A Bayesian K-PD model for synergy: a case study

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Co-administration of two or more compounds can alter both the pharmacokinetics (PK) and pharmacodynamics (PD) of individual compounds. While experiments on PD drug-drug interactions are usually performed in an in-vitro setting, this experiment focuses on an in-vivo setting. The change over time of a safety biomarker is modeled using an indirect response model, in which the virtual PK profile of one compound drives the effect of the other. Several experiments at different dose level combinations were performed sequentially. While a traditional frequentist analysis consists of estimating the model parameters based on all the data simultaneously, in this work we consider a Bayesian inference framework allowing to incorporate the results from a historical dose-response experiment.

KEYWORDS

Co-administration, Pharmacokinetics, Pharmacodynamics,
Bayesian inference, Indirect response model

1 | INTRODUCTION

For several years, the co-administration of multiple compounds has become standard practice in the treatment of patients. Nevertheless, the co-administration of two or more compounds can potentially - intentionally or unintentionally - alter the systemic exposure and/or the pharmacological effects of the individual compounds. The effect on the systemic exposure is commonly called the pharmacokinetic (or PK) interaction, whereas the pharmacological effect of the drug on the body is called the pharmacodynamic (or PD) interaction. Experiments on PD drug-drug interactions are usually performed in an in-vitro setting, but are rarely undertaken in an in-vivo framework, unless at late stage drug development (Phase 3). This manuscript will focus on an exceptional preclinical in-vivo example, where in vivo rat data

were used to investigate the synergy of two compounds (a marketed compound and a novel experimental compound) at a pharmacodynamic level.

The first pharmacodynamic models were introduced by Levy [1, 2], who showed that the intensity of the effect attributed to a compound can be linearly related to the logarithm of the concentration of the compound itself. In the late 1960s, recognizing that a linear relationship was only valid within a limited concentration range, Wagner [3] proposed a nonlinear concentration-response relationship, also known as the Emax model. The sigmoid Emax model is an extension of the Emax model that allows for different slopes of the concentration-effect relationship. The above models assume that effects are proportionally mediated by drug concentrations. However, sometimes a time lag between concentration and observed effect may occur. Such a delay can be caused by different factors (e.g. kinetics of receptor activation, active metabolites or change in baseline over time). More complex models were proposed to explain this phenomenon, also called hysteresis: biophase distribution models (Sheiner et al., 1979) [4], indirect response models (Dayneka et al., 1993) [5] and signal transduction models (Sun et al., 1998) [6].

The goal of this paper is to integrate knowledge from sequential in-vivo experiments using a Bayesian hierarchical semi-mechanistic model. An indirect response model with a virtual PK profile (also called K-PD model [7]) will be used, with a pharmacodynamic interaction at the IC_{50} (the drug amount that produces 50% of maximum inhibition). Random effects are incorporated to allow for differences between animals.

The structure of the paper is as follows. Section 2 describes the motivational case study. In Section 3, a K-PD model for drug-drug interaction is proposed, and the synergy between compounds is discussed in terms of the model. Details on the Bayesian specification of the model are given as well. Section 4 then describes the results of this method applied to the data of Section 2 and a final discussion is given in Section 5.

2 | CASE STUDY

The case study consists of 11 experiments as well as a historical experiment to be integrated in the analysis. It was part of the pre-clinical safety evaluation of a new experimental compound meant to be co-administered with a compound already available on the market [8].

For the marketed compound, historical experimental data were used to assess its safety [9]. Ten doses, ranging between 0 and 10 mg/kg, were investigated. A total of 55 rats were randomly allocated to receive a single dose of the marketed compound (Table 1). For each of those animals' body temperature, the safety biomarker of interest, was assessed up to 24 hours after oral administration. Dose related changes were observed for extremely high doses, indicating potential side effects (Figure 1).

Figure 1 shows the observed body temperature profiles for nine representative individuals. They show, for high dose levels, a rapid decline of body temperature, followed by a return to the baseline level within a few hours. Further, it is noted that both the maximal observed effect and the time to reach the maximal effect increase with increasing dose levels.

