SUPPLEMENTING INFORMATION

Quinolone amides as antitrypanosomal lead compounds with *in vivo* activity

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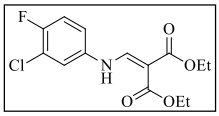
Content:

Syntheses

The compounds A, B, C, D, GHQ168, H, I, J, P, Q, R have already been published and can be found in refs 1, 2, 3, 4, 5, 6, 7, and 8.

Synthesis of *N*-Benzyl-1-butyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (GHQ168)

Synthesis of Diethyl 2-(((3-chloro-4-fluorophenyl)amino)methylene)malonate (A) (1)

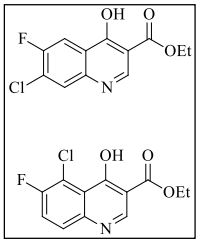


A solution of 5.00 g (34.4 mmol) 3-chloro-4-fluoroaniline, 8.91 g (41.2 mmol, 8.33 ml) diethyl ethoxymethylenemalonate and 5 Weflon®-discs were heated to 110 °C under microwave irradiation (heat-up rate: in 3 min to 110 °C, 800 W). After 1 h at 110 °C the solvent was removed under reduced pressure and the remaining oil was dissolved in 50 ml *n*-hexane. After storing the solution at -20 °C overnight the white precipitate was filtered off, washed with cold *n*-hexane and dried in vacuo.

Yield: 10.8 g (34.2 mmol, 99 %), mp: 60 - 62 °C (*n*-hexane).

Spectroscopical data were consistent with literature [1].

Synthesis of Ethyl 7-chloro-6-fluoro-4-hydroxyquinoline-3-carboxylate (B) (1)

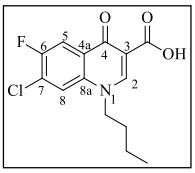


10.0 g (31.7 mmol) of diethyl 2-(((3-chloro-4-fluorophenyl)-amino)methylene)malonate (A) were dissolved in 5 ml Ph_2O. After addition of 3 Weflon®-discs the mixture was heated to 210 °C for 15 min under microwave irradiation (heat-up rate: in 5 min to 210 °C, 800 W). The resulting suspension was cooled to rt, diluted with 100 ml Et_2O and stirred for 1 h. The light grey solid was filtered off, washed with Et_2O and dried in vacuo to give the desired regioisomer in a 10:1 ratio.

Yield: 5.26 g (19.5 mmol, 62 %), mp: 315 - 318 °C (Ph_2O).

Spectroscopical data were consistent with literature (1).

Synthesis of 1-Butyl-7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (C) (2)

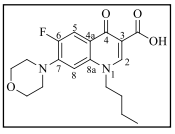


1.00 g (3.71 mmol) ethyl 7-chloro-6-fluoro-4-hydroxyquinoline-3-carboxylate (B) and 2.56 g (18.5 mmol) K_2CO_3 were suspended in 20 ml anhydrous DMF and heated to 60 °C for 1 h. 3.05 g (22.3 mmol, 2.38 ml) 1-bromobutane and a catalytic amount of KI were added and the mixture was stirred at 100 °C for 24 h. The solvent was removed in vacuo and the residue was partitioned between 60 ml water and 50 ml EA. After separation the aqueous layer was extracted with EA (3 x 50 ml). The combined organic layers were dried over Na_2SO_4, concentrated in vacuo and the crude ester was purified by normal phase column chromatography on silica gel ($CHCl_3$/*i*PrOH 150:1, R_f: 0.33). The resulting white solid was suspended in 45 ml of aqueous KOH-solution (3 M) and 5 ml EtOH and refluxed for 20 h. After cooling to rt the clear solution was acidified (pH = 3 - 4) with aqueous HCl-solution (2 M). The white precipitate was filtered off and recrystallized from EtOH.

Yield: 960 mg (3.23 mmol, 87 %), mp: 233 - 235 (EtOH).

Spectroscopical data were consistent with literature (2).

Synthesis of 1-Butyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (D) (2)

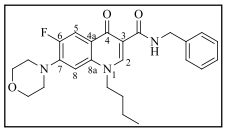


200 mg (672 µmol) 1-butyl-7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (C) were dissolved in 10 ml morpholine and heated to 110 °C for 4 h under microwave irradiation (heat-up rate: in 4 min to 110 °C, 600 W). The mixture was diluted with 30 ml water and acidified (pH = 5) with aqueous HCl-solution (2 M). The precipitate was collected by filtration and recrystallized form EtOH/$CHCl_3$ to give a light yellow solid.

Yield: 160 mg (459 µmol, 68 %), mp: 242 - 243 °C (EtOH/$CHCl_3$).

Spectroscopical data were consistent with literature [2].

Synthesis of N-Benzyl-1-butyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (GHQ168) (2)



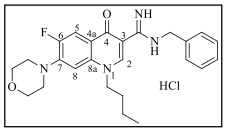
To a cooled solution (0 °C) of 150 mg (431 µmol) 1-butyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (D) and 218 mg (2.15 mmol, 237 µl) NMM in 10 ml anhydrous DMF 235 mg (1.72 mmol, 223 µl) isobutyl chloroformate were added dropwise under argon atmosphere. After 1 h at 0 °C

185 mg (2.90 mmol, 188 µl) benzylamine were added and stirring at rt was continued for 45 min. The solvent was removed in vacuo and the crude product was purified via normal phase column chromatography on silica gel ($CHCl_3$/MeOH 100:1, R_f: 0.79) followed by recrystallization from EA.

Yield: 135 mg (309 µmol, 72 %), mp: 157 - 159 (EA).

Spectroscopical data were consistent with literature (2).

Synthesis of *N-Benzyl-1-butyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboximidamide hydrochloride (GHQ232)*



200 mg (457 µmol) *N*-Benzyl-1-butyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (GHQ168) were dissolved in 4 ml $POCl_3$ and warmed to 40 °C for 24 h. The solution was diluted with 20 ml toluene and concentrated to dryness in vacuo. The residue was dissolved in 10 ml anhydrous ACN under argon atmosphere and cooled to 0 °C. A separately prepared solution of 49 mg (914 µmol) NH_4Cl and 118 mg (914 µmol, 159 µl) DIPEA dissolved in 5 ml anhydrous ACN, which was stirred at rt for 30 min under argon atmosphere, was added dropwise and the combined mixture was stirred at 0 °C for 3 h. After dilution with 20 ml water the pH value was adjusted to 8 - 9 using conc. NH_3-solution and the aqueous mixture was extracted with DCM (4 x 30 ml). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by means of normal phase column chromatography on silica gel ($CHCl_3$/MeOH/conc. NH_3 100:10:1, R_f: 0.31). The obtained solid was dissolved in 10 ml THF and treated with 1 ml of isopropanolic HCl-solution in order to achieve the corresponding hydrochloride salt. The resulting precipitate was filtered off and recrystallized from ACN/MeOH to give the desired product as a light yellow solid.

Yield: 56 mg (118 µmol, 26 %), mp: 311 - 312 °C (ACN/MeOH),

IR (ATR, $\tilde{\nu}$ [cm^{-1}]): 3223 (w), 3113 (w), 3060 (w), 2965 (w), 2872 (w), 1644 (s), 1626 (m), 1557 (m), 1509 (s), 1449 (m), 1370 (m), 1255 (s), 1118 (m), 929 (m), 823 (m), 737 (w), 694 (m).

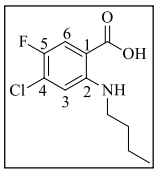
^{1}H-NMR (DMSO-d_6, δ [ppm], J [Hz]): 9.93 (s, 1H, CN**H**$_2$NHR$^+$), 9.67 (t, 1H, ^3J = 5.6, CNH$_2$N**H**R$^+$), 9.47 (s, 1H, CN**H**$_2$NHR$^+$), 9.34 (s, 1H, **H**-2), 8.56 (d, 1H, $^3J_{H,F}$ = 15.0, **H**-5), 7.42-7.25 (m, 5H, benzyl-C**H**$_{arom.}$), 7.20 (d, 1H, $^4J_{H,F}$ = 7.7, **H**-8), 4.59 (t, 2H, ^3J = 7.4, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 4.51 (d, 2H, ^3J = 5.6, benzyl-C**H**$_2$), 3.82-3.80 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.41-3.38 (m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 1.86 (quint, 2H, ^3J = 7.4,

$NCH_2C\mathbf{H}_2CH_2CH_3$), 1.41 (sext, 2H, $^3J = 7.4$, $NCH_2CH_2C\mathbf{H}_2CH_3$), 0.95 (t, 3H, $^3J = 7.4$, $NCH_2CH_2CH_2C\mathbf{H}_3$).

^{13}C-NMR (DMSO-d_6, δ [ppm], J [Hz]): 165.3 (1C, $\mathbf{C}NH_2NHR^+$), 155.7 (d, 1C, $^4J_{\mathbf{C,F}} = 2.7$, **C**-4), 152.8 (d, 1C, $^1J_{\mathbf{C,F}} = 251.7$, **C**-6), 146.4 (1C, **C**-2), 145.6 (d, 1C, $^2J_{\mathbf{C,F}} = 10.0$, **C**-7), 138.8 (1C, benzyl-$\mathbf{C}_q$), 135.7 (1C, **C**-8a), 128.3 (2C, benzyl-$\mathbf{C}H_{arom.}$), 127.5 (2C, benzyl-$\mathbf{C}H_{arom.}$), 126.9 (1C, benzyl-$\mathbf{C}H_{arom.}$), 111.6 (d, 1C, $^3J_{\mathbf{C,F}} = 9.6$, **C**-4a), 111.4 (d, 1C, $^2J_{\mathbf{C,F}} = 25.4$, **C**-5), 105.0 (d, 1C, $^3J_{\mathbf{C,F}} = 4.6$, **C**-8), 103.0 (1C, **C**-3), 65.7 (2C, morpholino-$\mathbf{C}H_2$-O-$\mathbf{C}H_2$), 54.2 (1C, $N\mathbf{C}H_2CH_2CH_2CH_3$), 49.5 (2C, $^4J_{\mathbf{C,F}} = 4.6$, morpholino-$\mathbf{C}H_2$-N-$\mathbf{C}H_2$), 42.5 (1C, benzyl-$\mathbf{C}H_2$), 30.1 (1C, $NCH_2\mathbf{C}H_2CH_2CH_3$), 19.1 (1C, $NCH_2CH_2\mathbf{C}H_2CH_3$), 13.4 (1C, $NCH_2CH_2CH_2\mathbf{C}H_3$).

Synthesis of Sodium 3-(benzylcarbamoyl)-1-butyl-6-fluoro-7-morpholino-2-oxo-1,2-dihydroquinoline-4-olate (GHQ215)

Synthesis of 2-(Butylamino)-4-chloro-5-fluorobenzoic acid (E)



2.00 g (9.57 mmol) 2,4-Dichloro-5-fluorobenzoic acid, 1.32 g (9.57 mmol) K_2CO_3 and 608 mg (9.57 mmol) cupper powder were suspended in 20 ml anhydrous DMF under argon atmosphere and stirred at rt for 10 min. After addition of 1.40 g (19.1 mmol, 1.89 ml) *n*-butylamine the mixture was heated to 80 °C for 5 h. The solvent was removed in vacuo and the residue was dissolved in 50 ml water. After the pH value was adjusted to 4 using aqueous HCL-solution (2 M) the suspension was extracted with DCM (4 x 50 ml). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by normal phase column chromatography on silica gel ($CHCl_3$/FA 100:1, R_f: 0.65) to give the desired product as light yellow solid.

Yield: 1.74 g (7.08 mmol, 74 %), mp: 138 - 139 °C ($CHCl_3$/FA).

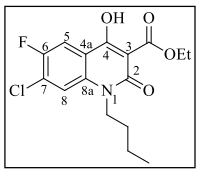
IR (ATR), $\tilde{\nu}$ [cm^{-1}]: 3380 (m), 2954 (m), 2929 (w), 2869 (m), 1660 (s), 1570 (s), 1510 (s), 1478 (m), 1406 (m), 1223 (s), 1191 (s), 997 (m), 840 (m).

^{1}H-NMR (DMSO-d_6, δ [ppm], J [Hz]): 11.7 (s, 1H, COO**H**), 7.74 (d, 1H, $^3J_{\mathbf{H,F}} = 9.9$, **H**-6), 7.45 (s, 1H, N**H**), 6.71 (d, 1H, $^4J_{\mathbf{H,F}} = 6.1$, **H**-3), 3.18 (t, 2H, $^3J = 7.3$, $NC\mathbf{H}_2CH_2CH_2CH_3$), 1.71 (quint, 2H, $^3J = 7.3$, $NCH_2C\mathbf{H}_2CH_2CH_3$), 1.49 (sext, 2H, $^3J = 7.3$, $NCH_2CH_2C\mathbf{H}_2CH_3$), 1.02 (t, 3H, $^3J = 7.3$, $NCH_2CH_2CH_2C\mathbf{H}_3$).

^{13}C-NMR (DMSO-d_6, δ [ppm], J [Hz]): 172.6 (d, 1C, $^4J_{\mathbf{C,F}} = 2.2$, **C**OOH), 148.9 (1C, **C**-2), 148.1 (d, 1C, $^1J_{\mathbf{C,F}} = 236.3$, **C**-5), 129.6 (d, 1C, $^2J_{\mathbf{C,F}} = 18.9$, **C**-4), 118.6 (d, 1C, $^2J_{\mathbf{C,F}} = 22.8$, **C**-

6), 112.7 (1C, **C**-3), 106.8 (d, 1C, $^{3}J_{\mathbf{C,F}}$ = 5.3, **C**-1), 43.0 (1C, N**C**$H_2CH_2CH_2CH_3$), 31.0 (1C, NCH_2**C**$H_2CH_2CH_3$), 20.3 (1C, NCH_2CH_2**C**H_2CH_3), 13.8 (1C, $NCH_2CH_2CH_2$**C**H_3).

