Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications.

Electronic Supplementary Information

Small-Molecule Phosphodiesterase Probes: Discovery of Potent and Selective CNS-Penetrable Quinazoline Inhibitors of PDE1.

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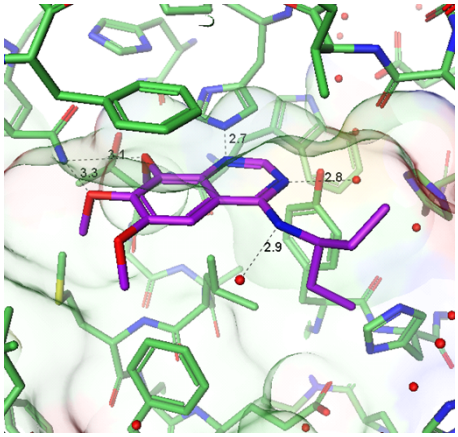
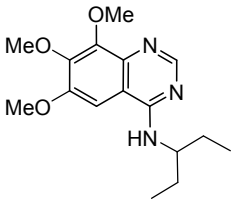
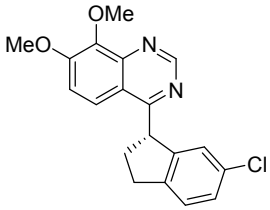




7a
(PF-04471141)
PDE1B IC_{50} = 35 nM



27
(PF-04822163)
PDE1B IC_{50} = 2.4 nM

This manuscript describes the discovery of a potent and selective series of brain penetrable quinazoline inhibitors of PDE1 that represent valuable new tools for the dissection of biological events linked to PDE1.

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Table S1. PDE 1B Binding Statistics for Key Compounds

Compound	PDE1B IC_{50} (nM)	n	STD
1	219	3	2.5
2	230	3	63
7a	35	3	0.07
11	47	8	28.7
21b	83	3	21.8
22	22	5	9.6
23b	5.6	3	0.06
27	2.4	4	0.08

Ethics Statement on the use of live animals:
All studies with live animals were conducted in accordance with the Institute for Laboratory Animal Resources (1996) and were approved by the Institutional Animal Care and Use Committee.

Table S2. PK data for quinazoline **7a** dosed subcutaneously

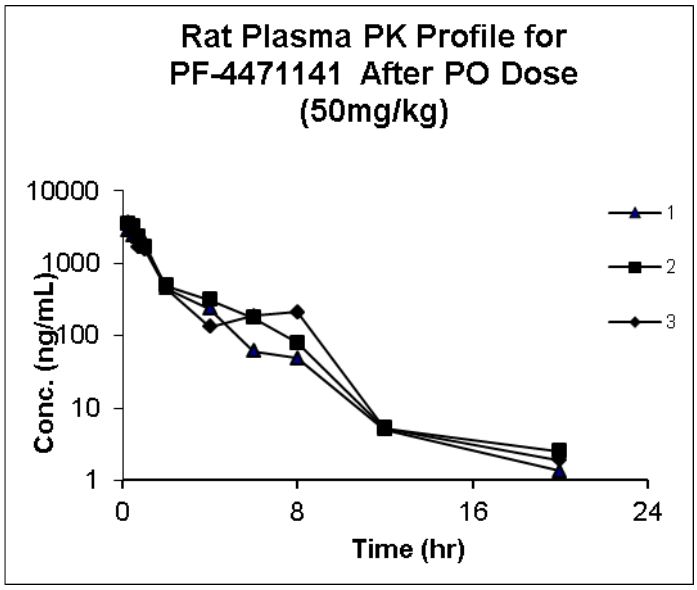
Species	Dose	Min	Total Plasma	Free Plasma	FP/IC50
Mouse	5 mpk sc	30	1120 nM	202 nM	4
Rat	10 mpk sc	30	6867 nM	714 nM	16
Rat	10 mpk sc	60	5017 nM	522 nM	12
Rat	50 mpk sc	30	40549	4217 nM	93

Table S3. PK data for quinazoline **27**, illustrating poor oral exposure vs. subcutaneous dosing

Species	Dose	Min	Total Plasma	Free Plasma	FP/IC50
Rat	10 mpk sc	30	1747 nM	50 nM	18
Rat	50 mpk po	30	806	23 nM	8

Rat PK data for PF-04471141 (7a) Dosed Orally

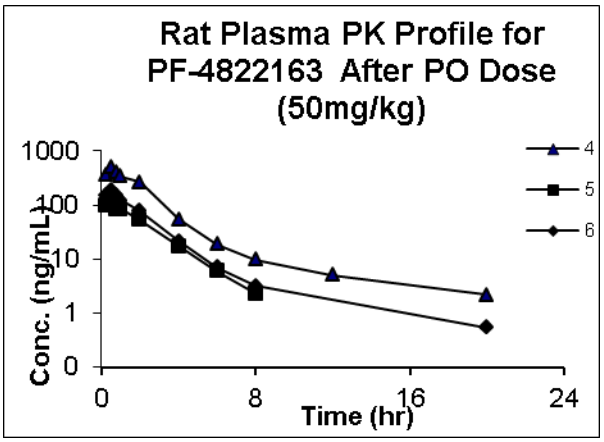
Compound	**PF-04471141**
MW Salt (g/mol)	341.8
MW Free Base (g/mol)	305.4
Route of adminstration	**PO**
Dose Free Base (mg/kg)	50
Species	Rat
Strain	Sprague-Dawley
Sex	M
Weight	300-350 g
Dose vehicle	Acid. 0.5% Mecellulose
Dose conc. (mg/ml)	5
Dose volume (ml/kg)	10
Animal number	3
Feeding Status	Fed



Sampling Time (hr)	1 (ng/mL)	2 (ng/mL)	3 (ng/mL)
0.25	2880	3450	3670
0.5	2420	3220	2670
0.75	2360	2330	1710
1	1940	1690	1560
2	453	490	450
4	234	311	133
6	61.3	177	189
8	48.8	79.6	213
12	5.11	5.30	5.21
20	1.35	2.52	1.91

Animal	**1**	**2**	**3**	**Mean**	**Stdev**
Tmax (hr)	0.25	0.25	0.25	**0.25**	**0.0**
Cmax (ng/ml)	2370	3450	3670	**3163**	**696**
t1/2 terminal(hr)	2.42	7.48	5.52	**5.14**	**2.55**
AUClast(hr*ng/ml)	4580	5300	4720	**4867**	**382**
AUCinf(hr*ng/ml)	4580	5330	4740	**4883**	**395**

Rat PK data for PF-04822163 (27), dosed Orally.



Compound	**PF-4822163**
Lot #	PF-04822163
MW Salt (g/mol)	
MW Free Base (g/mol)	340.6
	122060
Route of adminstration	**PO**
Dose Free Base (mg/kg)	50
Species	Rat
Strain	Sprague-Dawley
Sex	M
Weight	300-350 g
Dose vehicle	Acid. 0.5% MeCellulose
Dose conc. (mg/ml)	5
Dose volume (ml/kg)	10
Animal number	3
Feeding Status	Fed

Sampling Time (hr)	**4**	**5**	**6**
JVC Samples	**(ng/mL)**	**(ng/mL)**	**(ng/mL)**
0.25	366	100	149
0.5	511	120	190
0.75	399	85	148
1	337	84	122
2	268	53	77.6
4	54	17	21.6
6	18.9	6	7.08
8	9.8	2.3	3.18
12	5.03	<0.5	<0.5
20	2.19	<0.5	0.536

Animal	**1**	**2**	**3**	**Mean**	**Stdev**
Tmax (hr)	0.5	0.5	0.5	**0.50**	**0.0**
Cmax (ng/ml)	511	120	190	**274**	**208**
t1/2 terminal(hr)	5.70	6.02	4.67	**5.46**	**0.71**
AUClast(hr*ng/ml)	1150	274	397	**607**	**474**
AUCinf(hr*ng/ml)	1160	279	401	**613**	**477**

Rat PK Data for PF-04822163 (27) Dosed Subcutaneously.

