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Exploring Natural Product Chemistry and Biology with Multicomponent Reactions. 5. Discovery of a Novel Tubulin- Targeting Scaffold Derived from the Rigidin Family of Marine Alkaloids

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Abstract: We developed synthetic chemistry to access the marine alkaloid rigidins and over forty synthetic analogues based on the 7-deazaxanthine, 7-deazaadenine, 7-deazapurine and 7-deazahypoxanthine skeletons. Analogues based on the 7-deazahypoxanthine skeleton exhibited nanomolar potencies against cell lines representing cancers with dismal prognoses, tumor metastases and multidrug resistant cells. Studies aimed at elucidating the mode(s) of action of the 7-deazahypoxanthines in cancer cells revealed that they inhibited *in vitro* tubulin polymerization and disorganized microtubules in live HeLa cells. Experiments evaluating the effects of the 7-deazahypoxanthines on the binding of [³H]colchicine to tubulin identified the colchicine site on tubulin as the most likely target for these compounds in cancer cells. Because many microtubule-targeting compounds are successfully used to fight cancer in the clinic, we believe the new chemical class of antitubulin agents represented by the 7-deazahypoxanthine rigidin analogues have significant potential as new anticancer agents.

Introduction

In light of their fascinating novel structures, intriguing biological properties and the difficulty in obtaining large quantities from natural sources, marine pyrrole-derived alkaloids have attracted

considerable attention from organic and medicinal chemists.¹⁻⁸ At present, several marine natural products are in clinical trials, and one drug, trabectedin, was recently approved in Europe as the first ever marine-derived anticancer agent.⁹ In this context, the marine alkaloid rigidins A, B, C, D (Figure 1) and E isolated from the tunicate *Eudistoma cf. rigida* found near Okinawa and New Guinea have remained largely unexplored despite initial reports of promising biological activities.¹⁰⁻¹²

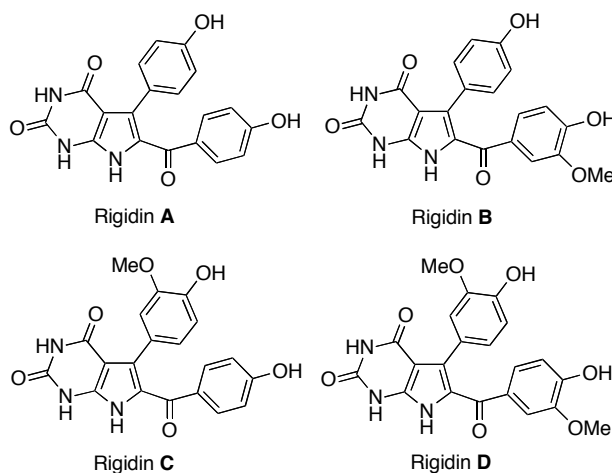


Figure 1. Structures of rigidins A, B, C and D

These compounds were shown to exhibit cytotoxicity against murine leukemia L1210 cells,¹¹ while rigidin A was also found to possess anticalmodulin activity.¹⁰ Further work in this area was hampered by the miniscule isolation yields of these natural products (0.0015 % for A, 0.00031 % for B, 0.00008 % for C, 0.00004 % for D, and 0.018 % for E based on dry weight). Up to 2011, the synthetic accomplishments in the rigidin area included three total syntheses of rigidin A¹³⁻¹⁶ and one of rigidin E.¹⁶ These approaches involved lengthy synthetic sequences and/or low overall yields and did not offer a solution to the natural product supply problem. More importantly, however, the lack of synthetic flexibility impeded their use for the preparation of analogues for structure-activity relationship (SAR)^a studies. Because our research program aims to develop multicomponent reactions (MCR) to access “privileged medicinal scaffolds,”¹⁷⁻²⁰ we became interested in the rigidins, which incorporate the purine-like pyrrolo[2,3-*d*]pyrimidine ring system, a common motif in natural products (e.g., tubercidin, toyocamycin, sangivamycin²¹) and medicinal agents with diverse bioactivities (e.g., anti-inflammatory,^{22,23} anticancer,²⁴⁻²⁹ antiviral,^{30,31}

adenosine A₁ and A₃ receptor inhibition,³² adenosine kinase inhibition,^{33,34} and dihydrofolate reductase inhibition³⁵).

In 2011, we reported in a communication format a general total synthesis of rigidins A, B, C and D, which involved only four steps from commercially available materials and was amenable to the production of synthetic rigidin analogues.³⁶ This work has led to further developments and discoveries, which we describe here. More specifically, first, we detail the synthesis of first-generation synthetic analogues based on the 7-deazaxanthine skeleton. Second, we describe an anticancer evaluation of synthetically prepared rigidins A, B, C and D that, together with the first generation analogues, fail to display any significant effects on cancer cell proliferation. Third, we disclose novel MCRs leading to the preparation of the second-generation rigidin analogues based on the 7-deazaadenine, 7-deazapurine and 7-deazahypoxanthine (Figure 2) skeletons. Fourth, we show that the 7-deazahypoxanthines display potent antiproliferative activities. Fifth, we demonstrate that these compounds maintain their activity against tumor cells derived from cancers associated with dismal prognoses, metastatic tissues as well as multidrug resistant cells. Finally, we show that the anticancer properties of these hypoxanthine-like compounds are most likely caused by their pronounced effects on microtubule organization in cancer cells.

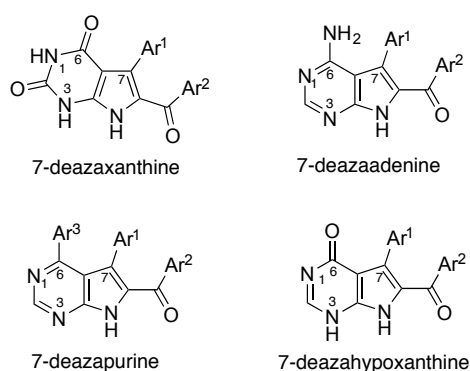


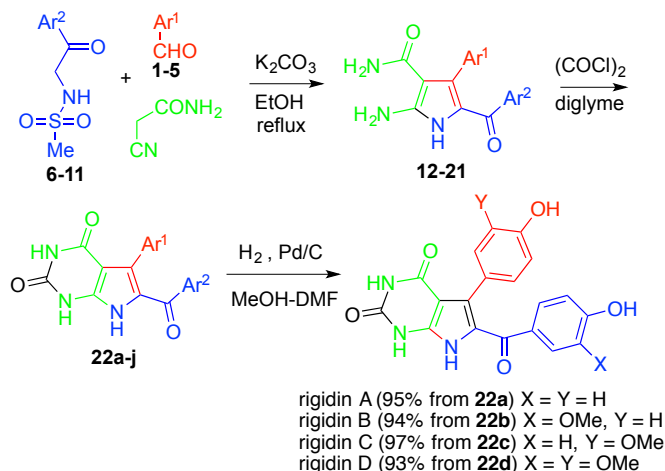
Figure 2. Structures of pyrrolo[2,3-*d*]pyrimidines prepared in this work

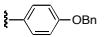
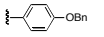
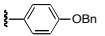
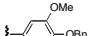
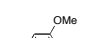
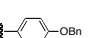


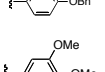
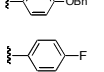
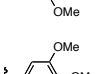
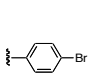
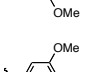
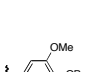
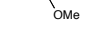

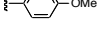
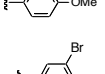
Results and Discussion

Chemistry

The synthesis of natural rigidins A, B, C and D, as well as synthetic 7-deazaxanthine analogues, is based on the chemistry shown in Table 1. Sulfonamides **6-11**, prepared by reacting commercially available aminoacetophenones with methanesulfonyl chloride, are utilized in a three-component reaction (3-CR) with various aromatic aldehydes **1-5** and cyanoacetamide in the presence of potassium carbonate in refluxing ethanol to give aminopyrroles **12-21** (Table 1). The synthesis of the 7-deazaxanthine framework is completed by carbonylation with oxalyl chloride in diglyme to give analogues **22a-j** possessing diversely substituted aromatic moieties Ar¹ and Ar². Compounds **22a-d** are further hydrogenolyzed to yield natural rigidins with overall yields of 61% for A, 58% for B, 60% for C and 53% for D from commercially available aminoacetophenones. This sequence constitutes the first synthesis of rigidins B, C and D, while rigidin A is accessed via a significantly improved method compared with the previously described routes.¹³⁻¹⁶ In addition, our reaction sequence leads to an expedient preparation of what we call the first-generation library of rigidin analogues based on the 7-deazaxanthine skeleton and possessing variable substituents in the aromatic moieties. Finally, in addition to these synthetic 7-deazaxanthines **22a-j**, we prepared a dithia variant **22k**, which is obtained by treating pyrrole **21** with carbon disulfide in pyridine (Figure 3).

Table 1. MCR-based total general synthesis of rigidins A-D and their first-generation 7-deazaxanthine synthetic analogues **22a-j**



aldehyde	methanes ulfonyl amide	pyrrole	7-deaza xanthine	Ar ¹	Ar ²	overall yield %
1	6	12	22a			64
1	7	13	22b			62
2	6	14	22c			62
2	7	15	22d			57
3	8	16	22e			74
3	9	17	22f			64
3	7	18	22g			59
4	10	19	22h			57
4	11	20	22i		Ph	62
5	11	21	22j		Ph	43

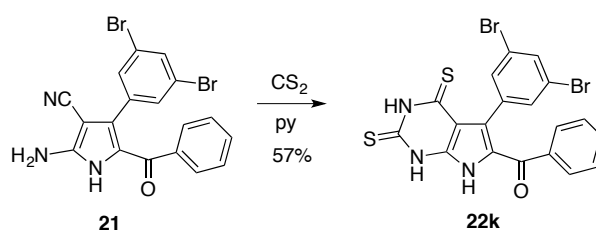


Figure 3. Preparation of an additional 7-deazapurine **22k**

Our further studies of the above-described three-component reaction led to the discovery of its four-component (4-CR) variants. Thus, we found that if the reaction is conducted in formamide as a solvent and cyanoacetamide is replaced with malononitrile (Table 2) or cyanomethylketones (Table 3), the entire pyrrolo[2,3-*d*]pyrimidine skeleton is assembled in one step giving rise to 7-deazaadenines **23** (Table 2) or 7-deazapurines **24** (Table 3), respectively. The success of these 4-CRs also depends on an optimized temperature regime, in which the reaction is first kept at 90 °C to allow for a clean formation of

intermediate aminopyrroles (similar to **12-21** in Table 1) and then at 150 °C to bring the fourth component formamide into the process, leading to closure of the pyrimidine portion of the molecule.

Table 2. 4-CR leading to the formation of the second-generation analogues based on the 7-deazaadenine skeleton.

Ar²

+

Ar¹

HCONH₂

Ar²

23a-f

K₂CO₃

1. 90 °C, 24 h

2. 150 °C, 3-6 h

7-deazaadenine	Ar ¹	Ar ²	% yield
23a		Ph	58
23b		Ph	66
23c	Ph	Ph	48
23d		Ph	85
23e			54
23f		Ph	72

Table 3. 4-CR leading to the formation of the second-generation analogues based on the 7-deazapurine skeleton.

Ar²

+

Ar¹

HCONH₂

Ar²

24a-e

K₂CO₃

1. 90 °C, 24 h

2. 150 °C, 3-6 h

6,7-dideazaadenine	Ar ¹	Ar ²	Ar ³	% yield
24a				63
24b		Ph	Ph	30

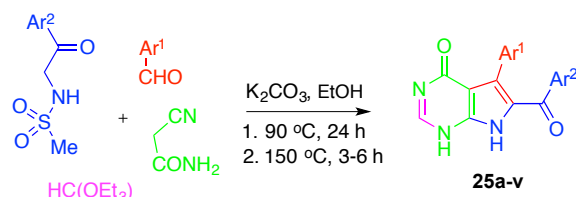
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



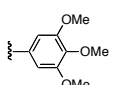







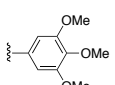


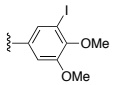

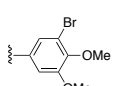

24c		Ph		33
24d		Ph	Ph	39
24e		Ph	Ph	22

Additionally, we discovered a 4-CR leading to the formation of a 7-deazahypoxanthine skeleton **25** (Table 4). In this modification, the starting sulfonamides, aldehydes and cyanoacetamide are reacted in a 1:1 mixture of EtOH and triethyl orthoformate under a similar temperature regime, involving reflux at 90 °C followed by a gradual increase in temperature to 150 °C, with the concomitant slow evaporation of ethanol.

