Supplementary Information

Novel 2,4-disubstituted quinazoline analogs as antibacterial agents with improved cytotoxicity profile: Modification of the benzenoid part

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Content

1. Experimental

1.1. Chemistry

1.1.1. Materials and methods

Solvents and reagents were obtained from commercial suppliers and were used without further purification. All organic solvents used were of pure analytical grade. Column chromatography was carried out using silica-gel 40-60 µm mesh with DCM/MeOH, EtOAc/Hexane or DCM/Hexane mixtures as eluents. Reaction progress was monitored by TLC using fluorescent pre-coated silica gel plates and detection of the components was made by short UV light (λ= 254 nm). ^{1}H-NMR and ^{13}C-NMR spectra were recorded at 500 MHz using a Bruker DRX-500 spectrometer, 400 MHz using Varian Mercury 400 Plus and 300 MHz using Bruker Fourier 300. ^{1}H shifts are referenced to the residual protonated solvent signal (δ 2.50 for DMSO-d_6 and δ 7.26 for $CDCl_3$) and ^{13}C shifts are referenced to the deuterated solvent signal (δ 39.5 for DMSO-d_6 and δ 77.0 for $CDCl_3$). Chemical shifts are given in parts per million (ppm), and all coupling constants (J) are given in Hz. The purities of the tested compounds were determined by HPLC coupled with mass spectrometry and were all higher than 95% purity. Mass spectrometric analysis (HPLC-ESI-MS) was performed on an MSQ plus (Thermo Fisher Scientific) instrument equipped with an ESI source and a single quadrupole mass detector. All samples were injected by autosampler (Surveyor, Thermo Finnigan) with an injection volume of 10 µL. A RP C18 NUCLEODUR 100-3 (125 mm x 3 mm) column (Macherey-Nagel) was used as stationary phase. The solvent system consisted of water containing 0.1% TFA (A) and 0.1% TFA in acetonitrile (B). HPLC-method: flow rate 400 µL/min. The percentage of B started at an initial of 5%, was increased up to 100% during 16 min, kept at 100% for 2 min, and flushed back to 5% in 2 min. All masses were reported as those of the parent ions. Melting points were determined on Buchi B-540 Melting Point apparatus and are uncorrected.

1.1.2. Synthesis of compounds **2a-f**

Different *ortho*- aminobenzonitrile derivatives **1a-f** (10 mmol) were added to 10 mL carbon disulfide and 10 mL pyridine. The reaction mixture was heated at 70 ºC for 6 hours. The mixture was then poured on ice water and the solid precipitate was filtered under vaccum.

1.1.2.1. ***7-Fluoroquinazoline-2,4(1H,3H)-dithione*** **(2a)**

The title compound was synthesized according to the general procedure by the cyclization of 2-amino-4-fluorobenzonitrile (**1a**) with carbon disulfide. Compound **2a** is commercially available.

1.1.2.2. ***6-Fluoroquinazoline-2,4(1H,3H)-dithione*** **(2b)**

The title compound was synthesized according to the general procedure by the cyclization of 2-amino-5-fluorobenzonitrile (**1b**) with carbon disulfide. Compounds **2b** is a reported compound.[1, 2]

1.1.2.3. **7-*Chloroquinazoline-2,4(1H,3H)-dithione*** **(2c)**

The title compound was synthesized according to the general procedure by the cyclization of 2-amino-4-chlorobenzonitrile (**1c**) with carbon disulfide. Compounds **2c** is a reported compound.[1, 2]

1.1.2.4. ***6-Chloroquinazoline-2,4(1H,3H)-dithione*** **(2d)**

The title compound was synthesized according to the general procedure by the cyclization of 2-amino-5-chlorobenzonitrile (**1d**) with carbon disulfide. Compounds **2d** is a reported compound.[1, 2]

1.1.2.5. ***7-Bromoquinazoline-2,4(1H,3H)-dithione*** **(2e)**

The title compound was synthesized according to the general procedure by the cyclization of 2-amino-4-bromobenzonitrile (**1e**) with carbon disulfide. Physical form: yellow powder; Yield= 91%; m.p.= 307-310 ºC; **^{1}H-**

NMR (DMSO-d_6, δ [ppm]): 13.75 (s, 1H), 13.07 (s, 1H), 8.56 (d, *J* = 1.7 Hz, 1H), 7.37 (dd, *J* = 4.3, 1.5 Hz, 1H), 7.35 (dd, *J* = 4.2, 1.5 Hz, 1H); **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 187.63, 171.37, 137.59, 132.51, 130.27, 128.58, 124.34, 122.31; **MS (ESI):** *m/z* 272.89 (M^++1), *m/z* 274.90 (M^++3); **TLC:** R_f= 0.16 (DCM).

1.1.2.6. ***6-Bromoquinazoline-2,4(1H,3H)-dithione* (2f)**

The title compound was synthesized according to the general procedure by the cyclization of 2-amino-5-bromobenzonitrile (**1f**) with carbon disulfide. Compounds **2f** is a reported compound.[1, 3]

1.1.3. Synthesis of compounds **3a-f**

A mixture of the 2,4-dithione quinazoline derivative **2a-f** (5mmol) and potassium carbonate (15 mmol) was dissolved in acetone (10 mL). 1-Bromobutane was then added in excess (20 mmol). The reaction was left to stir overnight under reflux. Afterwards, acetone was evaporated under vacuum. The residue was then extracted with DCM (3 × 20 mL), the combined organic layers were passed over anhydrous $MgSO_4$. The DCM was then evaporated under reduced pressure and the remaining residue was purified using column chromatography.

1.1.3.1. ***2,4-Bis(butylthio)-7-fluoroquinazoline* (3a)**

The compound was synthesized according to the general procedure for alkylation of 7-Fluoroquinazoline-2,4(1*H*,3*H*)-dithione (**2a**) using 1-bromobutane. Physical form: brown semisolid; Yield= 81%; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 8.02 (dd, *J* = 9.1, 6.0 Hz, 1H), 7.44 (dd, *J* = 10.1, 2.5 Hz, 1H), 7.42 – 7.37 (m, 1H), 3.30 – 3.26 (m, 2H), 3.19 – 3.15 (m, 2H), 1.71 – 1.67 (m, 2H), 1.67 – 1.63 (m, 2H), 1.47 – 1.42 (m, 2H), 1.42 – 1.37 (m, 2H), 0.91 (td, *J* = 7.3, 0.9 Hz, 6H).; **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 170.45, 167.07, 165.36 (d, *J* = 253.3 Hz), 149.99 (d, *J* = 14.1 Hz), 127.01 (d, *J* = 11.0 Hz), 117.81, 116.00 (d, *J* = 25.1 Hz), 110.86 (d, *J* = 20.9 Hz), 31.08, 30.51, 29.89, 28.62, 21.49, 21.47, 13.51, 13.44.; **MS (ESI):** *m/z* 325.05 $(M + H)^+$; **TLC:** R_f= 0.63 (DCM:Hexane, 2:1).

1.1.3.2. ***2,4-Bis(butylthio)-6-fluoroquinazoline* (3b)**

The compound was synthesized according to the general procedure for alkylation of 6-fluoroquinazoline-2,4(1*H*,3*H*)-dithione (**2b**) using 1-bromobutane. Physical form: yellow semisolid; Yield= 85%; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 7.77 (d, *J* = 2.1 Hz, 1H), 7.75 (d, *J* = 1.5 Hz, 1H), 7.63 – 7.60 (m, 1H), 3.29 – 3.25 (m, 2H), 3.18 – 3.15 (m, 2H), 1.70 – 1.66 (m, 2H), 1.65 (ddd, *J* = 9.9, 5.6, 3.1 Hz, 2H), 1.46 – 1.42 (m, 2H), 1.42 – 1.37 (m, 2H), 0.90 (t, *J* = 7.4 Hz, 6H); **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 170.11, 165.29 (d, *J* = 2.3 Hz), 158.72 (d, *J* = 247.2 Hz), 145.34, 129.85 (d, *J* = 8.7 Hz), 124.29 (d, *J* = 25.3 Hz), 120.76 (d, *J* = 9.1 Hz), 107.67 (d, *J* = 23.3 Hz), 31.09, 30.43, 29.84, 28.74, 21.50, 21.47, 13.51, 13.43; **MS (ESI):** *m/z* 325.08 $(M + H)^+$; **TLC:** R_f= 0.58 (DCM:Hexane, 2:1).

1.1.3.3. ***2,4-Bis(butylthio)-7-chloroquinazoline* (3c)**

The compound was synthesized according to the general procedure for alkylation of 7-chloroquinazoline-2,4(1*H*,3*H*)-dithione (**2c**) using 1-bromobutane. Physical form: yellow semisolid; Yield= 89%; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 7.94 (d, *J* = 8.8 Hz, 1H), 7.74 – 7.72 (m, 1H), 7.51 (dd, *J* = 8.8, 2.1 Hz, 1H), 3.30 – 3.26 (m, 2H), 3.19 – 3.15 (m, 2H), 1.71 – 1.67 (m, 2H), 1.67 – 1.64 (m, 2H), 1.47 – 1.43 (m, 2H), 1.42 – 1.38 (m, 2H), 0.91 (t, *J* = 7.4 Hz, 6H); **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 170.76, 167.17, 148.81, 139.20, 126.71, 125.82, 125.66, 119.10, 31.07, 30.45, 29.91, 28.66, 21.50, 21.48, 13.52, 13.45; **MS (ESI):** *m/z* 341.01 $(M + H)^+$; **TLC:** R_f= 0.73 (DCM:Hexane, 2:1).

