

Synthesis and biological evaluation of chromone-3-carboxamides

Allen T. Gordon, Isaiah D.I. Ramaite,^{*} and Simon. S. Mnyakeni-Moleele

Department of Chemistry, University of Venda, Private Bag X5050, Thohoyandou, Limpopo, South Africa
E-mail: Isaiah.Ramaite@univen.ac.za

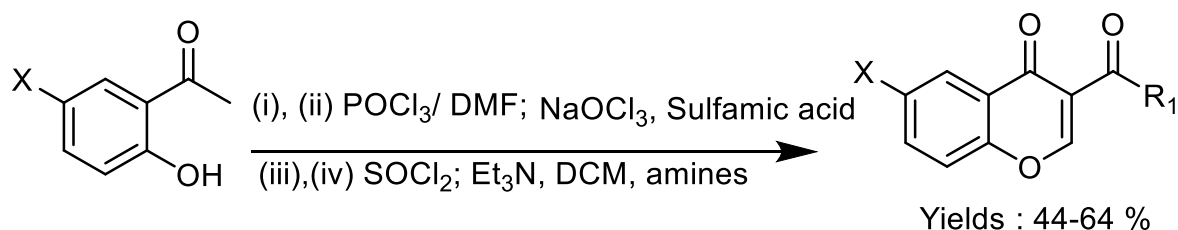
Received 09-05-2020

Accepted 11-30-2020

Published on line 12-15-2020

Abstract

The aim of our study was to synthesize novel chromone-3-carboxamides and to conduct biological evaluations in search for lead compounds for the treatment of a range of debilitating disease states. Corresponding 2-hydroxyacetophenones were subjected to Vilsmeier-Haack formylation to give chromone-3-carbaldehydes, which were subsequently oxidised to give chromone-3-carboxylic acids. Treatment of the carboxylic acids with thionyl chloride resulted in the in situ formation of the corresponding acid chlorides, which were reacted with various amines in the presence of triethylamine to give the corresponding novel chromone-3-carboxamides in good yields. Selected chromone derivatives were then evaluated for their anti-inflammatory, anti-trypanosomal and cytotoxic properties.



Keywords: Chromones, chromone-3-carboxylic acids, Vilsmeier-Haack formylation, anti-inflammatory, anti-trypanosomal, cytotoxicity

Introduction

Chromone (Chromen-4-one /4*H*-1-benzopyran-4-one) **1** is one of the most abundant classes of heterocyclic compounds with oxygen as a heteroatom.¹ The chromone moiety has become a very important structural unit in medicinal chemistry, with a wide variety of activities including antioxidant,² antiviral, anti-inflammatory, antibacterial, antitumor and tyrosine kinase inhibition properties.^{3,4} In addition to their biological activities, chromones have also been reported to have insecticidal activity and fluorescent properties.^{5,6}

These compounds are also used as an active pharmacophore in various therapeutic drugs including cromolyn, nedocromil, diosmin, flavoxate, and many others.⁷ Among chromone derivatives, chromone-3-carbaldehydes (3-formylchromones) **2** are valuable intermediates for the synthesis of various biologically-active compounds due to the presence of an unsaturated keto functional group, a conjugated second carbonyl group at the C-3 position, and an electrophilic centre at the C-2 position.⁸ The wide range of biological activities associated with the chromone compounds, and chemical reactivity of chromone-3-carbaldehydes, prompted us to synthesize novel chromone-3-carboxamide analogues. Herein, we report the results of the synthesis of novel chromone-3-carboxamide analogues from corresponding 2-hydroxyacetophenones in four steps, and their evaluation as potential anti-inflammatory, anti-trypanosomal and cytotoxicity agents.

Inflammation is a reaction to injuries which involves systemic and local responses. The main action of anti-inflammatory agents is the inhibition of cyclooxygenase enzymes which are responsible for the conversion of arachidonic acid to prostaglandins.¹⁵ Sodium cromoglycate **11** and nedocromil **12** (Figure 1) are part of the chromone family which have well-known anti-inflammatory activities. Sodium cromoglycate **11** inhibits zymosan-activated and platelet-activating-factor (PAF)-induced chemotaxis of human neutrophils,¹⁶ and nedocromil **12** improves symptoms, especially in moderate asthma, by decreasing bronchial obstruction and reducing bronchial hyperreactivity.¹⁷

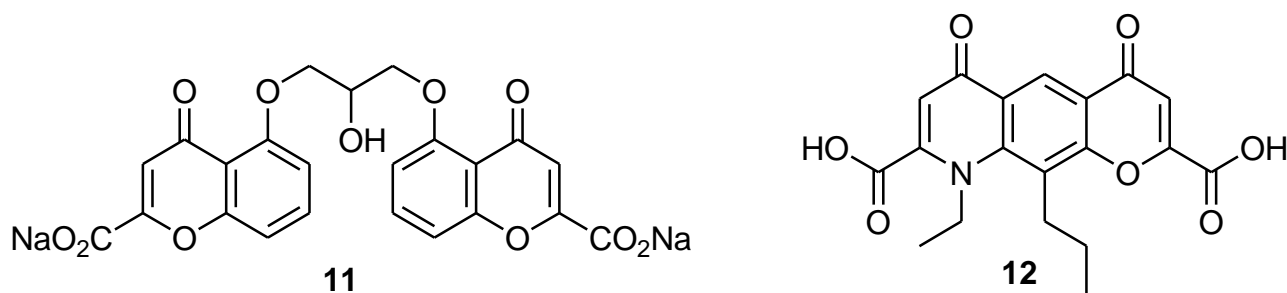


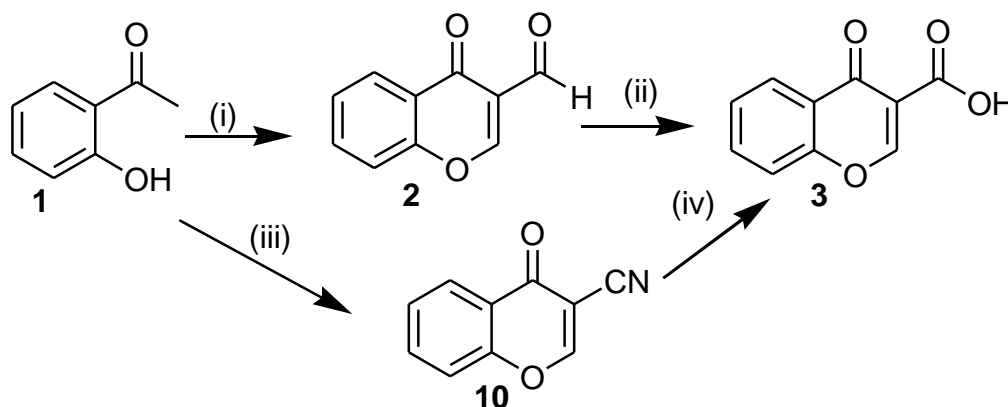
Figure 1. Sodium cromoglycate **11** and nedocromil **12**.

Trypanosomes are parasitic protozoa that cause Chagas disease in central and South America, and sleeping sickness in sub-Saharan Africa, leading to morbidity and mortality of millions of people.²⁰ American trypanosomiasis (also known as Chagas disease) is caused by the protozoan parasite *Trypanosoma cruzi*, and is endemic in 21 countries across Latin America.²¹ Human African Trypanosomiasis (also known as sleeping sickness), is caused by infection with *Trypanosoma brucei rhodesiense* (T.b.r) or *Trypanosoma brucei gambiense* (T.b.g) parasites. During the haemolymphatic phase, trypomastigotes circulate within the blood and lymphatic system. If not treated sufficiently, the neurological phase ensues as parasites penetrate the blood brain barrier, thus, infecting the central nervous system from which patient recovery is unlikely.²² Trypanosomiasis are among the most neglected diseases in the world, lacking desperately from financial

support for investigation.²³ Although the number of incidences of Trypanosomes has significantly declined in the past 20 years due to implementation of vector-control initiatives, the diseases still remain a major global health issue, especially, since the number of cases in non-endemic sites (United States, Australia, Europe and Japan) are rising, primarily due to international population migration and transfusions of contaminated blood.²¹

Results and Discussion

Several methods were employed for the synthesis of chromone-3-carboxylic acids (Scheme 1), however, in our laboratory all, but one, were unsuccessful. The first literature method attempted to make use of Jones' reagent as the oxidant, and is well documented in the literature,¹² however, this reaction did not work in our laboratory, and we could not establish or explain the reasons for the failure of the reaction. We then attempted another method which involved the synthesis of chromone-3-carbonitrile as a precursor to the chromone-3-carboxylic acid from the corresponding 2-hydroxyacetophenone.¹³ Several attempts to hydrolyse the carbonitrile **10** to the desired acid **3** were also unsuccessful.¹⁴ Several adjustments and/ or modifications to the reaction methodologies still did not yield the expected carboxylic acids. Our success was achieved when we decided to explore the Pinnick oxidation methodology, as shown in Scheme 2. Some of the compounds synthesized in our research laboratories (2d, 3d and 5e) gave melting points slightly to very different to those reported in literature which may be attributed to their purities. The same may be concluded for compounds 5c and 5f, the CHN values of which were not consistent with the calculated results.

