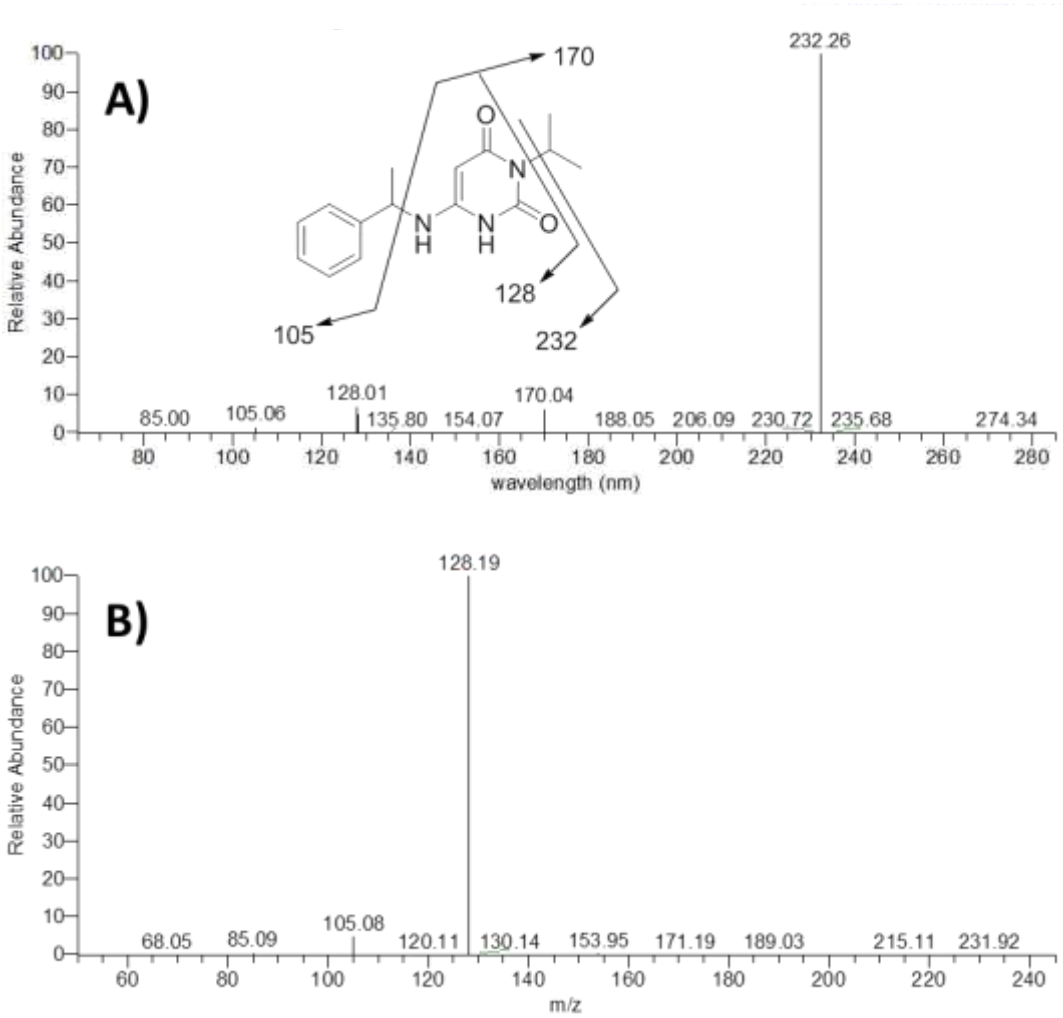
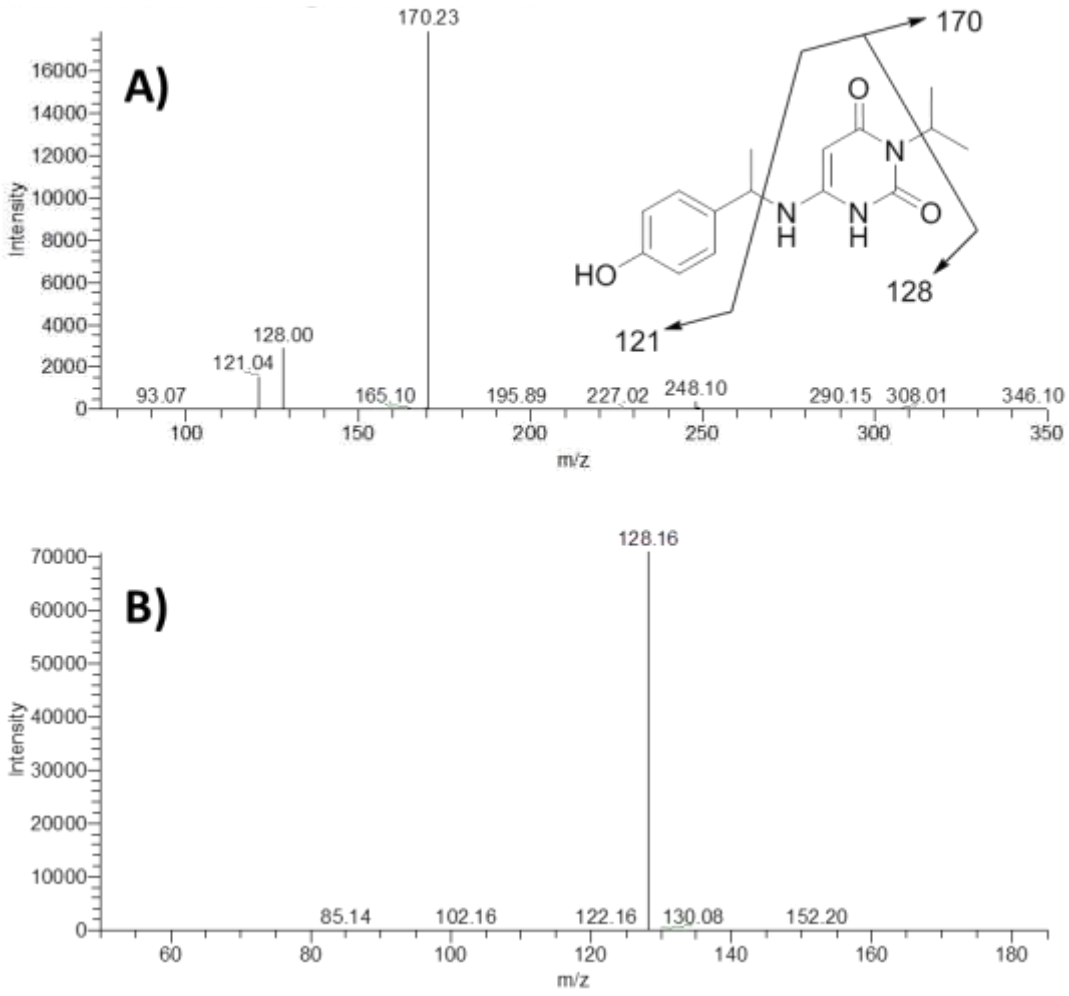
In vitro and i*n vivo* pharmacokinetic characterization of mavacamten, a first-in-class small molecule allosteric modulator of beta cardiac myosin

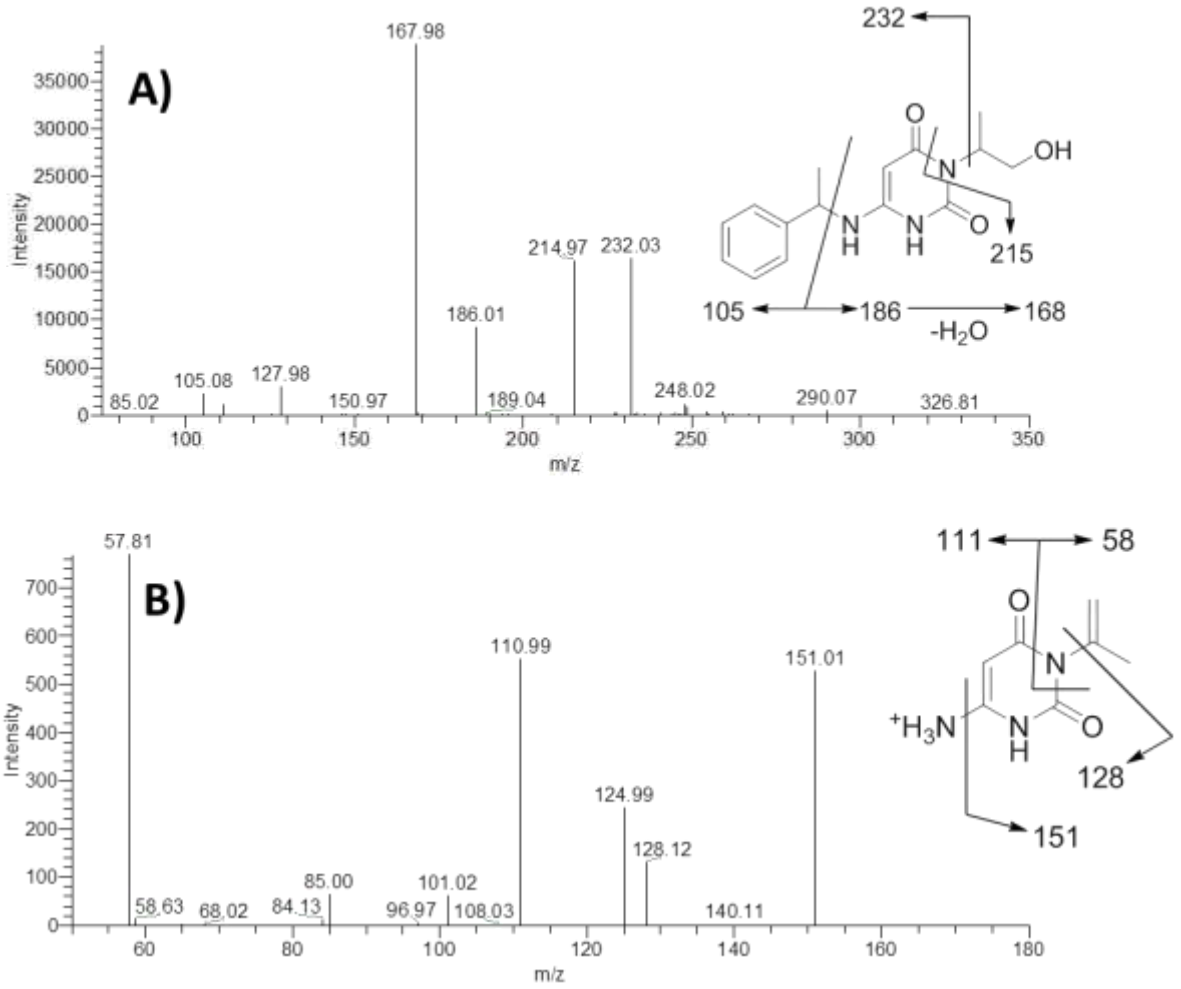
Supplemental Online Material



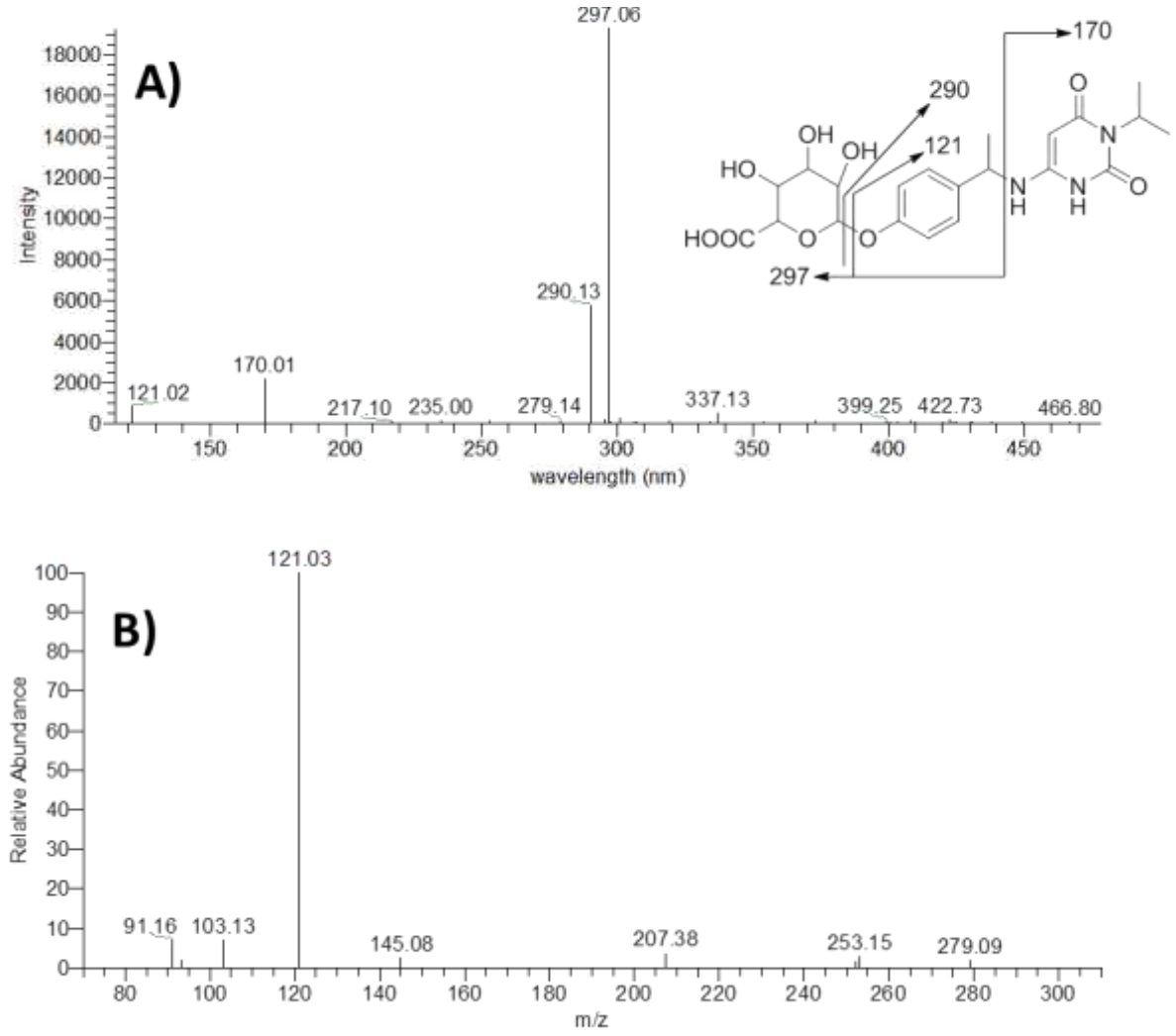
