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Design and synthesis of novel 5,6-bisindolylpyrimidin-4-ones structurally related to ruboxistaurin (LY333531)

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ABSTRACT

2,3-Bis(1-methyl-1*H*-indol-3-yl) methyl-3-oxopropionate is a key intermediate in the synthesis of a new family of LY333531 analogues. Base-mediated cyclocondensation with thiourea afforded novel 5,6-bis(1-methyl-1*H*-indol-3-yl)-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one, which was efficiently converted to the pyrimidin-2,4(1*H*,3*H*)-dione congener. Synthesis of a six-membered K-252c analogue, 5,6-bis(1-methyl-1*H*-indol-3-yl)pyrimidin-4(3*H*)-one, is also described.

1. Introduction

Protein kinases modulate cell signal transduction by phosphorylating tyrosine, threonine and serine residues inproteins implicated in a multitude of disease states, including cancer, diabetes and inflammation.[1] Misregulation of protein kinase C (PKC) activity and expression has been reported in several malignancies, and consequently, PKC has been established as a valid clinical target for the chemotherapeutic treatment of cancer.[2] Kinase inhibition has become a central target, along with DNA, at the forefront of anti-cancer drug development, due to an urgent need for research into more potent and selective, next generation anti-tumour therapies to overcome resistant cancers.[3]

Initial interest in indolocarbazole alkaloids as clinical anti-tumour agents began as a result of the extraction in 1977, of an antifungal natural product glycoside, staurosporine **1**, from cultures of *Streptomyces staurosporeus* (Fig. 1).[4] The broad spectrum of biological activity displayed by **1** was rationalized in 1986 to be due to its behaviour as a non-specific ATP-competitive kinase inhibitor, exhibiting strong cytotoxic activity against cancer cell lines due to its nanomolar PKC activity, as well as CDK inhibition, in vitro.[5,6] The closely related bisindolylmaleimide (3,4-di-1*H*-indol-3-yl-1*H*-pyrrole-2,5-dione) scaffold based on Arcyriarubin A **2**, isolated as the pigment from myxomycetes, such as *Arcyria denudata* and *Arcyria nutans*, represents a potential template for selective kinase inhibition reported to suppress the growth of cultured cancer cell lines while displaying a narrow therapeutic window.[7]

Recent progress in the development of PKC isoform-selective inhibitors has been highlighted by the new drug application submitted to the FDA by Eli Lilly, in February 2006, following successful

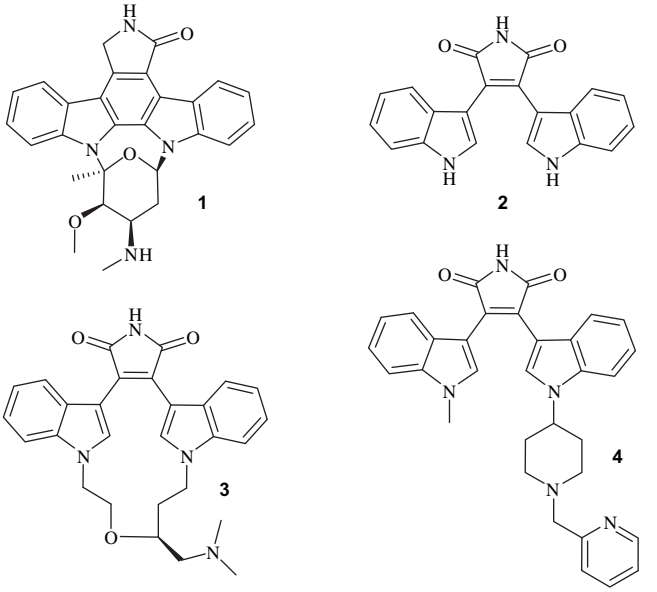

Fig. 1. Examples of clinically relevant indolocarbazoles and bisindolylmaleimides.

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clinical phase III trials, for the macrocyclic bisindolylmaleimide, ruboxistaurin mesylate (Arxxant®) **3**.[8] Interestingly, compound **3** was reported to confer quite discrete enzyme selectivity; IC_{50} values for PKC-β1 and β2 (4.7 and 5.9 nM, respectively), are more than 40-fold greater than for other PKC isoforms, and vastly more potent than against other ATP-dependent kinases, e.g., Src tyrosine kinase.[9,10] This clinical candidate possesses attractive efficacy in reducing vision loss in patients with diabetic peripheral retinopathy, and is currently defined as the first chemotherapeutic treatment for this serious complication of diabetes.

The orally-administered enzastaurin **4** has also been evaluated favourably in clinical trials for the treatment of glioblastoma and colo-rectal cancer, and is described to operate via induction of apoptosis, in addition to inhibition of VEGF-mediated angiogenesis.[11] Pre-clinical data published to date also supports the potential of **4** for the treatment of large B-cell lymphoma and Akt-mediated diseases, due to its highly attractive PKCβ and AKT/PI3 activity.[12] It has also been proposed that the bisindolylmaleimide framework constitutes an ABCG2 inhibitory pharmacophore, which may increase the oral bioavailability of co-administered ABCG2 substrate drugs, in order to potentiate an effective therapeutic response and significantly improve subsequent clinical outcome.[13]

SAR analysis suggests that application of precise structural features to probe interactions between the maleimide head group and essential residues within the conserved enzyme ATP-binding pocket is still challenging, requiring further elucidation of exact mechanisms of inhibition.[14] Accomplishing distinct kinase selectivity with sufficient potency (in the presence of intracellular ATP concentrations) represents the ultimate goal of vast research within this area; however, little work to date has assessed the protein backbone-interacting pyrrole-2,5-dione molecular domain as a viable target for inhibitory optimisation in order to achieve this overall aim. Natural indole alkaloids fused to a pyrimidine nucleus have recently been revealed to constitute an important pharmacological class, based on their ability to modulate important biological functions.

Meridianin D **5** isolated from the marine tunicate *Aplidium meridianum*, displayed cytotoxicity towards LMM3 (murine mammarian adenocarcinoma cell line), with an IC_{50} value of 33.9 μM, as well as being an inhibitor of several protein kinases, while a bisindolyl pyrimidine analog **6**, reported by Jiang et al., exhibited excellent inhibition against leukaemia CCRF-CEM cell line (GI_{50}=1.13 μM).[15,16] Extrapolating from this evidence, the design of inhibitors such as indolyluracil **7** was postulated by Časar et al. to be a putative scaffold capable of conferring significant cytotoxicity (Fig. 2).[17]

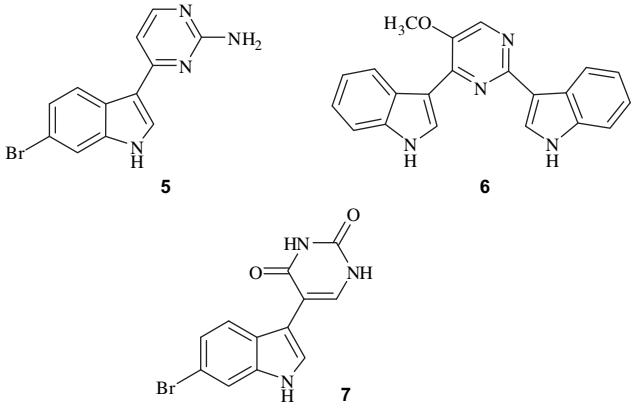


Fig. 2. Indole alkaloids with kinase inhibitory activity.

