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11a-*N*-tosyl-5-carbapterocarpans: Synthesis, antineoplastic evaluation and *in silico* prediction of ADMETox properties

Joseane A. Mendes[a,1], Eduardo J. Salustiano[b,c,1], Carulini de S. Pires[d], Thaís Oliveira[e], Julio C.F. Barcellos[f], Jhonny M.C. Cifuentes[a], Paulo R.R. Costa[f], Magdalena N. Rennó[d,*], Camilla D. Buarque[a,*]

[a] *Departamento de Química, Pontifícia Universidade Católica do Rio de Janeiro, Rua Marquês de São Vicente, 225, Gávea, Rio de Janeiro, RJ 22435-900, Brazil*
[b] *Laboratório de Imunologia Tumoral, Instituto de Bioquímica Médica Leopoldo de Meis, Centro de Ciências da Saúde, Bloco H sala 003, Universidade Federal do Rio de Janeiro, RJ 21941-590, Brazil*
[c] *Laboratório de Glicobiologia, Instituto de Biofísica Carlos Chagas Filho, Centro de Ciências da Saúde, Bloco C sala C1-042, Universidade Federal do Rio de Janeiro, RJ 21941-590, Brazil*
[d] *Laboratório de Modelagem Molecular e Pesquisa em Ciências Farmacêuticas, Núcleo em Ecologia e Desenvolvimento Sócio-Ambiental de Macaé, Universidade Federal do Rio de Janeiro Campus Macaé Professor Aloísio Teixeira, Macaé, RJ 27965-045, Brazil*
[e] *Laboratório de Bioquímica e Biologia Molecular do Câncer, Instituto de Bioquímica Médica Leopoldo de Meis, Centro de Ciências da Saúde, Bloco C sala E1-022, Universidade Federal do Rio de Janeiro, RJ 21941-590, Brazil*
[f] *Laboratório de Química Bioorgânica, Instituto de Pesquisas de Produtos Naturais, Centro de Ciências da Saúde, Bloco H, Universidade Federal do Rio de Janeiro, RJ 21941-590, Brazil*

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ABSTRACT

11a-*N*-tosyl-5-carbapterocarpans (**5a–c** and **6a–c**), 9-*N*-tosyl-4,4a,9,9a-tetrahydro-3H-carbazole (**7**), 11a-*N*-tosyl-5-carbapterocarpen (**8**) analogues of LQB-223 (**4a**), were synthesized through palladium catalyzed azaarylation of substituted dihydronaphtalenes (**14a–c**) and cyclohexadiene (**15**), respectively, with *N*-tosyl-*o*-iodoaniline (**11**). In order to understand the role of the *N*-tosyl moiety for the pharmacological activity, the azacarbapterocarpen (**9**) was also synthesized by Fischer indol reaction. The structural requirements at the A and D-rings for the antineoplastic activity toward human leukemias and breast cancer cells were evaluated as well. Substitutions on the A-ring of **4a** and analogues alter the effect on different breast cancer subtypes. On the other hand, A-ring is not essential for antileukemic activity since compound **7**, which does not contain the A-ring, showed efficacy with high selectivity indices for drug-resistant leukemias. On the other hand, substitutions on the D-ring of **4a** for fluorine or iodine did not improve the antileukemic activity. *In silico* studies concerning Lipinskís rule of five, ADMET properties and drug scores of those compounds were performed, indicating good physicochemical properties for all compounds, in special for compound **7**.

1. Introduction

Pterocarpans, the second most abundant sub-class of isoflavonoids and its derivatives are interesting sources of bioactive compounds [1]. For example, medicarpin (**1**), a pterocarpan isolated from *Medicago sativa*, presents antimitotic effects on sea urchin eggs [2] (Fig. 1). The 11a-aza-pterocarpan (**2**), recently isolated from roots of *black locust* (*Robinia pseudoacacia*) is the first example of a natural aza-pterocarpan. This compound was synthesized by Dejon et al. and showed moderate antineoplastic activity on HL-60 leukemia cell lines [3]. Von Angerer and co-workers reported the synthesis of some 6,11-dihydro-5*H*-benzo[*a*]carbazole as **3**, which inhibited the growth of breast cancer tumors in rats [4] due to their affinity to estrogen receptors [5]. The 11a-*N*-tosyl-5-carbapterocarpan LQB-223 (**4a**) was previously designed and prepared as racemate by our group, as part of a research aiming to the synthesis of new antineoplastic and antiparasitic drug candidates (Fig. 1). This compound presented antineoplastic activity towards multidrug resistant (MDR) leukemias and breast cancer cells [6–8], leishmanicidal properties *in vitro* [6], as well as *in vitro* and *in vivo* antimalarial activities [9]. The potential of **4a** as an antineoplastic prompted us to prepare derivatives for these studies. In this paper we report the synthesis of compounds **4–9** designed to evaluate, along with

