Supporting Information

Discovery of Novel Inhibitors Targeting Human O- GlcNAcase: Docking-Based Virtual Screening, Biological Evaluation, Structural Modification, and Molecular Dynamics Simulation

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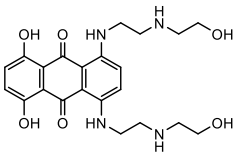
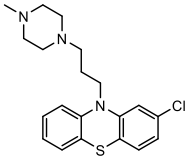
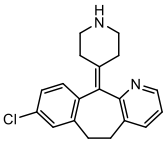
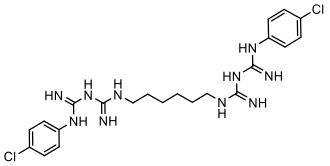
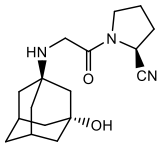
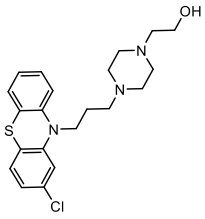
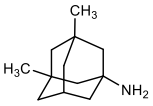
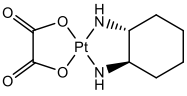
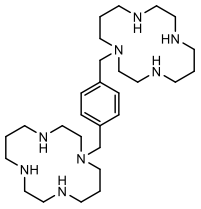
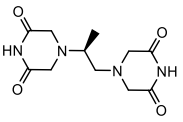
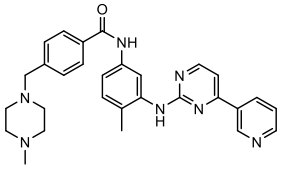
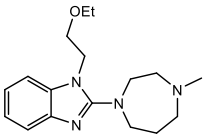
1. Structures and docking scores of the compounds.

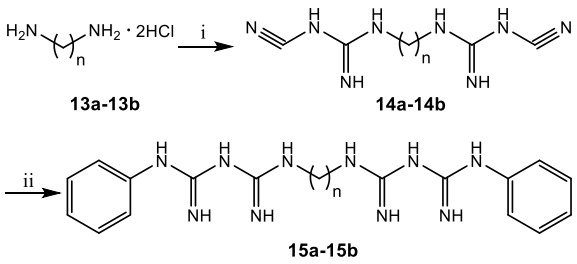
Table S1. Structures and docking scores of the hits selected for biological evaluation.

Sample NO.	Drugbank ID	Compound name	Structure	GeomX score	Autodock score
1	DB01204	Mitoxantrone		9.18	-8.43
2	DB00433	Prochlorperazine		7.52	-8.90
3	DB00967	Desloratadine		7.14	-7.55
4	DB00878	Chlorhexidine		7.49	-8.95
5	DB04876	Vildagliptin		7.12	-7.52
6	DB00850	Perphenazine		7.38	-8.21
7	DB01043	Memantine		7.90	-7.81
8	DB00526	Oxaliplatin		7.94	-8.60

9	DB06809	Plerixafor		7.55	-9.10
10	DB00380	Dexrazoxane		7.97	-7.66
11	DB00619	Imatinib		8.24	-8.71
12	DB01084	Emedastine		7.41	-7.82

2. Synthetic procedures and characterization of compounds

2.1 Synthesis of diaryl guanidines 15a-15b.[1,2]



15a: n=4; **15b**: n=6

Scheme S1. Synthesis of diaryl guanidines **15a-15b**. (i) sodium dicyanamide, *n*-butanol; (ii) aniline hydrochloride, ethoxyethanol.

A solution of **13a-13b** (10 mmol, 1 eq) and sodium dicyanamide (20 mmol, 2 eq) in *n*-butanol (20 mL) was refluxed for 16 h, until TLC (EtOAc: MeOH: H_2O = 8:1:1) indicated the reaction was completed. After the mixture cooling to room temperature, the suspension was filtered and washed with n-butanol. The resulting solid was further purified by recrystallization from water, which **14a-14b** were obtained.