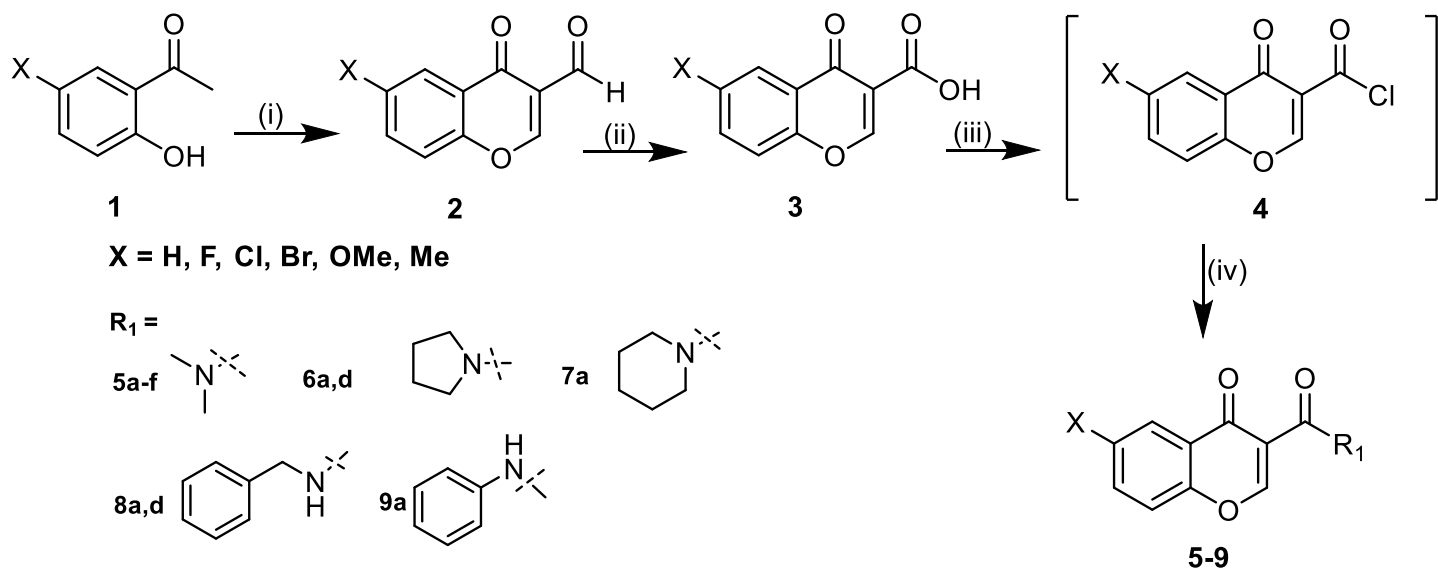


Reagents and conditions: (i) POCl₃, DMF, H₂O, 0-25 °C, 12 h (ii) Jones' reagent, acetone 0-30 °C (iii) POCl₃, DMF, NH₂OH·HCl, DCM, 0- 25°C (vi) HCl, H₂O, 100 °C, 2 h

Scheme 1. Attempted synthesis of chromone-3-carboxylic acids.

The synthetic route of the designed chromone-3-carboxamides **5-9** is outlined in Scheme 2. Chromone-3-carbaldehydes **2a-f** were synthesized from the corresponding 2-hydroxyacetophenones **1a-f** by Vilsmeier-Haack formylation.³ The yields ranged from 46-94% with good purity to enable us to proceed to the next step. Treatment of compounds **2** with sodium chlorite and sulfamic acid in a DCM-water mixture (Pinnick oxidation) afforded chromone-3-carboxylic acids **3** with yields ranging from 53-61%, and melting points comparable to reported literature values.¹⁰ The latter compounds were then activated with thionyl chloride (SOCl₂) in situ to

give the acid chlorides **4**, which were subsequently reacted with triethylamine (Et_3N) and an appropriate amine to afford the corresponding chromone-3-carboxamides, under mild reaction conditions, in 44 – 64% yields. In the literature, however, analogues **5a**, **5e** and **5f** were obtained as byproducts in the preparation of 3,3'-carbonyl-bis(chromones) derivatives.¹¹ Herein, we describe the direct synthesis of chromone-3-carboxamides **5** with some derivatives, **6-9**, with most of the synthesized compounds not previously reported in literature. All compounds were purified by recrystallization and characterized by NMR (^1H and ^{13}C), IR and elemental analysis for novel compounds.



Reagents and conditions: (i) POCl_3 , DMF, H_2O , 0-25 °C, 12 h (ii) NaOCl_2 , sulfamic acid, H_2O , DCM, 0-25 °C, 12 h (iii) SOCl_2 , DCM (iv) Et_3N , DCM, appropriate amine, 0-25 °C, 12 h

Scheme 2. Synthesis of chromone-3-carboxamides.

Anti-inflammatory activity

In this study, compounds **2a**, **2f**, **5a**, **5b**, **5c**, **5e**, **5f**, **6a**, **7a**, **8a**, **8d** and **9a** were screened in vitro against the known anti-inflammatory inhibitor, aminoguanidine. At the lowest concentration, **2f** was the only compound that produced meaningful inhibition, and was stronger than that of the positive control. Only **9a** negatively affected cell viability, however, the toxicity is considered to be modest. Negative inhibition observed for **9a** most likely reflects the toxicity of the compound. Compound **8a** was poorly soluble in DMSO, therefore, the accuracy of its subsequent inhibition and toxicity may have been compromised.

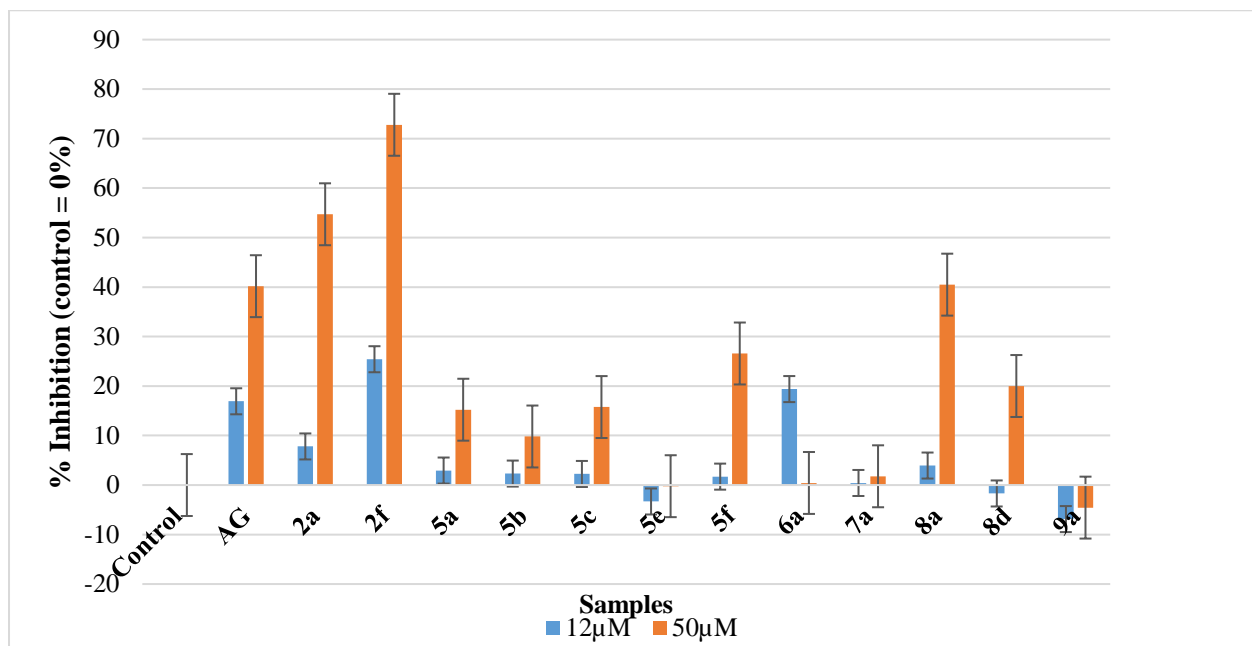


Figure 2. Inhibition of nitric oxide production in LPS activated RAW 264.7 macrophages. Data are expressed as a percentage of the LPS activated control. AG = aminoguanidine.

