PharmaExpert

user guide

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CHAPTER 1
ABOUT THIS MANUAL

ORGANISATION OF THIS DOCUMENT

This document consists of eight chapters.

- **Chapters 1 - 2** contain general information about the program.
- **Chapters 3** contains theoretical introduction to using terms and definitions.
- **Chapters 4 – 5** describe how to work with the program.
- **Chapter 6** contains detailed description of PharmaExpert interface.
- **Chapter 7** describes how to interpret the results obtained.
- **Chapter 8** describes what to do in case of trouble shooting.

ABBREVIATIONS

DBMS - Database Management System

IEP - Invariant Error of Prediction

MNA - Multilevel Neighbourhood of Atoms

PASS - Prediction of Activity Spectra for Substances

QSAR - Quantitative Structure-Activity Relationships

QSPR - Quantitative Structure-Property Relationships

SAR - Structure-Activity Relationships

MOLfiles - Molecule files: Each MOLfile describes a single molecular structure

SDfiles – Structure-data files: An SDfile contains structures and/or data for any number of molecules. SDF is the primary format for import/export of chemical data

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HOW TO CONTACT US

If you have any questions about PharmaExpert program, please contact us by E-mail: alexey.lagunin@ibmc.msk.ru or pass@ibmc.msk.ru

or by post:

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CHAPTER 2
PharmaExpert software product

ABOUT PharmaExpert

PharmaExpert software was developed to analyze the biological activity spectra of substances predicted by PASS program. This software has a flexible mechanism of selecting compounds with the desirable types of biological activity. It gives a quick answer about relationships between biological activities, drug-drug interactions and multiple targeting of chemical compounds. This analysis is based on the knowledge of "mechanism-effect(s)" and "effect-mechanism(s)" relationships.

All types of biological activity are divided into six classes: mechanisms of action, pharmacological effects, toxic and side effects, metabolic terms, transporter terms and gene expression terms. Typical mechanism of action is a description of the biologically active compound interactions with biological entities at macromolecular level, for example, *Acetylcholinesterase inhibitor*, *Acetylcholine release inhibitor* or *Alpha 1 adrenoreceptor agonist*. Pharmacological effect is a description of pharmaco-therapeutic action of compounds e.g., *Antiischemic*, *Anxiolytic* or *Alzheimer's disease treatment*. Toxic or side effect is description of specific toxic effect (e.g. *Mutagenic*, *Teratogenic* and so on) or side effect (*Arrhythmogenic*, *Anemic*, *Nauseant* and so on). Metabolic activities reflect interactions of chemical compounds with metabolic enzymes, for examples, *CYP2D6 inhibitor*, *CYP3A4 substrate*, *CYP 2C9 inducer*. Transporter terms are terms associated with transport of drugs (P-glycoprotein substrate, P-glycoprotein inhibitor, P-glycoprotein inductor, etc.). Gene expression terms are terms associated with gene expression (APOA1 expression enhancer, ErbB-2 expression inhibitor, etc.). PharmaExpert includes activity names database, a "mechanism-effect" relationships knowledgebase and a module for analysis of predicted biological activity spectra based on "mechanism-effect(s)" and "effect-mechanism(s)" relationships.

PharmaExpert is a commercially available software product.

HARDWARE AND SOFTWARE SYSTEM REQUIREMENTS

Processor	x86 family - Intel® Pentium® or compatible.
Operating environment	Microsoft® Windows® 98/NT/2000/XP/Vista/7.
Memory	512 MB of RAM (1024 MB or more recommended).
Hard disk	minimum 60 MB free hard disc space.
Display	1024x768 or higher resolution.

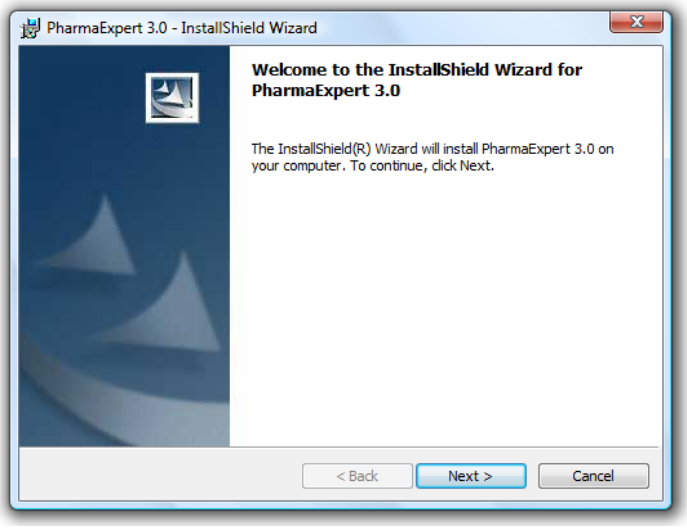
Mouse or other compatible pointing device is recommended.

Chemical structure information is represented as MOLfiles or SDfiles (formats of SYMYX MDL, http://www.symyx.com/index.jsp), which can be exported from many chemical Database Management Systems. The only requirement – these files should correspond with the ISIS/Base V2000 and/or V3000 standard.

PHARMAEXPERT INSTALLATION PROCEDURE AND ACTIVATION, CONFIGURATION FILES

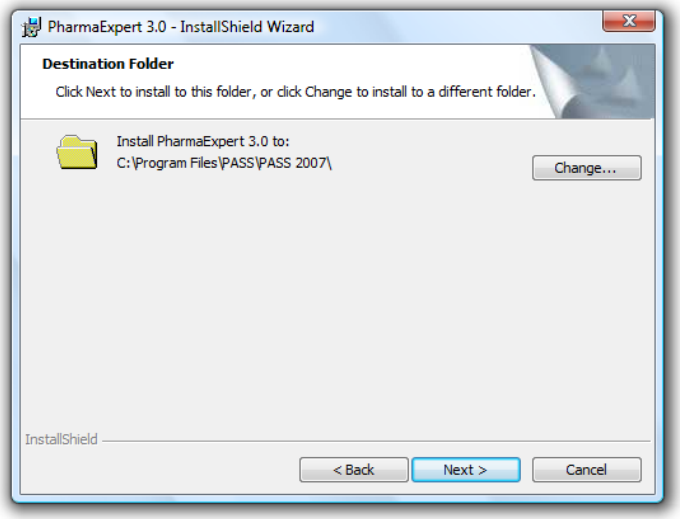
Installation procedure

1) Unpack and run up the archived file downloaded via Internet - setup.exe (double click on the left mouse button).
2) When the program starts the following screen appears:

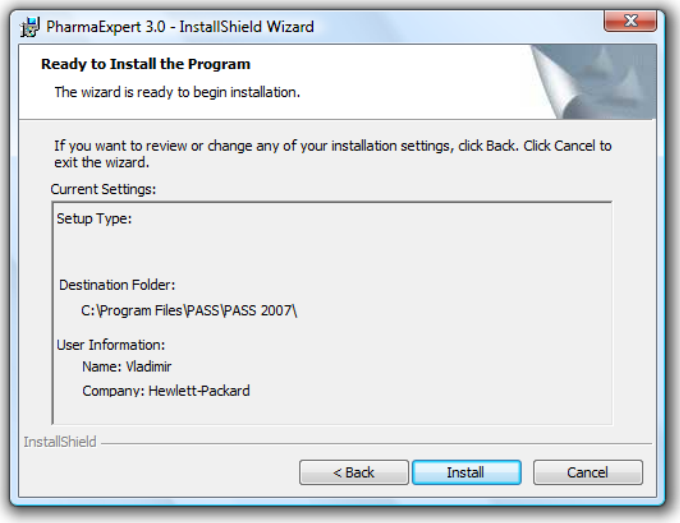


The program requires about 10 MB of free disc space for installation.

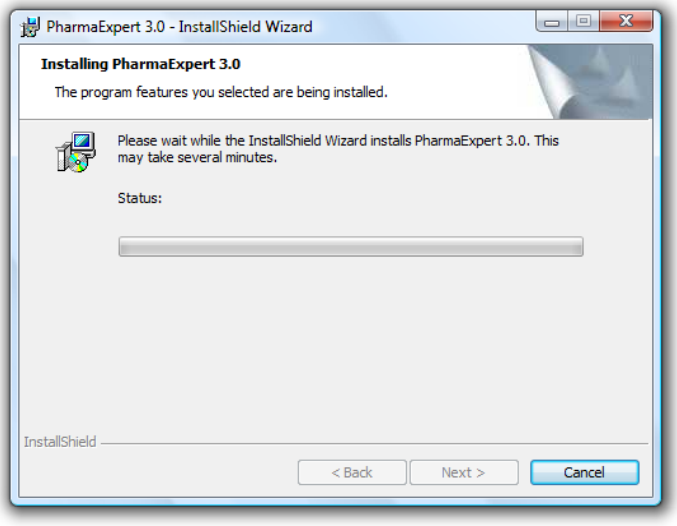
3) Please select a folder, into which the program will be installed. C:\Program Files\PASS\PASS 2009\ is the default folder (for the version of PharmaExpert delivered with PASS 2009, it may be changed according to the version of PASS).



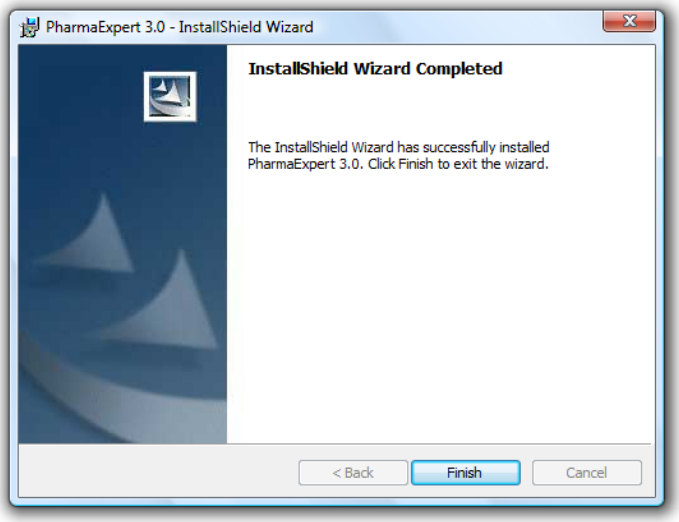
Please follow the instructions, which appear on the display, up to the following screen:



4) Press the «Install» button. After that the installation process starts:



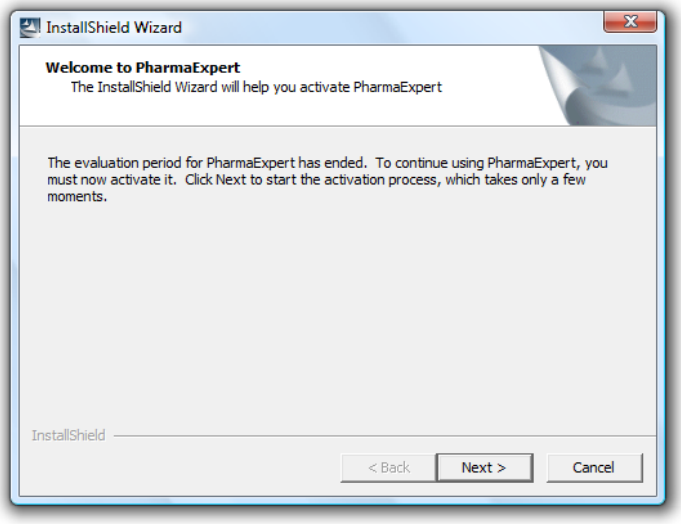
5) When the program has been installed, press the «Finish» button:



6) The folder appears with the installed PharmaExpert program. By default: C:\Program Files\PASS\PASS 2010\ (for the version of PharmaExpert delivered with PASS 2010, it may be changed according to the version of PASS).

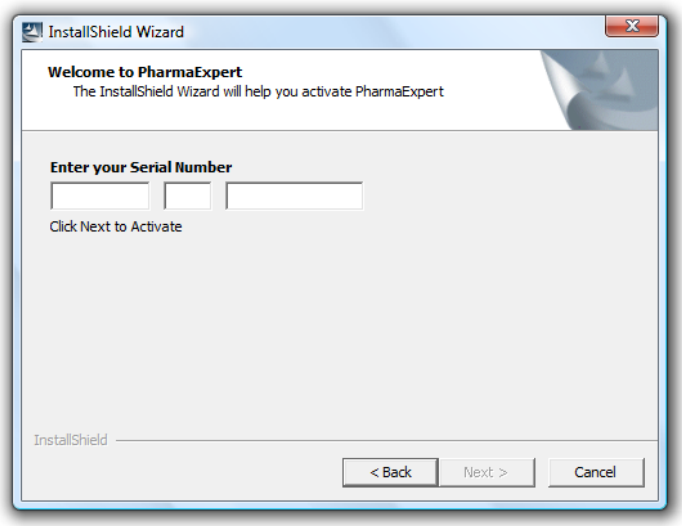
Run the program PharmaExpert.exe (double click on the left mouse button).

7) The following screen appears:



Press the «Next» button.

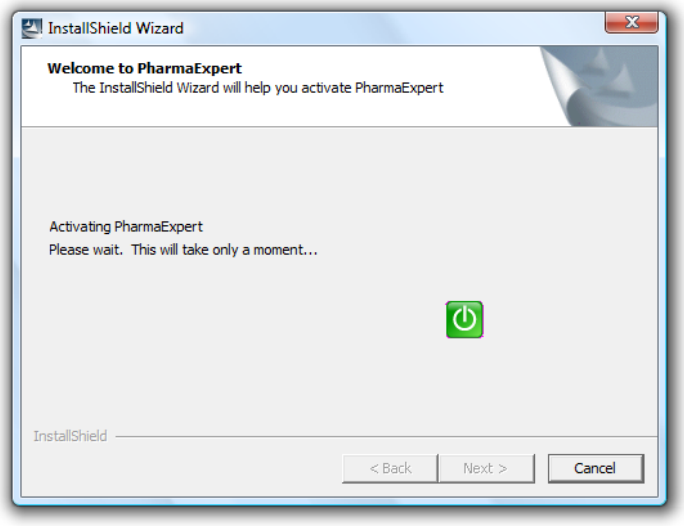
8) Please, enter the Serial Number e-mailed by PharmaExpert team, according to the picture:



For automatic activation of this program, computer MUST BE CONNECTED TO the INTERNET.

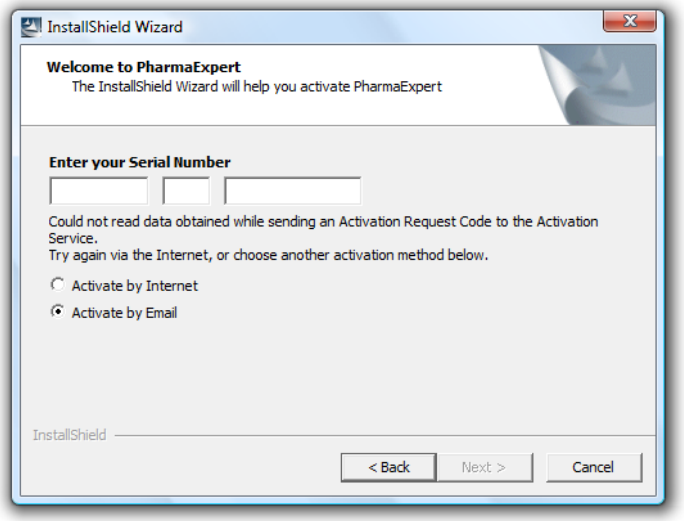
Press the «Next» button.

9) The program activates automatically:



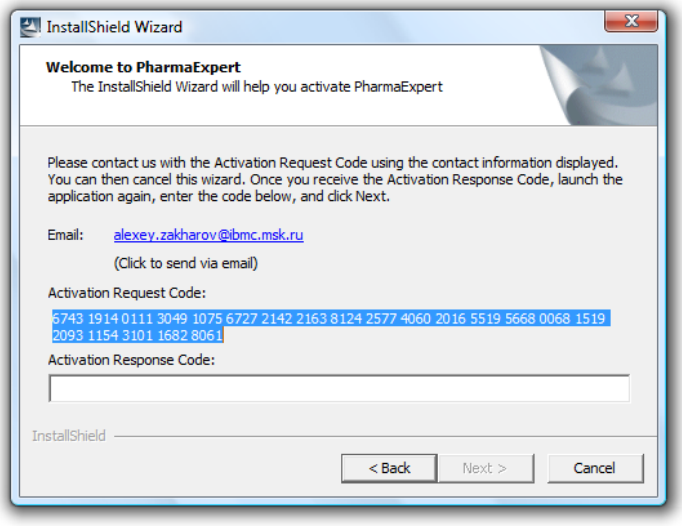
Note! If, for any reason, the computer on which you try to install the program is not connected to the Internet, please, contact us to activate the program using E-mail (according to the procedure described below).

10) If you do not have the INTERNET CONECTION, the following screen appears:



Choose «Activate by e-mail»

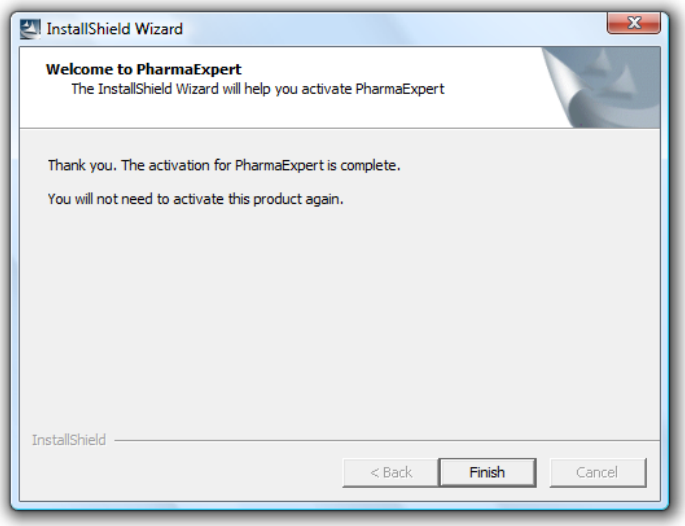
11) Then the «Activation Request Code» appears:



Please send this code to alexey.zakharov@ibmc.msk.ru with copy to vladimir.poroikov@ibmc.msk.ru

Then we shall e-mail you the «Activation Response Code». Please, enter this code and click the «Next» button.

12) After the activation press «Finish» button:



13) Later, you can use PharmaExpert without any restrictions, even if your computer is disconnected of the Internet.

