7.31 (m, 2H), 7.05 (s, 1H), 6.92 (brs,1H), 4.54 (d, 2H). MS APCI and ES show $[M+H]^+$ @ 332m/z and $[M-H]^-$ @ 330m/z.

PF-4150938

PF-4142196 (69mg, 0.2mmol) was dissolved in 7N NH_3 in methanol (2.5ml) in a reactivial. The vial was sealed and heated at 70°C overnight. TLC analysis 98:2 DCM:MeOH showed no SM (Rf 0.88) and a product was present (Rf 0.76). The solvent was removed in vacuo and the residue was treated with water (10ml) and extracted with EtOAc (20ml). The extract was washed with brine (10ml), dried over $MgSO_4$ and concentrated in vacuo to give the product (62mg) as a bright yellow solid. 1H NMR ($CDCl_3$,400MHz) δ 9.48 (brs, 1H, 7.44-7.33 (m, 5H), 6.44 (s, 1H), 4.56 (d, 2H), not all exchangeables observed. MS APCI and ES showed $[M+H]^+$ @ 313m/z.

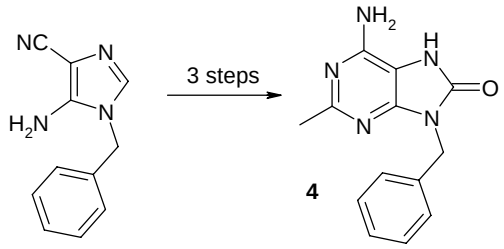
PF-4160892

PF-4150938 (55mg, 0.2mmol) was dissolved in AcOH:H_2O (4.6:0.4ml). The Fe powder (59mg, 1.1mmol) was added and the mixture was vigorously stirred at r.t under N_2 overnight. The yellow nitro colour had disappeared and an off-white precipitate had crashed out. LC-MS showed no SM and a new peak was seen for the desired product $[M+H]^+$ @ 283m/z. The reaction mixture was diluted with EtOAc (10ml) and water (10ml). The mixture was filtered through celite, washing through with EtOAc (~20ml). The phases were separated and the organic layer was washed with sat. $NaHCO_{3\ (aq)}$ (10ml) and brine (10ml), dried over $MgSO_4$ and concentrated in vacuo, azeotroping with toluene. The sample was dried in vacuo at 40°C overnight to give the product (48mg) as an off-white crystalline solid. 1H NMR ($CDCl_3$,400MHz) 7.41-7.31 (m, 5H), 6.59 (s, 1H), 4.67 (brs, 1H), 4.39 (d, 2H), not all exchangeable protons observed. MS ES and APCI shows $[M+H]^+$ @ 283m/z and $[M-H]^-$ @ 281m/z.