Synthesis of Ethyl 1-butyl-7-chloro-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylate (F)



To a cooled solution (0 °C) of 628 mg (2.12 mmol) triphosgene in 10 ml anhydrous $CHCl_3$, 400 mg (1.63 mmol) 2-(butylamino)-4-chlor-5-fluorobenzoic acid (E) dissolved in 5 ml anhydrous $CHCl_3$ were added dropwise under argon atmosphere. After 20 h of stirring at rt 5 ml sat. $NaHCO_3$-solution were added carefully and the biphasic system was stirred at rt for further 5 min. The organic layer was separated and the aqueous layer was extracted with $CHCl_3$ (2 x 30 ml). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo to give the crude isatoic anhydrid derivative. Under argon atmosphere 98 mg (2.45 mmol) sodium hydride (60 % dispersion in mineral oil) were suspended in 15 ml anhydrous DMF followed by addition of 522 mg (3.26 mmol, 493 µl) diethyl malonate. After stirring for 15 min at rt the mixture was cooled to 0 °C and the crude isatoic anhydride dissolved in 5 ml anhydrous DMF was added dropwise. After complete addition the solution was stirred at rt for 16 h followed by evaporation under reduced pressure. The residue was dissolved in 20 ml water, the pH value was adjusted to 4 using aqueous HCl-solution (2 M) and the resulting precipitate was collected via filtration. The crude product was purified via normal phase column chromatography on silica gel ($CHCl_3$, R_f: 0.94) to give a white solid.

Yield: 310 mg (907 µmol, 56 %), mp: 119 - 120 °C ($CHCl_3$).

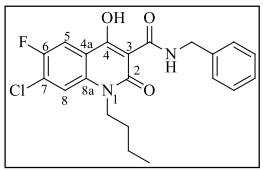
IR (ATR), $\tilde{\nu}$ [cm^{-1}]: 3079 (w), 2965 (m), 2934 (m), 2874 (w), 1666 (s), 1629 (s), 1593 (m), 1555 (m), 1500 (s), 1467 (m), 1410 (m), 1318 (s), 1207 (s), 1042 (m), 898 (m), 807 (m).

^{1}H-NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 14.2 (s, 1H, O**H**), 7.90 (d, 1H, $^{3}J_{\mathbf{H,F}}$ = 8.9, **H**-5), 7.31 (d, 1H, $^{4}J_{\mathbf{H,F}}$ = 5.9, **H**-8), 4.50 (q, 2H, ^{3}J = 7.1, OC**H**$_2CH_3$), 4.14 (t, 2H, ^{3}J = 7.5, NC**H**$_2CH_2CH_2CH_3$), 1.67 (quint, 2H, ^{3}J = 7.5, NCH_2C**H**$_2CH_2CH_3$), 1.49 (sext, 2H, ^{3}J = 7.5, NCH_2CH_2C**H**$_2CH_3$), 1.47 (t, 3H, ^{3}J = 7.1, OCH_2C**H**$_3$), 1.00 (t, 3H, ^{3}J = 7.5, $NCH_2CH_2CH_2$C**H**$_3$).

^{13}C-NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 172.9 (1C, **C**O_2Et), 170.6 (d, 1C, $^{4}J_{\mathbf{C,F}}$ = 2.8, **C**-4), 159.2 (1C, **C**-2), 153.5 (d, 1C, $^{1}J_{\mathbf{C,F}}$ = 245.8, **C**-6), 137.9 (d, 1C, $^{4}J_{\mathbf{C,F}}$ = 1.8, **C**-8a), 128.9 (d, 1C, $^{2}J_{\mathbf{C,F}}$ = 19.6, **C**-7), 116.6 (1C, **C**-8), 114.9 (d, 1C, $^{3}J_{\mathbf{C,F}}$ = 7.3, **C**-4a), 112.6 (d, 1C, $^{2}J_{\mathbf{C,F}}$ = 23.6, **C**-5), 99.1 (1C, **C**-3), 63.1 (1C, O**C**H_2CH_3), 43.1 (1C, N**C**$H_2CH_2CH_2CH_3$), 29.9 (1C,

$NCH_2\mathbf{C}H_2CH_2CH_3$), 20.7 (1C, $NCH_2CH_2\mathbf{C}H_2CH_3$), 14.7 (1C, $OCH_2\mathbf{C}H_3$), 14.2 (1C, $NCH_2CH_2CH_2\mathbf{C}H_3$).

Synthesis of N-Benzyl-1-butyl-7-chloro-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxamide (G)



To a solution of 280 mg (819 µmol) ethyl 1-butyl-7-chloro-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylate (F) in 100 ml toluene 220 mg (2.05 mmol, 223 µl) benzylamine were added and the mixture was refluxed for 1 h. Within that time 3 x 10 ml toluene were distilled of (Dean-Stark-apparatus) and replaced by an equal amount of fresh toluene. Afterwards the solvent was concentrated to a volume of about 5 ml. Upon cooling to rt the desired product crystallized as white solid which was dried in vacuo.

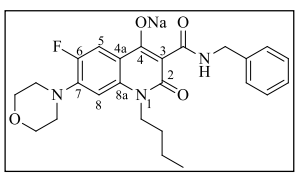
Yield: 264 mg (655 µmol, 80 %), mp: 111 - 112 °C (toluene).

IR (ATR), $\tilde{\nu}$ [cm^{-1}]: 3210 (w), 2957 (m), 2925 (w), 2867 (w), 1638 (s), 1585 (m), 1542 (s), 1498 (s), 1457 (m), 1397 (m), 1335 (m), 1218 (m), 1031 (m), 885 (m), 801 (m), 693 (m).

^{1}H-NMR (DMSO-d_6, δ [ppm], J [Hz]): 10.6 (t, 1H, ^3J = 6.0, CON**H**R), 7.87 (d, 1H, $^4J_{\mathbf{H,F}}$ = 4.8, **H**-8), 7.85 (d, 1H, $^3J_{\mathbf{H,F}}$ = 8.2, **H**-5), 7.37-726 (m, 5H, benzyl-C$\mathbf{H}_{arom.}$), 4.58 (d, 2H, ^3J = 6.0, benzyl-C$\mathbf{H}_2$), 4.17 (t, 2H, ^3J = 7.3, $NC\mathbf{H}_2CH_2CH_2CH_3$), 1.54 (quint, 2H, ^3J = 7.3, $NCH_2C\mathbf{H}_2CH_2CH_3$), 1.37 (sext, 2H, ^3J = 7.3, $NCH_2CH_2C\mathbf{H}_2CH_3$), 0.91 (t, 3H, ^3J = 7.3, $NCH_2CH_2CH_2C\mathbf{H}_3$).

^{13}C-NMR (DMSO-d_6, δ [ppm], J [Hz]): 170.1 (1C, **C**ONHR), 169.8 (d, 1C, $^4J_{\mathbf{C,F}}$ = 2.5, **C**-4), 161.0 (1C, **C**-2), 152.3 (d, 1C, $^1J_{\mathbf{C,F}}$ = 244.8, **C**-6), 137.8 (1C, benzyl-$\mathbf{C}_q$), 135.9 (d, 1C, $^4J_{\mathbf{C,F}}$ = 1.3, **C**-8a), 128.5 (2C, benzyl-$\mathbf{C}H_{arom.}$), 127.5 (2C, benzyl-$\mathbf{C}H_{arom.}$), 127.2 (1C, benzyl-$\mathbf{C}H_{arom.}$), 126.6 (d, 1C, $^2J_{\mathbf{C,F}}$ = 19.8, **C**-7), 117.4 (1C, **C**-8), 115.0 (d, 1C, $^3J_{\mathbf{C,F}}$ = 6.8, **C**-4a), 110.9 (d, 1C, $^2J_{\mathbf{C,F}}$ = 22.7, **C**-5), 96.4 (1C, **C**-3), 42.3 (1C, benzyl-$\mathbf{C}H_2$), 41.7 (1C, $N\mathbf{C}H_2CH_2CH_2CH_3$), 29.2 (1C, $NCH_2\mathbf{C}H_2CH_2CH_3$), 19.4 (1C, $NCH_2CH_2\mathbf{C}H_2CH_3$), 13.6 (1C, $NCH_2CH_2CH_2\mathbf{C}H_3$).

Synthesis of Sodium 3-(benzylcarbamoyl)-1-butyl-6-fluoro-7-morpholino-2-oxo-1,2-dihydroquinoline-4-olate (GHQ215)



420 mg (1.04 mmol) *N*-benzyl-1-butyl-7-chloro-6-fluoro-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxamide (G) were dissolved in 15 ml

morpholine and heated to 110 °C for 4 h under microwave irradiation (heat-up rate: in 3 min to 110 °C, 600 W). The solution was diluted with 50 ml water and the pH value was adjusted to 6 using aqueous HCl-solution (2 M). The aqueous solution was extracted with EA (4 x 50 ml), the combined organic layers were dried over Na_2SO_4 and the solvent was evaporated under reduced pressure followed by purified via normal phase column chromatography on silica gel (PE/EA 3:1, R_f: 0.57). In order to achieve the corresponding sodium salt the isolated white solid was suspended in 10 ml EtOH and treated with 114 µl (1.14 mmol) aqueous NaOH-solution (10 M). After temporary dissolution the desired sodium salt started to precipitate. After 15 min of stirring at rt the precipitate was filtered off, washed with a mixture of water and EtOH (1:1) and dried in vacuo to give a white solid.

Yield: 112 mg (236 µmol, 23 %), mp: 317 - 318 °C (water).

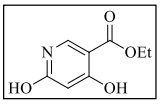
IR (ATR), $\tilde{\nu}$ [cm^{-1}]: 2956 (w), 2852 (w), 1617 (s), 1573 (m), 1496 (s), 1455 (m), 1406 (m), 1259 (m), 1122 (m), 909 (m), 807 (m), 697 (s).

^{1}H-NMR (DMSO, δ [ppm], *J* [Hz]): 11.5 (t, 1H, ^3J = 5.4, CON**H**R), 7.68 (d, 1H, $^3J_{\mathbf{H,F}}$ = 13.9, **H**-5), 7.32-7.21 (m, 5H, benzyl-C**H**$_{arom.}$), 6.64 (d, 1H, $^4J_{\mathbf{H,F}}$ = 7.0, **H**-8), 4.46 (d, 2H, ^3J = 5.4, benzyl-C**H**$_2$), 4.10 (br, 2H, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.77 (br, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.12 (br, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 1.56 (quint, 2H, ^3J = 7.2, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.37 (sext, 2H, ^3J = 7.2, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.95 (t, 3H, ^3J = 7.2, NCH$_2$CH$_2$CH$_2$C**H**$_3$).

^{13}C-NMR (DMSO, δ [ppm], *J* [Hz]): 173.7 (d, 1C, $^4J_{\mathbf{C,F}}$ = 1.5, **C**-4), 169.1 (1C, **C**ONHR), 164.0 (1C, **C**-2), 149.6 (d, 1C, $^1J_{\mathbf{C,F}}$ = 239.9, **C**-6), 141.9 (d, 1C, $^2J_{\mathbf{C,F}}$ = 9.6, **C**-7), 140.8 (1C, benzyl-**C**$_q$), 136.0 (1C, **C**-8a), 128.1 (2C, benzyl-**C**H$_{arom.}$), 127.2 (2C, benzyl-**C**H$_{arom.}$), 126.3 (1C, benzyl-**C**H$_{arom.}$), 117.4 (d, 1C, $^3J_{\mathbf{C,F}}$ = 7.2, **C**-4a), 111.9 (d, 1C, $^2J_{\mathbf{C,F}}$ = 20.5, **C**-5), 102.8 (1C, **C**-8), 97.7 (1C, **C**-3), 66.0 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 50.1 (2C, $^4J_{\mathbf{C,F}}$ = 3.5, morpholino-**C**H$_2$-N-**C**H$_2$), 41.6 (1C, benzyl-**C**H$_2$), 40.2 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 22.3 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.7 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.7 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$).

Synthesis of *N*-Benzyl-1-butyl-7-morpholino-4-oxo-1,4-dihydro-1,6-naphthyridine-3-carboxamide oxalate (GHQ237)

Synthesis of Ethyl 4,6-dihydroxynicotinate (H) (3)



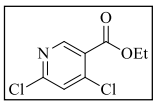
7.00 g (34.6 mmol) Diethyl 1,3-acetonedicarboxylate and 5.13 g (34.6 mmol,

5.70 ml) triethyl orthoformate were dissolved in 7.07 g (69.2 mmol, 6.55 ml) acetic anhydride and heated to reflux for 3 h. Excess of acetic anhydride was removed in vacuo and the residue was dissolved in 15 ml conc. NH_3-solution. The mixture was stirred at rt for 15 h followed by acidification (pH = 2) with aqueous HCl-solution (2 M). The yellow precipitate was filtered off, washed with water and dried in vacuo.

Yield: 4.68 g (26.5 mmol, 77 %), mp: 214 - 215 °C (water).

Spectroscopical data were consistent with literature (3).

Synthesis of Ethyl 4,6-dichloronicotinate (I) (4)

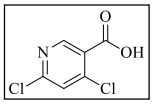


2.50 g (13.7 mmol) ethyl 4,6-dihydroxynicotinate (H) were dissolved in 21.0 g (0.14 mol, 12.6 ml) $POCl_3$ and heated to 80 °C for 24 h. After cooling to rt the dark brown solution was poured into 150 g ice and the aqueous mixture was extracted with EA (4 x 40 ml). The combined organic layers were washed with 50 ml Na_2CO_3-solution (10 %) and 50 ml brine. After drying over Na_2SO_4 the solvent was evaporated under reduced pressure and the crude product was purified via normal phase column chromatography on silica gel ($CHCl_3$/PE 2:1) to give a light yellow oil.

Yield: 2.14 g (9.73 mmol, 71 %).

Spectroscopical data were consistent with literature (4).

