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An explanation of the cytotoxic potential of arginine mimetics containing sulfo- and oxy- groups in their side chains

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ABSTRACT

L-Arginine is an amino acid involved in numerous essential metabolic pathways. It is catabolized by several enzymes to some important metabolites. Arginine is very important for mammals, and to ensure their supplies, mammalian cells can synthesize this amino acid from citrulline.This study deals with a series of arginine analogues and the activity of seven different enzymes of arginine metabolic pathway (nitric oxide synthases (inducible and endothelial), arginases, arginine:glycineamidinotransferase, arginine decarboxylase, arginine deiminase, and argininosuccinate synthase) in those analogues. The compounds were biologically tested and their in vitro effects are explained using docking. All investigated compounds inhibited five from the total of seven assessed enzymes, with norsulfoarginine (NsArg) and sulfoarginine (sArg) being more potent than the norcanaline (NCan) and norcanavanine (NCav) analogues due to the availability of more enzyme interactions sites in the first two compounds. Modifications with bis-(2-chloroetylhydrazine) and phenylhydrazine increased binding potential of the compounds. Computational methods can help in design of arginine mimetics, and are very useful for predicting the biological activities of newly synthesized compounds.

Key words: arginine mimetics, cytotoxic effects, docking, GOLD

INTRODUCTION

The guanidine functional group defines some medically interesting chemical and physicochemical properties of many compounds and guanidine-containing derivatives constitute a very important class of therapeutic agents used to treat diverse medical conditions. The cationic amino acid arginine (Arg), a natural guanidine containing compound, has attracted much attention due to its diverse pharmacological effects. Arginine is involved in numerous metabolic pathways in the human body. It is a precursor in the biosynthesis of proteins and also of ornithine, polyamines, nitric oxide, proline, glutamate, glutamine, creatine, agmatine and dimethylarginines [1]. In mammals, arginine is a substrate for 5 different enzymatic systems, including nitric oxide synthases (NOS; EC 1.14.13.39), arginases (EC 3.5.3.1), arginine:glycineamidinotransferase (EC 2.1.4.1), arginine decarboxylase (EC 4.1.1.19), and arginine deiminase (EC 3.5.3.6) [2]. The latter is not expressed by animal cells [3], but it takes part in arginine metabolism when expressed by resident pathogenic organisms. It may enter the mammalian host cells and disrupt host arginine metabolism (Fig 1). Arginine is a semi-essential amino acid for mammalian cells, because mammals can synthesize it from citrulline. Argininosuccinate synthase (ASS) is the one of the two enzymes that converts

citrulline to arginine (Fig. 1). The biosynthesis of L-arginine from L-citrulline is catalyzed by the cytosolic enzymes argininosuccinatesynthetase 1 (ASS1) and argininosuccinatelyase (ASL).

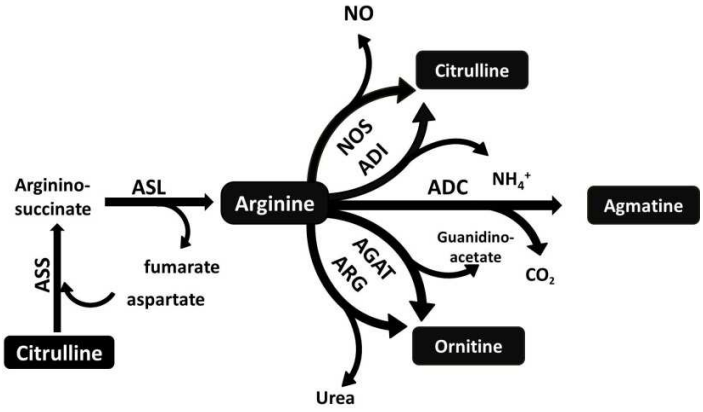
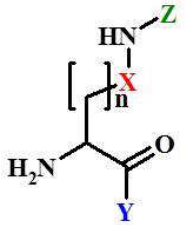


Fig-1 Biosynthesis and metabolic pathways of l-arginine: ASS – argininosuccinate synthase, ASL – argininosuccinatelyase, NOS – nitric oxide synthase, ADI – arginine deiminase, ADC – arginine decarboxylase, AGAT – arginine-glycine amidinotransferase, ARG – arginase