Compound 18

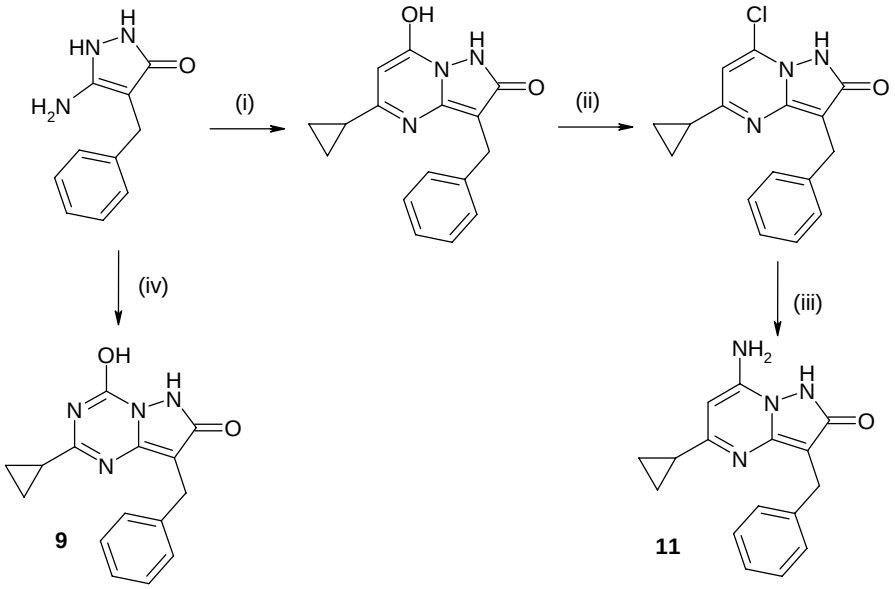
PF-4160892 (45mg, 0.2mmol) was dissolved in MeCN (2ml). CDI (52mg, 0.3mmol) was added and the mixture was heated at 80°C under N_2 for 2 days. TLC analysis 95:5 DCM:MeOH showed presence of SM (Rf 0.26) and three product spots (Rf 0.29, 0.21 and 0.15). Another 0.5eq CDI (13mg, 0.08mmol) was added and the mixture was heated at 80°C for another two days. TLC analysis still showed SM and the three products. The mixture was allowed to cool to r.t and the solvent was removed in vacuo. The residue was dissolved in EtOAc (10ml) and washed with 1N HCl (5ml), then water (5ml) and brine (5ml), dried over $MgSO_4$ and concentrated in vacuo to give the crude (140mg). A more concentrated TLC analysis showed that there were many trace impurities. Column chromatography through silica eluting with 80:20, then 70:30 DCM:EtOAc gave fractions 14-20 which contained a mixture of one possible product isomer and the SM (Rf 0.29 and 0.26 in 95:5 DCM:MeOH). Increasing the eluent to 60:40 DCM:EtOAc gave fractions 21-27 which contained the clean second product (Rf 0.21). LC-MS showed it had the desired molecular weight $[M+H]^+$ @ 309m/z. Combination and concentration in vacuo at 40°C gave the product (5mg) as an orange solid. 1H NMR (d6-DMSO, 400MHz) 10.46 (brs, 1H), 7.36-7.25 (m, 5H), 7.01 (s, 1H), 6.19 (brs, 2H), 5.04 (s, 2H). MS APCI and ES show $[M+H]^+$ @ 309m/z and $[M-H]^-$ @ 307m/z. Accurate mass: Adduct $[M+H]^+$, Error (ppm) -0.25, Exact 309.0958, Experimental 309.0957.

Compound 4



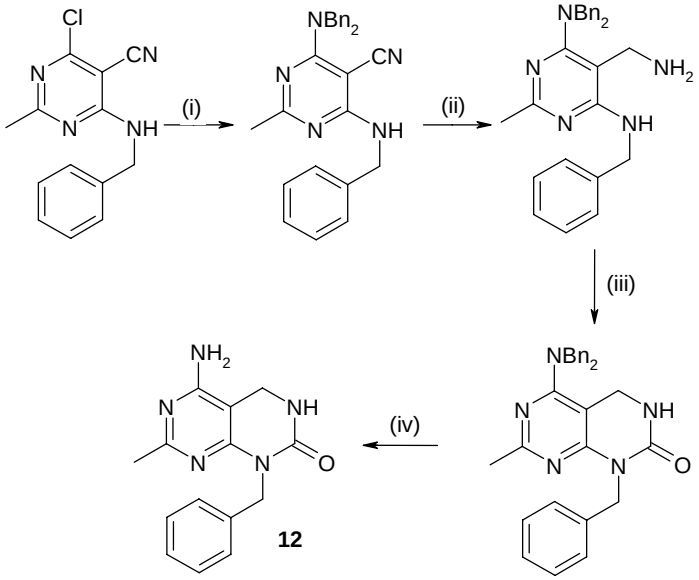
Synthesised as described in *Org. Biomol. Chem*., **2003**, 1, 1354-1365.

