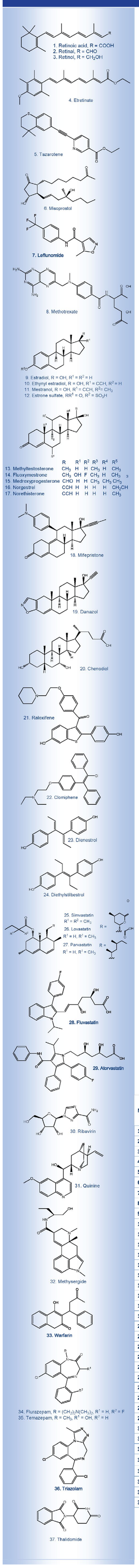
Retinoic acid activity on mamalian embryo in organ culture and evaluation of hazard and risk in correlation studies between molecular descriptors and ADMET parameters in a series of X-category drugs



Abstr. no. 815
Abstr. code PC-006

Jadrijević-Mladar Takač M.[1], Crnek Kunstelj V.[2], Barbarić M.[1] and Takač V.[3]

[1]Faculty of Pharmacy and Biochemistry, Department of Medicinal Chemistry, University of Zagreb, Croatia
[2]School of Medicine, Department of Biology, University of Zagreb, Croatia
[3]Health Pharmacy Institution Štefanić, Zagreb, Croatia

INTRODUCTION

The active derivative of vitamin A, retinoic acid (RA), as essential component of cell-cell signaling during vertebrate organogenesis is important for normal embryonic development. RA is an endogenous agonist for retinoic acid receptors (RARs), the type II of nuclear receptors (RARα, RARβ and RARγ) which are ligand-controlled transcription factors that function as heterodimers with retinoid X receptors (RXRs) to regulate cell growth, differentiation, survival and death. The concentration of RA must be within a very narrow range in order to avoid both deficiency and toxicity because the adding of vitamin A or RA to embryos can easily induce teratogenic effects including major alterations in organogenesis. (1-3) The results of many previous studies indicate that all endogenous retinoids (retinol, retinal, RA) are toxic and in some cases teratogenic (4, 5), therefore they are listed in the FDA Pregnancy category X-drugs among a very diverse group of drugs with different structural features, mechanisms of action and clinical indications. (6) The influence of RA on development of embryonic teratoma was also a subject of our previous *in vitro* investigations (7, 8). In addition, in this study we explored molecular features of selected pregnancy category X drugs, including retinol, retinal and retinoic acid, by correlation studies between computed molecular descriptors (MDs), predicted drug-likeness scores (dls) and ADMET properties.

Table 1. The results of teratoma developments investigation

Proportion of tissues in teratomas cultivated with RA. NGF and combination RA/NGF in serum suplemented medium (MEM/RS)				
Differentiated tissues	14 days	5 – 14 days of drugs exposure		
Treatmen and number (%) of explants	Control	RA (10^{-5} M)	NGF (100 ng/mL)	RA /NGF
	32	30	28	15
Keratinized epidermis	29 (90.0)	28 (93.0)	26 (93)	0 (0.0)
Imature epidermis	3 (9.0)	2 (6.0)	2 (7.0)	15 (100.0)
Neural tissue	20 (62.0)	20 (66.0)	14 (50.0)	15 (100.0)
Gut epithelium	25 (78.0)	29 (96.0)	18 (64.0)	14 (93.0)
Gland epithelium	7 (21.0)	3 (10.0)	6 (21.0)	0 (0.0)
Cartilage	24 (75.0)	1 (3.0)	19 (68.0)	0 (0.0)
Myotubes	6 (18.0)	8 (26.0)	9 (32.0)	7 (46.0)
Smuth muscles	5 (15.0)	7 (23.0)	7 (25.0)	7 (46.0)

Table 3. The number (%) of drugs with computed drug-likeness scores (dls) per each category

Drug-likeness scores (dls)	GPCR l dls	ICM dls	KI dls	NRL dls	PI dls	EI dls
	Number of drugs (%)					
0.50 – 1.70	3 (8.0)	1 (2.7)	0 (0.0)	17 (45.9)	2 (5.0)	17 (45.9)
0.20 – 0.49	16 (43.2)	9 (24.3)	2 (5.0)	7 (18.9)	4 (10.8)	9 (24.3)
≤ 0.19	18 (48.7)	27 (72.9)	35 (94.6)	13 (35.1)	31 (83.8)	11 (29.8)

