Cheburator

user manual

version 1.2.0
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1. Introduction and the purpose of this software

High-throughput *in vitro* screening of natural and synthetic compounds is an important primary stage in the selection of new anticancer drugs throughout the world. This approach was developed in the late 1980's and evolved from earlier screening programs based on the *in vivo* mouse leukemia models. The first large scale *in vitro* screening program was launched by the U.S. National Cancer Institute (NCI) in 1990 (http://www.dtp.nci.nih.gov). They selected sixty human cancer cell lines representing nine types of tumors as the test panel (NCI60 cancer cell line panel)[1]. Despite some criticism, the validity of such an approach was demonstrated by almost two decades of extensive usage. Nowadays, this approach has also been adopted in a number of laboratories working in the area of anticancer drug discovery.

The results of *in vitro* screening tests are often presented as an inhibitory concentration 50% (IC_{50}): the concentration of the tested agent that inhibits the proliferation of the cancer cell population to 50% of the theoretically possible effect (absolute IC_{50}) or maximum effect practically achieved by the drug (relative IC_{50}). The detailed description between the two types of IC_{50} values is provided below. The amount of data obtained during a screening program requires extensive processing and calculation. Therefore, the Cheburator software was developed for automated data processing and calculation of the IC_{50}. This software could substantially reduce the amount of time spent by scientific staff on data evaluation and could be utilized in numerous laboratories working in the area of the *in vitro* drug screening programs.

2. Implementation and availability

The Cheburator software for automated calculation of *in vitro* inhibitory concentrations was developed using the open-source Lazarus/FPC compiler of the Object Pascal programming language (http://www.lazarus.freepascal.org) and DMath 0.90 statistical library. The currently stable version has a user-friendly interface and runs under the Microsoft Windows family of operating systems. The software is standalone and does not require installation or any additional run-time third-party libraries. The latest version of the software, together with sample data files and documentation, can be downloaded from the following URL:

http://www.cheburator.nevozhay.com

The software is freely available for academic use, but a license is required for commercial use. Please send all inquiries or suggestions concerning this software to the author:

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3. Usage description

3.1. Prerequisites for the tests analyzed by the Cheburator

To standardize the calculation algorithm, several prerequisites have to be met for *in vitro* test results to be further processed by Cheburator. The software was designed to analyze results from the tests performed using 96-well plates. One row on each plate must contain appropriate control values – the first 9 wells of the row containing untreated cells and last 3 wells of the row have to represent background values for the cell culture medium without cells added to the plate. The second assumption is that the other rows on the plate have to represent data for the compounds of interest tested in 4 concentrations placed in the triplicates (4 concentrations x 3 wells = total 12 wells in the each row/compound used in the test). Concentrations have to be arranged from the greatest to the smallest from the left to the right, respectively.

Example: *Below is the sample plate with one control row (**H**), one row for reference drug (**A**) and four rows for four novel studied compounds (**B**, **C**, **D**, **E**, respectively) tested in four drug concentrations. Please note: you do not have to use all rows on the plate. You can also use different ranges of drug concentrations for different drugs as in this test (100, 10, 1 and 0.1 µM for the reference drug and 10, 1, 0.1 and 0.01 µM for the novel studied compounds, **Figure 1**).*

Figure 1. *Sample plate with four tested compounds and the reference drug. Concentrations are highlighted in red. Control of untreated cells (CC) and control of media (CM) are in blue and green colors, respectively.*

3.2. Data loading and built-in converter

Initially, the software was programmed to read the data files produced by a Multiskan RC photometer (Labsystems, Finland) used for acquiring the optical densities from colorimetric assays performed on the 96-well plates. However, to ensure wider use of the software, we have added a built-in converter for other data file formats. The software can import data from comma separated files produced by Excel or other spreadsheet programs. The converter is customizable to provide a wide range of possibilities for other format imports, even for any we are not aware of.

In the case that some data file format might not be directly imported into the Cheburator using the built-in converter, format examples are included with the installation package in the Samples directory, allowing users to program their own converters between third-party software and Cheburator. The software was tested on the results from both SRB[2] and MTT[3] assays. However, complete functionality is also possible with other assays based on measurement of optical densities.

