

Molecular iodine-promoted oxidative cyclization for the synthesis of 1,3,4-thiadiazole-fused-[1,2,4]-thiadiazole incorporating 1,4-benzodioxine moiety as potent inhibitors of α -amylase and α -glucosidase: *In vitro* and *in silico* study

Rafaqat Hussain¹, Mazloom Shah², Shahid Iqbal^{3*}, Wajid Rehman^{1*}, Shoaib Khan¹, Liaqat Rasheed¹, Haseena Naz¹, Hanan A. Al-ghulikah⁴, Eslam B. Elkaeed⁵, Rami Adel Pashameah⁶, Eman Alzahrani⁷, Abd-ElAzim Farouk⁸

¹Department of Chemistry, Hazara University, Mansehra-21300, Khyber Pakhtunkhwa, Pakistan

²Department of Chemistry, Abbottabad University of Science and Technology (AUST) Abbottabad, Pakistan.

³Department of Chemistry, School of Natural Sciences (SNS), National University of Science and Technology (NUST), H-12, Islamabad, 46000, Pakistan.

⁴Department of Chemistry, College of Sciences, Princess Nourahbint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia.

⁵Department of Pharmaceutical Sciences, College of Pharmacy, AlMaarefa University, Riyadh 13713, Saudi Arabia.

⁶Department of Chemistry, Faculty of Applied Science, Umm Al-Qura University, Makkah 24230, Saudi Arabia.

⁷Department of Chemistry, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia.

⁸Department of Biotechnology College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia.

***To whom corresponding should be addressed**

shahidgcs10@yahoo.com(Shahid Iqbal) and sono_waj@yahoo.com. (Wajid Rehman)

3.4. α -Glucosidase inhibition assay

The α -glucosidase inhibition activity was performed with slight modifications [43]. A total volume of 100 μ L reaction mixture contained, 70 μ L 50 mM phosphate buffer pH 6.8, 10 μ L (0.5 mM in methanol) test compound, followed by the addition of 10 μ L (0.057 units, Sigma Inc.) enzyme solution in the buffer. The contents were mixed, pre-incubated for 10 min at 37 °C and pre-read at 400 nm. The reaction was initiated by the addition of 10 μ L of 0.5 mM substrate (p-nitrophenyl glucopyranoside, Sigma Inc.). After 30 min of incubation at 37 °C, the absorbance of p-nitrophenol was measured at 400 nm using the Synergy HT 96-well plate reader, BioTek, USA. Acarbose was used as positive control. All experiments were carried out in triplicates (mean $\pm$ SEM, n = 3). Percent inhibition was calculated by the following equation: Inhibition (%) = (Abs of Control-Abs of Test/Abs of Control) $\times$ 100 Active compound solutions were

suitably diluted and their inhibition studies were determined. Data obtained was used for the determination of IC₅₀ values (concentration at which there is 50 % enzyme inhibition) using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

3.5. α -Amylase inhibition assay

The α -amylase inhibitory activity was determined by an assay modified from Kwon, Apostolidis and Shetty. A volume of 500 mL of α -amylase solution (0.5 mg/mL) in 0.2 mM phosphate buffer (pH 6.9) and 500 mL of test sample (100, 200, 400, 800, 1000 mg/mL) were incubated for 10 min at 25°C. After pre-incubation, 1% starch solution (500 mL) in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube and incubated at 25°C for 10 min. 1mL of dinitrosalicylic acid color reagent was then added to the reaction mixture and the tubes were incubated in boiling water for 5 min, and finally cooled to room temperature. The solutions were diluted by adding 10mL distilled water and the absorbance was measured at 540 nm [44]. The percentage inhibition was calculated as illustrated in given formula.

$$\% \text{inhibition} = (\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}) / \text{Absorbance}_{\text{control}} \times 100$$

3.6. Spectral analysis

3.6.1. (E)-2-((2,3-dihydrobenzo[b][1,4]dioxin-2-yl)methylene)hydrazine-1-carbothioamide (II)

Yield: 95%; mp 133-134°C; ¹H-NMR (500MHz, DMSO-*d*₆): δ 12.36 (s, 1H, NH), 10.23 (s, 2H, -NH₂), 9.88 (s, 1H, HC=N), 5.23 (t, J = 5.6 Hz, 1H, benzodioxine-H-2), 5.34 (d, J = 5.7 Hz, 2H, benzodioxine-H-3), 7.23 (dd, J = 6.8, 1.9Hz, 2H, benzodioxine-H-5/H-8), 6.98-7.08 (m, 2H, benzodioxine-H-6/H-7). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 185.5, 155.5, 144.4, 140.2, 123.4, 121.2, 118.3, 115.5, 85.7, 70.3; HREI-MS: *m/z* calcd for C₁₀H₁₁N₃O₂S [M]⁺ 237.0535, Found 237.0527.

