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Study of the reactivity of the generated liver metabolites of a newly synthesized derivative of bexarotene and paracetamol

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The present work is focused to predict probable hepatic metabolites (*in vivo* and *in vitro* rat) and to study their reactivity mechanism (DNA and protein binding). The parent structure of the newly synthesized compound of bexarotene and paracetamol can bind to DNA but it cannot bind to protein and experimental metabolic pathways of action were not observed for rat *in vivo* and *in vitro*. The reactivity of predicted hepatic metabolites for both conditions (*in vivo* and *in vitro* rat) have different mechanisms of action (A_N^2, non-covalent interaction, non-specific, radical mechanism, S_N^1 and S_N^2) by DNA binding. The protein reactivity of the bexarotene derivative has the following mechanisms of action (Michael addition, nucleophilic addition, Schiff base formation, S_N^2).

Keywords: bexarotene derivative, predict, metabolic activation, hepatic, QSAR Toolbox

INTRODUCTION

The search for new drugs and approaches in the treatment of oncological and infectious diseases is a leading goal in medical and pharmaceutical practice around the world.

Retinoids, a group of small lipophilic molecules, are essential for a variety of biological processes. Retinoids regulate gene transcription by binding to the nuclear receptors, the retinoic acid (RA) receptors (RARs), and the retinoid X receptors (RXRs). RARs and RXRs are ligand-activated transcription factors for the regulation of RA responsive genes. The actions of RARs and RXRs on gene transcription require a highly coordinated interaction with a large number of coactivators and corepressors [1].

Bexarotene, a third-generation retinoid, exhibits its pharmacological effects through its interaction with retinoid X receptors – RXR [1]. RXRs are located primarily in visceral organs such as the liver and kidneys. Activated RXRs form homodimers or heterodimers with RAR (retinoic acid receptors), vitamin D receptors, thyroid receptors or peroxisome proliferator activator receptors (PPAR) [1]. The ability of RXRs to form heterodimers with different nuclear receptors indicates that the biological activity of bexarotene may be much more diverse than that of compounds that activate only RARs [1].

In vitro, bexarotene inhibits the growth of tumor cell lines. *In vivo*, bexarotene causes tumor regression in some animal models and prevents tumor induction in others [1].

At the heart of the toxic effects of bexarotene is its retinoid nature. Like other members of the retinoid group, it is characterized by extreme teratogenicity. Some of the more specific side effects of bexarotene therapy include central hypothyroidism, elevated cholesterol and triglyceride levels [1].

Increases in liver function tests associated with bexarotene use have been reported. Based on data from ongoing clinical trials, elevations in liver function tests suffered back development within one month in 80% of patients after dose reduction or discontinuation of treatment. Temporary or permanent discontinuation of bexarotene should be considered if the test results reach values three times higher than the upper limit for normal values of SGOT / AST, SGPT / ALT or for bilirubin [2]. Bexarotene is also contraindicated for patients with hepatic failure [2].

Hydrazones have been demonstrated to possess, among other, antimicrobial, anticonvulsant, analgesic, antiinflammatory, antiplatelet, antitubercular and antitumoral activities [3].

Paracetamol (acetaminophen) is one of the most widely used of all drugs, with a wealth of experience clearly establishing it as the standard antipyretic and analgesic for mild to moderate pain states [4]. Systematic use of high doses of paracetamol can lead to increased liver failure and is now the leading cause of acute liver failure and is the second most common cause of liver failure requiring transplantation [5]. During the 1960s and 1970s, increasing concern was raised about the

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toxicity of nonprescription analgesics, but in normal use paracetamol exhibited a consistent safety profile [4].

Hepatic metabolic stability is a key parameter in drug discovery because it can prevent a drug from attaining sufficient *in vivo* exposure, producing short half-lives, poor oral bioavailability and low plasma concentrations. It is essential to identify metabolic liabilities early in drug discovery so they can be addressed during lead optimization. Metabolic stability is typically first measured *in vitro* using liver microsomes and data from this assay are used to guide structural modifications to improve stability or select the best compounds for *in vivo* pharmacokinetic (PK) and efficacy testing. Liver microsomes are enriched with cytochrome P (CYP) 450 enzymes, localized in the endoplasmic reticulum membrane, which are responsible for the metabolism of the majority (70–80%) of clinically approved drugs [6, 7].