At the outset of this work, a similar awareness of the intrinsic ability of related pyrimidin-4-one H-bonding networks to mimic and enhance specific spatial contacts between other potent kinase inhibitors, such as **3**, and essential kinase active site residues was developed. This structural optimisation could be envisaged to be due to its critical incorporation of a modified imide structural unit. In particular, the pyrimidin-2,4-dione (uracil) sub-unit at the 3-position of both indole moieties (X=OH; **8**), would be of interest since the uracil nucleus is an important constituent of nucleosides, which play a crucial role in biological systems (Fig. 3). Synthesis of a novel kinase inhibitory template possessing unique utility by displaying lactam H-bonding functionality, similar to staurosporine **1**, rather than the imide motif conserved in 3,4-disubstituted maleimide alkaloids, would also provide an interesting investigational compound, exploiting its close structural similarity to K-252c **9**—a cytotoxic anti-PKC indolocarbazole derived from the actinomycete strain, *Nocardiopsis* sp. K-290.[18]

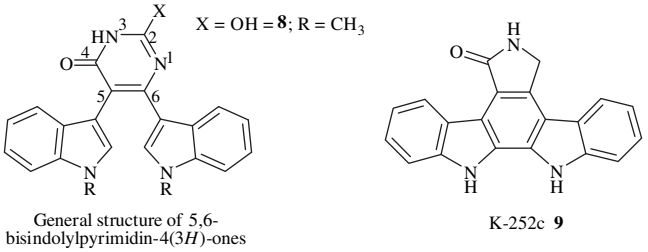


Fig. 3. Development of a novel kinase inhibitory template.

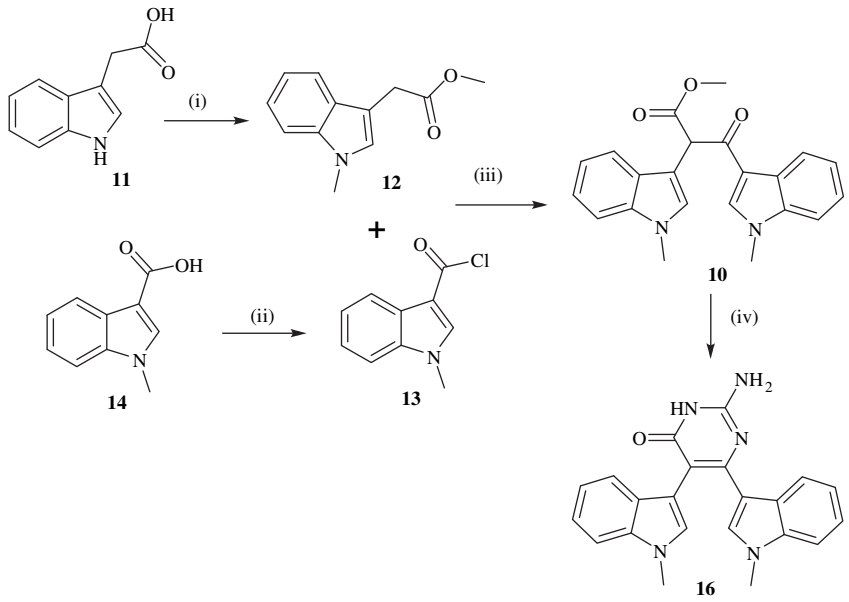
The primary method for converting simple alkyl β-ketoesters to the corresponding pyrimidin-4-one moiety involves exposure of the substrate to either urea or thiourea, in the presence of sodium alkoxide in alcohol, and heating of this mixture for an extended period.[19] In 2002, it was first reported that simple β-ketoester precursors could be converted directly to corresponding 5,6-alkyl substituted uracils by conventional microwave irradiation in the presence of urea, under solvent-free conditions.[20]

This report includes work carried out on the first reported conversion of a 2,3-bis(heteroaryl) methyl-3-oxopropionate to heterocyclic 5,6-disubstituted pyrimidin-4-ones. This heterocycle-forming process was optimised for an array of nucleophiles, affording derivatives of acute biological importance. Some strategic difficulties associated with conventional heterogeneous transformations performed in aqueous media also necessitated careful selection of novel reagent types, solvent nature and reaction times. Targeted synthesis of these title bisindolylpyrimidinones, displaying improved solubility profiles, can now be reported under mild and versatile conditions for oxidative and reductive desulfurisation.

2. Results and discussion

Recent advances in our laboratory have enabled a complete protocol for the synthesis of a series of novel 5,6-bisindolyl pyrimidin-4-one congeners, to be established, starting from cheap, readily available precursors. Initial investigations revealed that modification of pre-existing, classical chemical routes to this novel heterocyclic class was impractical. Extensive investigation was then carried out into the specific preparation of these derivatives by a convergent synthesis—it can now be reported that 2,3-bis(1-methyl-1*H*-indol-3-yl) methyl-3-oxopropionate **10** afforded access to the pyrimidinone nucleus via a cyclocondensation route (Scheme 1).

Starting from the commercially available indole-3-acetic acid **11**, the one-pot preparation of the *N*-methyl protected methyl indole-3-acetate ester **12**, was achieved in a yield of 82%, via reaction with dimethyl carbonate and potassium carbonate as base, at 130 °C, for 20 h.[21] *N*-Methyl indol-3-yl carbonyl chloride **13**, was obtained in quantitative yield from the corresponding acid **14**, in the presence of oxalyl chloride, suspended in dry DCM, and stirred at room temperature, for 75 min.[22] This method was highly advantageous



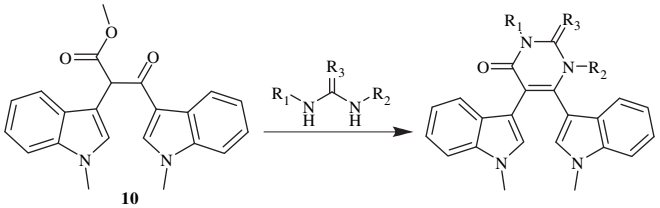
Scheme 1. Reaction conditions: (i) 1 equiv K_2CO_3, 3 equiv $(CH_3)_2CO_3$, DMF, 130 °C, 12 h, **12**=82%; (ii) 1.1 equiv $(COCl)_2$, DCM, rt, 75 min, **13**=100%, (iii) (a) 2.2 equiv LDA, 1.3 equiv *N*-methyl indole-3-carbonyl chloride (**13**), THF, −78 °C—rt, 16 h; (b) NH_4Cl/H_2O, **10**=80%; (iv) (i) (a) 20 equiv NaOMe, 5 equiv $[NH_2(C{=}NH_2)NH_2]_2CO_3$, MeOH, reflux, 24 h, (b) 10% HCl/H_2O, **16**=45%.

compared with the reported synthesis involving stirring of **14** with thionyl chloride, at room temperature for 20 h, which was limited by formation of an appreciable amount of the 2-chlorinated acid chloride side-product.[23] The novel β-dicarbonyl intermediate **10** was prepared by modified Claisen reaction of **12**, in the presence of LDA in THF, with acid chloride **13**, at −78 °C.

Synthesis of the corresponding β-enaminoester, a common precursor to pyrimidin-4-ones, was attempted via numerous approaches including imine formation from the β-ketoester **10**, modified Blaise reaction (using 1-methyl-3-cyanoindole and zinc chloride), etc., with none yielding a viable route to this intermediate.

However, it has been widely established that reaction of a dicarbonyl intermediate with an appropriate nitrogen nucleophile represents a viable route to heterocyclic systems bearing a pyrimidine moiety. The application of this reaction with related urea analogues under base-mediated conditions was studied and the results of this reactant study are described in Table 1.

Table 1
Reaction of **10** with panel of analogous urea nucleophiles[a]



R_1	R_2	R_3	Conditions[b]	Time	Result
H	H	O (**8**)	A	72 h	—
H	H	S (**17**)	A	24 h	24%
H	H	NH (**16**)	A	24 h	45%
H	—	H (**22**)	A	48 h	—
H	—	S–CH_3 (**18**)	A, C or D	24 h	—
H	—	O–CH_3 (**15**)	A or B	20 h	—
CH_3	CH_3	S (**19**)	A	24 h	—

[a] In each case, reaction was performed with 2,3-bis(1-methyl-1*H*-indol-3-yl) methyl-3-oxopropionate (**10**) under nitrogen atmosphere.

