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7-NITROBENZO[c] [1, 2, 5]OXADIAZOLE (NITROBENZOFURAZAN) DERIVATIVES WITH A SULFIDE GROUP AT THE 4-POSITION. SYNTHESIS AND PHYSICAL PROPERTIES

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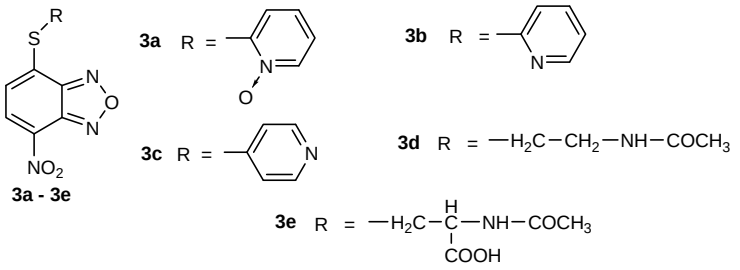
By applying a nucleophilic aromatic substitution process (S_NAr) between electrophile 4-chloro-7-nitrobenzo[c] [2.1.5]-oxadiazole **1** and mercaptoderivatives **2a**–**2e** as nucleophilic agents (**2a**, 2-pyridinethiol-1-oxide; **2b**, 2-pyridinethiol; **2c**, 4-pyridinethiol; **2d**, N-acetylcysteamine and **2e**, N-acetylcysteine) were obtained the corresponding thioethers **3a**–**3e**. The structure of compounds **3a**–**3e** was confirmed by ^{1}H and ^{13}C-NMR, IR and MS. For these sulfides with a 7-nitrobenzo[*c*] [1.2.5]-oxadiazole moiety, electron absorption and emission spectra, as well as their hydrophobic/hydrophilic properties are reported.



INTRODUCTION

7-Nitro-benzo[*c*] [1, 2, 5]oxadiazoles (NBD derivatives) are commonly called nitrobenzo-furazans, but the older numbering differs. Such derivatives with various electron donor substituents in position 4 have been used as fluorogenic and fluorescent reagents.[1-7] Some of them have biological applications such as antileukemic and immuno-suppressive drugs,[1,4,6] anticancer activity,[8-11] and antiviral as influenza A (H1N1) replication inhibitor.[12,13] The reaction of acetylcysteine and captopril with NBD-Cl via sulfhydryl groups leads to the derivatives which were spectrophotometrically and polarographically measured.[14] For sufides **3a** – **3e** having a nitrobenzofurazan moiety attached via sulfur atom ("pivot atom") we explored their physical properties. We reported in a previous paper about two NBD-sulfides prepared from NBD-Cl (**1**) and 2-benzoxazolethiol or 2-benzothiazolethiol, respectively.[2] The compounds **3a**,[10,15-18] **3b**[15] and **3d**[19] were known for the biological applications, but no data were provided on physico-chemical properties. Our previous studies with NBD derivatives involved reactions of NBD-Cl with other nucleophiles than thiols **2**.[20-22]

RESULTS AND DISCUSSION

Synthesis of compounds 3a – 3e

For the synthesis of thioethers **3a** – **3e** (Scheme 1) we applied an S_NAr process[2,20-22] involving the electrophile 4-chloro-7-nitrobenzo[c][2.1.5]-oxa-

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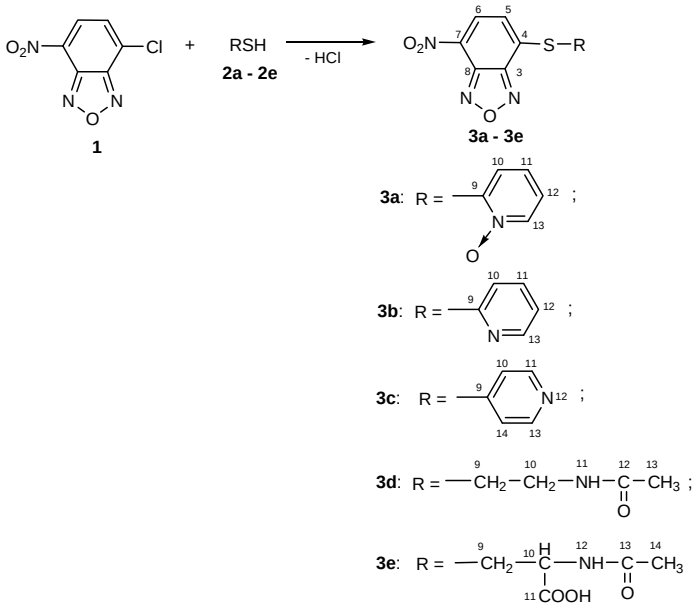
diazole **1** and thiolic nucleophiles **2a** – **2e** (RSH, Scheme 1) where: R = 2-pyridinethiol-1-oxide (**2a**); 2-pyridinethiol (**2b**); 4-pyridinethiol (**2c**); N-acetylcysteamine (**2d**) and N-acetylcysteine (**2e**) with sodium hydrogen carbonate in ethanol as solvent. In the case of compound **3a**, the reaction was carried out without a base, using acetone as solvent. The yields varied between 45% and 85%.