*__Example:__ Run Cheburator and click **Open data file** button. Locate and select file the **victor.csv** in the **Samples** directory. Since this is a comma separated file produced by Excel, it has to be converted before analysis. You will see the built-in converter window shown on **Figure 2**. Please select the first row of the plate data from the list box, as shown in the picture. Other rows have to follow immediately after the first row. All rows before and after the plate data will be discarded. Each read row is then divided into separate values using the delimiter specified in the combo box below (semicolon is the default, but other delimiters are also supported). Since each row could contain more values than the number of wells, you also have to specify in the edit box below which value in the row will be treated as the optical density value for the first well. Data for the subsequent wells have to follow immediately after, separated by the delimiters. Note that you can use either period or comma as decimal separator in your data files. Cheburator will automatically convert all decimal separators into periods, which are the default. After specifying the above parameters, click the **OK** button and data will be imported from the file for analysis.*
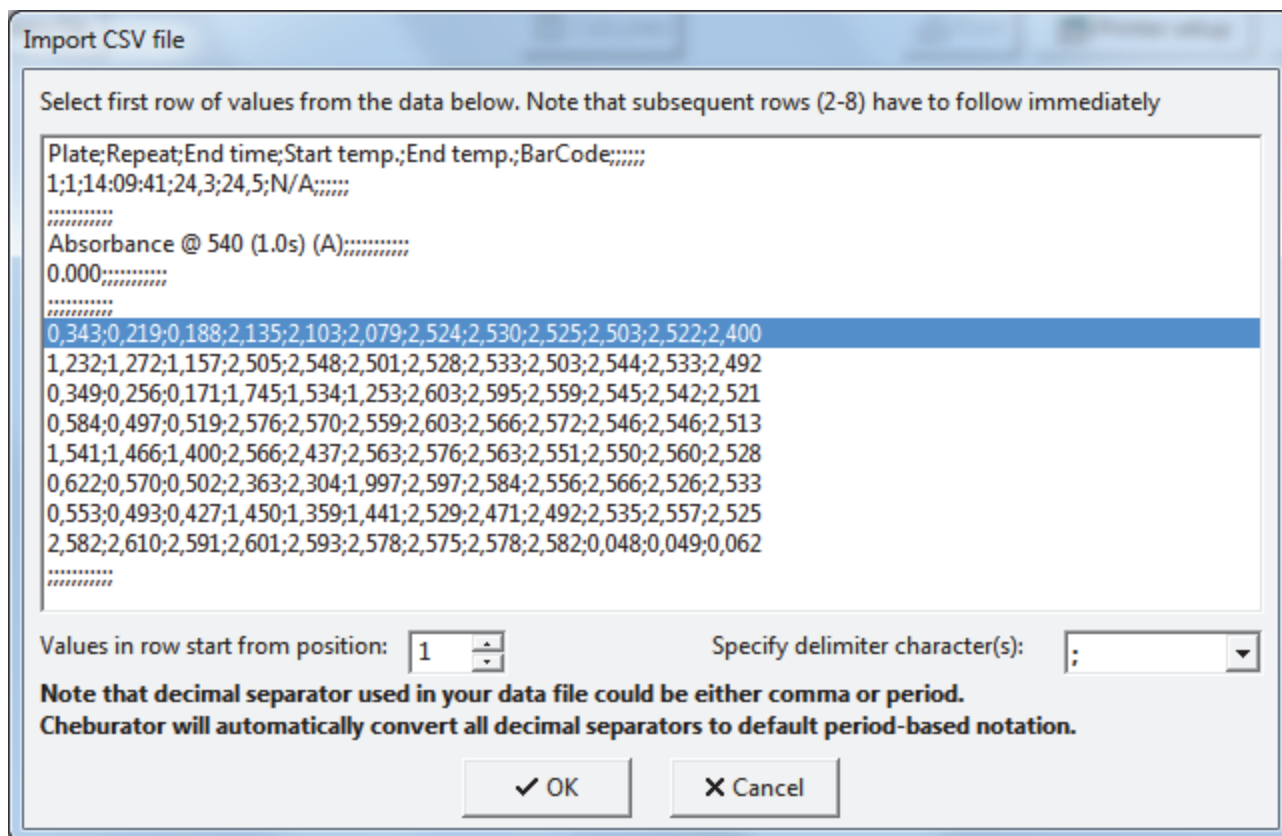

Figure 2. *Built-in converter window.*

3.3. Entering the test parameters

After the data is loaded, pages **Test data and information** and **Options** become visible. The optical density values are shown in the **Plate data** table at the bottom-right part of the window. One should specify optional test details on the first page, entering the following information: test name and date, name of cell line and cell concentration used, researcher, wave length at which optical densities were read, and test comments (**Figure 3**). Path, date, and the time of data file loaded will be shown below in blue. This information will also be shown in the final report produced by Cheburator (see *3.8 Printing the analysis report* section).

Figure 3. Main window of the program with test parameters.

The designated row containing control values (nine values for control of untreated cells and three values for control of the media) have to be selected in the **Control row** combo box (in this case, row H). Cell and medium control values are displayed in blue and green, respectively. After selection, optical densities in wells will be highlighted in color with the intensities proportional to the ratio between their respective values and control values. The higher the optical density value in the given well, the closer it is to the value of the control of untreated cells and the lower effect observed for the respective drug and the dose used in this well. This absorbance assessment feature is useful for quick visual data checking and comparing the relative potencies of tested chemical compounds. To turn this feature on/off, use the **Quick absorbance assessment** check box located below the **Control row** combo box in the right-upper part of the window (**Figure 3**).

