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Synthesis, Characterization and Antimicrobial Activity of Amidrazone Derivatives

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ABSTRACT

Six amidrazone derivatives were synthesized. Their structure was determined using modern analytical methods (TLC, mass spectrometry, elemental analysis, ^{1}H NMR spectrometry and infrared spectroscopy). As starting compounds for the preparation of substituted amidrazones hydrazonoyl chlorides commercially available compounds were used. Computer program PASS (Prediction of Activity Spectra for Substances) has been applied for prediction of biological activity spectra of synthetic substances with the aim to discover their pharmacological potential. Computer-aided prediction of interaction amidrazone derivatives with protein targets was carried out with specialized version of the computer program PASS - PASS Targets. Open- source program AutoDock Vina was used for docking of amidrazones to crystal structures of targets: *Candida albicans* glucan 1,3-β-glucosidase (PDB code 2PB1), *Candida albicans* ATP-dependent molecular chaperone HSP82 (PDB code 2IWX), *Staphylococcus aureus* dehydrosqualene synthase (PDB code 3ACX), *Bacillus subtillis* 4'-phosphopantetheinyl transferase sfp (PDB code 1QR0) and *Escherichia coli* cystathioninebeta-lyase metC (PDB code 1CL2). Antimicrobial activity was studied by two-fold serial dilutions of the sample in the meat-broth and Sabouraud medium. As a test cultures microorganisms were used: *Staphylococcus aureus* ATCC 6538-P, *Bacillus cereus* ATCC 6633, Escherichia coli ATCC 25922, *Candida albicans* NCTC 885-653, *Aspirgillus niger* ATCC 9642. The resulting compounds 4-Nitro-N'-phenylbenzenecarboximidohydrazide (2b) and N'-(4-Nitro- phenyl) – benzenecarboximidohydrazide (2c) showed a potation antimicrobial activity.

1. Introduction

Currently antimicrobial resistance among bacteria, viruses, parasites, and other microorganisms is a serious threat to infectious disease management globally [1]. This is related to increased resistance to pathogens of many commonly used antimicrobials. Therefore, it is very important to search and find new drugs that are effective against a broad spectrum of microorganisms – infectious agents.

Linear and cyclic amidrazones exhibit various types of biological activities. They are known to affect cholinesterase, nucleoside hydrolase and glycosidase, inhibit lipoxygenase and oxygenase which are responsible for important biological processes in microorganisms [2]. Amidrazones are available, highly reactive and a good intermediates of synthesis of new heterocyclic compounds having a broad spectrum of biological activity (anti-microbial, anti-inflammatory, analgesic).

Computational approaches such as SAR/QSAR/QSPR and docking have been widely applied to study the receptor – ligand relationship and are frequently used in the drug discovery process [3-7]. Since the majority of known pharmaceutical agents reveal pleiotropic activities, the concept of biological activity spectrum was introduced to describe the properties of biologically active substances. First, as a starting point for the identification potential biological activity for substances we apply fast and user-friendly computer program PASS (Prediction of Activity Spectra for Substances). PASS estimates biological activity spectra of new compounds on the basis of structure-activity relationships analysis of the training set, which currently includes about million known biologically active substances (drugs, drug-candidates, leads, and toxic compounds). PASS approach previously described in detail in papers [8, 9] and there are many publications where PASS predictions were confirmed by subsequent synthesis and biological testing [10-14].

In addition, the specialized version of the program PASS – PASS targets was used in the computer-aided assessment of the synthesized amidrazones interaction with the target proteins. PASS targets allows to predict the interaction of drug – like organic compounds with 3257 protein targets in the human body (1635 targets), animal models (857 targets), infectious agents (410 targets) and other organisms (355 targets). SAR base, obtained by training PASS targets, is based on the analysis of 348, 137 structures of unique compounds with known interaction with targets. The prediction average accuracy estimated by leave-one-out cross-validation (LOO CV) is 98.1%, and by 20-fold cross – validation (20-fold CV) is 97.9%. A slight difference between the prediction accuracy estimated by LOO CV and 20-fold CV indicates the robustness of "structure-activity" dependencies built in PASS targets [15].