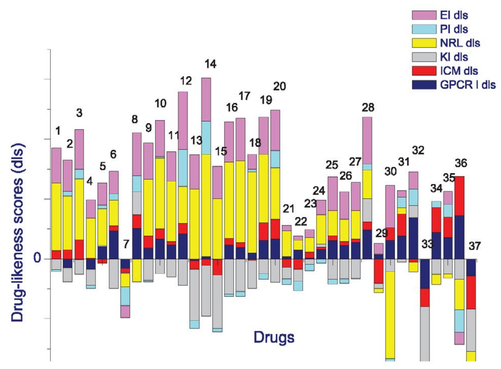


Fig 2. Drug-likeness scores per each drug **1 – 37** computed by Molinspiration Molecular Bioactivity Score engine (v2011.06)

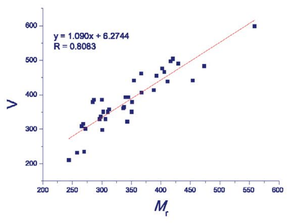


Fig. 3. Relationship between M_r and V

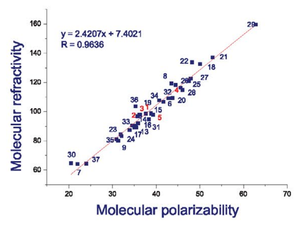


Fig. 4. Relationship between molecular polarizability and refractivity

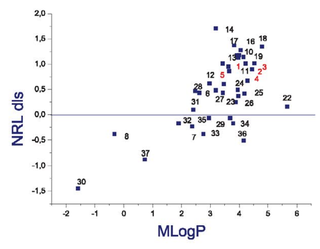


Fig. 5. Relationship between Log P and NRL dls

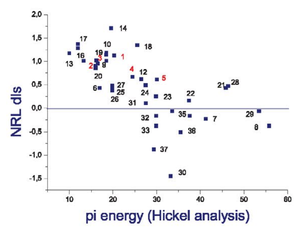


Fig. 6. Relationship between pI energy (Hickel anaysis) and NRL dls

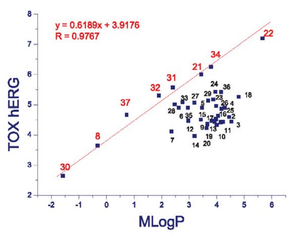


Fig. 7. The relationship between MLogP and TOX hERG in a group of investigated X drugs **1-37** with collinear relationship between drugs: **8**, **21-22**, **30-32**, **34** and **37**

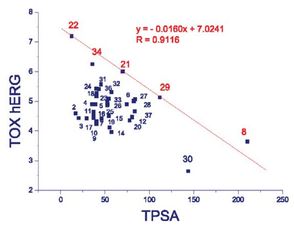


Fig. 8. The relationship between TPSA and TOX hERG in a group of investigated X drugs with collinear relationship between drugs: **8**, **21-22**, **29** and **34**

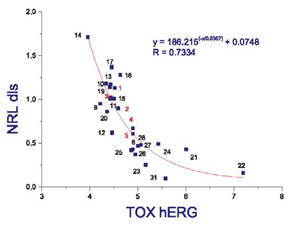


Fig. 9. Relationship between TOX hERG and NRL dls (computed positive scores)

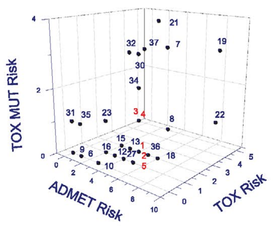


Fig. 10. Relationships between computed ADMET Risk, TOX Risk and TOX MUT Risk of investigated X drugs

