Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 13 (2005) 4228–4237
Bioorganic & Medicinal Chemistry

Synthesis of gossypol atropisomers and derivatives and evaluation of their anti-proliferative and anti-oxidant activity

Kalliopi Dodou,[a] Rosaleen J. Anderson,[a] W. John Lough,[a] David A. P. Small,[b] Michael D. Shelley[c] and Paul W. Groundwater[a,*]

[a]*Sunderland Pharmacy School, University of Sunderland, Wharncliffe Street, Sunderland SR1 3SD, UK*
[b]*Stiefel International R&D, Whitebrook Park, 68 Lower Cookham Road, Maidenhead, Berkshire SL6 8XY, UK*
[c]*Velindre NHS Trust, Whitchurch, Cardiff CF14 2TL, UK*

Received 3 December 2004; revised 7 April 2005; accepted 12 April 2005
Available online 4 May 2005

Abstract—Gossypol **1**, gossypolone **2**, and a series of bis **3** and half Schiff's bases **4** of gossypol were synthesised and tested for anti-proliferative and anti-oxidant activity. (−)-Gossypol (−)-**1** was the most potent inhibitor of the proliferation of the HPV-16 keratinocyte cell line (using an MTT viability assay) with a GI_{50} of 4.8 μM. The bis Schiff's base of (−)-gossypol with L-tyrosine ethyl ester (−)-**3b** was the most potent inhibitor of iron/ascorbate dependent lipid peroxidation (using the thiobarbituric acid test), with an IC_{50} of 11.7 μM, with (−)-gossypol being the next most potent of the series, with an IC_{50} of 13.1 μM. The results from these initial assays suggest that gossypol, as either a racemic mixture **rac-1**, or the individual atropisomers (−)-**1** or (+)-**1**, has potential for the treatment of psoriasis.
© 2005 Elsevier Ltd. All rights reserved.

1. Introduction

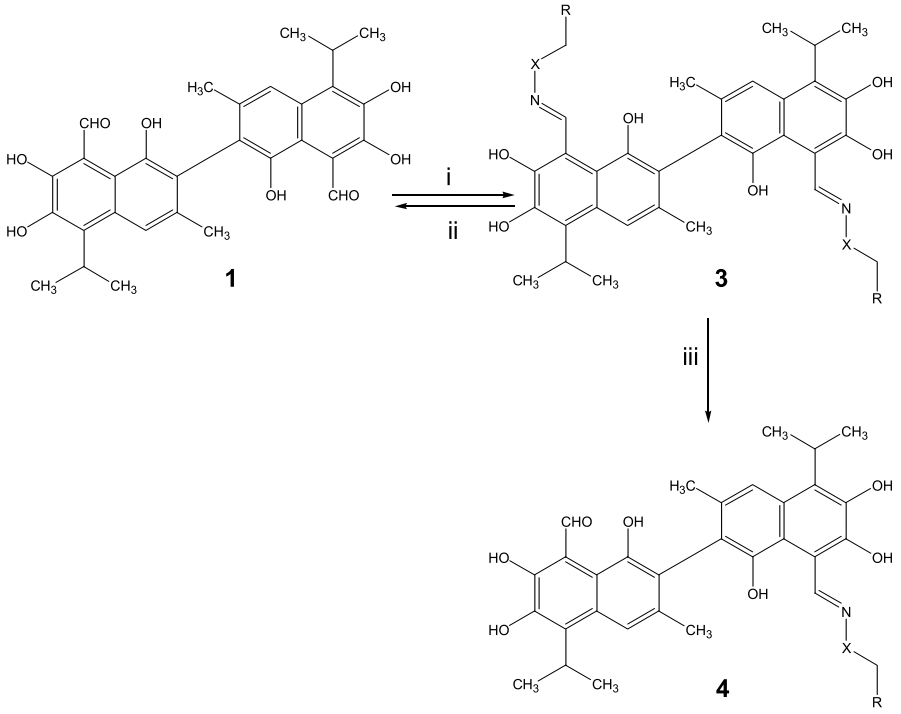
Psoriasis is a genetically heterogeneous skin condition, which is characterised by benign keratinocyte hyperproliferation, skin inflammation, altered dermal vasculature and defective keratinisation.[1,2] The cell-mediated immune mechanisms, which lead to the increased proliferation have been the subject of much attention, but the antigen responsible remains elusive.[3] Therapeutic interventions for psoriasis are usually based upon anti-proliferative, anti-inflammatory and differentiation-modifying activity or a combination of these effects,[4] but there is no cure for psoriasis. A number of selective immune response modifiers are in development.[3]

Gossypol **1**, a yellow anti-inflammatory compound that is present in members of the Malvaceae family, such as the cotton plant (*Gossypium* species) and the tropical tree *Thespesia populnea*, has been studied extensively since the discovery of its in vivo male anti-fertility activity in the late 1960's,[5–7] and has also been shown to exhibit in vitro anti-viral and anti-parasitic activities at micromolar levels,[8,9] and anti-tumour activity.[10–12] Gossypol exhibits atropisomerism, as a result of restricted rotation about the C_2–C_2' binaphthyl bond, resulting in two optically active forms, *l*- or (−), (−)-**1**, and *d*- or (+), (+)-**1**. The (−)-enantiomer is usually more potent in all these biological systems, when compared to the (+)-enantiomer and the racemic mixture **rac-1**. It has been suggested that the (−)-enantiomer in low concentrations affects cells in a stereospecific manner, whereas non-stereospecific interactions are found for (+)-gossypol and higher concentrations of (−)-gossypol.[13]

Gossypol **1** has been found to be reasonably potent against melanoma cell lines, with the (−)-isomer being more cytotoxic than cisplatin, melphalan and dacarbazine.[14] In addition, the anti-melanotic activity of gossypol **1**, its oxidation product gossypolone **2** (Scheme 2), the bis **3a** and half Schiff's base derivatives **4a** (Scheme 1) of gossypol with L-phenylalanine was studied in pigmented and non-pigmented melanoma cell lines. These studies showed that blocking both aldehyde groups [as bis Schiff's bases **3a**, R = Ph, X = (*S*)-$CHCO_2CH_3$] abolished activity against both cell lines, while retaining one aldehyde group (half Schiff's base with L-phenylalanine methyl ester **4a**) increased the activity against both the pigmented and non-pigmented cell lines, with the half

Keywords: Gossypol; Atropisomers; Psoriasis; Anti-oxidant; Anti-proliferative.
* Corresponding author. Tel.: +44 (0)191 515 2600; fax: +44 (0)191 515 3405; e-mail: paul.groundwater@sunderland.ac.uk

0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.04.026



Scheme 1. Reagents and conditions: (i) RCH_2XNH_2, DCM, IPA; (ii) AcOH, Et_2O, H_2SO_4, 0 °C, 16 h; (iii) AcOH, Et_2O, H_2SO_4, 0 °C, 4 h.

Schiff's base of (−)-gossypol, (−)-**4a**, being the most potent compound.[15]

Having considered the previously reported anti-inflammatory activity of gossypol,[16,17] we have investigated the activity of gossypol and its derivatives as novel agents for the treatment of psoriasis. We have tested these compounds as inhibitors of keratinocyte hyperproliferation (one of the hallmarks of psoriasis) and their antioxidant activity (based upon the 'oxidative stress' theory for the pathophysiology of psoriasis[18]). We have also investigated the importance of the aldehyde groups in gossypol **1**, in order to observe any structure–activity relationship correlations between the anti-melanotic and anti-psoriatic activities of gossypol and its derivatives.

2. Results and discussion

2.1. Chemistry

The bis Schiff's base diastereoisomeric mixtures of gossypol with amino acid esters **3** (Scheme 1), including two novel diastereoisomeric pairs with L-tyrosine ethyl ester and D-tryptophan methyl ester, were prepared by a reported method[19] and separated by chromatography on silica gel (Scheme 1, Table 1), using HPLC for reaction monitoring and the determination of diastereoisomeric purity. In solution, the bis Schiff's bases can exist as either the enamine **3** or imine tautomers **3′** (Fig. 1),[20] depending upon the amino acid ester used and the solvent, with the L-phenylalanine methyl ester **3a** and D- and L-tryptophan methyl ester **3c** derivatives existing as the enamines (with a doublet for the NH*CH*═), and the L-tyrosine ethyl ester derivative **3b′** existing as the imine tautomer (with a singlet for the imine *CH*═N) in $CDCl_3$. Interestingly, the Schiff's base with L-tyrosine ethyl ester exists as the enamine tautomer **3** in DMSO-d_6. For the first time, the ^1H and ^{13}C NMR spectra of the individual diastereoisomers of the bis **3** and half **4** Schiff's bases were fully assigned, using a variety of experiments, which included HMQC and HMBC spectra.