Based on the PASS targets prediction results, to obtain quantitative assessment of the target protein binding and to rank the amidrazone derivatives according to their affinity to active site of targets molecular docking was performed on: *C. albicans* glucan 1,3-β-glucosidase (PDB code 2PB1), *C. albicans* ATP-dependent molecular chaperone HSP82 (PDB code 2IWX), *S. aureus* dehydrosqualene synthase (PDB code 3ACX), Bac. subtillis 4'-phosphopantetheinyl transferase sfp (PDB code 1QR0) and *E. coli* Cystathionine beta-lyase metC (PDB code 1CL2).

Experimental investigations of antimicrobial activity of amidrazone derivatives were conducted at the Department of Microbiology of Saint-Petersburg Chemical Pharmaceutical Academy on the test cultures microorganisms: *S. aureus*, *Bac. cereus*, *E. coli*, *C. albicans*, *Asp. niger*.

2. Experimental Methods

2.1 Chemistry

2.1.1 Preparation of Amidrazones

Hydrazonoyl chlorides **1** [16], used as starting materials for the synthesis of **3** amidrazones hydrohalides, prepared from the corresponding hydrazides commercial without further purification using the method described in [17, 18]. ^{1}H-NMR spectra of solutions substance in DMSO-d_6 obtained on a Bruker AM-500, the operating frequency of 500 MHz at 25 °C. IR spectra of the samples synthesized compounds in KBr tablet recorded on a FTIR spectrometer FSM-1201. TLC of the reaction mixtures and pure sample substances carried on plates «Sorbfil»; eluent acetone-hexane, 1:2. MS spectra were run at electronic ionization at 70 eV

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on a MX-1321 spectrometer, the temperature of the ionizing chamber 200 °C, in the direct input mode. Elemental analysis was performed on Leco CHNS-932.

*Synthesis of Hydrohalides Amidrazones **3 (a – d)***

To a solution of 0.04 M hydrazonoyl chloride **(1)** in 65 mL of anhydrous THF at 3 – 4 °C for 40 minutes, ammonia was passed dried. The resultant precipitate of ammonium chloride was filtered off; the filtrate was evaporated using a rotary film evaporator under vacuum at a residual pressure of 50 mm Hg. The residue was dissolved in dichloromethane and the solution saturated with dry hydrogen chloride or hydrogen bromide at 3 – 4 °C. The formed salt was filtered, washed with dichloroethane 2 × 50 mL. The precipitate was dried at 40 – 50 °C and recrystallized from acetic acid (Table 1, Scheme 1).

*N'-Phenylbenzenecarboximidohydrazide Hydrochloride **(3a)***

^{1}H-NMR spectrum (δ, ppm, DMSO-d_6): 6.95 m, 7.28 t, 7.65 t, 7.78 t, 8.02 d (10H, 2Ph), 9.02 s (1H, NH), 9.42 br. s (1H, NH), 10.05 br. s (1H, NH), 11.95 s (1H, HCl). Calc. for $C_{13}H_{14}ClN_3$: C, 63.03%; H, 5.70%; N, 16.96%. Found: C, 63.12%; H, 5.62%; N, 16.88%.

*4-Nitro-N'-Phenylbenzenecarboximidohydrazide Hydrochloride **(3b)***

^{1}H-NMR spectrum (δ, ppm, DMSO-d_6): 6.99 m, 7.32 t, 8.20 d, 8.47 d (9H, 2Ph), 8.88 s (1H, NH), 10.21 br. s (2H, NH), 12.13 s (1H, NH). MS, m/z (Irelative, %): 292.07 (100%), 294.07 (32.2%) 293.08 (14.7%), 295.07 (5.1%), 293.07 (1.5%), 294.08 (1.4%). Calc. for $C_{13}H_{13}ClN_4O_2$: C, 60.93%; H, 4.72%; N, 21.86%. Found: C, 60.98%; H, 4.73%; N, 21.85%. (Scheme 2).

*4-Nitro-N'-Phenylbenzenecarboximidohydrazide Hydrobromide **(3b')***

^{1}H-NMR spectrum (δ, ppm, DMSO-d_6): 6.97 m, 7.39 t, 8.11 d, 8.43 d (9H, 2Ph), 8.87 s (1H, NH), 10.15 br. s (2H, NH), 12.09 s (1H, NH). Calc. for $C_{13}H_{13}BrN_4O_2$: C, 46.31%; H, 3.89%; N, 16.62%. Found: C, 46.38%; H, 3.78%; N, 16.69%.

