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Synthesis and antimitotic activity of novel 5-aminoisoxazoles bearing alkoxyaryl moieties

Dmitry A. Vasilenko,[a] Elena B. Averina,*[a,b] Nikolay A. Zefirov,[a] Birgit Wobith,[c] Yuri K. Grishin,[a] Victor B. Rybakov,[a] Olga N. Zefirova,*[a,b] Tamara S. Kuznetsova,[a] Sergei A. Kuznetsov[c] and Nikolay S. Zefirov[a,b]

[a] *Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation. Fax: +7 495 939 0290; e-mail: elaver@med.chem.msu.ru*

[b] *Institute of Physiologically Active Compounds, Russian Academy of Sciences, 142432 Chernogolovka, Moscow Region, Russian Federation. E-mail: olgaz@org.chem.msu.ru*

[c] *Institute of Biological Sciences, Cell Biology and Biosystems Technology, University of Rostock, D-18059 Rostock, Germany. E-mail: sergei.kuznetsov@uni-rostock.de*

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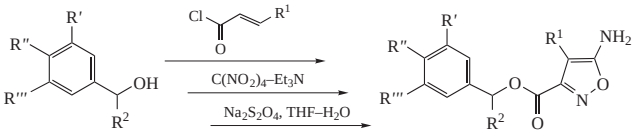
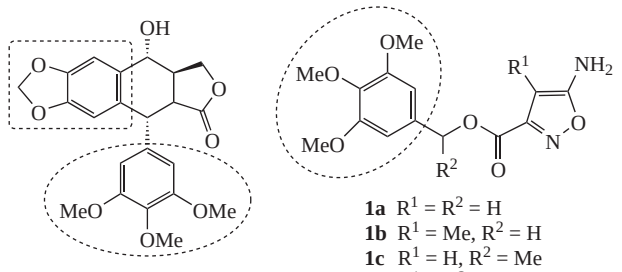
5-Aminoisoxazoles containing trimethoxy- and methylenedioxyphenyl moieties were prepared by heterocyclization of electrophilic alkenes with tetranitromethane in the presence of triethylamine with subsequent reduction of resulting 5-nitroisoxazoles. The crystal structure of 3,4,5-trimethoxybenzyl 5-aminoisoxazole-3-carboxylate was determined by X-ray analysis. Two novel 5-aminoisoxazoles demonstrated moderate antimitotic activity to human lung carcinoma A549 cell line.

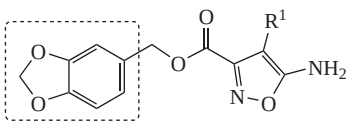


R′ = R″ = R‴ = OMe, R^1 = R^2 = H: EC_{50} (A549) = 38 μM
R′ = H, R″ + R‴ = OCH_2O, R^1 = R^2 = H: EC_{50} (A549) = 35 μM

Five-membered aromatic heterocycles are often applied in drug design as classical isosteric groups of substituted or non-substituted benzene rings of the lead compounds and quite often such a replacement leads to retention of biological activity.[1–3] Earlier we reported an easily accessible regioselective synthesis of 5-nitroisoxazoles by heterocyclization of electrophilic alkenes with tetranitromethane in the presence of triethylamine[4,5] and the following reduction ($SnCl_2$/EtOH or Zn/AcOH/Pr^iOH) of these compounds to the corresponding 5-aminoisoxazoles.[6] Elaboration of this method encouraged us to apply substituted 5-aminoisoxazole scaffolds in the design of compounds with different types of physiological activity, including putative antineoplastic agents.



1a R^1 = R^2 = H
1b R^1 = Me, R^2 = H
1c R^1 = H, R^2 = Me
1d R^1 = R^2 = Me



1e R^1 = H
1f R^1 = Me

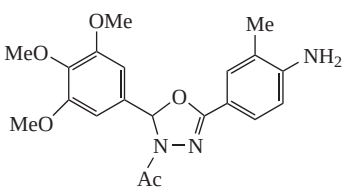
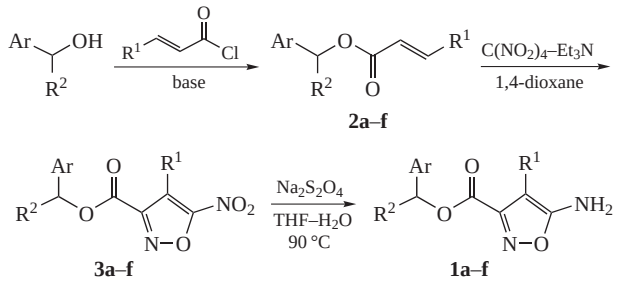


Figure 1 The structures of antineoplastic agents podophyllotoxin, A-105972 and compounds **1a**–**f** synthesized in the present work.

