Account

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Flavonoids as Inspiration for the Design and Synthesis of New Antiproliferative, Antiparasitic and Antiviral Compounds: An Account

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Modified flavonoids of the sub-group of pterocarpans, 5-carba-pterocarpans, 5-carba-pterocarpens, 1-carba-isoflavanones, coumestans, and aurones were synthesized and evaluated as antiproliferative, antileishmanial and antiviral. A new scaffold, called pterocarpanquinone, was discovered and **LQB-118**, the prototypical scaffold of its series, showed consistent antileishmanial (mice and hamster *in vivo*) and antineoplastic (respectively, human and mice tumors *ex vivo* and *in vivo*) activity. 5-Carpa-pterocarpan **LQB-485** is potent towards SF-295 cancer cells (CNS). **LQB-262** and **LQB-34** are new inhibitors of NB5S RdRp polymerase of hepatitis C virus (HCV) while **LQB-314** and **LQB-360** demonstrated potent activity and selectivity against HCV replicon reporter cells. **LQB-454** and **LQB-501** were evaluated for their *in vitro* anti-proliferative effects against human breast cancer and leukemia cell lines with diverse profiles of drug resistance. In breast cancer they present higher toxicity on multidrug resistant cells (collateral sensitivity). Aurone **LQB-814**, featuring a "lipophilic phenol" at A-ring", was a very potent and selective inhibitor of SARS-CoV-2 in Calu-3 cells. Other four aurones bearing EC_{50} (concentration required to inhibit 50% of cell growth) < 1 were also discovered.

Keywords: anti-proliferative, anti-leishmania, anti-viral, pterocarpans, MDR phenotype, carba-isoflavonoids

1. Introduction

Flavonoids, a diverse class of phenolic natural products, stand out as promising candidates in the realm of medicinal research due to their potential therapeutic properties.[1-3] Synthesized by higher plants with chalcones as their precursors, flavonoids exhibit a remarkable structural diversity based on their C_6-C_3-C_6 basic skeleton, making them a subject of growing interest in the scientific community (Figure 1). The intramolecular Michael-type addition of the hydroxyl group of chalcone into the β-carbon yields a singular flavanone enantiomer. Flavanones serve as precursors to diverse subclasses of flavonoids, characterized by an aryl group at the 2-position, such as flavones, flavonols, flavans, and catechins. Within the Leguminosae/Fabaceae family of plants, the rearrangement of the aryl group (depicted in green) to the 3-position occurs, giving rise to isoflavanones. Subsequently, isoflavanones lead to the formation of tricyclic isoflavonoids, including isoflavones and isoflavans, as well as

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This account is dedicated to Prof Eliezer J. Barreiro, whose contributions have significantly advanced the field of Medicinal Chemistry in Brazil.

tetracyclic compounds like pterocarpans and coumestans.[4,5] Moreover, chalcones can give rise to other minor subclasses characterized by unique structural frameworks. Examples include aurones, which feature an exocyclic C=C bond, and homoisoflavanones, distinguished by the incorporation of an additional carbon (C2) into their basic skeleton (Figure 1).[6,7] Rich natural sources of these compounds include fruits, soy, and legume seeds, where they exist in both aglycones and glycosylated forms at phenol groups.[8]

In vitro assessments of the aglycones of flavonoids, conducted through cell-based assays and enzymatic inhibition studies, have contributed significantly to the understanding of their potential biological actions. However, despite the wealth of *in vitro* data, a comprehensive exploration of flavonoids' biodistribution, metabolism, and proof of concept *in vivo* remains a crucial frontier in unraveling their full therapeutic potential. While *in vitro* studies have shed light on the intricate mechanisms underlying the pharmacological activities of flavonoids, translating these findings into effective *in vivo* applications is essential for advancing their development as anticancer, antiparasitic, and antiviral compounds.[9,10]

This account aims to explore the results obtained by our group over the years surrounding the synthesis and biological evaluation of modified flavonoids and their derivatives.

2. Pterocarpans and Pterocarpanquinones with Antiproliferative and Antiparasitic Activity

Pterocarpans represent a fascinating subgroup of natural flavonoids characterized by a 3,4-dihydro-2*H*-1-benzopyran skeleton.[11] These compounds exhibit a broad spectrum of biological effects, including antimicrobial, anti-inflammatory, and anticancer activities and have captured the attention of researchers due to their pharmacological significance.[12-14] In Figure 2a, the structural representation of (+/–)-**LQB-79**, the racemic variant of the dextrogyre natural pterocarpan isolated in 1995, is depicted. This natural compound has been documented to exhibit an antiproliferative effect on KB cells, a subline of the ubiquitous tumor cell line HeLa. Subsequently, (+/–)-**LQB-79** and various non-natural derivatives were synthesized by our group in racemic forms, and their respective antiproliferative effects on cultures of human chronic myeloid leukemias (CML) was studied.[15,16]

Remarkably, (+/–)-**LQB-79** demonstrated being equipotent on K562, representative of a CML with constitutive BCR/ABL tyrosine kinase activity, and on the multidrug resistant (MDR) variant Lucena-1, as evidenced in Table 1. The catechol moiety at the C3-C4 position within the A-ring was identified as pivotal, as removal of a single hydroxyl group or altering the position of the catechol moiety to C2-C3 resulted in inactive derivatives. Additionally, the introduction of a hydroxyl group into the D-aromatic ring led to a less active product, the structures of which are not presented herein.[15]

Despite the encouraging outcomes observed with (+/–)-**LQB-79**, apprehension arose concerning the potential *in vivo* metabolism of the catechol group in the A-ring, which could generate a toxic *o*-quinone. To address this concern, we synthesized the *o*-quinone (+/–)-**LQB-80** (Figure 2a), which showed enhanced activity against the studied cancer cell lines as compared to (+/–)-**LQB-79**. However, this profile was accompanied by increased

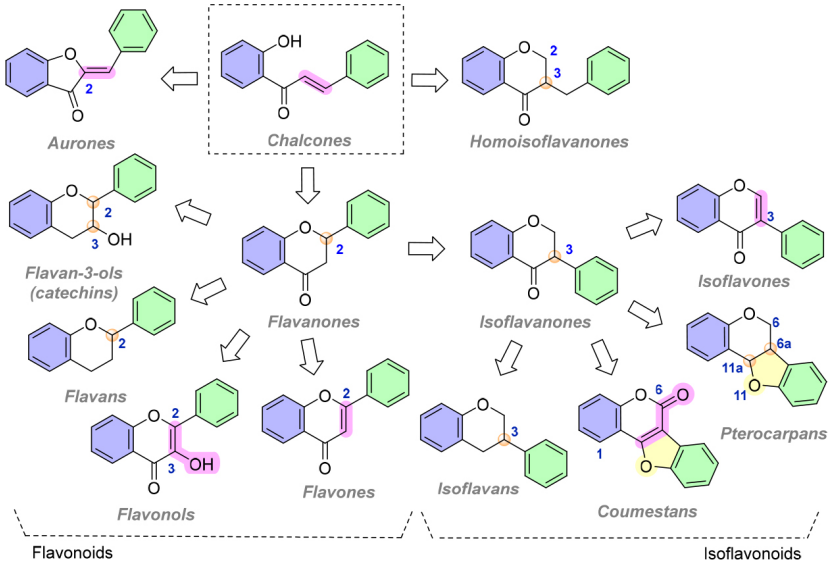


Figure 1. The flavonoid biosynthesis network.

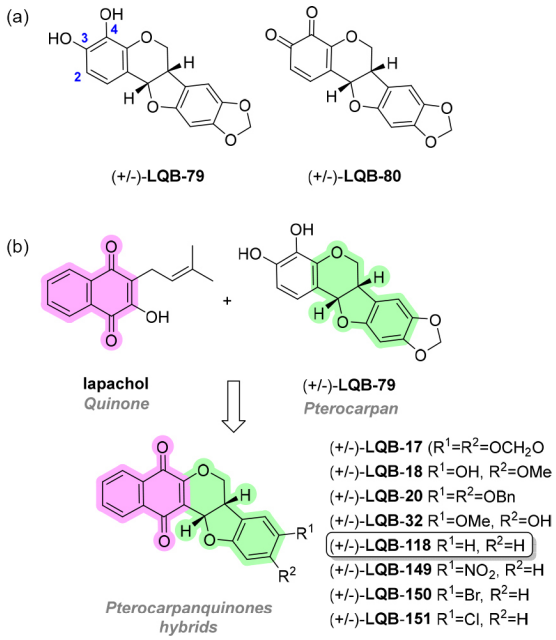


Figure 2. (a) Structures of pterocarpans (+/–)-**LQB-79** and (+/–)-**LQB-80**; (b) molecular hybridization strategy to obtain the pterocarpanquinones derivatives.

toxicity towards healthy peripheral blood mononuclear cells (PBMC), yielding a diminished selectivity index (SI), as detailed in Table 1.[15]

In response to this potential toxicity for *in vivo* studies, we employed the molecular hybridization strategy between (+/–)-**LQB-79** and lapachol (Figure 2b).[17] The latter, known for its mild cytotoxic effect on leukemia cells and PBMC, emerged as an ideal candidate. This hybridization resulted in the transformation of the pro-toxic catechol group (A-ring) in (+/–)-**LQB-79** into a 1,4-naphthoquinone moiety, yielding a novel scaffold termed pterocarpanquinones (Figure 2b). This innovative approach successfully mitigated concerns of potential *in vivo* toxicity and introduced a novel structural framework with promising implications for further development.

The hybridized product **LQB-18** has demonstrated a noteworthy antiproliferative effect on MDR Lucena-1 cells, comparable in potency to mitomycin C but exhibiting a superior SI when peripheral blood mononuclear cells (PBMC) are used as a reference. In contrast, mitomycin C exhibits higher potency to other leukemic cell lines, albeit employing a distinct mechanism that induces a cytostatic effect instead of apoptosis.[17] This marks the inaugural publication of the novel hybrid scaffold, pterocarpanquinone.

Remarkably, the antiproliferative activity of **LQB-18** extends to the B lymphoblastoid cell line Daudi, a rituximab-sensitive model of Burkitt's leukemia with a p53 mutation, commonly utilized in CAR-T cell studies and Raji, also bearing mutation in p53. Additionally, **LQB-18** and its counterpart **LQB-32** exhibit notable antiproliferative effects against imatinib-refractory CML cells obtained from patients undergoing treatment at the Brazilian National Cancer Institute (INCA-RJ). Intriguingly, the tested cell lines remain unresponsive to lapachol,[17] underscoring the significance of the newly introduced hybrid scaffold for the described antineoplastic effects.

