Interplay between α, β and γ-secretases determines biphasic Amyloid-β level in the presence of a γ-secretases inhibitor

Fernando Ortega, Jonathan Stott, Sandra A.G. Visser, Claus Bendtsen

Supplemental Sections and Tables

Supplemental Section 1

Secretases operating in the linear regime cannot produce an increase in Aβ production in response to a GSI

In this section, we present the steps that were followed to deduce equation 2. In this case all enzymes, α-, β- and γ-secretase, operate in the linear regime.
The expressions for the rate equations, operating in the linear regime, are the following:

$$v_{r_1,\alpha} = V_{m1}\ APP/K_{m1}$$
$$v_{r_2,\beta} = V_{m2}\ APP/K_{m2}$$
$$v_{r_3,\gamma} = V_{m3}\ C83/K_{m3}$$
$$v_{r_4,\gamma} = V_{m4}\ C99/K_{m4}$$
$$v_{r_5,\alpha} = V_{m5}\ C99/K_{m5}$$
$$v_{r_0} = constant$$

We consider that the γ-secretase inhibitor (GSI) follows an uncompetitive behaviour and it is introduced in the rates $v_{r_3,\gamma}$ and $v_{r_4,\gamma}$ as:

$$v_{r_3,\gamma} = V_{m3}\ C83/\left(K_{m3}\ \left(1 + X/K_X\right)\right)$$
$$v_{r_4,\gamma} = V_{m4}\ C99/\left(K_{m4}\ \left(1 + X/K_X\right)\right)$$

where X is the inhibitor concentration and K_X is the inhibitor dissociation constant. Introducing these expressions into equation 1 (main text) we obtain the the following for the system:

$$\begin{aligned} dAPP/dt &= v_{r_0} - V_{m2}\ APP/K_{m2} - V_{m1}\ APP/K_{m1} \\ dC99/dt &= V_{m2}\ APP/K_{m2} - V_{m5}\ C99/K_{m5} - V_{m4}\ C99/\left(K_{m4}\ \left(1 + X/K_X\right)\right) \\ dC83/dt &= V_{m1}\ APP/K_{m1} + V_{m5}\ C99/K_{m5} - V_{m3}\ C83/\left(K_{m3}\ \left(1 + X/K_X\right)\right) \end{aligned} \qquad \text{(S1.1)}$$

The steady-state expressions for the system variables, APP, C99 and C83, are deduced by equating the above system to zero. Here, it is shown the expression for C99 concentration:

$$C99 = \frac{v_{r_0}\ V_{m2}/K_{m2}\left(1 + X/K_X\right)}{\left(V_{m1}/K_{m1} + V_{m2}/K_{m2}\right)\left(V_{m4}/K_{m4} + V_{m5}/K_{m5}\ \left(1 + X/K_X\right)\right)} \qquad \text{(S1.2)}$$

We obtain the production rate of Aβ in the steady-state intoducing the expression S1.2 into $v_{r_4,\gamma}$ rate:

$$v_{r_4,\gamma} = \frac{V_{m4}/K_{m4}\ v_{r_0}\ V_{m2}/K_{m2}}{\left(V_{m1}/K_{m1} + V_{m2}/K_{m2}\right)\left(V_{m4}/K_{m4} + V_{m5}/K_{m5}\ \left(1 + X/K_X\right)\right)} \qquad \text{(S1.3)}$$

This equation shows the dependence of the production rate of Aβ with the inhibitor X (see equation 2, main text). To deduced the variation of $v_{r_4,\gamma}$ with respect to X its derivative is calculated and the following expression is obtained:

$$\frac{\partial v_{r_4,\gamma}}{\partial X} = -\frac{V_{m5}/K_{m5}\ V_{m4}/K_{m4}\ v_{r_0}\ V_{m2}/K_{m2}}{\left(V_{m1}/K_{m1} + V_{m2}/K_{m2}\right)\left(V_{m4}/K_{m4} + V_{m5}/K_{m5}\ \left(1 + X/K_X\right)\right)^2} \qquad \text{(S1.4)}$$

This expression shows that $v_{r_4,\gamma}$ value can only diminish increasing the concentration of the inhibitor X.

We analyzed the Aβ rise assuming all secretases following linear kinetics. Here, we show that the system, described by Eq. 1, doesn't display Aβ rebound when all the enzymes follow Michaelis-Menten kinetics with respect to their substrates but α-secretase shows non-saturation kinetics with respect to $C99$.