The next stage is the selection of the other rows which contain test data to be further analyzed. This can be done using the check boxes located on the left side of the **Plate data** table. The name of the compound and the dose ranges have to be specified for every selected row using the **Agent name** edit boxes and the **Dose used** combo boxes, respectively. Please note that the dose ranges available for selection in the **Dose used** combo boxes are dependent on the type of abscissa axis transformation used (see *3.5 Data analysis and the selection of the method* section). The units in which the doses are measured can be specified on the **Options** page (see below)

Example: *If we select dose range "10;1;0.1;0.01" µM it means that wells 1-3 in the current row represents triplicated data for the 10 µM dose, wells 4-6 in the same row represent data for the 1 µg/ml dose and so on. See 3.1 Prerequisites for the tests analyzed by the Cheburator*

It is also possible to discard or modify single values from the plate. To do this, the user must double click on the desired value in the plate table of the main window (**Figure 3**). The Edit data window will be shown (**Figure 4**). Plate values can be either modified in the edit box or discarded from further analysis using the **Discard this value from analysis** check box. Note that the cell will be either highlighted in pink or struck out if its value was changed or discarded, respectively (**Figure 5**). Such modified or discarded values will be also marked in the final report printed by the software after analysis.
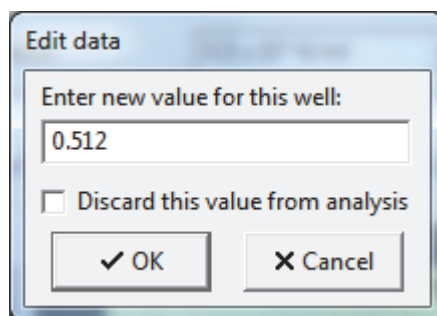

Figure 4. Window for modifying the data or discarding it from further analysis.

Figure 5. Example of modified (B2) or discarded (C3) values in the plate table of the main Cheburator window.

3.4. Absolute and relative IC_{50} values

Depending on the option selected in the program (**Options** page, please see below), Cheburator can estimate both absolute and relative IC_{50} values. To understand the difference between the two definitions of IC_{50}, let us consider a theoretical dose-response curve where concentration of the studied compound is shown on the abscissa axis and percent of inhibition is shown on ordinate axis (**Figure 6A**). The minimum effect is defined as 0% - complete uninhibited growth of the untreated cells without addition of a studied compound. The maximum inhibition effect achievable and measurable in the assay is 100% - complete inhibition of the cell growth (solid pink line in **Figure 6A**) due to the effect of a studied compound. In this case, absolute IC_{50} is defined as a concentration of studied compound which results in exactly 50% of the maximum inhibition effect achievable in assay (dashed pink line in **Figure 6A**). In practice, even the high concentration of very potent cytotoxic compounds rarely result in 100% inhibition of the cell population. Each drug has a maximum attainable inhibition effect which manifests in an upper plateau of the dose-response curve (solid orange line in **Figure 6A**). Relative IC_{50} is defined as the concentration of studied compound which results in exactly half of the maximum inhibition effect attainable for that particular compound (dashed orange line).

3.5. Data analysis and the selection of the method

After specifying all relevant details for the test, the transformations of ordinate axis (inhibition of proliferation in percents) and abscissa axis (doses used in the test) have to be specified. By default, only the abscissa axis is logarithmically transformed and therefore the concentrations are represented on the decimal log scale, which is common practice in the dose-response assays[4]. This often produces sigmoid-like dose-response curves. The final step before analysis is the selection of the method used for calculation of IC_{50} in the **Calculation method and options** box. Cheburator supports three methods, a simple two-point method previously described by *Reed and Muench*[5], a linear regression method, and a nonlinear regression method which are all described below.

After pressing the **Calculate** button, the mean and standard deviations (SD) of absorbance values are calculated for each triplicate scheduled for analysis. Absorbance values read for each cell well on the plate are proportional to the amount of the protein or to the mitochondrial activity of live cells in it, depending on the type of test used in the assay (SRB or MTT, respectively). Therefore, it can be used for evaluation of the cell proliferation. Then, the percentage of inhibition of cell proliferation by the tested compounds in each triplicate relative to untreated cells is calculated using the formula:

$$\text{inhibition of the proliferation (\%)} = 100(\%) - \left(\left(\frac{A_p - A_{cm}}{A_{cc} - A_{cm}}\right) \times 100(\%)\right) \tag{1}$$

where:
A_{cm} - mean background absorbance of wells without cells (control of the medium);
A_{cc} - mean absorbance in wells containing untreated cells (control of cells);
A_p - mean absorbance of the triplicate wells containing cells treated with compound and dose of interest.

The obtained percentages of inhibition of the cell proliferation are then used for calculation of the IC_{50} value, following the method chosen in the **Calculation method and options** box.

