Supplemental data for

Honokiol inhibits DNA polymerases β and λ and increases BLM sensitivity of human cancer cells

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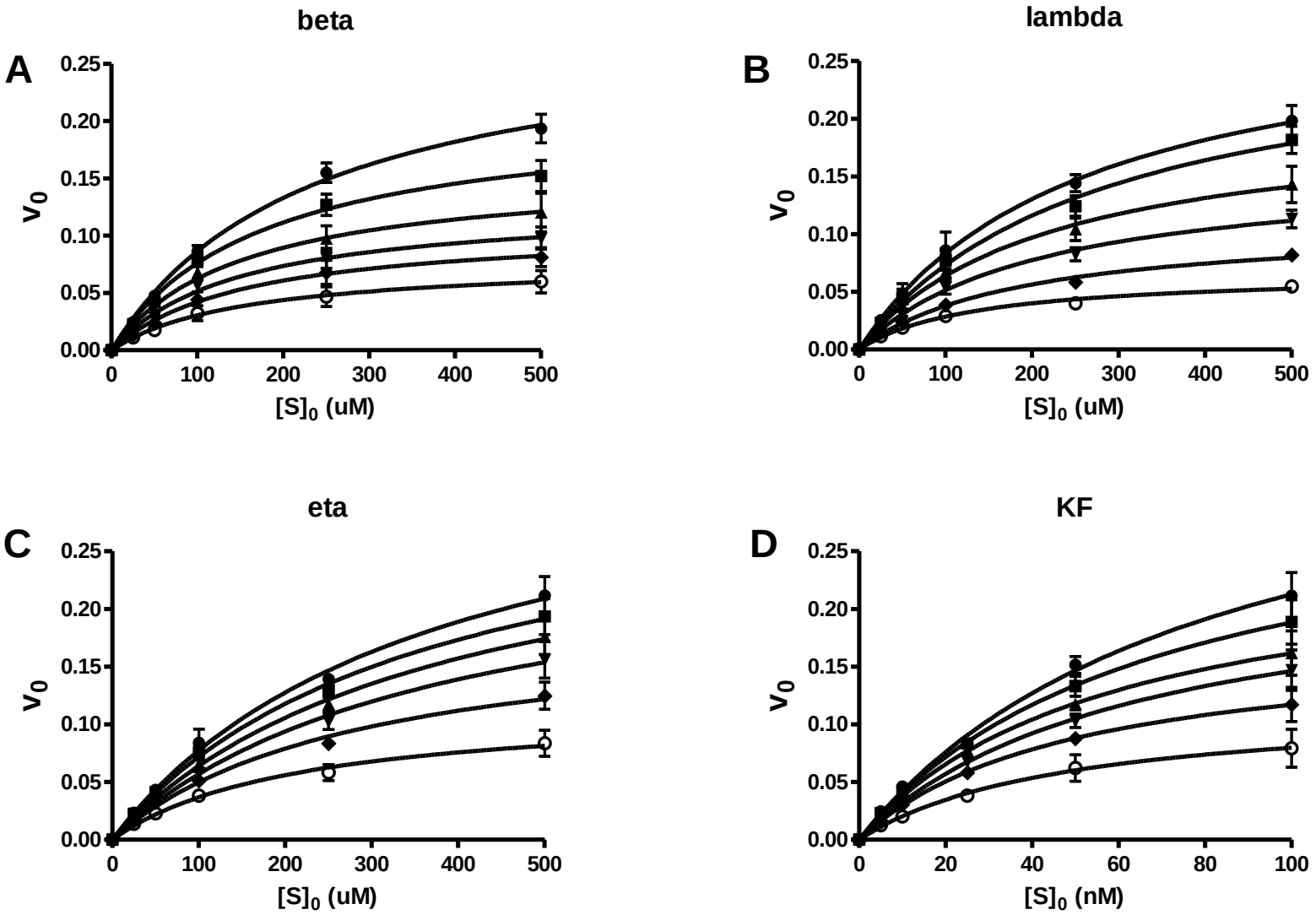


Figure S1 Initial rates plots of incorporation of dCTP opposite dG with 0 (open circle), 1, (diamond), 2 (down triangle), 5 (up triangle), 10 (square), 20 (circle) μM honokiol for (A) pol β, (B) pol λ,(C) pol η, and (D) Kf(exo-). The error bars are the SEM of three independent experiments. The lines are the nonlinear least-squares fit to $v_0 = \frac{V_{max}^{app}[S]_0}{K_m^{app}+[S]_0}$

