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5-Vinyl-3-pyridinecarbonitrile inhibitors of PKCθ: Optimization of enzymatic and functional activity

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ABSTRACT

PKCθ is a serine/threonine kinase involved in the regulation of IL2 production in T cells. It has recently become an attractive therapeutic target for a variety of immunological disorders. We describe the optimization of the enzymatic and cellular potency of a series of 5-vinyl-3-pyridinecarbonitrile inhibitors of PKCθ. A binding model was developed that explains much of the SAR observed for this series, including the enzymatic potency observed for **19**. An analysis of functional potency against various physiochemical parameters suggests that cellular potency is correlated with Log $D_{7.4}$, but not with *c* Log *P*, PAMPA permeability, or TPSA.

1. Introduction

Interleukin-2 (IL2) is a cytokine that plays an essential role in the growth, survival, and differentiation of T cells. IL2 expression in T cells is mediated by a variety of transcription factors which target the CD28 response element promoter. The activation of these transcription factors is carefully regulated and requires the co-stimulation of the T cell receptor (TCR) signaling complex and CD28. The CD28 mediated signal is thought to be transmitted by the PI3K/AKT pathway which ultimately results in PDK1 dependent phosphorylation and activation of protein kinase C theta (PKCθ). Activated PKCθ, in turn, is recruited to the TCR complex where it plays a critical role in the activation of the aforementioned transcription factors. Thus, PKCθ plays a unique role in the integration of signaling from the TCR complex and CD28 which results in immunostimulation via IL2 expression.[1]

PKCθ is a serine/threonine kinase which, as its role in IL2 production would suggest, is expressed primarily in lymphocytes and mast cells. Due to its important role in the activation and differentiation of T cells, PKCθ modulation has been extensively studied for its potential pharmacologic utility in various immunological disorders.[2] PKCθ knockout (KO) mice have been reported to have diminished responses in various T cell mediated disease models including the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis,[3,4] the type II collagen-induced arthritis (CIA) model,[5] and the ovalbumin challenge (OVA) model of asthma.[6,7] PKCθ KO mice have also showed significantly increased survival following a cardiac allograft transplantation, suggesting a potential utility of PKCθ inhibitors as immunosuppressives following transplantation.[8] In addition to lymphocytes and mast cells, PKCθ is also expressed in skeletal muscle where it is speculated to play a role in free fatty acid induced insulin resistance.[9] As such, PKCθ may also be emerging as an attractive target for diabetes mellitus. Interestingly, in spite of its crucial role in a variety of immune responses, PKCθ KO mice have been shown to have normal Th1 differentiation and normal viral clearance in spite of their defective T cell activation pathway.[6,10] This suggests that selective PKCθ inhibitors may be able to modulate immunological disorders without imparting severe immunodeficiency. Therefore, PKCθ has recently become an attractive target for pharmacological intervention of a variety of diseases.

PKCs are a broad family of structurally homologous serine/threonine kinases which play important roles in cell growth, differentiation, and apoptosis.[11] PKCs are generally grouped into three classes based upon their requirements for activation: classical PKCs (α, β, γ), novel PKCs (δ, ε, η, θ), and atypical PKCs (ζ, ι/λ).[12] Novel PKCs require only diacylglycerol (DAG) for activation while classical PKCs require both DAG and Ca^{2+}. Atypical PKCs do not require DAG or Ca^{2+} for activation. Among the PKC isoforms, PKCθ is most closely re-

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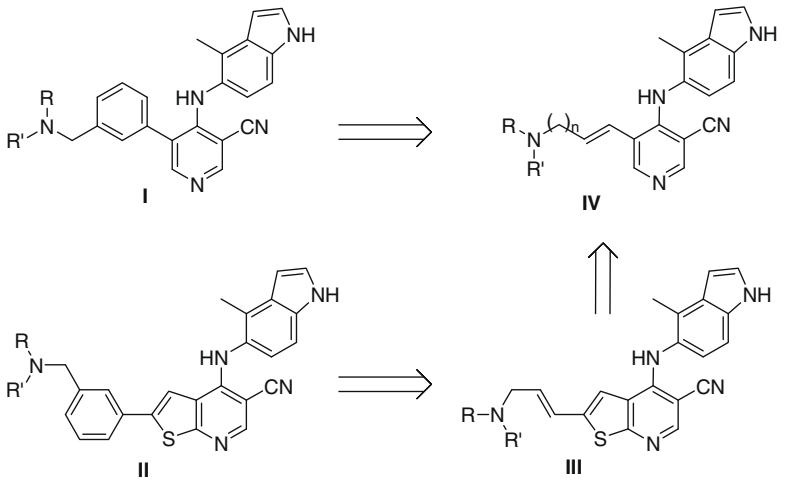


Figure 1.

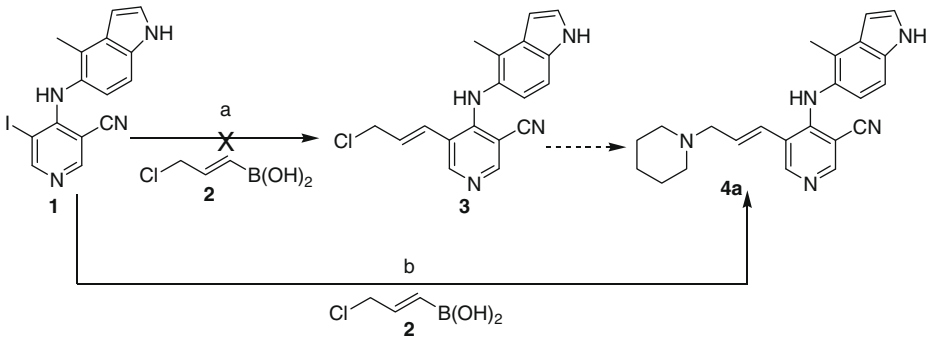
lated to PKCδ,[13] another novel PKC isoform which is thought to play a role in the regulation of B cell proliferation.[14,15] The PKCθ active site differs from PKCδ by only one amino acid: Tyr460 (Phe in PKCδ). While numerous inhibitors of PKCs have been described,[16,17] only a handful of θ-isoform selective inhibitors are known. These include 2,4-diaminopyridines,[18] thieno[2,3-*b*]pyridine-5-carbonitriles,[19–21] and 5-aryl-3-pyridinecarbonitrile.[22–25] While a 2.0 Å structure of the PKCθ kinase domain with the pan-kinase inhibitor staurosporine has been determined, no co-crystal structure with a selective PKCθ inhibitor has been reported.[26]

2. Results

We recently reported a series of 5-aryl-3-pyridinecarbonitrile PKCθ inhibitors (**I**).[22–25] We previously found that the analogous aryl group on a series of 2-arylthieno[2,3-*b*]pyridine-5-carbonitriles (**II**) could be replaced by a simple alkene tethered to an amine 'tail' that imparted improved solubility (**III**).[21] This resulted in compounds that retained the potency of parent aryl derivatives, but with significantly reduced molecular weight. In this paper, we report that the 5-aryl group of compounds (**I**) can be replaced by an alkene linker to give a new class of selective PKCθ inhibitors, 5-vinyl-3-pyridinecarbonitriles (**IV**) (Fig. 1). A detailed account of the optimization of potency, selectivity, functional activity, and pharmaceutical properties will be described.

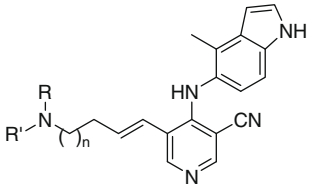
We initially reasoned that 5-allylamino-substituted 3-pyridinecarbonitriles could be easily obtained by a Suzuki coupling of the previously reported 5-iodonicotinonitrile **1**[23] with commercially available 3-chloroallylboronic acid **2** to give **3**. However, similar to our report on the related thieno[2,3-*b*]pyridine scaffold in 2008,[21] this reaction failed to give any observable product under a variety of reaction conditions. We suspected that the poor reactivity of this boronic acid may be due to a facile π-allyl insertion of the catalytic palladium which prevents the oxidative addition of the palladium into the aryl iodide. This being the case, we speculated that addition of a nucleophile should free the palladium from the π-allyl complex thereby allowing the Suzuki coupling to take place with the newly formed 3-amino substituted allylboronic acid. Gratifyingly, treatment of **1** with boronic acid **2** (1.5 equiv) in the presence of a slight excess of piperidine (2 equiv), Cs_2CO_3 (2 equiv) and $Pd(PPh_3)_2Cl_2$ (0.15 equiv) gave the desired 5-allylamino-3-pyridinecarbonitrile **4a**. The examples herein (vide infra) build upon our 2008 publication and further illustrate the versatility and attractiveness of this 'three-component-coupling' reaction for the addition of solubilizing tails to aryl halides (see Scheme 1).

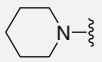
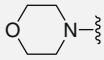
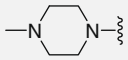
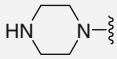
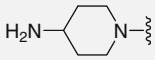
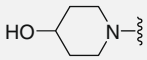
Compound **4a** proved to have an IC_{50} value of 190 nM against PKCθ (Table 1). Having established a baseline level of enzymatic activity for this new series, we set about exploring additional amine substituents and extensions of the alkene linker. The '3-component coupling' reaction described above was easily adapted to make a variety of amino-substituted prop-1-enyl derivatives such as **11a**–**15a** (Scheme 2, reaction 1). We next turned our attention to the extension of the alkene linker (Scheme 2, reactions 2 and 3) The above conditions were operationally attractive and we therefore initially explored the analogous reaction with homoallyl tosylate **5**.[28] While the desired product was formed, the appearance of two significant byproducts (Scheme 2, reaction 2) complicated the isolation of the product and adversely affected



Scheme 1. Initial synthesis of **4a**. Reagents and conditions: (a) Cs_2CO_3, $Pd(PPh_3)_2Cl_2$, DMSO, 90 °C, 16 h; (b) piperidine, Cs_2CO_3, $Pd(PPh_3)_2Cl_2$, DMSO, 90 °C, 16 h.

Table 1
Exploration of amine solubilizing group and linker length



X	Compd	*n*	PKCθ IC_{50}[a] (nM)	PKCδ IC_{50}[a] (nM)	Fold selectivity	T cell IC_{50}[b] (nM)
	**4a**	0	190 ± 1.1	690 ± 100	3.6	ND[c]
	4b	1	35 ± 1.7	260 ± 12	7.4	ND
	4c	2	19 ± 4.5	94 ± 6.1	4.9	ND
	**11a**	0	51 ± 6.1	67 ± 1.4	1.3	ND
	11b	1	16 ± 2.2	250 ± 18	16	ND
	11c	2	34 ± 11	120 ± 6.0	3.5	ND
	**12a**	0	31 ± 2.4	43 ± 5.9	1.4	330 ± 11
	12b	1	2.2 ± 0.66	2.5 ± 0.08	1.2	49 ± 29
	12c	2	6.4 ± 1.9	45 ± 1.8	7.0	77 ± 26
	**13a**	0	10 ± 2.4	22 ± 2.4	2.2	1600
	13b	1	4.0 ± 1.1	16 ± 1.7	4.0	2300
	13c	2	2.8 ± 0.6	27 ± 6.2	9.6	820 ± 190
	**14a**	0	3.6 ± 0.55	8.0 ± 1.9	2.2	2700
	14b	1	0.88 ± 0.04	5.8 ± 0.6	6.6	210 ± 59
	14c	2	5.2 ± 0.75	18 ± 3.4	3.4	ND
	**15a**	0	140 ± 5.8	760 ± 25	5.4	ND
	15b	1	6.0 ± 0.09	180 ± 16	30	1600
	15c	2	11 ± 0.12	62 ± 4.2	5.6	ND

[a] Represents the average of at least two measurements, ±standard error of the mean.
[b] Represents a single measurement, or the average of at least two measurements ±standard error of the mean.
[c] ND = not determined.

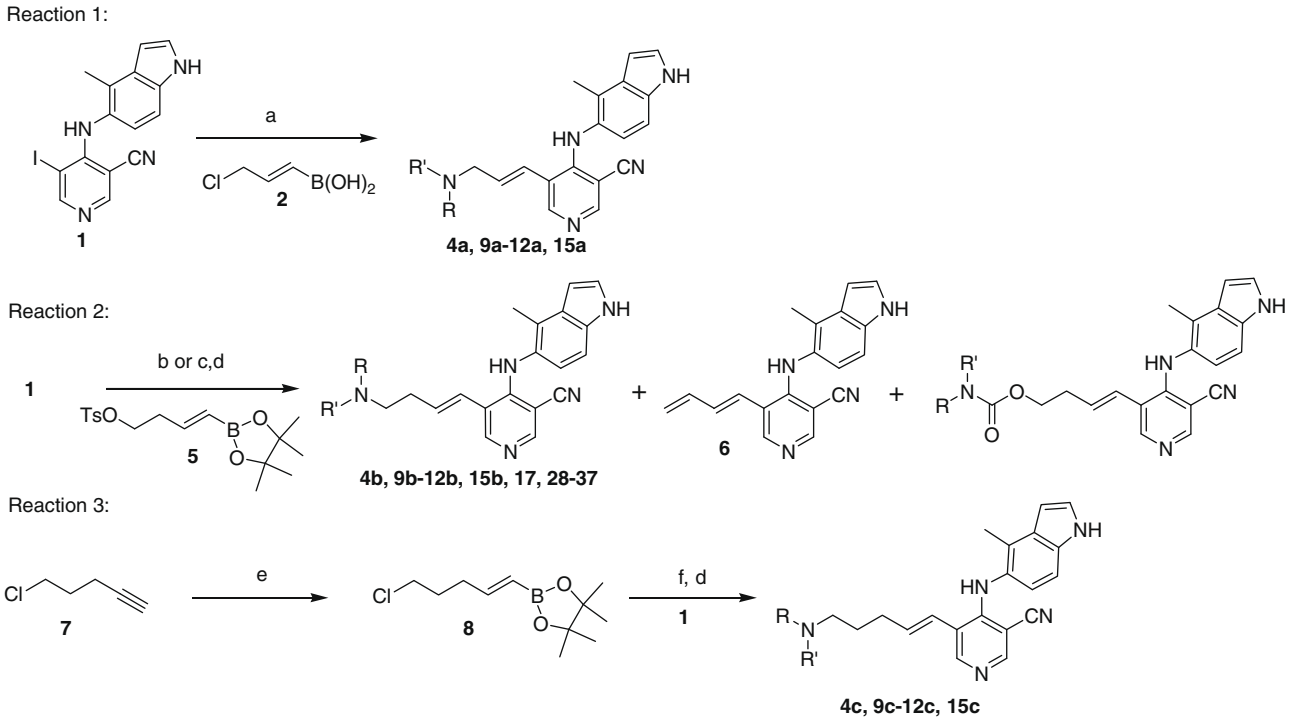
the yield. The first byproduct, (*E*)-5-(buta-1,3-dienyl)-4-(4-methyl-1*H*-indol-5-ylamino)-3-pyridinecarbonitrile **6**, presumably resulted from simple elimination of the tosylate. The mechanism for the formation of the second byproduct, a 4-carbamoyl-but-1-enyl derivative, remains unclear.