Table 1. % Yield and structure of the individual gossypol bis Schiff's bases **3**

Bis Schiff's base	R	X	% Yield
(−)-**3a**	Ph	(*S*)-$CHCO_2CH_3$	39
(+)-**3a**	Ph	(*S*)-$CHCO_2CH_3$	31
(−)-**3b**	C_6H_4OH-4	(*S*)-$CHCO_2CH_2CH_3$	16
(+)-**3b**	C_6H_4OH-4	(*S*)-$CHCO_2CH_2CH_3$	21
(−)-**3c(L)**	3-Indolyl	(*S*)-$CHCO_2CH_3$	25
(+)-**3c(L)**	3-Indolyl	(*S*)-$CHCO_2CH_3$	25
(−)-**3c(D)**	3-Indolyl	(*R*)-$CHCO_2CH_3$	24
(+)-**3c(D)**	3-Indolyl	(*R*)-$CHCO_2CH_3$	24

The half Schiff's bases **4** were prepared by the partial hydrolysis of their respective bis Schiff's bases **3** and the subsequent separation of the reaction mixtures (Scheme 1, Table 2). The method for this hydrolysis was a modification of previously reported methods.[15,21]

(−)-Gossypol (−)-**1** was prepared in 95% yield by the complete hydrolysis of (−)-gossypol bis(L-tyrosine ethyl ester) Schiff's base (−)-**3b** and (+)-gossypol (+)-**1** was prepared in 91.5% yield by the complete hydrolysis of (+)-gossypol bis(L-phenylalanine methyl ester) Schiff's base (+)-**3a** (Scheme 1). The oxidation of gossypol **rac-1** (Scheme 2) employed ferric chloride hexahydrate[22]

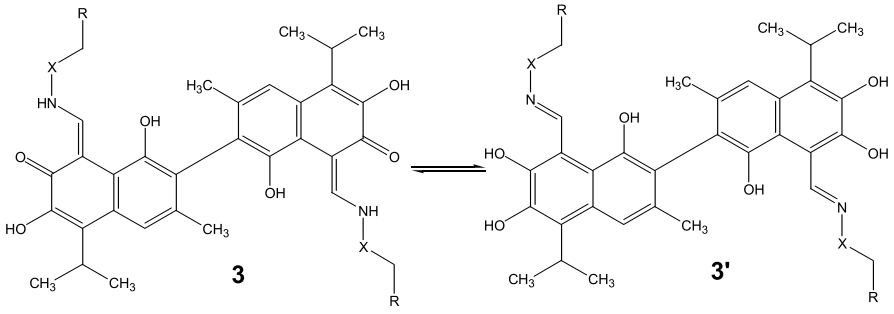
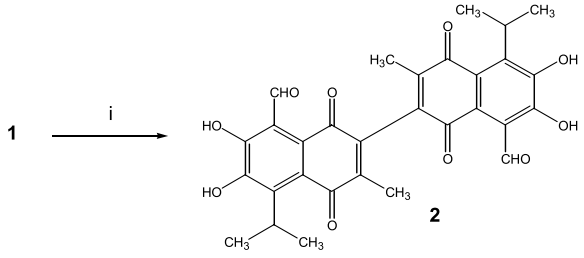


Figure 1.

Table 2. % Yield and structure of the gossypol half Schiff's bases **4**

Half Schiff's base	R	X	% Yield
(−)-**4a**	Ph	(*S*)-$CHCO_2CH_3$	51
(+)-**4a**	Ph	(*S*)-$CHCO_2CH_3$	13
(−)-**4b**	C_6H_4OH-4	(*S*)-$CHCO_2CH_2CH_3$	28
(−)-**4c(L)**	3-Indolyl	(*S*)-$CHCO_2CH_3$	29



Scheme 2. Reagents and conditions: (i) $FeCl_3$, acetone, AcOH, 100 °C, 2 h, then ether, 20% aq H_2SO_4.

and successfully produced pure gossypolone **rac-2** in 59% yield. This has been the most widely used method for the preparation of gossypolone[23,24] and has previously been shown to give racemic gossypolone **rac-2** even when starting with only one of the gossypol enantiomers—this epimerisation at the binaphthyl bond presumably arising as a result of the intermediacy of a radical species. The initial product from the gossypol oxidation is an iron(II) complex with the aldehyde and the 1-hydroxyl groups of gossypolone, which is decomposed after treatment with aqueous H_2SO_4.[22]

2.2. Biological evaluation

The in vitro anti-psoriatic activity of gossypol and its derivatives was evaluated using an anti-proliferative assay and an anti-oxidant assay. In the anti-proliferative study, the sensitivity of an HPV-16 keratinocyte cell line to each compound was determined using an MTT viability assay. HPV-16 is a rapidly dividing immortalised human keratinocyte cell line, which mimics the hyperproliferation of the epidermis, one of the pathological features of psoriasis. The assay relies upon the spectrophotometric measurement of solubilised purple formazan, produced by the mitochondrial reduction of MTT in viable cells. A comparison was made with the data from three MTT assays on HPV-16 keratinocyte cell lines using methotrexate **5**, which is used clinically for the treatment of psoriasis. The GI_{50} values for methotrexate **5** (148.6, 204.9 and 219 μM) and dithranol **6** (0.58 μM), Figure 2, show that methotrexate's inhibitory efficacy is similar to that of the bis Schiff's bases **3**, while dithranol **6** is an order of magnitude more active than (−)-gossypol (−)-**1**. Methotrexate **5** is an anti-proliferative, which is also an immunosuppressive,[3] while dithranol **6** is an anti-proliferative, which upregulates interleukin-10 receptor expression on keratinocytes.[4,25]

The compounds, which showed increased inhibition against keratinocyte proliferation were subsequently tested for their anti-oxidant effect against iron/ascorbate dependent lipid peroxidation, using the thiobarbituric acid (TBA) test.[26] There is growing evidence supporting the role of reactive oxygen species in psoriasis and a failure of the anti-oxidant defence system of the skin has been suggested.[18] In fact, the free fatty acid content is decreased by 46% in psoriatic scales compared to normal stratum corneum[27] and elevated malondialdehyde (MDA) levels, a lipid peroxidation product, have been detected in psoriatic lesions.[28] The TBA test utilises MDA as a marker for assessing the extent of lipid peroxidation and is based upon the spectrophotometric quantitation of the pink complex formed after reaction of MDA with two molecules of TBA. The absorbance of each reaction mixture was compared against a control mixture, consisting of the liposomes and the oxidising system in the absence of the test compound—the full

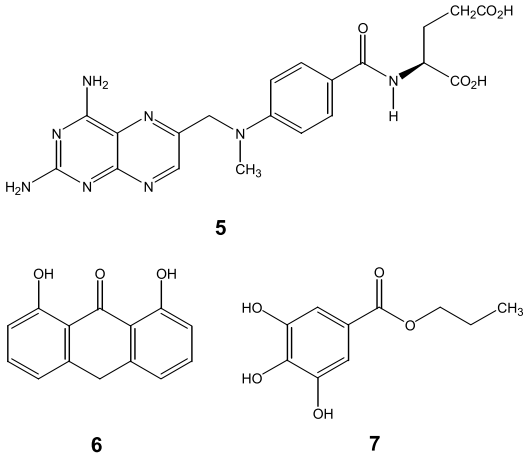


Figure 2.

reaction mixture (FRM). The assay was validated by a positive anti-oxidant control reaction, which involved the effect of the established anti-oxidant propyl gallate **7** on the full reaction mixture.

Racemic gossypol **rac-1** and (−)-gossypol (−)-**1** showed the highest anti-proliferative activity against HPV-16 keratinocytes, followed by the half Schiff's bases **4**, (+)-gossypol (+)-**1** and racemic gossypolone **rac-2**, and finally the bis Schiff's bases **3** (Table 3). The bis Schiff's bases **3** were the least active as inhibitors of keratinocyte proliferation. (−)-Gossypol (−)-**1** was tested twice in order to observe its effect within different concentration ranges; there is no significant difference between the two GI_{50} values when compared by Student's *t*-test for unpaired values ($p = 0.1156$). The fact that the GI_{50} values for the two gossypol enantiomers are different implies an enantioselective interaction with the site of action, in common with the other biological activities exhibited by gossypol, where the (−)-isomer (−)-**1** is usually more active than the (+)-isomer (+)-**1**. There is a noticeable increase in the GI_{50} values between the half **4** and the bis Schiff's bases **3**. This may indicate either that the aldehyde groups, which are derivatised upon Schiff's base formation, are essential for the anti-proliferative effect, or that the bis Schiff's bases have difficulty in entering the cell due to their size. Both these reasons could explain why the highest potency was observed for gossypol **1**, which has both aldehyde groups intact and the lowest molecular weight of all the compounds tested. Gossypol **1** is believed to exert its anti-proliferative action in different ways, depending upon the cellular environment, for example, the enzymes present, the lipidic composition of the cell membrane and the metabolic (mitochondrial) activity of the cell.[9,29–31] In pigmented (SK-mel-19) and non-pigmented (SK-mel-28) melanoma cell lines, the activity decreases on going from the half Schiff's bases to gossypol, with the bis Schiff's bases again being the least active.[15]

Table 3. Inhibition of proliferation of HPV-16 cell line by gossypol and its derivatives in order of decreasing potency

Compound	GI_{50} (μM)[a]	SD
(−)-**1**	4.8, 8.6	0.48, 0.04
rac-1	5.4	0.33
(+)-**1**	16.4	2.54
(−)-**4b**	21.4	11.15
(−)-**4c(L)**	37.1	14.58
rac-2	47.35	9.16
(−)-**4a**	49.4	0.324
(−)-**3b**	107.35	28.67
(−)-**3a**	155.85	7.67
(+)-**3c(D)**	166.5	58.38
(−)-**3c(L)**	171.0	23.08
(−)-**3c(D)**	188.2	20.57
(+)-**3a**	213.25	12.65
(+)-**3c(L)**	>219.0	—

[a] MTT cell viability assay, with absorbance readings at 570 nm. The GI_{50} values were obtained from LOWESS analysis of % growth versus logarithmic concentration (molar) of the compound. Six in-plate replicates of the eight concentrations were performed.

Table 4. Inhibition of iron/ascorbate dependent bovine brain lipid peroxidation

Compound	IC_{50} (μM)[a]
(−)-**3b**	11.7
(−)-**1**	13.1
rac-1	17.3
(+)-**1**	17.3
rac-2	28.0
7	$IC_{70} = 100$

[a] *n*-Butanol was used as an internal standard, and the absorbance of the MDA–TBA complex was determined at 532 nm. The IC_{50} values were obtained from LOWESS analysis of % inhibition of peroxidation versus logarithmic concentration (molar) of each inhibitor. Four replicates were carried out for each reaction mixture.

The anti-oxidant activity of all compounds tested was similar and they were all more active than the standard, propyl gallate **7** (Table 4). Gossypol **1**, in common with other aromatic phenols, is known for its anti-oxidant activity and its protective effect against lipid peroxidation has been attributed to its partition into the membrane and interruption of the chain reaction process.[32–34] This suggested mechanism is consistent with the lipophilic nature of the other compounds tested, and may explain the similarity of their IC_{50} values. Gossypol **1** is unable to scavenge superoxide radicals at the low concentrations at which it prevents peroxidation, and its iron chelating ability has not been shown to contribute to its anti-peroxidant effect.[33]

3. Conclusions

Gossypol **1** was shown to be the most potent inhibitor of keratinocyte proliferation in the anti-proliferative MTT assay, and a potent anti-oxidant. Considering the inhibitory activity of gossypol on cytoplasmic phospholipase-2,[16] its activity against endothelial cell growth[35] and its apoptotic effect on human lymphocytes,[36] an overall anti-psoriatic model of activity can be deduced, which encompasses both anti-proliferative and anti-inflammatory[16,17] features. This in vitro anti-psoriatic activity in conjunction with the low toxicity in humans,[37] lack of mutagenicity[38] and favourable comparison to methotrexate and dithranol, makes gossypol a good candidate for the topical treatment of psoriasis.