Table S4. Mean ± SD Plasma Concentrations (ng/mL) of PF-04822163-00 (**27**) on Days 1 and 4 in Sprague Dawley Rats (n = 4 per dose) Following Daily Subcutaneous Administration of 5, 50 & 200 mg/kg

Dose (mg/kg)	Day	Sex	Mean Plasma Concentrations (ng/mL)			
			1 h	4 h	7 h	24h
5	1	M	187±21	47.1±15.9	8.92±2.97	30.4
	4	M	N/A	21.2±9.7	N/A	N/A
50	1	M	744±107	272±56	84.4±36.7	17.5±11.1
	4	M	N/A	337±94	N/A	N/A
200	1	M	719±203	223±60	82.2±33.7	33.2±11.9
	4	M	N/A	402±95	N/A	N/A

N/A = Not Applicable

LLOQ = 0.98 ng/mL

Table S5. Mean Pharmacokinetic Parameters (ng/mL) of PF-04822163-00 (**27**) on Day 1 in Sprague Dawley Rats Following Daily Subcutaneous Administration of 5, 50 & 200 mg/kg

Dose (mg/kg)	Day	Sex	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-24h} (ng*h/mL)
5	**1**	**M**	1.0	187	546
50	**1**	M	1.0	744	2720
200	**1**	M	1.0	719	2960

Figure S1. Mean ± SD Plasma Concentrations (ng/mL) of PF-04822163-00 (**27**) on Day 1 in Sprague Dawley Rats Following Daily Subcutaneous Administration of 5, 50 and 200 mg/kg

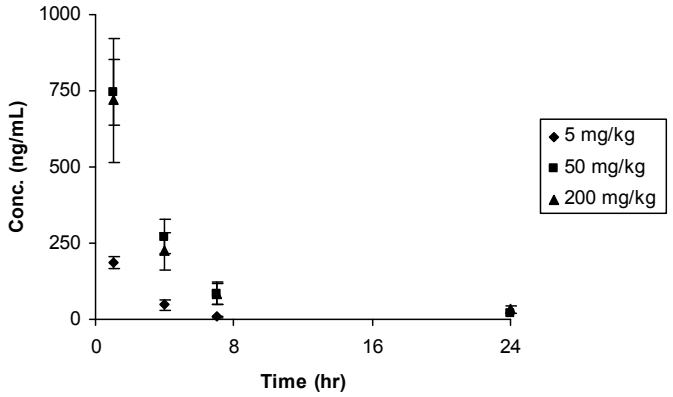


Figure S2. Mean AUC_{0-24} of PF-04822163-00 (**27**) on Day 1 in Sprague Dawley Rats Following Daily Subcutaneous Administration of 5, 50 & 200 mg/kg

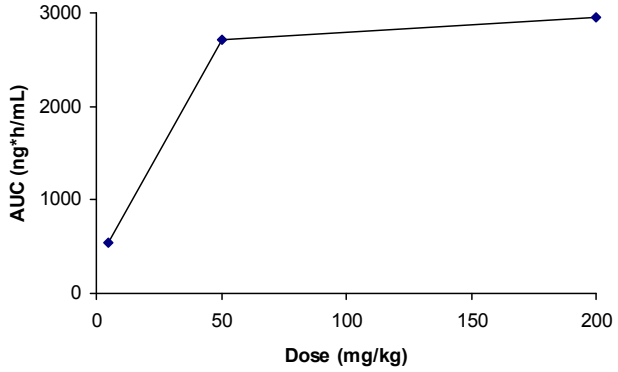


Figure S3. Mean C_{max} of PF-04822163 (**27**) on Day 1 in Sprague Dawley Rats Following Daily Subcutaneous Administration of 5, 50 & 200 mg/kg

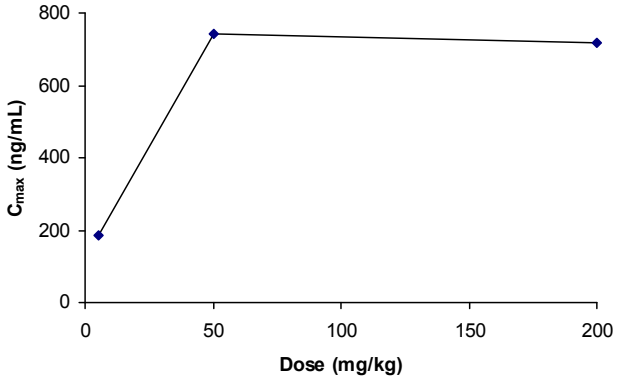


Table S6. Cerep Broad Receptor Protein Profiling of Compound 7a at 10 µM

Protein (human unless otherwise stated)	% inhibition	IC_{50} (nM)	Ki (nM)
5-HT	-3		
5-HT1A	18		
5-HT1B	-11		
5-HT1D (bovine)	4		
5-HT2A	9		
5-HT2A (agonist site)	-3		
5-HT2B (agonist site)	51	7700	7600
5-HT2C	0		
5-HT2C (agonist site)	-7		
5-HT3	4		
5-HT4e	14		
5-HT6	1		
5-HT7	34		
A1	52	1100	7100
A2A/3	39/17		
AbI kinase	-5		
Acetylcholinesterase	1		
Alpha 1	14		
Alpha 2A	11		
Alpha 2B	44		
Alpha 2C	14		
AMPA	-3		
Androgen	3		
AT1	-22		
ATPase (Na+/K+)	0		
Beta 1/2/3	0/5/1		
Ca2+	0		
CaMK2alpha	4		
Carbonic anhydrase	23		
Caspace-3	3		
CB1/2	2/6		
CCKA/B	7/6		
Choline Transporter	18		
Cl-channel	5		

Table S6 contd. Cerep Broad Receptor Protein Profiling of Compound 7a at 10 µM, Contd.

Protein (human unless otherwise stated)	% inhibition	IC_{50} (nM)	Ki (nM)
Cox1/2	0/13		
CXCR4	8		
CYP2C19	43		
CYP2C9	47		
CYP2D6	38		
CYP3A4	18		
D1/D2/D3/D4	10/2/-2/26		
DA transporter	22		
Delta 2	8		
ETA	3		
ETB	8		
FLT-1 kinase	16		
GABA A/B	-3/-12		
GABA transporter	-32		
Glucocorticoid	-25		
Glycine	14		
H1/2/3/4	0/5/2/0		
I1	33		
Kainate			
Kappa			
M1/2/3/4/5	11/11/1/14/3		
MAO-A	6		
ML1	-4		
MMP-9	-3		
mu (MOP)	0		
Na+ Channel	19		
NK1/2	13/1		
NMDA	-15		
PCP	-4		

Table S7. Cerep Broad Receptor Protein Profiling of Compound 27 at 10 µM

Protein (human unless otherwise stated)	% inhibition	IC_{50} (nM)	Ki (nM)
5-HT			
5-HT1A	7		
5-HT1B (rat)	3		
5-HT1D (rat)	-3		
5-HT2A	44		
5-HT2A (agonist site)	72	3600	2200
5-HT2B (agonist site)	94	250	250
5-HT2C	45		
5-HT2C (agonist site)	67	2200	1800
5-HT3	8		
5-HT4e	4		
5-HT6	37		
5-HT7	8		
A1 Adenosine	40	1100	7100
A2 Adenosoine	28		
A3 Adenosine	95	370	620
AbI kinase	-10		
ACE	-5		
Acetylcholinesterase	10		
Alpha 1 Adrenergic	4		
Alpha 2A Adrenergic	3		
Alpha 2B Adrenergic	10		
Angiotensin 1	-6		
Adrenergic alpha 2C	15		
AMPA (rat)	18		
Beta 3 adrenergic	13		
Ca2+ (verapamil/rat)	-19		
CaMK2alpha Kinase	7		
Carbonic anhydrase II	-13		
CB1/2	31/37		
CCK1/2	16/5		
Choline Transporter	22		

Table S7, contd. Cerep Broad Receptor Protein Profiling of Compound 27 at 10 µM, Contd.