Subsequently, reassessment of our project aiming to enhance the understanding of structure-activity relationships (SAR), led to the synthesis of a non-substituted product, **LQB-118**, and various 1,4-naphthoquinones featuring different substitutions at the D-ring (Scheme 1b). This expansion aimed to enhance our understanding of SAR. Surprisingly, **LQB-118** emerged as the most promising among all the pterocarpanquinones, and notably,

Table 1. Antiproliferative activity evaluation of pterocarpans and pterocarpanquinones

Compound	EC_{50} / μM							
	K562	Lucena-1	Daudi	Raji	HL-60	Jurkat	FEPS	PBMC
(+/–)-**LQB-79**	2.95	3.70	2.73	1.33	2.10	7.65	ND	24.13
(+/–)-**LQB-80**	1.49	3.70	0.89	0.75	0.24	1.86	ND	2.4
(+/–)-**LQB-17**	4.63	5.47	6.74	ND	ND	ND	ND	24.5
(+/–)-**LQB-18**	2.18	2.57	2.85	ND	ND	ND	ND	ND
(+/–)-**LQB-20**	3.43	3.46	6.31	ND	ND	ND	ND	24.5
(+/–)-**LQB-32**	4.50	4.49	6.08	ND	7.80	ND	ND	23.50
(+/–)-**LQB-118**	1.67	2.75	3.10	3.32	< 2	6.77	2.25	> 20
(+/–)-**LQB-149**	< 2.5	ND	ND	ND	< 1.2	ND	ND	ND
(+/–)-**LQB-150**	5.0	ND	ND	ND	5.0	ND	ND	ND
(+/–)-**LQB-151**	5.0	ND	ND	ND	5.0	ND	ND	ND
α-Lapachone	38.0	42.71	69.36	ND	ND	ND	ND	ND
Mitomycin C	0.47	2.75	0.45	ND	ND	ND	ND	4.3

EC_{50}: concentration required to inhibit 50% of cell growth; ND: not determined.

it is the easiest to synthesize, requiring only two steps from commercially available products. Despite moderate activity, the evaluation of **LQB-149**, **LQB-150**, and **LQB-151** in K562 cells was discontinued as they proved to be less potent than **LQB-118**.

The cytotoxicity of **LQB-118** was assessed against intracellular amastigotes of *Leishmania amazonensis*, in which (+/–)-**LQB-118** was effective with an EC_{50} (concentration required to inhibit 50% of cell growth) of 1.4 μM. This work[18] described a concentration-dependent reactive oxygen species (ROS) production in promastigotes within the initial 4 h, effects still observed after 24 h. Transmission electron microscopy (TEM) analysis also revealed various morphologic alterations characteristic of apoptosis in the treated cells yet keeping lower potency against macrophages (concentration required to reduce 50% of cell viability (CC_{50}) = 18.5 μM). Similar results were described towards *Toxoplasma gondii* tachyzoites *in vitro* as well.[19]

The antineoplastic effect of **LQB-118** was demonstrated across six models of human leukemias (Table 1), each displaying diverse adaptations to counteract drug-induced cell stress. These adaptations included elevated levels of glutathione and catalase (K562, Lucena-1 and FEPS), Bcl-2 (Jurkat), mutations in p53 (Daudi and Raji) and overexpression of the ABC transporters ABCB1 and ABCC1 (Lucena-1 and FEPS). The latter, pivotal in mitigating the efficacy of the gold standard CML treatment imatinib.[20] Significantly, **LQB-118** exhibited notable cytotoxicity (2 μM range) against Lucena-1 and FEPS MDR cells, which were later revealed to display resistance to imatinib, vincristine, daunorubicin, cisplatin, and clotrimazole, among others.[21-23] Analogous to its predecessor, the pterocarpanquinone **LQB-18** and **LQB-118** exhibited activity in CML cells from imatinib-refractory patients at the Brazilian National Cancer Institute (INCA-RJ)[24] and in ABC activity-positive cells from patients with acute myeloid leukemia (AML).[25]

This unique profile extended to non-hematologic malignancies, encompassing models of human lung and prostate cancers (Figure 3).

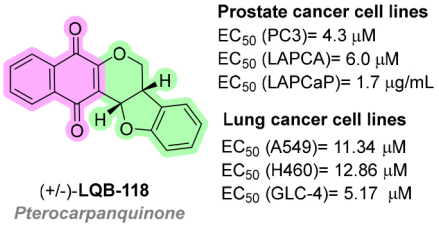
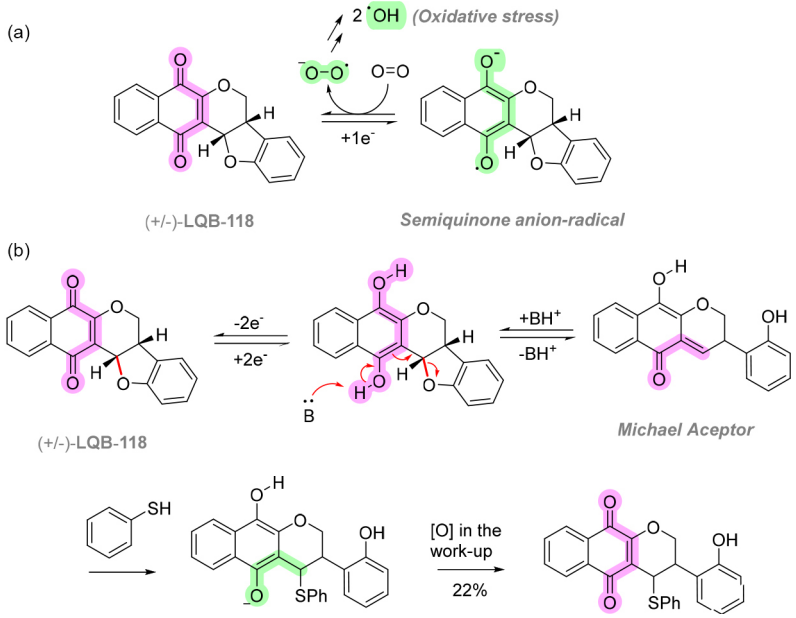


Figure 3. Antiproliferative activity of the pterocarpanquinone **LQB-118** in non-hematologic cancer cell lines.

The conventional molecular mechanism of action for quinones hinges on their redox potential. Once within the cell, xenobiotic quinones, exemplified by (+/–)-**LQB-118**, permeate through the mitochondrial membrane, disrupting the electron flow in the respiratory chain. The reduction of this quinone by a single electron yields the corresponding semiquinone anion-radical, a process wherein fumarate dehydrogenase and flavoprotein enzymes are implicated (see Scheme 1a). Upon re-oxidation, the semiquinone anion-radical regenerates the initial quinone while simultaneously reducing oxygen to the superoxide anion. This superoxide anion can subsequently transform into the



Scheme 1. (a) Reduction in mitochondria by one-electron transfer under high concentration of O_2; (b) activation by bioreduction (NQO1). *In vitro* reduction of **LQB-118** by dithionite revealed the formation of a Michael acceptor, indicative of a two-electron transfer.

hydroxyl radical, serving as the origin of oxidative stress within the mitochondria. This unique profile extended to non-hematologic malignancies, encompassing models of human lung, breast, colon, glioblastoma, and prostate cancers, as well as murine mammary carcinomas and melanomas, all of those posing specific challenges likely requiring the engagement of a complex mechanism of action that would explain the results (Figure 3).[26]

Bacelar *et al*.[27] demonstrated that the treatment of K562 and the immortalized T cell line Jurkat with 3 or 8 mmol L^{-1} **LQB-118** led to a rise in ROS levels, comparable to those induced by 10 mmol L^{-1} of hydrogen peroxide. Notably, this increase was more prolonged in Jurkat cells, likely attributable to their high expression of Bcl-2. Interestingly, when dicoumarol, an inhibitor of NQO1, was introduced, the EC_{50} on K562 increased to 4.3 mmol L^{-1}, suggesting that in these cells, **LQB-118** undergoes activation through reduction. Intriguingly, dicoumarol had no discernible effect on Jurkat cells. Subsequent confirmation of the importance of NQO1 was obtained when treatment with 50 μM dicoumarol impeded the cytotoxicity of **LQB-118**, resulting in a reduction in ROS production and apoptosis in the prostate cancer cell line PC3.[28] Consequently, these cell lines underwent apoptosis following the activation of caspase-12, caspase-9, and caspase-3.[24,27]

A co-occurring mechanism implicated in the antiproliferative activity of **LQB-118** involves the downregulation of the IAP family members Survivin and X-linked inhibitor of apoptosis protein (XIAP), which play a significant inhibitory role in apoptosis and autophagy. The participation of **LQB-118** in autophagy was also investigated, with non clear-cut results; though it synergizes with the autophagy promoter rapamycin, its toxicity failed to be suppressed by the inhibitor chloroquine in the murine melanoma B16F10[29] and to increase levels of Beclin-1 in human AML cells.[30] These effects would translate in a dysregulation of the cell cycle, as evidenced by flow cytometry studies[31] revealing arrest at the S/G2 phase in the androgen-independent prostate cancer cell line PC3. Remarkably, this effect surpassed the efficacy of the standard treatment paclitaxel in the androgen-dependent cell line LNCaP.[31] Intriguingly, this mechanism had previously been observed for the natural pterocarpan **LQB-79**.[32] This arrest can be partially attributed to **LQB-118** targeting the master cell cycle regulators FoxO3a and FoxM1 transcription factors, causing downregulation of c-Myc and of the cyclins D1 and B1, concurrent with an upregulation of the cell cycle inhibitor p21 demonstrated in AML cells. More recently, Maia and co-workers[33] reported the reduction of viability and cell migration of spheroids, three-dimensional models of human glioblastomas induced by (+/–)-**LQB-118**, as a monotherapy and combined with radiotherapy or temozolomide chemotherapy. Once again, the scope of action of this pterocarpanoquinone scope has broadened, now encompassing an organotypical model of cancer that helped in moving the studies towards *in vivo* models.

Following the successful demonstration of **LQB-118**'s efficacy across a diverse range of cells within the potency range of 0.75-16 μM,[26] our inquiry turned to the possibility of multiple concurrent mechanisms at play. Notably, like mitomycin, **LQB-118** and its derivatives feature a C–O bond at the benzylic position of the quinone ring (highlighted in red), a crucial structural element for activation through bioreduction (see Scheme 1b). *In vitro* reduction of **LQB-118** by dithionite revealed the formation of a Michael acceptor, indicative of a two-electron transfer. The resulting Michael acceptor was successfully intercepted with thiophenol, yielding an isolable intermediate in 22% yield. This procedure was replicated successfully for the earlier pterocarpanquinones, such as **LQB-32**.

