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ORIGINAL PAPER

Fluorescence Properties of Selected Benzo[c]phenanthridines

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Abstract The fluorescence properties of selected benzo[c]phenanthridines (BPs) were examined. The effect of structure, pH and solvent on the fluorescence properties has been investigated. It was found out that the presence of charged iminium nitrogen significantly decreased the fluorescence of the compounds. The fluorescence (intensity as well as emission spectra shape) of the investigated compounds was significantly dependent on pH as well as used solvent. The utilization in epigenetic modification mechanisms studies as demethylase probe and as possible pH indicator was suggested.

Keywords Benzo[c]phenanthridines · Fluorescence · Dual fluorescence

Introduction

The benzo[c]phenanthridines (BPs) have been studied especially for their wide biological activity [1]. For example, sanguinarine exhibits multiple effects, such as antimicrobial [2] and antifungal [3]. Another derivative, nitidine, is an inhibitor of topoisomerases I/II [4] and, further, shows antimalarial effects [5]. Chelerythrine is considered to be a potent protein kinase C inhibitor [6]. A growth-inhibitory activity against some drug-resistant tumor cell lines has been observed for NK109, O-demethylated analog of chelerythrine [7, 8].

Benzo[c]phenanthridines show interesting fluorescence properties as well. The fluorescence properties of BPs has been already described and suggested for labelling of biomolecules [9]. Generally, fluorescent probes allow researchers to detect particular components of complex biomolecular assemblies with exquisite sensitivity and selectivity as well as to observe interesting properties of the biosystem or biological processes. Nevertheless, fluorescence techniques require a suitable fluorescent label with optimal properties. Benzo[c]phenanthridines can occur in two basic forms depending on pH: quarternary (iminium) or pseudobases (hydroxyaduct) form [10]. Both forms could be present in biological environment, however, as pK values indicate the quarternary forms prevail. Both forms show different fluorescence properties. The excitation as well as the emission at higher wavelength was observed for quarternary form of sanguinarine and chelerythrine [11].

Using the fluorescence imaging the localization of BPs in plant as well as human tumor cells has been studied. In plant cells (species *Achillea*, *Artemisia* and *Gaillardia*) sanguinarine and chelerythrine was observed in secretory vesicles and on a cell surface. The reduction of BPs to dihydroderivatives is probably necessary for the penetration of BPs through the cell membrane [12]. In the case of human tumor cells the sanguinarine permeates quickly to the cells and it was observed in few minutes in the form of small cytoplasmic aggregates. Then the fluorescence intensity of the aggregates decreased, but on the other hand, the cell nucleus fluorescence appeared and finally predominated [13]. Further, the fluorescence of other BPs (chelerythrine, makarpine, sanguilutine and other) in living cells has been studied as well [14]. It was found that all studied BPs quickly penetrated into living cells and moreover, sanguinarine, makarpine and chelirubine were immediately incorporated into cell nuclei. An obtained cell image with bright red nucleus fluorescence was similar as commercial DNA probes (such as propidium iodide

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or DAPI) offer. Because the fluorescence of various colours in different cell locations has been observed the presence of both structural forms depending on pH can be expected.

This work describes fluorescences properties of selected BPs (Scheme 1) that differ by the substitution in the position of 7, 8 and at 5-nitrogen atom of benzo[c]phenanthridine skeleton. Because the BPs depending on pH can occur in two basic forms that can affect their fluorescence the effect of pH was studied. Further, the effect of various solvents was investigated as well. As mentioned above, the application of some BPs as fluorescent probes for the cell imaging as well as flow cytometry have been described and results of the work are a useful contribution to research and design of BPs as fluorescent probes.

