Supplementary Material

"Identification and optimization of PDE10A inhibitors using fragment-based screening by nanocalorimetry and X-ray crystallography"

Michael I. Recht[1*], Vandana Sridhar[2], John Badger[2], Pierre-Yves Bounaud[2], Cheyenne Logan[2], Barbara Chie-Leon[2], Vicki Nienaber[2], Francisco E. Torres[1]

[1]Palo Alto Research Center, 3333 Coyote Hill Road, Palo Alto, CA 94304

[2]Zenobia Therapeutics, 3550 General Atomics Court, Building 2, Room 435, San Diego, CA 92121

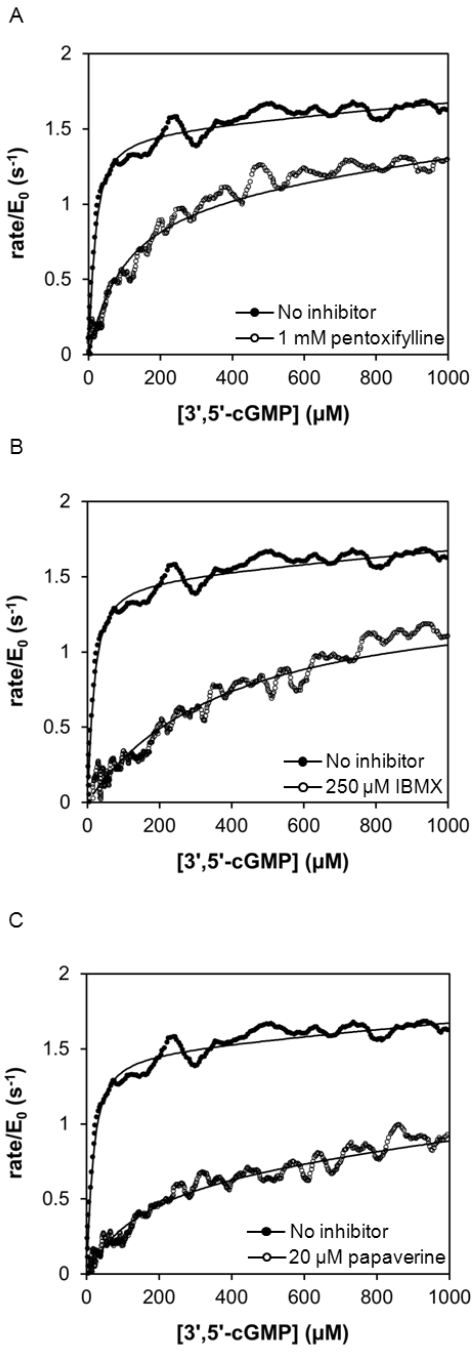


Figure S1 PDE10A hydrolysis of 3′,5′-cGMP in the absence (solid) and presence (open) of known general phosphodiesterase inhibitors. Reactions contained 15 µM PDE10A and 2 mM 3′,5′-cGMP. Solid curves are fit of data to equation 2. Fitting the solid black points (no inhibitor) yielded k_{cat}= 1.2 s^{-1} and K_M= 12 µM, compared to k_{cat}= 1.2 s^{-1} and K_M= 4.4 µM for the catalytic domain of PDE4A using a radioactivity-

based assay [1]. (A) Rate versus remaining 3′,5′-cGMP concentration in the absence and presence of 1 mM pentoxifylline. (B) Rate versus remaining 3′,5′- cGMP concentration in the absence and presence of 250 µM 3-isobutyl-1-methylxanthine (IBMX). (C) Rate versus remaining 3′,5′- cGMP concentration in the absence and presence of 20 µM papaverine.

