QSAR STUDY AND CYTOTOXIC ACTION OF ISATIN DERIVATIVES

Frosa Anastasova[1], Nataša Ristovska[1], Ivan Juranić[2],

[1]Institute of Chemistry, Faculty of Natural Science and Mathematics, "Sts. Cyril and Methodius" University, P.O. Box 162, MK-1001 Skopje, Republic of Macedonia

[2]Institute for Oncology and Radiology, Pasterova 14, 11000 Belgrade, Serbia

Abstract

The antiproliferative action of seven isatin derivatives: 5-fluoroisatin, 5-chloroisatin, 5-bromoisatin, 5-iodoisatin, 5-methylisatin, *N*-methylisatin and *N*-ethylisatin was investigated *in vitro* on two neoplastic cell lines, HeLa (human cervix carcinoma) and Fem-x (human malignant melanoma). Target cells were seeded (2000 cells per well) in the nutrient medium. Twenty hours later, five different concentrations of examined agents were added to cells. 48 hours after isatin derivative action, the cell survival was determined by the MTT test, and by the neutral red uptake test.

All the analyzed compounds affected target cells proliferation. Morphological examination of treated cells shows that halogen derivatives of isatin induced the fragmentation of HeLa cells. *N*-methyl and *N*-ethyl derivatives induced Fem-x cell vacuolization and cell necrosis. The neutral red uptake test was more reliable than the MTT test in IC50 determination. All the compounds showed marked Hansch-type relationship between IC50 values and molecular parameters of lipophylicity and substituent steric effect.

Introduction

Cancer is the leading disease-related cause of death of the human population in some areas of the world, and it is predicted to continue this trend within the coming years [1]. Chemotherapy, or the use of chemical agents to destroy cancer cells, is a mainstay in the treatment of malignancies. A major advantage of chemotherapy is its ability to treat widespread or metastatic cancers, whereas surgery and radiation therapies are limited. The chemotherapy has aroused many researchers' interests and a great deal of current efforts has been focusing on the design and development of varied anticancer drugs. The search for new

compounds that could be potential anticancer drugs is the ultimate goal in modern medicine.

The isatin molecule (1H-indole-2,3-dione) is a versatile moiety that displays diverse biological activities [2], including anticancer activity [3, 4]. *N*-alkylated indoles have also been reported to exhibit anticancer activity. For example, the indolyl amide D-24851 has been found to be block cell cycle progression in a variety of malignant cell line including those derived from the prostate, brain, breast, pancreas and colon [5].

The quantitative structure-activity relationship (QSAR) is a powerful tool for rationalization and understanding of the biological activity of chemical compounds. It enables to pick up the most interesting new compounds among innumerable organic substances. The type of QSAR depends on the class of the compound and on the target system. Therefore, there is a need for an extensive study of various classes of compounds and their effects on living tissues.

In this sense the aim of this work was to test the action of seven synthetic isatin derivatives: 5-fluoroisatin, 5-chloroisatin, 5-bromoisatin, 5-iodoisatin, 5-methylisatin, *N*-methylisatin and *N*-ethylisatin (Fig.1) towards survival of two human neoplastic cell lines *in vitro*.

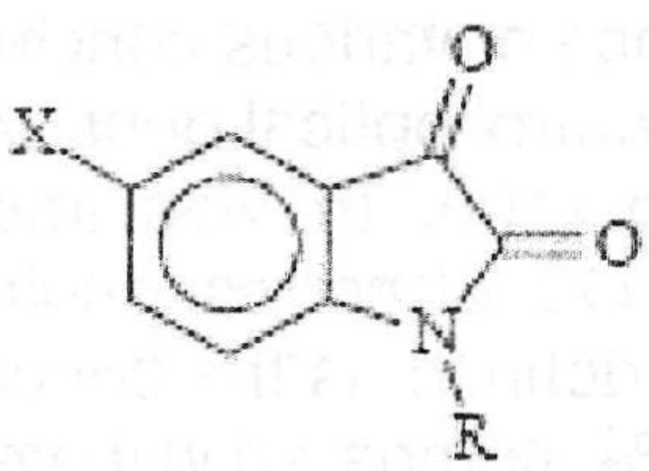


Fig. 1 Structure of isatin derivatives, X=F, Cl, Br, I, CH_3; R=H, CH_3, C_2H_5

Experimental

Synthesis of isatin derivatives was reported earlier [6].