In this case, the mass equations are the following:

$$dAPP/dt = v_{r_0} - V_{m2}\ APP/(K_{m2}+APP) - V_{m1}\ APP/(K_{m1}+APP) \quad \text{(S1.5a)}$$

$$dC99/dt = V_{m2}\ APP/(K_{m2}+APP) - V_{m5}\ C99 - V_{m4}\ (C99/K_{m4})/(1+r_X+C99/K_{m4}+C83/K_{m3}) \quad \text{(S1.5b)}$$

$$dC83/dt = V_{m1}\ APP/(K_{m1}+APP) + V_{m5}\ C99 - V_{m3}\ (C83/K_{m3})/(1+r_X+C99/K_{m4}+C83/K_{m3}) \quad \text{(S1.5c)}$$

where: r_X represents X/K_X.

From equation S1.5a, it is easily observed that the APP steady state concentration value remains constant irrespectively of X concentration value. To simplify, we use the following notation: $\alpha v_{r_0} = V_{m2}\ APP/(K_{m2}+APP)$ and $(1-\alpha)v_{r_0} = V_{m1}\ APP/(K_{m1}+APP)$ where APP is the steady-state concentration value and α varies in [0,1].

Subtituting these expressions in S1.5, the reduced system of equations is the following:

$$dC99/dt = \alpha v_{r_0} - V_{m5}\ C99 - V_{m4}\ (C99/K_{m4})/(1+r_X+C99/K_{m4}+C83/K_{m3}) \quad \text{(S1.6a)}$$

$$dC83/dt = (1-\alpha)v_{r_0} + V_{m5}\ C99 - V_{m3}\ (C83/K_{m3})/(1+r_X+C99/K_{m4}+C83/K_{m3}) \quad \text{(S1.6b)}$$

Considering steady-state conditions for equations S1.6, we deduced that Aβ rise conditions should be at the expense of a decrease in the C99 concentration (assuming that α and v_{r_0} remain constant). So, a necessary condition for Aβ rise is that $\partial A\beta/\partial r_X < 0$.

The solution of the system S1.6 in the steady-state has two potential solutions, $(C99_1, C83_1)$ and $(C99_2, C83_2)$.

$$C99_1 = -\frac{\alpha v_{r_0} V_{m3} - v_{r_0} V_{m4} + \alpha v_{r_0} V_{m4} + V_{m3} V_{m4} + K_{m4} V_{m3} V_{m5} + K_{m4} r_X V_{m3} V_{m5} + \sqrt{F_1}}{2(V_{m3}-V_{m4})V_{m5}}$$

$$C99_2 = \frac{\alpha v_{r_0} V_{m3} - v_{r_0} V_{m4} + \alpha v_{r_0} V_{m4} + V_{m3} V_{m4} + K_{m4} V_{m3} V_{m5} + K_{m4} r_X V_{m3} V_{m5} + \sqrt{F_1}}{2(V_{m3}-V_{m4})V_{m5}}$$

$$C83_1 = -K_{m3}\left(A1 - v_{r_0}\sqrt{F_1} + V_{m4}\sqrt{F_1}\right)/A_2$$

$$C83_2 = -K_{m3}\left(A1 + v_{r_0}\sqrt{F_1} - V_{m4}\sqrt{F_1}\right)/A_2$$

where

$$A_1 = \alpha v_{r_0}^2 + (1-\alpha) v_{r_0}^2 V_{m4} - (1+\alpha) v_{r_0} V_{m3} V_{m4} - (1-\alpha) v_{r_0} V_{m4}^2 + V_{m3} V_{m4}^2 + K_{m4}(1+r_x) v_{r_0} V_{m3} V_{m5} -$$
$$2 K_{m4}(1+r_x) v_{r_0} V_{m4} V_{m5} + K_{m4}(1+r_x) V_{m3} V_{m4} V_{m5}$$
$$A_2 = 2 K_{m4}(v_{r_0} - V_{m3})(V_{m3} - V_{m4}) V_{m5}$$
$$F_1 = 4\alpha K_{m4}(1+r_X) v_{r_0} V_{m3}(V_{m3} - V_{m4}) V_{m5} + \left(v_{r_0}(-\alpha V_{m3} + (-1+\alpha) V_{m4}) + V_{m3}(V_{m4} + K_{m4}(1+r_X) V_{m5})\right)^2$$

