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Small Molecule Antagonists of Cell-Surface Heparan Sulfate and Heparin–Protein Interactions

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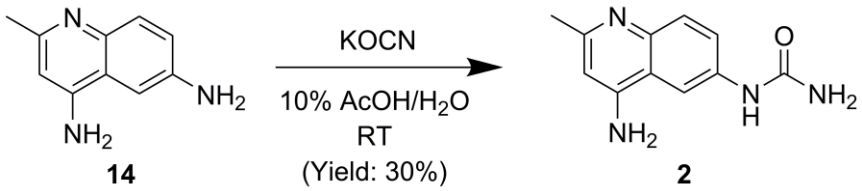
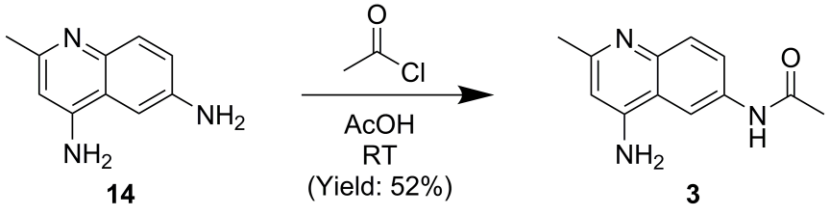
S.1 – Synthesis and characterization of surfen analogs

The synthesis for certain analogs (**3, 7–12**) was adapted from previously published procedures.[1]

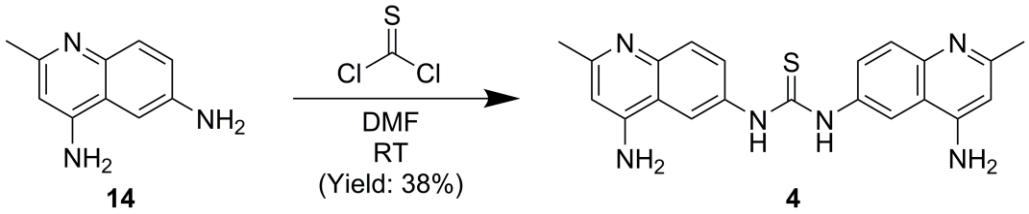


(4-amino-2-methyl-6-quinolyl)-urea (Hemisurfen) (2). 4,6-diamino-2-methylquinoline (**14**) (40 mg, 0.23 mmol) was suspended in water (1 ml). Potassium cyanate (22.5 mg, 0.28 mmol), dissolved in 0.8 ml of 10% acetic acid, was added dropwise to the suspension at room temperature and allowed to react overnight. Once the reaction was complete, 37% NaOH was added dropwise to the reaction mixture until basic. A precipitate formed overnight. The crude solid was filtered, washed with water, and dried overnight. The solid was recrystallized from water. Product: tan solid (15 mg, 0.069 mmol, 30%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.47 (s, 1H), 7.82 (d, J = 2 Hz, 1H), 7.60 (d, J = 2.4 Hz, 1H), 7.53 (d, J = 9.2 Hz, 1H), 6.38 (s, 1H), 6.29 (brd, 2H), 5.90 (brd, 2H), 2.36 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 156.13, 155.97, 150.67, 144.48, 135.53, 128.35, 122.85, 117.48, 108.81, 102.29, 24.60. HR-ESI-MS calculated for $C_{11}H_{13}N_4O$ $[M+H]^+$ 217.1084, found 217.1083.



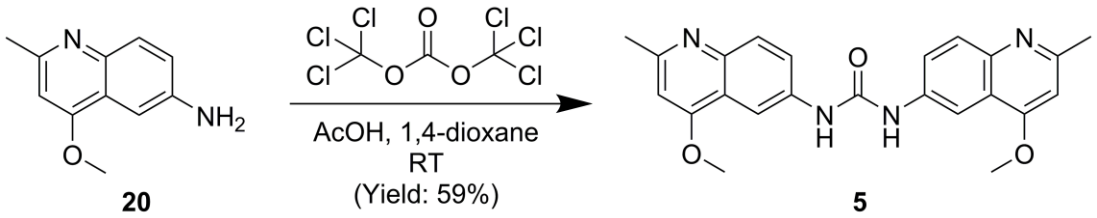
6-Acetamido-4-aminoquinaldine (Acetyl-hemisurfen) (3). 4,6-diamino-2-methyl-quinoline (**14**) (40 mg, 0.23 mmol) was dissolved in glacial acetic acid (0.5 ml) under argon. Acetyl

chloride (16.4 µl, 0.23 mmol) was slowly added. The mixture was stirred for 3 hours at room temperature and a heavy precipitate formed. Diethyl ether (3 ml) was added to the reaction mixture, and the solid was filtered and washed with diethyl ether. The hydrochloride salt was dissolved in 4 ml of warm water, cooled to room temperature, and made basic with 37% NaOH. The precipitated solid was filtered, washed with water, and dried under high vacuum overnight. The crude product was then recrystallized from water. Product: tan solid (25 mg, 0.12 mmol, 52%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.00 (s, 1H), 8.16 (s, 1H), 7.65-7.47 (m, 2H), 6.41 (s, 1H), 6.35 (brd, 2H), 2.37 (s, 3H), 2.07 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.19, 156.95, 151.07, 145.50, 134.03, 128.59, 123.46, 117.29, 111.25, 102.50, 24.77, 23.89. HR-ESI-MS calculated for $C_{12}H_{14}N_3O$ $[M+H]^+$ 216.1131, found 216.1132.



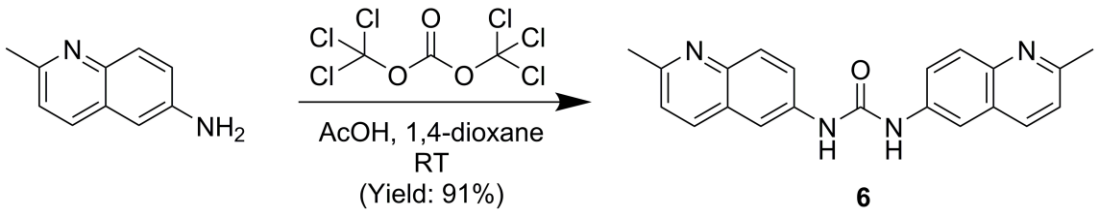
***N,N*'-bis-(4-amino-2-methyl-6-quinolyl)-thiourea (Thio Surfen) (4).** 4,6-diamino-2-methyl-quinoline (**14**) (100 mg, 0.58 mmol) was dissolved in dry DMF (0.6 ml) under argon. The solution was placed on ice and thiophosgene (TOXIC! 22.1 µl, 0.29 mmol) was slowly added. The mixture was stirred overnight and allowed to return to room temperature. A precipitate formed overnight with stirring. Methanol (2 ml) was added to quench any remaining thiophosgene. The reaction mixture was evaporated to dryness and dried under high vacuum overnight. The crude product was dissolved in 7.5 ml of warm water. The solution was cooled to room temperature and made basic with 37% NaOH. The precipitated solid was filtered, washed with water, and dried overnight under high vacuum. Recrystallization was accomplished by dissolving the solid in a minimum amount of hot DMF, filtering, and adding diethyl ether until

the solution became turbid. The crystals were collected by filtration, washed with ether, and dried under high vacuum. Product: dark yellow solid (43 mg, 0.11 mmol, 38%). IR spectrum: 1631 cm^{-1} (C=S). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.79 (s, 2H), 7.93 (s, 2H), 7.68–7.53 (m, 4H), 6.57 (brd, 4H), 6.41 (s, 2H), 2.39 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 180.74, 157.57, 151.24, 146.30, 134.07, 128.31, 127.85, 117.72, 116.92, 102.02, 24.55. HR-ESI-MS calculated for $C_{21}H_{21}N_6S$ $[M+H]^+$ 389.1543, found 389.1542.



