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Identification and SAR of squarate inhibitors of mitogen activated protein kinase-activated protein kinase 2 (MK-2)

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ABSTRACT

A novel series of inhibitors for mitogen activated protein kinase-activated protein kinase 2 (MK-2) are reported. These squarate based inhibitors were identified via a high-throughput screen. An MK2 co-structure with the starting ligand was obtained and a structure based approach was followed to optimize potency and selectivity.

1. Introduction

Tumor Necrosis Factor-alpha (TNF-α is an important cytokine with pro-inflammatory properties that has been associated with various inflammatory diseases such as rheumatoid arthritis (RA),[1] psoriasis,[2] inflammatory bowel disease[3] and others.[4] Anti-TNF-α therapies, such as TNF-α monoclonal antibodies (Remicade™ and Humira™) and TNF-α soluble receptors (Enbrel™)[5,6] have been shown to be efficacious for the treatment of various inflammatory diseases such as RA.

In the resting state, MAPKAP kinase 2 (MK-2) resides in the nucleus and upon stimulus it is phosphorylated by p38α and subsequently exported to the cytoplasm.[7–9] Once in the cytoplasm it is responsible for the induction of TNF-α production, as well as other pro-inflammatory cytokines.[10,11] Recent studies have helped to elucidate the role MK-2 plays in the upregulation of TNF-α. Induction of the translation of TNF-α mRNA is achieved by the phosphorylation of tristetraprolin (TTP), a protein which binds the adenosine/uridine-rich element (ARE).[12,13] MK-2 deficient mice have been shown to be protected against brain ischemic injury,[14] heart reperfusion injury[15] and are resistant to collagen-induced arthritis.[16]

Recently, there have been several reports of MK-2 inhibitors (Fig. 1). Anderson et al. have reported on two scaffolds, the aminocyanopyridines **1**[17] and the pyrrolopyridine inhibitors **3**.[18] The latter were shown to be potent and reasonably selective against a panel of kinases. More recently Trujillo et al. have reported on the tetrahydro-β-carboline carboxylic acids **2** as MK-2 inhibitors.[19] While not as potent as the former, they did demonstrate good selectivity for MK-2. Wu et al. of Boehringer–Ingelheim also reported a series of carboline (**4**) analogs as MK-2 inhibitors.[20] Interestingly, both the pyrrolopyridine **3** analogs as well as the carboline **4** analogs gain much of their affinity by interactions with

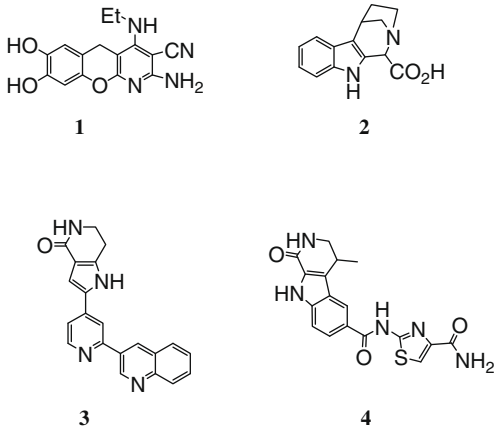

Figure 1. Recently published MK-2 inhibitors.

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the specificity surface.[21] Herein we wish to report our own progress on the identification of a series of MK-2 inhibitors.

Compound **17** was identified as an inhibitor of MK-2 (IC_{50} = 8.9 μM) as part of a high throughput screen. In order to take a structure based approach we obtained a co-crystal of **17** with MK-2 (Fig. 2, PDB code 3FPM). The key hinge binding interaction is between Leu141 and the pyridine nitrogen. One of the squarate oxygens makes a hydrogen bond with the side chain nitrogen of Lys93. The aryl ring tucks up under the glycine rich loop.

While **17** was selective against a number of kinases, it did inhibit several in our screening panel (see Table 7). It also proved to be an inhibitor of the Cytochrome P450 (Cyp450) isozymes 3A4 and 2C9 (IC_{50}'s of 1.3 and 0.6 μM respectively).[22] Thus as part of our routine screening paradigm, we needed to address potency and selectivity as well as identify approaches to reduce Cyp450 inhibition.

2. Chemistry

Compound **17** was originally prepared as part of an investigation into a previous project and much of the chemistry herein has been described.[24,25] Investigations on the right hand side of the molecule began with the addition of amines to compound **5**[24] (Scheme 1) in ethanol. If reactions were sluggish at room temperature or reflux, they were heated at 140 °C under microwave irradiation. Non-commercial amines were prepared by the condensation of ketones with hydroxylamine and the resulting oximes reduced with Raney nickel under a hydrogen atmosphere (Scheme 2). The resulting amines were then added to compound **5** in ethanol at 125 °C under microwave irradiation.

To further investigate the hinge binding pyridine we began by the addition of the appropriate heterocyclic amines to diethyl squarate, followed by the addition of (*R*)-1-phenylethanamine (Scheme 1). This approach allowed the preparation of compounds **36** and **39** as well as halogenated pyridines **13** and **40** (the 2-Br and 2-Cl pyridines utilized in Scheme 3). The halogenated pyridines provided access to 2-substituted pyridines. Most of the 2-pyridine analogs were prepared as illustrated in Scheme 3. Various modified Suzuki–Miyaura coupling conditions[26–28] were employed to effect C–C bond formation. Aryl and heteroaryl amines as well as amides were coupled using conditions developed by Buchwald[29] or Yin.[30]

Another disconnection was investigated wherein the functionalized 2-pyridine was coupled with the squarate nitrogen. Thus we found that aryl bromide **15** (Scheme 4) could be coupled with squarate amide **13** in the presence of copper using modified conditions developed by Buchwald[31] to afford **41**. Interestingly, we found that the recommended tribasic potassium phosphate gave a mixture of products, including di-arylated compounds. The weaker dibasic potassium phosphate afforded the mono-arylated **41**. This is likely due to the increased acidity of the squarate amide over alkyl amides.[32]

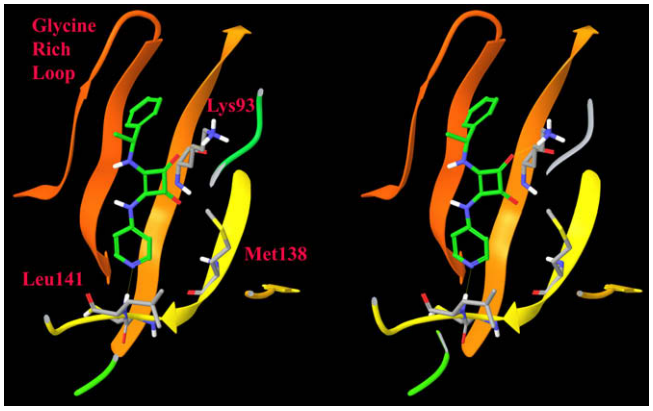
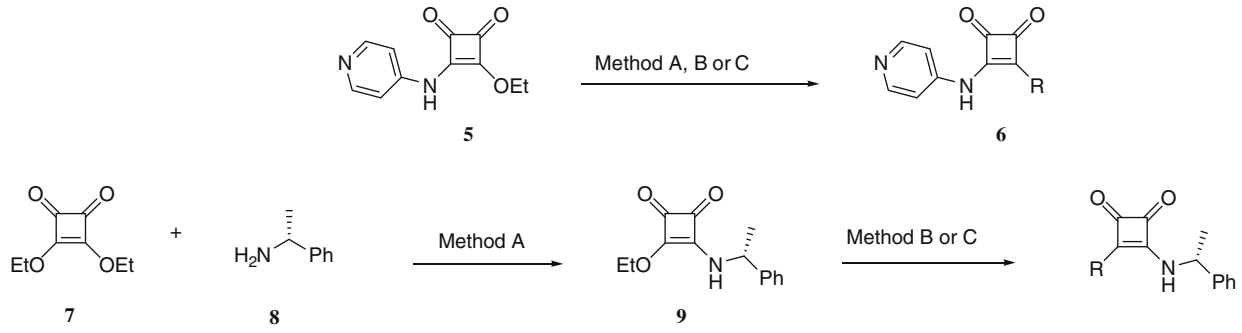


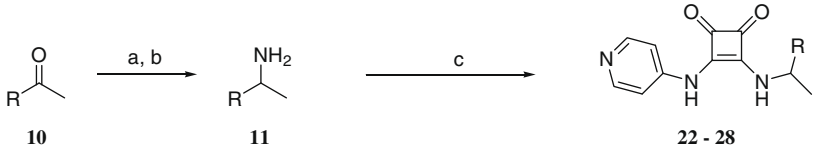
Figure 2. Stereo view of MK-2 cocrystal with compound **17** (PDB code 3FPM). The hinge peptide is yellow. The gatekeeper Met138 is shown at the back of the cavity above which is Lys93.[23]

3. Results and discussion

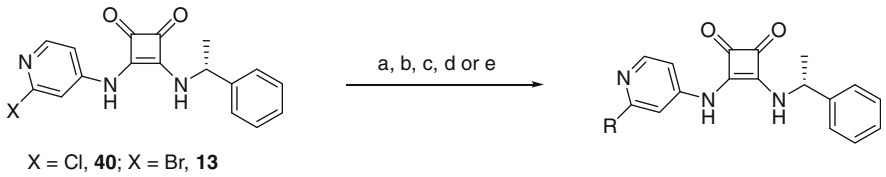
Examination of **17** in the protein crystal (Fig. 2) reveals the key interaction of these squarate inhibitors. The squarate ring is the central scaffold which interacts with the conserved Lys93 via one of the squarate oxygens (Fig. 2). These oxygens carry a significant negative charge due to a resonance form wherein the butadiene is aromatic with a +2 charge and each of the oxygens carry a −1



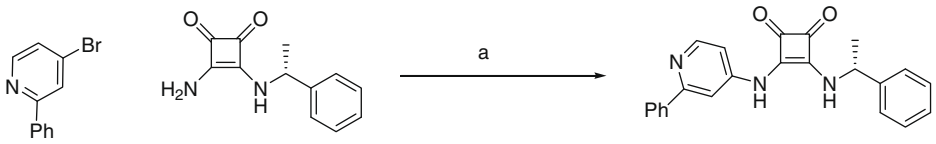
Scheme 1. For compounds **17**–**19**, **30**–**35** method A: amine, EtOH, reflux 15 min; for compounds **36**, **38**, **40** method B: amine, EtOH, reflux 5 h; for compounds **20**, **21**, **39** method C: amine, EtOH, microwave 140 °C, 15 min.



