Rational Approaches to Discovery of Orally Active and Brain Penetrable Quinazolinone Inhibitors of Poly(ADP-Ribose)Polymerase

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Supporting Information

Experimental method

Solid phase synthesis of quinazolinone derivatives.

1. A solution of anthranilic acid derivatives (5eq), pyridine (5eq), 1,3-diisopropylcarbodiimide(5eq), and DMAP(1eq) in DMF (5mL) was added to a reaction vessel containing Rink amide resin (support-bound secondary amine, 1.0g, 0.59mmol/g,loading). After the vessel was shaken for 12h at ambient temperature, the resin was washed with dichloromethane and THF, DMF, and dichloromethane.
2. Diisopropylethyamine(10eq), 4-bromobutyl chloride(10eq), and DMAP (1eq) were added to a mixture of the obtained resin in dichloromethane (10mL). After the vessel was shaken for 12h at ambient temperature, the resin was washed with dichloromethane and THF, DMF, and dichloromethane.
3. Cyclic amine derivatives (10eq), diisopropylethyamine(10eq), and DMAP (1eq) were added to a mixture of the obtained resin in N-methyl-2-pyrrolidinone (10mL). After the vessel was shaken for 12h at ambient temperature, the resin was washed with dichloromethane and THF, DMF, and dichloromethane.
4. Cleavage from resin was performed with 50% trifluoroacetic acid in dichloromethane (10mL) for 30min at ambient temperature. After the filtrated solvent was evaporated under pressure, the residue was dissolved in dioxane (5mL). An aqueous solution of sodium hydroxide (1M, 5mL) was added to the solution at room temperature, and the mixture was stirred at that temperature for 15h. The organic materials were extracted with chloroform, and the organic layer was washed with water and dried over sodium sulfate. Appropriate purification of the crude product by column chromatography on silica gel or HPLC gave to the desired product.

Liquid phase synthesis for quinazolinone derivatives 4.

1. Under a nitrogen atmosphere, a solution of 4-bromobutyryl chloride (4.9g, 26.4mmol) in dichloromethane (10mL) was added dropwise to a solution of 2-aminobenzamide (3.0g, 22mmol) in pyridine (18mL, 220mmol) and dichloromethane (15mL) at 0°C. The mixture was stirred for 1.5h at 0°C. The reaction mixture was poured into ice-cooled 1N hydrochloric acid, and the product was extracted with chloroform. The organic layer was washed with 1N hydrochloric acid and water and dried over sodium sulfate. The crude product was triturated with toluene to give 2-[(4-bromobutanoyl)amino]benzamide (5.11g, 81.3%) as a powder.

   1H NMR (200MHz, CDCl3) δ 2.29(2H, quint., J=6.8 Hz), 2.61(2H, t, J=7.2 Hz), 3.52(2H, t, J=6.4 Hz), 5.5-6.5(2H, br), 7.09(1H, dt, J=7.6, 1.1 Hz), 7.51(1H, t, J=7.6 Hz), 7.53(1H, d, J=7.6 Hz), 8.62(1H, d, J=8.5 Hz), 11.25(1H, s).

   API-ESMS: 307 (M^++Na).

2. Under a nitrogen atmosphere, triethylamine (0.73mL, 5.26mmol) was added to a solution of 2-[(4-bromobutanoyl)amino]benzamide (500mg, 1.75mmol) and 4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (412mg, 2.10mmol) in N,N-dimethylformamide (5mL) at 0°C. The mixture was allowed to warm to room temperature and stirred for 24h. The reaction was quenched with water, and the product was extracted with chloroform. The organic layer was washed with water and dried over sodium sulfate. Purification over silica gel chromatography gave 2-{[4-(4-phenyl-3,6-dihydro-1(2H)-pyridinyl)butanoyl]amino}benzamide (477mg, 74.8%) as a pale-yellow powder.

   1H NMR (200MHz, CDCl3) δ 2.01(2H, quint., J=7.3 Hz), 2.41-2.56(4H, m), 2.72(2H, t, J=5.4 Hz), 3.76(2H, d, J=5.7 Hz), 5.4-6.3(2H, br), 6.05(1H, m), 7.05(1H, t, J=7.0 Hz), 7.21-7.37(6H, m), 7.45-7.51(2H, m), 8.64(1H, d, J=8.6 Hz).

   API-ESMS: 364 (M^++H).