4. Experimental

4.1. Chemistry

Racemic gossypol acetic acid was a gift from Velindre NHS Trust, Cardiff, and was converted to the free racemic gossypol **rac-1** by dissolution in diethyl ether, double washing with equal amounts of distilled water to remove the acetic acid, drying of the ether layer over $MgSO_4$ and removal of the solvent under reduced pressure. The amino acid ester free bases were obtained by treatment of their corresponding hydrochloride salts with saturated $NaHCO_3$ solution and extraction into dichloromethane followed by removal of the solvent

under reduced pressure. Ferric chloride hexahydrate was obtained from Aldrich. $CDCl_3$ and DMSO-d_6 for NMR spectroscopy were obtained from Apollo Scientific Ltd. IR Spectra were recorded on an ATI Mattison Genesis Series FTIR and samples were examined as potassium bromide (KBr) discs. ^{1}H NMR Spectra were acquired on a JEOL GSX270, Bruker AVANCE 300 or Bruker AVANCE 500 at 270, 300 and 500 MHz, respectively. ^{13}C NMR Spectra were acquired on a Bruker AVANCE 300 or Bruker AVANCE 500 at 75 or 125 MHz, respectively. Coupling constants (*J*) are given in Hertz and all chemical shifts are relative to the chemical shift of the residual non-deuterated solvent. Thin-layer chromatography (TLC) was performed on silica gel-60 coated plastic plates obtained from Merck. Melting points were determined on a Reichert hot stage microscope and are uncorrected. Elemental analysis was performed by Medac Ltd., Brunel University, Uxbridge, United Kingdom.

Analytical HPLC work was performed with a Spectra–Physics SP8800 ternary HPLC Pump (Spectra–Physics, San Jose, California, USA) operating at a flow rate of 1.0 mL/min, at room temperature, connected to a Spectra–Physics SP8490 detector, operating at 254 nm for UV detection. Injections were performed using a Rheodyne 7125 manual injector equipped with a 20 μL loop (Anachem, Luton, Bedfordshire, UK). Data collection was performed on a Dionex advanced computer interface (Dionex, Camberley, Surrey, UK) and data processing on a computer equipped with Dionex A1-450 software. A C18 column was used, ODS-H15-19724 (15 cm × 3.2 mm i.d.), purchased from CAPITAL HPLC Ltd. The pH of the mobile phase was determined with a JENWAY 3030 pH meter by S.H. Scientific.

4.1.1. General procedure for the synthesis of diastereoisomeric gossypol bis Schiff's bases 3a–c. Racemic gossypol **rac-1** (1 equiv) was dissolved in DCM (50 mL/1 g of gossypol) and added to the chiral amino acid ester free base (6 equiv) and isopropanol (1 mL/1 g of gossypol). The reaction mixture was left to stir at room temperature, in the dark. Diastereoisomer formation was monitored by reverse phase HPLC, using a mobile phase of acetonitrile/aq 0.02 M $HCOONH_4$ (80:20, pH = 6.3) at λ = 254 nm and a flow rate of 1.0 mL/min. The diastereoisomeric reaction mixture was then evaporated to dryness, adsorbed onto silica and separated by column chromatography on silica, eluting with hexane/ether (100:0–33:66) for **3a**, and hexane/ethyl acetate (100:0–50:50) for **3b**, **3c(L)** and **3c(D)**.

4.1.1.1. Gossypol bis(L-phenylalanine methyl ester) Schiff's bases 3a. From the reaction of **rac-1** (1.18 g, 2.21 mmol) with L-Phe methyl ester (2.21 g, 11.83 mmol) and separation of the diastereoisomeric mixture by column chromatography, the (−)-gossypol bis(L-phenylalanine methyl ester) Schiff's base (−)-**3a** was isolated as a yellow powder in 39% yield; R_f = 0.53 in 1:3 hexane/ether; R_t (min) 6.08; $[\alpha]^{25}$ −838.9 (*c* 0.12, DCM); mp 112–116 °C; ν_{max} (KBr)/cm^{-1} 3478, 3438 (OH, NH), 1747 (C=O), 1612 (C=C); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.51 (12H, d, *J* = 7.3 Hz, CH(CH_3)$_2$), 2.06 (6H, s, Ar–CH_3), 3.17 (2H, dd, *J* = 13.85, 8.6 Hz, CH_2-a), 3.34 (2H, dd, *J* = 13.85, 4.6 Hz, CH_2-b), 3.69 (2H, septet, *J* = 7.3 Hz, C*H*(CH$_3$)$_2$), 3.77 (6H, s, COOCH_3), 4.30 (2H, m, α-CH), 5.35 (2H, s, 1,1′-O*H*), 7.11–7.26 (10H, m, Ar–H), 7.54 (2H, s, 4,4′-H), 7.94 (2H, s, 6,6′-O*H*), 9.37 (2H, d, *J* = 11.9 Hz, HN–C*H*=), 13.59 (2H, broad s, *H*N–CH=); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.4 (4 × CH_3), 20.7 (2 × Ar–CH_3), 27.8 (2 × CH), 40.4 (2 × CH_2), 53.3 (2 × OCH_3), 64.9 (2 × α-CH), 104.0 (2 × quat., C-8), 114.8 (2 × quat., C-8a), 116.2 (2 × quat., C-2), 118.6 (2 × CH, C-4), 127.7 (2 × CH, C-4″), 128.2 (2 × quat., C-4a), 129.3 (4 × CH, 2 × 2″,6″-CH), 129.6 (2 × quat., C-5), 129.8 (4 × CH, 2 × 3″,5″-CH), 132.4 (2 × quat., C-3), 135.5 (2 × quat., C-1″), 147.4 (2 × quat., C-1), 149.3 (2 × quat., C-6), 161.9 (2 × CH–NH), 170.5 (2 × quat., ester C=O), 174.0 (2 × quat., C=O, C-7). Anal. Calcd for $C_{50}H_{52}O_{10}N_2$: C, 71.4; H, 6.2; N, 3.3. Found: C, 71.1; H, 6.3; N, 3.3.

The (+)-gossypol bis(L-phenylalanine methyl ester) Schiff's base (+)-**3a** was isolated as a yellow-orange powder (0.57 g, 31%); R_f = 0.40 in 1:3 hexane/ether; R_t(min) 8.96; $[\alpha]^{25}$ +514.9 (*c* 0.09, DCM); mp 118–122 °C; ν_{max} (KBr)/cm^{-1} 3480 (OH, NH), 1745 (C=O), 1612 (C=C); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.52 (12H, d, *J* = 7.3 Hz, CH(CH_3)$_2$), 2.05 (6H, s, Ar–CH_3), 3.12 (2H, dd, *J* = 13.85, 8.6 Hz, CH_2-a), 3.32 (2H, dd, *J* = 13.85, 4.6 Hz, CH_2-b), 3.68 (2H, septet, *J* = 7.3 Hz, C*H*(CH$_3$)$_2$), 3.76 (6H, s, COOCH_3), 4.26 (2H, m, α-CH), 5.37 (2H, s, 1,1′-O*H*), 7.13–7.26 (10H, m, Ar–H), 7.54 (2H, s, 4,4′-H), 7.94 (2H, s, 6,6′-O*H*), 9.24 (2H, d, *J* = 11.9 Hz, HN–C*H*=), 13.60 (2H, broad s, *H*N–CH=); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.4 (4 × CH_3), 20.7 (2 × Ar–CH_3), 27.8 (2 × CH), 40.4 (2 × CH_2), 53.3 (2 × OCH_3), 64.9 (2 × α-CH), 104.1 (2 × quat., C-8), 114.8 (2 × quat., C-8a), 116.2 (2 × quat., C-2), 118.6 (2 × CH, C-4), 127.7 (2 × CH, C-4″), 128.2 (2 × quat., C-4a), 129.3 (4 × CH, 2 × 2″,6″-CH), 129.6 (2 × quat., C-5), 129.8 (4 × CH, 2 × 3″,5″-CH), 132.4 (2 × quat., C-3), 135.5 (2 × quat., C-1″), 147.4 (2 × quat., C-1), 149.3 (2 × quat., C-6), 161.9 (2 × CH–NH), 170.5 (2 × quat., ester C=O), 174.0 (2 × quat., C=O, C-7). Anal. Calcd for $C_{50}H_{52}O_{10}N_2 \cdot 1/2Et_2O$: C, 71.1; H, 6.5; N, 3.2. Found: C, 70.8; H, 6.2; N, 3.3.