Table 4. 4-CR leading to the formation of the second-generation analogues based on the 7-deazahypoxanthine skeleton.

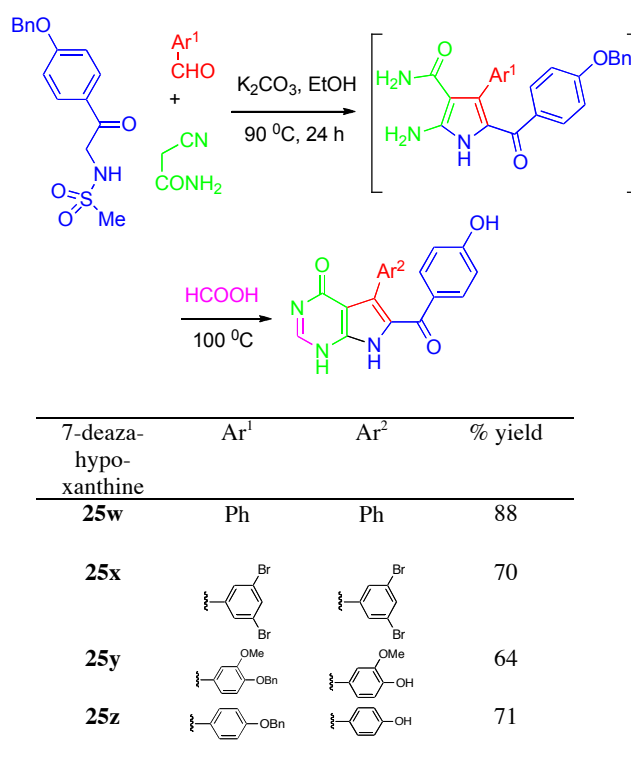


7-deaza-	Ar ¹	Ar ²	% yield	7-deaza-	Ar ¹	Ar ²	% yield
hypo-				hypo-			
xanthine				xanthine			
25a	Ph	Ph	71	25l		Ph	74
25b		Ph	68	25m		Ph	77
25c		Ph	61	25n		Ph	26
25d		Ph	54	25o		Ph	47

25e		Ph	53	25p			56
25f		Ph	60	25q			56
25g		Ph	88	25r			60
25h		Ph	61	25s			61
25i		Ph	64	25t			54
25j		Ph	37	25u	Ph		68
25k		Ph	51	25v		Ph	71

The analogues accessed by these 4-CRs (Tables 2, 3 and 4) are generally obtained in good yields given the complexity of the newly devised reactions. The product pyrrolopyrimidines contain diversely substituted aromatic moieties, including alkoxy (e.g., **22a-i**, **23e**, **24a**, **25i-k**, **25q**, etc), halogen (e.g., **23a,b**, **25b-g**, **25r-t**, etc), cyano (e.g., **25o**) and heterocycles (e.g., **23b**, **25f-h**, **25v**, etc). Additionally, the hydroxy-substituted pyrrolopyrimidines can be obtained by the use of precursors containing benzyl-protected phenolic oxygens, with subsequent hydrogenolysis (similar to the synthesis of rigidins A-D from **22a-d** in Table 1) or through another modification in the MCR chemistry involving the use of HCOOH as the fourth component (Table 5). Formic acid plays a dual role, providing the necessary carbon for pyrimidine ring-closure and promoting *in situ* debenzylation of the phenolic benzyl ethers, resulting in analogues **25w-z** (Table 5). Interestingly, a search of the literature reveals no examples of metal-free utilization of formic acid for deprotection of benzyl ethers. To our knowledge the described chemistry represents the first examples of construction of the pyrrolo[2,3-*d*]pyrimidine skeleton in a one-step MCR process.

Table 5. 4-CR with concomitant debenzoylation leading to the formation of hydroxyl-substituted 7-deazahypoxanthine analogues.



Pharmacology

(a) Antiproliferative Activities

The synthetically prepared natural products and their synthesized analogues were evaluated for antiproliferative activities using the HeLa cell line as a model for human cervical adenocarcinoma and MCF-7 cells as a model for breast adenocarcinoma. The cells were treated with each compound for 48 h, and cell viability was assessed using the MTT method³⁷ (Table 6). The natural alkaloids and synthetic 7-deazaxanthine **22e-k** analogues were inactive or weakly active, producing double-digit micromolar GI₅₀ values in some instances (e.g., analogue **22i**). Further, scaffold modifications, such as those resulting in 7-deazaadenine analogues **23** and 7-deazapurine compounds **24**, led to the discovery of micromolar potencies associated with selected compounds (e.g., **23c** and **24c**). An unexpected and most gratifying observation was, however, the discovery of nanomolar antiproliferative activity in compounds based on

the 7-deazahypoxanthine skeleton **25**. Thus, analogues **25a**, **25e**, **25g**, **25m** and **25u** all exhibited double-digit nanomolar potencies. A closer analysis of the SAR data indicates that the favored substitution pattern is the unsubstituted or para-substituted benzene ring at the sulfonamide-derived C-8 position (Ar²) and either the unsubstituted or meta-halogen-substituted benzene group at the aldehyde-derived C-7 position (Ar¹). These structural features are found in the most potent analogues **25a-g**, **25m**, **25r-u**. The replacement of the Ph group at C-7 (as in **25a**) with the pyridine moiety (as in **25h**) led to a drop in potency by a factor of 15. Together, these data indicate that the C-7 and, possibly, C-8 aromatic groups bind in a pocket where hydrophobic interactions predominate, and introduction of hydrophilic pyridine nitrogen (**25h**) or hydroxyl groups (**25n**, **25y,z**) leads to an unfavorable loss of solvation energy.

Table 6. Antiproliferative activities of the synthesized 7-deazapurines

7-deaza-purine	cell viability ^a GI ₅₀ , μM		7-deaza-purine	cell viability ^a GI ₅₀ , μM		7-deaza-purine	cell viability ^a GI ₅₀ , μM	
	HeLa	MCF-7		HeLa	MCF-7		HeLa	MCF-7
rigidin A	> 100	> 100	23f	> 100	> 100	25k	7.1 ± 1	10 ± 5
rigidin B	> 100	> 100	24a	20 ± 1	8.0 ± 1	25l	4.9 ± 0.3	2.2 ± 0.1
rigidin C	> 100	> 100	24b	70 ± 15	75 ± 11	25m	0.095 ± 0.040	0.095 ± 0.010
rigidin D	> 100	> 100	24c	9.0 ± 1	7.0 ± 1	25n	0.86 ± 0.06	1.09 ± 0.12
22e	> 100	> 100	24d	18 ± 10	10 ± 2	25o	0.19 ± 0.01	0.25 ± 0.02
22f	> 100	> 100	24e	45 ± 9	51 ± 2	25p	6.3 ± 1	0.80 ± 0.01
22g	> 100	> 100	25a	0.035 ± 0.007	0.040 ± 0.024	25q	>100	7.0 ± 1
22h	> 100	> 100	25b	0.2 ± 0.1	0.150 ± 0.060	25r	0.30 ± 0.07	0.11 ± 0.035
22i	80 ± 10	14 ± 1	25c	0.10 ± 0.07	0.06 ± 0.02	25s	0.40 ± 0.01	0.31 ± 0.01
22j	71 ± 12	47 ± 5	25d	0.13 ± 0.05	0.06 ± 0.02	25t	2.2 ± 1	0.6 ± 0.1
22k	> 100	> 100	25e	0.065 ± 0.010	0.070 ± 0.025	25u	0.045 ± 0.004	0.053 ± 0.005
23a	> 100	> 100	25f	0.15 ± 0.05	0.135 ± 0.075	25v	43 ± 6	56 ± 2
23b	> 100	> 100	25g	0.080 ± 0.025	0.087 ± 0.020	25w	0.4 ± 0.1	0.3 ± 0.07
23c	4.4 ± 0.4	3.9 ± 0.5	25h	0.88 ± 0.06	1.26 ± 0.18	25x	0.30 ± 0.01	0.26 ± 0.05
23d	28 ± 4	15 ± 0	25i	48 ± 8	33 ± 4	25y	5.0 ± 0.6	3.0 ± 0.6
23e	> 100	> 100	25j	8.2 ± 1	40 ± 4	25z	4.0 ± 0.6	2.8 ± 0.8

^a Concentration required to reduce the viability of cells by 50% after a 48 h treatment with the indicated compounds relative to a DMSO control \pm SD from two independent experiments, each performed in 4 replicates, as determined by the MTT assay.

(b) Cell Cycle Analysis

The structural similarities between the rigidin analogues and previously described podophyllotoxin analogues,¹⁹ as well as characteristic cellular morphological changes induced by the 7-deazahypoxanthines, led us to hypothesize that these compounds exert their antiproliferative properties through inhibition of tubulin dynamics, thus inducing mitotic arrest in cancer cells. To test this hypothesis, cell cycle analysis was performed using the cell permeable DNA binding dye Vybrant Orange. In a normal population, cells are distributed among the three major phases of the cell cycle, G₀/G₁, in which there is one set of paired chromosomes per cell (2n), followed by the S or synthesis phase, in which DNA is synthesized in preparation for division, and finally the G₂/M phase, in which the chromosomes of each cell have now been duplicated (4n). The DNA content can be measured by flow cytometry using fluorescent DNA selective probes such as Vybrant Orange that produce signals that are proportional to DNA mass per cell. Cell cycle analysis performed on HeLa cells using **25g** and **25m** with 0.1% dimethyl sulfoxide (DMSO) as a control revealed a pronounced cell cycle arrest in the G₂/M phase (Figure 4), and this effect is characteristic of antimitotic agents disrupting microtubule assembly.³⁸

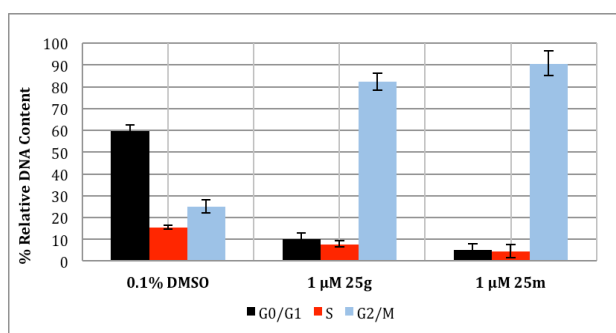


Figure 4. Flow cytometric cell cycle analysis using HeLa cells treated for 32 h with **25g** or **25m**. Error bars represent the standard error between triplicate experiments.

(c) Inhibition of tubulin assembly and colchicine binding to tubulin

To gain further insight into the possible antitubulin mechanism of action of the 7-deazahypoxanthines, the effects of these compounds on *in vitro* tubulin polymerization were assessed.³⁹ In our initial experiment, polymerization was followed by fluorescence enhancement due to the incorporation of a fluorescent reporter, in this case 4',6-diamidino-2-phenylindole (DAPI), into microtubules as polymerization occurs. The test compounds were compared to three different controls, a known microtubule stabilizer (taxol), a known microtubule destabilizer (colchicine) as well as a solvent control sample. Whereas taxol induced potent enhancement of microtubule formation relative to the effect of the DMSO control, 7-deazahypoxanthines **25a** and **25m** displayed potent inhibition of microtubule assembly in a manner similar to the known tubulin polymerization inhibitor colchicine (Figure 5).

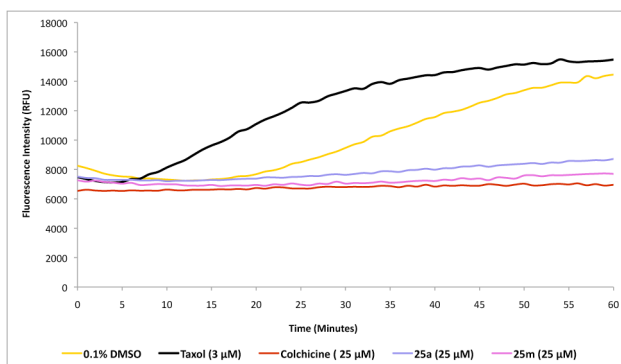


Figure 5. Effect of **25a** and **25m** on *in vitro* tubulin polymerization. Taxol (3 μ M) promotes microtubule formation relative to 0.1% DMSO control. **25a** (25 μ M), **25m** (25 μ M) and colchicine (25 μ M) completely suppress tubulin polymerization.

