# Screening of a custom-designed acid fragment library identifies 1-phenylpyrroles and 1-phenylpyrrolidines as inhibitors of Notum carboxylesterase activity.

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**Abstract**

The Wnt family of proteins are secreted signaling proteins that play key roles in regulating cellular functions. Recently, carboxylesterase Notum was shown to act as a negative regulator of Wnt signaling by mediating the removal of an essential palmitoleate. Here we disclose two new chemical scaffolds that inhibit Notum enzymatic activity. Our approach was to create a fragment library of 250 acids for screening against Notum in a biochemical assay followed by structure determination by X-ray crystallography. Twenty fragments were identified as hits for Notum inhibition and 14 of these fragments were shown to bind in the palmitoleate pocket of Notum. Optimization of 1-phenylpyrrole **20**, guided by structure-based drug design, identified **20z** as the most potent compound from this series. Similarly, optimization of 1-phenylpyrrolidine **8** gave acid **26**. This work demonstrates that inhibition of Notum activity can be achieved by small, drug-like molecules possessing favorable *in vitro* ADME profiles.

*Keywords:* Notum inhibitor, Wnt signaling, fragment library, structure-based drug design, 1-phenylpyrroles, 1-phenylpyrrolidines.

**INTRODUCTION**

The Wnt family of proteins are secreted signaling proteins that play key roles in regulating cellular functions in embryonic development and adult stem cell biology.[1] The Wnt pathways control cell proliferation, cell fate determination, cell polarity, cell migration and apoptosis. Wnt signaling is activated by the binding of a Wnt protein to a member of the Frizzled (FZD) family of cell surface receptors and to co-receptors such as the LDL-receptor-related protein (LRP) family. Upon binding, an intracellular signaling cascade is triggered by the pathway activating protein Disheveled (Dsh), which acts as a switch and branching point into multiple downstream Wnt pathways (canonical, noncanonical planar cell polarity, and noncanonical Wnt/calcium). Signaling by Wnt proteins is finely balanced and tightly regulated by a sophisticated network of modulators and feedback processes including secreted inhibitory proteins and post-translational modifications (PTM).[2-4] Conversely, misregulation of Wnt signaling disrupts normal development and cell homeostasis, and has been associated with cancer,[5] Alzheimer's disease (AD),[6] metabolic disorders and other diseases.[7]

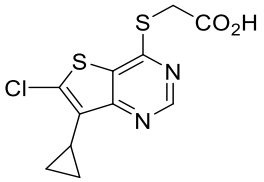
Recently, carboxylesterase Notum was shown to act as a negative regulator of the Wnt signaling pathway by mediating the removal of an essential palmitoleate group from a conserved serine residue of Wnt proteins.[8,9] This palimitoleate group is an important PTM required for efficient binding of the Wnt proteins to the FZD receptors. Hence, modulation of Notum activity represents a new approach to treat disease where dysregulation of Wnt signaling is an underlying cause and Notum has been implicated as the responsible agent.

There is an emerging understanding of the potential role Notum plays in disease. Notum is involved in the progression of colorectal cancer (CRC) as Notum expression was increased in human metastatic CRC cells and proliferation was suppressed by inhibiting expression of Notum.[10] Osteoblast-derived Notum reduces cortical bone mass in mice whereas Notum inhibition increases endocortical bone formation and bone strength, and so represents a potential treatment for preventing non-vertebral fractures.[11,12] Recent work has shown that the *Notum* gene is expressed and upregulated in endothelial cells in the hippocampus of APP/PS1 mice and AD patients compared to control, although the role of Notum in the mammalian central nervous system (CNS) has yet to be established.[13]

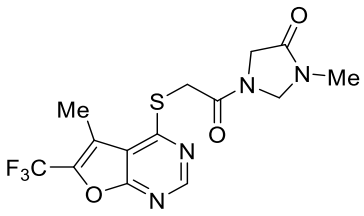
The Notum crystal structures reveal a well-defined, large (ca. 380 $Å^3$), hydrophobic active-site pocket adjacent to the catalytic triad (Ser232, His389, Asp340) that accommodates the palmitoleate group of Wnt (PDB: 4UZQ).[8] The *cis*-C9-10 double bond of the C16 palmitoleate tail is positioned at the base of the pocket between Ile291, Phe319 and Phe320, which helps explain substrate preference. The binding pocket can accommodate extended carbon tails up to C8/C10; however, longer fatty acid chains need to have a bend in their structure at C9 in order to fit. In addition, the Gly127–Trp128 amide participates in formation of the oxyanion hole along with the canonical Ser232–Ala233 and Gly126–Gly127 amides, thereby providing extra stabilization to the tetrahedral transition state during ester hydrolysis. The pocket entrance, lined by Ser232 and His389, is

relatively narrow but shows substantial flexibility. These structural features of Notum combine to render the palmitoleate pocket to be regarded as highly druggable.[14]

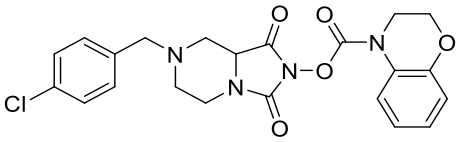
The number of reports of inhibitors of Notum carboxylesterase activity has gradually increased in recent years (**Figure 1**). Thieno[3,2-*d*]pyrimidine **LP-922056** (**1**) is a potent, orally active inhibitor of Notum that has been found to increase cortical bone thickness in mouse models of bone growth.[15] Scaffold-hopping from acid **1**, supported by X-ray structure determination, identified amide **2** as a potent inhibitor of Notum.[16] Pharmacokinetic studies in mouse with **2** showed good plasma exposure and reasonable brain penetration; in contrast, **1** had negligible brain exposure.[17] *N*-Hydroxyhydantoin carbamate **ABC99** is a potent and selective covalent irreversible inhibitor of Notum. [18] **ABC99** preserves Wnt-mediated cell signaling in the presence of Notum and has been used to show the role of Notum in the regeneration of aged intestinal epithelium.[19] A crystallographic fragment screen using the XChem platform at Diamond Light Source identified a number of fragment hits that bound in the palmitoleate pocket of Notum.[20,21] Phenoxyacetamide **3** was identified through the optimization of one of these hits and showed an unexpected flipped binding mode when compared to the structures of close analogues bound to Notum.[20] A second fragment hit from this screen was *N*-[2-(5-fluoro-1*H*-indol-3-yl)ethyl]acetamide (**4**), which is structurally related to the brain hormone melatonin. A detailed structural analysis of the binding of **4** (and close analogues) to Notum has been disclosed that may guide the development of new potent inhibitors.[21] Several of these inhibitors (**1**, **3**, **4** plus others) have been soaked into crystals of Notum and the structures solved to determine the inhibitor binding modes. Hence, under suitable conditions, Notum is very amenable to structure-based drug design (SBDD) to help guide progress towards the discovery of potent inhibitors.



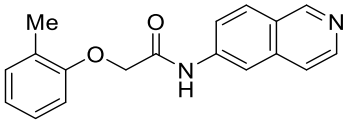
**1: LP-922056**
$IC_{50}$ 1.1 ± 0.4 nM[a]
PDB: 6T2K



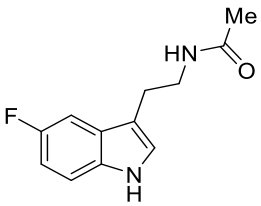
**2**
$IC_{50}$ 3.9 ± 0.8 nM[a]



**ABC99**
$IC_{50}$ 13 nM[b]
$IC_{50}$ 170 ± 20 nM[a,c]



**3**
$IC_{50}$ 85 ± 11 nM[a]
PDB: 6R8R



**4**
$IC_{50}$ 37,200 nM[d]
PDB: 6QCQ

**Figure 1:** Chemical structures and PDB codes of Notum inhibitors.

[a] Notum $IC_{50}$ data presented for comparison in a common assay format.
[b] Determined by competitive gel-based activity-based protein profiling (ABPP), see reference 18.
[c] As a covalent inhibitor of Notum, the $IC_{50}$ value will be time dependent.
[d] See reference 21.

Our objective was to identify alternative chemical scaffolds to the prior art that efficiently inhibit Notum enzymatic activity with the potential to deliver structurally orthogonal chemical tools for use in drug target validation experiments. We adopted a strategy to simultaneously explore multiple chemotypes in order to increase our chances of delivering a 'fit-for-purpose' chemical tool. Furthermore, diverse chemotypes, which share the same primary pharmacology, are likely to offer the benefit of a different secondary pharmacology fingerprint profile and so help identify any confounding off-target effects in disease models.

**RESULTS & DISCUSSION**

Our approach was to create a custom-designed fragment library of carboxylic acids for screening against Notum (**Figure 2**). Each compound of the library would have three distinct structural features: (1) a carboxylic acid to interact with the catalytic triad and to anchor the inhibitor through a polar interaction as observed previously;[16] (2) a suitable lipophilic group to be presented into the palmiteolate binding pocket of Notum; and (3) a linker of various lengths to join the acid with the lipophilic group (**Figure 2A**).

A single commercial provider was selected for ease and consistency of compound supply, and to simplify procurement. The Enamine Carboxylic Acids Fragment Library was provisionally selected because of its size (4350 compounds) and features of 'novelty and core diversity'; these features were assessed through evaluation of the library's content.[22] The full library was filtered using criteria for molecular properties and structural parameters as described in **Table 1**, and this virtual collection (514 compounds) was further manually curated by inspection to ensure a balanced representation of physicochemical property space (**Figure 2B**) along with structural diversity whilst minimizing repetition of a particular core. This process of design and selection gave an acid fragment library of 250 compounds (**Supplementary Table S1**), which was screened for inhibition of Notum activity (**Figure 2C**).

Inhibition of Notum carboxylesterase activity of this library was measured in a biochemical assay where test compounds (dispensed to give 10-point concentration-response-curves up to 100 μM) were incubated with Notum(81-451 Cys330Ser) and trisodium 8-octanoyloxypyrene-1,3,6-trisulfonate (OPTS) as the substrate for 40 minutes and fluorescence recorded. Twenty compounds showed inhibition of Notum activity with initial $IC_{50}$ < 25 μM (n = 2), although most of these hits showed a modest decrease in activity upon routine retesting (n = 5-10). All 20 of these putative hits were submitted to a Notum crystallographic screen by soaking crystals of *C*-terminal his-tagged Notum(81-451 Cys330Ser) with the compounds screened at 10 mM and 14 fragments were observed to bind in the palmitoleate pocket.

From the Notum biochemical and crystallographic screen, two preferred hit series were selected for further investigation: pyrrole-3-carboxylic acids **5**-**7** and pyrrolidine-3-carboxylic acids **8**-**10** (**Figure 3**)**.** These six hits shared a closely related structural motif and had excellent hit-like properties (LE = 0.45 - 0.57) with no obvious reactive groups. In addition, compounds **5**-**8** and **10** had been successfully crystallized with Notum to support a SBDD program (**Figure 4**).

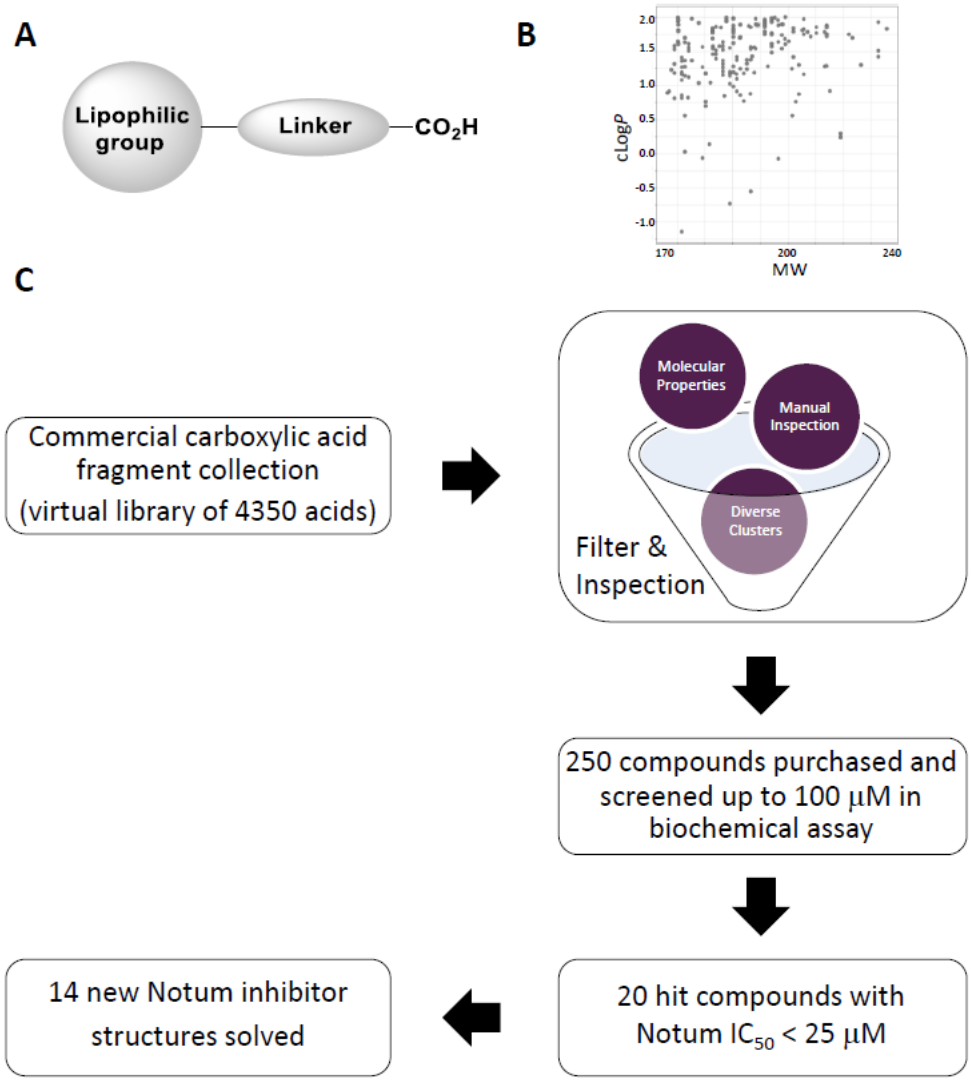
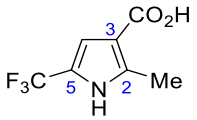



**Figure 2:** Design and screening of fragment library. (A) Compound structural features. (B) Plot of lipophilicity (clog*P*) verses molecular size (MW) for the acid fragment library. (C) Flow diagram showing key activities and results.

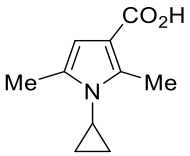
**Table 1:** Design and selection criteria for carboxylic acid fragment library.

| *Parameter (measure)* | *Criteria* |
|---|---|
| Molecular size (MW) | 170 ≤ MW ≤ 240 |
| Lipophilicity (clog*P*) | -2 ≤ clog*P* ≤ 2 |
| Polarity (TPSA) | ≤ 90 |
| H-bonding capacity (HBD, HBA)[a] | 1 ≤ HBD ≤ 2; HBA ≤ 3 |
| Aromatic rings | ≤ 2 |
| Minimize no. of stereocentres | ≤ 1 |
| Maximize no. of $sp^3$ carbons | ≥ 1 (where possible) |

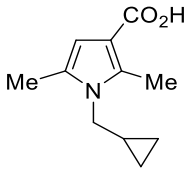
[a] Excluding the COOH group.



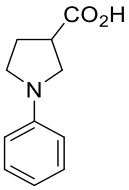

**5**
$IC_{50}$ 6.7 ± 3.6 μM



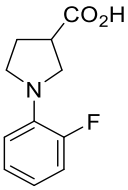

**6**
$IC_{50}$ 6.3 ± 0.5 μM



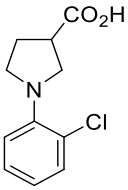

**7**
$IC_{50}$ 12.7 ± 6.2 μM




**8**
$IC_{50}$ 48 ± 31 μM




**9**
$IC_{50}$ 11.3 ± 8.9 μM




**10**
$IC_{50}$ 18.6 ± 14.1 μM

**Figure 3:** Pyrrole-3-carboxylic acids **5**-**7** and pyrrolidine-3-carboxylic acids **8**-**10** as fragment screening hits. Notum $IC_{50}$ (μM) (n = 5-10).

The original six hits **5**-**10** provided some insight into preliminary structure activity relationships (SARs), along with two structurally related inactive compounds, which were also present in the library (**Figure 3** and **Supplementary Figure S1**). The three pyrroles **5**-**7** all had 2- and 5- flanking groups either side of the *N*-atom supporting the inclusion of Me and/or $CF_3$ groups at these positions. There was some variation in the N1 substituent with both cPr and $CH_2$cPr represented and showing activity. The three pyrrolidines **8**-**10** had a single point change in the 2-position of the N1-phenyl ring showing activity could be improved with suitable groups (F, Cl > H). Despite these six hits providing limited SAR, it was encouraging that they represented a series of related hits (rather than singletons) with the potential for further optimization.

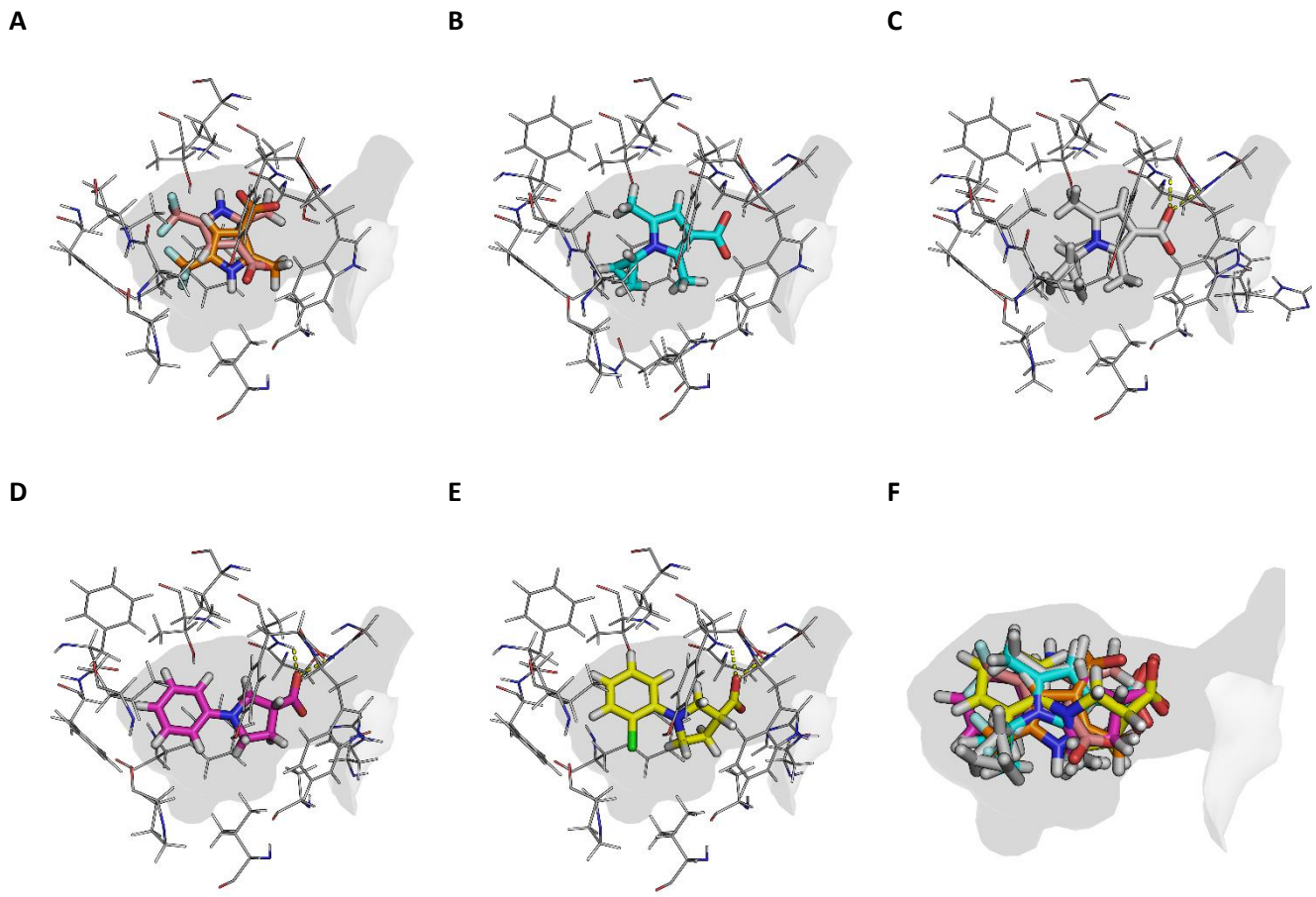


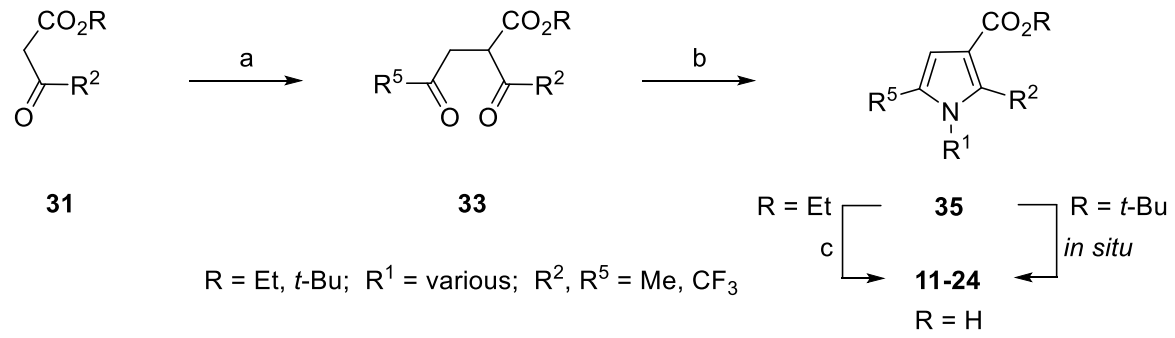

**Figure 4:** Notum structures with fragment screening hits **5**-**8** and **10**. Crystal structures of (A) **5** (orange), (B) **6** (teal), (C) **7** (gray), (D) **8** (magenta) and (E) **10** (yellow). (F) Overlay of all ligands. Binding site residues shown within 4Å of their respective ligands. Key hydrogen bond interactions are shown as dashed lines. Water molecules have been removed for clarity. The surface of the Notum palmitoleoate binding pocket outlined (gray) from the *O*-palmitoleoyl serine–Notum (S232A) structure (PDB: 4UZQ). Atomic coordinates have been deposited in the Protein Data Bank (PDB). PDB ID codes: **5**: 6YUY; **6**: 6YV4; **7**: 6YUW; **8**: 6YV2; **10**: 6YV0.

