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New 5-carba-pterocarpans: Synthesis and preliminary antiproliferative activity on a panel of human cancer cells

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ABSTRACT

Natural pterocarpans and synthetic 5-carba-pterocarpans are isosteres in which the oxygen atom at position 5 in the pyran-ring of pterocarpans is replaced by a methylene group. These 5-carba-analogues were obtained in good yields through the palladium-catalyzed oxyarylation of alcoxy-1,2-dihydronaphthalens with *o*-iodophenols in PEG-400. They were evaluated on human cancer cell lineages derived respectively from prostate tumor (PC3, IC_{50} = 11.84 μmol L^{-1}, SI > 12)) and acute myeloid leukemia (HL-60, IC_{50} = 8.81 μmol L^{-1}, SI > 16), highly incident cancer types presenting resistance against traditional chemotherapeutics. Compound **6c** (LQB-492) was the most potent (IC_{50} = 3.85 μmol L^{-1}, SI > 37) in SF-295 cell lineage (glioblastoma). Such findings suggest that 5-carba-pterocarpan can potentially be new hit compounds for further development of novel antiproliferative agents.

1. Introduction

Pterocarpans are the second-largest group of isoflavonoids and are isolated from *Fabaceaea*, *Leguminosae*, *Papilionaceae* and *Bituminaria* plant families [1,2], They act as phytoalexins, metabolites produced *de novo* in stressful conditions like resource deprivation, pathogen invasion and UV radiation-mediated injuries [3]. Pterocarpan-producing plants are extensively used in traditional medicines [4], some of their reported bioactivities are snake anti-venom [5], anti-HIV [6], antiprotozoal [7], antifungal [1,7], antibiotic [1], antioxidant anti-inflammatory [8] and antiproliferative [9–12]. Natural pterocarpans and synthetic 5-carba-pterocarpans are isosteres in which the oxygen atom at position 5 in the pyran-ring of pterocarpans is replaced by a methylene group. Despite this structural similarity, bioactive 5-carba-pterocarpans were poorly reported in the literature. We previously reported that the 11-aza-pterocarpan (**1**) and its isostere 5-carba-analogue (**2**) showed antiproliferative properties on some cell lineages and **2** was more potent (Fig. 1a) [12,13]. The isosteric concept was also successfully used by Miller [14] and co-workers (Fig. 1a) and pterocarpen (**3**) and its carba-analogue (**4**) presented similar affinity for α- and β-estrogenic human receptors, higher than estradiol, the natural ligand [14] (see Fig. 1c).

In this note we disclose the synthesis of a series of 5-carba-pterocarpans (**6**–**8**) structurally related to the natural (+)-2,3,9-trimethoxypterocarpan (**5**) (Fig. 2). This compound was isolated from *Platymiscium floribundum* in the northeast of Brazil [15–17] and its promising antiproliferative activity against four different leukemic cell lines was studied [15–17]. Pessoa and collaborators [16,17] reported that this pterocarpan blocked DNA synthesis in HL-60 cells [16,17]. Cancer is still the third most common death cause in the world [18], although new antineoplastic drugs have been developed in the last years. Several new aggressive tumor with MDR (multidrug resistance) phenotype have been identified, exihibiting a widespread resistance to known drugs, enhancing the need for constant research and development of new compounds and multidrug treatments [19].

In this work the comparison between the biological activities of compounds **5** and its 5-carba-analogues **6**–**8** is our main goal (Fig. 1c).

[1] Equal contribution to this work.
[2] Undergraduate students.

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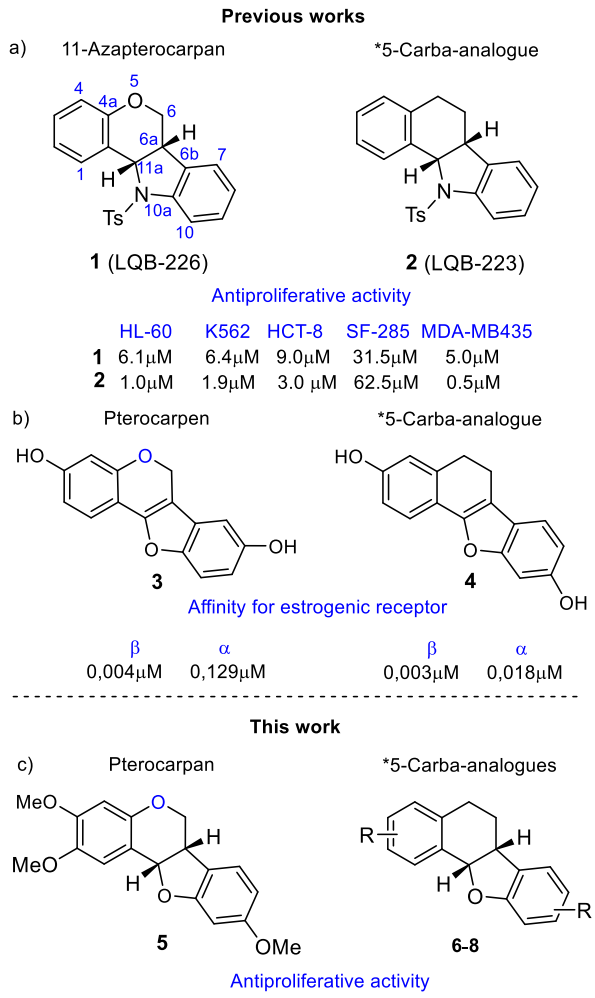


