Supplementary Data

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Infigratinib is a Reversible Inhibitor and Mechanism-based Inactivator of Cytochrome P450 3A4

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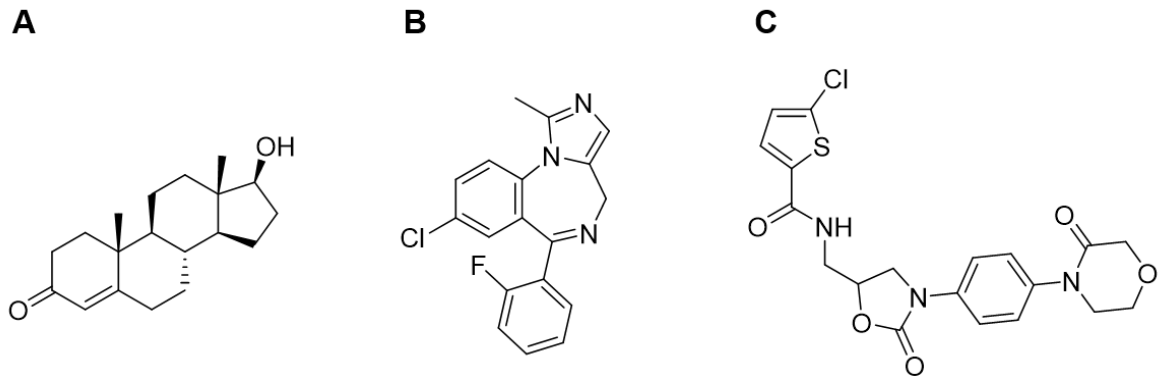
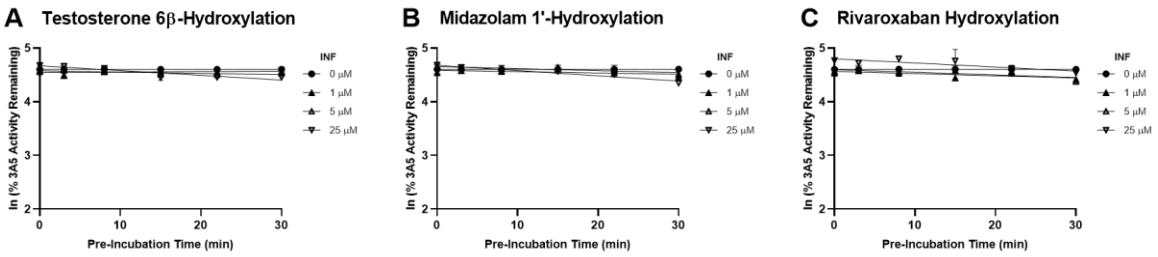
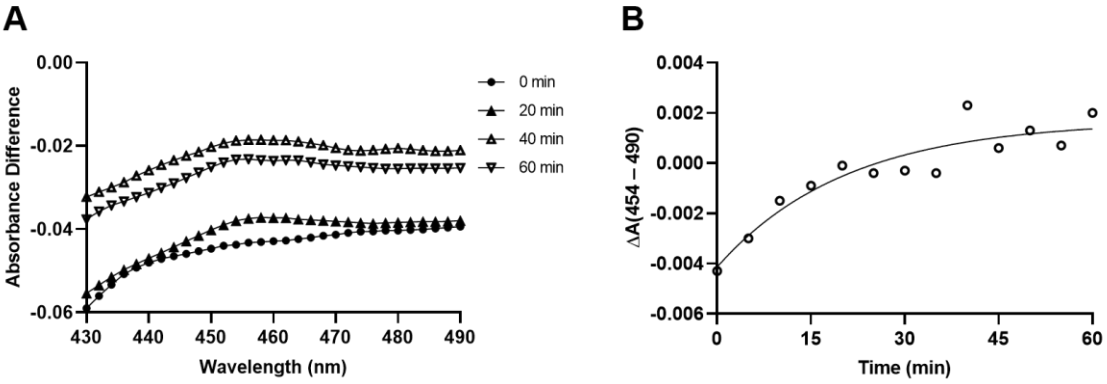
Supplementary Fig. 1. Chemical structures of (A) testosterone, (B) midazolam and (C) rivaroxaban. These three structurally-distinct compounds were employed in our assays as probe substrates of CYP3A.