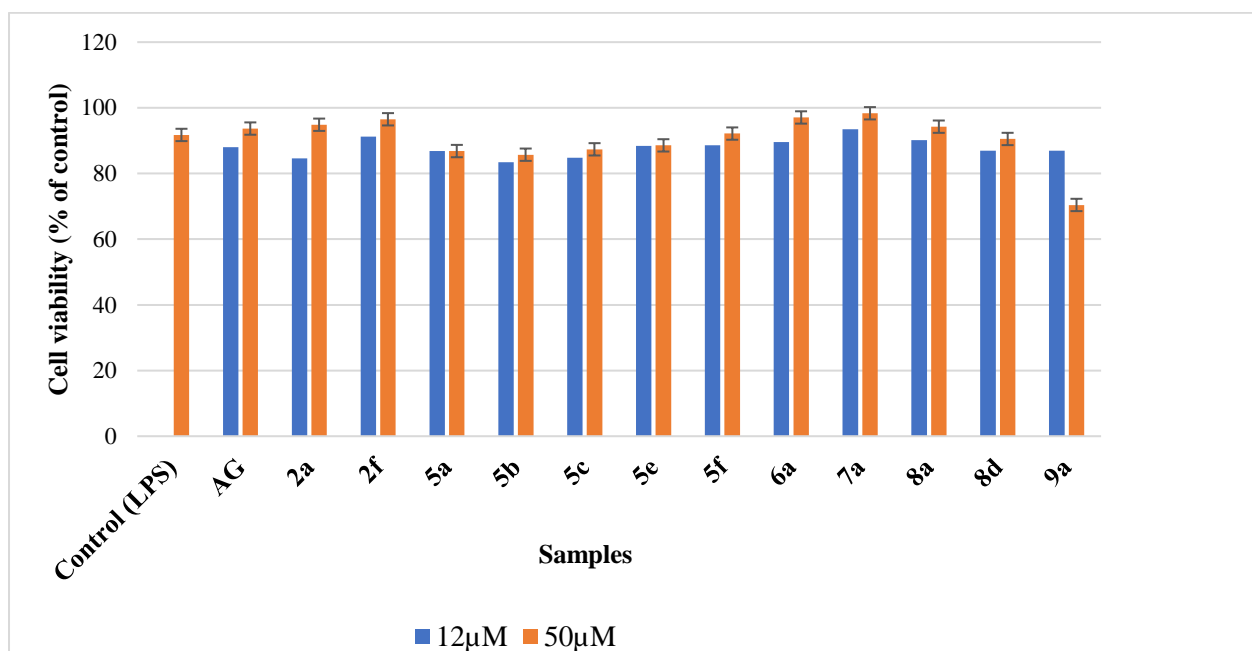


Figure 3. Relative cell viability determined using the MTT assay. Data are expressed as a percentage of the LPS activated control. AG = aminoguanidine.

Anti-trypanosomal activity

In vitro anti-trypanosomal effects of the investigated compounds were screened against pentamidine (an existing drug for treatment of trypanosomiasis). Compounds which reduced the parasite viability to <20% were put forward for further IC₅₀ testing. The screening data (Figure 5) show only two compounds, **2a** and **8d**, reduced parasite viability below the 20% threshold. Compound **2b** also showed significant anti-trypanosomal properties, however, the percentage viability observed was above the minimum percentage viability needed

for further IC₅₀ testing. The rest of the other compounds didn't have any notable effect on the cultures of *T.b. brucei*. The IC₅₀ testing for compounds **2a** and **8d** gave 4.3 and 1.3 µg/mL, respectively.

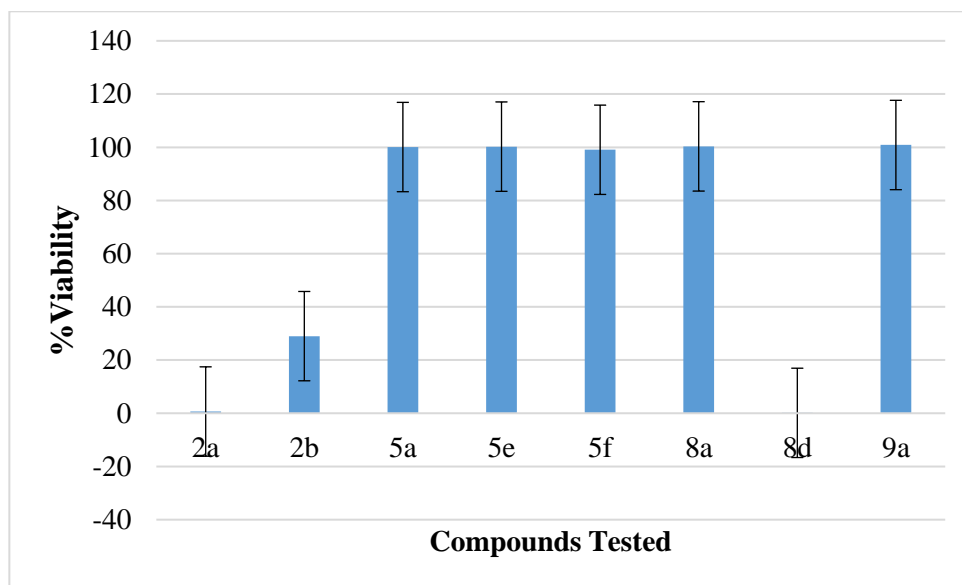


Figure 4. Anti-trypomonal activity of selected compounds 2a, 2b, 5a, 5e, 5f, 8a, 8d and 9a expressed as % parasite viability ± SD.

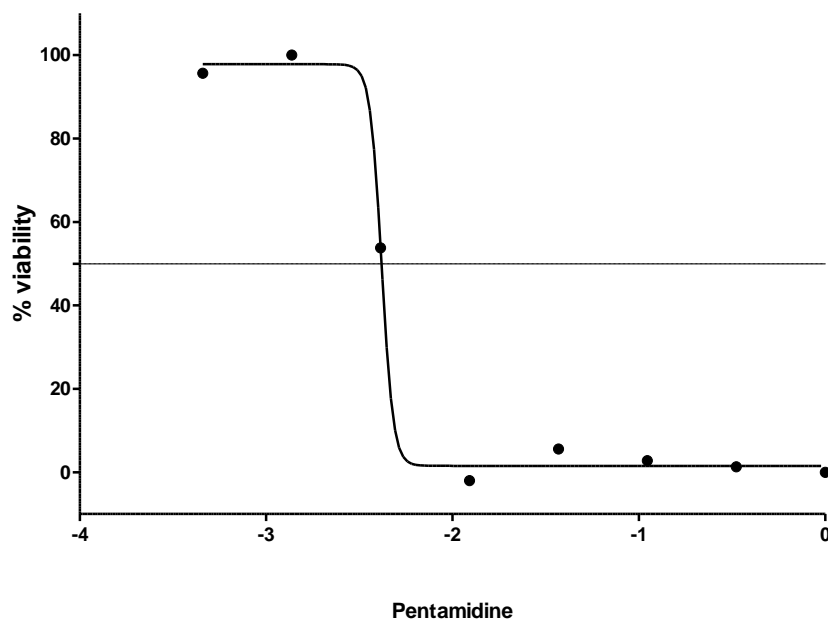


Figure 5. Dose-response curve for anti-trypomonal assay (Pentamidine standard: IC₅₀ – 0.04 µg/mL).