Protein (human unless otherwise stated)	% inhibition	IC_{50} (nM)	Ki (nM)
Cox2	27		
CXCR4	-17		
CYP2C19	76	870	
CYP2C9	51		
CYP2D6	12		
CYP3A4	44		
D1/D2/D3/D4	-14/2/-1/7		
DA transporter	20		
Delta 2 opioid	26		
Endothelin A/B	-6/2		
GABA A (rat)	4		
GABA B	6		
GABA transporter (rat)	11		
Glucocorticoid	-3		
Glutamate (rat kainate)	-12		
H1/2/3	1/4/0		
Kappa (rat)	35		
M1/2/3/4	17/8/4/11		
MAO-A (rat)	10		
mu opioid	11		
Na+ Channel	41		
nAChR	0		
NK1/NK2	29/6		
NMDA (rat)	-14		
Norepinephrine Transp.	8		
P38 alpha kinase	-4		
PCP (rat)	-2		
Rolipram (mouse)	51	5800	
Vasopressin 1A	-5		
VEGFR1	-13		

Xray crystal Experimental Information for compound **27.**

SUMMARY:

- The structure was solved in the P2(1) space group
- The asymmetric unit is comprised of two molecules of compound **27**.
- R value 5%
- Absolute configuration

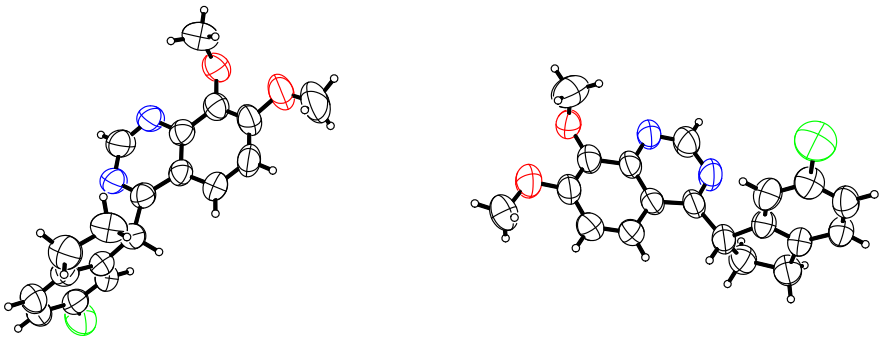


Figure 1. Asymmetric unit. ORTEP with ellipsoids drawn at 50% confidence level.

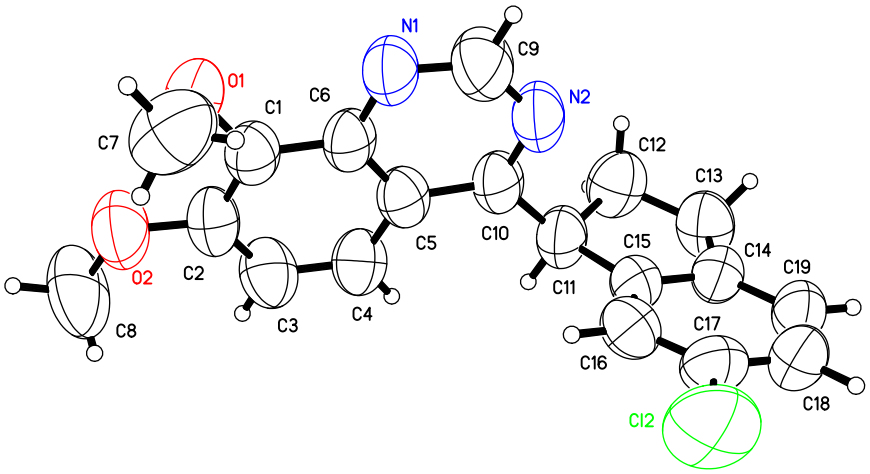


Figure 2. Molecule one in the asymmetric unit. ORTEP with ellipsoids drawn at 50% confidence level.

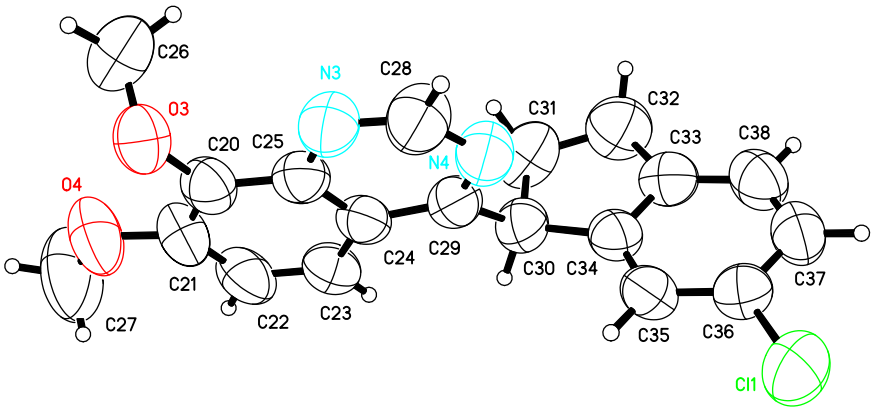


Figure 3. Molecule 2 in the asymmetric unit. ORTEP with ellipsoids drawn at 50% confidence level.

EXPERIMENTAL:

Data collection was performed on a Bruker APEX diffractometer at room temperature. Data collection consisted of 3 omega scans and low angle and three at high angle; each with 0.5 step. In addition, 2 phi scans were collected to improve the quality of the absorption correction.

The structure was solved by direct methods using SHELX software suite in the space group P2(1). The structure was subsequently refined by the full-matrix least squares method. All non-hydrogen atoms were found and refined using anisotropic displacement parameters.

All hydrogen atoms were placed in calculated positions and were allowed to ride on their carrier atoms. The final refinement included isotropic displacement parameters for all hydrogen atoms.

Absolute configuration was determined by examination of the Flack parameter. In this case, the Flack parameter = 0.0136 with esd 0.0256; within range for absolute configuration determination.

The final R-index was 5%. A final difference Fourier revealed no missing or misplaced electron density.

Pertinent crystal, data collection and refinement are summarized in table 1. Atomic coordinates, bond lengths, bond angles, Torsion angles and displacement parameters are listed in tables 2 – 6.

Software and References

SHELXTL, Version 5.1, Bruker AXS, 1997

PLATON, A.L. Spek, J. Appl. Cryst. 2003, 36, 7-13.

MERCURY, C.F. Macrae, P.R. Edington, P. McCabe, E. Pidcock, G.P. Shields, R. Taylor, M. Towler and J. van de Streek, *J. Appl. Cryst.* **39**, 453-457, 2006.

R.W.W. Hooft et al. *J. Appl. Cryst.* (2008). 41. 96-103.

H.D. Flack, *Acta Cryst.* 1983, **A**39, 867-881.

Table 1. Crystal data and structure refinement for compound **27**.

Identification code	z153	
Empirical formula	C19 H17 Cl N2 O2	
Formula weight	340.80	
Temperature	273(2) K	
Wavelength	1.54178 Å	
Crystal system	Monoclinic	
Space group	P2(1)	
Unit cell dimensions	a = 7.3534(3) Å	α= 90°.
	b = 25.5994(8) Å	β= 95.024(2)°.
	c = 9.1479(3) Å	γ = 90°.

Volume	1715.41(10) $Å^3$
Z	4
Density (calculated)	1.320 Mg/m^3
Absorption coefficient	2.078 mm^{-1}
F(000)	712
Crystal size	0.21 x 0.20 x 0.15 mm^3
Theta range for data collection	3.45 to 54.35°.
Index ranges	-7<=h<=7, -26<=k<=21, -9<=l<=9
Reflections collected	7617
Independent reflections	3195 [R(int) = 0.0248]
Completeness to theta = 54.35°	97.5 %
Absorption correction	Empirical
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3195 / 1 / 438
Goodness-of-fit on F^2	1.121
Final R indices [I>2sigma(I)]	R1 = 0.0502, wR2 = 0.1445
R indices (all data)	R1 = 0.0617, wR2 = 0.1638
Absolute structure parameter	0.01(3)
Extinction coefficient	0.0031(7)
Largest diff. peak and hole	0.247 and -0.196 $e.Å^{-3}$

Materials and Methods, Protein Crystallization Studies

Expression and purification of PDE1b for x-ray crystallography

The catalytic domain of PDE1B (residues 142-507) was modified by placement of a 6XHis tag, followed by a thrombin-cleavable linker, at the N-terminus of the protein. The protein was expressed in *E. coli*, and purified by Ni-NTA chromatography, followed by cleavage of the 6XHis tag using thrombin. The cleaved material further purified as described [1]. Briefly, the cleaved PDE1B was applied to a Blue Sepharose 6 Fast Flow (Amersham Biosciences) column (Amersham Biosciences, XK26/20; 26 mm id X 20 cm), and eluted using 10 mM cGMP. Further purification was achieved by separation on a MonoQ HR 1010 column (Amersham Biosciences), followed by gel filtration on a Superdex S200 column (Amersham Biosciences).