Assuming $V_{m3} > V_{m4}$, $(C99_2, C83_2)$ is the only potential solution. The Aβ condition is the following:

$$\frac{\partial C99_1}{\partial r_X} = K_{m4} V_{m3} \left(-1 + \frac{-v_{r_0}(-\alpha V_{m3} + V_{m4}(1+\alpha)) + V_{m3}(V_{m4} + K_{m4}(1+r_X) V_{m5})}{\sqrt{F_1}} \right) \Big/ \left(2(V_{m3} - V_{m4})\right)$$

S1.7

It can be show that this expression is always positive since $v_{r_0} < V_{m3}$ and $V_{m3} > V_{m4}$.

For the case that $V_{m3} < V_{m4}$, we have $(C99_1, C83_1)$ as a potential solution. The Aβ condition is the following:

$$\frac{\partial C99_2}{\partial r_X} = K_{m4} V_{m3} \left(-1 - \frac{-v_{r_0}(-\alpha V_{m3} + V_{m4}(1+\alpha)) + V_{m3}(V_{m4} + K_{m4}(1+r_X) V_{m5})}{\sqrt{F_1}} \right) \Big/ \left(2(V_{m3} - V_{m4})\right)$$

It can be showned that the expression is always positive.
In conclusion, the system with the kinetic properties that we have assumed in this section does not display Aβ rebound.

Supplemental Section 2

Conditions to display an increase in Aβ production

In this section, we derive an analytical expression that it is used in Figure 2. Now, it is assumed that only α-secretase can only be saturated by the intermediate C99. The expression for the rate equations for the steps catalyzed by alpha-secretase are the following:

$$v_{1,\alpha} = \frac{V_{m1}\ APP/K_{m1}}{1 + C99/K_{m5}}$$
$$v_{5,\alpha} = \frac{V_{m5}\ C99/K_{m5}}{1 + C99/K_{m5}}$$

As it was assumed in section 1, we consider that the GSI follows an uncompetitive behaviour and it is introduced in the rates $v_{r_3,\gamma}$ and $v_{r_4,\gamma}$.
The system is defined by the following equations:

$$dAPP/dt = v_{r_0} - V_{m2}\ APP/K_{m2} - V_{m1}\ APP/K_{m1}/(1 + C99/K_{m5}) \qquad \text{(S2.1)}$$
$$dC99/dt = V_{m2}\ APP/K_{m2} - V_{m5}\ C99/K_{m5}/(1 + C99/K_{m5}) - V_{m4}\ C99/(K_{m4}\ (1 + X/K_X))$$
$$dC83/dt = V_{m1}\ APP/K_{m1} + V_{m5}\ C99/K_{m5}/(1 + C99/K_{m5}) - V_{m3}\ C83/(K_{m3}\ (1 + X/K_X))$$

The intermediate steady-state concentrations values are deduced by solving the system defined by equation S2.1 equal to zero. However, in these conditions it is not possible to get analytical expression for the intermediate concentrations in the steady-state. To simplify the problem, we define that $V_{m1}/K_{m1} = V_{m2}/K_{m2} = V_{m3}/K_{m3} = V_{m4}/K_{m4} = V_{m6}/K_{m6} \equiv k$ and $V_{m5}/K_{m5} \equiv h * k$ where h is a positive real number. In addition, we change notation by defining the new parameter $r_v \equiv (V_{m2}/K_{m2})/v_{r_0} = k/v_{r_0}$. Introducing the simplification and notation changes above described into S2.1 and equating to zero, we obtained the following polynomial equation of order 3 in C99:

$$\begin{aligned} &-C99^3 + K_{m5}^2 r_v (1+r_X) + C99^2 (-h - 3K_{m5} + r_v (1+r_X) - h r_X) + \\ &C99\left(-2h K_{m5}(1+r_X) - 2K_{m5}^2 + 2K_{m5} r_v (1+r_X)\right) = 0 \end{aligned} \qquad \text{S2.2}$$

By solving S2.2 we obtained an analytical expression for the concentration of C99 in the steady-state:

$$C99 = -(1/3)(3K_{m5} + h(1+r_X) - r_v(1+r_X)) + (\sqrt[3]{2}/3)\left(3K_{m5}^2 + (1+r_x)^2 (h^2 - r_v)^2\right)/Z + \left(1/(3\sqrt[3]{2})\right)Z$$