While the above reaction conditions allowed for the preliminary exploration of 4-aminobut-1-enyl derivatives such as compound **4b** (Table 1), due to the potent activity of these 4-aminobut-1-enyl derivatives (vide infra), a more efficient synthesis was targeted. We found that separation of the nucleophilic displacement step from the Suzuki coupling step minimized the formation of both byproducts. Specifically, homoallyl tosylate **5** was treated with excess amine in DMSO at 50 °C for 16 h then treated with **1**, Cs_2CO_3, and $Pd(PPh_3)_2Cl_2$ and heated to 90 °C for an additional 16 h. Upon cooling, the reaction was generally filtered and the filtrate was purified by HPLC or silica gel chromatography to give the desired 4-aminobut-1-enyl pyridine. Only minimal amounts of the above two byproducts were observed when this two-step process was utilized. The same two-step, one-pot procedure was utilized for the synthesis of 5-aminopent-1-enyl-pyridines such as **4c**, **9c–12c**, and **15c** from 5-chloropent-1-enyl boronic ester **8**, as shown in Scheme 2, reaction 2. Primary and secondary amines could be easily introduced by utilizing the previously described reactions with Boc-protected piperazines and Boc-amino substituted piperidines followed by deprotection (Scheme 3).

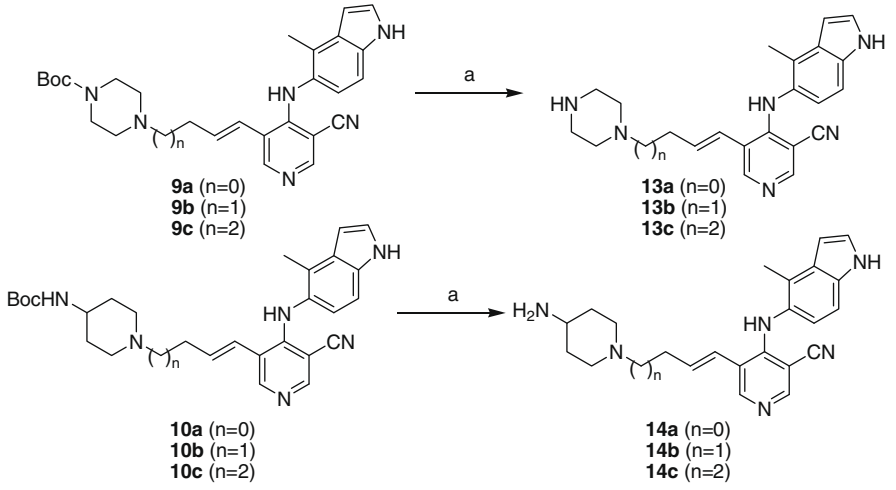
Table 1 illustrates the initial set of compounds that was synthesized in order to probe the SAR of the alkene linked solubilizing tail. As previously mentioned, compound **4a** was a 190 nM inhibitor of PKCθ with minimal selectivity over PKCδ. Changes in the alkyl amine (**11a**–**15a**) resulted in significant modulation of PKCθ activity. Importantly, the addition of a secondary basic amine (**12a**–**14a**) resulted in compounds with improved potency. Extension of the alkyl linker to two and three carbons served to increase activity against PKCθ regardless of the identity of the amine solubilizing group (NRR′). Based on this initial set of compounds, the optimal combination of linker and amine appeared to be a two-carbon linker to a 4-aminopiperidine (**14b**). This combination resulted in sub-nM potency against PKCθ (0.88 nM), but only moderate selectivity over PKCδ (6.6×). As described previously, selectivity over PKCδ is considered desirable due to the role this enzyme plays in the regulation of B cell proliferation. Interestingly, replacing the primary amine of **14b** with an alcohol (**15b**) resulted in a significant increase in selectivity over PKCδ. A comparison of activity and selectivity of the unsubstituted piperidine **4b** with the 4-substituted piperidines **14b** and **15b** clearly indicates that the distal position of the piperidine plays an important role in PKCθ activity and selectivity. Therefore, a larger set of compounds was prepared (Table 2, discussed below) in order to probe the importance of this position.

After an initial evaluation of PKCθ and PKCδ activity, compounds of interest were assayed for functional activity in T cells isolated from C57 mice. The T cells were stimulated with anti-CD3 and anti-CD28 antibodies in order to elicit the production of IL2. The functional activity of the compounds was determined as the IC_{50} value for the reduction of IL2 release. Perhaps not surprisingly, compounds of similar potency gave a wide range of functional activity (Table 1). For example, compound **12b** containing a methylpiperazine tail had an IC_{50} value of 49 nM in T cells while the related des methyl analog, **13b**, had an IC_{50} value of 2300 nM. Discrepancies such as this are frequently attributed to off-target activity or physiochemical properties such as solubility and permeability. Correlation of cellular potency with off-target activity and physiochemical parameters will be discussed in detail below.

In spite of the excellent PKCθ potency of this series of compounds, we were unable to obtain a co-crystal structure. Therefore, in order to better understand the emerging SAR, a PKCθ binding model of this series was developed by utilizing the published PKCθ



Scheme 2. Synthesis of 4-aminoalk-1-enyl pyridines. Reagents and conditions: (a) RR′NH, Cs_2CO_3, $Pd(PPh_3)_2Cl_2$, DMSO, 90 °C, 16 h; (b) RR′NH, **5**, Cs_2CO_3, $Pd(PPh_3)_2Cl_2$, DMSO, 90 °C, 16 h; (c) RR′NH, **5**, DMSO, 16 h, rt; (d) Cs_2CO_3, $Pd(PPh_3)_2Cl_2$, 90 °C, 16 h; (e) 4,4,5,5-tetramethyl-1,3,2-dioxaborolane, $Zr(cp)_2ClH$, Et_3N, rt; (f) RR′NH, DMSO, 16 h, rt.

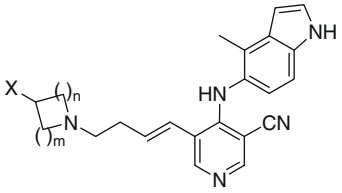


Scheme 3. Synthesis of piperazinyl and 4-aminopiperidine derivatives. Reagents and condition: (a) TFA/DCM, rt.

X-ray structure with staurosporine[26] and various in-house structures of 3-pyridinecarbonitriles with Lck, a related Src family kinase. The binding modes of several specific compounds were further optimized using QXP docking[29] against this PKCθ model. These experiments indicate that the pyridine nitrogen forms the critical hinge region hydrogen bond to Leu461 and the indole-NH forms a hydrogen bonding interaction with Glu428, which is part of Helix C (Fig. 2). The interaction of the indole-NH with this residue is incompatible with the PKCθ staurosporine X-ray structure, but was observed in X-ray structures with Lck. SAR around the indole 'headpiece'[22,27] agrees with the formation of the hydrogen bonding interaction of the indole with the Glu428. Therefore, in order to obtain the binding model described above, the PKCθ X-ray structure was optimized by utilizing the conformation of the glycine-rich loop of Lck in order to accommodate the headpiece in this position (see Section 4). This modification resulted in a PKCθ binding model which was consistent with the SAR observed around both the indole 'headpiece' and alkenyl 'tailpiece' (vide infra).

The SAR shown in Table 1 indicates that the length of the linker between the core and the saturated heterocycle plays an important role in enabling the formation of additional favorable interactions by the alkene tailpiece. Consistent with this SAR, docking studies of compound **4a** (Fig. 2, left panel) suggest that the piperidine nitrogen does not pick up any specific hydrogen bonding interactions with any of the polar or charged amino acids in the binding site. On the other hand, compound **14b** (Fig. 2, right panel) is able to form three additional hydrogen bonding interactions with Asp508 and Asn509 through its charged terminal amine group. These additional interactions result in a 200-fold enhancement of the potency. Interestingly, SAR suggests that this same interaction

Table 2
SAR of the cyclic amine tailpiece



Compd	*n*	*m*	X	PKCθ IC_{50}[a] (nM)	PKCδ IC_{50}[a] (nM)	Fold selectivity	T cell IC_{50}[b] (nM)
4b	2	2	H	35 ± 1.7	270 ± 12	7.5	ND[c]
14b	2	2	NH_2	0.88 ± 0.04	5.8 ± 0.6	6.6	210 ± 59
16	2	2	NHMe	9.3 ± 1.4	28 ± 4.6	3.0	ND
17	2	2	NMe_2	4.9 ± 0.38	56 ± 18	11	630 ± 200
18	2	2	CH_2NH_2	5.7 ± 0.59	33 ± 3.6	5.9	ND
19	1	3	(*R*)-NH_2	1.8 ± 0.35	26 ± 5.1	14	170 ± 34
20	1	3	(*S*)-NH_2	6.8 ± 0.31	29 ± 5.0	4.4	ND
21	1	1	NH_2	8.9 ± 2.2	120 ± 33	13	3700
22	1	2	(*R*)-NH_2	3.5 ± 0.47	15 ± 1.1	4.3	ND
23	1	2	(S)-NH_2	1.3 ± 0.38	18 ± 3.8	14	740 ± 220
24	2	2	$NHSO_2Me$	16 ± 3.3	200 ± 11	12	740 ± 125
25	2	2	$NHSO_2Et$	22 ± 3.0	240 ± 42	11	ND
26	2	2	$NHSO_2iPr$	22 ± 4.7	290 ± 86	13	900
27	2	2	$NHSO_2Ph$	23 ± 0.7	200 ± 67	8.8	ND
28	2	2	NHAc	31 ± 2.9	310 ± 39	10	ND
15b	2	2	OH	6.0 ± 0.09	180 ± 16	30	1600
29	2	2	OMe	42 ± 4.7	220 ± 58	5.2	ND
30	2	2	CH_2OH	39 ± 11	140 ± 11	3.5	ND
31	2	2	Ph	19 ± 6.2	77 ± 6.7	4.0	ND
32	1	2	(*S*)-OH	24 ± 3.9	120 ± 2.7	5.1	ND
33	1	2	(*R*)-OH	7.4 ± 1.5	120 ± 11	16	420 ± 76
34	1	3	(*S*)-OH	120 ± 34	1300 ± 130	11	ND
35	1	3	(*R*)-OH	71 ± 0.25	930 ± 98	13	ND

[a] Represents the average of at least two measurements, ± standard error of the mean.
[b] Represents a single measurement, or the average of at least two measurements ± standard error of the mean.
[c] ND = not determined.

is likely taking place in PKCδ. It is important to reiterate that (based on sequence homology) the only difference between the PKCθ and PKCδ binding pockets is Tyr460, shown adjacent to the hinge binding region in Figure 2. Attempts to exploit this difference by capturing a hydrogen bond from the terminal OH of Tyr460 have been unsuccessful to-date. As illustrated in Figure 2, the location of this residue in proximity to the hinge may make it challenging to access Tyr460 while retaining the key hinge interaction. This difficulty in accessing Tyr460 is likely the underlying reason for the relatively poor theta/delta selectivity of this series of compounds.

As explained above, the amino functionality of **14b** is thought to interact with Asp508/Asn509 resulting in a significant boost in

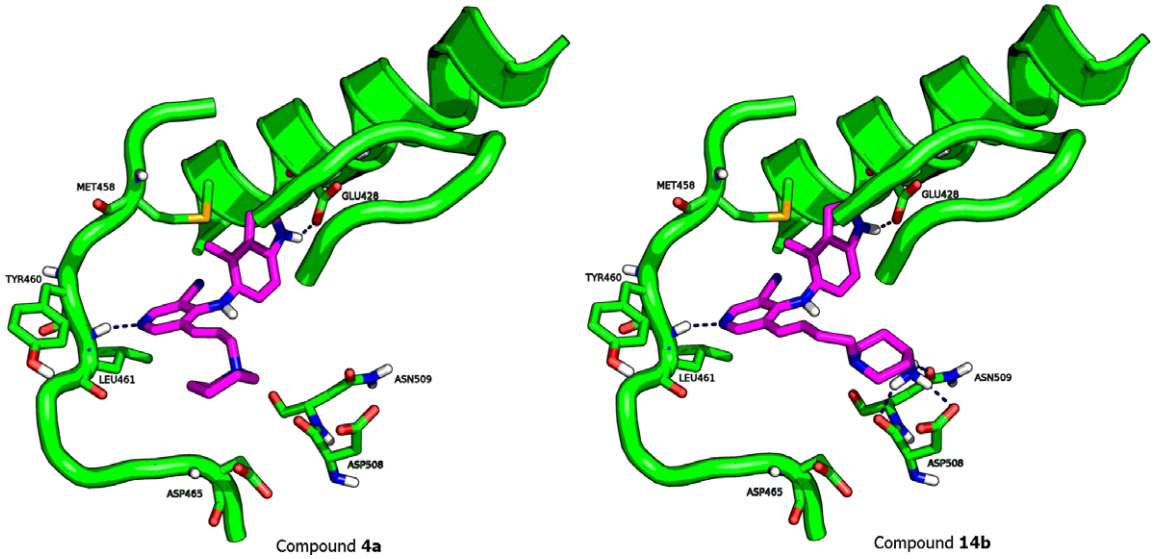
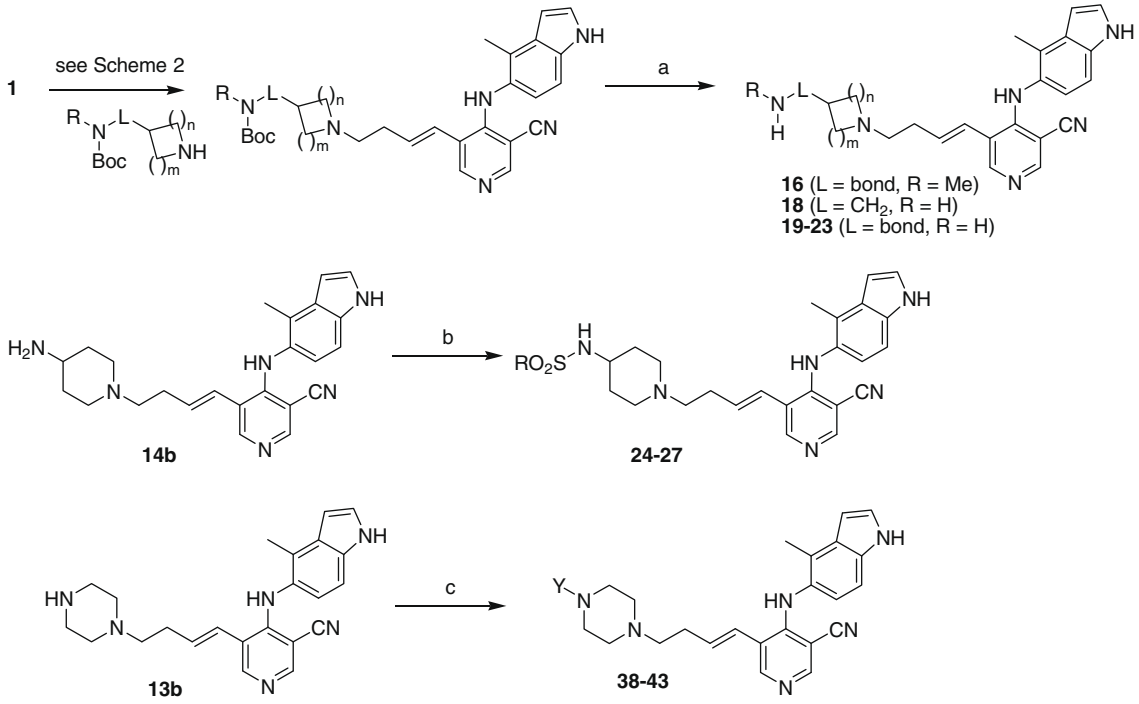


Figure 2. Predicted binding modes for compound **4a** (left) and **14b** (right) with hybrid model of PKCθ. The C Helix is shown in the back of the binding site and the hinge region is shown on the left hand side. Hydrogen bonding interactions are indicated by blue dashed lines. The pyridine core and indole headpiece form critical interactions with the enzyme.