1.1.3.4. ***2,4-Bis(butylthio)-6-chloroquinazoline* (3d)**

The compound was synthesized according to the general procedure for alkylation of 6-chloroquinazoline-2,4(1*H*,3*H*)-dithione (**2d**) using 1-bromobutane. Physical form: dark orange semisolid; Yield= 85%; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 7.90 (d, *J* = 2.3 Hz, 1H), 7.86 (dd, *J* = 8.9, 2.3 Hz, 1H), 7.70 (d, *J* = 8.9 Hz, 1H), 3.29 (t, *J* = 7.3 Hz, 2H), 3.20 – 3.16 (m, 2H), 1.72 – 1.68 (m, 2H), 1.68 – 1.63 (m, 2H), 1.48 – 1.43 (m, 2H), 1.43 – 1.37 (m, 2H), 0.91

(t, *J* = 7.4 Hz, 6H); **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 169.91, 166.32, 146.75, 135.01, 130.14, 129.12, 122.55, 121.11, 31.06, 30.41, 29.89, 28.78, 21.50, 21.46, 13.52, 13.45; **MS (ESI):** *m/z* 341.03 $(M + H)^+$; **TLC:** R_f = 0.6 (DCM:Hexane, 2:1).

1.1.3.5. ***2,4-Bis(butylthio)-7-bromoquinazoline*** **(3e)**

The compound was synthesized according to the general procedure for alkylation of 7-bromoquinazoline-2,4(1*H*,3*H*)-dithione (**2e**) using 1-bromobutane. Physical form: off white waxy solid; Yield= 84%; m.p.= 31-32 ºC; **IR, cm^{-1}:** ; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 7.91 (d, *J* = 1.7 Hz, 1H), 7.88 (d, *J* = 8.8 Hz, 1H), 7.65 (dd, *J* = 8.8, 2.0 Hz, 1H), 3.33 – 3.26 (m, 2H), 3.19 (t, *J* = 7.3 Hz, 2H), 1.76 – 1.63 (m, 4H), 1.51 – 1.37 (m, 4H), 0.93 (t, *J* = 7.3 Hz, 6H); **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 171.42, 167.61, 149.43, 129.85, 129.40, 128.77, 126.24, 119.87, 31.59, 30.96, 30.44, 29.18, 22.00, 21.98, 14.02, 13.95; **MS (ESI):** *m/z* 385.03 $(M + H)^+$; **TLC:** R_f = 0.73 (DCM:Hexane, 2:1).

1.1.3.6. ***2,4-Bis(butylthio)-6-bromoquinazoline*** **(3f)**

The compound was synthesized according to the general procedure for alkylation of 6-bromoquinazoline-2,4(1*H*,3*H*)-dithione (**2f**) using 1-bromobutane. Physical form: yellow semisolid; Yield= 87%; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 8.08 (d, *J* = 2.0 Hz, 1H), 8.00 (dd, *J* = 8.9, 2.2 Hz, 1H), 7.66 (d, *J* = 8.9 Hz, 1H), 3.31 (d, *J* = 7.3 Hz, 2H), 3.25 – 3.16 (m, 2H), 1.77 – 1.63 (m, 4H), 1.52 – 1.45 (m, 2H), 1.45 – 1.38 (m, 2H), 0.93 (t, *J* = 7.3 Hz, 6H).; **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 170.30, 166.91, 147.50, 138.15, 129.73, 126.26, 122.20, 118.96, 31.57, 30.93, 30.42, 29.31, 21.99, 21.94, 14.02, 13.95; **MS (ESI):** *m/z* 385.02 $(M + H)^+$; **TLC:** R_f = 0.62 (DCM:Hexane, 2:1).

1.1.4. Synthesis of compounds **4a-f**

The 2,4-bis(alkylthio)quinazoline derivatives (**3a-f**) were refluxed with n-butylamine (10 mL) overnight. After completion of the reaction (monitored by TLC), the n-butylamine was evaporated under vacuum. The remaining residue was then extracted with DCM (3 × 20 mL), then the combined organic layers were passed over anhydrous $MgSO_4$. The DCM was then evaporated under reduced pressure and the remaining residue was purified using column chromatography to give compounds **4a-f**.

1.1.4.1. ***N-butyl-2-(butylthio)-7-fluoroquinazolin-4-amine*** **(4a)**

The title compound was synthesized according to the general procedure by amination of 2,4-bis(butylthio)-7-fluoroquinazoline (**3a**) using n-butylamine. Physical form: beige powder; Yield= 90%; m.p.= 42-44 ºC; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 8.40 (t, *J* = 5.5 Hz, 1H), 8.23 (dd, *J* = 9.1, 6.1 Hz, 1H), 7.24 (td, *J* = 8.8, 2.7 Hz, 1H), 7.19 (dd, *J* = 10.5, 2.6 Hz, 1H), 3.49 (dd, *J* = 12.8, 7.1 Hz, 2H), 3.11 – 3.06 (m, 2H), 1.69 – 1.63 (m, 2H), 1.63 – 1.56 (m, 2H), 1.44 – 1.38 (m, 2H), 1.38 – 1.30 (m, 2H), 0.90 (td, *J* = 7.4, 6.0 Hz, 6H); **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 168.22, 164.61 (d, *J* = 249.3 Hz), 158.25, 151.72 (d, *J* = 13.4 Hz), 125.97 (d, *J* = 10.7 Hz), 113.03 (d, *J* = 24.1 Hz), 109.97 (d, *J* = 20.4 Hz), 109.95 (d, *J* = 1.0 Hz), 40.21, 31.71, 30.56, 29.58, 21.56, 19.72, 13.70, 13.55; **MS (ESI):** *m/z* 308.10 $(M + H)^+$; **TLC:** R_f = 0.32 (DCM).

1.1.4.2. ***N-butyl-2-(butylthio)-6-fluoroquinazolin-4-amine*** **(4b)**

The title compound was synthesized according to the general procedure by amination of 2,4-bis(butylthio)-6-fluoroquinazoline (**3b**) using n-butylamine. Physical form: off white powder; Yield= 92%; m.p.= 100-102 ºC; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 8.26 (t, *J* = 5.3 Hz, 1H), 8.02 (dd, *J* = 9.9, 2.6 Hz, 1H), 7.60 – 7.55 (m, 1H), 7.53 (dd, *J* = 9.1, 5.7 Hz, 1H), 3.49 (dd, *J* = 12.8, 7.0 Hz, 2H), 3.09 (t, *J* = 7.4 Hz, 2H), 1.66 (dd, *J* = 14.9, 7.4 Hz, 2H), 1.60 (dd, *J* = 14.6, 7.5 Hz, 2H), 1.45 – 1.38 (m, 2H), 1.38 – 1.31 (m, 2H), 0.93 – 0.87 (m, 6H); **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 166.21, 158.34 (d, *J* = 3.8 Hz), 158.13 (d, *J* = 241.9 Hz), 146.83, 128.54 (d, *J* = 8.4 Hz), 121.80 (d, *J* = 24.7 Hz),

113.13 (d, *J* = 8.6 Hz), 107.40 (d, *J* = 23.4 Hz), 40.28, 31.71, 30.47, 29.54, 21.56, 19.71, 13.69, 13.54; **MS (ESI):** *m/z* 308.11 $(M + H)^+$; **TLC:** R_f= 0.23 (DCM).

1.1.4.3. ***N-butyl-2-(butylthio)-7-chloroquinazolin-4-amine*** **(4c)**

The title compound was synthesized according to the general procedure by amination of 2,4-bis(butylthio)-7-chloroquinazoline (**3c**) using n-butylamine. Physical form: white powder; Yield= 92%; m.p.= 104-105 ºC; **^{1}H-NMR (DMSO-*d*$_6$, δ [ppm]):** 8.44 (t, *J* = 5.4 Hz, 1H), 8.18 (d, *J* = 8.8 Hz, 1H), 7.50 (d, *J* = 2.1 Hz, 1H), 7.39 (dd, *J* = 8.8, 2.2 Hz, 1H), 3.50 (dd, *J* = 12.7, 7.0 Hz, 2H), 3.15 – 3.03 (m, 2H), 1.72 – 1.55 (m, 4H), 1.44 (dd, *J* = 15.2, 7.6 Hz, 2H), 1.39 – 1.29 (m, 2H), 0.91 (tt, *J* = 7.6, 3.8 Hz, 6H); **^{13}C-NMR (DMSO-*d*$_6$, δ [ppm]):** 168.83, 158.82, 151.25, 137.86, 125.60, 125.23, 124.73, 112.07, 40.86, 32.20, 31.01, 30.11, 22.06, 20.22, 14.19, 14.04; **MS (ESI):** *m/z* 324.10 $(M + H)^+$; **TLC:** R_f= 0.25 (DCM).

