Synthesis and Evaluation of Novel Chromanone and Quinolinone Analogues of Uniflorol as Anti-Leishmanial agents

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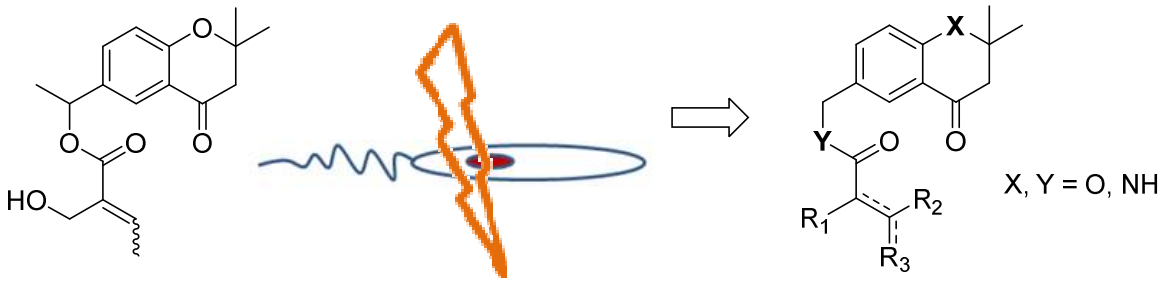
Graphical Abstract



Calea uniflorols

Chromanone and quinolinone analogues of the natural product uniflorol were designed, with the aim of improving activity against *Leishmania* while improving physicochemical properties, notably stability. (*E*)-*N*-((2,2-dimethyl-4-oxo-1,2,3,4-

tetrahydroquinolin-6-yl)methyl)-4-phenylbut-3-enamide (**13e**) showed weak inhibition (IC_{50}=10-20μM) of promastigote forms of *L. amazonensis*.

Abstract

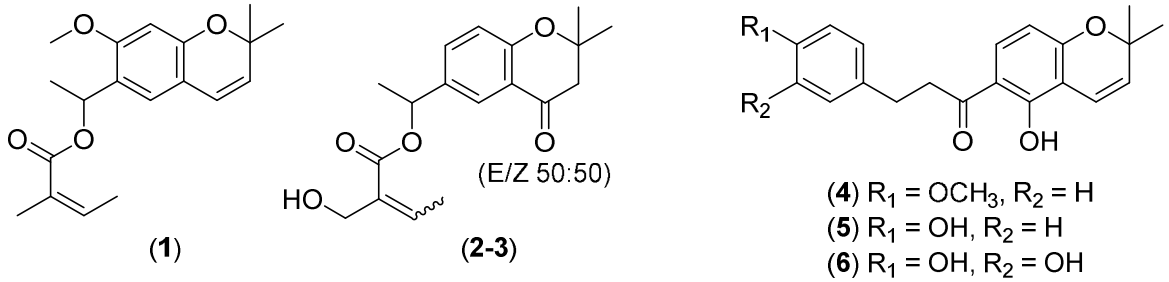
Within this work, we describe the design and synthesis of a range of novel chromanones and quinolinones, based on natural products reported to possess anti-leishmanial action. The target heterocycles were obtained either via classical or ionic liquid mediated Kabbe condensation in the case of chromanones, or aqueous Sonogashira based alkynylation followed by acid-catalysed cyclisation in the case of quinolinones. Upon testing in *Leishmania*-infected macrophages and against promastigotes of all three common *Leishmania* parasites, IC_{50} values in all cases were greater than 5μM against amastigotes, and only the quinolinone styryl derivative **13e** exhibited any activity against promastigotes.

Introduction

Leishmaniasis is a group of diseases caused by protozoa of the genus *Leishmania*, transmitted through the bite of infected sandflies. Various clinical presentations exist, notably visceral, cutaneous, and mucosal forms, with visceral disease carrying the most serious consequences. Although treatments exist, they are often toxic, expensive, or both; thus the development of novel therapies remains an important strategy [1]. One approach is to investigate the composition and activity of natural medicines used by cultures in areas of disease endemicity. Diverse natural products have been shown to exhibit anti-leishmanial activity, with much interest focussed on small molecule phenolics, a group that includes chromenes and chromanones. Several examples of these plant metabolites have been isolated from species used by indigenous peoples in ethnomedicine (Figure 1). For example, the chromene encecalol angelate **1** was isolated from *Ageratum conyzoides*, a plant traditionally used for parasitic infections [2]. Upon isolation and testing, this compound showed weak activity, including an IC_{50} of 14.6μg/mL against *L. donovani* (axenic amastigotes). Within the genus *Calea*, several species have been investigated for

anti-parasitic properties [3]. Among the bioactive constituents isolated from this genus are the chromanones, uniflorols A (**2**) and B (**3**), an E/Z mixture of which inhibited *L. major* promastigote growth by 55-89% between 25-100*μ*g/mL [4]. Among other phenolics, the chalcones are well established as having diverse pharmacological potential. Of these, the licochalcones and their derivatives have been widely explored. Interestingly, the reactive α,β-unsaturated chalcone system may not be a pre-requisite for activity, as evidenced by retention of activity in some dihydrochalcones. These include the chromene derivatives **4**-**6**, isolated from *Crotalaria ramosissima* [5].

Figure 1. Natural products with a chromene or chromanone skeleton with activity against *Leishmania*



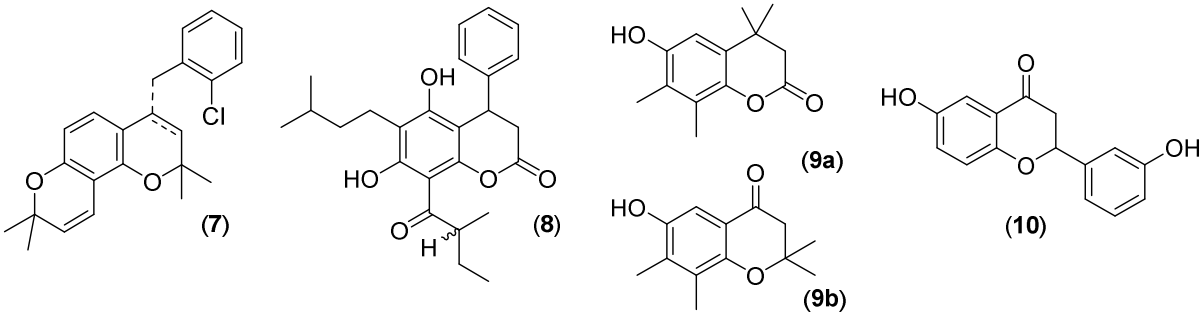
(**1**) (**2**-**3**)

(**4**) $R_1 = OCH_3$, $R_2 = H$
(**5**) $R_1 = OH$, $R_2 = H$
(**6**) $R_1 = OH$, $R_2 = OH$

Taking inspiration from natural product lead structures, some groups have developed analogues of chroman, chromene, flavonoid and coumarin systems (Figure 2). Drawing on the observed activity of the chromene glyasperin from *Smirnovia iranic*a, Alizadeh et al [6] prepared twelve chromenes, some of which, notably **7** were moderately active against *L. major*. Brenzan et al, in studying the structure activity relationship of coumarins ((-) mammea A/BB) isolated from *Calophyllum brasiliense*, prepared a series of analogues, of which the most active was chromanone **8**, inhibiting promastigote forms of *L. amazonensis* with an IC_{50} of 0.9μM and a CC_{50} of 85.4μM in macrophages [7]. A range of synthetic chromanols was developed by Monzote et al, some of which inhibited the growth of *Leishmania*. The observed activity of these compounds **9a**-**b**, essentially vitamin E derivatives, was considered to be at least partly due to cyt bc(1) inhibition in addition to antioxidative properties [8]. Notably however, in these latter two studies, activity was more prominent in 2-chromanones rather than in 4-chromanones. The importance of a chromene or chromanone system for anti-leishmanial activity in several natural products was

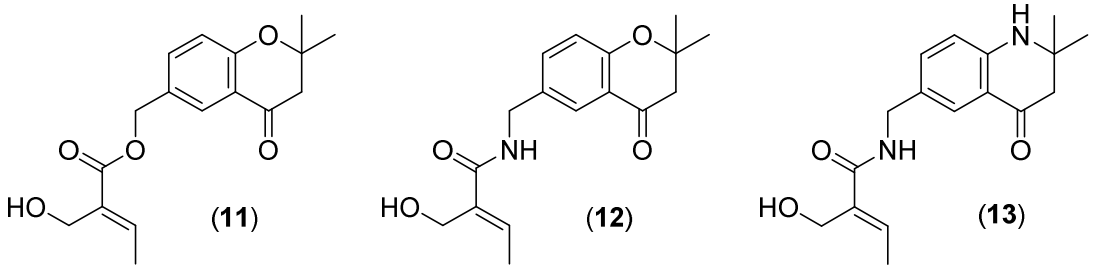
underlined by the work of Sandjo et al, who tested a series of six natural products alone and in combination and found that the presence of these intact ring systems enhanced activity compared to their ring-opened analogues [9]. Chroman-4-one analogues of flavonols have also been evaluated against *L. major*, with **10** showing binding to the enzyme *Lm*PTR1 and weak (31% at 50μM) inhibition of *L. infantum* [10].

Figure 2. Synthetic derivatives of naturally occurring chroman, chromene, flavonoid and coumarin systems with activity against *Leishmania*



Interested in probing the structural requirements of **2**-**3** for anti-leishmanial activity, we designed three distinct targets **11**-**13**, as shown in Figure 3. Firstly, we envisaged **11** as a more accessible, des-methyl analogue of uniflorol. Knowing that encecalol is unstable, and recognising similar potential within the uniflorols, we proposed to both omit the benzylic methyl group and replace the labile ester with a more stable amide functionality, as in **12**. Additionally, substitution of the heterocyclic oxygen for nitrogen was proposed to investigate the effect of additional H-bond donating capability and of altering the expected logP of the molecule, as depicted in **13** (and its analogues).

