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A facile metal-free approach to *N*,*N*'-bis(1-oxidopyrimidin-4-yl)diamines with promising biological activity

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General experimental details

^{1}H and ^{13}C NMR spectra were recorded on a spectrometer Agilent 400MR (400.0 MHz for ^{1}H and 100.6 MHz for ^{13}C) at room temperature; chemical shifts δ were measured with reference to the solvent ^{1}H ($CDCl_3$, δ = 7.26 ppm; CD_3OD, δ = 3.31 ppm) and ^{13}C ($CDCl_3$, δ = 77.1 ppm; CD_3OD, δ = 49.0 ppm). When necessary, assignments of signals in NMR spectra were made using 2D techniques. Accurate mass measurements (HRMS) were measured on JEOL GCMate II mass spectrometer (70 eV). Microwave synthesis was carried out in reactor Monowave-300, Anton Paar GmbH, operating pressure 30 bar. Analytical thin layer chromatography was carried out with silica gel plates (supported on aluminum); the detection was done by UV lamp (254 nm). Column chromatography was performed on silica gel (0.015–0.04 mm). Pyrimidine *N*-oxides **1a,b** were obtained as described: K. N. Sedenkova, E. B. Averina, Y. K. Grishin, A. B. Bacunov, S. I. Troyanov, I. V. Morozov, E. B. Deeva, A. V. Merkulova, T. S. Kuznetsova and N. S. Zefirov, *Tetrahedron Lett*., 2015, **56**, 4927.

***N*-(2-Methyl-1-oxido-5,6,7,8-tetrahydroquinazolin-4-yl)butane-1,4-diamine (2c).** Yield 45 mg (36%); colourless oil; R_f = 0.1 (MeOH); δ: 1.44–1.57 (m, 2H, CH_2, diamine), 1.60–1.73 (m, 2H, CH_2, diamine), 1.72–1.84 (m, 4H, $2CH_2$, cy-Hex), 2.27 (br t, 2H, $^{3}J_{HH}$ 6.2, CH_2, cy-Hex), 2.60 (s, 3H, CH_3), 2.75 (t, 2H, $^{3}J_{HH}$ 6.8, C**H**$_2$NH$_2$), 2.89 (br t, 2H, $^{3}J_{HH}$ 6.0, CH_2, cy-Hex), 3.46 (dt, 2H, $^{3}J_{HH}$ 5.3, $^{3}J_{HH}$ 6.8, C**H**$_2$NH), 5.05 (br t, 1H, $^{3}J_{HH}$ 5.3, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.2 (CH_3), 20.9 (CH_2, cy-Hex), 21.0 (CH_2, cy-Hex), 22.2 (CH_2, cy-Hex), 24.5 (CH_2, cy-Hex), 26.9 (CH_2), 30.7 (CH_2), 41.1 (CH_2N), 41.6 (CH_2N), 111.6 (C4a), 151.7 (C4), 153.1 (C8a), 154.7 (C2); HRMS (ESI$^+$, 70 eV, m/z): calcd. for $C_{13}H_{22}N_4O$ [M+H]: 251.1866, found: 251.1858.

***N*-(2-*tert*-Butyl-1-oxido-5,6,7,8-tetrahydroquinazolin-4-yl)ethane-1,2-diamine (2d).** Yield 46 mg (35%); yellow crystals; m.p. 154–157ºC (from MeOH); R_f = 0.4 (MeOH:NH_4OH 2:1); ^{1}H

NMR (400 MHz, CD_3OD) δ: 1.50 (s, 9H, $3CH_3$), 1.73–1.86 (m, 4H, $2CH_2$, cy-Hex), 2.41 (br t, 2H, $^3J_{HH}$ 5.9, CH_2, cy-Hex), 2.81 (br t, 2H, $^3J_{HH}$ 6.0, CH_2, cy-Hex), 2.99 (t, 2H, $^3J_{HH}$ 6.0, C**H**$_2$NH$_2$), 3.63 (t, 2H, $^3J_{HH}$ 6.0, C**H**$_2$NH); ^{13}C NMR (100 MHz, CD_3OD) δ: 20.4 (CH_2, cy-Hex), 20.9 (CH_2, cy-Hex), 21.8 (CH_2, cy-Hex), 24.2 (CH_2, cy-Hex), 26.4 ($3CH_3$), 38.5 (C, *t*-Bu), 39.6 (CH_2NH_2), 41.6 (CH_2NH), 113.2 (C4a), 153.5 (C4), 154.5 (C8a), 161.4 (C2); HRMS (ESI^+, 70 eV, m/z): calcd. for $C_{14}H_{24}N_4O$ [M+H]: 265.2023, found: 265.2021.