S2.3

where

$$Z = \sqrt[3]{9K_{m5} r_v + 2r_v^3 + 9K_{m5}^2 r_v r_X + 6r_v^3 r_X (1+r_X) + 2r_v^3 r_X^3 - 2h^3 (1+r_x)^3 + 6h^2 r_v (1+r_x)^3 - 6h(1+r_x)\left(-3K_{m5}^2 + r_v^2 (1+r_x)^2\right) + 3\sqrt{3H_0}}$$

and

$$H_0 = K_{m5}^2 \begin{pmatrix} -4h^4 (1+r_X)^4 + 12h^3 r_v (1+r_X)^4 - K_{m5}^2 \left(4K_{m5}^2 + r_v^2 (1+r_X)^2\right) + \\ 4h r_v (1+r_X)^2 \left(5K_{m5}^2 + r_v^2 (1+r_X)^2\right) - 4h^2 (1+r_X)^2 \left(-2K_{m5}^2 + 3r_v^2 (1+r_X)^2\right) \end{pmatrix}$$

To deduce the variation of $v_{r_4,\gamma}$ with respect to X we substitute the expression S2.3 into the rate $v_{r_4,\gamma}$ and its derivative is calculated and the following expression is obtained:

$$\left(\frac{\partial v_{r_4,\gamma}}{\partial r_X}\right)_{X\to 0} = \frac{1}{18} k_b \left(\begin{array}{l} 18K_{m5} + \dfrac{12\sqrt[3]{2}\left(h - r_v^2\right)^2}{\sqrt[3]{H_1}} - \dfrac{6\sqrt[3]{2}\left(h^2 + 3K_{m5}^2 - 2h r_v + r_v^2\right)}{\sqrt[3]{H_1}} - 3\sqrt[3]{4}\sqrt[3]{H_1} - \\ \dfrac{6\sqrt[3]{2}\left(h^2 + 3K_{m5}^2 - 2h r_v + r_v^2\right)\left(-2h^3 + 6h K_{m5}^2 + 6h^2 r_v + 3K_{m5}^2 r_v - 6h r_v^2 + 2r_v^3 + H_2\right)}{\sqrt[3]{H_1^4}} \\ + \dfrac{3\sqrt[3]{4}\left(-2h^3 + 6h K_{m5}^2 + 6h^2 r_v + 3K_{m5}^2 r_v - 6h r_v^2 + 2r_v^3 + H_2\right)}{\sqrt[3]{H_1^2}} \end{array} \right)$$

S2.4

where:

$$\begin{aligned} H_1 = &-2h^3 + 18h K_{m5}^2 + 6h^2 r_v + 9K_{m5}^2 r_v - 6h r_v^2 + 2r_v^3 + \\ &3\sqrt{3} K_{m5} \sqrt{-4h^4 + 12h^3 r_v + h^2\left(8K_{m5}^2 - 12r_v^2\right) - K_{m5}^2\left(4K_{m5}^2 + r_v^2\right) + 4h\left(5K_{m5}^2 r_v + r_v^3\right)} \end{aligned}$$

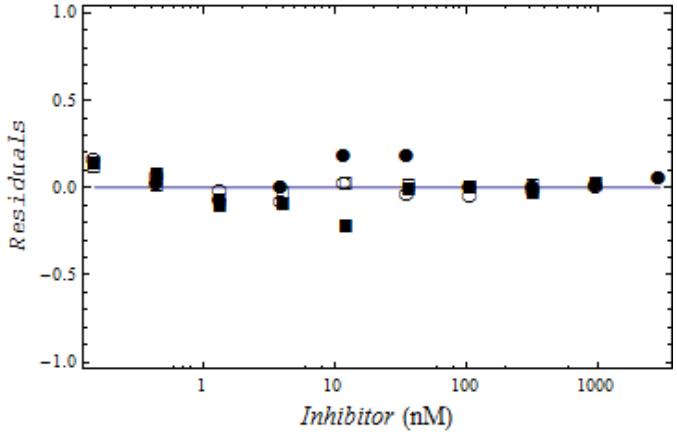
and

$$H_2 = \frac{\sqrt{3} K_{m5}\left(-8h^4 + 24h^3 r_v - K_{m5}^2 r_v^2 + 8h^2\left(K_{m5}^2 - 3r_v^2\right) + 4h\left(5K_{m5}^2 r_v + 2r_v^3\right)\right)}{\sqrt{-4h^4 + 12h^3 r_v + 4h^2\left(2K_{m5}^2 - 3r_v^2\right) - K_{m5}^2\left(4K_{m5}^2 + r_v^2\right) + 4h\left(5K_{m5}^2 r_v + r_v^3\right)}}$$

Finally, equation S2.4 is shown in Fig. 2 (main text) for different values of K_{m5}, r_v and h.