*N'-(4-Nitrophenyl)Benzenecarboximidohydrazide Hydrochloride **(3c)***

^{1}H-NMR spectrum (δ, ppm, DMSO-d_6): 7.08 d, 7.68 t, 7.81 t, 8.02 d, 8.18 d (9H, 2Ph), 9.89 br. s (2H, NH), 10.21 s (1H, NH), 12.31 s (1H, NH) (Fig. 1c). Calc. for $C_{13}H_{13}ClN_4O_2$: C, 53.34%; H, 4.48%; N, 19.14%. Found: C, 53.45%; H, 4.38%; N, 19.09%.

*N'-(4-Nitrophenyl)Benzenecarboximidohydrazide Hydrobromide **(3c')***

^{1}H-NMR spectrum (δ, ppm, DMSO-d_6): 7.06 d, 7.7 t, 7.81 t, 7.93 d, 8.19 d (9H, 2Ph), 9.73 s (3H, NH), 11.85 s (1H, NH). Calc. for $C_{13}H_{13}BrN_4O_2$: C, 46.31%; H, 3.89%; N, 16.62%. Found: C, 46.42%; H, 3.98%; N, 16.55%.

*4-Methoxy-N'-Phenylbenzenecarboximidohydrazide Hydrochloride **(3d)***

^{1}H-NMR spectrum (δ, ppm, DMSO-d_6): 3.74 s (3H, OCH_3), 7.07 m, 7.63 m, 7.75 m, 7.89 d, 8.1 m (9H, 2Ph), 9.85 s (2H, NH), 10.07 (H, NH), 12.18 s (1H, NH). Calc. for $C_{14}H_{16}ClN_3O$: C, 60.54%; H, 5.81%; N, 15.13%. Found: C, 60.47%; H, 5.88%; N, 15.20%.

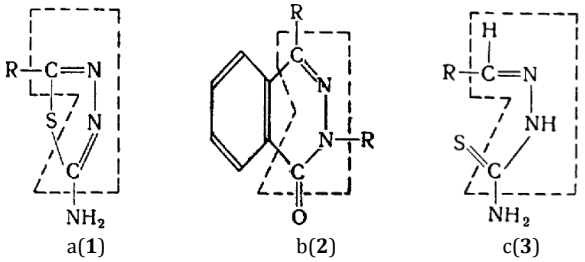


Fig. 1 Group «= N-NH-C-» which is present in amidrazones derivatives in other compounds with antimicrobial activity a) thiadiazole b) phthalazone and c) thiosemicarbazones

*2.2 Biological Activity of Amidrazones **3***

Antimicrobial activity of salts amidrazones (**3**) studied at the Department of Microbiology SPCPA. The antibacterial activity was determined against gram-positive cultures *S. aureus* ATCC 6538-P, spore forming bacteria – *Bac. cereus* ATCC 6633 and gram negative – *E. coli* ATCC 25922. To examine the antifungal activity as a test crop yeast *C. albicans* NCTC 885-653 and mycelial fungus *Asp. niger* ATCC 9642 were used. As a comparison drugs were took antifungal drug fluconazole and broad spectrum of antibacterial drug nifuroxazide. Antifungal and antibacterial drug were selected because of the functional group = N-NH-C which is present in amidrazones derivatives and in derivatives of isonicotinic acid, is probably responsible for the bactericidal and bacteriostatic activity. This functional group is present also in other compounds with antimicrobial activity such as thiadiazole derivatives (**1**) in phthalazone derivatives (**2**) and thiosemicarbazones (**3**) [19].

2.3 PASS Analysis

In silico predictions were carried out with PASS 2014 Refined version to get biological activity spectra of each compounds. PASS is software for prediction of biological activity spectra for organic compounds on the basis of their structural formulae. As input information PASS uses the information on the structural formula of the molecule represented as Molfile for a single structure or as SDfile for a set of structures (Accelrys Inc., http://accelrys.com). PASS output represents a list of probable activities with two estimates: P_a – probability to be active and P_i – probability to be inactive. Prediction of PASS is based on SAR analysis of the training set containing about one million compounds showing more than 7000 biological activities. Being probabilities, the Pa and Pi values vary from 0.000 to 1.000, however $P_a \neq P_i = 1$, since these probabilities are calculated independently.