Crystallization, data collection, structure solution, and refinement

Crystals of PDE-1B complexed with Compound 109 (5-(5-bromo-2-propoxyphenyl)-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7(6H)-one) [1] were grown by the vapor diffusion method. Large crystals (0.2 X 0.3 X 0.4 mm) appeared after 1-3 days when the protein (10 mg/ml PDE-1B with 250 uM compound 109) was mixed with an equal volume of reservoir (0.1 M Tris-HCl, pH 8.5, 0.2 M $MgCl_2$, 15% PEG 8000) at 22 ^oC. In order to obtain structures of PDE1b bound to compounds **7a**, crystals of PDE1b-13/Compound-109 were transferred to reservoir solution supplemented with 3 mM compound **7a** and allowed to equilibrate overnight at 22 C. Crystals were transferred to a cryoprotectant solution consisting of 85% reservoir solution, 15% glycerol, and flash-cooled in liquid nitrogen. X-ray diffraction data were collected at 100 K using the LS-CAT ID-21 beamline at the Advanced Photon Source. Data statistics are reported in table S1.

The structure was determined by rigid body refinement against the coordinates of PDE1B bound to compound 109, determined during the course of an ongoing research program [1]. The ligand coordinates of compound 109 were first removed, and the resulting "apo" coordinates were refined using the programs REFMAC [2, 3], followed by a final round of refinement using the program AUTOBUSTER [4]. Manual rebuilding was performed throughout the process using the program COOT [5], including automatic and manual placement of water molecules within 2.5-3.4 Å of hydrogen-bonding atoms on the protein. The final refined coordinates and structure factors have been deposited with the RCSB [6], under accession code 4NPV. Model quality indicators are indicated in table S1.

Use of the Advanced Photon Source was supported by the U. S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. DE-AC02-06CH11357. Use of the LS-CAT Sector 21 was supported by the Michigan Economic Development Corporation and the Michigan Technology Tri-Corridor for the support of this research program (Grant 085P1000817).

1. Pandit, J., *Crystal Structure of 3',5'-Cyclic Nucleotide Phosphodiesterase (PDE1B) and Uses Thereof*, USPTO, Editor. 2005, Pfizer Inc. p. 74.

2. Collaborative Computational Project Number 4, *The CCP4 Suite: Programs for Protein Crystallography.* Acta Crystallographica, 1994. **D50**: p. 760-763.

3. Murshudov, G.N., A.A. Vagin, and E.J. Dodson, *Refinement of Macromolecular Structures by the Maximum-Likelihood Method.* Acta Cryst., 1997. **D53**: p. 240-255.

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6. Berman, H.M., et al., *The Protein Data Bank.* Nucl. Acids Res., 2000. **28**(1): p. 235-242.

Compound	**7a**	
A. Data collection		
Space Group	$P4_32_12$	
Unit Cell	a=b=88.4 Å, c=135.0 Å	
Resolution (Å)	50-2.40 (2.49-2.40)	
Completeness (%)	100.0 (100.0)	
R_{sym}[b]	0.088 (0.861)	
χ^2	0.753 (0.635)	
Redundancy	15.8 (16.0)	
B. Refinement		
R_{work}	0.205	
R_{free}[c]	0.236	
Amino Acid Residues (#)	323	
Waters (#)	174	
Average B ($Å^2$)	57.4	
RMSD bond length (Å)	0.008	
RMSD angles (degrees)	0.99	
Ramachandran core (%)	99.4	
Ramachandran outliers (%)	0.6	

Table. Data collection and refinement statistics

[a] Values for the outer resolution shell are given in parentheses.

[b] $R_{sym} = \Sigma_{hkl}(|I_{hkl} - \langle I_{hkl}\rangle|)/\Sigma_{hkl}\langle I_{hkl}\rangle$, where I_{hkl} is the intensity of reflection hkl, and $\langle I_{hkl}\rangle$ is the average intensity of multiple observations.

[c] $R_{work} = \Sigma|Fo - Fc|/\Sigma Fo$, where Fo and Fc are the observed and calculated structure factor amplitudes, respectively. R_{free} is the R-factor for a randomly selected 5% of reflections which were not used in the refinement.

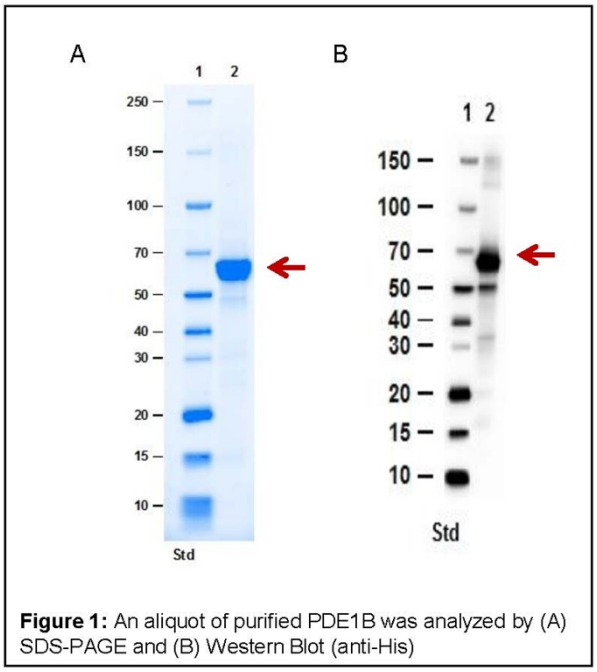


Figure 1: An aliquot of purified PDE1B was analyzed by (A) SDS-PAGE and (B) Western Blot (anti-His)

Phosphodiesterase 1B1 Enzyme Binding Assay. Compounds were profiled against the full length human phosphodiesterase 1B1 enzyme isoform (equivalent to UniProt Q01064 and NCBI NP_000915 reference sequences; amino acids 2-536) in a 384 well plate format. In brief, compounds (1µL in DMSO) were dispensed into wells. Enzyme was diluted to 10 ng/ml in enzyme assay buffer (Tris pH 7.5 (50 mM), $MgCl_2$ (1.3 mM), 21.8 mM $CaCl_2$ (21.8 mM), calmodulin (250 U/ml), Brij (0.01%)(v/v)). 30 µL of diluted enzyme was added to each well and incubated at 25 °C for 15 min. The enzymatic reaction was initiated by the addition of 20 µL of [2,8-^{3}H-] -cAMP Adenosine 3`,5`-Cyclic Phosphate (50 nM prepared in Tris pH 7.5 (50 mM), $MgCl_2$ (1.3 mM), 21.8 mM $CaCl_2$ (21.8 mM)). Following a 30 min incubation at 25 °C the reaction was terminated by addition of 20 µL slurry of PDE Yttrium silicate scintillation proximity assay beads (8 mg/ml in water) (Perkin Elmer, Waltham, Il, USA). The plates were incubated at room temperature for a further 8 h to allow the radiolabeled reaction product to bind to the beads and reach equilibrium. The plates were subsequently counted in a TopCount scintillation counter (Perkin Elmer, Waltham, Il, USA).

Modeling Methods for the Docking of 27 into PDE1B: Coordinates for the X-ray structure of **7a** bound to the catalytic domain of PDE1b (RCSB 4NPV) were used for the modeling studies of **27**. The quinazoline ring portion of **27** was overlaid with that of **7a** and the coordinates for the latter ligand were then deleted. The ligand was then conformationally minimized within the active site while restraining the quinazoline ring portion to its original position with a force constant of 20 Kcal/mol. Global and local ligand docking calculations were conducted using the program AGDOC[1] and the reported pose represents the lowest energy conformation.

(1) Gehlhaar, Daniel K., et al. "Molecular recognition of the inhibitor AG-1343 by HIV-1 protease: conformationally flexible docking by evolutionary programming."*Chemistry & biology* 2.5 (1995): 317-324.

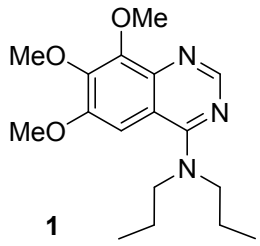
Organic Synthesis Experimental Section

Reagents and starting materials were obtained from commercial sources and used without purification unless otherwise indicated. Silica gel chromatography was performed using Biotage pre-packed cartridges, or glass columns hand packed with JT Baker 40 μM flash silica gel. NMR spectra are presented as chemical shifts relative to the solvent with multiplicities reported as s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), m (multiplet), comp (complicated pattern of overlapping resonances), app (apparent), and obsc (obscured peaks). Re-synthesized products were shown to be >95% by ^{1}H NMR analysis and by HPLC (Waters Acuity instrumentation, CSH C18 1.7 μM 2.1 x 50 mm column, mobile phase A: 99.9 acetonitrile/ 0.1 formic acid; mobile phase B: 99.9 water/0.1 formic acid. Runs operated as 1.5 min gradient beginning at 5:95 A/B and ending with 100% B) with compound identified by UV = 215 nM and ESI positive ion mass spectrometry.