Supplemental Online Material Figure 1. LC-MS/MS (A) and MS^3 (B) mass spectra of non-radiolabelled mavacamten.



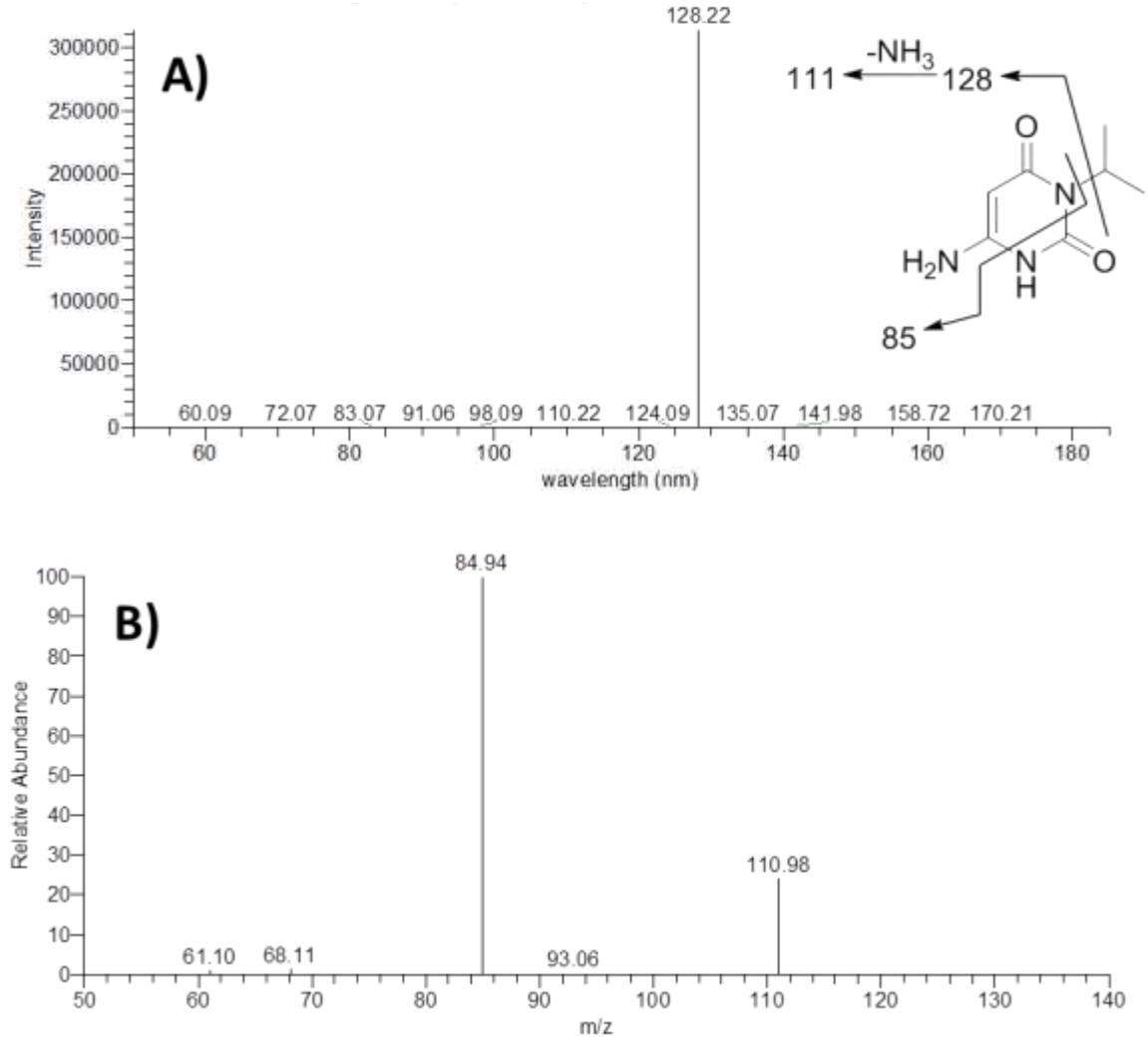
Supplemental Online Material Figure 2. LC-MS/MS (A) and MS^3 (B) mass spectra of non-radiolabelled mavacamten metabolite M1. Metabolite M1 eluted on reverse-phase LC-MS/MS on a Thermo Scientific LTQ Orbitrap-Velos instrument with a retention