1, 6-Di-(N^3-cyano-N^1-guanidino) butane (**14a**)[1]: white solid; (1.07 g, 48.1 %) yield; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.23 (br s, 2H), 6.75 (br s, 4H), 3.13 – 2.93 (m, 4H), 1.53 – 1.28 (m, 4H).

1, 6-Di-(N^3-cyano-N^1-guanidino) hexane(**14b**)[1,2]: white solid; (1.52 g, 60.8 %) yield; ^{1}H

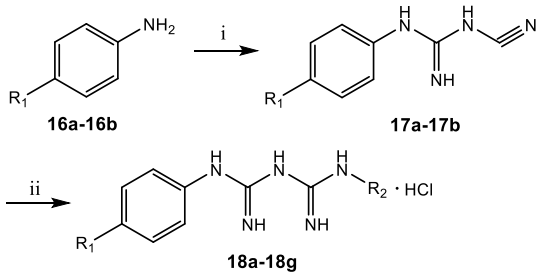
NMR (300 MHz, DMSO-d_6) δ 7.26 (br s, 2H), 6.96 (br s, 2H), 6.73 (br s, 2H), 3.19 – 2.87 (m, 4H), 1.47 – 1.33 (m, 4H), 1.30 – 1.15 (m, 4H).

A solution of **14a-14b** (5 mmol, 1 eq) and aniline hydrochloride (10 mmol, 2 eq) in ethoxyethanol (20 mL) was refluxed for 5 h, until TLC (EtOAc: MeOH: H_2O = 8:1:1) indicated the reaction was completed. The mixture was concentrated *in vacuo* and recrystallized from water, which resulted in **15a-15b**.

3,10-Diimino-1,12-bis(phenylamino)-2,4,9,11-tetraazatetradecane-1,12-diiminium (**15a**): white solid; (0.96 g, 47.0 %) yield; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.86 (s, 1H), 9.54 (s, 1H), 7.96 (s, 2H), 7.66 (s, 2H), 7.43 – 7.33 (m, 4H), 7.32 – 7.23 (m, 4H), 7.22 – 7.10 (m, 3H), 7.06 – 6.96 (m, 3H), 6.81 (s, 2H), 3.22 – 2.99 (m, 4H), 1.63 – 1.37 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.5, 154.4, 138.8, 128.5, 123.1, 120.4, 41.3, 25.6; HRMS (ESI) calcd for $C_{20}H_{29}N_{10}$ ($M+H^+$) 409.2577, found 409.2561.

3,12-Diimino-N^1,N^{14}-bis(phenyl)-2,4,11,13-tetraazatetradecane-1,14-diiminium (**15b**) [1,2]: white solid; (1.12 g, 51.4 %) yield; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.89 (s, 1H), 9.51 (s, 1H), 7.89 (s, 2H), 7.65 (s, 2H), 7.41 – 7.34 (m, 4H), 7.33 – 7.21 (m, 4H), 7.21 – 7.10 (m, 3H), 7.09 – 6.97 (m, 3H), 6.79 (s, 2H), 3.19 – 3.02 (m, 4H), 1.61 – 1.42 (m, 4H); 1.41 – 1.22 (m, 4H); HRMS (ESI) calcd for $C_{22}H_{33}N_{10}$ ($M+H^+$) 437.2890, found 437.2878.

2.2 Synthesis of biguanides 18a-18g.[3,4]



16a: R_1=H; **16b**: R_1=Cl; **17a**: R_1=H; **17b**: R_1=Cl

18a: R_1= H, R_2= Ph; **18b:** R_1= H, R_2= 4-F-Ph; **18c:** R_1= H, R_2= 4-OCH_3-Ph; **18d:** R_1= H, R_2= 4-NO_2-Ph; **18e:** R_1= H, R_2= 1-naphthyl; **18f:** R_1= H, R_2= propargyl; **18g:** R_1= Cl, R_2= H

Scheme S2. Synthesis of biguanides **18a-18g**. (i) sodium dicyanamide, hydrochloric acid (aq., 37%), H_2O; (ii) amine, hydrochloric acid (aq., 37%), EtOH, H_2O.

