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In Silico Predictions for Improving Permeability Properties of Principal Anticancer Anthracyclines through Structural Modification

Radu TAMAIAN, Violeta NICULESCU, Mihai ANGHEL

Research and Development Department, National Research and Development Institute for Cryogenics and Isotopic Technologies – ICIT Rm. Vâlcea, 4 Uzinei Street, VL 240050, Râmnicu Vâlcea, Romania; tradu@icsi.ro

Abstract. A method, probably the best one, to improve anticancer drugs permeability is structural modification on the base of "drug-likeness" fundamental concepts in the way of reducing ionizability and polarity, reducing number of hydrogen bond donors or acceptors and increasing lipophilicity. The present work looks forward to modify structure of major anticancer anthracyclines in order to improve their permeability, with a final aim to increase absorption and oral bioavailability. SmiLib v2.0 was used first to build a combinatorial library, by virtual reactions of building blocks (from antimetabolite and/or antineoplastic drugs) with scaffold molecules (the tetracene-5,12-dione moiety of anthracyclines). Second, ADME-Tox web-based software tool (hosted on the server of the Ressource Parisienne en Bioinformatique Structurale) was used to mass-computation of the most relevant physicochemical properties for "drug-likeness" and oral bioavailability for all anthracycline virtual derivates from combinatorial library. Qualified derivates as potential drugs were tested for genotoxicity and acute toxicity with Tox Boxes version 2.0. Sixteen from 160 derivates created with SmiLib v2.0 were qualified as "drug-like" compounds with good oral bioavailability. *In silico* toxicity tests showed that all derivates have good values for oral administration. One derivate presented a smaller genotoxicity and much better values for acute toxicity at oral, subcutaneous and intraperitoneal administration than any of anthracyclines. Only at intravenous administration this derivate showed certain acute toxicity, but the aim of the study was to improve the oral bioavailability.

Keywords: anthracycline drugs, genotoxicity, oral bioavailability, LD_{50}, permeability.

INTRODUCTION

Anthracyclines (anthracycline antibiotics) are the largest class of quinones with tetracene-5,12-dione moiety, effective against more types of cancer than any other class of chemotherapy agents and some of the most effective anticancer chemotherapy agents (Minotti *et al.*, 2004; Velíšek *et al.*, 2007; Weiss, 1992).

However, the anthracyclines-based chemotherapy has two major disadvantages: the low permeability (a determinant of intestinal absorption and oral bioavailability) and their high toxicity. Permeability is a necessary process for drug's absorption in the intestine, passage through restrictive organ barriers (especially blood-brain barrier), penetration into cells (reaching at intracellular therapeutic target), elimination by the liver and excretion by the kidneys (Kerns and Di, 2008). The most important permeability mechanism for drug discovery is passive diffusion, Mandagere *et al.* (2002) estimating that 95% of commercial drugs are predominantly absorbed in the gastrointestinal tract by passive diffusion.

In the last years, computational techniques have an increase development, due to the enormous cost to bring a drug to the market. Recent estimations have showed that typical cost of experiments per compound started from 10 USD for computer modeling, continuing with

400 USD for biochemical assay, and afterwards graduated increasing to 500,000,000 USD for human clinical trial (Young, 2009).

Aiming to increase absorption and bioavailability and decrease toxicity, current work attempts to improve anthracyclines anticancer drugs permeability by virtual structural modification. This study takes in consideration virtual structural modification of principal approved anthracyclines for cancer therapy: daunorubicin (DNM), doxorubicin (DOX), epirubicin (EPI), idarubicin (IDA), valrubicin (VAL) – DNA topoisomerase IIα (TOP2A) inhibitors.