Cyclic voltammetry studies[34,35] provided further confirmation of the transient quinonamethides (QM) intermediates generated from (+/–)-**LQB-118**. In the presence of dithionite as a reductant (reduction via 2e) and hexanethiol as a nucleophilic species, the Michael adduct generated in the reduction-rearrangement step was effectively trapped, consistent with our earlier findings.[34,35]

More recently, Maia and co-workers[33] reported on the antiproliferative and inhibitory effects on cell migration induced by (+/–)-**LQB-118** in glioblastomas (CNS cancer cells). This pterocarpanoquinone's scope has once again broadened, now encompassing an antiproliferative impact on cytarabine-resistant leukemia cell lines.[36]

2.1. *In vivo* studies with (+/–)-**LQB-118**

The comprehensive dataset available at the time strongly suggested that **LQB-118** held significant therapeutic value, being able to overcome a variety of adaptations to avoid chemotherapeutic stress through an elegant chain of events only possible given its unique chemical structure. After those encouraging findings, the progression to *in vivo* models became imperative.

In a seminal study by Torres-Santos and co-workers,[37] (+/–)-**LQB-118** was administered intralesionally, intraperitoneally, or orally in *L. amazonensis*-infected BALB/c mice. Remarkably, the compound demonstrated effective control over both lesion development and parasite burden, matching the efficacy of pentavalent antimonial, the standard treatment for this protozoan parasite. These outcomes were achieved without observable alterations in serological markers of toxicity, similar results obtained

by Da-Silva *et al.*[38] towards *L. braziliensis*-infected hamsters. Furthermore, the pterocarpanquinone induced enhancement of intradermal reactions to parasite antigens, phosphatidylserine exposure, increased production of reactive oxygen species, adenosine triphosphate (ATP) depletion, and deoxyribonucleic acid (DNA) fragmentation on promastigotes.

In 2016, Torres-Santos and co-workers[39] studied the subacute toxicity and therapeutic efficacy of **LQB-118** in experimental visceral leishmaniasis. They observed that oral treatment with 10 mg kg^{-1} of body weight *per* day of **LQB-118** inhibited the development of hepatosplenomegaly with a 99% reduction in parasite load. This *in vivo* toxicological analysis showed no change in the clinical, biochemical, or hematological parameters. Histologically, all the analyzed organs were normal, except for the liver where focal points of necrosis with leukocytic infiltration were observed at 5-fold higher treatment doses than the therapeutic one, but these changes were not accompanied by an increase in transaminases. Findings indicated that **LQB-118** is effective for treating different clinical forms of leishmaniasis and, more importantly, presents no relevant signs of toxicity at therapeutic doses, results that firmly established (+/–)-**LQB-118** as a strong candidate for those neglected diseases with a favorable safety profile.[39]

Inflammation is a critical component of tumor progression, with the microenvironment fostering proliferation, survival, and migration of neoplastic cells; as such, targeting inflammation represents a supporting strategy for cancer treatment. Cavalcante-Silva and co-workers[40] showed that (+/–)-**LQB-118** presented a great inhibitory effect on TNF-α release *in vitro*, since it reduced lipopolysaccharide (LPS)-induced lung inflammation in C57BL/6 mice. LPS inhalation induced a marked neutrophil infiltration to the lungs which was reduced by intraperitoneal treatment with (+/–)-**LQB-118** in a similar manner to that of dexamethasone and even better than that of acetylsalicylic acid. Moreover, administration of this product resulted in decrease of nuclear factor κB (NF-κB) activation and IL-8/keratinocyte-derived chemokine (KC) level in lungs, with a pronounced inhibitory effect on TNF-α release, as measured in the bronchoalveolar lavage fluid. Once again, the isoflavonoid origin of (+/–)-**LQB-118** might translate into the pharmacology, considering that molecular modeling showed that, as other isoflavonoids, it may bind to both α and β estrogen receptors with a similar orientation to 17-β-estradiol. More recently, its anti-inflammatory properties were further elucidated, where treating Swiss mice with 10 mg kg^{-1} (+/–)-**LQB-118** decreased the levels of TNF-α (26%), IL-1β (98%) and IL-6 (58%) after pro-inflammatory stimuli triggered by the *Saccharomyces cerevisiae* wall polysaccharide zymosan, with no toxicity to peritoneal macrophages.[40]

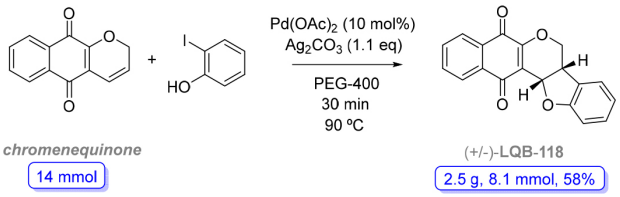
Concomitantly, toxicity toward the immune system was studied by Rumjanek and co-workers.[29] Immunotoxicity, which can render patients susceptible to infections or even secondary cancers, is an often-underappreciated subject that increases attrition rates during drug development, exerting great pressure on Medicinal Chemistry. As such, the possible toxicity of this pterocarpanquinone to primary and secondary immune system organs during antineoplastic treatment *in vivo* was first described by Salustiano *et al.*[29] Immunophenotyping of the bone marrow, thymus, spleen, draining and contralateral lymph nodes of Swiss and C57BL/6 mice after treatment regimens with either daily or *in bolus* $10 \times IC_{50}$ (half-maximal inhibitory concentration) (+/–)-**LQB-118** showed no detectable changes in the distribution of immune cells' subsets, all without any perceived behavioral alteration or common side effects such as fur loss. Adding to this, T lymphocytes were still capable of regular activation following concanavalin A treatment, a surrogate model for T cell stimulation by antigen-presenting cells. (+/–)-**LQB-118** mitigated the growth of B16F10 melanoma and Ehrlich mammary carcinoma in ascites or subcutaneous forms, inducing apoptosis *in vivo* while delaying the onset of cancer-induced cachexia, a common side effect closely related to inflammation mediated by NF-κB and TNF-α, and ultimately extending the lifespan of tumor-bearing mice.[29]

Lupold and co-workers[27] studied the antiproliferative activity of orally administered **LQB-118** in xenograft models of prostate cancer PC3 (4.3 μM), LAPC4 (6.0 μM), and LNCaP (1.7 μg mL^{-1}) with high levels of NQO1, Nrf2 and superoxide dismutase 1 (SOD1) in athymic male nude mice. In line with previously described results,[26] **LQB-118** increased SOD1 and its knockdown, either by siRNA or miRNA, enhanced (+/–)-**LQB-118** cytotoxicity levels indicating the activation of an antioxidant response. Conversely, dicoumarol, an inhibitor of NQO1 and *N*-acetylcysteine, a free radical scavenger, decreased the potency of this pterocarpanquinone and ROS production as quantified by flow cytometry, strongly suggesting this product may be activated by bioreduction followed by production of ROS.

It is worth mentioning that chirality can play an important role in the selectivity, potency, and metabolism of flavonoids. Accordingly, a variety of flavonoids with a high eudismic ratio have been reported in the literature.[41-46] Therefore, Cass and co-workers[47] reported the preparative chiral HPLC separation and absolute configuration determination of **LQB-118** by vibrational circular dichroism (VCD) and density functional theory (DFT)

calculations. The enantiomers were studied separately by Dr Eduardo Caio (FIOCRUZ-RJ) and Dr Eduardo Salustiano (studies conducted at UFRJ) and, surprisingly, showed the same potency as antileukemic and antileishmanial agents in the models studied.

In Scheme 2 is shown the synthesis of (+/–)-**LQB-118** in gram scale through an oxyarylation reaction between the chromenequinone and the *o*-iodophenol catalyzed by palladium.[48] The reaction is very fast and practical yielding the desired pure product by filtration of the reaction medium in a pad of silica gel using EtOAc as eluent.



Scheme 2. Obtaining (+/–)-**LQB-118** on a gram scale.

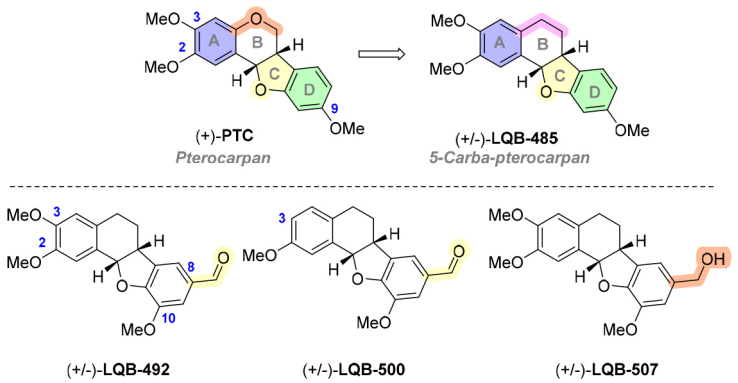
3. 5-Carba-Pterocarpans and 1-Carba-Isoflavanones with Antiproliferative Activity

The (+)-2,3,9-trimethoxypterocarpan (+)-**PTC** was isolated from *Platymiscium floribundum* and has been evaluated by Pessoa and co-workers[46,49-52] as cytotoxic agents against a panel of leukemia, breast, ovarian, prostate, colon, melanoma, and brain cancer cell lines, showing promising activity and bioselectivity. Structure-activity relationship investigations demonstrated that methoxy group at C2 position is key for the antiproliferative activity,[49] in contrast with the pterocarpan (+/–)-**LQB-79**, in which the presence of the catechol group at 3,4-position of the A-ring is essential for the anticancer potency. A study of cell viability and drug-induced morphological changes revealed the compound causes cell death through a mechanism characteristic of apoptosis. Besides, computational studies[46] suggested that the (+)-**PTC** bind to the kinesin-type protein Eg5 receptor with greater affinity than (*S*)-monastrol, through stabilizing interactions with the methoxy groups of the A-ring.

