

Synthetic and Biological Studies of Tubulin Targeting C2-Substituted 7-Deazahypoxanthines Derived from Marine Alkaloid Rigidins

Robert Scott,^[a] Menuka Karki,^[b] Mary R. Reisenauer,^[c] Roberta Rodrigues,^[a] Ramesh Dasari,^[a] W. Ross Smith,^[a] Stephen C. Pelly,^[d] Willem A. L. van Otterlo,^[d] Charles B. Shuster,^[b] Snezna Rogelj,^[c] Igor V. Magedov,^[c] Liliya V. Frolova,^{*,[c]} and Alexander Kornienko^{*,[a]}

C2-aryl- and C2-alkyl-7-deazahypoxanthines as analogues of marine alkaloid rigidins were prepared utilizing novel synthetic methods developed for the construction of the pyrrolo[2,3-*d*]pyrimidine ring system. The new compounds exhibited sub-micromolar to nanomolar antiproliferative potencies against a panel of cell lines including in vitro models for drug-resistant tumors, such as glioblastoma, melanoma and non-small-cell lung cancer. A selected representative C2-methyl-7-deazahypoxanthine was found to inhibit microtubule dynamics in cancer cells, lending evidence for tubulin targeting as a mode

of action for these compounds in cancer cells. The results of the docking studies utilizing the colchicine site on β -tubulin were consistent with the observed structure–activity relationship data, including an important finding that derivatization at C2 with linear alkyl groups leads to the retention of activity, thus permitting the attachment of a biotin-containing linker for the subsequent proteomics assays. Because many microtubule-targeting compounds are successfully used to fight cancer in the clinic, the reported antitubulin rigidin analogues have significant potential as new anticancer agents.

Introduction

Due to their useful biological properties, marine pyrrole-derived alkaloids have been the subject of numerous studies by organic and medicinal chemistry research groups.^[1–9] One such investigation conducted in our laboratories is aimed at the development of synthetic chemistry and elucidation of biological properties, associated with the marine alkaloid rigidins. This group of natural products, which includes rigidins A, B, C, D (Figure 1) and E, was isolated from the tunicate *Eudistoma cf. rigida* found near Okinawa and New Guinea and has remained scantily explored despite initial reports of promising biological activities.^[10–12] The first general total synthesis of rigidins A, B, C

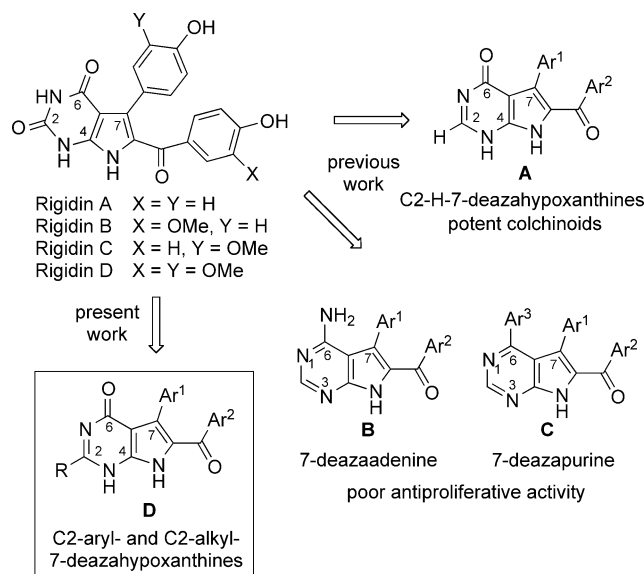


Figure 1. Structures of rigidins A, B, C, D, their synthetic analogues based on 7-deazahypoxanthine (A), 7-deazaadenine (B) and 7-deazapurine (C) skeletons, and proposed C2-substituted 7-deazahypoxanthines (D).

and D, which involved only four steps from commercially available materials, was reported by our group in 2011^[13] and allowed for a thorough investigation of their biological properties. However, despite the earlier reports of promising antiproliferative activity against murine leukemia L1210 cells,^[11] the rigidins were found to have very little, if any, activity against cultured human cancer cells.^[14] Further synthetic investigations

[a] R. Scott,⁺ R. Rodrigues, Dr. R. Dasari, W. R. Smith, Prof. Dr. A. Kornienko
Department of Chemistry and Biochemistry, Texas State University
San Marcos, TX, 78666 (USA)
E-mail: a_k76@txstate.edu

[b] Dr. M. Karki,⁺ Prof. Dr. C. B. Shuster
Department of Biology, New Mexico State University
Las Cruces, NM 88003 (USA)

[c] M. R. Reisenauer, Prof. Dr. S. Rogelj, Prof. Dr. I. V. Magedov,
Prof. Dr. L. V. Frolova
Departments of Chemistry and Biology
New Mexico Institute of Mining and Technology
Socorro, NM 87801 (USA)
E-mail: lfrolova@nmt.edu

[d] Prof. Dr. S. C. Pelly, Prof. Dr. W. A. L. van Otterlo
Department of Chemistry and Polymer Science, Stellenbosch University
Stellenbosch, Private Bag X1
Matieland, 7602 (South Africa)

[⁺] These authors contribute equally.

Supporting information for this article is available on the WWW under
<http://dx.doi.org/10.1002/cmdc.201300532>.

led to the development of approaches allowing for the modification of the 7-deazaxanthine skeleton present in the rigidins to obtain the corresponding 7-deazahypoxanthine (**A**), 7-deazaadenine (**B**) and 7-deazapurine (**C**) frameworks (Figure 1). These studies culminated in the discovery of potent antiproliferative activities associated with the synthesized 7-deazahypoxanthines (**A**) and their ability to disrupt microtubule organization in cancer cells by binding to the colchicine site of β -tubulin.^[14]

Because the only difference between the rigidins' 7-deazaxanthine skeleton and the newly discovered 7-deazahypoxanthines **A** is the absence of the carbonyl group at C2 in the structure of the latter, modifications at this position seemed crucial for activity. Thus, it was further decided to prepare a series of C2-aryl- and C2-alkyl-deazahypoxanthines (**D**, Figure 1) and evaluate these compounds for anticancer activities. Such a study was also warranted by the results of the computer modeling experiments revealing a favorable accommodation of the lipophilic C2 side chain at the colchicine binding site, as described below.

Results and Discussion

Synthesis and SAR of C2-aryl- and C2-alkyl-7-deazahypoxanthines

To develop synthetic access to the C2-substituted 7-deazahypoxanthines **D**, a previously discovered^[13] multicomponent reaction (MCR) of sulfonamidoacetophenones with aldehydes and cyanoacetamide was utilized to prepare pyrrole **1** (Table 1). This was followed by the pyrimidine ring closure using the reaction of **1** with a variety of aromatic and aliphatic esters catalyzed by sodium ethoxide in ethanol, which was reported for a related purine system.^[15] The desired 7-deazahypoxanthines **2–16** were obtained in variable but acceptable yields (Table 1), given that in most cases these products precipitated after the acidification of the reaction mixtures and required no further purification.