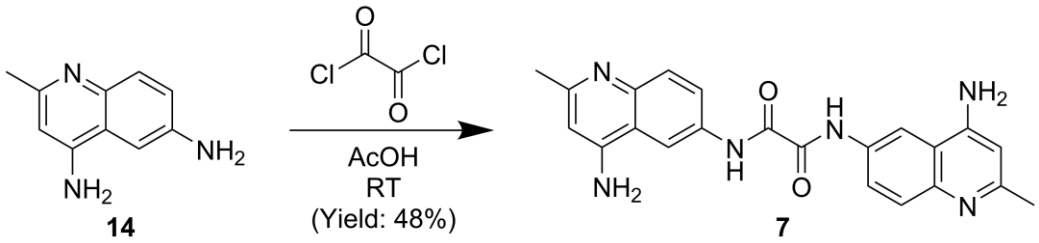
1,3-bis(4-methoxy-2-methyl-6-quinolyl)-urea (Methoxy Surfen) (5). 6-amino-4-methoxyquinaldine (**20**) (105 mg, 0.56 mmol) was dissolved in glacial acetic acid (3 mL) under argon. Triphosgene (TOXIC! 28 mg, 0.093 mmol) was dissolved in dioxane (150 µL) and slowly added to the reaction mixture. A heavy precipitate formed, and the reaction was stirred at room temperature for 2 hrs. Methanol (8 mL) was added to the solution to quench any unreacted triphosgene. The solution was then evaporated and the residue co-evaporated with toluene to azeotropically remove remaining acetic acid. The product was then dried under high vacuum. The hydrochloride salt was dissolved in warm water (2 ml). The solution was cooled to room temperature and was made basic with 37% NaOH. The precipitated solid was filtered, washed with water, and dried on high vacuum overnight. Recrystallization was accomplished by dissolving the solid in a minimum amount of hot DMF, filtering, and adding diethyl ether until the solution became turbid. The crystals were collected by filtration, washed with ether, and dried under high vacuum. Product: tan solid (22 mg, 0.055 mmol, 59%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.03 (s, 2H), 8.41 (d, J = 2.3 Hz, 2H), 7.78 (d, J = 9.0 Hz, 2H), 7.58 (dd, J = 9.0,

2.4 Hz, 2H), 6.89 (s, 2H), 4.04 (s, 6H), 2.58 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 160.95, 157.61, 152.56, 144.32, 136.28, 128.41, 122.70, 119.55, 107.37. 101.36, 55.80, 25.09. HR-ESI-MS calculated for $C_{23}H_{23}N_4O_3$ $[M+H]^+$ 403.1765, found 403.1766.

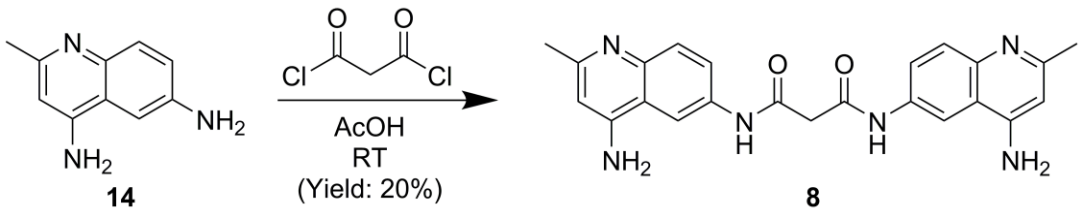


6

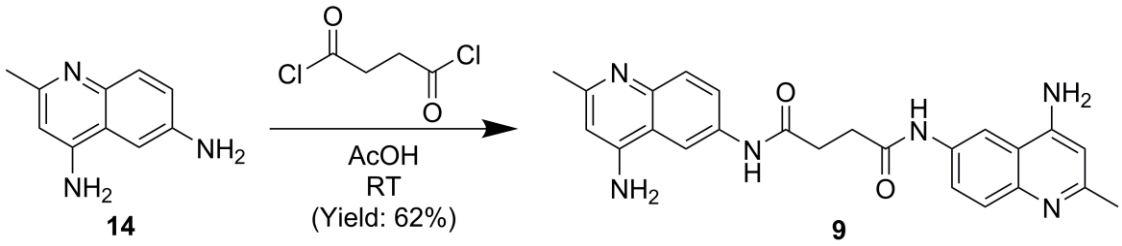
1,3-bis(2-methyl-6-quinolyl)-urea (Deaminated Surfen) (6). 6-Amino-2-methylquinoline (106 mg, 0.67 mmol) was dissolved in glacial acetic acid (2 mL) under argon. Triphosgene (TOXIC! 33 mg, 0.11 mmol) was dissolved in 150 µL of dioxane and slowly added dropwise to the reaction mixture. A heavy precipitate formed, and the reaction was stirred at room temperature for 2 hours. Next, 8 mL of methanol was added to the solution to quench any unreacted triphosgene. Solution was then rotoevaporated completely. Toluene was added to the reaction flask and rotoevaporated to aziotrope remaining acetic acid. The crude product was dried on high vacuum overnight. The hydrochloride salt was dissolved in 2 ml of warm water, the solution was cooled to room temperature, and it was made basic with 37% NaOH. The precipitated solid was filtered, washed with water, and dried on high vacuum overnight. Recrystallization was accomplished by dissolving the solid in a minimum amount of hot DMF, filtering, and adding diethyl ether until the solution became turbid. The crystals were collected by filtration, washed with diethyl ether, and dried under high vacuum. Product: tan solid (35 mg, 0.10 mmol, 91%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.09 (s, 2H), 8.17 (d, J = 4.9 Hz, 2H), 8.14 (s, 2H), 7.85 (d, J = 8.9 Hz, 2H), 7.67 (d, J = 8.9 Hz, 2H), 7.36 (d, J = 8.4 Hz, 2H), 2.62 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 156.62, 152.78, 143.82, 136.93, 135.46, 128.86, 126.82, 122.90, 122.50, 113.39, 24.71. HR-ESI-MS calculated for $C_{21}H_{19}N_4O$ $[M+H]^+$ 343.1553, found 343.1551.



Bis(4-amino-2-methyl-6-quinolyl)-oxalamide (Oxalyl Surfen) (7). 4-6-diamino-2-methyl-quinoline (**14**) (100 mg, 0.58 mmol) was dissolved in glacial acetic acid (0.6 ml) under argon. Oxalyl chloride (25 µl, 0.29 mmol) was slowly added to the solution. A heavy precipitate formed, and the mixture was stirred for 1 hour at room temperature. After addition of diethyl ether (2.5 ml), the solid was filtered, washed with diethyl ether and dried under vacuum overnight. The hydrochloride salt was dissolved in warm water (8 ml), the solution was cooled to room temperature, and it was made basic with 37% NaOH. The precipitated solid was filtered, washed with water, and dried on high vacuum. Recrystallization was accomplished by dissolving the solid in a minimum amount of hot DMF, filtering, and adding diethyl ether until the solution became turbid. The crystals were collected by filtration, washed with ether, and dried under high vacuum. Product: tan solid (54 mg, 0.14 mmol, 48%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.85 (s, 2H), 8.36 (d, J = 1.6 Hz, 2H), 7.91 (d, J = 9.0 Hz, 2H), 7.69 (d, J = 9.0 Hz, 2H), 6.49 (brd, 4H), 6.46 (s, 2H), 2.41 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 158.49, 157.68, 151.11, 146.07, 132.06, 128.52, 123.93, 116.99, 113.50, 102.43, 24.65. HR-ESI-MS calculated for $C_{22}H_{21}N_6O_2$ $[M+H]^+$ 401.1721, found 401.1722.