Scheme 4. Synthesis of amino-substituted heterocycles and substituted piperazines. Reagents and conditions: (a) TFA, DCM, rt; (b) RSO_2Cl, Et_3N, DMF, rt; (c) electrophile, Et_3N, DMF, rt.

PKCθ potency over the parent piperidine (**4b**). With this in mind, a further set of analogs were made containing functionalities amenable to potential H-bond and ionic interactions with Asp508/Asn509. These analogs were generally made by the previously described route (Scheme 2) or by minor modifications of the previously described route (Scheme 4). Movement of the 4-amino group of compound **14b** to the 3 position (**19–20**) and extension of the amino group (**18**) resulted in a slight to moderate loss of activity. Likewise, methylation of the amine (**16**, **17**) and contraction of the six-membered ring to a five- or four-membered ring (**21–23**) resulted in a modest loss of PKCθ activity. Enantiomer pairs show that there is a slight preference for the (*R*) enantiomer of 3-aminopiperidines (**19**, **20**) while there is a slight preference for the (*S*) enantiomer of the 3-aminopyrrolidines (**22**, **23**). Sulfonylation or acylation of the amine (**24–28**) were significantly deleterious to PKCθ activity, possibly indicative of a salt bridge between the amine moiety and Asp508. However, there appears to be no major size constraint as both large (**26**, **27**) and small (**24**, **28**) substituents at this position have similar enzymatic activity. Various other 4-substituted piperidines were also examined (**29–35**). Replacement of the amino functionality described above with hydroxy generally resulted in compounds with 10–20× reduced PKCθ activity (**15b**, **32–35**). These results are consistent with the loss of a salt bridge interaction between the amino functionality and Asp508. 4-Hydroxypiperidine **15b** is the only non-amino substituted piperidine exhibiting potency under 10 nM. Interestingly, for reasons that are unclear, this is the only compound of the series that exhibits >25-fold selectivity over PKCδ.

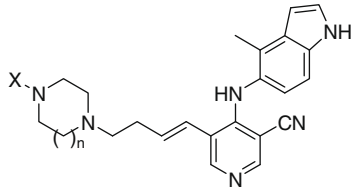
The potent cellular activity of the methylpiperazine analog **12b** prompted further exploration of the piperazinyl moiety (Table 3). It was previously shown (Table 1) that loss of the methyl group from the piperazine resulted in retention of enzymatic potency but loss of cellular potency. Likewise, expansion of the piperazine to a homopiperazine (**36**) resulted in a compound with good PKCθ inhibitory activity (3.6 nM) but poor functional activity (1900 nM). Interestingly, this compound possessed moderate selectivity (16-fold) over PKCδ. Unexpectedly, replacement of the methyl group of **12b** with a phenyl (**37**) resulted in a compound with dramatic loss of PKCθ activity but retention of PKCδ activity. As such, compound **37** was unexpectedly ~20-fold selective *towards PKC*δ. However, replacement of the phenyl group with amides and small sulfonamides (**38–41**) resulted in compounds with very weak potency against PKCδ, thereby possessing up to 39-fold selectivity for PKCθ. Unfortunately, these substituents also resulted in a ~10-fold loss of PKCθ activity and poor microsomal stability (data not shown). Increasing the size of the sulfonamide (**42–43**) resulted in loss of selectivity over PKCδ.

In the course of a related SAR study, we recently reported the synthesis of 6-methyl-3-pyridinecarbonitriles. The compounds were made via palladium mediated reactions with the intermediate **44**.[27] In order to probe the effect of 6-methyl substitution on this series of compounds, **44** was treated under the aforementioned conditions to give compounds **45** and **47** (Scheme 5). Treatment of compound **45** with methanesulfonyl chloride gave sulfonamide **46**. As illustrated in Table 4, substitution with a 6-methyl had a notably detrimental effect on PKCθ activity. No significant effects on selectivity over PKCδ were observed. Reduction of the alkene linker also resulted in a compound (**48**) with modestly reduced PKCθ activity as compared to the parent compound, **14b** (Scheme 6).

3. Discussion and conclusions

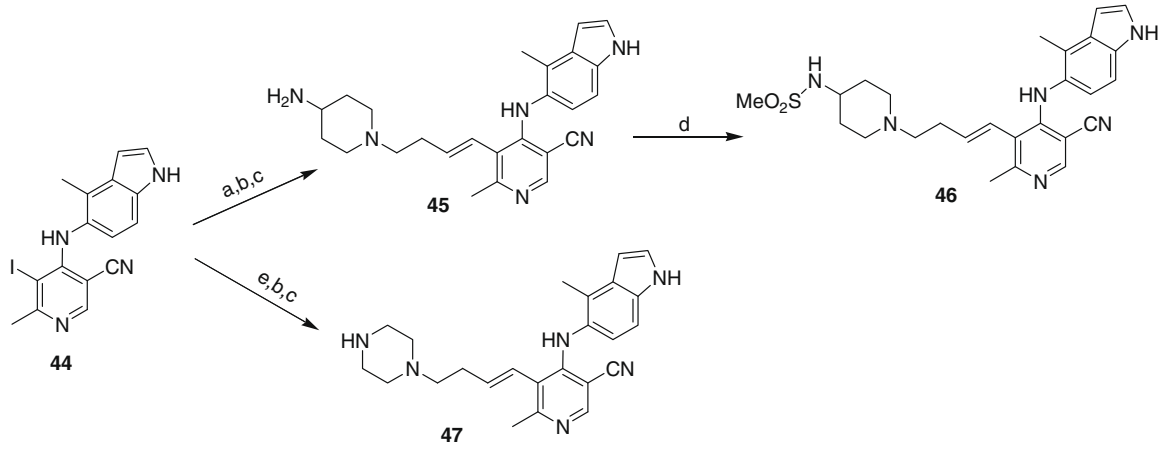
The modeling and SAR studies described above gave us a good understanding of the structural features necessary for potent PKCθ inhibition. However, an examination of the data described in Tables 1–3 illustrates that enzymatic activity alone is not sufficient to achieve functional (cellular) activity. Several compounds, particularly those containing primary and secondary amines such as **13b**, **14a**, and **21**, possessed sub-10 nM potency against the target, but gave only weak (μM) inhibition of IL2 release from T cells. First, in order to rule out activity due to non-PKCθ mediated effects

Table 3
SAR of piperazine tailpiece



Compd	*n*	X	PKCθ IC_{50}[a] (nM)	PKCδ IC_{50}[a] (nM)	Fold selectivity	T cell IC_{50}[b] (nM)
12b	1	Me	2.2 ± 0.66	2.5 ± 0.08	1.2	49 ± 29
13b	1	H	4.0 ± 1.1	16 ± 1.7	4.0	2300
36	2	H	3.6 ± 1.2	56 ± 7.6	16	1900
37	1	Ph	68 ± 13	3.4 ± 0.35	0.05	ND[c]
38	1	Ac	38 ± 1.2	390 ± 31	10	ND
39	1	Pivalate	51 ± 5.1	2000 ± 390	39	ND
40	1	SO_2Me	12 ± 0.68	240 ± 18	20	840
41	1	SO_2Et	12 ± 0.22	270 ± 100	23	ND
42	1	SO_2iPr	11 ± 0.44	81 ± 25	7.4	ND
43	1	SO_2Ph	50 ± 2.6	410 ± 140	8.2	ND

[a] Represents the average of at least two measurements, ± standard error of the mean.
[b] Represents a single meausrement, or the average of at least two measurements ± standard error of the mean.
[c] ND = not determined.



Scheme 5. Synthesis of 6-methyl-5-vinyl-3-pyridinecarbonitriles. Reagents and conditions: (a) 4-(*N*-Boc-amino)piperidine, **5**, 50 °C, DMSO, 16 h; (b) **44**, Cs_2CO_3, $Pd(PPh_3)_2Cl_2$, 90 °C, 16 h; (c) TFA, DCM; (d) $MeSO_2Cl$, Et_3N, DMF, rt; (e) *N*-Boc-piperazine, **5**, 50 °C, DMSO, 16 h.

('off-target' effects), the cellular efficacy of a variety of compounds was measured in T cells isolated from PKCθ knockout (KO) mice.[†] Gratifyingly, the compounds were dramatically less potent in the KO T cells than in the wild-type (WT) T cells (Table 5). This strongly suggests that the functional activity in WT T cells is due to inhibition of PKCθ rather than to off-target effects mediated by other enzymes in the IL2 pathway.

Having established the 'functional selectivity' of this series, it is still necessary to address the exceptionally weak cellular activity of some of the most potent PKCθ inhibitors. In order to gain a more quantitative assessment of this phenomenon, the T cell IC_{50} value was 'normalized' by dividing by the PKCθ IC_{50} thus giving a measure of 'cellular efficiency' ($Cell_{eff}$)

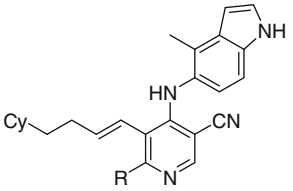
$$Cell_{eff} = T \text{ cell } IC_{50} \text{ (nM)} / PKC\theta \text{ } IC_{50} \text{ (nM)}$$

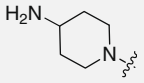
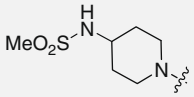
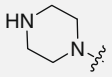
[†] Stimulation of PKCθ KO T cells requires much higher concentrations of anti-CD3 and anti-28 in order to elicit an IL2 response.

$Cell_{eff}$, therefore, is a measure of how effectively a particular inhibitor of PKCθ exerts functional effects on T cells. A low $Cell_{eff}$ indicates that an inhibitor efficiently inhibits functional activity while a high $Cell_{eff}$ indicates that an inhibitor displays a large discrepancy between enzymatic and functional activity. Other than off-target effects, discrepancies between enzymatic and functional activity are frequently attributed to poor physiochemical properties such as low permeability, low solubility, or high polarity. Polarity can be evaluated a number of ways, including 'total polar surface area' (TPSA), *c* Log *P*, and Log *D*. Table 5 illustrates several calculated and measured physiochemical properties of 18 compounds for which both enzymatic and functional activity were measured. The entire set of compounds is highly soluble (typically >100 μg/mL) and clearly this parameter does not play a role in $Cell_{eff}$. Figure 3 illustrates a plot of $Cell_{eff}$ against the remaining physiochemical parameters: *c* Log *P*, Log $D_{7.4}$, PAMPA permeability, and TPSA.

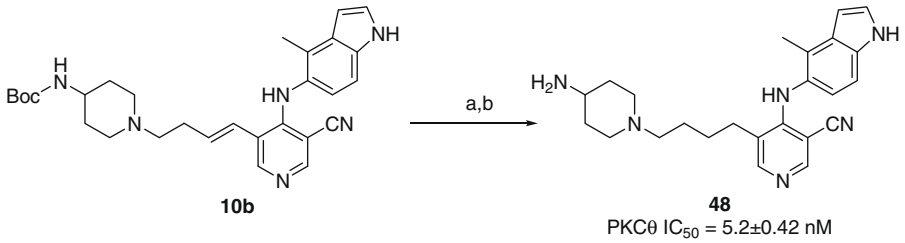
Parallel Artificial Membrane Permeability Assay (PAMPA) is a common model of permeability which measures the flux of a compound across an artificial membrane.[30] PAMPA permeability is fre-

Table 4
Activity of 6-methyl-5-vinyl-3-pyridinecarbonitriles



Compd	Cy	R	PKCθ IC_{50}[a] (nM)	PKCδ IC_{50}[a] (nM)	Fold selectivity
14b		H	0.88 ± 0.04	5.8 ± 0.6	6.6
45		Me	46 ± 0.21	ND[b]	ND
24		H	16 ± 3.3	200 ± 11	12
46		Me	57 ± 5.1	250 ± 26	4.4
13b		H	4.0 ± 1.1	16 ± 1.7	4.0
47		Me	17 ± 2.0	120 ± 12	7.0

[a] Represents the average of at least two measurements, ± standard error of the mean.
[b] ND = not determined.



Scheme 6. Synthesis of 5-alkyl-3-pyridinecarbonitriles. Reagents and conditions: (a) H_2, Pd/C (10%), EtOH, rt; (b) TFA/DCM, rt.

Table 5
Calculated and measured physiochemical properties of key compounds

Compd	PKCθ IC_{50} (nM)	T cell IC_{50} (nM)	$Cell_{eff}$	PKCθ KO T cell IC_{50} (nM)	Permeability (flux, 10^6 cm/s)	TPSA ($Å^2$)	Log $D_{7.4}$	*c* log *P*	Solubility (μg/mL)
12a	31	330	11	8900	0	71.0	2.7	2.8	>100
12b	2.2	49	22	4600	0.86	71.0	3.3	3.1	>100
12c	6.4	77	12	4300	0.07	71.0	3.7	3.6	>100
13a	12	1600	133	>10,000	0.02	79.8	0.8	3.3	>100
13b	4.0	2300	573	ND[a]	0.11	79.8	0.0	3.6	>100
13c	2.8	820	294	ND	0.16	79.8	2.1	4.2	>100
14a	3.6	2700	750	ND	0.03	93.8	1.1	2.9	>100
14b	0.88	210	239	>10,000	0.04	93.8	1.3	3.3	>100
15b	6.0	1600	267	ND	0.02	88.0	2.7	3.2	>100
17	4.9	630	128	>10,000	0.08	71.0	3.2	3.8	>100
19	1.8	170	94	>10,000	ND	93.8	2.1	4.1	>100
21	8.9	3700	415	ND	0.06	93.8	1.3	3.9	>100
23	1.3	740	575	>10,000	0.06	93.8	1.8	3.6	>100
24	16	740	45	ND	ND	113.9	2.4	3.1	>100
26	22	900	40	ND	0.05	113.9	3.4	3.7	>100
33	7.4	420	57	>10,000	0.07	88.0	3.1	3.5	>100
36	3.6	1900	531	ND	0.01	79.8	0.2	3.8	>100
40	12	840	70	>10,000	0.02	105.1	3.8	4.0	30

[a] ND = not determined.

quently utilized to predict absorption through GI membranes, cell membranes, and skin. Therefore, it is perhaps surprising that no discernable relationship was observed between $Cell_{eff}$ and PAMPA permeability. In fact, most compounds exhibited quite poor permeability regardless of their cellular efficacy. The reasons behind this poor permeability are unclear. Likewise, TPSA shows no correlation with cellular efficacy. TPSA is a calculated property that has frequently been shown to be correlated with passive membrane permeability.[31] The TPSA of the molecules in Table 5 is considered moderate (60–140 $Å^2$), which is generally predictive of acceptable permeability and absorption. It is clear from Figure 3 that PAMPA and TPSA play essentially no role in the cellular activity of this series.

