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Mendeleev Commun., 2021, **31**, 664–666

Mendeleev
Communications

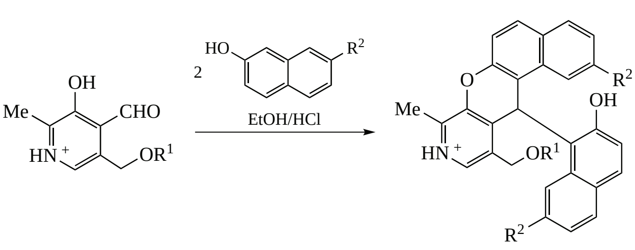
Anticancer activity of novel 3-azaxanthenes

Lyudmila K. Kibardina, Alexey V. Trifonov,* Alexey B. Dobrynin, Michail A. Pudovik, Alexander R. Burilov, Alexandra D. Voloshina, Anna G. Strelnik and Almir S. Gazizov

A. E. Arbuzov Institute of Organic and Physical Chemistry, FRC Kazan Scientific Center of the Russian Academy of Sciences, 420088 Kazan, Russian Federation. E-mail: alexey.trifonov@iopc.ru

DOI: 10.1016/j.mencom.2021.09.024

Novel 3-azaxanthenes have been synthesized *via* acid-catalyzed one-pot Friedel–Crafts reaction of pyridoxal/pyridoxal-5′-phosphate with naphthols followed by dehydration of the intermediate bis(2-hydroxyaryl)-(pyridin-4-yl)methane derivatives. The cytotoxicity of the obtained 3-azaxanthenes against M-HeLa and HuTu-80 tumor cell lines is comparable to that of tamoxifen, whereas the cytotoxicity against normal cell line is absent in the tested concentrations range, which makes this class of compounds interesting for further studies.



Keywords: 3-azaxanthenes, chromeno[2,3-*c*]pyridines, pyridoxal, naphthol, cyclization, cytotoxicity, anticancer activity.

The tricyclic xanthene is a major scaffold for a range of natural and synthetic products that exhibit various biological activities.[1,2] Azaxanthenes also modulate the physicochemical properties of xanthones. However, reports on azaxanthenes, especially 3-azaxanthenes (chromeno[2,3-*c*]pyridines) are relatively scarce. The approaches to substituted 3-azaxanthenes are limited to the intramolecular Friedel–Crafts acylation of 3-aryloxy-isonicotinic derivatives[3–5] or intramolecular nucleophilic substitution of phenyl(pyridin-4-yl)methanones[6] and 2-(pyridin-3-yloxy)benzamides.[7–9] Thus, the development of more convenient approach to these compounds is still on demand.

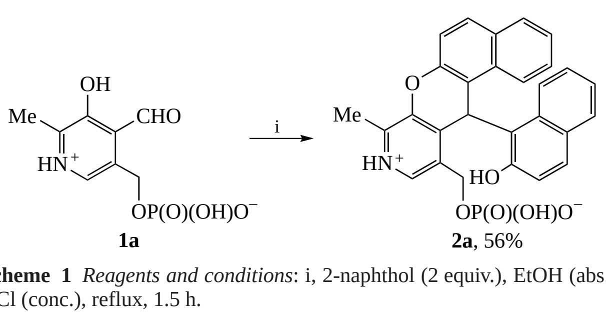
The most straightforward access to xanthenes involves the dehydration of bis(2-hydroxyaryl)methanes,[10–12] which are easily prepared by the Friedel–Crafts alkylation of phenols with aldehydes.[2,13,14] However, to the best of our knowledge, this approach has never been employed in the synthesis of 3-azaxanthenes.

