Discovery of Novel Inhibitors of UDP-N- Acetylenolpyruvylglucosamine Reductase (MurB) from *Pseudomonas aeruginosa*, an opportunistic infectious agent causing death in cystic fibrosis patients

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ABSTRACT: *Pseudomonas aeruginosa* is of major concern for cystic fibrosis patients where this infection can be fatal. With the emergence of drug-resistant strains there is an urgent need to develop novel antibiotics against *P. aeruginosa*. MurB is a promising target for novel antibiotic development as it is involved in the cell wall biosynthesis. MurB has been shown to be essential in *P. aeruginosa* and importantly no MurB homologue exists in eukaryotic cells. A fragment-based drug discovery approach was used to target *Pa* MurB. This led to the identification of a number of fragments which were shown to bind to MurB. One fragment, a phenylpyrazole scaffold was shown by ITC to bind with an affinity of K_d = 2.9 mM (LE 0.23). Using a structure guided approach different substitutions were synthesised and the initial fragment was optimised to obtain a small molecule with K_d = 3.6 μM (LE 0.35).

INTRODUCTION

Pseudomonas aeruginosa, a rod-shaped gram-negative bacterium, is a frequent opportunistic agent of hospital-acquired infections.[1] In cystic fibrosis (CF), *P. aeruginosa* is responsible for 80% of the lung infections of CF patients by the age of eighteen.[2] Moreover, acquisition of *P. aeruginosa* by CF patients leads to 2.6 times higher risk of death, making chronic infection by this pathogen the major cause of death in this type of patient.[3,4] This bacterium has become resistant to current antibiotics such as β-lactams due to its low membrane permeability, abundant efflux pumps and various antibiotic-degradative enzymes.[5] Currently, the antibiotics used against *P. aeruginosa* in the clinic are limited and resistant strains are increasing in the hospitals worldwide.[6] Consequently, *P. aeruginosa* has been classified as one of the six pathogens in the world that most require new antibacterial drugs.[6–9] Therefore, it is necessary to design new antibiotics that act on novel targets of *P. aeruginosa*.

In the cell wall of most bacteria, one of the main components is peptidoglycan, a peptide cross-linked polymer of alternating N-acetyl-glucosamine and N-acetyl-muramic acid (UNAM) units.[10–12] The bacterial cell wall offers osmotic stability, and as a result, the enzymes involved in peptidoglycan biosynthesis are essential for bacterium survival. Therefore, the inhibition of any of these enzymes results in loss of bacterial cell wall followed by cell lysis.[13] In recent decades, attention has been paid to the family of Mur enzymes,[14] which synthesise the cell wall from the nucleotide sugar uridine diphosphate N-acetylglucosamine. Antibiotics that block one of these enzymes are of interest and some have already been developed. One example is fosfomycin, which inhibits MurA (UDP-N-acetylglucosamine-enolpyruvyltransferase), the Mur enzyme that catalyzes the first step of the cell wall biosynthesis.[15] Unfortunately, *P. aeruginosa* has also developed resistance to this antibiotic by enzymatic deactivation or by decreasing its cellular uptake.[16]

The second enzyme in the pathway, UDP-*N*-acetylenolpyruvoylglucosamine reductase (MurB), has also proved to be of interest as a novel target in *P. aeruginosa* because no MurB homologue exists in eukaryotic cells. MurB catalyses the reduction of UDP-*N*-acetylglucosamine enolpyruvate (UNAGEP), the product of MurA, to *N*-acetyl-muramic acid (UNAM), using FADH2 (Figure 1).[17,18]

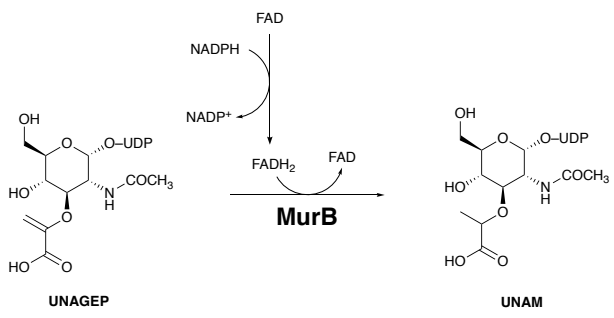


FIGURE 1. MurB enzymatic reaction.

P. aeruginosa MurB (*Pa* MurB) is a monomeric enzyme compromised of three different domains (PDB code 4JB1) (Figure 2). There are domain I (aminoacids 1–75 and 336–339), domain II (aminoacids 76–191) which binds the FAD cofactor, and domain III (aminoacids 192–335), which has binding sites for NADPH cofactor and substrate (UNAGEP).[12] The X-ray crystal structure of the complex of *Pa* MurB with FAD and $NADP^+$ (PDB code 4JB1) and the crystal structure of *E. coli* MurB in complex with the substrate UNAGEP (*Ec* MurB, PDB code 2MBR)[19] have very similar 3-dimensional structures with identical residues in the active site[12] and FAD bound in the same manner. Both enzymes are type I UNAGEP reductases, similar to the *S. aureus* MurB (*Sa*MurB, type IIa) and Thermus caldophilus MurB (type IIb) structures,[20,21] but lacking an α-helix and a protruding βαββ fold in the domain III (Figure 2). Extensive biochemical characterization of *Ec* MurB has led to the description of the reaction as a ping-pong bi-bi mechanism,[22] where first the NADPH transfers an hydrogen to FAD, followed by $NADP^+$ dissociation from the enzyme. Successively, UNAGEP binds and the hydride is transferred from $FADH_2$ to the vinyl ether of UNAGEP, becoming UNAM.

Several MurB inhibitors have been designed using structure-based approaches,[23] based on the co-crystal structures of *Ec* MurB and *Sa* MurB.[24–26] However, currently there are no inhibitors described against *Pa* MurB. In the present study, an in-house fragment library was screened against *Pa* MurB and this led to the identification of small molecules that bind to *Pa* MurB. X-ray crystallography of one of the fragment hits was shown that it binds at the interface of FAD and the substrate binding pockets, which offers a novel strategy for the development of *Pa* MurB inhibitors.

RESULTS

Chemical scaffold identification. A library of 960 rule-of-three compliant fragments was screened at concentration of 5.0 mM against MurB using differential scanning fluorimetry (DSF) (Table 1). Fourteen fragments were shown as positive hits and they were verified in triplicate at 1.0 mM concentration. As a result, nine hits still had a positive thermal shift compared to the negative control. In the screening, the MurB–FAD complex was used due to the high affinity of FAD for MurB.[12]

The fragment hits identified were validated by X-ray crystallography. However, only the pyrazole derivative **4** was successfully crystallised in complex with *Pa* MurB (Figure 3). X-ray crystallography showed that this fragment binds in the catalytic pocket in close proximity to FAD.[17] The binding affinity of fragment **4** was then determined using isothermal titration calorimetry (ITC) where the affinity was measured to be K_d = 2.8 mM.

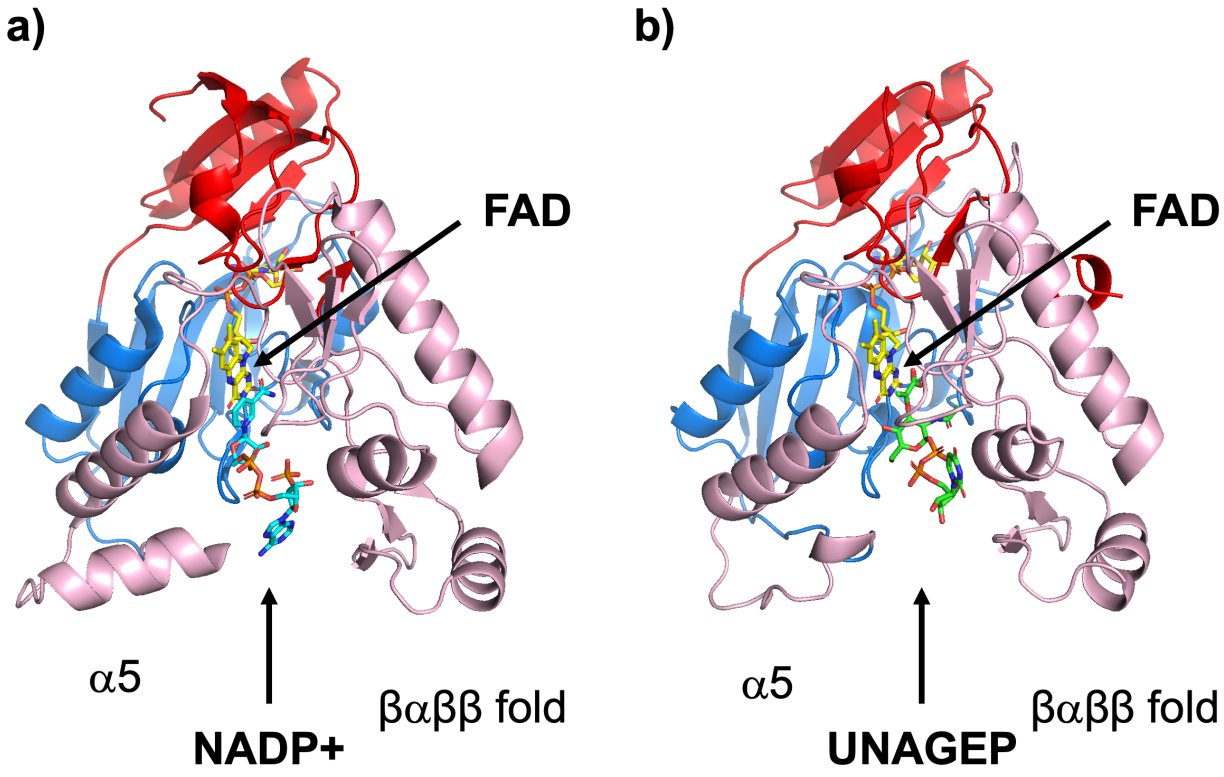


FIGURE 2. Catalytic pocket and domains distribution of MurB, illustrating domain I in red, domain II in blue and domain III in pink. (a) *Pa* MurB in complex with NADP+ (PDB code 4JB1).[12] (b) *Ec* MurB in complex with UNAGEP (PDB code 2MBR).[19] FAD is shown in yellow, NADP+ in cyan and UNAGEP in green.

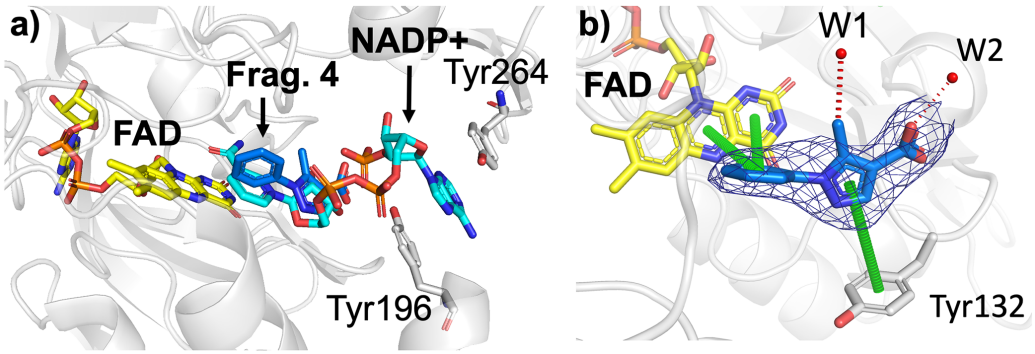
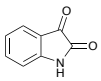
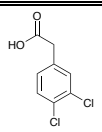
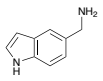
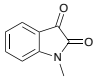
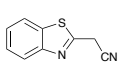
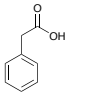
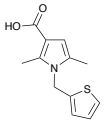
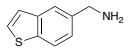
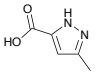
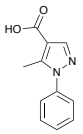
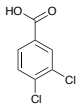
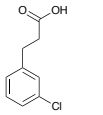
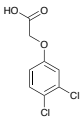
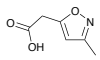


FIGURE 3. X-ray structure of fragment **4** (dark blue) (a, b, PDB code 7OR2) and NADP+ (cyan)(a, PDB code 4JB1)[12] bound to the active site of *Pa* MurB. (a) Superimposition of *Pa* MurB in complex with fragment **4** (dark blue) and *Pa* MurB in complex with NADPH (cyan). (b) X-ray structure of fragment 4 bound to the active site of *Pa* MurB. FAD is depicted in yellow. Arpeggio[27] was used to analyze the interactions. Hydrogen bonds are shown as red dashed lines, π–π interactions are indicated in green dashed lines and water molecules are shown as red dots. The final 2F0 − Fc map around the ligand is shown in blue at 1σ.

Table 1. Hits from differential scanning fluorimetry at a ligand concentration of 5.0 mM and 1.0 mM.

Fragment		ΔT_M (°C) (±SEM) 5 mM	1 mM	Fragment		ΔT_M (°C) (±SEM) 5 mM	1 mM	Fragment		ΔT_M (°C) (±SEM) 5 mM	1 mM
	**1**	+3.5	+1.0 (0)		**6**	+1.5	+1.0 (0)		**11**	+1.0	+0.2 (0.2)
	**2**	+4.0	+1.0 (0)		**7**	+1.0	0.0 (0)		**12**	+1.0	0.0 (0)
	**3**	+2.0	+1.5 (0)		**8**	+1.0	0.0 (0)		**13**	+1.0	0.0 (0)
	**4**	+1.5	+0.5 (0)		**9**	+1.0	+0.5 (0)		**14**	+1.0	+0.5 (0)
	**5**	+1.5	+1.0 (0)		**10**	+1.0	0.0 (0)				

Fragment hits show shifts in protein melting temperatures (ΔT_M) from DMSO-d_6 at 10 µM *Pa* MurB concentration to the two different ligand concentrations. Each ligand is screened at a concentration of 5.0 mM (n = 1) and at 1.0 mM (n = 3).

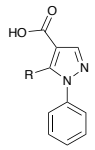
All the analogues were screened using two different biophysical techniques, DSF (at 5 mM and/or 1 mM) and surface plasmon resonance (SPR) at 1mM. Fragments with significant increases of ΔT_M or R higher than fragment **4** ($R>R_{f4}$) (see Figure 4) were validated by ITC. The goal was to identify potential binding analogues with higher binding affinity than fragment **4**.

Modification of the methyl group. The methyl group on the pyrazole ring of fragment **4** points into a small pocket that the $NADP^+$ does not fill (see Figure 3.B). This pocket contains mainly hydrophilic residues such as Glu335,

Asn243, Ser239 and Arg166. In addition, there is also a water molecule (W1, Figure 3.B) in this small pocket, which is tightly bound to the hydrophilic residues by several hydrogen bonds. In order to further explore the structure-activity relationships (SAR) with this pocket the methyl substituent was replaced by different groups that could possibly interact with the hydrophilic residues or with the water molecule (fragments **15**–**18**) (Table 2).

The substitution at the 5-position (R in Table 2) in the pyrazole ring with a trifluromethyl group (fragment **18**), was shown to give an increase in affinity where an K_d of 0.25 ± 0.04 mM was measured using ITC. This fragment was successfully crystallised in complex with *Pa* MurB and the X-ray crystal structure shows a similar binding mode to the original fragment hit **4** (Figure 5.A). However, the trifluromethyl group is shown to interacts with the water W1 in the small pocket and another water (W3) next to it, these interactions displace the rest of the fragment **18** closer to the alpha helix 6 (see Figure 5.A).

Table 2. Structure and biophysical data for fragments 4 and 15–18.

Fragment		R	ΔT_M (°C) (±SEM) 1.0 mM	SPR RU>RU_{f4} 1.0 mM	K_d (mM) ITC	LE
	**4**	Me	+0.5 (0)	–	2.88 ± 0.25	0.23
	15	NH_2	0.0 (0)	no	–	–
	16	OH	+0.7 (0.2)	–	No binding	–
	17	CH_2OH	+0.2 (0.2)	–	–	–
	18	**CF_3**	**+1.0 (0)**	**no**	**0.25 ± 0.04**	**0.27**

Shift in protein melting temperatures (ΔT_M) from DMSO-d_6 at 10 µM *Pa* MurB concentration and at 1.0 mM fragment concentrations (n = 3). SPR RU of each fragment at 1 mM in comparation with RU of fragment **4** (RU>RU_{f4}, RU=Response units) (n = 2). K_d calculated using ITC (50 µM *Pa* MurB, 3.0 mM fragment). Ligand efficiencies were calculated as LE = –(RTlnK_d)/(number of heavy atoms) and are reported in kcal/mol per heavy atom. Dash entries in the table mean not measured.

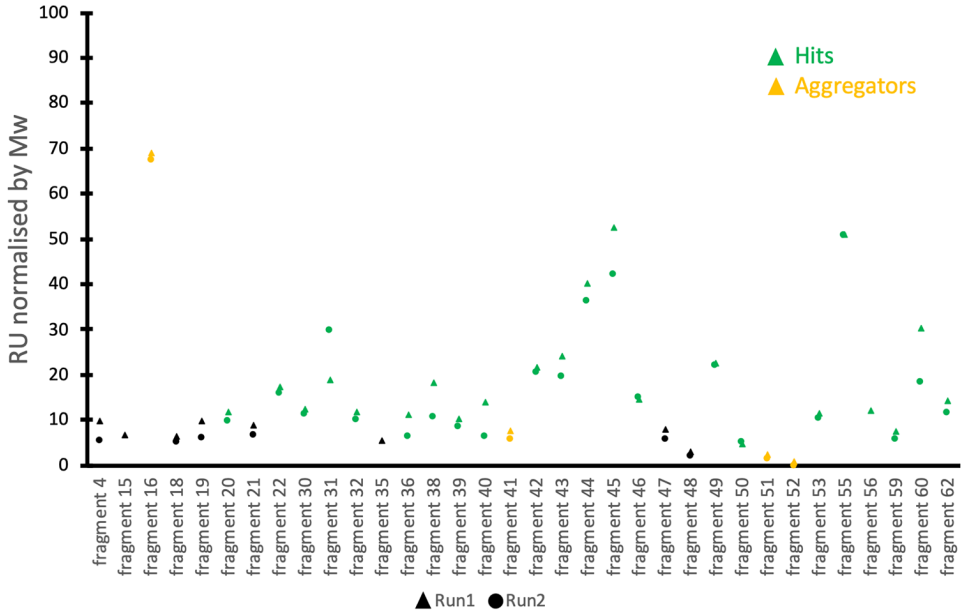


FIGURE 4. Hit plot displaying results of analogues in SPR assay. Hits were characterized as higher RU (Response units) normalized than fragment **4**. Analogues in yellow showed curves that they did not reach equilibrium indicating aggregation and were ruled out as hits. Mw, molecular weight.

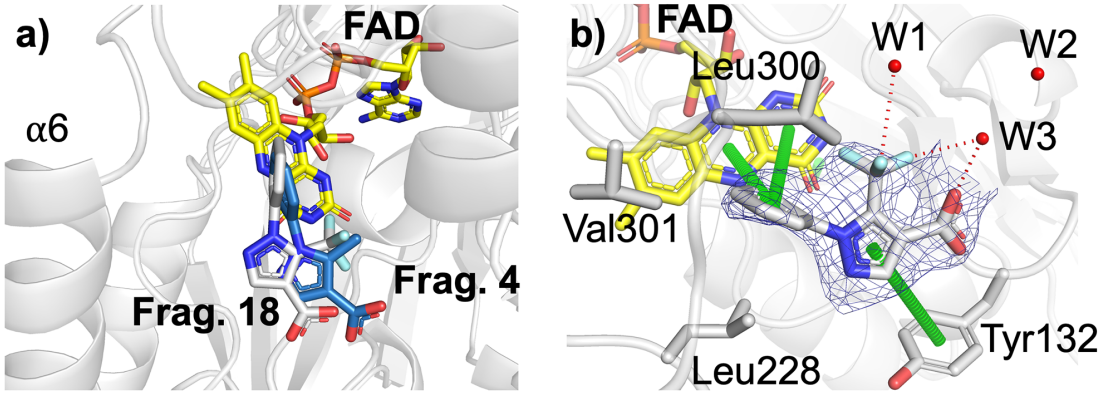


FIGURE 5. X-ray structures of fragment **4** and fragment **18** bound to the active site of *Pa* MurB. (a) Superimposition of *Pa* MurB in complex with fragment **4** (dark blue) and with fragment **18** (white). (b) X-ray structure of fragment **18** bound to the active site of *Pa* MurB. FAD is depicted in yellow. Arpeggio[27] was used to analyze the interactions. Hydrogen bonds are shown as red dashed lines, π–π interactions are indicated in green dashed lines and water molecules are shown as red dots. The final 2F0 − Fc map around the ligand is shown in blue at 1σ.

Modification of the pyrazole ring. The CH on the 3-position of the pyrazole ring of the fragment **4** was changed for a N to observe if a possible hydrogen bond interaction could be formed with residue Tyr132 (fragment **19**). This change did not give an increase in the melting temperature (Table 3). However, the X-ray crystal structure of *Pa* MurB in complex with fragment **19**, shows a 180º flip of the five-membered ring due to the formation of an interaction of the N at the 3-position of the triazole with Arg166 (see Figure 6). In addition, this methyl group points to a small pocket which mainly contains hydrophilic residues such as Lys227, Tyr132 and Arg196, and a highly bound water molecule. These observations could suggest that an analogue ring consisting of a pyrrole or imidazole containing two substituents at the 5- and 2-positions could also be of interest.

Therefore, the pyrrole (fragment **20**) and imidazole (fragment **21**) analogues were synthesised, screened and compared to fragment **4** using two different biophysical techniques, DSF and SPR (Table 3). Fragments with significant increase of ΔT_M or R higher than fragment **4** ($R>R_{f4}$) at 1.0 mM were validated again by ITC. The pyrrole analogue **20** showed a greater thermal shift. Interestingly, one pyrrole derivative (fragment **3**) was also identified in the initial library of hits. Subsequently, the methyl group at the 5-position was replaced with a CF_3 (fragment **22**) in order to compare it with fragment **18**. However, this modification showed no change in activity. Finally, the methyl at the 2-position was changed to an hydrophilic group to allow interaction with the hydrophilic residues or the water molecule of the small pocket (fragment **23**). Unfortunately, a lower affinity was observed (K_d = 208 ± 15 μM). As a result, two series of compounds were taken for further optimization, the pyrazole fragment **18** and the pyrrole fragments **20** and **22.**

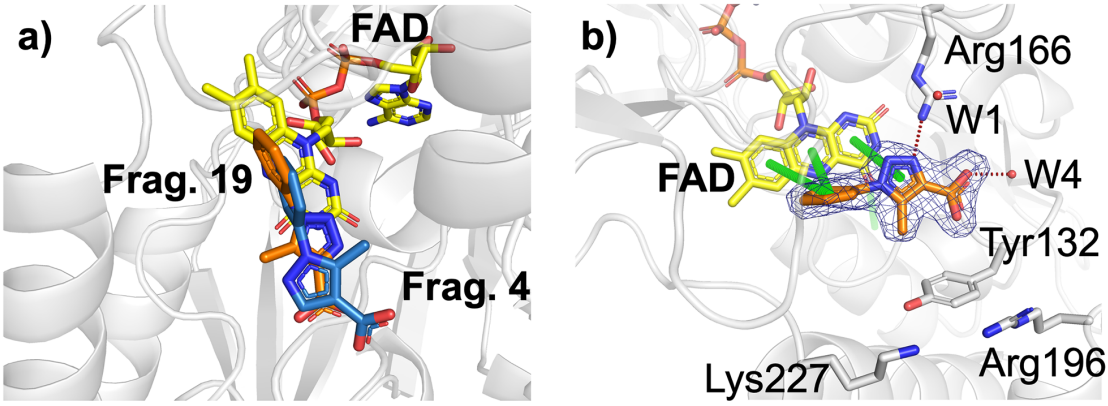
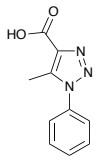
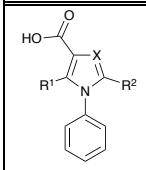


FIGURE 6. X-ray structure of fragment **4** and fragment **19** bound to the active site of *Pa* MurB. (a) Superimposition of *Pa* MurB in complex with fragment **4** (dark blue) and with fragment **19** (orange). (b) X-ray structure of fragment **19** bound to the active site of *Pa* MurB. FAD is depicted in yellow. Arpeggio[27] was used to analyze the interactions. Hydrogen bonds are shown as red dashed lines, π–π interactions are indicated in green dashed lines and water molecules are as red dots. The final 2F0 − Fc map around the ligand is shown in blue at 1σ.

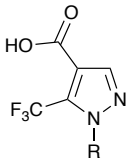
Table 3. Biophysical data for fragments 19–23.

Fragment		R^1	R^2	X	ΔT_M (°C) (±SEM) 1.0 mM	SPR RU>RU_{f4} 1.0 mM	K_d (μM) ITC	LE
	**19**	–			+0.5 (0)	no	877 ± 154	0.28
	**20**	Me	Me	CH	+2.0 (0)	yes	110 ± 14	0.34
	21	Me	Me	N	+0.7 (0.2)	no	–	–
	22	CF3	Me	CH	+2.0 (0)	yes	112 ± 4.4	0.28
	23	Me	CH_2CH_2OH	CH	+2.0 (0)	–	208 ± 15	0.28

Shift in protein melting temperatures (ΔT_M) from DMSO-d_6 at a ligand concentration of 1.0 mM and 10 μM MurB concentration (n=3). SPR RU of each fragment at 1 mM in comparation with RU of fragment **4 (**RU>$RU_{f4,}$ RU=Response units) (n = 2). Kd calculated using ITC (50 μM MurB, 3.0 mM fragment). Ligand efficiencies were calculated as LE = –(RTlnKd)/(number of heavy atoms) and are reported in kcal/mol per heavy atom. Dash entries in the table mean not measured.

