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Chimerically designed HDAC- and Tyrosine Kinase Inhibitors. A series of ErlotinibHybrides as dual-selective Inhibitors of EGFR, HER2 and Histone Deacetylases

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Abbreviations

BOP, [1-benzotriazolyloxy-tris-(dimethylamino)phosphonium-hexafluorophosphate]; EGFR, epidermal growth factor receptor; HDAC, histone deacetylase; HER2, human epidermal growth factor receptor 2/ErbB-2, avian erythroblastosis oncogene B 2; NMP, 1-methyl-2-pyrrolidinon; NSCLC, non small cell lung cancer

Experimental Part

Chemical procedures

General. NMR spectra: Bruker Avance 300 MHz spectrometer at 300 K,using the respective solven peak as internal standard. IR spectra (KBr or pure solid): Bruker Tensor 27 spectrometer. Melting points: Büchi B-545. MS spectra: Finnigan MAT 95 (EI, 70 eV), or Finnigan Thermo Quest TSQ 7000 (ESI) (DCM/MeOH + 10 mmol/l NH_4Ac), respectively. All reactions were carried out under nitrogen atmosphere. Elemental analyses: Analytical Lab. of the University of Regensburg. Analyses within ± 0.4 % of the calculated values if not stated otherwise.

6-nitroquinazoline-4(3*H*)-one (12a)[1]

7-nitroquinazoline-4(3*H*)-one (12b)[2]

Preparation of ***N*-(3-ethynylphenyl)-6-nitroquinazoline-4-amine (13a)** and ***N*-(3-ethynylphenyl)-7-nitroquinazoline-4-amine (13b)** according to Lit.[3]

Reduction to the compounds ***N*^{4}-(3-Ethynylphenyl)-quinazoline-4,6-diamine (14a)**[4] and ***N*^{4}-(3-ethynylphenyl)-quinazoline-4,7-diamine (14b)**[4] according to Lit.[1]

Preparation of carboxylic acid methyl esters 18a-d by amidation with mono-protected phenylenediamine (21)
The respective methoxycarbonylaroylic acid **17a-d** was dissolved in dry THF or DMF and 1.1 equ. BOP (benzotriazolyloxy-tris-(dimethylamino)phosphonium hexafluoro phosphate), 2.2 equ. NEt_3, and 1.1 equ. of the mono-protected phenylenediamine **(21)** were added. After stirring at room temperature for 24 h, the mixture was poured into water under stirring, the precipitated product was filtered off, dried in vacuo and purified by cc (CH_2Cl_2/MeOH = 10/1).

***N*-(2-*tert*-Butoxycarbonylaminophenyl)-terephthalamic acid methyl ester (18a)**[5]

Methyl 6-[2-(*tert*-butoxycarbonylamino)phenylcarbamoyl]nicotinate (18b)
White solid (9.23 mmol ,84 %): mp. 171.5-173.0 °C; ^{1}*H*-NMR (DMSO-[D_6]): δ (ppm) = 1.51 (s, 9H), 3.94 (s, 3H), 7.20 (m, 1H), 7.28 (m, 2H), 7.97 (dd, 1H, J = 1.3 Hz, J = 7.8 Hz), 8.31 (m, 1H), 8.56 (dd, 1H, J = 2.1 Hz, J = 8.1 Hz), 9.08 (d, 1H, J = 1.5 Hz), 9.19 (s, 1H), 10.55 (s, 1H). IR (KBr): ν (cm^{-1}) = 3342, 1732, 1690. pos. ESI m/z (%): 372 $[M+H^+]^+$ (100). Anal. caclcd. for $C_{19}H_{21}N_3O_5$: C 61.45, H 5.70, N 11.31, found: C 61.45, H 5.90, N 11.32.

Methyl-5-[2-(*tert*-butoxycarbonylamino)phenylcarbamoyl]thiophene-2-carboxylate (18c)[6]

Preparation of the protected and substituted carboxylic acids 19a-c by alkaline cleavage of the corresponding carboxylic acid methylesters 18a-c:
The respective carboxylic acid methyl ester **18a-c** was dissolved in the necessary amount of MeOH and 2 equ. LiOH in H_2O (2 % solution) were added. After stirring at room temperature overnight, MeOH was removed under reduced pressure, the aqueous layer extracted with ethyl acetate, cooled to 0°C and acidified with diluted acetic acid till pH 5-6. The precipitated product was collected by filtration and dried in vacuo.

4-[2-(*tert*-Butoxycarbonylamino)phenylcarbamoyl]benzoic acid (19a)[7]**:**

6-[2-(*tert*-Butoxycarbonylamino)phenylcarbamoyl]nicotinic acid (19b)

White crystals (8.62 mmol , 88 %): mp. 358.0-361.0 °C; ^{1}*H*-NMR (DMSO-[D_6]): δ (ppm) = 1.51 (s, 9H), 7.21 (dd, 1H, J_1 = 6.6 Hz, J_2 = 8.2 Hz), 7.28 (m, 2H), 7.98 (m, 1H), 8.29 (d, 1H, J_2 = 8.2 Hz), 8.53 (dd, 1H, J = 2.0 Hz, J = 8.1 Hz), 9.07 (d, 1H, J = 1.4 Hz), 9.18 (s, 1H), 10.55 (s, 1H), 13.74 (s, 1H).IR (KBr): ν (cm^{-1}) = 3345, 2986, 1689. + ESI m/z (%): 358 $[M+H^+]^+$ (100). Anal. calcd. for $C_{18}H_{19}N_3O_5$ x 1/2 H_2O: C 59.01, H 5.50, N 11.47, found: C 59.16, H 5.69, N 11.48.

5-(2-(*tert*-Butoxycarbonylamino)phenylcarbamoyl)thiophene-2-carboxylic acid (19c)[6]

Preparation of 15a-c and 16a-c by amidation of the respective carboxylic acids 19a-c with the respective amines 14a, b.

The respective carboxylic acid **19a-c** was dissolved in dry pyridine, and 1.1 equ. $SOCl_2$ were added. By the preparation of **15c**, **16a** and **16c**, $SOBr_2$ was used instead of $SOCl_2$.The mixture was stirred at room temp. for 2 h, and 1.0 equ. of the respective amine **14a**, **b** was added. After stirring at room temperature for 24 h, the mixture was poured into water, extracted with ethyl acetate, the solvent removed under reduced pressure, purified by cc (ethyl acetate) and crystallized from methanol. In case of **15b**, the crude product precipitated while pouring into water. The precipitated product was removed by filtration, dried in vacuo, purified by cc (DCM/MeOH = 10/1) and crystallized from methanol. For the preparation of **15a** the carboxylic acid **19a** was dissolved in dry THF and 1.1 equ. BOP, 2.0 equ. NEt_3, and 1.1 equ. of the amine **14a** were added. After stirring at room temperature for 72 h, the mixture was poured into water, extracted with ethyl acetate, the solvent removed under reduced pressure, and purified by cc (ethyl acetate).