[b] *Methods*: A 20 equiv NaOMe/MeOH, 5 equiv $R_1NH(CR_3)NHR_2$ reagent, reflux, 24–72 h; **B** $Ca(OH)_2$, 5 equiv $R_1NH(CR_3)NHR_2$ reagent H_2O, H_2SO_4 80 °C, 36 h; **C** 10% KOH/H_2O, 5 equiv $R_1NH(CR_3)NHR_2$ reagent, rt, 24 h; **D** 20 equiv. KOH, 5 equiv $R_1NH(CR_3)NHR_2$ reagent, THF, 65 °C, 24 h.

2.1. Reaction with bi-dentate *N*-nucleophiles—urea derivatives

As an obvious methodology for accessing 5,6-bis(1-methyl-1*H*-indol-3-yl) pyrimidine-2,4(1*H*,3*H*)-dione **8**, reaction of urea with **10**, in methanol, heated to reflux temperature, in the presence of sodium methoxide did not yield any anticipated product upon acid work-up, even after 72 h. Preliminary microwave reactions carried out have also been unpromising to date. Similarly, reaction with 5 equiv of *O*-methyl isourea yielded only starting material **10** following heating for 36 h. Utilising aqueous inorganic base-mediated conditions, as reported by Botta et al.,[24] we also attempted conversion to 2-methoxy-4(3*H*)-pyrimidinone **15** following reaction of **10** with 2.2 equiv of $Ca(OH)_2$ and *O*-methyl isourea, stirred in water at 70 °C for 48 h, prior to acidification and heating for a further 20 h. However, it was observed that no reaction had occurred under these conditions (Table 1).

2.2. Reaction with bi-dentate *N*-nucleophiles—guanidine derivatives

Following an initial lack of success with urea, it was determined that cyclisation with an alternative nucleophile to form 2-amino-5,6-bis(1-methyl-1*H*-indol-3-yl)pyrimidin-4(3*H*)-one **16** constituted a highly interesting target with potential biological activity. Compound **8** may also be accessed via chemical derivatisation of this precursor **16**. As shown in Scheme 1, reaction of β-ketoester **10** with guanidine (pre-formed in situ from the initial reaction of guanidine carbonate with excess sodium methoxide) afforded crude **16**, from which any remaining impurity was eliminated by chromatography, in order to derive pure compound **16** in a yield of 45% (Scheme 1).

It was initially postulated that a diazotisation route could introduce the hydroxyl group in order to convert isocytosine derivative **16** to the corresponding pyrimidine-2,4-dione **8**.[25] However, attempted reaction of the amino group of **16** with sodium nitrite/HCl, *tert*-butyl nitrite/acetic acid or *tert*-butyl nitrite/aqueous 2-propanol, to form a diazonium salt, which could be

hydrolysed to uracil **8**, yielded only unreacted **16**, in each case. Similarly, attempted hydrolytic reaction with aqueous 10% HCl at 85 °C resulted in detection of only starting material **16** upon investigation of the final reaction mixture after 7 days (Table 2).

Table 2
Conditions for attempted conversion of **16** to **8**[a]

Reagent	Equiv.	Conditions	Time	Result
$NaNO_2$	5.0	AcOH, 0 °C—rt	20 h	—
$NaNO_2$	5.0	10% HCl, 0 °C—70 °C	24 h	—
t-BuONO	5.0	AcOH, 0 °C—rt	20 h	—
t-BuONO	5.0	IPA/H_2O, 0 °C—rt	24 h	—
10% HCl	>100	H_2O, 85 °C	7d	—

[a] In each case, reaction was performed in presence of **16**, on 150 mg scale and assessed by qualitative ^{13}C NMR and ESI-MS analysis.

2.3. Reaction with bi-dentate *N*-nucleophiles—thiourea derivatives

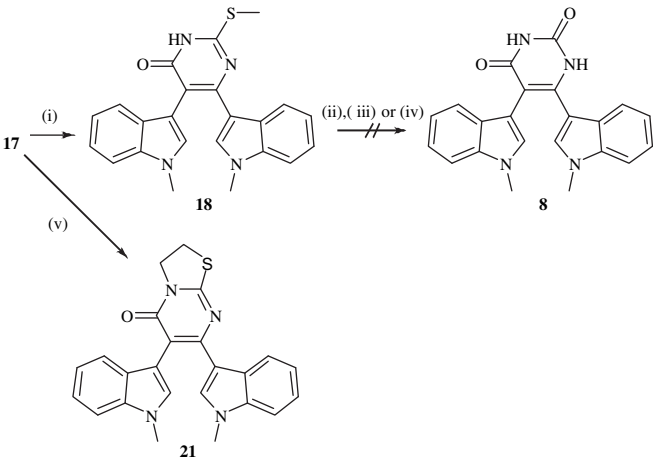
Formation of a thiouracil analogue **17** was also deemed to be an important derivative for comparison with target compound **8**. The use of thiourea was attractive due to its superior reactivity and the nature of the thiocarbonyl bond also permitted access to further secondary derivatives, including a possible route to **8**.

β-Ketoester **10** was converted to the corresponding 5,6-bis-(1-methyl-1*H*-indol-3-yl)-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one **17**, in the presence of sodium methoxide and excess thiourea following reflux in methanol for 24 h (method A; Table 1). The yield for this reaction was found to be 20—24%, independent of whether the reaction time was extended to 48 h or if further excess of base was employed. The crude residue was purified by trituration with boiling absolute ethanol, and filtration of this mixture yielded the novel **17**, as a pale yellow powder. Condensation of **10** was also attempted with a number of thiourea analogues (Table 1).

Reaction with 5 equiv of *N*,*N*′-dimethyl thiourea yielded only starting material following heating for 36 h. *S*-Methyl-isothiouronium hemisulfate was synthesised by reaction of thiourea with 2.5 equiv of dimethyl sulfate, in a yield of 87%. Reaction of 5 equiv of this salt under standard methoxide conditions did not result in formation of any product **18**, even following extended reflux for 72 h. Similarly, when reaction of 5 equiv of the salt was carried out, along with **10**, in 10% aqueous KOH, the absence of either **19** or 2-amino oxazin-4(5*H*)-one was observed (method C; Table 1).[19] Due to concerns over these heterogeneous reaction conditions, a modified reaction was attempted involving mixing of 20 equiv of pulverised KOH along with 5 equiv of this salt along with **10**, in THF, heated for 16 h (method D; Table 1), but without any success.

As an alternative route to target compound **8**, methylation of **17** was undertaken by treatment with iodomethane in methanol at 30 °C, in the presence of potassium carbonate to provide 2-methylthiopyrimidin-4(3*H*)-one **18** in a moderate yield of 35% (Scheme 2). Chromatography employing ethyl acetate and a few drops of methanol enabled elution of a small proportion of unreacted **17**, and following addition of a drop of triethylamine to the eluent, **18** could be rapidly isolated as a straw-coloured powder.

Subsequent hydrolytic conversion of 2-methylthiopyrimidin-4-one **18** to **8** was unsuccessful under conditions for an analogous transformation reported by Gibson et al., involving heating of **18** with Oxone® in aqueous 1,4-dioxane, for 3 days at 70 °C.[26] Similarly, treatment of sulfide **18** with either a solution of aqueous 30% NaOH or 10% HCl, in 1,4-dioxane, under similar conditions to Lu et al., yielded only starting material in each case, following investigation of the reaction mixture after heating for 24 h.[27] However, it was found that cycloalkylated analogues of thiouracil **17** could be synthesised under mild phase-transfer conditions.[28] Reaction with 1,2-dibromoethane in the presence of potassium carbonate, along with TBAB as catalyst, in 1,4-dioxane, for 16 h at room temperature, provided the *S*,*N*-dialkylated derivative, which was crystallised from 95% ethanol, to afford 2*H*-thiazolo[3,2-*a*]pyrimidin-5-one **21** as an yellow powder in a yield of 66% (Scheme 2).



