Received: 26 March 2018 | Revised: 2 August 2018 | Accepted: 3 August 2018
DOI: 10.1002/ardp.201800100

FULL PAPER

DPhG ARCH PHARM
Archiv der Pharmazie

Prospecting for cytotoxic and antiprotozoal 4-aryl-4*H*-chromenes and 10-aryldihydropyrano[2,3-*f*]chromenes

Erlon F. Martin[1] | Armelle T. Mbaveng[2,3] | Milene H. de Moraes[4] | Victor Kuete[2,3] | Maique W. Biavatti[1] | Mario Steindel[4] | Thomas Efferth[3] | Louis P. Sandjo[1]

[1] Department of Pharmaceutical Sciences, Universidade Federal de Santa Catarina, Florianópolis, Brazil

[2] Faculty of Science, Department of Biochemistry, University of Dschang, Dschang, Cameroon

[3] Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, Johannes Gutenberg University, Mainz, Germany

[4] Department of Microbiology, Immunology and Parasitology, Universidade Federal de Santa Catarina, Florianópolis, Brazil

Correspondence
Dr. Louis P. Sandjo, Department of Pharmaceutical Sciences, Universidade Federal de Santa Catarina, 88040-900 Florianópolis, SC, Brazil.
Email: p.l.sandjo@ufsc.br

Funding information
Alexander von Humboldt Foundation; CNPq/CAPES

Abstract

Different studies reported that genetic predisposition or metabolic dysfunction are the risk factors for cancer. Infectious parasitic diseases were listed among factors that predispose to cancer. Because of the resemblance between the life cycle of cancer cells and some parasites, this study aimed to prepare pyran derivatives with cytotoxic and antiprotozoal potencies. Therefore, 7 chromenes, 10 pyranocoumarins, and an unexpected intermediate were obtained from a multi-reagent one-pot reaction. These compounds were evaluated for their cytotoxicity on sensitive and resistant leukemia cancer cells lines and against two protozoan parasites, namely *Trypanosoma cruzi* and *Leishmania amazonensis* amastigote. Promising cytotoxicity (IC_{50} values of less than 1 μM) was obtained for two of the synthetic products (**12** and **15**). Compound **12** induced apoptosis and cell cycle arrest in CCRF-CEM leukemia cells in G0/G1 while compound **15** and doxorubicin induced apoptosis and arrest in the S and G2/M phases. Ten of these products showed trypanocidal activity, while only five of them were weakly active on *L. amazonensis*. Three of the obtained pyrans showed significant cytotoxicity and antitrypanocidal activity, simultaneously. Nevertheless, all antiparasitic compounds revealed potency with low selectivity toward THP-1 cells used as host.

KEYWORDS
4-aryl-4*H*-chromenes, 10-aryl[2,3-*f*]pyranocoumarins, antiprotozoal activity, cell cycle arrest, cytotoxicity

1 | INTRODUCTION

4-Aryl-4*H*-chromene-derived compounds such as MX-58151 (**1**) have been drawing a lot of attention in cancer drugs discovery because of their ability of inducing apoptosis in HL-60 promyelocytic leukemia cells. Their inhibitory effect is mediated by alterations in the G2-M phase of the cell cycle.[1,2] WHO reported that cancer caused about 8.8 million of deaths worldwide in 2015, and an important strategy to fight the disease is based on prevention and earlier detection.[3] Chemotherapy is one of the main treatment options, although patients

frequently relapse because of the development of drug resistance. Therefore, searching new molecules remains a rewarding task, in order to identify new lead entities for cancer treatment. Different studies reported

risk factors for cancer such as genetic predisposition or metabolic dysfunction.[4,5] Besides, these studies also showed that metabolic dysfunction is related to chemical (drugs), physical (UV-light, radiation), or biological (infections) exposure.[4,5] In the particular case of biological carcinogenesis, some patients previously infected with parasitic pathogens, including *Trypanosoma* and *Leishmania* species, have developed a type of cancer.[6,7] These kinetoplastid infections are endemic in certain regions of Latin America, Asia, and Africa.[8] There are about 14 *Leishmania* species in Latin America, which are responsible for cutaneous, mucocutaneous, and visceral leishmaniosis with the greatest incidence in Brazil.[8] The most prevalent *Trypanosoma* species (*Trypanosoma cruzi*) found in Latin America is responsible for Chagas' disease. Transmission occurs through contact with vector insects, infected animal, and other living conditions.[8]

Medicinal chemistry intensively contributed to new chemotherapies against cancer and parasitic diseases. The technical approach is usually based on small molecules screening. However, continuous efforts are needed because of drug resistance and high toxicity related to some treatments.[8] Small molecules for this purpose frequently originated from natural sources. In this regard, naturally occurring chromenes (mammea A/BB), pyranocoumarins (alloxanthoxyletin, seselin) and their synthetic analogs were described as antitumoral and antiprotozoal agents.[8–10] Therefore, parallels in differentiation existing between cancer cells and protozoan parasites prompted us to prepare chromenes derivatives which are potentially cytotoxic and antiparasitic agents. Although the structural shape of 4-aryl-4*H*-chromenes was successfully introduced in coumarin scaffolds by a known synthetic route to form 10-aryl-2,10-dipyranochromenes,[11,12] these latter have no biological record. Therefore, 18 compounds including 17 chromene derivatives and an unexpected methyl α-cyanophenyl pentanoate were prepared in a one-pot manner.

The obtained chromenes were comprised of seven 4-aryl-4*H*-chromenes and ten 10-aryl-2,10-dihydropyranochromenes. With the aim to identify biological multi-target compounds, all obtained chromenes were evaluated for their cytotoxicity on tumor cells and on intracellular amastigotes of *Leishmania amazonensis* and *T. cruzi*.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

A literature search revealed the 2-amino-7-(dimethylamino)-4*H*-chromene-3-carbonitrile scaffold as the most investigated bearing different aromatic substituents attached to the sp^3 CH group of the chromene core.[13] Moreover, other studies mostly prepared and extended the list of analogs by using different aromatic aldehydes.[14,15] Among the prepared compounds, MX-58151 (**1**) showed promising cytotoxic effects against cancer cells by inducing apoptosis via caspase activation.[1,13–15] Therefore, a series of related compounds were designed as shown in Figure 1 (drawing 2) by considering various structural changes characterized by other aryl substituents on the sp^3 CH group, the switch of the nitrile function to the methyl carboxylate group, and the introduction of a second chromene ring. Therefore, phloroglucinol was used as phenolic precursor for the coumarin synthesis via Pechmann condensation (Scheme 1) in a solvent-free condition.[16] Thereafter, the coumarin (**3**) was treated with malonitrile and different aromatic aldehydes in an alkaline medium (K_2CO_3) to afford 10-aryl-2,10-dipyranochromenes **4**–**9** in a one-pot manner (Scheme 2). Another set of pyranocoumarins derivatives (**10**–**14**) was obtained by using the same synthetic conditions and by treating **3** with methyl α-cyanoacetate ester and different aromatic aldehydes (Scheme 3).

The reaction between **3**, methyl α-cyanoacetate ester, and cinnamaldehyde did not form the desired product; in contrast, compound **15** was obtained from the Knoevenagel condensation of methyl α-cyanoacetate ester and cinnamaldehyde. Since the cyclization step might occur via a Michael addition mechanism,[17] the strong electron delocalization in **15** presumably reduced its reactivity in the presence of phloroglucinol. The reaction procedure herein used for the preparation of compounds **4**–**15** obtained with moderate to high yield was previously reported in the literature.[11]

A modified method formerly employed by Masesane and Mihigo[12] to prepare 4-aryl-4*H*-chromene derivatives was considered for the synthesis of compounds **16**–**22**. Methyl α-cyanoacetate ester was exclusively used to generate new 4-aryl-4*H*-chromenes (Scheme 4) in moderate to good yield.

