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Synthetic staurosporines via a ring closing metathesis strategy as potent JAK3 inhibitors and modulators of allergic responses

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ABSTRACT

The synthesis and biological evaluation of JAK3 based staurosporine compounds is described. The compounds are constructed completely de novo, and a ring closing metathesis strategy is used to assemble the sugar mimetic portion. These analogs show potent JAK3 activity against isolated enzyme and in T-cells. One analog (**32**) showed unique biological effects during in vitro and in vivo tests including inhibition of STAT5 phosphorylation, blockade of mast cell responses, and reduction of JAK3 based effects in mice models of allergic disease.

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The Janus kinases (JAK's) are a small family of cytoplasmic receptor associated protein tyrosine kinases that play pivotal roles in cytokine mediated biological responses by activation of the latent forms of STATs (signal transducers and activators of transcription). Activated JAK's phosphorylate the intracellular domain of cytokine receptors creating docking sites for the STAT signaling proteins, and further phosphorylation by the JAK's of the STAT's takes place.¹ Among the four members (JAK1, JAK2, JAK3, and Tyk2), JAK3 is segregated to the hematopoietic cells due to restricted tissue expression.² JAK3 is abundantly expressed in lymphoid cells and has a crucial role in T-cell development and homeostasis of the immune system because of association with the common gamma chain (γ c) of cytokine receptors. Disruption of JAK3 function results in quantitative and qualitative deficiencies in both B and T-cell compartments of the immune system of JAK3 deficient mice, and development of severe combined immunodeficiency (SCID) in humans with the JAK3 genetic aberration.³ Our studies have shown that JAK3 is also a key regulator of IgE receptor-mediated mast cell responses.⁴ Specifically, it has been shown that JAK3 knock out mast cells release substantially reduced amounts of inflammatory mediators upon IgE receptor crosslinking as compared to wild-type. Furthermore, JAK3 also plays a key role

in mast cell dependent innate immune responses to gram-negative bacteria.⁵ Inhibitors of JAK3 have shown utility in many different auto immune disorders, including allograft rejection during transplantation, acute lymphoblastic leukemia, type I diabetes, rheumatoid arthritis and allergic diseases.⁶ Thus, targeting JAK3 may be helpful in the treatment and prevention of immune system based inflammatory diseases.

Currently, several potent JAK3 inhibitors have been identified. These include naphthyl ketones, oxindole derivatives, pyrimidine heterocycles, and pyrimidine based purines.⁶ These inhibitors have been the focus of several reviews, and the two most notable developments have been the quinazoline WHIP-131 and the purine CP-690,550 (Fig. 1).^{7,8} Both have shown JAK3 based activity in animal models of JAK3 related disease, including allograft rejection. Our effort focused on the staurosporine class of natural products. Staurosporine is a potent inhibitor of many kinases including JAK3.⁹ Although both staurosporine and K-252a (Fig. 1) are broad spectrum protein kinase inhibitors, many of their derivatives have been found to selectively inhibit both serine/threonine and tyrosine protein kinase family members.¹⁰ Furthermore, small modifications on these scaffolds can have dramatic effects on target specificity. In fact, synthetic and medicinal chemistry studies have provided many types of semi-synthetic derivatives, of which some have progressed through various stages of human clinical testing (Fig. 1; ruboxistaurin/LY-333531,¹¹ UCN-01,¹² PKC412,¹³ lestaurtinib/CEP-701,¹⁴ and CEP-1347¹⁵). Most notably, it was recently

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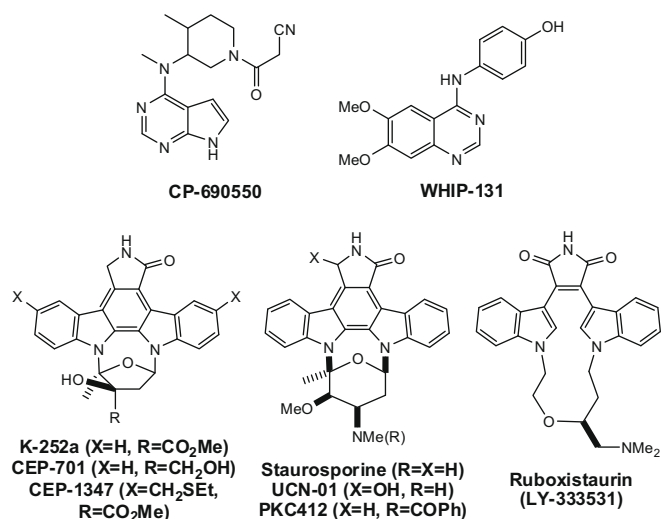


Figure 1. Small molecule JAK3 inhibitors, indole carbazole natural products and analogs.

shown that some of the anti-leukemic effects of the newly approved anti-cancer agent lestaurinib™ (CEP-701) are mediated through inhibition of the JAK2 enzyme pathway.¹⁴ In these cases it is worth noting that most of these materials are derived semi-synthetically directly from the natural products through fermentation, isolation, and derivation chemistries. Staurosporine and K-252a have both been used extensively to map ATP binding sites of protein kinases through X-ray studies.¹⁶ Natural product mimetics have the advantage of a high chance of target directed effects and have been estimated to be the basis for up to half of all new drugs in the past few decades.¹⁷

The overall goals of our efforts were to discover compounds that were more selective than staurosporine by modifying the natural products in three positions: the sugar portion, the pyrrolidinone ring, and also eventually on the indole ring. We devised an

approach to allow the inclusion of eight, nine, and ten member carbocyclic chains via the ring closing metathesis reaction scheme, and we previously communicated some of this chemistry.¹⁸ In principal, this would allow alkene type compounds to be produced, and also enable the ability to install oxygen functionality along the bottom portion. This strategy has been utilized in other natural product synthetic approaches.¹⁹ In this Letter, we provide how we utilized this chemistry to discover a series of unique natural product analogs through further modification and derivatization reaction pathways which has resulted in the discovery of potent and selective JAK3 inhibitors.

Our route started from construction of the bisindole precursor natural products based on acryrubin and acryflavin A (Scheme 1). The *N*-methyl-maleimide precursor **2** was assembled according to previous methodology from 2,3-dichloromaleic anhydride (**1**). Alkylation sequences provide both symmetrical (**3**) and unsymmetrical (**4**) RCM precursors. As previously reported, these substrates were exposed to the Grubbs 1st generation catalyst (**I**, Fig. 2) to give the ring closed eight (**5**) and nine member (**6**) ring products. Elaboration to final products that included hydroxyl groups consisted of hydroboration, and oxidation, and maleimide hydrolysis to give mono hydroxyl eight (**7**) and nine (**8**) member ring appended products. Most notable with the nine member ring (**8**) was the formation of a single isomer resulting from oxidation at the carbon closer to the indole nitrogen. Osmylation followed by maleimide hydrolysis resulted in eight (**9**) and nine (**10**) ringed syn diols.

Continuing the maleimide chemistry (Scheme 2), we next turned our attention to a more elaborate sugar mimetic substitution. First, we installed a more labile protecting group which would

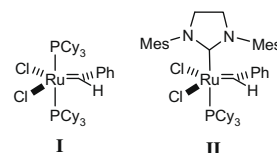
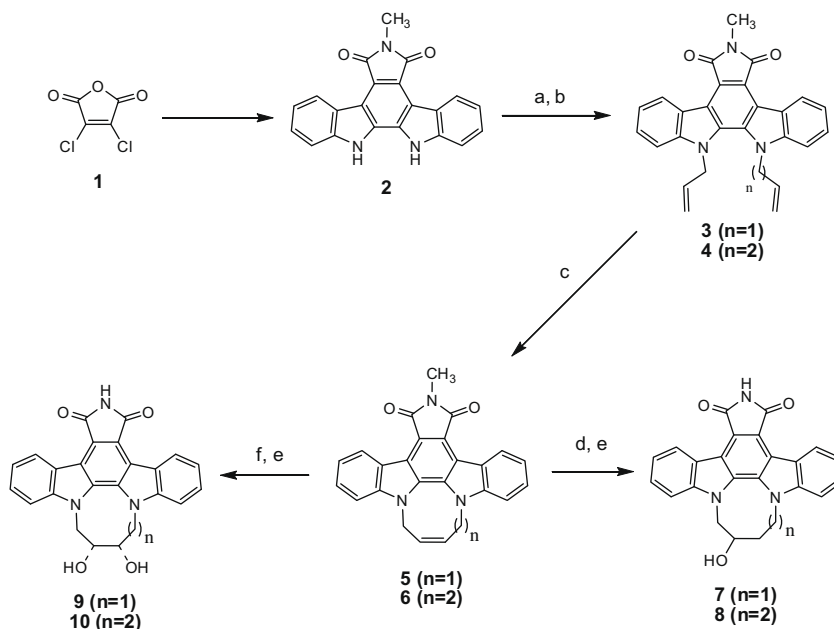
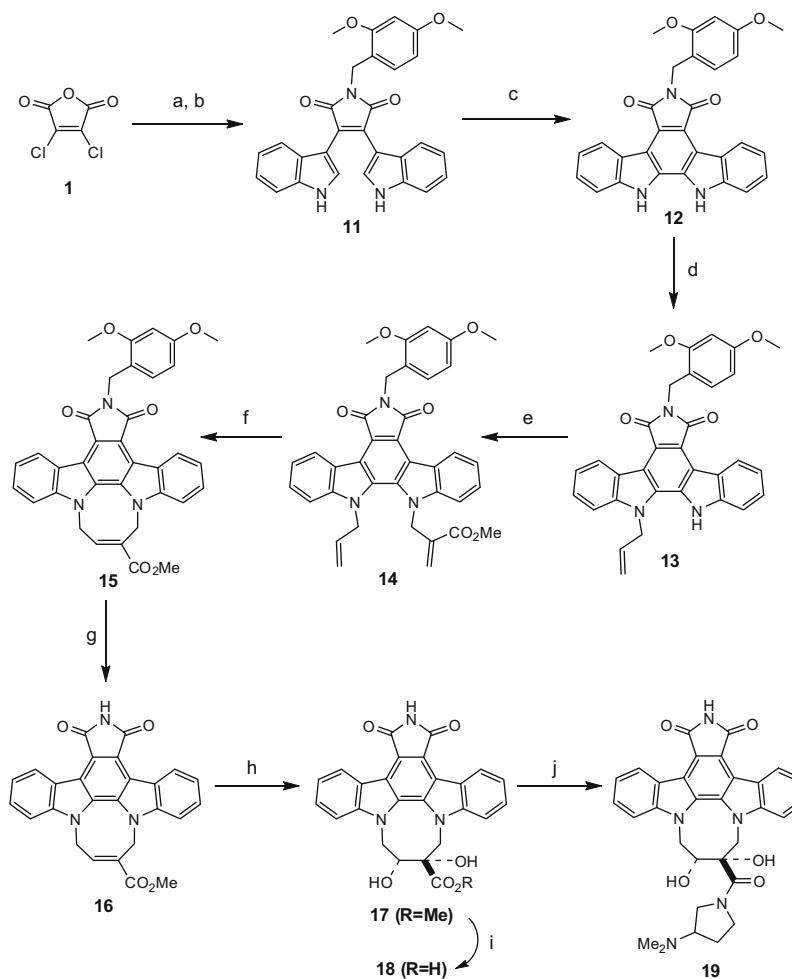


Figure 2. Ruthenium based olefin metathesis catalysts used in this study.