Supplementary Section 3

Parameter estimates for in vitro model



Supplemental Figure S1: Residual plot for the model of Aβ response across a range of inhibitor concentrations in HEK APPwt and HEK APPswe cell lines shown in Fig. 3. The symbols follow the nomenclature described in Fig. 3.

Supplemental Table S1a: α-, β- and γ-secretase parameter values used to fit HEKwt and HEKsw data as shown in Fig. 3.

	K_m	V_m
$v_{r_1,\alpha}$	0.186 [0.88, 1.19]	1.10 [0.97, 1.02]
$v_{r_2,\beta}$ (wt)	1.64 [0.84, 1.13]	0.153 [0.85, 1.16]
$v_{r_2,\beta}$ (sw)	0.266 [ind, 3.75]	6.95 [0.367, ind]
$v_{r_3,\gamma}$	28.8 [ind, ind]	14.6 [ind, ind]
$v_{r_4,\gamma}$	0.915 [0.69, 1.55]	1.71 [0.88, 1.04]
$v_{r_5,\alpha}$	0.0672 [0.62, 1.50]	0.0223 [ind, ind]

The K_m values are given in arbitrary concentration units and V_m in arbitrary concentration units/seconds.

Supplemental Table S1b: Inhibitor parameter values used to fit HEKwt and HEKsw data as shown in Fig. 3.

	K_{ic} (nM)	K_{iu1} (nM)	K_{iu2} (nM)
DAPT	0.173 [0.57, 1.9]	145 [ind, ind]	7.31 [0.84, 1.1]
DPH-111122	0.163 [0.42, 2.0]	94.3 [ind, ind]	2.54 [0.92, 1.2]

The inhibition constants are given in nM.

The number within the brackets are the factors by which the parameter value has to be multiplied to define the confidence interval for each individual parameter. The confidence region is defined as:

$$\left\{\theta |\, \chi^2(\theta) - \chi^2\left(\hat{\theta}\right) < \Delta_\alpha\right\}$$

where χ^2 is the objective function defined in method section (main text) and θ represent a parameter. The threshold Δ_α is the 1 – α quantile of the χ^2_{df} distribution. $df = 1$ gives confidence intervals that hold individually for each parameter (1). *Ind* means that it was not possible to obtain confidence interval in the region [0.05, 20] of the value estimated during the fitting procedure.

Model validation

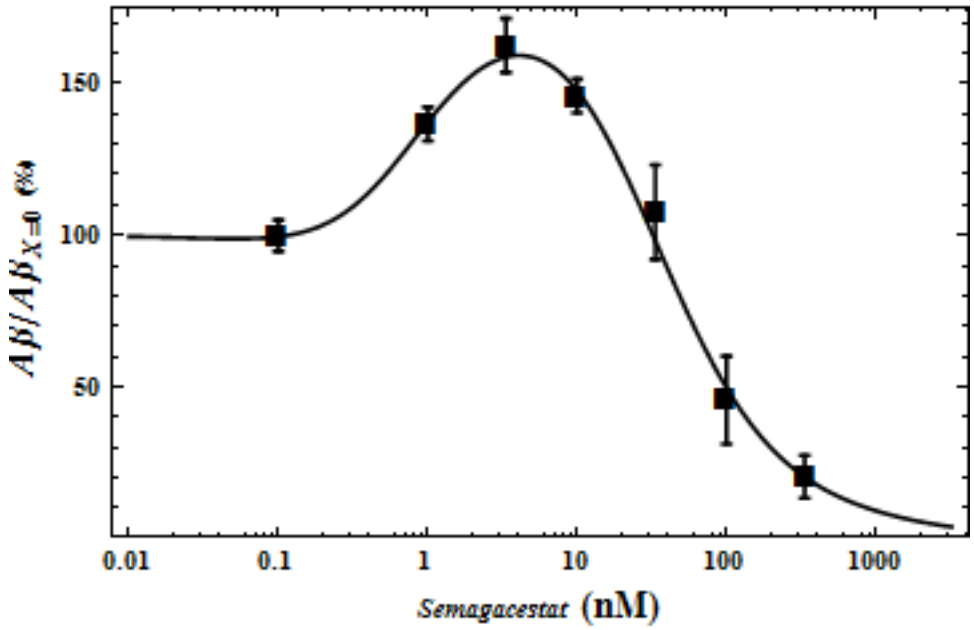
In the next paragraph we describe the minor change introduced to the model to simulate the increase in C99 basal level depicted in Fig. 4A. We do this by introducing a constant term for the production of C99, k_{99}, in the ordinary differential equation describing the the variation of this intermediate. The system is the following:

$$dAPP/dt = v_{r_0} - v_{r_1,\alpha} - v_{r_2,\beta}$$
$$dC99/dt = v_{r_2,\beta} - v_{r_5,\alpha} - v_{r_4,\gamma} + k_{99}$$
$$dC83/dt = v_{r_1,\alpha} + v_{r_5,\alpha} - v_{r_3,\gamma}$$
$$dA\beta/dt = v_{r_4,\gamma}$$
$$dp3/dt = v_{r_3,\gamma}$$

Supplemental Section 4

Prediction of human Aβ profile based on in vitro assay data

Adjusting the parameter values of the computational model proposed in the main text (V_{mi}, K_{mi} and K_i), we fit the model to the experimental in vitro profile of secreted Aβ in SH-SY5Y APPwt cells exposed to different Semagacestat concentrations (Jämsä, et al, 2011). The parameters values calculated are listed in Supplemental Table S2a and b. Supplemental Fig. S2 shows the experimental data used and the Aβ profile derived from the mathematical model. These parameters were used to adjust the plasma Aβ profile in humans.



Supplemental Figure S2: Quantitative modelling of the Aβ secretion response to semagacestat in SH-SY5Y APPwt cells. Experimental data are displayed as square (close symbols). The model simulation fits are shown as lines. The Aβ levels are represented as percentage change respect to Aβ level at the reference steady-state, i.e. Aβ(x=0). The experimental data used was extracted from the literature (2).

Supplemental Table S2a and b: Kinetic parameter values derived from fitting the computational model to the Aβ secretion profile to semagacestat in SH-SY5Y APPwt cells.

	K_m	V_m
$v_{r_1,\alpha}$	0.007834	1.003
$v_{r_2,\beta}$	4.283	0.03
$v_{r_3,\gamma}$	1.999	1.523
$v_{r_4,\gamma}$	3.68	4.947
$v_{r_5,\alpha}$	0.002928	0.7025

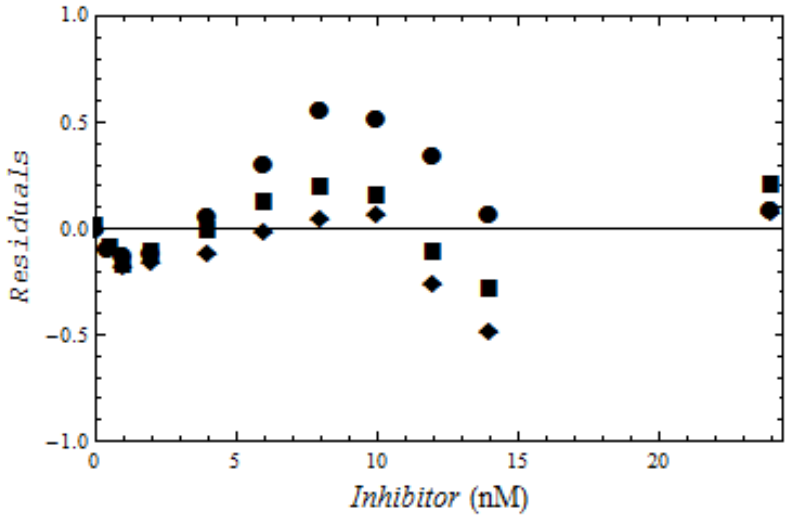
	K_{ic} (nM)	K_{iu1} (nM)	K_{iu2} (nM)
LY450139	0.1152	Large	Large

We use a two compartment model to describe the temporal profile of Semagacestat at three dosis: 40,100 and 140 mg (see method sections for details). The parameters that best fit these drug concentration profiles are shown in Supplemental Table S3.