Earlier, we described the reaction of pyridoxal with phenols, which resulted in 1-arylfuropyridines *via* intramolecular hemiacetal formation.[15] However, when 4-hydroxycoumarin was utilized, the reaction furnished the mixture of 1-ethoxyfuropyridine and benzochromenopyranopyridine.[16] The formation of the latter was best explained by the intramolecular dehydration of bis(4-hydroxycoumarin-3-yl)(2-hydroxy-pyridin-4-yl)methane derivative. We envisioned that the other bis(2-hydroxyaryl)(2-hydroxypyridin-4-yl)methanes could also be subjected to such a dehydration, thus providing easy access to 3-azaxanthenes. As a result of our investigations in this area, herein we report the simple synthesis of 3-azaxanthenes starting from commercially available pyridoxal or pyridoxal-5′-phosphate (PLP) and electron-rich phenols.

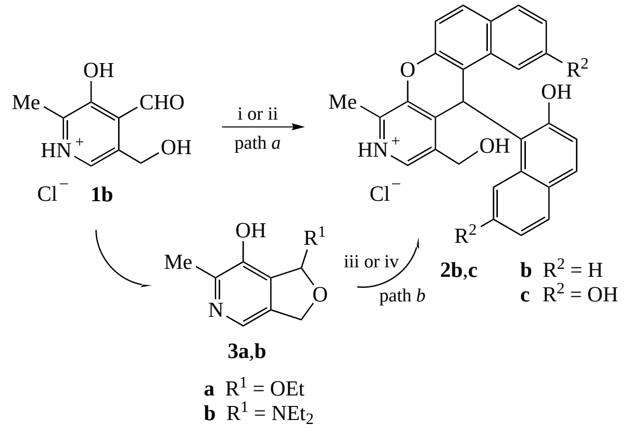
We initiated our studies with the synthesis of hydroxy-substituted diaryl(pyridin-4-yl)methanes. The simplest approach to these compounds would involve the Friedel–Crafts alkylation of phenols with pyridoxal, which is easily available isonicotin-aldehyde derivative bearing necessary hydroxyl group. Since the reaction of pyridoxal with electron-rich aromatics results in the furopyridines,[16] its O-protected derivative, pyridoxal-5′-phosphate (PLP) **1a**, was used as the starting compound. Pleasingly, the reaction of PLP with 2-naphthol under the conditions reported before[16] resulted in 3-azaxanthene derivative **2a** in 56% yield (Scheme 1). Interestingly, no hydrolysis of the phosphoryl group occurred in strongly acidic media. The ^{31}P NMR data suggests the presence of ionized phosphoryl group, and no chlorine counter-ion was found by elemental analysis; hence, the betaine structure was assigned to compound **2a**.

Surprisingly, the reaction of the O-unprotected pyridoxal **1b** with 2-naphthol and naphthalene-2,7-diol under the same conditions furnished analogous 3-azaxanthene derivatives **2b**,**c** (Scheme 2, path *a*). This fact is in a sharp contrast with other phenols, which gave 1-arylfuropyridines in the reaction with pyridoxal.[15] Compound **2c** in addition to the presence of chiral carbon atom exhibits the axial chirality similarly to BINOL derivatives due to restricted rotation around C(sp^3)–C(sp^2) bond. Thus, it was obtained as the 1:1 diastereomeric mixture. Notably, the replacement of the hydroxy group by hydrogen atom in the 3-azaxanthene **2b** results in lesser steric hindrance and the loss of axial chirality.

As was mentioned above, in contrast to 2-naphthols, other phenols react with pyridoxal to give 1-arylfuropyridine derivatives. We speculate that in case of 2-naphthols the reaction



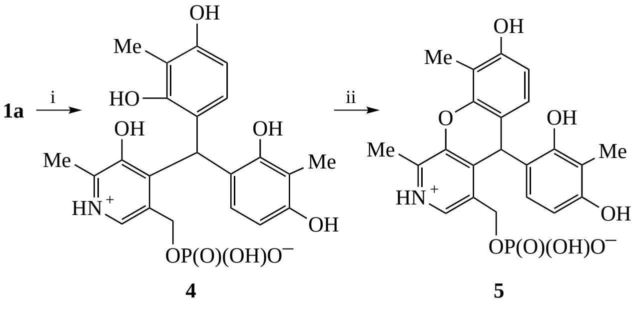
Scheme 1 *Reagents and conditions*: i, 2-naphthol (2 equiv.), EtOH (abs.)/HCl (conc.), reflux, 1.5 h.