*To facilitate further discussion, we will adopt the same numeration for ptercarpan and carbapterocarpan skeleton.

Fig. 1. Bioisosterism in pterocarpans and pterocarpenes.

The pattern of substitution at the A-ring and its relevance was evaluated through the comparison of the cytotoxic profile of compounds type **6,** dimethoxylated at the A-rind as the natural product, with compounds type **7** and **8**, respectively mono-methoxylated at position 2 or not substituted at the A-ring. Regarding the pattern of substitution at the D-ring, we have methoxy groups, as in the natural product. Alternatively, the presence of a hydroxymethyl group, that could mimic at some degree a phenol group [20,21] provides structural diversity at the D-ring. The aldehyde group present in the structure of some compounds can be used to prepare new derivatives or to connect the structure to carrier systems [20].

2. Results and discussion

Dihydronaphthalen (**11c**) is commercially available and the alkoxy-dihydronaphthalens **11a,b** were prepared from the corresponding α-tetralones **9a,b** (Scheme 1) [21]. On the other hand *o*-iodophenols **12a-c** were obtained according to standard literature procedures [22,23] (Scheme 2).

Olefins **11a-c** were oxyarylated with **12a-c** leading to 5-carba-pterocarpans **6a-c**, **7a-c** and **8c** in moderated to good yield (Scheme 3). It is worth to mention that while phenols containing electron releasing methoxy group, as in **12a** and **12c** reacts with 2*H*-chromenes leading to adducts in low chemical yields[10], we were pleased to know that dihydronaphthalens **11a,b** lead to adducts **6a,c** and **7a,c** in moderate to

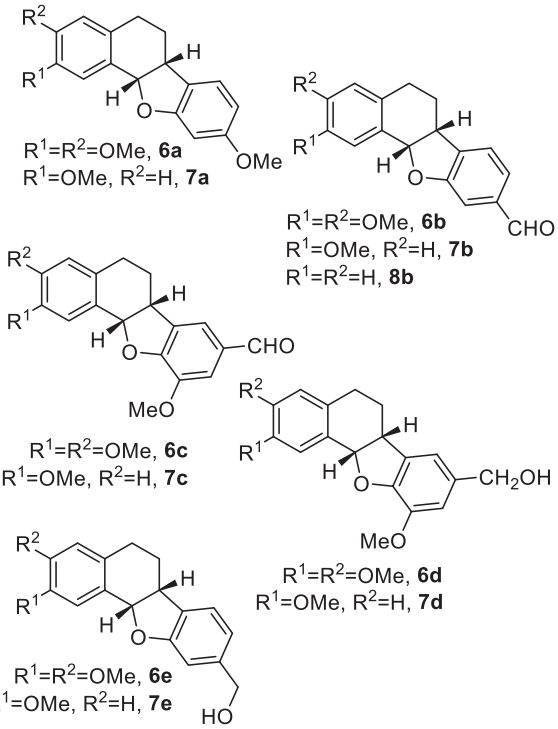
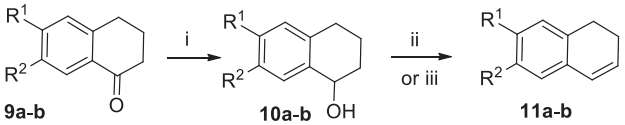


Fig. 2. 5-carbapterocarpans **6**, **7** and **8** synthesized in this work.



a, $R^1=R^2$=OMe 10a (96%) 11a (62%)
b, R^1=H, R^2=OMe 10b (98%) 11b (82%)

i. $NaBH_4$ 3 equiv., MeOH, r.t. ii **(10a).** H_3PO_4 1.1 equiv., DMF, 110 °C. iii (**10b**).. H_3PO_4 1.2 equiv.,THF, r.t. ,

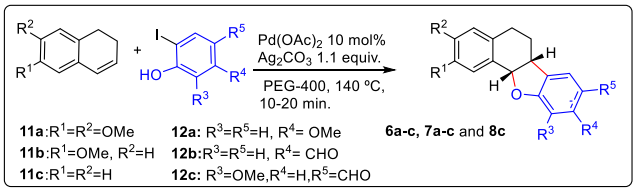
Scheme 1. Synthesis of Alkoxy-dihydronaphthalens.