***N*-(2-*tert*-Butyl-1-oxido-5,6,7,8-tetrahydroquinazolin-4-yl)propane-1,3-diamine (2e).** Yield 65 mg (47%); colourless crystals; m.p. 158–162ºC (from $CDCl_3$); R_f = 0.1 (MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.50 (s, 9H, $3CH_3$), 1.71–1.86 (m, 4H, $2CH_2$, cy-Hex), 1.92 (tt, 2H, $^3J_{HH}$ 6.6, $^3J_{HH}$ 6.2, CH_2, diamine), 2.26 (br t, 2H, $^3J_{HH}$ 5.9, CH_2, cy-Hex), 2.77 (t, 2H, $^3J_{HH}$ 6.1, CH_2, cy-Hex), 2.85–2.91 (m, 2H, C**H**$_2$NH$_2$), 3.58 (t, 2H, $^3J_{HH}$ 6.6, C**H**$_2$NH); ^{13}C NMR (100 MHz, CD_3OD) δ: 20.4 (CH_2, cy-Hex), 20.8 (CH_2, cy-Hex), 21.8 (CH_2, cy-Hex), 24.2 (CH_2, cy-Hex), 26.3 ($3CH_3$), 28.6 (CH_2), 37.7 (CH_2NH_2), 37.9 (CH_2NH), 38.5 (C, *t*-Bu), 112.8 (C4a), 153.4 (C), 154.4 (C), 161.5 (C2); HRMS (ESI^+, 70 eV, m/z): calcd. for $C_{15}H_{26}N_4O$ [M+H]: 279.2175, found: 279.2179.

***N*-(2-*tert*-Butyl-1-oxido-5,6,7,8-tetrahydroquinazolin-4-yl)butane-1,4-diamine (2f).** Yield 67 mg (46%); colourless crystals; m.p. 164–166ºC (from MeOH); R_f = 0.1 (MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.42 (s, 9H, $3CH_3$), 1.60–1.77 (m, 8H, $2CH_2$, cy-Hex + $2CH_2$, diamine), 2.33 (br t, 2H, $^3J_{HH}$ 5.8, CH_2, cy-Hex), 2.74 (br t, 2H, $^3J_{HH}$ 5.6, CH_2, cy-Hex), 2.89 (t, 2H, $^3J_{HH}$ 7.1, C**H**$_2$NH$_2$), 3.56 (t, 2H, $^3J_{HH}$ 6.2, C**H**$_2$NH); ^{13}C NMR (100 MHz, CD_3OD) δ: 20.6 (CH_2, cy-Hex), 21.1 (CH_2, cy-Hex), 21.9 (CH_2, cy-Hex), 24.3 (CH_2, cy-Hex), 24.6 (CH_2), 25.6 (CH_2), 27.0 ($3CH_3$), 38.8 (C, *t*-Bu), 38.9 (CH_2NH_2), 40.2 (CH_2NH), 112.6 (C4a), 153.3 (C), 154.2 (C), 161.7 (C2); HRMS (ESI^+, 70 eV, m/z): calcd. for $C_{16}H_{28}N_4O$ [M+H]: 293.2345, found: 293.2336.

