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Design, drug-likeness and cytotoxicity of some bromo-salicylaldehyde aroylhydrazones

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***Abstract*—Aroylhydrazones derived by the Schiff base condensation between salicylaldehyde and different hydrazides possess diverse pharmacological activities such as antimicrobial, antibacterial, anti-inflammatory, analgesic, antifungal, anti-tubercular, antiviral, anticancer, antioxidant etc. Various substitutions in the molecules have been made in order to improve their biological activity. Inclusion of a bromine atom in some hydrazones greatly increases the activity of the compounds.**

In this work we present the comparative evaluation of *in silico* biological activity of a series of nine various bromo-derivative hydrazones. The compounds were designed by varying the position of bromo-substituent in salicylaldehyde moiety and the type of substituents at 4th position of hydrazide moiety. The drug relevant properties of the studied compounds, important for drug pharmacokinetics in the human body, were evaluated with the Lipinski's rule of five. The value of logP and the remaining parameters of drug similarity were calculated by the method based on group contributions. The approach is used only as a first step in drug discovery, to find the lead candidates with encouraging properties for further elaboration.

Some of the investigated bromo-derivative hydrazones were tested for *in vitro* cytotoxicity on a HL-60 acute myeloid leukemia and SKW-3 T-cell leukemia cell lines by MTT-test. The bioassay results demonstrated that the compounds exhibit concentration-dependent cytotoxic effects at low micro molar concentrations. The values of IC_{50} for 5-bromosalicylaldehyde-4-hydroxy-benzoylhydrazone and 5-bromosalicylaldehyde isonicotinoyl hydrazone are comparable to these of Cisplatin, but lower to these of Melphalan. The results confirm that the compounds are potential candidates for future drug discovery study.

***Keywords*—bromo-salicylaldehyde benzoylhydrazone; cytotoxic activity; Lipinski's rule; lipophilicity**

I. INTRODUCTION

Hydrazones constitute a wide class of compounds in organic and medicinal chemistry, which demonstrate an extensive range of pharmacological properties, such as anti-inflammatory [1,2], analgesic [2], antituberculosis [3,4], antibacterial [5], antimicrobial [6], anti-HIV [6,7] and anticancer [6,8] activity. Iron chelators derived by condensation of salicylaldehyde and different acid hydrazides possess especially high and selective antiproliferative activities [9-11]. One of the compounds, salicylaldehyde benzoyl-hydrazone (SBH), has been shown to inhibit DNA synthesis and cell growth in a variety of cultured human and rodent cells [11]. Various derivatives of salicylaldehyde benzoylhydrazone have been synthesized in order to discover new bioactive compounds with antitumor activity [8-10, 12-14].

The interest in the development of novel "drug-like" compounds significantly increased the number of potential drug candidates requiring *in vitro* and *in vivo* evaluation but this is a long and costly process [15]. During the last years, the pharmaceutical chemists used some modern *in silico* tools in drug discovery to find the lead compounds and reduce the number of *in vivo* studies required [16]. *In silico* pharmacology enables the design of lots of compounds that can be screened against potential targets and determines those with appropriate physicochemical properties.

Optimization and modification of known drugs and bioactive structures by including many active groups and pharmacophores is a continuing challenge for the pharmacists and biochemists. One of the main disadvantages of this approach is that newly synthesized compounds tend to have higher molecular weight, high lipophilicity and low aqueous solubility which results in poor bioavailability. A widely accepted measure of lipophilicity is log P and compounds demonstrating log P > 3.5 usually have poor aqueous solubility [17]. Decreasing of lipophilicity will improve solvation potential by increasing solvent-solute interactions in aqueous media. A common method to reduction of lipophilicity is through the introduction of polar groups. In general, values of log P among 2 and 3 provide a good balance between water solubility and lipophilicity of the compounds and ensure a good permeability and bioavailability.

This article presents the comparative evaluation of *in silico* biological activity of nine bromo-derivative hydrazones designed by varying the position of bromo-substituent in salicylaldehyde moiety and the type of substituents at 4th position of hydrazide moiety. The important drug properties were calculated to reveal how the incorporation of bromo-substituent and other polar groups affect the lipophilicity of the compounds. Two of the most perspective hydrazones were synthesized and tested *in vitro* for their cytotoxic effects on the human leukemic cell lines HL-60 and SKW-3.

