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Rare Streptomyces sp. polyketides as modulators of K-Ras localisation

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Chemical investigations of a soil-derived Streptomyces sp. led to the isolation of five new polyketides, (+)-oxanthromicin, (±)-hemi-oxanthromicins A/B, (±)-spiro-oxanthromicin A and oxanthroquine, and the known alkaloid staurosporine, and the detection of four new metastable analogues, (±)-spiro-oxanthromicins B1/B2/C1/C2. Among the compounds tested, SAR investigations established the synthetic oxanthroquine ethyl ester and 3-O-methyl-oxanthroquine ethyl ester were optimal at mislocalising oncogenic mutant K-Ras from the plasma membrane of intact Madin-Darby canine kidney (MDCK) cells (IC50 4.6 and 1.2 µM), while a sub-EC50 dose of (±)-spiro-oxanthromicin A was optimal at potentiating (750%) the K-Ras inhibitory activity of staurosporine (IC50 60 pM). These studies demonstrate that a rare class of Streptomyces polyketide modulates K-Ras plasma membrane localisation, with implications for the future treatment of K-Ras dependent cancers.

Introduction

Ras GTPases are key molecular switches that regulate cell growth, proliferation and differentiation, and are ubiquitously expressed in mammalian cells as three isoforms (H-Ras, N-Ras and K-Ras).1 Constitutively activated oncogenic K-Ras is a key driver of oncogenesis in pancreatic adenocarcinomas (95%), colon adenomas (40%) and nonsmall cell lung cancer (15–20%).2 The key role played by K-Ras in these cancers is evidenced by experimental data, which demonstrate that inhibition of K-Ras membrane localisation blocks all oncogenic implications for the future treatment of K-Ras dependent cancers.

Results and discussion

Bioassay-guided fractionation of a solid phase (cracked wheat) cultivation of MST-134270 resulted in the isolation and characterisation of five new polyketides, (+)-oxanthromicin (1), (±)-hemi-oxanthromicin A (2), (±)-hemi-oxanthromicin B (3), (±)-spiro-oxanthromicin A (4), and oxanthroquine (9), as well as the detection and identification of four new metastable analogues, (±)-spiro-oxanthromicins B1/B2 (5/6), and (±)-spiro-oxanthromicins C1/C2 (7/8), and the isolation of the known indole alkaloid staurosporine (10) (Fig. 1).

HRESI(−)MS measurements on 1 established a molecular formula of C36H35O12 (Δmmu −0.2) while the NMR (DMSO-d6) data (Fig. 2 and ESI Table S1a) revealed only 18 carbon resonances, necessitating a degree of symmetry. Further analysis of the 1H NMR data revealed resonances for one tertiary methyl (δH 1.44), two benzylic methyis (δH 2.18 and 2.69), one isolated (δH 7.16) and two ortho coupled (δH 6.33 and 7.12, 7.8 Hz) aromatic protons, and a chelated hydroxy group (δH 13.46, s), with diagnostic 2D NMR correlations permitting assembly of a dimeric anthrone featuring a rare peroxide bridge. A search of the literature and comparison of NMR data with the published compound (ESI Table S1b) confirmed that 1 was (+)-oxanthromicin ([α]D25 +157, c 0.26, EtOH).5 A new enantiomer of the rare Streptomyces metabolite (+)-oxanthromicin ([α]D26−172, c 0.3, EtOH).5

