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Antitumor activity of phaeosphaeride A modified with nitrogen heterocyclic groups

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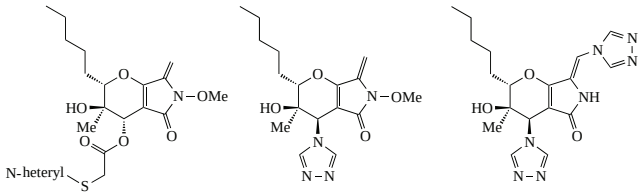
New C(6)-derivatives of natural phaeosphaeride A (PPA) modified with pharmacophoric nitrogen heterocyclic groups have been synthesized. The reaction of 1,2,4-triazole with PPA mesylate produced both the product of the expected substitution of the methanesulfonate group and the PPA derivative with two 1,2,4-triazole groups: at the C(6) atom and the exocyclic C=C bond. The synthesized compounds, with the exception of those containing two 1,2,4-triazole groups, are superior in cytotoxic activity to the original phaeosphaeride and the positive control, etoposide.



Keywords: antitumor activity, phaeosphaeride A, N-heterocyclic compounds, sulfides, natural products, *in vitro* studies, anticancer agents, etoposide.

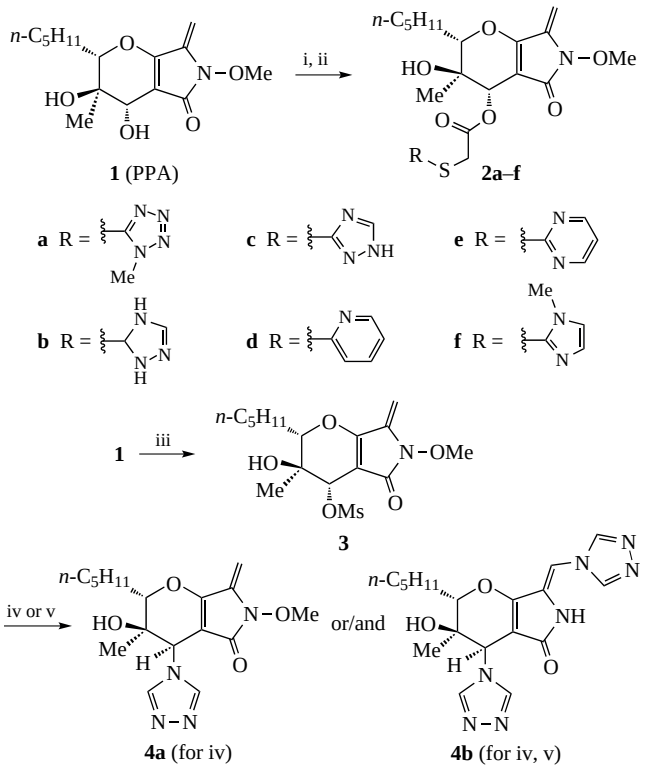
A significant part of the drugs used in clinical practice for chemotherapy of malignant tumors have been developed on the basis of natural compounds,[1–3] *e.g.*, about 40% of anticancer drugs approved by the FDA are either of natural origin or semisynthetic derivatives of natural compounds. Considering synthetic analogues of natural substances reaches 70%.[4]

One of the natural compounds on the platform of which semisynthetic anticancer substances can be obtained is phaeosphaeride A (PPA) **1**, 3,4,6,7-tetrahydro-2*H*-pyrano[2,3-*c*]-pyrrol-5-one. This compound was first isolated in 2006 from the endophytic fungus FA39 (*Phaeosphaeria avenaria*)[4] and showed ability[4] to inhibit STAT3/DNA binding with an IC_{50} cytotoxicity index of 0.61 mM, while demonstrating promising cell growth inhibition in STAT3-dependent U266 multiple myeloma cells with an EC_{50} of 6.7 mM. In 2011, an advanced method[5] for obtaining PPA from a solid culture of the fungus *Phoma* sp. N 19 opened the possibility of extensive research and chemical modification of this compound.[6–10] Some reactions proceeding through the nitrogen-containing PPA cycle afford compounds with lower cytotoxic activity compared to the initial substrate.[6,7] The introduction of various groups to the C(6) atom of the bicyclic PPA skeleton in many cases provides an increase in antitumor activity.[9,10] Thus, replacing the OH group at this position with pyrrolidine brought about a compound significantly superior in IC_{50} value on the six cell lines to both the original PPA and the control Etoposide.[10]

