Supplemental Data

DMD-AR-2021-000804

Direct and Sequential Bioactivation of Pemigatinib to Reactive Iminium Ion Intermediates Culminate in Mechanism-Based Inactivation of Cytochrome P450 3A

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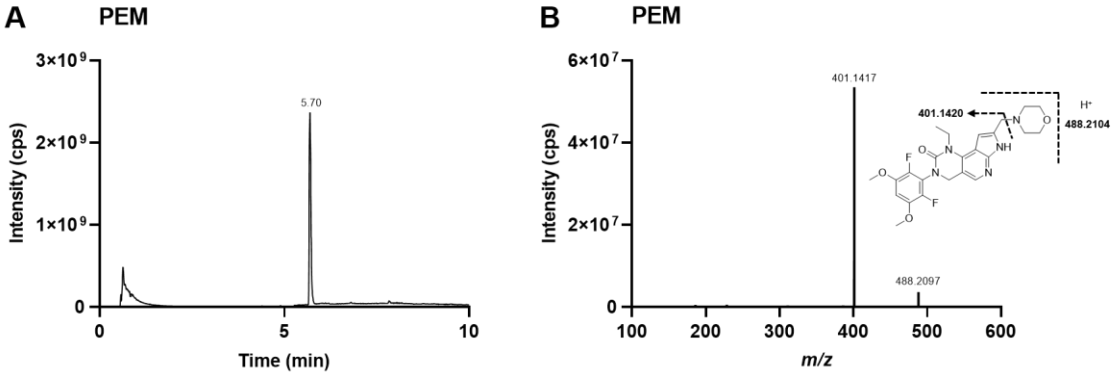
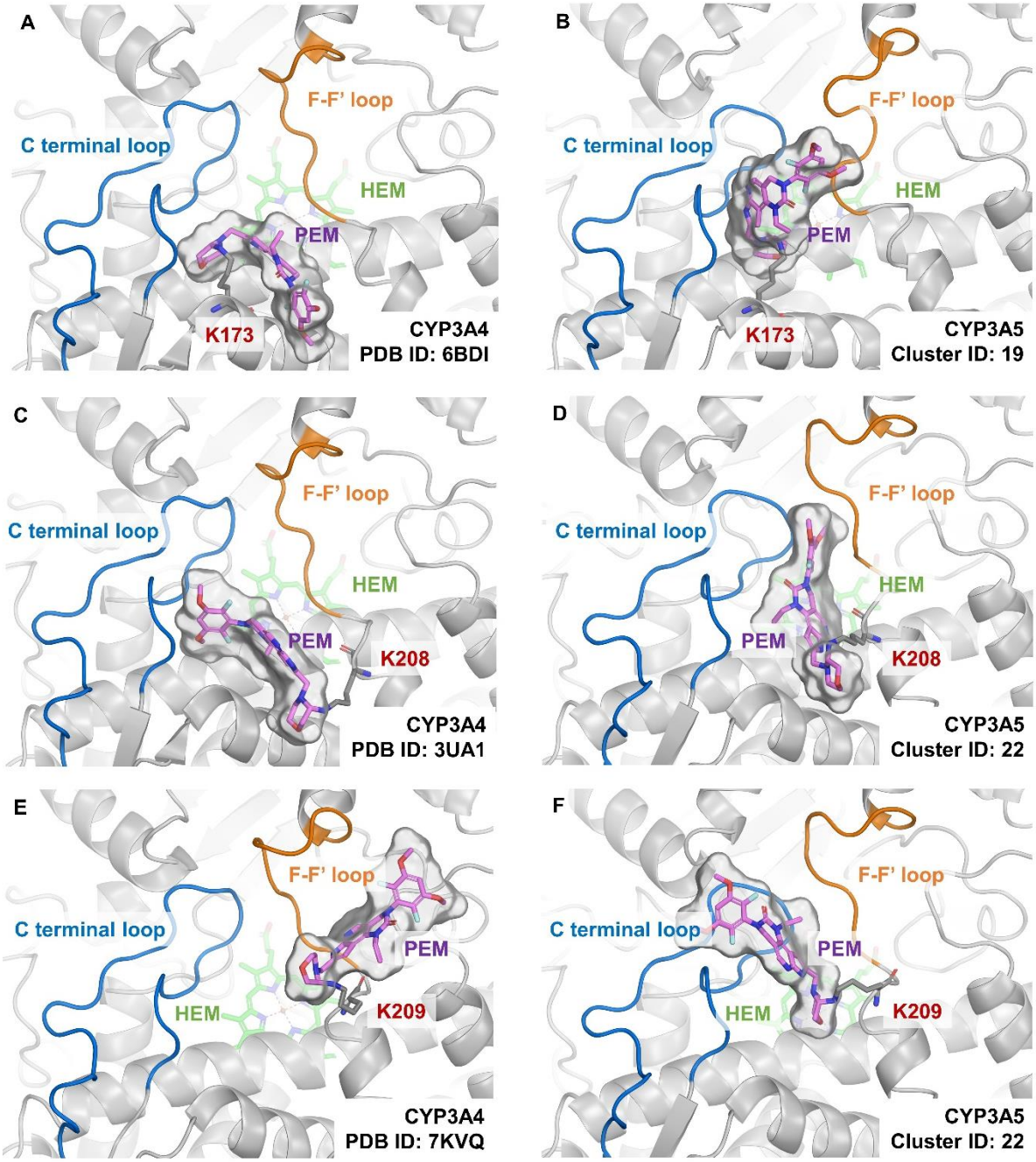
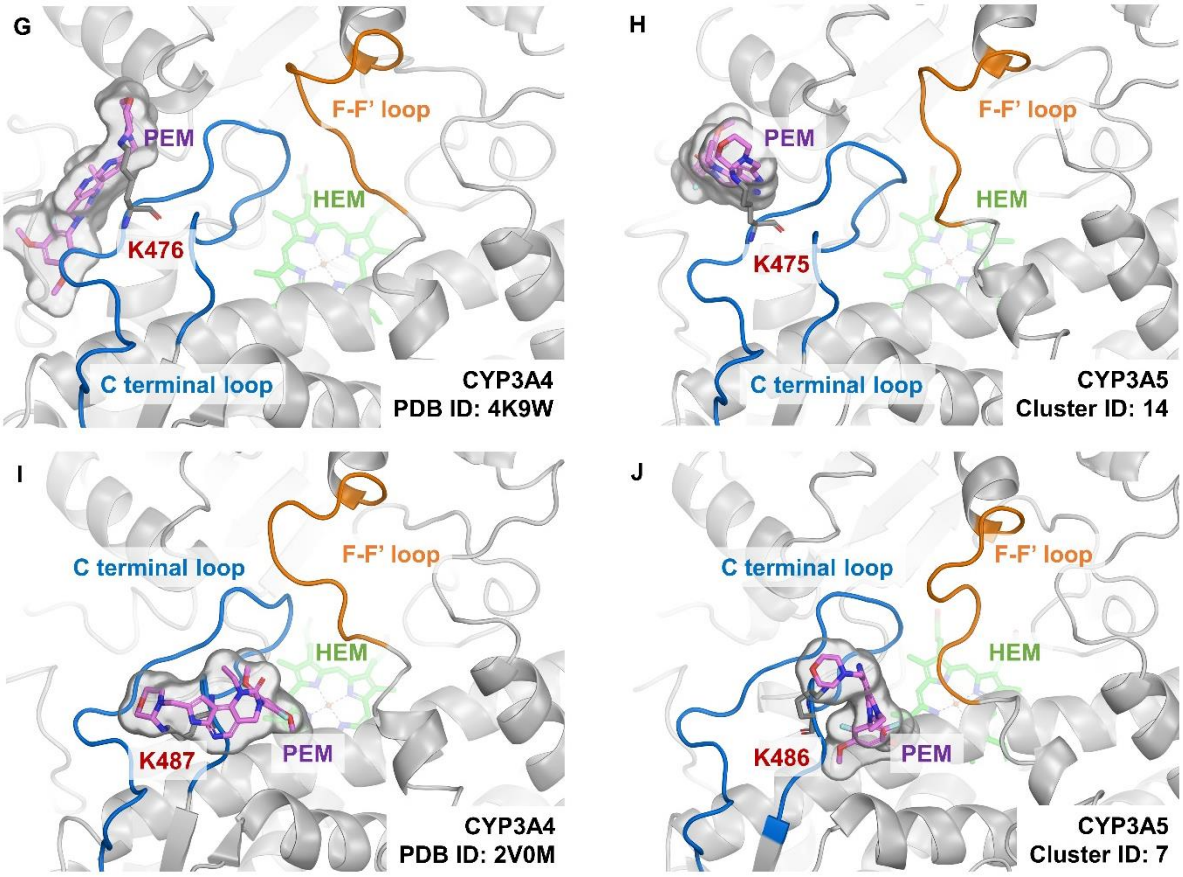
Supplemental Fig. 1. (A) Representative product ion chromatogram of PEM (retention time: 5.70 min). (B) Proposed accurate mass fragmentation pattern of PEM. The exact mass MS/MS spectra depict the experimental *m/z* values whereas the chemical structure (inset) illustrate the theoretical accurate *m/z* values of the parent and product ions as outlined in Supplementary Table 2 using a mass tolerance of 5 ppm.