Hybrid molecules are new class drugs. The advantages of these molecules are better bioavailability at the target site, better effect with minimal therapeutic doses, lower toxicity and cheap preclinical evaluation. The OECD QSAR Toolbox is a software designed to support hazard assessment of chemicals, as well as to increase mechanistic and other knowledge on chemical substances in a cost-efficient way. It promotes the use of assessment methods alternative to animals and minimizes unnecessary animal testing without reducing the safety of human health and environment [8].

At a glance, computational tools reduce the use of animals in laboratory tests, reduce the cost for testing and increase the number of chemicals which are assessed for their effects upon human health and the environment. The toxicity of substances can be predicted even before they are produced, facilitating sustainable product development and green chemistry [8].

The aim of this work was to study the probable reactivity of the parent structure of the newly synthesized compound of bexarotene and paracetamol and their generated hepatic metabolites (for both conditions (rat *in vivo* and *in vitro*)) with respect to DNA and protein binding.

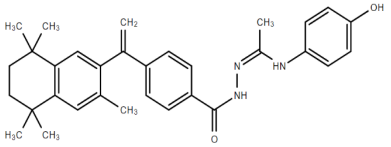
The newly synthesized hydrazone derivative was obtained according to the basic scheme of synthesis of bexarotene analogs and its structure was confirmed by its spectral data [9].

For the purpose of this study we synthesized a new hydrazone derivative of the retinoid bexarotene. The process consists of three major steps – esterification of the carboxylic group, hydrazinolysis and substitution of ketone to the newly formed hydrazone group. The result of the synthesis depends on the aldehyde or ketone used. For the purpose of this study we used paracetamol forming the new derivative of bexarotene shown in Table 1 [9]. Currently, there are no literature data of the mechanism of action of the newly synthesized derivative. The newly synthesized bexarotene derivative (N-[1-(4-hydroxyphenyl)amino ethilyden]-4-[1-(3,5,5,8,8-pentamethyl-6,7-dihydro naphtalen-2-yl)-ethenyl]phenylcarbohydrazide) is presented in Table 1 [9, 10].

Organisation for Economic Co-operation and Development (*OECD*) (*Q)SAR Toolbox (version 4.3)*. (Quantitative) structure-activity relationships [(Q)SARs] are methods for estimating properties of a chemical from its molecular structure and have the potential to provide information on the hazards of chemicals, while reducing time, monetary costs and animal testing currently needed. To facilitate practical application of (Q)SAR approaches in regulatory contexts by governments and industry and to improve their regulatory acceptance, the OECD (Q)SAR project has developed various outcomes such as the principles for the validation of (Q)SAR models, guidance documents as well as the QSAR Toolbox [11].

Observed rat in vivo metabolism. The observed (documented) metabolic pathways for 647 chemicals, extracted from the scientific literature, and associated with the *in vivo* biotransformations of xenobiotic chemicals in rodents (mostly rats) are stored in a database format that allows easy computer access to the metabolism information [11].

Table 1. Name and structural formula of the newly synthesized derivative.

Name of compound	Structural formula
N-[1-(4-hydroxyphenyl)aminoethilyden]-4-[1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphtalen-2-yl)-ethenyl]phenylcarbohydrazide	

MATERIAL AND METHODS

In vivo rat metabolism simulator. The current *in vivo* rat liver metabolic simulator (transformation table) represents electronically designed set of 671 structurally generalized, hierarchically arranged abiotic and enzymatic transformation reactions, which are characteristic for the metabolism for *in vivo* experimental systems such as rodent (mostly rat). The principal applicability of this simulator is associated with the reproduction, as well as the prediction of the metabolic activation reactions and pathways of xenobiotic chemicals, which may elicit *in vivo* genotoxicity effects [11].

Observed rat liver S9 metabolism. The documented metabolic pathways for 261 chemicals observed with the use of *in vitro* experimental systems such as rodent (mostly rat) liver microsomes and S9 fraction are stored in a database format that allows easy computer access to the metabolism information [11].