Synthesis of 4,6-dichloronicotinic acid (J) (5)

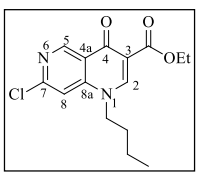


A solution of 441 mg (10.5 mmol) LiOH dissolved in 15 ml water was added dropwise to 1.93 g (8.77 mmol) ethyl 4,6-dichloronicotinate (I) dissolved in 15 ml THF. The mixture was stirred at rt for 15 h followed by evaporation of THF under reduced pressure. The remaining aqueous solution was acidified (pH = 3) using aqueous HCl-solution (2 M) and the resulting precipitate was collected by filtration. The solid was washed with water and dried in vacuo to give the desired product as a white solid.

Yield: 1.28 g (6.67 mmol, 76 %), mp: 151 - 152 (water).

Spectroscopical data were consistent with literature (5).

Synthesis of Ethyl 1-butyl-7-chloro-4-oxo-1,4-dihydro-1,6-naphthyridine-3-carboxylate (K)



900 mg (4.69 mmol) 4,6-dichloronicotinic acid (J) were dissolved in 15 ml $SOCl_2$ and heated to 80 °C for 2 h. Afterwards excess of $SOCl_2$ was distilled off

under reduced pressure and the residue was dissolved in 10 ml anhydrous toluene under argon atmosphere. 1.21 g (9.38 mmol, 1.63 ml) DIPEA and 1.01 g (7.03 mmol) Ethyl 3-(*N*,*N*-dimethylamino)acrylate were added and the mixture was heated to 90 °C for 2 h. After removing the solvent in vacuo the acrylate intermediate was purified by normal phase column chromatography on silica gel (EA/PE 2:1). The intermediate was dissolved in 40 ml EtOH and 20 ml Et_2O and 398 mg (5.45 mmol, 538 µl) *n*-butylamine were added. After 10 min of stirring at rt the mixture was evaporated to dryness under reduced pressure. The crude mixture was dissolved in 10 ml anhydrous DMF under argon atmosphere, 1.00 g (7.26 mmol) K_2CO_3 were added and the suspension was heated to 100 °C for 1 h. The solvent was removed in vacuo and the residue was partitioned between 50 ml water and 40 ml DCM. The organic layer was separated and the aqueous layer was extracted with DCM (3 x 40 ml). The combined organic layers were dried over Na_2SO_4, concentrated under reduced pressure and the resulting light brown solid was recrystallized from EA to give the desired product as a light yellow solid.

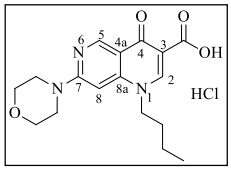
Yield: 862 mg (2.79 mmol, 77 %), mp: 161 - 162 °C (EA).

IR (ATR), $\tilde{\nu}$ [cm^{-1}]: 3071 (w), 2956 (w), 2933 (m), 2872 (w), 1724 (s), 1634 (m), 1577 (s), 1466 (s), 1318 (m), 1225 (m), 1190 (s), 1072 (s), 928 (m), 890 (m), 808 (m).

^{1}H-NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 9.36 (s, 1H, **H**-2), 8.40 (s, 1H, **H**-5), 7.27 (s, 1H, **H**-8), 4.37 (q, 2H, ^3J = 7.1, OC**H**$_2$CH$_3$), 4.09 (t, 2H, ^3J = 7.4, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 1.85 (quint, 2H, ^3J = 7.4, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.44 (sext, 2H, ^3J = 7.4, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 1.39 (t, 3H, ^3J = 7.1, OCH$_2$C**H**$_3$), 1.01 (t, 3H, ^3J = 7.4, NCH$_2$CH$_2$CH$_2$C**H**$_3$).

^{13}C-NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 173.1 (1C, **C**-4), 164.5 (1C, **C**O$_2$Et), 154.4 (1C, **C**-7), 152.3 (1C, **C**-5), 150.3 (1C, **C**-2), 145.8 (1C, **C**-8a), 122.8 (1C, **C**-4a), 114.6 (1C, **C**-3), 109.2 (1C, **C**-8), 61.3 (1C, O**C**H$_2$CH$_3$), 53.3 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 30.6 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.8 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 14.3 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$), 13.5 (1C, OCH$_2$**C**H$_3$).

Synthesis of 1-Butyl-7-morpholino-4-oxo-1,4-dihydro-1,6-naphthyridine-3-carboxylic acid hydrochloride (M)



Under microwave irradiation 400 mg (1.30 mmol) ethyl 1-butyl-7-chloro-4-oxo-1,4-dihydro-1,6-naphthyridine-3-carboxylate (K) dissolved in 15 ml morpholine were heated to 85 °C for 1.5 h (heat-up rate: in 2 min to 85 °C, 600 W). The mixture was diluted with 30 ml water and extracted with DCM (4 x 50 ml). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo.

The residue was suspended in 5 ml EtOH and 5 ml HCl-solution (6 M) and refluxed for 3 h. Afterwards the suspension was evaporated to dryness under reduced pressure and the crude product was recrystallized from EA/MeOH to give a yellow, crystalline solid.

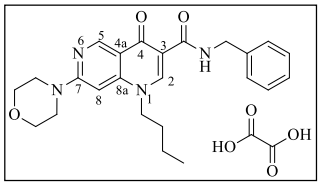
Yield: 356 mg (968 µmol, 75 %), mp: 257 - 259 °C (EA/MeOH).

IR (ATR), $\tilde{\nu}$ [cm^{-1}]: 2962 (w), 2856 (w), 2497 (br), 1710 (m), 1607 (s), 1525 (m), 1467 (s), 1418 (m), 1112 (m), 967 (m), 821 (m), 806 (m).

^{1}H-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 15.0 (s, 1H, COO**H**), 9.06 (s, 1H, **H**-5), 8.84 (s, 1H, **H**-2), 6.89 (s, 1H, **H**-8), 4.41 (t, 2H, ^3J = 7.3, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.73 (s, 8H, morpholino-C**H**$_2$), 1.72 (quint, 2H, ^3J = 7.3, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.33 (sext, 2H, ^3J = 7.3, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 1.01 (t, 3H, ^3J = 7.3, NCH$_2$CH$_2$CH$_2$C**H**$_3$).

^{13}C-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 177.2 (1C, **C**-4), 165.6 (1C, **C**OOH), 160.7 (1C, **C**-7), 151.2 (1C, **C**-2), 149.9 (1C, **C**-5), 146.1 (1C, **C**-8a), 112.2 (1C, **C**-4a), 107.6 (1C, **C**-3), 88.7 (1C, **C**-8), 65.7 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 52.1 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 44.8 (2H, morpholino-**C**H$_2$-N-**C**H$_2$), 30.1 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.0 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 14.4 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$).

Synthesis of N-Benzyl-1-butyl-7-morpholino-4-oxo-1,4-dihydro-1,6-naphthyridine-3-carboxamide oxalate (GHQ237)



To a cooled solution (0 °C) of 200 mg (544 µmol) 1-butyl-7-morpholino-4-oxo-1,4-dihydro-1,6-naphthyridine-3-carboxylic acid hydrochloride (M) and 275 mg (2.72 mmol, 299 µl) NMM in 10 ml anhydrous DMF 298 mg (2.18 mmol, 285 µl) isobutyl chloroformate were added dropwise under argon atmosphere. After 1 h at 0 °C 234 mg (2.18 mmol, 238 µl) benzylamine were added and stirring at rt was continued for 45 min. The solvent was removed in vacuo and the crude product was purified via normal phase column chromatography on silica gel ($CHCl_3$/EtOH 20:1, R_f: 0.49). The resulting solid was dissolved in 4 ml ACN and a solution of 245 mg (2.72 mmol) oxalic acid in 6 ml ACN was added dropwise. At -20 °C the oxalic salt precipitated and was recrystallized from ACN.

Yield: 217 mg (465 µmol, 85 %), mp: 178 - 179 °C (ACN).

CHN: *calculated* C: 64.50, H: 6.28, N: 12.04, O: 17.18 (for **API**/**Ox** 1:0.5), *found* C: 64.42, H: 6.29, N: 11.98.

Stoichiometry: **API**/**Ox** 1:0.5.

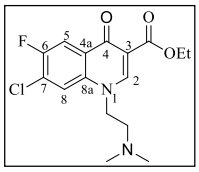
IR (ATR), $\tilde{\nu}$ [cm^{-1}]: 3171 (w), 3056 (m), 2954 (m), 2911 (m), 2867 (m), 2603 (w), 1734 (m), 1645 (s), 1610 (s), 1552 (s), 1467 (m), 1454 (m), 1440 (m), 1363 (m), 1192 (s), 1118 (m), 813 (m), 736 (m), 682 (m).

^{1}H-NMR (DMSO-d_6, δ [ppm], J [Hz]): 10.2 (t, 1H, ^3J = 5.8, CON**H**R), 9.02 (s, 1H, **H**-5), 8.70 (s, 1H, **H**-2), 7.37-7.24 (m, 5H, benzyl-C**H**$_{arom.}$), 6.63 (s, 1H, **H**-8), 4.53 (d, 2H, ^3J = 5.8, benzyl-C**H**$_2$), 4.34 (t, 2H, ^3J = 7.3, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 3.73-3.63 (m, 8H, morpholino-C**H**$_2$), 1.72 (quint, 2H, ^3J = 7.3, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.31 (sext, 2H, ^3J = 7.3, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.91 (t, 3H, ^3J = 7.3, NCH$_2$CH$_2$CH$_2$C**H**$_3$).

^{13}C-NMR (DMSO-d_6, δ [ppm], J [Hz]): 175.4 (1C, **C**-4), 163.7 (1C, **C**ONHR), 160.8 (1C, **C**-7), 160.5 (Oxalat), 149.9 (1C, **C**-5), 149.7 (1C, **C**-2), 146.0 (1C, **C**-8a), 139.2 (1C, benzyl-**C**$_q$), 128.4 (2C, benzyl-**C**H$_{arom.}$), 127.3 (2C, benzyl-**C**H$_{arom.}$), 126.8 (1C, benzyl-**C**H$_{arom.}$), 114.2 (1C, **C**-4a), 111.4 (1C, **C**-3), 88.5 (1C, **C**-8), 65.7 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 51.6 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 44.8 (2H, morpholino-**C**H$_2$-N-**C**H$_2$), 42.1 (1C, benzyl-**C**H$_2$), 30.1 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.0 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.5 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$).

Synthesis of N-Benzyl-1-(2-(dimethylamino)ethyl)-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide oxalate (GHQ243)

Synthesis of Ethyl 7-chloro-1-(2-(dimethylamino)ethyl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (N)



1.00 g (3.71 mmol) ethyl 7-chloro-6-fluoro-4-hydroxychinolin-3-carboxylate (B) and 769 mg (5.57 mmol) K_2CO_3 were suspended in 15 ml anhydrous DMF under argon atmosphere and stirred at rt for 30 min. A catalytic amount of KI and 588 mg (4.08 mmol) 2-chloro-*N*,*N*-dimethylethylamine hydrochloride were added and the mixture was heated to 100 °C for 16 h. The solvent was removed in vacuo and the residue was partitioned between 50 ml water and 50 ml EA. The organic layer was separated and the aqueous layer was extracted with EA (2 x 50 ml). After drying the combined organic layers over Na_2SO_4 and evaporating the solvent under reduced pressure the crude product was purified via normal phase column chromatography on silica gel ($CHCl_3$/MeOH/conc. NH_3 150:10:1, R_f: 0.43) to give a white solid.

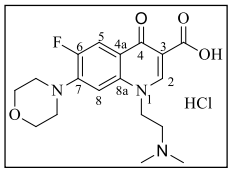
Yield: 480 mg (1.41 mmol, 38 %), mp: 140 - 141 °C ($CHCl_3$/MeOH/conc. NH_3).

IR (ATR), $\tilde{\nu}$ [cm^{-1}]: 2981 (w), 2933 (w), 2871 (w), 2840 (w), 2776 (w), 1716 (s), 1611 (m), 1594 (m), 1548 (m), 1487 (s), 1460 (m), 1384 (m), 1313 (m), 1209 (m), 1160 (s), 1040 (m), 893 (m), 800 (m), 770 (m).

^{1}H-NMR (DMSO-d_6, δ [ppm], J [Hz]): 8.61 (s, 1H, **H**-2), 8.18 (d, 1H, $^4J_{\mathbf{H,F}} = 6.0$, **H**-8), 8.03 (d, 1H, $^3J_{\mathbf{H,F}} = 9.4$, **H**-5), 4.47 (t, 2H, $^3J = 5.7$, NC**H**$_2$CH$_2$N(CH$_3$)$_2$), 4.23 (q, 2H, $^3J = 7.1$, OC**H**$_2$CH$_3$), 2.58 (t, 2H, $^3J = 5.7$, NCH$_2$C**H**$_2$N(CH$_3$)$_2$), 2.18 (s, 6H, NCH$_2$CH$_2$N(C**H**$_3$)$_2$), 1.28 (t, 3H, $^3J = 7.1$, OCH$_2$C**H**$_3$).

^{13}C-NMR (DMSO-d_6, δ [ppm], J [Hz]): 171.2 (d, 1C, $^4J_{\mathbf{C,F}} = 2.3$, **C**-4), 164.3 (1C, **C**OOEt), 154.2 (d, 1C, $^1J_{\mathbf{C,F}} = 246.9$, **C**-6), 150.5 (1C, **C**-2), 135.9 (d, 1C, $^4J_{\mathbf{C,F}} = 1.8$, **C**-8a), 128.6 (d, 1C, $^3J_{\mathbf{C,F}} = 5.4$, **C**-4a), 125.3 (d, 1C, $^2J_{\mathbf{C,F}} = 20.0$, **C**-7), 120.1 (1C,**C**-8), 112.4 (d, 1C, $^2J_{\mathbf{C,F}} = 22.4$, **C**-5), 109.1 (1C, **C**-3), 59.8 (1C, O**C**H$_2$CH$_3$), 56.9 (1C, N**C**H$_2$CH$_2$N(CH$_3$)$_2$), 50.4 (1C, NCH$_2$**C**H$_2$N(CH$_3$)$_2$), 45.2 (2C, NCH$_2$CH$_2$N(**C**H$_3$)$_2$), 14.2 (1C, OCH$_2$**C**H$_3$).

