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The synthesis and evaluation of indolylureas as PKCα inhibitors

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ABSTRACT

PKCα and PKA have crucial but opposing roles in the regulation of calcium handling within myocytes. Identification of compounds that inhibit PKCα, but not PKA, are potential therapeutic targets for the treatment of heart disease. The synthesis of indolylureas are described, and a compound displaying nanomolar inhibition towards PKCα with significant selectivity over PKA has been identified.

1. Introduction

Heart failure is one of the leading causes[1] of morbidity and mortality in developed nations. In the US, 5 million[2] people suffer from heart disease with over 700,000 new cases presenting every year. Over 1 million hospitalizations/year result from this condition in the US, with an estimated cost of $30–40 billion/annum.[2b,c,3] The mechanism of heart failure progression involves multiple causes, including hypertension, atherosclerosis and a variety of risk factors which lead to cardiac hypertrophy, impaired calcium handling by cardiomyocytes and ultimately heart failure.[4] While this disease is well characterised[5] and several therapies are available for treatment, patients' outcomes remain poor with a five-year mortality rate of approximately 50%.

A hallmark of heart failure is depressed cardiac function due to impaired calcium handling within the cells of the heart or cardiomyocytes.[6] The goal of several experimental therapies is to improve calcium handling and thus, restore the heart's function (inotropic/lusitropic therapy[7]) which would be beneficial in patients with emergent or acute heart failure. Recent evidence has revealed that PKCα and PKA play crucial, but opposing roles in the regulation of calcium handling within myocytes.[8a–d] The function of PKCα in cardiomyocytes appears to be to reduce calcium cycling capacity within the myocyte, leading to a reduction in cell function (impaired contraction and relaxation), in part via dephosphorylation of phospholamban[8a] and inhibition of the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA) or SR Ca^{2+} pump. This decrease in SERCA activity prolongs the time for cytosolic calcium removal (decreased cardiac relaxation time) and may lead to elevated cytosolic calcium concentrations (increased diastolic tension). In turn, as less cytosolic calcium is sequestered by SERCA, less calcium is available for subsequent contractions, diminishing contractile force. In the chronic setting, there is evidence that PKCα may be a key intracellular signalling kinase involved in activating signalling pathways which promote cardiomyocyte hypertrophy.[9] These data supported by reports[10] demonstrating that PKCα activity/expression is increased in the human failing heart, provide evidence that inhibition of this kinase may provide therapeutic benefit to heart failure patients.

A number of PKC inhibitors have been prepared[11] several of which have progressed to the clinical trial stage[12a–d] for the treatment of various disorders. Among them, the bis-indolylmaleimides **A** (Ruboxistaurin [LY333531], macular edema, diabetic kidney disease, PKCβ), **B** (Enzastaurin [LY317615], malignant glioma, pancreatic, colorectal and non-small cell lung cancer, PKCβ), **C** (Midostaurin [PKC412], acute myeloid leukaemia, aggressive systemic mastocytosis and mast cell leukaemia, PKCα, β, γ) and

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D (AEB071,[12e] transplant rejection, psoriasis, PKCα, β, θ) are noteworthy (Fig. 1). The role of PKC isoforms in disease processes have been the subject of a number of recent reviews.[12f–j]

Staurosporine is a broad spectrum kinase inhibitor, binding tightly to the ATP pocket.[13] We used the structure of staurosporine as a starting point to design a template that mimics some of the structural features of this molecule with the goal of achieving selectivity for the ATP binding pocket of PKCα over PKA. It was envisaged that indolylureas of type **1** would have the potential to form conformationally restricted structures via an intramolecular H-bond (Fig. 2), assuming a three dimensional structure with similarities to staurosporine. This paper describes our efforts to identify PKCα inhibitors based upon indolylurea template **1** in a programme focused on the development of new therapies for heart failure.

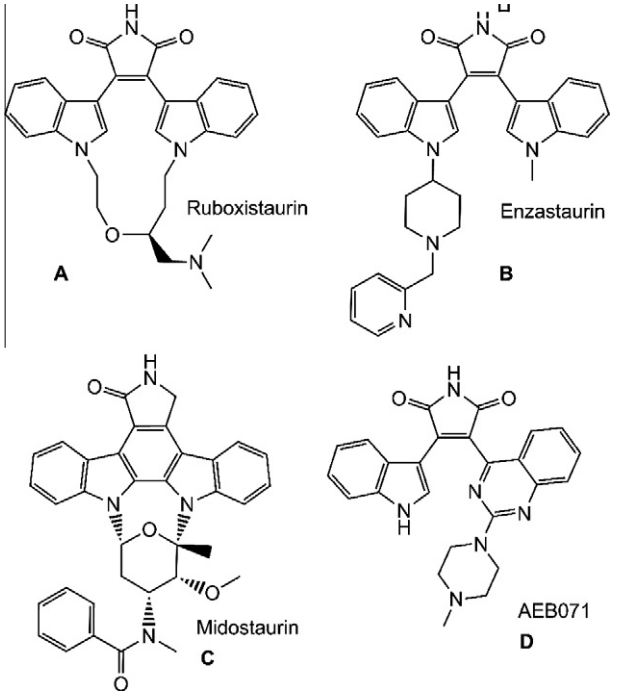


Figure 1. Bis-indolylmaleimide derived PKC inhibitors progressed to clinical trial stage.

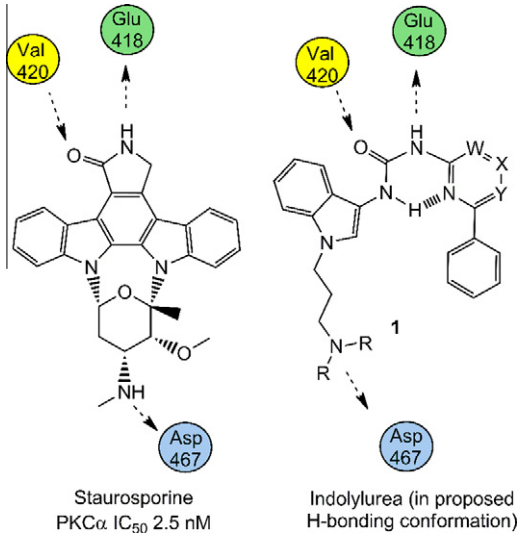
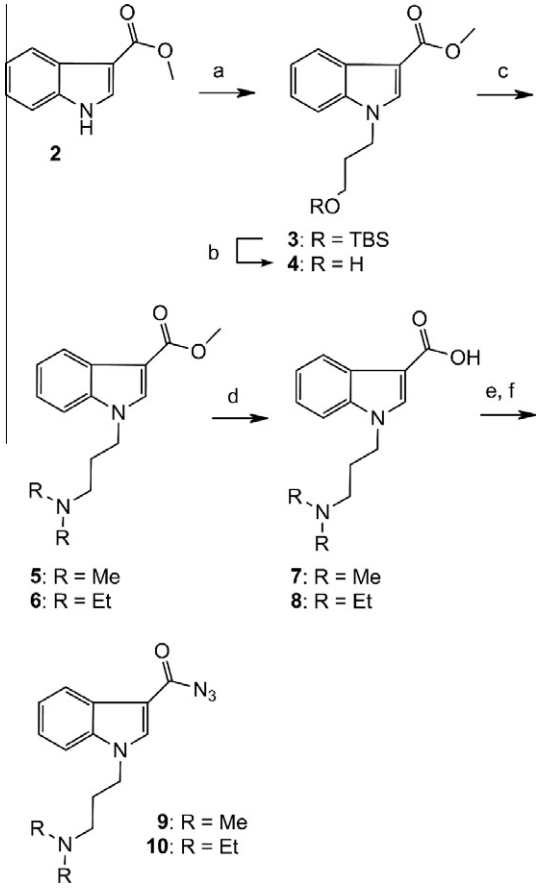


