Synthesis, photophysics and biomolecules interactive studies of new hybrid benzo-2,1,3-thiadiazoles

José S. S. Neto, a Roberta Krüger, a Renata A. Balaguez, a Mariana G. Fronza, b Thiago V. Acunha, c Robson S. Oliboni, d Lucielli Savegnago, b Bernardo A. Iglesias c* and Diego Alves a*

a Laboratório de Síntese Orgânica Limpa - LASOL - CCQFA - Universidade Federal de Pelotas - UFPel - P.O. Box 354 - 96010-900, Pelotas, RS, Brazil.
b Programa de Pós-Graduação em Biotecnologia (PPGB), Grupo de Pesquisa em Neurobiotecnologia - GPN, Universidade Federal de Pelotas, UFPel, Postal Code 96010-900, Pelotas, RS, Brazil.
c Departament of Chemistry, Laboratório de Bioinorgânica e Materiais Porfirínicos, Universidade Federal de Santa Maria, UFSM, 97115-900 Santa Maria - RS, Brazil.
d Grupo de Catálise e Estudos Teóricos, Universidade Federal de Pelotas, UFPel. CEP 96010-900, Pelotas, RS, Brazil.

e-mail: bernardoppg@gmail.com and diego.alves@ufpel.edu.br

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General information

The reactions were monitored by TLC carried out on Merck silica gel (60 F254) by using UV light as visualizing agent and 5% vanillin in 10% H₂SO₄ and heat as developing agents. Baker silica gel (particle size 0.040–0.063 mm) was used for flash chromatography. 4,7-diiodobenzo[c][1,2,5]thiadiazole was synthesized according previous literature.¹

Hydrogen nuclear magnetic resonance spectra (¹H NMR) were obtained at 400 MHz on Bruker Avance III HD spectrometer. Spectra were recorded in CDCl₃ or DMSO-d₆ solutions. Chemical shifts are reported in ppm, referenced to tetramethylsilane (TMS) as the external reference. Coupling constants (J) are reported in Hertz. Abbreviations to denote the multiplicity of the signals are s (singlet), d (doublet), dd (double doublet), t (triplet) and m (multiplet). Carbon-13 nuclear magnetic resonance spectra (¹³C NMR) were obtained at 100 MHz on Bruker Avance HD III spectrometer. Chemical shifts are reported in ppm, referenced to the solvent peak of CDCl₃ or DMSO-d₆. Low-resolution mass spectra were obtained with a Shimadzu GCMS-QP 2010 Plus mass spectrometer. High-resolution mass spectra (HRMS) were recorded on a Bruker Micro TOF-QII spectrometer 10416. UV-vis electronic absorption spectra were recorded using Shimadzu UV2600 spectrophotometer (data interval, 2.0 nm) using dichloromethane (DCM) or dimethyl sulfoxide (DMSO) as solvent at 250-800 nm range. Steady-state emission fluorescence spectra of samples in DCM or DMSO solutions were measured with a Varian Cary 50 fluorescence spectrophotometer (slit 2.0 mm/2.0 mm; emission/excitation). Fluorescence quantum yield values (Φ₁) of the derivatives in solutions were determined by comparing the corrected fluorescence spectra with that of 9,10-diphenylanthracene (DPA) in chloroform solution (Φ₁ = 0.65, λ(exc) = 366 nm) as the standard as the fluorescence yield.²

General procedure for the synthesis of 4,7-bis((2-methoxyphenyl)ethynyl)benzo[c][1,2,5]thiadiazole (1):³

To a 10 mL Schlenk tube containing the 4,7-diiodobenzo[c][1,2,5]chalcogenodiazole (0.5 mmol), were added 2-ethynylanisole (1.2 mmol), PdCl₂(PPh₃)₂ (10 mol%), CuI (10 mol%) and Et₃N (2.5 mL). Then, the reaction mixture was stirred at 70 °C for 12 h under N₂ atmosphere. After this time, the solution was cooled to room temperature, diluted with dichloromethane (20 mL), and

washed with water (3x 20 mL). The organic phase was separated, dried over MgSO₄ and concentrated under vacuum. The obtained product 1 was purified by column chromatography on neutral alumina using a mixture of ethyl acetate/hexane (10:90) as the eluent.