Quinazoline-amine libraries were prepared by the following procedure: To the designated quinazoline template (50 μmol) in a 4 mL reaction vessel was added the designated amine component (60 μmol) followed by isopropanol (500 μL) and triethylamine (150 μmol). The vials were capped and heated at 95 °C for 18 h. The mixtures were concentrated under vacuum and the residues purified via HPLC to yield the desired products.

Aminoquinazoline synthesis, General Procedure A: To the quinazoline component (1 mmol) in isopropanol (5 mL) is added the amine component (1.1 mmol) followed by triethylamine (3 mmol). The mixture is heated to reflux for 12 h or until complete by TLC analysis, at which point the solvent is removed under vacuum. The residue is chromatographed on silica gel with the appropriate eluent to provide the product. Alternatively the residue is partitioned between sodium bicarbonate and ethyl acetate. The organic portion is washed with brine, dried over $MgSO_4$, and concentrated. The residue is purified by silica gel chromatography or by conversion into a solid hydrochloride salt as described in General Procedure B.

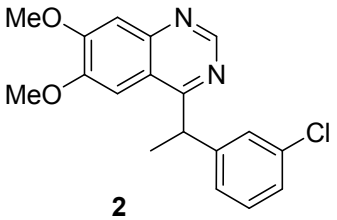
Aminoquinazoline synthesis, General Procedure B: To the quinazoline (0.30 mmol) in THF (3 mL) is added saturated aq $NaHCO_3$ (3 mL) and the amine component (0.33 mmol). The mixture is stirred at rt overnight, or until complete by TLC analysis. The mixture is partitioned between ethyl acetate and water, and the aqueous portion is extracted with an additional volume of ethyl acetate. Combined organic layers are washed with brine, dried with $MgSO_4$ and concentrated. The residue is either i) chromatographed to yield the free amine, or ii) dissolved in ethyl acetate or ethyl ether (~2 mL) and treated with methanolic HCl (0.3 mL of a 1 M solution) followed by stirring to yield the HCl salt as a filterable precipitate.



1

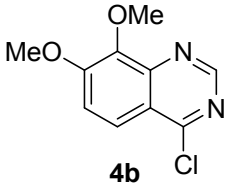
6,7,8-Trimethoxy-*N*,*N*-dipropylquinazolin-4-amine (1). To 4-chloro-6, 7, 8-trimethoxyquinazoline **4a** (16 mg, 0.063 mmol) in 2 mL DMF was added dipropylamine (139

mg, 1.38 mmol). The colorless solution was heated to 50 °C for 3 h, and was then partitioned between 1 N $KHSO_4$ and ether. The aqueous portion was basified to pH >10 with 5N NaOH and extracted twice with ethyl acetate. The combined extracts were washed with brine, dried with sodium sulfate, and concentrated. The residue was re-concentrated twice from hexane and placed under vacuum overnight to remove residual dipropylamine and give a colorless oil. The material was then dissolved in methanol and acidified with concentrated HCl (0.05 mL). The mixture was concentrated twice from ethyl acetate to remove residual HCl, and crystallized from ethyl acetate/methanol to give the title compound hydrochloride as 12 mg (60%) of a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (s, 1H), 7.27 (s, 1H), 4.13 (s, 3H), 4.11 (s, 3H), 4.06 (s, 3H), 3.93 (t, *J* = 9 Hz, 4H), 1.99 (m, 4H), 1.10 (t, *J* = 7.4 Hz, 6 h); MS *m/z* (ion) 320 (M + 1).



2

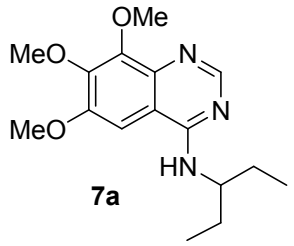
4-(1-(3-Chlorophenyl)ethyl)-6,7-dimethoxyquinazoline (2). To methyl 2-(3-chlorophenyl)propanoate (420 mg, 2.11 mmol) and 4-chloro-6,7-dimethoxyquinazoline (400 mg, 1.76 mmol) in DMF (8 mL) in an ice bath was added dropwise KHMDS (2.11 mmol, 2.11 mL of a 1 M solution in THF). The mixture was stirred at ice bath temperature for 20 min, at which point it was quenched by the addition of 20 mL of 0.5 N $KHSO_4$. The mixture was extracted twice with EtOAc and the combined extracts were washed with brine, dried with $MgSO_4$ and concentrated to yield 798 mg (98%) of a pale amber oil. The oil was dissolved in ethanol (5 mL) and heated to reflux. To the refluxing solution was added dropwise 5 N KOH (1.2 mL) and the mixture was refluxed for 45 min. The mixture was cooled to rt, diluted with 8 mL water, and acidified to pH 1 with 1 N $KHSO_4$. The mixture was extracted twice with ethyl acetate, washed with brine, dried with $MgSO_4$, and concentrated to give 679 mg of a crude amber oil. The material was chromatographed to yield 302 mg (43%) of the title compound as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.14 (s, 1H), 7.3-7.4 (comp, 2H), 7.1-7.2 (comp, 4H), 4.81 (q, *J* = 7.0 Hz, 1H), 3.99 (s, 3H), 3.88 (s, 3H), 1.78 (d, *J* = 7.0 Hz, 3H); MS *m/z* (ion) 329 (M + 1).



4b

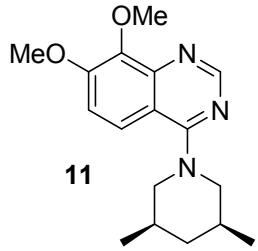
4-Chloro-7,8-dimethoxyquinazoline (4b). To a suspension of 7,8-dimethoxyquinazolin-4(3H)-one (**31**) (170 g, 0.825 mmol) in anhydrous acetonitrile (1.7 L) was added $POCl_3$ (377 g, 2.48 mol). The mixture was heated at reflux for 12 h. The mixture was concentrated under vacuum and residual $POCl_3$ was removed by re-concentrating from acetonitrile. The residue was dissolved in dichloromethane, washed sequentially with cold saturated aq $NaHCO_3$, water and brine (~1 L each). The organic portion was dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was purified via silica gel chromatography to provide

80.0 g (43%) of the title compound as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.97 (s, 1H), 8.03 (d, *J* = 9.2 Hz, 1H), 7.46 (d, *J* = 9.2 Hz, 1H), 4.11 (s, 3H), 4.07 (s, 3H); MS *m/z* (ion) 225 (M + 1).



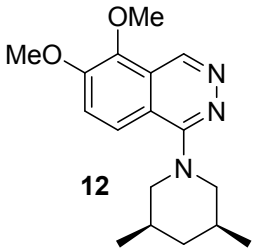
7a

6,7,8-Trimethoxy-*N*-(pentan-3-yl)quinazolin-4-amine (7a). (Note that this compound, also known as PF-04471141 or the hydrochloride salt PF-04471141-01, is now commercially available from Sigma Aldrich). To 4-chloro-6,7,8-trimethoxyquinazoline (5.00 g, 19.6 mmol) and 3-aminopentane (1.88 g, 21.6 mmol) in 2-propanol (40 mL) was added triethylamine and the solution was heated to reflux overnight. The solvent was removed under vacuum and the residue was re-concentrated from ethyl acetate/heptanes to remove residual triethylamine. The residue was reconstituted into 2-propanol and acidified to pH <1 with concentrated HCl. The solution was concentrated under vacuum and the residue was crystallized from 1:1 ethyl acetate/ether. The crystals were isolated via filtration to yield 6.71 g (67%) of a white solid. A sample was converted into the free base by partitioning between saturated $NaHCO_3$ and ethyl acetate. The extract was washed with brine, dried with $MgSO_4$, and concentrated. The residue was triturated with EtOAc/hexanes to produce a white solid: mp 175-176 C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (s, 1H), 6.67 (s, 1H), 5.20 (br s, 1H), 4.35 (m, 1H), 4.09 (s, 3H), 4.01 (s, 3H), 3.99 (s, 3H), 1.74 (m, 1H), 1.59 (m, 1H), 0.96 (t, *J* = 8 Hz, 6H); MS *m/z* (ion) 306 (M + 1).