Table S1. Crystallographic data collection and refinement statistics for crystals in which bound fragments were identified. Values given in parenthesis are for data in the highest resolution shells. The percentages of amino acids in the allowed region of the Ramachandran plot were calculated by the PDB Validation Server (http://validate.rcsb.org)

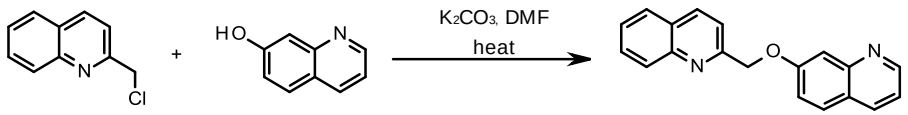
PDB ID	4LKQ	4MRW	4MSH	4LLJ	4LLK	4LLP	4MRZ	4LLX	4MS0
Compound ID	ZT017	ZT120	ZT143	ZT214	ZT217	ZT401	ZT429	ZT434	ZT443
Data Collection									
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Unit cell (a,b,c) (Å)	50.979, 82.100, 155.621	51.830, 82.231, 155.382	50.005, 82.068, 156.327	50.744, 82.057, 155.436	51.313, 82.160, 155.554	50.383, 82.558, 154.746	51.140, 82.133, 154.745	51.791, 82.141, 154.877	51.304, 82.380, 155.611
Resolution (Å)	1.62	1.96	2.30	1.56	1.55	1.75	1.58	1.76	1.79
R_{merge}	0.069 (0.591)	0.139 (0.544)	0.131 (0.706)	0.059 (0.566)	0.057 (0.591)	0.051 (0.571)	0.054 (0.583)	0.068 (0.573)	0.062 (0.585)
Completeness (%)	99.9 (99.6)	99.4 (99.9)	70.7 (64.8)	97.6 (82.3)	98.3 (93.3)	99.7 (99.6)	99.6 (99.2)	99.9 (100.0)	97.7 (96.6)
No. observed refs	497662	355501	104746	531234	524979	397997	411350	344342	301412
No. unique refs	83936	48142	20880	91255	94258	65994	89781	66913	61338
Mean I/σ(I)	22.4 (2.8)	11.9 (2.1)	9.0 (2.3)	26.2 (2.6)	24.9 (2.8)	30.7 (3.5)	24.2 (2.4)	21.7 (2.8)	22.4 (2.3)
Multiplicity	5.9 (5.4)	7.4 (6.3)	5.0 (5.9)	5.8 (4.8)	5.6 (5.3)	6.0 (6.0)	4.6 (4.5)	5.1 (5.2)	4.9 (4.4)
Refinement									
No. of work reflections	79657	45507	19755	86595	89457	62574	85147	63435	57938
No. of free reflections	4189	2426	1068	4567	4724	3344	4495	3384	3097
No. of atoms	5635	5619	5344	5598	5714	5318	5645	5698	5489
R_{work}	0.205	0.228	0.232	0.210	0.205	0.213	0.207	0.205	0.251
R_{free}	0.235	0.279	0.289	0.243	0.232	0.246	0.236	0.246	0.295
Mean B factor ($Å^2$)	30.2	22.6	29.7	32.5	25.3	34.7	31.2	29.6	38.0
R.m.s.d. bond lengths (Å)	0.014	0.011	0.007	0.015	0.014	0.012	0.011	0.014	0.013
R.m.s.d. bond angles (°)	1.389	1.203	0.995	1.436	1.364	1.276	1.226	1.337	1.324
Ramachandran plot (% allowed)	99.5	98.8	98.5	98.9	99.7	99.1	99.2	99.4	99.2

