Supporting Information (1, 2, 3)

Synthesis of tetrazole compounds as novel type of potential antimicrobial agents and their synergistic effects with clinical drugs and interaction with calf thymus DNA

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Supporting Information 1

1. General methods

Melting points were recorded on X-6 melting point apparatus and uncorrected. TLC analysis was done using pre-coated silica gel plates. FT-IR spectra were carried out on Bruker RFS100/S spectrophotometer (Bio-Rad, Cambridge, MA, USA) using KBr pellets in the 400–4000 cm^{-1} range. NMR spectra were recorded on a Bruker AV 300 or Varian 400 spectrometer using TMS as an internal standard. The chemical shifts were reported in parts per million (ppm), the coupling constants (*J*) were expressed in hertz (Hz) and signals were described as singlet (s), doublet (d), triplet (t), as well as multiplet (m). The following abbreviations were used to designate aryl groups: Tet, tetrazolyl; Ph, phenyl. The mass spectra were recorded on LCMS-2010A and the high-resolution mass spectra (HRMS) were recorded on an IonSpec FT-ICR mass spectrometer with TOF resource.

2. Spectral data of the prepared compounds

*2.1 General procedures for the preparation of intermediates **2a–c***

The intermediates **2a–c** were prepared according to the previously reported methods [1].

*2.2 2-(Hexyl(phenyl)amino)acetonitrile (**3a**)*

A mixture of compound **2a** (3.000 g, 0.023 mol), bromohexane (3.795 g, 0.023 mol) and potassium carbonate (3.456 g, 0.025 mol) in ethanol (25 mL) was refluxed. Upon the completion of reaction, the solvent was evaporated, and water (30 mL) was added. The residue was extracted with ethyl acetate (3 × 20 mL), and the organic phase was dried over anhydrous sodium sulphate and further purified by silica gel column chromatography (eluent, petroleum ether/ethyl acetate (15/1, V/V)) to afford the desired compound **3a** as colorless liquid. Yield: 48%. IR (KBr, cm^{-1}): 3021, 2956, 2896, 1626, 1573, 1519, 1509, 1385, 751, 700; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.21 (d, 2H, *J* = 7.5 Hz, Ph-3,5-*H*), 7.01–6.97 (m, H, Ph-4-*H*), 6.93 (d, 2H, *J* = 7.5 Hz, Ph-2,6-*H*), 4.13 (s, 2H, CH_2CN), 3.36–3.32 (t, 2H, *J* = 6.0 Hz, C$H_2C_4H_8$), 1.32–1.28 (m, 8H, $CH_2C_4H_8$), 0.88–0.85 (t, 3H, *J* = 4.5 Hz, CH_2CH_3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 150.19, 129.60, 117.23, 116.27, 114.18, 48.97, 42.13, 31.05, 28.51, 26.59, 21.37, 13.96 ppm; MS (*m/z*): 217 $[M+H]^+$; HRMS (TOF) calcd. for $C_{14}H_{20}N_2$: $[M+H]^+$, 217.1626; found, 217.1623.

*2.3 2-(Octyl(phenyl)amino)acetonitrile (**3b**)*

Compound **3b** was prepared according to the experimental procedure for compound **3a**, starting from compound **2a** (3.000 g, 0.023 mol), bromooctane (4.435 g, 0.023 mol) and potassium carbonate (3.450 g, 0.025 mol). The desired compound **3b** was obtained as colorless oil. Yield: 50%. IR (KBr, cm^{-1}): 3029, 2913, 2838, 1626, 1569, 1533, 1509, 1457, 1383, 751, 699; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.20 (d, 2H, *J* = 7.5 Hz, Ph-3,5-*H*), 7.03–7.00 (m, H, Ph-4-*H*), 6.95 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 4.15 (s, 2H, CH_2CN), 3.43-3.39 (t, 2H, *J* = 6.0 Hz, C$H_2C_6H_{12}CH_3$), 1.34−1.29 (m, 12H, $C_6H_{12}CH_3$), 0.89–0.86 (m, 3H, CH_2CH_3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 150.25, 129.51, 117.32, 116.31, 114.24, 48.89, 42.21, 31.33, 28.47, 28.16, 26.79, 21.37, 13.99 ppm; MS (*m/z*): 245 $[M+H]^+$; HRMS (TOF) calcd. for $C_{16}H_{24}N_2$: $[M+H]^+$, 245.1939; found, 245.1921.

*2.4 2-(Decyl(phenyl)amino)acetonitrile (**3c**)*

Compound **3c** was prepared according to the experimental procedure for compound **3a**, starting from compound **2a**

(2.500 g, 0.018 mol), bromodecane (3.975 g, 0.018 mol) and potassium carbonate (2.981 g, 0.022 mol). The desired compound **3c** was obtained as colorless oil. Yield: 44%. IR (KBr, cm^{-1}): 3019, 2935, 2874, 1624, 1567, 1513, 1463, 1385, 747, 696; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.21 (s, 2H, Ph-3,5-*H*), 6.99–6.95 (m, 3H, Ph-2,4,6-*H*), 4.13 (s, 2H, C*H*$_2$CN), 3.43–3.41 (t, 2H, *J* = 3.0 Hz, C*H*$_2$C$_8$H$_{16}$CH$_3$), 1.78–1.74 (m, 2H, C*H*$_2$C$_7$H$_{14}$C*H*$_3$), 1.31–1.29 (m, 14H, C$_7$*H*$_{14}$C*H*$_3$), 0.85–0.82 (t, 3H, *J* = 4.5 Hz, CH$_2$C*H*$_3$) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 150.23, 129.55, 117.36, 116.34, 114.26, 48.81, 42.25, 31.37, 28.47, 28.16, 26.77, 21.35, 14.02 ppm; MS (*m/z*): 245 $[M+H]^+$; HRMS (TOF) calcd. for $C_{18}H_{28}N_2$: $[M+H]^+$, 273.2252; found, 273.2261.

*2.5 2-(Dodecyl(phenyl)amino)acetonitrile (**3d**)*

Compound **3d** was prepared according to the experimental procedure for compound **3a**, starting from compound **2a** (2.000 g, 0.015 mol), bromododecane (3.735 g, 0.015 mol) and potassium carbonate (2.501 g, 0.022 mol). The desired compound **3d** was obtained as colorless oil. Yield: 41%. IR (KBr, cm^{-1}): 3034, 2913, 2840, 1622, 1607, 1573, 1459, 1383, 750, 699; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.21 (d, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 7.04–7.00 (m, H, Ph-4-*H*), 6.97 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 4.14 (s, 2H, C*H*$_2$CN), 3.45-3.40 (t, 2H, *J* = 7.5 Hz, C*H*$_2$C$_{10}$H$_{20}$C*H*$_3$), 1.78–1.72 (m, 4H, C$_2$*H*$_4$C$_8$H$_{16}$CH$_3$), 1.35–1.29 (m, 16H, C$_8$*H*$_{16}$CH$_3$), 0.86–0.84 (t, 3H, *J* = 3.0 Hz, CH$_2$C*H*$_3$) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 150.34, 129.62, 117.30, 116.27, 114.35, 48.77, 42.35, 31.35, 28.54, 28.17, 26.80, 21.37, 13.97 ppm; MS (*m/z*): 301 $[M+H]^+$; HRMS (TOF) calcd. for $C_{20}H_{32}N_2$: $[M+H]^+$, 301.2565; found, 301.2531.