It seemed expedient to synthesize new PPA derivatives modified with well-known pharmacophore nitrogen-containing cyclic groups and to evaluate their cytotoxic activity. In this work, substitution of chlorine in the chloroacetoxy group with heterylthio group gave compounds **2a**–**f** (Scheme 1). Substitution of the methanesulfonate group in PPA 9-*O*-mesylate by 1,2,4-triazole group afforded new derivatives **4a**,**b** with a C(6)–N bond. The reaction of compound **3** with triazole was carried out using different bases. Thus, under the action of NaH, along with the expected compound **4a**, product **4b** was also obtained. Compound **4b** contains two triazolyl groups, one of them being connected through a C=C bond to the nitrogen-containing PPA ring. When using Cs_2CO_3, only ditriazole product **4b** was formed (see Scheme 1). In the literature, there is a close example[12] of the addition of triazole to the terminal atom of the C=C bond of the C=C–N–C(O) system.

The selection of the reagents used in this work was dictated by the known general influence of the groups introduced on the antitumor properties of the modified substances. Thus, the presence of S-azaheterocyclic groups provides inhibition of the growth of colon cancer cells against HT-29 nearly equipotent with fluorouracil.[13] The 1,2,4-triazole cycle has been embedded into various anticancer drugs either in clinical use or in clinical trials.[14]

The structure of new compounds **2a**–**f** and **4a**,**b** was confirmed by HRMS data and IR and NMR (^{1}H, DEPT, ROESY, COSY, HMQC, HMBC) spectroscopy. HMBC spectroscopy and HRMS data unambiguously prove the structure of product **4b**. The ^{1}H NMR spectrum of **4b** is characterized by the absence of a signal for the methoxy protons at ~3.94 ppm and by the presence of the signal for the free NH-group at 8.70 ppm (^{1}H-^{13}C HMQC). The characteristic signals for the terminal olefinic protons at 5.18 and 5.12 ppm, which were present in the starting compound PPA, are absent in the spectrum of compound **4b**. Instead of them, there is a low-field singlet at 6.90 ppm related to the olefinic proton at the carbon atom bonded to the nitrogen atom of



Scheme 1 *Reagents and conditions*: i, $ClCH_2C(O)Cl$, Et_3N, CH_2Cl_2, 0 °C, 2 h; ii, RSH, K_2CO_3, NaI, MeCN, 55 °C, 4 h; iii, MsCl, Et_3N, CH_2Cl_2, 0 °C, 2 h; iv, 1,2,4-triazole, NaH, DMF, 50 °C, 24 h; v, 1,2,4-triazole, Cs_2CO_3, MeCN, 25 °C, 24 h.

the triazole ring. This is also confirmed by the ^{13}C DEPT spectrum, correlation with the carbon signal at 101.88 ppm in the spectrum of ^1H-^{13}C HMQC and correlation with quaternary carbon atoms C(3) and C(4) in the spectrum of ^1H-^{13}C HMBC.

The cytotoxic activity of PPA derivatives **2a**–**f** and **4a**,**b** was evaluated on human embryonic kidney cells and human prostate adenocarcinoma PC-3, human colorectal cancer HCT-116, human acute T-cell leukemia Jurkat, human breast cancer MCF-7, human lung cancer A549, human chronic myelogenous leukemia K562, human acute monocytic leukemia THP-1, human multiple myeloma NCI-H929 and human multiple myeloma RPMI8226 cell lines by MTT assays. All cells were incubated with different concentrations of PPA derivatives for 72 h, with etoposide used as reference compound. The anticancer activity of the tested compounds was described as the concentration of drug inhibiting 50% cell growth IC_{50} (Tables 1 and 2; data were expressed as inhibitory ratio ±SD based on three independent experiments, $n = 3$).

