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Influence of skin metabolites of the newly synthesized derivative of bexarotene and paracetamol on the potential antitumor effect

I. R. Iliev[2*], Y. K. Koleva[1], S. F. Georgieva[2]

[1]*University "Prof. Assen Zlatarov", Faculty of Natural Science, Department of Chemistry, Burgas, Bulgaria*
[2]*Medical university "Prof. Paraskev Stoyanov", Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Varna, Bulgaria*

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The present work is structured to predict probable skin metabolites, their DNA and protein binding of the newly synthesized compound of bexarotene and paracetamol. Predicted skin metabolites of the newly synthesized derivative of bexarotene and paracetamol are three in the following mechanistic domains - A_N^2, non-covalent interaction, non-specific, radical mechanism, S_N^1 and S_N^2 by DNA binding and two reactive metabolites in the mechanistic domain (Michael addition) by protein binding. Metabolites containing structural alerts with a potential toxic effect may complement the possible antitumor effect.

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

INTRODUCTION

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

In this regard retinoic acids play an important role in cell physiology. They are essential for embryonic development, regulating organogenesis, organ homeostasis and cell growth [1]. These compounds bind to and activate one or more nuclear retinoid receptors to modulate gene expression. There are two known classes of retinoid receptors, retinoic acid receptors (RARs) and retinoid X receptors (RXRs) [2].

In keeping with their ability to induce cell growth, differentiation and apoptosis, retinoic acids have been studied and used as anti-tumor or tumor preventive agents. Several retinoid acids are currently approved by the US Food and Drug Administration (FDA) for the treatment of certain malignancies, or are in clinical trials to assess their activities in various tumors [3, 4].

Bexarotene is a third generation retinoid. It is referred to as a rexinoid as it is the first KR-selective retinoid agonist to be studied in humans. It has been approved in the USA for the treatment of cutaneous T-cell lymphoma (CTCL) in patients who are refractory to at least one prior systemic therapy. There are also some data showing the potential use of bexarotene in combination with other currently available treatment modalities for CTCL. Finally, bexarotene has been assessed for potential use in solid tumors [2].

The use of some retinoids has been associated with photosensitivity. Patients should be advised to minimize exposure to sunlight and to avoid the use of tanning beds during treatment with bexarotene, as *in vitro* data suggest that bexarotene may have a potential photosensitizing effect [5].

The most common toxicity was dose-dependent skin toxicity, with the majority being mild skin dryness. Rarely skin dryness and peeling is dose limiting at 650 mg/m^2/day. Some patients report of cracking of the lips [2].

Hydrazones represent an important class of compounds for the development of new drugs. Therefore, many researchers synthesize these compounds as target structures and evaluate their biological activity. They have been demonstrated to possess, among other, antimicrobial, anticonvulsant, analgesic, antiinflammatory, antiplatelet, antitubercular and antitumoral activities, but from all significant effects the leading one is the antitumoral [6-9].

Hybrid molecules are defined as chemical entities with two or more structural domains having different biological functions and dual activity, indicating that a hybrid molecule acts as two distinct pharmacophores. Hybrid molecules can modify pharmacological effects of the parent structures. The advantages of these molecules are better bioavailability at the target site, better effect with minimal therapeutic doses, lower toxicity and cheap preclinical evaluation.

Paracetamol (acetaminophen) has become one of the most popular 'over-the-counter' non-narcotic analgesic. It is an effective mild analgesic, suitable

* To whom all correspondence should be sent:
E-mail: *i_iliev@abv.bg*

for treating mild to moderate pain although it does not appear to possess significant anti-inflammatory activity. Paracetamol's most troublesome side effect (methaemoglobinaemia) is due to another metabolite p-phenetidine. The mechanism of action of paracetamol is poorly defined, although it has been speculated that it may selectively inhibit prostaglandin production in the central nervous system, which would account for its analgesic/antipyretic properties. The lack of any significant influence on peripheral cyclooxygenase would explain the absence of anti-inflammatory activity [10].

There are no literature data of paracetamol being used as a topical treatment for any skin disease. There are no topical dosage forms.