Scheme 2. Reagents and conditions: (a) $HONH_2{\cdot}HCl$, Et_3N, EtOH, microwave, 120 °C, 20 min; (b) H_2, 50 PSI, Raney nickel, NH_4OH, EtOH; (c) **5**, ETOH, microwave, 125 °C, 30 min.



Scheme 3. Reagents and conditions: (a) **40**, $RB(OH)_2$ (1.2 equiv), $Pd(dppf)Cl_2$–CH_2Cl_2 (0.1 equiv), Cs_2CO_3 (2 M, 2 equiv), DMF, 80 °C, 13 h; compounds **43**, **44**, **47**; (b) **13**, $RB(OH)_2$ (1.3 equiv), $Pd(PPh_3)_4$ (0.2 equiv), Na_2CO_3 (2.05 equiv), DME/H_2O/EtOH (7:3:2), 150 °C, microwave, 5 min; compounds **42**, **45**, **46**; (c) **40**, RNH_2 (1.2 equiv), $Pd_2(dba)_3$ (0.1 equiv), XPHOS (0.3 equiv), NaO*t*-Bu (2.1 equiv), dioxane/DMF (5:1), 100 °C, 18 h; compounds **48**–**51**, **53**; (d) **40**, RNH_2 (2.0 equiv), Pd_2 $(dba)_3$ (0.1 equiv), XANTPHOS (0.2 equiv), K_3PO_3 (1.9 equiv), dioxane/DMF (5:1), 150 °C, microwave, 2 h; compound **54** (e) **40**, RNH_2 (1.2 equiv), $Pd_2(dba)_3$ (0.12 equiv), XANTPHOS (0.3 equiv), Na_2CO_3 (1.4 equiv), dioxane/DMF (5:1, 1.2 mL), 150 °C, microwave, 2 h; Compound **52**.



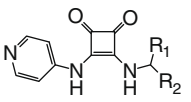
Scheme 4. Reagents and conditions: (a) CuI (1.03 equiv), K_2HPO_3, *N*,*N*′-dimethylethane diamine (1.5 equiv), DMF, 110 °C, 6.5 h.

charge.[33,34] The 4-pyridine provides the interaction with the NH of Leu141 in the hinge region. The aryl ring, which is important for potency, primarily makes van der Waals contacts with the glycine rich loop. Interestingly the glycine rich loop has moved significantly relative to our previously reported structures[35] in order to accommodate this moiety.

Analogs that investigated the right hand side indicated that an aryl ring was optimal (Table 1). The cyclohexyl **21**, as well as the thiazole **22** each displayed a reduction in potency. The configuration of the stereogenic center of the methylene was also important. While the *R* isomer **17** is 8.9 μM, its enantiomer **18** has an IC_{50} of >100 μM. The desmethyl **19** was 88 μM. Interestingly the gem-dimethyl **20** is 2.3 μM. The greater potencies of **17** and **20** over **18** is likely due to conformational effects. When a methyl is placed in the 'S' position, while maintaining the position of the aryl ring under the glycine rich loop, it is likely clashing somewhat with the squarate oxygen. The higher energy of this conformation is reflected by the lower potency of compound **18**. In the case of **20**, the option of a proton in the 'S' position no longer exists and thus neither does the lower energy conformer. Substitution off of either of the squarate amide nitrogens (**35**, **36**, Table 2) resulted in decreased potency.

Substitution around the aryl at the *ortho* and *para* positions did little to address potency (Table 1). However investigation of the *meta* position resulted in hydroxyl analog **27** which had improved potency significantly with an IC_{50} of 0.38 μM. It is noteworthy that neither the hydroxyl at the *ortho* nor *para* positions (**24** and **31**) nor other hydrogen bond donors (**26**, **28**–**29**) showed the same improvement in potency. Substitution off the methylene was also investigated. The amide **32** and alcohol **34** showed modest improvements over compound **17**. Compound **33** represents the combination of both the amide and the 3-OH. While potency did not improve over compound **27**, the Cyp450 profile was much improved over **17**. As can be seen in Table 3, the Cyp450 isozyme

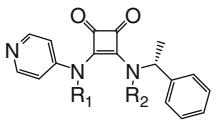
Table 1



Compound	R1	R2	MK-2[a]
17	(*R*)-Me	Phenyl	8.90
18	(*S*)-Me	Phenyl	>100
19	H	Phenyl	88.0
20	*gem*-Dimethyl	Phenyl	2.30
21	(*R*)-Me	Cyclohexane	65.0
22	Me	2-Thiazole	81.0
23	Me	2-F-Phenyl	9.30
24	Me	2-OH-Phenyl	33.00
25	Me	3 -Methyl-phenyl	49.00
26	Me	3-NH_2-Phenyl	14.0
27	Me	3-OH-Phenyl	0.38
28	Me	3-NHCOMe-Phenyl	5.50
29	Me	3-$NHSO_2$Me-Phenyl	24.00
30	Me	4-F-Phenyl	8.20
31	Me	4-OH-Phenyl	5.00
32	$CONH_2$	Phenyl	4.50
33	$CONH_2$	3-OH-Phenyl	0.39
34	CH_2OH	Phenyl	5.30

[a] IC50 (μM).

Table 2



Compound	R1	R2	MK-2[a]
35	H	Me	>100
36	Me	H	>100

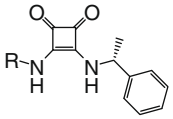
[a] IC50 (μM).

Table 3
Cytochrome P450 Inhibition

Compound	Cyp450 isozymes		
	3A4	2D6	2C9
17	1.3[a]	3.3[a]	0.6[a]
32	8.6[a]	>10[a]	5.1[a]
33	>10[a]	>10[a]	6.5[a]
37	16[b]	1[b]	26[b]
38	NT[c]	NT[c]	NT[c]
39	40[b]	0[b]	12[b]

[a] IC50 (μM).
[b] %Inhibition at 3 μM.
[c] Not tested.

Table 4



Compound	R	MK-2[a]
37		>100
38		>100
39		>100

[a] IC50 (μM).

IC_{50}'s for **33** were >10 μM for 3A4 and 2D6 and 6.5 μM for 2C9. This is a marked improvement compared to **17**.

It was hypothesized that the 4-amino pyridine may be a motif contributing to the high Cyp450 inhibition of the series as the corresponding 3-aminopyridine **37** (Table 4) did not inhibit 3A4 and 2C9 significantly (16% and 26% inhibition @ 3 μM, respectively). However it was a poor inhibitor of MK-2 (IC_{50} > 100 μM) as expected from the structural information. Efforts to identify other pyridine mimetics with reasonable MK-2 potency failed. The pyrimidine **38** as well as the pyrazole **39** each had IC_{50}'s greater than 100 μM.

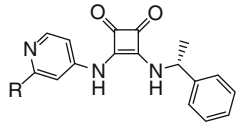
It was anticipated that exploration off the pyridine, in contrast to the aryl ring under the glycine rich loop, would better address potency. The reason for this optimism is several fold. It is difficult to utilize the structure in the design of compounds that interact with the glycine rich loop. The loop can move in a continuous manner and accommodate ligands with substituents of varying sizes.[36–38] In the case of MK-2, we have observed the loop in a somewhat closed position[35] to a more open position as illustrated herein. Thus designing ligands to interact optimally with this mobile loop would be challenging indeed. Furthermore, freezing out this dynamic portion of the protein is not without entropic costs and may limit opportunities for gaining greater energies of interaction and thus potency. Juxtaposed to this is the attractiveness of the specificity surface.[21]

In 2004 Anderson et al.[39] reported on a new series of pyrrolopyridine MK-2 inhibitors. Superposition of their structures (Fig. 3) over the squarates reported herein lead to a hypothesis of how these pyrrolopyridineones bound. It was envisioned that the pyrrolopyridine series gained significant improvements in potency by making interactions with the specificity surface,[21] particularly Leu70. Recent publications[18,40] indicate that this is indeed the case. In addition to addressing potency, it was expected that perturbations in this region would also address selectivity against other kinases.

As can be seen in Table 5, a variety of approaches to interacting with this region were explored. It is not surprising that **40** was >100 μM. The electronegative chlorine reduces the basicity of the pyridine nitrogen, thus compromising the hinge interaction. Compound **41** did show a modest improvement in activity (2.6 μM). Efforts to improve this activity further by growing off the aryl ring were not fruitful. Replacement of the aryl ring with a variety of heterocycles (compounds **45**–**47**) showed very similar activities. Other linkers were also investigated. Aryl amine linkers represented by compounds **48**–**52** and amide linkers represented by compounds **53** and **54** where explored. Like the direct aryl substituents, several of the compounds showed modest potency improvements. However only **50** had a greater than 10-fold improvement in the IC_{50} over **17**.