Exploration of substituents on the phenyl ring. Initially, the replacement of the phenyl group was studied (Table 4) and substitution for a benzyl or a thiophenemethyl group did not increase the affinity (fragments **24** and **25**). The change of the phenyl ring for a thiophenyl or pyridinyl showed lower thermal shifts (fragments **26**–**28**). Consequently, the phenyl ring was retained and the introduction of different substituents on the ring was explored (Table 4). An increase in the melting temperature was observed by adding large apolar groups such as halogens or methyl groups at the 2-substiuted-positions. It was observed that the larger the group, the higher the affinity (fragments **29**–**33**). Introduction of polar and apolar groups in other positions on the phenyl ring did not improve affinity (fragments **34**–**40**). The introduction of two apolar 3-substituted groups was shown to slightly increase the affinity (fragments **41** and **42**). It was observed that some of the initial fragment hits contained phenyl groups with 3,4-dichlorophenyl substituents (fragments **5**, **6** and **9**). Therefore, this modification was incorporated into the developed compounds and this change showed an increase in affinity, K_d = 26.1 ± 2.7 μM (compound **43**).

Table 4. Biophysical data for fragments 24–43.

Fragment		R	ΔT_M (°C) (±SEM) 1.0 mM	SPR RU>RU_{f4} 1.0 mM	K_d (μM) ITC	LE
	**24**	benzyl	+1.0 (0)	–	–	–
	25	(thiophen-2-yl)methyl	+1.0 (0)	–	–	–
	26	thiophen-2-yl	+0.3 (0.2)	–	–	–
	27	3-pyridinyl	+0.3 (0.2)	–	–	–
	28	4-pyridinyl	0.0 (0)	–	–	–
	29	2-fluorophenyl	+1.0 (0)	–	–	–
	30	2-chlorophenyl	+1.5 (0)	yes	148 ± 15	0.27
	31	2-bromophenyl	+2.0 (0)	yes	85.5 ± 7.7	0.29
	32	2-methylphenyl	+2.0 (0)	yes	44.6 ± 3.1	0.31
	33	2-trifluoromethylphenyl	+1.0 (0)	–	–	–
	34	4-trifluoromethylphenyl	+1.0 (0)	–	–	–
	35	4-methoxyphenyl	+0.2 (0.2)	no	–	–
	36	2-methyl-4-nitrophenyl	+1.7 (0.7)	yes	208 ± 28	0.23
	37	3-carbamoylphenyl	+0.5 (0)	–	–	–
	38	2,3-dimethylphenyl	+2.0 (0)	yes	99.0 ± 7.2	0.27
	39	2,4-dimethylphenyl	+2.3 (0.2)	yes	85.5 ± 8.0	0.28

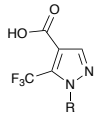
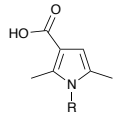
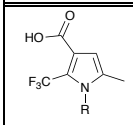
	40	2,5-dimethylphenyl	+2.0 (0)	yes	138 ± 16	0.26
	41	2,6-dimethylphenyl	+2.5 (0)	–	25.8 ± 5.5	0.31
	42	2,6-dichlorophenyl	2.5 (0)	yes	60.2 ± 4.4	0.29
	43	3,4-dichlorophenyl	+2.3 (0.2)	yes	26.1 ± 2.7	0.31

Shift in protein melting temperatures (ΔT_M) from DMSO at 1.0 mM fragment concentration and 10 μM MurB concentration (n=3). SPR RU of each fragment at 1 mM in comparation with RU of fragment **4 (**$RU>RU_{f4,}$ RU=Response units) (n = 2). K_d calculated using ITC (50 μM MurB, 3.0 mM fragment; except for fragment 41, where 200 μM MurB was used). Ligand efficiencies were calculated as LE = –($RTlnK_d$)/(number of heavy atoms) and are reported in kcal/mol per heavy atom. Dash entries in the table mean not measured.

The introduction of the 2-methylphenyl group could orientate the pyrazole ring and the phenyl ring at around 90º, which is the conformation observed in the crystal structure of fragment **18** (Figure 5). Molecular simulations were performed to examine this (Figure 8).[28] In the *Pa* MurB crystal structure, fragment **18** shows a dihedral angle of 86.5º between the pyrazole and the phenyl ring, whereas the minimum energy structure of fragment **18** has a dihedral angle of 120º. If an *ortho* substituent is added in the phenyl ring, the dihedral angle of the minimum energy structure becomes similar to the one in the crystal structure. Substituents that give closer angles to the crystal structure showed better affinity (59.8º for 2-fluorophenyl (**29**), 70.0º for 2-chlorophenyl (**30**), 69.4º for 2-bromophenyl (**31**), 79.6º for 2-methylphenyl (**32**) and 90.5º for the 2,6-dimethylphenyl (**41**) (Table 4). The 3,4-dichlorophenyl group could improve the binding by hydrophobic interactions with Leu300, Leu228 and Val301 (see Figure 5.B). Unfortunately, attempts to obtain X-ray crystal structures of these substituted compounds with *Pa* MurB were not successful.

As a result, the 2-methylphenyl substitution was merged with the 3,4-dichlorophenyl substitution (fragments **44** and **45**) (Table 5). This allowed the identification of the best substitution pattern, which was a phenyl ring substituted with a methyl group in the 2-position and two chlorines in the 4 and 5-position on the phenyl ring (fragment **44**). Subsequently, some of these modifications were successfully translated into the pyrrole fragments **20** and **22**, to yield fragments **46–52** and fragments **53–55**, respectively. Additionally, it was observed that both chlorine atoms are important for binding as there is a decrease in thermal shift if one of the chlorines is removed (fragments **49** and **50**). However, the fragment containing the pyrazole ring gave the lowest K_d of 3.57 ± 0.76 μM (fragment **44**) (see Figure 7.A).

Table 5. Biophysical data for fragments 44–55.

Fragment		R	ΔT_M (°C) (±SEM) 1.0 mM	SPR $RU>RU_{f4}$ 1.0 mM	K_d (μM) ITC	LE
	**44**	2-methyl-4,5-dichlorophenyl	+5.0 (0)	yes	3.57 ± 0.76	0.35
	45	2-methyl-3,4-dichlorophenyl	+3.7 (0.2)	yes	11.3 ± 2.5	0.32
	**46**	2-methylphenyl	+2.0 (0)	yes	64.5 ± 6.1	0.34
	47	2,6-dimethylphenyl	+3.0 (0)	no	62.5 ± 3.5	0.32
	48	3,4-dichlorophenyl	+2.3 (0.2)	no	47.8 ± 3.8	0.33
	49	3-chlorophenyl	+1.5 (0)	yes	–	–
	50	4-chlorophenyl	+1.5 (0)	no	–	–
	51	2-methyl-4,5-dichlorophenyl	+3.5 (0)	–	24.1 ± 4.0	0.33
	52	2-methyl-3,4-dichlorophenyl	+4.0 (0)	–	24.3 ± 7.4	0.33
	**53**	2-methylphenyl	+2.5 (0)	yes	40.2 ± 1.4	0.30
	54	2-methyl-4,5-dichlorophenyl	+4.7 (0.2)	–	8.00 ± 1.1	0.32
	55	2-methyl-3,4-dichlorophenyl	+2.7 (0.2)	yes	11.4 ± 3.9	0.31
NADP+		–	+3.5 (0)	–	23.6 ± 2.4	–
NADPH		–	+3.0 (0)	–		

Shift in protein melting temperatures (ΔT_M) from DMSO-d_6 at 1.0 mM fragment concentration and 10 μM *Pa* MurB concentration (n=3). SPR RU of each compound at 1.0 mM in comparation with RU of fragment **4 (**$RU>RU_{f4,}$ RU=Response units) (n = 2). K_d calculated using ITC (50 μM *Pa* MurB, 3.0 mM fragment; except for fragments **44**, **51** and **54**, which were tested at 1.0 mM and fragments **45** and **52**, which were tested at 0.5 mM). Ligand efficiencies were calculated as LE = –($RTlnK_d$)/(number of heavy atoms) and are reported in kcal/mol per heavy atom. Dash entries in the table mean not measured.

In the pyrrole series, the two substituents at the 2 and 5 position of the pyrrole ring can also affect the dihedral angle. Consequently, molecular simulations were also performed. Fragments **20** and **22**, which have an angle of 100º and 70.1º, respectively, have an angle more similar to the crystal structure (86.5º) than fragment **18**, which has an angle of 120º. Fragments **20** and **22** showed greater affinity (Table 3) (see Figure 7.B). However, these two fragments both had similar binding affinities. This suggests that the CF_3 group is not improving the affinity as in the pyrazoles. However, if a 2-methyl group is added in the phenyl ring of these fragments, both resulting fragments (fragments **46** and **53**) have an angle more similar to the one of the crystal structure (90.0º and 91.0º, respectively). Due to the fact that fragment **53** has a CF_3 group in the 2-position instead of the CH_3, a higher affinity is now observed. Consequently, once the dihedral angle is close to what is observed in the X-ray crystal structure, better affinity can be observed by the addition of the CF_3 group. Addition of a second *ortho*-methyl group (fragment **47**) did not change the dihedral angle (90.0º) as it did in the pyrazole series and no change in affinity was observed.

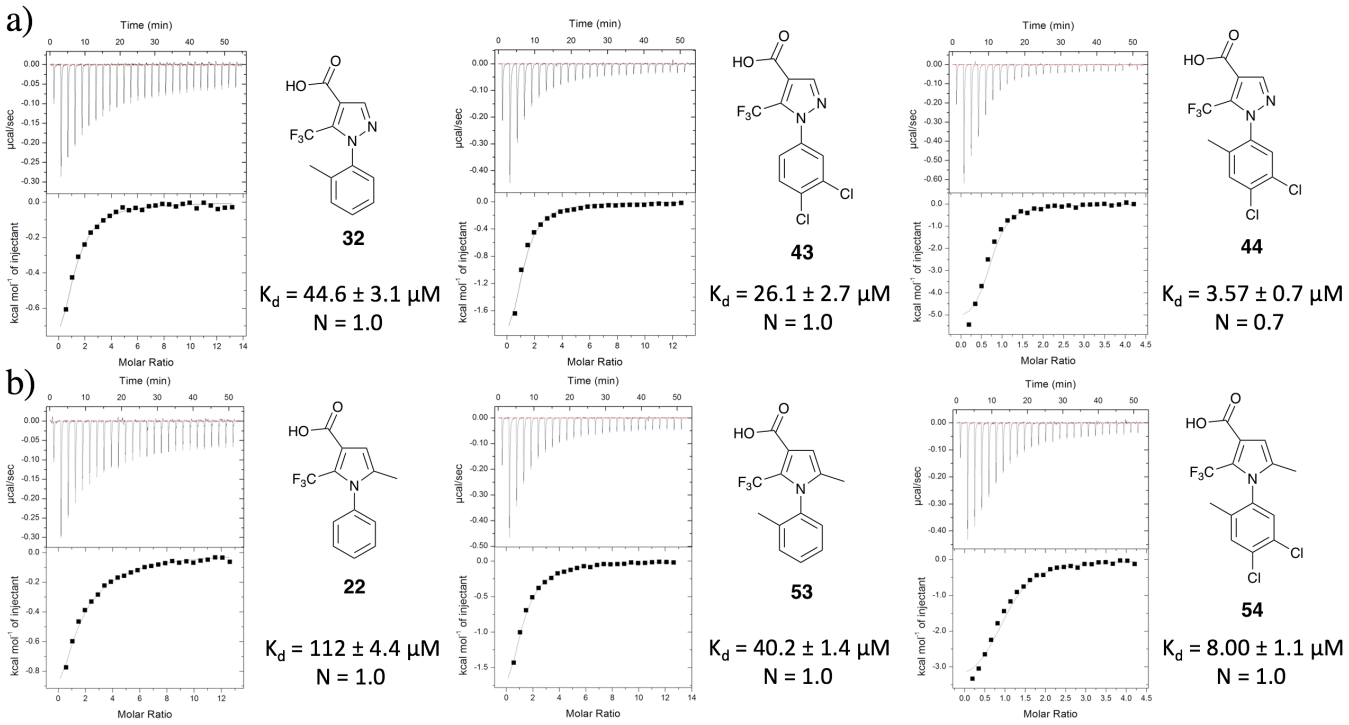


FIGURE 7. ITC titration curves at key stages of optimisation for the pyrazole (a) and pyrrole (b) series. Titrations performed at 50 µM *Pa* MurB with 3.0 mM fragments; except for fragments **44** and **54** were 1.0 mM was used. See SI for titration curves of other fragments and $NADP^+$.

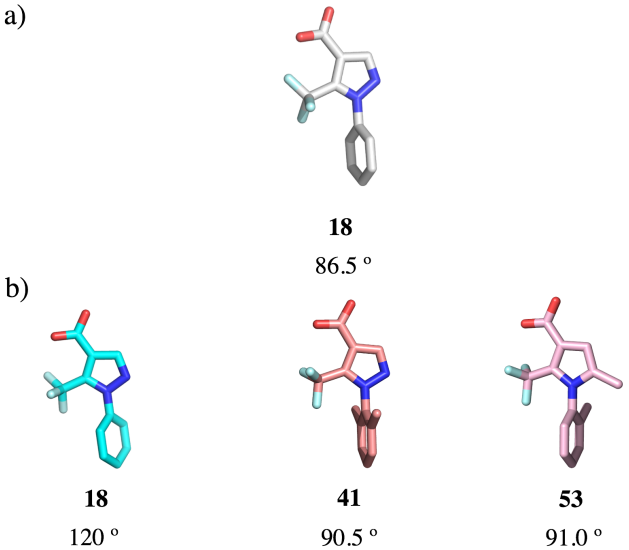


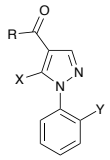
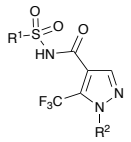
FIGURE 8. (a) Comparison of the dihedral angles between the conformation of fragment **18** in complex with *Pa* MurB and (b) the minimum energy conformations of fragments **18**, **41** and **53**. See SI for minimum energy conformations of other fragments.

Modifications at the carboxylic acid. Upon exploration of the SAR on the two rings, a further approach was explored to examine whether the compounds can be grown from the carboxylic acid moiety. $NADP^+$ was shown by X-ray crystallography to form a "sandwich" π-stacking interaction with its adenine ring to the Tyr196 and Tyr264 at the entrance of the binding pocket (see Figure 3a). In the absence of $NADP^+$, these two tyrosines residues are not stabilised and the α-helix and βαββ fold in the domain III (see Figure 2a) are flexible. The carboxylic acid of fragment **4** forms a polar interaction with the Tyr132 and it points in the same direction as the adenine ring of $NADP^+$ (see Figure 3B). Consequently, this is a good vector for developing these compounds. Therefore the carboxylic acids of some of the previously developed fragments were grown with different functional groups (Table 6). Amide and ester derivatives did not show any activity (fragments **56**–**58**). Only the sulfonamide derivatives were detected to be active by both DSF and ITC. If the methanesulfonyl group (fragment **59**) was changed for a benzenesulfonyl group (fragment **60**), a higher ΔT_M was observed (from +0.5 ºC to +1.0 ºC, respectively). However, fragment **60** (K_d = 0.32 ± 0.06 mM) showed similar binding affinity than the acid

analogue **18** (K_d = 0.25 ± 0.04 mM). The activity was lost when the phenyl group was changed for a benzyl group (fragment **61**).

Consequently, the effect in the binding affinity by the substitution of the acid moiety for a benzenesulfonyl group was investigated with the most optimised fragments such as **41** and **44**. After growing fragment **41**, a ΔT_M = +3.0 °C and a K_d = 25.6 ± 2.9 μM was observed (fragment **62**). The addition of a bromine substituent on this *N*-benzene sulfonyl group decreased the ΔT_M to +1.3 ºC (fragment **63**). In the case of fragment **44**, after the addition of the *N*-benzene sulfonyl group a ΔT_M of +2.7 ºC and a K_d = 12.0 ± 3.7 μM were observed (fragment **64**). Attempts to obtain X-ray crystal structures of these fragments proved unsuccessful.

Table 6. Biophysical data for fragments 56–64.

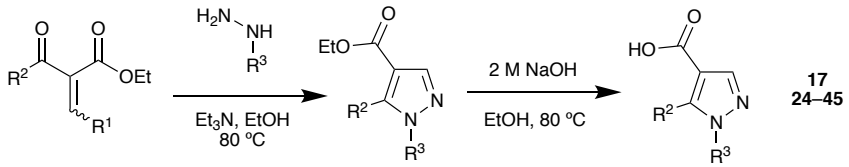
Fragment		R	X	Y	ΔT_M (°C) (±SEM) 1.0 mM	SPR RU>RU_{f4} 1.0 mM	K_d (μM) ITC	LE
	**56**	NH_2	CF_3	H	0.0 (0)	–	–	–
	57	OEt	CF_3	Cl	0.0 (0)	–	–	–
	58	*N*-benzylamino	Me	H	0.0 (0)	–	–	–
	59	*N*-(methanesulfonyl)amino	CF_3	H	+0.5 (0)	no	–	–
	60	*N*-(benzenesulfonyl)amino	CF_3	H	+1.0 (0)	yes	324 ± 59	0.18
	61	*N*-(benzylsulfonyl)amino	Me	H	0.0 (0)	–	–	–
		R^1	R^2					
	**62**	phenyl	2,6-dimethyl phenyl		+3.0 (0)	yes	25.6 ± 2.9	0.22
	63	3-bromophenyl			+1.3 (0.2)	–	–	–
	64	phenyl	2-methyl-4,5-dichlorophenyl		+2.7 (0.3)	–	12.0 ± 3.7	0.22

Shift in protein melting temperatures (ΔT_M) from DMSO-d_6 at 1.0 mM fragment concentration and 10 μM *Pa* MurB concentration (n=3). SPR RU of each fragment at 1.0 mM in comparation with RU of fragment **4** (RU>$RU_{f4,}$ RU=Response units) (n = 2). K_d calculated using ITC (50 μM *Pa* MurB, 3.0 mM fragment). Ligand efficiencies were calculated as LE = –(RTlnK_d)/(number of heavy atoms) and are reported in kcal/mol per heavy atom. Dash entries in the table mean not measured.

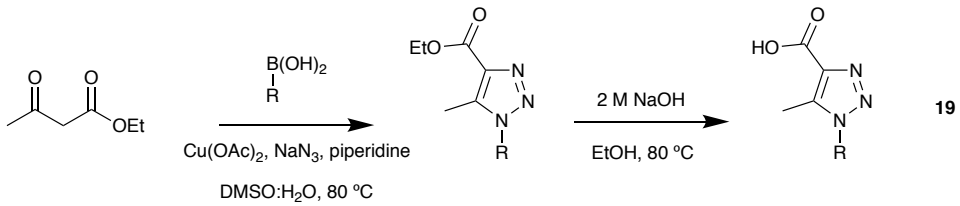
Synthetic Chemistry. Different synthetic routes were employed to prepare the different types of fragments (Scheme 1). Pyrazoles (fragments **17** and **24**–**45**) were prepared using two key steps. The first step involved the reaction of α,β-unsaturated keto esters with different hydrazines in the presence of Et_3N and EtOH at 80 ºC to yield the desired 4,5-substituted pyrazoles.[29] The 3,4-substituted regioisomers were only observed when aliphatic hydrazines were employed as starting materials (synthesis of fragments **24** and **25**). The second step involved the hydrolysis of the resulting esters with 2 M NaOH in EtOH at 80 ºC. The triazole **19** was prepared from ethyl acetoacetate and phenylboronic acid in the presence of sodium azide, copper acetate and catalytic amounts of piperidine.[30] No other major regioisomer was observed. The resulting product was hydrolised as previously. Pyrrole derivatives (**20**, **22**, **23** and **46**–**55**) were also prepared using two key steps. The first step involved the reaction of different α,β-keto esters with different amines using the Paal-Knorr reaction.[31,32] This reaction used acetic acid as solvent at 120 ºC when R^2 = CH_3. However, when R^2 = CF_3, *para*-toluenesulfonic acid in toluene at 110 ºC was used instead due to the degradation of the α,β-keto esters in the acetic acid. Finally, the resulting ester was hydrolysed as before. Imidazole **21** was also prepared in two main steps. In the first step an α,β-keto ester analogue reacted with aniline in the presence of trifluoroacetic acid and butyronitrile at 120 ºC.[33] The product was hydrolysed by 6 M HCl at 100 ºC. The basic conditions used previously were not used here due to the degradation of the product. Finally, the amide and sulfonamide derivatives (**58** and **59**–**64**, respectively) were prepared from the corresponding acids in a one-pot step. For the amide, the carboxylic acid was first converted into an acyl chloride by reaction with thionyl chloride at 80 ºC. This intermediate was then reacted with benzyl amine in the presence of pyridine in 1,4-dioxane at room temperature to yield the corresponding amide. For the sulfonamides, the carboxylic acid reacted with the desired sulfonamide through a coupling reaction involving the use of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and catalytic amounts of 4-dimethylaminopyridine in dichloromethane at room temperature.

Scheme 1. General synthetic routes for the synthesis of the tested fragments

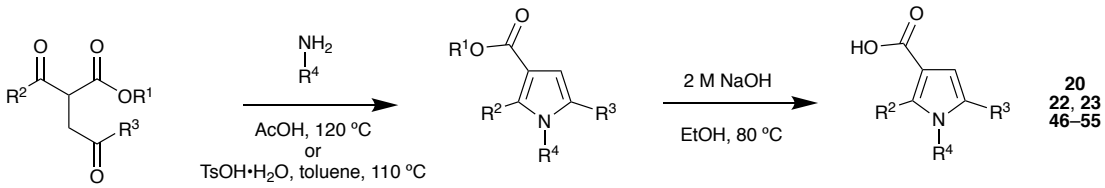
Pyrazoles



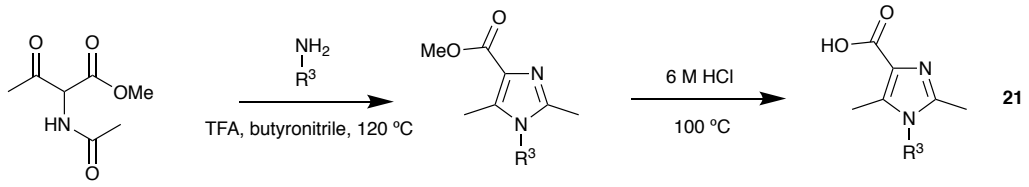
1,2,3-Triazoles



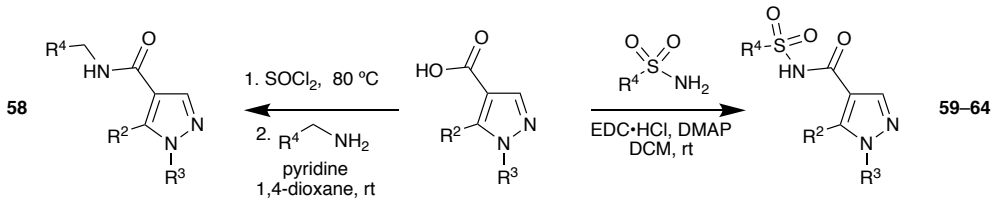
Pyrroles



Imidazoles



Amides and sulfonamides



DISCUSSION AND CONCLUSIONS

The development of inhibitors of peptidoglycan targeting MurB enzymes has proved exceptionally challenging and currently there are no approved antibiotics that target any of the nine subsequent steps after MurA. This study illustrates the successful application of a fragment-based approach to obtain for the first time a potent candidate that binds to the catalytic pocket of *Pa* MurB.

Screening of 960 fragments library by DSF and validation using X-ray crystallography allowed the identification of the identification of a pyrazole derived fragment **4**, which showed a K_d of 2.8 mM by ITC. This fragment was synthetically modified to improve its binding affinity. Different substitutions were tested in comparison with the initial fragment **4** using two different biophysical techniques, DSF and SPR at 1.0 mM. Binding parameters were calculated using ITC for those fragments with significant binding increase than the initial fragment.

The substitution of the 5-methyl group on fragment **4** for a 5-trifluoromethyl group on the pyrazole ring or the substitution of the 5-methylpyrazole for a 2,5-dimethylpyrrole or 2-methyl-5-trifluoromethylpyrrole ring increased the binding affinity to values of K_d around 0.1–0.3 mM (fragments **18**, **20** and **22**, respectively). Subsequently, the introduction of an *ortho*-methyl group or a 3,4-dichloro group on the phenyl ring decreased the K_d to around 50–20 μM, while maintaining the ligand efficiency. The initial library of hits contained fragments with a 2,5-dimethylpyrrole ring or 3,4-dichlolorophenyl groups, suggesting that it was important to look at these fragments for the optimisation of fragment **4**. The fact that the substitutions could be translated from pyrazole to pyrroles suggests that the binding mode could be similar; however, pyrazoles showed to bind tighter. Exploration of the SAR on the carboxylic showed that a phenylsulfonamide group was tolerated, but no gain in affinity was observed.

