Supporting Information

Optimization and Evaluation of Antiparasitic Benzamidobenzoic Acids as Inhibitors of a Kinetoplastid Hexokinase 1

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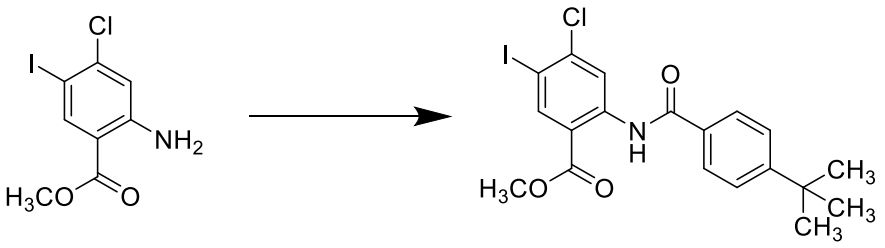
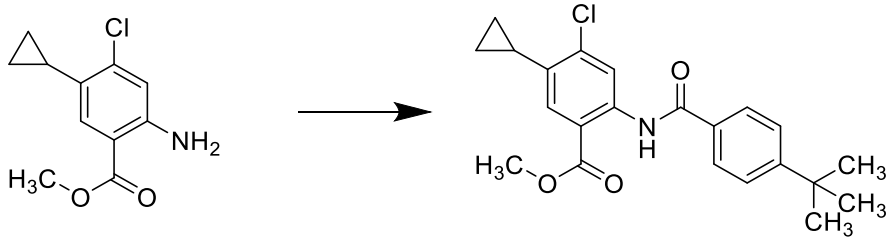
Chemical experimental and analytical details:

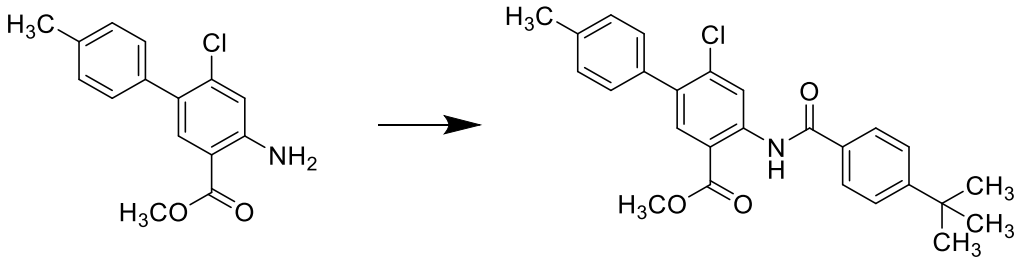
The synthesis and characterization of compounds **2, 4a** and **4b** have been previously described.[1]



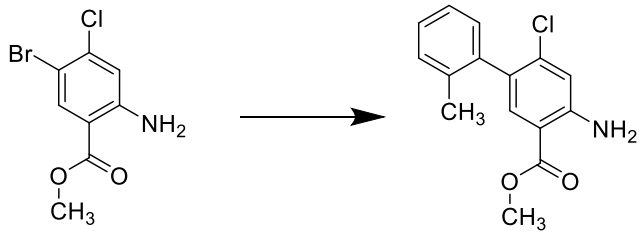
Methyl 2-(4-(*tert*-butyl)benzamido)-4-chloro-5-iodobenzoate. To a microwave vial was added methyl 2-amino-4-chloro-5-iodobenzoate (0.100 g, 0.321 mmol), 4-*tert*-butylbenzoyl chloride (0.064 ml, 0.353 mmol) and acetonitrile (5 mL). The reaction was heated at 150 °C in the microwave for 30 min. After cooling to rt, the reaction was diluted with saturated $NaHCO_3$ (3 mL), extracted with EtOAc (3x 5 mL), dried ($MgSO_4$), filtered and concentrated to produce methyl 2-(4-(*tert*-butyl)benzamido)-4-chloro-5-iodobenzoate (0.151 g, 0.320 mmol, 100 % yield). The product was used without further purification. ^{1}H NMR (400 MHz, CDCl3) δ 11.94 (s, 1H), 9.19 (s, 1H), 8.50 (s, 1H), 7.95 (d, J = 8.5 Hz, 2H), 7.55 (d, J = 8.5 Hz, 2H), 3.97 (s, 3H), 1.36 (s, 9H).



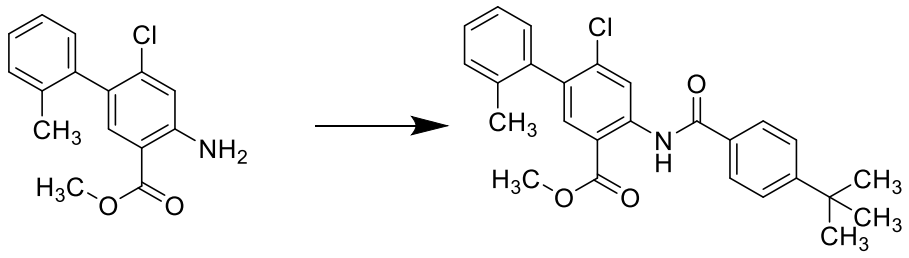
Methyl 2-(4-*tert*-butylbenzamido)-4-chloro-5-cyclopropylbenzoate. Prepared as described for compound **4d** (*step 2*). Purified by reverse-phase MPLC (10 - 100% MeCN:water) to produce methyl 2-(4-*tert*-butylbenzamido)-4-chloro-5-cyclopropyl benzoate (0.013 g, 0.034 mmol, 38% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.91 (s, 1H), 9.05 (s, 1H), 7.96 (d, *J* = 8.5 Hz, 2H), 7.66 (s, 1H), 7.54 (d, *J* = 8.5 Hz, 2H), 3.95 (s, 3H), 2.18 – 2.10 (m, 1H), 1.36 (s, 9H), 1.05 – 0.97 (m, 2H), 0.72 – 0.66 (m, 2H).



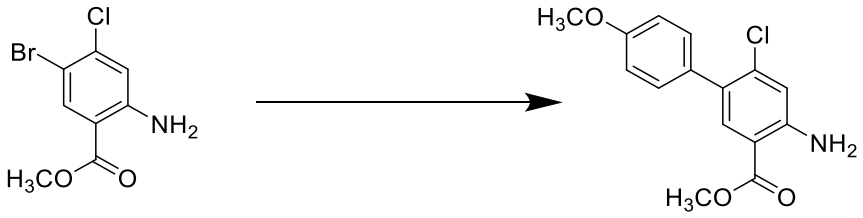
Methyl 4-(4-*tert*-butylbenzamido)-6-chloro-4'-methyl-[1,1'-biphenyl]-3-carboxylate. Prepared as described for compound **4d** (*step 2*). Purified by reverse-phase MPLC (10 - 100%, MeCN:water) to provide methyl 4-(4-*tert*-butylbenzamido)-6-chloro-4'-methyl-[1,1'-biphenyl]-3-carboxylate (0.055 g, 0.13 mmol, 79% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.01 (s, 1H), 9.17 (s, 1H), 8.06 (S, 1H), 7.99 (d, *J* = 8.6 Hz, 2H), 7.56 (d, *J* = 8.6 Hz, 2H), 7.35 (d, *J* = 8.1 Hz, 2H), 7.26 (d, *J* = 7.8 Hz, 2H), 3.95 (s, 3H), 2.42 (s, 3H), 1.37 (s, 9H).



Methyl 4-amino-6-chloro-2'-methyl-[1,1'-biphenyl]-3-carboxylate. Prepared as described for compound **4g** (*step 1*). The crude product was purified by reverse-phase MPLC (10 - 100% MeCN:water) to provide methyl 4-amino-6-chloro-2'-methyl-[1,1'-biphenyl]-3-carboxylate (0.097 g, 0.35 mmol, 93% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (s, 1H), 7.32 – 7.10 (m, 4H), 6.84 (s, 1H), 3.85 (s, 3H), 2.14 (s, 3H).

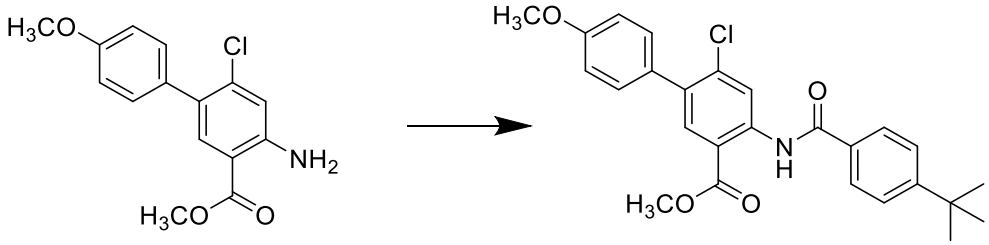


Methyl 4-(4-*tert*-butylbenzamido)-6-chloro-2'-methyl-[1,1'-biphenyl]-3-carboxylate. Prepared as described for compound **4d** (*step 2*). Purified by MPLC (0 - 15% EtOAc:hexanes) to produce methyl 4-(4-*tert*-butylbenzamido)-6-chloro-2'-methyl-[1,1'-biphenyl]-3-carboxylate (0.098 g, 0.23 mmol, 64% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.04 (s, 1H), 9.18 (s, 1H), 8.00 (d, J = 8.5 Hz, 2H), 7.97 (s, 1H), 7.57 (d, J = 8.5 Hz, 2H), 7.35 – 7.24 (m, 3H), 7.16 – 7.13 (m, 1H), 3.94 (s, 3H), 2.15 (s, 3H), 1.37 (s, 9H).