Data in Tables 1 and 2 demonstrate that compounds **2a**–**f**, **4a** are superior in cytotoxic activity to the original PPA in all tested cancer lines, and most of the products are superior to the control semi-natural etoposide on HCT-116-2, MCF-7, PC3, NCI-H929, RPMI8226 lines. Compound **4b** in which the triazole groups are present both at the C(6) atom and at the alkenyl carbon atom does not exhibit essential cytotoxic activity. This fact is in a good agreement with the previous assumption[8,9] of the importance of terminal multiple bond and the methoxy group in PPA derivatives for the manifestation of their biological activity.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.09.023.

Table 1 IC_{50} values for the respective compounds when studied on the suspension cell lines.

Compound	Suspension cell cultures, IC_{50}/μM				
	NCI-H929	THP-1	K562	RPMI8226	Jurkat
PPA **1**	6.7 ± 0.25	18 ± 0.25	25 ± 1.1	8.1 ± 0.2	12 ± 0.5
2a	1.3 ± 0.5	1.9 ± 0.3	4.2 ± 0.7	3.2 ± 0.2	1.9 ± 0.1
2b	1.5 ± 0.5	3.9 ± 0.2	6.3 ± 2.8	4.5 ± 0.2	1.9 ± 0.3
2c	1.0 ± 0.1	3.5 ± 0.4	5.5 ± 3.3	2.2 ± 0.2	2.1 ± 0.4
2d	1.2 ± 0.2	1.9 ± 0.3	4.1 ± 0.4	2.1 ± 0.2	2.0 ± 0.2
2e	1.7 ± 0.1	4.2 ± 0.5	7.3 ± 3.1	3.3 ± 0.2	3.5 ± 0.2
2f	1.8 ± 0.1	4.7 ± 0.5	3.9 ± 0.9	4.1 ± 0.2	3.1 ± 1.4
4a	2.0 ± 0.2	4.9 ± 0.4	8.5 ± 2.0	4.3 ± 0.2	3.4 ± 0.4
4b	5.6 ± 0.7	42.0 ± 5.2	45.0 ± 5.4	51.0 ± 0.2	94.0 ± 22.6
Etoposide	1.9	2	7.6	8.5	–

Table 2 IC_{50} values for the respective compounds when studied on the adhesive cell lines.

Compound	Adhesive cell cultures, IC_{50}/μM				
	HCT-116-2	MCF-7	PC3	A549	HEK293
PPA **1**	48 ± 0.5	19 ± 0.7	35 ± 0.5	42 ± 2.3	10 ± 0.4
2a	6.5 ± 2.1	4.9 ± 1.1	20.4 ± 1.6	15.0 ± 1.8	7.4 ± 0.5
2b	7.5 ± 0.7	6.0 ± 0.5	10.1 ± 2.1	15.0 ± 2.4	7.6 ± 0.4
2c	7.6 ± 0.5	3.4 ± 0.9	9.8 ± 2.1	19.0 ± 3.1	6.7 ± 0.3
2d	6.3 ± 1.6	3.7 ± 1.3	10.3 ± 1.3	26.0 ± 0.9	6.2 ± 0.9
2e	11.8 ± 3.2	4.7 ± 2.3	16.0 ± 1.3	37.0 ± 3.1	9.4 ± 0.7
2f	10.5 ± 5.0	6.1 ± 1.7	16.5 ± 1.3	20.0 ± 2.2	10.0 ± 1.1
4a	10.0 ± 7.1	6.1 ± 1.8	14.2 ± 2.8	22.0 ± 3.4	9.8 ± 0.2
4b	not active	49.0 ± 5.3	not active	not active	41.0 ± 2.1
Etoposide	43	8.9	55	14	2

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