The best fragments were obtained by merging the *ortho*-methyl group with the *meta*,*para*-dichloro groups and showed a LE = 0.35 (fragment **44**) and a LE = 0.32 (fragment **54)**. As a result, fragments with higher potency to that of the cofactors of *Pa* MurB have been designed. Consequently, these fragments can grow into *Pa* MurB inhibitors that would disrupt cell wall biosynthesis. This fragment is a good candidate because the binding is mediated by π- π interactions between FAD and Tyr132; thus, there is no possibility of *P. aeruginosa* becoming resistant by mutations in MurB. Although Tyr132 is quite conserved in bacteria (in MurB from *Escherichia coli* and *Staphylococcus aureus*), some bacteria such as *Thermus caldophilus* have R132. However, if Tyr132 were to mutate to another amino acid, then the π- π interaction would likely be replaced with a π-polar interaction. Future work can elaborate this fragment in order to increase the interactions with MurB in the catalytic pocket.

EXPERIMENTAL SECTION

Cloning, protein expression and purification. MurB gen of *P. aeruginosa* was designed based on the sequence available in NCBI database and synthesised using GeneArt (Invitrogen). The gen was cloned between the BamHI and HindIII sites in pET28a vector (Novagen) and modified with an N-terminal 6xHis-SUMO tag. The resulting plasmid was confirmed by DNA sequencing and transformed into *E. coli* BL21(DE3) strain (Invitrogen).

Transformed cells were grown to OD_{610} = 0.6 in LB media (Invitrogen) containing 30 mg L^{-1} kanamycin at 37 ºC. At this O.D., protein expression was induced using 0.5 mM isopropyl β–D-1-thiogalactopyranoside (IPTG) overnight at 18 ºC. Cells were harvested by centrifugation and re-suspended in 50 mM TRIS pH 8, 0.5 M NaCl, 5 mM $MgCl_2$ and 20 mM imidazole with protease inhibitor tablets (Roche) and DNAseI. Cells were lysed, sonicated and centrifuged at 30000 g for 45 min to collect the supernatant.

Pa MurB was purified with a HiTrap IMAC Sepharose FF column (GE-Healthcare), equilibrated with 50 mM TRIS pH 7.5, 0.5 M NaCl and 20 mM imidazole and the elution was performed in the same buffer with 500 mM imidazole. Imidazole was removed with overnight dialysis in 50 mM TRIS pH 8, NaCl 250 mM. Meanwhile, SUMO tag was cleaved by adding Ulp1 Protease at 1:100 ratio. SUMO tag, Ulp1 protease and *Pa* MurB were separated using Superdex 200 column equilibrated with 50 mM TRIS pH 8, NaCl 250 mM. Fraction purity was determined by SDS-page and purest fractions were pooled concentrated to 25.5 mg mL^{-1} in the same buffer, flash frozen in liquid nitrogen and stored at -80 ºC.

Differential scanning fluorimetry. Differential scanning fluorimetry was performed using a Bio-Rad CFX96 Touch PCR system, from 25 to 95 °C in 0.5 °C increments of 30 seconds duration. Samples were run in 96-well clear-bottomed plates. For these experiments each well contained a final volume of 25 μL, consisting of 25 mM Tris-HCl pH 8.0, 150 mM NaCl, 5x SyproOrange, 10 μM *Pa* MurB and either 5% DMSO-d_6 or ligand at 5.0 mM or 1.0 mM in DMSO-d_6 as specified. Controls were used for all experiments, with DMSO-d_6 (reference) and $NADP^+$ (positive control) used instead of the fragment. The resulting data (fluorescence intensity vs. temperature) was fitted to obtain the denaturing temperature T_M (point of sigmoidal inflection) as the maximum of each curve's derivative. This analysis was performed using Microsoft Office Excel. The reference unfolding temperature of *Pa* MurB in 5% DMSO-d_6 was subtracted from the values in the presence of the fragment to obtain the thermal shift. The thermal shifts at 5.0 mM were recorded once (n = 1), the thermal shifts at 1.0 mM were recorded three times (n = 3).

Crystallisation and data collection. Crystallisation of the complexes was carried out by seeding using *Pa* MurB:NADPH microcrystals as nucleation starting point. *Pa* MurB:NADPH crystals were set up manually mixing 1 μL of *Pa* MurB at 25.5 mg $μL^{-1}$ and 2 mM NADPH and 1 μL of crystallisation condition mix 160 mM $(NH_4)SO_4$, 80 mM sodium acetate pH 4.6, 20% PEG 4000 and 20% glycerol using the hanging-drop vapor-diffusion method in 24-well VDX greased plates (Hampton Research, Aliso Viejo, California, USA). Crystallisation of the fragment complexes or Apo crystal was prepared using sitting-drop vapor-diffusion method at 25 °C and the plates were mounted in the Mosquito Crystal robot (TPP Labtech, Hertfordshire, UK). In each crystallisation drop, 0.4 μL of reservoir solution and 0.05 μL microseeds were added to 0.2 μL protein solution. The protein droplets were equilibrated over 70 μL reservoir solution mix (30% glycerol and 22% PEG 1500). Suitable crystals for X-ray diffraction grew in 1 week.

Diffraction data were processed and reduced using XDS[30] and Aimless from the CCP4 suite.[31] All the structures crystallised in $P6_1$ space group with one protomer per asymmetric unit. Initial phases were determined using the previous published structure of *Pa* MurB (PDB code 4JB1)[12] as a model with PHASER[32] program from PHENIX software package.[33] Model building and structure validation were refined using PHENIX and Coot.[34]

All data sets were collected at stations I03 and I04-1 at Diamond Light Source (Oxford, UK). Data collection and refinement statistics are summarized in Table S1

General Chemistry. Commercially available starting materials and fragments **4**, **15**, **16**, **18** and **56** were obtained from Sigma−Aldrich, Acros, Fluorochem and Alfa Aesar. All non-aqueous reactions were performed under nitrogen atmosphere unless otherwise stated. Water-sensitive reactions were performed in anhydrous solvents in oven-dried glassware cooled under nitrogen before use. Petrol refers to petroleum spirit (b.p. 40–60 °C), THF refers to tetrahydrofuran and DCM refers to dichloromethane. A rotary evaporator was used to remove the solvents *in vacuo*.

Thin layer chromatography was performed using Merck glass-backed silica (Kieselgel 60 F_{254} 0.25 mm plates). Ultraviolet lamp (λ_{max} = 254 nm) and $KMnO_4$ were used for visualization. Flash column chromatography was performed using an automated Isolera Spektra One/Four purification systems and an appropriately sized Biotage SNAP column containing KP-silica gel (50 μm). Perkin-Elmer One FT-IR spectrometer was used to analyse the infrared spectra. Absorptions are reported in wavenumbers (cm^{-1}).

A SQD2 mass spectrometer detector (Waters) utilising electrospray ionization (ESI) was used for low-resolution mass spectrometry (MS). High-resolution mass spectrometry (HRMS) was recorded using a Waters LCT Premier Time of Flight (TOF) mass spectrometer or a Micromass Quadrapole-Time of Flight (Q-TOF) spectrometer.

The purity of tested fragments was determined by high performance liquid chromatography (HPLC). All final fragments had purity greater than 95% unless otherwise stated. HPLC was carried out using an Ultra Performance Liquid Chromatographic system (UPLC) Waters Acquity H-class. Samples were detected using a Waters Acquity TUV detector at 2 wavelengths (254 and 280 nm). Samples were run using an Acquity UPLC HSS column and a flow rate of 0.8 mL/min. The eluent consisted of 0.1% formic acid in water (A) and acetonitrile (B); gradient, from 95% A to 5% A over a period of 4 min.

Proton (^1H), carbon (^{13}C) and fluorine (^{19}F) NMR data were collected on a Bruker 400 MHz spectrometer. Data were collected at 300 K. Chemical shifts (δ) are given in parts per million (ppm) and they are referenced to the residual solvent peak. ^{19}F NMR spectra were references to TFA. Coupling constants (*J*) are reported in Hertz (Hz) and splitting patterns are reported in an abbreviated manner: app. (apparent), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad).

General Procedure A. A dispersion of 20% $Pd(OH)_2$ on carbon (0.5 eq) was added to a solution of the benzyl derivative (1.0 eq) in ethanol (0.1 M). The mixture was stirred under hydrogen at room temperature for 24 h. Subsequently, it was filtered through celite and concentrated *in vacuo* to give a crude product.

General Procedure B. An aqueous solution of 2 M NaOH (6.0 eq) was added dropwise to a solution of the ester derivative (1.0 eq) in ethanol (0.2 M) and the resulting mixture was stirred at 80 °C. After consumption of the starting material, the solvent was removed *in vacuo*, water (5 mL for each 1.00 mmol of the ester derivative) was added and the mixture was washed with ethyl acetate (5 mL for each 1.00 mmol of the ester derivative). Successively, the aqueous layer was acidified to pH 4 with an aqueous solution of 1 M HCl and extracted with ethyl acetate (3 × 5 mL for each 1.00 mmol of the ester derivative). The organic phases were combined, washed with brine (5 mL for each 1.00 mmol of the ester derivative), dried under anhydrous magnesium sulphate, filtered and concentrated *in vacuo* to yield a crude product or the corresponding acid derivative.

General Procedure C.[36] The amine derivative (1.0 eq) was added to a solution of the carbonyl derivative (1.0 eq) in acetic acid (0.27 M) and the mixture was stirred at 120 ºC until consumption of the starting material. After cooling to room temperature, water (4 mL for each 1.00 mmol of amine derivative) was added and the mixture was extracted with EtOAc (3 × 8 mL for each 1.00 mmol of amine derivative). The organic phases were combined, dried under anhydrous magnesium sulphate, filtered and concentrated *in vacuo* to give a crude product.

General Procedure D.[37] $TsOH•H_2O$ (0.5 eq) was added to a solution of the amine derivative (1.0 eq) and the dicarbonyl derivative **S9** (1.0 eq) in toluene (concentration of the amine derivative 0.27 M) and the mixture was stirred at 110 ºC. After consumption of the starting materials, the mixture was allowed to cool to room temperature and water (3 mL for each 1.00 mmol of the amine derivative) and EtOAc (3 mL for each 1.00 mmol of the amine derivative) were added. The phases were separated and the aqueous phase was extracted with EtOAc (3 × 3 mL for each 1.00 mmol of the amine derivative). The organic phases were combined, dried under anhydrous magnesium sulphate, filtered and concentrated *in vacuo* to give a crude product.

General Procedure E.[29] Triethylamine (1.2 eq or 2.4 eq if the hydrazine dihydrochloride salt derivative was used) was added dropwise to a stirred solution of ethyl-3-ethoxy-2-(2,2,2-trifluoroacetyl)acrylate (1.0 eq) and the hydrazine hydrochloride salt derivative (1.0 eq) in ethanol (concentration of acrylate derivative 0.4 M) and the resulting mixture was stirred at 80 °C. After consumption of the starting material, the mixture was allowed to cool to room temperature and the solvent evaporated under reduced pressure. EtOAc (2 mL for each 1.00 mmol of the hydrazine derivative) and water (2 mL for each 1.00 mmol of the hydrazine derivative) were added, the phases were separated and the aqueous phase was extracted with EtOAc (3 × 2 mL for each 1.00 mmol of the hydrazine derivative). The organic phases were combined, washed with brine (2 mL for each 1.00 mmol of the hydrazine derivative), dried under anhydrous magnesium sulphate, filtered and concentrated *in vacuo* to give a crude product.

General Procedure F. A solution of $NaNO_2$ (1.2 eq) in H_2O (1.8 M) was added dropwise to a solution of the amine derivative (1.0 eq) in concentrated HCl (0.3 M) at 0 ºC. The reaction was stirred for 30 min at 0 ºC and the insolubilities were removed. Successively, a solution of $SnCl_2•(H_2O)_2$ (3.0 eq) in 1:1 concentrated HCl–H_2O (1.1 M) was added. After stirring the reaction for 2.5 h at 0 ºC, the precipitate was filtered, washed with a cold aqueous solution of 6.0 M HCl, washed with hexane and dried *in vacuo* to give the hydrazine derivative.

General Procedure G. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.1 eq), the sulphonamide derivative (1.1 eq) and 4-dimethylaminopyridine (0.1 eq) were added to a solution of the carboxylic acid derivative (1.0 eq) in DCM (0.1 M). After stirring at room temperature for 18 h, water (8 mL for each 1.00 mmol of carboxylic acid derivative) was added, the phases were separated and the aqueous phase was extracted with DCM (3 × 8 mL for each 1.00 mmol of carboxylic acid derivative). The organic phases were combined, dried under anhydrous sodium sulphate, filtered and concentrated *in vacuo* to give a crude product or the sulphonamide derivative.

Ethyl 4-(benzyloxy)-3-oxobutanoate (S1). Benzyl alcohol (2.00 mL, 19.3 mmol) was added to a suspension of 60% NaH in mineral oil (1.16 g, 29.0 mmol) in THF (24 mL) at 0 °C. After stirring at room temperature for 2 h, ethyl 4-chloroacetoacetate (2.1 mL, 15.4 mmol) was added dropwise over 30 min and the reaction mixture was stirred at room temperature for 18 h. Successively, the mixture was acidified to pH 2 with an aqueous solution of 6.0 M HCl. Water (10 mL) and EtOAc

(10 mL) were added, the phases were separated and the aqueous phase was extracted with EtOAc (3 × 10 mL). The organic phases were combined, dried under anhydrous magnesium sulphate, filtered and concentrated *in vacuo* to yield a crude product. The crude product was purified by flash column chromatography (0% to 5% EtOAc in petrol) to give the benzyl derivative **S1**[39] as a yellow oil (2.50 g, 10.6 mmol, 55%). R_f 0.41 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40–7.27 (m, 5H), 4.58 (s, 2H), 4.16 (q, J = 7.1 Hz, 2H), 4.14 (s, 2H), 3.53 (s, 2H), 1.24 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.7, 167.0, 137.0, 128.6, 128.1, 127.9, 74.8, 73.5, 61.4, 46.1, 14.1 ppm. MS (ESI): $[M + H]^+$ 237.1. IR (neat) ν_{max} 3064–2871 (w, C–H), 1721 (s, C=O), 1656 (w, C=O), 1454 (m), 1393 (m), 1367 (m), 1317 (m), 1229 (m), 1098 (m), 1030 (m) cm^{-1}.

Ethyl 5-[(benzyloxy)methyl]-1-phenyl-1*H*-pyrazole-4-carboxylate (S2). The benzyl derivative **S1** (0.50 g, 2.11 mmol) and *N*,*N*-dimethylformamide dimethyl acetal (0.35 mL, 2.63 mmol) were stirred at room temperature for 18 h and concentrated *in vacuo* to yield the crude product. This was dissolved in EtOH (4 mL) and phenylhydrazine hydrochloride (0.30 g, 2.11 mmol) and Et_3N (0.35 mL, 2.53 mmol) were added and the reaction mixture was stirred at 80 ºC for 18 h. After allowing to cool to room temperature, the solvent was evaporated under reduced pressure and the residue was taken up in ethyl acetate (4 mL) and water (4 mL). The phases were separated and the aqueous phase was extracted with EtOAc (3 × 4 mL). The organic phases were combined, washed with brine (4 mL), dried under anhydrous magnesium sulphate, filtered and concentrated *in vacuo* to give a crude product. The crude product was purified using flash column chromatography (7% EtOAc in petrol) to give the benzyl derivative **S2** as a colourless oil (0.38 g, 1.13 mmol, 54%). R_f 0.50 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (s, 1H), 7.65 (d, J = 7.7 Hz, 2H), 7.50–7.40 (m, 3H), 7.36–7.25 (m, 5H), 4.81 (s, 2H), 4.63 (s, 2H), 4.35 (q, J = 7.1 Hz, 2H), 1.38 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.3, 142.0, 138.9, 137.6, 129.2, 128.7, 128.5, 128.0, 127.9, 125.1, 114.9, 73.1, 60.4, 60.3, 14.5 ppm. MS (ESI): $[M + H]^+$ 337.3. IR (neat) ν_{max} 3113–2868 (w, C–H), 1708 (s, C=O), 1549 (m), 1501 (m), 1379 (m), 1265 (s), 1241 (s), 1189 (m), 1093 (s), 1063 (s), 1022 (s) cm^{-1}.

Ethyl 5-(hydroxymethyl)-1-phenyl-1*H*-pyrazole-4-carboxylate (S3). According to General Procedure A, the benzyl derivative **S2** (0.32 g, 0.95 mmol) gave a crude product. The crude product was purified using flash column chromatography (5% EtOAc in petrol) to give the alcohol derivative **S3** as a colourless oil (0.22 g, 0.89 mmol, 94%). R_f 0.50 (50:50 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.53–7.42 (m, 5H), 4.76 (s, 2H), 4.37 (q, J = 7.1 Hz, 2H), 1.39 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.9, 146.6, 141.8, 138.3, 129.4, 129.1, 125.4, 114.0, 61.0, 55.1, 14.4 ppm. MS (ESI): $[M + H]^+$ 247.1. IR (neat) ν_{max} 3272 (s, O–H), 3057–2851 (w, C–H), 1698 (s, C=O), 1558 (m), 1497 (m), 1460 (m), 1410 (m), 1381(m), 1244 (s), 1207 (s), 1090 (s), 1026 (s) cm^{-1}.

5-(Hydroxymethyl)-1-phenyl-1*H*-pyrazole-4-carboxylic acid (17). According to General Procedure B, the alcohol derivative **S3** (56 mg, 0.23 mmol) was stirred for 4 h to give the acid derivative **17** as a white amorphous solid (47 mg, 0.22 mmol, 94%). R_f 0.16 (20:80 MeOH:DCM). ^{1}H NMR (400 MHz, CD_3OD) δ 8.05 (s, 1H), 7.65–7.47 (m, 5 H), 4.80 (s, 2H) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ 166.5, 146.9, 143.0, 139.8, 130.3, 130.2, 126.5, 115.2, 53.8 ppm. MS (ESI): $[M + H]^+$ 219.0. HPLC: retention time 1.31 min (>99%). IR (neat) ν_{max} 3295 (s, O–H), 3063–2548 (s, C–H), 1673 (s, C=O), 1560 (s), 1458 (m), 1416 (m), 1282 (s), 1262 (s), 1226 (m), 1096 (w), 1019 (s), 936 (s) cm^{-1}. HRMS: calculated for $C_{11}H_{10}N_2O_3$ $[M + H]^+$ = 219.0770, observed = 219.0765.

Ethyl 5-methyl-1-phenyl-1*H*-1,2,3-triazole-4-carboxylate (S4). According to a procedure,[35] sodium azide (0.26 g, 4.10 mmol) and copper(II) acetate (37.2 mg, 0.20 mmol) were added to a solution of phenylboronic acid (0.25 g, 2.05 mmol) in DMSO (10 mL) and water (1 mL). After stirring for 4 h at room temperature, ethyl acetoacetate (0.28 mL, 2.25 mmol) and piperidine (41 µL, 0.41 mmol) were added and the reaction mixture was stirred at 80 °C for 18 h. Successively, the reaction was allowed to cool to room temperature and an aqueous solution of 5% ammonium hydroxide (20 mL) and EtOAc (20 mL) were added. The phases were separated and the aqueous phase was extracted with EtOAc (3 × 10 mL). The organic phases were combined, washed with brine (3 × 30 mL), dried under anhydrous magnesium sulphate, filtered and concentrated *in vacuo* to give a crude product, which was purified by flash column chromatography (10%–20% EtOAc in petrol) to give the ester derivative **S4**[40] as a yellow oil (0.32 g, 1.38 mmol, 67%). R_f 0.43 (50:50 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58–7.48 (m, 3H), 7.45–7.38 (m, 2H), 4.43 (q, J = 7.1 Hz, 2H), 2.55 (s, 3H), 1.41 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.8, 138.9, 136.7, 135.5, 130.1, 129.7, 125.4, 61.1, 14.4, 10.0 ppm. MS (ESI): $[M + H]^+$ 232.1. IR (neat) ν_{max} 3061–2871 (w, C–H), 1712 (s, C=O), 1597 (w), 1566 (w), 1504 (m), 1423 (m), 1374 (m), 1350 (w), 1278 (w), 1244 (s), 1228 (s), 1207 (s), 1107 (s), 1010 (w), 980 (w) cm^{-1}.

5-Methyl-1-phenyl-1*H*-1,2,3-triazole-4-carboxylic acid (19). According to General Procedure B, the ester derivative **S4** (83 mg, 0.36 mmol) was stirred for 3 h to give the acid derivative **19**[41] as a white amorphous solid (70 mg, 0.34 mmol, 94%). R_f 0.29 (20:80 MeOH:DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.83 (br. s, 1H), 7.68–7.36 (m, 5H), 2.62 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.5, 140.0, 136.1, 135.3, 130.4, 129.8, 125.4, 10.2 ppm. MS (ESI): $[M + H]^+$ 204.1. HPLC: retention time 1.42 min (>99%). IR (neat) ν_{max} 3067 (s, O–H), 2928–2580 (w, C–H), 1681 (s, C=O), 1566 (m), 1494 (m), 1452 (m), 1270 (m), 1241 (m), 1229 (m), 1117 (m), 1090 (m) cm^{-1}.

Ethyl 2-acetyl-4-oxopentanoate (S5). According to a procedure,[42] chloroacetone (1.26 mL, 15.8 mmol) was added to a solution of ethyl acetoacetate (2.00 mL, 15.8 mmol) in triethylamine (15 mL) and the reaction mixture was stirred at 90 °C for 18 h. After allowing the mixture to cool to room temperature, the mixture was concentrated *in vacuo*. Water (10 mL) and DCM (10 mL) were added, the phases separated and the aqueous phase was extracted with DCM (3 × 10 mL). The organic phases were combined, washed with brine (10 mL), dried under anhydrous magnesium sulphate, filtered and concentrated *in vacuo* to give a crude product, which was purified by flash column chromatography (0%–10% EtOAc in petrol) to give the carbonyl derivative **S5**[37] as a yellow liquid (1.20 g, 6.44 mmol, 41%). R_f 0.51 (50:50 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.16 (q, J = 7.1 Hz, 2H), 3.98 (dd, J = 8.2, 5.7 Hz, 1H), 3.11 (dd, J = 18.5, 8.2 Hz, 1H), 2.92 (dd, J = 18.5, 5.7 Hz, 1H), 2.32 (s, 3H), 2.16 (s, 3H), 1.25 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 205.7, 202.2, 168.8, 61.8, 53.8, 41.6, 30.1, 29.7, 14.1 ppm. MS (ESI): [M +

H]$^+$ 187.1. IR (neat) ν_{max} 2984–2927 (w, C–H), 1739 (m, C=O), 1711 (s, C=O), 1359 (m), 1259 (m), 1229 (m), 1157 (m) cm^{-1}.

Ethyl 2,5-dimethyl-1-phenyl-1*H*-pyrrole-3-carboxylate (S6). According to General Procedure C, aniline (0.12 mL, 1.34 mmol) and the carbonyl derivative **S5** were stirred for 18 h to give a crude product, which was purified by flash column chromatography (5% EtOAc in petrol) to give the ester derivative **S6**[36] as a yellow oil (0.27 g, 1.11 mmol, 83%). R_f 0.70 (50:50 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52–7.41 (m, 3H), 7.17 (d, J = 7.6 Hz, 2H), 6.37 (s, 1H), 4.28 (q, J = 7.1 Hz, 2H), 2.29 (s, 3H), 1.97 (s, 3H), 1.35 (t, J = 7.1, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.8, 137.8, 136.3, 129.4, 128.8, 128.6, 128.3, 111.5, 107.6, 59.3, 14.7, 12.7, 12.5 ppm. MS (ESI): [M + H]$^+$ 244.2. IR (neat) ν_{max} 2981–2898 (w, C–H), 1738 (w), 1693 (s, C=O), 1540 (w), 1500 (w), 1408 (m), 1369 (w), 1226 (s), 1216 (s), 1079 (s), 1014 (m) cm^{-1}.

2,5-Dimethyl-1-phenyl-1*H*-pyrrole-3-carboxylic acid (20). According to General Procedure B, the ester derivative **S6** (0.10 g, 0.41 mmol) was stirred for 2 days to give the acid derivative **20**[41] as a white amorphous solid (70 mg, 0.32 mmol, 78%). R_f 0.54 (50:50 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.86 (br. s, 1H), 7.55–7.42 (m, 3H), 7.20 (d, J = 7.4 Hz, 2H), 6.44 (s, 1H), 2.32 (s, 3H), 1.99 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 137.8, 137.7, 129.5, 129.2, 128.7, 128.3, 110.8, 108.2, 12.8, 12.7 ppm. MS (ESI): [M + H]$^+$ 216.1. HPLC: retention time 1.72 min (>99%). IR (neat) ν_{max} 3056 (s, O–H), 2917–2584 (s, C–H), 1651 (s, C=O), 1579 (m), 1531 (m), 1494 (m), 1453 (m), 1400 (m), 1261 (s), 1084 (m) cm^{-1}.

Methyl 2-acetamido-3-oxobutanoate (S7). According to a procedure,[43] a solution of sodium nitrite (3.30 g, 48.0 mmol) in water (4 mL) was added dropwise to a solution of methyl acetoacetate (4.00 mL, 36.8 mmol) in acetic acid (10 mL). After stirring the solution at room temperature for 2 h, water (25 mL) was added and stirred for further 30 min. Successively, the solution was extracted with diethyl ether (3 × 50 mL). The organic phases were combined, washed with a saturated sodium bicarbonate aqueous solution (50 mL), dried under anhydrous sodium sulphate, filtered and concentrated *in vacuo*. Acetic acid (31.5 mL) and acetic anhydride (9.20 mL) were added and the mixture was cooled to 0 °C. Subsequently, zinc powder (12.0 g, 184 mmol) was slowly added and the reaction was stirred at room temperature for 18 h. After filtering the mixture through celite, water (30 mL) and DCM (50 mL) were added. The phases were separated and the aqueous phase was extracted with DCM (3 × 50 mL). Successively, the organic phases were combined, washed with a saturated sodium bicarbonate aqueous solution (50 mL), filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography (33% EtOAc in petrol) and triturated with Et_2O to give the carbonyl derivative **S7**[43] as a white solid (2.55 g, 14.7 mmol, 40%). R_f 0.36 (90:10 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.76 (app. br. s, 1H), 5.25 (d, J = 6.6 Hz, 1H), 3.78 (s, 3H), 2.35 (s, 3H), 2.04 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.6, 169.9, 166.7, 63.0, 53.3, 28.1, 22.7 ppm. MS (ESI): [M + H]$^+$ 174.1. IR (neat) ν_{max} 3231 (s, N–H), 3028–2956 (w, C–H), 1741 (s, C=O), 1724 (s, C=O), 1632 (s, C=O), 1524 (s), 1433 (m), 1375 (m), 1290 (m), 1223 (s), 1157 (s), 1140 (s) cm^{-1}.