3.6.2. 5-(2,3-dihydrobenzo[b][1,4]dioxin-2-yl)-1,3,4-thiadiazol-2-amine (III)

Yield: 81%; mp 151-152°C; ¹H-NMR (500MHz, DMSO-*d*₆): δ 11.13 (s, 2H, -NH₂), 5.83 (t, J = 7.6 Hz, 1H, benzodioxine-H-2), 5.54 (d, J = 7.5 Hz, 2H, benzodioxine-H-3), 7.34 (dd, J = 7.9, 2.4Hz, 2H, benzodioxine-H-5/H-8), 7.22-7.14 (m, 2H, benzodioxine-H-6/H-7). ¹³C-NMR (125

MHz, DMSO-*d*₆): δ 174.5, 167.5, 148.7, 143.8, 124.8, 122.8, 119.6, 116.3, 86.4, 71.5; HREI-MS: *m/z* calcd for C₁₀H₉N₃O₂S [M]⁺ 235.0388, Found 235.0373.

3.6.3. *(Z)*-6-(2,3-dihydrobenzo[*b*][1,4]dioxin-2-yl)-*N*-(4-nitrophenyl)-3*H*-[1,3,4]thiadiazolo[2,3-*c*][1,2,4]thiadiazol-3-imine (**1**)

Yield: 68%; mp 210-211°C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 8.21 (d, *J* = 7.3 Hz, 2H, ArH), 8.03 (d, *J* = 7.1 Hz, 2H, ArH), 7.31 (d, *J* = 6.7 Hz, 2H, ArH), 7.20 (d, *J* = 6.5 Hz, 2H, ArH), 5.50 (t, *J* = 5.7 Hz, 1H, ArH), 5.16 (d, *J* = 5.5 Hz, 2H, ArH). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 176.5, 166.1, 158.5, 150.2, 144.1, 143.3, 142.7, 139.8, 124.2, 124.2, 124.0, 124.0, 120.9, 120.9, 120.2, 115.0, 93.8; HREI-MS: *m/z* calcd for C₁₇H₁₁N₅O₂S₂ [M]⁺ 413.0247 Found 413.0242.

3.6.4. *(Z)*-4-((6-(2,3-dihydrobenzo[*b*][1,4]dioxin-2-yl)-3*H*-[1,3,4]thiadiazolo[2,3-*c*][1,2,4]thiadiazol-3-ylidene)amino)benzene-1,3-diol (**2**)

Yield: 66%; mp 201-202°C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 10.93 (s, 1H, OH), 10.57 (s, 1H, OH), 7.53 (d, *J* = 6.8 Hz, 1H, ArH), 7.47 (s, 1H, ArH), 7.25 (d, *J* = 6.4 Hz, 2H, ArH), 7.13 (d, *J* = 6.5 Hz, 2H, ArH), 6.31 (d, *J* = 6.7 Hz, 1H, ArH), 5.53 (t, *J* = 5.4 Hz, 1H, ArH), 5.17 (d, *J* = 5.3 Hz, 2H, ArH). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 177.5, 162.5, 162.2, 158.6, 146.0, 143.3, 142.7, 133.8, 120.9, 120.9, 120.2, 115.0, 111.1, 108.6, 103.7, 93.8, 66.1; HREI-MS: *m/z* calcd for C₁₇H₁₂N₄O₄S₂ [M]⁺ 400.0293 Found 400.0287.

3.6.5. *(Z)*-5-((6-(2,3-dihydrobenzo[*b*][1,4]dioxin-2-yl)-3*H*-[1,3,4]thiadiazolo[2,3-*c*][1,2,4]thiadiazol-3-ylidene)amino)benzene-1,3-diol (**3**)

Yield: 70%; mp 200-201°C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 9.19 (s, 2H, OH), 7.24 (d, *J* = 6.9 Hz, 2H, ArH), 7.25 (d, *J* = 6.7 Hz, 2H, ArH), 6.88 (d, *J* = 7.02 Hz, 2H, ArH), 6.37 (s, 1H, ArH), 5.58 (t, *J* = 5.6 Hz, 1H, ArH), 5.18 (d, *J* = 5.7 Hz, 2H, ArH). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 176.5, 163.0, 160.0, 158.4, 146.8, 143.3, 142.7, 136.5, 120.9, 120.9, 120.2, 115.0, 107.5, 107.5, 105.9, 93.8, 66.1; HREI-MS: *m/z* calcd for C₁₇H₁₂N₄O₄S₂ [M]⁺ 400.0293 Found 400.0287.

