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Exploiting stochastic Petri nets with fuzzy parameters to predict efficient drug combinations for Spinal Muscular Atrophy

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Abstract: Randomness and uncertainty are two major problems one faces while modeling nonlinear dynamics of molecular systems. Stochastic and fuzzy methods are used to cope with these problems, but there is no consensus among researchers regarding which method should be used when. This is because the areas of applications of these methods are overlapping with differences in opinions. In the present work, we demonstrate how to use stochastic Petri nets with fuzzy parameters to manage random timing of biomolecular events and deal with the uncertainty of reaction rates in biological networks. The approach is demonstrated through a case study of simulation-based prediction of efficient drug combinations for spinal muscular atrophy, for which we obtained very promising results. The feasibility of the approach is assessed through statistical analysis of deterministic, pure stochastic and fuzzy stochastic simulation results. Statistical analysis reveals that all three models produce significantly different results which, when coupled with the fact that fuzzy stochastic model provides the closest approximation of underlying biological network, successfully coping not only with randomness but also uncertainty, suggests that fuzzy stochastic model is the most appropriate choice for the present case study. The proposed approach can be adapted or extended to other biological networks.

Key words: Stochastic Petri nets, fuzzy logic, quantitative modelling, spinal muscular atrophy

1. Introduction

Quantitative modelling of biological networks has experienced renaissance for the past two decades. Quantitative description of molecular interactions in a complex biomolecular system is essential not only to understand the structure and properties of the system, but also to identify key molecular targets driving major biomolecular processes within this system. Such molecular targets can then be used to predict novel drugs or drug combinations in line with target-based drug discovery which has developed into an area of intense research. It is worth to mention that such a model is expected to reproduce the dynamic behavior of the system correctly. The important stages of quantitative modelling are (i) determination of the underlying biological network based on rigorous study of the biological databases and literature, (ii) development of the model using methods and techniques provided by computer science and/or model engineering, (iii) computational validation of the model

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based on known wet lab results, (iv) computer simulations aimed at identification of drugs or beneficial drug combinations that would play prominent role in the treatment of a disease.

Molecular interactions are characterized by a high degree of randomness and uncertainty. Despite the fact that randomness and uncertainty are both permissible within the same scope, these concepts essentially differ from one another. Randomness in biological networks may arise due to low molecular density, intrinsic random nature of phenomena, and noise in an experiment. An individual molecular event is subject to stochastic time delays as it takes place whenever the event conditions (availability of substrates, desired level of energy, temperature and pressure, etc.) are present, but not according to a predefined order. Stochastic nature of molecular interactions can be represented and analyzed using the Chapman–Kolmogorov equation [1], stochastic differential equations [2], the Gibson–Bruck algorithm [3], stochastic Pi-calculus [4], the Gillespie algorithm [5], stochastic process algebra [6], and stochastic Petri nets (SPNs) [7]. Expressive capabilities of the SPNs coupled with existing powerful analysis tools make SPN modeling approach an indispensable choice in the stochastic analysis of the biological systems.

It is quite regular that, identical wet lab experiments result in different observations at each time due to the inexactness of measurements and other technical noise. For instance, genetically identical cells even within the same tissue often exhibit different levels of gene expression, protein production, and different rates for biological phenomena. Usually, we deal with imprecise and incomplete knowledge about reaction rates which are often expressed by qualitative descriptions of parameters such as "is almost disrupted"or "occurs faster than". Fuzzy logic allows modelling reaction effects which can be derived from qualitative knowledge.

Mutation in the *Survival Motor Neuron 1 (SMN1)* gene leads to the absence or insufficient production of survival motor neuron (SMN) protein, which in turn causes spinal muscular atrophy (SMA), a motor neuron disease. In humans, there is a second copy of the gene, namely *SMN2*, which has the required genetic information to alleviate the disease. However, *SMN2* fails to compensate for the loss of *SMN1* because it results in only 10%–15% of the full length protein, while its 85%–90% present dysfunctional SMNΔ7 protein. Various drug candidates have been proposed as potential means of treating SMA or decreasing its severity by increasing SMN levels produced by *SMN2*. Increase in concentration of biological component is measured in folds, which is a ratio of current and nominal concentrations. These drug candidates provide 1.3- to 5-fold increase of SMN, which is not sufficient to cure SMA. In the present research, we explore the intermolecular interactions in SMN production network to predict the most efficient drug combinations which can result in maximum SMN levels from *SMN2*. Based on this motivation, we use SPNs with fuzzy parameters to develop a quantitative model of SMN production network, validate the model using biological data, and perform fuzzy stochastic simulations to predict drug combinations leading to the highest levels of SMN produced from *SMN2*. Simulation results allowed us to identify optimal combinations of drug candidates which can increase SMN concentration up to 149.9-folds over the control group, though this number for known drug candidates does not exceed 5-folds.

This paper has the following structure. In the following section, we outline the materials and methods, briefly reviewing key work to date, introducing SPNs with fuzzy parameters, and discussing biological context. In the next section, we provide results, describing the model of SMN production network, its validation through existing empirical data, simulation results, their analysis and comparison of deterministic, pure stochastic, and fuzzy stochastic models. The paper ends with a discussion and outlook regarding further work.