***N*-[(3-Aminomethyl-1-adamantyl)methyl]-2-methyl-5,6,7,8-tetrahydroquinazolin-4-amine 1-oxide (2g)**. Yield 99 mg (50%); yellow crystals; m.p. 156–159ºC (from MeOH); R_f = 0.4 (MeOH:NH_4OH 2:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.21 (br s, 2H, CH_2, Ad), 1.32–1.44 (m, 8H, $4CH_2$, Ad), 1.49 (s, 9H, $3CH_3$), 1.57 (br s, 2H, CH_2, Ad), 1.70–1.82 (m, 4H, $2CH_2$, cy-Hex), 2.04 (br s, 2H, 2CH, Ad), 2.28 (br t, 2H, $^3J_{HH}$ 5.8, CH_2, cy-Hex), 2.34 (br s, 2H, C**H**$_2$NH$_2$), 2.85 (br t, 2H, $^3J_{HH}$ 5.3, CH_2, cy-Hex), 3.27 (t, 2H, $^3J_{HH}$ 6.3, C**H**$_2$NH), 4.61–4.69 (m, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.8 (CH_2, cy-Hex), 21.2 (CH_2, cy-Hex), 22.1 (CH_2, cy-Hex), 24.5 (CH_2, cy-Hex), 26.9 ($3CH_3$), 28.3 (2CH, Ad), 33.9 (C, Ad), 34.2 (C, Ad), 36.4 (CH_2, Ad), 38.6 (C, *t*-Bu), 39.3 ($2CH_2$, Ad), 40.0 ($2CH_2$, Ad), 42.9 (CH_2, Ad), 51.9 (CH_2NH), 54.0 (CH_2NH_2), 111.4 (C4a), 151.3 (C), 154.6 (C), 160.5 (C2); HRMS (ESI^+, 70 eV, m/z): calcd. for $C_{24}H_{38}N_4O$ [M+H]: 399.3118, found: 399.3115.

4-[4-(Aminomethyl)benzylamino]-2-methyl-5,6,7,8-tetrahydroquinazoline 1-oxide (2i). Yield 76 mg (54%); yellow oil; R_f = 0.59 (MeOH); ^{1}H NMR (400 MHz, $CDCl_3$+CD_3OD) δ: 1.65–1.76 (m, 4H, 2CH_2, cy-Hex), 2.25 (t, 2H, ^3J 5.4 Hz, CH_2, cy-Hex), 2.49 (s, 3H, CH_3), 2.72–2.77 (m, 2H, CH_2, cy-Hex), 3.75 (s, 2H, C**H**$_2$NH), 4.56 (s, 2H, C**H**$_2$$NH_2$), 7.15–7.23 (m, 4H, 4CH, Ar); ^{13}C (100 MHz, $CDCl_3$+CD_3OD) δ: 19.9 (CH_3), 20.6 (CH_2, cy-Hex), 20.8 (CH_2, cy-Hex), 21.9 (CH_2, cy-Hex), 24.3 (CH_2, cy-Hex), 45.2 (CH_2), 49.8 (CH_2), 112.1 (C4a), 127.6 (2CH, Ar), 128.1 (2CH, Ar), 137.6 (C, Ar), 140.3 (C, Ar), 152.8 (C4), 153.3 (C8a), 155.1 (C2); HRMS (ESI$^+$, 70 eV, m/z): calcd. for $C_{17}H_{22}N_4O$ [M+H]: 299.1861, found: 299.1866.

***N*,*N*'-Bis(2-*tert*-butyl-1-oxido-5,6,7,8-tetrahydroquinazolin-4-yl)ethane-1,2-diamine (3d).** Yield 122 mg (52%); yellow crystals; m.p. 169–173ºC (from MeOH); R_f = 0.5 (MeOH:NH_4OH 2:1); ^{1}H NMR (400 MHz, CD_3OD) δ: 1.44 (s, 18H, 6CH_3) 1.72–1.88 (m, 8H, 4CH_2, cy-Hex), 2.32 (br t, 4H, $^3J_{HH}$ 5.8, 2CH_2, cy-Hex), 2.82 (br t, 4H, $^3J_{HH}$ 5.9, 2CH_2, cy-Hex), 3.81 (s, 4H, 2C**H**$_2$NH); ^{13}C NMR (100 MHz, CD_3OD) δ: 20.3 (2CH_2, cy-Hex), 20.8 (2CH_2, cy-Hex), 21.8 (2CH_2, cy-Hex), 24.1 (2CH_2, cy-Hex), 26.3 (6CH_3), 38.4 (2C, *t*-Bu), 40.2 (2CH_2N), 112.7 (2C4a), 153.7 (2C), 154.2 (2C), 161.4 (2C2); HRMS (ESI$^+$, 70 eV, m/z): calcd. for $C_{26}H_{40}N_6O_2$ [M+H]: 469.3286, found: 469.3296.