Compounds 9 and 11



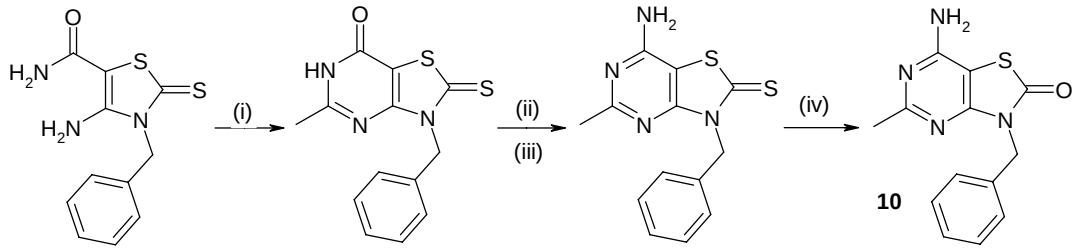
5-Amino-4-benzyl-1,2-dihydro-pyrazol-3-one was synthesised as described in patent WO 01/87889. (i) 3-Cyclopropyl-3-oxo-propionic acid ethyl ester (synthesised as described in *Synthesis*, **2004**, 16, 2629-2632), pTSA, dioxane, reflux, 88%; (ii) $POCl_3$, Et_3NCl, MeCN, reflux, 34%; (iii) NH_3, IPA, reflux, 21%; (iv) cyclopropylcarbamidine.HCl, NaOEt, EtOH reflux then diethylcarbonate, reflux, 5%.

Compound 12



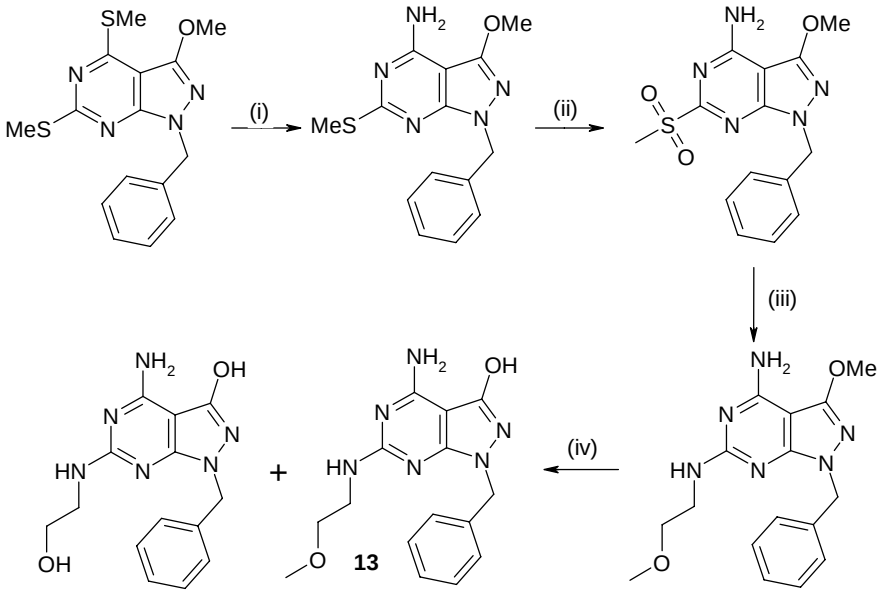
(i) Bn_2NH, Et_3N, THF, reflux, 58%; (ii) H_2, Ra-Ni, 2M NH_3 in MeOH, 50°C, 60psi, 99%; (iii) CDI, DME, 100°C, 46%; (iv) H_2, Pd-C, 50°C, 60psi, 1M HCl in MeOH, 56%.