Figure 2. Representation of staurosporine docked into the homology model of PKCα (showing key interactions) and design intention for indolylureas **1** (in proposed intramolecular H-bonding conformation) to make the same amino acid contacts.

2. Chemistry

The preparation of 3-indolylureas have been reported via Curtius rearrangement of indolyl-3-carbonyl azides[14] followed by addition of amines to the isocyanate, and it was hoped that this methodology would allow access to indolylureas of type **1**. Preparation of the acyl azide precursor (Scheme 1) commenced with the alkylation of methyl indole-3-carboxylate **2** with 3-(*tert*-butyldimethylsilyloxy)-1-bromopropane to surrender N-alkylated indole **3** in quantitative yield. A fluoride mediated deprotection of the TBS group furnished alcohol **4**, which was subsequently converted to the amines **5** and **6** via the *O*-triflate intermediate. Hydrolysis of the methyl ester group of **5** and **6** gave the corresponding amino acids **7** and **8** after neutralisation and evaporation. These amino acids were very hygroscopic and difficult to purify away from inorganic impurities without significant loss of material. Although the amino acids were isolated at neutral pH, observed ^{1}H NMR spectra were found to be very sensitive to the pH of isolation making it difficult to obtain consistent NMR spectra between different batches of the same compound. Conversion of the amino acids **7** and **8** to acyl azides **9** and **10** via the acid chloride derivatives was straightforward, and the acyl azide was found to be sufficiently stable and



Scheme 1. Preparation of acyl azides **9** and **10**. Reagents and conditions: (a) $TBSO(CH_2)_3Br$, K_2CO_3, DMF, 50 °C, 24 h, 100%; (b) TBAF, THF, rt, 93%; (c) Tf_2O, 2,6-lutidine, DCM, 0 °C then Me_2NH or Et_2NH, 87% (**5**), 59% (**6**); (d) NaOH, MeOH, H_2O, 60 °C, 44% (**7**), 100% (**8**); (e) $(COCl)_2$, DCM, rt, 4 h (f) NaN_3 aq, THF, 0 °C, 61% (**9**), 50% (**10**).

soluble in organic solvents to allow an aqueous workup to remove any inorganic impurities carried through from the ester hydrolysis step.

Preparation of biarylanilines **12**, **13**, **14** and **15** was undertaken by a heteroaromatic cyclisation, or by Suzuki coupling to a preformed aminoheterocyclic halide. Biarylaniline **12** was isolated in good yield from a two-step synthesis commencing from acetophenone (Scheme 2) according to literature precedents.[15a,b]

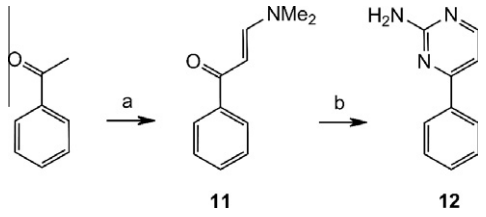
Biarylanilines **13**, **14** and **15** were prepared via Suzuki coupling (Scheme 3) with phenylboronic acid from commercially available 2-amino-6-chloropyridine and 2-amino-6-chloropyrazine. 4-Amino-2-chloropyrimidine was prepared according to literature precedent.[16] The Suzuki conditions employed[17] furnished the desired biarylanilines in good yield and circumvented the requirement for amino group protection often necessary when carrying out Suzuki couplings with substrates bearing basic NH functionality.

Having isolated the precursor acyl azides **9** and **10**, and biarylanilines **12**–**15**, preparation of the indolylureas was attempted. The Curtius rearrangement of both **9** and **10** was found to be facile at 100 °C in anhydrous toluene (Scheme 4), and the isocyanate formed proved sufficiently stable to allow reaction monitoring by LC–MS (mobile phase: water/acetonitrile gradient). Complete conversion to isocyanate was observed after 30–60 min at this temperature, whereupon the appropriate biarylaniline was introduced and heating continued for a further 2 h. The ureas **16**–**19** were then isolated chromatographically (silica-gel chromatography or prep-LC).

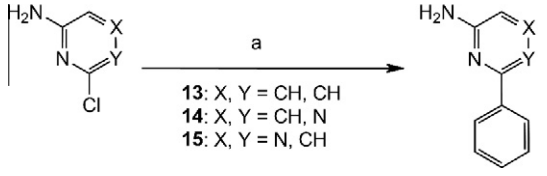
3. Results and discussion

The ureas **16**–**19** were screened for inhibition of PKCα and PKA (Table 1). Evaluation of PKA inhibition in the primary screen reflects the importance of this kinase in enhancing calcium transients within cardiomyocytes. Hence, selective inhibition of PKCα over PKA was a crucial hurdle to overcome for progression of compounds suitable for further biological evaluation. The primary screen was an isolated enzyme mass spectrometry (MS) assay[18a] evaluating the ability of test compounds to inhibit the phosphorylation of a pseudo-substrate peptide by the respective kinases.

During the course of our investigations to discover PKCα inhibitors, molecular modelling was used to evaluate new templates for



Scheme 2. Preparation of biarylaniline **12** from acetophenone. Reagents and conditions: (a) *N*,*N*-dimethylformamide dimethyl acetal, 115 °C, 20 h, 74%; (b) $(NH_2)_2CNH.HCl$, NaOEt, EtOH, rfx, 20 h, 84%.



Scheme 3. Preparation of biarylanilines **13**, **14** and **15** by Suzuki coupling. Reagents and conditions: (a) $PhB(OH)_2$, K_3PO_4, $Pd(OAc)_2$ cat., D*t*BPF cat., 1,4-dioxane, 100 °C, 82% (**13**), 36% (**14**), 55% (**15**).

synthesis, and as a tool to explain observed activity trends. In the absence of a publicly available crystal structure of human PKCα, it was necessary to construct a homology model. The 2.0 Å structure of the PKCθ isoform[19] was used as a template for this purpose and sequence alignment was performed in MOE.[20] The overall sequence homology was found to be 60%, but in the ATP binding pocket this homology value increased to 80%. Model building and refinement were also performed in MOE. Twenty-five intermediate models of PKCα were constructed using a Boltzman-weighted randomised modelling procedure[21a,b] and each minimised to a gradient of 0.1 using the MOE implementation of the AMBER94 forcefield.[22] The staurosporine ligand from the PKCθ structure was retained and fixed in the active site during this process in order to maintain the shape of the cavity. Each intermediate was scored with MOE's residue packing function and the highest ranking model selected. This was further optimised with the AMBER94 forcefield to remove unfavourable stereochemistry and atom contacts.