time of 37.2 min (LC-MS chromatogram not shown) and provided a protonated molecular ion ($[M+H]^+$) at *m/z* 290.1500, consistent with the elemental composition $C_{15}H_{19}N_3O_3$ (mass error −0.3 ppm). Cleavage of the carbon-amino-nitrogen bond with charge retention on nitrogen generated *m/z* 170 and *m/z* 128, which is identical to parent and consistent with an unmodified pyrimidinedione *N*-isopropyl group.



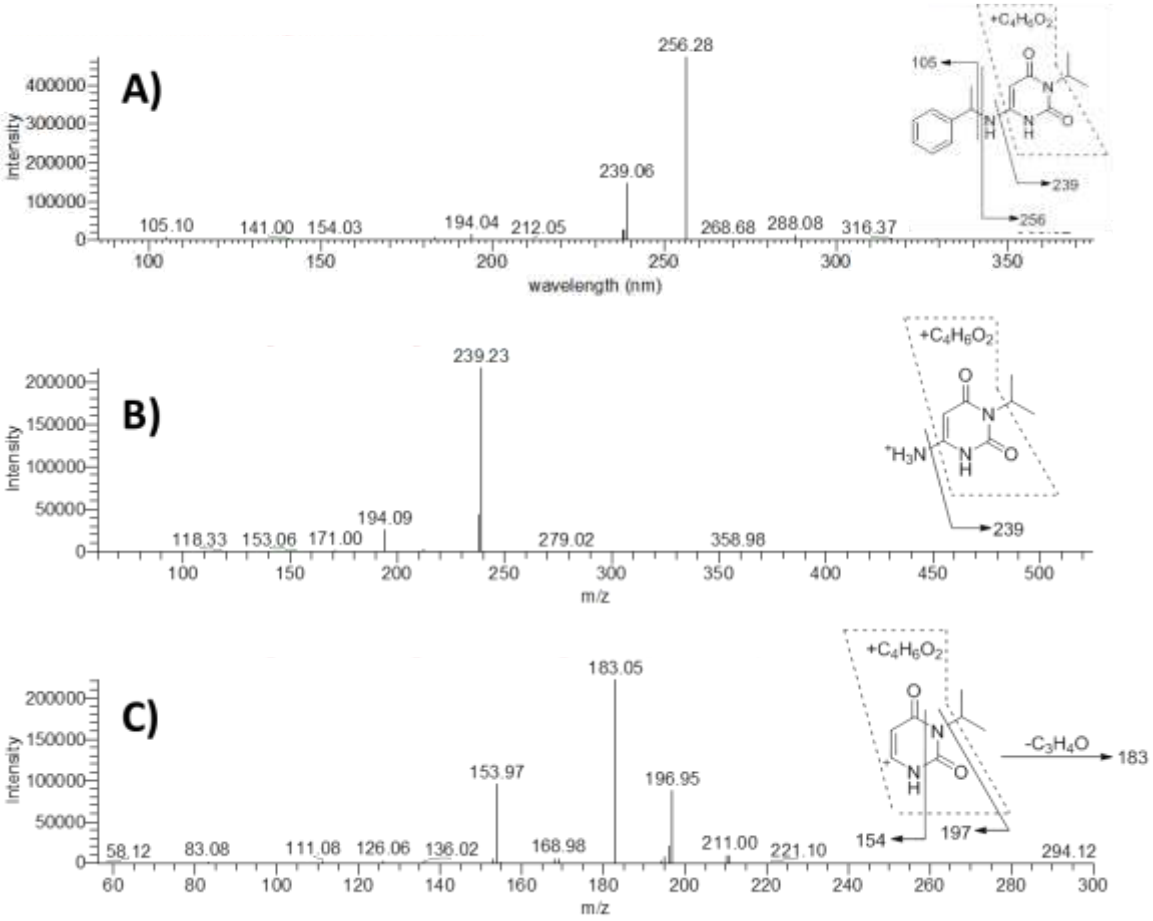
Supplemental Online Material Figure 3. LC-MS/MS (A) and MS^3 (B) mass spectra of non-radiolabelled mavacamten metabolite M2. Metabolite M2 eluted on reverse-phase LC-MS/MS on a Thermo Scientific LTQ Orbitrap-Velos instrument with a retention time of 39.9 min (LC-MS chromatogram not shown) and provided a protonated molecular ion ($[M+H]^+$) at *m/z* 290.1500, consistent with the elemental composition $C_{15}H_{19}N_3O_3$ (mass error -0.3 ppm). Cleavage of the carbon-amino-nitrogen bond with charge retention on the carbon atom generated *m/z* 105, identical to parent, indicating an unchanged phenylethyl ring. Loss of the isopropyl sidechain from the molecular ion generated *m/z* 232, identical to parent, indicating oxidation had occurred on the isopropyl group. Cleavage of the carbon amino nitrogen bond with charge retention on nitrogen generated *m/z* 186, 16 Da higher than parent, and together with observation of *m/z* 128 suggested an unchanged amino pyrimidinedione with an oxidized isopropyl group.



