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Synthesis of novel fluorescent styryl dyes based on the imidazo[1,2-*a*]pyridinium chromophore and their spectral-fluorescent properties in the presence of nucleic acids and proteins

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Abstract

The imidazo[1,2-*a*]pyridinium heterocyclic system was used to prepare styryl dyes. Improved synthetic methods were proposed for the parent imidazo[1,2-*a*]pyridines and their corresponding quaternary salts. The standard method for preparing styrylcyanines was modified for the synthesis of the target imidazo[1,2-*a*]pyridinium dyes. Spectral-luminescent properties of the obtained dyes in free state and in the presence of nucleic acids, BSA, and BSA/detergent system were studied. 7-[2-(4-Dihexylaminophenyl)-1-ethenyl]-2-(2,4-dimethoxyphenyl)-1-ethylimidazo[1,2-*a*]pyridin-1-ium iodide (SIP-8) containing C_6 aliphatic tails and 2,4-methoxy substituents in the 2-phenyl ring exhibited specificity to BSA.
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Keywords: Styrylcyanine dyes; Nucleic acids; Fluorescent detection

1. Introduction

Styrylcyanines are widely used fluorophores with unique physico-chemical properties that are applied in various biomedical techniques [1–4]. In a preceding paper, we reported a study of the design of fluorescent styrylcyanines as potential probes for the detection of nucleic acids and proteins [5]. Recently we synthesized and studied a series of homodimer styryls based on various *p*-dimethylaminostyryl chromophores and linked by an aliphatic chain. High DNA-binding affinity and significant DNA specificity was shown for *p*-(dimethylaminostyryl)pyridinium homodimers with 5–10 carbon atoms linkage group [6].

Present work is a continuation of researches aimed on the search of novel styrylcyanine dyes with attractive spectral-luminescent characteristics for biomedical applications, particularly for the usage as fluorescent probes in homogeneous assays of biological molecules. The fluorescent probes used for the homogenous assay are virtually nonfluorescent in free state, but increase the fluorescent intensity by orders of magnitude when bound to the biological molecule. Thus, it is not necessary to remove the nonbound probe during the assay [7].

The presented studies were aimed at producing a series of styryl dyes based on the imidazo[1,2-*a*]pyridinium heterocyclic system, and developing a convenient method for their synthesis. For this purpose, the

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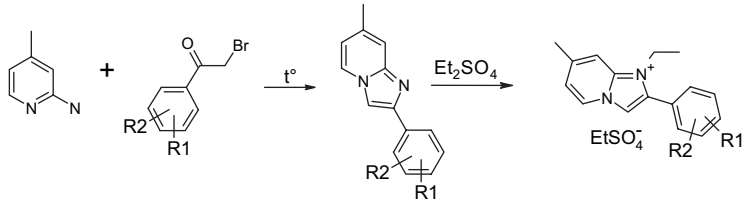


Scheme 1. Synthesis of quaternary imidazo[1,2-*a*]pyridinium salts.

standard method of preparation of styrylcyanines [8,9] was improved by using the high-boiling solvent *n*-butanol. We varied the substituents in 2-phenyl ring to study the influence of the nature of this substituent on the spectral characteristics of the dyes and their behavior in the presence of biological molecules. Spectral-luminescent properties of the novel dyes in the unbound state and in the presence of DNA, RNA, BSA as well as a BSA/detergent system (as detergent sodium dodecyl sulfate (SDS) was chosen) were studied and are reported below.

2. Results and discussion

2.1. Synthesis of styrylimidazo[1,2-a]pyridinium dyes SIP-1–SIP-8

As known, usually imidazo[1,2-*a*]pyridines are synthesized by the reaction of 2-aminopyridine with α-halocarbonyl compounds. This method requires separation of the intermediate quaternary salt and then its cyclization in polar solvent [10].

Here we propose a novel convenient procedure for the synthesis of various imidazo[1,2-*a*]pyridines. Reaction of 2-amino-4-picoline with corresponding phenacyl bromide in boiling ethanol in the presence of an acidic catalyst gave the substituted imidazo[1,2-*a*]pyridines. These compounds were precipitated as low-soluble hydroiodides. The salts were then transformed into their free bases and separated using triethylamine (Procedure 1, Scheme 1). Further treatment of the substituted imidazo[1,2-*a*]pyridines with an alkylating agent (diethyl sulfate or heptyl iodide) in boiling dioxane gave the corresponding quaternary salts (Procedure 2, Scheme 1).