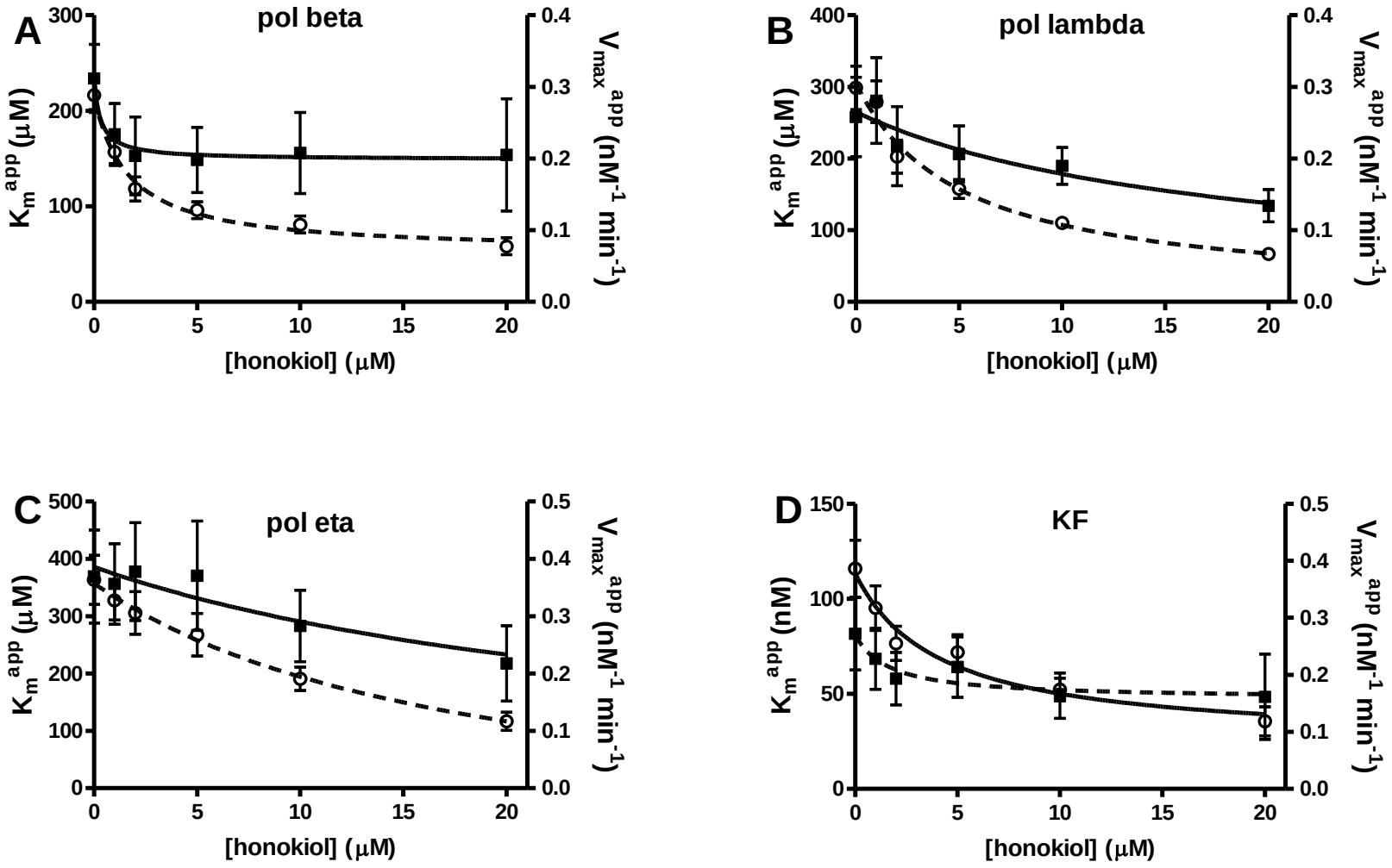


Figure S2. Plot of V_{max}^{app} and K_m^{app} versus honokiol concentration for the incorporation of the correct dNTP in to the DNA substrate for (A) pol β, (B) pol λ,(C) pol η, and (D) Kf(exo-). The data points V_{max}^{app}(circle) and K_m^{app}(square))are the mean ± SD of three determinations. The lines are the best fit of the data to equations below.

$$V_{max}^{app} = V_{max}\frac{\beta[I]+\alpha K_i}{[I]+\alpha K_i} \qquad K_m^{app} = \alpha K_s\frac{[I]+K_i}{[I]+\alpha K_i} \qquad (4)$$