MATERIALS AND METHODS

The research has been carried out *in silico* using combinatorial library software – SmiLib v2.0 (Schüller *et al.*, 2003) – to build a virtual library, for the derivates of the five anthracyclines: daunorubicin, doxorubicin, epirubicin, idarubicin, valrubicin. Virtual combinatorial library was created with the help of SmiLib v2.0, which works with three classes of molecules: scaffold molecules (Markush structures of molecules that contain R-groups – sites of variability), building blocks (small Markush molecules) and linkers (connectors between scaffold molecules and building blocks). In order to reduce molecular weight, a metric of oral bioavailability and of "drug-likeness" according to Lipinski *et al.* (1997), from the beginning of this study, only tetracene-5,12-dione moiety was used as backbone molecule for virtual derivates. Because of structural similarity of anthracyclines, it resulted only two types of backbone molecules with tetracene-5,12-dione moiety: one common to DNM, DOX, EPI and VAL, and another for IDA (Fig. 1).

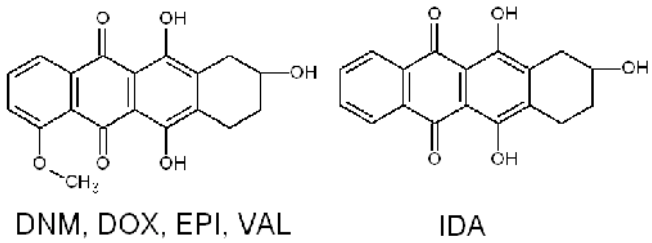

Fig. 1. The two types of backbone molecules with tetracene-5,12-dione moiety

For scaffold molecules there were taken in consideration two types of backbone molecules with tetracene-5,12-dione moiety (Fig. 1) and there were imposed two possible positions for sites of variability (R-groups: R_1 and R_2). Because the aim of the study was to improve permeability, the scaffold molecules were designed based on minimal molecular weight, assuming only one site of variability for each scaffold molecule – a total number of four structures had resulted (Tab. 1, Fig. 2).

Table 1

Enhanced SMILES strings for scaffold molecules (SM)

No.	Code	Enhanced SMILES strings for SM
1	SM DDEV-R1	COc:3:c:c:c:c:c:4:c(=O):c:2:c(O):c:1CC(O)CC([R1])c1:c(O):c2:c(=O):c34
2	SM DDEV-R2	COc:3:c:c:c:c:c:4:c(=O):c:2:c(O):c:1CC(O)([R1])CC(O)c1:c(O):c2:c(=O):c34
3	SM I-R1	O=c:3:c:1:c:c:c:c:c:c1:c(=O):c:4:c(O):c2:c(CC(O)CC2[R1]):c(O):c34
4	SM I-R2	O=c3c1ccccc1c(=O)c4c(O)c2c(CC(O)([R1])CC2O)c(O)c34

Note: SMILES is the abbreviation for Simplified Molecular Input Line Entry System . SmiLib v2.0 mandatory requires a [R1] label for each SM with a single site of variability.

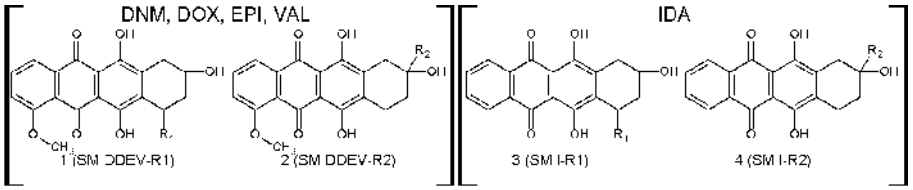

Fig. 2. The structure of scaffold molecules (SM). R_1 and R_2 marks the positions imposed to the sites of variability [R1]

To decrease molecular weight SmiLib v2.0 concatenated scaffolds with building blocks using a dummy (empty) linker: [A][R], where [A] is linker's attachment site and [R] is linker's site of variability. Building blocks were made on the base of structural motifs most frequently found in some antimetabolite/antineoplastic drugs (Fig. 3, Tab. 2).