The obtained compounds 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 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method (Table 1). The results revealed that in comparison with rigidin-related 7-deazaxanthine **2**, containing the carbonyl group at C2, most of the 7-deazahypoxanthines showed antiproliferative activity. Although the activity was found to be only double- to single-digit micromolar for C2-aryl (**5**, **6**), C2-OEt (**8**) and branched C2-alkyl (**9**), the linear C2-alkyl derivatives **10–12** showed potency at sub-micromolar, and the C2-methyl compound **13** at nanomolar concentrations. Of note, when fluorine was incorporated into the C2-Me group, the activity progressively dropped along with increasing steric size at the C2 position, that is, CH₃ (**13**)→CH₂F (**14**)→CHF₂ (**15**)→CF₃ (**16**).^[16] The activity of the C2-CF₃ derivative **16** is similar to that of C2-*i*Pr compound **9**, which is consistent with the compara-

ble Taft steric parameters of the CF₃ and *i*Pr groups.^[16,17] Because the CF₃ and *i*Pr groups differ significantly in their electronic properties, it is thus conceivable that steric rather than electronic properties of the C2 substituent govern the activity in this series of compounds.

MCR-based synthesis and SAR of C2-methyl-7-deazahypoxanthines

Potent activity of the C2-methyl analogue **13** led to further exploration of C2-methyl compounds by varying the aldehyde-derived C7 aromatic group. Because the previous work with 7-deazahypoxanthines **A** indicated that the best substitution pattern at the C7 and C8 positions (Figure 1) involved the use of the benzoyl group at C8 (Ar²=Ph) and a *meta*-halogen-substituted phenyl and 3-pyridinyl substituent (Ar¹) at C7, a small group of derivatives containing such structural features was prepared (Table 2).^[14] To facilitate the synthesis of these compounds, a new four-component cyclocondensation of methyl-sulfonamidoacetophenone, cyanoacetamide and triethyl orthoacetate with various aldehydes was developed (Table 2). In this reaction, the entire pyrrolo[2,3-*d*]pyrimidine skeleton is assembled in one step, and its success depended on an optimized temperature regime, in which the reaction was first kept at 90 °C to allow for a clean formation of intermediate aminopyrroles (similar to **1** in Table 1), and then at 150 °C to bring the fourth component orthoacetate into the process, leading to closure of the pyrimidine portion of the molecule.

The antiproliferative effects of compounds **13** and **17–22** were studied in a more challenging cell line panel that, in addition to the previously utilized HeLa and MCF-7, contained in vitro models for cancers known to be associated with poor prognoses, such as the U87 human glioblastoma and A549 human non-small-cell lung (NSCLC) carcinoma. Although the newly synthesized compounds **17–22** were found to be inferior to the original C7-phenyl analogue **13**, in general the sub-micromolar potencies were retained with the exception of the pyridine-containing variant **20** exhibiting single-digit micromolar potencies. The drop in activity of this analogue could be also explained by its polar character, impairing its cell permeability.

Microtubule organization in cells

Because the original 7-deazahypoxanthines were found to have strong effects on the microtubule cytoskeleton in cells,^[14] it was important to confirm that these properties remained unchanged in these novel analogues. To this end, cultured HeLa cells were treated with **13** over a range of concentrations, and microtubule morphology was examined (Figure 2a). Results revealed a pronounced change in mitotic microtubule organization at concentrations between 1 μ M and 2 μ M, where **13** caused a complete collapse of the mitotic spindle (panels J and K). The interphase microtubules were affected to a lesser extent at these concentrations (panels D and E), suggesting that **13** was primarily affecting dynamic microtubules. While there appeared to be little effect on stable interphase or spin-

Table 1. Synthesis and antiproliferative activities of C2-aryl and C2-alkyl-7-deazahypoxanthines 2–16.

$ \begin{array}{c} \text{Ph} \\ \\ \text{CH}=\text{O} \\ \\ \text{NH} \\ \\ \text{O}=\text{S}=\text{O} \\ \\ \text{Me} \end{array} + \begin{array}{c} \text{Ph} \\ \\ \text{CHO} \\ \\ \text{CN} \\ \\ \text{CONH}_2 \end{array} \xrightarrow[\text{reflux, 61\%}]{\text{K}_2\text{CO}_3, \text{EtOH}} \begin{array}{c} \text{Ph} \\ \\ \text{O}=\text{C} \\ \\ \text{H}_2\text{N} \\ \\ \text{H}_2\text{N} \end{array} \xrightarrow[\text{Na, EtOH}]{\text{RCO}_2\text{Et}} \begin{array}{c} \text{Ph} \\ \\ \text{O}=\text{C} \\ \\ \text{N} \\ \\ \text{R} \end{array} $								
Compd	Yield [%]	Cell Viability, GI ₅₀ [μM] ^[a]		Compd	Yield [%]	Cell Viability, GI ₅₀ [μM] ^[a]		
		HeLa	MCF-7			HeLa	MCF-7	
2	NA ^[b]	> 50	> 50	10	60	0.78 ± 0.05	0.93 ± 0.07	
3	41	> 50	> 50	11	42	0.34 ± 0.01	0.23 ± 0.01	
4	33	> 50	> 50	12	72	0.92 ± 0.11	1.1 ± 0.1	
5	18	2.7 ± 0.2	2.5 ± 0.3	13	49	0.029 ± 0.001	0.035 ± 0.003	
6	58	18.4 ± 1.8	23.1 ± 0.5	14	35	3.0 ± 0.2	3.2 ± 0.2	
7	41	> 50	> 50	15	47	5.1 ± 0.2	4.2 ± 0.5	
8	NA ^[c]	11.1 ± 0.4	10.4 ± 0.8	16	66	21.5 ± 0.4	21.1 ± 1.1	
9	21	9.1 ± 0.3	8.3 ± 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. Data represent the mean ± SD from two independent experiments performed in four replicates, as determined by the MTT assay. [b] Synthesized using the method described in ref. [13]. [c] Obtained as a side product of the reaction of 1 with CO(OEt)₂.

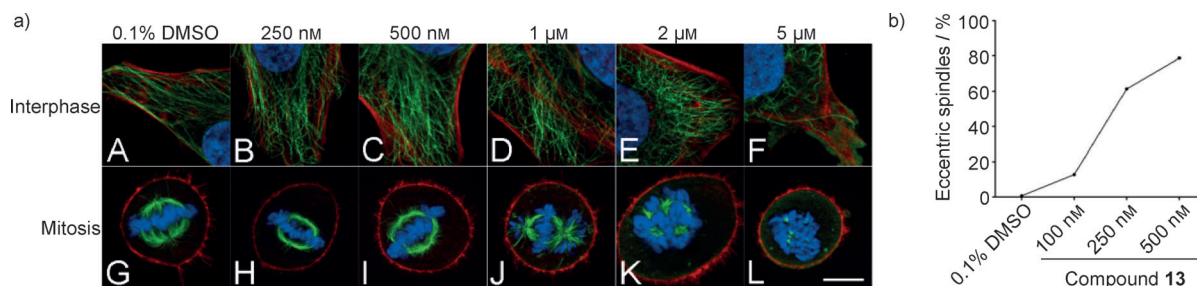
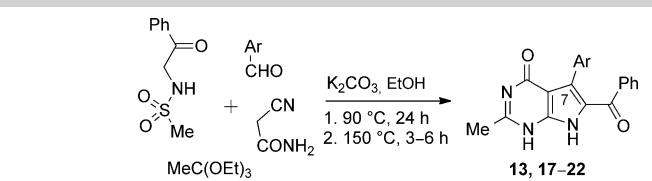


Figure 2. Microtubule organization is altered with increasing concentrations of 13. a) HeLa cells were treated with 0.1% DMSO or 13 at different concentrations for 3 h, fixed and probed for the presence of microtubules (green), F-actin (red) and DNA (blue). Scale bar, 10 μm. b) Line graph showing an increase in the eccentric spindles with the increasing concentrations of 13, 150 mitotic cells scored per condition.