The crystal structures reveal that the inhibitors preferably occupy the core of the pocket, interacting with the sidechains of Phe268 and Trp128, with some ligands (**7**, **8** and **10**) also extending to the oxyanion hole (backbone of Trp128, Gly127, Ala233) in proximity of the catalytic triad (**Figure 4**). Due to the fragment's modest size, they fail to occupy the pocket fully. The suboptimal occupation of the pocket aligns with the modest observed potencies.

Our general design strategy for new compounds was initially directed at exploring three principle areas of these hit structures: (1) the pyrrole-3-carboxylic acid ring **11**-**24** (**Table 2**); (2) the 1-phenylpyrrole ring that binds in the palmitoleate pocket **20** (**Table 3**); and (3) the 1-phenylpyrrolidine ring **25**-**27** (**Table 4**). Further bespoke modifications of preferred leads were then performed to improve activity and tune physicochemical properties **28**-**30** (**Table 5** and **Supplementary Figure S2**).

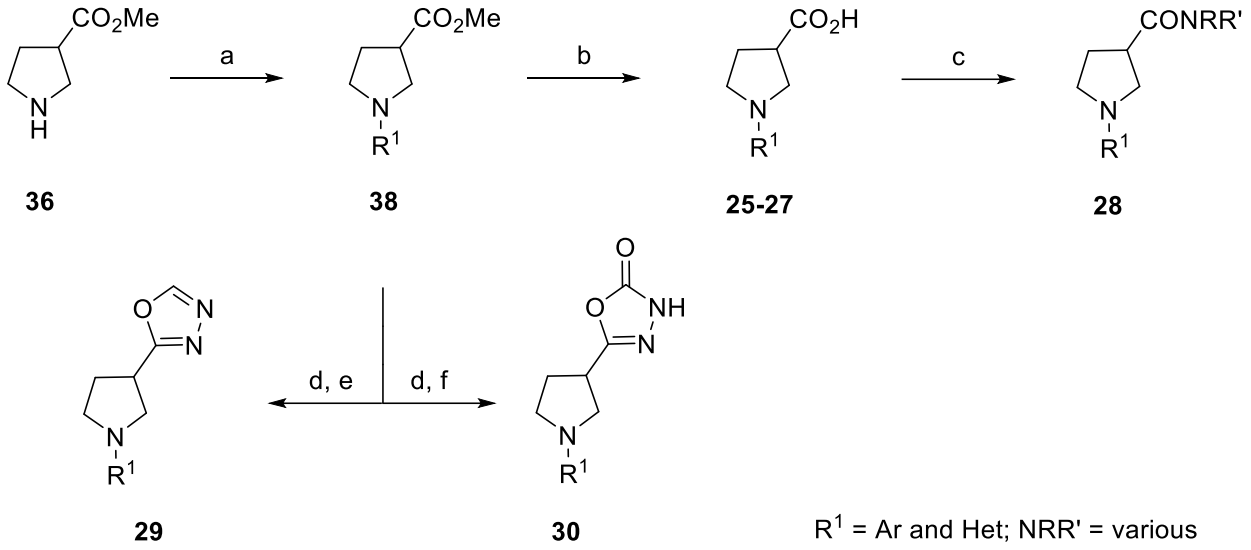
Minimizing lipophilicity is a well-established approach to improve ADME properties and target compounds were designed to have molecular and physicochemical properties consistent with drug-like space.[23] The palmitoleate binding pocket of Notum is a very lipophilic environment, and we were keen to avoid gains in activity simply through increased compound lipophilicity. A helpful design metric was lipophilic ligand efficiency (LLE),[24] used to track improvements in Notum inhibition activity against lipophilicity of the compounds as determined by calculated clog*P* and measured CHI $logD_{7.4}$ values.[25]

The synthetic routes to new inhibitors of Notum in the pyrrole-3-carboxylic acid (**11-24**) and pyrrolidine-3-carboxylic acid series (**25-30**) are presented in **Schemes 1** and **2** respectively.




**Scheme 1.** Preparation of pyrrole-3-carboxylic acids **11-24**. Representative reagents and conditions: (a) NaH (1.2 equiv.), THF, 0 °C then $R^5C(O)CH_2Cl$ **32** (1.0 equiv.), NaI (0.1 equiv.) 0 °C – RT, 24 h; (b) Amine **34** ($R^1NH_2$) (1.0 equiv.), *p*-TsOH·$H_2O$ (0.1 equiv.), EtOH, 80 °C; (c) LiOH·$H_2O$ (15 equiv.), 1,4-dioxane-$H_2O$, 100 °C.

Pyrrole-3-carboxylic acid target compounds **11-24** were either purchased or prepared using a classical Paal-Knorr pyrrole synthesis (**Scheme 1**). Alkylation of β-keto ester **31** with chloromethyl ketone **32** ($R^5C(O)CH_2Cl$) gave 1,4-diketo ester **33** and cyclocondensation of **33** with amine **34** ($R^1NH_2$) gave pyrrole ester **35**. Hydrolysis with LiOH afforded the pyrrole acid. Initially, ethyl esters (**31**: R = Et) were used but the corresponding pyrrole esters (**35**: R = Et) were found to be resilient to hydrolysis even under forcing conditions. Switching to the *t*-butyl esters (**31**: R = *t*-Bu) improved the synthetic route as the pyrrole esters (**35**: R = *t*-Bu) were found to hydrolyze under the conditions of the Paal-Knorr reaction giving directly the pyrrole-3-carboxylic acids.




**Scheme 2.** Preparation of pyrrolidine-3-carboxylic acids **25-27**, amides **28**, oxadiazole **29** and oxadiazolone **30**. Representative reagents and conditions: (a) **36** (1.2 equiv.), ArI **37** (1.0 equiv.), $Pd_2dba_3$ (2.5 mol%), Xantphos (5 mol%), $Cs_2CO_3$ (2.7 equiv.), 1,4-dioxane, 100 °C, 16 h; (b) aq. NaOH (1 M, 2.0 equiv.), MeOH, RT, 2 h; (c) HATU (1.1 equiv.), DMF, RT, 15 min; then $iPr_2NEt$ (2.2 equiv.), amine **39** (HNRR′)(1.5 equiv.); (d) $NH_2NH_2{\cdot}H_2O$ (4 equiv.), EtOH-PhMe, 80 °C, 16 h; (e) $HC(OEt)_3$ (4.5 equiv.), *p*-$TsOH{\cdot}H_2O$ (0.1 equiv.), 60 °C, 5 min then RT, 1 h; (f) triphosgene (0.4 equiv.), $iPr_2NEt$ (2.0 equiv.), $CH_2Cl_2$, 0 °C - RT, 15 min.

Pyrrolidine-3-carboxylic acid target compounds **25-27** were prepared using a simple two-step sequence (**Scheme 2**). 3-Pyrrolidine ester **36** was cross-coupled with the aryl iodide **37** under Pd-mediated catalysis to give the corresponding 1-arylpyrrolidine **38** and then hydrolysis of the ester gave acid **25**.[26] The single enantiomers (*S*)-**26** and (*R*)-**27** were prepared from the corresponding chiral esters, methyl (*S*)- and (*R*)-pyrrolidine-3-carboxylate (**36**) respectively as described above, to provide samples of high chemical and optical purity.[27] Amides **28** were prepared by activation of the acid **25** with HATU and subsequent reaction with the amine **39** (HNRR'). Heterocycles **29** and **30** were prepared from ester **38** by reaction with hydrazine to give the acyl hydrazide and then treatment with triethyl orthoformate gave 1,3,4-oxadiazole **29**, whereas treatment with triphosgene gave 1,3,4-oxadiazol-2(3*H*)-one **30**.

The design of new inhibitors with improved Notum activity initially focused on the SARs of the pyrrole-3-carboxylic acids by exploring the substitution on the pyrrole ring **11-24** (**Table 2**). This set allowed for the direct comparison of a number of matched pairs to identify beneficial changes. Deconstructing **5-7** to their components in order to identify the minimum pharmacophore was achieved with a new set of fragments **11-18**. However, all these smaller fragments had a significant decrease in Notum activity suggesting further reduction in size was not feasible (e.g. **5** v **11** v **12** v **15**). In contrast, the N1 substituent ($R^1$) proved to be quite

influential with the Ph group preferred: Ph (**20**) > cPr (**6**) > $CH_2$cPr (**7**), $CH_2$Ph (**23**) >> Me (**18**), H (**17**). There was a subtle balance of interplay between the $R^1$ and $R^5$ groups as shown by the significant decrease in activity when poorly partnered (e.g. $R^1$ = H prefers $R^5$ = $CF_3$ (**5**) >> Me (**17**); whereas $R^1$ = Ph prefers $R^5$ = Me (**20**) ≥ H (**19**) > $CF_3$ (**21**); and $R^1$ = $CH_2$Ph prefers $R^5$ = H (**22**) > Me (**23**) >> $CF_3$ (**24**)). From this limited set, 1-phenylpyrrole **20** emerged as having an attractive profile suitable for further optimization.

A wider range of *N*-aryls around **20** were then prepared to determine preferred substituents and substitution patterns whilst retaining the 2,5-dimethyl groups on the pyrrole (**Table 3**). The standard tactic of walking a F (**20b-d**), Me (**20e-g**) and Cl (**20h-j**) around the phenyl ring showed a clear preference for the 3-Cl group **20i** when compared to the unsubstituted Ph ring (**20**). Notum crystals were soaked with **20i** and the structure solved to show the ligand sitting deep in the lipophilic pocket with no direct interaction being made by the acid (**Figure 5A**). Additional single substituents were then introduced to the 3-position (**20k-q**) with a general trend emerging showing a preference for small lipophilic groups, and Notum inhibition tracking with lipophilicity: iPr (**20m**) ≥ Cl (**20i**), tBu (**20n**), cPr (**20l**), OEt (**20p**) > $CF_3$ (**20k**), OMe (**20o**), Me (**20f**), F (**20c**), H (**20**) ≥ CN (**20q**).

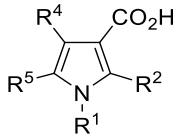
The combination of two substituents on the phenyl ring was initially explored by using two Cl atoms (**20r-w**) as a single 3- and 4-Cl atom had proved to be favorable (**20i**, **20j**) (**Table 3**) and the required dichloroanilines **34** were readily available. The 2,3- (**20r**) and 3,4-dichloro (**20v**) isomers were the most potent from this set and further improved Notum inhibition activity to ca. 200-300 nM. Additional SARs were then developed using a wider range of substituents at the 2,3- and 3,4-positions (**20x-ee**) by building on the earlier SAR whilst aiming to moderate overall lipophilicity of the compounds. A combination of a 2-F with preferred groups at the 3-position (iPr, cPr) gave some of the most potent compounds prepared so far: 2-F,3-iPr (**20z**) and 2-F,3-cPr (**20y**).

Through an exploration of the SARs within the pyrrole series, gains in Notum inhibition activity had been achieved when compared to the original fragment hits (e.g. for **20z** v **6**, 40-fold increase). However, the molecular properties of the most active inhibitors showed these compounds to be quite lipophilic (e.g. **20z**: $IC_{50}$ 0.15 μM, clog*P* 5.5). Despite our efforts to moderate compound properties, Notum inhibition activity tracked with lipophilicity in this series resulting in the most potent compounds having the highest clog*P* values.

Our focus then switched to the pyrrolidine-3-carboxylic acids hit series **8** as this template offered the advantage of significantly lower lipophilicity when compared to the matched pyrrole, albeit with weaker Notum inhibition (e.g. **8** vs **20**; $IC_{50}$ 48 vs 1.7 μM; Δclog*P* = -2.4). The application of preferred aryl groups from the pyrrole series to the 1-phenylpyrrolidines **25** showed the SARs were not directly transferable; there was a significant decrease in Notum inhibition activity and a change in the rank order (**Table 4**). However, the 2-Cl (**10**), 3-Cl (**25a**) and 4-Cl (**25b**) were again amongst the preferred single groups (2-F, 2-Cl, 3-$CF_3$, 3-Cl, 4-F, 4-Cl;

$IC_{50}$ <20 μM) and used to explore the effect of two substituents on Notum activity (**25j-o**) with the 3,4-dichloro **25n** as the most potent isomer within this set.

**Table 2:** Notum inhibition of pyrrole-3-carboxylic acids **11-24**. SAR of the pyrrole ring.[a]



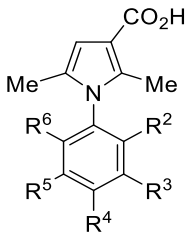

**11-24**

| **Compound** | R | | | | Notum $IC_{50}$ (μM) |
|---|---|---|---|---|---|
| | $R^1$ | $R^2$ | $R^4$ | $R^5$ | |
| **5** | H | Me | H | $CF_3$ | 6.7 ± 3.6 |
| **11** | H | H | H | H | >>100 [b] |
| **12** | H | Me | H | H | >>100 [b] |
| **13** | H | H | Me | H | ca. 100 [c] |
| **14** | H | H | H | Me | ca. 100 [c] |
| **15** | H | H | H | $CF_3$ | >>100 [b] |
| **16** | H | Me | Me | H | ca. 100 [c] |
| **17** | H | Me | H | Me | >> 100 [b] |
| **18** | Me | Me | H | Me | >100 [d] |
| **19** | Ph | Me | H | H | 3.8 ± 1.4 |
| **20** | Ph | Me | H | Me | 1.7 ± 0.6 |
| **21** | Ph | Me | H | $CF_3$ | 22 ± 3.1 |
| **22** | $CH_2Ph$ | Me | H | H | 3.1 ± 0.1 |
| **23** | $CH_2Ph$ | Me | H | Me | 14 ± 3.7 |
| **24** | $CH_2Ph$ | Me | H | $CF_3$ | >100 [d] |

[a] All values are mean ± s.d. of n = 2-4 experiments quoted to 2 s.f. Differences of <2-fold should not be considered significant. For details of the assay protocol, see reference 20.

[b] < 20 % I @ 100 μM; [c] ca. 50 % I @ 100 μM; [d] 20-40 % I @ 100 μM.

**Table 3:** Notum inhibition of 2,5-dimethyl-1-phenylpyrrole-3-carboxylic acids **20**. SAR of the phenyl ring.[a]




**20**

| Compound | $R^2$-$R^6$ | Notum $IC_{50}$ (μM) | Compound | $R^2$-$R^6$ | Notum $IC_{50}$ (μM) |
|---|---|---|---|---|---|
| **20** | H | 1.7 ± 0.6 | **20r** | 2-Cl, 3-Cl | 0.22 ± 0.09 |
| **20b** | 2-F | 3.3 ± 0.7 | **20s** | 2-Cl, 4-Cl | 0.43 ± 0.17 |
| **20c** | 3-F | 1.5 ± 0.2 | **20t** | 2-Cl, 5-Cl | 1.7 ± 0.15 |
| **20d** | 4-F | 1.9 ± 0.6 | **20u** | 2-Cl, 6-Cl | 2.3 ± 0.77 |
| **20e** | 2-Me | 2.2 ± 0.90 | **20v** | 3-Cl, 4-Cl | 0.27 ± 0.10 |
| **20f** | 3-Me | 1.5 ± 0.51 | **20w** | 3-Cl, 5-Cl | 0.85 ± 0.30 |
| **20g** | 4-Me | 2.0 ± 0.10 | **20x** | 2-F, 3-Cl | 0.38 ± 0.03 |
| **20h** | 2-Cl | 2.3 ± 0.74 | **20y** | 2-F, 3-cPr | 0.20 ± 0.09 |
| **20i** | 3-Cl | 0.43 ± 0.04 | **20z** | 2-F, 3-iPr | 0.15 ± 0.02 |
| **20j** | 4-Cl | 0.86 ± 0.10 | **20aa** | 2-F, 3-CN | 0.63 ± 0.23 |
| **20k** | 3-$CF_3$ | 1.5 ± 0.20 | **20bb** | 2-OMe, 3-Cl | 0.80 ± 0.25 |
| **20l** | 3-cPr | 0.58 ± 0.19 | **20cc** | 2-CN, 3-Cl | 2.0 ± 0.65 |
| **20m** | 3-iPr | 0.36 ± 0.20 | **20dd** | 3-Cl, 4-$CF_3$ | 1.5 ± 0.16 |
| **20n** | 3-tBu | 0.46 ± 0.09 | **20ee** | 3-$CF_3$, 4-Cl | 2.1 ± 0.12 |
| **20o** | 3-OMe | 1.5 ± 0.3 | | | |
| **20p** | 3-OEt | 0.64 ± 0.25 | | | |
| **20q** | 3-CN | 3.1 ± 1.1 | | | |

[a] See footnotes Table 2.