Scheme 2. Reaction conditions: (i) 2 equiv CH_3I, 1.1 equiv K_2CO_3, MeOH, 30 °C, 20 h, **18**=35%; (ii) 2 equiv Oxone®, 1,4-dioxane/H_2O, 70 °C, 3d; (iii) 30% aq NaOH, 1,4-dioxane, 70 °C, 24 h; (iv) 10% aq HCl, 1,4-dioxane, 70 °C, 24 h; (v) 3 equiv $Br(CH_2)_2Br$, 2 equiv K_2CO_3, cat. TBAB, 1,4-dioxane, rt, 16 h, **21**=66%.

2.4. Oxidative desulfurisation: pyrimidine-2,4-dione synthesis

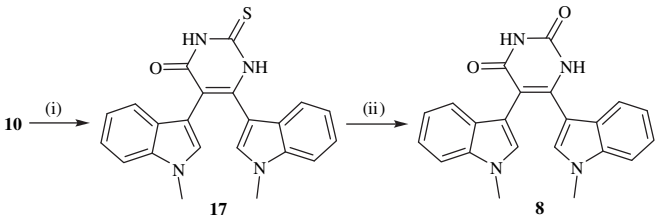
Synthesis of simple 5,6-dialkyl uracils from thiouracil precursors is known to be performed in the presence of 10% w/v chloroacetic acid, necessitating extended reaction times and high temperature, in moderate to good yields.[29] Following elimination of other alternative routes to **8**, work was undertaken in order to directly convert **17** to the attractive uracil ring system, **8** (Table 3). It appears that this is the first investigation of the oxidation conditions required for successful transformation of a 5,6-bis(heteroaryl)-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one to its corresponding pyrimidine-2,4(1*H*,3*H*)-dione analogue.

Table 3
Oxidative desulfurisation of 2-thiopyrimidin-4(3*H*)-one **17** to pyrimidine-2,4(1*H*,3*H*)-dione **8**

Reagent	Stoichiometry	Solvent	Reflux (time)	Product formation	
				X=SH (**17**)	X=OH (**8**)
$ClCH_2CO_2H$	10% w/v	H_2O	72 h	√	√
$ClCH_2CO_2H$	10% w/v	AcOH	72 h	√	√
$ClCH_2CO_2H$	10% w/v	DMF	72 h	√	√
$ClCH_2CO_2H$	5 equiv	MeOH	72 h	√	—
$BrCH_2CO_2Et$	1.5 equiv	DMF	24 h	√	—
$BrCH_2CO_2Et$	1.1 equiv	MeOH	24 h	—	18%
$BrCH_2CO_2Et$	2.5 equiv	MeOH/H_2O	48 h	—	45%

Treatment with aqueous chloroacetic acid for 72 h, afforded only an inseparable mixture of starting 2-thiopyrimidin-4-one **17**, along with a variable proportion of desired compound **8**, following ^{13}C NMR analysis of the crude reaction residue, under a range of conditions. It was postulated that reaction heterogeneity prevented full reactant conversion; however, when solvent conditions were altered in order to increase thiouracil solubility (e.g., DMF or methanol), these non-aqueous conditions then seemed to inhibit the oxidative desulfurisation process.Following the partial success of these initial efforts, further trials uncovered that a stoichiometric quantity of ethyl bromoacetate,[30] in dry methanol, heated at reflux for 16 h, allowed synthesis of the final product **8**, as an off-white

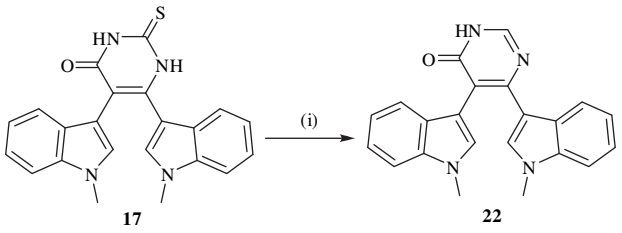
powder, in a moderate yield of 18%, following recrystallisation from DMF/H_2O (5:1). This yield could be improved once 2.5 equiv of ethyl bromoacetate were heated in the presence of **17**, in methanol, for 48 h, to provide **8** exclusively, in an enhanced yield of 45%, following crystallisation (Scheme 3).



Scheme 3. Reaction conditions: (i)(a) 10 equiv NaOMe, 5 equiv NH_2CSNH_2, MeOH, reflux, 24 h, (b) 10% HCl/H_2O, **17**=24%; (ii)(a) 2.5 equiv $BrCH_2CO_2Et$, MeOH, reflux, 48 h; (b) H_2O, **8**=45%.

2.5. Reaction with bi-dentate *N*-nucleophiles—amidine derivatives

Direct reaction of formamidine acetate with **10** under normal sodium methoxide conditions (Table 1; method A), for 24 h, failed to yield any of the desired reduced 2-(1*H*)-pyrimidine-4-one, **22**—postulated to be an attractive acyclic K-252c **9** analogue. The synthesis of this congener was subsequently effected by stirring of compound **17** overnight, with 5 equiv of nickel bromide in distilled methanol, in the presence of 15 equiv of sodium borohydride, to afford 5,6-bis(1-methyl-1*H*-indol-3-yl)pyrimidin-4(3*H*)-one **22**, in 52% yield, following filtration of the black reaction mixture and chromatographic purification (Scheme 4).[31]



Scheme 4. Reaction conditions: (i) 5 equiv $NiBr_2$, 15 equiv $NaBH_4$, MeOH, rt, 16 h, **22**=52%.

3. Conclusion

We now report that reaction of **10** with thiourea and guanidine carbonate affords the corresponding bisindolyl heterocyclic analogues and overall represents a validated route to a versatile range of analogues within this novel compound class. Key structural traits existing within the enzyme-interacting imide H-bonding domain of biologically significant bisindolylmaleimide analogues (**2**–**4**) were modified and augmented by incorporation of an analogueous pyrimidin-4-one pharmacophore (Fig. 3), in order to retain chemical efficacy, as well as to afford derivatives displaying improved biophysical parameters, e.g., enhanced aqueous solubility. Biological testing of these compounds will identify further structural refinements through modelling of their non-covalent interactions with key active site residues in silico, in comparison to the highly active, five-membered 3,4-bis(indol-3-yl)-1*H*-pyrrole-2,5-dione core **2**.

An initial study on overcoming challenges associated with the synthesis of novel uracil **8**, along with derivatisation of the 5,6-bis (1-methyl-1*H*-indol-3-yl)pyrimidin-4(3*H*)-one system has been completed and a successful route has been identified. A series of simplified ruboxistaurin derivatives has been produced and currently, the route to full macrocyclic ruboxistaurin derivatives is underway. In parallel, work is in progress to establish a full methodology for the synthesis of corresponding indolo[2,3-*a*]pyrimidino[5,6-*c*]carbazoles, via oxidative cyclisation, to form the central carbocyclic ring system from these acyclic precursors.

4. Experimental

4.1. General experimental procedures

Melting points were determined with a Thomas Hoover Capillary Melting Point apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer Paragon 1000 spectrophotometer. ^1H (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker Avance 300 NMR spectrometer. All spectra were recorded in deuterated chloroform ($CDCl_3$) or DMSO (DMSO-d_6), with tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on a Waters Quattro Micro (QAA1202) in electrospray ionization (ESI) positive or negative modes. High resolution mass spectra (HRMS) were recorded on a Waters Micromass LCT Premier mass spectrometer (Instrument number KD 160) in ESI positive mode. Thin layer chromatography (TLC) was carried out on pre-coated silica gel 60 (Merck PF_{254}) plates and compounds were visualised by UV (254 nm) light detection. Column chromatography was carried out using Merck PF_{254} silica gel 60A. DCM was distilled from P_2O_5, ethyl acetate was distilled from K_2CO_3, hexane was distilled prior to use, ethanol and methanol were distilled from magnesium/iodine and THF was distilled from benzophenone ketal. All commercial reagents, anhydrous DMF and 1,4-dioxane were purchased from Aldrich and were used without further purification.