Experimental

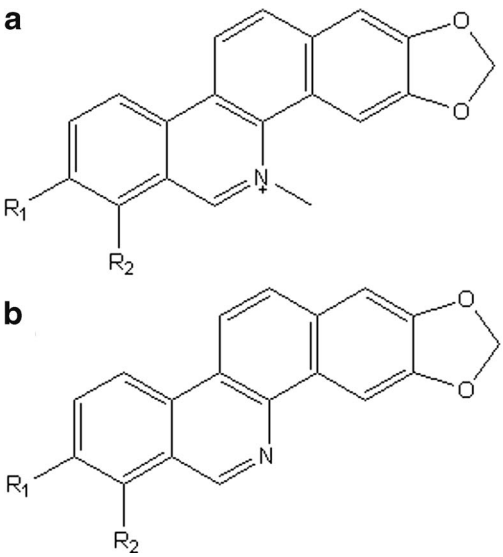
The synthesis of examined benzo[c]phenanthridines (Scheme 1) was described elsewhere (compounds **1** and **2** [15], **3** [16], **4** [8, 17], **5** [8], **6** [8, 16]). The absorbance, excitation and emission fluorescence spectra were measured by the spectrophotometer Cary 300 UV-VIS (Agilent Technologies) and the fluorescence spectrometer Cary Eclipse (Varian).

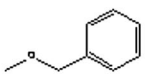
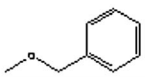
Results and Discussion

The excitation spectra (Fig. 1, Table 1) show that the excitation maximum wavelengths move in the range from 360 to 410 nm. From the BP excitation spectra, it is evident that an effectual excitation is possible in the wider range of excitation wavelengths. And moreover, some derivatives especially consisting of structure A (Scheme 1) can be excited at relatively high wavelengths around 400 nm. This fact is profitable with respect to the usability in biological applications when the fluorescence of intristic fluorophors as well as UV radiation effect on biosystems have to be taken into account. The difference (Stokes shift) between the longer excitation wavelength and the emission maxima wavelength remains still sufficient for the measurement of emission intensity at emission maxima.

Emission spectrum maxima of compounds **2**, **4** and **6** move in the wavelength range from 420 to 460 nm and show only one maximum. The shape of emission spectra of compounds **1**, **3** and **5** (structures containing N-methyl) is more complicated (Fig. 1). Emission spectra show two local maxima (Table 1). Moreover, the presence of the N-methyl group in the BP structure led to significant decrease of fluorescence intensity (see the quantum yield values in Table 1). Thus we found out, that quarternary salts (compounds **1**, **3**, **5**) exhibited lower intensity of fluorescence in comparison to N-unsubstituted derivatives (**2**, **4**, **6**). This fact can be explained by less efficient electron distribution caused by positive charge on nitrogen. The dual character of the spectra in case of quarternary salts can be then explained by equilibrium between ammonium form and pseudobase formed by attack of water to position 6. This fact is forced by the similar observation at sanguinarine [18] and the transformation to single emission spectrum, when the compound **1** or **5** is measured in alkaline pH (see Figs. 3 and 5), where pseudobase must be predominant. The substitution in position 7 and 8 is not crucial for fluorescence behaviour, because no significant change was observed within the set of derivatives **1**, **3**, **5** and **2**, **4**, **6**, respectively.

Scheme 1 The structures of the studied BPs



Compound	Structure	R_1	R_2
1	A	-OH	-OH
2	B	-OH	-OH
3	A	$-OCH_3$	-OH
4	B	$-OCH_3$	-OH
5	A	$-OCH_3$	
6	B	$-OCH_3$	