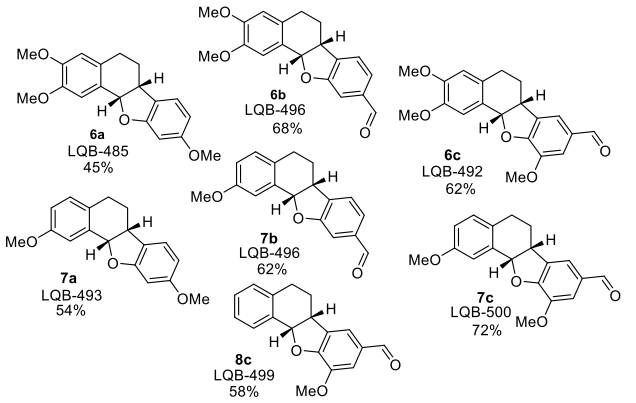
good yields when oxyarylated with the same *o*-iodophenols. These oxyarylations were accomplished under conditions previously described by our laboratory [21]: 10 mol% $Pd(OAc)_2$, 1.1 equiv. Ag_2CO_3 in PEG-400, at 140 °C 10–40 min. Under these conditions the reactions are faster and yields are better when compared to previous conditions using refluxing acetone [10]. The stereospecificity of the reaction was the same as observed in previous works, 5-carba-pterocarpans were obtained exclusively in the *cis* configuration, confirmed by the characteristically 1H NMR displacement and coupling constant of H11a (d, $J \cong$ 8.2 Hz, H11a - H6b) [21]. No regioisomeric products were observed as was recently identified by our group when oxyarylating electron-rich 2*H*-chromenes in similar conditions [24].

The aldehydes **6b,c** and **7b,c** were reduced by $NaBH_4$ to the corresponding hydroxymethyl derivatives **6d,e** and **7d,e** in excellent yields (Scheme 3). **8c** was not subjected to any further transformations as itself did not presented any interesting cytotoxicity (See Table 1).

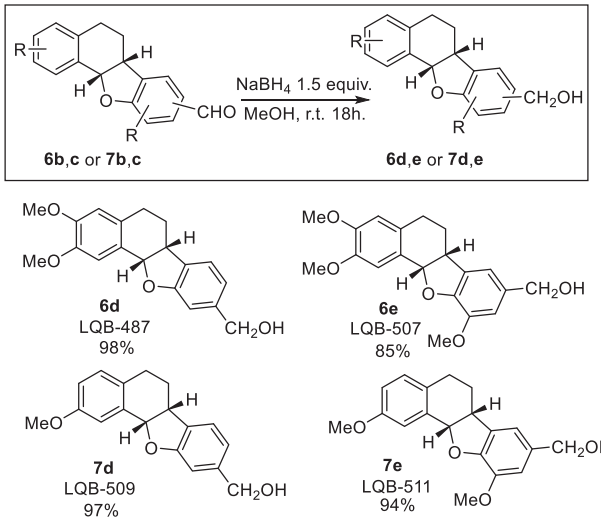
3. Preliminary antiproliferative evaluation

In Table 1 are shown the antiproliferative activity of the synthesized 5-carba-pterocarpans **6a–e, 7a–e**, **8c** and the pterocarpan (**5**) in its racemic and enantiomeric pure forms. The cytotoxic activity of these compounds was assessed through colorimetric MTT assay [25,26], performed to estimate the half-maximum inhibitory concentrations





Scheme 2. Synthesis of 5-carba-pterocarpans **6a-c**, **7a-c** and **8c.**



Scheme 3. Preparation of hydroxymethyl group **6d,e** and **7d,e.**

(IC_{50}) of these compounds against tumor and non-tumor cell lines (Table 1) (see Table 2).

We firstly focused our attention in comparing the activity of natural product **5,** previously studied by Pessoa's group [15–17] with its isostere **6a**. Surprisingly **6a** is inactive on those cell lines where **5** is active, suggesting the oxygen atom at the pyran ring in **5** may be involved in the interaction with the biological target.

Interestingly, compound 6b was less potent than 5 and showed higher potency than 6a, except for SF-295 cells, probably suggesting involvement of the aldehyde group is in the molecular recognition by the biological target". Compound **6c**, which presents a 2,3-dimethoxy group at the A-ring like the natural product and a methoxy group in C10 and an aldehyde at C8, was the most potent molecule (IC_{50} 3.85 μmol L^{-1}) against SF-295 in comparison to any other compound in the series, including the natural product itself. The presence of hydroxymethyl group in **7d** did not enhance or diminish its activity compared with **7b**. The hydroxymethyl group in the D ring of compound **6e** (LQB-507) substantially enhanced its biological activity against PC3, HL-60, and HCT-116 cell lineages, however, the inhibitory activity on SF-295 decreased.

