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PRACTICAL APPLICATION OF QSAR TECHNIQUE FOR PREDICTION OF BIOLOGICAL ACTIVITY OF SELECTED HYDRAZONES

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Abstract
One important class of organic compounds is hydrazones which find huge application in many scientific areas. They demonstrate *fascinating biological activities* like as antioxidant, anti-inflammatory, anticonvulsants, antidepressant, anxiolytic, antihypertensive, anticancer, antimicrobial, anti-tuberculosis, and antifungal activity. This wide palette of the useful medical properties has attracted considerable scientific interest for their synthesis. The biological activity and the changes in this activity depend on the substituents present in the hydrazone molecule. The hydrazones can be used in agriculture as herbicides, insecticides, and plant growth stimulants because of their physiological properties. A series of substituted aromatic hydrazones have been synthesized and evaluated for *in vitro* antimicrobial activity against: *Bacillus subtilis*. QSAR study was performed to estimate the quantitative effects of the selected descriptors of derivatives on their antibacterial activity. Topological and physicochemical descriptors were calculated for each molecule and a several two-parametric mathematical models have been selected for further discussion. The statistical significance of each model was measured by a few cross-validation parameters *(Q, PRESS/SSY;* S_{PRESS}*; PSE* andQ^2*)*. Statistical evaluation of the data used to test the quality of the obtained models, indicated that in statistically significant model both parameters AC (Atom count) and MAPA (Maximal projection area) have opposite input to the modeling of biological activity of the selected hydrazones. Following statistical parameters were obtained for this model: R^2 = 0.9444; Sd = 0.0097; F-test = 101.9875; R^2_{adj} = 0.9352; Q = 100.1858; PRESS/SSY = 0.0564; S_{PRESS} = 0.0098; PSE = 0.0088 and Q^2 = 0.9436.

Keywords: biological activity, *p*-substituted hydrazones, QSAR, statistical evaluation.

Introduction

Quantitative structure/property activity relationships (QSAR/QSPR) represent attempts to correlate activities/properties with structural descriptors of compounds.

Correlation and prediction of physical, chemical and biological activity/property from molecular structure is a very significant and an unsolved problem not only in various chemistry fields (theoretical, computational and environmental) but in life science, as well (Basak *et al*., 1999). The most important step in QSAR/QSPR is numerically transformation of the chemical structures of various molecules. Thus, how to accurately transfer the chemical formula (or molecular graph) into numerical format has been a major task for QSAR/QSPR researches. There are many methods to quantify the molecular structures in which topological index (descriptor) is the most accepted since it can be obtained directly from molecular structures and rapidly computed for large numbers of molecules. The obtained results demonstrated that the topological index is the first effective choice in QSAR research (Basak *et al*., 1999).

Hydrazones are important organic compounds with diverse application in many scientific areas. They possess a wide spectrum of biological activity and the changes in this activity depend on the substituents present in its molecule (Rollas and Küзükgüzel, 2007). The use of the hydrazones in medicine is due to their anticonvulsant, antidepressant, analgesic, anti-inflammatory, antiplatelet, antimicrobial, antitumoral, antischistosomiasis and antiviral activity [3]. Hydrazones are significant compounds for the development of novel drugs because they possess an *azomethine proton* (-NHN=CH-). *Both nitrogen atoms of the hydrazone group are nucleophilic, although while the carbon atom of hydrazone group has both electrophilic and nucleophilic character.* The wide palette of the useful properties has attracted considerable scientific interest for their synthesis (Kaymakçıoğlu, *et al*., 2009). There are a large number of methods known from the literature that have been developed for the synthesis of hydrazones (Ahmed *et al*., 2004; Leite *et al*., 2008). Aromatic hydrazones are also important for a number of synthetically useful transformations of carbonyl compounds and characterization of aldehydes and ketones (Juneja, *et al*., 2008). Some of the hydrazones are used as chelating agents, and their complexes with transition metals are used in various fields, including analytical, clinical and biological (Suarez-Iha *et al*., 1994). Additionally, hydrazones are group of the most useful spectrophotometric reagents (Terra *et al*., 1999). Combining appropriate starting materials such as carbonyl compounds and hydrazine, the sensitivity as analytical reagents could be improved and they could be used as analytical reagents for transition metal analysis and as catalyst for epoxidation of olefins. Hydrazones and their metal complexes exhibit a wide spectrum of physiological and pharmacological activities (Chohan and Sherazi, 1997). Because of their physiological activity, they are used in agriculture as herbicides, insecticides, and plant growth stimulants (Liu*et al*., 2010). Some aromatic hydrazones are DNA gyrase inhibitors (Sridhar *et al*., 2016). Furthermore, the hydrazones are also used in industry as plasticizers, polymer stabilizers, antioxidants, polymerization initiators (Sears and Darby, 2012).

