Article

Synthesis and Evaluation of Antileishmanial and Cytotoxic Activity of Benzothiopyrane Derivatives

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Abstract: As a part of our ongoing effort in the search for promising antileishmanial agents based on the thiochroman scaffold, we prepared a series of substituted 2H-thiochromenes. Thirty-three compounds were evaluated against intracellular amastigotes forms of *L. (V) panamensis*. Twelve compounds were active with EC_{50} values lower than 40 μM, and among those three compounds displayed the highest antileishmanial activity with EC_{50} values below 10 uM. Cytotoxicity was determined against human U-937 macrophages; thus, compounds having electrophilic alkenes (α,β-unsaturated carbonyl, or nitriles) displayed the highest antileishmanial activity but also moderate to high cytotoxicities. Based on SAR analysis, compounds **8d** and **10,** which differ only in the hydroxy group at C4, were selected as the most promising compounds in this library because good antiparasitic activity and Selectivity Index.

Keywords: *Leishmania*; thiochromenes; benzothiopyrans; cytotoxicity.

1. Introduction

Cutaneous leishmaniasis (CL) is a group of skin infections caused by intracellular protozoa belonging to the genus *Leishmania*. Parasites are spread by the bite of *lutzomia* and *phlebotomous* sand flies. CL is a poverty-associated disease considered by the World Health Organization, WHO, as one of the 17 neglected diseases due to lack of interest by the pharmaceutical industry to develop new drugs and the wide distribution around the world, with 310 million people at risk and about one million new cases occurring annually. Most CL cases occur in six countries, with most of the cases occurring in Afghanistan, Algeria, Brazil, Colombia, Iran, and Syria[1].

To date, only two therapies exist against leishmaniasis, pentavalent antimonials (meglumine antimoniate and sodium stibogluconate), and amphotericin B, which are unsatisfactory because of their high costs, toxicity, prolonged treatment, or lack of efficacy. Since current treatments present non-neglectable toxicity, there is an urgent need to develop new therapies against this disease with novel modes of action.

Chromenes are considered a privileged scaffold in medicinal chemistry as they exhibit a broad range of biological activities as antiemetic, anti-hypertensive, Anti-malarial and insecticidal activities among others[2–5] furthermore from a bioisosteric point of view the activities of chromene-like compounds could be improved with the replacement of the oxygen atom with sulfur[6] to give the corresponding thiochromenes. In addition, our previous findings showed that compounds bearing a thiochroman moiety display good antileishmanial activity[7,8].

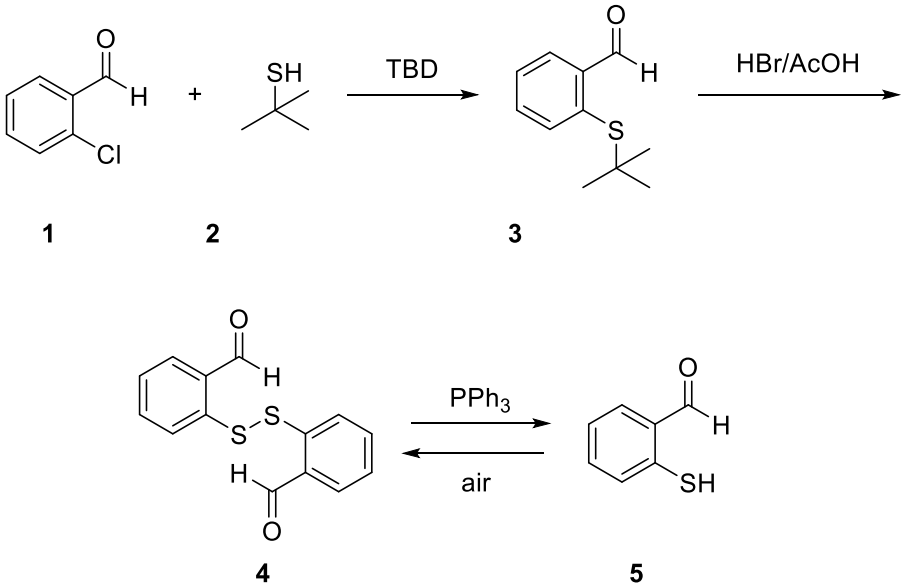
In our search for methods for the synthesis of 2H-thiochromene-like compounds, we first explore the synthetic methodologies of their oxygenated counterparts. Several methodologies have been proposed, which includes, among others, the Petasis reaction[9] of salicylaldehydes, tandem Michael addition[10], PPh_3-catalyzed domino reaction[11] and the domino oxa-Michael aldol reaction[12]. The latter approach seems to us to be of interest to be mimicked with the mercapto counterpart.

Other benzothiopyrane derivatives could be prepared by reaction of a suitable thiophenol with α,β-unsaturated carboxylic acids.

2. Results

2.1. Synthesis

Synthesis of the precursor 2-mercaptobenzaldehyde started from 2-chlorobenzaldehyde **1** and tert-butylthiol **2** (Scheme 1), following a modified procedure of Chemburkar[13] using TBD (1,5,7-triazabicyclo[4.4.0]dec-5-ene) in solvent-free conditions leading to high yields of the expected 2-(tert-butylthio)benzaldehyde (**3**). Treatment of **3** with a mixture of HBr/acetic acid in DMSO gave the 2,2'-dithiodibenzaldehyde **4**. Following the work of Humphrey and Hawkins[14], **4** was allowed to react with PPh_3 to give 2-mercaptobenzaldehyde **5**, which readily oxidizes in air to give again disulfide **4**.



Scheme 1. Synthesis of 2-mercaptobenzaldehyde

Considering the tedious procedure to obtain **5** and its ease for oxidation in the presence of air, we explored the synthesis of thiochromene compounds directly from aldehydes **3** or **4**. Thus, the reaction of **4** with activated alkenes **6a-e** in the presence of DBU and PPh_3 resulted in the formation of a mixture of diastereomeric thiochromanols **7a-e (Scheme 2).**

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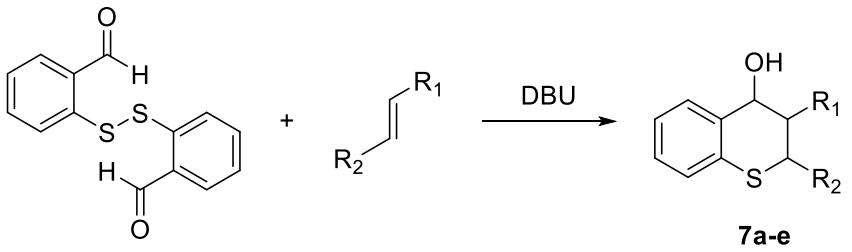
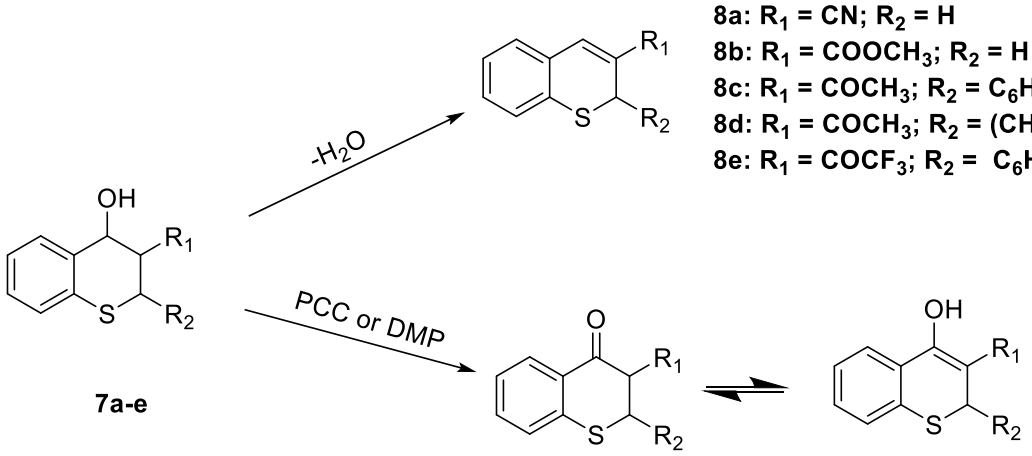


4 7a-e

6a: R_1 = CN; R_2 = H
6b: R_1 = $COOCH_3$; R_2 = H
6c: R_1 = $COCH_3$; R_2 = C_6H_5
6d: R_1 = $COCH_3$; R_2 = $(CH_2)_4CH_3$
6e: R_1 = $COCF_3$; R_2 = C_6H_5

Scheme 2. Synthesis of 2H-thiochromanols derivatives 7a-e

The above compounds **7a-e** were dehydrated to give 2H-thiochromenes **8a-e (Scheme 3)**. Oxidation of thiochromanols **7a,b,d** with PCC resulted in the formation of ketones **9a,b,d** which tautomerize to its enol form **10a,b,d (Scheme 3).**



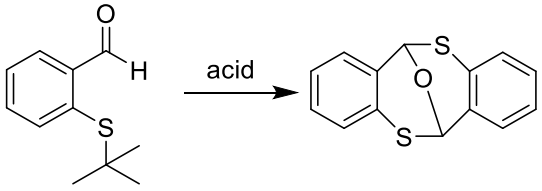
7a-e

8a: R_1 = CN; R_2 = H
8b: R_1 = $COOCH_3$; R_2 = H
8c: R_1 = $COCH_3$; R_2 = C_6H_5
8d: R_1 = $COCH_3$; R_2 = $(CH_2)_4CH_3$
8e: R_1 = $COCF_3$; R_2 = C_6H_5

9a, 10a: R_1 = CN; R_2 = H
9b, 10b: R_1 = $COOCH_3$; R_2 = H
9d, 10d: R_1 = $COCH_3$; R_2 = $(CH_2)_4CH_3$

Scheme 3. Synthesis of 2H-thiochromenes 8a-e and

Taking into account that S-tertbutyl groups are cleaved under acidic conditions[15,16], attempts to cleave the tertbutyl group in **3** with HCl or PTSA gave a dimeric hemithioacetal **11 (Scheme 4)** as previously reported by Dickmann[17] by treatment with HBr. Formation of compound **11** must occur through the formation of **5**, followed by dimerization.

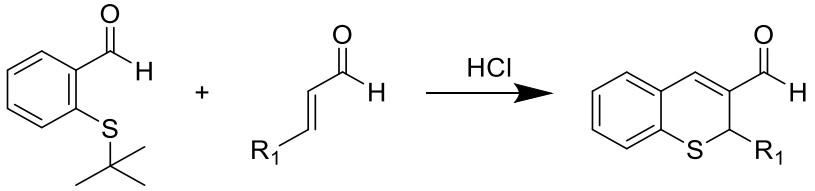


3 11

Scheme 4. Cleavage of the tert-butyl group

The above results led us to think that compound **5** formed in-situ could react with suitable activated alkenes to yield the addition product; thus, we explored the reaction of **3** in the presence of

cinnamaldehydes **12a-d** using 12M HCL to give substituted 2-phenyl-2H-thiochromene-3-carbaldehydes **13a-d** in moderate to good yields **Scheme 5**.