Stock solutions of investigated compounds were made in DMSO, in concentration range 5.8-9.9 mM. Afterwards were diluted by nutrient medium (RPMI 1640 medium supplemented with L-glutamine (3 mmol/L), streptomycin 100 µg/mL and penicillin 100 IU/mL, 10% heat inactivated fetal bovine serum, FBS and 25 mM Hepes, adjusted to pH 7.2 by bicarbonate solution.) to various final concentrations. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoliumbromide

(MTT) and neutral red (NR) were purchased from Sigma Chemicals (St. Luis, MO, USA) MTT was dissolved, 5 mg/mL in phosphate buffer saline pH 7.2 and filtrated through milipore filter, 0.22 µm, before use. RPMI

1640 cell culture medium and fetal bovine serum (FBS) were products of Gibco (Paisley, Scotland, UK)

Cell culture. Human malignant melanoma Fem-x cells and human cervix carcinoma HeLa cells were maintained as a monolayer culture in the same nutrient medium. The cells were grown at 37°C in 5% CO_2 and humidified air atmosphere by twice weekly subculture.

Treatment of Fem-x and HeLa cells. Target cells were seeded in triplicate (2000 cells per well), into 96-well microtiter flat-bottomed plates. Twenty hours later, five different concentrations of investigated compound were added to the wells to various final concentrations, except to the control wells where a nutrient medium only was added to the cell. All samples were set up in triplicate. Nutrient medium with corresponding concentrations of compounds, but void of cells was used as blank, in triplicate too.

Determination of HeLa and FEM-x cell survival. Cell survival was determined as reported earlier [7-9] by MTT test, 48 h after the addition of drug. Briefly, 50 µL of MTT solution (5 mg/mL PBS) was added to each well. Samples were incubated for further four hours at 37°C in 5% CO_2 and humidified air atmosphere. Then, 100 µL of 10% SDS in 0.01M HCl were added to the wells. Optical density (OD) at 570 nm was red the next day. To get cell survival (%), optical density at 570 nm of a sample with cells grown in the presence of various concentration of investigated agent (OD), was divided with control optical density ODc, (The OD of cell grown only in nutrient medium)×100. (It was implied that OD of blank was always subtracted from OD of corresponding sample with target cells.) Concentration IC50 was defined as the concentration of a drug needed to inhibit cell survival by 50%, compared with vehicle-treated control.

Neutral Red uptake test [10] was also used for determination of cell survival. Forty-eight hours after the agents' action nutrient medium was discarded and fresh medium with 40 µg/mL of neutral red was added to the cells. After three hours of the cell incubation with dye the medium was removed and the cells were washed with 1% $CaCl_2$ – 0.5% formaldehyde solution, which both removes the unincorporated dye and fixes the cells to the substratum. The dye was extracted into the supernatant by addition of 0.2 mL of 1% glacial acetic acid – ethanol solution. After 1 h at room temperature OD570 was red. Cell survival was calculated in the same way as in MTT test.

Methods of calculation. The quantitative correlation of the obtained IC50 values was done with lipophilicity, log (P), the substituent steric constants, Es, and with dipole moments. Estimation of logarithm of partition coefficient [n-Octanol/Water] log (P) =log (KOW) was done by Crippen's fragmentation method [11]. Substituent steric constants were taken from published compilations [12]. The geometries and dipole

moments of the molecules were determined by the AM1 method (using a MOPAC package, version 7.01 [13]), employing full geometry optimization and imposing no *a priory* symmetry constraints. The MNDO-AM1 method was proven to be accurate for the calculation of various molecular species [14-17].