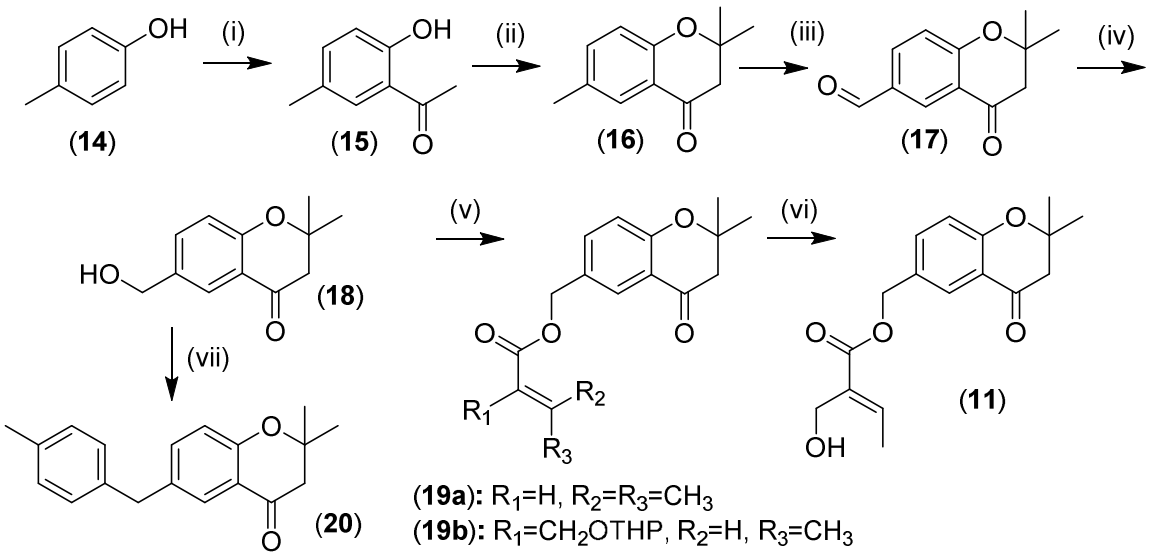
Figure 3. Synthetic targets based on lead compounds (**2**-**3**)



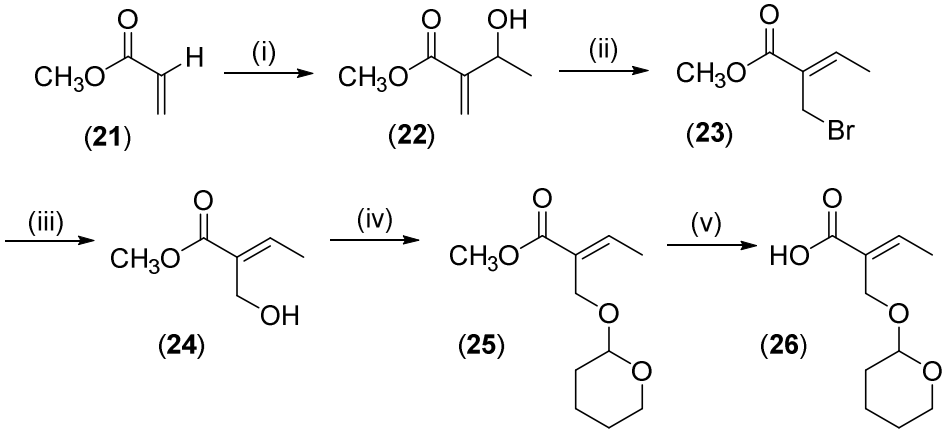
Results

Chemistry

Our synthetic approach towards **11** is outlined in Scheme 1. Friedel-Crafts acylation of cresol **14** with acyl chloride in neat TfOH [11] afforded the *o*-hydroxy acetophenone **15**. Cyclisation to afford the chromanone core was accomplished using ionic liquid-promoted Kabbe condensation [12], which produced the desired product **16** in significantly higher yield than under standard conditions. Oxidation of **16** with persulfate afforded **17**, lactarochromal, a known natural product [13]. Chemoselective bioreduction of **17** was achieved in excellent yield employing *D. carota* [14] with no evidence of the diol complicating other reportedly selective reduction protocols such as $Na_2CO_3/NaBH_4$ or $SnCl_2/NaBH_4$. This reaction represents a route to the natural product 6-hydroxymethyl-2,2-dimethylchroman-4-one (**18**), previously reported from submerged cultures of a *Stereum* sp. [15]. Prior to esterification, we were required to prepare a usable C5 synthon as the acid fragment. This is depicted in Scheme 2.



Scheme 1. Synthesis of compound **11**. Reagents and conditions: (i) CH_3COCl, CF_3SO_3H, 0°C-RT, 24h; (ii) CH_3COCH_3, [bbim]Br, 95–100°C, 8h; (iii) $K_2S_2O_8$, $CuSO_4$, H_2O/CAN (1:1), 75–80°C, 1h; (iv) *D. carota*, H_2O, RT, 72h; (v) (**26**), acrylic acid, EDC, DMAP, 2 weeks, RT; (vi) (**19b**), *p*-toluenesulfonic acid, MeOH, RT, 24h; (vi) acrylic acid, PFPAT, toluene, reflux.



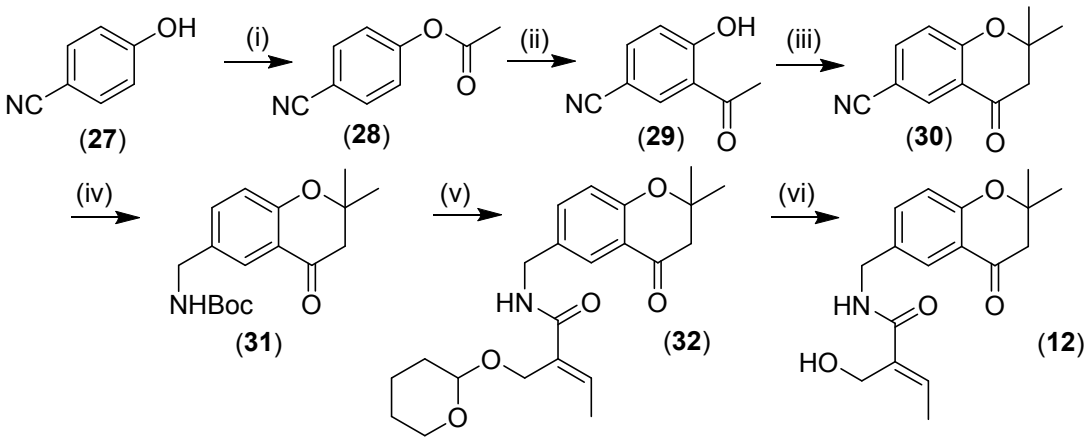
Scheme 2. Synthesis of acid synthon **26**. Reagents and conditions: (i) CH_3CHO, DABCO, RT, 7 days; (ii) NBS, Me_2S, DCM, RT, 17h; (iii) NaOAc, MeOH, Δ, 3.5h, then K_2CO_3, RT, 24h; (iv) 3,4-dihydropyran, DCM, pyridinium *p*-toluenesulfonate; (v) *p*-toluenesulfonic acid, MeOH, RT, 24h.

Baylis-Hillman reaction of methyl acrylate **21** with acetaldehyde, using 1,4-diazabicyclo-[2.2.2]octane (DABCO) as catalyst, provided the desired methyl 3-hydroxyl-2-methylenebutanoate (**22**) [16]. Reaction of **22** with *N*-bromosuccinimide/dimethylsulfide (NBS/Me_2S) afforded **23**; alternatively, this transformation can be achieved using LiBr/H_2SO_4 in acetonitrile at room temperature [17], affording *Z*-**23** in similar yield in our hands. Reaction of **23** with sodium acetate in refluxing methanol followed by K_2CO_3-mediated ester hydrolysis produced (2*E*)-2-(hydroxymethyl)-2-butenoate (**24**). Methyl ester hydrolysis of **24** was expected to afford the free acid; however this reaction was unsatisfactory, affording only small quantities of the acid using either LiOH or $Ba(OH)_2$ and with dimerization as the primary reaction. In any case, attempted direct esterification of the free acid with alcohol **18** failed. We therefore protected the primary alcohol of **24** via tetrahydropyranylation catalysed by pyridinium *p*-toluenesulfonate [18]. Finally, LiOH-mediated hydrolysis of the protected **25** afforded **26** in good yield.

Prior to utilisation of valuable acid synthon **26**, we attempted to establish suitable conditions for reaction of the related commercial acrylic acid 3,3-dimethylacrylic acid with alcohol **18**. Using either $ZrOCl_2.8H_2O$ [19] or tosyl chloride/*N*-methylimidazole [20] resulted in no reaction. Employing DCC/DMAP resulted in approximately 10% of ester as estimated by NMR, but it proved impossible to eliminate residual DCC

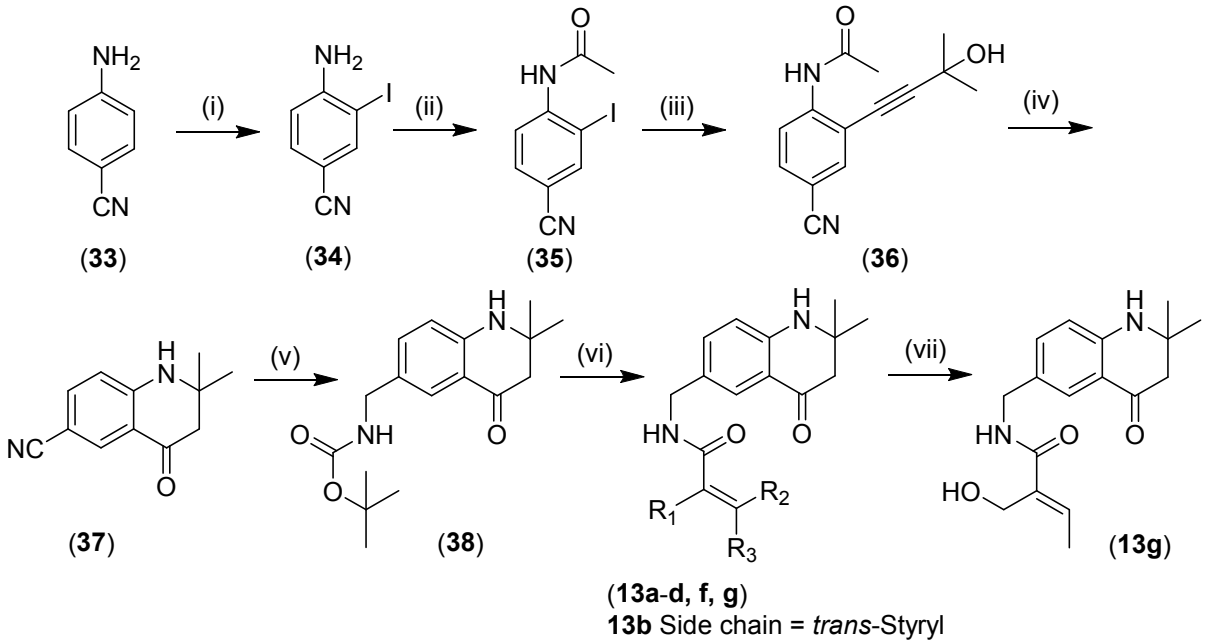
contamination. Interestingly, use of pentafluorophenylammonium triflate (PFPAT) in toluene [21] with various acrylic acids resulted in isolation of **20** in quantitative yield; this compound is novel to the literature. We finally identified that using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) with 4-dimethylaminopyridine (DMAP) catalysis under basic conditions afforded the desired ester(s) under acceptable yields. As a final step, deprotection of the primary alcohol in **19b** was accomplished using *p*-toluenesulfonic acid at room temperature, yielding **11**.