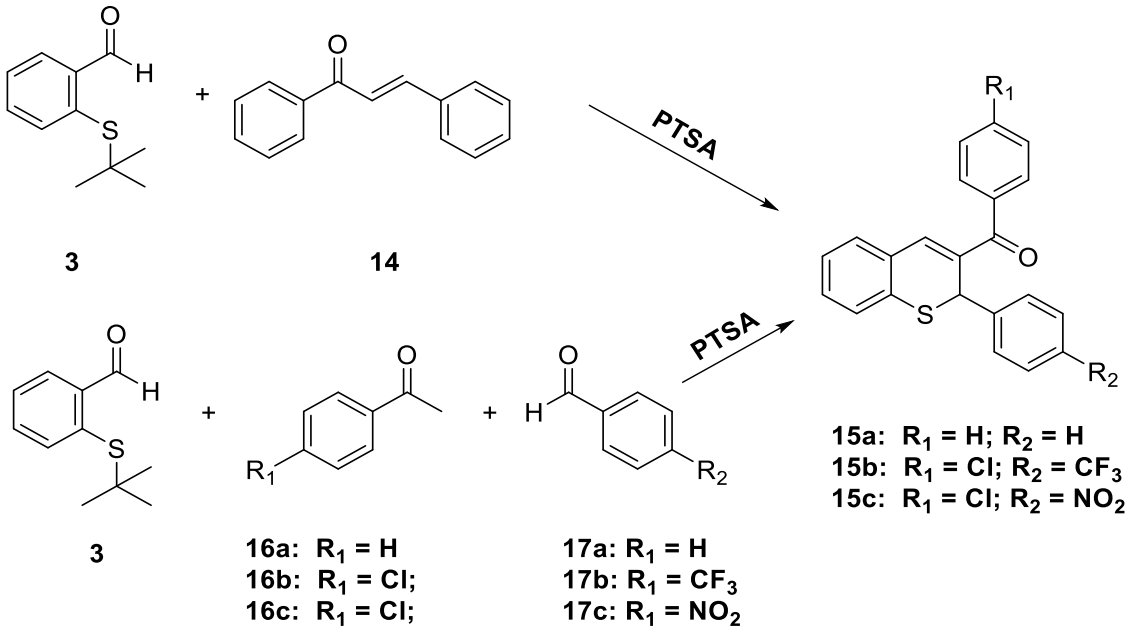
3

12a: $R_1 = C_6H_5$
12b: $R_1 = p\text{-}C_6H_4\text{-}F$
12c: $R_1 = p\text{-}C_6H_4\text{-}Cl$
12d: $R_1 = o\text{-}C_6H_4\text{-}NO_2$

13a: $R_1 = C_6H_5$
13b: $R_1 = p\text{-}C_6H_4\text{-}F$
13c: $R_1 = p\text{-}C_6H_4\text{-}Cl$
13d: $R_1 = o\text{-}C_6H_4\text{-}NO_2$

Scheme 5. direct synthesis of 2H-thiochromenes starting from 3

Similarly, trans-chalcone reacted with **3** in the presence of 4-toluenesulfonic acid in toluene to give phenyl(2-phenyl-2H-thiochromen-3-yl)methanone **16a** in 46% yield (**Scheme 6**). Acid-catalyzed *in situ* formation of the chalcones followed by reaction with 2-(tert-butylthio)benzaldehyde in a one-pot procedure also resulted in the formation of the desired thiochromenes **16b-c.**



3 **14**

15a: $R_1 = H$; $R_2 = H$
15b: $R_1 = Cl$; $R_2 = CF_3$
15c: $R_1 = Cl$; $R_2 = NO_2$

3

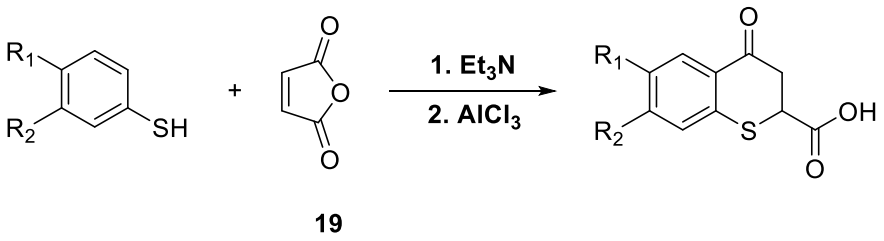
16a: $R_1 = H$
16b: $R_1 = Cl$;
16c: $R_1 = Cl$;

17a: $R_1 = H$
17b: $R_1 = CF_3$
17c: $R_1 = NO_2$

Scheme 6. Synthesis 2H-thiochromenes from chalcones

Synthesis of 4-oxothiochroman-2-carboxylic acid and its derivatives

4-Oxo-thiochroman-2-carboxylic acid **20** was obtained by reacting thiophenols **18a-d** with furan-2,5-dione (maleic anhydride) **19** in the presence of triethylamine and with subsequent treatment with $AlCl_3$ (**Scheme 7**).

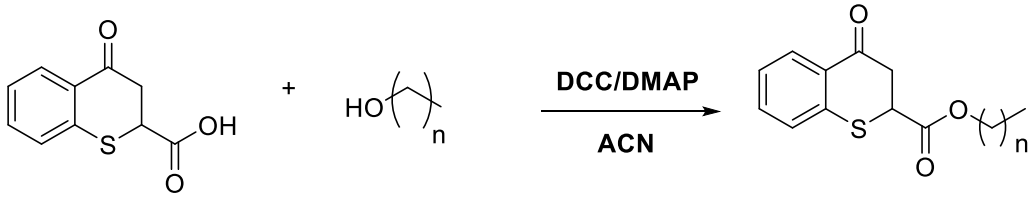


18a: R1 = H; R2 = H
18b: R1 = F; R2 = H
18c: R1 = OCH3; R2 = H
18d: R1 = H; R2 = OCH3

20a: R_1 = H; R_2 = H
20b: R_1 = F; R_2 = H
20c: R_1 = OCH_3; R_2 = H
20d: R_1 = H; R_2 = OCH_3

Scheme 7. Synthesis of 4-oxothiochroman-2-carboxylic acid

Esters of 4-Oxo-thiochroman-2-carboxylic acid **22a-d** were prepared by reaction with the corresponding alcohols **21a-d** using DCC as a coupling agent and DMAP as a catalyst (**Scheme 8**).



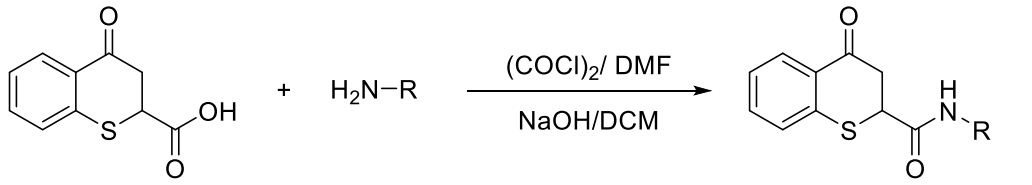
20a

21a: n = 1
21b: n = 3
21c: n = 5
21d: n = 9

22a: n = 1
22b: n = 3
22c: n = 5
22d: n = 9

Scheme 8. Esters of 4-Oxo-thiochroman-2-carboxylic acid

Amides **24a-c** were prepared by reacting the corresponding amine **23a-c** with 4-Oxo-thiochroman-2-carboxylic acid **20a** under Schotten-Baumann conditions (**Scheme 9**).



20a

23a:R = $(CH_2)_5CH_3$
23b:R = $(CH_2)_{11}CH_3$
23c: R = *o*-C_6H_4-CH_3

24a:R = $(CH_2)_5CH_3$
24b:R = $(CH_2)_{11}CH_3$
24c: R = *o*-C_6H_4-CH_3

Scheme 9. Synthesis of amides of 4-oxo-thiochroman-2-carboxylic acid

Acyl hydrazone derivatives **26a-c** were prepared by reaction of ethyl 4-oxothiochromane-2-carboxylate with acyl hydrazides **25a-c** in the presence of acetic acid (**Scheme 10**).

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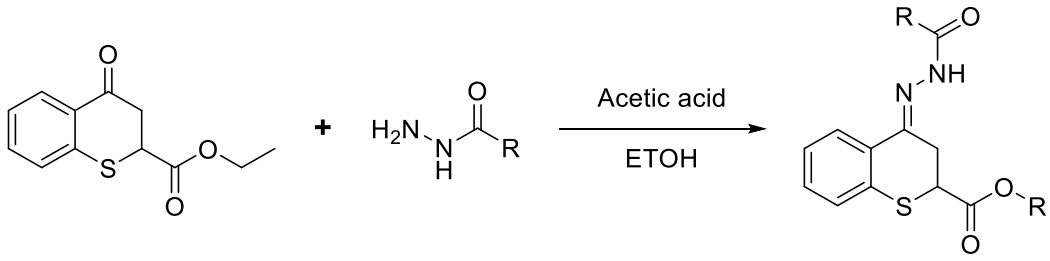
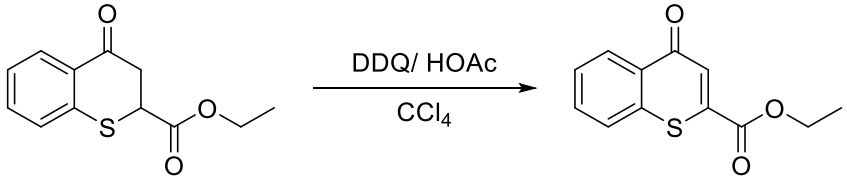


22a **25a: R = C_6H_5** **26a: R = C_6H_5**
25b: R = C_5NH_4 **26b: R = C_5NH_4**
25c: R = $CONH_2$ **26c: R = $CONH_2$**

Scheme 10. synthesis of acyl hydrazine derivatives

Dehydrogenation of **22a** was performed with DDQ in acetic acid to afford the desired ethyl 4-oxothiochromene-2-carboxylate **27 (Scheme 11)**.

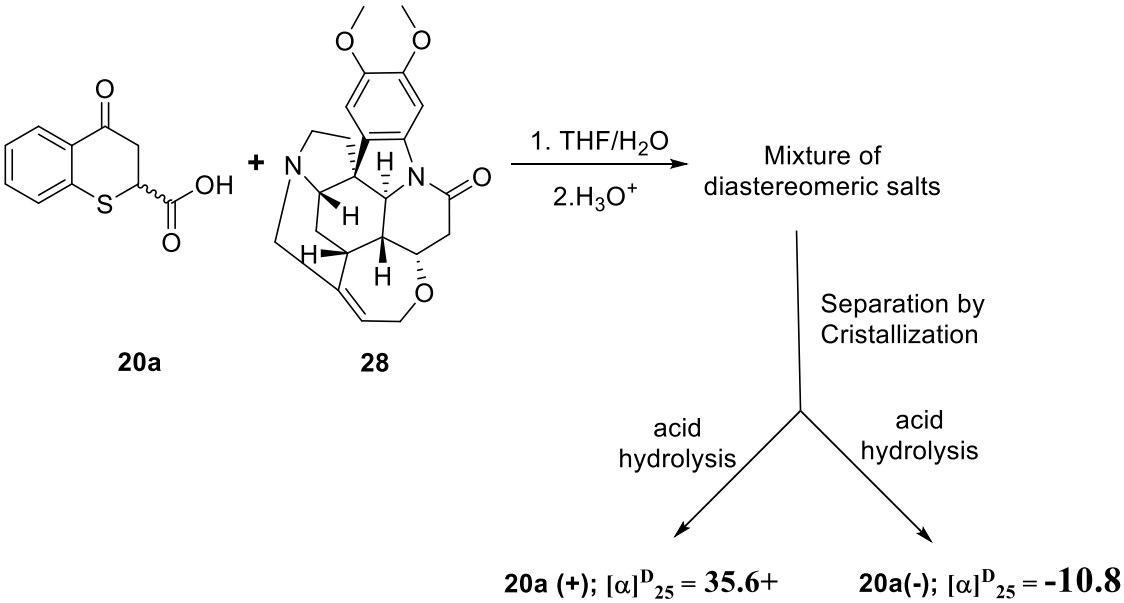


22a **27**

Scheme 11. dehydrogenation of 22a

Resolution of enantiomers of 4-oxo-thiochroman-2-carboxylic acid using brucine.

To explore the difference in antileishmanial activity of the enantiomers of 4-oxo-thiochroman-2-carboxylic acid **20**, we attempted the resolution by diastereomeric salt formation through reaction with optically pure (−)-brucine **28** as a chiral selector. One of the diastereoisomeric salts turned out to be more soluble in ethyl acetate than the other and, by allowing the mixture to crystallize, we were able to isolate samples enriched of this diastereoisomer. After evaporating the ethyl acetate from mother liquors, we obtained the less crystalline diastereoisomeric salt. Acid hydrolysis of the crystal and residue of the mother liquors resulted in non-racemic samples with specific rotation α_{25} = +35.6 for **20α(+)** with 45 % yield and $[\alpha]_{25}$ = -10.8 for **20α(-)**. We did not determine the enantiomeric excess since the compounds displayed low activity (**Scheme 12**).