Scheme 1. Reagents and conditions: (a) Cs₂CO₃, allyl bromide, DMF, rt (83%); (b) 4-bromo-1-butene, DMSO, rt, (55%) then (a, 99%); (c) Grubbs I catalyst (**I**), DCM, rt, 85/96%; (d) (i) BH₃/THF, 0 °C–rt; (ii) H₂O₂, NaOH; (e) (i) NaOH, EtOH; (ii) HCl; (iii) HMDS, DMF, 90 °C; (f) OsCl₃, Me₃NO, CHCl₃/THF.



Scheme 2. Reagents and conditions: (a) AcOH, 80 °C, 18 h, 2,4-(MeO)₂PhCH₂NH₂, (87%); (b) indole, EtMgBr, Et₂O, THF, reflux, 24 h (56%); (c) DDQ, *p*-TsOH (cat.), PhCH₃, 1,4-dioxane (100%); (d) NaH, THF, allyl bromide, –50 °C to rt (51%); (e) Cs₂CO₃, methyl-(2-bromomethyl)-acrylate, DMF, 96%; (f) Grubbs II catalyst (II, 0.125 equiv), μ wave, 150 °C, ClCH₂CH₂Cl, 30 min (59–74%); (g) TFA, anisole, reflux, 71%; (h) OsCl₃, Me₃NO, THF/CHCl₃, H₂O, 81%; (i) LiOH, THF (75%); (j) HOBt, DIPEA, EDAC, 3-(diethylamino)-pyrrolidine, DMF (45%).

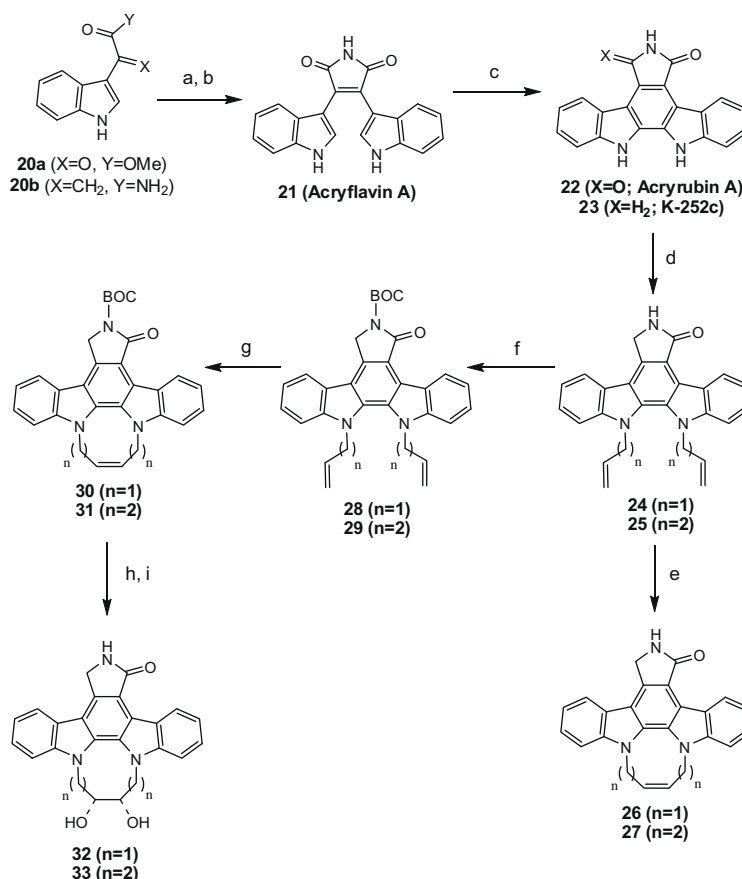
allow the installation of more sensitive functionality on the bottom portion. The 2,4-dimethoxy-bis-indole precursor **12** was synthesized by a similar sequence that was used for **2**. First, 2,3-dichloromaleimide (**1**) was converted to the bisindole compound **11** in two steps, followed by oxidation with DDQ. Selective alkylation was carried out by low temperature allylation to give **13**. Alkylation with bromomethyl acrylate gave **14**. Reaction with the Grubbs 2nd generation catalyst (**II**, Fig. 2) using microwave irradiation conditions we developed for this substrate gave a good yield of the corresponding acrylate gave the corresponding ring closed product **15**. Next, we removed the maleimide 2,4-dimethoxybenzyl protecting group utilizing acidic conditions to give **16**. We then performed dihydroxylation with osmium trichloride to give **17**. Hydrolysis of the ester gave dihydroxy-carboxylic acid **18**. Finally, amide bond formation could be completed to form secondary amide **19**.

The pyrrolidinone chemistry began with the synthesis of the staurosporine alglycon **23** (Scheme 3). Components of this synthesis were previously reported, and begin by condensation of indole precursors **20a** and **20b** to give Acrirubin A (**21**). Next, oxidation with palladium chloride was followed by Clemmensen reduction with tin metal to give **23**. Allylation was carried out to give bis-allyl compound **24**. In a similar fashion, bis-1-butenyl chains were attached (**25**) but required microwave irradiation. At this stage, ring closing metathesis was performed directly with **I** to give

eight (**26**) and ten member (**27**) cis-alkenyl ring systems. Alternatively, nitrogen protection with a *t*-butyl carbonate group (**28** and **29**), was followed by ring closing metathesis with **I** (**30** and **31**). Dihydroxylation with osmium trichloride followed by BOC deprotection gave the eight (**32**) and ten (**33**) member syn diols.

We then tested our compounds against the JAK3 enzyme along with staurosporine (Table 1). The SAR showed several trends. The maleimide derivatives had slightly better potency compared to the pyrrolidinones (**9** vs **32**). In all cases, the alglycon portion adds significant potency to the molecule resulting in up to 60-fold better potencies compared to the alglycon (K-252c). The dihydro-deoxy derivatives (**26** and **27**) showed activity better than K-252c and one derivative (**16**) was comparable to staurosporine, while the ring opened material lost almost all activity (**24**). The mono-hydroxyl (**7** and **8**) and diol chains (**9**, **10**, **32**, and **33**) showed equal or even greater activity (**17**) than staurosporine. Although the carboxymethyl group, which is also present on the natural product K-252a, seems to add some potency on these analogs (**17** versus **9**), the hydroxyl function seems to add equal and even more potency (**26** vs **32**). The diol functionality tolerates other substitutions such as a carboxylic acid (**18**) and a carboxamide (**19**) without much change in potency.

A computer based modeling study of one of the analogs (**32**) showed typical interactions of an ATP competitive inhibitor (Fig. 3).²⁰ Compound **32** was docked into a homology model of



Scheme 3. Reagents and conditions: (a) PdCl₂, DMF, 90 °C, (70%); (b) Sn⁰, HCl(conc.), THF, (86%); (c) allyl bromide, Cs₂CO₃, DMF, rt (72%); (d) 4-bromo-1-butene, Cs₂CO₃, AcCN, microwave, 150 °C (40%); (e) Grubbs I catalyst (**1**, 0.25 equiv.), Cl₂CH₂, reflux, 40 hrs., (55% for **26**, 31% for **27**); (f) (tBuOCO)₂O, MTBD, DMAP, THF, (83%); (g) Grubbs I catalyst (**1**, 0.125 equiv.), Cl₂CH₂, rt, (85%); (h) OsCl₃, Me₃NO, THF/CHCl₃, H₂O, (91%); (i) TFA, rt (67%).

Table 1
Janus kinase 3 (JAK3) enzyme and jurkat cell activity for derivatives and staurosporine

Compound	JAK3 IC ₅₀ ^a (nM)	IL2-stimulated jurkat cells IC ₅₀ ^b (nM)	Resting jurkat cells IC ₅₀ ^b (nM)
Staurosporine	6	71	311
7	5	160	5670
8	8	2860	>10,000
9	3	48	>10,000
10	9	227	>10,000
16	7	575	>10,000
17	1	23	>10,000
18	9	222	>10,000
19	15	57	1600
23	61	—	—
24	>200	—	—
26	39	111	>10,000
27	39	432	~10,000
32	6	98	~10,000
33	12	45	616

^a JAK3 enzyme inhibition was measured by inhibition of phosphorylation of biotinylated substrate in 96 well format.

^b Cell assays measuring JAK3 versus non-JAK3 inhibitory effects when stimulated by interleukin-2 (IL-2).

JAK3 generated from HCK sequence interchanging. The pyrrolidinone forms a donor acceptor complex with the backbone region with NH-O bonds to the Glu903 and Leu905 residues. The diol alglycon forms hydrogen bonding to the Arg953 residue in the flap region. The backbone interaction is consistent with the X-ray crystal structure determination of AFN491 with JAK3 and our own find-

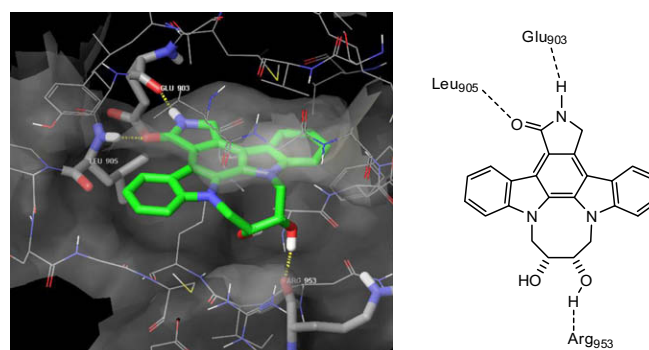


Figure 3. Compound **32** docked into the ATP site of JAK3.

ings after docking staurosporine, while the Arg953 binding is a new interaction.²¹

Since JAK3 plays a key role in the proliferation of T-cells through IL-2 mediated signaling,^{1–3} we hypothesized that a JAK3 inhibitor would inhibit clonogenic growth of JAK3-expressing human T-cells. In order to test this hypothesis, we exposed test compounds to resting or IL-2 stimulated Jurkat cells (Table 1) and their anti-proliferative effect was assessed. Most of these compounds showed modest to potent inhibition when the JAK3 based pathway is stimulated by IL-2, while showing little effect on resting cells ($\geq 10,000$ nM). In contrast, staurosporine shows lower selectivity in this assay (fourfold—71 nM vs 311 nM). The potency of inhibition in the IL2-Jurkat cell assay was mainly dependent upon the

alglycon portion. In general, the potencies increased relative to the increase in number of oxygen atoms in the molecule (**17** vs **32** vs **26**). The diols (**9**, **10**, **17**, **19**, **32**, and **33**) had the lowest IC₅₀ values in this assay (<100 nM), with equal or more potency than staurosporine in the IL-2 stimulated cells. Most notable were the compounds (**9**, **17**, **26**, and **32**) that were potent in the IL-2 stimulated pathway (<100 nM) and retained high selectivity (>100-fold) versus the non-stimulated Jurkat cells (>10,000 nM).