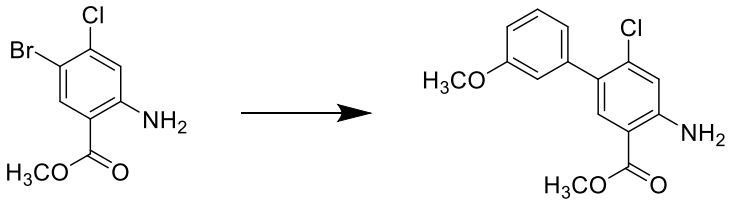


Methyl 4-amino-6-chloro-4'-methoxy-[1,1'-biphenyl]-3-carboxylate. Prepared as described for compound **4g** (*step 1*) to afford, after purification, methyl 4-amino-6-chloro-4'-

methoxy-[1,1'-biphenyl]-3-carboxylate (0.058 g, 0.20 mmol, 59% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.33 (d, *J* = 8.8 Hz, 2H), 6.94 (d, *J* = 8.8 Hz, 2H), 6.79 (s, 1H), 5.77 (brs, 2H), 3.86 (s, 3H), 3.85 (s, 3H).

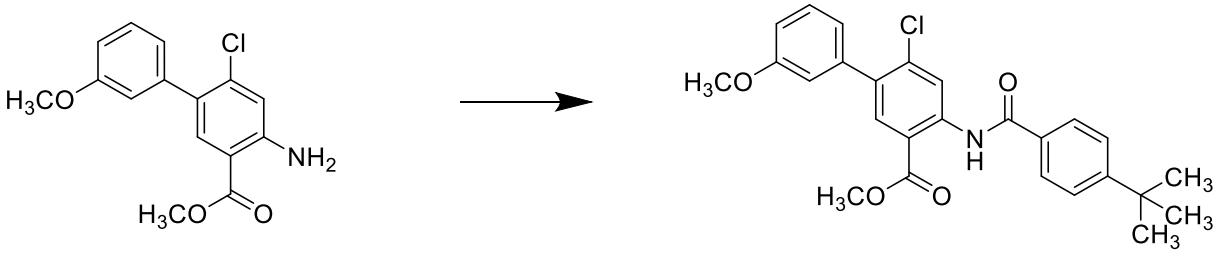


Methyl 4-(4-*tert*-butylbenzamido)-6-chloro-4'-methoxy-[1,1'-biphenyl]-3-carboxylate. Prepared as described for compound **4d** (*step 2*). Purified by MPLC (0 - 20% EtOAc:hexanes) to provide methyl 4-(4-*tert*-butylbenzamido)-6-chloro-4'-methoxy-[1,1'-biphenyl]-3-carboxylate (0.081 g, 0.18 mmol, 90% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.0 (s, 1H), 9.16 (s, 1H), 8.05 (s, 1H), 7.99 (d, *J* = 8.4 Hz, 2H), 7.56 (d, *J* = 8.5 Hz, 2H), 7.39 (d, *J* = 8.7 Hz, 2H), 6.98 (d, *J* = 8.7 Hz, 2H), 3.96 (s, 3H), 3.87 (s, 3H), 1.37 (s, 9H).

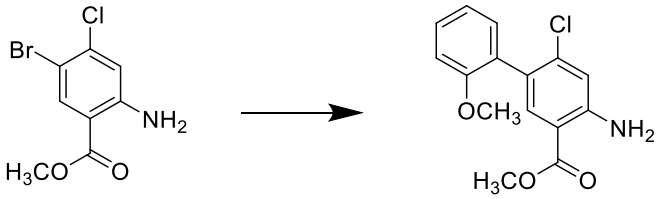


Methyl 4-amino-6-chloro-3'-methoxy-[1,1'-biphenyl]-3-carboxylate. Prepared as described for compound **4f** (*step 1*). Purified by reverse-phase MPLC (10 - 100% MeCN:water) to provide methyl 4-amino-6-chloro-3'-methoxy-[1,1'-biphenyl]-3-carboxylate

(0.084 g, 0.29 mmol, 81% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (s, 1H), 7.31 (t, *J* = 7.8 Hz, 1H), 7.01 – 6.88 (m, 3H), 6.81 (s, 1H), 3.86 (s, 3H), 3.84 (s, 3H).

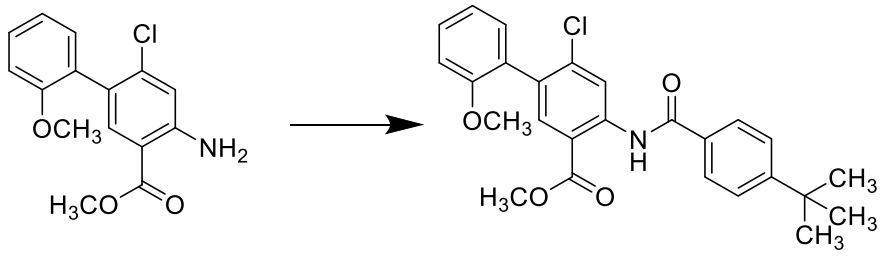


Methyl 4-(4-*tert*-butylbenzamido)-6-chloro-3'-methoxy-[1,1'-biphenyl]-3-carboxylate. Prepared as described for compound **4d** (*step 2*). Purified by MPLC (0 - 15% EtOAc:hexanes) to produce pure methyl 4-(4-*tert*-butylbenzamido)-6-chloro-3'-methoxy-[1,1'-biphenyl]-3-carboxylate (0.065 g, 0.14 mmol, 50% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.02 (s, 1H), 9.18 (s, 1H), 8.08 (s, 1H), 8.00 (d, *J* = 8.5 Hz, 2H), 7.56 (d, *J* = 8.7 Hz, 2H), 7.37 (t, *J* = 8.1 Hz, 1H), 7.04 – 6.93 (m, 3H), 3.96 (s, 3H), 3.86 (s, 3H), 1.37 (s, 9H).

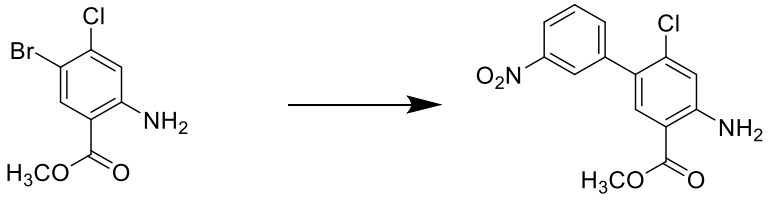


Methyl 4-amino-6-chloro-2'-methoxy-[1,1'-biphenyl]-3-carboxylate. Prepared as described for compound **4f** (*step 1*). Purified by reverse-phase MPLC (10 - 100% MeCN:water) provided methyl 4-amino-6-chloro-2'-methoxy-[1,1'-biphenyl]-3-carboxylate

(0.076 g, 0.26 mmol, 67% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.38 – 7.33 (m, 1H), 7.19 – 7.15 (m, 1H), 7.03 – 6.94 (m, 2H), 6.83 (s, 1H), 3.84 (s, 3H), 3.79 (s, 3H).

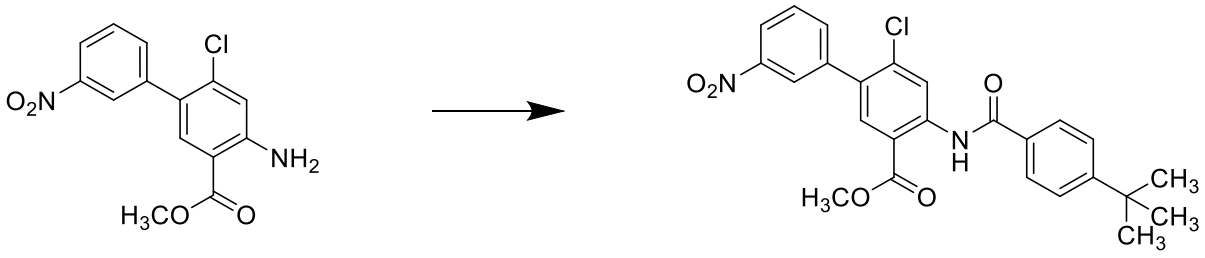


Methyl 4-(4-*tert*-butylbenzamido)-6-chloro-2'-methoxy-[1,1'-biphenyl]-3-carboxylate. Prepared as described for compound **4d** (*step 2*). Purified by MPLC (0 - 15% EtOAc:hexanes) to produce methyl 4-(4-*tert*-butylbenzamido)-6-chloro-2'-methoxy-[1,1'-biphenyl]-3-carboxylate (0.083 g, 0.18 mmol, 69% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.04 (s, 1H), 9.16 (s, 1H), 8.02 (s, 1H), 8.00 (d, J = 8.5 Hz, 2H), 7.56 (d, J = 8.6 Hz, 2H), 7.42 – 7.37 (m, 1H), 7.21 – 7.18 (m, 1H), 7.06 – 6.97 (m, 2H), 3.93 (s, 3H), 3.79 (s, 3H), 1.37 (s, 9H).