Considering these applications some *p*-substituted aromatic hydrazones were synthesized and characterized (Table 1). The aim of this paper was to introduce the

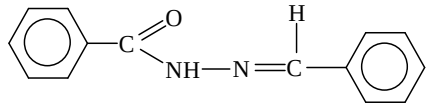
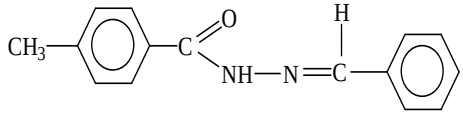
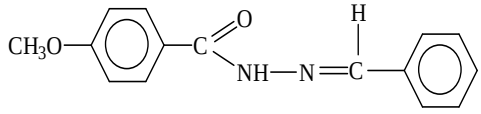
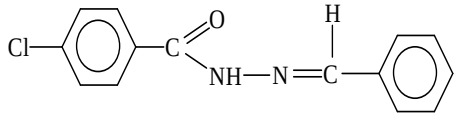
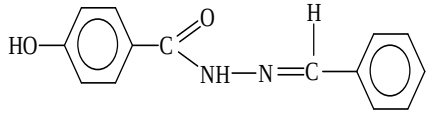
topological indices which are essentially numerical molecular descriptors associated with the molecular structure and to find mathematical equations relating the chemical structure of substituted hydrazones to a variety of their properties. Once a correlation between structure and activity/property is found, any number of compounds, including those not yet synthesized, can be readily screened on the computer in order to select structure with the desired properties. It is then possible to select the most promising compounds, to synthesize and to test in the laboratory.

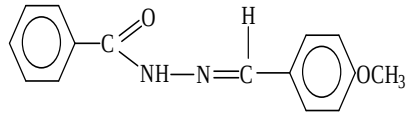
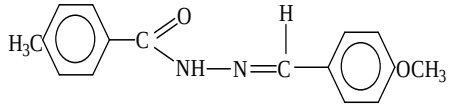
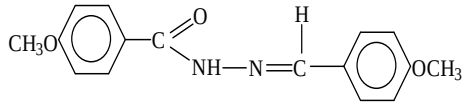
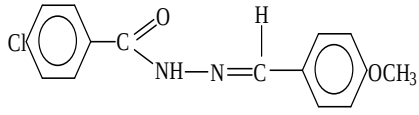
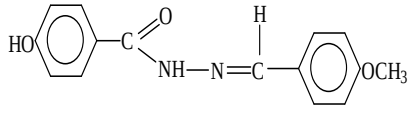
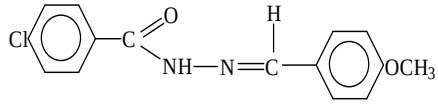
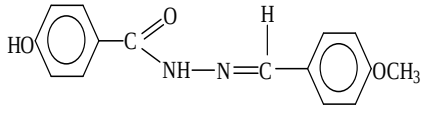
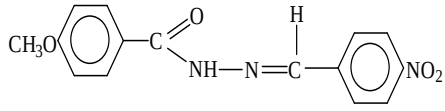
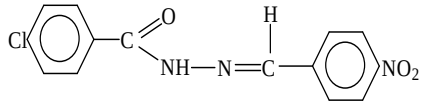
Material and methods