*2.6 N-((1H-Tetrazol-5-yl)methyl)-N-hexylaniline (**4a**)*

To a solution of compound **3a** (600 mg, 2.8 mmol) in DMF was added sodium azide (181 mg, 2.8 mmol) and ammonium chloride (1.487 g, 28 mmol), the mixture was stirred at 120 °C. After the reaction came to the end (monitored by TLC), solvents were removed under reduced pressure, the residue dissolved in 50 mL of water and acidified with concentrated hydrochloric acid to pH 2. The solution was cooled to 5 °C in an ice bath to give precipitate, and then the precipitate was recrystallized from ethanol to afford compound **4a** as yellow oil. Yield: 72%. IR (KBr, cm^{-1}): 3342, 3021, 2961, 2840, 1614, 1563, 1546, 1505, 1383, 750, 700; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.17 (t, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 6.72 (m, 1H, Ph-4-*H*), 6.65 (d, 2H, *J* = 4.5 Hz, Ph-2,6-*H*), 5.04 (s, 2H, Tet-C*H*$_2$), 3.38–3.35 (t, 2H, *J* = 4.5 Hz, C*H*$_2$C$_4$H$_8$), 1.35–1.29 (m, 8H, CH$_2$C$_4$*H*$_8$), 0.87–0.83 (t, 3H, *J* = 6.0 Hz, CH$_2$C*H*$_3$) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.32, 150.16, 129.68, 117.27, 116.39, 52.78, 48.99, 42.21, 31.12, 28.45, 26.50, 21.31, 14.05 ppm; MS (*m/z*): 260 $[M+H]^+$; HRMS (TOF) calcd. for $C_{14}H_{21}N_5$: $[M+H]^+$, 260.1797; found, 260.1791.

*2.7 N-((1H-Tetrazol-5-yl)methyl)-N-octylaniline (**4b**)*

Compound **4b** was prepared according to the experimental procedure for compound **4a**, starting from compound **3b** (600 mg, 2.4 mmol), sodium azide (159 mg, 2.4 mmol) and ammonium chloride (1.311 g, 24 mmol). The desired compound **4b** was obtained as yellow oil. Yield: 70%. IR (KBr, cm^{-1}): 3414, 3034, 2976, 2845, 1608, 1559, 1505, 1457, 1382, 750, 699; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.15 (t, 2H, *J* = 3.0 Hz, Ph-3,5-*H*), 6.70 (t, 1H, *J* = 4.5 Hz, Ph-4-*H*), 6.61 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 5.03 (s, 2H, Tet-C*H*$_2$), 3.42–3.39 (t, 2H, *J* = 4.5 Hz, C*H*$_2$C$_6$H$_{12}$CH$_3$), 1.36−1.28 (m, 12H, C$_6$*H*$_{12}$C*H*$_3$), 0.86−0.81 (m, 3H, CH$_2$C*H*$_3$) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.27, 150.19, 129.65, 117.22, 116.43, 52.75, 48.93, 31.09, 28.45, 26.52, 21.32, 14.04 ppm; MS (*m/z*): 288 $[M+H]^+$; HRMS (TOF) calcd. for $C_{16}H_{25}N_5$: $[M+H]^+$, 288.2110; found, 288.2107.

*2.8 N-((1H-Tetrazol-5-yl)methyl)-N-decylaniline (**4c**)*

Compound **4c** was prepared according to the experimental procedure for compound **4a**, starting from compound **3c** (800 mg, 2.9 mmol), sodium azide (191 mg, 2.9 mmol) and ammonium chloride (1.572 g, 29 mmol). The desired compound **91d** was obtained as yellow oil. Yield: 68%. IR (KBr, cm^{-1}): 3416, 3021, 2983, 2839, 1611, 1599, 1575, 1459, 1385, 749, 700; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.18 (t, 2H, *J* = 6.0 Hz, Ph-3,5-*H*), 6.75 (t, 1H, *J* = 7.5 Hz, Ph-4-*H*), 6.62 (d, 2H, *J* = 3.0 Hz, Ph-2,6-*H*), 5.04 (s, 2H, Tet-C*H*$_2$), 3.45–3.41 (t, 2H, *J* = 6.0 Hz, C*H*$_2$C$_8$H$_{16}$CH$_3$), 1.80–1.75 (m, 2H, C*H*$_2$C$_7$H$_{14}$C*H*$_3$), 1.33–1.28 (m, 14H, C$_7$*H*$_{14}$C*H*$_3$), 0.86–0.82 (t, 3H, *J* = 6.0 Hz, CH$_2$C*H*$_3$) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.20, 150.11, 129.69, 117.27, 116.49, 52.79, 48.87, 31.42, 28.53, 28.19, 26.79, 21.38,

14.03 ppm; MS (*m/z*): 316 $[M+H]^+$; HRMS (TOF) calcd. for $C_{18}H_{29}N_5$: $[M+H]^+$, 316.2423; found, 316.2427.

*2.9 N-((1H-Tetrazol-5-yl)methyl)-N-dodecylaniline (**4d**)*

Compound **4d** was prepared according to the experimental procedure for compound **4a**, starting from compound **3d** (800 mg, 2.7 mmol), sodium azide (173 mg, 2.7 mmol) and ammonium chloride (1.426 g, 27 mmol). The desired compound **4d** was obtained as yellow oil. Yield: 73%. IR (KBr, cm^{-1}): 3434, 3019, 2979, 2845, 1609, 1559, 1459, 1383, 753, 702; ^1H NMR (300 MHz, $CDCl_3$): δ 7.16 (t, 2H, *J* = 3.0 Hz, Ph-3,5-*H*), 6.74 (t, 1H, *J* = 4.5 Hz, Ph-4-*H*), 6.60 (d, 2H, *J* = 6.0 Hz, Ph-2,6-*H*), 5.04 (s, 2H, Tet-CH_2), 3.47–3.43 (t, 2H, *J* = 6.0 Hz, C$H_2C_{10}H_{20}CH_3$), 1.82–1.74 (m, 4H, $C_2H_4C_8H_{16}CH_3$), 1.39–1.30 (m, 16H, $C_8H_{16}CH_3$), 0.89–0.83 (t, 3H, *J* = 9.0 Hz, CH_2CH_3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.34, 150.23, 129.72, 117.16, 116.53, 52.71, 48.82, 31.39, 28.58, 28.24, 26.87, 21.32, 14.06 ppm; MS (*m/z*): 344 $[M+H]^+$; HRMS (TOF) calcd. for $C_{20}H_{33}N_5$: $[M+H]^+$, 344.2736; found, 344.2730.