As further proof of selectivity, three of the most potent JAK3 inhibitors (**Table 2**; **16**, **17**, and **32**) were tested against other protein tyrosine kinases known to be involved in T-cell and mast cell signaling (e.g., SYK, Zap70, MK2, and LCK). We found our analogs had a high level of selectivity for JAK3 from 30 to greater than 1000-fold against these other kinases. Although not definitive, these findings further suggest that the activity seen in the Jurkat assay is due to JAK3 inhibition.

We then selected the most potent diol compound **32** for further biological characterization. Because JAK3 is required for the activation and phosphorylation of STAT5 in T-cells following IL-2 stimulation,^{1–3} we hypothesized that a JAK3 inhibitor would inhibit phosphorylation of STAT5 in T-cells. The T-cells were incubated with differing amounts of compound **32** and STAT5 phosphorylation was assessed by western blot analysis after cell lysis. As we expected, compound **32** inhibited STAT5 phosphorylation with an IC₅₀ of 689 nM. The observed 100-fold decrease in potency over the isolated enzyme assay could be due to several factors, but is not unprecedented.

We next examined the effect of this JAK3 inhibitor on IL-2 induced phytohemagglutinin stimulated T-cell (PHA blast T-cells) proliferation. The T-cells were incubated with increasing concentrations of the compound and T-cell proliferation was assessed by a ³H-thymidine assay. In this assay the compound exhibited an IC₅₀ of 100 nM (**Table 3**). Because JAK3 also plays a key role in IgE as well as bacteria mediated mast cell responses,^{5,6} we examined the effect of the JAK3 inhibitor **32** on LPS induced TNF α and IgE/antigen induced mast cell degranulation. Compound **32** potently inhibited LPS induced TNF- α release with an IC₅₀ value of 18 nM and IgE/antigen induced hexosaminidase (granule associated enzyme) release with an IC₅₀ value of 55 nM. These results suggest that compound is a potent inhibitor of JAK3 based signaling pathways in different cell types.

We then tested compound **32** in mice models of allergy and asthma. We examined the effect of this compound on anti-ovalbumin (OVA) IgE production and OVA induced airway inflammation and airway-responsiveness. Immunoglobulin E (IgE) is a known

biomarker of and cause of allergic responses, including increased mast cell chemotaxis, and is the basis for the recently approved monoclonal antibody therapeutic.²² It has been postulated that IgE production is regulated mainly through the JAK3 pathway by B cells. Furthermore, mice with a JAK3 deficiency (JAK3 knockout) fail to produce sufficient levels of IgE. To examine the effect of compound **32** on ovalbumin induced IgE production, mice were challenged intraperitoneally with ovalbumin (OVA) and two doses of compound **32**. Quantization of plasma IgE levels was performed by ELISA analysis.²⁴ As shown in **Figure 4**, compound **32** caused 15% (10 mg/kg.i.p.) and ~70% (30 mg/kg.i.p.) reduction in OVA induced IgE production in mice.

Asthma is a chronic inflammatory condition of the lung. Mast Cells, T-cells and dendritic cells play a key role in the pathogenesis of the disease²³. JAK3 happens to be a key regulator of functional responses in these cells. We therefore, next examined the effect of compound **32** on airway inflammations and airway hyper-responsiveness in a mouse model of allergic asthma.²⁴ When compound **32** was tested in a mouse model of asthma (30 mg/kg ip dose), two effects were observed. First, a 54% reduction in eosinophils and neutrophils was observed in the bronchoalveolar lavage fluid. Second, a greater than 90% reduction was observed in methacholine induced airway constriction (**Fig. 5**). These results show that compound **32** could be useful in both the acute and chronic phases of asthma.

In summary, we have presented the synthesis and evaluation of novel staurosporine based JAK3 inhibitors. These compounds have potent activity against the isolated enzyme, and also against JAK3 induced pathways in T-cells. Several compounds showed reasonable selectivity when tested against other immune system related

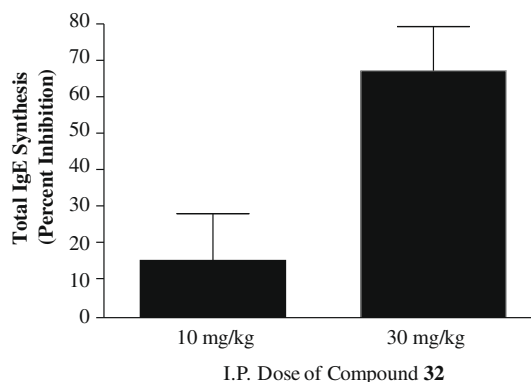


Figure 4. Effect of compound **32** on plasma IgE levels in a mouse model of ovalbumin (OVA) induced IgE synthesis.

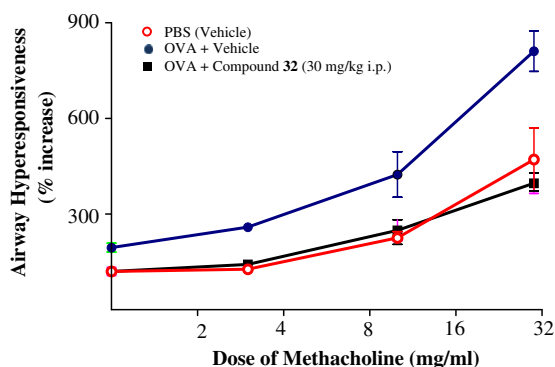


Figure 5. Reduction in airway hyper responsiveness by compound **32** in a mouse model of ovalbumin (OVA) induced asthma at the 30 mg/kg ip dose.

Table 2
Five enzyme protein kinase panel.

Compound	JAK3 ^b	IC ₅₀ ^a (nM)			
		SYK	Zap70	MK2 ^b	LCK
16	7	>1000	>1000	540	>1000
17	1	>1000	>1000	>1000	300
32	6	300	200	>1000	>1000

^a Enzyme assays were measured as inhibition of phosphorylation of biotinylated substrate in 96 well format.

^b Values for K-252a: JAK3–6 nM; MK2–9 nM.

Table 3
Outcome of JAK3 dependent in vitro assays performed on compound **32**

Bioassay	IC ₅₀ (nM)
STAT5 phosphorylation western blot in T-cells	689
IL2 induced PHA blast T-cell replication	100
LPS induced TNF- α release from mast cells	18
Inhibition of hexosaminidase release from IgE stimulated mast cells	55

kinases. One compound (**32**) showed broader activity in other biological systems and cell lines. This compound showed inhibition of the JAK3 phosphorylation substrate STAT5 in T-cells and potent activity in mast cells preventing chemotaxis. Furthermore, it showed potent activity in vivo lowering biomarkers of increased JAK3 activity, including IgE and asthmatic responses.

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Supplementary data

Supplementary data (synthetic procedures and characterization data for most compounds are provided, along with procedures for the some biological assays and animal experiments) associated with this article can be found, in the online version, at [doi:10.1016/j.bmcl.2009.04.039](https://doi.org/10.1016/j.bmcl.2009.04.039).

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Supplementary Material for Synthetic staurosporines via a ring closing metathesis strategy as potent JAK3 inhibitors and modulators of allergic responses

Chemistry

12,13-Dihydro-6-methyl-12,13-di(2-propenyl)-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6H)-dione (**3**). Compound **2** (2.37 g, 7 mmol; prepared from **1** according to procedures described by Slater, M. J. *Bioorganic & Medicinal Chemistry*, 1999, 7, 1067) was dissolved in DMF (200 mL) and Cs_2CO_3 (5 g) was added followed by allylbromide (1.8 mL). After stirring at 20 °C for 24h, the mixture was diluted with water (500 mL). The resulting brown precipitate was filtered, washed with water and methanol. After drying, compound **3** (2.447 g, 83%) was obtained as an orange-brown solid. ^1H NMR (CDCl_3) δ 3.03 (s, 3H), 4.81 (s, 4H); 5.43 (t, 4H, $J = 18$ Hz); 6.1 (m, 2H); 7.25-7.54 (m, 4H); 9.21 (d, 2H, $J = 8$ Hz); ^{13}C NMR δ 23.91, 51.17, 111.76; 117.85; 120.33; 120.7; 122.03; 123.37; 125.9; 127.86; 132.96; 133.22; 145.28; 169.98; MS 861 (2M+Na), 442 (M+Na), 420 (M+H⁺); HRMS Calcd. For $\text{C}_{27}\text{H}_{21}\text{N}_3\text{O}_2$: 419.1638; Found: 419.1623.

6-methyl-12,13-(but-2-en-1,4-yl)-12,13-dihydro-5,7-dioxo-6H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**5**). Compound **3** (1.26 g, 3 mmol) was dissolved in dichloromethane (500 mL) and benzylidenebis(tricyclohexylphosphine) dichlororuthenium (250 mg, 0.3 mmol) was added. The mixture was stirred at room temperature for 6h. Silica gel (5 g) was added and the mixture was filtered over a thin pad of silica gel. The silica gel was washed with 3 portions of 50 mL each of dichloromethane and ethyl acetate. The organic layers were combined and concentrated under vacuum. The resulting solids were washed with methanol, filtered and dried to give Compound **5** (1 g, 85%) as a brownish-yellow solid. ^1H NMR (CDCl_3) δ 2.91 (s, 3H); 4.77 (d, 4H, $J = 5$ Hz); 6.21 (quin, 2H, $J = 4$ Hz); 7.44 (t, 2H, $J = 5$ Hz); 7.48 (d, 2H, $J = 7$ Hz); 7.63 (t, 2H, $J = 6$ Hz); 9.39 (d, 2H, $J = 6$ Hz); ^{13}C NMR δ 23.37, 39.35, 77.07, 108.18, 118.51, 119.03, 120.66, 121.32, 125.87, 126.83; 128.95, 131.43, 140.68, 169.35; MS: 805 (2M+Na), 414 (M+Na), 392 (M+H⁺); HRMS Calcd. For $\text{C}_{25}\text{H}_{17}\text{N}_3\text{O}_2$: 391.1321; Found: 391.1331.

Preparation of 12-allyl-13-butenyl-6-methyl-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7(6H)-dione (**4**). Compound **2** (1.693 g, 5 mmol) was dissolved in DMF (100 mL) and NaH (95%, 625 mg) was added. The mixture was stirred for 1h and 4-bromo-1-butene (10 mL) was added. The mixture was stirred for 8h then quenched with aqueous NH_4Cl . The mixture was extracted with ethyl acetate and the organic extracts were washed with aqueous NH_4Cl and brine. The aqueous layers were back extracted and the combined organic layers were dried over Na_2SO_4 . After concentration, the residue was purified by flash column chromatography (hexane:ethyl acetate = 2:1) to give compound **4** (1.07 g) as a yellow solid. ^1H NMR (300 MHz, CDCl_3) δ 2.65 (quart, 2H, $J = 7$ Hz), 3.01 (s, 3H), 4.37 (t, 2H, $J = 7$ Hz), 5.13 (s, 1H), 5.14 (d, 1H, $J = 30$ Hz), 5.82 (m, 1H), 7.44 (m, 4H), 7.58 (quart, 2H, $J = 7$ Hz), 8.22 (s, 1H), 9.18 (d, 2H, $J = 8$ Hz); MS: 809 (2M+Na), 416 (M+Na), 394 (M+H⁺). A mixture of the butenyl compound (395 mg, 1 mmol), Cs_2CO_3 (975 mg, 3 mmol) and DMF (20 mL) was stirred for 20 min at room temperature and allyl bromide (500 μL , 2.95 mmol) was added. The mixture was stirred overnight at room temperature, quenched with aqueous NH_4Cl and extracted with ethyl acetate. The organic extracts were washed with aqueous NH_4Cl and brine. The aqueous layers were back extracted with ethyl acetate. The organic layers were combined, dried (Na_2SO_4) and concentrated in vacuo to give crude compound **4** as a yellow solid, which was suitable to be used in the next step without further purification.