Configuration file

*.**HLP** is a help file.

CHAPTER 3
TERMS AND DEFINITIONS

BIOLOGICAL ACTIVITY

In computer programs PharmaExpert and PASS biological activities are described qualitatively ("active" or "inactive"). Reflecting the result of chemical compound's interaction with a biological object, the biological activity depends on both the compound's molecular structure and the terms & conditions of the experiment. Therefore, structure-activity relationship analysis based on qualitative presentation of biological activity describes general "biological potential" of the molecule being studied. On the other hand, qualitative presentation allows integrating information concerning compounds collected from many different sources as in the PASS training set.

BIOLOGICAL ACTIVITY SPECTRUM

A Biological Activity Spectrum for a substance is the list of biological activity types for which the probability to be revealed (**Pa**) and the probability not to be revealed (**Pi**) are calculated. PASS calculates **Pa** and **Pi** for every activity type to be predicted. They are independent quantities and their values vary from 0 to 1.

DRUG-LIKENESS

The perspective compounds with desirable pharmacological activity may reveal side effects, toxicity or other characteristics that limit their bioavailability or influence to metabolism and excretion. Therefore biological activity should be balanced with characteristics of drugs, so-called drug-likeness. This is an integrative characteristic including peculiar to drugs features: bioavailability, absence of side/toxic effects, absence of toxic metabolites and acceptable excretion.

In PASS drug-likeness varies from 0 to 1, where 0 means that compounds is quite dissimilar to drugs and 1 means that compound is very similar to drugs. The prediction of drug-likeness is based on a special SAR Base that includes a set of 6842 drug-like compounds from World Drug Index and MDDR (MDL Drug Data Report) database that are marked and registered drugs. The SAR Base also includes a set of non-drugs including

14828 structures of compounds from Available Chemical Directory database. The accuracy of PASS drug-likeness prediction for training set calculated by leave-one-out cross-validation is about 92% [1].

(1) Anzali S, Barnickel G, Cezanne B, Krug M, Filimonov D, Poroikov V. Discriminating between drugs and nondrugs by prediction of activity spectra for substances (PASS). J Med Chem. 2001, 44(15), 2432-2437.

"MECHANISM-EFFECT" RELATIONSHIPS KNOWLEDGEBASE

The "Mechanism<->Effect" Relationships knowledgebase (MER Base) has been created for search, storing and analysis of data on cause-and-effect relationships between different types of biological activity. Each record of a biological activity type contains a parameter describing its belonging to the mechanism(s) of action or pharmacological effect(s) and a list of relationships with the other types of biological activity. Two categories of relationships are defined: "classification" and "mechanism-effect" relationships. The "classification" type of relationships shows that one type of biological activity is a subclass of another type (e.g. *5 Hydroxytryptamine 1A agonist* is a subclass of *5 Hydroxytryptamine 1 agonist*). The "mechanism<->effect" type of relationships shows that there is a cause-and-effect relationship between two types of biological activity (e.g., *5 Hydroxytryptamine 1A agonist* causes *Anxiolytic* effect). Each description of relationships includes a link to another type of biological activity, a parameter showing affiliation of the relationship type to the "classification" or "mechanism<->effect", and values of conditional probability of manifestations of one activity type on conditions that another type of activity is revealed (Figure 1).

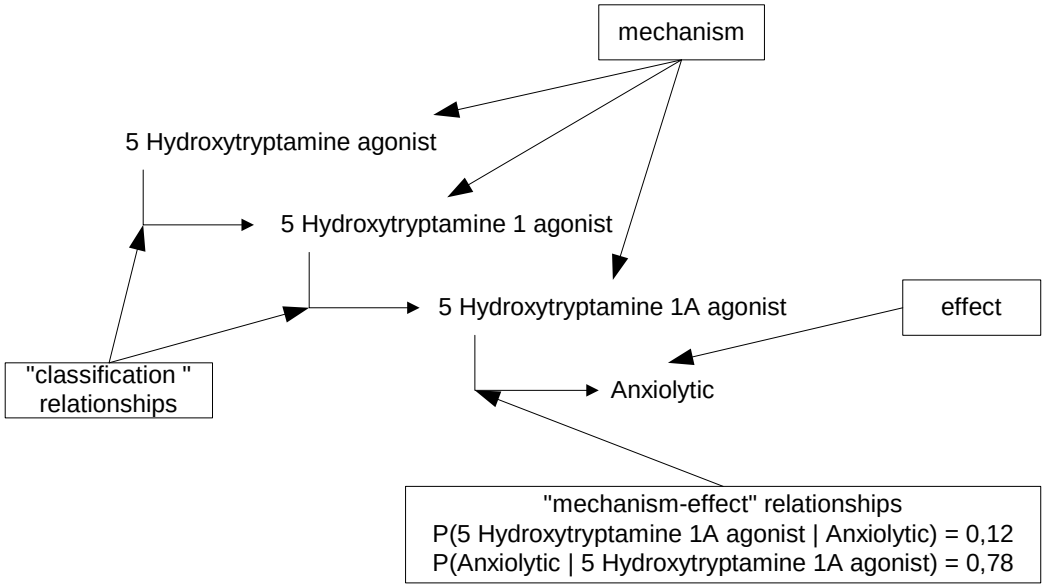


Figure 1. Description of the activity types and their relationships.

P(5 Hydroxytryptamine 1A agonist|Anxiolytic) – a conditional probability that compound acts by certain mechanism (5 Hydroxytryptamine 1A agonist) if it reveals the particular effect (Anxiolytic).
P(Anxiolitic|5 Hydroxytryptamine 1A agonist) - a conditional probability of revealing the particular effect (Anxiolytic) if compound acts by certain mechanism (5 Hydroxytryptamine 1A agonist).

The conditional probabilities are calculated based on PASS training set on the basis of the following equation:

$$\mathbf{P(E_i|M_j) = n_{ij}/n_i},$$

$$\mathbf{P(M_j|E_i) = n_{ij}/n_j}$$

where, $\mathbf{P(E_i|M_j)}$ is a conditional probability of the effect E_i revealing if compound acts by the particular mechanism M_j; $\mathbf{P(M_j|E_i)}$ is a conditional probability that compound acts by the particular mechanism M_j if the particular effect E_i is revealed; $\mathbf{n_{ij}}$ is a number of compounds which have both mechanism of action (M_i) and pharmacological effect (E_i) in their description; $\mathbf{n_i}$ is a number of a compounds in PASS training set with pharmacological effect (E_i) in its description and $\mathbf{n_j}$ is a number of compounds in PASS training set with mechanism of action (M_i) in its description.

The same structure of MER Base is used for relationships between the mechanisms, toxic/side effects and metabolic terms.

ANALYSIS OF "MECHANISM-EFFECT" RELATIONSHIPS

Two algorithms of the "mechanism-effect" relationships analysis have been developed and tested: (1) calculation of probability of effects if a particular mechanism of action is predicted; (2) calculation of probability of the mechanism of action if particular effects are predicted for a compound.

Calculation of probability of the effect goes on the suggestion that a compound acting in accordance with a particular mechanism (M_i) may cause a particular effect (E_i) that relates with this mechanism of action.

The function A_{ij} was introduced for mathematical description of activity-activity relationships:

$$\left.\begin{array}{l} M_i \xrightarrow{P=1} E_j \\ A_{ij} = 1 \end{array}\right\}$$
$$\left.\begin{array}{l} M_i \xrightarrow{P=0} E_j \\ A_{ij} = 0 \end{array}\right\}$$

If the relationship between the mechanism of action and effect exists, then A_{ij} is 1; otherwise A_{ij} is 0.

For each pharmacological effect of a particular compound the following probability values are calculated:

$$\bar{Pa}(Ej) = \max_i \{Pa(Ej), AijPa(Mi)\},$$
$$\bar{Pi}(Ej) = \min_i \{Pi(Ej), AijPi(Mi)\},$$

where, $\bar{Pa}(Ej)$ is a probability of a compound to be active by the effect E_j based on "mechanism-effect" relationship; $\bar{Pi}(Ej)$ is a probability of a compound to be inactive by the effect E_j based on "mechanism-effect" relationships; *Pa(Mi)* is a probability of a compound to be active by the mechanism M_i from PASS prediction result; *Pi(Mi)* is a probability of a compound to be inactive by the mechanism M_i from PASS prediction result; *Pa(Ej)* is a probability of a compound to have the effect E_j from PASS prediction result and *Pi(Ej)* is a probability of a compound does not have the effect E_j from PASS prediction result.

Calculation of probability for mechanisms of action based on the use of values of the conditional probabilities calculated on the basis of PASS training set. In other words, we answer the question: what is the value of probability for mechanism of action M_i if effect E_j is exhibited?

For each mechanism of action of a particular compound the following probability values are calculated:

$$\mathbf{P(M_i) = P_p(M_i) + P_c(M_i) - P_p(M_i)*P_c(M_i),}$$

where, $P_p(M_i)$ is a probability of a compound to have the mechanism M_i from PASS prediction result and $P_c(M_i)$ is a calculated probability of mechanism of action M_i:

$$\mathbf{P_c(M_i)=1-}\prod_{j=1}^{k}(1-P(E_j)\times P(M_i|E_j))\mathbf{,}$$

where, $P(M_i|E_j)$ is a conditional probability of revealing the particular effect E_j if a compound acts by particular mechanism M_i and $P(E_j)$ is probability of a compound to have the effect E_j from PASS prediction result.

MULTI-TARGETED ACTION

For many years, clinicians have treated patients by combining therapeutic mechanisms with combinations of drugs. It is being recognised that a balanced modulation of several targets can provide a superior therapeutic effect and side effect profile compared to the action of a selective ligand [1]. Compared with drug combinations, there are several advantages associated with ligands acting on several targets, such as the more predictable pharmacokinetic and pharmacodynamic properties that are a consequence of the administration of a single medicine, as well as improved patient compliance. Ligands that act on two different targets, usually called non-identical twin-drugs or dual-acting drugs, contain two pharmacophoric groups that may be combined by a linker, without linker or in overlap mode. Acetominosalol that is an association of salicylic acid and paracetamol is a good example of dual-acting drugs containing two pharmacophoric groups linked in an overlapped mode [2]. Dual-acting molecules may be obtained by associative synthesis or from a compound with an intrinsic acting profile. The molecular starting point for search of dual-acting molecules is generated using either rational design by a combination of pharmacophores or the screening of compound libraries or known drugs. The screening may be both *in vivo* and *in silico*. The study performed by Richard Morphy and co-authors [1] showed that the chance to discover dual-acting molecules with intrinsic biological profiles for related proteins from the same or similar protein families has the highest probability. The study of structures of ligands acting on different targets along with an analysis of protein similarity, size and features of their active centers can be used to discover potential targets for dual-acting drugs.

Research and development of new pharmaceuticals acting on single molecular targets is time-consuming and expensive. Furthermore, the cost of experimental studies of pharmaceutical agents acting on several molecular targets increases multiplicatively. It is known that traditional QSAR and 3D molecular modeling for predicting biological activity of chemical substances operate with a small number of activities and is usually for the same chemical series. In contrast to these approaches, the availability of several hundred types of biological activity already predicted and the data on the relationships between mechanisms of action and pharmacological effects allowed us to select compounds that act on different molecular targets and cause the same pharmacological effect. We studied the potential of this approach in finding antihypertensive compounds with a complex mechanism of antihypertensive action and (b) the possibilities of revealing both existing and new complex mechanisms of action [3]. The search for new dual inhibitors for the two proteolytic enzymes, ACE and NEP was made as an example of our approach for

finding new compounds with a complex mechanism of action. It was demonstrated that biological activity spectra prediction can be used for the selection of compounds with dual mechanisms of antihypertensive action. This approach offers an ability to identify probable combinations of mechanisms of action for the antihypertensive effect. The approach was used for the search of new ACE/NEP inhibitors in the databases of available chemical samples. Four compounds with a higher probability of inhibiting ACE and NEP were selected on the basis of the data of PASS prediction. Compounds predicted as dual inhibitors for ACE and NEP were tested for their interaction with the two enzymes. The experimental tests have confirmed that all these compounds are inhibitors of both ACE and NEP. The most effective compounds have IC_{50}) 10^{-7}-10^{-9} M for ACE and 10^{-5} M for NEP.

It appears that there is a high probability of finding compounds with new combinations of mechanisms of antihypertensive action such as antagonism to alpha1 adrenoreceptors and endothelin A receptors or calcium channel blocker and endothelin A receptor antagonist. Owing to the speed in predicting biological activity spectra and diversity of predicted biological activities, a similar approach can be applied not only in the search of new medicines with dual mechanisms of antihypertensive action but also for medicines used for treating other diseases with complex mechanisms of regulation, such as neoplastic growth, viral and bacterial infections, and others.

The similar study was made for search of dual COX/LOX inhibitors [4].

(1) Morphy, R.; Kay, C.; Rankovic, Z. From magic bullets to designed multiple ligands. *DDT,* **2004**, *9(15)*, 641-651.

(2) Contreras, J-M.; Bourguignon, J-J. Identical and non-identical twin drugs. In: The Practice of Medicinal Chemistry, Ed. by Wermouth C.G., Elsevier, **2003**, 251-273.

(3) Lagunin, A.A.; Gomazkov, O.A.; Filimonov, D.A.; Gureeva, T.A.; Dilakyan, E.A.; Kugaevskaya, E.V.; Elisseeva, Yu.E.; Solovyeva, N.I.; Poroikov, V.V. Computer-Aided Selection of Potential Antihypertensive Compounds with Dual Mechanism of Action. *J. Med. Chem.,* **2003**, *46,* 3326-3332.

(4) Geronikaki A.A., Lagunin A.A., Hadjipavlou-Litina D.I., Eleftheriou Ph.T., Filimonov D.A., Poroikov V.V., Alam I., Saxena A.K. Computer-aided discovery of anti-inflammatory thiazolidinones with dual cyclooxygenase/lipoxygenase inhibition. *J Med Chem.* **2008**, *51(6)*, 1601-1609.

DRUG-DRUG INTERACTIONS

Drug-drug interactions are possible whenever a person takes two or more medicines simultaneously. There are positive and negative drug-drug interactions. Positive ones may increase therapeutic action of each other. Negative ones lead to decrease therapeutic action of medicines and/or stimulate their toxic and side effects. Recent scientific developments—particularly in the area of cytochrome P450 drug metabolizing

enzymes—have revolutionized the study of drug interactions. The result has been a deluge of published drug interaction research that has overwhelmed most health care practitioners. While it is not possible for an individual health care practitioner to recognize all clinically significant drug interactions, it is possible to understand the important scientific principles and mechanisms that pertain to this topic. When discussing drug interactions, the drug affected by the interaction is called the "object drug," and the drug causing the interaction is called the "precipitant drug."

There are a number of mechanisms by which drugs interact with each other, and most of them can be divided into two general categories: pharmacokinetic and pharmacodynamic interactions. With pharmacokinetic drug interactions, one drug affects the absorption, distribution, metabolism, or excretion of another. When pharmacodynamic drug interactions occur, two drugs have additive or antagonistic pharmacologic effects. Either type of drug interaction can result in adverse effects in some individuals.

Due to the PASS abilities to predict many hundreds types of activity including interaction of compounds with drug-metabolizing enzymes, protein transporters, molecular targets, pharmacoterapeutic effects, different toxic and side effects, the analysis of PASS prediction results gives a unique opportunity to reveal different types of possible drug-drug interactions. The most important types of drug-drug interactions are described below.

Pharmacokinetic Drug Interactions

Inhibition of Absorption. Drugs that act as binding agents such as cholestyramine and colestipol can impair the bioavailability of other drugs. This will result in a reduction in the therapeutic effect of other drugs. The effect can be profound in case of some combinations such as cholestyramine and furosemide. Some drugs such as fluoroquinolone antibiotics (e.g., Ciprofloxacine) are susceptible to chelation with cations such as aluminum, magnesium, and iron. Other drugs such as itraconazole, ketoconazole, glipizide, glyburide, cefpodoxime, and cefuroxime demonstrate a pH dependent absorption. The amount of these drugs that is absorbed from the gut may be increased or decreased by drugs that increase the stomach pH.

This type of drug-drug interactions can by analyzed by PharmaExpert only for interaction with protein transporters participating in drug absorption. Look to the "Transporters and Blood proteins" part of Drug-Drug interaction window to see the possible interactions with protein transporters.

Enzyme Inhibition Increasing the Risk of Toxicity. Most drugs are metabolized to inactive or less active metabolites by enzymes in the liver and intestine. Inhibition of this metabolism can increase the effect of the object drug. If the increase in effect is large enough, drug toxicity may result. This is one of the most common mechanisms by which clinically important drug interactions occur. Since only a few different cytochrome P450 isozymes are the main enzymes of drug metabolism, competition between two drugs for

these isozymes will occasionally occur. This competition may result in one drug interfering with the metabolism of another drug.

For example, inhibitors of CYP1A2 can increase the risk of toxicity from clozapine or theophylline. Inhibitors of CYP2C9 can increase the risk of toxicity from phenytoin, tolbutamide, and oral anticoagulants such as warfarin. Inhibitors of CYP3A4 can increase the risk of toxicity from many drugs, including carbamazepine, cisapride, cyclosporine, ergot alkaloids, lovastatin, pimozide, protease inhibitors, rifabutin, simvastatin, tacrolimus, and vinca alkaloids.

This type of drug-drug interactions can by analyzed by PharmaExpert for interaction with drug-metabolizing enzymes. Look to "Metabolism" part of Drug-Drug interaction window to see the possible interactions with protein transporters.

Enzyme Inhibitors Resulting in Reduced Drug Effect. A small number of drugs are not active in the form administered to patients. These drugs are known as prodrugs and require activation by enzymes in the body before they can produce their effect. Inhibition of the metabolism of these prodrugs may reduce the amount of active drug formed, and decrease or eliminate the therapeutic effect. For example, the analgesic and toxic effects of codeine appear to result from its conversion to morphine by CYP2D6. Thus, CYP2D6 inhibitors can impair the therapeutic effect of codeine. CYP2D6 inhibitors may similarly affect the analgesic effect of hydrocodone.

This type of drug-drug interactions may by analyzed by PharmaExpert for interaction with drug-metabolizing enzymes. Look to "Metabolism" part of Drug-Drug interaction window to see the possible interactions with protein transporters.