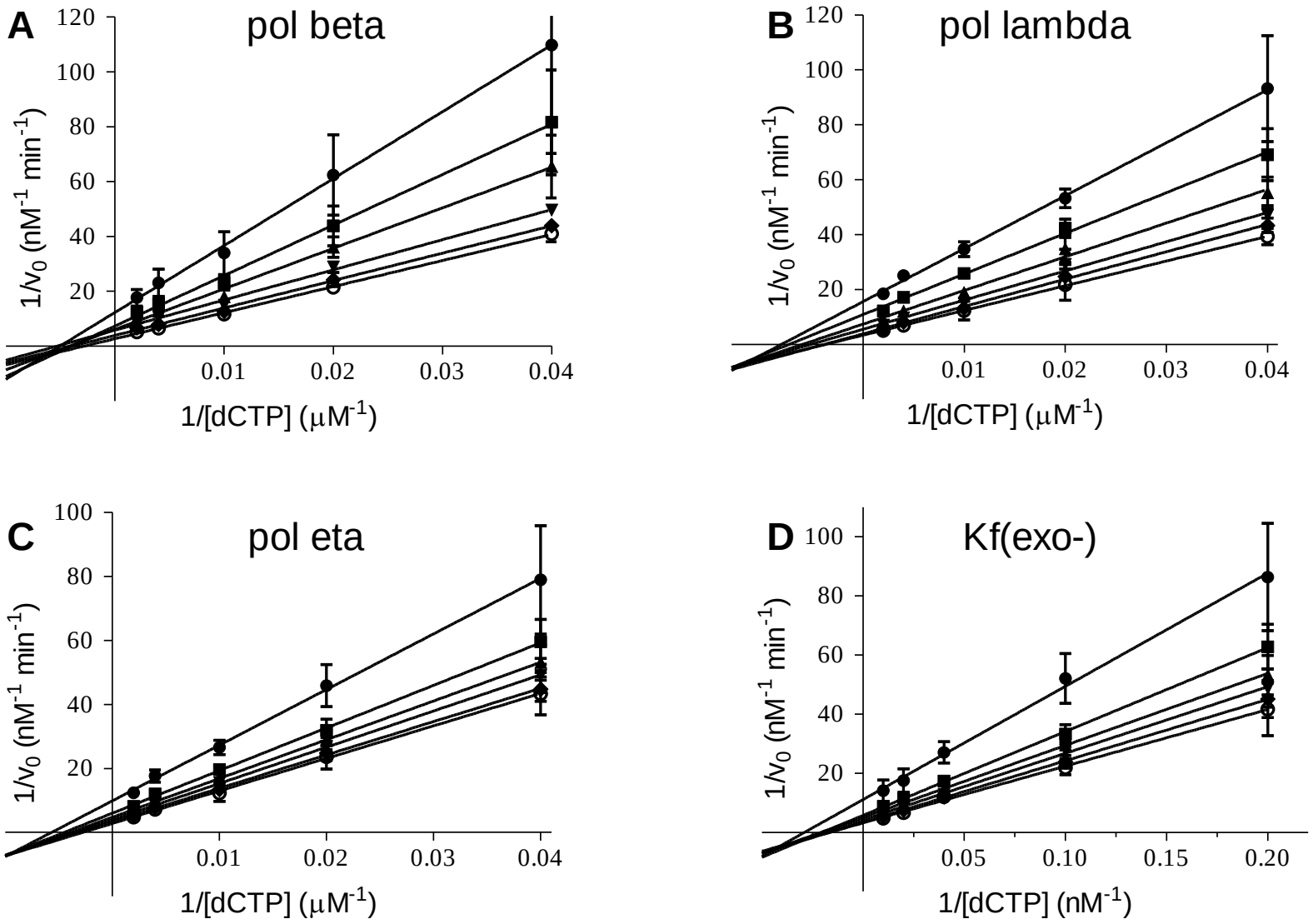


Figure S3. Lineweaver-Burk plots for the incorporation of dCTP opposite dG with 0 (O), 1, (diamond), 2 (down triangle), 5 (up triangle), 10 (square), 20 (circle) μM honokiol for (A) pol β, (B) pol λm (C) pol β, And (D) Kf(exo-). The error bars are the SD of three independent experiments. The lines are the linear least-squares fit to the data.

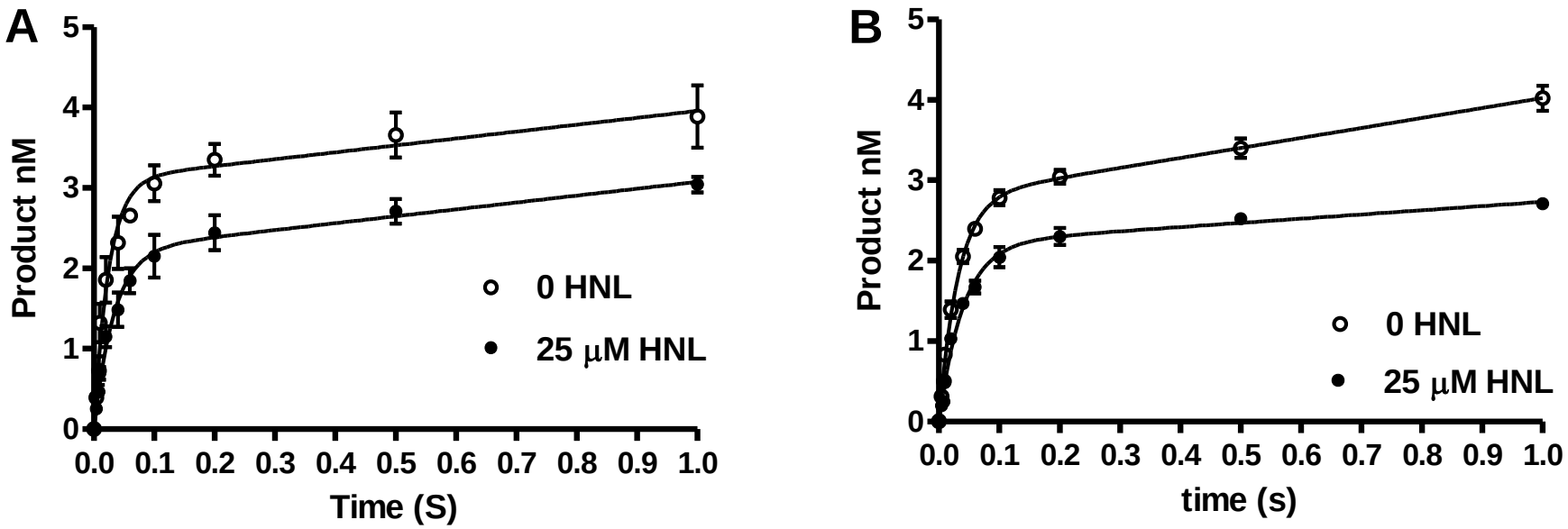


Figure S4. Reduced burst amplitudes in the presence of honokiol for (A) pol β and (B) pol λ. Time course for the insertion of 500 μM dCTP opposite dG in the presence of 0 (open circle) and 25 (solidcircle) μM honokiol with 10 nM DNA and 4 nM polymerase. The lines are the best fit to the burst equation. The data points are the mean ± SEM of three experiments.