3. 2-{[4-(4-Phenyl-3,6-dihydro-1(2H)-pyridinyl)butanoyl]amino}benzamide (475mg, 1.31mmol) was dissolved in dioxane (5mL). An aqueous solution of sodium hydroxide (1M, 3.92mL) was added to the solution at room temperature, and the mixture was stirred at that temperature for 15h. The organic materials were extracted with chloroform, and the organic layer was washed with water and dried over sodium sulfate. Recrystalization of the crude product from chloroform-methanol gave 2-{3-[4-phenyl-3,6-dihydro-1(2H)-pyridinyl]propyl}-4(3H)-quinazolinone **4** (329mg, 72.9%).

   1H NMR (200MHz, CDCl3) δ 2.05 (2H, quint., J=6.0 Hz), 2.66 (2H, t, J=6.0 Hz), 2.81-2.94 (4H, m), 3.31 (2H, d, J=3.2 Hz), 6.12 (1H, t, J=2.9 Hz), 7.21-7.49 (7H, m), 7.61-7.72 (2H, m), 8.23 (1H, d, J=6.6 Hz).

   API-ESMS: 346 (M^++H).

   Anal. Calcd for $C_{22}H_{23}N_3O$: C, 76.49; H, 6.71; N, 12.16. Found: C, 76.15; H, 6.75; N, 12.12.

3: 5-chloro-2-{3-[4-phenyl-3,6-dihydro-1(2H)-pyridinyl]propyl}-4(3H)-quinazolinone

1H NMR (200MHz, DMSO-d6): δ 1.8-2.0(2H, m), 2.2-2.8(8H, m), 2.9-3.1(2H, m), 6.05(1H, m), 7.1-7.8(7H, m), 8.01(1H, d, 1.6Hz)

API-ESMS: 380 (M^++H).

Anal. Calcd for $C_{22}H_{22}ClN_3O$: C, 69.56; H, 5.84; N, 11.06. Found: C, 69.54; H, 5.88; N, 11.03.

5: 5-fluoro-2-[3-(4-phenyl-3,6-dihydro-1(2H)-pyridinyl)propyl]-4(3H)-quinazolinone

1H NMR (200MHz, CDCl3) δ 2.04(2H, q., J=6.1 Hz), 2.66(2H, t, J=6.0 Hz), 2.7-3.0(6H, m), 3.31(2H, m), 6.10(1H, m), 7.04(1H, dd, J=10.5, 8.2 Hz), 7.2-7.5(6H, m), 7.63(1H, dt, J=8.1, 5.4 Hz).

API-ESMS: 364 (M^++H).

Anal. Calcd for $C_{22}H_{22}FN_3O + 0.12H_2O$: C, 72.28; H, 6.13; N, 11.49. Found: C, 72.26; H, 6.03; N, 11.44.

6: 6-chloro-2-{3-[4-phenyl-3,6-dihydro-1(2H)-pyridinyl]propyl}-4(3H)-quinazolinone

1H NMR (200MHz, DMSO-d6): δ 1.8-2.0(2H, m), 2.2-2.8(8H, m), 2.9-3.1(2H, m), 6.05(1H, m), 7.1-7.5(5H, m), 7.61(1H, d, J = 8Hz), 7.78(1H, dd, J = 8.0, 1.6Hz), 8.01(1H, d, 1.6Hz)

API-ESMS: 380 (M^++H).

Anal. Calcd for $C_{22}H_{22}ClN_3O + 0.19H_2O$: C, 68.94; H, 5.88; N, 10.96. Found: C, 68.94; H, 5.76; N, 10.96.

7: 7-chloro-2-{3-[4-phenyl-3,6-dihydro-1(2H)-pyridinyl]propyl}-4(3H)-quinazolinone

1H NMR (200MHz, DMSO-d6): δ 1.8-2.0(2H, m), 2.2-2.8(8H, m), 3.0-3.2(2H, m), 6.08(1H, m), 7.1-7.5(6H, m), 7.65(1H, d, J = 2.0Hz), 8.02(1H, d, J = 8.0Hz)

API-ESMS: 380 (M^++H).

Anal. Calcd for $C_{22}H_{22}ClN_3O$: C, 69.56; H, 5.84; N, 11.06. Found: C, 69.50; H, 5.81; N, 10.99.

8: 8-chloro-2-{3-[4-phenyl-3,6-dihydro-1(2H)-pyridinyl]propyl}-4(3H)-quinazolinone

1H NMR (200MHz, DMSO-d6): δ 1.8-2.1(2H,m), 2.3-2.8(8H,m), 3.05(2H, br.s), 6.07(1H, m), 7.2-7.5(5H, m), 7.86(1H, dd, J = 8.0,1.4Hz), 7.97(1H, dd, J = 8.0,1.4Hz)

API-ESMS: 380 (M^++H).