The structure of compounds **3a** – **3e** was confirmed by MS, IR, ^{1}H-NMR, and ^{13}C-NMR.



Scheme 1. – Synthesis of sulfides **3a** – **3e**.

The spectral and fluorescence characteristics which will be presented below brought additional arguments for the nitrobenzofurazan-sulfide structure.

For melting point of compound **3b** the literature provides[15] a lower value (81-82ºC) than the one found by us. By our synthetic procedure, after thin layer chromatography (TLC) purification, the melting point is 119-120ºC. The synthesis procedure from the literature[15] for **3b** does not indicate the purification method. By this procedure we obtained compound **3b** in a mixture; after TLC analysis and purification (silicagel GF_{254} Merck with dichloroethane-toluene mixture 1 : 1 v/v, twice) the melting point is 116-117ºC.

Electronic absorption and fluorescence measurements

Absorption and fluorescence characteristics of compounds **3a** – **3e** have been studied both in solid state as well as in solution. It is known that the presence of electron-donor substituents at position 4 of the nitrobenzofurazan moiety in the molecule leads to bathochromic effects and to fluorescence.[3,7]

On comparing the absorption wavelengths (Table 1) in propanol with those in the less polar solvent isopropanol, one can see that, for the absorption maximum around 400 nm, decreased solvent polarity leads to a small bathochromic effect. It is probable that this lowest electronic absorption energy is associated with a $\pi\rightarrow\pi^*$ transition in the nitro-benzofurazan moiety. In solid state absorption plots are less clear-cut. Figure 1 shows the absorption spectra of compounds **3a** – **3e** in isopropanol.

In solution, the absorption spectra of compounds **3a** – **3e** are independent of the solvent polarity, the $E_T(30)$ value[23] (Table 1). The strong bathochromic shift, irrespective of the solvent, was observed in the case of compound **3e** while, for the compounds **3a** – **3d** no significant changes in the absorption wavelengths are observed (Table 1). The peak centered around 400 nm for compounds **3a** – **3e** in all studied solvents is attributed to a polarizing $\pi\rightarrow\pi^*$ transition along the long axis of the compound.

The fluorescence emission spectra in solution (Figure 2) of compounds **3a** – **3e** exhibited a single maximum $\lambda_{em} \sim 530$ nm; fluorescence characteristics of compounds **3a** – **3e** are summarised in Table 2. Compounds **3a** – **3e** show fluorescence whose intensity varies from case to case depending on the quantum yield (ϕ with the standard quinine

bisulfate, see Experimental part) and on the solvent polarity (ϕ decreases when the polarity according to the $E_T(30)$ value increases). The obtained results are presented in Table 2.

Compound **3e** is the most fluorescent of the five compounds, whatever the solvent. It should be noted that the **3b** exhibits lowest values of ϕ in methanol (ϕ = 0.0050) and ethanol (ϕ = 0.0079).

Table 1

Absorption characteristics of compounds **3a** – **3e** in various solvents

Comp. (conc.)	Solvent	$E_T(30)$	λ_{abs} (nm)	$\varepsilon\times10^3$ ($L\times mole^{-1}\times cm^{-1}$)
3a (2.66×10^{-5} M)	Methanol	55.5	390	4.14
	Ethanol	51.9	392	4.51
	Propanol	50.7	395	5.64
	Isopropanol	48.6	396	3.08
3b (6.28×10^{-5} M)	Methanol	55.5	407	9.39
	Ethanol	51.9	407	10.35
	Propanol	50.7	409	9.87
	Isopropanol	48.6	407	10.35
3c (4.85×10^{-5} M)	Methanol	55.5	400	9.28
	Ethanol	51.9	400	10.30
	Propanol	50.7	400	14.23
	Isopropanol	48.6	399	11.13
3d (6.66×10^{-5} M)	Methanol	55.5	414	17.57
	Ethanol	51.9	414	11.41
	Propanol	50.7	414	13.81
	Isopropanol	48.6	414	12.01
3e (5.45×10^{-5} M)	Methanol	55.5	411	3.85
	Ethanol	51.9	416	3.49
	Propanol	50.7	414	4.59
	Isopropanol	48.6	420	4.04

Table 2

Fluorescence characteristics of the compounds **3a** – **3e** in various solvents