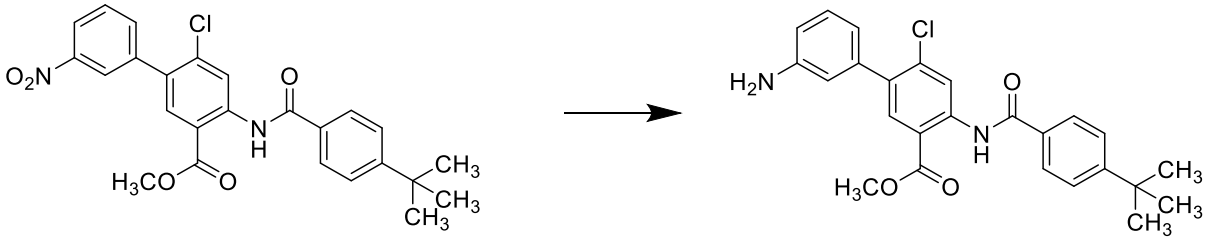


Methyl 4-amino-6-chloro-3'-nitro-[1,1'-biphenyl]-3-carboxylate. Prepared as described for compound **4f** (*step 1*). Purification by reverse-phase MPLC (10 - 100% MeCN:water) provided

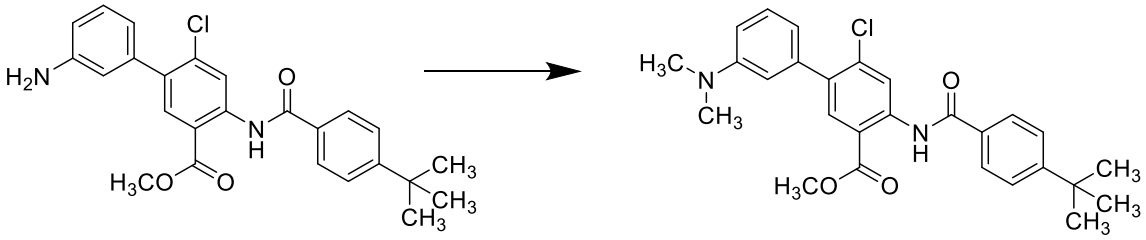
methyl 4-amino-6-chloro-3'-nitro-[1,1'-biphenyl]-3-carboxylate (0.17 g, 0.55 mmol, 74% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (t, *J* = 2.0 Hz, 1H), 8.22 – 8.18 (m, 1H), 7.87 (s, 1H), 7.75 – 7.45 (m, 1H), 7.57 (t, *J* = 8.2 Hz, 1H), 6.85 (s, 1H), 3.88 (s, 3H).



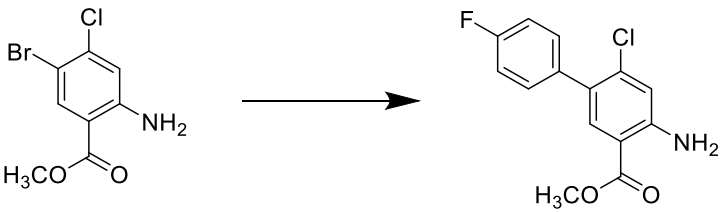
Methyl 4-(4-*tert*-butylbenzamido)-6-chloro-3'-nitro-[1,1'-biphenyl]-3-carboxylate. Prepared as described for compound **4d** (*step 2*). Purified by MPLC (0 - 15% EtOAc:hexanes) to produce methyl 4-(4-*tert*-butylbenzamido)-6-chloro-3'-nitro-[1,1'-biphenyl]-3-carboxylate (0.22 g, 0.46 mmol, 89% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.07 (s, 1H), 9.25 (s, 1H), 8.33 (t, *J* = 2.0 Hz, 1H), 8.29 – 8.25 (m, 1H), 8.09 (s, 1H), 8.00 (d, *J* = 8.5 Hz, 2H), 7.84 – 7.81 (m, 1H), 7.63 (t, *J* = 8.0 Hz, 1H), 7.57 (d, *J* = 8.6 Hz, 2H), 3.99 (s, 3H), 1.38 (s, 9H).



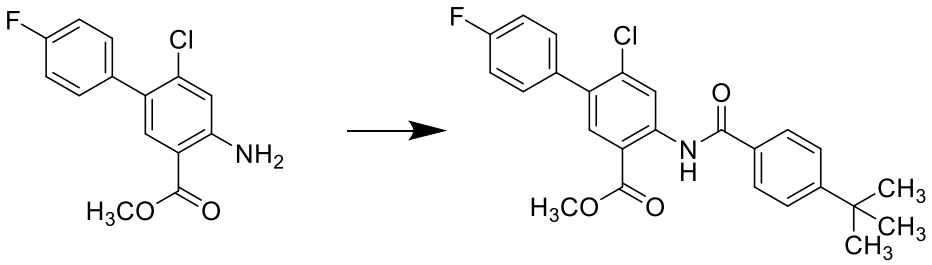
Methyl 3'-amino-4-(4-*tert*-butylbenzamido)-6-chloro-[1,1'-biphenyl]-3-carboxylate. Prepared as described for compound **4l** (*step 3*). Purified by MPLC (0 - 10% MeOH:CH_2Cl_2) to provide pure methyl 3'-amino-4-(4-*tert*-butylbenzamido)-6-chloro-[1,1'-biphenyl]-3-carboxylate (0.142 g, 0.325 mmol, 84% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.02 (s, 1H), 9.17 (s, 1H), 8.05 (s, 1H), 7.99 (d, *J* = 8.4 Hz, 2H), 7.55 (d, *J* = 8.6 Hz, 2H), 7.40 – 7.33 (m, 1H), 7.13 – 7.09 (m, 3H), 3.95 (s, 3H), 1.37 (s, 9H).



Methyl 4-(4-*tert*-butylbenzamido)-6-chloro-3'-(dimethylamino)-[1,1'-biphenyl]-3-carboxylate. Prepared as described for compound**4l** (*step 4*). Purified by reverse-phase MPLC (10 - 100% MeCN:water) to produce methyl 4-(4-*tert*-butylbenzamido)-6-chloro-3'-(dimethylamino)-[1,1'-biphenyl]-3-carboxylate (0.021 g, 0.045 mmol, 50% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.01 (s, 1H), 9.16 (s, 1H), 8.10 (s, 1H), 8.00 (d, *J* = 8.5 Hz, 2H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.30 (t, *J* = 8.8 Hz, 1H), 6.79 – 6.75 (m, 3H), 3.95 (s, 3H), 2.99 (s, 6H), 1.37 (s, 9H).

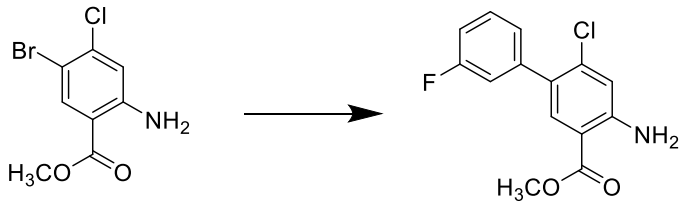


Methyl 4-amino-6-chloro-4'-fluoro-[1,1'-biphenyl]-3-carboxylate. Prepared as described for compound **4g** (*step 1*). Purification afforded methyl 4-amino-6-chloro-4'-fluoro-[1,1'-biphenyl]-3-carboxylate (0.093 g, 0.33 mmol, 41% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (s, 1H), 7.36 (d, *J* = 8.8 Hz, 1H), 7.35 (d, *J* = 8.8 Hz, 1H), 7.10 (d, *J* = 8.8 Hz, 1H), 7.07 (d, *J* = 8.8 Hz, 1H), 6.80 (s, 1H), 5.81 (br s, 2H), 3.86 (s, 3H).

