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New 7-azacoumarin-3-carboxamide phosphonium salts: cytotoxicity and the Wittig olefination

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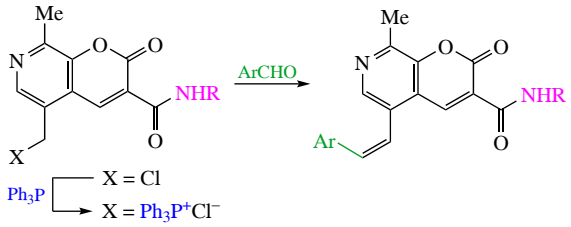
New phosphonium salts synthesized from 5-chloromethyl-8-methyl-7-azacoumarin-3-carboxamides and triphenylphosphine upon the Wittig reaction with aromatic aldehydes give the corresponding 5-styryl-7-azacoumarin derivatives. The cytotoxic activity of some phosphonium salts against cancer cell lines M-HeLa and HuTu-80 is equal to or superior to that of the reference 5-fluorouracil. The IC_{50} and SI values of the leading compounds exceed those for 5-fluorouracil by a factor of 3.7 and 2.2, respectively.



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Widespread in nature coumarins exhibit a wide range of biological activity,[1–3] in particular, anticancer activity.[4–6] A promising synthetic platform among them is coumarin-3-carboxamide whose derivatives have shown high activity against various types of cancer cell lines.[7–12] Derivatives of coumarin-3-carboxylic acid were employed for the fluorescent detection[13] of various compounds including cysteine and homocysteine[14] while coumarin-3-carboxamides served as fluorescent probes for metal ions, *e.g.* Al^{3+}, Ba^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, as well as β-oxygenated fatty acids.[15,16] One of the strategies for creating biologically active systems is based on modifying the basic structure of a molecule with known activity to obtain a new chemotype while maintaining the biological properties of the original compound.[17–19] The simplest modification of coumarins by replacing a carbon atom with a nitrogen atom in the ring leads to their aza analogues, which made it possible to reduce the susceptibility of the compounds to oxidative metabolism,[20] thereby improving their bioactivity profile.

The Schneider's concept[19] was confirmed in our publication[21] on the synthesis of a new biologically active 7-azacoumarin-3-carboxamide platform. We have obtained a large number of compounds and have shown that almost all of them have high antitumor activity and low toxicity.

The presence of an active chloromethyl group in the 7-azacoumarin-3-carboxamide platform opens up opportunities for further functionalization in the search for new substances with high antitumor activity. Thus, replacing the chlorine atom in the chloromethyl group with an azido substituent and further introducing it into the click reaction made it possible to obtain the corresponding 1,2,3-triazoles.[22] The cytotoxicity of most of these compounds was comparable to that of the drug 5-fluorouracil, and for some of them it was at the level of the comparison drug sorafenib. In development of these works on further functionalization of the 7-azacoumarin-3-carboxamide platform, it was of interest to introduce a triphenylphosphonium moiety into this molecule. Analysis of the literature has shown that in recent years there has been a rapid increase in the number of publications devoted to phosphonium salts.[23] This is due to their various practically important properties: antibacterial[24,25] and antitumor activity,[26,27] use as organocatalysts in asymmetric synthesis,[28] as well as in a number of other areas.[29] Herein, we assumed that the introduction of a phosphonium fragment into the 7-azacoumarin-3-carboxamide molecule could lead to increased cytotoxic activity.

First, we synthesized 7-azacoumarin-3-carboxamides **1a–d** by the previously described method.[21] The reaction of carboxamides with triphenylphosphine was carried out solvent-free by heating the reaction mixture at 150–170 °C under an argon atmosphere. New phosphonium salts **2a–d** (Scheme 1) were obtained with yields of 21–89%. The structures of the obtained compounds were confirmed by ^{1}H, ^{13}C, ^{31}P NMR, IR spectroscopy, ESI/MALDI mass spectrometry, and elemental analysis. In the mass spectra, one signal corresponding to the molecular ion of the expected product was observed. In the ^{31}P NMR spectra, signals in the region of 23 ppm typical of compounds with a four-coordinated phosphorus atom were detected. Characteristic changes occurred with the 5-positioned methylene group at the pyridine ring whose protons resonated as