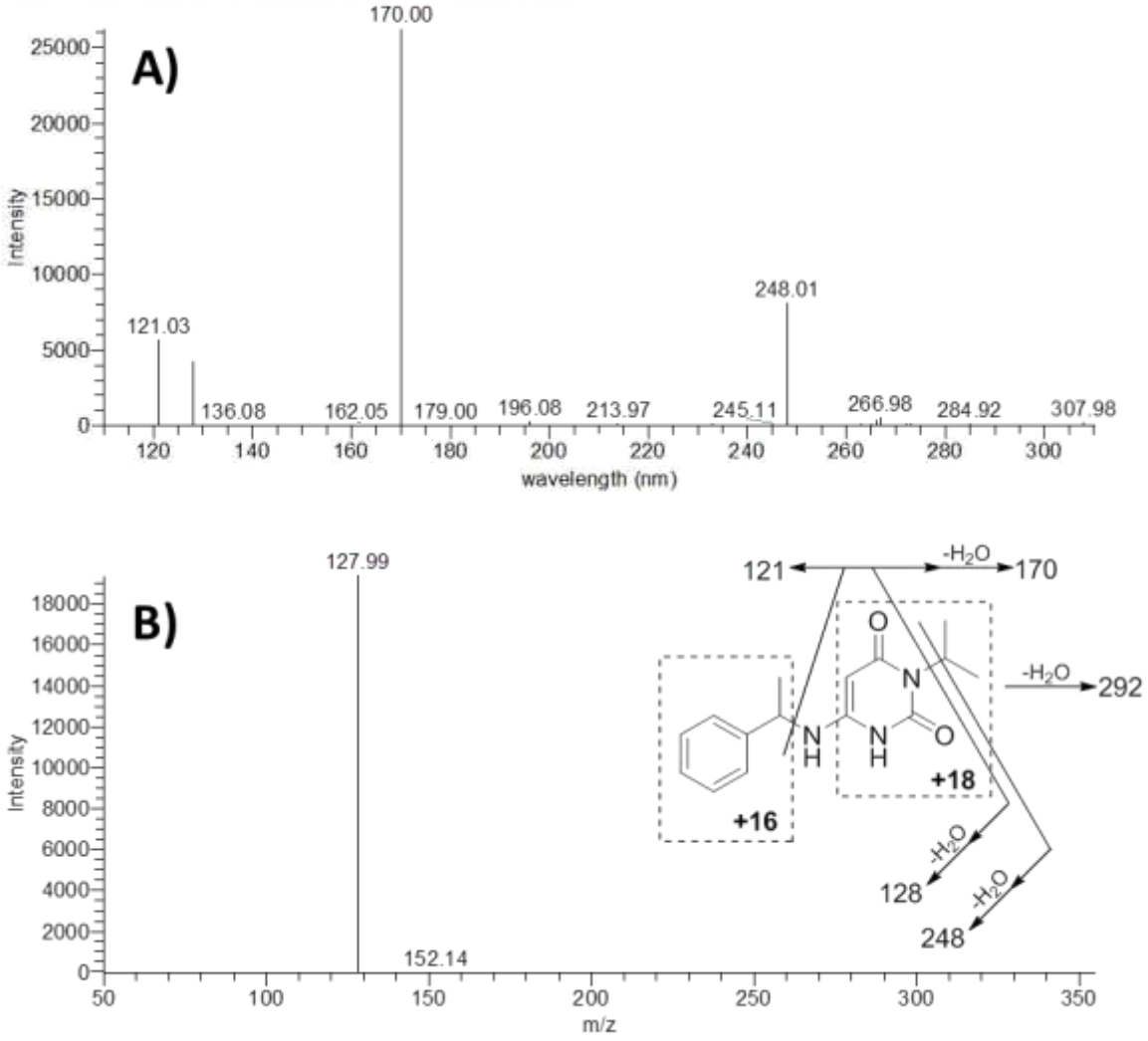
Supplemental Online Material Figure 4. LC-MS/MS (A) and MS^3 (B) mass spectra of non-radiolabelled mavacamten metabolite M4. Metabolite M4 eluted on reverse-phase LC-MS/MS on a Thermo Scientific LTQ Orbitrap-Velos instrument with a retention time of 31.8 min (LC-MS chromatogram not shown) and provided a protonated molecular ion ($[M+H]^+$) at *m/z* 466.1810, which is consistent with the elemental composition $C_{21}H_{28}N_3O_9$ (mass error 2.4 ppm). Cleavage of the carbon-amino-nitrogen bond with charge retention on carbon and loss of the glucuronide generated *m/z* 121, 16 Da higher than parent indicating oxidation of the phenylethyl ring. Cleavage of the carbon-amino-nitrogen bond with charge retention on nitrogen generated *m/z* 170 identical to parent consistent with an unmodified pyrimidinedione *N*-isopropyl group.