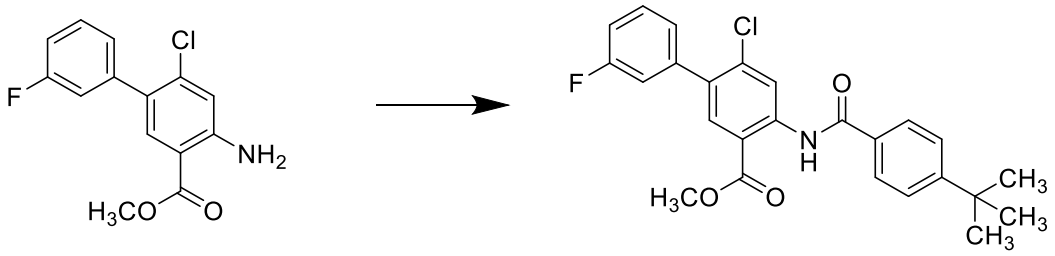


Methyl 4-(4-*tert*-butylbenzamido)-6-chloro-4'-fluoro-[1,1'-biphenyl]-3-carboxylate. Prepared as described for compound **4d** (s*tep 2*). Upon cooling the reaction mixture to rt, a precipitate formed. The solid was filtered, washed with MeCN and was shown to be pure by ^{1}H NMR. Isolated methyl 4-(4-*tert*-butylbenzamido)-6-chloro-4'-fluoro-[1,1'-biphenyl]-3-carboxylate (0.078 g, 0.177 mmol, 53% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.02 (s, 1H), 9.19 (s, 1H), 8.04 (s, 1H), 7.99 (d, *J* = 8.5 Hz, 2H), 7.56 (d, *J* = 8.5 Hz, 2H), 7.43 (d, *J* = 8.7

Hz, 1H), 7.41 (d, *J* = 8.7 Hz, 1H), 7.15 (d, *J* = 8.7 Hz, 1H), 7.12 (d, *J* = 8.7 Hz, 1H), 3.97 (s, 3H), 1.37 (s, 9H).

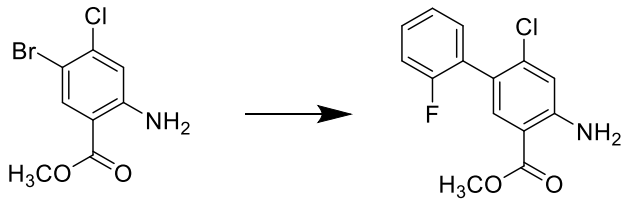


Methyl 4-amino-6-chloro-3'-fluoro-[1,1'-biphenyl]-3-carboxylate. Prepared as described for compound **4g** (*step 1*). Purification by reverse-phase MPLC (10 - 100% MeCN:water) provided methyl 4-amino-6-chloro-3'-fluoro-[1,1'-biphenyl]-3-carboxylate (0.051 g, 0.18 mmol, 52% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (s, 1H), 7.39 – 7.33 (m, 1H), 7.19 – 7.16 (m, 1H), 7.14 – 7.10 (m, 1H), 7.07 – 7.01 (m, 1H), 6.84 (s, 3H), 3.87 (s, 3H).

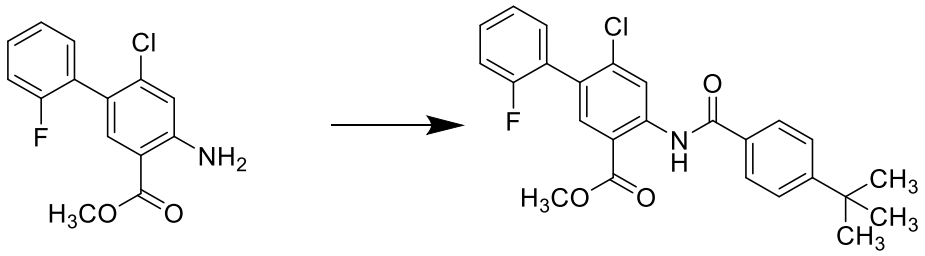


Methyl 4-(4-(*tert*-butyl)benzamido)-6-chloro-3'-fluoro-[1,1'-biphenyl]-3-carboxylate. Prepared as described for compound **4d** (s*tep 2*). Purified by MPLC (0 - 15% EtOAc:hexanes) to produce methyl 4-(4-*tert*-butylbenzamido)-6-chloro-3'-fluoro-[1,1'-biphenyl]-3-carboxylate (0.076 g, 0.17 mmol, 95% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.03 (s, 1H), 9.20 (s, 1H),

8.05 (s, 1H), 7.99 (d, *J* = 8.7 Hz, 2H), 7.56 (d, *J* = 8.6 Hz, 2H), 7.44 – 7.38 (m, 1H), 7.24 – 7.21 (m, 1H), 7.19 – 7.15 (m, 1H), 7.12 – 7.06 (m, 1H), 3.97 (s, 3H), 1.37 (s, 9H).

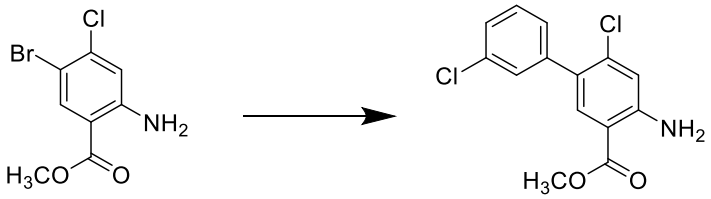


Methyl 4-amino-6-chloro-2'-fluoro-[1,1'-biphenyl]-3-carboxylate. Prepared as described for compound **4g** (*step 1*).Purification by reverse-phase MPLC (10 - 100% MeCN:water) provided methyl 4-amino-6-chloro-2'-fluoro-[1,1'-biphenyl]-3-carboxylate (0.077 g, 0.28 mmol, 76% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.38 – 7.26 (m, 2H), 7.21 – 7.10 (m, 2H), 3.85 (s, 3H).

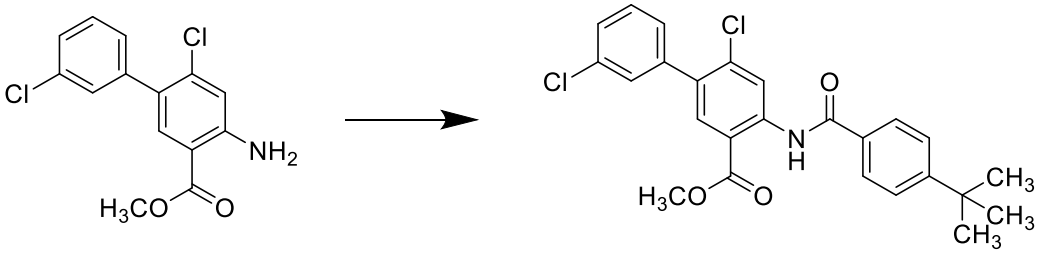


Methyl 4-(4-*tert*-butylbenzamido)-6-chloro-2'-fluoro-[1,1'-biphenyl]-3-carboxylate. Prepared as described for compound **4d** (s*tep 2*). Purified by MPLC (0 - 15% EtOAc:hexanes) to produce methyl 4-(4-*tert*-butylbenzamido)-6-chloro-2'-fluoro-[1,1'-biphenyl]-3-carboxylate (0.065 g, 0.15 mmol, 54% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.06 (s, 1H), 9.21 (s, 1H),

8.07 (s, 1H), 8.00 (d, *J* = 8.5 Hz, 2H), 7.56 (d, *J* = 8.5 Hz, 2H), 7.44 – 7.38 (m, 1H), 7.35 – 7.30 (m, 1H), 7.25 – 7.14 (m, 2H), 3.96 (s, 3H), 1.37 (s, 9H).

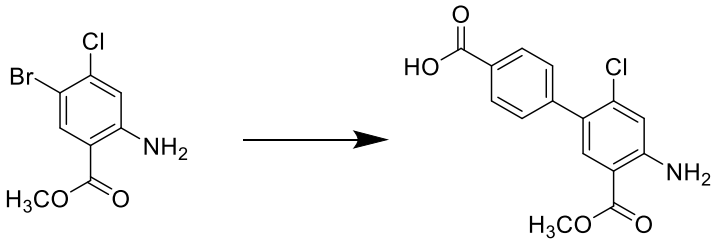


Methyl 4-amino-3',6-dichloro-[1,1'-biphenyl]-3-carboxylate. Prepared as described for compound **4f** (*step 1*). Purification by reverse-phase MPLC (10 - 100% MeCN:water) provide methyl 4-amino-3',6-dichloro-[1,1'-biphenyl]-3-carboxylate (0.040 g, 0.14 mmol, 33% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.40 – 7.39 (m, 1H), 7.33 – 7.27 (m, 3H), 6.81 (s, 1H), 3.87 (s, 3H).

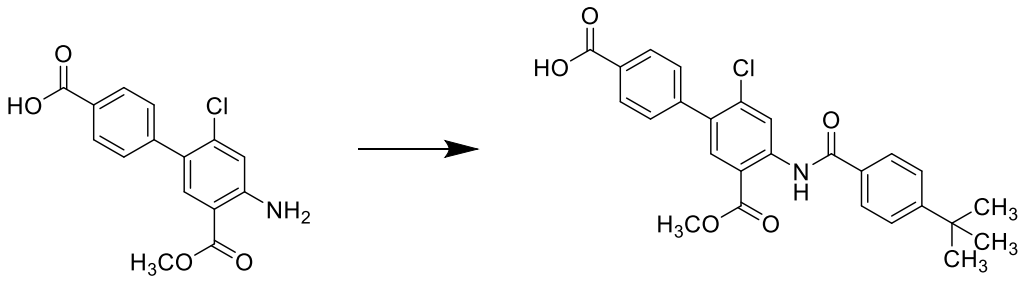


Methyl 4-(4-*tert*-butylbenzamido)-3',6-dichloro-[1,1'-biphenyl]-3-carboxylate. Prepared as described for compound **4d** (s*tep 2*). Purified by MPLC (0 - 15% EtOAc:hexanes) to produce methyl 4-(4-*tert*-butylbenzamido)-3',6-dichloro-[1,1'-biphenyl]-3-carboxylate (0.053 g, 0.12 mmol, 86% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.03 (s, 1H), 9.19 (s, 1H), 8.04 (s,

1H), 7.99 (d, *J* = 8.5 Hz, 2H), 7.56 (d, *J* = 8.7 Hz, 2H), 7.44 – 7.42 (m, 1H), 7.39 – 7.32 (m, 3H), 3.97 (s, 3H), 1.37 (s, 9H).