Methyl 2,5-dimethyl-1-phenyl-1*H*-imidazole-4-carboxylate (S8). According to a modified procedure,[38] trifluoroacetic acid (0.12 mL, 1.48 mmol) was added to a solution of the dicarbonyl derivative **S7** (0.20 g, 1.14 mmol) and aniline (0.14 mL, 1.48 mmol) in butyronitrile (4.40 mL). After stirring at 120 °C for 4 h, DCM (10 mL) was added, the solution was washed with a saturated sodium carbonate aqueous solution (10 mL), dried under anhydrous magnesium sulphate, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography (20% petrol in EtOAc) to give the ester derivative **S8**[44] as a white solid (0.11 g, 0.47 mmol, 41%). R_f 0.23 (80:20 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50–7.40 (m, 3H), 7.11 (d, J = 7.1 Hz, 2H), 3.81 (s, 3H), 2.23 (s, 3H), 2.13 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.2, 144.6, 136.9, 135.5, 129.8, 129.4, 127.3, 51.2, 13.7, 10.7 ppm. MS (ESI): [M + H]$^+$ 231.1. IR (neat) ν_{max} 3049–2925 (w, C–H), 1709 (s, C=O), 1543 (m), 1494 (m), 1434 (m), 1403 (m), 1372 (m), 1360 (s), 1186 (s), 1176 (s), 1092 (s) cm^{-1}.

2,5-Dimethyl-1-phenyl-1*H*-imidazole-4-carboxylic acid (21). A 6 M HCl aqueous solution (0.50 mL) was added to the ester derivative **S8** (10 mg, 43.4 μmol) and the mixture was stirred at 100 °C for 18 h. The solvent was removed *in vacuo* to yield the acid derivative **21**[44] as an hydrochloride salt (9 mg, 43.4 μmol, >99%). R_f 0.11 (20:80 MeOH:DCM). ^{1}H NMR (400 MHz, CD_3OD) δ 7.76–7.68 (m, 3H), 7.57–7.50 (m, 2H), 2.46 (s, 3H), 2.38 (s, 3H) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ 160.7, 147.8, 138.7, 133.5, 132.6, 131.7, 128.5, 121.4, 11.6, 10.4 ppm. MS (ESI): [M + H]$^+$ 217.1. HPLC: retention time 0.97 min (91%). IR (neat) ν_{max} 2761 (s, O–H, C–H), 1721 (s, C=O), 1444 (m), 1331 (m), 1220 (m), 1170 (s), 1117 (s) cm^{-1}.

Ethyl 4-oxo-2-(2,2,2-trifluoroacetyl)pentanoate (S9). Ethyl 4,4,4-trifluoroacetoacetate (2.31 mL, 15.8 mmol) was added dropwise to a suspension of 60% NaH in mineral oil (0.63 g, 15.8 mmol) in 1,2-dimethoxyethane (8.00 mL) at 0 ºC. After stirring for 30 min at 0 ºC, chloroacetone (1.45 mL, 18.2 mmol) in 1,2-dimethoxyethane (2 mL) and KI (32 mg, 0.19 mmol) were added and the reaction was stirred at 85 ºC for 18 h. The mixture was diluted with water (20 mL) and extracted with Et_2O (3 × 20 mL). The organic phases were combined, washed with brine (20 mL), dried under anhydrous magnesium sulphate, filtered and concentrated *in vacuo* to give a crude product. The crude product was purified by vacuum distillation to yield the carbonyl derivative **S9**[45] as an orange oil (2.31 g, 9.62 mmol, 61%). R_f 0.44 (50:50 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.34 (dd, J = 9.6, 4.7 Hz, 1H), 4.20 (app. qd, J = 7.2, 3.1 Hz, 2H), 3.28 (dd, J = 18.5, 9.6 Hz, 1H), 3.12 (dd, J = 18.5, 4.7 Hz, 1H), 2.20 (s, 3H), 1.25 (t, J = 7.2 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 204.1, 187.2 (q, J = 36.8 Hz), 166.7, 115.3 (q, J = 291.3 Hz), 62.7, 47.7, 42.1, 29.2, 13.9 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -78.0 ppm. MS (ESI): [M – H]$^-$ 239.0. IR (neat) ν_{max} 2988–2924 (w, C–H), 1718 (s, C=O), 13.70 (w), 1267 (m), 1157 (s), 1096 (m), 1041 (m) cm^{-1}.

Ethyl 5-methyl-1-phenyl-2-(trifluoromethyl)-1*H*-pyrrole-3-carboxylate (S10). According to General Procedure D, aniline (37.3 μL, 0.41 mmol) was stirred for 2.5 h to give a crude product, which was purified by flash column chromatography (0%–5% EtOAc in petrol) to give the ester derivative **S10** as a yellow oil (18.0 mg, 60.5 μmol, 15%). R_f 0.59 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51–7.44 (m, 3H), 7.25–7.20 (m, 2H), 6.47 (s, 1H), 4.33 (q, J = 7.1 Hz, 2H), 1.94 (s, 3H), 1.35 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.6, 137.6, 133.7, 129.3, 129.3, 127.8 (q, J = 0.7 Hz), 122.2 (q, J = 38.3 Hz), 120.5 (q, J = 269.4 Hz), 118.4 (q, J = 2.1 Hz), 110.0, 60.7, 14.2, 12.5 ppm. ^{19}F

NMR (376 MHz, $CDCl_3$) δ -54.4 ppm. MS (ESI): $[M + H]^+$ 298.1. IR (neat) ν_{max} 2981–2853 (w, C–H), 1721 (s, C=O), 1511 (m), 1495 (m), 1417 (m), 1276 (m), 1219 (s), 1174 (s), 1114 (s), 1039 (s), 996 (s) cm^{-1}.

5-Methyl-1-phenyl-2-(trifluoromethyl)-1*H*-pyrrole-3-carboxylic acid (22). According to General Procedure B, the ester derivative **S10** (8 mg, 26.9 μmol) was stirred for 4 h to give a crude product. The crude product was purified by flash column chromatography (30% EtOAc in petrol) to give the acid derivative **22** as a colourless amorphous solid (6 mg, 20.4 μmol, 76%). R_f 0.34 (50:50 EtOAc:petrol). ^1H NMR (400 MHz, $CDCl_3$) δ 7.55–7.49 (m, 3H), 7.31–7.24 (m, 2H), 6.60 (s, 1H), 1.99 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.6, 137.6, 133.8 (q, J = 1.3 Hz), 129.4, 129.3, 127.7 (q, J = 0.7 Hz), 123.4 (q, J = 38.5 Hz), 120.3 (q, J = 269.6 Hz), 116.9 (q, J = 2.0 Hz), 110.9, 12.6 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -53.5 ppm. MS (ESI): $[M + H]^+$ 270.0. HPLC: retention time 1.88 min (>99%). IR (neat) ν_{max} 3100–2600 (s, O–H, C–H), 1665 (s, C=O), 1517 (m), 1497 (m), 1456 (m), 1418 (m), 1255 (s), 1138 (s), 1000 (m) cm^{-1}. HRMS: calculated for $C_{13}H_{10}F_3NO_2$ $[M + H]^+$ = 270.0742, observed = 270.0735.

4-(Benzyloxy)butan-2-one (S11). Benzyl bromide (4.12 mL, 34.7 mmol) was added to a mixture of 4-hydroxy-2-butanone (2.00 mL, 23.15 mmol) and *N*,*N*-diisopropylethylamine (6.4 mL, 37.0 mmol) at room temperature. The reaction was stirred at 150 ºC for 2 h and allowed to cool to room temperature. EtOAc (10 mL) and an aqueous solution of 10% sodium bisulfate (10 mL) were added, the phases were separated and the aqueous phase was extracted with EtOAc (3 × 2 mL). The organic phases were combined, dried under anhydrous magnesium sulphate, filtered and concentrated *in vacuo* to yield a crude product. The crude product was purified by flash column chromatography (10% EtOAc in petrol) to give the benzyl derivative **S11**[46] as a yellow liquid (2.60 g, 14.6 mmol, 63%). R_f 0.67 (50:50 EtOAc:petrol). ^1H NMR (400 MHz, $CDCl_3$) δ 7.42–7.20 (m, 5H), 4.51 (s, 2H), 3.74 (t, J = 6.3 Hz, 2H), 2.71 (t, J = 6.3 Hz, 2H), 2.18 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.2, 138.1, 128.5, 127.8, 127.7, 73.3, 65.3, 43.8, 30.5 ppm. MS (ESI): $[M + H]^+$ 179.0. IR (neat) ν_{max} 2863 (m, C–H), 1714 (s, C=O), 1454 (w), 1363 (m), 1170 (w), 1104 (s), 1085 (s) cm^{-1}.

4-(Benzyloxy)-1-bromobutan-2-one (S12). Bromine (0.51 mL, 10.0 mmol) was added dropwise to a solution of the benzyl derivative **S11** (1.78 g, 10.0 mmol) in methanol (18 mL) at 0 ºC and the reaction was stirred at room temperature for 18 h. An aqueous solution of 1.0 M K_2CO_3 (20 mL) and Et_2O (20 mL) were added. The phases were separated and the aqueous phase was extracted with Et_2O (2 × 20 mL). The organic phases were combined, dried under anhydrous magnesium sulphate, filtered and concentrated *in vacuo* to give a crude product. The crude product was dissolved in THF (72 mL) and an aqueous solution of 1.0 M H_2SO_4 (36.0 mL) was added. After stirring for 2 h at 65 ºC and concentrating the mixture *in vacuo*, Et_2O (20 mL) and water (20 mL) were added and the phases were separated. The organic phase was washed with an aqueous solution of 2.0 M $KHCO_3$ (10 mL), dried under anhydrous magnesium sulphate, filtered and concentrated *in vacuo* to give a crude product. The crude product was purified by flash column chromatography (6% EtOAc in petrol) to give the bromine derivative **S12**[47] as a pale yellow oil (1.30 g, 5.05 mmol, 51%). R_f 0.44 (30:70 EtOAc:petrol). ^1H NMR (400 MHz, $CDCl_3$) δ 7.40–7.25 (m, 5H), 4.51 (s, 2H), 3.94 (s, 2H), 3.77 (t, J = 6.1 Hz, 2H), 2.92 (t, J = 6.1 Hz, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.4, 137.9, 128.5, 127.9, 127.8, 73.4, 65.4, 40.3, 35.0 ppm. MS (ESI): $[M + Na]^+$ 279.2. IR (neat) ν_{max} 3087–2865 (m, C–H), 1715 (s, C=O), 1495 (w), 1054 (w), 1390 (m), 1365 (m), 1326 (w), 1255 (w), 1205 (w), 1178 (w), 1095 (s), 1075 (s), 1026 (m) cm^{-1}.

Ethyl 2-acetyl-6-(benzyloxy)-4-oxohexanoate (S13). Ethyl acetoacetate (0.12 mL, 0.97 mmol) was added dropwise to a suspension of 60% NaH in mineral oil (39 mg, 0.97 mmol) in 1,2-dimethoxyethane (1 mL) at 0 ºC. After stirring for 10 min at 0 ºC, the halogen derivative **S12** (0.25 g, 0.97 mmol) was added and the reaction was stirred at room temperature for 18 h. Successively, an aqueous solution of 1.0 M HCl (1 mL) and EtOAc (1 mL) were added, the phases were separated and the aqueous phase was extracted with EtOAc (3 × 1 mL). The organic phases were combined, dried under anhydrous magnesium sulphate, filtered and concentrated *in vacuo* to give a crude product. The crude product was purified by flash column chromatography (10%–20% EtOAc in petrol) to give the benzyl derivative **S13**[48] as a colourless liquid (0.28 g, 0.91 mmol, 94%). R_f 0.50 (40:60 EtOAc:petrol). ^1H NMR (400 MHz, $CDCl_3$) δ 7.40–7.21 (m, 5H), 4.49 (s, 2H), 4.18 (q, J = 7.1 Hz, 2H), 4.02 (app. t, J = 6.9 Hz, 1H), 3.79–3.66 (m, 2H), 3.15 (dd, J = 18.5, 8.2 Hz, 1H), 2.96 (dd, J = 18.5, 5.7 Hz, 1H), 2.74 (t, J = 6.2 Hz, 2H), 2.34 (s, 3H), 1.26 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.3, 202.2, 168.8, 138.1, 128.5, 127.8, 127.7, 73.3, 65.1, 61.8, 53.7, 42.9, 41.3, 30.1, 14.1 ppm. MS (ESI): $[M + Na]^+$ 329.2. IR (neat) ν_{max} 2981–2869 (m, C–H), 1739 (m, C=O), 1712 (s, C=O), 1454 (w), 1399 (w), 1360 (m), 1256 (m), 1205 (m), 1147 (m), 1097 (m), 1022 (m) cm^{-1}.

Ethyl 5-[2-(benzyloxy)ethyl]-2-methyl-1-phenyl-1*H*-pyrrole-3-carboxylate (S14). According to General Procedure C, aniline (63 μL, 0.68 mmol) and the carbonyl derivative **S13** were stirred for 18 h to give a crude product, which was purified by flash column chromatography (5% EtOAc in petrol) to give the ester derivative **S14** as a white solid (0.16 g, 0.44 mmol, 65%). R_f 0.48 (30:70 EtOAc:petrol). ^1H NMR (400 MHz, $CDCl_3$) δ 7.50–7.40 (m, 3H), 7.36–7.22 (m, 5H), 7.17–7.10 (m, 2H), 6.45 (s, 1H), 4.44 (s, 2H), 4.29 (q, J = 7.1 Hz, 2H), 3.54 (t, J = 7.3 Hz, 2H), 2.64 (t, J = 7.3 Hz, 2H), 2.27 (s, 3H), 1.36 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 138.3, 137.5, 136.5, 130.0, 129.5, 128.8, 128.5, 128.4, 127.8, 127.7, 111.8, 107.6, 73.0, 69.1, 59.4, 27.3, 14.7, 12.3 ppm. MS (ESI): $[M + H]^+$ 364.3. IR (neat) ν_{max} 2981–2854 (m, C–H), 1685 (s, C=O), 1526 (w), 1494 (m), 1430 (m), 1379 (m), 1365 (m), 1221 (s), 1119 (m), 1084 (s), 1072 (s), 1024 (m), 1000 (m) cm^{-1}.

Ethyl 5-(2-hydroxyethyl)-2-methyl-1-phenyl-1*H*-pyrrole-3-carboxylate (S15). According to General Procedure A, the benzyl derivative **S14** (0.13 g, 0.36 mmol) gave a crude product. The crude product was purified using flash column chromatography (30% EtOAc in petrol) to give the alcohol derivative **S15** as a colourless oil (86.0 mg, 0.31 mmol, 87%). R_f 0.31 (50:50 EtOAc:petrol). ^1H NMR (400 MHz, $CDCl_3$) δ 7.52–7.39 (m, 3H), 7.16 (d, J = 7.2 Hz, 2H), 6.46 (s, 1H), 4.26 (q, J = 7.1 Hz, 2H), 3.60 (t, J = 6.7 Hz, 2H), 2.56 (t, J = 6.7 Hz, 2H), 2.25 (s, 3H), 2.04 (br. s, 1H), 1.33 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 137.4, 136.9, 129.6, 129.5, 128.8, 128.4, 111.8, 107.8, 61.2, 59.4, 30.0, 14.6, 12.3 ppm. MS (ESI): $[M + H]^+$ 274.2. IR (neat) ν_{max} 3447 (s, O–H), 2978–2875 (m, C–H), 1693 (s, C=O), 1674 (s), 1597 (w), 1572 (w), 1529 (m), 1498 (m),

1418 (m), 1378 (m), 1352 (w), 1219 (s), 1079 (s), 1047 (m), 1012 (m) cm^{-1}.

5-(2-Hydroxyethyl)-2-methyl-1-phenyl-1*H*-pyrrole-3-carboxylic acid (23). According to General Procedure B, the ester derivative **S15** (56 mg, 0.20 mmol) was stirred for 5 h to give a crude product. The crude product was purified by flash column chromatography (50% EtOAc in petrol) to give the acid derivative **23** as a white amorphous solid (36 mg, 0.15 mmol, 75%). R_f 0.50 (EtOAc). ^{1}H NMR (400 MHz, CD_3OD) δ 7.60–7.46 (m, 3H), 7.25 (d, J = 7.7 Hz, 2H), 6.42 (s, 1H), 3.55 (t, J = 7.2 Hz, 2H), 2.54 (t, J = 7.2 Hz, 2H), 2.23 (s, 3H) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ 169.4, 138.8, 137.8, 131.3, 130.6, 130.0, 129.6, 112.6, 109.2, 61.9, 31.0, 12.5 ppm. MS (ESI): $[M + H]^+$ 246.1. HPLC: retention time 1.50 min (>99%). IR (neat) ν_{max} 3326 (m, O–H), 2958–2850 (m, C–H), 1673 (s, C=O), 1568 (m), 1529 (m), 1492 (m), 1430 (m), 1375 (m), 1357 (m), 1217 (s), 1054 (s), 1025 (s) cm^{-1}. HRMS: calculated for $C_{14}H_{15}NO_3$ $[M + H]^+$ = 246.1130, observed = 246.1124.

Ethyl 1-benzyl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (S16). According to General Procedure E, benzylhydrazine dihydrochloride (0.50 g, 2.56 mmol) was stirred for 3.5 h to give a crude product. The crude product was purified by flash column chromatography (5% EtOAc in petrol) to give the ester derivative **S16** as a colourless oil (0.48 g, 1.60 mmol, 63%). R_f 0.56 (20:80 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.40–7.10 (m, 5H), 5.54 (s, 2H), 4.32 (q, J = 7.1 Hz, 2H), 1.35 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.0, 146.9, 142.0, 135.3, 131.9 (q, J = 40.1 Hz), 128.9, 128.4, 127.2, 119.6 (q, J = 271.0 Hz) 61.2, 56.7 (q, J = 3.2 Hz), 14.2 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -57.4 ppm. MS (ESI): $[M + H]^+$ 299.2. IR (neat) ν_{max} 2982 (w, C–H), 1735 (s, C=O), 1559 (m), 1477 (m), 1294 (s), 1222 (s), 1187 (s), 1152 (s), 1042 (s) cm^{-1}.

1-Benzyl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (24). According to General Procedure B, the ester derivative **S16** (0.20 g, 0.67 mmol) was stirred for 5.5 h to give the acid derivative **24** as a white amorphous solid (0.16 g, 0.60 mmol, 90%). R_f 0.18 (50:50 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.40–7.10 (m, 5H), 5.55 (s, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5, 146.0, 142.8, 135.1, 132.7 (q, J = 40.0 Hz), 128.9, 128.5, 127.2, 119.4 (q, J = 271.7 Hz), 56.8 (q, J = 3.2 Hz) ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -57.4 ppm. MS (ESI): $[M – H]^-$ 269.0. HPLC: retention time 1.79 min (>99%). IR (neat) ν_{max} 3040 (m, O–H), 2928–2525 (w, C–H), 1700 (m, C=O), 1562 (m), 1480 (m), 1410 (m), 1300 (s), 1231 (s), 1174 (s), 1136 (s), 1037 (s), 1014 (s) cm^{-1}. HRMS: calculated for $C_{12}H_9F_3N_2O_2$ $[M + H]^+$ = 271.0694, observed = 271.0682.

[(Thiophen-2-yl)methyl]hydrazine dihydrochloride (S17). *tert*-Butyl carbazate (1.50 g, 11.3 mmol) was added to a solution of 2-thiophenecarboxaldehyde (1.00 mL, 10.8 mmol) in MeOH (25 mL) at room temperature. After stirring the mixture at 65 ºC for 1 h, the solvent was removed *in vacuo* and the crude product was dissolved in THF (45 mL) and sodium cyanoborohydrate (1.00 g, 16.2 mmol) was added. Subsequently, AcOH (17.0 mL) was added dropwise and the mixture was stirred at room temperature for 24 h. Successively, a saturated aqueous solution of $NaHCO_3$ (20 mL) was slowly added and the mixture was extracted with EtOAc (2 × 10 mL). The organic phases were combined, washed with brine (10 mL), dried under anhydrous magnesium sulphate, filtered and concentrated *in vacuo* to give a crude product. EtOH (50 mL) and an aqueous solution of concentrated HCl (5 mL) were added. After stirring the mixture at 80 ºC for 18 h, the solvent was removed *in vacuo* to yield the hydrazine derivative **S17**[49] as a white amorphous solid (1.90 g, 9.44 mmol, 87%). R_f 0.65 (20:80 MeOH:DCM). ^{1}H NMR (400 MHz, CD_3OD) δ 7.49 (dd, J = 5.1, 1.1 Hz, 1H), 7.20 (dd, J = 3.5, 1.1 Hz, 1H), 7.06 (dd, J = 5.1, 3.5 Hz), 4.37 (s, 2H) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ 135.5, 130.4, 128.4, 128.3, 49.9 ppm. MS (ESI): $[M + H]^+$ 128.9. IR (neat) ν_{max} 3205 (m, N–H), 2990–2862 (s, N–H, C–H), 1582 (m), 1501 (m), 1375 (m), 1243 (w), 1047 (w), 1022 (w) cm^{-1}.

Ethyl 1-[(thiophen-2-yl)methyl]-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (S18). According to General Procedure E, the hydrazine derivative **S17** (0.25 g, 1.24 mmol) was stirred for 18 h to give a crude product. The crude product was purified by flash column chromatography (5%–30% EtOAc in petrol) to give the ester derivative **S18** as a pale brown oil (0.20 g, 0.66 mmol, 53%). R_f 0.56 (20:80 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.27 (dd, J = 5.1, 1.2 Hz, 1H), 7.04 (d, J = 3.5 Hz, 1H), 6.95 (dd, J = 5.1, 3.5 Hz, 1H), 5.67 (s, 2H), 4.31 (q, J = 7.1 Hz, 2H), 1.34 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.9, 142.2, 136.8, 131.4 (q, J = 40.3 Hz), 127.6, 127.0, 126.8, 119.6 (q, J = 271.1 Hz), 116.4 (q, J = 1.6 Hz), 61.2, 51.4 (q, J = 3.5 Hz), 14.2 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -57.1 ppm. MS (ESI): $[M + H]^+$ 305.1. IR (neat) ν_{max} 2983 (w, C–H), 1732 (s, C=O), 1558 (w), 1476 (w), 1408 (w), 1373 (w), 1292 (s), 1218 (s), 1188 (s), 1148 (s), 1039 (s), 1021 (s) cm^{-1}.

1-[(Thiophen-2-yl)methyl]-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (25). According to General Procedure B, the ester derivative **S18** (0.10 g, 0.33 mmol) was stirred for 6.5 h to give the acid derivative **25** as a white amorphous solid (72.0 mg, 0.26 mmol, 79%). R_f 0.29 (EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.02 (br. s, 1H), 8.09 (s, 1H), 7.29 (dd, J = 5.2, 1.2 Hz, 1H), 7.07 (d, J = 3.5 Hz, 1H), 6.97 (dd, J = 5.2, 3.5 Hz, 1H), 5.71 (s, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.2, 143.1, 136.4, 132.4 (q, J = 41.1 Hz), 127.8, 127.1, 127.0, 119.4 (q, J = 271.7 Hz), 115.1 (q, J = 1.4 Hz), 51.6 (q, J = 3.6 Hz) ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -57.2 ppm. MS (ESI): $[M – H]^-$ 275.0. HPLC: retention time 1.65 min (>99%). IR (neat) ν_{max} 2864–2557 (m, O–H, C–H), 1687 (s, C=O), 1565 (m), 1479 (m), 1422 (m), 1334 (m), 1302 (s), 1239 (s), 1129 (w), 1029 (s), 1009 (s) cm^{-1}. HRMS: calculated for $C_{10}H_7F_3N_2O_2S$ $[M – H]^-$ = 275.0102, observed = 275.0104.

***N*-(Thiophen-2-yl)(*tert*-butoxy)carbohydrazide (S19).** *tert*-Butyl carbazate (3.50 g, 26.8 mmol), Cs_2CO_3 (6.90 g, 21.2 mmol), CuI (0.25 g, 1.40 mmol), and *trans*-4-hydroxy-*L*-proline (0.35 g, 2.67 mmol) were added to a solution of 2-bromothiophene (1.00 mL, 10.3 mmol) in DMSO (50 mL) and the reaction was stirred at 80 ºC for 18 h. After allowing the mixture to cool to room temperature, water (40 mL) and EtOAc (10 mL) were added. The phases were separated and the aqueous phase was extracted with EtOAc (2 × 10 mL). The organic phases were combined, washed with brine (10 mL), dried under anhydrous magnesium sulphate, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography (10% EtOAc in petrol) to give the carbamate derivative **S19**[50] as a pale brown oil (0.52 g, 2.42 mmol, 24%). R_f 0.60 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.88 (br. s, 1H), 6.84–6.78 (m, 2H), 4.56 (s, 2H), 1.56 (s, 9H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.2, 146.8, 125.3, 117.3, 112.6, 83.2, 28.3 ppm. MS (ESI): $[M + H]^+$ 158.9 (^{t}Bu lost during MS). IR (neat) ν_{max} 3324 (m,

N–H), 3273 (w, N–H), 3201 (w, N–H), 2977–2930 (m, C–H), 1692 (s, C=O), 1621 (w), 1534 (m), 1473 (w), 1447 (m), 1368 (s), 1322 (s), 1280 (m), 1251 (m), 1224 (m), 1150 (s), 1082 (m), 1050 (m), 998 (s) cm^{-1}.