Table 2. MCR-based synthesis and antiproliferative activities of C2-methyl-7-deazahypoxanthines **13** and **17–22**.

					
Compd	Yield [%]	HeLa	MCF-7	in vitro GI ₅₀ [μM] ^[a] U-87	A549
13	40	0.029 ± 0.001	0.035 ± 0.003	0.077 ± 0.002	0.25 ± 0.01
17	61	0.27 ± 0.01	0.23 ± 0.00	0.90 ± 0.16	0.60 ± 0.23
18	40	0.29 ± 0.01	0.27 ± 0.03	0.94 ± 0.12	0.65 ± 0.19
19	43	0.29 ± 0.03	0.36 ± 0.02	1.7 ± 0.1	1.0 ± 0.9
20	65	3.1 ± 0.1	4.7 ± 0.8	9.23 ± 2.13	12.0 ± 1.4
21	63	0.49 ± 0.05	0.61 ± 0.01	2.4 ± 0.6	1.1 ± 0.4
22	58	0.29 ± 0.01	0.27 ± 0.01	0.72 ± 0.06	0.38 ± 0.08

[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. Data represent the mean ± SD from two independent experiments performed in four replicates, as determined by the MTT assay.

dle microtubules at lower concentrations, a distinct displacement of the mitotic spindle could be observed (panels H and I), suggesting a defect in astral microtubules. The central localization of the mitotic spindle is made possible by interactions between dynamic astral microtubules emanating from the spindle poles and the cell cortex, often described as Hertwig's rule.^[18] During mitosis, astral microtubules are the most dynamic microtubule population, and therefore, they would be most sensitive to tubulin-targeting drugs such as **13**. The quantification of spindle eccentricity with increasing concentrations of **13** is shown in Figure 2b. Together, these results

lend further evidence for tubulin-targeting being a likely mechanism responsible for the antiproliferative effects of the 7-deazahypoxanthines.

Molecular modeling

In our previous work, 7-deazahypoxanthines were proposed to target to the colchicine site on β -tubulin based on their potent inhibitory effects on the binding of [³H]colchicine to tubulin.^[14] In the current study, molecular docking simulations were performed on this series of compounds to understand the contribution of the C2 substituent toward the binding at this site. Figure 3 shows the proposed binding mode of the 7-deazahypoxanthines in the colchicine pocket.^[19] Of particular interest, is the accommodation of alkyl substituents at the C2 position. A small channel in the region of Asn258 and Lys352 accommodates the colchicine methoxy group protruding from the seven-membered C-ring system. Docking results of 7-deazahypoxanthine **13** suggest that the C2-methyl group is located in a nearly identical position to the above-mentioned colchicine methoxy group (Figure 3a). Moreover, this narrow channel is able to accommodate linear alkyl substituents at C2, such as the butyl group in **11** (Figure 3b). Sterically demanding substituents at C2 are, however, too bulky for the channel, perhaps explaining the lack of activity of **3–6**, **9** and **16**. A superimposition

of the co-crystallized colchicine and the docked 7-deazahypoxanthine **13** reveals the similar binding modes of the two compounds, with the C7-aryl substituent on the 7-deazahypoxanthine scaffold accommodated in a near identical fashion to that of the trimethoxy-aryl moiety of colchicine (Figure 3c).

Quantitative video microscopy

Poor prognoses of glioblastoma, melanoma and NSCLC patients are generally attributed to the drug-resistant nature of these malignancies^[20–22] and therefore it was encouraging that

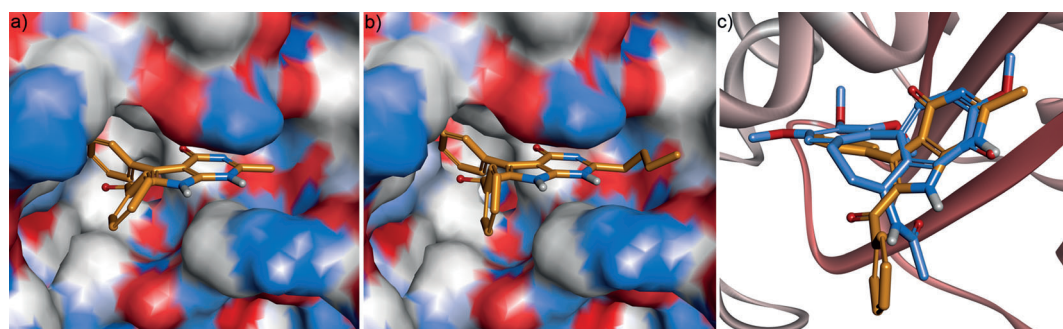


Figure 3. Docking studies (PDBID: 3UT5) of **13** (a) and **11** (b) reveal that linear C2-alkyl substituents on the 7-deazahypoxanthine scaffold can be accommodated in a small channel in the region of Asn258 and Lys352. Co-crystallized colchicine (blue) and **13** (orange) adopt similar binding poses in the colchicine pocket (c).

7-deazahypoxanthines **13** and **17–22** were active against the U87 human glioblastoma and A549 NSCLC carcinoma cell lines. Since tubulin-targeting agents are generally poorly effective against cancer cells resistant to the induction of apoptosis,^[23–25] compound **22** was further evaluated against U373 human glioblastoma and SKMEL human melanoma cells, reported to display apoptosis resistance.^[26,27] The obtained MTT-based GI_{50} value of $0.3\ \mu\text{M}$ in both of these cell lines indicates that this compound maintains similar levels of activity in these cell types (compare with GI_{50} values in Table 2). Computer-assisted phase-contrast microscopy (quantitative video microscopy) was utilized to confirm cell death as the principal mechanism of action associated with this compound's *in vitro* growth inhibitory effects in these cell lines. Figure 4 shows that **22** indeed inhibited cancer cell proliferation by inducing cell death when assayed at its MTT-based GI_{50} values in U373 glioblastoma and SKMEL melanoma cells.