Molecular formulae attributed to 2 (C34H34O10 Δmmu +0.8) and 3 (C34H32O9 Δmmu −0.2) on the basis of HRESI(−)MS measurements were suggestive that both compounds contain...
Detailed analysis of these NMR data, including consideration of the aromatic system possessing many structural features. HRESI-MS measurements established a molecular formula of $\text{C}_{16}\text{H}_{20}\text{O}_{10}$ ($\Delta$m $0.3$) for 4, while analysis of the NMR (DMSO-$d_6$) data (Fig. 3 and ESI Table S4), suggested a heavily substituted aromatic system possessing many structural characteristics in common with the co-metabolites 1–3. Detailed analysis of these NMR data, including consideration of diagnostic 2D NMR correlations, permitted assembly of the planar structure as indicated. More specifically, HMBC correlations required that the aromatic methyl H$_2$-13 be flanked by H-6 and the phenolic 8-OH, while additional correlations linked this fragment to the quaternary aromatic C-8a and C-10a, and the sp$^3$ spiro C-10’. COSY correlations established the H$_3$-14’ to H-14 fragment, while HMBC correlations linked this fragment to C-10’, C-5, C-10a and C-10. Further HMBC correlations required that the aromatic methyl H$_2$-11 be flanked by C-2 and C-9a, and para-disposed to H-4, which was in turn flanked by C-4a and C-5 (the latter bearing a hydroxy group). Additional HMBC correlations from H-4 to C-10, supported by ROESY correlations between (i) H-6 and H$_2$-13, (ii) H$_3$-13 and 8-OH, (iii) 8-OH and H$_2$-11 and (iv) H-4 and H-14, defined the ABCD ring system as indicated (Fig. 3). Comparable 2D NMR correlations defined the EFG ring system, with diagnostic HMBC correlations linking the spiro C-10’ to H-4’, H-5’ and H-6’. ROESY correlations between H-5’ and both H-6 and H$_2$-14’, and between H-4’ and both H-6 and H$_2$-14’, defined the orthogonal relationship between the ABCD and EFG ring systems (Fig. 3). Despite the presence of a chiral centre (C-10’), the lack of an optical rotation required that (+)-spiro-oxanthromicin A (4) be assigned the racemic structure as indicated. Further chemical studies supportive of this structure assignment are presented below.

HPLC-DAD-MS analysis of the crude MeOH extract of *Streptomyces* sp. MST-134270 confirmed the dominant cultivation/biosynthetic products as 1, 2 and 10, with 3 and 4 only detected at trace levels (ESI Fig. S10). Significantly, during SPE fractionation, the detected (and recovered) yields of 3 and 4 increased, as did levels of two hitherto undetected compounds 5 and 6. These observations strongly suggested that 3–6 were capable of being produced during handling (ESI Fig. S11). In support of this hypothesis, exposure of a pure sample of 2 to 0.1% TFA/MeOH (conditions comparable to those encountered during SPE fractionation) resulted in partial conversion to 3 and 4, while exposure to 0.1% TFA/MeCN yielded only 4 (ESI Fig. S12). Likewise, a pure sample of 3 was observed to undergo partial conversion to 2 during routine handling.

**Fig. 1** Structures of 1–10

**Fig. 2** Selected 2D NMR correlations for 1–3 and 9

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Finally, the acid (±)-spirooxanthromicin A (8) can undergo irreversible dehydration to yield (±)-spiro-oxanthromicin A (4) as a stable quinone methide. In addition to rationalizing the biosynthetic/chemical relationships between 1–9, we speculated that the polyketide precursor, oxanthroquinone (9), undergoes stereospecific methylation to a single (10R) enantiomer of 2, which in turn undergoes dimerisation to (+)-oxanthromicin (1). Acid-mediated dehydration of 2 could deliver an achiral carbocation intermediate that is reversibly quenched with either H₂O or MeOH to yield (±)-hemi-oxanthromicin A (2) or B (3) respectively. Significantly, the carbocation intermediate could also transform, via a mechanism foreshadowed in a 1979 study directed at the acid-mediated dimerisation of 10-methyleneanthrone,⁶ to yield a (±)-spiro-carbocation. The (±)-spiro-carbocation could in turn undergo reversible quenching with either MeOH or H₂O to deliver the diastereomeric (±)-spiro-oxanthromicin B₁ (5) and B₂ (6), or the diastereomeric (±)-spiro-oxanthromicin C₁ (7) and C₂ (8), respectively. Finally, the acid-labile doubly benzylic 10-OH moiety in 7 and 8 can undergo irreversible dehydration to yield (±)-spiro-oxanthromicin A (4) as a stable quinone methide. In addition to rationalising the biosynthetic/chemical relationships between 1–9, we speculated that the polyketide precursor, oxanthroquinone (9), undergoes stereospecific methylation to a single (10R) enantiomer of 2, which in turn undergoes dimerisation to (+)-oxanthromicin (1). Acid-mediated dehydration of 2 could deliver an achiral carbocation intermediate that is reversibly quenched with either H₂O or MeOH to yield (±)-hemi-oxanthromicin A (2) or B (3) respectively. Significantly, the carbocation intermediate could also transform, via a mechanism foreshadowed in a 1979 study directed at the acid-mediated dimerisation of 10-methyleneanthrone,⁶ to yield a (±)-spiro-carbocation. The (±)-spiro-carbocation could in turn undergo reversible quenching with either MeOH or H₂O to deliver the diastereomeric (±)-spiro-oxanthromicin B₁ (5) and B₂ (6), or the diastereomeric (±)-spiro-oxanthromicin C₁ (7) and C₂ (8), respectively. Finally, the acid-labile doubly benzylic 10-OH moiety in 7 and 8 can undergo irreversible dehydration to yield (±)-spiro-oxanthromicin A (4) as a stable quinone methide.