Ethyl 1-(thiophen-2-yl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (S20). A solution of 4.0 M HCl in 1,4-dioxane (1.00 mL) was added to a solution of the derived carbamate **S19** (0.10 g, 0.47 mmol) in DCM (1 mL). The reaction was stirred at room temperature for 3 days and the solvent was removed *in vacuo* to yield a crude product. The crude product was dissolved in EtOH (1.00 mL) and ethyl-3-ethoxy-2-(2,2,2-trifluoroacetyl)acrylate (0.10 mL, 0.53 mmol) and Et_3N (79 µL, 0.57 mmol) were added. The mixture was stirred at 80 ºC for 5 h. After allowing the reaction to cool to room temperature, the solvent was evaporated *in vacuo* and EtOAc (1 mL) and water (1 mL) were added. The phases were separated and the aqueous phase was extracted with EtOAc (3 × (1 mL)). The organic phases were combined, washed with brine (1 mL), dried under anhydrous magnesium sulphate, filtered and concentrated *in vacuo* to give a crude product. The crude product was purified by flash column chromatography (5% EtOAc in petrol) to give the ester derivative **S20** as a yellow oil (75 mg, 0.26 mmol, 55%). R_f 0.62 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.37 (dd, J = 5.6, 1.4 Hz, 1H), 7.17 (dd, J = 3.8, 1.4 Hz, 1H), 7.01 (dd, J = 5.6, 3.8 Hz, 1H), 4.37 (q, J = 7.1 Hz, 2H), 1.37 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.7, 142.8, 139.0, 133.9 (q, J = 40.1 Hz), 126.0, 125.6, 125.5 (q, J = 1.5 Hz), 118.9 (q, J = 271.7 Hz), 117.1 (q, J = 1.3 Hz), 61.5, 14.2 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -57.3 ppm. MS (ESI): $[M + H]^+$ 291.1. IR (neat) ν_{max} 3109–2907 (w, C–H), 1734 (s, C=O), 1566 (w), 1554 (w), 1466 (w), 1397 (w), 1377 (w), 1291 (s), 1230 (s), 1185 (s), 1140 (s), 1035 (s) cm^{-1}.

1-(Thiophen-2-yl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (26). According to General Procedure B, the ester derivative **S20** (60 mg, 0.21 mmol) was stirred for 5.5 h to give the acid derivative **26** as a white amorphous solid (50 mg, 0.19 mmol, 90%). R_f 0.33 (20:80 MeOH:DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.34 (br. s, 1H), 8.21 (s, 1H), 7.40 (dd, J = 5.6, 1.4 Hz, 1H), 7.21 (dd, J = 3.8, 1.4 Hz, 1H), 7.04 (dd, J = 5.6, 3.8 Hz) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.1, 143.6, 138.7, 134.9 (q, J = 40.7 Hz), 126.3, 125.8 (q, J = 1.5 Hz), 125.7, 118.7 (q, J = 272.1 Hz), 115.8 (q, J = 1.3 Hz) ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -56.3 ppm. MS (ESI): $[M – H]^-$ 261.0. HPLC: retention time 1.64 min (>99%). IR (neat) ν_{max} 2856–2583 (m, O–H, C–H), 1702 (m, C=O), 1568 (w), 1545 (w), 1418 (w), 1299 (m), 1254 (m), 1235 (m), 1187 (m), 1134 (s), 1026 (m) cm^{-1}. HRMS: calculated for $C_9H_5F_3N_2O_2S$ $[M + H]^+$ = 263.0102, observed = 263.0110.

Ethyl 1-(pyridin-3-yl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (S21). According to General Procedure E, 3-hydrazinopyridine dihydrochloride (0.30 g, 1.64 mmol) was stirred for 18 h to give a crude product. The crude product was purified by flash column chromatography (20%–40% EtOAc in petrol) to give the ester derivative **S21**[51] as a yellow oil (86 mg, 0.30 mmol, 18%). R_f 0.40 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (app. d, J = 4.9 Hz, 1H), 8.71 (s, 1H), 8.14 (s, 1H), 7.77 (app. dt, J = 8.2, 1.9 Hz, 1H), 7.46 (dd, J = 8.2, 4.9 Hz, 1H), 4.36 (q, J = 7.1 Hz, 2H), 1.36 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.7, 150.9, 146.8 (q, J = 1.1 Hz), 143.2, 136.2, 133.3 (q, J = 0.9 Hz), 133.1 (q, J = 40.1 Hz), 123.7, 119.0 (q, J = 271.5 Hz), 117.5 (q, J = 1.5 Hz), 61.5, 14.1 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -56.1 ppm. MS (ESI): $[M + H]^+$ 286.2. IR (neat) ν_{max} 2988 (w, C–H), 1710 (m, C=O), 1554 (w), 1491 (w), 1465 (w), 1411 (w), 1384 (w), 1295 (w), 1245 (m), 1223 (m), 1190 (s), 1148 (m), 1082 (s), 1027 (m) cm^{-1}.

1-(Pyridin-3-yl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (27). According to General Procedure B, the ester derivative **S21** (66 mg, 0.23 mmol) was stirred for 18 h to give the acid derivative **27**[47] as a white amorphous solid (57 mg, 0.22 mmol, 96%). R_f 0.16 (20:80 MeOH:DCM). ^{1}H NMR (400 MHz, CD_3OD) δ 8.75 (app. d, J = 4.9 Hz, 1H), 8.72 (d, J = 2.5 Hz, 1H), 8.21 (s, 1H), 8.02 (app. dt, J = 8.3, 2.0 Hz, 1H), 7.65 (dd, J 8.3, 4.9 Hz, 1H) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ 163.3, 151.7, 147.6 (q, J = 1.1 Hz), 144.5, 138.1, 135.7 (q, J = 1.0 Hz), 134.0, 125.5, 120.5 (q, J = 270.6 Hz), 119.1 (q, J = 1.4 Hz) ppm. ^{19}F NMR (376 MHz, CD_3OD) δ -57.5 ppm. MS (ESI): $[M + H]^+$ 258.1. HPLC: retention time 1.36 min (>99%). IR (neat) ν_{max} 3118–2413 (m, O–H, C–H), 1878 (w), 1718 (m, C=O), 1562 (m), 1489 (w), 1433 (m), 1378 (w), 1366 (w), 1299 (m), 1256 (m), 1225 (m), 1184 (s), 1144 (s), 1082 (m), 1045 (s), 1027 (s) cm^{-1}.

Ethyl 1-(pyridin-4-yl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (S22). According to General Procedure E, 4-hydrazinopyridine hydrochloride (20 mg, 0.14 mmol) was stirred overnight to give a crude product. The crude product was purified by flash column chromatography (30% EtOAc in petrol) to give the ester derivative **S22**[51] as a colourless oil (18 mg, 63.1 µmol, 45%). R_f 0.38 (60:40 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (d, J = 5.3 Hz, 2H), 8.15 (s, 1H), 7.43 (d, J = 5.3 Hz, 2H), 4.38 (q, J = 7.1 Hz, 2H), 1.38 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.6, 151.1, 146.4, 143.4, 132.6 (q, J = 40.7 Hz), 120.0, 119.0 (q, J = 271.6 Hz), 118.2 (q, J = 1.4 Hz), 61.7, 14.2 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -55.9 ppm. MS (ESI): $[M + H]^+$ 286.1. IR (neat) ν_{max} 1737 (s, C=O), 1590 (s), 1501 (w), 1299 (m), 1245 (s), 1148 (s), 1040 (m) cm^{-1}. HRMS: calculated for $C_{12}H_{10}F_3N_3O_2$ $[M + H]^+$ = 286.0803, observed = 286.0800.

1-(Pyridin-4-yl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (28). According to General Procedure B, the ester derivative **S22** (18 mg, 63.1 µmol) was stirred for 2 h to give the acid derivative **28**[51] as a white amorphous solid (15 mg, 59.3 µmol, 94%). R_f 0.13 (20:80 MeOH:DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.49 (br. s, 1H), 8.82 (d, J = 6.2 Hz, 2H), 8.32 (s, 1H), 7.64 (d, J = 6.2 Hz, 2H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.1, 151.6, 146.4, 143.7, 132.0, 131.7, 121.0, 118.4 (q, J = 1.4 Hz) ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -54.4 ppm. MS (ESI): $[M + H]^+$ 258.1. HPLC: retention time 1.34 min (>99%). IR (neat) ν_{max} 2447 (w, O–H), 1690 (s, C=O), 1601 (m), 1555 (m), 1400 (m), 1239 (m), 1209 (m), 1187 (m), 1126 (s), 1029 (m), 970 (m). HRMS: calculated for $C_{10}H_6F_3N_3O_2$ $[M+H]^+$ = 258.0490, observed = 258.0486.

Ethyl 1-(2-fluorophenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (S23). According to General Procedure E, 2-fluorophenylhydrazine hydrochloride (0.34 g, 2.10 mmol) was stirred for 18 h to give a crude product. The crude product was purified using flash column chromatography (30% EtOAc in petrol) to give the ester derivative **S23**[53] as a white amorphous solid (0.46 g, 1.53 mmol, 73%). R_f 0.56 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (s, 1H), 7.56–7.51 (m, 1H), 7.48 (td, J = 7.6, 1.7 Hz, 1H), 7.31 (tt, J = 7.6, 1.1 Hz, 1H), 7.28 (dt, J = 8.4, 1.1 Hz, 1H), 4.40 (q, J = 7.2 Hz, 2H), 1.40 (t, J = 7.2 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.9, 155.9 (d, J = 253.4 Hz), 133.3 (q, J =

40.4 Hz), 131.1 (d, J = 7.9 Hz), 127.6, 126.5 (d, J = 12.7 Hz), 123.8 (d, J = 4.0 Hz), 118.0 (q, J = 271.7 Hz), 115.7, 115.5 ppm. ^{19}F NMR (376 MHz, DMSO-d_6) δ -54.2, -60.4 ppm. MS (ESI): $[M + H]^+$ 303.1. IR (neat) ν_{max} 1733 (s, C=O), 1599 (w), 1567 (m), 1512 (s), 1302 (s), 1246 (s), 1147 (s), 1039 (s) cm^{-1}. HRMS: calculated for $C_{13}H_{10}F_4N_2O_2$ $[M + H]^+$ = 303.0756, observed = 303.0751.

1-(2-Fluorophenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (29). According to General Procedure B, the ester derivative **S23** (0.43 g, 1.41 mmol) was stirred for 2 h to give the acid derivative **29**[53] as a white amorphous solid (0.36 g, 1.30 mmol, 92%). R_f 0.32 (EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.58–7.50 (m, 1H), 7.47 (dd, J = 7.8, 1.7 Hz, 1H), 7.30 (t, J = 7.8 Hz, 1H), 7.25 (t, J = 5.3 Hz, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.6, 156.8 (d, J = 253.8 Hz), 143.9, 135.1 (q, J = 40.4 Hz), 132.1 (d, J = 7.8 Hz), 128.4, 127.3 (d, J = 12.6 Hz), 124.7 (d, J = 4.1 Hz), 120.1 (q, J = 271.8 Hz), 116.6, 115.4 (d, J = 1.3 Hz) ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -57.7, -62.2 ppm. MS (ESI): $[M - H]^-$ 273.1. HPLC: retention time 1.76 min (>99%). IR (neat) ν_{max} 2844 (w, O–H), 1704 (s, C=O), 1600 (w), 1572 (m), 1509 (m), 1419 (w), 1299 (s), 1261 (s), 1189 (m), 1135 (s), 1032 (s), 970 (m) cm^{-1}.

Ethyl 1-(2-chlorophenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (57). According to General Procedure E, 2-chlorophenylhydrazine hydrochloride (0.11 g, 0.63 mmol) was stirred for 2 h to give a crude product. The crude product was purified using flash column chromatography (5% EtOAc in petrol) to give the ester derivative **57**[53] as a yellow amorphous solid (60 mg, 0.19 mmol, 30%). R_f 0.69 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.55 (d, J = 8.0 Hz, 1H), 7.51–7.45 (m, 1H), 7.44–7.39 (m, 2H), 4.38 (q, J = 7.1 Hz, 2H), 1.38 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.8, 143.1, 137.3, 134.1 (q, J = 40.1 Hz), 132.2, 131.6, 130.4, 128.8 (q, J = 0.6 Hz), 127.6, 119.0 (q, J = 271.6 Hz), 116.3 (q, J = 1.4 Hz), 61.4, 14.2 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -58.2 ppm. MS (ESI): $[M + H]^+$ 319.1. HPLC: retention time 2.20 min (>99%). IR (neat) ν_{max} 3071–2850 (w, C–H), 1718 (s, C=O), 1562 (m), 1497 (m), 1444 (w), 1386 (w), 1299 (m), 1245 (s), 1221 (m), 1147 (s), 1097 (m), 1049 (m), 1017 (m) cm^{-1}.

1-(2-Chlorophenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (30). According to General Procedure B, the ester derivative **57** (45 mg, 0.14 mmol) was stirred for 1 h to give the acid derivative **30**[53] as a yellow amorphous solid (41 mg, 0.14 mmol, >99%). R_f 0.32 (EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.62 (br. s, 1H), 8.28 (s, 1H), 7.57 (d, J = 8.0 Hz, 1H), 7.51 (td, J = 8.0, 7.0, 2.8 Hz, 1H), 7.48–7.40 (m, 2H) ppm. ^{13}C NMR (125 MHz, CD_3OD) δ 163.4, 144.2, 138.5, 135.1 (q, J = 40.0 Hz), 133.1, 133.0, 131.2, 130.2, 129.0, 120.4 (q, J = 270.8 Hz), 117.9 (q, J = 1.5 Hz) ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -58.3 ppm. MS (ESI): $[M + H]^+$ 291.1. HPLC: retention time 1.91 min (>99%). IR (neat) ν_{max} 3000–2500 (w, O–H, C–H), 1702 (m, C=O), 1571 (m), 1495 (m), 1461 (w), 1424 (w), 1303 (m), 1268 (m), 1182 (s), 1143 (s), 1101 (m), 1050 (m), 1034 (s) cm^{-1}.

Ethyl 1-(2-bromophenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (S24). According to General Procedure E, 2-bromophenylhydrazine hydrochloride (0.37 g, 1.66 mmol) was stirred for 2 h to give a crude product. The crude product was purified using flash column chromatography (0%–10% EtOAc in petrol) to give the ester derivative **S24** as an orange oil (65 mg, 0.18 mmol, 11%). R_f 0.28 (25:75 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.73 (dd, J = 8.0, 1.6 Hz, 1H), 7.50–7.38 (m, 3H), 4.38 (q, J = 7.1 Hz, 2H), 1.39 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.9, 143.1, 138.9, 134.0 (q, J = 40.0 Hz), 133.5, 131.8, 128.9 (q, J = 0.7 Hz), 128.2, 121.9 (q, J = 0.8 Hz), 119.0 (q, J = 271.7 Hz), 116.3 (q, J = 1.5 Hz), 61.4, 12.2 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -58.0 ppm. MS (ESI): $[M + H]^+$ 365.1. IR (neat) ν_{max} 3070–2908 (w, C–H), 1716 (s, C=O), 1561 (m), 1494 (m), 1385 (w), 1298 (m), 1244 (s), 1221 (s), 1146 (s), 1092 (s), 1040 (s), 970 (s) cm^{-1}.

1-(2-Bromophenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (31). According to General Procedure B, the ester derivative **S24** (44 mg, 0.12 mmol) was stirred for 1 h to give the acid derivative **31**[54] as a yellow amorphous solid (37 mg, 0.11 mmol, 92%). R_f 0.30 (20:80 MeOH:DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1H), 7.77 (m, 1H), 7.55–7.40 (m, 3H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.1, 143.4, 138.8, 133.6, 132.9 (q, J = 39.5 Hz), 132.9, 129.9, 129.3, 121.3, 119.3 (q, J = 270.9 Hz), 117.3 (q, J = 2.9 Hz) ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -57.2 ppm. MS (ESI): $[M - H]^-$ 334.9. HPLC: retention time 1.80 min (95%). IR (neat) ν_{max} 2852 (w, O–H), 1702 (s, C=O), 1571 (m), 1493 (m), 1464 (m), 1447 (m), 1427 (m), 1144 (s) cm^{-1}. HRMS: calculated for $C_{11}H_6BrF_3N_2O_2$ $[M - H]^-$ = 334.9643, observed = 334.9635.

Ethyl 1-(*o*-tolyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (S25). According to General Procedure E, *o*-tolylhydrazine hydrochloride (0.10 g, 0.63 mmol) was stirred for 3 h to give a crude product. The crude product was purified using flash column chromatography (0%–10% EtOAc in petrol) to give the ester derivative **S25**[53] as yellow oil (0.11 g, 0.37 mmol, 59%). R_f 0.60 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.42 (td, J = 7.5, 1.5 Hz, 1H), 7.33 (d, J = 7.1 Hz, 1H), 7.30 (t, J = 7.4 Hz, 1H), 7.24 (d, J = 7.5 Hz, 1H), 4.38 (q, J = 7.1 Hz, 2H), 2.04 (s, 3H), 1.38 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.1, 142.6, 138.6, 135.4, 133.4 (q, J = 39.7 Hz), 131.0, 130.4, 127.1 (q, J = 0.9 Hz), 126.6, 119.1 (q, J = 271.5 Hz), 115.9 (q, J = 1.3 Hz), 61.3, 16.9, 14.2 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -57.8. MS (ESI): $[M + H]^+$ 299.2. IR (neat) ν_{max} 2984 (w, C–H), 1735 (s, C=O), 1561 (m), 1501 (m), 1467 (m), 1383 (m), 1298 (s), 1228 (s), 1147 (s), 1072 (m), 1040 (s) cm^{-1}.

1-(*o*-Tolyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (32). According to General Procedure B, the ester derivative **S25** (0.19 g, 0.63 mmol) was stirred for 1 h to give the acid derivative **32**[53] as a yellow oil (0.16 g, 0.58 mmol, 92%). R_f 0.20 (EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.45 (app. td, J = 7.5, 1.4 Hz, 1H), 7.36 (d, J = 7.0 Hz, 1H), 7.33 (app. t, J = 7.5 Hz, 1H), 7.26 (d, J = 7.2 Hz, 1H), 2.07 (s, 3H) ppm. ^{13}C NMR (125 MHz, CD_3OD) δ 163.6, 143.8, 139.9, 136.6, 134.3, 132.0, 131.6, 128.2 (q, J = 0.9 Hz), 127.7, 120.5 (q, J = 270.8 Hz), 117.6 (q, J = 1.3 Hz), 16.8 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -57.9 ppm. MS (ESI): $[M + H]^+$ 271.2. HPLC: retention time 1.92 min (>99%). IR (neat) ν_{max} 2927–2588 (m, O–H, C–H), 1701 (s, C=O), 1566 (m), 1500 (w), 1466 (w), 1421 (w), 1301 (m), 1255 (m), 1223 (m), 1185 (s), 1128 (s), 1068 (w), 1033 (s) cm^{-1}.

Ethyl 5-(trifluoromethyl)-1-(2-(trifluoromethyl)phenyl)-1*H*-pyrazole-4- carboxylate (S26). According to General Procedure E, 2-(trifluoromethyl)phenylhydrazine hydrochloride (0.35 g, 1.66 mmol) was stirred for 18 h to give a crude product. The crude product was purified by flash column chromatography (10%–20% EtOAc in petrol) to give the ester derivative **S26**[52] as a yellow oil (49 mg, 0.14 mmol, 8%). R_f

0.45 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (s, 1H), 7.91–7.82 (m, 1H), 7.79–7.67 (m, 2H), 7.49–7.39 (m, 1H), 4.40 (q, J = 7.1 Hz, 2H), 1.41 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.7, 142.7, 136.8, 134.5 (q, J = 40 Hz), 132.7, 130.8, 129.4, 127.9 (q, J = 32 Hz), 127.4 (q, J = 5.0 Hz), 122.5 (q, J = 274 Hz), 118.9 (q, J = 272 Hz), 116.4, 61.3, 14.1 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -56.5, -60.6 ppm. MS (ESI): $[M + H]^+$ 353.2. IR (neat) ν_{max} 1724 (s, C=O), 1563 (m), 1507 (m), 1319 (s), 1249 (s), 1161 (s), 968 (s) cm^{-1}. HRMS: calculated for $C_{14}H_{10}F_6N_2O_2$ $[M+H]^+$ = 353.0724, observed = 353.0721.

5-(Trifluoromethyl)-1-(2-(trifluoromethyl)phenyl)-1*H*-pyrazole-4-carboxylic acid (33). According to General Procedure B, the ester derivative **S26** (48.0 mg, 0.14 mmol) was stirred for 2 h to give the acid derivative **33**[52] as yellow amorphous solid (29.1 mg, 90.0 μmol, 64%). R_f 0.30 (20:80 MeOH:DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.05 (br. s, 1H), 8.26 (s, 1H), 7.87 (dd, J = 6.9, 2.5 Hz, 1H), 7.77–7.70 (m, 2H), 7.45 (dd, J = 6.9, 2.5 Hz, 1H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.7, 165.9, 143.4, 136.5, 135.4 (q, J = 41 Hz), 132.8, 131.0, 129.3, 127.5 (q, J = 32 Hz), 127.5 (q, J = 5 Hz), 122.4 (q, J = 274 Hz), 118.6 (q, J = 272 Hz) ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -56.6, -60.6 ppm. MS (ESI): $[M–H]^–$ 323.0. HPLC: retention time 1.80 min (>99%). IR (neat) ν_{max} 2924 (w, O–H), 1703 (s, C=O), 1573 (m), 1320 (s), 1258 (m), 1137 (s), 1034 (m) cm^{-1}. HRMS: calculated for $C_{12}H_6F_6N_2O_2$ $[M+H]^+$ = 325.0411, observed = 325.0400.

Ethyl 5-(trifluoromethyl)-1-[4-(trifluoromethyl)phenyl]-1*H*-pyrazole-4-carboxylate (S27). According to General Procedure E, 4-(trifluoromethyl)phenylhydrazine hydrochloride (0.25 g, 1.17 mmol) was stirred for 4 h to give a crude product. The crude product was purified by flash column chromatography (5% EtOAc in petrol) to give the ester derivative **S27**[52] as a yellow oil (0.27 g, 0.77 mmol, 66%). R_f 0.68 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.77 (d, J = 8.3 Hz, 2H), 7.57 (d, J = 8.3 Hz, 2H), 4.37 (q, J = 7.1 Hz, 2H), 1.37 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.8, 143.0, 142.2, 132.8 (q, J = 40.4 Hz), 132.1 (q, J = 33.1 Hz), 126.5 (q, J = 3.7 Hz), 126.4 (q, J = 1.1 Hz), 123.5 (q, J = 272.5 Hz), 119.1 (q, J = 271.5 Hz), 117.5 (q, J = 1.3 Hz), 61.5, 14.1 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -56.0, -63.7 ppm. MS (ESI): $[M + H]^+$ 353.2. IR (neat) ν_{max} 2991–2909 (w, C–H), 1737 (s, C=O), 1562 (w), 1466 (w), 1323 (m), 1300 (m), 1223 (s), 1175 (s), 1123 (s), 1062 (s), 1035 (s) cm^{-1}.

5-(Trifluoromethyl)-1-[4-(trifluoromethyl)phenyl]-1*H*-pyrazole-4-carboxylic acid (34). According to General Procedure B, the ester derivative **S27** (0.10 g, 0.28 mmol) was stirred for 4 h to give the acid derivative **34**[52] as an amorphous white solid (88 mg, 0.27 mmol, 96%). R_f 0.26 (20:80 MeOH:DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.81 (d, J = 8.3 Hz, 2H), 7.60 (d, J = 8.3 Hz, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 143.8, 141.9, 133.8 (q, J = 40.7 Hz), 132.4 (q, J = 33.1 Hz), 126.6 (q, J = 3.7 Hz), 126.5, (q, J = 1.1 Hz), 123.5 (q, J = 272.7 Hz), 118.9 (q, J = 271.9 Hz), 116.1 (q, J = 1.4 Hz) ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -56.1, -63.7 ppm. MS (ESI): $[M – H]^–$ 323.1. HPLC: retention time 1.99 min (>99%). IR (neat) ν_{max} 3100–2587 (m, O–H, C–H), 1701 (m, C=O), 1570 (w), 1467 (w), 1422 (w), 1407 (w), 1327 (m), 1295 (m), 1259 (m), 1224 (m), 1179 (m), 1141 (s), 1112 (s), 1063 (m), 1031 (m) cm^{-1}.