2. Materials and methods

2.1. Related work

SPNs bring together modeling power of regular Petri nets and results in stochastic molecular dynamics obtained by Gillespie [5]. SPNs can represent and analyze both the structure and stochastic dynamics of biological systems and therefore have attracted much of the attention since late 1990s. Over the last two decades, much research has been published on applications of SPNs in biological systems. Goss et al. [8] used SPNs to model plasmid *ColE1* replication. To our knowledge, this is the first account on the use of SPNs for modeling biological systems. Srivastava et al. [9] demonstrated with the example of *Escherichia coli* stress circuit, the appropriateness and conceptual simplicity of modeling gene regulatory networks using SPNs. Bahi-Jaber et al. [10] exploited colored SPNs to develop and investigate complex stochastic epidemic models. Marwan et al. [11] demonstrated on example of *Physarum polycephalum* how hierarchical-structured SPNs can be used to represent the gene regulatory pathway controlling the commitment and sporulation. Mura et al. [12] applied SPNs to model cell cycle in yeast. Lamprecht et al. [13] used SPNs to create model of Ca^{2+} release sites composed of a number of intracellular channels that have stochastic behavior, and Marwan et al. [14] investigated enteric bacteria phosphate regulation by using SPNs, while Castaldi et al. [15] developed SPN model of the tissue factor-induced coagulation cascade. Liu et al. [16] used fuzzy SPNs to create a yeast polarization model, and Bashirov et al. [17] presented stochastic simulation-based validation and analysis of the p16-mediated pathway, the disruption of which is among major causes of human cancers. Software tools used to conduct the above research include Snoopy [18], Möbius [19] and GreatSPN [20], while https://www.informatik.uni-hamburg.de/cgi-bin/TGI/tools/ collects links to 23 Petri net tools and software supporting SPNs.

Knowledge on kinetic parameters is vague than crisp and therefore is usually represented by natural language-based qualitative knowledge. Fuzzy logic is proved to be an efficient approach to deal with vagueness in biological models. Below we review the biological models developed using of Petri nets with fuzzy sets. Sokhansanj et al. [21] developed an algorithm that allows the creation of a model of intergenetic interactions based on the theory of fuzzy sets, which was later modified by Gintrowski [22] to reduce the search time in gene network. Hamed [23] introduced a quantitative model of a gene network in which imperfect kinetic data was reproduced in terms of fuzzy logic. Mehraei [24] exploited fuzzy stochastic hybrid Petri nets in modelling of mood disorder treatment. Liu et al. [16] combined SPNs and fuzzy logic in order to create a quantitative model of biological systems in which reaction rates are associated with fuzzy numbers. Bordon et al. [25] demonstrated how fuzzy logic and Petri nets can be used to deal with unknown or imprecise data arising from gene regulatory processes, and Liu et al. [26] represented interactions among genes with fuzzy rules implementing colored fuzzy Petri nets. Most of Petri net software and tools cannot handle fuzzy numbers. Once SPN model is created using one of the Petri net software such as Snoopy [18], it can be further fuzzified using MATLAB or similar software.

So far, there is no widespread evidence of the applications of both SPNs and fuzzy logic in biological models. To the best of our knowledge, the present paper is one of the first attempts [16, 25] to associate fuzzy numbers with reaction rates and concentrations in an SPN model of a biological network. Moreover, this work is the first account on the use of SPNs with fuzzy parameters in a target-based drug combination discovery.

2.2. Stochastic Petri nets with fuzzy parameters

For the last three decades, Petri nets have been extensively exploited for modelling biological systems. Below we briefly summarize the relationship between Petri net components and biological objects. A Petri net, sometimes

referred to as basic Petri net, is formally defined as 5-tuple $PN =< P, T, F, f, M_0 >$ such as

- $P = \{p_1, \dots p_n\}$ is a set of the places,
- $T = \{t_1, \dots t_m\}$ is a set of the transitions,
- $F \subseteq (P \times T) \cup (T \times P)$ is a set of directed arcs,
- $f : F \to \mathbb{N}^+$ is a function that assigns a weight to each arc $a \in F$,
- $M_0 : P \to \mathbb{N}_0$ is the initial marking,

where $P \cap T = \emptyset$.

While modelling biological systems with Petri nets, places, and transitions represent biological components (gene, protein, etc.) and biological processes (transcription, translation, binding, etc.), respectively, arcs determine the flow of biochemical reactions. The concentration of biological components changes according to the arc weights. The initial marking determines the initial state of a biological system being modelled.

2.2.1. Stochastic Petri nets

Basic Petri nets provide powerful modeling technique for learning logic behavior of dynamic systems but not their quantitative behavior. This is because basic Petri nets are not complete enough for modeling dynamic systems in which system activities are changing over time. In real world, however, almost every action is time dependent. There are many possible ways to associate time to basic Petri nets. Time Petri nets are time-dependent deterministic Petri nets that are gained wide application in scientific and engineering domains. In a time Petri net, each transition is associated with deterministic firing time interval so that enabled transitions may fire only during specified time intervals. The transitions must fire the latest at the end of their intervals if they are still enabled then. At any given moment, only one transition may fire. This firing does not take time.

Occurrence of a biochemical reaction is an entirely stochastic process which depends on the availability of the substrates and the presence of other conditions. Therefore, a regular Petri net as described above is not sufficient to create quantitative models of biological systems to the desired details of comprehension. In the present work, we use SPNs to associate random time delays with occurrences of transitions. In an SPN model, time from the enabling of a transition to its next occurrence is a random variable with negative exponential probability distribution function.

$$F(t) = 1 - e^{-\lambda t} \text{ if } t \geq 0 \text{ and } F(t) = 0 \text{ otherwise, } \lambda > 0.$$

We develop an SPN model of SMN production network and perform 38,000 stochastic replications for each combination of drug candidates, which are further averaged to obtain a reliable estimation of simulation-based behavior of SMN production network. This allows us to reach in stochastic simulations to a confidence level of 95% with the accuracy of 10^{-2}.