4.1.1.2. Gossypol bis(L-tyrosine ethyl ester) Schiff's bases 3b. From the reaction of **rac-1** (0.93 g, 1.79 mmol) with L-Tyr ethyl ester (3.2 g, 15.32 mmol) and separation of the diastereoisomeric mixture by column chromatography, the (−)-gossypol bis(L-tyrosine ethyl ester) Schiff's base (−)-**3b** was isolated and recrystallised from $CHCl_3$ as bright yellow crystals (0.27 g, 16%); R_t (min) 4.11; $[\alpha]^{22}$ −824.4 (*c* 0.09, CH_3OH); mp 129–134 °C; ν_{max} (KBr)/cm^{-1} 3478, 3436 (OH, NH), 1739 (C=O), 1612 (C=C); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.26 (6H, t, *J* = 7.3 Hz, $COOCH_2CH_3$), 1.51 (12H, d, *J* = 7.3 Hz, (CH_3)$_2$CH), 2.04 (6H, s, Ar–CH_3), 3.00 (2H, dd, *J* = 13.85, 9.2 Hz, CH_2-a), 3.24 (2H, dd, *J* = 13.85, 4.0 Hz, CH_2-b), 3.68 (2H, septet, *J* = 7.3 Hz, *C*H(CH$_3$)$_2$), 4.12 (4H, q, *J* = 7.3 Hz, $COOCH_2CH_3$), 4.20 (2H, t, *J* = 7.3 Hz, α-CH), 5.41 (2H, s, 1,1′-O*H*), 6.10 (2H, s, 2 × 4″-O*H*), 6.63 (4H, d, *J* = 7.9 Hz, 2 × 3″,5″-H), 6.96 (4H, d, *J* = 7.9 Hz, 2 × 2″,6″-H), 7.54

(2H, s, 4,4′-H), 7.88 (2H, broad s, 6,6′-OH), 9.31 (2H, s, C*H*=N), 13.56 (2H, broad s, 7,7′-OH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.18 (6H, t, J = 7.1 Hz, $COOCH_2CH_3$), 1.44 (12H, 2 × d, J = 7.0 Hz, $(CH_3)_2CH$), 1.94 (6H, s, Ar–CH_3), 3.11 (4H, m, CH_2), 3.69 (2H, septet, J = 7.0 Hz, $CH(CH_3)_2$), 4.15 (4H, q, J = 7.1 Hz, $COOCH_2CH_3$), 4.68 (2H, q, J = 8.0 Hz, α-CH), 6.64 (4H, d, J = 8.5 Hz, 2 × 3″,5″-H), 7.00 (4H, d, J = 8.5 Hz, 2 × 2″,6″-H), 7.45 (2H, s, 4,4′-H), 7.85 (2H, s, 2 × 4″-O*H*), 8.43 (2H, s, 6,6′-OH), 9.25 (2H, s, 1,1′-O*H*), 9.75 (2H, d, J = 12.5 Hz, =C*H*–NH), 13.43 (2H, dd, J = 12.5, 8.0 Hz, =CH–N*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.5 (2 × $COOCH_2CH_3$) 20.4 (4 × CH_3), 20.7 (2 × CH_3), 27.8 (2 × CH), 39.5 (2 × CH_2), 62.6 (2 × OCH_2), 64.8 (2 × α-CH), 104.1 (2 × quat., C-8), 114.8 (2 × quat., C-8a), 116.1 (4 × CH, 2 × 3″,5″-CH), 116.2 (2 × quat., C-2), 118.2 (2 × CH, C-4), 127.3 (2 × quat., C-4″), 128.3 (2 × quat., C-4a), 129.1 (2 × quat., C-5), 131.1 (4 × CH, 2 × 2″,6″-CH), 132.5 (2 × quat., C-3), 147.4 (2 × quat., C-1), 149.3 (2 × quat., C-6), 155.4 (2 × quat., C-1″), 156.0 (2 × quat., C-7), 162.0 (2 × CH=N), 170.2 (2 × quat., ester C=O). Anal. Calcd for $C_{52}H_{56}O_{12}N_2$·1/2EtOAc: C, 68.6; H, 6.35; N, 3.0. Found: C, 68.5; H, 6.2; N, 3.05.

The (+)-gossypol bis(L-tyrosine ethyl ester) Schiff's base (+)-**3b** was isolated an orange-brown powder (0.35 g, 21%); R_t (min) 2.56; $[\alpha]^{22}$ +393.6 (c 0.05, CH_3OH); mp 122–127 °C; ν_{max} (KBr)/cm^{-1} 3478 (OH), 1739 (ester C=O), 1612 (C=C); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.26 (6H, t, J = 7.3 Hz, $COOCH_2CH_3$), 1.50 (12H, 2 × d, J = 6.6 Hz, $(CH_3)_2CH$), 2.06 (6H, s, Ar–CH_3), 3.10 (2H, dd, J = 13.85, 7.9 Hz, CH_2-a), 3.20 (2H, dd, J = 13.85, 5.3 Hz, CH_2-b), 3.70 (2H, septet, J = 6.6 Hz, $CH(CH_3)_2$), 4.12 (4H, q, J = 7.3 Hz, $COOCH_2CH_3$), 4.20 (2H, dd, J = 7.9, 5.3 Hz, α-C*H*–CH_2), 5.49 (2H, s, 1,1′-O*H*), 6.10 (2H, s, 2 × 4″-O*H*), 6.67 (4H, d, J = 8.6 Hz, 2 × 3″,5″-H), 6.97 (4H, d, J = 8.6 Hz, 2 × 2″,6″-H), 7.55 (2H, s, Ar–H), 7.88 (2H, broad s, 6,6′-OH), 9.35 (2H, s, C*H*=N), 13.51 (2H, broad s, 7,7′-OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.5 (2 × $COOCH_2CH_3$), 20.4 (4 × CH_3), 20.7 (2 × CH_3), 27.8 (2 × CH), 39.5 (2 × CH_2), 62.6 (2 × O–CH_2), 64.8 (2 × α-CH), 104.1 (2 × quat., C-8), 114.8 (2 × quat., C-8a), 116.1 (4 × CH, 2 × 3″,5″-CH), 116.2 (2 × quat., C-2), 118.2 (2 × CH, C-4), 127.3 (2 × quat., C-4″), 128.3 (2 × quat., C-4a), 129.5 (2 × quat., C-5), 131.0 (4 × CH, 2 × 2″,6″-CH), 132.5 (2 × quat., C-3), 147.4 (2 × quat., C-1), 149.4 (2 × quat., C-6), 155.5 (2 × quat., C-1″), 156.0 (2 × quat., C-7), 162.0 (2 × CH=N), 170.2 (2 × quat., ester C=O). Anal. Calcd for $C_{52}H_{56}O_{12}N_2$· 2EtOAc: C, 66.9; H, 6.7; N, 2.6. Found: C, 66.7; H, 6.1; N, 3.0.

4.1.1.3. Gossypol bis(L-tryptophan methyl ester) Schiff's bases 3c(L). From the reaction of **rac-1** (0.91 g, 1.76 mmol) with L-Trp methyl ester (2.00 g, 9.16 mmol) and separation of the diastereoisomeric mixture by column chromatography, the (−)-gossypol bis(L-tryptophan methyl ester) Schiff's base (−)-**3c(L)** eluted first and recrystallised from hexane/ethyl acetate (70:30) as yellow crystals (0.4 g, 25%); R_t (min) 5.55; $[\alpha]^{21}$ −1175.2 (c 0.05, DCM); mp 230–235 °C; ν_{max} (KBr)/cm^{-1} 3475, 3388 (OH, NH), 1751 (ester C=O), 1733 (C=O), 1606 (C=C); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.53 (12H, d, J = 7.3 Hz, $(CH_3)_2CH$), 1.99 (6H, s, Ar–CH_3), 3.20 (2H, dd, J = 14.5, 9.9 Hz, CH_2-a), 3.60 (2H, dd, J = 14.5, 3.3 Hz, CH_2-b), 3.70 (2H, septet, J = 7.3 Hz, $CH(CH_3)_2$), 3.84 (6H, s, $COOCH_3$), 4.46 (2H, m, α-C*H*–CH_2), 4.66 (2H, s, 1,1′-O*H*), 6.73–7.03 (8H, m, Ar–*H*), 7.50 (2H, s, 4,4′-H), 7.57 (2H, d, J = 7.9 Hz, indole 2-H), 7.82 (2H, s, 6,6′-OH), 7.95 (2H, broad s, indole N*H*), 9.07 (2H, d, J = 9.9 Hz, =C*H*–NH), 13.48 (2H, broad, CH–N*H*); ^{1}H NMR (300 MHz, DMSO) δ 1.45 (12H, d, J = 6.9 Hz, $(CH_3)_2CH$), 1.95 (6H, s, Ar–CH_3), 3.4 (4H, broad m, CH_2), 3.67 (6H, s, $COOCH_3$), 3.73 (2H, m, $(CH_3)_2CH$), 4.82 (2H, m, α-C*H*), 6.95 (2H, t, J = 7.9 Hz, 2 × 5″-*H*), 7.04 (2H, t, J = 8.0 Hz, 2 × 6″-*H*), 7.16 (2H, d, J = 2.3 Hz, 2 × 2″-*H*), 7.33 (2H, d, J = 8.0 Hz, 2 × 7″-*H*), 7.46 (2H, s, 4,4′-*H*), 7.49 (2H, d, J = 7.9 Hz, 2 × 4″-*H*), 7.83 (2H, s, 1,1′-O*H*), 8.46 (2H, s, 6,6′-O*H*), 9.79 (2H, d, J = 12.5 Hz, C*H*–NH), 10.95 (2H, d, J = 2.1 Hz, indole N*H*), 13.51 (2H, dd, J = 12.5, 8.0 Hz, CH–N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.0 (2 × CH_3), 21.1 (4 × CH_3), 27.4 (2 × CH), 29.8 (2 × CH_2), 53.4 (2 × OCH_3), 62.9 (2 × α-CH), 104.7 (2 × quat., C-8), 108.6 (2 × quat., C-3″), 112.3 (2 × CH, C-5″), 116.5 (2 × quat., C-8a), 117.5 (2 × CH, C-4″), 118.9 (2 × CH, C-4), 119.4 (2 × CH, C-6″), 121.1 (2 × quat., C-2), 121.9 (2 × CH, C-7″), 125.1 (2 × CH, C-2″), 127.8 (2 × quat., C-3a''), 128.00 (2 × quat., C-5), 128.03 (2 × quat., C-4a), 132.4 (2 × quat., C-3), 136.9 (2 × quat., C-7a″), 147.1 (2 × quat., C-1), 150.5 (2 × quat., C-6), 162.1 (2 × CH–NH), 171.7 (2 × quat., ester C=O), 173.5 (2 × quat., C=O, C-7). Anal. Calcd for $C_{54}H_{54}O_{10}N_4$·1/2EtOAc: C, 69.85; H, 6.0; N, 5.8. Found: C, 69.6; H, 5.9; N, 5.9.

