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MINISTRY OF HEALTH OF UKRAINE
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QUALIFICATION WORK

on the topic: «**PREDICTION OF POSSIBLE METABOLIC PATHWAYS OF POTENTIAL API WITH SEDATIVE AND NOOTROPIC EFFECTS**»

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ANNOTATION

The computer prediction of the possible pathways of metabolism of a potential API with sedative and nootropic effects, 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinoline-4-one, was performed. It was proved, that the molecule of the test substance can be intensively metabolized by cytochrome P450 enzyme systems. The most likely biotransformation pathways are aromatic hydroxylation involving carbon atoms of both the quinolone heterocyclic system and the phenyl substituent, O-demethylation of the methoxyl group, and N-dealkylation of the aminomethyl fragment. The predicted direction of aliphatic hydroxylation at the methyl group at position 2 of the heterocycle to kynurenic acid derivatives indicates that the proven pharmacodynamic effects may be partially provided by these pharmacologically active metabolites.

Key words: 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinolin-4-one, metabolism, biotransformation, computer prediction, online systems, pharmacological activity.

АНОТАЦІЯ

Проведено комп’ютерне прогнозування ймовірних шляхів метаболізму потенційного АФІ седативної та ноотропної дії 2-метил-3-[(2-метоксианіліно)метил]-1Н-хінолін-4-ону. Доведено, що молекула досліджуваної речовини може інтенсивно метаболізуватись за участю ферментних систем цитохрому Р450. Найбільш імовірними шляхами біотрансформації є ароматичне гідроксилювання за участю атомів карбону як гетероциклічної системи хінолону, так і фенільного замісника, О-деметилювання метоксильної групи, N-деалкілування амінометильного фрагменту. Прогнозований напрямок аліфатичного гідроксилювання за метильною групою в положенні 2 гетероциклу до похідних кінуренової кислоти свідчить, що доведені фармакодинамічні ефекти можуть частково забезпечуватись саме цими фармакологічно активними метаболітами.

Ключові слова: 2-метил-3-[(2-метоксианіліно)метил]-1Н-хінолін-4-он, метаболізм, біотрасформація, комп’ютерне прогнозування, онлайн системи, фармакологічна активність.

CONTENTS

LIST OF ABBREVIATIONS

AA	Anti-amnestic activity
ADME/Tox	Parameters of absorption, distribution, metabolism, excretion and toxicity
API	Active pharmaceutical ingredient
AUC	Area under the concentration-time curve in blood plasma
CYP	Cytochrome P450 enzymes
EPM	Elevated plus maze
FDA	Food and Drug Administration, USA
IC_{50}	Average inhibitory concentration
in silico	Research methods using mathematical calculation methods
in vitro	Research methods using cell cultures
in vivo	Methods of study in a living organism
Ki	Inhibition constant
Km	Michaelis constant
LOO	Cross-validation without extraction
NHHH	Normobaric hypoxic hypoxia with hypercapnia
OFT	Open field test
PAT	Passive avoidance test
QSAR	Structure-activity relationship models
SOM	Metabolism site
UGT	Uridine diphosphate-glucuronosyltransferase

INTRODUCTION

Relevance of the topic. The qualification work is devoted to the study of possible metabolic pathways of 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinolin-4-one as a promising candidate for APIs with sedative and nootropic properties. *In vitro* and *in silico* drug metabolism models are regularly used in drug research and development as tools for assessing pharmacokinetic variability and the risk of drug interaction. The use of *in vitro* and *in silico* predictive approaches has such advantages as rational design of clinical drug interaction studies, minimization of human risk in clinical trials, and cost and time savings due to less exhaustion in the compound development process. That is why the use of computer prediction of possible metabolic pathways of a potential drug candidate at the initial stages is a fully justified and effective approach that allows to identify metabolic sites, predict the structures of the metabolites formed, the intensity of metabolism and the specificity of substrates to cytochrome P450 enzymes. The chosen topic of the qualification work is aimed at solving these issues, which determines its relevance.

Purpose of the study. Prediction of probable metabolic pathways of 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinoline-4-one as a promising candidate for APIs with sedative and nootropic properties.

To achieve the goal, the following **tasks** were set:

1. To systematize and analyze the scientific literature on the main mathematical and statistical approaches and methods used to predict possible pathways of chemical metabolism in the human body.

2. To perform a computer prediction of possible pathways of biotransformation of a promising compound – 2-methyl-3-[(2-methoxy-anilino)methyl]-1H-quinolin-4-one (laboratory code VAZ_07) using five different online resources that are freely available.

3. Based on the systematization of the obtained results, identify the main possible pathways of biotransformation of 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinolin-4-one. Summarize the data obtained by *in silico* methods and identify a potential range of metabolites for further *in vitro* and *in vivo* studies.

4. Based on the analysis of coincidences and discrepancies in the results obtained using different software products, determine the correlation of the main

trends in the directions of biotransformation.

Object of the study. A promising API with sedative and nootropic action 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinoline-4-one.

Subject of the study. Probable metabolic pathways of 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinoline-4-one in the human body.

Methods of the study:

1. Analysis and systematization of scientific and patent literature.
2. *In silico* prediction of possible pathways of xenobiotics biotransformation in the human body.
3. Methods of extrapolation and visualization of the results of prediction of possible metabolites.

The practical value of the results. The results of the study expand the knowledge of possible metabolic pathways of 2-methyl-3-[(2-methoxy-anilino)methyl]-1H-quinolin-4-one, a substance that is a promising API with sedative and nootropic effects. The results obtained can significantly expand and deepen the understanding of both pharmacodynamic and pharmacokinetic features of the promising API candidate, subject to further in-depth pharmacological research and introduction of the compound into medical practice.

Elements of scientific research. For the first time, a computer prediction of possible pathways of biotransformation of 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinolin-4-one as a promising candidate for APIs with sedative and nootropic properties.

Structure and scope of the qualification work. The qualification work consists of an introduction, 3 chapters, general conclusions, and a list of references (80 items). The total volume of the work is 51 pages. The work contains 1 scheme, 3 tables and 16 figures.

CHAPTER 1. MODERN APPROACHES TO COMPUTER PREDICTION OF MEDICINAL SUBSTANCE METABOLISM IN THE HUMAN BODY (Literature review)

1.1 Role of computational metabolism prediction methods in drug development

As our understanding of the metabolic reactions that determine the fate of drugs has recently deepened significantly, drug metabolism has attracted increasing attention as a critical factor in drug discovery [1, 2]. The fate of substances such as drugs and xenobiotics introduced into our bodies is largely governed by three phases of drug metabolism: phase I, the introduction of a reactive group by oxidation, reduction, or hydrolysis, among others; phase II, conjugation with various fragments; and phase III, the elimination of xenobiotics and metabolites from liver and intestinal cells. These transformation processes can turn compounds into inactive, active, or toxic metabolites. Not surprisingly, since it is responsible for the clearance of ~70% of clinical drugs, metabolism is intensively studied as part of drug development efforts [3].

Natural compounds have recently attracted considerable research attention due to their inherent advantages and high potential as drug candidates [4]. Moreover, the structural similarity of some natural compounds to metabolites found in the human body makes metabolism a critical factor in determining the efficacy of natural medicines [5]. For example, historical opioid drug candidates are metabolized into more potent metabolites, such as (dihydro)codeine, which in turn is metabolized into (dihydro)morphine [6]. Given the large number of endogenous enzymatic reactions that influence drug modification through (de)activation and (de)toxification, determining how a drug is metabolized is an important step in drug discovery.

In recent decades, numerous experimental technologies have been used to study drug metabolism and fate [7, 8]. The traditional method of drug discovery - target-to-target, target-to-ligand, and ligand optimization – is expensive, costing

more than $200 million for an average drug, and time-consuming, with a typical discovery period of 4-5 years [9]. In addition, due to the inability to accurately reproduce biological environments *in vivo*, such methods are relatively imprecise and are still considered low throughput, given the scale of combinatorial structural variations in chemical compounds.

As part of drug discovery efforts, numerous advances have been made in predicting drug metabolism using *in silico* approaches, and various aspects of these advances have been reviewed [10-14]. These include tools for predicting drug metabolism based on the interaction of drugs with cytochrome P450 (CYP450) enzymes and their metabolic endpoints [12, 14], tools for predicting ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties of drugs and their associated solubility permeability, and bioavailability [10], as well as approaches to predicting the inductance of drug-metabolizing enzymes and transporters that affect the concentration of drugs in blood plasma, which can cause undesirable or prolonged effects or side effects [13].

Because of these observations, *in silico* approaches are increasingly being used to predict the metabolic transformation of drugs [15] and as such are considered the best strategy to "fail early and fail cheap", which reduces costs, saves time, and thus reduces churn rates in the later stages of drug discovery.

1.2 *In silico* approaches to predicting molecular biotransformation

1.2.1 Prediction based on quantitative structure-activity relationships and machine learning approaches

The concept of quantitative structure-activity relationship (QSAR), developed in the early 1960s by Hansch/Fujita [16] and Free/Wilson [17] and widely used in drug discovery, suggests that molecules with similar structures potentially exhibit similar chemical and biological activities [18]. The initial concept of the structure-activity relationship dates back to 1868, when Cram-Brown and Fraser introduced the idea of correlating the chemical composition of a compound with its

physiological properties in biological systems [19]. QSAR-based models are widely used at the optimization stage of drug development to assess various drug properties (including toxicity) and, as a result, reduce the number of promising lead compounds identified through screening, which ultimately minimizes time, costs and labor. The European Commission's REACH (Registration, Evaluation and Authorization of Chemicals) regulation [20] allows the use of various approaches, such as QSAR, provided that the results are proven to be highly reliable [21].

The QSAR approach uses experimental datasets that include the biological activity of chemical compounds, their chemical and physical characteristics represented as molecular descriptors [22], and statistical methods to correlate these molecular descriptors with biological activity [23] (Fig. 1.1).

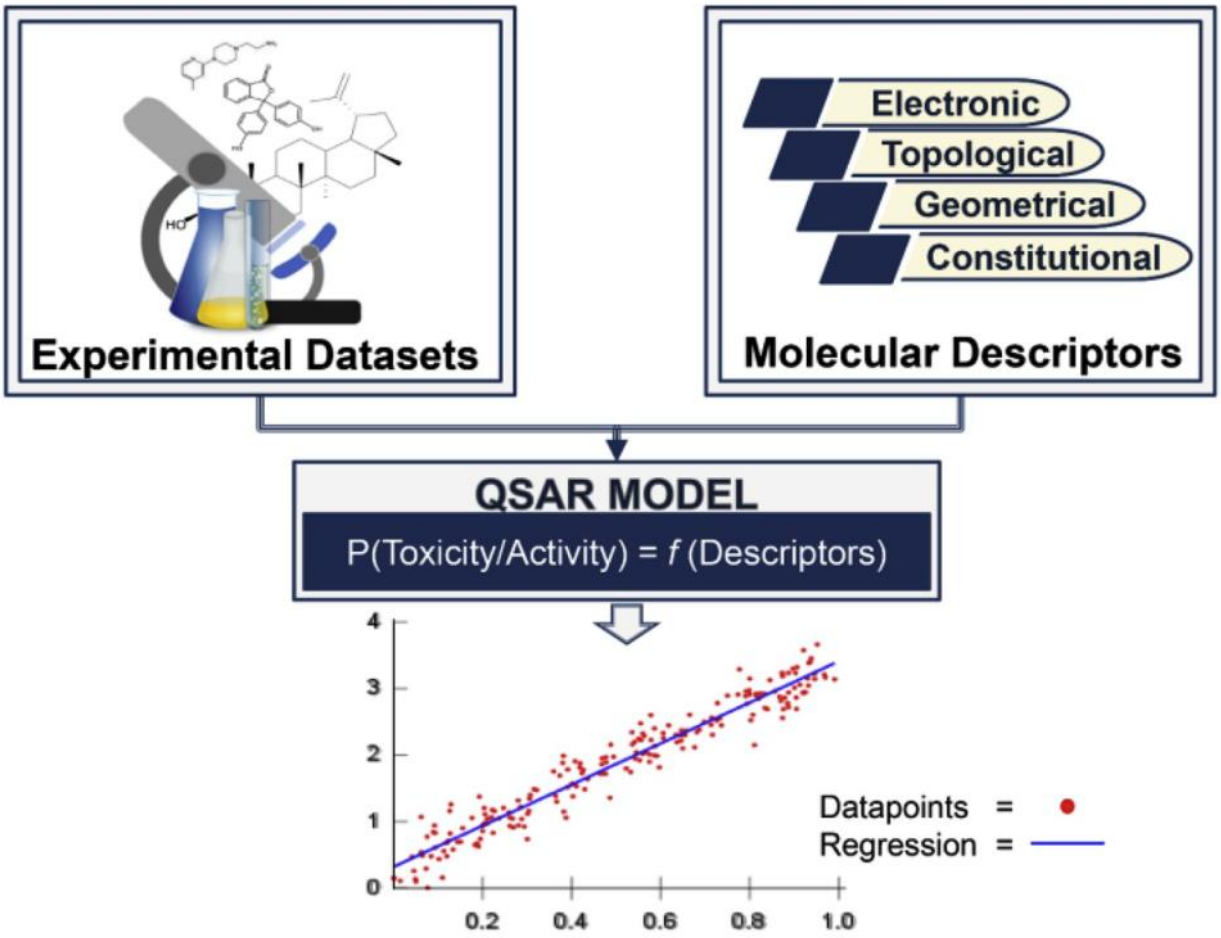


Figure 1.1 QSAR approach to *in silico* prediction

Table 1.1

List of the main online systems for calculating molecular descriptors

Software	Operating System	No. of available descriptors	License
ADAPT[a]	Unix/Linux	265	Free
ADMET Predictor[b] (PCB module)	Windows	297	Commercial
ChemAxon[c] (Calculator plugins)	Windows/Unix/Linux	> 500	Commercial
Codessa[d]	Windows	> 1400	Commercial
Corina Symphony[e]	Windows	786	Commercial
DRAGON 7.0[f]	Windows/Unix/Linux	5270	Commercial
E-Dragon[g]	Web service	1666	Free
MOE[h]	Windows/Unix/Linux	> 400	Commercial
Molconn-Z[i]	Windows/Unix/Linux	> 1000	Commercial
MOLGEN QSPR[j]	Windows	708	Commercial
PaDEL-descriptor (CDK)[k]	Java JRE	1875	Free
PowerMV[l]	Windows	122	Partially Free[m]
PreADMET[n]	Windows	955	Commercial
Open Babel[o]	Windows/Linux	> 20	Free
QikProp[p]	Windows/Linux	> 20	Commercial
ACD Labs/Percepta[q]	Web service & modules	> 40	Free
MOPAC[r]	Windows/Linux	24	Free
EPI Suite[s]	Windows	13	Free

[a] http://research.chem.psu.edu/pcjgroup/adapt.html
[b] http://www.simulations-plus.com/software/admet-property-prediction-qsar/
[c] https://www.chemaxon.com/products/
[d] http://www.codessa-pro.com/
[e] https://www.mn-am.com/products/corinasymphony
[f] https://chm.kode-solutions.net/products_dragon.php
[g] http://www.vcclab.org/lab/edragon/
[h] http://www.chemcomp.com/MOE-Cheminformatics_and_QSAR.htm
[i] http://www.edusoft-lc.com/molconn/
[j] http://molgen.de/download.html
[k] http://www.yapcwsoft.com/dd/padeldescriptor/
[l] https://www.niss.org/research/software/powermv
[m] Commercial affiliates available
[n] https://preadmet.bmdrc.kr/
[o] http://openbabel.org
[p] https://www.schrodinger.com/qikprop
[q] https://www.acdlabs.com/products/percepta/
[r] http://openmopac.net/
[s] https://www.epa.gov/tsca-screening-tools/download-epi-suitetm-estimation-program-interface-v411

Molecular descriptors are arithmetic values that reflect the physicochemical properties of compounds and can be classified as 1D, 2D, or 3D descriptors, depending on the amount/type of information provided. The most common types of descriptors used in QSAR are constitutional, electronic, topological, and geometric descriptors, which include molecular weight, total number of atoms, total number of carbon atoms, atomic lattice, total number of bonds, and Van der Waals area, among others. A wide range of software and web-based tools are available to calculate molecular descriptors, as shown in Table 1.1; there are also various QSAR systems with their own integrated descriptor generators, including CASE Ultra (http://www.multicase.com/case-ultra) and Leadscope (http://www.leadscope.com/).

