Supplemental Information

The trypanocidal Effect of Novel Quinolines: *In vitro* And *In vivo Studies*

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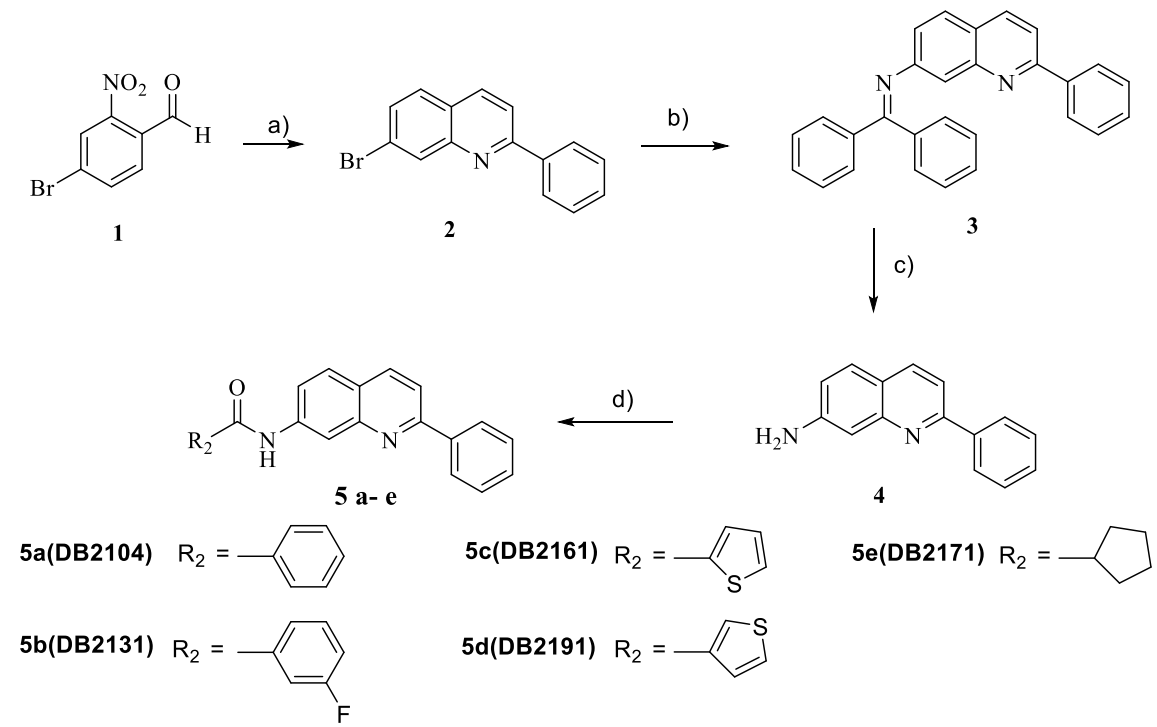

Scheme 1. Reagents and conditions: a) i. Fe, HCl, EtOH ii. acetophenone, KOH b) benzophenone imine-HCl, $Pd_2(dba)_3$, BINAP, NaO*t-Bu*, 1,4-dioxane c) *O*-methylhydroxylamine•HCl, NaOAc, H_2O,1,4-dioxane, EtOH, d) R_2COCl, Et_3N, CH_2Cl_2

