Non-Typical Fluorescence Effects and Biological Activity in Selected 1,3,4-thiadiazole Derivatives – Spectroscopic and Theoretical Studies on Substituent, Molecular Aggregation and pH Effects

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**Figure S1.** Fluorescence excitation spectra of TB (panel A) and TSF (panel B) dissolved in H$_2$O at different pH. The spectra were measured at room temperature.
Figure S2. Fluorescence excitation spectra of TS dissolved in H₂O at different pH (panel A pH=2, panel B pH=3, panel C pH=4, panel D pH=5, panel E pH=6, panel F pH=7). The spectra were measured at room temperature.
Figure S3. Intensity of resonance light scattering spectra in 436 nm of TB (black circles), TS (red circles) and TSF (blue circles) relative to change in pH.
Figure S4. The ratio of the maximum fluorescence intensity at 434/492 nm for TS and TSF dissolved in butan-1-ol in different excitation depending on the changes in concentration.
Figure S5. Stokes shift variation with normalized value of solvent polarity $E_N^T$ for TB, TS and TSF for various solvent (1 - cyclohexane, 2 - toluene, 3 - chloroform, 4 - ethyl acetate, 5 - butan-1-ol, 6 - propan-2-ol, 7 - ethanol, 8 - DMF, 9 - DMSO, 10 - methanol, 11 - acetonitrile).
Figure S6. Stokes shift versus $F(\varepsilon, n)$ for (panel A), $v_a + v_f$ versus $F(\varepsilon, n) + 2g(n)$ (panel B), for TB, TS, TSF dissolved in different solvents (1 - cyclohexane, 2 - toluene, 3 - chloroform, 4 - ethyl acetate, 5 - butan-1-ol, 6 - propan-2-ol, 7 - ethanol, 8 - DMF, 9 - DMSO, 10 - methanol, 11 - acetonitrile).
Figure S7. Fluorescence lifetimes (τ) and fractional intensities (%) measured for TSF, TS and TB relative to pH. Panel A – the main fluorescence lifetime component and panel B - the main fluorescence lifetime component + the second component when present.
Figure S8. DPPH• radicals (200 μM) absorption intensity decrease at λ_{max} 519 nm in the presence of increasing concentration of tested compounds after 30 min of reaction at 25 °C.

Figure S9. Percentage of reduced DPPH• radicals under the influence of increasing concentration of compounds tested after 30 minutes of reaction at 25 °C. The measurements were taken at λ_{max} 519 nm.
Figure S10. Tandem mass spectrometry (MS/MS) of the thiadiazole derivatives studied: A) TB, B) TS, and C) TSF. The corresponding MS-chromatographic traces are given in inserts. The MS/MS measurements were carried out using the collision energy of -30 eV.
Table S1. Spectroscopic data. Maximum absorbance, maximum fluorescence and Stokes shift in cm\(^{-1}\) for TB, TS, TSF.

| Solvents  | Absorbance [cm\(^{-1}\)] | Fluorescence [cm\(^{-1}\)] | Stokes shift [cm\(^{-1}\)] |
|-----------|--------------------------|-----------------------------|-----------------------------|
|           | TB    | TS    | TSF   | TB    | TS    | TSF   | TB    | TS    | TSF   |
| Methanol  | 33333 | 31949 | 31949 | 26 178 | 25 316 | 24 814 | 7 155 | 6 633 | 7 135 |
| Acetonitrile | 33557 | 31250 | 31447 | 26 738 | 24 752 | 24 631 | 6 819 | 6 498 | 6 816 |
| Ethanol   | 33113 | 31949 | 31746 | 26 316 | 25 189 | 24 938 | 6 797 | 6 760 | 6 808 |
| DMSO      | 31746 | 31250 | 30960 | 26 110 | 25 773 | 25 575 | 5 636 | 5 477 | 5 385 |
| Propan-2-ol | 33003 | 32051 | 31746 | 26 385 | 24 631 | 23 866 | 6 618 | 7 420 | 7 880 |
| DMF       | 32258 | 31056 | 31056 | 26 178 | 25 381 | 25 126 | 6 080 | 5 675 | 5 930 |
| Butan-1-ol | 33113 | 31746 | 31546 | 26 455 | 22 989 | 23 095 | 6 658 | 8 757 | 8 451 |
| Ethyl acetate | 33223 | 35971 | 31250 | 26316 | 24272 | 24876 | 6907  | 11699 | 6374 |
| Chloroform | 33898 | 37037 | 31447 | 26 667 | 27 174 | 24 510 | 7 231 | 9 863 | 6 937 |
| Cyclohexane | 35336 | 37313 | 35971 | 26 110 | 34 247 | 32 895 | 9 226 | 3 066 | 3 076 |
| Toluen    | 33670 | 31056 | 31056 | 26 810 | 27 933 | 25 126 | 6 860 | 3 123 | 5 930 |
Table S2. Physical constants of solvents. The average dipole molecular polarizability $\alpha$, dielectric constant $\varepsilon$, index of refraction $n$, functions $F(\varepsilon,n)$ and $F(\varepsilon+n)+2g(n)$ of the solvents.