3.6.6. *(Z)*-6-(2,3-dihydrobenzo[*b*][1,4]dioxin-2-yl)-*N*-(2-methoxyphenyl)-3*H*-[1,3,4]thiadiazolo[2,3-*c*][1,2,4]thiadiazol-3-imine (**4**)

Yield: 71%; mp 197-198°C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 7.79(d, J = 7.01 Hz, 1H, ArH), 7.44 (t, J = 6.9 Hz, 1H, ArH), 7.20 (d, J = 6.7 Hz, 1H, ArH), 7.17 (d, J = 6.6 Hz, 1H, ArH), 7.11 (d, J = 6.4 Hz, 2H, ArH), 7.08 (d, J = 6.5 Hz, 2H, ArH), 5.26 (t, J = 5.6 Hz, 1H, ArH), 5.03 (d, J = 5.4 Hz, 2H, ArH), 3.81 (s, 3H, OCH₃). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 174.5, 157.6, 158.7, 146.0, 143.3, 142.7, 132.0, 131.7, 121.1, 120.9, 120.9, 120.2, 116.9, 115.0, 111.2, 93.8, 66.1, 55.8; HREI-MS: m/z calcd for C₁₈H₁₄N₄O₃S₂ [M]⁺ 398.0502 Found 398.0497.

3.6.7. (Z)-4-((6-(2,3-dihydrobenzo[*b*][1,4]dioxin-2-yl)-3H-[1,3,4]thiadiazolo[2,3-*c*][1,2,4]thiadiazol-3-ylidene)amino)benzene-1,2-diol (**5**)

Yield: 69%; mp 201-202°C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 9.46(s, 2H, OH), 7.28 (s, 1H, ArH), 7.27 (d, J = 7.4 Hz, 1H, ArH), 7.16 (d, J = 6.7 Hz, 2H, ArH), 7.10(d, J = 6.8 Hz, 2H, ArH), 6.76 (d, J = 7.4 Hz, 1H, ArH), 5.37 (t, J = 5.6 Hz, 1H, ArH), 5.05 (d, J = 5.6 Hz, 2H, ArH). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 177.5, 158.8, 149.6, 146.8, 146.1, 143.3, 142.7, 131.3, 123.2, 120.9, 120.9, 120.2, 117.4, 116.3, 115.0, 93.8, 66.1; HREI-MS: m/z calcd for C₁₇H₁₂N₄O₄S₂ [M]⁺ 400.0293 Found 400.0287.

3.6.8. (Z)-2-((6-(2,3-dihydrobenzo[*b*][1,4]dioxin-2-yl)-3H-[1,3,4]thiadiazolo[2,3-*c*][1,2,4]thiadiazol-3-ylidene)amino)benzene-1,4-diol (**6**)

Yield: 67%; mp 199-200°C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 10.65 (s, 1H, OH), 9.47 (s, 1H, OH), 7.25 (d, J = 6.3 Hz, 2H, ArH), 7.19 (d, J = 6.1 Hz, 2H, ArH), 7.09 (s, 1H, ArH), 6.74 (d, J = 6.9 Hz, 1H, ArH), 6.67 (d, J = 6.9 Hz, 1H, ArH), 5.38 (t, J = 5.5 Hz, 1H, ArH), 5.13 (d, J = 5.3 Hz, 2H, ArH). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 174.5, 158.5, 153.7, 151.2, 146.0, 143.3, 142.7, 122.9, 120.9, 120.4, 122.2, 119.9, 118.6, 116.3, 115.0, 94.8, 66.1; HREI-MS: m/z calcd for C₁₇H₁₂N₄O₄S₂ [M]⁺ 400.0293 Found 400.0287.

