The Antimicrobial Acitvity and Quantitative Structure - Biological Activity Relationships Evaluation of Some Novel 2-Hydroxybenzamide Derivatives

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Based on our studies of the biological activity of 2-hydroxybenzamide derivatives, we evaluated the antimicrobial activity of some novel compounds from this class (esters, hydrazides, hydrazones), against bacterial (Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus) and fungical (Sacharomyces cerevisiae, Candida albicans) strains using the disc-diffusion method under standard conditions. It was found that all tested compounds present a good activity against the tested microorganisms and they were more active against Candida albicans. The established minimum inhibitory concentration (MIC) values were ranged between 10^{-2}- $3.6 \cdot 10^{-4}$ mol/L and were used to obtain some quantitative structure-biological activity relationships using HyperChem 5.01 program for molecular modelling and conformational analysis. The established equations using different parameters present correlation coefficients statistically ensured.

Key words: 2-hydroxybenzamide, esters, hydrazides, hydrazones, antimicrobial activity, minimum inhibitory concentration (MIC), quantitative structure-activity relationships (QSAR)

2-Hydroxybenzamide and its derivatives have a long history as antimicrobial agents. It was found that 2-hydroxybenzanilides, as well as *O*-substituted 2-hydroxybenzanilides, are a class of compounds with a large spectrum of biological activity [1, 2, 3]. Synthesis of 2-hydroxybenzamides fused to another halogen-substituted aromatic ring has attracted wide spread attention due to their diverse application as antibacterial-, antiviral-, anti-inflammatory and analgesic agents. Salicylamide-*O*-acetic hydrazide and its hydrazones show anti-inflammatory and analgesic activity superior to salicylamide itself and lower ulcerogenic activity [4,5]. Some *ortho*-substituted phenoxyalkanoic acids and their derivatives were synthesized [6,7] and tested for their antimicrobial activity [8]. Therefore the search for new antimicrobial active compounds represents one of the most important directions of current medicinal chemistry.

The goal of this research was to evaluate the biological activity of some new synthesized compounds, 2-hydroxybenzamide derivatives [9, 10], and to assess the relationships between the antimicrobial activity and the structure of these synthesized compounds. The influence of different substituents was also investigated.

Experimental part

Microbiology

Antimicrobial activity of compounds was tested *in vitro* against *Escherichia coli* (E.c.), *Staphylococcus aureus* (S.a.), *Bacillus subtilis* (B.s.), *Bacillus cereus* (B.c.), *Sacharomyces cerevisiae* (S.c.), *Candida albicans* (C.a.) using a specific nutrient medium (Sabouraud for yeasts and gelose for bacterial strains). The testing was performed in sterile, flat-bottom, Petri dishes, using the disc-diffusion method under standard conditions as described by NCCLS. The general structure of the tested compounds is presented in scheme 1. The compounds (**1-15**) were solved in dimethyl-sulphoxide to a concentration of 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.156, 0.078, 0.039 mmol/l. The minimum inhibitory concentration (MIC) was established visually as the lowest concentration that showed growth inhibition. The MIC values were read after 1, 2, 3 and 5 days of incubation.

Quantitative structure-activity relationships (QSAR)

Molecular modeling was achieved using HyperChem 5.01 for Windows software (MM+ program) and conformational analysis was established with Conformational Search program included in the same software package.

Results and discussions

The microbiological evaluation results are presented in table 2.

MICs values remain unchanged after the first two days of incubation.

Analizing the MIC values obtained for the tested compounds, it can be observed that the substitution of the aniline aromatic ring from salicylanilide, with 2-CF_3, provides compounds with bigger biological activity than the unsubstitued ones. Only in case of *Candida albicans*, the unsubstitued aniline aromatic ring possesses higher biological effect. The 3-CF_3 substitution of the aniline aromatic ring gives compounds more active against *Bacillus cereus*, *Escherichia coli*, *Staphilococcus aureus*, the rest of the microorganisms were easier inhibited by the unsubstitued salicylanilide. Comparing the activities obtained in case of 2-CF_3, respectively 3-CF_3, substituted aniline aromatic ring, it can be observed that the 2-CF_3-subtituted derivatives present a bigger activity against

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Table 1
THE TESTED COMPOUNDS