The skin, like other organs in the human body, contains numerous enzymes that are capable of metabolizing endogenous substances and xenobiotics. In the past, the skin has been considered only as a physical barrier. Nowadays, it is well known that the skin also has the potential to metabolize substances with a different nature. Although the topical administration of drugs offers several advantages compared to traditional routes it is necessary to be well aware of the possibility of metabolism in the skin. The study of skin metabolism is of major importance not only in the field of transdermal drug delivery systems but also for the safe and efficient local skin treatment with topically applied substances [11].

Skin metabolism, in turn, can affect a number of processes, including skin toxicity, absorption, maintenance of homeostasis, delivery of dermal dosage forms, and efficacy. Due to the potentially wide-ranging effects that skin metabolism may affect, interest in it is increasing. This has led to the development of *in vitro* methods for predicting the potential of various substances to produce skin metabolites [11].

Recently, the possibility of the metabolism of medicinal products in the skin has been the subject of numerous studies. The barrier functions, absorption, and distribution of chemicals into and through the skin have been studied long ago. Now it is known that skin metabolism plays a key role in toxicity processes [11].

To evaluate the risk of drug use, many factors have to be considered. Of these, the absorption and permeability of the skin to different molecules, as well as the possibility of metabolic changes, are of particular importance. The risk of dermal metabolite production should be thoroughly monitored in order to assess the safety and minimize the potential for toxic reactions [11].

The Organization for Economic Co-operation and Development (OECD) (Q)SAR Application Toolbox makes it possible to predict metabolic changes and assess the risk based on the chemical structure of the compounds. In many cases, the parent chemical is not responsible for the development of an adverse reaction or toxicity. They are the result of their transformation and activation (metabolic or chemical). When a chemical changes as a result of metabolism, it may form biologically active metabolites. Experimentally identifying metabolism opportunities is difficult, expensive, and often incomplete. Therefore, the use of mathematical models to predict metabolism opportunities is increasing [12].

The present work is structured to predict probable skin metabolites, their DNA and protein binding of the newly synthesized compound of bexarotene and paracetamol.

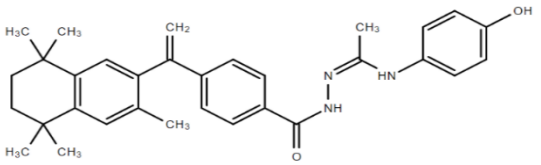
MATERIAL AND METHODS

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 [13,15].

For the purpose of this study we synthesized a new hydrazone derivative of the retinoid bexarotene. The process contains 3 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 [13].

Currently there's no literature data on the mechanism of action of the newly synthesized derivative. The compound (a newly synthesized bexarotene derivative (N-[1-(4-hydroxyphenyl) aminoethilyden]-4-[1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphtalen-2-yl)-ethenyl]phenylcarbo hydrazide)) is presented in Table 1 [13, 14].

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))	

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 [10].

Several profilers were used to predict hepatic and skin metabolic activation (observed and simulator) of the newly synthesized compound as well as DNA and protein binding:

Skin metabolism simulator. Skin metabolism simulator mimics the metabolism of chemicals in the skin compartment. Given the lack of reported skin metabolism data and the widespread hypotheses is that skin enzymes can metabolize absorbed xenobiotics *via* reactions analogous to those determined in liver, the simulator was developed as a simplified mammalian liver metabolism simulator. The skin metabolism simulator contains a list of hierarchically ordered principal transformations, which can be divided into two main types – rate-determining and non-rate-determining [10].

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 [10].

Protein binding by OASIS. The scope of the profiler is to investigate 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 [10].

RESULTS AND DISCUSSION

QSAR Toolbox software (version 4.3) has been 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 skin 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. After metabolic activation of the newly synthesized compound of bexarotene in the skin (skin metabolism simulator), three metabolites were predicted. Results of skin prediction of (N-[1-(4-hydroxyphenyl) amino-ethilyden]-4-[1-(3,5,5,8,8-pentamethyl-6,7-dihydro naphtalen-2-yl)-ethenyl]phenylcarbo hydrazide)) are presented in Table 2.