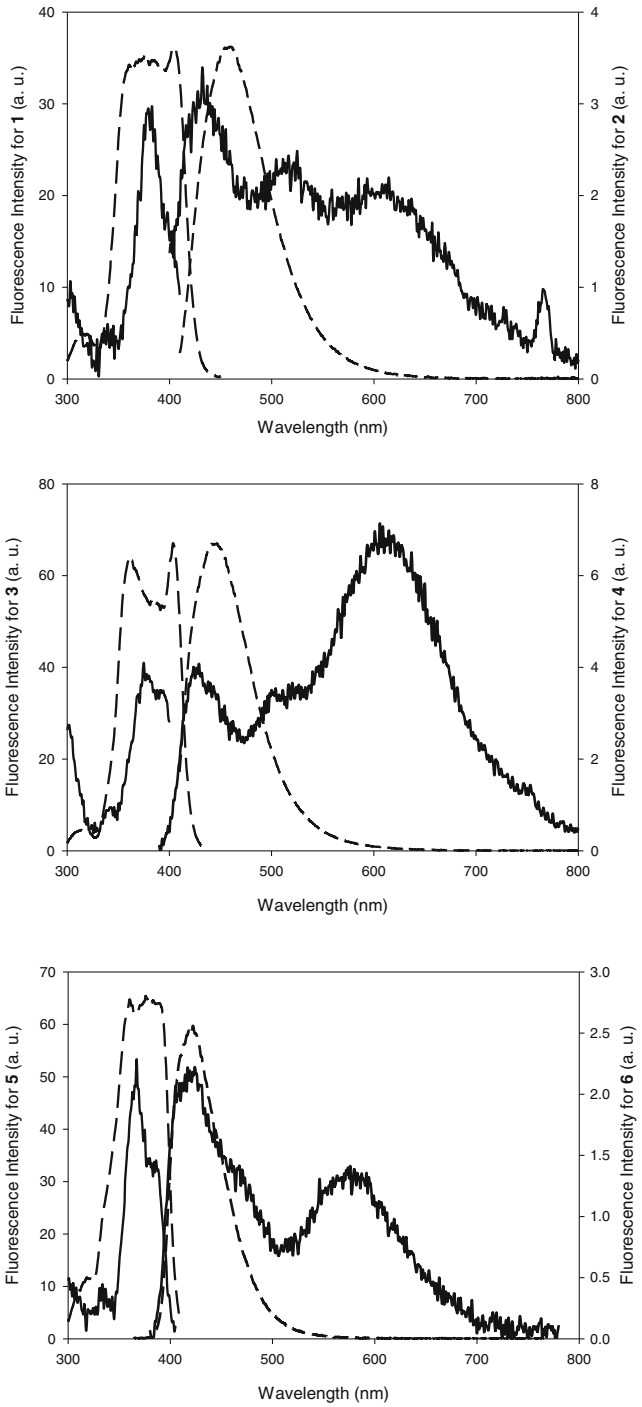


Fig. 1 Excitation and emission spectra of BPs in DMSO. (—) Compounds **1**, **3** and **5**; (– –) compounds **2**, **4** and **6**

Because BPs can occur in two basic forms depending on pH the fluorescence of studied compounds was investigated at various pH values (Table 2). The fluorescence intensity depending on pH for compounds **2**, **3**, **4** and **6** increased with increasing pH, then reached the maxima at pH of 7.33, 7.41, 5.12 and 5.81, respectively, and then decreased again

Table 1 Spectroscopic properties of the studied BPs in DMSO

Compound	$\lambda_{ex,max}$ (nm)[a]	$\lambda_{em,max}$ (nm)[b]	ϕ (%)[c]
1	379	432, 525	0.14
2	404	460	11.93
3	375	428, 610	0.19
4	404	444	2.35
5	367	468, 627	0.23
6	376	421	6.56

[a] λ_{ex}, excitation maximum wavelength

[b] $\lambda_{em,1}$, the fluorescence lower wavelength emission maximum

[c] φ, fluorescence quantum yield (determined with quinine sulphate in 0.5 M H_2SO_4 (φ=0.577[12]), taken as a reference fluorescence standard)