In an effort to assign structures to 5 and 6, we noted that their UV-vis (DAD) spectra were similar to those of 1–3, suggestive of closely related chromophores and molecular structures, while HPLC-HRESI(-)MS analysis suggested that 5 (C₁₇H₁₉O₁₁, Δmmu +2.4) and 6 (C₁₇H₁₉O₁₁, Δmmu +2.0) were isomeric MeOH adducts of 4. Attempts at purification of 5 and 6 by reversed phase HPLC proved problematic as immediately post-elution both underwent partial conversion to 7 and 8, a transformation that proceeded to near-completion after standing at r.t. for 3 h (ESI Figs. S13–S14). The transformation products 7 and 8 exhibited almost identical UV-vis (DAD) spectra to 5 and 6, with HPLC-HRESI(-)MS analysis suggesting that 7 (C₁₈H₂₀O₁₁, Δmmu +0.6) and 8 (C₁₈H₂₀O₁₁, Δmmu +0.0) were isomeric H₂O adducts of 4. On concentrating in vacuo and resuspending in MeOH, the mixture of 7 and 8 rapidly transformed to a complex mixture of 4–8, dominated by 4.

Fig. 3 Selected 2D NMR correlations for 4
1–9, this biosynthetic/chemical pathway demonstrates for the first time that a rare spiro dimerisation mechanism, first proposed in 1979,6 has a footprint in the natural world.

To support the structural assignments outlined above, and to provide material for a structure activity relationship (SAR) study, we embarked on the syntheses summarised in Scheme 1. Commercially available 2,4-dichloro-1,4-benzoquinone was treated with the Danishefsky diene derived from tiglic aldehyde7 to form a Diels-Alder adduct, which on Jones oxidation yielded 2-chloro-8-hydroxy-7-methylnaphthaquinone8 (60%). A subsequent Diels-Alder reaction with the Danishefsky diene derived from ethyl diacetoacetate9 yielded oxanthroquinone ethyl ester (11) (58%) (ESI Figs. S6a–S6b), which on hydrolysis returned oxanthroquinone (9) (88%). Treatment of synthetic 9 with MeMgBr resulted in regioselective addition to C-10 in preference to C-9, which is chelated to the adjacent 8-hydroxy, to yield (±)-hemi-oxanthromicin A (2) (45%). NMR data showed that synthetic samples 9 and 2 are identical in all respects to the natural products. As the stability studies discussed above had established a sequence of chemical transformations from 2 to 3–8, the total synthesis of 9 and 2 represents a formal synthesis of 3–8. To further explore SAR, we exploited the chelation of 8-OH to selectively monomethylate 11 with MeI (Scheme 1) to yield 3-O-methyl oxanthroquinone ethyl ester (14) (72%) (ESI Figs. S7a–S7b).

7-Me and 2-CO₂H moieties, we completed the syntheses outlined in Scheme 2, transforming 3-bromojuglone to 7-desmethyloxanthroquinone ethyl ester (12) (68%) (ESI Figs. S8a–S8b) and 7-desmethyloxanthroquinone (13) (90%) (ESI Figs. S9a–S9b).