Sixteen of the prepared compounds were newly reported, while compounds **3**, **15**, **20**, and **22** were known.[17–20]

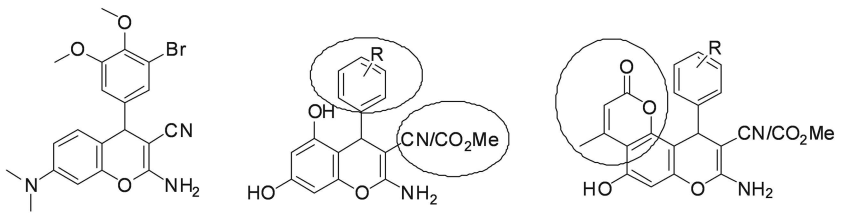
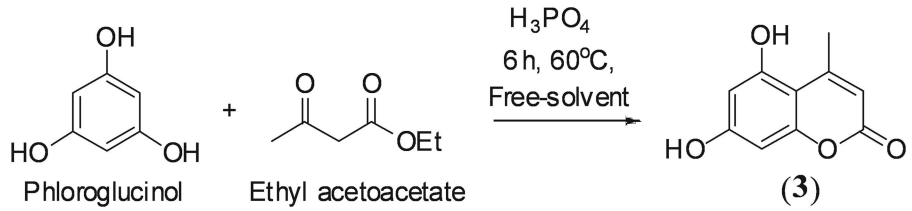


FIGURE 1 Modification on the 4-aryl-4*H*-chromene core



SCHEME 1 Preparation of the coumarin

This panel of compounds was evaluated for their antiproliferative effect on cancer cells and for their antiparasitic activity against the intracellular amastigote forms of *L. amazonensis* and *T. cruzi*.

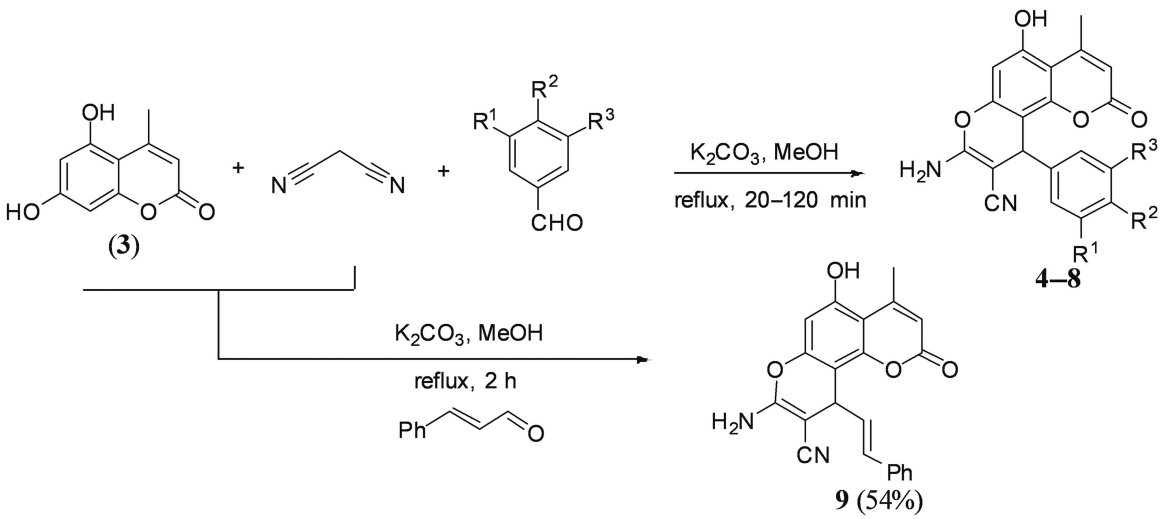
2.2 | Biological evaluation

2.2.1 | *In vitro* cytotoxicity

The cytotoxicity of **3**–**22** as well as doxorubicin (as control drug) was determined in drug-sensitive CCRF-CEM leukemia cells and their multidrug-resistant subline CEM/ADR5000. Compounds **10**–**12** and **15** displayed significant cytotoxic effects in both CCRF-CEM and CEM/ADR5000 cells (Table 1). Interestingly, IC_{50} values less than 1 μM were recorded with **12** (0.78 μM), **15** (0.61 μM), and doxorubicin (0.02 μM) toward CCRF-CEM cells, while compound **15** showed the lowest IC_{50} value (0.56 μM) toward CEM/ADR5000 cells. CEM/ADR5000 cells displayed hypersensitivity to compound **15** (Table 1) with a degree of resistance below 0.90, suggesting a potential inhibition of P-glycoprotein expression.[21]

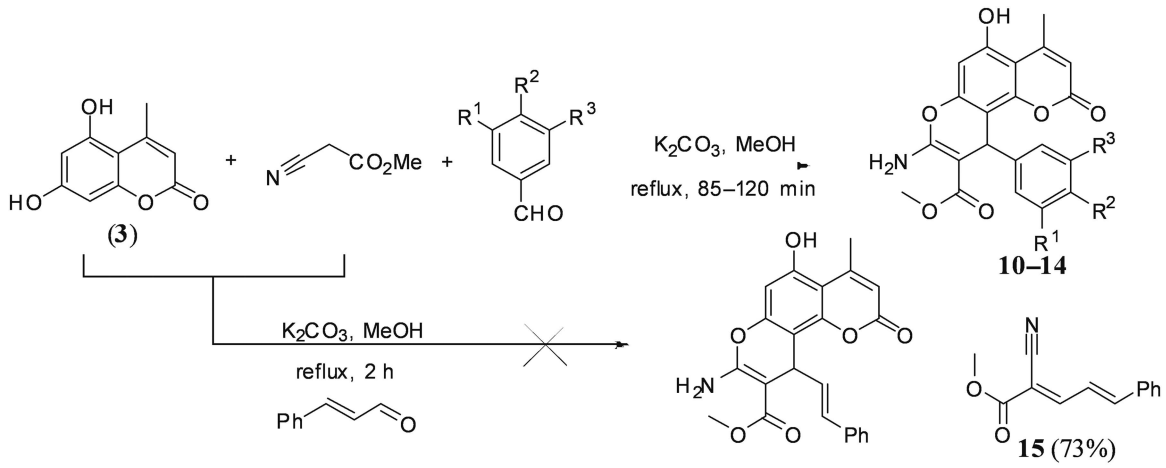
Apart from that, resistant CEM/ADR5000 cells were also moderately sensitive to **9**–**12** with IC_{50} values of 17.87, 5.65, 5.10, and 3.12 μM, respectively. These compounds also showed significant activity toward sensitive CCRF-CEM cells. However, their effects on the resistant cell line were lower than that observed for **15**. This observation was also noted from their resistance degrees (3.72, 3.71, 3.71, and 4.02, respectively). In addition, compounds **16** and **17** were almost equally active against both lines, although their cytotoxicity was moderate. More importantly, the resistant cells were more sensitive to compounds **4**, **5**, and **9**–**18** than to doxorubicin.

The high-conjugated system characterized by strong electrons delocalization in compound **15** might be the structural feature promoting the cytotoxicity against both sensitive and resistant cells; this also might explain why **15** was more active than others.



Compounds	R1	R2	R3	Yield
4	H	Cl	H	64%
5	H	Br	H	71%
6	H	NO_2	H	43%
7	OMe	OMe	H	56%
8	OMe	OMe	OMe	54%

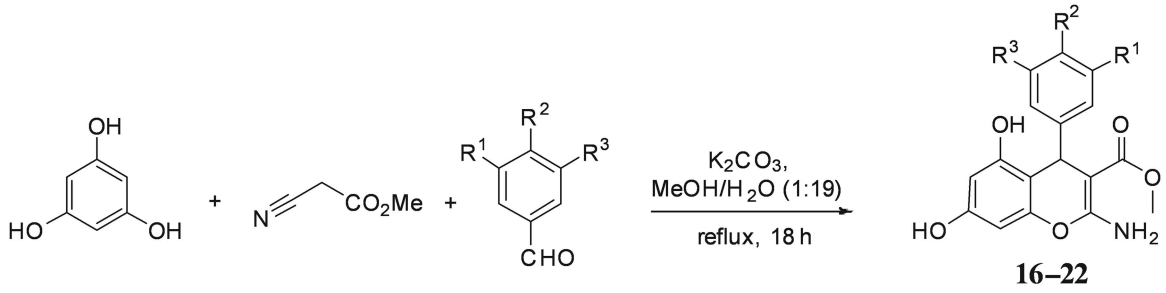
SCHEME 2 Preparation of the pyranocoumarin derivatives (part 1)