(2-{4-[4-(3-Ethynyl-phenylamino)-quinazoline-6-ylcarbamoyl]-benzoylamino}-phenyl)-carbamic acid *tert*-butyl ester (15a)

Yellow solid (1.4 mmol ,36 %): mp. 159 - 164 °C; ^1H-NMR (DMSO-[D_6]): δ (ppm) = 1.46 (s, 9H), 4.20 (s, 1H), 7.15 (m, 2H), 7.21 (m, 1H), 7.40 (t, 1H, J = 7.9 Hz), 7.60 (m, 3H), 7.82 (d, 1H, J = 9.0 Hz), 7.94 (d, 1H, J = 8.2 Hz), 8.09 (s, 1H), 8.15 (m, 4H), 8.23 (d, 2H, J = 8.4 Hz), 8.57 (s, 1H), 9.00 (d, 1H, J = 1.6 Hz), 10.25 (s, 1H). (KBr): ν (cm^{-1}) = 3319, 1681. ES-MS (CH_2Cl_2/CH_3OH/CH_3COONH_4) m/z (%): 599 $[M+H^+]^+$ (100). Anal. calcd. for $C_{35}H_{30}N_6O_4$ x H_2O: C 68.17, H 5.23, N 12.97, found: C 68.16, H 5.33, N 12.91.

***tert*-Butyl 2-(5-(4-(3-ethynylphenylamino)-quinazoline-6-ylcarbamoyl)picolinamido)-phenylcarbamate dihydrate (15b)**

Yellow solid (0.7 mmol ,50 %): mp. 163.0 °C; ^1H-NMR (DMSO-[D_6]): δ (ppm) = 1.52 (s, 9H), 4.22 (s, 1H), 7.25 (m, 4H), 7.42 (t, 1H, J = 7.9 Hz), 7.87 (d, 1H, J = 9.0 Hz), 7.91 (d, 1H, J = 8.2 Hz), 8.03 (m, 3H), 8.37 (d, 1H, J = 8.3 Hz), 8.62 (s, 1H), 8.68 (dd, 1H, J = 2.1 Hz, J = 8.1 Hz), 8.95 (d, 1H, J = 1.8 Hz), 9.21 (d, 1H, J = 1.6 Hz), 9.96 (s, 1H), 10.59 (s, 1H), 11.02 (s, 1H).IR (KBr): ν (cm^{-1}) = 3324, 1686. ES-MS (CH_2Cl_2/CH_3OH/CH_3COONH_4) m/z (%): 600 $[M+H^+]^+$ (100). Anal. calcd. for $C_{34}H_{29}N_7O_4$ x $2H_2O$: C 64.24, H5.23, N 15.42, found: C 64.13, H 5.39, N 15.31.

[2-({5-[4-(3-Ethynyl-phenylamino)-quinazoline-6-ylcarbamoyl]-thiophene-2-carbonyl}-amino)-phenyl]-carbamic acid *tert*-butyl ester (15c)

Yellow solid (1.32 mmol ,34 %): mp. 163 - 167 °C; ^1H-NMR (DMSO-[D_6]): δ (ppm) = 1.47 (s, 9H), 4.21 (s, 1H), 7.17 (dd, 1H, J = 1.6 Hz, J = 7.7 Hz), 7.24 (m, 2H), 7.41 (t, 1H, J = 7.9 Hz), 7.50 (dd, 1H, J_1 = 1.6 Hz, J_2 = 7.8 Hz), 7.58 (dd, 1H, J_1 = 1.4 Hz, J_2 = 8.0 Hz), 7.85 (d, 1H, J = 9.0 Hz), 7.90 (m, 1H), 8.01 (m, 2H), 8.05 (t, 1H, J = 1.7 Hz), 8.15 (d, 1H, J = 4.1 Hz), 8.61 (s, 1H), 8.78 (s, 1H), 8.90 (d, 1H, J = 2.0 Hz), 9.94 (s, 1H), 10.00 (s, 1H), 10.80 (s, 1H).IR (KBr): ν (cm^{-1}) = 3317, 1539. ES-MS (CH_2Cl_2/CH_3OH/CH_3COONH_4) m/z (%): 605 $[M+H^+]^+$. Anal. calcd. for $C_{33}H_{28}N_6O_4S$ x 3/2 H_2O: C 62.74, H 4.95, N 13.30, found: C 62.74, H 5.23, N 13.44.

(2-{4-[4-(3-Ethynyl-phenylamino)-quinazoline-7-ylcarbamoyl]-benzoylamino}-phenyl)-carbamic acid *tert*-butyl ester (16a)

Yellow solid (0.75 mmol ,27 %): mp. 167 - 171 °C; ^{1}*H*-NMR (DMSO-[D_6]): δ (ppm) = 1.46 (s, 9H), 4.21 (s, 1H), 7.20 (m, 4H), 7.42 (t, 1H, J = 8.0 Hz), 7.58 (d, 2H, J = 7.8 Hz), 7.94 (dd, 1H, J = 1.1 Hz, J = 8.0 Hz), 8.04 (dd, 1H, J = 1.9 Hz, J = 9.1 Hz), 8.11 (s, 1H), 8.17 (q, 3H, J = 8.7Hz), 8.38 (d, 1H, J = 1.9 Hz), 8.55 (d, 1H, J = 9.1 Hz), 8.63 (s, 1H), 8.74 (s, 1H), 9.78 (s, 1H), 10.00 (s, 1H), 10.82 (s, 1H). IR (KBr): ν (cm^{-1}) = 3285, 1679. ES-MS (CH_2Cl_2/CH_3OH/CH_3COONH_4) m/z (%): 599 $[M+H^+]^+$. Anal. calcd. for $C_{35}H_{30}N_6O_4$ x H_2O: C 69.76, H 4.68, N 16.27, found: C 69.85, H 5.02, N 16.54.

[2-({5-[4-(3-Ethynyl-phenylamino)-quinazoline-7-ylcarbamoyl]-pyridine-2-carbonyl}-amino)-phenyl]-carbamic acid *tert*-butyl ester (16b)

Yellow solid(0.7 mmol ,28 %): mp. 161 - 166 °C; ^{1}*H*-NMR (DMSO-[D_6]): δ (ppm) = 1.52 (s, 9H), 4.22 (s, 1H), 7.21 (dd, 2H, J_1 = 1.3 Hz, J_2 = 7.5 Hz), 7.27 (m, 2H), 7.42 (t, 1H, J = 6.9 Hz), 7.94 (d, 1H, J = 8.7 Hz), 8.02 (m, 2H), 8.11 (m, 2H), 8.36 (m, 2H), 8.56 (d, 1H, J = 6.8 Hz), 8.65 (m, 2H), 9.17 (d, 2H, J = 1.6 Hz), 9.80 (s, 1H), 10.58 (s, 1H), 11.04 (s, 1H). IR (KBr): ν (cm^{-1}) = 3291, 1685. ES-MS (CH_2Cl_2/CH_3OH/CH_3COONH_4) m/z (%): 600 $[M+H^+]^+$. Anal. calcd. for $C_{34}H_{29}N_7O_4$ x 3/2 H_2O: C 65.16, H 5.15, N 15.65, found: C 65.31, H 5.11, N 15.89.