The calculated partition coefficient between octanol and water (*c* Log *P*) has become a ubiquitous measure of lipophilicity of small

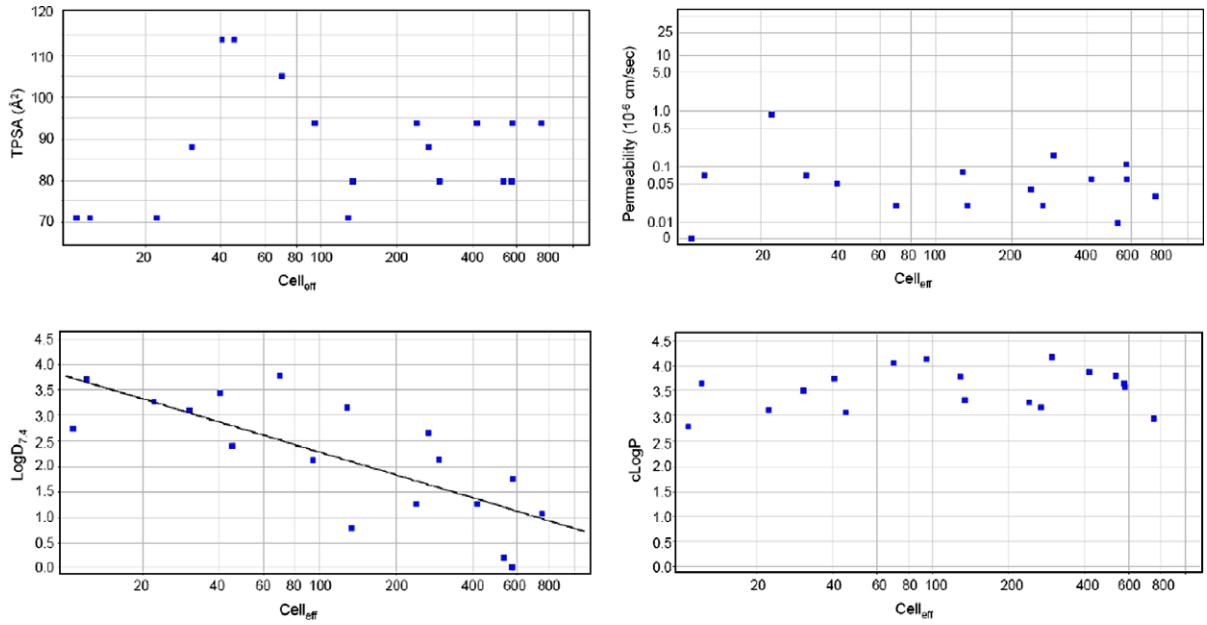


Figure 3. Plots of $Cell_{eff}$ against various physiochemical parameters.

organic molecules. While the focus of *c* Log *P* discussions tends to be on the *upper* limits of this parameter, it is also generally recognized that organic molecules must posses a minimal amount of lipophilicity (*lower* limit of *c* Log *P*) in order to facilitate membrane permeation and absorption.[32] For GI absorption, this lower limit of *c* Log *P* has been shown to be ~3.[33] It is generally accepted that *c* Log *P* accurately reflects the lipophilicity of neutral organic molecules. However, *c* Log *P* is of limited utility for ionizable compounds because the property calculation is made, by definition, on only the neutral form of the organic molecule and does not take into account ionization of various functionalities such as amines and carboxylic acids. Recent advances in computational chemistry have enabled the accurate and timely prediction of Log *D*, which is a partition coefficient which takes into account the ratio of various ionization states that are present a given pH.[34] Compounds that contain multiple ionizable amines, particularly primary and secondary amines, exhibit Log $D_{7.4}$ << *c* Log *P* (i.e., **13a–c**, **14a–b** and **36**). However, compounds with only a single ionizable group such as **12a** and **40** show nearly identical Log *D* and *c* Log *P* values. Therefore, within this data set, Log $D_{7.4}$ appears to be a more relevant measure of polarity than *c* Log *P*.

With this in mind, it is interesting to note that there is a reasonable positive correlation between Log $D_{7.4}$ and $Cell_{eff}$ while there is no measurable correlation between *c* Log *P* and $Cell_{eff}$ (Fig. 3). We believe that compounds with a very low Log $D_{7.4}$ such as **36** and **13b** are too polar to effectively cross cellular membranes and inhibit intracellular PKCθ. The Pearson's coefficient (R^2) for the Log *D*/$Cell_{eff}$ correlation is 0.57 which suggests that, as expected, there are factors other than Log $D_{7.4}$ that play a role in cellular potency (permeability, binding kinetics, etc.). Based on the correlation illustrated in Figure 3, it is our belief that optimal cellular efficacy can be achieved with molecules possessing Log $D_{7.4} \geqslant 2.5$.

As previously described, the PKCθ inhibitors were routinely screened against PKCδ due to concerns about possible deleterious effects upon B cells. In addition, select compounds of interest were screened against a broader panel of PKC isoforms containing representative examples of each of the three families of PKCs: novel, atypical, and classical. PKCθ and PKCδ are both novel PKC isoforms and, as such, it is hardly surprising that the compounds shown in Table 6 also have significant activity against the two remaining novel isoforms, PKCε and PKCη. Only compounds **14b** and **19** showed modest selectivity against all three novel isoforms. Of the novel isoforms, good selectivity (>100-fold) could only be achieved against PKCη. Perhaps not surprisingly, the compounds did not show significant inhibition of one representative example of the atypical and classical isoform families. The implications of activity against PKCε are unclear at this time. Overexpression of PKCε is associated with increased tumorogenesis in nude mice[35] and its inhibition is speculated to be a possible target for oncology drugs.[16]

3-Quinolinecarbonitriles and related scaffolds are known to inhibit Src family kinases.[36] Due to concerns about structural similarities between the 3-quinolinecarbonitriles and the 5-vinyl-3-pyridinecarbonitriles, the compounds in Table 6 were screened against two Src family kinases, Lck and Lyn. Lyn is of particular concern due to reports that Lyn KO mice possess a hyperresponsive B cell phenotype.[37] Gratifyingly, the compounds described in Table 6 generally showed well over 1000-fold selectivity over both Lyn and Lck. It is generally thought that kinase selectivity within the 3-quinolinecarbonitrile scaffold is largely driven by the group at

Table 6
Selectivity of select compounds against PKC isoforms and Src family kinases

Compd	Novel				Atypical	Classical	Src family	
	PKCθ IC_{50} (nM)	PKCδ IC_{50} (nM)	PKCε IC_{50} (nM)	PKCη IC_{50} (nM)	PKCζ IC_{50} (nM)	PKCβ IC_{50} (nM)	Lyn IC_{50} (nM)	Lck IC_{50} (nM)
13a	12	45	16	550	>100,000	>100	30,000	ND
13b	4.0	5.8	2.5	190	>100,000	2500	5100	1200
14b	0.88	27	6.5	100	>100,000	2300	9600	600
19	1.8	18	14	350	>100,000	>100,000	19,000	2900
24	16	26	8.0	88	>100,000	1400	20,000	47,000

the 4-position of the quinoline. This suggests that the 4-indolyl headpiece is not tolerated within the Src family ATP pocket or, alternatively, that the fused carbocyclic ring of the quinoline is necessary for Src family activity (note that compound **19** is selective over 3 additional Src family kinases, vide infra).

Compound **19** was screened against a broader panel of kinases as illustrated in Table 7. With the exception of modest activity against Src and PKA, only minimal inhibition was observed. Selectivity was generally well over 500-fold for 20 out of the 22 kinases tested. The selectivity against Src and PKA was >250-fold.

Based on enzymatic potency, kinase selectivity, and functional activity, compound **19** was selected for further evaluation in order to determine its suitability for advancement. The compound was shown to have no activity against CYP2C9 and modest activity against CYP3A4 and CYP2D6 (46% and 53% inhibition, respectively, at 3 μM). The compound proved to be exceptionally stable in rat and human microsomes ($T_{1/2}$ >30 min) and moderately stable in BALB/c mouse microsomes ($T_{1/2}$ = 22 min).[39] This suggests that first-pass metabolism is not likely to adversely affect oral exposure. However, the $CaCo_2$ permeability (A to B) for this compound was poor (0.5×10^{-6} cm/s), suggesting poor intestinal absorption. Moreover, the ratio of permeability (A to B vs B to A) was 16, strongly suggesting that **19** may be subject to active efflux, possibly mediated by PGP efflux pumps. PGP is widely expressed in intestine, kidney, and liver barriers and is frequently associated with poor intestinal absorption and biliary excretion.[40,41] Consistent with these observations, compound **19** was found to have high clearance (128 mL/min/kg, 2 mpk IV) and poor oral bioavailability (3%, 10 mpk PO) in BALB/c mice. The acceptable PK of related 5-heteroaryl-3-pyridinecarbonitriles[25] suggests that the poor characteristics of **19** are related to the vinyl side chain rather than the pyridine core or indole headpiece.

In summary, we have described 5-vinyl-3-pyridinecarbonitriles that exhibit excellent PKCθ inhibitory activity. SAR and molecular modeling studies indicate that an H-bond donor extending from the alkene side chain makes three key interactions with Asp508 and Asn509. Cellular activity of this series of compounds was found to be poorly correlated with enzymatic activity. However, a combination of good enzymatic activity and a high Log $D_{7.4}$ resulted in compounds with low nM cellular activity. Representative members of this series of compounds were found to have modest selectivity over other novel PKC isoforms. However, the compounds show excellent selectivity over a wide range of other kinases, including atypical and classical PKCs. Additional studies describing the advancement of this series of compounds will be disclosed in due course.

Table 7
Activity of 19 against a broad panel of kinases

Kinase	IC_{50} (nM)
Abl	3400
AuroraB	41,000
CDK1	26,000
CDK2	17,000
CHK1	4500
CK1 gamma	>50,000
ERK2	>50,000
Fyn	1700
Gck	2600
Hck	5500
IKKα	35,000
IKKβ	1000
Met	34,000
MK2	22,000
P38α	32,000
PDGFRα	10,000
PKA	490
PKBα	2400
ROCK1	>50,000
RSK1	11,000
Src	600
VEGFR2	3800

4. Experimental section

4.1. Modeling and docking experiments

The hybrid PKCθ model was developed with the PKCθ-Staurosporine X-ray structure as the starting point. The glycine-rich loop was modeled after an X-ray structure of Lck in complex with a compound from the related thienopyridine series using PRIME 1.2.[‡] The hybrid model was subsequently optimized further using the Induced Fit Docking protocol.[§]

Docking studies were conducted with the QXP docking algorithm[29] utilizing the mcdock+ or mcldock+ sampling and scoring scheme. Generally, 5000 Monte Carlo steps were used and if necessary a core constraint of the pyridine core and the indole headpiece was used through the mcldock+ procedure in QXP. The PKCθ hybrid model was used throughout all docking studies.

4.2. Calculated physiochemical properties

TPSA was calculated by the method described by Ertl et al.[38] Calculations assume sulfur and phosphorous are not polar atoms. *c* Log *P* and Log *D* were calculated using the Biobyte 4.3 algorithm as implemented in Daylight, version 4.81.

4.3. General experimental conditions

The following HPLC methods were utilized to evaluate compound purity.

4.3.1. Method A
1.0 mL/min, 20 min gradient ACN (10–95%) in H_2O/TFA, Prodigy ODS3, 0.46 × 15 cm column.

4.3.2. Method B
1.0 mL/min, 20 min gradient MeOH (10–95%) in H_2O/TFA, Prodigy ODS3, 0.46 × 15 cm column.

4.3.3. Method C
1.0 mL/min, 5 min gradient ACN (10–95%) in H_2O/formic acid, waters Xterra MS C18 3.5 μm 2.1 × 30 mm column.

4.3.4. Method D
1.0 mL/min, 5 min gradient ACN (10–95%) in H_2O/NH_4OH, waters Xterra MS C18 3.5 μm 2.1 × 30 mm column.

4.3.5. Method E
1.0 mL/min, 10 min gradient ACN (10–95%) in H_2O/TFA, Prodigy ODS3, 0.46 × 15 cm column.

4.4. Synthetic examples

4.4.1. 4-[(4-Methyl-1*H*-indol-5-yl)amino]-5-[(1*E*)-3-piperidin-1-ylprop-1-en-1-yl]nicotinonitrile (4a)
A mixture of **1**[23] (100 mg, 0.27 mmol), piperidine (70 mg, 0.81 mmol), *trans*-2-chloromethylvinylboronic acid (**2**) (64 mg, 0.53 mmol), cesium carbonate (173 mg, 0.53 mmol) and

[‡] Prime, version 1.2, Schrödinger, LLC, New York, NY 2005.
[§] Induced Fit Docking Using Glide and Prime; Glide version 3.5, Schrödinger, LLC, New York, NY, 2005; Prime version 1.2, Schrödinger, LLC, New York, NY, 2005.

$Pd(PPh_3)_2Cl_2$ (9.5 mg, 0.014 mmol) in 1 mL of DMSO was heated at 90 °C for 16 h, cooled to room temperature, and filtered. The filtrate was concentrated and purified by HPLC to give 97 mg (74% yield) of **4a** as its TFA salt. ^{1}H NMR (DMSO-d_6) δ 11.25 (1H, s), 9.65 (1H, br s), 9.47 (1H, s), 8.59 (1H, s), 8.54 (1H, s), 7.39 (1H, t, J = 4.2 Hz), 7.27 (1H, d, J = 6.0 Hz), 6.99 (1H, d, J = 11.4 Hz), 6.94 (1H, d, J = 6.3 Hz), 6.55 (1H, s), 6.33 (1H, dt, J = 5.5, 11.7 Hz), 3.82 (2H, t, J = 4.0 Hz), 3.49–3.44 (2H, m), 2.91 (2H, dd, J = 7.2, 16.2 Hz), 2.32 (3H, s), 1.88–1.35 (6H, m); HPLC 94.9% at 215 nm, 4.8 min (Method A); MS (ESI) m/z 372.2187 (M+H).