We next examined **25a** and **25m** in a quantitative turbidimetric assay,⁴⁰ in comparison with combretastatin A-4, a well-described potent inhibitor of the reaction (Table 7).⁴¹ While **25a** differed little from combretastatin A-4 in its inhibitory effect, **25m** was distinctly less active. We also examined **25a** and **25m** for potential inhibitory effects on the binding of [³H]colchicine to tubulin (Table 7).⁴² We found

25a to be almost as active as combretastatin A-4, while **25m** was less active, in accord with their relative activities in the assembly assay.

Table 7. Inhibition of tubulin assembly and colchicine binding to β -tubulin

compound	inhibition of tubulin assembly ^b		inhibition of colchicine binding ^c	
	IC ₅₀ (μ M) \pm SD		% inhibition \pm SD	
			5 μ M inhibitor	1 μ M inhibitor
CA-4^a	0.96 \pm 0.07		99 \pm 0.4	89 \pm 0.6
25a	1.2 \pm 0.1		88 \pm 0.7	58 \pm 4
25m	1.6 \pm 0.04		71 \pm 0.5	ND

^a CA-4 = combretastatin A-4. ^b Inhibition of tubulin polymerization by selected compounds. Tubulin was at 10 μ M. ^c % Inhibition of [³H]colchicine (5 μ M) binding to tubulin (1 μ M) by selected compounds.

There are three well-documented binding domains on tubulin, the first two of which include the pocket for taxanes and epothilones^{43,44} and the pocket for vinca alkaloids.⁴⁵ FDA approved tubulin inhibitors binding to these two domains are all derivatives of complex natural products and are characterized by high bulkiness, hydrophobicity, and complicated structure. In addition, many tend to be easily recognized by P-glycoprotein (P-gp) and pumped out of cancer cells, which causes drug resistance.⁴⁶ The third binding domain of tubulin is the pocket for colchicine and podophyllotoxin, small natural products with relatively simple structures. So far, there are no FDA approved anticancer drugs targeting this domain,⁴⁷ although many colchicine site agents inhibit the growth of multidrug resistant (MDR) tumor cell lines and have reduced neurotoxicity and good oral bioavailability. These properties should be advantageous compared to the currently FDA approved tubulin inhibitors.⁴⁸⁻⁴⁹ The results shown in Table 7 indicate that the 7-deazahypoxanthines, as represented by compounds **25a** and **25m**, constitute a new class of colchicine site agents.

(d) Microtubule organization in cells

To evaluate the effects of 7-deazahypoxanthines on the microtubule cytoskeleton in cells, we cultured HeLa cells in the presence of compounds **25a** and **25m** at a range of concentrations and examined microtubule morphology (Figure 6). At concentrations between 1 and 10 nM, interphase microtubule organization (Figure 6, panels A-C and M-O) was indistinguishable from DMSO controls (not shown). Similarly, mitotic spindle morphology and spindle length was unaffected at concentrations at or below 10 nM for both analogues. However, there was a pronounced change in both interphase and mitotic microtubule organization at concentrations between 10 and 100 nM, where both compounds caused a complete collapse of mitotic microtubule organization (Figure 6, Panel J-L and V-X). This was also the concentration range that encompassed the antiproliferative GI_{50} values for both compounds (GI_{50} (**25a**) = 35 nM, GI_{50} (**25m**) = 95 nM, Table 6). Additionally, while both compounds dramatically affected the polymerization and organization of dynamic mitotic microtubules, **25a** had a more drastic effect on interphase microtubule organization (Figure 6, panels D-F) in comparison with **25m** (Figure 6, Panels P-R), suggesting that **25a** was capable of affecting both dynamic and stable microtubule arrays. Together, these results lend further evidence indicating that tubulin-targeting is likely the main mechanism responsible for the antiproliferative effects of the 7-deazahypoxanthines.

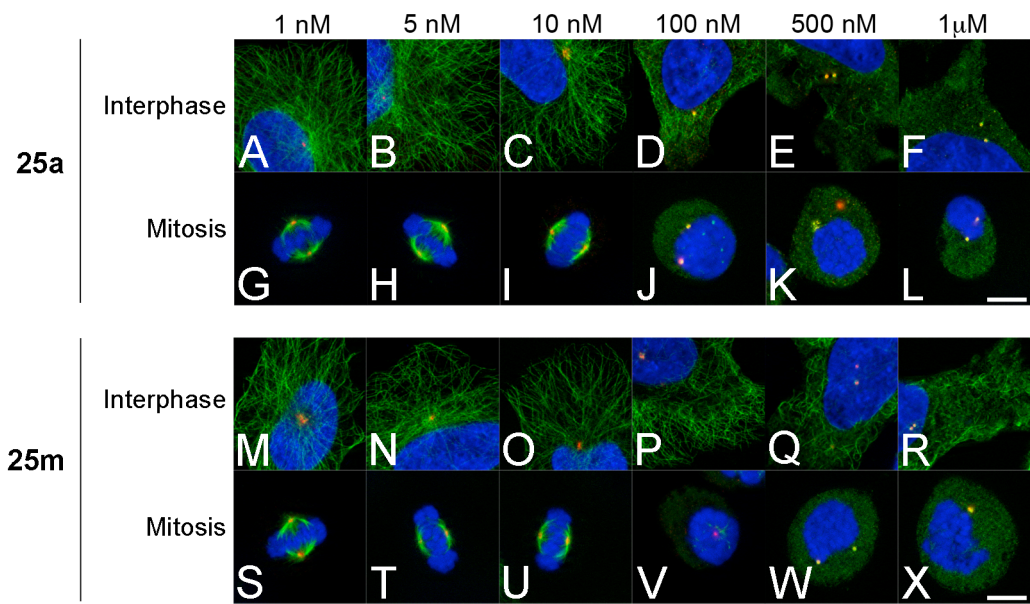


Figure 6. Microtubule organization in HeLa cells during interphase and mitosis. HeLa cells were treated for 3 h with compound **25a** (panels A-L) and **25m** (M-X) at a range of concentrations. Following drug treatment, cells were probed for microtubules (green), the centrosome marker pericentrin (red) and DNA (blue). Bar, 10 μm .

(e) Aqueous Solubility

Because low aqueous solubility is an intrinsic property of many natural and synthetic drug candidates and is usually associated with poor absorption and bioavailability,^{50,51} the water solubilities of the most potent nanomolar compounds **25a**, **25e**, **25g** and **25m** were determined with a UV/vis spectrophotometer at a wavelength of 335-336 nm. The solubility of compounds **25e**, **25g** and **25m** were 65 μM , 30 μM and 40 μM , respectively, while under the same conditions **25a** (10 μM) was less soluble. It appears that the *meta*-halogen substitution present in compounds **25e**, **25g** and **25m** is beneficial for the enhanced water solubility and will be considered for further development of this series of compounds. As a whole, these numbers fall in the range of water solubilities of the other reported colchicine-site targeting anti-tubulin compounds.⁵²

(f) Activity against MDR cells

Because it is known that colchicine site agents are often insensitive to the action of P-gp,⁵³ we tested potent 7-deazahypoxanthines against MDR cells. The MDR uterine sarcoma cell line MES-SA/Dx5 was utilized for this experiment.⁵⁴ This cell line was established from the parent uterine sarcoma MES-SA, grown in the presence of increasing concentrations of doxorubicin and is known to be resistant to a number of P-gp substrates. Both taxol and vinblastine displayed more than a thousand fold drop in potency when tested for antiproliferative activity against the MDR cell line as compared with the parent line (Table 8). In contrast, there was only a small variation in the sensitivities of the two cell lines towards

7-deazahypoxanthines **25a** and **25m**. Of note, the variation in sensitivity was slightly larger for the more lipophilic **25m** than that for **25a**, underscoring the idea that smaller, less lipophilic agents are poorer P-gp substrates.

Table 8. Antiproliferative effect of selected compounds against MDR cells

	GI ₅₀ <i>in vitro</i> Values (nM) ^a	
	MES-SA	MES-SA/Dx5
Taxol	7 ± 1	9800 ± 283
Vinblastine	6 ± 1	5000 ± 1414
25m	81 ± 6	394 ± 10
25a	30 ± 4	70 ± 4

^a Concentration required to reduce the viability of cells by 50% after a 48 h treatment with the indicated compounds relative to a DMSO control ± SD from two independent experiments, each performed in 4 replicates, as determined by the MTT assay.

(g) Activity against cancer cell lines representing cancers with dismal prognoses or derived from tumor metastases

Table 9. Antiproliferative properties of potent 7-deazahypoxanthines **25** against cancer cell lines representing cancers with dismal prognoses or derived from tumor metastases

compound	GI ₅₀ in vitro values (nM) ^a								
	glioma		melanoma		NSCLC	head and neck cancer		head and neck metastases	
	U373	Hs683	SKMEL-28	B16F10	A549	UM-	UM-	UM-	UM-
						SCC10A	SCC22A	SCC10B	SCC22B
25m	30	20	20	50	30	90	110	100	20
25g	30	20	20	40	20	90	120	130	20
25a	<10	10	<10	20	10	ND	ND	ND	ND

^a Concentration required to reduce the viability of cells by 50% after a 48 h treatment with the indicated compounds relative to a DMSO control, as determined by the MTT assay

To study the antiproliferative effects of our potent 7-deazahypoxanthines **25** further, we investigated whether they retained their potency against a panel of cancer cell lines, representing cancers known to be associated with dismal prognoses (Table 9). These include glioma (U373 and Hs683), melanoma (SKMEL-28 and B16F10), non-small-cell lung (NSCLC, A549) and head-and-neck cancers (UM-SCC10A and UM-SCC22A). In addition, because more than 90% of cancer deaths are caused by tumor metastases, two head-and-neck cancer cell lines derived from lymph node metastases (UM-SCC10B and UM-SCC22B) were added to the panel. 7-Deazahypoxanthines **25a**, **25g** and **25m** were found to retain their nanomolar potency against cells in this challenging *in vitro* panel (Table 9). Especially noteworthy are antiproliferative effects of **25a** against human glioma (U373) and melanoma (SKMEL-28) cells, which had single digit nanomolar GI₅₀ values.

Conclusion

The research reported in this paper involves the utilization of the pyrrolo[2,3-*d*]pyrimidine-based natural alkaloid rigidins to discover novel anticancer agents. We described the development of novel synthetic chemistry allowing rapid access to various structural variants of this heterocyclic scaffold,

which led to the generation of a library of diverse synthetic rigidin analogues. Although the natural products themselves were found to have little if any anticancer activity, synthetic analogues based on the 7-deazahypoxanthine skeleton were found to have promising double to single digit nanomolar antiproliferative potencies in human cancer cells. These compounds maintain potency in a panel of human cancer cells including those representing cancers with dismal prognoses as well as cells derived from metastatic lymph node tumors. All evidence obtained to date indicated that these rigidin analogues exert their antiproliferative action by inhibiting microtubule dynamics in cancer cells. Because many microtubule-targeting compounds are successfully used to fight cancer in the clinic, we believe the new chemical class of antitubulin agents represented by the 7-deazahypoxanthine rigidin analogues have significant potential as new anticancer agents.

Experimental Section

General Synthetic Methods

All reagents, solvents and catalysts were purchased from commercial sources (Acros Organics and Sigma-Aldrich) and used without purification. All reactions were performed in oven-dried flasks open to the atmosphere or under nitrogen and monitored by thin layer chromatography (TLC) on TLC precoated (250 μm) silica gel 60 F254 glass-backed plates (EMD Chemicals Inc.). Visualization was accomplished with UV light. Flash column chromatography was performed on silica gel (32-63 μm , 60 Å pore size). ^1H and ^{13}C NMR spectra were recorded on Jeol 300 or Bruker 400 spectrometers. Chemical shifts (δ) are reported in ppm relative to the TMS internal standard. Abbreviations are as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). HRMS analyses were performed at the Mass Spectrometry Facility, University of New Mexico or using Waters Synapt G2 LCMS. The synthesized compounds are at least 95% pure according to UPLC/MS analysis.