PDB ID	4LM0	4MSA	4LM1	4MSN	4LM2	4LM3	4LM4	4MSC	4MSE
Compound ID	ZT448	ZT449	ZT450	ZT451	ZT462	ZT464	ZT902	ZT1595	ZT1597
Data Collection									
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Unit cell (a,b,c) (Å)	50.598, 82.041, 155.687	51.202, 82.168, 155.075	52.127, 82.335, 154.846	49.859, 82.158, 156.130	52.184, 82.383, 155.130	51.893, 82.146, 155.085	50.892, 82.076, 155.512	51.380, 82.085, 155.926	50.652, 81.819, 156.850
Resolution (Å)	1.66	1.62	1.60	2.30	1.55	1.49	1.48	2.47	2.83
R_{merge}	0.067 (0.504)	0.076 (0.570)	0.061 (0.399)	0.120 (0.479)	0.058 (0.557)	0.060 (0.578)	0.067 (0.576)	0.085 (0.581)	0.084 (0.586)
Completeness (%)	98.6 (90.3)	96.1 (97.4)	97.6 (81.8)	95.8 (94.2)	96.2 (76.6)	99.5 (98.2)	99.6 (97.2)	99.9 (100.0)	99.9 (100.0)
No. observed refs	446626	327616	485902	114832	513852	641230	643853	134032	88461
No. unique refs	76392	80392	86792	28032	93768	108380	108799	24593	16439
Mean I/σ(I)	23.8 (2.5)	16.5 (2.5)	23.5 (2.8)	8.8 (2.5)	24.9 (2.0)	24.6 (2.7)	22.8 (2.6)	17.4 (3.4)	17.8 (3.3)
Multiplicity	5.8 (4.7)	4.1 (4.2)	5.6 (4.5)	4.1 (3.9)	5.5 (4.2)	5.9 (5.1)	5.9 (5.2)	5.5 (5.5)	5.4 (5.4)
Refinement									
No. of work reflections	72469	76291	82265	26584	88932	102889	103262	23287	15566
No. of free reflections	3835	4031	5342	1405	4693	5407	5428	1255	829
No. of atoms	5694	5681	5772	5390	5705	5856	5700	5283	5330
R_{work}	0.199	0.196	0.211	0.231	0.209	0.200	0.201	0.240	0.271
R_{free}	0.230	0.220	0.249	0.288	0.247	0.225	0.223	0.287	0.342
Mean B factor ($Å^2$)	30.9	24.9	28.0	31.4	27.3	26.3	24.5	61.9	87.026
R.m.s.d. bond lengths (Å)	0.014	0.012	0.014	0.008	0.016	0.011	0.011	0.009	0.007
R.m.s.d. bond angles (°)	1.373	1.264	1.436	1.087	1.485	1.275	1.265	1.133	1.029
Ramachandran plot (% allowed)	99.1	99.2	99.4	98.0	99.4	99.5	99.2	96.9	96.8

Table S2. Crystallographic data collection and refinement statistics for crystals in which no bound fragments were identified.

Compound ID	ZT0106	ZT0415	ZT0419	ZT0427	ZT0431	ZT0447	ZT0581	ZT0826
Resolution (Å)	1.76	1.85	1.93	1.5	1.99	1.8	1.48	1.46
R_{merge}	0.078	0.076	0.073	0.072	0.095	0.071	0.056	0.052
Mean I/σ(I)	21.9 (3.0)	18.8 (2.4)	18.6 (2.2)	22.3 (3.7)	15.9 (3.3)	19.7 (2.8)	27.9 (2.6)	29.2 (2.6)
R_{work}	0.2133	0.2285	0.2280	0.2130	0.2378	0.2224	0.2217	0.2174
R_{free}	0.2548	0.2646	0.2758	0.2344	0.2835	0.2545	0.2377	0.2408
R.m.s.d. bond lengths (Å)	0.009	0.012	0.013	0.006	0.015	0.010	0.007	0.007