1.1.4.4. ***N-butyl-2-(butylthio)-6-chloroquinazolin-4-amine*** **(4d)**

The title compound was synthesized according to the general procedure by amination of 2,4-bis(butylthio)-6-chloroquinazoline (**3d**) using n-butylamine. Physical form: white powder; Yield= 95%; m.p.= 142-144 ºC; **^{1}H-NMR (DMSO-*d*$_6$, δ [ppm]):** 8.39 (t, *J* = 5.4 Hz, 1H), 8.31 (d, *J* = 2.3 Hz, 1H), 7.68 – 7.65 (m, 1H), 7.48 (d, *J* = 8.9 Hz, 1H), 3.48 (dd, *J* = 12.7, 7.0 Hz, 2H), 3.11 – 3.06 (m, 2H), 1.69 – 1.63 (m, 2H), 1.60 (dd, *J* = 15.0, 7.9 Hz, 2H), 1.45 – 1.38 (m, 2H), 1.38 – 1.32 (m, 2H), 0.91 (td, *J* = 7.4, 6.0 Hz, 6H); **^{13}C-NMR (DMSO-*d*$_6$, δ [ppm]):** 167.34, 157.81, 148.41, 133.01, 128.06, 127.88, 122.27, 113.70, 40.32, 31.68, 30.41, 29.57, 21.56, 19.70, 13.70, 13.55; **MS (ESI):** *m/z* 324.06 $(M + H)^+$; **TLC:** R_f= 0.21 (DCM).

1.1.4.5. ***N-butyl-2-(butylthio)-7-bromoquinazolin-4-amine*** **(4e)**

The title compound was synthesized according to the general procedure by amination of 2,4-bis(butylthio)-7-bromoquinazoline (**3e**) using n-butylamine. Physical form: white solid; Yield= 92%; m.p.= 95-96 ºC; **^{1}H-NMR (DMSO-*d*$_6$, δ [ppm]):** 8.46 (t, *J* = 5.4 Hz, 1H), 8.10 (d, *J* = 8.8 Hz, 1H), 7.66 (t, *J* = 4.1 Hz, 1H), 7.52 (dd, *J* = 8.7, 2.0 Hz, 1H), 3.48 (dd, *J* = 12.8, 7.0 Hz, 2H), 3.13 – 3.05 (m, 2H), 1.66 (dd, *J* = 14.8, 7.3 Hz, 2H), 1.60 (dd, *J* = 14.6, 7.5 Hz, 2H), 1.42 (dt, *J* = 14.8, 7.4 Hz, 2H), 1.35 (dt, *J* = 14.4, 7.3 Hz, 2H), 0.91 (td, *J* = 7.4, 3.6 Hz, 6H); **^{13}C-NMR (DMSO-*d*$_6$, δ [ppm]):** 168.21, 158.39, 150.86, 127.92, 126.93, 126.30, 125.10, 111.81, 40.27, 31.70, 30.48, 29.60, 21.56, 19.71, 13.71, 13.56; **MS (ESI):** *m/z* 368.07 $(M + H)^+$; **TLC:** R_f= 0.31 (DCM).

1.1.4.6. ***N-butyl-2-(butylthio)-6-bromoquinazolin-4-amine*** **(4f)**

The title compound was synthesized according to the general procedure by amination of 2,4-bis(butylthio)-6-bromoquinazoline (**3f**) using n-butylamine. Physical form: white powder; Yield= 84%; m.p.= 149-150 ºC; **^{1}H-NMR (DMSO-*d*$_6$, δ [ppm]):** 8.45 (d, *J* = 2.2 Hz, 1H), 8.41 (t, *J* = 5.4 Hz, 1H), 7.78 (dd, *J* = 8.9, 2.2 Hz, 1H), 7.41 (d, *J* = 8.8 Hz, 1H), 3.48 (td, *J* = 7.1, 5.7 Hz, 2H), 3.11 – 3.06 (m, 2H), 1.69 – 1.63 (m, 2H), 1.63 – 1.57 (m, 2H), 1.45 – 1.38 (m, 2H), 1.38 – 1.32 (m, 2H), 0.91 (td, *J* = 7.4, 5.7 Hz, 6H); **^{13}C-NMR (DMSO-*d*$_6$, δ [ppm]):** 167.42, 157.67, 148.64, 135.65, 128.24, 125.37, 115.97, 114.27, 40.33, 31.67, 30.41, 29.58, 21.56, 19.70, 13.70, 13.55; **MS (ESI):** *m/z* 368.08 $(M + H)^+$; **TLC:** R_f= 0.15 (DCM:Hexane, 2:1).

1.1.5. Synthesis of compounds **5** and **6**

Compound **4e** (1 mmol) was added to a mixture of 10 mL toluene, 10 mL ethanol and 4 mL H_2O. The corresponding arylboronic acid was added to the mixture (2 mmol), in the presence of cesium carbonate (4 mmol) as a base and [1,1′-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) ($Pd(dppf)Cl_2$) (0.05%) as a catalyst. The reaction was then refluxed overnight. After the completion of the reaction, ethanol and toluene were evapourated under reduced pressure and the aqueous phase was extracted using ethylacetate (3 × 20 mL). The combined organic

layers were passed over anhydrous $MgSO_4$. The ethylacetate was then evaporated under vaccum and the remaining residue was purified using column chromatography to give compounds **5** and **6**.

1.1.5.1. ***N-butyl-2-(butylthio)-7-phenylquinazolin-4-amine*** **(5)**

The title compound was synthesized according to the general procedure by arylation of *N*-butyl-2-(butylthio)-7-bromoquinazolin-4-amine (**4e**) using phenylboronic acid. Physical form: beige powder; Yield= 76%; m.p.= 89-9 ºC; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 8.47 (t, *J* = 5.3 Hz, 1H), 8.27 (d, *J* = 8.4 Hz, 1H), 7.84 – 7.81 (m, 1H), 7.79 (s, 1H), 7.72 (dt, *J* = 8.4, 1.7 Hz, 2H), 7.57 – 7.48 (m, 2H), 7.47 – 7.39 (m, 1H), 3.54 (dd, *J* = 12.8, 6.9 Hz, 2H), 3.20 – 3.10 (m, 2H), 1.78 – 1.58 (m, 4H), 1.52 – 1.41 (m, 2H), 1.41 – 1.27 (m, 2H), 0.96 (dd, *J* = 7.7, 3.7 Hz, 3H), 0.93 – 0.87 (m, 3H); **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 167.56, 158.99, 150.28, 144.83, 139.45, 129.54, 128.80, 127.56, 124.24, 123.51, 123.31, 112.41, 40.86, 32.27, 31.13, 30.12, 22.09, 20.25, 14.23, 14.08; **MS (ESI):** *m/z* 366.28 $(M + H)^+$; **TLC:** R_f = 0.11 (DCM).

1.1.5.2. ***N-butyl-2-(butylthio)-7-(pyridin-3-yl)quinazolin-4-amine*** **(6)**

The title compound was synthesized according to the general procedure by arylation of *N*-butyl-2-(butylthio)-7-bromoquinazolin-4-amine (**4e**) using 3-pyridinylboronic acid. Physical form: light brown powder; Yield= 75%; m.p.= 100-101 ºC; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 9.03 (d, *J* = 1.8 Hz, 1H), 8.63 (dd, *J* = 4.7, 1.5 Hz, 1H), 8.42 (t, *J* = 5.5 Hz, 1H), 8.29 (d, *J* = 8.6 Hz, 1H), 8.23 (ddd, *J* = 8.0, 2.3, 1.7 Hz, 1H), 7.81 (d, *J* = 1.8 Hz, 1H), 7.76 (dd, *J* = 8.5, 1.9 Hz, 1H), 7.53 (ddd, *J* = 8.0, 4.8, 0.7 Hz, 1H), 3.53 (dd, *J* = 12.8, 6.9 Hz, 2H), 3.18 – 3.08 (m, 2H), 1.75 – 1.66 (m, 2H), 1.62 (dd, *J* = 9.8, 5.0 Hz, 2H), 1.51 – 1.41 (m, 2H), 1.36 (dd, *J* = 14.9, 7.7 Hz, 2H), 0.98 – 0.93 (m, 3H), 0.93 – 0.87 (m, 3H); **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 167.88, 158.98, 150.69, 149.70, 148.44, 141.68, 135.03, 134.99, 124.42, 124.09, 123.28, 112.87, 40.84, 32.27, 31.11, 30.10, 22.09, 20.24, 14.22, 14.07; **MS (ESI):** *m/z* 367.32 $(M + H)^+$; **TLC:** R_f = 0.13 (DCM:MeOH, 100:2).

1.1.6. Synthesis of compounds **7**-**16**

Compound **4f** (1 mmol) was added to a mixture of 10 mL toluene, 10 mL ethanol and 4 mL H_2O. The corresponding arylboronic acid was added to the mixture (2 mmol), in the presence of cesium carbonate (4 mmol) as a base and [1,1′-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) ($Pd(dppf)Cl_2$) (0.05%) as a catalyst. The reaction was then refluxed overnight. After the completion of the reaction, ethanol and toluene were evapourated under reduced pressure and the aqueous phase was extracted using ethylacetate (3 × 20 mL). The combined organic layers were passed over anhydrous $MgSO_4$. The ethylacetate was then evaporated under vaccum and the remaining residue was purified using column chromatography to give compounds **7-16**.

