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Some 1,2-Diphenylethane Derivatives as Inhibitors of Retinoic Acid—Metabolising Enzymes

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In a search for novel inhibitors of RA-metabolising enzyme inhibitors as potential anti-cancer agents some 1,2-ethandiones, 2-hydroxyethanones and 1-ethylenedioxyethanones based on aryl-substituted 1,2-diphenylethane have been examined. Several of the compounds were weak inhibitors of the non-specific rat liver microsomal P450 enzymes and moderate inhibitors of the RA-induced enzymes in cultured human genital fibroblasts, where the RA-specific enzyme CYP26 is probably expressed. The 2-hydroxyethanone (13) with a 1-(4-dimethylaminophenyl) substituent was overall the most potent compound for rat liver microsomal enzyme (IC_{50} = 52.1 μM; ketoconazole, 2.8 μM) and the RA-induced enzyme (100 μM, 65.9% inhibition; ketoconazole, 20 μM, 75.0%). Modification of the dimethylamino group in (13) with more hydrophobic dialkylamino functions or separate modification of the 2-(2,4-dichlorophenyl) function did not improve potency.

Keywords: Retinoic acid; RA; RA-metabolising enzyme inhibitors; Ketoconazole; RAMBAs; RA-metabolising blocking agents

INTRODUCTION

All-trans-retinoic acid (RA) is a naturally occurring retinoid, derived from vitamin A (retinol), responsible for growth and differentiation of mammalian epithelial tissues.[1] After binding to transcription-regulatory factors in the cell (RAR, retinoic acid receptor and RXR, retinoid X receptor) the activated receptor binds to its response element (RARE or RXRE) to transcriptionally regulate its target genes.[2–4] RA has been used clinically for the treatment of cancers and skin conditions. It has shown spectacular success in the treatment of acute promyelocytic leukaemia[5–7] although remission (1–3 months) is followed by relapse (4–12 months) due to increased RA-metabolism as a result of RA-induction of metabolising enzymes. Moderate success has been achieved with other cancer types especially in combination with other therapies.[8–9] RA has also been used for the treatment of hyperkeratinisation skin disorders e.g. cystic acne, psoriasis as well as photodamaged skin.[10–12]

RA is metabolised by cytochrome 450s present in liver and other tissues to the 4-hydroxylated form which is inactive. Further oxidation by dehydrogenases furnishes the partially active 4-keto form and then inactive polar metabolites.[13] Several reconstituted P450s, CYP1A2, 2B6, 2C8, 2D6, 2E1 and 3A4 can catalyse the reaction. The rat liver microsomal enzymes 1A1/2, 2A6 and 3A4 have been identified *in vitro* as catalysts.[14] More recently a specific RA-metabolising enzyme, CYP26, has been reported in mammalian tissues.[15–17]

A drug which can prolong and intensify the action of endogenous RA on epidermal cells by inhibiting the action of RA-metabolising enzymes would have potential as a clinical agent for the treatment of skin disorders and cancers. Well established inhibitors are the imidazoles, ketoconazole (**1**) and liarozole (**2**). Both lack specificity and inhibit P450s involved in steroidogenesis thus affecting the pathways for oestrogen and androgen synthesis which makes them unsuitable as orally active anticancer agents where

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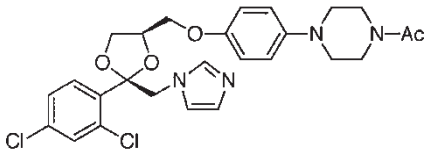
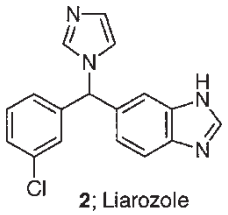
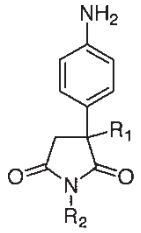
non-hormone dependent cancers are concerned. Liarozole (Liazal™) has been effective in clinical trials in psoriasis,[18,19] as well as icthyosis and hormone-dependent prostate cancer.[20–24]



1; Ketoconazole



2; Liarozole



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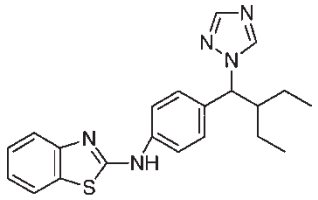
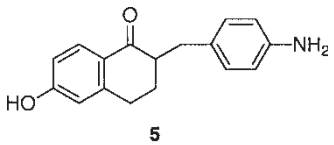


4; R115866



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More recently the triazole R115866 has been described as a potent inhibitor of RA-induced CYP26 ($IC_{50} = 4$ nM) with little effect on steroidogenic P450s.[25] After a single oral dose in rats endogenous RA levels were increased in tissues and R115866 mimicked RA in several of its biological actions.