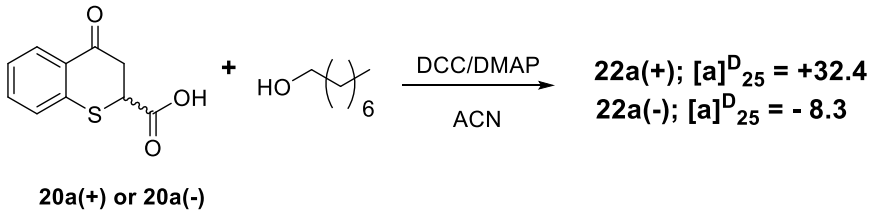


20a **28**

20a (+); $[\alpha]^D_{25}$ = 35.6+ **20a(-); $[\alpha]^D_{25}$ = -10.8**

Scheme 12. Chiral resolution of 4-oxothiochroman-2-carboxylic acid

Enantiomerically enriched samples of ethyl 4-oxothiochromane-2-carboxylate **22α(+)** and **22α(-)** were obtained from enantiomerically enriched samples 4-oxo-thiochroman-2-carboxylic acid **20a** to yield **22a(+)** with α_{25} = +32.4 and **22a(-)** with $[\alpha]_{25}$ = -8.3 (**Scheme 13**).



20a(+) or 20a(-)

Scheme 13. Enantiomerically enriched ethyl 4-oxothiochromane-2-carboxylate

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2.2. Antileishmanial and Cytotoxic Activities

Synthesized compounds, some precursors and intermediates were evaluated for their in vitro antileishmanial and cytotoxic activities (Table 1), following the methods of Pulido et al. [24]. Amphotericin B was used as a control with EC_{50} and LC_{50} values of 0.05 μM and 56.8 μM, respectively.

Table 1. In vitro antileishmanial and cytotoxic activities

Compound	EC_{50} (μM)[1]	LC_{50} (μM)[1]	SI[2]
3	111.9 ± 22.1	123.5 ± 23.2	1.1
4	**16.9 ± 5.5**	**18.6 ± 1.8**	**1.1**
8a	**6.1 ± 4.6**	**4.0 ± 0.6**	**0.7**
8b	126.0 ± 15.5	351.5 ± 47.5	2.8
8c	ND[3]	6.0 ± 0.4	-
8d	**8.3 ± 0.8**	**25.7 ± 4.6**	**3.1**
8e	29.6 ± 6.2	21.2 ± 3.4	0.7
9a	107.7 ± 34.3	770.5 ± 49.7	7.2
9b	133.6 ±27.0	485.0 ± 45.4	3.6
10	**12.5 ± 3.3**	**51.0 ± 9.0**	**4.1**
11	40.5 ± 5.8	774.1	19.1
13a	ND	54.7 ± 3.2	-
13b	29.7 ± 4.4	9.6 ± 0.4	0.3
13c	37.0 ± 5.6	160.8 ± 27.9	4.3
13d	**9.2 ± 2.7**	**23.9 ± 2.4**	**2.6**
15a	43.9 ± 11.6	>608.9	13.9
15b	37.9 ± 2.8	97.2 ± 14.2	2.6
15c	38.1 ± 11.3	151.3 ± 18.1	4.0
20a	155.3 ± 2.6	132.8 ± 67.7	0.5
20b	291.7 ± 1.4	199.5 ± 74.7	0.3
20c	493.6 ± 0.6	76.3 ± 8.6	0.2
20d	3339.5 ± 0.3	95.2 ± 17.3	0.1
22a	ND	238.8 ± 24.4	< 1.2
22b	ND	231.3 ± 44.5	< 1.0
22c	ND	213.3 ± 47.9	< 2.1
22d	ND	140.5 ± 20.7	< 1.9

24a	216.2 ± 2.5	157.0 ± 9.7	0.7
24b	ND	147.6 ± 45.4	< 2.8
24c	ND	118.7 ± 10.9	< 1.8
26a	28.5 ± 1.5	15.3 ± 0.2	0.5
26b	53.2 ± 0.8	15.4 ± 0.2	0.3
26c	ND	951.9 ± 279.5	< 6.1
27a	505.3 ± 132.6	------	ND
20a (+)	53.5 ± 10.8	33.2 ± 1.4	0.6
20a (-)	369.0 ± 24.2	74.9 ± 52.8	0.2
22a (+)	53.2 ± 10.9	28.9 ± 4.5	0.5
22a (-)	ND	42.2 ± 4.3	<2.0
Amphotericin B	0.05 ± 0.11	56.8 ± 3.9	1136

[1] Results reported as the mean value ± standard deviation of the half-maximum concentration in μM.

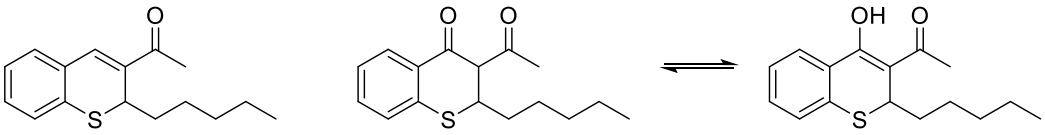
[2] Selectivity Index (IS) = LC_{50}/EC_{50}. Bold data represent compounds with high activity against amastigotes of *L. (V) panamensis.*

[3] ND = not determined

3. Discussion

2H-thiochromenes **8a, 8d**, **and 13d** were the most active compounds, with EC_{50} values lower than 10 uM; also, compound **10d** showed an EC_{50} 12,5 uM, later compound exhibit a keto-enol equilibrium where the enol form is favored.

Compounds **8d** and the enol form **10d** of the ketone **9d** (Figure 1) differ only in the hydroxy group at C4; the high activity of these compounds makes it possible to consider that the hydroxy group contribution to the antileishmanial activity is negligible.



8d **9d** **10d**

keto enol

EC50 = 8.3 μM
CL 50 = 25.7μM

enol form is favored
IC50 = 12.5 μM
CL50 = 51.0 μM

Figure 1. 2H-thiochromenes 8d, 9d, 10d

Except for compound **15a** with a selectivity index of 13.9, 2H-thiochromene compounds displayed low selectivity, In general, the higher the antileishmanial activity, the higher the cytotoxicity.

The compound with the highest activity of the thiochromene series is **8a**. However, its high toxicity makes it poorly selective (IS = 0.67). Compound **9a** bearing a cyano group in C3 displayed low cytotoxicity and low antileishmanial activity, its NMR spectra showed no presence of the enol form indicating that conjugation of the cyano group with the double bond is fundamental for activity and toxicity possibly by nucleophilic attack of Michael-donors at C4.

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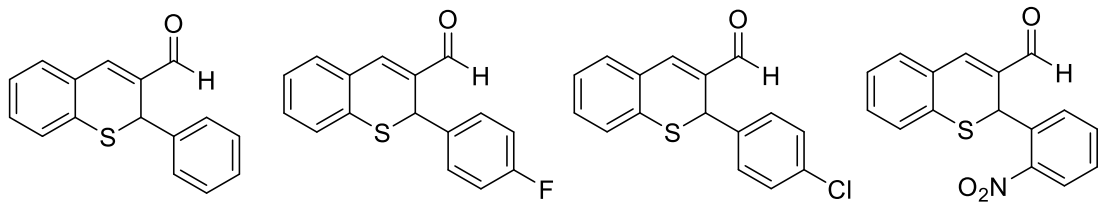

13a	13b	13c	13d
CE_{50} = ND	CE_{50} = 29.7	CE_{50} = 37.0	CE_{50} = 9.25
CL_{50} = 51.8	CL_{50} = 9.6	CL_{50} = 160.7	CL_{50} = 23.9

Figure 2. structure of 2-aryl-2H-thiochomene-3-carbaldehydes 13a-d

Compounds **13a-d** (Figure 2) shares the common formyl group at C3 and an aromatic ring at C2; a chlorine atom in the ***para*** position of the aromatic ring decreases the cytotoxicity considerably (18 times when comparing **13b** and **13c**); the nitro group in the ortho position (compound **13d**) significantly increases the leishmanicidal activity when compared to **13a**. Considering the enhance of activity due to the F, Cl, and NO_2 and that the size of these substituents is quite different, it seems reasonable that the increase in activity is due to an electronic effect, rather than a steric effect.

In general, it is appreciated that the carbonyl group (aldehyde or ketone) C3 favors antileishmanial activity. The conjugation with the C3-C4 double bond increases the electrophilicity at C4, resulting in a Michael type acceptor system, which is generally very reactive toward commons biological nucleophiles as the thiol groups of some proteins or glutathione (trypanothione in parasites). This fact can lead to adverse effects at the cellular level[18]; for this reason, the presence of α,β-unsaturated systems, is used as a warning of possible toxicity[19,20] causing many active compounds to be discarded in early stages of drug development.

The mechanism of toxicity of Michael type acceptors is, in many cases, related to their strong tendency to react with the thiol group of glutathione[21], which is the principal antioxidant agent of animal cells[22]. In the case of leishmania parasites, the antioxidant function is carried out by a similar mechanism that involves the enzyme trypanothione reductase, which is responsible for catalyzing the conversion of trypanothione between its reduced form (thiol) and its oxidized form (disulfide). The reaction of the thiol group of trypanothione with Michael type acceptors such as those present in the compounds of the 2H-thiochromenes series can alter the redox balance of the parasite causing its death.

Michael-type acceptors can also act as inhibitors of cysteine proteases[23], considering that these inhibitors have been reported as a therapeutic target in Leishmania[24–28] it is also possible that the mechanism of action of the compounds of the thiochromenes series is related to the inhibition of cysteine proteases.

In general, the derivatives of the 4-oxothiochromane-2-carboxylic acid, have low antileishmanial activity. However, structure-activity relationships show a strong correlation between electronic ring characteristics and biological activities. Compounds with electron-donating groups on the aromatic ring have an anti-leishmania activity lower than the compounds with electron-withdrawing groups.

On the other hand, ester and amide compounds have higher toxicity; as a consequence, EC_{50} was not calculated. This fact may be an outcome of the chemical nature of the *leishmania* infection, since the enzymatic expression of lyases and esterases during the phagocytosis and burns oxidative causes the hydrolysis of esters and amides of 4-oxothiochromane-2-carboxylic acid. Nevertheless, the performance of the chiral variations, both acid-free and an ester, suggest that the enzymatic hydrolysis reaction is strongly dependent on the asymmetric information of the substrate[29].

Worth highlighting the acyl hydrazone derivatives **26a-c**, which were significantly more active than other derivatives of 4-oxothiochromane-2-carboxylic acid **20a**. This result suggests that these compounds followed a different action mode. Hydrazone derivatives are well known for being an iron-selective metal complex agent. This metal is essential in the *Leishmania* infection progress; for this reason, these compounds may stop the infection through protein inactivation leading to iron

deficiency. However, it would also drive the toxicity mechanism to the host, for the high likelihood of reducing the hemoglobin concentration in blood plasma[30]

In conclusion, in search of new chemotherapeutic agents against leishmaniasis based on the benzothiopyran moiety, we synthesized thirty-three compounds with structural diversity. These compounds possess relevant *in vitro* antileishmanial activity with moderate cytotoxicity. Based on SAR study, compounds **8d** and **10,** which differ only in the hydroxy group at C4, were selected as the most promising compounds among this library with good antiparasitic activity and Selectivity Index. Biological studies with *Leishmania* parasite enzymes need to be performed to identify the potential targets. Structural modification on ring A of **8d** and **9d, 10d** could enhance the antileishmanial activity or reduce cytotoxicity. Overall, **8d** and **9d, 10d** represents a potential hit in the search for new chemotherapeutic agents for the treatment of leishmaniasis.