Figure 6. *Graphical explanation of data analysis algorithms used in Cheburator.* ***(A)*** *Two types of IC$_{50}$ which can be estimated by the software. Absolute IC$_{50}$ is defined as the concentration of tested compound which results in 50% inhibition of cell growth as defined by assay controls (pink lines). Relative IC$_{50}$ is defined as the concentration of tested compound (dashed orange line) which results in half of the maximum inhibition of cell growth attainable for that particular compound (solid orange line, also called I$_{max}$).* ***(B)*** *Example of a dose-response curve drawn on the basis of 4 values (black circles). The simple two-point method uses 2 data points bracketing 50% inhibition of proliferation (green lines) to estimate the IC$_{50}$. In the second method, a linear model is built with the intercept and the slope, which are calculated by linear regression analysis using data points which are >0% and <100% (red lines). These parameters are later used to estimate IC$_{50}$. In the third method, the software uses all data points to build nonlinear regression model and estimate IC$_{50}$ (blue lines). Absolute IC$_{50}$ values are shown on this plot, but software can also estimate relative IC$_{50}$ in linear and nonlinear regression analysis.*

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Two-points method. This is the simplest among the three methods implemented in our software and can only be used to calculate absolute IC_{50}. It assumes linear dose-response relationship in concentrations resulting in near 50% of inhibition and thus, its use is only recommended for preliminary assessment. Only percentages of inhibition >0% and <100% are taken for analysis. First, the average percent of inhibition is calculated for each tested concentration (triplicate). Then, the IC_{50} value is estimated by linear interpolation using the 2 data points representing averaged percents of inhibition bracketing 50%. The IC_{50} value or its logarithm, depending on the dose axis transformation, can be graphically represented as the abscissa value for a point of the line drawn between 2 data points where it reaches the value of 50% on the ordinate axis. For example, suppose we have 2 concentrations, 10 and 100 μg/ml, which caused 25% and 65% inhibitions of proliferation, respectively **(Figure 6B)**. One line could be drawn between these 2 data points (solid green line), and an additional horizontal line could be drawn for the ordinate value 50% (dashed pink line in **Figure 6B**). The abscissa value for the point where these 2 lines intersect is the concentration that causes 50% inhibition in the proliferation of the cell population (green arrow in **Figure 6B**). The program takes into account transformations for the abscissa or ordinate axes if they were applied. For example, when the abscissa is logarithmically transformed and the ordinate axis is not (the default), the IC_{50} value is calculated using the formula:

$$\lg IC_{50} = \lg C_{lower} + \frac{50\% - IP_{lower}}{IP_{higher} - IP_{lower}} \qquad (2)$$

where:
C_{lower} - concentration of tested preparation, which resulted in inhibition of proliferation less than 50%;
C_{higher} - concentration of tested preparation, which resulted in inhibition of proliferation more than 50%;
IP_{lower} - inhibition of the proliferation (%) calculated for C_{lower};
IP_{higher} - inhibition of the proliferation (%) calculated for C_{higher}.

It sometimes happens that one or both data points bracketing the 50% of inhibition of proliferation have themselves 0% or 100% of inhibition. This could occur if the applied dose range is too broad for a tested compound. In this case, the test has to be repeated with a narrower dose range. Cheburator supports such ranges.

Example: *Suppose (0.01; 0.1; 1; 10) μM dose range was applied. Two drug concentrations 0.1 μM and 1 μM bracketing the 50% of inhibition of proliferation have themselves resulted in 0% and 100% of inhibition of proliferation. In this case IC_{50} value will not be calculated due to uncertainty of the estimation. It is therefore reasonable to repeat the test for this compound using a narrower dose range, for example (0.1; 0.25; 0.5; 1) μM, which would provide better data for proper estimation of IC_{50} value.*

Another case in which proper estimation of IC_{50} value by the two-point method cannot be done is when there is very little difference between two data points bracketing the 50% of inhibition of proliferation. Therefore, IC_{50} value will not be estimated if one or both of the data points bracketing the 50% of inhibition are in the interval between 48% and 52%. The lowest and highest borders of this interval can be changed on the **Options** page in the **Thresholds for IC_{50} value counting** edit boxes.

Example: *Suppose two drug concentrations 0.1 μM and 1 μM bracketing the 50% of inhibition of proliferation have resulted in the 49% and 51% of inhibition of proliferation, respectively. In this case, the IC_{50} value will not be calculated due to the extremely narrow interval of percentage values and uncertainty of the estimation.*

Due to the simplicity of the IC_{50} calculation in this method, program does not report 95% confidence intervals for its value in this case.

Linear regression method. In this method of calculating the IC_{50} value, the linear regression line is built on the basis of all the data points for which the calculated percent of inhibition is more than 0% and less than 100% (solid red line in **Figure 6B**). The intercept and slope of this line are calculated and used to estimate the IC_{50} value (red arrow and IC_{50} value in **Figure 6B**). However, this method assumes a linear relationship in the entire dose-response curve, which is rarely the case as it typically has a sigmoidal shape. To employ this method for proper IC_{50} calculation, it usually has to be combined with the transformation of 1 or both axes to ensure the proper conversion of the dose-response curve into the linear approximation. The abscissa axis (doses) can be analyzed in either logarithmic scale with the base 2, 5, and 10 (default) or linear scale. The ordinate axis (percent of inhibition), which is linear by default, can also be analyzed using probit, logit, or logarithmic transformations. Goodness of fit in linear regression analysis and therefore the reliability of IC_{50} calculation for each particular compound can be assessed using r^2 values reported by the program. If linear regression method is chosen for the IC_{50} value calculation, our program also reports 95% confidence intervals for its value, estimated using the semi-parametric bootstrapping method.