General scheme of the synthesis of novel dyes (Fig. 1) is presented in Scheme 2. For obtaining the dyes SIP-1–SIP-7, SIP-9, SIP-10 and SIP-12 the mixture of corresponding quarternary salt and *p*-dimethylamonibenzaldehyde was boiled in *n*-butanol in the presence of piperidine (Procedure 3). Carrying out this reaction in ethanol according to standard condensation procedure gave low yields of the styryl products, and thus we used the high-boiling solvent *n*-butanol. For obtaining the dyes SIP-8 and SIP-11 correspondingly 4-dihexylaminobenzaldehyde or 4-hydroxybenzaldehyde was used. Reaction was carried out according to the same synthetic protocol (Procedure 3). All dyes except SIP-2, SIP-6 and SIP-11 were precipitated as iodides. Dyes SIP-2 and SIP-6 were precipitated as ethosulphates, dye SIP-11 was precipitated as perchlorate.

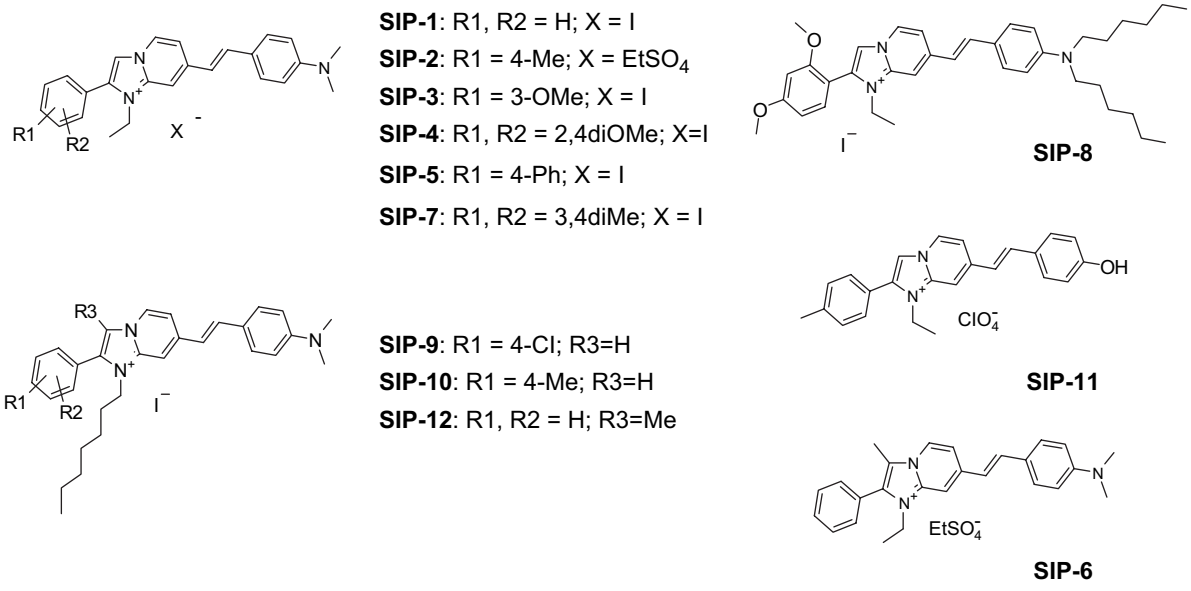
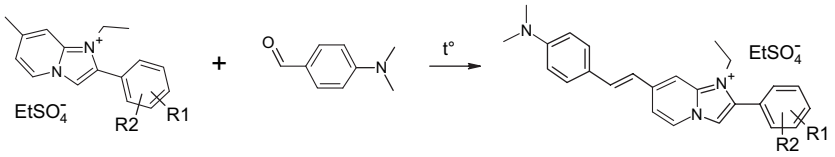


Fig. 1. Structures of novel styryl imidazo[1,2-*a*]pyridinium dyes.



Scheme 2. Synthesis of novel styryl imidazo[1,2-*a*]pyridinium dyes.