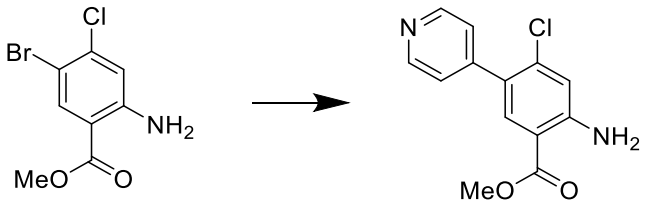


4'-Amino-2'-chloro-5'-(methoxycarbonyl)-[1,1'-biphenyl]-4-carboxylic acid. Prepared as described for compound **4f** (*step 1*). Purification by reverse-phase MPLC (10 - 100% MeCN:water) provided 4'-amino-2'-chloro-5'-(methoxycarbonyl)-[1,1'-biphenyl]-4-carboxylic acid (0.11 g, 0.36 mmol, 92% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.85 (d, *J* = 8.2 Hz, 2H), 7.68 (s, 1H), 7.22 (d, *J* = 8.2 Hz, 2H), 7.00 (s, 1H), 6.89 (br s, 2H), 3.80 (s, 3H).

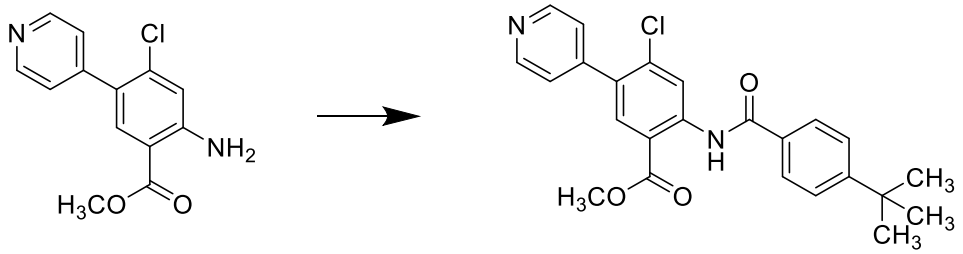


4'-(4-*tert*-Butylbenzamido)-2'-chloro-5'-(methoxycarbonyl)-[1,1'-biphenyl]-4-carboxylic acid. Prepared as described for compound **4d** (*step 2*). Purified by MPLC (0 - 10% MeOH:CH_2Cl_2) to produce 4'-(4-*tert*-butylbenzamido)-2'-chloro-5'-(methoxycarbonyl)-[1,1'-biphenyl]-4-carboxylic acid (0.045 g, 0.097 mmol, 25% yield) as an off-white solid. ^{1}H NMR

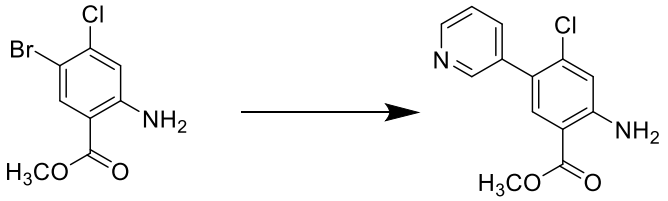
(400 MHz, $CDCl_3$) δ 9.10 (s, 1H), 8.12 (s, 1H), 8.11 (d, *J* = 8.7 Hz, 2H), 7.98 (d, *J* = 8.5 Hz, 2H), 7.63 (d, *J* = 8.5 Hz, 2H), 7.57 (d, *J* = 8.7 Hz, 2H), 4.00 (s, 3H), 1.39 (s, 9H).



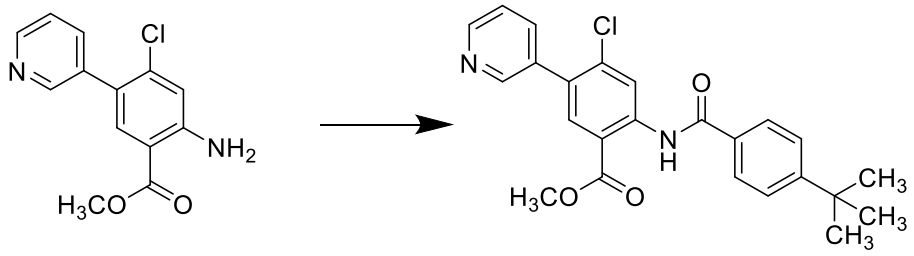
Methyl 2-amino-4-chloro-5-(pyridin-4-yl)benzoate. Prepared as described for compound **4g** (*step 1*). Purification by reverse-phase MPLC (10 - 100% MeCN:water) provided methyl 2-amino-4-chloro-5-(pyridin-4-yl)benzoate (0.028 g, 0.11 mmol, 60% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.63 (d, *J* = 6.1 Hz, 2H), 7.86 (s, 1H), 7.36 (d, *J* = 6.1 Hz, 2H), 6.85 (s, 1H), 6.08 (brs, 2H), 3.87 (s, 3H).



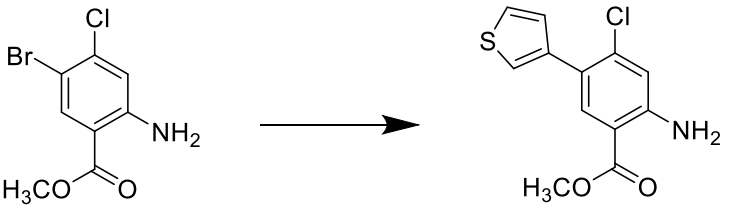
Methyl 2-(4-*tert*-butylbenzamido)-4-chloro-5-(pyridin-4-yl)benzoate. Prepared as described for compound **4d** (*step 2*). Purified by MPLC (0 - 10%, MeOH:CH_2Cl_2) to provide methyl 2-(4-*tert*-butylbenzamido)-4-chloro-5-(pyridin-4-yl)benzoate (0.036 g, 0.085 mmol, 80% yield). The product was carried into the saponification step without further characterization.



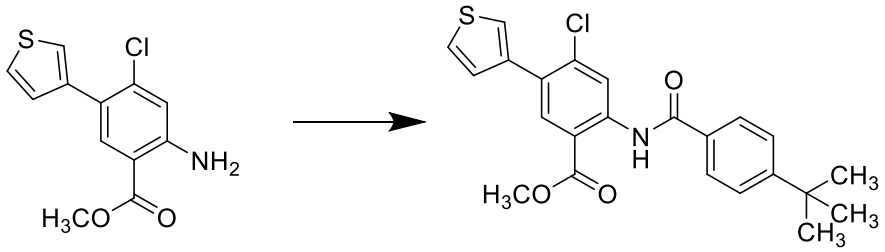
Methyl 2-amino-4-chloro-5-(pyridin-3-yl)benzoate. Prepared as described for compound **4f** (*step 1*). Purification by reverse-phase MPLC (10 - 100% MeCN:water) provided methyl 2-amino-4-chloro-5-(pyridin-3-yl)benzoate (0.092 g, 0.35 mmol, 56% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (br s, 1H), 8.56 (br d, J = 2.1 Hz, 1H), 7.84 (s, 1H), 7.77 – 7.35 (m, 1H), 7.35 – 7.30 (m, 1H), 6.82 (s, 1H), 5.95 (br s, 2H), 3.86 (s, 3H).



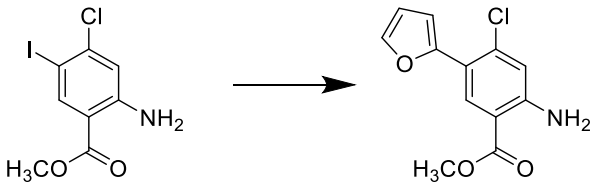
Methyl 2-(4-*tert*-butylbenzamido)-4-chloro-5-(pyridin-3-yl)benzoate. Prepared as described for compound **4d** (*step 2*). Purified by MPLC (0 - 15% EtOAc:hexanes) to produce methyl 2-(4-*tert*-butylbenzamido)-4-chloro-5-(pyridin-3-yl)benzoate (0.11 g, 0.26 mmol, 74% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.08 (s, 1H), 9.26 (s, 1H), 8.74 – 8.72 (m, 1H), 8.66 (dd, J_1 = 5.0 Hz, J_2 = 1.6 Hz, 1H), 8.09 (s, 1H), 8.00 (d, J = 8.5 Hz, 2H), 8.00 – 7.96 (m, 1H), 7.57 (d, J = 8.5 Hz, 2H), 7.54 – 7.49 (m, 1H), 3.99 (s, 3H), 1.37 (s, 9H).