*2.10 2-((3,4-Dichlorobenzyl)(phenyl)amino)acetonitrile （**5a**）*

A mixture of compound **2a** (2.986 g, 0.023 mol), 2,4-dichloro-1-(chloromethyl) benzene (4.507 g, 0.023 mol) and potassium carbonate (3.456 g, 0.025 mol) in ethanol (25 mL) was refluxed for 12 h. Upon completion of the reaction, the solvent was evaporated, and water (30 mL) was added. The residue was extracted with ethyl acetate (3 × 20 mL), and the organic phase was dried over anhydrous sodium sulphate and further purified by silica gel column chromatography (eluent, petroleum ether/ethyl acetate (10/1, V/V)) to afford the desired compound **5a** as white solid. Yield: 53%; mp: 79–81 °C. IR (KBr, cm^{-1}): 3029, 2985, 2243, 1658, 1597, 1561, 1504, 1449, 762, 747, 689; ^1H NMR (300 MHz, $CDCl_3$): δ 7.44 (s, 1H, 2,4-2ClPh-2,5,6-*H*), 7.34–7.29 (m, 2H, 2,4-2ClPh-5,6-*H*), 7.22 (s, 2H, Ph-3,5-*H*), 6.98–6.84 (m, 3H, Ph-2,4,6-*H*), 4.58 (s, 2H, 2,4-2ClPh-CH_2), 4.17 (s, 2H, CH_2CN) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 146.90, 134.08, 132.84, 129.72, 129.65, 129.42, 127.45, 120.69, 115.70, 114.85, 114.80, 53.59, 40.13 ppm; MS (*m/z*): 291 and 293 $[M+H]^+$; HRMS (TOF) calcd. for $C_{15}H_{12}Cl_2N_2$: $[M+H]^+$, 291.0378; found, 291.0453 and 293.0439.

*2.11 2-((3,4-Dichlorobenzyl)(phenyl)amino)acetonitrile (**5b**)*

Compound **5b** was prepared according to the experimental procedure for compound **5a**, starting from compound **2a** (3.006 g, 0.023 mol), 3,4-dichloro-1-(chloromethyl)benzene (4.497 g, 0.023 mol) and potassium carbonate (3.456 g, 0.025 mol). The desired compound **5b** was obtained as white solid. Yield: 53.2%; mp: 76–78 °C. IR (KBr, cm^{-1}): 3029, 2985, 2243, 1658, 1597, 1561, 1504, 1449, 762, 747, 689; ^1H NMR (300 MHz, $CDCl_3$): δ 7.43–7.33 (m, 3H, 3,4-2ClPh-2,5,6-*H*), 7.18 (d, 2H, J = 8.2 Hz, Ph-3,5-*H*), 7.00 (d, 2H, J = 7.3 Hz, Ph-2,4-*H*), 6.94 (t, 1H, Ph-4-*H*), 4.46 (s, 2H, 3,4-2ClPh-CH_2), 4.09 (s, 2H, CH_2CN) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 147.32, 137.61, 132.18, 130.16, 130.11, 129.40, 128.20, 127.67, 118.36, 116.24, 114.42, 54.80, 39.96 ppm; MS (*m/z*): 291 and 293 $[M+H]^+$; HRMS (TOF) calcd. for $C_{15}H_{12}Cl_2N_2$: $[M+H]^+$, 291.0378; found, 291.0453 and 293.0439.

*2.12 2-((2-Fluorobenzyl)(phenyl)amino)acetonitrile (**5c**)*

Compound **5c** was prepared according to the experimental procedure for compound **5a**, starting from compound **2a** (2.996 g, 0.023 mol), 1-(chloromethyl)-2-fluorobenzene (3.325 g, 0.023 mol) and potassium carbonate (3.456 g, 0.025 mol). The desired compound **5c** was obtained as colorless liquid. Yield: 50.1%; IR (KBr, cm^{-1}) ν: 3431, 3026, 2967, 2235, 1663, 1687, 1568, 1509, 1457, 762, 673; ^1H NMR (300 MHz, $CDCl_3$): δ 7.38–7.21 (m, 6H, 2-FPh-3,4,5,6-*H*, Ph-3,5-*H*), 6.96 (m, 3H, Ph-2,4,6-*H*), 4.48 (s, 2H, 2-FPh-CH_2), 4.07 (s, 2H, CH_2CN) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 161.32, 137.48 131.89, 129.74, 129.42, 127.75, 124.65, 120.29, 117.36, 115.24, 114.86, 50.39, 40.16 ppm; MS (ESI): m/z 241 $[M+H]^+$; HRMS (TOF) calcd. for $C_{15}H_{13}FN_2$ $[M+H]^+$, 241.1063; found, 241.1025.

*2.13 2-((3-Fluorobenzyl)(phenyl)amino)acetonitrile (**5d**)*

Compound **5d** was prepared according to the experimental procedure for compound **5a**, starting from compound **2a** (2.998 g, 0.023 mol), 1-(chloromethyl)-3-fluorobenzene (3.321 g, 0.023 mol) and potassium carbonate (3.449 g, 0.025 mol). The desired compound **5d** was obtained as colorless liquid. Yield: 53.3%; IR (KBr, cm^{-1}) ν: 3398, 3012,

2967, 2241, 1665, 1617, 1558, 1509, 1457, 758, 673; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40–7.34 (m, 3H, 3-FPh-2,4,6-*H*), 7.29–7.25 (m, 3H, 3-FPh-5-*H*, Ph-3,5-*H*), 6.97 (m, 3H, Ph-2,4,6-*H*), 4.47 (s, 2H, 2-FPh-CH_2), 4.03 (s, 2H, CH_2CN) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.38, 149.32, 139.32, 129.40, 128.83, 125.74, 118.36, 116.69, 116.24, 114.42, 112.43, 51.80, 40.06 ppm; MS (ESI): m/z 241 $[M+H]^+$; HRMS (TOF) calcd. for $C_{15}H_{13}FN_2$ $[M+H]^+$, 241.1063; found, 241.1045.

*2.14 2-((2,4-Difluorobenzyl)(phenyl)amino)acetonitrile (**5e**)*

Compound **5e** was prepared according to the experimental procedure for compound **5a**, starting from compound **2a** (2.995 g, 0.023 mol), 1-(chloromethyl)-2,4-difluorobenzene (3.721 g, 0.023 mol) and potassium carbonate (3.501 g, 0.025 mol). The desired compound **5e** was obtained as white liquid. Yield: 52.8%; mp: 76–79 ^{o}C. IR (KBr, cm^{-1}) ν: 3405, 3014, 2985, 2243, 1665, 1612, 1558, 1505, 1469, 748, 677; ^{1}H NMR (300 MHz, DMSO): δ 7.27 (m, 4H, 2,4-2FPh-3,6-*H*, Ph-3,5-*H*), 7.06 (t, 1H, *J* = 8.2 Hz, 2,4-2FPh-5-*H*), 6.90 (d, 2H, *J* = 8.2 Hz, Ph-2,5-*H*), 6.83 (t, 1H, *J* = 7.2 Hz, Ph-4-*H*), 4.61 (s, 2H, 2,4-2FPh-CH_2), 4.59 (s, 2H, CH_2CN)·ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.71, 162.09, 149.34, 130.53, 129.39, 123.54, 117.36 , 116.21, 114.42, 111.66, 104.82, 50.39, 39.96 ppm; MS (ESI): m/z 259 $[M+H]^+$; HRMS (ESI) calcd. for $C_{15}H_{13}FN_2$ $[M+H]^+$, 259.0969; found, 259.0956.