Inhibition of the production of Tumor Necrosis Factor-alpha (TNF-α) in THP cells upon exposure to LPS (lipopolysaccharide) was investigated (Table 6). The initial lead **17** had an IC_{50} of 10.8 μM. Compounds **27** and **33**, which were more potent in the enzymatic assay, showed a decrease in cell potency. This may be due to

Table 5



Compound	R	MK-2[a]
40	Cl	>100
41	Phenyl	2.6
42	2-F-Phenyl	5.8
43	3-F-Phenyl	6.6
44	3-MeO-Phenyl	3.7
45	4-Pyridine	3.6
46	2-Furan	5.3
47	3-Thiophene	5.4
48	NH-Phenyl	9.1
49	NH-(3-$CONH_2$-Phenyl)	2.4
50	NH-4-Pyrimidine	0.67
51	NH-2-Pyrazine	1.6
52	NH-2-Pyrimidine	2.3
53	NHCOMe	3.8
54	NHCO-(3-Pyridine)	1.3

[a] IC50 (μM).

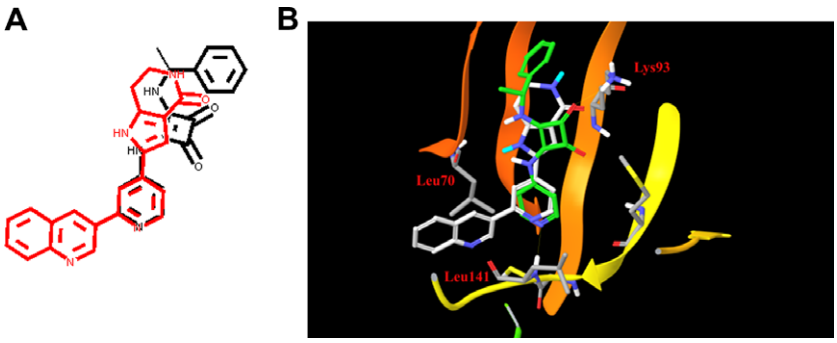


Figure 3. Overlays of **17** and **3**: (A) Chemdraw overlay of **17** and **3**, (B) Superposition of **17** and **3** based on protein alignment of 3FPM and 2JBO.

Table 6
Cell based data

Compound	IC50[a]	THP[a]	*ALog P*
17	8.9	10.8	1.4
27	0.38	34	1.1
32	4.5	>40	−0.03
33	0.39	17	−0.27
42	5.8	2.2	3.5
46	5.3	2.1	1.6
50	0.67	1.1	2.8

[a] IC50 (μM).

the increased polarity of the compound, as reflected by the *ALog P*[41] and its effect on cell penetration. We were pleased to find that the potency could be increased by working off the pyridine ring. Compounds **42**, **46** and **50** all showed significant improvements in cell activity. It should be noted that previous MK-2 inhibitors generally see a significant shift in IC_{50} going from the enzymatic assay to cell assays.[18,20,42] It is unclear if the increased inhibition of TNF-α is due solely to inhibition of MK-2 or a combination of MK-2 and other kinases involved in the regulation of TNF-α production. While it may be that other kinases are involved, it is gratifying to find that this approach endows the series with increased selectivity against a panel of kinases.

Selectivity data for selected compounds is shown in Table 7. The initial lead **17** was more potent for several of the kinases (Aurora B, CHK1, CK1-γ1, ERK2, PKA, ROCK1 and RSK1). Changes on the right hand side of the molecule did little to address the selectivity deficiencies. However it was anticipated that work off of the 2-position of the pyridine would address selectivity.[18,21,39] We were gratified to find that this approach did result in improved selectivities for the kinases tested. Compounds **42**, **46**, **47** all showed improvement in the selectivity profile. Compound **42** is >50 μM against all of the kinases tested except P38 alpha (21.7 μM).

In summary we have described the identification of a novel series of MK-2 inhibitors. The protein crystal structure has been obtained and illustrates the key interactions of this series. As seen in many kinase programs, selectivity for the initial lead needed to be improved. By working off the pyridine in the region of the specificity surface we were able to gain selectivity against a panel of kinases. While improvements in potency were made, the most potent compounds did not have improved potency in the cell based assay, likely due to permeability. Future efforts on the series will need to identify approaches to better take advantage of the area of Leu70 to gain better enzymatic and cell based potencies.

4. Experimental

4.1. Materials and methods for the MAPKAP kinase-2 kinase assay

MK-2 kinase activity was assessed using human recombinant MK-2 containing residues 41–353 used at 5 nM in an ELISA based assay. The kinase reaction was performed on 96-well streptavidin coated plates using a biotinylated 13-mer peptide derived from LSP1 at 200 nM in 20 mM Hepes pH 7.4, 10 mM $MgCl_2$, 3 mM DTT, 1 μM ATP. The reaction was stopped after 30 min incubation at room temperature and washed in PBS 0.05% Tween 20. Polyclonal Anti phospho-LSP1 antibodies was then added to the plate along with Goat anti-rabbit labeled with europium in 20 mM MOPS, 150 mM NaCl, 0.025% Tween 20, 0.02% gelatin, 1% BSA for 1 h at room temperature. The plate was then washed in PBS 0.05% Tween 20 and enhancement solution from Perkin Elmer was added before counting on an Envision reader from Perkin Elmer.

4.2. TNF-α production assay in THP-1 cells

Hundred microliters of THP-1 cells, at 1,000,000 cells/ml in RPMI + 0.5% FBS + 0.05 mM 2-mercaptoethanol, are plated on 96 well V-shaped plate (Costar 3894). Compounds are diluted in DMSO and added to the cell culture such that the final DMSO concentration is 1%. The cells are then incubated for 30 min at 37 °C in 5% CO_2. LPS (Sigma) at 1 mg/ml is then added to a final concentration of 40 μg/ml with further incubation for 3 h at 37 °C in 5% CO_2. The cells are then spun down and the supernatants are collected for analysis using the TNF-α kit (MA6000 Human TNF-α Base Kit) from Meso Scale Discovery.

4.3. Kinase selectivity

Kinases are run as off-chip mobility shift assay on Caliper LC3000, based on electrophoretic separation of peptide substrate and product. The assays were optimized to yield about 15–30% conversion in the presence of 1.5 μM peptide and an ATP concentration that equals the apparent Km. The kinases employed are either full-length or near full-length enzymes purchased from Invitrogen and Upstate. For reaction setup, 150 nl compound was stamped from a master compound plate to the assay plate, followed by sequential addition of 7.5 μL substrate mix and 7.5 μL kinase. The final DMSO concentration in the assay is 1%. The reactions are incubated at room temperature for 1–2 h before being quenched with 15 μL stop solution. The assay plates are then read on Caliper LC3000 using job files optimized for individual substrate/product pair.

4.4. Protien crystallization

The MK-2 protein (residues 41-364) was concentrated to ~5 mg/mL in a solution containing 20 mM HEPES pH 7.5, 200 mM NaCl, 10 mM DTT, and 5 mM $MgCl_2$. Prior to crystallization, compound (0.75 mM) was added from a DMSO stock. Diffraction quality co-crystals were obtained at 18 °C from 2.0 M ammonium sulfate. These bipyramidal crystals belong to space group F4132 with cell dimensions $a = b = c = 254.66$ Å and contain one molecule of MK-2 and one molecule of compound in the asymmetric unit. Data were collected at the Southeast Regional Collaborative Access

Table 7
Kinase selectivity data

Compound	MK-2[a]	Aurora B[a]	CHK1a	CK1-γ 1[a]	ERK2[a]	FYN[a]	IKK-α[a]	P38-α[a]	PDGFR-α[a]	PKA[a]	PKC-α[a]	ROCK1[a]	RSK1[a]	SRC[a]
17	8.9	3.2	0.69	0.25	4.6	>50	>50	>50	40.5	1.1	>50	0.06	0.81	>50
32	4.5	6.0	0.69	0.52	6.3	>50	>50	>50	44	0.71	ND	ND	0.78	>50
33	0.39	1.4	0.09	0.16	4.2	>50	>50	>50	11.1	0.27	33.6	0.06	0.29	>50
42	5.8	>50	>50	>50	>50	>50	>50	21.66	>50	>50	>50	>50	>50	>50
46	5.3	8.8	25.3	4.9	35	>50	>50	>50	27.1	>50	>50	>50	>50	>50
47	5.4	10.8	12.8	11.6	>50	15.3	>50	>50	36.2	>50	>50	>50	>50	>50

[a] IC50 (μM).

Team 22-ID beamline at the Advanced Photon Source, Argonne National Laboratory using a MAR300 CCD detector from a single crystal cooled to −180 °C. The data were processed using HKL2000.[43] The structure of the complex was solved using molecular replacement with PDB entry 1NY3 as the molecular replacement probe with AMORE.[44] The molecular replacement solution was then rebuilt into a 3.3 Å resolution solvent flattened map. The structure was refined using REFMAC[44] and converged after four+ rebuilding cycles with a R_{cryst} of 28.2% and a R_{free} of 33.7%. The final model consisted of residues 44-216, 226-263, 274-345, and compound. The coordinates for the structure described in this paper has been deposited in the PDB. The PDB ID code is 3FPM.

4.5. Chemistry

Reactions were run using commercially available starting materials and anhydrous solvents, without further purification. Unless otherwise stated, all reactions were performed under a nitrogen atmosphere. Proton NMR spectra were recorded on a 400 MHz Bruker AV-400 spectrometer using TMS (δ 0.0) as an internal reference. Microwave reactions were run on a Personal Chemistry Emrys Optimizer. High resolution mass spectra were obtained using a Bruker APEXIII Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer equipped with an actively shielded 7 T superconducting magnet (Magnex Scientific Ltd, UK) and an external Bruker APOLLO electrospray ionization (ESI) source. Preparative HPLC was run using a Waters reverse phase preparative HPLC (Xterra C18, 30 × 100 mm column; water/CH_3CN/0.1% formic acid). Purity in two solvent systems was determined using Agilent 1100 reverse phase HPLC with Agilent Zorbax SB-C18, 4.6 × 30 mm column at 254 nm [Gradient: 5–95% in 7 min @ 0.8 mL/min, H_2O/CH_3CN (method 1) and H_2O/MeOH (method 2)].