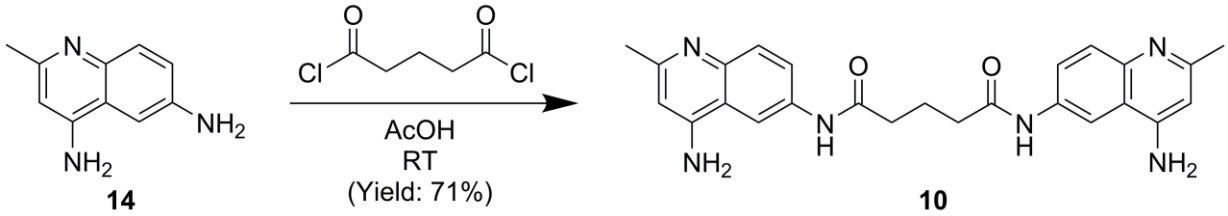


N,N'-bis(4-amino-2-methylquinolin-6-yl)malonamide (Malonyl Surfen) (8). 4-6-diamino-2-methyl-quinoline (**14**) (60 mg, 0.35 mmol) was dissolved in glacial acetic acid (0.3 ml) under argon. Malonyl chloride (16.8 µl, 0.17 mmol) was slowly added to the solution. A heavy precipitate formed, and the mixture was stirred for 1 hour at room temperature. After addition of diethyl ether (2 mL), the solid was filtered, washed with diethyl ether, and dried under vacuum overnight. The hydrochloride salt was dissolved in 4 ml of warm water, the solution was cooled to room temperature, and it was made basic with 37% NaOH. The precipitated solid was filtered, washed with water, and dried on high vacuum overnight. Recrystallization was accomplished by dissolving the solid in a minimum amount of hot DMF, filtering, and adding diethyl ether until the solution became turbid. The crystals were collected by filtration, washed with ether, and dried under high vacuum. Product: tan solid (14.1 mg, 0.034 mmol, 20%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.35 (s, 2H), 8.26 (s, 2H), 7.69-7.63 (m, 4H), 6.59 (brd, 4H), 6.43 (s, 2H), 3.58 (s, 2H), 2.39 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 165.41, 156.66, 151.53, 146.83, 133.86, 128.04, 123.45, 117.09, 111.22, 102.45, 45.47, 24.31. HR-ESI-MS calculated for $C_{23}H_{22}N_6O_2$ $[M+H]^+$ 415.1877, found 415.1874.



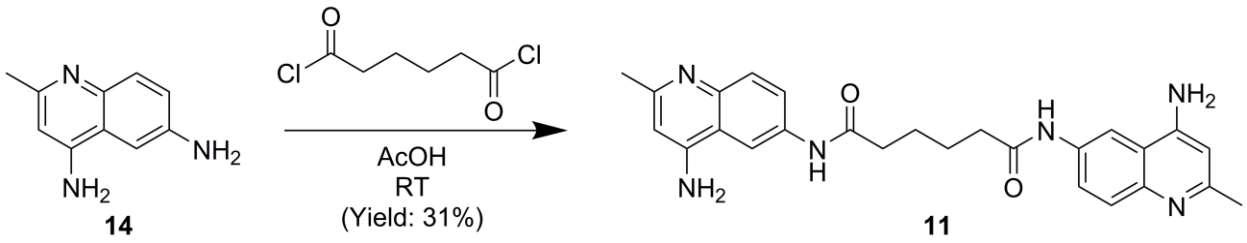
N,N'-bis(4-azanyl-2-methyl-6-quinolyl)-butanediamide (Succinyl Surfen) (9). 4-6-diamino-2-methyl-quinoline (**14**) (100 mg, 0.58 mmol) was dissolved in glacial acetic acid (0.6 ml) under argon. Succinyl chloride (32 µl, 0.29 mmol) was slowly added to this solution. A heavy precipitate formed, and the mixture was stirred for 2 hours at room temperature. After addition of diethyl ether (5 ml), the solid was filtered, washed with diethyl ether and dried under high

vacuum. The hydrochloride salt was dissolved in warm water (7.5 ml). The solution was cooled to room temperature and was made basic with 37% NaOH. The precipitated solid was filtered, washed with water, and dried on high vacuum. Recrystallization was accomplished by dissolving the solid in a minimum amount of hot DMF, filtering, and adding diethyl ether until the solution became turbid. The crystals were collected by filtration, washed with diethyl ether, and dried under high vacuum. Product: tan solid (75 mg, 0.18 mmol, 62%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.12 (s, 2H), 8.24 (s, 2H), 7.64–7.57 (m, 4H), 6.41 (brd, 6H), 2.74 (s, 4H), 2.38 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.53, 157.08, 151.28, 145.21, 134.31, 128.45, 123.33, 117.30, 110.70, 102.64, 31.14, 24.60. HR-ESI-MS calculated for $C_{24}H_{24}N_6O_2$ $[M+H]^+$ 429.2034, found 429.2031.



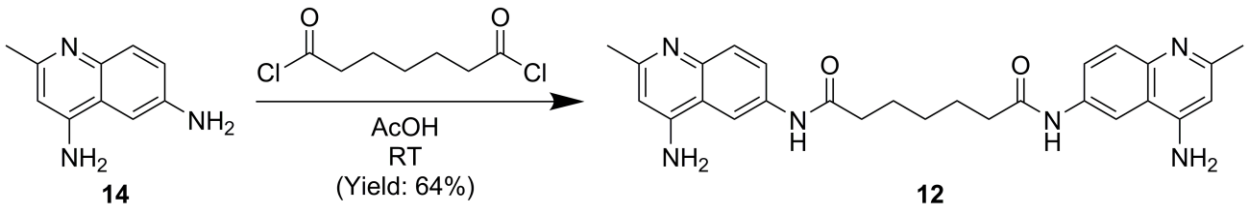
N,N'-bis(4-amino-2-methylquinolin-6-yl)glutaramide (Glutaryl Surfen) (10). 4-6-diamino-2-methyl-quinoline (**14**) (20 mg, 0.12 mmol) was dissolved in glacial acetic acid (0.3 ml) under argon. Glutaryl chloride (7.4 µl, 0.058 mmol) was slowly added to the solution. A heavy precipitate formed, and the mixture was stirred for 1 hour at room temperature. After addition of diethyl ether, the solid was filtered, washed with diethyl ether and dried under vacuum. The hydrochloride salt was dissolved in warm water, and the solution was cooled to room temperature and made basic with 37% NaOH. The precipitated solid was filtered, washed with water, and dried on high vacuum. Product: tan solid (18 mg, 0.041 mmol, 71%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.07 (s, 2H), 8.27 (s, 2H), 7.64–7.56 (m, 4H), 6.56 (s, 2H), 6.42 (brd, 4H), 2.45 (t, J = 7.2 Hz, 4H), 2.39 (s, 6H), 2.03-1.92 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ

170.78, 156.47, 151.56, 148.31, 134.27, 127.88, 123.74, 117.13, 111.17, 102.43, 35.46, 24.34, 21.04. HR-ESI-MS calculated for $C_{25}H_{26}N_6O_2$ $[M+H]^+$ 443.2190, found 443.2191.