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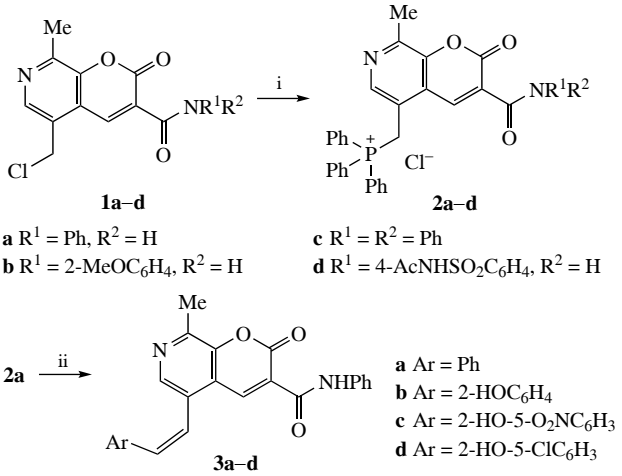

Scheme 1 *Reagents and conditions*: i, Ph_3P, argon, solvent-free, 150–170 °C; ii, ArCHO, Bu^tOK, argon, EtOH, 0 °C.

doublets at δ 5.7–5.8 ppm with coupling constant J_{PH} = 15.8 Hz, while in the original amides **1a–d** such protons appeared as singlets in the region of 5.08–5.29 ppm.

Phosphonium salts are known to be the effective reactants for the synthesis of unsaturated compounds under the Wittig reaction conditions.[30] To study the synthetic possibilities of the obtained phosphonium salts, we carried out a reaction of representative compound **2a** with benzaldehydes which provided olefins **3a–d**. The reaction was performed in an alcohol solution under argon at cooling, the yields were 15–36%. Olefins **3a–d** were formed as *Z*-isomers.

To study the effect of the phosphonium fragment on the cytotoxic activity of the synthesized compounds, we tested a series of compounds **1a–c** and **2a–c** against tumor cell lines of cervical carcinoma (M-HeLa) and human duodenal adenocarcinoma (HuTu-80) (Table 1). Analysis of the structure–activity relationship for the studied compounds showed that in the initial amides **1a** and **1b**, low cytotoxicity was observed towards M-HeLa cells (IC_{50} of >100 and 84.4 μM) and moderate cytotoxicity towards the HuTu-80 and Chang liver cell lines (IC_{50} = 59.4 and 56.6 μM for **1a**, and IC_{50} = 55.3 and 46.0 μM for **1b**).

Moving from *N*-phenyl- (**1a**) and *N*-(*o*-methoxyphenyl)-substituted (**1b**) derivatives to compound **1c** with NPh_2 moiety led to a significant increase in cytotoxicity against the M-HeLa and HuTu-80 cancer lines (IC_{50} = 32.0 and 17.4 μM). At the same time, the activity against the conditionally normal Chang liver cell line for compound **1c** changed insignificantly. Replacing chlorine atom in the chloromethyl fragment with a phosphonium group (compounds **2a–c**) led to a slight increase in the activity of derivatives **2a** and **2b** against cancer cell lines and a decrease in cytotoxicity towards the conditionally normal Chang liver cell line by a factor of 1.5 and 2, respectively.

Table 1 Cytotoxic effect of compounds **1a–c** and **2a–c** on cancer and normal human cell lines.

Compound	IC_{50}/μM[a]		
	Tumor cell line		Normal cell line
	M-HeLa[b]	HuTu-80[c]	Chang liver
1a	>100 (–)	59.4 ± 4.8 (–)	56.6 ± 4.5
1b	84.4 ± 5.5 (–)	55.3 ± 3.9 (–)	46.0 ± 3.2
1c	32.0 ± 2.2 (1.5)	17.4 ± 1.3 (2.8)	48.4 ± 3.3
2a	81.7 ± 2.9 (1.0)	63.6 ± 4.1 (1.3)	85.2 ± 3.4
2b	70.0 ± 4.7 (1.4)	43.1 ± 4.5 (2.3)	101.0 ± 4.1
2c	40.0 ± 1.2 (1.2)	17.2 ± 3.6 (2.8)	49.0 ± 4.2
5-Fluorouracil	62.0 ± 4.7 (1.4)	65.2 ± 5.4 (1.3)	86.3 ± 6.5

[a] Selectivity indices (SI values) are given in parentheses, (–) is non-selective. [b] M-HeLa is a human cervix epitheloid carcinoma. [c] HuTu-80 is a duodenal adenocarcinoma. The experiments were repeated three times, the results are expressed as the average ± standard deviation.

