1. Material and methods

1.1. Compounds and screening

A collection of ~50,000 chemically diverse, drug-like compounds from ChemDiv (SanDiego, CA, USA) was used for the primary screen. The fluorescence cell-based functional screen using MDCK cells expressing rat UT-A1 was described previously.[1,2]

1.2. Synthesis

All purchased materials, reagents and solvents were used without further purification. All solvents used for isolation of products and chromatography were reagent grade. Flash chromatography was done with silica gel columns. 1H and 13C NMR spectra were obtained in chloroform (CDCl3), methanol (CD3OD) or dimethylsulfoxide (DMSO-d6) using a 300 MHz Varian spectrometer referenced to tetramethylsilane (TMS). Chemical shifts are expressed in parts per million (ppm, δ) downfield from TMS. Splitting patterns are designated as brs (broad singlet), s (singlet), d (doublet), t (triplet), and m (multiplet), and coupling constants are designated as Hertz (Hz).Mass spectrometry was done using a Waters LC/MS instrument (Waters Micromass ZQ with HPLC Waters 2695). LC was done on Xterra MS C18 column (2.1mm x 100 mm, 3.5 μm) with 0.2 mL/min water/acetonitrile (containing 0.1 % formic acid), 16 min linear gradient, 5 to 95% acetonitrile. Mass spectra were acquired on a mass spectrometer (Waters 2695 + micromass ZQ; Waters) using electron spray (+) ionization, mass ranging from 200 to 1500 Da, 40-V cone voltage. UV absorbance was detected at 254 nm.

2,7-diamino-9-fluorenone (**2**)

To a solution of 2,7-dinitro-9-fluorenone (4.7 g, 17.5 mmol) in ethanol (180 ml) was added a solution of sodium sulfide nonahydrate (18.9 g, 78.8 mmol) and aqueous sodium hydroxide (7.5 g, 187 mmol) solution. The mixture was refluxed for 5 h, then cooled to 0 °C and the resulting precipitate collected by filtration. The crude product was washed with water, 5% aqueous NaOH, ether and hexane and dried in vacuo. Recrystallization from acetone-ethanol afforded **2** (2.86 g, 78%) as purple needles: ^{1}H NMR (DMSO-*d6*) δ 7.09 (d, 2H, *J* = 7.9 Hz), 6.71 (d, 2H, *J* = 2.0 Hz), 6.59 (dd, 2H, *J* = 2.2, 7.9 Hz), 5.31 (brs, 4H); ^{13}C NMR (DMSO-*d6*) δ 195.4, 148.6, 135.0, 133.7, 120.3, 119.1, 110.1; MS (ESI) for $C_{13}H_{10}N_2O$: 211 $(M+H)^+$.

Acylation procedure for **3**, **4-6**, **20, 22**

To a solution of 2,7-diamino-9-fluorenone **2** (1 eq) in THF was added triethylamine (2.5 eq) and the corresponding acyl halide (2.5-3 eq), and stirred for 2 h. The resulting precipitate was filtered and washed with EtOAc and Et_2O. The crude residue was triturated with EtOH-CH_2Cl_2 to give the 2,7-disubstituted amidofluorenone.

2,7-diacetamino-9-fluorenone (**3**)

Brown solid (62%): ^{1}H NMR (DMSO-*d6*) δ 10.2 (brs, 2H), 7.90 (brs, 2H), 7.67 (d, 2H, *J* = 7.9 Hz), 7.59 (d, 2H, *J* = 8.2 Hz), 2.07 (s, 6H); ^{13}C NMR (DMSO-*d6*) δ 193.4, 169.2, 140.1, 139.0, 134.6, 125.1, 121.3, 115.1, 24.4; LRMS (ESI) *m/z*295 $(M+H)^+$; HRMS (ESI) calcd for $C_{17}H_{15}N_2O_3$: 295.1004 $(M+H)^+$; found 295.1080.