Carcinogenesis is another area of growing interest in the role of arginine since the amino acid has been confirmed to be absolutely necessary for neoplastic cell growth. The effect of L-arginine is mainly due to its end-product, nitric oxide (NO). The L-arginine/NO pathway has been shown to play an important role in tumor development. Recent findings indicate that NO derived from L-arginine can influence angiogenesis factors, vascular permeability, perivascular-cell recruitment, and vessel remodeling and maturation. Additionally, the L-arginine/NO pathway can activate a broad array of genes that are functionally involved in proliferation, metastasis and apoptosis. Interestingly, this pathway affects both tumorogenesis and tumor killing [4]. On the other hand, it has been long known that various tumor cells are auxotrophic for arginine, such as breast carcinoma cells, pancreatic cancer cells [5], cervical carcinoma cells [6, 7], several types of melanoma cells [8], hepatocellular carcinoma cells [9], breast carcinoma, ovarian carcinoma, prostate carcinoma, colon carcinoma, lung carcinoma, osteosarcoma, glioma/astrocytoma, glioblastoma, premyelocytic leukemia, lymphoblastic leukemia [10], etc. ASS is not expressed or its expression is very low in those types of tumor cells [9]. This fact is used in the so called “deprivation therapy”, which is a very effective treatment strategy for some cancers. Another cancer treatment strategy is based on blocking the enzymes involved in arginine metabolism to stop tumor growth. Accordingly, significant effort is focused on the design and preparation of different arginine mimetics that have the potential to bind reversibly to the enzymes for which arginine is a substrate.

During the last 2 decades the scope of the research program of our laboratory included assessment of the biological activities of arginine mimetics. We have designed and synthesized series of arginine analogues with sulfo- and oxy-guanidino group in their side chain, as well as some other derivatives (Fig 2). All the compounds had enhanced growth-inhibiting activity on microorganisms, model plant systems and cultured tumor cell lines [11-14]. Our studies emphasize on the need to fully elucidate the mechanisms of action of these arginine mimetics.



n = 1,2;
X = O, SO_2;
Y = OH, NH_2, $NHNH_2$, $NHNHC_6H_5$, $NHN(CH_2CH_2Cl)_2$;
Z = H, $C(NH)NH_2$.

Fig-2 Structure of arginine mimetics

This study reports the structure-activity relationship for the synthesized arginine analogues and their cytotoxic activity, determined by docking of different enzymes involved in arginine metabolism. A hypothesis to explain action of the compounds based on the computational studies was proposed.

MATERIALS AND METHODS

- *Arginine analogues*

Sulfo- and oxy-arginine mimetics were synthesized as previously described [11-14].Abbreviations used for the compounds are the following: Can – canaline, NCan – norcanaline, Cav – canavanine, NCav – norcanavanine, sArg – sulfoarginine, NsArg – norsulfoarginine.

- *Enzymes*

Crystal structures of enzymes used were obtained from RCSB [15]: iNOS (id: 1nsi), eNOS (id: 1nod), ADI (id: 2a9g), ADC (id: 3n2o), AGAT (id: 5jdw), ARG (id: 3gmz), and ASS (id: 2nz2).

- *Computational tools*

Ligand preparation was done with Avogadro (an open-source molecular builder and visualization tool – Version 1.0.3) [16]; Docking studies were performed by using GOLD 5.1 (Genetic Optimization for Ligand Docking) [17], run on Scientific LINUX 5.5 operating system; Image generation and interaction studies were done after docking with Molegro Molecular Viewer (MMV) [18]. A GraphPad Prism 3.0 was used for the correlations.

- *Docking of arginine analogues*

Docking was carried out with GOLD 5.1 software. It uses a genetic algorithm and considers full ligand conformational flexibility and partial protein flexibility. Active centers of the enzymes were determined using substrate position in the crystal structures obtained from RCSB. GoldScore algorithm was used and Fitness scoring function was calculated for each compound. The conformations of the compounds with best scoring functions were selected and parameters of the scoring functions were used to find correlations between them and *in vitro* results.

RESULTS AND DISCUSSION

A total of 20 arginine mimetics including parent sulfo- and oxy-analogue, their amides and hydrazide derivatives were selected for this study. Their cytotoxicity for 3T3 (standard mouse embryotic cell line) and HepG2 (human liver hepatocellular carcinoma cell line) cells was studied and is reported elsewhere [11-14]. Mean cytotoxicity (%) for each compound for the different cell lines are shown in Table 1.