We have recently described, (1) some substituted 3-(4-aminophenyl) pyrrolidine-2,5-diones (**3**) as weak inhibitors of RA-metabolism in rat liver and other microsomal systems as well as RA-induced human male genital fibroblasts and HaCat cell cultures,[26] (2) the tetralone (**5**) which is more potent than or equipotent to ketoconazole in the cadavenous systems but less potent towards the RA-induced cell culture systems.[27] Here we describe a further series of compounds based on the 1,2-diphenylethane skeleton as inhibitors in these systems.

MATERIALS AND METHODS

Chemistry

All reagents and solvents were general purpose grade. Melting points were determined on a Gallenkamp digital apparatus and are uncorrected. IR spectra were obtained as KBr discs, as solids via a diffuse reflectance accessory using a KBr matrix, or between NaCl plates using a Perkin Elmer 1600 series FTIR. ^{1}H-NMR spectra were recorded on a Bruker DPX 300 (300 MHz) spectrometer as dilute solutions in deuteriochloroform, unless stated otherwise, with tetramethylsilane as internal standard. Chromatography was performed on Acros silica gel (pore size 0.035–0.07 nm) as the stationary phase. When required, chemical reactions were monitored by thin layer chromatography (TLC) using Merck silica gel 60 F_{254} precoated aluminium plates which were visualised with UV light or iodine.

1-(2,4-Dichlorophenyl)-2-(4′-nitrophenyl)-1-ethanol (6)

Freshly ground sodium hydroxide (0.2g) was added to a solution of 4-nitrotoluene (20.57g, 0.15 mol.) and 2,4-dichlorobenzaldehyde (8.75g, 0.05 mol.) in dry dimethylformamide (100 ml), and the reaction stirred under nitrogen at room temperature. After 2 h the reaction mixture was acidified with concentrated hydrochloric acid (1 ml), diluted with toluene and washed with water. After drying ($MgSO_4$), both solvent and excess 4-nitrotoluene were removed *in vacuo*, to yield a red oil which solidified on standing to give a yellow solid. The solid was recrystallised (ethanol) to give compound (**6**) (9.3g, 59.6% yield) as colourless crystals, m.p. 154°C; Found: C, 53.91; H, 3.56; N, 4.26, $C_{14}H_{11}Cl_2NO_3$ requires C, 53.87; H, 3.55; N, 4.49%. ν_{max} (KBr)/cm $^{-1}$ 3548 (OH), 1514 and 1343 (NO_2). δ_H (300 MHz, DMSO-d_6) 2.90 (1H, dd, $J_{AB} = 13.5$ Hz, $J_{XB} = 8.1$ Hz, $CH_AH_BCH_xOH$), 3.06 (1H, dd, $J_{BA} = 13.5$ Hz, $J_{XA} = 3.8$ Hz, CH_AH_B

CH_XOH), 5.15 (1H, ddd, $J_{BX} = 8.2$ Hz, $J_{AX} = 3.8$ Hz, $J = 4.7$ Hz, CH_XOH), 5.75 (1H, d, $J = 4.7$, CHO*H*), 7.46 (3 h, m, Ar C(3)*H*, C(5)*H*) and C(6)*H*), 7.7 (2H, d, $J =$ 8.7 Hz, Ar C(2′) *H* and C(6″)*H*), 8.2 (2H, d, $J = 8.7$ Hz, Ar C(3″)*H* and C(5″)*H*).

1-(2,4-Dichlorophenyl)-2-(4-nitrophenyl)-1-ethanone (7)

Compound (**6**) (4.6g, 0.015 mol.) in dry dichloromethane (40 ml) was added to a suspension of pyridinium chlorochromate (6.5g, 0.03 mol.) in dichloromethane (40 ml). After stirring for 2 h at room temperature, the reaction mixture was filtered through a silica gel plug which was then washed with copious amounts of ether. The filtrate was concentrated to give a solid which was recrystallised from ethanol (yield = 45%). M.p. = 83.0–83.8°C. (Found: C, 54.10; H, 2.88; N, 4.49. $C_{14}H_9Cl_2NO_3$ calculated C, 54.22; H, 2.93; N, 4.52%; ν_{max} (KBr)/cm^{-1} 1719 (C═O), 1515 and 1348 (NO_2). δ_H (300 MHz, $CDCl_3$) 4.40 (2H, s, CH_2), 7.31 (1H, dd, $J = 1.7$ Hz, $J = 8.3$ Hz, Ar C(5)*H*), 7.40 (2H, d, $J =$ 9.3 Hz, Ar C(2′)*H* and C(6′)*H*), 7.45 (2H, m, Ar C(3)*H* and C(6)*H*), 8.19 (2H, d, $J = 9$ Hz, Ar C(3′) *H* and C(5′)*H*).