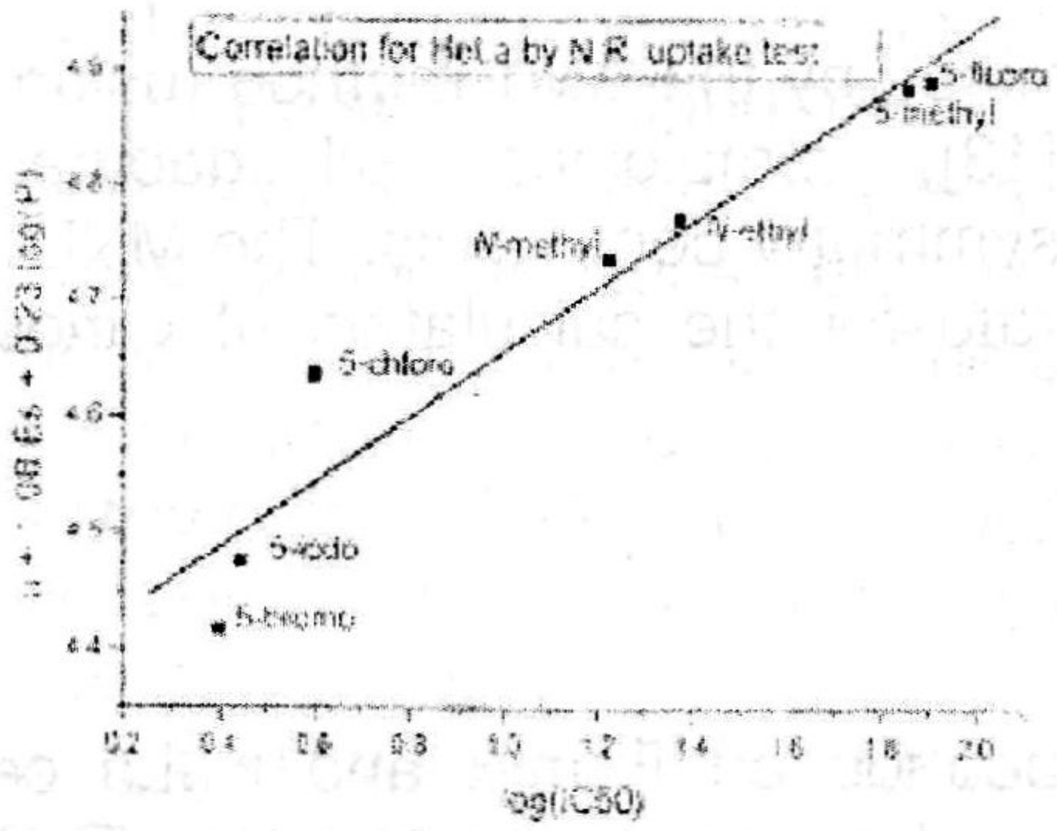
Results and discussion

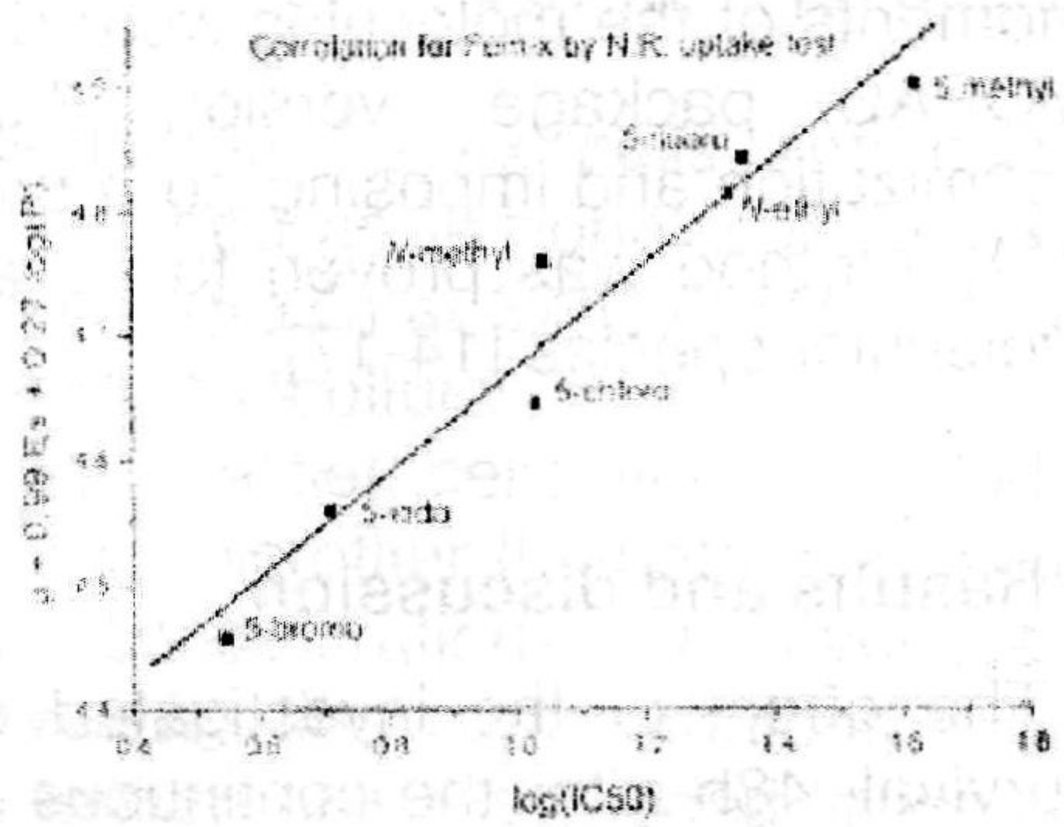
The effect of the investigated compounds on Fem-x and HeLa cell survival, 48h after the continuous action of agents, was shown on Fig.2, on graphs A-C. Investigated drugs expressed the dose dependent antiproliferative action toward investigated cell lines. In order to compare the extent of the antiproliferative action between members of this group of compounds, IC50 were determined under exactly the same conditions. HeLa cells were more sensitive to the cytotoxic action of investigated isatine derivatives. The sequence of cytotoxic potency determined by MTT test was 5-iodo- > 5-chloro- > 5-bromo- > 5-fluoroisatin > alkylisatin. The same sequence was obtained by MTT test from Fem-x cells. *N*-alkyl and 5-methyl isatins appeared to be without cytotoxic effect, having extremely high IC50 values.

Morphological examination of treated cells on inverted microscope showed that cytotoxic action of 5-halogeno- and 5-methyl-derivatives of isatin induced the fragmentation of HeLa cells. This fragmentation was accompanied by abortive mitosis in approx. 5% of cells in the presence of 5-iodo- and *N*-methylisatins. The main effect of the investigated isatin derivatives on Fem-x cells was necrosis. *N*-methyl, *N*-ethyl- and 5-iodo-derivatives were particularly efficient in cell vacuolization, cell enlargement and cell membranelysis. A morphological examination of Fem-x cells treated with alkylisatins showed the presence of many dead (necrotic) cells, while MTT test showed no cytotoxic effect. Therefore we used alternative test for evaluation of cytotoxic potency of chemicals, *i. e.* neutral red uptake test.

A neutral red uptake test was more consistent with morphological evidence than MTT test in IC50 determination.

The sequence of cytotoxic potency determined by NR uptake test was 5-bromo- > 5-iodo-> 5-chloro- > *N*-methyl >*N*-ethyl > 5-fluoro-isatine for HeLa and Fem-x cells.