Nonlinear regression method. This method is currently considered to be "state-of-the-art" for IC_{50} calculation and is used in our software by default. We strongly encourage users of the software to use this method of IC_{50} calculation as it does not require linear relationship between drug dose and percent of inhibition and uses all data points from the test, unless they were specifically discarded from the analysis (see below). Logistic model, which is the common way of analyzing sigmoidal dose-response relationships, is used for fitting (solid blue line in **Figure 6B**) and for estimation of the IC_{50} value (blue curve, arrow and IC_{50} value in **Figure 6B**) and I_{max}. Similarly to linear regression, program calculates R^2 value, which is used to assess goodness of fit. The closer R^2 value is to 1, the better the model fits the data and the higher the accuracy of IC_{50} estimation. In the case of nonlinear regression analysis, program also calculates and reports 95% confidence intervals for IC_{50} value, estimated using the semi-parametric bootstrapping method.

We recommend that the tests for each compound are repeated several times in independent assays, followed by the calculation of geometric mean of IC_{50} values and its 95% confidence interval. The geometric mean is statistically the most appropriate averaging method for IC_{50} values, due to the fact that they follow log-normal distribution.

3.6. Results of the analysis and their interpretation

After running the analysis by pressing the **Calculate** button, two additional **Results** and **Graphs** pages become available (**Figure 7, Figure 8**). There is a table for each compound included in the analysis, which contains the dose range applied, mean and standard deviations of absorbance values for every triplicate, and the calculated respective percentages of inhibition of cell population using the formula (1). The calculated IC_{50} values (if applicable), their types (absolute or relative), 95% confidence intervals, goodness of fit values (linear and nonlinear regression), and the method of estimation are shown below. Triplicates for which percentages of inhibition of the cell population were calculated to be 0 or 100% are highlighted in red (**Figure 7**).
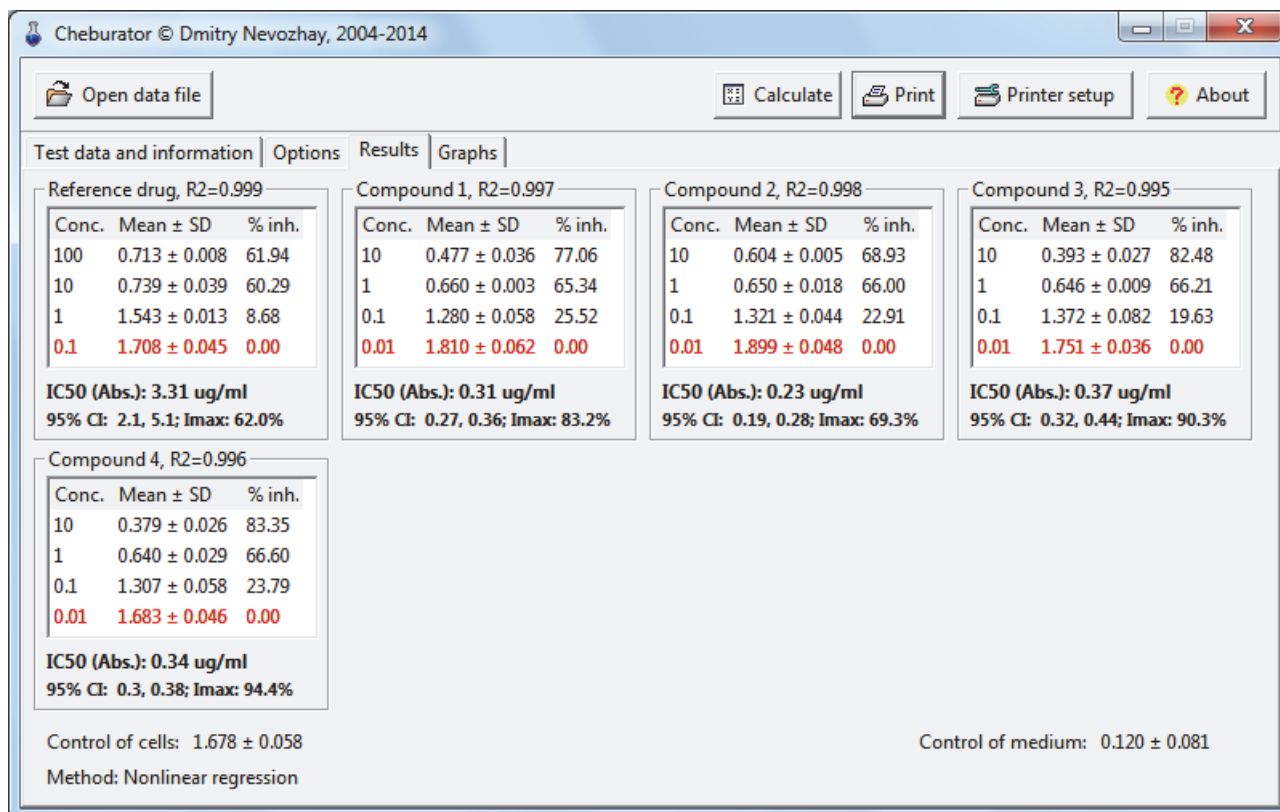

Figure 7. The Results tab after the analysis. It is showing the dose range applied, mean and standard deviations of absorbance values for every triplicate, calculated respective percentages of inhibition of cell proliferation, estimated IC_{50} values, its type, method of calculation, and 95% confidence intervals (for regression analysis).