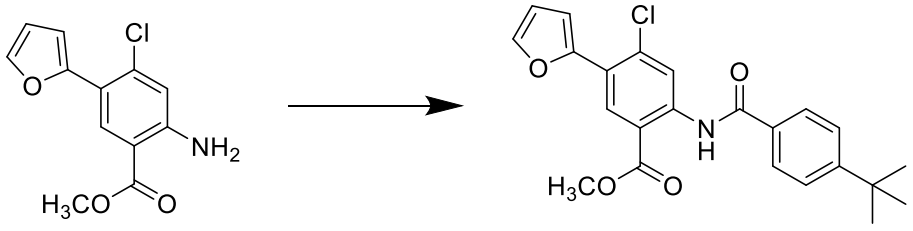
Methyl 2-amino-4-chloro-5-(thiophen-3-yl)benzoate. Prepared as described for compound **4d** (*step 1*). Purification by reverse-phase MPLC (10 - 100% MeCN:water) provided methyl 2-amino-4-chloro-5-(thiophen-3-yl)benzoate (0.049 g, 0.183 mmol, 64% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.39 - 7.31 (m, 2H), 7.26 - 7.24 (m, 1H), 6.79 (s, 1H), 3.87 (s, 3H).



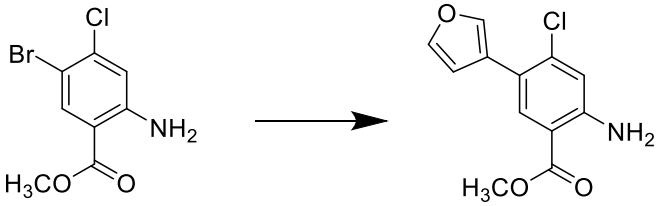
Methyl 2-(4-*tert*-butylbenzamido)-4-chloro-5-(thiophen-3-yl)benzoate. Prepared as described for compound **4d** (*step 2*). Purified by reverse-phase MPLC (10 - 100% MeCN:water) to produce methyl 2-(4-*tert*-butylbenzamido)-4-chloro-5-(thiophen-3-yl)benzoate (0.061 g, 0.143 mmol, 78% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.01 (s, 1H), 9.18 (s, 1H), 8.15 (s, 1H), 7.99 (d, J = 8.5 Hz, 2H), 7.56 (d, J = 8.5 Hz, 2H), 7.48 (dd, J = 3.0, 1.3 Hz, 1H), 7.39 (dd, J = 5.0, 3.0 Hz, 1H), 7.33 (dd, J = 5.0, 1.3 Hz, 1H), 3.97 (s, 3H), 1.37 (s, 9H).



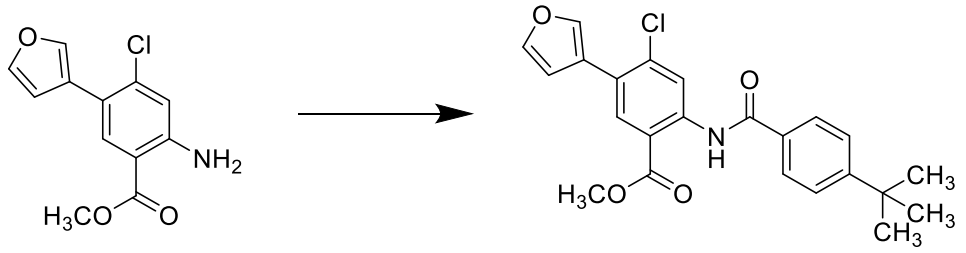
Methyl 2-amino-4-chloro-5-(furan-2-yl)benzoate. To a microwave vial was added methyl 2-amino-4-chloro-5-iodobenzoate **3e** (0.053 g, 0.170 mmol), 2-dicyclohexylphosphino-2',6'-di-i-propoxy-1,1'-biphenyl (9.5 mg, 0.020 mmol), palladium(II) acetate (2.3 mg, 10.2 μmol), potassium 2-furantrifluoroborate (0.031 g, 0.179 mmol) and sodium carbonate (0.036 g, 0.340 mmol). The vial was evacuated with argon 3 times and then degassed ethanol (1 mL) was added. The reaction was heated at 100 °C for 30 min in the microwave. After cooling to rt, the vial contents was diluted with EtOAc (10 mL) and washed with saturated $NaHCO_3$ (10 mL). The EtOAc layer was separated, dried with $MgSO_4$, filtered and concentrated. Reverse-phase MPLC (10 - 100% MeCN:water) purification afforded methyl 2-amino-4-chloro-5-(furan-2-yl)benzoate (0.031 g, 0.122 mmol, 72% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 1H), 7.46 (dd, J = 1.9, 0.8 Hz, 1H), 6.85 (dd, J = 3.4, 0.8 Hz, 1H), 6.77 (s, 1H), 6.48 (dd, J = 3.4, 1.8 Hz, 1H), 5.80 (br s, 2H), 3.90 (s, 3H).



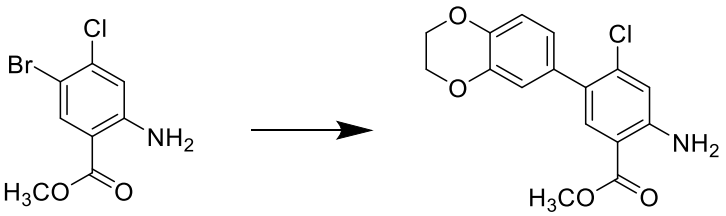
Methyl 2-(4-*tert*-butylbenzamido)-4-chloro-5-(furan-2-yl)benzoate. Prepared as described for compound **4d** (*step 2*). Purified by reverse-phase MPLC (10 - 100% MeCN:water) to produce methyl 2-(4-(*tert*-butyl)benzamido)-4-chloro-5-(furan-2-yl)benzoate (0.019 g, 0.045 mmol, 37% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.01 (s, 1H), 9.17 (s, 1H), 8.57 (s, 1H), 8.02 - 7.94 (m, 2H), 7.58 - 7.53 (m, 2H), 7.52 (dd, *J* = 1.8, 0.7 Hz, 1H), 7.12 (dd, *J* = 3.5, 0.7 Hz, 1H), 6.54 (dd, *J* = 3.5, 1.8 Hz, 1H), 4.00 (s, 3H), 1.37 (s, 9H).



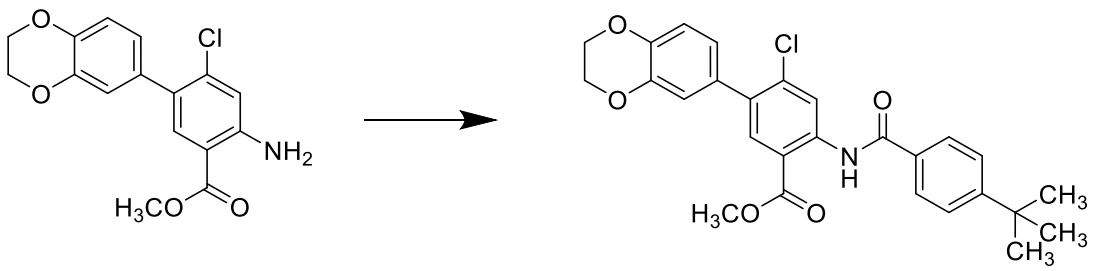
Methyl 2-amino-4-chloro-5-(furan-3-yl)benzoate. Prepared as described for compound **4f**. The crude product was purified by reverse-phase MPLC (10 - 100% MeCN:water) to produce methyl 2-amino-4-chloro-5-(furan-3-yl)benzoate (0.014 g, 0.056 mmol, 21% yield) as a brown solid. 1H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.73 (dd, *J* = 1.5, 0.9 Hz, 1H), 7.46 (t, *J* = 1.7 Hz, 1H), 6.80 (s, 1H), 6.66 (dd, *J* = 1.9, 0.9 Hz, 1H), 3.88 (s, 3H).



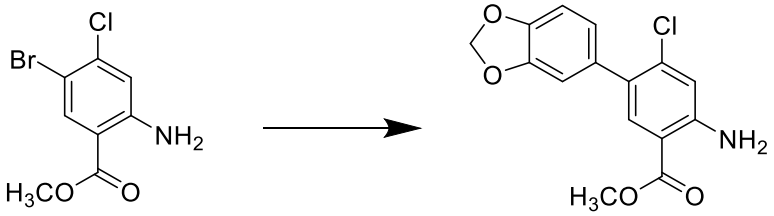
Methyl 2-(4-*tert*-butylbenzamido)-4-chloro-5-(furan-3-yl)benzoate. Prepared as described for compound **4d** (*step 2*). Purified by reverse-phase MPLC (10 - 100% MeCN:water) to produce methyl 2-(4-*tert*-butylbenzamido)-4-chloro-5-(furan-3-yl)benzoate (0.011 g, 0.027 mmol, 48% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.98 (s, 1H), 9.17 (s, 1H), 8.14 (s, 1H), 7.99 (d, *J* = 8.5 Hz, 2H), 7.86 (dd, *J* = 1.5, 0.9 Hz, 1H), 7.56 (d, *J* = 8.5 Hz, 2H), 7.52 - 7.48 (m, 1H), 6.74 (dd, *J* = 1.9, 0.9 Hz, 1H), 3.99 (s, 3H), 1.37 (s, 9H).