Typically, QSARs that predict the metabolic transformation of endogenous or exogenous compounds are built for hepatic enzymes of the CYP450 family (which metabolize most drugs into toxic chemical compounds [24]) and are known for their reliability in predicting toxicity; as such, they provide valuable information for large-scale virtual drug efficacy screening.

Table 1.2 lists common QSAR-based models built to predict drug metabolism reactions. Several of the other models listed in the table (e.g., IDsite, SMARTcyp) can also predict the site at which a metabolic transformation occurs in a chemical compound. In addition, the CQSAR database, created in 2003 and available to users [25], contains more than 18,000 QSAR equations and associated biophysical data.

The QSAR Data Bank, another repository that archives *in silico* descriptive and predictive models such as QSARs, allows the research community to share and present their QSAR data [26]. QSARs have been used since the early era of drug discovery, but their application has been limited to small linear datasets. However, advanced methods based on direct scoring and/or machine learning algorithms that can model complex nonlinear data sets have been applied recently [27].

Table 1.2

List of the main online systems for predicting drug metabolism

Software	Operating system	Target	License
ADME WORKS Predictor[a]	Windows/Linux	CYP450 isoforms[b]	Commercial
ADMET Predictor[c] (Metabolism module)	Windows	CYP450 isoforms[d]	Commercial
admetSAR[e]	Web service	CYP450 isoforms[f] and P-glycoprotein	Free
PreADMET[g]	Web service Windows (PC version)	CYP450 isoforms[h] and P-glycoprotein	Free (Web service) Commercial (PC version)
SMARTCyp[i]	Web service	CYP450 isoforms[j]	Free
SOMP (Way2Drug)[k]	Web service	CYP450 isoforms[l] and UDP-glucuronosyltransferase	Free
MetaSite[m]	Windows/Linux	CYP450, FMO3, and AOX1	Commercial
RS-WebPredictor[n]	Web service	CYP450 isoforms[o]	Free
Meteor Nexus[p]	Windows	User query structure	Commercial
ACD Labs/Percepta[q]	Windows/Linux	User 2D structure/SMILE	Commercial
MetabolExpert[r]	Windows/Linux	User 2D structure/SMILE	Commercial
Meta-PC[s]	Unix/Linux	Query chemical structure	Commercial
syGMa[t]	Windows/Linux	Query chemical structure	Free (for academic institutions)
TIMES[u]	Unix/Linux	User query structure	Commercial
MetaPath (OASIS)[v]	Unix/Linux	User query structure	Commercial
IDSite[w]	–	CYP isoform 2D6	–
Metabolizer (ChemAxon)[x]	Windows/Linux	User query structure	Commercial

a http://www.fqs.pl/en/chemistry/products/admeworks-predictor
b 2D and 3A4
c http://www.simulations-plus.com/software/admet-property-prediction-qsar/metabolism/
d 1A2, 2A6, 2B6, 2C8, 2C19, 2C9, 2D6, 2E1, and 3A4
e http://lmmd.ecust.edu.cn:8000/
f 1A1, 1A2, 2A5, 2C9, etc.
g https://preadmet.bmdrc.kr/
h 2C9, 2C19, 2D6, and 3A4
i https://smartcyp.sund.ku.dk/
j 3A4 isoform
k http://www.way2drug.com/SOMP/
l 1A2, 2C9, 2C19, 2D6 and 3A4
m http://www.moldiscovery.com/software/metasite/
n http://reccr.chem.rpi.edu/Software/RS-WebPredictor/
o 2C9, 2D6, 3A4, 1A2, 2A6, 2B6, 2C8, 2C19 and 2E1
p https://www.lhasalimited.org/products/meteor-nexus.htm
q https://www.acdlabs.com/products/percepta/
r http://www.compudrug.com/metabolexpert
s http://www.multicase.com/meta-pc
t https://sygma.readthedocs.io
u http://oasis-lmc.org/products/software/times.aspx
v http://oasis-lmc.org/products/software/metapath.aspx
w https://pubs.acs.org/doi/abs/10.1021/ct200462q
x https://docs.chemaxon.com/display/docs/Metabolizer
y https://portal.genego.com/

The rapid progress in the development of new machine learning methods in computer science has inspired the development of thousands of QSAR models for accurate drug metabolism prediction based on methods other than linear and multiple linear regression [20, 28]. Machine learning, defined as a computational method that is trained on a set of test data to build a model for classifying unknown data [29], was primarily used to develop QSAR models [30]. The application of machine learning approaches in modern drug discovery has accelerated the process of scanning and screening out ineffective compounds, achieving a significant reduction in time and cost compared to experimental screening methods [31]. Machine learning is better suited for extracting non-parametric and nonlinear relationships from data sets, which allows for the development of *in silico* models with better predictive performance [32].

Several machine learning methods (e.g., neural network, decision tree, support vector machine, k-nearest neighbor) have been successfully used to build more accurate QSAR models [33], which take a set of descriptors from a large data set as input and create a classification model that predicts the biological activity of the requested compound as output.

Currently, machine learning is widely used in the field of computer-assisted drug discovery, which allows predicting the interaction between a ligand and a target protein, and thus facilitates the development of new drugs [34]. It also aims to predict the ADMET properties of drugs, which ultimately facilitates the development of safe and promising agents [35]. Drug metabolism is divided into several phases, each of which has numerous enzymes that play a role in drug metabolism, so a large number of machine-learning models have been built to classify drug fate based on whether the drug will be metabolized by certain enzymes or not [35].

Recently, increased attention has been paid to predicting drug toxicity using other machine learning methods, such as neural networks and deep learning [36], which involve the use of powerful multilayer interconnected neural networks consisting of processing units, referred to as nodes [37]. Examples of architectures used to predict biological activity include convolutional, autoencoder, and recurrent

neural networks [38]. The rapid increase in pharmaceutical data and computing power has inspired the application of neural networks and deep learning in a variety of fields, including bioinformatics [39], chemical informatics [40], structure prediction [41], and drug discovery [42].

The emergence of computer-based prediction is an important turning point in the history of drug discovery, as a number of machine learning-based models are now available for predicting drug toxicity [43]. However, their application is still limited by such drawbacks as the tendency to overfit data and the difficulty in choosing the appropriate algorithm and descriptors for the problem from among the available ones [44]. An overfitted model occurs when the model is too complex or the number of features/descriptors is too large compared to the size of the data set. These problems result in a biased model that performs well on the training dataset used to build the model but is unable to accurately predict using external datasets [45].

1.2.2 Structural computing approaches

Identification of the structural properties of a protein provides insight into its biological activity and allows the development of effective ligands for its binding. Often, metabolic reactions occur at the site where the ligand binds to the target protein. This site tells us a lot about the metabolic fate of the drug, and thus whether the drug will be therapeutically active, inactive, or toxic. It also often provides information that helps to optimize lead compounds.

To date, structural approaches are among the most successful and recognized methodologies used in various fields of pharmaceutical research for drug development [46]. These modern methods include techniques such as computational docking and molecular dynamics, which are intensively used to study drug metabolism by identifying the metabolic site [47] and molecular interactions, information that contributes significantly to the drug discovery process [48].

The docking method considers the interaction between a small molecule and an active site on a target protein and predicts the affinity of their binding interactions

based on their docking orientation and the forces interacting between them. Protein-ligand interactions are modeled using powerful computational tools, such as docking algorithms [49] implemented in AutoDock Vina, GOLD, and DOCK software packages, which predict the most favorable response. The construction of these models is based on the assumption that structural information is closely related to the metabolic fate of the drug [50]. The docking approach is widely used for the rapid identification of promising lead compounds from large compound libraries. Ligand-protein interactions often require structural changes to achieve better interaction, and can be modeled using molecular dynamics simulations. Thus, molecular dynamics simulations are often used in conjunction with docking algorithms to further refine docking complexes by taking into account other parameters such as solvent effects, which allows for more accurate drug candidates; they are also used to predict the site of metabolism. Table 1.3 summarizes some of the most common tools used to model protein-ligand docking.

Despite their many advantages, structural approaches require high computational power to model structural flexibility. The processes of calculating the binding energy and estimating the docking conformation require different methods that can take from several seconds to several days, making these calculations computationally expensive. In addition, the target protein and its ligand may undergo structural changes to adapt their structures to the appropriate conformational state [51]; thus, obtaining an accurate model replica is still a challenging task. However, additional methods have been used to improve the accuracy of modeling, such as the use of rotamer libraries [52] or soft docking simulations [53].

Rotamer libraries are used to predict the most suitable side-chain conformations and remove unfavorable conformations, leading to the selection of low-energy side-chain conformations and thus increasing modeling accuracy and reducing modeling time. Soft docking can be performed using soft scoring functions to make minor changes to the conformation of protein receptors, an approach that is known to be computationally efficient [54].

Table 1.3

List of basic tools for modeling protein-ligand docking

Program	Operating system	License
AutoDock Vina[a]	Windows/Linux/Unix	Free Open source
BetaDock[c]	Linux	Free
BSP-SLIM[d]	Web service	Free
DOCK 6.8[e]	Windows/Linux/Unix	Free Open source
Docking Server[f]	Web service	Partially Free[g]
FlexAID[h]	Windows/Linux/Unix	Free Open source
Glide[j]	Windows/Linux/Unix	Commercial
GOLD Suite[k]	Windows/Linux	Commercial
idTarget[l]	Web service	Free
MOE[m]	Windows/Unix/Linux	Commercial
MOLS 2.0[n]	Java	Free Open source
ParDock[o]	Web service	Free
rDock[p]	Linux	Free Open source
SwissDock[q]	Web service	Free
Virtual ToxLab[r]	Windows/Unix/Linux	Free (for academic institutions)

a http://vina.scripps.edu/
b https://www.worldcommunitygrid.org/
c http://voronoi.hanyang.ac.kr/software.htm
d http://zhanglab.ccmb.med.umich.edu/BSP-SLIM/
e http://dock.compbio.ucsf.edu/DOCK_6/index.htm
f http://www.dockingserver.com/web
g Commercial premium licenses
h http://biophys.umontreal.ca/nrg/NRG/FlexAID.html
i http://sw16.im.med.umich.edu/databases/pdbbind/ index.jsp
j https://www.schrodinger.com/glide
k https://www.ccdc.cam.ac.uk/solutions/csd-discovery/components/gold/
l http://idtarget.rcas.sinica.edu.tw/
m https://www.chemcomp.com/MOE-Structure_Based_Design.htm
n https://sourceforge.net/projects/mols2-0/
o http://www.scfbio-iitd.res.in/dock/pardock.jsp
p http://rdock.sourceforge.net/
q http://www.swissdock.ch/
r http://www.biograf.ch/index.php?id=projects&subid=virtualtoxlab

Docking approaches have been thoroughly applied to predict drug toxicity *in silico*, allowing to identify the binding of lead compounds to adverse proteins and predict undesirable side effects and consequences [55]. For example, using docking simulations, Ji et al. searched for potential protein partners for binding 11 antiretroviral drugs among 147 known proteins associated with adverse reactions deposited in the DART (Drug Adverse Reaction Target) database to predict adverse drug effects. They confirmed that the predicted proteins associated with adverse drug reactions that caused side/toxic effects corresponded to the reported adverse reactions that occurred as a result of drug-target interaction [56]. In 2011, the same approach was used to predict the toxicity of melamine and its main derivative, cyanuric acid. This analysis identified potential target proteins associated with toxicity and provided a detailed understanding of the toxicity mechanism. In particular, in addition to nephrotoxicity, melamine was also predicted to have a toxic effect on the lungs [57]. Thus, a computational docking strategy can significantly facilitate the prediction of drug toxicity.

Despite the significant progress in drug discovery achieved through structure-based approaches, the widespread use of this strategy is hampered by numerous limitations, not the least of which is the problem of prodrugs and their metabolic conversion to another active compound(s) [58]. For example, reliable prediction of the metabolic fate of a particular drug requires high-resolution experimental structural data for all target proteins (e.g., enzymes). In addition, the analysis of protein-ligand complexes (and hence accurate reaction prediction) is hampered by the structural flexibility of proteins.

1.3 Application of *in silico* tools for predicting drug metabolic pathways

1.3.1 Prediction of the transformation of drugs into toxic metabolites

The metabolism of xenobiotics, such as drugs and other foreign substances, involves certain important enzymatic reactions, in particular those mediated by CYP450 enzymes expressed in the liver and small intestine. According to the

literature, ~90% of drugs can be efficiently metabolized by six CYP450 enzymes [59], whose activity can vary under the influence of factors such as genetic polymorphisms, cytokine regulation, disease state, gender, age, and hormones [60]. Another example is the membrane-bound P-glycoprotein (encoded by the multidrug resistance gene-1), which is expressed in various tissues, including intestinal epithelium, liver cells, and cells that form the blood-brain barrier. These tissues are known to act as biological barriers that limit the entry of various substances into cells, and thus affect the distribution of drugs for further metabolism [61].

As indicated in Tables 1.1 and 1.2, there are a large number of computer models for predicting enzymatic reactions, reflecting the strong influence of such reactions on the properties of ADMETs, which lead to a decrease or increase in the pharmaceutical effect of the drug [62].

1.3.2 Prediction of enzymatic reactions of drugs and enzymes

Endogenous enzymes in the human body can mediate the metabolic transformation of administered drugs into inactive, active, or toxic chemical compounds, which emphasizes the practical importance of predicting potential chemical modifications of drugs. Drug metabolism involves enzyme-catalyzed reactions; thus, a number of attempts have been made recently to predict enzyme-mediated reactions. An example of this is the reported prediction of hydrolysis and redox reactions. In this study, a machine learning-based model was built to predict classes/subclasses of hydrolysis reactions (EC 3.b.c.d, b - 1, 2, and 5) and redox reactions (EC 1.b.c.d, b - 1, 2, 3, 4, 5, 8, 13, and 14) [63], which allows predicting the metabolic transformations of a molecule.