Table 1 Arginine mimetics and their cytotoxicity for 3T3 and HepG2 cell lines after 24 hours at concentration 0.25 mM

No	Compounds	Cell cytotoxicity, %	
		3T3	HepG2
1	$NCanNHNH_2$	8.12	9.57
2	NCan	9.48	4.64
3	$NCanNH_2$	2.11	11.33
4	$NCanNHN(CH_2CH_2Cl)_2$	1.2	20.25
5	$NCanNHNHC_6H_5$	11.27	8.25
6	$NCavNHNH_2$	9.72	4.72
7	NCav	1.43	2.96
8	$NCavNH_2$	0.47	8.51
9	$NCavNHN(CH_2CH_2Cl)_2$	2.26	50.35
10	$NCavNHNHC_6H_5$	5.6	55.54
11	$NsArgNHNH_2$	6.43	-8.49
12	NsArg	0.68	8.39
13	$NsArgNH_2$	13.44	33.46
14	$NsArgNHN(CH_2CH_2Cl)_2$	3.8	34.56
15	$NsArgNHNHC_6H_5$	7.56	12.41
16	$sArgNHNH_2$	5.82	7.85
17	sArg	43.51	2.05
18	$sArgNH_2$	5.83	36.85
19	$sArgNHN(CH_2CH_2Cl)_2$	78.23	93.77
20	$sArgNHNHC_6H_5$	93.02	91.95

The ability of the 20 arginine analogues to form complexes with all 7 enzymes was examined using GOLD. First evaluation function for efficacy of docking of the ligand and receptor we used is the following:

$$E_{score}(Total\ energy) = E_{inter} + E_{intra},$$

where E_{score} is a docking scoring function or total energy, E_{inter} – ligand-protein interaction energy, and E_{intra} – internal energy of the ligand (MolDoc SE algorithm) [19]. This function was obtained from MMV. Values for the total energies are presented in Table 2.

Table 2 Total energies of enzyme-substrate complexes

Compounds	Total energies enzyme - substrate complexes						
	ARG	**eNOS**	**iNOS**	**ADI**	**ADC**	**AGAT**	**ASS**
$NCanNHNH_2$	-55.922	-64.029	-57.407	-48.254	-30.68	-57.912	-49.886
NCan	-35.945	-56.268	-71.904	-57.955	-33.006	-50.18	-38.773
$NCanNH_2$	45.111	-46.258	-59.56	-56.677	-32.583	-48.934	-46.184
$NCanNHN(CH_2CH_2Cl)_2$	-78.398	-64.728	-87.866	-59.47	-41.573	-33.934	-63.563
$NCanNHNHC_6H_5$	-77.294	-72.383	-90.544	-74.658	-25.089	-40.88	-53.78
$NCavNHNH_2$	-56.707	-69.302	-50.357	-84.563	-41.749	-39.921	-51.242
NCav	-60.649	-45.286	-69.235	-76.761	-33.249	-40.18	-50.294
$NCavNH_2$	-60.757	-75.483	-77.293	-77.681	-25.868	-32.76	-51.069
$NCavNHN(CH_2CH_2Cl)_2$	-72.225	-84.066	-122.474	-62.64	-48.256	0.832	-92.158
$NCavNHNHC_6H_5$	-57.157	-92.365	-76.499	-94.76	-57.944	-10.664	-93.234
$NsArgNHNH_2$	-49.407	-71.317	-56.936	-83.139	-43.588	-31.033	-53.839
NsArg	-55.56	-64.1	-87.365	-81.66	-15.902	-44.04	-66.276
$NsArgNH_2$	-59.664	-67.063	-47.553	-81.776	-49.468	-41.157	-65.712
$NsArgNHN(CH_2CH_2Cl)_2$	-51.61	-72.048	-131.188	-78.129	-51.88	3.553	-108.521
$NsArgNHNHC_6H_5$	-33.11	-55.41	-107.088	-94.576	-39.319	12.091	-83.182
$sArgNHNH_2$	-74.684	-70.077	-76.071	-71.235	-58.119	-48.31	-56.526
sArg	-74.799	-66.368	-102.044	-102.64	-49.723	-38.975	-74.627
$sArgNH_2$	-61.578	-89.457	-107.162	-72.359	-43.863	-34.821	-71.723
$sArgNHN(CH_2CH_2Cl)_2$	-55.96	-93.821	-107.47	-89.992	-67	12.558	-78.566
$sArgNHNHC_6H_5$	-36.311	-83.521	-100.241	-103.118	-43.462	29.24	-98.272
Natural ligands for the corresponding enzyme	-80.578	-25.4	-78.129	-116.586	-57.897	-39.879	-46.519