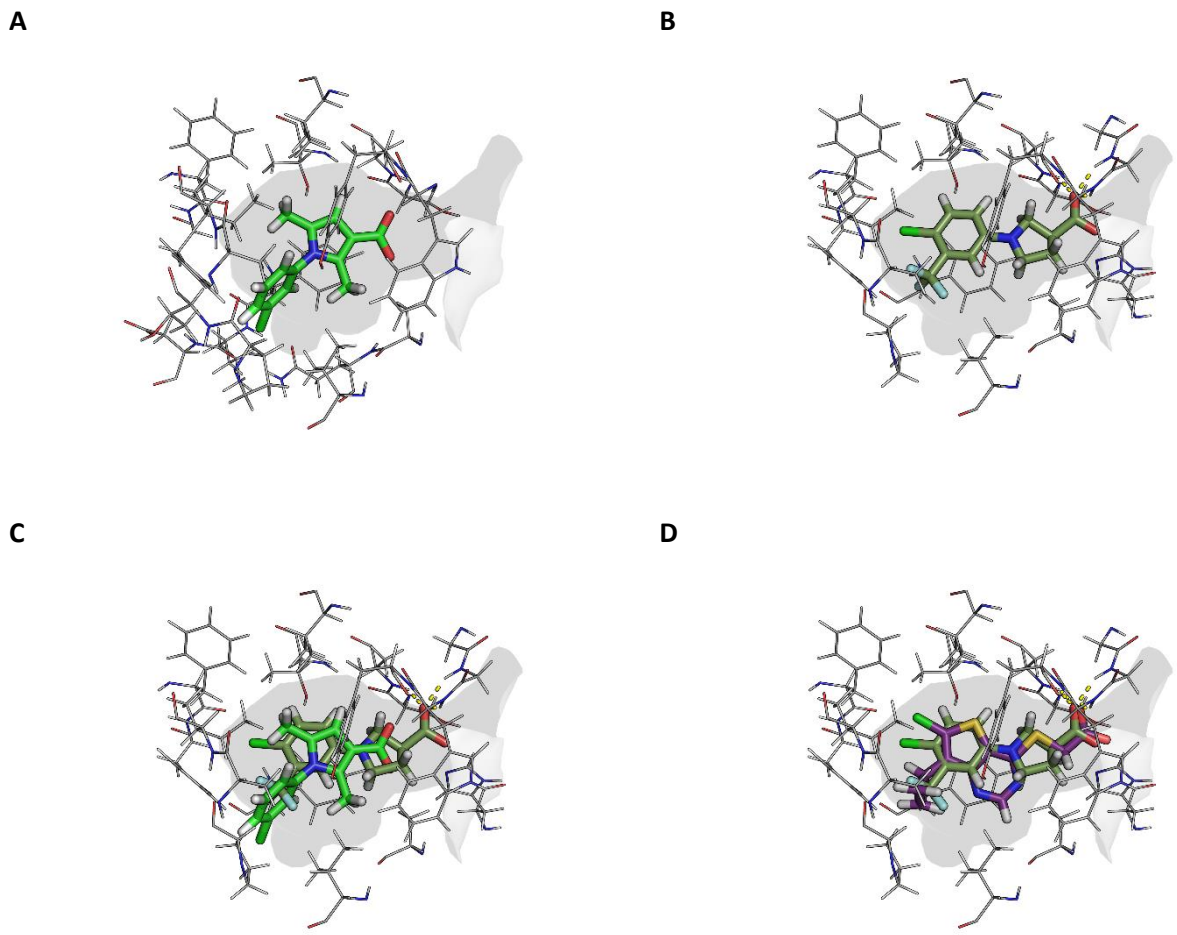
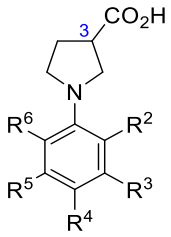
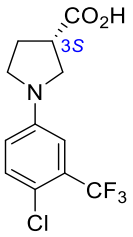



**Figure 5:** Notum structures with pyrrole and pyrrolidine inhibitors. Crystal structures of (A) **20i** (emerald) and (B) **26** (green). (C) Overlay of **20i** (emerald) and **26** (green) shows different binding modes. (D) Overlay of **26** (green) with **1** (purple; PDB: 6T2K) shows acid groups aligned. Binding site residues shown within 4Å of their respective ligands. Key hydrogen bond interactions are shown as dashed lines. Water molecules have been removed for clarity. The surface of the Notum palmitoleoate binding pocket outlined (gray) from the *O*-palmitoleoyl serine – Notum (S232A) structure (PDB: 4UZQ). Atomic coordinates have been deposited in the Protein Data Bank (PDB). PDB ID codes: **20i**: 6YXI; **26**: 6YSK.

**Table 4:** Notum inhibition of 1-phenylpyrrolidine-3-carboxylic acids **25-27**. SAR of the phenyl ring.[a]




**25**: *3RS*




**26**: *3S*
**27**: *3R*

| Compound | $R^2$-$R^6$ | Notum $IC_{50}$ (μM) | Compound | $R^2$-$R^6$ | Notum $IC_{50}$ (μM) |
|---|---|---|---|---|---|
| **8** | H | 48 ± 31 | **25j** | 2-Cl, 3-Cl | 8.0 ± 1.1 |
| **9** | 2-F | 11 ± 9 | **25k** | 2-Cl, 4-Cl | 10 ± 2.1 |
| **10** | 2-Cl | 19 ± 14 | **25l** | 2-Cl, 5-Cl | 7.9 ± 1.4 |
| **25a** | 3-Cl | 14 ± 0.9 | **25m** | 2-Cl, 6-Cl | 26 ± 8 |
| **25b** | 4-Cl | 19 ± 4.4 | **25n** | 3-Cl, 4-Cl | 1.2 ± 0.2 |
| **25c** | 3-Me | 49 ± 11 | **25o** | 3-Cl, 5-Cl | 11 ± 1.9 |
| **25d** | 3-$CF_3$ | 11 ± 0.6 | **25p** | 3-Cl, 4-$CF_3$ | 8.5 ± 0.77 |
| **25e** | 4-$CF_3$ | 50 ± 3.8 | **25q** | 3-$CF_3$, 4-Cl | 0.20 ± 0.02 |
| **25f** | 3-iPr | 57 ± 19 | **25r** | 3-$CF_3$, 4-F | 2.5 ± 0.5 |
| **25g** | 3-$OCF_3$ | 63 ± 13 | **25s** | 2-F, 3-$CF_3$ | 6.8 ± 0.9 |
| **25h** | 3-CN | 42 ± 12 | **25t** | 2-Cl, 3-Cl, 4-Cl | 0.60 ± 0.23 |
| **25i** | 4-F | 15 ± 1.2 | | | |
| | | | **26** | 3-$CF_3$, 4-Cl | 0.11 ± 0.01 |
| | | | **27** | 3-$CF_3$, 4-Cl | 0.28 ± 0.02 |

[a] See footnotes Table 2.

Extending this approach by exploring closely related halogenated analogues (**25p-s**) that incorporated a 3-$CF_3$ (cf. **25d**) identified 3-$CF_3$,4-Cl (**25q**) as a breakthrough compound due to its Notum inhibition activity and lower lipophilicity ($IC_{50}$ 0.20 μM, clog*P* 3.3). It is interesting to compare that the 3-$CF_3$,4-Cl substituents were significantly weaker in the pyrrole series (**20ee**, $IC_{50}$ 2.1 μM). Hence, despite the apparent structural similarity between the pyrrole **20** and pyrrolidine **25** templates, the SARs proved to be divergent. This was rationalized by the binding modes observed in the crystal structures of compounds **20i** and **8** where the pyrrole series sits deeper in the pocket compared to the pyrrolidine. The 2- and 5-methyl substituents on the pyrrole template also change the preferred conformation of the N1-Ar group. The 2,3,4-trichloro analogue **25s** ($IC_{50}$ 0.60 μM, clog*P* 3.7) was also a sub-micromolar inhibitor of Notum albeit with higher lipophilicity and was not pursued further at this time.

The two enantiomers of **25q** were then prepared to give (*S*)-**26 a**nd (*R*)-**27**.[27] Both enantiomers had good Notum activity with a slight preference for **26** ($IC_{50}$ 0.11 μM, clog*P* 3.3). Additional structural information was then sought to guide the next phase of design. Notum crystals were soaked with the most potent pyrrolidine **26** and the structure solved to show the compound binding in a similar mode as observed for hit **8** but with the 3-COOH more optimally positioned for interaction with the oxyanion hole (**Figure 5B** and **5C**). This is possibly due to the 3- and 4-aryl substituents allowing the inhibitor to both fully occupy the lipophilic pocket and make these interactions.

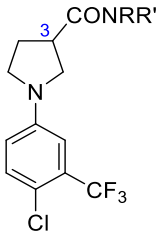
The X-ray structure of **26** in the active site of Notum was overlaid with the Notum structure of **1** (PDB: 6T2K) and showed very good alignment of the acid groups supporting the use of carboxamides at this position in the template (**Figure 5D**).[16] Initial SAR studies with amides **28** (derived from **25q**) suggested that Notum activity was particularly sensitive to the amide substituent as most groups were detrimental (**Table 5**). Only the 3,3-difluoroazetine **28e** and pyrrolidine **28f** amides retained activity similar to the corresponding acid **25q**.

An extension to this design strategy was to explore small heterocycles as bioisosteres for the carboxamide (**Table 5**). Oxadiazoles were initially selected due to their successful application in drug discovery programs and accessibility from ester **38**.[28] However, 1,3,4-oxadiazole **29** gave a significant drop in potency compared to acid **25q** and amides **28**. In contrast, switching to the corresponding weakly acidic 1,3,4-oxadiazol-2(3*H*)-one gave **30** ($IC_{50}$ 4.2 μM, clog*P* 3.2)(p$K_a$ ca 6.3),[29] which restored moderate inhibition of Notum activity. Oxadiazolone **30** was of particular interest as a lead to explore the potential of weak acids to penetrate the brain as molecules with these properties are under-represented in CNS drug discovery.[30,31,32]

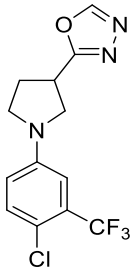
A set of preferred inhibitors (**20z**, **26**, **28e**, **30**) were then assessed in *in vitro* ADME assays to compare their aqueous solubility, microsomal stability (MLM) and cell permeability (MDCK-MDR1)(**Table 6**). These inhibitors were selected based on their Notum activity but also their chemical structural diversity and complementary physicochemical properties (clog*P*, p$K_a$). On balance, pyrrolidine-3-acid **26** demonstrated a superior profile

consistent with its properties: good solubility; good metabolic stability in MLM that predicts for low clearance; and good permeability without efflux by the P-glycoprotein (P-gp) transporter as measured by transit performance in the MDCK-MDR1 cell line. Oxadiazolone **30** also had a satisfactory combination of ADME results, whereas pyrrole-3-acid **20z** suffered from moderate MLM stability probably due to its higher lipophilicity. It is of note that there was evidence of non-CYP mediated metabolism in MLM for amide **28e** (i.e. loss of parent in an NADPH-independent manner).[20]

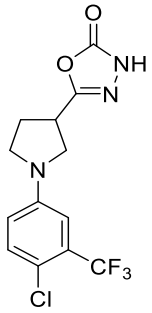
**Table 5:** Notum inhibition of 1-phenylpyrrolidine-3-carboxamides **28**, oxadiazole **29** and oxadiazolone **30**.[a]



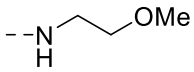
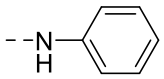
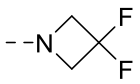
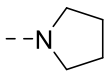
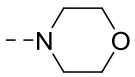
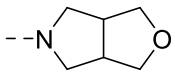
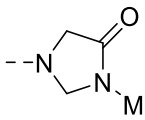
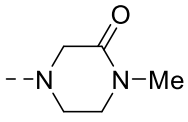

**28**




**29**

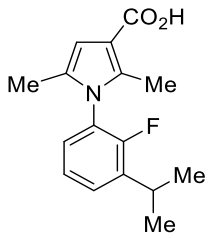



**30**

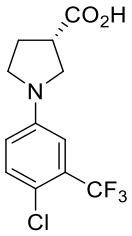
| **Compound** | **NRR′** | **Notum** $IC_{50}$ (μM) |
|---|---|---|
| **28a** | NHMe | 1.3 ± 0.17 |
| **28b** |  | 3.5 ± 0.25 |
| **28c** |  | 1.0 ± 0.05 |
| **28d** | $NMe_2$ | 1.2 ± 0.15 |
| **28e** |  | 0.26 ± 0.03 |
| **28f** |  | 0.41 ± 0.04 |
| **28g** |  | 9.7 ± 0.52 |
| **28h** |  | 3.1 ± 0.01 |
| **28i** |  | 1.4 ± 0.1 |
| **28j** |  | 3.1 ± 0.67 |
| **29** | - | 24 ± 0.1 |
| **30** | - | 4.2 ± 0.8 |

[a] See footnotes Table 2.

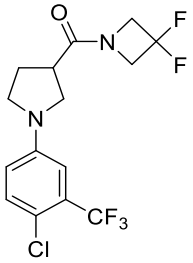
**Table 6.**
Summary of physicochemical and molecular properties, Notum inhibition and ADME data for **20z, 26, 28e** and **30**



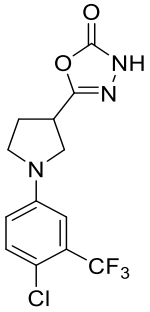
**20z**



**26**



**28e**



**30**

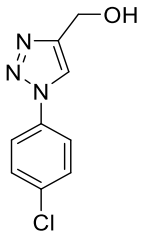
| | **20z** | **26** | **28e** | **30** |
|---|---|---|---|---|
| *Physicochemical and molecular properties* | | | | |
| mol wt | 275 | 293 | 368 | 333 |
| clog*P* | 5.5 | 3.3 | 4.1 | 3.2 |
| CHI $LogD_{7.4}$ | 3.1 | 1.5 | 4.0 | 3.5 |
| TPSA ($Å^2$) | 40.5 | 40.5 | 23.6 | 53.9 |
| Ligand efficiency (LE) | 0.48 | 0.51 | 0.39 | 0.34 |
| Lipophilic ligand efficiency (LLE) [a] | 3.7 | 5.4 | 2.5 | 1.9 |
| *Notum inhibition* [b] | | | | |
| OPTS, $IC_{50}$ (μM) | 0.15 ± 0.02 (n = 4) | 0.11 ± 0.01 (n = 6) | 0.26 ± 0.03 (n = 4) | 4.2 ± 0.8 (n = 4) |
| *ADME profile* [c] | | | | |
| Aq. solubility (μg/mL)/(μM) | 14/51 | >145/>595 | 7/19 | 23/69 |
| MLM, $Cl_i$ (μL/min/mg protein) | 52 | 2.5 | 21 [d] | 10 |
| MDCK-MDR1, AB/BA $P_{app}$ ($x10^{-6}$ cm/s) | 46/43 | 39/43 | 8/10 | 13/19 |
| MDCK-MDR1, efflux ratio (ER) | 0.93 | 1.1 | 1.2 | 1.5 |

[a] LLE based on calculation with measured CHI $LogD_{7.4}$ values.
[b] See footnotes Table 2.
[c] Studies were performed by GVK Biosciences (Hyderabad, India).
[d] Evidence of non-CYP mediated metabolism in MLM.

**CONCLUSION**

We have disclosed two new chemical scaffolds that efficiently inhibit Notum enzymatic activity. Our approach was to create a custom-designed fragment library of carboxylic acids for screening against Notum in a biochemical assay followed by structure determination by X-ray crystallography. Twenty fragments were identified as hits for Notum inhibition ($IC_{50}$ < 25 μM) with two preferred hit series selected for further investigation; these preferred fragments were shown by crystallography to bind in the palmitoleate pocket of Notum. Optimization of 1-phenylpyrrole **20** by SAR studies guided by SBDD identified **20z** ($IC_{50}$ 0.15 μM) as the most potent compound from this series. Similarly, optimization of 1-phenylpyrrolidine **8** gave acid **26** ($IC_{50}$ 0.11 μM), amide **28e** ($IC_{50}$ 0.26 μM) and oxadiazolone **30** ($IC_{50}$ 4.2 μM).

This work demonstrates that inhibition of Notum carboxylesterase activity can be achieved by small, drug-like molecules supported by attractive *in vitro* ADME profiles, some of which possess structural features and physicochemical properties consistent with CNS design space[33] including a favorable CNS MPO scores (e.g. **30**, CNS MPO 5.6/6.0).[34]




**40: ARUK3000123**

$IC_{50}$ 11.5 ± 3.0 μM (n = 9)

However, despite the significant progress in developing fragment hit **8** into lead **26** (>400-fold increase in activity), these compounds proved to be inferior to leads discovered by complementary screening strategies. In brief, screening of an alternative fragment library[20, 21] identified (1-(4-chlorophenyl)-1*H*-1,2,3-triazol-4-yl)methanol (**40**),[35] which was optimized to give a potent, selective and CNS penetrant inhibitor of Notum activity suitable for use in both cellular and *in vivo* models of disease.[36]

**EXPERIMENTAL SECTION**

**Chemistry**

**General Information:**

Unless preparative details are provided, all reagents were purchased from commercial suppliers and used without further purification. Solvents were of ACS reagent grade or higher and purchased from commercial suppliers without further purification. Anhydrous solvents were purchased as such from Acros Organics or Sigma Aldrich. Thin layer chromatography (TLC) was carried out on aluminum backed silica plates. The plates were visualized under UV (254 nm) light, followed by staining with phosphomolybdic acid dip or potassium permanganate and gentle heating.

Compound purification by column chromatography was performed using a Biotage Isolera using prepacked Biotage SNAP KP-Sil silica cartridges or Biotage SNAP Ultra C18 reverse phase cartridges. Chemical and chiral SFC analysis were performed by Reach Separation Ltd (Nottingham, UK) using conditions described below. Organic solvent layers were routinely dried with anhydrous $MgSO_4$ and concentrated using a Büchi rotary evaporator.

Melting points (mp) were determined using Stuart SMP20 melting point equipment using closed end glass capillary tubes and are uncorrected.

Infra-red (IR) spectra were recorded on a Shimadzu IRTracer-100 FT-IR spectrometer, using a Universal ATR accessory for sampling, with relevant absorbance quoted as $\nu_{max}$ in $cm^{-1}$.

$^{1}H$ NMR and $^{13}C$ NMR spectra were run in deuterated (≥99.5%) solvents on either a Bruker Avance 300 (300 MHz), Bruker Avance 400 (400 MHz), Bruker Avance 600 (600 MHz) or Bruker Avance 700 (700 MHz). Chemical shifts (δ) are reported as parts per million (ppm), coupling constants (*J*) are reported in Hz and signal multiplicities are reported as singlet (s), doublet (d), triplet (t), quartet (q), pentet (p), quintet (qu), sextet (sext), doublet of doublets (dd), doublet of triplets (dt), triplet of triplets (tt), multiplet (m), or broad singlet (br s).

LCMS analysis was performed on a Waters Acquity H-Class UPLC system with either an acidic (HSS C18 Column, $H_2O$:MeCN, 0.1% TFA) or basic (BEH C18 Column, $H_2O$:MeCN, 10 mM $NH_4OH$) mobile phase.

HRMS data acquisition was on a Waters Micromass LCT Premier electrospray time-of-flight (ESI-TOF) mass spectrometer. The observed mass and isotope pattern matched the corresponding theoretical values as calculated from the expected elemental formula.

Optical rotations $[\alpha]_D$ were measured on a Bellingham + Stanley ADP450 polarimeter.

Purity of screening compounds **11**-**30** was evaluated by NMR spectroscopy and LCMS analysis. All compounds had purity ≥ 95 %.

**General Methods:**

**1. Preparation of 1,4-diketo esters 33.**

General Method 1 adapted from Kazembe *et. al.*[37]

β-Keto ester **31** (8.0 mmol, 1.0 eq.) was added to a stirred solution of NaH (385 mg, 60% in mineral oil, 9.6 mmol, 1.2 eq.) in THF (10 mL) at 0 °C, and the solution was stirred at 0 °C for 30 min. A solution of 2-chloroacetone (0.66 mL, 8.0 mmol, 1.0 eq.) in THF (4 mL) was added dropwise and then NaI (120 mg, 0.8 mmol, 0.1 eq.). The reaction mixture was warmed to RT and stirred for 24 h. The mixture was quenched with dilute HCl (10 mL, 1 M) and extracted with EtOAc (3 x 30 mL). The combined organic phases were washed with $NaHCO_3$ (10 mL, 1 M), brine (10 ml), dried ($MgSO_4$) and concentrated *in vacuo*. The product was then purified by flash column chromatography on silica gel with gradient elution of EtOAc in cyclohexane.

**Ethyl 2-acetyl-4-oxo-pentanoate.**

Prepared by General Method 1 with ethyl acetoacetate (1.94 mL, 15.4 mmol). Purification by column chromatography (2-20% EtOAc:cyclohexane) gave the product as a yellow oil (859 mg, 4.61 mmol, 30 %).

$^1$H NMR (600 MHz, $CDCl_3$) δ 4.11 (q, *J* = 7.1 Hz, 2H), 3.93 (dd, *J* = 8.2, 5.7 Hz, 1H), 3.05 (dd, *J* = 18.5, 8.2 Hz, 1H), 2.87 (dd, *J* = 18.5, 5.7 Hz, 1H), 2.27 (s, 3H), 2.11 (s, 3H), 1.19 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 205.70, 202.27, 168.87, 61.83, 53.86, 41.64, 30.17, 29.79, 14.12.

***t*-Butyl 2-acetyl-4-oxo-pentanoate.**

Prepared by General Method 1 with *tert*-butyl acetoacetate (2.1 mL, 12.6 mmol). Purification by column chromatography (5-40% EtOAc:cyclohexane) gave the product as a yellow oil (759 mg, 3.54 mmol, 28 %).