Conclusions

In summary, we have successfully developed an efficient method to synthesize a range of novel chromone-3-carboxamides from corresponding chromone-3-carboxylic acids, thus, providing researchers with a rapid

arsenal for the synthesis of these compounds. Selected target compounds and intermediates were evaluated for their anti-inflammatory and anti-trypanosomal activities. Only compounds **2a** and **2f** displayed significant anti-inflammatory activity. Anti-trypanosomal properties were observed on compounds **2a** and **8d**.

Experimental Section

General. Commercially available 2-hydroxyacetophenones, N, N-dimethylformamide (DMF), sulfamic acid, sodium chlorite, and other reagents and solvents used were purchased from Sigma Aldrich and Merck. All purchased starting materials and reagents were used without further purification unless noted. All reactions were carried out using oven-dried glassware and reaction progress was monitored using analytical thin layer chromatography (TLC) on precoated Merck silica gel and the spots were detected under UV light ($\lambda = 254\text{-}365$ nm). ^1H - and ^{13}C -nuclear magnetic resonance (NMR) Spectra were recorded at 400 MHz and 100 MHz, respectively, with an Avance 400 spectrometer (Bruker, Fallanden, Switzerland) using residual nondeuterated solvent as the internal standard. The chemical shifts are reported downfield in ppm (δ) relative to internal TMS, and coupling constants are reported in Hertz (Hz). Splitting patterns describe apparent multiplicities, and are designated as s (singlet), d (doublet), t (triplet), m (multiplet), or bs (broad singlet), respectively. IR spectra were determined on a Perkin-Elmer 1420 spectrophotometer and were reported in wave number (cm^{-1}). The attenuated total reflection (ATR) infrared (IR) spectra were recorded on an Alpha Fourier transform infrared (FTIR) spectrometer (Bruker, Fallanden, Switzerland). CHNS analyses were run at Stellenbosch University, South Africa, and were recorded on an Elementar Vario EL Cube Analyzer (Elementar Analysensysteme GmbH, Frankfurt, Germany). We acknowledge that, for some compounds, the differences between the calculated and found results were greater than 2%. Therefore, ^1H and ^{13}C NMR spectra have been included in a Supplemental Material file to provide confirmation of the compounds' structures. Melting points were determined on a Büchi Melting Point B-540 apparatus using open capillary tubes and were uncorrected.

Synthesis of 4-oxo-4H-chromene-3-carbaldehyde analogues ⁹

2-hydroxyacetophenones **1a-f** (40 mmol) in DMF (23 mL) were cooled to 0 °C, then POCl_3 (150 mmol) was added gradually to the solution with constant stirring. The solution was then stirred at room temperature for 12 h, quenched with ice water (50 mL). The solid formed was filtered, dried and recrystallized from ethanol to afford the corresponding 4-oxo-4H-chromene-3-carbaldehyde analogues **2a-f**.

4-Oxo-4H-chromene-3-carbaldehyde (2a). Yield, 55%; yellow solid; mp 150-152 °C (lit.,¹² 151-152); IR $\nu_{\text{max}}/\text{cm}^{-1}$ 1635.18 (C=O), 1691.48 (HC=O), and 3054.82 (C-H aromatic); δ_{H} (400 MHz, $\text{DMSO-}d_6$) 7.58 (1H, ddd, J 7.6, 7.2 and 2.4 Hz, 6-H), 7.75 (1H, d, J 8.4 Hz, 7-H), 7.88 (1H, dd, J 1.6 and 8.4 Hz, 8-H) 8.14 (1H, dd, J 1.6 and 8.4 Hz, 5-H), 8.94 (1H, s, 2-H), 10.14 (1H, s, CHO); δ_{C} (100 MHz, $\text{DMSO-}d_6$) 163.9 (C-2), 120.4 (C-3), 175.4 (C-4), 127.2 (C-5), 125.1 (C-6), 135.7 (C-7), 119.4 (C-8), 156.07 (C-8a), 125.8 (C-4a) and 188.9 (CHO).

6-Fluoro-4-oxo-4H-chromene-3-carbaldehyde (2b). Yield, 54%; pale yellow solid; mp 155-157 °C (lit.,²⁴ 157 °C); IR $\nu_{\text{max}}/\text{cm}^{-1}$ 1688 (HC=O), 1651 (C=O); δ_{H} (400 MHz, CDCl_3) 7.51 (1H, m, 8-H), 7.53 (1H, m, 7-H), 7.96 (1H, dd, J 2.8 and 5.2 Hz, 5-H), 8.59 (1H, s, 2-H), 10.41 (1H, s, CHO); δ_{C} (100 MHz, CDCl_3) 160.69 (C-2), 119.68 (C-3), 175.26 (C-4), 120.90 (C-4a), 111.25 (C-5), 159.04 (C-6), 126.81 (C-7), 111.48 (C-8), 160.69 (C-8a) and 188.27 (CHO).

6-Chloro-4-oxo-4H-chromene-3-carbaldehyde (2c). Yield, 93.8%; yellow solid; mp 165-169 °C (lit.,²⁴ 163-164 °C, lit.,⁵ 170-172 °C); IR $\nu_{\text{max}}/\text{cm}^{-1}$ 1623 (C=O), 1714 (HC=O); δ_{H} (400 MHz, CDCl_3) 7.53 (1H, d, J 9.2 Hz, 8-H), 7.71 (1H, dd, J 2.4 and 9.2 Hz, 7-H), 8.26 (1H, d, J 2.4 Hz, 5-H), 8.57 (1H, s, 2-H), 10.38 (1H, s, CHO); δ_{C} (100 MHz,

CDCl₃) 160.77 (C-2), 120.34 (C-3), 174.85 (C-4), 125.62 (C-4a), 126.31 (C-5) J_{C,F} 47.79 Hz, 132.82 (C-6), 135.03 (C-7) J_{C,F} 32.23, 120.25 (C-8), 154.51 (C-8a) and 188.13 (CHO).

6-Bromo-4-oxo-4H-chromene-3-carbaldehyde (2d). Yield, 76.9%; whitish-yellowish solid; mp195-197 °C (lit.,²⁹ 202-204 °C, lit.,⁹ 189-192 °C); IR ν_{max}/cm⁻¹ 1647 (C=O), 1697 (C=O); δ_H (400 MHz, CDCl₃) 7.47 (1H, d, *J* 8.8 Hz, 8-H), 7.86 (1H, dd, *J* 2.4 and 8.8 Hz, 7-H), 8.44 (1H, d, *J* 2.4 Hz, 5-H), 8.56 (1H, s, 2-H), 10.39 (1H, s, CHO); δ_C (100 MHz, DMSO-*d*₆) 160.66 (C-2), 126.64 (C-3), 174.73 (C-4), 120.52 (C-4a), 128.85 (C-5), 120.36 (C-6), 137.83 (C-7), 154.98 (C-8a) and 188.12 (CHO).

6-Methoxy-4-oxo-4H-chromene-3-carbaldehyde (2e). Yield, 46%; brown solid; mp158-160 °C (lit.,²⁴ 158-159 °C); IR ν_{max}/cm⁻¹ 1651 (C=O), 1727 (C=O); δ_H (400 MHz, DMSO-*d*₆): 3.89 (3H, s, OCH₃), 7.46 (1H, d, *J* 9.2 Hz, 5-H), 8.91 (1H, s, 2-H), 10.14 (1H, s, CHO); δ_C (100 MHz, DMSO-*d*₆) 56.4 (OCH₃), 163.5 (C-2), 119.7 (C-3), 175.15 (C-4), 157.9 (C-4a), 105.9 (C-5), 126 (C-6), 124.3 (C-7), 121 (C-8), 150.8 (C-8a), 189.9 (CHO).

6-Methyl-4-oxo-4H-chromene-3-carbaldehyde (2f). Yield, 94%; yellow solid; mp173-176 °C (lit.,⁹ 173-174); IR ν_{max}/cm⁻¹1735 (C=O), 1691 (C=O); δ_H (400 MHz, CDCl₃) 2.49 (3H, s, CH₃), 7.42 (1H, d, *J* 8.4 Hz, 8-H), 7.56 (1H, dd, *J* 1.6 and 8.4 Hz, 7-H), 8.07 (1H, s, 5-H), 8.53 (1H, s, 2-H), 10.38 (1H, s, CHO); δ_C (100 MHz, DMSO-*d*₆) 20.99 (CH₃), 160.52 (C-2), 120.17 (C-3), 176.05 (C-4), 124.94 (C-4a), 125.50 (C-5), 136.92 (C-6), 135.96 (C-7), 118.34 (C-8), 154.47 (C-8a), 188.74 (HC=O).