2.2. Spectral properties of dyes SIP-1–SIP-12 in buffer solution

Characteristics of absorption, fluorescence excitation and emission spectra of the synthesized dyes in aqueous buffer solution are summarized in Table 1. The maximum wavelengths of absorption spectra (λ_{abs}) of dyes in buffer are situated between 390 and 402 nm, except the dyes SIP-8 and SIP-11 (416 nm and 362 nm, respectively). At the same time, fluorescence excitation spectra maximum wavelengths (λ_{ex}) are situated between 395 and 397 nm, except SIP-8 (427 nm) and SIP-11 (365 nm and 385 nm). The values of λ_{ex} are shifted up to 11 nm relatively to λ_{abs} to either short- or long-wave region. The maximum wavelengths of the fluorescence emission spectra (λ_{em}) are found in the region 570–582 nm (596 nm for SIP-8, 476 nm and 560 nm for SIP-11). For the dye SIP-11, two excitation maxima at 365 nm and 385 nm correspond to the emission maxima at 476 nm and 555 nm, respectively. The fluorescence intensity values of dyes in buffer solution (I_0) are moderate, are of the same order for all the dyes and lie between 255 arbitrary units (a.u.) and 414 a.u., except for the dyes SIP-8 and SIP-11 (19 and 36 a.u., respectively).

2.3. Spectral properties in the presence of nucleic acids

The spectral properties of the dyes in the presence of nucleic acids are summarized in Table 1. The addition of DNA shifts the absorption and excitation maxima of all the dyes to longer wavelength by up to 20 nm relative to free dye. In contrast, the emission maxima are virtually unchanged (shifts are not more than 3 nm) for the majority of the dyes, except SIP-4 and SIP-8, where λ_{em} shifts to the shorter wavelength by 6 and 7 nm, respectively. The fluorescence intensity value of the dyes in the presence of DNA (I^{DNA}) varies from dye to dye but is never more than twice I_0 or less than half I_0.

The presence of RNA in the dye buffer solution shifts the absorption and excitation maxima of the majority of the dyes bathochromically by up to 34 nm. The exceptions are the dyes SIP-6 (λ_{abs} stays unchanged), SIP-8 (λ_{abs} and λ_{ex} shift hypsochromically by 6 and 11 nm, respectively), and SIP-12 (λ_{ex} stays unchanged). The value of λ_{em} stays unchanged or shifted by not more than 3 nm for all the dyes except SIP-5 and SIP-9 (long-wave shift for 7 and 5 nm, respectively). The value of the fluorescence intensity change of the dyes in RNA presence as compared to the free dyes (I^{RNA}/I_0) lies

Table 1
Spectral-luminescent characteristics of the dyes SIP-1–SIP-12 in aqueous buffer in free state, and in the presence of DNA (6×10^{-5} M b.p.) and RNA (1.2×10^{-4} M b.)

Name	Free dye				In DNA presence					In RNA presence				
	λ_{abs} (nm)	λ_{ex} (nm)	λ_{em} (nm)	I_0 (a.u.)	λ_{abs} (nm)	λ_{ex} (nm)	λ_{em} (nm)	I^{DNA} (a.u.)	I^{DNA}/I_0	λ_{abs} (nm)	λ_{ex} (nm)	λ_{em} (nm)	I^{RNA} (a.u.)	I^{RNA}/I_0
SIP-1	393	397	578	352	397	407	576	327	0.93	396	416	575	312	0.87
SIP-2	392	396	575	255	397	415	572	245	0.96	397	410	575	240	0.94
SIP-3	392	397	577	281	399	413	577	286	1.02	397	407	577	243	0.86
SIP-4	394	397	576	414	400	409	570	420	1.01	401	407	576	323	0.78
SIP-5	401	396	582	319	420	409	581	164	0.51	435	427	589	69	0.22
SIP-6	390	397	572	305	392	406	570	301	0.99	390	404	570	273	0.90
SIP-7	394	396	576	365	414	413	573	274	0.75	402	410	576	294	0.81
SIP-8	416	427	596	19	424	433	603	25	1.32	410	416	597	22	1.16
SIP-9	402	396	577	336	416	415	578	184	0.55	419	402	582	111	0.33
SIP-10	398	395	574	260	412	412	575	143	0.55	416	412	575	111	0.43
SIP-11	362	365	476	36	365	376	474	67	1.86	363	379	479	56	1.56
		385	555	11										
SIP-12	394	396	570	316	400	397	570	263	0.83	400	396	570	372	1.18

Dye concentration, 5×10^{-6} M.
λ_{abs}, λ_{ex}, λ_{em} – Maximum wavelength of absorption, fluorescence excitation, and fluorescence emission, respectively.
I_0, I^{DNA}, I^{RNA} – Fluorescence intensity value (in arbitrary units, a.u.) of the dye in buffer in free form and in the presence of DNA and RNA, respectively.

between 0.22 and 1.56. It should be mentioned that the most significant intensity change takes place for dyes SIP-5, SIP-9 and SIP-10 (in 2–5-fold decrease). The value of I^{DNA}/I_0 for these dyes was the most noticeable of all the dyes studied as well.