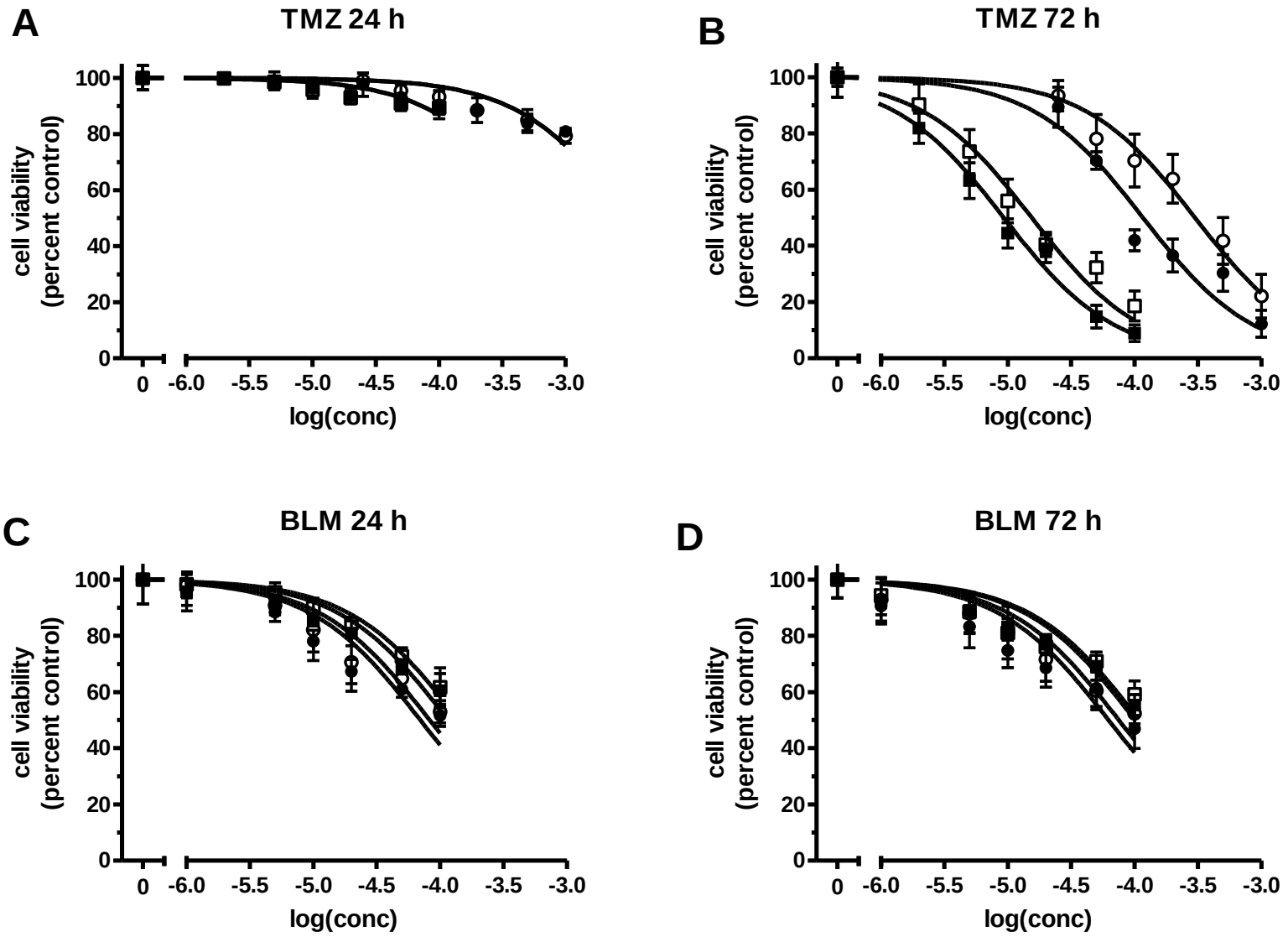


Figure S5. Cell viability for GM12787 cells as function of honokiol and (A and B) temozolomide or (C and D) bleomycin concentrations. The absorbance readings were normalized. Cell viability was determined 24 and 72 hours after treatment. A and B, TMZ (open circle) , TMZ + 10 μM HNL(solid circle), HNL(open square) HNL + 25 μM TMZ (solid square). A and B, BLM (open circle) , BLM + 10 μM HNL(solid circle), HNL(open square) HNL + 25 μM BLM (solid square). The markers represent the mean ± SEM of three experiments. The lines are the best fit to $Y = \frac{100}{1+10^{(logIC50-\log(X))}}$.