In light of the dose-response of the marketed compound and with the intent to develop a new compound for co-administration, a new experiment was designed to evaluate whether an interaction between both compounds occurs. A total of 20 animals were used in the experiment, with 5 animals randomized to each of four treatment groups. Group 1 received a single dose of the vehicle (0 mg/kg); group 2 received the maximum dose from the historical experiment (i.e. 10 mg/kg); group 3 obtained a high dose of the novel compound (40 mg/kg) and group 4 received a combination of both compounds (10 mg/kg of the marketed compound and 40 mg/kg of the new compound). Body temperature was assessed every hour after administration for four hours. High doses were chosen to maximize the chance of detecting a

PD interaction between both compounds. A PK interaction was not formally statistically tested. However, no major PK interaction was observed in an exploratory experiment. As expected, animals in the vehicle (group 1) and novel (group 3) treatment groups did not show any effect on body temperature, whereas animals attributed to the marketed compound (group 2) showed an effect in line with the historical dose response experiment of the marketed compound. Nevertheless, it was unexpected that animals assigned to the combination group (group 4) showed a more pronounced change in body temperature compared to the group treated with the marketed compound only (Figure 2). In order to further understand this synergetic behaviour and its potential impact on future drug development, the experiment was repeated 10 times, each time with a different combination of dose levels (Table 2, Figure 3). Because plasma samples could potentially impact body temperatures, plasma concentration-time profiles were not obtained.

3 | METHODS

In this section we first describe a K-PD model for the analysis of the historical experimental data of the marketed compound (Section 3.1). This model is then extended to describe the synergy of compounds in Section 3.2. Details on the Bayesian estimation are given in Section 3.3.

3.1 | Analysis of Historical Data Using a K-PD Model

Let us first focus on the analysis of the historical experiment of the marketed compound. Turnover models, also referred to as indirect pharmacodynamic response models, cope with profiles such as the ones observed in Figure 1. These models take into account the mechanism of action of the drug and other aspects of biological reality (Dayneka, 1993; Sharma and Jusko, 1998) [5, 10], and are therefore called semi-mechanistic models. An enzyme, protein or biomarker is produced and proportionally eliminated continuously over time. In the absence of the compound, the production and elimination processes are in equilibrium. The administration of a compound results in an increase of the drug concentration in plasma over time. This drug will bind to a receptor in a concentration dependent manner, which in turn can block or boost either of these processes pending the physiological pathway. Turnover models are appropriate when there is a time lag between exposure and the observed effect of the biomarker. For an extensive review of turnover models, we refer to Sharma and Jusko (1996) [11] and Gabrielson and Weiner (1997) [12].

Let R_{it} denote the observed body temperature for animal i at time t . We propose the use of a turnover model with an inhibition process operating on the production of body heat, namely:

$$R_{it} \sim N(\bar{R}_{it}, \sigma_R^2), \quad (1)$$

$$\frac{d\bar{R}_{it}}{dt} = k_{in} \left(1 - \frac{I_{max} C_{it}}{IC_{50} + C_{it}} \right) - k_{out} \bar{R}_{it}. \quad (2)$$

Equation (2) describes the body temperature change over time. $\bar{R}_{it}$ is the expected body temperature for animal i at time t . The parameters k_{in} and k_{out} are the rate for production and elimination of body heat, respectively. Inhibition of the production depends on the amount of the marketed compound in the central compartment C_{it} and is characterized by the maximal inhibition of the production rate, I_{max} ($0 \leq I_{max} \leq 1$) and the drug amount to inhibit the production to 50% (IC_{50}). In what follows we will refer to this IC_{50} value as the potency of the compound. The model takes into account residual error via equation (1) with σ_R^2 the measurement error variance.

As the amount C_{it} for animal i at time t could not be measured, a virtual PK profile is assumed to drive the inhibition of body heat [7]:

$$\frac{dA_{e,it}}{dt} = -k_a A_{e,it}, \quad (3)$$

$$\frac{dC_{it}}{dt} = k_a A_{e,it} - k_e C_{it}, \quad (4)$$

This represents a first-order one-compartment PK model with oral absorption. The amount of the marketed compound in the absorption depot is represented as $A_{e,it}$. The parameter k_a is the absorption constant whereas the parameter k_e is the elimination constant.

The full K-PD model is given by equations (1)-(4). At time $t = 0$, $A_{e,i0}$ corresponds to the dose of the marketed compound, $C_{i0} = 0$ as no drug amount is present in the central compartment before the actual administration of the compound and $R_{i0} = k_{in}/k_{out}$ corresponds to the fact that body temperature is in a steady state condition prior to administration of the compound.