Based on the promising structure of (+)-**PTC** we decided to explore the isosteric substitution of an oxygen at the pterocarpans' B-ring by a methylene group, resulting in the 5-carba-pterocarpens derivatives (Table 2). This type of isosterism was successfully employed previously for pterocarpens[53] and *aza*-pterocarpans.[54]

So, we prepared and evaluated a series of new 5-carba-pterocarpans and their antiproliferative activity against several cancer cell lines was evaluated and compared with (+/–)-**PTC** and its pure enantiomeric forms (Table 2).[55] Surprisingly, (+/–)-**LQB-485**, the isostere of (+)-**PTC**, was significantly less potent than the natural production in the four tested cell lines, suggesting the oxygen atom

Table 2. Antiproliferative activity of the 5-carbapterocarpans derivatives

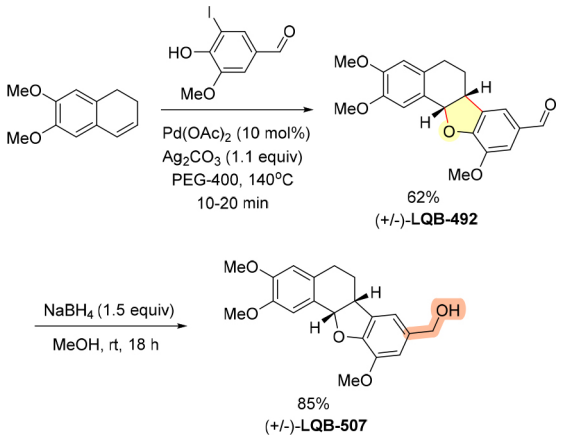


Compound	EC_{50} / μM			
	SF-295	PC3	HL-60	HCT-116
(+)-**PTC**	8.30	4.42	0.42	0.41
(–)-**PTC**	80.52	50.93	57.07	63.05
(+/–)-**PTC**	27.67	6.33	1.78	7.2
(+/–)-**LQB-485**	99.56	> 160	22.18	98.4
(+/–)-**LQB-492**	3.85	42.26	24.41	42.3
(+/–)-**LQB-500**	107.27	> 160	14.24	90.6
(+/–)-**LQB-507**	33.93	11.84	8.81	20.24

EC_{50}: concentration required to inhibit 50% of cell growth; PBMC: peripheral blood mononuclear cells.

at the pyran ring may be involved in the interaction with the biological target. However, (+/–)-**LQB-492** was more potent than (+)-**PCT** towards SF-295 cancer cells (CNS) and (+/–)-**LQB-507**, prepared by reduction of (+/–)-**LQB-492**, is more active than (+)-**PCT** in PC3 cells (prostate cancer). Interestingly, the presence of 3,2-dimethoxy group at the A-ring is essential for the activity, since **LQB-500** is up to 27 times less potent than (+/–)-**LQB-492** in three out of the four cancer cell lines tested.

The 5-carba-pterocarpans were synthesized in moderate to good yields (45-72%) through a palladium-catalyzed oxyarylation[56] of alkoxy-1,2-dihydronaphthalens with *o*-iodophenols at 140 °C in PEG-400 for 10-20 min, as indicated for (+/–)-**LQB-492** in Scheme 3. The reduction of the aldehyde group was achieved by treatment with $NaBH_4$, as indicated for (+/–)-**LQB-507**.[55]



Scheme 3. Synthesis of (+/–)-**LQB-492** and (+/–)-**LQB-507**.

Considering the substantial eudismic ratio observed, reaching up to 150 times, between (+)-**PTC** and (–)-**PTC**, as detailed in Table 2, coupled with the robust antiproliferative activities demonstrated by the racemic forms of 5-carbapterocarpans **LQB-492** and **LQB-507**, an ongoing endeavor in our laboratories involves the development of an enantioselective route to synthesize these derivatives based on asymmetric transfer hydrogenation (ATH) reactions.[41] We have already successfully applied this strategy to obtain isoflavanones,[57] pterocarpans,[57] flavans[58] and, homoisoflavanones[59] in their enantiomeric pure forms.

Building on the success achieved through the isosteric substitution of oxygen with a methylene group at the pyran ring of pterocarpans, resulting in the development of 5-carba-pterocarpans derivatives, we applied a similar strategy to the scaffold of isoflavanones. Our objective was to explore the antiproliferative activity of 1-carba-isoflavanones derivatives, as illustrated in Figure 4.

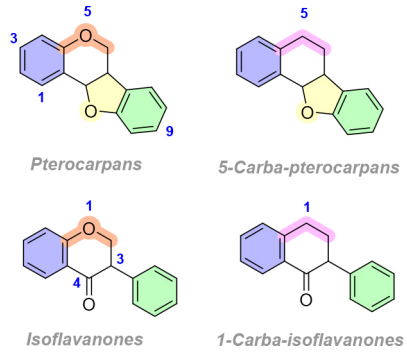
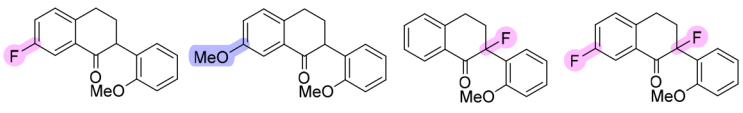


Figure 4. Isosteric substitution of oxygen by a methylene group: 5-carba-pterocarpans and 1-carba-isoflavanones derivatives.

A series of 1-carba-isoflavanones, also known as α-aryl-α-tetralones, and their α-fluorinated derivatives were systematically assessed for their anti-proliferative effects against human breast cancer and chronic myeloid leukemia cell lines with diverse multi-drug resistance profiles. The most promising compounds are summarized in Table 3.

In that work (+/–)-**LQB-454** and (+/–)-**LQB-501**

Table 3. Antineoplastic effect (IC_{50}) of synthesized 1-carba-isoflavanones on models of human breast cancer and chronic myeloid leukemia



(+/-)-**LQB-501** (+/-)-**LQB-309** (+/-)-**LQB-454** (+/-)-**LQB-556**

Compound	IC_{50} / μM						
	Breast cancer cell lines			Chronic myeloid leukemia cell lines			
	MCF-7	MCF-10	SI	K562	Lucena I	FEPS	RR
(+/–)-**LQB-501**	66.87	152.67	2.43	72.30	63.17	36.37	0.50
(+/–)-**LQB-309**	88.37	172.22	1.95	79.72	58.02	34.62	0.43
(+/–)-**LQB-454**	60.30	235.67	3.91	63.42	46.84	27.06	0.42
(+/–)-**LQB-556**	84.23	> 320	3.67	49.64	39.00	29.50	0.59

IC_{50}: half-maximal inhibitory concentration; SI: selective indexes, SI = MCF-10/MCF-7; RR: relative resistance indexes evaluated on chronic myeloid leukemias, RR = (IC_{50} resistant cell line, FEPS/(IC_{50} parental cell line, K562).

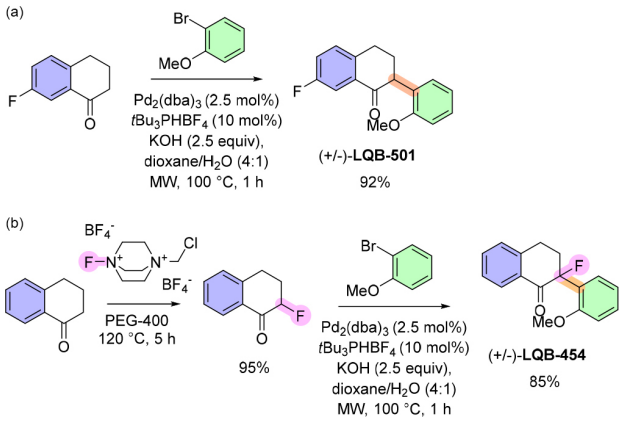
demonstrated significant inhibition of the mitochondrial reducing activity in MCF-7 cells, an invasive, endocrine therapy-sensitive breast ductal carcinoma model. Notably, both compounds incorporate a fluorine atom into their structures, suggesting a potential contribution to their efficacy against breast cancer. Remarkably, (+/–)-**LQB-454** exhibited the highest selectivity index (3.91) when compared to MCF-10A, an estrogen receptor-negative, non-tumorigenic human mammary cell widely used in studies regarding normal breast cell function and transformation. Concerning the CML models, (+/–)-**LQB-454** and (+/–)-**LQB-556** emerged as the most promising compounds, once again featuring α-fluorine atoms in their structures, while efflux activity assays indicated that these compounds may not be transported by the MDR-associated proteins ABCB1 and ABCC1.

Turning to chronic myeloid leukemias, (+/–)-**LQB-454** and (+/–)-**LQB-556** emerged as the most promising compounds, featuring α-fluorine atoms in their structures. The higher toxicity on multidrug-resistant cells suggests that these compounds may not serve as substrate for efflux transporters ABCB1 and ABCC1, as evidenced by the MCF-10/MCF-7 ratio exceeding 1 in breast cancer cell lines.

Interestingly, leukemic cells displayed lower EC_{50} values for most compounds compared to breast cancer cells, indicating potential distinctions in drug distribution or mechanisms of action between cells of epithelial and blood origins. These compounds were hypothesized to interact with aromatase, the final and rate-limiting step in estrogen biosynthesis.[60]

Additionally, compounds such as (+/–)-**LQB-501**, (+/–)-**LQB-309**, and (+/–)-**LQB-454** showed lower EC_{50} values for the MDR leukemia cell line FEPS, reflecting in the lower or equal to 0.5 relative resistance indexes (RR). The higher sensitivity of chemotherapeutic refractory cells, as compared to parental ones, corresponds to a form of synthetic lethality known as collateral sensitivity, a yet poorly understood mechanism of drug-induced stress. Intriguingly, halogenation has been previously linked to collateral sensitivity on a few of our works, as the addition of bromine, chlorine, and iodine to 5-carba-pterocarpans[61] and fluorine to azaspirodecanes[62] produced similar outcomes on the same cells. As fluorination is a ubiquitous modification that can change physicochemical properties[63,64] such as bioabsorption, binding affinity, chemical reactivity and metabolic stability, further investigation is required to understand its contribution to the increased selectivity.

The synthesis of 1-carba-isoflavanones (α-aryl-α-tetralones) involved the palladium-catalyzed direct α-arylation of readily available tetralones with *o*-alkoxybromoarenes, achieved under microwave irradiation for 1 h. This method is illustrated in Scheme 4a for (+/–)-**LQB-501**.[65,66] Conversely, the α-fluorinated derivatives, exemplified by (+/–)-**LQB-454**, underwent a two-step process. Initially, these derivatives were treated with Selectfluor™ in PEG-400 under heating,[67] followed by α-arylation under conditions identical to those employed for (+/–)-**LQB-501**, as depicted in Scheme 4b.[66]



Scheme 4. Synthesis of 1-carba-isoflavanones (a) (+/–)-**LQB-501**; (b) (+/–)-**LQB-454**.