Rat liver S9 metabolism simulator. The current *in vitro* rat liver metabolic simulator (transformation table) represents electronically designed set of 551 structurally generalized, hierarchically arranged biotransformation reactions, which are characteristic for the metabolism for *in vitro* experimental systems such as rodent (mostly rat) liver microsomes and S9 fraction. The principal applicability of this simulator is associated with the reproduction, as well as the prediction of the metabolic activation reactions and pathways of xenobiotic chemicals, which may elicit *in vitro* genotoxicity effects such as bacterial mutagenicity and chromosomal aberrations [11].

DNA binding by OASIS. The profiler is based on Ames mutagenicity model part of OASIS TIMES system. The profiler consists of 85 structural alerts responsible for interaction with DNA analyzed in Ames mutagenicity model. The scope of the profiler is to investigate presence of alerts within target molecules which may interact with DNA [11].

Protein binding by OASIS. The scope of the profiler is to investigate the presence of alerts within target molecules responsible for interaction with proteins. The list of 112 structural alerts has been separated into 11 mechanistic domains. Each of the mechanistic domains has been separated into more than 2 mechanistic alerts. The profiling result outcome assigns a target to the corresponding structural alert, mechanistic alerts and domain [11].

RESULTS AND DISCUSSION

QSAR Toolbox software (version 4.3) was used for predicting possible metabolites of N-[1-(4-hydroxyphenyl)aminoethilyden]-4-[1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphtalen-2-yl)-ethenyl]phenylcarbohydrazide in the liver (*in vivo* and *in vitro* rat) and its DNA and protein binding. The parent structure of N-[1-(4-hydroxyphenyl)aminoethilyden]-4-[1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphtalen-2-yl)-ethenyl] phenylcarbohydrazide can bind to DNA with mechanism of actions (A_N^2 (nucleophilic addition reaction with cycloisomerization (hydrazine derivatives)), non-covalent interactions (DNA intercalation (DNA intercalators with carboxamide and aminoalkylamine side chain)), radical mechanism *via* ROS formation (hydrazine derivatives) and S_N^2 (direct nucleophilic attack on diazonium cation (hydrazine derivatives))) and cannot bind to protein. Experimental metabolic pathways of activation were not observed for rat *in vivo* and *in vitro*. In the liver metabolism simulator (*in vivo* rat), twenty-four metabolites were predicted. Results of hepatic prediction (*in vivo* rat) of N-[1-(4-hydroxyphenyl)aminoethilyden]-4-[1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphtalen-2-yl)-ethenyl]phenylcarbohydrazide are present in Table 2. The possible DNA binding by OASIS (mechanism of reaction) of the predicted hepatic metabolites for N-[1-(4-hydroxyphenyl)amino ethilyden]-4-[1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphtalen-2-yl)-ethenyl]phenylcarbo hydrazide was estimated by QSAR Toolbox software. Results of DNA binding of the predicted hepatic metabolites for N-[1-(4-hydroxyphenyl) aminoethilyden]-4-[1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphtalen-2-yl)-ethenyl]phenylcarbo hydrazide are presented in Table 3.

Twenty-four metabolites are reactive, i.e. alerts are found by DNA binding. Structural alerts (quinoneimine, thionine and phenoxazinium derivatives, hydrazine derivatives, DNA intercalators with carboxamide and aminoalkylamine side chain, specific imine and thione derivatives, epoxides and aziridines) were identified for twenty four metabolites in the mechanistic domains (radical mechanism, A_N^2, non-covalent interaction and S_N^2, S_N^1, non-specific) with mechanistic alerts (Michael-type addition, quinoid structures, nucleophilic addition reaction with cycloisomerization, DNA intercalation, incorporation into DNA/RNA, due to structural analogy with nucleoside bases, radical mechanism *via* ROS formation, ROS formation after GSH depletion, nucleophilic substitution on diazonium ion, direct nucleophilic attack on diazonium cation and alkylation, direct acting epoxides and related).

Table 2. Number and structure of the predicted hepatic metabolites (*in vivo*) of N-[1-(4-hydroxyphenyl) aminoethilyden]-4-[1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphtalen-2-yl)-ethenyl]phenylcarbohydrazide by QSAR Toolbox.