[2-({5-[4-(3-Ethynyl-phenylamino)-quinazoline-7-ylcarbamoyl]-thiophene-2-carbonyl}-amino)-phenyl]-carbamic acid *tert*-butyl ester (16c)

Yellow solid(1.0 mmol ,27 %): mp. 203 - 208 °C; ^{1}*H*-NMR (DMSO-[D_6]): δ (ppm) = 1.47 (s, 9H), 4.21 (s, 1H), 7.16 (t, 1H, J = 7.4 Hz), 7.23 (m, 2H), 7.41 (t, 1H, J = 7.9 Hz), 7.52 (d, 1H, J = 7.4 Hz), 7.58 (d, 1H, J = 7.7 Hz), 7.98 (m, 3H), 8.11 (s, 1H), 8.15 (d, 1H, J = 3.8 Hz), 8.28 (d, 1H, J = 0.7 Hz), 8.55 (d, 1H, J = 9.1 Hz), 8.63 (s, 1H), 8.78 (s, 1H), 9.79 (s, 1H), 10.04 (s, 1H), 10.80 (s, 1H). IR (KBr): ν (cm^{-1}) = 3292, 1535. Anal. calcd. for $C_{33}H_{28}N_6O_4S$ x H_2O: C 63.65, H 4.86, N 13.50, found: C 63.86, H 4.52, N 13.83.

Preparation of 7a-c and 8a-c by cleavage of the *tert*-butyl phenylcarbamate-group

The respective acid-*tert*-butyl-ester **15a-c or 16a-c** was dissolved in the necessary amount of formic acid and stirred at room temp. for 24 h. In case of **7a** and **7b**, TFA was used instead of formic acid. The solution was poured into water under stirring and the mixture alkalized with NH_3 (pH 9). The precipitated product was filtered off, washed with H_2O, if necessary crystallized from methanol and dried in vacuo**.**

***N*-(2-Amino-phenyl)-*N*'-[4-(3-ethynyl-phenylamino)-quinazoline-6-yl]-terephthalamide (7a)**

Yellow solid (0.13 mmol ,52 %): mp. 253 - 258 °C; ^{1}*H*-NMR (DMSO-[D_6]): δ (ppm) = 4.22 (s, 1H), 4.98 (s, 2H), 6.62 (t, 1H, J = 7.4 Hz), 6.80 (d, 1H, J = 7.1 Hz), 7.00 (dt, 1H, J_1 = 1.1 Hz, J_2 = 7.8 Hz), 7.22 (m, 2H), 7.41 (t, 1H, J = 7.9 Hz), 7.85 (d, 1H, J = 8.9 Hz), 7.92 (d, 1H, J = 8.1 Hz), 8.06 (m, 2H), 8.18 (s, 4H), 8.61 (s, 1H), 8.94 (d, 1H, J = 1.5 Hz), 9.85 (s, 1H), 9.93 (s, 1H), 10.78 (s, 1H). IR (KBr): ν (cm^{-1}) = 3280, 1649. ES-MS (CH_2Cl_2/CH_3OH/CH_3COONH_4) m/z (%): 499 $[M+H^+]^+$ (100). HRMS-ESI: *m/z* $[M+H^+]^+$ calcd. for $C_{30}H_{23}N_6O_2$: 499.1877, found: 499.1880; Anal. calcd. for $C_{30}H_{22}N_6O_2$ x H_2O: C 65.15, H 4.94, N 14.55, found: C 65.12, H 4.87, N 14.33.

_N_-(2-aminophenyl)-N^5-(4-(3-ethynylphenylamino)-quinazoline-6-yl)pyridine-2,5-dicarboxamide (7b)

Yellow solid (0.2 mmol ,50 %): mp. 213 - 216 °C; ^{1}*H*-NMR (DMSO-[D_6]): δ (ppm) = 4.22 (s, 1H), 4.97 (s, 2H), 6.67 (dt, 1H, J = 1.4 Hz, J = 7.7 Hz), 6.85 (dd, 1H, J_1 = 1.3 Hz, J_2 = 8.0 Hz), 6.99 (dt, 1H, J_1 = 1.5 Hz, J_2 = 7.9 Hz), 7.24 (m, 1H), 7.42 (t, 1H, J = 7.9 Hz), 7.49 (dd, 1H, J = 1.3 Hz, J = 7.9 Hz), 7.87 (d, 1H, J = 9.0 Hz), 7.92 (m, 1H), 8.05 (m, 2H), 8.33 (dd, 1H, J_1 = 0.5 Hz, J_2 = 8.2 Hz), 8.62 (s, 1H), 8.65 (dd, 1H, J_1 = 2.2 Hz, J_2 = 8.2 Hz), 8.95 (d, 1H, J = 2.0 Hz), 9.31 (dd, 1H, J_1 = 0.6 Hz, J_2 = 2.2 Hz), 9.96 (s, 1H), 10.20 (s, 1H), 11.01 (s, 1H). IR (KBr): ν (cm^{-1}) = 3290, 1663. ES-MS (CH_2Cl_2/CH_3OH/CH_3COONH_4) m/z (%): 500 $[M+H^+]^+$ (100). HRMS-ESI: *m/z* $[M+H^+]^+$ calcd. for $C_{29}H_{22}N_7O_2$: 500.1829, found: 500.1832; Anal. calcd. for $C_{29}H_{21}N_7O_2$ x 3/2 H_2O: C 66.15, H 4.59, N 18.62, found: C 66.42, H 4.41, N 18.83.

_N_^{2}-(2-aminophenyl)-N^5-(4-(3-ethynylphenylamino)quinazolin-6-yl)thiophene-2,5-dicarboxamide (7c)

Yellow solid (0.4 mmol ,61 %): mp. 255 - 257 °C; ^{1}*H*-NMR (DMSO-[D_6]): δ (ppm) = 4.21 (s, 1H), 5.00 (s, 2H), 6.61 (dt, 1H, J_1 = 1.1 Hz, J_2 = 7.6 Hz), 6.80 (dd, 1H, J_1 = 1.0 Hz, J_2 = 8.0 Hz), 7.01 (m, 1H), 7.15 (dd, 1H, J_1 = 0.8 Hz, J_2 = 7.7 Hz), 7.23 (d, 1H, J = 7.6 Hz), 7.41 (t, 1H, J = 7.9 Hz), 7.85 (d, 1H, J = 8.9 Hz), 7.91 (d, 1H, J = 8.1 Hz), 8.02 (dd, 1H, J_1 = 1.9 Hz, J_2 = 9.1 Hz), 8.05 (m, 2H), 8.12 (d, 1H, J = 4.0 Hz), 8.61 (s, 1H), 8.90 (d, 1H, J = 1.3 Hz), 9.89 (s, 1H), 9.94 (s, 1H), 10.78 (s, 1H). IR (KBr): ν (cm^{-1}) = 3301, 1643. ES-MS (CH_2Cl_2/CH_3OH/CH_3COONH_4) m/z (%): 505 $[M+H^+]^+$ (100). HRMS-ESI: *m/z* $[M+H^+]^+$ calcd. for $C_{28}H_{21}N_6O_2S$: 505.1441, found: 505.1441; Anal. calcd. for $C_{28}H_{20}N_6O_2S$ x 1/2 H_2O: C 65.48, H 4.12, N 16.36, found: C 65.47, H 3.88, N 16.58.