To predict the enzymatic reactions involved in metabolic pathways, one study used a new approach to build a substrate-enzyme-product interaction network based on the k-nearest neighbor method to provide information related to toxicity in metabolic pathways. Substrate, enzyme, and products were encoded by molecular descriptors and physicochemical properties, and the k-nearest neighbor algorithm was used to build a predictive model. The substrate-enzyme-product interaction

networks were represented as the main factors, and the optimal features were selected using the maximum relevance, minimum redundancy and incremental feature selection (mRMR-IFS) method. Of the 290 features, 160 were selected and grouped into 10 different categories, including amino acid composition, predicted secondary structure, hydrophobicity, hydrophobicity and amino acid composition, predicted secondary structure, hydrophobicity, and polarity, among others [64].

In another study, a machine learning approach was recently used to computationally predict the potential reactions of 1449 enzymes (including CYP450 enzymes) deposited in the BRENDA (Braunschweig Enzyme Database) [65] and HMDB (Human Metabolome Database) [66] databases. In particular, it was assumed that if a known molecule interacts with a certain enzyme, then the query molecule should also interact with this enzyme if the physicochemical descriptors of the query molecule are similar to those of the known molecule. Interestingly, this model has shown the ability to predict enzymatic conversion by CYP450 enzymes and the concomitant formation of toxic metabolites, and therefore it was concluded that it is useful for predicting drug metabolism in terms of biological activity and toxicity [67]. Thus, the above methods of predicting potential enzymatic reactions have revolutionized *in silico* approaches and made a significant contribution to drug screening and identification of potential new drugs.

1.3.3 Prediction of drug molecule-target interactions based on the concept of pharmacological space

Based on the theory that proteins that mediate similar reactions are likely to have substrate similarity, Yamanishi et al. [68] proposed a QSAR-based model for predicting unknown drug-target interactions by introducing the concept of pharmacological space, which integrates chemical structure and information about the genomic profile of a protein. Based on the assumption that compounds with high structural similarity are more likely to interact with similar target proteins, chemical and genomic similarity was calculated and combined into a pharmacological space. The prediction model was built using three datasets: a dataset of drug-target

interactions obtained from various databases, a chemical dataset consisting of the structures of chemical compounds expressed as a similarity matrix between two compounds (chemical space), and a genomic dataset consisting of the amino acid sequences of target proteins expressed as a similarity matrix (genomic space). The chemical and genomic protein sequence datasets were combined into a pharmacological space and calculated using a bipartite graph learning model. The performance of the model was subsequently evaluated based on drug-target interaction data, and it was shown that the developed model predicts both enzyme-compound and protein interactions. enzyme-compound activity and protein interactions with other factors such as ion channels, G-protein-coupled receptors, and nuclear receptors. Thus, the model allowed us to reliably predict the interaction of a set of protein-compound pairs.

1.4. Problems associated with building predictive models

The inconsistency of available experimental data used to build *in silico* models is a major problem [69]. Predictive models rely heavily on experimental data to build the model; thus, high variability in experimental assays caused by biological variations and technical errors can lead to erroneous data and thus can introduce inaccuracy in predictive models. The inaccuracy of *in silico* models can also result from different experimental conditions for multiple resources collected, unbalanced datasets, and molecular descriptor values that differ from instrument to instrument [70]. The reliability of experimental data is confirmed if the results are consistent and accurate within a standardized experimental protocol over time. Therefore, in addition to considering the validity of *in silico* models, the quality of experimental data should also be considered. There have been several attempts to take into account the reliability of experimental data and their degree of uncertainty, efforts that often improve the accuracy of predictions [71]. This highlights the fact that low prediction accuracy may not only be the result of the *in silico* nature of the prediction tool, but may also reflect the nature of biological experiments. Comprehensive databases such as Drugbank, HMDB, and others such as MetaDrug and MetaCore are gradually

becoming more robust through human curation, improved data mining algorithms, and/or the addition of new experimentally validated data, which increases the reliability of the datasets used by *in silico* models and thereby improves the accuracy of the results. *In silico* methods have become a major innovation in attempts to predict the fate of drugs, but building reliable predictive models remains a challenge. Therefore, predictive models are tested and their accuracy, validity, and reliability are confirmed using external validation datasets to determine whether the model is acceptable for a particular purpose. For example, three web servers, SOMP, SMARTcyp, and RSWebPredictor, which are used to predict metabolic site, were compared for their prediction accuracy. Of these, the SOMP server was shown to have a higher invariant prediction accuracy (similar to AUC) than the others, with a score of 0.9, and thus is considered an adequate tool for drug metabolism prediction [72].

Thus, due to its central importance, metabolism in biological systems is intensively studied, especially in the field of drug development. The high influence of drug metabolism on drug efficacy and fate in biological systems has led to the emergence of numerous *in silico* approaches and tools for predicting metabolic reactions in recent decades. However, the limitations of these approaches cannot be ignored. In particular, the fact that these methods are heavily dependent on experimental data is a major concern, as inconsistent and erroneous data can lead to inaccurate prediction models. Although the prediction of metabolic reactions is an extremely challenging field, it has greatly facilitated the advancement of drug discovery, continuing to show rapid improvement with the development of computational methods and increased computing power.

Conclusions to the Chapter 1

1. The scientific literature on the main *in silico* approaches and methods used to predict possible pathways of chemical metabolism in the human body was systematized and analyzed.

2. The analysis confirms the prospects of using the software to predict possible metabolites of a potential drug at the early stages of its research.

CHAPTER 2. MATERIALS AND METHODS OF THE STUDY

The object of the study is 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinolin-4-one (laboratory code VAZ_07), synthesized by Associate Professor of the Department of Medicinal Chemistry, Doctor of Pharmaceutical Sciences Vadym Zubkov (Fig. 2.1).

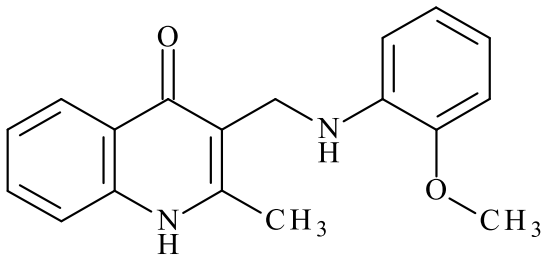


Fig. 2.1 Structural formula of 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinoline-4-one (VAZ_07)

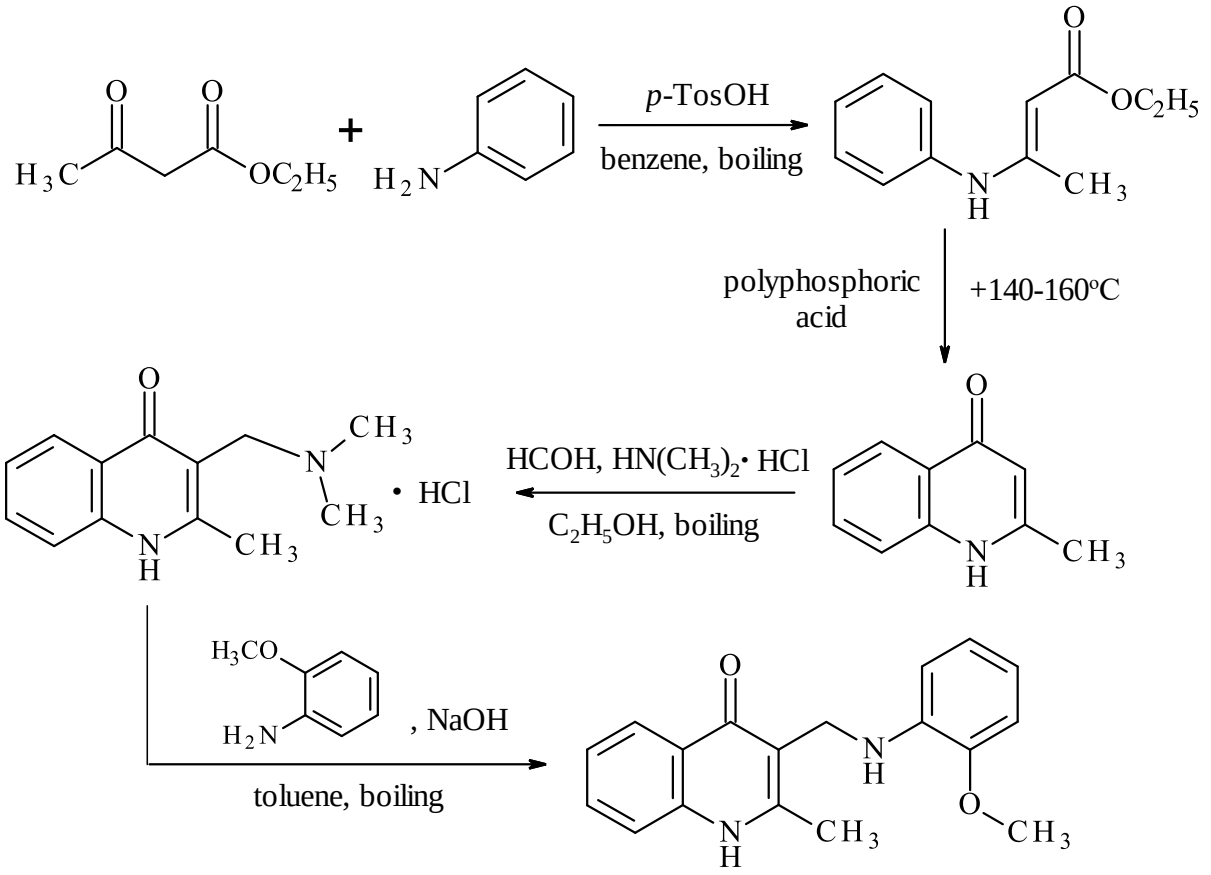
2.1 Synthesis of 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinoline-4-one

As a starting compound for the synthesis of 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinoline-4-one, 2-methylquinolin-4-one (starting compound) was used, which was aminomethylated under Mannich reaction conditions [73], and the resulting Mannich base (3-dimethylaminomethyl-2-methylquinolin-4-one hydrochloride) upon reamination with *ortho*-anisidine (2-methoxyaniline) forms 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinoline-4-one (Scheme 2.1).

It is known that the Mannich reaction is unambiguously carried out only when secondary amines are used, whereas ammonia and primary amines can react with the replacement of all hydrogen atoms adjacent to the nitrogen. It has been confirmed that the interaction of 2-methylquinolin-4-one with primary aliphatic amines, anilines, and diethylamine under classical Mannich reaction conditions leads to the formation of mostly by-products that are insoluble in most organic solvents. It is also known that Mannich bases can be used as alkylating agents in reactions with amines and methylenated compounds. Such alkylation is especially easy if the Mannich base

is formed by an amine, which can be easily cleaved off, for example, by dimethylamine. In this regard, the synthesis of 3-dimethylaminomethyl-2-methyl-1H-quinolin-4-one was carried out, as well as the subsequent synthesis of 3-arylamino derivatives of 2-methylquinolin-4-one on its basis.

Scheme 2.1



The hydrochloride of 3-dimethylaminomethyl-2-methyl-1H-quinoline-4-one was obtained in two ways: by boiling 2-methylquinoline-4-one with formaldehyde and dimethylamine hydrochloride in ethanol (method I), and by aminomethylation of 2-methylquinoline-4-one with N,N-dimethylimmonium chloride (method II). The use of imonium salts allows for an unambiguous synthesis, increases the yield of target products compared to the conventional Mannich reaction, and simplifies the reaction itself [44]. Thus, method II is more suitable for the synthesis of hydrochloride. The resulting salt, when boiled in toluene in the presence of powdered NaOH, readily undergoes a transamination reaction with primary aliphatic amines, anilines, and diethylamines to form the target 3-N-R-aminomethyl

quinolones. The end of the reaction is determined by the end of the release of dimethylamine from the reaction medium.

The key intermediate can be obtained by the interaction of the free base with primary amines and diethylamine in boiling toluene (method B). However, the total yield of the final products by this method in terms of hydrochloride was significantly lower than the yields of syntheses using hydrochloride itself. This is apparently due to the good solubility of 3-dimethylaminomethyl-2-methyl-1H-quinolin-4-one in water and, accordingly, to the loss of the compound at the stage of obtaining a free base [73].

The structure and identity of 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinoline-4-one were confirmed by ^{1}H NMR spectroscopy and thin-layer chromatography.

2.2 Pharmacological properties of 2-methyl-3-[(2-methoxy-anilino)methyl]-1H-quinoline-4-one

The investigated molecule became a promising object for pharmacological study based on the results of a comprehensive screening study of its psycho- and neurotropic properties conducted by Illya Podolsky, Associate Professor of the Department of Medicinal Chemistry, Doctor of Pharmaceutical Sciences.

The screening was performed on white nonlinear mice at doses of 10 and 100 mg/kg using open field, elevated plus maze, rotarod test, Porsolt's immobilization test, and conditional passive avoidance reaction against scopolamine-induced amnesia. At the end of the screening, the effect on the life expectancy of mice in a model of acute normobaric hypoxia with hypercapnia was studied [74].

The results of the VAZ_07 study in the open field test revealed the psychotropic indifference of the test compound. In animals injected with 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinolin-4-one at a dose of 10 mg/kg, a threefold decrease in the number of crossed squares ($p<0.01$) was observed compared to the intact control (Fig. 2.2). There was also a significant decrease in the number of

defecations ($p<0.05$) and, as a result, a twofold decrease in the total sum of all activities ($p<0.01$) (Fig. 2.3).