Many classical natural tubulin-targeted ligands, *e.g.* podophyllotoxin (Figure 1), interact with tubulin and inhibit its polymerization to microtubules.[7] 3,4,5-Trimethoxyphenyl and methylenedioxyphenyl moieties represent common structural fragments often found in the molecules of these ligands.[7,8] Therefore, we decided to introduce these moieties into 5-aminoisoxazole scaffolds and to synthesize compounds **1a**–**f** (see Figure 1). Interestingly, compounds **1a**–**d** can be considered as 'open' analogues of an antineoplastic agent A-105972 obtained using classical isosteric replacement of *o*-toluidine fragment by a methyl-substituted or unsubstituted 5-aminoisoxazole. The parent molecule A-105972 is known to possess noticeable cytotoxicity *in vitro* (EC_{50} = 0.003–0.2 μM depending on the cell line type) and to disrupt at high concentrations (100 μM) the tubulin polymerization to microtubules.[9]

The preliminary studies showed that compounds **1a**–**f** could not be obtained following strictly the described procedures.[4–6] Therefore, preparation of compounds **1a**–**f** was the purpose of the present work. The proposed method comprised acylation of the benzylic alcohols with appropriate acyl chloride followed by heterocyclization of thus obtained unsaturated esters **2**, and the final reduction of 5-nitroisoxazoles **3** into the corresponding 5-aminoisoxazoles **1** (Scheme 1).

To access the starting aryl-containing alkenes **2**, we used the synthetic protocol,[10,11] when the reaction between alcohols and acyl chlorides proceeded in the presence of Et_3N as a base. Under these conditions, acrylic esters **2a**,**c**,**e** were obtained in good



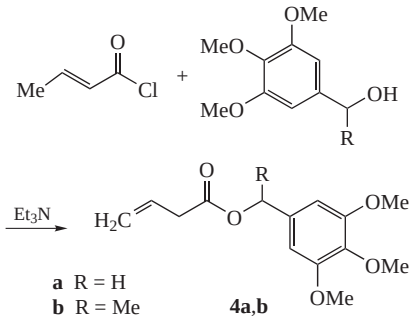
a Ar = 3,4,5-$(MeO)_3C_6H_2$, $R^1 = R^2 = H$
b Ar = 3,4,5-$(MeO)_3C_6H_2$, R^1 = Me, R^2 = H
c Ar = 3,4,5-$(MeO)_3C_6H_2$, R^1 = H, R^2 = Me
d Ar = 3,4,5-$(MeO)_3C_6H_2$, $R^1 = R^2$ = Me
e Ar = 3,4-methylenedioxyphenyl, $R^1 = R^2 = H$
f Ar = 3,4-methylenedioxyphenyl, R^1 = Me, R^2 = H

Scheme 1

Table 1 Synthesis of 5-aminoisoxazoles **1a**–**f**.

Compounds **1**–**3**	Isolated yields (%)		
	Ester **2** (base)	Nitroisoxazole **3**	Aminoisoxazole **1**
a	70 (Et_3N)	38	67
b	59 (Py)	33	50
c	64 (Et_3N)	29	30
d	34 (Py)	37	50
e	66 (Et_3N)	58	65
f	39 (Py)	57	58

preparative yields and no formation of side products was observed (Table 1).† However, the reaction of (3,4,5-trimethoxyphenyl)-methanol or 1-(3,4,5-trimethoxyphenyl)ethan-1-ol with crotonoyl chloride and Et_3N occurred with the double bond migration giving isomeric vinylacetates **4a**,**b** without traces of crotonates **2b** or **3d** (Scheme 2).



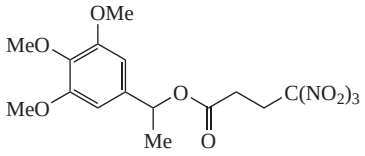
a R = H
b R = Me

4a,**b**

Scheme 2

To prepare the desired products **2b**,**d**,**f** we tried various known esterification conditions.[12–14] Such systems as Zn/benzene,[12] NaH/THF[13] or Al_2O_3 without solvent[14] were found to be unsuitable as they gave complex mixtures with a negligible amount of compound **2b**. We succeeded in obtaining ester **2b** as a sole product in a good preparative yield (59%, Table 1) using pyridine as a base in dichloromethane. This procedure was successfully extended onto esters **2d** and **2f** (Table 1).