Table 1
Cytotoxic activity of pterocapan **5** and 5-carbapterocarpans after a 72 h-exposure expressed by IC_{50} (μmol L^{-1}).

Compound*	SF-295	PC3	HL-60	HCT-116	L929
(+-)**5**	27.67 ± 1.94	6.33 ± 0.15	1.78 ± 0.06	7.2 ± 0.72	80.5± 11.05
(+)-**5**	8.3 ± 0.03	4.42 ± 0.01	0.42 ± 0.04	0.41 ± 0.06	62.16 ± 15.3
(-)-**5**	80.52 ± 2.24	50.93 ± 1.99	57.07 ± 4.05	63.05 ± 0.33	99.54 ± 6.22
LQB-485 (**6a**)	99.56 ± 3.24	> 160	22.18 ± 0.75	98.4 ± 4.03	> 160
LQB-488 (**6b**)	>160	76.24 ± 1.94	15.9 ± 1.1	32.04 ± 1.21	> 160
LQB-492 (**6c**)	3.85 ± 0.52	42.26 ± 2.26	24.41 ± 1.34	42.3 ± 2.11	>160
LBQ-487 (**6d**)	39.34 ± 2.62	76.77 ± 2.83	16.75 ± 1.12	28.01 ± 1.33	> 160
LBQ-507 (**6e**)	33.93 ± 1.82	11.84 ± 0.87	8.81 ± 0.68	20.24 ± 0.75	>160
LQB-493 (**7a**)	> 160	> 160	> 160	> 160	>160
LQB-496 (**7b**)	15.31 ± 4.36	29.55 ± 0.08	19.05 ± 0.33	29.6 ± 1.34	>160
LQB-500 (**7c**)	107.27 ± 6.01	>160	14.24 ± 1.35	90.6 ± 0.93	>160
LQB-509 (**7d**)	86.85 ± 5.2	106.22 ± 0.76	38.11 ± 0.81	64.7 ± 2.01	>160
LQB-511 (**7e**)	136.19 ± 1.32	46.73 ± 3.30	45.36 ± 0.95	101.39 ± 5.27	>160
LQB-499 (**8c**)	111.98 ± 4.14	>160	59.89 ± 0.24	53.7 ± 0.42	>160
Doxorubicin†	0.26 ± 0.03	0.43 ± 0.10	0.02 ± 0.004	0.14 ± 0.02	0.84 ± 0.06

*Estimated through nonlinear regression from three independent experiments with cancer and non-cancer cell lines.

Table 2
Selectivity index (SI) of 5-carbapterocarpans for selected tumour cell lineages.

Compound*	SF-295	PC3	HL-60	HCT116
(+/-)-**5**	2.91	12.72	45.22	11.18
(+)-**5**	7.47	14.03	147.62	151.22
(-)-**5**	1.23	1.94	1,73	1.57
LQB-488 (**6b**)	>1.01	>2.11	>10.13	>5.02
LQB-492 (**6c**)	>37.92	>3.45	>5.97	>3.45
LQB-487 (**6d**)	>4.07	>2.08	>9.55	>5.71
LQB-507 (**6e**)	>4.30	>12.33	>16.57	>7.21
LQB-500 (**7c**)	0.85	0.57	6.39	1.00
Doxorubicin (Positive control)	3.23	1.95	42.00	6.00

Compounds **7a-e** were in general far less potent than their **6** analogs, corroborating our suspicion that the methoxy groups in C2 and C3 are important for the biological activity. An exception was the compound **7b**, which was more potent than **6b** in almost all the cell lines, having the second lowest IC_{50} against SF-295, PC3 and HCT-116

It is worth mentioning that (+)-**5** is more potent than the racemate and the bioselectivity (+/-)-**5**/(+)-**5** ranged from 1.4 (PC3), 3.3 (SF-295), 4.2 (HL-60) to 17 (HCT-116). Therefore, we can reasonably speculate, based on the previous results, that enantiopure carba-analogues could be more potent than the corresponding racemates.

The selectivity indexes (SIs) of compounds with better anti-proliferative activities were calculated through the ratio between the IC_{50} on the L929 non-tumor cell line and the IC_{50} of the tumor cell line [26]. The natural product **5** presents high SI, specifically for HL-60 and HCT-116, and as expected the enantiomer (+)-**5** was more selective than the racemate in all cases. Concerning the 5-carba-analogues, **6c** is the most active compound against SF-295 cells, even when compared to the natural product, and presents a SI about five times higher than (+)-**5** for this cell line. The racemate **6e**, the more active carba-analogue in PC3, HL-60, and HCT-116, presented selectivity comparable to (+)-**5** and

(+/-)-**5** in PC3 linages.