Enzyme Induction Resulting in Reduced Drug Effect. Some drugs—called "enzyme inducers"—are capable of increasing the activity of drug metabolizing enzymes, resulting in a decrease in the effect of certain other drugs. Examples of enzyme inducers include aminoglutethimide, barbiturates, carbamazepine, glutethimide, griseofulvin, phenytoin, primidone, rifabutin, rifampin, and troglitazone. Some drugs, such as ritonavir, may act as either an enzyme inhibitor or an enzyme inducer, depending on the situation. Drugs metabolized by CYP3A4 or CYP2C9 are particularly susceptible to enzyme induction. In some cases, especially for drugs that undergo extensive first-pass metabolism by CYP3A4 in the gut wall and liver, the reduction in serum concentrations of the object drug can be profound.

This type of drug-drug interactions may by analyzed by PharmaExpert for interaction with drug-metabolizing enzymes. Look to "Metabolism" part of Drug-Drug interaction window to see possible interactions with protein transporters.

Enzyme Induction Resulting in Toxic Metabolites. Some drugs are converted to toxic metabolites by drug metabolizing enzymes. For example, the analgesic acetaminophen is converted primarily to non-toxic metabolites, but a small amount is converted to a

cytototoxic metabolite. Enzyme inducers can increase the formation of the toxic metabolite and increase the risk of hepatotoxicity as well as damage to other organs.

This type of drug-drug interactions may by analyzed by PharmaExpert for interaction with drug-metabolizing enzymes. Look to "Metabolism" part of Drug-Drug interaction window to see possible interactions with protein transporters.

Altered Renal Elimination. For some drugs, active secretion into the renal tubules is an important route of elimination. For example, digoxin is eliminated primarily through renal excretion, and drugs such as amiodarone, clarithromycin, itraconazole, propafenone, and quinidine can inhibit this process. Digoxin toxicity may result.

This type of drug-drug interactions may by analyzed by PharmaExpert only for interaction with renal protein transporters participating in drug secretion. Look to "Transporters and Blood proteins" part of Drug-Drug interaction window to see possible interactions with protein transporters.

Pharmacodynamic Drug Interactions

Additive Pharmacodynamic Effects. When two or more drugs with similar pharmacodynamic effects are given, the additive effects may result in excessive response and toxicity. Examples include combinations of drugs that prolong the QT interval resulting in ventricular arrhythmias, and combining drugs with hyperkalemic effects resulting in hyperkalemia.

This type of drug-drug interactions may by analyzed by PharmaExpert for additive pharmacotherapeutical, toxic and side effects. Look to "Additive or Synergistic effects and actions" and "Additive or Synergistic Toxic and Side effects" parts of Drug-Drug interaction window to see that.

Antagonistic Pharmacodynamic Effects. Drugs with opposite pharmacodynamic effects may reduce the response to one or both drugs. For example, drugs that tend to increase blood pressure (such as nonsteroidal anti-inflammatory drugs) may inhibit the antihypertensive effect of drugs such as ACE inhibitors. Another example would be inhibition of the response to benzodiazepines by the concurrent use of theophylline.

This type of drug-drug interactions may by analyzed by PharmaExpert only for a molecular level in "Pharmacodynamic Drug-Drug interaction" part of Drug-Drug interaction window to see that.

Although dramatic advances have been made in the study of drug interaction mechanisms over the past few decades, there is still much to learn. Thus, many of the mechanism concepts useful today could be refined in the future, yielding a picture closer to the truth. It also should be kept in mind that for some drug-drug interactions more than one mechanism may be occurring simultaneously.

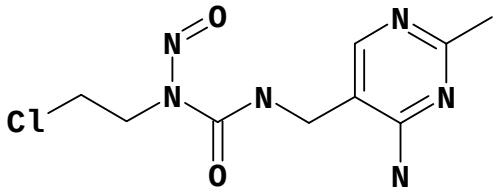
Chapter 4
HOW PharmaExpert WORKS

The description of interpretation process is based on the terms of Chapter 3. Please, read Chapter 3 carefully before proceeding.

INPUT DATE FORMATS

The software PharmaExpert uses MOLfiles or SDfiles as external sources of structural and activity data to prepare both the SAR Base and set of substances to be predicted. SDfiles can be exported either from ISIS/Base 2.0+ (SYMYX MDL, http://www.symyx.com) or from any other Molecular Editor or DBMS, which has the option of SDfiles' export.

The example of SDfile with PASS prediction results for Nimustine molecule:



Nimustine

```
  -ISIS-  10079915102D

 18 18  0  0  0  0  0  0  0  0999 V2000
    2.7250   -2.0667    0.0000 N   0  0  0  0  0  0  0  0  0  0  0  0
   -0.8417   -2.4833    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
   -1.5542   -2.0750    0.0000 N   0  0  3  0  0  0  0  0  0  0  0  0
    2.0125   -2.4792    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
    1.3042   -2.0667    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
   -1.5583   -1.2500    0.0000 N   0  0  0  0  0  0  0  0  0  0  0  0
   -0.1250   -2.0708    0.0000 N   0  0  0  0  0  0  0  0  0  0  0  0
    2.0125   -0.8292    0.0000 N   0  0  0  0  0  0  0  0  0  0  0  0
    2.7250   -1.2417    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
    1.3042   -1.2417    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
    0.5875   -2.4833    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
   -0.8375   -3.3083    0.0000 O   0  0  0  0  0  0  0  0  0  0  0  0
   -0.8417   -0.8333    0.0000 O   0  0  0  0  0  0  0  0  0  0  0  0
    2.0125   -3.3042    0.0000 N   0  0  0  0  0  0  0  0  0  0  0  0
   -2.2708   -2.4875    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
   -3.7000   -2.4875    0.0000 Cl  0  0  0  0  0  0  0  0  0  0  0  0
   -2.9833   -2.0750    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
    3.4417   -0.8333    0.0000 C   0  0  0  0  0  0  0  0  0  0  0  0
  2  7  1  0  0  0  0
  3  2  1  0  0  0  0
  4  1  1  0  0  0  0
  5  4  2  0  0  0  0
  6  3  1  0  0  0  0
  7 11  1  0  0  0  0
  8  9  1  0  0  0  0
  9  1  2  0  0  0  0
 10  8  2  0  0  0  0
 11  5  1  0  0  0  0
 12  2  2  0  0  0  0
 13  6  2  0  0  0  0
 14  4  1  0  0  0  0
 15  3  1  0  0  0  0
 16 17  1  0  0  0  0
 17 15  1  0  0  0  0
 18  9  1  0  0  0  0
  5 10  1  0  0  0  0
M  END
>  <ID> (689)
689

>  <PASS_DRUG_LIKENESS>
0.825

>  <PASS_MNA_COUNT>
 42

>  <PASS_MNA_NEW_COUNT>
  0

>  <PASS KNOWN ACTIVITIES>
```

```
              Alkylator
              Antineoplastic
              Antineoplastic, alkylator
              DNA synthesis inhibitor
              Teratogen

>  <PASS_RESULT_COUNT>
 79 of 2820 Possible Activities at Pa > Pi

>  <PASS_ACTIVITY_SPECTRUM>
0.846  0.005  Carcinogenic, male rats
0.843  0.004  DNA damaging
0.835  0.007  Antineoplastic
0.805  0.005  Cytostatic
0.757  0.005  Carcinogenic, female rats
0.746  0.009  DNA synthesis inhibitor
0.735  0.003  Alkylator
0.695  0.012  CYP2C9 substrate
0.669  0.007  Mutagenic
0.662  0.012  CYP1A2 substrate
0.653  0.006  Hematopoietic inhibitor
0.658  0.026  CYP2 substrate
0.639  0.011  Carcinogenic
0.613  0.007  Mutagenic, Salmonella
0.617  0.026  CYP2C substrate
0.588  0.006  Trypanothione-disulfide reductase inhibitor
0.576  0.010  Dopamine D4 agonist
0.610  0.049  Lysase inhibitor
0.607  0.053  Teratogen
0.579  0.035  Carcinogenic, group 1
0.543  0.002  Antineoplastic, alkylator
0.555  0.022  CYP1A substrate
0.537  0.004  Ribonucleoside triphosphate reductase inhibitor
0.527  0.010  Carcinogenic, group 2A
0.530  0.024  CYP1 substrate
0.504  0.004  Ribonucleoside diphosphate reductase inhibitor
0.550  0.052  CYP3A substrate
0.510  0.024  Carcinogenic, female mice
0.501  0.027  Carcinogenic, male mice
0.513  0.070  CYP3A4 substrate
0.410  0.006  ATPase stimulant
0.436  0.040  CYP2C19 substrate
0.455  0.061  Embryotoxic
0.396  0.022  Imidazoline I1 receptor agonist
0.374  0.002  Glutathione peroxidase stimulant
0.382  0.020  Growth stimulant
0.348  0.027  Glutathione-disulfide reductase inhibitor
0.329  0.019  Antiprotozoal (Trichomonas)
0.330  0.024  Lymphocytopoiesis inhibitor
0.379  0.138  Thrombocytopoiesis inhibitor
0.289  0.055  CYP3A7 substrate
0.334  0.102  Antiviral (Poxvirus)
0.309  0.088  Thiamine pyridinylase inhibitor
0.265  0.045  Carcinogenic, group 2B
0.243  0.037  Corticotropin releasing factor 1 receptor antagonist
0.271  0.067  Neuropeptide agonist
0.283  0.083  Antiprotozoal (Amoeba)
0.226  0.031  Ribonucleotide reductase inhibitor
0.312  0.118  CYP2D substrate
0.218  0.030  Antimetabolite
0.302  0.120  CYP2D6 substrate
0.232  0.053  CYP2C8 inducer
0.276  0.118  Granzyme B inhibitor
0.228  0.091  Neuropeptide Y antagonist
0.262  0.132  Interferon alpha agonist
0.376  0.262  Potassium sparing diuretic
0.171  0.059  Antineoplastic antimetabolite
```

```
0.231  0.124  Renal disease treatment
0.148  0.049  Radical formation agonist
0.128  0.034  Carbamoyl-serine ammonia-lyase inhibitor
0.114  0.028  Abl kinase inhibitor
0.241  0.171  Folate antagonist
0.339  0.284  (-)-limonene 3-monooxygenase inhibitor
0.339  0.284  (-)-limonene 6-monooxygenase inhibitor
0.339  0.284  (-)-limonene 7-monooxygenase inhibitor
0.069  0.018  Antiprotozoal (Histomonas)
0.288  0.237  Toxic
0.213  0.169  Tryptophan 2,3-dioxygenase inhibitor
0.182  0.141  CYP2E substrate
0.185  0.148  Channel-conductance-controlling ATPase inhibitor
0.213  0.185  CYP2A6 substrate
0.071  0.043  DNA repair enzyme inhibitor
0.184  0.156  Pancreatic ribonuclease inhibitor
0.102  0.077  Thymidylate synthase inhibitor
0.064  0.042  Interferon inducer
0.215  0.194  CYP2B6 substrate
0.022  0.008  Antibiotic Trimethoprim-like
0.252  0.250  Antiviral (Herpes)
0.227  0.226  Antiprotozoal (Trypanosoma)

>  <name_inn> (689)
Nimustine

>  <ACTIVITY> (689)
Antineoplastic, alkylator
DNA synthesis inhibitor
Teratogen

$$$$
```

A MOLfile can be prepared with chemical editor ISIS/Draw 2.00 and higher (SYMYX MDL, http://www.symyx.com).

PREDICTION RESULTS

A result of PASS prediction is saved in the appropriate SDfile. It presents the biological activity spectrum for each substance. It is the list of biological activity types for which the calculated probability to be revealed (**Pa**) exceed the calculated probability not to be revealed (**Pi**). Taking into account that if no data on activity is available, compound is considered as inactive. There are some other factors, which essentially influence on **Pa** absolute value: the number and diversity of substances revealing the activity in the training set, recall ratio, etc. In general, the higher **Pa** value is the higher probability for a studied substance to be structurally similar to known biologically active substances from the training set is.

The result of prediction is valuable at planning the experiment, but one should take into account some additional factors: particular interest to some kinds of activity, desirable novelty of a substance, available facilities for experimental testing, etc. Actually, each choice is always the compromise between the desirable novelty of studied substance and risk to obtain the negative result in testing.

PHARMAEXPERT INTERPRETATION OF PASS PREDICTION RESULTS

The results of PharmaExpert interpretations can be represented in two modes: text and graphic or can be saved as SDfile.

There are several types of prediction interpretations that are represented by PharmaExpert:

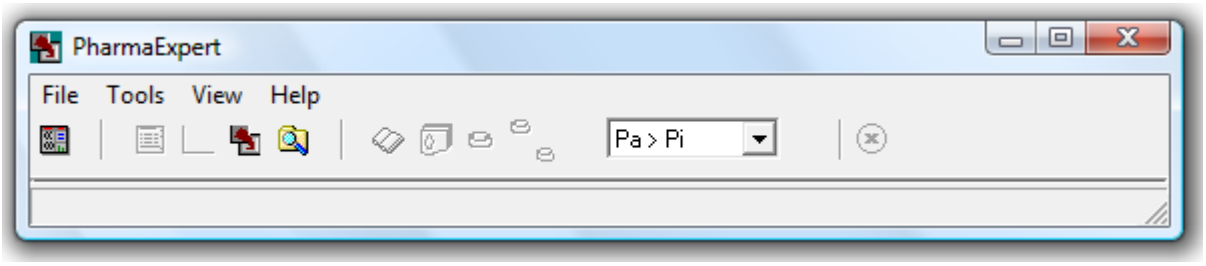
- The "classification" type of activity relationships (see for detailed in Chapter 3, MER Database) that one of two types of biological activity is a subclass of another (e.g. 5 Hydroxytryptamine 1A agonist is a subclass of 5 Hydroxytryptamine 1 agonist). The subclass has a left indentation. This type of PharmaExpert interpretation is represented in all sheets (Effects, Mechanisms, Toxicity, Metabolism, Gene Expression and Transporters).
- Effect -> mechanisms interpretations show that there is a cause-and-effect relationship between two types of biological activity (e.g., 5 Hydroxytryptamine 1A agonist causes Anxiolytic effect). The mechanism has a left indentation. This type of PharmaExpert interpretation is represented in the Effects and Toxicity sheets.
- Mechanisms -> effect interpretations show that there is a cause-and-effect relationship between two types of biological activity (e.g., 5 Hydroxytryptamine 1A agonist causes Anxiolytic effect). The effect has a left indentation. This type of PharmaExpert interpretation is represented in the Mechanisms sheets.

Chapter 5
GETTING STARTED WITH PharmaExpert

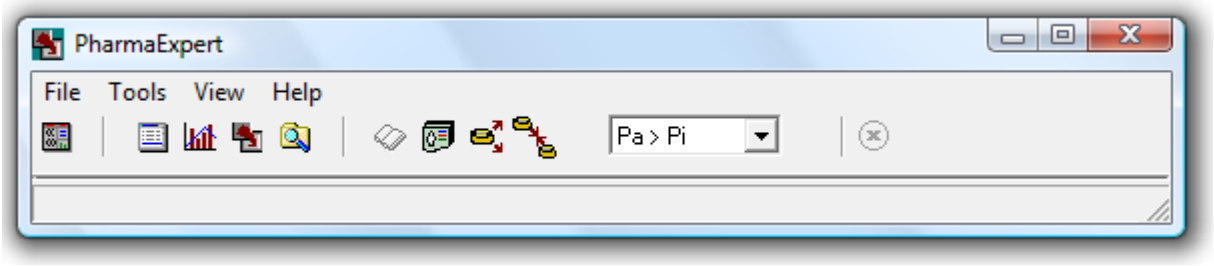
GETTING STARTED WITH PharmaExpert INTERFACE

To start **PharmaExpert** - double-click PharmaExpert icon (shortcut)  ;
or run **PharmaExpert.exe** from PASS folder.

The Main window of PharmaExpert interface appears and MER Base loading is started. After MER Base loading the main window of PharmaExper interface looks like this:



Seven buttons are inactive by default. When text or SD files are loaded five commands and buttons become active.



- Use **File|Open|Open PASS SDfile** menu command or the button  to open SDfile of the biological activity spectra prediction for substances.
- Use **File|Save|Save PASS SD file** menu command to save SDfile of the interpretation results of the biological activity spectra prediction for substances.
- Press the button  to save interpretation results. To recalculate (see page 13) the prediction results of PASS based on "Mechanism-Effect" Relationships mark the

Calculation checkbox in the **Predictions & Interpretation window**. Mark **Check not predicted activity** checkbox in the Predictions & Interpretation window if you want to display other activity types associated with the predicted type(s) of activity.

- Use **Tools|Statistics of Spectra** menu command or the button  to open the **Statistics window** with a table containing data on number of substances with a particular type(s) of biological activity in the set of compounds.

- **Use View|"Mechanism-Effect" Relationships** menu command or the button  to display the **"Mechanism-Effect" Relationships window**.

- Use **View|File Manager** menu command or the button  to display the **File Manager window** for choosing a file if several files with prediction results are loaded. The data from the current file will be displayed in **Prediction&Interpretation** window.

- Use **Tools|Comparative analysis** menu command or press the button  to open the window for the comparison of biological activity spectra for various substances in the set. The data from the current file will be displayed in the **Comparative analysis window**.

- Use **Tools|Multi-Targeting** menu command or press the button  to open the window for the search of compounds with multitargeted actions in the set. The **Multi-Targeting window** will be opened.

- Use **Tools|Drug-drug interactions** menu command or press the button  to open the window for the analysis of drug-drug interactions of the selected substances. The **Drug-drug interactions window** will be opened.

- Press the button  to stop any process.

- Use **View|Information** menu command to display the information about the current **"Mechanism-Effect" Relationships Database (MER Base)**.

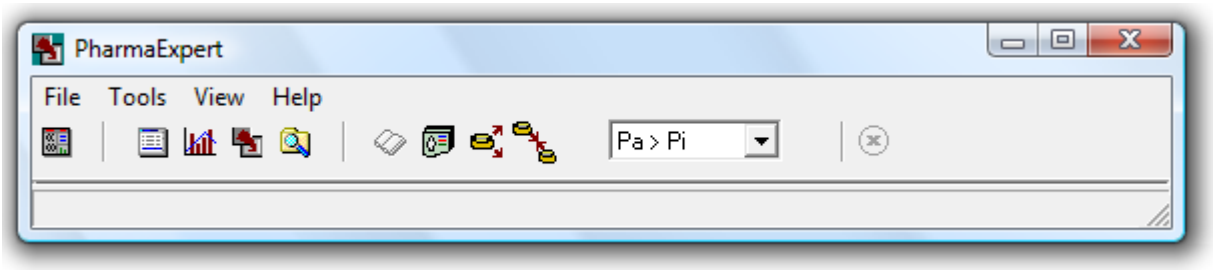
- Use **View|Predictions&Interpretation** menu command or press the button  to display the **Predictions & Interpretation window**.