Data in Table 2 allowed the following conclusions to be made:

1. None of the compounds had stronger affinity for ARG and ADI than the natural substrates, L-ornithine for ARG and L-arginine for ADI, respectively; the energies of enzyme complexes with analogueswere higher than those of complexes with the natural substrates.
2. All investigated compounds bound strongly to eNOS, and the resulting complexes had lower energies than the respective natural complex of eNOS and arginine. All of them were eNOS inhibitors.
3. Except for NCan and $NCanNH_2$, all compounds bound strongly to ASS. Their energies were lower than the total energy of the ASS-citrulline complex.
4. Several compounds bound strongly to iNOS, and the most effective binding occurred with $sArgNHN(CH_2CH_2Cl)_2$ and $NCavNHN(CH_2CH_2Cl)_2$.
5. Only three of the compounds in this series were inhibiting for ADC, and their complexes had lower energies than the ADC-arginine complex. These included $NCavNHNHC_6H_5$, $sArgNHNH_2$, and $sArgNHN(CH_2CH_2Cl)_2$.
6. With AGAT, some of compounds that acted as inhibitors, but in five of them the enzyme complexation had very high total energies. $NCavNHN(CH_2CH_2Cl)_2$, $NsArgNHN(CH_2CH_2Cl)_2$, $NsArgNHNHC_6H_5$, and $sArgNHNHC_6H_5$ complexes with AGAT had very high total energies.
7. According to the data presented in Table 2, $NCavNHNHC_6H_5$, NsArg, $sArgNHNH_2$, and $sArgNHN(CH_2CH_2Cl)_2$ were the best arginine mimetics because they were able to bind to four out of the seven enzymes. The rest of the compounds could be fairly good arginine mimetics, because they were inhibiting to at least two of the 7 enzymes.

After docking values for the Fitness function were obtained using a GoldScore algorithm and are listed in Table 3. The data indicated that $sArgNHN(CH_2CH_2Cl)_2$ bound effectively to eNOS and iNOS because its fitness function values were higher than those for the rest of the compounds, NsArg bound strongest to AGAT, $NsArgNHNHC_6H_5$ formed the best complex with ADC, and $sArgNHNHC_6H_5$ interacted with ASS better than all the other compounds in this series.

Table 3 Values of the Fitness function for the best pose of each compound with the corresponding enzyme

No	Compounds	Fitness funtion of the compound with corresponding enzyme						
		ARG	eNOS	iNOS	ADI	ADC	AGAT	ASS
1	$NCanNHNH_2$	42.08	38.48	49.03	41.63	35.89	33.87	36.32
2	NCan	36.94	34.74	44.61	39.19	32.07	31.38	31.71
3	$NCanNH_2$	39.15	34.57	46.74	40.58	32.47	33.87	31.45
4	$NCanNHN(CH_2CH_2Cl)_2$	56.20	51.53	63.85	50.24	38.63	32.88	44.46
5	$NCanNHNHC_6H_5$	56.17	47.36	59.23	54.14	44.05	32.45	45.89
6	$NCavNHNH_2$	49.66	46.12	52.06	56.20	41.41	39.64	39.63
7	NCav	46.13	41.73	46.77	54.56	40.49	40.06	40.23
8	$NCavNH_2$	52.46	45.71	49.61	53.57	39.90	42.48	37.83
9	$NCavNHN(CH_2CH_2Cl)_2$	55.48	56.77	74.10	62.15	52.18	28.47	51.40
10	$NCavNHNHC_6H_5$	54.19	49.69	76.36	70.54	47.04	32.30	50.82
11	$NsArgNHNH_2$	60.91	50.97	54.61	65.26	46.38	43.78	47.77
12	NsArg	55.49	46.07	54.85	60.47	43.54	44.41	45.83
13	$NsArgNH_2$	61.82	46.54	56.19	65.31	47.33	43.43	45.62
14	$NsArgNHN(CH_2CH_2Cl)_2$	63.94	63.26	77.04	69.96	44.70	18.65	54.92
15	$NsArgNHNHC_6H_5$	60.23	51.75	62.71	66.30	52.69	22.72	51.28
16	$sArgNHNH_2$	58.63	50.50	62.71	67.21	46.09	42.80	45.28
17	sArg	61.38	49.96	55.90	77.47	43.91	34.47	48.09
18	$sArgNH_2$	62.57	51.53	61.85	70.44	50.24	40.85	46.33
19	$sArgNHN(CH_2CH_2Cl)_2$	62.53	60.30	72.67	71.29	48.39	4.02	56.40
20	$sArgNHNHC_6H_5$	61.62	55.64	70.03	77.32	46.75	32.03	57.71