Synthesis of compound **12** is depicted in Scheme 3. Esterification of *p*-cyanophenol **27** followed by Fries rearrangement produced acetophenone **29**. Kabbe cyclisation under standard conditions afforded the known chromanone **30**. Reduction of the nitrile group was accomplished using nickel(II)/$NaBH_4$ [22] in the presence of di-*tert*-butyl dicarbonate; this reaction also reduced the benzylic ketone which was selectively re-oxidised using Jones reagent to afford the BOC-protected ketone **31**. Following carbamate cleavage with trifluoroacetic acid, EDC coupling was used to afford amide **32**. Removal of the tetrahydropyranyl group afforded the final product **12**.



Scheme 3. Synthesis of compound **12**. Reagents and conditions: (i) CH_3COCl, Et_3N, THF, 0^oC-RT, 24h; (ii) $AlCl_3$, 160^oC, 3h; (iii) CH_3COCH_3, pyrrolidine, toluene, 90°C, 3h; (iv) $NiCl_2.6H_2O$, Boc_2O, $NaBH_4$, MeOH, 15h, RT; then Jones reagent, CH_3COCH_3, 0^oC-RT, 2h; (v) CF_3CO_2H, DCM, 0^oC-RT, 3h, then **26**, EDC, Et_3N, DCM, 0^oC-RT, 3h; (vi) *p*-toluenesulfonic acid, MeOH, RT, 24h.

To produce quinolinone analogues (**13a**-**g**), an alternative strategy was adopted (Scheme 4). Iodination of cyanoaniline **33** afforded **34**, which was acetylated prior to Sonogashira coupling under aqueous conditions [23] to afford alkyne **36**. Acid-mediated cyclisation gave quinolinone **37**, whose nitrile group was reduced analogously to that of **30**, and similarly, any over-reduced alcohol could be selectively oxidised to **38** using Jones reagent. Following carbamate hydrolysis of **38**, amidation with various acrylic acids was facilitated by EDC to give **13a**-**f**. Compound **13g** was obtained via terminal deprotection of **13a**.



Scheme 4. Synthesis of compounds **13a**-**g**. Reagents and conditions: (i) ICl, CH_3CO_3H, RT, 30min; (ii) Ac_2O, H_2SO_4, 70^oC, 10min; (iii) H_2O, $PdCl_2$, 2-methylbut-3-yn-2-ol, pyrrolidine, 110°C, 5h; (iv) conc. HCl/H_2O, 80^oC, 5h; (v) $NiCl_2.6H_2O$, Boc_2O, $NaBH_4$, MeOH, 15h, RT; then Jones reagent, CH_3COCH_3, 0^oC-RT, 2h; (vi) CF_3CO_2H, DCM, 0^oC-RT, 3h, then RCOOH, EDC, Et_3N, DCM, 0^oC-RT, 3h; (vii) **13a**, *p*-toluenesulfonic acid, MeOH, RT, 24h.

Pharmacological activity

Selected compounds were evaluated for their leishmanicidal ability against *L. amazonensis* and *L. infantum*-infected macrophages (Table 1). Treatment was performed for 24h and results obtained through evaluation of the percentage of infected cells upon treatment when compared to control. In addition, compounds were tested for their cytotoxicity against bone marrow-derived macrophages (BMDM). Treatment was

performed for 24h and results obtained through evaluation of cell viability upon treatment when compared to control. Compounds were also evaluated against *L. amazonensis, L. major* and *L. infantum* promastigotes (Table 2). Treatment was performed for 24h and results assessed by resazurin assay. Results were obtained through evaluation of cell viability upon treatment when compared to control.

Table 1. Activity of chromanones and quinolinones against *Leishmania*-infected macrophages

	Compound			IC_{50} (μM)		CC_{50} (μM)
	R_1	R_2	R_3	*L. amazonensis*	*L. infantum*	BMDM
11				>5	>5	ND[a]
12				>5	>5	ND
20				>5	>5	ND
13b	H	H	CH_3	>40	>10	>40
13c	CH_3	H	CH_3	>40	>10	>40
13d	H	CH_3	CH_3	>40	>10	>40
13e	Amide = *trans*-Styryl			>40	>10	>40
13f	H	H	2-Thienyl	>40	>10	>40
13g	CH_2OH	H	CH_3	>5	>5	ND

[a]ND = not determined

Table 2. Activity of chromanones and quinolinones against *Leishmania* promastigotes

Compound	IC_{50} (μM)		
	L. amazonensis	*L. major*	*L. infantum*
11	>20	>20	>20
12	>20	>20	>20
20	>20	>20	>20
13b	>20	>20	>20
13c	>20	>20	>20
13d	>20	>20	>20
13e	>10	>20	ND[a]

13f	>20	>20	>20
13g	>20	>20	>20

[a]ND = not determined

Discussion

Our target compounds were designed based on reported activity of uniflorols A and B and related structures against Leishmania. Based on the results of Tables 1 and 2, disappointingly, none of our compounds showed strong anti-leishmanial activity. IC_{50}s recorded for all tested compounds against intracellular amastigotes exceeded 5μM, with **11**, **12**, **20** and **13g** having lower IC_{50}s than quinolinones **13b**-**f**. Introduction of the nitrogenated quinolinone heterocycle did not enhance activity per se, which may point towards a chroman or chromanone pharmacophore as optimal. None of the compounds showed toxicity towards uninfected macrophages. Only compound **13e**, the styryl derivative, showed any indicative activity against L. amazonensis promastigotes. This result in itself is interesting however, as Harel et al [24] synthesised chromane and chromene derivatives of another bioactive natural product, encecalin, and highlighted several chromane derived amines and amides as novel lead compounds, finding greater activity among the amines, and in particular, a phenylbutylamine derivative showed potent anti-leishmanial activity. Notably, our chromanones share some similarity with these structures but, in addition to the differential oxidation state of the heterocycle, they also lack hydroxyl or methoxyl substitution at C7. Whether our compounds may be further optimised to improve activity is the subject of ongoing work. It may well be that increased hydrophobicity may be important, as evidenced by the impact of isoprenyl and phenyl substituents in molecules such as **8**. To this end, variation of the α,β unsaturated side chain of the compounds **11** and **12** may yield derivatives of interest. We are particularly interested in pursuing compounds without the α,β unsaturated Michael acceptor functionality found within many of these natural product analogues, and in this regard are encouraged by the indicative activity of **13e**, which lacks this functionality.

Experimental

Chemistry

All required chemicals, solvents, and reagents were purchased from Sigma-Aldrich and were of reagent grade. Reaction progress was monitored on pre-coated thin layer chromatographic aluminum sheets (Silica Gel Merck 60 F_{254}), and TLC visualization was done using a UV lamp. Fourier transform infrared spectra were carried out with neat film coated samples on diamond using a Nicolet^{TM} iS^{TM} 10 FT-IR spectrophotometer (Thermo Fisher). Significant absorption peak (νmax) values are given in cm^{-1}. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 400 spectrometer at 400MHz and 100MHz, respectively, in $CDCl_3$ and CD_3OD using tetramethylsilane (TMS) as the internal standard. Chemical shift values are given on the δ (ppm) scale, with signals are described as follows: s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), br. (broad signal), m (multiplet), with coupling constants (*J*) expressed in Hz. Mass spectral analyses were recorded using a Waters LCT Premiere XE (ESI-TOF MS) instrument. All calculated exact mono isotopic mass distributions were calibrated against internal reference standards.

1-(2-hydroxy-5-methylphenyl)ethan-1-one (**15**) To a solution of *p*-cresol **14** (1g, 9.25mmol) in neat trifluoromethanesulfonic acid (5mL) at 0^{o}C was added acetyl chloride (0.66mL, 9.28mmol). The reaction was allowed to reach room temperature, and stirred overnight. The crude residue was poured into cold water and ethyl acetate. The organic layer was sequentially washed with 1N HCl, 5% $NaHCO_3$, 1N HCl, and saturated NaCl successively, dried over Na_2SO_4 and concentrated. The crude residue was purified by flash column chromatography to afford ketone (**15**), as a colourless oil that solidified on standing (700mg, 50.4%). ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 2.33 (3H, s, ArC**H**$_3$), 2.63 (3H, s, COC**H**$_3$), 6.90 (1H, d, *J*=8.5Hz, **H**3), 7.31 (1H, dd, *J*=8.5, 2.5Hz, **H**4), 7.52 (1H, d, *J*=2.5Hz, **H**6), 12.15 (1H, s, O**H**) [25].

2,2,6-trimethylchroman-4-one (**16**) A mixture of **15** (3.68g; 24.5mmol), acetone (3.6mL, 49.0mmol), and morpholine (1.1mL, 12.6mmol) was added to 5g of the ionic liquid[bbim]Br [26], and stirred at 95–100^{o}C for 8 hours. The reaction mixture was then extracted with EtOAc (3 x 10mL). The combined EtOAc extracts were

concentrated *in vacuo* and the resulting product was purified by flash column chromatography to yield the known [13] chromanone, as a colourless oil (2.21g, 47%). The ionic liquid was solubilized in acetonitrile, treated with charcoal, and filtered. The filtrate was evaporated and the ionic liquid dried under vacuum for further reuse. IR ν_{max} (neat) 1192, 1291, 1487, 1617, 1687, 2976cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 1.37 (6H, s, C(C**H**$_3$)$_2$), 2.23 (3H, s, C**H**$_3$), 2.63 (2H, s, C**H**$_2$), 6.75 (1H, d, *J*=8.3Hz, **H**8), 7.21 (1H, dd, *J*=8.3, 2.3Hz, **H**7), 7.58 (1H, dd, *J*=2, 0.8Hz, **H**5). ^{13}CNMR δ_C 20.3 (**C**H$_3$), 26.6 ((**C**H$_3$)$_2$), 48.9 (**C**H$_2$), 78.9 (**C**(CH$_3$)$_2$), 118.1 (Ar**C**H), 119.8 (Ar**C**), 126.1 (Ar**C**H), 130.0 (Ar**C**), 137.2 (Ar**C**H), 158.0 (Ar**C**), 192.8 (**C**=O).