General Procedure for the Synthesis of 7-Deazaxanthines (22a-j). A required pyrrole³⁶ (0.3478 mmol) was co-evaporated with 5 mL of toluene and dissolved in 3 mL of diglyme. Nitrogen gas was bubbled through the mixture for 5 min, and 0.20 mL (0.3825 mmol) of oxalyl chloride (2 M in CH₂Cl₂) was added dropwise while vigorously stirring the mixture. The mixture was refluxed for 10 h under nitrogen. After that time, the solvent was evaporated under reduced pressure, and 5 mL of 50% MeOH in CH₂Cl₂ was added to the solid in the flask. The precipitate was removed by filtration and rinsed sequentially with 2 mL of MeOH and 5 mL of diethyl ether. The mother liquor was chromatographed on silica gel using 30/1 to 30/5 CH₂Cl₂/MeOH gradient. The material from filtration and column chromatography was combined to give 7-deazaxanthines **22a-j**.

6-[4-(Benzyloxy)benzoyl]-5-[4-(benzyloxy)phenyl]-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione

(22a). 81% as a brown powder, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 5.02 (s, 2H), 5.06 (s, 2H), 6.69 (d, *J* = 8.8 Hz, 2H), 6.74 (d, *J* = 8.8 Hz, 2H), 7.07 (d, *J* = 8.7 Hz, 2H), 7.29-7.40 (m, 12H), 10.71 (bs, 1H), 11.31 (bs, 1H), 11.94 (bs, 1H). ¹³C NMR (DMSO-*d*₆) δ: 69.8, 70.0, 98.9, 113.8, 114.3, 128.0, 128.1, 128.3, 128.5, 128.9, 129.0, 131.7, 132.8, 136.8, 137.6, 151.3, 158.1, 160.3, 161.2, 185.7. HRMS *m/z* (ESI⁺) calc'd for C₃₃H₂₆N₃O₅ (M+H⁺) 542.1721, found 542.1714.

6-[4-(Benzyloxy)-3-methoxybenzoyl]-5-[4-(benzyloxy)phenyl]-1*H*-pyrrolo[2,3-*d*]pyrimidine-

2,4(3*H*,7*H*)-dione (22b). 74% as a brown powder, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 3.57 (s, 3H), 5.01 (s, 2H), 5.05 (s, 2H), 6.72 (s, 1H), 6.87-7.10 (m, 7H), 7.37 (m, 10H), 10.72 (bs, 1H), 11.27 (bs, 1H), 11.94 (bs, 1H). ¹³C NMR (DMSO-*d*₆) δ: 56.3, 70.3, 71.2, 99.1, 114.0, 114.1, 123.5, 125.4, 125.6, 127.9, 128.0, 128.2, 128.3, 128.9, 131.9, 132.8, 137.4, 137.8, 142.0, 149.2, 150.9, 151.7, 158.5, 160.1, 185.7. HRMS *m/z* (ESI⁺) calc'd for C₃₄H₂₇N₃NaO₆ (M+Na⁺) 596.1798, found 596.1792.

6-[4-(Benzyloxy)benzoyl]-5-[4-(benzyloxy)-3-methoxyphenyl]-1*H*-pyrrolo[2,3-*d*]pyrimidine-

2,4(3*H*,7*H*)-dione (22c). 76% as a yellow powder, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 3.60 (s, 3H), 4.97 (s, 2H), 5.03 (s, 2H), 6.66-6.74 (m, 5H), 7.34-7.38 (m, 12H), 10.70 (bs, 1H), 11.48 (bs, 1H), 11.96 (bs, 1H). ¹³C NMR (DMSO-*d*₆) δ: 55.9, 70.0, 70.6, 98.9, 113.1, 114.2, 116.2, 124.4, 125.5, 125.8, 128.1, 128.3, 128.5, 128.9, 129.0, 129.1, 131.2, 131.6, 137.0, 137.7, 151.3, 160.3, 161.5, 185.8. HRMS *m/z* (ESI⁺) calc'd for C₃₄H₂₇N₃NaO₆ (M+Na⁺) 596.1798, found 596.1804.

6-[4-(Benzyloxy)-3-methoxybenzoyl]-5-[4-(benzyloxy)-3-(methoxy)phenyl]-1*H*-pyrrolo[2,3-

***d*]pyrimidine-2,4(3*H*,7*H*)-dione (22d).** 72% as a yellow powder, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 3.52 (s, 3H), 3.55 (s, 3H), 4.98 (s, 2H), 5.04 (s, 2H), 6.74 (s, 1H), 6.78 (s, 1H), 6.88 (d, *J* = 8.4 Hz, 1H), 6.93 (d, *J* = 1.5 Hz, 1H), 7.10 (dd, *J* = 1.6 Hz, *J* = 8.3 Hz, 1H), 7.31-7.39 (m, 10H), 10.73 (bs, 1H), 11.27 (bs, 1H), 11.96 (bs, 1H). ¹³C NMR (DMSO-*d*₆) δ: 55.6, 55.7, 70.5, 70.7, 98.8, 112.7, 113.2, 123.3, 124.3, 125.5, 125.6, 128.0, 128.1, 128.2, 128.4, 128.8, 128.9, 131.4, 137.0, 137.6, 141.9, 147.7, 148.1, 148.3, 151.1, 151.2, 160.2, 185.7. HRMS *m/z* (ESI⁺) calc'd for C₃₅H₂₉N₃NaO₇ (M+Na⁺) 626.1903, found 626.1908.

6-(4-Fluorobenzoyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione

(22e): 74%, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 3.56 (s, 9H), 6.38 (s, 2H), 6.92 (m, 2H), 7.41 (m, 2H), 10.76 (bs, 1H), 11.32 (bs, 1H), 12.13 (bs, 1H). ¹³C NMR (DMSO-*d*₆) δ: 55.5, 60.3, 99.2, 109.2, 113.8, 114.9, 126.1, 127.6, 130.0, 131.5, 137.2, 151.1, 160.5, 156.3, 185.5. HRMS *m/z* (ESI⁺) calc'd for C₂₂H₁₈FN₃O₆ (M+Na⁺) 462.1077, found 462.1064.

6-(4-Bromobenzoyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione

(22f): 64%, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 3.50 (s, 9H), 6.33 (s, 2H), 7.23 (m, 4H). ¹³C NMR (DMSO-*d*₆) δ: 56.2, 60.7, 99.4, 109.7, 125.1, 125.7, 127.5, 130.7, 130.8, 130.9, 137.7, 138.0, 142.4,

151.1, 151.9, 160.0, 185.6. HRMS m/z (ESI⁺) calc'd for C₂₂H₁₈BrNaN₃O₆ (M+Na⁺) 524.0256, found 524.0259.

6-(4-Benzyloxy-3-methoxybenzoyl)-5-(3,4,5-trimethoxyphenyl)-1H-pyrrolo[2,3-*d*]pyrimidine-

2,4(3*H*,7*H*)-dione (22g): 59%, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ : 3.52 (s, 12H), 4.98 (s, 2H), 6.46 (m, 2H), 6.86 (m, 2H), 7.04 (m, 1H), 7.35 (m, 5H), 10.72 (bs, 1H), 11.25 (bs, 1H), 11.99 (bs, 1H). ¹³C NMR (DMSO-*d*₆) δ : 55.5, 56.0, 70.4, 98.8, 109.6, 112.6, 112.7, 122.9, 125.8, 127.7, 128.0, 128.4, 128.9, 129.1, 131.2, 131.6, 137.0, 137.3, 148.1, 151.0, 151.7, 160.5, 185.8. HRMS m/z (ESI⁺) calc'd for C₃₀H₂₇N₃NaO₈ (M+Na⁺) 580.1696, found 580.1697.

6-(4-Methoxybenzoyl)-5-(4-methoxyphenyl)-1H-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (22h).

76% as a yellow powder, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ : 3.66 (s, 3H), 3.70 (s, 3H), 6.60 (d, J = 8.8 Hz, 2H), 6.67 (d, J = 8.8 Hz, 2H), 7.06 (d, J = 8.8 Hz, 2H), 7.37 (d, J = 8.8 Hz, 2H), 10.70 (bs, 1H), 11.32 (bs, 1H), 11.93 (bs, 1H). ¹³C NMR (DMSO-*d*₆) δ : 55.7, 55.9, 70.0, 99.1, 113.1, 113.6, 125.0, 125.7, 128.9, 130.9, 131.6, 141.9, 151.1, 159.1, 160.2, 162.5, 185.8. HRMS m/z (ESI⁺) calc'd for C₂₁H₁₈N₃O₅ (M+H⁺) 392.1246, found 392.1245.

6-Benzoyl-5-(4-methoxyphenyl)-1H-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (22i). 81% as a

brown powder, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ : 3.63 (s, 3H), 6.57 (d, J = 8.8 Hz, 2H), 7.01-7.13 (m, 5H), 10.73 (bs, 1H), 11.30 (bs, 1H), 11.99 (bs, 1H). ¹³C NMR (DMSO-*d*₆) δ : 55.6, 99.4, 113.0, 124.7, 125.6, 128.1, 129.3, 130.1, 131.6, 132.8, 138.6, 142.3, 151.2, 159.1, 160.2, 186.9. HRMS m/z (ESI⁺) calc'd for C₂₀H₁₅N₃NaO₄ (M+Na⁺) 362.1141, found 362.1141.

6-Benzoyl-5-(3,5-dibromophenyl)-1H-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (22j): 43%, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ 7.14-7.35 (m, 8H), 10.8 (bs, 1H), 11.48 (bs, 1H), 12.37 (bs, 1H). ¹³C NMR (DMSO-*d*₆) δ: 99.3, 121.2, 126.3, 126.7, 128.2, 129.0, 132.1, 133.2, 136.5, 138.4, 142.2, 151.2, 160.1, 186.6. HRMS *m/z* (ESI⁺) calc'd for C₁₉H₁₁Br₂N₃NaO₃ (M+Na⁺) 511.9044, found 511.9052.

6-Benzoyl-5-(3,5-dibromophenyl)-1H-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dithione (22k): 57%, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 7.30-7.47 (m, 10H), 8.63 (bs, 1H). ¹³C NMR (DMSO-*d*₆) δ: 112.3, 120.8, 126.6, 128.1, 129.0, 132.1, 132.4, 133.9, 136.3, 138.1, 171.6, 187.1, 191.5. HRMS *m/z* (ESI⁺) calc'd for C₁₉H₁₂Br₂N₃OS₂ (M+H⁺) 519.8789, found 519.8788.

Synthesis of Rigidins A, B, C and D. A selected benzyl-protected 7-deazaxanthine **22a-d** (0.0735 mmol) and Pd/C (20 mg, 10%) were suspended in DMF (0.3 mL) and MeOH (1 mL). The suspension was degassed three times by the freeze-pump-thaw method at -196 °C and then stirred under a hydrogen balloon for 3 h. After that time, the solvent was removed from the frozen sample by lyophilization, and the crude residue was dissolved in MeOH/CH₂Cl₂ and subjected to silica gel column chromatography (MeOH/CH₂Cl₂/AcOH=10/50/1) to afford pure alkaloids.

6-(4-Hydroxybenzoyl)-5-(4-hydroxyphenyl)-1H-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (Rigidin A). 94.7% as a yellow solid, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 6.46 (d, *J* = 8.5 Hz, 2H), 6.47 (d, *J* = 8.6 Hz, 2H), 6.94 (d, *J* = 8.5 Hz, 2H), 7.29 (d, *J* = 8.6 Hz, 2H), 9.28 (bs, 1H), 10.02 (bs, 1H), 10.63 (bs, 1H), 11.32 (bs, 1H), 11.84 (bs, 1H). ¹³C NMR (CD₃OD) δ: 98.5, 113.6, 114.1, 123.9, 128.5, 129.3, 131.5, 132.3, 156.0, 160.2, 160.8, 162.9, 185.4. HRMS *m/z* (ESI⁺) calc'd for C₁₉H₁₃N₃NaO₅ (M+Na⁺) 386.0753, found 386.0742.

6-(4-Hydroxy-3-methoxybenzoyl)-5-(4-hydroxyphenyl)-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (Rigidin B). 95% as a brown solid, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 3.56 (s, 3H), 6.47 (d, *J* = 8.5 Hz, 2H), 6.57 (d, *J* = 8.2 Hz, 1H), 6.93 (d, *J* = 1.6 Hz, 1H), 6.99-7.01 (m, 3H), 9.30 (bs, 1H), 10.54 (bs, 1H), 11.99 (bs, 1H). ¹³C NMR (DMSO-*d*₆) δ: 55.7, 98.8, 113.7, 114.3, 115.0, 123.6, 124.2, 125.3, 128.8, 129.6, 133.0, 147.0, 150.7, 152.7, 156.9, 160.7, 185.8. HRMS *m/z* (ESI⁺) calc'd for C₂₀H₁₆N₃O₆ (M+H⁺) 394.1039, found 394.1032.