A similar modification of the structure of compound **2c** had absolutely no effect on its cytotoxicity against all cell lines. In the series tested, two lead compounds **1c** and **2c** were identified, which showed the highest cytotoxic activity against the human duodenal adenocarcinoma cell line (HuTu-80). The IC_{50} and selectivity indices (SI) values of the leading compounds exceed those for 5-fluorouracil by a factor of 3.7 and 2.2, respectively.

To summarize, new phosphonium salts were synthesized on the platform of 7-azacoumarin-3-carboxamides. These salts undergo the Wittig reaction with aromatic aldehydes to form new types of olefins. The resulting phosphonium salts were tested for cytotoxicity, and leading compounds were found among them.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.71267/mencom.7750.

References

1 Ł. Balewski, S. Szulta, A. Jalińska and A. Kornicka, *Front. Chem.*, 2021, **9**, 781779; https://doi.org/10.3389/fchem.2021.781779.
2 D. Srikrishna, C. Godugu and P. K. Dubey, *Mini-Rev. Med. Chem.*, 2018, **18**, 113; https://doi.org/10.2174/1389557516666160801094919.
3 F. Annunziata, C. Pinna, S. Dallavalle, L. Tamborini and A. Pinto, *Int. J. Mol. Sci.*, 2020, **21**, 4618; https://doi.org/10.3390/ijms21134618.
4 M. Kaur, S. Kohli, S. Sandhu, Y. Bansal and G. Bansal, *Anticancer Agents Med. Chem.*, 2015, **15**, 1032; https://doi.org/10.2174/1871520615666150101125503.
5 A. Thakur, R. Singla and V. Jaitak, *Eur. J. Med. Chem.*, 2015, **101**, 476; https://doi.org/10.1016/j.ejmech.2015.07.010.
6 X.-F. Song, J. Fan, L. Liu, X.-F. Liu and F. Gao, *Arch. Pharm.*, 2020, **353**, 2000025; https://doi.org/10.1002/ardp.202000025.
7 S. Awale, T. Okada, D. F. Dibwe, T. Maruyama, S. Takahara, T. Okada, S. Endo and N. Toyooka, *Bioorg. Med. Chem. Lett.*, 2019, **29**, 1779; https://doi.org/10.1016/j.bmcl.2019.05.010.
8 N. S. Reddy, K. Gumireddy, M. R. Mallireddigari, S. C. Cosenza, P. Venkatapuram, S. C. Bell, E. P. Reddy and M. V. R. Reddy, *Bioorg. Med. Chem.*, 2005, **13**, 3141; https://doi.org/10.1016/j.bmc.2005.02.051.
9 X. Yu, P. Teng, Y.-L. Zhang, Z.-J. Xu, M.-Z. Zhang and W.-H. Zhang, *Fitoterapia*, 2018, **127**, 387; https://doi.org/10.1016/j.fitote.2018.03.013.
10 H. Ji, Y. Tan, N. Gan, J. Zhang, S. Li, X. Zheng, Z. Wang and W. Yi, *Bioorg. Med. Chem.*, 2021, **29**, 115870; https://doi.org/10.1016/j.bmc.2020.115870.
11 W. Phutdhawong, A. Chuenchid, T. Taechowisan, J. Sirirak and W. S. Phutdhawong, *Molecules*, 2021, **26**, 1653; https://doi.org/10.3390/molecules26061653.
12 J. Ma, K. Huang, X. Ni, R. Chen, B. Xu and C. Wang, *Chem. Res. Chin. Univ.*, 2019, **35**, 410; https://doi.org/10.1007/s40242-019-8310-7.
13 C.-X. Jiao, C.-G. Niu, L.-X. Chen, G.-L. Shen and R.-Q. Yu, *Anal. Bioanal. Chem.*, 2003, **376**, 392; https://doi.org/10.1007/s00216-003-1909-y.
14 Y. Wang, G. Lu, Y. Tu and S. Pu, *J. Fluoresc.*, 2021, **31**, 599; https://doi.org/10.1007/s10895-021-02684-6.
15 S. Warrier and P. S. Kharkar, *Spectrochim. Acta, Part A*, 2018, **188**, 659; https://doi.org/10.1016/j.saa.2017.07.068.