(Table 2). The fluorescence of compounds **1** and **5** depending on pH showed different behavior. Both compounds exhibit dual emission spectra and intensities of local maxima changed at various pH (Table 2). The duality of emission spectra for **1** was not observed at lower pH values (<8). Fluorescence intensities of compound **1** measured at 474 nm (higher wavelength local maximum) significantly increased with pH (Fig. 2, the fluorescence intensity at 416 nm changed as well but not so perceptibly). On the contrary, in case of **5** the fluorescence intensity of lower wavelength local maximum (416 nm) significantly increased at higher pH (>8, Fig. 3). Unfortunately, the dependence of the ratio of intensities at emission maxima (I_2/I_1; I_2 measured at 563 nm; I_1 measured at 416 nm) on pH (Fig. 3) was not linear. It would be useful for a possible application of **1** or **5** as fluorescent pH indicator with dual emission spectrum. In the case of common single-band fluorescent labels the fluorescence intensity depends on the label concentration which can vary because of various biological processes in a sample. Dual fluorescence labels that exhibit two well-separated emission bands are not dependent on the concentration because the ratio of the intensities of the two bands can be applied as a signal [19, 20]. If the pH dependences for compounds **1** and **2**, **5** and **6** are compared the different course is evident. The high fluorescence intensities in alkaline solution can be probably caused by a formation of uncharged pseudobase in an alkaline solution. In contrast to [11] no significant emission maxima shift depending on pH was observed for any studied compound (for example Figs. 2 and 3).

The effect of solvents (such as acetonitrile, dimethylformamide, dimethyl sulfoxide, mixture of dimethylsulfoxide/water 1:1, chloroform, ethylacetate, methanol, tetrahydrofuran and toluene) on the fluorescence properties of compounds **5** and **6** was investigated too. In the case of compound **6** the solvents affects meaningly the fluorescence intensity as well as the emission spectra shape (Table 3, Fig. 4). The fluorescence intensity increased in the order

Table 2 Spectroscopic properties of compounds **3**, **4**, **5** and **6** at various pH

1		**2**		**3**		**4**		**5**		**6**	
pH	FI[a] (a.u.)	pH	FI (a.u.)	pH	FI (a.u.)	pH	FI (a.u.)	pH	FI (a.u.)	pH	FI (a.u.)
2.90	10.9[b]	2.72	8.72	3.03	6.81	2.19	29.4	2.47	7.8[b] 35.2[d]	2.61	34.4
4.67	7.8[b]	3.28	11.7	3.46	7.02	2.65	56.6	3.54	10.1[b] 32.4[d]	3.09	44.1
5.60	10.0[b]	4.79	16.7	4.81	7.27	3.38	76.4	4.21	10.4[b] 30.8[d]	5.81	56.1
6.73	10.8[b]	6.45	42.8	5.91	7.65	3.52	79.0	6.07	16.0[b] 28.1[d]	6.16	53.9
7.23	10.9[b]	7.33	52.9	6.64	7.86	5.12	80.3	6.48	21.6[b] 27.1[d]	7.03	52.4
8.28	30.1[b] 14.7[c]	8.34	52.8	7.41	8.63	6.16	69.0	6.83	42.0[b] 26.4[d]	9.74	37.3
9.15	17.1[b] 11.48[c]	9.41	48.4	8.27	8.39	6.74	70.8	7.21	82.7[b] 21.1[d]	11.10	26.8
9.86	26.0[b] 37.6[c]	10.71	22.4	9.76	5.97	7.07	69.4	7.83	200.5[b] 20.7[d]		
10.56	34.6[b] 94.1[c]	11.21	15.6	10.81	3.21	7.81	66.7	8.34	397.0[b] 3.8[d]		
11.40	72.5[b] 210.1[c]					9.51	60.6	8.56	505.0[b] 0.6[d]		
12.11	77.1[b] 228.3[c]					10.52	56.5	9.20	562.3[b] 0.5[d]		
								9.81	639.7[b] 0.2[d]		
								10.71	596.8[b] 0.9[d]		
								11.10	546.4[b] 0.6[d]		