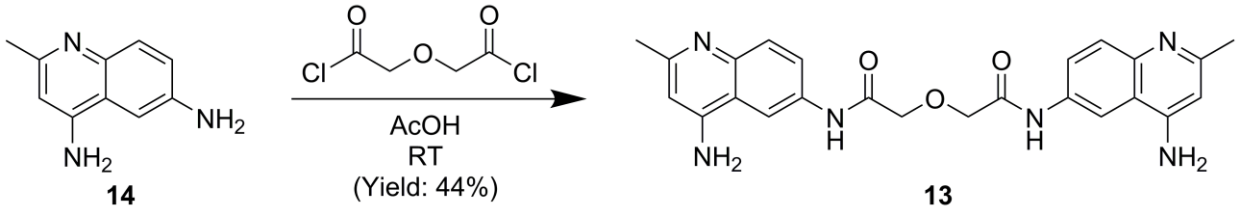
14 11

***N,N'-bis(4-amino-2-methylquinolin-6-yl)adipamide (Adipoyl Surfen) (11).** 4-6-diamino-2-methyl-quinoline (**14**) (50 mg, 0.29 mmol) was dissolved in glacial acetic acid (0.3 ml) under argon. Adipoyl chloride (21 µl, 0.14 mmol) was slowly added to this solution. A heavy precipitate formed, and the mixture was stirred for 1 hour at room temperature. After addition of diethyl ether (2.5 ml), the solid was filtered, washed with diethyl ether and dried under vacuum. The hydrochloride salt was dissolved in warm water (4 ml) and the solution was made basic with 37% NaOH. A solid precipitate formed, and the heterogeneous solution was allowed to cool to room temperature. The precipitated solid was filtered, washed with water, and dried on high vacuum overnight. Recrystallization was accomplished by dissolving the solid in a minimum amount of hot DMF, filtering, and adding diethyl ether until the solution became turbid. The crystals were collected by filtration, washed with ether, and dried under high vacuum. Product: white solid (19.5 mg, 0.043 mmol, 31%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.01 (s, 2H), 8.22 (s, 2H), 7.64–7.55 (m, 4H), 6.41 (brd, 6H), 2.43–2.39 (m, 4H), 2.37 (s, 6H), 1.77–1.64 (m, 4H).^{13}C NMR (125 MHz, DMSO-d_6): δ 168.22, 156.92, 151.13, 145.39, 134.06, 128.50, 123.50, 117.27, 111.23, 102.49, ~38.90 (hidden by DMSO solvent peak), 24.72, 23.89. HR-ESI-MS calculated for $C_{26}H_{28}N_6O_2$ $[M+H]^+$ 457.2347, found 457.2346.



14 12

N,N'-bis(4-amino-2-methylquinolin-6-yl)heptanediamide (Pimeloyl Surfen) (12). 4-6-diamino-2-methyl-quinoline (**14**) (32 mg, 0.19 mmol) was dissolved in glacial acetic acid (0.3 ml) under argon. Pimeloyl chloride (15 µl, 0.092 mmol) was slowly added to this solution. A heavy precipitate formed, and the mixture was stirred for 1 hour at room temperature. After addition of diethyl ether (2.5 ml), the solid was filtered, washed with diethyl ether and dried under vacuum. The hydrochloride salt was dissolved in warm water (3 ml). The solution was cooled to room temperature and made basic with 37% NaOH. The precipitated solid was filtered, washed with water, and dried on high vacuum. Product: maroon solid (28 mg, 0.059 mmol, 64%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.98 (s, 2H), 8.23 (s, 2H), 7.59 (brd, 4H), 6.46 (brd, 4H), 6.42 (s, 2H), 2.38 (brd, 10H), 1.77–1.57 (m, 4H), 1.49–1.30 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 171.21, 156.68, 151.46, 144.90, 134.27, 128.13, 123.68, 117.22, 111.15, 102.50, 36.18, 28.50, 25.11, 24.48. HR-ESI-MS calculated for $C_{27}H_{30}N_6O_2$ $[M+H]^+$ 471.2503, found 471.2504.



14 13

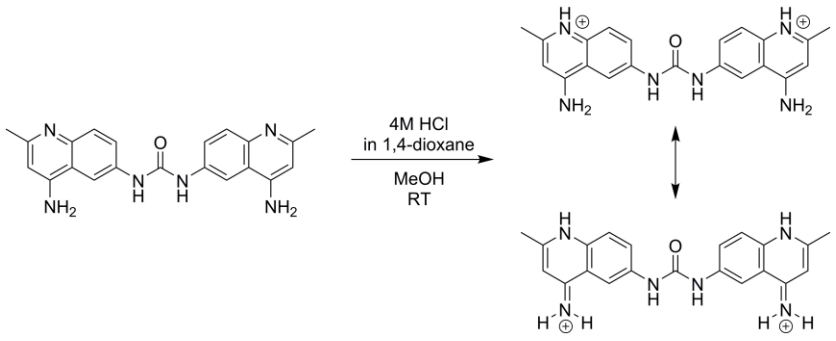
2,2'-oxybis(N-(4-amino-2-methylquinolin-6-yl)acetamide) (Diglycolyl Surfen) (13). 4-6-diamino-2-methyl-quinoline (**14**) (40 mg, 0.23 mmol) was dissolved in glacial acetic acid (0.3 ml) under argon. Diglycolyl chloride (13.7 µl, 0.12 mmol) was slowly added to this solution. A heavy precipitate formed, and the mixture was stirred for 1 hour at room temperature. After

addition of diethyl ether (2.5 ml), the solid was filtered, washed with diethyl ether and dried under vacuum. The hydrochloride salt was dissolved in warm water (4 ml) and the solution was made basic with 37% NaOH. The solution was allowed to cool to room temperature, and the precipitated solid was filtered, washed with water, and dried under high vacuum. Product: tan solid (23.5 mg, 0.053 mmol, 44%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.13 (s, 2H), 8.22 (s, 2H), 7.72 (d, J = 9.2 Hz, 2H), 7.65 (d, J = 9.2 Hz, 2H), 6.45 (s, 4H), 6.43 (s, 2H), 4.33 (s, 4H), 2.39 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 167.87, 157.41, 151.19, 145.89, 132.85, 128.66, 124.25, 117.22, 112.88, 102.49, 70.76, 24.79. HR-ESI-MS calculated for $C_{24}H_{24}N_6O_3$ $[M+H]^+$ 445.1983, found 445.1981.

S.2 – Synthesis and characterization of building blocks

The synthesis for ethyl-β-(*p*-acetamidophenylamino) crotonate (**16**), 6-acetamido-4-hydroxyquinaldine (**17**), 6-Acetamido-4-methoxyquinaldine (**18**), 4,6-diaminoquinaldine (**19**), and 6-amino-4-methoxyquinaldine (**20**) were previously reported.[1,2]

S.3 – General procedure for precipitation of HCl salts



Scheme S1. Example of synthesis of the HCl salt form.