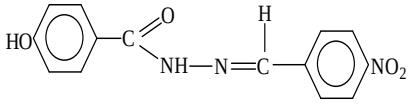
Structure of p-substituted aromatic hydrazones (AH_1-AH_{15})

A three series of *p*-substituted aromatic hydrazones have been synthesized by condensation of benzhydrazide or *p*-substituted benzhydrazides (–CH_3, –OCH_3, –Cl and –OH) with benzaldehyde or *p*-substituted benzaldehyde (–OCH_3 and –NO_2) [14]. The structure of the synthesized hydrazones was confirmed by the following techniques: ^{1}H NMR, ^{13}C NMR, IR, UV and elemental analysis (CNH) (Jankulovska *et al*., 2012). The structural formulas of investigated *p*-substituted aromatic hydrazones (AH_1-AH_{15}) are presented in Table 1. The calculated log $1/c_{MIC}$ values against *Bacillus subtilis* are also listed in the Table 1.

Table 1. Structure of investigated aromatic hydrazones (AH_1-AH_{15}) and calculated log $1/c_{MIC}$ values against *Bacillus subtilis*

Simbol	Structure	log1/c_{MIC}
AH_1		5.3500
AH_2		5.3766
AH_3		5.4048
AH_4		5.4116
AH_5		5.3802

AH_6		5.4048
AH_7		5.4280
AH_8		5.4533
AH_9		5.4594
AH_{10}		5.4149
AH_{11}		5.4297
AH_{12}		5.4517
AH_{13}		5.4756
AH_{14}		5.4814

AH_{15}		5.4548

Descriptor calculation and selection

The 2D structures of all investigated compounds were drawn and topological descriptors were calculated using softer packaging Marvinsketch (Marvin Sketch 6.2.1, www.chemaxon.com). All descriptors calculated were not relevant to the property considered. Therefore only descriptors with adequate values were chosen for analysis. Calculated topological descriptor (abbreviation and meaning) are presented in Table 2.

Table 2. Calculated values of selected topological descriptors

Symbol	AAC	ABC	AC	MAPA	CBC	RBC	Pl	χ	J	H	WW	Sz	W
AH_1	5	6	29	79.03	6	3	44	8.36	1.57	48.49	2179	866	614
AH_2	6	7	32	83.80	7	3	48	8.75	1.49	52.78	2694	1027	724
AH_3	7	8	33	85.30	8	4	50	9.29	1.42	56.86	3337	1206	852
AH_4	6	7	29	83.35	7	3	48	8.75	1.49	52.78	2694	1027	724
AH_5	6	7	30	81.19	7	3	48	8.75	1.49	52.78	2694	1027	724
AH_6	7	8	33	87.82	8	4	50	9.29	1.40	56.76	3388	1212	858
AH_7	8	9	36	93.02	9	4	54	9.69	1.56	61.20	4099	1412	995
AH_8	9	10	37	94.01	10	5	56	10.22	1.49	65.42	4967	1632	1152
AH_9	8	9	33	92.73	9	4	54	9.69	1.56	61.20	4099	1412	995
AH_{10}	8	9	34	90.00	9	4	54	9.69	1.56	61.20	4099	1412	995
AH_{11}	8	9	31	87.91	9	4	54	9.66	1.57	61.30	4061	1396	991
AH_{12}	9	10	34	91.82	10	4	58	10.06	1.50	65.81	4877	1616	1142
AH_{13}	10	11	35	95.82	11	5	60	10.60	1.43	70.10	5865	1857	1314
AH_{14}	9	10	31	92.39	10	4	58	10.06	1.50	65.81	4877	1616	1142
AH_{15}	9	10	32	90.49	10	4	58	10.06	1.50	65.81	4877	1616	1142

