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Antitumor activity of the marine natural product dibromophakellstatin in vitro

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Abstract—The pyrrole-imidazole alkaloid *rac*-dibromophakellstatin displayed selective antitumor activity in vitro when tested in 36 cell lines in a cell survival and proliferation assay. The ovarian cancer cell line OVXF 899L proved to be most sensitive (0.60 μM, IC_{50}), followed by the glioblastoma cell line CNXF 498NL (0.93 μM), the non-small lung cancer cell line LXF 529L (0.96 μM), and the uterus cancer cell line UXF 1138L (1.21 μM). The selectivity profile of *rac*-dibromophakellstatin may be indicative for a novel mechanism of action. Separation of the enantiomers on a chiral HPLC column revealed that only the naturally occurring (−)-dibromophakellstatin is antitumor active. Debromination of the pyrrole moiety leads to complete loss of activity.

Pyrrole-imidazole alkaloids constitute a unique class of marine alkaloids. Despite more than 30 years of structure elucidation and synthesis,[1] relatively little information is available on their biological activity. Usually, scattered data were gathered in course of the isolation of novel pyrrole-imidazole alkaloids.

When (−)-dibromophakellstatin (**1**) was isolated from the marine sponge *Phakellia mauritiana* by Pettit et al., it was discovered that the cyclic urea partial structure is necessary for antitumor activity in the high nanomolar range.[2] On replacement of the urea with a cyclic guanidine, the activity is lost. Since then, no other results on the biological activities of any enantiomer of dibromophakellstatin (**1**) have been reported.

Rac-dibromophakellstatin (*rac*-**1**) can be obtained by synthesis making it available for biological testing.[3] On the basis of our program on the total synthesis of the pyrrole-imidazole alkaloids, we have also become able to analyze the antitumor activity of close analogs of **1** with functionalized tri- and tetracyclic frameworks. We obtained racemates of the tetracyclic compounds **2**–**6**, differing from dibromophakellstatin (**1**) by their degrees of bromination, and by functionalization of the cyclic urea unit (Fig. 1).[3]

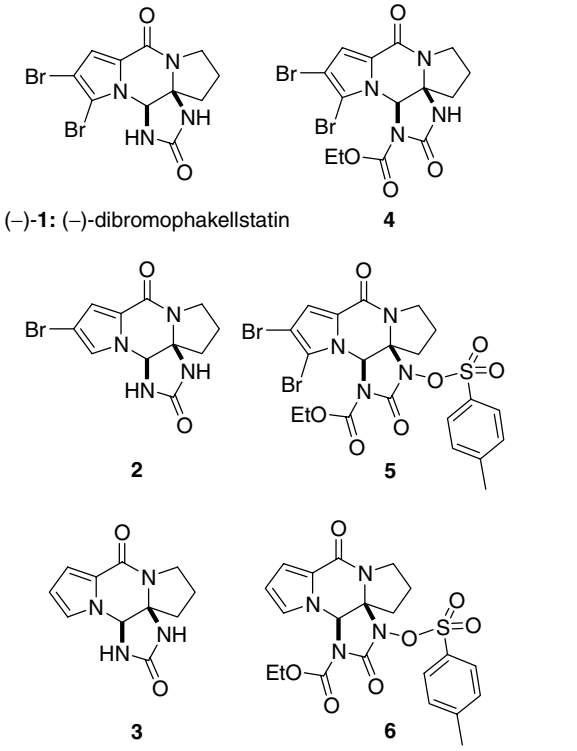

Figure 1. Tetracyclic analogs of the marine natural product (−)-dibromophakellstatin ((−)-**1**) investigated in this study.