1-(2,4-Dichlorophenyl)-1,1-ethylenedioxy-2-(4′-nitrophenyl)ethane (8)

A mixture of ethylene glycol (10 ml), *p*-toluene sulphonic acid (0.2g) and toluene (100 ml) was refluxed with a Dean–Stark trap for 2 days (until evolution of water ceased). Compound (**7**) (1.0g, 3.2 mmol) was added to the reaction mixture and refluxing continued for a further 2 days. On cooling the reaction mixture was concentrated to give a yellow oil, and a pure sample (15 mg) was obtained by preparative TLC with ethyl acetate: petroleum ether (3:7) as eluant. This pure sample was used to seed the remainder of the oil which crystallised. The crystals were filtered and recrystallised (ethanol) to give the *ketal* (yield = 29%). M.p. = 116.6–117.1°C. Found: C, 54.33; H, 3.69; N, 3.84. $C_{16}H_{13}Cl_2NO_4$ calculated C, 54.26; H, 3.70; N, 3.95%). ν_{max} (KBr)/cm^{-1}1584 (C═C), 1514 and 1342 (NO_2). δ_H (300 MHz, $CDCl_3$) 3.55 (2H, s, CH_2), 3.81 (2H, m, CH_2 *ketal*), 3.86 (2H, m, CH_2 *ketal*), 7.21 (1H, dd, $J = 8.4$ Hz, $J = 2.1$ Hz, Ar C(5)*H*), 7.42 (2H, d, $J = 8.6$ Hz, Ar C(2′)*H* and C(6′)*H*), 7.43 (1H, d, $J = 8.4$ Hz, Ar C(6)*H*), 7.49 (1H, d, $J = 2.1$ Hz, Ar C(3)*H*), 8.13 (2H, d, $J =$ 8.6 Hz, Ar C(3′)*H* and C (5′)*H*).

1-(2,4-Dichlorophenyl)-2-(4′-nitrophenyl)-1,2-ethandione (9)

Conc. nitric acid (30 ml, S.G. 1.42) was poured onto compound (**6**) (5g, 0.016 mol.) in a round-bottomed flask fitted with an air condenser. The reaction mixture was heated on a boiling water bath for 2.5 h until the evolution of brown fumes of nitric oxide ceased, whereupon the solution was poured onto ice and stirred. The resulting gum obtained after decantation, gave yellow crystals after crystallisation (ethanol), (2.90g, 55.9%). M.p. 133–4° Found: C, 51.86; H, 2.09; N, 4.19. $C_{14}H_7Cl_2NO_4$ requires C, 51.88; H, 2.18; N, 4.32%; ν_{max} (KBr)/cm^{-1} 1681 (C═O, α-diketone), 1529 and 1347 (NO_2). δ_H (300 MHz, $CDCl_3$) 7.43 (1H, dd, $J = 1.7$ Hz, $J =$ 8.8 Hz, Ar C(5)*H*), 7.44 (1H, d, $J = 1.8$ Hz, Ar C(3)*H*), 7.82 (1H, d, $J = 8.8$ Hz, Ar C(6)*H*), 8.17 (2H, d, $J = 8.9$ Hz, Ar C(2′)*H* and C(6′)*H*), 8.36 (2H, d, $J = 8.9$ Hz, Ar C(3′)*H* and C (5′)*H*).

N-(4-(2-(2′,4′-Dichlorophenyl)-2-hydroxyacetyl)phenyl) acetamide (10)

Potassium cyanide (1g) dissolved in water (10 ml) was added to a solution of 4-acetylaminobenzaldehyde, (1.63g, 0.01 mol.) and 2,4-dichlorobenzaldehyde (1.76g, 0.01 mol.) in ethanol (30 ml), and the mixture refluxed for 2 h. On cooling, the mixture was diluted with water, when an orange oily residue separated. The aqueous phase was decanted off, and the residue dissolved in dichloromethane, washed with water several times, and dried ($MgSO_4$). On evaporating the solvent, a dark orange oil was obtained which on standing gave a yellow solid. Crystallisation (ethanol) gave compound (**10**) (1.94g, 57.4%) as a yellow crystalline solid, m.p. 180–181°C. Found C: 56.78; H, 3.88; N 3.97. $C_{16}H_{13}Cl_2NO_3$ requires C, 56.82; H, 3.87; N, 4.14%. ν_{max} (KBr)/cm^{-1} 3352 (OH), 1673 and 1654 (C═O). δ (300 MHz, DMSO-d_6) 2.11 (3H, s, $COCH_3$), 6.23 (1H, d, $J =$ 7.0 Hz, C*H*OH or CHO*H*), 6.52 (1H, d, $J = 7.0$ Hz, C*H*OH or CHO*H*),7.45 (1H, dd, $J = 8.4$ Hz, $J =$ 1.7 Hz, Ar C(5′)*H*), 7.52 (1H, d, $J = 8.4$ Hz, Ar C(6′)*H*), 7.59 (1H, d, $J = 1.7$ Hz, Ar C(3′)*H*), 7.71 (2H, d, $J = 8.5$ Hz, Ar C(3)*H* and C(5)*H*), 7.97 (2H, d, $J = 8.4$ Hz, Ar C(2)*H* and C(6)*H*), 10.38 (1H, s, N*H*). m/e (APCI, low resolution) 162 and 163 ($CH_3CONHC_6H_4CO^+$) 175 and 177 ($C_6H_3Cl_2CHOH^+$).