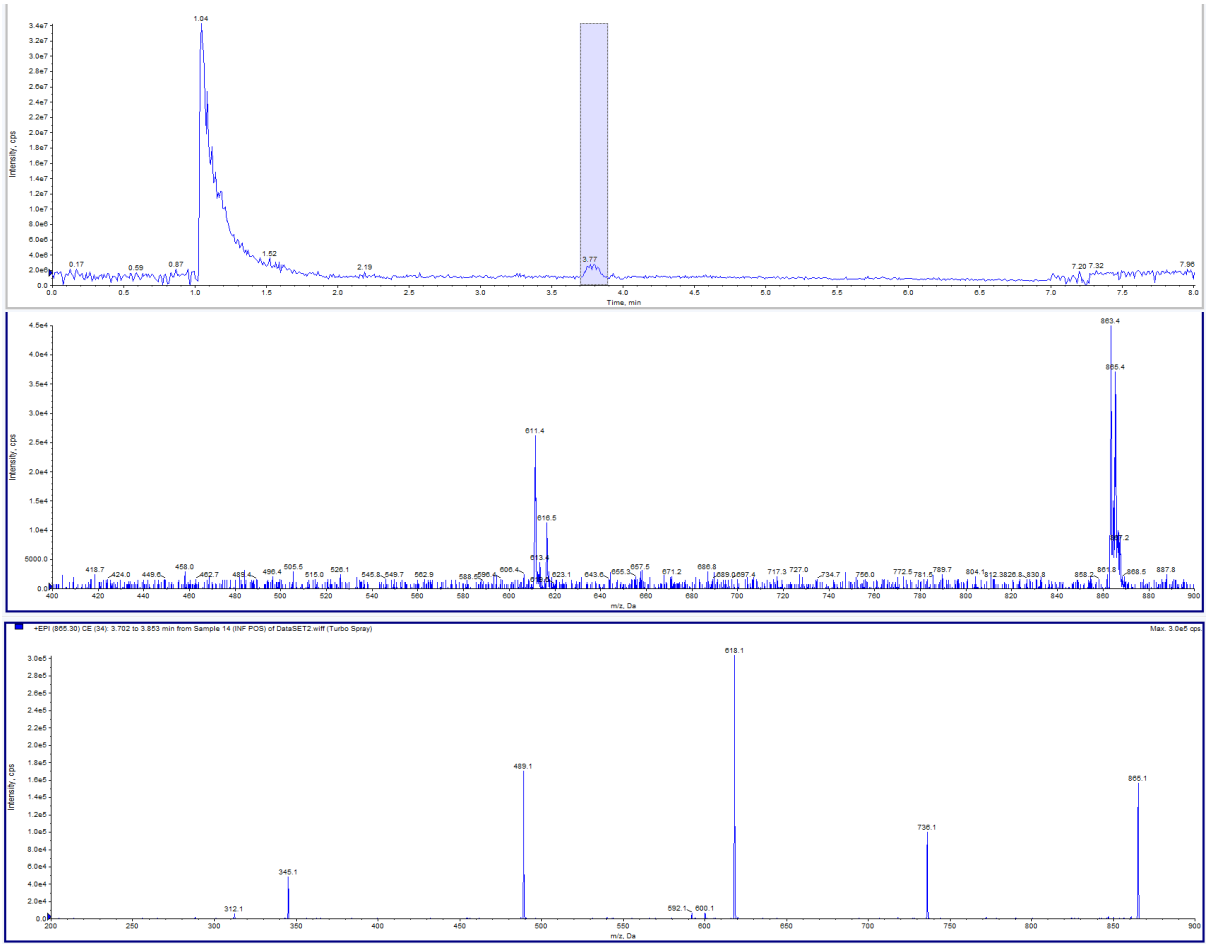
Supplementary Fig. 2. Screening for the MBI of CYP3A5 by INF using (A) testosterone, (B) midazolam and (C) rivaroxaban as probe substrates. No apparent time-dependent decrease in CYP3A5 activity elicited by INF is observed. Each point in (A to C) represents the mean and S.D of triplicate experiments.