The following abbreviations were used: XPHOS = 2-(dicyclohexylphosphino)-2′,4′,6′-tri-I-propyl-1,1′-biphenyl; XANTPHOS = 9,9-dimethyl-4,5-bis(diphenylphosphino)xanthene; $Pd_2(dba)_3$ = tris(dibenzylideneacetone)dipalladium.

4.5.1. 3-((*R*)-1-Phenyl-ethylamino)-4-(pyridin-4-ylamino)-cyclobut-3-ene-1,2-dione (17)

To a solution of 3-ethoxy-4-(pyridin-4-ylamino)-cyclobut-3-ene-1,2-dione [prepared as described in *J. Med. Chem.* **2000**, *43*, 1187] (50 mg, 0.229 mmol) in EtOH (2 mL) was added (*R*)-(+)-α-methylbenzylamine (31 μL, 0.236 mmol). The reaction mixture was heated to 100 °C for 3 h and then stirred at room temperature overnight. The reaction mixture was filtered and the precipitate collected and dried in vacuo to afford the title compound (43 mg, 64%). ^{1}H NMR (MeOH-d_4) δ ppm 1.67 (d, J = 6.82 Hz, 3H) 5.40 (q, J = 6.30 Hz, 1H) 7.25–7.35 (m, 1H) 7.35–7.46 (m, 4H) 7.52 (d, J = 4.29 Hz, 2H) 8.35 (d, J = 6.32 Hz, 2H); HPLC purity (method 1: 99.4%, method 2: 100%); HRMS: calcd for $C_{17}H_{15}N_3O_2$ $(M+H)^+$, 294.1237; found, 294.12395.

4.5.2. 3-{[(1*S*)-1-Phenylethyl]amino}-4-(pyridin-4-ylamino)-cyclobut-3-ene-1,2-dione (18)

The title compound was synthesized as outlined for **17** with (*S*)(−)-α-methylbenzylamine (yield = 55%). ^{1}H NMR (MeOH-d_4) δ ppm 1.66 (d, J = 6.83 Hz, 3H) 5.40 (q, J = 6.32 Hz, 1H) 7.24–7.35 (m, 1H) 7.35–7.45 (m, 4H) 7.52 (d, J = 4.31 Hz, 2H) 8.35 (d, J = 6.33 Hz, 2H); HPLC purity (method 1: 99.3%, method 2: 99.6%); HRMS: calcd for $C_{17}H_{15}N_3O_2$ $(M+H)^+$, 294.1237; found 294.12431.

4.5.3. 3-(Benzylamino)-4-(pyridin-4-ylamino)cyclobut-3-ene-1,2-dione (19)

The title compound was synthesized as outlined for **17** with benzylamine (yield = 70%); ^{1}H NMR (MeOH-d_4) δ ppm 4.89 (s, 2H) 7.29–7.35 (m, 1H) 7.38–7.39 (m, 4H) 7.39–7.41 (m, 2H) 7.51 (d, J = 5.56 Hz, 2H); HPLC purity (method 1: 99.7%, method 2: 100%);%); HRMS: calcd for $C_{16}H_{13}N_3O_2$ $(M+H)^+$, 280.10805; found 280.10904.

4.5.4. 3-[(1-Methyl-1-phenylethyl)amino]-4-(pyridin-4-ylamino)cyclobut-3-ene-1,2-dione (20)

A solution of 3-ethoxy-4-(pyridin-4-ylamino)cyclobut-3-ene-1,2-dione (100 mg, 0.5 mmol) and cumylamine (155 mg, 2.5 equiv) in EtOH (2 mL) was heated in a microwave reactor (140 °C, 15 min). The reaction mixture was cooled, concentrated and purified by reverse phase HPLC to afford the title compound as a fluffy yellow solid (60 mg, 42%). ^{1}H NMR (DMSO-d_6) δ ppm 1.79 (s, 6H) 7.28 (t, J = 7.07 Hz, 1H) 7.38 (t, J = 7.71 Hz, 2H) 7.43–7.51 (m, 4H) 8.43 (d, J = 5.81 Hz, 3H); HPLC purity (method 1: 99%, method 2: 99%); HRMS: calcd for $C_{18}H_{17}N_3O_2$ $(M+H)^+$, 308.13935; found 308.1397.

4.5.5. 3-{[(1*R*)-1-Cyclohexylethyl]amino}-4-(pyridin-4-ylamino)cyclobut-3-ene-1,2-dione (21)

3-Ethoxy-4-(pyridin-4-ylamino)cyclobut-3-ene-1,2-dione (200 mg, 0.9 mmol) and (*R*)-1-cyclohexylethanamine (152 mg, 1.3 equiv) were combined in EtOH (4 mL) and the reaction mixture was heated at 140 °C in a microwave for 15 min. The reaction mixture was cooled, concentrated and chromatographed (silica, 2% acetic acid/ethyl acetate to afford the title compound (145 mg, 54%) as a pale-yellow solid. ^{1}H NMR (DMSO- d_6) δ ppm 0.89–1.28 (m, 5H) 1.21 (d, J = 6.82 Hz, 3H) 1.32–1.47 (m, 1H) 1.63 (d, J = 12.13 Hz, 1H) 1.66–1.80 (m, 4H) 3.88–4.04 (m, 1H) 7.45 (d, J = 6.06 Hz, 2H) 7.75 (d, J = 9.09 Hz, 1H) 8.41 (d, J = 6.32 Hz, 2H) 9.79 (s, 1H); HPLC purity (method 1: 100%, method 2: 100%); HRMS: calcd for $C_{17}H_{21}N_3O_2$ $(M+H)^+$, 300.1707; found 300.1706.

4.5.6. 3-(Pyridin-4-ylamino)-4-{[1-(1,3-thiazol-2-yl)ethyl]-amino}cyclobut-3-ene-1,2-dione (22)

Step 1: To a microwave vial was added 2-acetylthiazole (1.0 g, 7.9 mmol), 95% EtOH (15 mL), hydroxylamine hydrochloride (0.82 g, 1.85 mmol, 1.5 equiv) and Et_3N (1.6 mL, 11.8 mmol, 1.5 equiv). The mixture was heated at 120 °C in a microwave reactor for 20 min. The reaction mixture was concentrated, taken up in EtOAc, washed with brine and dried ($MgSO_4$). Filtration and concentration afforded 1-(thiazol-2-yl)ethanone oxime (1.1 g, 100%) as a white solid which was used without further purification.

Step 2: To a Parr shaker bottle was added the above oxime (0.59 g, 4.15 mmol), 95% EtOH (20 mL), ammonium hydroxide (0.8 mL) and Raney nickel (398 mg). The mixture was shaken at 50 PSI for two days. The reaction mixture was filtered through Celite and concentrated to afford 1-(thiazol-2-yl)ethanamine (140 mg, 78%) as a white solid which was used without further purification.

Step 3: To a microwave vial was added 3-ethoxy-4-(pyridin-4-ylamino)-cyclobut-3-ene-1,2-dione (100 mg, 0.5 mmol), 95% EtOH (5 mL) and 1-(thiazol-2-yl)ethanamine (59 mg, 1.0 equiv) The mixture was heated at 125 °C in microwave reactor for 30 min. The reaction mixture was concentrated. Purification by chromatography (silica, 10–14% MeOH/DCM) afforded the title compound (52 mg, 38%) as a yellow solid. ^{1}H NMR (MeOH-d_4) δ ppm 1.82 (d, J = 7.1 Hz, 3H) 5.66–5.89 (m, 1H) 7.61 (d, J = 3.0 Hz, 2H) 7.66–7.94 (m, 2H) 8.18–8.35 (m, 2H) 8.44 (d, J = 6.3 Hz, 2H); HPLC purity (method 1: 100%, method 2: 99%); HRMS: calcd for $C_{14}H_{12}N_4O_2S$ $(M+H)^+$, 301.07537; found 301.0757.

4.5.7. 3-{[1-(2-Fluorophenyl)ethyl]amino}-4-(pyridin-4-ylamino)cyclobut-3-ene-1,2-dione (23)

The procedure outlined in steps 1–3 for compound **22** using 2-fluoroacetophenone was followed to afford the title compound (72 mg, 56%) as a yellow solid. ^{1}H NMR (MeOH-d_4) δ ppm 1.66

(d, J = 6.8 Hz, 3H) 5.56–5.71 (m, 1H) 7.04–7.27 (m, 2H) 7.26–7.38 (m, 1H) 7.38–7.53 (m, J = 1.8 Hz, 1H) 7.66 (d, J = 4.6 Hz, 2H) 8.07–8.25 (m, 2H) 8.36 (d, J = 6.3 Hz, 2H); HPLC purity (method 1: 100%, method 2: 100%); HRMS: calcd for $C_{17}H_{14}FN_3O_2$ $(M+H)^+$, 312.11428; found 312.1141.

4.5.8. 3-{[1-(2-Hydroxyphenyl)ethyl]amino}-4-(pyridin-4-ylamino)cyclobut-3-ene-1,2-dione (24)

The procedure outlined in steps 1–3 for compound **22** using 2-hydroxyacetophenone was followed to afford the title compound (34 mg, 23%) as a yellow solid. ^{1}H NMR (MeOH-d_4) δ ppm 1.78 (d, J = 6.8 Hz, 3H) 5.55–5.76 (m, 1H) 6.92 (dd, J = 8.1, 1.0 Hz, 2H) 7.22 (d, J = 1.5 Hz, 1H) 7.28–7.39 (m, 1H) 7.72 (d, J = 5.6 Hz, 2H) 8.23–8.42 (m, 1H) 8.46 (d, J = 5.6 Hz, 2H); HPLC purity (method 1: 100%, method 2: 100%); HRMS: calcd for $C_{17}H_{15}N_3O_3$ $(M+H)^+$, 310.11862; found 310.1189.