N,N′-(9-oxo-9H-fluorene-2,7-diyl)dipropionamide (**4**)

Yellowishpowder (34 mg, 44%): ^{1}H NMR (DMSO-*d6*) δ 10.12 (brs, 2H), 7.99-7.90 (m, 2H), 7.77-7.57 (m, 4H), 2.35 (q, 4H, *J* = 7.5 Hz), 1.09 (t, 6H, *J* = 7.5 Hz); ^{13}C NMR (DMSO-*d6*) δ 193.4, 172.7, 140.3, 138.9, 134.6, 125.1, 121.3, 115.1, 30.0, 10.0; LRMS (ESI) *m/z* 323$(M+H)^+$; HRMS (ESI) calcd for $C_{19}H_{19}N_2O_3$: 323.1317 $(M+H)^+$; found 323.1395.

N,N′-(9-oxo-9H-fluorene-2,7-diyl)bis(2-methylpropanamide) (**5**)

Yellowish powder (82 mg, 49%): ^{1}H NMR (DMSO-*d6*) δ 10.20 (brs, 2H), 8.81 (brs, 2H), 8.00 (d, 2H, *J* = 1.5 Hz), 7.63 (dd, 2H, *J* = 2.2 Hz, 8.0 Hz), 2.62 (q, 2H, *J* = 6.8 Hz), 1.12 (d, 6H, *J* = 6.8 Hz); ^{13}C NMR (DMSO-*d6*) δ 192.8, 176.1, 141.0, 138.2, 134.6, 126.0, 122.1, 115.4, 35.4, 19.8; LRMS (ESI) *m/z* 351$(M+H)^+$; HRMS (ESI) calcd for $C_{21}H_{23}N_2O_3$-CH_3: 336.1630 $(M+H\text{-}CH_3)^+$; found 336.1711.

N,N′-(9-oxo-9H-fluorene-2,7-diyl)dibutyramide (**6**)

Yellowishpowder (57 mg, 68%): ^{1}H NMR (DMSO-*d6*) δ 10.15 (brs, 2H), 7.91 (d, 2H, *J* = 1.9 Hz), 7.74-7.57 (m, 4H), 2.31 (t, 4H, *J* = 7.3 Hz), 1.63 (q, 4H, *J* = 7.3 Hz), 0.92 (t, 6H, *J* = 7.3 Hz); ^{13}C NMR (DMSO-*d6*) δ 192.8, 172.2, 140.1, 138.3, 134.5, 125.3, 121.4, 115.2, 44.0, 18.9, 14.0; LRMS (ESI) *m/z* 351$(M+H)^+$; HRMS (ESI) calcd for $C_{21}H_{23}N_2O_3$: 351.1630 $(M+H)^+$; found 351.1708.

N,N′-(9-oxo-9H-fluorene-2,7-diyl)bis(2,2,2-trifluoroacetamide) (**7**)

A mixture of 2,7-diamino-9-fluorenone **2** (170 mg, 0.8 mmol) and trifluoroacetic acid (0.7 ml, 5.4 mmol) was heated to 150 °C in a sealed tube for 6 h. The reaction mixture was cooled to room temperature, and then the solvent was evaporated. The resulting precipitate was filtered and then the crude residue was triturated with EtOH-CH_2Cl_2 to afford **7** (69 mg, 22%) as a yellow solid: ^{1}H NMR (DMSO-*d6*) δ 11.5 (brs, 2H), 7.97 (d, 2H, *J* = 1.8 Hz), 7.87 (dd, 2H, *J* = 1.9, 8.1 Hz), 7.82 (d, 2H, *J* = 8.1 Hz); ^{13}C NMR (DMSO-*d6*) δ 192.3, 155.1 (q, J^2_{CF} = 37 Hz), 140.8, 137.7, 134.7, 127.5, 122.1, 119.9 (q, J^1_{CF} = 286 Hz), 117.0; LRMS (ESI) *m/z* 403 $(M+H)^+$.

1,1'-(9-oxo-9*H*-fluorene-2,7-diyl)diurea (**8**)

To a solution of 2,7-diamino-9-fluorenone **2** (100 mg, 0.48 mmol) in AcOH (5 ml) was added a solution of KNCO (85 mg, 1.05 mmol) in water (2.5 ml) slowly. After 3h, the reaction mixture was diluted with EtOAc and resulting precipitate was filtered. Then the resulting solid was dried under high vacuum to give **8** (100 mg, 71%) as a brownish solid: ^{1}H NMR (DMSO-*d6*) δ 8.78 (brs, 2H), 7.76 (brs, 2H), 7.49-7.39 (m, 4H), 5.06 (brs, 4H); ^{13}C NMR (DMSO-*d6*) δ 193.0, 165.6, 139.6, 134.7, 125.5, 121.8, 115.3, 30.6; LRMS (ESI) *m/z* 297 $(M+H)^+$; HRMS (ESI) calcd for $C_{15}H_{13}N_4O_3$: 297.0909 $(M+H)^+$; found 297.0994.