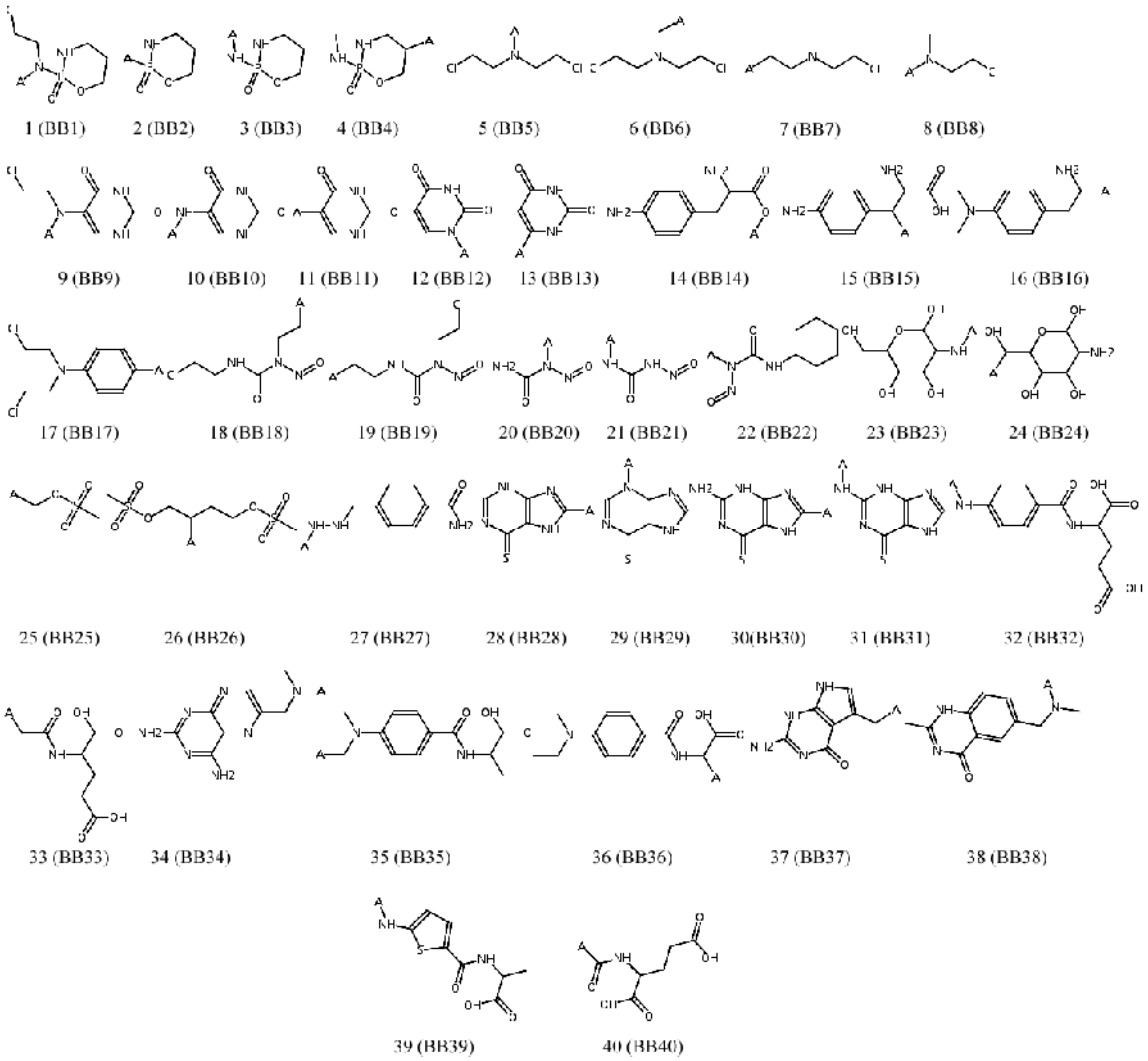

Fig. 3. The structure of building blocks (BB); [A] represents the attachment sites

