Novel inhibitors of breast cancer relevant kinases Brk and HER2

Electronic supplementary information

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Experimental protocols

1. Chemistry

Commercial reagents were used without further purification. The pyrido[2,3-*b*]indole was prepared in polyphosphoric acid from the pyridin-2-yl benzotriazole which resulted from the reaction of benzotriazole and 2-bromopyridine according to literature [21] in toluene under reflux. The 4-chloropyrido[2,3-*b*]indole was yielded with phosphorus oxychloride after formation of the *N*-oxide with hydrogen peroxide in acetic acid following literature [22]. The ^{1}H-NMR spectra (400 MHz) were measured using tetramethylsilane as internal standard. TLC was performed on E. Merck 5554 silica gel plates. The EI mass spectra were measured with an AMD 402 mass spectrometer, the ESI spectra were recorded on a Finnigan LCQ Classic mass spectrometer. IR spectra were recorded on a FT-IR spectrometer. Elemental analysis indicated by the symbols of the elements was within ± 0.4% of the theoretical values and was performed using a Leco CHNS-932 apparatus.

*1.1. General procedure for the formation of the 4-anilino-pyrido[2,3-b]indoles **5a-d***

0.25 g of the 4-chloropyrido[2,3-*b*]indole **4** (1.23 mmol, 1 eq.) and the corresponding aniline derivative (10 eq.) were dissolved in 5 mL of dried NMP. The solution was degassed under vacuum and then heated under argon atmosphere and under reflux until all of the starting product **4** disappeared as observed by TLC. After cooling the mixure was poured into 50 mL of ethyl acetate. Then 25 mL of water were added. After phase separation the aquous phase was extracted for three times with each 25 mL of ethyl acetate. The combined organic layers were dried over sodium sulfate and filtered. Then the solvent was removed in vaccuum

and the resulting oily residue was purified over silica gel by column chromatography using ethyl acetate as eluent.

1.1.1. N-o-Tolyl-9H-pyrido[2,3-b]indol-4-amine ***5a***

Yield 0.21 g (61%); light beige solid; mp 215-218 °C; ^{1}H NMR (DMSO-D_6) δ 11.52 (s, 1H, 9-*N*H), 8.15 (d, *J* = 7.9 Hz, 1H, 5-H), 8.00 (s, 1H, aniline *N*H), 7.94 (d, *J* = 5.6 Hz, 1H, 2-H), 7.40 (d, *J* = 8.0 Hz, 1H, 8-H), 7.33 (d, *J* = 6.3 Hz, 1H, 3´- H), 7.31 (dd, *J* = 8.0, 7.3 Hz, 1H, 7-H), 7.25-7.13 (m, 3H, 4´-, 5´-, 6´-H), 7.09 (dd, *J* = 7.9 , 7.3 Hz, 1H, 6-H), 6.06 (d, *J* = 5.6 Hz, 1H, 3-H), 2.2 (s, 3H, CH_3); MS (EI), *m/z* = 273 [M^+]; IR (KBr): 3422, 3252, 1692, 1593 cm^{-1}. Anal. ($C_{18}H_{15}N_3$) Calc. C 79.10, H 5.53, N 15.37; Found C 78.88, H 5.56, N 15.48.

1.1.2. 3-(9H-Pyrido[2,3-b]indol-4-ylamino)phenol ***5b***

Yield 0.14 g (41%); yellow solid; mp 225-227 °C; ^{1}H NMR (DMSO-D_6) δ 11.58 (s, 1H, 9-*N*H), 9.33, 8.35 (s, 2H, aniline *N*H, OH), 8.06 (d, *J* = 5.6 Hz, 1H, 2-H), 8.04 (d, *J* = 8.1 Hz, 1H, 5-H), 7.40 (d, *J* = 8.0 Hz, 1H, 8-H), 7.32 (dd, *J* = 8.0, 7.2 Hz, 1H, 7-H), 7.13-7.06 (m, 2H, 5´-H, 6-H), 6.78 (d, *J* = 5.5 Hz, 1H, 3-H), 6.72-6.64 (m, 2H, 2´-H, 6´-H), 6.44 (d, *J* = 7.1 Hz, 1H, 4´H); MS (EI), *m/z* = 275 [M^+]; IR (KBr): 3420, 1694, 1595, 1505, 1458, 1264 cm^{-1}. Anal. ($C_{17}H_{13}N_3O$) Calc. C 69.61, H 5.15, N 14.33; Found C 69.91, H 5.15, N 14.43.

1.1.3. N-(3-Chlorophenyl)-9H-pyrido[2,3-b]indol-amine ***5c***

Yield 0.34 g (78%); light brown solid; mp 241-244 °C; ^{1}H NMR (DMSO-D_6) δ 11.66 (s, 1H, 9-*N*H), 8.68 (s, 1H, aniline *N*H), 8.13 (d, *J* = 5.6 Hz, 1H, 2-H), 7.98 (d, *J* = 8.0 Hz, 1H, 5-

H), 7.43 (d, *J* = 8.1 Hz, 1H, 8-H), 7.34 (dd, *J* = 8.1, 7.2 Hz, 1H, 7-H), 7.31 (dd, *J* = 8.0 Hz, 7.8 Hz, 1H, 5´-H), 7.25 (s, 1H, 2´-H), 7.19 (dd, *J* = 7.8, 1.2 Hz, 1H, 4´-H), 7.11 (dd, *J* = 8.0, 7.2 Hz, 1H, 6-H), 7.02 (dd, *J* = 8.0, 1.2 Hz, 1H, 6´-H), 6.85 (d, *J* = 5.6 Hz, 1H, 3-H); MS (EI), *m/z* = 293 [M^+]; IR (KBr): 3412, 1694, 1584, 1506, 1457, 1258 cm^{-1}. Anal. ($C_{17}H_{12}ClN_3$) Calc. C 69.51, H 4.12, Cl 12.07, N 14.30; Found C 69.71, H 4.14, Cl 12.00, N 14.06.