IR $\nu_{max}$ (film) 1735, 1710, 1367, 1357, 1255, 1138 cm$^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$) δ 3.82 (dd, *J* = 8.1, 5.8 Hz, 1H), 2.97 (dd, *J* = 18.4, 8.1 Hz, 1H), 2.80 (dd, *J* = 18.4, 5.8 Hz, 1H), 2.23 (s, 3H), 2.07 (s, 3H), 1.40 – 1.33 (s, 9H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 205.71, 202.54, 167.83, 82.31, 54.88, 41.53, 29.95, 29.68, 27.85; LCMS (basic method) $R_t$ 1.65 min, m/z 214.3 $[M-H]^-$.

**2. Paal-Knorr pyrrole synthesis 35: R = Et.**

General Method 2 adapted from Kazembe *et. al.*[37]

Aniline **34** (1.2 eq.) and *p*-toluenesulfonic acid monohydrate (23 mg, 0.12 mmol, 0.1 eq.) were added to a solution of ethyl 2-acetyl-4-oxopentanoate (200 mg, 1.08 mmol, 1 eq.) in EtOH (5 mL) and the reaction mixture

was heated at 80 °C for 24 hr. The reaction was quenched with water (10 mL) and concentrated *in vacuo.* The residue was dissolved in dilute HCl (10 mL, 2 M) and extracted with EtOAc (3 x 15 mL). The combined organics were washed with NaOH (3 x 15 mL, 1 M), dried ($MgSO_4$) and concentrated *in vacuo* to give the 3-pyrrole ethyl ester.

**3. Hydrolysis of 3-pyrrole ethyl ester.**

Solid lithium hydroxide monohydrate (670 mg, 16 mmol, 15 eq.) was added to a solution of the 3-pyrrole ester (ca. 1.1 mmol, 1.0 eq.) in 1,4-dioxane (4.0 mL) and water (1.2 mL), and the reaction mixture heated to 100 °C for 24 h. The mixture was cooled to RT and quenched with water (5.0 mL). The mixture was acidified with conc. HCl (1 mL) to pH 1 and extracted with EtOAc (3 x 15 mL). The combined organic phases were concentrated *in vacuo* and the crude product was purified by reverse phase flash silica chromatography (0-100% MeCN:$H_2O$; 0.1% formic acid modifier) to give the 3-pyrrole acid.

**4. Paal-Knorr pyrrole synthesis 35: R = *t*-Bu.**

**4.1 DMSO as solvent:**

*t*-Butyl 2-acetyl-4-oxopentanoate (100 mg, 0.47 mmol) was dissolved in DMSO (2.5 mL) in a thick-walled reaction vial. The aniline **34** (0.47 mmol) and *p*-toluenesulfonic acid monohydrate (20 mg, 0.11 mmol) were added and the vial sealed with a Teflon-lined crimp cap. The vial was heated at 120 °C for 30 min under microwave irradiation to give directly the pyrrole-3-carboxylic acid. The crude product was purified by reverse phase flash silica chromatography (20-100% MeCN:$H_2O$; 0.1% formic acid modifier).

**4.2 Ethanol as solvent:**

*t*-Butyl 2-acetyl-4-oxopentanoate (100 mg, 0.47 mmol) was dissolved in EtOH (5.0 mL) in a thick-walled reaction vial. The aniline **34** (0.47 mmol) and *p*-toluenesulfonic acid monohydrate (20 mg, 0.11 mmol) were added and the vial sealed with a Teflon-lined crimp cap. The vial was heated at 80 °C thermally for 24 h to give directly the pyrrole-3-carboxylic acid. DMSO was added and the EtOH was evaporated under a flow of compressed air. The crude product was purified by reverse phase flash silica chromatography (20-100% MeCN:$H_2O$; 0.1% formic acid modifier).

**4.3 Xylenes as solvent:**

*t*-Butyl 2-acetyl-4-oxopentanoate (100 mg, 0.47 mmol) was dissolved in xylenes (5.0 mL) in a thick-walled reaction vial. The aniline **34** (0.47 mmol) and *p*-toluenesulfonic acid monohydrate (20 mg, 0.11 mmol) were added and the vial sealed with a Teflon-lined crimp cap. The vial was heated at 110 °C thermally for 24 h to give directly the pyrrole-3-carboxylic acid. DMSO was added and the xylenes were evaporated *in vacuo*. The crude product was purified by reverse phase flash silica chromatography (20-100% MeCN:$H_2O$; 0.1% formic acid modifier).

**5. *N*-Benzylation of 3-pyrrole esters**

General Method 5 adapted from Beard *et al.*[38]

3-Pyrrole ester (0.65 mmol, 1.0 eq.) was added to a solution of NaH (ca 31 mg, 60% in mineral oil, 0.78 mmol, 1.2 eq.) in THF (2.5 mL) stirring at 0 °C under nitrogen, and the solution was stirred at 0 °C for 15 min. Benzyl bromide (93 μL, 0.78 mmol, 1.2 eq.) was then added, and the reaction allowed to warm to RT and stirred for 24 h. The reaction was quenched with water (10 mL), extracted with EtOAc (3 x 15 mL), washed with brine (2 x 15 mL), dried (MgSO4) and concentrated *in vacuo*. The product was purified by column chromatography (0-15% EtOAc:cyclohexane) to give the 1-benzyl-3-pyrrole ester.

**6. Cross-coupling of 3-pyrrolidine ester 36 with aryl iodides 37.**

**6.1 Buchwald-Hartwig amination.**

To a thick-walled reaction vial was added methyl pyrrolidine-3-carboxylate (**36**) (320 mg, 2.0 mmol), tris(dibenzylideneacetone)dipalladium (0) (37 mg, 0.04 mmol), cesium(II) carbonate (1.4 g, 4.4 mmol) and Xantphos (47 mg, 0.08 mmol). The vial was sealed with a Teflon-lined crimp cap and charged with a solution of the aryl iodide **37** (1.6 mmol) in 1,4-dioxane (7.8 mL). The stirred reaction mixture was degassed with Ar and then heated to 100 $^{o}$C for 4 h. The reaction mixture was cooled to RT and diluted with $CH_2Cl_2$. The resulting suspension was filtered through a plug of sand and cotton wool and the filtrate concentrated under a continuous flow of air to give the ester **38**. This material was used directly in the next step without further purification.

**6.2 Alternative Buchwald-Hartwig amination.**

To a thick-walled reaction vial was added 2-dicyclohexylphosphino-2,4,6-triisopropylbiphenyl (18.4 mg, 0.03 mmol), water (0.76 uL, 0.04 mmol) and palladium acetate (2.4 mg, 0.01 mmol). The vial was purged with $N_2$ and the reagents dissolved in 1,4-dioxane (5mL). This mixture was stirred at 80 $^{o}$C under $N_2$ until the characteristic change in colour was seen (yellow to green). The catalyst mixture was then transferred to a second thick-walled reaction vial containing the aryl iodide **37** (1.06 mmol), cesium(II) carbonate (933.0 mg, 2.86 mmol) and methyl pyrrolidine-3-carboxylate hydrochloride (**36.HCl**) (211 mg, 1.3 mmol) in 1,4-dioxane (5 mL). The reaction was then stirred at 110 $^{o}$C for 16 h under $N_2$ until all the starting material was shown to be consumed by TLC. Reaction work-up and purification as General Method 6.1.

**7. Hydrolysis of 3-pyrrolidine methyl ester 38.**

To a solution of methyl 1-arylpyrrolidine-3-carboxylate **38** (0.32 mmol) in MeOH (3 mL) was added aqueous NaOH (0.65 mL, 1 N, 0.65 mmol). The reaction mixture was stirred at RT for 2 h and then quenched with dilute aqueous HCl (1 M, 2 eq.). The sample was concentrated under reduced pressure, redissolved in a minimum of DMSO and then purified by reverse phase chromatography (0-95% MeCN:$H_2O$; 0.1% formic acid modifier).

**8. Preparation of 3-pyrrolidine carboxamides 28.**

To a thick-walled reaction vial was added methyl 1-[4-chloro-3-(trifluoromethyl)phenyl]pyrrolidine-3-carboxylic acid (**25q**) (50 mg, 0.17 mmol). Anhydrous DMF (1.0 mL) and hexafluorophosphate azabenzotriazole tetramethyl uronium (HATU) (72 mg, 0.19 mmol) were added and the reaction mixture stirred at RT for 10 min. DIPEA (0.07 mL, 0.40 mmol) was added followed by the amine **39** (0.26 mmol) and the reaction was stirred at RT for 1 h. The reaction mixture was directly purified by reverse phase chromatography (0-100% MeCN:$H_2O$, 0.1% $NH_4OH$ modifier).

**Notum Inhibitors:**

**2-Methyl-5-(trifluoromethyl)-1*H*-pyrrole-3-carboxylic acid (5)**

Purchased from Enamine, EN300-38888

**1-Cyclopropyl-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (6)**

Purchased from Enamine, EN300-13229

**1-(Cyclopropylmethyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (7)**

Purchased from Enamine, EN300-69261

**1-Phenylpyrrolidine-3-carboxylic acid (8)**

Purchased from Enamine, EN300-226265

**1-(2-Fluorophenyl)pyrrolidine-3-carboxylic acid (9)**

Purchased from Enamine, EN300-217084

**1-(2-Chlorophenyl)pyrrolidine-3-carboxylic acid (10)**

Purchased from Enamine, EN300-218252

**1*H*-Pyrrole-3-carboxylic acid (11)**

Purchased from Fluorochem, 075258

**2-Methyl-1*H*-pyrrole-3-carboxylic acid (12)**

Purchased from Enamine, EN300-98523

**4-Methyl-1*H*-pyrrole-3-carboxylic acid (13)**

Available from multiple suppliers [CAS Reg. No. 64276-66-0]. Our sample was prepared by General Method 3 from methyl 4-methyl-1*H*-pyrrole-3-carboxylate. Isolated as a white solid (8 mg).

$^1$H NMR (600 MHz, $CDCl_3$) δ 8.23 (br s, 1H), 7.45 (m, 1H), 6.55 (m, 1H), 2.30 (s, 3H); LCMS (acidic method) $R_t$ 0.54 min, m/z 126.1 $[M+H]^+$.

**5-Methyl-1*H*-pyrrole-3-carboxylic acid (14)**

Purchased from Fluorochem, 091685

**5-(Trifluoromethyl)-1*H*-pyrrole-3-carboxylic acid (15)**

Purchased from Enamine, EN300-177045

**2,4-Dimethyl-1*H*-pyrrole-3-carboxylic acid (16)**

Available from multiple suppliers [CAS Reg. No. 17106-13-7]. Our sample was prepared by General Method 3 from ethyl 2,4-dimethyl-1*H*-pyrrole-3-carboxylate. Isolated as a yellow gum (7.4 mg)

$^1$H NMR (600 MHz, $CDCl_3$) δ 9.39 (br s, 1H), 7.91 (br s, 1H), 6.37 (m, 1H), 2.51 (s, 3H), 2.25 (d, *J* = 1.1 Hz, 3H); LCMS (acidic method) $R_t$ 0.81 min, m/z 140.1 $[M+H]^+$.

**2,5-Dimethyl-1*H*-pyrrole-3-carboxylic acid (17)**

Purchased from Alfa Aesar, B20635

**1,2,5-Trimethyl-1*H*-pyrrole-3-carboxylic acid (18)**

Purchased from Maybridge, CC08601

**2-Methyl-1-phenyl-1*H*-pyrrole-3-carboxylic acid (19)**

Prepared by General Methods 2 and 3. Isolated as a pale pink solid (33.4 mg, 54 %).

Mp 185 °C; $^1$H NMR (400 MHz, Methanol-$d_4$) δ 7.52 (t, *J* = 8.0 Hz, 2H), 7.45 (t, *J* = 8.0 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 2H), 6.74 (d, *J* = 3.0 Hz, 1H), 6.61 (d, *J* = 3.0 Hz, 1H), 2.40 (s, 3H); LCMS (acidic method) $R_t$ 1.55 min, m/z 202.2 $[M+H]^+$.

**2,5-Dimethyl-1-phenyl-1*H*-pyrrole-3-carboxylic acid (20)**

Prepared by General Methods 2 and 3. Isolated as a pink solid (7.7 mg, 32 %).

Mp 204 °C; $^1$H NMR (600 MHz, $CDCl_3$) δ 7.53 – 7.44 (m, 3H), 7.22 – 7.17 (m, 2H), 6.43 (d, *J* = 1.0 Hz, 1H), 2.31 (s, 3H), 1.98 (d, *J* = 0.5 Hz, 3H); LCMS (acidic method) $R_t$ 0.65 min, m/z 216.2 $[M+H]^+$.

**2,5-Dimethyl-1-(2-fluorophenyl)-1*H*-pyrrole-3-carboxylic acid (20b)**

Prepared by General Method 4.1 with 2-fluoroaniline. Isolated as a white solid (70 mg, 64 %).

$^1$H NMR (700 MHz, $CDCl_3$) δ 7.49 – 7.45 (m, 1H), 7.31 – 7.21 (m, 3H), 6.45 (d, *J* = 0.9 Hz, 1H), 2.31 (s, 3H), 1.98 (s, 3H); $^{13}$C NMR (176 MHz, $CDCl_3$) δ 170.59, 158.32 (d, *J* = 251.8 Hz), 138.17, 130.87 (d, *J* = 7.7 Hz), 130.49, 129.44, 125.42 (d, *J* = 13.2 Hz), 124.92 (d, *J* = 4.0 Hz), 117.01 (d, *J* = 19.9 Hz), 111.30, 108.48, 12.35, 12.25; LCMS (acidic method) $R_t$ 1.63 min, m/z 234.2 $[M+H]^+$.

**2,5-Dimethyl-1-(3-fluorophenyl)-1*H*-pyrrole-3-carboxylic acid (20c)**

Prepared by General Method 4.1 with 3-fluoroaniline. Isolated as a white solid (80 mg, 74 %).

$^1$H NMR (700 MHz, $CDCl_3$) δ 7.48 (m, 1H), 7.22 – 7.16 (m, 1H), 7.03 – 6.99 (m, 1H), 6.95 (dt, *J* = 9.0, 2.1 Hz, 1H), 6.43 (s, 1H), 2.32 (s, 3H), 2.00 (s, 3H); $^{13}$C NMR (176 MHz, CDCl3) δ 170.68, 162.96 (d, *J* = 249.3 Hz), 139.18 (d, *J* = 9.6 Hz), 137.62, 130.78 (d, *J* = 9.1 Hz), 129.11, 124.26 (d, *J* = 3.2 Hz), 116.05 (d, *J* = 19.9 Hz), 115.87, 111.09, 108.51, 12.77, 12.63; LCMS (acidic method) $R_t$ 1.70 min, m/z 234.2 $[M+H]^+$.

**2,5-Dimethyl-1-(4-fluorophenyl)-1*H*-pyrrole-3-carboxylic acid (20d)**

Prepared by General Method 4.1 with 4-fluoroaniline. Isolated as an off-white solid (60 mg, 55 %).

$^1$H NMR (600 MHz, $CDCl_3$) δ 7.18 (m, 4H), 6.42 (d, *J* = 0.8 Hz, 1H), 2.29 (s, 3H), 1.97 (s, 3H); $^{13}$C NMR (151 MHz, CDCl3) δ 171.11, 162.51 (d, *J* = 249.0 Hz), 137.88, 133.69 (d, *J* = 3.0 Hz), 130.02 (d, *J* = 8.6 Hz), 129.32, 116.61 (d, *J* = 22.9 Hz), 110.90, 108.26, 12.83, 12.68; LCMS (acidic method) $R_t$ 1.71 min, m/z 234.2 $[M+H]^+$.

**2,5-Dimethyl-1-(2-methylphenyl)-1*H*-pyrrole-3-carboxylic acid (20e)**

Prepared by General Method 4.2 with *o*-toluidine. Isolated as a light green solid (51 mg, 48 %).

$^1$H NMR (700 MHz, $CDCl_3$) δ 7.35 (m, 3H), 7.12 (d, *J* = 7.5 Hz, 1H), 6.45 (d, *J* = 0.8 Hz, 1H), 2.22 (s, 3H), 1.95 (s, 3H), 1.89 (s, 3H); $^{13}$C NMR (176 MHz, $CDCl_3$) δ 170.87, 137.36, 136.84, 136.72, 131.15, 129.24, 128.69, 128.63, 127.13, 110.65, 108.08, 17.19, 12.42, 12.29; LCMS (acidic method) $R_t$ 1.75 min, m/z 230.2 $[M+H]^+$

**2,5-Dimethyl-1-(3-methylphenyl)-1*H*-pyrrole-3-carboxylic acid (20f)**

Prepared by General Method 4.3 with *m*-toluidine. Isolated as light grey solid (97 mg, 57 %).

Mp 195-197 °C; IR $\nu_{max}$ (film) 1653, 1606, 1265, 1244, 771, 729 $cm^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$) δ 7.37 (t, *J* = 7.7 Hz, 1H), 7.27 (s, 1H), 6.99 (d, *J* = 8.4 Hz, 2H), 6.40 (d, *J* = 1.0 Hz, 1H), 2.42 (s, 3H), 2.30 (s, 3H), 1.97 (s, 3H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 169.44, 139.62, 137.75, 129.48, 129.26, 128.80, 125.25, 110.28, 108.01, 56.10, 32.10, 21.38, 12.78, 12.64; LCMS (acidic method) $R_t$ 2.04 min, m/z 230.2 $(M+H)^+$.

**2,5-Dimethyl-1-(4-methylphenyl)-1*H*-pyrrole-3-carboxylic acid (20g)**

Prepared by General Methods 2 and 3 with *p*-toluidine. Isolated as a white solid (123 mg, 37 %).

Mp 246 °C; $^{1}$H NMR (300 MHz, $CDCl_3$) δ 7.29 (d, *J* = 8.0 Hz, 2H), 7.07 (d, *J* = 8.0 Hz, 2H), 6.41 (s, 1H), 2.43 (s, 3H), 2.30 (s, 3H), 1.97 (s, 3H); LCMS (acidic method) $R_t$ 1.68 min, m/z 230.2 $[M+H]^+$.

**1-(2-Chlorophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (20h)**

Prepared by General Method 4.2 with 2-chloroaniline. Isolated as a white solid (60 mg, 52 %).

$^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 11.73 (s, 1H), 7.77 – 7.70 (m, 1H), 7.63 – 7.45 (m, 3H), 6.25 (d, *J* = 1.0 Hz, 1H), 2.11 (s, 3H), 1.85 (s, 3H); $^{13}$C NMR (176 MHz, DMSO-$d_6$) δ 166.23, 134.96, 134.72, 132.20, 131.07, 130.84, 130.33, 128.66, 127.82, 111.81, 107.79, 11.93, 11.66; LCMS (acidic method) $R_t$ 1.73 mins, m/z 250.2 $[M+H]^+$.

**1-(3-Chlorophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (20i)**

Prepared by General Methods 2 and 3 with 3-chloroaniline. Isolated as an amber oil (206 mg, 76 %).

IR $\nu_{max}$ (film) 3346, 3069-3087, 2834-2990, 2582, 1646, 1378-1594, 1330, 1250, 776 $cm^{-1}$; $^{1}$H NMR (700 MHz, $CDCl_3$) δ 7.45 (m, 2H), 7.22 (s, 1H), 7.10 (d, *J* = 8.0 Hz, 1H), 6.40 (s, 1H), 2.31 (s, 3H), 1.99 (s, 3H); $^{13}$C NMR (176 MHz, $CDCl_3$) δ 169.22, 138.86, 137.70, 135.22, 130.57, 129.20, 128.63, 126.64, 110.67, 108.49, 67.22, 12.79, 12.63; HRMS $C_{13}H_{12}ClNO_2$: calcd. 250.0635 $[M+H]^+$, found 250.0637.

**1-(4-Chlorophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (20j)**

Prepared by General Methods 2 and 3 with 4-chloroaniline. Isolated as a white solid (34 mg, 55 %).

Mp 236 °C; $^{1}$H NMR (600 MHz, $CDCl_3$) δ 7.48 (d, *J* = 9.0 Hz, 2H), 7.14 (d, *J* = 9.0 Hz, 2H), 6.42 (s, 1H), 2.31 (s, 3H), 1.98 (s, 3H); LCMS (basic method) $R_t$ 1.26 min, m/z 248.1 $[M-H]^-$.