Synthesis of 1-(2-(Dimethylamino)ethyl)-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride (O)



670 mg (1.97 mmol) ethyl 7-chloro-1-(2-(dimethylamino)ethyl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (N) were suspended in 5 ml EtOH and 5 ml aqueous HCl-solution (6 M) and heated to reflux for 4 h. The suspension was evaporated to dryness under reduced pressure and the residue was dissolved in 15 ml morpholine. The solution was heated to 100 °C for 4 h under microwave irradiation (heat-up rate: in 3 min to 100 °C, 600W). Excess of morpholine was removed in vacuo and the remaining oil was dissolved in 10 ml MeOH. After addition of 1.5 ml isopropanolic HCl-solution (6 M) the precipitate was filtered off and recrystallized from MeOH/water in order to give the desired product as hydrochlorid salt.

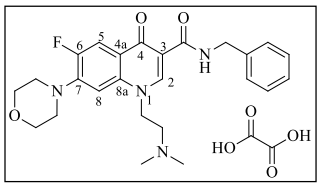
Yield: 276 mg (690 µmol, 35 %), 298 - 299 °C (MeOH/water).

IR (ATR), $\tilde{\nu}$ [cm^{-1}]: 3246 (w), 3046 (w), 2968 (w), 2903 (w), 2864 (m), 2373 (br), 1700 (s), 1622 (s), 1523 (m), 1488 (s), 1468 (m), 1385 (m), 1267 (s), 1118 (s), 1036 (m), 884 (m), 807 (m), 752 (m).

^{1}H-NMR (DMSO-d_6, δ [ppm], J [Hz]): 15.2 (s, 1H, COO**H**), 11.5 (br, 1H, N**H**$^+$), 9.03 (s, 1H, **H**-2), 7.94 (d, 1H, $^3J_{\mathbf{H,F}} = 13.4$, **H**-5), 7.26 (d, 1H, $^4J_{\mathbf{H,F}} = 7.1$, **H**-8), 5.03 (t, 2H, $^3J = 7.2$, NC**H**$_2$CH$_2$N(CH$_3$)$_2$), 3.79 (br, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.53 (br, 2H, NCH$_2$C**H**$_2$N(CH$_3$)$_2$), 3.40 (br, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 2.83 (s, 6H, CH$_2$CH$_2$N(C**H**$_3$)$_2$).

^{13}C-NMR (DMSO-d_6, δ [ppm], J [Hz]): 176.3 (d, 1C, $^{4}J_{\mathbf{C,F}}$ = 2.3, **C**-4), 165.7 (1C, **C**OOH), 152.7 (d, 1C, $^{1}J_{\mathbf{C,F}}$ = 249.5, **C**-6), 149.7 (1C, **C**-2), 145.5 (d, 1C, $^{2}J_{\mathbf{C,F}}$ = 9.9, **C**-7), 137.3 (1C, **C**-8a), 119.1 (d, 1C, $^{3}J_{\mathbf{C,F}}$ = 7.8, **C**-4a), 111.3 (d, 1C, $^{2}J_{\mathbf{C,F}}$ = 23.1, **C**-5), 107.3 (1C, **C**-3), 105.5 (d, 1C, $^{3}J_{\mathbf{C,F}}$ = 3.3, **C**-8), 65.8 (2C, morpholino-**C**H_2-O-**C**H_2), 53.0 (1C, N**C**$H_2$$CH_2N(CH_3)_2$), 50.0 (2C, $^{4}J_{\mathbf{C,F}}$ = 4.8, morpholino-**C**H_2-N-**C**H_2), 47.6 (1C, NCH_2**C**H_2N$(CH_3)_2$), 42.2 (2C, NCH_2CH_2N(**C**$H_3)_2$).

Synthesis of N-Benzyl-1-(2-(dimethylamino)ethyl)-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide oxalate (GHQ243)



To a cooled solution (0 °C) of 250 mg (625 µmol) 1-(2-(dimethylamino)ethyl)-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride (O) and 506 mg (2.50 mmol, 273 µl) NMM in 10 ml anhydrous DMF 342 mg (2.50 mmol, 326 µl) isobutyl chloroformate were added dropwise under argon atmosphere. After 1 h at 0 °C 268 mg (2.50 mmol, 273 µl) benzylamine were added and stirring at rt was continued for 45 min. The solvent was removed in vacuo and the crude product was purified via normal phase column chromatography on silica gel ($CHCl_3$/MeOH/conc. NH_3 100:10:1, R_f: 0.65). The resulting solid was dissolved in 4 ml ACN and a solution of 281 mg (3.13 mmol) oxalic acid in 6 ml ACN was added dropwise. After 30 min of agitating at rt the oxalic salt precipitated which was recrystallized from MeOH.

Yield: 145 mg (267 µmol, 43 %), mp: 227 - 228 °C (MeOH).

CHN: *calculated* C: 59.77, H: 5.76, F: 3.50, N: 10.33, O: 20.64 (for **API**/**Ox** 1:1), *found* C: 59.62, H: 5.62, N: 10.36.

Stoichiometry: **API**/**Ox** 1:1.

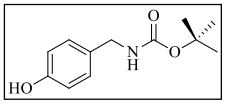
IR (ATR), $\tilde{\nu}$ [cm^{-1}]: 3175 (w), 3056 (m), 2981 (w), 2885 (w), 2815 (w), 2716 (br), 1717 (br), 1635 (s), 1572 (s), 1490 (s), 1450 (m), 1360 (m), 1251 (m), 1171 (m), 1122 (m), 1001 (m), 932 (m), 745 (m), 694 (m).

^{1}H-NMR (DMSO-d_6, δ [ppm], J [Hz]): 10.3 (t, 1H, ^{3}J = 5.8, CON**H**R), 8.86 (s, 1H, **H**-2), 7.88 (d, 1H, $^{3}J_{\mathbf{H,F}}$ = 13.6, **H**-5), 7.35-7.23 (m, 5H, benzyl-C$\mathbf{H}_{arom.}$), 7.12 (d, 1H, $^{4}J_{\mathbf{H,F}}$ = 7.0, **H**-8), 4.79 (t, 2H, ^{3}J = 6.8, N**C**$\mathbf{H}_2$$CH_2N(CH_3)_2$), 4.56 (d, 2H, ^{3}J = 5.8, benzyl-C$\mathbf{H}_2$), 3.79 (br, 4H, morpholino-C$\mathbf{H}_2$-O-C$\mathbf{H}_2$), 3.28 (br, 6H, morpholino-C$\mathbf{H}_2$-N-C$\mathbf{H}_2$, NCH_2C$\mathbf{H}_2$N$(CH_3)_2$), 2.68 (s, 6H, NCH_2CH_2N(C$\mathbf{H}_3)_2$).

^{13}C-NMR (DMSO-d_6, δ [ppm], J [Hz]): 174.2 (d, 1C, $^4J_{C,F}$ = 2.2, **C**-4), 163.9 (1C, **C**ONHR), 163.8 (Oxalat), 152.4 (d, 1C, $^1J_{C,F}$ = 247.5, **C**-6), 148.5 (1C, **C**-2), 144.6 (d, 1C, $^2J_{C,F}$ = 9.9, **C**-7), 139.3 (1C, benzyl-**C**$_q$), 136.6 (1C, **C**-8a), 128.3 (2C, benzyl-**C**H$_{arom.}$), 127.3 (2C, benzyl-**C**H$_{arom.}$), 126.8 (1C, benzyl-**C**H$_{arom.}$), 121.2 (d, 1C, $^3J_{C,F}$ = 7.1, **C**-4a), 111.5 (d, 1C, $^2J_{C,F}$ = 22.9, **C**-5), 110.4 (1C, **C**-3), 105.2 (d, 1C, $^3J_{C,F}$ = 2.3, **C**-8), 65.9 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 54.2 (1C, NCH$_2$**C**H$_2$N(CH$_3$)$_2$), 49.9 (2C, $^4J_{C,F}$ = 4.4, morpholino-**C**H$_2$-N-**C**H$_2$), 48.2 (1C, N**C**H$_2$CH$_2$N(CH$_3$)$_2$), 43.3 (2C, NCH$_2$CH$_2$N(**C**H$_3$)$_2$), 42.1 (1C, benzyl-**C**H$_2$).

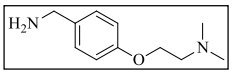
Synthesis of 1-Butyl-*N*-(4-(2-(dimethylamino)ethoxy)benzyl)-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide oxalate (GHQ250)

Synthesis of tert-Butyl-4-hydroxybenzylcarbamate (P) (6)



905 mg (7.35 mmol) 4-hydroxybenzylamine, 2.41 g (11.0 mmol) di-*tert*-butyl dicarbonate and 1.24 g (14.7 mmol) $NaHCO_3$ were suspended in 20 ml MeOH and stirred at rt for 16 h. The solvent was removed in vacuo, the residue was dissolved in 20 ml water and extracted with DCM (4 x 50 ml). After drying the combined organic layers over Na_2SO_4 and concentrating under reduced pressure the crude product was purified by normal phase column chromatography on silica gel (PE/EA 2:1, R_f: 0.69) to give a colorless oil.
Yield: 1.20 g (5.37 mmol, 73 %).
Spectroscopical data were consistent with literature (6).

Synthesis of 2-(4-(Aminomethyl)phenoxy)-N,N-dimethylethanamine (Q) (7)



Under argon atmosphere 600 mg (2.69 mmol) *tert*-butyl-4-hydroxybenzylcarbamate (P) were added potionwise to a cooled suspension (0 °C) of 215 mg (5.38 mmol) sodium hydride (60 % dispersion in mineral oil) in 10 ml anhydrous DMF. After 30 min at 0 °C 465 mg (3.23 mmol) 2-chloro-*N*,*N*-dimethylethylamine hydrochloride dissolved in 10 ml anhydrous DMF were added dropwise and the resulting solution was stirred at rt for 15 h. The solvent was removed in vacuo and the residue was partitioned between 50 ml water and 50 ml DCM. The organic layer was separated and the aqueous layer was extracted with DCM (3 x 50 ml). The combined organic layers were dried over Na_2SO_4, the solvent was evaporated under reduced pressure and the residue was purified by means of normal phase column chromatography on silica gel ($CHCl_3$/methanolic NH_3 (6 M) 40:1, R_f: 0.85). For

deprotection the intermediate was dissolved in 10 ml DCM and 4 ml TFA were added. After stirring the mixture for 2 h at rt the solvent was removed in vacuo and the residue was dissolved in 50 ml water. The pH was adjusted to 9 - 10 using aqueous NaOH-solution (2 M) and the mixture was extracted with DCM (4 x 50 ml). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to give the desired product as colorless oil.

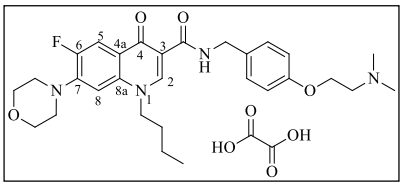
Yield: 193 mg (995 µmol, 37 %).

IR (ATR), $\tilde{\nu}$ [cm^{-1}]: 3361 (w), 3275 (w), 2940 (m), 2863 (m), 2821 (m), 2770 (m), 1610 (m), 1583 (m), 1510 (s), 1463 (m), 1239 (s), 1031 (m), 811 (m).

^{1}H-NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 7.21 (d, 2H, ^3J = 8.6, benzyl-C**H**$_{arom.}$), 6.89 (d, 2H, ^3J = 8.6, benzyl-C**H**$_{arom.}$), 4.06 (t, 2H, ^3J = 5.8, OC**H**$_2$$CH_2N$), 3.80 (s, 2H, benzyl-C**H**$_2$), 2.72 (t, 2H, ^3J = 5.8, OCH_2C**H**$_2$N), 2.33 (s, 6H, N(C**H**$_3$)$_2$), 1.54 (s, 2H, N**H**$_2$).

^{13}C-NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 157.8 (1C, benzyl-C_q), 135.7 (1C, benzyl-C_q), 128.2 (2C, benzyl-$CH_{arom.}$), 114.6 (2C, benzyl-$CH_{arom.}$), 66.1 (1C, OCH_2CH_2N), 58.3 (1C, OCH_2CH_2N), 45.9 (1C, benzyl-CH_2), 45.8 (2C, $N(CH_3)_2$).

Synthesis of 1-Butyl-N-(4-(2-(dimethylamino)ethoxy)benzyl)-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide oxalate (GHQ250)



To a cooled solution (0 °C) of 100 mg (287 µmol) 1-butyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (D) and 116 mg (1.15 mmol, 125 µl) NMM in 10 ml anhydrous DMF 118 mg (862 µmol, 112 µl) isobutyl chloroformate were added dropwise under argon atmosphere. After 1 h at 0 °C 168 mg (862 µmol) 2-(4-(aminomethyl)phenoxy)-*N*,*N*-dimethylethanamine (Q) were added and stirring at rt was continued for 45 min. The solvent was removed in vacuo and the crude product was purified via normal phase column chromatography on silica gel ($CHCl_3$/MeOH/ conc. NH_3 100:10:1, R_f: 0.81). The resulting solid was dissolved in 4 ml ACN and a solution of 129 mg (1.44 mmol) oxalic acid in 6 ml ACN were added dropwise. At -20 °C the oxalic salt precipitated which was recrystallized from ACN.

Yield: 103 mg (156 µmol, 54 %), mp: 172 - 173 °C (ACN).

CHN: *calculated* C: 58.26, H: 6.11, F: 2.88, N: 8.49, O: 24.25 (for **API**/**Ox** 1:1.5), *found* C: 58.57, H: 6.33, N: 8.71.

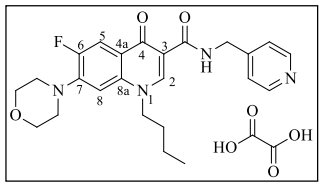
Stoichiometry: **API**/**Ox** 1:1.5.

