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Synthesis of 7-(4-methylphenyl)thiomethyl and 7-morpholylmethyl derivatives of natural phaeosphaeride A and their cytotoxic activity

Victoria V. Abzianidze,*[a] Ksenia P. Bolshakova,[b] Daria S. Prokofieva,[a] Alexander O. Berestetskiy,[c] Victor A. Kuznetsov[a] and Yuri G. Trishin[b]

[a] *Research Institute of Hygiene, Occupational Pathology and Human Ecology, 188663 St. Petersburg, Russian Federation. E-mail: vvaavv@mail.ru*
[b] *St. Petersburg State University of Industrial Technologies and Design, 198095 St. Petersburg, Russian Federation*
[c] *All-Russian Institute of Plant Protection, Pushkin, 196608 St. Petersburg, Russian Federation*

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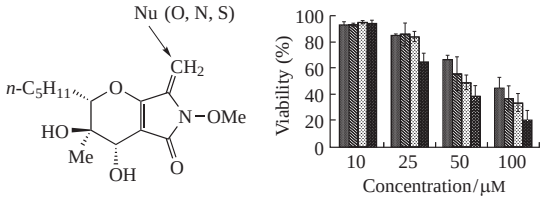
The herein synthesized 7-(4-methylphenyl)thiomethyl and 7-morpholylmethyl derivatives of natural phaeosphaeride A have lower cytotoxic activity towards the human lung adenocarcinoma A549 cell line compared to phaeosphaeride A. The presence of exocyclic C=C bond and N–OMe group in such compounds is suggested to be a factor in their antitumor activity.

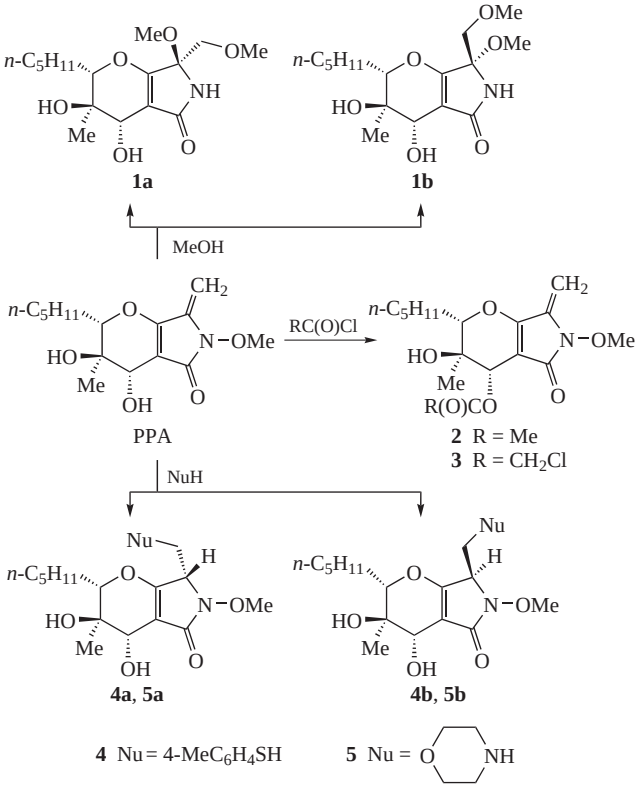


The search for novel drugs with antitumor activity is a high priority of modern medicinal chemistry. Among them, natural products and their analogues are of particular interest.[1,2] Thus, phaeosphaeride A (PPA) revealing a pronounced anticancer activity was isolated from the endophytic fungus, FA39 (*Phaeosphaeria avenaria*) in 2006.[3] This compound was selected for further detailed studies from a 10 000-member library, consisting primarily of natural product extracts, due to its ability to inhibit STAT3/DNA binding with an IC_{50} of 0.61 mM, while exhibiting cell growth inhibition in STAT3-dependent U266 multiple myeloma cells with an EC_{50} of 6.7 μM.[3] However, the study of chemical reactions of PPA was inhibited by the lack of reliable knowledge about its structure. Only in 2015 by means of X-ray crystallography we were able to establish the molecular structure of this compound, substituted 3,4,6,7-tetrahydro-2*H*-pyrano[2,3-*c*]pyrrol-5-one,[4] which opened up the prospects for its direct modification.