3.6.9. (Z)-3-((6-(2,3-dihydrobenzo[*b*][1,4]dioxin-2-yl)-3H-[1,3,4]thiadiazolo[2,3-*c*][1,2,4]thiadiazol-3-ylidene)amino)phenol (**7**)

Yield: 69%; mp 191-192°C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 9.76 (s, 1H, OH), 7.38 (d, J = 7.2 Hz, 1H, ArH), 7.30 (s, 1H, ArH), 7.23 (d, J = 6.4 Hz, 2H, ArH), 7.14 (t, J = 6.9 Hz, 1H, ArH), 7.13 (d, J = 6.3 Hz, 2H, ArH), 6.91 (d, J = 6.7 Hz, 1H, ArH), 5.40 (t, J = 5.4 Hz, 1H, ArH), 5.12 (d, J = 5.2 Hz, 2H, ArH). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 176.5, 158.6, 158.2, 146.8, 143.3,

142.7, 138.7, 130.2, 121.8, 120.9, 120.9, 120.2, 118.4, 115.0, 114.9, 96.8, 62.1; HREI-MS: m/z calcd for C₁₇H₁₂N₄O₃S₂ [M]⁺ 384.0378 Found 384.0373.

3.6.10. (Z)-2-((6-(2,3-dihydrobenzo[b][1,4]dioxin-2-yl)-3H-[1,3,4]thiadiazolo[2,3-c][1,2,4]thiadiazol-3-ylidene)amino)benzene-1,3,5-triol (**8**)

Yield: 72%; mp 214-215°C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 10.49 (s, 1H, OH), 10.20 (s, 2H, OH), 7.25 (d, J = 6.7 Hz, 2H, ArH), 7.22 (d, J = 6.8 Hz, 2H, ArH), 7.06 (s, 2H, ArH), 5.50 (t, J = 5.6 Hz, 1H, ArH), 5.12 (d, J = 5.7 Hz, 2H, ArH). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 175.9, 163.9, 163.6, 162.9, 158.6, 143.3, 142.7, 142.3, 120.9, 120.9, 120.2, 115.0, 106.2, 96.3, 96.3, 92.8, 66.1; HREI-MS: m/z calcd for C₁₇H₁₂N₄O₅S₂ [M]⁺ 416.0243 Found 416.0238.

3.6.11. (Z)-4-((6-(2,3-dihydrobenzo[b][1,4]dioxin-2-yl)-3H-[1,3,4]thiadiazolo[2,3-c][1,2,4]thiadiazol-3-ylidene)amino)-*N,N*-dimethylaniline (**9**)

Yield: 65%; mp 206-207°C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 7.51 (d, J = 6.9 Hz, 2H, ArH), 7.26 (d, J = 6.3 Hz, 2H, ArH), 7.14 (d, J = 6.3 Hz, 2H, ArH), 6.84 (d, J = 6.8 Hz, 2H, ArH), 5.55 (t, J = 5.7 Hz, 1H, ArH), 5.12 (d, J = 5.3 Hz, 2H, ArH), 3.03 (s, 6H, CH₃). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 172.5, 158.9, 151.4, 144.1, 143.3, 142.7, 128.3, 128.3, 123.3, 120.9, 120.9, 120.2, 115.0, 111.9, 111.9, 93.8, 66.1, 42.3, 41; HREI-MS: m/z calcd for C₁₉H₁₇N₅O₅S₂ [M]⁺ 411.0819 Found 411.0813.

3.6.12. (Z)-6-(2,3-dihydrobenzo[b][1,4]dioxin-2-yl)-*N*-(2-nitrophenyl)-3H-[1,3,4]thiadiazolo[2,3-c][1,2,4]thiadiazol-3-imine (**10**)

Yield: 66%; mp 209-210°C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 8.14 (d, J = 7.3 Hz, 1H, ArH), 7.97 (d, J = 7.2 Hz, 1H, ArH), 7.66 t, J = 7.2 Hz, 1H, ArH), 7.55 t, J = 7.1 Hz, 1H, ArH), 7.26 (d, J = 6.8 Hz, 2H, ArH), 7.18 (d, J = 6.4 Hz, 2H, ArH), 5.53 (t, J = 5.5 Hz, 1H, ArH), 5.07 (d, J = 5.2 Hz, 2H, ArH),). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 174.5, 158.1, 147.8, 143.3, 143.3, 142.7, 134.9, 131.9, 130.1, 128.4, 124.0, 120.9, 120.9, 120.2, 115.0, 93.8, 66.1; HREI-MS: m/z calcd for C₁₇H₁₁N₅O₂S₂ [M]⁺ 413.0247 Found 413.0242.