Compounds	R1	R2	R3	Yield
10	H	Cl	H	69%
11	H	Br	H	73%
12	H	NO_2	H	75%
13	OMe	OMe	H	70%
14	OMe	OMe	OMe	58%

SCHEME 3 Preparation of the pyranocoumarin derivatives (part 2)

Except for compound **13**, which was not active on sensitive cells, other pyranocoumarins were cytotoxic. Moreover, **4**–**12** and **14** were more active than their 4-aryl-4*H*-chromene-related compounds (**16**–**22**). Compounds **10**–**12** bearing the ester group (CO_2Me) were more potent than their congeners (**4**–**6**) flanked with a CN group at the same position. It is noteworthy to mention that **10**–**12** with significant effects against the sensitive cell line contained, respectively, 4-ClC_6H_4, 4-BrC_6H_4, and 4-$NO_2C_6H_4$ substituents on the sp^3 CH group. However, among the compounds with moderate activity, pyranocoumarins with methoxylated aryl groups adjacent to a CN group (**7** and **8**)



Compounds	R1	R2	R3	Yield
16	H	Cl	H	51.4%
17	H	Br	H	51%
18	H	NO_2	H	55%
19	OMe	OMe	H	56%
20	OMe	OMe	OMe	60%
21	OMe	OH	OMe	58%
22	OMe	OH	H	54%

SCHEME 4 Preparation of the 4-aryl-4*H*-chromene derivatives

TABLE 1 Cytotoxicity of compounds and doxorubicin toward leukemia cells as determined by resazurin assay

	Cell lines, IC_{50} values in μM, and degree of resistance[a] (in brackets)	
Compounds	**CCRF-CEM**	**CEM/ADR5000**
3	33.75 ± 0.93	>90 (nd)
4	1.65 ± 0.56	32.49 ± 0.04 (19.63)
5	2.02 ± 0.57	44.29 ± 5.20 (21.90)
6	7.22 ± 1.21	67.87 ± 8.09 (9.40)
7	6.75 ± 3.01	76.99 ± 2.12 (11.40)
8	6.24 ± 1.41	>90 (nd)
9	4.81 ± 2.35	17.87 ± 1.08 (3.72)
10	1.52 ± 0.39	5.65 ± 1.16 (3.71)
11	1.37 ± 0.22	5.10 ± 0.76 (3.71)
12	0.78 ± 0.11	3.12 ± 0.95 (4.02)
13	>90	29.33 ± 1.26 (nd)
14	10.43 ± 0.59	61.63 ± 7.41 (5.91)
15	0.61 ± 0.07	0.56 ± 0.03 (0.92)
16	10.70 ± 1.57	13.58 ± 1.28 (1.27)
17	15.46 ± 0.67	16.00 ± 1.03 (1.03)
18	11.46 ± 2.35	38.85 ± 2.25 (3.39)
19	27.40 ± 1.29	>90 (nd)
20	21.41 ± 7.17	>90 (nd)
21	27.22 ± 10.75	69.40 ± 4.36 (2.55)
22	22.71 ± 1.77	>90 (nd)
Doxorubicin	0.02 ± 0.00	66.83 ± 2.20 (3341)

[a]The degree of resistance was determined as the ratio of IC_{50} value in the resistant CEM/ADR5000 cells divided by the IC_{50} in the sensitive cell line, CCRF-CEM cells.

showed more pronounced cytotoxicity than those bearing the same methoxylated aryl group and a CO_2Me group (**13** and **14**). Moreover, we observed that MeO groups were unfavorable for the cytotoxic activity.

While compounds **3**, **8**, **19**, **20**, and **22** did not show cytotoxicity against resistant cells, the remaining heterocycles as well as the side product were active. Pyranocoumarins **10**–**12** bearing a CO_2Me group were more active against resistant cancer cells than all 4-aryl-4*H*-chromenes (**16**–**18** and **21**), which in turn were more potent than pyranochromenes (**4**–**7**) bearing a CN group. Interestingly, the most cytotoxic heterocyclic compounds (**10**–**12**) were still those containing 4-ClC_6H_4, 4-BrC_6H_4, and 4-$NO_2C_6H_4$ substituents alongside a CO_2Me group. Overall, the resistant CEM/ADR5000 cell line was more sensitive to pyranochromenes bearing a CO_2Me group than those with a CN group. The inactive and weakly active compounds were mainly found among the compounds bearing MeO groups on the aryl moiety (**7**, **8**, **13**, **14**, and **19**–**22**). However, among the CN group containing heterocycles, pyranochromenes bearing the styryl group revealed more impact on CEM/ADR5000 than those containing OH and MeO groups.

4-Aryl-4*H*-chromenes containing halogens and a NO_2 group (**16**–**18**) were cytotoxic against CCRF-CEM cells with almost the same strength. However, compound **18** bearing a NO_2 group was twofold less active on CEM/ADR5000 cells than **16** and **17**. Chromenes containing other substituents were about twofold less active on CCRF-CEM cells, while the same compounds showed weak or no cytotoxicity on CEM/ADR5000 cells.

2.2.2 | Cell cycle analysis

As a next step, the cell cycle distribution of CCRF-CEM was studied in the presence of the most cytotoxic compounds (**12** and **15**). Compounds **12**, **15**, and doxorubicin modified the cell cycle phases in a dose-dependent manner by increasing the cell population in the sub-G0/G1 phase. Compound **12** caused cell cycle arrest in G0/G1, which led to a decrease within the S and G2/M phases compared to the control. This suggested that cells did not undergo DNA synthesis and mitosis in the presence of **12** which acts as an apoptotic agent. In addition, **15** induced apoptosis in S and G2/M phases, whereas 2 × IC_{50} induced a decrease of the cell population in the G0/G1 phase compared to the control. As indication of apoptosis, percentages of cells in sub-G0/G1 phase were in a range of 4.76% (¼ × IC_{50}) to 18.84% (2 × IC_{50}) after treatment with **12**, of 4.56% (¼ × IC_{50}) to 25.40% (2 × IC_{50}) after treatment with **15**, and of 4.81% (¼ × IC_{50}) to 10.35% (2 × IC_{50}) upon treatment with doxorubicin. In non-treated cells, the percentage of cells in sub-G0/G1 phase was 1.78% (Figure 2).

It is also important to mention that some reported analogues of 4-aryl-4*H*-chromenes displayed moderate inhibitory percentage against the leukemia CCRF-CEM cell line.[22] However, other of the heterocycles were significantly cytotoxic toward other leukemic cancers such as AML and K562 cell lines.[23,24] The cytotoxicity herein reported revealed that compounds **10**–**12** can be explored to develop novel drugs to fight drug sensitive and multidrug-resistant leukemia.

Carcinogenesis has been repeatedly associated with parasitic infectious diseases, and the similar feature between cancer cells and protozoan parasites is that both live and multiply independently in a host.[25] Thus, studies focused simultaneously on chemotherapies against cancer and parasitic diseases revealed that compounds such as alsterpaullone reported as CDK inhibitor in cancer cells or aminobisphosphonates described as apoptotic agents on osteoclast showed excellent antiparasitic effects on *L. mexicana amazonensis* and *T. cruzi*.[25] Based on these previous observations, we found it appropriate also to evaluate the antiparasitic activity of compounds **3**–**22** on intracellular amastigotes of *L. amazonensis* and *T. cruzi*.