N,N'-(9-oxo-9H-fluorene-2,7-diyl)dimethanesulfonamide (**9**)

To a mixture of 2,7-diamino-9-fluorenone **2** (95 mg, 0.45 mmol) in THF (1.5 ml) was added pyridine (0.12 ml, 1.13 mmol) and MsCl (0.08 ml, 0.99 mmol), and stirred overnight. The reaction mixture was diluted with CH_2Cl_2 and washed with water and brine. The organic layer was dried over $MgSO_4$, then solvent was evaporated. Trituration with EtOH-CH_2Cl_2 afforded **9** (63 mg, 38%) as a yellow solid: ^{1}H NMR (DMSO-*d6*) δ 10.0 (brs, 2H), 7.68 (d, 2H, *J* = 8.0 Hz), 7.40-7.35 (m, 4H), 3.06 (s, 6H); ^{13}C NMR (DMSO-*d6*) δ 192.7, 139.57, 139.55, 135.0, 126.0, 122.2, 115.6, 39.9; LRMS (ESI) *m/z* 367 $(M+H)^+$; HRMS (ESI) calcd for $C_{15}H_{15}N_2O_5S_2$: 367.0344 $(M+H)^+$; found 367.0422.

2,7-bis(1,1-dioxido-1,2-thiazolidin-2yl)-9-H-fluoren-9-one (**10**)

To a solution of 2,7-diamino-9-fluorenone **2** (100 mg, 0.48mmol) in CH_2Cl_2 (2 ml) was added pyridine (1 ml, 17.4 mmol) and 3-chloropropansulfonyl chloride (0.11 ml, 0.95 mmol) and stirred overnight. The reaction mixture was diluted with CH_2Cl_2 and washed with water and brine. The organic layer was dried over $MgSO_4$, then solvent was evaporated. To a solution of crude product in CH_3CN (8 ml) was added potassium carbonate (365 mg, 2.65 mmol) and heated to reflux. After 5 h the reaction mixture was cooled, water was added and the resulting precipitate was filtered. Recrystallization from EtOH-CH_2Cl_2 afforded **10** as a yellowish powder (45 mg, 22% for 2 steps): ^{1}H NMR (DMSO-*d6*) δ 7.74 (d, 2H, *J* = 8.1 Hz), 7.40 (d, 2H, *J* = 8.1 Hz), 7.34 (dd, 2H, *J* = 8.1, 2.4 Hz), 3.82 (t, 4H, *J* = 6.5 Hz), 3.57 (t, 4H, *J* = 7.3 Hz), 2.43 (t, 4H, *J* = 7.3 Hz); ^{13}C NMR (DMSO-*d6*) δ 196.7, 159.7, 139.3, 138.8, 134.8, 124.1, 122.2, 113.7, 48.7, 47.1, 18.9; LRMS (ESI) *m/z* 419 $(M+H)^+$; HRMS (ESI) calcd for $C_{19}H_{19}N_2O_5S_2$: 419.0657 $(M+H)^+$; found 419.0739.

N,N'-(9-oxo-9*H*-florene-2,7-diyl)bis(2-bromoacetamide) (**11**)

To a solution of 2,7-diamino-9-fluorenone (150 mg, 0.71 mmol) in xylene (4 ml) was added bromoacetyl bromide (1ml, 11.5 mmol) and refluxed overnight. After cooling to 0 °C the resulting precipitate collected by filtration. The crude product was washed with ethanol, acetone and ether, and dried in vacuo. Trituraed with ethanol afforded **11** (128 mg, 40%) as a brownish solid: ^{1}H NMR (DMSO-*d6*) δ 10.64 (brs, 2H), 7.89 (d, 2H, *J*= 0.9 Hz), 7.67-7.66 (m, 4H), 4.06 (s, 4H); ^{13}C NMR (DMSO-*d6*) δ 193.0, 165.6, 139.6, 134.7, 125.6, 125.5, 121.8, 115.3, 30.6; LRMS (ESI) *m/z* 451 $(M+H)^+$.