**2,5-Dimethyl-1-(3-(trifluromethyl)phenyl)-1*H*-pyrrole-3-carboxylic acid (20k)**

Prepared by General Methods 2 and 3 with 3-(trifluoromethyl)aniline. Isolated as a white solid (71 mg, 70 %).

Mp 243 °C; IR $\nu_{max}$ (powder) 3460, 2582-3080, 2857-3080, 1739, 1435-1650, 1320, 1227, 1030-1163 $cm^{-1}$; $^{1}$H NMR (700 MHz, Methanol-$d_4$) δ 7.80 (d, *J* = 8.0 Hz, 1H), 7.75 (t, *J* = 8.0 Hz, 1H), 7.58 (s, 1H), 7.53 (d, *J* = 8.0 Hz, 1H), 6.32 (s, 1H), 2.25 (s, 3H), 1.96 (s, 3H); $^{13}$C NMR (175 MHz, Methanol-$d_4$) δ 150.9, 140.3, 135.3, 133.4, 132.8 (q, *J* = 32 Hz), 131.5, 128.8, 126.3 (q, *J* = 3.8 Hz), 126.2 (q, *J* = 3.8 Hz), 125.0 (q, *J* = 270 Hz), 109.8, 106.9, 12.6, 12.5; HRMS $C_{14}H_{12}F_3NO_2$: calcd. 284.0898 $[M+H]^+$, found 284.0893.

**1-(3-Cyclopropylphenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (20l)**

Prepared by General Method 4.2 with 3-cyclopropylaniline. Isolated as a solid (79 mg, 33 %).

Mp 120-122 °C; IR $\nu_{max}$ (film) 1651, 1533, 1490, 1427, 1259, 781, 474 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.36 (t, *J* = 7.8 Hz, 1H), 7.15 (d, *J* = 7.8 Hz, 1H), 6.95 (ddd, *J* = 7.7, 2.0, 1.0 Hz, 1H), 6.86 (t, *J* = 1.9 Hz, 1H), 6.40 (d, *J* = 1.0 Hz, 1H), 2.30 (s, 3H), 1.97 (s, 3H), 1.94 (ddd, *J* = 13.4, 8.4, 4.9 Hz, 1H), 1.04 (dd, *J* = 8.4, 1.7 Hz, 2H), 0.72 (dd, *J* = 4.9, 1.6 Hz, 2H); $^{13}C$ NMR (126 MHz, $CDCl_3$) 174.4, 149.4, 146.1, 137.7, 129.2, 129.2, 125.9, 125.3, 125.0, 108.0, 100.5, 15.3, 12.7, 12.6, 9.9.; LCMS (acidic method) $R_t$ 1.73 min, m/z 256.3 $[M+H]^+$.

**2,5-Dimethyl-1-(3-isopropylphenyl)-1*H*-pyrrole-3-carboxylic acid (20m)**

Prepared by General Method 4.2 with 3-isopropylaniline. Isolated as an off-white solid (51 mg, 43 %).
$^1H$ NMR (700 MHz, $CDCl_3$) δ 7.40 (t, *J* = 7.7 Hz, 1H), 7.31 (d, *J* = 7.7 Hz, 1H), 7.04 (s, 1H), 7.00 (d, *J* = 7.7 Hz, 1H), 6.42 (s, 1H), 2.97 (hept, *J* = 6.9 Hz, 1H), 2.31 (s, 3H), 1.98 (s, 3H), 1.28 (d, *J* = 6.9 Hz, 6H); $^{13}C$ NMR (176 MHz, $CDCl_3$) δ 170.1, 150.7, 137.7, 137.6, 129.3, 129.2, 126.8, 126.2, 125.4, 110.4, 108.0, 34.0, 24.0, 12.8, 12.7; LCMS (acidic method) $R_t$ 1.85 min, m/z 258.3 $[M+H]^+$.

**1-(3-(*tert*-Butyl)phenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (20n)**

Prepared by General Method 4.1 with 3-*tert*-butylaniline. Isolated as a white solid (84 mg, 66 %).
$^1H$ NMR (700 MHz, $CDCl_3$) δ 7.47 (ddd, *J* = 7.6, 1.8, 1.0 Hz, 1H), 7.41 (t, *J* = 7.8 Hz, 1H), 7.19 (t, *J* = 1.8 Hz, 1H), 7.00 (ddd, *J* = 7.6, 1.8, 1.0 Hz, 1H), 6.43 (d, *J* = 1.0 Hz, 1H), 2.32 (s, 3H), 1.99 (s, 3H), 1.34 (s, 9H); $^{13}C$ NMR (151 MHz, $CDCl_3$) δ 170.9, 153.0, 137.8, 137.4, 129.2, 129.0, 125.5, 125.4, 125.1, 110.5, 108.0, 34.9, 31.4, 12.9, 12.7; LCMS (acidic method) $R_t$ 1.88 min, m/z 272.3 $[M+H]^+$.

**2,5-Dimethyl-1-(3-methoxyphenyl)-1*H*-pyrrole-3-carboxylic acid (20o)**

Prepared by General Method 4.1 with *m*-anisidine. Isolated as a brown solid (56 mg, 49 %).
$^1H$ NMR (700 MHz, $CDCl_3$) δ 7.39 (t, *J* = 8.0 Hz, 1H), 7.00 (d, *J* = 8.0 Hz, 1H), 6.78 (d, *J* = 8.0 Hz, 1H), 6.72 (d, *J* = 1.9 Hz, 1H), 6.42 (s, 1H), 3.84 (s, 3H), 2.32 (s, 3H), 2.00 (s, 3H); $^{13}C$ NMR (176 MHz, $CDCl_3$) δ 170.7, 160.4, 138.8, 137.7, 130.2, 129.2, 120.5, 114.4, 114.0, 110.6, 108.1, 55.6, 12.7, 12.6; LCMS (acidic method) $R_t$ 1.65 min, m/z 246.2 $[M+H]^+$.

**2,5-Dimethyl-1-(3-ethoxyphenyl)-1*H*-pyrrole-3-carboxylic acid (20p)**

Prepared by General Method 4.1 with 3-ethoxyaniline. Isolated as an off-white solid (87 mg, 72 %).
$^1H$ NMR (700 MHz, $CDCl_3$) δ 7.38 (t, *J* = 8.1 Hz, 1H), 6.98 (dd, *J* = 8.1, 2.4 Hz, 1H), 6.76 (d, *J* = 8.1 Hz, 1H), 6.71 (t, *J* = 2.1 Hz, 1H), 6.41 (s, 1H), 4.05 (q, *J* = 7.0 Hz, 2H), 2.32 (s, 3H), 2.00 (s, 3H), 1.44 (t, *J* = 7.0 Hz, 3H); $^{13}C$ NMR (176 MHz, $CDCl_3$) δ 170.7, 159.8, 138.7, 137.7, 130.1, 129.2, 120.3, 115.0, 114.4, 110.6, 108.0, 63.9, 14.8, 12.7, 12.6; LCMS (acidic method) $R_t$ 1.73 min, m/z 260.3 $[M+H]^+$.

**1-(3-cyanophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (20q)**

Prepared by General Method 4.2 with 3-cyanoaniline. Isolated as an off-white solid (200 mg, 72 %).

Mp 201-203 °C; IR $\nu_{max}$ (film) 1645, 1533, 1261, 1238, 775, 727 $cm^{-1}$; $^{1}H$ NMR (500 MHz, $CDCl_3$) δ 7.80 – 7.77 (m, 1H), 7.66 (t, *J* = 7.9 Hz, 1H), 7.54 (t, *J* = 1.6 Hz, 1H), 7.48 (ddd, *J* = 8.0, 2.0, 1.1 Hz, 1H), 6.45 (d, *J* = 1.0 Hz, 1H), 2.31 (s, 3H), 1.99 (s, 3H); $^{13}C$ NMR (126 MHz, $CDCl_3$) δ 169.6, 138.7, 137.3, 132.9, 132.4, 131.8, 130.7, 128.9, 117.5, 114.1, 111.5, 109.0, 12.8, 12.6; LCMS (acidic method) $R_t$ 1.51 min, m/z 241.2 $[M+H]^+$.

**1-(2,3-Dichlorophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (20r)**

Prepared by General Method 4.2 with 2,3-dichloroaniline. Isolated as an off-white solid (150 mg, 71 %).

Mp 246-248 °C; IR $\nu_{max}$ (film) 1647, 1423, 1265, 1238, 783, 729 $cm^{-1}$; $^{1}H$ NMR (500 MHz, $CDCl_3$) δ 7.62 (dd, *J* = 8.2, 1.5 Hz, 1H), 7.36 (t, *J* = 8.0 Hz, 1H), 7.21 (dd, *J* = 7.8, 1.5 Hz, 1H), 6.45 (d, *J* = 1.0 Hz, 1H), 2.25 (s, 3H), 1.93 (s, 3H); $^{13}C$ NMR (126 MHz, $CDCl_3$) δ $^{13}C$ NMR (126 MHz, $CDCl_3$) δ 170.7, 144.2, 134.5, 132.9, 131.3, 128.8, 128.6, 127.8, 116.5, 111.0, 108.4, 12.2, 12.1; LCMS (acidic method) $R_t$ 1.70 min, m/z 284.1 $[M+H]^+$.

**1-(2,4-Dichlorophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (20s)**

Prepared by General Method 4.1 with 2,4-dichloroaniline. Isolated as a white solid (70 mg, 53 %).

$^{1}H$ NMR (700 MHz, $CDCl_3$) δ 7.60 (d, *J* = 2.3 Hz, 1H), 7.41 (dd, *J* = 8.4, 2.3 Hz, 1H), 7.22 (d, *J* = 8.4 Hz, 1H), 6.45 (d, *J* = 0.9 Hz, 1H), 2.25 (s, 3H), 1.93 (s, 3H); $^{13}C$ NMR (151 MHz, $CDCl_3$) δ 170.7, 137.7, 135.9, 134.7, 134.2, 131.1, 130.5, 129.0, 128.3, 111.3, 108.5, 12.3, 12.2; LCMS (acidic method) $R_t$ 1.79 min, m/z 284.1 $[M+H]^+$.

**1-(2,5-Dichlorophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (20t)**

Prepared by General Method 4.2 with 2,5-dichloroaniline. Isolated as a white solid (185 mg, 88 %).

IR $\nu_{max}$ (film) 3356, 1689, 1473, 1249, 634, 455 $cm^{-1}$; $^{1}H$ NMR (500 MHz, $CDCl_3$) δ 7.52 (d, *J* = 8.6 Hz, 1H), 7.44 (dd, *J* = 8.6, 2.5 Hz, 1H), 7.31 (d, *J* = 2.4 Hz, 1H), 6.44 (d, *J* = 1.0 Hz, 1H), 2.27 (s, 3H), 1.95 (s, 3H); $^{13}C$ NMR (126 MHz, $CDCl_3$) δ 169.4, 137.5, 136.6, 133.4, 132.3, 131.3, 130.7, 130.5, 128.8, 111.2, 108.5, 12.2, 12.1; LCMS (acidic method) $R_t$ 1.35 min, m/z 284.0 $[M+H]^+$.

**1-(2,6-Dichlorophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (20u)**

Prepared by General Method 4.2 with 2,6-dichloroaniline. Isolated as a white solid (10 mg, 8 %).

$^{1}H$ NMR (400 MHz, $CDCl_3$) δ 7.54 – 7.47 (m, 2H), 7.39 (dd, *J* = 8.8, 7.4 Hz, 1H), 6.49 (d, *J* = 1.0 Hz, 1H), 2.25 (s, 3H), 1.93 (s, 3H); $^{13}C$ NMR (176 MHz, $CDCl_3$) δ 169.6, 137.2, 135.7, 133.7, 130.8, 128.8, 128.3, 111.4, 108.6, 11.9, 11.7; LCMS (acidic method) $R_t$ 1.72 min, m/z 284.1 $[M+H]^+$.

**1-(3,4-Dichlorophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (20v)**

Prepared by General Method 4.2 with 3,4-dichloraniline. Isolated as an off-white solid (53 mg, 40 %). $^1$H NMR (700 MHz, $CDCl_3$) δ 7.59 (d, *J* = 8.4 Hz, 1H), 7.34 (d, *J* = 2.4 Hz, 1H), 7.08 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.42 (d, *J* = 0.8 Hz, 1H), 2.32 (s, 3H), 2.00 (s, 3H); $^{13}$C NMR (176 MHz, $CDCl_3$) δ 170.1, 137.5, 137.0, 133.6, 133.4, 131.3, 130.3, 129.0, 127.7, 111.3, 108.7, 12.8, 12.6; LCMS (acidic method) $R_t$ 1.82 min, m/z 284.1 $[M+H]^+$.

**1-(3,5-Dichlorophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (20w)**

Prepared by General Method 4.2 with 3,5-dichloroaniline. Isolated as a light pink solid (139 mg, 52 %). Mp 215-217 °C; IR $\nu_{max}$ (film) 1654, 1566, 1537, 1255, 1238, 418 $cm^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$) δ 7.49 (t, *J* = 1.9 Hz, 1H), 7.14 (d, *J* = 1.9 Hz, 2H), 6.41 (d, *J* = 1.0 Hz, 1H), 2.33 (s, 3H), 2.00 (s, 3H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 170.9, 139.6, 137.3, 135.8, 129.3, 128.9, 127.1, 116.8, 111.3, 108.8, 12.7, 12.6; LCMS (acidic method) $R_t$ 1.78 min, m/z 284.0 $[M+H]^+$.

**1-(3-Chloro-2-fluorophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (20x)**

Prepared by General Method 4.2 with 3-chloro-2-fluoroaniline. Isolated as an off-white solid (97 mg, 39 %). Mp 230-232 °C; IR $\nu_{max}$ (film) 1653, 1460, 1273, 1234, 1087, 781 $cm^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$) δ 7.54 (ddd, *J* = 8.2, 6.6, 1.7 Hz, 1H), 7.23 (td, *J* = 8.1, 1.4 Hz, 2H), 7.15 (ddd, *J* = 8.1, 6.6, 1.7 Hz, 1H), 6.45 (d, *J* = 1.0 Hz, 1H), 2.31 (s, 3H), 1.98 (s, 3H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 170.1, 154.4 (d, *J* = 253.9 Hz), 137.9, 131.5, 129.3, 128.8, 126.8 (d, *J* = 13.3 Hz), 124.8 (d, *J* = 5.3 Hz), 122.8 (d, *J* = 16.8 Hz), 111.6, 108.8, 12.3, 12.2; LCMS (acidic method) $R_t$ 2.01 min, m/z 268.2 $[M+H)]^+$.

**1-(3-Cyclopropyl-2-fluorophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (20y)**

Prepared by General Method 4.2 with 3-cyclopropyl-2-fluoroaniline. Isolated as a white solid (70 mg, 55 %). $^1$H NMR (700 MHz, $CDCl_3$) δ 7.14 (t, *J* = 7.8 Hz, 1H), 6.99 (dd, *J* = 15.0, 7.8 Hz, 2H), 6.45 (s, 1H), 2.31 (s, 3H), 2.20 – 2.10 (m, 1H), 1.98 (s, 3H), 1.07 (m, 2H), 0.84 – 0.76 (m, 2H); $^{13}$C NMR (176 MHz, $CDCl_3$) δ 170.3, 157.2 (d, *J* = 250.1 Hz), 138.2, 132.9 (d, *J* = 13.5 Hz), 129.4, 126.9, 126.5 (d, *J* = 4.2 Hz), 125.0 (d, *J* = 14.1 Hz), 124.2 (d, *J* = 4.6 Hz), 111.0, 108.3, 12.3, 12.3, 8.8 (d, *J* = 5.5 Hz), 8.72 (d, *J* = 3.8 Hz); LCMS (acidic method) $R_t$ 1.78 min, m/z 274.2 $[M+H]^+$.

**1-(2-Fluoro-3-isopropylphenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (20z)**

Prepared by General Method 4.1 with 2-fluoro-3-isopropylaniline. Isolated as an off-white solid (80 mg, 62 %). $^1$H NMR (700 MHz, $CDCl_3$) δ 7.37 (t, *J* = 6.7 Hz, 1H), 7.21 (m, 1H), 7.05 (t, *J* = 6.7 Hz, 1H), 6.45 (s, 1H), 3.29 (m, 1H), 2.30 (s, 3H), 1.98 (s, 3H), 1.30 (dd, *J* = 6.9, 3.0 Hz, 6H); $^{13}$C NMR (176 MHz, $CDCl_3$) δ 170.6 (d, *J* = 4.9 Hz), 156.0 (d, *J* = 250.2 Hz), 138.1, 137.3 (d, *J* = 13.9 Hz), 129.4, 128.0 (d, *J* = 5.2 Hz), 127.4, 125.2 (d, *J* = 14.5

Hz), 124.4 (d, *J* = 4.6 Hz), 111.1, 108.3, 27.4 (d, *J* = 2.0 Hz), 22.7 (d, *J* = 6.9 Hz), 12.3, 12.2; LCMS (acidic method) $R_t$ 1.83 min, m/z 276.2 $[M+H]^+$.

**1-(3-Cyano-2-fluorophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (20aa)**

Prepared by General Method 4.2 with 3-cyano-2-fluoroaniline. Isolated as an off-white solid (58 mg, 48 %). $^1$H NMR (700 MHz, $CDCl_3$) δ 7.78 (ddd, *J* = 7.9, 5.6, 1.7 Hz, 1H), 7.53 (td, *J* = 7.9, 1.7 Hz, 1H), 7.44 (t, *J* = 7.9 Hz, 1H), 6.48 (d, *J* = 1.0 Hz, 1H), 2.31 (s, 3H), 1.99 (s, 3H); $^{13}$C NMR (176 MHz, $CDCl_3$) δ 170.1, 159.0 (d, *J* = 264.4 Hz), 137.7, 135.6, 134.2, 129.2, 126.8 (d, *J* = 11.9 Hz), 125.7 (d, *J* = 4.9 Hz), 112.9, 112.2, 109.3, 103.6 (d, *J* = 14.6 Hz), 12.3, 12.2; LCMS (acidic method) $R_t$ 1.62 min, m/z 259.2 $[M+H]^+$.

**1-(3-Chloro-2-methoxyphenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (20bb)**

Prepared by General Method 4.2 with 3-chloro-2-methoxyaniline. Isolated as an off-white solid (124 mg, 62 %).

Mp 208-210 °C; IR $\nu_{max}$ (film) 1653; 1533, 1271, 1240, 783, 433 $cm^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$) δ 7.51 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.16 (t, *J* = 8.0 Hz, 1H), 7.08 (dd, *J* = 7.9, 1.7 Hz, 1H), 6.45 (d, *J* = 1.0 Hz, 1H), 3.47 (s, 3H), 2.32 (s, 3H), 1.99 (s, 3H); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 169.9, 152.9, 138.0, 131.8, 131.2, 129.5, 129.1, 128.7, 124.4, 111.1, 108.5, 60.4, 12.5, 12.4; LCMS (acidic method) $R_t$ 1.69 min, m/z 280.1 $[M+H]^+$.

**1-(3-Chloro-2-cyanophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (20cc)**

Prepared by General Method 4.2 with 3-chloro-2-cyanoaniline. Isolated as an off-white solid (59 mg, 30 %).

Mp 242-244 °C; IR $\nu_{max}$ (film) 1656, 1533, 1421, 1263, 1238, 783 $cm^{-1}$; $^1$H NMR (500 MHz, DMSO-$d_6$) δ 11.90 (br s, 1H), 7.98 – 7.91 (m, 2H), 7.65 (dd, *J* = 7.2, 1.8 Hz, 1H), 6.30 (app d, *J* = 1.0 Hz, 1H), 2.20 (s, 3H), 1.93 (s, 3H); $^{13}$C NMR (126 MHz, DMSO-$d_6$) δ 171.9, 151.2, 141.6, 136.0, 131.1, 129.5 (2C), 128.5, 113.6, 113.4, 111.3, 108.9, 12.2, 11.9; LCMS (acidic method) $R_t$ 1.57 min, m/z 275.1 $[M+H]^+$.

**1-(3-Chloro-4-(trifluoromethyl)phenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (20dd)**

Prepared by General Methods 2 and 3 with 3-chloro-4-(trifluoromethyl)aniline. Isolated as a white solid (102 mg, 59 %).