4. Flavonoid Derivatives with Antiviral Activity

4.1. Coumestans and coumarins as inhibitors of non-structural NB5S polymerase of HCV

Hepatitis C virus (HCV), a major public health concern with an estimated 58 million people with chronic infection worldwide, was identified as a causative agent of non-A, non-B viral hepatitis and belongs to the *Flaviviridae* family of viruses. Chronic HCV infection causes several changes within the host and can be associated with the risk of developing more serious conditions such as cirrhosis, steatosis, and hepatoma cellular carcinoma. The HCV NS5B is essential for viral ribonucleic acid (RNA) replication and is therefore a prime target for development of HCV replication inhibitors. Kaushic-Basu *et al.*[68] identified coumestans a new class of HCV NS5B inhibitors and reported the *in vitro* NS5B RNA-dependent RNA polymerase (RdRp) inhibition by wedelolactone, a naturally occurring coumestan, and four synthetic analogues prepared in our laboratory. Coumestans interfere at the step of NS5B-RNA binary complex formation and molecular docking of these compounds within the allosteric site of NS5B yielded significant correlation between their calculated binding energies and IC_{50} values. From this study **LQB-34** emerged as the best compound of the series, inhibiting the NB5S RdRp

polymerase of HCV with an IC_{50} value two times lower than wedelolactone (Figure 5).

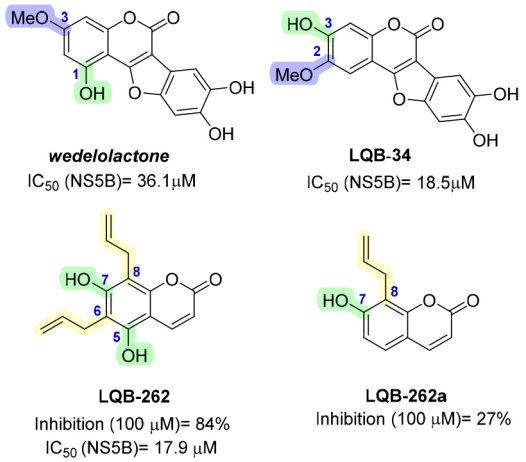
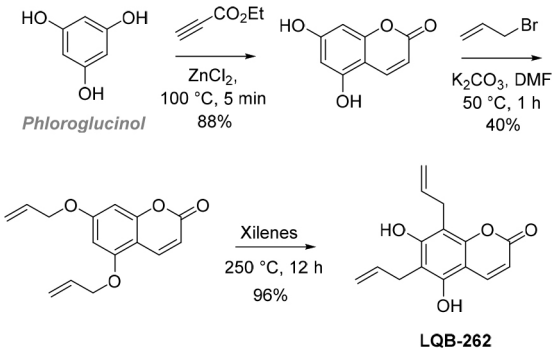


Figure 5. Coumestans, coumarins, and neoflavones evaluated as hepatitis C virus (HCV) NS5B inhibitors.

However, further efforts to optimize the structure of coumestans proved discouraging, as the synthesis of these compounds require an expensive multistep procedure. So, we sought to explore trimmed versions of these compounds, as coumarins and neoisoflavones, in which the C and D-rings are absent. Our strategy was to increase the affinity of A- and B-ring for the receptor by including the nonpolar allyl moiety at the A-ring.[69] Twenty-four products were evaluated as NS5B inhibitors and **LQB-262** was found as the most potent of the series (Figure 5). The compound binds at the NS5B PT-1 site, comprising two hydrophobic pockets, HP-1, and HP-2, and appears to span the entire site. Their allyl groups interact through hydrophobic contacts while the phenol groups stablish hydrogen bonding interactions. In **LQB-262a**, the absence of the phenol group at C5 and the allyl group at C6 led to a huge decrease in the NS5B inhibition. It is worth mentioning that **LQB-262** showed a potency equivalent to the coumestan **LQB-34** though being easier to prepare, requiring only three steps from phloroglucinol (Scheme 5).



Scheme 5. Synthesis of **LQB-262** from phloroglucinol.

4.2. 5-Carba-pterocarpens and 1-carba-isoflavanones as inhibitors of HCV in human reported cells

The structural resemblance exhibited in Figure 6 between coumestans and 5-carba-pterocarpens prompted our investigation into the antiviral potential of these compounds. We envisioned employing 1-carba-isoflavanones as synthetic intermediates for the synthesis of the desired 5-carba-pterocarpens. Given the interesting pharmacological properties previously reported for isoflavanones,[70] we decided to extend our screening efforts to evaluate the anti-HCV activity of the 1-carba-isoflavanones.[71,72]

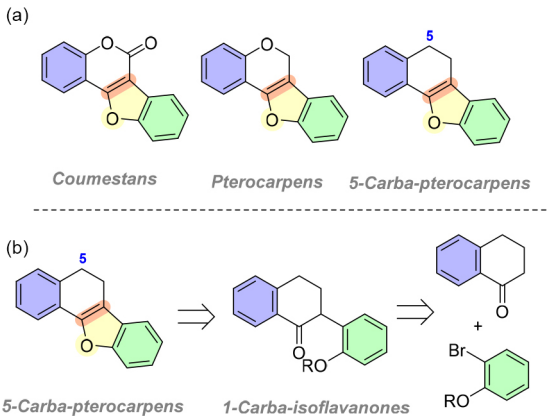
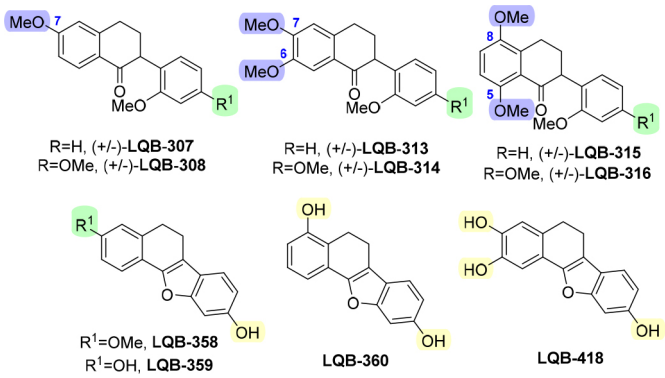


Figure 6. (a) Structural relationships between coumestans, pterocarpens and 5-carba-pterocarpens; (b) retrosynthetic analysis of 5-carba-pterocarpens.

Table 4 displays the anti-HCV activity of various 1-carba-isoflavanones and 5-carba-pterocapens in Huh7/Rep-Feo1b and Huh7.5-FGR-JC1-Rluc2A replicon systems.[71,72] Among these compounds, the (+/–)-**LQB-314**, bearing methoxy groups at C6 and C7 positions of the A-ring, emerges as the most potent in both replicon systems. Notably, this product exhibits a remarkable selectivity index, ranging from very high in Huh7/Rep-Feo1b to good in Huh7.5-FGR-JC1-Rluc2A replicon cells. Interestingly, the removal of the methoxy group at C6, as observed in (+/–)-**LQB-308**, or the repositioning of the methoxy groups to C5 and C8, as seen in both (+/–)-**LQB-315** and (+/–)-**LQB-316**, results in a decrease in both potency and selectivity index.[72]

The Huh7.5-FGR-JC1-Rluc2A replicon reporter cells exhibited heightened sensitivity to the 5-carba-pterocarpens, particularly those bearing phenolic groups on both the A- and D-ring of the structures. Notably, these compounds demonstrated elevated selective indexes, ranging from 15 to 70. Among this group, **LQB-360** stands out as particularly promising.[71]

Table 4. Inhibition of HCV RNA replication in Huh7/Rep-Feo1b and Huh7.5-FGR-JC1-Rluc2A replicon reporter cells

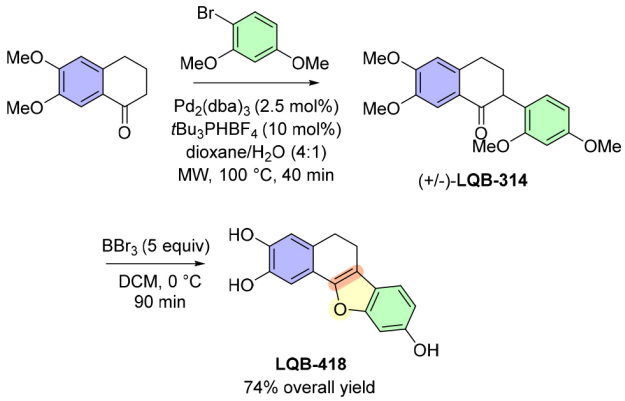


Compound	Huh7/Rep-Feo1b		Huh7.5-FGR-JC1-Rluc2A	
	EC_{50} / μM	SI	EC_{50} / μM	SI
(+/–)-**LQB-307**	10.6	14.3	1.5	101.4
(+/–)-**LQB-308**	7.5	< 6.6	3.3	< 15.3
(+/–)-**LQB-313**	42.5	2.9	4.8	26.2
(+/–)-**LQB-314**	1.8	> 111.1	4.3	> 46.2
(+/–)-**LQB-315**	8.0	> 24.9	6.8	> 29.5
(+/–)-**LQB-316**	8.2	< 6.0	21.5	< 2.3
LQB-358	19.9	5.3	1.5	70.6
LQB-359	12.3	6.8	1.9	44.4
LQB-360	5.9	10.1	2.4	24.7
LQB-418	5.5	20.9	6.1	18.8

EC_{50}: concentration required to inhibit 50% of viral replication; SI: selectivity index represents the ratio of CC_{50} to EC_{50}.

In exploring the underlying mechanism, the evaluation of NS5B inhibition was conducted for the 5-carba-pterocarpens. Interestingly, only **LQB-360** and **LQB-358** exhibited weak NS5B inhibition at a concentration of 50 μM. This suggests that NS5B inhibition is not the primary mechanism responsible for the inhibition of HCV replication in this context. Further investigation is warranted to elucidate the precise mechanisms underlying the observed anti-HCV activity of these compounds.[71]

The 1-carba-isoflavanones (α-aryl-α-tetralones) were obtained by the palladium catalyzed direct α-arylation of tetralones with *o*-alkoxybromoarenes. Then, a one-pot BBr_3-promoted *O*-demethylation and cyclization sequence gave the corresponding 5-carbapterocarpens as indicated in Scheme 6 for the compounds (+/–)-**LQB-314** and **LQB-418**.



Scheme 6. The synthesis of the 1-carba-isoflavanone (+/–)-**LQB-314** and its transformation into the 5-carbapterocarpen **LQB-418**.

4.3. Aurones as inhibitors of SARS-CoV-2 replication

The identification of natural polyphenolic flavonoids such as baicalein and myricetin as robust inhibitors of the 3CLpro enzyme of SARS-CoV-2 has prompted significant interest in their therapeutic potential for COVID-19 treatment (Figure 7).[73,74] The observed antiviral activity in cell-based systems underscores the promise of this class of natural products (NPs) in COVID-19 treatment.[73-77] This realization compelled us to explore new orally available small-molecule entities derived from the flavonoid skeleton, leading us to the intriguing yet unexplored class of aurones.