Table 2. Computed MDs and ADMET parameters of investigated molecules **1 – 37**

No.	Molecule	MLogP	S logP	TPSA	Mr	V	Rule of 5	ADMET Risk	S + Absn Risk	CYP Risk	TOX Risk	TOX MUT Risk	TOX hERG	TOX Rat	TOX BRM Rat	TOX BRM Mouse	GPCR l dls	ICM dls	KI dls	NRL dls	PI dls	EI dls	Molecular polarizability	Molecular refractivity
1	Retinoic acid	3.974	6.424	37.3	300.444	385	0	4	1	0	2	0	4.521	872.038	172.901	612.54	0.00	0.14	-0.18	1.13	-0.03	0.58	36.17	97.791
2	Retinal	4.449	6.621	17.07	284.445	378	1	4	2	0	2	0	4.592	2616.43	91.048	408.141	-0.15	0.15	-0.23	0.90	0.08	0.52	35.79	96.865
3	Retinol	4.531	6.329	20.23	286.461	385	1	4	2	0	2	1	4.435	986.011	107.927	271.047	-0.01	0.32	-0.25	1.02	0.16	0.66	36.35	97.923
4	Etretinate	4.297	6.974	35.53	354.493	441	1	4	2	0	2	1	4.9	1924.209	86.535	278.425	-0.17	0.01	-0.27	0.67	-0.05	0.31	44.48	118.201
5	Tazarotene	3.472	6.042	39.19	351.47	378	0	5	2	1	1	0	4.897	886.186	8.056	334.241	0.21	-0.07	0.02	0.61	0.06	0.37	39.30	97.882
6	Misoprostol	2.625	3.681	74.6	366.545	462	0	1	1	0	0	0	4.899	3770.578	15.334	1044.264	0.47	0.08	-0.49	0.43	0.11	0.38	41.66	106.662
7	Leflunomide	2.369	2.394	55.13	270.212	234.5	0	4	0	0	4	3	4.108	326.135	1.313	287.084	-0.16	-0.07	-0.01	-0.23	-0.31	-0.20	21.99	64.157
8	Methotrexate	-0.313	-0.691	210.54	454.448	441	2	7	4	1	2	1	3.643	1370.863	48.172	168.425	0.51	0.23	0.38	-0.38	0.27	0.72	43.52	119.211
9	Estradiol	3.635	3.619	40.46	272.39	301	0	0	0	0	0	0	4.221	2752.068	216.78	861.062	0.18	0.20	-0.36	0.95	-0.02	0.61	31.31	79.905
10	Ethynyl estradiol	4.004	3.679	40.46	296.412	329	0	3	1	2	0	0	4.336	1369.945	189.637	894.289	0.33	0.17	-0.24	1.18	0.03	0.61	33.90	87.374
11	Mestranol	4.227	4.101	29.46	310.439	350	1	5	2	2	1	0	4.428	1096.787	26.347	253.23	0.24	0.05	-0.30	1.01	0.00	0.49	33.90	87.374
12	Estron sulfate	2.975	1.534	80.67	350.436	350	0	3	0	1	1	0	4.468	872.248	100.863	1057.492	0.42	0.17	-0.44	0.62	0.62	0.96	35.49	89.074
13	Methyltestosteron	3.923	3.624	37.3	302.46	350	0	3	0	1	2	0	4.431	1549.547	13.374	604.365	-0.03	-0.14	-0.86	1.17	-0.13	0.57	35.10	89.068
14	Fluoxymestrone	3.2	2.557	57.53	336.45	360.5	0	3	0	1	2	0	3.96	733.015	2.047	620.704	0.04	-0.11	-0.85	1.71	0.55	0.72	35.43	90.189
15	Medroxyprogesterone	3.433	3.561	54.37	344.497	392	0	2	0	0	2	0	4.506	1047.676	4.555	854.445	-0.01	-0.26	-0.89	1.01	-0.06	0.54	38.86	98.654
16	Norgestrel	4.05	3.409	37.3	312.455	357	0	2	0	1	1	0	4.636	728.799	9.323	496.223	0.24	0.10	-0.59	1.28	-0.04	0.67	35.85	92.026
17	Norethisterone	3.827	2.995	37.3	298.428	336	0	2	0	1	1	0	4.458	807.728	7.31	502.914	0.19	0.08	-0.54	1.37	-0.09	0.71	34.00	87.424