IR (ATR), $\tilde{\nu}$ [cm^{-1}]: 3175 (w), 3034 (w), 2956 (w), 2925 (w), 2853 (w), 2593 (w), 1732 (br), 1645 (s), 1558 (m), 1490 (s), 1376 (w), 1241 (m), 1179 (m), 1117 (m), 934 (m), 802 (m), 681 (m).

^{1}H-NMR (DMSO-d_6, δ [ppm], J [Hz]): 10.3 (t, 1H, ^3J = 5.7, CON**H**R), 8.77 (s, 1H, **H**-2), 7.85 (d, 1H, $^3J_{\mathbf{H,F}}$ = 13.6, **H**-5), 7.29 (d, 2H, ^3J = 8.6, benzyl-C**H**$_{arom.}$), 7.08 (d, 1H, $^4J_{\mathbf{H,F}}$ = 7.2, **H**-8), 6.96 (d, 2H, ^3J = 8.6, benzyl-C**H**$_{arom.}$), 4.48-4.46 (m, 4H, benzyl-C**H**$_2$, NC**H**$_2$CH$_2$CH$_2$CH$_3$), 4.29 (t, 2H, ^3J = 5.0, OC**H**$_2$CH$_2$N(CH$_3$)$_2$), 3.79-3.77 (m, 4H, morpholino-C**H**$_2$-O-C**H**$_2$), 3.44 (t, 2H, ^3J = 5.0, OCH$_2$C**H**$_2$N(CH$_3$)$_2$), 3.25-2.24 (m, 4H, morpholino-C**H**$_2$-N-C**H**$_2$), 2.80 (s, 6H, OCH$_2$CH$_2$N(C**H**$_3$)$_2$), 1.75 (quint, 2H, ^3J = 7.3, NCH$_2$C**H**$_2$CH$_2$CH$_3$), 1.30 (sext, 2H, ^3J = 7.3, NCH$_2$CH$_2$C**H**$_2$CH$_3$), 0.91 (t, 3H, ^3J = 7.3, NCH$_2$CH$_2$CH$_2$C**H**$_3$).

^{13}C-NMR (DMSO-d_6, δ [ppm], J [Hz]): 174.1 (d, 1C, $^4J_{\mathbf{C,F}}$ = 2.5, **C**-4), 164.0 (1C, **C**ONHR), 163.4 (Oxalat), 156.6 (1C, benzyl-**C**$_q$), 152.5 (d, 1C, $^1J_{\mathbf{C,F}}$ = 247.5, **C**-6), 147.8 (1C, **C**-2), 144.4 (d, 1C, $^2J_{\mathbf{C,F}}$ = 10.3, **C**-7), 136.6 (1C, **C**-8a), 132.3 (1C, benzyl-**C**$_q$), 128.8 (2C, benzyl-**C**H$_{arom.}$), 121.5 (d, 1C, $^3J_{\mathbf{C,F}}$ = 7.0, **C**-4a), 114.7 (2C, benzyl-**C**H$_{arom.}$), 111.4 (d, 1C, $^2J_{\mathbf{C,F}}$ = 22.4, **C**-5), 110.0 (1C, **C**-3), 105.6 (d, 1C, $^3J_{\mathbf{C,F}}$ = 3.1, **C**-8), 65.9 (2C, morpholino-**C**H$_2$-O-**C**H$_2$), 62.4 (1C, O**C**H$_2$CH$_2$N(CH$_3$)$_2$), 55.3 (1C, OCH$_2$**C**H$_2$N(CH$_3$)$_2$), 52.9 (1C, N**C**H$_2$CH$_2$CH$_2$CH$_3$), 49.8 (2C, $^4J_{\mathbf{C,F}}$ = 4.4, morpholino-**C**H$_2$-N-**C**H$_2$), 42.8 (2C, OCH$_2$CH$_2$N(**C**H$_3$)$_2$), 41.6 (1C, benzyl-**C**H$_2$), 30.3 (1C, NCH$_2$**C**H$_2$CH$_2$CH$_3$), 19.2 (1C, NCH$_2$CH$_2$**C**H$_2$CH$_3$), 13.5 (1C, NCH$_2$CH$_2$CH$_2$**C**H$_3$).

Synthesis of 1-Butyl-6-fluoro-7-morpholino-4-oxo-*N*-(pyridin-4-ylmethyl)-1,4-dihydroquinoline-3-carboxamide oxalate (GHQ242)



To a cooled solution (0 °C) of 150 mg (431 µmol) 1-butyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (D) and 218 mg (2.16 mmol, 237 µl) NMM in 10 ml anhydrous DMF 235 mg (1.72 mmol, 224 µl) isobutyl chloroformate were added dropwise under argon atmosphere. After 1 h at 0 °C 186 mg (1.72 mmol, 175 µl) 4-(aminomethyl)pyridine were added and stirring at rt was continued for 45 min. The solvent was removed in vacuo and the crude product was purified via normal phase column chromatography on silica gel ($CHCl_3$/PE 100:1, R_f: 0.49). The resulting solid was dissolved in 4 ml ACN and a solution of 194 mg (2.16 mmol) oxalic acid in 6 ml ACN was added dropwise. After 30 min of agitating at rt the oxalic salt precipitated which was recrystallized from ACN.

Yield: 87 mg (151 µmol, 35 %), mp: 191 - 192 °C (ACN).

CHN: *calculated* C: 56.54, H: 5.27, F: 3.31, N: 9.77, O: 25.06 (for **API**/**Ox** 1:1.5), *found* C: 56.46, H: 5.28, N: 9.70.

Stoichiometry: **API**/**Ox** 1:1.5.

IR (ATR), $\tilde{\nu}$ [cm^{-1}]: 3235 (w), 3073 (w), 2957 (m), 2931 (w), 2871 (w), 2614 (br), 1905 (br), 1728 (br), 1660 (s), 1629 (s), 1530 (m), 1489 (s), 1451 (m), 1376 (w), 1258 (s), 1198 (m), 1114 (m), 932 (m), 799 (m), 692 (m).

^{1}H-NMR (DMSO-d_6, δ [ppm], J [Hz]): 10.5 (t, 1H, ^{3}J = 5.8, CON**H**R), 8.78 (s, 1H, **H**-2), 8.55 (d, 2H, ^{3}J = 5.2, Py-C**H**), 7.90 (d, 1H, $^{3}J_{\mathbf{H,F}}$ = 13.5, **H**-5), 7.39 (d, 2H, ^{3}J = 5.2, Py-C**H**), 7.10 (d, 1H, $^{4}J_{\mathbf{H,F}}$ = 7.2, **H**-8), 4.62 (d, 2H, ^{3}J = 5.8, C$\mathbf{H}_2$), 4.48 (t, 2H, ^{3}J = 7.3, NC$\mathbf{H}_2$$CH_2CH_2CH_3$), 3.80 (br, 4H, morpholino-C$\mathbf{H}_2$-O-C$\mathbf{H}_2$), 3.26 (br, 4H, morpholino-C$\mathbf{H}_2$-N-C$\mathbf{H}_2$), 1.77 (quint, 2H, ^{3}J = 7.3, $NCH_2C\mathbf{H}_2CH_2CH_3$), 1.32 (sext, 2H, ^{3}J = 7.3, $NCH_2CH_2C\mathbf{H}_2CH_3$), 0.92 (t, 3H, ^{3}J = 7.3, $NCH_2CH_2CH_2C\mathbf{H}_3$).

^{13}C-NMR (DMSO-d_6, δ [ppm], J [Hz]): 174.1 (d, 1C, $^{4}J_{\mathbf{C,F}}$ = 1.8, **C**-4), 164.5 (1C, **C**ONHR), 161.0 (Oxalat), 152.5 (d, 1C, $^{1}J_{\mathbf{C,F}}$ = 248.0, **C**-6), 149.9 (1C, Py-$\mathbf{C}_q$), 148.6 (2C, Py-**C**H), 147.8 (1C, **C**-2), 144.3 (d, 1C, $^{2}J_{\mathbf{C,F}}$ = 10.4, **C**-7), 136.6 (1C, **C**-8a), 122.4 (2C, Py-**C**H), 121.4 (d, 1C, $^{3}J_{\mathbf{C,F}}$ = 6.6, **C**-4a), 111.4 (d, 1C, $^{2}J_{\mathbf{C,F}}$ = 22.7, **C**-5), 109.7 (1C, **C**-3), 105.5 (d, 1C, $^{3}J_{\mathbf{C,F}}$ = 2.0, **C**-8), 65.8 (2C, morpholino-**C**H_2-O-**C**H_2), 52.8 (1C, N**C**$H_2CH_2CH_2CH_3$), 49.8 (d, 2C, $^{4}J_{\mathbf{C,F}}$ = 4.4, morpholino-**C**H_2-N-**C**H_2), 41.2 (1C, **C**H_2), 30.2 (1C, $NCH_2\mathbf{C}H_2CH_2CH_3$), 19.1 (1C, $NCH_2CH_2\mathbf{C}H_2CH_3$), 13.4 (1C, $NCH_2CH_2CH_2\mathbf{C}H_3$).

References:

1. **de la Cruz A EJ, Goya P, Martinez A., Pfleiderer W. 1992.** Tautomerism and Acidity in 4-Quinolone-3-carboxylic Acid Derivatives. Tetrahedron **48**: 6135-6150.
2. **Hiltensperger G, Jones NG, Niedermeier S, Stich A, Kaiser M, Jung J, Puhl S, Damme A, Braunschweig H, Meinel L, Engstler M, Holzgrabe U. 2012.** Synthesis and structure-activity relationships of new quinolone-type molecules against Trypanosoma brucei. J Med Chem **55**:2538-2548.
3. **Kellenberger JLD, J.; Reinelt, S. B. 2009.** Preparation of Clarithromycin Macrolide Derivatives as Antiinflammatory Agents.
4. **Kohlmann A, Zech SG, Li F, Zhou T, Squillace RM, Commodore L, Greenfield MT, Lu X, Miller DP, Huang WS, Qi J, Thomas RM, Wang Y, Zhang S, Dodd R, Liu S, Xu R, Xu Y, Miret JJ, Rivera V, Clackson T, Shakespeare WC, Zhu X, Dalgarno DC. 2013.**

Fragment growing and linking lead to novel nanomolar lactate dehydrogenase inhibitors. J Med Chem 56:1023-1040.

5. **Brunette SRK, J. M.; Lemieux, R. M.; Tschantz, M. A. 2006.** Preparation of Pyridine Derivatives Useful as Inhibitors of PKC-Theta for Treating Various Diseases.
6. **Chichak KS, Peters AJ, Cantrill SJ, Stoddart JF. 2005.** Nanoscale borromeates. J Org Chem **70**:7956-7962.
7. **Löber OB, C.; Ebel, K.; Johann, P. . 2004.** Verfahren zur Herstellung von Aminoalkoxybenzylaminen und Aminoalkoxybenzonitrilen als Zwischenprodukte.

Abbreviations:

mp: melting point

Ph_2O: diphenyl ether

rt: room temperature

EA: ethyl acetate

NMM: *N*-methyl morpholine

ACN: acetonitrile

DIPEA: diisopropylamine

DCM: dichloromethane

FA: formic acid

sat: saturated

TFA: trifluoroacetic acid

1. Physicochemical characterization

X-ray analysis

Method:

For single crystal structure analysis of GHQ168, a suited crystal with a size of 0,072 x 0, 112 x 0,19 mm^3 was selected. Data collection was carried out on a BRUKER AXS Apex II diffractometer (Mo-Kα radiation; λ=71.07 pm) at 100 K with Helios-mirror using the BRUKER AXS Apex Suite Software package (1). Data processing was done with the Olex2 software package (2). Structure solution was carried out with olex.solve (3) using charge flipping methods. The model was refined with the olex2.refine refinement package using Gauss-Newton minimization (4). Integrity of symmetry was checked using PLATON (5). Non-hydrogen atoms were refined anisotropically by least square techniques, hydrogen atoms were refined with geometrical constraints regarding their positions. Crystallographic data are summarized in Table S1.

References:

1. **Bruker.** SMART Apex Suite 2001, Bruker AXS Inc., Madison, Wisconsin, USA.
2. **Bourhis LJ, Dolomanov OV, Gildea RJ, Howard JA, Puschmann H.** 2015. The anatomy of a comprehensive constrained, restrained refinement program for the modern computing environment- Olex2 dissected. Acta Crystallogr A Found Adv **71:**59-75.
3. **L. J. Bourhis OVD, R. J. Gildea, J. A. K. Howard, H. Puschmann.** 2011. olex.solve.
4. **L. J. Bourhis OVD, R. J. Gildea, J. A. K. Howard, H. Puschmann.** 2011. olex.refine.
5. **A. L. Spek**. 2003. Single-crystal structure validation with the program *PLATON*. J. Appl. Cryst. 2003, **36:**7-13.

	GHQ168
Formula sum	$C_{25}H_{28}FN_3O_3$
Formula wgt /gmol^{-1}	437.514
a /pm	842.8(2)
b /pm	1708.3(3)
c /pm	1753.3(4)
α /°	61.06(3)
β /°	83.56(3)
γ /°	83.83(3)
V /10^{6}pm^3	2191(1)
Crystal system	Triclinic
Space group	$P\bar{1}$
Z	4
d_{calcd} /gcm^{-3}	1.3156
μ /cm^{-1}	0.93
T /K	173
Data range /°	$1.33 \leq 2\Theta \leq 26.59$
X-ray radiation	Mo-K$_\alpha$, λ = 71.073 pm
Diffractometer	Bruker APEX-II CCD
Reflections	21713
Unique reflections	9082
R(int)	0.0756
No. parameters ref.	575
R_1[a)] for n reflections with F_o>4σ (F_o), n	0.0732
R_1(all)	0.1652
wR_2[b)](all)	0.2304
S[c)]	1.0637
Rem. elec. density /10^{-6}epm	0.9876/-0.7158

a) $R_1 = \sum||F_o|-|F_c||/\sum|F_o|$. b) $wR_2 = [\sum(w(F_o^2-F_c^2)^2)/\sum(w(F_o^2)^2)]^{1/2}$.
c) GooF = $S = [\sum(w(F_o^2-F_c^2)^2)/(n-p)]^{1/2}$ with F_o, F_c = observed and calculated structure factors, n = number of observed reflections.

