ORIGINAL ARTICLE

ANTIMICROBIAL ACTIVITY SCREENING OF SOME HYDRAZINECARBOTHIOAMIDES AND HETEROCYCLIC COMPOUNDS

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Abstract

In this paper we present the antimicrobial activity screening of a series of eighteen compounds from hydrazinecarbothioamides (**1-3**), 1,2,4-triazole-3-thiones (**4-6**), S-alkylated 1,2,4-triazoles (**7-12**), 1,3,4-thiadiazoles (**13-15**) and 1,3,4-oxadiazoles (**16-18**) class which contain arylsulfonylphenyl and 2,4-difluorophenyl moieties in their molecule. The antimicrobial action of these compounds has been investigated against the following reference microbial strains: *Escherichia coli* ATCC 25922, *Enterobacter cloacae* ATCC 49141, *Acinetobacter baumannii* ATCC 19606, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* ATCC 13061, *Staphylococcus aureus* ATCC 29213, *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 64677, *Candida krusei* ATCC 14243 and *Candida parapsilosis* ATCC 22019, by the broth microdilution method. Comparing the values of the minimum inhibitory concentrations of the compounds it was noticed that triazoles **4-6** and hydrazinecarbothioamide **2** had in general the best antibacterial activity against some tested bacteria (MIC of 32 μg/mL) and oxadiazole **17** showed the best antifungal activity against *C. parapsilosis* (MIC of 32 μg/mL).

Rezumat

În această lucrare este prezentat *screening*-ul activității antimicrobiene a 18 compuși din clasa hidrazincarbotioamidelor (**1-3**), 1,2,4-triazol-3-tionelor (**4-6**), 1,2,4-triazolilor S-alchilați (**7-12**), 1,3,4-tiadiazolilor (**13-15**) și 1,3,4-oxadiazolilor (**16-18**) care conțin fragmentele arilsulfonilfenil și 2,4-difluorofenil în moleculă. Acțiunea antimicrobiană a acestor compuși a fost investigată pe următoarele tulpini microbiene de referință: *Escherichia coli* ATCC 25922, *Enterobacter cloacae* ATCC 49141, *Acinetobacter baumannii* ATCC 19606, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* ATCC 13061, *Staphylococcus aureus* ATCC 29213, *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 64677, *Candida krusei* ATCC 14243 și *Candida parapsilosis* ATCC 22019, prin metoda microdiluțiilor în bulion. În urma comparării valorilor concentrațiilor minime inhibitorii ale compușilor, s-a observat că triazolii **4-6** și hidrazincarbotioamida **2** au avut în general cea mai bună acțiune antibacteriană împotriva unor bacterii luate în lucru (MIC = 32 μg/mL), iar oxadiazolul **17** a avut cea mai bună acțiune antifungică împotriva *C. parapsilosis* (MIC = 32 μg/mL).

Keywords: hydrazinecarbothioamide, 1,2,4-triazole, 1,3,4-thiadiazole, 1,3,4-oxadiazole, antimicrobial activity

Introduction

The antibiotic resistance represents a major problem for the public health and the rapid spread of the multiresistant isolates among the opportunistic microorganisms such as: *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter* spp., *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Candida* became a serious threat. The methicillin-resistant *Staphylococcus aureus* isolates [1] and the carbapenem-resistant strains of: *Klebsiella pneumoniae* [2], *Escherichia coli* [3], *Enterobacter cloacae* [4] and *Pseudomonas aeruginosa* [5] are frequently associated with life-threatening infections, especially in hospitalized immune-compromised patients.

A. baumannii is an opportunistic non-fermentative Gram-negative with intrinsic resistance to many antibiotics, being able to acquire different antimicrobial resistance determinants [6]. At present, multi-drug resistant *A. baumannii* strains are disseminated worldwide, mainly in medical facilities and are involved in different types of infections, such as: bloodstream infections, respiratory infections, soft tissue infections, surgical associated infections [7].

It has been also noticed that the incidence of the fungal infections increased significantly. *Candida* represents the most frequent agent involved in local or sistemic fungal infections and multidrug isolates of *Candida albicans* and non-*albicans Candida* spp. have also been reported [8].