II. MATERIALS AND METHODS

A. Design of Bromo-Hydrazones

The common route for the synthesis of aroylhydrazones is the condensation of suitable aldehydes with acid hydrazides. A series of nine compounds was designed by consecutively

incorporation of a bromine atom on 3rd, 4th and 5th position in salicylaldehyde and replacement of H-atom on 4th position in benzhydrazide with hydroxyl-group or N-atom. The structures of bromo-derivatives are present in Table I.

B. Calculation of "Drug-Like" Properties

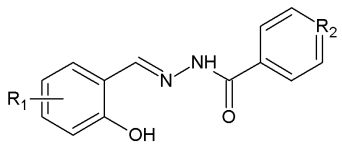
The molecular properties of bromo-salicylaldehyde derivative hydrazones, important for drug pharmacokinetics in the human body, were evaluated with the Lipinski's rule of five (RO5), formulated by the medical chemist Christopher A. Lipinski [18, 19]. He concluded that a compound is more likely to be membrane permeable and easily absorbed by the body if it matches the following criteria:

- Its molecular weight is less than 500;
- The compound's lipophilicity, expressed as a quantity known as log P (the logarithm of the partition coefficient between water and 1-octanol), is less than 5;
- The number of groups that can accept hydrogen atoms to form hydrogen bonds (estimated by the sum of oxygen and nitrogen atoms) is less than 10;
- The number of groups in the molecule that can donate hydrogen atoms to hydrogen bonds (usually the sum of hydroxyl and amine groups in a drug molecule) is less than 5.

Improved extensions of the RO5 related to the calculations of the Partition coefficient (log P range from −0.4 to +5.6) were also applied [20].

The value of log P and the remaining parameters of drug similarity were calculated by the method based on group contributions [21]. These have been obtained by fitting the values of the calculated log P with experimental log P for a set of more than twelve thousand, mostly drug-like molecules. The results were compared with the properties of salicylaldehyde benzoylhydrazone.

TABLE I. STRUCTURES OF BROMO-DERIVATIVE HYDRAZONES



№	*Compound*	R_1	R_2
1	**3BrSBH** 3-bromosalicylaldehyde benzoylhydrazone	3-Br	C-H
2	**3BrShBH** 3-bromosalicylaldehyde-4-hydroxybenzoylhydrazone	3-Br	C-OH
3	**3BrSIH** 3-bromosalicylaldehyde isonicotinoylhydrazone	3-Br	N
4	**4BrSBH** 4-bromosalicylaldehyde benzoylhydrazone	4-Br	C-H
5	**4BrShBH** 4-bromosalicylaldehyde-4-hydroxybenzoylhydrazone	4-Br	C-OH
6	**4BrSIH** 4-bromosalicylaldehyde isonicotinoylhydrazone	4-Br	N
7	**5BrSBH** 5-bromosalicylaldehyde benzoylhydrazone	5-Br	C-H
8	**5BrShBH** 5-bromosalicylaldehyde-4-hydroxybenzoylhydrazone	5-Br	C-OH
9	**5BrSIH** 5-bromosalicylaldehyde isonicotinoylhydrazone	5-Br	N

C. Cell Lines and Culture Conditions

The cell lines used in this study, namely HL-60 and SKW-3, were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). HL-60 is human acute myeloid leukemia cell line, established from the peripheral blood of a 35-year-old woman with acute myeloid leukemia in 1976. SKW-3 is human T-cell leukemia cell line, originally described to be established from the peripheral blood of a 61-year-old man with T cell chronic lymphocytic leukemia in 1977.

The cells were grown as a suspension-type cultures under standard conditions – RPMI 1640 liquid medium supplemented with 10 % fetal bovine serum (FBS) and 2 mM L-glutamine, in cell culture flasks, housed at 37 °C in an incubator "BB 16-Function Line" Heraeus with humidified atmosphere and 5 % carbon dioxide. Cell cultures were maintained in logarithmic growth phase by supplementation with fresh medium two or three times weekly.