As shown on Table 2 the hydrazone functional group doesn't make the molecule act as a prodrug. According to the QSAR the possible metabolic pathways are oxidation of different functional groups. The possible DNA binding by OASIS (mechanism of reaction) of the skin metabolites for (N-[1-(4-hydroxyphenyl)aminoethilyden]-4-[1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphtalen-2-yl)-ethenyl]phenylcarbohydrazide)) are predicted by QSAR Toolbox software. Results of DNA binding of the predicted skin 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 3.

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

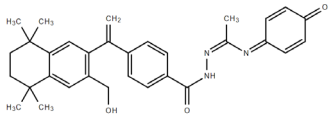
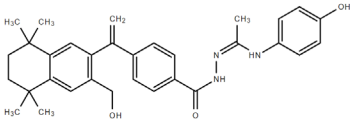
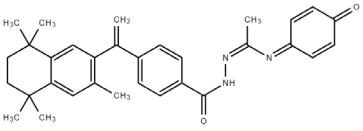
1	2	3
		

Table 3. DNA binding of skin metabolites for (N-[1-(4-hydroxyphenyl)aminoethilyden]-4-[1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphtalen-2-yl)-ethenyl]phenylcarbo hydrazide)) by QSAR Toolbox.

Number of metabolite	DNA binding by OASIS (Mechanism of reaction)		
	Structural alert	Mechanistic alert	Mechanistic domain
1,3	Quinoneimine, Thionine and Phenoxazinium derivatives	Michael-type addition, quinoid structures	A_N^2
1-3	Hydrazine derivatives	Nucleophilic addition reaction with cycloisomerization	A_N^2
1-3	DNA intercalators with Carboxamide and Aminoalkylamine side chain	DNA intercalation	Non-covalent interaction
1,3	Quinoneimine, Thionine and Phenoxazinium derivatives	DNA intercalation	Non-covalent interaction
1,3	-	Incorporation into DNA/RNA, due to structural analogy with nucleoside bases	Non-specific
1,3	Specific imine and thione derivatives	Incorporation into DNA/RNA, due to structural analogy with nucleoside bases	Non-specific
1-3	Specific imine and thione derivatives	Nucleophilic substitution on diazonium ion	S_N^2
1-3	Hydrazine derivatives	Radical mehanism via ROS formation (indirect)	Radical
1-3	Specific imine and thione derivatives	Radical mehanism via ROS formation (indirect)	Radical

Table 4. Protein binding of skin metabolites for (N-[1-(4-hydroxyphenyl)amino ethilyden]-4-[1-(3,5,5,8,8-pentamethyl-6,7-dihydronaphtalen-2-yl)-ethenyl]phenylcarbo hydrazide)) by QSAR Toolbox (skin metabolism simulator).

Number of metabolite	Protein binding by OASIS (Mechanism of reaction)		
	Structural alert	Mechanistic alert	Mechanistic domain
2	No alert found	-	-
1,3	Quinone methide(s)/imines; Quinoide oxime structure; Nitroquinones, Naphtaquinone(s)/imines	Michael addition on quinoid type compounds	Michael addition

Three 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 derivativess) were identified for three metabolites in the mechanistic domains (radical mechanism, A_N^2, non-covalent interaction and S_N^2, 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).

The results of protein binding of the predicted skin 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 4.

One metabolite is not reactive and two are reactive, i.e. alerts are found by protein binding. Structural alert (Quinone methide(s)/imines; Quinoide oxime structure; Nitroquinones, Naphtaquinone(s)/imines) was identified for two metabolites in the mechanistic domains (Michael addition) with mechanistic alerts (Michael Addition on quinoid type compounds).

The QSAR method is not yet validated with other softwares or through biological testing and the generated skin metabolites for the newly synthesized derivative have not yet been synthesized.

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 skin to the OECD) has been found to generate skin metabolites that exhibit different reactivity.

The possible adverse effects of skin active metabolites of the bexarotene derivative are with different mechanisms of action by protein and DNA binding.

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

A total of three metabolites were predicted as positive.

The three predicted metabolites belong to divers chemical classes, including quinoneimine, thionine, phenoxazinium derivatives, hydrazine derivatives, DNA intercalators with carboxamide and aminoalkylamine side chain, specific imine and thione derivatives, quinone methide(s)/imines, quinoide oxime structure, nitroquinones and naphtaquinone(s)/imines.

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

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