2.4. Spectral properties in the presence of BSA

The spectral characteristics of the dyes in the presence of BSA are presented in Table 2. The values of λ_{abs} of the dyes SIP-5, SIP-8, SIP-9, SIP-10 and SIP-18 in the presence of BSA are shifted to the long-wave region for 11–21 nm as compared to the corresponding values of the free dyes. The values of λ_{ex} for these dyes shift for 23–55 nm, and for the dye SIP-7 λ_{ex} shifts for 11 nm to the long-wave region. For the other dyes, λ_{abs} and λ_{ex} do not change for more than 5 nm. The value of λ_{em} of the dyes SIP-8, SIP-10 and SIP-12 in the presence of BSA shift to the short-wave region for 58 nm, 11 nm and 8 nm, respectively. For other dyes studied, λ_{em} does not change for more than 5 nm. For the dye SIP-11, in the presence of BSA, as well as for the free dye, two maxima are present in fluorescent excitation spectra (366 nm and 388 nm), corresponding to the emission maxima 476 nm and 560 nm, respectively. Besides, two peaks could be distinguished in absorption spectrum of SIP-11 in BSA presence as well.

The fluorescence intensity enhancement of the dyes upon BSA addition (I^{BSA}/I_0) lies between 0.71 and 1.57, being thus insignificant. The only exception is the dye SIP-8, for which I^{BSA} is 26.8 times more than I_0 (Fig. 2). As could be seen from Table 2, the only dye with long *N*-alkyl tails (SIP-8) has shown the significant fluorescence enhancement in BSA presence, thus we conclude that presence of aliphatic chains could increase the dye affinity to BSA and perhaps to other proteins.

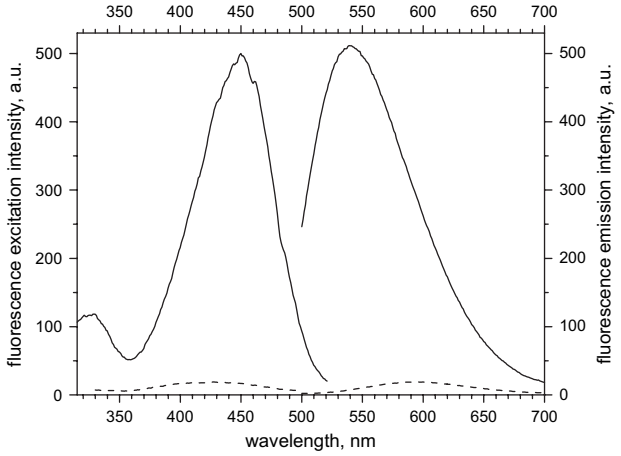


Fig. 2. Fluorescence excitation (left) and emission (right) spectra of SIP-8 (5×10^{-6} M) in aqueous pH 8.0 buffer in free state (dashed line) and in the presence of 0.2 mg/ml BSA (solid line).

2.5. Spectral properties in the presence of BSA–SDS

The spectral properties of the dyes were studied in the presence of a BSA–SDS mixture (Table 2). The SDS molecules denature the protein and form micelle-like structures on the denatured polypeptide chain. The presence of a BSA–SDS mixture in dye solutions results in a long-wave shift of λ_{abs} for 2–37 nm and λ_{ex} for 5–51 nm. The values of λ_{em} are shifted to the short-wave region for 2–28 nm, except the dye SIP-8 (long-wave

Table 2
Spectral-luminescent characteristics of the dyes SIP-1–SIP-12 in aqueous buffer in the presence of BSA (0.2 mg/ml), and in the presence of BSA–SDS mixture (0.2 mg/ml BSA and 0.05% SDS)