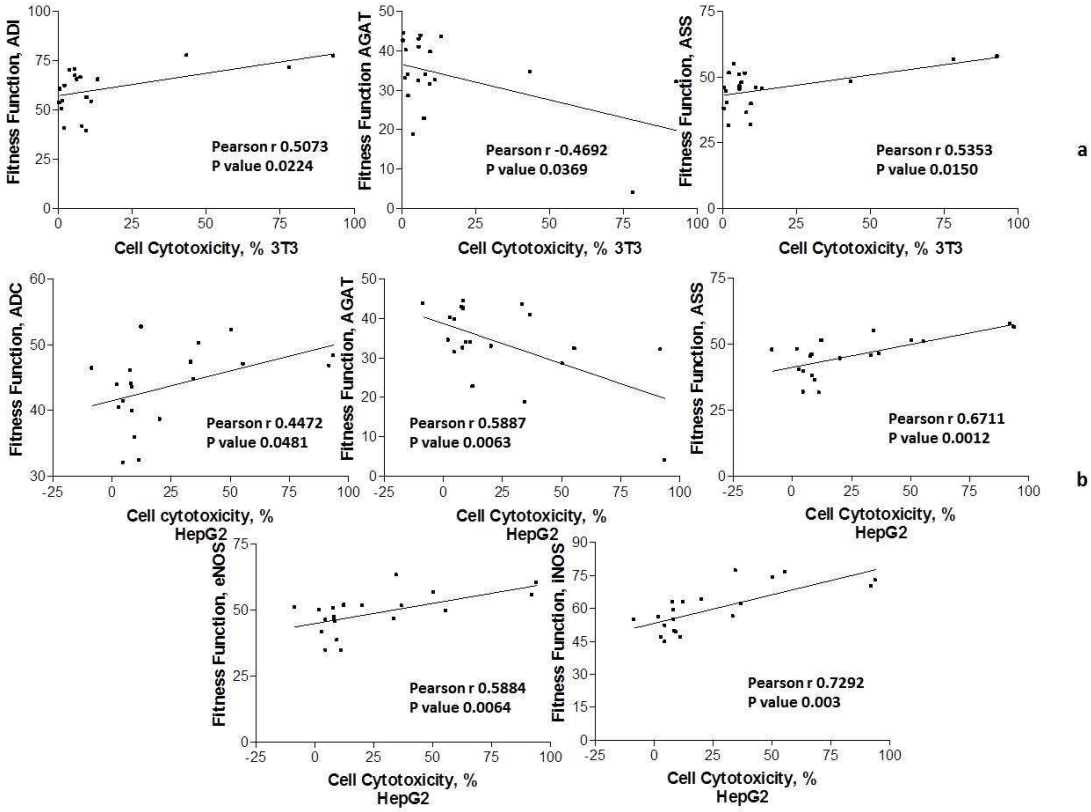


Fig-3 Correlations between cell cytotoxicity of the compounds to 3T3 and fitness functions of the compounds and enzymes: ADI, AGAT, and ASS (a) and cell cytotoxicity of the compounds to HepG2 and fitness functions of the compounds and enzymes: ADC, AGAT, ASS, eNOS, and iNOS (b)

To explain relationship between biological action of the compounds and their structure we found Pearson's correlation with linear regression of cytotoxicity data and fitness functions. For 3T3 cell line there were good correlations between cytotoxicity and fitness functions for ADI, AGAT, and ASS, as shown on Fig 3a. In the cases

of ADI and ASS, there were positive correlations, i.e. cytotoxicity increased with the value of the fitness function. With AGAT, there was a negative correlation, or in other words the lower the fitness function value, the higher the biological effect. The situation was similar for this enzyme, AGAT, with HepG2 cell cytotoxicity. Good correlations occurred between fitness functions of the compounds with ADC, ASS, eNOS, and iNOS and cytotoxicity of HepG2 cells (Fig 3b).