Our group had previously studied the effect of natural pterocarpan **5** in a large panel of prostatic cancer cells [27], considering the relevance of this cancer and its incidence around the world. For this reason, we were inclined to pursue our effort in the investigation of LQB-507 (**6e**). The mechanism of growth inhibition of **6e** in PC3 cell lines was initially investigated by flow cytometry.

The PC3 cells were treated with (+)-**5**, Taxol (Tx), and **6e** in five different concentrations (3, 7, 12, 18, and 24 μmol L^{-1}). The evaluation of tumor cells by flow cytometry after a 24 h exposure to **6e** indicated a pattern of concentration-dependent cell cycle arrest at the G2/M phase, starting with the 12 μmol L^{-1} concentration (Table 3 and Supplementary Fig. 1). This pattern was similar to that of (+)-**5** and Taxol both in this work and in previous assays evaluating the racemate of natural pterocarpan **5** [17,27]. These results indicated a disruption of the mitotic process in the PC3 cell line [27], the similar patterns found in the cell cycle evaluations of (+)-**5** and its derivative **6e** could indicate a resembling mechanism of action for these compounds.

4. Conclusions

5-Carba-pterocarpan is a new prototype for antiproliferative compounds. Further studies are in progress to better understanding the mechanism of action of the more promising compounds. 5-Carbapterocarpan **6c** is more potent against glioblastoma-derived tumor cells (SF-295) than the natural product, presenting a high SI (>37.92). On the other hand, **6e** showed cytotoxic and cytostatic activity against the prostate cancer-derived cells (PC3) and leukemia (HL-60) with SI > 12 and SI > 16, respectively. The aldehyde group in **6c** is probably responsible for the activity against SF-295 and although this function can be quickly metabolized *in vivo*, the conjugation of this group with carrier systems could further be explored.

5. Experimental

5.1. General procedure for oxyarylation reactions.

In a sealed reaction tube were added 0.25 mmol (0.048 g) of 6,7-dimethoxy-1,2-dihydronaphthalene (**11a**), 0.5 mmol (0.125 g) of 4-hydroxy-3-iodo-5-methoxybenzaldehyde (**12a**), 0.275 mmol (0.076 g) silver carbonate (1.1 equiv.), 10 mol% (0.0056 g) of palladium acetate and 2 mL of PEG-400 as solvent. Then reaction tube was sealed with the cap and stirred at 140 °C. The reaction was monitored by TLC till completion. The reaction was extracted with 5 mL EtOAc, washed with brine 5 × 10 mL, the organic phase was dried with anhydrous $MgSO_4$, filtered and evaporated under vacuum. The pure compound (45% yield) was obtained after flash chromatography (EtOAc/ hexane; 10/90).

Table 3
Flow cytometry profile of PC3 cells treated with (+)-5a, Taxol (Tx) and LQB-507 (**6e**) in 5 different concentrations (3, 7, 12, 18 and 24 μmol L^{-1}). SE, Standard error of the mean (*p < 0.05).

	Cell Cycle Phases					
	G0/G1		S		G2/M	
	Mean (%)	SE	Mean (%)	SE	Mean (%)	SE
Negative Control	55.4	0.8	22.3	1.5	22.3	1.6
(+)-**5**	16.26*	2.3	6.9*	1.4	76.84*	2.5
Taxol	18.45*	2.3	16.5	1.6	65.06*	1.8
6e 3.0 μmol L^{-1}	60.6	1.7	25.5	1.9	14.0	1.1
6e 7.3 μmol L^{-1}	61.1	2.0	25.2	2.5	13.7	1.5
6e 12 μmol L^{-1}	43.67*	2.3	11.44*	2.2	44.89*	3.3
6e 18 μmol L^{-1}	14.44*	2.0	31.6	2.6	53.96*	3.7
6e 24 μmol L^{-1}	22.86*	2.1	26.0	3.8	51.11*	2.2

Table 3. Patterns of cell cycle arrest in PC3 cells treated with vehicle (**a**), and natural pterocarpan (+)-**5** (**b**), Taxol (**c**), **6e** (**d**) at 18 μmol L^{-1}.

5.1.1. 2,3,9-trimethoxy-5,6,6a,11a-tetrahydronaphtho[1,2-b]benzofuran (6a)

White solid, (35.12 mg, 45% yield) mp 110–115 °C. **^{1}H NMR** (500 MHz, $CDCl_3$): δ 7.11 (d, *J* = 8.1 Hz, 1H), 7.03 (s, 1H), 6.63 (s, 1H), 6.44 (d, *J* = 8.3 Hz, 1H), 6.39 (s, 1H), 5.62 (d, *J* = 8.2 Hz, 1H), 3.93 (s, 3H), 3.87 (s, 3H), 3.76 (s, 3H), 3.57 (dd, *J* = 13.5, 8.1 Hz, 1H), 2.72 – 2.50 (m, 2H), 2.03 (d, *J* = 12.8 Hz, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$): δ 160.5, 149.2, 147.8, 144.9, 132.9, 131.3, 124.0, 120.7, 112.8, 111.6, 111.0, 84.5, 55.8, 55.9, 56.0, 40.6, 27.7, 26.8. **HRMS** (ESI). Calculated for $C_{38}H_{40}O_8$ (2 M+Na). Expected mass 2 M+Na 647, 1362; obtained mass 647.1358.