Scheme 2 *Reagents and conditions*: i, 2-naphthol (2 equiv.), EtOH (abs.)/HCl (conc.), reflux, 1.5 h, yield of **2b** 65%; ii, naphthalene-2,7-diol (2 equiv.), EtOH (abs.)/HCl (conc.), reflux, 1.5 h, yield of **2c** 63%; iii, 2-naphthol (2 equiv.), EtOH (abs.)/HCl (conc.), reflux, 1.5 h, yield of **2b** 65%; iv, naphthalene-2,7-diol (2 equiv.), EtOH (abs.)/HCl (conc.), reflux, 1.5 h, yield of **2c** 70%.

also may proceed through initial formation of 1-(2-hydroxynaphthyl)furopyridines followed by furan ring cleavage to afford bis(2-hydroxynaphthyl)(pyridyl)methanes. The subsequent dehydration of these intermediates may lead to final 3-azaxanthenes **2b**,**c**. With this in mind, we tried to obtain 1-(2-hydroxynaphthyl)furopyridines by the reaction of 1-ethoxyfuropyridine **3a** with 2-naphthol and naphthalene-2,7-diol under the same conditions. However, no desired compounds were observed in the reaction mixture, and 3-azaxanthenes **2b**,**c** were isolated in 65–70% yield instead (see Scheme 2, path *b*). Similarly, 1-diethylaminofuropyridine **3b** may also be employed into this reaction. Although these results do not prove the formation of 1-(2-hydroxynaphthyl)furopyridines in the course of the reaction, they indicate unequivocally the possibility of the furan ring cleavage.

Next, we employed 2-methylresorcinol in the reaction with PLP, which afforded diaryl(pyridyl)methane derivative **4** in 86% yield (Scheme 3). Notably, the simple recrystallization of the obtained compound from absolute methanol caused its dehydration and gave 3-azaxanthene **5** almost quantitatively. The obtained results suggest that bis(2-hydroxynaphthyl)(pyridyl)methanes similar to compound **4** may indeed be the intermediates in case of 2-naphthols.



Scheme 3 *Reagents and conditions*: i, 2-methylresorcinol (2 equiv.), EtOH (abs.)/HCl (conc.), reflux, 10 h, yield of **4** 86%; ii, EtOH (abs.), reflux, 5 min, yield of **5** 89%.

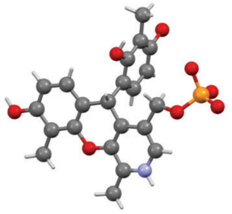


Figure 1 Molecular structure of compound **5** in crystals.

The structure of compound **5** was additionally confirmed by the X-ray analysis (Figure 1).[†] The X-ray data clearly indicates the closure of the pyran ring and the formation of the 3-azaxanthene skeleton as well as the retention of the phosphate group.

The cytotoxicity of 3-azaxanthenes **2a**–**c** was tested against normal and tumor cells. Evaluation of cytotoxicity was performed by counting viable cells using the CytellCellImaging multifunctional system (GE HelthcareLifeScience, Sweden) and the CellViabilityBioApp application, which allows one to accurately calculate the number of cells and evaluate their viability based on fluorescence intensity.[17] M-Hela clone 11 and HuTu-80 tumor cell lines and normal liver cells (Changliver) were used for experiments. The cytotoxicity was studied at concentrations recommended for screening for new antitumor agents (100-1 μM). Compound **2b** acts selectively on the M-Hela cell line (Table 1, entry 1). The IC_{50} value of the 3-azaxanthene **2b** for the M-HeLa cell line is comparable with that of tamoxifen reference drug. At the same time, in contrast with tamoxifen, compound **2b** appeared to be non-toxic to the normal cell line in the studied concentration range. Interestingly, the replacement of the hydrogen atom by the hydroxy group reverses the activity profile. Thus, compound **2c** exhibited noticeable cytotoxicity against normal cell line, whilst being completely inactive towards M-HeLa tumor cells. At the same time, its cytotoxicity against HuTu-80 cell line increased compared to compound **2b** (entry 2). The absence of the cytotoxicity against M-HeLa tumor cell line for compound **2a** also indicates the importance of the hydroxyl group (entry 3).