Name	In BSA presence					In BSA and SDS presence				
	λ_{abs} (nm)	λ_{ex} (nm)	λ_{em} (nm)	I^{BSA} (a.u.)	I^{BSA}/I_0	λ_{abs} (nm)	λ_{ex} (nm)	λ_{em} (nm)	I^{SDS} (a.u.)	I^{SDS}/I_0
SIP-1	397	397	576	308	0.88	427	444	559	800	2.3
SIP-2	392	400	575	270	1.06	429	444	560	491	1.93
SIP-3	394	402	577	268	0.95	425	443	562	613	2.2
SIP-4	392	397	574	312	0.75	427	442	554	555	1.34
SIP-5	421	424	583	244	0.76	404	435	610	73	0.23
SIP-6	392	396	571	348	1.14	425	441	558	668	2.2
SIP-7	399	407	575	379	1.04	418	447	563	528	1.45
SIP-8	427	450	538	510	26.8	414	422	599	93	4.9
SIP-9	421	451	572	238	0.71	408	434	575	156	0.46
SIP-10	419	443	563	397	1.53	416	445	560	251	0.97
SIP-11	360	366	476	37	1.03	375	380	472	156	4.33
	369[a]	388	560	18	1.64					
SIP-12	412	427	562	496	1.57	420	438	561	396	1.25

Dye concentration, 5×10^{-6} M.
λ_{abs}, λ_{ex}, λ_{em} – Maximum wavelength of absorption, fluorescence excitation, and fluorescence emission, respectively.
I^{BSA}, I^{SDS} – Fluorescence intensity value (in arbitrary units, a.u.) of the dye in buffer in the presence of BSA and BSA–SDS mixture.
[a] Peak is manifested as a shoulder.

shift for 3 nm). The fluorescence intensity enhancement value (I^{SDS}/I_0) lies between 0.23 and 4.9.

3. Conclusions

For the first time series of styrylcyanines containing a 2-aryl imidazo[1,2-*a*]pyridinium moiety and with different substituents at 2-phenyl ring was synthesized using a modified method for synthesis of styrylcyanines.

Spectral-luminescent properties of imidazo[1,2-*a*]pyridinium dyes in the free state and in the presence of DNA, RNA, BSA and a BSA/detergent mixture were studied. Moderate level of intrinsic fluorescence was found for the majority of dyes in their free state. The wavelength change in the absorption, excitation and fluorescence maxima in the presence of DNA, RNA, protein or BSA–SDS mixture points to specific binding of the dye molecule with the corresponding biological molecule.

In addition, in the majority of dyes, irrespective of the nature of substituents, fluorescence intensity in the presence of biological molecules were either unchanged or decreased. Such behavior makes these dyes unsuitable for homogeneous fluorescent assays.

However, the imidazo[1,2-*a*]pyridinium dye SIP-8 was found to bind to BSA with a resultant 26-fold increase in fluorescence intensity. Unlike the other dyes, SIP-8 contains C_6 *N*-alkyl tails that in our opinion are responsible for the fluorescence intensity increase. This dye is a suitable candidate for homogeneous BSA detection.

4. Experimental

Novel styryl dyes were synthesized according to the procedures described below. Structures of obtained compounds were confirmed with ^{1}H NMR and element analysis.

4.1. Procedure 1. Synthesis of substituted imidazo[1,2-a]pyridines (Scheme 1)

A mixture of 2.16 g (0.02 mol) of 2-amino-4-picoline and (0.02 mol) of corresponding phenacyl bromide was boiled in ethanol for 4 h. Then 0.2 ml of concentrated HCl was added, and the mixture was boiled for 2 h more. To the warm reaction mixture, 3.4 g of KI dissolved in 3 ml of water was added. After cooling, hydroiodide was filtered off and washed with ethanol. Obtained substance was dissolved in boiling water and 3 ml of Et_3N was added. The precipitate was filtered off and washed with water (yield, 70–83%).

4.2. Procedure 2. Synthesis of quaternary imidazo[1,2-a]pyridinium salts (Scheme 1)

A mixture of 0.01 mol of corresponding imidazo[1,2-*a*]pyridine and 0.011 mol of alkylation agent (diethyl sulfate or heptyl iodide) in 3 ml of dioxane was boiled for 3–10 h. Then diethyl ether was added and the mixture was filtered off. Precipitate was washed with ether and used without additional purification (yield, 90–95%).

4.3. Procedure 3. Synthesis of styryl imidazo[1,2-a]pyridinium salts (Scheme 2)

A mixture of 0.001 mol of quaternary salt and 0.0011 mol of corresponding aldehyde and 3 drops of piperidine in 5 ml of *n*-butanol was boiled for 6 h. If necessary, by adding KI or $NaClO_4$ the dye could be converted into corresponding salt. The precipitate was filtered off, washed with water, alcohol and ether. Dye was recrystallized from DMF–ethanol mixture (yield, 58–73%).

