ANTIMICROBIAL EVALUATION OF SOME HYDRAZONE DERIVATIVES

Jankulovska, M.S.[1*], Dimova, V.[2], Doneva-Sapceska, D.[2]

[1]Ss. Cyril and Methodius University in Skopje, Faculty of Agricultural Sciences and Food-Skopje, Republic of Macedonia
[2]Ss Cyril and Methodius University in Skopje, Faculty of Technology and Metallurgy, Republic of Macedonia

[*]Corresponding author: jankulovska_m@yahoo.com

Abstract
Hydrazone derivatives represent one of the most active classes of compounds possessing a broad spectrum of biological activity. The use of the hydrazones is due to their anti-inflammatory, antimicrobial, antidepressant, antitumoral, analgesic, antiplatelet, anticonvulsant, antischistosomiasis and antiviral activity. Due to their physiological activity, they are also used in agriculture as herbicides, insecticides, fungicides and plant growth regulators. Furthermore, hydrazone derivatives possessing an *azomethine proton* (-NH-N=CH-) constitute a significant class of compounds for new drug development in order to synthesize effective agents against microbial activity. Considering these applications some *p*-substituted aromatic hydrazones were previously synthesized and characterized. In this study a series of aromatic hydrazones were evaluated for their *in vitro* growth and inhibitory activity against *Bacillus subtilis, Aspergillus niger* and *Candida utilis,* using filter paper disc method. Stock solutions of compounds were prepared in DMSO, as inert medium in three different concentration levels: 1, 5 and 10 mg/mL. A control disc using DMSO without any test compound was included and there was no inhibitory activity in those disks. The diameter of zone of inhibition (mm) was measured. Every test was done in triplicate to confirm the findings. The screening results indicate that not all investigated compounds exhibited antimicrobial activities. It can be noted that compounds with N-*p*-methoxy substitute group showed the greatest inhibitory effect against *Bacillus subtilis* (max zone of inhibition of 14.3 mm) and *Candida utilis* (max zone of inhibition of 16 mm). All investigated hydrazones showed no inhibitory effects against *Aspergillus niger*.

Keywords: hydrazones, antimicrobial activity, *Bacillus subtilis*, *Aspergillus niger*, *Candida utilis.*

Introduction

Hydrazones are special group of compounds in the Schiff bases family. The presence of two inter-linked nitrogen atoms (-C=N-N-C=O) constitute a significant

class of compounds for new drug development (Eissa, 2015). These compounds possess diverse biological and pharmacological properties such as antimicrobial, anti-inflammatory, analgesic, antifungal, anti-tubercular, antiviral, anticancer, antiplatelet, antimalarial, anticonvulsant, cardio protective, antiprotozoal, antischistosomiasis etc. (Khan, 2008). Hydrazones contain C = N bond, which is conjugated with a lone pair of electrons of the functional nitrogen atom. The nitrogen atoms of the hydrazones are nucleophilic and the carbon atom has both electrophilic and nucleophilic nature. The combination of hydrazones with other functional group leads to compounds with unique physical and chemical character. Owing to their biological and pharmacological properties, they are considered important for the synthesis of heterocyclic compounds (Verma *et al*., 2014). As biologically active compounds, hydrazones find applications in the treatment of diseases such anti-tumor, tuberculosis, leprosy and mental disorder (Richardson and Bernhardt, 1999; Yadawe and Patil, 1997). For example, tuberculostatic activity is attributed to the formation of stable chelates with transition metals present in the cell. Thus many vital enzymatic reactions catalyzed by these transition metals cannot take place in the presence of hydrazones (Darnell and Richardson, 1999; Murukan and Mohanan, 2007). Hydrazone Schiff bases of acyl, aroyl and heteroacroyl compounds have additional donor sites like C=O. The additional donor sites make them more flexible and versatile. This versatility has made hydrazones good polydentate chelating agents that can form a variety of complexes with various transition and inner transition metals and have attracted the attention of many researchers. Hence, hydrazones can be used in analytical chemistry as analytical reagents (Sclafani *et al*., 1996; Corhnelissen *et al*., 1992). Hydrazones also act as herbicides, insecticides, nematicides, rodenticides, plant growth regulators growth regulators (Liu *et al*., 2010).