TABLE S1 Crystallographic data of single crystal X-ray diffraction of GHQ168. Deviations are given in brackets.

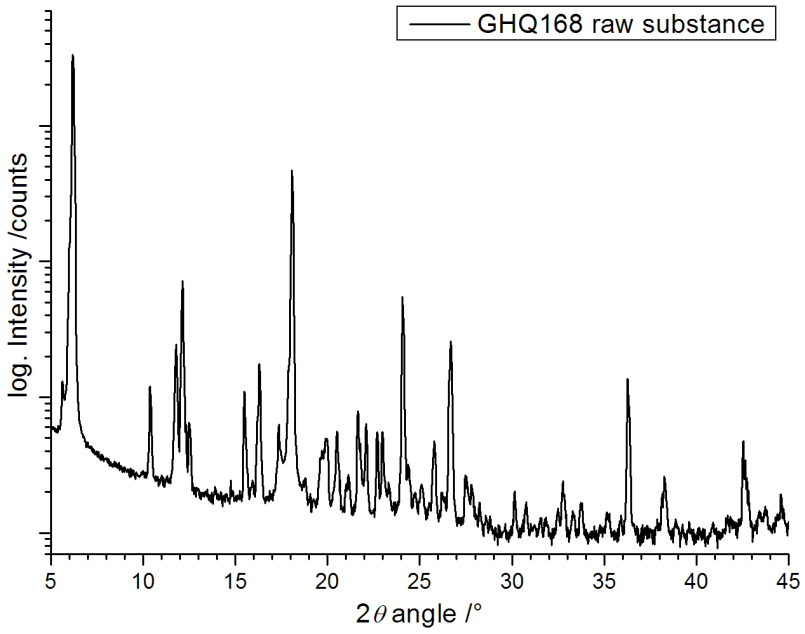


FIG S1A XRPD (Bruker D8 Discover, Billerica, USA) data show characteristic crystal reflexes for GHQ168.

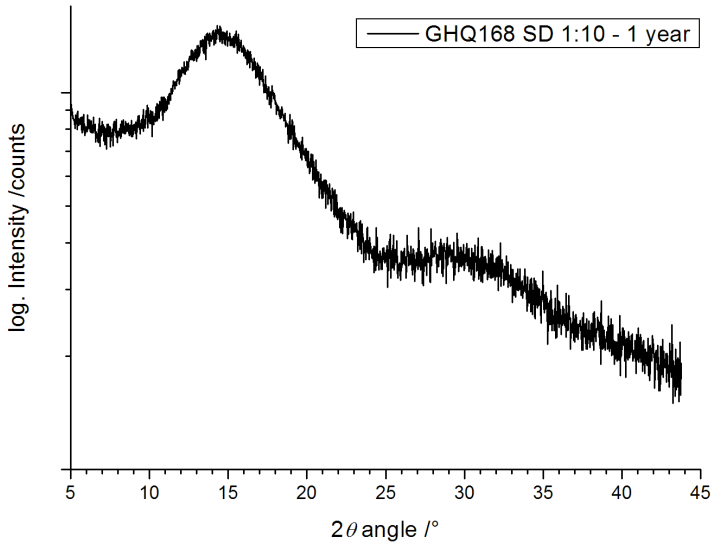


FIG S1B The spray dried formulation of GHQ168 and Eudragit L100 shows a broad amorphous signal. No recrystallization events occurred after 12 month.

Scanning electron microscopy (SEM)

Method:

For the characterization of particle morphology, scanning electron microscopy (JSM-7500F, Jeol, Japan) was used with an accelerating voltage of 2.0 kV and a 1000-fold magnification at a working distance of 8.6 mm.

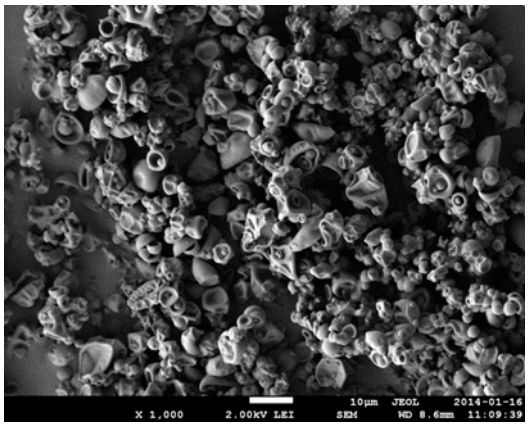
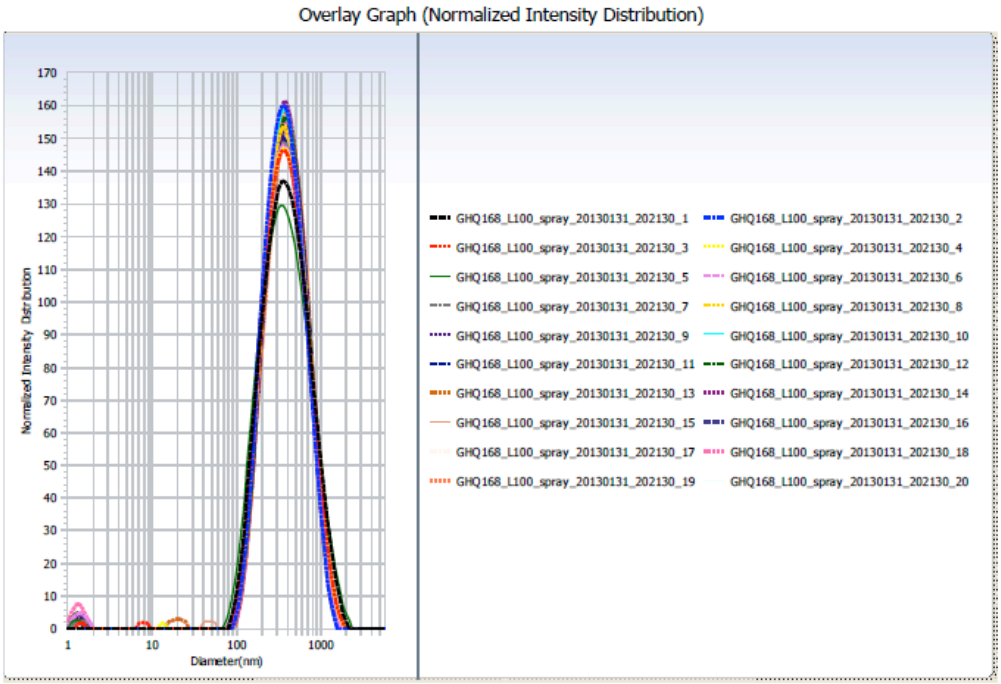


FIG S2 Scanning electron micrographs of spray dried particles of GHQ168 and Eudragit® L100 (1+10 w/w) revealed that the particle size distribution was within a narrow range. The morphology complies with desired specifications such as smooth surface and spherical form.

Dynamic Light Scattering (DLS)

Method:

A solution of GHQ168 spray dried with Eudragit L100 (2 g/l) was prepared in PBS buffer and filtered using syringe filters (5 µm).



No	Data	Repet. No	pH	Ave. Diameter(nm)	Polydispersity Index	D (10%) (nm)	D (50%) (nm)	D (90%) (nm)
1	GHQ168_L100_spray_20130131_202130_1	1	NA	331.7	0.236	164.5	363.8	842.3
2	GHQ168_L100_spray_20130131_202130_2	2	NA	340.4	0.226	175.6	349.2	712.2
3	GHQ168_L100_spray_20130131_202130_3	3	NA	330.3	0.223	169.7	359.0	786.7
4	GHQ168_L100_spray_20130131_202130_4	4	NA	330.1	0.226	171.4	360.5	785.9
5	GHQ168_L100_spray_20130131_202130_5	5	NA	317.7	0.256	150.7	349.3	859.0
6	GHQ168_L100_spray_20130131_202130_6	6	NA	325.8	0.218	164.6	352.5	754.6
7	GHQ168_L100_spray_20130131_202130_7	7	NA	328.5	0.218	167.4	357.8	758.6
8	GHQ168_L100_spray_20130131_202130_8	8	NA	329.7	0.204	173.3	355.4	744.4
9	GHQ168_L100_spray_20130131_202130_9	9	NA	331.7	0.212	171.0	362.8	743.1
10	GHQ168_L100_spray_20130131_202130_10	10	NA	329.4	0.203	177.2	356.9	723.2
11	GHQ168_L100_spray_20130131_202130_11	11	NA	333.0	0.227	168.9	349.1	753.6
12	GHQ168_L100_spray_20130131_202130_12	12	NA	333.1	0.213	174.4	363.3	738.4
13	GHQ168_L100_spray_20130131_202130_13	13	NA	332.5	0.214	176.5	362.5	734.5
14	GHQ168_L100_spray_20130131_202130_14	14	NA	337.3	0.195	179.0	360.0	721.3
15	GHQ168_L100_spray_20130131_202130_15	15	NA	337.8	0.218	176.0	374.3	763.3
16	GHQ168_L100_spray_20130131_202130_16	16	NA	341.3	0.208	178.6	369.0	771.1
17	GHQ168_L100_spray_20130131_202130_17	17	NA	341.5	0.204	179.5	368.1	768.0
18	GHQ168_L100_spray_20130131_202130_18	18	NA	342.0	0.204	176.1	363.8	755.8
19	GHQ168_L100_spray_20130131_202130_19	19	NA	338.4	0.222	176.2	374.5	770.5
20	GHQ168_L100_spray_20130131_202130_20	20	NA	342.4	0.202	180.6	366.1	778.9

FIG S3 Subsequent DLS measurements were performed in order to monitor the pattern in solution (20 subsequent measurements are depicted above) and particle size did not change significantly over time.

Caco-2 monolayers

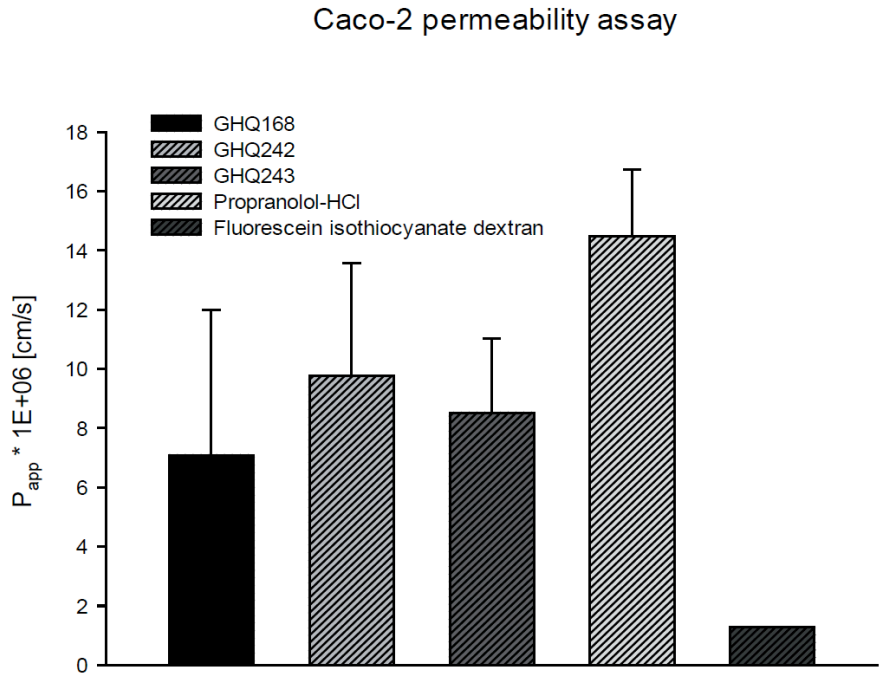


FIG S4 Caco-2 Cell assays illustrating the equal permeability of GHQ168, GHQ242, GHQ243, and Propranolol-HCl.

	P_{app} * 1E+06 cm/s				
	Propranolol-HCl	GHQ168	GHQ242	GHQ243	Fluorescein
MV [cm/s]	14.47	7.09	9.77	8.52	1.27
SD [cm/s]	2.28	4.90	3.80	2.51	0.02
RSD [%]	15.75	69.09	38.96	29.48	1.70

TABLE S2 Results for permeability testing in Caco-2 cells.

Calculation of P_{app}:

$$P_{app} = \frac{V_A}{Area\ x\ Time} x \frac{[drug]_{acceptor}}{[drug]_{initial,donor}}$$

$V_A = 0.6$, Area = 0.33, Time = 3600 s, $[drug]_{initial,\ donor} = 100$

In vitro activity and Cytotoxicity

In vitro activity

The anti-trypanosomal activity was recorded essentially as developed by Räz *et al.* (with some modifications, cf. Ilkay 2010). The compounds were dissolved in DMSO (20 mM).

In brief, *T.b. brucei* TC 221 were incubated at a cell density of 1 x 10^5 cells/mL in Complete Baltz medium (Baltz, 1985) with serial dilutions of the compounds at 37 °C and 5 % CO_2 for 72 h. The final DMSO concentration was 1 %. After 24 h of incubation, AlamarBlue was added to a final concentration of 1.1 mg/L and the plates were incubated further on for 48 h. Untreated cells and cells incubated with the solvent only were used as controls to check for parasite viability. The IC_{50} value was calculated with respect to negative controls (without compounds) from the absorbance values measured at λ=550nm using a microplate reader (Asys Expert 96, Biochrome, Cambridge, UK). Reference wavelength was set to λ=630nm.
T. brucei rhodesiense STIB 900 BSF trypomastigotes were seeded at 1 x 10^3 cells/mL in 200µL of growth medium (composition: Minimum Essential Medium 50 µL, 25 mM HEPES, 1g/L additional glucose, 1% MEM non-essential amino acids (100x), 0.2 mM 2-mercaptoethanol, 1 mM sodium pyruvate and 15% heat inactivated horse serum) with serial dilutions of the compounds. After incubation for 3 days at 37°C and 5% CO_2, 20µL of AlamarBlue (12.5mg/ 100mL) were added to each well and the plates were incubated for further 16h.