N-(4-(2-(2′,4′-Dichlorophenyl)-2-oxoacetylphenyl) acetamide (11)

Copper sulphate (1.5g), pyridine (3 ml) and water (1 ml) were heated for 10 min, until a homogeneous solution was obtained when compound (**10**) (0.84g, 0.0025 mol.) was added and the mixture heated at 100°for 2 h with stirring. On pouring onto water a yellow oil separated which was extracted with dichloromethane, and the extract washed with HCl (2 M) and water. After drying ($MgSO_4$), the solvent was removed to give a yellow powder.

Crystallisation (ethanol) gave compound (**11**) (0.44g, 52.4%) as fine yellow crystals, m.p. 177°C. Found C: 57.20; H, 3.44; N, 4.01. $C_{16}H_{11}Cl_2NO_3$ requires C, 57.17; H, 3.30; N, 4.17%. ν_{max} (KBr)cm^{-1} 1681 and 1661 (C═O). δ_H (300 MHz, DMSO-d_6) 2.13 (3H, s, COCH_3), 7.69 (1H, dd, J = 8.4 Hz, J = 1.8 Hz, Ar C(5′)*H*), 7.82 (2H, d, J = 8.4 Hz, Ar C(3)*H* and C(5)*H*), 7.87 (1H, d, J = 1.6 Hz, Ar C(3′)*H*), 7.92 (1H, d, J = 8.6 Hz, Ar C(6′)*H*), 7.96 (2H, d, J = 8.3 Hz, Ar C(2)*H* and C(6)*H*), 10.49 (1H, s, N*H*).

2-(4′-Aminophenyl)-1-(2,4-dichlorophenyl)-1,2-ethandione (12)

Compound (**11**) (0.38g, 0.001 mol.) was refluxed in conc. hydrochloric acid (20 ml) and methanol (10 ml) for 1 h. On cooling the HCl salt precipitated from solution. The mixture was concentrated to half volume and the precipitate collected. The salt was redissolved in warm methanol (30 ml) and sodium hydroxide (2 M, 10 ml) and the solution evaporated to dryness. The residue was dissolved in dichloromethane, washed with water and the organic phase dried ($MgSO_4$) and evaporated to give a yellow oil. Trituration (ether) gave compound (**12**) as a yellow solid (0.24g, 81.6%) m.p. 92–93°C. Found: C: 57.04; H, 2.97; N 4.51. $C_{14}H_9Cl_2NO_2$ requires C, 57.17; H, 3.08; N, 4.76%. ν_{max} (KBr)/cm^{-1} 3448 (NH), 1675 (C═O, diketone). δ_H (300 MHz, $CDCl_3$) 4.43 (2H, br, s, NH_2), 6.73 (2H, d, J = 8.7 Hz, Ar C(3)*H* and C(5)*H*), 7.43 (1H, dd, J = 8.4 Hz, J = 1.9 Hz, Ar C(5′)*H*), 7.49 (1H, d, J = 1.9 Hz, Ar C(3′)*H*), 7.87 (1H, d, J = 8.3 Hz, Ar C(6′)*H*), 7.89 (2H, d, J = 8.7 Hz, Ar C(2)*H* and C(6)*H*).