2.4 PASS Targets Analysis

ChEMBL is a database with free access regularly updated by members of the European Bioinformatics Institute (EBI), containing information about chemical compound structures and experimental data on their interaction with pharmacological targets [20]. We used the 19-th version of ChEMBL (ChEMBL_19), from which we extracted the training set consisted of 589107 structural formulae of organic compounds with the appropriate information about biological activity. PASS targets trained on this training set allows predicting the interaction of drug-like compounds with 2501 protein targets with average accuracy of about 97%.

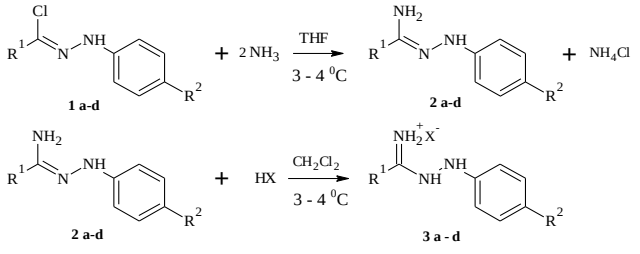
2.5 Docking Using AutoDock Vina

Molecular docking of obtained compounds **2 a-d** (Table 6 and 7; Scheme 1) and the reference compounds (fluconazole and nifuroxazide) was performed using the open – source software AutoDock Vina. AutoDock Vina needs receptor and ligand representations in a pdbqt file format, which is a modified protein data bank format containing atomic charges, atom type definitions and, for ligands, topological information (rotatable bonds). Targets taken from the collection of experimentally determined 3D-structures of biological macromolecules database PDB. As a targets, we used *C. albicans* glucan 1,3-β-glucosidase [20] (PDB code 2PB1), *C. albicans* ATP-dependent molecular chaperone HSP82 [21] (PDB code 2IWX), *S. aureus* dehydrosqualene synthase [22] (PDB code 3ACX), *Bac. subtillis* 4'-phosphopantetheinyl transferase sfp [23] (PDB code 1QR0), *E. coli* cystathionine β-lyase metC (PDB code 1CL2) (Table 5 and 6). The docking poses in AutoDock Vina are ranked according to their docking scores and both the ranked list of docked ligands and their corresponding binding poses were analyzed.

3. Results and Discussion

3.1 Synthesis and Structure Hydrohalides N'- Rylbenzenecarboximido-hydrazide

Amidrazones **3** were obtained in a two-step synthesis starting by N – arylbenzenecarbohydrazonoyl chlorides **1** according the Scheme 1.



2a, R^1 = C_6H_5, R^2 = H; **2b**, R^1 = 4-$O_2NC_6H_4$, R^2 = H;
2c, R^1 = C_6H_5, R^2 = NO2; **2d**, R^1 = 4-$H_3COC_6H_4$, R^2 = H;
3a, R^1 = C_6H_5, R^2 = H, X = Cl; **3b**, R^1 = 4-$O_2NC_6H_4$, R^2 = H, X = Cl;
3b', R^1 = 4-$O_2NC_6H_4$, R^2 = H, X = Br; **3c**, R^1 = C_6H_5, R^2 = NO_2, X = Cl;
3c', R^1 = C_6H_5, R^2 = NO_2, X = Br; **3d**, R^1 = 4-$H_3COC_6H_4$, R^2 = H, X = Cl.