2.2.3 | Antiprotozoal evaluation

Antitrypanosomal activity

Twelve of the twenty tested compounds were active on *T. cruzi*, while five of them were effective on *L. amazonensis*. Compounds **4**, **10**, and **12** showed significant trypanocidal activity with IC_{50} of 4.42, 8.16, and 9.31 μM, respectively (Table 2). These compounds were more potent

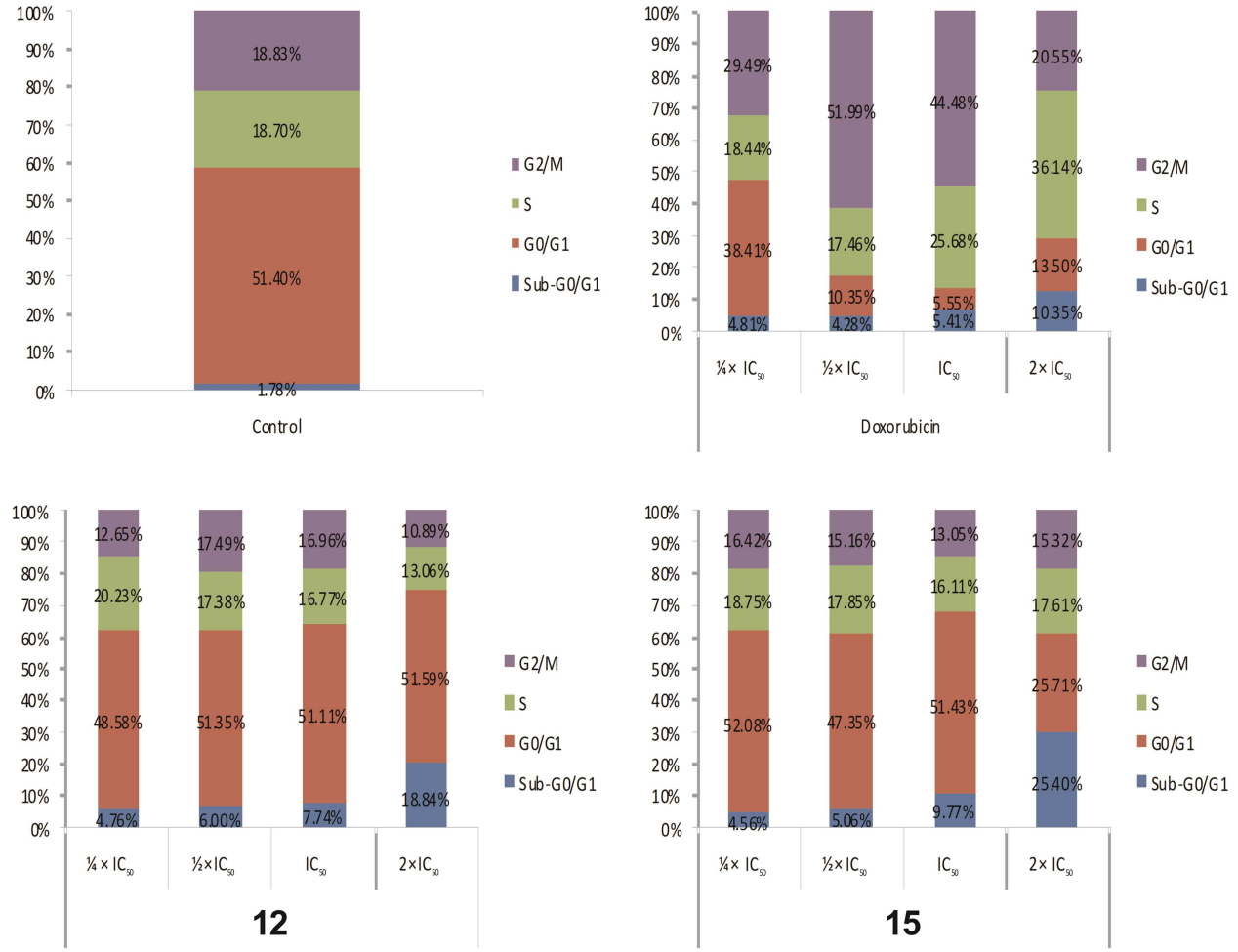


FIGURE 2 Cell cycle distribution of CCRF-CEM leukemia cells treated with compounds **12**, **15**, and doxorubicin for 24 h

than the control drug benznidazole, which inhibited 50% of the parasites with a concentration of 11.05 μM. Antitrypanosomal activities of compounds **5**, **6**, **11**, **13**–**18** were weak or moderate with IC_{50} values varying between 11.43 and 42.21 μM. All tested compounds were cytotoxic to the host cell with selectivity indices (SI) lower than 6.

Antileishmanial activity

Antileishmanial activities were observed with compounds **10**–**12**, **15**, and **18** with IC_{50} in a range of 33.10–53.75 μM. These activities were weak, if compared to the control drug amphotericin B. In addition, these antileishmanial compounds showed only weak selectivity to THP-1 cells.

The most active compound **4** on *T. cruzi* contained a 4-chlorophenyl moiety adjacent to a carbonitrile group. This activity drops twofold if the CN group was replaced by a CO_2Me group (compound **10**). However, an opposite feature was observed with compounds **5** and **6** if their trypanocidal activities were compared to those of **11** and **12**, respectively. The styryl group in **18** reduced its toxicity to THP-1 cells, although the obtained trypanocidal activity (15.45 μM) was moderate.

By comparing the biological activities of the investigated compounds herein reported, pyranocoumarins were more active on *T. cruzi* than their lighter analogues 4-aryl-4*H*-chromenes. Moreover, an interesting fact was observed between compounds **4**–**6** structures which differed from each to another by the aryl substituent at C-10. Compound **4** the most potent heterocycle contained a 4-ClC_6H_4 group and when this portion was replaced by a 4-BrC_6H_4 (compound **5**), the trypanocidal was lost by 11-fold. The activity dropped by almost ninefold when the 4-ClC_6H_4 group was switched with a 4-$NO_2C_6H_4$ group (compound **6**).

3 | CONCLUSION

In this work, the preparation of 20 chromene derivatives in a one-pot manner and their biological activities are presented. One of the reactions did not afford the expected compound instead the intermediate (**15**) was obtained. Compounds **4**, **10**–**12**, and **15** showed significant cytotoxic activity on the drug-sensitive cancer cell line while **10**–**12** and **15** were the most potent on both sensitive and resistant cancer cell lines. Compounds **12** and **15** caused alterations in G0/G1, S,

TABLE 2 Inhibition effect of compounds **3**–**22** against *T. cruzi* and *L. amazonensis* and their selectivity index toward THP-1 cells

	T. cruzi		THP-1	*L. amazonensis*	
Compounds	**IC_{50} (μM)**	**SI**	**CC_{50} (μM)**	**IC_{50} (μM)**	**SI**
3	–	–	–	–	
4	4.42 ± 1.51	<3.53	<15.60	–	
5	42.21 ± 1.96	<0.37	<15.60	–	
6	34.20 ± 3.61	<0.45	<15.60	–	
7	–	–	–	–	
8	–	–	–	–	
9	–	–	–	–	
10	8.16 ± 1.75	<1.91	<15.60	38.43 ± 4.20	<0.40
11	21.73 ± 3.78	<0.72	<15.60	33.21 ± 1.72	<0.47
12	9.31 ± 1.56	<1.67	<15.60	33.10 ± 1.52	<0.47
13	11.43 ± 1.37	<1.36	<15.60	–	
14	34.70 ± 2.30	0.74	25.65 ± 8.77	–	
15	28.42 ± 2.51	<0.55	<15.60	53.75 ± 1.85	<0.29
16	35.54 ± 0.11	2.13	75.62 ± 7.18	–	
17	39.58 ± 1.52	1.95	77.45 ± 8.94	–	
18	15.45 ± 0.60	5.78	89.26 ± 9.96	52.27 ± 2.07	1.71
19	–	–	–	–	
20	–	–	–	–	–
21	–	–	–	–	–
22	–	–	–	–	–
Benznidazole	11.05 ± 0.22	>45.24	>500	–	–
Amphotericin B	–	–	27.86 ± 0.98	0.14 ± 0.02	199

and G2/M phases of CCRF-CEM. Moreover, **4**, **10**, and **12** were more active than benznidazole on *T. cruzi* and represent three candidates that can be explored to develop further anticancer and antitrypanosomal agents. Nevertheless, their selectivity toward the parasites host cell need to improve. Only five compounds showed weak leishmanicidal effect on *L. amazonensis*.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

Thin layer chromatography (TLC) was performed on silica gel GF254 (Merck) plates while the spots were visualized using ultraviolet light at 254 and 365 nm. NMR spectra were recorded on a 300 MHz Bruker Avance operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C. The purity grade was performed on an Acquity UPLC system class H (Waters) equipped with a photodiode array (PDA) detector, sample manager, and a quaternary solvent manager as well as a BEH C18 column: 50 mm, 1.0 mm, particle size 1.7 m (Waters, São Paulo-Brazil). The high accuracy mass spectra were recorded on a Xevo G2-S QTOF machine (Waters, São Paulo-Brazil) equipped with an electrospray probe. The temperatures of the column and the sample tray were 40 and 20°C, respectively. The gradient employed at flow rate of 0.3 mL/min was 95% A (water/formic acid 99.9:0.1 v/v) and 5% B (acetonitrile); 1–2 min, 95% of A; 2–10 min 55% of A; 14–15 min 5% of A; 15–20 min 95% of A. The injection volume amounted to 5 μL. The wavelength interval used for the detection was 190–400 nm while the positive mode ionization was used for mass detection. The melting points were measured on a MQAPF301 apparatus (Microquímica, Palhoça-Brazil) and are uncorrected.