[a] FI, fluorescence intensity

[b] FI at emission wavelength of 413 nm

[c] FI at emission wavelength of 474 nm

[d] FI at emission wavelength of 562 nm

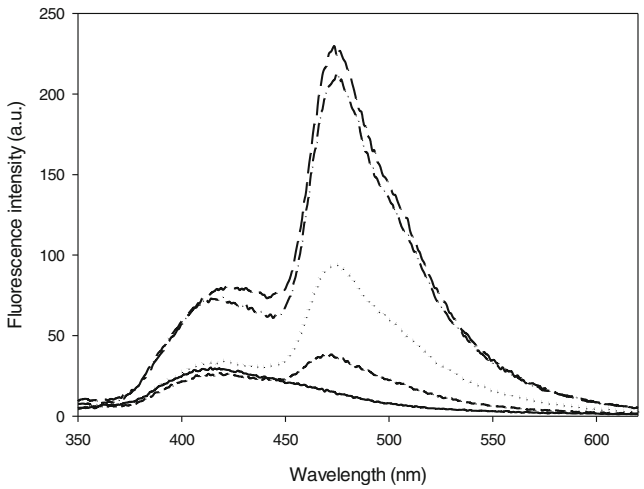


Fig. 2 Emission spectra of **1** depending on pH (for better lucidity emission spectra for selected pH values are depicted, for complete data see Table 2). (—) pH 8.28; (—) pH 9.86; (•••) pH 10.56; (– • –) pH 11.40; (– –) pH 12.11

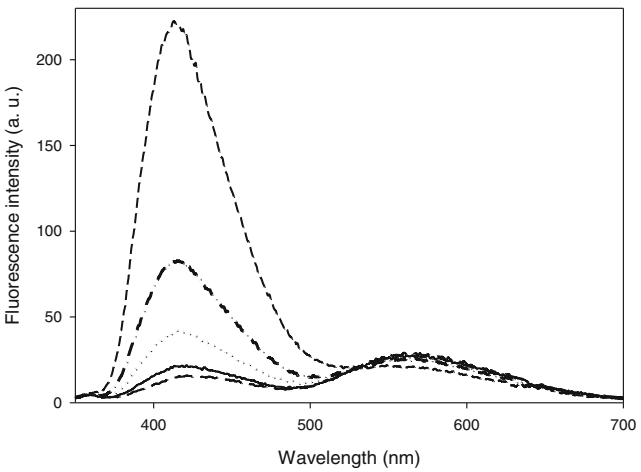


Fig. 3 Emission spectra of **5** depending on pH (for better lucidity emission spectra for selected pH values are depicted, for complete data see Table 2). (– –) pH 6.07; (—) pH 6.48; (•••) pH 6.83; (– • –) pH 7.21; (—) pH 7.83

Table 3 Fluorescence properties of compounds **5** and **6** in various solvents (excitation wavelength 325 nm for both **5** and **6**; 30 μmol.L^{-1})

Solvent	**5**		**6**	
	$\lambda_{em,max}$ (nm)	FI (a.u.)	$\lambda_{em,max}$ (nm)	FI (a.u.)
Chloroform	419;563	4.3;26.7	418	49.2
Dimethylformamide	420	37.7	421	62.2
Dimethyl sulfoxide	418	12.2	421	60.4
Dimethyl sulfoxide:water (1:1)	423	16.0	436	56.4
Ethylacetate	415;563	13.5;3.8	417	55.9
Methanol	416	17.3	429	55.9
Tetrahydrofurane	412	42.1	416	50.4
Toluene	416	35.0	418	53.4

dimethyl sulfoxide, ethylacetate, dimethyl sulfoxide:water (1:1), methanol, chloroform, toluene, dimethylformamide and tetrahydrofurane. Moreover, the shape of emission spectra of **5** changed in especially in ethyl acetate and chloroform when the second emission maximum at 651 nm appeared (Fig. 4). Tested solvents did not affect significantly the fluorescence of compound **6**. The fluorescence intensities and emission spectra were similar for all tested solvents (Table 3). Only slight shift of emission maxima depending on used solvent was observed in the range from 416 to 436 nm (Fig. 5).