1.1.4. N-(4-Chlorophenyl)-9H-pyrido[2,3-b]indol-amine ***5d***

Yield 0.23 g (64%); beige solid; mp 274-277 °C; ^{1}H NMR (DMSO-D_6) δ 11.63 (s, 1H, 9-*N*H), 8.55 (s, 1H, aniline *N*H), 8.09 (d, *J* = 5.6 Hz, 1H, 2-H), 7.98 (d, *J* = 7.9 Hz, 1H, 5-H), 7.42 (d, *J* = 8.0 Hz, 1H, 8-H), 7.37-7.31 (m, 3H, 7-H, 3´-, 5´-H), 7.25-7.22 (m, 2H, 2´-, 6´-H), 7.11 (dd, *J* = 7.9, 7.0 Hz, 1H, 6-H), 6.77 (d, *J* = 5.6 Hz, 1H, 3-H); MS (EI), *m/z* = 293 [M^+]; IR (KBr): 3424, 3073, 1588, 1493, 1457, 1261 cm^{-1}. Anal. ($C_{17}H_{12}ClN_3$) Calc. C 69.51, H 4.12, Cl 12.07, N 14.30; Found C 69.55, H 4.09, Cl 12.17, N 14.36.

1.2. General procedure for the formation of the 4-chloro sulfonamide compounds ***7****,* ***8*** *and* ***9*** *via the crude chlorosulfonic compound* ***6*** *intermediate*

1.0 g of the 4-chloropyrido[2,3-*b*]indole **4** (4.94 mmol, 1 eq.) was cooled down and then treated dropwise with 1.5 mL of chlorosulfonic acid (2.62 g, 59.1 mmol) under stirring which continued for 2 h at rt at the low temperature. Then the excess of the used acid was hydrolyzed with crushed ice. The precipitate of the crude compound **6** was filtered off, washed with cold water and dried by sucking. Then the solid was resuspended in 25 mL of THF and stirred with 10 eq. of the respective amine. Stirring continued overnight at rt. Then

the solvent was removed in vacuum and the oily residue was mixed with 50 mL of water. After stirring for 24 h at rt the solid was filtered off, washed with water and dried on air.

*1.2.1. 4-(4-Chloro-9H-pyrido[2,3-b]indol-6-ylsulfonyl)morpholine **7***

Yield 0.60 g (35%); beige solid; mp 285-292 °C; ^{1}H NMR (DMSO-D_6) δ 12.21 (s, 1H, 9-*N*H), 8.62 (d, *J* = 1.5 Hz, 1H, 5H), 8.46 (d, *J* = 5.3 Hz, 1H, 2-H), 7.86 (dd, *J* = 8.6 Hz, 1.5 Hz, 1H, 7-H), 7.76 (d, *J* = 8.6 Hz, 1H, 8-H), 7.42 (d, *J* = 5.3 Hz, 1H, 3-H), 3.60 (t, *J* = 4.4 Hz, 4H, CH_2-O), 2.87 (t, *J* = 4.4 Hz, 4H, CH_2-N); MS (EI), *m/z* = 351 [M^+]; IR (KBr): 3428, 3208, 3128, 2963, 2897, 2860, 1624, 1598, 1570, 1455, 1306, 1160, 1114 cm^{-1}. Anal. ($C_{15}H_{14}ClN_3O_3S$) Calc. C 51.21, H 4.01, Cl 10.08, N 11.94, S 9.11; Found C 51.06, H 4.10, Cl 10.18, N 11.77, S 8.81.

*1.2.2. 4-Chloro-6-(piperazin-1-yl-sulfonyl)-9H-pyrido[2,3-b]indole **8***

Yield 0.79 g (50%); light beige solid; mp > 320 °C; ^{1}H NMR (DMSO-D_6) δ 12.00 (s, 1H, 9-*N*H), 8.62 (s, 1H, 5H), 8.49 (d, *J* = 5.2 Hz, 1H, 2-H), 7.86 (d, *J* = 8.4 Hz, 1H, 7-H), 7.78 (d, *J* = 8.4 Hz, 1H, 8-H), 7.45 (d, *J* = 5.2 Hz, 1H, 3-H), 3.60 (br s, 1H, piperazino *N*H), 2.84-2.78 (m, 4H, CH_2-N-SO_2), 2.75-2.68 (m, 4H, C**H**$_2$-NH); MS (EI), *m/z* = 350 [M^+]; IR (KBr): 3323, 3121, 2949, 2856, 2743, 1624, 1596, 1570, 1456, 1306, 1159 cm^{-1}. Anal. ($C_{15}H_{15}ClN_4O_2S$) Calc. C 51.35, H 4.31, Cl 10.11, N 15.97, S 9.14; Found C 50.95, H 3.97, Cl 10.30, N 15.65, S 9.15.

*1.2.3. 2-(4-(4-Chloro-9H-pyrido[2,3-b]indol-6-ylsulfonyl)piperazin-1-yl)ethanol **9***

Yield 0.94 g (48%); light beige solid; mp > 213-215 °C; ^{1}H NMR (DMSO-D_6) δ 12.59 (br s, 1H, 9-*N*H), 8.63 (d, *J* = 1.7 Hz, 1H, 5H), 8.48 (d, *J* = 5.4 Hz, 1H, 2-H), 7.87 (dd, *J* = 8.6, 1.7 Hz, 1H, 7-H), 7.78 (d, *J* = 8.6 Hz, 1H, 8-H), 7.44 (d, *J* = 5.4 Hz, 1H, 3-H), 4.29 (br s, 1H, OH), 3.40-3.28 (m, 2H, C**H**$_2$-OH), 2.95-2.84 (m, 4H, CH_2-N-SO_2), 2.50-2.44 (m, 4H, C**H**$_2$-N-CH_2-CH_2-OH), 2.31 (t, *J* = 6.1 Hz, 2H, N-C**H**$_2$-CH_2-OH); MS (EI), *m/z* = 394 [M^+]; IR (KBr): 3430, 2947, 2822, 1624, 1596, 1569, 1456, 1306, 1161 cm^{-1}. Anal. ($C_{17}H_{19}ClN_4O_3S$) Calc. C 51.71, H 4.85, Cl 8.98, N 14.19, S 8.12; Found C 51.44, H 5.00, Cl 8.65, N 13.89, S 8.50.

*1.3. General procedure for the formation of the 4-anilino substituted compound series **10**, **11** and **12***

The procedure followed the above described synthesis of the compound series **5** using each 0.25 g of the respective 4-chloro 6-sulfonamides **7**-**9**, respectively, (1 eq.) and the tenfold amount of the respective aniline (10 eq.).