2.2.2. Fuzzy logic

Fuzzy logic proposed by Zadeh [27] provides a systematic framework for dealing with uncertainty, imprecision, and vagueness of kinetic parameters. In this theory, a fuzzy set $\widetilde{\zeta}$ defined on a universal set $\mathbb{X}$ is determined by its membership function $\mu_{\widetilde{\zeta}} : \mathbb{X} \to [0, 1]$, which assigns to each element $x \in \mathbb{X}$ a real value $\mu_{\widetilde{\zeta}}$ in $[0, 1]$.

Fuzzy numbers are the fuzzy sets that are normalized and convex. A fuzzy number can be represented in different formats such as triangular and trapezoidal. We associate triangular fuzzy numbers with reaction rates and concentrations. A triangular fuzzy number $\widetilde{\zeta}$ is defined by three numbers $a < b < c$ where $[a, b]$ is the base of the triangle and $x = c$ is its vertex. Triangular fuzzy number represented in Figure 1 is monotonically increasing in interval [a,b], and it is monotonically decreasing in interval [b,c]. A triangular fuzzy number $\mu_{\widetilde{\zeta}} = (a, b, c), a \leq b \leq c$ is formally defined as follows:

$$\widetilde{\zeta} = \begin{cases} 0, & \text{if } x \leq a; \\ \frac{x-a}{b-a}, & \text{if } a \leq x \leq b; \\ \frac{c-x}{c-b}, & \text{if } b \leq x \leq c; \\ 0, & \text{if } x \geq c. \end{cases}$$

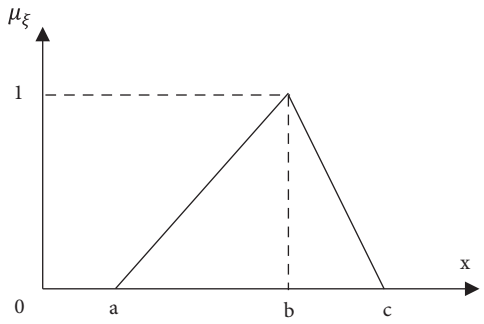

Figure 1. A triangular fuzzy number $\widetilde{\zeta}$.

2.3. Biological context

SMA is the leading genetic cause of infant mortality and the second most common fatal autosomal recessive disorder after cystic fibrosis. The disease affects 1 in 6000–10,000 newborns. The disease is caused by the deletion of or mutations in the *SMN1* gene. Several approaches have been suggested for treating SMA or decreasing its severity. These approaches can be classified into four approaches, namely, promoting *SMN2* transcription through use of histone deacetylase (HDAC) inhibitors, increasing correct splicing of the *SMN2* transcript, upregulating promoter activity of *SMN2*, increasing *SMN2* activity through deoxyribonucleic acid (DNA) demethylation. In what follows, we briefly review existing approaches. The present work combines all these approaches to account for the total efficacy of various combinations of potential drugs with existing qPCR data.

2.3.1. Inhibition of the inhibitor

One way to treat SMA is through the inhibition of HDAC activity that is known to suppress the *SMN2* expression. We have found that ValProic Acid (VPA), TrichoStatin A (TSA), Dacinostat, and Resveratrol are the only HDAC inhibitors reported so far in the biological literature for which there are available qPCR and protein data on increased levels of SMN produced from *SMN2*.

Brichta et al. reported on 2- to 4-fold increase of SMN levels in fibroblast cultures derived from SMA patients treated with 0.5-–500 μM of VPA. VPA is a well-known drug that has regularly used in a long-term epilepsy treatment, and has recently been shown to yield therapeutic effects in mood disorders and migraine.

Avila et al. [28] observed that TSA treatment in SMA model mice results in 1.5- to 2-fold increase of SMN protein levels in the brain, liver, and spinal cord. Dayangaç-Erden et al. [29] noticed on 1.3-fold increase in SMN protein levels relative to untreated cultures after treatment with 100 μM of Resveratrol.

2.3.2. Regulating pre-mRNA splicing

While all the genetic information for functional SMN protein is present in the *SMN2* gene, a translationally silent C to T change in *SMN2* exon 7 results in exon skipping. This causes the production of a truncated, unstable SMNΔ7 protein. Hastings et al. [30] showed that treatment with the tetracycline derivative PTMK-SMA1 in type III SMA mice promotes the inclusion of exon 7 into *SMN2* mRNA during the splicing step, eliciting nearly 5-fold increase in SMN protein concentrations compared to untreated animals. Hastings et al. [30] reported that PTMK-SMA1 is the only chemical identified to date that has been demonstrated to alter splicing by directly targeting the splicing reaction to promote a specific splicing pathway.

2.3.3. Upregulating promoter activity

Jarecki [31] suggested to enhance SMN transcription arising from *SMN2* through the manipulation of the SMN2 promoter activity. It is reported in the same study that treatment with Indole in patient-derived cells demonstrates direct effect on *SMN2* promoter activity, increasing SMN transcription by 3-fold over the controls.

2.3.4. Targeting DNA methylation

Hauke et al. [32] demonstrated that *SMN2* is subject to gene silencing by DNA methylation. In this sense, inhibition of *SMN2* silencing conferred by DNA methylation represents a promising strategy for pharmacologic SMA therapy. AZA is a potential drug that positively affects SMN protein production by inhibiting methylation of *SMN2* gene transcription factors. Hauke et al. [32] reported on 2-fold increase of SMN protein levels in SMA patients treated with AZA.

