Supporting Information

2-Aminopyridines with a Truncated Side Chain to Improve Human Neuronal Nitric Oxide Synthase Inhibitory Potency and Selectivity

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Table S1. Crystallographic data collection and refinement statistics.

Data set [1]	nNOS-**10a**	nNOS-**14a**	nNOS-**14b**	nNOS-**19a**
Data collection				
PDB code	4UGZ	4UH0	4UH1	4UH2
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions a, b, c (Å)	51.8 111.2 164.9	52.2 111.7 165.6	52.2 111.1 164.1	51.7 111.0 164.4
Resolution (Å)	2.08 (2.11-2.08)	2.03 (2.07 -2.03)	1.80 (1.83-1.80)	1.99 (2.02-1.99)
Rmerge	0.096 (>1.00)	0.063 (0.392)	0.058 (0.458)	0.096 (0.931)
Rpim	0.061 (0.721)	0.037 (0.456)	0.034 (0.285)	0.039 (0.577)
CC 1/2	n/a (0.575)	n/a (0.933)	n/a (0.925)	n/a (0.871)
I / σI	16.5 (0.8)	25.7 (2.0)	28.4 (1.8)	23.3 (1.0)
No. unique reflections	57,430	61,372	88,754	64,181
Completeness (%)	99.1 (94.5)	98.0 (93.3)	99.5 (99.2)	97.9 (91.0)
Redundancy	3.5 (3.0)	3.8 (3.7)	3.9 (3.4)	5.2 (3.2)
Refinement				
Resolution (Å)	2.08	2.04	1.80	1.99
No. reflections used	57,220	61,196	88,343	63,889
R_{work} / R_{free} [2]	0.190/0.227	0.181/0.224	0.200/0.234	0.179/0.216
No. atoms				
Protein	6673	6660	6671	6659
Ligand/ion	173	183	173	181
Water	256	377	456	297
R.m.s. deviations				
Bond lengths (Å)	0.008	0.008	0.015	0.007
Bond angles (deg)	1.14	1.13	1.76	1.14

Data set 1	nNOS-**19b**	nNOS-**19c**	HnNOS-**14b**	HnNOS-**19c**
Data collection				
PDB code	4UH3	4UH4	4UH5	4UH6
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions a, b, c (Å)	51.7 110.6 164.6	52.1 111.5 165.2	52.4 122.6 164.0	52.3 122.7 165.0
Resolution (Å)	2.03 (2.07-2.03)	1.95 (1.98-1.95)	1.98 (2.05-1.98)	1.98 (2.05-1.98)
Rmerge	0.079 (0.982)	0.088 (0.882)	0.095 (1.710)	0.077 (0.922)
Rpim	0.040 (0.874)	0.040 (0.395)	0.086 (1.555)	0.070 (0.828)
CC 1/2	n/a (0.874)	n/a (0.932)	0.995 (0.335)	0.996 (0.398)
I / σI	24.1 (1.6)	28.5 (2.5)	5.3 (0.4)	8.2 (1.1)
No. unique reflections	62,031	69,615	71,749	71,459
Completeness (%)	99.6 (99.6)	99.4 (99.9)	97.0 (90.1)	96.3 (91.8)
Redundancy	4.8 (4.2)	6.0 (5.9)	3.9 (3.6)	4.1 (4.0)
Refinement				
Resolution (Å)	2.03	1.95	1.98	1.98
No. reflections used	61,974	66,076	71,655	71,391
R_{work} / R_{free} [2]	0.185/0.234	0.191/0.224	0.195/0.247	0.173/0.213
No. atoms				
Protein	6686	6665	6735	6716
Ligand/ion	175	177	167	171
Water	226	280	297	512
R.m.s. deviations				
Bond lengths (Å)	0.017	0.011	0.007	0.008
Bond angles (deg)	1.85	1.96	1.15	1.18

Data set [1]	eNOS-**10a**	eNOS-**14b**	eNOS-**19b**	eNOS-**19c**
Data collection				
PDB code	4UH7	4UH8	4UH9	4UHA
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions a, b, c (Å)	58.0 106.4 157.2	57.9 106.3 156.9	58.2 106.8 157.9	57.9 106.3 156.5
Resolution (Å)	2.24 (2.28-2.24)	2.30 (2.34-2.30)	2.14 (2.23-2.14)	2.20 (2.30-2.20)
Rmerge	0.057 (0.660)	0.060 (0.738)	0.069 (1.063)	0.102 (1.741)
Rpim	0.033 (0.374)	0.035 (0.431)	0.034 (0.614)	0.091 (1.531)
CC 1/2	n/a (0.815)	n/a (0.725)	0.999 (0.535)	0.995 (0.346)
I / σI	29.9 (2.3)	26.8 (1.9)	14.8 (1.2)	7.6 (0.8)
No. unique reflections	47,727	43,858	54,517	49,840
Completeness (%)	99.6 (99.9)	99.6 (100.0)	98.5 (98.5)	99.7 (98.4)
Redundancy	4.0 (4.0)	3.9 (3.9)	4.9 (3.6)	4.0 (4.0)
Refinement				
Resolution (Å)	2.24	2.30	2.14	2.20
No. reflections used	47,588	43,089	54,405	49,581
R_{work} / R_{free} [2]	0.159/0.211	0.161/0.217	0.174/0.227	0.181/0.240
No. atoms				
Protein	6431	6438	6418	6408
Ligand/ion	234	191	234	213
Water	338	294	243	233
R.m.s. deviations				
Bond lengths (Å)	0.008	0.007	0.017	0.008
Bond angles (deg)	1.16	1.18	1.90	1.20

[1] See Figure 3 for the inhibitor chemical formula.

[2] R_{free} was calculated with the 5% of reflections set aside throughout the refinement. The set of reflections for the R_{free} calculation were kept the same for all data sets of each isoform according to those used in the data of the starting model.

Table S2. HPLC and MS Conditions for DMPK study

Chromatographic Mode **:**	LC/MS/MS
MS System Used **:**	AB Sciex API-4000
Software Version **:**	Analyst 1.5
Scan Type **:**	MRM
Polarity **:**	Positive
Ion Source **:**	Turbospray
Mobile Phase **:**	A**:** 0.1% Formic Acid in Water B**:** 0.1% Formic Acid in Acetonitrile
Flow Rate (mL/min) **:**	0.8
Needle Stroke **:**	52
Splitter **:**	Approximately 75% out
Probe Position **:**	5 mm vertical, and 5mm horizontal
Injection Volume (µL) **:**	5
Auto Sampler Temperature (°C) **:**	4
Column Oven Temperature (°C) **:**	40
Column Used (length x width in mm, Particle size)**:**	WATERS Xterra, MX C18, (50 x 3.0, 5 µ)
Retention Time (in min) **:**	**19c**: 1.11
Glipizide (IS):	1.44
Run Time (in min) **:**	3.20

Table S3. MRM Transitions at DMPK:

Q1 Mass (Da)	**Q3 Mass (Da)**	**I.D.**	**Dwell time (msec)**	**DP**	**CE**	**CXP**
324.5	122.3	**19c**	80	80	26	6
446.3	347.0	GLIPIZIDE_POS	50	40	22	12
Source Parameters						
CAD	6					
CUR	25					
GS1	60					
GS2	40					
Ion Spray Voltage	5500					
Temperature	500					
Interface Heater	ON					
EP	10					