***N*-(2-Amino-phenyl)-*N*'-[4-(3-ethynyl-phenylamino)-quinazoline-7-yl]-terephthalamide dihydrate (8a)**

Yellow powder (0.4 mmol ,53 %): mp. 240 – 246 °C; ^{1}*H*-NMR (DMSO-[D_6]): δ (ppm) = 4.22 (s, 1H), 4.98 (s, 2H), 6.62 (t, 1H, J = 7.4 Hz), 6.80 (dd, 1H, J_1 = 0.8 Hz, J_2 = 7.9 Hz), 7.00 (dt, 1H, J_1 = 1.3 Hz, J_2 = 7.9 Hz), 7.21 (m, 2H), 7.42 (t, 1H, J = 7.9 Hz), 7.94 (d, 1H, J = 8.2 Hz), 8.04 (dd, 1H, J_1 = 2.0 Hz, J_2 = 9.1 Hz), 8.11 (m, 1H), 8.17 (m, 5H), 8.38 (d, 1H, J = 1.9 Hz), 8.55 (d, 1H, J = 9.0 Hz), 8.62 (s, 1H), 9.89 (s, 1H), 10.62 (s, 1H).IR (KBr): ν (cm^{-1}) = 3367, 1662. ES-MS (CH_2Cl_2/CH_3OH/CH_3COONH_4) m/z (%): 499 $[M+H^+]^+$ (100). HRMS-ESI: *m/z* $[M+H^+]^+$ calcd. for $C_{30}H_{23}N_6O_2$: 499.1877, found: 499.1882; Anal. calcd. for $C_{30}H_{22}N_6O_2$ x 2 H_2O: C 67.40, H 4.90, N 15.72, found: C 67.23, H 5.03, N 15.55..

***N*^{2}-(2-aminophenyl)-*N*^{5}-(4-(3-ethynylphenylamino)quinazolin-7-yl)pyridine-2,5-dicarboxamide (8b)**

Yellow powder (0.5 mmol ,71 %): mp. 248 - 251 °C; ^{1}*H*-NMR (DMSO-[D_6]): δ (ppm) = 4.22 (s, 1H), 4.96 (s, 2H), 6.67 (t, 1H, J = 7.5 Hz), 6.85 (d, 1H, J = 7.8 Hz), 6.99 (t, 1H, J = 7.5 Hz), 7.24 (d, 1H, J = 7.5 Hz), 7.42 (t, 1H, J = 7.9 Hz), 7.50 (d, 1H, J = 7.7 Hz), 7.94 (d, 1H, J = 8.1 Hz), 8.02 (dd, 1H, J_1 = 1.3 Hz, J_2 = 8.9 Hz), 8.11 (m, 1H), 8.32 (d, 1H, J = 8.2 Hz), 8.36 (d, 1H, J = 1.6 Hz), 8.57 (d, 1H, J = 9.1 Hz), 8.62 (m, 2H), 9.28 (d, 1H, J = 1.2 Hz), 9.80 (s, 1H), 10.20 (s, 1H), 11.03 (s, 1H). IR (KBr): ν (cm^{-1}) = 3278, 1671. ES-MS (CH_2Cl_2/CH_3OH/CH_3COONH_4) m/z (%): 500 $[M+H^+]^+$ (100). HRMS-ESI: *m/z* $[M+H^+]^+$ calcd. for $C_{29}H_{22}N_7O_2$: 500.1829, found: 500.1825; Anal. calcd. for $C_{29}H_{21}N_7O_2$ x H_2O: C 67.30, H 4.48, N 18.94, found: C 67.06, H 4.25, N 19.02.

N^2-(2-aminophenyl)-N^5-[4-(3-ethynylphenylamino)quinazolin-7-yl]thiophene-2,5-dicarboxamide trihydrate (8c)

Yellow powder (0.6 mmol ,60 %): mp. 220 – 228 °C; ^1H-NMR (DMSO-[D_6]): δ (ppm) = 4.22 (s, 1H), 5.02 (s, 2H), 6.61 (dt, 1H, J_1 = 1.2 Hz, J_2 = 7.7 Hz), 6.80 (dd, 1H, J_1 = 1.2 Hz, J_2 = 8.0 Hz), 7.01 (dt, 1H, J_1 = 1.4 Hz, J_2 = 7.8 Hz), 7.15 (dd, 1H, J_1 = 1.1 Hz, J_2 = 7.8 Hz), 7.23 (m, 1H), 7.42 (t, 1H, J = 7.9 Hz), 7.93 (dd, 1H, J_1 = 1.5 Hz, J_2 = 7.8 Hz), 8.01 (dd, 1H, J_1 = 1.8 Hz, J_2 = 9.2 Hz), 8.05 (d, 1H, J = 3.9 Hz), 8.11 (m, 2H), 8.28 (d, 1H, J = 2.0 Hz), 8.55 (d, 1H, J = 9.2 Hz), 8.62 (s, 1H), 9.79 (s, 1H), 9.90 (s, 1H), 10.77 (s, 1H). IR (KBr): ν (cm^{-1}) = 3371, 1662. ES-MS (CH_2Cl_2/CH_3OH/CH_3COONH_4) m/z (%): 505 $[M+H^+]^+$ (100). HRMS-ESI: *m/z* $[M+H^+]^+$ calcd. for $C_{28}H_{21}N_6O_2S$: 505.1441, found: 505.1447; Anal. Calcd. for $C_{28}H_{20}N_6O_2S$ x $3H_2O$: C 60.20, H 4.69, N 15.04, found: C 60.64, H 4.39, N 15.17. .

Preparation of **6-Methoxyquinazoline-4(3*H*)-one (26)** according to Lit.[8],[9]

Preparation of ***N*-(3-Bromophenyl)-6-methoxyquinazoline-4-amine (27)** according to Lit.[3]:

Preparation of **4-(3-Bromophenylamino)quinazoline-6-ol (29)**

Modified preparation analogous to *Barker*[10] as follows: A mixture of *N*-(3-bromophenyl)-6-methoxyquinazoline-4-amine (**28**), sodium ethanethiolate (10 equ.) and DMF was stirred and heated to 140°C for 8 h. The solvent was removed in vacuo, the remaining solid dissolved in water, the aqueous layer extracted with ethyl acetate, acidified with acetic acid untill pH 5 and the precipitated product removed by filtration.

Beige owder (18 mmol ,69 %): mp. 297 – 300 °C; ^1H-NMR (DMSO-[D_6]): δ (ppm) = 7.27 (m, 1H), 7.34 (t, 1H, J = 8.0 Hz), 7.45 (dd, 1H, J = 2.5 Hz, J = 9.0 Hz), 7.71 (d, 1H, J = 9.0 Hz), 7.80 (d, 1H, J = 2.5 Hz), 7.94 (m, 1H), 8.28 (t, 1H, J = 1.9 Hz), 8.52 (s, 1H), 9.59 (s, 1H), 10.12 (s, 1H). IR (KBr): ν (cm^{-1}) = 3402, 1633. CI-MS (NH_3) *m/z* (%): 316 (100) $[M+H^+]^+$. Anal. calcd. for $C_{14}H_{10}BrN_3O$: C 53.19, H 3.19, N 13.29, found: C 53.26, H 3.29, N 13.02.

tert-Butyl 2-(4-(bromomethyl)benzamido)phenylcarbamate (32)

4-(Bromomethyl)benzoic acid (**31**, Acros) was dissolved in $SOCl_2$ and the mixture refluxed for 30 min. Excess of thionyl chloride was removed in vacuo, the resulting carboxylic acid chloride dissolved in methylene chloride, *tert*-butyl 2-aminophenylcarbamate (1.0 equ.) and pyridine (1.2 equ.) were added, and the mixture was stirred at room temperature for 30 min. The mixture was directly subjected to cc (SiO_2, CH_2Cl_2/ethyl acetate = 10/1) with subsequent crystallisation from CH_2Cl_2/hexane.