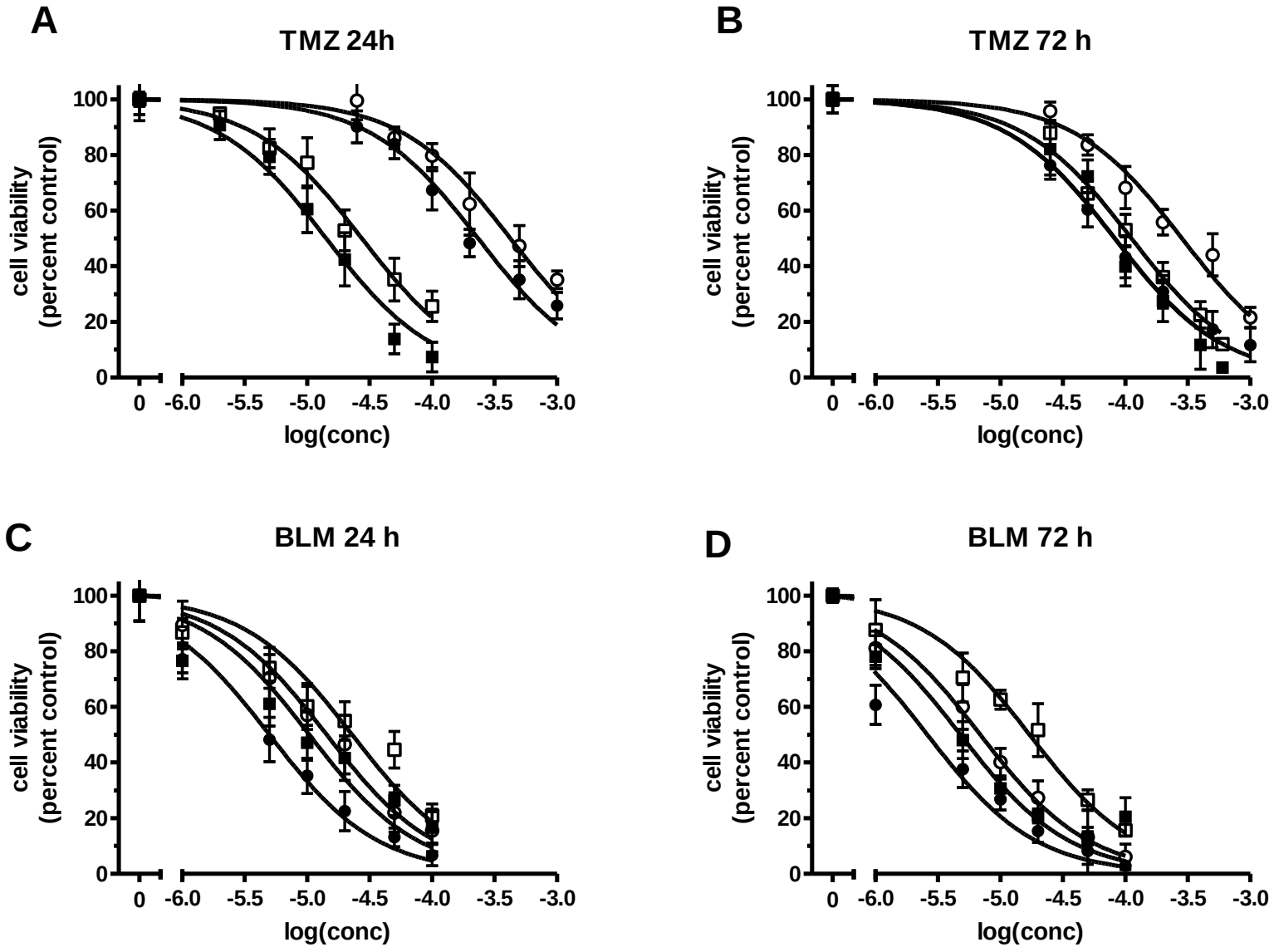


Figure S6. Cell viability for A549 cells as function of honokiol and (A and B) temozolomide or (C and D) bleomycin concentrations. The absorbance readings were normalized. Cell viability was determined 24 and 72 hours after treatment. A and B, TMZ (open circle) , TMZ + 10 μM HNL(solid circle), HNL(open square) HNL + 25 μM TMZ (solid square). A and B, BLM (open circle) , BLM + 10 μM HNL(solid circle), HNL(open square) HNL + 25 μM BLM (solid square). The markers represent the mean ± SEM of three experiments. The lines are the best fit to $Y = \frac{100}{1+10^{(logIC50-\log(X))}}$.