The search for new synthetic compounds useful as therapeutic agents in patients with bacterial or fungal infections still remain a challenge for many researchers in the medical field. A lot of studies have been focused on the heterocyclic compounds with potential antimicrobial action [9-20].

Hydrazinecarbothioamides and their derivatives from 1,2,4-triazole, 1,3,4-thiadiazole and 1,3,4-oxadiazoles class have attracted considerable interest not only because of their chemical synthesis, but also because of their biological properties. Among the numerous biological properties of these compounds it is to be noted the antibacterial and antifungal activity of some derivatives from these classes [9-17, 21, 22].

The results of the previous investigation studies on antimicrobial activity of some heterocyclic compounds from 1,2,4-triazoles, 1,3,4-thiadiazoles and 1,3,4-oxadiazoles class, as well as of the acyclic compounds from hydrazinecarbothioamides class from which they were obtained [23-27], encouraged the authors of the present study to continue the research of screening of other derivatives from these classes for their antimicrobial activity, in order to obtain antimicrobial agents.

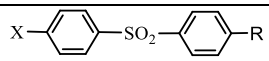
Materials and Methods

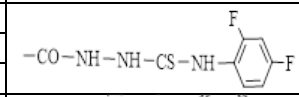
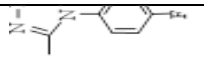
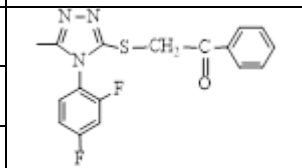
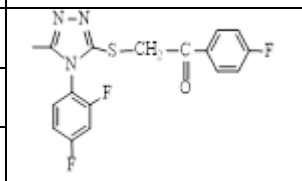
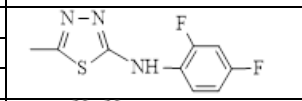
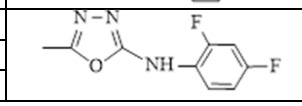
A number of 18 newly synthesized compounds were screened in this study for their antimicrobial action. The synthesis and physicochemical characterization of these compounds have been already reported [28, 29]. The hydrazinecarbothioamides **1-3**, 1,2,4-triazole-3-thione **4-6** and S-alkylated 1,2,4-triazoles **7-12** have been reported in a previous paper [28] and the 1,3,4-thiadiazoles **13-15** and 1,3,4-oxadiazoles **16-18** have been reported in another previously published paper [29].

The structures of the compounds tested for their antimicrobial activity are presented in the Table I.

Table I

The chemical structure and name of the tested compounds



No	X	R	Name of the compounds
1	H		N-(2,4-difluorophenyl)-2-(4-(phenylsulfonyl) benzoyl)hydrazinecarbothioamide
2	Cl		2-(4-(4-chlorophenylsulfonyl)benzoyl)-N-(2,4-difluorophenyl)hydrazinecarbothioamide
3	Br		2-(4-(4-bromophenylsulfonyl)benzoyl)-N-(2,4-difluorophenyl)hydrazinecarbothioamide
4	H		4-(2,4-difluorophenyl)-5-(4-(phenylsulfonyl)-phenyl)-2H-1,2,4-triazole-3(4H)-thione
5	Cl		5-(4-(4-chlorophenylsulfonyl)phenyl)-4-(2,4-difluorophenyl)-2H-1,2,4-triazole-3(4H)-thione
6	Br		5-(4-(4-bromophenylsulfonyl)phenyl)-4-(2,4-difluorophenyl)-2H-1,2,4-triazole-3(4H)-thione
7	H		2-(4-(2,4-difluorophenyl)-5-(4-(phenylsulfonyl)-phenyl)-4H-1,2,4-triazol-3-ylthio)-1-phenylethanone
8	Cl		2-(5-(4-(4-chlorophenylsulfonyl)phenyl)-4-(2,4-difluorophenyl)-4H-1,2,4-triazol-3-ylthio)-1-phenylethanone
9	Br		2-(5-(4-(4-bromophenylsulfonyl)phenyl)-4-(2,4-difluorophenyl)-4H-1,2,4-triazol-3-ylthio)-1-phenylethanone
10	H		2-(4-(2,4-difluorophenyl)-5-(4-(phenylsulfonyl)-phenyl)-4H-1,2,4-triazol-3-ylthio)-1-(4-fluorophenyl)ethanone
11	Cl		2-(5-(4-(4-chlorophenylsulfonyl)phenyl)-4-(2,4-difluorophenyl)-4H-1,2,4-triazol-3-ylthio)-1-(4-fluorophenyl)ethanone
12	Br		5-(4-(4-bromophenylsulfonyl)phenyl)-4-(2,4-difluorophenyl)-4H-1,2,4-triazol-3-ylthio)-1-(4-fluorophenyl)ethanone
13	H		N-(2,4-difluorophenyl)-5-(4-(phenylsulfonyl)-phenyl)-1,3,4-thiadiazol-2-amine
14	Cl		5-(4-(4-chlorophenylsulfonyl)phenyl)-N-(2,4-difluorophenyl)-1,3,4-thiadiazol-2-amine
15	Br		5-(4-(4-bromophenylsulfonyl)phenyl)-N-(2,4-difluorophenyl)-1,3,4-thiadiazol-2-amine
16	H		N-(2,4-difluorophenyl)-5-(4-(phenylsulfonyl)-phenyl)-1,3,4-oxadiazol-2-amine
17	Cl		5-(4-(4-chlorophenylsulfonyl)phenyl)-N-(2,4-difluorophenyl)-1,3,4-oxadiazol-2-amine
18	Br		5-(4-(4-bromophenylsulfonyl)phenyl)-N-(2,4-difluorophenyl)-1,3,4-oxadiazol-2-amine