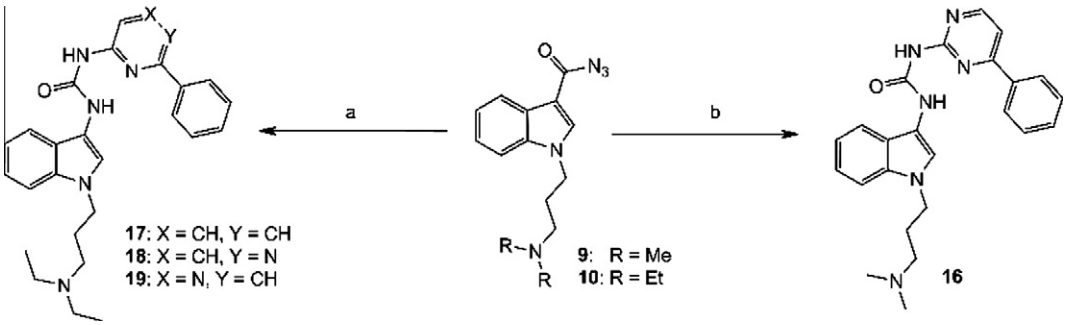
The urea compounds **16**–**19** were prepared for docking in MOE. Protonation states were assigned using in-house EVOwash software and a low energy 3D conformer generated for each molecule using the MOE implementation of the MMFF94x force field.[23] All molecules were docked into the hinge region[24] of the PKCα homology model with GOLD v3.0.1,[25] using the ChemScore[26] scoring function, with the active site defined as a 12 Å sphere centred in the binding pocket. The 20 highest scoring poses for each molecule were retained and clustered at an RMSD of 1.5 Å to remove redundancy in the set of conformations for each ligand.

This docking study suggested a possible explanation for the observed PKCα activity profile. Our initial assumption that the urea carbonyl would form an H-bond with Val420 (NH) and the urea N^3H would H-bond with Glu418 (C:O) in a manner analogous to staurosporine proved invalid (Supplementary data, Figs. 7 and 8 show key domain contact representations of staurosporine and **16** after docking into the PKCα homology model to illustrate the relative shift of **16** in the binding pocket). As illustrated for urea **16** (Figs. 4 and 5), the homology model suggests H-bonds exist between N^3H and Val420 (C:O) and the urea carbonyl to Val420 (NH), with the phenyldiazine unit orientated out of the ATP binding pocket rather than occupying the hydrophobic pocket according to our initial assumption. In this binding orientation an (ionic) interaction between ammonium N^5 and Asp467 carboxylate can still occur (as it could if bound in a staurosporine like mode), but additional interactions between diazine N^1 and Tyr419 and a possible π-stacking effect between the phenyl ring and Tyr427 may stabilise the 4-phenylpyrimidine group in this conformation whilst maintaining an intramolecular (N^2–N^4H) H-bond to the urea. The alternative 4-phenylpyrimidine conformation in which pyrimidine N^1 is H-bonded to urea N^4H orientates the phenyl towards the lower part of the ATP pocket (i.e., towards Asp467) which contains several carboxylate residues (Asp424 and Asp481). The electrostatic repulsion between these residues and the π-system may disfavour this conformation.

Ureas **17**–**19** can also adopt the same preferred conformation as **16**, but at the expense of the intramolecular H-bonding between urea N^4H and the pyridine/diazine ring nitrogen N^1. The alternative conformation (as shown in Fig. 3) in which intramolecular H-bonding to urea N^4H can occur, brings the phenyl groups of **17**–**19** into juxtaposition with carboxylate residues Asp424 and Asp467, resulting in repulsive interactions, and loss of H-bonding to Tyr419.

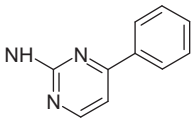
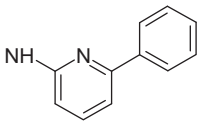
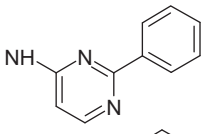
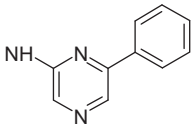
Hence in the optimum orientation shown in Figure 5, urea **16** is stabilised by two H-bonds (intramolecular[27] N^2–N^4H and intermolecular N^1–Tyr419), whereas ureas **17**–**19** are stabilised by a single N^1 intermolecular H-bond to Tyr 419.

Compounds **17**–**19** also show distortion of the planarity of the biaryl group with respect to the indolylurea moiety, presumably



Scheme 4. Preparation of indolylureas via Curtius rearrangement of acylazides **9** and **10**. Reagents and conditions: (a) (i) **10**, toluene, 100 °C, 60 min; (ii) **13**, **14** or **15**, 2 h, 100 °C, 11% (**17**), 6% (**18**), 3% (**19**); (b) (i) **9**, toluene, 100 °C, 30 min; (ii) **12**, 2 h, 100 °C, 33%.

Table 1
In vitro inhibition of PKCα and PKA by indolylureas **16**–**19**

Compound	Biaryl substituent	PKCα IC_{50}[a] (nM)	PKA IC_{50}[a] (nM)	PKA/PKCα
16		95	23550	248
17		2685	nt[b]	
18		4810	nt	
19		8070	nt	
Staurosporine[c]		3	33	11

[a] Data collected in triplicate from three successive days for a total of nine dose curves per test compound ($n = 9$). Estimated error of ±10 nM associated with PKCα assay used for compounds **16**–**19**.
[b] nt: not tested.
[c] IC_{50} determination of staurosporin (PKCα and PKA) using this MS assay, discussion of errors and comparison to literature values have been described.[18a]

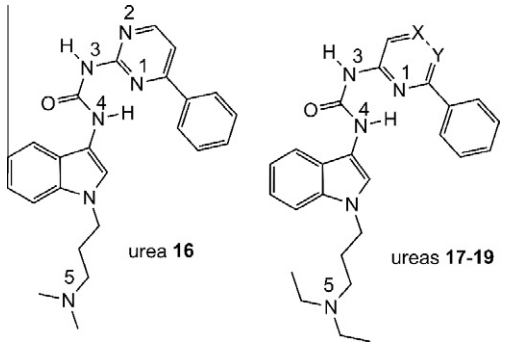


Figure 3. Numbering of nitrogen atoms in ureas **16**–**19** for discussion purposes.