To a solution of **16a-16b** (20 mmol, 1 eq) in water (40 mL), then hydrochloric acid (aq., 37 %, 1.7 mL, 20 mmol) and sodium dicyanamide (1.78 g, 20 mmol) were added. The reaction was stirred for 4 h at 60 °C, until TLC (EtOAc) indicated that the reaction was complete. After cooling to room temperature, the solid was filtered off and recrystallized from water to obtain **17a-17b**.

N-Cyano-*N'*-phenyl-guanidine (**17a**)[3]: white solid; (2.15 g, 67.2 %) yield; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.12 (s, 1H), 7.42 – 7.25 (m, 4H), 7.14 – 7.05 (m, 1H), 7.04 – 6.92 (m, 2H).

N-(4-Chlororphenyl)-*N'*-cyanoguanidine (**17b**)[3]: white solid; (2.41 g, 61.9 %) yield; ^{1}H NMR

(300 MHz, CD_3OD) δ 7.40 – 7.35 (m, 2H), 7.34 – 7.29 (m, 2H).

To a solution of amine (6 mmol, 1.2 eq) in EtOH (20 mL) and H_2O (4 mL), then hydrochloric acid (aq., 37%, 0.6 mL, 7 mmol) and **17a-17b** (5 mmol, 1 eq) were added. The reaction was stirred for 20 h, until TLC (EtOAc) indicated that the reaction was complete. The mixture was concentrated *in vacuo* and recrystallized from water to afford **18a-18g**.

N, *N*'-Diphenyl-imidodicarbonimidic diamide hydrochloride (**18a**)[4,5]: white solid; (1.06 g, 73.1 %) yield; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.99 (s, 2H), 7.47 (s, 4H), 7.34 – 7.24 (m, 8H), 7.12 – 7.02 (m, 2H).

N-(4-Fluorophenyl)-*N*'-phenyl-imidodicarbonimidic diamide hydrochloride (**18b**): white solid; (1.07 g, 69.9 %) yield; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.18 (s, 2H), 7.75 – 7.43 (m, 4H), 7.39 – 7.25 (m, 6H), 7.24 – 7.09 (m, 2H); ^{13}C NMR (75 MHz, DMSO) δ 157.37, 157.09, 138.10, 134.40, 128.91, 124.16, 123.93, 123.82, 121.54, 115.42; HRMS (ESI) calcd for $C_{14}H_{15}FN_5$ ($M+H^+$) 272.1311, found 272.1319.

N-(4-Methoxyphenyl)-*N*'-phenyl-imidodicarbonimidic diamide hydrochloride (**18c**): white solid; (0.98 g, 61.3 %) yield; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.03 (s, 1H, NH), 9.91 (s, 1H), 7.54 – 7.37 (m, 4H), 7.35 – 7.27 (m, 4H), 7.24 – 7.02 (m, 3H), 6.89 (d, J = 9.0 Hz, 2H), 3.72 (s, 3H); ^{13}C NMR (75 MHz, DMSO) δ 157.77, 156.41, 138.38, 130.50, 128.85, 124.30, 123.85, 121.26, 114.22, 55.39; HRMS (ESI) calcd for $C_{15}H_{18}N_5O$ ($M+H^+$) 284.1511, found 284.1534.

N-(4-Nitrophenyl)-*N*'-phenyl-imidodicarbonimidic diamide hydrochloride (**18d**): white solid; (1.02 g, 61.1 %) yield; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.87 (s, 1H), 10.44 (s, 1H), 8.28 – 8.10 (m, 2H), 7.95 (s, 2H), 7.81 – 7.55 (m, 4H), 7.44 – 7.27 (m, 4H), 7.23 – 7.07 (m, 1H); ^{13}C NMR (75 MHz, DMSO) δ 158.50, 155.14, 145.40, 142.06, 137.31, 129.12, 125.10, 124.96, 122.50, 119.47; HRMS (ESI) calcd for $C_{14}H_{15}N_6O_2$ ($M+H^+$) 299.1256, found 299.1247.