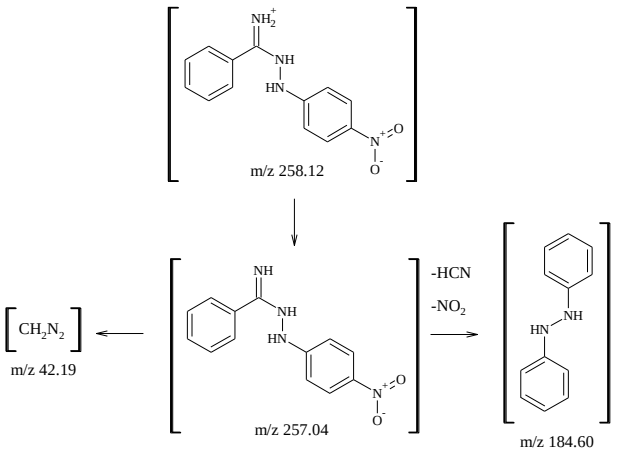
Scheme 1 Preparation of derivatives amidrazone (**3 a – d**)

Table 1 Characterization data of synthesized novel amidrazonesderivatives

Comp.	Molecular formula	M.P. (°C)	Mol. Wt.	App.	*Rf* (acetone-hexane, 1: 2)	Yield (%)
3a	$C_{13}H_{14}ClN_3$	135 – 137	247.72	Yellow	0.65	82
3b	$C_{13}H_{13}ClN_4O_2$	153 – 155	292.72	Yellow	0.701	80
3b`	$C_{13}H_{13}BrN_4O_2$	169 – 171	337.17	Orange	0.703	80
3c	$C_{13}H_{13}ClN_4O_2$	170 – 173	292.72	Yellow	0.625	85
3c`	$C_{13}H_{13}BrN_4O_2$	175 – 177	337.17	Orange	0.716	85
3d	$C_{14}H_{16}ClN_3O$	195 – 197	277.75	Yellow	0.609	80

Salts **3 a-d** of obtained amidrazones are crystalline solids or yellow orange, readily soluble in water. The yields of amidrazones ranged from 80 to 85%. Structure and identity of the obtained compounds were established using physic-chemical methods of analysis (^{1}H-NMR and IR spectroscopy, TLC, elemental analysis). The IR spectra of amidrazones 3a-d presence of absorption bands in the 1540-1600 and 2980-3130 cm^{-1} due to stretching vibrations of aromatic moieties and C-H bonds, respectively, and the characteristic absorption bands in the 3400-3440 and 1660 cm^{-1} corresponds to the valence N-H and vibrations of (N)C=N.

In the mass spectrum obtained for hydrochloride salt **3c** molecular ion peaks of the free base (m/z 257.04) and $[M + H]^+$ (m/z 258.12) are formed by dissociation of salt in a recording range (Scheme 2). Fragmentation of this molecular ion is accompanied by cleavage of molecular HCN and NO_2 to form a splinter radical cation 1, 2 – diphenylhydrazine (m/z 184.60). In the spectrum also observed signal radical cation carbodiimide (m/z 42.19).



Scheme 2 The Mass spectrometry of *N'*-(4-nitrophenyl) benzenecarboximido-hydrazide hydrochloride **(3c)**

3.2 The Study of the Antibacterial Activity of DerivativesAmidrazone

Open – chain and cyclic amidrazones are compounds which exhibit various types of biological activity. There is evidence that amidrazones can affect cholinesterase activity, inhibit lipoxygenases and oxygenases which are responsible for important biological processes [2]. For example, 5-lipoxygenase is involved in the biosynthesis of leukotrienes, which are in turn responsible for susceptibility to painful effects and can therefore be used in anesthesia. To increase the probability of finding unexplored type of activities and detecting potential drug–target interactions for test amidrazones we have applied the in silico prediction based on commercially available software products - PASS 2014 Refined and PASS targets [8, 15]. The proposed approach consists of two steps: (i) prediction of pharmacological effects from chemical structures of given compounds and (ii) inference of unknown drug–target interactions based on the pharmacological effect similarity.

In order to predict the biological activity profiles the structural formulas of the test amidrazones **2 a-d** were presented as SDF-file. The predicted results for the compounds **2 a-d** are shown in Table 2. It should be noted that the probability P_a reflects, above all, the similarity of molecular structure of the substance with the structures of the molecules of the most typical in the corresponding subset of the "active" compounds in the training set. Therefore, as a rule, there is no direct correlation values Pa with quantitative characteristics of the activity. The PASS prediction results usually are interpreted and used in a flexible manner: (I) only activities with $P_a > P_i$ are considered as possible for a particular compound; (II) if $P_a > 0.7$, the chance to find the activity experimentally is high; (III) if $0.5 < P_a < 0.7$, the chance to find the activity experimentally is less, but the compound is probably not so similar to known pharmaceutical agents and (IV) if $P_a < 05$, the chance to find the activity experimentally is even more less, but if the prediction is confirmed, the compound may be the progenitor of a new chemical class for the given type of biological activity (New Chemical Entity).