4.4.2. 4-[(4-Methyl-1*H*-indol-5-yl)amino]-5-[(1*E*)-4-piperidin-1-ylbut-1-en-1-yl]nicotinonitrile (4b)

A mixture of piperidine (104 mg, 1.2 mmol) and (*E*)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)but-3-enyl 4-methylbenzenesulfonate **5** (200 mg, 0.57 mmol) was stirred in 2 mL of DMSO. After stirring for 16 h at room temperature, **1** (108 mg, 0.29 mmol), cesium carbonate (186 mg, 0.57 mmol) and $Pd(PPh_3)_4$ (17 mg, 0.015 mmol) were added. After heating to 70°C for 16 h, the reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated and purified by HPLC to give 73 mg (50 % yield) of **4b** as its TFA salt; ^{1}H NMR (DMSO-d_6) δ 11.25 (1H, s), 9.44 (1H, br s), 9.23 (1H, br s), 8.56 (1H, s), 8.38 (1H, s), 7.40 (1H, t, J = 2.0 Hz), 7.28 (1H, d, J = 6.3 Hz), 9.95 (1H, d, J = 6.3 Hz), 6.68 (1H, d, J = 11.7 Hz), 6.55 (1H, s), 6.24 (1H, dt, J = 5.0, 12 Hz), 3.44 (2H, d, J = 9 Hz), 3.19–3.14 (2H, m), 2.88 (2H, q, J = 8.3 Hz), 2.61 (2H, q, J = 5.3 Hz), 2.32 (3H, s), 1.87–1.37 (6H, m); HPLC 96% at 254 nm, 4.2 min (Method E); MS (ESI) m/z 386.2340 (M+H).

4.4.3. 4-[(4-Methyl-1*H*-indol-5-yl)amino]-5-[(1*E*)-5-piperidin-1-ylpent-1-en-1-yl]nicotinonitrile (4c)

A mixture of piperidine (90 mg, 1.06 mmol) and (*E*)-2-(5-chloropent-1-enyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane **8** (122 mg, 0.53 mmol) was stirred in 1 mL of DMSO. After stirring for 16 h at room temperature, **1** (100 mg, 0.27 mmol), cesium carbonate (173 mg, 0.53 mmol) and $Pd(PPh_3)_4$ (16 mg, 0.014 mmol) were added to the reaction mixture. The resulting suspension was heated at 70°C for 16 h. After cooling to room temperature and filtering, the filtrate was concentrated and purified by HPLC to give 53 mg (38 % yield) of **4c** as its TFA salt; ^{1}H NMR (DMSO-d_6) δ 11.25 (1H, s), 9.60 (1H, br s), 9.35 (br s, 1H), 8.60 (1H, s), 8.40 (1H, s), 7.40 (1H, t, J = 2.8 Hz), 7.28 (1H, d, J = 8.4 Hz), 6.95 (1H, d, J = 8.4 Hz), 6.64 (1H, d, J = 16 Hz), 6.55 (1H, s), 6.33 (1H, dt, J = 6.6, 15.6 Hz), 3.46–3.41 (2H, m), 3.09–3.05 (2H, m), 2.91–2.83 (2H, m), 2.32 (3H, s), 2.26 (2H, q, J = 6.8 Hz), 1.88–1.36 (8H, m); HPLC 94.5% at 215 nm, 5.1 min (Method A); MS (ESI) m/z 400.2500 (M+H).

4.4.4. (3*E*)-4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)but-3-en-1-yl-4-methylbenzenesulfonate (5)

A mixture of but-3-ynyl 4-methylbenzenesulfonate (5 g, 22.3 mmol), 4,4,5-tetramethyl-1,3,2-dioxaborolane (4.3 g, 33.5 mmol), bis(cyclopentadienyl)-zirconium(IV) chloride hydride (864 mg, 3.35 mmol) and triethylamine (225 mg, 2.23 mmol) were stirred neat at room temperature for 24 h. The reaction was dissolved in DCM, filtered through a pad of silica gel and washed with DCM. The eluent was removed to give 5.62 g (72% yield) of **5** as a white solid; ^{1}H NMR (DMSO-d_6) δ 11.28 (1H, s), 10.07 (1H, s), 8.69 (1H, s), 8.56 (1H, s), 7.41 (1H, t, J = 2.6 Hz), 7.29 (1H, d, J = 8.4 Hz), 7.08–6.91 (3H, m), 6.59–6.49 (2H, m), 5.52 (1H, d, J = 17.2 Hz), 5.37 (1H, d, J = 11.2 Hz), 2.33 (3H, s); MS 353.2 (M+H).

4.4.5. 5-[(1*E*)-Buta-1,3-dien-yl]-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (6)

A mixture of **1** (100 mg, 0.27 mmol), indoline (95 mg, 0.80 mmol), (*E*)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-but-3-enyl 4-methylbenzenesulfonate (**5**) (187 mg, 0.53 mmol), cesium carbonate (173 mg, 0.53 mmol) and $Pd(PPh_3)_4$ (16 mg, 0.014 mmol) in 3 mL of DMSO was heated at 70 °C for 16 h, cooled to room temperature, diluted with EtOAc and washed with water. The crude material was purified by silica gel chromatography with an EtOAc/hexane gradient to give 21 mg (26% yield) of **6**; MS 301.2 (M+H).

4.4.6. (*E*)-2-(5-Chloropent-1-enyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (8)

A mixture of 5-chloro-1-pentyne **7** (500 mg, 4.9 mmol), 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (947 mg, 7.4 mmol), bis(cyclopentadienyl)-zirconium(IV) chloride hydride (191 mg, 0.74 mmol) and triethylamine (49.5 mg, 0.49 mmol) were stirred neat at room temperature for 24 h. The reaction was dissolved in DCM, filtered through a pad of silica gel and washed with DCM. The eluent was removed to give 824 mg (73% yield) of **8** as a clear yellow liquid; ^{1}H NMR (DMSO-d_6) δ 6.50 (1H, dt, J = 6.4, 18 Hz), 5.37 (1H, dt, J = 1.6, 18 Hz), 3.61 (2H, t, J = 6.6 Hz), 2.29–2.18 (2H, m), 1.88–1.78 (2H, m), 1.19 (12H, s); MS 231.2 (M+H).

4.4.7. *tert*-Butyl 4-[(2*E*)-3-{5-cyano-4-[(4-methyl-1*H*-indol-5-yl)-amino]pyridine-3-yl}prop-2-en-1-yl]piperazine-1-carboxylate (9a)

Compound **9a** was prepared from **1** and *tert*-butyl piperazine-1-carboxylate using the procedure described for **4a** and purified by silica gel chromatography using a MeOH/DCM gradient to give 239 mg (96% yield) as a solid. ^{1}H NMR (DMSO-d_6) δ 11.14 (1H, s), 8.59 (1H, s), 8.37 (1H, s), 8.25 (1H, s), 7.34 (1H, t, J = 2.6 Hz), 7.22 (1H, d, J = 8.8 Hz), 6.89 (1H, d, J = 8.8 Hz), 6.73 (1H, d, J = 15.2 Hz). 6.50 (1H, s), 6.25–6.17 (m, 1H), 3.33–3.28 (4H, m), 3.08 (2H, d, J = 6.4 Hz), 2.36–2.27 (4H, m), 2.28 (3H, s), 1.38 (9H, s); MS 473.4 (M+H).

4.4.8. *tert*-Butyl 4-[(3*E*)-4-{5-cyano-4-[(4-methyl-1*H*-indol-5-yl)amino]pyridin-3-yl}but-3-en-1-yl]piperazine-1-carboxylate (9b)

Compound **9b** was prepared from **1** and *tert*-butyl piperazine-1-carboxylate using the procedure described for **4b** and purified by HPLC to give 83 mg (40% yield) TFA salt; ^{1}H NMR (DMSO-d_6) δ 11.22 (1H, s), 9.69 (1H, br s), 9.24 (1H, br s), 8.50 (1H, s), 8.38 (1H, s), 7.39 (1H, t, J = 2.6 Hz), 7.28 (1H, d, J = 8.4 Hz), 6.94 (1H, d, J = 8.4 Hz), 6.70 (1H, d, J = 15.6 Hz), 6.55 (1H, s), 6.29–6.18 (1H, m), 4.08–3.99 (4H, m), 3.55–2.90 (8H, m), 2.32 (3H, s), 1.42 (9H, s); MS 487.4 (M+H).

4.4.9. *tert*-Butyl 4-[(4*E*)-5-{5-cyano-4-[(4-methyl-1*H*-indol-5-yl)amino]pyridin-3-yl}pent-4-en-1-yl]piperazine-1-carboxylate (9c)

Compound **9c** was prepared from **1** and *tert*-butyl piperazine-1-carboxylate using the procedure described for **4c** and purified by silica gel chromatography using a MeOH/DCM gradient to give 130 mg (49% yield) of **9c** as a solid. ^{1}H NMR (DMSO-d_6) δ 11.12 (1H, s), 8.52 (1H, s), 8.30 (1H, s), 8.21 (1H, s) , 7.34 (1H, t, J = 2.8 Hz) 7.22 (1H, d, J = 8.4 Hz), 5.88 (1H, d, J = 8.8 Hz), 6.59 (1H, d, J = 15.6 Hz), 6.50 (1H, s), 6.28–6.19 (1H, m), 3.32–3.26 (2H, m), 2.35–2.25 (8H, m), 2.18 (2H, q, J = 7.0 Hz). 1.60 (2H, quint, J = 7.0 Hz), 1.39 (9H, s); MS 501.5 (M+H).

4.4.10. *tert*-Butyl {1-[(2*E*)-3-{5-cyano-4-[(4-methyl-1*H*-indol-5-yl)amino]pyridine-3-yl}prop-2-en-1-yl]piperidin-4-yl}carbamate (10a)

Compound **10a** was prepared from **1** and *tert*-butyl piperidin-4-ylcarbamate using the procedure described for **4a** and purified by silica gel chromatography using a MeOH/DCM gradient to give 110 mg (42% yield) of **10a** as a solid. ^{1}H NMR (DMSO-d_6) δ 11.13 (1H, s), 8.60 (1H, s), 8.37 (1H, s), 8.24 (1H, s), 7.34 (1H, t,

J = 2.6 Hz), 7.22 (1H, d, *J* = 8.4 Hz), 6.89 (1H, d, *J* = 8.4 Hz), 6.73 (1H, d, *J* = 15.6 Hz), 6.50 (1H, s), 6.21 (1H, dt, *J* = 6.6, 15.6 Hz), 3.35–2.78 (7H), 2.28 (3H, s), 2.03–1.88 (2H, m), 1.73–1.65 (2H, m), 1.38 (9H, s); MS 487.5 (M+H).

4.4.11. *tert*-Butyl {1-[(3*E*)-4-{5-cyano-4-[(4-methyl-1*H*-indol-5-yl)amino]pyridin-3-yl}but-3-en-1-yl]piperidin-4-yl}carbamate (10b)

Compound **9b** was prepared from **1** and *tert*-butyl piperidin-4-ylcarbamate using the procedure described for **4b** and purified by HPLC to give 122 mg (39% yield) of **10b** as its TFA salt; ^{1}H NMR (DMSO-d_6) δ 11.24 (1H, s), 9.39 (1H, br s), 9.21 (1H, br s), 8.54 (1H, 8.38 (1H, s), 7.40 (1H, t, *J* = 2.8 Hz), 7.28 (1H, d, *J* = 8.4 Hz), 7.10 (1H, t, *J* = 5.6 Hz), 6.94 (1H, d, *J* = 8.4 Hz), 6.67 (1H, d, *J* = 15.6 Hz), 6.55 (1H, s), 6.23 (1H, dt, *J* = 6.8, 15.6 Hz), 3.51 (2H, d, *J* = 11.2 Hz) 3.23–2.93 (5H, m), 2.62–2.45 (2H, m), 2.32 (3H, s), 1.96 (2H, d, *J* = 12 Hz), 1.60 (2H, q, *J* = 12.3 Hz), 1.39 (9H, s); MS 501.4 (M+H).

4.4.12. *tert*-Butyl {1-[(4*E*)-5-{5-cyano-4-[(4-methyl-1*H*-indol-5-yl)amino]pyridin-3-yl}pent-4-en-1-yl]piperidin-4-yl}carbamate (10c)

Compound **10c** was prepared from **1** and *tert*-butyl piperidin-4-ylcarbamate using the procedure described for **4c** and purified by silica gel chromatography using a MeOH/DCM gradient to give 114 mg (29% yield) of **10c** as a solid. ^{1}H NMR (DMSO-d_6) δ 11.12 (1H, s), 8.53 (1H, s) 8.29 (1H, s), 8.21 (1H, s), 7.34 (1H, t, *J* = 2.6 Hz), 7.22 (1H, d, *J* = 8.4 Hz), 6.89 (1H, d, *J* = 8.0 Hz), 6.75 (1H, br s), 6.59 (1H, d, *J* = 15.2 Hz), 6.50 (1H, s), 6.23 (1H, dt, *J* = 6.4, 15.6 Hz), 3.36–3.13 (3H, m), 2.85–2.72 (2H, m), 2.28 (3H, s), 2.16 (1H, q, *J* = 7.2 Hz), 1.89–1.52 (6H, m), 1.37 (9H, s); MS 515.5 (M+H).

4.4.13. 4-[(4-Methyl-1*H*-indol-5-yl)amino]-5-[(1*E*)-3-morpholin-4-ylprop-1-en-1-yl]nicotinonitrile (11a)

Compound **11a** was prepared from **1** and morpholine using the procedure described for **4a** and purified by HPLC. The HCl salt was generated to give 33 mg (30% yield) of **11a** as a solid; ^{1}H NMR (DMSO-d_6) δ 11.81 (1H, s), 11.34 (1H, s), 10.30 (1H, s), 8.79 (1H, s), 8.57 (1H, s), 7.41 (1H, t, *J* = 2.6 Hz), 7.30 (1H, d, *J* = 8.8 Hz), 7.11 (1H, d, *J* = 15.6 Hz), 6.99 (1H, d, *J* = 8.8 Hz), 6.57 (1H, s), 6.48 (1H, dt, *J* = 7.2, 15.6 Hz), 4.02–3.78 (6H, m), 3.50–3.42 (2H, m), 3.17–3.07 (2H, m), 2.35 (3H, s); HPLC 97.4% at 215 nm, 3.95 min (Method E); MS (ESI) *m*/*z* 394.1980 (M+H).

4.4.14. 4-[(4-Methyl-1*H*-indol-5-yl)amino]-5-[(1*E*)-4-morpholin-4-ylbut-1-en-1-yl]nicotinonitrile (11b)

A mixture of **1** (150 mg, 0.40 mmol), morpholine (104 mg, 1.2 mmol), (*E*)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)but-3-enyl 4-methylbenzenesulfonate (**5**) (281 mg, 0.80 mmol), cesium carbonate (261 mg, 0.80 mmol) and Pd(PPh$_3$)$_4$ (23 mg, 0.02 mmol) in 4 mL of DMSO was heated at 70 °C for 16 h, cooled to room temperature, diluted with EtOAc and washed with water. The crude extracts were purified by silica gel chromatography with a MeOH/DCM gradient to give **11b**. The product was treated with excess HCl/dioxane giving 53 mg of the HCl salt of **11b** (31% yield); ^{1}H NMR (DMSO-d_6) δ 11.36 (1H, br s), 11.31 (1H, s), 10.07 (1H, s), 8.71 (1H, s), 8.57 (1H, s), 7.41 (1H, t, *J* = 2.6 Hz), 7.29 (1H, d, *J* = 8.4 Hz), 6.98 (1H, d, *J* = 8.4 Hz), 6.75 (1H, d, *J* = 15.2 Hz), 6.57 (1H, s), 6.37 (1H, dt, *J* = 6.8, 15.6 Hz), 3.99–3.82 (6H, m), 3.29–3.23 (2H, m), 3.25–3.01 (2H, m), 2.72 (2H, q, *J* = 6.8 Hz), 2.33 (3H, s); HPLC 100% at 215 nm, 4.14 min (Method E); MS (ESI) *m*/*z* 386.2 (M−H).