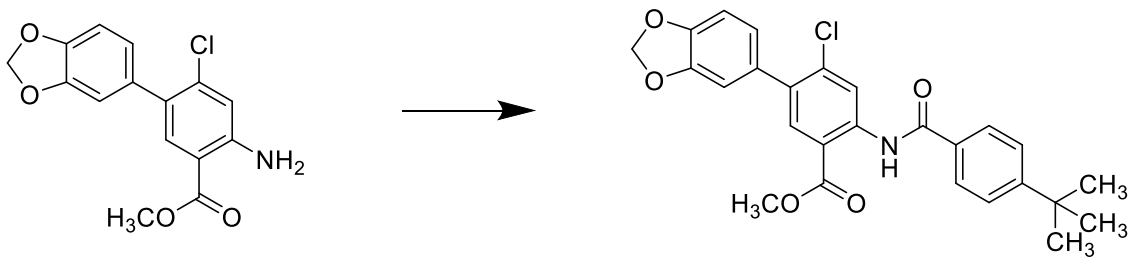
Methyl 2-amino-4-chloro-5-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)benzoate. Prepared as described for compound **4f** (*step 1*). Purification by reverse-phase MPLC (10 - 100% MeCN:water) provided methyl 2-amino-4-chloro-5-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)benzoate (0.047 g, 0.147 mmol, 51% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (s, 1H), 6.93 (dd, *J* = 1.5, 0.9 Hz, 1H), 6.91 - 6.85 (m, 2H), 6.78 (s, 1H), 5.78 (s, 2H), 4.29 (s, 4H), 3.85 (s, 3H).



Methyl 2-(4-*tert*-butylbenzamido)-4-chloro-5-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)benzoate. Prepared as described for compound **4d** (*step 2*). Purified by reverse-phase MPLC (10 - 100% MeCN:water) to produce methyl 2-(4-*tert*-butylbenzamido)-4-chloro-5-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)benzoate (0.047 g, 0.098 mmol, 66% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.00 (s, 1H), 9.15 (s, 1H), 8.04 (s, 1H), 7.99 (d, *J* = 8.5 Hz, 2H), 7.56 (d, *J* = 8.5 Hz, 2H), 7.00 - 6.96 (m, 1H), 6.95 - 6.90 (m, 2H), 4.31 (s, 4H), 3.95 (s, 3H), 1.37 (s, 9H).



Methyl 2-amino-5-(benzo[d][1,3]dioxol-5-yl)-4-chlorobenzoate. Prepared as described for compound **4f (***step 1*). The crude product was purified by reverse-phase MPLC (10 - 100% MeCN:water) to provide methyl 2-amino-5-(benzo[d][1,3]dioxol-5-yl)-4-chlorobenzoate (0.042 g, 0.137 mmol, 48% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 1H), 6.89 (dd, *J* = 1.3, 0.9 Hz, 1H), 6.87 - 6.81 (m, 2H), 6.78 (s, 1H), 6.00 (s, 2H), 5.78 (s, 2H), 3.86 (s, 3H).



Methyl 5-(benzo[d][1,3]dioxol-5-yl)-2-(4-*tert*-butylbenzamido)-4-chlorobenzoate. Prepared as described for compound **4d** (*step 2*). Purified by reverse-phase MPLC (10 - 100% MeCN:water) to produce methyl 5-(benzo[d][1,3]dioxol-5-yl)-2-(4-*tert*-butylbenzamido)-4-chlorobenzoate (0.028 g, 0.060 mmol, 44% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.00 (s, 1H), 9.16 (s, 1H), 8.04 (s, 1H), 7.99 (d, *J* = 8.5 Hz, 2H), 7.56 (d, *J* = 8.5 Hz, 2H), 6.96 - 6.91 (m, 1H), 6.91 - 6.81 (m, 2H), 6.02 (s, 2H), 3.96 (s, 3H), 1.37 (s, 9H).

Recombinant *Leishmania major* Hexokinase 1 (*r*LmHK1) characterization

The recombinant enzyme displayed Michaelis-Menten kinetics when substrate levels were increased, and had apparent K_m values for glucose and ATP of 0.13 ± 0.01 and 0.16 ± 0.04 mM, respectively. These values are similar to those from yeast hexokinase and *T. brucei* TbHK1. Interestingly, the enzyme could utilize mannose as an alternative to glucose, with an apparent K_m value indistinguishable from that of glucose (0.11 ± 0.02 mM).[2]

Using glucose as a substrate, the LmHK1 k_{cat} value was 3.6 x 10^4 min^{-1}, similar to that reported for the characterized TbHK1 (2.9 x 10^4 min^{-1}).[2] LmHK1 required a divalent cation, being most active with $MgCl_2$ and having minimal activity when $MnCl_2$ or $CaCl_2$ were included in place of $MgCl_2$ in the standard assay. The enzyme functioned across a broad pH range, with values between 6.2 and 8.0 supporting at least 50% of the activity found at the optimal pH of ~7.4. Basic pH above 8.0 was strongly inhibitory. In addition to ATP, LmHK1 could also use the purine nucleoside triphosphate GTP as a phosphoryl donor, though the enzyme affinity was lower for this substrate (with an apparent K_m value for GTP of 2.13 ± 0.10 mM). The pyrimidine nucleoside triphosphates CTP and UTP also supported activity with apparent K_m values of 3.74 ± 0.59 and 1.07 ± 0.08 mM. TTP, but not ITP or PP_i, was also a competent substrate. While the broad spectrum of nucleoside triphosphates used as substrates differed from that used by TbHK1, both enzymes were similarly inhibited by high concentrations of ATP (LmHK1 K_i value of 9.78 ± 0.97 mM. Additionally, both enzymes were inhibited by ADP and not by AMP, although LmHK1 ADP inhibition (84 ± 5.1% at 10 mM) was observed under pH conditions distinct from the acidic ones required for ADP inhibition of TbHK1.[3] The other

product, G6-P, had minimal impact on LmHK1 activity inhibiting the enzyme 22 ± 5.1% at 10 mM.

Solubility Assessment Protocol

Compound solubility in aqueous solution was measured using an automated kinetic solubility method at the Sanford Burnham Medical Research Institute at Lake Nona. The concentration of the compound in a saturated pH-buffered aqueous solution was determined by UV absorbance (250-498 nm) and compared to the spectra of a precipitation-free reference solution. Aqueous solubility was measured in phosphate buffered saline (PBS) at room temperature (23°C). PBS by definition is 137 mM NaCl, 2.7 mM KCl, 10 mM sodium phosphate dibasic, 2 mM potassium phosphate monobasic and a pH of 7.4.

In vitro ADME assay protocols

Plasma Stability – Stability of the compound in human plasma and mouse plasma (BioChemed Services) was determined. All liquid dispense and transfer steps were performed with the Freedom Evo automated liquid handler (Tecan US). Plasma was allowed to thaw at room temperature prior to preparing the assay solution of plasma:1X PBS (1:1). The assay solution was warmed up at 37 °C prior of adding the compound. Immediately after compounds were added, time 0 min aliquots were promptly collected and mixed with cold acetonitrile (spiked with an internal standard). The remainder of the reaction volume was incubated at 37 °C with shaking. Additional aliquots were collected 180 min after the start of the reaction and promptly quenched with cold acetonitrile (spiked with an internal standard). Samples were centrifuged at 3000 rpm for 10 min. The amount of compound in the supernatant was determined by LC/MS/MS (Applied Biosystems, Sciex API4000 Q-Trap) and the percent of parent compound remaining after 180 min was calculated by the following formula:

$$\% \text{ parent compound remaining} = \left[\frac{\text{Concentration at 180 min}}{\text{Concentration at 0 min}} \text{ X } 100 \right]$$

Results reported are the mean of each reaction duplicate, normalized to the internal standard, and expressed as a percent of compound remaining after the incubation time.

Assay details:

- Mouse Plasma in K3 EDTA
- Procaine and Procainamide were used as standards. Procaine is highly unstable, Procainamide is highly stable.
- Assay concentrations of standards and test compound: 1 µM
- Incubation Time: 3 hrs
- Reaction pH: 7.4
- Assay DMSO final concentration: 2.5%

Plasma Protein Binding – Teflon® Base Plate wells were rinsed with 20% ethanol for 10 minutes. Ethanol was then removed and wells were rinsed with ultrapure water and allowed to dry. RED (rapid equilibrium dialysis) inserts from Thermo Scientific (Pierce) were placed (open end up) into the wells of the base plate. All liquid dispense and transfer steps were performed with the Freedom Evo automated liquid handler (Tecan US). The sample chambers (red ring) contained 300 µl of a mixture of plasma and compound, and the buffer chambers received 500 µl of dialysis buffer (1X PBS, pH 7.4). Duplicate inserts were made for each concentration tested. The base plate was covered with sealing tape and incubated at 37˚C on an orbital shaker at 350 rpm for 4 hours. After the incubation time, equal volume from both chambers were removed and transferred to a 96 well plate containing either plasma or buffer. To precipitate proteins and release compounds, ice cold acetonitrile (with an internal standard) was added. Samples were vortexed and centrifuged at 3700 rpm for 10 min. The amount of compound in the supernatant was determined by LC/MS/MS (Applied Biosystems, Sciex API4000 Q-Trap). The percent of free and bound compounds were calculated with the following formula:

$$\% \text{ of bound parent compound} = \left[\frac{\text{amount of compound in donor - receiver}}{\text{amount of compound in donor}} \right] \times 100$$

Results reported are the mean of each reaction duplicate, normalized to the internal standard, and expressed as a percent compound bound after the incubation time.