3.6.13. ((Z)-6-(2,3-dihydrobenzo[b][1,4]dioxin-2-yl)-*N*-(3-nitrophenyl)-3H-[1,3,4]thiadiazolo[2,3-c][1,2,4]thiadiazol-3-imine (**11**)

Yield: 69%; mp 208-209°C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 8.46 (s, 1H, ArH), 8.09 (d, J = 7.4 Hz, 1H, ArH), 8.01 (d, J = 7.1 Hz, 1H, ArH), 7.73 (t, J = 7.1 Hz, 1H, ArH), 7.29 (d, J = 6.3 Hz, 2H, ArH), 7.18 (d, J = 6.5 Hz, 2H, ArH), 5.51 (t, J = 5.4 Hz, 1H, ArH), 5.08 (d, J = 5.1 Hz, 2H, ArH). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 174.5, 158.2, 148.0, 143.3, 142.8, 142.7, 134.6, 130.5, 129.7, 126.2, 121.9, 121.6, 120.9, 120.2, 115.0, 93.8, 66.1; HREI-MS: m/z calcd for C₁₇H₁₁N₅O₂S₂ [M]⁺ 413.0247 Found 413.0242.

3.6.14. (Z)-4-((6-(2,3-dihydrobenzo[b][1,4]dioxin-2-yl)-3H-[1,3,4]thiadiazolo[2,3-c][1,2,4]thiadiazol-3-ylidene)amino)phenol (**12**)

Yield: 70%; mp 189-190°C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 9.75 (s, 1H, OH), 7.62 (d, J = 6.8 Hz, 2H, ArH), 7.25 (d, J = 6.2 Hz, 2H, ArH), 7.12 (d, J = 6.9 Hz, 2H, ArH), 6.82 (d, J = 6.7 Hz, 2H, ArH), 5.44 (t, J = 5.6 Hz, 1H, ArH), 5.11 (d, J = 5.2 Hz, 2H, ArH). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 175.0, 162.8, 158.8, 144.1, 143.9, 143.7, 132.6, 131.6, 126.3, 120.9, 120.9, 120.5, 116.0, 116.0, 115.0, 93.8, 66.1; HREI-MS: m/z calcd for C₁₇H₁₂N₄O₃S₂ [M]⁺ 384.0378 Found 384.0373.

3.6.15. ((Z)-N-(4-chlorophenyl)-6-(2,3-dihydrobenzo[b][1,4]dioxin-2-yl)-3H-[1,3,4]thiadiazolo[2,3-c][1,2,4]thiadiazol-3-imine (**13**))

Yield: 74%; mp 204-205°C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 7.77 (d, J = 7.5 Hz, 2H, ArH), 7.54 (d, J = 7.5 Hz, 2H, ArH), 7.21 (d, J = 6.6 Hz, 2H, ArH), 7.12 (d, J = 6.5 Hz, 2H, ArH), 5.43 (t, J = 5.7 Hz, 1H, ArH), 5.04 (d, J = 5.6 Hz, 2H, ArH). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 176.5, 158.5, 144.1, 143.3, 142.7, 136.6, 131.8, 130.6, 130.6, 128.9, 128.9, 121.9, 121.9, 120.2, 115.0, 93.8, 72.1; HREI-MS: m/z calcd for C₁₇H₁₁ClN₄O₂S₂ [M]⁺ 402.0007 Found 402.0002.

3.6.16. methyl(Z)-4-((6-(2,3-dihydrobenzo[b][1,4]dioxin-2-yl)-3H-[1,3,4]thiadiazolo[2,3-c][1,2,4]thiadiazol-3-ylidene)amino)benzoate (**14**)

Yield: 70%; mp 216-217°C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 8.18 (d, J = 7.2 Hz, 2H, ArH), 7.83 (d, J = 6.8 Hz, 2H, ArH), 7.31 (d, J = 6.6 Hz, 2H, ArH), 7.21 (d, J = 6.5 Hz, 2H, ArH), 5.52 (t, J = 5.7 Hz, 1H, ArH), 5.16 (d, J = 5.6 Hz, 2H, ArH), 3.87 (s, 3H, OCH₃). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 175.5, 165.9, 158.4, 144.1, 143.3, 142.7, 138.0, 132.4, 130.0, 130.0, 129.1,

129.1, 120.9, 120.9, 120.2, 115.0, 93.8, 66.1, 51.5; HREI-MS: m/z calcd for C₁₉H₁₄N₄O₄S₂ [M]⁺ 426.0451 Found 426.0446.