Mp 186 °C; IR $\nu_{max}$ (powder) 3459, 2584-3068, 1739, 1441-1645, 1313, 1228, 1029, 776 $cm^{-1}$; $^1$H NMR (700 MHz, Methanol-$d_4$) δ 7.96 (d, *J* = 8.3 Hz, 1H), 7.64 (s, 1H), 7.42 (d, *J* = 8.3 Hz, 1H), 6.34 (s, 1H), 2.29 (s, 3H), 2.00 (s, 3H); $^{13}$C NMR (176 Hz, Methanol-$d_4$) δ 170.2, 143.5, 136.4 (m), 134.1, 132.6, 129.8 (q, *J* = 5 Hz), 129.4, 129.2 (q, *J* = 31 Hz), 128.6, 123.9 (q, *J* = 272 Hz), 110.7, 109.9, 12.5, 12.4; HRMS $C_{14}H_{11}ClF_3NO_2$: calcd. 316.0352 $[M-H]^-$, found 316.0352.

**1-(4-Chloro-3-(trifluoromethyl)phenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (20ee)**

Prepared by General Methods 2 and 3 with 4-chloro-3-(trifluoromethyl)aniline. Isolated as a white solid (21 mg, 49 %).

Mp 178 °C; IR $\nu_{max}$ (powder) 3460, 3080, 2945-3004, 1739, 1324-1533, 1217, 1109, 1035, 794 $cm^{-1}$; $^{1}H$ NMR (700 MHz, Methanol-$d_4$) δ 7.77 (d, *J* = 8.3 Hz, 1H), 7.66 (s, 1H), 7.51 (d, *J* = 8.3 Hz, 1H), 6.29 (s, 1H), 2.25 (s, 3H), 1.96 (s, 3H); $^{13}C$ NMR (176 MHz, $CDCl_3$) 169.3, 137.4, 136.5, 133.1 (m), 132.9, 132.7, 129.9 (q, *J* = 32 Hz), 129.0, 127.5 (q, *J* = 5 Hz), 122.2 (q, *J* = 272 Hz), 111.4, 109.0, 12.8, 12.6; HRMS $C_{14}H_{11}ClF_3NO_2$: calcd. 318.0509 $[M+H]^+$, found 318.0508.

**2-Methyl-1-phenyl-5-(trifluoromethyl)-1*H*-pyrrole-3-carboxylic acid (21)**

Prepared by General Methods 2 and 3. Isolated as a pink solid (35 mg, quant.).

Mp 188 °C; IR $\nu_{max}$ (powder) 3384, 3164, 2852-2920, 2538-2663, 1660, 1444-1568, 1305, 1249, 1118 $cm^{-1}$; $^{1}H$ NMR (700 MHz, $CDCl_3$) δ 7.53-7.48 (m, 3H), 7.29 (d, *J* = 7.5 Hz, 2H), 7.09 (s, 1H), 2.45 (s, 3H); $^{13}C$ (175 MHz, $CDCl_3$) δ 168.3, 140.6, 138.0, 129.8 (2C), 129.2, 126.6 (2C), 123.7 (q, *J* = 7 Hz), 124.5 (q, *J* = 268 Hz), 115.3 (q, *J* = 37 Hz), 109.4, 12.9; HRMS $C_{13}H_{10}F_3NO_2$: calcd. 270.0742 $[M+H]^+$, found 270.0743.

**1-Benzyl-2-methyl-1*H*-pyrrole-3-carboxylic acid (22)**

Prepared by General Methods 5 and 3. Isolated as an amber oil (52 mg, 49 %).

$^{1}H$ NMR (600 MHz, $CDCl_3$) δ 7.34-7.31 (m, 2H), 7.28 (m, 1H), 7.01 (app d, *J* = 7.9 Hz, 2H), 6.66 (d, *J* = 3.1 Hz, 1H), 6.57 (d, *J* = 3.1 Hz, 1H), 5.06 (s, 2H), 2.47 (s, 3H); LCMS (basic method) $R_t$ 0.63 min, m/z 216.2 $[M+H]^+$.

**1-Benzyl-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (23)**

Prepared by General Methods 5 and 3. Isolated as a white solid (91 mg, 61 %).

Mp 199 °C; $^{1}H$ NMR (400 MHz, Methanol-$d_4$) δ 7.33-7.29 (m, 2H), 7.24 (m, 1H), 6.92-6.89 (m, 2H), 6.28 (app d, *J* = 0.8 Hz, 1H), 5.13 (s, 2H), 2.41 (s, 3H), 2.11 (s, 3H); LCMS (acidic method) Rt 1.60 min, m/z 230.2 $[M+H]^+$.

**1-Benzyl-2-methyl- 5-(trifluoromethyl)-1*H*-pyrrole-3-carboxylic acid (24)**

Prepared by General Methods 5 and 3. Isolated as a white solid (67 mg, 96 %).

Mp 202 °C; IR $\nu_{max}$ (film) 3386, 3016-3030, 2854-2970, 2582-2806, 1739, 1313-1669, 1216, 1129, 746 $cm^{-1}$; $^{1}H$ NMR (700 MHz, Methanol-$d_4$) δ 7.35 (t, *J* = 7.4 Hz, 2H), 7.28 (t, *J* = 7.4 Hz, 1H), 7.18 (s, 1H), 7.07 (d, *J* = 7.4 Hz, 2H), 5.18 (s, 2H), 2.40 (s, 3H); $^{13}C$ NMR (175 MHz, Methanol-$d_4$) δ 167.3, 139.7, 138.0, 129.9 (2C), 128.8, 127.6 (2C), 124.6 (q, *J* = 265 Hz), 124.2 (q, *J* = 6.5 Hz), 114.9 (q, *J* = 37 Hz), 111.5, 51.5, 11.4; HRMS $C_{14}H_{12}F_3NO_2$: calcd. 282.0742 $[M-H]^-$, found 282.0737.

**1-(3-Chlorophenyl)pyrrolidine-3-carboxylic acid (25a)**

Prepared by General Methods 6.1 and 7 with 1-chloro-3-iodobenzene. Isolated as a white gum (71 mg, 36 %). IR $\nu_{max}$ (film) 3100, 2923, 2849, 1700, 1592, 755 $cm^{-1}$; $^1H$ NMR (600 MHz, DMSO-$d_6$) δ 7.13 (t, *J* = 8.1 Hz, 1H), 6.57 (app dd, *J* = 7.7, 1.7, Hz, 1H), 6.48 (app t, *J* = 2.0 Hz, 1H), 6.45 (dd, *J* = 8.1, 2.0 Hz, 1H), 3.27-3.18 (m, 4H), 3.02 (quin, *J* = 7.0 Hz, 1H), 2.12 (q, *J* = 7.0 Hz, 2H); $^{13}C$ NMR (151 MHz, DMSO-$d_6$) δ 175.3, 148,7, 133.8, 130.4, 114.5, 110.8, 110.3, 50.4, 47.0, 40.0, 28.7; HRMS $C_{11}H_{12}ClNO_2$: calcd 226.0629 $[M+H]^+$, found 226.0629.

**1-(4-Chlorophenyl)pyrrolidine-3-carboxylic acid (25b)**

Prepared by General Methods 6.1 and 7 with 1-chloro-4-iodobenzene. Isolated as a brown gum (44 mg, 92 %). IR $\nu_{max}$ (film) 3100, 2923, 2849, 1700, 1592, 755 $cm^{-1}$; $^1H$ NMR (600 MHz, DMSO-$d_6$) δ 7.15-7.13 (m, 2H), 6.40-6.46 (m, 2H), 3.36 (dd, *J* = 9.3, 6.3 Hz, 1H), 3.31 (dd, *J* = 9.3, 8.0 Hz, 1H), 3.23-3.19 (m, 1H), 3.17-3.13 (m, 1H), 2.92 (quin, *J* = 7.2 Hz, 1H), 2.15 - 2.05 (m, 2H); HRMS $C_{11}H_{12}ClNO_2$: calcd 226.0629 $[M+H]^+$, found 226.0629.

**1-(3-Methylphenyl)pyrrolidine-3-carboxylic acid (25c)**

Prepared by General Methods 6.1 and 7 with 1-iodo-3-methylbenzene. Isolated as a yellow solid (68 mg, 29 %).

Mp 105 °C; IR $\nu_{max}$ (film) 3036, 2920, 2835, 1690, 1600 $cm^{-1}$; $^1H$ NMR (700 MHz, DMSO-$d_6$) δ 12.45 (s, 1H), 7.02 (t, *J* = 7.7 Hz, 1H), 6.42 (d, *J* = 7.4 Hz, 1H), 6.35 (app s, 1H), 6.33 (dd, *J* = 8.0, 2.2 Hz, 1H), 3.40 (dd, *J* = 9.3, 8.0 Hz, 1H), 3.36 (dd, *J* = 9.3, 6.2 Hz, 1H), 3.25 (m, 1H), 3.21 (m, 1H), 3.15 (m, 1H), 2.22 (s, 3H), 2.17 (m, 1H), 2.12 (m, 1H); $^{13}C$ NMR (151 MHz, DMSO-$d_6$) δ 175.0, 147.6, 137.9, 128.8, 116.5, 112.5, 109.2, 49.9, 46.9, 42.4, 28.12, 21.5; HRMS $C_{12}H_{15}NO_2$: calcd 206.1176 $[M+H]^+$, found 206.1176.

**1-(3-(Trifluoro)phenyl)pyrrolidine-3-carboxylic acid (25d)**

Prepared by General Methods 6.1 and 7 using 1-iodo-3-(trifluoromethyl)benzene. Isolated as a yellow solid (113 mg, 53 %).

Mp 101 °C; IR $\nu_{max}$ (film) 2849, 1703, 1608, 782; $^1H$ NMR (700 MHz, DMSO-$d_6$) δ 12.51 (s, 1H), 7.35 (t, *J* = 7.9 Hz, 1H), 6.88 (d, *J* = 7.5 Hz, 1H), 6.80 (dd, *J* = 8.3, 2.1, Hz, 1H), 6.71 (app s, 1H), 3.48 (dd, *J* = 9.5, 8.0 Hz, 1H), 3.43 (dd, *J* = 9.5, 6.0 Hz, 1H), 3.35 - 3.27 (m, 2H), 3.20 (m, 1H), 2.22 (m, 1H), 2.16 (m, 1H); $^{13}C$ NMR (151 MHz, DMSO-$d_6$) δ 174.7, 147.5, 129.9, 129.9 (q, *J* = 31 Hz), 124.6 (q, *J* = 272 Hz), 115.41, 111.4 (q, *J* = 4 Hz), 107.3 (q, *J* = 4 Hz), 49.8, 46.9, 42.4, 28.2; HRMS $C_{12}H_{12}F_3NO_2$: calcd 260.0893 $[M+H]^+$, found 260.0904.

**1-(4-(Trifluoro)phenyl)pyrrolidine-3-carboxylic acid (25e)**

Prepared by General Methods 6.1 and 7 with 1-iodo-4-(trifluoromethyl)benzene. Isolated as an off-white solid (73 mg, 33 %).

Mp 105 °C; IR $\nu_{max}$ (film) 3047, 2911, 2849, 1710, 1612, 815 $cm^{-1}$; $^1H$ NMR (700 MHz, DMSO-$d_6$) δ 12.52 (s, 1H), 7.44 (d, *J* = 8.8 Hz, 2H), 6.63 (d, *J* = 8.8 Hz, 2H), 3.50 (dd, *J* = 9.7, 7.8 Hz, 1H), 3.45 (dd, *J* = 9.7, 6.3 Hz, 1H), 3.36-3.29 (m, 2H) 3.20 (m, 1H), 2.22 (m, 1H), 2.16 (m, 1H); $^{13}C$ NMR (176 MHz, DMSO-$d_6$) δ 174.6, 149.6, 126.2 (q, *J* = 4 Hz, 2C), 125.5 (q, *J* = 270 Hz), 115.1 (q, *J* = 33 Hz), 111.3 (2C), 49.6, 46.8, 42.4, 28.2; HRMS $C_{12}H_{12}F_3NO_2$: calcd 260.0893 $[M+H]^+$, found 260.0899.

**1-(3-Isopropylphenyl)pyrrolidine-3-carboxylic acid (25f)**

Prepared by General Methods 6.1 and 7 with 1-iodo-3-isopropylbenzene. Isolated as a yellow oil (52.1 mg, 16 %).

IR $\nu_{max}$ (film) 3040, 2957, 2866, 1703, 1601 $cm^{-1}$; $^1H$ NMR (600 MHz, DMSO-$d_6$) δ 12.49 (s, 1H), 7.06 (t, *J* = 7.7 Hz, 1H), 6.50 (d, *J* = 7.7 Hz, 1H), 6.39 (t, *J* = 1.9 Hz, 1H), 6.35 (ddd, *J* = 8.1, 2.4, 0.6 Hz, 1H), 3.43 (dd, *J* = 9.2, 8.1 Hz, 1H), 3.38 (dd, *J* = 9.2, 6.0 Hz, 1H), 3.30 - 3.21 (m, 2H), 3.16 (m, 1H), 2.79 (sept, *J* = 6.9 Hz, 1H), 2.22 - 2.10 (m, 2H), 1.18 (d, *J* = 6.9 Hz, 6H); $^{13}C$ NMR (151 MHz, DMSO-$d_6$) δ 175.0, 149.2, 147.6, 128.9, 113.6, 109.9, 109.5, 49.9, 46.9, 42.4, 33.9, 28.2, 24.1 (2C); HRMS $C_{14}H_{19}NO_2$: calcd 234.1489 $[M+H]^+$, found 234.1487.

**1-(3-(Trifluoromethoxy)phenyl)pyrrolidine-3-carboxylic acid (25g)**

Prepared by General Methods 6.1 and 7 with 1-iodo-3-(trifluoromethoxy)benzene. Isolated as an amber oil (90.8 mg, 40 %).

IR $\nu_{max}$ (film) 3082, 2911, 2860, 1699, 1615, 822 $cm^{-1}$; $^1H$ NMR (700 MHz, DMSO-$d_6$) δ 12.50 (s, 1H), 7.23 (t, *J* = 8.2 Hz, 1H), 6.53 (dd, *J* = 8.3, 2.3 Hz, 1H), 6.51 (d, *J* = 8.3 Hz, 1H), 6.39 (app s, 1H), 3.44 (dd, *J* = 9.6, 7.8 Hz, 1H), 3.39 (dd, *J* = 9.6, 6.0 Hz, 1H), 3.29 - 3.23 (m, 2H), 3.18 (m, 1H), 2.20 (m, 1H), 2.14 (m, 1H); $^{13}C$ NMR (176 MHz, DMSO-$d_6$) δ 174.7, 149.6 (q, *J* = 1.6 Hz), 148.8, 130.4, 120.2 (q, *J* = 256 Hz), 110.7, 106,8, 103.8, 49.8, 46.9, 42.4, 28.2; HRMS $C_{12}H_{12}F_3NO_3$: 276.0842 $[M+H]^+$, found 276.0841.

**1-(3-Cyanophenyl)pyrrolidine-3-carboxylic acid (25h)**

Prepared by General Methods 6.1 and 7 with 3-iodobenzonitrile. Isolated as an off-white solid (72 mg, 27 %).

Mp 139 °C; IR $\nu_{max}$ (film) 3038, 2915, 2850, 1702, 1554 $cm^{-1}$; $^1H$ NMR (700 MHz, DMSO-$d_6$) δ 12.53 (s, 1H), 7.32 (app t, *J* = 8.0 Hz, 1H), 6.97 (app d, *J* = 7.5 Hz, 1H), 6.88 (t, *J* = 2.2 Hz, 1H), 6.84 (dd, *J* = 8.3, 2.2 Hz, 1H), 3.46 (dd, *J* = 9.6, 7.9 Hz, 1H), 3.41 (td, *J* = 9.6, 6.0 Hz, 1H), 3.34 - 3.26 (m, 2H), 3.19 (m, 1H), 2.21 (m, 1H), 2.14 (m, 1H); $^{13}C$ NMR (176 MHz, DMSO-$d_6$) δ 174.7, 147.4, 130,1, 119.6, 118.5, 116.4, 114.1, 111.8, 49.7, 46.8, 42.4, 28.2; LCMS (acidic method) Rt 1.53 min, m/z 217.2 $[M+H]^+$.

**1-(4-Fluorophenyl)pyrrolidine-3-carboxylic acid (25i)**

Prepared by General Methods 6.1 and 7 with 1-fluoro-4-iodobenzene. Isolated as a brown solid (51.3 mg, 32 %).

Mp 105 °C; IR $\nu_{max}$ (film) 3031, 2910, 2852, 1697, 1518, 809 $cm^{-1}$; $^1$H NMR δ (600 MHz, DMSO-$d_6$) 12.50 (s, 1H), 7.02-6.98 (m, 2H), 6.54-6.50 (m, 2H), 3.40 (dd, *J* = 9.3, 8.1 Hz, 1H), 3.36 (dd, *J* = 9.3, 6.1 Hz, 1H), 3.27 - 3.15 (m, 3H), 2.19 (m, 1H), 2.13 (m, 1H); $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 175.0, 154.2 (d, *J* = 231 Hz), 144.5, 115.4 (d, *J* = 22 Hz, 2C), 112.5 (d, *J* = 7.2 Hz, 2C), 50.3, 47.4, 42.5, 28.3 ppm; HRMS $C_{11}H_{12}FNO_2$: calcd 210.0925 $[M+H]^+$, found 210.0927.

**1-(2,3-Dichlorophenyl)pyrrolidine-3-carboxylic acid (25j)**

Prepared by General Methods 6.1 and 7 with 1,2-dichloro-3-iodobenzene. Isolated as an orange solid (84 mg, 50 %).

IR $\nu_{max}$ (film) 3074, 2946, 2855, 1699, 1578, 775 $cm^{-1}$; $^1$H NMR (600 MHz, DMSO-$d_6$) δ 12.50 (s, 1H), 7.20 (t, *J* = 8.2 Hz, 1H), 7.10 (dd, *J* = 7.9, 1.4, Hz, 1H), 6.96 (dd, *J* = 8.2, 1.4, Hz, 1H), 3.56 (dd, *J* = 9.6, 6.4 Hz, 1H), 3.48 (dd, *J* = 9.6, 7.9 Hz, 1H), 3.37 (m, 1H), 3.31 (m, 1H), 3.11 (quin, *J* = 7.3 Hz, 1H), 2.15 (m, 1H), 2.08 (m, 1H); $^{13}$C NMR (151 MHz, DMSO-$d_6$) 174.8, 148.4, 133.0, 128.1, 121.3, 120.6, 116.4, 53.2, 50.2, 42.3, 28.1; HRMS $C_{11}H_{11}Cl_2NO_2$: calcd 260.0240 $[M+H]^+$, found 260.0240.

**1-(2,4-Dichlorophenyl)pyrrolidine-3-carboxylic acid (25k)**

Prepared by General Methods 6.1 and 7 with 2,4-dichloro-1-iodobenzene. Isolated as a brown oil (69 mg, 38 %).

IR $\nu_{max}$ (film) 3030, 2951, 2840, 1703, 1475, 800 $cm^{-1}$; $^1$H NMR (600 MHz, DMSO-$d_6$) δ 12.50 (s, 1H), 7.42 (d, *J* = 2.5 Hz, 1H), 7.24 (dd, *J* = 8.8, 2.5, Hz, 1H), 6.95 (d, *J* = 8.8 Hz, 1H), 3.54 (dd, *J* = 9.5, 6.6 Hz, 1H), 3.47 (dd, *J* = 9.5, 7.8 Hz, 1H), 3.34 (m, 1H), 3.29 (m, 1H), 3.11 (quin, *J* = 7.4 Hz, 1H), 2.14 (m, 1H), 2.07 (m, 1H); $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 174.8, 145.3, 130.0, 127.5, 123.3, 122.7, 118.7, 53.0, 50.0, 42.3, 28.0; HRMS $C_{11}H_{11}NO_2Cl_2$: calcd 260.0240 $[M+H]^+$, found 260.0241.

**1-(2,5-Dichlorophenyl)pyrrolidine-3-carboxylic acid (25l)**

Prepared by General Methods 6.1 and 7 using 1,4-dichloro-2-iodobenzene. Isolated as an orange oil (88 mg, 39 %).