On reviewing the polyketide natural products literature, we noted 1–9 possess unique 1-Me/7-Me and 2-CO₂H substitutions. To explore the possible SAR significance of the

![Scheme 1. Synthesis of 2, 9, 11, and 14.](image)

![Scheme 2. Synthesis of 12 and 13.](image)

We next set out to use quantitative confocal imaging to measure the ability of 1–2, 4, 9 and 11–14 to mislocalise oncogenic mutant K-Ras (mGFP-K-RasG12V) from the PM of intact Madin Darby canine kidney (MDCK) cells following a previously published protocol.4 The results (Table 1) revealed that the natural product (+)-oxanthromicin (1), the dimeric transformation product (±)-spiro-oxanthromicin A (4) and the synthetic ethyl ester analogues 12, 11 and 14 (in increasing order of potency) were effective at mislocalising K-Ras from the PM. An SAR analysis of these data suggests that the monomers 11 and 12–14 are more active than the dimers 1 and 4, and that esterification of the 2-CO₂H moiety improves K-Ras mislocalisation. Activity is further enhanced by the presence of a 7-Me, and substitution (methylation) of the 3-OH.

![Table 1. Summary of K-Ras mislocalisation studies](image)

To further our investigations into oxanthromicin/oxanthroquinone chemistry and biology, we analysed our in-house database of the HPLC-DAD secondary metabolite profiles of ~50,000 microbial extracts, to detect additional Streptomyces capable of producing examples of this
structure class. This study revealed two *Streptomyces* that were subsequently re-cultivated and subjected to detailed chemical analysis. *Streptomyces* sp. MST-RA9773 isolated from a soil sample collected near Barellan, New South Wales (NSW), and *Streptomyces* sp. MST-104069 isolated from a soil sample collected near Broken Hill, NSW, produced 1 and related *hemi-* and *spiro-*oxanthromicins, only the former produced 10. This analysis established that oxanthromicins/oxanthroquinone are exceptionally rare with an incidence (in our library) of ~1:17,000, in contrast to staurosporine with an incidence of ~1:100. The repeated co-production of oxanthromicins/oxanthroquinone and staurosporine is noteworthy, and raised the possibility that these structurally diverse microbial metabolites may exhibit synergistic biological properties. To probe this hypothesis, we quantified the K-Ras mislocalising properties of 10 when exposed to sub-IC₅₀ doses of 1–2, 4, 9 or 12–13 (4 µM), 11 (1.45 µM) and 14 (0.60 µM), revealing significant levels of synergism by 1 (130%), 4 (750%), 11 (410%) and 14 (470%).

**Conclusions**

In conclusion, this report describes a successful high-throughput, high-content microbial biodiscovery approach to detect and identify novel small molecule inducers of K-Ras PM mislocalisation. Our chemical investigations of *Streptomyces* sp. MST-134270 yielded a suite of new polyketides 1–9, interconnected by an array of biosynthetic and chemical transformations, inclusive of the first natural occurrence of a rare *spiro* dimerisation reaction. Structure elucidations were supported by detailed spectroscopic analysis, chemical interconversion and total synthesis. SAR studies established the synthetic anthraquinone 14 as a potent selectable candidate, capable of mislocalising oncogenic mutant K-Ras (mGFP-K-RasG12V) from the PM of intact MDCK cells. We established the natural occurrence of oxanthromicins/oxanthroquinone (in our library) as being exceptionally low, and correlated with the co-production of staurosporine (10). Co-treatment of 10 with sub-EC₅₀ doses of selected oxanthroquinones resulted in a significant synergism of K-Ras PM mislocalisation. Collectively, these studies establish that a rare class of *Streptomyces* polyketides, and analogues inspired by these compounds, can induce significant K-Ras PM mislocalisation (IC₅₀ 1.2 µM), and can synergise the K-Ras PM mislocalisation properties of staurosporine (IC₅₀ 60 pM). A detailed account of the biological properties and mechanism of action of these polyketides will be reported elsewhere.

**Experimental section**

**Microbial Cultivation and Extraction**

A *Streptomyces* sp. (MST-134270) cultivation was incubated for 10 days at 28 °C in 40 Erlenmeyer flasks (250 mL each) containing sterilised cracked wheat (50 g) hydrated in water (30 mL), and inoculated with 5 mL of a ISP2 media seed fermentation. The resulting ferment (3.14 kg) was extracted with acetone (6 L), filtered and concentrated *in vacuo* to an aqueous concentrate (800 mL). The aqueous concentrate was extracted with EtOAc (1.5 L) and concentrated *in vacuo* to yield a crude EtOAc extract (7.2 g), which was subsequently partitioned between hexane and MeOH to give hexane-soluble (2.4 g) and MeOH-soluble (4.8 g) extracts (ESI Scheme S1).