Supplemental Fig. 2. Molecular plots illustrating the top scored binding poses for PEM adducts on (A and B) Lys173, (C and D) Lys208, (E and F) Lys209, (G and H) Lys476/475 and (I and J) Lys487/486 in CYP3A4 and CYP3A5 respectively. Color code: F-F' loop, orange; C terminal loop, blue; heme moiety, green; PEM, pink stick with white surface.




G
PEM
F-F' loop
HEM
K476
C terminal loop
CYP3A4
PDB ID: 4K9W
H
PEM
F-F' loop
HEM
K475
C terminal loop
CYP3A5
Cluster ID: 14
I
C terminal loop
F-F' loop
HEM
K487
PEM
CYP3A4
PDB ID: 2V0M
J
C terminal loop
F-F' loop
HEM
K486
PEM
CYP3A5
Cluster ID: 7

Supplemental Table 1. Optimized compound-dependent MS parameters for LC/MS/MS analysis

Compound	Q1 Mass (*m/z*)	Q3 Mass (*m/z*)	DP (V)	EP (V)	CE (V)	CXP (V)
Pemigatinib (PEM)	488	401	78	11	26	12
Erdafitinib	447	362	48	10	31	15
Hydroxylated Rivaroxaban	452	406	90	10	25	9
Dexamethasone	393	355	112	10	14	14

DP: declustering potential, EP: entrance potential, CE: collision energy, CXP: collision exit potential

Supplemental Table 2. Accurate mass measurement of the parent and product ions of PEM using a mass tolerance of 5 ppm.

Proposed Elemental Composition	**Theoretical *m/z***	**Experimental *m/z***	**Mass Accuracy**	
			∆Da	**∆ppm**
$C_{24}H_{28}O_4N_5F_2$	488.2104	488.2097	-0.0007	-1.4
$C_{20}H_{19}O_3N_4F_2$	401.1420	401.1417	-0.0003	-0.8

Supplemental Table 3. Summary of covalent docking scores and distances to F-F' loop of the top scored binding poses for PEM adducts on lysine residues in CYP3A4 and CYP3A5.

CYP3A4				CYP3A5			
Residue	PDB/Cluster ID	Docking Score	Minimum distance between PEM and F-F' loop (Å)	Residue	PDB/Cluster ID	Docking Score	Minimum distance between PEM and F-F' loop (Å)
Lys173	6BDI	-5.3	3.4	Lys173	Cluster 19	-5.1	3.3
Lys208	3UA1	-5.3	3.5	Lys208	Cluster 22	-5.4	3.2
Lys209	7KVQ	-4.7	3.1	Lys209	Cluster 22	-3.4	3.8
Lys476	4K9W	-5.1	10.6	Lys475	Cluster 14	-5.4	13.4
Lys487	2V0M	-2.5	2.9	Lys486	Cluster 7	-2.7	3.7