5.1.2. 2,3-dimethoxy-5,6,6a,11a-tetrahydronaphtho[1,2-b]benzofuran-9-carbaldehyde (6b)

White solid, (53 mg, 68% yield) mp 140 °C. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 9.88 (s, 1H), 7.41 (d, *J* = 7.4 Hz, 1H), 7.35 (d, *J* = 7.5 Hz, 1H), 7.23 (d, *J* = 5.1 Hz, 1H), 7.02 (s, 1H), 6.61 (s, 1H), 5.66 (d, *J* = 8.3 Hz, 1H), 3.91 (s, 1H), 3.84 (s, 1H), 3.67 (dd, *J* = 13.8, 8.4 Hz, 1H), 2.62 (dd, *J* = 10.0, 5.1 Hz, 1H), 2.07 (dd, *J* = 12.8, 6.2 Hz, 1H), 1.88 – 1.76 (m, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 191.7, 159.9, 149.0, 147.8, 139.2, 137.8, 124.4, 124.3, 124.2, 112.4, 111.0, 110.1, 109.0, 82.4, 56.0, 55.9, 55.8, 41.0, 29.6, 27.4. **HRMS** (ESI): Calculated for $C_{19}H_{18}O_4$ (M + Na). Expected mass M + Na 333,1003; obtained mass: 333,1010.

5.1.3. 2,3,10-trimethoxy-5,6,6a,11a-tetrahydronaphtho[1,2-b]benzofuran-8-carbaldehyde (6c)

White solid, (52 mg, 54% yield) mp 135 °C. **^{1}H NMR** (500 MHz, $CDCl_3$): δ 1,93–1.89 (m, 1H), 2.11–2.05 (m, 1H), 2.63 – 2.55 (m, 2H), 3.77–3.62 (m, 1H), 3.79 (s, 3H), 3.87 (s, 3H), 3.92 (s, 3H), 5.84 (d, *J* = 8.6 Hz, 1H), 6.63 (s, 1H), 7.09 (s, 1H) 7.32 (s, 1H), 7.43 (s, 1H), 9.84 (s, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ: 26.8, 27.7, 40.6, 55.8, 55.9, 56.0, 84.5, 111.0, 111.6, 112.8, 120.7, 124.0, 131.2, 131.3, 132.9, 144.9, 147.8, 149.2, 153.7, 190.6.). **HRMS** (ESI): Calculated for $C_{20}H_{20}O_5$ (M + H) 341, 1354; obtained mass: 341.1384.

5.1.4. 2-methoxy-5,6,6a,11a-tetrahydronaphtho[1,2-b]benzofuran-9-carbaldehyde (7b)

White solid, (43 mg, 62% yield) mp 130 °C. **^{1}H NMR** (500 MHz, $CDCl_3$): δ 1.77–1,82 (m, 1H), 2.18 – 2.04 (m, 1H), 2.70 – 2.59 (m, 2H), 3.66–3.60 (m, 1H), 3.90 (s, 3H), 5.90 (d, *J* = 8.8 Hz, 1H), 6.8 (d, *J* = 7.5 Hz, 1H 1H), 7.14 (d, *J* = 7.5 Hz, 1H), 7.20(s, 1H), 7.32 (s, 1H), 7.49 (d, *J* = 7.3 Hz 1H) 7.52 (d, *J* = 7.4 Hz, 1H) 9.89 (s, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 26.8, 27.7, 40.6, 55.8, 84.5, 109.0, 114.2, 115.2, 124.2, 124.6, 129.4, 130.4, 133.5, 137.4, 139.1, 158.4, 160.0, 190.6 . **HRMS.** Calculated for $C_{18}H_{16}NaO_3$ (M + Na). Expected mass M + Na 303,0997; obtained mass: M + Na) + 303.1002.