The InChI codes of the investigated compounds together with some biological activity data are provided as Supporting Information.

4.1.2 | Preparation of 5,7-dihydroxy-4-methyl-2*H*-chromen-2-one (3)

One equivalent of ethyl acetoacetate (2.1 mL, 1 equiv., purchased from Sigma–Aldrich) was added to phloroglucinol (2.0 g, 15.9 mmol, purchased from Sigma–Aldrich). The reaction was promoted by 0.05 mL of polyphosphoric acid (purchased from Sigma–Aldrich) and the mixture was stirred at 60°C under solvent-free conditions. The preparation was complete within 6 h based on the TLC profile (hexane/ethyl acetate 1:1 v/v). The medium was poured onto a solution of $NaHCO_3$ and filtered under reduced pressure to afford a pale white

solid. This solid was once again poured onto EtOH and after sonication the mixture was filtered under reduced pressure yielding compound **3**.

5,7-Dihydroxy-4-methyl-2*H*-chromen-2-one (3)

Pale white solid (1.687 g, yield 56%); m.p. 274–276°C, purity 99%. ^{1}H NMR (300 MHz, $(CD_3)_2CO$) δ 6.35 (d, *J* = 2.3 Hz, 1H), 6.27 (d, *J* = 2.3 Hz, 1H), 5.83 (q, *J* = 1.1 Hz, 1H), 2.56 (d, *J* = 1.1 Hz, 3H), ^{13}C NMR (75 MHz, $(CD_3)_2CO$) δ 160.9, 159.9, 157.7, 157.1, 154.4, 109.6, 102.6, 99.0, 95.0, 23.0; HRESIMS *m/z* 193.0507 ($[C_{10}H_8O_4+H]^+$ calcd. for *m/z* 193.0501).

4.1.3 | Preparation of 10-aryl-2,10-dihydropyrano[2,3-*f*]chromenes (4–14)

An amount of 0.066 g of malononitrile (1 mmol) (purchased from Sigma–Aldrich) was mixed with 1.0 equivalent of different aromatic aldehydes (purchased from Sigma–Aldrich) and calcium carbonate (0.014 g, 0.14 mmol) (purchased from Spectrum®) in 3 mL of methanol. The mixture was stirred for 5 min under reflux and 1.0 equivalent of compound **3** was added. This procedure related to the previously reported[11] afforded compounds **4**–**9**. The preparation of compounds **10**–**14** was performed by using the same method except that malononitrile was replaced by 0.100 g of methyl α-cyanoacetate (1 mmol) (purchased for Sigma–Aldrich) with one equivalent of the other reagents. The completion of these reactions was characterized by a precipitation and compounds **4**–**14** were obtained by filtration *in vacuo* and the solids were washed during the filtration with EtOH/H_2O (5:1).

8-Amino-10-(4-chlorophenyl)-5-hydroxy-4-methyl-2-oxo-2,10-dihydropyrano[2,3-*f*]chromene-9-carbonitrile (4)

White solid (0.2432 g, yield 64%); m.p. 258.3°C; reaction time 20 min; purity 98.5%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.34 (d, *J* = 8.4 Hz, 2H), 7.15 (d, *J* = 8.4 Hz, 2H), 7.08 (s, 2H, exchangeable), 6.49 (s, 1H), 6.09 (d, *J* = 1.1 Hz, 1H), 4.63 (s, 1H), 2.60 (br s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.1, 159.9, 158.0, 155.0, 153.9, 148.0, 144.7, 131.6, 129.5 (2C), 128.9 (2C), 120.4, 112.1, 108.5, 102.4, 99.1, 57.4, 36.3, 24.5; HRESIMS *m/z* 381.0639 ($[C_{20}H_{13}N_2O_4{}^{35}Cl+H]^+$ calcd. for *m/z* 381.0642) and *m/z* 383.0627 ($[C_{20}H_{13}N_2O_4{}^{37}Cl+H]^+$ calcd. for *m/z* 383.0608).

8-Amino-10-(4-bromophenyl)-5-hydroxy-4-methyl-2-oxo-2,10-dihydropyrano[2,3-*f*]chromene-9-carbonitrile (5)

Orange solid (0.3010 g, yield 71%); m.p. 297.0°C; reaction time 30 min; purity: 85.5%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.47 (d, *J* = 8.4 Hz, 2H), 7.10 (d, *J* = 8.40 Hz, 2H), 7.08 (s, 2H, exchangeable), 6.49 (s, 1H), 6.09 (d, *J* = 0.90 Hz, 1H), 4.62 (s, 1H), 2.60 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.5, 159.4, 157.5, 154.5, 153.4, 147.5, 144.6, 131.3 (2C), 129.4 (2C), 119.9, 119.6, 111.6, 107.9, 101.9, 98.6, 56.9, 35.9, 24.0; HRESIMS *m/z* 425.0123 ($[C_{20}H_{13}N_2O_4{}^{79}Br+H]^+$ calcd. for *m/z* 425.0137) and *m/z* 427.0131 ($[C_{20}H_{13}N_2O_4{}^{81}Br+H]^+$ calcd. for *m/z* 427.0111).

8-Amino-5-hydroxy-4-methyl-10-(4-nitrophenyl)-2-oxo-2,10-dihydropyrano[2,3-*f*]chromene-9-carbonitrile (6)

Orange solid (0.2658 g, yield 43%); m.p. 242.8°C; reaction time 25 min; purity 97.7%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.18 (d, *J* = 8.5 Hz, 2H), 7.56 (d, *J* = 8.5 Hz, 2H), 6.48 (s, 2H, exchangeable), 6.56 (s, 1H), 6.03 (br s, 1H), 4.79 (s, 1H), 2.71 (br s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.2, 160.0, 155.4, 153.9, 153.4, 148.0, 146.6, 128.9 (2C), 124.2 (2C), 120.3, 111.1, 109.4, 107.9, 101.4, 99.2, 56.7, 36.8, 24.4; HRESIMS *m/z* 392.0894 ($[C_{20}H_{13}N_3O_6+H]^+$ calcd. for 392.0883).

8-Amino-10-(3,4-dimethoxyphenyl)-5-hydroxy-4-methyl-2-oxo-2,10-dihydropyrano[2,3-*f*]chromene-9-carbonitrile (7)

Yellowish solid (0.2273 g, yield 56%); m.p. 204.8°C; reaction time 40 min; purity 93.5%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.92 (s, 2H, exchangeable), 6.85 (d, *J* = 1.8 Hz, 1H), 6.83 (d, *J* = 8.4 Hz, 1H), 6.59 (dd, *J* = 8.4, 1.8 Hz, 1H), 6.30 (s, 1H), 5.90 (s, 1H), 4.57 (s, 1H), 3.68 (s, 6H), 2.56 (br s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.8, 160.6, 160.3, 155.2, 153.9, 148.8, 148.0, 147.9, 138.5, 120.9, 119.2, 112.3, 111.7, 110.1, 110.0, 100.4, 99.2, 58.0, 55.9, 55.9, 36.1, 24.4; HRESIMS *m/z* 407.1254 ($[C_{22}H_{18}N_2O_6+H]^+$ calcd. for 407.1243).

8-Amino-5-hydroxy-4-methyl-2-oxo-10-(3,4,5-trimethoxyphenyl)-2,10-dihydropyrano[2,3-*f*]chromene-9-carbonitrile (8)

Pale white solid (0.2354 g, yield 54%); m.p. 238.9°C; reaction 40 min; purity 95.4%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.00 (s, 2H, exchangeable), 6.47 (s, 2H), 6.42 (s, 1H), 5.97 (s, 1H), 4.64 (s, 1H), 3.69 (s, 6H), 3.61 (s, 3H), 2.59 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.2, 160.7, 160.1, 155.1, 153.9, 153.1 (2C), 148.1, 141.1, 136.7, 121.0, 110.4, 109.7, 104.7 (2C), 101.0, 99.2, 60.3, 57.6, 56.2 (2C), 36.7, 24.4; HRESIMS *m/z* 437.1348 ($[C_{23}H_{20}N_2O_7+H]^+$ calcd. for 437.1349).