| Solvent    | $\alpha$ [$10^{-24}$ cm$^3$] | $\varepsilon$ | $n$  | $E_T$ (30) | $F(\varepsilon,n)$ | $F(\varepsilon+n)+2g(n)$ |
|------------|-------------------------------|---------------|------|------------|-------------------|-----------------------------|
| Methanol   | 3,29                          | 33,00         | 1,3265| 55,5       | 0,765             | 0,856                       | 1,301                      |
| Acetonitrile | 4,40                          | 36,64         | 1,3416| 45,6       | 0,460             | 0,862                       | 1,327                      |
| Ethanol    | 5,41                          | 25,30         | 1,3594| 51,9       | 0,654             | 0,817                       | 1,307                      |
| DMSO       | 7,30                          | 47,24         | 1,4773| 45,1       | 0,444             | 0,842                       | 1,487                      |
| Propan-2-ol | 7,61                          | 20,18         | 1,3772| 48,6       | 0,552             | 0,781                       | 1,294                      |
| DMF        | 7,81                          | 36,70         | 1,4305| 43,2       | 0,386             | 0,836                       | 1,420                      |
| Butan-1-ol | 8,88                          | 17,80         | 1,3993| 49,7       | 0,586             | 0,753                       | 1,296                      |
| Ethyl acetate | 8,62                          | 6,08          | 1,3723| 38,1       | 0,228             | 0,493                       | 0,999                      |
| Chloroform | 9,50                          | 4,81          | 1,4429| 39,1       | 0,259             | 0,372                       | 0,973                      |
| Cyclohexane | 11,00                         | 2,02          | 1,4262| 30,9       | 0,006             | -0,003                      | 0,575                      |
| Toluene    | 11,80                         | 2,38          | 1,4969| 33,9       | 0,099             | 0,029                       | 0,700                      |
Table S3. Lifetimes ($\tau$) and fractional intensities (f) measured for TSF, TS and TB depending for pH.