Interpretation results may be imported back to the ISIS/Base if they were saved as SDfile.

Chapter 6
PharmaExpert INTERFACE and FUNCTIONS

MAIN WINDOW

The main window contains menu, speed buttons, drop down list for the selection criterion, progress bar, status panel.



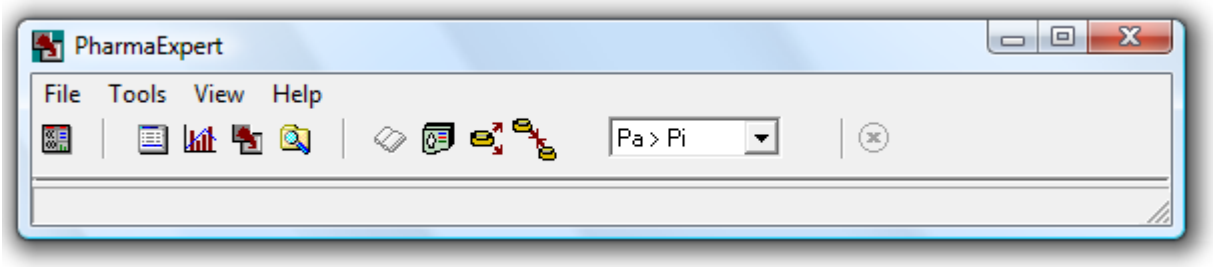
You can use either **menu commands** or the speed buttons to execute **PharmaExpert** system procedures.

The main menu items are: **File, Tools, View** and **Help.** All these item menus are described in more detail below.

Use the mouse, the keyboard (**F10**) or key combinations: **Alt+F**, **Alt+T, Alt+V, Alt+H** to choose the particular menu item.

- Press F1 to call the context sensitive **Help** in the main window, in all dialog boxes and sub-windows of the PASS software.
- The speed buttons' hints will appear when the mouse cursor points to the button.
- Use Help menu command for more information you need.
- Use Alt+F4 shortcut, **File|Exit** (**Alt+X**) menu command or button ✕ in upper right corner of PharmaExpert main window to quit PharmaExpert.

An existing MER Base is loading by default. After that the most of the commands become available (see the Figure below).

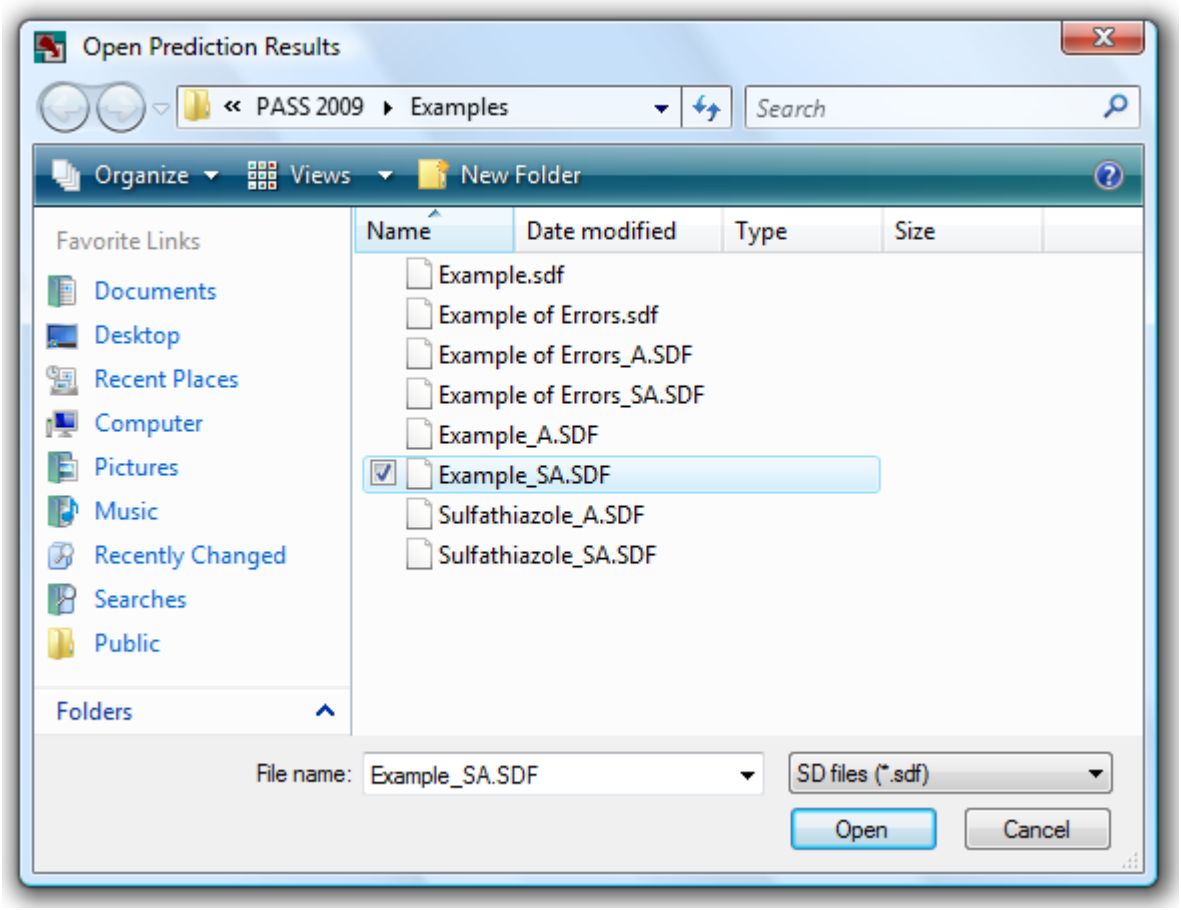


The buttons have the following functions:

- Opens SDfile.
- Saves the interpretation results of PharmaExpert in SDFile.
- Opens the **Statistics window** with a table containing the number of substances with a particular type(s) of biological activity in the set of compounds.
- Opens the **"Mechanism-Effect" Relationships window.**
- Opens the **File Manager window** for choosing a file if several files with prediction results are loaded.
- Opens the **Predictions & Interpretation window**.
- Opens the **Comparative analysis** window for the comparison of biological activity spectra of substances in the set.
- Opens the **Multi-Targeting** window for the search of compounds with multitarget actions.
- Open the **Drug-drug interactions** window for the analysis of drug-drug interactions of the selected substances.
- Stop any process of PharmaExpert

OPENING SDFILE

Use **File|Open|PASS SDfile** menu command or the button  to open SDfile (*.sdf) of the biological activity spectra prediction for substances. The following dialog box appears:



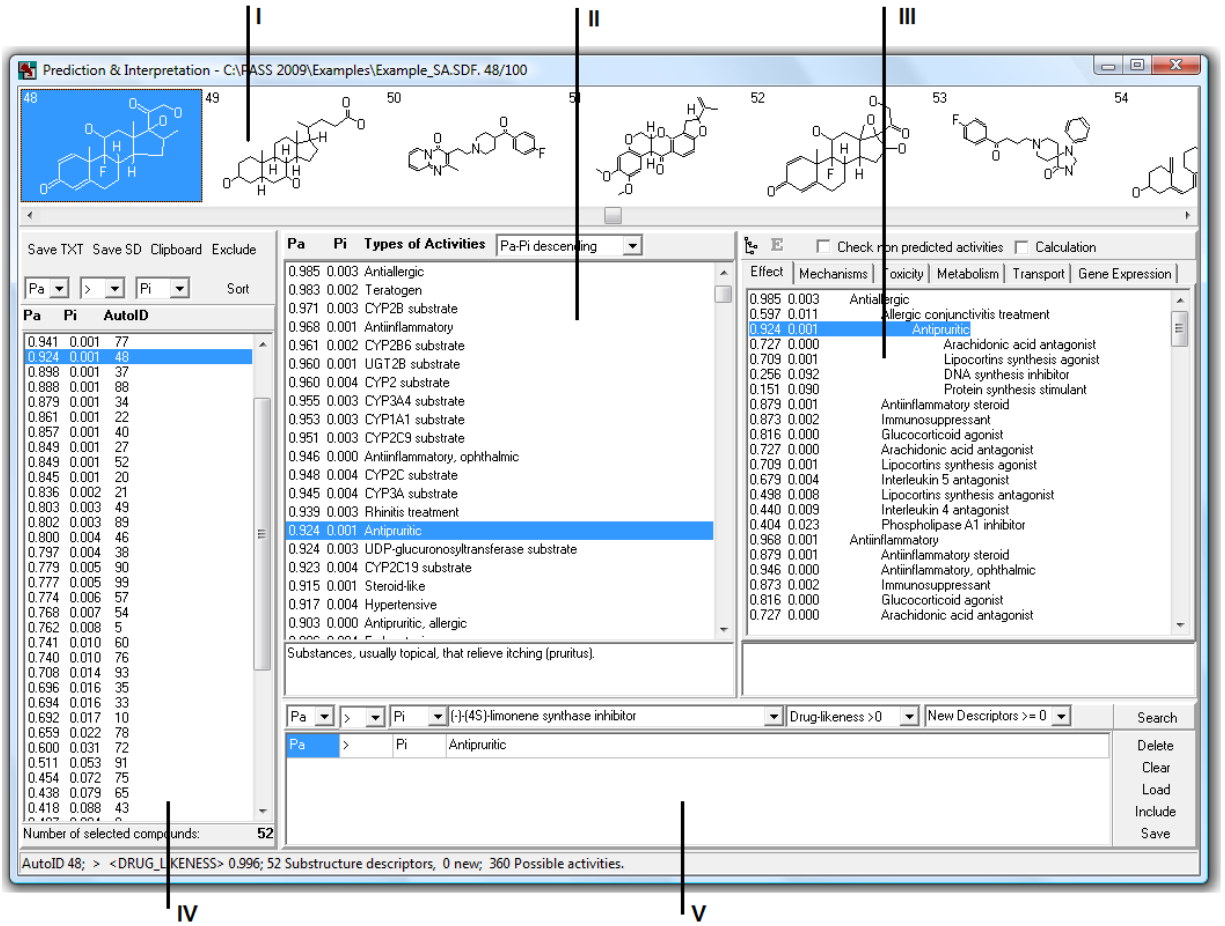
The **Prediction&Interpretation window** with prediction results will be shown. (its description is given below).

Note!

You can analyze several SDfiles with PASS prediction results simultaneously. For that you should use **File|Add|PASS SDfile** menu command.

VIEWING PREDICTION AND INTERPRETATION RESULTS

To analyze the prediction results, **PharmaExpert** provides the special interactive tool. It



allows one to view structures, prediction and interpretation results, and also to search for compounds with desirable biological activity profiles.

Use **View|Prediction&Interpretation** menu command or press the button  to display the **Prediction&Interpretation window**.

Prediction&Interpretation window consists of 5 parts:

I. The structure representation and navigation.

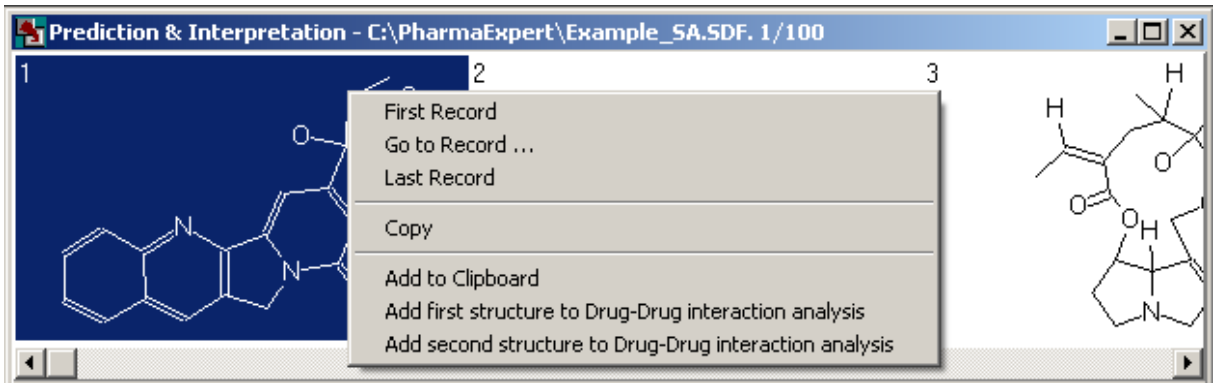
II. PASS prediction results.

III. PharmaExpert interpretations.

IV. The search for compounds with the desirable types of biological activities.

V. The result of the search.

I. The structure representation and navigation



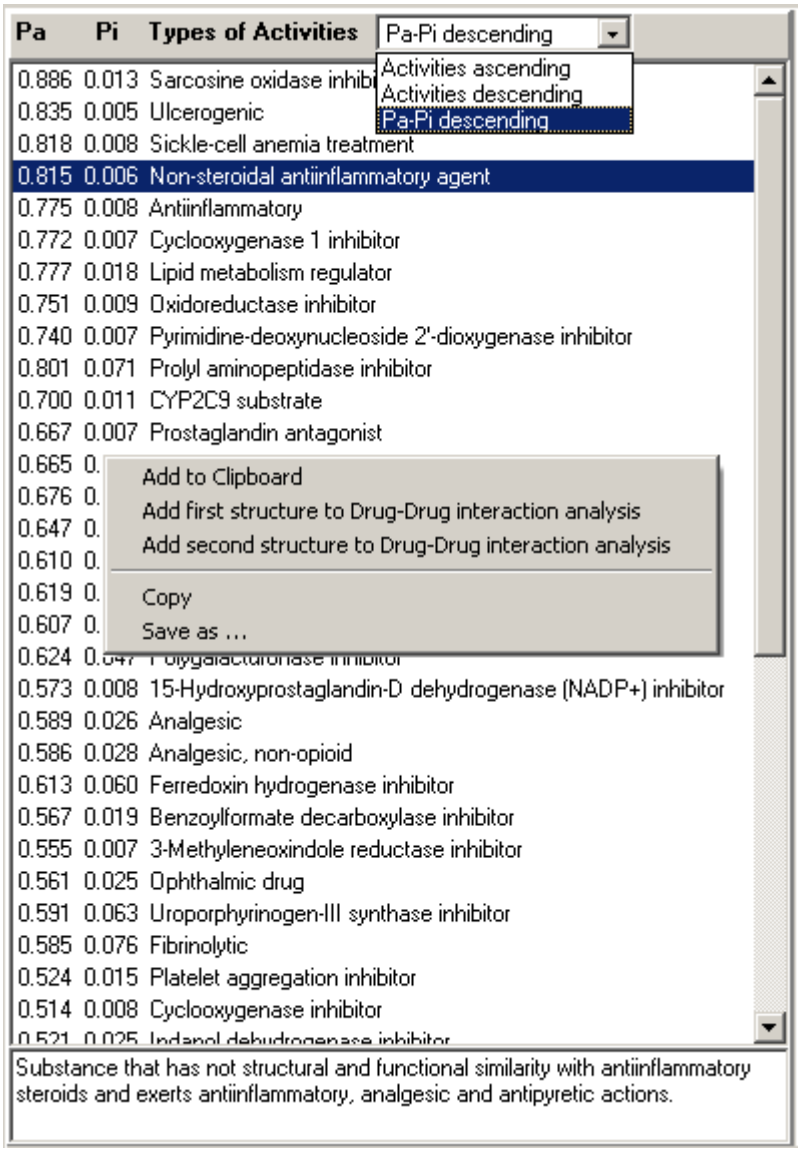
This part of the window is destined for representation and copy of the structures also as for navigation through PASS prediction results.

Press the right button on the structure to call the Pop-Up menu with commands (see the previous figure):

- Choose **First Record** command to go to the first record.
- Choose **Go to Record** command to go to the chosen record. The **Go to** dialog box will appear. You should type the Record Number and press **OK** to go to this record.

- Choose **Last Record** command to go to the last record.
- Choose **Copy** command to copy the current structure to the Microsoft Windows' clipboard. It is saved as a bitmap (.bmp file). This format is not used in ISIS Base.
- Choose **Add to Clipboard** command to add the substance to the **PharmaExpert Clipboard** window. The "Substance ID" will be added.
- Choose **Add first structure to Drug-Drug interaction analysis** command to add the selected structures for Drug-Drug interaction analysis. The structure appears in the left top corner of the **Drug-drug interactions window**.
- Choose **Add second structure to Drug-Drug interaction analysis** command to add the selected structures for Drug-Drug interaction analysis. The structure appears in the right top corner of the **Drug-drug interactions window**.

II. PASS prediction results



The names of activities in the PASS prediction results can be sorted using the drop-down box:

Activities ascending	Alphabetically.
Activities descending	Alphabetically (reverse order).
Pa-Pi descending	Descending order of Pa-Pi values for biological activities.

Press the right button on the prediction results to call the Pop-Up menu with following commands:

- Choose **Add to Clipboard** command to add the substance to **PharmaExpert Clipboard**. The "Substance ID" will be added.

- Choose **Add first structure to Drug-Drug interaction analysis** command to add the selected structure for Drug-Drug interaction analysis. The structure appears in the left top corner of the **Drug-drug interactions window**.
- Choose **Add second structure to Drug-Drug interaction analysis** command to add the selected structure for Drug-Drug interaction analysis. The structure appears in the right top corner of the **Drug-drug interactions window**.
- Choose **Copy** command to copy the PASS prediction results for the selected compound to the clipboard.
- Choose **Save As…** command to save the PASS prediction results for the selected compound to a text file.

The lower element of the part shows information (Activity Description) about the selected activity type.