Colorless crystals (4.3 mmol ,31 %): mp. 282 – 283 °C; ^1H-NMR (DMSO-[D_6]): δ (ppm) = 1.45 (s, 9H), 4.81 (s, 1H), 4.82 (s, 1H), 7.18 (m, 2H), 7.55 (m, 2H), 7.61 (dd, 2H, J_1 = 1.8 Hz, J_2 = 8.3 Hz), 7.96 (dd, 2H, J_1 = 6.6 Hz, J_2 = 8.2 Hz), 8.69 (s, 1H), 9.85 (s, 1H). IR (KBr): ν (cm^{-1}) = 3327, 1656. CI-MS (NH_3) *m/z* (%): 327 (100), 405 $[M+H^+]^+$ (8).

***tert*-Butyl 2-(4-{[4-(3-bromophenylamino)quinazoline-6-yloxy]methyl}-benzamido)phenylcarbamate (30)**

4-(3-Bromophenylamino)quinazoline-6-ol (**29**) was dissolved in DMF, the solution cooled to 0 °C and NaH (1.1 equ.; 60 % in paraffine) was added by stirring. After 30 min. *tert*-butyl 2-(4-(bromomethyl)benzamido)phenylcarbamate (**32**) (1.1 equ.) was added and the mixture stirred for 48 h at room temp. The solution was poured into water under stirring, the precipitated product collected by filtration, dried in vacuo and purified by cc (SiO_2, CH_2Cl_2/ethyl acetate:1:2). The solid which remained after removal of the solvent was crystallized by dissolving in a small amount of CH_2Cl_2 and drop wise adding to diethyl ether

whilst stirring. Yellow solid (1.9 mmol ,60 %): mp. 208 - 210 °C; ^1H-NMR (DMSO-[D_6]): δ (ppm) = 1.44 (s, 9H), 5.40 (s, 2H), 7.19 (m, 2H), 7.32 (m, 1H), 7.39 (t, 1H, J = 8.0 Hz), 7.56 (ddd, 2H, J_1 = 1.7 Hz, J_2 = 6.2 Hz, J_3 = 8.0 Hz), 7.65 (dd, 1H, J_1 = 2.5 Hz, J_2 = 9.1 Hz), 7.72 (d, 1H, J = 8.3 Hz), 7.81 (d, 1H, J = 9.1 Hz), 7.95 (m, 1H), 8.03 (d, 2H, J = 8.2 Hz), 8.11 (d, 1H, J = 2.5 Hz), 8.22 (t, 1H, J = 1.9 Hz), 8.60 (s, 1H), 8.70 (s, 1H), 9.69 (s, 1H), 9.86 (s, 1H). IR (KBr): ν (cm^{-1}) = 3251, 1652. ES-MS (CH_2Cl_2/CH_3OH/CH_3COONH_4) m/z (%): 640 $[M+H^+]^+$ (100). Anal. calcd. for $C_{33}H_{30}BrN_5O_4$: C 61.88, H 4.72, N 10.93, found: C 61.83, H 4.84, N 10.81.

Preparation of ***N*-(2-aminophenyl)-4-{[4-(3-bromophenylamino)quinazoline-6-yloxy]-methyl}benzamide (9a)**

tert-butyl 2-(4-{[4-(3-Bromophenylamino)quinazoline-6-yloxy]methyl}benzamido)phenylcarb-amate (**30**) was dissolved in trifluoroacetic acid and the solution stirred for 1 h at room temp. The solution was poured into water whilst stirring, the mixture alkalized with conc. NH_3 untill pH 9, the precipitated product removed by filtration, washed with water and dried in vacuo. Purification by cc (SiO_2, ethyl acetate), removing most of the solvent under reduced pressure and storage of the solution at -18°C over night led to crystallization of **9a**. Yellow solid (1.4 mmol ,71 %): mp. 215 - 218 °C; ^1H-NMR (DMSO-[D_6]): δ (ppm) = 4.93 (s, 2H), 5.38 (s, 2H), 6.61 (dt, 1H, J_1 = 1.2 Hz, J_2 = 7.8 Hz), 6.79 (dd, 1H, J_1 = 1.3 Hz, J_2 = 8.0 Hz), 6.98 (dt, 1H, J_1 = 1.5 Hz, J_2 = 7.9 Hz), 7.18 (dd, 1H, J_1 = 0.8 Hz, J_2 = 8.1 Hz), 7.32 (m, 1H), 7.39 (t, 1H, J = 8.0 Hz), 7.64 (d, 1H, J = 2.5 Hz), 7.69 (d, 2H, J = 8.6 Hz), 7.81 (d, 1H, J = 9.1 Hz), 7.94 (m, 1H), 8.05 (d, 1H, J = 8.2 Hz), 8.12 (d, 1H, J = 2.5 Hz), 8.22 (t, 1H, J = 1.9 Hz), 8.59 (s, 1H), 9.70 (d, 2H, J = 2.1 Hz). IR (KBr): ν (cm^{-1}) = 3286, 1643. EI-MS (70eV) *m/z* (%): 539 $[M]^{+\cdot}$ (2). HRMS-ESI: *m/z* $[M+H^+]^+$ calcd. for $C_{28}H_{23}BrN_5O_2$: 540.1030, found:: 540.1025; Anal. calcd. for $C_{28}H_{22}BrN_5O_2$: C 62.23, H 4.10, N 12.96, found: C 61.81, H 4.16, N 12.96.

***N*-(2-Aminophenyl)-4-{[4-(3-ethynylphenylamino)quinazoline-6-yloxy]methyl}benzamide (9b)**

A mixture of *N*-(2-aminophenyl)-4-{[4-(3-bromophenylamino)quinazoline-6-yloxy]methyl}benzamide (**9a**), bis(triphenylphoshine)palladium(II)chloride (0.5 equ.), CuI (0.5 equ.), trimethylsilylacetylene (7 equ.) and triethylamine (10 equ.) in 1-methyl-2-pyrrolidinone was stirred in a sealed tube under nitrogen atmosphere at 40 °C for 24 h. Then the reaction mixture was cooled to room temp. and poured into water in a separatory funnel and the aqueous layer extracted with ethyl acetate. The combined organic layers were washed with water (3x), dried with sodium sulfate and the solvent was removed in vacuo. The resulting intermediate, *N*-(2-aminophenyl)-4-[(4-{3-[(trimethylsilyl)ethynyl]phenyl amino}quinazoline-6-yloxy)methyl]benzamide, was purified by cc (SiO_2, ethyl acetate) and the product characterized by ^1H-NMR spectroscopy. Then the TMS protected alkyne was in the following dissolved in THF, MeOH and K_2CO_3 (1.1 equ.) were added and the mixture was stirred over night at room temp. The mixture was filtered, the residue passed through a plug of silica gel by washing with THF, and the solvent was removed to afford the terminal alkyne **9b**.Yellow solid (1.2 mmol ,92 %): mp. 164 – 168 °C; ^1H-NMR (DMSO-[D_6]): δ (ppm) = 4.23 (s, 1H), 4.92 (s, 2H), 5.38 (s, 2H), 6.60 (dt, 1H, J_1 = 1.2 Hz, J_2 = 7.8 Hz), 6.79 (dd, 1H, J_1 = 1.2 Hz, J_2 = 8.0 Hz), 6.98 (dt, 1H, J_1 = 1.4 Hz, J_2 = 7.8 Hz), 7.18 (m, 1H), 7.24 (td, 1H, J_1 = 1.1 Hz, J_2 = 7.6 Hz), 7.44 (t, 1H, J = 7.9 Hz), 7.64 (dd, 1H, J_1 = 2.4 Hz, J_2 = 9.1 Hz), 7.69 (d, 2H, J = 8.2 Hz), 7.79 (d, 1H, J = 9.1 Hz), 7.95 (m, 1H), 8.05 (m, 2H), 8.13 (d, 1H, J = 2.3 Hz), 8.57 (s, 1H), 9.70 (s, 2H). ES-MS (DCM/MeOH + 10 mmol/l NH_4Ac) *m/z* (%): 486 $[M+H^+]^+$ (100). HRMS-ESI: *m/z* $[M+H^+]^+$ calcd. for $C_{30}H_{24}N_5O_2$: 486.1925, found:

486.1928; Anal. calcd. for $C_{30}H_{23}N_5O_2$ x 4/3 H_2O: C 70.71, H 5.08, N 13.74, found: C 71.08, H 4.73, N 13.45..