![Chemical Structure](image)

**4,7-bis((2-methoxyphenyl)ethynyl)benzo[c][1,2,5]thiadiazole (1):** Yield: 0.143 g (72%); yellow solid; mp: 137-139 °C. ¹H NMR (DMSO-ｄ₆, 400 MHz) δ: 7.90 (s, 2H), 7.58 (dd, J = 7.6 and 1.7 Hz, 2H), 7.50 – 7.43 (m, 2H), 7.15 (d, J = 8.1 Hz, 2H), 7.04 (m, 2H), 3.90 (s, 6H). ¹³C NMR (DMSO-ｄ₆, 100 MHz) δ: 159.90, 153.71, 133.34, 132.68, 131.24, 120.66, 116.38, 111.58, 110.71, 93.79, 89.22, 55.81. MS (relative intensity) m/z: 396 (100); 361 (12); 277 (23); 190 (4); 131(10). HRMS calcd. for C₂₄H₁₆N₂O₂S: [M]+ 396.0932. Found: 396.0909.

General procedure for the synthesis of 4,7-bis(3-(arylselanyl)benzofuran-2-yl)benzo[c][1,2,5]thiadiazoles (3a-d):

To a 10 mL round bottom flask containing an appropriated diaryl diselenide 2a-d (0.50 mmol) and ethanol (3 mL), was added TCICA (trichloroisocyanuric acid) (0.75 mmol). Then, the reaction mixture was stirred at room temperature for 15 minutes under air. After this, we added the bis-alkynyl BTD 1 (0.25 mmol) and the homogenous reaction mixture was stirred at 60 °C for additional 3 hours. After this time, the solution was cooled to room temperature, diluted with dichloromethane (20 mL) and washed with water (3x 20 mL). The organic phase was separated, dried over MgSO₄ and concentrated under vacuum. The obtained products 3a-d were purified by chromatography on neutral alumina using a mixture of ethyl acetate/hexane (10:90) as the eluent.

![Chemical Structure](image)
4,7-bis(3-(phenylselanyl)benzofuran-2-yl)benzo-2,1,3-thiadiazole (3a): Yield: 0.530 g (78%); yellow solid; mp: 245-247 °C. $^1$H NMR (CDCl$_3$, 400 MHz) δ: 8.15 (s, 2H), 7.65 (d, $J = 8.2$ Hz, 2H), 7.64 – 7.37 (m, 4H), 7.34 – 7.24 (m, 6H), 6.86 (t, $J = 8.6$ Hz, 6H). $^{13}$C NMR (CDCl$_3$, 100 MHz) δ: 155.19, 154.23, 153.06, 130.96, 130.74, 130.06 (2C), 130.02, 129.22 (2C), 126.55, 125.87, 124.07, 123.60, 121.75, 111.68, 105.49. MS (relative intensity) m/z: 680 (70), 520 (76), 443 (38), 366 (100), 44(84). HRMS calcd. for C$_{34}$H$_{20}$N$_2$O$_2$S$\text{Se}_2$: [M]$^+$ 679.9576. Found: 679.9553.

![Diagram of 4,7-bis(3-(phenylselanyl)benzofuran-2-yl)benzo-2,1,3-thiadiazole (3a)](image)

4,7-bis(3-(4-fluorophenylselanyl)benzofuran-2-yl)benzo-2,1,3-thiadiazole (3b): Yield: 0.372 g (52%); orange solid; mp: 233-235 °C. $^1$H NMR (CDCl$_3$, 400 MHz) δ: 8.15 (s, 2H), 7.65 (d, $J = 8.2$ Hz, 2H), 7.45 – 7.36 (m, 4H), 7.34 – 7.29 (m, 4H), 7.27 – 7.21 (m, 2H), 6.86 (t, $J = 8.5$ Hz, 4H). $^{13}$C NMR (CDCl$_3$, 100 MHz) δ: 161.9 (d, $J = 246.8$ Hz) 155.2, 153.8, 153.0, 132.4 (d, $J = 7.8$ Hz, 2C), 130.5, 129.8, 125.9, 125.2 (d, $J = 3.2$ Hz, 2C), 124.1, 123.6, 121.6, 116.4 (d, $J = 21.8$ Hz), 111.7, 106.0. MS (relative intensity) m/z: 716 (68), 556 (81), 461 (31), 366 (100), 278 (27). HRMS calcd. for C$_{34}$H$_{18}$F$_2$N$_2$O$_2$S$\text{Se}_2$: [M]$^+$ 715.9387. Found: 715.9355.