(*E*)-8-Amino-5-hydroxy-4-methyl-2-oxo-10-styryl-2,10-dihydropyrano[2,3-*f*]chromene-9-carbonitrile (9)

Brownish solid (0.2008 g, yield 54%); m.p. 195.9°C; reaction time 45 min; purity 88.7%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.39 (dd, *J* = 7.3, 1.2 Hz, 2H), 7.28 (dd, *J* = 7.3, 1.2 Hz, 2H), 7.20 (m, 1H), 7.06 (s, 2H, exchangeable), 6.51 (s, 1H), 6.40 (d, *J* = 15.6 Hz, 1H), 6.20 (dd, *J* = 15.6, 7.1 Hz, 1H), 5.99 (br s, 1H), 4.24 (d, *J* = 7.1 Hz, 1H), 2.56 (br s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 160.9, 160.0, 154.9, 153.8 (2C), 147.8, 136.9, 131.4, 129.0 (2C), 128.8, 127.8, 126.7 (2C), 120.7, 111.0, 108.1, 101.7, 99.2, 54.8, 34.3, 24.4; HRESIMS *m/z* 373.1172 ($[C_{22}H_{16}N_2O_4+H]^+$ calcd. for *m/z* 373.1188).

Methyl 8-amino-10-(4-chlorophenyl)-5-hydroxy-4-methyl-2-oxo-2,10-dihydropyrano[2,3-*f*]chromene-9-carboxylate (10)

A pale white solid (0.2849 g, yield 69%), m.p. 197.6°C; reaction time 85 min; purity 91.6%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.62 (s, 2H, exchangeable), 7.40 (d, *J* = 8.4 Hz, 2H), 7.13 (d, *J* = 8.4 Hz), 6.52 (s, 1H), 6.10 (d, *J* = 1.1 Hz, 1H), 4.98 (s, 1H), 3.58 (s, 3H), 2.66

(br s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.5, 160.9, 159.9, 157.7, 154.5, 153.7, 148.1, 145.8, 130.9, 129.6 (2C), 128.4 (2C), 111.9, 111.5, 102.4, 98.9, 77.2, 51.1, 33.9, 24.4; HRESIMS *m/z* 414.0750 ($[C_{21}H_{16}NO_6{}^{35}Cl+H]^+$ calcd. for *m/z* 414.0744) and *m/z* 416.0750 ($[C_{21}H_{16}NO_6{}^{37}Cl+H]^+$ calcd. for *m/z* 416.0710).

Methyl 8-amino-10-(4-bromophenyl)-5-hydroxy-4-methyl-2-oxo-2,10-dihydropyrano[2,3-*f*]chromene-9-carboxylate (11)

White solid (0.3307 g, yield 73%); m.p. 201.3°C; reaction time 100 min; purity 95.6%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.61 (s, 2H, exchangeable), 7.39 (d, *J* = 8.4 Hz, 2H), 7.13 (d, *J* = 8.4 Hz, 2H), 6.51 (s, 1H), 6.08 (d, J = 0.8 Hz, 1H), 4.97 (s, 1H), 3.57 (s, 3H), 2.65 (br s, 3H). ^{13}C NMR (75 MHz, DMSO-d$_6$) δ168.5, 160.9, 159.9, 157.7, 154.5, 153.7, 148.1, 146.2, 131.3 (2C), 130.1 (2C), 119.4, 111.9, 111.5, 102.4, 98.9, 77.1, 51.1, 34.0, 24.4; HRESIMS *m/z* 458.0271 ($[C_{21}H_{16}NO_6{}^{79}Br+H]^+$ calcd. for *m/z* 458.0239) and *m/z* 460.0252 ($[C_{21}H_{16}NO_6{}^{81}Br+H]^+$ calcd. for *m/z* 460.0214).

Methyl 8-amino-5-hydroxy-4-methyl-10-(4-nitrophenyl)-2-oxo-2,10-dihydropyrano[2,3-*f*]chromene-9-carboxylate (12)

Pale white solid (0.3180 g, yield 75%); m.p. 209.6°C; reaction time 100 min; purity 89.3%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.11 (d, *J* = 8.6 Hz, 2H), 7.45 (d, *J* = 8.6 Hz, 2H), 6.52 (s, 1H), 6.11 (br s, 1H), 5.12 (s, 1H), 3.59 (s, 3H), 2.69 (br s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.3, 160.8, 159.8, 157.8, 154.8, 154.4, 153.7, 148.1, 146.2, 129.2 (2C), 123.8 (2C), 111.9, 110.5, 102.4, 98.9, 76.4, 51.1, 34.8, 24.4; HRESIMS *m/z* 425.0966 ($[C_{21}H_{16}N_2O_8+H]^+$ calcd. for *m/z* 425.0985).

Methyl 8-amino-10-(3,4-dimethoxyphenyl)-5-hydroxy-4-methyl-2-oxo-2,10-dihydropyrano[2,3-*f*]chromene-9-carboxylate (13)

White solid (0.3073 g, yield 70%); m.p. 223.5°C; reaction time 120 min; purity 86.1%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.50 (s, 2H, exchangeable), 7.02 (d, *J* = 1.7 Hz, 1H), 6.76 (d, *J* = 8.4 Hz, 1H), 6.63 (dd, *J* = 8.4, 1.7 Hz, 1H), 6.21 (s, 1H), 5.75 (br s, 1H), 5.06 (s, 1H), 3.65 (s, 3H), 3.64 (s, 3H), 3.58 (s, 3H), 2.59 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.1, 161.8, 160.7, 155.4, 153.4, 153.9, 148.4, 148.3, 147.3, 140.3, 119.0, 113.9, 112.2, 112.0, 107.7, 99.3, 98.7, 78.2, 55.9, 55.7, 50.9, 33.3, 24.4; HRESIMS *m/z* 440.1331 ($[C_{23}H_{21}NO_8+H]^+$ calcd. for *m/z* 440.1345).

Methyl 8-amino-5-hydroxy-4-methyl-2-oxo-10-(3,4,5-trimethoxyphenyl)-2,10-dihydropyrano[2,3-*f*]chromene-9-carboxylate (14)

Yellowish solid (0.2720 g, yield 58%); m.p. 223.5°C; reaction time 120 min; purity 85.9%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.61 (s, 2H, exchangeable), 6.57 (s, 1H), 6.50 (s, 2H), 6.08 (s, 1H), 5.06 (s, 1H), 3.70 (s, 6H), 3.65 (s, 3H), 3.61 (s, 3H), 2.68 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) data: δ 168.7, 161.4, 160.0, 157.9, 154.4, 153.7, 152.9, 148.4, 142.4, 136.4, 112.1, 111.8, 104.9, 102.4, 77.5, 60.3, 56.2, 51.1, 34.2, 24.4; HRESIMS *m/z* 470.1472 ($[C_{24}H_{23}NO_9+H]^+$ calcd. for *m/z* 470.1451).

(2*E*,4*E*)-Methyl 2-cyano-5-phenylpenta-2,4-dienoate (15)

Yellow bright solid (0.1913 g, yield 89.2%); m.p. 183°C; reaction time 2 h; purity 86%. ^{1}H NMR (300 MHz, DMSO-d_6) data: δ 8.05 (m, 1H), 7.61 (m, 2H), 7.42–7.44 (m, 3H), 7.31 (m, 1H), 7.29 (m, 1H), 3.91 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.8, 155.7, 149.1, 134.6, 131.2, 129.1 (2C), 128.5 (2C), 123.0, 114.5, 104.0, 53.1; HRESIMS *m/z* 214.0850 ($[C_{13}H_{11}NO_2+H]^+$ calcd. for *m/z* 214.0868).