*4.1.1. Methyl (1-methyl-1H-indol-3-yl) acetate (**12**)*[21]. Dimethyl carbonate (8.6 mL, 101.6 mmol) was added to a mixture of indole-3-acetic acid **11** (6.002 g, 29.6 mmol), anhydrous potassium carbonate (6.007 g, 43.2 mmol) and DMF (70 mL), which was then stirred at 130 °C for 16 h. Following solvent removal, the product residue was extracted with ethyl acetate (150 mL), which was washed with water (2×150 mL) and brine (1×100 mL). The combined organic layers were dried using magnesium sulfate, filtered and evaporated in vacuo. The pure ester **12** was obtained, as a straw-coloured oil, following column chromatography, using an 80:20 mixture of hexane and ethyl acetate (5.722 g, 82%); δ_H (300 MHz) $CDCl_3$ 3.70 [3H, s, O–CH_3], 3.76 [3H, s, N–CH_3], 3.77 [2H, s, CH_2], 7.02 [1H, s, aromatic C–H_2], 7.09–7.15 [1H, t, J=7.86 Hz, aromatic C–H_5], 7.20–7.25 [1H, t, J=7.98 Hz, aromatic C–H_6], 7.27–7.30 [1H, d, J=8.07 Hz, aromatic C–H_7], 7.58–7.61 [1H, d, J=7.86 Hz, aromatic C–H_4]; δ_C (75 MHz) $CDCl_3$ 31.0 [CH_2], 32.7 [N–CH_3], 52.0 [O–CH_3], 106.7 [aromatic quat. C], 109.3 [aromatic C–H], 118.9 [aromatic C–H], 119.2 [aromatic C–H], 121.8 [aromatic C–H], 126.6 [aromatic quat. C], 127.7 [aromatic C–H], 136.9 [aromatic quat. C], 172.6 [–CO_2CH_3]; ν_{max}/cm^{-1} (KBr) 2951, 1732, 1616, 1556; m/z (ESI) 204.1 [$(M+H)^+$, 100%].

*4.1.2. 1-Methyl-1H-indol-3-yl carbonyl chloride (**13**).* *N*-Methyl indole-3-carboxylic acid **14** (0.502 g, 2.85 mmol) was suspended in dry DCM (15 mL), followed by the careful addition of oxalyl chloride (0.25 mL, 2.86 mmol) over several minutes. Following stirring at ambient temperature for 75 min, gas evolution had ceased, and the solvent was evaporated under reduced pressure—yielding a light red solid, (0.540 g, 98%), which was used without any further purification, following IR analysis; ν_{max}/cm^{-1} (KBr) 1748 (C=O).

*4.1.3. Methyl 2,3-bis(1-methyl-1H-indol-3-yl)-3-oxopropanoate (**10**).* A solution of LDA (36.2 mL, 65.1 mmol, 2.2 equiv) was added over 10 min to a flask containing dry THF (50 mL), followed by cooling

to −78 °C. A mixture of *N*-methyl ester **12** (6.005 g, 29.6 mmol) in THF (3 mL) was then added slowly to the flask, while stirring, under inert atmosphere. The acid chloride **13** (38.5 mmol, 1.3 equiv, based on corresponding acid **14**) was dissolved in THF (15 mL), and then added dropwise to the reaction vessel. The reaction was allowed to warm up to room temperature, while stirring was maintained overnight. Following solvent removal, the product was extracted with ethyl acetate (3×150 mL), followed by washing with saturated ammonium chloride (250 mL), water (4×250 mL) and brine (2×150 mL). The combined organic layers were dried using magnesium sulfate, filtered and evaporated in vacuo. The optimised yield of the β-ketoester **10**—a dark yellow crystalline solid, following gradient column chromatography employing ethyl acetate/hexane, was 8.815 g (82%); mp 142–144 °C; δ_H (300 MHz) $CDCl_3$ 3.74 [3H, s, N–CH_3], 3.74 [3H, s, N–CH_3], 3.76 [3H, s, O–CH_3 (ester)], 5.70 [1H, s, –C–H_α], 7.12–7.17 [1H, td, *J*=6.90, 1.35 Hz, aromatic C–H_5], 7.19–7.25 [1H, td, *J*=8.16, 1.26 Hz, aromatic C–$H_{5'}$], 7.28–7.30 [4H, m, aromatic C–$H_{6,6',7,7'}$], 7.32 [1H, s, aromatic C–H_2], 7.66–7.68 [1H, d, *J*=7.65 Hz, aromatic C–H_4], 7.78 [1H, s, aromatic C–$H_{2'}$], 8.42–8.45 [1H, m, aromatic C–$H_{4'}$]; δ_C (75 MHz) $CDCl_3$ 32.9 [N–CH_3], 33.6 [N–CH_3], 52.7 [O–CH_3], 53.2 [–C–H_α] 107.5 [aromatic quat. C], 109.6 [aromatic C–H], 109.7 [aromatic C–H], 115.0 [aromatic quat. C], 118.3 [aromatic C–H], 119.5 [aromatic C–H], 121.8 [aromatic C–H], 122.8 [aromatic C–H], 122.9 [aromatic C–H], 123.7 [aromatic C–H], 126.8 [aromatic quat. C], 127.2 [aromatic quat. C], 129.0 [aromatic C–H], 136.2 [aromatic C–H], 136.7 [aromatic quat. C], 137.4 [aromatic quat. C], 170.3 [–*C*=O(–OCH_3)], 188.0 [C–*C*=O(–C)]; ν_{max}/cm^{-1} (KBr) 2927, 1732, 1644, 1529; *m*/*z* (ESI) 361.3 [$(M+H)^+$, 100%], 259.3 (80); HRMS—(ESI^+) requires: 361.1548, found: 361.1552 ($C_{22}H_{21}N_2O_3$).

*4.1.4. 2-Amino-5,6-bis(1-methyl-1H-indol-3-yl)pyrimidin-4(3H)-one (**16**).* Sodium metal (1.742 g, 76 mmol, 20 equiv) was dissolved in dry methanol (60 mL) under an inert atmosphere followed by stirring at room temperature for 20 min. Guanidinium carbonate (3.421 g, 19 mmol, 5 equiv) was then added to the flask in a single portion, and the heterogeneous mixture was stirred vigorously for 25 min at ambient temperature. Methyl 2,3-bis(1-methyl-1*H*-indol-3-yl)-3-oxopropanoate **10** (1.366 g, 3.8 mmol) was carefully added to this suspension and the dark orange reaction was then heated to reflux for 24 h. Following solvent removal under reduced pressure, the residue was dissolved in a minimum volume of water (30 mL), and subsequently acidified with 10% HCl to pH 3. The crude 2-amino-5,6-bis(1-methyl-1*H*-indol-3-yl)pyrimidin-4(3*H*)-one **16** was then filtered, washed successively with water and diethyl ether and dried. Gradient chromatography afforded a fraction, eluting with 0.5% methanol/DCM, which was evaporated to provide the isocytosine derivative **16** as a light brown amorphous powder (0.627 g, 45%); mp 195–197 °C; δ_H (300 MHz) DMSO-d_6 3.40 [3H, s, N–CH_3], 3.80 [3H, s, N–CH_3], 6.41 [2H, br s, NH_2], 6.65 [1H, s, aromatic C–H_2], 6.78–6.83 [1H, t, *J*=7.47 Hz, aromatic C–H_5], 6.98–7.13 [4H, m, aromatic C–$H_{5',6,6',7}$], 7.17 [1H, s, aromatic C–$H_{2'}$], 7.27–7.29 [1H, d, *J*=8.04 Hz, aromatic C–$H_{7'}$], 7.39–7.42 [1H, d, *J*=8.19 Hz, aromatic C–H_4], 8.32–8.35 [1H, d, *J*=7.98 Hz, aromatic C–$H_{4'}$], 10.83 [1H, br s, NH]; δ_C (75 MHz) DMSO-d_6 32.4 [N–CH_3], 32.7 [N–CH_3], 105.5 [aromatic quat. C], 106.6 [aromatic quat. C], 109.0 [2×aromatic quat. C], 109.5 [aromatic C–H], 110.0 [aromatic C–H], 118.5 [aromatic C–H], 119.6 [aromatic C–H], 119.9 [aromatic C–H], 120.7 [aromatic C–H], 121.2 [aromatic C–H], 121.8 [aromatic C–H], 125.6 [aromatic quat. C], 127.1 [aromatic quat. C], 130.5 [aromatic C–H], 132.2 [aromatic C–H], 136.2 [aromatic quat. C], 136.3 [aromatic quat. C], 152.7 [–*C*=N(NH_2)], 161.8 [–*C*=O(NH)]; ν_{max}/cm^{-1} (KBr) 3384, 2925, 1686, 1627, 1517, 1462; *m*/*z* (ESI) 370.1 [$(M+H)^+$, 100%], 371.1 (25); HRMS—(ESI^+) requires: 370.1677, found: 370.1668 ($C_{22}H_{20}N_5O$).