The plant pathogenic fungus causes devastating disease in agriculture. The pathogenic fungus is responsible for billions of dollars in economic losses worldwide each year. In order to discover new fungicidal molecule with good fungicidal activity the active sub-structure of hydrazone and pyrazole amide derivatives was combined together in order to synthesize novel pyrazole amide derivatives containing a hydrazone moiety (Wu *et al*., 2012). Fungal infections are generally observed as superficial or systemic infections in humans, animals, as well as in plants. The development of antifungal agents has surpassed the development of antibacterials. A novel hydrazine derivative was developed and evaluated for *in-vitro* anti-*Candidal* activity (Secci *et al*., 2012). Virus is a small infectious agent, which can replicate only inside the living cell of an organism. It infects all types of organisms-humans, animals and plants. In the literature there are a lot of results obtained testing antiviral activity of hydrazone derivatives (El-Sabbagh and Rady, 2009; Ortiz *et al*., 2016; Backes *et al*., 2014).

Taking into consideration the relation between the structure and biological activity the aim of this work was evaluation of antimicrobial activity of some newly synthesized *p*-substituted aromatic hydrazones with differences in the structure

against *Bacillus subtilis, Candida utilis,* and *Aspergillus niger* using filter paper disc method.

Material and methods

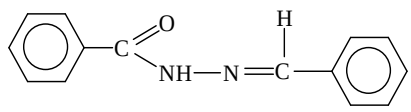
Structure of investigated p-substituted aromatic hydrazones (H_1 - H_{15})

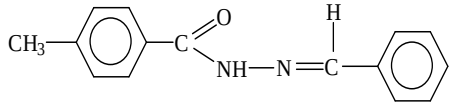
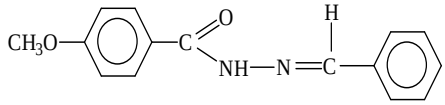
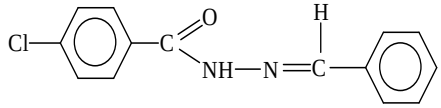
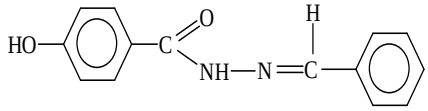
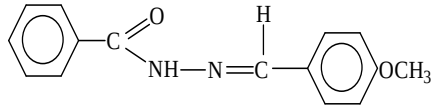
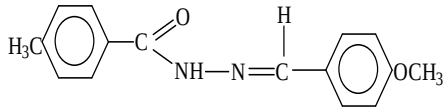
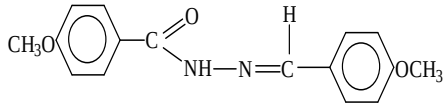
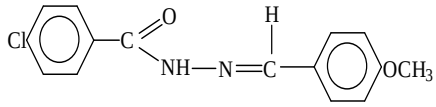
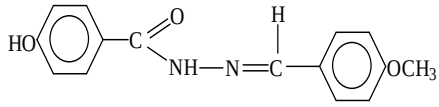
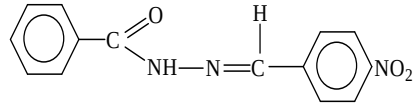
The hydrazones were prepared by condensation of the *p*-substituted hydrazides with benzaldehyde, *p*-methyl benzaldehyde and *p*-metoxy benzaldehyde (Jankulovska *et al*., 2012). The structure of synthesized hydrazones was confirmed by different techniques such as: elemental analysis, UV, IR, ^{1}H NMR, ^{13}C NMR spectra and elemental analysis (Jankulovska *et al*., 2012). The structural formulas, molecular formulas and molecular weights of the synthesized hydrazones are given in Table 1.