It should be noted that the lack of information on the exposure-time profile causes a form of model overspecification, as it does not allow the identifiability of some parameters. In particular, IC_{50} could not be estimated and was set to 1, as discussed in Section 3.2. In Jacqmin et al. [7] an intravenous (IV) infusion model was used, so that the underlying exposure is fully corresponding to the dose level. In our context an oral absorption model is implemented, where the bioavailability cannot be estimated as such. As a result, IC_{50} becomes not estimable (confounded up to a factor). The absence of information on the absorption phase of the marketed compound further causes non-identifiability of k_a : the compound first needs to reach the site of action to induce a physiological downstream reaction. Hence, the unobserved absorption needs to be faster than the change in response. This implies that k_{out} is a lower boundary of the absorption rate, so the true absorption rate is censored. In order to overcome this identifiability problem k_a was set to e^5 , which reflects a fast change in body temperature during the first hour. This assumption of an extremely fast absorption process makes the model comparable to the model with IV bolus administration illustrated by Jacqmin et al. [7]. A sensitivity analysis confirms that the IV bolus and our proposed model give the same results.

To allow for heterogeneity amongst animals, a random effect was added for body temperature at baseline R_{i0} ,

$$R_{i0} \sim N(\bar{R}_0, \sigma_{R_0}^2).$$

Inclusion of random effects on other parameters (k_{in} and k_{out}) was also evaluated.

3.2 | Synergy

Up to this point, only the effect of the marketed compound has been accounted for. In this section, the model is extended to include the effect of the novel compound onto the marketed compound.

Given the absence of a pharmacokinetic interaction between the two compounds and the inactivity of the novel compound on body temperature, and given the fact that the co-administration did not affect the maximal effect (I_{max}) observed in the historical experiment on the marketed compound, it is assumed that the presence of the novel compound increases the potency of the marketed compound with respect to body temperature. Therefore, the following definition

for the potency is proposed:

$$\begin{aligned} IC_{50new} &= \exp(\alpha_0 + \alpha A_{n,i0} + \beta A_{e,i0} A_{n,i0}) \\ &= IC_{50} \exp(\alpha A_{n,i0} + \beta A_{e,i0} A_{n,i0}), \end{aligned} \quad (5)$$

where $A_{n,i0}$ and $A_{e,i0}$ are the doses of the novel and marketed compound for the i^{th} individual.

Although, as mentioned above, $IC_{50} = \exp(\alpha_0)$ is fixed to 1 due to the lack of PK information, this parameterization allows to generalize the model to a non-virtual PK profile, from which IC_{50} can be estimated.

The main effect α represents how the novel compound influences the binding of the marketed compound at the receptor. Note that in the particular scenario of $A_{e,i0} = 0$, $A_{n,i0} > 0$ and $\alpha \neq 0$, one obtains $IC_{50new} = IC_{50} \exp(\alpha A_{n,i0})$. Hence in this case $IC_{50new} \neq IC_{50}$, which makes sense since binding cannot be observed when no dose of the marketed compound is administered.

The interaction coefficient β can be initially considered as controversial, as in this way the potency of a compound is made dependent on its dose. However, also the amount of marketed compound near the receptor can modify the impact of the novel compound on the potency of the marketed compound itself. This interaction is expressed by β .

An extensive publication list on models for co-administration of compounds can be found in Harbron et al. (2010) [13]. However, such models measure in-vitro synergy. One of the most widely used methods is the Loewe Additivity model [13]:

$$\frac{d_1}{D_{y,1}} + \frac{d_2}{D_{y,2}} \begin{cases} = 1 & \text{Loewe Additivity} \\ < 1 & \text{Loewe Synergy} \\ > 1 & \text{Loewe Antagonism} \end{cases} \quad (6)$$

where y represents the effect that two compounds produce when they are administered as monotherapy with doses $D_{y,1}$ and $D_{y,2}$ respectively; d_1 and d_2 represent the doses of the two compounds that produce the same effect as $D_{y,1}$ and $D_{y,2}$ when both compounds are co-administered. In the specific case where one compound is inactive if administered as monotherapy, the in-vitro equation (6) simplifies to:

$$\frac{d_1}{D_{y,1}} \begin{cases} = 1 & \text{Inertism} \\ < 1 & \text{Synergy} \\ > 1 & \text{Antagonism.} \end{cases} \quad (7)$$

Thus, an in-vitro synergistic behavior is observed if the dose of the active compound required to achieve the effect in combination is less than required in monotherapy [14]. Note that, according to the so-called Saariselkä agreement [15], the terms synergism/antagonism (without a leading adjective) are used for an increased/decreased effect following the co-administration when only one compound is active.