*1.3.1. 3-(6-Morpholinosulfonyl)-9H-pyrido[2,3-b]indol-4-ylamino)phenol **10a***

Yield 0.11 g (37%); dark brown solid; mp > 300 °C; ^{1}H NMR (acetone-D_6) δ 11.15 (br s, 1H, 9-*N*H), 8.32 (s, 1H, aniline *N*H), 8.22 (d, *J* = 5.4 Hz, 1H, 2-H), 8.21 (s, 1H, 5-H), 7.79-7.71 (m, 3H, OH, 7-H, 8-H), 7.18 (t, *J* = 8.0 Hz, 1H, 5´-H), 6.93 (d, *J* = 5.4 Hz, 1H, 3-H), 6.77-6.72 (m, 2H, 4´-, 6´-H), 6.59 (d, *J* = 1.8 Hz, 1H, 2´- H), 3.63 (t, *J* = 4.6 Hz, 4H, CH_2-O), 2.86 (t, *J* = 4.6 Hz, 4H, CH_2-N); MS (ESI), *m/z* = 425 [M+H^+]; IR (ATR): 3330, 3070, 2956, 2921, 2852, 1630, 1598, 1502, 1455, 1295, 1155 cm^{-1}. Anal. ($C_{21}H_{20}N_4O_4S$) Calc. C 59.42, H 4.75, N 13.20, S 7.55; Found C 59.15, H 4.58, N 12.95, S 7.29.

1.3.2. N-(3-Methoxyphenyl)-(6-morpholinosulfonyl)-9H-pyrido[2,3-b]indol-4-amine ***10b***

Yield 0.13 g (44%); brown solid; mp 230-240 °C; ^{1}H NMR (acetone-D_6) δ 11.21 (br s, 1H, 9-*N*H), 8.32 (d, *J* = 1.5 Hz, 1H, 5-H), 8.25-8.22 (m, 2H, 2-H, aniline *N*H), 7.76-7.74 (m, 2H, 7-, 8-H), 7.27 (t, *J* = 8.1 Hz, 1H, 5´-H), 6.93 (d, *J* = 5.6 Hz, 1H, 3-H), 6.86-6.82 (m, 2H, 4´-, 6´-H), 6.69 (d, *J* = 1.6 Hz, 1H, 2´- H), 3.75 (s, 3H, OCH_3), 3.65-3.63 (m, 4H, CH_2-O), 2.87-2.85 (m, 4H, CH_2-N); MS (ESI), *m/z* = 439 [M+H^+]; IR (ATR): 3410, 3040, 2960, 2922, 2850. 1602, 1579, 1508, 1456, 1298, 1151, 1113 cm^{-1}. Anal. ($C_{22}H_{22}N_4O_4S$) Calc. C 60.24, H 5.06, N 12.78, S 7.31; Found C 59.93, H 4.88, N 12.58, S 7.15.

1.3.3. N-(3-Ethoxyphenyl)-(6-morpholinosulfonyl)-9H-pyrido[2,3-b]indol-4-amine ***10c***

Yield 0.16 g (50%); beige solid; mp 237-241 °C; ^{1}H NMR ($CDCl_3$) δ 11.49 (br s, 1H, 9-*N*H), 8.28 (d, *J* = 5.8 Hz, 1H, aniline *N*H), 8.22 (s, 1H, 5-H), 7.79 (d, *J* = 5.6 Hz, 1H, 2-H), 7.61 (d, *J* = 8.5 Hz, 1H, 7-H), 7.32 (t, *J* = 8.1 Hz, 1H, 5´-H), 6.95-6.86 (m, 3H, 4´-, 3-H, 8-H), 6.74 (dd, *J* = 8.4 Hz, 1.8 Hz, 1H, 6´-H), 6.86 (s, 1H, 2´-H), 4.02 (q, *J* = 6.8 Hz, 2H, OC**H**$_2$$CH_3$), 3.72 (t, *J* = 4.6 Hz, 4H, CH_2-O), 3.0 (t, *J* = 4.6 Hz, 4H, CH_2-N), 1.41 (t, *J* = 6.8 Hz, 3H, OCH_2C**H**$_3$); MS (ESI), *m/z* = 453 [M+H^+]; IR (ATR): 3414, 3060, 2960, 2921, 2852, 1604, 1580, 1507, 1451, 1298, 1152, 1112 cm^{-1}. Anal. ($C_{23}H_{24}N_4O_4S$) Calc. C 61.05, H 5.35, N 12.38, S 7.08; Found C 60.75, H 5.15, N 12.13, S 7.04.

1.3.4. N-(3-(Benzyloxy)phenyl)-(6-morpholinosulfonyl)-9H-pyrido[2,3-b]indol-4-amine ***10d***

Yield 0.11 g (30%); brown solid; mp 100-110 °C; ^{1}H NMR (acetone-d_6) δ 11.60 (br s, 1H, 9-*N*H), 8.30 (d, *J* = 8.4 Hz, 1H, aniline *N*H), 8.21 (d, *J* = 5.6 Hz, 1H, 2-H), 7.77-7.75 (m,

2H, 7-, 5-H), 7.42-7.26 (m, 8H, $OCH_2C_6\mathbf{H}_5$, 3-, 8-, 5´-H), 6.92-6.86 (m, 2H, 4´-, 6´-H), 6.78 (dd, *J* = 8.4, 2.2 Hz, 1H 2´-H), 5.08 (s, 2H, $OC\mathbf{H}_2C_6H_5$), 3.65 (t, *J* = 4.7 Hz, 4H, CH_2-O), 2.86 (t, *J* = 4.7 Hz, 4H, CH_2-N); MS (ESI), *m/z* = 515 [$M+H^+$]; IR (ATR): 3214, 3062, 2955, 2922, 2853, 1579, 1508, 1453, 1325, 1154, 1111 cm^{-1}. Anal. ($C_{28}H_{26}N_4O_4S$) Calc. C 65.35, H 5.09, N 10.89, S 6.23; Found C 65.22, H 5.30, N 10.64, S 5.99.