Anal. Calcd for $C_{22}H_{22}ClN_3O + 0.29H_2O$: C, 68.61; H, 5.91; N, 10.91. Found: C, 68.60; H, 5.79; N, 10.91.

9: 6,8-dichloro-2-{3-[4-phenyl-3,6-dihydro-1(2H)-pyridinyl]propyl}-4(3H)-quinazolinone

1H NMR (200MHz, DMSO-d6): δ 1.8-2.0(2H, m), 2.2-2.8(8H, m), 3.0-3.2(2H, m), 5.99(1H, m), 7.2-7.4(5H, m), 7.80(1H, d, J = 1.4Hz), 8.02(1H, d, J = 1.2Hz)

API-ESMS: 415 (M^++H).

Anal. Calcd for $C_{22}H_{21}Cl_2N_3O + 1.10H_2O$: C, 60.86; H, 5.39; N, 9.68. Found: C, 60.52; H, 4.92; N, 9.75.

10: 8-methyl-2-[3-(4-phenyl-3,6-dihydro-1(2H)-pyridinyl)propyl]-4(3H)-quinazolinone

1H NMR (200MHz, CDCl3) δ 2.05 (2H, q, J=6.0 Hz), 2.54(3H, s), 2.66 (2H, t, J=6.0 Hz), 2.81-2.94 (4H, m), 3.30 (2H, d, J=3.2 Hz), 6.12 (1H, t, J=2.9 Hz), 7.21-7.72 (8H, m), 8.10 (1H, d, J=7.0 Hz).

API-ESMS: 360 (M^++H).

Anal. Calcd for $C_{23}H_{25}N_3O$: C, 76.85; H, 7.01; N, 11.69. Found: C, 76.83; H, 7.19; N, 11.53.

11: 8-ethyl-2-[3-(4-phenyl-3,6-dihydro-1(2H)-pyridinyl)propyl]-4(3H)-quinazolinone

1H NMR (200MHz, CDCl3) δ 1.29(3H, t, J = 7.4Hz), 2.05 (2H, q, J=6.0 Hz), 2.66 (2H, t, J=6.0 Hz), 2.81-2.94 (4H, m), 3.09(2H, q, J = 7.4Hz), 3.20 (2H, d, J=3.2 Hz), 6.10 (1H, t, J=2.9 Hz), 7.21-7.72 (8H, m), 8.09 (1H, d, J=7.0 Hz).

API-ESMS: 374 (M^++H).

Anal. Calcd for $C_{24}H_{27}N_3O + 0.26H_2O$: C, 76.22; H, 7.33; N, 11.11. Found: C, 76.23; H, 7.09; N, 11.10.

12: 8-methoxy-2-[3-(4-phenyl-3,6-dihydro-1(2H)-pyridinyl)propyl]-4(3H)-quinazolinone

1H NMR (200MHz, CDCl3) δ 2.05 (2H, q, J=6.0 Hz), 2.68 (2H, t, J=6.0 Hz), 2.81-2.94 (4H, m), 3.30 (2H, d, J=3.2 Hz), 4.01 (3H, s), 6.10 (1H, t, J=2.9 Hz), 7.21-7.72 (8H, m7.82 (1H, d, J=8.0 Hz).

API-ESMS: 376 (M^++H).

Anal. Calcd for $C_{23}H_{25}N_3O2$: C, 73.58; H, 6.71; N, 11.19. Found: C, 73.26; H, 6.65; N, 11.03.

13: 2-[3-(4-phenyl-1-piperidinyl)propyl]-4(3H)-quinazolinone

1H NMR (200MHz, CDCl3) δ 1.4-1.8(6H, m), 1.9-2.1(2H, m), 2.7-3.2(8H, m), 7.44(1H, d, J = 8.0Hz), 7.5-7.8(2H, m), 8.27(1H, d, J = 8.0Hz).

API-ESMS: 348(M^++H).

Anal. Calcd for $C_{22}H_{25}N_3O + 0.25H_2O$: C, 75.08; H, 7.30; N, 11.94. Found: C, 74.99; H, 7.26; N, 11.85.