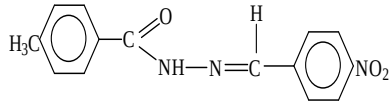
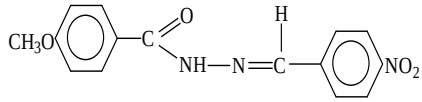
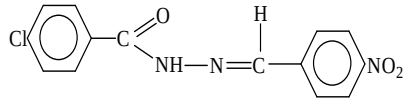
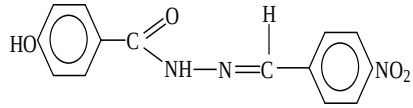
Antimicrobial activity

The antimicrobial activity of investigated compounds were screened against gram positive bacteria *Bacillus subtilis* and two fungal strains, yeast *Candida utilis* and mould *Aspergillus niger* from own microbial collection. Antibacterial activity of the test compounds was assayed by spread plate method using nutrient agar medium and for antifungal activity Sabouraud dextrose agar. These agar media were inoculated with 0.5 mL cells suspension. Inocula were prepared by picking and suspending of colonies in 5 ml of a solution containing 0.145 mol of saline per liter. Bacterial culture was 24 hours old and grown at 37^{o}C on nutrient slant, while fungal species *Candida utilis* and *Aspergillus niger* were 48 hours and 72 hours old, respectively. Both were grown at 28^{o}C on Sabouraud dextrose slants. Filter paper discs (5 mm diameter) saturated with each compound solution (1 mg/mL, 5 mg/mL and 10 mg/mL in DMSO) were placed on the indicated agar mediums. Discs with dimethyl sulfoxide (DMSO) were used as control. Antimicrobial activity was determined by measuring the diameter of the zone showing growth inhibition (mm) after appropriate incubation of plates (Leboffe and Pierce, 2008). The tests were repeated 3 times to confirm the findings.

Table 1. Structural formula, molecular formulas and molecular weights of investigated *p*-substituted hydrazones

Group	Compound	Structural formula	Mol. formula/ Mol. weights [g/mol]
I	H_1		$C_{14}H_{12}ON_2$ 224

	H_2		$C_{15}H_{14}N_2O$ 238
	H_3		$C_{15}H_{14}O_2N_2$ 254
	H_4		$C_{14}H_{11}ON_2Cl$ 258
	H_5		$C_{14}H_{12}O_2N_2$ 240
II	H_6		$C_{15}H_{14}O_2N_2$ 254
	H_7		$C_{16}H_{16}O_2N_2$ 268
	H_8		$C_{16}H_{16}O_3N_2$ 284
	H_9		$C_{15}H_{13}O_2N_2Cl$ 288
	H_{10}		$C_{15}H_{14}O_3N_2$ 260
III	H_{11}		$C_{14}H_{11}O_3N_3$ 269

	H_{12}		$C_{15}H_{13}O_3N_3$ 283
	H_{13}		$C_{15}H_{13}O_4N_3$ 299
	H_{14}		$C_{14}H_{10}O_3N_3Cl$ 303
	H_{15}		$C_{14}H_{11}O_4N_3$ 285

Results and discussion

The dramatically rising prevalence of multi-drug resistant microbial infections in the past few decades has become a serious health care problem. The search for new antimicrobial agents will consequently always remain as an important and challenging task for medicinal and organic chemists. Hydrazones, related to ketones and aldehydes belong to a class of organic compounds with the structure, -NH-N=CH-. These compounds contain N = C bond, which is conjugated with a lone pair of electrons of the functional nitrogen atom. The nitrogen atoms of the hydrazones are nucleophilic and the carbon atom has both electrophilic and nucleophilic nature (Verma *et al.*, 2014). The combination of hydrazones unit with other functional group/atom leads to compounds with unique physical and chemical character. According the literature those compounds possess diverse biological and pharmacological properties (Khan, 2008).