* Corresponding authors.
E-mail addresses: mnrenno@macae.ufrj.br (M.N. Rennó), camilla-buarque@puc-rio.br (C.D. Buarque).
[1] These authors contributed equally to this work.

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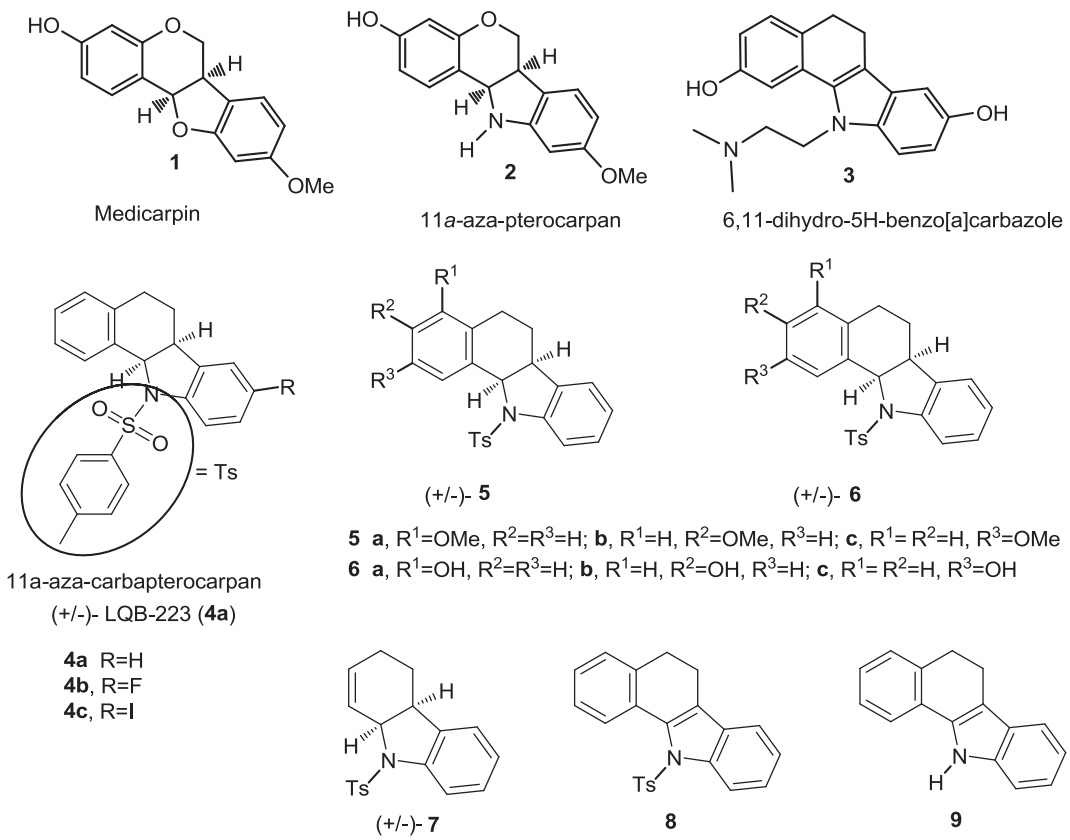


Fig. 1. Pterocapan, Aza-pterocarpan and 6,11-dihydro-5*H*-benzo[*a*]carbazole derivatives.

compound **4a**, the effect of substitutions at A and D-rings on the cytotoxicity against models of human leukemias (K562, Lucena-1 and FEPS) and breast cancers (MCF-7 and MDA-MB-231) presenting diverse phenotypes of drug resistance. Additionally, ADMET properties were predicted *in silico*. Since the exchange of the *N*-tosyl group for mesyl or benzoyl groups led to a drastic reduction in cytotoxic effect [7], we decided to further investigate the role of tosyl group for the toxicity exerted by the aza-carbapterocarpan derivatives.