5.1.5. 2,10-dimethoxy-5,6,6a,11a-tetrahydronaphtho[1,2-b]benzofuran-8-carbaldehyde (7c)

White solid, (55.6 mg, 72% yield) mp 142 °C. **^{1}H NMR** (500 MHz, $CDCl_3$): δ 1.90 – 1.72 (m, 1H), 2.18 – 2.04 (m, 1H), 2.70 – 2.59 (m, 2H), 3.90 (s, 3H), 3.84 (s, 3H), 5.90 (d, *J* = 8.8 Hz, 1H), 6.8 (dd, *J* = 7.4, 5.5 Hz, 1H), 7.14 (d, *J* = 7.4 Hz, 1H), 7.20 (d, *J* = 5.6 Hz, 1H), 7.32 (s, 1H), 7.52 (s,1H) 9.89 (s, 1H).**^{13}C NMR** (101 MHz, $CDCl_3$): δ (ppm): 27.1, 28.0, 40.7, 56.0, 84.5, 111.7, 120.8, 126.8, 128.4, 128.8, 130.6, 131.2, 132.1, 132.9, 138.9, 144.8, 153.9, 190.5. **HRMS**. Calculated for $C_{19}H_{18}O_4$ (M + Na). Expected mass M + Na 333,1103; obtained mass: 333.1097.

5.1.6. 10-methoxy-5,6,6a,11a-tetrahydronaphtho[1,2-b]benzofuran-8-carbaldehyde (8c)

White solid, (40 mg, 58% yield) mp 150 °C. **^{1}H NMR** (500 MHz, $CDCl_3$): δ 1.90–1.72 (m, 1H), 2.18 – 2.04 (m, 1H), 2.76 – 2.59 (m, 2H), 3.84 (m, 1H), 3.90 (s, 3H), 5.90 (d, *J* = 8.8 Hz, 1H), 7.30 – 7.25 (m, 1H), 7.14 (d, *J* = 6.8 Hz, 1H), 7.43 (s, 1H), 7.32 (s, 1H), 7.59 (d, *J* = 6.6 Hz, 1H), 9.83 (s, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ (ppm): 27.1, 28.0, 40.7,

55.9, 84.4, 111.7, 120.8, 126.8, 128.3, 128.8, 130.6, 131.2, 132.1, 132.9, 138.9, 144.8, 153.9, 190.5. **HRMS.** Calculated for $C_{18}H_{16}NaO_3$ (M + Na). Expected mass M + Na 303,0997; obtained mass: (M + Na) + 303.09916.

5.2. General procedure for reduction of aldehydes

To 34,1 mg (0.1 mmol) (of 5-carbapterocarpan (**6c**) in 1 mL of methanol, 5.7 mg (0.15 mmol) of $NaBH_4$ was added. After total consumption of starting materials observed by TLC analysis (10–20 min), 5 mL of water was added, extractions with ethyl acetate (3x10 mL) were performed. The organic layers were evaporated in vacuum giving the respective alcohols. The pure compound was obtained after flash chromatography (EtOAc/ hexane;30/70).

5.2.1. (2,3-dimethoxy-5,6,6a,11a-tetrahydrobenzo[d]naphtho[1,2-b] furan-9-yl)methanol(**6d**)

White solid, (41 mg, 98% yield) mp 112–115 °C. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.20 (d, J = 7.5 Hz, 1H), 7.02 (s, 1H), 6.88 (d, J = 7.5 Hz, 1H), 6.79 (s, 1H), 6.61 (s, 1H), 5.61 (d, J = 8.3 Hz, 1H), 4.61 (s, 2H), 3.91 (s, 3H), 3.85 (s, 3H), 3.60 (dd, J = 14.4, 7.2 Hz, 1H), 2.68 – 2.53 (m, 2H), 2.03 (dt, J = 11.5, 5.0 Hz, 1H), 1.79 (dtd, J = 12.8, 8.5, 4.3 Hz, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 159.5, 148.8, 147.7, 141.6, 131.1, 130.9, 125.0, 124.1, 119.2, 112.4, 111.0, 108.3, 82.1, 65.3, 55.9, 55.9, 55.8, 55.8, 40.7, 29.6, 27.6, 27.1.

5.2.2. 2,3,10-trimethoxy-5,6,6a,11a-tetrahydrobenzo[d]naphtho[1,2-b] furan-8-yl)methanol (**6e**)

White solid, (43 mg, 85% yield) mp −138–140 °C. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.13 – 7.05 (m, 1H), 6.86 (s, 1H), 6.79 (d, J = 14.9 Hz, 1H), 6.60 (s, 1H), 5.67 (d, J = 8.3 Hz, 1H), 4.61 (d, J = 4.9 Hz, 2H), 3.88 (s, 3H), 3.83 (s, 6H), 3.69 – 3.57 (m, 1H), 2.72 – 2.49 (m, 2H), 2.02 (dt, J = 12.1, 5.2 Hz, 1H), 1.80 (ddd, J = 17.6, 8.7, 4.5 Hz, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 148.8, 147.6, 147.0, 144.2, 134.2, 132.6, 131.1, 124.7, 115.4, 112.7, 110.8, 110.4, 82.8, 65.6, 56.0, 55.8, 55.8, 41.4, 27.7, 27.2. **HRMS** (ESI)**:** Calculated for $C_{20}H_{20}O_5$ (M + Na) calculated for 363,1202; found: 363,1259.