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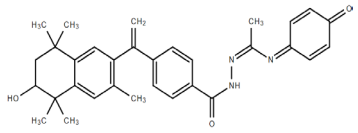	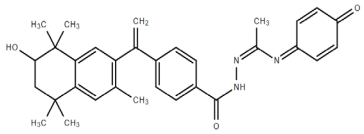	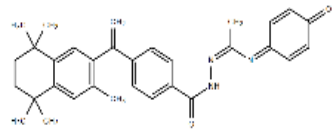
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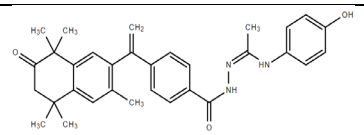	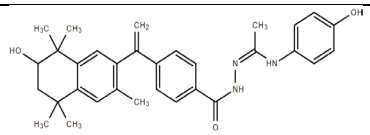	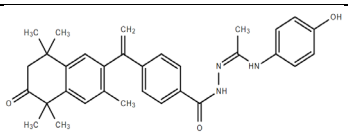
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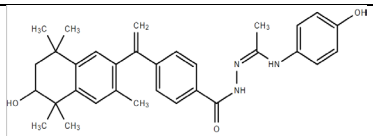	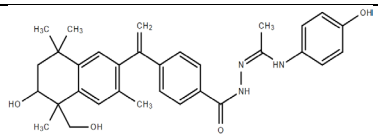	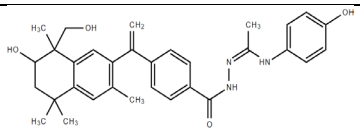
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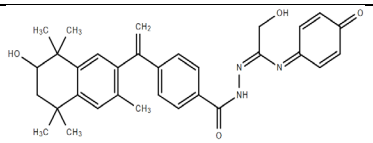	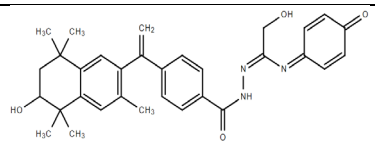	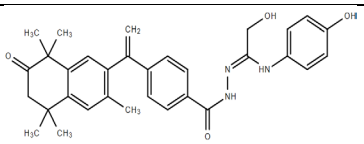
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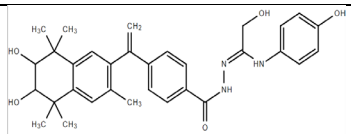	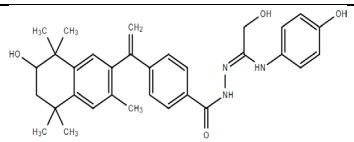	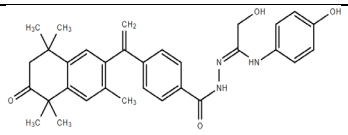
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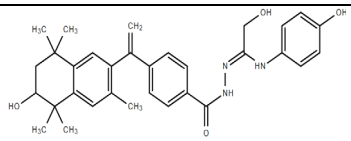	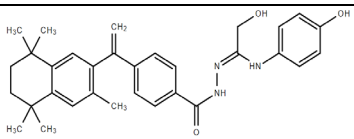	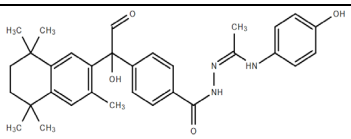
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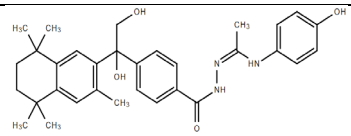	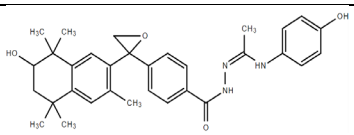	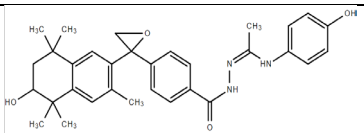
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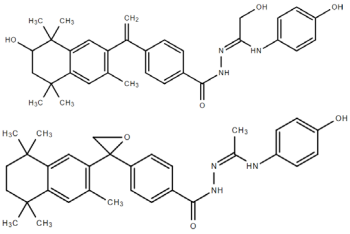
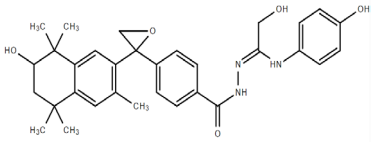
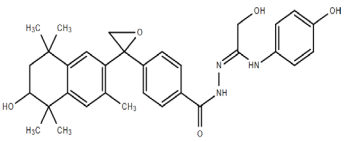
		