2-(2′,4′-Dichlorophenyl)-1-(4-dimethylaminophenyl)-2-hydroxy-1-ethanone (13)

Potassium cyanide (3g) in water (60 ml) was added to a solution of 4-dimethylaminobenzaldehyde (14.9g, 0.1 mol.) and 2,4-dichlorobenzaldehyde (17.6g, 0.1 mol.) in 95% ethanol (80 ml). After refluxing for 1 h, the mixture was allowed to cool when an oil separated from the solution. A further portion of potassium cyanide (1.0g) was added to the reaction mixture and refluxing was continued for 1 h. The reaction mixture was then allowed to cool and then extracted with dichloromethane (100 ml) and then again with dichloromethane (3 × 50 ml). The combined organic layers were washed with water (3 × 50 ml) then dried ($MgSO_4$) and concentrated to give a clear brown oil which crystallised with ethanol. The solid was recrystallised twice from ethanol to give the product (**13**) as yellow crystals (38%). M.p. 110.0–110.5°C Found: C, 59.20; H, 4.77; N, 4.35. $C_{16}H_{15}Cl_2NO_2$ requires C, 59.27; H, 4.66; N, 4.32%. ν_{max} (KBr)/cm^{-1} 3419 (OH), 2920 (N–Me), 1654 (C═O). δ_H (300 MHz, $CDCl_3$) 3.11 (6H, s, N $(CH_3)_2$), 4.93 (1H, d, J = 5.1 Hz, C*H*OH), 6.27 (1H, d, J = 4.6 Hz, CHO*H*), 6.62 (2H, d, J = 9.1 Hz, Ar C(3)*H* and C(5)*H*), 7.11 (1H, d, J = 8.4 Hz, Ar C(6′)*H*), 7.18 (1H, dd, J = 8.4 Hz, J = 1.9 Hz, Ar C(5′)*H*), 7.48 (1H, d, J = 2.0 Hz, Ar C(3′)*H*), 7.84 (2H, d, J = 9.1 Hz, Ar C(2)*H* and C(6)*H*).

The following analogues of (**13**) were made using the same general method.

2-(2,4-Dichlorophenyl)-1-[4-(diethylamino)phenyl]-2-hydroxy-1-ethanone (15)

With 4-diethylaminobenzaldehyde and 2,4-dichlorobenzaldehyde, a cream coloured solid was formed which was recrystallised with ethanol (yield = 28%). M.p. = 118.5–119.3°C. Found: C, 61.40; H, 5.47; N, 4.00. $C_{18}H_{19}Cl_2NO_2$ requires C, 61.37; H, 5.44; N, 3.98%. ν_{max} (KBr)/cm^{-1} 3418 (OH), 1660 (C═O);δH ($CDCl_3$) 7.84 (2H, d, J = 9.1 Hz, Ph-*H*), 7.48 (1H, d, J = 2 Hz, Ph-*H*), 7.18 (1H, dd, J = 8.4 Hz, J = 2 Hz, Ph*H*), 7.11 (1H, d, J = 8.4 Hz, Ph-*H*), 6.62 (2H, d, J = 9.1, Ph-*H*), 6.27 (1H, d, J = 4.6 Hz, CHO*H*), 4.93 (1H, d, J = 5.1 Hz C*H*OH), 3.54 (4H, q, J = 7.2 Hz, 2 × CH_2), 1.2 (6H, t, J = 7.2 Hz, 2 × CH_3).

1-[4-(Dibutylamino)phenyl]-2-(2,4-dichlorophenyl)-2-hydroxy-1-ethanone (16)

With 4-dibutylaminobenzaldehyde and 2,4-dichlorobenzaldehyde, a crystalline solid was formed which was recrystallised with ethanol (yield = 17%). M.p. = 106.8–108.1°C. Found: C, 64.56; H, 6.72; N, 3.41. $C_{22}H_{27}Cl_2NO_2$ requires C, 64.71; H, 6.66; N, 3.43%). ν_{max} (KBr)/cm^{-1} 3377 (OH), 1644 (C═O); δH ($CDCl_3$) 7.84 (2H, d, J = 9.1 Hz, Ph-*H*), 7.48 (1H, d, J = 2 Hz, Ph-*H*), 7.18 (1H, dd, J = 8.4 Hz J = 2 Hz, Ph-*H*), 7.11 (1H, d, J = 8.4 Hz, Ph-*H*), 6.62 (2H, d, J = 9.1, Ph-*H*), 6.27 (1H, d, J = 4.6 Hz, CHO*H*), 4.93 (1H, d, J = 5.1 Hz C*H*OH), 3.3 (4H, t, J = 7.2 Hz, 2 × CH_2), 1.6 (4H, quin, J = 7 Hz, 2 × CH_2), 1.4 (4H, quin, J = 7 Hz, 2 × CH_2), 1.0 (6H, t, J = 7 Hz, 2 × CH_3).