6-(4-Hydroxybenzoyl)-5-(4-hydroxy-3-methoxyphenyl)-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (Rigidin C). 96% as a dark yellow solid, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 3.58 (s, 3H), 6.53 (m, 3H), 6.69-6.76 (m, 2H), 7.33 (d, *J* = 8.5 Hz, 2H), 8.69 (bs, 1H), 9.66 (bs, 1H), 10.21 (bs, 1H). ¹³C NMR (DMSO-*d*₆) δ: 56.2, 98.7, 114.8, 115.6, 116.4, 123.5, 125.5, 129.3, 131.7, 132.1, 135.4, 141.8, 147.1, 148.1, 151.1, 161.1, 185.7. HRMS *m/z* (ESI⁺) calc'd for C₂₀H₁₆N₃O₆ (M+H⁺) 394.1039, found 394.1028.

6-(4-Hydroxy-3-methoxybenzoyl)-5-(4-hydroxy-3-methoxyphenyl)-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (Rigidin D). 93% as a maroon solid, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 3.52 (s, 3H), 3.54 (s, 3H), 6.49 (d, *J* = 8.1 Hz, 1H), 6.58 (d, *J* = 8.2 Hz, 1H), 6.66 (dd, *J* = 1.7 Hz, *J* = 8.1 Hz, 1H), 6.73 (d, *J* = 1.6 Hz, 1H), 6.93 (d, *J* = 1.6 Hz, 1H), 7.05 (dd, *J* = 1.6 Hz, *J* = 8.2 Hz, 1H), 8.89 (bs, 1H), 9.65 (bs, 1H), 10.60 (bs, 1H), 11.83 (bs, 1H). ¹³C NMR (DMSO-*d*₆) δ: 55.8, 55.8, 98.8, 113.7, 114.7, 116.5, 123.7, 124.0, 124.8, 125.3, 128.8, 129.7, 146.3, 146.6, 146.9, 150.8, 160.6, 185.8. HRMS *m/z* (ESI⁺) calc'd for C₁₄H₇N₃NaO₇ (M+Na⁺) 446.0964, found 446.0964.

General procedure for the synthesis of 4-amino-7(*H*)pyrrolo[2,3-*d*]pyrimidines (7-deazaadenines 23a-f). To a solution of *N*-(2-oxo-2-arylethyl)methanesulfonamide (2 mmol), the selected aldehyde (2.6 mmol) and malononitrile (2.6 mmol) in formamide (2 mL) was added anhydrous granulated K₂CO₃ (1.2

mmol) in one portion. The mixture was purged with nitrogen for 5 min and then heated at 100 °C for 24 hours under the nitrogen atmosphere. The formation of the intermediate pyrrole was monitored by TLC. After that, the reaction temperature was increased to 150 °C, and the reaction mixture was heated for 3-6 h. The mixture was cooled to room temperature and poured into water (30 mL). The formed precipitate was collected by filtration and washed with EtOH (2 mL) and diethyl ether (2 mL) to give the desired 7-deazaadenine **23a-f**.

4-Amino-6-benzoyl-5-(3,5-dibromophenyl)-7(*H*)pyrrolo[2,3-*d*]pyrimidine (23a): 58%, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 7.23 (t, *J* = 7.41, 2H), 7.39 (m, 5H), 7.45 (d, *J* = 7.2 Hz, 2H), 8.24 (s, 1H), 12.63 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 101.4, 119.7, 121.9, 127.5, 128.6, 129.2, 131.9, 132.2, 132.3, 137.1, 137.7, 150.9, 155.0, 158.8, 187.7. HRMS *m/z* (ESI⁺) calcd for C₁₉H₁₃Br₂N₄O (M+H⁺) 470.9456, found 470.9455.

4-Amino-6-benzoyl-5-(6-bromopyridin-2-yl)-7(*H*)pyrrolo[2,3-*d*]pyrimidine (23b): 66%, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 6.37 (bs, 2H), 7.20 (m, 2H), 7.38 (m, 3H), 7.79 (s, 1H), 8.23 (s, 1H), 8.32 (s, 1H), 8.42 (s, 1H), 12.66 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 102.2, 118.0, 120.0, 128.3, 129.3, 130.1, 131.5, 132.4, 138.1, 140.5, 149.1, 149.2, 151.6, 155.5, 159.5, 188.1. HRMS *m/z* (ESI⁺) calcd for C₁₈H₁₃BrN₅O (M+H⁺) 394.0303, found 394.0312.

4-Amino-6-benzoyl-5-phenyl-7(*H*)pyrrolo[2,3-*d*]pyrimidine (23c): 48%, mp 284 °C. ¹H NMR (DMSO-*d*₆) δ: 7.13 (m, 2H), 7.29 (m, 5H), 7.31 (m, 1H), 7.45 (d, *J* = 6.4 Hz, 2H), 7.97 (d, *J* = 8.8 Hz, 2H), 8.23 (s, 1H), 12.52 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 101.7, 122.5, 127.5, 127.6, 128.1, 128.9, 130.3, 131.7, 133.5, 137.5, 150.7, 154.9, 158.9, 162.9, 187.8. HRMS *m/z* (ESI⁺) calcd for C₁₉H₁₅N₄O (M+H⁺) 315.1246, found 315.1238.

4-Amino-6-benzoyl-5-(thiophen-2-yl)-7(*H*)pyrrolo[2,3-*d*]pyrimidine (23d): 85%, mp 260 °C. ¹H NMR (DMSO-*d*₆) δ: 6.92 (m, 1H), 7.03 (s, 1H), 7.25 (t, *J* = 6.8 Hz, 2H), 7.4 (m, 3H), 7.58 (d, *J* = 7.4 Hz, 2H), 7.98 (d, *J* = 8.9 Hz, 1H), 8.24 (s, 1H), 12.65 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 102.0, 113.2, 127.5, 127.9, 128.2, 128.8, 129.5, 132.2, 133.6, 137.4, 150.6, 154.9, 158.8, 162.8, 187.7. HRMS *m/z* (ESI) calcd for C₁₇H₁₃N₄OS (M+H⁺) 321.0810, found 321.0801.

4-Amino-6-(3-cyanobenzoyl)-5-(4-benzyloxyphenyl)-7(*H*)pyrrolo[2,3-*d*]pyrimidine (23e): 54%, mp 252 °C. ¹H NMR (DMSO-*d*₆) δ: 5.12 (s, 2H), 6.82 (d, *J* = 8.08 Hz, 2H), 7.4 (m, 7H), 7.47 (d, *J* = 7.3 Hz, 2H), 7.67 (d, *J* = 6.5 Hz, 2H), 8.24 (s, 1H), 12.60 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 69.5, 69.5, 109.8, 114.1, 118.8, 119.6, 127.6, 128.0, 128.5, 129.4, 130.1, 131.3, 131.6, 131.9, 136.4, 138.7, 150.9, 154.5, 158.6, 161.6, 186.3. HRMS *m/z* (ESI⁺) calcd for C₂₇H₂₀N₅O₂ (M+H⁺) 446.1617, found 446.1609.

4-Amino-6-benzoyl-5-(2,6-dichlorophenyl)-7(*H*)pyrrolo[2,3-*d*]pyrimidine (23f): 72%, mp 262 °C. ¹H NMR (DMSO-*d*₆) δ: 7.22 (m, 3H), 7.35 (m, 3H), 7.49 (d, *J* = 6.7 Hz, 2H), 8.22 (s, 1H), 12.69 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 102.5, 116.5, 128.1, 128.4, 128.8, 129.5, 131.4, 131.9, 132.4, 135.7, 138.3, 151.9, 154.3, 155.8, 159.4, 187.7. HRMS *m/z* (ESI⁺) calcd for C₁₉H₁₃Cl₂N₄O (M+H⁺) 383.0466, found 383.0474.

General procedure for the synthesis of 4-aryl-7(*H*)pyrrolo[2,3-*d*]pyrimidines (7-deazapurines 24a-e). To a solution of the selected *N*-(2-oxo-2-arylethyl)methanesulfonamide (3 mmol), the selected arylcyanomethyl ketone (3.9 mmol), the selected aldehyde (3.9 mmol) in formamide (2 mL) was added anhydrous granulated K₂CO₃ (1.8 mmol). The mixture was purged with nitrogen and stirred overnight at 110 °C. The TLC monitoring of the reaction indicated the disappearance of the sulfonamide and the formation of the intermediate pyrrole (bright yellow spot on TLC). The temperature was then raised to 160 °C and the reaction was stirred for 3-6 h under nitrogen. After the pyrrole disappeared (TLC), the reaction mixture was cooled to room temperature and poured in a beaker with water (40 mL). The

precipitate was collected by filtration and purified by column chromatography ($\text{CH}_2\text{Cl}_2/\text{EtOAc}$, 10:1) to yield the desired 7-deazapurine **24a-e**.

6-(4-Methoxybenzoyl)-4-(4-methoxyphenyl)-5-(4-Methoxyphenyl)-7(*H*)pyrrolo[2,3-*d*]pyrimidine

(24a): 63%, mp 224 °C. ^1H NMR ($\text{DMSO-}d_6$) δ : 3.64 (s, 3H), 3.78 (s, 3H), 3.83 (s, 3H), 6.65 (s, 1H), 6.84 (d, $J = 7.9$ Hz, 2H), 6.96 (d, $J = 8.0$ Hz, 2H), 7.03 (d, $J = 7.9$ Hz, 2H), 7.38 (d, $J = 8.0$ Hz, 2H), 7.82 (d, $J = 7.9$ Hz, 2H), 8.13 (d, $J = 7.9$ Hz, 2H), 8.80 (s, 1H); ^{13}C NMR ($\text{DMSO-}d_6$) δ : 54.9, 55.1, 55.3, 113.7, 114.1, 115.4, 125.8, 126.9, 127.8, 129.1, 131.2, 133.3, 134.8, 146.8, 157.0, 158.6, 161.2, 173.7; HRMS m/z (ESI^+) calc'd for $\text{C}_{28}\text{H}_{24}\text{N}_3\text{O}_4$ ($\text{M}+\text{H}^+$) 466.1767, found 466.1761.

(5-(4-Methoxyphenyl)-4-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)(phenyl)methanone (24b): 30%, mp 208 °C. ^1H NMR ($\text{DMSO-}d_6$) δ : 3.56 (s, 3H), 6.37 (d, $J = 6.8$ Hz, 1H), 6.66 (d, $J = 6.8$ Hz, 1H), 7.06 (dd, $J = 8.2$ Hz, 2H), 7.23 (m, 5H), 7.39 (t, $J = 7.4$ Hz, 1H), 7.53 (dd, $J = 7.4$ Hz, 2H), 8.99 (s, 1H); 13.03 (s, 1H). ^{13}C NMR ($\text{DMSO-}d_6$) δ : 55.4, 113.1, 114.5, 120.8, 125.4, 127.5, 128.4, 129.1, 129.7, 129.8, 132.1, 133.1, 137.1, 137.3, 152.14, 153.5, 158.5, 162.3, 189.8. HRMS m/z (ESI^+) calc'd for $\text{C}_{26}\text{H}_{19}\text{N}_3\text{O}_2$ ($\text{M}+\text{H}^+$) 406.1556, found 406.1557.

(5-(3,5-Dibromophenyl)-4-(*p*-tolyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)(phenyl)methanone (24c): 33%, mp 250 °C. ^1H NMR ($\text{DMSO-}d_6$) δ : 2.27 (s, 3H), 6.90 (d, $J = 1.7$ Hz, 2H), 6.93 (d, $J = 7.8$ Hz, 1H), 7.10 (dd, $J = 8.0$ Hz, 2H), 7.25 (dd, $J = 8.0$ Hz, 2H), 7.37 (t, $J = 1.7$ Hz, 1H), 7.45 (t, $J = 7.4$ Hz, 1H), 7.55 (dd, $J = 8.1$ Hz, 2H), 9.01 (s, 1H); 13.25 (s, 1H). ^{13}C NMR ($\text{DMSO-}d_6$) δ : 20.9, 114.0, 118.0, 120.8, 127.8, 128.0, 128.9, 129.2, 131.1, 132.6, 132.9, 133.9, 136.6, 137.0, 138.9, 151.5, 151.5, 162.1, 188.8. HRMS m/z (ESI^+) calc'd for $\text{C}_{26}\text{H}_{18}\text{Br}_2\text{N}_3\text{O}$ ($\text{M}+\text{H}^+$) 545.9817, found 545.9819.