4.4.15. 4-[(4-Methyl-1*H*-indol-5-yl)amino]-5-[(1*E*)-5-morpholin-4-ylpent-1-en-1-yl]nicotinonitrile (11c)

Compound **11c** was prepared from **1** and morpholine using the procedure described for **4c** giving 86 mg (58% yield) as its TFA salt; ^{1}H NMR (DMSO-d_6) δ 11.25 (1H, s), 10.03 (1H, br s), 9.58 (1H, s), 8.59 (1H, s), 8.40 (1H, s), 7.40 (1H, t, *J* = 2.8 Hz), 7.28 (1H, d, *J* = 8.4 Hz), 6.95 (1H, d, *J* = 8.0 Hz), 6.65 (1H, d, *J* = 15.2 Hz), 6.55 (1H, s), 6.32 (1H, dt, *J* = 6.6, 15.6 Hz), 4.00 (2H, d, *J* = 11.6 Hz), 3.67 (2H, t, *J* = 1.8 Hz), 3.49–4.50 (2H, m), 3.19–3.01 (4H, m), 2.32 (3H, s), 2.28 (2H, q, *J* = 6.8 Hz), 1.90–1.80 (2H, m); HPLC 100% at 215 nm, 4.22 min (Method E); MS (ESI) *m*/*z* 402.2293 (M+H).

4.4.16. 4-[(4-Methyl-1*H*-indol-5-yl)amino]-5-[(1*E*)-3-(4-methylpiperazin-1-yl)prop-1-en-1-yl]nicotinonitrile (12a)

Compound **12a** was prepared from **1** and 1-methylpiperazine using the procedure described for **4a** giving 54 mg (44% yield) as its HCl salt; ^{1}H NMR (DMSO-d_6) δ 11.30 (1H, s), 10.08 (1H, br s), 8.74 (1H, s), 8.54 (1H, s) 7.41 (1H, t, *J* = 2.8 Hz), 7.29 (1H, d, *J* = 8.4 Hz), 7.08 (1H, d, *J* = 14.8 Hz), 6.98 (1H, d, *J* = 8.4 Hz), 6.56 (1H, s), 6.42 (1H, dt, *J* = 7.4, 15.6 Hz), 3.95–3.27 (10H, m), 2.83 (3H, s), 2.34 (3H, s); HPLC 100% at 215 nm, 3.90 min (Method E); MS (ESI) *m*/*z* 387.2297 (M+H).

4.4.17. 4-[(4-Methyl-1*H*-indol-5-yl)amino]-5-[(1*E*)-3-(4-methylpiperazin-1-yl)but-1-en-1-yl]nicotinonitrile (12b)

A mixture of **1** (100 mg, 0.27 mmol), 1-methylpiperazine (80 mg, 0.80 mmol), (*E*)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-but-3-enyl 4-methylbenzenesulfonate (**5**) (187 mg, 0.53 mmol), cesium carbonate (173 mg, 0.53 mmol) and Pd(PPh$_3$)$_4$ (16 mg, 0.014 mmol) in 3 mL of DMSO was heated at 70 °C for 16 h, cooled to room temperature, and filtered. The filtrate was concentrated and purified by HPLC to give **12b** which was converted to its HCl by addition of HCl/dioxane (13 mg, 10% yield); ^{1}H NMR (DMSO-d_6) δ 11.29 (1H, s), 9.99 (1H, br s), 8.69 (1H, s) 8.51 (1H, s), 7.41 (1H, t, *J* = 2.8 Hz), 7.29 (1H, d, *J* = 8.4 Hz), 6.97 (1H, d, *J* = 8.4 Hz), 6.73 (1H, d, *J* = 12.4 Hz), 6.55 (1H, s), 6.35 (1H, dt, *J* = 7.2, 15.6 Hz), 3.65–3.20 (10H, m), 2.82 (3H, s), 2.72–2.62 (2H, m), 2.33 (3H, s); HPLC 97.7% at 215 nm, 5.1 min (Method B); MS (ESI) *m*/*z* 401.2450 (M+H).

4.4.18. 4-[(4-Methyl-1*H*-indol-5-yl)amino]-5-[(1*E*)-5-(4-methylpiperazin-1-yl)pent-1-en-1-yl]nicotinonitrile (12c)

Compound **12c** was prepared from **1** and 1-methylpiperazine using the procedure described for **4c** giving 13 mg (10% yield) as its TFA salt; ^{1}H NMR (DMSO-d_6) δ 11.26 (1H, s), 9.66 (1H, s), 8.61 (1H, s), 8.39 (1H, s), 7.40 (1H, t, *J* = 2.8 Hz), 7.29 (1H, d, *J* = 8.4 Hz), 6.96 (1H, d, *J* = 8.4 Hz), 6.64 (1H, d, *J* = 15.2 Hz), 6.55 (1H, s), 6.35 (1H, dt, *J* = 6.8, 15.2 Hz), 3.55–2.92 (10H, m), 2.80 (3H, s), 2.32 (3H, s), 2.26 (2H, q, *J* = 6.8 Hz), 1.81–1.76 (2H, m); HPLC 92.9% at 215 nm, 5.9 min (Method B); MS (ESI) *m*/*z* 415.2608 (M+H).

4.5. General procedure for the synthesis of (13a–c) and (14a–c)

Boc-protected amines **9a–c** and **10a–c** were dissolved in 10% TFA/DCM. After stirring at room temperature for 1–3 h, the solvents were removed and the residue was purified by HPLC or flash chromatography.

4.5.1. 4-[(4-Methyl-1*H*-indol-5-yl)amino]-5-[(1*E*)-3-piperazin-1-ylprop-1-en-1-yl]nicotinonitrile (13a)

33.6 mg (27% yield); ^{1}H NMR (DMSO-d_6) δ 11.13 (1H, s), 8.58 (1H, s), 8.36 (1H, s), 8.24 (1H, s), 7.34 (1H, t, *J* = 2.6 Hz), 7.22 (1H, d, *J* = 8.8 Hz), 6.89 (1H, d, *J* = 8.4 Hz), 6.50 (1H, s), 6.20 (1H, dt, *J* = 6.8, 15.2 Hz), 3.02 (2H, d, *J* = 6.4 Hz), 2.68 (4H, t, *J* = 4.8 Hz), 2.42–2.25 (4H, m), 2.29 (3H, s); HPLC 98.7% at 215 nm, 3.7 min (Method A); MS (ESI) *m*/*z* 373.2136 (M+H).

4.5.2. 4-[(4-Methyl-1*H*-indol-5-yl)amino]-5-[(1*E*)-4-piperazin-1-ylbut-1-en-1-yl]nicotinonitrile (13b)

28 mg (41% yield) as its TFA salt; ^{1}H NMR (DMSO-d_6) δ 11.25 (1H, s), 9.51 (1H, br s), 9.01 (1H, br s), 8.58 (1H, s), 8.37 (1H, s),

7.40 (1H, t, J = 2.8 Hz), 7.28 (1H, d, J = 8.4 Hz), 6.95 (1H, d, J = 8.4 Hz), 6.69 (1H, d, J = 15.6 Hz), 6.28 (1H, dt, J = 6.6, 15.6 Hz), 3.36–3.05 (10H, m), 2.59–2.51 (2H, m), 2.32 (3H, s); HPLC 98.3% at 215 nm, 2.8 min (Method A); MS (ESI) m/z 387.2298 (M+H).

4.5.3. 4-[(4-Methyl-1*H*-indol-5-yl)amino]-5-[(1*E*)-5-piperazin-1-ylpent-1-en-1-yl]nicotinonitrile (13c)

71 mg (60% yield) as its TFA salt; ^{1}H NMR (DMSO-d_6) δ 11.26 (1H, s), 9.67 (1H, s), 9.22 (1H, s), 8.62 (1H, s), 8.40 (1H, s), 7.40 (1H, t, J = 2.6 Hz), 7.28 (1H, d, J = 8.4 Hz), 6.96 (1H, d, J = 8.4 Hz), 6.65 (1H, d, J = 15.6 Hz), 6.56 (1H, s), 6.34 (1H, dt, J = 6.8, 15.6 Hz), 3.45–3.10 (10H, m), 2.32 (3H, s), 2.28 (2H, q, J = 6.8 Hz), 1.86–1.76 (2H, m); HPLC 100% at 215 nm, 4.01 min (Method E); MS (ESI) m/z 401.2456 (M+H).

4.5.4. 5-[(1*E*)-3-(4-Aminopiperidin-1-yl)prop-1-en-1-yl]-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (14a)

19 mg (37% yield); ^{1}H NMR (DMSO-d_6) δ 11.13 (1H, s), 8.58 (1H, s), 8.36 (1H, s), 8.23 (1H, s), 7.34 (1H, t, J = 2.6 Hz), 7.22 (1H, d, J = 8.4 Hz), 6.89 (1H, d, J = 8.4 Hz), 6.72 (1H, d, J = 15.6 Hz), 6.50 (1H, s), 6.22 (1H, dt, J = 6.8, 15.6 Hz), 3.52–3.38 (1H, m), 3.04 (2H, d, J = 6.0 Hz), 2.77 (2H, d, J = 11.6 Hz), 2.28 (3H, s), 1.91 (2H, t, J = 10.2 Hz), 1.66 (2H, d, J = 8.0 Hz), 1.28–1.18 (2H, m); HPLC 96.2% at 215 nm, 2.9 min (Method A); MS (ESI) m/z 387.2293 (M+H).

4.5.5. 5-[(1*E*)-4-(4-Aminopiperidin-1-yl)but-1-en-1-yl]-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (14b)

53 mg (46% yield) as its TFA salt; ^{1}H NMR (DMSO-d_6) δ 11.25 (1H, s), 9.81 (1H, bsw), 9.43 (1H, br s), 8.56 (1H, s), 8.38 (1H, s), 8.14 (3H, br s), 7.40 (1H, t, J = 2.8 Hz), 7.28 (1H, d, J = 8.4 Hz), 6.95 (1H, d, J = 84 Hz), 6.68 (1H, d, J = 15.6 Hz), 6.55 (1H, s), 6.25 (1H, dt, J = 6.8, 15.6 Hz), 3.60 (2H, d, J = 12.4 Hz), 3.35–3.16 (3H, m), 3.00 (2H, q, J = 8.4 Hz), 2.60 (2H, q, J = 6.8 Hz), 2.32 (3H, s), 2.12 (2H, d, J = 12.4 Hz), 1.76 (2H, q, J = 11.6 Hz); HPLC 94.7% at 215 nm, 2.3 min (Method A); MS (ESI) m/z 401.2452 (M+H).

4.5.6. 5-[(1*E*)-5-(4-Aminopiperidin-1-yl)pent-1-en-1-yl]-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (14c)

84 mg (84% yield) as its TFA salt; ^{1}H NMR (DMSO-d_6) δ 11.25 (1H, s), 9.88 (1H, br s), 9.56 (1H, s), 8.58 (1H, s), 8.39 (1H, s), 8.19 (3H, br s), 7.39 (1H, t, J = 2.8 Hz), 7.28 (1H, d, J = 8.4 Hz), 6.94 (1H, d, J = 8.4 Hz), 6.65 (1H, d, J = 16 Hz), 6.55 (1H, s,), 6.31 (1H, dt, J = 6.6, 15.6 Hz), 3.55 (2H, d, J = 12 Hz), 3.30–3.27 (1H, m), 3.15–2.97 (4H, m), 2.32 (3H, s), 2.26 (2H, q, J = 6.7 Hz), 2.09 (2H, d, J = 6.0 Hz), 1.89–1.71 (4H, m); HPLC 100% at 215 nm, 3.95 min (Method E); MS (ESI) m/z 415.2610 (M+H).

4.5.7. 5-[(1*E*)-3-(4-Hydroxypiperidin-1-yl)prop-1-en-1-yl]-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (15a)

Compound **15a** was prepared from **1** and piperidine-4-ol using the procedure described for **4a** giving 19 mg (18% yield) as its HCl salt; ^{1}H NMR (DMSO-d_6) δ 11.27 (1H, s), 10.61 (1H, br s), 9.94 (1H, br s), 8.70 (1H, s), 8.53 (1H, d, J = 7.6 Hz), 7.40 (1H, t, J = 2.8 Hz), 7.29 (1H, d, J = 7.6 Hz), 7.04 (1H, dd, J = 4.8, 10 Hz), 6.97 (1H, dd, J = 2.8, 8.8 Hz), 6.56 (1H, s), 6.46–6.39 (1H, m), 3.96–2.95 (7H, m), 2.34 (3H, s), 2.01–1.66 (4H, m); HPLC 100% at 215 nm, 3.86 min (Method E); MS (ESI) m/z 388.2132 (M+H).

4.5.8. 5-[(1*E*)-4-(4-Hydroxypiperidin-1-yl)but-1-en-1-yl]-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (15b)

Compound **15b** was prepared from **1** and piperidin-4-ol using the procedure described for **4b** giving 55 mg (40% yield) as its TFA salt; HPLC 100% at 215 nm, 1.25 min (Method C); MS (ESI) m/z 402.2 (M+H).

4.5.9. 5-[(1*E*)-5-(4-Hydroxypiperidin-1-yl)pent-1-en-1-yl]-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (15c)

Compound **15c** was prepared from **1** and piperidin-4-ol using the procedure described for **4c** and purified by HPLC to give 101 mg (71% yield) TFA salt; ^{1}H NMR (DMSO-d_6) δ 11.27 (1H, s), 9.71 (1H, d, J = 7.6 Hz), 9.47 (1H, br s), 8.62 (1H, d, J = 2.0 Hz), 8.41 (1H, d, J = 3.6 Hz), 7.40 (1H, t, J = 2.8 Hz), 7.28 (1H, d, J = 8.4 Hz), 6.95 (1H, dd, J = 2.0, 8.8 Hz), 6.65 (1H, d, J = 14.4 Hz), 6.55 (1H, s), 6.38–6.29 (1H, m), 3.66–2.89 (7H, m), 2.32 (3H, s), 2.27–1.52 (8H, m); HPLC 95.5% at 215 nm, 4.08 min (Method E); MS (ESI) m/z 416.2452 (M+H).