N-(1-Naphthalenyl)-*N*'-phenyl-imidodicarbonimidic diamide hydrochloride (**18e**): white solid; (1.13 g, 66.5 %) yield; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.20 (s, 1H), 10.04 (s, 1H), 8.34 – 8.11 (m, 1H), 8.04 – 7.89 (m, 1H), 7.86 – 7.70 (m, 3H), 7.66 – 7.39 (m, 6H), 7.32 – 7.13 (m, 4H), 7.11 – 6.94 (m, 1H); ^{13}C NMR (75 MHz, DMSO) δ 158.80, 156.52, 138.27, 133.93, 133.12, 128.88, 128.78, 128.26, 126.41, 126.38, 125.69, 123.80, 123.30, 122.89, 121.15; HRMS (ESI) calcd for $C_{18}H_{18}N_5$ ($M+H^+$) 304.1562, found 304.1589.

N-Phenyl-*N*'-(prop-2-yn-1-yl)-imidodicarbonimidic diamide hydrochloride (**18f**): white solid; (0.54 g, 42.8 %) yield; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.11 (s, 1H), 8.00 (s, 1H), 7.52 (s, 1H), 7.46 – 7.34 (m, 3H), 7.33 – 7.14 (m, 4H), 7.10 – 7.00 (m, 1H), 4.07 – 3.88 (m, 2H), 3.29 (s, 1H); HRMS (ESI) calcd for $C_{11}H_{14}N_5$ ($M+H^+$) 216.1249, found 216.1232.

N-(4-Chlorophenyl)-*N*'-phenyl-imidodicarbonimidic diamide hydrochloride (**18g**)[4]: white solid; (0.85 g, 52.5 %) yield; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.30 (s, 1H), 10.23 (s, 1H), 7.65 (s, 2H), 7.55 (s, 2H), 7.41 – 7.34 (m, 4H), 7.34 – 7.27 (m, 4H), 7.16 – 7.02 (m, 1H).