3.6.17. (Z)-6-(2,3-dihydrobenzo[b][1,4]dioxin-2-yl)-N-(o-tolyl)-3H-[1,3,4]thiadiazolo[2,3-c][1,2,4]thiadiazol-3-imine (**15**)

Yield: 74%; mp 188-189°C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 7.70 (d, J = 7.1 Hz, 1H, ArH), 7.27 (m, 3H, ArH), 7.14 (d, J = 6.4 Hz, 2H, ArH), 7.03 (d, J = 6.4 Hz, 2H, ArH), 5.34 (t, J = 5.6 Hz, 1H, ArH), 5.03 (d, J = 5.6 Hz, 2H, ArH), 2.45 (s, 3H, CH₃), ¹³C-NMR (125 MHz, DMSO-*d*₆) δ 176.5, 158.5, 146.3, 143.3, 142.7, 135.3, 131.1, 130.9, 129.0, 126.5, 125.8, 121.9, 121.9, 120.2, 115.0, 95.8, 65.1, 18.9; HREI-MS: m/z calcd for C₁₈H₁₄N₄O₂ S₂ [M]⁺ 382.0553 Found 382.0548.

3.6.18. (Z)-6-(2,3-dihydrobenzo[b][1,4]dioxin-2-yl)-N-(p-tolyl)-3H-[1,3,4]thiadiazolo[2,3-c][1,2,4]thiadiazol-3-imine (**16**)

Yield: 71%; mp 187-188°C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 7.86 (d, J = 7.4 Hz, 1H, ArH), 7.36 (d, J = 6.8 Hz, 2H, ArH), 7.07 (d, J = 6.3 Hz, 2H, ArH), 7.02 (d, J = 6.4 Hz, 2H, ArH), 5.38 (t, J = 5.7 Hz, 1H, ArH), 5.06 (d, J = 5.4 Hz, 2H, ArH), 2.36 (s, 3H, CH₃). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 173.5, 158.7, 144.1, 143.3, 142.7, 140.7, 130.7, 128.1, 126.1, 126.1, 123.9, 122.9, 122.1, 120.2, 115.0, 93.8, 43.1, 21.3; HREI-MS: m/z calcd for C₁₈H₁₄N₄O₂ S₂ [M]⁺ 382.0553 Found 382.0548.

3.6.19. (Z)-N-(3-chlorophenyl)-6-(2,3-dihydrobenzo[b][1,4]dioxin-2-yl)-3H-[1,3,4]thiadiazolo[2,3-c][1,2,4]thiadiazol-3-imine (**17**)

Yield: 73%; mp 203-204°C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 7.64 (s, 1H, ArH), 7.61 (d, J = 7.1 Hz, 1H, ArH), 7.51 (d, J = 6.8 Hz, 1H, ArH), 7.41 (t, J = 6.9 Hz, 1H, ArH), 7.23 (d, J = 6.4 Hz, 2H, ArH), 7.14 (d, J = 6.4 Hz, 2H, ArH), 5.45 (t, J = 5.6 Hz, 1H, ArH), 5.06 (d, J = 5.5 Hz, 2H, ArH). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 175.5, 158.3, 146.8, 143.3, 141.7, 136.1, 134.4, 132.1, 130.2, 127.9, 125.3, 125.1, 124.9, 120.2, 116.0, 96.8, 43.1; HREI-MS: m/z calcd for C₁₇H₁₁ClN₄O₂S₂ [M]⁺ 402.0007 Found 402.0002.

3.6.20. ((Z)-2-((6-(2,3-dihydrobenzo[b][1,4]dioxin-2-yl)-3H-[1,3,4]thiadiazolo[2,3-c][1,2,4]thiadiazol-3-ylidene)amino)phenol (**18**)

Yield: 67%; mp 190-191°C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 10.66 (s, 1H, OH), 7.44 (d, J = 7.3 Hz, 1H, ArH), 7.31 (t, J = 7.1 Hz, 1H, ArH), 7.19 (d, J = 6.5 Hz, 2H, ArH), 7.10 (d, J = 6.4 Hz, 2H, ArH), 6.88 (d, J = 6.9 Hz, 1H, ArH), 6.85 (t, J = 6.9 Hz, 1H, ArH), 5.39 (t, J = 5.5 Hz, 1H, ArH), 5.13 (d, J = 5.3 Hz, 2H, ArH). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 173.5, 158.2, 156.7, 146.0, 143.3, 142.7, 132.4, 126.5, 122.2, 121.9, 121.4, 120.9, 118.5, 117.8, 115.0, 93.8, 66.1; HREI-MS: m/z calcd for C₁₇H₁₂N₄O₃S₂ [M]⁺ 384.0378 Found 384.0373.