2-(2,4-Dichlorophenyl)-2-hydroxy-1-(4-tetrahydro-1H-1-pyrrolylphenyl)-1-ethanone (17)

With 4-tetrahydro-1*H*-1-pyrrolylbenzaldehyde and 2,4-dichlorobenzaldehyde, a brown oil was formed which was purified by flash, gradient column chromatography (yield = 15%). Found: C, 61.57; H, 5.07; N, 4.13. $C_{18}H_{17}Cl_2NO_2$ requires C, 61.73; H, 4.89; N, 4.00%). ν_{max} (KBr)/cm^{-1} 3328 (OH), 1662 (C═O); δH ($CDCl_3$) 7.84 (2H, d, J = 9.1 Hz, Ph-*H*), 7.48 (1H, d, J = 2 Hz, Ph-*H*), 7.18 (1H, dd, J = 8.4 Hz J = 2 Hz, Ph-*H*), 7.11 (1H, d, J = 8.4 Hz, Ph-*H*), 6.62 (2H, d, J = 9.1, Ph-*H*), 6.27 (1H, d, J = 4.6 Hz, CHO*H*), 4.93 (1H, d, J = 5.1 Hz C*H*OH), 3.4 (4H, q, J = 7.2 Hz, 2 × CH_2), 2.1 (4H, t, J = 7.2 Hz, 2 × CH_2).

1-[4-(Dimethylamino)phenyl]-2-[2,5-di(trifluoromethyl)phenyl]-2-hydroxy-1-ethanone (18)

With 4-dimethylaminobenazaldehyde and 2,5-bis-(trifuoromethyl) benzaldehyde a dark brown solid was formed which solidified upon standing, and was recrystallised from ethanol to give a yellow solid (yield = 18%). M.p. = 169.2–170.8°C. Found: C, 55.25; H, 3.89; N, 3.42. $C_{18}H_{15}F_6NO_2$ requires C, 55.25; H, 3.86; N, 3.58%. ν_{max} (KBr diffusion)/cm^{-1} 3460 (OH), 1649 (C═O); δH 7.9 (1H, d, J = 8.2 Hz, Ph-*H*), 7.8 (2H, d, J = 9.1 Hz, Ph-*H*), 7.7 (1H, d, J = 8.2 Hz, Ph-*H*), 7.4 (1H, s, Ph-*H*), 6.6 (2H, d, J = 9.1 Hz, Ph-*H*), 6.2 (1H, d, J = 5.3 Hz, CHCO*H*), 4.9 (1H, d, J = 5.4 Hz, C*H*COH), 3.1 (6H, s, N(CH_3)$_2$).

1-[4-(Dimethylamino)phenyl]-2-hydroxy-2-(1-naphthyl)-1-ethanone (19)

With 4-dimethylaminobenzaldehyde and 1-napthaldehyde an oil was formed which was crystallised and then recrystallised with methanol (yield = 6%). M.p. = 195.7–206.8°C. Found: C, 78.50; H, 6.31; N, 4.44. $C_{20}H_{19}NO_2$ requires C, 78.66; H, 6.27; N, 4.59%. ν_{max} (KBr diffusion)/cm^{-1} 3429 (OH), 1650 (C═O); δH 8.45 (1H, d, J = 8.5 Hz, Ph-*H*), 7.93 (1H, d, J = 8.0 Hz, Ph-*H*), 7.86 (1H, d, J = 6.7 Hz, Ph-*H*), 7.82 (2H, d, J = 9 Hz, Ph-*H*), 7.68 (1H, dt, J = 1.2 Hz J = 6.8 Hz, Ph-*H*), 7.58 (1H, t, J = 8 Hz, Ph-*H*), 7.4 (1H, t, J = 7.2 Hz, Ph-*H*), 7.3 (1H, d, J = 7 Hz, Ph-*H*), 6.55 (1h, d, J = 5.9 Hz, CHCO*H*), 6.53 (2H, d, J = 9.3 Hz, Ph-*H*), 4.9 (1H, d, J = 5.4 Hz, C*H*COH). 3.0 (6H, s, N(CH_3)$_2$).

1-[4-(Dimethylamino)phenyl]-2-hydroxy-2-(2-naphthyl)-1-ethanone (20)

With 4-dimethylaminobenzaldehyde and 2-naphthaldehyde an oil was formed which was crystallised with ether and then recrystallised with ethanol (yield = 10%). Found: C, 79.05; H, 6.42; N, 4.49. $C_{20}H_{19}NO_2$ requires C, 78.66; H, 6.27; N, 4.59%. ν_{max} (KBr diffusion)/cm^{-1} 3440 (OH), 1623 (C═O); δH 8.0–7.8 (6H, m, 6 × Ph − *H*), 7.5 (3H, m, 3 × Ph − *H*), 6.6 (2H,d, J = 8.0 Hz, 2 × Ph − *H*), 6.1 (1H, d, J = 5.7 Hz, CHCO*H*), 5.0 (1H, d, J = 5.7 Hz, C*H*COH), 3.0 (6H, s, N(CH_3)$_2$.