4.4. Spectroscopic studies

Absorption spectra were obtained with Specord M40 spectrophotometer (Germany). Fluorescence excitation and emission spectra were obtained with a Cary Eclipse fluorescence spectrophotometer (Australia). All measurements were carried out at room temperature.

4.5. Preparation of working solutions

The dye stock solutions with a concentration of 2×10^{-3} M were prepared in DMF. Concentration of dye in working solutions was 5×10^{-6} M. A 0.05 M Tris–HCl (pH = 8.0) buffer was used for the measurements. Total DNA from chicken erythrocytes, total yeast RNA, and BSA were purchased from Sigma. The nucleic acid concentrations in the working solutions were 6×10^{-5} M (b.p.) for DNA and 1.2×10^{-4} M (b.) for RNA. The used concentration of BSA in working solutions was 0.2 mg/ml. For preparation of protein-detergent solution, concentrations of 0.2 mg/ml BSA and 0.05% SDS were used.

4.6. ^{1}H NMR spectra and CHN characteristics of synthesized dyes

4.6.1. 7-[2-(4-Dimethylaminophenyl)-1-ethenyl]-1-ethyl-2-phenylimidazo[1,2-a]pyridin-1-ium iodide (SIP-1)

Yield: 71%; m.p.: 277–279 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.32 (3H, t, $J = 8.0$), 3.01 (6H, s), 4.41 (2H, q, $J = 8.3$), 6.79 (2H, d, $J = 9.3$), 7.21 (1H, d, $J = 18.5$), 7.55 (2H, d, $J = 9.3$), 7.70 (6H, m), 7.85 (1H, d,

$J = 8.0$), 8.27 (1H, s), 8.43 (1H, s), 8.82 (1H, d, $J = 7.3$). Anal. calcd. for $C_{25}H_{26}IN_3$: C, 60.61; H, 5.29; N, 8.48. Found: C, 60.43; H, 5.27; N, 8.35.

4.6.2. 7-[2-(4-Dimethylaminophenyl)-1-ethenyl]-1-ethyl-2-(4-methylphenyl)imidazo[1,2-a]pyridin-1-ium ethosulfate (SIP-2)

Yield: 68%; m.p.: 186–188 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.11 (3H, t, $J = 8.0$), 1.31 (3H, t, $J = 8.3$), 2.45 (3H, s), 3.01 (6H, s), 3.75 (2H, q, $J = 8.0$), 4.40 (2H, q, $J = 8.3$), 6.81 (2H, d, $J = 9.6$), 7.20 (1H, d, $J = 18.0$), 7.48 (2H, d, $J = 8.7$), 7.57 (4H, m), 7.72 (1H, d, $J = 18.0$), 7.84 (1H, d, $J = 7.0$), 8.24 (1H, s), 8.38 (1H, s), 8.80 (1H, d, $J = 8.0$). Anal. calcd. for $C_{28}H_{33}N_3O_4S$: C, 66.25; H, 6.55; N, 8.28. Found: C, 65.97; H, 6.58; N, 8.11.

4.6.3. 7-[2-(4-Dimethylaminophenyl)-1-ethenyl]-1-ethyl-2-(3-methoxyphenyl)imidazo[1,2-a]pyridin-1-ium iodide (SIP-3)

Yield: 64%; m.p.: 346–347 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.33 (3H, t, $J = 7.7$), 3.01 (6H, s), 3.88 (3H, s), 4.43 (2H, q, $J = 8.0$), 6.79 (2H, d, $J = 9.7$), 7.21 (4H, m), 7.57 (3H, m), 7.73 (1H, d, $J = 18.3$), 7.85 (1H, d, $J = 9.0$), 8.27 (1H, s), 8.44 (1H, s), 8.82 (1H, d, $J = 7.7$). Anal. calcd. for $C_{26}H_{28}IN_3O$: C, 59.43; H, 5.37; N, 8.00. Found: C, 59.15; H, 5.31; N, 7.87.