Comp. (conc.)	Solvent	$E_T(30)$	λ_{ex}, nm	λ_{em} , nm	ϕ
3a *(2.66×10^{-5} M)*	Methanol	55.5	400	533 bb**	-
	Ethanol	51.9		537 bb**	-
	Propanol	50.7		532 sh*	-
	Isopropanol	48.6		537	0.0656
3b *(6.28×10^{-5} M)*	Methanol	55.5	400	522	0.0050
	Ethanol	51.9		522	0.0079
	Propanol	50.7		526	0.0110
	Isopropanol	48.6		526	0.0114
3c *(4.85×10^{-5} M)*	Methanol	55.5	400	521	0.0084
	Ethanol	51.9		526	0.0127
	Propanol	50.7		525	0.0133
	Isopropanol	48.6		526	0.0205
3d *(6.66×10^{-5} M)*	Methanol	55.5	400	516	0.0473
	Ethanol	51.9		513	0.0585
	Propanol	50.7		514	0.0439
	Isopropanol	48.6		513	0.0440
3e *(5.45×10^{-5} M)*	Methanol	55.5	480	542	0.2467
	Ethanol	51.9		539	0.5259
	Propanol	50.7		538	0.4691
	Isopropanol	48.6		537	0.5158

Note: *sh - shoulder; **bb - broad band

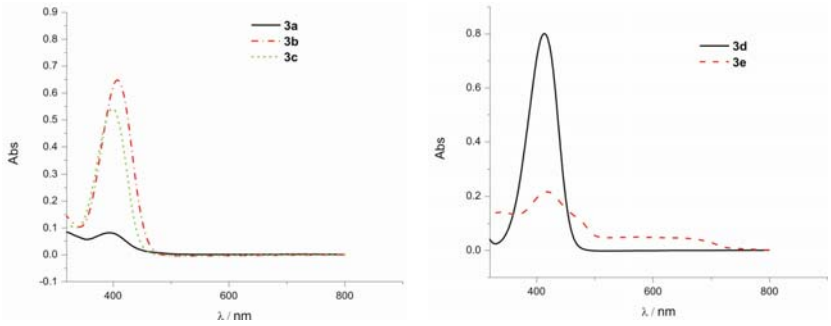


Fig. 1 – Absorption spectra in isopropanol of derivatives **3a** – **3e**.

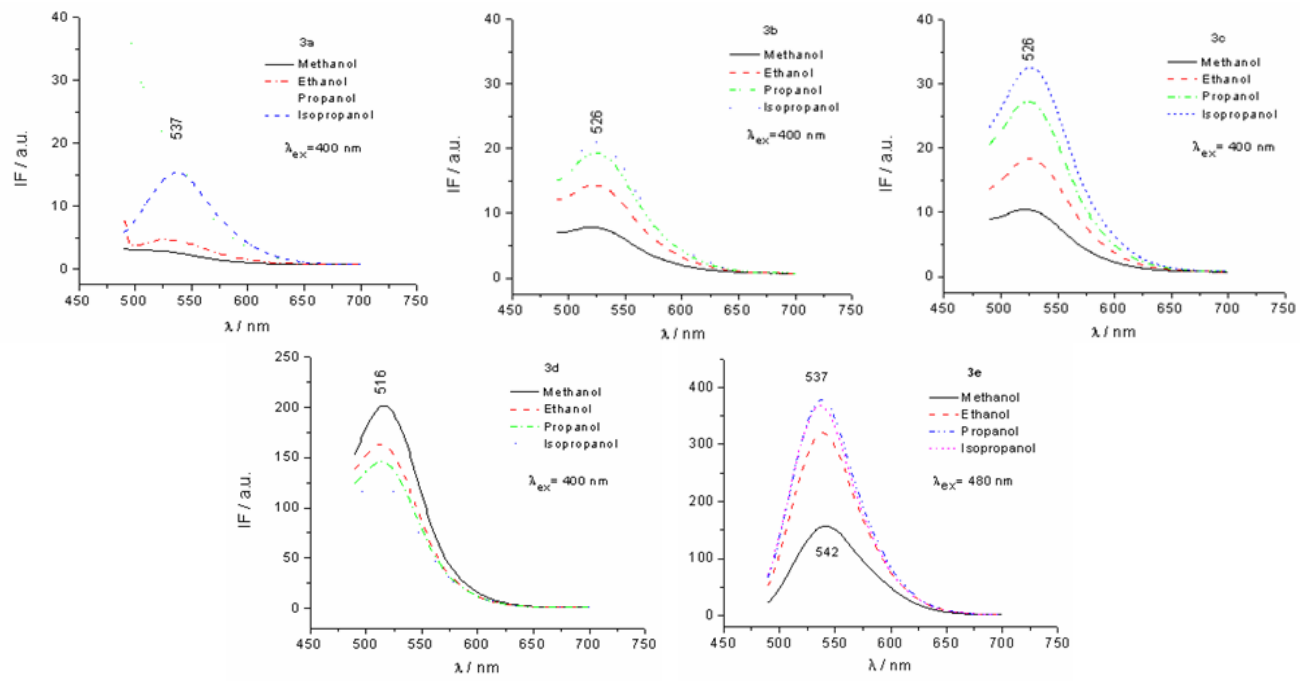


Fig. 2 – Fluorescence emission spectra in various solvents of compounds **3a** – **3e**.