Surfen analog (1 eq.) was dissolved in a minimum amount of methanol. In some cases, the solid did not initially dissolve. HCl in dioxane (4M solution, 1 eq.) was added generating a

homogeneous solution. The solution was stirred until the HCl salt precipitated out. Diethyl ether was added to the reaction mixture to further assist in precipitation. The product was filtered, washed with diethyl ether, and dried on high vacuum overnight.

Hemisurfen HCl (2a). Product: tan solid (4.8 mg, .019 mmol, 95%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.04 (s, 1H), 8.53 (s, 2H), 8.21 (s, 1H), 7.89 (dd, J = 9.1, 1.9 Hz, 1H), 7.76 (d, J = 9.1 Hz, 1H), 6.53 (s, 1H), 6.15 (s, 2H), 2.55 (s, 3H). HR-ESI-MS calculated for $C_{11}H_{13}N_4O$ $[M+H]^+$ 217.1085, found 217.1084.

Acetyl-hemisurfen HCl (3a). Product: tan solid (11.8 mg, 0.047 mmol, 97%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.48 (s, 1H), 8.71 (brd, 2H), 8.56 (s, 1H), 7.86 (d, J = 2.2 Hz, 2H), 6.57 (s, 1H), 2.57 (s, 3H), 2.12 (s, 3H). HR-ESI-MS calculated for $C_{11}H_{13}N_4O$ $[M+H]^+$ 217.1085, found 217.1084.

Thio Surfen HCl (4a). Product: yellow solid (15 mg, 0.035 mmol, 46%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.10 (s, 2H), 8.23 (s, 2H), 7.59 (d, J = 2.4 Hz, 4H), 6.41 (s, 2H), 6.34 (s, 4H), 2.37 (s, 6H). HR-ESI-MS calculated for $C_{21}H_{21}N_6S$ $[M+H]^+$ 389.1543, found 389.1545.

Methoxy Surfen HCl (5a). Product: tan solid (4.9 mg, 0.01 mmol, 86%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.28 (s, 2H), 8.61 (d, J = 2.3 Hz, 2H), 8.10 (d, J = 9.2 Hz, 2H), 7.96 (dd, J = 9.2, 2.0 Hz, 2H), 7.48 (s, 2H), 4.28 (s, 6H), 2.84 (s, 6H). HR-ESI-MS calculated for $C_{23}H_{23}N_4O_3$ $[M+H]^+$ 403.1766, found 403.1765.

Deaminated Surfen HCl (6a). Product: yellow solid (11 mg, 0.026 mmol, 76%). ^{1}HNMR (400 MHz, DMSO-d_6): δ 10.26 (s, 2H), 8.88 (d, J = 7.5 Hz, 2H), 8.49 (s, 2H), 8.22 (d, J = 9.1 Hz, 2H), 8.03 (d, J = 9.1 Hz, 2H), 7.83 (d, J = 8.2 Hz, 2H), 2.88 (s, 6H). HR-ESI-MS calculated for $C_{21}H_{19}N_4O$ $[M+H]^+$ 343.1553, found 343.1556.

Oxalyl Surfen HCl (7a). Product: tan solid (16 mg, 0.04 mmol, 56%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.22 (s, 2H), 8.64 (brd, 6H), 8.17 (d, J = 9.3 Hz, 2H), 7.94 (d, J = 9.3 Hz, 2H), 6.63 (s, 2H), 2.60 (s, 6H). HR-ESI-MS calculated for $C_{22}H_{21}N_6O_2$ $[M+H]^+$ 401.1721, found 401.1718.

Malonyl Surfen HCl (8a). Product: white solid (4.1 mg, 0.008 mmol, 87%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.72 (s, 2H), 8.59 (brd, 6H), 7.91 (d, J = 9.6 Hz, 2H), 7.83 (d, J = 9.3 Hz, 2H), 6.56 (s, 2H), 3.66 (s, 2H), 2.56 (s, 6H). HR-ESI-MS calculated for $C_{23}H_{23}N_6O_2$ $[M+H]^+$ 415.1877, found 415.1879.

Succinyl Surfen HCl (9a). Product: tan solid (4 mg, 0.01 mmol, 84%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.54 (s, 2H), 8.61 (s, 6H), 7.95–7.73 (m, 2H), 6.56 (s, 2H), 2.79 (s, 4H), 2.56 (s, 6H). HR-ESI-MS calculated for $C_{24}H_{24}N_6O_2$ $[M+H]^+$ 429.2034, found 429.2036.

Glutaryl Surfen HCl (10a). Product: tan solid (2.6 mg, 0.005 mmol, 80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.49 (s, 2H), 8.63 (brd, 6H), 7.85 (s, 4H), 6.56 (s, 2H), 2.57 (s, 6H), 2.53–2.51 (m, 4H), 2.04–1.94 (m, 2H). HR-ESI-MS calculated for $C_{25}H_{27}N_6O_2$ $[M+H]^+$ 443.2190, found 443.2192.

Adipoyl Surfen HCl (11a). Product: white solid (14.6 mg, 0.0276 mmol, 98%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.40 (s, 1H), 8.57 (s, 1H), 8.36 (s, 1H), 7.88–7.73 (m, J = 0.5 Hz, 1H), 6.54 (s, 1H), 2.54 (s, 2H), 2.48–2.40 (m, 1H), 1.75–1.66 (m, 1H). HR-ESI-MS calculated for $C_{26}H_{29}N_6O_2$ $[M+H]^+$ 457.2347, found 457.2346.

Pimeloyl Surfen HCl (12a). Product: white solid (7.2 mg, 0.014 mmol, 100%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.44 (s, 2H), 8.60 (s, 6H), 7.91–7.77 (m, 4H), 6.56 (s, 2H), 2.57 (s, 6H), 2.42 (t, J = 7.3 Hz, 4H), 1.74–1.61 (m, 4H), 1.47–1.34 (m, 2H). HR-ESI-MS calculated for $C_{27}H_{31}N_6O_2$ $[M+H]^+$ 471.2503, found 471.2501.

Diglycolyl Surfen HCl (13a). Product: white solid (13.2 mg, 0.0255 mmol, 97%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.52 (s, 2H), 8.62 (brd, 6H), 8.03 (dd, J = 9.1, 2.0 Hz, 2H), 7.88 (d, J = 9.1 Hz, 2H), 6.59 (s, 2H), 4.39 (s, 4H), 2.58 (s, 6H). HR-ESI-MS calculated for $C_{24}H_{25}N_6O_3$ $[M+H]^+$ 445.1983, found 445.1984.

S.4 – X-ray crystal structures

Experimental Summary

The single crystal X-ray diffraction studies were carried out on a Bruker Kappa APEX-II CCD diffractometer equipped with Mo K_α radiation (λ = 0.71073 Å) at the UCSD Chemistry and Biochemistry Small Molecule X-ray Facility. Crystals were mounted on a Cryoloop with Paratone oil. Data were collected in a nitrogen gas stream at 100(2) K using ϕ and ϖ scans. The data were integrated using the Bruker SAINT software program and scaled using the SADABS

software program. Solution by direct methods (SHELXT) produced a complete phasing model consistent with the proposed structure. All nonhydrogen atoms were refined anisotropically by full-matrix least-squares (SHELXL-2014). All hydrogen atoms were placed using a riding model. Their positions were constrained relative to their parent atom using the appropriate HFIX command in SHELXL-2014. Crystallographic data are summarized in Table S1−S4.