Table 3. DNA binding of hepatic metabolites for N-[1-(4-hydroxyphenyl) aminoethilyden]-4-[1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphtalen-2-yl)-ethenyl]phenylcarbohydrazide by QSAR Toolbox (liver *in vivo* metabolism simulator)

Number of metabolite	DNA binding by OASIS (Mechanism of reaction)		
	Structural alert	Mechanistic alert	Mechanistic domain
1-1-3,10,11 3,10,11	Quinoneimine, thionine and phenoxazinium derivatives	Michael-type addition, quinoid structures	A_N^2
1-24	Hydrazine derivatives	Nucleophilic addition reaction with cycloisomerization	A_N^2
1-24	DNA intercalators with carboxamide and aminoalkylamine side chain	DNA intercalation	Non-covalent interaction
1-3, 10,11	Quinoneimine, thionine and phenoxazinium derivatives	DNA intercalation	Non-covalent interaction
1-3, 10,11	Specific imine and thione derivatives	Incorporation into DNA/RNA, due to structural analogy with nucleoside bases	Non specific
1-24	Hydrazine derivatives	Radical mechanism via ROS formation	Radical
1-3, 10,11	Specific imine and thione derivatives	Radical mechanism via ROS formation	Radical
1-3, 10,11	Quinoneimine, thionine and phenoxazinium derivatives	ROS formation after GSH depletion	Radical
1-3, 10,11	Specific imine and thione derivatives	Nucleophilic substitution on diazonium ion	S_N^1
1-24	Hydrazine derivatives	Direct nucleophilic attack on diazonium cation	S_N^2
20-24	Epoxides and aziridines	Alkylation, direct acting epoxides and related	S_N^2

The results of protein binding of the predicted hepatic (liver *in vivo*) metabolites for N-[1-(4-hydroxyphenyl)aminoethilyden]-4-[1-(3,5,5,8,8-pentamethyl-6,7-dihydro naphtalen-2-yl)-ethenyl] phenylcarbohydrazide are presented in Table 4. Nine metabolites are not reactive and fifteen are reactive, i.e. alerts are found by protein binding. Structural alerts (quinone methide(s)/imines; quinoide oxime structure; nitroquinones, naphtaquinone(s)/imines, aldehydes, epoxides, aziridines and sulfuranes) were identified for fifteen metabolites in the mechanistic domains (Michael addition, Nucleophilic addition, Schiff base formation and S_N^2) with mechanistic alerts (Michael addition on quinoid type compounds, Addition to carbon-hetero double bond, Schiff base formation with carbonyl compounds and Ring opening S_N^2 reaction). The possible liver metabolites of N-[1-(4-hydroxyphenyl) aminoethilyden]-4-[1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphtalen-2-yl)-ethenyl]phenylcarbo hydrazide that have been predicted by QSAR Toolbox (*in vitro* rat metabolism simulator) are thirteen. Results of hepatic prediction (*in vitro* rat) of N-[1-(4-hydroxyphenyl)aminoethilyden]-4-[1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphtalen-2-yl)-ethenyl] phenylcarbohydrazide are presented in Table 5. Results of DNA binding of the predicted hepatic (liver *in vitro*) metabolites for N-[1-(4-hydroxyphenyl)aminoethilyden]-4-[1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphtalen-2-yl)-ethenyl] phenylcarbohydrazide are presented in Table 6.