In solid state the absorption spectra of compounds **3a** – **3e** exhibited also maxima around 400 nm, due to $\pi \rightarrow \pi^*$ transitions. By excitation at 450 nm of compounds **3a** – **3e** in solid state, except for compounds **3a** and **3e** which are poorly fluorescent, the other three compounds **3b** – **3d** show fluorescence with different λ_{em} values.

Hydrophobic/hydrophilic properties

The hydrophobic/hydrophilic property of organic compounds due to their structure is important theoretically and also practically, for physico-chemical and biomedical applications.[24] Currently this property is defined by the parameter log P (*n*-octanol – water partition coefficient), which is determined experimentally or is calculated with various programs.[25-27] Reverse phase thin-layer chromatography (RP-TLC) has found application in determining the molecular hydrophobicity (R_{M0} value) based on eq. (1) and eq. (2).[28,29]

$$R_M = \log (1/R_f - 1) \quad (1)$$

$$R_M = R_{M0} + bK \quad (2)$$

The R_{M0} value[28,29] for compounds **3a** – **3d** was determined by applying RP-TLC in experimental conditions and based on eq. (1) and eq. (2) (Table 3). For the compound **3e**, the hydrophobicity could not be determined because the spot of this compound moved with the solvent front. In conclusion, depending on the R_{M0} values: (i) the hydrophobicity decreases in order of compounds **3c** > **3b** > **3d** > **3a** (hydrophility increases in reverse order); (ii) excepting the presence of relatively polar N-oxide group (compound **3a**), the pyridine moiety increases the hydrophobicity.

Table 3

Experimental hydrophobicity (R_{M0}, b)[a] for **3a** – **3d**

Compound	R_M in aqueous ethanol, conc.(v/v)				R_{M0}	b	Statistical parameters		
	70%	60%	50%	40%			*R*	*F*	*SD*
3a	-0.586	-0.367	-0.124	0.189	1.190	-0.025	-0.996	276.066	0.034
3b	-0.263	0.058	0.397	0.564	1.741	-0.028	-0.990	101.803	0.062
3c	-0.154	0.116	0.528	0.662	1.864	-0.028	-0.984	61.188	0.081
3d	-0.586	-1.145	-1.217	-1.430	1.607	-0.031	-0.991	112.653	0.066

[a)]Silica gel RP-18 F_{254} (Merck); R_{M0} = molecular hydrophobicity (eq. 2); b = change in R_M value caused by increasing the concentration (K) of the organic component in the mobile phase (eq. 1); R = correlation coefficient for parameters R_{M0} and b in eq. (2).[28,29]

EXPERIMENTAL PART

1. General. ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Varian Gemini 300BB (300 MHz for ^{1}H and 75 MHz for ^{13}C); the numbering of the compounds **3a** – **3e** as that from Scheme 1. FTIR spectra were recorded on a Bruker Vertex 70 spectrometer with horizontal device for attenuated reflectance and diamond crystal, on a spectral window ranging from 4000 to 400 cm^{-1}.

In solution, the absorption spectra were recorded using a spectrophotometer Perkin Elmer, Lambda 35, at a scan rate of 480 nm/min and slit, 1 nm. In solid, the absorption spectra were recorded with the same spectrophotometer, equipped with an integrating sphere. As reference, a certified reflectance standard, spectralon, has been used and the measurements were carried out in the range 900-200 nm. The sample holder is 8º wedge and the used parameters were: data interval, 1 nm; scan speed, 120 nm/min and slits, 4 nm.

The fluorescence emission spectra were recorded: i) in solution, with Jasco FP-6500 Spectrofluorometer, using 5 nm bandpasses for the excitation and the emission monochromators, the detector response of 1 sec, data pitch of 1 nm, the scanning speed of 100 nm/min; ii) in solid, on the same device, using the EpiFluorescence attachment (EFA - 383).

Melting points (uncorrected) have been recorded in open capillary with Electrothermal IA 9000 Series of digital melting point instruments.

Mass spectra were recorded with Varian 1200 L/MS/MS Triple Quadrupole with ESI interface both positive and negative ionization, fragmentation by collision with argon at 1.5 mTorr.

Starting compounds for synthesis: **1** from Merck, **2a** – **2e** from Aldrich. Silicagel plates $60F_{254}$ (for TLC) and silica gel plates RP-$18F_{254S}$ (for RP-TLC) were from Merck. Methanol (spectrophotometric grade) and 1-propanol was purchased from Flucka; ethanol (absolute spectral) and isopropyl alcohol was purchased from Sigma-Aldrich.