Crystal structures were deposited in the Cambridge Crystallographic Data Centre.

The data have been assigned the following deposition numbers:

Summary of Data CCDC 1057474
Compound Name: **adipoyl surfen (11)**
Formula: C26 H28 N6 O2,2(H2 O1)
Unit Cell Parameters: a 8.5132(5) b 21.0477(13) c 7.2003(4) P21/c

Summary of Data CCDC 1057475
Compound Name: **oxalyl surfen (7)**
Formula: C22 H20 N6 O2,4(C1 H4 O1)
Unit Cell Parameters: a 6.2877(7) b 8.8655(9) c 12.0399(13) P-1

Summary of Data CCDC 1057476
Compound Name: **diglycolyl surfen•2HCl (13)**
Formula: C24 H25 N6 O3 1+,H2 Cl1 O1,H2 O1,Cl1 1-
Unit Cell Parameters: a 5.0339(9) b 37.994(7) c 7.1360(11) P21/m

Summary of Data CCDC 1057477
Compound Name: **surfen•2CF_3COOH (1)**
Formula: 2(C21 H22 N6 O1 1+),(C3 H8 F6 Na1 O7)n,2(C3 F6 O3)n,2(C2 F3 O2 1-),x(F1),x(C1 F1),F1,4(C1 O2)
Unit Cell Parameters: a 14.0292(11) b 22.3313(16) c 9.5059(10) C2/m

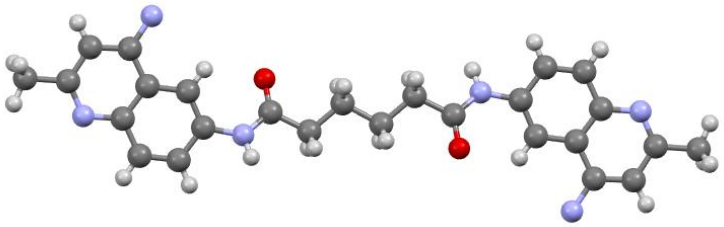


Table S4.1. Crystal data and structure refinement for Tor86 (**adipoyl surfen**).

Identification code	Tor86	
Empirical formula	C26 H28 N6 O4 (with 2 H_2O)	
Formula weight	488.54	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 21/c	
Unit cell dimensions	a = 8.5132(5) Å	α= 90°.
	b = 21.0477(13) Å	β= 113.595(2)°.
	c = 7.2003(4) Å	γ = 90°.
Volume	1182.31(12) $Å^3$	
Z, Z'	2, 0.5	
Density (calculated)	1.372 Mg/m^3	
Absorption coefficient	0.095 mm^{-1}	
F(000)	516	
Crystal size	0.300 x 0.100 x 0.070 mm^3	
Theta range for data collection	2.611 to 26.402°.	
Index ranges	-10<=h<=10, -26<=k<=26, -9<=l<=9	
Reflections collected	20966	
Independent reflections	2413 [R(int) = 0.0491]	
Completeness to theta = 25.000°	99.8 %	
Absorption correction	Multi-scan	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2413 / 0 / 172	
Goodness-of-fit on F^2	1.064	
Final R indices [I>2sigma(I)]	R1 = 0.0463, wR2 = 0.1290	
R indices (all data)	R1 = 0.0502, wR2 = 0.1325	
Extinction coefficient	n/a	

Largest diff. peak and hole	0.812 and -0.199 e.Å^{-3}

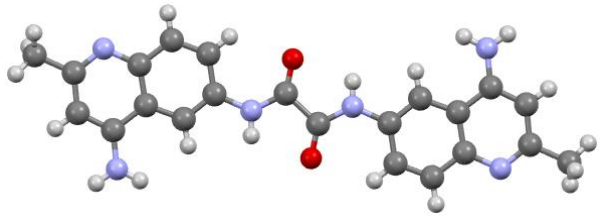


Table S4.2. Crystal data and structure refinement for Tor88 (**oxalyl surfen**).

Identification code	Tor88	
Empirical formula	C13 H18 N3 O3	
Molecular formula	C11 H10 N3 O, 2(C H4 O)	
Formula weight	264.30	
Temperature	100.0 K	
Wavelength	1.54178 Å	
Crystal system	Triclinic	
Space group	P -1	
Unit cell dimensions	a = 6.2877(7) Å	α= 102.993(5)°.
	b = 8.8655(9) Å	β= 91.548(6)°.
	c = 12.0399(13) Å	γ = 92.630(6)°.
Volume	652.79(12) Å^3	
Z	2	
Density (calculated)	1.345 Mg/m^3	
Absorption coefficient	0.800 mm^{-1}	
F(000)	282	
Crystal size	0.317 x 0.031 x 0.015 mm^3	
Crystal color, habit	Light Yellow Needle	
Theta range for data collection	3.770 to 69.433°.	
Index ranges	-7<=h<=7, -10<=k<=10, -12<=l<=14	
Reflections collected	20226	
Independent reflections	2357 [R(int) = 0.0331]	
Completeness to theta = 68.000°	98.3 %	
Absorption correction	Semi-empirical from equivalents	

Max. and min. transmission	0.7532 and 0.6330
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2357 / 5 / 195
Goodness-of-fit on F^2	1.059
Final R indices [I>2sigma(I)]	R1 = 0.0427, wR2 = 0.1156
R indices (all data)	R1 = 0.0490, wR2 = 0.1203
Extinction coefficient	n/a
Largest diff. peak and hole	0.283 and -0.214 e.$Å^{-3}$

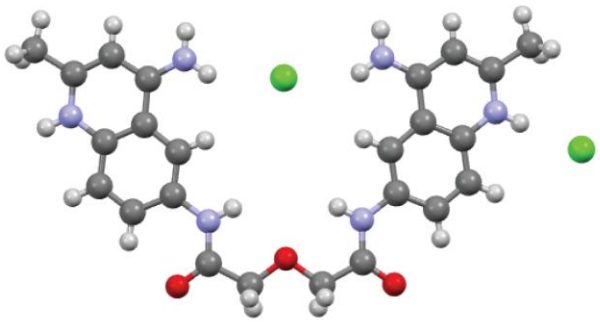


Table S4.3. Crystal data and structure refinement for Tor90 (**diglycolyl surfen•2HCl**).