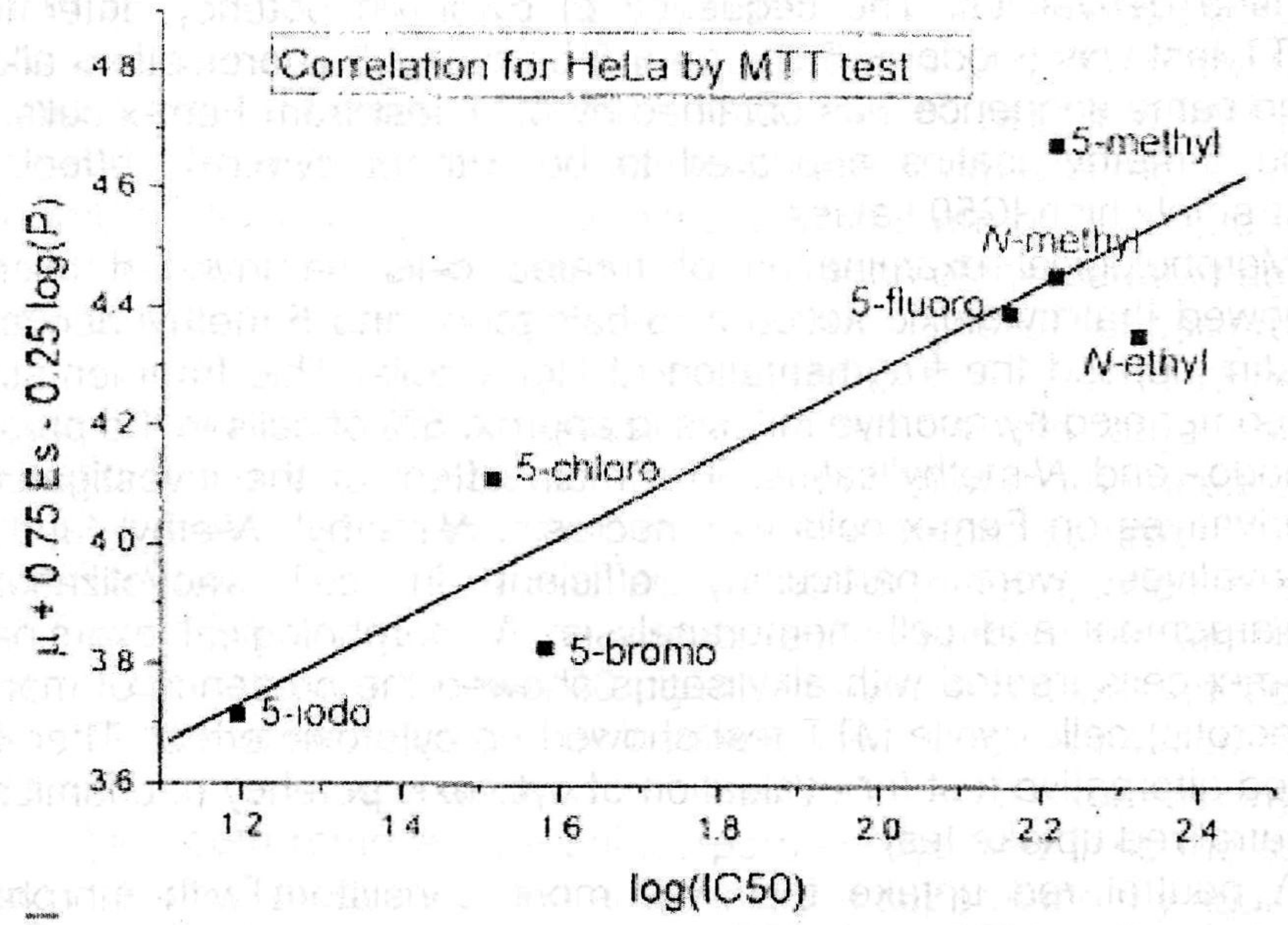


Fig.2 a) Correlation for HeLa by NR uptake test, b) Correlation for Fem-x by NR uptake test, c) Correlation for HeLa by MTT test

Structurally analogous derivatives have excellent correlations with single molecular parameter, such as the regression coefficients for various linear regressions of log (IC50) with calculated molecular properties for the investigated molecules. It could be seen that different behavior of halogen- and alkyl-substituted isatins, inferred from

morphological evidences, is mirrored in their different regression patterns. Generally, halogen derivatives correlate with Es and log (P) values, while alkyl-derivatives have best correlation with dipole moments.

However, using quadriparametric Hanch-type equation:

$$\log (IC50) = A \cdot (\mu + B \cdot Es + C \cdot \log (P)) + D \quad (1)$$

a very good correlation for all sets of experiments could be obtained.

An explanation for the observed discordance between data obtained by MTT test, NR uptake test and morphological evidence could be the production of some substances, during the cell necrosis, capable to reduce the MTT to formazan. Due that effect, the erroneously high cell survival values were obtained by MTT test.

Conclusions

Isatin is reported to be an endogenous natural inhibitor of monoamine oxidase B [18].

This study shows that IC50 values obtained by different methods produce different QSAR equation. This must be taken in account when results from various sources are compared.

Acknowledgement

The authors would like to thanks d-r Zorica Juranić and her coworkers who contributed to this study: S. Radulović, Lj. Pantelić and T. Stanojković, for providing facilities to carry out the experiment treatments in Institute for Oncology and Radiology in Belgrade, and help in data collection and analysis.