2. Chemistry

As previously described, compounds **4a**, **5a–c** and **7** were prepared in moderate to good yields by a palladium-catalyzed azaarylation of substituted dihydronaphthalenes (**14a–c**) or cyclohexadiene (**15**) by *N*-tosyl-*o*-iodoaniline (**11a**) using 10 mol% of $Pd(OAc)_2$, 1.2 eq. of Ag_2CO_3 in PEG-400 at 130 °C or in refluxing acetone (Scheme 1, conditions iii or iv) [10]. Compounds **5a–c** were subsequently purified using flash chromatography followed by recrystallization with methanol, resulting in yields up to 55%. In this work, PEG was also used at 220 °C (condition vi) in order to obtain 11a-*N*-tosyl-5-carbapterocarpen (**8**). In this case, a mixture of products **4** and **8** was obtained in which the compound **8** was obtained in 30% yield. The dihydronaphthalenes (**14a–c**) were prepared in excellent yields by reduction of commercially available tetralones (**12a–c**) followed by water elimination in the resulting alcohols **13a–c** [11]. *N*-tosyl-*o*-iodoaniline (**11a**) was prepared in good yields (88%) by the reaction of tosyl chloride and *o*-iodoaniline (**10a**) in solution of pyridine/dichloromethane (10% v/v) [12]. Aiming to achieve the synthesis of new analogues for biological assays analogues for biological assays, these compounds were submitted to *O*-demethylation in the presence of five equivalents of BBr_3 in an inert atmosphere leading to **6a–c** in almost quantitative yields (88–98%) [13].

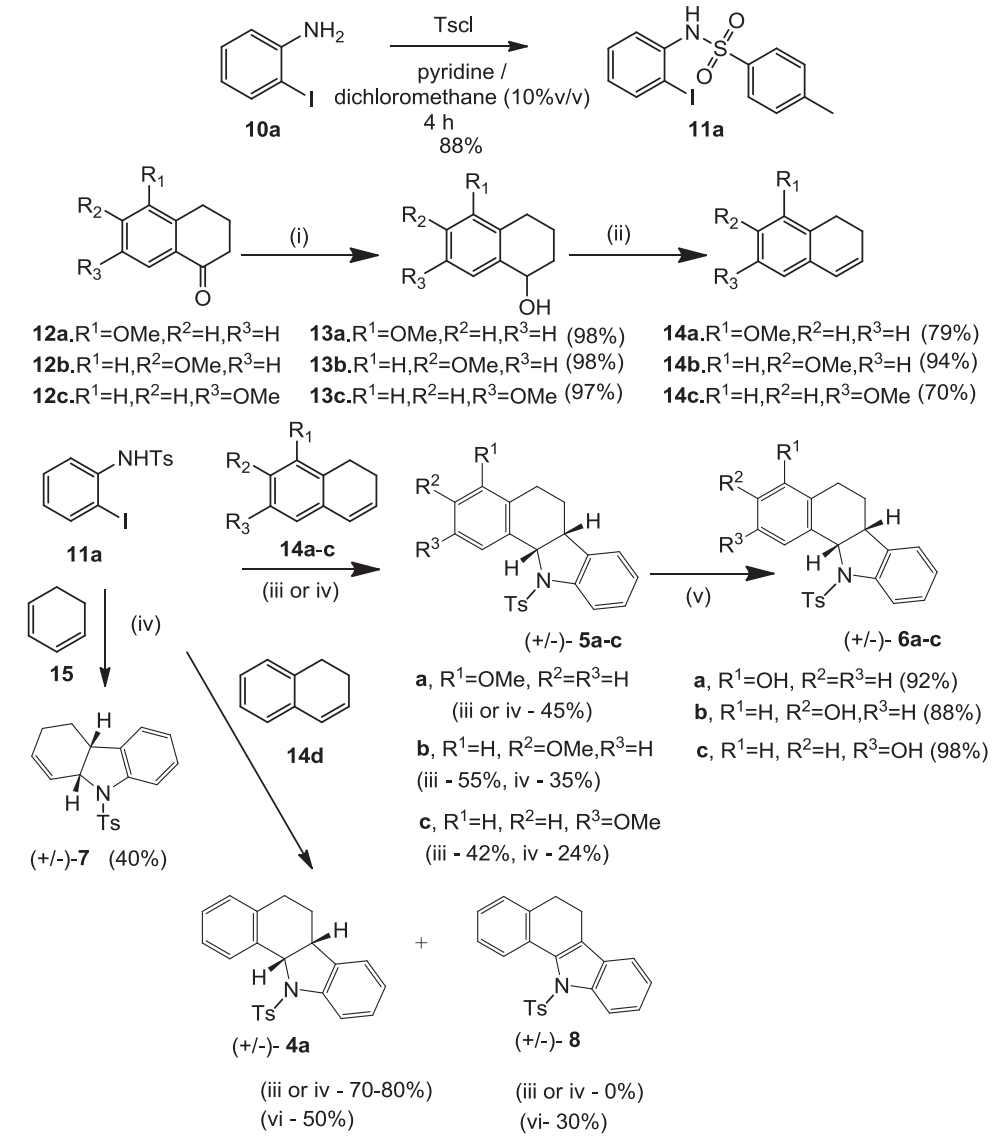
Aza-carbapterocarpan **4b** was synthesized in moderate yields (56%) by palladium-catalyzed Aza-Heck arylation in PEG 400 between dihydronaphthalene and *N*-tosyl-4-fluoro-*o*-iodoaniline (**11b**), previously prepared in good yield (86%) by reaction of tosyl chloride and 4-fluoro-*o*-iodoaniline (**10b**) employing similar conditions as to obtain **11a**. Compound **4c** was obtained in 88% yield by the reaction of compound **4a** with *N*-iodosuccinimide (NIS) catalyzed by $FeCl_3$ in dichloromethane (Scheme 2) [14].