III. PharmaExpert interpretations

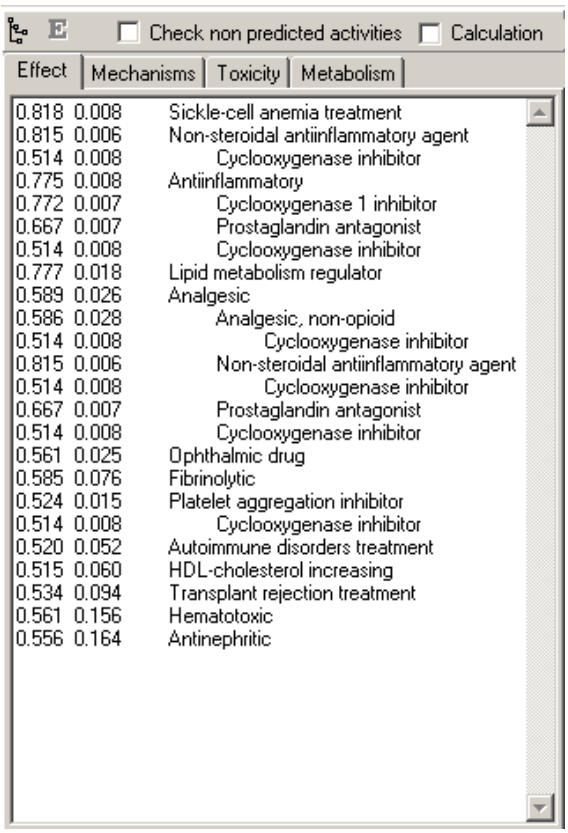
The results of PharmaExpert interpretations can be represented in two modes: text and graphic. You should press the button  to change the mode of the interpretation result from text to graphic and vice versa. The button  expands and collapses the tree of the interpretation result.

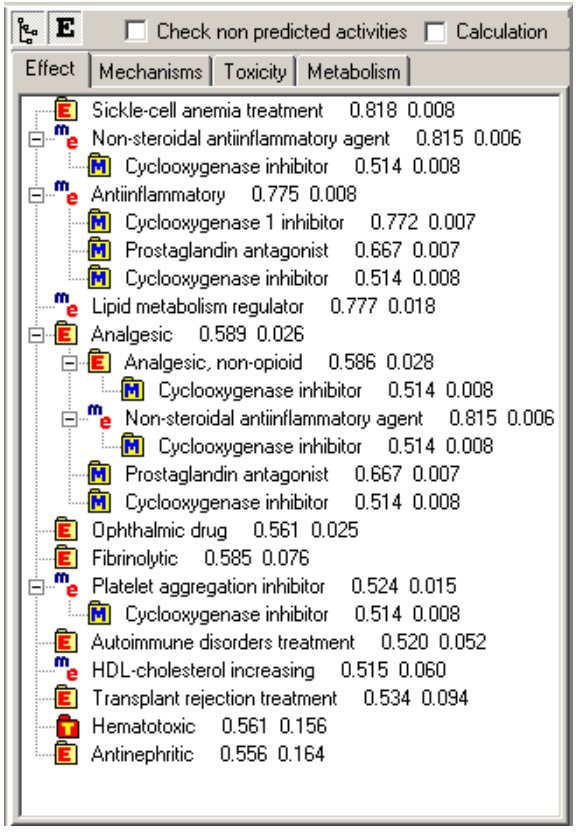
To recalculate the prediction results of PASS using known "Mechanism-Effect" Relationships you should mark the **Calculation** checkbox. You should mark **Check not predicted activity** checkbox if you want to display other activity types associated with predicted type(s) of activity.

There are several supplementary sheets in the PharmaExpert interpretation part:

- **Effect** - all predicted pharmacotherapeutic effects and their relationships with predicted mechanisms of action in the PASS prediction results that correspond to the criterion specified by the user.

Text **Graphic**





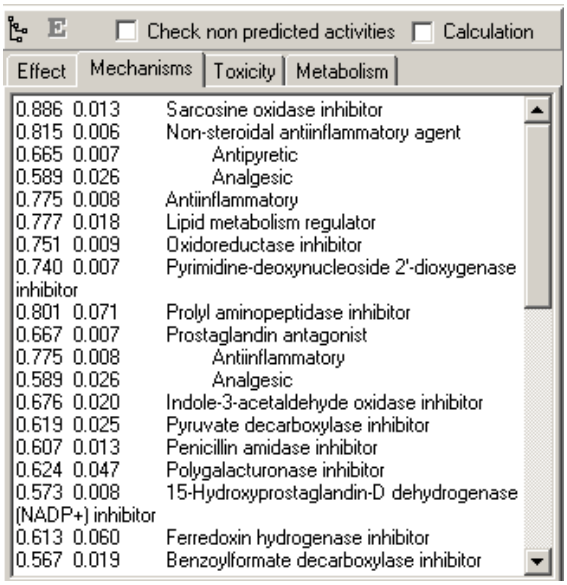
The icon E indicates that this type of activity is a pharmacological effect.

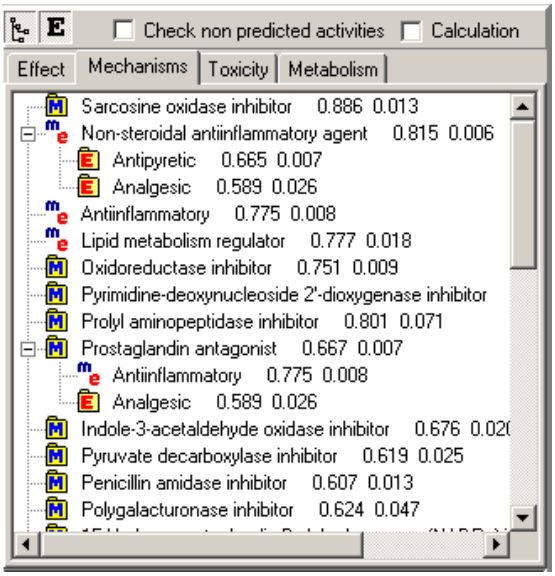
The icon M indicates that this type of activity is a mechanism of action.

The icon me indicates that this type of activity is both a pharmacological effect and a mechanism of action for the upper-class pharmacological effect (for example, 'Non-steroidal anti-inflammatory agent' is a mechanism of action for Analgesic effect).

- **Mechanisms** - all predicted mechanisms of action and their relationships with predicted pharmacotherapeutic effects in the PASS prediction results that meet the criterion specified by the user.

Text **Graphic**





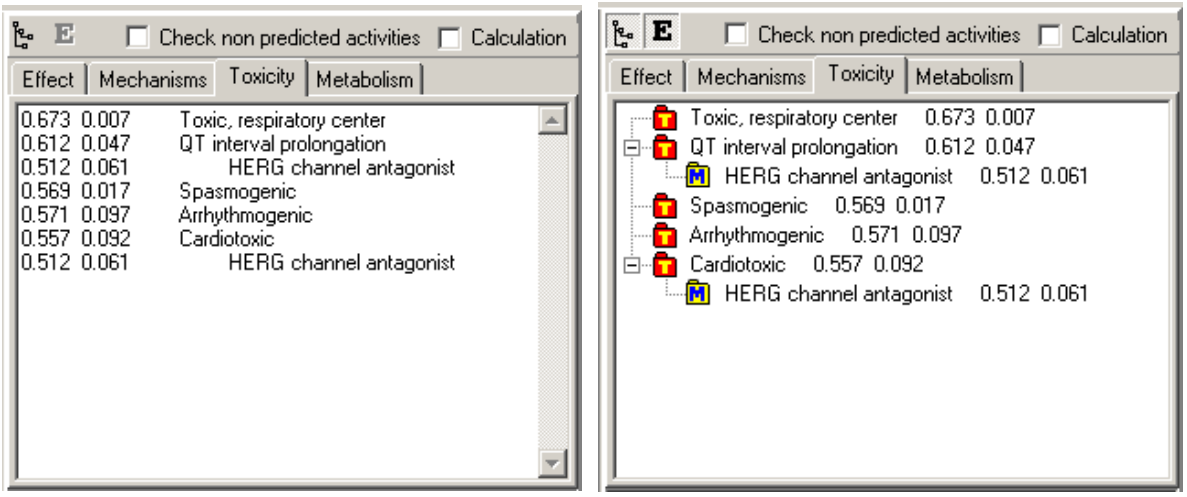
The icon indicates that this type of activity is a pharmacological effect.

The icon indicates that this type of activity is a mechanism of action.

The icon indicates that this type of activity is both a pharmacological effect and a mechanism of action for the upper-class pharmacological effect (for example, 'Non-steroidal anti-inflammatory agent' is a mechanism of action for Analgesic and Antipyretic effects).

- **Toxicity** – all predicted adverse and toxic effects and their relationships with predicted mechanisms of action in the PASS prediction results that meet the criterion specified by the user.

Text **Graphic**



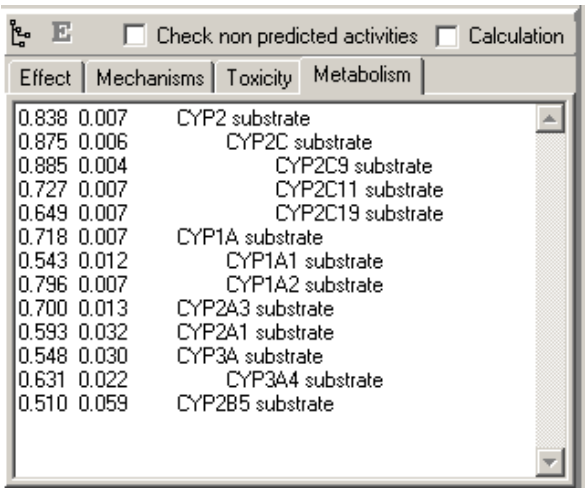
The icon indicates that this type of activity is a toxic or adverse effect.

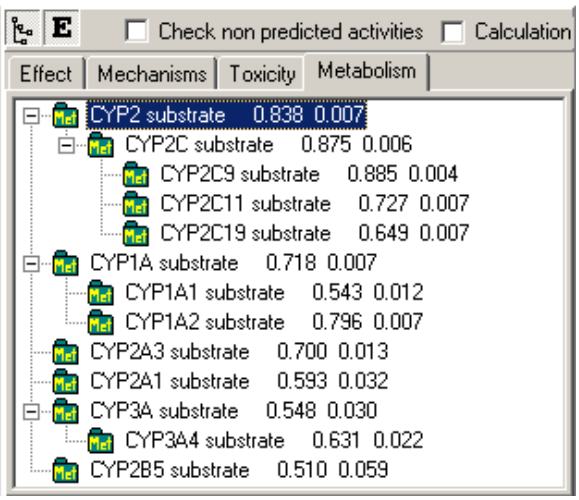
The icon indicates that this type of activity is a mechanism of action.

For example, 'HERG channel antagonist' is a mechanism of action for QT interval prolongation.

- **Metabolism** - all predicted metabolic terms for which the substance is a substrate/inhibitor or inducer for drug-metabolizing enzymes that meet the criterion specified by user.

Text **Graphic**

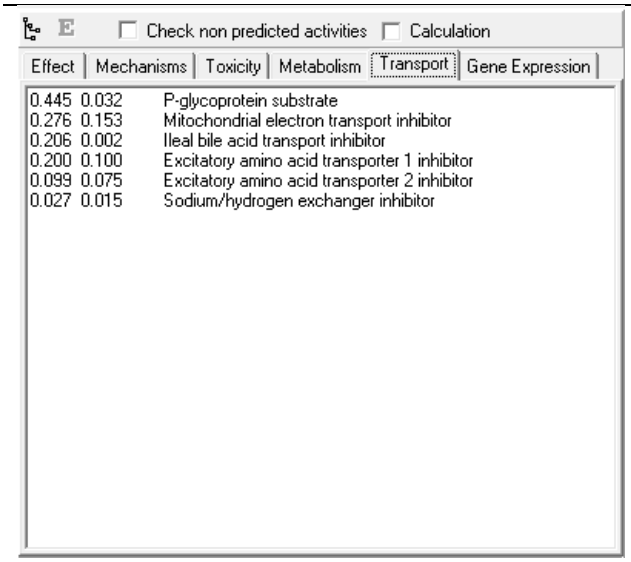


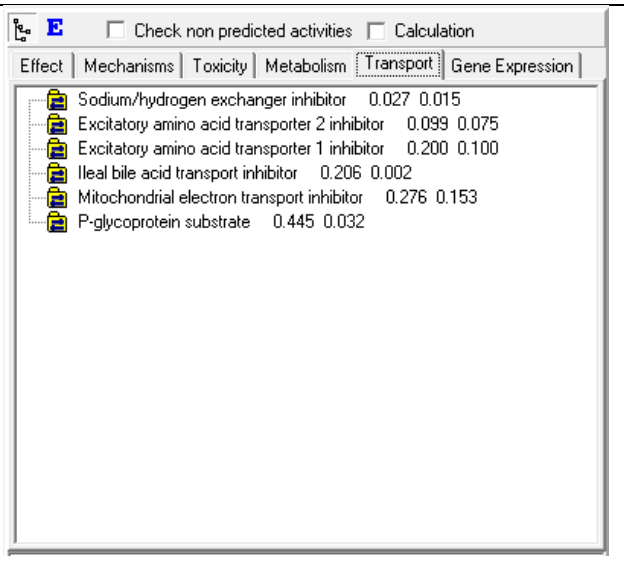


The icon Met indicates that this type of activity is a substrate/inhibitor or inducer of drug-metabolizing enzymes.

- **Transport** - all predicted transporter-related actions that meet the criterion specified by user.

Text **Graphic**

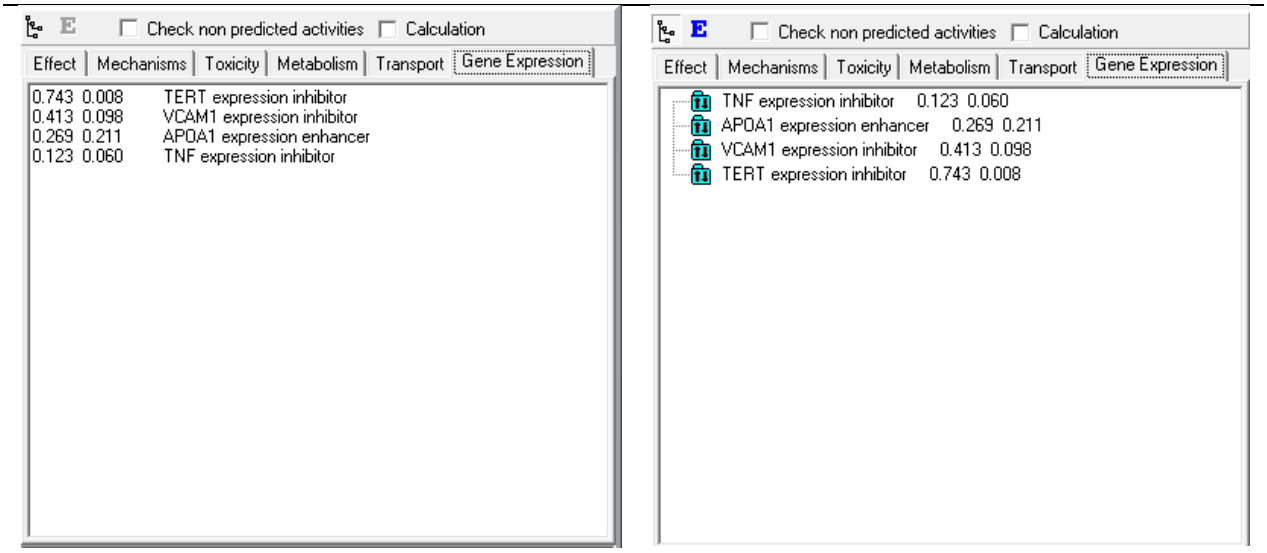




The icon indicates that this type of activity is a substrate/inhibitor or inducer of transport protein.

- **Gene Expression** - all predicted gene expression regulation terms that meet the criterion specified by user.

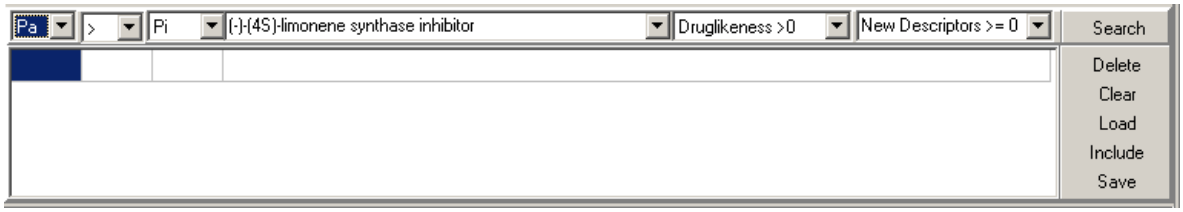
Text **Graphic**



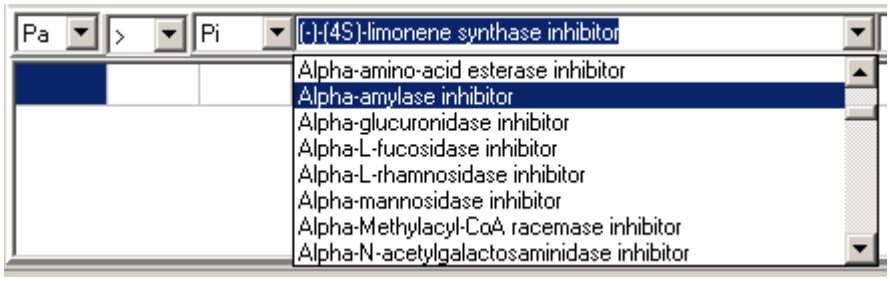
The icon indicates that this type of activity is inhibitor or enhancer for gene expression.

IV. The search for compounds with the desirable types of biological activities

The Search part of The Prediction & Interpretation window provides the means for search of compounds with the desirable types of biological activity, drug-likeness and number of new descriptors in the loaded PASS prediction results.