*2.15 2-((4-Fluorobenzyl)(phenyl)amino)acetonitrile (**5f**)*

Compound **5f** was prepared according to the experimental procedure for compound **5a**, starting from compound **2a** (2.999 g, 0.023 mol), 1-(chloromethyl)-4-fluorobenzene (3.321 g, 0.023 mol) and potassium carbonate (3.449 g, 0.025 mol). The desired compound **5f** was obtained as colorless liquid. Yield: 53.3%; IR (KBr, cm^{-1}) ν: 3412, 3018, 2983, 2242, 1661, 1619, 1568, 1504, 1457, 753, 669; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40–7.34 (m, 3H, 3-FPh-2,4,6-*H*), 7.29–7.25 (m, 3H, 3-FPh-5-*H*, Ph-3,5-*H*), 6.97 (m, 3H, Ph-2,4,6-*H*), 4.47 (s, 2H, 2-FPh-CH_2), 4.03 (s, 2H, CH_2CN) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.65, 149.32, 131.79, 130.69, 129.59, 129.43, 117.26, 115.64, 114.80, 54.69, 40.17 ppm; MS (ESI): m/z 241 $[M+H]^+$; HRMS (TOF) calcd. for $C_{15}H_{12}F_2N_2$ $[M+H]^+$, 241.1063; found, 241.1045.

*2.16 2-((2-Chlorobenzyl)(phenyl)amino)acetonitrile (**5g**)*

Compound **5g** was prepared according to the experimental procedure for compound **5a**, starting from compound **2a** (3.009 g, 0.023 mol), 1-(chloromethyl)-2-chlorobenzene (3.711 g, 0.023 mol) and potassium carbonate (3.509 g, 0.025 mol). The desired compound **5g** was obtained as colorless liquid. Yield: 55.3%; IR (KBr, cm^{-1}) ν: 3392, 3012, 2986, 2245, 1671, 1629, 1601, 1554, 1468, 761, 665; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.34–7.27 (m, 2H, 2-ClPh-3,5-*H*), 7.26–7.19 (m, 4H, 2-ClPh-4,6-*H*, Ph-3,5-*H*), 6.97 (m, 3H, Ph-2,4,6-*H*), 4.43 (s, 2H, 2-FPh-CH_2), 4.07 (s, 2H, CH_2CN) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 149.32, 136.11, 133.24, 131.03, 130.27, 129.68, 129.40, 127.15, 118.36, 116.24, 114.42, 52.69, 40.07 ppm; MS (ESI): m/z 257 $[M+H]^+$; HRMS (TOF) calcd. for $C_{15}H_{13}ClN_2$ $[M+H]^+$, 257.0767; found, 257.0752.

*2.17 2-((4-Chlorobenzyl)(phenyl)amino)acetonitrile (**5h**)*

Compound **5h** was prepared according to the experimental procedure for compound **5a**, starting from compound **2a** (3.009 g, 0.023 mol), 1-(chloromethyl)-2-chlorobenzene (3.711 g, 0.023 mol) and potassium carbonate (3.509 g, 0.025 mol). The desired compound **5h** was obtained as colorless liquid. Yield: 52.7%; IR (KBr, cm^{-1}) ν: 3402, 3019, 2986, 2243, 1659, 1629, 1551, 1504, 1476, 763, 670; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39 (dd, 2H, *J* = 8.3 Hz, 4-ClPh-3,5-*H*), 7.32 (dd, 2H, *J* = 8.3 Hz, 4-ClPh-2,6-*H*), 6.94 (t, 2H, *J* = 7.9 Hz, Ph-3,5-*H*), 6.75–6.67 (m, 3H, Ph-2,4,6-*H*), 4.48 (s, 2H, 4-ClPh -CH_2), 4.03 (s, 2H, CH_2CN) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 149.32, 136.07, 132.68, 129.49, 129.45, 128.21, 121.29, 118.36, 116.24, 114.42, 50.09, 40.19 ppm; MS (TOF): m/z 257 $[M+H]^+$; HRMS (ESI) calcd. for $C_{15}H_{13}ClN_2$ $[M+H]^+$, 257.0767; found, 257.0752.

*2.18 2-((2,4-Dichlorobenzyl)(p-tolyl)amino)acetonitrile (**5i**)*

Compound **5i** was prepared according to the experimental procedure for compound **5a**, starting from compound **2b**

(3.009 g, 0.021 mol), 1-(chloromethyl)-2,4-dichlorobenzene (3.414 g, 0.021 mol) and potassium carbonate (3.189 g, 0.023 mol). The desired compound **5i** was obtained as colorless liquid. Yield: 56.0%. IR (KBr, cm^{-1}) ν: 3401, 3012, 2986, 2886, 2244, 1650, 1619, 1557, 1407, 1461, 768, 669; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39 (s, 1H, 2,4-ClPh-3-*H*), 7.30–7.24 (m, 2H, 2,4-ClPh-5,6-*H*), 7.19 (dd, 2H, *J* = 8.4 Hz, 4-CH_3Ph-3,5-*H*), 6.93 (s, 2H, dd, 2H, *J* = 8.4 Hz, 4-CH_3Ph-2,4-*H*), 4.41 (s, 1H, 2,4-ClPh-CH_2), 3.96 (s, 1H, CH_2CN), 2.39 (s, 3H, 4-Ph-CH_3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 147.20, 134.50, 134.24, 131.07, 130.90, 130.19, 129.87, 127.19, 126.57, 117.09, 116.24, 50.13, 39.96, 21.15 ppm; MS (ESI): m/z 305 $[M+H]^+$; HRMS (TOF) calcd. for $C_{15}H_{12}Cl_2N_2$ $[M+H]^+$, 305.2018; found, 305.2009.

*2.19 2-((2,4-Difluorobenzyl)(p-tolyl)amino)acetonitrile (**5j**)*

Compound **5j** was prepared according to the experimental procedure for compound **5a**, starting from compound **2b** (3.001 g, 0.021 mol), 1-(chloromethyl)-2，4-difluorobenzene (4.011 g, 0.021 mol) and potassium carbonate (3.189 g, 0.023 mol). The desired compound **5j** was obtained as white solid. Yield: 55.4%; mp: 84-85 °C.IR (KBr, cm^{-1}) ν: 3411, 3011, 2956, 2885, 2247, 1649, 1609, 1561, 1497, 1466, 767, 671; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30 (m, 1H, 2,4-FPh-3-*H*), 6.96–6.81 (m, 4H, 2,4-FPh-5,6-*H*, 4-CH_3Ph-3,5-*H*), 6.63 (d, 2H, 4-CH_3Ph-2,4-*H*), 4.53 (s, 1H, 2,4-FPh-CH_2), 4.41 (s, 1H, CH_2CN), 2.37 (s, 3H, 4-Ph-CH_3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 147.20, 134.50, 134.24, 131.07, 130.90, 130.19, 129.87, 127.19, 126.57, 117.09, 116.24, 52.23, 40.16, 21.15 ppm; MS (ESI): m/z 273 $[M+H]^+$; HRMS (TOF) calcd. for $C_{15}H_{12}F_2N_2$ $[M+H]^+$, 273.1125; found, 273.1109.