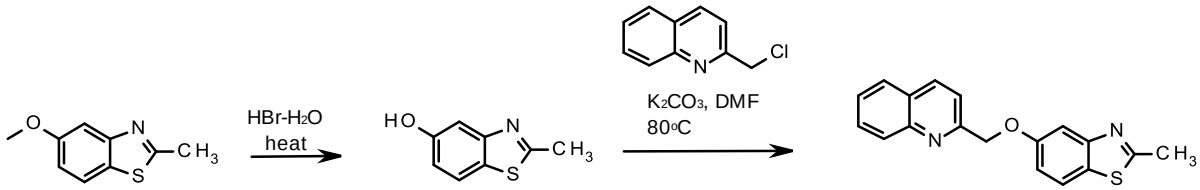
Synthesis of ZT1595



ZT1595

A mixture of 2-(chloromethyl)quinoline (80 mg, 0.38 mmol), quinolin-7-ol (50 mg, 0.34 mmol) and K_2CO_3 (143 mg, 1.02 mmol) in DMF (5.0 mL) was heated at 90°C for 4 h. The mixture was cooled to room temperature, poured into water and extracted with ethyl acetate. The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by flash column chromatography on silica gel (Pet.Ether:EtOAc = 4:1 to 1:1) to give **ZT1595** (48 mg, 49% yield) as a light yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.81 (m, 1H), 8.42 (m, 1H), 8.37 (m, 1H), 8.02-7.83 (m, 3H), 7.82-7.75 (m, 2H), 7.65-7.54 (m, 1H), 7.43-7.35 (m, 3H), 5.56 (s, 2H). MS (ESI): *m/z* 287.0 $[M+H]^+$.

Synthesis of ZT1597



ZT1597

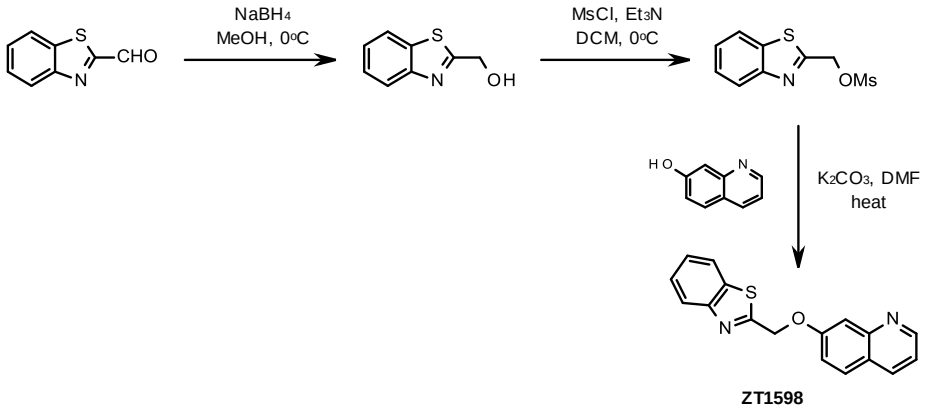
Step 1: 2-Methylbenzo[*d*]thiazol-5-ol

A suspension of 5-methoxy-2-methylbenzo[*d*]thiazole (1.0 g, 5.7 mmol) in 48% HBr/H_2O (10 mL) was heated at 105°C overnight. The mixture was cooled to room temperature and the precipitate was collected by filtration, washed with acetone and dried under vacuum to give 2-methylbenzo[*d*]thiazol-5-ol (580 mg, 62% yield) as a white solid.

Step 2: ZT1597

A mixture of 2-methylbenzo[*d*]thiazol-5-ol (165 mg, 1.0 mmol), 2-(chloromethyl)quinoline (212 mg, 1.32 mmol) and K_2CO_3 (346 mg, 2.5 mmol) in DMF (8.0 mL) was heated at 80°C for 3h. The mixture was cooled to room temperature and poured into water. The resulting mixture was extracted with ethyl acetate and the combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by flash column chromatography on silica gel (DCM/MeOH= 100:1) to afford **ZT1597** (100 mg, 33% yield) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.20 (m, 2H), 7.88-7.70 (m, 3H), 7.62-7.56 (m, 2H), 7.16 (dd, *J*= 2.5, 8.8 Hz, 1H), 5.51 (s, 2H), 2.82 (s, 3H); MS (ESI): *m/z* 307.0 $[M+H]^+$.