4.5.9. 3-{[1-(3-Methylphenyl)ethyl]amino}-4-(pyridin-4-ylamino)cyclobut-3-ene-1,2-dione (25)

The procedure outlined in steps 1–3 for compound **22** using 3-methylacetophenone was followed to afford the title compound (115 mg, 81%) as a yellow solid. ^{1}H NMR (MeOH-d_4) δ ppm 1.85 (d, 3H) 2.48–2.65 (m, 3H) 5.50–5.68 (m, J = 6.6 Hz, 1H) 7.33 (d, J = 7.3 Hz, 1H) 7.38–7.56 (m, 3H) 7.93 (s, 2H) 8.46 (s, 2H) 8.60 (d, J = 6.1 Hz, 2H); HPLC purity (method 1: 100%, method 2: 100%); HRMS: calcd for $C_{18}H_{17}N_3O_2$ $(M+H)^+$, 308.13935; found 308.1398.

4.5.10. 3-{[1-(3-Aminophenyl)ethyl]amino}-4-(pyridin-4-ylamino)cyclobut-3-ene-1,2-dione (26)

The procedure outlined in steps 1–3 for compound **22** using 3-aminoacetophenone was followed to afford the title compound (30 mg, 23%) as a yellow solid. ^{1}H NMR (MeOH-d_4) δ ppm 1.53 (d, J = 6.8 Hz, 3H) 5.09–5.32 (m, 1H) 6.40–6.71 (m, J = 2.0 Hz, 3H) 6.86–7.13 (m, 1H) 7.26–7.65 (m, 2H) 8.24 (d, J = 6.3 Hz, 2H); HPLC purity (method 1: 98%, method 2: 100%); HRMS: calcd for $C_{17}H_{16}N_4O_2$ $(M+H)^+$, 309.13460; found 309.1348.

4.5.11. 3-{[1-(3-Hydroxyphenyl)ethyl]amino}-4-(pyridin-4-ylamino)cyclobut-3-ene-1,2-dione (27)

The procedure outlined in steps 1–3 for compound **22** using 3-hydroxyacetophenone was followed to afford the title compound (75 mg, 56%) as a white solid. ^{1}H NMR (DMSO-d_6) δ ppm 1.56 (d, J = 6.8 Hz, 3H) 5.08–5.31 (m, 1H) 6.70 (dd, J = 8.1, 1.8 Hz, 1H) 6.76–6.89 (m, 2H) 7.19 (t, J = 7.8 Hz, 1H) 7.45 (d, J = 5.8 Hz, 2H) 8.07–8.24 (m, 2H) 8.41 (d, J = 6.1 Hz, 3H); HPLC purity (method 1: 100%, method 2: 100%); HRMS: calcd for $C_{17}H_{15}N_3O_3$ $(M+H)^+$, 310.11862; found 310.1186.

4.5.12. *N*-[3-(1-{[3,4-Dioxo-2-(pyridin-4-ylamino)cyclobut-1-en-1-yl]amino}ethyl)phenyl]acetamide (28)

The procedure outlined in steps 1–3 for compound **22** using 3-acetamidoacetophenone was followed to afford the title compound (19 mg, 12%) as a yellow solid. ^{1}H NMR (CH_3CO_2H-d_4) δ ppm 1.71 (d, J = 6.8 Hz, 3H) 2.20–2.30 (m, 3H) 5.39–5.56 (m, 1H) 7.18–7.30 (m, 1H) 7.37 (t, J = 7.9 Hz, 1H) 7.54–7.72 (m, J = 22.2 Hz, 2H) 8.11 (s, 2H) 8.52 (d, J = 7.3 Hz, 2H); HPLC purity (method 1: 100%, method 2: 99%); HRMS: calcd for $C_{19}H_{18}N_4O_3$ $(M+H)^+$, 351.14517; found 351.1453.

4.5.13. *N*-[3-(1-{[3,4-Dioxo-2-(pyridin-4-ylamino)cyclobut-1-en-1-yl]amino}ethyl)phenyl]methanesulfonamide (29)

To a solution of 3-aminoacetophenone (1.0 g, 7.4 mmol) and Et_3N (1.2 mL, 8.9 mmol, 1.2 equiv) in DCM (20 mL) was added methanesulfonyl chloride (0.7 mL, 8.9 mmol, 1.2 equiv) at 0 °C. The mixture was stirred for 2.5 h. Purification by chromatography (silica, 30–50% EtOAc/hexanes) afforded *N*-(3-acetylphenyl)methanesulfonamide (0.93 g, 59%) as a colorless oil. The procedure outlined in steps 1–3 for compound **6** using the above intermediate was followed to afford the title compound (63 mg, 35%) as a yellow solid. ^{1}H NMR (MeOH-d_4) δ ppm 1.67 (d, 3H) 2.94–3.05 (m, 3H) 5.30–5.52 (m, 1H) 7.14–7.32 (m, 3H) 7.32–7.44 (m, 2H) 7.70 (s, 2H) 8.25 (s, 2H) 8.40 (d, J = 6.1 Hz, 2H); HPLC purity (method 1: 100%, method 2: 100%); HRMS: calcd for $C_{18}H_{18}N_4O_4S$ $(M+H)^+$, 387.11215; found 387.1124.

4.5.14. 3-{[1-(4-Fluorophenyl)ethyl]amino}-4-(pyridin-4-ylamino)cyclobut-3-ene-1,2-dione (30)

The title compound was synthesized as outlined for **17** with (d, l)-4-fluoro-α-methylbenzylamine (55 mg, 77% yield). ^{1}H NMR (MeOH-d_4) δ ppm 1.66 (d, J = 6.82 Hz, 3H) 5.40 (q, J = 6.91 Hz, 1H) 7.12 (t, J = 8.84 Hz, 2H) 7.45 (dd, J = 8.59, 5.31 Hz, 2H) 7.52 (d, J = 5.31 Hz, 2H) 8.35 (d, J = 6.57 Hz, 2H); HPLC purity (method 1: 99.2%, method 2: 99.6%); HRMS: calcd for $C_{17}H_{14}FN_3O_2$ $(M+H)^+$, 312.11428; found 312.11433.

4.5.15. 3-{[1-(4-hydroxyphenyl)ethyl]amino}-4-(pyridin-4-ylamino)cyclobut-3-ene-1,2-dione (31)

The title compound was synthesized as outlined for **17** with 4-(1-aminoethyl)phenol (yield = 70%); ^{1}H NMR (MeOH-d_4) δ ppm 1.64 (d, J = 6.82 Hz, 3H) 5.31 (q, J = 5.81 Hz, 1H) 6.80 (d, J = 8.59 Hz, 2H) 7.25 (d, J = 8.59 Hz, 2H) 7.52 (d, J = 5.05 Hz, 2H) 8.35 (d, J = 6.06 Hz, 2H); HPLC purity (Method 1: 98.3%, Method 2:99.3%); HRMS: calcd for $C_{17}H_{15}N_3O_3$ $(M+H)^+$, 310.11862; found 310.11867.

4.5.16. 2-{[3,4-Dioxo-2-(pyridin-4-ylamino)cyclobut-1-en-1-yl]amino}-2-phenylacetamide (32)

The title compound was synthesized as outlined for **17** with 2-amino-2-phenylacetamide (yield = 57%); ^{1}H NMR (MeOH-d_4) δ ppm 5.90 (s, 1H) 7.32–7.44 (m, 3H) 7.50–7.58 (m, 4H) 8.36 (dd, J = 4.93, 1.64 Hz, 2H); HPLC purity (method 1: 99.2%, method 2: 100%); HRMS: calcd for $C_{17}H_{14}N_4O_3$ $(M+H)^+$, 323.11387; found 323.11399.

4.5.17. 2-{[3,4-Dioxo-2-(pyridin-4-ylamino)cyclobut-1-en-1-yl]amino}-2-(3-hydroxyphenyl)acetamide (33)

A solution of methyl amino(3-hydroxyphenyl)acetate (*Eur. J. Org. Chem.* **2003**, 3131) (6.90 g, 38 mmol) was dissolved in aq NH_4OH (200 mL) and stirred in a sealed tube at 80 °C overnight. The solution was evaporated to afford 2-amino-2-(3-hydroxyphenyl)acetamide (6.35 g, 100%) which was used without further purification. ^{1}H NMR (DMSO-d_6) δ ppm 4.18 (br s, 1H), 6.56–6.66 (m, 1H), 6.77–6.84 (m, 2H), 6.99 (br s, 1H), 7.03–7.11 (m, 1H), 7.41 (s, 1H), 9.29 (s, 1H). A solution of 3-ethoxy-4-(pyridin-4-ylamino)cyclobut-3-ene-1,2-dione (100 mg, .50 mmol) and 2-amino-2-(3-hydroxyphenyl)acetamide (76 mg, 1.0 equiv) in EtOH (5 mL) was stirred at reflux overnight. The reaction mixture was concentrated and chromatographed (silica, 5% MeOH/DCM) to afford the title compound (136 mg, 87%) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ ppm 5.72 (d, J = 8.6 Hz, 1H), 6.65–6.74 (m, 1H), 6.83–6.93 (m, 2H), 7.17 (t, J = 7.8 Hz, 1H), 7.41–7.49 (m, 3H), 8.03 (s, 1H), 8.42 (d, J = 6.1 Hz, 1H), 8.75 (d, J = 8.6 Hz, 2H), 9.55 (s, 1H), 10.30 (s, 1H); HPLC purity (method 1: 98%, method 2: 94%); HRMS: calcd for $C_{17}H_{14}N_4O_4$ $(M + H)^+$, 339.1088; found 339.1087.