The antibacterial activity of all these compounds has been tested against the following reference strains: *Escherichia coli* ATCC 25922, *Enterobacter cloacae* ATCC 49141, *Acinetobacter baumannii* ATCC 19606, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* ATCC 13061 and *Staphylococcus aureus* ATCC 29213, while the antifungal activity has been tested against 4 yeast reference strains: *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 64677, *Candida krusei* ATCC 14243 and *Candida parapsilosis* ATCC 22019.

The antimicrobial activity testing of the compounds was performed in duplicate using the broth microdilution method. Dimethyl sulfoxide (DMSO) showed no antimicrobial activity against the tested strains and was used as solvent for preparing the stock solutions of the compounds (2048 μg/mL). Binary dilution series of the compounds were performed in 50 μL broth per well (Mueller-Hinton broth for the antibacterial activity testing and Sabouraud broth for the antifungal activity testing, respectively). The initial microbial inoculum was adjusted at 0.5 Mc.Farland turbidity and then diluted to 1/100 in Mueller-Hinton broth (in case of the bacterial inoculum) or in Sabouraud broth (for obtaining a fungal density of 1 x 10^5 CFU/mL). Aliquots of 50 μL of the obtained inoculum dilutions were added in the wells containing the tested compounds and in the positive growth control wells (which already contained 50 μL compound-free broth). The negative growth control (the sterility control) wells contained 100 μL compound-free broth. Inoculum controls were performed for all the strains. The minimum inhibitory concentrations (MIC) were determined after the microplate was incubated at 37°C for 24 h. The MIC value was considered the lowest concentration of the tested compound which inhibited the microbial growth and it was indicated by the last well of the respective dilutions series showing no turbidity or growth button.

The following reference strains: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 29213 were tested for quality control against amikacin and *C. parapsilosis* ATCC 22019 was tested against fluconazole (as quality control) by the same microdilution broth method. The MIC value of both amikacin and fluconazole was of 2 μg/mL when tested against the above mentioned strains.

Results and Discussion

The results of the antimicrobial screening of compounds from hydrazinecarbothioamides (**1-3**), 1,2,4-triazole-3-thiones (**4-6**), S-alkylated 1,2,4-triazoles (**7-12**), 1,3,4-thiadiazoles (**13-15**) and 1,3,4-oxadiazoles (**16-18**) class expressed as the MIC values are summarized in the Table II and Table III.