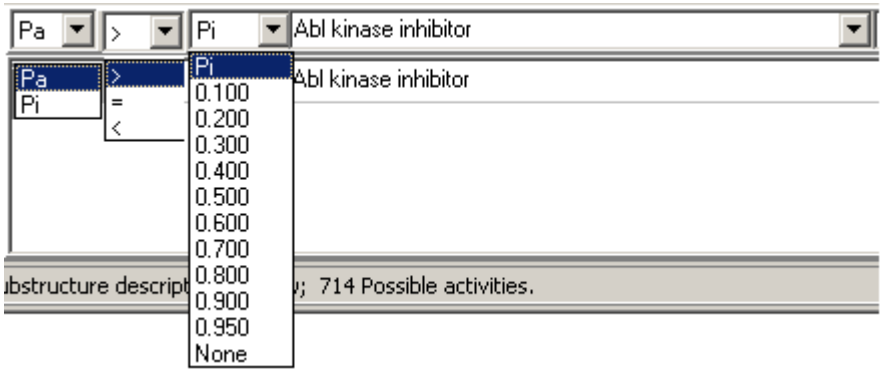


Select the required activity type(s) (desirable or undesirable) in the biological activity spectra using the drop-down box:



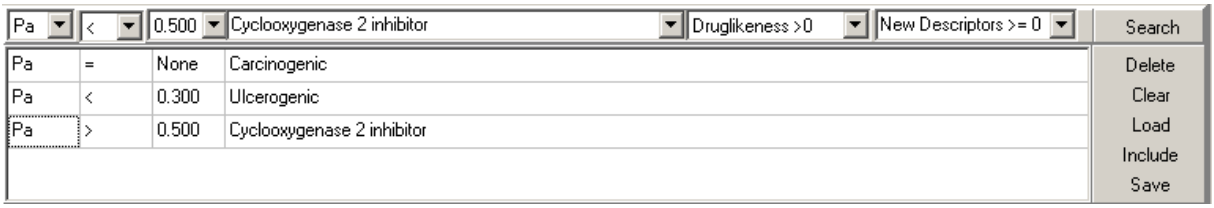
Type the first symbols of the activity type to quick selection of the required type of activity in this drop-down box. You should press "Enter" after selecting an activity to put it to the query table.

Choose the **Pa** or **Pi** value to specify the threshold for the selected types of activity using the drop-down boxes:



Choose 'Pa = None' for a particular type(s) of biological activity to find compounds for which one(s) is (are) not predicted.

For instance, to find compounds, which are predicted to be *Cyclooxygenase 2 inhibitor* with Pa>0.5, *Ulcerogenic* activity with Pa<0.1% and does not have *Carcinogenic* activity in their biological activity spectra we should specify the following query:



- Use "**Drug-likeness**" parameter for selection of compounds on the basis of PASS predicted Drug-likeness values (see page 12). It may be combined with activities query.
- Use "**New Descriptors**" parameters for selection of compounds with an appropriate number of new descriptors (new descriptors are descriptors, which are not found in any substance from PASS SAR Base during the prediction). It may be combined with activities query.

- Choose the **Delete** button to delete the marked type of activity.
- Choose the **Clear** button to clear the query table.
- Choose the **Load** button to load the txt file with parameters of the search.
- Choose the **Include** button to add the txt file with parameters of the search.
- Choose the **Save** button to save parameters of the search as txt file.

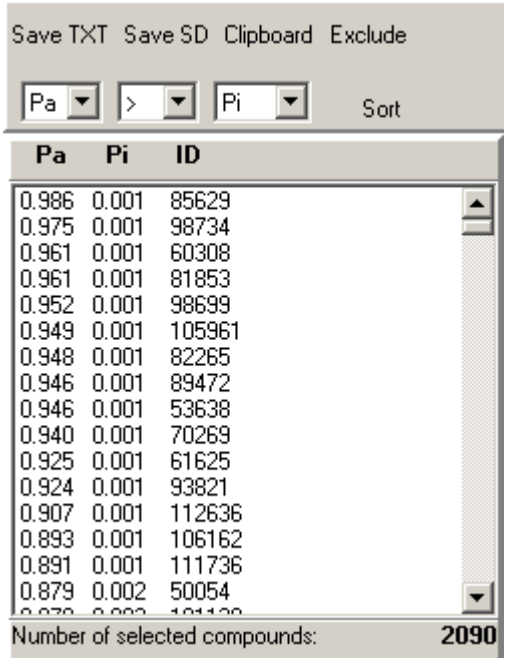
Press the button  in the Main window to open the "Mechanism-Effect" Relationships window to look up the associated activities of the particular type of activity.

Press the "Search" button to search the substances with requested properties.

The search results are appeared in the **results part of the search in Prediction & Intrpretation window**.

V. The result of the search

The Result part of the search in **Prediction & Intrpretation window** displays the ID with appropriate Pa and Pi values (Druglikeness or number of New Descriptors) of selected compounds.



Pa column displays either a probability of revealing this type of activity by the compound (if the search was carried out for one type of activity) or arithmetical mean of all Pa for the required types of activity.

ID column displays a structure identifier.

If a query in the Search window contains a type of activity with the search parameter 'None' (an unwanted type of activity) then the substances will be added to the Result window if the list with the predicted activity types does not contain this type of activity.

Choose the **Pa** value to define the threshold for the selection of the results using the drop-down box in the top of the Result window. The threshold is Pa > Pi by default.

Number of compounds displays the number of selected compounds.

- Press **Save TXT** button to save the search results as TXT file. The list with Pa, Pi and ID values will be saved.
- Press **Save SD** button to save the search results as SDfile. The selected structure with prediction results will be saved.
- Press **Clipboard** button to add the selected compounds to **Clipboard** window.
- Press **Exclude** button to exclude equivalent structures (Structures having the same set of MNA descriptors and atomic composition) from the selected compounds. It is useful by analysis of several SDfiles having the same structures.

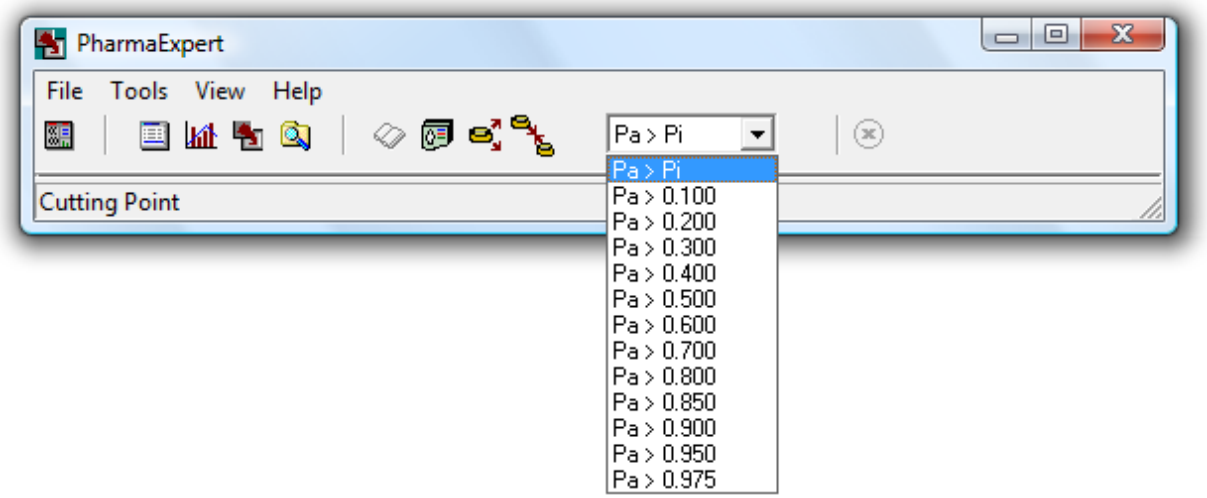
Click on the record to look on the structure and prediction & interpretation results.

Press the right button on a structure to call the Pop-Up menu with commands (see fig.):

The commands are similar to the earlier described buttons.

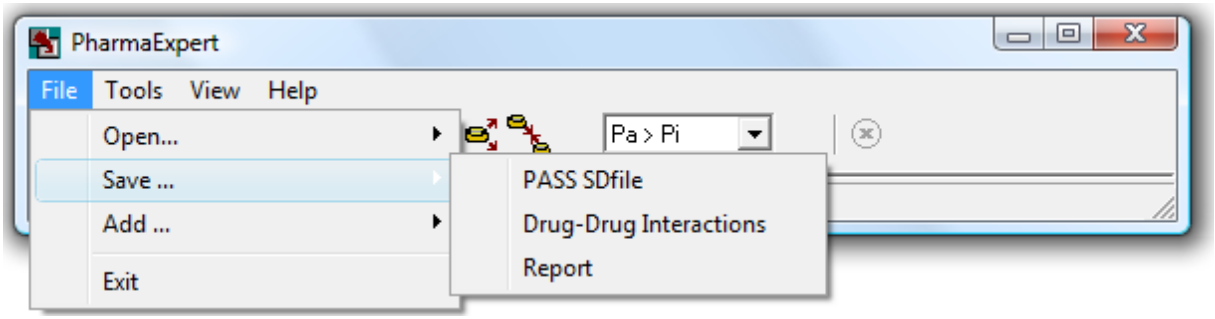
DROP DOWN LIST FOR CUTTING POINT

PharmaExpert interface provides a possibility to choose the selection criterion for activity types in the PASS prediction results. Only activities with **Pa** exceeded than the chosen threshold will be shown in predicted activity spectra in all PharmaExpert windows.



Pa value – the probability to find a particular type of activity for the substance. By default it is Pa > Pi (*Pi* - the probability that a particular substance does not have this type of activity. See also description of Biological Activity Spectrum on page 11).

SAVING PREDICTION RESULTS



Use **File|Save|PASS SDfile** menu command to save prediction and interpretation results as SDfile. The button  should be down before to save interpretation result otherwise only PASS prediction results would be saved.

Use **File|Save|Drug-drug interactions** to save .txt file with possible drug-drug interactions between all compounds in the current SD file with PASS prediction results.

Note! You may choose the Cutting Point for possible drug-drug interactions by **drop down list for cutting point** before saving (p. 48).

Example of txt file with potential drug-drug interactions for the first four compounds from example.sdf is presented:

Drug-drug interaction between compounds with ID: 1,2,3,4 – *Structure identifications*

Synergistic effects and actions - *compounds may lead to the same effects*

Antineoplastic – *name of synergistic effect*
Antineoplastic (1):1 - *name of synergistic effect (number of compounds with the effect): Structure identifications*
Antineoplastic enhancer (1):2
Phosphatase inhibitor (1):3
Antineoplastic 0.923 0.007 ID:1 Antineoplastic enhancer 0.835 0.005 ID:2
Antineoplastic 0.923 0.007 ID:1 Phosphatase inhibitor 0.627 0.121 ID:3
name of synergistic effect Pa Pi ID: Structure identificator of the compound name of synergistic effect Pa Pi ID: Structure identificator of the compound

Synergistic toxic and side effects - *compounds may lead to the same toxic or side effects*

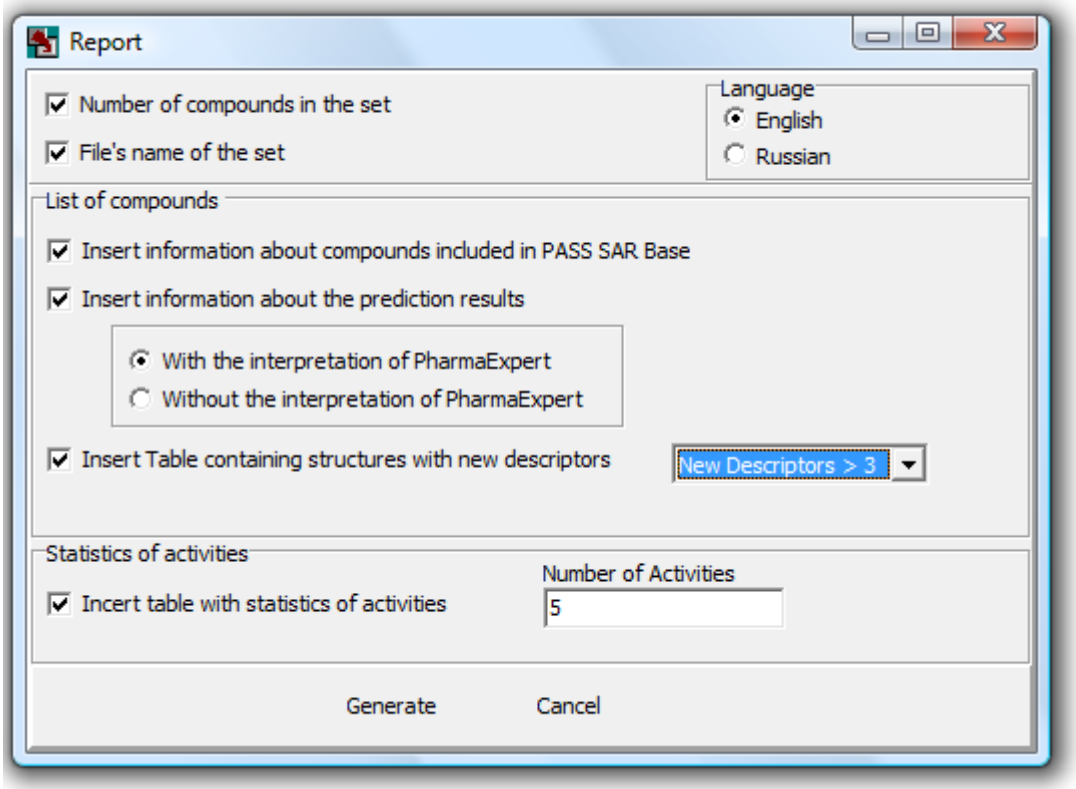
Metabolic drug-drug interactions - *compounds may lead to metabolic drug-drug interactions*

CYP3A1 substrate
CYP3A1 substrate (2):1,3
CYP3A1 substrate 0.552 0.102 ID:1 CYP3A1 substrate 0.948 0.004 ID:3

Pharmacodynamic drug-drug interactions - *compounds may lead to pharmacodynamic drug-drug interactions*

Dopamine release stimulant
Dopamine release stimulant (2):2,4
Dopamine release stimulant 0.614 0.081 ID:2 Dopamine release stimulant 0.656 0.063 ID:4

Use **File|Save|Report** menu command to save TXT file with the report about current SDfile.
The Report window will appeared to select parameters of the report.



You may select different characteristics of structures and SDfile for saving them in the report TXT file:

- **Number of compounds in the set** means that the information about the number of structures in appropriate SDfile will be included in the report.
- **File's name of the set** - the name of appropriate SDfile will be included in the report.
- **Language** – what language will be used in the report (English by default).
- **Insert information about compounds included in PASS SAR Base** – If the structure has an equivalent in the SAR Base its ID and known biological activities will be added to the report.
- **Insert the information about the prediction results** – the prediction results for all structures will be added to the report (the information is the same as one saved by command **File|Save|PASS SDfile**).
- **Insert Table containing structures with new descriptors** – the table with ID of structures and number of knew descriptors will be added to the report (you may select how many descriptors should be at the structure to include one into the table).
- **Insert Table with statistics of activity** – the table with activities arranged by descending order of number of compounds for activities that are predicted with probability Pa>0.7 will be added to the report (you may limit the number of activities in the table, all activities are included if the field "Number of Activities" is empty).

Example of TXT file with the report about example.sdf is presented:

General information about the set of compounds, for which the PASS predictions were obtained, and the version of PASS, with which the predictions were obtained:

Number of compounds in the set: 100.
The prediction results were obtained for all compounds by PASS version, that predicts 3750 types of biological activity including 418 Pharmacological Effects, 3032 Molecular Mechanisms, 196 Metabolism-Related Actions, 11 Gene Expression Regulation, 35 Transporters-Related Actions, 58 Side Effects and Toxicity.
The prediction results are in the file Example_SA.SDF.

If molecule under prediction has the same set of MNA descriptors as any molecule from the training set of PASS, this compound is considered as an equivalent. To provide more objective prediction results, equivalent structures and all associated information about biological activities are excluded from PASS SAR Base during the prediction. The list of such molecules with the appropriate information about biological activity is given below.

Compounds that are included into the PASS training set:

> <AutoID> (1)
1

> <PASS_KNOWN_ACTIVITIES>
Antineoplastic
Antiviral

> <AutoID> (2)
2

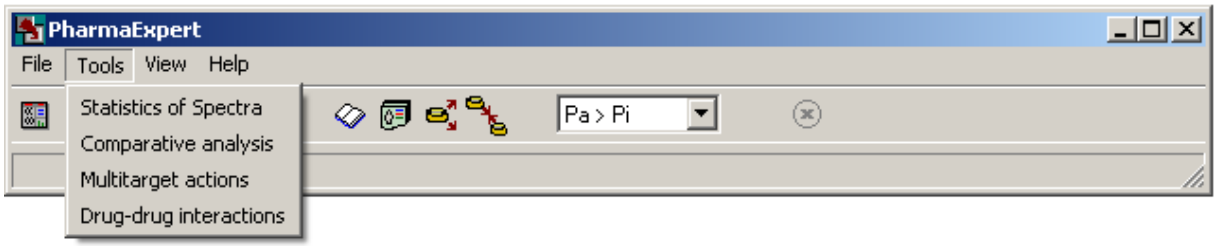
> <PASS_KNOWN_ACTIVITIES>
Antiarrhythmic
Antihypertensive
Cardiotonic
Phosphodiesterase inhibitor
Psychotropic
Spasmolytic
Teratogen

The set does not include structures with the selected number of new descriptors.

For the particular set of molecules under prediction the statistics of different biological activities is calculated. Here the statistics is arranged descending order of number of compounds for activities that are predicted with probability Pa>0.7.