In an urge to develop new antimicrobial compound, a number of hydrazones were tested for their antimicrobial activities because of the evolution of drug-resistant microbial pathogens (Singh and Raghav, 2011). Rollas *et al*. synthesized a series of hydrazones as potential antimicrobial agents and tested these compounds for their antibacterial and antifungal activities against *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans* (Rollas *et al*., 2007). A compound with halogen substituent showed equal activity as ceftriaxone against *S. aureus*. Küçükgüzel *et al*. synthesized di-halo - hydroxy substituted derivatives, which shown activity against *S. epidermis* HE-5 and *S. aureus* HE-9 at 18.75 µg/mL and 37.5 µg/mL, respectively (Rollas et al., 2007). The results from all these investigations showed that the biological and pharmacological activity is clearly linked with the structure of the organic compounds. Hence, the goal of this research was to evaluate the biological activity

of some newly synthesized *p*-substituted hydrazones (H_1-H_{15}) and to assess the relationships between the antimicrobial activity and the structure of these synthesized compounds. The influence of different substituents was also investigated and discussed. Investigated *p*-substituted aromatic hydrazones were divided in three groups according the presence or absence of substituent on the second benzoic ring: Group I –H; Group II $–CH_3O$ and Group III $–NO_2$ group (H_1 - H_{15}, Table 1)**.** In our work, *in vitro* antimicrobial activity of hydrazones was investigated against *Bacillus subtilis*, *Candida utilis* and *Aspergillus niger*.

a) Antimicrobial activity against *Bacillus subtilis*

The results of antibacterial tests against *Bacillus subtilis* indicated that all hydrazones exhibited activities in all three concentration levels (Table 2). Compounds H_1, H_5, H_{12} and H_{13} were the most active in the smallest concentration (1 mg/mL), while compounds H_2, H_3, H_4, H_7, H_8, H_9 and H_{14} were most active in the highest concentration of 10 mg/mL. The most active compounds with 14.3 mm zone of inhibition was compound H_6 which is compound without substituent in the first benzoic ring and methoxy group, a strongly activation group in *p*-position in the second benzoic ring. The lowest zone of inhibition of 8.3 mm was obtained in the most concentrated solution of compound H_5 (*p*-hydroxy substituted hydrazone). According to the results of Singh and Raghav's investigation, the synthesized hydrazones possessed activity against methicillin-resistant *Staphylococcus aureus* strain probably due to the presence of carbonyl region and hydroxyl group in their structure (Singh and Raghav, 2011).

Table 2. Antimicrobial activity of investigated three different concentration levels of *p*-substituted hydrazones (H_1 - H_{15}) against *Bacillus subtilis*

Compound	Zone of growth inhibition (mm)		
	1 [mg/mL]	5 [mg/mL]	10 [mg/mL]
H_1	11.0	10.3	9.0
H_2	9.3	11.0	12.0
H_3	11.0	10.0	13.0
H_4	10.3	11.6	13.3
H_5	9.6	9.0	8.3
H_6	9.3	14.3	9.7
H_7	9.5	9.7	11.7
H_8	9.3	9.0	12.3
H_9	8.7	10.5	13.5
H_{10}	9.0	10.2	9.2
H_{11}	11.2	12.0	10.7
H_{12}	12.0	10.8	11.0
H_{13}	11.5	11.0	10.3
H_{14}	10.3	10.0	11.0
H_{15}	10.7	11.0	10.3

b) Antimicrobial activity against *Candida utilis*

Results obtained from the antifungal activity of *p*-substituted hydrazones (H_1 - H_{15}) against *Candida utilis* are presented in Table 3. It should be noted that all compounds of Group III, compounds (H_{11} - H_{15}) with *p*-substituted $-NO_2$ group in the second benzoic ring, did not inhibit the growth of *Candida utilis.*