3. Binding modes of 4, 15a, 15b, and 18d with hOGA revealed by molecular docking.

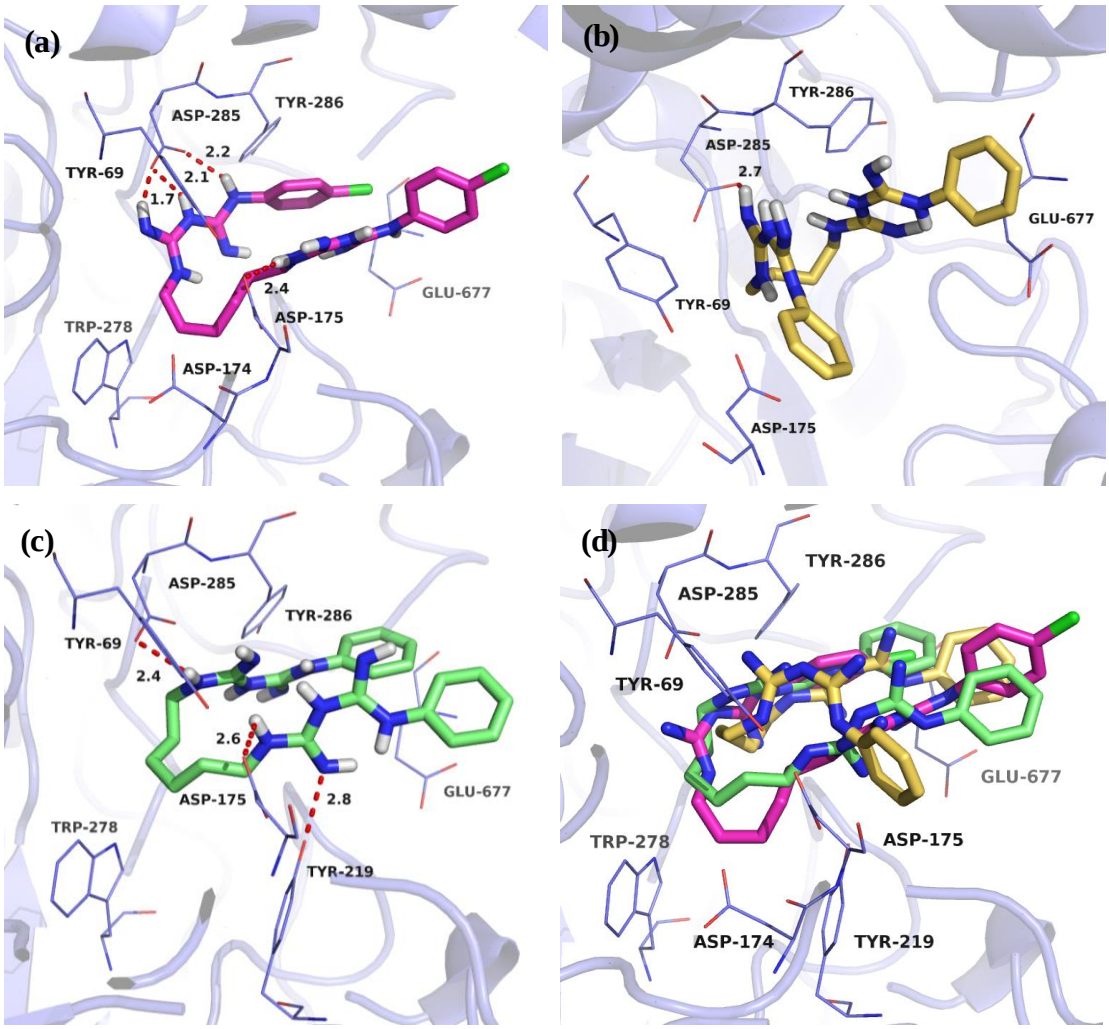


Figure S1. Predicted binding modes of **4**, **15a**, and **15b** with hOGA revealed by molecular docking. Specific binding modes of **4**-hOGA (**a**), **15a**-hOGA (**b**), and **15b**-hOGA (**c**) systems. (**d**) Superimposition of conformations of **4**, **15a**, and **15b** with hOGA. Compound **4** is shown in pink, **15a** is shown in yellow, **15b** is shown in green (colored according to the element).

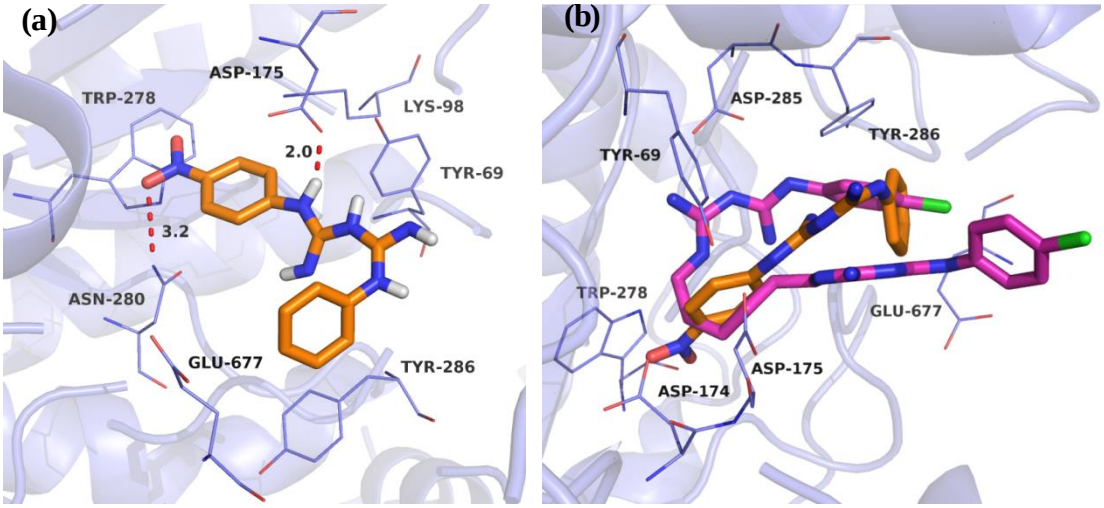


Figure S2. (**a**) Specific binding modes of **18d** in complex with hOGA revealed by molecular docking. (**b**) Superimposition of conformations of **18d** and **4** with hOGA. Compound **18d** is shown in orange, **4** is shown in pink (colored according to the element).