Synthesis of ZT1598



Step 1: Benzo[*d*]thiazol-2-ylmethanol

Sodium borohydride (0.38 g, 10.0 mmol) was added slowly to a solution of 1,3-benzothiazole-2-carbaldehyde (1.63 g, 10.0 mmol) in methanol (10.0 mL) at 0°C. The reaction mixture was stirred at 0°C for 4 h and saturated NH_4Cl solution was added. The resulting mixture was extracted with dichloromethane. The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated to give benzo[*d*]thiazol-2-ylmethanol (1.47 g, 89% yield).

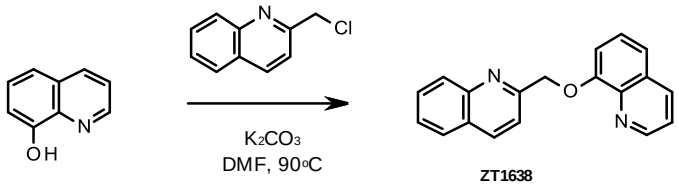
Step 2: Benzo[*d*]thiazol-2-ylmethyl methanesulfonate

To a solution of benzo[*d*]thiazol-2-ylmethanol (1.0 g, 6.02 mmol) in DCM (10.0 mL) at room temperature was added triethylamine (1.68 mL, 12.04 mmol). After the mixture was cooled to 0°C, a solution of methanesulfonyl chloride (0.60 mL, 7.75 mol) in DCM (5.0 mL) was added dropwise over 0.5 h. The reaction mixture was stirred at 0°C for an additional hour and saturated $NaHCO_3$ solution was added. The resulting mixture was extracted with dichloromethane and the combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated to give benzo[*d*]thiazol-2-ylmethyl methanesulfonate (1.1 g, 75% yield), which was used in the next step without further purification.

Step 3: ZT1598

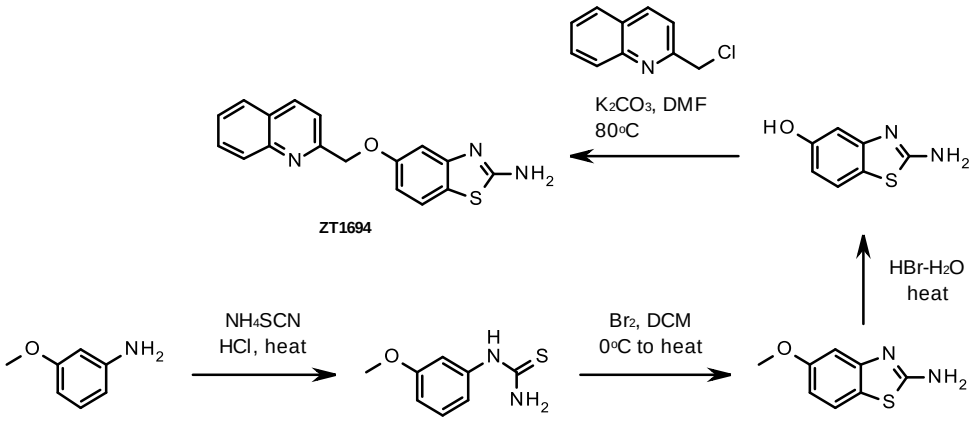
A mixture of benzo[*d*]thiazol-2-ylmethyl methanesulfonate (140 mg, 0.58 mmol), quinolin-7-ol (75 mg, 0.52 mmol) and K_2CO_3 (159 mg, 1.15 mmol) in DMF (5.0 mL) was heated at 90°C for 12 h. The mixture was cooled to room temperature, poured into water and extracted with ethyl acetate. The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by flash column chromatography on silica gel (Pet.Ether:EtOAc = 4:1 to 1:1) to give **ZT1598** (23 mg, 14% yield) as a light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.82 (m, 1H), 8.26 (m, 1H), 8.16 (m, 1H), 7.89 (m, 1H), 7.821 (m, 1H), 7.64 (m, 1H), 7.46-7.33 (m, 4H), 5.38 (s, 2H). MS (ESI): *m/z* 293.0 $[M+H]^+$.