Prediction results of activity spectra for test amidrazones **2 a-d** (Table 2) showed that among already well-known activities (anti-inflammatory, anesthetic general) there was high probability of antiprotozoal, antibacterial and antifungal activities (Table 2) for these compounds. The number of substances in the training set and accuracy of prediction (%) for pharmacological effects obtained for test amidrazones by PASS 2014 Refined are given in Table 3.

Table 2 PASS predicted activities for amidrazones

	Compound	Activities predicted by PASS ($P_a > P_i$)		
		$P_a > 0.7$	$0.5 < P_a < 0.7$	$P_a < 0.5$
2a	*N'*-Phenylbenzene carboxi midohydrazide	Antiinflammatory	Antiprotozoal	Anesthetic general, Antibacterial
2b	4-Nitro-*N'*-phenyl benzenecarboxi midohydrazide	Antiinflammatory	Antiprotozoal	Anesthetic general, Antibacterial, Antifungal
2c	*N'*-(4-Nitrophenyl) benzenecar boximidohydrazide	Antiinflammatory	Antiprotozoal	Anesthetic general, Antibacterial, Antifungal
2d	4-Methoxy-*N'*-phenyl benzenecarboxi midohydrazide	Antiinflammatory	none	Anesthetic general, Antifungal

Table 3 List of pharmacological effects projected for amidrazones by PASS

S.No.	Effect Name	Number of active compounds in the training set	Average accuracy of prediction %
1	Antiinflammatory	5983	84.7
2	Antiprotozoal	22185	88.2
3	Anesthetic general	151	92.5
4	Antibacterial	8342	92.2
5	Antifungal	2470	92.5

During the training procedure PASS generates a knowledge base containing information on the relationship between the structure of chemical compounds and their effects on target proteins, which is later used for prediction of drug-target interaction. The results prediction PASS Targets for each of the analyzed molecules contain lists of targets with estimates of probable interaction - Pa and the probability of a lack of interaction - P_i (Table 4).

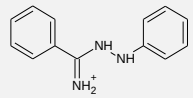
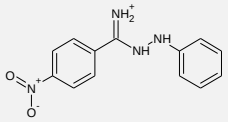
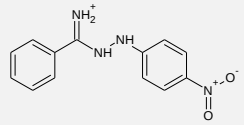
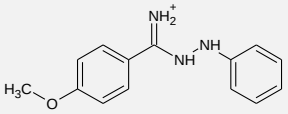
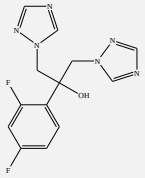
Based on the results of the PASS and PASS Targets prediction, molecular docking was applied to some of the selected targets with known 3D structures. The predicted values of the binding energy (scoring function) by AutoDock Vina for antifungal targets glucan 1,3-β-glucosidase and test compounds were higher (-8.0; - 8.5; -8.8 kcal/mol) than the binding energy for antifungal drug fluconazole (-7.9 kcal/mol) except of compound **2d** having a donor substituent in the benzene ring (-6.9 kcal/mol). The binding energy for antifungal targets ATP-dependent molecular chaperone HSP82 and test compounds were higher (-6.7; -6.9; -6.8 kcal/mol) than the binding energy for fluconazole (-6.6 kcal/mol) except of base **2d** having a donor substituent in the benzene ring (-6.5 kcal/mol) (Table 5).

The predicted values of the binding energy by AutoDock Vina for antibacterial targets dehydrosqualene synthase and test compounds were higher (-8.4, -8.5, -8.6, -8.3 kcal/mol), than the binding energy for wide effect antimicrobial drug nifuroxazide (-7.5 kcal/mol). The binding energy for 4'-phosphopantetheinyl transferase sfp and test compounds **2b** and **2c** were higher (-7.5, -7.3 kcal/mol), than the binding energy for antimicrobial drug nifuroxazide (-7.1 kcal/mol). The binding energy for antibacterial targets cystathionine beta – lyase metC and test compounds **2b** and **2c** were higher (-7.4, -8.2 kcal/mol), than the binding energy for antimicrobial drug nifuroxazide (-7.3 kcal/mol) (Table 6).