14: 2-[3-(4-phenyl-1-piperazinyl)propyl]-4(3H)-quinazolinone

1H NMR (200MHz, CDCl3) δ 2.05(2H, q, J=6.0 Hz), 2.62(2H, t, J=5.8 Hz), 2.78(4H, t, J=5.0 Hz), 2.8-3.0(2H, m), 3.45(4H, t, J=5.0 Hz), 6.87(1H, t, J=7.2 Hz), 6.98(2H, d, J=7.8 Hz), 7.28(2H, t, J=8.0 Hz), 7.42(1H, t, J=7.4 Hz), 7.6-7.8(2H, m), 8.23(1H, d, J=8.0 Hz), 12.92(1H, br s).

API-ESMS: 349 (M^++H).

Anal. Calcd for $C_{21}H_{24}N_4O$: C,72.39; H, 6.94; N, 16.08. Found: C, 72.35; H, 7.05; N, 15.94.

15: 2-[3-(4-phenyl-3-cyclohexen-1-yl)propyl]-4(3H)-quinazolinone

1H NMR (200MHz, CDCl3) δ 1.2-2.0(9H, m), 2.3-2.6(3H, m), 2.80(2H, t, J = 8.0H), 6.07(1H, m), 7.1-7.5(7H, m), 7.6-6.8(2H, m), 8.28(1H, d, J = 8.8Hz)

API-ESMS: 345 (M^++H).

Anal. Calcd for $C_{23}H_{24}N_2O$ + 0.16H_2O: C, 79.53; H, 7.06; N, 8.07. Found: C, 79.53; H, 7.07; N, 8.06.

16: 2-[3-(1,4,5,6-tetrahydrobenzo[f]isoquinolin-3(2H)-yl)propyl]-4(3H)-quinazolinone

1H NMR (200MHz, CDCl3) δ 2.06(2H, q, J=6.4 Hz), 2.20(2H, t, J=7.9 Hz), 2.65(2H, t, J=6.2 Hz), 2.7-3.0(8H, m), 3.20(2H, br s), 7.1-7.3(4H, m), 7.41(1H, t, J=7.3 Hz), 7.63(1H, d, J=6.9 Hz), 7.72(1H, t, J=7.4 Hz), 8.22(1H, d, J=7.8 Hz).

API-ESMS: 372. (M^++H).

Anal. Calcd for $C_{24}H_{25}N_3O$ + 0.12H_2O: C, 77.15; H, 6.81; N, 11.25. Found: C, 77.14; H, 6.84; N, 11.24.

17: 2-[3-(1,3,4,9-tetrahydro-2H-beta-carbolin-2-yl)propyl]-4(3H)-quinazolinone

1H NMR (200MHz, CDCl3) δ 2.05 (2H, q, J=6.0 Hz, 2.66 (2H, t, J=6.0 Hz), 2.81-2.94 (4H, m), 3.30 (2H, d, J=3.2 Hz), 6.12 (1H, t, J=2.9 Hz), 7.21-7.72 (8H, m), 8.10 (1H, d, J=7.0 Hz).

API-ESMS: 359 (M^++H).

Anal. Calcd for $C_{22}H_{22}N_4O$ + 2.05H_2O: C, 66.83; H, 6.65; N, 14.07. Found: C, 66.83; H, 6.35; N, 13.68.

18: 2-[3-(1,3,4,9-tetrahydro-2H-beta-carbolin-2-yl)propyl]-4(3H)-quinazolinone

1H NMR (200MHz, CDCl3) δ 1.4-1.8(6H, m), 1.9-2.1(2H, m), 2.7-3.2(8H, m), 7.44(1H, d, J = 8.0Hz), 7.5-7.8(2H, m), 8.27(1H, d, J = 8.0Hz).

API-ESMS: 272 (M^++H).

HRMS (EI) 272.1761 [calcd for $C_{16}H_{21}N_3O$ 272.1765]

19: 2-{3-[4-(4-fluorophenyl)-3,6-dihydro-1(2H)-pyridinyl]propyl}-4(3H)-quinazolinone

1H NMR (200MHz, CDCl3) δ 2.05(2H, q, J=6.1 Hz), 2.66(2H, t, J=5.9 Hz), 2.79-3.20 (8H, d, J=3.0 Hz), 7.1-7.7(7H, m), 8.21(1H, d, J = 6.6Hz)

API-ESMS: 364 (M^++H).

Anal. Calcd for $C_{22}H_{22}FN_3O$ + 0.25H_2O: C, 71.82; H, 6.16; N, 11.42. Found: C, 71.99; H, 6.14; N, 11.51.