As it could be seen in Table 1, compounds **4a** and **8** presented similar biological activity against the cell lines studied in this work. In order to understand the role of the *N*-tosyl moiety we decided to synthesize compound **9** (Fig. 1), which lacks the tosyl group, using the classic Fischer indol synthesis [15]. Compound **9** is known as an antifungal [15] and anticancer agent with immunoregulatory properties [16], and this framework is present in some derivatives with anticancer and antiestrogen activities as described in Fig. 1 [4,5].

3. Results and discussion

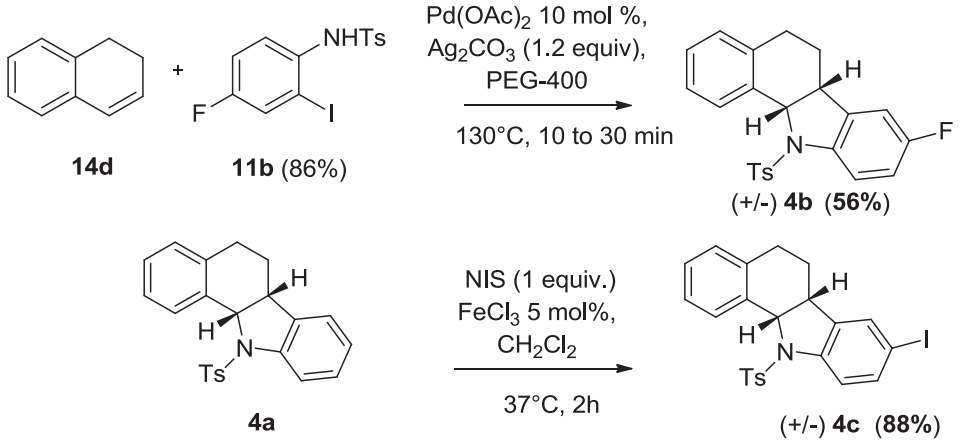
3.1. Cytotoxicity towards neoplastic cells

All compounds were evaluated for their effect against a panel of human cell lines with diverse phenotypes of drug resistance and the results compared with those obtained for **4a**. For breast cancers, MCF-7 is a model of an invasive, endocrine therapy-sensitive breast ductal carcinoma, estrogen and progesterone receptors positive and Her2/neu overexpression negative. MDA-MB-231 cell line is an invasive, endocrine therapy-resistant breast ductal carcinoma, p53 mutant and negative for estrogen, progesterone and Her2/Neu receptors. For leukemias, K562 is representative of a chronic myeloid leukemia from erythroid origin with constitutive BCR/ABL tyrosine kinase activity, whereas Lucena-1 and FEPS are multidrug resistant (MDR) cell lines selected by either vincristine or the anthracycline daunorubicin (**DNR**) exposure [17,18]. The latter was employed as a standard due to being widely used in many chemotherapy regimens, including for cancers