1.1.6.1. ***N-butyl-2-(butylthio)-6-phenylquinazolin-4-amine*** **(7)**

The title compound was synthesized according to the general procedure by arylation of *N*-butyl-2-(butylthio)-6-bromoquinazolin-4-amine (**4f**) using phenylboronic acid. Physical form: off white powder; Yield= 75%; m.p.= 114-115 ºC; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 8.53 (t, *J* = 5.2 Hz, 1H), 8.48 (d, *J* = 5.4 Hz, 1H), 8.01 (dd, *J* = 8.7, 2.0 Hz, 1H), 7.82 (dd, *J* = 8.8, 7.5 Hz, 2H), 7.56 (d, *J* = 8.7 Hz, 1H), 7.51 (t, *J* = 6.7 Hz, 2H), 7.44 – 7.33 (m, 1H), 3.55 (dd, *J* = 12.7, 6.9 Hz, 2H), 3.18 – 3.08 (m, 2H), 1.71 (dt, *J* = 5.6, 5.2 Hz, 2H), 1.67 – 1.58 (m, 2H), 1.52 – 1.42 (m, 2H), 1.37 (dd, *J* = 14.2, 7.2 Hz, 2H), 0.96 (dd, *J* = 7.5, 4.0 Hz, 3H), 0.93 – 0.88 (m, 3H).; **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 167.26, 159.26, 149.67, 139.72, 136.22, 131.77, 129.41, 127.98, 127.18, 127.05, 120.97, 113.64, 40.86, 32.29, 31.16, 30.11, 22.09, 20.28, 14.24, 14.07; **MS (ESI):** *m/z* 366.30 $(M + H)^+$; **TLC:** R_f = 0.6 (DCM:MeOH, 100:2).

1.1.6.2. ***4-(4-(Butylamino)-2-(butylthio)quinazolin-6-yl)benzoic acid*** **(8)**

The title compound was synthesized according to the general procedure by arylation of *N*-butyl-2-(butylthio)-6-bromoquinazolin-4-amine (**4f**) using 4-carboxyphenylboronic acid. Physical form: beige powder; Yield= 64%; m.p.= 250-252 ºC; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 12.86 (s, 1H), 8.65 (t, *J* = 5.4 Hz, 1H), 8.60 (d, *J* = 1.8 Hz, 1H), 8.13 (s, 1H), 8.09 (dd, *J* = 8.7, 1.8 Hz, 1H), 8.07 (s, 1H), 8.05 (s, 1H), 7.94 (d, *J* = 5.0 Hz, 1H), 7.57 (d, *J* = 8.7 Hz, 1H), 3.55 (dd, *J* = 12.8, 6.9 Hz, 2H), 3.14 (t, *J* = 7.4 Hz, 2H), 1.67 (qd, *J* = 14.8, 7.5 Hz, 4H), 1.41 (ddq, *J* = 22.0, 14.6, 7.3 Hz, 4H), 0.93 (q, *J* = 7.3 Hz, 6H); **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 167.12, 167.08, 162.98, 158.71, 143.18, 134.54, 131.50, 129.93, 129.60, 126.68, 121.21, 113.02, 40.40, 31.71, 30.62, 29.66, 21.57, 19.76, 13.73, 13.56; **MS (ESI):** *m/z* 410.09 $(M + H)^+$; **TLC:** R_f = 0.13 (DCM:MeOH, 100:4).

1.1.6.3. ***3-(4-(Butylamino)-2-(butylthio)quinazolin-6-yl)benzoic acid* (9)**

The title compound was synthesized according to the general procedure by arylation of *N*-butyl-2-(butylthio)-6-bromoquinazolin-4-amine (**4f**) using 3-carboxyphenylboronic acid. Physical form: beige powder; Yield= 62%; m.p.= 145-146 ºC; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 13.11 (s, 1H), 8.60 (t, *J* = 5.4 Hz, 1H), 8.54 (d, *J* = 1.8 Hz, 1H), 8.34 (s, 1H), 8.03 (dd, *J* = 8.6, 2.0 Hz, 2H), 7.96 (d, *J* = 7.8 Hz, 1H), 7.67 – 7.59 (m, 2H), 3.54 (dd, *J* = 12.9, 6.9 Hz, 2H), 3.15 – 3.08 (m, 2H), 1.66 (tt, *J* = 14.9, 7.5 Hz, 4H), 1.47 – 1.34 (m, 4H), 0.92 (td, *J* = 7.3, 5.9 Hz, 6H); **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 167.28, 167.01, 158.73, 149.36, 139.64, 134.82, 131.55, 131.49, 131.39, 131.11, 129.29, 127.36, 126.72, 120.77, 113.16, 40.28, 31.77, 30.67, 29.60, 21.58, 19.78, 13.75, 13.58; **MS (ESI):** *m/z* 410.08 $(M + H)^+$; **TLC:** R_f = 0.11 (DCM:MeOH, 100:4).

1.1.6.4. ***(3-(4-(Butylamino)-2-(butylthio)quinazolin-6-yl)phenyl)methanol* (10)**

The title compound was synthesized according to the general procedure by arylation of *N*-butyl-2-(butylthio)-6-bromoquinazolin-4-amine (**4f**) using 4-(hydroxymethyl)phenylboronic acid. Physical form: yellowish powder; Yield= 71%; m.p.= 121-123 ºC; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 8.52 (dd, *J* = 13.8, 8.2 Hz, 1H), 8.00 (dd, *J* = 8.7, 2.0 Hz, 1H), 7.73 (s, 1H), 7.66 (d, *J* = 7.8 Hz, 1H), 7.56 (d, *J* = 8.7 Hz, 1H), 7.46 (dd, *J* = 9.5, 5.7 Hz, 1H), 7.39 – 7.31 (m, 1H), 7.09 (t, *J* = 7.8 Hz, 1H), 5.28 (t, *J* = 5.7 Hz, 1H), 4.61 (d, *J* = 5.5 Hz, 2H), 3.54 (dd, *J* = 12.8, 7.0 Hz, 2H), 3.16 – 3.09 (m, 2H), 1.72 – 1.60 (m, 4H), 1.45 (dt, *J* = 14.9, 7.4 Hz, 2H), 1.38 (dt, *J* = 14.7, 7.4 Hz, 2H), 0.93 (td, *J* = 7.4, 5.8 Hz, 6H); **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 166.67, 158.74, 157.21, 143.25, 139.03, 135.94, 128.91, 128.69, 126.51, 125.66, 125.13, 124.75, 120.36, 113.45, 62.94, 40.28, 31.78, 30.68, 29.60, 21.58, 19.78, 13.75, 13.58; **MS (ESI):** *m/z* 396.13 $(M + H)^+$; **TLC:** R_f = 0.19 (DCM:MeOH, 100:2).

1.1.6.5. ***3-(4-(Butylamino)-2-(butylthio)quinazolin-6-yl)benzonitrile* (11)**

The title compound was synthesized according to the general procedure by arylation of *N*-butyl-2-(butylthio)-6-bromoquinazolin-4-amine (**4f**) using 3-cyanophenylboronic acid. Physical form: off white solid; Yield= 69%; m.p.= 82-83 ºC; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 8.27 (t, *J* = 1.5 Hz, 1H), 8.15 (ddd, *J* = 8.0, 1.9, 1.1 Hz, 1H), 8.11 (dd, *J* = 8.7, 2.0 Hz, 1H), 7.88 – 7.82 (m, 1H), 7.71 (dt, *J* = 10.8, 7.9 Hz, 1H), 7.57 (d, *J* = 8.7 Hz, 1H), 7.12 – 7.11 (m, 2H), 3.56 (dd, *J* = 12.9, 7.1 Hz, 2H), 3.18 – 3.10 (m, 2H), 1.67 (qd, *J* = 14.8, 7.5 Hz, 4H), 1.44 (dt, *J* = 12.7, 6.3 Hz, 2H), 1.39 (dd, *J* = 9.4, 5.5 Hz, 2H), 0.96 – 0.90 (m, 6H); **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 158.67, 157.76, 140.13, 131.31, 131.04, 130.85, 130.20, 130.00, 122.74, 121.16, 120.72, 118.78, 118.16, 112.14, 111.98, 40.42, 31.70, 30.65, 29.68, 21.57, 19.76, 13.73, 13.56; **MS (ESI):** *m/z* 391.11 $(M + H)^+$; **TLC:** R_f = 0.41 (DCM:MeOH, 100:2).

1.1.6.6. ***3-(4-(Butylamino)-2-(butylthio)quinazolin-6-yl)phenol* (12)**

The title compound was synthesized according to the general procedure by arylation of *N*-butyl-2-(butylthio)-6-bromoquinazolin-4-amine (**4f**) using 3-hydroxyphenylboronic acid. Physical form: light brown solid; Yield= 67%; m.p.= 170-171 ºC; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 9.56 (s, 1H), 8.51 (t, *J* = 5.0 Hz, 1H), 8.45 (d, *J* = 1.7 Hz, 1H),

7.93 (dd, *J* = 8.7, 1.9 Hz, 1H), 7.53 (d, *J* = 8.7 Hz, 1H), 7.33 – 7.25 (m, 1H), 7.23 – 7.14 (m, 2H), 6.80 (dd, *J* = 8.0, 1.5 Hz, 1H), 3.53 (dd, *J* = 12.8, 6.9 Hz, 2H), 3.12 (t, *J* = 7.4 Hz, 2H), 1.73 – 1.58 (m, 4H), 1.49 – 1.34 (m, 4H), 0.93 (dd, *J* = 13.7, 7.3 Hz, 6H); **^{13}C-NMR (DMSO-*d*$_6$, δ [ppm]):** 166.62, 158.73, 157.84, 140.66, 135.96, 131.28, 129.87, 126.38, 120.39, 117.51, 114.48, 113.62, 113.05, 106.15, 40.29, 31.78, 30.63, 29.60, 21.58, 19.76, 13.74, 13.57; **MS (ESI):** *m/z* 382.15 $(M + H)^+$; **TLC:** R_f = 0.22 (DCM:MeOH, 100:2).