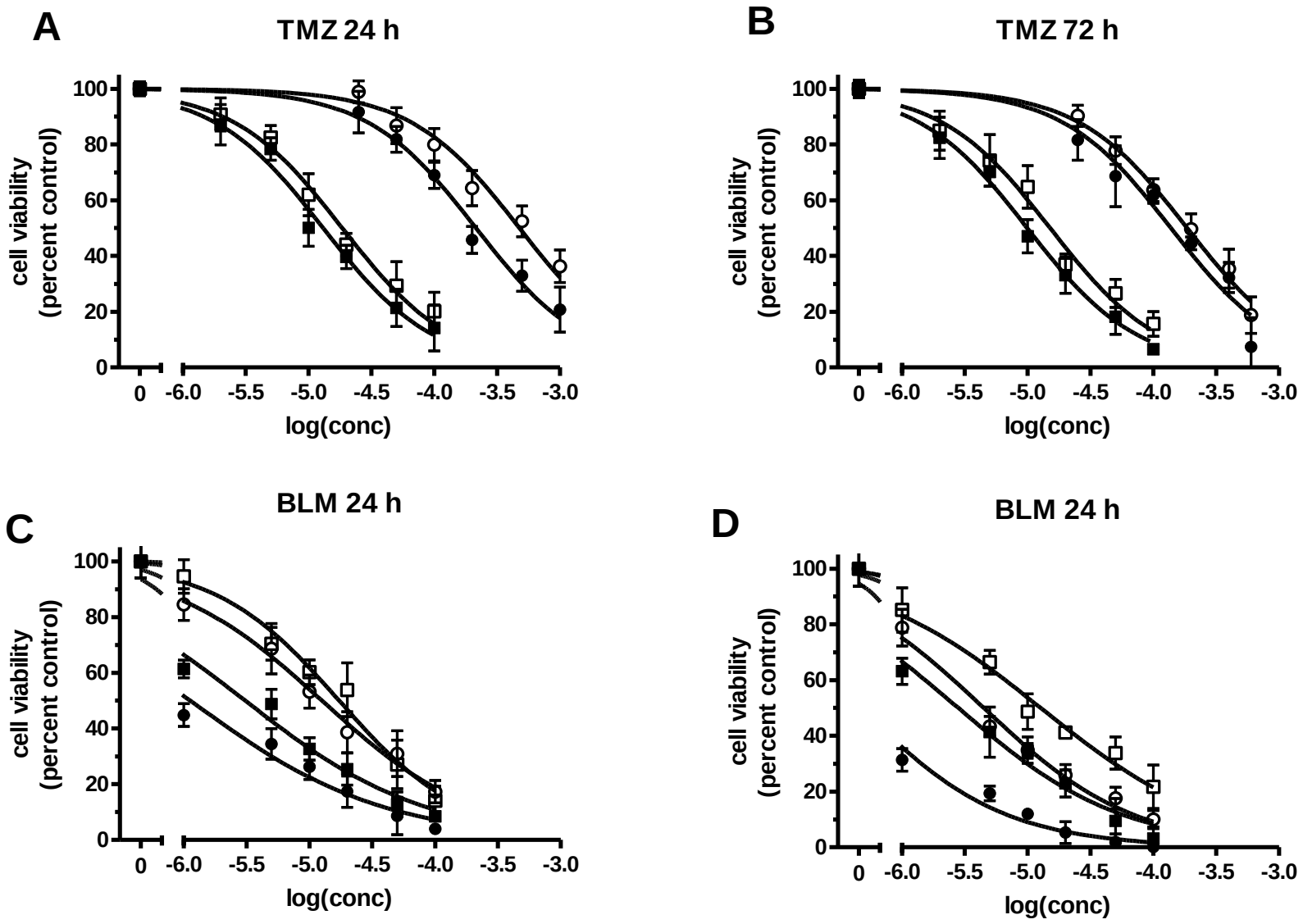


Figure S7. Cell viability for MCF7 cells as function of honokiol and (A and B) temozolomide or (C and D) bleomycin concentrations. The absorbance readings were normalized. Cell viability was determined 24 and 72 hours after treatment. A and B, TMZ (open circle) , TMZ + 10 μM HNL(solid circle), HNL(open square) HNL + 25 μM TMZ (solid square). A and B, BLM (open circle) , BLM + 10 μM HNL(solid circle), HNL(open square) HNL + 25 μM BLM (solid square). The markers represent the mean ± SEM of three experiments. The lines are the best fit to $Y = \frac{100}{1+10^{(logIC50-\log(X))}}$.