Conditions: (i) $NaBH_4$, MeOH, rt, 30 min; (ii): H_3PO_4, THF, reflux, 2h; (iii) $Pd(OAc)_2$ 10 mol %, Ag_2CO_3 (1.2 equiv), PEG-400, 130°C, 10 to 30 min. (iv) $Pd(OAc)_2$ 10 mol %, Ag_2CO_3 (1.2 equiv), acetone, reflux, 20 h,(v) BBr_3, CH_2Cl_2, 0^0 C, 2h, (vi) $Pd(OAc)_2$ 10 mol% Ag_2CO_3 (1.5 equiv), PEG-

Scheme 1. Synthesis of compounds **5a-d**, **6a–c**, **7** and **8**.



Scheme 2. Synthesis of compounds **4b** and **4c**.

Table 1
Antineoplastic effect (IC_{50}) of **4**, **5**, **6**, **7**, **8**, **9** and daunorubicin (**DNR**) on the cell viability of human breast cancer and chronic myeloid leukemia cells.

Compound	MDA-MB-231	MCF-7	K562	Lucena-1	FEPS
4a	31.71 ± 3.68	17.96 ± 5.76	2.90 ± 0.65[a]	2.49 ± 0.14[a]	2.12 ± 0.73[a]
4b	NA	NA	15.95 ± 1.26	28.30 ± 3.30	14.05 ± 2.89
4c	NA	NA	23.53 ± 5.46	14.35 ± 4.48	3.92 ± 0.40
5a	> 40	27.45 ± 3.67	11.29 ± 1.66	12.75 ± 0.28	11.16 ± 0.70
5b	8.29 ± 2.08	5.93 ± 3.58	2.91 ± 0.40	4.54 ± 0.28	2.58 ± 0.44
5c	27.06 ± 7.31	34.56 ± 6.13	8.09 ± 2.51	10.05 ± 0.02	7.90 ± 2.06
6a	22.08 ± 5.77	26.57 ± 8.25	11.27 ± 1.78	6.55 ± 1.62	7.39 ± 1.11
6b	> 40	10.39 ± 3.99	2.87 ± 0.76	3.56 ± 0.60	2.90 ± 0.76
6c	4.88 ± 1.18	16.97 ± 3.90	13.31 ± 2.75	26.46 ± 3.76	8.83 ± 0.74
7	12.17 ± 1.18	> 40	1.85 ± 0.87	4.14 ± 1.35	1.68 ± 0.62
8	33.37 ± 3.98	> 40	1.93 ± 0.88	2.18 ± 1.47	2.89 ± 0.92
9	> 40	> 40	> 40	34.73 ± 7.35	25.28 ± 8.16
DNR[b]	104.20 ± 8.73	27.40 ± 4.93	108.53 ± 13.26	799.67 ± 115.59	> 1200

Results are reported as IC_{50} values ± SD in μM. Data represent means obtained from three independent experiments, with each concentration tested in triplicate. Assays were performed as described in the Experimental Section [20].

[a] IC_{50} obtained from our previous work [7]. NA = Not analyzed.

[b] For daunorubicin (**DNR**) only, results are reported as IC_{50} values ± SD in nM.

from both breast and blood origins [19]. The effect of the various compounds on our cell panel, as measured by their IC_{50} values, are summarized in Table 1.