D. Cytotoxicity Assessment (MTT-dye Reduction Assay)

The cytotoxic activity of the tested compounds was assessed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] dye reduction assay as described by Mossman [22]. The method is based on the reduction of the yellow tetrazolium salt MTT to a violet formazan via the mitochondrial succinate dehydrogenase in viable cells. In brief, exponentially growing cells were seeded in 96-well flat-bottomed microplates (100 µl/well) at a density of 1×10^5 cells per ml and after 24 h incubation at 37 °C they were exposed to various concentrations of the tested compounds for 72 h. For each concentration at least 8 wells were used. After the incubation with the test compounds 10 µl MTT solution (10 mg/ml in PBS) aliquots were added to each well. The microplates were further incubated for 4 h at 37 °C and the MTT-formazan crystals formed were dissolved by adding 100 µl/well 5 % HCOOH in 2-propanol. The MTT-formazan absorption was determined using a microprocessor controlled microplate reader (Labexim LMR-1) at 580 nm.

E. Data Processing and Statistics

The cell survival data were normalized as percentage of the untreated control (set as 100 % viability). The statistical processing of biological data included the Student's t-test whereby values of $p \leq 0.05$ were considered as statistically significant. In addition IC_{50} values were derived from the concentration-response curves using non-linear regression analysis.

III. RESULTS AND DISCUSSION

A. In silico Evaluation of Drug Likeness

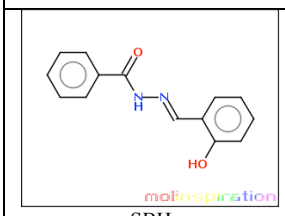
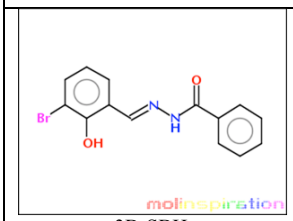
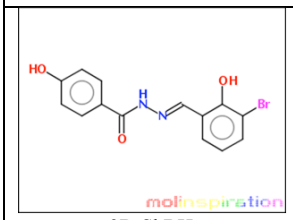
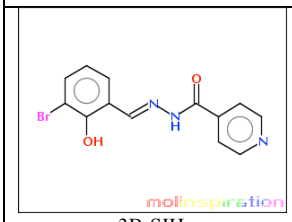
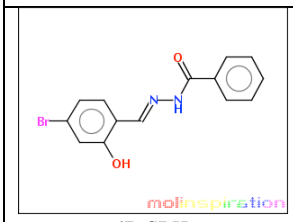
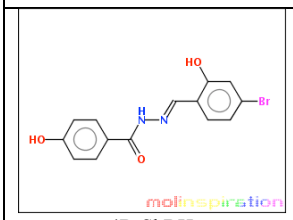
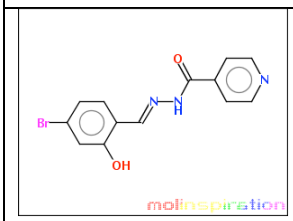
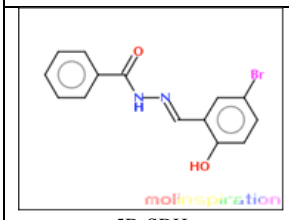
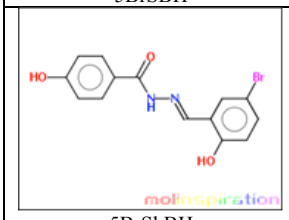
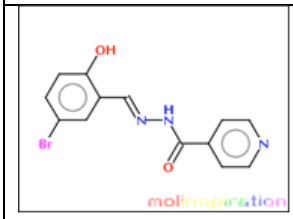
Lipinski's rule is a rule for evaluation of drug-likeness of compounds based on the observation that most medication drugs are relatively small and lipophilic molecules. The value

of log P is one criterion used in medicinal chemistry to assess the drug-likeness of a given molecule and to predict the solubility of a potential drug.

The compounds and their characteristics used for evaluation of drug similarity on the bases of Lipinski's rule are presented in Table II. The calculations show that all 3-bromo-, 4-bromo- and 5-bromosalicylaldehyde hydrazone derivatives observed boundary conditions of the "rule of Lipinski" and did not violate any of the listed criteria. Incorporation of various substituents in the molecule of SBH affects the value of log P, i.e. modify the lipophilicity of the compounds.