18	Mifepristone	4.795	5.027	40.54	429.606	490	1	6	2	2	2	0	5.265	329.739	3.381	373.834	0.10	-0.02	-0.47	1.35	0.05	0.25	50.04	132.581
19	Danazol	4.154	3.604	46.26	337.465	364	1	8	2	1	5	3	4.416	139.981	2.197	90.709	0.31	0.30	-0.24	1.14	-0.10	0.62	37.66	98.535
20	Chenodiol	3.661	3.772	77.76	392.583	455	0	2	1	0	1	0	4.361	2133.29	139.943	553.347	0.33	0.33	-0.39	0.86	0.29	0.68	43.77	109.274
21	Raloxifene	3.437	5.485	70	473.594	483	0	6	2	1	2	4	6.007	907.258	205.835	76.848	0.04	-0.14	-0.19	0.43	-0.10	0.09	52.98	136.997
22	Chlomiphene	5.67	7.001	12.47	405.971	465.5	1	9	1	2	4	1	7.196	658.548	78.846	17.415	0.15	-0.17	-0.20	0.16	-0.16	0.07	48.23	133.761
23	Dienestrol	3.879	4.506	40.46	266.342	308	0	2	1	0	1	1	5.168	1683.752	263.432	418.767	0.02	0.09	-0.09	0.25	-0.11	0.13	31.89	84.357
24	Diethylstilbestrol	3.956	4.746	40.46	268.358	315	0	1	1	0	0	0	5.424	1849.518	268.131	832.918	0.16	0.04	0.05	0.49	-0.07	0.24	32.12	83.241
25	Simvastatin	4.184	6.876	52.6	416.605	497	1	3	2	1	0	0	4.862	1686.334	58.286	618.311	0.30	0.09	-0.34	0.42	-0.08	0.56	47.48	120.760
26	Lovastatin	3.98	6.459	52.6	402.578	476	0	3	2	1	0	0	4.954	2080.163	69.868	588.499	0.23	0.06	-0.33	0.37	-0.08	0.46	45.63	116.259
27	Parvastatin	3.192	5.301	83.83	420.593	504	0	4	3	0	1	0	5.065	1096.966	141.852	785.705	0.28	0.05	-0.32	0.48	-0.01	0.47	47.95	122.630
28	Fluvastatin	2.476	3.592	82.69	411.476	437.5	0	1	0	0	0	0	5.007	320.844	79.321	156.445	0.48	0.14	0.39	0.47	0.11	0.78	46.04	114.855
29	Atorvastatin	3.692	4.749	111.79	558.654	598.5	1	6	2	1	2	0	5.133	378.333	52.325	135.165	0.07	-0.41	-0.08	-0.07	0.01	0.18	62.83	159.623
30	Ribavirin	-1.583	-1.805	143.72	244.208	210	0	4	2	0	2	3	2.642	1660.253	25.191	31.524	0.31	0.21	-0.21	-1.45	-0.20	0.71	20.51	64.574
31	Quinine	2.415	3.624	45.59	340.468	392	0	0	0	0	0	1	5.574	422.812	6.634	90.607	0.38	0.37	-0.05	0.10	0.18	0.11	38.35	94.694
32	Methysergide	1.904	2.393	57.5	367.494	406	0	3	0	1	2	3	5.306	303.561	2.638	96.91	0.69	-0.05	0.20	-0.17	0.28	0.28	42.90	109.111
33	Warfarin	2.767	3.034	54.37	306.364	329	0	3	0	1	1	0	5.084	486.019	24.42	517.755	-0.49	-0.31	-0.92	-0.38	-0.39	-0.16	34.51	90.586
34	Flurazepam	3.794	4.192	35.91	387.887	413	0	4	0	2	2	2	6.251	915.972	43.045	16.424	0.45	0.41	-0.26	-0.17	0.10	0.00	40.72	107.545
35	Temazepam	2.953	2.278	52.9	300.746	297.5	0	1	0	1	0	1	4.893	1723.908	11.206	33.083	0.36	0.34	-0.25	-0.07	0.21	0.22	30.95	81.010
36	Triazolam	4.16	3.074	43.07	343.217	322	1	5	2	1	2	0	5.414	1776.856	17.612	5.256	0.73	0.65	-0.34	-0.51	-0.38	-0.19	35.28	103.684
37	Thalidomide	0.735	0.172	83.55	258.235	231	0	3	0	0	3	3	4.661	715.799	7.644	527.637	-0.28	-0.56	-0.70	-0.88	-0.13	-0.12	24.01	64.325