Table 4 The spectrum of interaction amidrazones with the target made using PASS Targets (threshold $P_a > P_i$)

S. no.	Compound	Activities predicted by PASS Targets ($P_a > P_i$)		
		$P_a > 0.7$	$0.5 < P_a < 0.7$	$P_a < 0.5$
2a	*N*'-Phenylbenzenecarboximidohydrazide	Serine/threonine-protein phosphatase – *Candida dubliniensis* (strain CD36/CBS7987/NCPF 3949/NRRLY-17841) (Yeast)	mRNA interferase MazF – *E. coli* K – 12; Endonuclease 4 – *E. coli* K – 12	ATP-dependent molecular chaperone HSP82 – *C. albicans* (strain WO-1) (Yeast); Endonuclease 4 – *E. coli* K-12; Beta-galactosidase *E. coli*; 4'-phosphopantetheine inyl transferase sfp – *Bac. Subtilis*; Cystathionine beta-lyase metC – *E. coli* K-12; Cytochrome P450 14 alpha-demethylase – *Pen. digitatum*; 3-oxoacyl-[acyl-carrier-protein] synthase 3 – *E. coli*; Modulator of drug activity A – *E. coli* O157:H7
2b	4-Nitro-*N*'-phenylbenzenecarboximidohydrazide			
2c	*N*'-(4-Nitrophenyl)benzenecarboximidohydrazide			
2d	4-Methoxy-*N*'-phenylbenzenecarboximidohydrazide			

Table 5 The value of the binding energy (kcal/mol) for test compounds on antifungaltargets: i) glucan 1,3-β-glucosidase; ii) ATP-dependent molecular chaperone HSP82.

Antifungal targets		i	ii
	**2a**	-8	-6.7
	**2b**	-8.5	-6.9
	**2c**	-8.8	-6.8
	**2d**	-6.9	-6.5
	Fluconazole	-7.9	-6.6

Thus, computational docking results broadly corresponds to the PASS prediction, and allows to determine the binding modes of the studied compounds. Based on the result of in silico prediction 6 novel amidrazones was studied against fungal organism's *C. albicans* and *Asp. niger* and bacterial organisms *E. coli*, *S. aureus* and B. cereus using the opportunities available in Saint-Petersburg State Chemical Pharmaceutical Academy. During these studies it was found that amidrazones **3a-d** were virtually inactive against these of bacterial cultures whereas has a pronounced anti-staphylococcal and antifungal activity (Table 7).

Table 6 The value of the binding energy (kcal/mol) for test compounds on the antibacterial targets: i) dehydrosqualene synthase, ii) 4'-phosphopantetheinyl transferase sfp, iii) Cystathionine beta-lyase met C

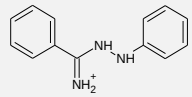
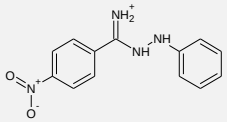
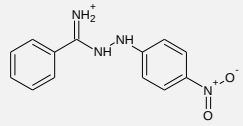
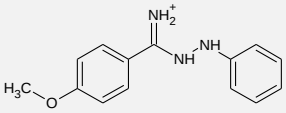
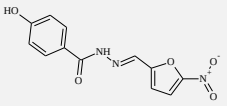
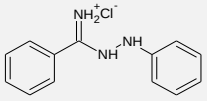
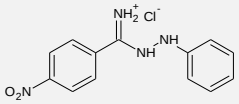
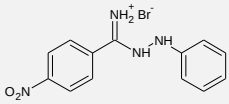
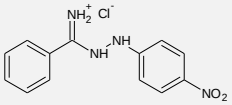
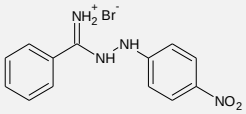
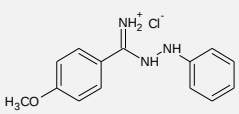
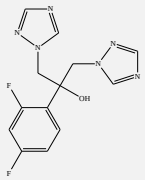
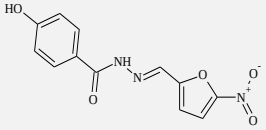
Antifungal targets		i	ii	iii
	**2a**	-8.4	-7	-6.7
	**2b**	-8.5	-7.5	-7.4
	**2c**	-8.6	-7.3	-8.2
	**2d**	-8.3	-6.7	-7.2
	Nifuroxazide	-7.5	-7.1	-7.3