due to repulsive interactions between the aromatic C–H and N4–H in the preferred binding conformation (Fig. 6). The effect of this change can be observed by comparison of **16** (near planar orientation of phenylpyrimidine group with respect to the indolylurea moiety imposed by the N4–H to N2 H-bond) and **17** (repulsive aryl C–H N4–H steric clash, skewing the phenylpyridine group 20° out

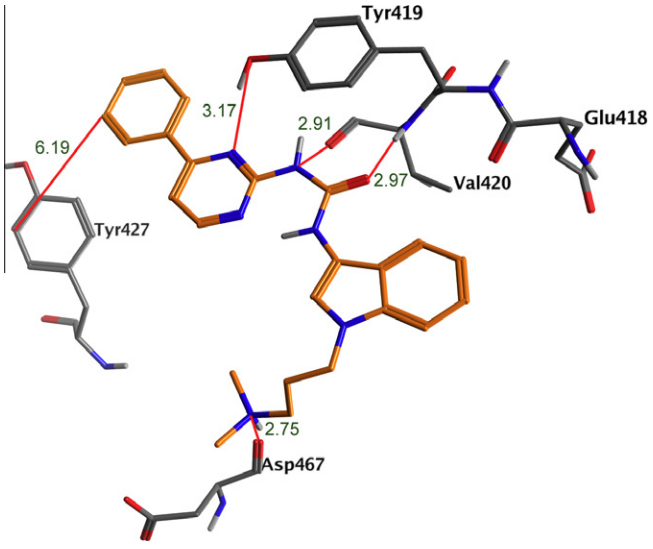


Figure 4. Homology model of urea **16** in optimum binding mode with ATP pocket of PKCα (key interactions only shown).

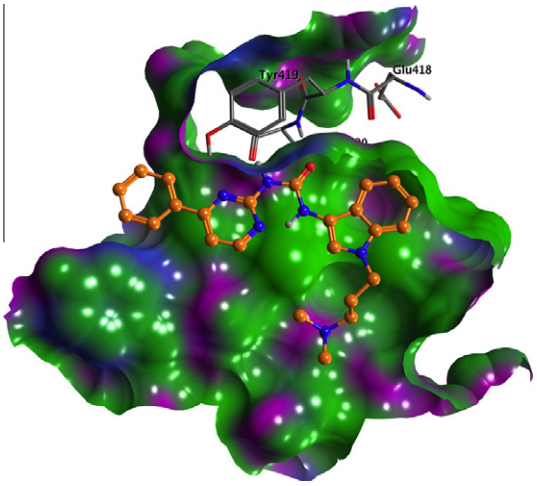


Figure 5. Homology model of urea **16** in optimum binding mode with ATP pocket of PKCα showing surface polarities (green: hydrophobic; blue: polar; purple: charged residues).

of planarity) in their side-on docking views (Figs. 9–11, Supplementary data). This skewing has little effect on the N1 to Tyr419 (OH) bond length (3.07 Å for **17** compared to 3.17 Å for **16**) but C4 of the pyridine ring of **17** is now placed in close proximity to the top of the binding pocket with the potential for steric interactions destabilising this skewed orientation relative to the planar **16**.

The decrease in IC_{50} of **16** versus **17**–**19** could also be ascribed to the change in substitution of the N5 group (Me_2N (**16**) to Et_2N (**17**–**19**)). Examination of the homology model of this pocket, however, demonstrates that the region surrounding Asp467 is sterically unencumbered (Figs. 9 and 10, Supplementary data, show side-on views of docking **16** and **17** into the ATP pocket) and should be able to accommodate a diethylamino group as easily as dimethylamino. The possibility that the more hydrophobic diethylamino group may seek out a less solvent exposed area of the binding pocket, which could have an influence upon binding affinity cannot be excluded, although substrate docking suggests a similar spatial orientation of both dimethylamino and diethylamino substituted chains. Hence the change in substitution at N5 is unlikely to cause the change in inhibition observed between **16** and **17**–**19**, but cannot be entirely discounted as the origin of the IC_{50} trends.

We propose the activity of urea **16** can be assigned to the conformational rigidity imposed by the two H-bonding interactions of the pyrimidine nitrogen atoms.

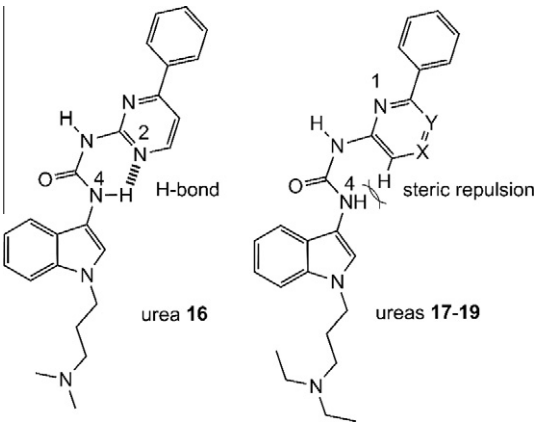


Figure 6. Compounds **16** and **17**–**19** in preferred binding conformation.

4. Conclusion

A number of indolylureas bearing heterobiaryl side chains on the urea moiety were prepared during this study. One of these compounds demonstrated nanomolar inhibition of PKCα and an acceptable selectivity profile against PKA. Using a homology model of the PKCα ATP binding site, a rationale was developed to explain the activity trend observed with the series of compounds prepared.

5. Experimental

5.1. General methods

Thin-layer chromatography was performed on Merck Kieselgel 60 F_{254} silica gel plates and visualised by UV (254 nm). Flash chromatography was conducted using Silicycle silica gel (40–60 μM, pH 6.5–7.0). IR was recorded on a Nicolet 360 FTIR as a thin film or with a golden-gate ATR device if solid. ^1H and ^{13}C NMR were recorded on Bruker Avance 400 or Avance 250 systems operating at 400 or 250 MHz (^1H), 100.6 or 62.9 Hz (^{13}C). LC–MS were analysed on an Atlantis C18 reverse phase column using a water/acetonitrile mobile phase (0.1% formic acid) linked to a Waters Micromass® ZQ™ mass spectrometer. Accurate mass measurements were recorded on an LC–MS system incorporating a Waters 1525 binary HPLC pump, a Waters 2488 UV detector and a Waters LCT Premier mass spectrometer. Melting points were determined on a Gallenkamp (model: MPD350) apparatus and are uncorrected.