4. Materials and Methods

4.1. Chemistry

4.1.1. General

All commercially available reagents and solvents were obtained from commercial suppliers and used without further purification. The reaction progress was monitored with thin layer chromatography on silica gel TLC aluminum sheets (Merck, $60F_{254}$). The melting points were determined using an Electrothermal Mel-Temp apparatus and are uncorrected. FTIR spectra were obtained on a Bruker Alpha FTIR spectrometer (Bruker Optic GmbH, Ettlingen. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded using Bruker 300 and 400 spectrometers (300 and 400 MHz for ^{1}H, 75, and 100 MHz for ^{13}C). Chemical shifts were reported relative to internal tetramethyl silane (δ 0.00 ppm) for ^{1}H, and $CDCl_3$ (δ 77.0 ppm) for ^{13}C. HRMS was obtained using Q-TOF quadrupole/orthogonal spectrometry (Waters, Milford, MA) in positive mode (reported as $[M + H]^+$ or $[M + Na]^+$).

4.1.2. Synthesis of the benzothiopyranes and derivatives.

2-(tert-butylthio)benzaldehyde **(3)** In a screw-capped flask equipped with a stir bar, DMSO (2.0mL, 0.028mol, 4 equiv.) and potassium hydroxide (KOH; 470 mg, 8.4 mmol,1.1 equiv.) were mixed under nitrogen and stirred at room temperature. After 30min, the KOH was crushed with a spatula, and 2-methyl-2-propanethiol (610 µL, 0.0106mol, 1.5 eq) was added, and the mixture was stirred for 20min. 2-Chlorobenzaldehyde (810 µL, 7.2 mmol, 1.0 equiv.) was added, and the reaction was heated to 110°C for 90min. After that, the reaction mixture was diluted with water (20mL) and extracted with ethyl acetate (2x30mL). The combined organic layers were washed with water (30mL) and brine (30mL), dried (anhydrous Na_2SO_4), and concentrated on a rotary evaporator the residue was purified by chromatography using a mixture of 10% ethyl acetate in hexanes to afford 2-(tert-butylthio)benzaldehyde 15 980 mg, 70% yield (along with some unreacted 2-Chlorobenzaldehyde. ^{1}H RMN (300 MHz, $CDCl_3$) 10.78 (s, 1H), 7.98 (d, J = 4.3 Hz, 1H), 7.62 – 7.50 (m, 1H), 7.45 – 7.30 (m, 1H), 1.30 (s, 9H). GC-MS: m/z (%) = 194 (27) [M^+], 161 (8), 138 (100), 77 (5).

2,2'-disulfanediyldibenzaldehyde **(4).** In a 25-mL round-bottom flask, 2-(tert-butylthio)benzaldehyde **3** 194 mg, (1.0 mmol, 1 equiv.) was placed in an ice bath. Acetic acid (343 µL, 6 mmol, 6.0 equiv.),48% aqueous HBr (343 µL, 3 mmol, 3.0 equiv.) and DMSO (73µL, 1.0 mmol, 1.0 equiv.) were added to the resulting cooled mixture. The reaction was then allowed to warm to room temperature and then was stirred overnight. The reaction mixture, containing a solid precipitate, was diluted with cold water (2 mL), filtered, dried and purified by column chromatography using 20% ethyl acetate in hexanes as eluent to afford 2,2-disulfanediyldibenzaldehyde **4**. Yield 73%. Mp: 149-151°C. ^{1}H RMN (300 MHz, $CDCl_3$) δ 10.19 (s, 2H), 7.85 (dd, J = 7.5, 1.4 Hz, 2H), 7.75 (d, J = 7.8 Hz, 2H), 7.47 (td, J = 7.7, 1.6 Hz, 2H), 7.37 (dd, J = 10.6, 4.2 Hz, 2H). ^{13}C RMN (75 MHz, $CDCl_3$) δ 192.1, 140.2,

135.0, 134.5, 134.0, 126.8, 126.5. **IR ν:** 1664, 1582, 1555. GC-MS: m/z (%) = 274 (14) [M+], 137 (100), 109 (54). HRMS-ESI (m/z): calcd. for $C_{14}H_{10}O_2S_2$ [M + Na]+ 297.0014, found: 297.0025.

2-mercaptobenzaldehyde **(5).** In a 25 mL round bottom flask, 2,2-disulfanediyl-dibenzaldehyde (**4)** 177 mg (0.65 mmol, 1 equiv.), was dissolved in a mixture of 5.4 mL of DMF, 5.4mL MeOH and 3.0 mL of water (all solvents were deoxygenated before use) then 253 mg (0.975 mmol, 1.5 equiv.) of triphenylphosphine is added, and the mixture is stirred a room temperature for 30 min, After that the reaction mixture was diluted with water (20mL) and extracted with deoxygenated diethyl ether (2x30mL). The combined organic layers were washed with deoxygenated water (30mL) and brine (30mL), dried (anhydrous Na_2SO_4), and concentrated on a rotary evaporator the residue was purified by chromatography using a mixture of 10% ethyl acetate in hexanes to afford 2-mercaptobenzaldehyde **(5)**, in 73% yield. During the whole process, it was avoided the presence of air in the mixture to prevent oxidation to the disulfide.

^{1}H RMN (300 MHz, Chloroform) δ 10.02 (s, 1H), 7.73 (dd, *J* = 8.5, 7.7 Hz, 1H), 7.38 – 7.20 (m, 3H), 5.48 (s, 1H).

2H-thiochromene-3-carbonitrile **(8a).** In a round-bottom flask were placed 2,2-disulfanediyl-dibenzaldehyde (**4**) 137 mg (0.5 mmol, 1 equiv.), acrylonitrile 58 mg (0.55 mmol, 2.2 equiv.) and DBU 84 mg (0.55 mmol, 1.1 equiv.) under nitrogen gas, and heated with stirring at 80°C for 24 h, After cooling to room temperature, the crude reaction mixture was then purified by column chromatography to afford **7a** as a white solid (77 mg, 45%)**.**

Alternatively, 2H-thiochromene-3-carbonitrile (**7a**) was prepared by a different methodology, as follows. Step 1. In a screw-capped flask, 2,2-disulfanediyl-dibenzaldehyde **(4)** 70 mg (0.25 mmol, 1 equiv.); triphenylphosphine 131 mg (0.5 mmol, 2 equiv.) were dissolved in THF, the mixture was stirred at room temperature under argon atmosphere, after 20 min and excess of acrylonitrile (80 mg, 1.5 mmol, 6 equiv.) was added and after another 10 min of reaction a TLC plate showed the formation of 2-mercaptobenzaldehyde (**5**) and the mixture of stereoisomers of 4-hydroxythiochromane-3-carbonitrile. The reaction mixture is stirred overnight, and after that time, the only products are 4-hydroxythiochromane-3-carbonitrile.

Step 2. The mixture of 4-hydroxythiochromane-3-carbonitrile is heated until reflux in dichloroethane with 20 % mol of Amberlyst 15 overnight after that, the solvent was evaporated in a rotary evaporator and diluted in the minimum amount of CH_2CL_2 and purified by column chromatography purified by chromatography using a mixture of 20% ethyl acetate in hexanes to afford 2H-thiochromene-3-carbonitrile **7a** in 68 % yield (calculated from the dimer). **mp.** 96-98°C. ^{1}H RMN (400 MHz, $CDCl_3$) δ 7.30 – 7.19 (m, *J* = 7.5 Hz, 2H), 7.20 – 7.10 (m, 3H), 3.57 (s, 2H). ^{13}C RMN (101 MHz, $CDCl_3$) δ 142.4, 132.9, 131.1, 130.3, 130.3, 127.7, 126.5, 118.5, 103.9, 26.0. **IR ν**: 2207, 1615, 1435, 753. HRMS-ESI (m/z): calcd. for $C_{10}H_7NS$ [M + H]+ 174.0372, found: 174.0380.

Methyl 2H-thiochromene-3-carboxylate **(8b).** In a screw-capped flask, 2,2-disulfanediyldibenzaldehyde (**4)**137 mg (0.5 mmol, 1 equiv.), methylacrylate 130 mg (1.5 mmol, 3.0 equiv.) and DBU 230 mg (1.5 mmol, 3.0 equiv.) were mixed under argon atmosphere and then heated to 80°C for 24 h, After cooling to room temperature the reaction mixture was dissolve with CH_2Cl_2 and poured directly to the column chromatography using 10% ethyl acetate in hexanes as eluent to afford methyl 2H-thiochromene-3-carboxylate in 48% yield as a yellowish solid with mp. 34-35°C. ^{1}H RMN (400 MHz, $CDCl_3$) δ 7.55 (s, 1H), 7.30 – 7.16 (m, 3H), 7.13 (d, *J* = 7.4 Hz, 1H), 3.84 (s, 3H), 3.73 (s, 2H). ^{13}C RMN (100 MHz, $CDCl_3$) δ 166.4, 137.3, 134.0, 131.3, 130.6, 130.2, 127.1, 125.8, 123.0, 52.2, 24.0. **IR ν:** 2952, 1703, 1434, 1235, 751. HRMS-ESI (m/z): calcd. for $C_{11}H_{10}O_2S$ [M + H]+ 207.0474, found: 207.0185.

1-(2-phenyl-2H-thiochromen-3-yl)ethan-1-one **(8c).** In a 10 mL round bottom flask equipped with a reflux condenser were added (*E*)-4-phenylbut-3-en-2-one (146 mg, 1.0 mmol) and 2-(tert-butylthio)benzaldehyde **3** (250 mg, 1.3 mmol) to this mixture was added 2.0 mL of a concentrated

hydrochloric acid, 12M, and the mixture was placed in a preheated oil bath at 110°C and held for two hours, thereafter 10 mL of water was added and the mixture was extracted with dichloromethane 3x15 mL, the combined organic layers were washed with saturated $NaHCO_3$ solution. After drying over Na_2S0_4, the solvent was evaporated, and the residue was purified by column chromatography to yield 106 mg of 1-(2-phenyl-2H-thiochromen-3-yl)ethan-1-one **27**, 40% as a yellowish solid. M. p: 115-116°C. ^{1}H RMN (400 MHz, $CDCl_3$) δ 7.67 (s, 1H), 7.38 (d, *J* = 7.8 Hz, 1H), 7.25 (dd, *J* = 3.9, 2.8 Hz, 3H), 7.21 – 7.13 (m, 5H), 5.36 (d, *J* = 1.6 Hz, 1H), 2.49 (s, 3H). ^{13}C RMN (101 MHz, $CDCl_3$) δ 196.5, 142.0, 137.4, 134.0, 132.8, 131.1, 130.7, 130.4, 128.5, 127.6, 127.6, 126.5, 125.7, 38.8, 25.6. **IR ν:** 3031, 1656, 1622, 1196, 900. HRMS-ESI (m/z): calcd. for $C_{17}H_{14}OS$ $[M + H]^+$ 267.0838, found: 267.0345.