Synthesis of a biotinylated protein pull-down reagent

Microtubules represent an important target for a number of clinically successful drugs, but so far, there are no US Food and

Drug Administration (FDA)-approved anticancer agents targeting the colchicine binding domain on β -tubulin, although many colchicoids inhibit the growth of multidrug-resistant (MDR) tumor cell lines and have reduced neurotoxicity and good oral bioavailability. A number of colchicoids have been examined in human clinical trials with generally promising results, but having drawbacks that include narrow therapeutic windows and unacceptable toxicities.^[28] It is thus important to understand and predict possible causes of potential toxicity in the clinic by elucidating other intracellular targets of these agents during their preclinical evaluation. The importance of identifying additional targets interacting with the 7-deazahypoxanthines could also help understand their effectiveness against drug-resistant cancer cell lines used in the current study, since sole tubulin targeting may not be sufficient to induce death of these cancer cells. The current study revealed that the linear alkyl substitution at C2 of the 7-deazahypoxanthine skeleton (such as C2-Bu, see **11** in Table 1) is tolerated, and thus this position appeared ideal for the probe preparation to be used in proteomic pull-down assays. To this end, using the developed chemistry, pyrrole **1** was successfully converted to the C2-alkyne-containing 7-deazahypoxanthine **23**, which was then coupled with a commercially available biotin-azide reagent utilizing click chemistry (Scheme 1).^[29] Mode of action studies utilizing the prepared biotinylated probe **24** are in progress and will be reported in due course.

Conclusions

A synthetic route was developed to access C2-aryl- and C2-alkyl-deazahypoxanthines as analogues of the marine alkaloid rigidins containing a carbon group instead of the carbonyl present in the natural products. The new 7-deazahypoxanthines were found to have sub-micromolar to nanomolar antiproliferative potencies and to inhibit microtubule dynamics in cancer cells. This activity was most plausibly explained by binding of these compounds to the colchicine site on β -tubulin, a proposal consistent with the experimental findings of their effects on microtubule organization and a theoretical docking model developed for these compounds. These agents are also effective against cancer cells representing drug-resistant cancers with poor prognoses, raising the possibility that additional

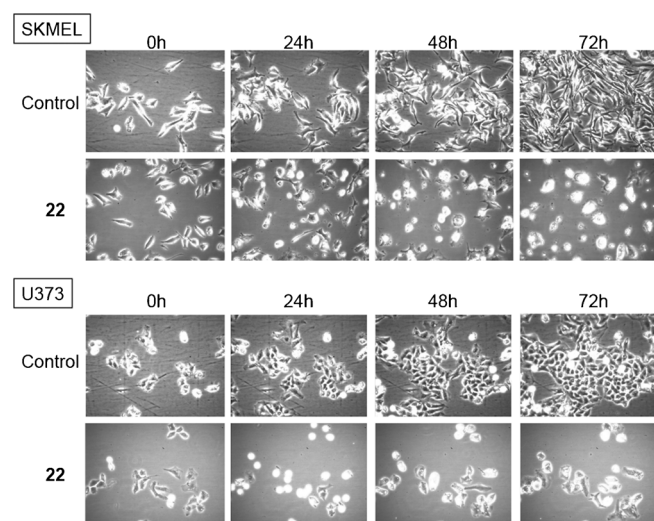
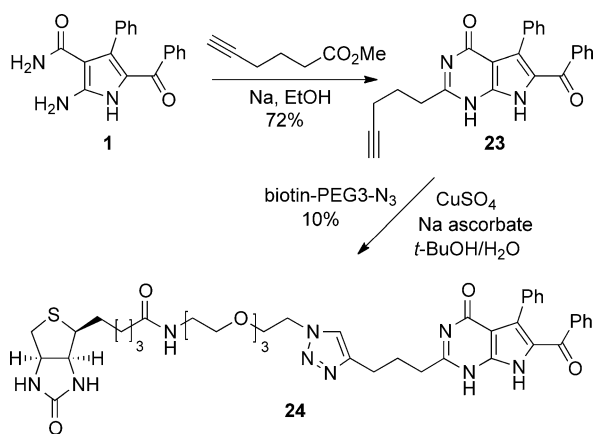


Figure 4. Cellular imaging of **22** against U373 glioblastoma and SKMEL melanoma cells illustrating cell death at MTT colorimetric assay-related GI_{50} value of $0.3\ \mu\text{M}$.



Scheme 1. Synthesis of the biotinylated 7-deazahypoxanthine probe **24**.

intracellular targets may be involved. The current investigation sets the stage for further mode-of-action studies utilizing biotinylated protein pull-down probes. Since many microtubule-targeting compounds have been successfully used to fight cancer in the clinic, the antitubulin agents represented by the 7-deazahypoxanthine rigidin analogues have significant potential as new anticancer agents.

Experimental Section

Chemistry

General: 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 μ m) 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 μ m, 60 Å pore size). ^1H and ^{13}C NMR spectra were recorded on a Bruker 400 spectrometer. 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 using Waters Synapt G2 LCMS. The >95% purity of the synthesized compounds was ascertained by UPLC–MS analyses.

2-Amino-5-benzoyl-4-phenyl-1H-pyrrole-3-carboxamide (1): Anhydrous granulated K_2CO_3 (0.028 g, 0.2 mmol) was added to a stirred solution of *N*-methylsulfonamidoacetophenone^[13] (0.084 g, 0.4 mmol), benzaldehyde (0.055 g, 0.52 mmol) and cyanoacetamide (0.044 g, 0.52 mmol) in EtOH (3 mL). The mixture was held at reflux for 14 h. The reaction mixture was then cooled to RT and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel using $\text{CH}_2\text{Cl}_2/\text{AcOEt}$ (2:1) as eluent to obtain pyrrole **1** as a yellow powder (74 mg, 61%): ^1H NMR ($[\text{D}_6]\text{DMSO}$): δ = 10.89 (brs, 1H), 7.18–7.05 (m, 8H), 6.97 (t, J = 7.8 Hz, 2H), 6.70 (brs, 1H), 6.39 (s, 2H), 4.59 ppm (brs, 1H); ^{13}C NMR ($[\text{D}_6]\text{DMSO}$): δ = 184.3, 167.6, 149.8, 139.7, 134.6, 132.6, 130.9, 129.9, 128.3, 128.2, 128.0, 127.5, 121.5, 99.4 ppm; HRMS (ESI) m/z [$M + \text{H}$] $^+$ calcd for $\text{C}_{18}\text{H}_{16}\text{N}_3\text{O}_2$: 306.1243, found 306.1248.

General procedure for the synthesis of deazahypoxanthines 3–16, 23: A selected ethyl ester (1.28 mmol) and pyrrole **1** (50 mg,

0.16 mmol) were added to the solution of EtONa in EtOH prepared by dissolving Na (30 mg, 1.3 mmol) in EtOH (2 mL). The mixture was held at reflux for 10 h. The reaction mixture was then diluted with H_2O and neutralized with 1 M HCl. The formed precipitate was collected by filtration and dried under vacuum overnight. Although in most cases the product deazahypoxanthines were >95% pure, they could be further purified using column chromatography (5% MeOH in CHCl_3).