6-methyl-12,13-(pent-2-en-1,5-yl)-12,13-dihydro-5,7-dioxo-6H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**6**). The crude compound **4** (~1 mmol) was dissolved in dichloromethane (100 mL) and benzylidenebis(tricyclohexylphosphine) dichlororuthenium (120 mg, 0.146 mmol) was added. The mixture was stirred for 1h at room temperature then filtered over a pad of silica gel with additional dichloromethane. The silica gel was washed with ethyl acetate (200 mL) to elute remaining product. The washings were combined and concentrated under vacuum. The resulting solids were diluted with methanol, filtered, washed and dried to give compound **6** (387 mg, 96% yield over 2 steps) as a greenish-yellow solid. ¹H NMR (300 MHz, CDCl₃) δ 3.05 (quart, 2H, J = 6 Hz), 3.13 (s, 3H), 4.88 (bs, 2H), 5.16 (d, 2H, J = 9 Hz), 5.36 (quart, 1H, J = 9 Hz), 5.65 (quint, 1H, J = 6 Hz), 7.43 (m, 3H), 7.52 (d, 1H, J = 8 Hz), 7.61 (quart, 2H, J = 7 Hz), 9.32 (d, 1H, J = 8 Hz), 9.36 (d, 1H, J = 8 Hz); MS: 833 (2M+Na), 428 (M+Na), 406 (M+H⁺).

12,13-(2-hydroxy-butan-1,4-yl)-12,13-dihydro-5,7-dioxo-6H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**7**). Compound **5** (586 mg, 1.5 mmol) was slurried in THF (40 mL) and BH₃-THF solution (1M, 7.5 mL) was added at 0 °C over 10 minutes. The reaction mixture was stirred for 45 minutes at 0 °C, then warmed to room temperature over 1 hour. The mixture was then cooled to 0 °C and aqueous H₂O₂ solution (50%, 35 mL) was added over 10 minutes. Subsequently 10% aqueous NaOH (105 mL) was added slowly over 20 minutes. The resulting mixture was stirred at 0°C for 45 minutes, then extracted with ethyl acetate and washed twice with dilute NaOH solution. The aqueous layers were then back extracted with ethyl acetate. The organic layers were combined, dried (Na₂SO₄) and concentrated. The residue was diluted with methanol and the resulting solids were collected by filtration to give (579 mg, 94%) an orange solid. ¹H NMR (d₆-DMSO) δ 1.89 (m, 1H), 2.31 (m, 1H), 3.01 (s, 3H), 4.29 (m, 1H), 4.39-4.59 (m, 3H), 4.72 (dd, 1H, J = 9, 14 Hz), 7.37 (t, 1H, J = 7 Hz), 7.38 (t, 1H, J = 7 Hz), 7.59 (t, 1H, J = 7 Hz), 7.61 (t, 1H, J = 7 Hz), 7.73 (d, 1H, J = 9 Hz), 7.77 (d, 1H, J = 9 Hz), 9.08 (d, 1H, J = 8 Hz), 9.13 (d, 1H, J = 8 Hz); MS 841 (2M+Na), 432 (M+Na), 410 (M+H). The N-methyl compound (579 mg, 1.43 mmol) was diluted with 1,4-dioxane (20 mL), EtOH (20 mL) and aqueous 30% KOH (40 mL). The mixture was heated to 95 °C for 7 days, then cooled and acidified with HCl (conc.) until pH = 1. The resulting mixture was then extracted with ethyl acetate, washed with H₂O and aqueous Na₂SO₄ solution. The aqueous layers were back extracted with ethyl acetate and the organic layers were combined, dried (Na₂SO₄) and concentrated. The resulting brownish residue was dissolved in N,N-dimethylformamide (80 mL) and heated with 1,1,3,3-hexamethyldisilazane (9 mL) and methanol (1 mL) for 4 hours. The mixture was cooled then aqueous NaHCO₃ solution was added and extracted with ethyl acetate. The organic extracts were evaporated and the residue was diluted with tetrahydrofuran (100 mL), methanol (10 mL) and trifluoroacetic acid (1 mL). The mixture was stirred for 2 hours at room temperature and the volatiles were removed under vacuum. The residue was diluted with methanol and filtered to give compound **7** (464 mg, 83%) as an orange solid. ¹H NMR (d₆-DMSO) δ 2.03 (bs, 1H), 2.43 (bs, 1H), 4.35 (bs, 1H), 4.53-4.72 (m, 3H), 4.84 (dd, 1H, J = 9, 15 Hz), 5.44 (d, 1H, J = 4 Hz), 7.4 (t, 1H, J = 7 Hz), 7.41 (t, 1H, J = 7 Hz), 7.64 (t, 1H, J = 8 Hz), 7.65 (t, 1H, J = 8 Hz), 7.82 (d, 1H, J = 13 Hz), 7.85 (d, 1H, J = 13 Hz), 9.16 (d, 1H, J = 8 Hz), 9.22 (d, 1H, J = 8 Hz), 11.08 (s, 1H); ¹³C NMR (d₆-DMSO) δ 25.8, 49.33, 67.68, 117.57, 118.5, 120.32, 120.99, 121.08, 121.55, 121.64, 125.01, 125.1, 127.66, 129.39, 131.7, 143.62, 171.39, 171.48; MS 396 (M+H), 394 (M-H); HRMS calcd. For C₂₄H₁₇N₃O₃: 395.1274; Found: 395.1270.

12,13-(2-hydroxy-pentan-1,5-yl)-12,13-dihydro-5,7-dioxo-6H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**8**). Compound **6** (880 mg, 2.17 mmol) was mixed with tetrahydrofuran (150 mL) and BH₃-THF solution (1M, 12 mL) was added dropwise at 0° C over a period of 20 minutes. The reaction mixture was stirred at 0° C for 3.5 hours and methanol (20 mL) was added to quench excess borane reagent. Aqueous H₂O₂ solution (50%, 20 mL) was added slowly over a period of 15 minutes, followed by addition of aqueous 10% NaOH solution (30 mL) over a period of 30 minutes. The mixture was then diluted with water (50 mL), warmed to room temperature and extracted with ethyl acetate. The organic layer was washed with brine, dried over Na₂SO₄ and concentrated. The residue was diluted with methanol and filtration gave 865 mg (94%) a brownish-yellow-orange solid. ¹H NMR (d₆-

DMSO) δ 1.43 (bs, 2H), 1.93 (bs, 1H), 2.24 (bs, 1H), 3.06 (s, 3H), 4.08 (bs, 1H), 4.84 (m, 3H), 4.93 (m, 1H), 7.34 (m, 2H), 7.61 (m, 2H), 7.74 (d, 2H, $J = 7$ Hz), 9.27 (d, 2H, $J = 7$ Hz); ^{13}C NMR DEPT (d_6 -DMSO) δ 24.38 (up), 24.99 (down), 28.02 (down), 46.11 (down), 52.52 (down), 70.50 (up), 110.51 (up), 111.69 (up), 121.13 (up), 121.22 (up), 125.19 (up), 125.47 (up), 127.82 (up), 128.07 (up); MS: 869 (2M+Na), 446 (M+Na), 424 (M+H⁺). The N-methyl compound (423 mg, 1 mmol) was heated with KOH (6.57 g) in anhydrous ethanol (50 mL) at 95 °C for 3 days. The reaction mixture was cooled and the pH was adjusted to 1 by adding HCl (conc.). The resulting solids were filtered, dried then mixed with N,N-dimethylformamide (50 mL), 1,1,3,3-hexamethyldisilazane (10 mL) and methanol (1 mL). The resulting mixture was heated to 95 °C for 48 hours, then cooled and quenched with methanol (10 mL). Additional methanol (40 mL) was added along with trifluoroacetic acid (20 mL). The mixture was stirred at room temperature overnight. The solids were filtered, washed with tetrahydrofuran and methanol, dried under vacuum to give compound **8** (306 mg, 75%) as a yellow solid. ^1H NMR (300 MHz, d_6 -DMSO) δ 1.54 (bs, 2H), 2.02 (bs, 1H), 2.27 (bs, 1H), 4.15 (bs, 1H), 5.04 (m, 3H), 5.12 (m, 1H), 7.4 (quart, 2H, $J = 7$ Hz), 7.65 (m, 2H), 7.87 (m, 2H), 9.38 (d, 2H, $J = 8$ Hz), 11.09 (s, 1H); MS: 841 (2M+Na), 432 (M+Na), 410 (M+H⁺).

12,13-(2,3-cis-dihydroxy-butan-1,4-yl)-12,13-dihydro-5,7-dioxo-6H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**9**). A mixture of compound **5** (984 mg, 2.5 mmol), trimethylamine-N-oxide, (410 mg, 5.46 mmol) and anhydrous osmium trichloride (27 mg, 0.09 mmol) was diluted with chloroform (60 mL) and tetrahydrofuran (30 mL), along with 5 drops of water. The mixture was stirred at room temperature for 24 hours. The solvent was removed under vacuum and the resulting solids were filtered off and washed with water, methanol and chloroform (4 x each). The solids were dried in a vacuum oven to give the N-methyl compound (1.055 g, 99% yield) as an orange solid. ^1H NMR (d_6 -DMSO, 400 MHz) δ 3.01 (s, 3H), 4.26 (s, 2H), 4.53 (d, 2H, $J = 13$ Hz), 4.61 (d, 1H, $J = 9$ Hz), 4.65 (d, 1H, $J = 9$ Hz), 5.45 (s, 2H), 7.4 (t, 2H, $J = 8$ Hz), 7.64 (t, 2H, $J = 8$ Hz), 7.76 (d, 2H, $J = 8$ Hz), 9.11 (d, 2H, $J = 8$ Hz); ^{13}C NMR (d_6 -DMSO, 400 MHz) δ 24.39, 49.03, 70.51, 111.08, 118.26, 119.63, 121.52, 121.6, 125.23, 128.26, 170.16; MS (ES) 873 (2M+Na), 426 (M+H). A mixture of the N-methyl compound (1 g, 2.35 mmol), KOH (30 g), 1,4-dioxane (20 mL) and absolute ethanol (100 mL) was heated at 105 °C for 5 days. The mixture was cooled to room temperature, diluted with H₂O and acidified with HCl (conc.) to pH=2. The mixture was extracted with ethyl acetate and the organic extracts were washed with water and brine solution. The aqueous layers were back extracted two times with ethyl acetate and the combined organic layers were concentrated under vacuum. The residue was mixed with 1,4-dioxane (50 mL), N,N-dimethylformamide (25 mL) and 1,1,3,3-hexamethyldisilazane (25 mL). The resulting mixture was heated to 95 °C for 36 hours and concentrated in vacuo. The residue was stirred with methanol (40 mL) and trifluoroacetic acid (15 mL) and the resulting solids were filtered. The solids were washed with methanol and ethyl acetate (3 times each) to give compound **9** (550 mg, 57%) as a yellow solid. ^1H NMR (d_6 -DMSO, 300 MHz) δ 4.31 (bs, 2H), 4.59 (d, 2H, $J = 14$ Hz), 4.71 (d, 1H, $J = 9$ Hz), 4.76 (d, 1H, $J = 9$ Hz), 5.45 (bs, 2H), 7.41 (t, 2H, $J = 8$ Hz), 7.65 (t, 2H, $J = 8$ Hz), 7.83 (d, 2H, $J = 8$ Hz), 9.17 (d, 2H, $J = 8$ Hz), 11.09 (s, 1H, NH); ^{13}C NMR DEPT (d_6 -DMSO, 400 MHz) δ 49.09 (CH₂), 70.55 (CH), 111.17 (CH), 121.58 (CH), 125.41 (CH), 128.24 (CH); MS (ES): 821 (2M-H), 410 (M-H); HRMS calcd. For C₂₄H₁₇N₃O₄: 411.1219; Found: 411.1207.