4. Binding modes of 1 and 4 with hOGA revealed by molecular docking and MD simulations.

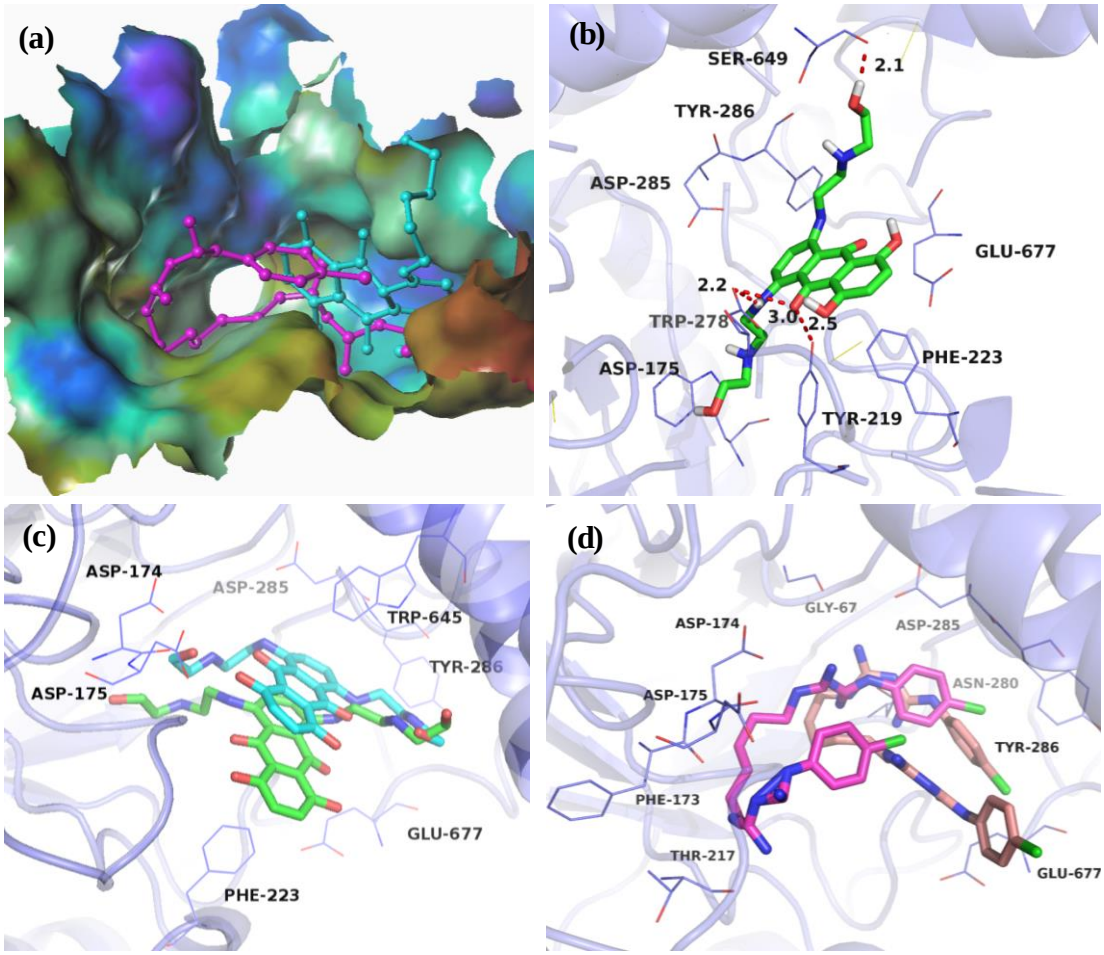


Figure S3. (**a**) Superimposition of conformations of **1** and **4** in hOGA active pocket at 30 ns MD simulations (the enzyme is presented as surface form). (**b**) Specific binding modes of **1** with hOGA revealed by molecular docking. (**c**) Superimposition of conformations of **1** with hOGA revealed by molecular docking (colored in green) and MD simulations (colored in cyan). (**d**) Superimposition of conformations of **4** with hOGA revealed by molecular docking (colored in brown) and MD simulations (colored in pink).

References

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