Comparative evaluation of *in silico* and *in vitro* pharmacological activity of some 5-nitrosalicylaldehyde-derived hydrazones

B. Nikolova-Mladenova[1], *Ts. Gerassimova[2], M. Topashka-Ancheva[2], G. Momekov[3]

[1]Department of Chemistry, Faculty of Pharmacy, Medical University of Sofia, 2 Dunav Str., 1000 Sofia, Bulgaria
[2]Institute of Biodiversity and Ecosystems Research, Bulgarian Academy of Sciences, Sofia, Bulgaria
[3]Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University-Sofia, 2 Dunav Str., 1000 Sofia, Bulgaria

Aroylhydrazones derived from salicylaldehyde are compounds with interesting biological properties including a high anticancer activity. Salicylaldehyde benzoylhydrazone (SBH) is an unusually potent inhibitor of DNA synthesis and cell growth in a variety of cultured human and rodent cells. Various derivatives of SBH have been synthesized in order to discover new more effective antiproliferative agents.

In the present paper we report the comparative evaluation of *in silico* and *in vitro* biological activity of three recently synthesized 5-nitrosalicylaldehyde-derived hydrazones, namely 5-nitrosalicylaldehyde benzoylhydrazone (5nSBH), 5-nitrosalicylaldehyde-4-hydroxy-benzoylhydrazone (5nShBH) and 5-nitrosalicylaldehyde isonicotinoylhydrazone (5nSIH). The molecular 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. All 5-nitro-derivatives observed boundary conditions of the rule and theoretically can be successful oral drugs. The rule is used only as a first step in drug discovery, to find the lead candidates for further elaboration and quickly eliminate the compounds with poor pharmacological properties.

In order to examine the antiproliferative effects of the investigated compounds they were tested for *in vitro* cytotoxicity on a HL-60 acute myeloid leukaemia and BV-173 chronic myeloid leukaemia cell lines. 5-nitro-derivatives exhibited concentration dependent cytotoxic effect on the leukaemia cell lines. The values of IC_{50} for 5-nitrosalicylaldehyde benzoylhydrazone are comparable with these for the referent cytotoxic drug cisplatin, whereas the IC_{50} values for 5nShBH and 5nSIH are higher. Based on the results of the MTT-dye reduction assay the compound 5nSBH deserves more detailed toxicological and pharmacological investigation for the development of new anticancer drug.

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were carried out in order to establish their value as potential drug candidates.

Stimulated by this we designed SBH analogues derived from 3-methoxysalicylaldehyde and three acid hydrazides in order to receive new compounds with high cytotoxic activity.

These promising results with salicylaldehyde benzoylhydrazone prompted us to design new SBH analogues derived from 3-methoxysalicylaldehyde and three acid hydrazides in order to receive new compounds with high cytotoxic activity. The structures of the new aroylhydrazones are shown on Fig.1.

The value of logP and the remaining parameters of drug similarity were calculated by the method based on group contributions. Compounds …….. observed boundary conditions of the "rule of Lipinski" and can be successful oral drugs according the rule.

The compounds were tested for *in vitro* cytotoxicity on a panel of seven human epithelial tumour cell lines …. The analysis showed that the -based ……..demonstrate high cytotoxic…. activity against tumour cell lines.

Based on the results of the MTT-dye reduction assay the compounds ….. deserve more detailed toxicological and pharmacological investigation for the development of new anticancer drugs.

It was discovered that aroylhydrazones of the type R-CO-NH-N=CH-R′, an effective iron chelators, may be useful for the treatment of cancer because Fe is critical for cellular growth and proliferation[1-3]. Previous studies have shown that salicylaldehydebenzoylhydrazone (SBH) is an unusually potent inhibitor of DNA synthesis and cell growth in a variety of cultured human and rodent cells[2-4]. Various derivatives of salicylaldehyde benzoylhydrazone have been synthesized in order to discover new more effective antiproliferative agents[3-7].

Salicylaldehyde benzoylhydrazone (SBH) is an unusually potent inhibitor of DNA synthesis and cell growth in a variety of cultured human and rodent cells. Aroylhydrazones derived from salicylaldehyde are compounds with interesting biological properties including a high anticancer activity.

This rule of thumb is used as a first step in drug discovery to quickly eliminate lead candidates with poor bioavailability properties.

This article presents the potential of PLGA nanoparticles for the oral administration of drugs. Different strategies are used to improve oral absorption of these nanoparticles.

Oral administration is one of the most commonly-used routes for drug administration because of its non-invasive nature and the fact that it avoids the pain and discomfort associated with injections. However, it is not possible when the actives present unfavourable characteristics: inadequate physicochemical properties for intestinal absorption, stability or solubility problems and a clear decrease in bioavailability by first-pass hepatic effects [1].