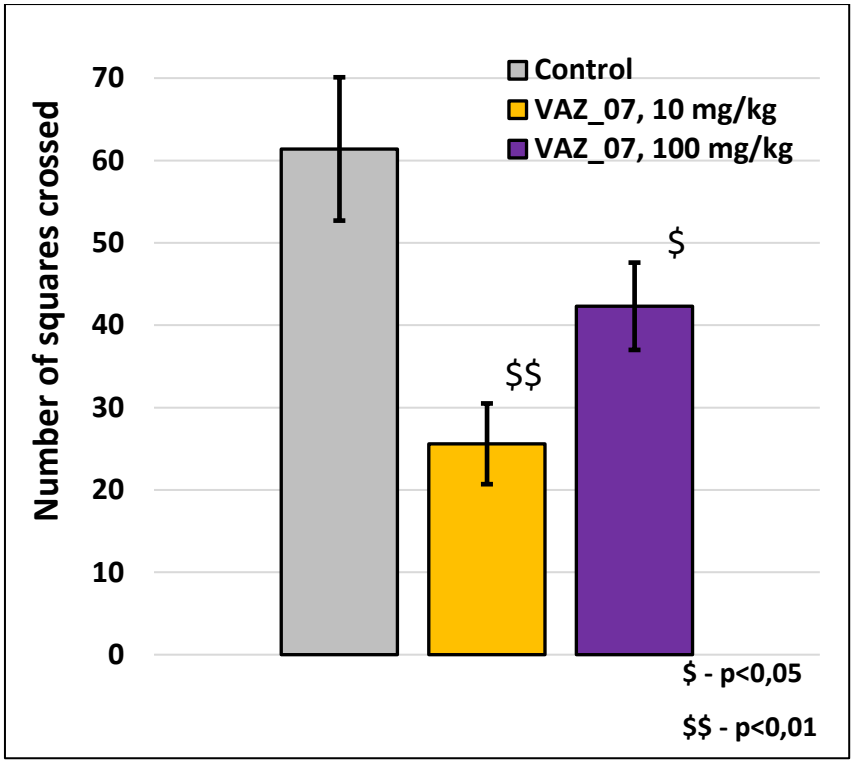


Fig. 2.2 Effect of VAZ_07 on the number of squares crossed by animals in the open field test

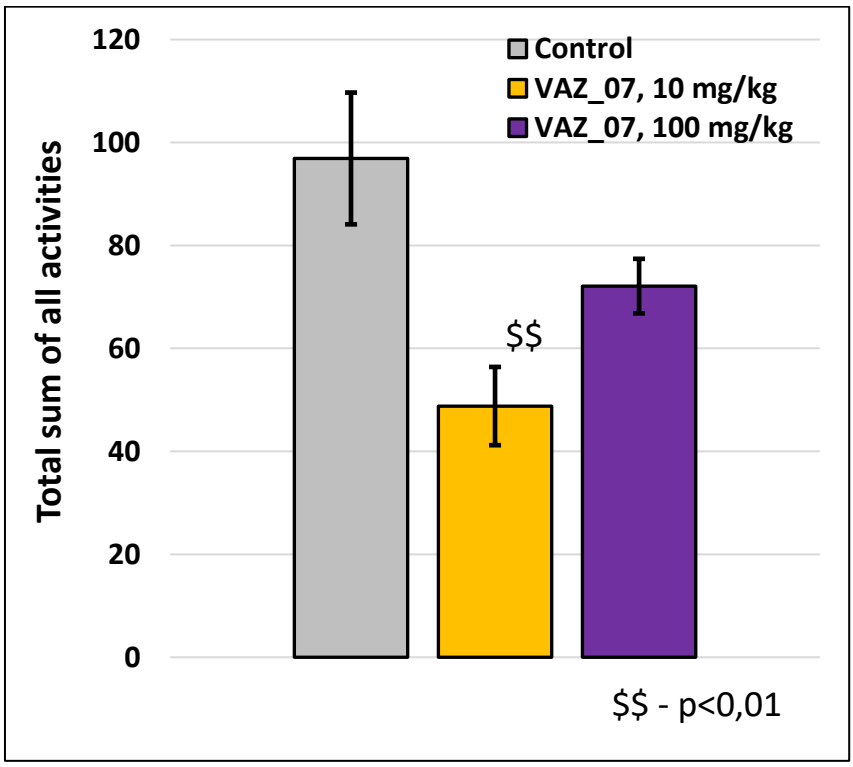


Fig. 2.3 Effect of VAZ_07 on the total sum of all animal activities in the open field test

However, these changes in locomotor activity and emotional reactions were not accompanied by impaired research activity. This reflects a certain selectivity of sedation, which may be a positive feature of this compound. At a dose of 100 mg/kg, the effect on behavioral reactions was similar, but less pronounced.

According to the results of the conditioned passive avoidance reaction test against scopolamine-induced amnesia, VAZ_07 only at a dose of 10 mg/kg significantly showed an anti-amnestic effect, and the anti-amnestic activity was 87.9 % ($p<0.05$). At a dose of 100 mg/kg, the test substance also had a protective effect against M-cholinergic blocker administration at the level of 78.7 %, but the difference with the amnesia control group did not reach a significant level (Fig. 2.4).

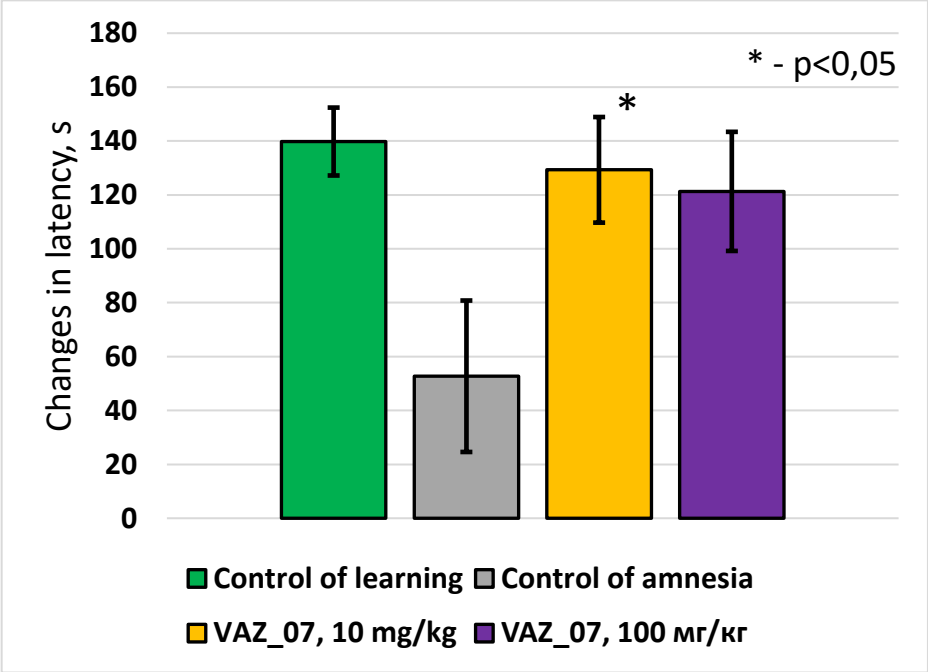


Fig. 2.4 Results of the study of VAZ_07 in the test of passive avoidance conditioned response against scopolamine-induced amnesia

The anxiolytic properties of VAZ_07 were studied in the elevated plus maze test (Fig. 2.4). However, no significant differences in the behavior of animals were found in terms of indicators of anxiety. It should be noted that a significant decrease in the number of transitions between the maze arms in animals administered VAZ_07 at a dose of 10 mg/kg (Fig. 2.5) is more in favor of the sedative properties, which were also found in the open field test.

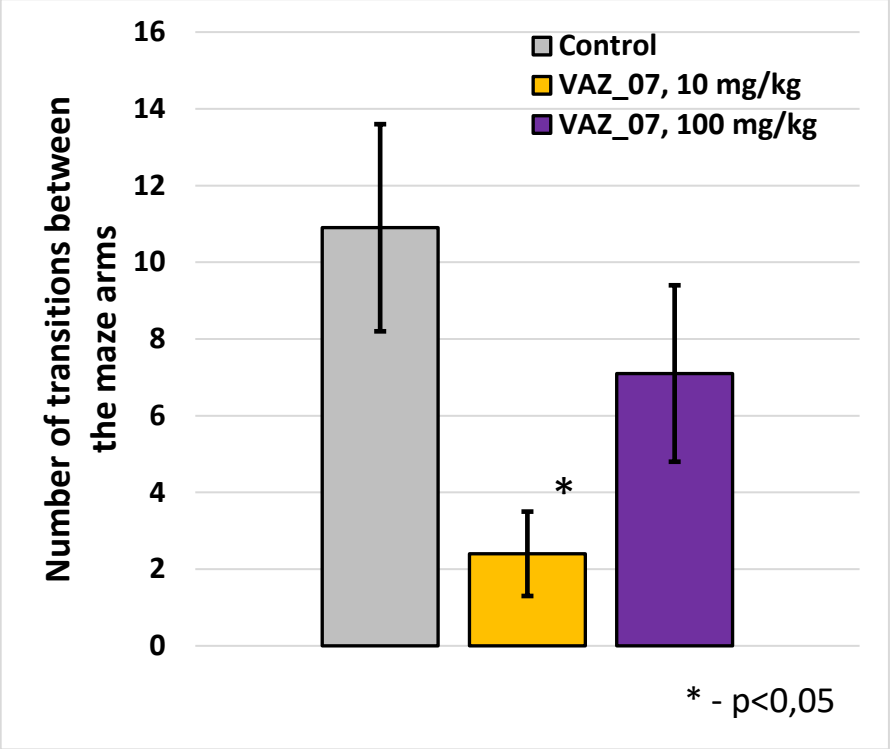


Fig. 2.5 Results of the VAZ_07 study in the elevated plus maze test

Animals treated with a dose of 10 mg/kg VAZ_07 made more than 4-times ($p<0.05$) fewer transitions between maze compartments compared to mice in the intact control group, which, when compared with the almost unchanged latency time of the first pass and the total time spent in the illuminated arms, indicates that the compound under study has no effect on animal anxiety.

Thus, the results of a comprehensive screening study [74] outlined the prospects for an in-depth study of VAZ_07 at a dose of 10 mg/kg as a promising API with sedative and nootropic properties.

2.3 Online computerized metabolism prediction systems used

Xenosite (https://xenosite.org)

XenoSite is a neural network-based CYP SOM prediction model that improves on RSP in a number of ways [75]. XenoSite uses the sets of substrates and descriptors generated by RSP as a starting point and makes the following improvements:

1. New molecular-level descriptors have been developed that allow machine learning methods to internally determine which atomic descriptors are most relevant for a particular substrate in prediction.

2. Neural networks are used to build the models, rather than the SVM technology used by RSP. One of the advantages of neural networks is that they have a much faster training model execution time than SVMs. The second advantage is that their output oxidation probability coefficient has a quantitative expression in a numerical format that can be interpreted as a probability, unlike RSP SVMs, which only provide a rank ordering of SOMs contained in the same substrate. The SOM score obtained from the neural network is significantly correlated with the probability of SOM oxidation, while the SOM score obtained from the RSP rank orderings is not. Thus, XenoSite scores serve as a reflection of both the model's prediction validity and prediction accuracy. This means that consumers can view SOM scores for an entire substrate and make informed decisions about the reliability of the prediction [75].

Xenosite utilizes a pre-assembled set of 680 CYP substrates distributed across nine CYP enzymes: 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4. In addition, a human liver microsome (HLM) set is analyzed, considering all 680 substrates and all observed metabolites, regardless of metabolizing isoform. This HLM set does not represent all metabolic functions of liver microsomes, but reflects the totality of known CYP metabolism [75].

In a molecule, every atom that can be metabolized in a CYP substrate is a potential SOM. Each atom is associated with a vector of numbers, with each number encoding the chemical properties of that SOM; these codes of chemical information are known as descriptors. Machine learning algorithms then analyze these encoded SOM descriptors to determine a scoring function that gives experimentally observed CYP-mediated SOMs high scores and unobserved SOMs low scores. A combination of previously defined descriptors - topological (TOP) and quantum chemical (QC) descriptors, SMARTCyp reactivity descriptor (SCR) in addition to a refined subset of QC descriptors (SQC), molecular (MOL) descriptors and fingerprint similarity

(FP) descriptors - are used. MOL and FP descriptors have recently been used for SOM prediction and encode information about molecules as a whole in addition to the local atomic environment [75].

All models are built using a standard neural network with five hidden nodes, calibrated using cross-validation without outliers (LOO) with gradient descent on the cross-entropy error. LOO cross-validation in this case means that all SOMs for one test substrate are predicted using models calibrated with all SOMs from the rest of the substrate set. This process is repeated with each substrate that is considered as a test once. The models created by this protocol produce output scores from 0 to 1, which can be interpreted as probabilities. For each training cycle, three random re-runs were performed, selecting the model with the best accuracy to the training set before testing. Unique SOM prediction models were built from each of the 10 sets of SOM substrates represented by the TOP and SCR descriptors in combination with various combinations of the QC, SQC, MOL, and FP descriptors. XenoSite's optimal models are on average 87 % accurate for all analyzed substrate sets, a performance level 3% higher than the previously existing optimal RSP method. This performance improvement comes from representing the predicted SOMs with two new molecular-level descriptor classes and pruning the descriptor composition of the previously developed atomic-level descriptor class to remove noise while preserving signal; neither of these improvements is responsible for the full increase in prediction accuracy.

SMARTCyp (https://smartcyp.sund.ku.dk/mol_to_som)

Most of the previously published methods for predicting CYP metabolism require experimental data to create models. Such data are incomplete because they always include sites that are "false negatives" (reactive sites for which no metabolites are found because a metabolite is found for an even more reactive site) and often include compounds with missing metabolites, leading to significant "noise" in the training data.

SMARTCyp does not require three-dimensional structures of the molecule, and although it is supported by experimental data, its implementation is not

dependent on them [76]. The idea behind SMARTCyp is that the activation energies of CYPs reacting with ligand fragments calculated by quantum chemical methods are the best possible reference to the reactivity of a fragment. The reference data from quantum chemical calculations for substances have a very high signal-to-noise ratio, as the data are free of experimental errors or so-called "false negatives". The results are very easy to interpret, as the lower the activation energy, the more likely the site is to be metabolized.

Atoms that do not match any pattern are not considered reactive. The accessibility descriptor, A, is the coefficient of the SPAN descriptor as defined by Sheridan et al. It is defined as the longest bonding distance from a given atom divided by the longest bonding distance in the entire molecule. It is a measure of how far from the 2D center of the molecule an atom is located and is always a number between 0.5 and 1. So, it is not a measure of available surface area, but it describes how the atoms at the end of the molecule are likely to get close to the reactive heme group in the active center of CYP. Finally, the S score is calculated for each atom as S = E-8A, where a lower score indicates a higher probability of being a SOM. The constant 8 is chosen so that availability can change the score, corresponding to a maximum energy of 4 kJ/mol (which is slightly higher than the average standard deviation among the calculated energies using our rules, which is 3.2 kJ/mol). This allows somewhat less reactive atoms to be scored higher if their availability A is significantly higher [76].

The development of SMARTS rules is based on a dataset of 475 cytochrome P450 substrates from the literature. Procedures for determining activation energies within the framework of density functional theory (DFT), energy differences between the transition state and the reagent complex have already been described. While the original SMARTCyp program is based on Java using the Chemistry Development Kit (CDK) library, SMARTCyp 3.0 is based on Python using the RDKit library. CDK and RDKit perceive aromaticity in a molecule's structure differently, and thus there is a difference in which atoms SMARTS models match, for example, due to a different set of atoms. To ensure backward compatibility, the

differences in SMARTS rules were identified by CDK and RDKit for all sites for the test set of 475 3A4 substrates. Each SMARTS rule with a discrepancy was analyzed individually, compared to the corresponding molecule and substructure from which it was generated, and corrected as necessary [76].

The calculated different sites can be divided into six different types, which represent one or more types of P450 reactions. The distribution of activation energies varies quite a bit between the different types, with phosphorus desulfurization and S-oxidation yielding the lowest energies and N-oxidation and N-dealkylation of peptide groups yielding the highest energies. To obtain the activation energies, the reaction step with the highest activation energy in the respective reactions is calculated. For aliphatic hydroxylation, aldehyde hydroxylation, and dealkylation reactions, this is the abstraction of hydrogen from a carbon atom, while for other reaction types it is the attack of oxygen on the corresponding atom.

One of the new features implemented in SMARTCyp 3.0 is the "Similarity" function, which compares the similarity of the matched substructure to the full molecule fragment for which the DFT calculation was performed based on Morgan fingerprints. A score of "1.0" indicates a perfect match, while a score of "0.0" means that there is no matching fragment, which means that the atom is either not considered reactive or the assigned reactivity is not based on calculated data and therefore not as reliable [76].