Conclusion

The fluorescence properties of selected BPs were studied. Especially the fluorescence effect of structure, pH and solvent has been investigated. It was found out that (1) the presence of charged iminium nitrogen significantly decreased the fluorescence of the molecule (expressed by the quantum yield), (2) the fluorescence (intensity as well as emission spectra shape) especially of the compound **5** was significantly dependent on pH and (3) for compound 5 an interesting effect of solvents was observed as well. The effect of N-methyl group on the fluorescence intensity of compound **5** (if N-methyl group is present in the molecule the fluorescence intensity greatly decreased) could be utilized in studies of epigenetic modification mechanisms where demethylases play an important role. Nevertheless, the extensive studies of such possible application of studied compounds as demethylase fluorescent probe would have to be performed. Further, in spite the fact that the dependence of the ratio of emission maxima for compound **5** on pH was not linear the usage of compound **5** as pH indicator is possible, especially in the pH range 6.07–7.83 when the intensity of higher wavelength emission maxima is (in comparison with lower wavelength emission maxima) substantial.

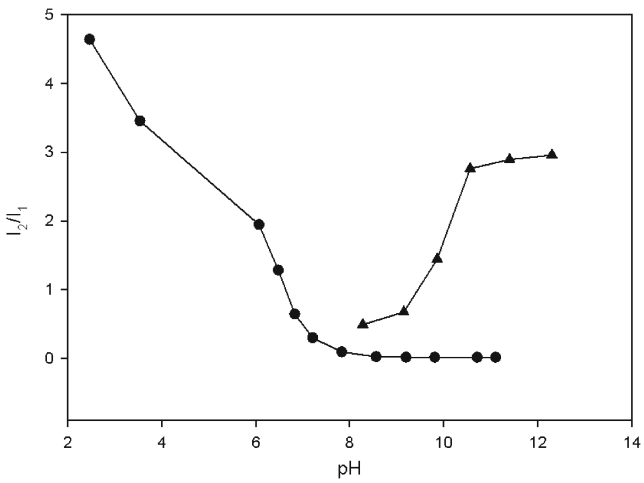


Fig. 4 The ratio of fluorescence intensities at local maxima of emission spectra for compounds **1** (▲; I_1 413 nm; I_2 474 nm) and **5** (●; I_1 413 nm; I_2 562 nm) depending on pH

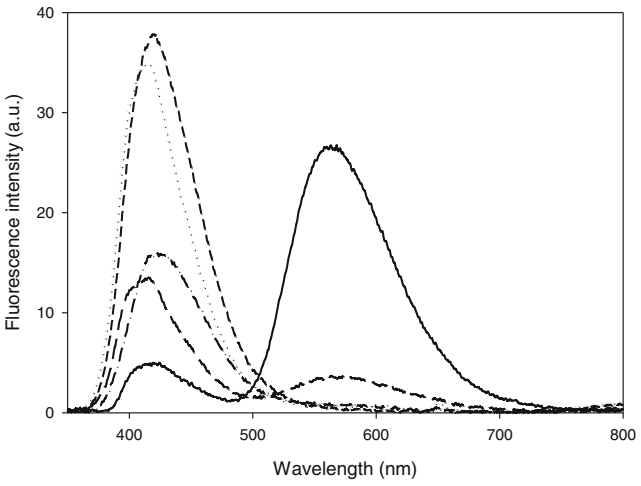


Fig. 5 Emission spectra of **5** in various solvents (for better lucidity emission spectra for selected solvents are depicted, for complete data see Table 3). (– –) ethylacetate; (—) chloroform; (–•–) DMSO/water 1/1; (—) dimethylformamide; (•••) toluene