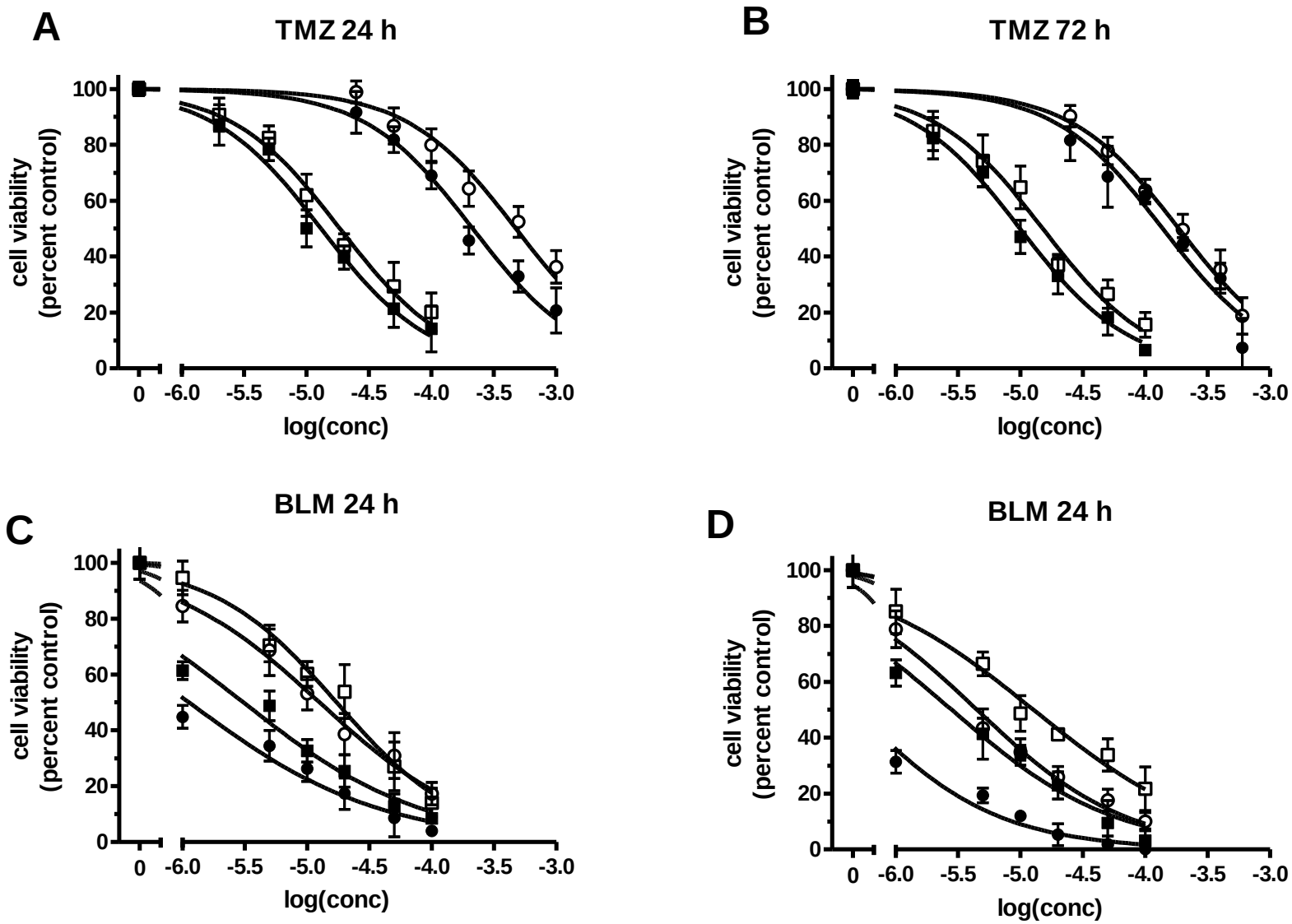


Figure S8. Cell viability for PANC1 cells as function of honokiol and (A and B) temozolomide or (C and D) bleomycin concentrations. The absorbance readings were normalized. Cell viability was determined 24 and 72 hours after treatment. A and B, TMZ (open circle) , TMZ + 10 μM HNL(solid circle), HNL(open square) HNL + 25 μM TMZ (solid square). A and B, BLM (open circle) , BLM + 10 μM HNL(solid circle), HNL(open square) HNL + 25 μM BLM (solid square). The markers represent the mean ± SEM of three experiments. The lines are the best fit to $Y = \frac{100}{1+10^{(logIC50-\log(X))}}$.

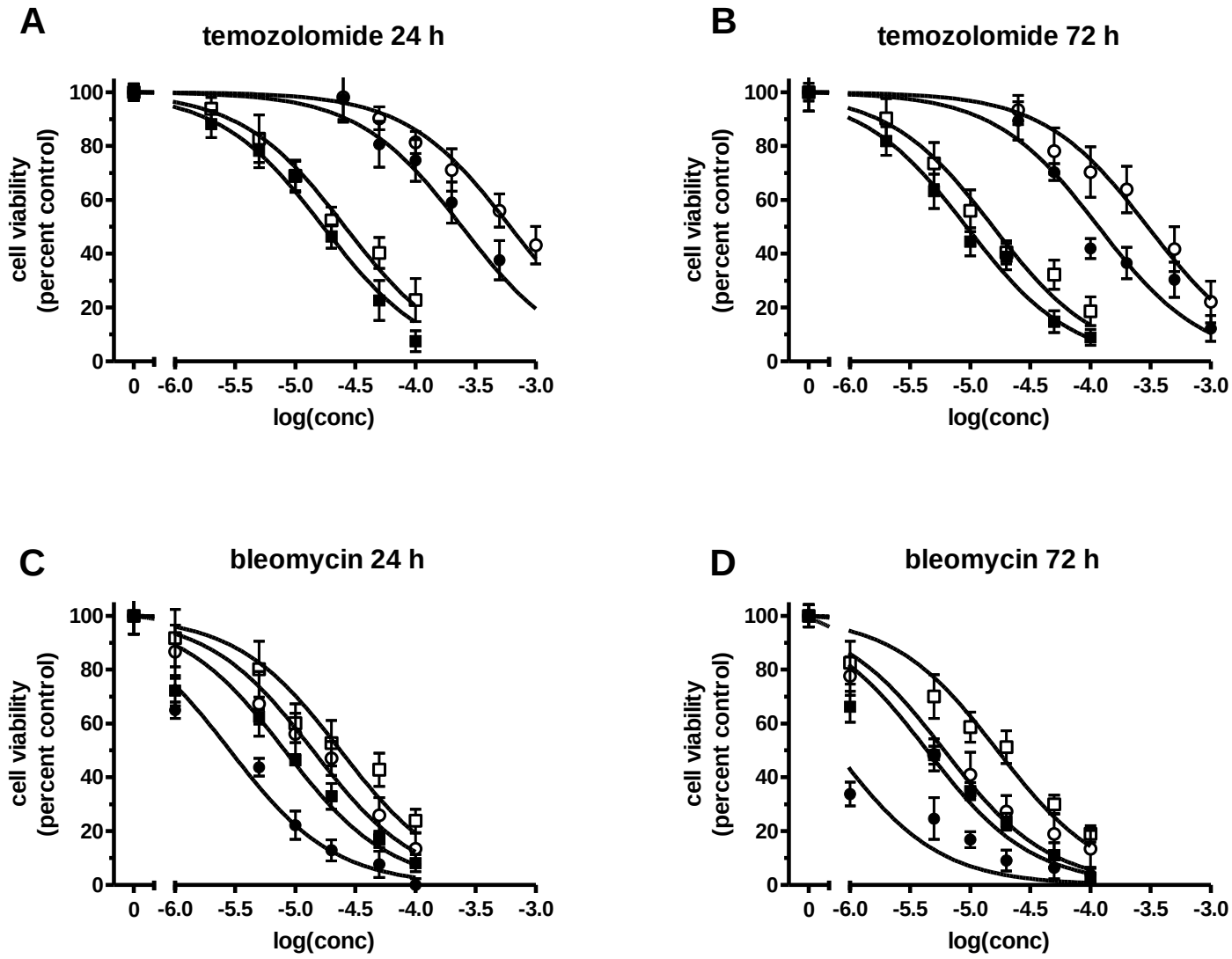


Figure S9. Cell viability for UACC903 cells as function of honokiol and (A and B) temozolomide or (C and D) bleomycin concentrations. The absorbance readings were normalized. Cell viability was determined 24 and 72 hours after treatment. A and B, TMZ (open circle) , TMZ + 10 μM HNL(solid circle), HNL(open square) HNL + 25 μM TMZ (solid square). A and B, BLM (open circle) , BLM + 10 μM HNL(solid circle), HNL(open square) HNL + 25 μM BLM (solid square). The markers represent the mean ± SEM of three experiments. The lines are the best fit to $Y = \frac{100}{1+10^{(logIC50-\log(X))}}$.