1-(2-pentyl-2H-thiochromen-3-yl)ethan-1-one **(8d).** In a 10 mL round bottom flask were added 2,2-disulfanediyldibenzaldehyde **4** (140 mg, 0.5 mmol), 2-nonanono, (145 mg, 1.0 mmol) and triphenylphosphine (262 mg, 1.0 mmol), were dissolved in THF and the mixture was stirred under argon atmosphere at room temperature overnight. Whereupon column chromatography of the crude gave 195 mg (70%) of the mixture of stereoisomers of 1-(4-hydroxy-2-pentylthiochroman-3-yl)ethan-1-one. Later 140 mg of the isomeric alcohols was dissolved in THF and Amberlyst 15 in a 10 mL round bottom flask equipped with a reflux condenser and the mixture was heated to reflux overnight during 20 h after cooling to room temperature the reaction mixture was dissolve with CH_2Cl_2 and the crude was purified by column chromatography to afford 40 mg of 1-(2-pentyl-2H-thiochromen-3-yl)ethan-1-one **8d** (29%) as a yellowish oil. ^{1}H RMN (400 MHz, CDCl3) δ 7.45 (s, 1H), 7.37 (d, *J* = 8.0 Hz, 1H), 7.34 – 7.24 (m, 2H), 7.20 (td, *J* = 7.3, 1.4 Hz, 1H), 4.11 (dd, *J* = 8.0, 5.6 Hz, 1H), 2.52 (s, 3H), 1.47 (dt, *J* = 10.8, 3.7 Hz, 2H), 1.35 – 1.15 (m, 6H), 0.88 (t, *J* = 6.8 Hz, 3H^{13}C NMR (75 MHz, Chloroform-*d*) δ 196.8, 136.4, 133.3, 130.8, 130.6, 128.1, 125.5, 36.2, 34.4, 31.2, 25.7, 25.5, 22.5, 14.1. **IR ν:** 2927, 1635, 1407, 1388, 756. HRMS-ESI (m/z): calcd. for $C_{16}H_{20}OS$ $[M + H]^+$ 261.1308, found: 261.1320.

Note: Chemical shift at 130.6 and 133.3 each correspond to two overlapped peaks.

4-oxothiochromane-3-carbonitrile **(9a).** According with the step 1 of the procedure for the synthesis of compound **8a** we prepared a mixture of stereoisomers of 4-hydroxythiochromane-3-carbonitrile, this mixture, (191 mg, 1.0 mmol) were dissolved in 5.0 mL of dichloromethane and mixed with the Dess-Martin periodinane reagent (430 mg, 1 mmol). After 1 h of stirring at room temperature the crude mixture was purified by column chromatography to afford the desired 4-oxothiochromane-3-carbonitrile **9a** (60 mg, 30%) as a white solid with mp: 79-81°C. ^{1}H RMN (300 MHz, $CDCl_3$) δ ^{1}H RMN (300 MHz, $CDCl_3$) δ 8.17 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.61 – 7.44 (m, 1H), 7.42 – 7.21 (m, 2H), 4.18 (dd, *J* = 11.6, 3.7 Hz, 1H), 3.67 (dd, *J* = 13.5, 11.7 Hz, 1H), 3.57 – 3.40 (m, 1H). ^{13}C RMN (75 MHz, $CDCl_3$) δ 184.6, 141.0, 134.7, 130.5, 128.9, 127.7, 126.0, 115.1, 41.8, 29.4. **IR ν:** 2918, 2256, 1678, 1582, 1430. HRMS-ESI (m/z): calcd. for $C_{10}H_7NOS$ $[M + Na]^+$ 212.0141, found: 212.0153.

Methyl 4-oxothiochromane-3-carboxylate **(9b, 10b)** In a screw-capped flask, 2,2-disulfanediyl-dibenzaldehyde **4** 70 mg (0.25 mmol, 1 equiv.); triphenylphosphine 131 mg (0.5 mmol, 2 equiv.), were dissolved in THF, the mixture was stirred at room temperature under argon atmosphere, after 20 min and excess of acrylonitrile (80 mg, 1.5 mmol, 6 equiv.) was added and after another 10 min of reaction a TLC plate showed the formation of 2-mercaptobenzaldehyde **5** and the mixture of isomers of 3-Cyano-4-hydroxy-2H-thiochroman. The reaction mixture was stirred overnight, and after that, the only products are the mixture of stereoisomers of 3-Cyano-4-hydroxy-2H-thiochroman. 112 mg of the above mixture was dissolved in 5.0 mL of dichloromethane and mixed with the Dess-Martin periodinane reagent (430 mg, 1 mmol). After 1 h of stirring at room temperature, the crude mixture was purified by column chromatography to afford 40 mg, 36% of the desired methyl 4-oxothiochromane-3-carboxylate as a white solid with mp: 85-86°C. The methyl 4-oxothiochromane-3-carboxylate exists in solution in equilibria with its tautomeric enol form methyl 4-hydroxy-2H-thiochromene-3-carboxylate.

^{1}H NMR spectrum of **9b** in $CDCl_3$ revealed the existence of the tautomer **9b** with **10b** in 1:5 ratio. Proton H5 appears as a doublet at 8.17 ppm, *J* = 7.9 Hz, the proton H5 of the enol form has a chemical shift of 7.89 d , J = 7.7 Hz the integration areas are 0.2 and 1.0 respectively, which indicates that the enol form corresponds to 83% of the mixture. The same ratio can be calculated with the protons of the methoxy group of the ester.

^{1}H RMN (300 MHz, $CDCl_3$) δ 12.71[a] (s), 8.17[b] (d, *J* = 7.9 Hz), 7.89[a] (d, *J* = 7.7 Hz), 7.44[b] (t, *J* = 7.6 Hz), 7.37 – 7.19[a,b] (m), 3.89[a] (s), 3.85[b] (s), 3.76[a] (s,), 3.38[b] (dd, *J* = 13.5, 3.6 Hz)

[a] is the enol form

[b] is the keto form

^{13}C RMN (75 MHz, $CDCl_3$) δ 171.6, 165.9, 137.2, 131.0, 129.8, 129.2, 127.3, 126.8, 125.6, 125.3, 93.7, 77.5, 77.1, 76.7, 54.0, 52.7, 52.1, 23.3.

IR ν: 3012, 2951, 1718, 1681, 1645, 1608, 1583, 1553, 1437. HRMS-ESI (m/z): calcd. for $C_{11}H_{10}O_3S$ $[M + Na]^+$ 245.0243, found: 245.0254.

1-(4-hydroxy-2-pentyl-2H-thiochromen-3-yl)ethan-1-one **(9d, 10d)** In a 10 mL round bottom flask were added 2,2-disulfanediyldibenzaldehyde **17** (140 mg, 0.5 mmol), 2-nonanono, (145 mg, 1.0 mmol) and triphenylphosphine (262 mg, 1.0 mmol), and the mixture was stirred under argon atmosphere at room temperature overnight. Whereupon column chromatography of the crude gave 195 mg (70%) of the mixture of stereoisomers of 1-(4-hydroxy-2-pentylthiochroman-3-yl)ethan-1-one. Later, 90 mg of the above mixture were dissolved in 5.0 mL of dichloromethane and mixed with the Dess-Martin periodinane reagent (192 mg, 0.45 mmol) and 1.0 mL of water. After 1 h of stirring at room temperature the crude mixture was purified by column chromatography to afford 55 mg, 60% of the desired 3-acetyl-2-pentylthiochroman-4-one as yellowish oil. The 3-acetyl-2-pentylthiochroman-4-one exists in solution only as its enol form 1-(4-hydroxy-2-pentyl-2H-thiochromen-3-yl)ethan-1-one.

^{1}H RMN (400 MHz, $CDCl_3$) δ 7.97 (ddd, *J* = 7.9, 1.4, 0.5 Hz, 1H), 7.36 – 7.25 (m, 2H), 7.22 (ddd, *J* = 7.9, 7.0, 1.6 Hz, 1H), 3.67 (dd, *J* = 9.6, 5.0 Hz, 1H), 2.31 (s, *J* = 1.8 Hz, 3H), 1.77 – 1.65 (m, 1H), 1.63 – 1.43 (m, 2H), 1.36 – 1.09 (m, 5H), 0.85 (t, *J* = 7.0 Hz, 3H). ^{13}C RMN (101 MHz, MeOH) δ 195.4, 174.0, 135.8, 132.0, 129.5, 128.2, 127.7, 125.5, 108.8, 39.6, 36.4, 31.1, 26.6, 24.4, 22.5, 14.0. **IR ν:** 2928, 2856, 1634, 1593, 1545, 1380. HRMS-ESI (m/z): calcd. for $C_{16}H_{20}O_2S$ $[M + Na]^+$ 299.1076, found: 299.1088.

6H,12H-6,12-epoxydibenzo[b,f][1,5]dithiocine **(11).** In a 10 mL round bottom flask equipped with a reflux condenser containing 3.0 mL of concentrated hydrochloric acid, 12M, was added 2-(tert-butylthio)benzaldehyde **3** (582 mg, 3.0 mmol). Thus, the mixture was placed in a preheated oil bath at 110°C and held for two hours, after that 10 mL of water was added and the mixture was extracted with dichloromethane 3x15 mL, the combined organic layers were washed with saturated $NaHCO_3$ solution. After drying over Na_2S0_4, the solvent was evaporated, and the residue was purified by column chromatography to yield 282 mg, 72% of 6H,12H-6,12-epoxydibenzo[b,f][1,5]dithiocine with m.p 160-161°C. ^{1}H RMN (300 MHz, $CDCl_3$) δ 7.36 – 7.31 (m, 1H), 7.17 – 7.05 (m, 3H), 6.40 (s, 1H). ^{13}C RMN (75 MHz, $CDCl_3$) δ 132.7, 129.0, 128.3, 127.9, 125.2, 74.7. **IR ν:** 3051, 1433, 1262, 1083, 956, 726. GC-MS: m/z (%) = 258 (40) [M^+], 153 (100), 121 (15), 77 (28).

4.1.3. General procedure for the synthesis of 2-aryl-2H-thiochromene-3-carbaldehydes, 13a-d.

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In a 10 mL round bottom flask equipped with a reflux condenser were added the corresponding cinnamaldehyde (1.0 mmol) and 2-(tert-butylthio)benzaldehyde (**3**) (1.3 mmol) to this mixture was added 2.0 mL of concentrated hydrochloric acid, 12M. Then, the mixture was placed in a preheated oil bath at 110°C and held for two hours, thereafter 10 mL of water was added and the mixture was extracted with dichloromethane 3x15 mL, the combined organic layers were washed with saturated $NaHCO_3$ solution. After drying over Na_2S0_4, the solvent was evaporated, and the residue was purified by column chromatography to yield the corresponding 2-aryl-2H-thiochromene-3-carbaldehyde **13a-d**.

2-phenyl-2H-thiochromene-3-carbaldehyde **(13a).** Starting from cinnamaldehyde, according to the general procedure described above to yield 175 mg of 2-phenyl-2H-thiochromene-3-carbaldehyde **13a**, 70% as a yellowish solid with m.p: 195-196°C. ^{1}H RMN (200 MHz, $CDCl_3$) δ 9.70 (s, 1H), 7.55 (s, 1H), 7.43 (m, 1H), 7.35 – 7.19 (m, 5H), 7.10 (s, 1H), 4.80 (s, 1H). **IR ν:** 3040, 2820, 1660, 1622, 1139. HRMS-ESI (m/z): calcd. for $C_{16}H_{12}OS$ $[M + Na]^+$ 275.0501, found: 275.0510.

2-(4-fluorophenyl)-2H-thiochromene-3-carbaldehyde **(13b).** Starting from 4-Fluorocinnamaldehyde (150mg, 1.0 mmol) according to the general procedure described above to yield 2-(4-fluorophenyl)-2H-thiochromene-3-carbaldehyde (160 mg, 59% yield) as a yellow solid with m.p 96-98°C. ^{1}H RMN (400 MHz, $CDCl_3$) δ 9.66 (s, 1H), 7.47 (s, 1H), 7.42 (d, *J* = 7.6 Hz, 1H), 7.35 – 7.25 (m, 2H), 7.24 – 7.12 (m, 3H), 6.86 (dd, *J* = 12.6, 4.8 Hz, 2H), 5.19 (s, 1H). ^{13}C RMN (101 MHz, $CDCl_3$) δ 191.3, 162.5 (d, *J* = 246.5 Hz), 145.4, 137.7, 134.9, 134.0, 132.1, 131.3, 129.9, 128.4 (d, *J* = 8.2 Hz), 127.9, 126.2, 115.7 (d, *J* = 21.6 Hz), 37.8. **IR ν:** 3050, 2826, 1665, 1625, 1503, 1139, 845. HRMS-ESI (m/z): calcd. for $C_{16}H_{11}FOS$ $[M + Na]^+$ 293.0407, found: 293.0416.