1.3.5. N-(3-Methoxyphenyl)-6-(piperazin-1-yl-sulfonyl)-9H-pyrido[2,3-b]indol-4-amine ***11a***

Yield 0.10 g (30%); dark brown solid; mp 137-140 °C; ^{1}H NMR (DMSO-D_6) δ 12.27 (s, 1H, 9-*N*H), 8.87 (s, 1H, aniline *N*H), 8.16 (d, *J* = 5.6 Hz, 1H, 2-H), 8.15 (s, 1H, 5H), 7.41-7.05 (m, 2H, 7-, 8-H), 7.41-7.05 (m, 1H, 5´-H), 6.81 (m, 2H, 4´, 6´-H), 6.71 (d, *J* = 5.6 Hz, 1H, 3-H), 6.61 (d, *J* = 1.8 Hz, 1H, 2´-H), 3.67 (s, 3H, OCH_3), 2.65 (s, 4H, CH_2-N-SO_2), 2.04 (s, 1H, piperazino *N*H), 1.76 (s, 4H, $C\mathbf{H}_2$-NH); MS (ESI), *m/z* = 438 [$M+H^+$]; IR (ATR): 3212, 2920, 2851, 1663, 1579, 1491, 1453, 1318, 1156, 1131 cm^{-1}. Anal. ($C_{22}H_{23}N_5O_3S$) Calc. C 60.40.35, H 5.30, N 16.00, S 7.33; Found C 60.15, H 4.98, N 15.79, S 7.18.

1.3.6. N-(3-Ethoxyphenyl)-6-(piperazin-1-yl-sulfonyl)-9H-pyrido[2,3-b]indol-4-amine ***11b***

Yield 0.10 g (31%); light beige solid; mp 280-286 °C; ^{1}H NMR (DMSO-D_6) δ 12.45 (s, 1H, 9-*N*H), 8.92 (s, 1H, aniline *N*H), 8.19 (d, *J* = 5.6 Hz, 1H, 2-H), 8.16 (s, 1H, 5H), 7.65 (d, *J* = 8.1 Hz, 1H, 7-H), 7.21 (t, *J* = 8.0 Hz, 1H, 5´-H), 6.82 (d, *J* = 8.0 Hz, 1H, 4´-H), 6.80 (d, *J* = 8.0 Hz, 1H, 6´-H), 6.73 (d, *J* = 8.1 Hz, 1H, 8-H), 6.69 (s, 1H, 2´-H), 6.61 (d, *J* = 5.6 Hz, 1H, 3-H), 3.91 (q, *J* = 6.9 Hz, 2H, $OC\mathbf{H}_2CH_3$), 2.68 (s, 8H, CH_2-N-SO_2, $C\mathbf{H}_2$-NH), 2.07 (s, 1H, piperazino *N*H), 1.27 (t, *J* = 6.9 Hz, 3H, $OCH_2C\mathbf{H}_3$); MS (ESI), *m/z* = 452 [$M+H^+$]; IR (ATR): 3335, 2956, 2853, 1661, 1576, 1505, 1453, 1318, 1155, 1113 cm^{-1}. Anal. ($C_{23}H_{25}N_5O_3S$) Calc. C 61.18, H 5.58, N 15.91, S 7.10; Found C 60.95, H 5.32, N 15.68, S 7.14.

1.3.7. 2-(4-(4-3-Methoxyphenylamino)-9H-pyrido[2,3-b]indol-6-ylsulfonyl)piperazin-1-yl)ethanol ***12a***

Yield 0.17 g (56%); brown solid; mp 138-143 °C; ^{1}H NMR ($CDCl_3$) δ 11.05 (br s, 1H, 9-*N*H), 8.24-8.21 (m, 2H, 2-H, aniline *N*H), 7.74 (d, *J* = 8.7 Hz, 1H, 7-H), 7.54 (d, *J* = 8.7 Hz, 1H, 8-H), 7.32 (t, *J* = 8.2 Hz, 1H, 5´-H), 6.92-6.87 (m, 4H, OH, 5-, 4´-, 6´-H), 6.77-6.74 (m, 2H, 2´-H, 3-H), 3.81 (s, 3H, OCH_3), 3.54 (t, *J* = 5.2 Hz, 2H, N-CH_2-C**H**$_2$-OH), 3.03 (s, 4H, SO_2-N-CH_2), 2.58 (s, 4H, OH-CH_2-CH_2-N-C**H**$_2$), 3.54 (t, *J* = 5.2 Hz, 2H, N-C**H**$_2$-CH_2-OH); MS (ESI), *m/z* = 482 [M+H$^+$]; IR (KBr): 3340, 2958, 2852, 1580, 1455, 1322, 1157 cm^{-1}. Anal. ($C_{24}H_{27}N_5O_4S$) Calc. C 59.86, H 5.65, N 14.54, S 6.66; Found C 59.65, H 5.33, N 14.36, S 6.45.

1.3.8. 2-(4-(4-3-Ethoxyphenylamino)-9H-pyrido[2,3-b]indol-6-ylsulfonyl)piperazin-1-yl)ethanol ***12b***

Yield 0.11 g (35%); beige solid; mp 148-155 °C; ^{1}H NMR ($CDCl_3$) δ 10.98 (br s, 1H, 9-*N*H), 8.78 (s, 1H, aniline *N*H), 8.42 (d, *J* = 5.2 Hz, 1H, 2-H), 8.21 (s, 1H, 5-H), 7.75 (d, *J* = 8.7 Hz, 1H, 7-H), 7.58 (d, *J* = 8.7 Hz, 1H, 8-H), 7.31-7.23 (m, 2H, OH, 5´-H), 6.93-6.86 (m, 3H, 3-, 4´-, 6´-H), 6.74 (d, *J* = 1.9 Hz, 1H, 2´-H), 4.01 (q, *J* = 69 Hz, 2H, OC**H**$_2$$CH_3$), 3.57 (t, *J* = 5.0 Hz, 2H, OH-C**H**$_2$-CH_2-N), 3.9-3.05 (m, 4H, SO_2-N-CH_2), 2.65-2.53 (m, 6H, OH-CH_2-C**H**$_2$-N, OH-CH_2-CH_2-N-C**H**$_2$), 1.41 (t, *J* = 6.9 Hz, 3H, OCH_2C**H**$_3$); MS (ESI), *m/z* = 496 [M+H$^+$]; IR (KBr): 3208, 3123, 2923, 2851, 1661, 1579, 1454, 1324, 1155 cm^{-1}. Anal. ($C_{25}H_{29}N_5O_4S$) Calc. C 60.57, H 5.90, N 14.13, S 6.47; Found C 60.35, H 5.85, N 14.43, S 6.51.