1.1.6.7. ***N-butyl-2-(butylthio)-6-(3-methoxyphenyl)quinazolin-4-amine*** **(13)**

The title compound was synthesized according to the general procedure by arylation of *N*-butyl-2-(butylthio)-6-bromoquinazolin-4-amine (**4f**) using 3-methoxyphenylboronic acid. Physical form: pink solid; Yield= 62%; m.p.= 81-82 ºC; **^{1}H-NMR (DMSO-*d*$_6$, δ [ppm]):** 8.52 (t, *J* = 5.4 Hz, 1H), 8.49 (d, *J* = 1.9 Hz, 1H), 8.03 (dd, *J* = 8.7, 2.0 Hz, 1H), 7.54 (d, *J* = 8.7 Hz, 1H), 7.42 (t, *J* = 7.9 Hz, 1H), 7.39 – 7.33 (m, 2H), 6.97 (ddd, *J* = 8.1, 2.5, 1.0 Hz, 1H), 3.85 (s, 3H), 3.54 (dd, *J* = 12.8, 7.1 Hz, 2H), 3.16 – 3.09 (m, 2H), 1.72 – 1.61 (m, 4H), 1.48 – 1.34 (m, 4H), 0.93 (td, *J* = 7.4, 6.1 Hz, 6H); **^{13}C-NMR (DMSO-*d*$_6$, δ [ppm]):** 159.81, 158.72, 140.60, 131.43, 130.00, 129.78, 120.54, 119.05, 112.68, 112.61, 107.73, 104.54, 101.13, 55.20, 54.78, 40.32, 31.76, 30.65, 29.61, 21.58, 19.77, 13.74, 13.57; **MS (ESI):** *m/z* 396.12 $(M + H)^+$; **TLC:** R_f = 0.52 (DCM:MeOH, 100:2).

1.1.6.8. ***N-butyl-2-(butylthio)-6-(pyridin-3-yl)quinazolin-4-amine*** **(14)**

The title compound was synthesized according to the general procedure by arylation of *N*-butyl-2-(butylthio)-6-bromoquinazolin-4-amine (**4f**) using 3-pyridinylboronic acid. Physical form: yellow powder; Yield= 72%; m.p.= 89-90 ºC; **^{1}H-NMR (DMSO-*d*$_6$, δ [ppm]):** 9.05 (d, *J* = 1.8 Hz, 1H), 8.63 – 8.52 (m, 2H), 8.48 (t, *J* = 5.1 Hz, 1H), 8.22 – 8.15 (m, 1H), 8.07 (dd, *J* = 8.7, 1.9 Hz, 1H), 7.57 (d, *J* = 8.6 Hz, 1H), 7.53 (dd, *J* = 7.9, 4.7 Hz, 1H), 3.54 (dd, *J* = 12.7, 6.8 Hz, 2H), 3.12 (t, *J* = 7.4 Hz, 2H), 1.66 (tt, *J* = 15.1, 7.4 Hz, 4H), 1.49 – 1.32 (m, 4H), 0.99 – 0.85 (m, 6H); **^{13}C-NMR (DMSO-*d*$_6$, δ [ppm]):** 167.22, 158.68, 149.47, 148.46, 147.68, 134.60, 133.87, 132.50, 131.17, 126.77, 123.81, 120.94, 113.16, 40.32, 31.74, 30.62, 29.61, 21.58, 19.76, 13.74, 13.57; **MS (ESI):** *m/z* 367.13 $(M + H)^+$; **TLC:** R_f = 0.28 (DCM:MeOH, 100:2).

1.1.6.9. ***N-butyl-2-(butylthio)-6-(pyridin-4-yl)quinazolin-4-amine*** **(15)**

The title compound was synthesized according to the general procedure by arylation of *N*-butyl-2-(butylthio)-6-bromoquinazolin-4-amine (**4f**) using 4-pyridinylboronic acid. Physical form: yellow powder; Yield= 65%; m.p.= 160-162 ºC; **^{1}H-NMR (DMSO-*d*$_6$, δ [ppm]):** 8.69 – 8.68 (m, 1H), 8.67 (d, *J* = 1.6 Hz, 1H), 8.66 (d, *J* = 2.0 Hz, 1H), 8.55 (t, *J* = 5.5 Hz, 1H), 8.13 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.83 (d, *J* = 1.7 Hz, 1H), 7.82 (d, *J* = 1.7 Hz, 1H), 7.58 (d, *J* = 8.7 Hz, 1H), 3.55 (dd, *J* = 12.8, 7.1 Hz, 2H), 3.16 – 3.08 (m, 2H), 1.72 – 1.59 (m, 4H), 1.48 – 1.35 (m, 4H), 0.96 – 0.89 (m, 6H); **^{13}C-NMR (DMSO-*d*$_6$, δ [ppm]):** 167.79, 158.74, 150.24, 150.20, 145.92, 132.35, 130.88, 126.81, 121.38, 120.89, 113.10, 40.33, 31.73, 30.64, 29.63, 21.58, 19.76, 13.73, 13.56; **MS (ESI):** *m/z* 367.14 $(M + H)^+$; **TLC:** R_f = 0.19 (DCM:MeOH, 100:2).

1.1.6.10. ***N-butyl-2-(butylthio)-6-(1H-pyrazol-4-yl)quinazolin-4-amine*** **(16)**

The title compound was synthesized according to the general procedure by arylation of *N*-butyl-2-(butylthio)-6-bromoquinazolin-4-amine (**4f**) using 1*H*-pyrazole-4-boronic acid. Physical form: beige powder; Yield= 37%; m.p.= 237-238 ºC; **^{1}H-NMR (DMSO-*d*$_6$, δ [ppm]):** 13.00 (s, 1H), 8.38 (d, *J* = 1.8 Hz, 1H), 8.24 (t, *J* = 5.5 Hz, 2H), 8.01 (t, *J* = 5.3 Hz, 1H), 7.94 (dd, *J* = 8.6, 1.9 Hz, 1H), 7.46 (d, *J* = 8.6 Hz, 1H), 3.54 (dd, *J* = 12.8, 7.0 Hz, 2H), 3.14 – 3.08 (m, 2H), 1.72 – 1.66 (m, 2H), 1.66 – 1.61 (m, 2H), 1.44 (dt, *J* = 11.8, 5.9 Hz, 2H), 1.41 – 1.34 (m, 2H), 0.94 (t, *J* = 5.3 Hz, 3H), 0.92 (t, *J* = 5.3 Hz, 3H); **^{13}C-NMR (DMSO-*d*$_6$, δ [ppm]):** 165.69, 158.48, 148.15, 136.27, 130.51, 128.97,

126.34, 125.49, 120.78, 117.84, 113.13, 40.23, 31.76, 30.76, 29.55, 21.58, 19.78, 13.75, 13.57; **MS (ESI):** *m/z* 356.09 $(M + H)^+$; **TLC:** R_f= 0.17 (DCM:MeOH, 4:100).

1.1.7. **Synthesis of *thieno[2,3-d]pyrimidine-2,4(1H,3H)-dithione* (18)**

2-Aminothiophene-3-carbonitrile **17** (10 mmol) was added to 10 mL carbon disulfide and 10 mL pyridine. The reaction mixture was heated at 70 ºC for 6 hours. The mixture was then poured on ice water and the solid precipitate was filtered under vaccum. Compound **18** is a reported compound.[4, 5]

1.1.8. **Synthesis of *2,4-bis(butylthio)thieno[2,3-d]pyrimidine* (19)**

A mixture of compound **18** (5mmol) and potassium carbonate (15 mmol) was dissolved in acetone (10 mL). 1-Bromobutane was then added in excess (20 mmol). The reaction was left to stir overnight under reflux. Afterwards, acetone was evaporated under vacuum. The residue was then extracted with DCM (3 × 20 mL), the combined organic layers were passed over anhydrous $MgSO_4$. The DCM was then evaporated under reduced pressure and the remaining residue was purified using column chromatography to give compound **19**. Physical form: yellow semisolid; Yield= 88%; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 7.70 (d, *J* = 6.0 Hz, 1H), 7.31 (d, *J* = 6.0 Hz, 1H), 3.31 – 3.26 (m, 2H), 3.19 – 3.15 (m, 2H), 1.71 – 1.67 (m, 2H), 1.67 – 1.62 (m, 2H), 1.46 – 1.42 (m, 2H), 1.42 – 1.37 (m, 2H), 0.90 (td, *J* = 7.4, 1.9 Hz, 6H); **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 166.07, 165.09, 163.77, 125.51, 124.00, 118.74, 31.09, 30.89, 30.05, 28.22, 21.48, 21.43, 13.52, 13.45; **MS (ESI):** *m/z* 313.06 $(M + H)^+$; **TLC:** R_f = 0.67 (DCM:Hexane, 2:1).