The results also demonstrated that only two compounds form Group I were active in all three concentration levels (hydrazones H_4 and H_5). This could be explained by the presence of -*ortho* and -*para* directing: deactivator atom (chlorine atom in H_4) and activator group (hydroxyl group in H_5) in the *p*-position in the first benzene nucleus. The rest of compounds of this Group (H_1, H_2 and H_3) were inactive in smallest concentration. This can be explained by the fact that compounds which were bearing highly electronegative -chloro and -fluoro substituents at the -*para* position of phenyl ring exhibited good activity as compared to those compounds having these atoms at either -*ortho* or -*meta* position or the other compounds containing the less electronegative/electropositive substituent at these positions (Singh and Raghav, 2011). Hydrazones H_6 and H_7 were active only in the smallest concentration (1 mg/mL) with 7.0 and 7.5 mm zone of inhibition, respectively. The most active compounds with 16.0 mm zone of inhibition was hydrazone H_8 which has the same type of substituent ($-CH_3O$) in the both aromatic rings. Probably as a result of the opposite direction of action of two different types of substituents in compounds H_9 ($-Cl$ and $-CH_3O$), antimicrobial activity against *Candida utilis* was the lowest with 5.0 mm zone of inhibition in the most concentrated solution of 10 mg/mL.

Table 3. Antimicrobial activity of three different concentrations of *p*-substituted hydrazones (H_1 - H_{15}) against *Candida utilis*

Compound	Zone of inhibitions (mm)		
	1 [mg/mL]	5 [mg/mL]	10 [mg/mL]
H_1	-	8.0	9.6
H_2	-	-	8.0
H_3	-	7.0	8.0
H_4	7.0	9.0	15
H_5	7.6	7.6	13.6
H_6	7.0	-	-
H_7	7.5	-	-
H_8	-	-	16.0
H_9	-	-	5.0
H_{10}	-	7.0	6.33
H_{11}	-	-	-
H_{12}	-	-	-
H_{13}	-	-	-
H_{14}	-	-	-
H_{15}	-	-	-

c) Antimicrobial activity against *Aspergillus niger*
Using the filter paper disc method none of investigated hydrazones did not inhibit the growth of *Aspergillus niger*.

Conclusions

In vitro antimicrobial activity of some newly synthesized *p*-substituted hydrazones was investigated against *Bacillus subtilis*, *Candida utilis* and *Aspergillus niger* using the filter paper disc method. The obtained data indicated that the zone of inhibition depends on the structure of investigated compounds (Wu *et al*., 2012). The results of this investigation demonstrated that compounds with N-*p*-methoxy substitute group ($-CH_3O$) showed the greatest inhibitory effect against *Bacillus subtilis* and *Candida utilis*, while all investigated *p*-substituted hydrazones did not demonstrated antifungal activity against *Aspergillus niger*.

References

Backes, G. L., Neumann, D. M., Jursic, B. (2014). Synthesis and antifungal activity of substituted salicylaldehyde hydrazones, hydrazides and sulfohydrazides. Bioorganic & Medicinal Chemistry, 22(17): 4629-4636.

Corhnelissen, J. P., Van Diemen, J. H., Groeneveld, L. R,, Haasnoot, J. G., Spek, A. L. (1992). Synthesis of Co(II), Ni(II) and Cu(II) Complexes from Schiff base. Inorganic Chemistry, 31: 198-202.

Darnell, G., Richardson, D. R. (1999). The potential of iron chelators of the pyridoxalisonicotinoyl hydrazone class as effective antiproliferative agents III: the effect of the ligands on molecular targets involved in proliferation. Blood, 94: 781-792.

Eissa, H. H. (2015). Synthesis, Characterization, Anticorrosion Activity and *Antibacterial* Activity of Macrocyclic Schiff Bases Based on 1,3-Dithiocarbonyl Phenyl Dihydrazide, Organic Chemistry: Current Research, 4(4): 151-163.

El-Sabbagh, O. I., Rady, H. M. (2009). Synthesis of new acridines and hydrazones derived from cyclic beta-diketone for cytotoxic and antiviral evaluation. European Journal of Medicinal Chemistry, 44(9): 3680-3686.