The fact that SMARTCyp performs quite well shows that reactivity is a major factor in CYP 3A4 metabolism and emphasizes the importance of using accurate methods to generate reactivity rules. SMARTCyp is good at detecting compounds with a metabolic position that ranks highest, in part because it is a pure 2D method that gives extremely fast predictions. The two main advantages of the method are that the creation of the method makes physical sense and the low signal-to-noise ratio in the input data. Both of these stem from the fact that the reactivity model is calculated based on highly skilled quantum chemical calculations of the activation energy for oxidation reactions. Other methods often use a larger number of descriptors, which leads to a significant amount of noise in the input data, and the

relative influence of descriptors is often difficult to understand. Another advantage of the method is that it is easy to implement using any of the available chemical programming libraries, free or commercial, and can be integrated into workflows used by other software.

Way2Drug SOMP/RA (http://www.way2drug.com/RA)

To determine the SOM, machine learning approaches must take into account the basic mechanisms of enzyme action. However, such information is not always available, and the results of SOM predictions can be properly interpreted to understand the structure of reaction products. For example, in many cases, researchers prefer to consider the carbon atom of the leaving group adjacent to the nitrogen as the SOM for N-dealkylation. This assumption is based on the mechanism of abstraction of the hydrogen atom, but does not take into account other possible one-electron transfer mechanisms of the N-dealkylation reaction [77]. We consider nitrogen as a "reacting atom" in the case of the N-dealkylation reaction. Another problem with the uncertainty of detecting the site of the molecule attacked by cytochromes P450 is related to the mechanism of aromatic hydroxylation, which can be realized by the formation of an epoxy intermediate or "NIH shift". Therefore, the direct determination of SOMs to create training sets in machine learning approaches is problematic, and the interpretation of the predicted results is ambiguous.

In the Way2Drug approach, SOMP and RA [77] do not attempt to model or simulate the hypothetical process of intermediate formation implemented by P450. Only known information about the substrate and metabolite structures of the reactions is used to create training sets for predicting the reacting atoms of nine classes of reactions. The Way2Drug SOMP and RA approach considers the reaction classes of aliphatic and aromatic hydroxylation, N-, S-, and C-oxidation, N- and O-dealkylation, which, according to the Biovia Metabolite database, cover approximately 70 % of all reactions catalyzed by the five major P450 isoenzymes (CYP1A2, CYP3A4, CYP2D6, CYP2C9, CYP2C19). In addition, the reactions of N- and O-glucuronidation are discussed, which cover almost all reactions catalyzed by the UDP-glucuronyltransferase family.

The use of the term "reactive atom" and its definition as the portion of the substrate molecule to which a specific structural fragment is added (or removed) allows the identification of metabolite structures based on the prediction of the reactive atom. Structural fragments added to reactive atoms include hydroxyl (hydroxylation reactions), carbonyl or carboxyl (C oxidation reactions), hydroxyl or oxo groups (N- and S-oxidation reactions), and glucuronyl (glucuronidation reactions) groups. In the case of dealkylation reactions, the alkyl group is considered as a fragment that is removed from the reacting atom represented by an oxygen or nitrogen [77].

In the Way2Drug SOMP and RA approach, the reacting atoms are automatically identified in each substrate structure from selected biotransformations. The APGL and python-igraph libraries are used to automatically identify the reacting atoms. First, all subisomorphisms between substrate and product are detected. Then the algorithm checks whether the graphical difference in the structures of the substrate and the reaction product is related to the process under study. If so, it looks for atoms with a changed number of neighbors in the isomorphic environment. Oxidation reactions are catalyzed by cytochromes P450 and are mainly realized by oxidation by heteroatoms (N- and S-oxidation) or hydroxylation of carbon (aliphatic or aromatic hydroxylation). The aliphatic hydroxylation reaction is understood as the hydroxylation of a carbon atom that is not part of the aromatic rings. In the case of C oxidation reactions, the formation of carbonyl or carboxyl groups is considered. N- and O-glucuronidation is catalyzed by UDP-glucuronosyltransferases.

Biotransformer (http://biotransformer.ca)

BioTransformer is an open-source software tool and a freely available web service for accurate and comprehensive *in silico* metabolism prediction and metabolite identification [78].

BioTransformer consists of a metabolism prediction tool (BMPT) and a metabolite identification tool (BMIT). BMPT consists of five independent prediction modules called "transformers", namely:

1) an enzyme-directed transformer;

2) CYP450 transformer (phase I);

3) phase II transformer;

4) transformer of human intestinal microbiota;

5) microbial environment transformer.

For metabolite prediction, BioTransformer uses two approaches - a rule-based or knowledge-based approach and a machine learning approach. The knowledge-based system in BioTransformer consists of three main components: (1) a biotransformation database (called MetXBioDB) containing detailed annotations of experimentally validated metabolic reactions, (2) a reaction knowledge base containing general biotransformation rules, preference rules, and other constraints for metabolite prediction, and (3) a selection engine that implements both general and transformer-specific algorithms for metabolite prediction and selection. BMPT's machine learning system uses a set of random forest and ensemble prediction models to predict CYP450 substrate selectivity and to filter out phase II molecules. The BioTransformer metabolite identification tool relies on BMPT to identify specific metabolites using mass spectrometry (MS) data, namely precise mass or chemical formula information [78].

MetXBioDB is a database consisting of a collection of more than 2000 experimentally validated biotransformations derived from the literature. It was developed to assist in (1) developing biotransformation rules, (2) training and validating machine learning metabolism prediction models, and (3) developing preference rules. Each biotransformation in MetXBioDB includes a starting reagent (structure and identifiers), a reaction product (structure and identifiers), the name or type of enzyme catalyzing the biotransformation, a reaction type, and one or more citations. For the purposes of this article, a reagent is defined as a small molecule that binds to a specific enzyme and undergoes a metabolic transformation catalyzed by that enzyme. Biotransformation describes the chemical conversion or molecular transformation of a reactant into one or more products by a specific enzyme (or class of enzymes) through a specific chemical reaction. Cytochrome P450 enzymes

(CYP450s) are responsible for >90% of phase I oxidative reactions and >75% of drug metabolism, while UDP-glucuronosyltransferases (UGTs) and sulfotransferases (SULTs) are responsible for phase II metabolism of most xenobiotics [49]. In the gut microbiota, enzymatic reactions are mostly reductive and are carried out by anaerobic bacteria due to the very low oxygen concentration.

The BioTransformer Reaction Knowledge Base contains chemical reaction descriptions and rules encoded in SMARTS and SMIRKS strings that are used by the selection engine to predict biotransformation. This knowledge base encodes information about five different concepts and contains data representing: (1) biosystem, (2) metabolic enzyme, (3) metabolic reaction, (4) metabolic pathway, and (5) chemical class.

The BMPT reasoning system uses rules in the reaction knowledge base to select the most likely of all applicable metabolic biotransformations or pathways. In general, two types of considerations are used to select and rank predicted metabolites: absolute and relative [49]. Absolute considerations focus exclusively on the probability of biotransformation and are used to select biotransformations with an occurrence rate above a given threshold.

GLORYx (https://nerdd.zbh.uni-hamburg.de)

GLORY includes a new set of reaction rules for CYP-mediated metabolism, which distinguishes common reaction types from more unusual reactions [79]. Importantly, GLORY investigated how SoM prediction can be effectively used in the context of metabolite structure prediction.

The SoM prediction software used in GLORY was FAME 2, a machine learning-based SoM prediction program that uses highly randomized tree classifiers combined with two-dimensional (2D) circular descriptors to predict SoMs for CYP-mediated metabolism. Since the development of GLORY, a successor to FAME 2 has become available. FAME 3 continues to utilize the concept of additional tree classifiers and 2D circular descriptors developed in FAME 2 and applies this approach to create comprehensive SoM prediction models for both phase 1 and phase 2 metabolism.

Based on the extended approach developed in GLORY, a new tool called GLORYx has been created that combines SoM prediction with a set of reaction rules to predict metabolites from both phase 1 and phase 2 metabolism. GLORYx uses FAME 3 to perform SoM prediction, the results of which are used to score and rank the predicted metabolites. Compared to GLORY, GLORYx requires a larger number of reaction rules to cover non-CYP phase 1 metabolic reactions as well as phase 2 metabolic reactions. GLORYx is freely available via a web server at https://nerdd.zbh.uni-hamburg.de/.

A reference dataset of combined metabolite pairs was compiled from freely available metabolism data in the DrugBank (drug group "All") and MetXBioDB databases to serve as a basis for method evaluation during GLORYx development. For each metabolic reaction in either database, the reactant was considered the starting molecule and the product was considered the metabolite. Thus, the reference dataset is presented in the format of a map of each parent molecule to its first-generation metabolites, regardless of whether the parent molecule is itself a metabolite of another molecule.

GLORYx applies the reaction rules to all relevant positions in the molecule, which is determined by where each SMIRKS reaction rule matches, if at all. Within the program, the main parameters are predicted using FAME 3, and the predicted probabilities are used to score and rank the predicted metabolites. The software is written in Java and uses CDK version 2.0. GLORYx performs an initial preprocessing step on all input molecules to check that the input molecule can be successfully analyzed by the CDK, does not have multiple components, and does not contain element types other than C, N, S, O, H, F, Cl, Br, I, P, B, and Si (allowed FAME 3 element types). If any of these checks fail, no predictions are made for the input molecule [79].

SoM prediction in GLORYx is performed using FAME 3. FAME 3 is trained on SoM data from the MetaQSAR database and offers three SoM prediction models:

1) model P1 predicts SoMs corresponding to phase 1 metabolic reactions;

2) the P2 model predicts SoMs corresponding to phase 2 metabolic reactions;

3) the P1 + P2 model predicts SoMs corresponding to both phases of metabolism.

The FAME 3 code includes preprocessing of the input molecules, including nitro group standardization, aromaticity detection, and automatic hydrogen addition if the hydrogen of the input molecule is not explicitly specified. Since the SoM prediction step occurs before the application of reaction rules within the GLORYx program, the standardization of the molecules described here remains in place for the next transformation step.

The reaction rules are applied using Ambit-SMIRKS. For GLORY, any product containing less than three heavy atoms is not included in the set of predicted metabolites. In order to apply the reaction rules correctly, i.e. to achieve the same predicted metabolites as SyGMa using the same rules, it was necessary to use an aromaticity model that could recognize aromaticity in rings with exocyclic heteroatoms.

In GLORYx, a weighting factor of 1 is used for reaction rules labeled as "common" and a weighting factor of 0.2 is used for reaction rules labeled as "unusual". Thus, these weights maintain the same 5:1 ratio, but are scaled so that the final priority score is more reflective of the probabilistic concept, with values ranging from 0 to 1.

Conclusions to the Chapter 2

1. The methods for the synthesis of 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinoline-4-one (laboratory code VAZ_07) are presented.

2. The prospects for in-depth pharmacological study of 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinoline-4-one as a potential API with sedative and nootropic effects are substantiated.

3. The choice and analysis of the calculation algorithms used in the work of online computer prediction systems for possible metabolic pathways in the human body was substantiated.

CHAPTER 3. PREDICTION OF PROBABLE METABOLIC PATHWAYS OF 2-METHYL-3-[(2-METHOXYANILINO)METHYL]-1H-QUINOLINE-4-ONE

In order to predict the possible pathways of biotransformation of a promising compound – 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinolin-4-one (laboratory code VAZ_07), five different online resources that are freely available were used, namely:

1) Xenosite *(https://xenosite.org/)*

2) SMARTCyp *(https://smartcyp.sund.ku.dk/mol_to_som)*

3) Way2Drug RA *(http://www.way2drug.com/RA)*

4) Biotransformer 3.0 *(http://biotransformer.ca)*

5) GLORYx *(https://nerdd.zbh.uni-hamburg.de)*

According to the results of prediction of possible pathways of VAZ_07 metabolism using the online service Xenosite, the most likely direction is unstable oxidation, i.e. O-demethylation with the formation of the corresponding 4′-hydroxy derivative. Oxidative deamination of the amino methyl fragment at position 3 of the quinolone ring is also possible by the classical mechanism, i.e. formation of the corresponding aldehyde and amine. Stable oxidation, i.e., aromatic or aliphatic hydroxylation, is also a possible direction of biotransformation of the molecule (Fig. 3.1).

According to the prediction results, the methyl group at position 2 of the quinolone cycle is the most reactive. In such a scenario, as a result of further oxidation of the hydroxymethyl group to the carboxyl group, the generation of metabolites with new pharmacological properties – kynurenic acid derivatives – is predicted. Kynurenic acid (4-hydroxyquinoline-2-carboxylic acid) is a tryptophan metabolite and is formed from kynurenine under the action of kynurenine aminotransferase [80].

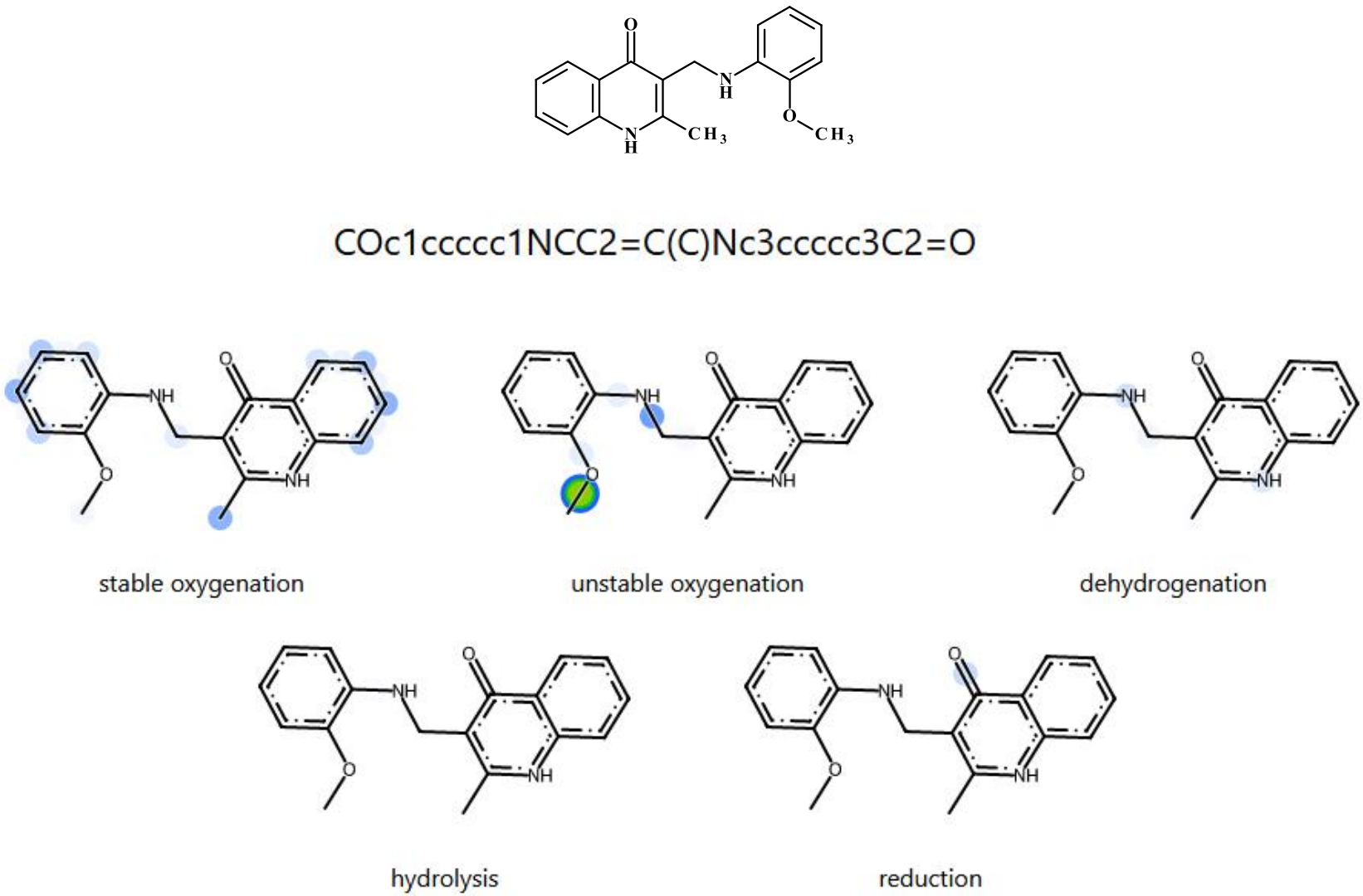


Fig. 3.1 Results of prediction of possible metabolic pathways of VAZ_07 using the online service Xenosite

In the brain, kynurenic acid acts as an endogenous antagonist of the glycine site of NMDAR, which determines the interest in kynurenic acid as a potential pharmacocorrector of pathological conditions accompanied and burdened by excitotoxicity. A significant problem in *in vivo* studies was the low permeability of this molecule through the blood-brain barrier [80], so researchers focused on its chemical modification to find kynurenic acid derivatives with physicochemical properties that can overcome this limitation.