Table 2

Enhanced SMILES strings for building blocks (BB)

No.	Code	Enhanced SMILES strings for BB	BB origin / anticancer drug category
1	BB1	O=P1(N([A])CCCl)NCCCO1	cyclophosphamide / AnAg-Alk
2	BB2	O=P1([A])NCCCO1	cyclophosphamide / AnAg-Alk
3	BB3	O=P1(N[A])NCCCO1	cyclophosphamide / AnAg-Alk
4	BB4	CNP1(=O)NCC([A])CO1	cyclophosphamide / AnAg-Alk
5	BB5	ClCCN([A])CCCl	mechlorethamine / AnAg-Alk
6	BB6	ClCCN(C[A])CCCl	mechlorethamine / AnAg-Alk
7	BB7	CN(CCCl)CC[A]	mechlorethamine / AnAg-Alk
8	BB8	CN([A])CCCl	mechlorethamine / AnAg-Alk
9	BB9	O=c:1:[nH]:c:c(N([A])CCCl):c(=O):[nH]1	uramustine / AnAg-Alk
10	BB10	O=c:1:[nH]:c:c(N[A]):c(=O):[nH]1	uramustine / AnAg-Alk
11	BB11	O=c:1:[nH]:c:c([A]):c(=O):[nH]1	uramustine / AnAg-Alk
12	BB12	O=c:1:c:c:n([A]):c(=O):[nH]1	uramustine / AnAg-Alk
13	BB13	O=c:1:c:c([A]):[nH]:c(=O):[nH]1	uramustine / AnAg-Alk
14	BB14	Nc:1:c:c:c(CC(N)C(=O)O[A]):c:c1	melphalan / AnAg-Alk
15	BB15	Nc:1:c:c:c(C([A])C(N)C(=O)O):c:c1	melphalan / AnAg-Alk
16	BB16	CN(C)c:1:c:c:c(CC(N)[A]):c:c1	melphalan / AnAg-Alk
17	BB17	ClCCN(CCCl)c:1:c:c:c([A]):c:c1	melphalan / AnAg-Alk
18	BB18	O=NN(CC[A])C(=O)NCCCl	carmustine / AnAg-Alk
19	BB19	O=NN(CCCl)C(=O)NCC[A]	carmustine / AnAg-Alk
20	BB20	NC(=O)N([A])N=O	carmustine / AnAg-Alk
21	BB21	O=NNC(=O)N[A]	carmustine / AnAg-Alk
22	BB22	O=NN([A])C(=O)NC1CCCCC1	lomustine / AnAg-Alk
23	BB23	OCC1OC(O)C(N[A])C(O)C1O	streptozotocin / AnAg-Alk
23	BB24	NC1C(O)OC(C(O)[A])C(O)C1O	streptozotocin / AnAg-Alk
25	BB25	CS(=O)(=O)OC[A]	busulfan / AnAg-Alk
26	BB26	CS(=O)(=O)OCCC([A])COS(C)(=O)=O	busulfan / AnAg-Alk
27	BB27	NC(=O)c:1:c:c:c(CNN[A]):c:c1	procarbazine / AnAg-Alk
28	BB28	S=c:1:n:c:[nH]:c:2:n:c([A]):[nH]:c12	mercaptopurine / AmAnAg-Imm
29	BB29	S=c:1:n:c:n([A]):c:2:n:c:[nH]:c12	mercaptopurine / AmAnAg-Imm
30	BB30	Nc:2:n:c(=S):c:1:[nH]:c([A]):n:c1:[nH]2	thioguanine / AmAnAg
31	BB31	S=c:1:n:c(N[A]):[nH]:c:2:n:c:[nH]:c12	thioguanine / AmAnAg
32	BB32	O=C(O)CCC(NC(=O)c:1:c:c:c(N[A]):c:c1)C(=O)O	methotrexate / AmAnAg-Imm
33	BB33	O=C(O)CCC(NC(=O)C[A])C(=O)O	methotrexate / AmAnAg-Imm
34	BB34	CN([A])Cc:2:c:n:c:1:n:c(N):n:c(N):c1:n2	methotrexate / AmAnAg-Imm
35	BB35	CC(NC(=O)c:1:c:c:c(N(C)C[A]):c:c1)C(=O)O	methotrexate / AmAnAg-Imm
36	BB36	CCN(C)c:1:c:c:c(C(=O)NC([A])C(=O)O):c:c1	methotrexate / AmAnAg-Imm
37	BB37	Nc:2:n:c(=O):c:1:c(C[A]):c:[nH]:c1:[nH]2	pemetrexed / AmAnAg
38	BB38	Cc:2:n:c(=O):c:1:c:c(CN(C)[A]):c:c:c1:[nH]2	raltitrexed / AmAnAg
39	BB39	CC(NC(=O)c:1:c:c:c(N[A]):s1)C(=O)O	raltitrexed / AmAnAg
40	BB40	O=C(O)CCC(NC(=O)[A])C(=O)O	raltitrexed / AmAnAg