4.1.4 | Preparation of 4-aryl-4*H*-chromenes (16–22)

Phloroglucinol (purchased from Sigma–Aldrich) (0.126 g, 1 mmol) was mixed with a methanol solution (1 mL) of 1.0 equivalent of different benzaldehyde derivatives. Methyl α-cyanoacetate (1.0 equivalent) (purchased from Sigma–Aldrich) and an aqueous solution (19 mL) of K_2CO_3 (0.09 g, 0.65 mmol) (purchased from Spectrum®) were added to the mixture. The medium was stirred at room temperature and the reaction was complete within 18 h based on the TLC profile. The precipitate observed in the medium was purified by filtration and washed during this separation process with mixture water/MeOH (1:1).

Methyl 2-amino-4-(4-chlorophenyl)-5,7-dihydroxy-4*H*-chromene-3-carboxylate (16)

Yellow solid (0.1804 g, yield 51.4%); m.p. 195.7°C; purity 58.2%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.64 (s, 1H, exchangeable), 9.43 (s, 1H, exchangeable), 7.55 (s, 2H, exchangeable), 7.23 (d, *J* = 8.3 Hz, 2H), 7.12 (d, *J* = 8.3 Hz, 2H), 6.04 (br s, 1H), 5.96 (br s, 1H), 4.83 (s, 1H), 3.52 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.9, 161.9, 157.5, 155.4, 150.8, 147.2, 130.3, 129.5 (2C), 128.1 (2C), 105.3, 99.0, 94.0, 77.5, 50.8, 33.8; HRESIMS *m/z* 348.0642 ($C_{17}H_{14}NO_5{}^{35}Cl+H]^+$ calcd. for *m/z* 348.0639) and *m/z* 350.0642 ($C_{17}H_{14}NO_5{}^{37}Cl+H]^+$ calcd. for *m/z* 350.0604).

Methyl 2-amino-4-(4-bromophenyl)-5,7-dihydroxy-4*H*-chromene-3-carboxylate (17)

Yellow solid (0.1994 g, yield 51%); m.p. 220.9°C; purity 69.6%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.65 (s, 1H, exchangeable), 9.44 (s, 1H, exchangeable), 7.55 (s, 2H, exchangeable), 7.37 (d, *J* = 8.2 Hz, 2H), 7.08 (d, *J* = 8.2 Hz, 2H), 6.05 (d, *J* = 1.6 Hz, 1H), 5.97 (d, *J* = 1.6 Hz, 1H), 4.83 (s, 1H), 3.52 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.9, 161.8, 157.6, 155.5, 150.8, 147.6, 131.0 (2C), 130.0 (2C), 118.8, 105.2, 99.0, 94.0, 77.4, 50.8, 33.9; HRESIMS *m/z* 392.0124 ($[C_{17}H_{14}NO_5{}^{79}Br+H]^+$ calcd. *m/z* 392.0134) and *m/z* 394.0113 ($[C_{17}H_{14}NO_5{}^{81}Br+H]^+$ calcd. *m/z* 394.0108).

Methyl 2-amino-5,7-dihydroxy-4-(4-nitrophenyl)-4*H*-chromene-3-carboxylate (18)

Yellow solid (0.1969 g, yield 55%); m.p. 211.4°C; purity 56.6%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.65 (br s, 2H exchangeable), 8.08 (d, *J* = 8.6 Hz, 2H), 7.64 (br s, 2H, exchangeable), 7.37 (d, *J* = 8.6 Hz, 2H), 6.04 (d, *J* = 2.0 Hz, 1H), 5.99 (d, *J* = 2.0 Hz, 1H), 4.95 (s, 1H), 3.52 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.8, 161.8, 158.0, 156.0, 155.7, 150.8, 145.9, 129.1 (2C), 123.7 (2C), 104.3, 99.1, 94.2, 76.8, 50.9, 34.8; HRESIMS *m/z* 359.0893 ($[C_{17}H_{14}N_2O_7+H]^+$ calcd. for *m/z* 359.0879).

Methyl 2-amino-4-(3,4-dimethoxyphenyl)-5,7-dihydroxy-4*H*-chromene-3-carboxylate (19)

Brown solid (0.2088 g, yield 56%); m.p. 227.1°C; purity 85.2%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.60 (s, 1H, exchangeable), 9.39 (s, 1H, exchangeable), 7.49 (s, 2H), 6.82 (d, J = 1.2 Hz, 1H), 6.75 (d, J = 8.4 Hz, 1H), 6.55 (dd, J = 8.4, 1.2 Hz, 1H), 6.05 (d, J = 1.7 Hz, 1H), 5.95 (d, J = 1.7 Hz, 1H), 4.83 (s, 1H), 3.66 (s, 3H), 3.65 (s, 3H), 3.55 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.1, 162.0, 157.2, 155.4, 151.2, 148.4, 147.2, 140.9, 119.2, 112.1, 112.0, 106.2, 99.0, 94.0, 78.1, 55.9, 55.8, 50.8, 33.5; HRESIMS m/z 374.1252 ([$C_{19}H_{19}NO_7$+H]$^+$ calcd. for m/z 374.1240).

Methyl 2-amino-5,7-dihydroxy-4-(3,4,5-trimethoxyphenyl)-4*H*-chromene-3-carboxylate (20)

White solid (0.2418 g, yield 60%); m.p. 177.5°C; purity 88.4%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.65 (s, 1H, exchangeable), 9.41 (s, 1H, exchangeable), 7.51 (s, 2H, exchangeable), 6.42 (s, 2H), 6.07 (d, J = 1.8 Hz, 1H), 5.97 (d, J = 1.8 Hz, 1H), 4.86 (s, 1H), 3.86 (s, 3H), 3.65 (s, 3H), 3.57 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.1, 162.2, 157.4, 155.4, 152.7 (2C), 151.3, 143.9, 136.0, 105.8, 104.8 (2C), 99.1, 94.1, 77.8, 60.3, 56.1 (2C), 50.8, 34.2; HRESIMS m/z 404.1350 ([$C_{20}H_{21}NO_8$+H]$^+$ calcd. for m/z 404.1345).

Methyl 2-amino-5,7-dihydroxy-4-(4-hydroxy-3,5-dimethoxyphenyl)-4*H*-chromene-3-carboxylate (21)

Brown solid (0.2256 g, yield 58%); m.p. 242.5°C; purity 90.6%; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.60 (s, 1H, exchangeable), 9.38 (s, 1H, exchangeable), 8.06 (s, 1H, exchangeable), 7.48 (s, 2H, exchangeable), 6.38 (s, 2H), 6.06 (d, J = 1.7 Hz, 1H), 5.96 (d, J = 1.7 Hz, 1H), 4.82 (s, 1H), 3.63 (s, 6H), 3.57 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.2, 162.1, 157.2, 155.4, 151.3, 147.8 (2C), 138.4, 134.1, 106.3, 105.3 (2C), 99.1, 94.0, 78.2, 56.4 (2C), 50.7, 33.7; HRESIMS m/z 390.1197 ([$C_{19}H_{19}NO_8$+H]$^+$ calcd. for m/z 390.1189).

Methyl 2-amino-5,7-dihydroxy-4-(4-hydroxy-3-methoxyphenyl)-4*H*-chromene-3-carboxylate (22)

Brownish solid (0.1938 g, yield 54%); m.p. 224.4°C; purity 89.4%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.57 (s, 1H, exchangeable), 9.35 (s, 1H, exchangeable), 8.62 (s, 1H, exchangeable), 7.46 (s, 2H, exchangeable), 6.78 (d, J = 1.5 Hz, 1H), 6.56 (d, J = 1.5 Hz, 1H), 6.43 (dd, J = 8.2, 1.5 Hz, 1H), 6.05 (d, J = 1.9 Hz, 1H), 5.94 (d, J = 1.9 Hz, 1H), 4.79 (s, 1H), 3.67 (s, 3H), 3.57 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.2, 162.1, 157.1, 155.4, 151.2, 147.1, 144.8, 139.3, 119.5, 115.3, 112.3, 106.5, 99.0, 94.0, 78.4, 55.9, 50.8, 33.4; HRESIMS m/z 360.1092 ([$C_{18}H_{17}NO_7$+H]$^+$ calcd. for m/z 360.1083).