**Fractionation and characterisation of compounds**

A portion of MeOH-soluble extract (206 mg) was fractionated using a C₁₈-max SPE cartridge (5 g) eluting with a stepwise gradient from 90% H₂O/MeOH to 100% MeOH with isocratic 0.01% TFA modifier to give Fractions A–G. SPE Fraction E (62 mg), eluting at 30% H₂O/MeOH, was further fractionated by semi-preparative HPLC (Agilent Zorbax XDB-C₅, 5 µm, 9.4 × 250 mm column, 10 min gradient elution at 3.5 mL/min from 70–20% H₂O/MeCN, then 100% MeCN for 5 min, with isocratic 0.01% TFA modifier) to afford staurosporine (10) (tᵣ 4.8 min, 16.0 mg), (α)-*hemi*-oxanthromicin A (2) (tᵣ 8.1 min, 17.1 mg) and (α)-*hemi*-oxanthromicin B (3) (tᵣ 10.1 min, 2.9 mg). SPE Fraction F (40 mg), eluting at 15% H₂O/MeOH, was further fractionated by semi-preparative HPLC (Agilent Zorbax XDB-C₅, 5 µm, 9.4 × 250 mm column, 12 min gradient elution at 3.5 mL/min, from 50–20% H₂O/MeCN with isocratic 0.01% TFA modifier) to afford staurosporine (10) (tᵣ 2.8 min, 7.5 mg), (α)-*hemi*-oxanthromicin A (2) (tᵣ 5.2 min, 8.0 mg), oxanthroquinone (9) (tᵣ 6.5 min, 2.0 mg), (α)-*spiro*-oxanthromicin B (3) (tᵣ 7.5 min, 5.1 mg), (α)-*spiro*-oxanthromicin B₁ (5)* (tᵣ 9.0 min, 2.0 mg), (α)-*spiro*-oxanthromicin A (4) (tᵣ 9.8 min, 1.8 mg), (α)-*spiro*-oxanthromicin B₂ (6)* (tᵣ 10.6 min, 1.5 mg). (*Note: 5 and 6 transformed to the more stable 4 during the removal of HPLC solvents.) SPE Fraction G (55 mg), eluting with MeOH, was further fractionated by semi-preparative HPLC (Agilent Zorbax XDB-C₅, 5 µm, 9.4 × 250 mm column, 12 min gradient elution at 3.5 mL/min, from 60% H₂O/MeCN to 100% MeCN with isocratic 0.01% TFA modifier) to afford staurosporine (10) (tᵣ 3.5 min, 5.5 mg), (α)-*hemi*-oxanthromicin A (2) (tᵣ 6.2 min, 0.7 mg), (α)-*hemi*-oxanthromicin B (3) (tᵣ 8.2 min, 1.8 mg), (α)-*spiro*-oxanthromicin A (4) (tᵣ 9.7 min, 2.3 mg) and (α)-*oxanthromicin* (1) (tᵣ 11.4 min, 13.7 mg) (ESI Scheme S1).

Based on the above, the estimated % yield from the crude culture extract is 1 (0.99%), 2 (1.9%), 3 (0.71%), 4 (0.30%), 5 (0.14%), 6 (0.11%), 9 (0.15%) and 10 (2.1%) (Note: these yields do not take into account the transformation of 2 to 3 and 4, 3 to 2; 5 and 6 to 4 after HPLC purification). (ESI Figs. S13–S14).

(+)-*oxanthromicin* (1): Yellow amorphous solid; [α]D⁺²² +157 (c=0.26, EtOH); UV (MeOH) λmax (log ε) 259 (4.34), 321 (4.31), 355 (4.22) nm; NMR (DMSO-d₆) see ESI Table S1 and Figs. S1a–S1b; HRESI(+)-MS m/z 653.1662 [M+H⁺] (calced for C₁₉H₁₄O₇).