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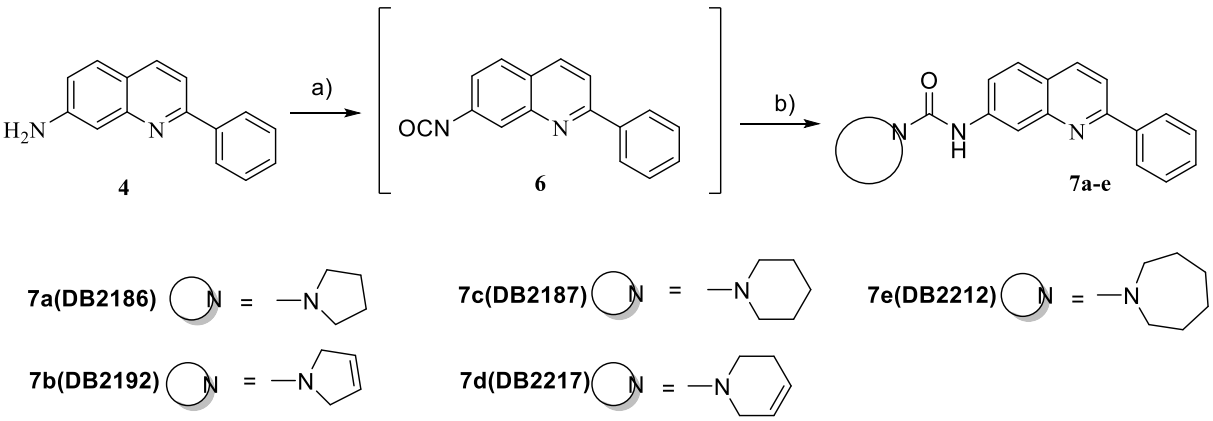

Scheme 2. Reagents and conditions: a) triphosgene, Et_3N, CH_2Cl_2, 0°C b) appropriate amine, rt

All commercial reagents were used without further purification. All melting points were determined on a Mel-Temp 3.0 melting point instrument, and are uncorrected. TLC analysis was carried out on silica gel 60 F254 precoated aluminum sheets using UV light for detection. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer using the indicated solvents. Mass spectral data were obtained from the Georgia State University Mass Spectrometry Laboratory, Atlanta, GA. If the compounds are reported contain hydrates or solvates, these components were detected in HNMR spectra. Elemental analysis were performed by Atlantic Microlab Inc., Norcross, GA.

7 –Amino-2-phenylquinoline. (4). A literature method was used to prepare 7-bromo-2-phenylquinoline **(2)** (yellow solid) in a 39% yield which gave both ^{1}H NMR and ^{13}C NMR data in good agreement with that previously reported [1]. ^{1}H NMR ($CDCl_3$): δ 8.36 (s, 1H), 8.15 (d, *J* = 7.2 Hz, 3H), 7.87 (d, *J* = 8.6 Hz, 1H), 7.66 (d, *J* = 8.6 Hz, 1H), 7.59 (d, *J* = 8.6 Hz, 1H), 7.55–7.46 (m, 3H). ^{13}C NMR (DMSO- d_6): δ 158.4, 149.1, 139.4, 136.8, 132.3, 129.9, 129.8, 129.1, 128.9, 127.8, 125.9, 123.9, 119.4. Tris(dibenzylideneacetone)dipalladium(0) (184 mg, 0.201 mmol) and 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (375 mg, 0.603 mmol) were added to a mixture of 7-bromo-2-phenylquinoline (11.4 g, 40.2 mmol), benzophenone imine hydrochloride (13.1 g, 60.4 mmol) and sodium *tert*-butoxide (9.3 g, 96.4 mmol) in 1,4-dioxane (250 mL). The reaction mixture was refluxed at 100 °C overnight under nitrogen [2]. The reaction mixture was cooled to room temperature and diluted with ether (500 mL), filtered and dried. The solid was recrystallized in hot methanol, filtered and dried to provide *N*-(diphenylmethylene)-2-phenylquinolin-7-amine **(3**) (10.6 g, 70%). ^{1}H NMR (DMSO-d_6): δ 8.27 (d, *J* = 8.6 Hz, 1H), 8.18 (d, *J* = 7.3 Hz, 2H), 7.95 (d, *J* = 8.6 Hz, 1H), 7.77 (d, *J* = 8.6 Hz, 1H), 7.72 (d, *J* = 7.3 Hz, 2H), 7.57–7.46 (m, 6H), 7.26–7.25 (m, 6H), 7.07 (d, *J* = 8.6 Hz, 1H). ^{13}C NMR (DMSO- d_6): δ 168.1, 156.3, 152.7, 152.6, 148.1, 138.7, 136.7, 135.7, 131.1, 129.3, 128.7, 128.3, 128.1, 127.1, 127.0, 123.3, 121.9, 117.1. A premixed suspension of methylhydroxylamine hydrochloride (3.87 g, 46.4 mmol) and sodium hydroxide (4.75 g, 58.0 mmol) in water (10 mL) was added to a mixture of *N*-(diphenylmethylene)-2-phenylquinolin-7-amine (5.6 g, 14.5 mmol,) in 1,4-dioxane (90 mL) and ethanol (60 mL). The reaction mixture was stirred under nitrogen, protected from light, overnight. The reaction mixture was diluted with 0.5 N HCl till acidic, and extracted with ethyl acetate (2 $\times$ 250 mL). The aqueous layer was basified with 5.0 N NaOH and extracted with ethyl acetate (2 $\times$ 200 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to provide 7-amino-2-phenylquinoline **(4)** (1.8 g, 58%, (**4**) as a pale yellow solid. ^{1}H NMR (DMSO-d_6): δ 8.17 (d, *J* = 7.3 Hz, 2H), 8.09 (d, *J* = 8.3 Hz, 1H),7.65 (dd, *J* = 12.2, 8.4 Hz, 2H), 7.50 (t, *J* = 7.3 Hz, 2H), 7.45 (d, *J* = 7.2 Hz, 1H), 6.99 (s, 1H), 6.97 (s, 1H), 5.77 (s, 2H); ^{13}C NMR (DMSO- d_6): δ 155.5, 150.2, 149.7, 139.3, 136.1, 128.8, 128.0, 126.7, 119.6, 118.6, 113.5, 106.6; HRMS (ESI) calcd for $C_{15}H_{12}N_2$ (M^+ +H) 221.1079, found 221.1078.