12,13-(2,3-cis-dihydroxy-pentan-1,5-yl)-12,13-dihydro-5,7-dioxo-6H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**10**). Compound **6** (153 mg, 0.38 mmol) was dissolved in chloroform (30 mL) and tetrahydrofuran (20 mL). OsCl₃ (20 mg, 0.067 mmol) was added followed by water (0.25 mL) and trimethylamine-N-oxide (150 mg, 2 mmol). The mixture was stirred at room temperature overnight. The solvent was removed under vacuum and the resulting solids were diluted with water and filtered. The filter cake was washed with water and methanol (3 times each) and dried to give (153 mg, 93%) a yellow solid. MS: 901 (2M+Na), 462 (M+Na), 440 (M+H⁺). The N-methyl compound (100 mg, 0.228 mmol) was mixed with ethanol (25 mL) and KOH (6 g) was added. The mixture was heated to 94 °C for 48 hours then cooled, diluted with water and the pH was adjusted to 1 by adding HCl

(conc.). The yellow precipitate was extracted with ethyl acetate and washed with brine twice. The aqueous layers were back extracted. The organic layers were combined and the volatile components were removed under vacuum. The resulting residue was mixed with N,N-dimethylformamide (20 mL) and 1,1,3,3-hexamethyldisilazane (10 mL) and methanol (1 mL) were added. The resulting mixture was heated to 95 °C for 18 hours then cooled and methanol (15 mL) and trifluoroacetic acid (10 mL) were added. The resulting mixture was stirred at room temperature for two hours. The volatiles were removed under vacuum, followed by addition of water and filtration of the resulting solids. The solids were washed with water and methanol, then dissolved in DMSO and filtered over silica gel eluting with tetrahydrofuran. After solvent removal, the residue was diluted with methanol, filtrated and dried under vacuum to give compound **10** (57 mg, 59%). ¹H NMR (300 MHz, d₆-DMSO) δ 1.88 (m, 1H), 2.61 (m, 1H), 3.58 (d, 1H, J = 8 Hz), 4.14 (bs, 1H), 4.5 (m, 1H), 4.91 (m, 4H), 5.52 (d, 1H, J = 4 Hz), 7.4 (t, 2H, J = 6 Hz), 7.65 (quart, 2H, J = 7 Hz), 7.76 (d, 1H, J = 8 Hz), 7.87 (d, 1H, J = 8 Hz), 9.34 (quart, 2H, J = 5 Hz), 11.05 (s, 1H); ¹³C NMR DEPT (d₆-DMSO, 400 MHz) δ 32.28 (down), 44.1 (down), 49.61 (down), 66.4 (up), 72.42 (up), 109.74 (up), 110.25 (up), 120.83 (up), 120.97 (up), 125.05 (up), 125.1 (up), 127.68 (up), 127.79 (up); MS: 873 (2M+Na), 426 (M+H⁺).

N-(2,4-Dimethoxy benzyl)-2,3-bis-(3-indolyl)-maleimide (**11**). Neat 3,4-dichloro-furan-2,5-dione (6.7 g, 40 mmol) was mixed with 2,4-dimethoxy-benzylamine (6.25 mL) in glacial acetic acid (120 mL). The mixture was heated to 80°C for 18 hrs. Upon cooling, the mixture was poured over ice and the precipitate was collected by filtration, then washed with water and NaHCO₃ (aq.) and dried in a vacuum oven to provide 3,4-dichloro-1-(2,4-dimethoxy-benzyl)-pyrrole-2,5-dione (11.08 g, 87%) as a light orange solid. ¹H NMR (d₆-DMSO, 300 MHz) δ 3.73 (s, 3H), 3.76 (s, 3H), 4.54 (s, 2H), 6.44 (d, 1H, J=8 Hz), 6.57 (s, 1H), 7.12 (d, 1H, J=8 Hz); MS m/z 340 (M+2+Na), 338 (M+Na), 318 (M+2H), 316 (M+H). A solution of ethylmagnesium bromide (1M, tetrahydrofuran) was added to a solution of indole (4 equivalents) in tetrahydrofuran. The reaction was stirred at room temperature for 1 hour, followed by addition of N-(2,4-Dimethoxybenzyl)-2,3-dichloro-maleamide in tetrahydrofuran. The mixture was then heated to 65°C for 24 hrs. Upon cooling, the mixture was quenched with aqueous NH₄Cl and extracted with ethyl acetate. The organic layer was washed with aqueous NH₄Cl and brine. The aqueous layer was back extracted and the organic extracts were combined and concentrated. The resulting residue was diluted with DCM, the solids were collected by filtration, then washed four times with DCM and dried in a vacuum oven to provide 1-(2,4-dimethoxy-benzyl)-3,4-bis-(1H-indol-3-yl)-pyrrole-2,5-dione (**11**, 4.66 g, 56%). ¹H NMR (d₆-DMSO, 300 MHz) δ 3.73 (s, 3H), 3.82 (s, 3H), 4.68 (s, 2H), 6.48 (d, 1H, J=8 Hz), 6.58 (s, 1H), 6.23 (t, 2H, J=8 Hz), 6.81 (d, 2H, J=9 Hz), 7.11 (quart, 3H, J=8 Hz), 7.38 (d, 2H, J=9 Hz), 7.85 (s, 2H), 11.68 (s, 2H); MS m/z 977 (2M+Na), 478 (M+H).

6-(2,4-dimethoxy-benzyl)-12,13-dihydro-5,7-dioxo-6H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**12**). Compound **11** (1.7 g, 3.56 mmol) was dissolved in 1,4-dioxane (85 mL) and toluene (255 mL). The mixture was heated to 100 °C with stirring for 15 min., then DDQ (889 mg, 3.91 mmol) and p-toluenesulfonic acid (34 mg, 0.18 mmol) were added. The resulting mixture was stirred at 120 °C for 1 hr, then the heat was removed and the mixture was allowed to cool and was stirred at 20°C overnight. After concentration, the residue was dissolved in ethyl acetate and washed with 5% aqueous NaHCO₃ and brine. After the layers were separated, the organic phase was concentrated to provide 6-(2,4-dimethoxy-benzyl)-12,13-dihydro-5,7-dioxo-6H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**12**, 2.11 g) as a dark brown solid. ¹H NMR (d₆-DMSO, 300 MHz) δ 3.72 (s, 3H), 3.86 (s, 3H), 4.82 (s, 2H), 6.44 (d, 1H, J=8 Hz), 6.60 (s, 1H), 7.08 (d, 1H, J=8 Hz), 7.34 (t, 2H, J=7 Hz), 7.58(t, 2H, J=7 Hz), 7.80 (d, 2H, J=8 Hz), 8.90 (d, 2H, J=8 Hz), H), 11.97; MS m/z 973 (2M+Na), 489 (M+Na), 474 (M-H).

6-(2,4-dimethoxy-benzyl)-12-allyl-12,13-dihydro-5,7-dioxo-6H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**13**). Compound **12** (3.3 g, 6.94 mmol) was dissolved in DMF and sodium hydride (555 mg, 13.88 mmol) was added. The mixture was stirred at 20°C for 90 min, cooled at -52 °C for 1 hr and 3-bromo-

prop-1-ene (600 μ L, 6.94 mmol) was added. The mixture was stirred at -52°C for 3 hrs, then at 20°C overnight. The reaction was quenched with methanol and diluted with water. The mixture was extracted with ethyl acetate twice and the organic layers were washed with brine. The organic layers were combined and dried (Na_2SO_4), then concentrated and purified via flash column chromatography (CH_2Cl_2 :MeOH = 500:1) to provide 6-(2,4-dimethoxy-benzyl)-12-allyl-12,13-dihydro-5,7-dioxo-6H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**13**, 1.82 g, 51% yield) as a brown solid. ^1H NMR (CDCl_3) 3.73 (s, 3H), 3.84 (s, 3H), 4.85 (s, 2H), 5.05-5.20 (m, 3H), 5.35 (d, 1H, $J=10$ Hz), 6.31 (m, 1H), 6.37 (d, 1H, $H=8$ Hz), 6.43 (s, 1H), 7.19 (d, 1H, $J=8$ Hz), 7.38 (m, 3H), 7.46-7.62 (m, 3H), 8.56 (s, 1H), 9.24 (t, 2H, $J=9$ Hz); MS m/z 1053 ($2\text{M}+\text{Na}$), 538 ($\text{M}+\text{Na}$), 516 ($\text{M}+\text{H}$).

6-(2,4-dimethoxy-benzyl)-12-allyl-13-[(2-methylene-3-oxo-3-methoxy)prop-1-yl]-12,13-dihydro-5,7-dioxo-6H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**14**). The mono allyl compound (**13**, 1.53 g, 2.97 mmol) was dissolved in DMF (76 mL) and Cs_2CO_3 (2.42 g, 7.42 mmol) and (3-bromo-2-methylene-1-oxo-1-methoxy)propane (393 μ L, 3.27 mmol) were added. The reaction mixture was stirred at 20°C for 2 hrs and quenched with water (200 mL). The precipitate was collected by filtration, rinsed with methanol and dried in a vacuum oven to provide 6-(2,4-dimethoxy-benzyl)-12-allyl-13-[(2-methylene-3-oxo-3-methoxy)prop-1-yl]-12,13-dihydro-5,7-dioxo-6H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**14**, 1.75 g, 96%) as a yellow solid. ^1H NMR (CDCl_3) 3.75 (s, 3H), 3.86 (s, 6H), 4.88 (s, 2H), 5.01 (s, 2H), 5.22 (s, 2H), 5.43 (m, 2H), 5.81 (s, 1H), 6.07 (m, 1H), 6.41 (d, 1H, $J=8$ Hz), 6.47 (s, 1H), 6.62 (s, 1H), 7.22 (s, 1H), 7.36 (d, 1H, $J=7$ Hz), 7.43 (m, 2H), 7.52 (m, 3H), 9.37 (t, 2H, $J=7$ Hz); MS m/z 1249 ($2\text{M}+\text{Na}$), 636 ($\text{M}+\text{Na}$), 614 ($\text{M}+\text{H}$).