Compound 10



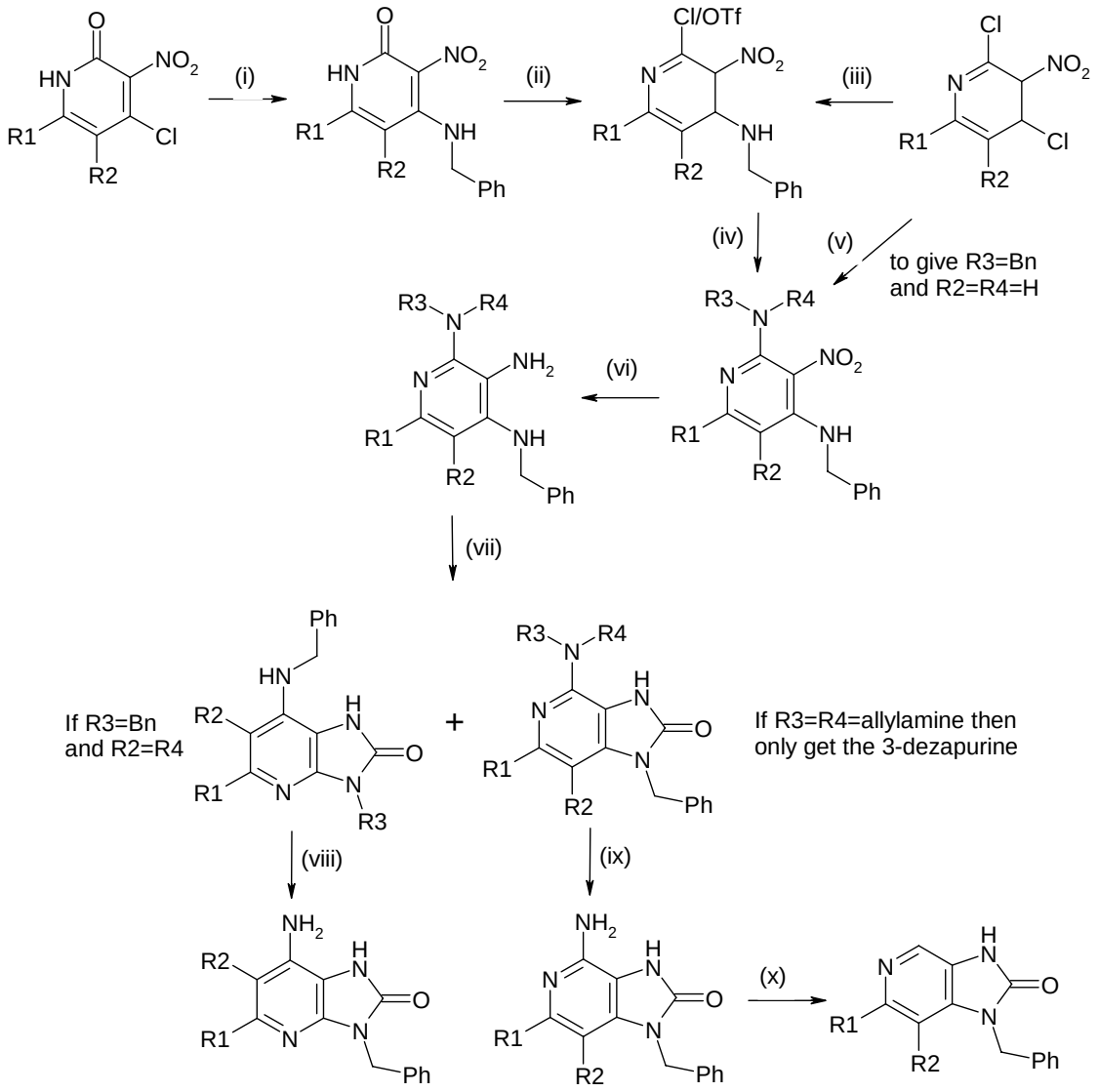
Thiazole-carboxamide was synthesised as described in *Arzneimettel-Forschung*, **1977**, 27 (9), 1652-1655. (i) Triethyl-ortho-acetate, Ac_2O, 140°C, 75%; (ii) $POCl_3$, reflux, 90%; (iii) 2M NH_3 in IPA, reflux, 80%; (iv) dimethyl sulphate, 130°C then H_2O reflux, 21%.

Compound 13



(i) 7N NH_3 in MeOH, 130°C, 40%; (ii) *m*CPBA, DCM, r.t, 70%; (iii) 2-methoxyethylamine, *n*-BuOH, DMSO, 90°C, 97%; (iv) c.HCl, r.t, 6% and 3% (methoxy and hydroxy ethylamine products).