The (+)-gossypol bis(L-tryptophan methyl ester) Schiff's base (+)-**3c(L)** eluted second and recrystallised from $CHCl_3$ as yellow crystals (0.4 g, 25%); R_t (min) 3.89; $[\alpha]^{18.5}$ +250 (c 0.02, CH_3OH); mp 148–153 °C; ν_{max} (KBr)/cm^{-1} 3471, 3434 (OH, NH), 1743 (ester C=O), 1612 (C=O); ^{1}H NMR (300 MHz, DMSO) δ 1.43 (6H, d, J = 6.65 Hz, $(CH_3)_2CH$), 1.45 (6H, d, J = 6.0 Hz, $(CH_3)_2CH$), 1.93 (6H, s, Ar–CH_3), 3.38 (4H, broad m, CH_2), 3.67 (6H, s, $COOCH_3$), 3.73 (2H, m, $(CH_3)_2CH$), 4.77 (2H, m, α-C*H*), 6.94 (2H, t, J = 7.9 Hz, 2 × 5″-*H*), 7.00 (2H, t, J = 7.8 Hz, 2 × 6″-*H*), 7.15 (2H, d, J = 2.3 Hz, 2 × 2″-*H*), 7.30 (2H, d, J = 7.9 Hz, 2 × 7″-*H*), 7.45 (2H, s, 4,4′-*H*), 7.48 (2H, d, J = 7.8 Hz, 2 × 4″-*H*), 7.55 (2H, s, 1,1′-O*H*), 8.41 (2H, s, 6,6′-O*H*), 9.65 (2H, d, J = 12.5 Hz, C*H*–NH), 10.90 (2H, d, J = 2.1 Hz, indole N*H*), 13.47 (2H, dd, J = 12.5, 8.0 Hz, CH–N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.7 (2 × CH_3), 20.8 (4 × CH_3), 27.1 (2 × CH), 29.6 (2 × CH_2), 53.1 (2 × OCH_3), 62.6 (2 × α-CH), 104.3 (2 × quat., C-8), 108.3 (2 × quat., C-3″), 112.0 (2 × CH, C-5″), 116.1 (2 × quat., C-8a), 117.2 (2 × CH, C-4″), 118.6 (2 × CH, C-4), 119.1 (2 × CH, C-6″), 120.6 (2 × quat., C-2), 121.6 (2 × CH, C-7″), 124.9 (2 × CH, C-2″), 127.5 (2 × quat., C-3a″), 127.6 (2 × quat., C-5), 127.7 (2 × quat., C-4a), 132.1 (2 × quat., C-3), 136.7 (2 × quat., C-7a″), 146.8 (2 × quat., C-1), 150.2 (2 × quat., C-6), 161.9 (2 × CH–NH), 171.4 (2 × quat.,

ester C=O), 173.2 (2 × quat., C=O, C-7). Anal. Calcd for $C_{54}H_{54}O_{10}N_4 \cdot 1/2EtOAc$: C, 69.85; H, 6.0; N, 5.8. Found: C, 69.8; H, 5.9; N, 5.95.

4.1.1.4. Gossypol bis(D-tryptophan methyl ester) Schiff's bases 3c(D). From the reaction of **rac-1** (0.98 g, 1.89 mmol) with D-Trp methyl ester (2.37 g, 10.86 mmol) and separation of the diastereoisomeric mixture by column chromatography, the (+)-gossypol bis(D-tryptophan methyl ester) Schiff's base (+)-**3c(D)** eluted first and recrystallised from hexane/ethyl acetate (70:30) as yellow crystals (0.4 g, 24%); R_t (min) 5.33; $[\alpha]^{16.5}$ +1180 (*c* 0.05, DCM); mp 230–235 °C; ν_{max} (KBr)/cm^{-1} 3471, 3421 (OH, NH), 1793 (ester C=O), 1733 (C=O), 1608 (C=C); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.53 (12H, 2 × d, *J* = 7.3 Hz, $(CH_3)_2CH$), 1.99 (6H, s, Ar–CH_3), 3.24 (2H, dd, *J* = 14.5, 9.9 Hz, CH_2-a), 3.60 (2H, dd, *J* = 14.5, 4.0 Hz, CH_2-b), 3.70 (2H, septet, *J* = 7.3 Hz, $CH(CH_3)_2$), 3.83 (6H, s, $COOCH_3$), 4.47 (2H, m, α-C*H*–CH_2), 4.67 (2H, s, 1,1′-O*H*), 6.73–7.02 (8H, m, Ar–*H*), 7.50 (2H, s, 4,4′-H), 7.55 (2H, d, *J* = 7.9 Hz, indole 2-H), 7.81 (2H, s, 6,6′-OH), 7.95 (2H, broad s, indole N*H*), 9.07 (2H, d, *J* = 11.2 Hz, =C*H*–NH), 13.49 (2H, broad s, CH–N*H*); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.44 (12H, d, *J* = 6.8 Hz, $(CH_3)_2CH$), 1.94 (6H, s, Ar–CH_3), 3.37 (4H, broad m, CH_2), 3.67 (6H, s, $COOCH_3$), 3.72 (2H, m, $(CH_3)_2CH$), 4.80 (2H, m, α-C*H*), 6.95 (2H, t, *J* = 7.8 Hz, 2 × 5″-*H*), 7.04 (2H, t, *J* = 8.0 Hz, 2 × 6″-*H*), 7.16 (2H, d, *J* = 2.2 Hz, 2 × 2″-*H*), 7.32 (2H, d, *J* = 8.0 Hz, 2 × 7″-*H*), 7.45 (2H, s, 4,4′-*H*), 7.48 (2H, d, *J* = 7.8 Hz, 2 × 4″-*H*), 7.82 (2H, s, 1,1′-O*H*), 8.45 (2H, s, 6,6′-O*H*), 9.78 (2H, d, *J* = 12.6 Hz, C*H*–NH), 10.94 (2H, d, *J* = 2.1 Hz, indole N*H*), 13.50 (2H, dd, *J* = 12.5, 7.9 Hz, CH–N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.1 (6 × CH_3), 27.4 (2 × CH), 29.8 (2 × CH_2), 53.4 (2 × OCH_3), 62.9 (2 × α-CH), 104.7 (2 × quat., C-8), 108.7 (2 × quat., C-3″), 112.3 (2 × CH, C-5″), 116.5 (2 × quat., C-8a), 117.6 (2 × CH, C-4″), 118.9 (2 × CH, C-4), 119.4 (2 × CH, C-6″), 121.1 (2 × quat., C-2), 121.9 (2 × CH, C-7″), 125.1 (2 × CH, C-2″), 127.8 (2 × quat., C-3a″), 128.01 (2 × quat., C-5), 128.06 (2 × quat., C-4a), 132.4 (2 × quat., C-3), 136.9 (2 × quat., C-7a″), 147.1 (2 × quat., C-1), 150.5 (2 × quat., C-6), 162.1 (2 × CH–NH), 171.7 (2 × quat., ester C=O), 173.5 (2 × quat., C=O, C-7). Anal. Calcd for $C_{54}H_{54}O_{10}N_4 \cdot 1/2EtOAc$: C, 69.85; H, 6.0; N, 5.8. Found: C, 69.95; H, 5.9; N, 5.9.

The (−)-gossypol bis(D-tryptophan methyl ester) Schiff's base (−)-**3c(D)** eluted second and recrystallised from $CHCl_3$ as yellow crystals (0.4 g, 24%); R_t (min) 3.68; $[\alpha]^{18.5}$ −250 (*c* 0.04, CH_3OH); mp 147–152 °C; ν_{max} (KBr)/cm^{-1} 3471, 3442 (OH, NH), 1741 (ester C=O), 1612 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.43 (6H, d, *J* = 6.0 Hz, $(CH_3)_2CH$), 1.45 (6H, d, *J* = 5.9 Hz, $(CH_3)_2CH$), 1.93 (6H, s, Ar–CH_3), 3.38 (4H, broad m, CH_2), 3.68 (6H, s, $COOCH_3$), 3.73 (2H, m, $(CH_3)_2CH$), 4.77 (2H, m, α-C*H*), 6.95 (2H, t, *J* = 7.7 Hz, 2 × 5″-*H*), 7.00 (2H, t, *J* = 7.8 Hz, 2 × 6″-*H*), 7.15 (2H, d, *J* = 2.4 Hz, 2 × 2″-*H*), 7.31 (2H, d, *J* = 7.9 Hz, 2 × 7″-*H*), 7.45 (2H, s, 4,4′-*H*), 7.49 (2H, d, *J* = 7.8 Hz, 2 × 4″-*H*), 7.55 (2H, s, 1,1′-O*H*), 8.41 (2H, s, 6,6′-O*H*), 9.65 (2H, d, *J* = 12.6 Hz, C*H*–NH), 10.90 (2H, d, *J* = 2.1 Hz, indole N*H*), 13.47 (2H, dd, *J* = 12.5, 7.9 Hz, CH–N*H*); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.1 (6 × CH_3), 27.4 (2 × CH), 29.8 (2 × CH_2), 53.4 (2 × OCH_3), 62.9 (2 × α-CH), 104.6 (2 × quat., C-8), 108.5 (2 × quat., C-3″), 112.3 (2 × CH, C-5″), 116.4 (2 × quat., C-8a), 117.5 (2 × CH, C-4″), 118.9 (2 × CH, C-4), 119.4 (2 × CH, C-6″), 121.1 (2 × quat., C-2), 121.9 (2 × CH, C-7″), 125.2 (2 × CH, C-2″), 127.8 (2 × quat., C-3a″), 127.9 (2 × quat., C-5), 128.06 (2 × quat., C-4a), 132.4 (2 × quat., C-3), 137.0 (2 × quat., C-7a″), 147.1 (2 × quat., C-1), 156.0 (2 × quat., C-6), 162.2 (2 × CH–NH), 171.7 (2 × quat., ester C=O), 173.2 (2 × quat., C=O, C-7). Anal. Calcd for $C_{54}H_{54}O_{10}N_4 \cdot 1/2EtOAc$: C, 69.85; H, 6.0; N, 5.8. Found: C, 69.8; H, 5.9; N, 5.9.

4.1.2. General procedure for the synthesis of gossypol half Schiff's bases 4a–c by partial hydrolysis and gossypol atropisomers 1 by complete hydrolysis. The corresponding gossypol bis Schiff's base **3** was dissolved in the minimum amount of diethyl ether and glacial acetic acid and stirred at 0 °C in the dark. To the cooled solution, concentrated H_2SO_4 and distilled H_2O were added and the reaction mixture was left to stir at 0 °C in the dark. The progress of the hydrolysis was monitored by reverse-phase HPLC, using a mobile phase of acetonitrile/aq $HCOONH_4$ (80:20, pH = 6.3) at λ = 254 nm and a flow rate of 1.0 mL/min. The reaction was stopped by the addition of aqueous saturated $NaHCO_3$ solution, in order to neutralise the acid. The ether layer was separated, washed with distilled H_2O, dried over $MgSO_4$, filtered and evaporated to dryness to give either the partial hydrolysis mixture or the gossypol atropisomer. The half Schiff's base was separated from the partial hydrolysis product mixture by column chromatography on silica, eluting with hexane/ether (100:0–25:75) for (−)-**4a**, and hexane/ethyl acetate (100:0–0:100) for (+)-**4a**, (−)-**4b** and (−)-**4c(L)**.