6-(2,4-dimethoxy-benzyl)-12,13-(2-methoxycarbonyl-but-2-en-1,4-yl)-12,13-dihydro-5,7-dioxo-6H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**15**). A mixture of compound **14** (122.6 mg, 0.2 mmol), 1,3-bis-[(2,4,6-trimethylphenyl)-2-imidazolidinylidene] dichloro(phenylmethylene)-(tricyclohexylphosphine) ruthenium (21.2 mg, 0.025 mmol) and 1,2-dichloroethane (4 mL) was heated to 150°C in a microwave oven for 30 min. The mixture was cooled to room temperature and the resulting precipitate was collected by filtration to provide 6-(2,4-dimethoxy-benzyl)-12,13-(2-methoxycarbonyl-but-2-en-1,4-yl)-12,13-dihydro-5,7-dioxo-6H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**15**, 75 mg, 64%) as a yellow solid: ^1H NMR (CDCl_3) δ 3.62 (s, 3H), 3.73 (s, 3H), 3.86 (s, 3H), 4.77 (s, 2H), 5.62 (d, 2H, $J=8$ Hz), 5.7 (s, 2H), 6.43 (dd, 1H, $J=2$ Hz, $J=8$ Hz), 6.6 (d, 1H, $J=2$ Hz), 7.04 (d, 1H, $J=8$ Hz), 7.41 (t, 1H, $J=8$ Hz), 7.42 (t, 1H, $J=8$ Hz), 7.52 (t, 1H, $J=8$ Hz), 7.7 (m, 2H), 8.03 (d, 1H, $J=5$ Hz), 8.06 (d, 1H, $J=5$ Hz), 9.28 (d, 1H, $J=5$ Hz), 9.3 (d, 1H, $J=5$ Hz); MS m/z 585.9 ($\text{M}+\text{H}$), 584 ($\text{M}-\text{H}$); HRMS Calcd. For $\text{C}_{35}\text{H}_{27}\text{N}_3\text{O}_6$: 585.1899; Found: 585.1907.

12,13-[2-carbomethoxy-1,4-but-cis-2-enyl]-6,7,12,13-tetrahydro-5,7-dioxo-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**16**). Trifluoroacetic acid (5.7 mL) and anisole (8 mL) were added to compound **15** (155 mg, 0.265 mmol). The reaction mixture was heated to 90°C for 8 hours, cooled and concentrated. The residue was mixed with hexane and the resulting solids were filtered, washed with hexane three times and dried under vacuum. The orange solid was dissolved in THF, filtered and the filtrate was concentrated to dryness. The resulting solids were diluted with methanol, filtered, washed with methanol and dried to give 12,13-[2-carbomethoxy-1,4-but-cis-2-enyl]-6,7,12,13-tetrahydro-5,7-dioxo-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**16**, 82 mg, 71%) as a yellow solid. ^1H NMR (CDCl_3) δ 3.63 (s, 3H), 5.68 (d, 2H, $J=8$ Hz), 5.76 (s, 2H), 7.42 (t, 1H, $J=8$ Hz), 7.43 (t, 1H, $J=8$ Hz), 7.53 (t, 1H, $J=8$ Hz), 7.69 (t, 1H, $J=8$ Hz), 7.7 (t, 1H, $J=8$ Hz), 8.05 (d, 1H, $J=4$ Hz), 8.08 (d, 1H, $J=4$ Hz), 9.33 (t, 2H, $J=7$ Hz), 11.17 (s, 1H); MS: 893 ($2\text{M}+\text{Na}$), 436 ($\text{M}+\text{H}^+$); HRMS Calcd. For $\text{C}_{26}\text{H}_{17}\text{N}_3\text{O}_4$: 435.1219; Found: 435.1209.

12,13-[2-carboxymethyl-cis-2,3-dihydroxy)-1,4-butyl]-6,7,12,13-tetrahydro-5,7-dioxo-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**17**). The nitrogen deprotected compound (**16**, 70 mg, 0.161 mmol) was suspended in CHCl_3 (12 mL) and tetrahydrofuran (6 mL). Trimethylamine-N-oxide (30 mg) and OsCl_3 (8 mg) were added, followed by 8 drops of H_2O . The mixture was stirred at room temperature for 7 hours and the solvent was removed under vacuum. The resulting residue was stirred

with water, filtered then washed with water, methanol and chloroform (twice each). The solids were dried under vacuum to give compound **17** (61 mg, 81%) as an orange solid. ^1H NMR (CDCl_3): δ 3.47 (s, 3H), 4.6 (m, 2H), 4.8 (quart, 2H, $J=14$ Hz), 5.09 (quart, 1H, $J=6$ Hz), 5.82 (d, 1H, $J=5$ Hz), 6.31 (s, 1H), 7.42 (quart, 2H, $J=8$ Hz), 7.67 (quint, 2H, $J=7$ Hz), 7.88 (d, 1H, $J=8$ Hz), 9.18 (d, 1H, $J=8$ Hz), 9.26 (d, 1H, $J=8$ Hz), 11.15 (s, 1H); MS: 961 ($2\text{M}+\text{Na}$), 470 ($\text{M}+\text{H}^+$), 468 ($\text{M}-\text{H}^+$); HRMS Calcd. For $\text{C}_{26}\text{H}_{19}\text{N}_3\text{O}_6$: 469.1273; Found: 469.1288.

12,13-[2-carboxy-cis-2,3-dihydroxy-1,4-butyl]-6,7,12,13-tetrahydro-5,7-dioxo-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**18**). Compound **17** (50 mg, 0.106 mmol) was suspended in tetrahydrofuran (5 mL). H_2O (2.5 mL) and LiOH (5.2 mg) were added. The mixture was stirred at room temperature for 2 hours and then concentrated under vacuum. The residue was diluted with water and extracted with ethyl acetate. The mixture was acidified with 1N HCl solution. The organic layer was washed with brine solution, dried over Na_2SO_4 and concentrated. The resulting solids were dried under vacuum to give compound **18** (36 mg, 75%). ^1H NMR (CDCl_3): δ 4.57 (d, 2H, $J=7$ Hz), 4.66 (d, 1H, $J=16$ Hz), 4.84 (d, 1H, $J=16$ Hz), 5.06 (m, 1H), 7.38 (t, 1H, $J=8$ Hz), 7.42 (t, 1H, $J=8$ Hz), 7.61 (m, 2H), 7.68 (d, 1H, $J=7$ Hz), 7.85 (d, 1H, $J=8$ Hz), 9.16 (d, 1H, $J=8$ Hz), 9.24 (d, 1H, $J=8$ Hz), 11.12 (s, 1H); MS: 933 ($2\text{M}+\text{Na}$), 456 ($\text{M}+\text{H}^+$), 454 ($\text{M}-\text{H}^+$); HRMS Calcd. For $\text{C}_{25}\text{H}_{17}\text{N}_3\text{O}_6$: 455.1117; Found: 455.1138.

Preparation of Acryflavin A (**22**). Palladium dichloride (7.4 g, 41.6 mmoles) was added to a solution of acryrubin A (**21**, 2.9 g, 8.86 mmol; prepared as described in Faul M.M., Winneroski L.L. and Krumrich C.A., *Jornal of Organic Chemistry*, **1998**, 63, 6053-6058 from **19** and **20**) in DMF (100 mL) at 90°C . The reaction temperature was kept at 90°C for 1 hr. The mixture was cooled and conc. HCl (50 mL), then water (50 mL) were added. The mixture was poured over ice and the resulting precipitate was filtered off. The solids were washed with H_2O and MeOH, then dissolved in THF (200 mL) and acetone (200 mL) and the remaining solids were filtered off. The solution was filtered through a plug of silica gel and the solvent was removed under vacuum. The resulting residue was diluted with MeOH, the solids were filtered and washed with MeOH then dried to provide Acryflavin A (**22**, 2 g, 70%) as a brown solid.

Preparation of K-252c (**23**). Hydrochloric acid (conc.) (45 mL) was added dropwise over a 2 hr period to a solution of compound **22** (2 g, 6.15 mmoles) and tin (18 g) in acetic acid (100 mL) at 100°C . The mixture was heated for an additional 2 hrs at 100°C , and THF (75 mL) was added. The mixture was cooled to room temperature, then filtered over celite and the filter cake was washed with THF (200 mL) and acetone (200 mL). The washings were combined and poured over ice. The resulting precipitate was filtered and washed sequentially with H_2O and Et_2O (4 times each), then dried to provide **23** (1.27 g) as a light brown solid. The filter cake was rewashed with additional portions of MeOH (200 mL), THF (100 mL) and acetone (100 mL). The solvent was removed from the combined additional washings and the resulting residue was diluted with Et_2O and filtered to provide additional compound **23** (0.37 g) (combined yield 1.64 g, 86%). ^1H NMR (300 MHz, d^6 -DMSO): δ 4.97 (s, 2H), 7.24 (t, 1H, $J=7.5$ Hz), 7.32 (t, 1H, $J=7.8$ Hz), 7.44 (quin, 2H, $J=8.4$ Hz), 7.73 (d, 1H, $J=8.1$ Hz), 7.8 (d, 1H, $J=8.1$ Hz), 8.05 (d, 1H, $J=7.8$ Hz), 8.47 (s, 1H), 9.23 (d, 1H, $J=7.8$ Hz), 11.35 (s, 1H), 11.52 (s, 1H); MS m/z 645 ($2\text{M}+\text{Na}$), 334 ($\text{M}+\text{Na}$), 312 ($\text{M}+\text{H}$).

12,13-diallyl-6,7,12,13-tetrahydro-5-oxo-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**24**). Cs_2CO_3 (2.5 g) was added to a solution of compound **23** (650 mg, 2.09 mmoles) in DMF (30 mL). The mixture was stirred for 1 hr at room temperature, then 3-bromo-propene (1 mL) was added. The mixture was stirred for 6 hrs, then additional 3-bromo-propene (1 mL) was added. The mixture was stirred for overnight, then the reaction mixture was extracted with EtOAc and sequentially washed with NH_4Cl (aq.) and NaCl (aq.). The organic layers were separated and dried over Na_2SO_4 (s), then filtered and the solvent was removed. The resulting brown residue was diluted with Et_2O and the solids were filtered off, then washed with Et_2O and dried to provide 12,13-diallyl-6,7,12,13-tetrahydro-5-oxo-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**24**, 584 mg, 72%) as a yellowish-brown solid. ^1H NMR (300

MHz, CDCl₃): δ 4.95 (m, 4H), 5.48 (m, 4H), 6.2 (m, 2H), 7.43 (m, 2H), 7.55 (m, 4H), 7.92 (d, 1H, $J=7.8$ Hz), 9.57 (d, 1H, $J=7.8$ Hz); MS m/z 805 (2M+Na), 783 (2M+H), 392 (M+H).