1.3.9. 3-(6-(4-2-Hydroxyethyl)piperazin-1-ylsulfonyl)-9H-pyrido[2,3-b]indol-4-ylamino)-phenol ***12c***

Yield 0.11 g (35%); brown solid; mp 173-179 °C; ^{1}H NMR (acetone-d_6) δ 11.21 (br s, 1H, 9-*N*H), 8.30, 8.31 (s, 2H, C_6H_5-O**H**, aniline *N*H), 8.22 (d, *J* = 5.6 Hz, 1H, 2-H), 8.19 (s, 1H, 5-H), 7.78-7.70 (m, 3H, HO-CH_2-CH_2-N, 7-, 8-H), 7.18 (t, *J* = 8.0 Hz, 1H, 5´-H), 6.93 (d, *J* = 5.6 Hz, 1H, 3-H), 6.74, 6.73 (d, *J* = 8.0 Hz, 2H, 4´-, 6´- H), 6.59 (s, 1H, 2´-H), 3.52 (t, *J* = 5.6 Hz, 2H, OH-C**H**$_2$-CH_2-N), 2.53 (t, *J* = 4.8 Hz, 4H, SO_2-N-CH_2), 2.44 (t, *J* = 5.6 Hz, 2H, HO-CH_2-C**H**$_2$-N), 1.26 (t, *J* = 4.8 Hz, 4H, HO-CH_2-CH_2-N-C**H**$_2$); MS (ESI), *m/z* = 468 [M+H$^+$]; IR (KBr): 3214, 2922, 2852, 1582, 1455, 1323, 1155 cm^{-1}. Anal. ($C_{23}H_{25}N_5O_4S$) Calc. C 59.09, H 5.39, N 14.98, S 6.86; Found C 58.95, H 5.15, N 14.73, S 6.67.

1.4. Formation of the 6-bromo 4-chloropyrido[2,3-b]indole ***13***

1.0 g (4.93 mmol) of compound **4** was dissolved in 30 mL of acetic acid and then 300 µmL of bromine (0.94 g, 5.89 mmol) were added dropwise under stirring that continued for 24 h at rt. Then 50 mL of a sodium thiosulfate solution (1 M) were added. The mixture was cooled on an ice bath and alkalized with an ammonia solution. Extractions with 50 mL of dichloromethane and with ethylacetate each for three times followed. The unified organic layer was then dried over sodium sulfate, filtered and the solvent was removed in vacuum. The crude product was used for further derivatization reactions.

1.4.1. 6-Bromo-4-chloro-9H-pyrido[2,3-b]indole ***13***

Yield 0.95 g (69%); beige solid; mp 200-208 °C; ^{1}H NMR (DMSO-D_6) δ 11.65 (s, 1H, 9-*N*H), 8.40 (d, *J* = 5.2 Hz, 1H, 2-H), 8.39 (s, 1H, 5-H), 8.62 (d, *J* = 7.5 Hz, 1H, 7-H), 8.60 (d, *J*

= 7.5 Hz, 1H, 8-H), 7.33 (d, *J* = 5.2 Hz, 1H. 3-H), 7.31; MS (EI), *m/z* = 282 [M^+]; IR (KBr): 3436, 3224, 3137, 3074, 1619, 1585, 1566, 1456 cm^{-1}. Anal. ($C_{11}H_6BrClN_3$) Calc. C 46.93, H 2.15, Cl 12.59, N 9.95; Found C 46.73, H 1.95, Cl 12.35, N 9.78.

*1.5. General procedure for the formation of the 4-anilino substituted 6-bromo compound series **14***

The procedure followed the above described synthesis for the compound series **5** using 0.25 g of the 6-bromo 4-chloro derivative **13** (0.89 mmol, 1 eq.) and the tenfold amount (8.90 mmol, 10 eq.) of the respective aniline.

*1.5.1. 3-(6-Bromo-9H-pyrido[2,3-b]indol-4-ylamino)phenol **14a***

Yield 0.06 g (19%); dark beige solid; mp 264-266 °C; ^{1}H NMR (DMSO-D_6) δ 11.77 (s, 1H, 9-*N*H), 9.41, 8.49 (s, 2H, aniline *N*H, OH), 8.33 (d, *J* = 5.6 Hz, 1H, 2-H), 8.08 (s, 1H, 5-H), 7.48-7.32 (m, 2H, 7-, 8-H), 7.17-7.09 (m, 1H, 5´-H), 6.79-6.69 (m, 3H, 4´-, 3-, 6´-H), 6.45 (d, *J* = 1.8 Hz, 1H, 2´-H); MS (EI), *m/z* = 354 [M^+]; IR (ATR): 3427, 3262, 3055, 2922, 1875, 1586, 1520, 1453 cm^{-1}. Anal. ($C_{17}H_{12}BrN_3O$) Calc. C 57.65, H 3.41, N 11.86; Found C 57.35, H 3.25, N 11.56.

*1.5.2. 6-Bromo-N-(3-methoxyphenyl)-9H-pyrido[2,3-b]indol-4-amine **14b***

Yield 0.06 g (18%); white solid; mp 312-318 °C; ^{1}H NMR (DMSO-D_6) δ 11.80 (s, 1H, 9-*N*H), 8.59 (s, 1H, aniline *N*H), 8.35 (d, *J* = 1.9 Hz, 1H, 5-H), 8.09 (d, *J* = 5.6 Hz, 1H, 2-H), 7.47 (dd, *J* = 8.5, 1.9 Hz, 1H, 7-H), 7.38 (d, *J* = 8.5 Hz, 1H, 8-H), 7.28 (t, *J* = 7.9 Hz, 1H, 5´-H), 6.91-6.88 (m, 2H, 4´-, 6´-H), 6.79 (d, *J* = 5.6 Hz, 1H, 3-H), 6.67 (d, *J* = 1.9 Hz, 1H, 2´-H),

3.75 (s, 3H, OCH_3); MS (ESI), *m/z* = 369 [$M+H^+$]; IR (ATR): 3443, 3132, 3029, 2920, 1892, 1579, 1510, 1452 cm^{-1}. Anal. ($C_{18}H_{14}BrN_3O$) Calc. C 58.71, H 3.83, N 11.41; Found C 58.55, H 3.64, N 11.13.