Arginase (ARG) is a binuclear manganese metalloenzyme that hydrolyzes L-arginine to L-ornitine and urea. Different kinds of interactions are present in the ornithine complex, including hydrogen bonds (H-bonds), electrostatic, and steric interactions. Important residues in the active site of ARG, involved in interactions with the active compound, include His126, Asp128, Asn130, Ser137, His141, and Asp183 [20]. The investigated compounds bind to the enzyme by multiple H-bonds, electrostatically and sterically. Many of the important residues were involved in those interactions, but no arginine analogue was found to bind to all of them. This might be the reason why all complexes of the arginine analogues have higher total energies than ARG-Orn.

Important residues in the active site of ADI are: Leu41, Asp166, Arg185, Arg243Asp280, and Gly400 [21]. All compounds interacted with enzymes by electrostatic and steric interactions and by forming H-bonds, but none interacted with Arg185. Maybe this residue plays crucial role in the enzyme activity and no complexes are favored, because analogues do not bind to it. All of the complexes had higher energies than ADI-Arg.

Nitric oxide is a key signaling molecule in many biological processes, making regulation of nitric oxide levels highly desirable for human medicine and for advancing our understanding of basic physiology. Designing inhibitors to specifically target one of the three nitric oxide synthase (NOS) isozymes that form nitric oxide from the L-arginine poses a significant challenge due to overwhelmingly conserved active site. It is known [22, 23] that a good inhibitor must not form bidentate hydrogen bonds such as L-arginine makes with Glu371 in eNOS. The guanidinium group of arginine binds to a Heme. This interaction is stronger than with the rest of the compounds. Glu371 also interacted more strongly with arginine than with its analogues. Nevertheless all of the compounds interacted electrostatically and sterically and by forming many H-bonds thus inactivating eNOS. $NsArgNHN(CH_2CH_2Cl)_2$ interacted with eNOS by a greater range of interactions than other compounds (Fig 4a). The same situation occurred with iNOS, but in that case $NsArgNHNHC_6H_5$ formed a greater number of bonds with the enzyme than the other arginine analogues (Fig 4b).

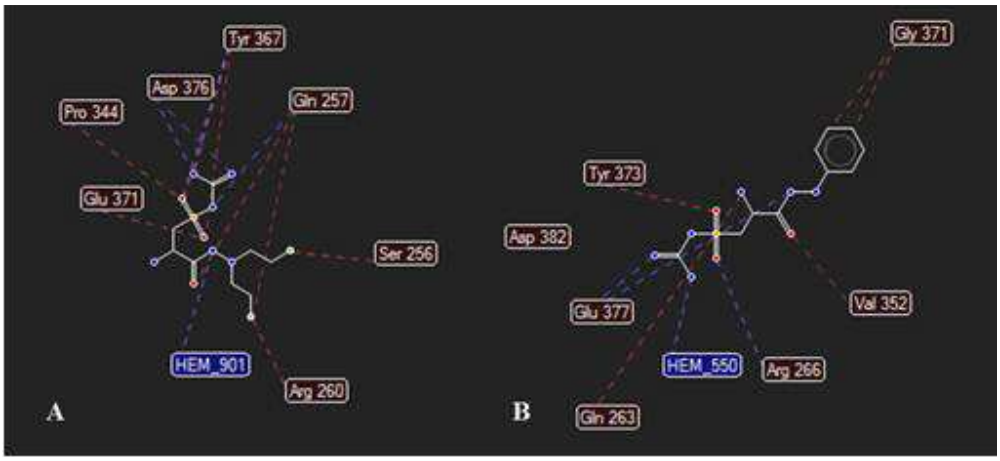


Fig-4 Ligand map generated in Molegro Molecular Viewer for (a) $NsArgNHN(CH_2CH_2Cl)_2$ with eNOS and (b) $NsArgNHNHC_6H_5$ with iNOS:blue – hydrogen bonds; red – steric interactions