5.2. PKCα and PKA inhibition assay

All enzyme inhibition assays were performed as described previously.[18] Briefly, 10 point enzyme inhibitor dilution series were prepared as 10 × 10 arrays in the centre of 384 well plates. Compound dilutions and the peptide substrate containing the necessary cofactors (i.e., ATP, lipids, etc.) were prepared on separate plates using a Beckman–Coulter Biomek 2000 workstation. The reactions were initiated, allowed to proceed for 30–45 min, stopped by removing 5 μL of the reaction mixture into 50 μL of MALDI-MS matrix solution and transferred onto a MALDI-TOF MS target plate, maintaining the original 10 × 10 array. The specific assay conditions for each enzyme reaction were as follows: PKA—enzyme activity was measured using 2.7 units/well PKA (Upstate Biotech #V5161) in 40 mM Tris–HCl, pH 7.5 containing 20 mM $MgCl_2$, 0.1 mg/mL BSA, 50 μM ATP, and 50 μM kemptide substrate. Standard incubations for PKA were 30 min at rt. PKCα—enzyme activity was measured using 6 ng/well PKCα (Promega #12,946) in Promega activation buffer, plus 0.1 mg/mL BSA, 50 μM ATP, 200 μM EDTA, 400 μM $CaCl_2$, and 25 μM neurogranin substrate. Standard incubation conditions for PKCα were 45 min at rt. A cellular assay for PKC utilising MALDI-TOF was developed (see Supplementary data).

5.3. Mass spectrometry readout

All spectra were collected on an Applied Biosystems (Foster City, CA) 4700 Proteomics Analyzer, Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometer (MALDI-TOF MS) equipped with a Nd–YAG laser (355 nm, 3 ns pulse width, 200 Hz repetition rate) in negative ion reflector mode as indicated. The system was operated with the 4700 Explorer software, version 2.0 or 3.0. Automated acquisition parameters were adjusted to capture and average only those individual spectra within defined success criteria. Specifically, signal intensities for the substrate peptide were set to a minimum threshold of 3000 counts and a maximum intensity of 65,000 counts. This ensured that neither

null spectra nor saturated spectra were averaged into the final readout. Depending on the substrate peptide used, between 1000 and 2000 laser shots were averaged from a minimum of 25 random positions from each sample spot. Data were collected in triplicate from three successive days (unless otherwise noted) to capture the maximum variability related to preparation of the enzyme reactions, transfer of samples to MALDI target plates, data collection, and data extraction. For data analysis, the isotope cluster areas for the peptide substrate and product peaks were extracted into a Microsoft Excel worksheet with the relative inhibition plotted as % Maximal Activity as described previously.[18a,b]

5.3.1. Methyl 1-[3-(*tert*-butyldimethylsilyloxy)propyl]-1*H*-indole-3-carboxylate 3

A solution of methyl indole-3-carboxylate (1.87 g, 10.68 mmol), 1-bromo-3-*tert*-butyldimethylsilyloxypropane (5.0 mL, 21.5 mmol), cesium carbonate (10.43 g, 32 mmol) and anhydrous DMF (90 mL) was heated to 50 °C for 16 h under a nitrogen atmosphere. After cooling, the reaction contents were poured into water (90 mL) and extracted with EtOAc (2 × 100 mL). The combined extracts were dried over $MgSO_4$, filtered and evaporated. The residue was purified by flash column chromatography to yield the N-alkylated indole **3** (3.74 g, 10.68 mmol, 100%) as a colourless oil: R_f 0.24 (9:1 heptane/EtOAc); IR (film) 1696, 1533, 1465, 1375, 1228, 1172, 1088 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.10–8.14 (1H, m), 7.78 (1H, s), 7.33–7.36 (1H, m), 7.19–7.22 (2H, m), 4.22 (2H, t, J = 6.8 Hz), 3.85 (3H, s), 3.49 (2H, t, J = 5.6 Hz), 1.96 (2H, quint., J = 6.2 Hz), 0.88 (9H, s), 0.00 (6H, s); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 165.5, 136.5, 134.7, 126.7, 122.4, 121.8, 121.7, 110.1, 106.8, 59.1, 50.9, 43.3, 32.5, 26.0, 18.4, −5.5; LC–MS: 100% UV (215 nm), MS 348 $(M+1)^+$ 100%; HRMS $M+H^+$ calcd for $C_{19}H_{30}NO_3Si$ 348.1955, found 348.1990.

5.3.2. Methyl 1-(3-hydroxypropyl)-1*H*-indole-3-carboxylate 4

To a solution of silylether **3** (3.693 g, 10.64 mmol) in anhydrous THF (75 mL) at room temperature was introduced TBAF (10.64 mL of a 1.0 M solution in THF, 10.64 mmol). After 30 min stirring, the solution was evaporated to dryness. The residual oil was purified by flash column chromatography (1:1–2:1 EtOAc/heptanes gradient) to furnish alcohol **4** (2.303 g, 9.88 mmol, 93%) as a colourless waxy solid: R_f 0.30 (EtOAc); mp 58–59 °C; IR (ATR) 3493, 1674, 1527, 1459, 1375, 1223, 1161, 1099, 1065, 1026 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.09–8.12 (1H, m), 7.79 (1H, s), 7.32–7.36 (1H, m), 7.19–7.23 (1H, m), 4.25 (2H, t, J = 6.7 Hz), 3.83 (3H, s), 3.53 (2H, dt, J = 5.7, 4.7 Hz), 2.01 (2H, quint., J = 6.3 Hz), 1.72 (1H, t, J = 4.5 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 165.9, 136.5, 134.8, 126.6, 122.8, 121.9, 121.7, 110.1, 106.8, 58.7, 51.1, 43.3, 32.1; LC–MS: 100% UV (215 nm), MS 234 $(M+1)^+$ 100%; HRMS $M+H^+$ calcd for $C_{13}H_{16}NO_3$ 234.1130, found 234.1124.

5.3.3. Methyl 1-(3-dimethylaminopropyl)-1*H*-indole-3-carboxylate 5

To a solution of alcohol **4** (0.245 g, 1.05 mmol) in anhydrous DCM (7.0 ml) at 0 °C under an atmosphere of nitrogen was introduced trifluoromethanesulfonic anhydride (0.26 mL, 1.6 mmol). After 2–3 min, a pre-cooled (0 °C) solution of 2,6-lutidine (0.25 mL, 2.1 mmol) in anhydrous DCM (10.0 mL) was added via canula over several minutes. The reaction solution was kept at 0 °C for 5 h. Dimethylamine (6.85 mL of a 2.0 M solution in THF, 13.7 mmol) was then introduced and the reaction mixture warmed to room temperature for 16 h. The reaction solution was evaporated to dryness and purified by flash column chromatography (EtOAc containing a 1–10% gradient of triethylamine) to yield amine **5** (0.237 g, 0.91 mmol, 87%) as a yellow oil: R_f 0.17 (10% Et_3N in EtOAc); IR (film) 2952, 1769, 1696, 1532, 1460, 1375, 1268, 1234, 1189, 1150, 1087, 1031 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.10–8.13 (1H, m), 7.78 (1H, s), 7.31–7.33 (1H, m), 7.21–7.25 (2H, m), 4.17 (2H, t, J = 6.9 Hz), 3.81 (3H, s), 2.17 (2H, t, J = 6.9 Hz), 2.16 (6H, s), 1.94 (2H, quint., J = 6.9 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 165.5, 136.5, 134.6, 126.7, 122.6, 122.0, 121.8, 110.1, 106.8, 55.9, 50.9, 45.4, 44.4, 27.8; LC–MS: 99% UV (215 nm), MS 261 $(M+1)^+$ 100%; HRMS $M+H^+$ calcd for $C_{15}H_{21}N_2O_2$ 261.1603, found 261.1597.

