Supporting Information for

Catalytic Mechanism of Cytochrome P450 for *N*-methylhydroxylation of Nicotine: Reaction Pathways and Regioselectivity of the Enzymatic Nicotine Oxidation

Dongmei Li,*[a,b] Xiaoqin Huang,[b] Jianping Lin[a] and Chang-Guo Zhan*[b]

[a] *College of Pharmacy, State Key Laboratory of Medicinal Chemical Biology and Tianjin Key Laboratory of Molecular Drug Research, Nankai University, Tianjin 300071, China; Fax: +86-22-23507760; Tel: +86-22-23506290; E-mail: dongmeili@nankai.edu.cn*

[b] *Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, 789 South Limestone Street, Lexington, Kentucky 40536, USA; Tel: 859-323-3943; E-mail: zhan@uky.edu*

Table of Contents:

Table S1. Relative free energies (in kcal/mol) calculated at the QM/MM(B1:AMBER), QM/MM(B2:AMBER) and QM/MM-FEP(B2:AMBER) levels including zero-point and thermal corrections for the QM subsystem.

	QM/MM (B1:AMBER)+ZPE	QM/MM (B2:AMBER)+ZPE	QM/MM-FEP (B2:AMBER) + ZPE
CYP2A6-SR_t complex			
^{4}RC	0.0	0.0	0.0
^4TS_H	18.8	17.9	18.0
^{4}IM	1.6	-2.4	-1.8
$^4TS_{reb}$	1.9	-2.9	-2.3
^{4}PC	-42.7	-45.7	-45.3
^{2}RC	0.0	0.0	0.0
^2TS_H	16.8	16.2	15.5
^{2}IM	3.4	1.3	0.7
$^2TS_{reb}$	3.4	1.1	-0.2
^{2}PC	-37.0	-41.5	-41.6
CYP2A6-SR_c complex			
^{4}RC	0.0	0.0	0.0
^4TS_H	21.2	20.9	19.4
^{4}PC	-45.2	-49.1	-46.0
^{2}RC	0.0	0.0	0.0
^2TS_H	19.6	19.5	18.0
^{2}PC	-38.4	-43.3	-42.6

Table S2. Imaginary vibrational frequencies calculated for the transition states

CYP2A6-SR_t complex				CYP2A6-SR_c complex	
^4TS_H	*i*1638	$^4TS_{reb}$	*i*85	^4TS_H	*i*1738
^2TS_H	*i*1199	$^2TS_{reb}$	*i*79	^2TS_H	*i*1091