Compounds 5, 7, 8, 14-17, 19



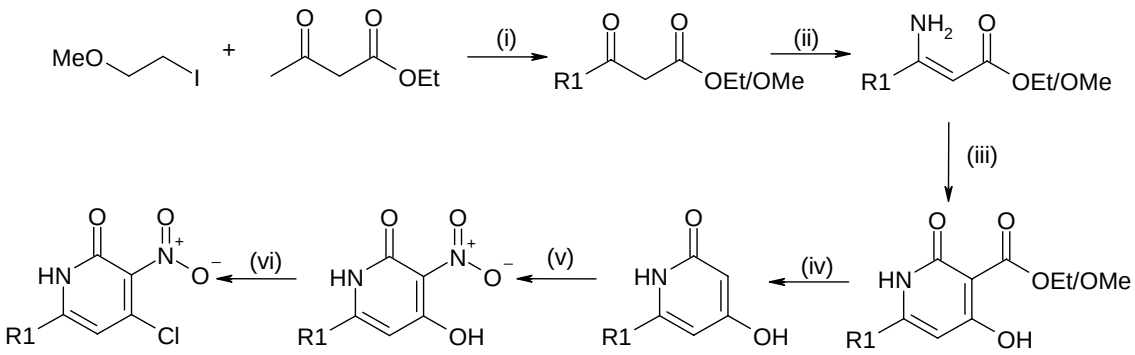
(i) $BnNH_2$, Et_3N or K_2CO_3, THF or MeCN, r.t, or 60°C, 94-96%; (ii) Tf_2O, Et_3N, DCM, 0°C-r.t, 100% or $POCl_3$, Et_3NCl, MeCN or propionitrile, reflux, 93-99%; (iii) 1eq $BnNH_2$, K_2CO_3, MeCN or THF, r.t-55°C 37-75%; (iv) diallylamine, K_2CO_3 or Et_3N or DIPEA, THF or MeCN or $EtOCH_2CH_2OH$, reflux,48-97% or 7M NH_3 in MeOH, 100°C, 100%; (v) 2eq $BnNH_2$, THF, r.t, 100%; (vi) Fe filings, 1N HCl, EtOH, 50-70°C, 70-87% or H_2, Ra-Ni, EtOH or MeCN, r.t, 50psi, 85-100%; (vii) CDI, MeCN, reflux, for R3=H or Bn and R2=R4=H get mixture of isomers which are separated by chromatography, 5%-100%; (viii) $Pd(OH)_2$, NH_4CO_2H, EtOH, 70°C, 5%; (ix) $BF_3.OEt_2$, 10% Pd-C, EtOH, reflux, 27% or $RhCl(PPh_3)_3$, MeCN, H_2O, reflux, 5-33%.

See **Table A** for details of compounds synthesised and source of starting materials.

Table A

Compound	R1	R2	Description	SM Source
5	Me	H	3-deazapurine	dichloropyridine is synthesised by treating commercially available hydroxynitropyridone with $POCl_3$, Et_4NCl in refluxing MeCN.
8	Me	H	1-deazapurine	
7	Me	Me	3-deazapurine	dichloropyridine is synthesised as described in Patent WO 2007093901.
14	*i*Pr	H	3-deazapurine	monochloropyridone is synthesised as described in patent WO 02/50062.
15	*n*Pr	H	3-deazapurine	monochloropyridone is synthesised as described in scheme X.
16	$MeOCH_2CH_2CH_2$	H	3-deazapurine	monochloropyridone is synthesised as described in scheme X.
17	H	H	3-deazapurine	dichloropyridine is synthesised by treating the commercially available hydroxynitropyridone with $POCl_3$, Et_4NCl in refluxing MeCN.
19	CF_3	H	6-des-amino 3-deazapurine	Compound 18