Quinazolin-4(3*H*)-one (33)[3]

4-Oxo-3,4-dihydroquinazoline-6-sulfonic acid (34)[11]

4-Oxo-3,4-dihydroquinazoline-6-sulfonyl chloride (35)
4-Oxo-3,4-dihydroquinazoline-6-sulfonic acid (**34**) (25 g) was refluxed in a mixture from $SOCl_2$ (100 mL) and DMF (1.0 mL) for 7 h and stirred overnight at room temperature. Excess of $SOCl_2$ was distilled off, the residue suspended in CH_2Cl_2, filtered off and dried in vacuo.
Beige powder (8.1 mmol ,69 %): mp. > 400 °C; ^1H-NMR (DMSO-[D_6]): δ (ppm) = 9.07 (s, 1H), 8.34 (d, J = 1.9 Hz, 1H), 8.13 (dd, J_1 = 8.3 Hz, J_2 = 1.9 Hz, 1H), 7.77 (d, J = 8.3 Hz, 1H). IR (KBr): ν (cm^{-1}) = 3428, 2623, 1712. ES-MS (CH_2Cl_2, CH_3OH, CH_3COONH_4) *m/z* (%): 243 $[M-H^+]^-$ (100).

3-(1*H*-Pyrrol-3-yl)acrylic acid ethyl ester (39)[12]

3-[1-(4-Oxo-3,4-dihydroquinazoline-6-sulfonyl)-1*H*-pyrrol-3-yl]acrylic acid ethyl ester (36). 3-(1*H*-pyrrol-3-yl)acrylic acid ethyl ester (**39**) was dissolved in 20 mL THF, cooled to -20 °C, NaH (1.2 equ.) was added and the mixture stirred for 1h. Then 4-oxo-3,4-dihydroquinazoline-6-sulfonyl chloride (**35,** 1.0 eq) was added in small portions, and the mixture was stirred over night while warming up to room temperature. Afterwards, the solution was treated with sat. aqu. NH_4Cl and extracted with ethyl acetate, dried with sodium sulfate and subjected to cc (SiO_2, ethyl acetate). Beige solid (1.15 mmol ;23 %): mp. 207.4 – 209.6 °C; ^1H-NMR (DMSO-[D_6]): δ (ppm) = 8.60 (d, J = 2.5 Hz, 1H), 8.33-8.26 (m, 2H), 7.96-7.92 (m, 1H), 7.87 (d, J = 8.7 Hz, 1H), 7.54-7.44 (m, 2H), 6.87-6.83 (m, 1H), 6.35 (d, J = 15.9 Hz, 1H), 4.12 (q, J = 7.1 Hz, 2H), 1.20 (t, J = 7.1 Hz, 3H). IR (KBr): ν (cm^{-1}) = 3431, 2868, 1685. CI-MS (NH_3) *m/z* (%): 391 $[M+NH_4^+]^+$ (39), 374 $[M+H^+]^+$ (2). Anal. calcd. for $C_{17}H_{15}N_3O_5S$: C 54.68, H 4.05, N 11.25,ffound: C 54.17, H 4.23, N 11.27.

3-{1-[4-(3-Ethynylphenylamino)quinazoline-6-sulfonyl]-1*H*-pyrrol-3-yl}acrylic acid ethyl ester (37)
A mixture of 3-[1-(4-oxo-3,4-dihydroquinazoline-6-sulfonyl)-1*H*-pyrrol-3-yl]acrylic acid ethyl ester (**36**) (1.0 g), $POCl_3$ (0.5 g), triethylamine (0.33 g) and toluene (1 mL) was stirred for 2 h at 75 °C. After cooling to room temperature, 3-ethynylaniline (1.1 equ.) in 4-methyl-2-oxopentane (0.8 mL) was added and the mixture was stirred for 1 h at 70 °C. The resulting precipitate was filtered off and the residue was stirred for 30 min in 15 mL of 1M NaOH aqu. The product was filtered off and dried in vacuo. Yellow solid (2.1 mmol ,78 %): mp. 153.5 – 156.2 °C; ^1H-NMR (DMSO-[D_6]): δ (ppm) = 10.50 (s, 1H), 9.42 (s, 1H), 8.71 (s, 1H), 8.25 (dd; J_1 = 8.8 Hz, J_2 = 1.9 Hz, 1H), 8.05-7.82 (m, 4H), 7.55-7.38 (m, 3H), 7.30 (d, J = 7.4 HZ, 1H), 6.90-6.84 (m, 1H), 6.35 (d, J = 15.9 Hz, 1H), 4.25 (s, 1H), 4.12 (q, J = 7.1 Hz, 2H), 1.20 (t, J = 7.1 Hz, 3H). IR (KBr): ν (cm^{-1}) = 3424, 3285, 1707. CI-MS (NH_3) *m/z* (%): 473 $[MH^+]^+$ (14), 183 (100). Anal. calcd. for $C_{25}H_{20}N_4O_4S$ x H_2O: C 61.21, H 4.52, N 11.42, found: C 61.05, H 4.60, N 11.25.

3-{1-[4-(3-Ethynylphenylamino)quinazoline-6-sulfonyl]-1*H*-pyrrol-3-yl}acrylic acid (38)
A mixture of 3-{1-[4-(3-ethynylphenylamino)quinazoline-6-sulfonyl]-1*H*-pyrrol-3-yl}acrylic acid ethyl ester (**37**) (470 mg; 1.0 mmol), LiOH (40 mg) in THF (10 mL) and H_2O (5.0 mL)

was stirred for 15 h at 75 °C. After cooling down to room temperature, 150 mg acetic acid was added slowly and the resulting precipitate was collected by filtration. The solid was treated with THF and removed by filtration. The THF-solution was directly subjected to cc (SiO_2 ethyl acetate). Yellow solid (0.25 mmol ,25 %): mp. 286.4 – 289.0 °C; ^1H-NMR (DMSO-[D_6]): δ (ppm) = 12.30 (bs, 1H), 10.45 (s, 1H), 9.41 (s, 1H), 8.72 (s, 1H), 8.25 (dd, J_1 = 8.8 Hz, J_2 = 1.9 Hz, 1H), 8.03-7.79 (m, 4H), 7.50-7.38 (m, 3H), 7.30 (d, J = 8.5 Hz, 1H), 6.87-6.81 (m, 1H), 6.25 (d, J = 15.9 Hz, 1H), 4.25 (s, 1H). IR (KBr): ν (cm^{-1}) = 3420, 3295, 1668. CI-MS (NH_3) *m/z* (%): 445 $[MH^+]^+$ (12), 155 (100). Anal. calcd. for $C_{23}H_{16}N_4O_4S$ x 2/3 H_2O: C 60.52, H 3.83, N 12.27, found: 60.39, H 3.68, N 12.44.