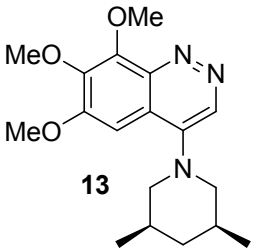
11

4-((3R,5S)-3,5-Dimethylpiperidin-1-yl)-7,8-dimethoxyquinazoline (11). This compound was prepared from 4-chloro-7,8-dimethoxyquinazoline and *cis*-3,5-dimethylpiperidine according to General Procedure B to yield 87 mg (61%) of the free base after chromatography: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H), 7.61 (d, *J* = 9.1 Hz, 1H), 7.16 (d, *J* = 9.1 Hz, 1H), 4.28 (br d, *J* = 12 Hz, 2H), 4.07 (3, 3H), 4.01 (s, 3H), 2.59 (app t, *J* = 12 Hz, 2H), 1.84-1.95 (comp, 4 H), 0.95 (d, *J* = 6.7 Hz, 6H), 0.87 (app q, *J* = 11.9 Hz, 1H). A portion was converted into the hydrochloride salt: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (s, 1H), 7.62 (d, *J* = 9.5 Hz, 1H), 7.23 (d, *J* = 9.5 Hz, 1H), 4.76 (br, 2H), 4.15 (s, 3H), 4.04 (s, 3H), 2.85 (br, 2H), 2.01 (br m, 2H), 1.85 (br m, 2H), 1.02 (d, *J* = 6.6 Hz, 6H), 0.67 (br m, 1H); MS *m/z*, (ion) 302 (M + 1).



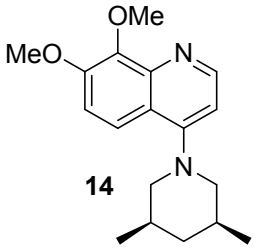
12

1-((3R,5S)-3,5-Dimethylpiperidin-1-yl)-5,6-dimethoxyphthalazine (12). To 1-chloro-5,6-dimethoxyphthalazine[34] (75 mg, 0.33 mmol) in 2-propanol was added cis-3,5-dimethylpiperidine (100 mg, 0.67 mmol) and triethylamine (68 mg, 0.67 mmol). The mixture was heated at reflux overnight. The solvent was removed under vacuum and the residue was chromatographed over silica gel to yield 54 mg (54%) of a yellow solid. This was converted to the hydrochloride salt as in General Procedure B to yield 35 mg (35%) of a pale yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 9.15 (br s, 1H), 8.13 (d, *J* = 9.6 Hz, 1H), 7.91 (d, *J* = 9.6 Hz, 1H), 4.13 (obsc m, 2 H), 4.12 (s, 3H), 4.09 (s, 3H), 2.97 (br t, 2H), 2.02 (comp, 3H), 1.02 (d, *J* = 7.0 Hz, 6H), 0.97-1.07 (obsc m, 1H); MS m/z (ion) 302 (M + 1).



13

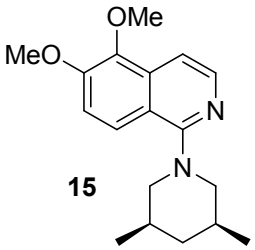
4-((3R,5S)-3,5-Dimethylpiperidin-1-yl)-6,7,8-trimethoxycinnoline (13). Prepared from 4-chloro-6,7,8-trimethoxycinnoline[34] and cis-3,5-dimethylpiperidine in 10% unoptimized yield according to General Procedure B. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (s, 1H), 6.86 (s, 1H), 4.24 (s, 3H), 4.06 (s, 3H), 3.98 (s, 3H), 3.57 (m, 2H), 2.46 (t, *J* = 11.5 Hz, 2H), 1.91-2.01 (comp, 3H), 0.96 (d, *J* = 6.5 Hz, 6H), 0.83 (app q, *J* = 11.8 Hz, 1H); MS m/z (ion) 332 (M + 1).



14

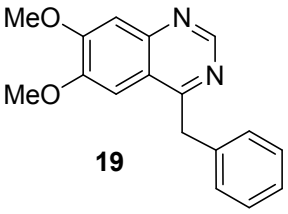
4-((3R,5S)-3,5-Dimethylpiperidin-1-yl)-7,8-dimethoxyquinoline (14). To a solution of 4-chloro-7,8-dimethoxyquinoline (76 mg, 0.338 mmol) and cis-3,5-dimethylpiperidine (101 mg, 0.675 mmol) in isopropanol (4 mL) was added triethylamine (68 mg, 0.675 mmol), and the solution was heated to reflux for 14 h. The solvent was then removed under vacuum and the residue chromatographed (EtOAc/MeOH) to yield 20 mg (20%) of the free base: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (d, *J* = 5.1 Hz, 1H), 7,7 (d, *J* = 9.1 Hz, 1H), 7.25 (d, *J* = 9.1 Hz, 1H), 6.71 (d, *J* = 5.1 Hz, 1H), 4.09 (s, 3H), 4.01 (s, 3H), 3.53 (m, 2H), 1.89-2.04 (comp, 3H), 0.94 (d, *J* =

6.7 Hz, 3H), 0.79 (app q, *J* = 11.7 Hz, 1H). The material was converted into the HCl salt similarly to General Procedure B to afford 20 mg of a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ 8.27 (d, *J* = 7.1 Hz, 1H), 7.90) d, *J* = 9.8 Hz, 1H), 7.54 (d, *J* = 9.8 Hz, 1H), 7.01(d, *J* = 7.1 Hz, 1H), 4.16 (m, 2H), 4.07 (s, 3H), 4.04 (s, 3H), 2.98 (t, *J* = 11.9 Hz, 2H), 1.97 (m, 3H) 1.00 (d, *J* = 6.5 Hz, 6H) 1.03 (obsc m, 1H); MS *m/z* (ion) 300 (M + 1).



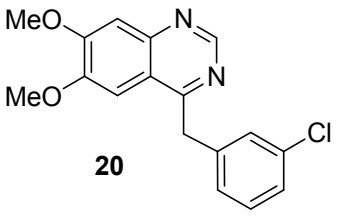
15

1-((3R,5S)-3,5-Dimethylpiperidin-1-yl)-5,6,7-trimethoxyisoquinoline (15). This compound was prepared according to General Procedure A from 1-chloro-5,6-dimethoxyisoquinoline and cis-3,5-dimethylpiperidine. Chromatography (2.5% MeOH in CH_2Cl_2) yielded 15 mg (11%, unoptimized) of the title compound. This was converted into the hydrochloride salt according to General Procedure B to yield 7 mg of a white solid. 1H NMR (400 MHz, CD_3OD) δ 8.07 (d, *J* = 9.5 Hz, 1H), 7.57-7.66 (m, 2H), 4.09 (s, 3H), 4.04 (m, 2H) 3.96 (s, 3H), 2.96 (t, *J* = 12 Hz, 2H), 1.98-2.09 (m, 3H), 1.02 (d, *J* = 6.0 Hz, 6H), 0.96-1.05 (obsc m, 1H); MS *m/z* (ion) 301 (M + 1).



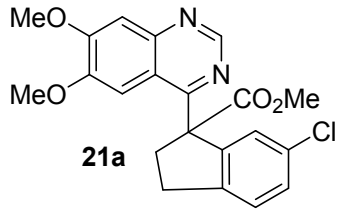
19

4-benzyl-6,7-Dimethoxyquinazoline (19). To methyl-2-phenylacetate (287 mg, 1.45 mmol) and 4-chloro-6,7-dimethoxyquinazoline (250 mg, 1.11 mmol) in DMF (4 mL) at 0 °C was added potassium tert-butoxide (162 mg, 1.45 mmol). The mixture was warmed to rt for 1.5 h, and was then quenched with 5 N sodium hydroxide (5 mL) and heated to 80 °C for 10 min with vigorous stirring. The mixture was acidified with 1M $KHSO_4$ and extracted twice with ethyl acetate. The combined extracts were washed with brine, dried with $MgSO_4$, and concentrated. The solid residue was recrystallized from a stirred solution in ethyl acetate/hexanes to yield 55 mg (20%) of a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.06 (s, 1H), 7.16-7.27 (comp, 7H), 4.51 (s, 2H), 3.99 (s, 3H), 3.87 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 166.0, 155.6, 153.6, 150.1, 148.4, 138.0, 128.8, 128.7, 126.8, 119.6, 107.2, 102.5, 56.4, 56.1, 41.8; MS *m/z* (ion) 281 (M + 1).