2. Synthesis of the compounds 3a – 3e

2-(7-Nitro-benzo[c][2.1.5]oxadiazol-4-yl) thiopyridine-1-oxide (**3a**). 4-Chloro-7-nitrobenzo[c][2.1.5]-oxadiazole (NBD-Cl) **1** and 2-thiopyridine-1-oxide **2a** (molar ratio 1 : 1) were stirred for 10 min. at room temperature in acetone (10 mL /0.1 g of **1**) when a precipitate was formed. The reaction mixture was filtered and the precipitate retained after filtration through a G3 glass filter was purified by preparative TLC (silica gel GF_{254} with methylene chloride : methanol = 10 : 0.2 v/v, three times). 46% yield, orange solid, m.p. 198-199°C (lit. m.p. 201-202°C [1]); ESI-MS, (*m/z*); for $C_{11}H_6N_4SO_4$ (**3a**, M=290), in positive: $[M+H]^+$ 291; Anal.: Calcd. for $C_{11}H_6N_4SO_4$: C 45.52; H 2.08; N 19.3; S 11.05; found C 45.45; H 2.03; N 19.26; S 11.01%; ^{1}H-NMR (DMSO-d6, δ ppm, *J* Hz): 8.70 (d, 1H, H-6, 7.4); 8.44 (bd, 1H, H-13, 6.5); 8.05 (d, 1H, H-5, 7.4); 7.39 (td, 1H, H-12, 6.5, 3.1); 7.27÷7.22 (m, 2H, H-10, H-11); ^{13}C-NMR (DMSO-d6, δ ppm): 150.73 (C-7); 146.07 (C-9); 143.47 (C-4); 136.98 (C-3); 128.52 (C-8); 138.61 (C-13); 136.63 (C-5); 131.88 (C-6); 125.98 (C-10 or C-11); 125.84 (C-10 or C-11); 124.32 (C-12); FT-IR (ATR in solid, ν cm^{-1}): 3101w; 3076m; 3039w; 1518vs; 1469m;1422s; 1363m; 1330s; 1275m; 1250s; 1227m; 1140m; 1044w; 1000w; 958w; 890m; 843w; 808w; 758m; 733w; 704w.

2-(2-[(7-Nitro-benzo[c][2.1.5]oxadiazol-4-yl)) thiopyridine (**3b**). (NBD-Cl) **1** and 2-thiopyridine **2b** (molar ratio 1 : 1) in ethanol (about 10 mL / 0.2 g of compound **1**) and sodium hydrogen carbonate (about 1.1 mol / 1 mol of compound **1**) was refluxed for half an hour. The red solution was diluted with water, then the mixture was acidified with 1N hydrochloric acid to *p*H = 1 till a precipitate was formed. The precipitate was filtered off (G3 glass filter) and was obtained in pure state by preparative TLC using silica gel Merck GF_{254} with dichloroethane, three times. 85% yield, brown solid, m.p. 119-120°C (lit. m.p. 81-82°C [1]); ESI-MS, (*m/z*); for $C_{11}H_6N_4SO_3$ (**3b**, M=274), in positive: $[M+H]^+$ 275; Anal.: Calcd. for $C_{11}H_6N_4SO_3$: C 48.17; H 2.21; N 20.43; S 11.69; found C 48.12; H 2.16; N 20.39; S 11.66%; ^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 8.60 (ddd, 1H, H-13, 5.0, 1.9, 0.9); 8.38 (d, 1H, H-6, 8.0); 7.81 (td, 1H, H-11, 7.4, 7.4, 1.9); 7.64 (dt, 1H, H-10, 7.4, 1.1, 0.9); 7.58 (d, 1H, H-5, 8.0); 7.37 (ddd, 1H, H-12, 7.4, 5.0, 1.1); ^{13}C-NMR ($CDCl_3$, δ ppm): 152.13 (C-9); 151.18 (C-13); 149.59 (C-7); 142.75 (C-4); 138.29 (C-11); 137.02 (C-8); 134.52 (C-3); 130.66 (C-6); 127.76 (C-10); 126.40 (C-5); 124.02 (C-12); FT-IR (ATR in solid, ν cm^{-1}): 3128w; 3081m; 2924w; 2853w; 1568m; 1518vs; 1506vs; 1433s; 1415s; 1362m; 1319vs; 1253m; 1218m; 1098w; 1045s; 1004w; 954m; 895m; 863m; 805m; 765m; 732m; 672w.