Heterocyclization of electrophilic alkenes **2a**–**f** was performed by the action of *in situ* generated tetranitromethane–triethylamine complex [$C(NO_2)_4$–Et_3N] in 1,4-dioxane at 70 °C. The resulting 5-nitroisoxazoles **3a**–**d** were obtained in moderate yields owing to partial oxidation of alkenes **2a**–**d** leading to 3,4,5-trimethoxybenzaldehyde or 1-(3,4,5-trimethoxyphenyl)ethanone. Lowering the reaction temperature to ambient did not raise the yield due to the formation of 3,3,3-trinitropropanoic esters as the main products. For example, compound **5** was isolated from the reaction mixture of ester **2c** and $C(NO_2)_4$–Et_3N complex in 1,4-dioxane at 25 °C.



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Although we previously[6] prepared a series of various 5-aminoisoxazoles in good yields, the attempts to reduce 5-nitroisoxazoles **3a**–**f** into target 5-aminoisoxazoles **1a**–**f** under previously established conditions (*i.e.* $SnCl_2$/EtOH or Zn/AcOH/Pr^iOH) gave complex mixtures and no identified products were isolated. The variations of the reaction time and temperature or the amounts of reducing agents ($SnCl_2$ or Zn) in the reaction of model 5-nitroisoxazole **3e** did not significantly influence the formation of the target products. The application of a neutral reducing system Zn/NH_4Cl[15] was also shown to be inefficient. Luckily, system $Na_2S_2O_4$/THF/H_2O[16] allowed us to obtain the target 5-aminoisoxazoles in satisfactory yields (see Table 1). All synthesized compounds were characterized and identified using ^1H and ^{13}C NMR spectroscopy and HRMS or elemental analysis.‡

The structure of **1a** was unambiguously proved by X-ray diffraction analysis (Figure 2).§ Within the crystal lattice the molecules of **1a** arrange in a chain along the *a*-axis due to intermolecular hydrogen bonds between amino group and sp^2-nitrogen atom of isoxazole ring (see Online Supplementary Materials).

† For the synthesis and characteristics of all novel compounds, see Online Supplementary Materials.

‡ *Compounds* **1a**–**f** *(general procedure)*. To a stirred solution of 5-nitroisoxazole (0.15 mmol) in a mixture (1 : 1) THF–H_2O (2 ml), $Na_2S_2O_4$ (75 mg, 0.45 mmol) was added in one portion at room temperature. The resulting mixture was stirred for 1 h at 90 °C. The mixture was cooled, diluted with water (5 ml) and extracted with EtOAc (3 × 10 ml). The organic layers were dried over $MgSO_4$ and evaporated *in vacuo*; the residue was purified by column chromatography on silica gel (light petroleum–EtOAc, 1:1).

3,4,5-Trimethoxybenzyl 5-aminoisoxazole-3-carboxylate **1a**: colourless solid, mp 144–145 °C, R_f 0.4 (light petroleum–EtOAc, 1 : 1). ^1H NMR (400 MHz, $CDCl_3$–CD_3OD) δ: 3.20 (br. s, 2H, NH_2), 3.74 (s, 3H, OMe), 3.77 (s, 6H, 2OMe), 5.19 (s, 2H, CH_2), 5.39 (s, 1H, CH), 6.58 (s, 2H, $2CH_{Ar}$). ^{13}C NMR (101 MHz, $CDCl_3$–CD_3OD) δ: 56.0 (2OMe), 60.7 (OMe), 67.5 (CH_2), 79.6 (CH), 105.8 ($2CH_{Ar}$), 130.6 (C_{Ar}), 137.9 (C_{Ar}), 153.1 ($2C_{Ar}$), 156.9 (C), 160.4 (C=O), 170.8 (CNH_2). HRMS (ESI), *m*/*z*: 331.0901 $[M + Na]^+$ (calc. for $C_{14}H_{16}N_2O_6Na^+$, *m*/*z*: 331.0901). Found (%): C, 54.62; H, 5.27; N, 8.70. Calc. for $C_{14}H_{16}N_2O_6$ (%): C, 54.54; H, 5.23; N, 9.09. All other analytical data of obtained compounds are described in Online Supplementary Materials.