4.5.18. 3-{[(1*S*)-2-Hydroxy-1-phenylethyl]amino}-4-(pyridin-4-ylamino)cyclobut-3-ene-1,2-dione (34)

The title compound was synthesized as outlined for **1** with (*S*)-(+)-2-phenylglycinol (yield = 28%). ^{1}H NMR (MeOH-d_4) δ ppm 3.85 (dd, J = 11.36, 7.07 Hz, 1H) 3.95 (dd, J = 11.62, 4.55 Hz, 1H) 5.32–5.40 (brm, 1H) 7.28–7.44 (m, 5H) 7.55 (d, J = 5.05 Hz, 2H) 8.36 (d,

J = 6.32 Hz, 2H); HPLC purity (method 1: 100%, method 2: 100%); HRMS: calcd for $C_{17}H_{15}N_3O_3$ $(M+H)^+$, 310.11862; found 310.11905.

4.5.19. 3-{Methyl[(1*R*)-1-phenylethyl]amino}-4-(pyridin-4-ylamino)cyclobut-3-ene-1,2-dione (35)

The title compound was synthesized as outlined for **17** with (*R*)-(+)-*N*-α-dimethylbenzylamine (yield = 65%). ^{1}H NMR (MEOH-d_4) δ ppm 1.72 (d, J = 7.07 Hz, 3H) 3.07 (s, 3H) 5.99–6.10 (br m, 1H) 7.25 (d, J = 6.06 Hz, 2H) 7.31–7.46 (m, 5H) 8.27 (d, J = 4.80 Hz, 2H); HPLC purity (method 1: 98.4%, method 2: 100%);%); HRMS: calcd for $C_{18}H_{17}N_3O_2$ $(M+H)^+$, 308.13935; found 308.13943.

4.5.20. 3-[Methyl(pyridin-4-yl)amino]-4-{[(1*R*)-1-phenylethyl]amino}cyclobut-3-ene-1,2-dione (36)

A solution of diethyl squarate (500 mg, 2.9 mmol) in EtOH (15 mL) was heated at reflux and a solution of and 4-(methylamino)pyridine (316 mg, 1.0 equiv) in EtOH (5 mL) was added via syringe pump at a rate of 1 mL/h. The solution was cooled and concentrated. Chromatography (silica, ethyl acetate, acetonitrile, methanol, water (70/10/5/5)) afforded 3-ethoxy-4-(methyl(pyridin-4-yl)amino)cyclobut-3-ene-1,2-dione (43 mg, 6.4%) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ ppm 1.38 (t, J = 7.1 Hz, 3H), 3.64 (s, 3H), 4.75 (q, J = 7.1 Hz, 2H), 7.28 (dd, J = 4.7, 1.6 Hz, 2H), 8.55 (dd, J = 4.7, 1.6 Hz, 2H). A solution of 3-ethoxy-4-(methyl(pyridin-4-yl)amino)-cyclobut-3-ene-1,2-dione (0.04 g, 1 equiv) and (*R*)-1-phenylethanamine (66 μL, 3 equiv) in EtOH (0.5 mL) was stirred at room temperature overnight. Evaporation and flash chromatography (silica, 5% MeOH/DCM) afforded the title compound (47 mg, 90%). ^{1}H NMR (DMSO-d_6) δ ppm 1.54 (d, J = 6.82 Hz, 3H) 3.56 (s, 3H) 6.92–6.98 (m, 2H) 7.23–7.32 (m, 1H) 7.33–7.47 (m, 4H) 8.11 (s, 1H) 8.44 (d, J = 6.06 Hz, 2H); HPLC purity (method 1: 100%, method 2: 99%); HRMS: calcd for $C_{18}H_{17}N_3O_2$ $(M+H)^+$, 308.1394; found 308.1394.

4.5.21. 3-{[(1*R*)-1-Phenylethyl]amino}-4-(pyrimidin-4-ylamino)cyclobut-3-ene-1,2-dione (38)

A solution of diethyl squarate (500 mg, 2.9 mmol) in EtOH (15 mL) was heated at reflux and a solution of 4-aminopyrimidine (291 mg, 1.0 equiv) in EtOH (5 mL) was added via syringe pump at a rate of 1 mL/h. The solution was cooled, evaporated, and flash chromatographed (silica, ethyl acetate, acetonitrile, methanol, water (70/10/5/5)) to afford 3-ethoxy-4-(pyrimidin-4-ylamino)cyclobut-3-ene-1,2-dione (16 mg, 2.3%) as a yellow solid. The 3-ethoxy-4-(pyrimidin-4-ylamino)cyclobut-3-ene-1,2-dione (16 mg, 0.07 mmol) and (*R*)-1-phenylethanamine (28 μL, 3 equiv) in EtOH (0.1 mL) were stirred overnight at room temperature. The reaction mixture was concentrated and chromatographed (silica, 5% MeOH/DCM) to afford the title compound (5 mg, 23%). ^{1}H NMR (MeOH-d_4) δ ppm 1.74 (d, J = 7.07 Hz, 3H) 5.57 (q, J = 6.99 Hz, 1H) 7.19 (d, J = 5.31 Hz, 1H) 7.31–7.38 (m, 1H) 7.44 (t, J = 7.71 Hz, 2H) 7.48–7.53 (m, 2H) 8.53 (d, J = 4.80 Hz, 1H) 8.83 (s, 1H); HPLC purity (method 1: 100%, method 2: 96%); HRMS: calcd for $C_{16}H_{14}N_4O_2$ $(M+H)^+$, 295.1190; found 295.1189.

4.5.22. 3-{[(1*R*)-1-Phenylethyl]amino}-4-(1*H*-pyrazol-3-ylamino)cyclobut-3-ene-1,2-dione (39)

Diethylsquarate (400 mg, 2.4 mmol) and 3-aminopyrazole (235 mg, 1.2 equiv) were combined as described for **38** to afford 3-ethoxy-4-(1*H*-pyrazol-3-ylamino)cyclobut-3-ene-1,2-dione (458 mg, 92%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ ppm 1.39 (t, J = 7.1 Hz, 3H), 4.72 (q, J = 7.1 Hz, 2H), 6.28 (s, 1H), 7.61–7.71 (m, 1H), 11.03 (s, 1H), 12.52 (s, 1H). To a solution of 3-ethoxy-4-(1*H*-pyrazol-3-ylamino)cyclobut-3-ene-1,2-dione 200 mg, 1.0 mmol) in EtOH (4 mL) was added (*R*)-1-phenylethanamine (160 μL, 1.3 equiv) and the reaction mixtures was heated at 140 °C in a microwave for 15 min. The reaction mixture was cooled, concentrated and chromatographed (silica, 2% acetic acid/ethyl acetate) to afford the title compound (268 mg, 95%). ^{1}H NMR (DMSO- d_6) δ ppm 1.57 (d, J = 6.82 Hz, 3H) 5.25–5.46 (m, 1H) 7.25–7.35 (m, 1H) 7.35–7.44 (m, 4H) 7.67 (d, J = 2.27 Hz, 1H) 12.52 (s, 1H); HPLC purity (method 1: 96%, method 2: 98%); HRMS: calcd for $C_{15}H_{14}N_4O_2$ $(M+H)^+$, 283.1190; found 283.1189.

4.5.23. 3-[(2-Chloropyridin-4-yl)amino]-4-{[(1*R*)-1-phenylethyl]amino}cyclobut-3-ene-1,2-dione (40)

Diethyl squarate (1.0 g, 6.0 mmol) and 2-chloro-4-aminopyridine (756 mg, 1.0 equiv) were combined as described for **38** to afford 3-[(2-chloropyridin-4-yl)amino]-4-ethoxycyclobut-3-ene-1,2-dione (938 mg, 21%) after flash chromatography (silica, 50% ethyl acetate/hexanes). ^{1}H NMR (DMSO- d_6) δ ppm 1.45 (t, J = 7.1 Hz, 3H), 4.81 (q, J = 7.1 Hz, 2H), 7.39 (dd, J = 5.6, 2.0 Hz, 1H), 7.55 (d, J = 2.0 Hz, 1H), 8.28 (d, J = 5.8 Hz, 1H), 11.17 (s, 1H). The squarate ester (250 mg, 1 mmol) and (*R*)-1-phenylethanamine (256 μL, 2.0 equiv) were stirred in EtOH (4 mL) at room temperature overnight. The reaction mixture was concentrated and chromatographed (silica, 5% MeOH/DCM) to afford the title compound (270 mg, 83%). ^{1}H NMR (DMSO-d_6) δ ppm 1.56–1.65 (m, J = 6.82 Hz, 3H) 5.24–5.37 (m, 1H) 7.23–7.46 (m, 6H) 7.65 (s, 1H) 8.18–8.32 (m, 2H) 10.01 (br s, 1H); HPLC purity (method 1: 99%, method 2: 99%); HRMS: calcd for $C_{17}H_{14}ClN_3O_2$ $(M+H)^+$, 328.08473; found 328.0848.