Aurones, structural isomers of flavones and flavonols, feature a unique C_6-C_3-C_6 skeleton comprising benzofuranone and phenyl moieties connected by an exocyclic carbon-carbon double bond with (*Z*)-geometry.[78]

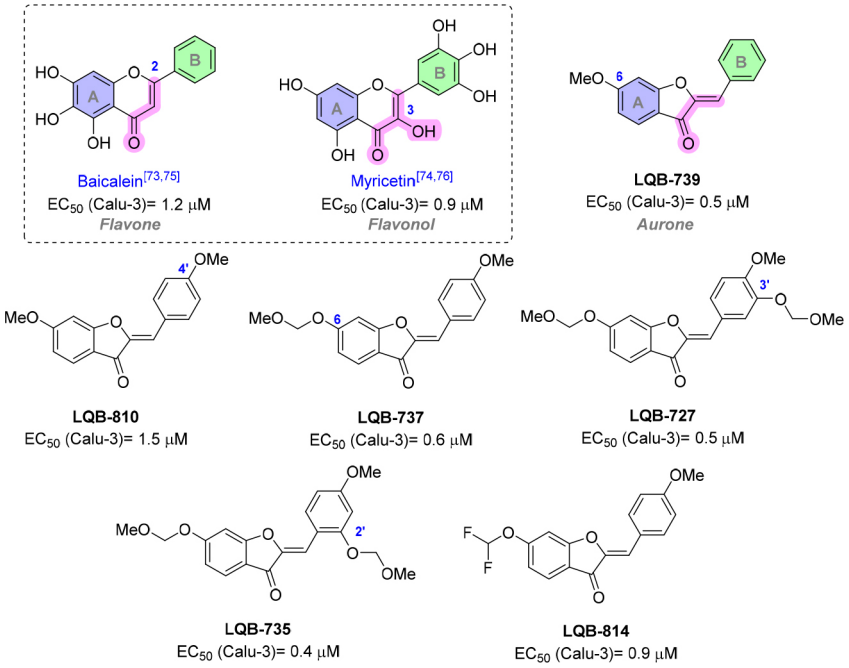


Figure 7. Flavonoids as inhibitors of SARS-CoV-2 replication in infected Calu-3 cells.

Surprisingly, literature analysis revealed a notable absence of evaluations of aurones against SARS-CoV-2 replication. Consequently, we embarked on a comprehensive investigation, involving the design, synthesis, and evaluation of the anti-SARS-CoV-2 activity of 25 aurones. The primary aim was to establish a preliminary structure-activity relationship (SAR) through a phenotypic-based screening approach.[79]

The synthetic route involved the oxidative cyclization of 2'-hydroxychalcones, incorporating diverse oxygenated groups at A- and/or B-rings. A limitation of the number of phenolic groups was employed in the design of the compounds to preclude potential bioavailability and extensive metabolization issues commonly observed in natural flavonoids during preclinical *in vivo* evaluations.[80-82]

Remarkably, the results unveiled that 12 out of the 25 compounds exhibited EC_{50} values below 3 μM, with five demonstrating even more remarkable EC_{50} values below 1 μM and exhibiting no apparent cytotoxic effects. Substituting A- and B-rings with OMe and OMOM (OCH_2OCH_3) groups proved beneficial for activity, while corresponding phenolic derivatives showed a significant reduction in anti-SARS-CoV-2 potency. Intriguingly, the introduction of the "lipophilic phenol" OCF_2H at the 6-position of the A-ring (**LQB-814**, EC_{50} = 0.9 μM) significantly enhanced antiviral potency, surpassing its phenolic counterpart (EC_{50} > 10 μM) by more than 10 times. The most potent compound in the series, aurone **LQB-735** (EC_{50} = 0.4 μM, SI = 2441.3), demonstrated a two-to-three-fold greater efficacy in inhibiting SARS-CoV-2 replication in Calu-3 cells compared to polyphenolic flavonoids myricetin and baicalein, respectively (Figure 7).[79]

An initial exploration of the mechanism of action for the five most active compounds, as inhibitors of SARS-CoV-2 3CLpro, based on molecular dynamic calculations, suggests that these aurones may detach from the active site of 3CLpro. Ongoing efforts involve computational calculations and experimental enzymatic assays to further elucidate these interactions, adding depth to our understanding of the therapeutic potential of aurones in the context of SARS-CoV-2 inhibition.[79]

5. Conclusions

LQB-118 has been shown to exhibit anti-inflammatory and consistent anti-cancer properties *in vivo* and *ex vivo*. It also presents antileishmanial effects, cutaneous and visceral, in mice and hamsters. *In vivo* studies have demonstrated no toxicity for the immune system and no changes to the clinical, biochemical, hematological, or histological parameters. These results indicate that **LQB-118** is a strong lead for the development of successful anti-parasitic and anti-cancer drug candidates. In addition, carba-flavonoids were explored for the first time as a source of bioactive products, with one 5-carba-pterocarpan showing promising toxicity against CNS cancer models. Fluorinated 1-carba-isoflavanones were active in drug-resistant models of breast cancers and chronic myeloid leukemias. 5-Carba-pterocarpens and

1-carba-isoflavanones demonstrated potent activity and selectivity against HCV replicon reporter cells as well. Finally, aurones were reported for the first time by our group as strong inhibitors of SARS-CoV-2 replication in cell-based essays. Five new promising compounds were discovered, bearing EC_{50} < 1 μM. Currently, ADMET of the more promising compounds are now being evaluated to select the better ones for *in vivo* studies using healthy animals. Overall, data obtained in-house or in collaboration not only helped in advancing the knowledge of medicinal chemistry of flavonoids and in bridging gaps between studies *in vitro* and validations *in vivo*, but ultimately in creating a strong network of researchers with diverse backgrounds, all focused-on tackling neglected, recent and future challenges with Brazilian technology.

Acknowledgments

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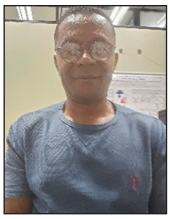
Author Contributions

Guilherme S. Caleffi was responsible for formal analysis, writing (original draft, review and editing); Alcides J. M. da Silva for writing review and editing; Chaquip Daher Netto for writing review and editing; Jorge L. O. Domingos for writing (original draft, review and editing); Eduardo J. Salustiano for formal analysis, writing (original draft, review and editing); Paulo R. R. Costa for conceptualization, project administration, resources, supervision, writing (original draft, review and editing).

Guilherme S. Caleffi *initiated, in 2016, his doctoral studies under the guidance of Prof Paulo Costa at the Federal University of Rio de Janeiro (UFRJ). Following a research period at the University of Alicante (UA) under the mentorship of Prof Carmen Nájera, he successfully earned his PhD in 2020. As a postdoctoral fellow in a CAPES emergency program, he focused on the synthesis of SARS-CoV-2 protease inhibitors. Currently, as a visiting professor at UFRJ's Natural Products Research Institute, he works in the areas of natural products synthesis, asymmetric catalysis, and medicinal chemistry.*



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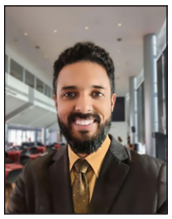
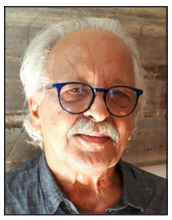
Jorge L. O. Domingos *graduated in Chemical Engineering from the State University of Rio de Janeiro (UERJ) in 1997. He obtained his PhD in 2004 focused on synthetic organic chemistry and medicinal chemistry in Prof Paulo R. R. Costa´s group at the Federal University of Rio de Janeiro (UFRJ). From 2005 to 2009 he worked as a researcher at FarManguinhos in Oswaldo Cruz Foundation (FIOCRUZ) and he had a postdoctoral stay at University of Alicante in 2011. He joined to the Chemistry Institute*

of UERJ as a professor in 2012 and since 2023 he became an associate professor.



Eduardo J. Salustiano *is a seasoned professional and an accomplished science communicator, with diverse contributions to cancer biology and preclinical drug development. He has produced one patent, one book, and over 20 papers investigating the major challenge in cancer treatment, multidrug resistance. After graduating from UFRJ in 2013, he worked as a peer reviewer for over 10 journals, as an R&D analyst and lecturer, mentoring numerous graduate students. In 2022, he joined the University of Utah as a Flow Cytometry Specialist, providing critical analysis, training, and assistance to researchers and key opinion leaders in generating and sharing their knowledge and innovations.*



Paulo R. R. Costa *joined Federal University of Rio de Janeiro (UFRJ) in 1975 as an Assistant Professor and became a Full Professor in Organic Chemistry in 1995. He was a visiting professor at the Universities of Paris-Sud (1996) and Alicante (2011), director of IPPN-UFRJ (1992-1996), director of the Organic Chemistry Division of the SBQ (2001-2004) and a member of the CAPES Graduate Evaluation Committee (1998-2002). He had postdoctoral stays at the Université Joseph Fourier (UJF, 1984, Grenoble) and École Supérieure de Physique et de Chimie Industrielles de la Ville de Paris (ESPCI, 1988, Paris). He is a co-author of two textbooks of chemistry (ARTMED/Bookman) and a book "The Chemistry of Carbonyl Compounds and Derivatives" edited by the Royal Society of Chemistry in 2022. He is a co-author of more than 170 papers and has supervised 30 PhD students.*