5.3.4. Methyl 1-(3-diethylaminopropyl)-1*H*-indole-3-carboxylate 6

Prepared on a 0.64 mmol scale according to procedure described for dimethylamine analogue **5**. The diethylamine **6** was isolated (0.110 g, 0.38 mmol, 59%) by flash column chromatography as a yellow oil: R_f 0.38 (10% Et_3N in EtOAc); IR (film) 2969, 1696, 1532, 1465, 1381, 1234, 1194, 1155, 1093, 1037 cm^{-1}; ^1H NMR (400 MHz, MeOH-d_4) δ 8.07 (1H, d, J = 7.7 Hz), 7.94 (1H, s), 7.45 (1H, d, J = 8.1 Hz), 7.18–7.26 (2H, m), 4.19 (2H, t, J = 7.0 Hz), 3.86 (3H, s), 2.49 (4H, q, J = 7.3 Hz), 2.40–2.44 (2H, m), 1.92–1.99 (2H, m), 0.95 (6H, t, J = 7.3 Hz); ^{13}C (100.6 MHz, MeOH-d_4) δ 167.3, 138.0, 136.2, 128.1, 123.9, 122.9, 122.4, 111.5, 107.5, 51.5, 50.5, 47.7, 45.7, 27.5, 11.3; LC–MS: 98% UV (215 nm), MS 289 $(M+1)^+$ 100%; HRMS $M+H^+$ calcd for $C_{17}H_{25}N_2O_2$ 289.1916, found 289.1903.

5.3.5. 1-(3-Dimethylaminopropyl)-1*H*-indole-3-carboxylic acid 7

To a solution of methyl ester **5** (0.105 g, 0.40 mmol) in methanol/water (10 mL of a 4:1 methanol/water mixture) was introduced sodium hydroxide (0.081 g, 2.00 mmol). The solution was heated to 60 °C for 36 h. After neutralisation of the reaction solution with 2 M aq HCl and evaporation to dryness, the residue was diluted with methanol (2.0 mL) and the insoluble material (NaCl) removed by filtration. The filtrate was evaporated to furnish acid **7** as a tan amorphous solid (0.170 g, 0.69 mmol, 172%) containing inorganic residues. Typically the material was carried through to subsequent steps at this level of purity, but could be purified by flash column chromatography (MeCN/MeOH gradient neat MeCN to 1:1 MeCN/MeOH), evaporation, re-dilution in MeCN and filtration. The filtrate was evaporated to surrender the acid **7** (0.043 g, 0.17 mmol, 44%) as a tan solid: R_f 0.27 (methanol); mp 140–180 °C (sample slowly darkens but does not melt up to 400 °C); IR (ATR) 3113, 2903, 1672, 1526, 1275, 1199, 1135, 1018 cm^{-1}; ^1H (400 MHz, MeOH-d_4) δ 8.25 (1H, dd, J = 7.0, 1.1 Hz), 7.73 (1H, s), 7.38 (1H, d, J = 7.7 Hz), 7.17, (1H, dt, J = 6.9, 1.4 Hz), 7.12 (1H, dt, J = 7.4, 1.1 Hz), 4.19 (2H, t, J = 6.8 Hz), 2.31–2.27 (2H, m), 2.19 (6H, s), 1.99 (2H, quint., J = 6.9 Hz); ^{13}C (100.6 MHz, MeOH-d_4) δ 168.9, 138.0, 136.2, 128.3, 124.1, 123.0, 122.6, 111.42, 108.8, 56.3, 44.7, 43.6, 26.5; LC–MS: 100% UV (215 nm), MS 247 $(M+1)^+$ 100%; HRMS $M+H^+$ calcd for $C_{14}H_{19}N_2O_2$ 247.1446., found 247.1442.

5.3.6. 1-(3-Diethylaminopropyl)-1*H*-indole-3-carboxylic acid 8

Prepared on a 42 mmol scale according to procedure described for dimethylaminocarboxylic acid **7**. The crude carboxylic acid **8** was isolated (22.27 g, 81 mmol, 192%) containing inorganic residues. A small sample was purified by flash column chromatography (as carboxylic acid **7**) for analysis purposes only, furnishing acid **8** as a viscous orange/brown oil: R_f 0.34 (methanol), IR (film) 3493 (br), 1550, 1431, 1262, 1166, 1110, 1037 cm^{-1}; ^1H NMR, MeOH-d_4) δ 8.21 (1H, dd, J = 7.1, 1.6 Hz), 7.76 (1H, s), 7.46 (1H, dd, J = 7.0, 1.6 Hz), 7.16 (2H, app. d quint., J = 7.1, 1.4 Hz), 4.29 (2H, t, J = 6.8 Hz), 2.91 (4H, q, J = 7.3 Hz), 2.82–2.89 (2H, m), 2.11–2.23 (2H, m), 1.11 (6H, t, J = 7.3 Hz); ^{13}C (62.9 MHz, MeOH-d_4) δ 173.9, 137.9, 134.1, 129.1, 123.15, 123.10, 121.8, 114.5, 110.8, 49.3, 48.0, 44.7, 26.2, 9.6; LC–MS: 97% UV (215 nm), MS 275 $(M+1)^+$ 100%; HRMS $M+H^+$ calcd for $C_{16}H_{23}N_2O_2$ 275.1759, found 275.1749.

5.3.7. 1-(3-Dimethylaminopropyl)-1*H*-indole-3-carbonylazide 9

To a suspension of carboxylic acid **7** (0.115 g, 0.47 mmol) in anhydrous DCM (30 mL) under an atmosphere of nitrogen was introduced oxalyl chloride (0.122 mL, 1.40 mmol) and catalytic DMF (~10 mg). The reaction mixture was stirred at room temperature for 5 h, then evaporated to dryness. The residue was immediately re-dissolved in anhydrous THF (3.0 mL) and cooled to 0 °C. An aqueous solution of sodium azide (0.151 g, 2.33 mmol in 0.5 mL of water) was then introduced dropwise over several minutes. After 4 h at 0 °C, the reaction mixture was cautiously evaporated (bath temp <21 °C) to remove THF and the aqueous residue transferred to a separating funnel. Water (10 mL) was introduced and the pH adjusted to 7.0 (if required), then the aqueous solution was extracted with EtOAc (2 × 10 mL). The combined extracts were dried over $MgSO_4$, filtered and evaporated (bath <21 °C) to furnish the crude acyl azide **9** (0.077 g, 0.28 mmol, 61%) as an oil. The purity of this oil was found to be acceptable for use in subsequent steps and no attempts were made to purify this substrate further [note: this material was stored in a freezer at −25 °C]. IR (film) 2944, 2769, 2133, 1666, 1520, 1462, 1380, 1188, 1141, 1059 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.24–8.28 (1H, m), 7.88 (1H, s), 7.40–7.43 (1H, m), 7.30–7.34 (2H, m), 4.26 (2H, t, J = 6.7 Hz), 2.24 (6H, s), 2.23 (2H, t, J = 6.6 Hz), 2.01 (2H, quint., J = 6.6 Hz); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 167.6, 136.7, 135.8, 126.3, 123.1, 122.4, 121.5, 110.1, 108.1, 55.4, 44.9, 44.3, 27.1; LC–MS: 100% UV (215 nm), MS 272 $(M+1)^+$ 100%. HRMS $M+H^+$ calcd for $C_{14}H_{18}N_5O$ 272.1511, found 272.1501.