3. Results

3.1. Developing and validating the model

Petri net is some kind of high-level programming language. Petri net software tools usually provide engine with GUI simulator, which in turn converts program code into network of places, transitions, and arcs similar to the one illustrated in Figure 2. In this work, we use Snoopy [18] framework to create Petri net model of SMN production network based on four approaches discussed in Section 1 and Subsection 2.3. Our model comprises 7 discrete places (Dacinostat, TSA, Resveratrol, VPA, AZA, PTMKSMA1 and Indole), 11 continuous places (HDAC_premRNA, HDAC, Methyl, TF_producer, TF, SMN2_gene, SMN2_premRNA, SMN2_mRNA, SMN2, SMNDelta7mRNA, SMNDelta7), 25 transitions (T1–T19, d1–d6), 2 read, 7 inhibitory, and 32 regular arcs. Discrete places stand for a drug candidate, while continuous places represent genes and gene products whose concentration changes smoothly over the time. Treatment by a drug candidate is simulated by introducing an inhibitory arc directed from discrete place to transition. Treatment by a drug candidate is enabled if discrete place is empty, disabled otherwise. To simulate treatment by a combination of drug candidates, we keep empty corresponding discrete places. A Boolean variable is used to check absence/presence of a drug treatment for the drug candidate. In this model, transitions represent transcription, translation, binding, gene activation, methylation, and degradation processes. Snoopy snapshot of the model is demonstrated in Figure 2.

To run the application, we simply set the initial data, that is, choose composition of the drug candidates by placing tokens in corresponding places.

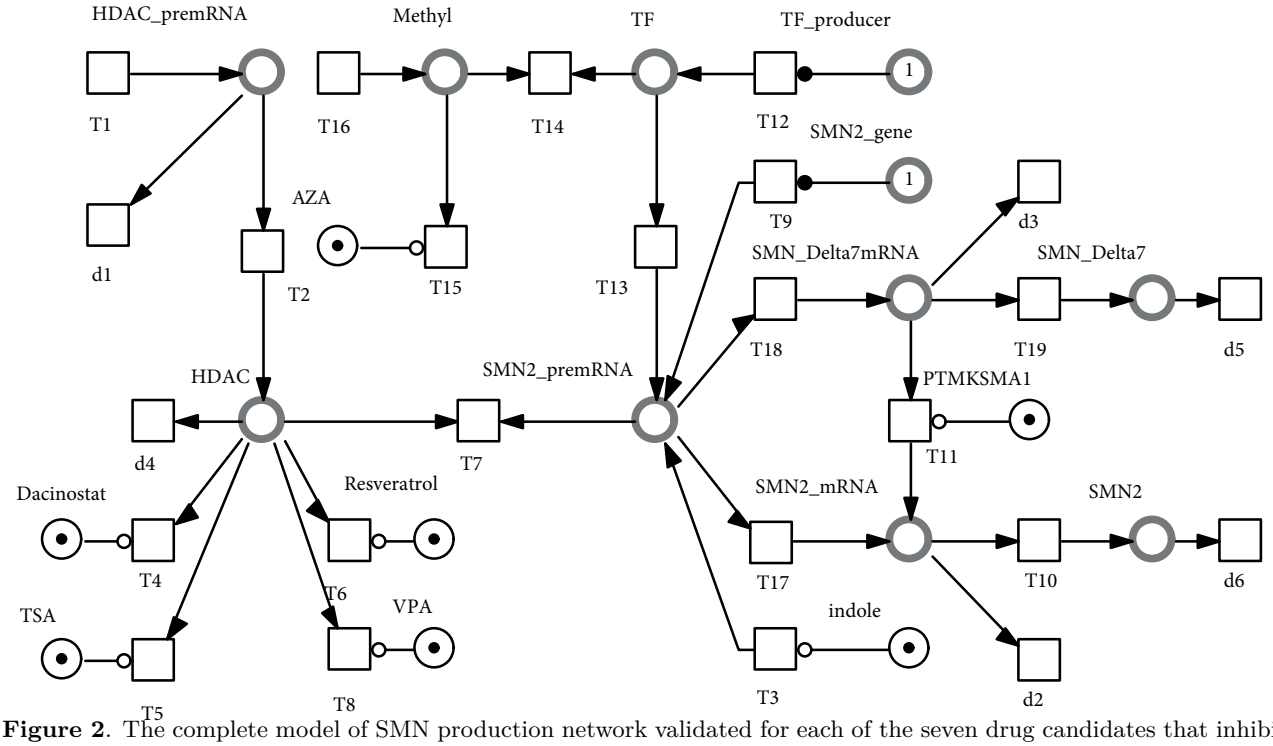

Figure 2. The complete model of SMN production network validated for each of the seven drug candidates that inhibit HDAC (TSA, VPA, Dacinostat, and Resviratrol), modulate pre-mRNA splicing (PTK-SMA1), upregulate promoter activity (Indole), and target DNA methylation (AZA). Read arcs and inhibitor arcs are respectively represented by a black dot and hollow dot as arc head. Read arcs are used to ensure continuous expression of SMN2 gene and production of transcription factor, while inhibitory arcs are used to simulate enabling/disabling a drug treatment.