Identification code	Tor90	
Empirical formula	C24 H29 Cl2 N6 O5	
Molecular formula	C24 H25 N6 O3, 2(H2 O), 2(Cl)	
Formula weight	552.43	
Temperature	100.0 K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	P 1 21/m 1	
Unit cell dimensions	a = 5.0339(9) Å	α= 90°.
	b = 37.994(7) Å	β= 110.533(11)°.
	c = 7.1360(11) Å	γ = 90°.
Volume	1278.1(4) $Å^3$	
Z	2	
Density (calculated)	1.435 Mg/m^3	
Absorption coefficient	0.302 mm^{-1}	

F(000)	578
Crystal size	0.24 x 0.2 x 0.08 mm^3
Crystal color, habit	Colorless Plate
Theta range for data collection	2.144 to 26.490°.
Index ranges	-6<=h<=6, -47<=k<=45, -8<=l<=8
Reflections collected	7858
Independent reflections	2635 [R(int) = 0.0438]
Completeness to theta = 25.000°	99.3 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.0932 and 0.0478
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2635 / 3 / 189
Goodness-of-fit on F^2	1.188
Final R indices [I>2sigma(I)]	R1 = 0.0751, wR2 = 0.1702
R indices (all data)	R1 = 0.0944, wR2 = 0.1780
Extinction coefficient	n/a
Largest diff. peak and hole	0.380 and -0.316 e.$Å^{-3}$

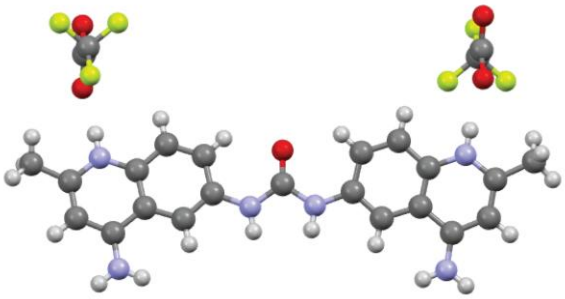


Table S4.4. Crystal data and structure refinement for Tor99 (**surfen•2CF_3COOH**).

Identification code	Tor99
Empirical formula	C26 H26 F7.50 N6 Na0.50 O9
Molecular formula	C2 F3 O2, C21 H22 N6 O, 0.5(C6 H8 F9 Na O12)
Formula weight	720.52
Temperature	100.0 K
Wavelength	0.71073 Å
Crystal system	Monoclinic

Space group	C 1 2/m 1	
Unit cell dimensions	a = 14.0292(11) Å	α= 90°.
	b = 22.3313(16) Å	β= 92.950(2)°.
	c = 9.5059(10) Å	γ = 90°.
Volume	2974.2(4) $Å^3$	
Z	4	
Density (calculated)	1.609 Mg/m^3	
Absorption coefficient	0.157 mm^{-1}	
F(000)	1476	
Crystal size	0.053 x 0.021 x 0.016 mm^3	
Crystal color, habit	Colorless Block	
Theta range for data collection	1.716 to 25.356°.	
Index ranges	-16<=h<=16, -26<=k<=26, -11<=l<=11	
Reflections collected	20193	
Independent reflections	2807 [R(int) = 0.0924]	
Completeness to theta = 25.000°	100.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.0917 and 0.0617	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2807 / 99 / 298	
Goodness-of-fit on F^2	1.041	
Final R indices [I>2sigma(I)]	R1 = 0.1119, wR2 = 0.2863	
R indices (all data)	R1 = 0.1943, wR2 = 0.3458	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.891 and -0.599 $e.Å^{-3}$	

S.5 – FGF2 binding inhibition curves

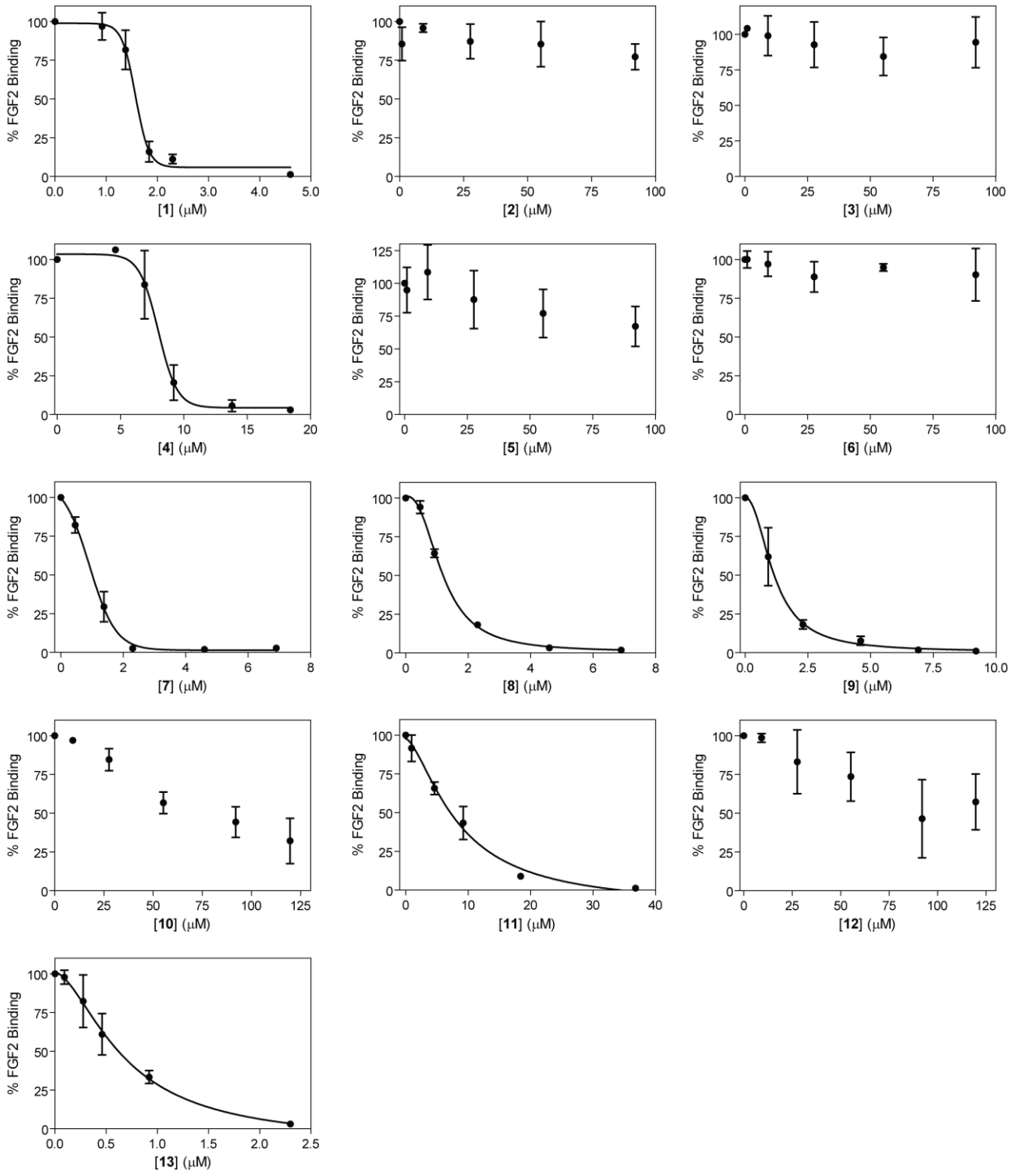


Figure S1. FGF2 binding inhibition curves for surfen (**1**), hemisurfen (**2**), acetyl-hemisurfen (**3**), thio surfen (**4**), methoxy surfen (**5**), deaminated surfen (**6**), oxalyl surfen (**7**), malonyl surfen (**8**), succinyl surfen (**9**), glutaryl surfen (**10**), adipoyl surfen (**11**), pimeloyl surfen (**12**), and diglycolyl surfen (**13**).