Keywords: Antitumor activity; Pyrrole-imidazole alkaloids; Enantiomer; Marine natural products; Dibromophakellstatin.
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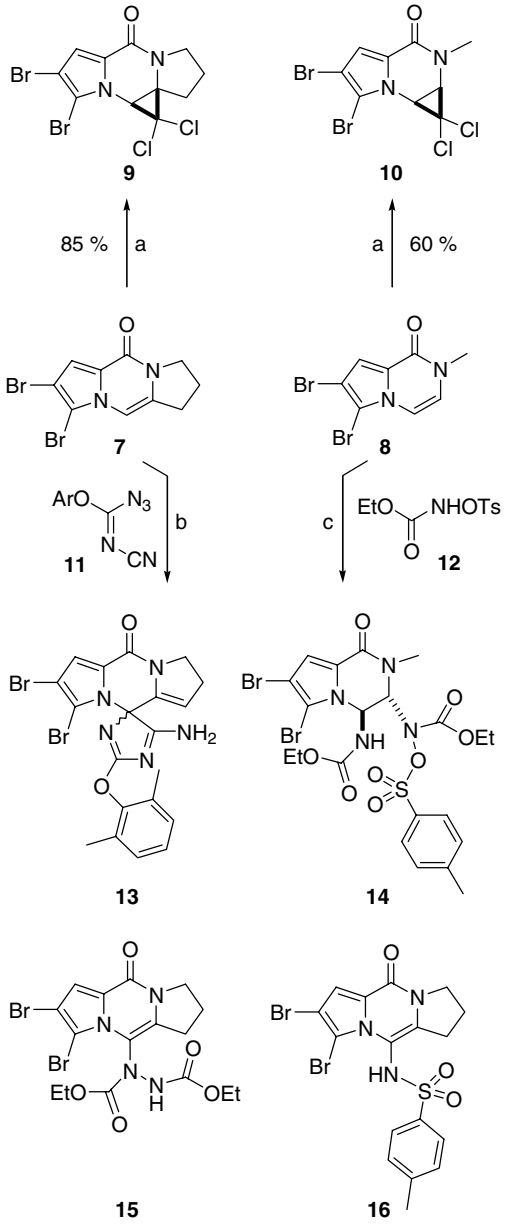
Constitutions and relative configurations of compounds **9**, **10**, **13**–**16** (Scheme 1) have been confirmed by X-ray analysis.[4] In brief, racemic tetracycle **9** was obtained from **7** in 85% yield by treatment of **7** with $CHCl_3$/NaOH under phase transfer conditions (Scheme 1).

The synthesis of the dichlorocarbene adduct **9** shows that cyclopropanation of **7** is possible without ring opening. This is a difference to our epoxidation and aziridination reactions which afforded ring-opened acyliminium ions further reacting with various nucleophiles.[3] As tricyclic analog of **9**, we obtained cyclopropane **10** starting from the N-methylated pyrrolopyrazinone **8**.[5]



Scheme 1. Analogs of the marine natural product dibromophakellstatin (**1**). Reagents and conditions: (a) NaOH (50%), $CHCl_3$, 0 °C, 2 h, 85% (**9**) and 60% (**10**), respectively; (b) **11** (1 equiv, Ar = 2-*m*-xylyl), 1,4-dioxane, reflux, 12 h, 55%; (c) **12** (7 equiv), CaO (7 equiv), DCM, rt, 24 h, 44%.

Tetracyclic spirocycle **13** was obtained from **7** on reaction with $N_3C(OAr)NCN$ (**11**, Ar = 2-*m*-xylyl) in refluxing dioxane. Compound **14** was obtained from the bicyclic precursor **8** on reaction with EtOC(O)NHOTs (**12**)/CaO. Compounds **15** and **16** were synthesized from the core dipyrrolopyrazinone unit **7** by reaction with DEAD and chloramine T, respectively.

Compounds were tested in a monolayer cell survival and proliferation assay in vitro in a panel of 36 human tumor cell lines at five concentrations in triplicates.[6] The read-out is propidium iodide based fluorescence which is a measure of viable cell number. The 36 cell line panel comprises 14 different tumor types, each represented by 1–6 cell lines. Twenty-four out of the 36 cell lines were established from patient tumors passaged as subcutaneous xenografts in nude mice.[7–9] To get clues as to their mechanisms of action, following analysis in the 36 cell line panel of the monolayer assay, novel compounds can be subjected to Compare Analysis. For this, the compounds' IC_{70} activity profiles in the 36 cell line panel are compared to the available corresponding activity profiles determined in the same cell lines for more than 100 reference compounds representing diverse mechanisms of action. High correlation (Spearman correlation coefficient $\rho > 0.6$) is indicative of a similar mechanism of action.[10]

Table 1 gives the antitumor activities (IC_{50}, μM) of the marine natural product *rac*-dibromophakellstatin (*rac*-**1**) and of its structural analog **5**. The other compounds displayed in Figure 1 only showed activity at concentrations higher than 10 μM or, mostly, were inactive.