General Procedure for 5 a-e. The appropriate acid chloride (1.06 mmol) was added to a stirred solution of 7-amino-2-phenylquinoline (1.06 mmol) and

triethylamine (1.16 mmol) in dichloromethane (20 mL) at 0 °C under nitrogen gas. The reaction mixture was allowed to reach room temperature by stirrring overnight, after which it was concentrated to dryness. Purification was by column chromatography on silicia gel eluting with hexanes/ ethyl acetate (4:1, then 1:1) or recrystallization as noted.

***N*-(2-phenylquinolin-7-yl)benzamide (DB2104, 5a).** Off-white solid (192 mg, 56%); mp = 201 –202 °C. ^{1}H NMR (DMSO-d_6): δ 8.65 (s, 1H), 8.38 (d, *J* = 8.4 Hz, 1H), 8.27 (d, *J* = 8.4 Hz, 2H), 8.04 (d, *J* = 8.8 Hz, 3H), 7.96 (d, *J* = 2.4 Hz, 2H), 7.63–7.50 (m, 7H); ^{13}C NMR (DMSO-d_6): δ 166.0, 156.4, 148.2, 140.4, 138.7, 136.6, 134.8, 131.7, 129.5, 128.8, 128.4, 127.9, 127.8, 127.1, 123.7, 121.0, 117.4; HRMS (ESI) calcd for $C_{24}H_{22}N_4O_2$ (M^+ +H) 399.1821, found 399.1833; Anal. Calcd for $C_{22}H_{16}N_2O$: C, 81.46, H, 4.97; N, 8.64. Found: C, 81.32; H, 5.00; N, 8.58.

3-Fluoro-*N*-(2-phenylquinolin-7-yl)benzamide (DB2131, 5b). The reaction mixture was filtered and recrystallized in ethyl acetate to give an off-white solid (209 mg, 52%); mp 184 –186 °C. ^{1}H NMR (DMSO-d_6): δ 10.66 (s, 1H), 8.67 (s, 1H), 8.39 (d, *J* = 8.4 Hz, 1H), 8.28 (d, *J* = 8.4 Hz, 2H), 8.06 (d, *J* = 8.4 Hz, 1H), 8.00–7.84 (m, 5H), 7.65 (q, *J* = 8.4, 5.5 Hz, 1H), 7.58–7.47 (m, 4H); ^{13}C NMR (DMSO-d_6): δ 164.6, 156.7, 148.3, 140.1. 138.9. 137.1, 136.6, 130.5, 129.4, 128.7, 127.9, 127.0, 123.9, 121.2, 118.6, 118.4, 117.6, 114.6, 114.4; HRMS (ESI) calcd for $C_{22}H_{15}N_2OF$ (M^+ +H) 343.1247, found 343.1249; Anal. Calcd for $C_{20}H_{15}N_2OF$: C, 77.18, H, 4.42; N, 8.18. Found: C, 77.00; H, 4.36; N, 8.14.