4.6.4. 2-(2,4-Dimethoxyphenyl)-7-[2-(4-dimethylaminophenyl)-1-ethenyl]-1-ethylimidazo[1,2-a]pyridin-1-ium iodide (SIP-4)

Yield: 67%; m.p.: 190–192 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.35 (3H, t, $J = 8.3$), 3.01 (6H, s), 3.87 (3H, s), 3.89 (3H, s), 4.44 (2H, q, $J = 8.0$), 6.80 (2H, d, $J = 8.6$), 7.24 (4H, m), 7.57 (2H, d, $J = 8.2$), 7.74 (1H, d, $J = 16.3$), 7.86 (1H, d, $J = 6.0$), 8.27 (1H, s), 8.40 (1H, s), 8.82 (1H, d, $J = 6.3$). Anal. calcd. for $C_{27}H_{30}IN_3O_2$: C, 58.38; H, 5.44; N, 7.56. Found: C, 58.51; H, 5.47; N, 7.39.

4.6.5. 7-[2-(4-Dimethylaminophenyl)-1-ethenyl]-1-ethyl-2-(4-phenylphenyl)imidazo[1,2-a]pyridin-1-ium iodide (SIP-5)

Yield: 61%; m.p.: >300 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.37 (3H, t, $J = 8.0$), 3.01 (6H, s), 4.46 (2H, q, $J = 6.3$), 6.81 (2H, d, $J = 9.7$), 7.21 (1H, d, $J = 17.3$), 7.52 (5H, m), 7.75 (6H, m), 7.97 (2H, d, $J = 9.3$), 8.28 (1H, s), 8.47 (1H, s), 8.83 (1H, d, $J = 8.3$). Anal. calcd. for $C_{31}H_{30}IN_3$: C, 65.15; H, 5.29; N, 7.35. Found: C, 65.01; H, 5.31; N, 7.22.

4.6.6. 7-[2-(4-Dimethylaminophenyl)-1-ethenyl]-1-ethyl-3-methyl-2-phenylimidazo[1,2-a]pyridin-1-ium ethosulfate (SIP-6)

Yield: 75%; m.p.: 183–185 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.11 (3H, t, $J = 8.0$), 1.24 (3H, t, $J = 8.0$), 2.48 (3H, s), 3.01 (6H, s), 3.75 (2H, q, $J = 8.0$), 4.33 (2H, q, $J = 8.0$), 6.81 (2H, d, $J = 9.7$), 7.21 (1H, d, $J = 18.3$), 7.56 (2H, d, $J = 9.3$), 7.68 (6H, m), 7.89 (1H, d, $J = 8.0$), 8.26 (1H, s), 8.77 (1H, d, $J = 8.3$). Anal. calcd. for $C_{29}H_{35}N_3O_4S$: C, 61.30; H, 5.54; N, 8.25. Found: C, 61.07; H, 5.49; N, 8.12.

4.6.7. 7-[2-(4-Dimethylaminophenyl)-1-ethenyl]-2-(3,4-dimethylphenyl)-1-ethylimidazo[1,2-a]pyridin-1-ium iodide (SIP-7)

Yield: 65%; m.p.: 284–286 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.32 (3H, t, $J = 7.5$), 2.36 (1H, s), 3.01 (1H, s), 4.42 (2H, q, $J = 7.7$), 6.79 (2H, d, $J = 9.2$), 7.21 (1H, d, $J = 15.9$), 7.45 (3H, m), 7.56 (2H, d, $J = 8.8$), 7.73 (1H, d, $J = 16.5$), 7.85 (1H, d, $J = 7.3$), 8.27 (1H, s), 8.39 (1H, s), 8.82 (1H, d, $J = 7.1$). Anal. calcd. for $C_{27}H_{30}IN_3$: C, 61.95; H, 5.78; N, 8.03. Found: C, 61.73; H, 5.80; N, 7.91.

4.6.8. 7-[2-(4-Dihexylaminophenyl)-1-ethenyl]-2-(2,4-dimethoxyphenyl)-1-ethylimidazo[1,2-a]pyridin-1-ium iodide (SIP-8)

Yield: 58%; m.p.: 236–238 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 0.89 (6H, t br), 1.32 (15H, m), 1.54 (4H, m), 3.33 (4H, m), 3.89 (6H, d s, $J = 4.7$), 4.43 (2H, q br), 6.72 (2H, d, $J = 9.0$), 7.05–7.38 (4H, m), 7.52 (2H, d, $J = 7.3$), 7.70 (1H, d, $J = 18.0$), 7.83 (1H, m), 8.23 (1H, s), 8.37 (1H, s), 8.79 (1H, m). Anal. calcd. for $C_{37}H_{50}IN_3O_2$: C, 63.88; H, 7.24; N, 6.04. Found: C, 63.52; H, 7.20; N, 5.89.