Table 1 The cytotoxicity of compounds **2a**–**c**.

Entry	Compound	IC_{50}/μM		
		Cancer cell lines		Normal cell line
		M-HeLa	HuTu-80	Chang liver
1	**2b**	26.1 ± 1.9	83.5 ± 7.3	>100
2	**2c**	>100	43.2 ± 3.8	51.3 ± 4.5
3	**2a**	>100	69.2 ± 5.7	>100
4	Tamoxifen	28.0 ± 2.5	–	46.2 ± 3.5
5	5-Fluorouracil	–	65.2 ± 5.6	–

[†] *Crystal data for* **5**. $C_{22}H_{21}NO_8P \cdot 4(C_2H_6O)$, $M = 642.64$, monoclinic, space group $P2_1/c$, at 100 K: $a = 12.448(2)$, $b = 22.191(5)$ and $c = 12.727(3)$ Å, $\beta = 107.399(10)°$, $V = 3354.8(12)$ Å^3, $Z = 4$, $d_{calc} = 1.272$ g cm^{-3}, $\mu = 0.142$ mm^{-1}, $F(000) = 1372$. The cell parameters and the experimental data were obtained on an automatic Bruker Smart APEX II CCD diffractometer [$\lambda(\mathrm{Mo}K_\alpha) = 0.71073$ Å, ω-scanning], $2\theta < 52°$, $R_{int} = 0.148$. 27790 reflections were collected, 6202 of them were independent; the number of the observed reflections with $I > 2\sigma(I)$ was 2671. The absorption correction was applied using the SADABS program.[18] The structure was solved by the direct method using the SIR program,[19] and it was refined by the full-matrix least-squares method using the SHELXL97 program package.[20] The hydrogen atoms of the hydroxyl groups were revealed by means of the difference electron density maps and refined in the isotropic approximation. The coordinates of the other hydrogen atoms were calculated geometrically and refined in a riding model. All the calculations were carried out using the WinGX[21] and APEX2[22] programs; the final values of the divergence factors were $R = 0.0808$, $wR_2 = 0.2359$, GOF = 1.02; the number of parameters to be refined was 412.

CCDC 2084681 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.

In conclusion, we have developed the simple one-pot procedure for the synthesis of 3-azaxanthene derivatives from readily available 2-naphthols and pyridoxal/PLP. The cytotoxicity of the obtained compounds **2a–c** was studied to evaluate their feasibility as novel anti-tumor agents. Notably, the cytotoxicity of 3-azaxanthene **2b** against M-HeLa tumor cell line is comparable to that of tamoxifen. At the same time, it is non-toxic to the normal cell line, which makes it promising candidate for the further studies.

The work was supported by the Russian Science Foundation (grant no. 21-13-00022), https://rscf.ru/project/21-13-00022/. The authors are grateful to the Assigned Spectral-Analytical Center of FRC Kazan Scientific Center of RAS for technical assistance in research. The authors are grateful to the Collective Spectral-Analytical Center of Physicochemical studies of structure, properties, and composition of substances and materials of the Federal Research Center of the Kazan Scientific Center of RAS for technical support of the studies. X-ray studies were performed using the equipment of the Federal Research Center Kazan Scientific Center of RAS.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.09.024.

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Received: 14th May 2021; Com. 21/6560