Table 7 Antimicrobial activity of derivatives hydrohalides amidrazones **3**

Compound	Formula	Test-organisms	Concentration (mcg/mL)	
			MICst	MICcd
3a		*St. aureus*	-	16
		C. albicans	8	16
		Asp. niger.	-	125
3b		*St. aureus*	-	16
		C.albicans	4	8
		Asp. niger.	-	16
3b'		*St. aureus*	-	16
		C. albicans	16	32.5
3c		*St. aureus*	-	4
		C.albicans	-	250
3c'		*St. aureus*	-	8
		C.albicans	-	250
3d		*St. aureus*	-	62.5
		C.albicans	-	62.5
Fluconazole		*C.albicans*	-	32.5
Nifuroxazide		*St. aureus*	-	16

According to experimental study anti-staphylococcal activity of amidrazones **3a-d** depended on the nature of the input substituting molecule. Chloride and bromide (**3b, 3b'**) having nitro group in the *p*-position one of the benzene ring had the greatest antimicrobial activity. Compounds **3b**, **3b'**, **3c** and **3c'** had a pronounced bactericidal action (MICcd 4–16 mcg/mL). Cidal and static concentrations of these compounds were abreast or lower than that of the reference drug nifuroxazide (MICcd = 16 mcg/mL). Replacing the electron-acceptor substituents in acyl moiety (e.g. compound **3b, 3b'**) with electron-donating (methoxy group, compound **3d**) leads to a significant decrease in bactericidal activity (MICcd 62.5 mcg/ mL).

Some of the compounds possess a potent antifungal activity against *C. albicans*: unsubstituted compound **3a** (MICst = 8 mcg/mL, MICcd = 16 mcg/mL) and compounds with strong electron-acceptor substituent at the *para*-position of the aryl moieties of such compound **3b** (MICst = 4 mcg/mL, MICcd = 8 mcg/mL), **3b'** (MICst

= 16 mcg/mL, MICcd = 32.5 mcg/mL). Cidal and static concentrations of these compounds were lower than that of the reference drug fluconazole (MICcd = 32 mcg/mL).

Based on the results obtained during the study of the antimicrobial action amidrazone derivatives, two of the most active compounds **3a** and **3b** was selected, which were used for further study their activity against micellar fungi *Asp. niger.*

4. Conclusion

A series of novel amidrazones possessing antimicrobial activity were prepared in a two-step synthesis starting by *N*-arylbenzene-carbohydrazonoyl chlorides. Interaction of hydrazones with phosphorus pentachloride in carbon tetrachloride to give *N*- arylbenzenecarbo-hydrazonoyl chlorides followed ammination is an effective method for the synthesis of amidrazones. Application of computer-aided drug design approaches (PASS, AutoDoc Vina) allowed to reveal the pharmacological potential of the studied compounds, and to identify their probable molecular targets. Amidrazone - 4-nitro-*N'*-phenylbenzenecarboximido-hydrazide hydrochloride and 4-nitro-*N'*-phenylbenzenecarboximido-hydrazide hydrobromide with a strong acceptor substituent (nitro group), in one of the benzene rings have antibacterial activity against *S. aureus*. The strongest anti-staphylococcal effect has *N'*-(4-nitrophenyl) benzene-carboximidohydrazide hydro-chloride. Amidrazone derivative of 4-nitro-*N'*-phenylbenzene-carboximidohydrazide, provide effective antifungal activity against yeasts *C. albicans* and filamentous fungi *Asp. niger*. Found that the antimicrobial activity is dependent on the amidrazones administered electronic nature of the substituent. Electron withdrawing substituents (nitro group) in one of the benzene rings increase the antimicrobial activity of the compound and the electron- donating substituents (methoxy group) in acyl moiety is reduced.

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