Ethyl 1-(4-methoxyphenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (S28). According to General Procedure E, 4-methoxyphenyl hydrazine hydrochloride (0.22 g, 1.24 mmol) was stirred for 2 h to give a crude product. The crude product was purified by flash column chromatography (10% EtOAc in petrol) to give the ester derivative **S28**[55] as an amorphous orange solid (0.19 g, 0.60 mmol, 48%). R_f 0.53 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.33 (d, J = 9.0 Hz, 2H), 6.98 (d, J = 9.0 Hz, 2H), 4.37 (q, J = 7.1 Hz, 2H), 3.87 (s, 3H), 1.38 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.2, 160.6, 142.3, 132.7 (q, J = 39.8 Hz), 132.4, 127.2, 119.2 (q, J = 271.4 Hz), 116.5 (q, J = 1.0 Hz), 114.3, 61.3, 55.7, 14.2 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -56.5 ppm. MS (ESI): $[M + H]^+$ 315.1. IR (neat) ν_{max} 3117–2843 (w, C–H), 1735 (m, C=O), 1565 (w), 1516 (s), 1465 (w), 1446 (w), 1372 (w), 1302 (s), 1223 (s), 1185 (m), 1129 (s), 1080 (m), 1043 (s), 1018 (s), 971 (m) cm^{-1}.

1-(4-Methoxyphenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (35). According to General Procedure B, the ester derivative **S28** (62 mg, 0.20 mmol) was stirred for 1 h to give the acid derivative **35**[55] as an amorphous white solid (50 mg, 0.18 mmol, 90%). R_f 0.30 (20:80 MeOH:DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.43 (br. s, 1H), 8.19 (s, 1H), 7.35 (d, J = 8.5 Hz, 2H), 7.00 (d, J = 8.5 Hz, 2H), 3.87 (s, 3H) ppm. ^{13}C NMR (400 MHz, $CDCl_3$) δ 166.4, 160.7, 143.1, 133.6 (q, J = 40.2 Hz), 132.1, 127.2, 119.0 (q, J = 271.6 Hz), 115.2 (q, J = 1.2 Hz), 114.4, 55.7 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -56.5 ppm. MS (ESI): $[M – H]^–$ 285.0. HPLC: retention time 1.80 min (>99%). IR (neat) ν_{max} 2968–2591 (m, O–H, C–H), 1700 (s, C=O), 1679 (m), 1563 (w), 1517 (s), 1466 (w), 1423 (w), 1302 (s), 1249 (s), 1175 (m), 1140 (s), 1075 (m), 1028 (s) cm^{-1}.

(2-Methyl-4-nitrophenyl)hydrazine (S29). According to a procedure,[56] hydrazine monohydrate (2.00 mL, 40.6 mmol) was added to a solution of 1-fluoro-2-methyl-4-nitrobenzene (3.00 g, 19.3 mmol) in isopropyl alcohol (30.0 mL). After stirring the mixture at 90 °C for 2 h, additional hydrazine monohydrate (2.00 mL, 40.6 mmol) was added and the mixture was kept at 90 °C for a further 2 h. The mixture was allowed to cool to room temperature and diethyl ether (25 mL) was added. The precipitate was collected by filtration, washed with water (12 mL) and diethyl ether (12 mL) and dried *in vacuo*. Subsequently, a 6 M HCl aqueous solution (18 mL) was added. After stirring the mixture at room temperature for 1 h, the precipitate was collected by filtration, washed with a 6 M HCl aqueous solution and dried *in vacuo* to yield the hydrochloride salt of the hydrazine derivative **S29**[56] as a white-yellow amorphous solid (2.00 g, 9.86 mmol, 51%). R_f 0.75 (20:80 MeOH:DCM). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.56 (br. s, 2H), 8.88 (s, 1H), 8.09 (dd, J = 9.0, 2.7 Hz, 1H), 8.02 (d, J = 2.7 Hz, 1H), 7.04 (d, J = 9.0 Hz, 1H), 2.27 (s, 3H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ 149.4, 140.2, 125.4, 124.4, 123.0, 111.2, 17.2 ppm. MS (ESI): $[M + H]^+$ 168.0. IR (neat) ν_{max} 3280 (s, N–H), 3074 (m, N–H), 2817–2607 (m, N–H, C–H), 1593 (m), 1584 (m), 1495 (s), 1335 (s), 1298 (m), 1253 (m), 1216 (m), 1102 (m) cm^{-1}.

Ethyl 1-(2-methyl-4-nitrophenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (S30). According to General Procedure E, the hydrazine derivative hydrochloride **S29** (0.25 g, 1.22 mmol) was stirred for 18 h to give a crude product. The crude product was purified using flash column chromatography (0%–10% EtOAc in petrol) to give the ester derivative **S30** as a yellow oil (0.24 g, 0.70 mmol, 57%). R_f 0.40 (20:80 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, J = 2.5 Hz, 1H), 8.18 (s, 1H), 8.17 (dd, J = 8.6, 2.5 Hz, 1H),

7.44 (d, J = 8.6 Hz, 1H), 4.37 (q, J = 7.1 Hz, 2H), 2.16 (s, 3H), 1.37 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.6, 148.6, 143.4, 138.0, 133.6 (q, J = 40.0 Hz), 128.6, 128.5, 126.1, 121.9, 118.9 (q, J = 271.6 Hz), 116.8 (q, J = 1.4 Hz), 61.6, 17.3, 14.1 ppm. ^{19}F NMR (396 MHz, $CDCl_3$) δ -57.5 ppm. MS (ESI): $[M + H]^+$ 344.2. IR (neat) ν_{max} 2984–2873 (w, C–H), 1726 (s, C=O), 1566 (w), 1534 (m), 1499 (w), 1354 (m), 1300 (m), 1229 (s), 1180 (s), 1139 (s), 1101 (s), 1039 (s) cm^{-1}.

1-(2-Methyl-4-nitrophenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (36). According to General Procedure B, the ester derivative **S30** (0.10 g, 0.29 mmol) was stirred for 6 h to give the acid derivative **36** as a white amorphous solid (76.0 mg, 0.24 mmol, 83%). R_f 0.33 (20:80 MeOH:DCM). ^{1}H NMR (400 MHz, CD_3OD) δ 8.32 (d, J = 2.5 Hz, 1H), 8.24 (s, 1H), 8.23 (dd, J = 8.6, 2.5 Hz, 1H), 7.62 (d, J = 8.6 Hz, 1H), 2.16 (s, 3H) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ 163.3, 150.1, 144.6, 139.3, 134.6 (q, J = 40.1 Hz), 129.9, 126.9, 122.9, 120.4 (q, J = 270.9 Hz), 118.4 (d, J = 1.0 Hz), 17.1 ppm. ^{19}F NMR (396 MHz, CD_3OD) δ -58.8 ppm. MS (ESI): $[M - H]^-$ 314.0. HPLC: retention time 1.84 min (>99%). IR (neat) ν_{max} 3100–2580 (w, O–H, C–H), 1705 (m, C=O), 1686 (m), 1572 (m), 1527 (m), 1496 (m), 1347 (m), 1293 (m), 1263 (m), 1228 (m), 1179 (m), 1140 (s), 1098 (m), 1034 (m) cm^{-1}. HRMS: calculated for $C_{12}H_8F_3N_3O_4$ $[M + H]^+$ = 316.0545, observed = 316.0542.

3-Hydrazinylbenzamide hydrochloride (S31). According to General Procedure F, 3-aminobenzamide (1.00 g, 7.34 mmol) gave the hydrazine derivative **S31**[57] as a pale brown amorphous solid (1.36 g, 7.25 mmol, 99%). R_f 0.20 (20:80 MeOH:DCM). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.3 (br. s, 3H), 7.93 (br. s, 1H), 7.53 (s, 1H), 7.44 (app. d, J = 7.7 Hz, 1H), 7.34 (app. t, J = 8.0, 1H), 7.13 (dd, J = 8.0 and 2.4 Hz, 1H), 4.83 (br. s, 2H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.7, 145.6, 135.1, 128.8, 120.1, 117.1, 113.7 ppm. MS (ESI): $[M + H]^+$ 152.0. IR (neat) ν_{max} 3458 (w, N–H), 3358 (w, N–H), 3271 (m, N–H), 3176–2700 (s, N–H, C–H), 1646 (s, C=O), 1561 (s), 1540 (s), 1456 (s) cm^{-1}.

Ethyl 1-(3-carbamoylphenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (S32). According to General Procedure E, the hydrazine derivative **S31** (0.25 g, 1.33 mmol) was stirred for 6 h to give a crude product. The crude product was purified using flash column chromatography (40%–60% EtOAc in petrol) to give the ester derivative **S32** as a white amorphous solid (0.20 g, 0.61 mmol, 46%). R_f 0.53 (90:10 EtOAc:petrol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (s, 1H), 8.16 (br. s, 1H), 8.11 (app. dt, J = 7.5, 1.6 Hz, 1H), 8.02 (s, 1H), 7.75–7.65 (m, 2H), 7.61 (br. s, 1H), 4.32 (q, J = 7.1 Hz, 2H), 1.30 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.2, 160.2, 142.3, 138.9, 135.3, 131.4, 129.4, 129.11, 128.8, 125.1, 118.9 (q, J = 271.1 Hz), 116.3 (q, J = 1.5 Hz), 61.1, 13.9 ppm. ^{19}F NMR (376 MHz, DMSO-d_6) δ -55.5 ppm. MS (ESI): $[M + H]^+$ 328.1. IR (neat) ν_{max} 3442 (m, N–H), 3199 (m, N–H), 2984 (w, C–H), 1729 (s, C=O), 1697 (s, C=O), 1623 (w), 1566 (w), 1449 (w), 1374 (m), 1299 (s), 1248 (s), 1224 (s), 1190 (s), 1133 (s), 1079 (m), 1038 (s), 986 (m) cm^{-1}.

1-(3-Carbamoylphenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (37). According to General Procedure B, the ester derivative **S32** (85 mg, 0.26 mmol) was stirred for 6 h to give the acid derivative **37** as a white amorphous solid (70 mg, 0.23 mmol, 88%). R_f 0.10 (20:80 MeOH:DCM). ^{1}H NMR (400 MHz, CD_3OD) δ 8.22 (app. dt, J = 7.4, 1.6 Hz, 1H), 8.18 (s, 1H), 8.08 (s, 1H), 7.75–7.64 (m, 2H), 5.05 (br. s, 2H) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ 167.9, 163.5, 144.0, 140.9, 133.4, 132.1, 131.4, 130.6, 128.2 (q, J = 0.8 Hz), 124.6, 120.5 (q, J = 270.7 Hz), 118.6 (q, J = 1.4 Hz) ppm. ^{19}F NMR (376 MHz, CD_3OD) δ -56.6 ppm. MS (ESI): $[M - H]^-$ 298.0. HPLC: retention time 1.53 min (94%). IR (neat) ν_{max} 3100–2555 (s, N–H, O–H, C–H), 1689 (s, C=O), 1571 (m), 1472 (w), 1448 (w), 1418 (m), 1398 (w), 1298 (s), 1265 (s), 1220 (m), 1196 (m), 1117 (s), 1135 (s), 1125 (s), 1069 (m), 1032 (s) cm^{-1}. HRMS: calculated for $C_{12}H_8F_3N_3O_3$ $[M + H]^+$ = 300.0596, observed = 300.0592.

Ethyl 1-(2,3-dimethylphenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (S33). According to General Procedure E, 2,3-dimethylphenylhydrazine hydrochloride (0.25 g, 1.44 mmol) was stirred for 18 h to give a crude product. The crude product was purified using flash column chromatography (5% EtOAc in petrol) to give the ester derivative **S33** as yellow oil (0.30 g, 0.96 mmol, 67%). R_f 0.66 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.29 (d, J = 7.7 Hz, 1H), 7.18 (t, J = 7.7 Hz, 1H), 7.08 (d, J = 7.7, 1H), 4.37 (q, J = 7.2, 2H), 2.33 (s, 3H), 1.87 (s, 3H), 1.38 (t, J = 7.2, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.1, 142.5, 138.6, 138.4, 134.0, 133.5 (q, J = 39.9 Hz), 131.7, 125.9, 124.7, 119.1 (q, J = 271.5 Hz), 115.8 (d, J = 1.3 Hz), 61.2, 20.2, 14.1, 13.8 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -57.8 ppm. MS (ESI): $[M + H]^+$ 313.1. IR (neat) ν_{max} 2995–2924 (w, C–H), 1713 (s, C=O), 1554 (m), 1481 (m), 1384 (m), 1293 (m), 1244 (s), 1230 (s), 1176 (s), 1144 (s), 1038 (s), 1017 (m) cm^{-1}.

1-(2,3-Dimethylphenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (38). According to General Procedure B, the ester derivative **S33** (0.12 g, 0.38 mmol) was stirred for 4.5 h to give the acid derivative **38** as a yellow amorphous solid (95 mg, 0.33 mmol, 88%). R_f 0.22 (EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.44 (br. s, 1H), 8.25 (s, 1H), 7.33 (d, J = 7.7 Hz, 1H), 7.22 (t, J = 7.7 Hz, 1H), 7.12 (d, J = 7.7 Hz, 1H), 2.36 (s, 3H), 1.91 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5, 143.4, 138.6, 138.5, 134.5 (q, J = 40.2 Hz), 134.0, 131.9, 126.0, 124.7, 118.9 (q, J = 271.8 Hz), 114.6 (d, J = 1.1 Hz), 20.3, 13.8 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) -56.9 ppm. MS (ESI): $[M + H]^+$ 285.1. HPLC: retention time 1.88 min (>99%). IR (neat) ν_{max} 2926–2580 (m, O–H, C–H), 1698 (s, C=O), 1681 (s), 1564 (m), 1482 (w), 1456 (w), 1417 (w), 1299 (s), 1258 (s), 1225 (s), 1186 (s), 1138 (s), 1033 (s), 968 (m) cm^{-1}. HRMS: calculated for $C_{13}H_{11}F_3N_2O_2$ $[M + H]^+$ = 285.0851, observed = 285.0851.

Ethyl 1-(2,4-dimethylphenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (S34). According to General Procedure E, 2,4-dimethyphenylhydrazine hydrochloride (0.21 g, 1.22 mmol) was stirred for 18 h to give a crude product. The crude product was purified using flash column chromatography (15% EtOAc in petrol) to give the ester derivative **S34** as a pale red amorphous solid (0.25 g, 0.81 mmol, 66%). R_f 0.44 (15:85 EtOAc:petrol). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.18–7.06 (m, 3H), 4.38 (q, J = 7.2 Hz, 2H), 2.39 (s, 3H), 2.00 (s, 3H), 1.39 (t, J = 7.2 Hz, 3H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.1, 142.4, 140.4, 136.0, 134.9, 133.4 (q, J = 39.7 Hz), 131.5, 127.1, 126.7, 119.1 (q, J = 271.4 Hz), 115.7, 61.2, 21.2, 16.7, 14.1 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -56.9 ppm. MS (ESI): $[M + H]^+$ 313.2. IR (neat) ν_{max} 1715 (s, C=O), 1553 (m), 1550 (w), 1389 (m), 1255 (m), 1241 (s), 1149 (s), 1144 (s), 1041 (w), 1038 (m), 972 (m) cm^{-1}.

HRMS: calculated for $C_{15}H_{15}F_3N_2O_2$ $[M + H]^+$ = 313.1163, observed = 313.1153.

1-(2,4-Dimethylphenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (39). According to General Procedure B, the ester derivative **S34** (0.22 g, 0.70 mmol) was stirred for 2 h to give the acid derivative **39** as a red amorphous solid (0.19 g, 0.67 mmol, 96%). R_f 0.22 (EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.74 (s, 1H), 8.25 (s, 1H), 7.21–7.11 (m, 3H), 2.40 (s, 3H), 2.02 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 143.3, 140.7, 135.8, 134.8, 134.3 (q, J = 40.2 Hz), 131.6, 127.2, 126.7 (q, J = 0.7 Hz), 118.9 (q, J = 271.8 Hz), 114.6 (d, J = 1.2 Hz), 21.2, 16.7 ppm. ^{19}F NMR (396 MHz, $CDCl_3$) δ -57.0 ppm. MS (ESI): $[M + H]^+$ 285.1. HPLC: retention time 1.95 min (>99%). IR (neat) ν_{max} 2927 (w, O–H), 1720 (s, C=O), 1558 (m), 1506 (m), 1305 (s), 1233 (s), 1163 (s), 1068 (m), 1031 (s) cm^{-1}. HRMS: calculated for $C_{13}H_{11}F_3N_2O_2$ $[M + H]^+$ = 285.0851, observed = 285.0841.

Ethyl 1-(2,5-dimethylphenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (S35). According to General Procedure E, 2,5-dimethylphenylhydrazine hydrochloride (0.25 g, 1.44 mmol) was stirred for 16 h to give a crude product. The crude product was purified using flash column chromatography (5% EtOAc in petrol) to give the ester derivative **S35** as a yellow amorphous solid (0.28 g, 0.90 mmol, 63%). R_f 0.70 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.24–7.16 (m, 2H), 7.05 (s, 1H), 4.36 (q, J = 7.1 Hz, 2H), 2.34 (s, 3H), 1.98 (s, 3H), 1.37 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.1, 142.5, 138.4, 136.6, 133.4 (q, J = 39.8 Hz), 132.1, 131.1, 130.7, 127.5, 119.1 (q, J = 271.4 Hz), 115.8 (d, J = 1.4 Hz), 61.2, 20.7, 13.4, 14.2 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -57.9 ppm. MS (ESI): $[M + H]^+$ 313.1. IR (neat) ν_{max} 3122–2926 (w, C–H), 1717 (s, C=O), 1553 (m), 1468 (m), 1384 (w), 1298 (m), 1234 (s), 1174 (s), 1135 (s), 1070 (w), 1036 (m) cm^{-1}.

1-(2,5-Dimethylphenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (40). According to General Procedure B, the ester derivative **S35** (0.11 g, 0.35 mmol) was stirred for 4.5 h to give the acid derivative **40** as a yellow oil (83.0 mg, 0.29 mmol, 83%). R_f 0.37 (EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 1H), 7.26–7.20 (m, 2H), 7.08 (s, 1H), 2.37 (s, 3H), 2.01 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5, 143.4, 138.3, 136.7, 134.4 (q, J = 40.1 Hz), 132.1, 131.4, 130.8, 127.5, 118.9 (q, J = 271.8 Hz), 114.6, 20.8, 16.5 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -57.0 ppm. MS (ESI): $[M – H]^-$ 283.0. HPLC: retention time 1.90 min (>99%). IR (neat) ν_{max} 2929–2600 (m, O–H, C–H), 1708 (m C=O), 1679 (m), 1559 (m), 1510 (w), 1458 (m), 1421 (w), 1297 (m), 1259 (s), 1232 (m), 1182 (m), 1143 (s), 1033 (m) cm^{-1}. HRMS: calculated for $C_{13}H_{11}F_3N_2O_2$ $[M + H]^+$ = 285.0851, observed = 285.0855.

Ethyl 1-(2,6-dimethylphenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (S36). According to General Procedure E, 2,6-dimethylphenylhydrazine hydrochloride (0.32 g, 1.87 mmol) was stirred for 18 h to give a crude product. The crude product was purified using flash column chromatography (30% EtOAc in petrol) to give the ester derivative **S36** as a white amorphous solid (93.7 mg, 0.30 mmol, 16%). R_f 0.59 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 1H), 7.31 (t, J = 7.7 Hz, 1H), 7.17 (d, J = 7.7 Hz, 2H), 4.40 (q, J = 7.1 Hz, 2H), 1.99 (s, 6H), 1.41 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.0, 143.1, 138.0, 135.5, 133.5 (q, J = 40.0 Hz), 130.0, 128.3, 119.1 (q, J = 271.0 Hz), 115.6 (q, J = 1.5 Hz), 61.2, 16.9, 14.1 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -58.4 ppm. MS (ESI): $[M + H]^+$ 313.2. IR (neat) ν_{max} 1736 (s, C=O), 1560 (s), 1484 (s), 1297 (s), 1222 (s), 1145 (s), 1039 (s), 966 (s) cm^{-1}. HRMS: calculated for $C_{15}H_{15}F_3N_2O_2$ $[M + H]^+$ = 313.1163, observed = 313.1155.

1-(2,6-Dimethylphenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (41). According to General Procedure B, the ester derivative **S36** (92.0 mg, 0.29 mmol) was stirred for 2 h to give the acid derivative **41** as a white amorphous solid (59.0 mg, 0.21 mmol, 70%). R_f 0.38 (20:80 MeOH:DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.32 (t, J = 7.6 Hz, 1H), 7.17 (d, J = 7.6 Hz, 2H), 1.99 (s, 6H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.3, 144.0, 138.0, 135.5, 134.5 (q, J = 40.2 Hz), 130.2, 128.4, 118.9 (q, J = 271.8 Hz), 114.5 (q, J = 1.2 Hz), 17.0 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -58.5 ppm. MS (ESI): $[M + H]^+$ 285.2. HPLC: retention time 1.90 min (>99%). IR (neat) ν_{max} 2924 (w, O–H), 1704 (s, C=O), 1571 (m), 1485 (m), 1303 (m), 1266 (m), 1249 (m), 1149 (s), 1035 (s), 967 (s) cm^{-1}. HRMS: calculated for $C_{13}H_{11}F_3N_2O_2$ $[M + H]^+$ = 285.0851, observed = 285.0847.

Ethyl 1-(2,6-dichlorophenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (S37). According to General Procedure E, 2,6-dichlorophenylhydrazine hydrochloride (0.13 g, 0.63 mmol) was stirred for 2 h to give a crude product. The crude product was purified using flash column chromatography (10% EtOAc in petrol) to give the ester derivative **S37** as a pale yellow amorphous solid (0.14 g, 0.40 mmol, 63%). R_f 0.47 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.50–7.45 (m, 2H), 7.42 (dd, J = 9.5, 6.4 Hz, 1H), 4.39 (q, J = 7.1 Hz, 2H), 1.39 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.7, 143.9, 135.5, 134.5 (q, J = 40.4 Hz), 134.4 (q, J = 0.7 Hz), 131.8, 128.7, 118.9 (q, J = 271.6 Hz), 116.4 (q, J = 1.6 Hz), 61.4, 14.2 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -59.6 ppm. MS (ESI): $[M + H]^+$ 353.1. IR (neat) ν_{max} 3090–2942 (w, C–H), 1729 (s, C=O), 1571 (m), 1496 (m), 1442 (m), 1294 (m), 1240 (s), 1221 (s), 1149 (s), 1084 (m), 1040 (s) cm^{-1}.

1-(2,6-Dichlorophenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (42). According to General Procedure B, the ester derivative **S37** (88.3 mg, 0.25 mmol) was stirred for 1 h to give the acid derivative **42** as a yellow amorphous solid (78.0 mg, 0.24 mmol, 98%). R_f 0.25 (EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.01 (br. s, 1H), 8.35 (s, 1H), 7.53–7.41 (m, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 144.6, 135.5 (q, J = 41.2 Hz), 134.3, 134.3 (q, J = 0.6 Hz), 132.0, 128.7, 118.6 (q, J = 272.0 Hz), 115.2 (q, J = 1.5 Hz) ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -59.7 ppm. MS (ESI): $[M – H]^-$ 323.0. HPLC: retention time 1.90 min (94%). IR (neat) ν_{max} 2873 (m, O–H), 2599–2565 (w, C–H), 1699 (s, C=O), 1572 (m), 1494 (m), 1444 (m), 1424 (m), 1302 (m), 1259 (m), 1223 (m), 1148 (s), 1033 (m) cm^{-1}. HRMS: calculated for $C_{11}H_5Cl_2F_3N_2O_2$ $[M + H]^+$ = 324.9758, observed = 324.9758.

Ethyl 1-(3,4-dichlorophenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (S38). According to General Procedure E, 3,4-dichlorophenylhydrazine hydrochloride (0.25 g, 1.17 mmol) was stirred for 18 h to give a crude product. The crude product was purified using flash column chromatography (5% EtOAc in petrol) to give the ester derivative **S38**[52] as a yellow-white amorphous solid (0.21 g, 0.59 mmol, 51%). R_f 0.38 (10:90 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H), 7.62–7.56 (m, 2H), 7.29 (d, J = 8.7 Hz, 1H), 4.38 (q, J = 7.1 Hz, 2H), 1.38 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.8, 143.0, 138.4, 134.7, 133.5 (q, J = 40.3 Hz), 130.9, 128.1 (q, J = 1.1 Hz), 125.2 (q, J = 1.1 Hz),

122.2, 119.1 (q, J = 271.6 Hz), 117.5, 61.6, 14.2 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -55.2 ppm. MS (ESI): $[M + H]^+$ 353.1. IR (neat) ν_{max} 3068–2906 (w, C–H), 1702 (s, C=O), 1595 (w), 1557 (m), 1486 (m), 1417 (w), 1403 (w), 1385 (m), 1356 (w), 1295 (m), 1254 (s), 1221 (m), 1174 (m), 1149 (s), 1135 (s), 1084 (m), 1039 (m), 1013 (m), 982 (m) cm^{-1}.

1-(3,4-Dichlorophenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (43). According to General Procedure B, the ester derivative **S38** (0.10 g, 0.28 mmol) was stirred for 4 h to give the acid derivative **43**[52] as a light brown amorphous solid (40 mg, 0.12 mmol, 43%). R_f 0.30 (20:80 MeOH:DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.57 (br. s, 1H), 8.22 (s, 1H), 7.65–7.55 (m, 2H), 7.31 (dd, J = 8.6, 2.5 Hz, 1H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 143.7, 138.2, 135.0, 133.8 (q, J = 40.6 Hz), 133.6, 131.0, 128.1 (q, J = 1.2 Hz), 125.2 (q, J = 1.2 Hz), 118.8 (q, J = 272.0 Hz), 116.1 (q, J = 1.2 Hz) ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -55.2 ppm. MS (ESI): $[M – H]^–$ 323.0. HPLC: retention time 2.06 min (>99%). IR (neat) ν_{max} 3102–2872 (m, O–H, C–H), 1703 (s, C=O), 1562 (m), 1484 (m), 1424 (w), 1297 (m), 1265 (m), 1249 (m), 1216 (m), 1185 (s), 1152 (s), 1132 (s), 1076 (m), 1031 (s) cm^{-1}.