2,2-dimethyl-4-oxochromane-6-carbaldehyde (**17**) To a solution of **16** (0.51g, 2.68mmol) in acetonitrile (20mL) was added a solution of potassium persulfate (1.6g, 5.92mmol) in water (20mL), and copper sulfate (0.16g, 1.00mmol). The resulting solution was stirred at 75-80°C for 1h and then cooled. The cold mixture was extracted with diethyl ether (3 x 50mL). The diethyl ether extract was extracted with 60% sodium bicarbonate solution (3 x 30mL). Acidification of the bicarbonate extract with dilute HCl gave a colourless crystalline solid (0.26g, 47%), whose experimental data agreed with the literature [13]. IR ν_{max} (neat) 1192, 1204, 1291, 1316, 1488, 1617, 1689, 2924cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 1.44 (6H, s, C(C**H**$_3$)$_2$), 2.72 (2H, s, C**H**$_2$), 6.99 (1H, d, *J*=8.8Hz, **H**8), 7.96 (1H, dd, *J*=8.8, 2.1Hz, **H**7), 8.29 (1H, d, *J*=2.3Hz, **H**5), 9.86 (1H, s, C**H**O). ^{13}CNMR δ_C 25.6 ((**C**H$_3$)$_2$), 47.5 (**C**H$_2$), 79.7 (**C**(CH$_3$)$_2$), 118.7 (Ar**C**H), 118.9 (Ar**C**), 128.9 (Ar**C**), 130.2 (Ar**C**H), 133.8 (Ar**C**H), 163.4 (Ar**C**), 189.2 (**C**HO), 190.2 (**C**=O).

6-(hydroxymethyl)-2,2-dimethylchroman-4-one (**18**) To **17** (100mg, 0.49mmol) in distilled water (50mL) was added freshly cut slices of *D. carota* (10g). The resulting mixture was stirred vigorously at room temperature for 72h. The reaction was filtered, and the filtrate washed with ethyl acetate (50mL). The water/ethyl acetate mixture was separated, and the ethyl acetate extract dried over Na_2SO_4. The crude orange oil was purified by flash column chromatography to afford the benzylic alcohol **18** (76mg, 75%) [15]. IR ν_{max} (neat) 1188, 1488, 1616, 1685, 2976, 3404cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 1.38 (6H, s, C(C**H**$_3$)$_2$), 2.64 (2H, s, C**H**$_2$), 4.56 (2H, s, C**H**$_2$OH), 6.85 (1H, d, *J*=8.5Hz, **H**8), 7.44 (1H, dd, *J*=8.5, 2.5Hz, **H**7), 7.74 (1H, d,

J=2.3Hz, **H**5). ^{13}CNMR δ_C 25.5 (($\underline{\mathbf{C}}H_3)_2$), 47.7 ($\underline{\mathbf{C}}H_2$), 63.4 ($\underline{\mathbf{C}}H_2OH$), 78.3 ($\underline{\mathbf{C}}(CH_3)_2$), 117.7 (Ar**C**H), 118.7 (Ar**C**), 124.0 (Ar**C**H), 132.3 (Ar**C**), 134.5 (Ar**C**H), 158.5 (Ar**C**), 191.7 (**C**=O). HRMS $(M+H)^+$ 207.0950, $C_{12}H_{15}O_3$ requires 207.1021.

(2,2-dimethyl-4-oxochroman-6-yl)methyl 3-methylbut-2-enoate (**19a**) To a solution of 3,3-dimethylacrylic acid (100mg, 1.0mmol) in dichloromethane (10mL) was added EDC HCl (327mg, 1.71mmol) and DMAP (5mg). To this solution was added **18** (206mg, 1.0mmol). The reaction was stirred for 1 week at room temperature. The residual solvent was removed *in vacuo*, and the crude residue purified by flash column chromatography to afford ester **19a** as a colourless oil (125mg, 43%). ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 1.38 (6H, s, ($C\underline{H}_3)_3$), 1.82 (3H, s, ($C\underline{\mathbf{H}}_3$), 2.11 (3H, s, $C\underline{\mathbf{H}}_3$), 2.64 (2H, s, $C\underline{\mathbf{H}}_2CO$), 4.99 (2H, s, $ArC\underline{\mathbf{H}}_2O$), 5.64 (1H, m, C**H**=C), 6.85 (1H, d, *J*=8.3Hz, **H**8), 7.41 (1H, dd, *J*=8.5, 2.3Hz, **H**7), 7.79 (1H, d, *J*=2.3Hz, **H**5). ^{13}CNMR δ_C 20.3, 26.6 (2**C**), 27.5, 48.8, 64.6, 79.4, 115.7, 118.7, 119.9, 126.4, 128.9, 136.3, 157.6, 159.8, 166.3, 192.3.

(2,2-dimethyl-4-oxochroman-6-yl)methyl (*E*)-2-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)but-2-enoate (**19b**) To a solution of **26** (60mg, 0.3mmol) in dichloromethane (10mL) was added EDC HCl (100mg, 0.52mmol) and DMAP (2mg). To this solution was added **18** (60mg, 0.29mmol). The reaction was stirred for 2 weeks at room temperature. The residual solvent was removed *in vacuo*, and the crude residue purified by flash column chromatography to afford ester **19** as a colourless oil (37mg, 33%). IR ν_{max} (neat) 1232, 1490, 1618, 1692, 1712, 2938cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 1.46 (6H, s, ($C\underline{H}_3)_3$), 1.48-1.83 (6H, m, ($C\underline{\mathbf{H}}_2)_3$), 1.94 (3H, d, *J*=7.2Hz, $C\underline{\mathbf{H}}_3C$=C), 2.72 (2H, s, $C\underline{\mathbf{H}}_2CO$), 3.50 (1H, m, H of $OC\underline{\mathbf{H}}_2$ pyran), 3.88 (1H, m, H of $OC\underline{\mathbf{H}}_2$ pyran), 4.23 (1H, d, *J*=11Hz, 1H of $OC\underline{\mathbf{H}}_2$=C), 4.51 (1H, d, *J*=11Hz, 1H of $OC\underline{\mathbf{H}}_2$=C), 4.66 (1H, m, O**C**HO), 5.14 (2H, br. s, $OC\underline{\mathbf{H}}_2Ar$), 6.92 (1H, d, *J*=8.4Hz, **H**8), 7.13 (1H, q, *J*=7.2Hz, C**H**=C), 7.51 (1H, dd, *J*=8.5, 2.4Hz, **H**7), 7.86 (1H, d, *J*=2.3Hz, **H**5). ^{13}CNMR δ_C 14.6, 19.4, 25.4, 26.6 (2**C**), 30.5, 48.8, 60.5, 62.1, 65.6, 79.4, 98.4, 118.7, 119.9, 126.4, 128.7, 129.8, 136.4, 143.6, 159.8, 166.8, 192.3. HRMS $(M+Na)^+$ 411.1786, $C_{22}H_{28}O_6Na$ requires 411.1784.

2,2-dimethyl-6-(4-methylbenzyl)chroman-4-one (**20**) To a solution of **26** (49mg, 0.24mmol) in toluene (10mL) was added **18** and a few crystals of PFPAT. The reaction was heated to reflux for 5h, cooled and allowed to stir at room temperature overnight. Excess solvent was removed *in vacuo*, and the crude residue purified by flash column chromatography to afford a white crystalline solid (34mg). IR ν_{max} (neat) 1372, 1484, 1610, 1684, 2924cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 1.48 (6H, s, (C**H**$_3$)$_2$), 2.28 and 2.34 (3H, 2 x s, ArC**H**$_3$), 2.73 (2H, s, C**H**$_2$CO), 3.91 and 3.96 (2H, 2 x s, ArC**H**$_2$Ar), 6.87 (1H, d, *J*=8.3Hz, Ar**H**), 7.09-7.21 (4H, m, 4 x Ar**H**), 7.25 and 7.31 (1H, 2 x dd, *J*= 8.4, 1.9Hz, Ar**H**), 7.70 and 7.75 (1H, 2 x d, *J*=1.5Hz, Ar**H**). ^{13}CNMR δ_C 19.8 & 21.1, 26.7 (2**C**), 38.5 & 40.6, 48.9, 79.1, 118.4 & 118.5, 119.9, 126.1 x 2, 126.2 & 126.7, 128.7, 129.3, 129.9, 130.4, 132.8 & 133.9, 135.7 & 136.5, 136.7 & 136.9, 137.8 & 138.6, 158.4 & 158.5, 192.7 x 2. HRMS $(M+H)^+$ 281.1547, $C_{19}H_{21}O_2$ requires 281.1542.

(2,2-dimethyl-4-oxochroman-6-yl)methyl (*E*)-2-(hydroxymethyl)but-2-enoate (**11**) *p*-Toluenesulfonic acid monohydrate (5mg, 0.026mmol) was added to a solution of **19b** (10mg, 0.026mmol, and MeOH (5mL) at room temperature. This solution was maintained for 24h at room temperature, quenched with saturated aqueous $NaHCO_3$ (10mL), and then concentrated. The resulting mixture was extracted with EtOAc (2 x 10mL). The combined organic extracts were washed with brine (10mL), dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography to give 6.1mg of the corresponding alcohol (78%). IR ν_{max} (neat) 1129, 1193, 1262, 1490, 1690, 2852, 2922, 3454cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 1.47 (6H, s, C(C**H**$_3$)$_2$), 1.9 (3H, d, *J*=7.3Hz, C**H**$_3$CH=C), 2.73 (2H, s, C**H**$_2$C=O), 4.37 (2H, br. s, HOC**H**$_2$), 5.15 (2H, s, OC**H**$_2$Ar), 6.94 (1H, d, *J*=8.4Hz, **H**8), 7.02 (1H, q, *J*=7.2Hz, C=C**H**), 7.50 (1H, dd, *J*=8.5, 2.4Hz, **H**7), 7.87 (1H, d, *J*=2.4Hz, **H**5). ^{13}CNMR δ_C 14.3 (**C**H$_3$C=C), 26.6 ((**C**H$_3$)$_2$), 48.8 (**C**H$_2$C=O), 57.0 (**C**H$_2$OH), 65.8 (COOC**H**$_2$), 79.5 (**C**2), 118.8 (Ar**C**H), 120.0 (Ar**C**), 126.7 (Ar**C**H), 128.3 (Ar**C**), 131.6 (Ar**C**), 136.4 (Ar**C**H), 141.3 (Ar**C**H), 160.0 (Ar**C**), 167.2 (O**C**=O), 192.3 (**C**4). HRMS $(M+Na)^+$ 327.1193, $C_{17}H_{20}O_5Na$ requires 327.1208.