12,13-diallyl-6,7,12,13-tetrahydro-5-oxo-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-6-carboxylic acid tert-butyl ester (**28**). Compound **24** (615 mg, 1.57 mmol), di-*t*-butoxy-carbonyl anhydride (450 mg) and DMAP (250 mg) were dissolved in THF (20 mL). MTBD (0.23 mL) was added and the mixture was stirred at room temperature for 2 hrs. Additional di-*t*-butoxy-carbonyl anhydride (400 mg) was added and the mixture was stirred for 2 hrs. The reaction was quenched with NH₄Cl(aq.), then extracted with EtOAc and washed with a solution of NaCl (aq.). The aqueous layers were re-extracted, the organic layers were combined and dried over Na₂SO₄(s), then filtered and the solvents removed. The resulting residue was triturated with ethyl ether and filtered, then washed with ether and dried to provide 12,13-diallyl-6,7,12,13-tetrahydro-5-oxo-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-6-carboxylic acid tert-butyl ester (**28**, 644 mg, 83%) as a brown-orange solid. ¹H NMR (300 MHz, CDCl₃): δ 1.74 (s, 9H), 4.95 (m, 2H), 5.05 (m, 2H), 5.28 (s, 2H), 5.48 (m, 4H), 6.19 (m, 2H), 7.42 (m, 2H), 7.51 (m, 2H), 7.56 (m, 2H), 7.99 (d, 1H, $J=8.8$ Hz), 9.55 (d, 1H, $J=7.9$ Hz); MS m/z 1005 (2M+Na), 514 (M+Na).

12,13-Di-(4-butenyl)-6,7,12,13-tetrahydro-5-oxo-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**25**). A total of 368 mg of K-252c (**23**) was split into five microwave tubes equally. To each tube was added 600-650 mg of Cs₂CO₃ and 4 mL of acetonitrile. The tubes were sealed with a crimped septa, and incubated at 120°C for 4 minutes in a Personal ChemistryTM microwave. Next, 0.3 mL of 4-butenyl bromide was added to each tube and then irradiation was continued at 160°C for 30 minutes. The contents of each tube were combined, and the mixture extracted with ethyl acetate followed by washing with NH₄Cl (aq.) and NaCl(aq.) solutions. The organic layers were separated and dried over Na₂SO₄. Filtration, solvent removal, and column chromatography gave 198 mg of an orange solid (40% yield), after fraction combination, solvent removal, and drying. Physical data for **25**: ¹H NMR (300 MHz, CDCl₃): δ 2.23 (quart, 2H, $J=7.5$ Hz), 2.33 (quart, 2H, $J=6.6$ Hz), 4.69 (m, 4H), 4.87 (m, 4H), 5.04 (s, 2H), 5.49 (m, 2H), 6.68 (s, 1H), 7.43 (m, 2H), 7.57 (m, 3H), 7.66 (t, 1H, $J=8.4$ Hz), 7.95 (d, 1H, $J=7.8$ Hz), 9.54 (d, 1H, $J=7.8$ Hz); MS: 861 (2M+Na), 839 (2M+H), 420 (M+H).

6-*t*-Butoxycarbonyl-12,13-di-(4-butenyl)-6,7,12,13-tetrahydro-5-oxo-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**29**). To a stirred solution of 246 mg of bis-butenyl-indole **25** in 10 mL of THF was added 150 mg of 4-dimethylamino pyridine, 240 mg of di-*t*-butyl-dicarbonate, and 0.1 mL of 7-methyl-1,5,7-triazabicyclo-(4.4.0)-dec-5-ene. The reaction was stirred at room temperature for 1 hour. The material was then extracted with ethyl acetate, and washed with NH₄Cl(aq) and NaCl(aq) solutions. The organic layers were separated and dried over Na₂SO₄(s). Filtration, solvent removal, dilution with hexanes and filtration of the resulting solids gave 278 mg of a light orange solid (91% yield). ¹H NMR (300 MHz, CDCl₃): δ 1.73 (s, 9H), 2.22 (quart, 2H, $J=7.4$ Hz), 2.34 (quart, 2H, $J=6.6$ Hz), 4.67 (t, 2H, $J=11$ Hz), 4.73 (t, 2H, $J=7.8$ Hz), 4.82 (m, 2H), 5.34 (s, 2H), 5.47 (m, 2H), 7.43 (m, 2H), 7.59 (m, 3H), 7.67 (t, 1H, $J=8$ Hz), 8.01 (d, 1H, $J=8$ Hz), 9.52 (d, 1H, $J=7$ Hz); MS: 1061 (2M+Na), 542 (M+Na).

12,13-(but-2-en-1,4-yl)-6,7,12,13-tetrahydro-5-oxo-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-6-carboxylic acid tert-butyl ester (**30**). Benzylidene-bis (tricyclohexylphosphine)dichlororuthenium (125 mg) was added to a solution of compound **28** (350 mg, 0.71 mmol) in dichloromethane (70 mL). The reaction was stirred at room temperature for 20 hrs, then filtered over silica gel with excess DCM. The DCM layers were discarded and the silica gel was washed with EtOAc. The EtOAc layers were removed under vacuum, the resulting residue was diluted with ethyl ether and the solids were filtered and dried to provide 12,13-(but-2-en-1,4-yl)-6,7,12,13-tetrahydro-5-oxo-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-6-carboxylic acid tert-butyl ester (**30**, 282 mg, 85%) as a gray solid. ¹H NMR (300 MHz, CDCl₃): δ 1.75 (s, 9H), 4.19 (d, 2H, $J=7.2$ Hz), 4.23 (s, 2H), 4.95 (d, 2H, $J=5.1$ Hz), 6.08 (m, 2H), 7.31 (m, 1H), 7.39 (m, 2H), 7.57 (m, 3H), 7.66 (d, 1H, $J=7.2$ Hz), 9.72 (d, 1H, $J=8.1$ Hz); MS m/z 949 (2M+Na).

6-t-Butoxycarbonyl-12,13-(1,6-hex-cis-3-enyl)-6,7,12,13-tetrahydro-5-oxo-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**31**). Two vessels were loaded each with 105 mg of N-BOC-bis-4-butenyl compound **29**, 30 mL of dichloromethane, and 27 mg of benzylidene bis(tricyclohexylphosphine) dichlororuthenium. The vessels were heated to 45°C for 4 hours. The vessels were cooled, combined, and filtered over silica gel with excess dichloromethane. The washings were discarded, and the silica gel eluted with ethyl acetate. The ethyl acetate washings were combined, and the solvent was removed. The residue was diluted with hexanes, and the solids were filtered and washed with hexanes to give 138 mg of a brown solid. An additional 35 mg of solid was recovered from the hexane washings by filtration. Total amount was 173 mg (88% yield). ¹H NMR (300 MHz, CDCl₃): δ 1.73 (s, 9H), 2.79 (m, 2H), 2.92 (m, 2H), 4.48 (m, 2H), 4.74 (s, 2H), 4.85 (m, 2H), 5.43 (m, 2H), 7.38 (t, 1H, J=8 Hz), 7.45 (m, 1H), 7.53 (d, 1H, J=8 Hz), 7.6 (m, 4H), 7.82 (d, 1H, J=8 Hz), 9.64 (d, 1H, J=8 Hz); ¹³C NMR (75.5 MHz, HETCOR, CDCl₃): δ 31.12, 46.76, 50.69, 84.82, 111.27, 112.16, 117.57, 119.18, 120.69, 122.62, 123.07, 123.26, 124.93, 128.38, 128.55, 128.85, 130.19, 130.44, 130.80, 132.7, 133.39, 143.33, 152.87, 170.01; MS: 1005 (2M+Na), 514 (M+Na).

12,13-(1,4-but-cis-2-enyl)-6,7,12,13-tetrahydro-5-oxo-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**26**). To a solution of 200 mg of **24** in 55 mL of dichloromethane was added 60 mg of benzylidene bis(tricyclohexylphosphine) dichlororuthenium. The mixture was heated at 40°C for 4 hours. The reaction was then cooled, and the majority of the solvent was removed by evaporation. The resulting solids were then filtered and washed three times each with dichloromethane, diethyl ether, ethyl acetate, methanol, and diethyl ether. Drying gave 102 mg of a light gray solid (55%) yield. ¹H NMR (300 MHz, d⁶-DMSO): δ 4.93 (s, 2H), 5.39 (d, 2H, J=5.4 Hz), 5.44 (d, 2H, J=5.4 Hz), 6.51 (bs, 2H), 7.27 (t, 1H, J=7.2 Hz), 7.37 (t, 1H, J=7.5 Hz), 7.55 (quint, 2H, J=7.8 Hz), 7.89 (d, 1H, J=8.7 Hz), 7.99 (d, 1H, J=8.4 Hz), 8.03 (d, 1H, J=8.1 Hz), 8.57 (s, 1H), 9.59 (d, 1H, J=7.8 Hz); MS: 749 (2M+Na), 727 (2M+H), 364 (M+H).

12,13-(1,6-hex-cis-3-enyl)-6,7,12,13-tetrahydro-5-oxo-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**27**). To a solution of 42 mg (0.1 mmole) of bis-4-butenyl compound **25** in 10 mL of dichloromethane was added 44 mg of benzylidene bis(tricyclohexylphosphine) dichlororuthenium. The reaction was stirred at room temperature for 5 days. The solvent was removed, and the mixture purified by thin layer chromatography. The bands containing the compound were combined, and the compound eluted off the silica gel with ethyl acetate. The solvent was removed, and the residue diluted with ethyl ether, and the solids filtered and washed with ether to give 12 mg of a brownish-gray solid (31% yield). ¹H NMR (300 MHz, d⁶-DMSO): δ 2.52 (bs, 4H), 4.98 (s, 2H), 5.14 (m, 4H), 5.36 (m, 2H), 7.29 (t, 1H, J=7.5 Hz), 7.39 (t, 1H, J=7.5 Hz), 7.56 (quint, 2H, J=7.2 Hz), 7.8 (d, 1H, J=8.7 Hz), 7.89 (d, 1H, J=8.1 Hz), 8.05 (d, 1H, J=7.8 Hz), 8.58 (s, 1H), 9.6 (d, 1H, J=7.8 Hz); MS: 783 (2M+H), 392 (M+H).