1.5.3. 6-Bromo-N-(3-chlorophenyl)-9H-pyrido[2,3-b]indol-4-amine ***14c***

Yield 0.06 g (19%); dark grey solid; mp 244-249 °C; ^1H NMR (DMSO-D_6) δ 11.87 (s, 1H, 9-*N*H), 8.75 (s, 1H, aniline *N*H), 8.31 (d, *J* = 1.9 Hz, 1H, 5-H), 8.15 (d, *J* = 5.6 Hz, 1H, 2-H), 7.48 (dd, *J* = 8.6, 1.9 Hz, 1H, 7-H), 7.41-7.33 (m, 3H, 6´-, 8-, 5´-H), 7.25 (d, *J* = 8.2 Hz, 1H, 4´-H), 7.09 (d, *J* = 1.6 Hz, 1H, 2´-H), 6.83 (d, *J* = 5.6 Hz, 1H, 3-H); MS (ESI), *m/z* = 474 [$M+H^+$]; IR (ATR): 3419, 3094, 3034, 2915, 1883, 1587, 1507, 1450 cm^{-1}. Anal. ($C_{17}H_{11}BrClN_3$) Calc. C 54.79, H 2.98, Cl 9.51, N 11.16; Found C 54.71, H 2.83, Cl 9.45, N 11.07.

1.5.4. 6-Bromo-N-(3-nitrophenyl)-9H-pyrido[2,3-b]indol-4-amine ***14d***

Yield 0.06 g (17%); yellow solid; mp 211-218 °C; ^1H NMR (DMSO-D_6) δ 11.09 br s, 1H, 9-*N*H), 8.44 (s, 1H, aniline *N*H), 8.27 (d, *J* = 1.1 Hz, 1H, 5-H), 8.19 (d, *J* = 5.3 Hz, 1H, 2-H), 7.95 (d, *J* = 8.0 Hz, 1H, 4´-H), 7.81 (d, *J* = 8.4 Hz, 1H, 8-H), 7.66 (t, *J* = 8.0 Hz, 1H, 5´-H), 7.55-7.46 (m, 2H, 7-H, 6´-H), 7.04 (d, *J* = 5.3 Hz, 1H, 3-H), 7.02 (s, 1H, 2´-H); MS (EI), *m/z* = 383 [M^+]; IR (ATR): 3420, 3093. 3030, 2921, 1898, 1594, 1522, 1451 cm^{-1}. Anal. ($C_{17}H_{11}BrN_3O_2$) Calc. C 53.28, H 2.89, N 14.62; Found C 53.22, H 2.75, N 14.32.

1.6. Formation of the 6-acetyl 4-chloropyrido[2,3-b]indole ***15***

3.0 g (14.8 mmol) of compound **4** and 8.9 g (66.6 mmol) of aluminium chloride were suspended in 75 mL of dry dichloromethane. The mixture was cooled down and 2.1 mL (2.32 g, 29.6 mmol) of acetyl chloride were added dropwise under stirring. Then the mixture was heated for 4 h under argon atmosphere and reflux conditions. After cooling to rt and then on an ice bath 120 mL of water were added dropwise under further stirring for 15 min at rt. The formed precipitate was filtered off, washed with water and dried overnight under air conditions.

*1.6.1. 1-(4-Chloro-9H-pyrido[2,3-b]indol-6-yl)ethanone **15***

Yield 3.12 g (86%); light yellow solid; mp 297-302 °C; ^{1}H NMR (DMSO-D_6) δ 12.58, s, 1H, 9-*N*H), 8.88 (d, *J* = 1.4 Hz, 1H, 5-H), 8.42 (d, *J* = 5.3 Hz, 1H, 2-H), 8.13 (dd, *J* = 8.6, 1.4 Hz, 1H, 7-H), 7.60 (d, *J* = 8.6 Hz, 1H, 8-H), 7.38 (d, *J* = 5.3 Hz, 1H, 3-H), 2.65 (s, 3H, $COCH_3$); MS (EI), *m/z* = 244 [M^+]; IR (KBr): 3435. 3209, 3124, 3004, 2959, 2836, 2769, 1671, 1574, 1299, 1218 cm^{-1}. Anal. ($C_{13}H_9ClN_2O$) Calc. C 63.82, H 3,71, N 11.45; Found C 63.57, H 3.45, N 11.38.

*1.7. General procedure for the formation of the 4-anilino substituted 6-acetyl compound series **16a-c***

The procedure followed the above described synthesis for the compound series **5** using 0.25 g of the 6-acetyl 4-chloro derivative **15** (1.02 mmol, 1 eq.) and the tenfold amount (10.2 mmol, 10 eq.) of the respective aniline.

*1.7.1. 1-(4-(3-Hydroxyphenylamino)-9H-pyrido[2,3-b]indol-6-yl)ethanone **16a***

Yield 0.14 g (42%); dark orange solid; mp 250-260 °C; ^{1}H NMR (acetone-D_6) δ 11.15 (br s, 1H, 9-*N*H), 8.49 (d, *J* = 1.1 Hz, 1H, aniline *N*H), 8.06 (d, *J* = 5.7 Hz, 1H, 2-H), 7.99 (s, 1H, 5-H), 7.92 (d, *J* = 8.5, 1H, 7-H), 7.46 (d, *J* = 8.5 Hz, 1H, 8-H), 7.11-7.07 (m, 1H, 5´-H), 6.80 (d, *J* = 5.7 Hz, 1H, 3-H), 6.71-6-69 (m, 3H, 4´-, 6´-H, OH), 6.50 (d, *J* = 1.1 Hz, 1H, 2´-H), 2.41 (s, 3H, $COCH_3$); MS (ESI), *m/z* = 318 [M+H^+]; IR (ATR): 3217, 3144, 2923, 2851, 2764, 1652, 1583, 1300, 1217 cm^{-1}. Anal. ($C_{19}H_{15}N_3O_2$) Calc. C 71.91, H 4.76, N 13.24; Found C 71.75, H 4.84, N 13.17.

1.7.2. 1-(4-(3-Nitrophenylamino)-9H-pyrido[2,3-b]indol-6-yl)ethanone ***16b***

Yield 0.14 g (39%); yellow brownish solid; mp 290-300 °C; ^{1}H NMR (acetone-D_6) δ 12.19 (s, 1H, 9-*N*H), 9.33 (s, 1H, aniline *N*H), 8.53 (d, *J* = 0.8 Hz, 1H, 5-H), 8.24 (d, *J* = 5.6 Hz, 1H, 2-H), 8.06-8.01 (m, 2H, 8-, 4´-H), 7.84 (dd, *J* = 8.0, 0.8 Hz, 1H, 7-H), 7.67 (d, *J* = 1.1 Hz, 1H, 2´-H), 7.58 (t, *J* = 8.0 Hz, 1H, 5´-H), 7.52 (d, *J* = 8.0 Hz, 1H, 6´-H), 6.99 (d, *J* = 5.5 Hz, 1H, 3-H), 2.67 (s, 3H, $COCH_3$); MS (ESI), *m/z* = 347 [M+H^+]; IR (ATR): 3393, 3293, 3095, 3038, 2956, 2851, 2783, 1657, 1565, 1299, 1238 cm^{-1}. Anal. ($C_{19}H_{14}N_4O_3$) Calc. C 65.89, H 4.07, N 16.18; Found C 65.67, H 4.23, N 16.08.