We validate the model according to the knowledge derived from biological literature (see Section 1 and Subsection 2.3), and adjust rates of transitions $T_7, T_{10}, T_{12}, T_{13}, T_{17}, T_{18}$, and T_{19} in order to strike the balance between two protein types produced from *SMN2*, namely 85 percent SMNΔ7 and 15 percent SMN. To validate the model for each of seven drug candidates, we calibrate rates of $T_3, T_4, T_5, T_6, T_8, T_{11}$, and T_{15} one at a time until reaching desired level of SMN concentration for treatment with specified drug candidate. Rates of transitions representing mRNA and protein degradations are set to those used in previous works [17, 33]. The reaction rates are calibrated in terms of stochastic replications by further averaging obtained results.

Once the model is validated, we change kinetic parameter in the hazard function of each transition from a crisp value b to a fuzzy number (a, b, c). Then we perform 38,000 separate stochastic runs for a, b, and c. After that, we measure average mean for each parameter with the confidence level of 95% and the accuracy of 10^{-2}.

3.2. Simulation results

For each of the deterministic, stochastic and fuzzy stochastic models and for each of the 127 drug and drug combinations we run independent replications and then compute the sample mean to measure SMN concentration. Let C_n be a set of all possible combinations of n drugs for $n = 2, \ldots, 6$, where each $c \in C_n$ is recognized by fuzzy interval, (x_c, y_c). The following algorithm creates a set of effective combinations of n drugs, E_n.

```
algorithm create set of effective drug combinations
input: set of n-combinations, C_n
output: set of effective n-combinations, E_n
for n:= 2 to 6 do
set E_n = ∅
remove c with the maximum y_c from C_n and add it in E_n
set y_max = y_c and x_min = x_c
while E_n does not contain all effective n-combinations
if y_c > y_max then
remove c from C_n and add it in E_n
y_max = y_c
if x_c < x_min then x_min = x_c
return E_n
```

This algorithm splits the set of n-combinations into two disjoint subsets of effective n-combinations and remaining n-combinations, with the property that the lower limit of any effective n-combination is grater than the upper limit of any other n-combination for all n. This algorithm allows us to determine a small set of potentially beneficial drug combinations. Fuzzy intervals of any two effective n-combinations are either overlapping or one of them contains the other. For instance, this algorithm finds six 3-combinations that span the interval (16.1, 46) (see Figure 3). Based on the procedure described above, we find that only 35 out of 120 possible n-combinations are effective. All effective n-combinations are represented by Table 1.

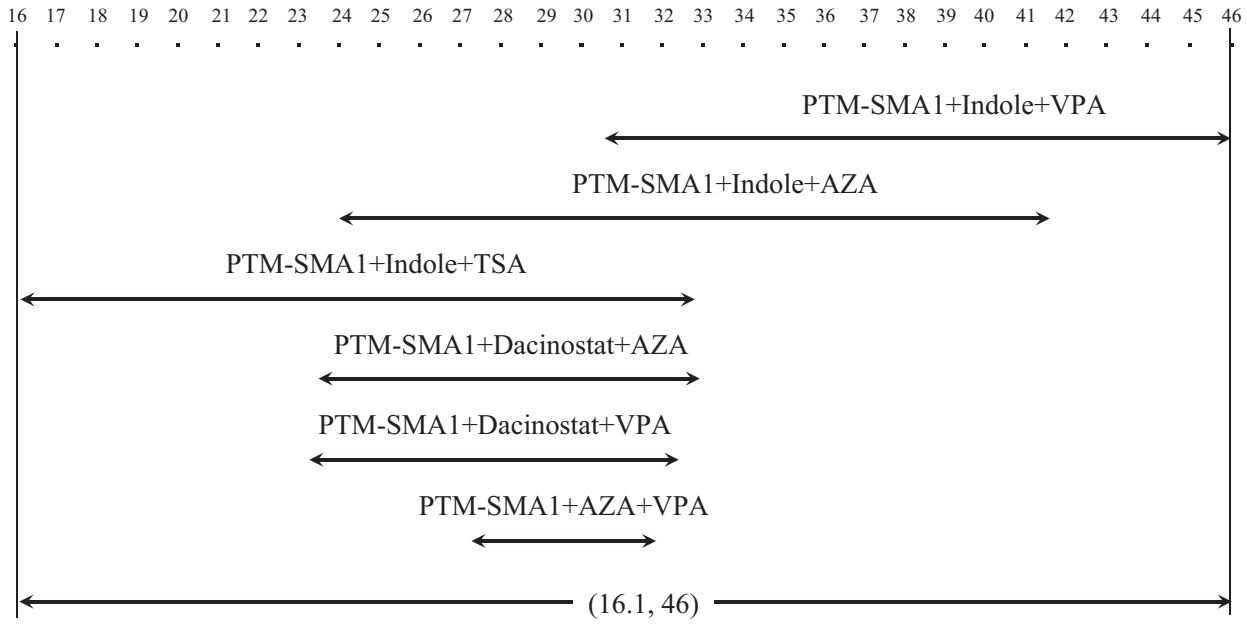

Figure 3. There are 6 effective 3-combinations out of 35 possible ones. Folds of SMN variation of effective 3-combinations span the interval (16.1, 46).

According to Table 1, the combination of all seven drug candidates results in the maximum increase of SMN concentration produced from *SMN2* (149.9-fold increase over the control). Although all seven chemicals are compatible, they can cause serious side effects when used in combination. One important aspect of the present research is that we determine the scope of effective drug combinations to be examined by pharmacological

groups. If a drug combination causes unavoidable side effects, there can be still other effective combinations that can be tested.