3.6.21. (Z)-6-(2,3-dihydrobenzo[*b*][1,4]dioxin-2-yl)-*N*-(*m*-tolyl)-3*H*-[1,3,4]thiadiazolo[2,3-*c*][1,2,4]thiadiazol-3-imine (**19**)

Yield: 75%; mp 186-187 °C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 7.71 (s, 1H, ArH), 7.63 (d, J = 7.1 Hz, 1H, ArH), 7.36 (t, J = 7.1 Hz, 1H, ArH), 7.22 (d, J = 6.7 Hz, 1H, ArH), 7.11 (d, J = 6.5 Hz, 2H, ArH), 7.04 (d, J = 6.5 Hz, 2H, ArH), 5.39 (t, J = 5.5 Hz, 1H, ArH), 5.04 (d, J = 5.3 Hz, 2H, ArH), 2.42 (s, 3H, CH₃). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 175.5, 158.5, 145.8, 143.3, 142.7, 136.5, 132.6, 131.3, 129.4, 128.7, 126.2, 120.9, 120.9, 120.2, 115.0, 93.8, 66.1, 21.3; HREI-MS: m/z calcd for C₁₈H₁₄N₄O₂ S₂ [M]⁺ 382.0553 Found 382.0548.

3.6.22. (Z)-5-((6-(2,3-dihydrobenzo[*b*][1,4]dioxin-2-yl)-3*H*-[1,3,4]thiadiazolo[2,3-*c*][1,2,4]thiadiazol-3-ylidene)amino)-2-methoxyphenol (**20**)

Yield: 71%; mp 212-213°C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 9.33 (s, 1H, OH), 7.41 (s, 1H, ArH), 7.23 (d, J = 6.5 Hz, 2H, ArH), 7.17 (d, J = 6.3 Hz, 2H, ArH), 7.08 (d, J = 6.8 Hz, 1H, ArH), 6.92 (d, J = 6.9 Hz, 1H, ArH), 5.48 (t, J = 5.7 Hz, 1H, ArH), 5.16 (d, J = 5.6 Hz, 2H, ArH), 3.82 (s, 3H, OCH₃). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 175.5, 158.7, 152.4, 147.3, 146.8, 143.3, 142.7, 131.0, 122.8, 120.9, 120.9, 120.2, 115.9, 115.0, 112.3, 93.8, 66.8, 56.1; HREI-MS: m/z calcd for C₁₈H₁₄N₄O₄ S₂ [M]⁺ 414.0452 Found 414.0447.

3.6.23. (Z)-6-(2,3-dihydrobenzo[*b*][1,4]dioxin-2-yl)-*N*-(2,4-dimethoxyphenyl)-3*H*-[1,3,4]thiadiazolo[2,3-*c*][1,2,4]thiadiazol-3-imine (**21**)

Yield: 69%; mp 218-219°C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 7.58 (d, J = 6.9 Hz, 1H, ArH), 7.14 (d, J = 6.3 Hz, 2H, ArH), 7.04 (d, J = 6.4 Hz, 2H, ArH), 6.69 (s, 1H, ArH), 6.64 (d, J = 6.6 Hz, 1H, ArH), 5.37 (t, J = 5.4 Hz, 1H, ArH), 5.08 (d, J = 5.14 Hz, 2H, ArH), 3.84 (s, 3H, OCH₃), 3.82 (s, 3H, OCH₃). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 171.5, 163.9, 159.5, 158.5, 146.0, 145.4,

142.7, 133.0, 120.9, 120.9, 120.2, 115.0, 111.2, 106.7, 101.5, 90.8, 66.1, 55.8, 55.8; HREI-MS: m/z calcd for C₁₉H₁₆N₄O₄S₂ [M]⁺ 428.0608 Found 428.0603.

3.6.24. *(Z)*-3-((6-(2,3-dihydrobenzo[*b*][1,4]dioxin-2-yl)-3*H*-[1,3,4]thiadiazolo[2,3-*c*][1,2,4]thiadiazol-3-ylidene)amino)benzene-1,2-diol (**22**)

Yield: 70%; mp 198-199°C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 10.94 (s, 1H, OH), 9.65 (s, 1H, OH), 7.22 (d, *J* = 6.9 Hz, 2H, ArH), 7.18 (d, *J* = 6.7 Hz, 2H, ArH), 6.97 (d, *J* = 6.4 Hz, 1H, ArH), 6.78 (d, *J* = 6.2 Hz, 1H, ArH), 6.70 (t, *J* = 6.3 Hz, 1H, ArH), 5.39 (t, *J* = 5.5 Hz, 1H, ArH), 5.14 (d, *J* = 5.4 Hz, 2H, ArH). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 175.5, 159.5, 151.7, 146.9, 146.0, 143.3, 142.7, 124.7, 122.8, 120.9, 120.9, 120.2, 119.9, 119.6, 115.0, 93.8, 66.3; HREI-MS: m/z calcd for C₁₇H₁₂N₄O₄S₂ [M]⁺ 400.0293 Found 400.0287.