1.7.3. 1-(4-(3-Chlorophenylamino)-9H-pyrido[2,3-b]indol-6-yl)ethanone ***16c***

Yield 0.09 g (25%); dark yellow solid; mp 233-236 °C; ^{1}H NMR (DMSO-D_6) δ 11.39 (s, 1H, 9-*N*H), 8.85 (s, 1H, aniline *N*H), 8.33 (d, *J* = 7.6 Hz, 1H, 7-H), 8.23 (d, *J* = 5.6 Hz, 1H, 2-H), 8.09 (d, *J* = 7.6 Hz, 1H, 8-H), 7.37-7.25 (m, 4H, 4´-, 5´-, 6´-, 5-H), 7.07 (d, *J* = 1.9 Hz, 1H, 2´-H), 6.94 (d, *J* = 5.6 Hz, 1H, 3-H), 2.48 (s, 3H, $COCH_3$); MS (ESI), *m/z* = 336 [M+H^+]; IR (ATR): 3223, 3152, 2922, 2855, 2770, 1654, 1585, 1219 cm^{-1}. Anal. ($C_{19}H_{14}ClN_3O$) Calc. C 67.96, H 4.20, Cl 10.56, N 12.51; Found C 67.76, H 4.18, Cl 10.23, N 12.35.

*1.8. Formation of the 3-aminophenylamino compound **16d***

0.24 g (0.7 mmol) of derivative **16c** was suspended in 15 mL of a hydrochloric acid solution (10%). 0.8 g (4.21 mmol) of tin-II chloride were added and the reaction mixture was heated for 80 h under reflux. After no more starting compound **16c** was detectable by TLC the mixture was cooled and poured into 25 mL of water. The pH was adjusted to 12 using a potassium hydroxide solution in water (10 M). The water phase was extracted with 25 mL of ethyl acetate for five times and the unified organic layer was then dried over sodium sulfate. After filtration the solvent was removed in vacuum and compound **16d** remained.

*1.8.1. 1-(4-(3-Aminophenylamino)-9H-pyrido[2,3-b]indol-6-yl)ethanone **16d***

Yield 0.21 g (95%); dark brown solid; mp > 300 °C; ^{1}H NMR (DMSO-D_6) δ 11.99 (s, 1H, 9-*N*H), 8.60 (s, 1H, aniline *N*H), 8.55 (s, 1H, 5-H), 8.09 (d, *J* = 5.6 Hz, 1H, 2-H), 7.96 (d, *J* = 8.6 Hz, 1H, 7-H), 7.46 (d, *J* = 8.6 Hz, 1H, 8-H), 7.02 (t, *J* = 7.8 Hz, 1H, 5´-H), 6.75 (d, *J* = 5.6 Hz, 1H, 3-H), 6.51 (s, 1H, 2´-H), 6.43 (d, *J* = 7.8 Hz, 1H, 4´-H), 6.34 (d, *J* = 7.8 Hz, 1H, 6´-H), 5.11 (br s, 2H, NH_2), 2.31 (s, 3H, $COCH_3$); MS (ESI), *m/z* = 317 [M+H^+]; IR (ATR): 3476, 3310, 3084, 2959, 2923, 2852, 2688, 1643, 1578, 1306, 1248 cm^{-1}. Anal. ($C_{19}H_{16}N_4O$) Calc. C 72.14, H 5.10, N 17.71; Found C 71.85, H 4.96, N 17.65.

2. Protein kinase inhibition determination

The protein kinases were all expressed in baculovirus Sf9 insect cells as human recombinant GST fusion proteins and purified by affinity chromatography using GSH-agarose. The kinase identity was confirmed by mass spectrometry using LC-ESI-MS/MS technique.

2. 1. Assay conditions for inhibition determinations

The measuring of protein kinase activity was performed in 96-well FlashPlates^{TM} in a 50 µL reaction volume. The reaction mixture consisted of 20 µL of assay buffer solution, 5 µL of ATP solution in water, 5 µL of used test compound in a 10% dmso solution and finally a premixture of each 10 µL of used substrate and enzyme solutions. The assay buffer solution contained 70 mM of HEPES-NAOH, each 3 mM of magnesium chloride and manganese(II) chloride, 3 µM of sodium orthovanadate, 1.2 mM of DTT, 50 µg/mL of PEG20000 and finally 1 µM of [γ-^{33}P]-ATP making approximately 1.2 x 10^6 cpm per well.

The final kinase concentrations have been 6.1 nM for Brk and 5.3 nM for HER2. The used substrate was Poly(Glu,Tyr)$_{4:1}$ in an amount of 125 ng/50 µL. The final kinase concentrations in the proceeded screening studies have been 25 ng/50 µL for AKT1 and ALK , 200 ng/50 µL for AKT2, Aurora C and JAK3, 50 ng/50 µL for AKT3, Aurora A, PAK3 and VRK1, 20 ng/50 µL for PAK3, 10 ng/mL for EGFR and ERK2, 5 ng/50 µL for ERK1, JNK1 and 3 and 75 ng/50 µL for PAK1. The used substrates have been GSK3 (14-27) (AKT1-3) in concentrations of 1 µg/50 µL (AKT2, 3) and of 2 µg/50 µL (AKT1), Poly(Glu,Tyr)$_{4:1}$ in a concentration of 125 ng/50 µL (ALK, EGFR), tetra(LRRWSLG) in concentrations of 0.5 µg/50 µL for Aurora A and PAK3, of 0.25 µg/50 µL for Aurora C and PAK2 and of 1 µg/50 µL for PAK1, RBER-CHKtide in a concentration of 2 µg/50 µL for ERK1 and 2 and for VRK1, Poly(Ala,Glu,Lsy,Tyr)$_{6:2:5:1}$ in a concentration of 0.125 µg/50 µL for JAK3 and, finally, ATF2 for JNK1 and 3 in a concentration of 0.25 µg/50 µL.