MLogP - lipophiliciy, TPSA - topological polar surface area, M_r - relative molecular mass, V – volume, Rule of 5 - absorption filter designed by Chris Lipinski et. al. , ADMET Risk - a global ris k score, S+Absn Risk - low absorption risk, CYP Risk - MET Risk with inclusion of the CYP substrate rules, TOX Risk - risk of overall toxicity, including mutagenicity, TOX MUT Risk - risk of mu tagenicity in *S. typhimurium*, TOX hERG - inhibition of the hERG potassium channel in human expressed as pIC50 in mol/L), TOX RAT - acute lethal toxicity in rat, TOX BRM Rat, - TD50 value of a particular compound in units of mg/kg/day, TD 50 - dose of a au bstance administered orally to rats over the course of their lifetimes that results in the appearance of tumors in 50 percent o f their population) , TOX BRM Mouse - TD50 value in mice mg/kg/day, dls – drug-likeness score, GPCR l = G protein-coupled receptor l igand; ICM = ion channel modulator; KI = kinase inhibitor; NRL = nuclear receptor ligand; PI = protease inhibitor; EI = enzyme inhibitor.

METHODS

A) Teratoma development

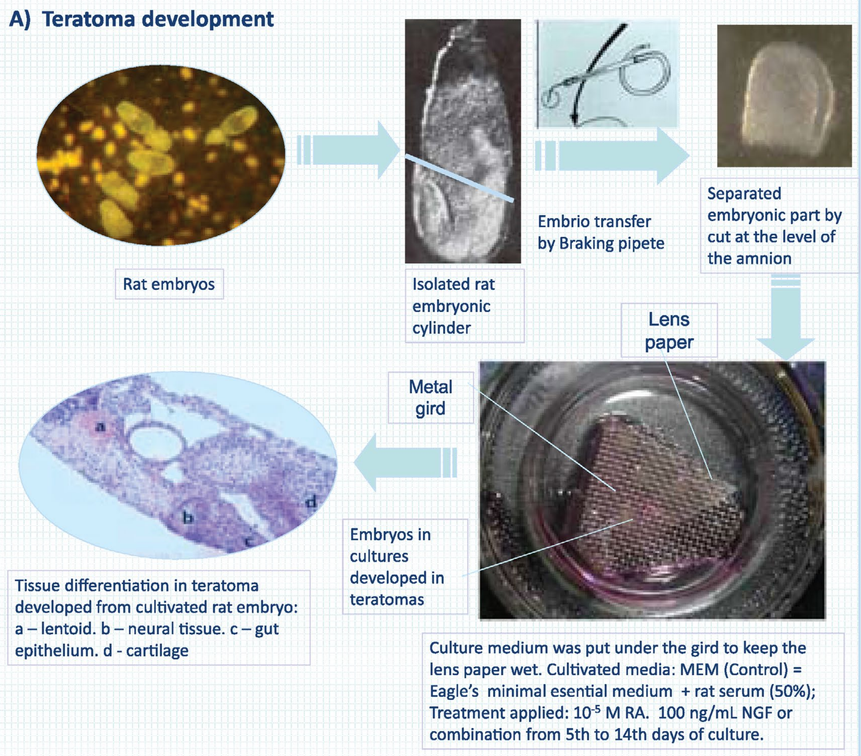


Fig. 1. Schematic display of teratoma development

B) Correlation studies

Drugs (n = 37) in a series of pregnancy category X-drugs. labelled by FDA as contraindicated in pregnancy. were used in this study. Computed MDs and ADMET parameters of toxicity of investgated molecules were predicted by ADMET Predictor Module™ 6.5 (Simulations Plus. Inc. USA). Drug-likeness scores were computed by Molinspiration Bioactivity Score (v2011.06) (www-molinspiration.com). Molecular refractivity and molecular polarizability, as well as Hickel analysis (pI energy) were computed by ChemAxon software (www.chemicalize.org). All correlation analyses were performed using OriginPro 8.0 software (Origin Laboratories. USA).