The proposed parametrization given in (5) is consistent with the Loewe Additivity model in the special situation of one active compound. The synergy is built into the model as follows:

$$\begin{aligned} \frac{I_{max} C_{it}}{IC_{50new} + C_{it}} &= \frac{I_{max} C_{it}}{IC_{50} \exp(\alpha A_{n,i0} + \beta A_{e,i0} A_{n,i0}) + C_{it}} \\ &= \frac{I_{max} C_{it} \exp(-\alpha A_{n,i0} - \beta A_{e,i0} A_{n,i0})}{IC_{50} + C_{it} \exp(-\alpha A_{n,i0} - \beta A_{e,i0} A_{n,i0})}. \end{aligned} \quad (8)$$

Based on this rearrangement of the equation and under the assumption of linear kinetics, one obtains:

$$d_1 = D_{y,1} \exp(\alpha A_{ni0} + \beta A_{e,i0} A_{n,i0}),$$

which reflects how much one needs to adjust the dose in the combination therapy to obtain the same effect y as under monotherapy. Furthermore, one can easily see that:

$$\frac{d_1}{D_{y,1}} = \exp(\alpha A_{ni0} + \beta A_{e,i0} A_{n,i0}) = \frac{IC_{50_{new}}}{IC_{50}}. \quad (9)$$

Hence, in case $A_{n,i0} = 0$ it follows that $IC_{50_{new}} = IC_{50}$. This is comparable to the Loewe Additivity model where $d_2 = 0$ implies that $d_1 = D_{y,1}$ or, in other words, that the compound is additive to itself.

So, when two compounds are co-administered, the evaluation whether the interaction is additive, synergistic or antagonistic depends on the value of $IC_{50_{new}}$:

- $IC_{50_{new}} = IC_{50}$, means that the effect of the marketed compound remains independent of the novel compound and corresponds to the definition of additivity;
- $IC_{50_{new}} < IC_{50}$ means that a lower exposure for the marketed compound is required in the presence of the novel compound to obtain the same effect, hence synergy;
- $IC_{50_{new}} > IC_{50}$ means that a higher exposure for the marketed compound is required in the presence of the novel compound to obtain the same effect, hence antagonism.

This illustrates that the model presented in this paper can be considered as an extension of the Loewe additivity approach to an in-vivo framework in the special situation when a compound is inactive if administered as a monotherapy.

3.3 | Bayesian data integration

The data of all (sub)experiments were collected at the same laboratory by the same scientist. This justifies to pool the experiments and analyze all data altogether.

Initially, a likelihood-based model was used to estimate the parameters based on all pooled experiments (1-11) using NONMEM software (version 7.3.0) [16]. This however resulted in convergence issues even with different starting values and different estimation methods (SAEM and Laplace). Therefore, a Bayesian model was considered as it allows to take into account prior knowledge from the historical dose-response experiment on the marketed compound, thus avoiding potential performance issues and facilitating convergence by restricting the exploration of the parameter space. Prior distributions were chosen reflecting the knowledge on the marketed compound. In particular, expected values were set equal to point estimates obtained from the historical data. The standard errors were doubled to down-weight the information derived from the previous analysis (since the historical experiment was conducted two decades before the new experiments [9]). The priors for k_e and k_{out} were assumed lognormally distributed, I_{max} was assumed to follow a beta distribution and a uniform distribution with parameters -10 and 10 was used as a prior for α and β . An inverse gamma distribution was used as a prior for the measurement error variance σ_R^2 , and lognormal and inverse gamma distributions were used as priors for the mean and variance of the random effect ($\bar{R}_0$ and $\sigma_{R_0}^2$). Table 3 summarizes the set of prior distributions for the parameters of the above presented model.

The use of uninformative priors was also evaluated, but it led to convergence issues due to the high model complexity

relatively to the amount of data.

The Bayesian model was estimated using the No-U-Turn Sampler (NUTS) algorithm in Stan (rstan version 2.14.1) [17]. Specifically, three Markov chains were run, with a total length equal to 3000 (2000 draws for each chain, of which 1000 warmup). The correlation among Markov chain samples and the degree of convergence were also assessed through the effective sample size and the R-hat statistic respectively.