Comparative evaluation of *in silico* and *in vitro* biological activity of …-derived …..were carried out in order to establish their value as potential drug candidates. The compounds were tested for *in vitro* cytotoxicity on a panel of seven human epithelial tumour cell lines. Safety testing was performed both *in vitro* (3T3 NRU test) and *in vivo* on ICR mice for genotoxicity and antiproliferative activity.
The structure-activity relationship analysis showed that the anthracen-based aminophosphonates demonstrate high cytotoxic activity against tumour cell lines. Compounds …….. can be successful oral drugs according the rule of Lipinski. Bis-aminophosphonates tested exerted lower cytotoxicity towards human tumour cell lines than the aminophosphonates. The copolymer **7** showed excellent antiproliferative activity against cell lines HBL-100, MDA-MB-231, MCF-7 and HepG2. However, the *in vitro* safety testing revealed significant toxicity against Balb/c 3T3 mouse embryo cells. In contrast, the copolymer **6** showed complete absence of cytotoxicity against Balb/c 3T3 cells, but it inhibited the growth of breast cancer cells, cervical carcinoma cells (HeLa) and hepatocellular carcinoma cell cultures after prolonged (72 h) exposure.

The molecular properties of the 5-nitro-salicylaldehyde benzoylhydrazone and its derivatives, important for drug pharmacokinetics in the human body, were evaluated with the Lipinski's rule of five (RO5) [14, 15] which states, that most "drug-like" molecules have logP $\leq$ 5, molecular weight $\leq$ 500, number of hydrogen bond acceptors $\leq$ 10 and number of hydrogen bond donors $\leq$ 5. Improved extensions of the RO5 related to the calculations of the Partition coefficient (logP range from −0.4 to +5.6) [16] were also applied.
The value of logP and the remaining parameters of drug similarity were calculated by the method based on group contributions [13]. These have been obtained by fitting calculated logP with experimental logP for a training set more than twelve thousand, mostly drug-like molecules. The results were compared with the properties of salicylaldehyde benzoylhydrazone SBH.

The calculations show that all 5-nitro hydrazone derivatives observed boundary conditions of the "rule of Lipinski" and did not violate any of the listed criteria. Incorporation of various substituents on the molecule of SBH affects the value of logP, i.e. modify the lipophilicity of the compounds.

SBH has an average value of lipophilicity and logP = 3.039. The presence of the nitro group in salicylaldehyde moiety slightly decreases lipophilicity. Much more noticeable is the influence of the substituents on hydrazine ring. There is a clear trend – lipophilicity of hydrazone

derived from benzhydrazide is the highest, it is lower in the derivative obtained from the 4-hidroxybenzhidrazide and lowest for compound containing a pyridine nucleus.

It is important to note that new hydrazones have lipophilicity and intermediate values of logP of between 1 and 3 making them suitable for oral drugs. However, the rule can not predict if a compound is pharmacologically active and we use it only as a preliminary screening.

Evaluation of Drug Likeness

Lipinski's rule or simply the Rule of five (RO5) is a Rule of thumb for evaluation of drug likeness to similar compounds or for determination of probability for oral activity of a chemical compound with certain pharmacological or biological activity. The rule was formulated by Christopher A. Lipinski in 1997, based on the observation that most medication drugs are relatively small and lipophilic molecules.

The results from molecular evaluations of anthracene-based α-aminophosphonate and bis-aminophosphonate derivatives for drug similarity on the basis of Lipinski's rule of five are presented in Table 1.

The calculations show that aminophosphonates observed the boundary conditions of the Lipinski's rule of five. Incorporation of various substituents in the molecules affects the value of logP, i.e. modify the lipophilicity of the compounds. Compounds APhA-t and APhA-f did not violate any of the listed criteria. After substitution of methoxy groups in APhA-t with ethoxy groups in APhEA-t one violation of the rule appears (logP=6.154) which indicates a slightly decreased lipophilicity of the tested compound (Table 1).

Much more noticeable is the influence of the substituents in bis-aminophosphonates. In order to improve the affinity of the new derivatives lipophilicity, the molecular weight of the compounds is increased. The novel molecules have more than one violation of the Lipinski's rule. The compounds are unlikely to be further pursued as potential oral drugs, because they would likely lack properties important for their absorption, distribution, metabolism, and excretion.

Although this rule provides a powerful and simple tool to find potential drugs, sometimes it could exclude the compound that would have proven the successful drug. For example, two bisphosphonate drugs - Alendronic acid and Pamidronic acid and Doxorubicin in general do not follow the rule too. However, the rule does not predict if a compound is pharmacologically active and we use it only as a preliminary screening.

Cancer, known medically as a malignant neoplasm, is the second largest cause of death in developed countries. It contains a broad group of various diseases, all involving unregulated cell growth. Due to the impact of this illness, chemotherapy has been intensively studied and huge amount of compounds have been synthesized over the last 30 years.
In the past few decades, photodynamic therapy has received