N,N'-(9-oxo-9H-fluorene-2,7-diyl)bis(2-hydroxyacetamide) (**12**)

To a solution of **11** (100 mg, 0.22 mmol) in ethanol (2 ml) was added 1N NaOH aqueous solution (0.6 ml, 0.6 mmol) and stirred for 3 h. The resulting mixture was filtered. The crude product was triturated with EtOH-CH_2Cl_2 to give **12** (62 mg, 86%) as a white solid: ^{1}H NMR (DMSO-*d6*) δ 10.66 (brs, 2H), 7.91 (brs, 2H), 7.68 (brs, 4H), 4.07 (brs, 4H); ^{13}C NMR (DMSO-*d6*) δ 193.5, 171.7, 139.6, 134.7, 125.4, 121.5, 115.8, 115.2, 62.3; LRMS (ESI) *m/z* 327 $(M+H)^+$; HRMS (ESI) calcd for $C_{17}H_{15}N_2O_5$: 327.0903 $(M+H)^+$; found 327.0984.

N,N'-(9-oxo-9H-fluorene-2,7-diyl)bis(2-(2-methoxyethoxy)acetamide) (**13**)

To a suspension of NaH (8 mg, 0.18 mmol) in CH_3CN (2 ml) was added methoxyethanol (0.06 ml, 0.18 mmol) and stirred for 0.5 h. To the mixture was added 2,7-bisbromoacetamino-9-fluorenone **11** (33 mg, 0.073 mmol) and stirred overnight. The reaction mixture was diluted with EtOAc and washed with water, brine, dried over $MgSO_4$ and evaporated in vacuo. Triturated with EtOH-CH_2Cl_2 afforded **13** (10 mg, 18 %) as a yellow solid: ^{1}H NMR (MeOD) δ 7.87 (d, 2H, *J* = 2.0 Hz), 7.74 (dd, 2H, *J* = 2.0, 8.1 Hz), 7.53 (d, 2H, *J* = 8.1 Hz), 4.15 (s, 4H), 3.80-3.77 (m, 4H), 3.68-3.65 (m, 4H), 3.40 (s, 6H); ^{13}C NMR (DMSO-*d6*) δ 193.4, 169.4, 138.6, 135.2, 134.4, 133.7, 120.3, 119.1, 77.8, 69.8, 63.5;LRMS (ESI) *m/z* 443 $(M+H)^+$; HRMS (ESI) calcd for $C_{23}H_{27}N_2O_7$: 443.1740 $(M+H)^+$; found 443.1815.

N,N'-(9-oxo-9H-fluorene-2,7-diyl)bis(2-azidoacetamide) (**14**)

Brownish solid (10 mg, 24%): ^{1}H NMR (DMSO-*d6*) δ 10.41 (brs, 2H), 7.90 (brs, 2H), 7.69-7.67 (m, 4H), 4.08 (brs, 4H); ^{13}C NMR (DMSO-*d6*) δ 193.1, 167.1, 139.5, 134.7, 125.5, 121.7, 115.4, 51.7; LRMS (ESI) *m/z* 377$(M+H)^+$; HRMS (ESI) calcd for $C_{17}H_{13}N_8O_3$: 377.1032 $(M+H)^+$; found 377.1112.

N,N'-(9-oxo-9H-fluorene-2,7-diyl)bis(2-(benzyloxy)acetamide) (**15**)

To a solution of 2,7-diamino-9-fluorenone **2** (190 mg, 0.9 mmol) in xylene (7 ml) was added benzyloxyacetyl chloride (0.35 ml, 2.26 mmol), refluxed overnight and cooled to 0 °C, and the resulting precipitate collected by filtration. The crude product was washed with ethanol, acetone and ether, and dried in vacuo. Triturated with ethanol afforded **15** (120 mg, 26%) as a brownish solid: ^{1}H NMR ($CDCl_3$) δ 7.90-7.87 (m, 2H), 7.66 (d, 2H, *J* = 2.0 Hz), 7.46-7.38 (m, 12H), 4.69 (s, 4H), 4.13 (s, 4H); ^{13}C NMR (DMSO-*d6*) δ 193.9, 168.8, 139.5, 139.4, 138.1, 134.6, 128.7, 128.1, 125.9, 121.4, 115.9, 72.9, 69.8; MS (ESI) for $C_{31}H_{27}N_2O_5$: 507.5 $(M+H)^+$.