Note: [A] marks the attachment sites from building blocks. AnAg-Alk stands for antineoplastic agents, alkylating. AmAnAg-Imm stands for antimetabolite antineoplastic agents, immunosuppressant. AmAnAg stands for antimetabolite antineoplastic agents.

All the three classes of molecules (scaffold molecules, empty linker and building blocks) were manually loaded in SmiLib v2.0 as enhanced SMILES strings in order to build the combinatorial library of virtual derivatives. Resulted combinatorial library was tested for "drug-likeness" and oral bioavailability with ADME-Tox web-based software tool (hosted on the server of Ressource Parisienne en Bioinformatique Structurale, RPBS:

http://bioserv.rpbs.jussieu.fr/). ADME-Tox web-based tool software allowed selecting tested parameters and imposing a series of conditions for "drug-likeness" and oral bioavailability. Tested parameters referred to molecular weight (MW), number of hydrogen donors (Drs), number of hydrogen acceptors (Ars), number of flexible (rotatable) bonds (FB), calculated octanol/water partition coefficient (logP) and polar surface area (PSA). For calculated octanol/water partition coefficient it was used the method described by Wang *et al.* (1997, 2000). For PSA calculation, ADME-Tox web-based tool software used the method of a topological polar surface area (Ertl *et al.*, 2000). Based on "drug-likeness" metrics from literature, including the Lipinski rule of fives (Lipinski *et al.*, 1997) for oral bioavailability, there were imposed six conditions for the virtual derivatives. The conditions represent a combination between the most tolerant values and limits from literature for physicochemical properties: MW (Lipinski *et al.*, 1997), Drs (Lipinski *et al.*, 1997; Oprea *et al.*, 2005), Ars (Lipinski *et al.*, 1997), FB (Oprea *et al.*, 2005), logP (Lipinski *et al.*, 1997) and PSA (Palm *et al.*, 1997). For comparative reasons, the same conditions were imposed to all five anthracyclines. All derivates qualified as potential drugs with a good permeability (increased absorption and oral bioavailability) were in depth analyzed for toxicity. Tox Boxes version 2.0, web-based tool software from Pharma Algorithms, Inc., (http://pharma-algorithms.com/webboxes/) predicted probabilities for basic toxicity endpoints of each anthracycline and qualified derivate.

RESULTS AND DISCUSSION

The combinatorial library, created by virtual reactions of the four SM with forty BB connected themselves by empty linkers, contains 160 virtual anthracycline derivates, saved as SMILES strings and SD files (data not showed for unqualified derivates). SmiLib v2.0 created all the virtual derivates using the following syntax: *SMno.linker1_BBno*, where *SMno* is the identifier (number) assigned to each SM (see Tab. 1 and Fig. 2), *linker1* represents the empty linker, and *BBno* comes from the identifier (number) assigned to each BB (see Tab. 2 and Fig. 3). SM identifiers and linker/building block groups were separated by ".", linker and building block identifiers were separated by "_". ADME-Tox web-based tool software calculated molecular properties of anthracyclines (Tab. 3) and qualified, using imposed conditions, as potential drug candidates with good oral bioavailability only 16 of 160 derivatives from combinatorial library (Tab. 4).