*4.1.5. 5,6-Bis(1-methyl-1H-indol-3-yl)-2-thioxo-2,3-dihydropyrimidin-4(1H)-one (**17**).* To a sodium methoxide solution sodium metal (1.595 g, 69 mmol, 10 equiv) in dry methanol (120 mL), under inert atmosphere, was added thiourea (2.627 g, 35 mmol, 5 equiv) followed by vigorous stirring for 15 min. Methyl 2,3-bis(1-methyl-1*H*-indol-3-yl)-3-oxopropanoate **10** (2.508 g, 6.9 mmol) was then charged to the reaction mixture and heated at reflux for 24 h. Following solvent evaporation, water (100 mL) was added to the complex residue, prior to acidification with 10% HCl to pH 3. Filtration of the resultant dark brown solid, followed by trituration of the crude pyrimidinone **17** with boiling absolute ethanol (40 mL), produced a slurry, which, following filtration and successive washing of the yellow powder with water and diethyl ether, was identified as the pure thiouracil product **17** (0.640 g, 24%); mp 317–320 °C; δ_H (300 MHz) DMSO-d_6 3.63 [3H, s, N–CH_3], 3.70 [3H, s, N–CH_3], 6.74–6.80 [2H, m, aromatic C–$H_{5,5'}$], 6.96–7.01 [2H, m, aromatic C–$H_{7,7'}$], 7.02–7.07 [2H, t, *J*=7.85 Hz, aromatic C–$H_{6,6'}$], 7.21 [1H, s, aromatic C–H_2], 7.24–7.27 [1H, d, *J*=8.19 Hz, aromatic C–H_4], 7.30–7.33 [1H, d, *J*=8.19 Hz, aromatic C–$H_{4'}$], 7.65 [1H, s, aromatic C–$H_{2'}$]; 12.15 [1H, br s, thioamide N–H]; 12.51 [1H, br s, imide N–H]; δ_C (75 MHz) DMSO-d_6 32.4 [N–CH_3], 32.7 [N–CH_3], 105.7 [aromatic quat. C], 106.3 [aromatic quat. C], 109.1 [aromatic quat. C], 109.4 [aromatic C–H], 110.0 [aromatic C–H], 118.5 [aromatic C–H], 119.5 [aromatic C–H], 119.6 [aromatic C–H], 119.7 [aromatic C–H], 120.6 [aromatic C–H], 121.4 [aromatic C–H], 125.1 [aromatic quat. C], 127.0 [aromatic quat. C], 130.7 [aromatic C–H], 132.4 [aromatic C–H], 135.9 [aromatic quat. C], 136.0 [aromatic quat. C], 144.9 [aromatic quat. C], 161.3 [–C=O], 174.3 [–C=S]; ν_{max}/cm^{-1} (KBr) 2922, 1651, 1541, 1452; *m*/*z* (ESI) 387.2 [$(M+H)^+$, 20%], 115.0 (75), 73.9 (100); HRMS—(ESI^+) requires: 387.1280, found: 387.1272 ($C_{22}H_{19}N_4OS$).

*4.1.6. 5,6-Bis(1-methyl-1H-indol-3-yl)-2-(methylthio)pyrimidin-4(3H)-one (**18**).* Distilled methanol (15 mL) was added to a flask containing anhydrous potassium carbonate (0.203 g, 1.42 mmol, 1.1 equiv), along with **17** (0.507 g, 1.29 mmol). Iodomethane (0.16 mL, 2.58 mmol, 2 equiv) was then added and the mixture was stirred vigorously at 30 °C for a further 20 h. Following solvent removal, the residue was taken up in ethyl acetate (15 mL) and filtered, to remove any unreacted base. This ethyl acetate solution was evaporated to yield a light beige solid, which was subjected to column chromatography, employing a gradient from 100% ethyl acetate, to 90% ethyl acetate/10% methanol, and finally with the addition of a few drops of Et_3N, to isolate polar **18**. The product fractions were combined and evaporated to a light yellow powder, under reduced pressure (0.180 g, 35%); mp 273–276 °C; δ_H (300 MHz) DMSO-d_6 2.66 [3H, s, S–CH_3], 3.46 [3H, s, N–CH_3], 3.82 [3H, s, N–CH_3], 6.76–6.81 [1H, t, *J*=7.65 Hz, aromatic C–H_5], 6.87 [1H, s, aromatic C–H_2], 6.93–6.95 [1H, d, *J*=7.86 Hz, aromatic C–H_7], 7.00–7.05 [1H, t, *J*=7.35 Hz, aromatic C–$H_{5'}$], 7.05–7.10 [1H, t, *J*=7.26 Hz, aromatic C–H_6], 7.10–7.15 [1H, t, *J*=7.02 Hz, aromatic C–$H_{6'}$], 7.32–7.35 [1H, d, *J*=8.10 Hz, aromatic C–$H_{7'}$], 7.37 [1H, s, aromatic C–$H_{2'}$], 7.41–7.44 [1H, d, *J*=8.16 Hz, aromatic C–H_4], 8.06–8.09 [1H, d, *J*=7.95 Hz, aromatic C–$H_{4'}$], 12.50 [1H, br s, N–H]; δ_C (75 MHz) DMSO-d_6 12.92 [S–CH_3], 32.5 [N–CH_3], 32.6 [N–CH_3], 107.5 [aromatic quat. C], 109.6 [aromatic C–H], 109.8 [aromatic C–H], 112.7 [aromatic quat. C], 118.6 [aromatic C–H], 119.7 [aromatic C–H], 119.7 [aromatic quat. C], 119.9 [aromatic C–H], 120.7 [aromatic quat. C], 120.8 [aromatic C–H], 121.5 [aromatic C–H], 122.1 [aromatic C–H], 126.3 [aromatic quat. C], 126.7 [aromatic quat. C], 130.3 [aromatic C–H], 132.7 [aromatic C–H], 136.3 [aromatic quat. C], 136.5 [aromatic quat. C], 156.6 [N=*C*(SCH_3)], 161.9 [C=O]; ν_{max}/cm^{-1} (KBr) 3434, 2923, 1628, 1521, 1461; *m*/*z* (ESI) 401.1 [$(M+H)^+$, 30%]; HRMS—(ESI^+) requires: 401.1436, found: 401.1430 ($C_{23}H_{21}N_4OS$).