*2.20 2-((2,4-Difluorobenzyl)(4-methoxyphenyl)amino)acetonitrile (**5k**)*

Compound **5k** was prepared according to the experimental procedure for compound **5a**, starting from compound **2c** (3.001 g, 0.018 mol), 1-(chloromethyl)-2-chlorobenzene (3.011 g, 0.018 mol) and potassium carbonate (2.736 g, 0.020 mol). The desired compound **5k** was obtained as white solid. Yield: 58.2%; mp: 84–85 °C.IR (KBr, cm^{-1}) ν: 3427, 3010, 2968, 2883, 2242, 1619, 1654, 1551, 1467, 1406, 710, 674; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32 (m, 1H, 2,4-FPh-3-*H*), 7.00 (d, 2H, *J* = 9.0 Hz, 4-CH_3OPh-3,5-*H*), 6.92–6.80 (m, 4H, 4-CH_3OPh-3,5-*H*, 2,4-FPh-5,6-*H*), 4.38 (s, 2H, 2,4-FPh-CH_2), 3.98 (s, 2H, CH_2CN), 3.78 (s, 3H, 4-Ph-OCH_3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.14, 155.16, 141.61, 131.03, 119.63, 115.67, 114.76, 111.61, 111.28, 104.17, 77.02, 55.51, 50.07, 41.51 ppm; MS (ESI): m/z 289 $[M+H]^+$; HRMS (TOF) calcd. for $C_{15}H_{12}F_2N_2$ $[M+H]^+$, 289.1074; found, 289.1089.

*2.21 2-((2,4-Dichlorobenzyl)(4-methoxyphenyl)amino)acetonitrile (**5l**)*

Compound **5l** was prepared according to the experimental procedure for compound **5a**, starting from compound **2c** (3.000 g, 0.018 mol), 1-(chloromethyl)-2,4-dichlorobenzene (3.514 g, 0.018 mol) and potassium carbonate (2.734 g, 0.020 mol). The desired compound **5l** was obtained as colorless liquid. Yield: 56.0%. IR (KBr, cm^{-1}) ν: 3396, 3016, 2986, 2883, 2239, 1641, 1609, 1552, 1405, 1471, 768, 669; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41 (s, 1H, 2,4-2ClPh-3-*H*), 7.32–7.27 (m, 2H, 2,4-2ClPh-5,6-*H*), 6.98 (s, 2H, 4-CH_3OPh-3,5-*H*), 6.88-6.80 (m, 3H, Ph-2,4,6-*H*), 4.40 (s, 2H, 2,4-2ClPh-CH_2), 4.01 (s, 2H, CH_2CN), 3.78 (s, 3H, 4-Ph-OCH_3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ 151.50, 142.22 , 134.50, 134.24, 131.03, 130.94, 130.19, 127.19, 116.24, 115.79, 114.26, 56.08, 50.13, 41.96 ppm; MS (ESI): m/z 321 $[M+H]^+$; HRMS (TOF) calcd. for $C_{15}H_{12}Cl_2N_2$ $[M+H]^+$, 321.0483; found, 321.0509.

*2.22 N-((1H-tetrazol-5-yl)methyl)-N-(2,4-dichlorobenzyl) aniline (**6a**)*

To a solution of compound **5a** (1.228 g, 4.2 mmol) in DMF was added sodium azide (0.273 g, 4.2 mmol), ammonium chloride (2.247 g, 42 mmol), the mixture was stirred for 7 h at 120 °C. After the reaction came to the end (monitored by TLC), solvents were removed under reduced pressure, the residue dissolved in 80 mL of water and acidified with concentrated hydrochloric acid to pH 2. The solution was cooled to 5 °C in an ice bath to give precipitate, and then the precipitate was recrystallized from ethanol to afford compound **6a** as yellow solid. Yield: 78%; mp: 188–191 °C. IR (KBr, cm^{-1}): 3440, 3112, 3029, 2986, 1602, 1563, 1546, 1505, 1470, 762, 747, 692, 619; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.67 (s, 1H, 2,4-2ClPh-3-*H*), 7.37 (d, 1H, *J* = 7.2 Hz, 2,4-2ClPh-5-*H*), 7.20 (d, 1H, *J* = 8.4 Hz, 2,4-2ClPh-6-*H*), 7.13 (t, 2H, *J* = 7.6 Hz, Ph-3,5-*H*), 6.67 (t, 1H, *J* = 7.0 Hz, Ph-4-*H*), 6.59 (d, 2H, *J* = 7.8 Hz, Ph-2,6-*H*), 5.06 (s, 2H, Tet-CH_2), 4.78 (s, 2H, 2,4-2ClPh-CH_2) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ 155.36,

147.21, 135.00, 133.19, 132.55, 129.60, 129.53, 129.40, 127.81, 117.80, 112.76, 52.80, 45.09 ppm; MS (*m/z*): 334 and 336 $[M+H]^+$; HRMS (TOF) calcd. for $C_{15}H_{13}Cl_2N_5$: $[M+H]^+$, 334.0548; found, 334.0625 and 336.0613.

*2.23 N-((1H-Tetrazol-5-yl)methyl)-N-(3,4-dichlorobenzyl) aniline (**6b**)*

Compound **6b** was prepared according to the experimental procedure for compound **6a**, starting from compound **5b** (1.228 g, 4.2 mmol), sodium azide (0.273 g, 4.2 mmol) and ammonium chloride (2.247 g, 42 mmol). The desired compound **6b** was obtained as colorless liquid. Yield: 76.3%; mp: 182–184 ^oC. IR (KBr, cm^{-1}): 3441, 3112, 3029, 2986, 1602, 1563, 1546, 1505, 1470, 762, 747, 692, 619; ^1H NMR (300 MHz, DMSO-d_6): δ 7.63–7.53 (m, 2H, 3,4-2ClPh-2,5-*H*), 7.28 (d, 1H, *J* = 8.3 Hz, 3,4-2ClPh-6-*H*), 7.13 (t, 2H, *J* = 7.7 Hz, Ph-3,5-*H*), 6.68 (m, 3H, Ph-2,4,6-*H*), 5.02 (s, 2H), 4.74 (s, 2H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 155.40, 147.48, 140.66, 131.57, 131.04, 129.66, 129.50, 129.05 (s), 127.41, 117.84, 113.27, 53.97, 45.16 ppm; MS (*m/z*): 334 and 336 $M+H]^+$, HRMS (TOF) calcd. for $C_{15}H_{13}Cl_2N_5$: $[M+H]^+$, 334.0548; found, 334.0625 and 336.0613.

*2.24 N-((1H-Tetrazol-5-yl)methyl)-N-(2-fluorobenzyl)aniline (**6c**)*

Compound **6c** was prepared according to the experimental procedure for compound **6a**, starting from compound **5c** (500 mg, 2.8 mmol), sodium azide (135 mg, 2.8 mmol) and ammonium chloride (1.498 g, 28 mmol). The desired compound **6c** was obtained as yellow solid. Yield: 78.3%; mp: 184–186 ^oC. IR (KBr, cm^{-1}): 3441, 3112, 3029, 2986, 1602, 1563, 1546, 1505, 1470, 762, 747, 692, 619; ^1H NMR (300 MHz, DMSO-d_6): δ 7.37 (m, 1H, 2-FPh-3-*H*), 7.13–7.04 (m, 5H, Ph-3,5-*H*, 2-FPh-4,5,6-*H*), 6.71–6.64 (m, 3H, Ph-2,4,6-*H*), 5.01 (s, 2H, Tet-CH_2), 4.76 (s, 2H, 2-FPh-CH_2) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 164.50, 161.27,155.43, 147.65, 142.42, 142.33, 130.94,130.83, 129.46, 123.00, 122.97, 117.67, 114.14, 113.86, 113.56, 113.20, 54.46 ppm; MS (*m/z*): 284 $M+H]^+$, HRMS (TOF) calcd. for $C_{15}H_{14}FN_5$: $[M+H]^+$, 284.1233; found, 284.1203.