***N*,*N*'-Bis(2-*tert*-butyl-1-oxido-5,6,7,8-tetrahydroquinazolin-4-yl)propane-1,3-diamine (3e).** Yield 84 mg (35%); brown crystals; m.p. 171–175ºC; R_f = 0.6 (MeOH:NH_4OH 2:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.46 (s, 18H, 6CH_3), 1.70–1.84 (m, 8H, 4CH_2, cy-Hex), 1.94 (quint, 2H, $^3J_{HH}$ 6.2, CH_2, diamine), 2.35–2.47 (m, 4H, 2CH_2, cy-Hex), 2.82 (br t, 4H, $^3J_{HH}$ 6.0, 2CH_2, cy-Hex), 3.61 (dt, 4H, $^3J_{HH}$ 6.2, $^3J_{HH}$ 5.9, 2C**H**$_2$NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.8 (2CH_2, cy-Hex), 21.2 (2CH_2, cy-Hex), 22.5 (2CH_2, cy-Hex), 24.6 (2CH_2, cy-Hex), 27.0 (6CH_3), 38.5 (2C, *t*-Bu), 38.8 (2CH_2N), 112.6 (2C4a), 154.5 (4C), 161.6 (2C2); HRMS (ESI$^+$, 70 eV, m/z): calcd. for $C_{27}H_{42}N_6O_2$ [M+H]: 483.3442, found: 483.3442.

***N*,*N*'-Bis(2-*tert*-butyl-1-oxido-5,6,7,8-tetrahydroquinazolin-4-yl)butane-1,4-diamine (3f).** Yield 119 mg (48%); brown crystals; m.p. 174–177ºC; R_f = 0.6 (MeOH:NH_4OH 2:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.46 (s, 18H, 6CH_3), 1.64–1.84 (m, 12H, 4CH_2, cy-Hex + 2CH_2, diamine), 2.49–2.57 (m, 4H, 2CH_2, cy-Hex), 3.01 (br t, 4H, $^3J_{HH}$ 5.5, 2CH_2, cy-Hex), 3.64–3.72 (m, 4H, 2C**H**$_2$NH), 8.25 (br s, 2H, 2NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.3 (2CH_2, cy-Hex), 20.6 (2CH_2, cy-Hex), 22.7 (2CH_2, cy-Hex), 24.9 (2CH_2, cy-Hex), 27.3 (2CH_2), 28.2 (6CH_3), 38.7 (2C, *t*-Bu), 39.6 (2CH_2N), 113.7 (2C), 154.1 (2C), 157.7 (2C), 165.2 (2C2); HRMS (ESI$^+$, 70 eV, m/z): calcd. for $C_{28}H_{44}N_6O_2$ [M+H]: 497.3587, found: 497.3599.

***N*,*N*'-[Tricyclo[3.3.1.1^{3,7}]decane-1,3-diylbis(methylene)]bis(2-methyl-5,6,7,8-tetrahydroquinazolin-4-amine) 1,1'-dioxide (3g).** Yield 140 mg (38%); yellow crystals; m.p. 178–181ºC;

R_f = 0.7 (petroleum ether:ethyl acetate:MeOH 3:1:2.5); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.31 (br s, 2H, CH_2, Ad), 1.43–1.55 (m, 8H, 4CH_2, Ad), 1.48 (s, 18H, 6CH_3), 1.58 (s, 2H, CH_2, Ad), 1.72–1.85 (m, 8H, 4CH_2, cy-Hex), 2.01 (2H, 2CH, Ad), 2.39 (br t, 4H, 2CH_2, $^3J_{HH}$ 5.7, cy-Hex), 2.80 (br t, 4H, 2CH_2, $^3J_{HH}$ 5.7, cy-Hex), 3.33 (s, 4H, 2C**H**$_2$NH); ^{13}C NMR (100 MHz, CD_3OD) δ: 20.4 (2CH_2, cy-Hex), 20.9 (2CH_2, cy-Hex), 21.9 (2CH_2, cy-Hex), 24.2 (2CH_2, cy-Hex), 26.5 (6CH_3), 28.6 (2CH, Ad), 35.4 (CH_2, Ad), 35.9 (2C, Ad), 38.6 (2C, *t*-Bu), 39.8 (4CH_2, Ad), 43.6 (CH_2, Ad), 51.4 (2CH_2N), 112.3 (2C4a), 154.1 (2C6), 154.2 (2C4), 161.2 (2C2); HRMS (ESI$^+$, 70 eV, m/z): calcd. for $C_{36}H_{54}N_6O_2$ [M+H]: 603.4381, found: 603.4374.