<u>Synthesis of ZT1638</u>



A mixture of 2-(chloromethyl)quinoline (200 mg, 0.93 mmol), quinolin-8-ol (136 mg, 0.93 mmol) and K_2CO_3 (258 mg, 1.87 mmol) in DMF (10.0 mL) was heated at 90°C for 1 h. The mixture was cooled to room temperature and poured into water. The resulting mixture was extracted with ethyl acetate and the combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by flash column chromatography on silica gel (Pet.Ether:EtOAc = 10:1 to 5:1) to give compound **ZT1638** (205 mg, 77% yield) as a light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.12 (m, 1H), 8.13 (m, 3H), 7.82-7.42 (m, 3H), 7.59 (m, 1H), 7.54 (m, 1H), 7.51-7.38 (m, 2H), 7.09 (m, 1H), 5.77 (s, 2H); MS (ESI): *m/z* 287.0 $[M+H]^+$.

Synthesis of ZT1694



Step 1: 3-Methoxyphenylthiourea

A mixture of 3-methoxyaniline (10.0 g, 81.3 mmol) and NH_4SCN (6.18 g, 81.3 mmol) in 1 M HCl solution (82.0 mL) was heat at 100°C for 16 h. After the mixture was cooled to room temperature, it was diluted with water. The precipitate was collected by filtration, washed with water and ether and dried to give 3-methoxyphenylthiourea (7.36 g, 50% yield) as a yellow solid.

Step 2: 5-Methoxybenzo[*d*]thiazol-2-amine

To a suspension of 3-methoxyphenylthiourea (3.36 g, 18.46 mmol) in dichloromethane (37.0 mL) at 0°C was added dropwise a solution of bromine (7.66 g, 20.31 mmol) in dichloromethane (10.0 mL) over 30 min. The reaction mixture was stirred for 3 h and then heated under reflux for another 3 h. The precipitate was collected by filtration and washed with dichloromethane. The solid was suspended in saturated $NaHCO_3$ solution and extracted with dichloromethane. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated to afford 5-methoxybenzo[*d*]thiazol-2-amine (2.95 g, 89% yield) as a white solid.

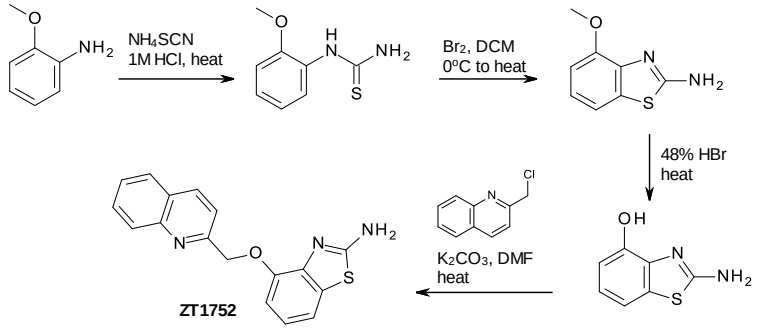
Step 3: 2-Aminobenzo[*d*]thiazol-5-ol

A suspension of 5-methoxybenzo[*d*]thiazol-2-amine (1.5 g, 8.3 mmol) in 48% HBr/H_2O (14.0 mL) was heated at 105°C overnight. The mixture was cooled to room temperature and the precipitate was collected by filtration and washed with acetone to give 2-aminobenzo[*d*]thiazol-5-ol (0.8 g, 58% yield) as a white solid.