Thirteen metabolites are reactive, i.e. alerts are found by DNA binding. Structural alerts (quinoneimine, thionine and phenoxazinium derivatives, hydrazine derivatives, DNA intercalators with carboxamide and aminoalkylamine side chain, specific imine and thione derivatives) were identified for thirteen metabolites in the mechanistic domains (radical mechanism, A_N^2, non-covalent interaction, S_N^2, nonspecific, S_N^1) with mechanistic alerts (Michael-type addition, quinoid structures, nucleophilic addition reaction with cycloisomerization, DNA intercalation, incorporation into DNA/RNA, due to structural analogy with nucleoside bases, radical mechanism via ROS formation, ROS formation after GSH depletion, nucleophilic substitution on diazonium ion and direct nucleophilic attack on diazonium cation).

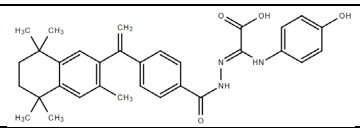
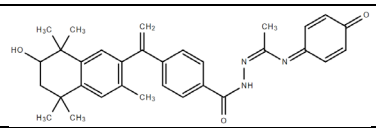
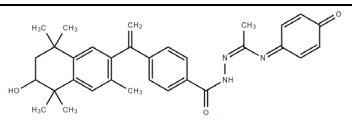
Prediction results of protein binding of the predicted hepatic (liver *in vitro*) metabolites for N-[1-(4-hydroxyphenyl)aminoethilyden]-4-[1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphtalen-2-yl)-ethenyl]phenylcarbohydrazide are presented in Table 7.

Seven metabolites are not reactive and six are reactive, i.e. alerts are found by protein binding. Structural alerts (quinone methide(s)/imines; quinoide oxime structure; nitroquinones, naphtaquinone(s)/imines, ketones and aldehydes) were identified for six metabolites in the mechanistic domains (Michael addition, Schiff base formation and nucleophilic addition) with mechanistic alerts (Michael addition on quinoid type compounds, addition to carbon-hetero double bond and Schiff base formation with carbonyl compounds).

Table 4. Protein binding of hepatic metabolites for N-[1-(4-hydroxyphenyl) aminoethilyden]-4-[1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphtalen-2-yl)-ethenyl] phenylcarbohydrazide by QSAR Toolbox (liver *in vivo* metabolism simulator)

Number of metabolite	Protein binding by OASIS (Mechanism of reaction)		
	Structural alert	Mechanistic alert	Mechanistic domain
5,7-9,13,14,16,17,19	No alert found	-	-
1-3,10,11	Quinone methide(s)/imines; Quinoide oxime structure; Nitroquinones, Naphtaquinone(s)/imines	Michael Addition on quinoid type compounds	Michael addition
4,6,12,15	Ketones	Addition to carbon-hetero double bond	Nucleophilic addition
18	Aldehydes	Schiff base formation with carbonyl compounds	Schiff base formation
20-24	Epoxides, Aziridines and Sulfuranes	Ring opening S_N2 reaction	S_N2

Table 5. Number and structure of the predicted hepatic metabolites (*in vitro*) of N-[1-(4-hydroxyphenyl)aminoethilyden]-4-[1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphtalen-2-yl)-ethenyl]phenylcarbohydrazide by QSAR Toolbox.

1	2	3
		
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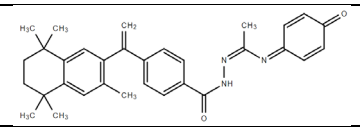	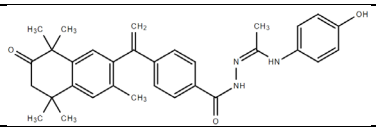	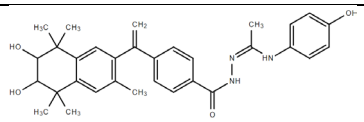
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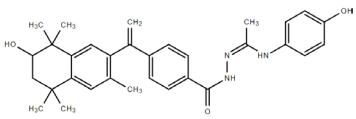
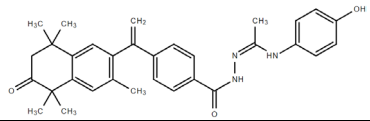
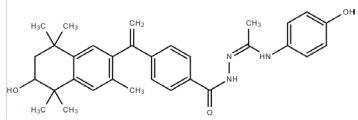
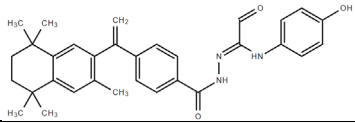
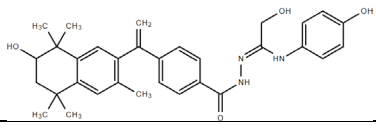
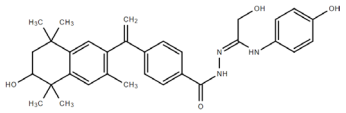
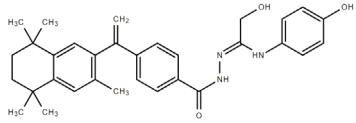
		