4,4'-{[1,4-Phenylenebis(methylene)]bis(azanediyl)}bis(2-methyl-5,6,7,8-tetrahydro-quinazoline) 1,1'-dioxide (3i). Yield 124 mg (53%), reaction time 80 min; brown oil; R_f = 0.59 (MeOH); ^{1}H NMR (400 MHz, $CDCl_3$+CD_3OD) δ: 1.70–1.83 (m, 8H, 2CH_2, cy-Hex), 2.28 (t, 4H, ^3J 5.6 Hz, 2CH_2, cy-Hex), 2.57 (s, 3H, CH_3), 2.82 (t, 2H, ^3J 5.7 Hz, CH_2, cy-Hex), 4.63 (s, 4H, 2CH_2), 7.26 (s, 4H, 4CH, Ar); ^{13}C (100 MHz, $CDCl_3$+CD_3OD) δ: 19.9 (2CH_3), 20.6 (2CH_2, cy-Hex), 20.8 (2CH_2, cy-Hex), 21.95 (2CH_2, cy-Hex), 24.3 (2CH_2, cy-Hex), 44.5 (2CH_2), 112.0 (2C4a), 128.0 (4CH, Ar), 137.9 (2C, Ar), 152.52 (2C4), 153.5 (2C8a), 155.2 (2C2); HRMS (ESI$^+$, 70 eV, m/z): calcd. for $C_{26}H_{32}N_6O_2$ [M+H]: 461.2660, found: 461.2648.

Cells and viruses

Porcine embryo kidney (PEK) cell line was maintained at 37 °C in medium 199 (Chumakov FSC R&D IBP RAS, Russia) supplemented with 5% fetal bovine serum (Invitrogen). TBEV strain Absettarov (GenBank access no. KU885457.1) was from laboratory collection of Chumakov FSC R&D IBP RAS.

Cell toxicity assay

Protocol for cytotoxicity test in PEK cells was adopted as reported: K. N. Sedenkova, E. V. Dueva, E. B. Averina, Y. K. Grishin, D. I. Osolodkin, L. I. Kozlovskaya, V. A. Palyulin, E. N. Savelyev, B. S. Orlinson, I. A. Novakov, G. M. Butov, T. S. Kuznetsova, G. G. Karganova and N. S. Zefirov, *Org. Biomol. Chem*., 2015, **13**, 3406. In brief, PEK cells were seeded and incubated for 72 hours at 37 °C. Stock solutions of the compounds with the concentration of 25 mM were prepared in 100% DMSO (Sigma). Two-fold dilutions of studied compounds were prepared in medium 199 on Earle solution to obtain final concentrations starting from 500 μM. Equal volumes of compound dilutions were added in three replicates to the cells. Cell control was treated with the same sequential concentrations of DMSO, as in compounds dilutions, and the culture medium with no additions the possible non-specific effect of DMSO on the cell line. After incubation at 37 °C on days 1 or 7 the cultural supernatant was gently removed and the

cells were washed with phosphate buffered saline (PBS, Sigma) twice. A solution of 0.0002% neutral red in PBS was added to the washed cells, and the cells were incubated for 30 minutes at 37 °C, so the live cells would consume dye in endosomes. Afterwards cells were gently washed with PBS twice and fixed with 96% ethanol. Dye absorption was counted using MultiScan FC (Thermo) at 450 nm. CC_{50} was calculated according to the Reed and Muench method.

50% Plaque reduction test

Plaque reduction test protocol was adopted from the reference: A. A. Orlov, A. A. Chistov, L. I. Kozlovskaya, A. V. Ustinov, V. A. Korshun, G. G. Karganova and D. I. Osolodkin, *Med. Chem. Commun*., 2016, **7**, 495. In brief, four fold dilutions of the compounds were preincubated with the virus (20–40 PFU) in 96-well plates at 37°C in CO_2-incubator for 1 h and then added to PEK cell monolayers in 24-well plates (seeded and incubated for 72 h at 37°C). Virus control was treated with the same sequential concentrations of DMSO, as it was in compounds dilutions. The plates were incubated for 1 h and overlaid with 1.26% methylcellulose. After 6 days, cells were fixed with ethanol and stained with 0.4% gentian violet. EC_{50} values were calculated according to the Reed-and-Muench method.