*4.1.7. 6,7-Bis(1-methyl-1H-indol-3-yl)-2H-thiazolo[3,2-a]pyrimidin-5(3H)-one (**21**).* To a solution of compound **17** (0.101 g, 0.26 mmol)

in 1,4-dioxane (10 mL)were added anhydrous potassium carbonate (0.072 g, 0.52 mmol, 2 equiv) and TBAB (0.025 g, 0.078 mmol, 0.33 equiv). 1,2-Dibromoethane (0.07 mL, 0.78 mmol, 3 equiv) was then syringed into the reaction flask, with vigorous stirring of the mixture allowed to proceed overnight. The dioxane solution was filtered, prior to washing with DCM (10 mL). The clarified filtrate was evaporated in vacuo, and the residue was then washed with a small volume of hexane, to remove any excess starting reagent. The resultant beige solid was dissolved in hot absolute ethanol (8 mL), followed by cooling to room temperature and subsequently to 0 °C. The heterocyclic product **21** was then filtered and dried to provide a light yellow powder (0.071 g, 66%); mp>300 °C; δ_H (300 MHz) DMSO-d_6 3.52 [3H, s, N–CH_3], 3.64–3.69 [2H, t, J=7.56 Hz, S–CH_2–CH_2–N], 3.87 [3H, s, N–CH_3], 4.46–4.51 [2H, t, J=7.56 Hz, S–CH2–CH_2–N], 6.84–6.89 [1H, t, J=7.59 Hz, aromatic C–H_5], 6.89 [1H, s, aromatic C–H_2], 7.02–7.04 [1H, d, J=7.89 Hz, aromatic C–H_7], 7.02–7.07 [1H, t, J=7.62 Hz, aromatic C–$H_{5'}$], 7.11–7.17 [1H, t, J=9.03 Hz, aromatic C–H_6], 7.14–7.19 [1H, t, J=7.20 Hz, aromatic C–$H_{6'}$], 7.36–7.39 [1H, d, J=8.16 Hz, aromatic C–H_7], 7.39 [1H, s, aromatic C–$H_{2'}$], 7.47–7.50 [1H, d, J=8.22 Hz, aromatic C–H_4], 7.97–8.00 [1H, d, J=7.98 Hz, aromatic C–$H_{4'}$]; δ_C (75 MHz) DMSO-d_6 26.3 [N–CH_2*C*H_2–S], 32.5 [N–*C*H_3], 32.7 [N–*C*H_3], 49.0 [N–*C*H_2*C*H_2-S], 107.4 [aromatic quat. C], 109.0 [aromatic quat. C], 109.6 [aromatic C–H], 109.8 [aromatic C–H], 112.2 [aromatic quat. C], 118.6 [aromatic C–H], 119.7 [aromatic C–H], 119.9 [aromatic C–H], 120.8 [aromatic C–H], 121.5 [aromatic C–H], 122.1 [aromatic C–H], 126.4 [aromatic quat. C], 126.5 [aromatic quat. C], 130.3 [aromatic C–H], 132.7 [aromatic C–H], 136.2 [aromatic quat. C], 136.6 [aromatic quat. C], 155.5 [aromatic quat. C], 160.8 [N–*C*=N], 161.1 [–*C*=O]; ν_{max}/cm^{-1} (KBr) 2923, 1644, 1562, 1520, 1479, 1460; *m*/*z* (ESI) 413.0 [$(M+H)^+$, 100%]; HRMS—(ESI^+) requires: 413.1436, found: 413.1432 ($C_{24}H_{21}N_4OS$).

*4.1.8. 5,6-Bis(1-methyl-1H-indol-3-yl)pyrimidine-2,4(1H,3H)-dione (**8**).* The bisindolyl-2-thiopyrimidin-4-one derivative **17** (0.100 g, 0.26 mmol) was stirred in the presence of methanol (15 mL) and ethyl bromoacetate (0.108 g, 0.07 mL, 0.65 mmol, 2.5 equiv) at reflux, for 24 h, prior to the addition of a few drops of water and heating for a further 24 h. Following evaporation of the solvent under reduced pressure, a hot DMF/H_2O solution (5:1) (15 mL) was added to the resultant beige residue. The milky slurry was allowed to cool to room temperature and then filtered under reduced pressure. The resulting off-white powder was washed with water and diethyl ether successively, prior to drying under high vacuum overnight, to afford the pure 5,6-bisindolyl uracil **8** (0.044 g, 45%); mp>320 °C; δ_H (300 MHz) DMSO-d_6 3.63 [3H, s, N–CH_3], 3.67 [3H, s, N–CH_3], 6.75–6.81 [2H, m, aromatic C–$H_{5,5'}$], 6.96–7.08 [4H, m, aromatic C–$H_{6,6',7,7'}$], 7.11 [1H, s, aromatic C–$H_{2'}$], 7.24–7.27 [1H, d, J=8.19 Hz, aromatic C–H_4], 7.30–7.33 [1H, d, J=8.25 Hz, aromatic C–$H_{4'}$], 7.50 [1H, s, aromatic C–H_2], 10.69 [1H, br s, amide N–H], 11.12 [1H, br s, imide N–H]; δ_C (75 MHz) DMSO-d_6 32.3 [N–*C*H_3], 32.7 [N–*C*H_3], 103.8 [aromatic quat. C], 106.6 [aromatic quat. C], 107.4 [aromatic quat. C], 109.2 [aromatic C–H], 109.9 [aromatic C–H], 118.3 [aromatic C–H], 119.5 [aromatic C–H], 119.6 [aromatic C–H], 119.8 [aromatic C–H], 120.5 [aromatic C–H], 121.5 [aromatic C–H], 125.1 [aromatic quat. C], 127.6 [aromatic quat. C], 130.5 [aromatic C–H], 131.6 [aromatic C–H], 136.0 [2×aromatic quat. C], 145.2 [aromatic quat. C], 151.1 [NH–*C*=O(–NH)], 164.2 [NH–*C*=O(–C=C)]; ν_{max}/cm^{-1} (KBr) 3425, 2925, 1703, 1639, 1555, 1453; *m*/*z* (ESI) 371.1 [$(M+H)^+$, 80%], 369.1 (40%); HRMS—(ESI^+) requires: 371.1508, found: 371.1506 ($C_{22}H_{19}N_4O_2$).

*4.1.9. 5,6-Bis(1-methyl-1H-indol-3-yl)pyrimidin-4(3H)-one (**22**).* Thiouracil **17** (0.105 g, 0.26 mmol) was added to a flame-dried flask along with nickel bromide (0.290 g, 1.3 mmol, 5 equiv) and dry methanol (10 mL). Sodium borohydride (0.152 g, 3.89 mmol, 15 equiv) was added cautiously to the reaction mixture portionwise, and the reaction was allowed to stir for 2 h at room temperature. TLC analysis indicated full consumption of starting material had occurred at this point. Following stirring at room temperature for a further 14 h, the reaction mixture was then filtered through a pad of Celite® and washed with additional methanol (2×20 mL), followed by ethyl acetate (3×15 mL). Following evaporation of these combined extracts, the light orange residue was dissolved in ethyl acetate (70 mL). This material was then washed with water (75 mL), and the aqueous layer further extracted with ethyl acetate (2×25 mL). The ethyl acetate layers were combined and washed with water (3×100 mL) and brine (100 mL). The organic layer was dried over magnesium sulfate, filtered and evaporated in vacuo. The pyrimidin-4(3*H*)-one derivative **22** was then purified by flash chromatography (1% methanol/DCM), to yield a dark brown powder (0.049 g, 52%); mp 218–221 °C; δ_H (300 MHz) DMSO-d_6 3.38 [3H, s, N–CH_3], 3.74 [3H, s, N–CH_3], 6.68–6.73 (1H, t, J=7.05 Hz, C–$H_{5'}$), 6.80 (1H, s, C–$H_{2'}$), 6.84–6.86 (1H, d, J=7.89 Hz, C–$H_{7'}$), 6.88–6.93 (1H, t, J=7.83Hz, C–H_5), 6.96–7.01 (1H, t, J=7.56 Hz, C–$H_{6'}$), 7.01–7.06 (1H, t, J=6.99 Hz, C–H_6), 7.23–7.25 (1H,d, J=8.01 Hz, C–H_7), 7.33–7.35 (1H, d, J=5.85 Hz, C–H_4), 7.35 (1H, s, C–H_2), 7.95–7.98 (1H, d, J=7.86 Hz, C–$H_{4'}$), 8.13 (1H, s, N=C–*H*), 12.23 (1H, br s, N–H); δ_C (75 MHz) DMSO-d_6 32.5 [N–*C*H_3], 32.6 [N–*C*H_3], 107.5 [aromatic quat. C], 109.6 [aromatic C–H], 109.7 [aromatic C–H], 112.7 [aromatic quat. C], 114.7 [aromatic quat. C], 118.6 [aromatic C–H], 119.8 [2×aromatic C–H], 120.8 [aromatic C–H], 121.4 [aromatic C–H], 122.3 [aromatic C–H], 126.1 [aromatic quat. C], 126.6 [aromatic quat. C], 130.5 [aromatic C–H], 132.5 [aromatic C–H], 136.3 [aromatic quat. C]; 136.5 [aromatic quat. C], 146.3 [aromatic quat. C], 155.7 [–N=*C*–H(NH)], 161.7 [–*C*=O(NH)]; ν_{max}/cm^{-1} (KBr) 3426, 2926, 1633, 1528, 1465, 1372; *m*/*z* (ESI) 355.1 [$(M+H)^+$, 100%]; HRMS—(ESI^+) requires: 355.1559, found: 355.1569 ($C_{22}H_{19}N_4O$).