4.5.10. 5-{(1*E*)-4-[4-(Methylamino)piperidin-1-yl]but-1-en-1-yl}-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (16)

tert-Butyl methyl(piperidin-4-yl)carbamate (113 mg; 0.53 mmol) and (*E*)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)but-3-enyl 4-methylbenzenesulfonate **5** (186 mg, 0.53 mmol) were stirred in 1 mL of DMSO. After 16 h at room temperature, **1** (100 mg, 0.27 mmol), cesium carbonate (173 mg, 0.53 mmol) and Pd(PPh_3)$_4$ (16 mg, 0.014 mmol) were added and the reaction was heated at 70 °C for 16 h. The mixture was cooled to room temperature, diluted with EtOAc and washed with water to give the Boc-protected intermediate. The crude organic extracts were concentrated and treated with 10% trifluoroacetic acid/DCM for 2 h at room temperature. Concentration and purification by HPLC gave 10 mg (5.7% yield) of **16** as its TFA salt. HPLC 100% at 215 nm, 1.26 min (Method D); MS (ESI) m/z 401.2 (M+H).

4.5.11. 5-{(1*E*)-4-[4-(Dimethylamino)piperidin-1-yl]but-1-en-1-yl}-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (17)

Compound **15b** was prepared from **1** and *N*,*N*-dimethylpiperidin-4-amine using the procedure described for **4b** giving 57 mg (32% yield) as its TFA salt; HPLC 97.5% at 215 nm, 3.56 min (Method E); MS (ESI) m/z 429.3 (M+H).

4.5.12. 5-{(1*E*)-4-[4-(Aminomethyl)piperidin-1-yl]but-1-en-1-yl}-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (18)

Compound **18** was prepared from **1** and *tert*-butylpiperidin-4-ylmethylcarbamate using the procedure described for **16** to give 53 mg (31% yield) as its TFA salt; HPLC 100% at 215 nm, 1.09 min (Method C); MS (ESI) m/z 415.3 (M+H).

4.5.13. 5-{(1*E*)-4-[(3*R*)-3-Aminopiperidin-1-yl]but-1-en-1-yl}-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (19)

Compound **19** was prepared from **1** and (*R*) *tert*-butyl piperidin-3-ylcarbamate using the procedure described for **16** to give 65 mg (38% yield) as its TFA salt; ^{1}H NMR (DMSO-d_6) δ 11.13 (1H, s), 8.52 (1H, br s), 8.27 (1H, s), 8.21 (1H, s), 7.35 (1H, t, J = 2.8 Hz), 7.22 (1H, d, J = 8.4 Hz), 6.88 (1H, d, J = 8.4 Hz), 6.58 (1H, d, J = 15.2 Hz), 6.50 (1H, s), 6.22–6.15 (1H, m), 2.73–2.27 (7H, m), 2.28 (3H, s), 1.97–0.99 (6H, m); HPLC 100% at 215 nm, 1.19 min (Method C); MS (ESI) m/z 401.2452 (M+H).

4.5.14. 5-{(1*E*)-4-[(3S)-3-Aminopiperidin-1-yl]but-1-en-1-yl}-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (20)

Compound **20** was prepared from **1** and (*S*) *tert*-butyl piperidin-3-ylcarbamate using the procedure described for **16** to give 58 mg (34% yield) as its TFA salt; HPLC 100% at 215 nm, 3.58 (Method E); MS (ESI) m/z 401.2 (M+H).

4.5.15. 5-[(1*E*)-4-(3-Aminoazetidin-1-yl)but-1-en-1-yl]-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (21)

Compound **21** was prepared from **1** and *tert*-butyl azetidin-3-ylcarbamate using the procedure described for **16** to give 41 mg (25% yield) as its TFA salt; HPLC 100% at 215 nm, 1.25 min (Method D); MS (ESI) m/z 373.3 (M+H).

4.5.16. 5-{(1*E*)-4-[(3*R*)-3-Aminopyrrolidin-1-yl]but-1-en-1-yl}-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (22)

Compound **22** was prepared from **1** and (*R*)-*tert*-butyl pyrrolidin-3-ylcarbamate using the procedure described for **16** to give 49 mg (30% yield) as its TFA salt; HPLC 100% at 215 nm, 1.04 min (Method D); MS (ESI) *m*/*z* 387.2 (M+H).

4.5.17. 5-{(1*E*)-4-[(3*S*)-3-Aminopyrrolidin-1-yl]but-1-en-1-yl}-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (23)

Compound **23** was prepared from **1** and (*S*)-*tert*-butyl pyrrolidin-3-ylcarbamate using the procedure described for **16** to give 47 mg (28% yield) TFA salt; HPLC 100% at 215 nm, 1.05 min (Method D); MS (ESI) *m*/*z* 387.2 (M+H).

4.5.18. *N*-{1-[(3*E*)-4-{5-Cyano-4-[(4-methyl-1*H*-indol-5-yl)amino]-pyridin-3-yl}but-3-en-1-yl]piperidin-4-yl}methanesulfonamide (24)

A mixture of **14b** (75 mg, 0.10 mmol), methanesulfonyl chloride (15 mg, 0.13 mmol) and triethylamine (41 mg, 0.40 mmol) in 1 mL of DMF were stirred at room temperature for 1 h. The reaction mixture was filtered and the filtrate was purified by HPLC to give **24** as its TFA salt; ^{1}H NMR (DMSO-d_6) δ 11.25 (1H, s), 9.46 (1H, br s), 9.38 (1H, br s), 8.56 (1H, s), 8.38 (1H, s), 7.41–7.37 (2H, m), 7.28 (1H, d, *J* = 8.8 Hz), 6.94 (1H, d, *J* = 8.8 Hz), 6.67 (1H, d, *J* = 15.2 Hz), 6.55 (1H, s), 6.24 (1H, dt, *J* = 6.8, 16 Hz), 3.68 (2H, d, *J* = 4 Hz), 3.55–3.38 (2H, m), 3.27–3.12 (3H, m), 2.96 (3H, s), 2.62–2.50 (2H, m), 2.32 (3H, s), 2.08–1.62 (4H, s); HPLC 96.7% at 216 nm, 4.4 min (Method A); MS (ESI) *m*/*z* 479.2223 (M+H).

4.5.19. *N*-{1-[(3*E*)-4-{5-Cyano-4-[(4-methyl-1*H*-indol-5-yl)amino]-pyridin-3-yl}but-3-en-1-yl]piperidin-4-yl}ethanesulfonamide (25)

Compound **25** was prepared from **14b** and ethanesulfonyl chloride using the procedure described for **24** to give 6.3 mg (8.3% yield) as its TFA salt; HPLC 98.0% at 215 nm, 4.27 min (Method E); MS (ESI) *m*/*z* 493.4 (M+H).

4.5.20. *N*-{1-[(3*E*)-4-{5-Cyano-4-[(4-methyl-1*H*-indol-5-yl)amino]-pyridin-3-yl}but-3-en-1-yl]piperidin-4-yl}propane-2-sulfonamide (26)

Compound **26** was prepared from **14b** and propane-2-sulfonyl chloride using the procedure described for **24** to give 14 mg (19% yield) as its TFA salt; HPLC 97.9% at 215 nm, 1.45 min (Method C); MS (ESI) *m*/*z* 507.2 (M+H).

4.5.21. *N*-{1-[(3*E*)-4-{5-Cyano-4-[(4-methyl-1*H*-indol-5-yl)amino]-pyridin-3-yl}but-3-en-1-yl]piperidin-4-yl}benzenesulfonamide (27)

Compound **27** was prepared from **14b** and benzenesulfonyl chloride using the procedure described for **24** to give 6.2 mg (8% yield) as its TFA salt; HPLC 97.8% at 215 nm, 4.88 min (Method E); MS (ESI) *m*/*z* 541.4 (M+H).

4.5.22. *N*-{1-[(3*E*)-4-{5-Cyano-4-[(4-methyl-1*H*-indol-5-yl)amino]-pyridin-3-yl}but-3-en-1-yl]piperidin-4-yl}acetamide (28)

Compound **28** was prepared from **14b** and acetyl chloride using the procedure described for **24** to give 48 mg (55% yield) TFA salt; ^{1}H NMR (DMSO-d_6) δ 11.26 (1H, s), 9.61 (1H, br s), 9.43 (1H, br s), 8.61 (1H, s), 8.39 (1H, s), 7.98 (1H, d, *J* = 7.6 Hz), 7.41 (1H, t, *J* = 2.6 Hz), 7.28 (1H, d, *J* = 8.0 Hz), 6.96 (1H, d, *J* = 8.0 Hz), 6.66 (1H, d, *J* = 14.8 Hz), 6.56 (1H, s), 6.26 (1H, dt, *J* = 6.6, 15.6 Hz), 3.82–3.75 (1H, m), 3.53 (2H, d, *J* = 11.6 Hz), 3.25–2.98 (4H, m), 2.63–2.49 (2H, m), 2.32 (3H, s), 1.96 (2H, d, *J* = 11.6 Hz), 1.81 (3H, s), 1.58 (2H, q, *J* = 12.4 Hz); HPLC 94.9% at 215 nm, 4.1 min (Method A); MS (ESI) *m*/*z* 443.2558 (M+H).

4.5.23. 5-[(1*E*)-4-(4-Methoxypiperidin-1-yl)but-1-en-1-yl]-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (29)

Compound **29** was prepared from **1** and 4-methoxypiperidine using the procedure described for **4b** and purified by HPLC to give 45 mg (40% yield); ^{1}H NMR (DMSO-d_6) δ 11.12 (1H, s), 8.52 (1H, s), 8.27 (1H, s), 8.81 (1H, s), 7.34 (1H, t, *J* = 2.8 Hz), 7.22 (1H, d, *J* = 8.8 Hz), 6.88 (1H, d, *J* = 8.8 Hz), 6.59 (1H, d, *J* = 15.2 Hz), 6.50 (1H, s), 6.17 (1H, dt, *J* = 6.6, 15.6 Hz), 3.21 (3H, s), 3.13 (1H, sept., *J* = 4.4 Hz), 2.69–2.66 (2H, m), 2.38–2.25 (4H, m), 2.28 (3H, s), 2.05 (2H, t, *J* = 9.2 Hz), 1.80 (2H, d, *J* = 9.6 Hz), 1.41–1.33 (2H, m); HPLC 97.6% at 215 nm, 4.8 min (Method A); MS (ESI) *m*/*z* 416.2446 (M+H).

4.5.24. 5-{(1*E*)-4-[4-(Hydroxymethyl)piperidin-1-yl]but-1-en-1-yl}-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (30)

Compound **30** was prepared from **1** and piperidin-4-ylmethanol using the procedure described for **4b** giving 64 mg (57% yield); ^{1}H NMR (DMSO-d_6) δ 11.12 (1H, s), 8.51 (1H, s), 8.26 (1H, s), 8.21 (1H, s), 7.34 (1H, t, *J* = 2.8 Hz), 7.21 (1H, d, *J* = 8.0 Hz), 6.87 (1H, d, *J* = 8.0 Hz), 6.59 (1H, d, *J* = 15.6 Hz), 6.50 (1H, s), 6.18 (1H, dt, *J* = 6.4, 15.6 Hz), 4.37 (1H, t, *J* = 5.4 Hz), 3.23–3.17 (3H, m), 2.85 (2H, d, *J* = 11.2 Hz), 2.40–2.29 (4H, m), 2.28 (3H, s), 1.84 (2H, dt, *J* = 2.4, 11.6 Hz), 1.60 (2H, d, *J* = 12 Hz), 1.07 (2H, dq, *J* = 3.2, 12 Hz); HPLC 97.8% at 215 nm, 3.97 min (Method E); MS (ESI) *m*/*z* 416.2450 (M+H).

4.5.25. 4-[(4-Methyl-1*H*-indol-5-yl)amino]-5-[(1*E*)-4-(4-phenylpiperidin-1-yl)but-1-en-1-yl]nicotinonitrile (31)

Compound **31** was prepared from **1** and 4-phenylpiperidine using the procedure described for **4b** and purified by HPLC to give 58 mg (37% yield) as its TFA salt; HPLC 100% at 215 nm, 1.56 min (Method C); MS (ESI) *m*/*z* 462.3 (M+H).

4.5.26. 5-{(1*E*)-4-[(3*S*)-3-Hydroxypyrrolidin-1-yl]but-1-en-1-yl}-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (32)

Compound **32** was prepared from **1** and (*S*)-pyrrolidin-3-ol using the procedure described for **4b** and purified by HPLC to give 42 mg (40% yield); ^{1}H NMR (DMSO-d_6) δ 11.12 (1H, s), 8.53 (1H, s), 8.27 (1H, s), 8.21 (1H, s), 7.34 (1H, t, *J* = 2.8 Hz), 7.22 (1H, d, *J* = 8.4 Hz), 6.88 (1H, d, *J* = 8.4 Hz), 6.60 (1H, d, *J* = 15.6 Hz), 6.51 (1H, s), 6.20 (1H, dt, *J* = 6.8, 16 Hz), 4.64 (1H, d, *J* = 4.4 Hz), 4.15 (1H, oct., *J* = 3.5 Hz), 2.73–2.68 (1H, m), 2.57–2.28 (6H, m), 2.28 (3H, s), 1.98–1.89 (1H, m), 1.54–1.47 (1H, m), 1.17–1.07 (1H, m); HPLC 93% at 215 nm, 3.8 min (Method A); MS (ESI) *m*/*z* 388.2137 (M+H).

4.5.27. 5-{(1*E*)-4-[(3*R*)-3-Hydroxypyrrolidin-1-yl]but-1-en-1-yl}-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (33)

Compound **33** was prepared from **1** and (*R*)-pyrrolidin-3-ol using the procedure described for **4b** and purified by HPLC to give 37 mg (35% yield); ^{1}H NMR (DMSO-d_6) δ 11.12 (1H, s), 8.53 (1H, s), 8.27 (1H, s), 8.21 (1H, s), 7.34 (1H, t, *J* = 2.8 Hz), 7.22 (1H, d, *J* = 8.4 Hz), 6.88 (1H, d, *J* = 8.4 Hz), 6.60 (1H, d, *J* = 15.6 Hz), 6.51 (1H, s), 6.20 (1H, dt, *J* = 6.8, 16 Hz), 4.64 (1H, d, *J* = 4.4 Hz), 4.15 (1H, oct., *J* = 3.5 Hz), 2.73–2.68 (1H, m), 2.57–2.28 (6H, m), 2.28 (3H, s), 1.98–1.89 (1H, m), 1.54–1.47 (1H, m), 1.17–1.07 (1H, m); HPLC 93.6% at 215 nm, 3.89 min (Method E); MS (ESI) *m*/*z* 388.2136 (M+H).

4.5.28. 5-{(1*E*)-4-[(3*S*)-3-Hydroxypiperidin-1-yl]but-1-en-1-yl}-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (34)

Compound **34** was prepared from **1** and (*S*)-piperidin-3-ol using the procedure described for **4b** and purified by HPLC to give 8 mg (5.7% yield); HPLC 100% at 215 nm, 1.33 min (Method C); MS (ESI) *m*/*z* 402.2 (M+H).