3.3 General synthesis of 4-oxo-4H-chromene-3-carboxylic acid analogues¹⁰

A solution of sodium chlorite (47.87 mmol) in water (8 mL) was stirred at 0 °C for 10 min. To this solution, 4-oxo-4H-chromene-3-carbaldehyde **2** (13.67 mmol) and sulfamic acid (54.69 mmol) were added, followed by gradual addition of DCM (15 mL). The resulting mixture was stirred for 3 h at room temperature and then quenched with water (25 mL), and extracted with DCM (3 x 25 mL). The combined organic phases were dried over Na₂SO₄ and concentrated under reduced pressure. The solid obtained was recrystallized from 80:20 methanol-water to give 4-oxo-4H-chromene-3-carboxylic acids **3a-f**.

4-Oxo-4H-chromene-3-carboxylic acid (3a). Yield, 53%; white solid; mp200-203 °C (lit.,¹² 204-205 °C); IR ν_{max}/cm⁻¹ 3078 (OH), 1737 (C=O); δ_H(400 MHz, DMSO-*d*₆), 7.64 (1H, t, *J* 7.6 Hz, 6-H), 7.82 (1H, d, *J* 8.8 Hz, 8-H), 7.95 (1H, dd, *J* 8.8 and 1.2 Hz, 7-H), 8.19 (1H, d, *J* 8 Hz, 5-H), 9.16 (1H, s, 2-H) 13.28 (1H, s, OH); δ_C (100 MHz, DMSO-*d*₆) 164.2 (C-2), 114.7 (C-3), 176.6 (C-4), 123.8 (C-4a), 127.3 (C-5), 125.9 (C-6), 135.9 (C-7), 119.3 (C-8), 156.2 (C-8a), 164.3 (C-OH).

6-Fluoro-4-oxo-4H-chromene-3-carboxylic acid (3b). Yield, 57%; white solid; mp210-213 °C; IR ν_{max}/cm⁻¹ 1694 and 1634 (C=O), δ_H (400 MHz, DMSO-*d*₆) 7.80-8.34 (2H, m, 7-H and 5-H), 7.64 (1H, d, *J* 8.4 Hz, 8-H), 9.10 (1H, s, 2-H), 13.15 (1H, bs, OH); δ_C (100 MHz, DMSO-*d*₆) 164.17 (C-2), 114.68 (C-3), 175.09 (C-4), 123.68 (C-4a), 122.21 (C-5) J_{C,F} 17.63 Hz, 152.55 (C-6), 123.93 (C-7) J_{C,F} 30.34 Hz, 110.19 (C-8), 158.76 (C-8a), 164.07 (C-OH).

6-Chloro-4-oxo-4H-chromene-3-carboxylic acid (3c). Yield, 61.3%; white solid mp230-231 °C (lit.,¹² 231-232 °C); IR ν_{max}/cm⁻¹ 3087 (OH); δ_H (400 MHz, DMSO-*d*₆) δ_C 8.89 (1H, s, 2-H), 7.39 (1H, d, *J* 7.2 Hz, 8-H), 7.80 (1H, d, *J* 8.8 Hz, 7-H), 8.03 (1H, s, 5-H); δ_C (100 MHz, DMSO-*d*₆) 160.69 (C-2) 118.72 (C-3), 174.68 (C-4), 124.82 (C-4a), 129.87 (C-5), 130.70 (C-6) 134.92 (C-7), 121.57 (C-8), 154.17 (C-8a), 162.2 (C-OH).

6-Bromo-4-oxo-4H-chromene-3-carboxylic acid (3d). Yield, 55.2%; as a pale yellow solid; mp236-240 °C (lit.,⁹ 245-249 °C); IR ν_{max}/cm⁻¹ 3071 (OH), 1691 (C=O), 1651. (C=O) δ_H (400 MHz, DMSO-*d*₆) 7.71 (1H, d, *J* 9.2 Hz, 8-H), 8.00 (1H, dd, *J* 2.4 and 9.2 Hz, 7-H), 8.20 (1H, d, *J* 2.4 Hz, 5-H); δ_C (100 MHz, DMSO-*d*₆) 160.67 (C-2), 119.02 (C-3), 174.59 (C-4), 121.73 (C-4a), 134.95 (C-5), 129.90 (C-6), 137.65 (C-7), 118.84 (C-8) 154.59 (C-8a), 162.12 (C-OH)

6-Methoxy-4-oxo-4H-chromene-3-carboxylic acid (3e). Yield, 55.6%; brown solid; mp172-173 °C (lit.,¹² 173-174 °C); IR ν_{max}/cm⁻¹ 3068 (OH), 1694 (C=O), δ_H (400 MHz, DMSO-*d*₆) 3.88 (3H, s, CH₃), 7.47 (1H, d, *J* 3.2 Hz, 8-H), 7.51 (1H, d, *J* 3.2 Hz, 7-H), 7.74 (1H, d, *J* 9.2 Hz, 5-H), 9.10 (1H, s, 2-H), 13.32 (1H, bs, OH); δ_C (100 MHz,

DMSO-*d*₆) 56.39 (OCH₃), 163.94 (C-2), 113.73 (C-3), 176.53 (C-4), 124.53 (C-4a), 120.93 (C-5), 151.02 (C-6), 125.06 (C-7), 105.53 (C-8), 157.90 (C-8a), 164.37 (C-OH)

6-Methyl-4-oxo-4H-chromene-3-carboxylic acid (3f). Yield, 57.2%; yellow solid; mp240-247 °C; IR $\nu_{\max}/\text{cm}^{-1}$ 3069 (OH), 1745 (C=O)); δ_{H} (400 MHz, DMSO-*d*₆) 7.67 (1H, d, *J* 8.8 Hz, 8-H), 7.74 (1H, d, *J* 8.8 Hz, 7-H), 7.94 (1H, d, *J* 8 Hz, 5-H), 9.11 (1H, s, 2-H); δ_{C} (100 MHz, DMSO-*d*₆) 20.00 (CH₃) 164.19 (C-2), 114.42 (C-3), 176.77 (C-4), 123.43 (C-4a), 125.05 (C-5), 136.63 (C-6), 137.13 (C-7), 119.09 (C-8), 154.57 (C-8a), 164.36 (C-OH).

General synthesis of chromone-3-carboxamide analogues 5-9²⁵

The synthetic strategy to chromone-3-carboxamide **5-9** was implemented by adding to a suspension of corresponding 4-oxo-4H-chromene-3-carboxylic acid analogues **3** (3 mmol), SOCl₂ (3 mmol) and dry DCM (30 mL). The solution was stirred for 1 h under N₂ gas to give *in situ* product 4-oxo-4H-chromene-3-carbonyl chloride **4**. The latter product was cooled to 0 °C, followed by the addition of Et₃N (9 mmol) and an appropriate amine (3 mmol) then stirred overnight. The resultant mixture was quenched with water and extracted with DCM (2x 25 mL). The combined organic layers were washed extensively with saturated 25% ammonia solution (2x 10 mL), washed with water (2 x 10 mL) then dried over anhydrous MgSO₄ and concentrated under reduced pressure to afford solid products. The crude products were directly crystallized from Methanol to give corresponding 4-oxo-4H-chromene-3-carboxamide analogues **5-9**.

N,N-Dimethyl-4-oxo-4H-chromene-3-carboxamide (5a). Yield, 53.8%; white solid; mp190-192 °C (lit.,¹¹ 195-196 °C); IR $\nu_{\max}/\text{cm}^{-1}$ 2930 (CH), 1721 (C=O), 1625 (C=O), δ_{H} (400 MHz, CDCl₃) 3.01 (3H, s, NCH₃), 3.13 (3H, s, NCH₃), 7.44-7.51 (2H, m, 6-H and 8-H), 7.72 (1H, m, 7-H), 8.25 (1H, dd, *J* 8 and 1.2 Hz, 5-H), 8.17 (1H, s, 2-H); δ_{C} (100 MHz, DMSO-*d*₆) 35.40 (NCH₃), 38.58 (NCH₃), 118.25 (C-8), 125.84 (C-6), 126.22 (C-5), 134.21 (C-7), 156.30 (C-2), 123.42 (C-3), 124.39 (C-4a), 156.11 (C-8a), 164.45 (C-N), 173.76 (C-4).