The graphic representation was made using the statistical software package R version 3.3.3 (Foundation for Statistical Computing, Vienna, Austria).

4 | RESULTS

In this section, we present the results of the above described synergy model. We first show the evaluation of model performance, followed by model parameter estimation and model prediction.

Our Bayesian model seemed to fit the data well. Individual estimates of body temperature for data of experiment 1 are shown in Figure 4. Columns 1 and 2 show the observed and estimated time profiles of rats in treatment group 1 (no treatment) and group 3 (novel compound), respectively. Clearly, when the novel compound is administered as monotherapy, there are no temperature variations over time. Column 3 displays the profiles in treatment group 2 (marketed compound), showing a substantial decrease in body temperature, which reaches its maximum one hour after oral administration, followed by an increase that reaches the initial steady state four hours after administration. Column 4 shows the profiles for rats in treatment group 4 (co-administration): it is possible to observe a later maximal effect and an increase in potency as the decrease is much more pronounced, reaching its maximum approximately 2 or 3 hours after administration. Body temperature takes longer than four hours before reaching the steady state. The estimated time profiles in experiments 2 to 11 are given in the Appendix A.

Table 4 shows posterior mean, 95% credible intervals, effective sample size and R-hat statistic for the parameters in the model. The value of R-hat is equal to unity for all parameters, meaning that the chains are well-mixed and they are all exploring the same regions of parameter space. The effective sample size is equal to its maximum for all parameters except for I_{max} , β and $\sigma_{R_0}^2$. Particularly, the lower value for β indicates a higher correlation among the Markov chain samples for this parameter. This was to be expected since only the observations belonging to the combination treatment group actually contribute to the estimation of β .

The maximal inhibition of the production rate is likely between 16% and 22%. The negative posterior means of α and β (with credible intervals not including 0) confirm the presence of a pharmacodynamic synergy between the study compounds, as $IC_{50_{new}} < IC_{50}$.

The model estimates are used to illustrate $IC_{50_{new}}$ (Figure 5) and maximum predicted temperature change (Figure 6) as a function of both the marketed and the novel compound. It can be observed that the synergistic behaviour affects body temperature only for high doses of the marketed compound (higher than 2.5 mg/kg), whereas low doses produce negligible deviations in body temperature independently of the novel compound dose. This illustrates that the synergistic behaviour between the two compounds is only observed at dose level combinations well above the clinical range (until 0.1 mg/kg) and that clinically relevant dose levels remain without effect.

5 | DISCUSSION

In this paper a novel K-PD model for the estimation of synergy is presented. The use of a physiologically inspired model enables translating the scientific questions of interest directly into tangible parameters, leading to clearly interpretable

conclusions. Our proposed model directly quantifies the impact of the new compound on the safety of the existing compound in one or two parameters. It is therefore easier to interpret when compared to a model such as a spline or polynomial. It has been demonstrated that the model is an extension of the in-vitro synergy methodology to in-vivo data using the Loewe Additivity Model in the situation when a compound is inactive if administered as a monotherapy.

Unlike the frequentist framework, the Bayesian framework allows to borrow information from the historical experiment, thus facilitating convergence. The model has proven to work well when all data coming from different experiments conducted in different time periods were pooled. Further work is being devoted to keeping the sequential nature of the experiments, i.e., fitting a Bayesian model in a sequential manner, so that the posterior distributions resulting from an experiment are used to determine the hyperparameters of the prior distributions of the experiment which follows.

As mentioned previously in the Methods section, several models were performed by including random effects on different parameters. The choice of random effects highly affected the model performance: as an example, substantially biased parameter estimates were observed when the random effect was allocated to the parameter k_{out} . A possible explanation of such behaviour could be related to the highly correlated parameter space, which leads to overcompensated estimates. A highly correlated parametric space is a typical property of nonlinear hierarchical models. Nevertheless, the parameter correlation becomes particularly high in models with virtual variables [18].

Prior elicitation represented another challenge in fitting the presented model: the use of uninformative priors led to convergence issues due to the combination of a complex, but physiologically inspired model and a highly correlated parameter space. When data is scarce, there is a lack of information which leads to a higher correlated parametric space, where parameters compensate each other. Increasing the informativeness of priors, on the other hand, gives the possibility to add prior information, causing more stability and less convergence problems. In such context, the information coming from the historical experiment was particularly useful, because it helped choosing the prior distributions for analyzing the data under study. These evaluations will be more deeply explored in future work. The stiffness of prior choice did not allow the consideration of multivariate prior distributions, which incorporate the intercorrelations among parameters, as this would further increase the computational burden and convergence issues.