IR $\nu_{max}$ (film) 3038, 2953, 2848, 1704, 1584, 785 $cm^{-1}$; $^1$H NMR (600 MHz, DMSO-$d_6$) δ 12.51 (s, 1H), 7.31 (d, *J* = 8.4 Hz, 1H), 6.91 (d, *J* = 2.4 Hz, 1H), 6.85 (dd, *J* = 8.4, 2.4 Hz, 1H), 3.59 (dd, *J* = 9.6, 6.5 Hz, 1H), 3.52 (dd, *J* = 9.6, 7.7 Hz, 1H), 3.40-3.31 (m, 2H), 3.11 (app quin, *J* = 7.3, 1H), 2.14 (m, 1H), 2.07 (m, 1H); $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 174.7, 147.3, 132.2 (2C), 120.2, 119.7, 116.8, 52.9, 49.9, 42.4, 28.1; HRMS $C_{11}H_{11}Cl_2NO_2$: calcd 260.0240 $[M+H]^+$, found 260.0240.

**1-(2,6-Dichlorophenyl)pyrrolidine-3-carboxylic acid (25m)**

Prepared by General Methods 6.1 and 7 using 1,3-dichloro-2-iodobenzene. Isolated as a brown oil (82 mg, 42 %).

IR $\nu_{max}$ (film) 3028, 2915, 2850, 1702, 1554, 775 $cm^{-1}$; $^{1}H$ NMR (600 MHz, DMSO-$d_6$) δ 12.41 (s, 1H), 7.45 (d, *J* = 8.1 Hz, 2H), 7.22 – 7.17 (m, 1H), 3.46 (t, *J* = 8.2 Hz, 1H), 3.41 (dd, *J* = 8.3, 7.0 Hz, 1H), 3.34 – 3.30 (m, 1H), 3.27 (q, *J* = 7.3 Hz, 1H), 3.20 – 3.10 (m, 1H), 2.20 – 2.13 (m, 2H); $^{13}C$ NMR (151 MHz, DMSO-$d_6$) δ 174.9, 141.6, 135.6, 129.4, 127.6, 52.1, 49.3, 43.5, 29.0; HRMS $C_{11}H_{11}NO_2Cl_2$: calcd 260.0240 $[M+H]^+$, found 260.0241.

**1-(3,4-Dichlorophenyl)pyrrolidine-3-carboxylic acid (25n)**

Prepared by General Methods 6.1 and 7 with 1,2-dichloro-4-iodobenzene. Isolated as an off-white solid (70.8 mg, 36 %).

Mp 138 °C; IR $\nu_{max}$ (film) 3300, 2954, 2846, 1700, 1596, 804 $cm^{-1}$; $^{1}H$ NMR (700 MHz, DMSO-$d_6$) δ 12.51 (s, 1H), 7.33 (d, *J* = 8.9 Hz, 1H), 6.70 (d, *J* = 2.8 Hz, 1H), 6.52 (dd, *J* = 8.9, 2.8 Hz, 1H), 3.47 – 3.41 (m, 1H), 3.41 – 3.36 (m, 1H), 3.30 – 3.23 (m, 2H), 3.21 – 3.15 (m, 1H), 2.24 – 2.10 (m, 2H); $^{13}C$ NMR (176 MHz, DMSO-$d_6$) δ 174.6, 147.1, 131.4, 130.4, 116.4, 112.6, 112.1, 49.8, 47.0, 42.4, 28.2; HRMS $C_{11}H_{11}Cl_2NO_2$: calcd 260.0240 $[M+H]^+$, found 260.0240.

**1-(3,5-Dichlorophenyl)pyrrolidine-3-carboxylic acid (25o)**

Prepared by General Methods 6.1 and 7 with 1,3-dichloro-5-iodobenzene. Isolated as a brown solid (63.9 mg, 32 %).

Mp 135 °C; $^{1}H$ NMR (600 MHz, DMSO-$d_6$) δ 12.56 (s, 1H), 6.68 (apparent t, *J* = 1.8 Hz, 1H), 6.52 (d, *J* = 1.8 Hz, 2H), 3.43 (ddd, *J* = 15.9, 9.9, 7.0 Hz, 2H), 3.32 – 3.24 (m, 2H), 3.22 – 3.14 (m, 1H), 2.25 – 2.10 (m, 2H); $^{13}C$ NMR (151 MHz, DMSO-$d_6$) δ 174.6, 149.0, 134.5, 114.1, 110.0, 49. 8, 46.9, 42.3, 28.2; HRMS $C_{11}H_{11}Cl_2NO_2$: 260.0240 $[M+H]^+$, found 260.0240.

**1-(3-Chloro-4-(trifluoromethyl)phenyl)pyrrolidine-3-carboxylic acid (25p)**

Prepared by General Methods 6.1 and 7 with 3-chloro-4-(trifluoromethyl)-1-iodobenzene except purification by reverse phase chromatography (0-95% MeCN:$H_2O$; 0.1% $NH_4OH$ modifier). Isolated as a white solid (7 mg, 7 %).

$^{1}H$ NMR (700 MHz, DMSO-$d_6$) δ 7.47 (d, *J* = 8.9 Hz, 1H), 6.60 (d, *J* = 2.1 Hz, 1H), 6.48 (dd, *J* = 8.9, 2.1 Hz, 1H), 3.47 (dd, *J* = 9.4, 6.1 Hz, 1H), 3.30 (m, 2H), 3.26 (dd, *J* = 14.7, 8.2 Hz, 1H), 3.21 (dd, *J* = 16.1, 7.3 Hz, 1H), 2.75 – 2.67 (m, 1H), 2.13 (td, *J* = 14.1, 7.1 Hz, 1H), 1.99 (td, *J* = 12.9, 7.1 Hz, 1H); $^{13}C$ NMR (176 MHz, DMSO-$d_6$) δ 175.4,

150.6, 131.7, 128.3 (q, *J* = 5.0 Hz), 124.2 (q, *J* = 269.8 Hz), 112.5, 111.1 (d, *J* = 31.2 Hz), 109.2, 51.6, 47.3, 46.0, 29.5; LCMS (acidic method) $R_t$ 1.89 min, m/z 294.1 $[M+H]^+$.

**1-(4-Chloro-3-(trifluoromethyl)phenyl)pyrrolidine-3-carboxylic acid (25q)**

Prepared by General Methods 6.1 and 6.2, and then 7 with 2-chloro-4-iodo-1-(trifluoromethyl)benzene. Isolated as an off-white solid (84 mg, 47 %).

Mp 158 °C; IR $\nu_{max}$ (film) 3047, 2915, 2861, 1707, 1608, 1130, 812 $cm^{-1}$; $^1H$ NMR (700 MHz, DMSO-$d_6$) δ 12.53 (s, 1H), 7.41 (d, *J* = 8.8 Hz, 1H), 6.81 (d, *J* = 2.9 Hz, 1H), 6.78 (dd, *J* = 8.8, 2.8 Hz, 1H), 3.51 – 3.47 (m, 1H), 3.46 – 3.42 (m, 1H), 3.36 – 3.27 (m, 2H), 3.23 – 3.17 (m, 1H), 2.26 – 2.20 (m, 1H), 2.19 – 2.13 (m, 1H); $^{13}C$ NMR (176 MHz, DMSO-$d_6$) δ 174.6, 146.0, 131.9, 126.9 (q, *J* = 30.0 Hz), 123.1 (q, *J* = 273.1 Hz), 116.2 (s), 115.3, 109.7 (q, *J* = 5.5 Hz), 49.8, 47.0, 42.4, 28.2; HRMS $C_{12}H_{11}ClF_3NO_2$: calcd 294.0503 $[M+H]^+$, found 294.0503.

**1-(4-Fluoro-3-(trifluoromethyl)phenyl)pyrrolidine-3-carboxylic acid (25r)**

Prepared by General Methods 6.1 and 7 with 1-fluoro-4-iodo-2-(trifluoromethyl)benzene. Isolated as an oil (88 mg, 49 %).

IR $\nu_{max}$ (film) 3014, 2965, 2853, 1706, 1514, 812 $cm^{-1}$; $^1H$ NMR (700 MHz, DMSO-$d_6$) δ 12.50 (s, 1H), 7.28 (t, *J* = 9.8 Hz, 1H), 6.85 – 6.79 (m, 1H), 6.69 (dd, *J* = 5.7, 3.1 Hz, 1H), 3.48 – 3.45 (m, 1H), 3.43 – 3.39 (m, 1H), 3.34 – 3.25 (m, 2H), 3.23 – 3.17 (m, 1H), 2.27 – 2.19 (m, 1H), 2.18 – 2.12 (m, 1H); $^{13}C$ NMR (176 MHz, DMSO-$d_6$) δ 174.7, 150.9 (d, *J* = 2.0 Hz), 149.5 (d, *J* = 2.1 Hz), 144.2, 123.0 (q, *J* = 272.0 Hz), 117.6 (d, *J* = 21.2 Hz), 117.0 – 116.2 (m), 108.0 (q, *J* = 4.8 Hz), 50.1, 47.2, 42.4, 28.3; HRMS $C_{12}H_{11}F_4NO_2$: calcd 278.0799 $[M+H]^+$, found 278.0798.

**1-(2-Fluoro-3-(trifluoromethyl)phenyl)pyrrolidine-3-carboxylic acid (25s)**

Prepared by General Methods 6.1 and 7 with 2-fluoro-1-iodo-3-(trifluoromethyl)benzene. Isolated as an oil (90 mg, 59 %).

IR $\nu_{max}$ (film) 3051, 2915, 2853, 1704, 1498, 780 $cm^{-1}$; $^1H$ NMR (700 MHz, DMSO-$d_6$) δ 12.51 (s, 1H), 7.17 (t, *J* = 8.0 Hz, 1H), 7.03 (t, *J* = 8.3 Hz, 1H), 6.96 (t, *J* = 6.5 Hz, 1H), 3.57 (dd, *J* = 7.0, 2.7 Hz, 2H), 3.48 – 3.37 (m, 2H), 3.15 (p, *J* = 7.1 Hz, 1H), 2.20 – 2.14 (m, 1H), 2.14 – 2.08 (m, 1H); $^{13}C$ NMR (176 MHz, DMSO-$d_6$) δ 174.7, 147.9 (dd, *J* = 249.3, 2.0 Hz), 137.3 (d, *J* = 7.8 Hz), 124.0 (d, *J* = 3.5 Hz), 123.1 (q, *J* = 272.3 Hz), 120.2 (d, *J* = 5.8 Hz), 117.5 (qd, *J* = 31.3, 11.1 Hz), 113.6 (q, *J* = 4.7 Hz), 51.9 (d, *J* = 5.7 Hz), 49.0 (d, *J* = 4.8 Hz), 42.2 (d, *J* = 1.4 Hz), 28.0 (d, *J* = 1.1 Hz); LCMS (acidic method) $R_t$ 1.79 min, m/z 278.1 $[M+H]^+$.

**1-(2,3,4-Trichlorophenyl)pyrrolidine-3-carboxylic acid (25t)**

Prepared by General Methods 6.1 and 7 with 1,2,3-trichloro-4-iodobenzene. Isolated as an orange solid (56 mg, 53 %).

Mp 111 °C; IR $\nu_{max}$ (film) 3058, 2920, 2852, 1580, 1702, 789 $cm^{-1}$; $^{1}H$ NMR (600 MHz, DMSO-$d_6$) δ 12.52 (s, 1H), 7.44 (d, *J* = 9.1 Hz, 1H), 6.99 (d, *J* = 9.1 Hz, 1H), 3.61 – 3.55 (m, 1H), 3.52 – 3.45 (m, 1H), 3.41 – 3.30 (m, 2H), 3.17 – 3.05 (m, 1H), 2.19 – 2.11 (m, 1H), 2.11 – 2.04 (m, 1H); $^{13}C$ NMR (151 MHz, DMSO-$d_6$) δ 174.7, 147.3, 131.2, 128.4, 122.4, 121.8, 116.8, 53.2, 50.2, 42.3, 28.1; HRMS $C_{11}H_{10}Cl_3NO_2$: calcd 293.9850 $[M+H]^+$, found 293.9851.

**(*S*)-1-(4-Chloro-3-(trifluoromethyl)phenyl)pyrrolidine-3-carboxylic acid (26)**

Prepared by General Methods 6.1 and 7. Isolated as an off-white solid (258 mg, 54 %) (SFC chemical purity 99.78 %).

Mp 155 °C; IR $\nu_{max}$ (film) 3059, 2915, 2861, 1708, 1608, 812 $cm^{-1}$; $^{1}H$ NMR (700 MHz, DMSO-$d_6$) δ 12.53 (s, 1H), 7.41 (d, *J* = 8.8 Hz, 1H), 6.81 (d, *J* = 2.9 Hz, 1H), 6.78 (dd, *J* = 8.8, 2.9 Hz, 1H), 3.49 (dd, *J* = 9.6, 8.0 Hz, 1H), 3.44 (dd, *J* = 9.6, 6.1 Hz, 1H), 3.36 – 3.27 (m, 2H), 3.24 – 3.17 (m, 1H), 2.23 (dtd, *J* = 13.0, 7.4, 5.7 Hz, 1H), 2.19 – 2.12 (m, 1H); $^{13}C$ NMR (176 MHz, DMSO-$d_6$) δ 174.6, 146.0, 132.0, 126.9 (q, *J* = 30.0 Hz), 123.2 (q, *J* = 273.1 Hz), 116.3, 115.3, 109.7 (q, *J* = 5.5 Hz), 49.9, 47.0, 42.4, 28.3; LCMS (acidic method) $R_t$ 1.90 min, m/z 294.1 $[M+H]^+$. $[\alpha]_D^{20}$ +0.29 (c 1, $CHCl_3$); Chiral SFC analysis: *R*:*S* 1.5:98.5; 97.0 enantiomeric excess.

**(*R*)-1-(4-Chloro-3-(trifluoromethyl)phenyl)pyrrolidine-3-carboxylic acid (27)**

Prepared by General Methods 6.1 and 7. Isolated as a white solid (291 mg, 61 %)(SFC chemical purity 99.80 %).

Mp 157 °C. IR, $^{1}H$ NMR (700 MHz, DMSO-$d_6$) and $^{13}C$ NMR (176 MHz, DMSO-$d_6$) were consistent with **26**. LCMS (acidic method) $R_t$ 1.86 min, m/z 294.1 $[M+H]^+$.

Chiral SFC analysis: *R*:*S* 98.2:1.8; 96.4 enantiomeric excess.

**1-(4-Chloro-3-(trifluoromethyl)phenyl)-*N*-methylpyrrolidine-3-carboxamide (28a)**

Prepared by General Method 8 with methylamine. Isolated as a colourless gum (37 mg, 71 % yield).

IR $\nu_{max}$ (film) 3310, 2917, 2824, 1638, 1566, 797 $cm^{-1}$; $^{1}H$ NMR (600 MHz, DMSO-$d_6$) δ 7.99 (q, *J* = 4.5 Hz, 1H), 7.41 (d, *J* = 8.8 Hz, 1H), 6.79 (d, *J* = 2.8 Hz, 1H), 6.76 (dd, *J* = 8.8, 2.9 Hz, 1H), 3.47 – 3.43 (m, 1H), 3.41 – 3.25 (m, 3H), 3.05 (p, *J* = 7.6 Hz, 1H), 2.60 (d, *J* = 4.5 Hz, 3H), 2.21 – 2.11 (m, 1H), 2.11 – 1.97 (m, 1H); $^{13}C$ NMR (151 MHz, DMSO-$d_6$) δ 172.5, 146.0, 131.9, 126.8 (q, *J* = 29.9 Hz), 123.2 (q, *J* = 273.1 Hz), 116.2, 115.1 – 115.0 (m), 109.6 (q, *J* = 5.6 Hz), 50.5, 47.3, 43.2, 29.0, 25.7; HRMS $C_{13}H_{14}ClF_3N_2$: calcd 307.0820 $[M+H]^+$, found 307.0820.

**1-(4-Chloro-3-(trifluoromethyl)phenyl)-*N*-(2-methoxyethyl)pyrrolidine-3-carboxamide (28b)**

Prepared by General Method 8 with 2-methoxyethylamine. Isolated as a white solid (49 mg, 82%).

Mp 112 °C ; IR $\nu_{max}$ (film) 3297, 2915, 2827, 1642, 1117, 801 $cm^{-1}$; 1H NMR (600 MHz, DMSO-$d_6$) δ 8.14 (t, *J* = 5.4 Hz, 1H), 7.41 (d, *J* = 8.8 Hz, 1H), 6.79 (d, *J* = 2.8 Hz, 1H), 6.76 (dd, *J* = 8.8, 2.8 Hz, 1H), 3.45 (t, *J* = 8.7 Hz, 1H), 3.40 – 3.21 (m, 10H), 3.14 – 3.06 (m, 1H), 2.20 – 2.12 (m, 1H), 2.11 – 1.99 (m, 1H); $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 172.3, 146.1, 131.9, 126.8 (q, *J* = 29.8 Hz), 123.2 (q, *J* = 273.1 Hz), 116.2, 115.1, 109.6 (q, *J* = 5.5 Hz), 70.6, 58.0, 50.5, 47.3, 43.1, 38.5, 29.1; HRMS $C_{15}H_{18}ClF_3N_2O_2$: calcd 351.1082 $[M+H]^+$, found 351.1082.

**1-(4-Chloro-3-(trifluoromethyl)phenyl)-*N*-phenylpyrrolidine-3-carboxamide (28c)**

Prepared by General Method 8 with aniline. Isolated as an off-white solid (54 mg, 86 %).

Mp 115 °C; IR $\nu_{max}$ (film) 3296, 2864, 1658, 1434, 1114, 753 $cm^{-1}$; $^1$H NMR (600 MHz, DMSO-$d_6$) δ 10.13 (s, 1H), 7.61 (d, *J* = 7.6 Hz, 2H), 7.43 (d, *J* = 8.8 Hz, 1H), 7.30 (t, *J* = 7.9 Hz, 2H), 7.04 (q, *J* = 7.6 Hz, 1H), 6.83 (d, *J* = 2.8 Hz, 1H), 6.80 (dd, *J* = 8.9, 2.8 Hz, 1H), 3.63 – 3.53 (m, 1H), 3.51 – 3.39 (m, 2H), 3.39 – 3.26 (m, 2H), 2.36 – 2.24 (m, 1H), 2.25 – 2.12 (m, 1H); $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 171.2, 146.1, 139.1, 132.0, 131.8 – 131.6 (m), 128.8, 128.2 (q, *J* = 11.8 Hz), 126.9 (q, *J* = 30.0 Hz), 123.3, 123.2 (q, *J* = 273.2 Hz), 119.2, 116.2, 115.2, 109.7 (q, *J* = 5.6 Hz), 50.5, 47.3, 44.2, 29.2; HRMS $C_{18}H_{17}ClF_3N_2O$: calcd 369.0981 $[M+H]^+$, found 369.0976.

**1-(4-Chloro-3-(trifluoromethyl)phenyl)-*N,N*-dimethylpyrrolidine-3-carboxamide (28d)**

Prepared by General Method 8 with dimethylamine. Isolated as an orange gum (29 mg, 53 %).

IR $\nu_{max}$ (film) 2932, 2855, 1640, 1115 $cm^{-1}$; $^1$H NMR (600 MHz, DMSO-$d_6$) δ 7.42 (d, *J* = 8.8 Hz, 1H), 6.81 (d, *J* = 2.9 Hz, 1H), 6.78 (dd, *J* = 8.8, 2.9 Hz, 1H), 3.57 – 3.48 (m, 2H), 3.40 – 3.27 (m, 3H), 3.07 (s, 3H), 2.84 (s, 3H), 2.25 – 2.14 (m, 1H), 2.12 – 1.99 (m, 1H); $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 171.9, 146.1, 131.9, 126.8 (q, *J* = 30.0 Hz), 123.2 (q, *J* = 273.2 Hz), 116.2, 115.1, 109.7 (q, *J* = 4.8 Hz), 50.4, 47.3, 36.8, 35.2, 28.6; LCMS (basic method) $R_t$ 1.98 min, m/z 321.1 $[M+H]^+$.

**(1-(4-Chloro-3-(trifluoromethyl)phenyl)pyrrolidin-3-yl)(3,3-difluoroazetidin-1-yl)methanone (28e)**

Prepared by General Method 8 with 3,3-difluoroazetidine. Isolated as a white solid (62 mg, 99 % yield).