No	Pa>Pi	Pa>30%	Pa>50%	Pa>70%	Types of Activity
1	70	65	46	37	Toxic
2	61	50	42	32	Teratogen
3	57	55	37	31	Antiinflammatory
4	60	51	36	29	CYP3A4 substrate
5	59	55	37	28	Reproductive dysfunction

TOOLS OF PHARMAEXPERT

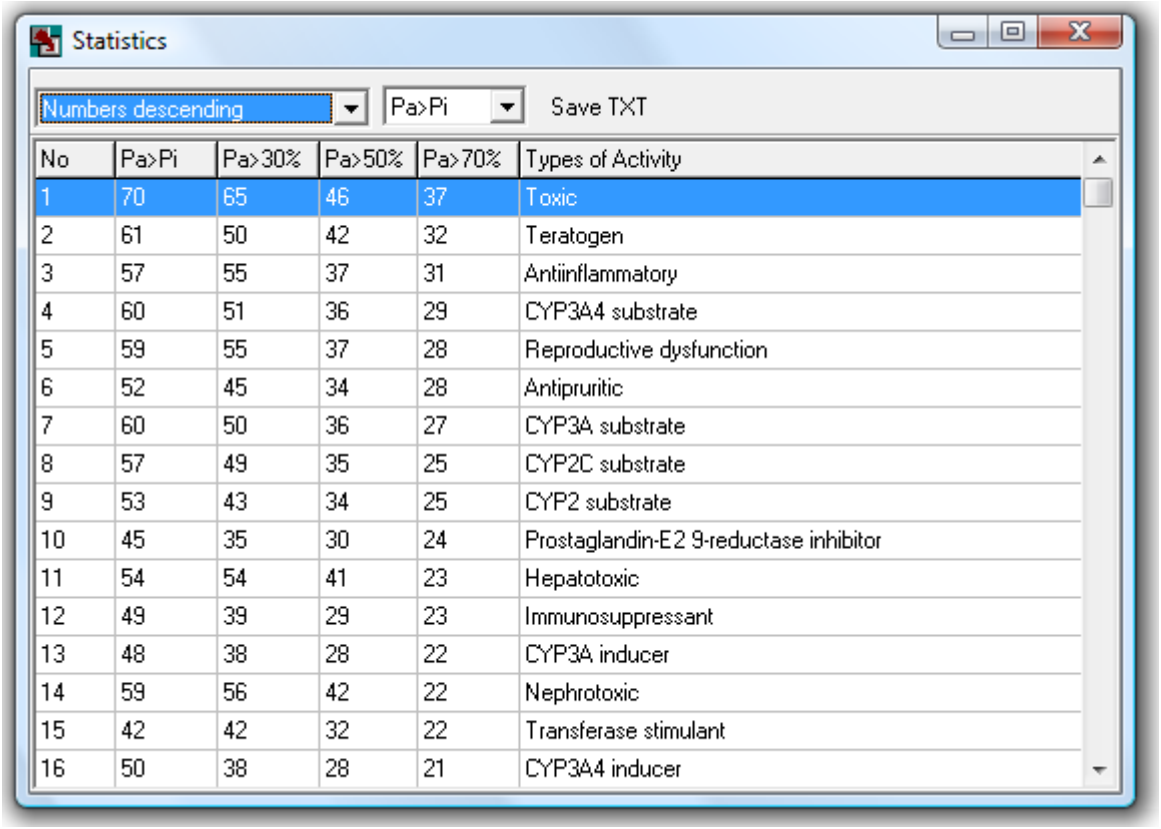


PharmaExpert provides the following tools for analysis of PASS prediction results:

Predict menu commands	Description
Tools|Statistics of Spectra	Use **Tools|Statistics of Spectra** menu command or press the button to open the window with a table containing data on the number of substances with a particular type(s) of biological activity. The **Statistics window** will be opened.
Tools|Comparative analysis	Use **Tools|Comparative analysis** menu command or press the button to open the window for comparison of biological activity spectra of substances. The **Comparative analysis** window will be opened.
Tools|Multitargeted actions	Use **Tools|Multi-targeted actions** menu command or press the button to open the window for search compounds with multitarget actions. The **Multi-Targeting** window will be opened.
Tools|Drug-drug interactions	Use **Tools|Drug-drug interactions** menu command or press the button to open the window for analysis of drug-drug interactions of selected substances. The **Drug-drug interactions** window will be opened.

STATISTICS OF PASS PREDICTION RESULTS

Use **Tools|Statistica of Spectra** menu command or the button  to open this window.



No	Pa>Pi	Pa>30%	Pa>50%	Pa>70%	Types of Activity
1	70	65	46	37	Toxic
2	61	50	42	32	Teratogen
3	57	55	37	31	Antiinflammatory
4	60	51	36	29	CYP3A4 substrate
5	59	55	37	28	Reproductive dysfunction
6	52	45	34	28	Antipruritic
7	60	50	36	27	CYP3A substrate
8	57	49	35	25	CYP2C substrate
9	53	43	34	25	CYP2 substrate
10	45	35	30	24	Prostaglandin-E2 9-reductase inhibitor
11	54	54	41	23	Hepatotoxic
12	49	39	29	23	Immunosuppressant
13	48	38	28	22	CYP3A inducer
14	59	56	42	22	Nephrotoxic
15	42	42	32	22	Transferase stimulant
16	50	38	28	21	CYP3A4 inducer

- The **Pa>Pi** column displays the number of substances for which Pa>Pi for a particular type of biological activity.
- The **Pa>30%** column displays the number of substances for which Pa>30% for a particular type of biological activity.
- The **Pa>50%** column displays the number of substances for which Pa>50% for a particular type of biological activity.
- The **Pa>70%** column displays the number of substances for which Pa>70% for a particular type of biological activity.
- The **Types of Activity** column displays activity names.

The names of activities are sorted by descending order of the number of substances for which Pa>Pi for a particular type of biological activity (by default).

The names of activities can be sorted using the drop-down box in the left top corner of the Statistics window:

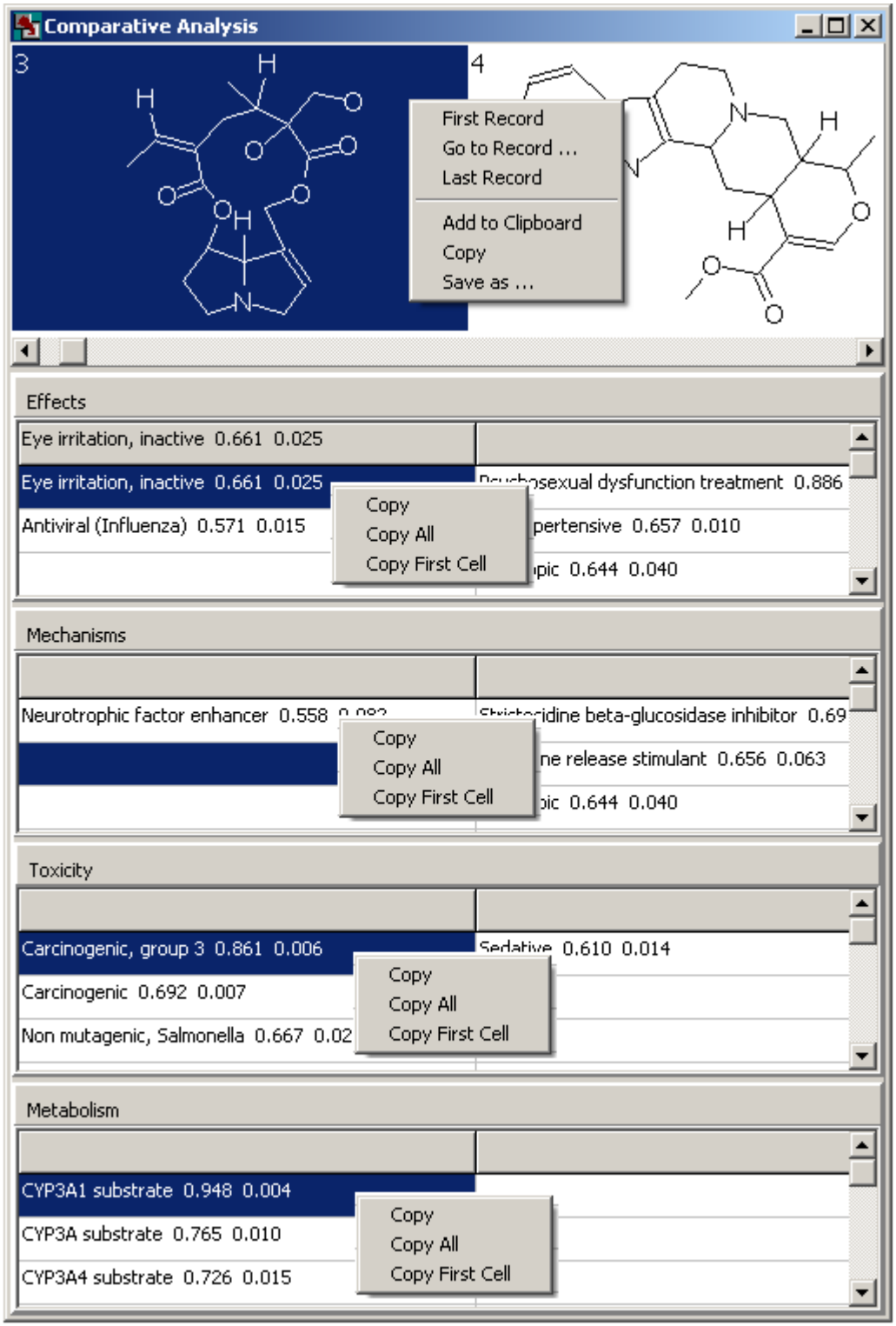
Activities ascending	Alphabetically.
Activities descending	Alphabetically (reverse order).
Number ascending	Ascending order of number of the substances, which have particular types of biological activity, respectively for Pa>Pi, Pa>30%, Pa>50% or Pa>70% according to the drop-down box in the right top corner of the **Statistics** window.
Number descending	Descending order of number of the substances, which have particular types of biological activity, respectively for Pa>Pi, Pa>30%, Pa>50% or Pa>70% according to the drop-down box in the right top corner of the **Statistics** window.

Press 'Save TXT' button to save the table as *.txt file.

Note! Double click on the left mouse button on the threshold of the particular type of activity to open the **Search part of The Prediction & Intrpretation window** and find the substances, which have this type of biological activity in the predicted biological activity spectrum.

COMPARATIVE ANALYSIS

Use **Tools|Comparative analysis** menu command or press the button  to open the window for comfortable comparison of biological activity spectra of several substances from the set.

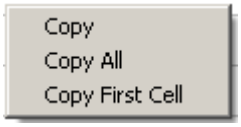


Press the right button on a structure to call the Pop-Up menu with the following commands:

- Choose **First Record** command to go to the first record.
- Choose **Go to Record** command to go to the chosen record. The **Go to** dialog box will appear. You should type the Record Number and press **OK** to go to this record.

- Choose **Last Record** command to go to the last record.
- Choose **Add to Clipboard** command to add the substance to **Clipboard.** The "Substance ID" will be added.
- Choose **Copy** command to copy the current structure to the clipboard. It is saved as a bitmap (.bmp file). This format is not used in ISIS Base.
- Choose **Save As...** command to save the current PASS prediction results to a text file.

The prediction results for each structure are divided into four sections: effects, mechanisms, toxicity and metabolism. This separation is similar to those in **Prediction&Interpretation window**. Click right mouse button to call the Pop-Up menu with commands:

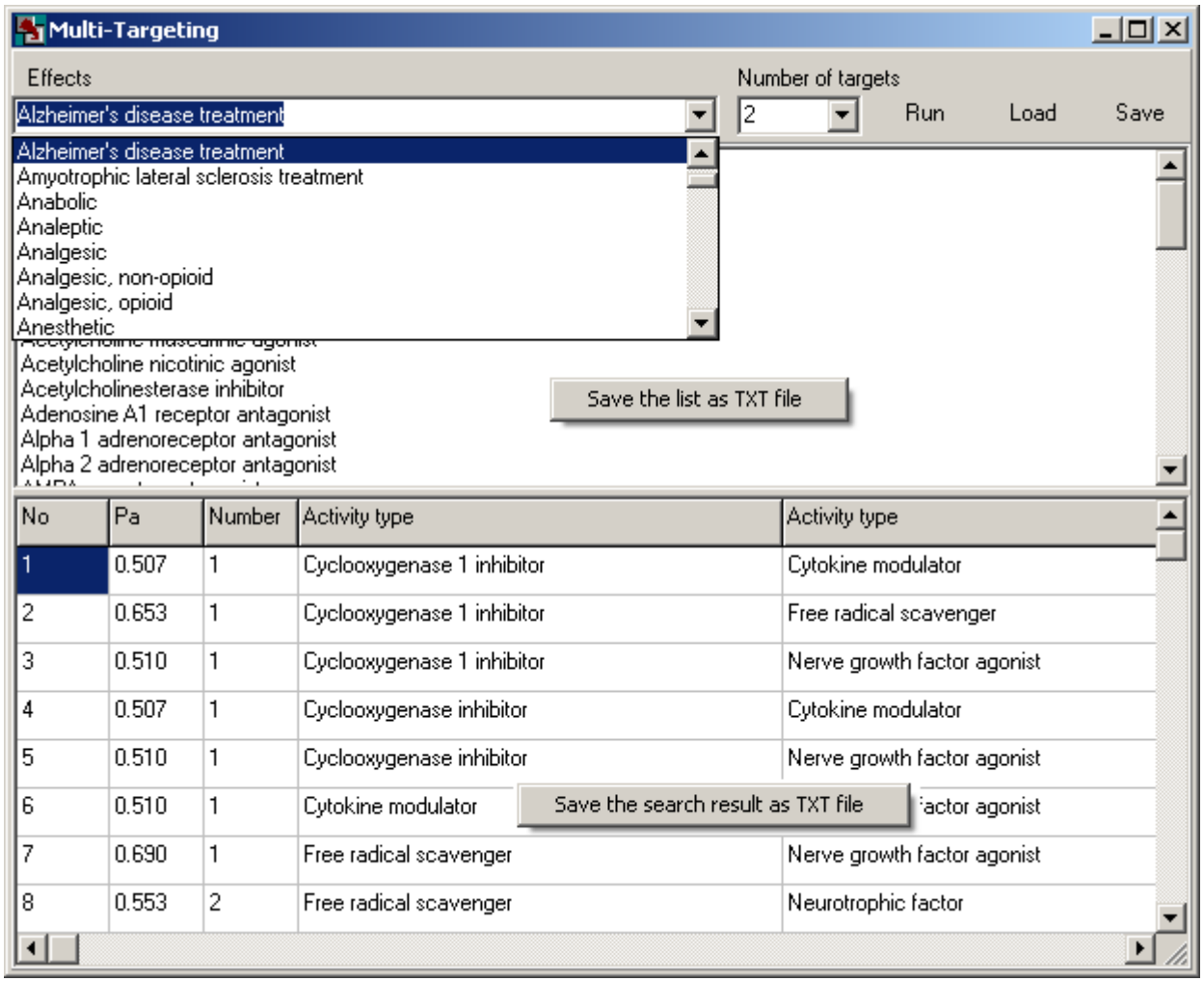


- Choose **Copy** command to copy a cell;
- Choose **Copy All** command to copy a content of the column;
- Choose **Copy First Cell** command to copy a content of the first cell.

Note! Use the left double click to move the selected type of activity to the first row in all columns.

MULTI-TARGETING ANALYSIS

Use **Tools|Multitargeted actions** menu command or press the button  to open the window for the search of compounds with multitarget actions.



The window provides a tool for the search of compounds with multitarget actions on the basis of the analysis of PASS prediction results.

First of all you should select a desirable effect in the "Effects" drop-down box. Its mechanisms of action will appear. You should limit the number of mechanisms of action that may be included in biological activity spectra simultaneously (use "Number of targets" drop-down box for this). For example, choose "2" if you would like to find compounds with dual-mechanisms of action. You may also establish the threshold value for **Pa** by **drop down list for cutting point**.

Press the "Run" button to start search.

The search result will be displayed at the table below. The table has the following colons:

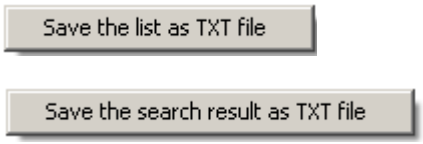
- **No** – number of the row in the table;

- **Pa** – maximal Pa values for the mechanisms from the selected compounds;
- **Number** – the number of compounds with appropriate mechanisms of action;
- **Activity type** – a name of the mechanism of action.

The **Multi-Targeting window** has following buttons:

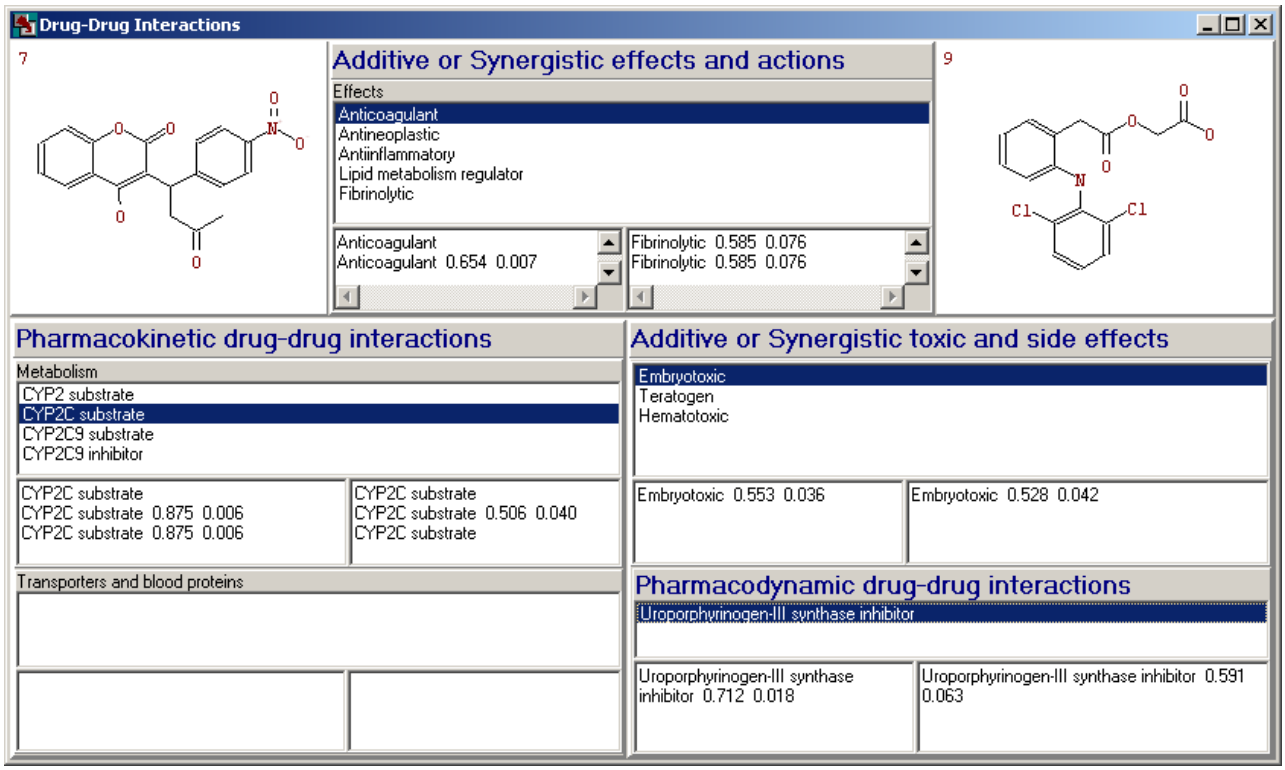
- **Run -** starts the search of compounds with multitarget actions;
- **Load –** loads a text file with list of activity types;
- **Save -** saves the search results to the text file.