Arginine decarboxylase (ADC) is a member of the pyridoxal-5-phosphate (PLP) – dependent basic amino acid decarboxylases family. They are found in most organisms and catalyze the decarboxylation of diverse substrates essential for polyamine and lysine biosynthesis [24]. Very important residues involved in catalytic action were Asp480 and Asp512 which are capable of strong electrostatic interactions. All investigated compounds bound strongly to Asp512, but did not bind to Asp480. Additionally they interacted by forming H-bonds, electrostatically

and sterically with many amino acid residues around the active site and caused blocking of enzyme activity. Many interactions with these enzymes occurred in the case of $NCavNHNHC_6H_5$ (Fig 5a).

A very important residue in AGAT was Asn300, which played the key role [25]. This enzyme interacted very strongly with all compounds and this most probably underlies the blocking of enzyme action. The most potent AGAT inhibitor was likely to be the $sArgNHNHC_6H_5$ (Fig. 5b), because it interacted stronger than all the other compounds.

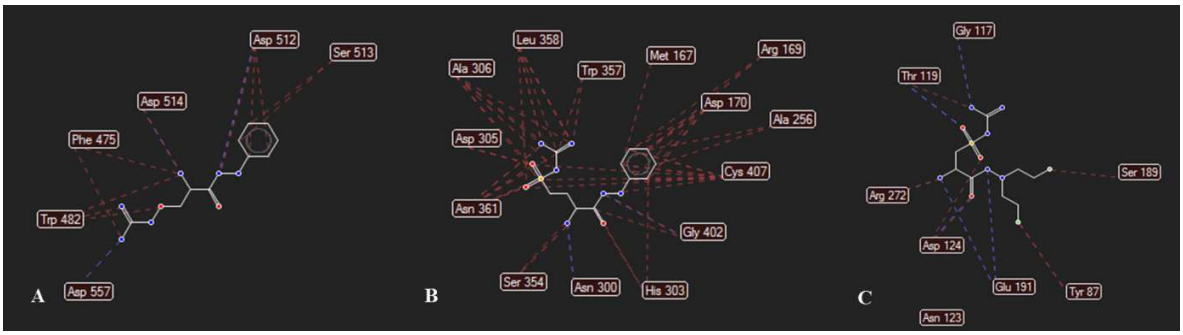


Fig-5 Ligand map generated in Molegro Molecular Viewer for (A) $NCavNHNHC_6H_5$ with ADC, (B) $sArgNHNHC_6H_5$ with AGAT, and (C) $NsArgNHN(CH_2CH_2Cl)_2$ with ASS: blue – hydrogen bonds; red – steric interactions

Argininesuccinatesynthetase catalyzes the transformation of citrulline and aspartate into argininosuccinate and pyrophosphate using the hydrolysis of ATP to AMP and pyrophosphate. This enzymatic process is the rate limiting step in both the urea and arginine cycles [26].

Noteworthy interactions in the active site of the enzyme were the electrostatic interactions between the COOH group of the substrate and Arg127 from the enzymatic sequence. Most of the arginine analogues bound to this residue but also interacted strongly with other residues in the enzyme active site, thus blocking the action of ASS. $NsArgNHN(CH_2CH_2Cl)_2$ (Fig 5c) bound to ASS by a stronger interaction than the other investigated compounds.
The number of interactions with all enzymes is presented in Table 4. Check for correlations between these interactions and cytotoxicity, we found correlations for iNOS and AGAT (Fig 6).