4.5.24. 3-{[(1*R*)-1-Phenylethyl]amino}-4-[(2-phenylpyridin-4-yl)amino]cyclobut-3-ene-1,2-dione (41)

To a reaction vessel was added (*R*)-3-amino-4-(1-phenylethylamino)cyclobut-3-ene-1,2-dione (0.103 g, .48 mmol), 4-bromo-2-phenyl pyridine (0.112 g, 1.0 equiv), K_2HPO_4 (0.171 g, 2.05 equiv), CuI (0.094 g, 1.03 equiv) and *N*,*N*-dimethylethane-1,2-diamine (0.16 mL, 0.132 mg, 1.5 equiv). The vessel was evacuated and purged with nitrogen twice. DMF (degassed, 2 mL) was then added. The reaction was stirred at 110 °C for 6.5 h, filtered through a Celite plug and taken up in THF. The solution was then adsorbed onto silica. Chromatography (silica, 5%MeOH–95%DCM) afforded the title compound (0.046 g, 32%) as a pale orange solid. ^{1}H NMR (DMSO-d_6) δ ppm 1.62 (d, J = 7.07 Hz, 3H) 5.08–5.47 (m, 1H) 7.17–7.27 (m, 1H) 7.28–7.58 (m, 8H) 8.00–8.10 (m, 3H) 8.20–8.35 (m, 1H) 8.50 (d, J = 5.56 Hz, 1H) 9.94 (s, 1H); HPLC purity (method 1: 99%, method 2: 99%); HRMS: calcd for $C_{23}H_{19}N_3O_2$ $(M+H)^+$, 370.15500; found 370.1562.

4.5.25. 3-{[2-(2-Fluorophenyl)pyridin-4-yl]amino}-4-{[(1*R*)-1-phenylethyl]amino}cyclobut-3-ene-1,2-dione (42)

2-Bromo-4-aminopyridine (5 g, 29 mmol) and diethyl squarate (4.9 g, 1.0 equiv) were combinded as described for **38** to afford 3-[(2-bromopyridin-4-yl)amino]-4-{[(1*R*)-1-phenylethyl]amino}cyclobut-3-ene-1,2-dione (1.51 g, 18%) as a yellow foam after flash chromatography (silica, 40% ethyl acetate/hexanes). ^{1}H NMR (DMSO- d_6) δ ppm 1.45 (t, J = 7.1 Hz, 3H), 4.81 (q, J = 7.1 Hz, 2H), 7.39–7.43 (m, 1H), 7.69 (d, J = 2.0 Hz, 1H), 8.22–8.32 (m, 1H), 11.15 (s, 1H). To a reaction vessel was added the above bromopyridine (0.74, 0.20 mmol), 2-fluoroboronic acid (0.036, 1.3 equiv), Na_2CO_3 (0.034 g, 2.05 equiv) and $Pd(PPh_3)$ (0.024 g, 0.2 equiv). The vessel was evacuated and purged with nitrogen twice. A solvent mixture of DME/H_2O/EtOH (7:3:2) (degassed, 4 mL) was then added. The reaction was microwaved at 150 °C for 300 s, filtered through a Celite plug and concentrated to give crude residue. Purification by RP-HPLC (CH_3CN/H_2O with 0.1% formic acid) afforded the title compound (0.036 g, 18%) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ ppm 1.61 (d, J = 7.07 Hz, 3H) 5.23–5.40 (m, 1H) 7.23–7.57 (m, 9H) 7.78 (s, 1H) 7.94 (t, J = 7.71 Hz, 1H) 8.27 (s, 1H) 8.55 (d, J = 5.56 Hz, 1H) 10.01 (s, 1H); HRMS: calcd for $C_{23}H_{18}FN_3O_2$ $(M+H)^+$, 388.14558; found 388.1458.

4.5.26. 3-{[2-(3-Fluorophenyl)pyridin-4-yl]amino}-4-{[(1*R*)-1-phenylethyl]amino}cyclobut-3-ene-1,2-dione (43)

To a reaction vessel was added 3-[(2-chloropyridin-4-yl)-amino]-4-{[(1*R*)-1-phenylethyl]amino}cyclobut-3-ene-1,2-dione (0.111, 0.34 mmol), 3-fluorophenylboronic acid (0.065 g, 1.2 equiv), Cs_2CO_3 (2 M, 0.7 mL, 2.05 equiv) and Pd(dppf)Cl_2:DCM (0.033 g, 0.10 equiv). The vessel was evacuated and purged with nitrogen twice. DMF (degassed, 4 mL) was then added. The reaction was stirred at 80 °C for 13 h, filtered through a Celite plug and concentrated to give a crude residue. Purification by RP-HPLC (CH_3CN/H_2O with 0.1% formic acid) afforded the title compound (0.012 g, 18%) as a light orange solid. ^{1}H NMR (DMSO-d_6) δ ppm 1.62 (d, J = 6.82 Hz, 3H) 5.19–5.54 (m, 1H) 7.21–7.36 (m, 3H) 7.37–7.49 (m, 4H) 7.50–7.60 (m, 1H) 7.78–7.86 (m, 1H) 7.89 (d, J = 8.08 Hz, 1H) 8.18 (s, 1H) 8.30 (s, 1H) 8.51 (d, J = 5.56 Hz, 1H) 10.01 (s, 1H); HPLC purity (method 1: 100%, method 2: 100%); HRMS: calcd for $C_{23}H_{18}FN_3O_2$ $(M+H)^+$, 388.14558; found 388.1453.

4.5.27. 3-{[2-(3-Methoxyphenyl)pyridin-4-yl]amino}-4-{[(1*R*)-1-phenylethyl]amino}cyclobut-3-ene-1,2-dione (44)

The procedure outlined for 27 was followed using 3-methoxyphenylboronic acid to afford the title compound (0.39 g, 36%) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ ppm 1.62 (d, J = 6.6 Hz, 4H), 3.83 (s, 3H), 5.25–5.39 (m, 1H), 7.02 (d, J = 8.1 Hz, 1H), 7.24 (d, J = 5.1 Hz, 1H), 7.28–7.69 (m, 8H), 8.17 (s, 1H), 8.30 (d, J = 9.3 Hz, 1H), 8.50 (d, J = 5.3 Hz, 2H), 9.99 (s, 1H); HPLC purity (method 1: 99%, method 2: 98%); HRMS: calcd for $C_{24}H_{21}N_3O_3$ $(M+H)^+$, 400.16557; found 400.1661.

4.5.28. 3-(2,4′-Bipyridin-4-ylamino)-4-{[(1*R*)-1-phenylethyl]-amino}-cyclobut-3-ene-1,2-dione (45)

The title compound was prepared as outlined for **42** using 4-pyridine boronic acid to afford the title compound (0.008 g, 14%) as a brown solid. ^{1}H NMR (DMSO-d_6) δ ppm 1.61 (d, J = 6.82 Hz, 6H) 5.18–5.44 (m, 1H) 7.24–7.50 (m, 9H) 7.99 (d, J = 6.06 Hz, 2H) 8.21–8.34 (m, 1H) 8.50 (d, J = 7.83 Hz, 1H) 8.57 (d, J = 5.56 Hz, 1H) 8.73 (d, J = 6.06 Hz, 2H) 10.20–10.36 (m, 1H); HPLC purity (method 1: 99%, method 2: 92%); HRMS: calcd for $C_{22}H_{18}N_4O_2$ $(M+H)^+$, 371.15025; found 371.1502.

4.5.29. 3-{[2-(2-Furyl)pyridin-4-yl]amino}-4-{[(1*R*)-1-phenylethyl]amino}cyclobut-3-ene-1,2-dione (46)

The title compound was prepared as outlined for **42** using 2-furanboronic acid to afford the title compound (0.035 g, 62%) as a dark yellow solid. ^{1}H NMR (DMSO-d_6) δ ppm 1.60 (d, J = 6.8 Hz, 3H) 5.11–5.66 (m, 1H) 6.65 (s, J = 1.0 Hz, 1H) 7.04 (d, J = 2.8 Hz, 1H) 7.19–7.62 (m, 6H) 7.85 (d, J = 4.3 Hz, 2H) 8.39 (d, J = 5.6 Hz, 1H) 8.83 (s, 1H) 10.54 (s, 1H); HPLC purity (method 1: 99%, method 2: 99%); HRMS: calcd for $C_{21}H_{17}N_3O_3$ $(M+H)^+$, 360.13427; found 360.1343.

4.5.30. 3-{[2-(3-Thienyl)pyridin-4-yl]amino}-4-{[(1*R*)-1-phenylethyl]amino}cyclobut-3-ene-1,2-dione (47)

The title compound was prepared as outlined for **43**, but using 3-thiophene boronic acid, to afford 0.032 g (28%) as an off white solid. ^{1}H NMR (DMSO-d_6) δ ppm 1.62 (d, J = 6.8 Hz, 3H), 5.25–5.37 (m, 1H), 7.17–7.25 (m, 1H), 7.29–7.36 (m, 1H), 7.37–7.47 (m, 5H), 7.67 (d, J = 2.0 Hz, 1H), 7.99 (s, 1H), 8.06 (t, J = 2.1 Hz, 1H), 8.27 (d, J = 8.3 Hz, 1H), 8.42 (d, J = 5.6 Hz, 1H), 9.93 (s, 1H); HPLC purity (method 1: 99%, method 2: 99%); HRMS: calcd for $C_{21}H_{17}N_3O_2S$ $(M+H)^+$, 376.11142; found 376.1124.