References

1. Jucá, M. M.; Cysne Filho, F. M. S.; de Almeida, J. C.; Mesquita, D. S.; Barriga, J. R. M.; Dias, K. C. F.; Barbosa, T. M.; Vasconcelos, L. C.; Leal, L. K. A. M.; Ribeiro, J. E.; Vasconcelos, S. M. M.; *Nat. Prod. Res.* **2020**, *34*, 692. [Crossref]
2. Dias, M. C.; Pinto, D. C. G. A.; Silva, A. M. S.; *Molecules* **2021**, *26*, 5377. [Crossref]
3. Ullah, A.; Munir, S.; Badshah, S. L.; Khan, N.; Ghani, L.; Poulson, B. G.; Emwas, A. H.; Jaremko, M.; *Molecules* **2020**, *25*, 5243. [Crossref]
4. Dixon, R. A.; Steele, C. L.; *Trends Plant. Sci.* **1999**, *4*, 394. [Crossref]
5. Liu, W.; Feng, Y.; Yu, S.; Fan, Z.; Li, X.; Li, J.; Yin, H.; *Int. J. Mol. Sci.* **2021**, *22*, 12824. [Crossref]
6. Boucherle, B.; Peuchmaur, M.; Boumendjel, A.; Haudecoeur, R.; *Phytochemistry* **2017**, *142*, 92. [Crossref]
7. Dewick, P. M.; *Phytochemistry* **1975**, *14*, 983. [Crossref]
8. Shen, N.; Wang, T.; Gan, Q.; Liu, S.; Wang, L.; Jin, B.; *Food. Chem.* **2022**, *383*, 132531. [Crossref]
9. Chen, L.; Cao, H.; Huang, Q.; Xiao, J.; Teng, H.; *Crit. Rev. Food. Sci. Nutr.* **2022**, *62*, 7730. [Crossref]
10. Khan, H.; Ullah, H.; Martorell, M.; Valdes, S. E.; Belwal, T.; Tejada, S.; Sureda, A.; Kamal, M. A.; *Semin. Cancer. Biol.* **2021**, *69*, 200. [Crossref]
11. Goel, A.; Kumar, A.; Raghuvanshi, A.; *Chem. Rev.* **2013**, *113*, 1614. [Crossref]
12. Selvam, C.; Jordan, B. C.; Prakash, S.; Mutisya, D.; Thilagavathi, R.; *Eur. J. Med. Chem.* **2017**, *128*, 219. [Crossref]
13. Coelho, A. L.; Vasconcellos, M. L. A. A.; Simas, A. B. C.; Rabi, J. A.; Costa, P. R. R.; *Synthesis* **1992**, *1992*, 914. [Crossref]
14. Lichtenfels, R. A.; Coelho, A. L.; Costa, P. R. R.; *J. Chem. Soc., Perkin Trans. 1* **1995**, 949. [Crossref]
15. Netto, C. D.; Santos, E. S. J.; Castro, C. P.; da Silva, A. J. M.; Rumjanek, V. M.; Costa, P. R. R.; *Eur. J. Med. Chem.* **2009**, *44*, 920. [Crossref]
16. da Silva, A. J. M.; Netto, C. D.; Costa, P. R. R.; *J. Braz. Chem. Soc.* **2004**, *15*, 979. [Crossref].
17. Salustiano, E. J. S.; Netto, C. D.; Fernandes, R. F.; da Silva, A. J. M.; Bacelar, T. S.; Castro, C. P.; Buarque, C. D.; Maia, R. C.; Rumjanek, V. M.; Costa, P. R. R.; *Invest. New Drugs* **2010**, *28*, 139. [Crossref]
18. Portes, J. A.; Netto, C. D.; da Silva, A. J. M.; Costa, P. R. R.; DaMatta, R. A.; dos Santos, T. A. T.; De Souza, W.; Seabra, S. H.; *Vet. Parasitol.* **2012**, *186*, 261. [Crossref]
19. da Silva, L. L. R.; Portes, J. A.; de Araújo, M. H.; Silva, J. L. S.; Rennó, M. N.; Netto, C. D.; da Silva, A. J. M.; Costa, P. R. R.; De Souza, W.; Seabra, S. H.; DaMatta, R. A.; *Parasitol. Int.* **2015**, *64*, 622. [Crossref]
20. Maia, R. C.; Vasconcelos, F. C.; Souza, P. S.; Rumjanek, V. M.; *Molecules* **2018**, *23*, 119. [Crossref]
21. Oliveira, T.; Lemos, D.; Jean, L.; Kawashima, J. M.; de Azevedo, V. R.; Salustiano, E. J.; Rumjanek, V. M.; Monteiro, R. Q.; *Front. Oncol.* **2022**, *12*, article 852985. [Crossref]
22. Salustiano, E. J.; da Costa, K. M.; Freire-de-Lima, L.; Mendonça-Previato, L.; Previato, J. O.; *J. Biol. Chem.* **2020**, *295*, 6457. [Crossref]
23. Daflon-Yunes, N.; Pinto-Silva, F. E.; Vidal, R. S.; Novis, B. F.; Berguetti, T.; Lopes, R. R. S.; Polycarpo, C.; Rumjanek, V. M.; *Mol. Cell. Biochem.* **2013**, *383*, 123. [Crossref]
24. Maia, R. C.; Vasconcelos, F. C.; Bacelar, T. S.; Salustiano, E. J.; da Silva, L. F. R.; Pereira, D. L.; Moellman-Coelho, A.; Netto, C. D.; da Silva, A. J.; Rumjanek, V. M.; Costa, P. R. R.; *Invest. New Drugs* **2011**, *29*, 1143. [Crossref]

25. Reis, F. R. S.; de Faria, F. C. C.; Castro, C. P.; de Souza, P. S.; Vasconcelos, F. C.; Bello, R. D.; da Silva, A. J.; Costa, P. R. R.; Maia, R. C.; *Anti-Cancer Agents Med. Chem.* **2013**, *13*, 341. [Crossref]
26. Rumjanek, V. M.; Maia, R. C.; Salustiano, E. J.; Costa, P. R. R.; *Anti-Cancer Agents Med. Chem.* **2019**, *19*, 29. [Crossref]
27. Bacelar, T. S.; da Silva, A. J.; Costa, P. R. R.; Rumjanek, V. M.; *Anti-Cancer Drugs* **2013**, *24*, 73. [Crossref]
28. Martino, T.; Kudrolli, T. A.; Kumar, B.; Salviano, I.; Mencalha, A.; Coelho, M. G. P.; Justo, G.; Costa, P. R. R.; Sabino, K. C. C.; Lupold, S. E.; *Prostate* **2018**, *78*, 140. [Crossref]
29. Salustiano, E. J.; Dumas, M. L.; Silva-Santos, G. G.; Netto, C. D.; Costa, P. R. R.; Rumjanek, V. M.; *Invest. New Drugs* **2016**, *34*, 541. [Crossref]
30. de Moraes, G. N.; Castro, C. P.; Salustiano, E. J.; Dumas, M. L.; Costas, F.; Lam, E. W.-F.; Costa, P. R. R.; Maia, R. C.; *Int. J. Oncol.* **2014**, *45*, 1949. [Crossref]
31. Martino, T.; Magalhães, F. C. J.; Justo, G. A.; Coelho, M. G. P.; Netto, C. D.; Costa, P. R. R.; Sabino, K. C. C.; *Bioorg. Med. Chem.* **2014**, *22*, 3115. [Crossref]
32. Salustiano, E.: *Anti-Tumor Effect of New Synthetic Compounds on Multidrug Resistant Cell Lines*; MSc Dissertation, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, 2008. [Link]
33. Bernardo, P. S.; Guimarães, G. H. C.; De Faria, F. C. C.; Longo, G. M. C.; Lopes, G. P. F.; Netto, C. D.; Costa, P. R. R.; Maia, R. C.; *Oncol. Rep.* **2020**, *43*, 346. [Crossref]
34. Silva, T. L.; da Silva, J. C. S.; Lima, D. J. P.; Ferreira, F. R.; de Vasconcelos, C. C.; Santos, D. C.; Netto, C. D.; Costa, P. R. R.; Goulart, M. O. F.; *J. Braz. Chem. Soc.* **2019**, *30*, 2438. [Crossref]
35. Silva, T. L.; Ferreira, F. R.; de Vasconcelos, C. C.; da Silva, R. C.; Lima, D. J. P.; Costa, P. R. R.; Netto, C. D.; Goulart, M. O. F.; *ChemElectroChem* **2016**, *3*, 2252. [Crossref]
36. Hancio, T.; Mazzoccoli, L.; Guimarães, G.; Robaina, M.; Mendonça, B. S.; de Moraes, G. N.; Monte-Mor, B. C. R.; Gutiyama, L. M.; de Carvalho, L. O.; Netto, C. D.; Costa, P. R. R.; de Faria, F. C. C.; Maia, R. C.; *Int. J. Oncol.* **2021**, *58*, article number 24. [Crossref]
37. da Cunha-Júnior, E. F.; Pacienza-Lima, W.; Ribeiro, G. A.; Netto, C. D.; do Canto-Cavalheiro, M. M.; da Silva, A. J. M.; Costa, P. R. R.; Rossi-Bergmann, B.; Torres-Santos, E. C.; *J. Antimicrob. Chemother.* **2011**, *66*, 1555. [Crossref]
38. Costa, L.; Pinheiro, R. O.; Dutra, P. M. L.; Santos, R. F.; Cunha-Júnior, E. F.; Torres-Santos, E. C.; da Silva, A. J. M.; Costa, P. R. R.; Da-Silva, S. A. G.; *PLoS One* **2014**, *9*, e109672. [Crossref]
39. Cunha-Júnior, E. F.; Martins, T. M.; Canto-Cavalheiro, M. M.; Marques, P. R.; Portari, E. A.; Coelho, M. G. P.; Netto, C. D.; Costa, P. R. R.; Sabino, K. C. C.; Torres-Santos, E. C.; *Antimicrob. Agents Chemother.* **2016**, *60*, 3794. [Crossref]
40. Lima, É. A.; Cavalcante-Silva, L. H. A.; Carvalho, D. C. M.; Netto, C. D.; Costa, P. R. R.; Rodrigues-Mascarenhas, S.; *Int. Immunopharmacol.* **2020**, *83*, 106399. [Crossref]
41. Caleffi, G. S.; Demidoff, F. C.; Nájera, C.; Costa, P. R. R.; *Org. Chem. Front.* **2022**, *9*, 1165. [Crossref]
42. Brand, W.; Shao, J.; Hoek-Van Den Hil, E. F.; van Elk, K. N.; Spenkelink, B.; de Haan, L. H. J.; Rein, M. J.; Dionisi, F.; Williamson, G.; van Bladeren, P. J.; Rietjens, I. M. C. M.; *J. Agric. Food Chem.* **2010**, *58*, 6119. [Crossref]
43. Lu, W. J.; Ferlito, V.; Xu, C.; Flockhart, D. A.; Caccamese, S.; *Chirality* **2011**, *23*, 891. [Crossref]
44. Quaglia, M. G.; Desideri, N.; Bossù, E.; Sestili, I.; Conti, C.; *Chirality* **1992**, *4*, 65. [Crossref]
45. Mayo, B.; Vázquez, L.; Flórez, A. B.; *Nutrients* **2019**, *11*, 2231. [Crossref]
46. Farias, K.; da Costa, R. F.; Meira, A. S.; Diniz-Filho, J.; Bezerra, E. M.; Freire, V. N.; Guest, P.; Nikahd, M.; Ma, X.; Gardiner, M. G.; Banwell, M. G.; de Oliveira, M. C. F.; de Moraes, M. O.; Pessoa, C. Ó.; *ACS Med. Chem. Lett.* **2020**, *11*, 1274. [Crossref]
47. Scatena, G. S.; Cassiano, N. M.; Netto, C. D.; Costa, P. R. R.; Cass, Q. B.; Batista Jr., J. M.; *Chirality* **2017**, *29*, 167. [Crossref]
48. Buarque, C. D.; Domingos, J. L. O.; Netto, C. D.; Costa, P. R. R.; *Curr. Org. Synth.* **2015**, *12*, 772. [Crossref]
49. Falcão, M. J. C.; Pouliquem, Y. B. M.; Lima, M. A. S.; Gramosa, N. V.; Costa-Lotufo, L. V.; Militão, G. C. G.; Pessoa, C.; de Moraes, M. O.; Silveira, E. R.; *J. Nat. Prod.* **2005**, *68*, 423. [Crossref]
50. Militão, G. C. G.; Dantas, I. N. F.; Pessoa, C.; Falcão, M. J. C.; Silveira, E. R.; Lima, M. A. S.; Curi, R.; Lima, T.; Moraes, M. O.; Costa-Lotufo, L. V.; *Life Sci.* **2006**, *78*, 2409. [Crossref]
51. Militão, G. C. G.; Bezerra, D. P.; Pessoa, C.; de Moraes, M. O.; da Ponte, F. A. F.; Lima, M. A. S.; Silveira, E. R.; Costa-Lotufo, L. V.; *J. Nat. Med.* **2007**, *61*, 196. [Crossref]
52. de Farias, K. M.; Rosa-Ribeiro, R.; Souza, E. E.; Kobarg, J.; Banwell, M. G.; Vieira Neto, J. B.; Sales, S. L. A.; Costa, P. R. R.; dos Santos, R. C.; Gaspar, F. V.; Barreto Jr., A. G.; Oliveira, M. C. F.; de Moraes, M. O.; Furtado, C. L. M.; Carvalho, H. F.; Pessoa, C.; *Chem. Biodiversity* **2022**, *19*, e202200102. [Crossref]
53. Miller, I. C. P.; Collini, M. D.; Morris, R.; Singhaus, R.; *US pat. 20060004087* **2006**.
54. Buarque, C. D.; Salustiano, E. J.; Fraga, K. C.; Alves, B. R. M.; Costa, P. R. R.; *Eur. J. Med. Chem.* **2014**, *78*, 190. [Crossref]
55. Gaspar, F. V.; Ribeiro, S. M.; Barcellos, J. C. F.; Monteiro, S.; Domingos, J. L. O.; Luciano, M. C. S.; Paier, C. R. K.; Pessoa, C.; Costa, P. R. R.; *Bioorg. Chem.* **2021**, *107*, 104584. [Crossref]
56. de Moraes, P. F.; Gaspar, F. V.; Borges, R. H. F.; Netto, C. D.; Leão, R. A. C.; Nájera, C.; Costa, P. R. R.; *Synthesis* **2015**, *47*, 3505. [Crossref]
57. Gaspar, F. V.; Caleffi, G. S.; Costa-Júnior, P. C. T.; Costa, P. R. R.; *ChemCatChem* **2021**, *13*, 5097. [Crossref]
58. Demidoff, F. C.; Caleffi, G. S.; Figueiredo, M.; Costa, P. R. R.; *J. Org. Chem.* **2022**, *87*, 14208. [Crossref]