4.1.2.1. (−)-Gossypol half(L-phenylalanine methyl ester) Schiff's base (−)-4a. This was obtained from the reaction of (−)-**3a** (0.17 g, 0.20 mmol) in ether (10 mL), glacial acetic acid (2 mL), concentrated H_2SO_4 (0.34 mL) and distilled H_2O (0.68 mL), for 7 h. Yellow solid (0.07 g, 51%); R_t (min) 0.90 (94% purity); $[\alpha]^{24}$ −383.3 (*c* 0.06, DCM); mp 100–105 °C; ν_{max} (KBr)/cm^{-1} 3480 and 3436 (OH, NH), 1745 (ester C=O), 1612 (C=O); ^{1}H NMR (270 MHz, $CDCl_3$) δ −4.72 (1H, s, 7′-O*H*), 1.54 (6H, d, *J* = 7.25 Hz, $(CH_3)_2CH$), 1.57 (6H, d, *J* = 6.6 Hz, $(CH_3)_2CH$), 2.08 (3H, s, Ar–CH_3), 2.13 (3H, s, Ar–CH_3), 3.15 (1H, dd, *J* = 13.85, 8.6 Hz, CH_2-a), 3.31 (1H, dd, *J* = 13.85, 8.6 Hz, CH_2-b), 3.71 (1H, m, $(CH_3)_2CH$), 3.77 (3H, s, $COOCH_3$), 3.90 (1H, m, $(CH_3)_2CH$), 4.30 (1H, m, α-C*H*), 5.28 (1H, s, 1-O*H*), 5.72 (1H, s, 1′-O*H*), 6.45 (1H, s, 6′-O*H*), 7.11–7.27 (5H, m, Ar–*H*), 7.58 (1H, s, 4-*H*), 7.75 (1H, s, 4′-*H*), 7.94 (1H, s, 6-O*H*), 9.31 (1H, d, *J* = 11.9 Hz, HN–C*H*=), 11.18 (1H, s, C*H*O), 13.61 (1H, broad s, *H*N–CH=); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.4 (4 × CH_3), 20.7 (Ar–CH_3), 20.8 (Ar–CH_3), 28.0 (2 × CH), 40.5 (CH_2), 53.4 (OCH_3), 64.9 (α-CH), 104.1 (quat., C-8), 112.2 (quat., C-8′), 114.9 (quat., C-8a),

115.7 (quat., C-8′a), 116.5 (2 × quat., 2,2′-C), 118.0 (2 × CH, 4,4′-CH), 127.7 (CH, C-4″), 128.2 (2 × quat., C-4a), 129.3 (2 × CH, 2″,6″-CH), 129.8 (2 × CH, 3″,5″-CH), 129.9 (2 × quat., 5,5′-C), 132.2 (2 × quat., 3,3′-C), 135.5 (quat., C-1″), 147.5 (quat., C-1), 149.2 (quat., C-6), 150.8 (quat., C-1′), 150.9 (quat., C-6′), 156.5 (quat., C-7′), 162.0 (CHNH), 170.5 (quat., ester C=O), 173.9 (quat., C=O, C-7), 199.9 (CHO). HRMS: calcd for $C_{40}H_{42}O_9N$: 680.2854; found 680.2849.

4.1.2.2. (+)-Gossypol half(L-phenylalanine methyl ester) Schiff's base (+)-4a. This was obtained from the reaction of (+)-**3a** (0.37 g, 0.44 mmol) in ether (15 mL), glacial acetic acid (4.5 mL), concentrated H_2SO_4 (1.5 mL) and distilled H_2O (1.5 mL), for 7 h. Dark yellow solid (0.04 g, 13%); R_t (min) 1.05 (96% purity); $[\alpha]^{20}$ +266.7 (*c* 0.03, DCM); mp 113–117 °C; ν_{max} (KBr)/cm^{-1} 3484 and 3434 (OH, NH), 1747 (ester C=O), 1614 (C=O); ^{1}H NMR (270 MHz, $CDCl_3$) δ −4.84 (1H, s, 7′-O*H*), 1.53 (12H, 2 × d, *J* = 7.25 Hz, $(CH_3)_2CH$), 2.07 (3H, s, Ar–C*H*$_3$), 2.13 (3H, s, Ar–C*H*$_3$), 3.11 (1H, dd, *J* = 13.85, 8.6 Hz, CH_2-a), 3.31 (1H, dd, *J* = 13.85, 4.6 Hz, CH_2-b), 3.67 (1H, septet, *J* = 7.25 Hz, $(CH_3)_2CH$), 3.74 (3H, s, COOC*H*$_3$), 3.90 (1H, septet, *J* = 7.25 Hz, $(CH_3)_2CH$), 4.28 (1H, m, α-C*H*), 5.41 (1H, s, 1-O*H*), 5.92 (1H, s, 1′-O*H*), 6.45 (1H, s, 6′-O*H*), 7.12–7.27 (5H, m, Ar–*H*), 7.56 (1H, s, 4-*H*), 7.78 (1H, s, 4′-*H*), 7.89 (1H, s, 6-O*H*), 9.26 (1H, d, *J* = 7.9 Hz, HN–C*H*=), 11.11 (1H, s, C*H*O), 13.55 (1H, broad s, *H*N–CH=); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.4 (4 × CH_3), 20.7 (Ar–CH_3), 20.8 (Ar–CH_3), 25.7 (2 × CH), 40.5 (CH_2), 53.4 (OCH_3), 64.9 (α-CH), 104.1 (quat., C-8), 112.2 (quat., C-8′), 114.9 (quat., C-8a), 115.7 (quat., C-8′a), 116.5 (2 × quat., 2,2′-C), 118.0 (2 × CH, 4,4′-CH), 127.7 (CH, C-4″), 128.2 (2 × quat., C-4a), 129.3 (2 × CH, 2″,6″-CH), 129.8 (2 × CH, 3″,5″-CH), 129.9 (2 × quat., 5,5′-C), 132.2 (2 × quat., 3,3′-C), 135.5 (quat., C-1″), 147.5 (quat., C-1), 149.2 (quat., C-6), 150.8 (quat., C-1′), 150.9 (quat., C-6′), 156.5 (quat., C-7′), 162.0 (CH–NH), 170.5 (quat., ester C=O), 173.9 (quat., C=O, C-7), 199.9 (CHO). Anal. Calcd for $C_{40}H_{41}O_9N$.3EtOAc: C, 66.2; H, 6.9; N, 1.5. Found: C, 66.6; H, 6.4; N, 1.65; HRMS: calcd for $C_{40}H_{42}O_9N$: 680.2854. Found: 680.2852.

4.1.2.3. (−)-Gossypol half(L-tyrosine ethyl ester) Schiff's base (−)-4b. This was obtained from the reaction of (−)-**3b** (0.63 g, 0.70 mmol) in ether (110 mL), glacial acetic acid (7.5 mL), concentrated H_2SO_4 (5.1 mL) and distilled H_2O (7.0 mL), for 7 h. Dark orange solid (0.14 g, 28%); R_t (min) 0.80 (92% purity); $[\alpha]^{20}$ −514 (*c* 0.14, DCM); mp 90–95 °C; ν_{max} (KBr)/cm^{-1} 3438 (OH, NH), 1739 (ester C=O), 1614 (aldehyde C=O); ^{1}H NMR (270 MHz, $CDCl_3$) δ −4.84 (1H, s, 7′-O*H*), 1.26 (3H, t, *J* = 7.3 Hz, $COOCH_2CH_3$), 1.53 (12H, 2 × d, *J* = 6.6 Hz, $(CH_3)_2CH$), 2.07 (3H, s, Ar–C*H*$_3$), 2.13 (3H, s, Ar–C*H*$_3$), 3.00 (1H, dd, *J* = 13.85, 8.6 Hz, CH-a), 3.20 (1H, dd, *J* = 13.85, 4.6 Hz, CH_2-b), 3.69 (1H, septet, *J* = 6.6 Hz, $(CH_3)_2CH$), 3.90 (1H, septet, *J* = 6.6 Hz, $(CH_3)_2CH$), 4.12 (2H, q, *J* = 7.3 Hz, $COOCH_2CH_3$), 4.22 (1H, m, α-C*H*), 5.43 (1H, s, 1-O*H*), 5.75 (1H, broad, 4″-O*H*), 5.91 (1H, s, 1′-O*H*), 6.46 (1H, s, 6′-O*H*), 6.65 (2H, d, *J* = 7.9 Hz, 3″,5″-*H*), 6.97 (2H, d, *J* = 7.9 Hz, 2″,6″-*H*), 7.57 (1H, s, 4-*H*), 7.75 (1H, s, 4′-*H*), 7.93 (1H, s, 6-O*H*), 9.30 (1H, d, *J* = 9.2 Hz, =C*H*–NH), 11.15 (1H, s, C*H*O), 13.55 (1H, broad s, =CH–N*H*); ^{13}C NMR (75 MHz, $CDCl_3$) 14.5 ($COOCH_2CH_3$), 20.4 (4 × CH_3), 20.7 (2 × CH_3), 28.0 (2 × CH), 42.0 (CH_2), 62.6 (OCH_2), 67.0 (α-CH), 104.1 (quat., C-8), 112.2 (quat., C-8′), 114.8 (quat., C-8a), 115.8 (quat., C-8a′), 116.1 (2 × CH, 3″,5″-CH), 116.6 (2 × quat., 2,2′-C), 118.0 (2 × CH, 4,4′-C), 127.0 (quat., C-4″), 128.0 (2 × quat., 4a,4a′-C), 129.1 (2 × quat., 5,5′-C), 131.1 (2 × CH, 2″,6″-CH), 132.0 (2 × quat., 3,3′-C), 147.5 (quat., C-1), 149.4 (quat., C-6), 150.8 (quat., C-1′), 150.9 (quat., C-6′), 155.5 (quat., C-1″), 156.0 (quat., C-7), 156.5 (quat., C-7′), 162.0 (CH=N), 170.0 (quat., ester C=O), 199.2 (CHO). HRMS: calcd for $C_{41}H_{44}O_{10}N$: 710.2959. Found: 710.2957.