Aliphatic atom count (AAC); Aliphatic bond count (ABC); Atom count (AC); Maximal projection area (MAPA); Chain bond count (CBC); Ring bond count (RBC); Platt index (Pl); Randic index (χ); Balaban index (J); Harary index (H); Hyper Wiener index (WW); Szeged index (Sz); Wiener index (W);

Antibacterial Investigation

All investigated hydrazones were tested for their *in vitro* growth inhibitory activity against *Bacillus subtilis* using filter paper disc method. Stock solutions of the compounds were prepared in DMSO as inert medium in three concentration levels: 1, 5 and 10 mg/mL DMSO. A control discs using DMSO without any test

compound were included and there was no inhibitory activity in those disks. The diameter of zone of inhibition (mm) was measured. Inhibitory activity data determined as μg/mL were transformed to the negative logarithms of molar MICs ($\log 1/c_{MIC}$), (Table 1). Every test was done in triplicate in order to confirm the findings.

Statistical analysis

The statistical evaluation of the data was performed using STATISTICA program package (STATISTICA, www.statsoft.com). With the aim to test the quality of the regression models the following statistical parameters were used: Correlation coefficient (R^2), Standard deviation of the estimate (Sd), Fisher test for significance of the equation (F-test), Adjusted R^2(R^2_{adj}), Quality factor (Q), Uncertainty of Prediction (S_{PRESS}), Predictive Square Error (PSE) and Cross-validation squared correlation coefficient (Q^2).

Results and discussion

The development the QSAR models of investigated hydrazones was performed in two steps. In the first step, selected aromatic hydrazones were evaluated for *in vitro* antimicrobial activity against *Bacillus subtilis*. In the second step, efforts were focused on developing the QSAR models of compounds with antibacterial activity against *Bacillus subtilis,* using a set of topological descriptors together with some physicochemical descriptors.

Experimentally obtained inhibitory activity data were first transformed to the negative logarithms of molar MICs ($\log 1/c_{MIC}$), (Table 1) which were used as a dependent variable in the QSAR study. In the present work, selected topological descriptors (Table 2), were used as independent variable in correlation with antibacterial activity ($\log 1/c_{MIC} = \log 1/c_{MICobs.}$).

Two-parametric models where descriptors were not correlated between themselves have been selected for further discussion. In bivariate correlation analysis, applying stepwise multiple linear regression method, 11 QSAR models were obtained. The selection was based on the preliminary statistical quality of the models (*R*, Sd, F-test, R^2_{adj} and p-level). According to preliminary statistical results (R^2, Sd, R^2_{adj} and F-test) it can be noticed that excellent correlation was obtained when in bivariate correlation AC and MAPA were used. This correlation is expressed by the following equation:

$$\log 1/c_{MIC} = 4.8172 - 0.0091 * AC + 0.0102 * MAPA \quad \text{(Eq. 1)}$$

$$R^2 = 0.9444 \quad Sd = 0.0097 \quad \text{F-test} = 101.9875 \quad R^2_{adj} = 0.9352$$

Antimicrobial activity against *B. subtilis* predicted by the Eq. 1 ($\log 1/c_{MICpred.}$) was compared with the corresponding observed values ($\log 1/c_{MICobs.}$) reported in Table 1. A plot between the observed and predicted $\log 1/c_{MIC}$ values is shown in Fig. 1. It can be seen that within experimental error the values agree well. The predictive

correlation coefficient (R_{pre}) also had been calculated using the Eq. 1 The obtained value was 0.944 (Fig. 1).