No.	Compound name	R_1	R_2	R_3
1	2-Hydroxy-*N*-phenyl-benzamide (**M**)	H	H	H
2	2-Hydroxy-*N*-(2-trifluoromethyl-phenyl)-benzamide	H	o-CF_3	H
3	[2-(2-Trifluoromethyl-phenylcarbamoyl)-phenoxy]-acetic acid ethyl ester	H	o-CF_3	$CH_2COOC_2H_5$
4	2-Hydrazinocarbonylmethoxy-*N*-(2-trifluoromethyl-phenyl)-benzamide	H	o-CF_3	$CH_2CONHNH_2$
5	2-(4-Chloro-benzylidene-hydrazinocarbonylmethoxy)-*N*-(2-trifluoromethyl-phenyl)-benzamide	H	o-CF_3	$CH_2CONHN{=}CH(4\text{-}Cl)C_6H_4$
6	2-(2-Chloro-benzylidene-hydrazinocarbonylmethoxy)-*N*-(2-trifluoromethyl-phenyl)-benzamide	H	o-CF_3	$CH_2CONHN{=}CH(2\text{-}Cl)C_6H_4$
7	2-Hydroxy-*N*-(3-trifluoromethyl-phenyl)-benzamide	H	m-CF_3	H
8	[2-(3-Trifluoromethyl-phenylcarbamoyl)-phenoxy]-acetic acid ethyl ester	H	m-CF_3	$CH_2COOC_2H_5$
9	2-Hydrazinocarbonylmethoxy-*N*-(3-trifluoromethyl-phenyl)-benzamide	H	m-CF_3	$CH_2CONHNH_2$
10	2-(4-Chloro-benzylidene-hydrazinocarbonylmethoxy)-*N*-(3-trifluoromethyl-phenyl)-benzamide	H	m-CF_3	$CH_2CONHN{=}CH(4\text{-}Cl)C_6H_4$
11	2-(2-Chloro-benzylidene-hydrazinocarbonylmethoxy)-*N*-(3-trifluoromethyl-phenyl)-benzamide	H	m-CF_3	$CH_2CONHN{=}CH(2\text{-}Cl)C_6H_4$
12	5-Chloro-2-hydroxy-*N*-phenyl-benzamide	5-Cl	H	H
13	(4-Chloro-2-phenylcarbamoyl-phenoxy)-acetic acid ethyl ester	5-Cl	H	$CH_2COOC_2H_5$
14	5-Chloro-2-(4-chloro-benzylidene-hydrazinocarbonylmethoxy)-*N*-phenyl-benzamide	5-Cl	H	$CH_2CONHN{=}CH(4\text{-}Cl)C_6H_4$
15	5-Chloro-2-(2-chloro-benzylidene-hydrazinocarbonylmethoxy)-*N*-phenyl-benzamide	5-Cl	H	$CH_2CONHN{=}CH(2\text{-}Cl)C_6H_4$

Table 2
THE MINIMUM INHIBITORY CONCENTRATION (MIC) VALUES

	MIC (mmol/l)					
	B.s.	**B.c.**	**E.c.**	**S.a.**	**S.c.**	**C.a.**
No.	**24h/48h**	**24h/48h**	**24h/48h**	**24h/48h**	**24h/48h**	**24h/48h**
1(M)	10/10	2,5/5	10/10	10/10	5/5	0.3125/0.3125
2	5/10	0.625/1.25	5/5	2.5/2.5	1.25/1.25	1.25/1.25
3	10/10	1.25/5	10/10	0.3125/0.3125	10/10	5/5
4	5/5	10/10	5/5	10/10	1.25/2.5	0.3125/0.625
5	5/5	5/5	10/10	10/10	5/10	10/10
6	5/5	2.5/2.5	10/10	10/10	5/5	5/5
7	5/10	2.5/5	5/5	0.3125/0.625	10/10	0.3125/0.3125
8	10/10	2.5/10	10/10	10/10	10/10	0.3125/0.625
9	2.5/5	5/5	10/10	1.25/1.25	0.625/2.5	0.625/1.25
10	10/10	2.5/2.5	10/10	10/10	5/10	0.3125/0.3125
11	5/5	1.25/2.5	10/10	5/5	10/10	2.5/5
12	1.25/1.25	2.5/2.5	1.25/1.25	2.5/5	2.5/5	0.3125/0.3125
13	10/10	1.25/2.5	10/10	10/10	10/10	5/10
14	10/10	2.5/5	10/10	10/10	10/10	0.3125/0.3125
15	10/10	1.25/1.25	10/10	2.5/2.5	5/10	5/5

1(M)-salicylanilide-control substance

Escherichia coli °i *Sacharomyces cerevisiae,* meantime the 3-CF_3-subtituted derivatives are more active against *Staphilococcus aureus, Candida albicans*. The 5-chloro-2-hydroxy-*N*-phenyl-benzamide derivatives possess higher biological effect than salicylanilide, only *Sacharomyces cerevisiae* and *Candida albicans* species were easier inhibited by the salicylanilide itself. The MIC values obtained for ethyl esters were comparable to those for salicylanilide, but the hydrazides and hydrazones are more active comparatively to the control substance.