59. Caleffi, G. S.; Brum, J. O. C.; Costa, A. T.; Domingos, J. L. O.; Costa, P. R. R.; *J. Org. Chem.* **2021**, *86*, 4849. [Crossref]
60. Bonfield, K.; Amato, E.; Bankemper, T.; Agard, H.; Steller, J.; Keeler, J. M.; Roy, D.; McCallum, A.; Paula, S.; Ma, L.; *Bioorg. Med. Chem.* **2012**, *20*, 2603. [Crossref]
61. Mendes, J. A.; Salustiano, E. J.; Pires, C. S.; Oliveira, T.; Barcellos, J. C. F.; Cifuentes, J. M. C.; Costa, P. R. R.; Rennó, M. N.; Buarque, C. D.; *Bioorg. Chem.* **2018**, *80*, 585. [Crossref]
62. Mendes, J. A.; Merino, P.; Soler, T.; Salustiano, E. J.; Costa, P. R. R.; Yus, M.; Foubelo, F.; Buarque, C. D.; *J. Org. Chem.* **2019**, *84*, 2219. [Crossref]
63. Böhm, H.-J.; Banner, D.; Bendels, S.; Kansy, M.; Kuhn, B.; Müller, K.; Obst-Sander, U.; Stahl, M.; *ChemBioChem* **2004**, *5*, 637. [Crossref]
64. Purser, S.; Moore, P. R.; Swallow, S.; Gouverneur, V.; *Chem. Soc. Rev.* **2008**, *37*, 320. [Crossref]
65. Fernandes, T. A.; Domingos, J. L. O.; da Rocha, L. I. A.; de Medeiros, S.; Nájera, C.; Costa, P. R. R.; *Eur. J. Org. Chem.* **2014**, *2014*, 1314. [Crossref]
66. de Souza, L. G.; Salustiano, E. J.; da Costa, K. M.; Costa, A. T.; Rumjanek, V. M.; Domingos, J. L. O.; Rennó, M. N.; Costa, P. R. R.; *Bioorg. Chem.* **2021**, *110*, 104790. [Crossref]
67. Souza, L. G.; Domingos, J. L. O.; Fernandes, T. A.; Renno, M. N.; Sansano, J. M.; Najera, C.; Costa, P. R. R.; *J. Fluorine Chem.* **2019**, *217*, 72. [Crossref]
68. Kaushik-Basu, N.; Bopda-Waffo, A.; Talele, T. T.; Basu, A.; Costa, P. R. R.; da Silva, A. J. M.; Sarafianos, S. G.; Noël, F.; *Nucleic Acids Res.* **2008**, *36*, 1482. [Crossref]
69. Nichols, D. B.; Leão, R. A. C.; Basu, A.; Chudayeu, M.; de Moraes, P. F.; Talele, T. T.; Costa, P. R. R.; Kaushik-Basu, N.; *Chem. Biol. Drug Des.* **2013**, *81*, 607. [Crossref]
70. Emami, S.; Ghanbarimasir, Z.; *Eur. J. Med. Chem.* **2015**, *93*, 539. [Crossref]
71. Fernandes, T. A.; Manvar, D.; Domingos, J. L. O.; Basu, A.; Nichols, D. B.; Kaushik-Basu, N.; Costa, P. R. R.; *Eur. J. Med. Chem.* **2016**, *112*, 33. [Crossref]
72. Manvar, D.; Fernandes, T. A.; Domingos, J. L. O.; Baljinnyam, E.; Basu, A.; Junior, E. F. T.; Costa, P. R. R.; Kaushik-Basu, N.; *Eur. J. Med. Chem.* **2015**, *93*, 51. [Crossref]
73. Su, H.-x.; Yao, S.; Zhao, W.-f.; Li, M.-j.; Liu, J.; Shang, W.-J.; Xie, H.; Ke, C.-q.; Hu, H.-c.; Gao, M.-n.; Yu, K.-q.; Liu, H.; Shen, J.-s.; Tang, W.; Zhang, L.-k.; Xiao, G.-f.; Ni, L.; Wang, D.-w.; Zuo, J.-p.; Jiang, H.-l.; Bai, F.; Wu, Y.; Ye, Y.; Xu, Y.-C.; *Acta Pharmacol. Sin.* **2020**, *41*, 1167. [Crossref]
74. Su, H.; Yao, S.; Zhao, W.; Zhang, Y.; Liu, J.; Shao, Q.; Wang, Q.; Li, M.; Xie, H.; Shang, W.; Ke, C.; Feng, L.; Jiang, X.; Shen, J.; Xiao, G.; Jiang, H.; Zhang, L.; Ye, Y.; Xu, Y.; *Nat. Commun.* **2021**, *12*, 3623. [Crossref]
75. Zandi, K.; Musall, K.; Oo, A.; Cao, D.; Liang, B.; Hassandarvish, P.; Lan, S.; Slack, R. L.; Kirby, K. A.; Bassit, L.; Amblard, F.; Kim, B.; AbuBakar, S.; Sarafianos, S. G.; Schinazi, R. F.; *Microorganisms* **2021**, *9*, 893. [Crossref]
76. Chaves, O. A.; Fintelman-Rodrigues, N.; Wang, X.; Sacramento, C. Q.; Temerozo, J. R.; Ferreira, A. C.; Mattos, M.; Pereira-Dutra, F.; Bozza, P. T.; Castro-Faria-Neto, H. C.; Russo, J. J.; Ju, J.; Souza, T. M. L.; *Viruses* **2022**, *14*, 1458. [Crossref]
77. Leal, C. M.; Leitão, S. G.; Sausset, R.; Mendonça, S. C.; Nascimento, P. H. A.; Cheohen, C. F. A. R.; Esteves, M. E. A.; da Silva, M. L.; Gondim, T. S.; Monteiro, M. E. S.; Tucci, A. R.; Fintelman-Rodrigues, N.; Siqueira, M. M.; Miranda, M. D.; Costa, F. N.; Simas, R. C.; Leitão, G. G.; *Rev. Bras. Farmacogn.* **2021**, *31*, 658. [Crossref]
78. Sui, G.; Li, T.; Zhang, B.; Wang, R.; Hao, H.; Zhou, W.; *Bioorg. Med. Chem.* **2021**, *29*, 115895. [Crossref]
79. Caleffi, G. S.; Rosa, A. S.; de Souza, L. G.; Avelar, J. L. S.; Nascimento, S. M. R.; de Almeida, V. M.; Tucci, A. R.; Ferreira, V. N.; da Silva, A. J. M.; Santos-Filho, O. A.; Miranda, M. D.; Costa, P. R. R.; *J. Nat. Prod.* **2023**, *86*, 1536. [Crossref]
80. Williamson, G.; Kay, C. D.; Crozier, A.; *Compr. Rev. Food Sci. Food Saf.* **2018**, *17*, 1054. [Crossref]
81. Zhang, M.; Zhu, S.; Yang, W.; Huang, Q.; Ho, C.-T.; *Food Funct.* **2021**, *12*, 3307. [Crossref]
82. Jannat, K.; Paul, A. K.; Bondhon, T. A.; Hasan, A.; Nawaz, M.; Jahan, R.; Mahboob, T.; Nissapatorn, V.; Wilairatana, P.; Pereira, M. L.; Rahmatullah, M.; *Pharmaceutics* **2021**, *13*, 1895. [Crossref]

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