1-(2,4-Dichlorophenyl)-2-[4-(dimethylamino)phenyl]-1,2-ethanedione (14)

Compound (**13**) (0.27g, 0.83 mmol) was dissolved in dichloromethane (20 ml) and the solution was stirred with PCC (0.39g, 1.83 mmol) under N_2 for 45 min. The reaction mixture was diluted with dichloromethane (20 ml), filtered through silica gel and the silica gel was washed with dichloromethane. The organic washings were concentrated to form a solid which was crystallised from ethanol to give compound (**14**) as a bright yellow solid (30%). M.p. = 153.5–153.9°C. Found: C, 59.98; H, 4.13; N, 4.26. $C_{16}H_{13}Cl_2NO_2$ requires C, 59.65; H, 4.07; N, 4.35%. ν_{max} (KBr diffusion)/cm^{-1} 1683 (C═O); δH 7.96 (2H, d, J = 9.1 Hz, Ph-*H*), 7.88 (1H, d, J = 8.4 Hz, Ph-*H*), 7.51 (1H, d, J = 1.9 Hz, Ph-*H*), 7.45 (1H, dd, J = 1.9 Hz J = 8.4 Hz, Ph-*H*), 6.76 (2H, d, J = 9.1 Hz, 2 × Ph − *H*), 3.17 (6H, s, N(CH_3)$_2$).

4-Nitrobenzylpyridinium Bromide (21)

Pyridine (16.2 ml, 0.2 mol) was added dropwise to a solution of 4-nitrobenzyl bromide (21.6g, 0.1 mol) in dry dichloromethane (200 ml) with maintenance of the reaction temperature below 30°C. After 30 min a heavy white precipitate was formed, and 2 h later was filtered off, washed with dichloromethane and dried under vacuum to give compound (**21**) (24g 83%) as a dense white solid, m.p. 221–222°C. A second crop was obtained after standing overnight, to give a total yield of 97%. ν_{max} (KBr)/cm^{-1} 1518 and 1349 (NO_2). δ_H (300 MHz, $CDCl_3$) 6.21 (2H, s, CH_2), 7.88 (2H, d, J = 8.9 Hz, Ar C(2)*H* and C(6)*H*), 8.2 (2H, d, J = 8.9 Hz, Ar C(3)*H* and C(5)*H*), 8.41 (2H, dd, J = 7.3 Hz, J = 8.0 Hz Pyr C(3″)*H* and C(5″)*H*), 8.7 (1H, t, J = 8.1 Hz, Pyr C(4″)*H*), 9.4 (2H, d, J = 7.2 Hz, Pyr C(2″)*H* and C(6″)*H*).

N-[2-(2′,4′-Dichlorophenyl)-1-(4-nitrophenyl)-1-ethenyl]pyridinium Bromide (22)

A mixture of compound (**21**) (24.0g, 0.08 mol.), 2,4-dichlorobenzaldehyde (14.0g, 0.08 mol), anhydrous potassium acetate (6.4g), acetic acid (10 ml), and acetic anhydride (120 ml) was refluxed at 75–80°C for 48 h, allowed to cool to room temperature, and the excess potassium acetate removed by filtration. The filtrate was diluted with water (100 ml) and allowed to stand at room temperature for 48 h with stirring. The mixture was washed with diethyl ether and the aqueous layer acidified with hydrobromic acid (33% in glacial acetic acid), before concentrating. The bright yellow crystals which precipitated from the solution were filtered off, and the mother liquors concentrated to obtain further crops. The crops were combined and recrystallised (ethanol) to yield compound (**22**) (5.8, 16%) as yellow crystals, m.p. >250°C. ν_{max} (KBr)/cm^{-1} 1514 and 1346 (NO_2). δ_H (300 MHz, $CDCl_3$) 6.94 (1H, d, J = 8.4 Hz, Ar C(6′)*H*), 7.31 (1H, dd, J = 8.4 Hz, J = 2.1 Hz, Ar C(5′)*H*), 7.79 (1H, d, J = 2.2 Hz, Ar C(3′)*H*), 7.81 (2H, d, J = 8.9 Hz, Ar C(2)*H* and C(6)*H*), 8.12 (1H, s, = C*H*–), 8.32 (2H, d, J = 8.9 Hz, Ar C(3)*H* and C(5)*H*), 8.34 (2H, t, J = 6.4 Hz, Pyr C(3″)*H* and C(5″)*H*), 8.89 (1H, t, J = 7.9 Hz, Pyr C(4″)*H*), 9.24 (2H, d, J = 5.5 Hz, Pyr C(2″)*H* and C(6″)*H*).