It is also possible to examine the dose-response curves for each compound analyzed on the *Graphs* tab (**Figure 8**). This option is useful for visually assessing the validity of curves and the method for estimating IC_{50} values. Here, individual data points are plotted in black, which helps to see the experimental variability between absorbance values from different wells. In cases when either the linear or nonlinear regression method is used for estimation, the regression line is also shown in the respective graph. Calculated IC_{50} values (if reached) are shown as green points on the graphs, together with a fuchsia line marking respective percent of inhibition for either absolute or relative IC_{50}.
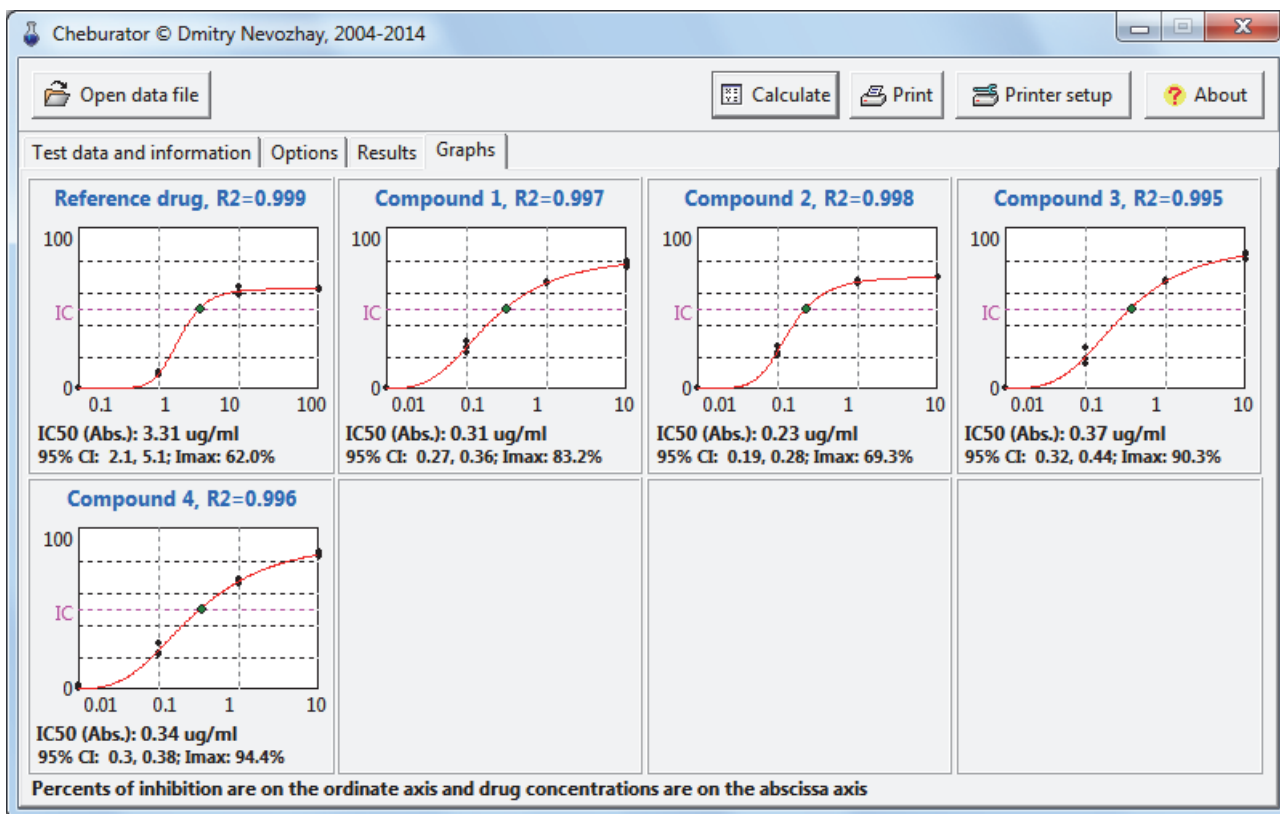
Figure 8. *The Graphs tab after the analysis. It is showing the dose-response curves, individual values of percentages of inhibition for each data point, method of calculation used, IC$_{50}$ values, its type, 95% confidence intervals, and goodness of fit (for regression analysis).*
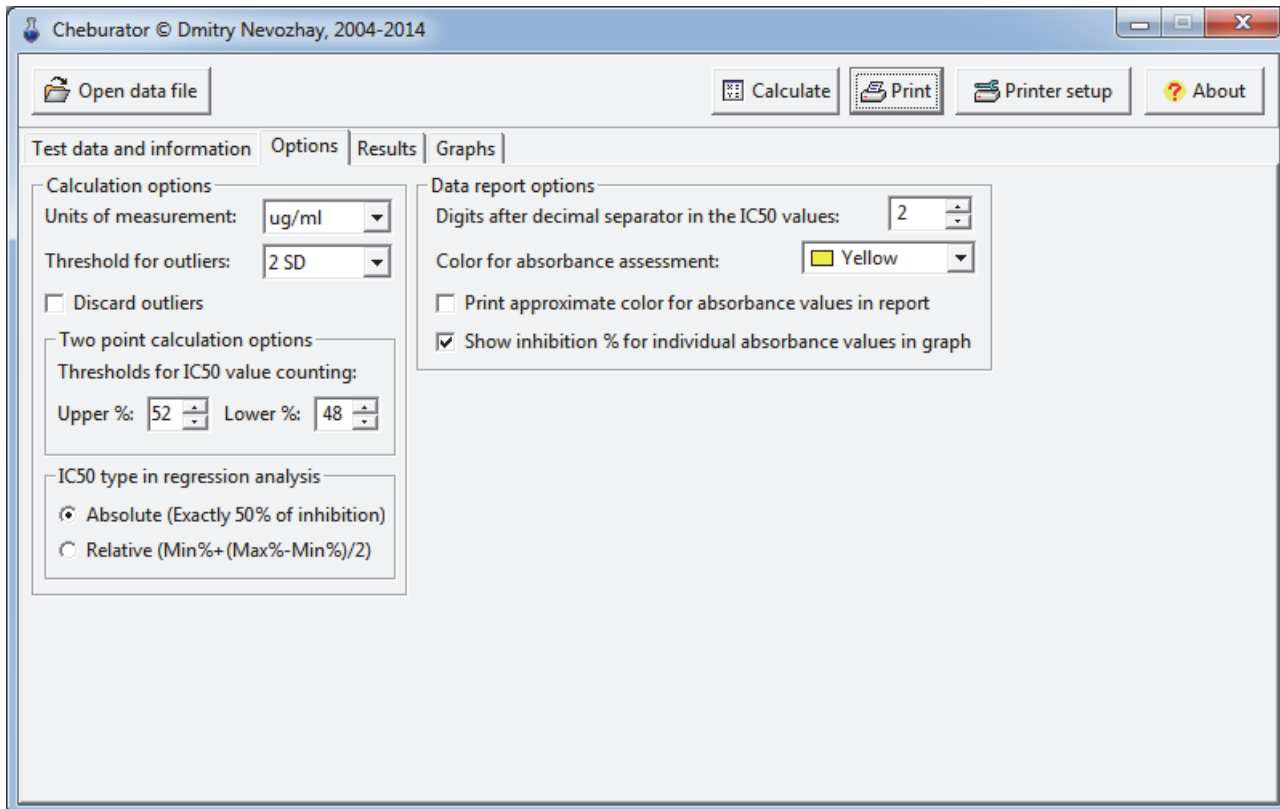