***N*-(2-phenylquinolin-7-yl)thiophene-2-carboxamide (DB2161, 5c).** The reaction mixture was filtered to give an off-white solid (550 mg, 92%); mp 282 – 283 °C. ^{1}H NMR (DMSO-d_6): δ 10.56 (s, 1H), 8.59 (s, 1H), 8.38 (d, *J* = 8.4 Hz, 1H), 8.27 (d, *J* = 8.4 Hz, 2H), 8.16 (s, 1H), 8.05 (d, *J* = 8.4 Hz, 1H), 7.98 (d, *J* = 12.2 Hz, 1H), 7.92 (s, 2H), 7.56–7.48 (m, 3H), 7.28 (t, *J* = 4.0 Hz, 1H); ^{13}C NMR (DMSO-d_6): δ 160.3, 145.6, 148.3, 140.0, 139.8, 139.0, 136.4, 131.7, 129.5, 129.3, 128.6, 127.8, 127.2, 127.1, 123.8, 121.1. 117.9, 117.4 ; HRMS (ESI) calcd for $C_{20}H_{14}N_2OS$ (M^+ +H) 331.0905, found 331.0920; Anal. Calcd for $C_{20}H_{14}N_2OS$: C, 72.70, H, 4.27; N, 8.48. Found: C, 72.60; H, 4.10; N, 8.46.

***N*-(2-Phenylquinolin-7-yl)thiophene-3-carboxamide (DB2191, 5d).** The reaction mixture was filtered to provide a white solid (230 mg, 77%); mp 228 – 229 °C. ^{1}H NMR (DMSO-d_6): δ 10.3 (s, 1H), 8.62 (s, 1H), 8.45 (s, 1H), 8.38 (d, *J* = 8.4 Hz, 1H), 8.27 (d, *J* = 7.2 Hz, 2H), 8.04 (d, *J* = 8.4 Hz, 1H), 7.98–7.90 (m, 2H), 7.70 (s, 2H), 7.57–7.48 (m,3H); ^{13}C NMR (DMSO-d_6): δ 161.2, 156.3, 148.2, 140.2, 138.7, 137.6, 136.6, 130.1, 129.5, 128.8, 127.9, 127.2, 127.1, 127.0, 123.6, 120.9, 117.4, 117.2 ; HRMS (ESI) calcd for $C_{20}H_{14}N_2OS$ (M^+ +H) 331.0905, found 331.0894; Anal. Calcd for $C_{20}H_{14}N_2OS$, 0.2 H_2O: C, 71.92, H, 4.17; N, 8.39. Found: C, 71.93; H, 4.20; N, 8.39.

***N*-(2-Phenylquinolin-7-yl)cyclopentanecarboxamide (DB2171, 5e).** The reaction mixture was filtered and recrystallized from hexanes/dichloromethane to provide a yellow solid (50 mg, 23%); mp 184 –186 °C. ^{1}H NMR (DMSO-d_6): δ 10.2 (s, 1H), 8.50 (s, 1H), 8.33 (d, *J* = 8.4 Hz, 1H), 8.24 (d, *J* = 7.2 Hz, 2H), 8.00 (d, *J* = 8.4 Hz, 1H), 7.90 (d, *J* = 8.4 Hz, 1H), 7.69 (d, *J* = 8.4 Hz, 1H), 7.56–7.49 (m, 3H), 2.93–2.60 (m, 1H), 1.93–1.90 (m, 2H), 1.80–1.71 (m, 4H), 1.66–1.58 (m, 2H) ; ^{13}C NMR (DMSO-d_6): δ 174.9, 156.2, 148.3, 140.3, 138.8, 136.6, 129.4, 128.8, 128.0, 127.1, 123.2, 120.1, 117.1, 116.0, 45.4, 30.1, 25.69 ; HRMS (ESI) calcd for $C_{20}H_{20}N_2O$ (M^+ +H) 317.1654, found 317.1646; Anal. Calcd for $C_{20}H_{20}N_2O$, 0.3 CH_2Cl_2: C, 79.72, H, 6.37; N, 8.85. Found: C, 79.49; H, 6.43; N, 8.73.