Anticancer activity of some natural products can be sometimes enhanced by their derivatization.[5,6] In this context, it seemed reasonable to synthesize a series of PPA derivatives and evaluate the influence of the structural fragments in the molecules of PPA and its derivatives on cytotoxic activity (Scheme 1).

We have previously shown[7] that compounds **2** and **3**, obtained by acylation of the hydroxyl group of PPA at the C^4 atom, retaining the pyrrolone cycle, have equal or higher cytotoxic activity as compared with PPA. At the same time, the reaction of PPA with methanol in the presence of water and lithium hydroxide leads to diastereomers **1a** and **1b**[7] which lose the ability to influence the viability of the human lung adenocarcinoma A549 cell line as compared with parent PPA. Having conducted special experiments using ethanol and methanol-d_4,[7] we have shown that the formation of compounds such as **1a** and **1b** is the result of addition of two alkoxy groups (primary to the exocyclic bond C=C and then to the intermediate endocyclic bond C=N) and removal of methoxy group from N-atom of the heterocycle. It seemed important to obtain the product of mono addition of a nucleophile to exocyclic C=C bond without removal of methoxy group, and thus determine whether the preservation of Weinreb-amide moiety is a necessary prerequisite for PPA derivatives to exhibit anticancer activity.

The goal has been achieved using reagents other than methoxide ion (O-nucleophile). In this work, we synthesized products of addition of 4-methylbenzenethiol (S-nucleophile) and morpholine (N-nucleophile) to the exocyclic C=C bond of PPA **4a**,**b** and **5a**,**b**. The reaction with 4-methylbenzenethiol proceeded



Scheme 1

under mild conditions in the presence of triethylamine.[8] The obtained diastereomers, substituted 3,4,6,7-tetrahydro-2*H*-pyrano-[2,3-*c*]pyrrol-5-ones **4a**,**b** (1 : 1), were separated by preparative HPLC. For the synthesis of a morpholine derivative, a number of catalysts were tested, *i.e.* $FeCl_3$,[9] BuLi,[10] PEG 400,[11] $PdCl_2(MeCN)_2/SnCl_2$.[12] The highest yield was achieved on using $PdCl_2(MeCN)_2/SnCl_2$.†

We could not separate pure diastereomers **5a**,**b** and determine their optical implementation.

The structure of new compounds **4a**,**b** and **5a**,**b** was confirmed by HRMS data and IR and NMR (^{1}H, ROESY, COSY) spectroscopy. For instance, ^{1}H NMR spectra of compounds **4a**,**b** and **5a**,**b** contain an ABX spin system as a doublet of doublets and a multiplet. The configuration of the C^7 atom in isomers **4a** and **4b** was established by 2D techniques using the Nuclear Overhauser effect (ROESY) (see Figure S1, Online Supplementary Materials). Thus, the spectrum of compound **4a** contains a cross-peak between the methine protons at 4.31 ppm (m, 1H, C*H*NOMe) and 4.07 ppm (m, 1H, *H*CO), which corresponds to the *R*-configuration of the C^7 atom. In the spectrum of compound **4b**, under similar experimental conditions, such an effect was not observed, which is characteristic for the *S*-configuration of the C^7 atom.

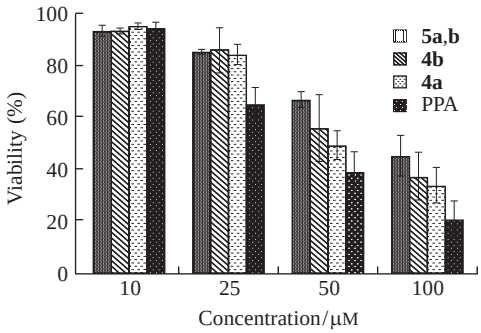


Figure 1 Effect of PPA, **4a**, **4b** and **5a**,**b** on A549 viability after 48 h exposure.

The cytotoxic activity of PPA and its derivatives was evaluated on the human lung adenocarcinoma A549 cell line.‡

The statistically significant influence of the experimental samples on viability of the cells begins with a concentration of 25 μM (Figure 1). Two-factor ANOVA revealed derivatives **4a**,**b** and **5a**,**b** to be less toxic compared to the parent compound, PPA. Arylthio diastereomers **4a** and **4b** turned out to have the same effect on A549 cells. In addition, they have a greater effect on the viability of these cells compared to morpholine derivatives **5a**,**b**.