N,N-Dimethyl-6-fluoro-chromone-3-carboxamide (5b). Yield, 45.7%; yellow solid; mp170-172 °C ; IR $\nu_{\max}/\text{cm}^{-1}$ 1694 (C=O), δ_{H} (400 MHz, DMSO-*d*₆) 2.89 (3H, s, NCH₃), 2.98 (3H, s, NCH₃), (1H, dd, *J* 4.4 and 2.8 Hz, 8-H), 7.78 (1H, d, *J* 2.4 Hz, 7-H), 7.83 (1H, dd, *J* 4.4 and 2.8 Hz, 5-H), 8.57 (1H, s, 2-H); δ_{C} (100 MHz, DMSO-*d*₆) 35.94 (CH₃), 38.26 (CH₃), 110.45 (C-8), 121.93 (C-5) $J_{\text{C,F}}$ 39.81, 123.36 (C-7) $J_{\text{C,F}}$ 38.74 , 156.89 (C-2), 161.74 (C-6), 125.40 (C-3), 158.40 (C-8a), 163.75 (C-N), 173.20 (C-4).

N,N-Dimethyl-6-chloro-chromone-3-carboxamide (5c). Yield, 48.9%; yellow solid; mp155-157°C IR $\nu_{\max}/\text{cm}^{-1}$ 2929 (CH), 1705 (C=O), 1624.25 (C=O) δ_{H} (400 MHz, DMSO-*d*₆) 2.89 (3H, s, NCH₃), 2.97 (3H, s, NCH₃), 7.35(1H, d, 2.4 Hz, 8-H), 7.38-8.03 (2H, m, 5-H and 7-H), 8.35 (1H, s, 2-H); δ_{C} (100 MHz, DMSO-*d*₆) 34.75 (NCH₃), 38.14 (NCH₃), 112.67 (C-3), 156.91 (C-8a), 156.12 (C-2), 172.78 (C-4), 124.62 (C-4a), 127.64 (C-5), 129.33 (C-6), 134.22 (C-7), 119.33 (C-8), 154.77 (C-8a), 163.30 (C-N); Anal. Calc. for C₁₂H₁₀ClNO₃: C 57.27; H 4.01; N 5.57. Found: C 55.66; H 4.02; N 4.99.

N,N-Dimethyl-6-bromo-chromone-3-carboxamide (5d). Yield, 50.5%; yellow solid; mp165-167 °C; IR $\nu_{\max}/\text{cm}^{-1}$ 2927 (CH aromatic), 1643 (C=O) ; δ_{H} (400 MHz, acetic acid-*d*₄) 2.89 (3H, s, NCH₃), 2.95 (3H, s, NCH₃), 7.45 (1H, d, *J* 8.8 Hz, 8-H), 7.79 (1H, dd, *J* 2 and 8.8 Hz, 7-H), 8.14 (1H, d, *J* 2.4 Hz, 5-H), 8.29 (1H, s, 2-H); δ_{C} (100 MHz, acetic acid-*d*₄) 34.61 (NCH₃), 37.86 (NCH₃), 120.83 (C-8), 127.97 (C-5), 137.28 (C-7), 157.28 (C-2), 118.71 (C-3), 122.23 (C-4a), 125.45 (C-6), 164.98 (C-N), 172.55 (C-4); Anal. Calc. for C₁₂H₁₀BrNO₃: C 48.67; H 3.40; N 4.73. Found: C 48.57; H 3.71; N 4.87.

N,N-Dimethyl-6-methoxy-chromone-3-carboxamide (5e). Yield, 53.93%; yellow solid; mp158-160°C (lit.,¹¹ 127-128°C); IR $\nu_{\max}/\text{cm}^{-1}$ 2925 (CH aromatic), 1638 (C=O); δ_{H} (400 MHz, DMSO-*d*₆) 2.95 (3H, s, NCH₃), 3.04 (3H, s, NCH₃), 3.94 (3H, s, OCH₃), 7.50-7.52 (2H, m, 8-H and 7-H), 7.73 (1H, d, *J* 9.6 Hz, 5-H), 8.57 (1H, s, 2-H); δ_{C} 100 MHz, DMSO-*d*₆) 34.94 (NCH₃), 38.27 (NCH₃), 56.25 (OCH₃), 105.33 (C-8), 120.70 (C-5), 124.24 (C-7), 156.32 (C-2), 122.47 (C-3), 124.85 (C-4a), 150.97 (C-6), 157.30 (C-8a), 164.15 (C-N), 173.44 (C-4); Anal. Calc. for C₁₄H₁₃NO₃: C 63.15; H 5.30; N 5.67. Found: C 63.77; H 5.40; N 5.40.

***N,N*-6-Trimethyl-4-oxo-4*H*-chromene-3-carboxamide (5f).** Yield, 50.3%; yellowish-orange solid; mp121-124 °C (lit.,¹¹ 118-119 °C); IR $\nu_{\max}/\text{cm}^{-1}$ 2924 (CH aromatic), 1643 (C=O); δ_{H} (400 MHz, DMSO-*d*₆) 2.44 (3H, s, CH₃), 2.89 (3H, s, NCH₃), 2.98 (3H, s, NCH₃), 7.59 (1H, d, *J* 8.4 Hz, 8-H), 7.66 (1H, d, *J* 8.4 Hz, 7-H), 7.88 (1H, s, 2-H) 8.49 (1H, d, *J* 2.4 Hz); δ_{C} (100 MHz, DMSO-*d*₆) 20.88 (ArCH₃), 34.92 (NCH₃), 38.25 (NCH₃), 118.84 (C-8), 124.92 (C-5), 136.13 (C-7), 156.41 (C-2), 123.13 (C-3), 123.82 (C-4a), 136.04 (C-6), 154.49 (C-8a), 164.11 (C-N), 173.70 (C-4); Anal. Calc. for C₁₃H₁₃NO₃: C 67.52; H 5.67; N 6.06. Found: C 65.97; H 5.936; N 6.07.

3-(Pyrrolidine-1-carbonyl)-4*H*-chromen-4-one (6a). Yield, 44%; brown semi-solid. IR $\nu_{\max}/\text{cm}^{-1}$ 1641 (C=O), 1605 (C=O); δ_{H} (400 MHz, CDCl₃) 1.89 (4H, m, CH₂), 3.46 (2H, dd, *J* 6.8 and 6.4 Hz, CH₂), 3.64 (2H, m, CH₂), 7.45 (1H, m, 6-H), 7.49 (1H, d, *J* 8.4 Hz, 8-H), 7.70 (1H, m, 7-H), 8.22 (1H, s, 2-H), 8.23 (1H, dd, *J* 1.6 and 8 Hz, 5-H); δ_{C} (400 MHz, CDCl₃) 24.40 and 25.93 (C-2'), 156.53 (C-2), 46.29 and 47.64 (C-3'), 123.99 (C-3), 173.65 (C-4), 125.53 (C-4a), 134.18 (C-5), 125.79 (C-6), 126.10 (C-7), 118.24 (C-8), 156.55 (C-8a), 162.75 (C=O); Anal. Calc. for C₁₄H₁₃NO₃: C 69.12; H 5.39; N 5.76. Found: C 66.78; H 5.57; N 6.13.

6-Bromo-3-(pyrrolidine-1-carbonyl)-4*H*-chromen-4-one (6d). Yield, 64.15%; yellow solid; mp234-236 °C as a yellow solid; IR $\nu_{\max}/\text{cm}^{-1}$ 2962 (CH aromatic), 1625 (C=O) δ_{H} 1.78 (4H, m, 2'-H), 3.32 (2H, t, *J* 6.4 Hz, CH₂), 3.47 (2H, t, 6.8 Hz, CH₂), 7.39 (1H, d, *J* 8.8 Hz, 8-H), 7.75 (1H, dd, *J* 2.4 and 8.8 Hz, 7-H), 8.20 (1H, d, *J* 2.4 Hz), 8.34 (1H, s, 2-H); δ_{C} (100 MHz, acetic acid-*d*₄) 24.01 and 25.42 (CH₂), 158.25 (C-2), 46.54 and 47.88 (CH₂), 119.19 (C-3), 172.92 (C-4), 122.43 (C-4a), 128.31 (C-5), 125.40 (C-6), 137.51 (C-7), 120.61 (C-8), 155.12 (C-8a), 163.53 (C=O); Anal. Calc. for C₁₄H₁₂BrNO₃: C 52.20; H 3.75; N 4.35. Found: C 52.54; H 3.80; N 4.50.