20: 2-{3-[4-(3-fluorophenyl)-3,6-dihydro-1(2H)-pyridinyl]propyl}-4(3H)-quinazolinone

1H NMR (200MHz, CDCl3) δ 2.05(2H, quint., J=7.1 Hz), 2.66(2H, t, J=6.0 Hz), 2.7-3.0(6H, m), 3.30(2H, q, J=3.2 Hz), 6.13(1H, m), 6.95(1H, t, J=8.2 Hz), 7.1-7.5(4H, m), 7.6-7.8(2H, m), 8.23(1H, d, J=7.9 Hz), 12.55(1H, br).

API-ESMS: 364 (M^++H).

Anal. Calcd for $C_{22}H_{22}FN_3O$: C, 72.71; H, 6.10; N, 11.56. Found: C, 72.37; H, 6.07; N, 11.49.

21: 2-{3-[4-(2-fluorophenyl)-3,6-dihydro-1(2H)-pyridinyl]propyl}-4(3H)-quinazolinone

1H NMR (200MHz, CDCl3) δ 2.05(2H, q, J=6.4 Hz), 2.67(2H, t, J=6.2 Hz), 2.7-3.0(6H, m), 3.31(2H, q, J=3.2 Hz), 6.02(1H, m), 7.0-7.5(5H, m), 7.6-7.8(2H, m), 8.25(1H, d, J=7.8 Hz), 12.64(1H, br).

API-ESMS: 364 (M^++H).

Anal. Calcd for $C_{22}H_{22}FN_3O$: C, 72.71; H, 6.10; N, 11.56. Found: C, 72.56; H, 6.11; N, 11.51.

22: 2-{3-[4-(4-chlorophenyl-3,6-dihydro-1(2H)-pyridinyl)propyl]-4(3H)-quinazolinone

1H NMR (200MHz, DMSO-d6): δ 2.0-2.2(2H, m), 2.3-2.8(8H, m), 3.0-3.2(2H, m), 6.12(1H, m), 7.0-7.8(8H, m)

API-ESMS: 380 (M^++H).

Anal. Calcd for $C_{22}H_{22}ClN_3O$: C, 69.56; H, 5.84; N, 11.06. Found: C, 69.51; H, 5.90; N, 11.01.

23: 2-{3-[4-(4-methylphenyl)-3,6-dihydro-1(2H)-pyridinyl]propyl}-4(3H)-quinazolinone

1H NMR (200MHz, CDCl3) δ 2.05(2H, q, J=6.2 Hz), 2.35(3H, s), 2.65(2H, t, J=6.0 Hz), 2.78-2.93(6H, m), 3.30(2H, d, J=3.2 Hz), 6.06(1H, m), 7.15(2H, d, J=8.1 Hz), 7.35(2H, d, J=8.2 Hz), 7.43(1H, d, J=6.5 Hz), 7.65(1H, t, J=6.9 Hz), 7.71(1H, t, J=8.2 Hz), 8.24(1H, dd, J=8.0, 1.2 Hz).

API-ESMS: 360 (M^++H).

Anal. Calcd for $C_{23}H_{25}N_3O + 0.48H_2O$: C, 75.04; H, 7.11; N, 11.41. Found: C, 75.05; H, 6.89; N, 10.98.

24: 2-{3-[4-(4-hydroxyphenyl)-3,6-dihydropyridin-1(2H)-yl]propyl}quinazolin-4(3H)-one 2.1-2.4(2H, m), 2.65-2.95(4H, m), 3.2-3.5(3H, m), 3.6-4.2(3H, m), 6.03(1H, s), 6.77(2H, d, J = 8.7Hz), 7.32(2H, d, J=8.7 Hz), 7.56(1H, t, J=7.3 Hz), 7.67(1H, d, J=8.1 Hz), 7.85(1H, t, J=7.4 Hz), 8.14(1H, dd, J=7.8, 1.2 Hz).

API-ESMS: 362 (M^++H).

HRMS (EI) 362.1862 [calcd for $C_{22}H_{23}N_3O_2$ 362.1869]

25: 2-{3-[4-(4-methoxylphenyl)-3,6-dihydro-1(2H)-pyridinyl]propyl}-4(3H)-quinazolinone

1H NMR (200MHz, CDCl3) δ 2.04(2H, quint., J=6.0 Hz), 2.65(2H, t, J=6.0 Hz), 2.79-2.93(6H, m), 3.29(2H, d, J=3.2 Hz), 3.82(3H, s), 6.01(1H, m), 6.88(2H, d, J=8.8 Hz), 7.37-7.46(3H, m), 7.63(1H, d, J=7.0 Hz), 7.71(1H, t, J=7.8 Hz), 8.23(1H, d, J=7.8 Hz).

API-ESMS: 376 (M^++H).