The ovarian cancer cell line OVXF 899L proved to be most sensitive against *rac*-dibromophakellstatin (0.60 μM, IC_{50}), followed by the glioblastoma cell line CNXF 498NL (0.93 μM), the non-small lung cancer cell line LXF 529L (0.96 μM), and the uterus cancer cell line UXF 1138L (1.21 μM). The sensitivity pattern observed for *rac*-dibromophakellstatin in the 36 cell lines did not reveal similarities ($\rho < 0.6$) with the profiles of any of 100 reference compounds that represent different mechanisms of action (compare analysis.[10]) This observation suggests of a novel mechanism of action for *rac*-dibromophakellstatin.

Among the compounds shown in Figure 1, tetracycle **5** was the only analog of dibromophakellstatin (**1**) showing antitumor activities at concentrations below 10 μM (IC_{50}). However, the selectivity profile is different and less pronounced compared to *rac*-dibromophakellstatin (Table 1).

Interestingly, partial or full debromination of dibromophakellstatin affording compounds **2** and **3** leads to complete loss of antitumor activity. Both bromine substituents of the pyrrole ring are necessary. Bromination seems to be even important for the doubly N-functionalized analog **5** which shows antitumor activity at concentrations below 10 μM against some of the investigated cell lines, while the debrominated analog **6** was completely inactive.

Table 1. Proliferation inhibition of 36 human cancer cell lines by *rac*-dibromophakellstatin (**1**) and its N-functionalized analog **5** (IC_{50}, μM)

Tumor type	Cell line	*rac*-**1**[a]	*rac*-**5**
Bladder	BXF 1218L	>10	7.42
	T24	>10	9.98
Glioblastoma	CNXF 498NL	0.93	>10
	SF268	2.28	6.45
Colon	HCT116	>10	>10
	HT29	>10	>10
Stomach	GXF 251L	>10	>10
Head and neck	HNXF 536L	>10	7.24
Lung, non-small cell	LXF 1121L	>10	>10
	LXF 289L	>10	>10
	LXF 526L	>10	7.96
	LXF 529L	0.96	>10
	LXF 629L	>10	6.52
	H460	>10	>10
Breast	MAXF 401NL	>10	8.16
	MCF7	>10	>10
Melanoma	MEXF 276L	>10	9.44
	MEXF 394NL	1.37	4.02
	MEXF 462NL	>10	>10
	MEXF 514L	4.41	>10
	MEXF 520L	>10	>10
Ovary	OVXF 1619L	2.23	4.83
	OVXF 899L	0.60	8.89
	OVCAR3	>10	>10
Pancreas	PAXF 1657L	>10	>10
	PANC1	4.06	5.00
Prostate	22RV1	>10	5.92
	DU145	>10	>10
	LNCAP	>10	>10
	PC3M	2.42	8.60
Pleuramesothelio	PXF 1752L	>10	>10
Kidney	RXF 1781L	>10	>10
	RXF 393NL	>10	>10
	RXF 486L	>10	>10
	RXF 944L	>10	6.30
Uterus body	UXF 1138L	1.21	8.96

XF Xenograft Freiburg derived cell line.
[a] Values are calculated based on the median test/control values of three independent experiments.

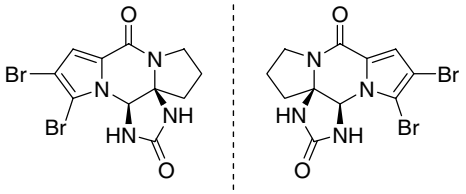


Figure 2. Enantiomers of dibromophakellstatin (**1**).

Among the less related analogs shown in Figure 2, only the tetracyclic dichlorocarbene adduct **9** shows antitumor activity at concentrations below 10 μM, while the smaller analog **10** had almost no effect (Table 2). Again, the selectivity profile of **9** is different and much less pronounced than for the natural product dibromophakellstatin. However, the average antitumor activity of **9** against all 36 cell lines was greater (7 μM, IC_{50}) than for dibromophakellstatin (>30 μM, IC_{50}).