*2.25 N-((1H-Tetrazol-5-yl)methyl)-N-(3-fluorobenzyl)aniline (**6d**)*

Compound **6d** was prepared according to the experimental procedure for compound **6a**, starting from compound **5d** (501 mg, 2.8 mmol), sodium azide (135 mg, 2.8 mmol) and ammonium chloride (1.501 g, 28 mmol). The desired compound **6d** was obtained as yellow solid. Yield: 76.3%; mp: 185–187 ^oC. IR (KBr, cm^{-1}): 3447, 3112, 3029, 2986, 1602, 1563, 1546, 1505, 1470, 762, 747, 692, 619; ^1H NMR (300 MHz, DMSO-d_6): δ 7.35–7.09 (m, 6H, Ph-3,5-*H*, 3-FPh-2,4,5,6-*H*), 6.68 (m, 3H, Ph-2,4,6-*H*), 5.02 (s, 2H, Tet-CH_2), 4.81 (s, 2H, 3-FPh-CH_2) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 162.28, 159.05, 155.45, 129.49, 129.17, 128.84, 125.47, 124.82, 117.70, 115.61, 113.02, 49.01, 45.05 ppm; MS (*m/z*): 284 $M+H]^+$, HRMS (TOF) calcd. for $C_{15}H_{14}FN_5$: $[M+H]^+$, 284.1233; found, 284.1203.

*2.26 N-((1H-Tetrazol-5-yl)methyl)-N-(2,4-difluorobenzyl)aniline (**6e**)*

Compound **6e** was prepared according to the experimental procedure for compound **6a**, starting from compound **5e** (500 mg, 1.9 mmol), sodium azide (123 mg, 1.9 mmol) and ammonium chloride (1. 016 g, 19 mmol). The desired compound **6e** was obtained as yellow solid. Yield: 76.8%; mp: 188–190 ^oC. IR (KBr, cm^{-1}): 3447, 3112, 3029, 2986, 1602, 1563, 1546, 1505, 1470, 762, 747, 692, 619; ^1H NMR (300 MHz, DMSO-d_6): δ 7.35–7.20 (m, 2H, 2,4-FPh-5,6-*H*), 7.14 (t, 2H, *J* = 7.6 Hz, Ph-3,5-*H*), 7.03 (t, 1H, 2,4-FPh-3-*H*), 6.71-6.65 (m, 3H, Ph-2,4,6-*H*), 5.00 (s, 2H, Tet-CH_2), 4.77 (s, 2H, 2,4-FPh-CH_2) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 163.33, 162.26, 160.03, 158.83, 155.42, 129.52, 121.82, 117.82 (s), 113.10, 111.82, 111.55, 104.42, 48.70, 45.00 ppm; MS (*m/z*): 320 $M+H]^+$, HRMS (TOF) calcd. for $C_{15}H_{13}F_2N_5$: $[M+H]^+$, 302.1139; found, 302.1124.

*2.27 N-((1H-Tetrazol-5-yl)methyl)-N-(4-fluorobenzyl)aniline (**6f**)*

Compound **6f** was prepared according to the experimental procedure for compound **6a**, starting from compound **5f** (501 mg, 2.8 mmol), sodium azide (135 mg, 2.8 mmol) and ammonium chloride (1.500 g, 28 mmol). The desired compound **6f** was obtained as yellow solid. Yield: 77.2%; mp: 188–190 ^oC. IR (KBr, cm^{-1}): 3447, 3112, 3029, 2986, 1602, 1563, 1546, 1505, 1470, 762, 747, 692, 619; ^1H NMR (300 MHz, DMSO-d_6): δ 7.35–7.27 (m, 2H, Ph-3,5-*H*), 7.17 (dd, 2H, *J* = 8.4 Hz, 4-ClPh-3,5-*H*), 7.11 (dd, 2H, *J* = 8.3 Hz, 4-FPh-2,6-*H*), 6.74-6.62 (m, 3H, Ph-2,4,6-*H*), 4.97

(s, 2H, Tet-CH_2), 4.72 (s, 2H, 4-FPh-CH_2) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 161.96, 155.06, 149.32, 131.79, 130.69, 129.40, 128.81, 125.47, 118.36, 114.80, 55.27, 45.23 ppm; MS (*m/z*): 284 M+H]$^+$, HRMS (TOF) calcd. for $C_{15}H_{14}FN_5$: [M+H]$^+$, 284.1233; found, 284.1203.

*2.28 N-((1H-Tetrazol-5-yl)methyl)-N-(2-chlorobenzyl)aniline (**6g**)*

Compound **6g** was prepared according to the experimental procedure for compound **6a**, starting from compound **5g** (500 mg, 2.0 mmol), sodium azide (127 mg, 2.0 mmol) and ammonium chloride (1.043 g, 20 mmol). The desired compound **6g** was obtained as yellow solid. Yield: 77.2%; mp: 190–192 ^{o}C. IR (KBr, cm^{-1}): 3447, 3112, 3029, 2986, 1602, 1563, 1546, 1505, 1470, 762, 747, 692, 619; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.50 (m, 1.9 Hz, 1H, 2-ClPh-5-*H*), 7.33–7.24 (m, 2H, 2-ClPh-3,6-*H*), 7.22–7.17 (m, 1H, 2-ClPh-4-*H*), 7.13 (t, 2H, *J* = 7.9 Hz, Ph-3,5-*H*), 6.66 (t, 1H, *J* = 7.3 Hz, Ph-4-*H*), 6.60 (d, 2H, *J* = 8.2 Hz, Ph-2,6-*H*), 5.06 (s, 2H, Tet-CH_2), 4.81 (s, 2H, 2-ClPh-CH_2) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 155.46 (s), 147.37, 135.63, 132.26, 129.95, 129.56, 128.98, 128.11, 127.69, 117.62, 112.69, 53.06, 45.11 ppm; MS (*m/z*): 300 M+H]$^+$, HRMS (TOF) calcd. for $C_{15}H_{14}ClN_5$: [M+H]$^+$, 300.0938; found, 300.1203.