Anal. Calcd for $C_{23}H_{25}N_3O_2$: C, 73.58; H, 6.71; N, 11.19. Found: C, 73.41; H,6.81; N, 11.11.

26: 2-{3-[4-(3-methoxylphenyl)-3,6-dihydro-1(2H)-pyridinyl]propyl}-4(3H)-quinazolinone

1H NMR (200MHz, CDCl3) δ 2.05(2H, quint., J=7.2 Hz), 2.66(2H, t, J=6.0 Hz), 2.79-2.93(6H, m), 3.30(2H, d, J=1.6 Hz), 3.84(3H, s), 6.10(1H, m), 6.82(1H, dd, J=8.1, 2.6 Hz), 7.00(1H, t, J =2.3Hz), 7.06(1H, d, J = 7.9Hz), 7.26(1H, t, J = 7.9Hz), 7.41(1H, t, J = 7.3Hz), 7.6--7.8(2H, m), 8.23(1H, d, J=7.9 Hz).

API-ESMS: 376 (M^++H).

Anal. Calcd for $C_{23}H_{25}N_3O_2 + 0.25H_2O$: C, 72.70; H, 6.76; N, 11.06. Found: C, 72.72; H, 6.62; N, 11.20.

27: 2-{3-[4-(2-methoxylphenyl)-3,6-dihydro-1(2H)-pyridinyl]propyl}-4(3H)-quinazolinone

1H NMR (200MHz, CDCl3) δ 2.04(2H, quint., J=6.2 Hz), 2.66(2H, t, J=6.2 Hz), 2.79-2.93(6H, m), 3.29(2H, q, J=2.6 Hz), 3.84(3H, s), 5.83(1H, m), 6.88(2H, d, J=8.8 Hz), 6.95(1H, t, J = 7.4Hz), 7.2-7.3(2H, m), 7.42(1H, t, J = 7.3Hz), 7.6-7.8(2H, m), 8.23(1H, d, J=11.2 Hz).

API-ESMS: 376 (M^++H).

Anal. Calcd for $C_{23}H_{25}N_3O_2$: C, 73.58; H, 6.71; N, 11.19. Found: C, 73.18; H. 6.70; N, 11.06.

28: 2-{3-[4-(4-trifluoromethylphenyl)-3,6-dihydro-1(2H)-pyridinyl]propyl}-4(3H)-quinazolinone

1H NMR (200MHz, CDCl3) δ 2.06(2H, q, J=6.1 Hz), 2.68(2H, t, J=5.9 Hz), 2.83-2.94(6H, m), 3.33(2H, d, J=3.1 Hz), 6.18(1H, m), 7.41(1H, t, J=7.3 Hz), 7.53-7.76(6H, m), 8.23(1H, d, J=6.6 Hz).

API-ESMS: 413 (M^++H).

Anal. Calcd for $C_{23}H_{22}F_3N_3O + 0.34H_2O$: C, 65.86; H, 5.45; N, 10.02. Found: C, 65.96; H, 5.26; N, 9.98.

29: : 2-{3-[4-(4-cyanophenyl)-3,6-dihydro-1(2H)-pyridinyl]propyl}-4(3H)-quinazolinone

1H NMR (200MHz, CDCl3 δ 2.03(2H, q, J=6.0 Hz), 2.68(2H, t, J=5.9 Hz), 2.78-2.94(6H, m), 3.33(2H, q, J=3.3 Hz), 6.21(1H, m), 7.43(1H, t, J=8.1 Hz), 7.51-7.72(6H, m), 8.22(1H, dd, J=7.8, 1.1 Hz).

API-ESMS: 370 (M^++H).

Anal. Calcd for $C_{23}H_{22}N_4O + 0.19H_2O$: C, 73.89; H, 6.03; N, 14.99. Found: C, 73.90; H, 6.04; N, 15.01.

30: 2-{3-[4-(1,1'-biphenyl-4-yl)-3,6-dihydro-1(2H)-pyridinyl]propyl}-4(3H)-quinazolinone

1H NMR (200MHz, DMSO-d6) δ 1.97(2H, q, J=6.0 Hz), 2.4-2.5(4H, m), 2.6-2.8(4H, m), 3.12(2H, br s), 6.20(1H, m), 7.3-7.5(6H, m), 7.5-7.8(6H, m), 8.06(1H, d, J=7.9 Hz), 12.49(1H, br s).

API-ESMS: 422 (M^++H).

Anal. Calcd for $C_{28}H_{27}N_3O + 0.80H_2O$: C, 77.14; H, 6.61; N, 9.64. Found: C, 77.14; H, 6.19; N, 9.47.