1.1.9. **Synthesis of *N-butyl-2-(butylthio)thieno[2,3-d]pyrimidin-4-amine* (20)**

The compound **19** was refluxed with n-butylamine (10 mL) overnight. After completion of the reaction (monitored by TLC), the n-butylamine was evaporated under vacuum. The remaining residue was then extracted with DCM (3 × 20 mL), then the combined organic layers were passed over anhydrous $MgSO_4$. The DCM was then evaporated under reduced pressure and the remaining residue was purified using column chromatography to give compound **20**. Physical form: beige solid; Yield= 89%; m.p.= 52-54 ºC; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 8.02 (t, *J* = 5.6 Hz, 1H), 7.50 (d, *J* = 6.0 Hz, 1H), 7.34 (d, *J* = 6.0 Hz, 1H), 3.48 – 3.43 (m, 2H), 3.09 – 3.05 (m, 2H), 1.68 – 1.61 (m, 2H), 1.61 – 1.54 (m, 2H), 1.45 – 1.38 (m, 2H), 1.38 – 1.31 (m, 2H), 0.90 (q, *J* = 7.3 Hz, 6H); **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 166.16, 165.56, 156.16, 120.13, 119.23, 113.16, 39.97, 31.59, 30.92, 29.76, 21.55, 19.68, 13.70, 13.55; **MS (ESI):** *m/z* 296.03 $(M + H)^+$; **TLC:** R_f = 0.25 (DCM:Hexane, 2:1).

1.1.10. **Synthesis of *2,7-dihydro-4H-pyrazolo[3,4-d]pyrimidine-4,6(5H)-dithione* (22)**

Compound **22** is a reported compound.[6, 7]

1.1.11. **Synthesis of *4,6-bis(methylthio)-2H-pyrazolo[3,4-d]pyrimidine* (23a)**

A mixture of compound **22** (5mmol) and potassium carbonate (15 mmol) was dissolved in acetone (10 mL). Methyliodide was then added in excess (20 mmol). The reaction was left to stir overnight under reflux. Afterwards, acetone was evaporated under vacuum. The residue was then extracted with DCM (3 × 20 mL), the combined organic layers were passed over anhydrous $MgSO_4$. The DCM was then evaporated under reduced pressure and the remaining residue was purified using column chromatography, to give compound **23a**. Physical form: white powder; Yield= 68%; m.p.= 163-165 ºC; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 13.83 (s, 1H), 8.17 (s, 1H), 2.66 (s, 3H), 2.57 (s, 3H); **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 168.15, 164.84, 153.56, 132.88, 108.82, 14.23, 11.95; **MS (ESI):** *m/z* 213.06 $(M + H)^+$; **TLC:** R_f = 0.29 (DCM:MeOH, 100:2).

1.1.12. Synthesis of compounds **23a-c** and **24**

A mixture of compound **22** (5mmol) and potassium carbonate (15 mmol) was dissolved in acetone (10 mL). n-Butylbromide was then added in excess (20 mmol). The reaction was left to stir overnight under reflux. Afterwards, acetone was evaporated under vacuum. The residue was then extracted with DCM (3 × 20 mL), the combined organic layers were passed over anhydrous $MgSO_4$. The DCM was then evaporated under reduced pressure and the remaining residue was purified using column chromatography, to give compounds **23b, 23c** and **24** (as a mixture).

1.1.12.1. ***4,6-Bis(butylthio)-2H-pyrazolo[3,4-d]pyrimidine*** **(23b)**

The title compound was synthesized according to the procedure by alkylation of 2,7-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidine-4,6(5*H*)-dithione (**22**) using n-butylbromide. Physical form: white powder; Yield= 20%; m.p.= 123-124 ºC; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 13.82 (s, 1H), 8.17 (s, 1H), 3.35 (t, *J* = 7.3 Hz, 2H), 3.20 (t, *J* = 7.3 Hz, 2H), 1.78 – 1.65 (m, 4H), 1.54 – 1.47 (m, 2H), 1.47 – 1.39 (m, 2H), 0.95 (t, *J* = 7.3 Hz, 6H); **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 167.66, 164.38, 153.57, 132.82, 109.01, 31.55, 31.45, 30.45, 28.35, 22.01, 21.87, 14.01, 13.92; **MS (ESI):** *m/z* 297.03 $(M + H)^+$; **TLC:** R_f = 0.44 (EtOAc:Hexane, 1:4).

1.1.12.2. ***2-Butyl-4,6-bis(butylthio)-2H-pyrazolo[3,4-d]pyrimidine*** **(23c)**

The title compound was synthesized according to the procedure by alkylation of 2,7-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidine-4,6(5*H*)-dithione (**22**) using n-butylbromide. Physical form: yellowish semisolid; Yield= 32%; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 8.64 (s, 1H), 4.33 (t, *J* = 6.9 Hz, 2H), 3.30 (dd, *J* = 13.8, 6.6 Hz, 2H), 3.13 (t, *J* = 7.3 Hz, 2H), 1.92 – 1.80 (m, 2H), 1.73 – 1.61 (m, 4H), 1.50 – 1.42 (m, 2H), 1.42 – 1.35 (m, 2H), 1.29 – 1.22 (m, 1H), 1.22 – 1.15 (m, 1H), 0.89 (q, *J* = 7.5 Hz, 9H); **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 166.68, 166.20, 158.21, 125.85, 108.87, 53.31, 31.94, 31.58, 31.36, 30.26, 28.38, 22.03, 21.84, 19.53, 14.02, 13.91, 13.78; **MS (ESI):** *m/z* 353.16 $(M + H)^+$; **TLC:** R_f = 0.46 (EtOAc:Hexane, 1:4).

1.1.12.3. ***1-Butyl-4,6-bis(butylthio)-1H-pyrazolo[3,4-d]pyrimidine*** **(24)**

The title compound was synthesized according to the procedure by alkylation of 2,7-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidine-4,6(5*H*)-dithione (**22**) using n-butylbromide. Physical form: yellow semisolid; Yield= 34%; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 8.14 (s, 1H), 4.30 (t, *J* = 6.8 Hz, 2H), 3.31 (dd, *J* = 10.2, 4.2 Hz, 2H), 3.21 – 3.13 (m, 2H), 1.86 – 1.74 (m, 2H), 1.70 (dd, *J* = 8.5, 2.8 Hz, 2H), 1.66 (dd, *J* = 9.7, 5.1 Hz, 2H), 1.50 – 1.43 (m, 2H), 1.42 – 1.35 (m, 2H), 1.26 – 1.12 (m, 2H), 0.96 – 0.82 (m, 9H); **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 167.72, 164.71, 151.71, 131.94, 109.34, 46.51, 31.72, 31.42, 31.33, 30.49, 28.38, 22.06, 21.85, 19.65, 13.97, 13.91, 13.74; **MS (ESI):** *m/z* 353.17 $(M + H)^+$; **TLC:** R_f = 0.5 (EtOAc:Hexane, 1:20).

1.1.13. Synthesis of compounds **25a-c** and **26**

The corresponding pyrazolo[3,4-*d*]pyrimidine derivative **(23a-c** and **24**) was refluxed with n-butylamine (10 mL) overnight. After completion of the reaction (monitored by TLC), the n-butylamine was evaporated under vacuum. The remaining residue was then extracted with DCM (3 × 20 mL), the combined organic layers were passed over anhydrous $MgSO_4$. The DCM was then evaporated under reduced pressure and the remaining residue was purified using column chromatography to give compounds **25a-c** and **26**.

1.1.13.1. ***N-butyl-6-(methylthio)-2H-pyrazolo[3,4-d]pyrimidin-4-amine*** **(25a)**

The title compound was synthesized according to the general procedure by amination of 4,6-bis(methylthio)-2*H*-pyrazolo[3,4-*d*]pyrimidine (**23a**) using n-butylamine. Physical form: beige solid; Yield= 92%; m.p.= 208-210 ºC; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 13.09 (s, 1H), 8.18 (t, *J* = 5.3 Hz, 1H), 8.02 (s, 1H), 3.46 (dd, *J* = 12.7, 6.9 Hz, 2H), 2.46 (s, 3H), 1.64 – 1.52 (m, 2H), 1.37 (dq, *J* = 14.3, 7.2 Hz, 2H), 0.96 – 0.88 (m, 3H); **^{13}C-NMR (DMSO-d_6, δ [ppm]):**

168.61, 155.85, 155.63, 132.73, 98.19, 31.40, 20.13, 14.18, 13.98; **MS (ESI):** *m/z* 238.16 $(M + H)^+$; **TLC:** R_f = 0.37 (DCM:MeOH, 100:4).

1.1.13.2. ***N-butyl-6-(butylthio)-2H-pyrazolo[3,4-d]pyrimidin-4-amine*** **(25b)**

The title compound was synthesized according to the general procedure by amination of 4,6-bis(butylthio)-2*H*-pyrazolo[3,4-*d*]pyrimidine (**23b**) using n-butylamine. Physical form: white powder; Yield= 85%; m.p.= 160-161 ºC; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 13.13 (s, 1H), 8.19 (t, *J* = 5.3 Hz, 1H), 8.00 (d, *J* = 1.0 Hz, 1H), 3.46 (dd, *J* = 12.7, 7.0 Hz, 2H), 3.16 – 3.00 (m, 2H), 1.67 (dd, *J* = 14.8, 7.2 Hz, 2H), 1.57 (dd, *J* = 14.5, 7.3 Hz, 2H), 1.44 (dd, *J* = 14.9, 7.5 Hz, 2H), 1.34 (dd, *J* = 14.3, 7.2 Hz, 2H), 0.97 – 0.92 (m, 3H), 0.91 – 0.86 (m, 3H); **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 168.18, 155.86, 155.55, 132.89, 98.24, 32.07, 31.47, 30.17, 22.07, 20.16, 14.17, 14.06; **MS (ESI):** *m/z* 280.15 $(M + H)^+$; **TLC:** R_f = 0.19 (DCM:MeOH, 100:2).