Table II
Antibacterial activities of compounds **1-18**

No	Value of MIC (μg/mL)					
	Gram-negative bacteria				Gram-positive bacteria	
	E. coli ATCC 25922	***E. cloacae*** ATCC 49141	***A. baumannii*** ATCC 19606	***P. aeruginosa*** ATCC 27853	***B. cereus*** ATCC 13061	***S. aureus*** ATCC 29213
1	128	128	64	128	64	128
2	128	128	64	128	64	32
3	256	256	128	256	256	256
4	128	128	64	128	32	64
5	128	64	32	128	32	32
6	128	64	32	128	32	32
7	512	512	512	512	> 512	512
8	512	512	512	256	256	> 512
9	512	128	512	256	256	128
10	512	512	256	512	> 512	128
11	256	128	32	256	> 512	256
12	256	128	32	256	> 512	256
13	256	256	128	256	256	> 512
14	> 512	> 512	256	> 512	256	> 512
15	> 512	> 512	> 512	> 512	> 512	> 512
16	256	256	128	256	128	256
17	64	128	128	256	64	256
18	256	256	128	256	128	512
Amikacin	2	-	-	2	-	2

The results of the antibacterial screening against the Gram-negative reference bacteria showed a better activity of some tested compounds against *A. baumannii* with MIC values of 32 and 64 μg/mL.

The most active compounds against this strain were found to be triazole-3-thiones **5** and **6** (with a MIC value of 32 μg/mL), compounds containing either a chlorine or a bromine atom on the diphenylsulfone moiety. The same value of MIC against this strain showed the S-alkylated triazoles **11** and **12**, that contained a chlorine or a bromine atom in the same position on the diphenylsulfone moiety as triazole-3-thiones **5** and **6**. Hydrazinecarbothioamides **1**, **2** and triazole **4** showed a higher MIC value against this strain, of 64 μg/mL. It can be noticed that seven of the eighteen compounds tested against this strain showed a better activity, the MIC value being of 32-64 μg/mL. The lowest activity against *A. baumannii* was presented by the S-alkylated triazoles **7-9** and thiadiazole **15** (with a MIC value of ≥ 512 μg/mL). The same MIC value, of the 64 μg/mL, was detected when the triazoles **5** and **6** were tested against *E. cloacae*. The only compound that had better activity against *E. coli* was oxadiazole **17**, with a MIC value of 64 μg/mL.

The results of testing the compounds against Gram-positive bacterial strains indicated that the triazoles **5** and **6** had the most intense activity against *B. cereus* and *S. aureus* (the MIC value of 32 μg/mL). The triazole **4** presented the same MIC value only against *B. cereus* and 64 μg/mL against *S. aureus* strain. From hydrazinecarbothioamide class, compound **2** had the best activity (a MIC value of 32 μg/mL) against *S. aureus*. The same derivative **2** had better activity against *B. cereus* (the MIC value of 64 μg/mL) and equal with the one of derivatives **1** and oxadiazole **17**.

All eighteen compounds showed a poor antifungal activity, except for compound **17**, which showed a lower MIC value (of 32 μg/mL), when tested against C. *parapsilosis* reference strain. The values of the MIC for the other compounds against the studied strains were equal or higher than 128 μg/mL.

The compounds showed the lowest antifungal activity against *C. glabrata* and only the hydrazine-carbothioamide **1**, triazole **4** and oxadiazole **17** were slightly more active, following a MIC value of 128 μg/mL.

Table III

Antifungal activities of compounds **1-18**

No	**Value of MIC (μg/mL)**			
	C. albicans ATCC 90028	***C. glabrata*** ATCC 64677	***C. krusei*** ATCC 14243	***C. parapsilosis*** ATCC 22019
1	128	128	128	128
2	128	512	512	512
3	128	512	512	512
4	128	128	128	128
5	128	512	128	128
6	128	512	128	128
7	128	512	512	512
8	128	512	512	512
9	128	512	512	512
10	128	512	512	512
11	128	512	512	256
12	128	512	512	512
13	512	512	256	256
14	> 512	> 512	> 512	512
15	> 512	> 512	> 512	> 512
16	256	256	256	128
17	256	128	128	32
18	> 512	> 512	128	> 512
Fluconazole	-	-	-	2

The calculation of the molecular descriptors relevant for the bioavailability of the compounds defined a group of highly lipophilic compounds (logP values between 4.27 and 6.48, Table IV). This is the single parameter indicating limitations of the oral absorption, based on the Lipinski rule of five [30]. The hydrogen bonding ability is within the proposed limits, i.e. less than 5 hydrogen bond donors and less than 10 hydrogen bond acceptors. A possible consequence is the high value of the protein bounded fraction, limiting the permeability across the biological barriers, despite the lipophilic character. No correlation between the *in-silico* estimated parameters and the antifungal profiles was registered. This represents a common observation, since the relationship between the molecular structure and the intimate mechanism of action is presumably more complicated than a direct relationship. Moreover, the calculated parameters represent the main determinants of absorption and distribution profiles.