10	11	12
		
13		
		

Table 6. DNA binding of the hepatic metabolites for N-[1-(4-hydroxyphenyl) aminoethilyden]-4-[1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphtalen-2-yl)-ethenyl] phenylcarbohydrazide by QSAR Toolbox (liver *in vitro* metabolism simulator)

Number of metabolite	DNA binding by OASIS (Mechanism of reaction)		
	Structural alert	Mechanistic alert	Mechanistic domain
2-4	Quinoneimine, thionine and phenoxazinium derivatives	Michael-type addition, quinoid structures	A_N^2
1-13	Hydrazine derivatives	Nucleophilic addition reaction with cycloisomerization	A_N^2
1-13	DNA intercalators with carboxamide and aminoalkylamine side chain	DNA intercalation	Non-covalent interaction
2-4	Quinoneimine, thionine and phenoxazinium derivatives	DNA intercalation	Non-covalent interaction
2-4		Incorporation into DNA/RNA, due to structural analogy with nucleoside bases	Non specific
2-4	Specific imine and thione derivatives	Incorporation into DNA/RNA, due to structural analogy with nucleoside bases	Non specific
1-13	Hydrazine derivatives	Radical mechanism *via* ROS formation	Radical
2-4	Specific imine and thione derivatives	Radical mechanism *via* ROS formation	Radical
2-4	Quinoneimine, thionine and phenoxazinium derivatives	ROS formation after GSH depletion	Radical
2-4	Specific imine and thione derivatives	Nucleophilic substitution on diazonium ion	S_N^1
1-13	Hydrazine derivatives	Direct nucleophilic attack on diazonium cation	S_N^2

Table 7. Protein binding of hepatic metabolites for N-[1-(4-hydroxyphenyl) aminoethilyden]-4-[1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphtalen-2-yl)-ethenyl] phenylcarbohydrazide by QSAR Toolbox (liver *in vitro* metabolism simulator)

Number of metabolite	Protein binding by OASIS (Mechanism of reaction)		
	Structural alert	Mechanistic alert	Mechanistic domain
1,6,7,9,11-13	No alert found		
2-4	Quinone methide(s)/imines; Quinoide oxime structure; Nitroquinones, Naphtaquinone(s)/imines	Michael Addition on quinoid type compounds	Michael addition
5,8	Ketones	Addition to carbon-hetero double bond	Nucleophilic addition
10	Aldehydes	Schiff base formation with carbonyl compounds	Schiff base formation

CONCLUSIONS

The parent (basic) structure of the newly synthesized derivative of bexarotene and paracetamol after application of *in silico* methods (QSAR Toolbox software for metabolic activation in the liver of rats (*in vivo* and *in vitro*) to the OECD) was found to generate hepatic metabolites that exhibit different reactivity.

The metabolites were mainly formed through different types of mechanism – Michael type addition, nucleophilic addition, non-covalent interaction, radical mechanism and both types of nucleophilic substitution.

A total of twenty-four metabolites were predicted as positive.

The twenty-four predicted metabolites belong to diverse chemical classes, including quinoneimine, thionine, phenoxazinium derivatives, hydrazine

derivatives, DNA intercalators with carboxamide and aminoalkylamine side chain, specific imine and thione derivatives, epoxides and aziridines.

The probable active metabolites (hepatic) may be cytotoxic and enhance the potential antitumor effect of the newly synthesized compound.

It was also predicted that the parent structure of the newly synthesized derivative may be a substrate for different CYP450 enzymes.

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