Table 2. Antitumor activity of enantiomers and structural analogs of dibromophakellstatin (IC_{50}, μM) against five selected human cancer cell lines

Cell line	(−)-**1**	(+)-**1**	*rac*-**9**	*rac*-**10**
CNXF 498NL	0.72	>10	5.84	>10
LXF 529L	1.62	>10	9.84	>10
OVXF 899L	0.05	>10	5.37	>10
SF268	0.55	>10	3.91	>10
MEXF 394NL	1.35	>10	7.87	>10

Values are calculated based on the median test/control values of three or two independent experiments.

To shed some light on the question if the effect of dibromophakellstatin may be specific, we resolved a lower milligram amount of the racemate into the enantiomers employing a Chiralpak AD-H/45 HPLC column. Testing against the whole panel was not conducted, because either of the enantiomers would hardly be active against a cell line insensitive to the racemate.

Table 2 gives the activities of both enantiomers (−)-**1** and (+)-**1** against five of the cell lines which proved to be sensitive against *rac*-dibromophakellstatin (*rac*-**1**). The (+)-enantiomer was inactive against all investigated cell lines. Only the (−)-enantiomer showed antitumor effects.

In summary, the antitumor activity of the pyrrole-imidazole alkaloid dibromophakellstatin has been determined comprehensively against a panel 36 human cancer cell lines. Overall, the in vitro antitumor effects of (−)-dibromophakellstatin are weaker than those of the clinically relevant agents, such as doxorubicin. However, two of the most important anticancer agents used in the clinic, cisplatin and 5-fluorouracil, also showed only moderate in vitro antitumor activities in the low micromolar range when test against our 36 cell lines.[11]

What makes (−)-dibromophakellstatin ((−)-**1**) particularly interesting is the antitumor activity of only the (−)-enantiomer against a limited number of cell lines, together with the negative results of the compare analysis. This indicates that (−)-dibromophakellstatin ((−)-**1**) acts by a novel, specific mechanism. Thus, we consider it important to further investigate the mechanism of action of (−)-dibromophakellstatin.

Regarding structural variation, our results agree with the frequent observation that it is very difficult to enhance the biological activity of natural products. The importance of bromination for selective anticancer activity has also been observed for other marine natural products such as bromofascaplysine.[12] Perhaps, dichlorocarbene adduct **9** points at a new class of anticancer agents.[12]

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4. CCDC-618899 (**9**), CCDC-618900 (**10**), CCDC-614042 (**13**), CCDC-614039 (**14**), CCDC-614045 (**15**), and CCDC-614046 (**16**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; fax: +44 1223 336 033; or deposit@ccdc.cam.ac.uk).
5. Bu_4NBr (97.0 mg, 0.30 mmol) was added to a solution of pyrazinone **8** (306 mg, 1.00 mmol) in $CHCl_3$ (20 ml). At 0 °C, aqueous NaOH (50%, 5 ml) was added. After 2 h, saturated aqueous NH_4Cl (50 ml) was added and the brown mixture was extracted thrice with DCM (100 ml). The unified organic phases were extracted with water, dried with $MgSO_4$, and concentrated. The residue was chromatographed (silica, ethyl acetate/isohexane, 1:1) affording tricycle **10** (237 mg, 61%) as a colorless solid which was recrystallized from MeOH. ^1H NMR (400 MHz, $CDCl_3$): $\delta = 3.26$ (s, 3H, NCH_3), 3.68 (d, $^3J = 9.9$ Hz, 1H, MeNCHCCl$_2$), 4.25 (d, $^3J = 9.9$ Hz, 1H, NCHCCl$_2$), 6.98 (s, 1H, CBrCBrCH). ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 33.8$ (*C*H$_3$), 41.5 (MeN*C*HCCl$_2$), 47.4 (N*C*HCCl$_2$), 56.2 (*C*Cl$_2$), 102.0 (CBr*C*BrCHC), 108.2 (*C*BrCBrCHC), 116.5 (CBrCBr*C*HC), 123.9 (CBrCBrCH*C*), 155.1 (*C*O). MS (EI, 70 eV): *m*/*z* (%) = 351/3/53/355/357 (27.5/62.0/41.7/8.18) [M^+−Cl], 109/111 (28.4/42.0), 69.0/71.0 (55.9/66.7), 55.0/57.0 (53.9/100). UV ($CHCl_3$): λ_{max} ($\log\varepsilon$) = 287 nm (3.14). HRMS (EI) calcd for $C_9H_6{}^{79}Br_2{}^{35}Cl_2N_2O$–Cl 350.8535, found 350.8535.
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