(α)-*hemi*-oxanthromicin A (2): Yellow amorphous solid; [α]D⁺²² 0 (c=0.10, EtOH); UV (MeOH) λmax (log ε) 259 (4.05), 325 (4.02), 341 (3.99), 356 (3.96) nm; NMR (DMSO-d₆) ESI Table S2 and Figs. S2a–S2b; HRESI(+)-MS m/z 327.0882 [M+H⁺] (calced for C₁₄H₁₂O₅).
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(+)–hemi-oxanthromicin B (3): Yellow amorphous solid; \([\text{rt}]_{D}^{22} = 0 (c=0.13, \text{EtOH}); \text{UV (MeOH)} \lambda_{\text{max}} (\log \varepsilon) = 258 (4.12), 323 (4.10), 344 (4.04), 355 (4.03) \text{ nm;} \text{NMR (DMSO-d}_6) \text{ESI Table S3 and Figs. S3a–S3b;} \text{HRESI-MS } m/z = 341.1029 [M–H]– (calcd for C_{19}H_{11}O_{6}, 341.1031).

(±)-spiro-oxanthromicin A (4): Yellow amorphous solid; \([\text{rt}]_{D}^{22} = 0 (c=0.13, \text{EtOH}); \text{UV (MeOH)} \lambda_{\text{max}} (\log \varepsilon) = 239 (4.55), 258 (4.48), 303 (4.38), 356 (4.18) \text{ nm;} \text{NMR (DMSO-d}_6) \text{ESI Table S4 and Figs. S4a–S4b;} \text{HRESI-MS } m/z = 617.1450 [M–H]– (calcd for C_{32}H_{25}O_{10}, 617.1453).

(±)-spiro-oxanthromicin B1 (5): UV (MeCN/H_2O) \lambda_{\text{max}} = 235, 275, 320, 360 nm; \text{HRESI-MS } m/z = 649.1739 [M–H]– (calcd for C_{32}H_{25}O_{10}, 649.1715).

(±)-spiro-oxanthromicin B2 (6): UV (MeCN/H_2O) \lambda_{\text{max}} = 235, 275, 320, 360 nm; \text{HRESI-MS } m/z = 649.1735 [M–H]– (calcd for C_{32}H_{25}O_{10}, 649.175).

(±)-spiro-oxanthromicin C1 (7): UV (MeCN/H_2O) \lambda_{\text{max}} = 235, 275, 320, 360 nm; \text{HRESI-MS } m/z = 635.1565 [M–H]– (calcd for C_{28}H_{21}O_{9}, 635.1559).

(±)-spiro-oxanthromicin C2 (8): UV (MeCN/H_2O) \lambda_{\text{max}} = 235, 275, 320, 360 nm; \text{HRESI-MS } m/z = 635.1559 [M–H]– (calcd for C_{28}H_{21}O_{9}, 635.1559).

oxanthroquinone (9): Orange amorphous solid; UV (MeOH) \lambda_{\text{max}} (\log \varepsilon) = 221 (4.19), 284 (4.14), 412 (3.53) nm; \text{NMR (DMSO-d}_6) m/z = 221 (4.50), 273 (4.58), 414 (3.93) nm; \text{1H NMR (600 MHz, CDCl}_3); \delta_{H} = 12.06 (s, 1H), 7.56 (d, J = 7.7 Hz, 1H), 7.53 (d, J = 7.7 Hz, 1H), 7.17 (s, 1H), 2.37 (s, 3H).

oxanthroquinone ethyl ester (11): A solution of 2-chloro-8-hydroxy-7-methylnapthaquinone (80 mg, 0.36 mmol) and the Danishefsky diene derived from ethyl diazoacetate\(^{2}\) (230 mg, 0.72 mmol) in toluene (5 mL) was refluxed for 3 days. After concentrating in vacuo the residue was extracted with CH_2Cl_2 (5 mL) and stirred in the presence of silica gel (240 mg) at r.t. for 5 min. The reaction mixture was then concentrated in vacuo and the residue purified by silica gel chromatography (isocratic elution 1:10 EtOAc:light petroleum) to afford oxanthroquinone ethyl ester (11) (R_f 0.4; 71 mg, 58%). UV (MeOH) \lambda_{\text{max}} (\log \varepsilon) = 221 (4.50), 273 (4.58), 414 (3.93) nm; \text{1H NMR (600 MHz, CDCl}_3); \delta_{H} = 13.28 (s, 1H), 10.49 (s, 1H), 7.78 (s, 1H), 7.69 (d, J = 7.6 Hz, 1H), 7.49 (d, J = 7.6 Hz, 1H), 4.53 (q, J = 7.2 Hz, 2H), 2.99 (s, 3H), 2.38 (s, 3H), 1.48 (t, J = 7.2 Hz, 3H); \text{13C NMR (150 MHz, DMSO-d}_6); \delta_{C} = 189.6, 181.6, 166.8, 160.0, 158.9, 141.0, 136.8, 136.5, 134.6, 130.2, 129.8, 122.4, 118.1, 115.7, 112.0, 61.5, 19.9, 15.9, 14.1 (ESI Figs. S6a–S6b); \text{HRESI-MS } m/z = 339.0874 [M+]– (calcd for C_{19}H_{18}O_4, 339.0874).