5.3.8. 1-(3-Diethylaminopropyl)-1*H*-indole-3-carbonylazide 10

Prepared on a 5.46 mmol scale according to procedure outlined for acyl azide **9**. The crude acyl azide **10** (0.81 g, 2.73 mmol, 50%) was isolated as a brown oil of acceptable purity for subsequent reactions and stored in a freezer at −25 °C until used. IR (film) 2135, 1668, 1527, 1465, 1375, 1262, 1189, 1026 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.20–8.23 (1H, m), 7.89 (1H, s), 7.41–7.44 (1H, m), 7.30–7.34 (2H, m), 4.30 (2H, t, J = 6.9 Hz), 3.00 (4H, q, J = 7.3 Hz), 2.92–2.95 (2H, m), 2.28 (2H, quint., J = 7.3 Hz), 1.20 (6H, t, J = 7.2 Hz); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 166.9, 135.8, 134.9, 125.5, 122.5, 121.7, 120.7, 109.3, 107.5, 48.3, 45.7, 43.6, 25.2, 9.3; LC–MS: 92% UV (215 nm), MS 300 $(M+1)^+$ 100%; HRMS $M+H^+$ calcd for $C_{16}H_{22}N_5O$ 300.1824, found 300.1832.

5.3.9. General procedure for the synthesis of compounds 13, 14 and 15

To a solution of the requisite chloro-amino-substituted heteroaromatic in anhydrous 1,4-dioxane (30 volumes wrt chloride) in a sealed tube was introduced phenylboronic acid (1.5 equiv) and finely ground potassium phosphate (2.0 equiv). The solution was degassed (N_2 bubbling) for 5 min, $Pd(OAc)_2$ (5 mol % wrt chloride) and di-*tert*-butylphosphinoferrocene (5 mol % wrt chloride) introduced and degassing continued for a further 5 min. The tube was sealed under nitrogen and heated with rapid stirring at 100 °C for 5 h. After cooling, the reaction mixture was filtered in vacuo through a celite pad and the precipitated material washed with 1,4-dioxane. The combined filtrates were evaporated and purified by flash column chromatography (neat hexane to 1:1 hexane/EtOAc gradient containing 2.5% by volume Et_3N) to furnish the biarylanilines **13**, **14** and **15**.

5.3.9.1. 2-Amino-6-phenylpyridine 13. Prepared on a 7.8 mmol scale. Purified product isolated as a viscous orange oil (1.092 g, 6.4 mmol, 82%): R_f 0.51 (neat EtOAc); IR (film): 3475, 3387, 3311, 3177, 1608, 1567, 1444, 1345, 1269, 1177, 1018 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 7.94 (2H, d, J = 7.3 Hz), 7.51 (1H, t, J = 7.9 Hz), 7.45 (2H, t, J = 7.5 Hz), 7.38 (1H, t, J = 7.3 Hz), 7.10 (1H, d, J = 7.4 Hz), 6.46 (1H, d, J = 8.1 Hz), 4.7 (2H, br s); ^{13}C NMR (100.6 MHz, $CDCl_3$) 158.8, 156.6, 140.0, 138.5, 128.6, 126.9, 110.9, 107.8 [note: DEPT 135 experiment shows have 3 quaternary carbons, but only 5 methine carbons, assumed two methines with coincident δ's]; LC–MS: 95% UV (215 nm), MS 171 $(M+1)^+$ 100%; HRMS $M+H^+$ calcd for $C_{11}H_{11}N_2$ 171.0922, found 171.0920.

5.3.9.2. 4-Amino-2-phenylpyrimidine 14. Prepared on a 7.8 mmol scale. Purified product isolated as a colourless wax (0.477 g, 2.78 mmol, 36%): mp 111–112 °C; R_f 0.38 (neat EtOAc); IR (ATR): 3393, 3323, 3177, 1654, 1584, 1532, 1479, 1392, 1246 cm^{-1}; ^1H NMR (250 MHz, MeOH-d_4) δ 8.18–8.22 (2H, m), 8.14 (1H, d, J = 6.0 Hz), 7.41–7.47 (3H, m), 6.44 (1H, d, J = 6.0 Hz); ^{13}C NMR (100.6 MHz, MeOH-d_4) δ 162.3, 162.1, 152.4, 135.9, 127.8, 126.0, 125.7, 125.2, 100.8; LC–MS: 96% UV (215 nm), MS 172 $(M+1)^+$ 100%; HRMS $M+H^+$ calcd for $C_{10}H_{10}N_3$ 172.0874, found 172.0874.

5.3.9.3. 2-Amino-6-phenylpyrazine 15. Prepared on a 3.9 mmol scale. Purified product isolated as a tan, amorphous solid (0.365 g, 2.1 mmol, 55%): mp 127.5–128.5 °C; R_f 0.32 (EtOAc); IR (ATR): 3369, 3095, 3048, 1631, 1573, 1532, 1415, 1333, 1269, 1211, 1071 cm^{-1}; ^1H NMR (400 MHz, MeOH-d_4) δ 8.18 (1H, s), 7.92–7.95 (2H, m), 7.84 (1H, s), 7.39–7.47 (3H, m); ^{13}C NMR (100.6 MHz, MeOH-d_4) δ 157.1, 151.8, 138.2, 131.4, 130.5, 129.8, 129.6, 128.1; LC–MS: 100% UV (215 nm), MS 172 $(M+1)^+$ 100%; HRMS $M+H^+$ calcd for $C_{10}H_{10}N_3$ 172.0874, found 172.0857.