(2Z,2'Z)-4,4'-(9-oxo-9H-fluorene-2,7-diyl)bis(azanediyl)bis(4-oxobut-2-enoic acid) (**16**)

To a solution of 2,7-diamino-9-fluorenone **2** (50 mg, 0.24 mmol) in chloroform (2 ml) was added maleic anhydride (46 mg, 0.24 mmol). After stirring overnight at 50 °C water was added and the resulting precipitate was filtered. Trituration of the crude residue with EtOH-CH_2Cl_2 afforded **16** as a brown solid (25 mg, 26%): ^{1}H NMR (DMSO-*d6*) δ 10.78 (brs, 2H), 7.95 (brs, 2H), 7.76 (d, 1H, *J* = 7.1 Hz), 7.67 (d, 2H, *J* = 8.3 Hz), 6.51 (d, 2H, *J* = 12.1 Hz), 6.34 (d, 2H, *J* = 12.1 Hz); ^{13}C NMR (DMSO-*d6*) δ 193.1, 167.4, 163.9, 139.8,

139.5, 134.6, 131.6, 131.0, 122.5, 121.7, 115.5;LRMS (ESI) *m/z* 407 $(M+H)^+$; HRMS (ESI) calcd for $C_{21}H_{15}N_2O_7$: 407.0801 $(M+H)^+$; found 407.0880.

N,N′-(9-(hydroxyimino)-9H-fluorene-2,7-diyl)diacetamide (**17**)

To a solution of 2,7-diacetamino-9-fluorenone **3** (54 mg, 0.18mmol) in DMSO (1.9 ml) was added a solution of hydroxylamine hydrochloride (14 mg, 0.2mmol) in water (0.1 ml). The reaction mixture stirred at 80 °C overnight to give **17** (25 mg, 22%): ^{1}H NMR (DMSO-*d6*) δ 8.55 (d, 1H, *J* = 1.9 Hz), 7.98 (d, 1H, *J* = 1.4 Hz), 7.75 (dd, 1H, *J* = 8.1, 1.9 Hz), 7.65-7.59 (m, 1H), 7.52 (dd, 1H, *J* = 8.1, 1.9 Hz), 2.16 (brs, 6H); ^{13}C NMR (DMSO-*d6*) δ 170.2, 151.5, 138.0, 136.58, 136.52, 135.7, 130.8, 121.9, 121.0, 120.9, 119.4, 119.1, 112.7, 22.4, 22.3; LRMS (ESI) *m/z* 310 $(M+H)^+$; HRMS (ESI) calcd for $C_{17}H_{16}N_3O_3$: 310.1113 $(M+H)^+$; found 310.1191.

N,N′-(9-hydroxy-9H-fluorene-2,7-diyl)diacetamide (**18**)

To a suspension of 2,7-diacetamino-9-fluorenone **3** (200 mg, 0.68 mmol) in CH_3CN (10 ml) was added a solution of $NaBH_4$ (40 mg, 1.02 mmol) in MeOH (1 ml). The reaction mixture was stirred for 30 min. The crude product was filtered and triturated with EtOH-CH_2Cl_2 to give **18** (56 mg, 28%) as a yellow solid: ^{1}H NMR (CD_3OD) δ 7.87 (brs, 2H), 7.59 (d, 2H, *J* = 8.1 Hz), 7.53-7.50 (m, 2H), 5.49 (s, 1H), 2.16 (s, 6H); ^{13}C NMR (DMSO-*d6*) δ 168.5, 148.0, 138.7, 134.7, 119.8, 119.3, 116.4, 74.0, 24.5; LRMS (ESI) *m/z* 297 $(M+H)^+$; HRMS (ESI) calcd for $C_{17}H_{17}N_2O_3$: 297.1161 $(M+H)^+$; found 297.1241.