2-(4-chlorophenyl)-2H-thiochromene-3-carbaldehyde **(13c).** Starting from 4-chlorocinnamaldehyde (174 mg, 1.0 mmol) according to the general procedure described above to yield 2-(4-chlorophenyl)-2H-thiochromene-3-carbaldehyde (250 mg, 87% yield) as a yellow solid with m.p 119-120°C. ^{1}H RMN (400 MHz, $CDCl_3$) δ 9.67 (s, 1H), 7.48 (s, 1H), 7.42 (d, *J* = 7.4 Hz, 1H), 7.34 – 7.25 (m, 2H), 7.21 (td, *J* = 7.3, 1.1 Hz, 1H), 7.17 – 7.11 (m, 4H), 5.17 (s, 1H). ^{13}C RMN (101 MHz, $CDCl_3$) δ 191.1, 145.3, 140.3, 140.1, 134.5, 133.6, 132.0, 131.1, 129.7, 128.8, 127.9, 127.7, 126.1, 37.7. **IR ν:** 2845, 1667, 1627, 1580. 1138, 761. HRMS-ESI (m/z): calcd. for $C_{16}H_{11}ClOS$ $[M + Na]^+$ 309.0111, found: 309.0120.

2-(2-nitrophenyl)-2H-thiochromene-3-carbaldehyde **(13d).** Starting from 2-nitrocinnamaldehyde (1.0 mmol) according to the general procedure described above to yield 2-(4-chlorophenyl)-2H-thiochromene-3-carbaldehyde (410 mg, 69% yield) as a yellow solid with m.p 130-132°C. ^{1}H RMN (400 MHz, CDCl3) δ 9.66 (d, *J* = 1.7 Hz, 1H), 8.01 (d, *J* = 7.1 Hz, 1H), 7.67 (s, 1H), 7.45 (d, *J* = 7.4 Hz, 1H), 7.35 (p, *J* = 7.5 Hz, 2H), 7.29 (d, *J* = 7.5 Hz, 1H), 7.26 – 7.15 (m, 2H), 7.10 (d, *J* = 7.1 Hz, 1H), 6.01 (s, 1H). ^{13}C RMN (101 MHz, $CDCl_3$) δ 190.7, 147.1, 146.4, 135.8, 133.9, 133.5, 133.2, 132.3, 131.2, 129.2, 128.5, 128.4, 127.8, 126.2, 125.9, 33.4. **IR ν:** 2992, 1661, 1623, 1519, 1142, 740. GC-MS: m/z (%) = 297 (4) $[M^+]$, 280 (43), 251 (60), 235 (44), 221 (100.). HRMS-ESI (m/z): calcd. for $C_{16}H_{11}NO_3S$ $[M + Na]^+$ 320.0352, found: 320.0362.

phenyl(2-phenyl-2H-thiochromen-3-yl)methanone **(15a).** In a 10 mL round bottom flask equipped with a reflux condenser were added chalcone (1 mmol), 2-(tert-butylthio)benzaldehyde (**3**) (1.5 mmol) and *p*-toluenesulfonic acid monohydrate (60 mg) in toluene (2 mL). The reaction mixture was stirred at reflux temperature for 2 h and progress of the reaction was monitored with TLC. Upon the consumption of chalcone, the reaction mixture was directly subjected to column chromatography to afford the desired compound as a yellow solid (45 mg, 46%) mp. 151-153°C. ^{1}H RMN (400 MHz, $CDCl_3$) δ 7.71 (dd, *J* = 8.3, 1.3 Hz, 2H), 7.57 (ddd, *J* = 6.7, 2.7, 1.3 Hz, 1H), 7.51 – 7.44 (m, 2H), 7.41 (s, 1H), 7.33 – 7.18 (m, 8H), 7.14 (tdd, *J* = 7.7, 5.9, 1.6 Hz, 1H), 5.49 (s, 1H). ^{13}C RMN (101 MHz, $CDCl_3$) δ 195.9, 142.1, 140.2, 138.3, 133.4, 132.9, 132.1, 131.3, 131.0, 130.6, 129.4, 128.9, 128.6, 127.9, 127.8, 126.8, 125.9, 40.4. **IR ν:** 3126, 2918, 1640, 1596, 1488, 1087, 756. HRMS-ESI (m/z): calcd. for $C_{22}H_{16}OS$ $[M + H]^+$ 329.0995, found: 329.1002.

4.1.4. General procedure for the synthesis of (4-chlorophenyl)(2-(aryl)-2H-thiochromen-3-yl)methanones, 15b,c.

In a 10 mL round bottom flask equipped with a reflux condenser were mixed 4'-chloroacetophenone (155mg, 1.0 mmol) and the substituted benzaldehyde (1.0 mmol) with Amberlyst-15 (250 mg) in toluene (5 mL). The reaction mixture was stirred at room temperature for

two h, and the progress of the reaction was monitored with TLC until the formation of the corresponding chalcone. Afterward 2-(tert-butylthio)benzaldehyde (**3**) was added, and the mixture was allowed to react for another 2 hours at 110°C and then cooled to room temperature and subjected to column chromatography to afford the desired (4-chlorophenyl)(2-(aryl)-2H-thiochromen-3-yl)-methanones**, 15b,c.**

(4-chlorophenyl)(2-(4-(trifluoromethyl)phenyl)-2H-thiochromen-3-yl)methanone **(15b).** Following the general procedure described above, starting from 4-(trifluoromethyl)benzaldehyde (175 mg, 1 mmol) to afford the desired **15b** as a yellow solid (200 mg, 46%) %) mp. 105-106°C. ^{1}H RMN (400 MHz, $CDCl_3$) δ 7.66 (d, *J* = 8.6 Hz, 2H), 7.47 (d, *J* = 8.5 Hz, 4H), 7.43 (s, 1H), 7.37 (d, *J* = 8.2 Hz, 2H), 7.28 (t, *J* = 7.5 Hz, 3H), 7.18 (ddd, *J* = 7.2, 5.3, 3.7 Hz, 1H), 5.43 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.4, 145.6, 141.0, 138.7, 136.1, 132.33, 132.26, 131.7, 131.2, 130.8, 130.2, 129.9 (q, *J* = 32.7 Hz), 129.0, 127.9, 127.0, 126.3, 125.8 (q, *J* = 3.9 Hz), 123.8 (q, *J* = 270.4 Hz), 40.0. **IR ν:** 2952, 1612, 1585, 1323, 1114. HRMS-ESI (m/z): calcd. for $C_{23}H_{14}ClF_3OS$ $[M + Na]^+$ 453.0298, found: 453.0304.

(4-chlorophenyl)(2-(4-nitrophenyl)-2H-thiochromen-3-yl)methanone **(15c).** Following the general procedure described above, starting from 4-nitrobenzaldehyde (152 mg, 1 mmol to afford the desired **15c** as a yellow solid (180 mg, 45%) %) mp. 93-94°C. ^{1}H RMN (400 MHz, $CDCl_3$) δ 8.07 (d, *J* = 8.7 Hz, 2H), 7.66 (d, *J* = 8.4 Hz, 2H), 7.52 – 7.38 (m, 5H), 7.34 – 7.25 (m, 3H), 7.22 – 7.16 (m, 1H), 5.42 (s, 1H). ^{13}C RMN (75 MHz, $CDCl_3$) δ ^{13}C RMN (101 MHz, $CDCl_3$) δ 194.3, 148.9, 147.4, 141.3, 138.9, 136.3, 135.9, 132.0, 131.9, 131.4, 130.8, 130.2, 129.1, 128.1, 127.6, 126.6, 124.2, 40.1. **IR ν:** 3026, 2849, 1656, 1607, 1585, 1515, 1338, 821. HRMS-ESI (m/z): calcd. for $C_{22}H_{14}ClNO_3S$ $[M + Na]^+$ 408.0456, found: 408.0461.

4.1.5. General procedure for the synthesis of the 4-oxothiochromane-2-carboxylic acids 20a-d

A round bottom flask equipped with a magnetic stirrer was loaded with a mixture of anhydride maleic 1.718g (17.5mmol) and thiophenols 2,0 mL (19.3mmol) in acetonitrile dry and then ten triethylamine drops were slowly added. The reaction flask was closed with a glass-stopper and stirred at 50°C for 2h. The reaction was quenched for room temperature, the solvent was removed under reduced pressure, and the black oil residue was cooled at 0°C in bath ice, and re-dissolved with DCM dry, after that a significant excess of $AlCl_3$ was added. The mixture reaction was stirred at room temperature overnight. After the reaction was completed as determinate by TLC, the mixture reaction was treated with a cold solution of chloride acid to 5 % and extracted with CH_2Cl_2 (3x25 mL) for three times. The combined organic layers were dried over anhydrous Na_2SO_4, the residue after solvent evaporated was filtered through silica gel column using mobile phase hexanes /ethyl acetate with 5 % of acetic acid as an additive (80:20 v/v) to give pure the compound set **20a-d** with 55-70 % global yield.

4-oxothiochromane-2-carboxylic acid **(20a).** Following the general procedure described above. White solid, m.p = 151-152 °C. ^{1}H NMR (300 MHz, DMSO-d6) δ 7.95 (dd, J = 7.9, 1.5 Hz, 1H), 7.49 (td, J = 7.6, 1.5 Hz, 1H), 7.35 (d, J = 7.8 Hz, 1H), 7.25 (t, J = 7.6 Hz, 1H), 4.39 (dd, J = 6.1, 4.3 Hz, 1H), 3.19 – 2.97 (m, 2H). ^{13}C NMR (75 MHz, DMSO) δ 192.63, 172.14, 138.99, 134.25, 130.52, 128.37, 127.82, 125.94, 41.71, 41.26. **IR** ν (cm^{-1}) = 2918.37, 1695.44, 1681.12, 881.15, 768.35. HRMS-ESI (m/z): calcd. for $C_{10}H_8O_3S$ $[M+H]^+$ 209.0267, found 209.0282.

6-fluoro-4-oxothiochromane-2-carboxylic acid **(20b)**. Following the general procedure described above. White solid, m.p = 135-137°C. ^{1}H NMR (300 MHz, DMSO-d6) δ 7.66 (d, J = 11.0 Hz, 1H), 7.44 (s, 1H), 7.23 (t, J = 8.8 Hz, 1H), 4.40 (t, J = 5.1 Hz, 1H), 3.10 (t, J = 4.9 Hz, 2H).^{13}C NMR (75 MHz, DMSO) δ 191.92, 172.33, 162.10, 158.86, 134.47, 127.97, 122.07, 116.53, 114.27, 41.62, 40.92. **IR** ν (cm^{-1}) = 3498.15, 2895.35, 1723.95, 1684.65, 805.21, 238.57. HRMS-ESI (m/z): calcd. for $C_{10}H_7O_3FS$ $[M+H]^+$ 227.0173, found 227.0173.

6-methoxy-4-oxothiochromane-2-carboxylic acid **(20c).** Following the general procedure described. White solid, m.p = 165-166 °C ^{1}H NMR (300 MHz, DMSO-d6) δ 7.45 (d, J = 2.8 Hz, 1H), 7.27 (d, J = 8.7 Hz, 1H), 7.12 (d, J = 8.7 Hz, 1H), 3.77 (s, 3H), 3.15 - 2.96 (m, 2H). ^{13}C NMR (75 MHz, DMSO) δ 192.60, 172.21, 157.64, 131.43, 129.80, 129.28, 122.05, 111.46, 55.83, 41.70, 40.44. **IR** ν (cm^{-1}) =2888.72, 1714.93, 1626.73, 881.75, 807.73. HRMS-ESI (m/z): calcd. for $C_{11}H_{10}O_4S$ $[M+H]^+$ 239.0373, found 239.0387.