SBH possesses balanced lipophilicity and a value of log P equal to 3.039. The inclusion of bromine atom in salicylaldehyde moiety in 3BrSBH, 4BrSBH and 5BrSBH increases a little the value of log P to 3.593 – 3.824. The values showed that the position of bromo-substituent has no significant importance. Much more noticeable is the influence of the substituents in the hydrazide ring. A clear trend exists in each group of bromo-derivatives – lipophilicity of hydrazones derived from benzhydrazide is the highest, it is lower in the derivatives obtained from the 4-hidroxy-benzhidrazide and the lowest for compounds containing a pyridine nucleus. The substitution of H-atom on 4th position in benzhydrazide with hydroxyl-group slightly decreases the value of log P with 0.3 – 0.5 units and the lipophilicity of 3BrShBH, 4BrShBH and 5BrShBH is similar to this of SBH. The replacement of benzene nuclei in hydrazides with pyridine in 3BrSIH, 4BrSIH and 5BrSIH notably reduces the log P with 1.3 units.

TABLE II. CHEMICAL STRUCTURE AND PARAMETERS OF EVALUATION OF BROMO-HYDRAZONE DERIVATIVES WITH LIPINSKI'S RULE OF FIVE

Compound	LogP <5	M_w <500	O,N <10	OH,NH <5
 SBH	3.039	240.262	4	2
 3BrSBH	3.593	319.158	4	2
 3BrShBH	3.113	335.157	5	3
 3BrSIH	2.303	320.146	5	2
 4BrSBH	3.824	319.158	4	2
 4BrShBH	3.345	335.157	5	3
 4BrSIH	2.535	320.146	5	2
 5BrSBH	3.824	319.158	4	2
 5BrShBH	3.345	335.157	5	3
 5BrSIH	2.535	320.146	5	2

Nevertheless of the small differences, all hydrazones have suitable lipophilicity with values of log P between 2.5 and 3.8 and potentially good permeability across cell membrane. However, the rule cannot predict the pharmacological activity of the compounds and we use it only as a preliminary screening.

B. In Vitro Cytotoxicity

Two of the hydrazones, 5-bromosalicylaldehyde-4-hydroxybenzoylhydrazone (5BrShBH) and 5-bromo-salicylaldehyde isonicotinoylhydrazone (5BrSIH) were synthesized and tested for *in vitro* cytotoxicity. The cytotoxic potential of the compounds against the human leukemic cell lines HL-60 (human acute myeloid leukemia) and SKW-3 (T-cell leukemia) was studied using the standard MTT-dye reduction assay for cell viability.

Throughout the screening investigation the data about the new compounds were compared with the referent agent Cisplatin and the clinically utilized antineoplastic drug Melphalan (2-amino-3-[4-bis(2-chloroethyl) amino] phenylpropanoic acid).

The constructed concentration-response curves and the corresponding IC_{50} values obtained are shown in Figs.1-4 and in Table III, respectively. Each data point represents the arithmetic mean ± standart deviation (sd) of at least eight independent experiments. IC_{50} values were calculated as concentrations of the tested compounds causing 50 % decrease of cell survival.

TABLE III. CYTOTOXIC ACTIVITY OF THE TESTED HYDRAZONES AFTER 72 H TREATMENT (MTT-ASSAY)

Compounds	IC_{50} (µmol/l) ± sd	
	HL-60	***SKW-3***
5BrShBH	3.14 ± 1.1	3.02 ± 1.05
5BrSIH	4.13 ± 1.2	2.53 ± 1.06
Cisplatin	8.70 ± 2.4	11.4 ± 2.1
Melphalan	18.5 ± 2.1	31.3 ± 2.9

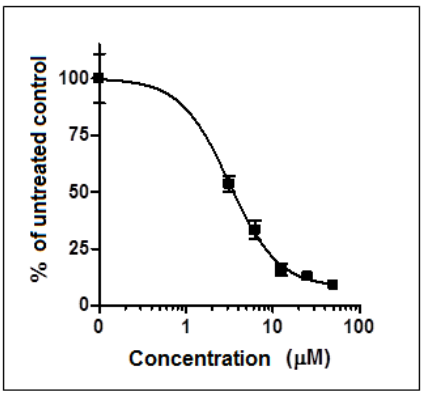


Fig. 1. Cytotoxic effect of 5BrShBH as assessed by the MTT-dye reduction assay on HL-60 cells.