N,N'-(5,5-dioxidodibenzo[*b*,*d*]thiene-3,7-diyl)diacetamide (**20**)

White solid (130 mg, 65%): ^{1}H NMR (DMSO-*d6*) δ 10.4 (brs, 2H), 8.26 (d, 2H, *J* = 1.8 Hz), 7.97 (d, 2H, *J* = 8.4 Hz), 7.73 (dd, 2H, *J* = 1.9, 8.4 Hz), 2.11 (s, 6H); ^{13}C NMR (DMSO-*d6*) δ 169.5, 141.2, 137.9, 125.6, 124.4, 123.1, 111.6, 24.5; LRMS (ESI) *m/z* 331 $(M+H)^+$; HRMS (ESI) calcd for $C_{16}H_{15}N_2O_4S$: 331.0674 $(M+H)^+$; found 331.0757.

N,N′-(3,3'-carbonylbis(3,1-phenylene))diacetamide (**22**)

Yellowishpowder (87 mg, 62%): ^{1}H NMR (DMSO-*d6*) δ 10.17 (s, 2H), 7.97 (brs, 2H), 7.91 (d, 2H, *J* = 8.0 Hz), 7.49 (t, 2H, *J* = 7.8 Hz), 7.39 (d, 2H, *J* = 7.7 Hz), 2.06 (s, 6H); ^{13}C NMR (DMSO-*d6*) δ 182.7, 169.1, 139.9, 137.9, 129.4, 124.6, 123.3, 120.2, 24.4; LRMS (ESI) *m/z* 297 $(M+H)^+$; HRMS (ESI) calcd for $C_{17}H_{17}N_2O_3$: 297.1161 $(M+H)^+$; 297.1238.

N,N′-(3,3'-carbonylbis(3,1-phenylene))bis(2-(benzyloxy)acetamide) (**23**)

Yellowish powder (110 mg, 46%): ^{1}H NMR (DMSO-*d6*) δ 10.17 (brs, 2H), 8.15 (brs, 2H), 8.04 (d, 2H, *J* = 7.9 Hz), 7.55-7.31 (m, 16H), 4.64 (brs, 4H), 4.15 (brs, 4H); ^{13}C NMR (DMSO-*d6*) δ 195.9, 168.9, 139.2, 138.1, 137.9, 129.4, 128.7, 128.3, 128.1, 125.2, 124.1, 121.1, 72.9, 69.8; LRMS (ESI) *m/z* 509 $(M+H)^+$; HRMS (ESI) calcd for $C_{31}H_{29}N_2O_5$: 509.1998 $(M+H)^+$; found 509.2078.

N,N′-(3,3'-carbonylbis(3,1-phenylene))dimethanesulfonamide (**24**)

Off-white powder (20 mg, 23%): ^{1}H NMR (DMSO-*d6*) δ 8.05-7.70 (m, 8H), 3.56 (brs, 6H); ^{13}C NMR (DMSO-*d6*) δ 193.5, 138.0, 135.9, 134.3, 132.5, 131.8, 130.5, 43.4; LRMS (ESI) *m/z* 369 $(M+H)^+$; HRMS (ESI) for $C_{15}H_{17}N_2O_5S_2$: 369.0501 $(M+H)^+$; found 369.0583.

N,N'-2H-fluoren-7-yl-2-ylidenediacetamide (**25**)

To a solution of 2,7-diacetamino-9-fluorenol **18** (10 mg, 0.034 mmol) in CH_3CN/MeOH (1:1, 1 ml) was added a 1 N aqueous solution of NaOH (0.04 ml, 0.04 mmol). The reaction mixture was stirred for 1 h, then added water and evaporated solvent under reduced pressure. The mixture was extracted with CH_2Cl_2 and the organic layer was washed with brine and the solvent was evaporated under reduced pressure. ^{1}H NMR (DMSO-d_6) δ 10.13 (brs, 1H), 7.78 (brs, 1H), 7.57 (d, 1H, *J* = 8.0 Hz), 7.37 (d, 1H, *J* = 7.8 Hz), 7.28 (d, 1H, *J* = 8.0 Hz), 6.79 (brs, 1H), 6.66 (d, 1H, *J* = 8.1 Hz), 5.57 (brs, 1H), 2.55 (s, 3H), 2.05 (s, 3H); LRMS (ESI) *m/z* 279 $(M+H)^+$.