16 S. Uchinomiya, N. Matsunaga, K. Kamoda, R. Kawagoe, A. Tsuruta, S. Ohdo and A. Ojida, *Chem. Commun*., 2020, **56**, 3023; https://doi.org/10.1039/C9CC09993J.
17 Y. Hu, D. Stumpfe and J. Bajorath, *J. Med. Chem*., 2017, **60**, 1238; https://doi.org/10.1021/acs.jmedchem.6b01437.
18 H. Sun, G. Tawa and A. Wallqvist, *Drug Discov. Today*, 2012, **17**, 310; https://doi.org/10.1016/j.drudis.2011.10.024.
19 G. Schneider, W. Neidhart, T. Giller and G. Schmid, *Angew. Chem., Int. Ed*., 1999, **38**, 2894; https://doi.org/10.1002/(SICI)1521-3773(19991004)38:19%3C2894::AID-ANIE2894%3E3.0.CO;2-F.
20 P. R. Lazzara and T. W. Moore, *RSC Med. Chem*., 2020, **11**, 18; https://doi.org/10.1039/C9MD00396G.
21 A. V. Trifonov, A. S. Gazizov, A. S. Tapalova, L. K. Kibardina, N. O. Appazov, A. D. Voloshina, A. S. Sapunova, A. P. Luybina, G. M. Abyzbekova, A. B. Dobrynin, I. A. Litvinov, A. K. Tauekel, S. O. Yespenbetova, A. R. Burilov and M. A. Pudovik, *Int. J. Mol. Sci.*, 2023, **24**, 9927; https://doi.org/10.3390/ijms24129927.
22 A. V. Trifonov, R. K. Bagautdinova, L. K. Kibardina, M. A. Pudovik, A. P. Lyubina, A. D. Voloshina, A. S. Gazizov and A. R. Burilov, *Mendeleev Commun*., 2024, **34**, 688; https://doi.org/10.1016/j.mencom.2024.09.020.
23 M. Grymel, M. Zawojak and J. Adamek, *J. Nat. Prod*., 2019, **82**, 1719; https://doi.org/10.1021/acs.jnatprod.8b00830.
24 I. Yu. Strobykina, M. G. Belenok, M. N. Semenova, V. V. Semenov, V. M. Babaev, I. Kh. Rizvanov, V. F. Mironov and V. E. Kataev, *J. Nat. Prod*., 2015, **78**, 1300; https://doi.org/10.1021/acs.jnatprod.5b00124.
25 O. V. Tsepaeva, A. V. Nemtarev, T. I. Abdullin, L. R. Grigor'eva, E. V. Kuznetsova, R. A. Akhmadishina, L. E. Ziganshina, H. H. Cong and V. F. Mironov, *J. Nat. Prod*., 2017, **80**, 2232; https://doi.org/10.1021/acs.jnatprod.7b00105.
26 M. Grymel, A. Lalik, A. Kazek-Kęsik, M. Szewczyk, P. Grabiec and K. Erfurt, *Molecules*, 2022, **27**, 5156; https://doi.org/10.3390/molecules27165156.
27 J. S. Modica-Napolitano and K. Singh, *Expert Rev. Mol. Med*., 2002, **4**, 1; https://doi.org/10.1017/s1462399402004453.
28 L. Chen, Y. Deng, T. Li, D. Hu, X. Ren and T. Wang, *CCS Chem*., 2024, **6**, 2110; https://doi.org/10.31635/ccschem.024.202404039.
29 N. Noroozi-Shad, M. Gholizadeh and H. Sabet-Sarvestani, *J. Mol. Struct*., 2022, **1257**, 132628; https://doi.org/10.1016/j.molstruc.2022.132628.
30 M. Selva, A. Perosa and G. Fiorani, in *Organophosphorus Chemistry*, eds. D. W Allen, D. Loakes and J. C. Tebby, Royal Society of Chemistry, 2019, vol. 48, pp. 145–198; https://doi.org/10.1039/9781788016988.

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