Step 4: ZT1694

A mixture of 2-aminobenzo[*d*]thiazol-5-ol (200 mg, 1.2 mmol), 2-(chloromethyl)quinoline (283 mg, 1.32 mmol) and K_2CO_3 (415 mg, 3.0 mmol) in DMF (10.0 mL) was heated at 80°C for 3 h. The mixture was cooled to room temperature and poured into water. The resulting mixture was extracted with ethyl acetate and the combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by flash column chromatography on silica gel (DCM:MeOH = 60:1) to afford **ZT1694** (0.21 g, 58% yield) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.42 (d, 1H), 8.12 (d, 1H), 7.94 (d, 1H), 7.81-7.76 (m, 2H), 7.63 (m, 1H), 7.58 (m, 1H), 7.16 (m, 1H), 6.86 (m, 3H), 5.40 (s, 2H); MS (ESI): *m/z* 308.0 $[M+H]^+$.

Synthesis of ZT1752



Step 1: 2-Methoxyphenylthiourea

A solution of 2-methoxyaniline (10.0 g, 81.3 mmol) and NH_4SCN (6.18 g, 81.3 mmol) in 1 M HCl (82.0 mL) was stirred at 100°C for 16 h. After the mixture was cooled to room temperature, it was diluted with water and stirred at room temperature for 2 h. The precipitate was collected by filtration, washed with water and ether and dried under vacuum to give 2-methoxyphenylthiourea (7.5 g, 51% yield) as a yellow solid.

Step 2: 4-Methoxybenzo[*d*]thiazol-2-amine

To a suspension of 2-methoxyphenylthiourea (4.0 g, 21.98 mmol) in dichloromethane (37.0 mL) at 0°C was added a solution of bromine (3.9 g, 24.0 mmol) in dichloromethane (10.0 mL) dropwise over 30 min. The reaction mixture was stirred for 3 h and then heated under reflux for another 3 h. The precipitate was collected by filtration and washed with dichloromethane. The solid was suspended in saturated $NaHCO_3$ solution and the resulting mixture was extracted with dichloromethane. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated to afford 4-methoxybenzo[*d*]thiazol-2-amine (1.0 g, 25% yield) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.23 (d, *J* = 8.1 Hz, 1H), 7.10 (m, 1H), 6.83 (d, *J* = 8.1 Hz, 1H), 5.63 (br s, 2H), 3.91 (s, 3H). MS (ESI): *m/z* 180.9 $[M+H]^+$.

Step 3: 2-Aminobenzo[*d*]thiazol-4-ol

A suspension of 4-methoxybenzo[*d*]thiazol-2-amine (500 mg, 2.78 mmol) in 48% HBr (10.0 mL) was heated at 105°C overnight. The mixture was cooled to room temperature and the precipitate was collected by filtration, washed with acetone and dried under vacuum to give 2-aminobenzo[*d*]thiazol-4-ol (320 mg, 69% yield) as a white solid.

Step 4: ZT1752

A mixture of 2-aminobenzo[*d*]thiazol-4-ol (320 mg, 1.93 mmol), 2-(chloromethyl)quinoline (454 mg, 2.1 mmol) and K_2CO_3 (665 mg, 3.8 mmol) in DMF (10.0 mL) was heated at 80^{o}C for 3 h. The mixture was cooled to room temperature, poured into water and extracted with ethyl acetate. The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by flash column chromatography on silica gel (DCM/MeOH = 30:1) to afford **ZT1752** (200 mg, 34% yield) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d6): δ 8.45 (d, *J* = 8.1 Hz, 1H), 8.07 (m, 2H), 7.90 (m, 2H), 7.76 (m, 1H), 7.63 (m, 2H), 7.27 (m, 1H), 6.95 (m, 2H), 5.48 (s, 2H). MS (ESI): *m/z* 308.0 $[M+H]^{+}$.

Supplementary Material Reference

1. Wang H, Liu Y, Hou J, Zheng M, Robinson H, Ke H. Structural insight into substrate specificity of phosphodiesterase 10. *Proc. Natl. Acad. Sci. U. S. A.* **2007**; 104:5782-5787.