6-Benzoyl-2-phenyl-5-phenyl-1H-pyrrolo[2,3-*d*]pyrimidin-4(7H)-one (3): White powder (27 mg, 41%): ^1H NMR ($[\text{D}_6]\text{DMSO}$): δ = 12.21 (brs, 1H), 11.64 (brs, 1H), 8.01–7.96 (m, 2H), 7.74 (t, J = 7.3 Hz, 1H), 7.67 (t, J = 7.5 Hz, 2H), 7.37–7.09 ppm (m, 10H); ^{13}C NMR ($[\text{D}_6]\text{DMSO}$): δ = 185.8, 167.5, 165.5, 138.4, 133.6, 133.1, 132.6, 131.3, 131.0, 129.8, 129.7, 129.0, 128.7, 128.6, 128.5, 127.9, 127.6, 123.3, 103.4 ppm; HRMS (ESI) m/z [$M + \text{NH}_4$] $^+$ calcd for $\text{C}_{25}\text{H}_{20}\text{N}_4\text{O}_2$: 410.1727, found: 410.1722.

6-Benzoyl-2-(2-ethoxycarbonyl-ethyl)-5-phenyl-1H-pyrrolo[2,3-*d*]pyrimidin-4(7H)-one (10): Red powder (41 mg, 60%): ^1H NMR ($[\text{D}_6]\text{DMSO}$): δ = 12.59 (brs, 1H), 11.91 (brs, 1H), 7.42 (d, J = 8.0 Hz, 2H), 7.32 (t, J = 8.0 Hz, 1H), 7.20–6.99 (m, 7H), 4.09 (q, J = 8.0 Hz, 2H), 2.98–2.75 (m, 4H), 1.15 ppm (t, J = 8.0 Hz, 3H); ^{13}C NMR ($[\text{D}_6]\text{DMSO}$): δ = 188.1, 173.9, 172.4, 159.7, 159.6, 158.6, 150.1, 138.0, 133.0, 131.6, 129.5, 128.1, 127.3, 104.9, 60.5, 31.1, 30.4, 14.5 ppm; HRMS (ESI) m/z [$M + \text{Na}$] $^+$ calcd for $\text{C}_{24}\text{H}_{21}\text{N}_3\text{NaO}_4$: 438.1430, found: 438.1431.

6-Benzoyl-2-butyl-5-phenyl-1H-pyrrolo[2,3-*d*]pyrimidin-4(7H)-one (11): Red powder (25 mg, 42%): ^1H NMR ($[\text{D}_6]\text{DMSO}$): δ = 12.54 (brs, 1H), 11.86 (brs, 1H), 7.43 (d, J = 6.7 Hz, 2H), 7.30 (t, J = 6.8 Hz, 1H), 7.23–6.85 (m, 7H), 2.62 (t, J = 6.9 Hz, 2H), 1.81–1.60 (m, 2H), 1.36–1.28 (m, 2H), 0.92 ppm (t, J = 7.0 Hz, 3H); ^{13}C NMR ($[\text{D}_6]\text{DMSO}$): δ = 189.1, 160.7, 138.9, 133.9, 133.0, 132.4, 130.3, 128.9, 128.0, 105.6, 35.0, 30.4, 22.9, 14.9 ppm; HRMS (ESI) m/z [$M + \text{Na}$] $^+$ calcd for $\text{C}_{23}\text{H}_{21}\text{N}_3\text{NaO}_2$: 394.1531, found: 394.1528.

6-Benzoyl-2-methyl-5-phenyl-1H-pyrrolo[2,3-*d*]pyrimidin-4(7H)-one (13): White powder (26 mg, 49%): ^1H NMR ($[\text{D}_6]\text{DMSO}$): δ = 12.57 (brs, 1H), 11.90 (brs, 1H), 7.42 (dd, J = 6.7, 0.4 Hz, 2H), 7.31 (t, J = 7.4 Hz, 1H), 7.17–7.01 (m, 7H), 2.36 ppm (s, 3H); ^{13}C NMR ($[\text{D}_6]\text{DMSO}$): δ = 188.1, 159.8, 156.8, 150.5, 138.1, 133.0, 132.1, 131.6, 129.5, 128.1, 127.6, 127.4, 127.2, 127.1, 104.7, 21.5 ppm; HRMS (ESI) m/z [$M + \text{Na}$] $^+$ calcd for $\text{C}_{20}\text{H}_{15}\text{N}_3\text{NaO}_2$: 352.1062, found: 352.1068.

6-Benzoyl-2-(difluoromethyl)-5-phenyl-1H-pyrrolo[2,3-*d*]pyrimidin-4(7H)-one (15): White powder (28 mg; 47%): ^1H NMR ($[\text{D}_6]\text{DMSO}$): δ = 13.12 (brs, 1H), 12.80 (brs, 1H), 7.46 (d, J = 7.0 Hz, 2H), 7.35 (t, J = 7.4 Hz, 1H), 7.24–7.05 (m, 7H); ^{13}C NMR ($[\text{D}_6]\text{DMSO}$): δ = 188.3, 159.0, 148.2, 137.6, 132.6, 132.5, 131.6, 129.6, 129.2, 128.2, 127.4, 127.0, 112.9, 110.5, 108.1, 106.9 ppm; HRMS (ESI) m/z [$M + \text{H}$] $^+$ calcd for $\text{C}_{20}\text{H}_{14}\text{F}_2\text{N}_3\text{O}_2$: 366.1054, found: 366.1054.

6-Benzoyl-5-phenyl-2-(trifluoromethyl)-1H-pyrrolo[2,3-*d*]pyrimidin-4(7H)-one (16): White/brown powder (63 mg; 66%): ^1H NMR ($[\text{D}_6]\text{DMSO}$): δ = 13.34 (brs, 1H), 13.25 (brs, 1H), 7.48 (d, J = 7.0 Hz, 2H), 7.36 (t, J = 7.4 Hz, 1H), 7.22–7.04 ppm (m, 7H); ^{13}C NMR ($[\text{D}_6]\text{DMSO}$): δ = 188.4, 137.4, 132.8, 132.4, 131.5, 131.3, 131.1, 130.2, 129.6, 129.2, 128.9, 128.3, 128.1, 128.0, 127.5, 127.2 ppm; HRMS (ESI) m/z [$M + \text{Na}$] $^+$ calcd for $\text{C}_{20}\text{H}_{12}\text{F}_3\text{N}_3\text{NaO}_2$: 406.0779, found: 406.0777.

6-Benzoyl-2-(pent-4-ynyl)-5-phenyl-1H-pyrrolo[2,3-*d*]pyrimidin-4(7H)-one (23): White/brown powder (45 mg 72%): ^1H NMR ($[\text{D}_6]\text{DMSO}$): δ = 12.57 (brs, 1H), 11.91 (brs, 1H), 7.42 (d, J = 7.4 Hz, 2H), 7.30 (t, J = 7.4 Hz, 1H), 7.19–6.97 (m, 7H), 2.82 (s, 1H), 2.72 (t,

$J=6.9$ Hz, 2H), 2.28 (t, $J=6.8$ Hz, 2H), 1.98–1.85 ppm (m, 2H); ^{13}C NMR ($[\text{D}_6]\text{DMSO}$): $\delta=187.6, 159.4, 158.8, 149.8, 137.6, 132.6, 131.7, 131.1, 129.0, 127.6, 127.3, 126.8, 126.6, 104.5, 83.8, 71.7, 32.9, 25.7, 17.3$ ppm; HRMS (ESI) m/z $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{24}\text{H}_{19}\text{N}_3\text{NaO}_2$: 404.1375, found: 404.1374.