4.5.29. 5-{(1*E*)-4-[(3*R*)-3-Hydroxypiperidin-1-yl]but-1-en-1-yl}-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (35)

Compound **35** was prepared from **1** and (*R*)-piperidin-3-ol using the procedure described for **4b** and purified by HPLC to give 7 mg (5.0% yield); HPLC 100% at 215 nm, 1.33 min (Method C); MS (ESI) *m*/*z* 402.2 (M+H).

4.5.30. 5-[(1*E*)-4-(1,4-Diazepan-1-yl)but-1-en-1-yl]-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (36)

Compound **36** was prepared from **1** and *tert*-butyl 1,4-diazepane-1-carboxylate using the procedure described for **16** to give 66 mg (39% yield) TFA salt; HPLC 100% at 215 nm, 1.06 min (Method D); MS (ESI) *m*/*z* 401.2 (M+H).

4.5.31. 4-[(4-Methyl-1*H*-indol-5-yl)amino]-5-[(1*E*)-4-(4-phenylpiperazine-1-yl)but-1-en-1-yl]nicotinonitrile (37)

Compound **37** prepared from **1** and 1-phenylpiperazine using the procedure described for **11b** to give 27 mg (22% yield); ^{1}H NMR (DMSO-d_6) δ 11.13 (1H, s), 8.54 (1H, s), 8.28 (1H, s), 8.22 (1H, s), 7.35 (1H, t, J = 2.6 Hz), 7.23–7.17 (3H, m), 6.91–6.88 (3H, m), 6.76 (1H, t, J = 7.4 Hz), 6.64 (1H, d, J = 15.6 Hz), 6.50 (1H, s), 6.21 (1H, dt, J = 6.6, 15.2 Hz), 3.11 (4H, t, J = 4.8 Hz), 2.53 (4H, t, J = 5.0 Hz), 2.49–2.34 (4H, m), 2.29 (3H, s); HPLC 98.9% at 215 nm, 6.9 min (Method A); MS (ESI) *m*/*z* 463.2612 (M+H).

4.5.32. 5-[(1*E*)-4-(4-Acetylpiperazin-1-yl)but-1-en-1-yl]-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (38)

Compound **38** was prepared from **1** and 1-(piperazin-1-yl)ethanone using the procedure described for **4b** and purified by HPLC to give 98 mg (67% yield); ^{1}H NMR (DMSO-d_6) δ 11.24 (1H, s), 9.88 (1H, br s), 9.37 (1H, br s), 8.54 (1H, s), 8.39 (1H, s), 7.39 (1H, t, J = 2.8 Hz), 7.28 (1H, d, J = 8.8 Hz), 6.94 (1H, d, J = 8.8 Hz), 6.70 (1H, d, J = 15.6 Hz), 6.55 (1H, s), 6.25 (1H, dt, J = 6.6, 16 Hz), 4.03–4.00 (2H, m), 3.51–2.88 (10H, m), 2.63 (2H, q, J = 7.2 Hz), 2.31 (3H, s), 2.06 (3H, s); HPLC 95.4% at 215 nm, 4.2 min (Method A); MS (ESI) *m*/*z* 429.2393 (M+H).

4.5.33. 5-{(1*E*)-4-[4-(2,2-Dimethylpropanoyl)piperazin-1-yl]but-1-en-1-yl}-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (39)

A solution of **13b** (75 mg, 0.12 mmol), pivaloyl chloride (19 mg, 0.16 mmol) and triethylamine (36 mg, 0.36 mmol) in 1 mL of DMF was stirred at room temperature for 1 h. The reaction mixture was filtered and the filtrate was purified by HPLC to give 25 mg (30% yield) of **39** as its TFA salt; ^{1}H NMR (DMSO-d_6) δ 11.24 (1H, s), 10.02 (1H, br s), 9.43 (1H, br s), 8.56 (1H, s), 8.40 (1H, s), 7.39 (1H, t, J = 2.8 Hz), 7.28 (1H, d, J = 8.4 Hz), 6.94 (1H, d, J = 8.4 Hz), 6.70 (1H, d, J = 15.6 Hz), 6.55 (1H, s), 6.26 (1H, dt, J = 6.6, 15.2 Hz), 4.49–4.36 (2H, m), 3.57–3.49 (2H, m), 3.31–2.91 (m, 6H), 2.68–2.53 (2H, m), 2.53 (3H, s), 1.21 (9H, s); HPLC 98.9% at 215 nm, 5.4 min (Method A); MS (ESI) *m*/*z* 471.2870 (M+H).

4.5.34. 4-[(4-Methyl-1*H*-indol-5-yl)amino]-5-{(1*E*)-4-[4-(methylsulfonyl)piperazin-1-yl]but-1-en-1-yl}nicotinonitrile (40)

Compound **40** was prepared from **1** and 1-(methylsulfonyl)piperazine using the procedure described for **4b** and purified by HPLC to give 80 mg (48% yield) as its TFA salt; ^{1}H NMR (DMSO-d_6) δ 11.24 (1H, s), 9.88 (1H, br s), 9.37 (1H, br s), 8.54 (1H, s), 8.39 (1H, s), 7.40 (1H, t, J = 2.6 Hz), 7.28 (1H, d, J = 8.8 Hz), 6.94 (1H, d, J = 8.4 Hz), 6.70 (1H, d, J = 15.2 Hz), 6.55 (1H, s), 6.25 (1H, dt, J = 6.6, 15.6 Hz), 3.82–3.53 (4H, m), 3.37–3.05 (6H, m), 3.03 (3H, s), 2.66–2.59 (2H, m), 2.32 (3H, s); HPLC 98.1% at 215 nm, 4.16 min (Method E); MS (ESI) *m*/*z* 465.2065 (M+H).

4.5.35. 5-{(1*E*)-4-[4-(Ethylsulfonyl)piperazin-1-yl]but-1-en-1-yl}-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (41)

Compound **41** was prepared from **13b** and ethanesulfonyl chloride using the procedure described for **39** and purified by HPLC to give 30 mg (35% yield) as its TFA salt; ^{1}H NMR (DMSO-d_6) δ 11.24 (1H, s), 9.95 (1H, br s), 9.47 (1H, br s), 8.57 (1H, s), 8.39 (1H, s), 7.40 (1H, t, J = 3.0 Hz), 7.28 (1H, d, J = 8.8 Hz), 6.95 (1H, d, J = 8.8 Hz), 6.70 (1H, d, J = 15.6 Hz), 6.55 (1H, s), 6.25 (1H, dt, J = 6.6, 15.6 Hz), 3.83–3.52 (4H, m), 3.35–3.05 (6H, m), 3.20 (2H, q, J = 7.3 Hz), 2.62 (2H, q, J = 6.9 Hz), 2.32 (3H, s), 1.32 (3H, t, J = 7.4 Hz); HPLC 100% at 215 nm, 4.34 min (Method E); MS (ESI) *m*/*z* 479.2227 (M+H).

4.5.36. 5-{(1*E*)-4-[4-(Isopropylsulfonyl)piperazin-1-yl]but-1-en-1-yl}-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (42)

Compound **42** was prepared from **13b** and propane-2-sulfonyl chloride using the procedure described for **39** and purified by HPLC to give 10 mg (12% yield) as its TFA salt; HPLC 94.2% at 215 nm, 1.42 min (Method C); MS (ESI) *m*/*z* 493.2 (M+H).

4.5.37. 4-[(4-Methyl-1*H*-indol-5-yl)amino]-5-{(1*E*)-4-[4-(phenylsulfonyl)piperazin-1-yl]but-1-en-1-yl}nicotinonitrile (43)

Compound **43** was prepared from **13b** and benzenesulfonyl chloride using the procedure described for **39** and purified by HPLC to give 32 mg (42% yield) as its TFA salt; ^{1}H NMR (DMSO-d_6) δ 11.24 (1H, s), 9.75 (1H, br s), 9.45 (br s, 1H), 8.56 (1H, s), 8.36 (1H, s), 7.83–7.70 (5H, m), 7.40 (1H, t, J = 2.8 Hz), 7.27 (1H, d, J = 8.0 Hz), 6.92 (1H, d, J = 8.0 Hz), 6.67 (1H, d, J = 15.6 Hz), 6.56 (1H, s), 6.20 (1H, dt, J = 6.6, 15.2 Hz), 3.73–3.48 (6H, m), 3.35–3.10 (4H, m), 2.54 (2H, q, J = 6.9 Hz), 2.28 (3H, s); HPLC 98.5% at 215 nm, 6.2 min (Method A); MS (ESI) *m*/*z* 527.2228 (M+H).

4.5.38. 5-[(1*E*)-4-(4-Aminopiperidin-1-yl)but-1-en-1-yl]-6-methyl-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (45)

A mixture of *tert*-butyl piperidin-4-ylcarbamate (520 mg, 2.6 mmol) and (*E*)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)but-3-enyl 4-methylbenzenesulfonate (**5**) (454 mg, 1.29 mmol) in 2.5 mL of DMSO was stirred for 60 h at room temperature. Compound **44** (250 mg, 0.64 mmol), cesium carbonate (421 mg, 1.29 mmol) and Pd(PPh_3)$_4$ (37 mg, 0.032 mmol) were added to the same pot and the resulting suspension was heated at 70° for 16 h. After cooling to room temperature, the reaction was diluted with EtOAc and washed with water. The organic extracts were concentrated and purified by silica gel chromatography with a MeOH/DCM gradient to give 179 mg of the desired intermediate tert-butyl {1-[(3*E*)-4-{5-cyano-2-methyl-4-[(4-methyl-1*H*-indol-5-yl)amino]-pyridin-3-yl}but-3-en-1-yl]piperidin-4-yl}carbamate. A solution of this intermediate (137 mg, 0.27 mmol) in 10% trifluoroacetic acid/DCM was stirred for 1 h at room temperature. The reaction was concentrated and the residue was purified by HPLC to give 140 mg (95% yield) of **45** as its TFA salt; ^{1}H NMR (DMSO-d_6) δ 11.29 (1H, s), 10.18 (1H, br s), 9.52 (1H, s), 8.67 (1H, s), 8.22 (3H, br s), 7.41 (1H, t, J = 2.8 Hz), 7.28 (1H, d, J = 8.4 Hz), 6.94 (1H, d, J = 8.4 Hz), 6.55 (1H, s), 6.33 (1H, d, J = 16.4 Hz), 6.03 (1H, dt, J = 6.6, 16 Hz), 3.60 (2H, d, J = 12.8 Hz), 3.37–3.21 (3H, m), 3.10–2.08 (2H, m), 2.73–2.63 (2H, m), 2.47 (3H, s), 2.31 (3H, s), 2.12 (2H, d, J = 12.8 Hz), 1.80 (2H, q, J = 11.7 Hz); HPLC 100% at 215 nm, 3.54 min (Method E); MS (ESI) *m*/*z* 415.2610 (M+H).

4.5.39. *N*-{1-[(3*E*)-4-{5-Cyano-2-methyl-4-[(4-methyl-1*H*-indol-5-yl)amino]pyridin-3-yl}but-3-en-1-yl]piperidin-4-yl}methanesulfonamide (46)

A solution of compound **45** (92 mg, 0.22 mmol), methane sulfonyl chloride (36 mg, 0.31 mmol) and triethylamine (89 mg, 0.88 mmol) in 1 mL DMF was stirred for 1 h at room temperature. The reaction was filtered and the filtrate was purified by HPLC to

give 50 mg (32% yield) of **46** as its TFA salt; ^{1}H NMR (DMSO-d_6) δ 11.25 (1H, s), 9.46 (1H, br s), 9.28 (1H, br s), 8.59 (3H, br s), 7.40 (1H, t, J = 2.8 Hz), 7.30 (1H, d, J = 8.0 Hz), 6.92 (1H, d, J = 8.0 Hz), 6.55 (1H, s), 6.34 (1H, d, J = 16 Hz), 6.01 (1H, dt, J = 6.8, 16.4 Hz), 3.53 (2H, d, J = 12 Hz), 3.31–2.96 (5H, m), 2.96 (3H, s), 2.66–2.60 (2H, m), 2.49 (3H, s), 2.31 (3H, s), 2.07 (2H, d, J = 12.4 Hz), 1.72–1.62 (2H, m); HPLC 98.0% at 215 nm, 4.8 min (Method A); MS (ESI) m/z 493.2384 (M+H).

4.5.40. 6-Methyl-4-[(4-methyl-1*H*-indol-5-yl)amino]-5-[(1*E*)-4-piperazin-1-ylbut-1-en-1-yl]nicotinonitrile (47)

Compound **47** was prepared from **44** and *tert*-butyl piperazine-1-carboxylate using the procedure described for **45** and purified by HPLC to give 300 mg (60% yield) as its TFA salt; ^{1}H NMR (DMSO-d_6) δ 11.29 (1H, s), 9.64 (1H, s), 9.13 (2H, br s), 8.73 (1H, s), 7.41 (1H, t, J = 2.8 Hz), 7.29 (1H, d, J = 8.6 Hz), 6.94 (1H, d, J = 8.6 Hz), 6.56 (1H, s), 6.31 (1H, d, J = 16 Hz), 6.06 (1H, dt, J = 6.8, 16 Hz), 3.28–3.05 (10H, m), 2.65–2.59 (2H, m), 2.49 (3H, s), 2.32 (3H, s); HPLC 100% at 215 nm, 3.69 min (Method E); MS (ESI) m/z 401.2455 (M+H).

4.5.41. 5-[4-(4-Aminopiperidin-1-yl)butyl]-4-[(4-methyl-1*H*-indol-5-yl)amino]nicotinonitrile (48)

Compound **10b** (200 mg, 0.40 mmol) and palladium (20 mg, 10%; wet) in 20 mL of EtOH was stirred rapidly under H_2 at 1 atm for 16 h at room temperature. The solvent and palladium were removed to give *tert*-butyl 1-(4-(5-cyano-4-(4-methyl-1*H*-indol-5-ylamino)pyridin-3-yl)butyl)piperidin-4-ylcarbamate. Ten percent of TFA/DCM (5.5 mL) were added to the crude intermediate. After stirring for 2 h at room temperature the reaction was concentrated and the residue was purified by HPLC to give 201 mg (78% yield) of **48** as its TFA salt; ^{1}H NMR (DMSO-d_6) δ 11.30 (1H, s), 9.93 (2H, br s), 8.74 (1H, s), 8.29 (1H, s), 8.20 (3H, br s), 7.42 (1H, t, J = 2.8 Hz), 7.30 (1H, d, J = 8.0 Hz), 6.99 (1H, d, J = 8.0 Hz), 6.57 (1H, s), 3.57 (2H, d, J = 11.6 Hz), 3.32–3.29 (1H, m), 3.08–2.96 (4H, m), 2.83–2.77 (2H, m), 2.34 (3H, s), 2.14 (2H, d, J = 9.2 Hz), 1.85–1.59 (6H, m); HPLC 95.3% at 254 nm, 3.00 min (Method E); MS (ESI) m/z 403.2612 (M+H).

4.6. Assay conditions

The PKCθ and PKCδ IMAP assays and T cell IL2 production assays were performed as previously described.[22]

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