Figure 9. *Options page for Cheburator customization.*

3.7. Options page

On the **Options** page, users can specify the units of measurement used in the test (**Figure 9**). Turning on the **Discard outliers** checkbox will result in removing the outliers from the analysis (this option is not recommended and is off by default). Outliers are defined as the values which are outside the mean ± SD for the current triplicate. SD limits can be specified in the **Threshold for outliers** combo box. Below are the edit boxes in which the upper and lower threshold percentage of inhibition values for simple method of IC_{50} calculation can be specified (See *3.5. Data analysis and the selection of the method* section for details). **IC$_{50}$ type in regression analysis** selection panel allows user to choose whether absolute or relative IC_{50} is calculated during the linear or nonlinear regression analysis.

On this page, users can also change the number of digits displayed after the decimal separator in the counted IC_{50} values. In addition, color highlighting of absorbance values can also be printed in the final hard-copy report if **Print approximate color for absorbance values in report** checkbox was turned on. The default color for this highlighting can be selected in the combo box above.

3.8. Printing the analysis report

Following the calculation, the user can print a detailed report on the test. An example of such report is shown in **Figure 10**. Specific details on the test such as the description, surname of the researcher, name of the cell line and concentration of cells, date of the test and optional comments are printed at the top of the report. Below are the IC_{50} values for every compound tested, I_{max}, their dose-response curves, dose ranges used, absorbances obtained, and percentages of inhibition counted. The data are identical to those shown on the **Results** and **Graphs** pages described previously (see *3.6. Results of the analysis and their interpretation* section). Information on the control row section contains the average absorbances and their SD for the control of untreated cells and the medium background, respectively. The type of the method used for the estimation of the IC_{50} value and axes transformations are also specified.

A table with raw plate data is printed at the bottom part of the report. Values are highlighted in color depending on whether they were used in the calculation or not and if they were noted as outliers or not. In case of manual modification of some raw data before analysis, both the original and the modified values are printed. In addition, color absorbance highlighting for easy visual data assessment could also be printed in the report if the user specified it on the **Options** page. Even if an original file was accidentally lost, a hard copy of the report could be used to restore the raw data and repeat the analysis if necessary. Finally, the path to the original file containing the raw data along with its date and time are printed below the table with absorbance values.