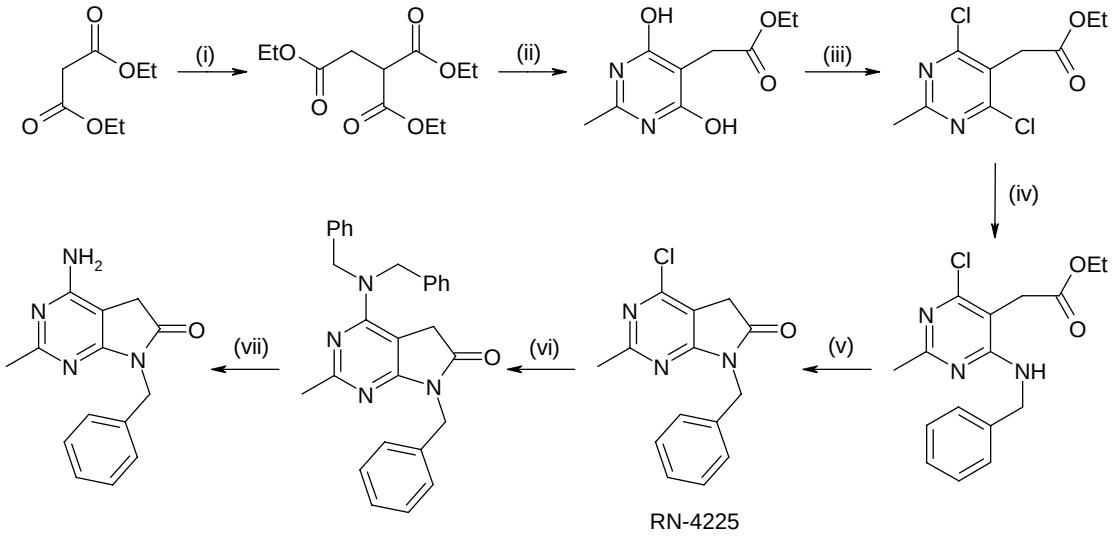
Scheme X



For R1= $MeOCH_2CH_2CH_2$: starting from ethyl acetoacetate. (i) NaH, THF, 0°C, then *n*-BuLi, 0°C-r.t, 40%, (ii) NH_4OAc, MeOH, r.t, 91%; (iii) diethylmalonate, Na/EtOH, Toluene, 60°C, 33%; (iv) NaOH, H_2O, reflux, 100%; (v) HNO_3/H_2SO_4, 0°C, 100%; (vi) $POCl_3$, Bu_4NCl, MeCN, 60°C, 41%.

For R1=nPr: starting from commercially available methyl n-butyrylacetate (ii) NH_4OAc, MeOH, r.t, 100%; (iii) diethylmalonate, Na/EtOH, 60°C, 58% mixture of ethyl and methyl esters; (iv) c.HCl, reflux, 95%; (v) HNO_3/AcOH, 0°C, 96%; (vi) $POCl_3$, $BnEt_3NCl$, MeCN, reflux, 17%.

Compound 6



RN-4225 prepared as described for similar compounds in *J. Prakt. Chem.* **2000**, 342(5), 504-507 and patent WO1995033750(A1) 36-37.
(i) Ethyl bromoacetate, NaOEt; (ii) acetamidine, NaOEt;, (ii) $POCl_3$; (iv) $BnNH_2$; (v) pTSA, Dean-Stark; (vi) Bn_2NH 100°C, 7%; (vii) H_2, Pd, EtOH, 50°C, 60psi, 28%.

TLR selectivity profiling data performed at Invivogen with Compound 18

I/ METHOD :

293-TLR cell lines :
Samples and controls are tested in duplicate on recombinant HEK-293 cell line that functionally express a given TLR protein as well as a reporter gene driven by NFKB promoter.
TLR activation results are given as optical density values (OD).