12,13-(2,3-Cis-dihydroxy-1,4-butyl)-6,7,12,13-tetrahydro-5-oxo-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**32**). Compound **30** (162 mg, 0.35 mmoles) and trimethylamine-N-oxide (155 mg) were dissolved in chloroform (15 mL) and THF (15 mL), then water (10 drops) and osmium trichloride (9 mg) were added. The mixture was stirred for 4 hrs at room temperature., then the solvent was removed under vacuum and water was added. The resulting solids were filtered, sequentially washed with water and ethyl ether (4 times each) and dried to provide 12,13-(2,3-dihydroxy-butan-1,4-yl)-6,7,12,13-tetrahydro-5-oxo-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole-6-carboxylic acid tert-butyl ester (158 mg, 91%) as a gray solid. ¹H NMR (300 MHz, d⁶-DMSO): δ 1.64 (s, 9H), 4.28 (d, 2H, J=3.3 Hz), 4.62 (m, 3H), 4.73 (m, 1H), 5.27 (quart, 2H, J=17.1 Hz), 5.43 (s, 2H), 7.32 (t, 1H, J=7.5 Hz), 7.43 (t, 1H, J=7.5 Hz), 7.59 (quint, 2H, J=7.8 Hz), 7.73 (d, 1H, J=8.4 Hz), 7.84 (d, 1H, J=8.4 Hz), 7.99 (d, 1H, J=7.5 Hz), 9.29 (d, 1H, J=7.5 Hz); MS m/z 1017 (2M+Na), 520 (M+Na). The diol compound (158 mg) was dissolved in anhydrous TFA (10 mL) and the mixture was stirred for 2 hrs at room temperature. The solvent was removed under vacuum and the resulting residue was diluted with ethyl ether, the solids were filtered and sequentially washed with DCM, MeOH and EtOAc (3 times each). The solvent was removed from the combined washings and then diluted with THF and filtered to

provide compound **32** (54 mg). The wash procedure was repeated to provide additional **32** (31 mg) (combined yield 85 mg, 67%). ¹H NMR (300 MHz, d⁶-DMSO): δ 4.33 (bs, 2H), 4.62 (m, 1H), 4.64 (s, 2H), 4.79 (m, 1H), 4.99 (s, 2H), 5.47 (s, 2H), 7.31 (t, 1H, J=5 Hz), 7.40 (t, 1H, J=5 Hz), 7.55 (t, 1H, J=5 Hz), 7.6 (t, 1H, J=5 Hz), 7.75 (d, 1H, J=6 Hz), 7.84 (d, 1H, J=6 Hz), 8.08 (d, 1H, J=5 Hz), 8.62 (s, 1H), 9.45 (d, 1H, J=6 Hz); MS m/z 817 (2M+Na), 795 (2M+H), 398 (M+H); Anal.Calc. For C₂₄H₁₉N₃O₃+0.33 C₂F₃HO₂: C, 68.04; H, 4.48, N, 9.65; Found: C, 68.11, H, 4.61, N, 9.70.

12,13-(3,4-Cis-dihydroxy-1,6-hexyl)-6,7,12,13-tetrahydro-5-oxo-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**33**). To a solution of 122 mg of N-BOC compound **31** in 15 mL of CHCl₃ was added 160 mg of trimethylamine-N-oxide, 0.2 mL of H₂O, and 4 mg of osmium trichloride. The mixture was stirred at room temperature for 3 hours, and the solvent was removed under vacuum. The material was diluted with H₂O, and the solids were filtered, washed four times each with H₂O and ethyl ether, then followed by drying. The solids were then taken back up in 10 mL of dichloromethane, and 1 mL of trifluoroacetic acid was added. The mixture was stirred for 1 hour, and then the solvent was removed under vacuum. The residue was diluted with ethyl ether, and the precipitates filtered, followed by washing three times with ethyl ether and drying. The solids were then taken up in DMSO, and purified by reverse phase HPLC. Combination of the fractions, followed by lyophilization gave 18 mg of an off-white solid (17% yield). ¹H NMR (300 MHz, d⁶-DMSO): δ 3-4.4 (m, 12H), 4.91 (s, 2H), 7.34 (t, 1H, J=8 Hz), 7.42 (t, 1H, J=8 Hz), 7.58 (quint, 2H, J=7 Hz), 7.71 (d, 1H, J=8 Hz), 7.78 (d, 1H, J=8 Hz), 8.06 (d, 1H, J=8 Hz), 8.59 (s, 1H), 9.39 (d, 1H, J=8 Hz); MS: 851 (2M+H), 426 (M+H).

12,13-(5-hydroxyl-1,5-pentanyl)-6,7,12,13-tetrahydro-5-oxo-5H-indolo[2,3-a]pyrrolo[3,4-a]carbazole (**35**). K-252c (**23**, 50 mg, 0.16 mmol), and Cs₂CO₃ (312 mg, 0.96 mmol) were added into a microwave reaction vessel containing acetonitrile (4 mL) and stirring bar, followed by the addition of ditosylate **34** (104 mg, 0.2 mmol). The vessel was stirred for 30 seconds and then irradiated at 150°C for 30 min. The reaction mixture was diluted with ethyl acetate, washed with sat. NaHCO₃, brine, and dried over Na₂SO₄. Removal of solvent gave the silyl ether compound (659 mg, 93%) as a yellow solid; MS 510 (M+H⁺). This compound (235 mg, 0.45 mmol) was stirred with a 1 M solution of t-butylammonium fluoride/tetrahydrofuran (4.5 mL) for 1 hour at room temperature. The reaction mixture was concentrated. The residue was diluted with water (15 mL), resulting in the formation of precipitates. The mixture was then filtered, washed with water, and MeOH (2 mLx3). The filter cake was dried over vacuum oven at 55°C overnight to give compound **35** as light brown solid (145 mg, 82%). ¹H NMR (d⁶-DMSO, 300 MHz) δ 2.07-2.26 (m, 4H), 4.96 (s, 2H), 5.05-5.18 (m, 5H), 7.27 (t, 1H, J=8 Hz), 7.37 (t, 1H, J=8 Hz), 7.54 (quintet, 2H, J=8 Hz), 7.76 (d, 1H, J=8 Hz), 7.86 (d, 1H, J=8 Hz), 8.04 (d, 1H, J=8 Hz), 9.61 (d, 1H, J=8 Hz); MS: 791 (2M+ H⁺), 396(M+ H⁺).

12,13-(2,3-O-isopropylidene-2,3-trans-dihydroxy-1,4-butyl)-6,7,12,13-tetrahydro-5-oxo-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**37**). To a microwave tube was added 65 mg of K-252c, 315 mg of Cs₂CO₃, 122 mg of 1,4-Di-O-toluenesulfonyl-2,3-O-isopropylidene-L-threitol (**36**), and 4 mL of acetonitrile. The tube was placed inside a microwave instrument and irradiated for 3400 seconds at 150°C. The process was repeated seven more times, and a total of 521 mg of K-252c was used. The contents of all eight vessels were combined, extracted with ethyl acetate, and washed with NH₄Cl(aq.) and NaCl(aq.) solutions. The organic layers were separated and the solvent removed under vacuum. Column chromatography (ethyl acetate/hexanes gradient) gave a light brown solid, which was diluted with ethyl acetate, followed by filtration of the solids, and washing three times each with ethyl acetate, ethyl ether, and hexanes. After drying, 150 mg of an off-white solid was obtained. An additional 184 mg of an-orange waxy solid was obtained from the supernatant (total = 334 mg, 45% yield). ¹H NMR (300 MHz, CDCl₃): δ 1.44 (s, 3H), 1.45 (s, 3H), 4.55 (s, 2H), 4.59 (m, 1H), 4.68 (m, 3H), 4.98 (s, 2H), 7.32 (t, 1H, J=8 Hz), 7.4 (t, 1H, J=8 Hz), 7.56 (quint, 2H, J=7 Hz), 7.73 (d, 1H, J=8 Hz), 7.81 (d, 1H, J=8 Hz), 8.06 (d, 1H, J=8 Hz), 8.66 (s, 1H), 9.45 (d, 1H, J=8 Hz); MS: 897 (2M+Na), 875 (2M+H), 438 (M+H).

Preparation of 12,13-(2,3-trans-(S,S)-dihydroxy-1,4-butyl)-6,7,12,13-tetrahydro-5-oxo-5H-indolo[2,3-a]pyrrolo[3,4-c]carbazole (**38**). To a solution of 66 mg of compound **37** in 2 mL of tetrahydrofuran and 1 mL of methanol was added 9 drops of a 6% *p*-toluenesulfonic acid (aq.) solution. The reaction was heated to 75°C for 2.5 hours. The reaction was cooled, and the resulting precipitate was filtered and washed three times each with methanol, ethyl acetate, water, methanol, and dichloromethane. Drying gave 52 mg of a beige solid (87% yield). ¹H NMR (300 MHz, d⁶-DMSO): δ 3.99 (m, 2H), 4.72 (m, 4H), 4.97 (s, 2H), 5.47 (s, 2H), 7.28 (t, 1H, J=8 Hz), 7.37 (t, 1H, J=7 Hz), 7.54 (quint, 2H, J=8 Hz), 7.74 (d, 1H, J=8 Hz), 7.82 (d, 1H, J=8 Hz), 8.05 (d, 1H, J=8 Hz), 8.57 (s, 1H), 9.44 (d, 1H, J=8 Hz); MS: 795 (2M+H), 398 (M+H).

Biology

JAK3 Enzyme Assay. JAK3 enzyme was purified from Sf21 cells (San Diego, CA) infected with a baculovirus expression vector for JAK3 (JH1 and JH2 domain). Enzyme activity of JAK3 was determined in terms of enzyme phosphorylation by the following method. JAK3 enzyme solution (48 µL) in 1.25 X TK buffer (62.5 mM HEPES pH 7.5, 12.5 mM MgCl₂) containing 42 mM DTT (Sigma, St. Louis, MO) was added into the each well of a polypropylene 96 well plate. Next, 5 µL of diluted compounds in DMSO and 48 µL of biotinylated peptide enzyme substrate (5 µg diluted in TK buffer containing 10 µM ATP) was added to each well. Control wells received equal volume of vehicle. The contents of the wells were mixed and the reaction mixture was incubated for 1 h at room temperature. Post incubation, 90 µL of reaction mixture was transferred into a washed Neutravidin coated plate (Pierce Neutravidin Biotin-binding Protein 31000; 1mg/mL 1:100). The plate was then incubated for 15 minutes at room temperature and washed 3 times with PBS-T. PY99 anti-phosphotyrosine antibody (Santa Cruz #sc-7020HRP) was added into each well and the plate was incubated for 40 minutes at room temperature. After incubations, the plate was washed 3 times and 100 µL TMB (Sigma, St. Louis, MO) was added to the each well. The plate was incubated for another 40 min at room temperature in the dark. The reaction was stopped by the addition of 1 M H₂SO₄ (50 µL/well) and the optical density was read at 450/650 nm.

PHA Blast Assay. Blood was obtained from healthy male donors, 22–42 years old. PBMC were isolated from heparinized blood using Ficoll-Paque gradient (Pharmacia, Uppsala, Sweden) as described previously. Cells were washed twice with Dulbecco's phosphate-buffered saline (DPBS), resuspended in culture medium or DPBS and used immediately. The T cells were incubated with increasing concentrations of the compound and T cell proliferation was assessed by a ³H-thymidine assay.

Mouse Model of IgE Stimulation. Compound **32** was administered i.p. at 10 and 30 mg/kg dose on day 1, 3 and 5 after 1 h after the OVA challenge. Additional drug treatment were made to the mice on day 2 and 4. Control mice were injected with vehicle alone (2% DMSO). Mice were bled on day 8 for quantization of plasma IgE by ELISA and compared to the control group.

Mouse Model of Allergic Asthma. To examine the effect of the JAK3 inhibitor on allergic asthma in mice, BALB/c mice were injected i.p. with 20 µg of ovalbumin (OVA) in alum on days 0 and 14. On days 28, 29, and 30, mice were challenged for 5 min with 2% OVA via their airways by ultrasonic nebulization. Mice were assessed for "airway responsiveness" on day 32. Following airway reactivity measurements, their lungs were lavaged with cold saline.