S.6 – sRAGE Binding Inhibition Data

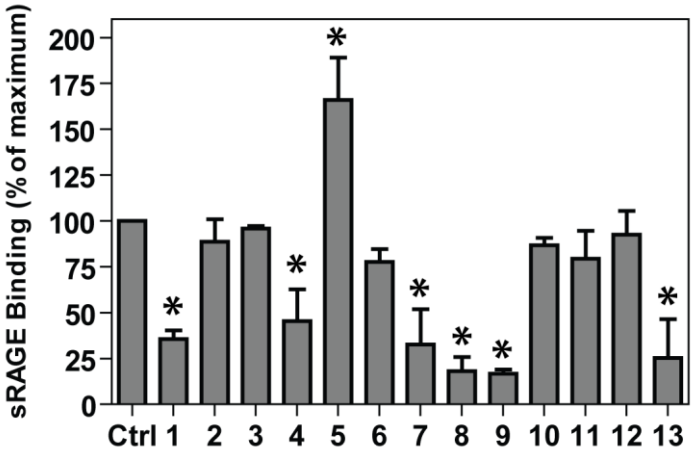


Figure S2. sRAGE binding inhibition of surfen compounds (10 μM). The values represent the means ± SD. **P* < 0.0001 compared with the control (absence of antagonist). The enhanced sRAGE binding of compound **5** (methoxy surfen) has been attributed to an artifact caused by potential Förster resonance energy transfer (FRET) between the emissive methoxy analog and the cyanine dye (Cy5) used in this assay.

S.7 – Biotinylation of FGF2

150 μg of bFGF (35 μl in H_2O, E. coli recombinant, Peptrotech) was mixed at room temperature for 2 hours with 10 μl of heparin (20 mg/ml), 50 ul of HEPES buffer (200 mM, pH 8.4), and 5 μl of Sulfo-NHS-LC-Biotin (4 mg/ml in H_2O, Thermo Scientific). Next, 20 μl of glycine (10 mg/ml) was added to stop the reaction. A 500 μl heparin-Sepharose HP column was equilibrated with wash buffer (0.5 M NaCl, 0.2% BSA, 20 mM HEPES, pH 7.4). The reaction mixture was diluted in 10 ml of wash buffer, loaded onto the column, and subsequently eluted with 2 ml of elution buffer (3 M NaCl, 0.2% BSA, 20 mM HEPES, pH 7.4). Biotinylated FGF2 was stored at 4°C for further use.

S.8 – Biotinylation of sRAGE

Recombinant soluble RAGE protein, generated in *Escherichia coli*,[3] was diluted in 3 ml of PBS and was loaded onto a 200-µl heparin-Sepharose column. Sulfo-NHS-LC-biotin in PBS (500 µl of a 1 mM solution, pH 8) was then applied to the column, and biotinylation was allowed to proceed for 30 min at room temperature. The reaction was stopped by applying 600 µl of PBS containing 100 mM glycine, pH 7. After washing the column with 1 ml of 20 mM HEPES buffer (pH 7.1) containing 150 mM NaCl, the column was eluted sequentially with 600 mM NaCl and 1 M NaCl in 20 mM HEPES buffer (800 µl). Protein eluted by 1 mM NaCl was used for all binding experiments.

S.9 – Example of histograms for FACS binding inhibition experiments

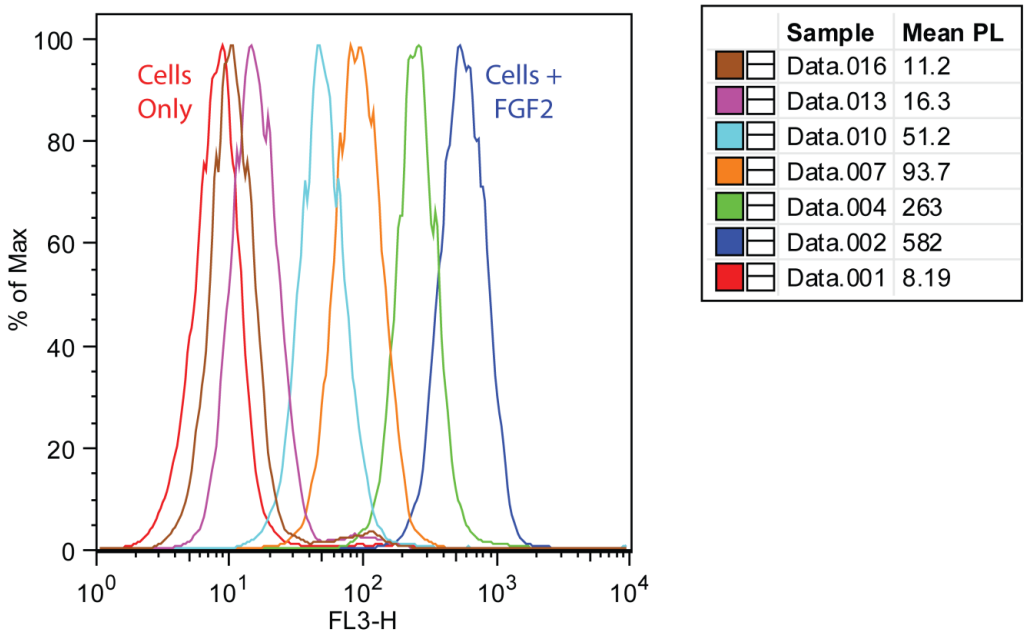


Figure S3. Example of a histogram from FGF2 binding experiments with succinyl surfen (**9**) and biotinylated-FGF2 . Data sets 001 and 002 refer to cells only and cells + biotin-FGF2 conjugated to streptavidin PE-Cy5 (absence of surfen analog), respectively. Data sets 004–016 refer to to histograms obtained in the presence of **9** at 1 µM, 2.5 µM, 5 µM, 7.5 µM, 10 µM, respectively.

S.10 – Neutralization of heparinoids *in vitro*

Table S10.1 Neutralization of Heparinoids by Surfen Analogs (IC_{50}).[a]

Compound	Heparin (μM)	Enoxaparin (μM)	Fondaparinux (μM)
Surfen (**1**)	2 ± 0.06	6.4 ± 0.4	10.2 ± 0.2
Oxalyl Surfen (**7**)	1.2 ± 0.04	8 ± 0.2	11.9 ± 0.2
Succinyl Surfen (**9**)	4.6 ± 0.07	13.7 ± 2.7	38.9 ± 2.1
Diglycolyl Surfen (**13**)	2 ± 0.03	13.6 ± 0.6	39.5 ± 1.6

[a] Values represent the mean ± SD of n = 2 experiments.

S.11 – References

1. (a) Lanza, T. J.; Durette, P. L.; Rollins, T.; Siciliano, S.; Cianciarulo, D. N.; Kobayashi, S. V.; Caldwell, C. G.; Springer, M. S.; Hagmann, W. K., Substituted 4,6-Diaminoquinolines as Inhibitors of C5a Receptor-Binding. *J Med Chem* **1992,** *35* (2), 252-258; (b) Pratt, M. G.; Archer, S., The Preparation of Some Amides of 4,6-Diaminoquinaldine. *J Am Chem Soc* **1948,** *70* (12), 4065-4069.

2. Iensch, H., New chemotherapeutical of the 4-amino-quinoline-series*). *Angew Chem-Ger Edit* **1937,** *50*, 0891-0895.

3. Xu, D.; Young, J.; Song, D. Y.; Esko, J. D., Heparan Sulfate Is Essential for High Mobility Group Protein 1 (HMGB1) Signaling by the Receptor for Advanced Glycation End Products (RAGE). *Journal of Biological Chemistry* **2011,** *286* (48), 41736-41744.