The reaction mixtures were incubated at 30 °C for 60 min. The reaction was stopped with 50 µL of a 2% (v/v) solution of phosphoric acid. Then the plates were aspirated and washed twice with 200 µL of water or 0.9% solution of sodium chloride. The incorporation of ^{33}Pi was determined with a microplate scintillation counter. Ten different inhibitor concentrations were measured in a range of 3 nM to 100 µM. In the case of the lower nanomolar active

compound subnanomolar concentrations were additionally measured. The residual activity and the IC_{50} values were finally calculated.

3. Inhibition of STAT3 phosphorylation in cells

3.1. Cell culture conditions

The T47D cell line was obtained from DSMZ (Braunschweig, Germany) and cultivated in RPMI 1640 medium supplemented with 10% fetal calf serum at 37°C in a humidified 5% carbon dioxide atmosphere. For suspension growth, cultivation plates were coated with a 5 mg/ml solution of poly(2-hydroxy-ethyl-methacrylic acid) (polyHEMA) in 96% ethanol by evaporation in a 37°C air-tight environment over three days. Cells were plated in the appropriate cellular density and cultured in medium described above. For incubation experiments stock solutions of **5b** were prepared in DMSO in a concentration of 10 mM and working solutions were diluted thereof with culture medium resulting in final incubation solutions containing not more than 0.1% DMSO.

3.2. Expression of proteins STAT3 and pSTAT3

After cultivation under respective culture conditions cells were washed with ice-cold PBS, lysed using standard lysis buffer containing 50 mM Tris pH 7.6, 100 mM NaCl, 5 mM EDTA, 0.2 mM sodium vanadate, 0.1% triton X-100 and 1 µl protease inhibitor cocktail per ml lysis buffer (Sigma-Aldrich, Deisenhofen), and protein content was assessed by a modified Bradford assay (Roti(R)-Nanoquant, Carl Roth GmbH, Karlsruhe). Proteins were separated on 10% SDS-polyacrylamide gels transferred to nitrocellulose membranes. Specific protein bands were visualized by incubating membranes with primary antibodies against STAT3, and

pSTAT3 (Cell Signaling Technologies, Danvers, MA, USA, 1:500 dilution) and a secondary horseradish peroxidase-conjugated anti-rabbit IgG antibody (Cell Signaling Technologies, Danvers, MA, USA, 1:3000 dilution). Chemoluminescence was generated using Immun-StarTM WesternCTM Kit (Bio Rad Laboratories, München) and detected by ChemoCam Imaging System (Intas, Göttingen).

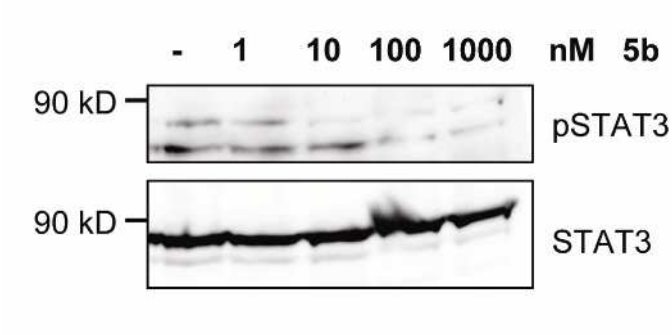


Figure S1. T47D cells were plated in 6-well plates at 10^5 cells/well and treated with depicted concentrations of **5b**. Phosphorylation of STAT3 (pSTAT3) was detected by Western Blot analysis as described.

4. Docking results

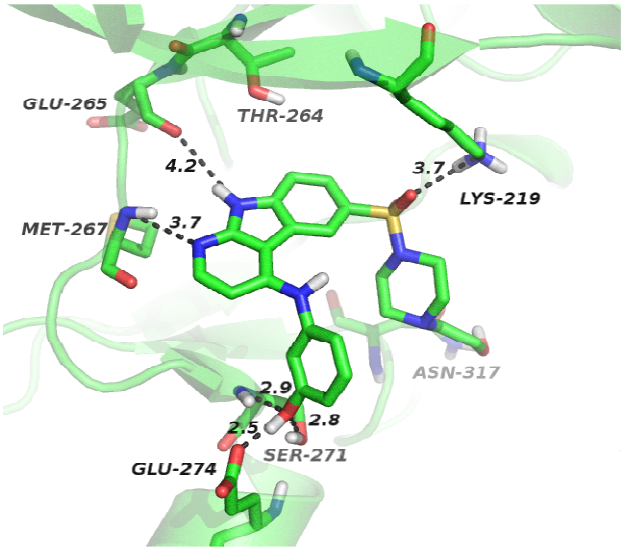
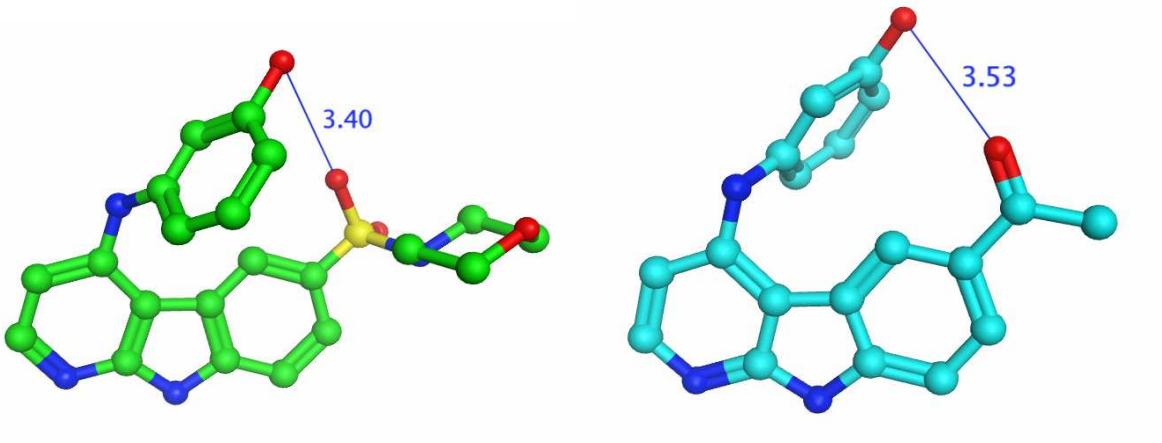


Figure S2. Docking solution for the Brk-inactive compound **12c** (green sticks). An inverted binding mode is observed. Distances are given in Å.



(a) (b)

Figure S3. Intermolecular hydrogen bond observed for the lowest energy conformation of inhibitor a) **10a** and b) **16a**. The hydrogen bonds are shown as blue lines with distances given in Å.