After reading the plates using an excitation wavelength of 536nm and an emission wavelength of 588nm (Multiskan Ascent, Thermo Fisher Scientific, Braunschweig, Germany), data were analysed and the IC50 value was calculated.

References:

1. **Räz B, Iten M, Grether-Bühler Y, Kaminsky R, Brun R.** 1997. The Alamar Blue® assay to determine drug sensitivity of African trypanosomes (T.b. rhodesiense and T.b. gambiense) in vitro. Acta Tropica **68:**139-147.

2. **Baltz T Baltz D, Giroud C, Crockett J.** 1985. Cultivation in a semi-defined medium of animal infective forms of Trypanosoma brucei, T. equiperdum, T. evansi, T. rhodesiense and T. gambiense. EMBO J **4:**1273-1277.

3. **Papadopoulou MV, Bloomer WD, Lepesheva GI, Rosenzweig HS, Kaiser M, Aguilera-Venegas B, Wilkins**

4. **on SR, Chatelain E, Ioset J-R**. 2015. Novel 3-nitrotriazole-based amides and carbinols as bifunctional antichagasic agents. Journal of medicinal chemistry **58**:1307-1319.

Cytotoxicity.

Cell viability was measured as described before (Larson 1997, Muth 2007). Briefly, the compounds were dissolved in DMSO to a concentration of 20 mM and serially diluted in DMSO. A defined number of mammalian cells (see below) were incubated in a volume of 200 µl in 96-well cell culture plates in the respective medium without phenol red with serial compound dilutions at 37 °C and 5 % CO_2. The final concentration of DMSO was 1 %. After 24 h of incubation, 10 % of an AlamarBlue solution was added. The CC_{50} value was calculated with respect to negative controls (without compounds) from the absorbance values measured at λ=550 nm using a microplate reader (Multiskan Ascent, Thermo Fisher Scientific, Braunschweig, Germany). The reference wavelength was set to λ=630 nm.

References:

1. **Larson EM, Doughman DJ, Gregerson DS, Obritsch WF.** 1997. A new, simple, nonradioactive, nontoxic in vitro assay to monitor corneal endothelial cell viability. Investigative Ophthalmology & Visual Science **38:**1929-1933

2. **Muth M, Hoerr V, Glaser M, Ponte-Sucre A, Moll H, Stich A, Holzgrabe U.** 2007. Antitrypanosomal activity of quaternary naphthalimide derivatives. Bioorganic & medicinal chemistry letters **17:**1590-1593.

4. PBPK modelling

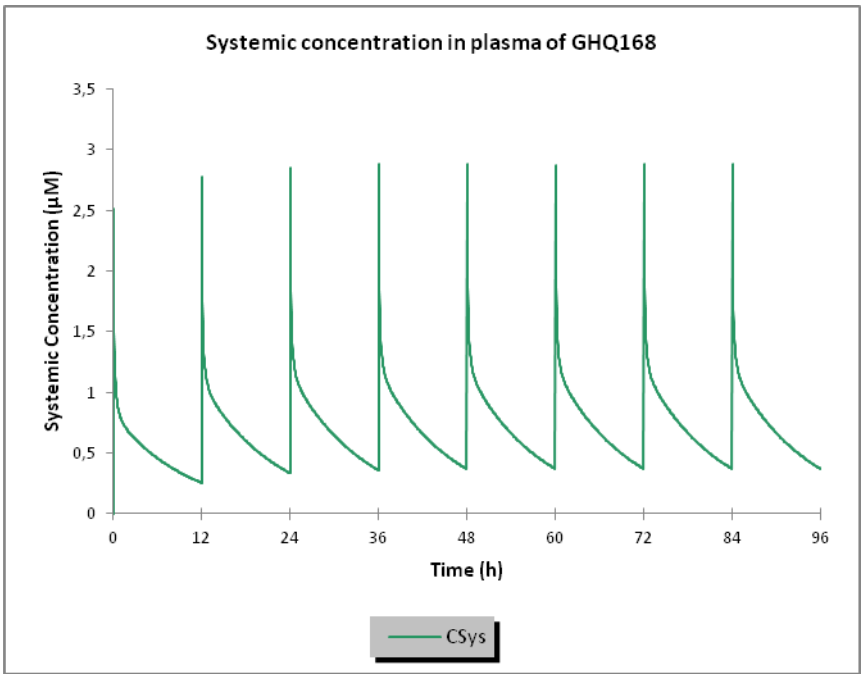


FIG S5A Simcyp plasma concentration prediction for GHQ168

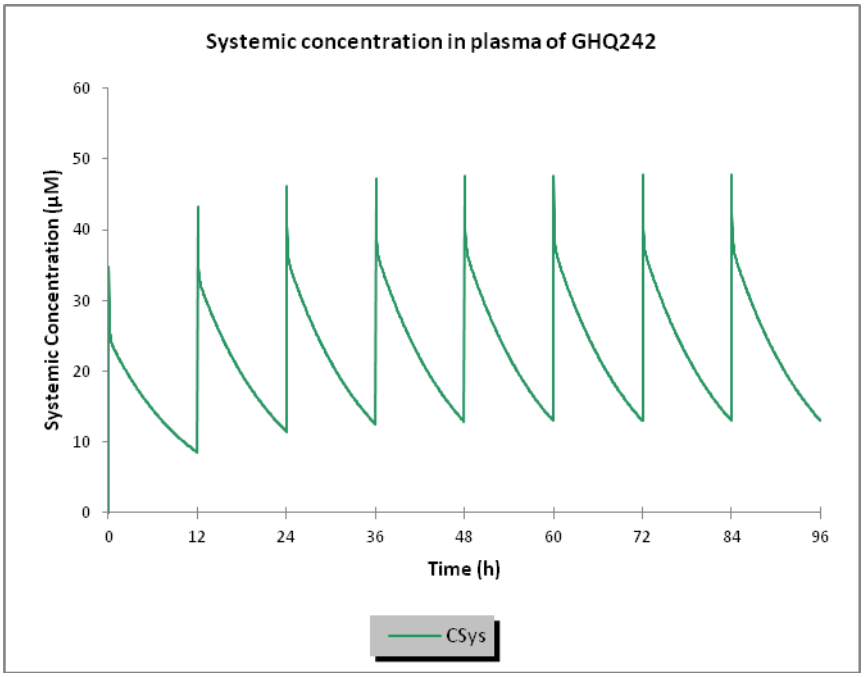


FIG S5B: Simcyp plasma concentration prediction for GHQ242

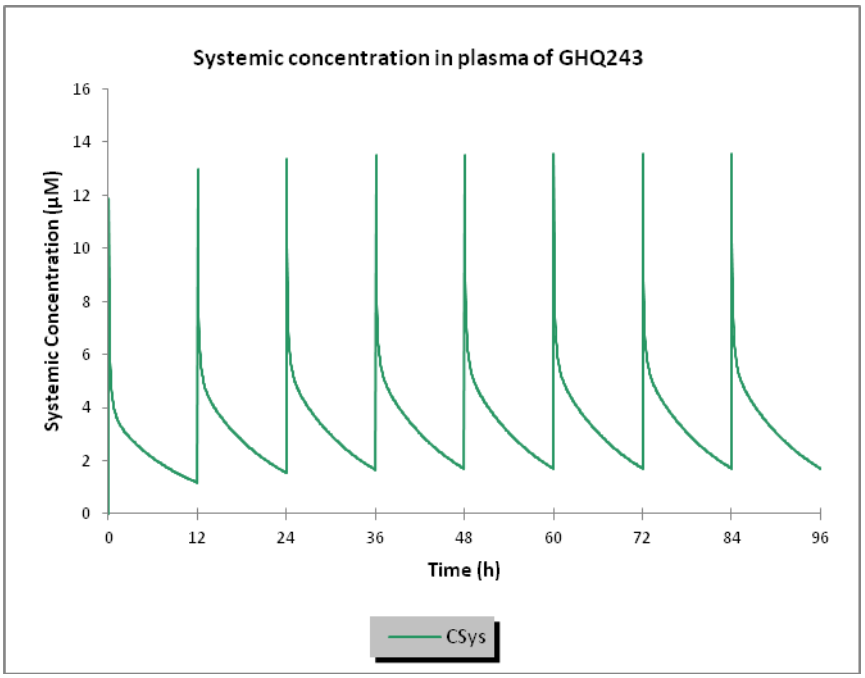


FIG S5C Simcyp plasma concentration prediction for GHQ243

5. Pharmacokinetics

Analytical Method Validation

GHQ168:

Sample Name	Concentration [ng/ml]	Peak Area GHQ168	Peak Area Int. standard	Ratio of Peak Areas	Weighting (1/c²)	Conc. calc. [ng/ml]	RD [%]
E08	500.000	1990000	23400000	0.0850427	4.00E-06	495.08	-1.0
E07	400.000	1720000	24600000	0.0699187	6.25E-06	407.05	1.8
E06	200.000	829000	23000000	0.0360435	2.50E-05	209.87	4.9
E05	100.000	398000	22300000	0.0178475	1.00E-04	103.96	4.0
E04	20.000	79200	22500000	0.0035200	2.50E-03	20.56	2.8
E03	5.000	16300	22400000	0.0007277	4.00E-02	4.31	-13.8
E02	2.000	6820	21100000	0.0003232	2.50E-01	1.96	-2.0
E01	1.000	3590	21700000	0.0001654	1.00E+00	1.04	4.0
Standard curve parameters		Slope 0.0001718		Intercept -0.000012760		Coefficient of Correlation 0.9977	

TABLE S3A Linearity data for GHQ168 in the concentration range of 1.0 - 500.0 ng/ml

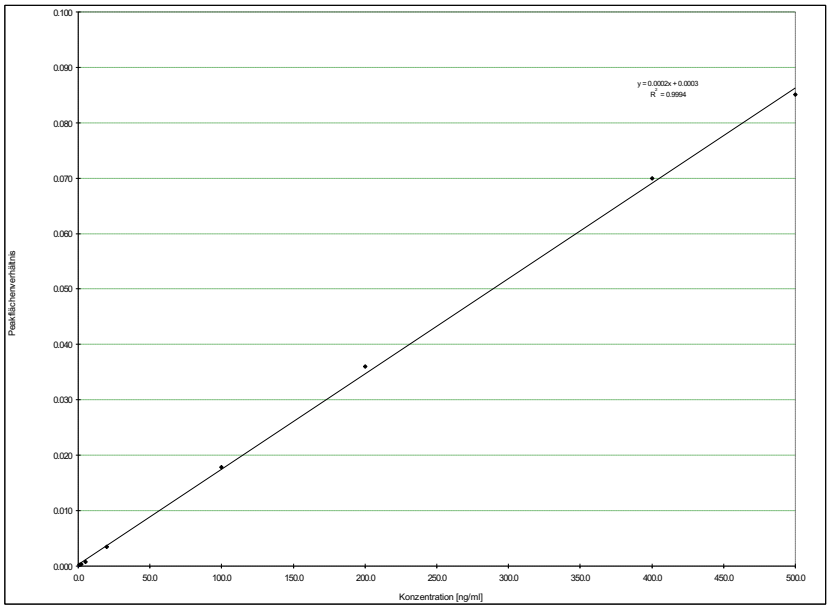


FIG S6A Linearity curve of GHQ168 in the concentration range of 1.0 - 500.0 ng/ml

Sample Name	High conc. [ng/ml]	Peak Area GHQ168	Peak Area Int. standard	Ratio of Peak Areas	Conc. calc. [ng/ml]	RD [%]
QC-H1	400.000	1620000	23100000	0.07013	408.281	2.1
QC-H2	400.000	1640000	24000000	0.06833	397.804	-0.5
QC-H3	400.000	1650000	23200000	0.07112	414.044	3.5
QC-H4	400.000	1730000	23300000	0.07425	432.263	8.1
QC-H5	400.000	1730000	22600000	0.07655	445.651	11.4
QC-H6	400.000	1690000	25200000	0.06706	390.412	-2.4
arithm. mean				0.07124	414.743	
±SD				0.003590	20.8970	
CV [%]				5.0	5.0	
RD [%]					3.7	

Sample Name	Medium conc. [ng/ml]	Peak Area GHQ168	Peak Area Int. standard	Ratio of Peak Areas	Conc. calc. [ng/ml]	RD [%]
QC-M1	100.000	402000	24000000	0.01675	97.571	-2.4
QC-M2	100.000	368000	24000000	0.01533	89.306	-10.7
QC-M3	100.000	374000	21600000	0.01731	100.831	0.8
QC-M4	100.000	393000	23000000	0.01709	99.550	-0.5
QC-M5	100.000	361000	21000000	0.01719	100.132	0.1
QC-M6	100.000	362000	21200000	0.01708	99.492	-0.5
arithm. mean				0.01679	97.814	
±SD				0.000740	4.3070	
CV [%]				4.4	4.4	
RD [%]					-2.2	

Sample Name	Low conc. [ng/ml]	Peak Area GHQ168	Peak Area Int. standard	Ratio of Peak Areas	Conc. calc. [ng/ml]	RD [%]
QC-L1	5.000	15800	21400000	0.00074	4.382	-12.4
QC-L2	5.000	16600	21200000	0.00078	4.614	-7.7
QC-L3	5.000	16700	20500000	0.00081	4.789	-4.2
QC-L4	5.000	15900	21000000	0.00076	4.498	-10.0
QC-L5	5.000	15700	20000000	0.00079	4.673	-6.5
QC-L6	5.000	17100	21100000	0.00081	4.789	-4.2
arithm. mean				0.00078	4.624	
±SD				0.000028	0.1621	
CV [%]				3.6	3.5	
RD [%]					-7.5	

TABLE S3B Precision data for GHQ168 at high, medium and low concentrations

GHQ242:

Sample Name	Concentration [ng/ml]	Peak Area GHQ168	Peak Area Int. standard	Ratio of Peak Areas	Weighting (1/c²)	Conc. calc. [ng/ml]	RD [%]
E08	500.000	1320000	21500000	0.0613953	4.00E-06	527.61	5.5
E07	400.000	993000	21400000	0.0464019	6.25E-06	398.80	-0.3
E06	200.000	491000	20500000	0.0239512	2.50E-05	205.92	3.0
E05	100.000	242000	21400000	0.0113084	1.00E-04	97.31	-2.7
E04	20.000	43700	20100000	0.0021741	2.50E-03	18.83	-5.9
E03	5.000	10700	19400000	0.0005515	4.00E-02	4.89	-2.2
E02	2.000	4660	20900000	0.0002230	2.50E-01	2.07	3.5
E01	1.000	2040	21000000	0.0000971	1.00E+00	0.99	-1.0
Standard curve parameters		Slope 0.0001164		Intercept -0.000018075		Coefficient of Correlation 0.9991	

TABLE S3C Linearity data for GHQ242 in the concentration range of 1.0 - 500.0 ng/ml

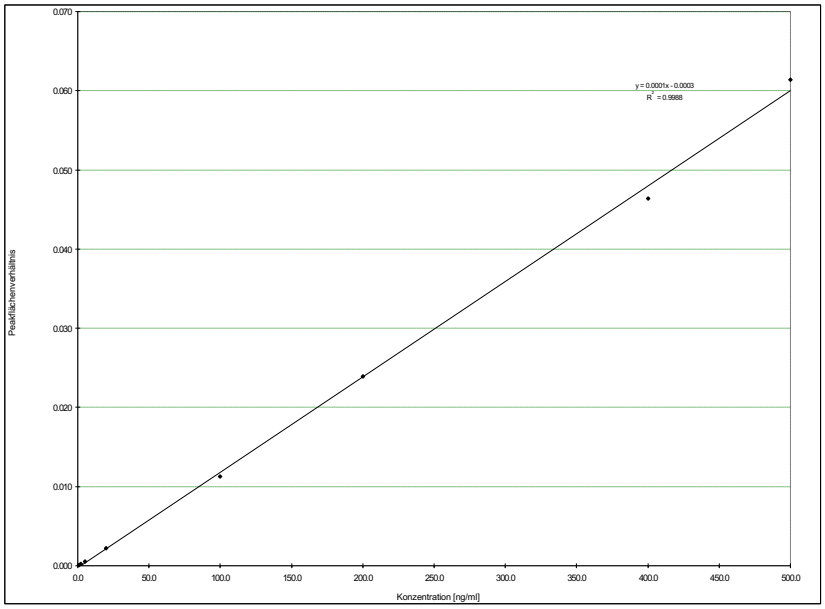


FIG S6B Linearity curve of GHQ242 in the concentration range of 1.0 - 500.0 ng/ml

Sample Name	High conc. [ng/ml]	Peak Area GHQ168	Peak Area Int. standard	Ratio of Peak Areas	Conc. calc. [ng/ml]	RD [%]
QC-H1	400.000	976000	20700000	0.04715	405.224	1.3
QC-H2	400.000	1020000	21900000	0.04658	400.327	0.1
QC-H3	400.000	985000	20700000	0.04758	408.918	2.2
QC-H4	400.000	978000	20000000	0.04890	420.258	5.1
QC-H5	400.000	1030000	21700000	0.04747	407.973	2.0
QC-H6	400.000	966000	20600000	0.04689	402.990	0.7
arithm. mean				0.04743	407.615	
±SD				0.000810	6.9555	
CV [%]				1.7	1.7	
RD [%]					1.9	

Sample Name	Medium conc. [ng/ml]	Peak Area GHQ168	Peak Area Int. standard	Ratio of Peak Areas	Conc. calc. [ng/ml]	RD [%]
QC-M1	100.000	230000	19700000	0.01168	100.499	0.5
QC-M2	100.000	231000	19200000	0.01203	103.506	3.5
QC-M3	100.000	232000	20500000	0.01132	97.406	-2.6
QC-M4	100.000	238000	21000000	0.01133	97.492	-2.5
QC-M5	100.000	249000	21400000	0.01164	100.155	0.2
QC-M6	100.000	238000	20500000	0.01161	99.898	-0.1
arithm. mean				0.01160	99.826	
±SD				0.000262	2.2544	
CV [%]				2.3	2.3	
RD [%]					-0.2	

Sample Name	Low conc. [ng/ml]	Peak Area GHQ168	Peak Area Int. standard	Ratio of Peak Areas	Conc. calc. [ng/ml]	RD [%]
QC-L1	5.000	11000	19800000	0.00056	4.966	-0.7
QC-L2	5.000	11200	21000000	0.00053	4.709	-5.8
QC-L3	5.000	11300	19900000	0.00057	5.052	1.0
QC-L4	5.000	11000	20000000	0.00055	4.880	-2.4
QC-L5	5.000	10600	20500000	0.00052	4.623	-7.5
QC-L6	5.000	10700	20400000	0.00052	4.623	-7.5
arithm. mean				0.00054	4.809	
±SD				0.000021	0.1833	
CV [%]				3.9	3.8	
RD [%]					-3.8	

TABLE S3D Precision data for GHQ242 at high, medium and low concentrations

GHQ243:

Sample Name	Concentration [ng/ml]	Peak Area GHQ168	Peak Area Int. standard	Ratio of Peak Areas	Weighting (1/c²)	Conc. calc. [ng/ml]	RD [%]
E08	500.000	516000	21400000	0.0241121	4.00E-06	519.76	4.0
E07	400.000	404000	20900000	0.0193301	6.25E-06	416.70	4.2
E06	200.000	189000	20700000	0.0091304	2.50E-05	196.88	-1.6
E05	100.000	98600	20900000	0.0047177	1.00E-04	101.78	1.8
E04	20.000	18300	20300000	0.0009015	2.50E-03	19.53	-2.3
E03	5.000	4570	21200000	0.0002156	4.00E-02	4.75	-5.0
E02	2.000	1830	21800000	0.0000839	2.50E-01	1.91	-4.5
E01	1.000	895	20700000	0.0000432	1.00E+00	1.03	3.0
Standard curve parameters		Slope 0.0000464		Intercept -0.000004727		Coefficient of Correlation 0.9991	

TABLE S3E Linearity data for GHQ243 in the concentration range of 1.0 - 500.0 ng/ml

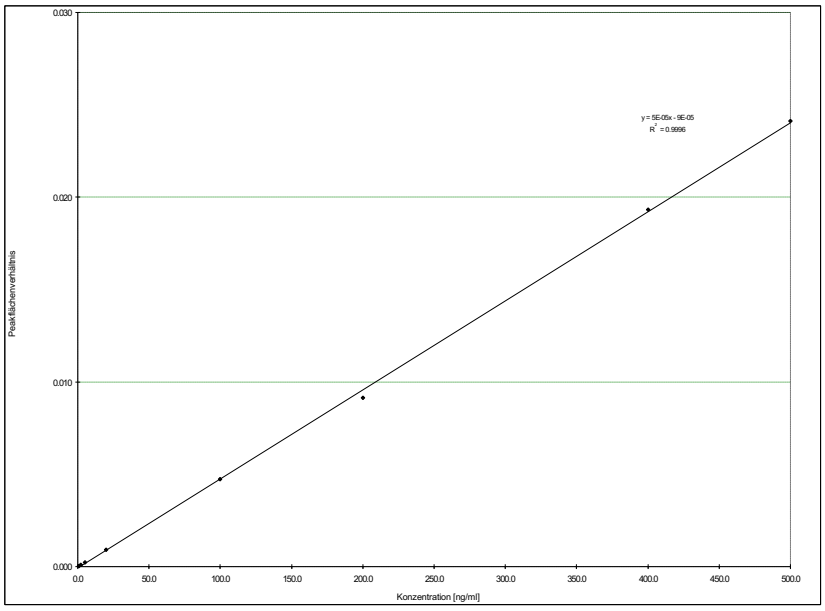


FIG S6C Linearity curve of GHQ243 in the concentration range of 1.0 - 500.0 ng/ml

Sample Name	High conc. [ng/ml]	Peak Area GHQ168	Peak Area Int. standard	Ratio of Peak Areas	Conc. calc. [ng/ml]	RD [%]
QC-H1	400.000	402000	21000000	0.01914	412.602	3.2
QC-H2	400.000	401000	19600000	0.02046	441.050	10.3
QC-H3	400.000	387000	20500000	0.01888	406.998	1.7
QC-H4	400.000	405000	20900000	0.01938	417.774	4.4
QC-H5	400.000	411000	20600000	0.01995	430.059	7.5
arithm. mean				0.01956	421.697	
±SD				0.000639	13.7716	
CV [%]				3.3	3.3	
RD [%]					5.4	

Sample Name	Medium conc. [ng/ml]	Peak Area GHQ168	Peak Area Int. standard	Ratio of Peak Areas	Conc. calc. [ng/ml]	RD [%]
QC-M1	100.000	102000	21400000	0.00477	102.904	2.9
QC-M2	100.000	97900	20600000	0.00475	102.473	2.5
QC-M3	100.000	99800	19800000	0.00504	108.723	8.7
QC-M4	100.000	98200	20200000	0.00486	104.843	4.8
QC-M5	100.000	96700	20700000	0.00467	100.748	0.7
QC-M6	100.000	98200	20600000	0.00477	102.904	2.9
arithm. mean				0.00481	103.766	
±SD				0.000128	2.7567	
CV [%]				2.7	2.7	
RD [%]					3.8	

Sample Name	Low conc. [ng/ml]	Peak Area GHQ168	Peak Area Int. standard	Ratio of Peak Areas	Conc. calc. [ng/ml]	RD [%]
QC-L1	5.000	4650	20000000	0.00023	5.059	1.2
QC-L2	5.000	4610	21600000	0.00021	4.628	-7.4
QC-L3	5.000	4290	21000000	0.00020	4.412	-11.8
QC-L4	5.000	4420	21100000	0.00021	4.628	-7.4
QC-L5	5.000	4790	21900000	0.00022	4.843	-3.1
QC-L6	5.000	5110	21400000	0.00024	5.274	5.5
arithm. mean				0.00022	4.807	
±SD				0.000015	0.3172	
CV [%]				6.8	6.6	
RD [%]					-3.9	

TABLE S3F Precision data for GHQ168 at high, medium and low concentrations

Blood concentration levels

Compound	Animal	Time [h]	Conc. [ng/ml]	Conc. [μM]
GHQ168	1	1	293.0	0.669714
	2		195.9	0.447771
	3		46.2	0.105600
	1	4	17.6	0.040229
	2		27.1	0.061943
	3		75.7	0.173029
	1	16	0.3	0.000686
	2		1.7	0.003886
	3		1.0	0.002286
GHQ242	1	1	11.7	0.022137
	2		17.4	0.032921
	3		3.2	0.006055
	4		49.8	0.094224
	1	4	2.9	0.005487
	2		8.4	0.015893
	3		5.5	0.010406
	4		10.1	0.019110
	1	16	14.6	0.027624
	2		1.0	0.001892
	3		2.2	0.004162
	4		< 1.0	0.000946
GHQ243	1	1	376.1	0.693195
	2		191.1	0.352219
	3		223.0	0.411014
	4		202.6	0.373415
	1	4	41.8	0.077042
	2		14.3	0.026357
	3		22.8	0.042023
	4		13.2	0.024329
	1	16	4.0	0.007372
	2		5.5	0.010137
	3		2.0	0.003686
	4		< 1.0	0.000922

TABLE S4: Blood concentration levels of GHQ168, GHQ242 and GHQ243 in mice.

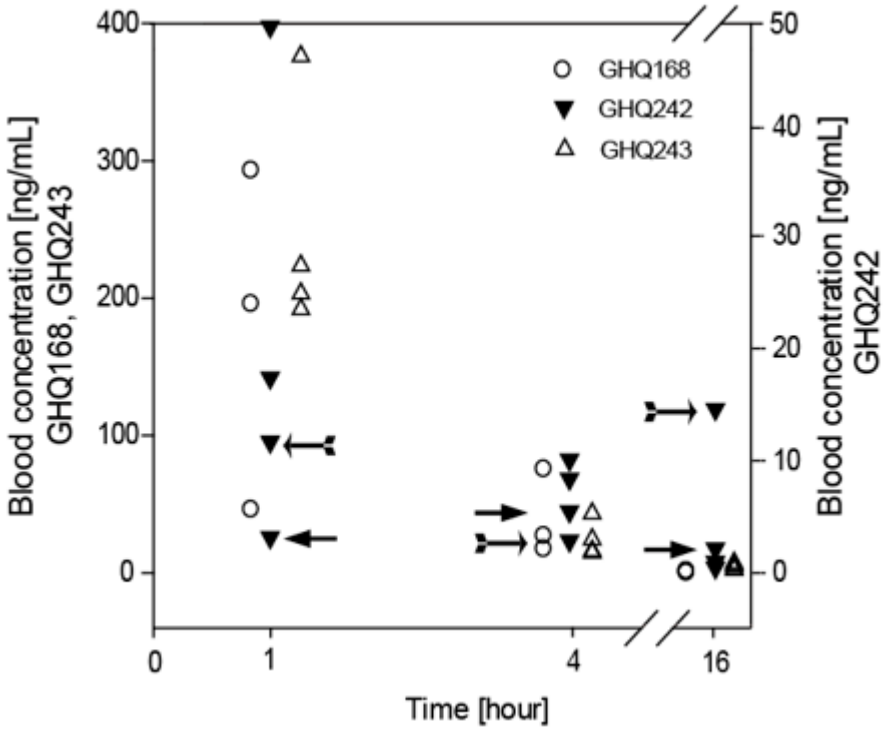


FIGURE S7: Blood concentration [ng/mL] versus time profiles [hour]. Arrows highlight two individual mice exposed to GHQ242 for which concentration results from subsequent samplings exceeded those of previous samples. The left axis is for GHQ168, and GHQ 243, the right axis for GHQ242.