4.5.31. 3-[(2-Anilinopyridin-4-yl)amino]-4-{[(1*R*)-1-phenylethyl]amino}cyclobut-3-ene-1,2-dione (48)

To a reaction vessel was added 3-[(2-chloropyridin-4-yl)-amino]-4-{[(1*R*)-1-phenylethyl]amino}cyclobut-3-ene-1,2-dione (0.1 g, 0.3 mmol), $Pd_2(dba)_3$ (0.028 g, 0.031 mmol), XPHOS (0.044 g, 0.09 mmol), sodium *tert*-butoxide (0.06 g, 0.63 mmol) and aniline (0.034 g, 0.36 mmol). The vessel was evacuated and purged with nitrogen 3X. Dioxane (degassed, 1 mL) and DMF (degassed, 0.2 mL) was then added. The reaction was stirred at 100 °C overnight, filtered through a plug of silica and concentrated. Purification (RP-HPLC, CH_3CN/H_2O/0.1% formic acid) afforded the title compound (0.03 g, 17%) as an off white solid. ^{1}H NMR (DMSO-d_6) δ ppm 1.60 (d, J = 6.8 Hz, 3H), 5.25–5.36 (m, 1H), 6.74 (s, 1H), 6.88 (app t, J = 7.3 Hz, 1H), 7.00 (d, J = 4.5 Hz, 1H), 7.23 (d, J = 8.3 Hz, 2H), 7.25–7.27 (m, 1H), 7.28–7.46 (m, 10 H), 7.62 (dd, J = 8.6, 1.0 Hz, 2H), 8.02 (d, J = 5.6 Hz, 1H), 8.27 (d, J = 8.1 Hz, 1H), 8.96 (s, 1H), 9.78 (s, 1H); HPLC purity (method 1: 97%, method 2: 97%); HRMS: calcd for $C_{23}H_{20}N_4O_2$ $(M+H)^+$, 385.16590; found 385.1664.

4.5.32. 3-({4-[(3,4-Dioxo-2-{[(1*R*)-1-phenylethyl]amino}-cyclobut-1-en-1-yl)amino]pyridin-2-yl}amino)benzamide (49)

The procedure outlined for **48** was followed using 3-aminobenzamide to afford the title compound (0.025 g, 19%) as an off white powder. ^{1}H NMR (DMSO-d_6) δ ppm 1.60 (d, J = 7.1 Hz, 3H), 5.26–5.35 (m, 1H), 6.74 (d, J = 1.3 Hz, 1H), 7.05 (d, J = 5.6 Hz, 1H), 7.25–7.46 (m, 9H), 7.84–7.90 (m, 2H), 7.99–8.02 (m, 1H), 8.04 (d, J = 5.8 Hz, 1H), 8.37 (d, J = 10.6 Hz, 1H), 9.13 (s, 1H), 9.89 (s, 1H); HPLC purity (method 1: 100%, method 2: 100%); HRMS: calcd for $C_{24}H_{21}N_5O_3$ $(M+H)^+$, 428.17172; found 428.1723.

4.5.33. 3-{[(1*R*)-1-Phenylethyl]amino}-4-{[2-(pyrimidin-4-ylamino)pyridin-4-yl]amino}cyclobut-3-ene-1,2-dione (50)

The procedure outlined for **48** was followed using 4-aminopyrimidine to afford the title compound (0.004 g, 3%) as an off white powder. ^{1}H NMR (DMSO-d_6) δ ppm 1.60 (d, J = 7.1 Hz, 3H), 5.26–5.37 (m, 1H), 7.28–7.35 (m, 1H), 7.37–7.46 (m, 4H), 7.52 (d, J = 4.0 Hz, 1H), 7.58–7.65 (m, 2H), 8.17 (d, J = 5.6 Hz, 1H), 8.40 (d, J = 6.1 Hz, 1H), 8.65 (d, J = 6.6 Hz, 1H), 8.70 (d, J = 1.0 Hz, 1H), 10.23 (s, 1H), 10.32 (s, 1H); HPLC purity (method 1: 99%, method 2: 99%); HRMS: calcd for $C_{21}H_{18}N_6O_2$ $(M+H)^+$, 387.15640; found 387.1561.

4.5.34. 3-{[(1*R*)-1-Phenylethyl]amino}-4-{[2-(pyrazin-2-ylamino)pyridin-4-yl]amino}cyclobut-3-ene-1,2-dione (51)

The procedure outlined for **48** was followed using aminopyrazine to afford the title compound (0.022 g, 19%) as an off white powder. ^{1}H NMR (DMSO-d_6) δ ppm 1.61 (d, J = 6.8 Hz, 3H), 5.25–5.35 (m, 1H), 7.29–7.50 (m, 6H), 7.61 (d, J = 2.0 Hz, 1H), 8.07 (d, J = 2.8 Hz, 1H), 8.16 (d, J = 5.8 Hz, 1H), 8.21 (dd, J = 2.8, 1.5 Hz, 1H), 8.25 (d, J = 8.3 Hz, 1H), 8.89 (d, J = 1.3 Hz, 1H), 9.91 (s, 1H), 10.09 (s, 1H); HPLC purity (method 1: 95%, method 2: 95%); HRMS: calcd for $C_{21}H_{18}N_6O_2$ $(M+H)^+$, 387.15640; found 387.1564.

4.5.35. 3-{[(1*R*)-1-Phenylethyl]amino}-4-{[2-(pyrimidin-2-ylamino)pyridin-4-yl]amino}cyclobut-3-ene-1,2-dione (52)

To a reaction vessel was added 3-[(2-chloropyridin-4-yl)-amino]-4-{[(1*R*)-1-phenylethyl]amino}cyclobut-3-ene-1,2-dione (0.106 g, 0.33 mmol), $Pd_2(dba)_3$ (0.037 g, 0.040 mmol), XANTPHOS (0.059 g, 0.10 mmol), sodium carbonate (0.047 g, 0.45 mmol) and pyrimidin-2-amine (0.038 g, 0.40 mmol). The vessel was evacuated and purged with nitrogen twice. Dioxane (degassed, 2 mL) and DMF (degassed, 0.2 mL) was then added. The reaction was microwaved for 1 h at 150 °C, filtered through a plug of Celite and concentrated. Purification (RP-HPLC, CH_3CN/H_2O with 0.1% formic acid) afforded 0.048 g (38%) the title compound as an orange solid. ^{1}H NMR (DMSO-d_6) δ ppm 1.60 (d, J = 6.8 Hz, 3H) 5.02–5.66 (m, 1H) 6.97 (t, J = 4.8 Hz, 1H) 7.22–7.62 (m, 6H) 7.93–8.29 (m, 2H) 8.37 (d, J = 6.8 Hz, 1H) 8.56 (d, J = 4.8 Hz, 2H) 9.82 (s, 1H) 10.07 (s, 1H);

HPLC purity (method 1: 99%, method 2: 99%); HRMS: calcd for $C_{21}H_{18}N_6O_2$ $(M+H)^+$, 387.15640; found 387.1563.

4.5.36. *N*-{4-[(3,4-Dioxo-2-{[(1*R*)-1-phenylethyl]amino}-cyclobut-1-en-1-yl)amino]pyridin-2-yl}acetamidedione (53)

A reaction vessel containing 3-[(2-chloropyridin-4-yl)amino]-4-{[(1*R*)-1-phenylethyl]amino}cyclobut-3-ene-1,2-dione (0.1 g, 0.3 mmol), acetamide (0.08 g, 1.4 mmol), Pd_2dba (0.04 g, 0.04 mmol), XPHOS (0.04 g, 0.08 mmol) and cesium carbonate (0.2 g, 0.62 mmol) was purged with nitrogen. Dioxane/DMF (1.2 mL, 5:1, degassed) was then added. The reaction was stirred under microwave irradiation at 150 °C for 2 h. The reaction mixture was filtered through a plug of silica (1% triethylamine/10%MeOH/DCM) and concentrated. Purification by RP-HPLC (CH_3CN/H_2O) afforded the title compound as an off white solid (0.02 g, 19%). ^1H NMR (DMSO-d_6) δ ppm 1.59 (d, J = 6.8 Hz, 3H), 5.21–5.34 (m, 1H), 7.25–7.47 (m, 5H), 7.64 (d, J = 3.3 Hz, 1H), 7.81 (s, 1H), 8.17 (d, J = 6.1 Hz, 1H), 8.34 (s, 1H), 10.13 (s, 1H), 10.62 (s, 1H); HPLC purity (method 1: 97%, method 2: 98%); HRMS: calcd for $C_{19}H_{18}N_4O_3$ $(M+H)^+$, 351.14517; found 351.1453.

4.5.37. *N*-{4-[(3,4-Dioxo-2-{[(1*R*)-1-phenylethyl]amino}-cyclobut-1-en-1-yl)amino]pyridin-2-yl}nicotinamide (54)

A reaction vessel containing 3-[(2-chloropyridin-4-yl)amino]-4-{[(1*R*)-1-phenylethyl]amino}cyclobut-3-ene-1,2-dione (0.1 g, 0.3 mmol), nicotinamide (0.07 g, 0.6 mmol), Pd_2dba (0.28 g, 0.03 mmol), XANTPHOS (0.036 g, 0.06 mmol) and potassium phosphate (tribasic, fluka brand, 0.12 g, 0.57 mmol) was purged with nitrogen. Dioxane/DMF (1.2 mL, 5:1, degassed) was then added. The reaction was stirred under microwave irradiation at 150 °C for 2 h. The reaction mixture was filtered through a plug of silica (1% triethylamine/10%MeOH/DCM) and concentrated. Purification by RP-HPLC (CH_3CN/H_2O) afforded the title compound (0.028 g, 22%) as an off white solid. ^1H NMR (DMSO-d_6) δ ppm 1.61 (d, J = 6.82 Hz, 3H) 5.28–5.34 (m, 1H) 7.29–7.46 (m, 5H) 7.54 (ddd, J = 7.89, 4.86, 0.88 Hz, 1H) 7.68 (d, J = 4.29 Hz, 1H) 8.03 (d, J = 1.77 Hz, 1H) 8.27 (d, J = 5.56 Hz, 1H) 8.33 (ddd, J = 8.08, 2.02, 1.77 Hz, 1H) 8.75 (dd, J = 4.80, 1.52 Hz, 1H) 9.12 (dd, J = 2.27, 0.76 Hz, 1H) 10.13 (s, 1H) 11.08 (s, 1H) HPLC purity (method 1: 100%, method 2: 100%); HRMS: calcd for $C_{23}H_{19}N_5O_3$ $(M+H)^+$, 414.15607; found 414.1562.

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