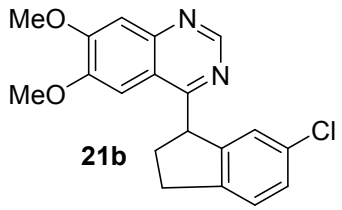
20

4-(3-Chlorobenzyl)-6,7-dimethoxyquinazoline (20). Prepared similarly to compound **19** to afford a pale yellow solid in the amount of 189 mg (40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.06 (s, 1H), 7.28 (s, 1H), 7.1-7.25 (comp, 5 H), 4.47 (s, 2H), 4.00 (s, 3H), 3.90 (s, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 165.2, 155.9, 153.7, 150.5, 148.7, 140.0, 134.8, 130.1, 129.1, 127.2, 127.1, 119.7, 107.4, 102.4, 56.6, 56.3, 41.3; MS *m/z* (ion) 315 (M + 1).



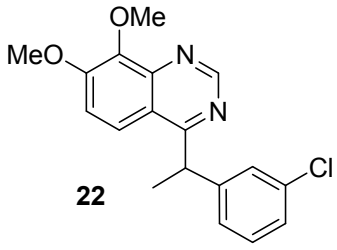
21a

(±) Methyl 6-chloro-1-(6,7-dimethoxyquinazolin-4-yl)-2,3-dihydro-1*H*-indene-1-carboxylate (21a). To a slurry of methyl 6-chloro-2,3-dihydro-1H-indene-1-carboxylate 300 mg, 1.42 mmol) and 4-chloro-6,7-dimethoxyquinazoline (255 mg, 1.13 mmol) in 6 mL THF in an ice bath was added dropwise KHMDS (1.4 mL, 1.4 mmol as a 1M solution in THF). The mixture was stirred for 30 min during which time the slurry became a clear orange solution. The solution was quenched with saturated NH_4Cl (5 mL), diluted with water and extracted twice with ether. The combined extract was washed with brine, dried with $MgSO_4$, treated with decolorizing carbon, filtered and concentrated to give a pale amber oil. The oil was crystallized from ethyl acetate/hexane (~1:1) to give 321 mg (81%) of the title compound as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.07 (s, 1H), 7.46 (d, *J* = 1.5 Hz, 1H,), 7.37 (s, 1H), 7.28-7.35 (comp, 2H), 6.91 (s, 1H), 4.08 (s, 3H), 3.78 (s, 3H), 3.74 (s, 3H), 3.54 (m, 1H), 3.25 (ddd, *J* = 7.9, 7.9, 16 Hz, 1H), 3.07 (ddd, *J* = 4.1, 9.1, 16.1 Hz, 1H), 2.57(m, 1H); MS *m/z* (ion) 399 (M + 1), 400 (M + 2).



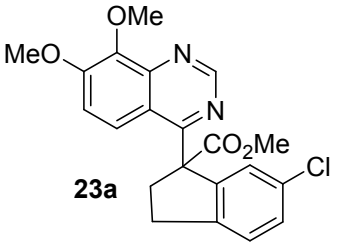
21b

(±) 4-(6-Chloro-2,3-dihydro-1*H* -inden-1-yl)-6,7-dimethoxyquinazoline (21b). Prepared similarly to compound **23b**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.10 (s, 1H), 7.40 (s, 1H), 7.20-7.30 (comp, 2H), 7.21 (dd, *J* = 1.7, 8.2 Hz, 1H), 6.87 (s, 1H), 5.20 (t, *J* = 8.4 Hz, 1H), 4.10 (s, 3H), 3.98 (s, 3H), 3.25 (m, 1H), 3.11 (m, 1H), 2.67-2.72 (comp, 2H); MS *m/z* (ion) 342 (M + 1).



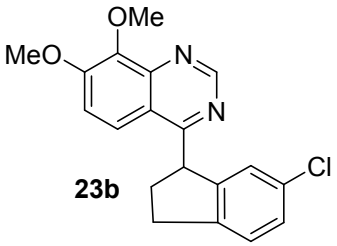
22

(±) 4-(1-(3-Chlorophenyl)ethyl)-7,8-dimethoxyquinazoline (22). To methyl 2-(3-chlorophenyl)propanoate (174 mg, 0.876 mmol) and 4-chloro-7,8-dimethoxyquinazoline (164 mg, 0.73 mmol) in DMF (4 mL) at 0 °C was added KHMDS (0.876 mmol, 0.876 mL dropwise, 1 M solution in THF). After 15 min the mixture was warmed to rt for 30 min. The mixture was quenched with 4 mL saturated $NaHCO_3$ and partitioned between water and ethyl acetate. The aqueous layer was extracted with an additional quantity of ethyl acetate and the combined organic material was washed with brine, dried with $MgSO_4$ and concentrated to yield an oil. The oil was dissolved in 5 mL ethanol and heated to reflux, at which point (1 mL) 5 N NaOH (1 mL) was added. After 1 h the mixture was diluted with water and extracted with ether. The extract was washed with brine, dried with $MgSO_4$, and concentrated. Silica gel chromatography eluting with 20:80 EtOAc/hexanes afforded 302 mg (43%) of the title compound as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.27 (s, 1H), 7.85 (d, *J* = 9.7 Hz, 1H), 7.28-7.32 (comp, 2H), 7.11-7.18 (comp, 3H), 4.92 (q, *J* = 7.2 Hz, 1H), 4.07 (s, 3H), 3.99 (s, 3H), 1.76 (d, *J* = 7.93 Hz, 3H); MS *m/z* (ion) 329 (M + 1).



23a

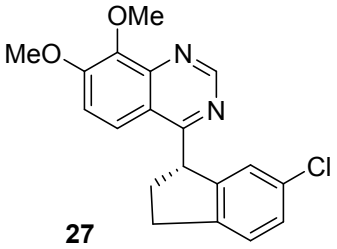
(±) Methyl 6-chloro-1-(7,8-dimethoxyquinazolin-4-yl)-2,3-dihydro-1*H* -indene-1-carboxylate (23a). To 4-chloro-7,8-dimethoxyquinazoline (549 mg, 2.61 mmol) and methyl 6-chloro-2,3-dihydro-1H-indene-1-carboxylate **34** (500 mg, 2.00 mmol) in THF (8 mL) at 0 °C was added dropwise a solution of KHMDS (2.72 mL, 2.72 mmol, 1M in THF). The mixture was stirred for 30 min and was then quenched by the addition of saturated NH_4Cl (10 mL). The mixture was partitioned twice between ether and water. The organic portion was washed with brine, dried with $MgSO_4$, treated with decolorizing carbon, filtered and concentrated to give a yellow solid. Recrystallization from a stirred solution of EtOAc/heptane gave 500 mg (53%) of the title compound as a white powder. The mother liquor was chromatographed to provide an additional 93 mg for a combined yield of 559 mg (63%). A portion was recrystallized from EtOAc/hexanes without stirring to give colorless prisms: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.13 (s, 1H), 7.53 (d, *J* = 9.8 Hz, 1H), 7.40 (d, *J* = 2.1 Hz, 1H), 7.19-7.30 (comp, 3 H), 4.08 (s, 3H), 4.00 (s, 3H), 3.65 (s, 3H), 3.44 (m, 1H), 3.16 (m, 1H), 2.98 (m, 1H), 2.50 (m, 1H), MS *m/z* (ion) 399 (M + 1).