(5-(3,5-Dibromophenyl)-4-phenyl-7H-pyrrolo[2,3-d]pyrimidin-6-yl)(phenyl)methanone (24d): 39%, mp 257 °C. ¹H NMR (DMSO-*d*₆) δ: 6.94 (d, *J* = 1.8 Hz, 2H), 7.13 (dd, *J* = 8.0 Hz, 2H), 7.25 (m, 5H), 7.35 (t, *J* = 1.7 Hz, 1H), 7.45 (t, *J* = 7.4 Hz, 1H), 7.55 (dd, *J* = 8.2 Hz, 2H), 9.03 (s, 1H); 13.29 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 114.4, 118.4, 121.3, 127.7, 128.4, 129.4, 129.6, 130.0, 131.7, 133.0, 133.3, 133.4, 137.1, 137.4, 152.0, 153.9, 162.4, 189.2. HRMS *m/z* (ESI⁺) calc'd for C₂₅H₁₆Br₂N₃O (M+H⁺) 531.9660, found 531.9653.

Phenyl(4-phenyl-5-(pyridin-3-yl)-7H-pyrrolo[2,3-d]pyrimidin-6-yl)methanone (24e): 22%, mp 232 °C. ¹H NMR (DMSO-*d*₆) δ: 6.82 (m, 1H), 7.03 (dd, *J* = 8.0 Hz, 2H), 7.20 (m, 6H), 7.42 (t, *J* = 7.4 Hz, 1H), 7.55 (dd, *J* = 8.2 Hz, 2H), 7.96 (d, *J* = 1.08 Hz, 1H), 8.10 (dd, *J* = 4.8 Hz, 2H), 9.04 (s, 1H); 13.28 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 114.3, 117.2, 121.9, 127.3, 128.1, 129.0, 129.1, 129.2, 129.5, 132.9, 133.2, 132.9, 133.2, 136.6, 136.7, 137.5, 147.3, 150.5, 151.8, 153.4, 162.0, 188.9. HRMS *m/z* (ESI⁺) calc'd for C₂₄H₁₇N₄O (M+H⁺) 377.1402, found 377.1403.

General procedure for the synthesis of 1H-pyrrolo[2,3-d]pyrimidin-4(7H)-ones (7-deazahypoxanthines 25a-v). To a solution of *N*-(2-oxo-2-arylethyl)methanesulfonamide (0.676 mmol), the selected aldehyde (0.879 mmol) and cyanoacetamide (0.072 g, 0.879 mmol) in a mixture of EtOH (2.5 mL) and HC(OEt)₃ (2.5 mL) was added anhydrous granulated K₂CO₃ (0.052g, 0.372 mmol) in one portion. The mixture was purged with nitrogen for 5 min and then heated at 90 °C for 24 hours under the nitrogen atmosphere. The formation of the intermediate pyrrole was monitored by TLC. After that the reaction temperature was increased to 150 °C, and the reaction mixture was heated for 3-6 h. The mixture was cooled to room temperature, and the formed precipitate was collected by filtration and washed with EtOH (2 mL) and diethyl ether (2 mL) to give the desired 7-deazahypoxanthine **25a-v**. An additional amount of the product was obtained by the evaporation of the mother liquor and purification of the residue by column chromatography with MeOH/CH₂Cl₂=1/40 to 1/20 gradient.

6-Benzoyl-5-phenyl-1H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (25a): 71%, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 7.04 (m, 3H), 7.15 (m, 4H), 7.33 (t, *J* = 6.8 Hz, 1H), 7.43 (d, *J* = 7.12 Hz, 2H), 8.02 (s, 1H), 12.01 (s, 1H), 12.79 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 106.3, 126.6, 126.7, 126.8, 127.5, 127.7, 129.0, 131.1, 131.8, 132.4, 137.4, 146.7, 149.3, 158.6, 187.7. HRMS *m/z* (ESI⁺) calcd for C₁₉H₁₄N₃O₂ (M+H⁺) 316.1086, found 316.1087.

6-Benzoyl-5-(3-iodophenyl)-1H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (25b): 68%, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 6.87 (t, *J* = 7.52 Hz, 1H), 7.19 (t, *J* = 7.44 Hz, 2H), 7.25 (d, *J* = 7.52 Hz, 1H), 7.42 (m, 5H), 8.04 (s, 1H), 12.90 (s, 1H), 12.09 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 93.0, 106.3, 125.0, 127.8, 128.7, 128.8, 130.4, 131.9, 134.6, 135.2, 137.4, 139.5, 146.9, 149.4, 158.5, 187.6. HRMS *m/z* (ESI⁺) calcd for C₁₉H₁₃IN₃O₂ (M+H⁺) 443.0086, found 443.0080.

6-Benzoyl-5-(3-chlorophenyl)-1H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (25c): 61%, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 7.04 (t, *J* = 7.48 Hz, 1H), 7.11 (d, *J* = 7.52 Hz, 2H), 7.19 (m, 3H), 7.38 (t, *J* = 6.2 Hz, 1H), 7.46 (d, *J* = 7.4 Hz, 2H), 8.04 (s, 1H), 12.05 (s, 1H), 13.00 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 106.4, 125.0, 126.5, 127.7, 128.5, 128.9, 129.7, 130.7, 131.5, 132.0, 134.6, 137.5, 146.8, 149.4, 158.5, 187.6. HRMS *m/z* (ESI⁺) calcd for C₁₉H₁₃ClN₃O₂ (M+H⁺) 350.0696, found 350.0694.

6-Benzoyl-5-(3-bromophenyl)-1H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (25d): 54%, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 7.00 (t, *J* = 7.08 Hz, 1H), 7.19 (m, 3H), 7.25 (d, *J* = 7.76 Hz, 1H), 7.34 (s, 1H), 7.38 (t, *J* = 6.96 Hz, 1H), 7.45 (d, *J* = 7.32 Hz, 2H), 8.04 (s, 1H), 12.05 (s, 1H), 12.90 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 106.4, 120.1, 124.9, 127.7, 127.8, 128.8, 128.9, 129.4, 132.0, 130.1, 133.6, 134.8,

137.4, 146.9, 149.3, 158.5, 187.5. HRMS m/z (ESI⁺) calcd for C₁₉H₁₃BrN₃O₂ (M+H⁺) 394.0191, found 394.0183.

6-Benzoyl-5-(3-fluorophenyl)-1H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (25e): 53%, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 7.08-6.88 (m, 2H), 7.19 (m, 1H), 7.36 (m, 1H), 7.46 (m, 2H), 7.61 (d, *J* = 7.2 Hz, 1H), 7.96 (d, *J* = 11.5 Hz, 1H), 8.01 (d, *J* = 7.2 Hz, 1H), 12.30 (s, 1H), 12.96 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 105.6, 114.1, 114.9, 115.1, 118.3, 124.5, 127.8, 129.0, 129.5, 131.2, 131.3, 132.5, 137.9, 159.8, 188.0. HRMS m/z (ESI⁺) calcd for C₁₉H₁₃FN₃O₂ (M+H⁺) 334.0992, found 395.0999.

6-Benzoyl-5-(6-bromopyridin-2-yl)-1H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (25f): 60%, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 7.19 (d, *J* = 8.0 Hz, 2H), 7.23 (d, *J* = 7.2 Hz, 2H), 7.40 (t, *J* = 6.4 Hz, 1H), 7.49 (d, *J* = 7.6 Hz, 2H), 7.61 (t, *J* = 7.6 Hz, 1H), 8.03 (s, 1H), 8.09 (d, *J* = 8.0 Hz, 1H), 12.17 (s, 1H), 13.05 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 105.5, 122.1, 124.6, 125.4, 128.0, 128.5, 129.7, 132.2, 137.8, 138.7, 139.3, 146.8, 149.3, 152.7, 158.8, 188.5. HRMS m/z (ESI⁺) calcd for C₁₈H₁₂BrN₄O₂ (M+H⁺) 395.0144, found 395.0125.

6-Benzoyl-5-(5-bromopyridin-3-yl)-1H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (25g): 88%, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 7.21 (d, *J* = 7.2 Hz, 2H), 7.38 (t, *J* = 6.4 Hz, 1H), 7.46 (d, *J* = 6.4 Hz, 2H), 7.81 (s, 1H), 8.05 (s, 1H), 8.31 (s, 1H), 8.35 (s, 1H), 12.12 (s, 1H), 13.01 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 107.3, 119.0, 121.9, 128.4, 129.1, 129.6, 131.1, 132.6, 138.0, 140.8, 147.6, 148.3, 150.0, 150.2, 159.2, 187.7. HRMS m/z (ESI⁺) calcd for C₁₈H₁₁BrN₄NaO₂ (M+Na⁺) 416.9963, found 416.9965.

6-Benzoyl-5-(pyridin-3-yl)-1H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (25h): 61%, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 7.08 (m, 1H), 7.18 (t, *J* = 7.24 Hz, 2H), 7.36 (t, *J* = 6.88 Hz, 1H), 7.46 (d, *J* =

7.12 Hz, 2H), 7.60 (d, $J = 7.72$ Hz, 1H), 8.06 (s, 1H), 8.23 (m, 1H), 8.31 (s, 1H), 12.12 (s, 1H), 13.00 (s, 1H). ^{13}C NMR (DMSO- d_6) δ : 106.7, 121.9, 123.0, 127.8, 127.9, 128.6, 129.2, 132.0, 137.3, 138.0, 147.0, 147.3, 149.5, 150.8, 158.6, 187.3. HRMS m/z (ESI $^+$) calcd for $\text{C}_{18}\text{H}_{13}\text{N}_4\text{O}_2$ ($\text{M}+\text{H}^+$) 317.1039, found 317.1038.

6-Benzoyl-5-(3,4,5-trimethoxyphenyl)-1H-pyrrolo[2,3-*d*]pyrimidin-4(7H)-one (25i): 64%, mp > 300 °C decomp., ^1H NMR (DMSO- d_6) δ : 3.32 (s, 3H), 3.54 (s, 3H), 3.56 (s, 3H), 6.46 (s, 2H), 7.16 (t, $J = 8.0$ Hz, 2H), 7.32 (t, $J = 8.0$ Hz, 1H), 7.44 (d, $J = 7.7$ Hz, 2H), 8.02 (m, 1H), 11.98 (s, 1H), 12.78 (s, 1H). ^{13}C NMR (DMSO- d_6) δ : 55.6, 59.7, 106.3, 109.4, 127.1, 127.5, 127.7, 128.8, 131.6, 137.8, 146.8, 149.4, 151.3, 158.5, 187.7. HRMS m/z (ESI $^+$) calcd for $\text{C}_{22}\text{H}_{20}\text{N}_3\text{O}_5$ ($\text{M}+\text{H}^+$) 406.1403, found 406.1390.

6-Benzoyl-5-(3-iodo-4,5-dimethoxyphenyl)-1H-pyrrolo[2,3-*d*]pyrimidin-4(7H)-one (25j): 37%, mp > 300 °C decomp. ^1H NMR (DMSO- d_6) δ : 3.58 (s, 6H), 6.82 (s, 1H), 7.07 (s, 1H), 7.16 (d, $J = 7.6$ Hz, 2H), 7.33 (t, 1H), 7.40 (d, $J = 7.2$ Hz, 2H), 8.02 (s, 1H), 12.00 (s, 1H), 12.85 (s, 1H). ^{13}C NMR (DMSO- d_6) δ : 56.1, 60.0, 91.3, 106.8, 117.2, 125.7, 128.1, 128.3, 129.2, 130.9, 132.2, 133.2, 138.2, 147.4, 147.6, 149.9, 150.9, 159.0, 188.1. HRMS m/z (ESI $^+$) calcd for $\text{C}_{21}\text{H}_{17}\text{IN}_3\text{O}_4$ ($\text{M}+\text{H}^+$) 503.0297, found 503.0280.