Table 3

Molecular (physicochemical) properties of anthracyclines

Property	MW	Drs	Ars	FB	logP	PSA
DNM	527.3	5	11	4	0.56	185.84
DOX/EPI	543.3	6	12	5	-0.04	206.07
VAL	723.4	5	14	12	2.63	215.22
IDA	497.3	5	10	3	0.65	176.61

Note: DOX and EPI have the same SMILES strings and 2D structures; the difference between the two anthracyclines is the spatial orientation of the hydroxyl group at the 4' carbon of the sugar.

Table 3 shows that only IDA accomplishes at least three conditions of Lipinski rule of fives (those for MW, Drs and logP), indicating a good bioavailability according to Lipinski *et al.* (1997). In addition, values for PSA over-exceed, for all anthracyclines, the limit imposed by Palm *et al.* (1997). However, values of metrics from Lipinski rule of fives and PSA predict only intestinal passive absorption and not the active transport and this is reason why

anthracyclines have, in general, a low oral bioavailability, being not enough lipophilic to enter the blood-stream through passive intestinal absorption in small intestine, according to Kerns and Di (2008) considerations.

Table 4

Molecular (physicochemical) properties of qualified virtual anthracycline derivates

Property and limit	MW ≤ 500 Da	Drs ≤ 5	Ars ≤ 10	FB ≤ 10	logP ≤5	PSA ≤ 140Å^2
1.1_5	480.2	3	7	6	3.13	107.30
1.1_6	494.2	3	7	7	3.05	107.30
1.1_7	459.7	3	7	6	2.64	107.30
1.1_8	431.7	3	7	4	2.36	107.30
2.1_5	496.2	4	8	6	2.55	127.53
2.1_7	475.7	4	8	6	2.01	127.53
2.1_8	447.7	4	8	4	1.78	127.53
3.1_5	450.2	3	6	5	3.21	98.07
3.1_6	464.2	3	6	6	3.14	98.07
3.1_7	429.7	3	6	5	2.73	98.07
3.1_8	401.7	3	6	3	2.45	98.07
3.1_16	472.3	4	7	4	3.09	124.09
4.1_5	466.1	4	7	5	2.63	118.30
4.1_6	480.2	4	7	6	2.50	118.30
4.1_7	445.7	4	7	5	2.09	118.30
4.1_8	417.7	4	7	3	1.87	118.30

Table 4 indicates that all four SM were equally used in structures of qualified derivates. In the same time, the two types of backbone molecules with tetracene-5,12-dione moiety were equally used in resulted qualified derivates. Analyzing BB usage, it can be observed an unequal distribution of the forty structures in resulted qualified derivates: identifiers 5, 7 and 8 appear each of them in four structures; identifier 6 appears in three structures; identifier 16 appears only in one new structure. Comparing Tab. 4 with Tab. 2 and Fig. 3, it can be remarked that all BB have origin in two alkylating antineoplastic agents: mechlorethamine and melphalan. Moreover, BB from mechlorethamine appeared in fifteen new structures and melphalan in only one structure. All 16 derivates accomplishes at least three of conditions of Lipinski rule of fives (those for MW, Drs and logP) and for PSA limit, indicating a good bioavailability according to Lipinski *et al.* (1997) and Palm *et al.* (1997).

Table 5 presents Tox Boxes version 2.0 results for each qualified derivates for genotoxicity predictions and acute toxicity (LD_{50}). Qualified derivates with DNM, DOX, EPI and VAL common backbone were tested against corresponding anthracyclines; qualified derivates with IDA backbone were tested against IDA.