4.6.9. 2-(4-Chlorophenyl)-7-[2-(4-dimethylaminophenyl)-1-ethenyl]-1-heptylimidazo[1,2-a]pyridin-1-ium iodide (SIP-9)

Yield: 65%; m.p.: >300 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 0.819 (3H, t, $J = 8.2$), 1.11 (8H, m), 1.62 (2H, m), 3.01 (3H, s), 4.40 (2H, t, $J = 8.5$), 6.80 (2H, d, $J = 9.0$), 7.22 (1H, d, $J = 16.1$), 7.57 (2H, d, $J = 9.2$), 7.75 (5H, m), 7.87 (2H, d, $J = 7.1$), 8.29 (1H, s), 8.46 (1H, s), 8.84 (1H, d, $J = 7.7$). Anal. calcd. for $C_{30}H_{35}ClIN_3$: C, 60.06; H, 5.88; N, 7.00. Found: C, 60.25; H, 5.84; N, 6.91.

4.6.10. 7-[2-(4-Dimethylaminophenyl)-1-ethenyl]-1-heptyl-2-(4-methylphenyl)imidazo[1,2-a]pyridin-1-ium iodide (SIP-10)

Yield: 70%; m.p.: 254–256 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 0.81 (3H, t, $J = 7.5$), 1.14 (8H, m), 1.63 (2H, t br), 2.45 (3H, s), 3.01 (6H, s), 4.39 (2H, t, $J = 7.0$), 6.80 (2H, d, $J = 10.0$), 7.21 (1H, d, $J = 17.7$), 7.47 (2H, d, $J = 8.7$), 7.57 (4H, m), 7.72 (1H, d, $J = 17.7$), 7.85 (1H, d, $J = 8.0$), 8.26 (1H, s), 8.39 (1H, s), 8.80 (1H, d, $J = 7.3$). Anal. calcd. for $C_{31}H_{38}IN_3$: C, 64.24; H, 6.61; N, 7.25. Found: C, 64.09; H, 6.64; N, 7.15.

4.6.11. 1-Ethyl-7-[2-(4-hydroxyphenyl)-1-ethenyl]-2-(4-methylphenyl)imidazo[1,2-a]pyridin-1-ium perchlorate (SIP-11)

Yield: 62%; m.p.: 176–178 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 1.32 (3H, t, $J = 8.0$), 2.46 (3H, s), 4.42 (2H, q, $J = 8.0$), 6.88 (2H, d, $J = 10.0$), 7.27 (1H, d, $J = 17.7$), 7.48 (2H, d, $J = 9.3$), 7.58 (4H, m), 7.73 (1H, d, $J = 18.3$), 7.86 (1H, d, $J = 6.7$), 8.29 (1H, s), 8.41 (1H, s), 8.84 (1H, d, $J = 7.7$), 9.6–10.2 (1H, s br). Anal. calcd. for $C_{24}H_{23}ClN_2O_5$: C, 63.37; H, 5.10; N, 6.16. Found: C, 63.21; H, 5.16; N, 6.21.

4.6.12. 7-[2-(4-Dimethylaminophenyl)-1-ethenyl]-1-hepthyl-3-methyl-2-phenylimidazo[1,2-a]pyridin-1-ium iodide (SIP-12)

Yield: 73%; m.p.: 203–205 °C; ^{1}H NMR (DMSO-d_6) δ (ppm): 0.80 (3H, t, $J = 8.0$), 1.11 (8H, m), 1.56 (2H, t br), 2.48 (3H, s), 3.01 (6H, s), 4.33 (2H, t, $J = 8.0$), 6.80 (2H, d, $J = 9.7$), 7.22 (1H, d, $J = 18.7$), 7.56 (2H, d, $J = 9.7$), 7.65 (5H, m), 7.76 (1H, d, $J = 17.7$), 7.89 (H, d, $J = 8.3$), 8.28 (1H, s), 8.77 (1H, d, $J = 8.0$). Anal. calcd. for $C_{32}H_{40}IN_3$: C,64.24; H, 6.61; N, 7.25. Found: C, 64.06; H, 6.58; N, 7.16.

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