Concerning breast cancer, compounds **5b**, **6c** and **7** presented higher cytotoxicity to the MDA-MB-231 cell line whereas **5b** and **6b** were the most active against MCF-7. Compounds **5c** and **6a** were equipotent on both breast cancer cells. The presence of the A-ring with oxygenated groups in position 3 of the aza-carbapterocarpan was associated with toxicity on MCF-7 cells, although such correlation was not valid for MDA-MB-231 since removal of the A-ring on **7** did not diminish the cytotoxicity. Nevertheless, compound **5b** was the most potent on breast cancer cells, deserving further studies. The aza-carbapterocarpen **8** acted in a comparable fashion as **4a**, which is consistent with their structural similarity**,** and the removal of *N*-tosyl in **9** drastically reduced the activity towards breast cancer. Concerning leukemias, all compounds, except for compound **9**, were active on K562, Lucena-1 and FEPS, being **5b**, **6b**, **7** and **8** the most active. These data suggest a correlation with the oxygenation pattern at the A-ring as well as the presence of *N*-tosyl reinforcing what we have indicated before for LQB-223 (**4a**) [7]. Accordingly, this is proven by the synthesis of compound **9**, since removal of the *N*-tosyl group abrogated its cytotoxicity showing the highest IC_{50} toward all cell types. Furthermore, the A-ring was not essential for the antileukemic activity considering the results of compound **7**. The fluoro- (**4b)** or iodo-**(4c)** substitution of D-ring did not improve the antileukemic activity. Due to increased level of antioxidants, resistant cells usually have lower reactive oxygen species (ROS) levels compared to their sensitive counterparts [21]. Chronic myeloid leukemias such as K562 are tolerant to oxidative stress, and the MDR counterpart Lucena-1 is described as even more resistant due to overexpression of catalase and glutathione [18,22]. ROS levels are reported to be lower in MDA-MB-231 than in MCF-7 [23], and since glutathione is also present in high levels in the latter [24], our results suggest that compounds **4a**, **5b** and **6b** act regardless of resistance to oxidative stress. In addition, these compounds manifested similar effects on cells expressing varying degrees of ATP-binding cassette proteins, a feature associated to MDR [25]. ABCB1 genomic region is reported to be 5 and 30-fold amplified, respectively, in Lucena-1 and FEPS cells over K562 [26], leading to high levels of protein expression and to an increase in its activity [17]. FEPS, MDA-MB-231 and MCF-7 express functional ABCC1 [17,27], indicating that **4a**, **5b** and **6b** were active in the presence of those active transporters. The prototype compound **4a** was previously demonstrated to be similarly effective on MCF-7 Dox^R, an ABCB1-overexpressing cell line derived from MCF-7 after exposure to the **DNR** analogue doxorubicin [8], adding depth to this observation. Compound **6c**, however, was more active on MDA-MB-231 and FEPS. As for **DNR**, anthracyclines induce toxicity by a dual mechanism, inhibition of DNA topoisomerase II and ROS generation [19]. Resistance to this drug depends on the level of expression of ABC proteins [28], a fact that reflected on its IC_{50} for the various cells evaluated in this work.

3.2. Effect on non-neoplastic cells and calculation of selectivity indices

For drugs to be successfully used in the chemotherapeutic practice, it is important to assess their toxicity on healthy cells. To accomplish this, we employed cells from breast and blood origins. As a model of non-neoplastic breast cells we used MCF-10A, a human epithelial cell line derived from benign breast tissue that does not express the estrogen receptor [29], whereas fresh human peripheral blood mononuclear cells (PBMC) were employed as controls for leukemias. Both cells were stimulated to proliferate, the first with epidermal growth factor and insulin, and the latter with phytohemagglutinin. The cytotoxicity exerted by the most potent compounds on the corresponding models of normal cells, as measured by their IC_{50} values, are reported in Table 2.

Results in Table 3 indicate that selectivity indices (SI) were, in general, higher to leukemias than to breast cancer cells, mainly for compounds **4a**, **5b** and **6b**. Compound **4a** was previously demonstrated to be selective to leukemia cells, not affecting normal proliferating murine lymphocytes [7]. This profile was maintained on human PBMC and a similar outcome was observed for **5b**, **6c** and **7**. For breast cells, in

Table 2
Cytotoxicity of **4a**, **5b**, **6b**, **6c**, **7** and daunorubicin (**DNR**) on models of non-neoplastic breast cells and peripheral blood mononuclear cells (PBMC).

Compound	MCF-10A	PBMC
4a	6.15 ± 1.80	> 30
5b	21.94 ± 6.85	> 40
6b	33.83 ± 5.90	23.83 ± 4.03
6c	NA	> 40
7	NA	> 40
DNR[a]	70.46 ± 8.45	28.67 ± 4.19

PBMC and MCF-10A: Results are reported as IC_{50} values ± SD in μM. Data represent means obtained from three independent experiments, with each concentration tested in triplicate. Assays were performed as described in the Experimental Section. NA = Not analyzed.

[a] For DNR only, results are reported as IC_{50} values ± SD in nM.

Table 3
Selectivity indices (SI) of **4a**, **5b**, **6b**, **6c**, **7** and daunorubicin (**DNR**).