1.1.13.3. ***N,2-dibutyl-6-(butylthio)-2H-pyrazolo[3,4-d]pyrimidin-4-amine*** **(25c)**

The title compound was synthesized according to the general procedure by amination of 2-butyl-4,6-bis(butylthio)-2*H*-pyrazolo[3,4-*d*]pyrimidine **(23c**) using n-butylamine. Physical form: off white powder; Yield= 93%; m.p.= 128-130 ºC; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 8.21 (s, 1H), 8.13 (t, *J* = 5.3 Hz, 1H), 4.26 (t, *J* = 6.9 Hz, 2H), 3.45 (d, *J* = 6.9 Hz, 1H), 3.41 (d, *J* = 6.9 Hz, 1H), 3.09 – 2.98 (m, 2H), 1.89 – 1.75 (m, 2H), 1.72 – 1.61 (m, 2H), 1.56 (dd, *J* = 14.4, 7.2 Hz, 2H), 1.44 (dd, *J* = 15.2, 7.4 Hz, 2H), 1.34 (dd, *J* = 15.4, 7.6 Hz, 2H), 1.25 (t, *J* = 5.6 Hz, 1H), 1.23 – 1.16 (m, 1H), 0.92 (dt, *J* = 5.9, 2.9 Hz, 6H), 0.90 – 0.85 (m, 3H); **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 167.65, 160.50, 156.81, 124.92, 99.99, 52.72, 32.14, 32.06, 31.36, 29.98, 22.10, 20.16, 19.57, 14.17, 14.08, 13.85; **MS (ESI):** *m/z* 336.19 $(M + H)^+$; **TLC:** R_f = 0.29 (DCM:MeOH, 100:2).

1.1.13.4. ***N,1-dibutyl-6-(butylthio)-1H-pyrazolo[3,4-d]pyrimidin-4-amine*** **(26)**

The title compound was synthesized according to the general procedure by amination of 1-butyl-4,6-bis(butylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidine (**24**) using n-butylamine. Physical form: yellow semisolid; Yield= 90%; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 8.23 (t, *J* = 5.4 Hz, 1H), 7.99 (s, 1H), 4.19 (t, *J* = 6.8 Hz, 2H), 3.45 (dd, *J* = 12.7, 6.9 Hz, 2H), 3.12 – 3.01 (m, 2H), 1.82 – 1.71 (m, 2H), 1.66 (dd, *J* = 14.8, 7.5 Hz, 2H), 1.56 (dd, *J* = 14.6, 7.4 Hz, 2H), 1.44 (dd, *J* = 15.0, 7.4 Hz, 2H), 1.34 (dd, *J* = 15.0, 7.3 Hz, 2H), 1.27 – 1.20 (m, 1H), 1.19 – 1.12 (m, 1H), 0.97 – 0.91 (m, 3H), 0.90 (d, *J* = 5.1 Hz, 3H), 0.86 (t, *J* = 6.2 Hz, 3H); **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 168.23, 155.91, 153.69, 131.95, 98.56, 46.06, 32.27, 31.51, 31.45, 30.24, 22.12, 20.15, 19.67, 14.15, 14.00, 13.79; **MS (ESI):** *m/z* 336.19 $(M + H)^+$; **TLC:** R_f = 0.34 (EtOAc:Hexane, 1:4).

1.1.14. **Synthesis of *2,4-bis(butylthio)quinazolin-6-amine* (30)**

A mixture of the nitro derivative **29** (1 mmol) and Fe powder (10 mmol) was stirred at room temperature in ethanol/water (2:1), in the presence of 6 mmol conc. HCl, for 5 h. The reaction mixture was then refluxed for 1 h at 65 ºC. The reaction mixture was then filtered and the ethanol was evaporated. The remaining solution was justified at pH 9 using ammonia and extracted with methylene chloride (mobile phase: 0.5% methanol in methylene chloride). Yield = 70%; **^{1}H-NMR (DMSO-d_6, δ [ppm]):** 7.48 – 7.44 (m, 1H), 7.24 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.90 – 6.86 (m, 1H), 5.82 (s, 2H), 3.29 – 3.25 (m, 2H), 3.17 – 3.13 (m, 2H), 1.71 – 1.67 (m, 2H), 1.67 – 1.63 (m, 2H), 1.47 – 1.43 (m, 2H), 1.43 – 1.39 (m, 2H), 0.92 (td, *J* = 7.4, 4.4 Hz, 6H). **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 166.67, 159.63, 147.18, 141.46, 127.66, 125.51, 122.15, 100.92, 31.39, 30.76, 29.71, 28.28, 21.50, 21.48, 13.54, 13.48; **MS (ESI):** *m/z* 322.31 $(M + H)^+$.

1.1.15. **Synthesis of *1-(2,4-bis(butylthio)quinazolin-6-yl)-3-ethylurea* (31)**

A mixture of the amino derivative **30** and ethyl isocyanate (2 equivalent) in DMF was stirred at room temperature for 2 hours. The reaction mixture was then poured onto ice-cooled water, and then extracted with ethyl acetate. Yield = 73%; **^{1}H-NMR ($CDCl_3$, δ [ppm]):** 8.94 (s, 1H), 8.32 – 8.27 (m, 1H), 7.72 – 7.69 (m, 1H), 7.64 – 7.59 (m, 1H), 6.25 (t, *J* = 5.6 Hz, 1H), 3.31 – 3.27 (m, 2H), 3.21 – 3.16 (m, 2H), 3.14 (tt, *J* = 7.2, 3.5 Hz, 2H), 1.73 – 1.69 (m, 2H), 1.69 – 1.64 (m, 2H), 1.49 – 1.44 (m, 2H), 1.44 – 1.38 (m, 2H), 1.10 – 1.05 (m, 3H), 0.92 (td, *J* = 7.4, 4.0 Hz, 6H). **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 169.01, 162.75, 154.92, 143.72, 138.53, 127.32, 126.96, 121.09, 34.01, 31.27, 30.58, 29.78, 28.47, 21.49, 21.47, 15.35, 13.52, 13.46; **MS (ESI):** *m/z* 393.22 $(M + H)^+$.

1.1.16. **Synthesis of *N-(2,4-bis(butylthio)quinazolin-6-yl)ethanesulfonamide* (32)**

A mixture of the amino derivative **30** and ethanesulfonylchloride (2 equivalent) in pyridine was stirred at room temperature for 2 hours. The reaction solvent was then evaporated. Yield = 88%; **^{1}H-NMR ($CDCl_3$, δ [ppm]):** 10.28 (s, 1H), 7.78 (dd, *J* = 1.7, 1.3 Hz, 1H), 7.73 (d, *J* = 0.7 Hz, 1H), 7.72 (d, *J* = 4.1 Hz, 1H), 3.30 (d, *J* = 7.4 Hz, 2H), 3.22 – 3.15 (m, 4H), 1.74 – 1.69 (m, 2H), 1.67 (dd, *J* = 11.8, 4.5 Hz, 2H), 1.48 – 1.44 (m, 2H), 1.44 – 1.40 (m, 2H), 1.20 (t, *J* = 7.3 Hz, 3H), 0.92 (td, *J* = 7.4, 5.0 Hz, 6H). **^{13}C-NMR (DMSO-d_6, δ [ppm]):** 169.57, 164.33, 144.96, 136.27, 128.41, 127.57, 120.94, 110.42, 45.36, 31.16, 30.55, 29.83, 28.59, 21.48, 21.47, 13.52, 13.45, 8.00; **MS (ESI):** *m/z* 414.25 $(M + H)^+$.

1.2. γ-Irradiation of the synthesized compounds

The tested compounds (**4a**, **7** and **25b**), in solid state, were collected in polypropylene vials wrapped with an aluminium sheet. The vials were subjected to γ-irradiation at an absorbed dose of 25 kGy. Irradiation was achieved utilizing a ^{60}Co source at a dose rate of 0.950 kGy/h, using Indian-Gamma Cell (Ge 4000 A).

1.3. Antibacterial assays

All bacterial isolates (*S. aureus*, *S. pneumoniae*, *E. faecalis, E. faecium, E. coli* and *M. smegmatis*) were handled under conditions recommended by the depositor. MIC values were determined by 2-fold dilutions of tested compounds according to guidelines of the Clinical & Laboratory Standards Institute (CLSI). Briefly, serial dilutions of tested compounds (0.06-128 µg/mL) were prepared in a sterile 96-well plate. Single colonies of bacterial strains were suspended in 0.9% NaCl and McFarland was adjusted to 0.5 using a Densitometer. The bacterial suspension was diluted 1:100 in the corresponding growth media to achieve a final inoculum of ~10^4 CFU/mL. Growth inhibition and MIC determination was performed after overnight incubation (18-24 h) at optimum temperature.

1.4. Cytotoxicity evaluation

HepG2 cells (hepatocellular carcinoma cell line-ACC 180) were cultured under conditions recommended by the depositor. Briefly, serial dilution of tested compounds was performed and transferred to already seeded cells (6 x 10^3 cells/well) in a 96-well plate. After 5 days of incubation, 20 µL of 5 mg/ml MTT (thiazolyl blue tetrazolium bromide) in PBS was added per well and cells were further incubated for 2 h at 37°C. The medium was then discarded and cells were washed with 100 µL PBS before adding 100 µL 2-propanol/10 N HCl (250:1) in order to dissolve formazan granules. The absorbance at 570 nm was measured using a microplate reader (Tecan Infinite M200Pro), and cell viability was expressed as percentage relative to the respective methanol control. IC_{50} values were determined as average value (SD <10%).