(4,5-Dichloro-2-methylphenyl)hydrazine hydrochloride (S39). According to General Procedure F, 4,5-dichloro-2-methylaniline (0.69 g, 3.91 mmol) gave the hydrazine derivative **S39** as a pale brown amorphous solid (0.49 g, 2.15 mmol, 55%). R_f 0.47 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, CD_3OD) δ 7.34 (s, 1H), 7.05 (s, 1H), 2.22 (s, 3H) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ 143.7, 133,1, 131.0, 127.5, 126.2, 115.2, 16.5 ppm. MS (ESI): $[M + H]^+$ 228.0. IR (neat) ν_{max} 3273 (m, N–H), 2872 (s, N–H), 2693 (s, N–H, C–H), 1586 (w), 1536 (s), 1493 (s), 1422 (m), 1380 (m), 1219 (w), 1174 (m), 1149 (m), 1118 (m), 941 (m) cm^{-1}.

Ethyl 1-(4,5-dichloro-2-methylphenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (S40). According to General Procedure E, the hydrazine derivative **S39** (0.49 g, 2.15 mmol) was stirred for 4 h to give a crude product. The crude product was purified using flash column chromatography (0%–5% EtOAc in petrol) to give the ester derivative **S40** as a yellow amorphous solid (0.43 g, 1.17 mmol, 54%). R_f 0.63 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.45 (s, 1H), 7.38 (s, 1H), 4.38 (q, J = 7.1 Hz, 2H), 2.00 (s, 3H), 1.38 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.7, 143.1, 137.6, 135.8, 134.7, 133.6 (q, J = 40.1 Hz), 132.4, 130.4, 129.0, 119.0 (q, J = 271.6 Hz), 116.5 (q, J = 1.5 Hz), 61.5, 16.5, 14.2 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -57.6 ppm. MS (ESI): $[M + H]^+$ 367.1. IR (neat) ν_{max} 3122–2869 (w, C–H), 1716 (m, C=O), 1555 (m), 1488 (m), 1419 (w), 1400 (w), 1383 (m), 1352 (w), 1294 (m), 1245 (m), 1149 (s), 1132 (s), 1077 (m), 1035 (m), 1008 (m), 984 (m) cm^{-1}.

1-(4,5-Dichloro-2-methylphenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (44). According to General Procedure B, the ester derivative **S40** (0.43 g, 1.17 mmol) was stirred for 5 h to give a crude product. The crude product was triturated with hexane to give the acid derivative **44** as a white amorphous solid (0.32 g, 0.94 mmol, 80%). R_f 0.38 (20:80 MeOH:DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.12 (br. s, 1H), 8.26 (s, 1H), 7.47 (s, 1H), 7.40 (s, 1H), 2.03 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 143.9, 137.4, 135.6, 135.0, 134.6 (q, J = 40.4 Hz), 132.5, 130.6, 129.0, 118.7 (q, J = 272.0 Hz), 115.2, 16.5 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -57.7 ppm. MS (ESI): $[M – H]^–$ 337.0. HPLC: retention time 2.15 min (>99%). IR (neat) ν_{max} 2924–2600 (m, O–H, C–H), 1701 (m, C=O), 1681 (m), 1561 (m), 1490 (m), 1452 (m), 1418 (w), 1300 (m), 1260 (m), 1223 (m), 1178 (m), 1149 (s), 1074 (w), 1032 (m) cm^{-1}. HRMS: calculated for $C_{12}H_7Cl_2F_3N_2O_2$ $[M + H]^+$ = 338.9915, observed = 338.9915.

(3,4-Dichloro-2-methylphenyl)hydrazine hydrochloride (S41). According to General Procedure F, 3,4-dichloro-2-methylaniline (0.20 g, 1.13 mmol) gave the hydrazine derivative **S41** as a pale brown amorphous solid (0.23 g, 0.63 mmol, 56%). R_f 0.54 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, CD_3OD) δ 7.41 (d, J = 8.7 Hz, 1H), 6.90 (d, J = 8.7 Hz, 1H), 2.37 (s, 3H) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ 143.6, 134.2, 128.8, 127.7, 127.5, 113.5, 14.9 ppm. MS (ESI): $[M + H]^+$ 228.1. IR (neat) ν_{max} 3181–3156 (m, N–H), 2990–2653 (s, N–H, C–H), 1556 (s), 1517 (m), 1483 (w), 1456 (s), 1401 (w), 1190 (m), 1170 (s), 1062 (m) cm^{-1}.

Ethyl 1-(3,4-dichloro-2-methylphenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate (S42). According to General Procedure E, the hydrazine derivative **S41** (0.12 g, 0.53 mmol) was stirred for 5.5 h to give a crude product. The crude product was purified using flash column chromatography (5% EtOAc in petrol) and triturated with hexane to give the ester derivative **S42** as a colourless amorphous solid (0.11 g, 0.30 mmol, 57%). R_f 0.58 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.45 (d, J = 8.5 Hz, 1H), 7.15 (d, J = 8.5 Hz, 1H), 4.38 (q, J = 7.1, 2H), 2.07 (s, 3H), 1.39 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.8, 143.1, 137.7, 136.8, 135.5, 134.1, 133.7 (q, J = 40.0 Hz), 127.9, 126.1, 119.0 (q, J = 271.6 Hz), 116.5 (q, J = 1.4 Hz), 61.5, 16.1, 14.2 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -57.6 ppm. MS (ESI): $[M + H]^+$ 367.1. IR (neat) ν_{max} 3120 (w, C–H), 2980 (w, C–H), 1715 (s, C=O), 1555 (m), 1476 (m), 1385 (m), 1295 (m), 1248 (s), 1150 (s), 1086 (m), 1039 (m), 1013 (m), 974 (m) cm^{-1}.

1-(3,4-Dichloro-2-methylphenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (45). According to General Procedure B, the ester derivative **S42** (0.11 g, 0.30 mmol) was stirred for 5 h to give a crude product. The crude product was triturated with hexane to give the acid derivative **45** as a white amorphous solid (90 mg, 0.27 mmol, 90%). R_f 0.25 (20:80 MeOH:DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.43 (br. s, 1H), 8.26 (s, 1H), 7.47 (d, J = 8.5 Hz, 1H), 7.17 (d, J = 8.5 Hz, 1H), 2.10 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 143.8, 137.5, 136.7, 135.8, 134.7 (q, J = 40.4 Hz), 134.2, 127.9, 126.1, 118.8 (q, J = 272.0 Hz), 115.2, 16.1 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -57.7 ppm. MS (ESI): $[M – H]^–$ 337.0. HPLC: retention time 2.08 min (>99%). IR (neat) ν_{max} 3127–2592 (m, O–H, C–H), 1678 (m, C=O), 1561 (m), 1459 (m), 1293 (m), 1254 (m), 1228 (m), 1183 (m), 1147 (s), 1088 (m), 1035 (m) cm^{-1}. HRMS: calculated for $C_{12}H_7Cl_2F_3N_2O_2$ $[M + H]^+$ = 338.9915, observed = 338.9910.

Ethyl 2,5-dimethyl-1-(2-methylphenyl)-1*H*-pyrrole-3-carboxylate (S43). According to General Procedure C, 2-methylaniline (57 µL, 0.54 mmol) and the carbonyl derivative **S5** were stirred for 7 h to give a crude product. The crude product was purified by flash column chromatography (0%–5% EtOAc in petrol) to give the ester derivative **S43**[58] as a colourless oil (0.12 g, 0.47 mmol, 87%). R_f 0.53 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42–7.27 (m, 3H), 7.10 (d, J = 7.6 Hz, 1H), 6.39 (s, 1H), 4.28 (q, J = 7.1 Hz, 2H), 2.19 (s, 3H), 1.93 (s, 3H), 1.87 (s, 3H), 1.35 (t, J = 7.1, 3H), ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.9, 136.9, 136.7, 135.8, 131.0, 129.1, 128.6, 128.2, 127.0, 111.4, 107.4, 59.3, 17.1, 14.7, 12.3, 12.0 ppm. MS (ESI): $[M + H]^+$ 258.1. IR (neat) ν_{max} 2979–2921 (w, C–H), 1695 (s, C=O), 1579 (w),

1534 (m), 1495 (m), 1411 (m), 1335 (w), 1241 (m), 1215 (s), 1198 (m), 1121 (w), 1073 (s), 1002 (w) cm^{-1}.

2,5-Dimethyl-1-(2-methylphenyl)-1*H*-pyrrole-3-carboxylic acid (46). According to General Procedure B, the ester derivative **S43** (30 mg, 0.11 mmol) was stirred for 2 days to give a crude product. The crude product was purified using flash column chromatography (30% EtOAc in petrol) to give the acid derivative **46**[58] as a white amorphous solid (21 mg, 92 µmol, 83%). R_f 0.21 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43–7.28 (m, 3H), 7.13 (d, J = 7.6 Hz, 1H), 6.46 (s, 1H), 2.23 (s, 3H), 1.95 (s, 3H), 1.89 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 137.3, 136.8, 136.7, 131.1, 129.4, 128.7, 128.6, 127.1, 110.8, 108.1, 17.1, 12.4, 12.3 ppm. MS (ESI): $[M + H]^+$ 230.1. HPLC: retention time 1.90 min (>99%). IR (neat) ν_{max} 3033–2850 (s, O–H, C–H), 2746–2509 (m, C–H), 1652 (s, C=O), 1575 (w), 1530 (m), 1494 (m), 1425 (m), 1401 (w), 1257 (s), 1197 (w), 1122 (w), 1078 (m), 1026 (w), 1004 (w), 931 (m) cm^{-1}.

Ethyl 1-(2,6-dimethylphenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylate (S44). According to General Procedure C, 2,6-dimethylaniline (65 µL, 0.53 mmol) and the carbonyl derivative **S5** were stirred for 18 h to give a crude product. The crude product was purified by flash column chromatography (5% EtOAc in petrol) to give the ester derivative **S44** as a colourless oil (0.13 g, 0.48 mmol, 91%). R_f 0.71 (50:50 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31–7.23 (m, 1H), 7.19 (d, J = 7.5 Hz, 2H), 6.45 (s, 1H), 4.30 (q, J = 7.1 Hz, 2H), 2.18 (s, 3H), 1.93 (s, 6H), 1.86 (s, 3H), 1.38 (t, J = 7.1, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 136.7, 135.9, 135.0, 128.8, 128.4, 127.2, 111.6, 107.7, 59.3, 17.5, 14.7, 12.0, 11.7 ppm. MS (ESI): $[M + H]^+$ 272.5. IR (neat) ν_{max} 2978–2920 (w, C–H), 1696 (s, C=O), 1533 (w), 1478 (w), 1410 (m), 1380 (w), 1214 (s), 1098 (m), 1073 (s), 1001 (w) cm^{-1}.

1-(2,6-Dimethylphenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (47). According to General Procedure B, the ester derivative **S44** (61 mg, 0.22 mmol) was stirred for 2 days to give the acid derivative **47** as a white amorphous solid (40 mg, 0.16 mmol, 73%). R_f 0.70 (EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31–7.26 (m, 1H), 7.20 (d, J = 7.6 Hz, 2H), 6.52 (s, 1H), 2.21 (s, 3H), 1.96 (s, 6H), 1.87 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 136.7, 136.5, 135.9, 128.9, 128.5, 127.7, 111.0, 108.4, 17.5, 12.1, 11.9 ppm. MS (ESI): $[M - H]^-$ 242.8. HPLC: retention time 2.01 min (>99%). IR (neat) ν_{max} 3033–2573 (O–H, C–H), 1649 (s, C=O), 1574 (w), 1530 (m), 1475 (w), 1425 (m), 1376 (w), 1330 (w), 1265 (s), 1250 (s), 1101 (w), 1078 (m), 1025 (w), 1001 (w), 972 (w), 935 (m) cm^{-1}. HRMS: calculated for $C_{15}H_{17}NO_2$ $[M + H]^+$ = 244.1338, observed = 244.1335.

Ethyl 1-(3,4-dichlorophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylate (S45). According to General Procedure C, 3,4-dichloroaniline (0.11 g, 0.67 mmol) and the carbonyl derivative **S5** were stirred for 18 h to give a crude product. The crude product was purified by flash column chromatography (0%–5% EtOAc in petrol) to give the ester derivative **S45** as a colourless oil (0.15 g, 0.48 mmol, 72%). R_f 0.57 (20:80 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, J = 8.4 Hz, 1H), 7.32 (s, 1H), 7.05 (d, J = 8.4 Hz, 1H), 6.36 (s, 1H), 4.27 (q, J = 7.1 Hz, 2H), 2.29 (s, 3H), 1.98 (s, 3H), 1.34 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.5, 137.2, 136.0, 133.5, 133.2, 131.2, 130.3, 128.6, 127.7, 112.3, 108.2, 59.5, 14.6, 12.7, 12.4 ppm. MS (ESI): $[M + H]^+$ 312.1. IR (neat) ν_{max} 2976–2858 (w, C–H), 1690 (s, C=O), 1585 (w), 1533 (m), 1473 (m), 1454 (m), 1410 (m), 1352 (m), 1324 (w), 1253 (w), 1219 (s), 1129 (m), 1084 (s), 1028 (s) cm^{-1}.

1-(3,4-Dichlorophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (48). According to General Procedure B, the ester derivative **S45** (28.0 mg, 89.7 µmol) was stirred for 18 h to give a crude product. The crude product was purified using flash column chromatography (30% EtOAc in petrol) to give the acid derivative **48**[37] as a white amorphous solid (15 mg, 52.8 µmol, 59%). R_f 0.22 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.7 (br. s, 1H), 7.81 (d, J = 8.5 Hz, 1H), 7.77 (s, 1H), 7.37 (dd, J = 8.5, 2.4 Hz, 1H), 6.24 (s, 1H), 2.22 (s, 3H), 1.95 (s, 3H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.0, 136.9, 135.0, 131.8, 131.5, 131.2, 130.2, 128.7, 128.0, 111.8, 108.0, 12.3, 12.0 ppm. MS (ESI): $[M + H]^+$ 284.0. HPLC: retention time 1.99 min (>99%). IR (neat) ν_{max} 3069–2574 (s, O–H, C–H), 1644 (s, C=O), 1583 (w), 1530 (m), 1465 (s), 1403 (m), 1383 (m), 1327 (w), 1255 (s), 1241 (s), 1129 (m), 1091 (m), 1030 (m), 927 (m) cm^{-1}.

Ethyl 1-(3-chlorophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylate (S46). According to General Procedure C, 3-chloroaniline (57 µL, 0.54 mmol) and the carbonyl derivative **S5** were stirred for 18 h to give a crude product. The crude product was purified by flash column chromatography (0%–5% EtOAc in petrol) to give the ester derivative **S46**[37] as a colourless oil (0.12 g, 0.43 mmol, 80%). R_f 0.54 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47–7.40 (m, 2H), 7.21 (s, 1H), 7.12–7.05 (m, 1H), 6.37 (s, 1H), 4.28 (q, J = 7.1 Hz, 2H), 2.29 (s, 3H), 1.98 (s, 3H), 1.34 (t, J = 7.1 Hz, 3H) ppm. ^{1}C NMR (100 MHz, $CDCl_3$) δ 165.6, 139.0, 136.1, 135.1, 130.4, 129.0, 128.6, 126.6, 125.1, 112.0, 108.0, 59.4, 14.6, 12.7, 12.4 ppm. MS (ESI): $[M + H]^+$ 278.2. IR (neat) ν_{max} 2978–2850 (m, C–H), 1695 (s, C=O), 1592 (m), 1581 (m), 1535 (m), 1479 (m), 1409 (m), 1373 (m), 1354 (w), 1216 (s), 1098 (m), 1077 (s), 1026 (w) cm^{-1}.

1-(3-Chlorophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (49). According to General Procedure B, the ester derivative **S46** (30 mg, 0.11 mmol) was stirred for 18 h to give a crude product. The crude product was purified using flash column chromatography (30% EtOAc in petrol) to give the acid derivative **49**[37] as a colourless amorphous solid (19 mg, 76.1 µmol, 69%). R_f 0.22 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50–7.41 (m, 2H), 7.23 (s, 1H), 7.15–7.08 (m, 1H), 6.43 (s, 1H), 2.32 (s, 3H), 1.99 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 138.9, 137.6, 135.2, 130.5, 129.2, 129.1, 128.6, 126.6, 111.2, 108.5, 12.8, 12.6 ppm. MS (ESI): $[M + H]^+$ 250.1. HPLC: retention time 1.95 min (>99%). IR (neat) ν_{max} 3086–2586 (s, O–H, C–H), 1646 (s, C=O), 1584 (m), 1534 (m), 1466 (m), 1430 (m), 1399 (w), 1378 (w), 1330 (w), 1271 (m), 1250 (s), 1122 (w), 1079 (w), 1034 (w), 945 (w) cm^{-1}.

Ethyl 1-(4-chlorophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylate (S47). According to General Procedure C, 4-chloroaniline (0.10 g, 0.54 mmol) and the carbonyl derivative **S5** were stirred for 18 h to give a crude product. The crude product was purified by flash column chromatography (0%–5% EtOAc in petrol) to give the ester derivative **S47**[37] as a colourless amorphous solid (0.11 g, 0.40 mmol, 74%). R_f 0.57 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, J = 8.2 Hz, 2H), 7.12 (d, J = 8.2 Hz, 2H), 6.37 (s, 1H), 4.27 (q, J = 7.1 Hz, 2H), 2.28 (s, 3H), 1.97 (s, 3H), 1.34 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 136.3, 136.1, 134.6, 129.7, 129.6, 128.7, 111.9, 107.9, 59.4, 14.6, 12.7, 12.4 ppm. MS (ESI): $[M + H]^+$ 278.2. IR (neat) ν_{max} 2922

(s, C–H), 2852 (m, C–H), 1694 (s, C=O), 1535 (w), 1492 (m), 1415 (m), 1371 (w), 1221 (s), 1081 (s), 1000 (m) cm^{-1}.

1-(4-Chlorophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (50). According to General Procedure B, the ester derivative **S47** (30 mg, 0.11 mmol) was stirred for 18 h to give a crude product. The crude product was purified using flash column chromatography (30% EtOAc in petrol) to give the acid derivative **50**[32] as a white amorphous solid (18 mg, 72.1 μmol, 66%). R_f 0.22 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, J = 8.4 Hz, 2H), 7.14 (d, J = 8.4 Hz, 2H), 6.43 (s, 1H), 2.30 (s, 3H), 1.98 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 137.6, 136.2, 134.8, 129.8, 129.6, 129.1, 111.1, 108.5, 12.8, 12.6 ppm. MS (ESI): $[M + H]^+$ 250.1. HPLC: retention time 1.95 min (>99%). IR (neat) ν_{max} 3089–2508 (s, O–H, C–H), 1646 (s, C=O), 1580 (w), 1538 (m), 1495 (m), 1468 (w), 1428 (m), 1401 (m), 1366 (w), 1329 (w), 1258 (s), 1083 (m), 1018 (w), 1002 (w), 942 (m) cm^{-1}.

Ethyl 1-(4,5-dichloro-2-methylphenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylate (S48). According to General Procedure C, 4,5-dichloro-2-methylaniline (95 mg, 0.54 mmol) and the carbonyl derivative **S5** were stirred for 5 h to give a crude product. The crude product was purified by flash column chromatography (5% EtOAc in petrol) to give the ester derivative **S48** as a pale yellow oil (0.15 g, 0.46 mmol, 85%). R_f 0.48 (20:80 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (s, 1H), 7.25 (s, 1H), 6.39 (s, 1H), 4.27 (q, J = 7.1 Hz, 2H), 2.20 (s, 3H), 1.90 (s, 3H), 1.89 (s, 3H), 1.34 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.6, 137.1, 136.3, 135.5, 133.3, 132.4, 130.6, 130.4, 128.0, 112.2, 108.1, 59.5, 16.7, 14.6, 12.3, 12.0 ppm. MS (ESI): $[M + H]^+$ 326.1. IR (neat) ν_{max} 2979–2922 (w, C–H), 1695 (s, C=O), 1581 (w), 1535 (w), 1479 (s), 1414 (m), 1331 (w), 1216 (s), 1187 (s), 1132 (m), 1077 (s), 1028 (m) cm^{-1}.

1-(4,5-Dichloro-2-methylphenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (51). According to General Procedure B, the ester derivative **S48** (0.12 g, 0.36 mmol) was stirred for 1 day to give a crude product. The crude product was purified using flash column chromatography (0%–30% EtOAc in petrol) to give the acid derivative **51** as an amorphous white solid (81 mg, 0.27 mmol, 75%). R_f 0.20 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.75 (s, 1H), 7.62 (s, 1H), 6.25 (s, 1H), 2.10 (s, 3H), 1.83 (s, 6H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.0, 137.6, 136.2, 134.6, 132.3, 131.7, 130.4, 129.0, 127.4, 111.7, 107.9, 15.9, 11.8, 11.6 ppm. MS (ESI): $[M + H]^+$ 298.0. HPLC: retention time 2.18 min (>99%). IR (neat) ν_{max} 2918–2579 (m, O–H, C–H), 1646 (s, C=O), 1581 (w), 1536 (w), 1477 (m), 1429 (w), 1406 (w), 1383 (w), 1327 (w), 1260 (s), 1233 (m), 1187 (w), 1134 (m), 1083 (w), 1028 (m), 951 (w) cm^{-1}. HRMS: calculated for $C_{14}H_{13}Cl_2NO_2$ $[M + H]^+$ = 298.0402, observed = 298.0399.

Ethyl 1-(3,4-dichloro-2-methylphenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylate (S49). According to General Procedure C, 3,4-dichloro-2-methylaniline (0.10 g, 0.54 mmol) and the carbonyl derivative **S5** were stirred for 3 h to give a crude product. The crude product was purified by flash column chromatography (5% EtOAc in petrol) to give the ester derivative **S49** as an amorphous white solid (0.15 g, 0.46 mmol, 85%). R_f 0.68 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, J = 8.4 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 6.39 (s, 1H), 4.28 (q, J = 7.1 Hz, 2H), 2.19 (s, 3H), 1.99 (s, 3H), 1.87 (s, 3H), 1.35 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.6, 137.9, 136.3, 135.8, 134.2, 134.0, 128.3, 128.2, 127.6, 112.1, 108.0, 59.5, 16.0, 14.6, 12.3, 12.0 ppm. MS (ESI): $[M + H]^+$ 328.0. IR (neat) ν_{max} 3075–2917 (w, C–H), 1696 (s, C=O), 1577 (w), 1535 (m), 1467 (m), 1411 (m), 1395 (m), 1372 (m), 1351 (w), 1333 (w), 1249 (m), 1219 (s), 1194 (s), 1127 (w), 1080 (s), 1046 (m), 1001 (m) cm^{-1}.

1-(3,4-Dichloro-2-methylphenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (52). According to General Procedure B, the ester derivative **S49** (0.12 g, 0.36 mmol) was stirred for 1 day to give a crude product. The crude product was purified using flash column chromatography (0%–30% EtOAc in petrol) to give the acid derivative **52** as an amorphous white solid (90 mg, 0.30 mmol, 83%). R_f 0.15 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.68 (d, J = 8.4 Hz, 1H), 7.31 (d, J = 8.4 Hz, 1H), 6.27 (s, 1H), 2.09 (s, 3H), 1.92 (s, 3H), 1.82 (s, 3H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.0, 137.4, 136.0, 134.8, 132.6, 132.5, 128.6, 128.4, 127.7, 111.8, 107.9, 15.6, 11.8, 11.6 ppm. MS (ESI): $[M + H]^+$ 298.1. HPLC: retention time 2.20 min (>99%). IR (neat) ν_{max} 3067–2521 (m, O–H, C–H), 1655 (s, C=O), 1577 (w), 1534 (m), 1469 (m), 1427 (m), 1392 (m), 1379 (m), 1359 (w), 1331 (w), 1261 (s), 1239 (m), 1194 (m), 1126 (w), 1087 (m), 1046 (w), 999 (m) cm^{-1}. HRMS: calculated for $C_{14}H_{13}Cl_2NO_2$ $[M + H]^+$ = 298.0402, observed = 298.0394.

Ethyl 5-methyl-1-(2-methylphenyl)-2-(trifluoromethyl)-1*H*-pyrrole-3-carboxylate (S50). According to General Procedure D, 2-methylaniline (0.10 g, 0.41 mmol) was stirred for 4.5 h to give a crude product. The crude product was purified by flash column chromatography (0%–5% EtOAc in petrol) to give the ester derivative **S50** as a yellow oil (27 mg, 86.7 μmol, 21%). R_f 0.57 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44–7.24 (m, 3H), 7.14 (d, J = 7.8 Hz, 1H), 6.53 (s, 1H), 4.33 (q, J = 7.1 Hz, 2H), 1.97 (s, 3H), 1.86 (s, 3H), 1.36 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.5, 136.8, 136.1, 133.1 (q, J = 1.4 Hz), 130.9, 129.6, 128.1 (q, J = 0.75 Hz), 126.8, 121.8 (q, J = 38.1 Hz), 120.5 (q, J = 269.3 Hz), 118.2 (q, J = 2.1 Hz), 110.3, 60.7, 16.9, 14.2, 12.1 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -55.8 ppm. MS (ESI): $[M + H]^+$ 312.1. IR (neat) ν_{max} 2925 (m, C–H), 2853 (w, C–H), 1727 (m, C=O), 1511 (m), 1495 (m), 1460 (w), 1422 (m), 1378 (w), 1274 (m), 1227 (s), 1199 (m), 1159 (s), 1116 (s), 1035 (m) cm^{-1}.