Table IV

The molecular descriptors of compounds **1-18**

No	logP	MP	MR	PSA	SAS	RBC	MV	MaxPA	MinPA	HBD	HBA
1	4.27	43.73	115.09	87.3	541	5	352.44	112.18	65.71	3	3
2	4.88	45.66	119.895	87.3	557.52	5	366.42	116.95	66.3	3	3
3	5.04	46.61	122.713	87.3	561.58	5	370.73	118.42	67.9	3	3
4	5.31	42.69	110.466	61.77	506.41	3	333.03	97.79	54.64	1	3
5	5.91	44.61	115.271	61.77	522.49	3	346.85	102.15	55.47	1	3
6	6.07	45.54	118.089	61.77	526.63	3	351.17	103.23	55.42	1	3
7	5.55	56.58	165.571	81.92	685.99	7	440.96	121.34	70.55	0	5
8	6.07	58.48	170.376	81.92	702.11	7	454.71	124.59	71.84	0	5
9	6.34	59.36	173.193	81.92	706.26	7	459.04	125.92	72.3	0	5
10	5.69	56.27	165.787	81.92	692.9	7	445.76	123.52	69.56	0	5
11	6.21	58.19	170.592	81.92	709.26	7	459.64	126.71	72.3	0	5
12	6.48	59.11	173.41	81.92	713.39	7	463.93	128.02	72.26	0	5
13	5.14	41.68	118.415	71.95	516.39	5	333.94	110.45	52.01	0	5
14	5.74	43.61	132.22	71.95	532.76	5	347.8	114.7	58.52	0	5
15	5.91	44.57	126.038	71.95	536.98	5	352.12	117.3	59.97	0	5
16	4.34	39.89	114.01	85.09	509.2	5	323.79	106.75	56.36	0	5
17	4.95	41.83	118.814	85.09	525.74	5	337.63	112.02	56.08	0	5
18	5.11	42.79	121.633	85.09	529.55	5	341.83	113.26	57.18	0	5

logP = n-octanol / water partition coefficient; **MP =** Molecular polarizability; **MR** = Molecular refractivity; PSA = Polar surface area (A^2); **SAS** = Solvent accessible surface area (A^2); **RBC** = Rotatable bond count; **MV** = Volume (cm^3/mol); **MaxPA =** Maximal projection area (A^2); **MinPA =** Minimal projection area (A^2); **HBD =** number of hydrogen bond donors; **HBA** = number of hydrogen bond acceptors

Conclusions

Analysing the results it can be observed that the triazole-3-thiones **4-6** and in most cases the key intermediates from hydrazinecarbothioamides class **1-3** had the best antibacterial activity. This behaviour could be explained by the presence of the thiourea group –NH-CS-N<, a recognized pharmacophore centre. The presence of this group can generate extended delocalization of the electrons by conjugation effects and by creating a triazolic aromatic system. The dissolution of the thione group in the case of S-alkylated triazoles **7-9** could explain the decrease of the antibacterial activity in this case. For the S-alkylated triazoles **11** and **12**, the increase of activity (against *A. baumannii*) can be explained by the presence of another fluorine atom introduced on the phenacyl fragment. The substitution of the nitrogen atom from triazoles with the sulfur atom in thiadiazoles led to a more significant decrease of antimicrobial activity than in the case of oxadiazoles where the sulfur atom from thiadiazoles was replaced with an oxygen atom. It can be stated that in general the antimicrobial activity of the tested heterocyclic compounds decreased in the following order: triazoles > oxadiazoles > thiadiazoles. The presence of the bromine atom and especially of chlorine atom on the diphenylsulphone fragment improved the antimicrobial activity in most cases. The triazoles **4-6** and hydrazinecarbothioamide **2** have been remarked for the best antibacterial activity against some tested bacteria, while oxadiazole **17** for the antifungal activity against *C. parapsilosis.*

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Chemicalize.org was used for name to structure generation/prediction of molecular descriptors on November 28th, 2011, chemicalize.org and ChemAxon (http://www.chemaxon.com).

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