Mp 104 °C; IR $\nu_{max}$ (film) 2929, 2849, 1654, 1110; $^1$H NMR (600 MHz, DMSO-$d_6$) δ 7.42 (d, *J* = 8.8 Hz, 1H), 6.82 (d, *J* = 2.9 Hz, 1H), 6.79 (dd, *J* = 8.8, 2.9 Hz, 1H), 4.85 – 4.62 (m, 2H), 4.31 (t, *J* = 12.6 Hz, 2H), 3.52 (dd, *J* = 9.2, 8.3 Hz, 1H), 3.41 – 3.36 (m, 1H), 3.35 – 3.21 (m, 3H), 2.26 – 2.16 (m, 1H), 2.16 – 2.00 (m, 1H); $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 172.6, 146.0, 132.0, 126.9 (q, *J* = 30.1 Hz), 123.2 (q, *J* = 273.1 Hz), 116.3, 116.2 (t, *J* = 272.1 Hz), 115.3, 109. 8 (q, *J* = 5.5 Hz), 61.3 (t, *J* = 28.0 Hz), 59.5 (t, *J* = 27.8 Hz), 49.9, 47.2, 28.1; HRMS $C_{15}H_{14}ClF_5N_2O$: calcd 369.0788 $[M+H]^+$, found 369.0787.

**(1-(4-Chloro-3-(trifluoromethyl)phenyl)pyrrolidin-3-yl)(pyrrolidin-1-yl)methanone (28f)**

Prepared by General 8 with pyrrolidine. Isolated as a yellow gum (51 mg, 87 %).

IR $\nu_{max}$ (film) 2977, 2873, 1632, 1129, 847 $cm^{-1}$; $^{1}H$ NMR (700 MHz, DMSO-$d_6$) δ 7.41 (d, *J* = 8.8 Hz, 1H), 6.81 (d, *J* = 2.9 Hz, 1H), 6.78 (dd, *J* = 8.8, 2.9 Hz, 1H), 3.58 – 3.48 (m, 3H), 3.42 – 3.33 (m, 3H), 3.33 – 3.26 (m, 3H), 2.25 – 2.17 (m, 1H), 2.16 – 2.04 (m, 1H), 1.90 (p, *J* = 6.7 Hz, 2H), 1.78 (p, *J* = 6.9 Hz, 2H); $^{13}C$ NMR (176 MHz, DMSO-$d_6$) δ 170.3, 146.1, 131.9, 126.8 (q, *J* = 30.0 Hz), 123.2 (q, *J* = 273.1 Hz), 116.2, 115.1, 110.5 – 109.3 (m), 50.2, 47.3, 45.9, 45.6, 41.4, 28.3, 25.6, 23.8; LCMS (basic method) $R_t$ 1.95 min, m/z 347.1 $[M+H]^+$.

**(1-(4-Chloro-3-(trifluoromethyl)phenyl)pyrrolidin-3-yl)(morpholin-4-yl)methanone (28g)**

Prepared by General Method 8 with morpholine. Isolated as a clear gum (54 mg, 87 %).

IR $\nu_{max}$ (film) 2965, 2856, 1639, 1112 $cm^{-1}$; $^{1}H$ NMR (700 MHz, DMSO-$d_6$) δ 7.42 (d, *J* = 8.8 Hz, 1H), 6.81 (d, *J* = 2.9 Hz, 1H), 6.78 (dd, *J* = 8.8, 2.9 Hz, 1H), 3.64 – 3.43 (m, 8H), 3.41 – 3.33 (m, 2H), 3.41 – 3.33 (m, 2H), 3.30 (dd, *J* = 9.1, 7.4 Hz, 1H), 2.18 (dtd, *J* = 12.3, 7.2, 5.1 Hz, 1H), 2.15 – 2.06 (m, 1H); $^{13}C$ NMR (176 MHz, DMSO-$d_6$) δ 170.7, 146.1, 131.9, 126.8 (q, *J* = 30.0 Hz), 123.1 (q, *J* = 273.1 Hz), 116.2, 115.1, 109.7 (q, *J* = 5.4 Hz), 66.2 (d, *J* = 26.1 Hz), 50.3, 47.2, 45.5, 41.9, 28.5; HRMS $C_{16}H_{18}ClF_3N_2O_2$: calcd 363.1082 $[M+H]^+$, found 363.1081.

**(1-(4-Chloro-3-(trifluoromethyl)phenyl)pyrrolidin-3-yl)(tetrahydro-1*H*-furo[3,4-*c*]pyrrol-5(3*H*)-yl)methanone (28h)**

Prepared by General Method 8 with hexahydro-1*H*-furo[3,4-*c*]pyrrole. Isolated as a clear gum (63.0 mg, 95 %).

IR $\nu_{max}$ (film) 2962, 2863, 1635, 1114 $cm^{-1}$; $^{1}H$ NMR (600 MHz, DMSO-$d_6$) δ 7.42 (d, *J* = 8.8 Hz, 1H), 6.81 (d, *J* = 2.6 Hz, 1H), 6.80 – 6.76 (m, 1H), 3.84 – 3.71 (m, 3H), 3.61 – 3.45 (m, 5H), 3.40 – 3.33 (m, 3H), 3.33 – 3.25 (m, 2H), 3.02 – 2.94 (m, 1H), 2.90 – 2.83 (m, 1H), 2.24 – 2.14 (m, 1H), 2.13 – 2.03 (m, 1H); $^{13}C$ NMR (151 MHz, DMSO-$d_6$) δ 170.3 (d, *J* = 3.4 Hz), 146.1, 131.9, 126.8 (q, *J* = 30.0 Hz), 123.2 (q, *J* = 273.0 Hz), 116.3, 115.1, 109.7 (q), 73.0 (d, *J* = 7.1 Hz), 72.7 (d, *J* = 10.4 Hz), 50.5 (d, *J* = 3.5 Hz), 50.2, 50.2 (d, *J* = 8.2 Hz), 47.3 (d, *J* = 2.1 Hz), 43.5, 41.6, 41.3, 28.4 (d, *J* = 13.1 Hz); LCMS (basic method) $R_t$ 1.90 min, m/z 389.1 $[M+H]^+$.

**(1-(4-Chloro-3-(trifluoromethyl)phenyl)pyrrolidine-3-carbonyl)-3-methylimidazolidin-4-one (28i)**

Prepared by General Method 8 with 3-methylimidazolidin-4-one. Isolated as a clear gum (47 mg, 74 %).

IR $\nu_{max}$ (film) 2956, 2868, 1702, 1634, 1113 $cm^{-1}$; $^{1}H$ NMR (600 MHz, DMSO-$d_6$) δ rotamers 7.48 – 7.40 (m, 1H), 6.88 – 6.81 (m, 1H), 6.81 – 6.73 (m, 1H), 5.04 (dd, *J* = 16.7, 5.4 Hz, 0.5H), 4.73 (s, 1.5H), 4.22 (q, *J* = 14.9 Hz, 1.5H), 3.84 (s, 0.5H), 3.59 – 3.54 (m, 1H), 3.45 – 3.23 (m, 4H), 2.86 – 2.75 (m, 3H), 2.31 – 2.19 (m, 1H), 2.18 – 2.09 (m, 1H); $^{13}C$ NMR (151 MHz, DMSO-$d_6$) δ rotamers 170.5, 169.9, 167.2, 167.0, 146.0, 132.0, 131.9, 126.9 (q, *J* = 30.0 Hz), 123.2 (q, *J* = 273.1 Hz), 116.3, 109.8 (q, *J* = 5.8 Hz), 63.7, 50.1, 49.9, 47.8, 47.4, 47.3, 47.2, 41.0, 28.2, 27.9, 27.11, 27.08; HRMS $C_{16}H_{17}ClF_3N_3O_2$: calcd 376.1040 $[M+H]^+$, found 376.1029.

**(1-(4-Chloro-3-(trifluoromethyl)phenyl)pyrrolidine-3-carbonyl)-1-methylpiperazin-2-one (28j)**

Prepared by General Method 8 with 1-methylpiperazin-2-one. Isolated as a white solid (64 mg, 97 %).

IR $\nu_{max}$ (film) 2954, 2847, 1636, 1132 $cm^{-1}$; $^1H$ NMR (700 MHz, DMSO-$d_6$) δ 7.42 (dd, *J* = 8.8, 3.8 Hz, 1H), 6.86 – 6.80 (m, 1H), 6.80 – 6.76 (m, 1H), 4.30 – 4.13 (m, 1H), 4.01 (s, 1H), 3.93 – 3.77 (m, 1H), 3.77 – 3.66 (m, 1H), 3.66 – 3.45 (m, 2H), 3.45 – 3.25 (m, 5H), 2.87 (s, 2H), 2.28 – 2.04 (m, 3H); $^{13}C$ NMR (176 MHz, DMSO-$d_6$) δ rotamers 170.8, 170.6, 164.6, 164.2, 146.0, 131.9, 126.8 (q, *J* = 29.9 Hz), 123.1 (q, *J* = 273.1 Hz), 116.2, 115.2, 115.1, 109.7 (q, *J* = 5.5 Hz), 50.3, 50.2, 48.4, 47.9, 47.2, 47.1, 45.9, 41.8, 38.5, 33.7, 33.5, 28.5, 28.4; LCMS (basic method) $R_t$ 1.80 min, m/z 390.2 $[M+H]^+$; HRMS $C_{17}H_{19}ClF_3N_3O_2$: calcd 390.1191 $[M+H]^+$, found 390.1190.

**2-(1-(4-Chloro-3-(trifluoromethyl)phenyl)pyrrolidin-3-yl)- 1,3,4-oxadiazole (29)**

Hydrazine monohydrate (0.05 mL, 1.0 mmol) was added to a thick-walled reaction vial (20 mL) containing a solution of methyl 1-[4-chloro-3-(trifluoromethyl)phenyl]pyrrolidine-3-carboxylate (100 mg, 0.30 mmol) in EtOH (2.0 mL) and PhMe (1.0 mL). The vial was then sealed and heated to 80 °C for 16 h. The reaction mixture was concentrated under reduced pressure, azeotroped with EtOH-PhMe (1:1, 4 x 20 mL) and 1-(4-chloro-3-(trifluoromethyl)phenyl)pyrrolidine-3-carbohydrazide was isolated as an off-white solid, which was carried forward without further purification.

Triethyl orthoformate (0. 52 mL, 3.4 mmol) was added to a thick-walled reaction vial (10 mL) containing 1-[4-chloro-3-(trifluoromethyl)phenyl]pyrrolidine-3-carbohydrazide (0.76 mmol) and *p*-toluenesulfonic acid monohydrate (0.08 mmol) under $N_2$ and the mixture warmed to 60 °C for 1 h. The cooled mixture was diluted with saturated aq. $NaHCO_3$ (5 mL), extracted with $CH_2Cl_2$ (x3), and the combined organic extracts dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by column chromatography (0-5% MeOH:$CH_2Cl_2$) to give **29** as a yellow oil (125 mg, 52 %).

IR $\nu_{max}$ (film) 2920, 2855, 1609, 1114, 808 $cm^{-1}$; $^1H$ NMR (600 MHz, DMSO-$d_6$) δ 9.21 (s, 1H), 7.44 (d, *J* = 8.8 Hz, 1H), 6.89 – 6.81 (m, 2H), 4.00 – 3.90 (m, 1H), 3.76 (dd, *J* = 9.7, 7.6 Hz, 1H), 3.60 (dd, *J* = 9.8, 6.0 Hz, 1H), 3.48 – 3.38 (m, 2H), 2.51 – 2.42 (m, 1H), 2.36 – 2.25 (m, 1H); $^{13}C$ NMR (151 MHz, DMSO-$d_6$) δ 166.7, 154.7, 145.9, 132.0, 126.9 (q, *J* = 30.1 Hz), 123.2 (q, *J* = 273.1 Hz), 116.5, 115.6, 109.9 (q, *J* = 5.6 Hz), 50.7, 46.8, 34.4, 29.1; HRMS $C_{13}H_{11}ClF_3N_3O$: calcd 318.0616 $[M+H]^+$, found 318.0610.

**5-(1-(4-Chloro-3-(trifluoromethyl)phenyl)pyrrolidin-3-yl)-1,3,4-oxadiazol-2(3*H*)-one (30)**

To a solution of 1-(4-chloro-3-(trifluoromethyl)phenyl)pyrrolidine-3-carbohydrazide (0.30 mmol) and DIPEA (0.11 mL, 0.65 mmol) in $CH_2Cl_2$ (5 mL) at 0 °C was added dropwise a solution of triphosgene (39 mg, 0.10 mmol) in $CH_2Cl_2$ (5 mL). The reaction mixture was warmed to RT and stirred for 10 minutes. The reaction was diluted with sat. aq. $NaHCO_3$ solution (2 mL) and the organic phase were separated, dried ($MgSO_4$) and

concentrated under reduced pressure. The residue was and dissolved in the minimum volume of DMSO and purified by column chromatography (0-100% MeCN:$H_2O$, 0.1 % $NH_4OH$ modifier) to give **30** as a white solid (104 mg, 34 %).

Mp 98 °C; IR $\nu_{max}$ (film) 3166, 2900, 2850, 1771, 1608, 1115, 808 $cm^{-1}$; $^1H$ NMR (600 MHz, DMSO-$d_6$) δ 12.20 (s, 1H), 7.44 (d, *J* = 8.8 Hz, 1H), 6.85 (d, *J* = 2.8 Hz, 1H), 6.82 (dd, *J* = 8.8, 2.8 Hz, 1H), 3.65 – 3.57 (m, 2H), 3.57 – 3.48 (m, 1H), 3.45 – 3.35 (m, 2H), 2.36 – 2.28 (m, 1H), 2.26 – 2.16 (m, 1H); $^{13}C$ NMR (151 MHz, DMSO-$d_6$) δ 157.2, 155.0, 145.9, 132.0, 126.9 (q, *J* = 30.0 Hz), 123.2 (q, *J* = 273.2 Hz), 116.4, 115.6, 109.9 (q, *J* = 5.5 Hz), 49.7, 46.7, 35.3, 28.0; HRMS $C_{13}H_{11}ClF_3N_3O_2$: calcd 334.0570 $[M+H]^+$, found 334.0564.

**Notum protein expression and purification**

Methods have been described in detail in references 20 and 21**.** In brief, human Notum (UniProtKB ID: Q6P988) enzyme core sequence comprising amino acids S81–T451 with a C330S mutation was cloned into a stable cell line vector pNeo_sec. A stable HEK293S GNTI- cell line was obtained and used for protein production as described previously. The cells were expanded and grown in roller bottles (Greiner). The conditioned media were dialyzed and passed through a 5 ml HisTrap Excel column (GE Healthcare), followed by 20 mM imidazole PBS wash. Notum protein was eluted with 300 mM imidazole PBS. To remove flexible glycans, the protein was deglycosylated with endo-β-N-acetylglucosaminidase F1 (37°C, 1 hour) and further purified by size-exclusion chromatography (Superdex 200 16/60 column, GE Healthcare) in 10 mM Hepes, pH 7.4, 150 mM NaCl buffer.

**Notum OPTS biochemical assay**

Methods have been described in detail in reference 20**.** A Labcyte Echo 550 acoustic liquid handler was used to dispense 500 nL of test compound into 384-well plates (Greiner catalog #781076). 25 µL of 2 µM trisodium 8-octanoyloxypyrene-1,3,6-trisulfonate (OPTS, Sigma #74875) solution in 50 mM Tris, 5 mM $CaCl_2$, 0.5 mM $MgCl_2$, pH 7.4 assay buffer was added to each well, followed by 25 µL of 2.38 nM Notum carboxyesterase enzyme solution. Plates were incubated for 40 minutes at room temperature, and endpoint fluorescence was measured on a PheraSTAR FSX microplate reader with an excitation wavelength of 485 nm and emission wavelength of 520 nm. Plates were quality controlled based on a signal window greater than three-fold between DMSO and positive control compound, and a Z prime greater than 0.5.

Dose-response curve analysis: Plate layout and measured fluorescence values were input into Dotmatics Studies data management tool. After implementing Quality Control with a cut-off of Z prime values higher than 0.5, compound $IC_{50}$ values were calculated from curves using a 4PL fit and compared between technical replicates. Final potency values implied an $n \geq 2$ with $IC_{50}$ within 0.5-2x between replicates.

Data analysis: $IC_{50}$ values presented in Figure 3 and Tables 2-5 are arithmetic mean ± SD (n = 2-10).

**In vitro ADME screens**

Selected compounds were screened for aqueous solubility, transit performance in MDCK-MDR1 cell lines for permeability, and metabolic stability in mouse liver microsomes as a measure of clearance. Assay protocols and additional data is presented in the Supporting Information Figure S4.

ADME studies reported in this work were performed by GVK Biosciences (Hyderabad, India).
GVK Biosciences, India. See: https://www.gvkbio.com/discovery-services/biology-services/dmpk-services/

## ASSOCIATED CONTENT

### Supporting Information

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

Acid fragment library of 250 compounds (catalogues numbers and SMILES). X-ray structure determination: PDB codes, data collection and refinement. Chemical structures for inactive pyrrole and pyrrolidine-3-carboxylic acids present in the acid fragment library. Inhibition of Notum activity of additional pyrroles and pyrrolidines. Spectroscopic and analytical data for (*S*)-**26**. ADME protocols and data. Notum protein production, crystallization, compound soaking, data collection and structure determination (PDF).

Molecular formula strings (CSV)

### Accession Codes

Coordinates for X-ray structures of Notum crystallized with **5** (6YUY), **6** (6YV4), **7** (6YUW), **8** (6YV2), **10** (6YV0), **20i** (6YXI) and **26** (6YSK) have been deposited in the Protein data Bank.

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### Notes

The authors declare no competing financial interest.

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**ABBREVIATIONS**

AD, Alzheimer's disease; ADME, absorption distribution metabolism elimination; CHI, chromatographic hydrophobicity index; CNS, central nervous system; CRC, colorectal cancer; DIPEA, *N,N*-diisopropylethylamine; DMF, *N,N*-dimethylformamide; DMSO, dimethylsulfoxide; ER, efflux ratio; FZD, Frizzled receptors; HATU, (1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate; HPLC, high performance liquid chromatography; LE, ligand efficiency; LLE, lipophilic ligand efficiency; MLM, mouse liver microsomes; MPO, multiparameter optimization; OPTS, trisodium 8-octanoyloxypyrene-1,3,6-trisulfonate; P-gp, P-glycoprotein; PMT, post-translational modifications; RT, room temperature; SAR, structure activity relationship; SBDD, structure based drug design; SFC, supercritical fluid chromatography; THF, tetrahydrofuran; TPSA, topological polar surface area.

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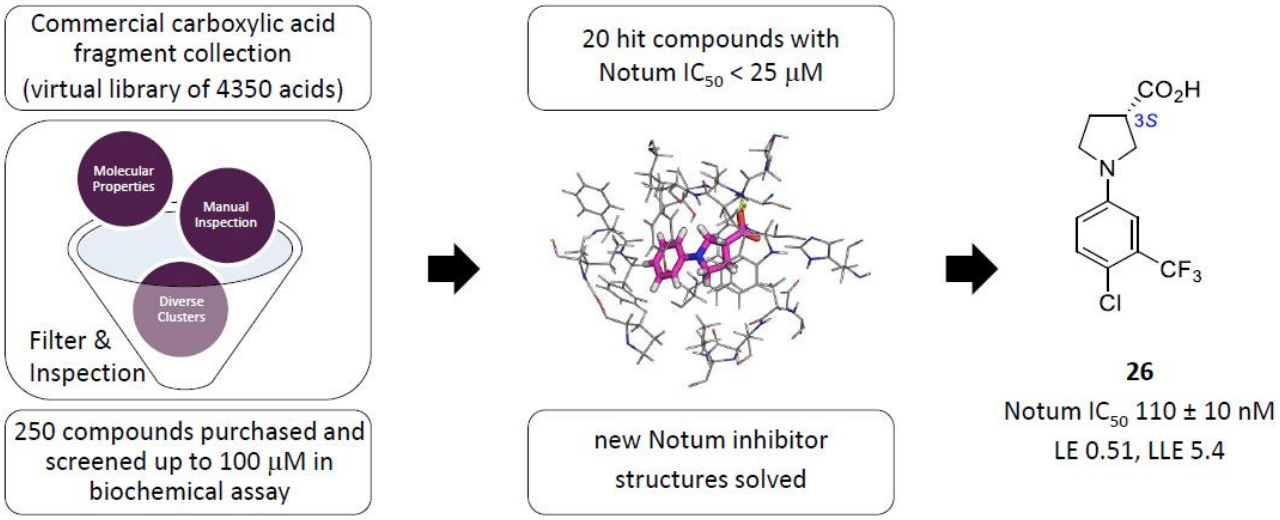
**Table of Contents graphic**

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