4.1.2.4. (−)-Gossypol half(L-tryptophan methyl ester) Schiff's base (−)-4c(L). This was obtained from the reaction of (−)-**3c(L)** (0.36 g, 0.39 mmol) in ether (7 mL), glacial acetic acid (4.3 mL), concentrated H_2SO_4 (1 mL) and distilled H_2O (1.8 mL) at −20 °C for 23 h. Red-orange powder (0.08 g, 29%); R_t (min) 0.88 (93% purity); $[\alpha]^{20}$ −321 (*c* 0.02, DCM); mp 115–120 °C; ν_{max} (KBr)/cm^{-1} 3458 (OH, NH), 1749 (ester C=O), 1631 (aldehyde C=O), 1610 (C=C); ^{1}H NMR (270 MHz, $CDCl_3$) δ −4.76 (1H, s, 7′-O*H*), 1.50 (6H, d, *J* = 6.6 Hz, $(CH_3)_2CH$), 1.58 (6H, d, *J* = 7.3 Hz, $(CH_3)_2CH$), 2.04 (6H, s, Ar–C*H*$_3$), 3.20 (1H, dd, *J* = 14.5, 9.2 Hz, CH_2-a), 3.50 (1H, dd, *J* = 14.5, 4.0 Hz, CH_2-b), 3.70 (1H, m, $(CH_3)_2CH$), 3.80 (3H, s, OCH_3), 3.90 (1H, m, $(CH_3)_2CH$), 4.43 (1H, broad, α-C*H*), 4.70 (1H, s, 1-O*H*), 5.69 (1H, s, 1′-O*H*), 6.45 (1H, s, 6′-O*H*), 6.76–7.02 (4H, m, Ar–H), 7.52 (3H, m, 4,4′-*H* and 2″-C*H*), 7.75 (1H, s, 6-O*H*), 8.0 (1H, broad s, indole N*H*), 8.96 (1H, s, C*H*=N), 11.22 (1H, s, C*H*O), 13.43 (1H, s, 7-O*H*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.4 (4 × CH_3), 20.7 (Ar–CH_3), 20.8 (Ar–CH_3), 28.0 (2 × CH), 30.1 (CH_2), 56.0 (OCH_3), 65.0 (α-CH), 104.1 (quat., C-8), 112.2 (quat., C-8′), 113.0 (quat., C-3″), 114.9 (quat., C-8a), 115.8 (quat., C-8a′), 116.1 (CH, C-5″), 116.2 (quat., C-2), 116.7 (quat., C-2′), 118.6 (2 × CH, 4,4′-C), 122.5 (CH, C-4″), 123.5 (CH, C-6″), 127.4 (quat., C-3a″), 128.3 (2 × quat., 4a,4a′-C), 129.7 (quat., C-5), 129.9 (quat., C-5′), 130.1 (CH, C-7″), 131.1 (CH, C-2″), 132.2 (2 × quat., 3,3′-C), 134.5 (quat., C-7a″), 147.5 (quat., C-1), 149.3 (quat., C-6), 150.8 (quat., C-1′), 150.9 (quat., C-6′), 155.4 (quat., C-7), 156.5 (quat., C-7′), 162.0 (CH=N), 170.1 (quat., ester C=O), 199.8 (CHO). HRMS: calcd for $C_{42}H_{43}O_9N_2$: 719.2963. Found: 719.2965.

4.1.2.5. (−)-Gossypol (−)-1. This was obtained from the complete hydrolysis of (−)-**3b** (0.35 g, 0.39 mmol) in ether (80 mL), glacial acetic acid (4.2 mL), concentrated H_2SO_4 (5 mL) and distilled H_2O (6 mL), for 21 h. Yellow-orange crystalline solid (0.19 g, 95%); $[\alpha]^{18.5}$ −358.5 (*c* 0.05, CH_3OH) (lit. $[\alpha]^{29}$ −363.6; *c* 0.20, CH_3OH[39]); mp 135–140 °C (lit. mp 166–167 °C[40]); (Found: C, 69.3; H, 6.0. $C_{30}H_{30}O_8$ requires C, 69.5; H, 5.8); ν_{max} (KBr)/cm^{-1} 3498 and 3434 (OH), 1617 (C=O), 1614 (C=C); ^{1}H NMR (270 MHz,

$CDCl_3$) δ −4.86 (2H, s, 7,7′-O*H*), 1.55 (12H, d, J = 7.25 Hz, CH(CH_3)$_2$), 2.15 (6H, s, Ar–CH_3), 3.89 (2H, septet, J = 7.25 Hz, C*H*(CH$_3$)$_2$), 5.86 (2H, s, 1,1′-O*H*), 6.42 (2H, s, 6,6′-O*H*), 7.78 (2H, s, 4,4′-*H*), 11.13 (2H, s, C*H*O). Enantiomeric identification was based upon the sign of the optical rotation, and reverse-phase HPLC using a packed analytical chiral column (250 mm × 4.6 mm i.d.) with a mobile phase of acetonitrile/aq 0.01 M KH_2PO_4 (70:30) at λ = 254 nm and a flow rate of 1.0 mL/min (R_t = 7.09 min). Racemic gossypol in this HPLC method gave two peaks at R_t = 7.09 min and R_t = 9.01 min.

4.1.2.6. (+)-Gossypol (+)-1. This is obtained from the complete hydrolysis of (+)-**3a** (0.16 g, 0.19 mmol) in concentrated H_2SO_4/acetic acid/ether (2.5:1:1) (10 mL) for 20 h. Dark green solid (0.09 g, 91.5%); $[\alpha]^{19}$ +348.25 (c 0.02, CH_3OH) (lit. $[\alpha]^{22}$ +359; c 0.05, $CHCl_3$[40]); mp. 135–140 °C (lit. mp 166–167 °C[40]); (Found: C, 69.2; H, 6.0. $C_{30}H_{30}O_8$ requires C, 69.5; H, 5.8); ν_{max} (KBr)/cm^{-1} 3496 and 3436 (OH), 1617 (C=O); ^{1}H NMR (270 MHz, $CDCl_3$) δ −4.84 (2H, s, 7,7′-O*H*), 1.55 (12H, d, J = 7.3 Hz, (CH_3)$_2$CH), 2.16 (6H, s, Ar–CH_3), 3.90 (2H, septet, J = 7.3 Hz, C*H*(CH$_3$)$_2$), 5.84 (2H, s, 1,1′-O*H*), 6.43 (2H, s, 6,6′-O*H*), 7.79 (2H, s, 4,4′-*H*), 11.14 (2H, s, C*H*O). Reverse-phase HPLC with the chiral column and method used previously gave an R_t (min.) of 9.01.

4.1.3. Preparation of gossypolone rac-2. A solution of racemic gossypol acetic acid **1** (0.5 g, 0.865 mmol) in acetone (25 mL) and acetic acid (50 mL) was heated on a steam bath at 100 °C during the careful addition of a 10% aq solution of $FeCl_3 \cdot 6H_2O$ (30 mL, 15 mmol). The mixture was left to stir at 100 °C for 2 h, then allowed to cool. Distilled water (63 mL) was added to precipitate a dark iron-containing compound, which was removed by filtration under gravity, and treated with a mixture of ether (200 mL) and 20% aq H_2SO_4 (200 mL). The liberated phenol partitioned into the ether layer, which was separated, dried over $MgSO_4$ and evaporated to dryness (0.43 g, 91%). This product was recrystallised from aq acetic acid and the resulting orange crystals of gossypolone **rac-2** were collected by filtration under vacuum (0.28 g, 59%); mp 256–260 °C (lit.[22] mp 264 °C); (Found: C, 65.5; H, 5.2. $C_{30}H_{26}O_{10}$ requires C, 65.9; H, 4.8%); ν_{max} (KBr)/cm^{-1} 3492 (OH), 1635 (C=O); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.45 (12H, 2 × d, J = 6.6 Hz, CH(CH_3)$_2$), 2.15 (6H, s, Ar–CH_3), 4.11 (2H, septet, J = 6.6 Hz, C*H*(CH$_3$)$_2$), 6.67 (2H, s, 6,6′-OH), 10.57 (2H, s, C*H*O), 13.00 (2H, s, 7,7′-O*H*).

4.2. Biological testing

4.2.1. Anti-proliferative MTT assay. Chemicals were purchased from Sigma–Aldrich and PBS from Gibco. Manipulation of the cells was conducted in a Class II Safety Cabinet under aseptic conditions, at Stiefel Laboratories, Maidenhead, UK. The HPV-16 cells (American Tissue Culture Collection) were cultured in a mixture of Epilife liquid (Cascade Ltd., Mansfield, UK) with keratinocyte growth supplement, consisting of bovine pituitary extract, epidermal growth factor, hydrocortisone, insulin and transferrin. Cells were allowed to grow to 60–80% confluence under conditions of 5% CO_2 at 37 °C.

A stock solution of each compound was prepared in 1 mL of DMSO followed by dilution with fresh medium. Two 96 well plates were set up for each compound with six in-plate replicates of the eight concentrations on the plate. Cell solution (200 μL, 5×10^4 cells/mL, 1×10^4 cells/well) was plated (rows 2–10, B–G) and initially incubated for 24 h, after which the media were removed and 100 μL of media containing 1% DMSO was added. Stock solution (200 μL) was then added to row 2 and serially half diluted across to row 9. Plates were incubated at 5% CO_2 at 37 °C for 3 days, after which the cells were washed with 200 μL PBS, 200 μL of 0.5 mg/mL MTT solution was added to each well, and the cells were incubated for 2 h at 37 °C at 5% CO_2. The MTT solution was then removed, the wells were washed again with PBS, 200 μL of 90% IPA/10% DMSO solution was added and the plates incubated in the dark for 10 min. Absorbance readings were taken at 570 nm using an Anthos III microplate spectrophotometer. The data were processed with the aid of Microsoft Excel 2000 and the % growth at each compound concentration was calculated as follows:

$$\%\text{Growth} = 100\left[\frac{(\text{Compound} - \text{Blank})}{(\text{Control} - \text{Blank})}\right]$$

Compound is the mean absorbance of the inhibitor and the cell culture, blank is the absorbance of the media alone and control is the absorbance of the media with cells. GI_{50} was defined as the compound concentration, which caused 50% inhibition of the growth of the treated cells compared to the control cells. The GI_{50} values were obtained from LOWESS analysis of % growth versus logarithmic concentration (molar) of the compound, using PRISM GraphPad.