oxanthroquinone (9): A solution of 11 (70 mg, 0.21 mmol) inaq. LiOH (1 M; 2 mL) was stirred at 100 °C overnight. The dark red solution was then acidified by addition ofaq. HCl (1 M; 2.1 mL) and extracted with EtO (3 × 5 mL). The organic phase was dried over anhydrous MgSO_4, concentrated in vacuo and purified using a C_18 SFE cartridge (stepwise gradient of 90% H_2O/MeCN to 100% MeCN) to afford synthetic oxanthroquinone (9; 56 mg, 88%) identical in all respects to natural 9.

(+)-hemi-oxanthromicin A (2). A solution of 9 (20 mg, 0.064 mmol) in anhydrous THF (1 mL) was cooled to 0 °C and MeMgBr (3 M in EtO; 430 µL, 1.3 mmol) was added. The mixture was allowed to reach r.t. and then stirred overnight. After quenching with sat. aqueous NH_4Cl, the reaction mixture was acidified to pH 4 with 0.1 M HCl and extracted with EtO (3 × 3 mL), after which the organic phase was dried over anhydrous MgSO_4, concentrated in vacuo, and purified by semi-preparative HPLC [Agilent Zorbax RX-C_8, 5 µm, 9.4 × 250 mm column, 15 min gradient elution at 3.5 mL/min from 90% H_2O/MeCN to 100% MeCN with isocratic 0.01% TFA modifier] to afford synthetic (+)-hemi-oxanthromicin A (2; R_t 10.9 min, 8.3 mg, 45%), identical in all respects to natural 2, and recovered oxanthroquinone (9; R_t 11.9 min, 9 mg, 43%).

3-O-methyl oxanthroquinone ethyl ester (14): A solution of 11 (6.0 mg, 0.018 mmol) and K_2CO_3 (5.0 mg, 0.035 mmol) in acetone (1 mL) was treated with MeI (6.0 mg, 0.042 mmol) and stirred overnight at r.t. The filtered reaction mixture was acidified to pH 4 with 0.1 M HCl and extracted with EtO (3 × 3 mL), and the organic phase dried over anhydrous MgSO_4, concentrated in vacuo, and purified by preparative HPLC [Phenomenex Luna C_18 (2), 10 µm, 21.2 × 250 mm column, 15 min gradient elution at 20 mL/min from 90% H_2O/MeCN to 100% MeCN with isocratic 0.01% TFA modifier] to afford 3-O-methyl-oxanthroquinone ethyl ester (14; R_t 16.9 min, 4.5 mg, 72%). UV (MeOH) \lambda_{\text{max}} (\log \varepsilon) = 222 (4.70), 270 (4.72), 298
(4.28), 415 (3.94) nm; 1H NMR (600 MHz, CDCl3): δH = 13.25 (s, 1H), 7.75 (s, 1H), 7.69 (d, J = 7.7 Hz, 1H), 7.48 (d, J = 7.7 Hz, 1H), 4.46 (q, J = 7.2 Hz, 1H), 4.01 (s, 3H), 2.76 (s, 3H), 2.37 (s, 3H), 1.41 (t, J = 7.2 Hz, 3H); 13C NMR (150 MHz, CDCl3): δC = 190.3, 182.5, 167.3, 161.1, 160.0, 141.4, 137.8, 136.5, 135.8, 131.5, 130.6, 125.0, 118.8, 116.2, 107.6, 62.1, 56.6, 20.2, 16.5, 14.3 (ESI Figs. S7a–S7b); HRESI(+MS m/z 377.0997 [M+Na]+ (calcd for C20H15O2Na2, 377.0996).