5.3.10. 1-[1-(3-Dimethylaminopropyl)-1*H*-indol-3-yl]-3-(4-phenylpyrimidin-2-yl)urea 16

A solution of acyl azide **9** (0.020 g, 0.074 mmol) in anhydrous toluene (1.5 mL) was heated in a sealed tube at 100 °C for 30 min. After cooling, aniline **12** (0.025 g, 0.15 mmol) was added, the tube re-capped and the solution heated to 100 °C for a further 2 h. The solution was evaporated, re-dissolved in DMSO and purified by mass directed prep-LC. The urea **16** was isolated (0.013 g, 0.024 mmol, 33%) as an amorphous pale yellow solid: mp 172 °C; IR (ATR) 3142, 2938, 1683, 1586, 1544, 1404, 1322, 1293, 1230, 1187, 1007, 826, 767, 727 cm^{-1}; R_f 0.17 (20% Et_3N in EtOAc); ^1H NMR (400 MHz, MeOH-d_4) δ 8.69 (1H, d, J = 5.5 Hz), 8.15 (2H, d, J = 7.0 Hz), 7.62 (1H, s), 7.50–7.60 (5H, m), 7.45 (1H, d, J = 8.0 Hz), 7.24 (1H, t, J = 7.7 Hz), 7.06 (1H, t, J = 7.5 Hz), 4.30 (2H, t, J = 6.6 Hz), 3.05–3.16 (2H, m), 2.83 (6H, s), 2.26 (2H, quint., J = 6.9 Hz); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 165.3, 158.7 (br), 158.1, 151.6, 136.3, 133.9, 131.4, 129.1, 127.5, 127.1, 121.9, 121.0, 118.4, 118.0, 117.2, 114.3, 110.8, 109.5, 56.5, 45.3, 44.1, 28.2; LC–MS: 100% UV (215 nm), MS 415 $(M+1)^+$ 100%; HRMS $M+H^+$ calcd for $C_{24}H_{27}N_6O$ 415.2246, found 415.2233.

5.3.11. Generic procedures for synthesis of compounds 17, 18 and 19

A solution of acyl azide **10** (0.640 g, 2.14 mmol) in anhydrous toluene (30 mL) was heated at 100 °C for 45 min. After cooling, the solution was divided equally between three sealed tubes and anilines **13**, **14** and **15** (1.42 mmol, 2 equiv) added to the appropriate sealed tube. The tubes were re-capped and heated to 100 °C for a further 2 h. After cooling the toluene was evaporated and the residue purified by flash column chromatography (1:1 hexane/EtOAc to neat EtOAc gradient containing 2.5% by volume Et_3N) to furnish the ureas **17**, **18** and **19**.

5.3.11.1. 1-[1-(3-Diethylaminopropyl)-1*H*-indol-3-yl]-3-(6-phenylpyridin-2-yl)-urea 17. Purified product isolated as an orange wax (0.034 g, 0.077 mmol, 11%): R_f 0.23 (20% Et_3N in EtOAc); IR (ATR): 2956, 1643, 1579, 1433, 1386, 1158, 1071 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ δ 11.97 (1H, br s), 8.79 (1H,

br s), 7.96–7.99 (2H, m), 7.76 (1H, s), 7.72 (1H, t, J = 7.8 Hz), 7.49–7.54 (4H, m), 7.35 (1H, d, J = 8.4 Hz), 7.21 (1H, t, J = 7.3 Hz), 6.93 (1H, t, J = 7.5 Hz), 6.90 (1H, br d, J = 7.7 Hz), 4.21 (2H, t, J = 7.0 Hz), 2.60 (4H, q, J = 7.0 Hz), 2.55 (2H, t, J = 7.0 Hz), 2.09 (2H, quint., J = 6.9 Hz), 1.05 (6H, t, J = 7.1 Hz); ^{13}C NMR (62.9 MHz, MeOH-d_4) δ 156.6, 155.0, 154.6, 140.54, 140.48, 135.5, 130.3, 130.1, 128.3, 123.2, 122.7, 119.6, 119.3, 118.7, 115.7, 115.4, 111.7, 110.6, 50.8, 47.6, 45.1, 27.9, 11.2; LC–MS: 98% UV (215 nm), MS 442 (M+1)$^+$ 100%; HRMS M+H$^+$ calcd for $C_{27}H_{32}N_5O$ 442.2607, found 442.2589.

5.3.11.2. 1-[1-(3-Diethylaminopropyl)-1*H*-indol-3-yl]-3-(2-phenylpyrimidin-4-yl)-urea 18. Purified product isolated as an orange oil (0.018 g, 0.041 mmol, 6%): R_f 0.25 (20% Et_3N in EtOAc); IR (film): 3212, 2961, 1678, 1549, 1468, 1427, 1380, 1211, 1065 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.61 (1H, br s), 9.67 (1H, br s), 8.58 (1H, d, J = 5.9 Hz), 8.38 (2H, d, J = 6.6 Hz), 7.75 (1H, br s), 7.66 (1H, d, J = 8.0 Hz), 7.52–7.57 (3H, m), 7.40 (1H, d, J = 8.0 Hz), 7.26 (1H, t, J = 7.5 Hz), 7.06 (1H, t, J = 7.5 Hz), 6.88 (1H, br s), 4.24 (2H, t, J = 7.0 Hz), 2.60 (4H, q, J = 7.1 Hz), 2.55 (2H, t, J = 7.1 Hz), 2.09 (2H, quint., J = 6.5 Hz), 1.05 (6H, t, J = 7.3 Hz); ^{13}C NMR (62.9 MHz, MeOH-d_4) δ 163.2, 158.6, 156.4, 151.8, 136.7, 133.6, 130.1, 127.7, 127.3, 121.3, 120.8, 117.91, 117.8, 116.3, 112.9, 108.73, 105.7, 48.8, 45.8, 43.1, 25.8, 9.1; LC–MS: 98% UV (215 nm), MS 443 (M+1)$^+$ 100%; HRMS M+H$^+$ calcd for $C_{26}H_{31}N_6O$ 443.2559, found 443.2559.

5.3.11.3. 1-[1-(3-Diethylaminopropyl)-1*H*-indol-3-yl]-3-(6-phenylpyrazin-2-yl)-urea 19. Purified product isolated as an yellow wax (0.008 g, 0.018 mmol, 3%): R_f 0.25 (20% Et_3N in EtOAc); IR (ATR): 3200, 2967, 1678, 1584, 1549, 1501, 1398, 1287, 1182, 1147, 1065 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ11.53 (1H, br s), 9.78 (1H, br s), 8.62 (1H, s), 8.44 (1H, br s), 8.00–8.04 (2H, m), 7.78 (1H, br s), 7.56–7.59 (3H, m), 7.55 (1H, d, J = 8.1 Hz), 7.39 (1H, d, J = 8.5 Hz), 7.24 (1H, t, J = 7.9 Hz), 7.02 (1H, br t, J = 7.3 Hz), 4.23 (2H, t, J = 7.1 Hz), 2.57 (4H, q, J = 7.3 Hz), 2.52 (2H, t, J = 7.0 Hz), 2.06 (2H, quint., J = 7.0 Hz), 1.03 (6H, t, J = 7.1 Hz); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 153.4, 148.9, 136.3, 134.4, 134.21, 134.15, 133.9, 130.1, 129.2, 127.5, 122.0, 120.9, 118.5, 118.1, 117.4, 113.9, 109.64, 50.0, 46.7, 44.5, 27.8, 11.6; LC–MS: 99% UV (215 nm), MS 443 (M+1)$^+$ 100%; HRMS M+H$^+$ calcd for $C_{26}H_{31}N_6O$ 443.2559, found 443.2549.

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Supplementary data

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