| Compound | pH | $\tau_1$ | f$_1$ | $\tau_2$ | f$_2$ |
|----------|----|----------|------|----------|------|
| TSF      | 2  | 0.37±0.10 | 1.0  |          |      |
|          | 3  | 4.03±0.02  | 1.0  |          |      |
|          | 4  | 5.22±0.01  | 1.0  |          |      |
|          | 5  | 5.41±0.04  | 1.0  |          |      |
|          | 6  | 5.43±0.04  | 1.0  |          |      |
|          | 7  | 5.34±0.01  | 1.0  |          |      |
|          | 8  | 5.37±0.01  | 1.0  |          |      |
|          | 9  | 5.25±0.06  | 1.0  |          |      |
|          | 10 | 5.24±0.02  | 1.0  |          |      |
|          | 11 | 5.16±0.05  | 1.0  |          |      |
|          | 12 | 4.97±0.05  | 1.0  |          |      |
| TS       | 2  | 1.64±0.06  | 0.72±0.03 | 0.29±0.16 | 0.28±0.03 |
|          | 3  | 4.09±0.08  | 1.0  |          |      |
|          | 4  | 4.70±0.07  | 1.0  |          |      |
|          | 5  | 4.87±0.03  | 1.0  |          |      |
|          | 6  | 4.75±0.05  | 1.0  |          |      |
|          | 7  | 4.55±0.11  | 1.0  |          |      |
|          | 8  | 4.59±0.04  | 1.0  |          |      |
|          | 9  | 4.50±0.09  | 1.0  |          |      |
|          | 10 | 4.53±0.03  | 1.0  |          |      |
|          | 11 | 4.34±0.08  | 1.0  |          |      |
|          | 12 | 4.1±0.05   | 1.0  |          |      |
| TB       | 2  | 0.10±0.02  | 0.80±0.05 | 1.17±0.37 | 0.20±0.05 |
|          | 3  | 0.07±0.03  | 0.76±0.05 | 0.65±0.30 | 0.25±0.05 |
|          | 4  | 0.09±0.02  | 0.90±0.03 | 1.04±0.37 | 0.10±0.02 |
|          | 5  | 0.07±0.01  | 0.78±0.01 | 0.73±0.05 | 0.21±0.02 |
|          | 6  | 0.11±0.02  | 0.90±0.05 | 1.14±0.28 | 0.10±0.05 |
|          | 7  | 0.14±0.01  | 1.0  |          |      |
|          | 8  | 0.16±0.01  | 1.0  |          |      |
|          | 9  | 0.15±0.02  | 1.0  |          |      |
|          | 10 | 0.15±0.01  | 1.0  |          |      |
|          | 11 | 0.09±0.02  | 0.91±0.04 | 1.29±0.01 | 0.11±0.04 |
|          | 12 | 0.10±0.03  | 0.83±0.02 | 1.31±0.12 | 0.17±0.02 |
Table S4. Thiadiazole derivatives MICs for 9 *Candida* species.

| Organism                      | MIC (μg/ml) |   |   |
|-------------------------------|-------------|---|---|
|                               | TB          | TS | TSF|
| *Candida krusei* (Polish isolate) | 256         | >256 | 256 |
| *Candida fructus* (JCM 1513)  | 128         | 4  | 128 |
| *Candida fragicola* (JCM 1589) | 128         | 32 | 128 |
| *Candida butyri* (JCM 1501)   | 64          | 8  | 128 |
| *Candida tropicalis* (ATCC 1369) | >256       | >256 | >256 |
| *Candida shehatae* (ATCC 22984) | 256         | 64 | 256 |
| *Candida fluviatilis* (CBS 6776) | 128        | 32 | 128 |
| *Candida freyschussi* (CBS 3562) | 128        | 128 | 256 |
| *Candida parapsilopsis* (DSM 70125) | >256       | >256 | >256 |
**Table S5.** MIC curves interpolations of thiadiazole derivatives against *Candida* species.