Thus, the presence of the exocyclic C=C bond and N–OMe group in PPA derivatives is probably a necessary prerequisite for them to reveal high cytotoxic 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.2017.01.026.

† Synthesis of compounds **1a**,**b**, **2** and **3** was previously described.

Synthesis of diastereomers **4a**,**b**. 4-Methylbenzenethiol (30 mg, 0.24 mmol) and then triethylamine (24 mg, 0.24 mmol) were added to a solution of PPA (55 mg, 0.185 mmol) in anhydrous toluene (5 ml). The mixture was stirred at room temperature overnight. On completion of the reaction, the mixture was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (Merck 60 Å 70–230 mesh silica gel, elution with dichloromethane–methanol, 20 : 1). The ratio of the resulting diastereomers (**4a** : **4b**) was estimated as 1 : 1 from LCMS spectrum of the crude mixture, yield 67%. Diastereomers **4a**,**b** were separated by preparative HPLC (elution with acetonitrile–0.1% formic acid, 45 : 55).

*(2*S*,3*R*,4*S*,7*R*)-3,4-Dihydroxy-6-methoxy-3-methyl-7-(4-methylphenylthiomethyl)-2-pentyl-3,4,6,7-tetrahydropyrano[2,3-c]pyrrol-5(2*H*)-one* **4a**: white powder, mp 156 °C, $[\alpha]_D^{20}$ –34.5 (*c* 0.267, CH_2Cl_2). IR (KBr, ν/cm^{-1}): 3480, 3318, 2949, 2924, 2856, 1692, 1655, 1493, 1448, 1422, 1223, 1040, 922. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.33 (d, 2H, Ar, *J* 8.1 Hz), 7.13 (d, 2H, Ar, *J* 7.9 Hz), 4.41 (s, 1H, C*H*OH), 4.31 (m, 1H, C*H*NOMe), 4.07 (m, 1H, HCO), 3.80 (s, 3H, NOMe), 3.60 (br.s, 1H, CHO*H*), 3.44 (dd, 1H, CH_2S, *J* 13.5, 4.2 Hz), 3.22 (dd, 1H, CH_2S, *J* 13.5, 5.0 Hz), 2.82 (br.s, 1H, MeCO*H*), 2.34 (s, 3H, *Me*Ar), 1.95–1.28 (m, 8H, C_4H_8Me), 1.32 (s, 3H, *Me*COH), 0.90 (t, 3H, C_4H_8*Me J* 6.1 Hz). HRMS, *m*/*z*: 422.19917 $[M+H]^+$ (calc. for $C_{22}H_{31}NO_5S$, *m*/*z*: 422.19957).

*(2*S*,3*R*,4*S*,7*S*)-3,4-Dihydroxy-6-methoxy-3-methyl-7-(4-methylphenylthiomethyl)-2-pentyl-3,4,6,7-tetrahydropyrano[2,3-c]pyrrol-5(2*H*)-one* **4b**: yellow oil, $[\alpha]_D^{24}$ –126 (*c* 0.350, CH_2Cl_2). IR (KBr, ν/cm^{-1}): 3418, 2955, 2926, 2855, 1707, 1659, 1493, 1439, 1377, 1261, 1047, 1016, 918, 808. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.33 (d, 2H, Ar, *J* 8.1 Hz), 7.12 (d, 2H, Ar, *J* 8.0 Hz), 4.43 (s, 1H, C*H*OH), 4.32 (m, 1H, C*H*NOMe), 3.79 (s, 3H, NOMe), 3.75 (m, 1H, HCO), 3.49 (dd, 1H, CH_2S, *J* 13.9, 4.0 Hz), 3.26 (dd, 1H, CH_2S, *J* 13.9, 3.9 Hz), 2.33 (s, 3H, *Me*Ar), 1.79–1.24 (m, 8H, C_4H_8Me), 1.24 (s, 3H, *Me*COH), 0.91 (t, 3H, C_4H_8*Me*, *J* 6.8 Hz). HRMS, *m*/*z*: 422.19846 $[M+H]^+$ (calc. for $C_{22}H_{31}NO_{5S}$, *m*/*z*: 422.19957).