General procedure for the synthesis of deazahypoxanthines 13 and 17–22: Anhydrous granulated K_2CO_3 (0.052 g, 0.372 mmol) was added in one portion 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 $\text{MeC}(\text{OEt})_3$ (2.5 mL). The mixture was purged with N_2 for 5 min and then heated at 90°C for 24 h under nitrogen atmosphere. The formation of the intermediate pyrrole was monitored by TLC. The reaction temperature was then increased to 150°C , and the reaction mixture was heated for 3–6 h. The mixture was cooled to RT, and the formed precipitate was collected by filtration and washed with EtOH (2 mL) and Et_2O (2 mL) to give the desired 7-deazahypoxanthine **13** and **17–23**. An additional amount of the product was obtained by evaporation of the mother liquor and purification of the residue by column chromatography with $\text{MeOH}/\text{CH}_2\text{Cl}_2$ (1/40 $\rightarrow$ 1/20).

6-Benzoyl-5-(3-chlorophenyl)-2-methyl-1H-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one (17): White powder (150 mg, 61%): ^1H NMR ($[\text{D}_6]\text{DMSO}$): $\delta=12.67$ (brs, 1H), 11.93 (brs, 1H), 7.44 (d, $J=7.2$ Hz, 2H), 7.36 (t, $J=7.4$ Hz, 1H), 7.17 (t, $J=7.3$ Hz, 2H), 7.13–7.09 (m, 3H), 7.04 (t, $J=7.6$ Hz, 1H), 2.37 ppm (s, 3H); ^{13}C NMR ($[\text{D}_6]\text{DMSO}$): $\delta=187.9, 159.7, 156.9, 150.5, 138.1, 135.2, 132.3, 132.0, 131.2, 130.2, 129.4, 129.0, 128.2, 127.9, 126.9, 125.7, 104.8, 21.5$ ppm; HRMS (ESI) m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{20}\text{H}_{15}\text{ClN}_3\text{O}_2$: 364.0853, found: 364.0851.

6-Benzoyl-5-(3-bromophenyl)-2-methyl-1H-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one (18): White/brown powder (90 mg, 40%): ^1H NMR ($[\text{D}_6]\text{DMSO}$): $\delta=12.67$ (brs, 1H), 11.93 (brs, 1H), 7.43 (d, $J=7.0$ Hz, 2H), 7.36 (t, $J=7.5$ Hz, 1H), 7.30 (t, $J=1.8$ Hz, 1H), 7.25–7.15 (m, 4H), 6.99 (t, $J=7.8$ Hz, 1H), 2.37 ppm (s, 3H); ^{13}C NMR ($[\text{D}_6]\text{DMSO}$): $\delta=187.9, 159.7, 157.0, 150.4, 138.1, 135.4, 134.0, 132.3, 130.6, 129.8, 129.3, 129.2, 128.2, 127.9, 125.6, 120.5, 104.8, 21.6$ ppm; HRMS (ESI) m/z $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{20}\text{H}_{14}\text{BrN}_3\text{NaO}_2$: 430.0167, found: 430.0168.

6-Benzoyl-5-(3-fluorophenyl)-2-methyl-1H-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one (19): White/brown powder (107 mg, 43%): ^1H NMR ($[\text{D}_6]\text{DMSO}$): $\delta=12.66$ (brs, 1H), 11.93 (brs, 1H), 7.44 (d, $J=7.0$ Hz, 2H), 7.35 (t, $J=7.4$ Hz, 1H), 7.17 (t, $J=7.8$ Hz, 2H), 7.06–6.99 (m, 2H), 6.94 (dt, $J=7.8, 1.2$ Hz, 1H), 6.91–6.85 (m, 1H), 2.37 ppm (s, 3H); ^{13}C NMR ($[\text{D}_6]\text{DMSO}$): $\delta=187.3, 162.0, 159.6, 159.1, 156.2, 149.8, 137.5, 134.8, 131.6, 128.8, 128.3, 127.5, 127.2, 125.1, 117.6, 113.3, 104.1, 20.9$ ppm; HRMS (ESI) m/z $[\text{M}+\text{K}]^+$ calcd for $\text{C}_{20}\text{H}_{14}\text{FN}_3\text{O}_2$: 386.0707, found: 386.0708.

6-Benzoyl-2-methyl-5-(pyridin-3-yl)-1H-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one (20): White/yellow powder (146 mg, 65%): ^1H NMR ($[\text{D}_6]\text{DMSO}$): $\delta=12.74$ (brs, 1H), 11.97 (brs, 1H), 8.30 (dd, $J=2.2, 0.7$ Hz, 1H), 8.22 (dd, $J=4.8, 1.6$ Hz, 1H), 7.61–7.57 (m, 1H), 7.47–7.42 (m, 2H), 7.38–7.33 (m, 1H), 7.20–7.14 (m, 2H), 7.07 (ddd, $J=7.8, 4.8, 0.8$ Hz, 1H), 2.37 ppm (s, 3H); ^{13}C NMR ($[\text{D}_6]\text{DMSO}$): $\delta=187.2, 159.4, 156.5, 150.8, 150.2, 147.2, 138.0, 137.5, 131.9, 129.1, 128.7, 127.8, 127.6, 123.2, 121.9, 104.6, 21.1$ ppm; HRMS (ESI) m/z $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{19}\text{H}_{14}\text{N}_4\text{NaO}_2$: 353.1014, found: 353.1019.

6-Benzoyl-5-(5-bromopyridin-3-yl)-2-methyl-1H-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one (21): White powder (60 mg, 63%): ^1H NMR ($[\text{D}_6]\text{DMSO}$): $\delta=12.90$ (s, 1H), 12.09 (s, 1H), 8.36 (d, $J=9.0$ Hz, 2H),

7.81 (s, 1H), 7.52–7.11 (m, 5H), 2.40 ppm (s, 3H); ^{13}C NMR ($[\text{D}_6]\text{DMSO}$): $\delta=187.6, 159.9, 157.4, 150.7, 150.0, 148.4, 140.8, 138.1, 132.6, 131.2, 129.6, 128.5, 122.2, 119.1, 105.2, 21.7$ ppm; HRMS (ESI) m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{19}\text{H}_{14}\text{BrN}_4\text{O}_2$: 409.0300, found: 409.0287.