It should be noted that only a limited design space was assessed in this case study. This is due to its explorative nature: in a preclinical context, practical constraints such as limitation of animal facilities and workload of laboratory technicians prohibit the design of an optimal set of dose combinations. Furthermore, the studies were designed without the consultation with a statistician. The case study illustrated in this manuscript highlights the importance of involving statisticians at the experimental design phase, so that both scientists and statisticians can benefit from such a collaboration. Nevertheless, the compound doses assessed in this study were sufficient for the calculation of accurate parameter estimates and good model fitting.

Lastly, it needs to be emphasized that expressing the potency in function of treatment doses is solely used in the absence of the plasma concentration-time profiles. The latter would be a more suitable covariate to describe the activity at the receptor and therefore the synergy. Hence, the dose levels are only an approximation of the true exposure at the receptor levels. As previously described, the lack of PK information causes non-identifiability of IC_{50} . It should be noted, however, that the scientific question of interest focuses on the change of IC_{50} in the presence of the novel compound, rather than quantifying the value itself. This proposed method enables to answer this question through a restricted number of parameters.

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TABLE 1 Dose levels used in historical experiment of marketed compound.

Dose (mg/kg)	0	0.04	0.08	0.16	0.31	0.63	1.25	2.5	5	10
Group size	5	5	5	5	5	5	5	5	5	10

TABLE 2 Dose levels used in current experiments of drug-drug interactions.

Experiment	1	2	3	4	5	6	7	8	9	10	11
Marketed compound (mg/kg)	10	2.5	10	0.63	10	0.16	2.5	0.63	0.16	0.04	0.04
Novel compound (mg/kg)	40	40	10	40	2.5	40	10	10	10	10	40

TABLE 3 Priors relative to the parameters of the Bayesian K-PD model for synergy.

Parameter	Prior distribution
k_e	$\log N(-0.115, 0.514)$
k_{out}	$\log N(1.93, 6.84)$
I_{max}	$Beta(19.574, 174.532)$
$\bar{R}_0$	$\log N(3.61, 0.002)$
α	$Unif(-10, 10)$
β	$Unif(-10, 10)$
$\sigma^2_{\bar{R}_0}$	$Inv\text{-}Gamma(54.441, 24.476)^*$
σ^2_R	$Inv\text{-}Gamma(2.022, 1.186)^*$

* The inverse-gamma distribution is parametrized with α and β , i.e., the PDF is: $\frac{\beta^\alpha}{\Gamma(\alpha)} x^{-\alpha-1} \exp\left(\frac{-\beta}{x}\right)$.

TABLE 4 Results from the Bayesian model: posterior mean, 95% credible intervals, effective sample size and R-hat statistic.

Parameter	Posterior mean	95% CI	ESS*	R-hat
k_e	0.50	(0.41, 0.58)	3000	1
k_{out}	1.26	(1.01, 1.57)	3000	1
I_{max}	0.19	(0.16, 0.22)	2509	1
$\bar{R}_0$	37.16	(37.10, 37.22)	3000	1
α	-1.17	(-1.51, -0.80)	3000	1
β	-0.67	(-1.46, -0.06)	1792	1
$\sigma^2_{\bar{R}_0}$	0.14	(0.11, 0.19)	2322	1
σ^2_R	0.30	(0.28, 0.33)	3000	1

* Effective Sample Size.

FIGURE 1 Time profiles of nine individuals from the historical experiment.

FIGURE 2 Time profiles of the individuals from experiment 1.

FIGURE 3 Dose scheme used in current set of experiments of drug-drug interactions (in logarithmic scale).

FIGURE 4 Individual estimates (solid line) and credible intervals (grey bands), experiment 1.

FIGURE 5 Change in $IC_{50_{new}}$ depending on the marketed compound dose (in logarithmic scale), according to three different doses of the novel compound: 0 mg/kg (solid line), 10 mg/kg (dashed line) and 40 mg/kg (dotted line).

FIGURE 6 Maximum Predicted temperature change from baseline depending on the marketed compound dose (in logarithmic scale), according to three different doses of the novel compound: 0 mg/kg (solid line), 10 mg/kg (dashed line) and 40 mg/kg (dotted line).