We observed that increase in number of drugs does not always result in increase of SMN levels. For instance, the most effective 5-combination (PTK-SMA1&Dacinostat&Indole&VPA&AZA) yields more SMN (57.5- to 125.6-fold) than many 6-combinations. Similarly, the most effective 4-combination (PTK-SMA1&Dacinostat&AZA&Indole) leads to 40.6- to 88.9-fold increase of SMN levels, which is more than in case of five effective 5-combinations. It turns out that PTK-SMA1 is the most promising among the seven chemicals as it is present in all 35 effective combinations, while Indole is present in 23, AZA in 21, Dacinostat in 20, VPA in 19, TSA in 16, and Resviratrol in 14 effective combinations.

3.2.1. Comparison of deterministic, stochastic and fuzzy stochastic models

Figures 4–6 graphically compare the simulation results for deterministic, stochastic, and fuzzy stochastic models. The solid curves superimposing on the data for the fuzzy stochastic model were obtained by fitting interval (x, y) in the linear regime. For all n-combinations, both deterministic and stochastic simulations result in the same most effective drug combination while fuzzy stochastic case in general demonstrates different behavior. For instance, Indole&PTK-SMA1 is the most efficient 2-combination in deterministic and stochastic models, respectively resulting in 15.03- and 15.367-fold increase of SMN levels. However, it turns out that PTK-SMA1&Dacinostat is the most efficient in fuzzy stochastic case, leading to 18.2-fold increase of SMN concentration (see Figure 4a). Similarly, deterministic and stochastic models agree that Indole&PTK-SMA1&AZA is the most efficient 3-combination, respectively resulting in 39,523- and 39.111-fold increase of SMN levels, with 46-fold over the control group VPA&Indole&PTK-SMA1 is the most efficient in fuzzy stochastic case (see Figure 4b).

We performed statistical analysis on SPSS Statistics Software Package to characterize more accurately how much the deterministic, stochastic, and fuzzy stochastic models agree or differ. We conducted normality tests for all data sets. As a result, neither of these data sets is found to be normally distributed. Thus, we needed to apply a nonparametric statistical test to pairwise compare corresponding data sets picked up from distinct models. Appropriate tests are based on the following hypotheses:

$$H_0 : Median(x) = Median(y),$$
$$H_1 : Median(x) \neq Median(y),$$

where x and y are variables created for deterministic, stochastic, and fuzzy stochastic data sets such that $x \neq y$.

First, Friedman test is conducted to compare the data sets. For all seven drug combinations, Friedman test resulted in the rejection of the null hypotheses, H_0, with a $P < 0.001$, which indicates that there is a significant difference between the medians of data sets determined by the deterministic, stochastic, and fuzzy stochastic models. Then we performed a paired difference test called the Wilcoxon Signed Rank Test which compares two related data sets on a single sample to assess whether data sets have the same distribution. Pairwise comparison of data sets yielded a P-value of < 0.001, which leads us to reject the hypothesis H_0. Hence, we can conclude that there is an essential difference between distribution of related values in deterministic, stochastic, and fuzzy stochastic models. Moreover, statistical analysis reveals that values in stochastic case are significantly higher than corresponding values in deterministic case, and that values in fuzzy stochastic model are substantially higher compared to related values in stochastic model. Therefore, we conclude that fuzzy stochastic model

Table. Effective drug combinations determined by deterministic, stochastic, and fuzzy stochastic models.

Effective drug combinations	Model		
	Deter.	Stoch.	Fuzzy stoch.
PTKSMA1	**5.000**	**5.020**	**(4.300,7.100)**
PTKSMA1&Dacinostat	**12.716**	**13.278**	**(10.800,18.200)**
PTKSMA1&VPA	13.442	14.263	(13.100,17.500)
PTKSMA1&Indole	15.030	15.367	(14.600,17.000)
PTKSMA1&AZA	9.926	11.007	(11.700,12.100)
PTKSMA1&Indole+VPA	**35.101**	**36.522**	**(30.500,46.000)**
PTKSMA1&Indole&AZA	39.523	39.111	(24.000,41.600)
PTKSMA1&Indole&TSA	23.936	25.822	(16.100,32.800)
PTKSMA1&Dacinostat&AZA	23.977	26.689	(23.500,32.900)
PTKSMA1&Dacinostat&VPA	20.621	23.971	(23.300,32.400)
PTKSMA1&AZA&VPA	25.217	28.690	(27.300, 31.900)
PTKSMA1&Dacinostat&AZA&Indole	**84.907**	**64.852**	**(40.600,88.900)**
PTKSMA1&VPA&Indole&AZA	86.877	67.669	(45.800,87.400)
PTKSMA1&Dacinostat&TSA&Indole	40.452	43.963	(27.200,63.800)
PTKSMA1&Dacinostat&Resviratrol&Indole	36.586	39.217	(24.100,55.100)
PTKSMA1&VPA&Resviratrol&Indole	38.095	41.023	(26.400,53.600)
PTKSMA1&Dacinostat&TSA&AZA	29.515	35.135	(22.800,45.700)
PTKSMA1&VPA&AZA&TSA	30.682	36.712	(25.900,44.800)
PTKSMA1&Dacinostat&Indole&VPA&AZA	**104.399**	**86.020**	**(57.500,125.600)**
PTKSMA1&Dacinostat&Indole&AZA&TSA	93.782	75.767	(48.100,110.700)
PTKSMA1&Indole&VPA&AZA&TSA	95.532	77.820	(52.200,108.800)
PTKSMA1&Dacinostat&Indole&Resviratrol&AZA	39.016	39.016	(44.800,99.500)
PTKSMA1&Dacinostat&Indole&VPA&Resviratrol	52.068	56.240	(35.600,82.800)
PTKSMA1&Indole&AZA&Resviratrol&TSA	77.251	60.768	(37.800,79.100)
PTKSMA1&Dacinostat&Indole&TSA&Resviratrol	43.384	48.289	(31.000,70.800)
PTKSMA1&Indole+VPA&Resviratrol&TSA	44.773	49.285	31.700,69.600)
PTKSMA1&Dacinostat&VPA&TSA&AZA	41.874	50.427	(38.100,68.400)
PTKSMA1&Dacinostat&VPA&Resviratrol&AZA	39.150	46.747	(32.500,62.000)
PTKSMA1&Dacinostat&Indole&TSA&VPA&AZA	**111.086**	**94.647**	**(62.900,143.800)**
PTKSMA1&Dacinostat&Indole&Resviratrol&VPA&AZA	107.707	90.298	(60.000,133.900)
PTKSMA1&Dacinostat&Indole&Resviratrol&TSA&AZA	97.150	80.664	(51.100,119.400)
PTKSMA1&Indole&Resviratrol&VPA&TSA&AZA	98.842	82.862	(55.500,117.300)
PTKSMA1&Dacinostat&Indole&Resviratrol&VPA&TSA	57.601	63.841	(40.500,97.300)
PTKSMA1&Dacinostat&Resviratrol&VPA&TSA&AZA	43.876	53.701	(37.100,74.400)
7-combination	**113.799**	**98.142**	**(65.400,149.900)**