6-Benzoyl-5-(3,5-dibromophenyl)-2-methyl-1H-pyrrolo[2,3-*d*]pyrimidin-4(7*H*)-one (22): White powder (66 mg, 58%): ^1H NMR ($[\text{D}_6]\text{DMSO}$): $\delta=12.82$ (s, 1H), 11.94 (s, 1H), 7.48 (s, 1H), 7.44 (d, $J=6.0$ Hz, 2H), 7.37 (t, $J=7.2$ Hz, 1H), 7.33 (s, 2H), 7.21 (t, $J=6.8$ Hz, 2H), 2.28 (s, 3H); ^{13}C NMR ($[\text{D}_6]\text{DMSO}$): $\delta=187.9, 160.0, 157.0, 151.1, 138.2, 137.2, 133.2, 132.5, 131.8, 129.2, 128.2, 124.2, 121.1, 104.8, 21.7$ ppm; HRMS (ESI) m/z $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{20}\text{H}_{14}\text{Br}_2\text{N}_3\text{O}_2$: 485.9453, found: 485.9438.

***N*-(2-(2-(2-(4-(3-(6-benzoyl-4-oxo-5-phenyl-4,7-dihydro-1H-pyrrolo[2,3-*d*]pyrimidin-2-yl)propyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethyl)-5-((3*a*,4*s*,5,6*a**R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamide (24):** A freshly prepared aq 1 M sodiumascorbate solution (4 μL , 4.0 μmol), and freshly prepared aq 0.2 M $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ solution (4 μL , 0.8 μmol) were added to a solution of **23** (5 mg, 0.013 mmol) and biotin-PEG3-azide (5.8 mg, 0.013 mmol) in $t\text{BuOH}/\text{H}_2\text{O}$ (1:2, 1.5 mL). The mixture was stirred at RT for 48 h and concentrated in vacuo. The crude product was purified by preparative TLC (1.5:8.5 $\text{MeOH}/\text{CHCl}_3$) to obtain **24** as a white solid (1.1 mg, 10%): ^1H NMR ($[\text{D}_6]\text{DMSO}$): $\delta=12.58$ (brs, 1H), 11.89 (brs, 1H), 7.87 (s, 1H), 7.79 (t, $J=5.6$ Hz, 1H), 7.41 (dd, $J=8.3, 1.3$ Hz, 2H), 7.31 (t, $J=7.4$ Hz, 1H), 7.18–6.99 (m, 7H), 6.36 (d, $J=21.2$ Hz, 1H), 5.02 (s, 1H), 4.47 (t, $J=5.2$ Hz, 2H), 4.32–4.26 (m, 1H), 4.15–4.07 (m, 1H), 3.80 (t, $J=5.5$ Hz, 2H), 3.55–3.41 (m, 8H), 3.19–3.15 (m, 4H), 3.11–3.06 (m, 3H), 2.82 (dd, $J=12.4, 5.1$ Hz, 2H), 2.68–2.65 (m, 2H), 2.58–2.52 (m, 2H), 2.46–2.42 (m, 1H), 2.35–2.31 (m, 1H), 1.62–1.41 ppm (m, 6H); HRMS (ESI) m/z $[\text{M}+\text{Na}]^+$ calcd for $\text{C}_{42}\text{H}_{51}\text{N}_9\text{NaO}_7\text{S}$: 848.3530, found: 848.3528.

Biology

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). Human cervical adenocarcinoma HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Human mammary carcinoma MCF-7 cells were cultured in RPMI supplemented with 10% FBS. U87 and U373 cells were cultured in DMEM culture medium (Lonza code 12–136F, Vervier, Belgium), while 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 $\mu\text{g mL}^{-1}$ gentamicin (Lonza code 17–5182), and penicillin-streptomycin (200 units mL^{-1} and 200 $\mu\text{g mL}^{-1}$) (Lonza code 17–602E). All cell lines were cultured in T25 flasks, maintained and grown at 37°C , 95% humidity, 5% CO_2 .

Antiproliferative properties: To evaluate antiproliferative properties of the synthesized compounds, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used. The cell lines were assessed by trypsinizing each cell line and seeding 4×10^3 cells per well into 96-well plates. All compounds were dissolved in dimethyl sulfoxide (DMSO) at a concentration of either 100 mM or 50 mM prior to cell treatment. Cells were grown for 24 h and then treated with compounds at concentrations ranging from 0.04 μM to 100 μM and incubated for 48 h in 200 μL media. 20 μL of MTT

reagent in serum-free medium (5 mg mL⁻¹) was added to each well and incubated further for 2 h. Media was removed, and the resulting formazan crystals were re-solubilized in 200 µL of DMSO. A₄₉₀ 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 1 µM phenyl arsine oxide (PAO) was used as a positive killing control.

Morphological analysis of microtubule organization in HeLa cells: HeLa cells were cultured in Eagle's minimal essential medium (EMEM; Lonza, Walkersville, MD, USA) supplemented with 10% FBS (Atlanta Biologicals, Lawrenceville, GA, USA), sodium pyruvate and sodium bicarbonate. Cells were treated for 3 h with either carrier (0.1% DMSO) or **13** solubilized in DMSO and pre-diluted in media. Following fixation by immersion in MeOH at -20 °C for 30 min, cells were subsequently rehydrated in phosphate-buffered saline (1×PBS), and blocked by incubation in blocking buffer (3% bovine serum albumin dissolved in 1×PBS) for 1 h at RT. Cells were then incubated overnight at 4 °C with mouse anti-tubulin antibody (Sigma) and rabbit anti-pericentrin antibody (Abcam, Cambridge, MA, USA) 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 Structured Illumination module (Carl Zeiss, Thornwood, NY, USA). Acquired 8-bits images were exported, and figures were prepared using Adobe Photoshop CS5 software.

Quantitative video microscopy: The effects of **22** on the viability of human U373 glioblastoma and SKMEL melanoma cells were characterized in vitro using computer-assisted phase contrast video microscopy, as described elsewhere.^[30]

Molecular modeling

Multiple crystal structures of tubulin co-crystallized with ligands at the colchicine binding site were downloaded from the Protein Data Bank (PDB) and compared in terms of resolution and incomplete residues. The structure PDBID: 3UT5 was found to be the most suitable receptor for modeling purposes. From this structure, chain B was retained, along with the corresponding co-crystallized colchicine ligand. All other chains, ligands and water molecules were deleted. A minimization was performed on the receptor ligand complex using Accelrys Discovery Studio 4.0 (Smart Minimizer) and the CHARMM forcefield. Fixed atom constraints were applied to all non-hydrogen atoms, and a GBSW solvent model was employed. Docking studies were performed using the CDocker algorithm in Accelrys Discovery Studio 4.0, employing 150 starting ligand conformations and 75 structures for refinement per ligand.

Acknowledgements

This project was supported by the Texas State University start-up funding (USA) and National Institute of General Medical Sciences (USA) (P20GM103451). The modeling studies were made possible by the Centre for High Performance Computing (Rosebank, Cape Town), who provided the access to Accelrys Discovery Studio. S.C.P. and W.A.L.v.O. acknowledge funding from the National Research Foundation (NRF, RSA) and the University of Stellenbosch (South Africa).

