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New triazole-containing 7-azacoumarin-3-carboxamides: synthesis and biological properties

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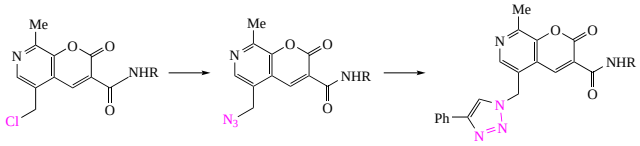
New azidomethyl-substituted 7-azacoumarin-3-carboxamides were converted into the corresponding 1,2,3-triazole hybrids by the copper-catalyzed click reaction with phenylacetylene. Cytotoxicity and antimicrobial activity were evaluated, and leading compounds were identified.



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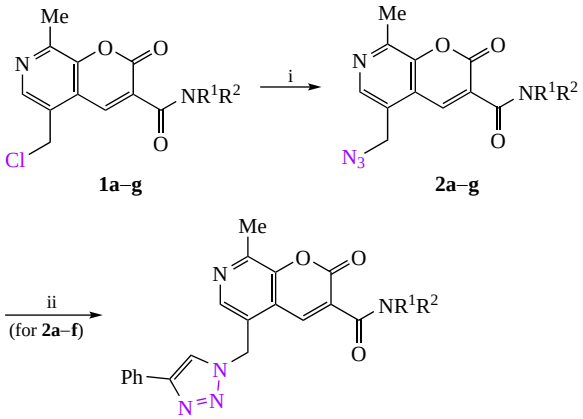
A current and important task of modern organic chemistry is the targeted synthesis of new types of effective drugs. This task has become especially important recently due to the resistance of microorganisms to known antimicrobial and antiviral drugs, as well as due to the rapid increase in the number of cancer diseases, which poses a serious threat to human health around the world. More than 70% of compounds among the large library of organic compounds used as drugs are cyclic and heterocyclic ones. A special place among them is occupied by substances containing coumarin and azacoumarin fragments responsible for antibacterial,[1–3] antimicrobial,[4] fungicidal,[5] antioxidant,[6–8] antitumor[2,9–12] and anti-HIV[13–16] activities. These compounds can also be used as fluorescent probes[17–19] and insecticides.[20] It should be especially noted that 7-hydroxycoumarin-3-carboxamides are biologically active against physiologically significant human carbonic anhydrases and can serve as a promising basis for the development of antitumor drugs.[21]

1,2,3-Triazole[22–24] is another important heterocyclic fragment used in the development of biologically active compounds which exhibit antiviral (including anti-HIV) and antibacterial effects.[25] Compounds incorporating 1,2,3-triazole are also used as ligands in the synthesis of new catalysts for intramolecular hydroamination of alkynes.[26]

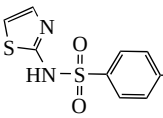
One of the promising methods for the molecular design of effective drugs is the combination of several pharmacophore groups in one molecule, that is, the creation of 'hybrid' structures. Previously, we proposed a method for the synthesis of 7-azacoumarin-3-carboxamides[27] which demonstrated high antitumor activity. The presence of a chloromethyl group in the position 5 of the azacoumarin system allows one to use these compounds in the synthesis of biologically active 'hybrid' molecules. The introduction of a triazole fragment into the 7-azacoumarin-3-carboxamide molecule will make it possible to obtain new 'hybrid' compounds for evaluating their antimicrobial and antitumor activity.

One of the most common syntheses of 1,2,3-triazoles is the azide–alkyne copper-catalyzed cycloaddition reaction (click reaction, CuAAC). The advantages of this method are mild reaction conditions, stereospecificity, high yields, and easy isolation of the product.[28]

In this work, the synthesis of target triazoles was carried out as a two-step one. The first step involved azidation of 5-chloromethyl-7-azacoumarin-3-carboxamides **1a–g**, which resulted in the corresponding azides **2a–g** (Scheme 1). The reaction proceeds easily in acetone in the presence of a minimal amount of water. The product yields were 57–82%.† The IR spectra of compounds **2a–g** contained intense absorption bands in the



1a–g **2a–g** **3a–f**

a R^1 = Ph, R^2 = H
b R^1 = 2-$MeOC_6H_4$, R^2 = H
c R^1 = R^2 = Ph
d R^1 = PhC(O)NH, R^2 = H
e R^1 = 4-$(H_2NSO_2)C_6H_4$, R^2 = H
f R^1 =  , R^2 = H
g R^1 = 4-$(AcNHSO_2)C_6H_4$, R^2 = H

Scheme 1 *Reagents and conditions*: i, NaN_3, acetone/water, room temperature; ii, PhC≡CH, $CuSO_4$, sodium ascorbate, MeCN, reflux, argon atmosphere.

† *General procedure for the synthesis of compounds* **2a–g**. A twofold excess of sodium azide dissolved in a minimal amount of water was added at stirring to a suspension of the corresponding acid amide in acetone (3 ml) at room temperature. The next day, the precipitate was separated and washed with water (5 ml) and then with acetone (5 ml) (in the case of compounds **2a–c**, the reaction mixture was heated at 85 °C for 6.5 h, then the precipitate was filtered off, washed with water (5 ml), then with acetone (5 ml), and dried under reduced pressure).