El-Sayed, N. N. E., Alafeefy, A. M., Bakht, M. A., Masand, V. H., Aldalbahi A., Chen, N., Fan, C., Bacha, A. B. (2016). Synthesis, Antiphospholipase A2, Antiprotease, Antibacterial Evaluation and Molecular Docking Analysis of Certain Novel Hydrazones. Molecules, 21: 1664.

Jankulovska, M., Colanceska-Ragenovic, K., Dimova, V., Spirevska, I., Makreski, P. (2012). Synthesis and characterization of new *p*-substituted aromatic hydrazones. Organic Chemistry: An Indian Journal, 8(9): 326-334.

Khan, S. A. (2008). Synthesis, characterization and in vitro antibacterial activity of new steroidal 5-en-3-oxazolo and thiazoloquinoxaline. European Journal of Medicinal Chemistry, 43(9): 2040-2044.

Leboffe, M. J., Pierce, B. E. (2008). Microbiology: Laboratory Theory and Application Brief edition. Morton Publishing Company, U.S.A.

Liu, M., Wang, Y., WangYang, W. Z., Liu, F., Cui, Y. L., Duan, Y. S., Wang, M., Liu, S. Z., Rui, C. H. (2010). Design, synthesis, and insecticidal activities of phthalamides containing a hydrazone substructure. Journal of Agriculture and Food Chemistry, 58: 6858–6863.

Murukan, B., Mohanan, K. (2007). Synthesis, characterization and antibacterial properties of some trivalent metal complexes with [(2-hydroxy-1-naphthaldehyde)-3-isatin]-bishydrazone. Journal of Enzyme Inhibition and Medicinal Chemistry, 22(1): 65-70.

Ortiz, S., Nelson, A. R., Kesternich, A. V, Pérez-Fehrmann, A. M., Christenb, A. P., Marcourt, L. B. (2016). Synthesis and antifungal activity of diaryl hydrazones from 2,4-dinitrophenylhydrazine. Journal of Chilean Chemical Society, 61(3): o*n-line:* ISSN 0717-9707.

Richardson, D. R., Bernhardt, P. V. (1999). Crystal and molecular structure of 2-hydroxy-1-naphthaldehyde isonicotinoyl hydrazone (NIH) and its iron(III) complex: an iron chelator with anti-tumour activity. Journal of Biological Inorganic Chemistry, 4(3): 266-273.

Rollas, S., Küçükgüzel, G. Ş. (2007). Biological Activities of Hydrazone Derivatives. Molecules, 12: 1910-1939.

Sclafani, J. A., Maranto, M. T., Sisk, T. M., Van Arman, S. A. (1996). Terminal Alkylation of Linear Polyamines. Journal of Organic Chemistry, 61: 3221-3222.

Secci, D., Bizzarri, B., Bolasco, A., Carradori, S., D'Ascenzio, M., Rivanera, D., Mari, E., Polletta, L., Zicari, A. (2012). Synthesis, anti-Candida activity, and cytotoxicity of new (4-(4-iodophenyl)thiazol-2-yl)hydrazine derivatives. European Journal of Medicinal Chemistry, 53: 246-253.

Singh, M., Raghav, N. (2011). Biological activities of hydrazones: A review. International Journal of Pharmacy and Pharmaceutical Sciences, 3(4): 26-32.

Verma, G., Marella, A., Shaquiquzzaman, M., Akhtar, M., Ali, M. R., Alam, M. M. (2014). A review exploring biological activities of hydrazones. Journal of Pharmacy and Bioallied Science, 6(2): 69–80.

Wu, J., Wang, J., Hu, D., He, M., Jin, L., Song, B. (2012). Synthesis and antifungal activity of novel pyrazolecarboxamide derivatives containing a hydrazone moiety. Chemistry Central Journal, 6: 51.

Yadawe, M. S., Patil, S. A. (1997). Synthesis, characterization and biological studies of cobalt(II) and nickel(II) complexes with new Schiff bases. Transition Metal Chemistry, 22(3): 220-224.