Supplemental Table S3: Parameters used in the PK model for Semagacestat.

	unit	*Value*
k_A	$l\ min^{-1}$	0.0006193
k_{Cl}	min^{-1}	0.0000783
V	l	58.14

The two additional parameter values corresponding to the extended model, f_{eff} and $k_{\deg}$, are given in the main text. These parameters were calculated by fitting the plasma Aβ profile.

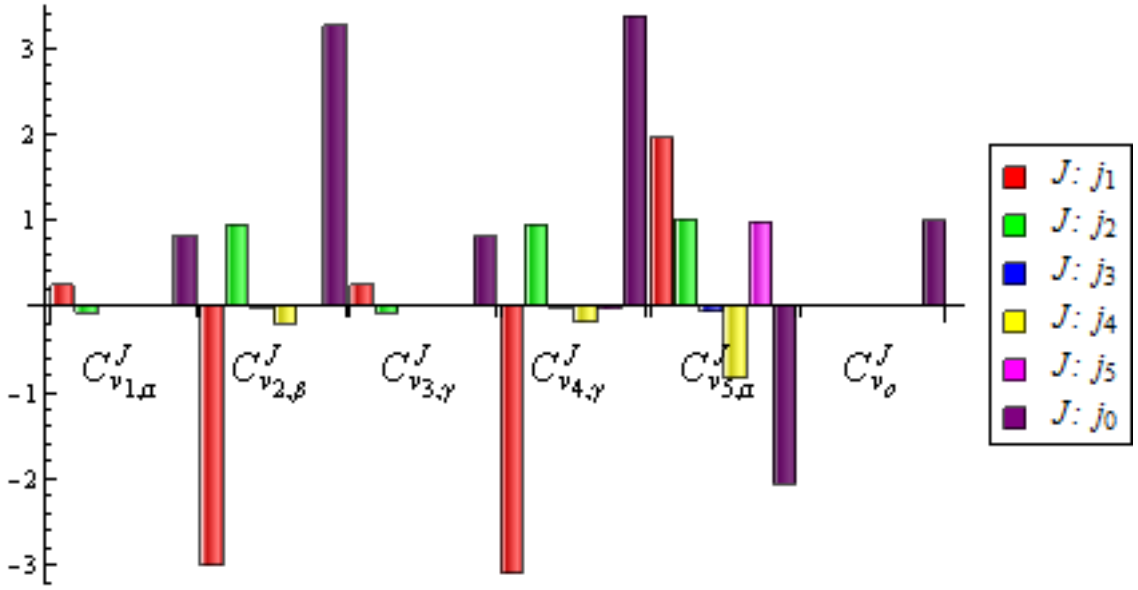


Supplemental Figure S3: Residual plot for the model of Aβ response for three semagacestat concentrations over 24 hours shown in Fig. 5. The symbols are described in Fig. 5.

Supplemental Section 5

Flux sensitivity analysis in the reference state (*GSI* = 0)

Flux control coefficients (FCCs) were calculated for the HEK APPwt model. The values are shown in Supplemental Fig. S4. It is observed that an increase in the activity of the step 3 will not increase significantly the production rate of its product (i.e. p3 or AICD) where $C^{J_3}_{v_{3,\gamma}} = 0.001$.



Supplemental Figure S4: Flux control coefficients in the reference state. The total number of FCCs is 36. The FCCs, $C^J_v = (d \ln J)/(d \ln v)$, describe the effect that the change in the rate (vi) of the process has on the steady-state flux (J), being independent of the particular effector that is canged (see 3 and reference therein). These values were calculated using the kinetic parameters in Supplemental Table S1.

References

1. Meeker, W. and Escobar, L. (1995) Teaching about approximate confidence regions based on maximum likelihood estimation. *Am. Stat.* **49**, 48–53.
2. Jämsä, A., Belda, O. Edlund, M., Lindström, E. (2011) BACE-1 inhibition prevents the γ-secretase inhibitor evoked Aβ rise in human neuroblastoma SH-SY5Y cells. *J. Biomed. Sci.* **18**, 76.
3. Ortega, F., Acerenza, L., Westerhoff, H. V., Mas, F. & Cascante, M. (2002) Product dependence and bifunctionality compromise the ultrasensitivity of signal transduction cascades. *Proc. Natl. Acad. Sci. USA* **99**, 1170-1175.