Thus, the results of the calculations indicate that some of the effects of VAZ_07 pharmacodynamics, in particular its anti-amnesic properties, may be related not only to the direct action of the compound on certain receptor systems in the brain, but also to active metabolites formed as a result of biotransformation.

In addition to predicting the possible directions of biotransformation of molecules within the first phase of metabolism, the Xenosite software package allows to assess the safety of a promising compound in terms of reactivity, as well as the possibility of formation of toxic metabolites.

According to the results of the prediction, VAZ_07 has low probability of formation of quinones or epoxides (Fig. 3.2).

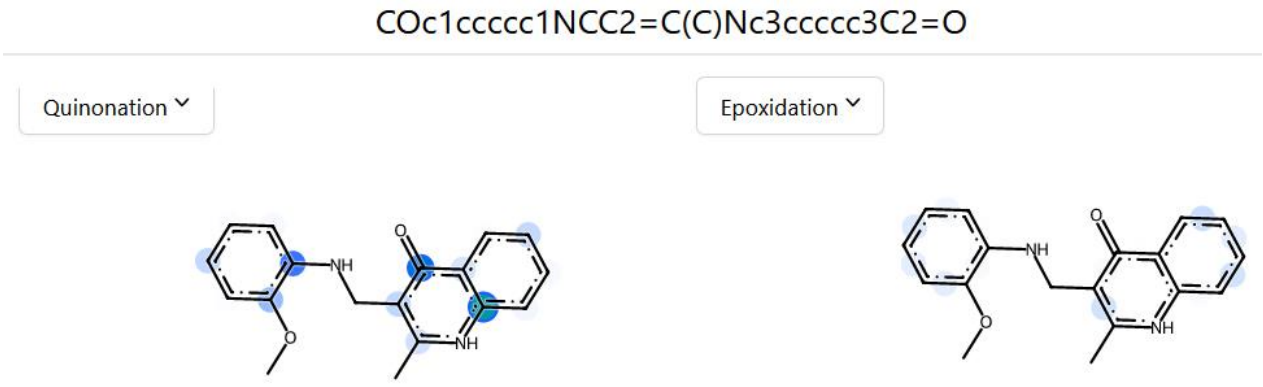


Fig. 3.2 Results of prediction of the possibility of formation of highly reactive quinones and epoxides as metabolites of VAZ_07 (online service Xenosite)

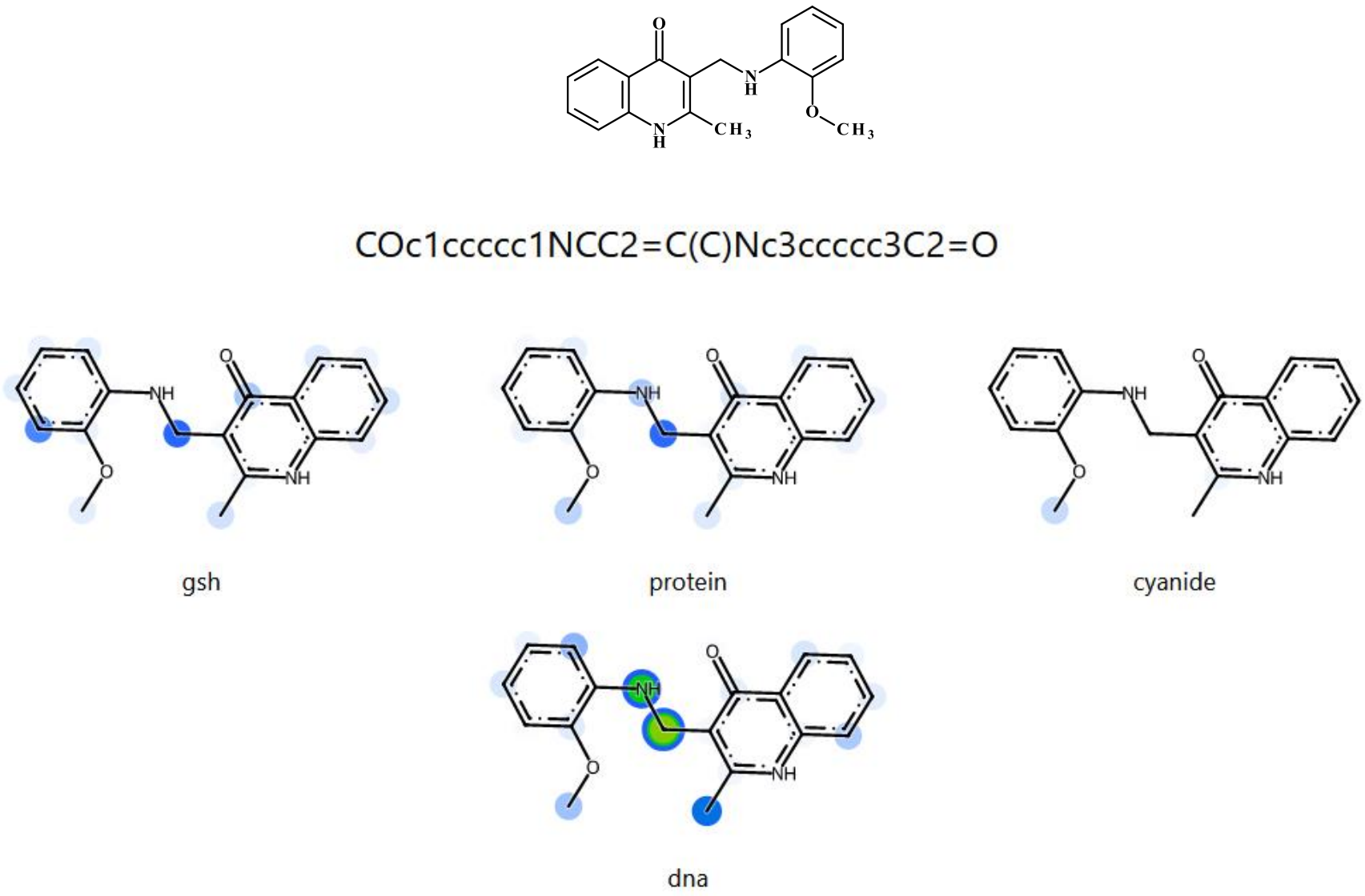


Fig. 3.3 Results of predicting the reactivity of VAZ_07 in the human body using the online service Xenosite

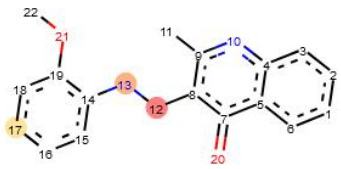
Fig. 3.3 shows the results of predicting the reactivity of the test molecule, i.e., the potential interaction with certain structures in the human body. It is shown that VAZ_07 has low indicators of potential interaction with the reduced glutathione system, proteins, and low potential for cyanide formation. Nevertheless, there is a certain probability of interaction with DNA material, which is unlikely to occur *in vivo*, since it is unlikely that this molecule will be able to penetrate directly into the cell nucleus, at least in unchanged form.

The analysis of the prediction results using the online SMARTCyp system showed that different CYP isoforms can catalyze oxidation processes, namely O-demethylation, oxidative deamination and aromatic hydroxylation at different positions (Figs. 3.4, 3.5 and 3.6).

With the participation of the CYP3A4 isoform, the most likely directions of biotransformation of VAZ_07 are oxidative deamination of the aminomethyl fragment at position 3 of the quinolone ring and O-demethylation, aromatic hydroxylation of the *para*- and *ortho*-positions of the phenyl substituent and aliphatic hydroxylation of the methyl group at position 2 of the heterocycle (Fig. 3.4).

3A4 2D6 2C9

1

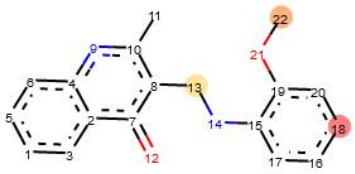


3A4 Ranking	Atom	3A4 Score	Energy	2DSASA	Span2end	Relative Span	Similarity
1	C.12	35.9	41.1	20.0	5	0.5	0.4
2	N.13	48.6	54.1	9.5	4	0.6	0.3
3	C.17	50.2	59.5	33.5	0	1.0	1.0
4	C.22	51.6	62.2	64.3	0	1.0	1.0
5	C.15	51.9	59.5	27.2	2	0.8	0.7
6	C.11	58.4	66.4	54.4	3	0.7	0.7
7	C.1	59.6	68.2	32.7	1	0.9	1.0
8	C.16	65.5	74.1	33.5	1	0.9	1.0
9	C.18	68.8	77.2	29.4	1	0.9	0.7
10	N.10	69.2	75.6	13.5	3	0.7	0.3
11	C.2	71.5	80.8	33.5	0	1.0	1.0
12	C.6	73.2	80.8	26.9	2	0.8	1.0
13	C.3	77.9	86.3	28.5	1	0.9	1.0

Fig. 3.4 Results of predicting possible pathways of VAZ_07 metabolism involving CYP3A4 (SMARTCyp software package)

3A4 2D6 2C9

1

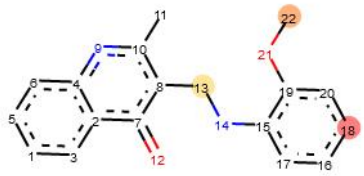


2C9 Ranking	Atom	2C9 Score	Energy	2DSASA	Span2end	COO-Dist	Similarity
1	C.18	58.2	59.5	33.5	0	0	1.0
2	C.22	59.6	62.2	64.3	0	0	1.0
3	C.13	64.0	41.1	20.0	5	0	0.4
4	C.17	70.2	59.5	27.2	2	0	0.7
5	C.1	72.8	68.2	32.7	1	0	1.0
6	N.14	77.4	54.1	9.5	4	0	0.3
7	C.16	78.7	74.1	33.5	1	0	1.0
8	C.5	79.5	80.8	33.5	0	0	1.0
9	C.20	81.9	77.2	29.4	1	0	0.7
10	C.11	81.9	66.4	54.4	3	0	0.7
11	C.6	91.1	86.3	28.5	1	0	1.0
12	C.3	91.5	80.8	26.9	2	0	1.0
13	N.9	92.8	75.6	13.5	3	0	0.3

Fig. 3.5 Results of predicting possible pathways of VAZ_07 metabolism involving CYP2C9 (SMARTCyp software package)

3A4 2D6 2C9

1



2D6 Ranking	Atom	2D6 Score	Energy	2DSASA	Span2end	N+dist	Similarity
1	C.18	58.2	59.5	33.5	0	0	1.0
2	C.22	59.6	62.2	64.3	0	0	1.0
3	C.13	67.2	41.1	20.0	5	0	0.4
4	C.17	71.8	59.5	27.2	2	0	0.7
5	C.1	73.6	68.2	32.7	1	0	1.0
6	C.5	79.5	80.8	33.5	0	0	1.0
6	C.16	79.5	74.1	33.5	1	0	1.0
7	N.14	80.6	54.1	9.5	4	0	0.3
8	C.20	82.7	77.2	29.4	1	0	0.7
9	C.11	84.3	66.4	54.4	3	0	0.7
10	C.6	91.9	86.3	28.5	1	0	1.0
11	C.3	93.1	80.8	26.9	2	0	1.0
12	N.9	95.2	75.6	13.5	3	0	0.3

Fig. 3.6 Results of predicting possible pathways of VAZ_07 metabolism involving CYP2D6 (SMARTCyp software package)

If oxidative deamination predictably leads to a complete loss of the original molecular architecture, O-demethylation does not significantly affect the activity profile and can be considered as an intermediate step, aromatic hydroxylation is also unlikely to be interesting in terms of pharmacological activity of metabolites, then aliphatic hydroxylation of the methyl group again opens the prospect of further oxidation to biologically active kynurenic acid derivatives.

The results of predicting the possible pathways of VAZ_07 metabolism involving CYP2C9 and CYP2D6 indicate mainly the same directions as those involving CYP3A4 (Figs. 3.5 and 3.6).

It should be noted that the results of predicting the directions of biotransformation of VAZ_07 using different systems with different algorithms largely coincide or correlate well with each other.

Fig. 3.7 shows a fragment of the protocol for predicting possible pathways of VAZ_07 metabolism using the online Biotransformer system. In total, the system calculated the possibility of formation of 11 different metabolites, the vast majority of which are products of aromatic hydroxylation at different positions of both the quinolone heterocyclic system and the phenyl substituent. This is quite predictable given the biochemical nature of the processes catalyzed by CYP enzymes. One of the possible directions is also the O-demethylation of the methoxyl substituent in the para-position of the phenyl substituent of the aminomethyl fragment. Among the predicted metabolites is also a 2-hydroxymethyl derivative, which is a product of aliphatic hydroxylation of a reactive methyl group at position 2 of the quinolone ring. Also, some of the predicted metabolites indicate the possibility of oxidative deamination. Thus, it can be stated that the main directions of biotransformation predicted by the Biotransformer system completely coincide with the results of previous programs, despite the differences in calculation algorithms.