*2.29 N-((1H-Tetrazol-5-yl)methyl)-N-(4-chlorobenzyl)-4-methylaniline (**6h**)*

Compound **6h** was prepared according to the experimental procedure for compound **6a**, starting from compound **5h** (500 mg, 2.0 mmol), sodium azide (127 mg, 2.0 mmol) and ammonium chloride (1.043 g, 20 mmol). The desired compound **6h** was obtained as yellow solid. Yield: 78.2%; mp: 190–193 ^{o}C. IR (KBr, cm^{-1}): 3447, 3112, 3029, 2986, 2885, 1602, 1563, 1546, 1505, 1470, 762, 747, 692, 619; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.39 (dd, 2H, *J* = 8.4 Hz, 4-ClPh-3,5-*H*), 7.30 (dd, 2H, *J* = 8.3 Hz, 4-ClPh-2,6-*H*), 7.12 (t, 2H, *J* = 7.9 Hz, Ph-3,5-*H*), 6.71–6.64 (m, 3H, Ph-2,4,6-*H*), 4.98 (s, 2H, Tet-CH_2), 4.73 (s, 2H, 4-ClPh-CH_2) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 155.06, 147.68, 138.07, 131.73, 129.46, 128.95, 128.86, 117.68, 113.25, 54.19, 45.04 ppm; MS (*m/z*): 300 M+H]$^+$; HRMS (TOF) calcd. for $C_{15}H_{14}ClN_5$: [M+H]$^+$, 300.0938; found, 300.1203.

*2.30 N-((1H-Tetrazol-5-yl)methyl)-N-(2,4-dichlorobenzyl)-4-methylaniline (**6i**)*

Compound **6i** was prepared according to the experimental procedure for compound **6a**, starting from compound **5i** (500 mg, 1.6 mmol), sodium azide (107 mg, 1.6 mmol) and ammonium chloride (0.843 g, 20 mmol). The desired compound **6i** was obtained as yellow solid. Yield: 77.6%; mp: 196–198 ^{o}C. IR (KBr, cm^{-1}): 3447, 3112, 3029, 2986, 2891, 1612, 1563, 1546, 1505, 1470, 762, 747, 692, 619; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.65 (s, 1H, 2,4-2ClPh-3-*H*), 7.21 (d, 2H, *J* = 6.2 Hz, 2,4-2ClPh-5-*H*), 7.07 (d, 1H, *J* = 7.2 Hz, 2,4-2ClPh-6-*H*), 6.73-6.53 (m, 4H, 4-CH_3Ph-2,3,5,6-*H*), 4.84 (s, 1H, Tet-CH_2), 4.55 (s, 1H, 2,4-2ClPh-CH_2), 2.35 (s, 3H, 4-Ph-CH_3) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 155.72, 147.20, 133.32, 133.21, 132.49, 130.90, 130.19, 129.87, 127.19, 126.57, 117.09, 50.53, 45.23, 21.15 ppm; MS (*m/z*): 348 M+H]$^+$; HRMS (TOF) calcd. for $C_{16}H_{15}Cl_2N_5$: [M+H]$^+$, 348.0705; found, 348.0801.

*2.31 N-((1H-Tetrazol-5-yl)methyl)-N-(2,4-difluorobenzyl)-4-methylaniline (**6j**)*

Compound **6j** was prepared according to the experimental procedure for compound **6a**, starting from compound **5j** (500 mg, 1.8 mmol), sodium azide (119 mg, 1.8 mmol) and ammonium chloride (0.967 g, 18 mmol). The desired compound **6j** was obtained as yellow solid. Yield: 77.6%; mp: 184–186 ^{o}C. IR (KBr, cm^{-1}): 3447, 3112, 3029, 2986, 2891, 1612, 1563, 1546, 1505, 1470, 762, 747, 692, 619; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.31–7.21 (m, 2H, 2,4-FPh-5,6-*H*), 7.00 (t, 1H, *J* = 7.6 Hz, 2,4-FPh-3-*H*), 6.93 (dd, 2H, *J* = 8.3 Hz, 4-CH_3Ph-2,6-*H*), 6.63 (dd, 2H, *J* = 8.4 Hz, 4-CH_3Ph-3,5-*H*), 4.86 (s, 2H, Tet-CH_2), 4.69 (s, 2H, 2,4-FPh-CH_2), 2.89 (s, 3H, 4-Ph-CH_3) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 162.80, 160.17, 156.18, 145.59, 129.86, 126.26, 121.97, 118.19, 113.46, 111.46, 104.30 , 48.66, 45.41, 20.25 ppm; MS (*m/z*): 316 M+H]$^+$; HRMS (TOF) calcd. for $C_{16}H_{15}F_2N_5$: [M+H]$^+$, 316.1396; found, 316.1375.

*2.32 N-((1H-Tetrazol-5-yl)methyl)-N-(2,4-difluorobenzyl)-4-methoxyaniline (**6k**)*

Compound **6k** was prepared according to the experimental procedure for compound **6a**, starting from compound **5k** (500 mg, 1.7 mmol), sodium azide (113 mg, 1.7 mmol) and ammonium chloride (0.907 g, 17 mmol). The desired

compound **6k** was obtained as yellow solid. Yield: 77.3%; mp: 184–186 °C. IR (KBr, cm^{-1}): 3447, 3112, 3029, 2986, 2891, 1612, 1563, 1546, 1505, 1470, 762, 747, 692, 619; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.41 (m, 1H, 2,4-FPh-3-*H*), 7.29–6.98 (m, 2H, 2,4-FPh-5,6-*H*), 6.85 (dd, 2H, *J* = 8.3 Hz, 4-CH_3OPh-2,6-*H*), 6.57 (m, 2H, dd, 2H, *J* = 8.3 Hz, 4-CH_3OPh-3,5-*H*), 4.98 (s, 2H, 2,4-FPh-CH_2), 4.72 (s, 2H, Tet-CH_2), 3.81 (s, 3H, 4-Ph-OCH_3) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 163.29, 163.02, 156.18, 145.44, 129.92, 126.26, 123.57, 115.34, 114.57, 111.67, 103.36 , 47.69, 44.92, 39.25 ppm; MS (*m/z*): 332 M+H]$^+$; HRMS (TOF) calcd. for $C_{16}H_{15}F_2N_5$: [M+H]$^+$, 332.1245; found, 332.1253.

*2.33 N-((1H-Tetrazol-5-yl)methyl)-N-(2,4-dichlorobenzyl)-4-methoxyaniline (**6l**)*

Compound **6l** was prepared according to the experimental procedure for compound **6a**, starting from compound **5l** (500 mg, 1.5 mmol), sodium azide (103 mg, 1.5 mmol) and ammonium chloride (0.837 g, 15 mmol). The desired compound **6l** was obtained as yellow solid. Yield: 78.9%; mp: 189–191 °C. IR (KBr, cm^{-1}): 3447, 3112, 3029, 2986, 2891, 1612, 1563, 1546, 1505, 1470, 762, 871, 795, 708; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.47 (m, 1H, 2,4-FPh-3-*H*), 7.26–6.97 (m, 2H, 2,4-FPh-5,6-*H*), 6.71 (dd, 2H, *J* = 8.4 Hz, 4-CH_3OPh-2,6-*H*), 6.59 (m, 2H, dd, 2H, *J* = 8.4 Hz, 4-CH_3OPh-3,5-*H*), 4.89 (s, 2H, 2,4-FPh-CH_2), 4.62 (s, 2H, Tet-CH_2), 3.79 (s, 3H, 4-Ph-OCH_3) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ 155.33, 147.61, 142.22, 134.50, 134.24, 130.90, 130.19, 129. 84, 127.19, 115.79, 114.98, 46.79, 44.13, 39.85 ppm; MS (*m/z*): 364 M+H]$^+$; HRMS (TOF) calcd. for $C_{16}H_{15}F_2N_5$: [M+H]$^+$, 364.0654; found, 364.1203.