Keywords: 7-deazapurines • alkaloids • drug discovery • privileged structures • pyrrolo[2,3-d]pyrimidines

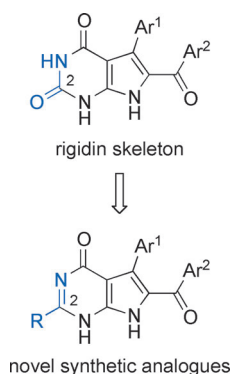
- [1] S. Urban, S. J. H. Hickford, J. W. Blunt, M. H. G. Munro, *Curr. Org. Chem.* **2000**, *4*, 765.
- [2] R. A. Hill, *Annu. Rep. Prog. Chem. Sect. B* **2005**, *101*, 124.
- [3] T. L. Simmons, E. Andrianasolo, K. McPhail, P. Flatt, W. H. Gerwick, *Mol. Cancer Ther.* **2005**, *4*, 333.
- [4] V. M. Dembitsky, T. A. Glorizova, V. V. Poroikov, *Mini-Rev. Med. Chem.* **2005**, *5*, 319.
- [5] Y. Nakao, N. Fusetani, *J. Nat. Prod.* **2007**, *70*, 689.
- [6] M. Sugumaran, W. E. Robinson, *Mar. Drugs* **2010**, *8*, 2906.
- [7] H. Fan, J. Peng, M. T. Hamann, J. F. Hu, *Chem. Rev.* **2008**, *108*, 264.
- [8] M. Chittchang, P. Batsomboon, S. Ruchirawat, P. Ploypradith, *ChemMedChem* **2009**, *4*, 457.
- [9] S. Mabuchi, T. Hisamatsu, C. Kawase, M. Hayashi, K. Sawada, K. Mimura, K. Takahashi, T. Takahashi, H. Kurachi, T. Kimura, *Clin. Cancer Res.* **2011**, *17*, 4462.
- [10] J. Kobayashi, J. Cheng, Y. Kikuchi, M. Ishibashi, S. Yamamura, Y. Ohizumi, T. Ohta, S. Nozoe, *Tetrahedron Lett.* **1990**, *31*, 4617.
- [11] M. Tsuda, K. Nozawa, K. Shimbo, J. Kobayashi, *J. Nat. Prod.* **2003**, *66*, 292.
- [12] R. A. Davis, L. V. Christensen, A. D. Richardson, R. M. da Rocha, C. M. Ireland, *Mar. Drugs* **2003**, *1*, 27.
- [13] L. V. Frolova, N. M. Evdokimov, K. Hayden, I. Malik, S. Rogelj, A. Kornienko, I. V. Magedov, *Org. Lett.* **2011**, *13*, 1118.
- [14] L. V. Frolova, I. V. Magedov, A. E. Romero, M. Karki, I. Otero, K. Hayden, N. M. Evdokimov, L. M. Y. Banuls, S. K. Rastogi, W. R. Smith, S. L. Lu, R. Kiss, C. B. Shuster, E. Hamel, T. Betancourt, S. Rogelj, A. Kornienko, *J. Med. Chem.* **2013**, *56*, 6886.
- [15] Y. Isobe, M. Tobe, H. Ogita, A. Kurimoto, T. Ogino, H. Kawakami, H. Takaku, H. Sajiki, K. Hirota, H. Hayashi, *Bioorg. Med. Chem.* **2003**, *11*, 3641.
- [16] J. A. Macphee, A. Panaye, J. E. Dubois, *Tetrahedron* **1978**, *34*, 3553.
- [17] G. Bott, L. D. Field, S. Sternhell, *J. Am. Chem. Soc.* **1980**, *102*, 5618.
- [18] T. E. Gillies, C. Cabernard, *Curr. Biol.* **2011**, *21*, R599.
- [19] F. M. Ranaivoson, B. Gigant, S. Berritt, M. Joullie, M. Knossow, *Acta Crystallogr. Sect. D* **2012**, *68*, 927.
- [20] C. P. Haar, P. Hebbbar, G. C. Wallace IV, A. Das, W. A. Vandergrift III, J. A. Smith, P. Giglio, S. J. Patel, S. K. Ray, N. L. Banik, *Neurochem. Res.* **2012**, *37*, 1192.
- [21] M. A. Gallego, C. Ballot, J. Kluza, N. Hajji, A. Martoriat, L. Castera, C. Cuevas, P. Formstecher, B. Joseph, G. Kroemer, C. Bailly, P. Marchetti, *Oncogene* **2008**, *27*, 1981.
- [22] M. S. Soengas, S. W. Lowe, *Oncogene* **2003**, *22*, 3138.
- [23] For an example of apoptosis resistant glioma cells, see: A. Mircic, U. Vili-manovic, G. Brajuskovic, V. Bumbasirevic, *Acta Veterinaria* **2012**, *62*, 17.
- [24] For an example of apoptosis resistant melanoma cells, see: S. Merighi, P. Mirandola, K. Varani, S. Gessi, S. Capitani, E. Leung, P. G. Baraldi, M. A. Tabrizi, P. A. Borea, *Biochem. Pharmacol.* **2003**, *66*, 739.
- [25] For an example of induction of a non-apoptotic cell death in colon cancer and melanoma cells, see: J. W. Biggers, T. Nguyen, X. Di, J. T. Gupton, S. C. Henderson, S. M. Emery, M. Alotaibi, K. L. White Jr., R. Brown, J. Almenara, D. A. Gewirtz, *Cancer Chemother. Pharmacol.* **2013**, *71*, 441.
- [26] F. Branle, F. Lefranc, I. Camby, J. Jeuken, A. Geurts-Moespot, S. Sprenger, F. Sweep, R. Kiss, I. Salmon, *Cancer* **2002**, *95*, 641.
- [27] V. Mathieu, C. Pirker, E. M. de Lasalle, M. Vernier, T. Mijatovic, N. De Neve, J. F. Gaussin, M. Dehoux, F. Lefranc, W. Berger, R. Kiss, *J. Cell. Mol. Med.* **2009**, *13*, 3960.
- [28] Y. Lu, J. Chen, M. Xiao, W. Li, D. D. Miller, *Pharm. Res.* **2012**, *29*, 2943.
- [29] M. D. Best, *Biochemistry* **2009**, *48*, 6571.
- [30] O. Debeir, V. Megalizzi, N. Warze, R. Kiss, C. Decaestecker, *Exp. Cell Res.* **2008**, *314*, 2985.

Received: December 16, 2013

Published online on ■ ■ ■, 0000

FULL PAPERS

Rigid(in) development! Novel analogues of marine alkaloid rigidins were prepared utilizing new synthetic methods developed for the construction of the pyrrolo[2,3-*d*]pyrimidine ring system. The compounds exhibited sub-micromolar to nanomolar antiproliferative potencies against drug-resistant cells, such as glioblastoma, melanoma and non-small-cell lung cancer.



R. Scott, M. Karki, M. R. Reisenauer, R. Rodrigues, R. Dasari, W. R. Smith, S. C. Pelly, W. A. L. van Otterlo, C. B. Shuster, S. Rogelj, I. V. Magedov, L. V. Frolova, A. Kornienko**

■■ – ■■

Synthetic and Biological Studies of Tubulin Targeting C2-Substituted 7-Deazahypoxanthines Derived from Marine Alkaloid Rigidins