31: 2-[3-(3,6-dihydro-4,4'-bipyridin-1(2H)-yl)propyl]-4(3H)-quinazolinone

1H NMR (200MHz, CDCl3) δ 2.06(2H, q, J=6.1 Hz), 2.68(2H, t, J=6.0 Hz), 2.7-3.0(6H, m), 3.33(2H, d, J=3.3 Hz), 6.33(1H, br s), 7.33(2H, d, J=6.2 Hz), 7.41(1H, t, J=7.4 Hz), 7.64(1H, d, J=7.0 Hz), 7.72(1H, t, J=7.5 Hz), 8.22(1H, d, J=7.9 Hz), 8.57(2H, d, J=6.2 Hz), 12.49(1H, br).

M API-ESMS: 347 (M^++H).

Anal. Calcd for $C_{21}H_{22}N_4O$: C, 72.81; H, 6.40; N, 16.17. Found: C, 72.62; H, 6.36; N, 16.15.

32: 8-chloro-2-{3-[4-(4-fluorophenyl)-3,6-dihydro-1(2H)-pyridinyl]propyl}-4(3H)-quinazolinone

1H NMR (200MHz, DMSO-d6): δ 1.8-2.1(2H,m), 2.2-2.8(8H,m), 3.3(2H, br.s), 6.03(1H, m), 7.0-7.2(2H, m), 7.3-7.6(2H, m), 7.42(1H, t, J=8.0Hz), 7.90(1H,dd, J=8.0, 1.4Hz), 7.99(1H,dd, J = 8.0,1.4Hz)

API-ESMS: 398 (M^++H).

Anal. Calcd for $C_{22}H_{21}FClN_3O + 1.19H_2O$: C, 63.02; H, 5.42; N, 10.02. Found: C, 63.01; H, 5.10; N, 9.92.

Crystallography of 1/PARP. Cocrystals of human recombinant PARP were obtained using procedures similar to those previously described.[11] In this study, 2.1-2.2 M ammonium sulphate, 2% PEG400, 0.1 M Tris-HCl at pH 8.5 were used as precipitant. X-ray diffraction data were collected from this *C*2 form: a = 179.96 Å, b = 53.27 Å, c = 91.47 Å β = 113.8°, at SP-ring8 beamline 32B2. Data

resolution is from 30.0-3.0 Å; 86444 observations were scaled and merged into 15831 unique reflections using Crystal Clear (RIGAKU). The overall *R*-merge is 7.0 %, the ration $I/\sigma(I)$ is 9.4, and the data is 99.3 % complete. Corresponding values for the high-resolution data shell (3.18-3.00 Å) are 22.7 %, 2.2, and 98.7 %, respectively. The structure of the **1**/PARP complex was solved and refined using this data, program AmoRe[19] and X-PLOR (Accelrys), and the protein model 1UK0 from the Protein Data Bank[11]. The conventional and free R-factors after refinement are 24.2 % and 27.4 %. The rms deviations between model and ideal bond distances, bond angles, dihedral angles, and improper angles are 0.018 Å, 3.8 °, 28.8 °, and 2.58 °. The model coordinates have been deposited in the Protein Data Bank with the code 1UK1.

(19) Navaza, J. On the computation of the fast rotation function. *Acta. Crystallogr.* **1993**, *D49*, 588-591.

PARP-1 Inhibition Assay

To assess PARP-1 inhibitory activity, the previously described method of Banasik and co-workers[5b] with minor modifications was used. Purified human-PARP (0.2 units, TREVIGEN, Gaithersburg, MD) was added to 96-well plates containing assay buffer [50mM Tris, pH 8.0, 25mM $MgCl_2$, 1mM Dithiothreitol], 0.05mM NAD (SIGMA), 1μCi/mL ^{32}P-NAD (NEN), 1 μg/mL activated DNA (TREVIGEN, Gaithersburg, MD) and various concentrations of inhibitor, 5% (v/v) DMSO. Fifteen minutes after mixing the plate at room temperature, the reaction was stopped by addition of 2 volumes of ice-cold 20% (v/v) trichloroacetic acid and the plate was kept at 4°C until measurement. The sample was transferred to the UniFilter™-96, GF/B (Packard, Meriden, CT) using the harvester for a 96-well plate, UniFilter™-96 Packard, Meriden, CT). This filter was washed with 10% (v/v) trichloroacetic acid once before and 2-times after this transfer. After drying for at least 2 hours, 50 μL of liquid scintillator was added to each well and radioactivity was measured using a Liquid Scintillattion Counter, Top-Count™ (Packard, Meriden, CT). IC_{50} values for inhibition of PARP activity were calculated using GraphPad Software, Prism (Version 3.0).