is the most adequate model for the case study as this model creates the closest approximation of underlying biological network.

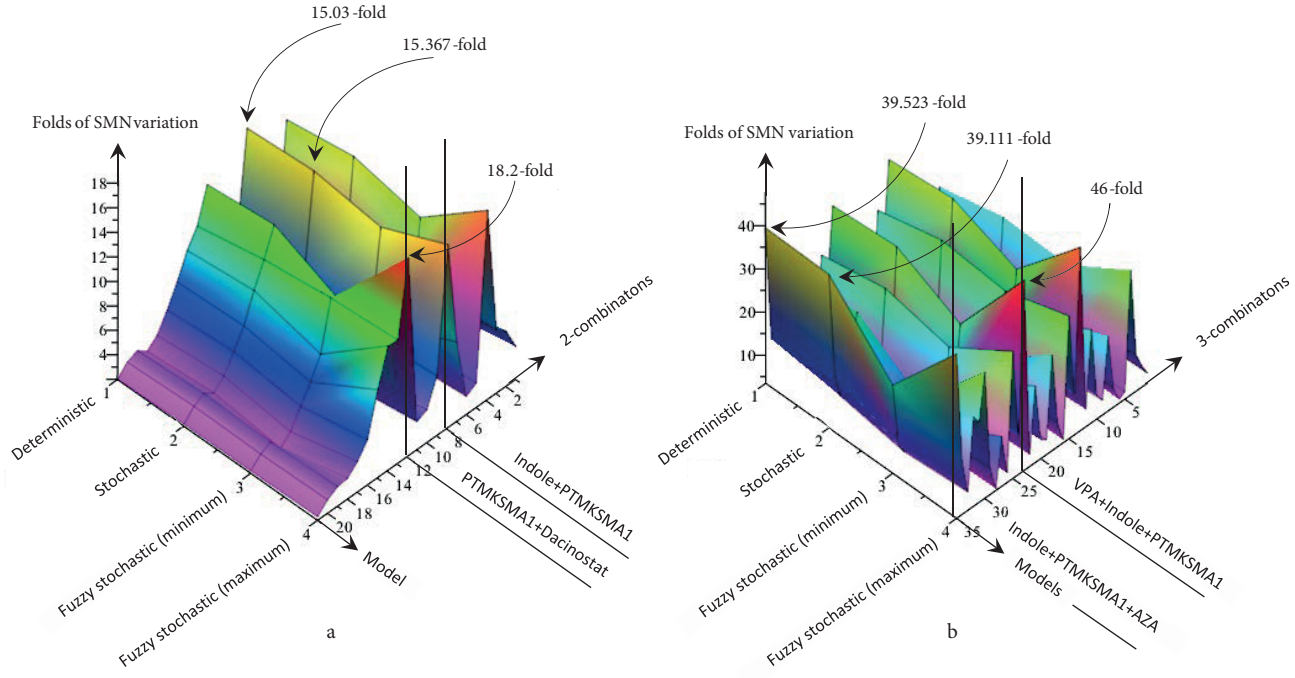

Figure 4. Graphical representation of simulation results for (a) 2-combinations and (b) 3-combinations.