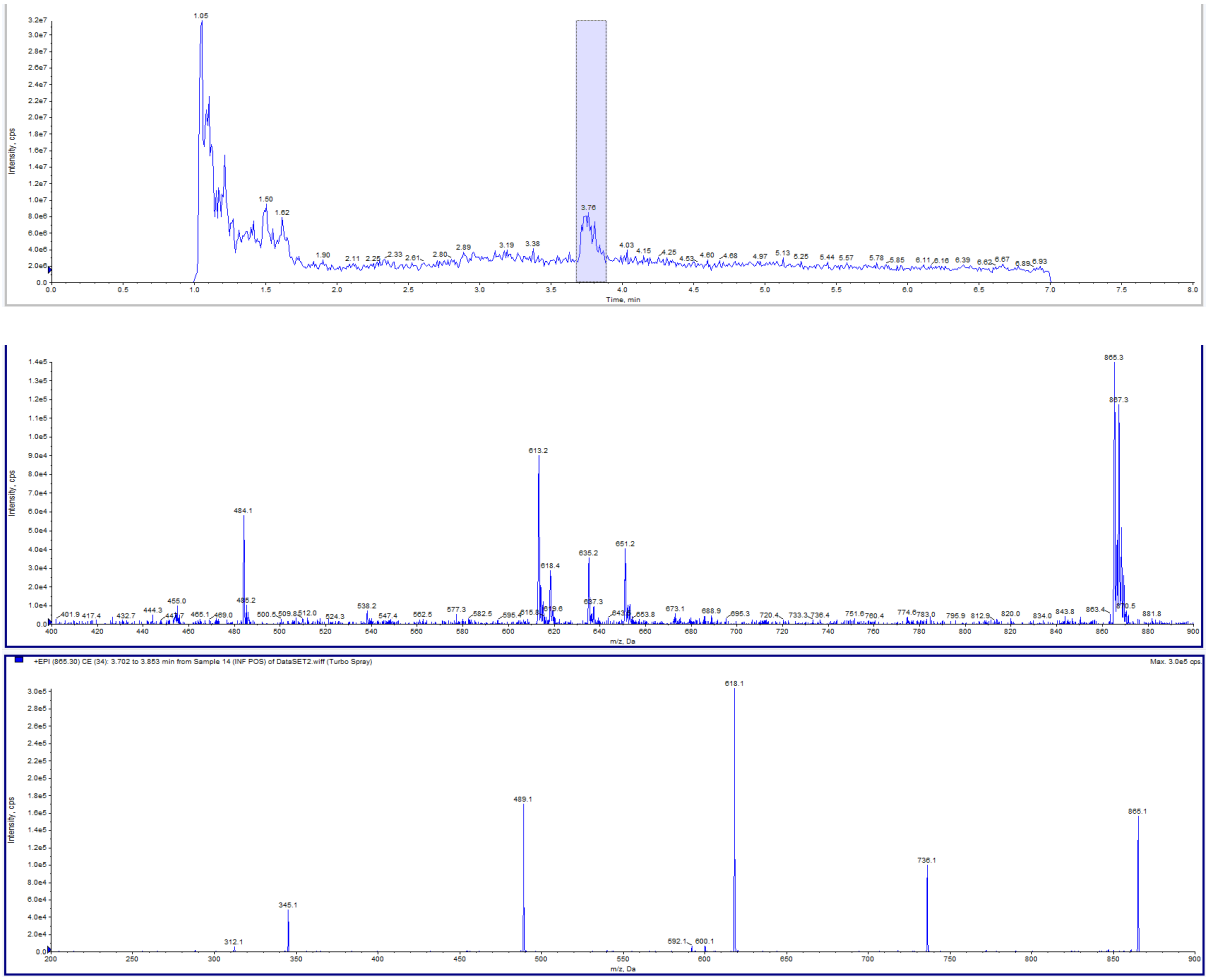
Supplementary Fig. 3. (A) Spectral difference measured over 60 min elucidated a Soret peak in the absorbance ranges of 448 – 458 nm for CYP3A4 incubated with 10 µM verapamil. (B) A comparison of the absorbance at the reference of 454 nm against the isosbestic point at 490 nm demonstrated an increase in the extent of MI complex formation over time.



Supplementary Fig. 4. Precursor ion scan (*m/z* 272 in negative ESI mode)-information-dependent acquisition-enhanced product ion (PIS-IDA-EPI) scan of INF incubated with rCYP3A4 and enriched with GSH.



Supplementary Fig. 5. Neutral loss scan (*m/z* 129 in positive ESI mode)-information-dependent acquisition-enhanced product ion (NL-IDA-EPI) scan of INF incubated with rCYP3A4 and enriched with GSH.



Supplementary 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)
Infigratinib (INF)	560	368	69	10	38	13
Erdafitinib	447	362	48	10	31	15
Hydroxylated Rivaroxaban	452	406	90	10	25	9
6β-hydroxytestosterone	305	269	52	6	20	3
1'-Hydroxymidazolam	342	203	130	10	38	11
Dexamethasone	393	355	112	10	14	14
Prednisolone	361	343	60	11	12	13

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