6-Benzoyl-5-(3-bromo-4,5-dimethoxyphenyl)-1H-pyrrolo[2,3-*d*]pyrimidin-4(7H)-one (25k): 51%, mp > 300 °C decomp. ^1H NMR (DMSO- d_6) δ : 3.59 (s, 6H), 6.77 (s, 1H), 6.95 (s, 1H), 7.16 (d, $J = 7.2$ Hz, 2H), 7.34 (t, 1H), 7.41 (d, $J = 7.2$ Hz, 2H), 8.04 (s, 1H), 12.01 (s, 1H), 12.87 (s, 1H). ^{13}C NMR (DMSO- d_6) δ : 56.3, 60.2, 106.9, 115.5, 116.3, 125.8, 127.4, 128.0, 128.2, 129.2, 130.2, 132.2, 138.2, 145.0, 147.5, 150.0, 152.0, 159.0, 188.0. HRMS m/z (ESI $^+$) calcd for $\text{C}_{21}\text{H}_{17}\text{BrN}_3\text{O}_4$ ($\text{M}+\text{H}^+$) 456.0382, found 456.0370.

6-Benzoyl-5-(2,6-dichlorophenyl)-1H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (25l): 74%, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 7.12 (d, *J* = 6.8 Hz, 2H), 7.20 (m, 3H), 7.34 (m, 1H), 7.47 (d, *J* = 6.0 Hz, 2H), 8.02 (s, 1H), 12.05 (s, 1H), 13.01 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 107.6, 120.2, 127.4, 127.5, 127.9, 128.2, 130.0, 132.0, 132.2, 135.3, 137.8, 147.6, 150.0, 158.0, 187.1. HRMS *m/z* (ESI) calcd for C₁₉H₁₂Cl₂N₃O₂ (M+H⁺) 384.0307, found 384.0299.

6-Benzoyl-5-(3,5-dibromophenyl)-1H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (25m): 77%, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 7.23 (t, *J* = 7.24 Hz, 2H), 7.36 (s, 2H), 7.41 (t, *J* = 7.68 Hz, 1H), 7.46 (d, *J* = 7.68 Hz, 2H), 7.51 (s, 1H), 8.06 (s, 1H), 12.10 (s, 1H), 13.01 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 107.0, 107.1, 124.0, 128.3, 128.6, 129.3, 131.9, 132.6, 133.3, 137.0, 138.0, 147.6, 149.9, 159.0, 187.9. HRMS *m/z* (ESI⁺) calcd for C₁₉H₁₂Br₂N₃O₂ (M+H⁺) 471.9296, found 471.9301.

6-Benzoyl-5-(3-hydroxyphenyl)-1H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (25n): 26%, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 6.43 (s, 1H), 6.51 (s, 1H), 6.65 (s, 1H), 6.76 (s, 1H), 7.17 (s, 2H), 7.34 (s, 1H), 7.45 (s, 2H), 7.98 (s, 1H), 9.08 (s, 1H), 11.97 (s, 1H), 12.72 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 106.7, 114.2, 118.6, 122.6, 127.0, 128.0, 128.2, 129.4, 132.4, 134.1, 138.0, 147.1, 149.7, 156.5, 159.0, 188.4. HRMS *m/z* (ESI⁺) calcd for C₁₉H₁₄N₃O₃ (M+H⁺) 332.1035, found 332.1032.

3-(6-Benzoyl-4-oxo-4,7-dihydro-1H-pyrrolo[2,3-d]pyrimidin-5-yl)benzonitrile (25o): 47%, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 7.19 (t, *J* = 7.64 Hz, 1H), 7.26 (t, *J* = 7.2 Hz, 1H), 7.38 (t, *J* = 6.64 Hz, 1H), 7.44 (d, *J* = 6.0 Hz, 2H), 7.51 (d, *J* = 6.96 Hz, 2H), 7.59 (s, 1H), 8.06 (s, 1H), 12.11 (s, 1H), 12.99 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 106.5, 109.9, 118.6, 118.6, 127.8, 128.0, 129.0, 130.2, 131.9, 133.8, 134.5, 135.8, 137.4, 147.0, 149.4, 158.6, 187.3. HRMS *m/z* (ESI⁺) calcd for C₂₀H₁₃N₄O₂ (M+H⁺) 341.1039, found 341.1041.

5-(3,5-Dibromophenyl)-6-(4-fluorobenzoyl)-1H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (25p): 56%, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 7.03 (t, *J* = 8.0 Hz, 2H), 7.35 (s, 2H), 7.52 (t, *J* = 6.4 Hz, 2H), 7.56 (s, 1H), 8.05 (s, 1H), 12.11 (s, 1H), 13.02 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 80.3, 107.1, 115.5, 121.3, 124.4, 128.7, 132.0, 133.5, 134.9, 137.1, 147.9, 150.3, 159.4, 163.2, 166.1, 186.4. HRMS *m/z* (ESI⁺) calcd for C₁₉H₁₁Br₂FN₃O₂ (M+H⁺) 489.9202, found 489.9182.

6-(4-Methoxybenzoyl)-5-(3,4,5-trimethoxyphenyl)-1H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (25q): 56%, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 3.32 (s, 3H), 3.56 (s, 6H), 3.71 (s, 3H), 6.51 (s, 2H), 6.71 (d, *J* = 9.6 Hz, 2H), 7.46 (d, *J* = 9.6 Hz, 2H), 8.01 (s, 1H), 11.97 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 56.0, 56.3, 60.4, 106.5, 110.0, 113.6, 126.4, 128.4, 131.9, 136.6, 146.9, 149.8, 152.1, 159.3, 162.8, 187.0. HRMS *m/z* (ESI⁺) calcd for C₂₃H₂₂N₃O₆ (M+H⁺) 436.1509, found 436.1501.

5-(3,5-Dibromophenyl)-6-(4-methoxybenzoyl)-1H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (25r): 60%, mp > 300 °C decomp., ¹H NMR (DMSO-*d*₆) δ: 3.77 (s, 3H), 6.79 (d, *J* = 8.36 Hz, 2H), 7.39 (s, 2H), 7.50 (d, *J* = 8.36 Hz, 2H), 7.57 (s, 1H), 8.04 (s, 1H), 12.01 (s, 1H), 12.92 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 55.5, 106.1, 113.3, 120.8, 122.3, 128.5, 129.9, 131.2, 131.4, 132.7, 136.6, 146.7, 149.1, 158.5, 162.5, 186.1. HRMS *m/z* (ESI⁺) calcd for C₂₀H₁₃Br₂N₃NaO₃ (M+Na⁺) 523.9221, found 523.9221.

6-(4-(Benzyloxy)benzoyl)-5-(3,5-dibromophenyl)-1H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (25s): 61%, mp 252 °C. ¹H NMR (DMSO-*d*₆) δ: 5.14 (s, 2H), 6.86 (d, *J* = 7.92 Hz, 2H), 7.36 (m, 3H), 7.41 (m, 4H), 7.56 (m, 3H), 8.04 (s, 1H), 12.15 (s, 1H), 12.95 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 69.4, 106.1, 106.2, 120.8, 122.3, 127.5, 127.9, 128.5, 130.0, 131.2, 131.4, 132.7, 136.5, 136.6, 146.7, 149.1, 158.5, 161.6, 186.0. HRMS *m/z* (ESI⁺) calcd for C₂₆H₁₇Br₂N₃NaO₃ (M+Na⁺) 599.9534, found 599.9532.

6-(4-Bromobenzoyl)-5-(3,5-dibromophenyl)-1H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (25t): 54%, mp 256 °C. ¹H NMR (DMSO-*d*₆) δ: 7.03 (d, *J* = 8.66 Hz, 2H), 7.36 (s, 2H), 7.52 (d, *J* = 8.06 Hz, 2H), 7.56 (s, 1H), 8.04 (s, 1H), 12.08 (s, 1H), 13.00 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 107.3, 114.9, 115.2, 121.2, 124.3, 131.4, 132.7, 132.9, 133.6, 135.4, 159.1, 163.2, 165.7, 186.1. HRMS *m/z* (ESI⁺) calcd for C₁₉H₁₁Br₃N₃O₂ (M+H⁺) 549.8401, found 549.8395.

5-Benzoyl-6-(4-benzyloxyphenyl)-1H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (25u): 68%, mp 246 °C. ¹H NMR (DMSO-*d*₆) δ: 5.07 (s, 1H), 6.77 (d, *J* = 8.3 Hz, 2H), 7.06 (m, 3H), 7.20 (m, 2H), 7.38 (m, 4H), 7.45 (d, *J* = 8.3 Hz, 2H), 7.98 (d, *J* = 3.7 Hz, 1H), 11.97 (s, 1H), 12.71 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 69.7, 106.6, 114.5, 125.8, 127.0, 127.4, 128.1, 128.2, 128.4, 128.9, 130.5, 131.5, 132.0, 133.1, 136.9, 146.8, 149.5, 159.1, 161.9, 186.9. HRMS *m/z* (ESI⁺) calcd for C₂₆H₂₀N₃O₃ (M+H⁺) 422.1505, found 422.1502.

6-Benzoyl-5-(4-bromothiophen-2-yl)-1H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (25v): 71%, mp 300 °C decomp., ¹H NMR (DMSO-*d*₆) δ: 6.95 (s, 1H), 7.33 (m, 2H), 7.48 (m, 2H), 7.58 (d, *J* = 6.9 Hz, 2H), 8.04 (s, 1H), 12.11 (s, 1H), 12.99 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 79.6, 79.6, 108.0, 117.0, 125.0, 128.5, 129.0, 129.3, 132.2, 133.0, 135.2, 137.9, 147.5, 149.8, 158.9, 187.9. HRMS *m/z* (ESI⁺) calcd for C₁₇H₁₀BrN₃NaO₂S (M+Na⁺) 421.9575, found 421.9568.

General procedure for the synthesis of 5-aryl-6-(4-hydroxybenzoyl)-1H-pyrrolo[2,3-d]pyrimidin-4(7H)-ones (7-deazahypoxanthines 25w-z). To a solution of *N*-(2-oxo-2-arylethyl)methanesulfonamide (2 mmol), the selected aldehyde (2.6 mmol) and 2-cyanoacetamide (2.6 mmol) in ethanol (2 mL) was added anhydrous granulated K₂CO₃ (1.2 mmol) in one portion. The mixture was purged with nitrogen for 5 min and then refluxed for 24 hours under the nitrogen atmosphere. The formation of the intermediate pyrrole was monitored by TLC. After that, ethanol was evaporated under reduced pressure. To the dry

residue, formic acid (4 mL) was added, and the mixture was refluxed for 24 hours. The formation of the 7-deazahypoxanthines **25w-z** was monitored by TLC. The mixture was cooled to room temperature and purified by flash chromatography to give the desired product.

6-(4-Hydroxybenzoyl)-5-phenyl-1H-pyrrolo[2,3-*d*]pyrimidin-4(7H)-one (25w): 88%, mp > 300 °C decomp., ¹H NMR (DMSO-*d*₆) δ: 6.52 (d, *J* = 7.88 Hz, 2H), 7.11 (m, 3H), 7.22 (m, 2H), 7.40 (d, *J* = 7.4 Hz, 2H), 7.99 (s, 1H), 10.17 (br.s, 1H), 11.93 (s, 1H), 12.66 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 106.0, 114.6, 124.6, 126.5, 126.9, 127.9, 128.3, 131.0, 131.9, 132.7, 146.2, 148.9, 158.8, 161.5, 186.7. HRMS *m/z* (ESI⁺) calcd for C₁₉H₁₃KN₃O₃ (M+K⁺) 370.0594, found 370.0595.

5-(3,5-Dibromophenyl)-6-(4-hydroxybenzoyl)-1H-pyrrolo[2,3-*d*]pyrimidin-4(7H)-one (25x): 70%, mp > 300 °C decomp. ¹H NMR (DMSO-*d*₆) δ: 7.42 (m, 4H), 7.57 (s, 1H), 7.61 (d, *J* = 7.8 Hz, 2H), 8.03 (s, 1H), 10.26 (s, 1H), 12.06 (s, 1H), 12.88 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 106.4, 115.2, 121.3, 122.0, 128.7, 129.1, 131.6, 132.3, 133.2, 137.2, 147.0, 149.4, 159.0, 162.2. HRMS *m/z* (ESI⁺) calcd for C₁₉H₁₂Br₂N₃O₃ (M+H⁺) 489.9225, found 489.9220.