Note! The list of mechanisms of action or the search results may be saved by clicking the right mouse button on the list of activity or on the table with the search results. The following Pop-Up menu will appear:



DRUG-DRUG INTERACTIONS ANALYSIS

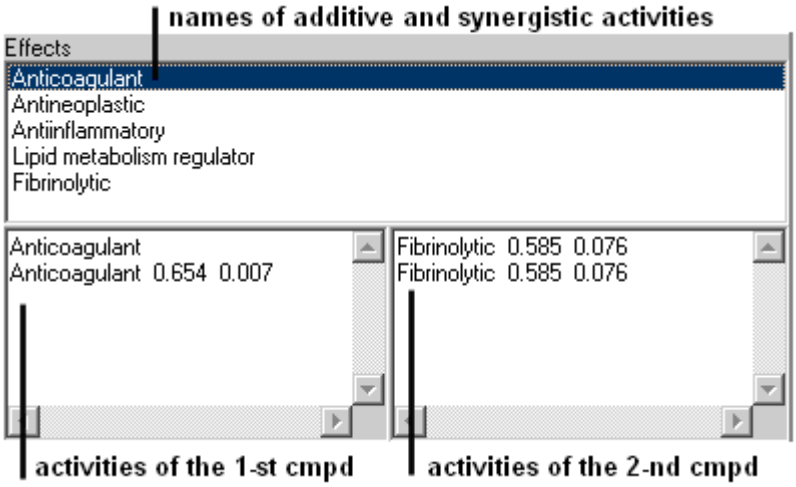
Use **Tools|Drug-drug interactions** menu command; press the button  or use **Add first structure to Drug-Drug interaction analysis** and **Add second structure to Drug-Drug interaction analysis** commands of Pop-up menu in **Prediction&Interpretation** and **Comparative analysis** windows to open the window for analysis of drug-drug interactions of selected substances.



The window provides information about probable types of drug-drug interactions between two selected structures:

- **Additive or Synergistic effects and actions** means that both compounds may lead to the same effect.
- **Pharmacokinetic drug-drug interactions** include interaction on metabolism and transport levels. It means that both compounds are a substrate/inhibitor/inducer of the same izoform of drug-metabolizing enzymes or transport proteins;
- **Synergistic toxic and side effects** mean that both compounds may lead to the same toxic or side effect;
- **Pharmacodynamic drug-drug interactions** mean that both compounds act on the same drug-target.

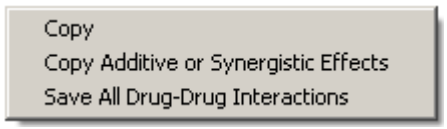
All types of interaction are represented in a similar view. For example:



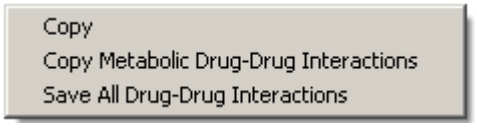
The field of names of additive or synergistic activities includes the name of activities that could be increased if both compounds are used simultaneously. It could be due to several reasons, for example both compounds reveal the same effect or one compounds acts on mechanism causing the effect of another compound.

Click on the name of the type of biological activity to look at the reason of drug-drug interaction, which is shown in the list boxes below. The absence of Pa and Pi at the name of activity type means that the structure with this activity is in the PASS training set.

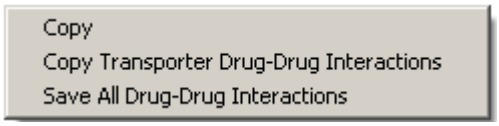
You can save or copy the results of drug-drug interactions analysis by clicking the right mouse button on the appropriate list box. The following Pop-Up menus will appear:



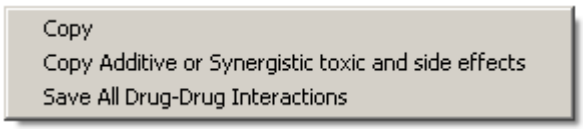
for the **Additive or Synergistic Effects and Actions** list box;



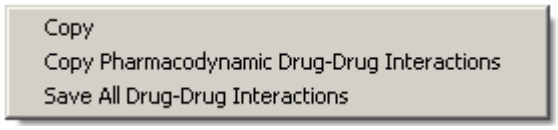
for the **Metabolic Drug-Drug Interactions** list box;



for the **Transporter Drug-Drug Interactions** list box;



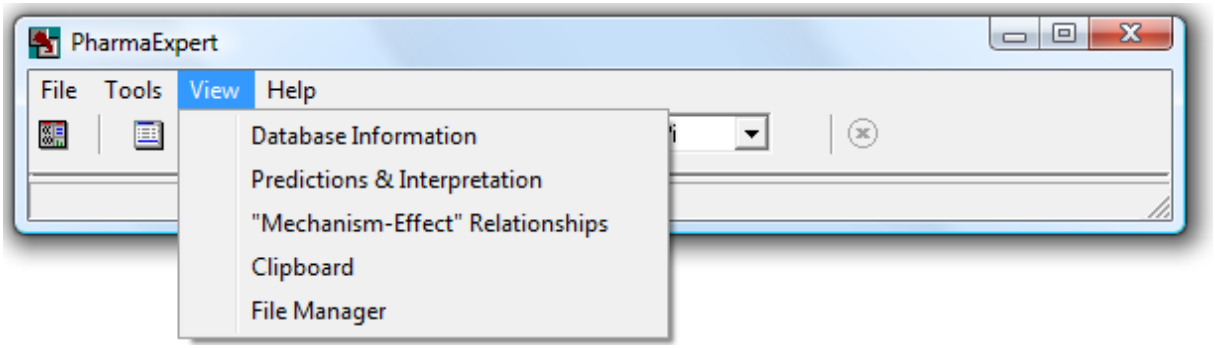
for the **Additive or Synergistic Toxic and Side Effects** list box;



for the **Pharmacodynamic Drug-Drug Interactions** list box.

- **Copy** means a copying of the selected activity type with the related drug-drug interactions to the Microsoft Windows' clipboard.
- **Copy appropriate types of drug-drug interactions** means the copying of all activity types in the current list box with the related drug-drug interactions to the Microsoft Windows' clipboard.
- **Copy appropriate types of drug-drug interactions** means saving to .txt file all possible drug-drug interactions between two compounds.

VIEWING BASIC INFORMATION

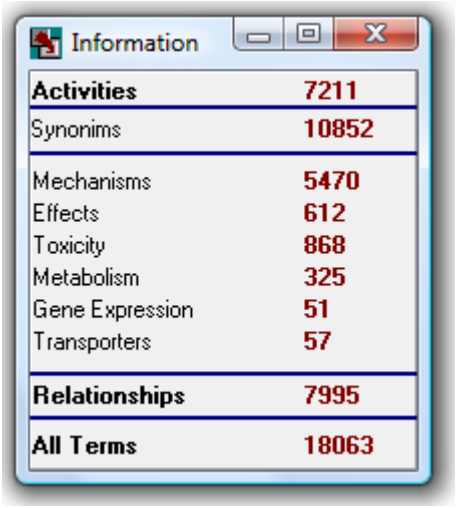


Use **View** menu command to display the following information: current status of the MER Base, prediction results and their interpretation; window of MER Base, Clipboard and File Manager window:

View menu options	Description
Database Information	Use **View|Database Information** menu command to display the current status of MER Base.
Prediction & Intrpretation	Use **View|Prediction&Interpretation** menu command or press the button  to display the **Prediction & Interpretation** window.
"Mechanism-Effect" Relationships	Use **View|"Mechanism-Effect" Relationships** menu command to display the **"Mechanism-Effect" Relationships window**.
Clipboard	Use **View|Clipboard** menu command to display the **Clipboard window** for editing and saving the data for compounds, which have been added to Clipboard.
File Manager	Use **View|File Manager** menu command or press the button  to display the **File Manager window** for switching between the prediction results files, which have been loaded. The current file is displayed in **Prediction&Interpretation** window.

DATABASE INFORMATION

This command displays the window with current status of "Mechanism-Effect" Relationships Database (MER Base).

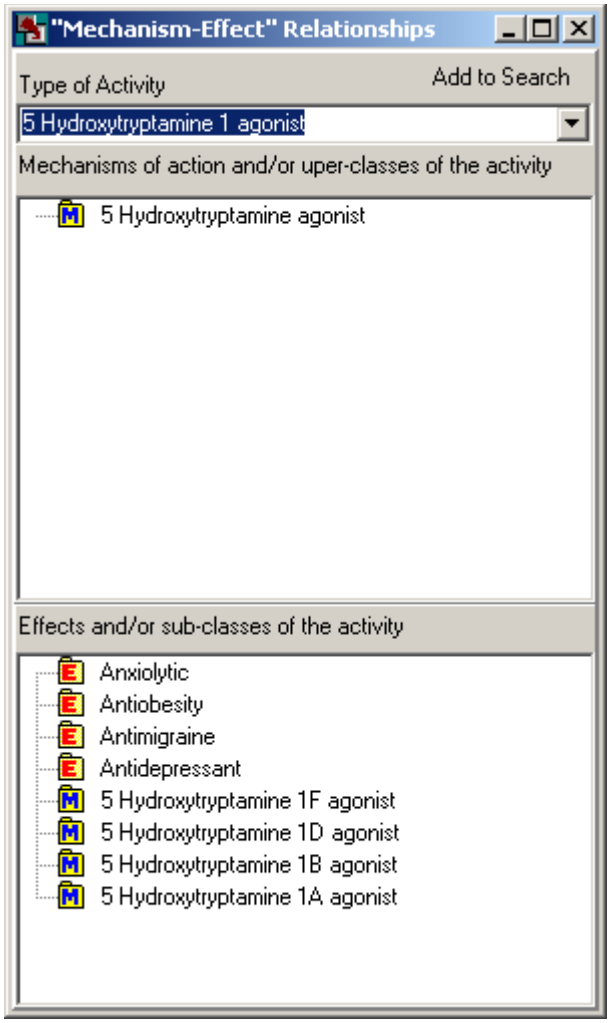


Where,

Activities - Number of main names of biological activity in MER Base
Synonyms - Number of synonyms of biological activity in MER Base
Mechanisms - Number of mechanisms in MER Base
Effects - Number of pharmacological effects in MER Base
Toxicity - Number of toxic and side effects in MER Base
Metabolism - Number of metabolic terms in MER Base
Gene Expression - Number of gene expression regulation in MER Base
Transporters - Number of transporter-related actions in MER Base
Relationships - Number of "Mechanism-Effect" Relationships in MER Base
All Terms - Number of all names of biological activity in MER Base

MECHANISM-EFFECT RELATIONSHIPS

This command displays **"Mechanism-Effect" Relationships window** that includes information about relationships between types of biological activity on the basis of MER Base.



Select the particular type of activity in the "Type of Activity" drop-down list to display the associated types of activity.

"Mechanisms of action and/or upper-classes of the activity"

The "Mechanisms of action and/or upper-classes of the activity" list displays either mechanisms of action which cause the selected type of activity in the "Type of Activity" drop-down list or the type of activity belonging to the upper-class for the selected type of activity (for example, 5 Hydroxytryptamine agonist is the upper-class of the 5 Hydroxytryptamine 1 agonist).

"Effects and/or sub-classes of the activity"

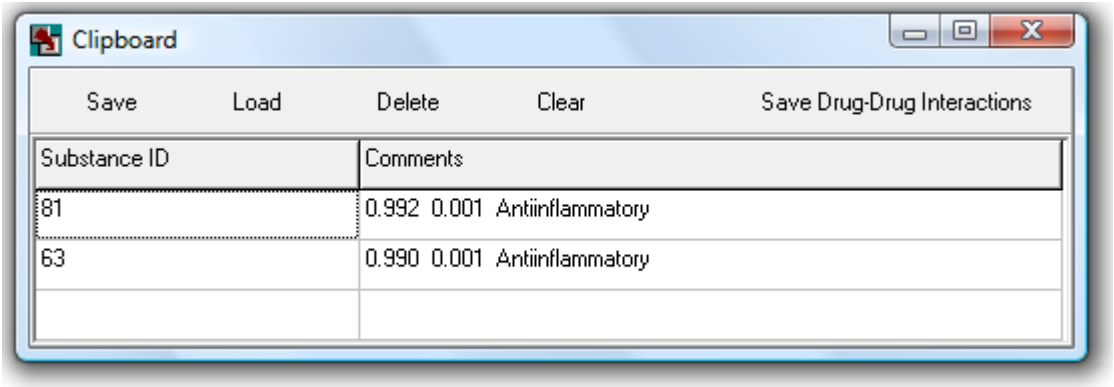
The "Effects and/or sub-classes of the activity" list displays either biological effects which may be caused by the selected type of activity in the "Type of Activity" drop-down list or the type of activity belonging to the sub-class for the selected type of activity.

Select the type of activity and press the Add to Search button to add it in the **Search part of Prediction & Interpretation window**.

Note! You should double click on the activity in "**Mechanisms of action and/or upper-classes of the activity**" or "**Effects and/or sub-classes of the activity**" lists to go to its associated types of activity.

CLIPBOARD

The Clipboard provides a place to collect the selected substances.



The Clipboard provides the following options:

1. **Save** button - saving the data on structure of compound, PASS prediction results and PharmaExpert interpretations as SDfile;
2. **Load** button - load a text file with IDs of structures;
3. **Delete** button - delete the record in the table;
4. **Clear** button - clear Clipboard;
5. **Save Drug-Drug Interactions** button - save a text file with drug-drug interactions between the structures in the Clipboard.

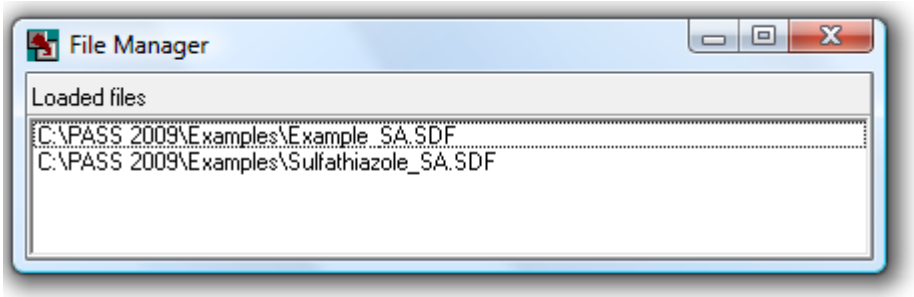
To place an item to the Clipboard, select the ID in the **Results part of Prediction&Interpretation window**, click the left mouse button and select the Add to Clipboard command. You may also place an item to the Clipboard from the **Prediction&Interpretation window**, just click on the left mouse button and select the Add to Clipboard command.

Substance ID is the previously defined identifier of the compounds.

Comments – the comments, which are added automatically when you add ID using the Results part or Prediction&Interpretation part of **Prediction&Interpretation window**. For example, 0.981 is **Pa** and 0.003 is **Pi** values in the first row. Antiinflammatory is a type of activity which is a parameter for search in the Search part **Prediction&Interpretation window** or if you select the activity type in the prediction result from the Prediction&Interpretation part of **Prediction&Interpretation window**.)

FILE MANAGER

This command displays the window with loaded files with PASS prediction result to PharmaExpert.



Choose the file name to display the data from the file.

CHAPTER 7

INTERPRETING PREDICTION RESULTS

The result of prediction for the substance is returned in the form of a table containing the list of biological activities with appropriate probability values - i.e. the likelihood for the given activity to be either revealed (Pa) or not revealed (Pi)**.** Their values vary from 0.000 to 1.000.

The more is Pa value, the less is the probability of false positives in the set of compounds selected for biological testing. For example, if one selects for testing only compounds for which a particular activity is predicted with Pa $\geq$ 90%, the expected probability to find inactive compounds in the selected set is very low, but about 90% of active compounds are missed. If only compounds with Pa $\geq$ 80% are chosen, the probability to find inactive compounds is also low, but about 80% of active compounds are missed; etc. By default, in PASS Pa=Pi value is chosen as a threshold, therefore all compounds with Pa>Pi are suggested to be active.

Another criterion for selection is the compounds' novelty. If Pa value is high, sometimes one may find close analogs of known biologically active substances among the tested compounds. For example, if Pa > 0.7 the chance to find the activity in experiment is high, but in some cases the compound may occur to be the close analogue of known pharmaceutical agents. If 0.5 < Pa < 0.7 the chance to find the activity in experiment is less, but the compound is not so similar to known pharmaceutical agents. If Pa < 0.5 the chance to find the activity in experiment is even more less, but if it will be confirmed the compound might occur to be a New Chemical Entity.

The quality of predictions is the main criterion of the program power. The mean accuracy of the prediction is about 92% (leave one out cross validation). There is an appropriate table where the maximum error of prediction for each type of activity is shown (see Supplement). In "Prediction Results" window a user also obtains the total sum of chemical descriptors of the substance. Reported are the number of descriptors, which are new compared with the PASS training set descriptors.

It should be mentioned that prediction of biological activity spectra is possible only for low molecular weight (drug-like) substances. Prediction of biological activity spectra for synthetic or biopolymers and inorganic substances will not provide reasonable results.

In some cases substance is predicted simultaneously as agonist and antagonist (stimulator and blocker, activator and inhibitor) for the same receptors (enzymes, etc.). It means that PASS could not make differentiation of intrinsic activity of substance and indicate only its affinity to this receptor (enzymes, etc.).

It is necessary to stress that PASS can't predict if the concrete substance becomes drug, because it depends on many other factors. Prediction, however, can help to define what

kind of tests are adequate for studying of biological activity of concrete chemical substance and which substances more probable will reveal the required effects.

Based on these criteria, you may choose which activities have to be tested in your compounds on the basis of compromise between the novelty of pharmacological action and the risk to obtain the negative result in experimental testing.

Certainly, you will also take into account your particular interest to some kinds of activity, experimental facilities, etc.

CHAPTER 8

TROUBLESHOOTING

The best practice is to create regular backup from all newly created or modified data files:

- SAR Base files,
- generates SDF files

In case of any trouble researcher can reinstall PharmaExpert from distributive and restore generated data files.

Please contact us by e-mail: pass@ibmc.msk.ru for PharmaExpert support.

CHAPTER 8

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