3-(Piperidine-1-carbonyl)-4*H*-chromen-4-one (7a). Yield, 57%; brown solid; mp136-140 °C; IR $\nu_{\max}/\text{cm}^{-1}$ 1716 (C=O), 1698 (C=O) δ_{H} (400 MHz, DMSO-*d*₆) 1.47 (6H, m, CH₂), 3.24 (2H, s, CH₂), 3.43 (2H, s, CH₂), 7.50 (1H, t, *J* 7.2 Hz, 6-H), 7.66 (1H, d, *J* 8 Hz, 8-H), 7.82 (1H, dd, *J* 7.2 and 6.8 Hz, 7-H), 8.07 (1H, d, *J* 7.6 Hz, 5-H), 8.51 (1H, s, 2-H); δ_{C} (100 MHz, DMSO-*d*₆) 24.39 (CH₂), 25.73 (CH₂), 26.45 (CH₂), 42.59 (CH₂), 48.10 (CH₂), 118.99 (CH), 125.69 (CH), 126.30 (CH), 135 (CH), 156.06 (C-2), 123.20 (C-3), 124.04 (C-4a), 156.19 (C-8a), 162.14 (C-N), 173.86 (C-4); Anal. Calc. for C₁₅H₁₅NO₃: C 70.02; H 5.88; N 5.44. Found: C 69.02; H 5.85; N 5.64.

***N*-Benzyl-4-oxo-4*H*-chromene-3-carboxamide (8a).** Yield, 60%; white solid; δ_{H} mp170-172 °C; IR $\nu_{\max}/\text{cm}^{-1}$ 3262 (NH), 1716 (C=O), 1652.18 (C=O) δ_{H} (400 MHz, CDCl₃) 4.64 (2H, d, *J* 7.6 Hz, CH₂), 7.39 (4H, m, Ar-H), 7.45 (1H, dd, *J* 7.2 and 8 Hz, 6-H), 7.53 (1H, d, *J* 4.4 Hz, 8-H), 7.70 (1H, dd, *J* 1.2 and 7.2 Hz, 7-H), 8.22 (1H, dd, *J* 1.2 and 7.2 Hz, 5-H), 9.00 (1H, s, 2-H), 9.69 (1H, s, NH); (100 MHz, CDCl₃) 43.27 (CH₂), 162.41 (C-2), 118.39 (C-3), 124.17 (C-4a), 177.00 (C-4), 134.62 (C-7), 127.30 (2Ar-C), 126.35 (2Ar-C).

***N*-Benzyl-6-bromo-4-oxo-4*H*-chromene-3-carboxamide (8d).** Yield, 64%; white solid; mp179-182 °C IR $\nu_{\max}/\text{cm}^{-1}$ 3296 (NH), 1668 (C=O); δ_{H} (400 MHz, CDCl₃) 4.64 (2H, d, *J* 5.6 Hz, CH₂), 7.29 (1H, d, *J* 11.6 Hz, Ar-H), 7.34 (4H, m, Ar-H), 7.45 (1H, d, *J* 8.8 Hz, 8-H), 7.82 (1H, dd, *J* 2.4 and 8.8 Hz, 7-H), 8.37 (1H, d, *J* 2.4 Hz, 5-H), 9.00 (1H, s, 2-H), 9.55 (1H, bs, NH); δ_{C} (100 MHz, CDCl₃) 43.37 (CH₂), 160.82 (C-2), 115.98 (C-3), 138.02 (Ar-C), 175.93 (C-4), 125.55 (C-4a), 128.72 (2Ar-C), 128.82 (Ar-C), 120.33 (C-6), 137.67 (C-7), (2Ar-C), 154.86 (C-8a), 162.58 (C-N).

4-Oxo-*N*-phenyl-4*H*-chromene-3-carboxamide (9a). Yield, 61%; pale yellow solid; mp224-226 °C (lit.,²⁶ 218-220 °C); IR $\nu_{\max}/\text{cm}^{-1}$ 3081 (NH), 1672 (C=O), δ_{H} (400 MHz, CDCl₃) 7.14 (1H, t, *J* 7.2 Hz, 8-H), 7.32 (2H, m, Ar-H), 7.77-7.81 (2H, m, 6-H and 7-H), 7.75 (1H, d, *J* 8 Hz, Ar-H), 8.33 (1H, dd, *J* 1.6 and 6.4 Hz, 5-H), 9.08 (1H, s, 2-H), 11.40 (1H, bs, NH); δ_{C} (100; MHz, CDCl₃) 160.71 (Ar-C), 162.85(C-2), 137.98 (Ar-C) 120.54 (Ar-C), 115.99 (C-3), 129.01 (Ar-C), 174.44 (C-4); 126.25 (C-4a), 126.52 (C-5), 124.47 (Ar-C), 129.01 (Ar-C), (C-6) 124.07 120.54 (Ar-C), 134.89 (C-7), 118.53 (C-8), 156.17 (C-8a), Anal. Calc. for C₁₆H₁₁NO₃: C 72.45; H 4.18; N 5.28. Found: C, 73.69; H, 4.31; N, 5.55.

Chromone-3-carbonitrile (10a)¹³

2-hydroxyacetophenone **1** (3.4 mL, 28.24 mmol) in DMF (15.5 mL, 200 mmol) was cooled to 0 °C and POCl₃ (9.3 mL, 100 mmol) was gradually added to the solution. The mixture was stirred at room temperature for 4 h and then a solution of NH₂OH·HCl (5.22 g, 75 mmol) in DCM (34 mL) added to the reaction mixture at 5 °C. The resulting mixture was then stirred for 6 h at room temperature and quenched with water (30 mL); extracted with DCM (3 x 25 mL), washed with water (1 x 10 mL), washed with saturated NaHCO₃ solution (1 x 10 mL) and dried over Na₂SO₄. The solution was concentrated under reduced pressure to give a crude solid, which was directly recrystallized from methanol to afford chromone-3-carbonitrile **10a** (3.8 g, 78%) as pale yellow solid; mp 174-176 °C (lit.,¹³ 175-177 °C); IR $\nu_{\max}/\text{cm}^{-1}$ 3083 (CH), 2239. (CN), 1658. (C=O); ¹H NMR δ_{H} (400 MHz, DMSO-*d*₆) 7.60 (1H, dt, *J* 7.2 and 8.8 Hz, 8-H), 7.76 (1H, d, *J* 8.8 Hz, H-6), 7.91 (1H, m, 7-H), 8.08 (1H, dd, *J* 1.2 and 7.2 Hz, 5-H), 9.26 (1H, s, 2-H); δ_{C} (100 MHz, DMSO-*d*₆) 165.86 (C-2), 101.49 (C-3), 172.99 (C-4), 123.12 (C-4a), 127.54 (C-5), 125.56 (C-6), 136.09 (C-7), 119.33 (C-8), 155.82 (C-8a) and 113.66 (CN); Anal. Calc. for C₁₀H₅NO₂; C 70.18; H 2.94; N 8.18. Found: C 69.09; H 2.75; N 7.95.

Anti-inflammatory screening²⁷

Compounds were solubilized in DMSO to a final concentration of 50 mM and immediately used to test anti-inflammatory activity. RAW 264.7 cells were seeded into 96-well plates at a density of 25 000 cells per well and allowed to attach overnight. The following day spent culture medium was removed and the samples (diluted in DMEM complete medium) added to give final concentrations of 12.5 and 50 μM (50 μL per well at double the desired final concentration). To assess the anti-inflammatory activity, 50 μL of LPS containing medium was added to the corresponding wells. Aminoguanidine, a known inhibitor of iNOS expression served as a positive control. Cells were then returned to the incubator for a further 20 hr. To quantify NO production, 50 μL of the spent culture medium was transferred to a new 96-well plate and 50 μL Griess reagent added. Absorbance was measured at 510 nm and the results expressed relative to the appropriate untreated control. To confirm the absence of toxicity as a contributory factor, cell viability was assessed using MTT.