7-methoxy-4-oxothiochromane-2-carboxylic acid **(20d).** Following the general procedure described above. White solid, m.p = 162-164 °C ^{1}H NMR (300 MHz, Acetone-d6) δ 7.99 (d, J = 8.6 Hz, 1H), 6.83 (d, J = 2.4 Hz, 1H), 6.83 -6.78 (m, 1H), 4.36 (dd, J = 6.4, 4.7 Hz, 1H), 3.88 (s, 3H), 3.08 (dd, J = 5.6, 3.5 Hz, 2H). ^{13}C NMR (75 MHz, Acetone-d6) δ 190.56, 170.91, 163.51, 141.17, 130.44, 124.13, 112.67, 110.55, 55.29, 41.97, 40.89. **IR** ν (cm^{-1}) = 3419.98, 2906.14, 1698.81, 1671.88, 870.22, 824.56. HRMS-ESI (m/z): calcd. for $C_{11}H_{10}O_4S$ $[M+H]^+$ 239.0373, found =239.0375.

4.1.6. General procedure for the synthesis of the esters from 4-oxothiochromane-2-carboxylic acids 22a-d

A mixture of 4-oxothiochromane-2-carboxylic acid **(20a)** 100 mg (0.48 mmol), N,N´dicyclohexylcarbodiimide (DCC) 150 mg and small amount of 4-dimethylaminopyridine (DMAP) dissolved in acetonitrile (ACN) was heated under microwave irradiation at 70 °C by 20 minutes, then 1.3 equivalents of the aliphatic alcohol were added, after that the reaction vial was sealed and heated to 70°C for 40 minutes. When the reaction was completed determinate by TLC, the reaction mixture was diluted with ethyl acetate and washed with brine (2 x 20mL). The combined organic layers were dried over anhydrous Na_2SO_4, the residue after solvent evaporated was purified through flash chromatography using hexanes /ethyl acetate (80:20 v/v) to give **22a-d** in 65-80 % yield.

ethyl 4-oxothiochromane-2-carboxylate **(22a).** Yellowish oil - ^{1}H NMR (300 MHz, Chloroform-d) δ 8.14 (d, *J* = 9.2 Hz, 1H), 7.42 (d, *J* = 8.7 Hz, 1H), 7.31 – 7.19 (m, 2H), 4.27 – 4.12 (m, 3H), 3.21 (d, *J* = 6.5 Hz, 1H), 1.25 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, CDCl3) δ 192.24, 169.85, 138.45, 133.71, 130.45, 128.85, 127.37, 126.22, 125.77, 77.55, 77.13, 76.70, 62.27, 42.34, 41.34, 13.97. **IR** ν (cm^{-1}) = 2981.39, 1731.29, 1684.23, 761.18. HRMS-ESI (m/z): calcd. for $C_{12}H_{12}O_3S$ $[M+H]^+$ 237.0580, found 237.0592.

butyl 4-oxothiochromane-2-carboxylate **(22b).** Yellowish oil ^{1}H NMR (300 MHz, Chloroform-d) δ 8.14 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.43 (ddd, *J* = 8.0, 7.2, 1.6 Hz, 1H), 7.34 – 7.18 (m, 2H), 4.24 – 4.04 (m, 3H), 3.21 (dd, *J* = 5.6, 1.6 Hz, 2H), 1.66 – 1.45 (m, 2H), 1.40 – 1.22 (m, 2H), 0.90 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (75 MHz, CDCl3) δ 192.18, 169.94, 141.93, 138.49, 137.47, 133.68, 132.32, 130.47, 129.25, 129.14, 128.84, 128.69, 128.30, 127.36, 125.74, 77.55, 77.12, 76.70, 67.08, 66.06, 42.46, 41.34, 30.38, 19.16, 18.94, 13.71, 13.63. **IR** ν (cm^{-1}) = 2960.28, 2933.24, 1731.63, 1683.60, 758.76. HRMS-ESI (m/z): calcd. for $C_{14}H_{16}O_3S$ $[M+Na]^+$ 287.0712, found 287.0709.

hexyl 4-oxothiochromane-2-carboxylate **(22c**). Yellowish oil ^{1}H NMR (300 MHz, Chloroform-d) δ 8.13 (d, *J* = 7.9 Hz, 1H), 7.41 (t, *J* = 7.5 Hz, 1H), 7.28 – 7.16 (m, 2H), 4.14 (q, *J* = 6.5, 5.8 Hz, 3H), 3.20 (d, *J* = 5.8 Hz, 2H), 1.57 (p, J = 6.4 Hz, 3H), 1.25 (s, 6H), 0.88 (t, J = 6.0 Hz, 3H). ^{13}C NMR (75 MHz, CDCl3) δ 192.14, 169.95, 138.48, 133.67, 130.45, 128.81, 127.33, 125.72, 66.33, 42.42, 41.30, 31.31, 28.34, 25.35, 22.49, 14.01. **IR** ν (cm^{-1}) = 3056.06, 2959.62, 1730.43, 1684.96, 738.80. HRMS-ESI (m/z): calcd. for $C_{16}H_{20}O_3S$ $[M+Na]^+$ 315.1025, found 315.1005

decyl 4-oxothiochromane-2-carboxylate **(22d).** Yellowish oil ^{1}H NMR (300 MHz, Chloroform-d) δ 8.14 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.43 (td, *J* = 7.7, 1.6 Hz, 1H), 7.31 – 7.20 (m, 2H), 4.19 – 4.11 (m, 3H), 3.25 – 3.18 (m, 2H), 1.58 (d, *J* = 6.6 Hz, 2H), 1.27 (d, *J* = 5.7 Hz, 16H), 0.95 – 0.87 (m, 3H).^{13}C NMR (75 MHz, CDCl3) δ 192.15, 169.94, 138.50, 133.67, 130.47, 128.85, 127.34, 125.73, 77.53, 77.11, 76.68, 66.36, 42.45,

41.32, 31.91, 29.53, 29.48, 29.33, 29.17, 28.40, 25.70, 22.71, 14.16. **IR** ν (cm^{-1}) = 2954.83, 2925.62, 1734.22, 1686.36, 759.28. HRMS-ESI (m/z): calcd. for $C_{20}H_{28}O_3S$ $[M+H]^+$ 349.1832, found 349.1864

4.1.7. General procedure for the synthesis of the -4-oxothiochromane-2-carboxamides 24a-c

A mixture of 1.0g (4.8 mmol), 4-oxothiochromane-2-carboxylic acid (1a) in DCM anhydrous, at 0 °C was added a small amount of DMF, followed by 10.0 mmol oxalyl chloride. Once the effervesce was stopped, the solution was quenched to room temperature for 2h. FT-IR analyses have been determined that the acid chloride was formed as a brownish oil since fermi resonance was observed. On the other hand, other solution of 10.0 mmol amines in DCM (5mL) and 5 ml of NaOH (2.0M) was slowly added to the initial mixture, under Schotten-Baumann condition and stirred to room temperature overnight to give to amides. When the reaction was completed determinate by TLC, the reaction mixture was diluted with ethyl acetate and washed with brine (2 x 20mL). The combined organic layers were dried over anhydrous Na2SO4, the residue after solvent evaporated was purified through flash chromatography using hexanes /ethyl acetate (80:20 v/v) to give pure molecules set **3i-k** in 30-35 % yield.

N-hexyl-4-oxothiochromane-2-carboxamide **(24a)**. White solid. mp= 128-129 °C. ^{1}H NMR (300 MHz, Chloroform-d) δ 8.13 (d, *J* = 7.9 Hz, 1H), 7.44 (t, J = 7.6 Hz, 1H), 7.33 – 7.22 (m, 2H), 6.56 (t, J = 5.9 Hz, 1H), 4.05 (t, *J* = 5.4 Hz, 1H), 3.48 (dd, *J* = 16.8, 6.5 Hz, 1H), 3.36 – 3.05 (m, 3H), 1.41 (p, *J* = 7.1 Hz, 2H), 1.23 (td, *J* = 12.1, 10.4, 5.6 Hz, 6H), 0.87 (t, *J* = 6.6 Hz, 3H). ^{13}C NMR (75 MHz, CDCl3) δ 192.52, 168.50, 138.06, 133.66, 131.04, 129.19, 127.47, 126.00, 77.53, 77.11, 76.68, 43.57, 41.75, 40.07, 31.39, 29.29, 26.31, 22.52, 14.04. **IR** ν (cm^{-1}) = 3331.59, 2926.30, 2859.39, 1658.69, 755.77. HRMS-ESI (m/z): calcd. for $C_{16}H_{21}NO_2S$ $[M+H]^+$ 292.1366, found 292.1393.

N-dodecyl-4-oxothiochromane-2-carboxamide **(24b).** White solid, mp = 125-127 °C. ^{1}H NMR (300 MHz, Chloroform-d) δ 8.14 (d, *J* = 7.9 Hz, 1H), 7.44 (t, *J* = 7.6 Hz, 1H), 7.34 – 7.22 (m, 2H), 6.50 (d, *J* = 7.2 Hz, 1H), 4.05 (t, *J* = 5.4 Hz, 1H), 3.49 (dd, *J* = 16.7, 6.5 Hz, 1H), 3.37 – 3.08 (m, 3H), 1.49 – 1.14 (m, 19H), 0.97 – 0.86 (m, 3H). ^{13}C NMR (75 MHz, CDCl3) δ 192.44, 168.41, 138.00, 133.66, 131.08, 129.24, 127.48, 126.04, 77.52, 77.09, 76.67, 43.59, 41.75, 40.09, 31.96, 29.68, 29.68, 29.61, 29.53, 29.40, 29.40, 29.34, 29.24, 29.24, 26.66, 22.74, 14.18. **IR** ν (cm^{-1}) = 3365.75, 2979.88, 2913.42, 1686.63, 779.84. HRMS-ESI (m/z): calcd. for $C_{22}H_{33}NO_2S$ $[M+H]^+$ 376.2305, found 376.2314.

4-oxo-N-(o-tolyl)thiochromane-2-carboxamide **(24c).** Yellowish solid, mp =144-146 °C. ^{1}H NMR (300 MHz, Chloroform-d) δ 7.61 (s, 1H), 7.40 (d, *J* = 7.8 Hz, 1H), 6.95 (d, *J* = 7.9 Hz, 1H), 6.71 (t, *J* = 7.7 Hz, 1H), 6.64 – 6.36 (m, 5H), 6.33 (d, *J* = 7.3 Hz, 1H), 3.48 (d, *J* = 5.5 Hz, 1H), 2.88 (dd, *J* = 17.1, 4.9 Hz, 1H), 2.45 (dd, *J* = 16.9, 4.0 Hz, 1H), 1.33 (s, 3H). ^{13}C NMR (75 MHz, CDCl3) δ 192.00, 166.95, 137.28, 134.97, 133.87, 131.26, 130.54, 129.57, 129.51, 129.41, 127.64, 126.82, 126.64, 126.46, 125.71, 122.97, 77.54, 77.11, 76.69, 44.11, 41.51, 17.43. **IR** ν (cm^{-1}) = 3261.48, 1673.35, 1650.48, 748.51. HRMS-ESI (m/z): calcd. for $C_{17}H_{15}NO_2S$ $[M+H]^+$ 298.0896, found 298.0919

4.1.8. General procedure for the synthesis of the thiochromane hydrazones

Ethyl 4-oxothiochromane-2-carboxylate (**22a**) 500 mg were dissolved in anhydrous methanol (25 mL). The mixture was heated at reflux by 12 h with the correspond hydrazide (1.6 mmol) and glacial acetic acid (100 μL). When the reaction was completed, determinate by the resulting precipitate, it was collected through filtration and washed with methanol. Acyl hydrazones were obtained as white solids to give pure **26a-c** with a 90-98 % yield.

ethyl 4-(2-benzoylhydrazineylidene)thiochromane-2-carboxylate **(26a).** White solid mp= 170-171 °C. ^{1}H NMR (300 MHz, DMSO-d6) δ 11.20 (s, 1H), 8.77 (d, *J* = 5.3 Hz, 3H), 8.15 (d, *J* = 7.9 Hz, 1H), 7.80 (d, *J* = 5.1 Hz, 2H), 7.29 (tt, *J* = 15.7, 7.4 Hz, 4H), 4.38 (dd, *J* = 6.7, 4.5 Hz, 1H), 4.08 (q, *J* = 7.1 Hz, 3H), 3.22 (dd,