[2-(3-{1-[4-(3-Ethynylphenylamino)quinazoline-6-sulfonyl]-1*H*-pyrrol-3-yl}acryloylamino)phenyl]carbamic acid *tert*-butyl ester (40)

3-{1-[4-(3-Ethynylphenylamino)quinazoline-6-sulfonyl]-1*H*-pyrrol-3-yl}acrylic acid (**38**) was dissolved together with *tert*.-butyl-2-aminophenylcarbamate (1.1 equ.) in acetonitrile (7.5 mL) and DMF (2.0 mL) After addition of BOP (1.1 equ.) and triethylamine (1.1 equ.) the solution was stirred over night, concentrated in vacuo, treated with water and filtered. The collected solid was dried in vacuo. Yellow solid (0.44 mmol ,89 %): mp. 146.2 – 149.8 °C; ^1H-NMR (DMSO-[D_6]): δ (ppm) = 10.50 (s, 1H), 9.60 (s, 1H), 9.45 (s, 1H), 8.76 (s, 1H), 8.30 (d, J = 7 Hz, 1H), 8.05-7.80 (m, 6H), 7.63-7.40 (m, 5H), 7.34 (d, J = 7 Hz, 1H), 7.10 (m, 1H), 6.70 (d, 3.5 Hz, 1H), 6.58 (d, J = 12 Hz, 1H), 4.27 (s, 1H), 1.40 (s, 9H). IR (KBr): ν (cm^{-1}) = 3288, 1781, 1729. ES-MS (CH_2Cl_2, CH_3OH, CH_3COONH_4) *m/z* (%): 635 $[M+H^+]^+$ (100). Anal. calcd. for $C_{34}H_{30}N_6O_5S$: C 64.34, H 4.76, N 13.24, found: 64.11, H 4.76, N 13.49.

***N*-(2-Amino-phenyl)-3-{1-[4-(3-ethynylphenylamino)quinazoline-6-sulfonyl]-1*H*-pyrrol-3-yl}acrylamide (10)**

[2-(3-{1-[4-(3-ethynylphenylamino)quinazoline-6-sulfonyl]-1*H*-pyrrol-3-yl}acryloylamino) phenyl]carbamic acid *tert*-butyl ester (**40**) was dissolved in the neccasary amount of formic acid and stirred over night. The solution was poured into sat aqu. NaCl solution and neutralized with aqu. ammonia. The resulting precipitate was filtered, dissolved in ethyl acetate and subjected to cc (SiO_2, ethyl acetate). Yellow crystals (0.13 mmol ,18 %): mp. 149.5 – 153.2 °C; ^1H-NMR (DMSO-[D_6]): δ (ppm) = 10.45 (s, 1H), 9.43 (d, J = 1.9 Hz, 1H), 9.26 (s, 1H), 8.74 (s, 1H), 8.28 (dd, J_1 = 8.8 Hz, J_2 = 1.9 Hz, 1H), 8.02-7.87 (m, 3H), 7.79-7.77 (m, 1H), 7.50-7.26 (m, 5H), 6.92-6.86 (m, 1H), 6.75-6.51 (m, 4H), 4.90 (bs, 2H), 4.25 (s, 1H). IR (KBr): ν (cm^{-1}) = 3419, 1619. ES-MS (CH_2Cl_2, CH_3OH, CH_3COONH_4) *m/z* (%): 535 $[M+H^+]^+$ (100). HRMS-ESI: *m/z* $[M+H^+]^+$ calcd. for $C_{29}H_{23}N_6O_3S$: 535.1547, found: 535.1553; Anal. calcd. for $C_{29}H_{22}N_6O_3S$: C 65.15, H 4.15, N 15.72, found: 65.29, H 3.80, N 15.19.

3-{1-[4-(3-Ethynylphenylamino)quinazoline-6-sulfonyl]-1*H*-pyrrol-3-yl}-*N*-(tetrahydro-pyran-2-yloxy)acrylamide (41). 3-{1-[4-(3-Ethynylphenylamino)quinazoline-6-sulfonyl]-1*H*-pyrrol-3-yl}acrylic acid (**38**) (220 mg) was dissolved together with O-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (1.1 equ.) in acetonitrile (7.5 mL) and DMF (2.5 mL) After addition of BOP (1.1 equ.) and triethylamine (200 mg) the mixture was stirred over night. Volatiles were removed in vacuo and the residue was treated with water. The resulting precipitate was collected by filtration and dried in vacuo. Yellow solid (0.4 mmol ;82 %): mp. 152.0 – 155.3 °C; ^1H-NMR (DMSO-[D_6]): δ (ppm) = 11.12 (s, 1H), 10.45 (s, 1H), 9.40 (s, 1H), 8.72 (s, 1H), 8.26 (dd, J_1 = 8.8 Hz, J_2 = 1.9 Hz, 1H), 8.05-7.72 (m, 4H), 7.50-7.25 (m, 4H), 6.62 (s, 1H), 6.20 (d, J = 15.9 Hz, 1H), 4.85 (s, 1H), 4.25 (s, 1H), 3.98-3.85 (m, 1H),

3.57-3.44 (m, 1H), 1.75-1.40 (m, 6H). IR (KBr): ν (cm^{-1}) = 3422, 3289, 1661. ES-MS (CH_2Cl_2, CH_3OH, CH_3COONH_4) *m/z* (%): 544 $[M+H^+]^+$ (100). Anal. calcd. for $C_{28}H_{25}N_5O_5S$ x 1.25 H_2O) C 59.41, H 4.90, N 12.27, found: C 59.00, H 5.08, N 12.93.

3-{1-[4-(3-Ethynylphenylamino)quinazoline-6-sulfonyl]-1*H*-pyrrol-3-yl}-*N*-hydroxy-acrylamide dihydrochloride hydrate (11)

3-{1-[4-(3-Ethynylphenylamino)quinazoline-6-sulfonyl]-1*H*-pyrrol-3-yl}-*N*-(tetrahydropyran-2-yloxy)acrylamide (**41**) (259 mg) was dissolved in MeOH (10 mL), HCl in methanole (10 mL 0.5 N solution) was added and the mixture stirred over night. The solution was concentrated in vacuo and the residue washed with diethylether. Yellow crystals (0.37 mmol ,82 %): mp. 184.4 °C (decomp.); ^{1}*H*-NMR (DMSO-[D_6]): δ (ppm) = 12.28 (bs, 1H), , 9.80 (s, 1H), 9.00 (s, 1H), 8.52 (d, J = 8.5 Hz, 1H), 8.12 (d, J = 8.8 Hz, 1H), 7.96-7.77 (m, 3H), 7.61-7.48 (m, 2H), 7.46-7.22 (m, 2HH), 6.60 (s, 1H), 6.19 (d, J = 15.9 Hz, 1H), 4.30 (s, 1H). IR (KBr): ν (cm^{-1}) = 3425, 3271, 2542, 1612. ES-MS (CH_2Cl_2, CH_3OH, CH_3COONH_4) *m/z* (%): 460 $[M+H]^+$ (95). HRMS-ESI: *m/z* $[M+H^+]^+$ calcd. for $C_{23}H_{18}N_5O_4S$: 460.1074, found: 460.1079; Anal. calcd. for $C_{23}H_{17}N_5O_4S$ x 2 HCl x 1.5 H_2O: C 49.38, H 3.96, N 12.52, found: C 49.04, H 4.36, 13.11.