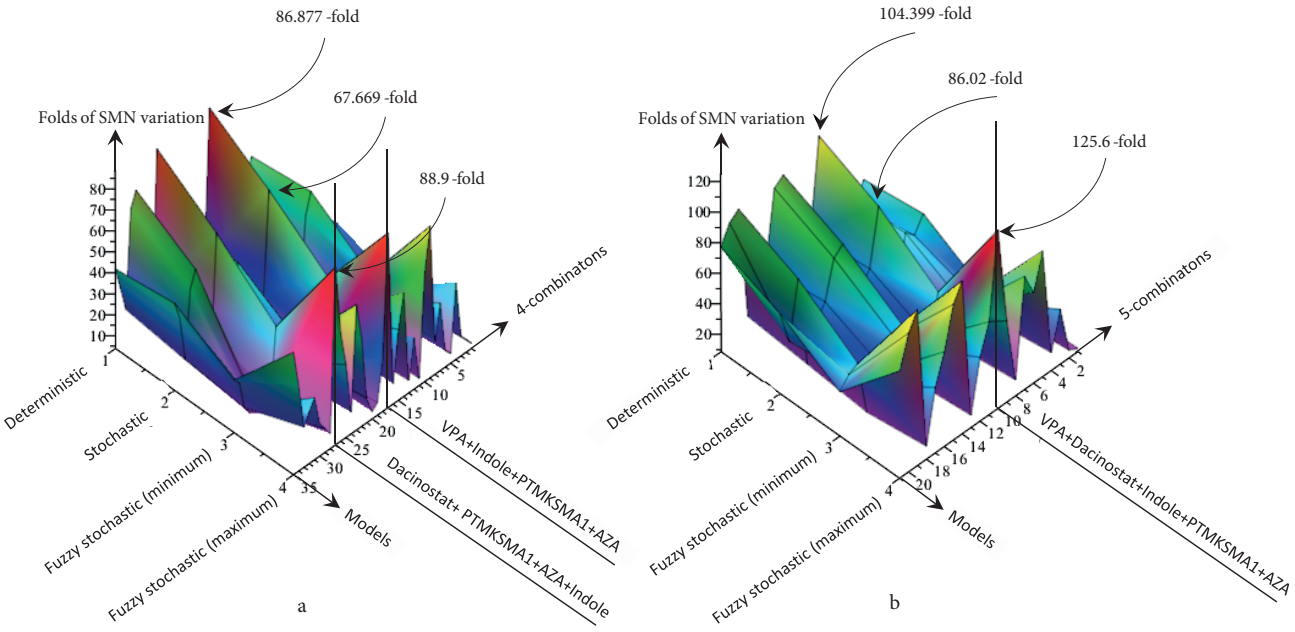

Figure 5. Graphical representation of simulation results for (a) 4-combinations and (b) 5-combinations.

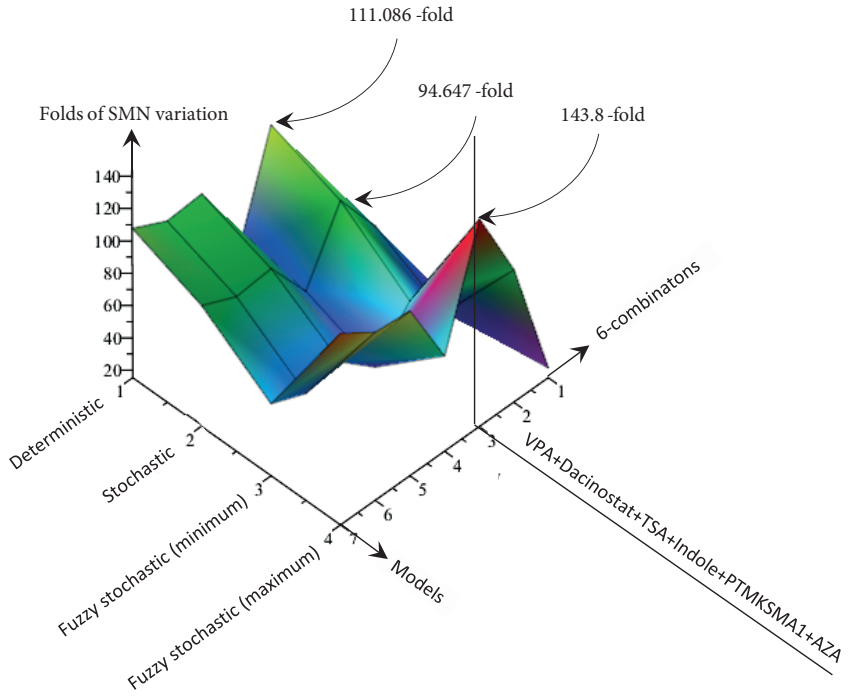

Figure 6. Graphical representation of simulation results for 6-combinations.

4. Discussions and further work

A question of practical interest may be the issue of determining precise fuzzy numbers to be assigned to kinetic parameters with uncertain or unknown experimental values. In [16], the authors adopted the following scheme for fuzzy parameter estimation: A fuzzy number is initially represented as a union of its α-cuts. The α-cut for each output is obtained by decomposing all fuzzy parameters into their α-cuts and then running stochastic simulations at each α level. Following this step, the membership function for each output is obtained by composing all the α-cuts. Unfortunately, there are some complications preventing the applicability of this approach in the present work. Firstly, this approach increases the number of simulation runs by the number of α levels. In the present study, we perform 127 simulation runs for the drugs and their combinations. Application of the above scheme for even 10 α levels would require 1270 simulation runs and any further decrease in the step size would result in substantial increase in the number of the simulation runs. Next, the approach suggests the step size of the α levels be determined carefully according to the nature of the problem, it is not quite clear, however, how to determine the step size based on the nature of the current case study.

We are aware that medications may have side effects and that, if a medication possesses side effects, its release, when not required, poses an extra burden on the metabolic system. A combination of multiple medications may even complicate the situation in the sense that it may cause unexpected side effects. As further work, in collaboration with pharmacogenetics groups, we propose the in vitro analysis of the current results to determine the practical applicability of the in silico models in established disease model tissues.

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Contribution of authors

The paper is a product of a tight collaboration and cooperation between all five authors. RB determined the topic, gave valuable insights, and managed the research project. Modelling and simulation in Snoopy have been conducted by RD during the work on his PhD thesis. The case study has been proposed by AS who was also in charge of the soundness of biological context. MM has performed fuzzification of the model. Comparison of deterministic, stochastic, and fuzzy models has been done by NA. The editorial work has been mainly done by RB, but all authors have contributed to the final composition of the paper.

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