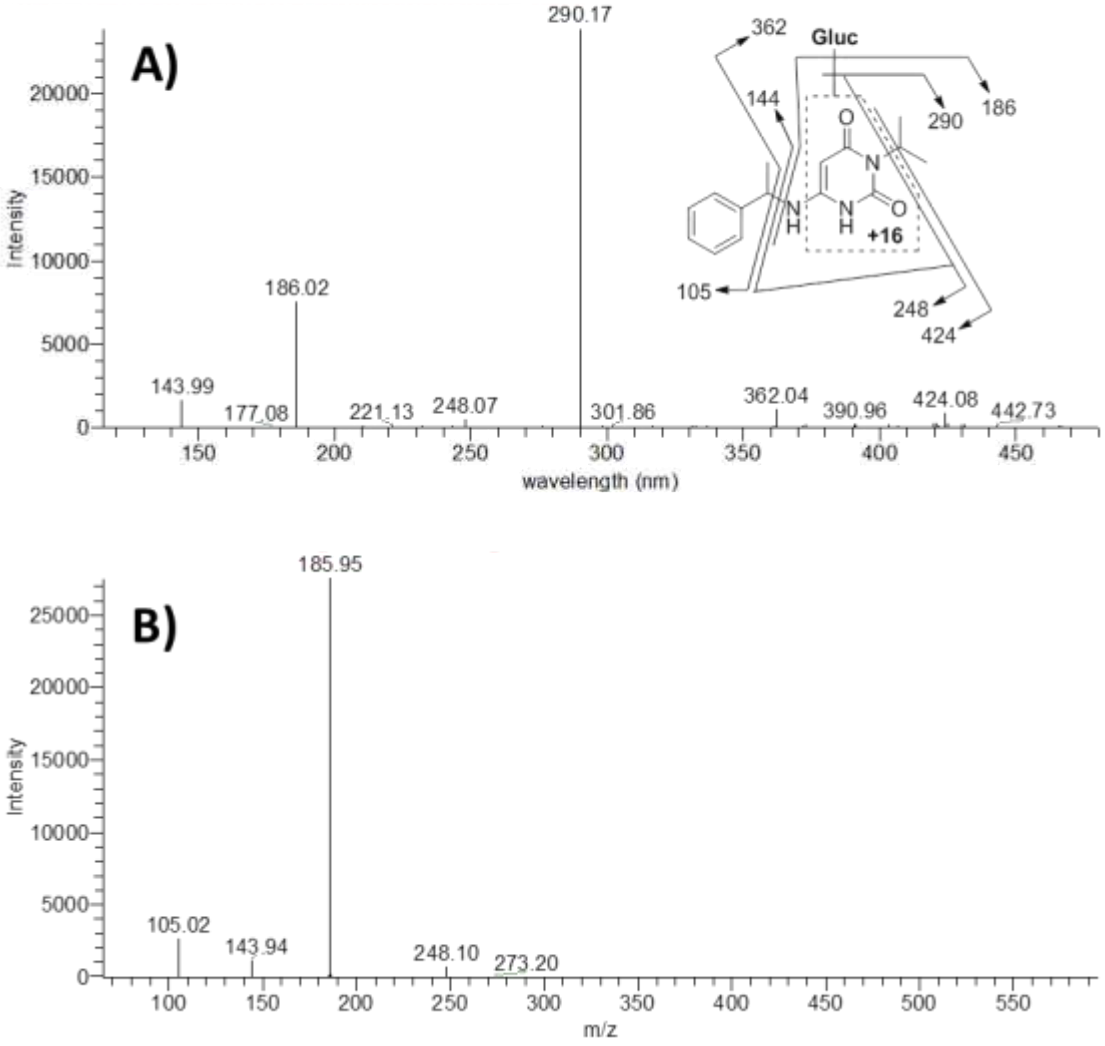
Supplemental Online Material Figure 5. LC-MS/MS (A) and MS^3 (B) spectra of non-radiolabelled mavacamten metabolite M6. Metabolite M6 eluted on reverse-phase LC-MS/MS on a Thermo Scientific LTQ Orbitrap-Velos instrument with a retention time of 16.6 min (LC-MS chromatogram not shown) and provided a protonated molecular ion ($[M+H]^+$) at *m/z* 170.0924, consistent with the elemental composition $C_7H_{11}N_3O_2$ (no mass error). Loss of the isopropyl sidechain from the molecular ion generated *m/z* 128. Cleavage through the pyrimidinedione ring generated the product ion at *m/z* 85.



Supplemental Online Material Figure 6. LC-MS/MS (A), MS^3 (B), and MS^4 (C) mass spectra of non-radiolabelled mavacamten metabolite M10.



Supplemental Online Material Figure 7. LC-MS/MS (A) and MS^3 (B) spectra of non-radiolabelled mavacamten metabolite M11.



Supplemental Online Material Figure 8. LC-MS/MS (A) and MS^3 (B) spectra of non-radiolabelled mavacamten metabolite M13.