3.6.25. *((Z)*-6-(2,3-dihydrobenzo[*b*][1,4]dioxin-2-yl)-*N*-(2-fluorophenyl)-3*H*-[1,3,4]thiadiazolo[2,3-*c*][1,2,4]thiadiazol-3-imine (**23**)

Yield: 72%; mp 193-194°C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 7.74 (d, *J* = 7.4 Hz, 1H, ArH), 7.41 (t, *J* = 7.2 Hz, 1H, ArH), 7.33 (d, *J* = 7.1 Hz, 1H, ArH), 7.29 (d, *J* = 6.7 Hz, 2H, ArH), 7.25 (t, *J* = 7.1 Hz, 1H, ArH), 7.21 (d, *J* = 6.4 Hz, 2H, ArH), 5.49 (t, *J* = 5.8 Hz, 1H, ArH), 5.13 (d, *J* = 5.5 Hz, 2H, ArH). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 176.6, 160.6, 159.2, 143.3, 142.7, 142.3, 132.6, 130.8, 124.4, 123.2, 122.9, 122.9, 120.2, 115.6, 115.0, 93.8, 66.1; HREI-MS: m/z calcd for C₁₇H₁₁FN₄O₄S₂ [M]⁺ 386.0302 Found 386.0294.

3.6.26. *((Z)*-6-(2,3-dihydrobenzo[*b*][1,4]dioxin-2-yl)-*N*-(3-fluorophenyl)-3*H*-[1,3,4]thiadiazolo[2,3-*c*][1,2,4]thiadiazol-3-imine (**24**)

Yield: 74%; mp 194-195 °C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ 7.80 (s, 1H, ArH), 7.63 (t, *J* = 6.9 Hz, 1H, ArH), 7.55 (d, *J* = 6.9 Hz, 1H, ArH), 7.39 (d, *J* = 6.7 Hz, 1H, ArH), 7.33 (d, *J* = 6.5 Hz, 2H, ArH), 7.21 (d, *J* = 6.3 Hz, 2H, ArH), 5.51 (t, *J* = 5.7 Hz, 1H, ArH), 5.13 (d, *J* = 5.4 Hz, 2H, ArH). ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 175.5, 163.0, 158.5, 143.3, 142.7, 135.3, 130.4, 124.8, 120.9, 120.9, 120.2, 117.8, 115.0, 114.0, 93.8, 66.1, 146.8; HREI-MS: m/z calcd for C₁₇H₁₁FN₄O₄S₂ [M]⁺ 386.0302 Found 386.0294.

3.6.27. *((Z)*-6-(2,3-dihydrobenzo[*b*][1,4]dioxin-2-yl)-*N*-(4-fluorophenyl)-3*H*-[1,3,4]thiadiazolo[2,3-*c*][1,2,4]thiadiazol-3-imine (**25**)

Yield: 73%; mp 195-196°C; ^1H -NMR (500 MHz, $\text{DMSO-}d_6$): δ 7.79 (d, J = 7.1 Hz, 2H, ArH), 7.35 (d, J = 6.6 Hz, 2H, ArH), 7.56 (d, J = 6.6 Hz, 2H, ArH), 7.28 (d, J = 6.5 Hz, 2H, ArH), 7.25 (d, J = 7.0 Hz, 2H, ArH), 5.51 (t, J = 5.7 Hz, 1H, ArH), 5.13 (d, J = 5.5 Hz, 2H, ArH). ^{13}C -NMR (125 MHz, $\text{DMSO-}d_6$): δ 174.5, 165.2, 158.3, 144.1, 143.3, 142.7, 130.8, 130.8, 129.3, 120.9, 120.9, 120.2, 115.6, 115.6, 115.0, 93.8, 66.1; HREI-MS: m/z calcd for $\text{C}_{17}\text{H}_{11}\text{FN}_4\text{O}_4\text{S}_2$ $[\text{M}]^+$ 386.0302 Found 386.0294.