General procedures for urea derivatives (7a-e) Triphosgene (70 mg, 0.23 mmol) was added to a reaction mixture of 2-phenylquinolin-7-amine (150 mg, 0.68 mmol, **4**) , triethylamine (0.19 mL, 1.36 mmol) in dichloromethane (20 mL) at 0 °C. Concentration of reactants is very important to minimize formation of symmetrical ureas [3]. The reaction mixture was stirred at 0 °C for 30 minutes, after which it was warmed to room temperature for 15 minutes. The appropriate amine was added (1.36 mmol) and the reaction mixture was stirred for 2 hours at room temperature. Accept as noted, the reaction mixture was concentrated under reduced pressure and purified by column chromatography on silica gel eluting with hexanes/ ethyl acetate (4:1 then 1:1, then 0:100 (w/5% MeOH) to provide solid product.

***N*-(2-Phenylquinolin-7-yl) pyrrolidine-1-carboxamide (DB2186, 7a).** An off-white solid (440 mg, 61%); mp 228 – 230 °C. ^{1}H NMR (DMSO-d_6): δ 8.47 (s, 1H), 8.32 (s, 1H), 8.28 (d, *J* = 8.4 Hz, 1H), 8.23 (d, *J* = 8.4 Hz, 2H), 7.94 (d, *J* = 8.4 Hz, 1H), 7.84–7.76 (m, 2H), 7.55–7.48 (m, 3H), 3.44 (bs, 4H), 1.88 (bs, 4H); ^{13}C NMR (DMSO-d_6): δ 155.9, 153.6, 148.4, 142.0, 138.9, 136.4, 129.3, 128.7, 127.4, 127.0, 122.5, 120.9, 116.4, 115.2, 45.8, 25.0; HRMS (ESI) calcd for $C_{20}H_{19}N_3O$ (M^+ +H) 318.1606, found 318.1594; Anal. Calcd for $C_{20}H_{19}N_3O$, 0.15 EtOAc: C, 74.84, H, 6.16, N, 12.71. Found: C, 74.85; H, 6.15; N, 12.47.

***N*-(2-Phenylquinolin-7-yl)-2,5-dihydro-1*H*-pyrrole-1-carboxamide (DB2192, 7b).** The reaction mixture was filtered to provide a white solid (155 mg, 72%); mp 266 – 269 °C dec,. ^{1}H NMR (DMSO-d_6): δ 8.41 (s, 1H), 8.31 (m, 1H), 8.28 (d, *J* = 8.4 Hz, 1H), 8.23 (d, *J* = 8.4 Hz, 2H), 7.92 (d, *J* = 8.4 Hz, 1H), 7.84–7.79 (m, 2H), 7.55–7.47 (m, 3H), 5.95 (s, 2H), 4.28 (s, 4H); ^{13}C NMR (DMSO-d_6): δ 155.8, 153.2. 148.2, 141.6, 138.8, 136.0, 128.9, 128.3, 127.0, 126.7, 125.4, 122.3, 120.7, 116.2, 115.4, 52.6 ; HRMS (ESI) calcd for $C_{20}H_{18}N_3O$ (M^+ +H) 316.1450, found 316.1447; Anal. Calcd for $C_{20}H_{17}N_3O$, 0.2 H_2O: C, 75.31, H, 5.50, N, 13.17. Found: C, 75.15; H, 5.38; N, 13.07.