BioTransformer 3.0 Metabolism Prediction About Download Contact Help

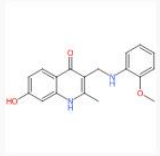
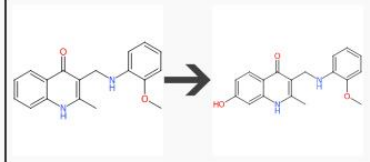
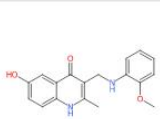
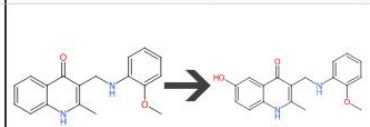
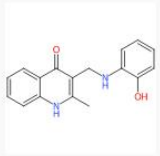
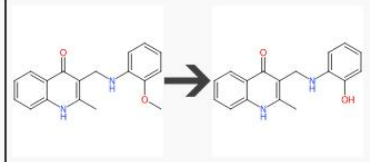
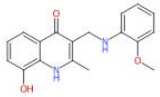
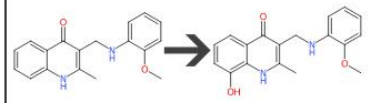
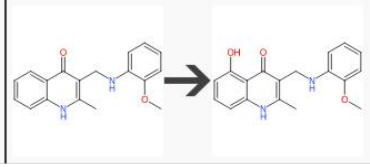
Result ID	Predicted Result	SMILES	Chemical Formula	Isotope Mass (Da)	Reaction Type	Reaction Info	Biotransformation Reaction
1		COC1=CC=CC=C1NCC2=C(C)NC3=CC(=CC=C3C2=O)O	C18H18N2O3	310.1317	Aromatic hydroxylation of fused benzene ring AndFromCyProduct	Enzyme: Cytochrome P450 1A2 BioSystem: HUMAN	
2		COC1=CC=CC=C1NCC2=C(C)NC3=CC=C(C=C3C2=O)O	C18H18N2O3	310.1317	Aromatic hydroxylation of fused benzene ring AndFromCyProduct	Enzyme: Cytochrome P450 1A2 BioSystem: HUMAN	
3		OC1=CC=CC=C1NCC2=C(C)NC3=CC=CC=C3C2=O	C17H16N2O2	280.1211	O-Dealkylation AndFromCyProduct	Enzyme: Cytochrome P450 1A2 BioSystem: HUMAN	
4		COC1=CC=CC=C1NCC2=C(C)NC3=C(C=CC=C3C2=O)O	C18H18N2O3	310.1317	Aromatic hydroxylation of fused benzene ring	Enzyme: Cytochrome P450 1A2 BioSystem: HUMAN	
5		COC1=CC=CC=C1NCC2=C(C)NC3=CC=CC(=C3C2=O)O	C18H18N2O3	310.1317	Aromatic hydroxylation of fused benzene ring	Enzyme: Cytochrome P450 1A2 BioSystem: HUMAN	

Fig. 3.7 Excerpt from the protocol for predicting possible metabolic pathways of VAZ_07 using the online system Biotransformer

The analysis of the predicted metabolites of VAZ_07 using the GLORYx online system confirms that the main pathways of metabolic transformations of the studied molecule are O-demethylation (which begins with hydroxylation of the corresponding methoxyl group), aromatic hydroxylation, oxidative deamination of the amino methyl fragment at position 3 and oxidation of the methyl group at position 2 of the quinolone ring (Fig. 3.8).

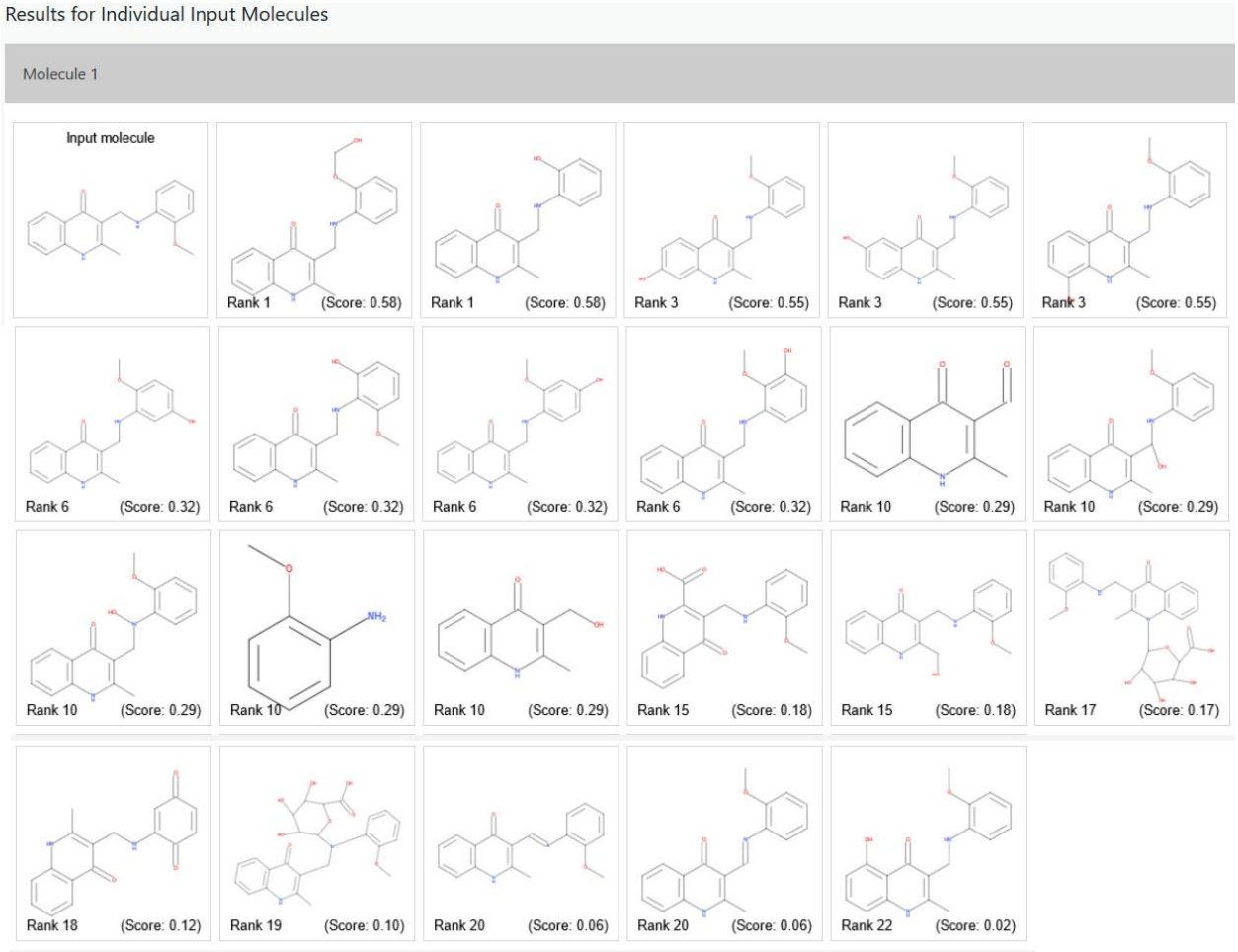


Fig. 3.8 Results of predicting possible metabolic pathways of VAZ_07 using the online GLORYx system

One of the most informative is the forecasting results using the online service Way2Drug RA, which are graphically represented in Fig. 3.9. This software product provides only indicators of the probability of a particular process, so visualization of the results requires a certain expert understanding of the nature of biotransformation changes to extrapolate specific processes that may occur with respect to the compound under study.

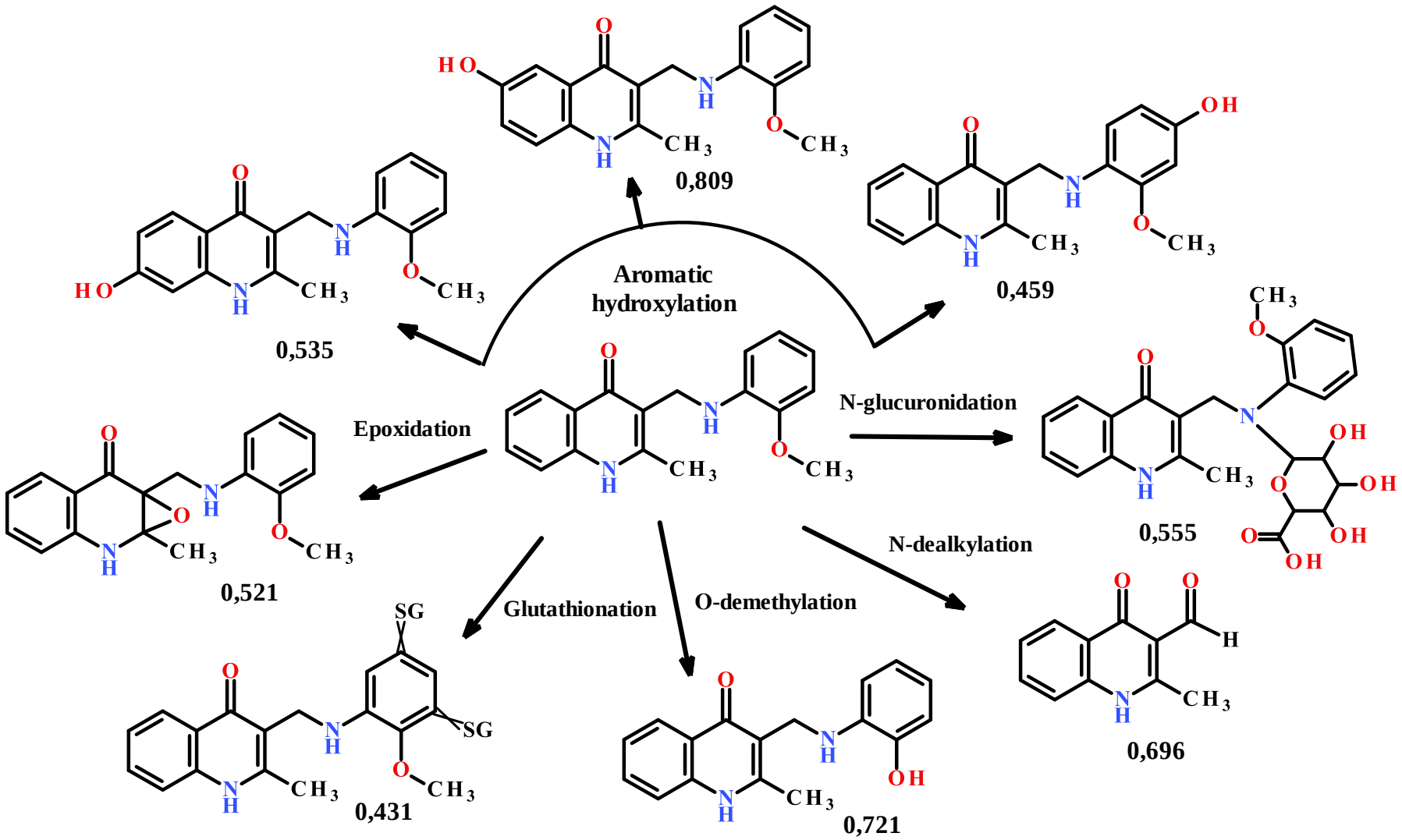


Fig. 3.9 Results of predicting possible metabolic pathways of VAZ_07 using the Way2Drug RA system (only the pathways with the highest DeltaP values are shown)

The prediction results are shown in Fig. 3.9, and the main pathways predicted are the variants of aromatic hydroxylation involving carbon atoms at positions 6, 7, and 8 of the quinolone heterocyclic system as well as *para*-position of the phenyl substituent. The most reactive in terms of aromatic hydroxylation is position 6 of the quinolin-4-one system (Fig. 3.9). It should be noted that according to the forecast of the Way2Drug RA program, the directions of O-demethylation and N-dealkylation also have a high probability. In addition, in addition to the reactions of the first phase of metabolism, this system suggests the processes of the synthetic phase – conjugation with glucuronic acid at the nitrogen atom of the aminomethyl fragment and with glutathione with the participation of ortho-positions to the methoxyl group in the phenyl substituent.

Special attention should be paid to the GLORYx module of the system, which allows predicting the substrate specificity of a compound to certain CYP isoforms (Fig. 3.10).

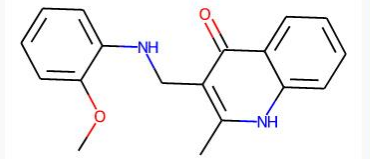
2D structure	CYP1A2	CYP2A6	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A4
	Substrate	Non-substrate	No prediction	Non-substrate	No prediction	No prediction	Substrate	Non-substrate	No prediction

Fig. 3.10 Substrate specificity of VAZ_07 to cytochrome P450 isoforms according to the results of the online GLORYx system

Such an assessment makes it possible to predict possible metabolic interactions of a substance with known cytochrome substrates at the early stages of research on promising molecules when used simultaneously. As can be seen from Fig. 3.10, VAZ_07 is highly likely to be metabolized by cytochromes CYP1A2 and CYP2D6.

Thus, a comprehensive analysis of the results of predicting the possible pathways of VAZ_07 metabolism using five different online systems allows us to conclude that the molecule 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinolin-4-one can be intensively metabolized with the participation of cytochrome P450

enzyme systems. The main directions are aromatic hydroxylation of the test substance molecule with the participation of carbon atoms of the quinolone heterocyclic system, O-demethylation of the methoxy group, N-dealkylation of the amino methyl fragment. In this case, the predicted metabolites are unlikely to significantly affect the overall pharmacological activity profile of the parent molecule. However, the possible directions of aliphatic hydroxylation at the methyl group at position 2 of the heterocycle to kynurenic acid derivatives suggest that the proven pharmacodynamic effects of VAZ_07, namely nootropic and sedative, may be at least partially provided by these pharmacologically active metabolites.

The general regularities of biotransformation transformations of 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinolin-4-one completely coincide and are fully consistent with the current views of medicinal chemistry on the reactivity of xenobiotics under the influence of cytochrome P450 enzyme systems in the human body. The results obtained using different systems differ somewhat, which is fully explained by the difference in the calculation algorithms underlying the software products.

Conclusions to the Chapter 3

1. A computer prediction of possible pathways of biotransformation of a promising compound - 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinolin-4-one (laboratory code VAZ_07) was performed using five different online resources that are freely available.

2. The results obtained indicate that the molecule 2-methyl-3-[(2-methoxy-anilino)methyl]-1H-quinoline-4-one in the human body can be intensively metabolized with the participation of cytochrome P450 enzyme systems.

3. The most probable pathways of metabolism of the test compound are aromatic hydroxylation of the test substance molecule with the participation of carbon atoms of both the quinolone heterocyclic system and the phenyl substituent, O-demethylation of the methoxyl group, N-dealkylation of the amino methyl fragment.

4. The predicted direction of aliphatic hydroxylation at the methyl group at position 2 of the heterocycle to kynurenic acid derivatives suggests that the proven pharmacodynamic effects of VAZ_07 may be partially provided by these pharmacologically active metabolites.

5. According to the results of the GLORYx system module, which allows predicting the substrate specificity of a compound to certain CYP isoforms, the investigated compound is most likely to be metabolized by cytochromes CYP1A2 and CYP2D6.

6. According to the results of the Xenosite program, 2-methyl-3-[(2-methoxy-anilino)methyl]-1H-quinolin-4-one has low potential interaction with the reduced glutathione system, proteins and low potential for the formation of cyanides, quinones or epoxides, However, there is a certain probability of interaction with cell DNA.

GENERAL CONCLUSIONS

1. The article systematizes and analyzes the current scientific literature on the main *in vitro* and *in silico* methods used to predict possible pathways of chemical metabolism in the human body. The analysis confirms the prospects of using the software to predict possible metabolites of a potential drug at the early stages of its development.

2. The methods for the synthesis of 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinolin-4-one (laboratory code VAZ_07) are presented. The prospects of in-depth pharmacological study of VAZ_07 as a potential API with sedative and nootropic properties are substantiated. The choice and analysis of the calculation algorithms used in the work of online computer prediction systems for possible metabolic pathways in the human body was substantiated.