Name of test: Sample test
Comments: For educational purposes
Cell line: A549
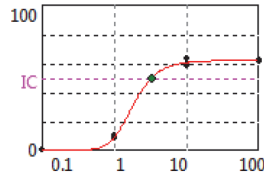
Date of test: 28.07.2014
Researcher: Dmitry Nevozhay
Cell concentration: 0.5 x 10^4/ml   Filter wavelength: 540nm

Reference drug; IC50 (Abs.): 3.31 ug/ml; 95% CI: 2.15, 5.10; Imax: 62.0%

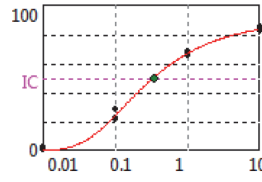| ug/ml	Avg ± SD	% inh
100	0.713 ± 0.008	61.94
10	0.739 ± 0.039	60.29
1	1.543 ± 0.013	8.68
0.1	*1.708 ± 0.045*	*0.00*

Method: nonlinear regression   R2: 0.999

Compound 4; IC50 (Abs.): 0.34 ug/ml; 95% CI: 0.30, 0.38; Imax: 94.4%

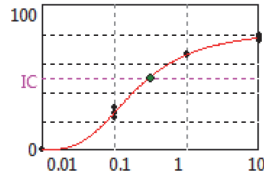| ug/ml	Avg ± SD	% inh
10	0.379 ± 0.026	83.35
1	0.640 ± 0.029	66.60
0.1	1.307 ± 0.058	23.79
0.01	*1.683 ± 0.046*	*0.00*

Method: nonlinear regression   R2: 0.996

Compound 1; IC50 (Abs.): 0.31 ug/ml; 95% CI: 0.27, 0.36; Imax: 83.2%

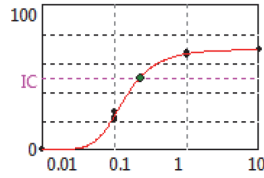| ug/ml	Avg ± SD	% inh
10	0.477 ± 0.036	77.06
1	0.660 ± 0.003	65.34
0.1	1.280 ± 0.058	25.52
0.01	*1.810 ± 0.062*	*0.00*

Method: nonlinear regression   R2: 0.997

Compound 2; IC50 (Abs.): 0.23 ug/ml; 95% CI: 0.19, 0.28; Imax: 69.3%

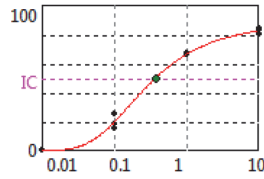| ug/ml	Avg ± SD	% inh
10	0.604 ± 0.005	68.93
1	0.650 ± 0.018	66.00
0.1	1.321 ± 0.044	22.91
0.01	*1.899 ± 0.048*	*0.00*

Method: nonlinear regression   R2: 0.998

Compound 3; IC50 (Abs.): 0.37 ug/ml; 95% CI: 0.32, 0.44; Imax: 90.3%

ug/ml	Avg ± SD	% inh
10	0.393 ± 0.027	82.48
1	0.646 ± 0.009	66.21
0.1	1.372 ± 0.082	19.63
0.01	*1.751 ± 0.036*	*0.00*

Method: nonlinear regression   R2: 0.995

Control of cells: 1.678 ± 0.058
Control of medium: 0.120 ± 0.081
Outlier threshold: 2 SD   Discard: NO
Calculation method: nonlinear regression
Y axis: Linear   X axis: Logarithmic (10)

Legend:		1	2	3	4	5	6	7	8	9	10	11	12
Accepted	A	0.721	0.713	0.705	0.695	0.753	0.768	1.547	1.528	1.553	1.678	1.760	1.686
Outliers	B	0.479	0.512	0.441	0.656	0.662	0.662	1.337	1.282	1.222	1.739	1.835	1.855
Unused	C	0.608	0.599	0.605	0.666	0.631	0.652	1.329	1.360	1.274	1.899	1.947	1.851
CC	D	0.424	0.381	0.374	0.655	0.637	0.647	1.392	1.442	1.282	1.746	1.718	1.789
CM	E	0.405	0.379	0.354	0.671	0.636	0.614	1.343	1.338	1.241	1.731	1.680	1.639
Values changed manually:	F	*0.039*	*0.038*	*0.037*	*0.039*	*0.039*	*0.042*	*0.042*	*0.039*	*0.038*	*0.048*	*0.041*	*0.039*
o: original absorbance	G	*0.038*	*0.041*	*0.038*	*0.039*	*0.038*	*0.041*	*0.038*	*0.042*	*0.040*	*0.043*	*0.042*	*0.041*
n: new absorbance	H	1.561	1.705	1.676	1.690	1.687	1.691	1.782	1.672	1.637	0.072	0.075	0.213

Data file: X:\Export to Houston\Dropbox\Cheburator\Samples\normal.dat   [04.05.2005 09:22]

***Figure 10**. Example of test report printed. It contains the original file's name, date and time, the name and date of the test, the calculated IC50 values, Imax, its type, 95% confidence intervals, and goodness of fit (for regression analysis), the percentages of growth inhibition for every concentration and compound, the dose-response curves, the mean optical densities and their standard deviations, the test control values, and the type of the method used for calculation. Raw plate data is printed at the bottom part of the report.*

4. References

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