J = 17.5, 4.6 Hz, 1H), 1.13 (q, *J* = 8.2, 7.1 Hz, 4H).^{13}C NMR (75 MHz, DMSO) δ 170.41, 163.11, 152.94, 150.62, 141.41, 133.54, 131.33, 130.66, 128.21, 127.05, 126.27, 122.39, 61.85, 40.72, 40.58, 40.44, 40.17, 39.89, 39.61, 39.34, 39.05, 30.28, 14.32. **IR** ν (cm^{-1}) = 3173.40, 2978.57, 1718.42, 1652.27, 754.99, 734.77. HRMS-ESI (m/z): calcd. for $C_{19}H_{18}N_2O_3S$ $[M+H]^+$ 355.1111, found 355.1135.

ethyl 4-(2-isonicotinoylhydrazineylidene)thiochromane-2-carboxylate **(26b).** White solid, mp=190-192 °C. ^{1}H NMR (300 MHz, DMSO-d6) δ 10.97 (s, 1H), 8.14 (s, 1H), 7.88 (d, *J* = 7.4 Hz, 2H), 7.55 (dt, *J* = 14.8, 7.2 Hz, 3H), 7.25 (d, *J* = 18.9 Hz, 3H), 4.38 (t, *J* = 5.5 Hz, 1H), 4.08 (q, *J* = 7.2 Hz, 2H), 1.12 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, DMSO) δ 170.43, 164.52, 134.38, 134.37, 133.29, 132.09, 131.61, 130.34, 128.79, 128.46, 128.18, 126.92, 126.21, 61.81, 30.11, 14.33. **IR** ν (cm^{-1}) = 3145.28, 2947.51, 1717.64, 1651.30, 759.40, 708.68. HRMS-ESI (m/z): calcd. for $C_{18}H_{17}N_3O_3S$ $[M+H]^+$ 356.1063, found 356.1096.

4-(2-carbamoylhydrazineylidene)thiochromane-2-carboxylic acid **(26C).** White solid, mp= 238°C. ^{1}H NMR (300 MHz, DMSO-d6) δ 9.44 (s, 1H), 8.73 (s, 1H), 8.23 (d, *J* = 7.8 Hz, 1H), 7.21 (d, *J* = 4.0 Hz, 2H), 7.14 (dq, *J* = 8.3, 4.3 Hz, 1H), 6.58 (s, 2H), 4.18 (dd, *J* = 6.8, 4.7 Hz, 1H), 3.12 (dd, *J* = 17.6, 7.0 Hz, 1H), 2.99 (dd, *J* = 17.6, 4.9 Hz, 1H).^{13}C NMR (75 MHz, DMSO-d6) δ 171.99, 157.60, 141.08, 132.46, 132.16, 129.08, 128.17, 126.80, 126.10, 41.16, 30.31. **IR** ν (cm^{-1}) = 3469.94, 3237.11, 2906.14, 1713.23, 1645.36, 757.97, 736.96. HRMS-ESI (m/z): calcd. for $C_{11}H_{11}N_3O_3S$ $[M+H]^+$ 266.0594, found 266.0595.

4.1.8. General procedure for the synthesis of the ethyl 4-oxo-4H-thiochromene-2-carboxylate

200 mg of ethyl 4-oxothiochromane-2-carboxylate **22a** dissolved in carbon tetrachloride (3 mL), was stirred with DDQ (2 equivalents) and HOAc (0.3 mL). The reaction mixture was heated under microwave irradiation at 150°C for one h. The resultant mixture was quenched with saturated brine (20 mL) and was extracted with CH_2Cl_2 (10 mL × 3). The organic layers were combined, washed with brine, and dried with anhydrous Na_2SO_4. After it was concentrated in vacuo, the resultant residue was purified by flash chromatography (ethyl acetate/n-hexane = 1: 10) to give pure **27** with a 50 % yield.

ethyl 4-oxo-4H-thiochromene-2-carboxylate **(27).** Yellowish solid, mp= 98-100 °C. ^{1}H NMR (300 MHz, Chloroform-d) δ 8.44 (d, *J* = 7.9 Hz, 1H), 7.70 (s, 1H), 7.68 – 7.55 (m, 2H), 7.53 (ddd, *J* = 8.3, 4.7, 3.4 Hz, 1H), 4.52 – 4.36 (m, 16H), 1.42 (t, *J* = 7.1 Hz, 3H).^{13}C NMR (75 MHz, Chloroform-d) δ 181.04, 162.82, 141.77, 137.37, 132.27, 131.22, 129.19, 128.55, 128.23, 127.29, 63.28, 14.10. FTIR ν (cm^{-1}) = 3054.19, 1716.19, 1625.88, 756.11, 748.32. HRMS-ESI (m/z): calcd. for $C_{12}H_{10}O_3S$ $[M+H]^+$ 235.0423, found 235.0452.

4. 2. Resolution of the racemic acids and derivatives

1,0 g of compound 4-oxothiochromane-2-carboxylic acid (**22a**) dissolved in Tetrahydrofuran (THF)/water in ratio 4:1 (5 mL), was mixture with brucine (2 equivalents). The reaction mixture was overnight stirred at room temperature. The solvent was evaporated under reduced pressure, and the resulting solid was re-dissolved in hot ethyl acetate. A crystal phase was generated which was filtered off resulting in two news fractions (solid and liquid diastereomers), over both fraction was made acid extraction (HCl 10%) The resultant mixture was quenched with saturated brine (20 mL) and was extracted with hot ethyl acetate three-time (10 mL × 3). The organic layers were combined, washed with brine, and dried with anhydrous Na_2SO_4. After it was concentrated in vacuo. The resultant residue was purified by flash chromatography (ethyl acetate/n-hexane = 1: 10) to give pure **20a (+)** with 10 % yield, and specific rotation [α]25D = 35.6 as same to **20a (-)** with 45 % yield with [α]25D = -10.8.

Each one acid compound in respect excess enantiomerical was esterified according with general methodology before describe as General procedure for the synthesis of the esters from 4-

oxothiochromane-2-carboxylic acids to give compounds **22a (+)** in 9 % yield and specific rotation [α]25D = 32.4 also the compound **22a (-)** in 85 %yield with specific rotation [α]25D = -8.3.

4. 3. *Biological Activity*

4. 3. 1. Cytotoxic Activity

Cytotoxicity of the compounds was evaluated over human monocytes (U-937 ATCC CRL-1593.2) in an exponential growth phase and adjusted at 1 × 105 cells/mL in RPMI-1640 enriched with 10% fetal bovine serum (FBS). One hundred microliters of cell suspension were dispensed in each well of a 96-wells microplate, and then 100 µL of 200-50-12.5 and 3.125 µg/mL concentration of each compound or standard drug (amphotericin B) were added dissolved in PBS with 0.5% DMSO. Cell exposed to compounds or standard drugs were incubated 72 h at 37 °C and 5% of CO_2. Cytotoxic activity of each compound was determined according to the effect on the cell viability by the MTT microenzymatic method in which 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide is reduced to a purple product named formazan by mitochondrial enzyme succinate dehydrogenase. Thus, 10 µL/well of MTT solution (5 mg/mL) was added to each well of exposed and unexposed cells, and plates were incubated at 37 °C, 5% CO_2 during 3 h. The reaction was stopped by adding 100 µL/well of isopropanol with 50% and 10% of SDS (sodium dodecyl sulfate). The concentration of formazan was determined spectrophotometrically at 570 nm (Varioskan, Thermo) and intensity of color (absorbance) was registered as O.D. Cells exposed to control drug (amphotericin B) were used as control for toxicity (positive control) while cell incubated in the absence of any compound or drug were used as control for viability (negative control). Non-specific absorbance was corrected by subtracting absorbance (O.D) of the blank. Determinations were done by triplicate in at least two independent experiments [31].

4. 3. 2. Antileishmanial Activity

Antileishmanial activity of compounds was determined according to the ability of the compound to reduce the infection by *L. panamensis* parasites. For this, the antileishmanial activity was tested on intracellular amastigotes of *L. panamensis* transfected with the green fluorescent protein gene (MHOM/CO/87/UA140-EGFP strain)[32]. Briefly, U-937 human cells at a density of 3 × 105 cells/mL in RPMI 1640 and 0.1 µg/mL of PMA (phorbol-12-myristate-13-acetate) were dispensed on 24-wells microplate and then infected with stationary phase growing *L. panamensis* promastigotes in 15:1 parasites per cell ratio. Plates were incubated at 34 °C and 5% CO_2 for 3 h, and then cells were washed twice with phosphate buffer solution (PBS) to eliminate not internalized parasites. Fresh RPMI-1640 was added into each well (1 mL), and plates were incubated again. After 24 h of infection, the RPMI-1640 medium was replaced by fresh culture medium containing each compound at four serial dilutions (50, 12.5, 3.125 and 0.78 µg/mL) and plates were then incubated at 37 °C and 5% CO_2 during 72 h, then, cells were removed from the bottom plate with 100 uL of EDTA/Trypsin (250 mg) solution. The cells were centrifuged at 1100 rpm during 10 min at 4 °C, the supernatant was discarded, and cells were washed with 1 mL of cold PBS and centrifuged at 1100 rpm for 10 min at 4 °C. Cells were washed two times employing PBS, as previously, and after the last wash, the supernatant was discarded, and cells were suspended in 500 µL of PBS.

Cells were analyzed by flow cytometry employing a flow cytometer (Cytomics FC 500MPL) reading at 488 nm (exciting) and 525 nm (emitting) over an argon laser and counting 10,000 events. Infected cells were determined according to the events for green fluorescence (parasites). All determinations for each compound and standard drug were carried out by triplicate, in two experiments. Infected cells exposed to control drug (amphotericin B) were used as the control for antileishmanial activity (positive control), while infected cells incubated in the absence of any compound or drug were used as another control for infection (negative control). Nonspecific fluorescence was corrected by

subtracting the fluorescence of unstained cells. Determinations were done by triplicate in at least two independent experiments[32,33].

4. 3. 3. Statistical Analysis

Cytotoxicity was determined according to viability and mortality percentages obtained for each experimental condition (synthesized compounds, amphotericin B, and culture medium). Results were expressed as the mean lethal concentrations (LC_{50}), the concentration necessary to kill 50% of cells, calculated by the parametric method of linear regression that permits doses-response analysis (Probit analysis) [33].

Initially, viability percentages were calculated by Equation (1), where the O.D of control well, corresponds to 100% of viability.

(1) % viability = (O.D exposed cells / O.D unexposed cells) x 100

Then, the percentage of cell growth inhibition was calculated using Equation (2):

(2) % inhibition = 100- (% Viability)

The toxicity was defined according to LC_{50} values, using the follow scale: Toxic; LC_{50} < 100 μM; moderately toxic; LC_{50} > 100 μM and <200 μM and potentially nontoxic; LC_{50} > 200 μM.

The antileishmanial activity was determined according to the reduction of the percentage of fluorescent parasites determined according to the median fluorescence intensity (MFI) obtained for each experimental condition by cytometry. The parasite values for each concentration of compound were calculated by Equation (3), where the % of parasites in control well, corresponds to 100% of parasites.

(3) % parasites = (MFI exposed parasites/ MFI unexposed parasites) x 100

Then, the inhibition percentage was calculated with Equation (4):

(4) % inhibition of parasites = 100 – (% parasites)

Results of antileishmanial activities were expressed as the median effective concentrations (EC_{50}) measured by Probit method. The activity of each compound was established according to EC_{50} values as: high activity: EC_{50} < 25 μM; moderate activity: EC_{50} >25 μM and <100 μM and low activity: EC_{50} > 100 μM.

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