3. The computer prediction of possible pathways of biotransformation of a promising compound with sedative and nootropic action – 2-methyl-3-[(2-methoxy-anilino)methyl]-1H-quinolin-4-one (laboratory code VAZ_07) using five different online resources that are freely available.

4. The most probable pathways of the metabolism of the test compound are aromatic hydroxylation of the test substance molecule with the participation of carbon atoms of both the quinolone heterocyclic system and the phenyl substituent, O-demethylation of the methoxyl group, N-dealkylation of the amino methyl fragment. Predicted directions of aliphatic hydroxylation at the methyl group at position 2 of the heterocycle to kynurenic acid derivatives support the assumption that the proven pharmacodynamic effects of VAZ_07 may be partially provided by these pharmacologically active metabolites.

5. According to the results of the GLORYx system module, which allows predicting the substrate specificity of a compound to certain CYP isoforms, the tested compound is most likely metabolized by cytochromes CYP1A2 and CYP2D6.

6. According to the results of the Xenosite program, 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinolin-4-one has low potential interaction with the reduced glutathione system, proteins and low potential for the formation of cyanides, quinones or epoxides, however, there is a certain probability of interaction with cell DNA.

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Ф А 2.2.1–32-366

National University of Pharmacy

Faculty for foreign citizens' education
Department medicinal chemistry
Level of higher education master
Specialty 226 Pharmacy, industrial pharmacy
Educational program Pharmacy

APPROVED
The Head of Department
of medicinal chemistry

Lina PEREKHODA
"_22nd_" of August 2022

ASSIGNMENT
FOR QUALIFICATION WORK
OF AN APPLICANT FOR HIGHER EDUCATION

Abdelilah ELHARRAB

1. Topic of qualification work: « Prediction of possible metabolic pathways of potential API with sedative and nootropic effects », supervisor of qualification work: Illya Podolsky, DSc approved by order of NUPh from "6th" of February 2023 № 35

2. Deadline for submission of qualification work by the applicant for higher education: April 2023.

3. Outgoing data for qualification work: biotransformation of drugs in the human body, promising biologically active substance, pharmacological activity, computer online systems for predicting xenobiotic metabolism, reactions of the first phase of metabolism, reactions of the second phase of metabolism, oxidation, aromatic hydroxylation, aliphatic hydroxylation, dealkylation, metabolic site, biologically active metabolites.

4. Contents of the settlement and explanatory note (list of questions that need to be developed): substantiation of the need to study possible ways of biotransformation of a potential API with sedative and nootropic properties; analysis and selection of computer prediction systems; characterization of materials and research methods used in the experiment; computer prediction; the processing of the results.

5. List of graphic material (with exact indication of the required drawings):

Tables – 3, figures – 16, scheme – 1.

6. Consultants of chapters of qualification work

Chapter	Name, SURNAME, position of consultant	Signature, date	
		assignment was issued	assignment was received
1	Illya PODOLSKY, associate professor of higher education institution of department of medicinal chemistry	September 2022	September 2022
2	Illya PODOLSKY, associate professor of higher education institution of department of medicinal chemistry	November 2022	November 2022
3	Illya PODOLSKY, associate professor of higher education institution of department of medicinal chemistry	January 2023	January 2023

7. Date of issue of the assignment: "_22nd_" of August 2022

CALENDAR PLAN

№	Name of stages of qualification work	Deadline for the stages of qualification work	Notes
1	Selection and study of information sources for writing a qualification work, a compilation of a bibliographic list of information sources	Sept-Nov 2022	**done**
2	Review and analysis of algorithms of online computer systems for predicting xenobiotic metabolism. Choosing software products and analyzing the features of working with them	Dec 2022 - Jan 2023	**done**
3	Prediction of probable metabolic pathways of 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinoline-4-one, a potential API with antihypoxic and sedative effects	Jan-Feb 2023	**done**
4	Analysis of the results obtained	March 2023	**done**
5	Preparation of qualification work and submission to the Examination Commission	April 2023	**done**

An applicant of higher education _____ Abdelilah ELHARRAB

Supervisor of qualification work _____ Illya PODOLSKY

ВИТЯГ З НАКАЗУ № 35
По Національному фармацевтичному університету
від 06 лютого 2023 року

нижченаведеним студентам 5-го курсу 2022-2023 навчального року, навчання за освітнім ступенем «магістр», галузь знань 22 охорона здоров'я, спеціальності 226 – фармація, промислова фармація, освітня програма – фармація, денна форма здобуття освіти (термін навчання 4 роки 10 місяців та 3 роки 10 місяців), які навчаються за контрактом, затвердити теми кваліфікаційних робіт:

Прізвище студента	Тема кваліфікаційної роботи		Посада, прізвище та ініціали керівника	Рецензент кваліфікаційної роботи
• **по кафедрі медичної хімії**				
Ельгарраб Абделілах	Прогнозування ймовірних шляхів метаболізму потенційного АФІ седативної та ноотропної дії	Prediction of possible metabolic pathways of potential API with sedative and nootropic effects	доц. Подольський І.М.	проф. Власов С.В.

Підстава: подання декана, згода ректора

Ректор

Вірно. Секретар

Ф А2.8-47-110

ВИСНОВОК

Комісії з академічної доброчесності про проведену експертизу
щодо академічного плагіату у кваліфікаційній роботі
здобувача вищої освіти

№ 112832 від « 1 » травня 2023 р.

Проаналізувавши випускну кваліфікаційну роботу за магістерським рівнем здобувача вищої освіти денної форми навчання Ельгарраб Абделілах, 5 курсу, _____ групи, спеціальності 226 Фармація, промислова фармація, на тему: «Прогнозування ймовірних шляхів метаболізму потенційного АФІ седативної та ноотропної дії / Prediction of possible metabolic pathways of potential API with sedative and nootropic effects», Комісія з академічної доброчесності дійшла висновку, що робота, представлена до Екзаменаційної комісії для захисту, виконана самостійно і не містить елементів академічного плагіату (компіляції).

Голова комісії,
професор **Інна ВЛАДИМИРОВА**

9%
31%

Ф А 2.2.1-32-353

REVIEW

of scientific supervisor for the qualification work of the master's level of higher education of the specialty 226 Pharmacy, industrial pharmacy

Abdelilah ELHARRAB

on the topic: «Prediction of possible metabolic pathways of potential API with sedative and nootropic effects»

Relevance of the topic. To reduce the risk of withdrawal of drug candidates at the clinical trial stage due to unfavorable metabolic characteristics of molecules, effective and reliable methods of predicting the metabolism of a biologically active compound *in silico*, *in vitro,* and *in vivo* are needed. Experimental studies of possible pathways of biotransformation of new molecules *in vitro* and *in vivo* are always non-trivial and resource-intensive tasks. That is why the use of computer prediction of possible metabolic pathways of a potential drug candidate at the initial stages is a fully justified and effective approach that allows identifying metabolic sites, predict the structures of the formed metabolites, metabolic rate, and specificity of substrates to cytochrome P450 enzymes. The chosen topic of the qualification work is aimed at solving these issues, which determines its relevance.

Practical value of conclusions, recommendations and their validity. The obtained results of the study expand the knowledge of possible metabolic pathways of 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinolin-4-one, a substance that is a promising API with sedative and nootropic properties. The results obtained can significantly expand and deepen the understanding of both pharmacodynamic and pharmacokinetic features of the promising API candidate, subject to further in-depth pharmacological research and the introduction of the compound into medical practice.

Assessment of work. The qualification work has a classical structure: an introduction, 3 chapters (literature review and 2 chapters of experimental research), conclusions and a list of references. The work thoroughly substantiates the relevance

of the topic, describes in detail the materials and research methods, consistently presents the results of computer forecasting, conducts a thorough analysis of the results, and logically formulates conclusions. The research is performed at a modern and high level, and the conclusions drawn are not in doubt.

General conclusion and recommendations on admission to defend. The qualification work of Abdelilah ELHARRAB meets the requirements for qualification works in terms of the relevance and scope of the performed research, the novelty of the obtained results, their theoretical and practical significance and can be recommended for defense at the Examination Commission.

Scientific supervisor _____ Illya PODOLSKY

«7th» of April 2023

Ф А 2.2.1-32-356

REVIEW

for qualification work of the master's level of higher education, specialty 226 Pharmacy, industrial pharmacy

Abdelilah ELHARRAB

on the topic: «Prediction of possible metabolic pathways of potential API with sedative and nootropic effects »

Relevance of the topic. properties, 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinolin-4-one. During metabolic transformations of biologically active molecules in the human body, metabolites with physicochemical and pharmacological properties that differ significantly from those of the parent compounds may be produced, which is important both in terms of efficacy and safety of medicines. Experimental studies of possible pathways of biotransformation of new molecules *in vitro* and *in vivo* are always non-trivial and resource-intensive tasks. That is why the use of computer prediction of possible metabolic pathways of a potential drug candidate at the initial stages is a fully justified and effective approach that allows identifying metabolic sites, predict the structures of the formed metabolites, metabolic rate, and specificity of substrates to cytochrome P450 enzymes. Such studies are of particular importance at the early stages of studying the properties of an API candidate in order to reduce the risk of withdrawal of drug candidate compounds at the stage of clinical trials due to the metabolic characteristics of the molecules. The chosen topic of the qualification work is aimed at addressing such issues, which determines its relevance.

Theoretical level of work. The qualification work was performed at a high theoretical level, since its results, in addition to their practical significance, have significant methodological potential. The methodological approach to predicting possible pathways of xenobiotics metabolism in the human body using various algorithms developed in the course of the work should be recommended for use by scientists in their applied research.

Author's suggestions on the research topic. The results obtained by the author indicate that the molecule 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinolin-4-

one can be intensively metabolized by cytochrome P450 enzyme systems. The most likely pathways of metabolism of the compound under study are aromatic hydroxylation involving carbon atoms of both the quinolone heterocyclic system and the phenyl fragment. The predicted direction of aliphatic hydroxylation at the methyl group at position 2 of the heterocycle to kynurenic acid derivatives suggests that the proven pharmacodynamic effects of 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinolin-4-one may be partially provided by these pharmacologically active metabolites.

Practical value of conclusions, recommendations and their validity. The obtained results of the study expand the knowledge of possible metabolic pathways of 2-methyl-3-[(2-methoxyanilino)methyl]-1H-quinolin-4-one, a substance that is a promising compound with sedative and nootropic properties. The results obtained can significantly expand and deepen the understanding of both pharmacodynamic and pharmacokinetic features of a promising candidate for APIs, subject to further in-depth pharmacological research and implementation of the compound in medical practice. The conclusions are logically formulated on the basis of the data obtained and do not raise any doubts.

Disadvantages of work. The qualification paper contains grammatical errors, incorrect hyphenations of chemical names, and some mistakes in the formatting of references, but they are minor and do not reduce the overall value of the paper.

General conclusion and assessment of the work. The qualification work of Abdelilah ELHARRAB in terms of relevance, scientific novelty of the obtained results, methodological level, theoretical and practical significance, volume of performed research meets the requirements of the Regulation on the Procedure for the Preparation and Defense of Qualification Works at the National Pharmaceutical University and can be recommended for defense at the Examination Commission.

Reviewer _____ prof. Serhiy VLASOV

«14th» of April 2023

Ф А 2.2.1-91-287

ВИТЯГ
з протоколу засідання кафедри медичної хімії
№ 10 від 21 квітня 2023 р.

ПРИСУТНІ:

проф. Ліна ПЕРЕХОДА, проф. Андрій ФЕДОСОВ, доц. Вадим ЗУБКОВ, доц. Ірина СИЧ, доц. Віталій ЯРЕМЕНКО, доц. Ілля ПОДОЛЬСЬКИЙ, доц. Наталія КОБЗАР, доц. Марина РАХІМОВА, доц. Маргарита СУЛЕЙМАН, ас. Олена БЕВЗ, ас. Ольга ВІСЛОУС

ПОРЯДОК ДЕННИЙ:

Звіт про стан виконання кваліфікаційної роботи здобувача вищої освіти факультету з підготовки іноземних громадян Фм18(5,0д)англ-02 групи, спеціальності «226 Фармація, промислова фармація», освітньої програми «Фармація» Абделілаха ЕЛЬГАРРАБА на тему: «Прогнозування ймовірних шляхів метаболізму потенційного АФІ седативної та ноотропної дії / Prediction of possible metabolic pathways of potential API with sedative and nootropic effects»

СЛУХАЛИ: доповідь здобувача вищої освіти факультету з підготовки іноземних громадян Фм18(5,0д)англ-02 групи, спеціальності «226 Фармація, промислова фармація», освітньої програми «Фармація» Абделілаха ЕЛЬГАРРАБА на тему: «Прогнозування ймовірних шляхів метаболізму потенційного АФІ седативної та ноотропної дії / Prediction of possible metabolic pathways of potential API with sedative and nootropic effects», керівник – доцент закладу вищої освіти кафедри медичної хімії, д.фарм.н., доцент Ілля ПОДОЛЬСЬКИЙ.

УХВАЛИЛИ: рекомендувати кваліфікаційну роботу Реди БЕНОТМАНА до офіційного захисту в Екзаменаційній комісії.

Завідувачка кафедри медичної хімії,
професор **Ліна ПЕРЕХОДА**

Секретар кафедри медичної хімії,
доцент **Марина РАХІМОВА**

Ф А 2.2.1-32-042

НАЦІОНАЛЬНИЙ ФАРМАЦЕВТИЧНИЙ УНІВЕРСИТЕТ

ПОДАННЯ
ГОЛОВІ ЕКЗАМЕНАЦІЙНОЇ КОМІСІЇ
ЩОДО ЗАХИСТУ КВАЛІФІКАЦІЙНОЇ РОБОТИ

Направляється здобувач вищої освіти Абделілах ЕЛЬГАРРАБ до захисту кваліфікаційної роботи
за галуззю знань 22 Охорона здоров'я
спеціальністю 226 Фармація, промислова фармація
освітньою програмою Фармація
на тему: «Прогнозування ймовірних шляхів метаболізму потенційного АФІ седативної та ноотропної дії».

Кваліфікаційна робота і рецензія додаються.

Декан факультету _____ / Світлана КАЛАЙЧЕВА /

Висновок керівника кваліфікаційної роботи

Здобувач вищої освіти Абделілах ЕЛЬГАРРАБ у повному обсязі виконав кваліфікаційну роботу. За актуальністю, методичним рівнем, теоретичним та практичним значенням, об'ємом виконаних досліджень кваліфікаційна робота відповідає вимогам і допускається до захисту в Екзаменаційній комісії.

Керівник кваліфікаційної роботи

_____ Ілля ПОДОЛЬСЬКИЙ

«07» квітня 2023 р.

Висновок кафедри про кваліфікаційну роботу

Кваліфікаційну роботу розглянуто. Здобувач вищої освіти Абделілах ЕЛЬГАРРАБ допускається до захисту даної кваліфікаційної роботи в Екзаменаційній комісії.

Завідувачка кафедри
медичної хімії

_____ Ліна ПЕРЕХОДА

«21» квітня 2023 р.

Qualification work was defended

of Examination commission on

« » of June 2022

With the grade _____

Head of the State Examination commission,

DPharmSc, Professor

_____ / Oleh SHPYCHAK /