Trypanosoma brucei Assay²⁸

To assess trypanocidal activity, compounds were added to cultures of *T.b. brucei* in 96-well plates at a fixed concentration of 20 μM . After a 48-hour incubation, parasites surviving drug treatment were enumerated by adding a resazurin based reagent. Resazurin is reduced to resorufin (a fluorophore (Ex_{C560}/Em₅₉₀)) in viable cells and was thus quantified in a Spectramax M3 microplate reader. Results were expressed as % parasite viability – the resorufin fluorescence in compound-treated wells relative to untreated controls. Compounds were tested in duplicate and standard deviations (SD) derived.

Acknowledgements

The authors are thankful to Sasol Inzalo Foundation, National Research Foundation (NRF), University of Venda Research and Publication Committee (RPC) for financial support. We also wish to thank Rhodes University (RU) and Nelson Mandela University (NMU) for biological screening of the compounds.

Supplementary Material

Supplementary material associated with this manuscript, consisting of copies of ¹H NMR, ¹³C NMR and IR spectra of selected compounds, can be found in the online version.

References

1. Tome, S. M.; Silva, A.M.S.; Santos, C.M.M. *Curr. Org. Synth.* **2014**, *11*, 317-341.
<https://doi.org/10.2174/15701794113109990063>
2. Wang, Q.; Yang, Z.-Y.; Qi, G.-F.; Qin, D.-D. *Eur. J. Med. Chem.* **2009**, *44*, 2425-2433.
<https://doi.org/10.1016/j.ejmech.2008.10.023>
3. Kabalka, G. W.; Mereddy, A. R. *Tetrahedron Lett.* **2005**, *46*, 6315-6317.
<https://doi.org/10.1016/j.tetlet.2005.07.038>
4. Houghton, P. J. *J. Chromatogr. A* **2002**, *967*, 75-84.
[https://doi.org/10.1016/S0021-9673\(01\)01555-2](https://doi.org/10.1016/S0021-9673(01)01555-2)
5. Zhao, P.-L.; Li, J.; Yang, G.-F. *Bioorg. Med. Chem.* **2007**, *15*, 1888-1895.
[https://doi.org/10.1016/S0021-9673\(01\)01555-2](https://doi.org/10.1016/S0021-9673(01)01555-2)
6. Parveen, M.; Malla, A. M.; Yaseen, Z.; Ali, A.; Alam, M. *J. Photochem. Photobiol. B* **2014**, *130*, 179-187.
<https://doi.org/10.1016/j.jphotobiol.2013.11.019>
7. Santos, C. M.; Silva, V. L.; Silva, A. *Molecules* **2017**, *22*, 1665.
<https://doi.org/10.3390/molecules22101665>
8. Tiwari, S. V.; Seijas, J. A.; Vazquez-Tato, M. P.; Sarkate, A. P.; Karnik, K. S.; Nikalje, A. P. G. *Molecules* **2018**, *23*, 440.
<https://doi.org/10.3390/molecules23020440>
9. Cao, L.; Zhang, L.; Cui, P. *Chem. Heterocycl. Compd.* **2004**, *40*, 635-640.
10. Patel, M. C.; Ng, N.; Rajani, D. *Der Pharma Chemica* **2011**, *3*, 422-432.
11. Miliutina, M.; Ejaz, S. A.; Iaroshenko, V. O.; Villinger, A.; Iqbal, J.; Langer, P. *Org. Biomol. Chem.* **2016**, *14*, 495-502.
<https://doi.org/10.1039/C5OB01350J>
12. Nohara, A.; Umetani, T.; Sanno, Y. *Tetrahedron Lett.* **1973**, *14*, 1995-1998.
[https://doi.org/10.1016/S0040-4039\(01\)96102-7](https://doi.org/10.1016/S0040-4039(01)96102-7)
13. Reddy, G. J.; Latha, D.; Thirupathaiah, C.; Rao, K. S.; *Tetrahedron Lett.* **2004**, *45*, 847-848.
<https://doi.org/10.1016/j.tetlet.2003.11.023>
14. Al Houari, G.; Baba, B. F.; Bennani, B.; Larbi, N. B.; Kerbal, A.; Laude, B.; Vebrel, J. *J.Mar.Chim.Heterocycl.*, **2002**, *1*, 22.
15. Saleem, T. M.; Azeem, A.; Dilip, C.; Sankar, C.; Prasanth, N.; Duraisami, R.; *Asian Pacific Journal of Tropical Biomedicine*, **2011**, *1*, 147-149.
[https://doi.org/10.1016/S2221-1691\(11\)60014-2](https://doi.org/10.1016/S2221-1691(11)60014-2)
16. Edwards, A. *Clin. Exp. Allergy* **1994**, *24*, 612-623.
<https://doi.org/10.1111/j.1365-2222.1994.tb00964.x>
17. Braunstein, G. *Allergie et immunologie* **1995**, *27*, 50-54.
18. Kashyap, A.; Chetia, D.; Rudrapal, M. *Indian J. Pharm. Sci.* **2017**, *78*, 801-809.
<https://doi.org/10.4172/pharmaceutical-sciences.1000186>
19. Nethavhani, S. A.; van Ree, T.; *Arab. J. Sci. Eng.* **2014**, *39*, 6595-6598.
<https://doi.org/10.1007/s13369-014-1193-5>
20. Ugwu, D. I.; Okoro, U. C.; Mishra, N. K.; *Plos One*, **2018**, *13*, 1-27.
21. Papadopoulou, M. V.; Bloomer, W. D.; Lepesheva, G. I.; Rosenzweig, H. S.; Kaiser, M.; Aguilera-Venegas, B.; Wilkinson, S. R.; Chatelain, E.; Ioset, J.-R. *J. Med. Chem.* **2015**, *58*, 1307-1319.
<https://doi.org/10.1021/jm5015742>

22. Bhambra, A. S.; Edgar, M.; Elsegood, M. R.; Li, Y.; Weaver, G. W.; Arroo, R. R.; Yardley, V.; Burrell-Saward, H.; Krystof, V. *Eur. J. Med. Chem.* **2016**, *108*, 347-353.
<https://doi.org/10.1016/j.ejmech.2015.11.043>
23. Dardonville, C. *Expert Opinion on Therapeutic Patents* **2005**, *15*, 1241-1257.
<https://doi.org/10.1517/13543776.15.9.1241>
24. Su, W.; Li, Z.; Zhao, L. *Org. Prep. Proced. Int.* **2007**, *39*, 495-502.
<https://doi.org/10.1080/00304940709458601>
25. Kim, M.; Sharma, S.; Mishra, N. K.; Han, S.; Park, J.; Kim, M.; Shin, Y.; Kwak, J. H.; Han, S. H.; Kim, I. S.; *Chem. Commun.* **2014**, *50*, 11303-11306.
<https://doi.org/10.1039/C4CC03354J>
26. Machida, Y.; Nomoto, S.; Negi, S.; Ikuta, H.; Saito, I. *Synth. Commun.* **1980**, *10*, 889-895.
<https://doi.org/10.1080/00397918008061846>
27. Soonthornsit, N.; Pitaksutheepong, C.; Hemstapat, W.; Utaisincharoen, P.; Pitaksuteepong, T. *Evidence-Based Complementary and Alternative Medicine* **2017**, 1-8.
<https://doi.org/10.1155/2017/3928956>
28. Lima, L. A.; Alves, T.; Zani, C. L.; Sales, Jr., P. A.; Romanha, A. J.; Johann, S.; Cisalpino, P. S.; Pimenta, L. P.; Boaventura, M. A. D. *Anais da Academia Brasileira de Ciências* **2014**, *86*, 829-839.
<https://doi.org/10.1590/0001-3765201420130048>
29. Shelke, K. F.; Madje, B. R.; Sapkal, S. B.; Shingate, B. B.; Shingare, M. S. *Green Chem. Lett. Rev.* **2009**, *2*, 3-7.30.
<https://doi.org/10.1080/17518250902763101>

This paper is an open access article distributed under the terms of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>)