§ *Crystallographic data*. Crystals of **1a** (from $CDCl_3$), $C_{14}H_{16}N_2O_6$, M = 308.29, monoclinic, space group $P2_1/c$, at 295(2) K: a = 5.9317(3), b = 20.9814(6) and c = 11.9352(5) Å, β = 94.615(4), V = 1480.58(11) Å^3, Z = 4, d_{calc} = 1.383 mg m^{-3}, μ = 0.929 mm^{-1}, $F(000)$ = 648. Intensities of 2909 reflections were measured with STADI VARI Pilatus-100 K diffractometer, using focusing mirrors CuKα radiation (λ = 1.5418 Å, $4.21 < \theta < 72.79°$) and 2909 independent reflections (R_{int} = 0.0000) were used in a further refinement. The structure was solved by direct method and refined by the full-matrix least-squares technique against F^2 with anisotropic thermal parameters. All hydrogen atoms were refined with a riding model and a mutual isotropic thermal parameter. The refinement converged to wR_2 = 0.1368 and GOF = 0.923 for all independent reflections [R_1 = 0.0553 was calculated against F for reflections with $I > 2\sigma(I)$]. All calculations were performed using SHELX-97 and SHELXL-2014. The drawings were created with the PLATON program.

CCDC 1474822 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* http://www.ccdc.cam.ac.uk.

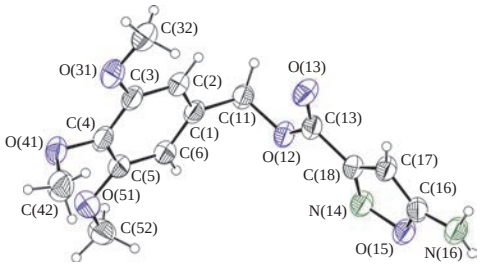

Figure 2 The general view of 3,4,5-trimethoxybenzyl 5-aminoisoxazole-3-carboxylate **1a** in representation of atoms by thermal ellipsoids (p = 50%).

Novel substituted 5-aminoisoxazoles were examined for their cytotoxicity to epithelial lung carcinoma cancer cells A549 in a standard colorimetric MTT assay using the procedure described[17–19] (Table 2; for details, see Online Supplementary Materials). Their cytotoxicity turned out to be inferior to that of podophyllotoxin or A-105972. Nevertheless, two compounds of the series, namely **1a** and **1e**, were moderately cytotoxic with EC_{50} values in the micromolar concentration range. The data obtained demonstrated that the introduction of methyl substituent either to the isoxazole or to the linker chain (compounds **1b**–**d**,**f**) led to a decrease in cytotoxicity. Interestingly, the equal EC_{50} values for **1a** and **1e** indicated the equivalence of the trimethoxyphenyl and methylenedioxyphenyl rings for the cytotoxic activity of these compounds.

We also studied the effect of compounds **1a**, **1e** and **1f** (100 μM) on cell growth using direct cell counting by microscopy over 24, 48 and 72 h of culturing (Figure 3). The compounds strongly inhibited cell proliferation, and the extent of inhibition correlated with the cytotoxicity of compounds **1a**,**e**,**f** (see Table 2). Thus, cell growth experiments confirmed the MTT data.

In conclusion, a three-step synthetic protocol was accomplished for the preparation of substituted 5-aminoisoxazoles with polymethoxyphenyl- and methylenedioxyphenyl moieties. The optimization of the first and the third steps of the protocol was required in comparison with the earlier established conditions. A gentle reagent was found for the reduction of nitro group in isoxazole core. Two biotests revealed moderate antimitotic activity of compounds **1a** and **1e** to human lung carcinoma A549 cell line. These compounds are of interest for additional investigation of the mechanism of their antiproliferative properties.

Table 2 Cytotoxicity of compounds **1a**–**f** to human lung carcinoma cell line A549.

Compound	Cytotoxicity (EC_{50}, μM)	Cell growth inhibition (%)[a]
1a	38±4	91
1b	>100	–
1c	>100	–
1d	>100	–
1e	35±5	100
1f	93±7	55
Podophyllotoxin	0.04	–

[a]After 48 h of culturing.

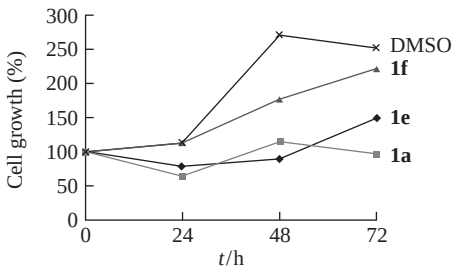

Figure 3 Effect of compounds **1a**, **1e** and **1f** on cell growth in culture. The cells were incubated with 100 μM of each compound and after resuspension were counted by phase-contract microscopy using hemocytometer. 1% DMSO was used as a control.

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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.2017.05.003.

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