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Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2010.10.020.

References and notes

1. Kishimoto, A.; Takai, Y.; Mori, T.; Kikkawa, U.; Nishizuka, Y. *J. Biol. Chem.* **1980**, *255*, 2273–2276.
2. Nettleton, D. E.; Doyle, T. W.; Krishnan, B.; Matsumoto, G. K.; Clardy, J. *Tetrahedron Lett.* **1985**, *26*, 4011–4014.
3. Powis, G. *Trends Pharmacol. Sci.* **1991**, *12*, 188–194.
4. Omura, S.; Iwai, Y.; Hirano, A.; Nakagawa, A.; Awaya, J.; Tsuchiya, M.; Takahashi, Y.; Masuma, R. *J. Antibiot.* **1977**, *30*, 275–282.
5. Tamaoki, T.; Nomoto, H.; Takahashi, I.; Kato, Y.; Morimoto, M.; Tomita, F. *Biochem. Biophys. Res. Commun.* **1986**, *135*, 397–402.
6. Sanchez-Martinez, C.; Shih, C.; Zhu, G.; Li, T.; Brooks, H. B.; Patel, B. K. R.; Schultz, R. M.; DeHahn, T. B.; Spencer, C. D.; Watkins, S. A.; Ogg, C. A.; Considine, E.; Dempsey, J. A.; Zhang, F. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3841–3846.
7. Courage, C.; Snowden, R.; Gescher, A. *Br. J. Cancer* **1996**, *74*, 1199–1205.
8. Aiello, L. P.; Clermont, A.; Arora, V.; Davis, M. D.; Sheetz, M. J.; Bursell, S. E. *Invest. Ophthalmol. Visual Sci.* **2006**, *47*, 86–92.
9. Jirousek, M. R.; Gillig, J. R.; Gonzalez, C. M.; Heath, W. F.; McDonald, J. H., III.; Neel, D. A.; Rito, C. J.; Singh, U.; Stramm, L. E.; Melikian-Badalian, A.; Baevsky, M.; Ballas, L. M.; Hall, S. E.; Winneroski, L. L.; Faul, M. M. *J. Med. Chem.* **1996**, *39*, 2664–2671.
10. Way, K. J.; Chou, E.; King, G. L. *Trends Pharmacol. Sci.* **2000**, *21*, 181–187.
11. Pearse, H. L.; Miller, M. A. *Adv. Enzyme Regul.* **2005**, *45*, 229–255.
12. Robertson, M. J.; Kahl, B. S.; Vose, J. M.; De Vos, S.; Laughlin, M.; Flynn, P. J.; Rowland, K.; Cruz, J. C.; Goldberg, S. L.; Musib, L.; Darstein, C.; Enas, N.; Kutok, J.

L.; Aster, J. C.; Neuberg, D.; Savage, K. J.; LaCasce, K.; Thornton, D.; Slapak, C. A.; Shipp, M. A. *J. Clin. Oncol.* **2007**, *25*, 1741–1746.
13. Robey, R. W.; Shukla, S.; Steadman, K.; Obrzut, T.; Finley, E. M.; Ambudkar, S. V.; Bates, S. E. *Mol. Cancer Ther.* **2007**, *6*, 1877–1885.
14. Cohen, P. *Curr. Opin. Chem. Biol.* **1999**, *3*, 459–465.
15. Franco, L. H.; Joffé, E. B. K.; Puricelli, L.; Tatian, M.; Seldes, A. M.; Palermo, J. A. J. *J. Nat. Prod.* **1996**, *61*, 1130–1132.
16. Jiang, B.; Yang, C.-G.; Xiong, W.-N.; Wang, J. *Bioorg. Med. Chem.* **2001**, *9*, 1149–1154.
17. Časar, Z.; Bevk, D.; Svete, J.; Stanovnik, B. *Tetrahedron* **2005**, *61*, 7508–7519.
18. Nakanishi, S.; Matsuda, Y.; Iwahashi, K.; Kase, H. *J. Antibiot.* **1986**, *39*, 1066–1071.
19. Larsen, J. S.; Christensen, L.; Ludvig, G.; Jùrgensen, P. T.; Pedersen, E. B.; Nielsen, C. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3035–3038.
20. Mojtahedi, M. M.; Saidi, M. R.; Shirzi, J. S.; Bolourtchian, M. *Synth. Commun.* **2002**, *32*, 851–855.
21. (a) Jiang, X.; Tiwari, A.; Thompson, M.; Chen, Z.; Cleary, T. P.; Lee, T. B. K. *Org. Process Res. Dev.* **2001**, *5*, 604–608; (b) Bergman, J.; Norrby, P.; Sand, P. *Tetrahedron* **1990**, *46*, 6113–6124.
22. Hutchins, M.; Sainsbury, M.; Scopes, D. *Tetrahedron* **1984**, *40*, 1511–1515.
23. Braña, M. F.; Gradillas, A.; Ovalles, A. G.; Lopez, B.; Acero, N.; Llinares, F.; Mingarro, D. M. *Bioorg. Med. Chem.* **2006**, *14*, 9–16.
24. Botta, M.; Artico, M.; Massa, S.; Gambacorta, A. *J. Heterocycl. Chem.* **1988**, *26*, 883–884.
25. Holmes, R. E.; Robins, R. K. *J. Am. Chem. Soc.* **1964**, *86*, 1242–1245.
26. Gibson, C. L.; La Rosa, S.; Suckling, C. J. *Org. Biomol. Chem.* **2003**, *1*, 1909–1918.
27. Lu, Y.; Xiang, T.; Bartberger, M. D.; Bernard, C.; Bostick, T.; Huang, L.; Liu, L.; Siegmund, A.; Sukay, G.; Guo, G.; Silva Elipe, M.; Tormos, W.; Dominguez, C.; Koch, K.; Burgess, L. E.; Basil, T. C.; Ibrahim, P.; Hummel, C. *Tetrahedron* **2006**, *62*, 11714–11723.
28. Hassan, M. A.; Mohamed, M. M.; Shiba, S. A.; El-Regal, M. K. A.; Khalil, A. *Phosphorus, Sulfur Silicon Relat. Elem.* **2003**, *178*, 2497–2504.
29. Macchia, M.; Barontini, S.; Bertini, S.; Di Bussolo, V.; Fogli, S.; Giovannetti, E.; Grossi, E.; Minutolo, F.; Danesi, R. *J. Med. Chem.* **2001**, *44*, 3994–4000.
30. Abdel Aal, M. T. *Synth. Commun.* **2002**, *32*, 1365–1377.
31. Khurana, J. M.; Agrawal, A.; Kukreja, G. *Heterocycles* **2006**, *68*, 1885–1892.