Table S1: MIC values of the synthesized analogs in μM concentration units.

Compound*	Minimum Inhibitory Concentration[a] (μM)									
	S. aureus			*S. pneumoniae*		*E. faecium* DSM-20477	*E. faecalis* DSM-20478	*E. coli*		*M. smegmatis* mc^{2}155
	Newman	N315 (MRSA)	Mu50 (MRSA/ VISA)	DSM-20566	DSM-11865 (PRSP)			DSM-1116	K12 Δ*tolC*	
9	20	20	20	10	39	20	20	>313	>313	>313
12	5	>336	>336	3	42	11	5	>336	>336	42
20	27	108	>433	27	27	>433	>433	>433	27	54
25a	270	270	270	>539	>539	>539	>539	>539	270	>539
25b	>458	>458	>458	>458	>458	>458	>458	>458	14	>458
26	>382	>382	>382	24	>382	>382	>382	>382	>382	>382
30	25	12	12	12	25	25	100	>398	>398	100
32	>310	>310	>310	10	10	19	19	>310	>310	>310
I	28	28	14	14	14	14	14	>442	7	28
Vancomycin	0.34	0.69	6	0.34	0.69	0.69	0.34	> 44)	> 44	6
Linezolid	3	6	6	2	6	12	6	> 190	47	12

[a] Values are from two independent experiments.

* Values were calculated from their corresponding values in "μg/mL". Values were rounded for simplification.

2D Spectra

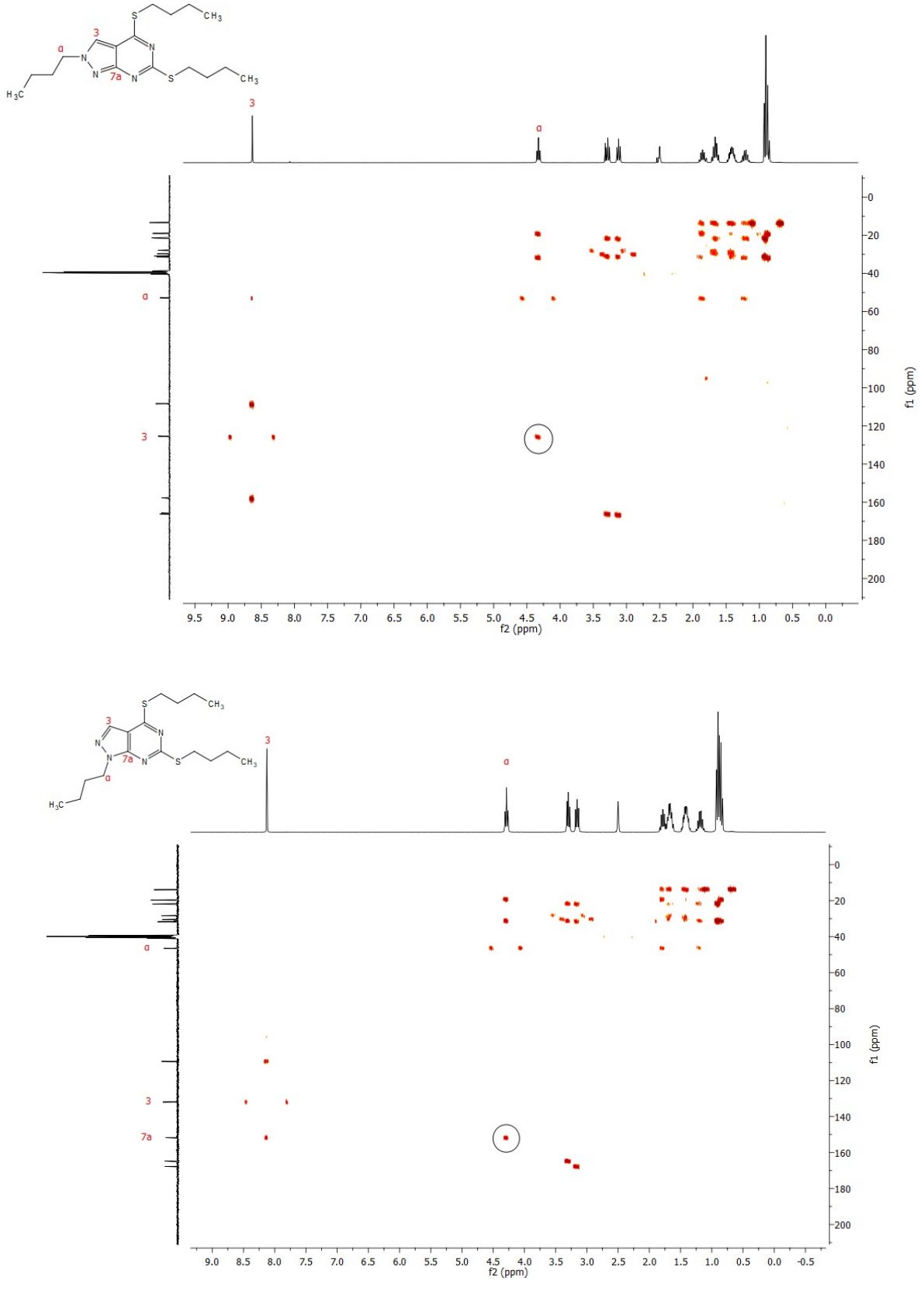

Figure S1: 2D-HMBC spectra of compounds **23c** and **24**

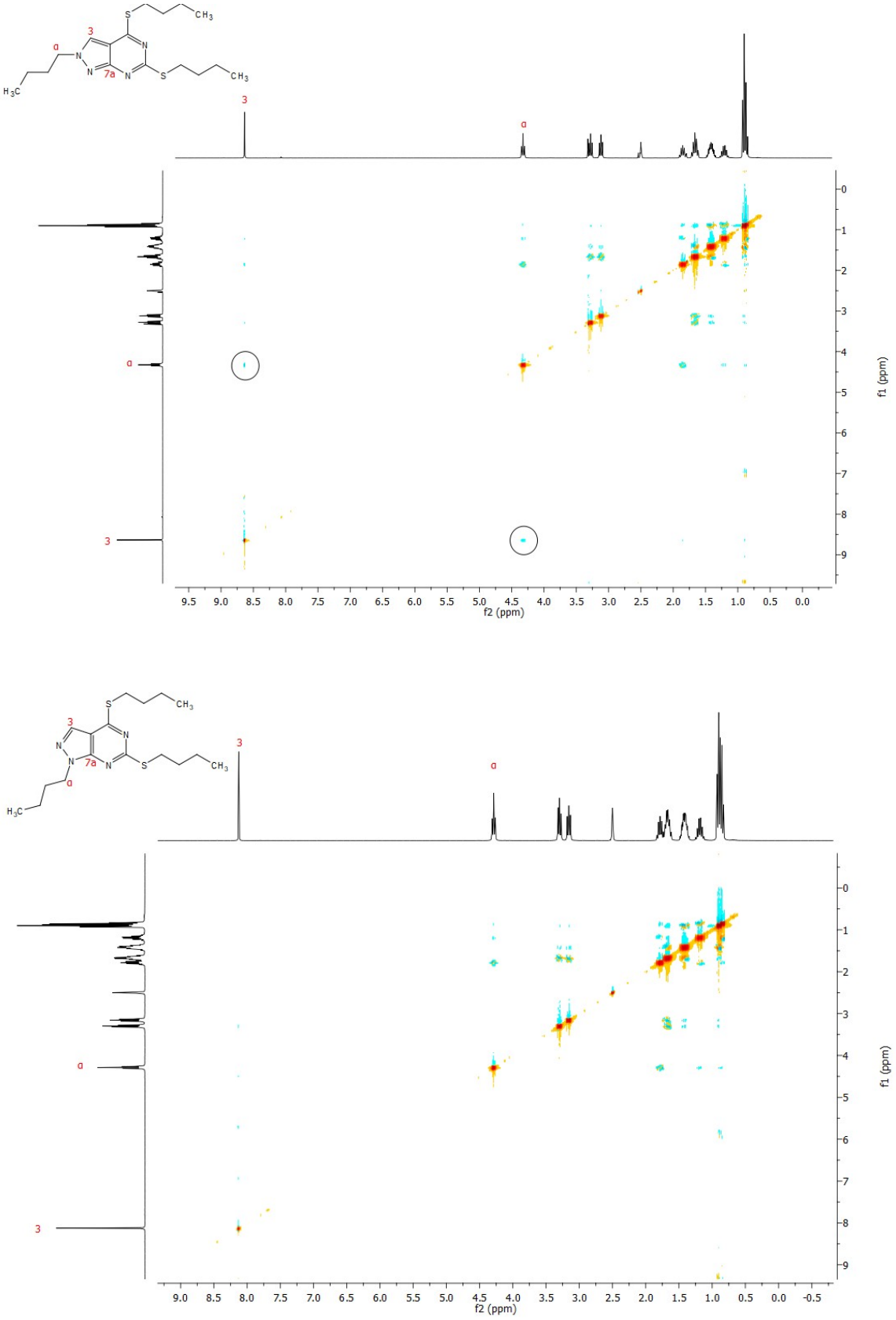

Figure S2: 2D-NOESY spectra of compounds **23c** and **24**

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