Supplementary Material for

Novel nanomolar Imidazopyridines as selective Nitric Oxide Synthase (iNOS) inhibitors: SAR and structural insights

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Crystallization and Refinement

iNOS (10 mg/ml) in a solution of 6.7 mM DTT, 6.7 mM BH4. and 5% beta-octyl glycoside was mixed in a 1:1 (v/v) ratio with the reservoir solution (1000 mM ammonium sulfate, 33.3 mM Mes buffer at pH 6.0, 6.7 mM DTT, 6.7 mM BH4 and 5% beta-octyl glycoside). Crystals were obtained by the hanging drop vapour diffusion method by equilibrating against 1 ml of the reservoir solution for 5 days at 18°C. After adding 20% (v/v) glycerol for cryo protection, they were harvested with loops and frozen in liquid nitrogen. Complete X-ray datasets were obtained at the synchrotrons DESY (Hamburg, Germany), ESRF (Grenoble, France) and SLS (Villigen, Switzerland) on Mar CCD detectors and using cryogenic temperature. Datasets were processed with the XDS package.[1] Crystallographic refinement was performed with CNS version 1.1.[2] The same set of 5% of the total number of reflections was used throughout all refinement rounds for the calculation of R_{free}. Model building was performed in O.[3]

Starting point of the refinement was the structure of murine iNOS (PDB code 1NOD). After a first energy minimization, the inhibitor was built into difference density, and the refinement was continued. Ideal geometry libraries for the inhibitors were established manually.

Strong non-crystallographic restraints were employed throughout the refinement.

The coordinates of the co-crystal structure of iNOS in complex with BYK191023 **17** have been deposited with RCSB Protein Data Bank under the accession code 3NW2.

Table 1. Data collection and refinement statistics of the iNOS complex with the inhibitor BYK191023 **17**.

Space group	P6(1)22
Cell constant a, b, c (Å)	212.6, 212.6, 111.5
Resolution (Å)	50 – 2.8
No. of observations	345021
No. of unique reflections	36767
Completeness of data (%)	99.4
R_{sym}[a] for data (%)	21.6
<I/σ (I)>	8.2
R_{free}[b] (%)	32.3
R-factor[b] (%)	27.9
No. of protein atoms (non-hydrogens)	6813
No. of water molecules	84
No. of other atoms (BH4, heme, SO_4, inhibitor)	163
r.m.s. dev., bond (Å)	0.009
r.m.s. dev., angle (deg)	1.5

[a] $R_{sym} = \Sigma |I - \langle I \rangle| / \Sigma I$, where I is the observed intensity and $\langle I \rangle$ is the average intesity for multiple measurements. [b] The R_{free} was calculated from a random selection of 1824 reflections (~5% of the data); the R-factor was calculated with the remaining intensities.

REFERENCES

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