methyl 3-hydroxy-2-methylenebutanoate (**22**) A solution of acetaldehyde (6.25mL, 111.4mmol), methyl acrylate (15.0mL, 166.6mmol), and 1,4-

diazabicyclo[2.2.2loctane (1.5g, 13.4mmol) was stirred for 7 days at 25°C under an atmosphere of nitrogen before being diluted with diethylether (200mL) and washed with H_2O (200mL). The aqueous layer was separated, acidified to pH 6 with 1M HCl, and extracted with Et_2O (200mL). The combined ethereal layers were washed with brine, dried over $MgSO_4$, and concentrated *in vacuo* to yield the alcohol as a pungent, colourless oil (6.46g, 45%) [16]. ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 1.33 (3H, d, *J*=6.5Hz, CHC**H**$_3$), 3.07 (1H, br., O**H**), 3.74 (3H, s, COOC**H**$_3$), 4.59 (1H, m, C**H**CH_3), 5.81 (1H, d, *J*=1Hz, 1H of C**H**$_2$), 6.17 (1H, s, 1H of C**H**$_2$). ^{13}CNMR δ_C 22.2, 51.8, 66.8, 124.0, 143.7, 167.0.

methyl (*Z*)-2-(bromomethyl)but-2-enoate (**23**) To a 0°C solution of *N*-bromosuccinimide (9.8g, 55.1mmol) in CH_2Cl_2 (50mL) under nitrogen was slowly added dimethyl sulphide (4.4mL, 59.7mmol) in dry CH_2Cl_2 (30 mL), over 10 minutes. A solution of **22** (6.46g, 49.6mmol) in dry CH_2Cl_2 (30mL) was then added dropwise over 15min. The reaction was stirred for 17h at 25°C before being diluted with pentane (200mL) and poured into a chilled mixture of H_2O and brine (1:1). The aqueous layer was separated and extracted with Et_2O (3 x 80mL). The combined ethereal layers were dried (Na_2SO_4) and concentrated *in vacuo* before purification by flash chromatography to yield the known [16] bromide as a pale oil (7.59g, 79%). IR ν_{max} (neat) 1263, 1436, 1647, 1709cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 1.86 (3H, d, *J*=7.3Hz, C**H**$_3$CH=C), 3.73 (3H, s, COOC**H**$_3$), 4.17 (2H, s, C**H**$_2$), 7.01 (1H, q, *J*=7.3Hz, C=C**H**). ^{13}CNMR δ_C 14.5 (**C**H_3C=C), 24.0 (**C**H_2), 52.1 (COO**C**H_3), 130.3 (**C**CH_2Br), 143.3 (C=**C**H), 165.9 (**C**=O).

methyl (*E*)-2-(hydroxymethyl)but-2-enoate (**24**) A solution of **23** (8.20g, 21.8mmol) and sodium acetate (5.3g, 63.7mmol) in anhydrous MeOH (90mL) was heated to reflux for 3.5 h under N_2. The mixture was cooled, and then anhydrous K_2CO_3 (3.00g, 21.8mmol) was added. The resulting slurry was stirred for 15 h at 25°C before being filtered. The filtrate was concentrated to ca. 25% of the original volume, diluted with EtOAc (200mL), and washed with H_2O (50mL). The aqueous layer was separated, acidified to pH = 3 with 1M HCl, and extracted with EtOAc (3 x 50mL). The combined organic extracts were washed with H_2O and brine, dried (Na_2SO_4), and concentrated *in vacuo* to yield the crude product (2.18g, 77%) which was not

purified further [16]. IR ν_{max} (neat) 1278, 1436, 1651, 1706, 3420cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 1.84 (3H, d, *J*=7.3Hz, C=CHC**H**$_3$), 3.70 (3H, s, COOC**H**$_3$), 4.29 (2H, s, C**H**$_2$), 6.92 (1H, q, *J*=7.3Hz, C=C**H**). ^{13}CNMR δ_C 14.1 (**C**H_3C=C), 51.8 (COO**C**H_3), 56.6 (**C**H_2), 131.7 (**C**CH_2OH), 141.1 (C=**C**H), 167.9 (**C**=O).

methyl (*E*)-2-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)but-2-enoate (**25**) To a solution of **24** (0.24g, 1.85mmol) in DCM (10mL) was added 3,4-dihydropyran (0.47g, 5.60mmol) and a few crystals of pyridine *p*-toluenesulfonate. The reaction was allowed to stir at room temperature for 72 hours, after which time the reaction was partitioned between saturated sodium bicarbonate/ethyl acetate, the organic solvent evaporated, and the crude residue purified by flash chromatography to yield the product as a pale oil (0.27g, 68%): IR ν_{max} (neat) 905, 974, 1022, 1117, 1235, 1436, 1716, 2946cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 1.42-1.78 (6H, m, 3 x C**H**$_2$), 1.87 (d, *J*=7.0Hz, C**H**$_3$CH=C), 3.44-3.50 (1H, m, H of OC**H**$_2CH_2$), 3.69 (3H, s, OC**H**$_3$), 3.82-3.88 (1H, m, H of OC**H**$_2CH_2$), 4.18 (1H, d, *J*=11Hz, H of C=CC**H**$_2$O), 4.43 (1H, d, *J*=11Hz, H of C=CC**H**$_2$O), 4.60 (1H, dd, *J*=3.9, 3.1Hz, OC**H**O), 7.04 (1H, q, *J*=7.2Hz, C=C**H**).

(*E*)-2-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)but-2-enoic acid (**26**) To a solution of **25** (1.163g, 5.43mmol) in THF/H_2O (2:1, 15mL) was added lithium hydroxide (0.13g, 5.43mmol). The reaction was stirred at room temperature overnight, diluted with 1M HCl and extracted twice with ethyl acetate (2 x 50mL). The organic layers were combined, dried over Na_2SO_4 and concentrated, to afford the acid as a colourless oil (0.83g, 76%). IR ν_{max} (neat) 1021, 1438, 1689, 2941cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 1.40-1.81 (6H, m, 3 x C**H**$_2$), 1.89 (d, *J*=7.3Hz, C**H**$_3$CH=C), 3.44-3.50 (1H, m, H of OC**H**$_2CH_2$), 3.81-3.87 (1H, m, H of OC**H**$_2CH_2$), 4.17 (1H, d, *J*=11Hz, H of C=CC**H**$_2$O), 4.42 (1H, d, *J*=11Hz, H of C=CC**H**$_2$O), 4.62 (1H, dd, *J*=3.5Hz, OC**H**O), 7.15 (1H, q, *J*=7.3Hz, C=C**H**). HRMS $(M+H)^+$ 199.0962, $C_{10}H_{17}O_4$ requires 199.0970.

4-cyanophenyl acetate (**28**) To a solution of 4-cyanophenol **27** (10g, 83.9mmol) in tetrahydrofuran (250mL) at 0^{o}C was added, dropwise, triethylamine (17.6mL, 125.9mmol) and acetyl chloride (9.0mL, 125.9mmol). The solution was stirred for 24

hours, allowing it to reach room temperature. The reaction was extracted with saturated ammonium chloride (2 x 50mL), and the aqueous layer further extracted with ethyl acetate (3 x 30mL). The combined organic layers were dried over sodium sulfate and the residual solvent removed *in vacuo* to afford the ester **28**, in quantitative yield (13.5g), as a pale brown oil that solidified on standing [27]. IR ν_{max} (neat) 1162, 1181, 1370, 1498, 1601, 1760, 2229cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 2.33 (3H, s, C**H**$_3$), 7.24 (2H, br. d, *J*~8Hz, 2 x Ar**H**), 7.69 (2H, br. d, *J*~8Hz, 2 x Ar**H**). ^{13}CNMR δ_C 21.1, 109.7, 118.3, 122.8 (2**C**), 133.7 (2**C**), 153.9, 168.6.

3-acetyl-4-hydroxybenzonitrile (**29**) To **28** (8.9g, 55.3mmol) was added freshly powdered aluminium chloride (26g, 195mmol). The solid mixture was heated at 160^{o}C under N_2 for 3 hours. The reaction was then cooled to room temperature, and the resultant black solid crushed. The black solid was cooled on ice, and slowly acidified (pH 1-2) with 1M HCl to afford a brown precipitate. The precipitate was dissolved in dichloromethane and dried with Na_2SO_4. The residual solvent was removed *in vacuo*, and the crude residue purified by flash column chromatography to afford the product (**29**) as a white solid (6.15g, 55.4%). IR ν_{max} (neat) 1195, 1302, 1480, 1647, 2225, 3073cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 2.62 (3H, s, C**H**$_3$), 7.01 (1H, d, *J*=8.5Hz, **H**5), 7.65 (1H, dd, *J*=8.7, 2Hz, **H**6), 8.02 (1H, d, *J*=2Hz, **H**2), 12.64 (1H, s, O**H**). ^{13}CNMR δ_C 26.7, 102.7, 118.2, 119.8, 120.1, 135.8, 138.8, 165.5, 203.6 [27].

2,2-dimethyl-4-oxochromane-6-carbonitrile (**30**) To a solution of **29** (2.74g, 17.0mmol) in toluene (30mL) was added pyrrolidine (0.5mL, 5.99mmol) and acetone (1.7mL, 23.2mmol). The resulting solution was stirred at 90°C for 3 hours in a Dean Stark apparatus and then cooled. The cold mixture was evaporated *in vacuo*, and the residue purified by flash column chromatography to afford known [28] chromanone (**30**) as an off-white solid (1.95g, 57%). IR ν_{max} (neat) 1179, 1292, 1606, 1691, 2225, 2920cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 1.42 (6H, s, C(C**H**$_3$)$_2$), 2.71 (2H, s, C**H**$_2$), 6.96 (1H, d, *J*=8.8Hz, **H**8), 7.63 (1H, dd, *J*=8.8, 2.3Hz, **H**7), 8.11 (1H, d, *J*=2.3Hz, **H**5). ^{13}CNMR δ_C 26.6, 48.2, 80.9, 104.6, 118.2, 119.9, 120.4, 131.9, 138.5, 162.6, 190.4.