Assay details:

- Mouse in K3 EDTA
- Propranolol and Metoprolol were used as standards. Propranolol is highly bound, Metoprolol is poorly bound
- Assay concentrations of standards and test cpd: 1 µM and 10 µM
- Incubation Time: 4 hrs
- Reaction pH: 7.4
- Assay DMSO final concentration: 1%

Hepatic Microsome Stability – Metabolic stability was assessed in the presence of mouse liver mirosomes (XenoTech, P/N M1000). All liquid dispense and transfer steps were performed with the Freedom Evo automated liquid handler (Tecan US). NADPH, a required cofactor for CYP450 metabolism, was provided by the NADPH Regenerating System, Solutions A (BD Biosciences, P/N 451220) and B (BD Biosciences, P/N 451200). Compound stock solutions were initially prepared in 100% DMSO and subsequently diluted in acetonitrile for the assay. The pH of the reactions was kept at ~ 7.4 with potassium phosphate buffer (BD Biosciences, P/N 451201). The reactions were started after adding NADPH to the reaction plate containing microsomes and compounds and time 0 min aliquots were promptly collected and mixed with ice cold acetonitrile (spiked with internal standards) to quench the reactions. The remainder of the reaction volume was incubated at 37 °C with shaking. Additional aliquots were collected 60 min after the start of the reaction and promptly quenched with ice cold acetonitrile (spiked with an internal standard). Samples were centrifuged at 3000 rpm for 10 min. The amount of compound in the supernatant was determined by LC/MS/MS (Applied Biosystems, Sciex API4000 Q-Trap) and the percent of parent compound remaining after 60 min was calculated by the following formula:

$$\% \text{ parent compound remaining} = \left[\frac{\text{Concentration at 60 min}}{\text{Concentration at 0 min}} \times 100 \right]$$

All reactions were run in triplicate, except negative controls (no NADPH) which were performed as single reactions. Results reported are the mean of each reaction triplicate, normalized to the internal standard, and expressed as a percent compound remaining after the incubation time.

Assay details:

- Mouse Liver Microsomes: 0.5 mg/mL protein concentration
- NADPH Regenerating System: 1.55 mM NADP+, 1.33 mM glucose-6-phosphate,1.33 mM Magnesium chloride, and 0.4 U/mL glucose-6 phosphate dehydrogenase
- Incubation Temperature: 37 °C
- Incubation Time: 60 min
- Standards: Verapamil-HCl and Testosterone, at 20 µM and 50 µM, respectively
- Test compound at 1 µM
- Assay DMSO final concentration: ≤ 0.5%
- Assay ACN final concentration: ≤ 1.2%

Supplemental Table 1. Eurofins PanLabs LeadProfiling Results for Compound **4f** – part 1

Catalog #	Assay name	Batch	Species	Replicates	Concentration	% inhibition
200510	Adenosine A_1	326378	hum	2	10 µM	0
200610	Adenosine A_{2A}	326337	hum	2	10 µM	-17
200720	Adenosine A_3	326379	hum	2	10 µM	-9
203100	Adrenergic α_{1A}	326306	rat	2	10 µM	32
203200	Adrenergic α_{1B}	326307	rat	2	10 µM	53
203400	Adrenergic α_{1D}	326308	hum	2	10 µM	-9
204010	Adrenergic β_1	326303	hum	2	10 µM	24
204110	Adrenergic β_2	326304	hum	2	10 µM	-9
285010	Androgen (Testosterone) AR	326285	rat	2	10 µM	12
212510	Bradykinin B_1	326321	hum	2	10 µM	11
212620	Bradykinin B_2	326280	hum	2	10 µM	-9
214510	Calcium Channel L-Type, Benzothiazepine	326310	rat	2	10 µM	1
214600	Calcium Channel L-Type, Dihydropyridine	326311	rat	2	10 µM	21
216000	Calcium Channel N-Type	326279	rat	2	10 µM	0
217030	Cannabinoid CB_1	326381	hum	2	10 µM	3
219500	Dopamine D_1	326312	hum	2	10 µM	57
219700	Dopamine D_{2S}	326313	hum	2	10 µM	25
219800	Dopamine D_3	326314	hum	2	10 µM	86
219900	Dopamine $D_{4.2}$	326315	hum	2	10 µM	18
224010	Endothelin ET_A	326350	hum	2	10 µM	25
224110	Endothelin ET_B	326351	hum	2	10 µM	3
225510	Epidermal Growth Factor (EGF)	326352	hum	2	10 µM	24
226010	Estrogen ERα	326353	hum	2	10 µM	-2
226600	$GABA_A$, Flunitrazepam, Central	326322	rat	2	10 µM	-19
226500	$GABA_A$, Muscimol, Central	326370	rat	2	10 µM	11
228610	$GABA_{B1A}$	326335	hum	2	10 µM	11
232030	Glucocorticoid	326328	hum	2	10 µM	19
232700	Glutamate, Kainate	326325	rat	2	10 µM	0
232810	Glutamate, NMDA, Agonism	326326	rat	2	10 µM	28
232910	Glutamate, NMDA, Glycine	326327	rat	2	10 µM	12
233000	Glutamate, NMDA, Phencyclidine	326283	rat	2	10 µM	1
239610	Histamine H_1	326316	hum	2	10 µM	42
239710	Histamine H_2	326317	hum	2	10 µM	98
239820	Histamine H_3	326282	hum	2	10 µM	-1

Supplemental Table 1. Eurofins PanLabs LeadProfiling Results for Compound **4f** – part 2

Catalog #	Assay name	Batch	Species	Replicates	Concentration	% inhibition
241000	Imidazoline I_2, Central	326329	rat	2	10 µM	-17
243520	Interleukin IL-1	326355	mouse	2	10 µM	7
250460	Leukotriene, Cysteinyl $CysLT_1$	326620	hum	2	10 µM	87
251600	Melatonin MT_1	326356	hum	2	10 µM	12
252610	Muscarinic M_1	326408	hum	2	10 µM	33
252710	Muscarinic M_2	326409	hum	2	10 µM	-4
252810	Muscarinic M_3	326348	hum	2	10 µM	9
257010	Neuropeptide Y Y_1	326330	hum	2	10 µM	-2
257110	Neuropeptide Y Y_2	326331	hum	2	10 µM	0
258590	Nicotinic Acetylcholine	326298	hum	2	10 µM	6
258700	Nicotinic Acetylcholine α1, Bungarotoxin	326300	hum	2	10 µM	-7
260130	Opiate δ_1 (OP1, DOP)	326357	hum	2	10 µM	-7
260210	Opiate κ(OP2, KOP)	326371	hum	2	10 µM	15
260410	Opiate µ(OP3, MOP)	326372	hum	2	10 µM	25
264500	Phorbol Ester	326373	mouse	2	10 µM	12
265010	Platelet Activating Factor (PAF)	326383	hum	2	10 µM	11
265600	Potassium Channel [K_{ATP}]	326384	ham	2	10 µM	7
265900	Potassium Channel hERG	326385	hum	2	10 µM	-7
268420	Prostanoid EP_4	326442	hum	2	10 µM	9
268700	Purinergic P_{2X}	326287	rabbit	2	10 µM	-19
268810	Purinergic P_{2Y}	326288	rat	2	10 µM	32
270000	Rolipram	326374	rat	2	10 µM	1
271110	Serotonin (5-Hydroxytryptamine) 5-HT_{1A}	326289	hum	2	10 µM	90
271700	Serotonin (5-Hydroxytryptamine) 5-HT_{2B}	326386	hum	2	10 µM	6
271910	Serotonin (5-Hydroxytryptamine) 5-HT_3	326387	hum	2	10 µM	-26
278110	Sigma σ_1	326284	hum	2	10 µM	-2
279510	Sodium Channel, Site 2	326318	rat	2	10 µM	17
255520	Tachykinin NK_1	326382	hum	2	10 µM	-1
285900	Thyroid Hormone	326388	rat	2	10 µM	65
220320	Transporter, Dopamine (DAT)	326376	hum	2	10 µM	88
226400	Transporter, GABA	326281	rat	2	10 µM	22
204410	Transporter, Norepinephrine (NET)	326375	hum	2	10 µM	91
274030	Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	326377	hum	2	10 µM	7

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