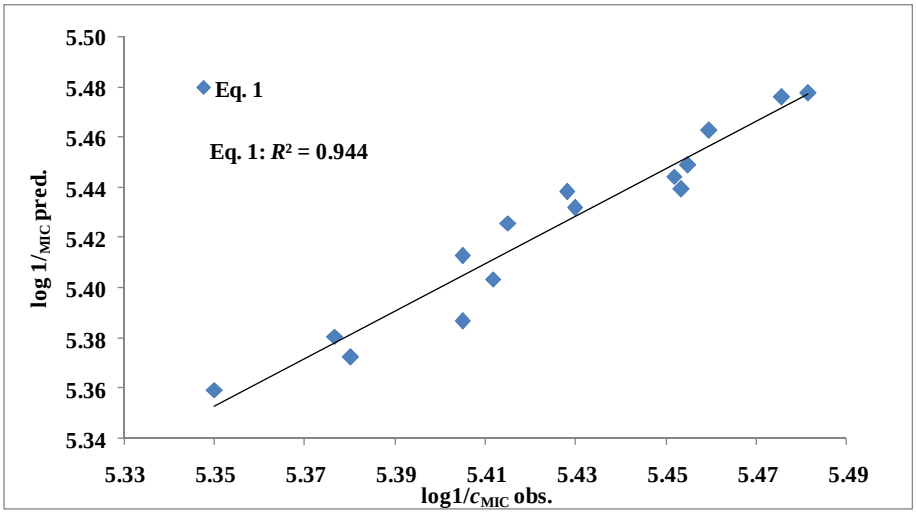


Fig. 1. Correlation between the observed log $1/c_{MICobs.}$ and predicted (log $1/c_{MICpred.}$) values

Statistical evaluation

Statistical evaluation for Eq. 1 was measured by several cross-validation parameters: *Q*, PRESS/SSY, S_{PRESS}, *PSE* and Q^2. Following statistical parameters were obtained for this equation:

$$Q = 100.1858;\ \text{PRESS/SSY} = 0.0564;\ S_{PRESS} = 0.0098;$$
$$PSE = 0.0088 \text{ and } Q^2 = 0.9436.$$

Equations with PRESS/SSY < 0.4 (Singh *et al*, 2008; Thakur *et al*., 2005), as in this case were reliable QSAR models (Singh *et al*., 2008; Thakur, 2005). Good S_{PRESS} and *PSE* values were obtained for these models confirming the assumption that the model can be used as a tool for predicting the inhibition of *Bacillus subtilis* (Bansal *et al*., 2007). For a reliable model the Cross-validation squared correlation coefficient values (Q^2) should be greater than 0.6 (Singh *et al*, 2008) and the difference between R^2 and Q^2 should not be more than 0.3 (Veerasamy *et al*., 2011). Due to this fact the Eq. 1 was statistically satisfactory describing model in which parameters have opposite input to the modeling of biological activity of selected hydrazones (AC-negative; MAPA-positive). Slightly higher influence associated with Maximal projection area (MAPA) coefficient (52.85%) compared to other descriptor coefficient (AC = 7.15%) indicate their positive role towards antibacterial activity. In this model, the coefficient of AC and MAPA were higher

than their standard deviation, which is another confirmation for statistical significance of Eq. 1.

Conclusions

A series of *p*-substituted aromatic hydrazones have been synthesized and evaluated for *in vitro* antimicrobial activity against *Bacillus subtilis*. QSAR study was performed in order to estimate the quantitative effects of the selected topological descriptors of derivatives on their antibacterial activity. Topological and physicochemical descriptors were calculated for each molecule and a two-parametric model has been selected for further discussion.

The statistical significance of the developed model was measured by a few cross-validation parameters *(Q, PRESS/SSY;* S_{PRESS}*; PSE* and Q^2*)*. Statistical evaluation of the data used to test the quality of the obtained models indicated that the selected model (Eq. 1) was statistically significant when all parameters were summarized. The both parameters AC (Atom count) and MAPA (Maximal projection area) contributing to the statistically best model (Eq. 1) have opposite input to the modeling of biological activity of the selected hydrazones.

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