Compound	MCF-10A/ MDA-MB-231	MCF-10A/ MCF-7	PBMC/ K562	PBMC/LUC	PBMC/ FEPS
4a	0.19	0.34	10.34	12.04	14.15
5b	2.65	3.70	13.75	8.81	15.50
6b	0.85	3.26	8.30	6.69	8.22
6c	NA	NA	3.00	1.51	4.53
7	NA	NA	21.62	9.66	23.81
DNR	0.68	2.57	0.26	0.04	0.02

Selectivity indices (SI) were calculated as a ratio of the IC_{50} in non-neoplastic cells (PBMC or MCF-10A, Table 2) to the IC_{50} in the corresponding neoplastic cell (breast cancer or leukemias, Table 1). When IC_{50} was expressed on Tables 1 or 2 as being higher than a concentration (e.g. > 30), this value, 30 μM, was used for the SI calculation (e.g. SI of **4** towards K562: IC_{50} PBMC/IC_{50} K562 = 30/2.90 = 10.34). NA = Not analyzed.

contrast, **4a** reduced the viability of non-tumor MCF-10A in lower IC_{50} than in tumor cells. Conversely, the non-neoplastic breast human cell line HB4a was resistant to the action of **4a** in another work [8], suggesting that the SI for breast cancer could vary depending on the subtypes of both the tumor and the normal cell. This is especially true for **5b**, that despite presenting lower IC_{50} than **6b** on MCF-10A, it was more effective toward breast cancer cells. Results toward normal blood cells indicate that substitutions in the A-ring were associated with loss of selectivity. In this regard, removal of the A-ring in **7** led to the highest SI of all compounds for K562 and FEPS cells. Overall, results on models of normal cells indicate **5b** and **7**, respectively, as interesting drug candidates for breast cancer and leukemias.

3.3. Admetox predictions

In order to describe the potential for good oral bioavailability and to evaluate the druglikeness and drug score values of the synthesized compounds, physicochemical properties were predicted *in silico* and compared with the descriptors obtained for daunorubicin (**DNR**). The physicochemical and toxicological descriptors were calculated using the Molinspiration Cheminformatics version 2016.10 server (http://www.molinspiration.com) and OSIRIS Property Explorer (https://www.organic-chemistry.org/prog/peo/) using DataWarrior version 4.7.2 software (http://www.openmolecules.org/datawarrior/) [30,31] The Lipinskís 'rule of five' descriptors were analyzed, which describes physicochemical properties of a compound required to estimate important pharmacokinetic parameters, such as absorption or permeation of the molecule, distribution, metabolism and excretion, being those parameters associated with permeability and solubility [32]. For the Lipinski's rule the compounds **4(a,b)**, **5(a–c)**, **6a** and **8** presented only one violation. Additionally, compounds **6b**, **6c**, **7** and **9** did not violate any criteria. These results showed that the compounds have structures that would present good absorption properties, therefore permeability across the cell membrane, and good theoretical oral bioavailability. However, the positive control **DNR** and compound **4c** showed at least three and two violations, including acceptor, donor hydrogen, molecular weight and miLogP, respectively. The data for other physicochemical parameters such as Topological Polar Surface Area (TPSA) and number of rotatable bonds is presented in Table 4. The TPSA methodology described by Ertl et al. using published data of various types of drug transport properties shown to be a very good descriptor to characterize drug absorption, including intestinal absorption, bioavailability, blood-brain barrier penetration, and Caco-2 cell permeability [33]. Compounds that present TPSA values equal to or less than 140 $Å^2$ and fewer rotatable bonds would show good potential for oral bioavailability [34]. The results indicate that all compounds presented lower TPSA values than 140 $Å^2$ and lower number of rotatable bonds when compared to **DNR** (Table 4). Furthermore, TPSA has been positively correlated to ABCC1-mediated drug efflux, in a way that xenobiotics with low values of this descriptor present low affinity for this transporter as well [35]. This is in accordance to the IC_{50} values on cells such as FEPS (Table 1), whose ABCC1 activity has been well described [17]. For the Lipinskís rule, all compounds presented parameters for good oral bioavailability, except **DNR** and compound **4c**.

Table 4
Physicochemical parameters of the compounds and **DNR**.