***tert*-butyl ((2,2-dimethyl-4-oxochroman-6-yl)methyl)carbamate** (**31**) To a stirred solution of **30** (1.58g, 7.86mmol) in dry methanol (30mL) at 0^{o}C and under N_2, were added Boc_2O (3.43g, 15.7mmol) and $NiCl_2{\cdot}6H_2O$ (190mg, 0.80mmol). $NaBH_4$ (2.08g, 55.0mmol) was then added in small portions over 20min. The reaction was exothermic and effervescent. The resulting reaction mixture containing a finely divided black precipitate was allowed to warm to room temperature and left to stir for 15hr, at which point diethylenetriamine (0.75mL, 6.94mmol) was added. The mixture was allowed to stir for 30 min before solvent evaporation. The purple residue was dissolved in EtOAc (50mL) and extracted with saturated $NaHCO_3$ (2 x 50mL).The organic layer was dried (Na_2SO_4) and the solvent removed *in vacuo* to yield a mixture of products, with upon purification by flash column chromatography afforded as the predominant product the carbamate alcohol: ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 1.47 (3H, s, C(C**H**$_3$), 1.60 (3H, s, C(C**H**$_3$), 1.62 (9H, s, C(C**H**$_3$)$_3$), 2.02 (1H, dd, *J*=13.3, 8.8Hz, 1H of **H**3), 2.33 (1H, dd, *J*=13.3, 6.1Hz, 1H of **H**3), 4.40 (2H, d, *J*=6.0Hz, NC**H**$_2$), 4.94 (1H, br. s, N**H**), 4.99 (1H, m, H**4**), 6.91 (1H, d, *J*=8.5Hz, **H**8), 7.26 (1H, br. d, **H**7), 7.53 (1H, d, *J*=2.0Hz, **H**5). ^{13}CNMR δ_C 25.9, 28.4, 28.9, 42.7, 63.6, 75.4, 117.5, 124.3, 128.8, 128.7, 130.6, 152.5, 155.9). To a stirred solution of this alcohol (0.5g, mmol) in acetone (30mL) at 0^{o}C, was added the Jones reagent (2mL). The resulting reaction was allowed to warm to room temperature and left to stir for 2hr. On appearance of the green $Cr_2(SO_4)_3$, anhydrous sodium sulphate (0.30g, 2.12mmol) was added. After 2 hours, the solvent was evaporated, and the reaction separated using ether/water. After three washings with ether (3 x 20mL), the combined organic extracts were filtered and the solvent removed *in vacuo*. The residue was purified by flash column chromatography on silica gel to yield the amide as a clear oil, which solidified on standing (0.41g, 83%). IR ν_{max} (neat) 1165, 1250, 1270, 1492, 1525, 1685, 2975, 3392cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 1.38 (6H, s, C(C**H**$_3$)$_2$), 1.39 (9H, s, C(C**H**$_3$)$_3$), 2.64 (2H, s, C**H**$_2$), 4.20 (3H, d, *J*=6.0Hz, NHC**H**$_2$), 4.80 (1H, br. s, N**H**), 6.83 (1H, d, *J*=8.5Hz, **H**8), 7.35 (1H, br. d, *J*=8.5, **H**7), 7.67 (1H, d, **H**5). ^{13}CNMR δ_C 26.6 ((**C**H$_3$)$_2$), 28.4 C(C**H**$_3$)$_3$), 43.8 (**C**H$_2$), 48.8 (**C**H$_2$), 79.3 (**C2**), 118.8 (Ar**C**H), 119.9 (Ar**C**), 124.9 (Ar**C**H), 131.4 (Ar**C**), 135.7 (Ar**C**H), 155.8 (Ar**C**), 159.3 (NH**C**=O), 192.5 (**C**4).

(*E*)-*N*-((2,2-dimethyl-4-oxochroman-6-yl)methyl)-2-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)but-2-enamide (**32**) To a solution of **31** (542mg, 1.78mmol) in DCM (5ml) at 0^{o}C was added trifluoroacetic acid (5mL). The reaction was stirred overnight, allowing the reaction to reach room temperature. When TLC analysis showed completion of the reaction, the solvent was removed *in vacuo*, removing any remaining TFA azeotropically. After washing with base, extracting with ether and removing the solvent gave the free amine, a dark oil, which was reacted without further purification: 0.05g, 0.24mmol of this amine was added to a solution of **26** (50mg, 0.25mmol) in dichloromethane (10mL) at 0^{o}C containing EDC HCl (0.07g, 0.37mmol) and triethylamine (0.046mL, 0.33mmol). The reaction was allowed to reach room temperature and stirred for 24 hours under a N_2 atmosphere. The residual solvent was removed *in vacuo*, and the crude residue purified by flash column chromatography to afford amide **32** as a colourless oil (45mg, 38%). IR ν_{max} (neat) 1189, 1257, 1300, 1487, 1532, 1616, 1667, 2975, 3338cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 1.33-1.49 (4H, m, C**H**$_2$C**H**$_2$ pyran), 1.38 (6H, s, C(C**H**$_3$)$_2$), 1.57-1.69 (2H, m, C**H**$_2$ pyran), 1.80 (3H, d, *J*=7.3Hz, C**H**$_3$CH=C), 2.63 (2H, s, C**H**$_2$C=O), 3.40 (1H, m, H of OC**H**$_2$ pyran) 3.68 (1H, m, H of OC**H**$_2$ pyran), 4.33 (2H, q, *J*=10.5Hz, OC**H**$_2$C=C), 4.39 (2H, dd, *J*=5.8, 1.8Hz, NHC**H**$_2$), 4.52 (1H, dd, *J*=5.1, 2.6Hz, OC**H**O), 6.82 (1H, d, *J*=8.5Hz, **H**8), 6.95 (1H, q, *J*=7.3Hz, C=C**H**), 7.21 (1H, br., N**H**), 7.38 (1H, dd, *J*=8.5, 2.5Hz, **H**7), 7.69 (1H, d, *J*=2.3Hz, **H**5).

(*E*)-*N*-((2,2-dimethyl-4-oxochroman-6-yl)methyl)-2-(hydroxymethyl)but-2-enamide (**12**) *p*-Toluenesulfonic acid monohydrate (5mg, 0.026mmol) was added to a solution of **32** (10mg, 0.026mmol, and MeOH (5mL) at room temperature. This solution was maintained for 7h at room temperature, quenched with saturated aqueous $NaHCO_3$ (10 mL), and then concentrated. The resulting mixture was extracted with EtOAc (2 x 10mL). The combined organic extracts were washed with brine (10mL), dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography to give the alcohol (5.2mg, 66%). IR ν_{max} (neat) 1257, 1299, 1488, 1531, 1616, 1667, 1686, 2925, 3334cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 1.38 (6H, s, C(C**H**$_3$)$_2$), 1.77 (3H, d, *J*=7.0Hz, C**H**$_3$CH=C), 2.63 (2H, s, C**H**$_2$C=O), 2.76 (1H, br., O**H**), 4.34 (2H, br. s, HOC**H**$_2$), 4.38 (2H, d, *J*=5.8Hz, NHC**H**$_2$), 6.58 (1H, q, *J*=7Hz, C=C**H**), 6.82 (1H, d, *J*=8.5Hz, **H**8), 6.88 (1H, br., N**H**), 7.37 (1H, dd, *J*=8.5,

2.3Hz, **H**7), 7.66 (1H, d, *J*=2.3Hz, **H**5). ^{13}CNMR δ_C 13.6 (**C**H_3C=C), 26.6 ((**C**H_3)$_2$), 42.7 (NH**C**H_2), 48.8 (**C**H_2C=O), 57.6 (**C**H_2OH), 79.3 (**C2**), 118.9 (Ar**C**H), 119.9 (Ar**C**), 125.2 (Ar**C**H), 130.9 (Ar**C**), 134.0 (Ar**C**), 134.7 (Ar**C**H), 136.0 (Ar**C**H), 159.4 (Ar**C**), 168.7 (NH**C**=O), 192.6 (**C**4). HRMS $(M+H)^+$ 302.1404, $C_{17}H_{22}NO_4$ requires 302.1392.

4-amino-3-iodobenzonitrile (**34**) To a solution of 4-aminobenzonitrile **33** (5g, 42.3mmol) in glacial acetic acid (25mL), was slowly added a solution of iodine monochloride (6.9g, 42mmol) in glacial acetic acid (5mL). The mixture was stirred at room temperature for 30 minutes. The reaction mixture was then poured into 250mL of cold water and stirred vigorously for 10 minutes to yield a suspension of brown solids. The suspension was filtered and recrystallized from methanol/water to give a pale pink lustrous solid which was dried *in vacuo*. IR ν_{max} (neat) 1293, 1386, 1512, 1665, 2235cm^{-1}. ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 4.56 (2H, br. s, N**H**$_2$), 6.64 (1H, d, *J*=8.4Hz, **H**5), 7.33 (1H, dd, *J*=8.4, 1.9Hz, **H**6), 7.83 (1H, d, *J*=1.9Hz, **H**2) [29].

***N*-(4-cyano-2-iodophenyl)acetamide** (**35**) To a solution of **30** (8.5g, 35mmol) in acetic anhydride (20mL, 0.21mol) was added 6 drops of conc. H_2SO_4. The reaction was stirred with gentle heating to 50^{o}C for 30 minutes, before pouring over 400mL of cold water and stirring for 10 minutes at room temperature to yield a suspension of a white solid. The solid was filtered and dried to yield the acetamide as white needles. IR ν_{max} (neat) 1296, 1384, 1514, 1660, 2230, 3272cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 2.22 (3H, s, COC**H**$_3$), 7.54-7.57 (2H, m, **H**5 & N**H**), 7.98 (1H, d, *J*=1.8Hz, **H**3), 8.40 (1H, d, *J*=8.8Hz, **H**6) [29].

N-(4-cyano-2-(3-hydroxy-3-methylbut-1-yn-1-yl)phenyl)acetamide (**36**) To 5g (17.5mmol) of **35** were added water (25mL), $PdCl_2$ (30mg, 1mol%) and pyrrolidine (7.3mL, 87.5mmol) under aerobic conditions. The resulting mixture was stirred at 50°C for 5 min. To this mixture was added 2-methylbut-3-yn-2-ol (1.7mL, 21mmol), and the reaction mixture was stirred at 110 °C for 5h. The reaction was then extracted with EtOAc (3 x 10 mL), and the combined organic layers dried with anhydrous sodium sulfate. The solvent was removed *in vacuo* to give a crude residue, which was reacted as such in the next step. IR ν_{max} (neat) 1454, 1508,

1614, 2217, 2876, 2978, 3341cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 1.65 (6H, s, (C**H**$_3$)$_2$), 2.26 (3H, s, C**H**$_3$CO), 7.52 (1H, dd, *J*=8.7, 1.9Hz, **H**5), 7.60 (1H, d, *J*=2Hz, **H**3), 8.44 (1H, d, *J*=8.5Hz, **H**6), 8.60 (1H, br. s, N**H**).

2,2-dimethyl-4-oxo-1,2,3,4-tetrahydroquinoline-6-carbonitrile (**37**) To crude **36** (3g, 12.4mmol) was added a 1:1 mixture of conc. HCl/H_2O (20mL). The reaction was heated at 80^{o}C for 5 hours, after which the reaction was extracted with ethyl acetate/water (3 x 50 mL), and the combined organic layers dried with anhydrous sodium sulfate. The solvent was removed *in vacuo*, and the crude residue purified by flash chromatography to give (**37**) as a brown oil (1.1g, 44%). IR ν_{max} (neat) 804, 1514, 1612, 1669, 2219, 2922, 3322cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 1.29 (6H, s, C**H**$_3$CC**H**$_3$), 2.55 (2H, s, C**H**$_2$), 6.57 (1H, d, *J*=8.8Hz, **H**8), 7.39 (1H, dd, *J*=8.5, 2Hz, **H**7), 8.03 (1H, d, *J*=2Hz, **H**5). ^{13}CNMR δ_C 27.7 (**C**H$_3$C**C**H$_3$), 49.9 (**C**3), 53.7 (**C**2), 100.0, 116.3 (Ar**C**H), 117.5, 119.1, 132.8 (Ar**C**H), 137.3 (Ar**C**H), 151.7, 191.8 (**C**=O).