Tab. 5

Genotoxicity predictions and acute toxicity for anthracyclines and qualified derivates

Codes	G_A	Acute toxicity on mouse							
		LD_{50} (mg/kg)				Log LD_{50} (pLD_{50})			
		ip	o	iv	sc	ip	o	iv	sc
DNM	1.000	5.8	100	16	22	1.96	0.71	1.53	1.39
DOX/EPI	1.000	19	210	35	66	1.46	0.41	1.19	0.91
VAL	1.000	170	590	91	210	0.64	0.09	0.90	0.53
1.1_5	1.000	20	200	19	61	1.37	0.38	1.40	0.90
1.1_6	1.000	7.6	120	11	29	1.81	0.61	1.65	1.23
1.1_7	1.000	21	190	19	57	1.34	0.38	1.39	0.91
1.1_8	1.000	28	240	17	81	1.19	0.25	1.41	0.73
2.1_5	1.000	12	140	20	53	1.61	0.56	1.39	0.97
2.1_7	0.999	27	210	16	95	1.25	0.36	1.48	0.70
2.1_8	1.000	31	200	36	95	1.16	0.34	1.09	0.67
IDA	0.999	6.5	100	18	26	1.88	0.64	1.43	1.28
3.1_5	1.000	20	220	23	76	1.35	0.30	1.29	0.77
3.1_6	1.000	9	140	13	36	1.71	0.53	1.55	1.11
3.1_7	0.999	23	220	22	69	1.28	0.30	1.28	0.79
3.1_8	1.000	41	270	34	99	0.99	0.18	1.08	0.61
3.1_16	0.987	200	780	49	430	0.38	-0.22	0.99	0.04
4.1_5	1.000	15	160	24	65	1.51	0.48	1.28	0.85
4.1_6	1.000	7.7	110	9.1	41	1.80	0.62	1.72	1.06
4.1_7	0.999	31	230	34	120	1.16	0.28	1.12	0.58
4.1_8	0.999	36	230	43	120	1.07	0.26	0.98	0.55

Note: G_A represents genotoxicity (probability of positive Ames test). LD_{50} and pLD_{50} were calculated for intraperitoneal (ip), oral (o), intravenous (iv) and subcutaneous (sc) administration. Underline values represents the highest toxicity values.

Table 5 shows that all seven derivates with DNM, DOX, EPI and VAL common backbone have good values for acute toxicity for oral administration. However, VAL presents a smaller acute toxicity for all acute toxicity tests that any of other anthracyclines and corresponding derivates does. Genotoxicity predictions were the same for DNM, DOX, EPI, VAL and their derivates. On the other hand, the IDA's derivate 3.1_16 presented a small improvement of genotoxicity value. Acute toxicity predictions for 3.1_16 showed a very low toxicity for subcutaneous and oral predictions (better than IDA and VAL). A good value has been achieved for intraperitoneal administration of 3.1_16, also better than IDA and VAL. At intravenous administration, 3.1_16 presents certain toxicity, being less toxic than IDA, but having a greater value than VAL. All nine IDA's derivates present good values for oral and subcutaneous administration.

CONCLUSIONS

Structural modification of anthracyclines' backbone to improve their permeability generated a combinatorial library with 160 derivates. Sixteen derivates have shown "drug-like" characteristics and a good oral bioavailability. All those 16 derivates are safe for oral administration, according to computational predictions. Moreover, all IDA's derivates present

good values for subcutaneous administration. One of IDA's derivates (3.1_16) shows better values that any of the anthracyclines for oral, subcutaneous and intraperitoneal administration and certain toxicity for intravenous administration does. *In silico* docking studies will be considered for the evaluation of the new derivates binding in the active site of TOP2A (because of their backbone molecules with tetracene-5,12-dione moiety found in anthracyclines) or DNA binding (because of their BB origins from mechlorethamine and melphalan).

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