Supporting Information 2

1. Biological assays procedures

Minimal inhibitory concentration (MIC, μg/mL) is defined as the lowest concentration of the new compounds that completely inhibited the growth of bacteria, by means of standard two folds serial dilution method in 96-well microtest plates according to the National Committee for Clinical Laboratory Standards (NCCLS). [2] The tested microorganism strains were provided by the School of Pharmaceutical Sciences, Southwest University and the College of Pharmacy, Third Military Medical University. Chloromycin, Norfloxacin and Fluconazole were used as control drugs. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment. All the bacteria and fungi growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimal inhibitory concentration (MIC).

1.1 Antibacterial Assays

The prepared compounds **3–6** were evaluated for their antibacterial activities against *Methicillinresistant staphylococcus aureus* N315, *Staphylococcus aureus* ATCC25923, *Micrococcus luteus* ATCC 4698 and *Bacillus subtilis* as Gram-positive, *Escherichia coli* DH52, *Shigella dysenteriae* ATCC51252, *Pseudomonas aeruginosa* ATCC27853, *Bacillus proteus* ATCC13315 as Gram-negative bacteria. The bacterial suspension was adjusted with sterile saline to a concentration of 1×10^5 CFU/mL. The compounds were dissolved in DMSO to prepare the stock solutions. The compounds and reference drugs were prepared in Mueller–Hinton broth (Guangdong huaikai microbial sci.& tech co., Ltd, Guangzhou, Guangdong, China) by twofold serial dilution to obtain the required concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5 μg/mL. These dilutions were inoculated and incubated at 37 ºC for 24 h. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment.

1.2 Antifungal Assays

The synthesized compounds **3–6** were also evaluated for their antifungal activities against *Candida albicans*, *Candida mycoderma*, *Candida utilis* and *Aspergillus flavus*. A spore suspension in sterile distilled water was prepared from 1-day old culture of the fungi growing on Sabouraud agar (SA) media. The final spore concentration was 1–5×10^3 spore/mL. From the stock solutions of the tested compounds and reference antifungal Fluconazole, dilutions in sterile RPMI 1640 medium (Neuronbc Laboraton Technology CO., Ltd, Beijing, China) were made resulting in

eleven wanted concentrations (0.5-512 μg/mL) of each tested compounds. These dilutions were inoculated and incubated at 35 ºC for 24 h. The drug's MIC was defined as the first well with an approximate 80% reduction in growth compared to the growth of the drug-free well.

1.3 Synergistic effects

The combination studies were screened by microdilution checkerboard method as described by Fivelman and co-workers (FIC (fractional inhibitory concentration) = MIC of compound A in mixture/MIC of compound A alone + MIC of compound B in mixture/MIC of compound B alone, FIC ≤ 1 suggests synergistic effect, FIC > 1 and < 2 suggests additive interaction, FIC > 2 suggests antagonistic effect). [3]

Supporting Information 3

3.1 Measurement of the log*p* values

The partition coefficient (P) is defined as the ratio of the equilibrium concentrations of a dissolved substance in a two-phase system (*n*-octanol and water) which are two largely immiscible solvents. Octanol represents a substitute for biotic lipid and hence gives an approximation to a biotic lipid-water partition coefficient. The ratio is reported as a logarithm usually as log P_{ow} or log K_{ow}.

Usually, the data of logp are analyzed according to the following equation:

$$\log P_{OW} = \log(\frac{C_o}{C_w}) \quad (1)$$

Where C_o is the concentration of compound in the *n*-octanol phase and C_w is the concentration in the aqueous phase when the system is at equilibrium.

In this experiment, we employed the traditional saturation shake flask approach and UV−vis spectrophotometric methods to measure the values of log p_{ow} as below:

n-Octanol and doubly distilled water were shaken for 24 h under 25℃, and then separated and stored for next steps. The synthesized target compounds were dissolved in *n*-octanol−saturated water to prepare solutions at concentrations of 1.0×10^{-4}, 1.2×10^{-4}, 1.4×10^{-4}, 1.6×10^{-4}, 1.8×10^{-4} M, respectively. The absorbance of these solutions was measured by UV−spectrophotometer. Solutions of 3×10^{-4} M compounds were prepared in *n*-octanol-saturated water. Then 5.0 mL of *n*-octanol was added to 5.0 mL of the aqueous compounds solution in glass flasks. The mixtures were then shaked for 24 h under 25℃. After that, the aqueous phases were isolated and the concentrations were determined by measuring the UV absorbance. All the partition coefficients experiments were carried out in triplicate.

3.2 UV-vis spectra and standard curves of some compounds for the measurement of the log*p* values

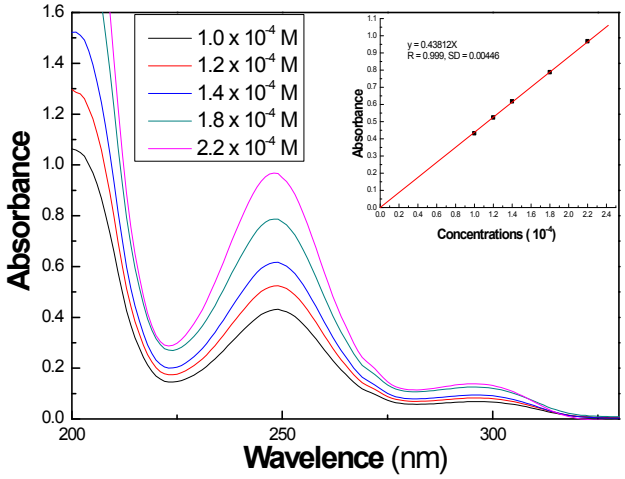

Fig. S1 UV-vis spectra of compound **6e** of various concentrations, the standard curves were depicted in the inset.

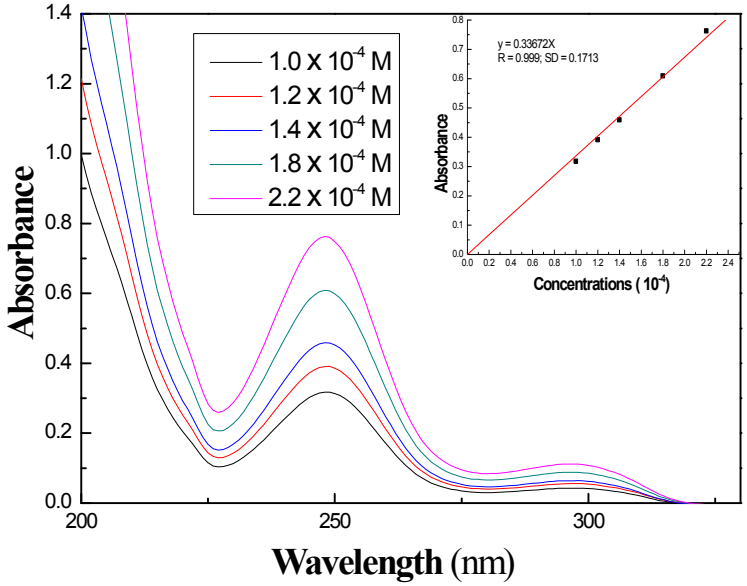

Fig. S2 UV-vis spectra of compound **6g** various concentrations, the standard curves were depicted in the inset.

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