***tert*-butyl ((2,2-dimethyl-4-oxo-1,2,3,4-tetrahydroquinolin-6-yl)methyl)carbamate** (**38**) To a stirred solution of **37** (0.5g, 2.5mmol) in dry methanol (20mL) at 0^{o}C, were added Boc_2O (0.6g, 2.75mmol) and $NiCl_2{\cdot}6H_2O$ (0.06g, 0.25mmol). $NaBH_4$ (0.47g, 12.4mmol) was then added in small portions over 10 min. The reaction was exothermic and effervescent. The resulting reaction mixture containing a finely divided black precipitate was allowed to warm to room temperature and left to stir for 15hr, at which point diethylenetriamine (0.26mL, 2.4mmol) was added. The mixture was allowed to stir for 30 min before solvent evaporation. The purple residue was dissolved in EtOAc (50mL) and extracted with saturated $NaHCO_3$ (2 x 50mL).The organic layer was dried (Na_2SO_4) and the solvent removed *in vacuo* to yield a mixture of products, including carbamate (**38**) (0.25g, 33%). IR ν_{max} (neat) 645, 802, 1162, 1274, 1515, 1623, 1651, 1695, 3316cm^{-1}. ^{1}H NMR ($CDCl_3$, 400MHz) δ_H 1.24 (6H, s, C**H**$_3$CC**H**$_3$), 1.38 (9H, s, (C**H**$_3$)$_3$C), 2.50 (2H, s, C**H**$_2$), 4.12 (3H, m, NC**H**$_2$ and N**H**(CH$_3$)$_2$), 4.73 (1H, br. s, N**H**CO), 6.52 (1H, d, *J*=8.5Hz, **H**8), 7.18 (1H, br. d, *J*=8.5Hz, **H**7), 7.61 (1H, d, *J*=2Hz, **H**5). ^{13}CNMR δ_C 27.6 (2**C**), 28.4 (3**C**), 44.0, 50.5, 53.6, 79.4, 116.3, 117.6, 125.8, 127.8, 135.2, 149.3, 155.9, 193.8.

General method (**13a**-**f**)

To a solution of appropriate acid (0.49mmol) in dichloromethane (10mL) at 0^{o}C was added EDC (0.08g, 0.52mmol) and triethylamine (0.08mL, 0.57mmol). After 30 minutes, the primary amine liberated via trifluoroacetic acid treatment of **38** (100mg, 0.49mmol) was added to this solution. The reaction was allowed to reach room temperature and stirred for 24 hours. The residual solvent was removed *in vacuo*, the residue extracted with saturated bicarbonate/DCM, and the crude organic residue purified by flash column chromatography (Pet. Ether:EtOAc 1:1) to afford the amide.

(*E*)-*N*-((2,2-dimethyl-4-oxo-1,2,3,4-tetrahydroquinolin-6-yl)methyl)-2-(((tetrahydro-2H-pyran-2-yl)oxy)methyl)but-2-enamide (**13a**) Yellow oil, (89mg, 47%). IR ν_{max} (neat) 1021, 1506, 1619, 1662, 2923, 3306cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) 1.31 (6H, s, C**H**$_3$CC**H**$_3$), 1.40-1.72 (6H, m, (C**H**$_2$)$_3$), 1.86 (3H, d, *J*=7.2Hz, C**H**$_3$), 2.57 (2H, s, C**H**$_2$CO), 3.47 (1H, m, H of OC**H**$_2$ pyran), 3.74 (1H, m, H of OC**H**$_2$ pyran), 4.12 (1H, m, N**H**), 4.34-4.42 (4H, m, OC**H**$_2$C=C and NHC**H**$_2$, 4.59 (1H, m, OC**H**O), 6.58 (1H, d, *J*=8.4Hz, **H**8), 7.01 (1H, q, *J*=7.2Hz, CH=C**H**), 7.19 (1H, br., N**H**), 7.29 (1H, dd, *J*=8.4, 2.1Hz, **H**7), 7.71 (1H, d, *J*=2Hz, **H**5). ^{13}CNMR δ_C 13.8, 19.7, 25.2, 27.7 (2**C**), 30.5, 43.0, 50.6, 53.6, 60.7, 63.0, 97.7, 116.3, 117.7, 126.2, 127.6, 130.4, 135.7, 138.7, 149.2, 167.5, 193.8. HRMS $(M+H)^+$ 387.2265, $C_{22}H_{31}N_2O_4$ requires 387.2284.

(*E*)-*N*-((2,2-dimethyl-4-oxo-1,2,3,4-tetrahydroquinolin-6-yl)methyl)but-2-enamide (**13b**) Yellow oil, (88mg, 66%); ^{1}H NMR ($CDCl_3$, 400MHz) 1.31 (6H, s, C**H**$_3$CC**H**$_3$), 1.85 (3H, dd, *J* = 6.9, 1.7Hz, CHC**H**$_3$), 2.57 (2H, s, C**H**$_2$), 4.37 (2H, d, *J*=5.7Hz, NHC**H**$_2$, 5.77 (1H, br. s, N**H**), 5.79 (1H, m, C**H**=CHCH$_3$), 6.59 (1H, d, *J*=8.3Hz, **H**8), 6.87 (1H, m, CH$_3$C**H**=C), 7.28 (1H, dd, *J*=2.2Hz, **H**7), 7.68 (1H, d, *J*=2.3Hz, **H**5). ^{13}CNMR δ_C 17.8, 27.6 (2**C**), 42.8, 50.5, 53.6, 116.4, 117.6, 124.9, 126.1, 127.3, 135.7, 140.3, 149.4, 165.8, 193.9. HRMS $(M+H)^+$ 273.1601, $C_{16}H_{21}N_2O_2$ requires 273.1603.

(*E*)-*N*-((2,2-dimethyl-4-oxo-1,2,3,4-tetrahydroquinolin-6-yl)methyl)-2-methylbut-2-enamide (**13c**) Yellow solid, (87mg, 62%). IR ν_{max} (neat) 634, 1190, 1278, 1289,

1516, 1604, 1656, 3263, 3347cm^{-1}. ^{1}H NMR ($CDCl_3$, 400MHz) 1.32 (6H, s, C**H**$_3$CC**H**$_3$), 1.74 (3H, dd, *J* = 7.0, 1.0Hz, CHC**H**$_3$), 1.84 (3H, m, C**H**$_3$C=CH), 2.58 (2H, s, C**H**$_2$), 4.37 (2H, d, *J*=5.6Hz, NHC**H**$_2$, 5.96 (1H, br. s, N**H**), 6.45 (1H, m, CH_3C**H**=C), 6.59 (1H, d, *J*=8.4Hz, **H**8), 7.29 (1H, dd, *J*=8.4, 2.2Hz, **H**7), 7.69 (1H, d, *J*=2.3Hz, **H**5). ^{13}CNMR δ_C 12.5, 13.9, 27.7 (2**C**), 43.1, 50.5, 53.6, 116.5, 117.6, 126.2, 127.4, 131.0, 131.6, 135.7, 149.4, 169.2, 193.9. HRMS $(M+H)^+$ 287.1707, $C_{17}H_{23}N_2O_2$ requires 287.1760.

***N*-((2,2-dimethyl-4-oxo-1,2,3,4-tetrahydroquinolin-6-yl)methyl)-3-methylbut-2-enamide** (**13d**) Yellow oil, (77mg, 55%). IR ν_{max} (neat) 599, 654, 683, 1163, 1252, 1517, 1618, 3251cm^{-1}. ^{1}H NMR ($CDCl_3$, 400MHz) 1.31 (6H, s, C**H**$_3$CC**H**$_3$), 1.83 (3H, d, *J* = 0.9Hz, C=CC**H**$_3$), 2.17 (3H, d, *J*=0.9Hz, C=CC**H**$_3$), 2.56 (2H, s, C**H**$_2$), 4.33 (2H, d, *J*=5.7Hz, NHC**H**$_2$, 5.57 (1H, br., C=C**H**), 5.75 (1H, br. s, N**H**), 6.59 (1H, d, *J*=8.4Hz, **H**8), 7.27 (1H, dd, *J*=8.4, 2.2Hz, **H**7), 7.67 (1H, d, *J*=2.2Hz, **H**5). ^{13}CNMR δ_C 19.9, 27.2, 27.6 (2**C**), 42.5, 50.5, 53.6, 116.4, 117.5, 118.3, 126.0, 127.5, 135.6, 149.4, 151.4, 166.8, 193.9. HRMS $(M+H)^+$ 287.1751, $C_{17}H_{23}N_2O_2$ requires 287.1760.

(*E*)-*N*-((2,2-dimethyl-4-oxo-1,2,3,4-tetrahydroquinolin-6-yl)methyl)-4-phenylbut-3-enamide (**13e**) Yellow oil, (129mg, 75%). IR ν_{max} (neat) 693, 966, 1163, 1302, 1506, 1620, 1645, 3297cm^{-1}. ^{1}H NMR ($CDCl_3$, 400MHz) 1.30 (6H, s, C**H**$_3$CC**H**$_3$), 2.55 (2H, s, C**H**$_2$), 3.18 (2H, d, *J*=7.2Hz, C**H**$_2$C=C), 4.24 (1H, br., N**H**), 4.31 (2H, d, *J*=5.6Hz, NHC**H**$_2$, 5.99 (1H, br. s, N**H**), 6.29 (1H, m, C**H**=CH), 6.52 (1H, d, *J*=15.9Hz, CH=C**H**), 6.58 (1H, d, *J*=8.4Hz, **H**8), 7.21-7.37 (6H, m, 6 x Ar**H**), 7.66 (1H, d, *J*=2.1Hz, **H**5). ^{13}CNMR δ_C 27.7 (2C), 40.9, 43.1, 50.5, 53.6, 116.5, 117.6, 122.3, 126.2, 126.4 (2**C**), 127.0, 127.8, 128.6 (2C), 134.7, 135.6, 136.6, 149.4, 170.6, 193.9. HRMS $(M+H)^+$ 349.1902, $C_{22}H_{25}N_2O_2$ requires 349.1916.