References

[1] Gibbs, J. B. (2000) Mechanism-based target identification and drug discovery in cancer research, Science, 287, 1969-1973.

[2] Pandeya, S. N, Smitha, S., Jyoti, M., Sridhar, S. K. (2005) Biological activities of isatin and its derivatives , Acta Pharmaecutica, 55, 27-46.

[3] Cane, A., Tournaire, M. C., Barritault, D., Crumeyrolle-Arias, M. (2000) The endogenous oxindoles 5-hydroxyoxindole and isatin are antiproliferative and proapoptotic, Biochemistry and Biophysics Research Commuication, 276, 379-384.

[4] Vine, K. L., Locke, J. M., Ranson, M., Benkendorff, K., Pyne, S. G., Bremner, J. B. (2007) In vitro cytotoxicity evaluation of some substituted isatin derivatives, Bioorganic and Medicinal Chemistry, 15, 931-938.

[5] Bacher, G., Nickel, B., Emig, P., Vanhoefer, U., Seeber, S., Klenner, A. S., Beckers, T., (2001) D-24851, a novel synthetic microtubule inhibitor, exerts curative antitumoral activity in vivo, shows efficacy toward multidrug-resistant tumor cells, and lacks neurotoxicity, Cancer Research, 61, 392-399.

[6] Anastasova, V.F. (1997) Ph D. Thesis, Faculty of Science, Skopje, Macedonia.

[7] Juranić, Z., Radulović, S., Joksimović, J., Juranić, I. (1998) The mechamism of 8-Cl-cAMP action, J. Exp.Clin.Cancer Res., 17, 269-275.

[8] Mosmann, T. (1983) Rapid colorimetric assay for cellular growth and survival: Aplication to proliferation and cytotoxicity assays, J. Immunol. Methods, 65, 55-63.

[9] Ohno, M., Abe, T. (1991) Rapid colorimetric assay for quantification of leukemia inhibitory factor (LIF) and interleukin-6 (IL-6), J. Immunol. Methods, 145, 199.

[10] Kumar, R., Bansal, C.R., Mahmood, A. (1994) Inhibition of rat brain monoamine oxidase by indole-2,3-dione (isatin) and its structural analogs, Biogenic Amines, 10, 473-485.

[11] Ghose, A.K., Crippen, G.M. (1987) Atomic physicochemical parameters fot three-dimensional-structure-directed quantitative structure-activity relationships. 2. Modeling dispersive and hydrophobic interactions, J. Chem.Inf.Comput.Sci., 27, 21-35.

[12] Charton, M. (1981) Progress in Physical Organic Chemistry, Ed. Taft R.W., New York vol.13, 119-252.

[13] Stewart, J.J.P. (1989) Optimization of parameters for semiempirical methods. II. Aplications., J. Comput.Chemistry, 10, 221-264.

[14] Wyttenbach, T., Vonhelden, G., Bowers, M.T. (1996) Gas-phase conformation of biological molecules: Bradykinin, J. Am. Chem. Soc., 118, 8355-8364.

[15] Bock, H., Nick, S., Seitz, W., Nather, C., Bats, J.W. (1996) Structures of charge-perturbed or sterically overcrowed molecules Part 80. Structural changes of p-benzoquinone by donor and acceptor substituents, Z. Naturforsch B - J.Chem.Sci., 51, 153-171.

[16] Pelsherbe, G.H., Wang, H.B., Hase, W.L. (1996) Trajectory studies of SN2 nucleophilic substitution. V. Semiempirical direct dynamics of Cl-CH_3Br unimolecular decomposition, J. Am. Chem. Soc, 118, 2257-2266.

[17] Hu, W.P., Truhlar, D.G. (1996) Factors affecting competitive ion-molecule reactions: Cl^- + C_2H_5Cl and C_2D_5Cl via E2 and SN2 channels, J. Am. Chem. Soc., 118, 860-869.

[18] Babich, H., Borenfreund, E., Stern, A. (1993) Comparative cytotoxicities of selected minor dietary non-nutrients with chemopreventive properties, Cancer Letters, 73, 127-133.