4-(7-Nitro-benzo[c][2.1.5]oxadiazol-4-yl) thiopyridine (**3c**). (NBD-Cl) **1** and 4-thiopyridine **2c** (molar ratio 1 : 1) in ethanol (about 15 mL / 0.2 g of **1**) and sodium hydrogen carbonate (about 1.1 mol / 1 mol of **1**) was stirred at 50°C for 15 min; then the reaction mixture was refluxed for two hours.

The precipitate was filtered off (G3 glass filter) and washed with ethanol. The product was dried and it was obtained in pure state by preparative TLC using silica gel Merck GF_{254} with dichloroethane, three times. 52% yield, yellow dark solid, m.p. 145-146°C; ESI-MS, (*m/z*); for $C_{11}H_6N_4SO_3$ (**3c**, M=274), in positive: $[M+H]^+$ 275; Anal.: Calcd. for $C_{11}H_6N_4SO_3$: C 48.17; H 2.21; N 20.43; S 11.69; found C 48.11; H 2.16; N 20.39; S 11.66%; ^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 8.76 (d, 2H, H-11, H-13, 5.2); 8.32 (d, 1H, H-6, 8.2); 7.52 (d, 2H, H-10, H-14, 5.2); 7.05 (d, 1H, H-5, 8.2); ^{13}C-NMR ($CDCl_3$, δ ppm): 151.59 (C-11, C-13); 149.07 (C-7); 142.65 (C-4); 138.61 (C-9); 137.71 (C-3); 134.52 (C-8); 130.45 (C-6); 128.11 (C-10, C-14); 124.76 (C-5); FT-IR (ATR in solid, ν cm^{-1}): 3120w; 3071w; 3041w; 3005w; 2924w; 2853w; 1569m; 1508vs; 1434s; 1406s; 1361m; 1321vs; 1214m; 1045m; 988w; 953m; 893m; 860m; 810s; 770w; 731m; 706m; 670m; 504m.

N-Acetyl-cysteamino (7-nitrobenzo[c][2.1.5]oxadiazol-4-yl) sulfide (**3d**). Starting from 4-Chloro-7-nitrobenzo[c] [2.1.5]-oxadiazole **1** and **2d** (molar ratio 1 : 1) in ethanol (about 15 mL / 0.2 g of **1**) and sodium hydrogen carbonate (about 1.1 mol / 1 mol of **1**). The reaction mixture was stirred at room temperature for 24h. The precipitate was filtered off (G3 glass filter) and washed with ethanol. The product was dried and was obtained in pure state by preparative TLC using silica gel Merck GF_{254} with methylene chloride: methanol 10 : 0.2 v/v, twice. 67% yield, yellow solid, m.p. 163-163.5°C; ESI-MS, (*m/z*); for $C_{10}H_{10}N_4SO_4$ (**3d**, M=282), in positive: $[M+H]^+$ 283; Anal.: Calcd. for $C_{10}H_{10}N_4SO_4$: C 42.55; H 3.57; N 19.85; S 11.36; found C 42.50; H 3.54; N 19.80; S 11.34%; ^{1}H-NMR (DMSO-d6, δ ppm, *J* Hz): 8.57 (d, 1H, H-6, 8.0); 8.27 (bs, 1H, H-11, deuterable); 7.68 (d, 1H, H-5, 8.0); 3.43(bs, 4H, 2H-9, 2H-10); 1.83 (s, 3H, H-13). Because the amide is not soluble in chloroform, it was used DMSO. Isochronous protons, H-9 and H-10, form an enlarged singlet signal; ^{13}C-NMR (DMSO-d6, δ ppm): 169.74 (C-12); 149.19 (C-7); 142.63 (C-4); 139.51 (C-3); 132.23 (C-6); 122.26 (C-5); 36.98 (C-9); 30.46 (C-10); 22.47 (C-13). One of C signals is probable to coalescence and is not visible. In the case of compound 4-Chloro-7-nitrobenzo[c][2.1.5]-oxadiazole it has been observed that the signal at δ = 135.69 is a broad singlet, indicating the presence of an internal reorientation process dependent on the temperature; FT-IR (ATR in solid, ν cm^{-1}): 3259s; 3075m; 2952m; 2924m; 2853m; 1643s; 1553s; 1539s; 1511vs; 1432s; 1394w; 1362s; 1325vs; 1305vs; 1289vs; 1246m; 1217m; 1195m; 1113m; 1091m; 1049m; 1029m; 961m; 889m; 850s; 802w; 730m; 601m.