| Control/MIC | Species            | Lag Time (hours) | Max Specific Growth Rate (hours\(^{-1}\)) | Doubling Time (hours) | Max OD | Min OD | R\(^2\) |
|-------------|--------------------|-----------------|------------------------------------------|----------------------|--------|--------|---------|
| Control     | *C. krusei*        | 3.765           | 0.034                                    | 4.450                | 1.635  | 0.057  | 0.998   |
| TB/MIC      | *C. krusei*        | 5.389           | 0.069                                    | 8.767                | 1.456  | 0.044  | 0.997   |
| TS/MIC      | *C. krusei*        | n.d.            | n.d.                                     | n.d.                 | n.d.   | n.d.   | n.d.    |
| TSF/MIC     | *C. krusei*        | 5.869           | 0.071                                    | 9.733                | 1.574  | 0.042  | 0.990   |
| Control     | *C. fructus* (ICM 1513) | 3.051         | 0.107                                    | 6.446                | 1.368  | 0.120  | 0.999   |
| TB/MIC      | *C. fructus* (ICM 1513) | 31.858       | 0.033                                    | 20.986               | 0.533  | 0.048  | 0.992   |
| TS/MIC      | *C. fructus* (ICM 1513) | 27.497       | 0.029                                    | 23.596               | 0.478  | 0.022  | 0.097   |
| TSF/MIC     | *C. fructus* (ICM 1513) | 23.582       | 0.026                                    | 25.708               | 0.499  | 0.018  | 0.996   |
| Control     | *C. fragicola* (ICM 1589) | 2.856        | 0.049                                    | 13.976               | 1.55   | 0.065  | 0.999   |
| TB/MIC      | *C. fragicola* (ICM 1589) | 23.252       | 0.022                                    | 31.131               | 0.613  | 0.063  | 0.998   |
| TS/MIC      | *C. fragicola* (ICM 1589) | 29.016       | 0.017                                    | 40.526               | 0.384  | 0.024  | 0.985   |
| TSF/MIC     | *C. fragicola* (ICM 1589) | 12.780       | 0.038                                    | 17.822               | 1.072  | 0.044  | 0.999   |
| Control     | *C. butyri* (ICM 1501) | 14.981         | 0.087                                    | 7.911                | 1.569  | 0.028  | 0.999   |
| TB/MIC      | *C. butyri* (ICM 1501) | 18.077         | 0.045                                    | 15.129               | 1.180  | 0.042  | 0.998   |
| TS/MIC      | *C. butyri* (ICM 1501) | 30.018         | 0.013                                    | 51.816               | 0.241  | 0.000  | 0.970   |
| TSF/MIC     | *C. butyri* (ICM 1501) | 10.356         | 0.044                                    | 15.465               | 1.218  | 0.043  | 0.998   |
| Control     | *C. tropicalis* (ATCC 1369) | 3.431        | 0.187                                    | 3.692                | 1.626  | 0.048  | 0.995   |
| TB/MIC      | *C. tropicalis* (ATCC 1369) | n.d.         | n.d.                                     | n.d.                 | n.d.   | n.d.   | n.d.    |
| TS/MIC      | *C. tropicalis* (ATCC 1369) | n.d.         | n.d.                                     | n.d.                 | n.d.   | n.d.   | n.d.    |
| TSF/MIC     | *C. tropicalis* (ATCC 1369) | n.d.         | n.d.                                     | n.d.                 | n.d.   | n.d.   | n.d.    |
| Control     | *C. shehatae* (ATCC22984) | 1.107         | 0.071                                    | 9.652                | 1.469  | 0.037  | 0.997   |
| TB/MIC      | *C. shehatae* (ATCC22984) | 12.234         | 0.056                                    | 11.564               | 0.085  | 0.064  | 0.0995  |
| Control/MIC | Species                  | Lag Time (hours) | Specific Growth Rate (hours$^{-1}$) | Doubling Time (hours) | Max OD | Min OD | $R^2$ |
|------------|--------------------------|------------------|------------------------------------|-----------------------|--------|--------|-------|
| Control    | *C. fluviatilis* (CBS 6776) | 9.848            | 0.067                              | 10.267                | 1.558  | 0.073  | 0.998 |
| TB/MIC     | *C. fluviatilis* (CBS 6776) | 72.941           | 2.905                              | 20.238                | 0.921  | 0.082  | 0.985 |
| TS/MIC     | *C. fluviatilis* (CBS 6776) | 27.873           | 0.024                              | 28.008                | 0.542  | 0.042  | 0.993 |
| TSF/MIC    | *C. fluviatilis* (CBS 6776) | 99.999           | 0.322                              | 2.146                 | 0.977  | 0.038  | 0.991 |
| Control    | *C. freyschussi* (CBS 3562) | 10.356           | 0.065                              | 4.561                 | 1.674  | 0.065  | 0.992 |
| TB/MIC     | *C. freyschussi* (CBS 3562) | 36.112           | 0.123                              | 5.463                 | 1.431  | 0.0441 | 0.995 |
| TS/MIC     | *C. freyschussi* (CBS 3562) | 37.546           | 0.124                              | 5.566                 | 1.449  | 0.042  | 0.996 |
| TSF/MIC    | *C. freyschussi* (CBS 3562) | 99.999           | 0.151                              | 4.583                 | 0.392  | 0.057  | 0.992 |
| Control    | *C. parapsilosis* (DSM 70125) | 1.000            | 0.062                              | 11.024                | 1.370  | 0.095  | 0.998 |
| TB/MIC     | *C. parapsilosis* (DSM 70125) | n.d.             | n.d.                               | n.d.                  | n.d.   | n.d.   | n.d.  |
| TS/MIC     | *C. parapsilosis* (DSM 70125) | n.d.             | n.d.                               | n.d.                  | n.d.   | n.d.   | n.d.  |
| TSF/MIC    | *C. parapsilosis* (DSM 70125) | n.d.             | n.d.                               | n.d.                  | n.d.   | n.d.   | n.d.  |

n.d. not detected