Table 1 Cytotoxic effect (IC_{50}/μM values) for compounds **2a**–**g** and **3a**–**f** against cancer and normal human cell lines.[a]

Compound	Tumor cell line[b]		Normal cell line
	M-HeLa	HuTu 80	Chang liver
2a	82.4 ± 17	44.1 ± 10	54.5 ± 3.6
2b	90.7 ± 6.3	62.9 ± 3.0	72.0 ± 2.1
2c	84.7 ± 6.7	80.8 ± 14	78.3 ± 17
2d	55.8 ± 3.9	28.9 ± 2.0	53.3 ± 3.7
2e	85.0 ± 12	77.2 ± 7.7	72.8 ± 10
2f	58.6 ± 4.1 (1.9)	80.2 ± 6.4	110.0 ± 8.3
2g	67.1 ± 5.4	99.3 ± 7.0	93.0 ± 6.5
3a	82.8 ± 14	8.2 ± 0.7 (4.5)	36.5 ± 3.1
3b	86.1 ± 23	25.5 ± 10 (3.1)	79.6 ± 5.5
3c	74.6 ± 10	45.5 ± 2.5 (1.6)	73.5 ± 23
3d	52.6 ±3.7	88.2 ± 6.4	66.6 ± 4.6
3e	83.0 ± 10	97.8 ± 0.1	98.1 ± 4.1
3f	72.4 ± 5.5 (1.4)	70.1 ± 5.2 (1.5)	104.0 ± 7.3
5-Fluorouracil	62.0 ± 4.7	65.2 ± 5.4	86.3 ± 6.5
Sorafenib	35.6 ± 2.8	5.0 ± 0.4	35.0 ± 2.6

[a] Three independent experiments were performed. [b] Selectivity index (SI) is given in parentheses.

region of 2086–2151 cm^{-1} characteristic of the azide group. The ^1H NMR spectra of chlorides **1a**–**g** contain the singlet for the methylene protons at the position 5 of the azacoumarin ring in the region of 5.22–5.29 ppm, while for azides **2a**–**g** this signal is manifested in the somewhat upfield region of 4.77–4.98 ppm.

As the second step, azides **2a**–**f** were coupled with phenylacetylene under the conditions of the CuAAC reaction to afford the corresponding 1,2,3-triazoles **3a**–**f** (see Scheme 1). The reaction proceeded in acetonitrile solution under argon atmosphere and 6 h boiling, the yields of triazoles **3a**–**f** were 77–96%.‡ The composition and structure of the obtained products were confirmed by ESI mass spectrometry, elemental analysis, ^1H and ^{13}C NMR spectroscopy. In the ^1H NMR spectra of compounds **3a**–**f**, along with the signals for the protons of the 7-azacoumarin-3-carboxamide fragment, a signal of the methine proton of the triazole ring was detected, and a downfield shift of the signals of the methylene group was also observed (δ 5.99–6.16 ppm).

The resulting compounds were tested for cytotoxicity against tumor cell lines of cervical carcinoma and duodenal adenocarcinoma, and their toxicity was also tested. Cytotoxicity in human cancer and normal cell lines was determined at concentrations of 1–100 μM. Table 1 contains IC_{50} data for the compounds under study. All of them showed biological activity, for most of them it is comparable to that of the reference drug 5-Fluorouracil. The cytotoxic effect of the tested compounds against cancer cell lines was observed in the concentration range of 8.2–90.7 μM. The undoubted leader in this series is compound **3a**. Its cytotoxicity and toxicity are at the level of the reference drug Sorafenib.

The antimicrobial (bacteriostatic and fungistatic) activity of the synthesized compounds was studied *in vitro*. The results obtained show that within the concentration range of 0.97–500 μg ml^{-1} most of the tested compounds do not manifest antimicrobial activity. The exceptions are **2d**,**f**, **3d**, data for them are given in Table 2.

Table 2 Antimicrobial activity of the studied compounds as minimum inhibitory concentrations.

Compound	MIC/μg ml^{-1}	
	S. aureus[a]	*E. faecalis*[b]
2d	62.5 ± 5.2	62.5 ± 4.5
2f	62.5 ± 4.8	250.0 ± 19
3d	125.0 ± 9	125.0 ± 11
Chloramphenicol	62.5 ± 5.2	62.5 ± 5.2

[a] *Staphylococcus aureus* ATCC 6538P FDA 209P. [b] *Enterococcus faecalis* ATCC 29212.

In summary, new 'hybrid' compounds were obtained, namely, azides and 1,2,3-triazoles containing a 7-azacoumarin-3-carboxamide fragment. These compounds were tested for antimicrobial (bacteriostatic and fungistatic) activity and cytotoxicity against tumor cell lines M-HeLa and HuTu 80. Among tested compounds, antimicrobial activity was exhibited only by **2d**,**f** and **3d**. At the same time, the cytotoxicity of most of the studied compounds is comparable to that of the reference drug 5-Fluorouracil. The leading compound in this series is **3a** whose cytotoxicity and toxicity are at the level of the reference drug Sorafenib.

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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.2024.09.020.

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‡ *General procedure for the synthesis of compounds* **3a**–**f**. To a suspension of the corresponding azide **2** in dry acetonitrile (3 ml), under argon copper sulfate and sodium ascorbate were added at a ratio of 1 : 0.5 : 1 at stirring, then an equimolar amount of phenylacetylene was added dropwise and the reaction mixture was heated at 90 °C (bath) for 6 h. The next day, the precipitate was separated, washed with 10 ml of dry acetonitrile, and dried *in vacuo*.

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