4.2 | Biological activities

4.2.1 | Cytotoxicity assay

Cell cultures

The drug-sensitive CCRF-CEM leukemia and its multidrug-resistant P-glycoprotein-overexpressing subline CEM/ADR5000 used for this assay as well as their origin were previously reported.[26,27]

Methodology

Resazurin reduction assay used to evaluate the samples cytotoxicity was applied as previously described.[28,29]

Cell cycle analysis and detection of apoptotic cells by flow cytometry

Compounds **12**, **15**, and doxorubicin or DMSO (solvent control) were used to treat CCRF-CEM cells (1×10^6) at various concentrations followed by the cell cycle analysis after 24 h incubation as previously reported.[30] The propidium iodide fluorescence of individual nuclei was measured using a BD Accury C6 flow cytometer (BD Biosciences, Heidelberg, Germany).

4.2.2 | Antiparasitic assay

Human macrophage cell line THP-1 (ATCC TIB202) was used as host for the intracellular amastigote forms of *T. cruzi* and *L. amazoneneis*. Cell viability assay (MTT) and antiparasitic *in vitro* evaluation were performed as we described.[31]

ACKNOWLEDGMENTS

EFM, MWB, and MS would like to thank CNPq/CAPES. ATM is thankful to Alexander von Humboldt Foundation for an 18 months fellowship Germany through the "Georg Foster Research Fellowship for Experienced Researcher" program. VK is very grateful to the Alexander von Humboldt Foundation for funding through the Linkage program (2015–2018). The work is part of the activities of the Research Network Natural Products against Neglected Diseases (ResNetNPND) http://www.ResNetNPND.org).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ORCID

Louis P. Sandjo http://orcid.org/0000-0001-5840-7733

REFERENCES

[1] S. Kasibhatla, H. Gourdeau, K. Meerovitch, J. Drewe, S. Reddy, L. Qiu, H. Zhang, F. Bergeron, D. Bouffard, Q. Yang, J. Herich, S. Lamothe, S. X. Cai, B. Tseng, *Mol. Cancer Ther.* **2004**, *3*, 1365.
[2] S. X. Cai, J. Drewe, W. Kemnitzer, *Anticancer Agents Med. Chem.* **2009**, *9*, 437.
[3] Cancer. http://www.who.int/cancer/en/. Browsed on 07.02.2018.
[4] D. S. Wishart, *EBioMedicine* **2015**, *2*, 478.
[5] Y. Sun, Z. Shi, H. Lian, P. Cai, *Clin. Transl. Med.* **2016**, *5*, https://doi.org/10.1186/s40169-016-0094-5.
[6] H. van Tong, P. J. Brindley, C. G. Meyer, T. P. Velavan, *EBioMedicine* **2017**, *15*, 12.
[7] M. A. N. Al-Kamel, *Clin. Skin Cancer* **2017**, *2*, 54.

[8] A. S. Nagle, S. Khare, A. B. Kumar, F. Supek, A. Buchynskyy, C. J. N. Mathison, N. K. Chennamaneni, N. Pendem, F. S. Buckner, M. H. Gelb, V. Molteni, *Chem. Rev*. **2014**, *114*, 11305.
[9] K. Ostrowska, W. Olejarz, M. Wrzosek, A. Głuszko, G. Nowicka, M. Szczepański, I. B. Materek, A. E. Kozioł, M. Struga, *Biomed. Pharmacother*. **2017**, *95*, 1412.
[10] D. Harel, D. Schepmann, H. Prinz, R. Brun, T. J. Schmidt, B. Wünsch, *J. Med. Chem*. **2013**, *56*, 7442.
[11] B. Karami, S. Khodabakhshi, K. Eskandari, *Tetrahedron Lett*. **2012**, *53*, 1445.
[12] I. B. Masesane, S. O. Mihigo, *Synthetic Commun*. **2015**, *45*, 1546.
[13] W. Kemnitzer, J. Drewe, S. Jiang, H. Zhang, Y. Wang, J. Zhao, S. Jia, J. Herich, D. Labreque, R. Storer, K. Meerovitch, D. Bouffard, R. Rej, R. Denis, C. Blais, S. Lamothe, G. Attardo, H. Gourdeau, B. Tseng, S. Kasibhatla, S. X. Cai, *J. Med. Chem*. **2004**, *47*, 6299.
[14] W. Kemnitzer, S. Kasibhatla, S. Jiang, H. Zhang, J. Zhao, S. Jia, L. Xu, C. Crogan-Grundy, R. Denis, N. Barriault, L. Vaillancourt, S. Charron, J. Dodd, G. Attardo, D. Labrecque, S. Lamothe, H. Gourdeau, B. Tseng, J. Drewe, S. X. Cai, *Bioorg. Med. Chem. Lett*. **2005**, *15*, 4745.
[15] W. Kemnitzer, J. Drewe, S. Jiang, H. Zhang, C. Crogan-Grundy, D. Labrecque, M. Bubenick, G. Attardo, R. Denis, S. Lamothe, H. Gourdeau, B. Tseng, S. Kasibhatla, S. X. Cai, *J. Med. Chem*. **2008**, *51*, 417.
[16] A. S. Zambare, F. A. K. Khan, S. P. Zambare, S. D. Shinde, J. N. Sangshetti, *Curr. Org. Chem*. **2016**, *20*, 798.
[17] M. Farahi, B. Karami, R. Keshavarz, F. Khosravian, *RSC Adv*. **2017**, *7*, 46644.
[18] A. Parhami, A. Khalafi-Nezhad, S. M. Haghighi, R. Bargebid, A. Zare, A. R. Moosavi-Zare, M. Nikrooza, *Arkivoc* **2012**, *IX*, 111.
[19] S. Balalaie, M. Bararjanian, S. Hekmat, P. Salehi, *Synthetic Commun*. **2006**, *36*, 3703.
[20] B. Karami, S. Khodabakhshi, K. Eskandari, *Tetrahedron Lett*. **2012**, *53*, 1445.
[21] A. T. Mbaveng, V. Kuete, T. Efferth, *Front. Pharmacol*. **2017**, *8*, 343.
[22] A. Fallah-Tafti, R. Tiwari, A. N. Shirazi, T. Akbarzadeh, D. Mandal, A. Shafiee, K. Parang, A. Foroumadi, *Med. Chem*. **2011**, *7*, 466.
[23] H. K. Keerthy, M. Garg, C. D. Mohan, V. Madan, D. Kanojia, R. Shobith, S. Nanjundaswamy, D. J. Mason, A. Bender, Basappa, K. S. Rangappa, H. P. Koeffler, *PLoS ONE* **2014**, *9*, e107118.
[24] H. Aryapour, M. Mahdavi, S. R. Mohebbi, M. R. Zali, A. Foroumadi, *Arch. Pharm. Res*. **2012**, *35*, 1573.
[25] M.-Q. Klinkert, V. Heussler, *Mini-Rev. Med. Chem*. **2006**, *6*, 131.
[26] J. Gillet, T. Efferth, D. Steinbach, J. Hamels, F. de Longueville, V. Bertholet, J. Remacle, *Cancer Res*. **2004**, *64*, 8987.
[27] A. Kimmig, V. Gekeler, M. Neumann, G. Frese, R. Handgretinger, G. Kardos, H. Diddens, D. Niethammer, *Cancer Res*. **1990**, *50*, 6793.
[28] V. Kuete, P. D. Tchakam, B. Wiench, B. Ngameni, H. K. Wabo, M. F. Tala, M. L. Moungang, B. T. Ngadjui, T. Murayama, T. Efferth, *Phytomedicine* **2013**, *20*, 528.
[29] J. O'Brien, I. Wilson, T. Orton, F. Pognan, *Eur. J. Biochem*. **2000**, *267*, 5421.
[30] V. Kuete, B. Ngameni, B. Wiench, B. Krusche, C. Horwedel, B. T. Ngadjui, T. Efferth, *Planta Med*. **2011**, *77*, 1984.
[31] L. A. E. Pollo, M. H. de Moraes, J. Cisilotto, T. B. Creczynski-Pasa, M. W. Biavatti, M. Steindel, L. P. Sandjo, *Parasitol. Int*. **2017**, *66*, 789.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Martin EF, Mbaveng AT, de Moraes MH, et al. Prospecting for cytotoxic and antiprotozoal 4-aryl-4*H*-chromenes and 10-aryldihydropyrano[2,3-*f*]chromenes. *Arch Pharm Chem Life Sci*. 2018;1–11. https://doi.org/10.1002/ardp.201800100