(*E*)-*N*-((2,2-dimethyl-4-oxo-1,2,3,4-tetrahydroquinolin-6-yl)methyl)-3-(thiophen-2-yl)acrylamide (**13f**) Yellow solid, (110mg, 69%). IR ν_{max} (neat) 704, 820, 959, 1211, 1526, 1614, 3252cm^{-1}. ^{1}H NMR ($CDCl_3$, 400MHz) 1.25 (6H, s, C**H**$_3$CC**H**$_3$), 2.51 (2H, s, C**H**$_2$), 4.37 (2H, d, *J*=5.6Hz, NHC**H**$_2$, 5.76 (1H, br. s, N**H**), 6.12 (1H, d, *J*=15.3Hz, C**H**=CH), 6.53 (1H, d, *J*=8.4Hz, **H**8), 6.96 (1H, m, Ar**H**), 7.13 (1H, d, *J*=3.7Hz, Ar**H**), 7.22-7.25 (2H, m, 2 x Ar**H**), 7.65 (1H, d, *J*=2.0Hz, **H**5), 7.70 (1H, d,

J=15.2Hz, CH=C**H**). ^{13}CNMR δ_C 26.6 (2**C**), 28.7, 42.1, 49.5, 52.6, 115.5, 116.6, 118.3, 125.2, 126.1, 126.3, 127.0, 129.3, 133.1, 134.7, 138.9, 148.4, 164.4, 192.8. HRMS $(M+H)^+$ 341.1310, $C_{19}H_{21}N_2O_2S$ requires 341.1324.

(*E*)-*N*-((2,2-dimethyl-4-oxo-1,2,3,4-tetrahydroquinolin-6-yl)methyl)-2-(hydroxymethyl)but-2-enamide (**13g**) Prepared as for (**12**) using 50mg (**13a**); Yellow oil, (22mg, 56%). IR ν_{max} (neat) 1272, 1294, 1506, 1600, 1664, 2921, 3276cm^{-1}; ^{1}H NMR ($CDCl_3$, 400MHz) 1.30 (6H, s, C**H**$_3$CC**H**$_3$), 1.80 (3H, d, *J*=7.2Hz, C**H**$_3$), 2.54 (2H, s, C**H**$_2$CO), 4.34 (2H, d, *J*=5.7Hz, NHC**H**$_2$, 4.38 (2H, s, C**H**$_2$OH), 4.44 (1H, br. s, N**H**), 6.58 (1H, d, *J*=8.4Hz, **H**8), 6.64 (1H, q, *J*=7.1Hz, C=C**H**), 7.16 (1H, br., N**H**), 7.24 (1H, dd, *J*=8.5, 2.3Hz, **H**7), 7.64 (1H, d, *J*=2.3Hz, **H**5). ^{13}CNMR δ_C 13.6, 27.6 (2**C**), 42.8, 50.5, 53.5, 57.3, 116.5, 117.4, 125.9, 127.2, 134.0, 134.8, 135.6, 149.5, 168.8, 194.2. HRMS $(M+Na)^+$ 325.1517, $C_{17}H_{22}N_2O_3Na$ requires 325.1528.

Bioassay procedures

Ethics statement

BALB/c mice were obtained from the animal breeding facility at the Instituto de Investigação e Inovação em Saúde (i3S), University of Porto. The animals were maintained in pathogen free conditions, in individually ventilated cages and were fed with sterilized food and water *ad libitum*. All animal procedures were performed in compliance with the Local Animal Ethics Committee of i3S, licensed by DGAV (Direção Geral de Alimentação e Veterinária, Govt. of Portugal – DGAV). Animals were handled in strict accordance with good animal practice as defined by national authorities (DGAV, directive 113/2013 from 7th August) and European legislation (directive 2010/63/EU, revising directive 86/609/EEC).

Parasite culture

Leishmania parasites used in this work were obtained from infected mice splenocytes by amastigote to promastigote differentiation as described by Sereno *et al* [30]. To ensure infectivity, promastigotes were cultured at 25ºC for a maximum of 10 passages. The insect stage of *L. infantum* (strain MHOM/MA/67/ITMAP263) was

maintained in RPMI 1640 GlutaMAX™-I medium (Gibco® Life Technologies Thermo Fisher) supplemented with 10% heat inactivated Fetal Bovine Serum (FBSi, Gibco® Life Technologies Thermo Fisher), 50U/mL penicillin and 50µg/mL streptomycin (Gibco® Life Technologies Thermo Fisher) and 5 mM HEPES sodium salt pH 7.4. Promastigotes from both *L. major* (strain MHOM/SA/85/JISH118) and *L. amazonensis* (strain MHOM/BR/LTB0016) were kept in Schneider's medium (Sigma-Aldrich) supplemented with 10% FBSi, 100U/mL penicillin and 100µg/mL streptomycin, 2% human urine, 5mg/mL phenol red and 5mM HEPES sodium. For drug assays, the three parasite species were kept in culture at 25ºC for 5 to 7 days without medium renewal to promote the differentiation from the exponential to the stationary phase.

Determination of IC_{50} against *Leishmania* intracellular amastigotes

Macrophages were obtained from BALB/c mice bone-marrow precursors by differentiation with L929 cell conditioned medium (LCCM) as described in Vale-Costa *et al.* [31]. Briefly, bone-marrow cells were seeded onto 96-well plates at a density of $3x10^5$ cells/mL and maintained in culture for 7 days at 37ºC with 5% CO_2 in DMEM medium with GlutaMAX™-I (Gibco® Life Technologies Thermo Fisher) supplemented with 10% FBSi, 50U/mL penicillin and 50µg/mL streptomycin, 1% MEM-NEAA and 10% LCCM. Macrophages were then co-incubated with a rate of 10:1 stationary-phase promastigotes for 3 hours at 37ºC with 5% CO_2, after which non-phagocytosed parasites were removed by washing. Twenty four hours later, test compounds, diluted in in DMEM medium, were added to the infected monolayers, and cells were maintained in culture for a further 24 hours. Infected macrophages subjected to 0.5% DMSO were used as positive control and amphotericin B was used as a standard drug. Activity against *L. infantum* and *L. amazonensis* intracellular amastigotes was determined according to Gomes-Alves *et al.* [32]. For this, cells were fixed, stained with a DAPI (Sigma-Aldrich)/HCS CellMask™ (Invitrogen) solution and photographed in a IN Cell Analyzer 2000 microscope (GE Healthcare). Upon image acquisition, both the total number of macrophages as well as the number of infected cells was determined with the IN Cell Investigator Developer Toolbox v. 1.9.2 software (GE Healthcare). The percentage of infected cells was then used to assess the IC_{50} values determined with Graph Pad Prism software.

Cytotoxicity in mammalian cells

Bone marrow-derived macrophages, obtained as above, were incubated at 37ºC and 5% CO_2 with test compounds, up to a highest concentration of 40 μM for 24 hours. Cell viability was determined using the resazurin assay [31]. The 50% cytotoxic concentration (CC_{50}) was calculated from viability percentage plots in relation to control using Graph Pad Prism software.

Evaluation of compound activity against *Leishmania* promastigotes

Promastigotes in the late exponential phase were seeded at $3x10^6$ cells/mL, in a final volume of 100 μL of either RPMI or Schneider's medium as referred to above. Test compounds were dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich) and diluted in a final volume of 100 μL of RPMI or Schneider medium. The positive control consisted of promastigotes incubated with 0.5% DMSO, and amphotericin B was used as a standard comparator drug. All experimental conditions were performed in triplicate. Parasite viability was evaluated upon 24h exposure to each compound and was assessed using the resazurin assay [31]. The fluorescence intensity was read in a Sinergy™2 Microplate Reader (BioTek Instruments). The 50% inhibitory concentration (IC_{50}) values obtained were determined with GraphPad Prism 5.0 software (GraphPad Software Inc., La Jolla, CA).

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Conflict of Interest

The authors declare no conflicts of interest.

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Figure legends

Figure 1. Natural products with a chromene or chromanone skeleton with activity against *Leishmania*

Figure 2. Synthetic derivatives of naturally occurring chroman, chromene, flavonoid and coumarin systems with activity against *Leishmania*

Figure 3. Synthetic targets based on lead compounds (**2**-**3**)

Schemes sub-text

Scheme 1. Synthesis of compound **11**. Reagents and conditions: (i) CH_3COCl, CF_3SO_3H, 0^{o}C-RT, 24h; (ii) CH_3COCH_3, [bbim]Br, 95–100^{o}C, 8h; (iii) $K_2S_2O_8$, $CuSO_4$, H_2O/CAN (1:1), 75–80^{o}C, 1h; (iv) *D. carota*, H_2O, RT, 72h; (v) (**26**), acrylic acid, EDC, DMAP, 2 weeks, RT; (vi) (**19b**), *p*-toluenesulfonic acid, MeOH, RT, 24h; (vi) acrylic acid, PFPAT, toluene, reflux.

Scheme 2. Synthesis of acid synthon **26**. Reagents and conditions: (i) CH_3CHO, DABCO, RT, 7 days; (ii) NBS, Me_2S, DCM, RT, 17h; (iii) NaOAc, MeOH, Δ, 3.5h, then K_2CO_3, RT, 24h; (iv) 3,4-dihydropyran, DCM, pyridinium *p*-toluenesulfonate; (v) *p*-toluenesulfonic acid, MeOH, RT, 24h.

Scheme 3. Synthesis of compound **12**. Reagents and conditions: (i) CH_3COCl, Et_3N, THF, 0^{o}C-RT, 24h; (ii) $AlCl_3$, 160^{o}C, 3h; (iii) CH_3COCH_3, pyrrolidine, toluene, 90°C, 3h; (iv) $NiCl_2.6H_2O$, Boc_2O, $NaBH_4$, MeOH, 15h, RT; then Jones reagent,

CH_3COCH_3, 0°C-RT, 2h; (v) CF_3CO_2H, DCM, 0°C-RT, 3h, then **26**, EDC, Et_3N, DCM, 0°C-RT, 3h; (vi) *p*-toluenesulfonic acid, MeOH, RT, 24h.

Scheme 4. Synthesis of compounds **13a**-**g**. Reagents and conditions: (i) ICl, CH_3CO_3H, RT, 30min; (ii) Ac_2O, H_2SO_4, 70°C, 10min; (iii) H_2O, $PdCl_2$, 2-methylbut-3-yn-2-ol, pyrrolidine, 110°C, 5h; (iv) conc. HCl/H_2O, 80°C, 5h; (v) $NiCl_2.6H_2O$, Boc_2O, $NaBH_4$, MeOH, 15h, RT; then Jones reagent, CH_3COCH_3, 0°C-RT, 2h; (vi) CF_3CO_2H, DCM, 0°C-RT, 3h, then RCOOH, EDC, Et_3N, DCM, 0°C-RT, 3h; (vii) **13a**, *p*-toluenesulfonic acid, MeOH, RT, 24h.