Biological Methods

Biological Methods were used identical to the methods described in Lit[13]. Biochemical HDAC Assays. For rHDAC1 and rHDAC6 expression, a clonal HEK293 (ATCC CRL1573) human kidney cell line expressing the human rHDAC1 isoenzyme bearing a C-terminal Flag epitope was provided by E. Verdin, The Gladstone Institute/San Francisco, CA, USA. Human HDAC3-Flag was coexpressed with the SMRT DAD domain in Sf21 insect cells, whereas human HDAC8-Flag was expressed without cofactor in Sf21 cells. The rHDAC proteins were purified by M2-affinity gel chromatography according to the manufacture protocol (Sigma no. A2220). Purified protein samples were routinely analyzed by SDS-PAGE (12.5% or 10% Laemmli gels) followed by Coomassie stain and Western blotting using a FLAG-specific antibody (anti M2-POX antibody, Sigma no. A8592) followed by ECL-detection (GE Healthcare). In addition, protein batches were analyzed by Western blotting for various HDAC isoenzymes including HDAC1, 3, 6, and 8. The biochemical HDAC activity assay was essentially done as described by Wegener et al.[14]. Briefly 40µl of a rHDAC1 dilution (about 10ng/well rHDAC1) or a 1:100 dilution (= 0.4 µl) of a nuclear HeLa extract), 29 µl enzyme buffer (15mM Tris HCl pH 8.1, 0.25mM EDTA, 250mM NaCl, 10% v:v glycerol) and 1µl test compound were added to a well of a 96well microtiter plate and reaction started by addition of 30µl substrate (Ac-NH-GGK(Ac)-AMC; final concentration 25µM and final volume). After incubation for 180min at 30°C, the reaction was terminated by the addition of 25µl stop solution (50mM Tris HCl pH 8, 100mM NaCl, 0.5mg/ml trypsin and 2µM trichostatin A /TSA). After incubation at room temperature for further 40min, fluorescence was measured using a Wallac Victor 1420 multilabel counter (excitation λ=355nm, emission λ=460nm) for quantification of AMC (7-amino-4-methylcoumarin) generated by trypsin cleavage of the deacetylated peptide. For the calculation of IC50 values the fluorescence in wells without test compound (1% DMSO, negative control) was set as 100% enzymatic activity and the fluorescence in wells with 2µM TSA (positive control) were set at 0% enzymatic activity. The corresponding IC50 values of the compounds for HDAC inhibitory activity were determined from the concentration-effect curves by means of non-linear regression analysis using the program GraphPad prism (Version 4.0).

To assess the cellular potency of histone deacetylase inhibitors, an assay recently described was applied.[15] 5x103 HeLa cervical carcinoma cells/well (ATCC CCL-2) were cultivated in 200µl/well DMEM (containing 10% FCS) in cell culture microtiter plates (white, 96well flat bottom plates suitable for fluorimetric analysis; Costar Art. No. 3917). Cells were cultivated for 24h under standard cell culture conditions at 37°C and 5% CO_2 to allow cell attachment and proliferation. The compounds of the present investigations were added at different concentrations (dilutions done in DMSO) and incubation continued for 4h at 37°C under cell culture conditions (each concentration was tested in quadruplicate). For determination of the background activity defined as deacetylase activity not associated with HDAC class I and II enzymes, 10µM TSA were added to control wells. After discarding the culture medium, 100µl/well substrate solution (200µM Boc-K(Ac)-AMC in DMEM (containing 10% FCS), stock solution diluted 1:250) were added and incubation continued at 37°C under cell culture conditions for further 3h. After discarding the substrate solution, HeLa cells were washed once with 200µl/well PBS before addition of 100µl/well developer solution (50mM TRIS pH 8, 100mM NaCl with 0.5mg/ml trypsin and 10µM TSA). After incubation for 5min at 37°C, cells were lysed by addition of 100 µl/well lysis buffer (50mM TRIS pH 8, 137mM NaCl, 2,7mM KCl, 1mM MgCl2, 1vol% NP-40) and further incubation for 20min at 37°C. Finally, the amount of AMC was quantified using the Wallac Victor2 device (extinction λ=355nm, emission at λ=460nm). For the calculation of IC50 values, the fluorescence in wells without test compound (1% DMSO, negative control) was set as 100% enzymatic activity and the fluorescence in wells with 10µM TSA (background control) were set at 0% enzymatic activity. Biochemical Protein Kinase Assays. Active kinase proteins were either obtained from commercial suppliers (ProQinase, Freiburg/Germany; InvitroGen/Panvera, Carlsbad, CA, USA) or prepared in-house. Experimental details of the flash-plate based radioactive enzyme assay have been described previously.[16] Cellular Histone H3 Hyperacetylation Assay. Cellular Proliferation Assay. The antiproliferative activity of selected compounds was evaluated using the tumor cell lines HeLa (cervical carcinoma, ATCC CCL-2), A549 (NSCLC, ATCC CCL-185), SKBR-3 (breast carcinoma, ATCC HTB-30), SKOV-3 (ovarian carcinoma, ATCC HTB-77), Cal27 (tongue carcinoma, ATCC CRL-2095), and A-431 (vulva carcinoma, ATCC CRL-2592). For quantification of cellular proliferation/ cytotoxicity, the Alamar Blue (Resazurin) cell viability assay was applied.[39]

The combined effect of SAHA and erlotinib treatment was analyzed by median effect analysis according to the method of Chou and Talalay [17] Combination Index (CI) values were expressed at fraction affected of 0.5 (ED50 level) using the CalcuSyn Software (Biosoft, Cambridge, UK).[18] CI<1, CI=1, and CI>1 indicates synergism, additive or antagonistic interaction, respectively. For analysis of combination treatment, antiproliferative activity / cytotoxicity was determined by using a fluorescence based propidium iodide assay. [19]

Western Blot Analysis. For Western blot analysis, about 4 x 10^5 Cal27 cells/well in 6-well cell culture plates were treated with the test compounds for 16 h. Next, cells were stimulated with 100 ng/mL recombinant EGF for 5 min at room temperature before cell lysis. Cells were lysed in lysis buffer (50mMTris HCl pH8, 150 mMNaCl, 1v/v NP-40, 0.5% w/v sodium desoxycholate, 0.2% w/v disodium dodecylsulfate (SDS), 0.02% w/v NaN_3, 1 mM sodium vanadate, 20 mMNaF, 100 µg/mL PMSF, 10 mM sodium pyrophosphate, protease inhibitor mix/Roche and 50U/mL Benzonase) at 4 °C. Respective equal amounts of protein were separated by SDS-PAGE before transfer to polyvinylidendifluoride (PVDF) membrane (Biorad art. no. 162-0177) by semidry blotting. The following antibodies were used: monoclonal mouse antibody specific for β-actin (clone AC-12, Sigma art. no. A-5441), phosphorylated EGFR (Y1068, Cell Signaling), EGFR (Upstate), acetylated histone H3 (Cell Signaling), and histone H3 (Cell Signaling). As secondary antibodies, goat antirabbit IgG-

HRP conjugated (Biorad: 170-6515), goat-antisheep (Santa Cruz) and goatantimouse IgG-HRP conjugated (Biorad: 170-6516) were used. [13-14, 16, 20]

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