![Diagram of 4,7-bis(3-(4-fluorophenylselanyl)benzofuran-2-yl)benzo-2,1,3-thiadiazole (3b)](image)

4,7-bis(3-(4-methoxyphenylselanyl)benzofuran-2-yl)benzo-2,1,3-thiadiazole (3c): Yield: 0.481 g (65%); orange solid; mp: 204-206 °C. $^1$H NMR (CDCl$_3$, 400 MHz) δ: 8.16 (s, 2H), 7.64 (d, $J = 8.3$ Hz, 2H), 7.45 (d, $J = 7.6$ Hz, 2H), 7.37 (t, $J = 7.7$ Hz, 2H), 7.31 (d, $J = 8.8$ Hz, 4H), 7.28 – 7.19 (m,
4,7-bis(3-(p-tolylyphenylselanyl)benzofuran-2-yl)benzo-2,1,3-thiadiazole (3d): Yield: 0.375 g (53%); yellow solid; mp: 239-241 °C. $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$: 8.15 (s, 2H), 7.66 (d, $J = 8.2$ Hz, 2H), 7.47 (d, $J = 7.7$ Hz, 2H), 7.38 (t, $J = 8.4$ Hz, 2H), 7.31 – 7.16 (m, 6H), 6.96 (d, $J = 7.9$ Hz, 4H), 2.24 (s, 6H). $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$: 155.14, 153.92, 153.07, 136.58, 130.82, 130.48 (2C), 130.01 (3C), 126.98, 125.76, 124.06, 123.51, 121.77, 111.63, 105.90, 20.98. MS (relative intensity) m/z: 708 (72), 548 (100), 455 (43), 366 (83), 266 (24). HRMS calcd. for C$_{36}$H$_{24}$N$_2$O$_2$SSe$_2$: [M]$^+$ 707.9889. Found: 707.9869.
**FIGURES**

**Figure S1.** UV–vis titration absorption spectra of 3b derivative, in a DMSO/Tris-HCl buffer (pH 7.4) mixture. The concentration of CT-DNA ranged from 0 to 150 μM. *Insert graph* shows the plot of $[\text{DNA}]/(\varepsilon_a - \varepsilon_f)$ versus [DNA].

**Figure S2.** UV–vis titration absorption spectra of 3c derivative, in a DMSO/Tris-HCl buffer (pH 7.4) mixture. The concentration of CT-DNA ranged from 0 to 150 μM. *Insert graph* shows the plot of $[\text{DNA}]/(\varepsilon_a - \varepsilon_f)$ versus [DNA].
**Figure S3.** UV–vis titration absorption spectra of 3d derivative, in a DMSO/Tris-HCl buffer (pH 7.4) mixture. The concentration of CT-DNA ranged from 0 to 150 μM. *Insert graph* shows the plot of [DNA]/(εₐ – εᵢ) versus [DNA].

**Figure S4.** Steady-state emission fluorescence spectra of EB-DNA adduct in the presence of 3b, in a DMSO/Tris-HCl pH 7.4 buffer mixture at λₑₓᶜᵉ ᵐ = 510 nm. The arrow indicates the changes in fluorescence intensities at increasing concentrations of samples. *Insert graph* shows the plot of F₀/F versus [compound].
**Figure S5.** Steady-state emission fluorescence spectra of EB-DNA adduct in the presence of 3c, in a DMSO/Tris-HCl pH 7.4 buffer mixture at $\lambda_{exc} = 510$ nm. The arrow indicates the changes in fluorescence intensities at increasing concentrations of samples. Insert graph shows the plot of $F_0/F$ versus [compound].

**Figure S6.** Steady-state emission fluorescence spectra of EB-DNA adduct in the presence of 3d, in a DMSO/Tris-HCl pH 7.4 buffer mixture at $\lambda_{exc} = 510$ nm. The arrow indicates the changes in fluorescence intensities at increasing concentrations of samples. Insert graph shows the plot of $F_0/F$ versus [compound].
Figure S7. Steady-state HSA-emission fluorescence spectra of 3a, in a DMSO/Tris-HCl buffer (pH 7.4) mixture at 310.15 K. The concentration of compounds ranged from 0 to 150 μM. *Insert graph* shows the plot of $F_0/F$ versus [compound].

Figure S8. Steady-state HSA-emission fluorescence spectra of 3a, in a DMSO/Tris-HCl buffer (pH 7.4) mixture at 315.15 K. The concentration of compounds ranged from 0 to 150 μM. *Insert graph* shows the plot of $F_0/F$ versus [compound].
Figure S9. Steady-state HSA-emission fluorescence spectra of 3b, in a DMSO/Tris-HCl buffer (pH 7.4) mixture at 305.15 K. The concentration of compounds ranged from 0 to 150 μM. *Insert graph* shows the plot of $F_0/F$ versus [compound].