***N*-(2-Phenylquinoline-7-yl)piperidine-1-carboxamide (DB2187, 7c).** An off-white solid (219 mg, 73%); mp 187 –188 °C. ^{1}H NMR (DMSO-d_6): δ 8.84 (s, 1H), 8.28 (d, *J* = 8.4 Hz, 1H), 8.23 (d, *J* = 8.4 Hz, 1H), 7.96 (d, *J* = 8.4 Hz, 1H), 7.82 (d,

J = 8.4 Hz, 1H), 7.71 (d, *J* = 8.4 Hz, 1H), 7.55–7.45 (m, 3H), 3.48 (bs, 4H), 1.59 (bs, 2H), 1.53 (bs, 4H); ^{13}C NMR (DMSO-d_6): δ 155.9, 154.6, 148.4, 142.2, 138.9, 136.4, 129.3, 128.7, 127.3, 127.0, 122.4, 121.0, 116.4, 115.3, 44.7, 25.5, 24.0 ; Anal. Calcd for $C_{21}H_{21}N_3O$: C, 76.11, H, 6.39, N, 12.68. Found: C, 76.31; H, 6.55; N, 12.71.

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***N*-(2-Phenylquinolin-7-yl)-5,6-dihydropyridine-1(2*H*)-carboxamide (DB2217, 7d). 7d).** A yellow solid (80 mg, 26%); mp 190 –191 °C. ^{1}H NMR (DMSO-d_6): δ 8.85 (s, 1H), 8.29 (d, *J* = 8.4 Hz, 1H), 8.26–8.23 (m, 3H), 7.95 (d, *J* = 8.4 Hz, 1H), 7.84 (d, *J* = 8.4 Hz, 1H), 7.73 (d, *J* = 8.4 Hz, 1H), 7.56–7.48 (m, 3H), 5.87 (d, *J* = 6.2 Hz, 1H), 5.78 (d, *J* = 6.2 Hz, 1H), 4.02 (s, 2H), 3.59 (t, *J* = 5.2 Hz, 2H), 2.17 (bs,, 2H) ; ^{13}C NMR (DMSO-d_6): δ 156.0, 154.8, 148.4, 142.0, 138.9, 136.5, 129.3, 128.8, 127.4, 127.0, 125.2, 124.6, 122.6, 121.0, 116.5, 115.6, 43.7, 40.3, 24.9; Anal. Calcd for $C_{21}H_{19}N_3O$: C, 76.57, H, 5.81, N, 12.76. Found: C, 76.37; H, 5.68; N, 12.70.

***N*-(2-Phenylquinolin-7-yl)azepane-1-carboxamide (DB2212, 7e).** A yellow solid (175 mg, 68%); mp 227 –228 °C. ^{1}H NMR (DMSO-d_6): δ 8.55 (s, 1H), 8.31–8.23 (m, 4H), 7.95 (d, *J* = 8.4 Hz, 1H), 7.83 (d, *J* = 8.4 Hz, 1H), 7.76 (d, *J* = 8.4 Hz, 1H), 7.56–7.47 (m, 3H), 3.52 (t, *J* = 12.4 Hz, 4H), 3.37 (s, 2H), 1.71 (bs, 4H), 1.53 (bs. 4H); ^{13}C NMR (DMSO-d_6): δ 160.2, 156.4, 155.3, 142.7, 136.9, 131.2, 129.8, 129.2, 127.8, 127.5, 123.0, 121.7, 116.9, 116.1, 46.7, 28.6, 27.7; HRMS (ESI) calcd for $C_{22}H_{23}N_3O$ (M^+ +H) 346.1919, found 346.1927; Anal. Calcd for $C_{22}H_{23}N_3O$: C, 76.49, H, 6.71, N, 12.16. Found: C, 76.41; H, 6.71; N, 12.11.

References

1. Li, A-H.; Beard, D.J.; Coate, H.; Honda, A.; Kadalbajoo, M.; Kleinberg, A.; Laufer, R.; Mulvihil, K. m.;Nigro, A.; Rastogi, P.; Sherman, D.; Siu, K. W.;Steinig, A. G.; Wang, T.; Werner, D.; Crew, A. P.; Mulvihill, M. J. *Synthesis*, **2010**, 1678-1986.

2. Wolfe, J. P.; Ahaman, J.; Sadighi, J. P.; Singer, R. A.; Buchwald, S. L. *Tetrahedron Lett.* **1997**, 38, 6367-6370.

3. Majer, P; Randad, R. S. *J. Org. Chem.* **1994**, 59, 1937-1938.