1.3. Cell culture

Triply transfected MDCK cells expressing rat UT-A1, yellow fluorescent protein (YFP)-H148Q/V163S and human aquaporin-1 (AQP1) were grown in Dulbecco's modified Eagle medium, 10% FBS and three selection antibiotics (zeocin, geneticin and hygromycin B), as described.[2]

1.4. UT-A1 inhibition assay

MDCK-UT-A1-AQP1-YFP cells were used for UT-A1 inhibition assay as described.[2] Briefly, after incubation for 15 min with compounds the cells were subjected to a 800 mM urea gradient and cellular YFP fluorescence was continuously measured with a plate reader (model Infinite M1000, Tecan Trading AG, Switzerland). UT-A1 inhibition alters the profile of the curve, increasing the initial shrinkage (decreased fluorescence) and slowing reswelling (increased fluorescence). Percentage UT-A1 inhibition was computed as 100% $(F_{neg} - F_{test})/(F_{neg} - F_{pos})$, where F is fluorescence measured 7 s after urea injection for the negative control (F_{neg}), test compound (F_{test}) and positive control (F_{pos}).

1.5. UT-B inhibition assay

As described,[3] whole rat blood was diluted to a hematocrit of ~1.5% in PBS containing 1.25 M acetamide. Erythrocyte suspensions were incubated for 15 min with test compounds and then rapidly mixed with PBS. Percentage lysis was quantified from absorbance at 710 nm as: 100% $(A_{neg} - A_{test})/(A_{neg} - A_{pos})$, where A is absorbance for the negative control (A_{neg}), test compound (A_{test}) and positive control (A_{pos}) at 710 nm.

1.6. Functional studies

Reversibility was tested by incubation of inhibition at a concentration near their IC_{50} and then washing with PBS before UT-A1 inhibition assay. Competition with urea was studied using different concentrations of urea(80 to 1,600 mM) in the UT-A1 inhibition assay.

1.7. In vitro metabolic stability

Rat microsomes (1 mg protein/mL; Sigma-Aldrich, St. Louis, MO) in potassium phosphate buffer containing 1 mM NADPH was incubated with compounds for specified times at 37 °C and as described.[4] Reverse-phase HPLC separations were carried out using aXterra MS C18 column (2.1 mm x 100 mm, 3.5 μm) equipped with a solvent delivery system (Waters model 2695, Milford, MA). The solvent system consisted of a linear gradient from 5 to 95% acetonitrile containing 0.1% formic acid, run over 16 min (0.2 mL/min flow rate). Mass spectra were acquired on a mass spectrometer (Waters 2695 + micromass ZQ; Waters) using electron spray (+) ionization, mass ranging from 200 to 1500 Da, 40-V cone voltage.

1.8. Homology modeling and docking

Homology models of rat UT-A1 and UT-B were generated, as described.[1, 2] The models were generated using the coordinates of the X-ray crystal structure of bovine UT-B (PDB 4EZC)[5] as a homology template. Notably, UT-A1 is a heterodimer of UT-A3 linked to UT-A2 (on the N- and C- termini, respectively). The homology model was based on the C-terminal domain of UT-A1, which corresponds to the UT-A2 protein sequence. The homology models were prepared for docking simulations with the FRED-RECEPTOR utility (Version 2.2.5, OpenEye Scientific, Santa Fe, NM, http://www.eyesopen.com), using the cytoplasmic domain defined with a 10 cubic Å box. Inhibitor structures were drawn in ChemDraw (Cambridge Software, Cambridge, MA), converted to SMILES strings, transformed to three-dimensional conformations, and minimized using PIPELINE PILOT (Accelrys, San Diego, CA). The single conformations were passed through MOLCHARGE (Version 1.5.0, OpenEye Scientific) to apply MMFF charges, and through OMEGA (Version 2.4.6, OpenEye Scientific Software)[6, 7] to generate multi-conformational libraries. The inhibitor conformational libraries were docked using FRED (Version 2.2.5, OpenEye Scientific Software),[8] free of pharmacophore restraint. FRED was configured to use consensus scoring, with scoring functions ChemGauss3, ChemScore, OEChemScore, ScreenScore, ShapeGauss, PLP, and ZapBind. The final protein-inhibitor complexes were visualized using PYMOL (Schrödinger, San Diego, CA).

References

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