Figure S10. Steady-state HSA-emission fluorescence spectra of 3b, in a DMSO/Tris-HCl buffer (pH 7.4) mixture at 310.15 K. The concentration of compounds ranged from 0 to 150 μM. *Insert graph* shows the plot of $F_0/F$ versus [compound].
Figure S11. Steady-state HSA-emission fluorescence spectra of 3b, in a DMSO/Tris-HCl buffer (pH 7.4) mixture at 315.15 K. The concentration of compounds ranged from 0 to 150 μM. Insert graph shows the plot of $F_0/F$ versus [compound].

Figure S12. Steady-state HSA-emission fluorescence spectra of 3c, in a DMSO/Tris-HCl buffer (pH 7.4) mixture at 305.15 K. The concentration of compounds ranged from 0 to 150 μM. Insert graph shows the plot of $F_0/F$ versus [compound].
Figure S13. Steady-state HSA-emission fluorescence spectra of 3c, in a DMSO/Tris-HCl buffer (pH 7.4) mixture at 310.15 K. The concentration of compounds ranged from 0 to 150 μM. Insert graph shows the plot of $F_0/F$ versus [compound].

Figure S14. Steady-state HSA-emission fluorescence spectra of 3c, in a DMSO/Tris-HCl buffer (pH 7.4) mixture at 315.15 K. The concentration of compounds ranged from 0 to 150 μM. Insert graph shows the plot of $F_0/F$ versus [compound].
Figure S15. Steady-state HSA-emission fluorescence spectra of 3d, in a DMSO/Tris-HCl buffer (pH 7.4) mixture at 305.15 K. The concentration of compounds ranged from 0 to 150 μM. Insert graph shows the plot of $F_0/F$ versus [compound].

Figure S16. Steady-state HSA-emission fluorescence spectra of 3d, in a DMSO/Tris-HCl buffer (pH 7.4) mixture at 310.15 K. The concentration of compounds ranged from 0 to 150 μM. Insert graph shows the plot of $F_0/F$ versus [compound].
Figure S17. Steady-state HSA-emission fluorescence spectra of 3d, in a DMSO/Tris-HCl buffer (pH 7.4) mixture at 315.15 K. The concentration of compounds ranged from 0 to 150 μM. *Insert graph* shows the plot of $F_0/F$ versus [compound].

Figure S18. Modified Stern-Volmer plots for HSA:3b at three different temperatures.
Figure S19. Modified Stern-Volmer plots for HSA:3c at three different temperatures.

Figure S20. Modified Stern-Volmer plots for HSA:3d at three different temperatures.
Figure S21. Molecular docking obtained binding modes and protein-ligand of molecules (A) 3a and (B) 3c in the minor groove of DNA structure.
Figure S22. Molecular docking obtained binding modes and protein-ligand interactions of molecules (A) 3c and (B) 3d in the pocket atoms of HSA structure.
**Table S1.** Selected geometrical parameters for compounds 3a–3d. All distances are in Angstroms and angles in degrees.

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**Figure S23.** Atom indexes description for compounds 3a–3d.
Figure S24. $^1$H NMR (400 MHz) spectrum for compound 1 in DMSO-$d_6$.

Figure S25. $^{13}$C NMR (100 MHz) spectrum for compound 1 in DMSO-$d_6$. 

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Figure S26. $^1$H NMR (400 MHz) spectrum for compound 3a in CDCl$_3$.

Figure S27. $^{13}$C NMR (100 MHz) spectrum for compound 3a in CDCl$_3$. 
Figure S28. $^1$H NMR (400 MHz) spectrum for compound 3b in CDCl$_3$.

Figure S29. $^{13}$C NMR (100 MHz) spectrum for compound 3b in CDCl$_3$. 
**Figure S30.** $^1$H NMR (400 MHz) spectrum for compound 3c in CDCl$_3$.

**Figure S31.** $^{13}$C NMR (100 MHz) spectrum for compound 3c in CDCl$_3$. 
Figure S32. $^1$H NMR (400 MHz) spectrum for compound 3d in CDCl$_3$.

Figure S33. $^{13}$C NMR (100 MHz) spectrum for compound 3d in CDCl$_3$. 