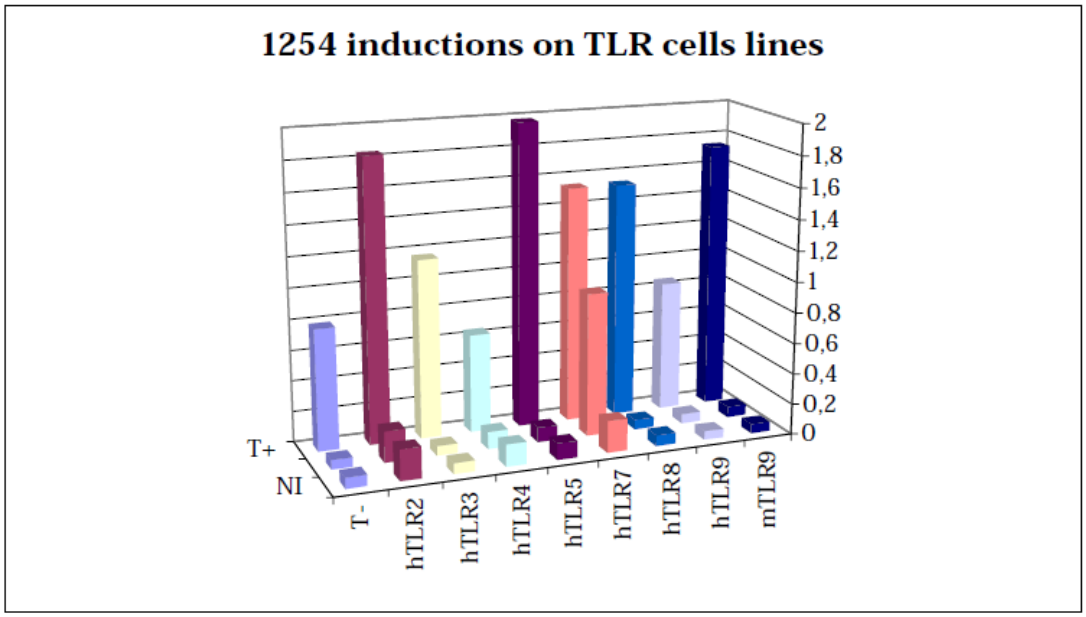
Positive controls :
Each 293-TLR cell line is induced with a known specific ligand as a positive control. Samples are tested in parallel.
Positive control ligands are tested as followed:

For the 293-hTLR2 cell line the ligand tested is : PAM2 (10 ng/ml)
For the 293-hTLR3 cell line the ligand tested is : Poly I :C (100 ng/ml)
For the 293-hTLR4 cell line the ligand tested is LPS K12 à 100 ng/ml
For the 293-hTLR5 cell line the ligand tested is : Flagellin (1µg/ml)
For the 293-hTLR7 cell line the ligand tested is : R848 (10 µg/ml)
For the 293-hTLR8 cell line the ligand tested is : R848 (10 µg/ml)
For the 293-hTLR9 (human) cell line the ligand tested is : ODN 2006 (10 µg/ml)
For the 293-hTLR9 (mouse) cell line the ligand tested is : ODN 1826 (10 µg/ml)
Each of these ligands solution contain

Negative control :
A recombinant HEK-293 cell line for the reporter gene only was used as a negative control for the TLR cell lines.
The negative control value for each clone is the background signal of these non induced clones.

Sample testing :
Samples have been re suspended in DMSO as required then diluted 100 times in water. This 100 fold dilution will be considered as the "direct solution" for all the TLR screening experiment.
20 µl of each "direct solution" is used to stimulate all the cell lines in a 200µl of reaction volume (an additional ten times dilution).

Résults :
Results are given under a histogram presentation as followed



	T-	hTLR2	hTLR3	hTLR4	hTLR5	hTLR7	hTLR8	hTLR9	mTLR9
T+	0,79	1,84	1,166	0,644	1,982	1,539	1,534	0,852	1,739
d	0,06	0,202	0,062	0,11	0,096	0,933	0,063	0,061	0,071
NI	0,071	0,203	0,07	0,144	0,106	0,209	0,072	0,057	0,061

Stimulation with 1254 gives specific activation on the 293 hTLR7 clone only