Synthesis of diastereomers **5a**,**b**. PPA (50 mg, 0.168 mmol) was added to a suspension of $PdCl_2(MeCN)_2$ (0.73 mg, 0.0028 mmol), $SnCl_2$ (0.53 mg, 0.0028 mmol) in dry acetonitrile (2 ml) under argon. Morpholine (12.2 mg, 0.14 mmol) was then added, and the mixture was stirred at room temperature for 24 h. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (Merck 60 Å 70–230 mesh silica gel, elution with dichloromethane–methanol, 30 : 1). The ratio of the resulting diastereomers (**5a** : **5b**) was estimated as 1 : 1 from ^{1}H NMR spectrum of the crude mixture, yield 46%. Diastereomers **5a**,**b** were separated from PPA by preparative HPLC (elution with methanol–water, 60 : 40).

*(2*S*,3*R*,4*S*,7R)-3,4-Dihydroxy-6-methoxy-3-methyl-7-(morpholinomethyl)-2-pentyl-3,4,6,7-tetrahydropyrano[2,3-*c*]pyrrol-5(2*H*)-one and its (2*S*,3*R*,4*S*,7*S*)-diastereomer* **5a**,**b**: colorless oily powder. IR (KBr, ν/cm^{-1}): 3418, 2955, 2924, 2855, 1703, 1661, 1636, 1456, 1117. ^{1}H NMR (400 MHz, $CDCl_3$) δ, first diastereomer: 4.42 (s, 1H, C*H*OH), 4.20 (m, 1H, C*H*NOMe), 4.04 (m, 1H, HCO), 3.94 (s, 3H, NOMe), 3.71 [m, 4H, $N(CH_2CH_2)_2O$], 2.77 [dd, 1H, $CH_2N(CH_2CH_2)_2O$, *J* 14.2, 2.6 Hz], 2.71–2.36 [m, 3H, $CH_2N(CH_2CH_2)_2O$], 2.49–2.44 [m, 2H, $N(CH_2CH_2)_2O$], 1.86–1.26 (m, 8H, C_4H_8Me), 1.26 (s, 3H, *Me*COH), 0.92 (t, 3H, C_4H_8*Me J* 6.4 Hz); second diastereomer: 4.35 (s, 1H, C*H*OH), 4.18 (dd, 1H, C*H*NOMe, *J* 5.0, 2.4 Hz), 4.04 (m, 1H, HCO), 3.92 (s, 3H, NOMe), 3.64 [m, 4H, $N(CH_2CH_2)_2O$], 2.89 [dd, 1H, $CH_2N(CH_2CH_2)_2O$, *J* 13.7, 2.3 Hz], 2.73 [dd, 1H, $CH_2N(CH_2CH_2)_2O$, *J* 13.8, 5.3 Hz], 2.69–2.64 [m, 2H, $N(CH_2CH_2)_2O$], 2.48–2.43 [m, 2H, $N(CH_2CH_2)_2O$], 1.89–1.32 (m, 8H, C_4H_8Me), 1.32 (s, 3H, *Me*COH), 0.92 (m, 3H, C_4H_8*Me*). HRMS, *m*/*z*: 385.23324 $[M+H]^+$ (calc. for $C_{19}H_{32}N_2O_6$, *m*/*z*: 385.23331).

‡ A549 cancer cells were seeded at a density of 7500 cells/well into 96-well microplates and cultured for 24 h prior to exposure. The stock solutions were prepared in acetonitrile at a concentration of 10 mM and stored at –20 °C for no more than 2 weeks. Working solutions were prepared in complete medium just prior to the experiments. Cells were treated with PPA and its derivatives within the range of concentrations from 10 to 100 μM for 48 h (n = 3). Cells exposed to appropriate concentrations of acetonitrile solutions served as controls in each experiment though it was found that these concentrations did not affect the cells. Cell viability was evaluated by measuring the total protein content of wells using Sulforhodamine assay kit according to the manufacturer protocol (TOX6, Sigma). The data were obtained from three independent experiments. Statistical calculations were performed using GraphPad Prizm 5.0 software. Since the data were normally distributed (Kolmogorov−Smirnov test), further processing was carried out using parametric ANOVA. The Dunnett's test was used to compare the experimental data with the control values. Two-factor ANOVA was used to assess the nature and degree of influence of the two factors (the concentration and type of compound) on the viability assay data. The critical significance level (P) for the testing of statistical hypotheses was taken to be 0.05.

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