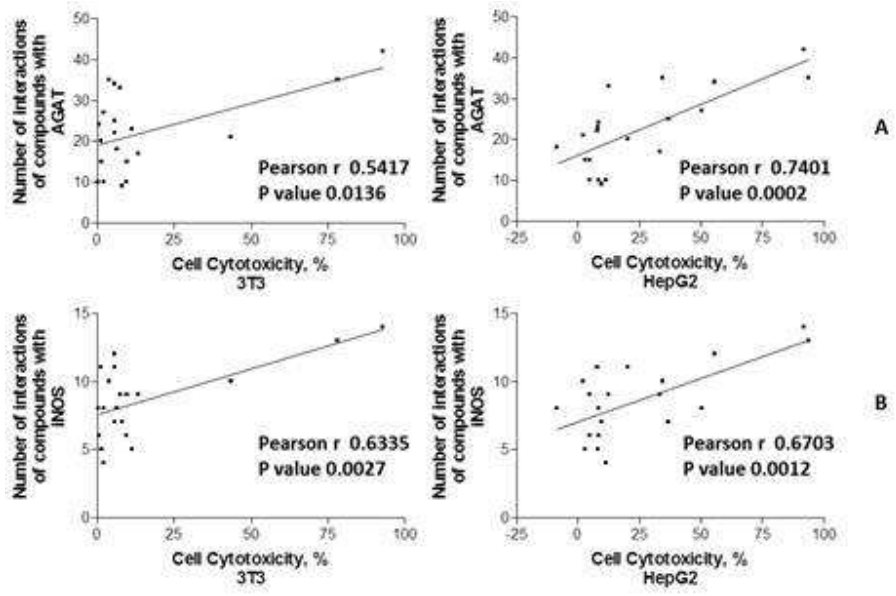


Fig-6 Correlations between cell cytotoxicity of the compounds and number of interactions of compounds and (a) AGAT and (b) iNOS

According to the data listed in Table 4, the following conclusions can be drawn:
1. NCan and NCav analogues bind to the enzymes more weakly, because they do not have enough binding sites. Oxy-guanidinium and oxy-amino groups are less polar and the oxygen atom itself is less electronegative.
2. NsArg and sArg analogues bind more strongly to the enzymes, because they have more binding sites available in their molecules.
3. *Bis*-(2-chloroetylhydrazide) and phenylhydrazide derivatives are even more potent because they have additional binding sites in their structures.
4. iNOS and AGAT may be proposed to be the most important in those cell lines because of their life cycle. Impairing arginine metabolism by disturbing those two enzymes is likely to cause cell death.

Table 4 Number of interactions of each compound with the enzymes

Compounds	Number of interaction of enzymes						
	ARG	**eNOS**	**iNOS**	**ADI**	**ADC**	**AGAT**	**ASS**
$NCanNHNH_2$	7	6	7	12	5	9	7
NCan	11	5	6	11	7	10	7
$NCanNH_2$	10	3	4	9	6	10	4
$NCanNHN(CH_2CH_2Cl)_2$	10	9	11	11	6	20	7
$NCanNHNHC_6H_5$	16	9	5	8	10	23	6
$NCavNHNH_2$	12	6	9	10	7	15	8
NCav	6	9	5	8	10	15	8
$NCavNH_2$	5	7	8	9	10	10	8
$NCavNHN(CH_2CH_2Cl)_2$	21	10	8	12	11	27	7
$NCavNHNHC_6H_5$	13	9	12	9	14	34	4
$NsArgNHNH_2$	10	12	8	10	9	18	10
NsArg	12	12	6	8	13	24	6
$NsArgNH_2$	11	9	9	10	7	17	6
$NsArgNHN(CH_2CH_2Cl)_2$	15	14	10	14	8	35	11
$NsArgNHNHC_6H_5$	28	9	9	10	13	33	8
$sArgNHNH_2$	16	10	11	10	6	22	5
sArg	14	6	10	15	12	21	8
$sArgNH_2$	13	9	7	16	11	25	9
$sArgNHN(CH_2CH_2Cl)_2$	10	11	13	9	10	35	10
$sArgNHNHC_6H_5$	25	6	14	15	12	42	6

CONCLUSION

The present results suggest that all compounds studied could act as inhibitors for five of the total of seven enzymes tested in this study: eNOS, iNOS, ADC, AGAT, and ASS. NCan and NCav are not effective inhibitors, due to the lack of binding sites to the active centers of the enzymes. Nevertheless, they may be interesting as potential effectors for cells when incorporated in some other proteins. NsArg and sArg analogues, and *bis*-(2-chloroethylhydrazide) and phenylhydrazide derivatives possess structures with numerous interaction sites that could interact with enzymes, and bind stronger than natural substrates. Thus they could block the metabolic pathways. In line with this, computational methods are very useful tools for determination of the structure-activity relationship. Using data from the *in vitro* tests, it is possible to explain the observed effects of examined compounds, as well as to design new compounds with desired action.

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