5.2.3. 2-methoxy-5,6,6a,11a-tetrahydrobenzo[d]naphtho [1,2-b]furan-9-yl)metanol (**7d**)

White solid, (41 mg, 97% yield) mp 120 °C. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.20 (d, J = 7.5 Hz, 1H), 7.06 (dd, J = 10.9, 5.5 Hz, 2H), 6.88 (d, J = 7.6 Hz, 1H), 6.83 – 6.76 (m, 2H), 5.63 (d, J = 8.5 Hz, 1H), 4.61 (s, 2H), 3.82 (s, 3H), 3.65 (dd, J = 13.7, 8.3 Hz, 1H), 2.69 – 2.50 (m, 2H), 2.11 – 1.95 (m, 1H), 1.77 – 1.42 (m, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 159.6, 158.2, 141.6, 134.1, 130.8, 130.7, 129.3, 124.2, 119.3, 114.9, 114.9, 114.2, 108.2, 108.2, 82.2, 82.2, 65.3, 55.4, 55.3, 40.8, 40.7, 29.6, 28.0, 26.6. **HRMS** (ESI): Calculated for $C_{17}H_{14}NaO_4$:305.0789; found: 305.0788.

5.2.4. 2,10-dimethoxy-5,6,6a,11a-tetrahydrobenzo[d]naphtho[1,2-b] furan-8-yl)methanol (**7e**)

White solid, (43 mg, 94% yield) mp 140 °C. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 7.15 (d, J = 2.5 Hz, 2H), 7.05 (d, J = 8.4 Hz, 2H), 6.87 (d, J = 7.2 Hz, 2H), 6.86 – 6.77 (m, 4H), 5.71 (d, J = 8.6 Hz, 1H), 4.63 (s, 2H), 3.87 (s, 3H), 3.83 (s, 3H), 3.68 (dd, J = 13.8, 8.2 Hz, 1H), 2.69 – 2.51 (m, 2H), 2.10 – 2.01 (m, 1H), 1.81 (ddd, J = 17.3, 8.5, 4.2 Hz, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$): δ 159.6, 158.2, 141.6, 134.1, 130.8, 130.7, 129.3, 124.2, 119.3, 114.9, 114.9, 114.2, 108.2, 108.2, 82.2, 82.2, 65.3, 55.4, 55.3, 40.8, 40.7, 29.6, 28.0, 26.6.

5.3. Biological activities

5.3.1. Cell lines

The cell lines were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/ streptomycin, incubated at 37 °C at a 5% CO_2 atmosphere. For cytotoxicity assays, PC3 (prostate cancer), SF295 (glioblastome), HL-60 (acute myeloid leukemia), HCT-116 (colorectal cancer), and L929 (murine non-tumor fibroblasts) cell lines were plated in 96-well plates respectively at 1.0×10^4, 1.0×10^4, 3.0×10^4, 7.0×10^4 and 1.0×10^4 cells/ml. These cells were provided by the National Cancer Institute (USA), except for L929 obtained from BCRJ (Banco de Células do Rio de Janeiro).

5.3.2. MTT assay for cytotoxicity assessment

This method is based on the reduction of the yellow tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), in a formazan purple crystal by the mitochondrial dehydrogenases and other NADPH-dependent cellular oxidoreductases [25]. The cells were plated in 96-well plates and incubated for 72 h as previously described. At the end of the treatment time the formazan precipitate was solubilized in DMSO and the solution was read in a spectrophotometer at 595 nm.

5.3.3. Flow cytometry analysis

The PC3 cells at a density of 5×10^5 cells/mL were seeded in a 6-well plate and let to adhere on to the surface overnight. Cells were exposed to different concentrations (3, 7.3, 12, 18 and 24 μM) of pterocarpan derivative LQB-507 for 24 h. The vehicle DMSO was used as negative control, Taxol (Tx) and the natural dextrorotatory pterocarpan were used as positive controls. Cells were washed thoroughly with PBS. Processed cells were incubated at room temperature in propidium iodide (50 μg mL^{-1}) for 30 min. The analysis was performed in the Guava flow cytometry system (Merk, Darmstadt, Germany).

5.4. Data analysis

Cell viability was calculated as a percentage of cell growth in vehicle-treated cell culture (negative control) and IC_{50} was estimated through non-linear regression using the GraphPad Prism version 6.0.

The cell cycle data were exported and analyzed using the ModFit 4.1 trial version. All the experiments were accomplished for at least three independent times. The mean of cell cycle experiments was used for statistical analysis in GraphPad Prism 6.0. The results were presented by the mean of ±SE (standard error of the mean). Entire statistic differences were calculated after one-way anova followed by the Tukey's test for post-hoc analysis (*$p < 0.05$).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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