In order to establish the QSAR equations, the logarithm of the reversed MIC has been used.

Using minimum energy conformations the significant structural parameters were calculated [11] which are presented in table 3.

Table 3
THE SIGNIFICANT STRUCTURAL PARAMETERS FOR ANTIMICROBIAL ACTIVITY

Cod	Description
SIC1	structural information content (neighborhood symmetry of 1-order)
ASP	asphericity
Mor25m	3D-MoRSE - signal 25 / weighted by atomic masses
Mor27e	3D-MoRSE - signal 27 / weighted by atomic Sanderson electronegativities
Mor31e	3D-MoRSE - signal 31 / weighted by atomic Sanderson electronegativities
Km	K global shape index / weighted by atomic masses
HATS8v	leverage-weighted autocorrelation of lag 8 / weighted by atomic van der Waals volumes
R3v+	R maximal autocorrelation of lag 3 / weighted by atomic van der Waals volumes
R4v+	R maximal autocorrelation of lag 4 / weighted by atomic van der Waals volumes
nN-N	number of N hydrazines (aliphatic)
H-050	H attached to heteroatom

There have been obtained several equations for each strain, but we present here only the most statistically significant ones. We have established two types of equations: mono- and multiparametrial (equations 1-9).

B.s. $\hat{pA_i} = 0.42(\pm0.34) + 26.2(\pm4.85)\cdot(R3v+)_i$ (1)

n=15; r=0.831; s=0.15; F=29

B.c. $\hat{pA_i} = 2.68(\pm0.057) + 1.15(\pm0.31)\cdot(Mor31e)_i$ (2)

n=15; r=0.720; s=0.21; F=14

E.c. $\hat{pA_i} = 1.0(\pm0.26) + 7.36(\pm1.70)\cdot(HATS8v)_i$ (3)

n=15; r=0.770; s=0.17; F=19

$\hat{pA_i} = -1.28(\pm0.51) + 4.26(\pm0.89)\cdot(SIC1)_i + 6.03(\pm1.08)\cdot(HATS8v)_i$ (4)

n=15; r=0.927; s=0.1; F=36

S.a. $\hat{pA_i} = 0.12(\pm0.6) + 41.1(\pm11.1)\cdot(R4v+)_i$ (5)

n=15; r=0.720; s=0.39; F=14

S.c. $\hat{pA_i} = 2.75(\pm0.21) + 1.08(\pm0.4)\cdot(Mor27e)_i + 0.56(\pm0.2)\cdot(nN-N)_i$ (6)

n=15; r=0.845; s=0.23; F=15

$\hat{pA_i} = 2.23(\pm0.3) + 0.92(\pm0.39)\cdot(Mor27e)_i + 0.26(\pm0.08)\cdot(H-050)_i$ (7)

n=15; r=0.867; s=0.21; F=18

C.a. $\hat{pA_i} = 1.6(\pm0.29) + 2.61(\pm0.53)\cdot(Km)_i$ (8)

n=15; r=0.810; s=0.37; F=24

$\hat{pA_i} = 3.16(\pm0.93) + 1.5(\pm1.0)\cdot(ASP)_i - 0.8(\pm0.74)\cdot(Mor25m)_i$ (9)

n=15; r=0.796; s=0.39; F=10

Conclusions

Many of the tested compounds present good activities against the microorganisms, especially against *Candida albicans*, *Bacillus cereus*, *Staphilococcus aureus* and *Sacharomyces cerevisiae*.

Generally, the presence of groups like CF_3, Cl, in different positions of the basic structure (salicylanilide) increases the antimicrobial activity, proved by the decrease of MICs. Activity of ethyl esters was generally unchanged comparatively to salicylanilide. Conversion into hydrazides and hydrazones leads to a decreasing of MIC values.

Some equations were statistically significant, but the parameters included in these relations are not the most relevant ones for establishing the substituents contribution to the antimicrobial effect.

The new compounds may constitute a potential antimicrobial group. Their structure-activity relationships varied for different strains.

Abreviations

B.s. - *Bacillus subtilis*
B.c. - *Bacillus cereus*
E.c. - *Escherichia coli*
S.a. - *Staphylococcus aureus*
S.c. - *Sacharomyces cerevisiae*
C.a. - *Candida albicans*
MIC - minimum inhibitory concentration
QSAR - quantitative structure-activity relationships

Acknowledgement: Authors are grateful to Professor Mircea Mracec, Ph.D. ("Coriolan Drãgulescu" Institute of Chemistry, Timiºoara) for permission to use the HyperChem molecular modeling package.

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Manuscript received: 24.09.2007