Compound	miLogP	nON	nOHNH	MW	TPSA	nrotb	nviolations
4a	5.27	3	0	375.49	37.38	2	1
4b	5.41	3	0	393.48	37.38	2	1
4c	6.33	3	0	501.39	37.38	2	2
5a	5.28	4	0	405.52	46.61	3	1
5b	5.30	4	0	405.52	46.61	3	1
5c	5.30	4	0	405.52	46.61	3	1
6a	5.21	4	1	391.49	57.61	2	1
6b	4.77	4	1	391.49	57.61	2	0
6c	4.77	4	1	391.49	57.61	2	0
7	4.33	3	0	325.43	37.38	2	0
8	5.36	3	0	373.48	39.08	2	1
9	3.93	1	1	219.29	15.79	0	0
DNR	0.90	11	6	527.53	185.85	4	3

miLogP: octanol/water partition coefficient; nON: number of hydrogen bond acceptors; nOHNH: number of hydrogen bond donors; MW: Molecular weight, TPSA: Topological Polar Surface Area; nrotb: Number of Rotatable Bonds; nviolations: number of violations from Lipinski's rule.

Our study correlated *in silico* physicochemical properties (druglikeness and drug scores), to potential toxicity risks, such as mutagenicity, tumorigenicity, irritating effects and reproductive effects. Prediction of toxicity risks showed that the structure with the lowest risk of undesirable effects is compound **7**, which would likely not manifest risks of tumorigenicity, mutagenicity, irritant nor reproductive effects. However, all other synthetic compounds presented high risk for reproductive effect, though compounds **8** and **9** were predicted to present tumorigenic effects. Results suggest that these risks may be linked to the presence of the aromatic A-ring in all substances and its absence in compound **7** (Table 4). The standard drug **DNR** was predicted to manifest both reproductive and irritant effects. A high drug score suggest that the structure is a promising lead for future development of an efficient drug, and all compounds were in the range of 0.0693–0.3208, while the positive control presented 0.1889. Additionally, **DNR** presented the highest druglikeness value in relation to the compounds, and this drug is commercially available as an injectable pharmaceutical formulation (see Table 5).

Table 5
In silico predicted druglikeness, drug score and toxicity risks of the synthesized compounds and **DNR**.

Compound	Druglikeness	Drug score	Toxicity risks[a] (high)
4a	−4.7053	0.1435	Reproductive
4b	−6.505	0.1304	Reproductive
4c	−4.6745	0.0942	Reproductive
5a	−4.4787	0.1402	Reproductive
5b	−4.4811	0.1402	Reproductive
5c	−4.4811	0.1402	Reproductive
6a	−4.778	0.1565	Reproductive
6b	−4.8167	0.1565	Reproductive
6c	−4.8167	0.1565	Reproductive
7	−8.8552	0.3208	None
8	−4.5446	0.0693	Reproductive tumorigenic
9	−0.4097	0.1761	Reproductive tumorigenic
DNR	6.1633	0.1889	Reproductive irritant

[a] Mutagenicity, tumorigenicity, irritating effects, reproductive effects.

4. Conclusion

In conclusion, the new 11a-*N*-tosyl-5-carbapterocarpans present efficacy on different neoplastic cell subtypes in accordance with the presence or absence of the A-ring as well as the pattern of substitution at A-ring. The *N*-tosyl group is essential for the antineoplastic activity to manifest. Compounds **5b** and **7** showed to be the best candidates for treating the majority of the cancer cell lines studied in this work, with higher potencies and the best selectivity indices for leukemia cell lines. The A-ring was important for targeting breast cancer cells, and the position of methoxyl or hydroxyl groups altered the efficacy on subtypes of these tumors. Furthermore, our results confirmed the structural importance of the *N*-tosyl moiety for the design of more effective *N*-tosyl-carbapterocarpan drug candidates, since its removal in **9** abolished the effect on both cancer cell types studied in this work. In addition, compounds **5b**, **6b**, **7** and **8** exerted toxicity in similar levels to cells with diverse phenotypes commonly associated with resistance to chemotherapy.

In silico studies suggest that the compounds present descriptors for good oral bioavailability, low or absent toxicity risks and drug score values comparable with the one for daunorubicin. Further investigations will be carried out aiming to clarify the mechanism of action exerted by the most promising compounds. Results obtained in this work propose the 11a-*N*-tosyl-5-carbapterocarpans, notably compound **7**, as antineoplastic drug candidates effective on drug-resistant cancer subtypes, with reasonable selectivity indices and interesting physicochemical properties.

Acknowledgements

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bioorg.2018.07.004. These data include MOL files and InChiKeys of the most important compounds described in this article.

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