6-(4-Hydroxybenzoyl)-5-(4-hydroxy-3-methoxyphenyl)-1H-pyrrolo[2,3-*d*]pyrimidin-4(7H)-one (25y): 64%, mp > 300 °C decomp., ¹H NMR (DMSO-*d*₆) δ: 3.54 (s, 3H), 6.76-6.47 (m, 5H), 7.39 (d, *J* = 8.1 Hz, 2H), 8.81 (s, 1H), 7.95 (s, 1H), 10.12 (s, 1H), 11.88 (s, 1H), 12.52 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ: 121.9, 115.7, 115.1, 114.4, 128.8, 134.4, 141.3, 146.6, 149.9, 154.4, 157.1, 159.8, 162.5, 168.1, 187.6. HRMS *m/z* (ESI⁺) calcd for C₂₀H₁₅KN₃O₅ (M+K⁺) 400.0909, found 400.0917.

6-(4-Hydroxybenzoyl)-5-(4-hydroxyphenyl)-1H-pyrrolo[2,3-*d*]pyrimidin-4(7H)-one (25z): 71%, mp > 300 °C decomp., ¹H NMR (DMSO-*d*₆) δ: 6.46 (d, *J* = 7.9 Hz, 2H), 6.51 (d, *J* = 7.9 Hz, 2H), 7.00 (d, *J* = 7.7 Hz, 2H), 7.37 (d, *J* = 7.7 Hz, 2H), 7.94 (s, 1H), 9.24 (s, 1H), 10.13 (s, 1H), 11.89 (s, 1H),

12.51 (s, 1H). ¹³C NMR (DMSO-d₆)
δ: 106.3, 114.3, 115.0, 123.8, 125.6, 127.8, 128.8, 129.0, 132.4, 132.7, 146.5, 149.3, 156.7, 159.2, 161.7,
186.0. HRMS *m/z* (ESI⁺) calcd for C₁₉H₁₄N₃O₄ (M+H⁺) 348.0984, found 348.0987

Cell Culture

Human cancer cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA), the European Collection of Cell Culture (ECACC, Salisbury, UK) and the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). The origin and histological type of each human cell line analyzed are as follows: glioma model lines were the Hs683 oligodendroglioma (ATCC code HTB-138) and the U373 glioblastoma (ECACC code 89081403) cell lines, melanoma model was the SKMEL-28 (ATCC code HTB-72) cell line, and carcinoma model was the A549 NSCLC (DSMZ code ACC107) cell line. Human cervical adenocarcinoma HeLa cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS). Human mammary carcinoma MCF-7 cells were cultured in DMEM supplemented with 10% FBS. Human uterine sarcoma MES-SA and MES-SA/Dx5 cells were cultured in RPMI-1640 medium supplemented with 10% FBS. The U373 cells were cultured in MEM culture medium (Lonza code 12-136F, Vervier, Belgium), while the Hs683, SKMEL-28 and A549 cells were cultured in RPMI culture medium (Lonza; code 12-115F) supplemented with 10% heat-inactivated FBS (Lonza, FBS South America code DE14-801F). Cell culture media were supplemented with 4 mM glutamine (Lonza code BE17-605E), 100 µg/mL gentamicin (Lonza code 17-5182), and penicillin-streptomycin (200 units/ml and 200 µg/ml) (Lonza code 17-602E). All cell lines were cultured in T25 flasks, maintained and grown at 37° C, 95% humidity, 5% CO₂.

Human head and neck cancer cell lines UMSCC10A, 10B, 22A and 22B were provided by Dr. Thomas Carey of University of Michigan under the MTA agreement with the University of Colorado. UMSCC10A and 22A were originated from the primary tumors of two individual head and neck

squamous cell carcinoma patients, and 10B and 22B were from their corresponding lymph node metastases. Cells were cultured in DMEM medium supplemented with 10% FBS, and antibiotics.

Antiproliferative Properties

To evaluate antiproliferative properties of the rigidins and rigidin analogues, the MTT assay was used. The HeLa, MCF-7, MES-SA, and MES-SA/Dx5 cell lines were assessed by trypsinizing each cell line and seeding 4×10^3 cells per well into microtiter plates. All compounds were dissolved in DMSO at a concentration of either 100 mM or 25 mM prior to cell treatment. The cells were grown for 24 h before treatment at concentrations ranging from 0.004 to 100 μ M and incubated for 48 h in 200 μ L media. 20 μ L of MTT reagent in serum free medium (5 mg/mL) was added to each well and incubated further for 2 h. Media was removed, and the resulting formazan crystals were re-solubilized in 100 μ L of DMSO. A_{490} was measured using a Thermomax Molecular Device plate reader. The experiments were performed in quadruplicate and repeated at least twice for each compound per cell line. Cells treated with 0.1% DMSO were used as a control, and phenyl arsine oxide (PAO) was used as a positive killing control.

For UMSCC-10A, 10B, 22A and 22B cells, the MTT assay was performed in a 24-well plate with 40000 cells in 500 μ L media. The cells were grown for 24 h before treatment at concentrations ranging from 0.025 to 0.4 μ M and incubated for additional 4 days. MTT was added for 3 h before harvest. A_{560} was measured using a BioTek plate reader. The experiments were performed in quadruplicate and repeated at least twice for each compound per cell line. Cells treated with 0.1% DMSO were used as a control.

Cell Cycle Analysis

To assess the effects of the analogues on the cell cycle, cell cycle analysis was performed by flow cytometry. 70-80 % confluent HeLa cells were trypsinized and seeded at an initial density of 2×10^5 cell per well into 6 well plates in DMEM supplemented with 10% FBS, 100 mg/L penicillin G and 100 mg/L

streptomycin. The cells were allowed to adhere overnight before treatment with **25g** or **25m** at 1 μ M. After 32 h, the cells were washed 3x with DMEM, trypsinized, pelleted, and re-suspended in 200 μ L of culture medium containing 10 μ M (2 μ L/mL) Vybrant Orange dye. The samples in the labeling solution were transferred into Falcon tubes and incubated in a water bath for 30 min at 37 °C. The samples were then analyzed using a Becton Dickinson FACscan flow cytometer with CellQuest software. Cells treated with 0.1% DMSO were used as a control.

***In Vitro* Tubulin Polymerization Assay**

To determine whether the compounds bind to tubulin, initial experiments were performed with the tubulin polymerization assay obtained from Cytoskeleton Inc. A 10x stock solution of each test compound (0.1% DMSO, taxol, colchicine, **25a** and **25m**) was prepared using molecular grade water. The tubulin reaction mix was prepared by mixing 243 μ L of buffer 1 [80 mM PIPES sequisodium salt; 2.0 mM MgCl_2 ; 0.5 mM ethylene glycol-bis(2-aminoethyl ether)-N,N,N',N'-tetraacetic acid, pH 6.9, 10 μ M DAPI], 112 μ L tubulin glycerol buffer (80 mM PIPES sequisodium salt; 2.0 mM MgCl_2 ; 0.5 mM ethylene glycol-bis(2-aminoethyl ether)-N,N,N',N'-tetraacetic acid, 60% v/v glycerol, pH 6.9), 1 mM GTP (final concentration) and 2 mg/mL tubulin protein (final concentration). The reaction mixture was kept on ice until needed, but it was used within an hour of preparation. The 10x test compounds were pipetted into the corresponding wells and warmed in the plate reader for 1 min, after which time they were diluted with the reaction mixture to their final 1x concentrations and placed in the fluorescent plate reader. The test compounds were incubated with the tubulin reaction mixture at 37 °C each in a separate experiment. The effect of each agent on tubulin polymerization was monitored in a temperature-controlled TECAN GENios Multifunction Fluorescence TRF, FI, FRET, Absorbance and Luminescence Microplate Reader for one hour, with readings acquired every 60 s.

Quantitative Effects on Tubulin Polymerization and on Colchicine Binding to Tubulin.

To evaluate the quantitative effect of the compounds on tubulin assembly in vitro, varying concentrations of compounds were preincubated with 10 μ M (1.0 mg/mL) bovine brain tubulin in 0.8 M monosodium glutamate (pH 6.6 in 2M stock solution) at 30 °C for 15 min and then cooled to 0 °C. After addition of 0.4 mM GTP, the mixtures were transferred to 0 °C cuvettes in recording spectrophotometers equipped with electronic temperature controller and rapidly (less than one minute) warmed to 30 °C. Tubulin assembly was followed turbidimetrically at 350 nm. The IC₅₀ was defined as the compound concentration that inhibited the extent of assembly by 50% after a 20 min incubation. The methodology was described in detail previously.⁴⁰ The capacity of the test compounds to inhibit colchicine binding to tubulin was measured as described.⁵⁵ The reaction mixtures contained 1 μ M tubulin, 5 μ M [³H]colchicine, and 5 or 1 μ M test compound. Combretastatin A-4 was generously provided by Dr. G. A. Pettit, Arizona State University.

Morphological Analysis of Microtubule Organization in HeLa Cells

HeLa cells were cultured in EMEM (Lonza, Walkersville, MD) supplemented with 10% FBS (Atlanta Biologicals, Lawrenceville, GA), sodium pyruvate and sodium bicarbonate. For compound treatments, cells were treated for 3 h with either carrier (0.1% DMSO) or compounds solubilized in DMSO and prediluted in media prior to fixation by immersion in methanol at –20°C for 30 min. Cells were subsequently rehydrated in phosphate-buffered saline (1XPBS), and blocked by incubation in 3% bovine serum albumin (dissolved in 1XPBS) for one hour at room temperature. Cells were then incubated overnight at 4 °C with mouse anti-tubulin antibody (Sigma) and rabbit anti-pericentrin antibody (Abcam, Cambridge, MA) in blocking buffer. Primary antibodies were detected using Alexafluor-conjugated secondary antibodies (Molecular Probes), while DNA was detected using Hoechst 33342 (Invitrogen). All images were acquired using a Zeiss Axiovert 200M inverted microscope equipped with epifluorescence optics and an Apotome module (Carl Zeiss). Acquired images were then exported into eight bit tiff files, and figures were prepared using Adobe Photoshop software.

Water Solubility

A known mass of each molecule was dispersed in ultrapure deionized water (Millipore) at a concentration > 0.5 mg/mL. The supersaturated mixtures were sonicated for 1 minute in a bath sonicator and allowed to equilibrate for > 24 hours at room temperature. The supersaturated mixtures were then centrifuged for 10 minutes at 10,000 rpm ($9,391 \times g$) in an Eppendorf microcentrifuge to remove the insoluble fraction of the drug. The supernatant was centrifuged further in a separate tube and the centrifugation process was repeated until no pellet was visible. The solution of the drug was then diluted in dimethyl sulfoxide (DMSO) at a 1:2 volume ratio of solution-to-DMSO. The concentration of the molecule in the water/DMSO solution was determined based on a concentration-absorbance calibration line created with solutions of the drug in a 1:2 water/DMSO co-solvent system. The peak absorbance of the drug (335-336 nm) was used to create the calibration. The water solubility of the drug was then determined by taking into account the dilution performed with the addition of DMSO.

Selection of Doxorubicin Resistant Cells

To assess the effects of the hypoxanthine-like rigidin analogues on MDR cells, the MES-SA/Dx5 cell line had to first be selected for. This was done according to Harker et al.⁵² The cells were split and allowed to adhere overnight. The next day cells were initially exposed to a DOX concentration of 100 nM, which represented the GI_{50} concentration. The cells were maintained at this DOX concentration until their growth rate reached that of the untreated cells. The DOX concentration was then increased in two-fold increments and following the same growth criteria at each concentration. A final DOX concentration of 500 nM was used as the stopping point. Each new DOX concentration required approximately 2 passages to reach the growth rate of the untreated cells.

Supporting Information Available: Copies of ^1H and ^{13}C NMR spectra of all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Abbreviations Used. ATCC, American Type Culture Collection; 3-CR, three-component reaction; 4-CR, four-component reaction; DAPI, 4',6-diamidino-2-phenylindole; DMEM, Dulbecco's modified Eagle's medium; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; DSMZ, Deutsche Sammlung von Mikroorganismen and Zellkulturen; ECACC, European Collection of Cell Culture; EDTA, diaminoethanetetraacetic acid; EtOH, ethanol; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; FOG, Ficol Orange G; HEPES, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid; HHB, Heinz-HEPES buffer; HRMS, high resolution mass spectrometry; MCR, multicomponent reaction; MDR, multidrug resistant; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PAO, phenyl arsine oxide; P-gp, P-glycoprotein; SAR, structure-activity relationship; TLC, thin layer chromatography; SD, standard deviation.

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