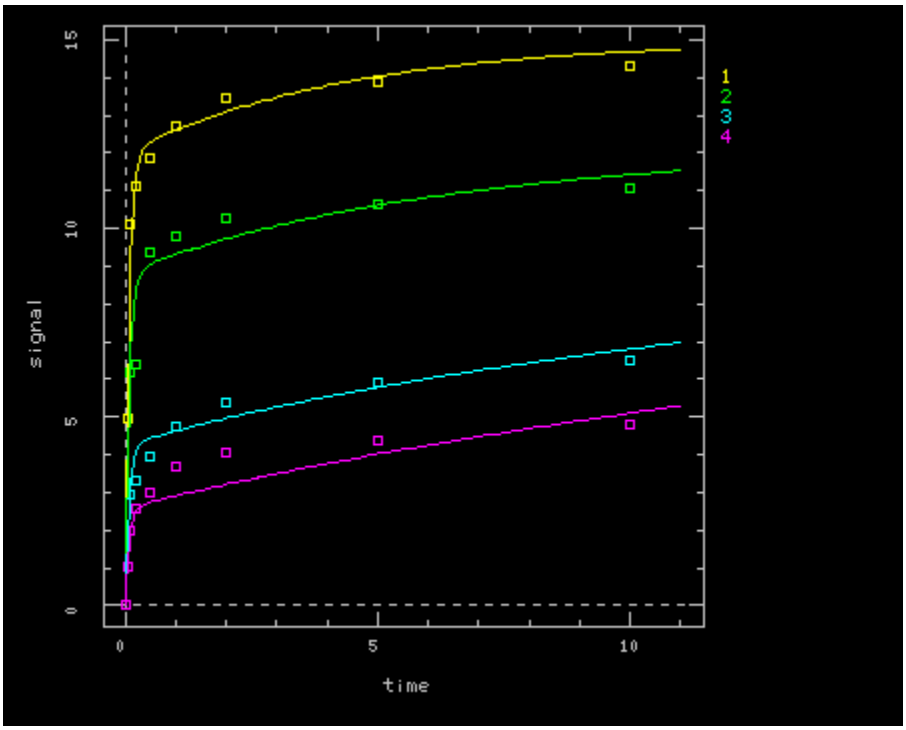
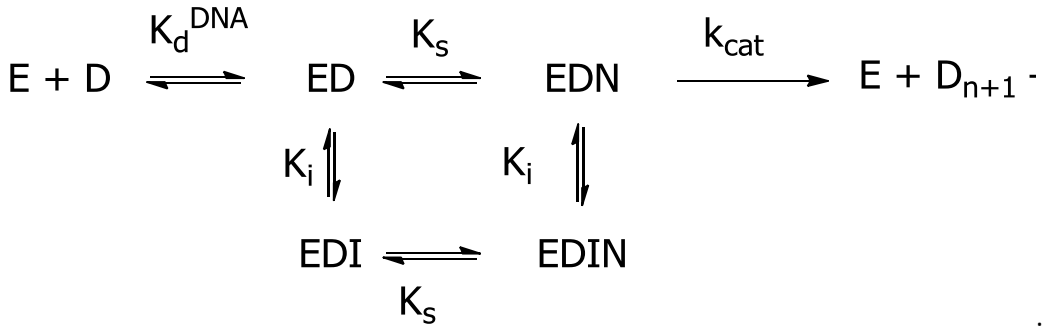


Figure S10. Modeling of the HNL inhibition of pol β

DynaFit V4 was used to fit the honokiol inhibition of the pol β catalyzed dNTP incorporation to a simplified for of Scheme 1, in which α = 1 and β = 0.

Initial Parameters
k1 = 0.01?, k-1 = 0.1
k2 = 1 , k-2 = 20000
k3 = 18,
k4 = 0.001? , k-4 = 0.01?



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Dynafit Script

```
-------------------------------------------------

; DNA polymerase beta Honokiol Inhibition
; time course
: inactive complex
;-------------------------------------------------

[task]

  task = fit
  data = progress
  model = pol non-competitive

[mechanism]

  E + D <==> ED          :  k1   k-1
  ED + N  <==> EDN     :  k2   k-2
  EDN --> E + P          :  k3
  ED + I <==> EDI        :  k4   k-4
  EDN + I <==> EDNI    :  k4   k-4
  EDI + N <==> EDNI    :  k2   k-2

 [constants]

   k1 = 0.01?,       k-1 = 0.1
   k2 = 1  ,        k-2 = 20000
   k3 = 18,
   k4 = 0.001? ,       k-4 = 0.01?

[responses]

  P = 1

[data]
  directory ./honokiol
  extension     txt
  file       h00 | equilibrate E = 300, D = 30, I =      0, dilute 0.5| concentration N = 50000
  file       h01 | equilibrate E = 300, D = 30, I =  20000, dilute 0.5| concentration N = 50000
  file       h05 | equilibrate E = 300, D = 30, I = 100000, dilute 0.5| concentration N = 50000
  file       h10 | equilibrate E = 300, D = 30, I = 200000, dilute 0.5| concentration N = 50000

[output]

  directory ./honokiol/output
```