Ex vivo Assay (Measurement of brain and plasma concentrations)

Compounds were administered (po or ip; 10 or 32mg/kg) to nine-week-old male C57BL/6NCrj mice (Charles River, Atsugi, Japan). After 0.5 or 2 hours, blood was collected into a heparinized tube on ice, and saline-perfused brains were removed, and placed on dry ice. Plasma was separated from blood by centrifugation at 11,000g for 10 minutes at 4°C; samples were stored at –80°C until use. 500μL of homogenized brain tissue added 1mL of saline or 200μL from plasma was admitted into a tube. Various concentration of inhibitors were added to untreated tissue samples (500 μL homogenized brain tissue; 200μL plasma) to generate a standard curve. All tissue samples were sonicated for 20 minutes after adding 5mL of ethanol. After centrifugation at 1,400g. for 10 minutes at 15°C, 4mL of supernatant was taken and dried under nitrogen gas at 50°C. Samples were redissolved in 1mL of DMSO and tissue concentration was determined in the PARP inhibition assay by comparison with the standard curve (Prism 3.0, GraphPad Software).

In Vitro Metabolism by liver microsomes.

32 was incubated with a reaction mixture consisting of mouse, rat, dog, human liver microsomal protein and NADPH-generating system (2 mmol/L $NADP^+$, 10mmol/L glucose-6-phophate, 0.4 Unit/mL glucose-6-phosphate dehydrogenase and 5mmol/L $MgCl_2$) in the presence of 100 mmol/L potassium phosphate buffer (PH 7.4). After pre-incubation at 37°C for 5 min, enzyme reactions were initiated by addition of **32**. The final concentration of **32** was 1μmol/L. The microsomal protein concentration was 1 mg/mL. After incubation at 37°C for various time periods, aliquots of the reaction mixture were withdrawn. They were quickly added into two times volume of methanol to terminate the reactions. After they were centrifuged at 14000 rpm for 5 min, the supernatants were injected into HPLC to measure the unchanged **32** concentration. Clint values were calculated from **32** disappearance rate in microsomes.

Table

Compound	PARP-1 IC50±SE (nM)	Brain±SE (μg/g tissue) 0.5h	2.0h	Plasma±SE (μg/mL) 0.5h	2.0h
1	60±0.12				
2	1200±60				
3	65±1.0	4.60±0.43	2.82±0.26	4.18±0.78	2.17±0.26
4	16±0.55	1.90±0.17	0.37±0.072	0.68±0.26	0.15±0.05
5	16±0.2	1.62±0.67	0.22±0.059	0.37±0.019	0.14±0.030
6	27±0.9	18.6±2.62	14.2±0.56	16.2±1.10	15.5±0.040
7	250±5	6.12±1.52	2.66±0.34	0.08±0.037	0.51±0.49
8	26±0.85	7.99±1.20	8.43±0.083	1.28±0.032	1.20±0.030
9	39±1.55				
10	14±0.25	2.54±0.46	0.73±0.075	0.94±0.19	0.40±0.090
11	66±0.4				
12	26±0.15	1.15±0.51	0	0.24±0.14	0
13	46±0.6	0.37±0.10	0.10±0.02	0.25±0.087	0
14	71±0.6	2.85±0.034	0.30±0.041	2.27±0.31	0.53±0.12
15	>1000				
16	12±0.25				
17	74±0.35	0.58±0.21	0.15±0.017	77.2±2.75	9.51±3.75
18	1100±12				
19	8.9±0.35	3.54±0.64	1.16±0.27	6.83±0.11	3.48±0.24
20	23±0.85				
21	37±0.9				
22	23±0.7	23.9±1.82	9.41±3.13	12.1±3.4	2.78±0.68
23	17±0.6	5.31±0.048	2.34±0.55	5.40±0.25	0.84±0.52
24	12±0.6				
25	8.3±0.4	5.61±0.44	3.75±0.41	6.05±0.51	1.67±0.44
26	1045±55				
27	170±2.5				
28	25±1.15	23.4±2.04	19.5±2.3	6.80±2.99	3.27±0.33
29	6.0±0.15	4.50±0.074	0.54±0.35	20.0±2.79	5.20±0.48
30	34±0.55				
31	12±0.25	2.76±0.52	0.30±0.21	14.1±1.71	0.62±0.072
3-AB	11200±200				