RESULTS AND CONCLUSIONS

- Mammalian embryo-derived teratomas investigation in organ culture (Fig. 1) treated with with either retinoic acid (RA, $c = 10^{-5}$ mol/L) or neural growth factor (NGF, 100 ng/mL) showed no influence on neural tissue differentiation.
- The combination of RA ($c = 10^{-5}$ mol/L) and NGF (100 ng/mL) influenced the neural tissue differentiation (100%) and the total inhibition of epidermis, gland epithelium and cartilage. (Table 1)
- The same effects were observed in experiments of teratoma development treated with RA alone in concentration, $c = 10^{-4}$ mol/L.
- The results in this *in vitro* model revealed the potential teratogenicity of RA in higher concentrations (c = 10^{-4} mol/L) or in combination with NGF.
- Computed MDs and ADMET parameters are listed in Table 2.
- Drug-likeness scores (dls) analysis revealed that 73 % of investigated molecules are with positive NRL dls and EI dls. Out of total, 45.9% of molecules were with 0.5 – 1.7 NRL dls and EI dls, while with scores 0.2 – 0.49 were 19% of NRL dls and 24% of EI dls (Table 3, Fig. 2). GPCR l dls with dls > 0.5 (8%) and dls between 0.2 and 049 (44%) were also computed.
- Linear correlations were obtained between M_r and V (Fig. 3), also between molecular polarizability and molecular refractivity (Fig. 4). Insignificant collinearities were obtained with NRL dls *v.s.* MLogP and pI energy. However NRL dls found to increase with increasing of MLogP and decreasing of pI (Fig. 5 and Fig. 6, respectively).
- In correlations of MLog P and TPSA with TOX hERG, a two sets of molecules were observed, a small subgroup with high collinear relationship between TOX hERG either with MLogP (Fig. 7) or TPSA (Fig. 8), while the other set represents a cluster of diverse molecules including retinoids. It was also found the exponential decrease of TOX hERG with the NRL dls decreasing (Fig. 9).
- For all retinoids (**1** – **5**) included in this study the predicted ADMET Risk was 4, CYP Risk 0 and TOX Risk 2, while TOX MUT Risk 1 were predicted for retinol (**3**) and etretinate (**4**) (Table 2, Fig. 10).
- Pregnancy category X drugs have a potential not only for teratogenicity but also for other ADRs, therefore their prescription and the use in treatment of patients should be with extreme caution.

References

1. Kam, R. K. T., Deng, Y., Chen, Y., Zhao, H., 2012, Retinoic acid synthesis and functions in early embryonic development. Cell & Bioscience **2**, 1-14.
2. Duester, G., 2008, Retinoic acid synthesis and signaling during early organogenesis. Cell 134, 921-931.
3. Germain, P., Chambon, P., Eichele, G., Evans, R. M., Lazar, M. A., Leid, M., De Lera, A. R., Lotan, R., Mangelsdorf, D. J., Gronemeyer, H., 2006, International Union of Pharmacology. LX. Retinoic acid receptors. Pharmacol Rev 58, 712–725.
4. Turton, J. A., Willars, G. B., Haselden, J. N., Ward, S. J., Steele, C. E., Hickst, R. M., 1992, Comparative teratogenicity of nine retinoids in the rat. Int J. Exp. Path. 73, 551-563.
5. Teelmann, K., 1998, Retinoids: Toxycology and Teratogenicity to date, Pharmac. Ther. 40, 29-42.
6. Briggs, G. G., Freeman, R. K., Yaffe, S. J., 2011, Drugs in pregnancy and lactation: A reference guide to fetal and neonatal risk. 9th ed., Lippincott Williams & Wilkins Publishers.
7. Crnek-Kunstelj, V., Ester, K., Stipic, J., 2002, Role of retinoic acid and DNA methylation in differentiation of mammalian embryo *in vitro*. Period. Biol. 104, 55-66.
8. Crnek-Kunstelj, V., Stipić, J., Stipić-Marković, A., 2001, Differentiation in embryo-derived teratomas *in vitro* can be mediated by retinoic acid and dibutyryl-cAMP. Libri. Oncol. 29, 133-141.

ACKNOWLEDGEMENTS - The authors acknowledge to the Ministry of Science, Education and Sports of the Repubic of Croatia, Grant no. 006-0982929-2940 and for the financial support of the University of Zagreb.

Contact e-mail: jmtmilena@gmail.com