N-Acetyl-cysteino (7-Nitrobenzo[c][2.1.5]oxadiazol-4-yl)-sulfide (**3e**). 4-Chloro-7-nitrobenzo[c][2.1.5]-oxadiazole **1** was treated with **2e**, in molar ratio 1 : 1. The reaction medium was ethanol, about 10 mL / 0.2 g of **1**. A small excess of sodium hydrogen carbonate was used (about 1.1 / 1 mol of **1**). The mixture was stirred seven days at room temperature and the solution was concentrated under reduced pressure. Compound **3e** was isolated from the concentrated solution by repeated preparative TLC, then it was purified TLC using silica gel Merck GF_{254} and the elution solvent methylene chloride : methanol : acetic acid glacial = 8 : 2 : 0.5 v/v, twice). 85% yield, yellow solid, m.p. 87-88°C; ESI-MS, (*m/z*); for $C_{11}H_{10}N_4SO_6$ (**3e**, M=326), in negative: $[M]^{-\cdot}$ 325; Anal.: Calcd. for $C_{11}H_{10}N_4SO_6$: C 40.49; H 3.09; N 17.17; S 9.83; found C 40.46; H 3.05; N 17.14; S 9.79%. ^{1}H-NMR (DMSO-d6, δ ppm, *J* Hz): 8.50 (d, 1H, H-6, 8.0); 8.02 (bd, 1H, H-12, 6.8, deuterable); 7.56 (d, 1H, H-5, 8.0); 4.46(bs, 1H, H-10); 3.80 (d, 1H, H-9A, 4.4, 12.4); 3.55 (dd, 1H, H-9B, 6.6, 12.4); 1.86 (s, 3H, H-14). Because the amide is not soluble in chloroform, we used DMSO. H-10 protons form an AB system. H-9A proton is enlarged doublet due to lower coupling (unresolved) with H-10; the angle between the two protons mentioned before is near 90°. H-COSY spectra show the coupling H-12 connected to the amide nitrogen with H-10. ^{13}C-NMR (DMSO-d6, δ ppm): 172.61 (C-11); 169.44 (C-12); 149.07 (C-7); 142.49 (C-4); 140.70 (C-3); 132.40 (C-6); 131.82 (C-8); 122.00 (C-5); 52.46 (C-9); 34.64 (C-10); 22.69 (C-13). FT-IR (ATR in solid, ν cm^{-1}): 3366m; 3085w; 2957m; 2924m; 2854m; 1713w; 1598s; 1509vs; 1421s; 1363m; 1326vs; 1304s; 1217m; 1122w; 1048m; 963m; 896w; 852w; 807w; 732w. The proton spectra were carried out at 50°C and 70°C. By heating, H-N signal moves to a higher field, therefore at 70°C it appears as a doublet with δ = 7.76 ppm. Methylene protons signals from the AB system solve better and indicate hyperfine structure.

3. Electronic absorption and fluorescence measurements

The stock solutions of the compound **3a** – **3e** were prepared in methanol. Aliquots from stock solutions were dried at room temperature and then diluted with various solvents.

The fluorescence quantum yield (ϕ) was determined by comparison to dilute quinine bisulfate solution in 0.1N H_2SO_4 with 0.55 absolute quantum yield.[30]

CONCLUSIONS

Through a S_NAr process of the electrophile **1** (electrophile 4-chloro-7-nitrobenzo[c][2.1.5]-oxadiazole) with nucleophiles **2a** – **2e**, the corresponding thioethers **3a** – **3e** resulted. Except for the compound **3a**, which is weak fluorescent, the other four compounds **3b** – **3e** are fluorescent. The hydrophobicity of compounds **3a** – **3d** depends on the R_{M0} value (estimated by RP-TLC) as follows: **3c** > **3b** > **3d** > **3a**. The biological tests of all studied compounds **3a** – **3e** will be described in a separate paper. Studies of the cytotoxic activity of these compounds, in order to be used as antitumoral drugs, are currently in progress.