7-desmethyloxanthroquinone ethyl ester (12): A solution of 3-bromojuglone10 (100 mg, 0.40 mmol) and the Danishefsky diene derived from ethyl diaacetate9 (250 mg, 0.79 mmol) in toluene (5 mL) was refluxed for 3 days. After concentrating in vacuo the residue was dissolved in CH2Cl2 (5 mL), stirred with silica gel (250 mg) at rt for 5 min, concentrated in vacuo and purified by silica gel chromatography (isocratic elution 1:1 EtOAc:light petroleum) to afford 7-desmethyloxanthroquinone ethyl ester (12; Rf 0.4; 88 mg, 68%).11 UV (MeOH) λmax (log ε) 220 (4.20), 272 (4.26), 409 (3.47) nm; 1H NMR (600 MHz, CDCl3): δH = 12.95 (s, 1H), 10.53 (s, 1H), 7.79 (s, 1H), 7.78 (dd, J = 7.5, 1.0 Hz, 1H), 7.62 (dd, J = 8.3, 7.5 Hz, 1H); 7.31 (dd, J = 8.3, 1.0 Hz, 1H), 4.54 (q, J = 7.2 Hz, 1H), 2.99 (s, 3H), 1.48 (t, J = 7.2 Hz, 3H); 13C NMR (150 MHz, CDCl3): δC = 189.6, 182.2, 170.1, 163.6, 162.5, 148.0, 138.7, 135.8, 132.7, 125.0, 124.5, 121.2, 118.9, 117.5, 115.1, 62.9, 21.9, 14.1 (ESI Figs. S8a–S8b); HRESI(+MS m/z 325.0719 [M+H]+ (calcd for C13H13O2, 325.0718).

7-desmethyloxanthroquinone (13): A solution of 12 (48 mg, 0.15 mmol) inaq. LiOH (1 M; 1 mL) was stirred at 100 °C overnight. The dark red solution was acidified byaq. HCl (1 M; 1.05 mL) and extracted with Et2O (2 × 5 mL), after which the organic phase was dried over anhydrous MgSO4, concentrated in vacuo, and purified using a C18 SPE cartridge (stepwise gradient of 90% H2O/MeCN to 100% MeCN) to afford 7-desmethyloxanthroquinone (13; 39 mg, 90%).12 UV (MeOH) λmax (log ε) 217 (4.68), 280 (4.72), 411 (4.08) nm; 1H NMR (600 MHz, DMSO-d6): δH = 12.89 (s, 1H), 7.76 (dd, J = 8.3, 7.5 Hz, 1H), 7.66 (dd, J = 7.5, 1.1 Hz, 1H), 7.60 (s, 1H), 7.36 (dd, J = 8.3, 1.1 Hz, 1H), 2.71 (s, 3H); 13C NMR (150 MHz, DMSO-d6): δC = 189.4, 182.1, 168.3, 161.5, 159.2, 140.9, 136.4, 136.2, 132.5, 131.2, 124.5, 122.5, 118.4, 116.9, 112.2, 20.0 (ESI Figs. S9a–S9b); HRESI(+MS m/z 297.0405 [M+H]+ (calcd for C13H13O2, 297.0405).

K-Ras bioassay

Madin-Darby canine kidney (MDCK) cells stably co-expressing monomeric green fluorescent protein (mGFP) coupled to the N-terminus of oncogenic K-Ras (K-RasG12V) and mCherry-CAAX, a red fluorescent fusion protein that decorates endomembranes,4 were plated at 150,000 cells/well on 12-well plates. After 24 h, cells were treated with compounds and incubated for another 48 h. Each compound was tested in 3 independent experiments. At the end of incubation time, cells were fixed with 4% paraformaldehyde and imaged in a Nikon A1R confocal microscope. Ras mislocalisations from the plasma membranes were calculated using Manders coefficients, by measuring the fraction of mCherry-CAAX co-localizing with mGFP-K-RasG12V.4 IC50 values and two-tailed t-tests were calculated using Prism software (Ver. 5.0c, GraphPad). Emax quantifies the maximum extent of mislocalisation of K-Ras from the plasma membrane to endomembrane.

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Notes and references

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Electronic Supplementary Information (ESI) available: General experimental details, full details of microbial collection and taxonomy, tabulated 2D NMR data and NMR spectra and LCMS stability studies. See DOI: 10.1039/b000000x/