5-Methyl-1-(2-methylphenyl)-2-(trifluoromethyl)-1*H*-pyrrole-3-carboxylic acid (53). According to General Procedure B, the ester derivative **S50** (27.0 mg, 86.7 μmol) was stirred for 4 h to give a crude product. The crude product was purified using flash column chromatography (30% EtOAc in petrol) to give the acid derivative **53** as an amorphous white solid (16.0 mg, 56.5 μmol, 65%). R_f 0.67 (20:80 MeOH:DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43–7.28 (m, 3H), 7.17 (d, J = 7.7 Hz, 1H), 6.63 (s, 1H), 1.99 (s, 3H), 1.88 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.5, 136.8, 136.0, 133.2 (q, J = 1.4 Hz), 131.0, 129.7, 128.0, 126.9, 123.0 (q, J = 38.5 Hz) 120.3 (q, J = 269.9 Hz), 116.7 (q, J = 1.5 Hz), 111.2, 16.9, 12.2 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -54.9 ppm. MS (ESI): $[M + H]^+$ 284.1. HPLC: retention time 1.88 min (>99%). IR (neat) ν_{max} 2920–2850 (s, O–H, C–H), 1690 (m, C=O), 1666 (m), 1519 (m), 1496 (w), 1460 (w), 1413 (m), 1336 (w), 1284 (m), 1252 (m), 1206 (w), 1172 (m), 1117 (s), 1046 (w), 1018 (m), 1002 (m) cm^{-1}. HRMS: calculated for $C_{14}H_{12}F_3NO_2$ $[M + H]^+$ = 284.0898, observed = 284.0896.

Ethyl 1-(4,5-dichloro-2-methylphenyl)-5-methyl-2-(trifluoromethyl)-1*H*-pyrrole-3-carboxylate (S51). According to General Procedure D, 4,5-dichloro-2-methylaniline (0.22 g, 1.25 mmol) was stirred for 24 h to give a crude product. The

crude product was purified by flash column chromatography (0%–5% EtOAc in petrol) to give the ester derivative **S51** as a yellow oil (0.13 g, 0.34 mmol, 27%). R_f 0.68 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (s, 1H), 7.29 (s, 1H), 6.53 (s, 1H), 4.32 (q, J = 7.1 Hz, 2H), 1.93 (s, 3H), 1.89 (s, 3H), 1.35 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.2, 136.5, 136.1, 133.9, 132.9 (q, J = 1.5 Hz), 132.3, 130.5, 129.8 (q, J = 1.1 Hz), 121.8 (q, J = 38.3 Hz), 120.3 (q, J = 269.6 Hz), 118.9 (q, J = 2.3 Hz), 110.8, 60.9, 16.5, 14.2, 12.2 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -55.6 ppm. MS (ESI): $[M + H]^+$ 380.2. IR (neat) ν_{max} 2983–2929 (w, C–H), 1726 (m, C=O), 1513 (m), 1477 (m), 1423 (m), 1276 (m), 1234 (s), 1194 (s), 1164 (s), 1121 (s), 1039 (m), 1027 (m), 1001 (m) cm^{-1}.

1-(4,5-Dichloro-2-methylphenyl)-5-methyl-2-(trifluoromethyl)-1*H*-pyrrole-3-carboxylic acid (54). According to General Procedure B, the ester derivative **S51** (0.10 g, 0.26 mmol) was stirred for 18 h to give a crude product. The crude product was purified using flash column chromatography (0%–30% EtOAc in petrol) and triturated with petrol to give the acid derivative **54** as an amorphous white solid (71 mg, 0.22 mmol, 85%). R_f 0.58 (EtOAc). ^{1}H NMR (400 MHz, CD_3OD) δ 7.63 (s, 1H), 7.51 (s, 1H), 6.55 (s, 1H), 1.95 (s, 3H), 1.92 (s, 3H) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ 166.2, 138.3, 137.6, 134.7, 133.4, 133.4, 131.2, 131.1, 122.6 (q, J = 38.1 Hz), 121.7 (q, J = 268.7 Hz), 120.3 (q, J = 2.1 Hz), 111.9, 16.3, 12.0 ppm. ^{19}F NMR (376 MHz, CD_3OD) δ -56.8 ppm. MS (ESI): $[M - H]^-$ 350.0. HPLC: retention time 2.23 min (>99%). IR (neat) ν_{max} 2958–2559 (w, O–H, C–H), 1673 (m, C=O), 1517 (m), 1475 (m), 1420 (m), 1272 (m), 1205 (m), 1159 (m), 1130 (s), 1035 (m), 1006 (m) cm^{-1}. HRMS: calculated for $C_{14}H_{10}Cl_2F_3NO_2$ $[M + H]^+$ = 352.0119, observed = 352.0114.

Ethyl 1-(3,4-dichloro-2-methylphenyl)-5-methyl-2-(trifluoromethyl)-1*H*-pyrrole-3-carboxylate (S52). According to General Procedure D, 4,5-dichloro-2-methylaniline (0.22 g, 1.25 mmol) was stirred for 1 day to give a crude product. The crude product was purified by flash column chromatography (0%–5% EtOAc in petrol) to give the ester derivative **S52** as a yellow oil (80 mg, 0.21 mmol, 17%). R_f 0.67 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, J = 8.5 Hz, 1H), 7.07 (d, J = 8.5 Hz, 1H), 6.53 (s, 1H), 4.32 (q, J = 7.2 Hz, 2H), 2.01 (s, 3H), 1.87 (s, 3H), 1.35 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.2, 137.3, 136.0, 134.6, 134.1, 133.2 (q, J = 1.4 Hz), 128.0, 127.1 (q, J = 0.7 Hz), 121.9 (q, J = 38.2 Hz), 120.3 (q, J = 269.5 Hz), 118.9 (q, J = 2.1 Hz), 110.7, 60.9, 16.0, 14.2, 12.2 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -55.6 ppm. MS (ESI): $[M + H]^+$ 380.2. IR (neat) ν_{max} 3121–2851 (w, C–H), 1721 (m, C=O), 1512 (w), 1460 (w), 1420 (w), 1274 (m), 1234 (m), 1187 (m), 1124 (s), 1073 (m), 1053 (m), 1030 (m), 999 (m) cm^{-1}.

1-(3,4-Dichloro-2-methylphenyl)-5-methyl-2-(trifluoromethyl)-1*H*-pyrrole-3-carboxylic acid (55). According to General Procedure B, the ester derivative **S52** (80.0 mg, 0.21 mmol) was stirred for 24 h to give a crude product. The crude product was purified using flash column chromatography (0%–30% EtOAc in petrol) and triturated with petrol to give the acid derivative **55** as an amorphous white solid (52.0 mg, 0.15 mmol, 71%). R_f 0.27 (30:70 EtOAc:petrol). ^{1}H NMR (400 MHz, CD_3OD) δ 7.58 (d, J = 8.5 Hz, 1H), 7.25 (d, J = 8.5 Hz, 1H), 6.56 (s, 1H), 2.02 (s, 3H), 1.90 (s, 3H) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ 166.3, 138.5, 137.5, 135.4, 134.9 (q, J = 1.4 Hz), 134.6, 129.3, 128.8 (q, J = 1.0 Hz), 122.7 (q, J = 38.1 Hz), 121.7 (q, J = 268.7 Hz), 120.3 (q, J = 2.1 Hz), 111.9, 16.0, 12.0 ppm. ^{19}F NMR (376 MHz, CD_3OD) δ -56.7 ppm. MS (ESI): $[M - H]^-$ 350.0. HPLC: retention time 2.26 min (>99%). IR (neat) ν_{max} 3084–2592 (m, O–H, C–H), 1689 (s), 1579 (w), 1519 (m), 1467 (m), 1424 (m), 1391 (w), 1334 (w), 1282 (m), 1257 (s), 1178 (m), 1158 (m), 1124 (s), 1052 (m), 1012 (m), 1000 (m) cm^{-1}. HRMS: calculated for $C_{14}H_{10}Cl_2F_3NO_2$ $[M + H]^+$ = 352.0119, observed = 352.0112.

***N*-Benzyl-5-methyl-1-phenyl-1*H*-pyrazole-4-carboxamide (58).** Thionyl chloride (1.40 mL) was added to the carboxylic acid derivative **4** (0.13 g, 0.63 mmol) and the solution was stirred at 80 °C for 3 h. After concentrating the solution *in vacuo*, 1,4-dioxane (3.1 mL), benzylamine (0.10 mL, 0.95 mmol) and pyridine (77 µL, 0.95 mmol) were added and the resulting mixture was stirred at room temperature for 18 h. Successively, the mixture was concentrated *in vacuo* and EtOAc (6 mL) was added. The organic phase was washed with a 0.1 M HCl aqueous solution (3 × 10 mL) and brine (5 mL), dried under anhydrous magnesium sulphate and concentrated *in vacuo* to yield a crude product, which was purified by flash column chromatography (EtOAc) to give the amide derivative **58**[59] as a pale brown amorphous solid (76 mg, 0.26 mmol, 42%). R_f 0.49 (50:50 EtOAc:petrol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.53–7.48 (m, 2H), 7.47–7.40 (m, 3H), 7.39–7.35 (m, 4H), 7.33–7.28 (m, 1H), 6.07 (br. s, 1H), 4.63 (d, J = 5.7 Hz, 2H), 2.61 (s, 3H) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ 163.4, 142.3, 138.8, 138.3, 137.9, 129.1, 128.7, 128.5, 127.8, 127.5, 125.4, 115.3, 43.3, 11.8 ppm. MS (ESI): $[M + H]^+$ 292.2. HPLC: retention time 1.84 min (>99%). IR (neat) ν_{max} 3315 (m, N–H), 1629 (s, C=O), 1593 (m), 1567 (s), 1536 (m), 1503 (s), 1453 (m), 1394 (s), 1353 (w), 1287 (s), 1262 (w), 1138 (w), 938 (s) cm^{-1}.

***N*-Methanesulfonyl-1-phenyl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (59).** According to General Procedure G, the carboxylic acid derivative **18** (44 mg, 0.17 mmol) and methanesulfonamide gave the sulfonamide derivative **59** as a white amorphous solid (40 mg, 0.12 mmol, 70%). R_f 0.29 (20:80 MeOH:DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.08 (s, 1H), 8.09 (s, 1H), 7.58–7.48 (m, 3H), 7.45–7.38 (m, 2H), 3.45 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.0, 140.1, 138.8, 132.8 (q, J = 40.4 Hz), 130.4, 129.4, 125.8 (q, J = 1.0 Hz), 118.9 (q, J = 271.6 Hz), 117.3 (q, J = 1.2 Hz), 41.9 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -56.4 ppm. MS (ESI): $[M + H]^+$ 334.1. HPLC: retention time 1.80 min (>99%). IR (neat) ν_{max} 3276 (m, N–H), 3137–2940 (w, C–H), 1703 (m, C=O), 1559 (w), 1502 (w), 1433 (m), 1405 (m), 1326 (m), 1294 (m), 1227 (m), 1158 (s), 1132 (s), 1082 (m), 1073 (m), 1022 (m), 973 (m) cm^{-1}. HRMS: calculated for $C_{12}H_{10}F_3N_3O_3S$ $[M + H]^+$ = 334.0473, observed = 334.0475.

***N*-(Benzenesulfonyl)-1-phenyl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (60).** According to General Procedure G, the carboxylic acid derivative **18** (44 mg, 0.17 mmol) and benzenesulfonamide gave the sulphonamide derivative **60** as a white amorphous solid (38 mg, 95.2 µmol, 56%). R_f 0.40 (20:80 MeOH:DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79 (s, 1H), 8.16 (d, J = 7.6 Hz, 2H), 8.00 (s, 1H), 7.69 (t, J = 7.4 Hz, 1H), 7.59 (t, J = 7.6 Hz, 2H), 7.55–7.46 (m, 3H), 7.41–7.35 (m, 2 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.7, 140.1, 138.1, 134.5, 133.0, 130.4, 129.4, 129.2, 128.7, 126.5, 125.8 (q, J = 0.8 Hz), 118.9 (q, J = 271.7 Hz), 117.7 (q, J = 1.0 Hz) ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -56.2 ppm. MS (ESI): $[M + H]^+$ 396.2. HPLC: retention time 1.96 min (>99%). IR (neat) ν_{max} 3247 (m, N–H), 2921–2850 (w, C–H), 1717 (m, C=O),

1563 (w), 1501 (w), 1448 (w), 1425 (m), 1408 (m), 1335 (m), 1294 (m), 1219 (m), 1169 (s), 1147 (s), 1135 (s), 1084 (s), 1020 (m) cm^{-1}. HRMS: calculated for $C_{17}H_{12}F_3N_3O_3S$ $[M + H]^+$ = 396.0630, observed = 396.0636.

1-Phenyl-*N*-phenylmethanesulfonyl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (61). According to General Procedure G, the carboxylic acid derivative **4** (0.11 g, 0.55 mmol) and benzylsulfonamide gave a crude product. The crude product was purified by flash column chromatography (30% EtOAc in petrol) to give the ester derivative **61** as a pale yellow amorphous solid (11.7 mg, 33.0 µmol, 6%). R_f 0.31 (50:50 EtOAc:petrol). ^{1}H NMR (400 MHz, CD_3OD) δ 8.00 (s, 1H), 7.65–7.32 (m, 10H), 4.79 (s, 2H), 2.58 (s, 3H) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ 164.6, 146.4, 141.0, 139.6, 132.0, 130.6, 130.5, 130.4, 129.8, 129.7, 129.5, 126.9, 59.6, 12.1 ppm. MS (ESI): $[M + H]^+$ 356.2. HPLC: retention time 1.83 min (>99%). IR (neat) ν_{max} 3330 (w, N–H), 1682 (s, C=O), 1598 (w), 1549 (m), 1503 (m), 1455 (m), 1403 (m), 1337 (s), 1231 (m), 1154 (s), 1135 (m), 1056 (m) cm^{-1}. HRMS: calculated for $C_{18}H_{17}N_3O_3S$ $[M + H]^+$ = 356.1068, observed = 356.1064.

***N*-(Benzenesulfonyl)-1-(2,6-dimethylphenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (62).** According to General Procedure G, the carboxylic acid derivative **41** (0.11 g, 0.39 mmol) and benzenesulfonamide gave a crude product. The crude product was purified by flash column chromatography (30%–100% EtOAc in petrol) to give the sulfonamide derivative **62** as a white amorphous solid (38 mg, 89.7 µmol, 23%). R_f 0.34 (EtOAc). ^{1}H NMR (400 MHz, CD_3OD) δ 8.14 (s, 1H), 8.05 (d, J = 7.6 Hz, 2H), 7.66–7.50 (m, 3H), 7.33 (t, J = 7.6 Hz, 1H), 7.20 (d, J = 7.6 Hz, 2H), 1.93 (s, 6H) ppm. ^{13}C NMR (100 MHz, CD_3OD) δ 165.6, 143.4, 142.4, 139.0, 137.2, 133.3, 133.2 (q, J = 39.8 Hz), 131.3, 129.5, 129.3, 128.6, 123.0, 120.6 (q, J = 270.5 Hz), 17.0 ppm. ^{19}F NMR (376 MHz, CD_3OD) δ -60.6 ppm. MS (ESI): $[M + H]^+$ 424.2. HPLC: retention time 2.10 min (96%). IR (neat) ν_{max} 3443 (w, N–H), 1701 (m, C=O), 1605 (m), 1560 (m), 1485 (m), 1448 (m), 1375 (m), 1304 (s), 1136 (s), 1087 (m), 1048 (m), 969 (m) cm^{-1}. HRMS: calculated for $C_{19}H_{16}F_3N_3O_3S$ $[M + H]^+$ = 424.0942, observed = 424.0947.

***N*-(3-Bromobenzenesulfonyl)-1-(2,6-dimethylphenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (63).** According to General Procedure G, the carboxylic acid derivative **41** (0.15 g, 0.54 mmol) and 3-bromobenzene-1-sulfonamide gave a crude product. The crude product was purified by flash column chromatography (30% petrol in EtOAc) to give the sulfonamide derivative **63** as a yellow amorphous solid (21.7 mg, 43.2 µmol, 8%). R_f 0.12 (EtOAc). ^{1}H NMR (400 MHz, acetone-d_6) δ 8.22 (s, 1H), 8.18 (s, 1H), 8.05 (d, J = 7.9 Hz, 1H), 7.74 (d, J = 7.6 Hz, 1H), 7.47 (t, J = 7.9 Hz, 1H), 7.33 (t, J = 7.6 Hz, 1H), 7.21 (d, J = 7.6 Hz, 2H), 1.91 (s, 6H) ppm. ^{13}C NMR (125 MHz, acetone-d_6) δ 147.4, 143.6, 139.8, 136.9, 135.8, 134.9 (q, J = 39.2 Hz), 131.7, 131.0, 130.9, 129.3, 129.0, 128.9, 127.1, 122.8, 120.8 (q, J = 270.2 Hz), 17.3 ppm. ^{19}F NMR (376 MHz, acetone-d_6) δ -59.0 ppm. MS (ESI): $[M – H]^–$ 502.1. HPLC: retention time 2.30 min (>99%). IR (neat) ν_{max} 3457 (w, N–H), 1706 (s, C=O), 1559 (m), 1364 (s), 1294 (s), 1255 (m), 1222 (s), 1175 (s), 1139 (vs), 1098 (m), 968 (m) cm^{-1}. HRMS: calculated for $C_{19}H_{15}BrF_3N_3O_3S$ $[M + H]^+$ = 500.9969, observed = 500.9985.

***N*-(Benzenesulfonyl)-1-(4,5-dichloro-2-methylphenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide (64).** According to General Procedure G, the carboxylic acid derivative **44** (40 mg, 0.12 mmol) and benzenesulfonamide gave a crude product. The crude product was purified by flash column chromatography (0%–50% EtOAc in petrol) to give the sulfonamide derivative **64** as a white amorphous solid (18 mg, 37.6 µmol, 31%). R_f 0.40 (EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, J = 7.8 Hz, 2H), 8.07 (s, 1H), 7.63 (t, J = 7.5 Hz, 1H), 7.52 (app. t, J = 7.8 Hz, 2H), 7.42 (s, 1H), 7.29 (s, 1H), 1.93 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.5, 141.3, 139.0, 137.8, 136.9, 135.8, 135.0, 134.0 (q, J = 3.1 Hz), 133.0 (q, J = 39.8 Hz), 132.4, 130.4, 129.1, 129.0, 128.2, 118.8 (q, J = 271.7 Hz), 16.5 ppm. ^{19}F NMR (376 MHz, $CDCl_3$) δ -57.1 ppm. MS (ESI): $[M – H]^–$ 476.1. HPLC: retention time 2.12 min (>99%). IR (neat) ν_{max} 3267 (m, N–H), 2925 (w, C–H), 1704 (m, C=O), 1559 (w), 1492 (w), 1432 (m), 1411 (m), 1335 (w), 1293 (m), 1236 (m), 1165 (s), 1153 (s), 1134 (s), 1080 (m), 1022 (m), 987 (m) cm^{-1}. HRMS: calculated for $C_{18}H_{12}Cl_2F_3N_3O_3S$ $[M + H]^+$ = 478.0007, observed = 478.0001.

Surface plasmon resonance. Experiments were performed using as a running buffer consisted of 10 mM $NaPO_4$, pH 7, 150 mM NaCl and 2% DMSO at 25 °C in a Biacore T200 (GE Healthcare). For data analyses, bulk effects were corrected using solvent correction and were performed through the Biacore T200 evaluation software 2.0 (GE Healthcare). *Pa* MurB was covalently coupled to a CM5 chip (GE Healthcare) by standard amine coupling protocol.

For the single concentration experiment, all fragments were diluted to 1 mM in running buffer, injected for 30 s at 30 µL s^{-1} and the dissociation was for 320 s. All fragments were tested two times in reverse orders. Sensograms were visually inspected, and fragments with significant signal increase comparing with the original fragment were selected for affinity study by ITC.

Isothermal titration calorimetry. Isothermal titration calorimetry experiments to quantify ligand binding to *Pa* MurB were performed using Malvern MicroCal Auto-iTC200 system at 25 °C. Titrations consisted of an initial injection of 0.4 µL, which was discarded during data processing, followed by 28 further injections of 1.5 µL separated by a 120 s interval. The *Pa* MurB protein was dialysed overnight at 4 °C in 25 mM Tris-HCl pH 8.0, 150 mM NaCl. Sample cell and syringe solutions were prepared using the same buffer, with a final DMSO-d_6 concentration of 5%. *Pa* MurB concentrations of 200–50 µM were used, with ligand concentrations of 3.0–0.5 mM. The protein well had a volume of 400 µL, the ligand well a volume of 200 µL and the blank well a volume of 400 µL. Titrations were fitted with Origin software (OriginLab, Northampton, MA, USA), using a one site binding model. All ITC titration curves are shown in the SI.

Dihedral angle calculations. The global ground state conformations and dihedral angle calculations were performed using Schrödinger Maestro 11.[60] The scanning of the dihedral angles was performed using a MacroModel coordinate scan (force field: OPLS-2005, solvent: water, default settings).

ANCILLARY INFORMATION

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Data collection and refinement statistics of *Pa* MurB X-ray crystal structures, ITC titration curves, Dihedral angle calculations, 1H & 13CNMR spectra of tested novel compounds, HPLC spectra of tested compounds and minimum inhibitory concentrations (MIC) experiments (PDF)

Molecular formula strings (CSV)

Accession Codes

Atomic coordinates for the X-ray structures of fragment 4 (PDB code 7OR2), 18 (PDB code 7ORZ), 19 (PDB code 7OSQ), are available from the RCPB Protein Data Bank (www.rcpb.org). Authors will release the atomic coordinates and experimental data upon article publication.

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Author Contributions

± M.A.G.D.E. and J.M.L. contributed equally. M.A.G.D.E. carried out the structural biology and biochemical studies. J.M.L., M.H. and C.M. synthesised the compounds used in this study. S.Y.K. cloned *Pa* MurB and designed the purification protocol. S.Y.K. and O.D.P. carried out the fragment screening of the 960-fragment library by thermal shift. J.M.L. carried out thermal shift and ITC and M.A.G.D.E. carried out SPR. J.H. carried out the computational studies. M.A.G.D.E. and J.M.L. wrote the manuscript with contributions from A.G.C. and T.L.B. C.A., A.G.C., T.L.B., V.M., and R.A.F. supervised the project. All authors have given approval to the final version of the manuscript.

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ABBREVIATIONS

CF, Cystic fibrosis; DSF, Differential scanning fluorimetry; EDC, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide; *Ec, Escherichia coli*; FAD, Flavin adenine dinucleotide; ITC, Isothermal titration calorimetry; LE, Ligand efficiency; MIC, Minimum inhibitory concentration; MurB, UDP-*N*-acetylenolpyruvoylglucosamine reductase; Mw, molecular weight; $NADP^+$, Nicotinamide adenine dinucleotide phosphate; *Pa, Pseudomonas aeruginosa;* PBPs, penicillin-binding proteins; RU, Response units; *Sa, Staphylococcus aureus*; SAR, Structure activity relationship; SPR, Surface plasmon resonance; UDP, Uridine diphosphate; UNAGEP, UDP-*N*-acetylglucosamine enolpyruvate; UNAM, *N*-acetyl-muramic acid.

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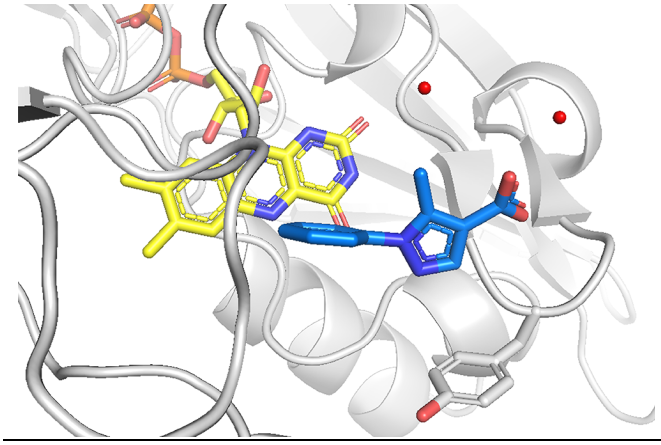
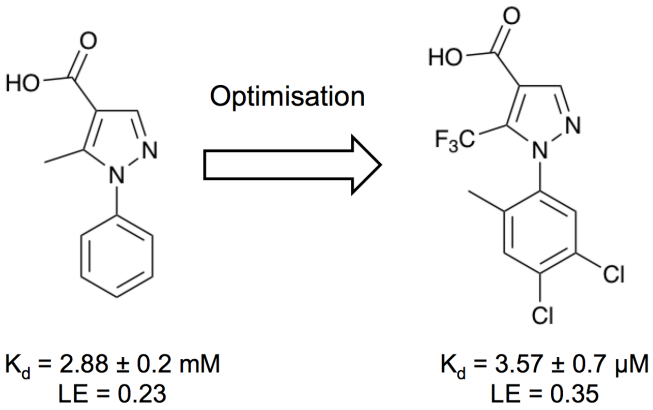
Table of Contents graphic (TOC)





K_d = 2.88 ± 0.2 mM
LE = 0.23

K_d = 3.57 ± 0.7 µM
LE = 0.35