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REFERENCES

1. P. B. Ghosh and M. W. Whitehouse, *Biochem. J.*, **1968**, *108*, 155-156.
2. O. Oprea, M. Bem, M. T. Caproiu, C. Draghici, R. D. Baratoiu, A. C. Radutiu, A. Beteringhe, C. Enache, G. Ionita, T. Constantinescu and A. T. Balaban, *Rev. Roum. Chim.*, **2013**, *58*, 327-334.
3. M. Bem, F. Badea, C. Draghici, M. T. Caproiu, M. Vasilescu, M. Voicescu, A. Beteringhe, A. Caragheorgheopol, M. Maganu, T. Constantinescu and A. T. Balaban, *Arkivoc*, **2007**, *xiii*, 87-104.
4. P. B. Ghosh and M. W. Whitehouse, *J. Med. Chem.*, **1968**, *11*, 305-311.
5. D. J. Birkett, N. C. Price, G. K. Radda and A. G. Salmon, *FEBS Letters*, **1970**, *6*, 346-348.
6. D. Castagnolo, M. Pagano, M. Bernardini and M. Botta, *Tetrahedron Letters*, **2012**, *53,* 5008-5011.
7. S. Uchiyama, T. Santa, N. Okiyama, T. Fukushima and K. Imai, *Biomed. Chromatogr.*, **2001**, *15*, 295-318.
8. E. V. Patridge, E. S. E. Eriksson, P. G. Penketh, R. P. Baumann, R. Zhu, K. Shyam, L. A. Eriksson and A. C. Sartorelli, *Arch. Toxicol.*, **2012**, *86*, 1613-1625.
9. Y. L. Yang Yang and X. Guan, *Anal. Chem.*, **2012**, *84*, 6877-6883.
10. V. Sakanyan, M. Angelini, M. Le Béchec, M. F. Lecocq, F. Benaiteau, B. Rousseau, A. Gyulkhandanyan, L. Gyulkhandanyan, C. Logé, E. Reiter, C. Roussakis and F. Fleury, *Sci. Rep.*, **2014**, *4*, 1-11.
11. G. Ricci, F. De Maria, G. Antonini, P. Turella, A. Bullo, L. Stella, G. Filomeni, G. Federici and A. M. Caccuri, *J. Biol. Chem.*, **2005**, *280*, 26397-26405.
12. U. Kessler, D. Castagnolo, M. Pagano, D. Deodato, M. Bernardini, B. Pilger, C. Ranadheera and M. Botta, *Bioorg.&Med. Chem. Lett.*, **2013**, *23*, 5575-5577.
13. M. Pagano, D. Castagnolo, M. Bernardini, A. L. Fallacara, I. Laurenzana, D. Deodato, U. Kessler, B. Pilger, L. Stergiou, S. Strunze, C. Tintori and M. Botta, *Chem. Med. Chem.*, **2014**, *9*, 129-150.
14. R. Haggag, S. Belal and R. Shaalan, *Sci. Pharm.*, **2008**, *76*, 33-48.
15. F. Xie, B. X. Li, C. Broussard and X. Xiao, *Bioorg. Med. Chem. Lett.*, **2013**, *23*, 5371-5375.
16. S. Jasial, Y. Hu and J. Bajorath, *J. Med. Chem.*, **2017**, *60*, 3879-3886.
17. Y. Hu and J. Bajorath, *The AAPS J.*, **2013**, *15,* 808-815.
18. S. Cosconati, J. A. Hong, E. Novellino, K. S. Carroll, D. S. Goodsell and A. J. Olson, *J. Med. Chem.*, **2008**, *51*, 6627-6630.
19. D. Rotili, A. De Luca, D. Tarantino, S. Pezzola, M. Forgione, B. M. Della Rocca, M. Falconi, A. Mai and A. Caccuri, M. *Eur. J. Med. Chem.*, **2015**, *89*, 156-171.
20. M. Bem, M. T. Caproiu, D. Stoicescu, T. Constantinescu and A. T. Balaban, *Central Eur. J. Chem.*, **2003**, *3*, 260-276.
21. M. Bem, M. Vasilescu, M. T. Caproiu, C. Draghici, A. Beteringhe, T. Constantinescu, M. D. Banciu and A. T. Balaban, *Central Eur. J. Chem.*, **2004**, *4*, 672-685.
22. M. Bem, F. Badea, C. Draghici, M. T. Caproiu, M. Vasilescu, M. Voicescu, G. Pencu, A. Beteringhe, M. Maganu, I. C. Covaci, T. Constantinescu and A. T. Balaban, *Arkivoc*, **2008**, *ii*, 218-234.
23. C. Reichardt, "Solvents and Solvent Effects in Organic Chemistry", 3th edition, Wiley-VCH: Weinheim, 2003, p. 418.
24. C. Hansch, A. Leo, "Substituent Constants for Correlation Analysis in Chemistry and Biology", Wiley, New York, 1979.
25. A. C. Radutiu, I. Baciu, M. T. Caproiu, C. Draghici, A. Beteringhe, G. Ionita, P. Ionita, T. Spataru, N. Spataru, R. D. Baratoiu, T. Constantinescu and A. T. Balaban, *Arkivoc*, **2009**, *xiii*, 342-362.
26. R. D. Baratoiu, L. Mutihac, M. T. Caproiu, C. Draghici, F. Dumitrascu, R. Socoteanu, A. Beteringhe, M. Maganu, I. C. Covaci, M. Bem, T. Constantinescu and A. T. Balaban, *Arkivoc*, **2008**, *xi*, 307-321.
27. R. Mannhold, G. I. Poda, C. Ostermann and I. V. Tetko, *J. Pharm. Sci.*, **2009**, *98,* 861-893.
28. A. D. Kossoy, D. S. Risley, R. M. Kleyle and D. Nurok, *Anal. Chem.*, **1992**, *64,* 1345-1349.
29. E. Soczewinski, *Anal. Chem.*, **1969**, *41*, 179-182.
30. W. H. Melhuish, *J. Phys. Chem.*, **1961**, *65*, 229-235.