23b

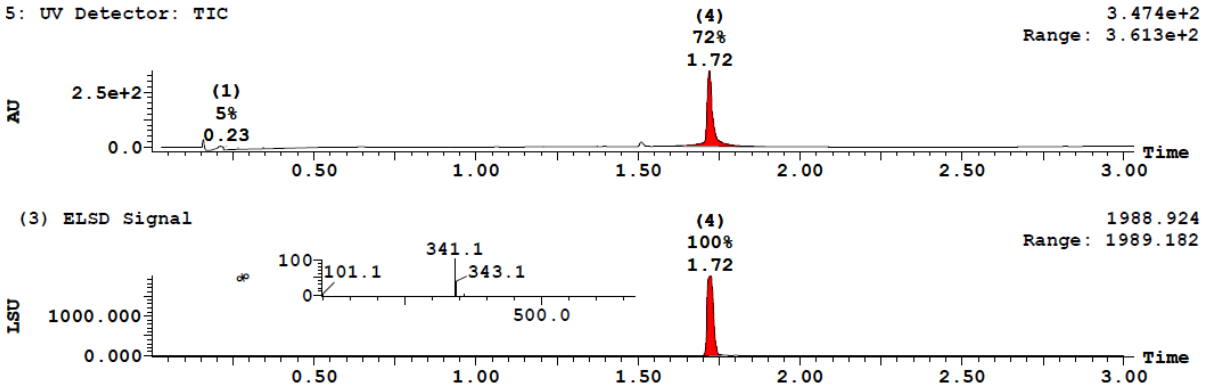
(±) 4-(6-Chloro-2,3-dihydro-1*H*-inden-1-yl)-7,8-dimethoxyquinazoline (23b). A mixture of methyl 6-chloro-1-(7,8-dimethoxyquinazolin-4-yl)-2,3-dihydro-1H-indene-1-carboxylate (**23a**) (340 mg, 0.85 mmol) in 1N NaOH (5 mL) and acetonitrile (5 mL) was irradiated in a Biotage

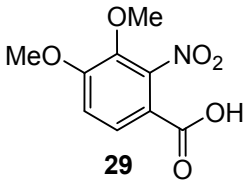
Initiator microwave at 130 °C for 10 min. The crude mixture was extracted twice with ethyl acetate. The organic layer was washed with brine, treated with $MgSO_4$ and decolorizing carbon, filtered, and concentrated under reduced pressure. The residue was purified via crystallization from ethyl acetate to afford 173 mg (60%) of the title compound as a white powder: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.17 (s, 1H), 7.95 (d, *J* = 8.0 Hz, 1H), 7.41 (d, *J* = 8.0 Hz, 1H), 7.23 (obsc d, 1H), 7.15 (dd, 1H, *J* = 8.0, 2.0 Hz), 6.83 (s, 1H), 5.23 (t, *J* = 8.0 Hz, 1H), 4.12 (s, 3H), 4.06 (s, 3H), 3.17-3.25 (m, 1H), 3.00-3.08 (m, 1H), 2.61-2.67 (m, 2H); MS *m/z* (ion) 341 (M + 1), 343 (M + 2).



27

(S)-4-(6-Chloro-2,3-dihydro-1*H*-inden-1-yl)-7,8-dimethoxyquinazoline (27). Chiral resolution was accomplished on a Chiralpak AD-H column eluting with 70:30 CO_2/methanol. The product was then crystallized from ethyl acetate/hexanes to give white needles: mp 121-123 °C; $[a]^{20}_D$ -121.6° (c 0.80, $CHCl_3$); LCMS trace below:

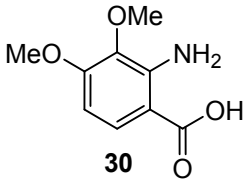




29

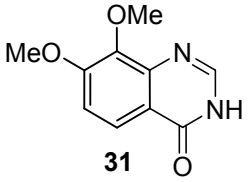
3,4-Dimethoxy-2-nitrobenzoic acid (29). A suspension of 3,4-dimethoxy-2-nitrobenzaldehyde (180 g, 853 mmol) in acetone (1.8 L) and water (1.7 mL) was heated to 70 °C. $KMnO_4$ (276 g, 1.4 mol) was added in portions while keeping the internal temperature between 70 and 90 °C. The mixture was stirred at 80 °C for an additional 2 h and filtered through Celite. The filter cake was rinsed with water, the filtrate was acidified with concentrated HCl and the

resultant slurry was cooled in an ice bath for 1 h. The precipitate was collected via filtration, washed with water (400 mL), and dried to yield 150 g (74%) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.77 (d, *J* = 8.0 Hz, 1H), 7.3 (d, *J* = 8 Hz, 1H), 3.98 (s, 3H), 3.77 (s, 3H).



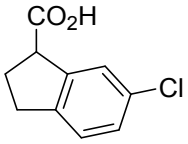
30

2-Amino-3,4-dimethoxybenzoic acid (30). A mixture of 3,4-dimethoxy-2-nitrobenzoic acid and 5% palladium on carbon in ethanol was hydrogenated at 50 psi for 16 h. The mixture was carefully filtered and the filter cake was rinsed with ethanol. The solvent was removed under vacuum to yield 76 g (87%) of the title compound as a brown solid that was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.49 (d, *J* = 8.8 Hz, 1 H), 6.30 (d, *J* = 8.8 Hz, 1H), 3.79 (s, 3H), 3.62 (s, 3H).



31

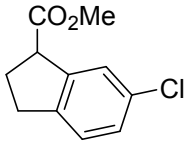
7,8-Dimethoxyquinazolin-4(3*H*)-one (31). A mixture of 2-amino-3,4-dimethoxybenzoic acid (115 g, 0.59 mol), trimethylorthoformate (253 g, 2.4 mol) and ammonium acetate (220 g, 2.86 mol) was heated to 160 °C in a 5 L autoclave for 12 h. The mixture was cooled and the resultant off white solid was filtered and rinsed with methanol to afford 85 g (71%) of the title compound as an off-white solid that was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.05 (br s, 1H), 8.01 (s, 1H), 7.07 (d, *J* = 8.4 Hz, 1H), 7.02 (d, *J* = 8.4 Hz, 1H), 3.90 (s, 3H), 3.82 (s, 3H).



33

(±) 6-Chloro-2,3-dihydro-1*H*-indene-1-carboxylic acid (33). To 2,3-dihydro-1H-indene-1-carboxylic acid (16.4 g, 101 mmol) and *N*-chlorosuccinimide (16.2 g, 121 mmol) in TFA (50 mL) at -20 °C was added dropwise concd HCl at a rate to keep the internal temperature between -10 °C and -20 °C (~20 min; caution, an exotherm is possible with fast addition). The mixture was kept at ice bath temperature for 20 min, and then warmed to rt. The resultant thick slurry was partitioned between 100 mL each of ether and water. The organic portion was washed three times with 1 M HCl to remove succinimide, and once with 1:9 1M HCl/brine. The organic layer was dried over $MgSO_4$ and concentrated under vacuum to a thin yellow oil which solidified on

stirring. After stirring overnight, the precipitate was collected via filtration and rinsed with cold ether to provide 6.0 g (30%) of a pale yellow solid (NMR ratio 7:1:0.5, title compound vs. unknown regioisomers). Recrystallization from acetonitrile/isooctane (1:1) gave 4.71 g (24%) of the desired product as a >20:1 ratio (^{1}H NMR) with an uncharacterized minor regioisomer. Title compound: ^{1}H NMR ($CDCl_3$): δ 7.4 (s, 1H), 7.2 (t, 2H), 4.1 (t, 1H), 3.1 (m, 1H), 2.9 (m, 1H), 2.4-2.5 (m, 2H).



34

(±) Methyl 6-chloro-2,3-dihydro-1*H*-indene-1-carboxylate (34). To a solution of 6-chloro-2,3-dihydro-1H-indene-1-carboxylic acid **31** (25.0 g, 110 mmol) in methanol (250 mL), was added H_2SO_4 (12.5 mL) and the solution was heated at reflux for 3 h. The reaction mixture was then concentrated to remove the bulk of the methanol and slowly added to a mixture of methylene chloride and saturated $NaHCO_3$ while ensuring a basic mixture throughout the addition. The phases were separated and the organic layer was dried and concentrated. Silica gel chromatography provided 20.8 g (80%) of the title compound as a pale yellow liquid. IR (thin film): 3068, 2950, 2902, 2851, 1736, 1595, 1471, 1438, 1339, 1265, 1202, 1163, 1069, 1028 cm^{-1}. ^{1}HNMR ($CDCl_3$): δ 7.4 (s, 1H), 7.1 (s, 2H), 4.0 (t, 1H), 3.8 (s, 3H), 3.0-3.1 (m, 1H), 2.8-2.9 (m, 1H), 2.3-2.5 (m, 2H); MS m/z (ion) 211 (M + 1).