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References

1. Dvořák Z, Kubáň V, Klejdus B, Hlaváč J, Vičar J, Ulrichová J, Šimánek V (2006) Quaternary benzo[*c*]phenanthridines sanguinarine and chelerythrine: a review of investigations from chemical and biological studies. Heterocycles 68(11):2403–2422
2. Liu H, Wang J, Zhao J, Lu S, Wang J, Jiang W, Ma Z, Zhou L (2009) Isoquinoline alkaloids from Macleaya cordata active against plant microbial pathogens. Nat Prod Commun 4(11):1557–1560
3. Zdařilová A, Malíková J, Dvořák Z, Ulrichová J, Šimánek V (2006) Quaternary isoquinoline alkaloids sanguinarine and chelerythrine. In vitro and in vivo effects. Chem List 100:30–41
4. Wang LK, Johnson RK, Hecht SM (1993) Inhibition of topoisomerase I function by nitidine and fagaronine. Chem Res Toxicol 6(6): 813–818
5. Gakanju DMN, Mberu EK, Dossaji SF, Gray AI, Waigh RD, Waterman PG, Watkins WM (1995) Potent antimalarial activity of the alkaloid nitidine, isolated from a Kenyan herbal remedy. Antimicrob Agents Chemother 39(12):2606–2609
6. Chmura SJ, Dolan ME, Cha A, Mauceri HJ, Kufe DW, Weichselbaum RR (2000) In vitro and in vivo activity of protein kinase C inhibitor chelerythrine chloride induces tumor cell toxicity and growth delay in vivo. Clin Cancer Res 6(2):737–742
7. Nakanishi T, Masuda A, Suwa M, Akiyama Y, Hoshino-Abe N, Suzuki M (2000) Synthesis of derivatives of NK109, 7-OH benzo[c]phenanthridine alkaloid, and evaluation of their cytotoxicities and reduction-resistant properties. Bioorg Med Chem Lett 10(20):2321–2323
8. Nakanishi T, Suzuki M, Mashiba A, Ishikawa K, Yokotsuka T (1998) Synthesis of NK109, as anticancer benzo[c]phenanthridine alkaloid. J Org Chem 63(13):4235–4239
9. Slaninová I, Slanina J, Táborská E (2008) Fluorescence properties of quarternary benzo[c]phenantridine alkaloids and their use as supravital DNA probes. Chem List 102(6):427–433
10. Dostál J, Slavík J (2000) Recent knowledge on sanguinarine and related alkaloids. Chem List 94(1):15–20
11. Maiti M, Das S, Sen A, Das A, Kumar GS, Nandi R (2002) Influence of DNA structures on the conversion of sanguinarine alkanolamine form to iminium form. J Biomol Struct Dyn 20(3):455–464
12. Roshchina VV (2005) Allelochemicals as fluorescent markers, dyes and probes. Allelopath J 16(1):31–46
13. Holy J, Lamont G, Perkins E (2006) Disruption of nucleocytoplasmic trafficking of cyclin D1 and topoisomerase II by sanguinarine. BMC Cell Biol 7:13
14. Slaninova I, Slanina J, Taborska E (2007) Quaternary benzo[c]phenanthridine alkaloids—novel cell permeant and red fluorescing DNA probes. Cytometry A 71A(9):700–708
15. Stýskala J, Hlaváč J, Cankař P (2013) Synthesis of oxidative dihydroxy metabolites of benzo[*c*]phenanthridines. Tetrahedron 69(23): 4670–4678
16. Stýskala J, Cankař P, Soural M, Hlaváč J, Hradil P, Vičar J, Šimánek V (2007) Synthesis of isodecarine. Heterocycles 73:769–775
17. Nakanishi T, Suzuki M (1998) Revision of the structure of Fagaridine based on the comparison of UV and NMR data of synthetic compounds. J Nat Prod 61(10):1263–1267
18. Janovská M, Kubala M, Šimánek V, Ulrichová J (2009) Fluorescence of sanguinarine: fundamental characteristic and analysis of interconversion between variol forms. Anal Bioanal Chem 395:235–240
19. Motyka K, Hlavac J, Soural M, Funk P (2010) Fluorescence properties of 2-aryl-3-hydroxyquinolin-4(1H)-one-carboxamides. Tetrahedron Lett 51(38):5060–5063
20. Motyka K, Hlaváč J, Soural M, Hradil P, Krejčí P, Kvapil L, Weiss M (2011) Fluorescence properties of some 2-(4-amino-substituted-3-nitrophenyl)-3-hydroxyquinolin-4(1H)-ones. Tetrahedron Lett 52(6):715–717