4.2.2. Anti-oxidant assay. Chemicals were purchased from BDH and Sigma–Aldrich. The liposomes were prepared as a 5 mg/mL suspension of bovine brain extract (Sigma) in PBS and were stored at −80 °C. Ascorbic acid and ferric chloride (1 mM aqueous solutions) were freshly prepared before each test. A stock solution of each compound was prepared in 10 mL ethanol and six serial 1 in 10 dilutions were made. Four replicates were carried out for each reaction mixture, resulting in 68 tubes (Sterilin) per assay (blank, full reaction mixture (FRM), positive control and seven compound concentrations with and without the FRM).

Stock solution (0.1 mL) and each of the six dilutions were added into eight consecutive tubes each, arranged into two rows of four replicates. Liposomes (0.2 mL), 0.5 mL PBS, 0.1 mL $FeCl_3$ solution and 0.1 mL of ascorbic acid solution were added in the first row of each concentration (test compound). Distilled water (0.4 mL) and 0.5 mL of PBS were added in the second row of each concentration (compound alone). The positive control tubes contained 0.1 mL propyl gallate, 0.2 mL of liposomes, 0.5 mL of PBS, 0.1 mL $FeCl_3$ solution and

0.1 mL of ascorbic acid solution. The FRM tubes contained 0.1 mL of ethanol, 0.2 mL of liposomes, 0.5 mL of PBS, 0.1 mL $FeCl_3$ solution and 0.1 mL of ascorbic acid solution. The blank reaction mixture tubes contained 0.3 mL of distilled water, 0.2 mL of liposomes and 0.5 mL of PBS. The tubes were incubated at 37 °C, covered with foil paper, for 30 min and then 0.1 mL of BHT, 0.5 mL of TBA and 0.5 mL of HCl solutions were added. The tubes were heated in a water-bath (Gallenkamp) at 85–90 °C for 30 min and, after they had cooled, 2.5 mL of *n*-butanol was added. They were then shaken individually with a vortex mixer (Gallenkamp) and centrifuged at 3500 rpm (MISTRAL-3000i) at room temperature, for 10 min. The top, *n*-butanol, layer (2.5 mL) was removed from each tube into a cuvette, and its absorbance was measured with a UNICAM UV/VIS spectrophotometer operating at 532 nm, using *n*-butanol as internal standard. The data were processed with the aid of Microsoft Excel 2000 and the % inhibition of each compound, at a given concentration, was calculated as follows:

$$\%\text{Inhibition} = 100\left[\frac{((\text{FRM} - \text{Blank}) - (\text{Test} - \text{Compound} - \text{Blank}))}{(\text{FRM} - \text{Blank})}\right]$$

The IC_{50} values were obtained from LOWESS analysis of %inhibition of peroxidation versus logarithmic concentration (molar) of each inhibitor, using PRISM GraphPad.

Acknowledgements

We would like to thank Stiefel International R&D for their generous financial support for this project and Mrs. Yu Gong for the data on dithranol.

References and notes

1. Boehncke, W. H. *Trends Microbiol.* **1996**, *4*, 485.
2. Bundu-Kamara, S. *Hosp. Pharmacist* **2002**, *9*, 191.
3. Mendonça, C. O.; Burden, A. D. *Pharmacol. Ther.* **2003**, *99*, 133.
4. Pol, A.; Bergers, M.; Schalkwijk, J. *In Vitro Cell Dev. Anim* **2003**, *39*, 36.
5. Cass, Q. B.; Tiritan, E.; Matlin, S. A.; Freire, E. *Phytochemistry* **1991**, *30*, 2655.
6. Jaroszewski, J. W.; Strom-Hansen, T.; Hansen, S. H.; Thastrup, O.; Kofod, H. *Planta Med.* **1992**, *58*, 454.
7. Wu, D. *Drugs* **1989**, *38*, 333.
8. Lin, T.-S.; Schinazi, R.; Griffith, B. P.; August, E. M.; Eriksson, B.; Zheng, D.-K.; Huang, L.; Prusoff, W. *Antimicrob. Agents Chemother.* **1989**, *33*, 2149.
9. Van der Jagt, D. L.; Deck, L. M.; Royer, R. E. *Curr. Med. Chem.* **2000**, *7*, 479.
10. Stein, R. C.; Joseph, A. E. A.; Matlin, S. A.; Cunningham, D. C.; Ford, H. T. *Cancer Chemother. Pharmacol.* **1992**, *30*, 480.
11. Bushunow, P.; Reidenberg, M. M.; Wasenko, J.; Winfield, J.; Lorenzo, B.; Lemke, S.; Himpler, B.; Corona, R.; Coyle, T. *J. Neuro-Oncol.* **1999**, *43*, 79.
12. Van Poznak, C.; Seidman, A. D.; Reidenberg, M. M.; Moasser, M. M.; Sklarin, N.; Van Zee, K.; Borgen, P.; Gollub, M.; Bacotti, D.; Yao, T.-J.; Bloch, R.; Ligueros, M.; Sonenberg, M.; Norton, L.; Hudis, C. *Breast Cancer Res. Treat.* **2001**, *66*, 239.
13. Qiu, J.; Levin, L. R.; Buck, J.; Reidenberg, M. M. *Exp. Biol. Med.* **2002**, *227*, 398.
14. Blackstaffe, L.; Shelley, M. D.; Fish, R. G. *Melanoma Res.* **1997**, *7*, 364.
15. Shelley, M. D.; Hartley, L.; Groundwater, P. W.; Fish, R. G. *Anti-Cancer Drugs* **2000**, *11*, 209.
16. Yu, B.-Z.; Rogers, J.; Ranadive, G.; Baker, S.; Wilton, D.; Apitz-Castro, R.; Jain, M. K. *Biochemistry* **1997**, *36*, 12400.
17. Benhaim, P.; Mathes, S.; Hunt, T. K.; Scheuenstuhl, H.; Benz, C. C. *Inflammation* **1994**, *18*, 443.
18. Drewa, G.; Krzyzynska-Malinowska, E.; Wozniak, A.; Protas-Drozd, F.; Mila-Kierzenkowska, C.; Rozwodwska, M.; Kowaliszyn, B.; Czajkowski, R. *Med. Sci. Monit.* **2002**, *8*, BR338.
19. Shelley, M. D.; Hartley, L.; Fish, R. G.; Groundwater, P.; Morgan, J. J. G.; Mort, D.; Mason, M.; Evans, A. *Cancer Lett.* **1999**, *135*, 171.
20. Brzezinski, B.; Przyblyski, P. *Biopolymers* **2002**, *67*, 61.
21. Matlin, S. A.; Belenguer, A.; Tyson, R. G.; Brookes, A. N. *J. High Res. Chrom. Chrom. Commun.* **1987**, *10*, 86.
22. Haas, R. H.; Shirley, D. *J. Org. Chem.* **1965**, *30*, 4111.
23. Dao, V.-T.; Dowd, M. K.; Gaspard, C.; Martin, M.-T.; Hemez, J.; Laprevote, O.; Mayer, M.; Michelot, R. *Bioorg. Med. Chem.* **2003**, *11*, 2001.
24. Gilbert, N. E.; O'Reilly, J. E.; Chang, C. J.; Lin, Y. C.; Brueggemeier, R. W. *Life Sci.* **1995**, *57*, 61.
25. Muller, K.; Prinz, H.; Gawlik, I.; Ziereis, K.; Huang, H. S. *J. Med. Chem.* **1997**, *40*, 3773.
26. Hong, Y. L.; Yeh, S. L.; Chang, C. Y.; Hu, M. L. *Clin. Biochem.* **2000**, *33*, 619.
27. Motta, S.; Sesana, S.; Monti, M.; Guiliani, A.; Caputo, R. *Acta Derm. Venereol. Suppl.* **1994**, *186*, 131.
28. Utas, S.; Kose, K.; Yazici, C.; Akdas, A.; Kelestimur, F. *Clin. Biochem.* **2002**, *35*, 241.
29. Tang, F.; Tsang, A. Y. F.; Lee, C.-P.; Wong, P. Y. D. *Contraception.* **1982**, *26*, 515.
30. Cuellar, A.; Ramirez, J. *Int. J. Biochem.* **1993**, *25*, 1149.
31. Benz, C. C.; Keniry, M. A.; Ford, J. M.; Townsend, A. J.; Cox, F. W.; Palayoor, S.; Matlin, S. A.; Hait, W. N.; Cowan, K. H. *Mol. Pharmacol.* **1990**, *37*, 840.
32. Janero, D. R.; Burghardt, B. *Biochem. Pharmacol.* **1988**, *37*, 3335.
33. Laughton, M. J.; Halliwell, B.; Evans, P. J.; Hoult, J. R. *Biochem. Pharmacol.* **1989**, *38*, 2859.
34. Li, A. S-H.; Bandy, B.; Tsang, S.-S.; Davison, A. *Free Radical. Res.* **2000**, *33*, 551.
35. Wong, K.-P.; Wong, M.-C. US Patent 6,608,107 B2, 2003.
36. Yurtcu, E.; Ergun, M. A.; Menevse, A. *Cell Biol. Int.* **2003**, *27*, 791.
37. Coutinho, E. M. *Contraception* **2002**, *65*, 259.
38. Li, Y. F.; Booth, G. M.; Seegmiller, R. E. *Reproductive Toxicol.* **1989**, *3*, 59.
39. Matlin, S. A.; Zhou, R. *J. High Res. Chrom. Chromat. Commun.* **1984**, *7*, 629.
40. Si, Y. K.; Zhou, J.; Huang, L. *Sci. Sinica (Ser. B)* **1987**, *30*, 297.