The evaluation of the cell viability following 72 h treatment with 5-bromohydrazone derivatives revealed that compounds exhibited concentration-dependent cytotoxic effects at low micro molar concentrations of both HL-60 and SKW-3 cells.

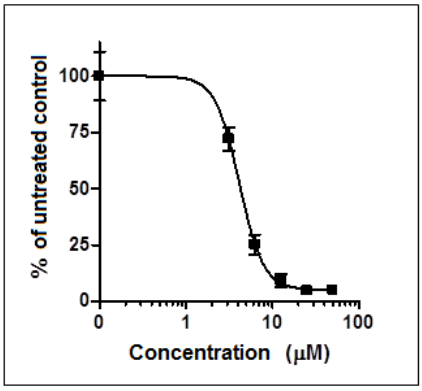


Fig. 2. Cytotoxic effect of 5BrSIH as assessed by the MTT-dye reduction assay on HL-60 cells.

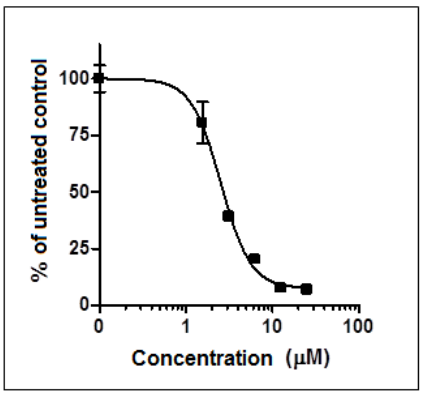


Fig. 3. Cytotoxic effect of 5BrShBH as assessed by the MTT-dye reduction assay on SKW-3 cells.

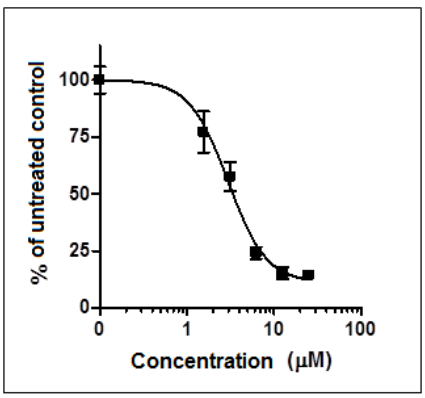


Fig. 4. Cytotoxic effect of 5BrSIH as assessed by the MTT-dye reduction assay on SKW-3 cells.

As evident from the results obtained, 5BrShBH and 5BrSIH exerted similar cytotoxic effects against the myeloid HL-60 cells with IC_{50} value of 3.14 – 4.13 μmol/l (Figs. 1-2, Table III). At the highest concentration of 50 μmol/l applied, both compounds caused a drastic decrease of the viability of the malignant cells by approximately 91 – 95 %. IC_{50} values are lower, but comparable to this of Cisplatin and much lower to this of Melphalan.

T-cell leukemic line SKW-3 exhibits high sensitivity to 5-bromohydrazone derivatives. 5-bromo hydrazones reduced the percentage of viable cells by 20-23 % even at the lowest applied concentration 1.56 μmol/l of the compounds. At the highest applied concentration of 25 μmol/l reduction of the vital cells was 86 % and 93 % for 5BrShBH and 5BrSIH respectively (Figs. 3 and 4). IC_{50} values are lower than this of Melphalan and even less than this of Cisplatin.

IV. CONCLUSION

The values of log P and the remaining parameters of drug similarity were calculated by the "rule of Lipinski" for a series of nine bromo-derivative hydrazones. The rule was used as a first step in drug discovery to quickly find the lead candidates with encouraging bioavailability properties. The results of *in silico* investigations indicated that all compounds observed boundary conditions of the method and the position of bromo-substituent has no significant importance on lipophilicity and permeability of bromo-hydrazones.

The compounds were tested for in vitro cytotoxicity on two human leukemia cell lines HL-60 and SKW-3. The analysis showed that 5-bromo-hydrazones demonstrate high cytotoxic activity in micro molar concentrations against leukemia cell lines. Based on the results of the MTT-dye reduction assay the compounds deserve more detailed toxicological and pharmacological investigations for the development of new anticancer drugs.

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