2-(2′,4′-Dichlorophenyl)-1-(4-nitrophenyl)-1-ethanone (23)

Compound (**22**) (4.52g, 0.01 mol.), pyridine (40 ml), piperidine (8 ml), and water (16 ml) were combined and heated at 80°C for 1 h. The resulting deep red solution was cooled, evaporated, and ethanol (14 ml) added to the residue. The resulting mixture was then diluted with water (40 ml), acidified with hydrobromic acid (50 ml, 33% in glacial acetic acid) and shaken for 2 min. The solid material was filtered off and dried. Crystallisation (ethanol) furnished compound (**23**) as white crystals (2.77g, 89.3%), m.p. 141–142°C. Found: C, 53.61; H, 2.98; N, 4.19. $C_{14}H_9Cl_2NO_3$ requires C, 54.22; H, 2.93; N, 4.52%. ν_{max} (KBr)/cm^{-1} 1692 (C═O), 1520 and 1348 (NO_2). δ_H (300 MHz; $CDCl_3$) 4.51 (2H, s, CH_2), 7.26 (1H, d, J = 8.2 Hz, ArC(6′)H), 7.31 (1H, dd, J = 2.1 Hz, J = 8.2 Hz, ArC(5′)*H*), 7.50 (1H, d, J = 2.0 Hz, ArC(3′)*H*), 8.23 (2H, d, J = 8.9 Hz, ArC(2)*H* and C(6)*H*), 8.40 (2H, d, J = 8.8 Hz, Arc(3)*H* and C(5)*H*).

2-(2′,4′-Dichlorophenyl)-1,1-ethylenedioxy-1-(4-nitrophenyl)ethane (24)

Compound (**23**) (0.78g, 0.0025 mol.), was reacted with ethylene glycol (10 ml) in the presence of *p*-toluene sulphonic acid (0.2g) in toluene (80 ml) under reflux with a Dean–Stark trap for 24 h. The above work-up, yielded compound (**24**) (0.29g, 32.8%) as a white solid, m.p. 122°C. Found: C, 54.50; H, 3.63; N, 4.04. $C_{16}H_{13}Cl_2NO_4$ requires C, 54.26; H, 3.70; N, 3.95%. ν_{max} (KBr)/cm^{-1} 1584 (C═O), 1519 and 1354 (NO_2). δ_H (300 MHz, $CDCl_3$) 3.29 (2H, s, CH_2), 3.71 (2H, m, CH_2 ketal), 3.87 (2H, m, CH_2 ketal), 7.12 (1H, dd, J = 8.3 Hz, J = 2.1 Hz, Ar C(5′)*H*), 7.24 (1H, d, J = 8.4 Hz, Ar C(6′)*H*), 7.49 (1H, d, J = 2.3 Hz, Ar C(3′)*H*), 7.52 (2H, d, J = 8.8 Hz, Ar C(2)*H* and C(6)–*H*), 8.12 (2H, d, J = 8.8 Hz, Ar C(3)*H* and C(5)*H*).

Biochemistry

[11,12-^{3}H]-RA (1.92 TBq/mmol) and RA were purchased from NEN (Hounslow, UK) and Sigma Chemical Co. (Poole, UK) respectively. Dulbecco's Modified Eagle's medium, ketoconazole and butylated hydroxyanisole were from Sigma. All solvents were HPLC grade and were obtained from Fisher (Leicestershire, UK).

General Assay for Metabolism of RA

Tubes in triplicate, with a total volume of 400 µl containing [11,12-^{3}H]-retinoic acid 10 nM (10 µl of 400 nM stock), unlabelled retinoic acid in methanol (10 µl of 120 µM stock to give 3 µM), inhibitor (8 µl of 5 mM ethanol stock to give 100 µM concentration in final assay volume), phosphate buffer 50 (pH = 7.4, 312 µl), NADPH solution (50 µl of 16 mg/ml) were prepared, and the tubes vortexed and preheated in a water bath for 4 min. The enzymic reaction was initiated by addition of the respective tissue, and the mixture incubated at 37°C for 30 min. The enzyme action was arrested by addition of 100 µl of 1% formic acid and the tubes were placed in ice for 5 min. Then 3 ml of ethyl acetate containing 0.02% butylated hydroxy anisole was added and the tubes vortexed for 10 s. The tubes were then left for 5 min at room temperature and the organic layer (2 ml) was removed from each tube, transferred to another set of tubes and the ethyl acetate extracts evaporated using a Christ centrifuge connected to a vacuum pump and a multitrap at −80°C. After 60 min the tubes were removed and the residue was reconstituted in methanol (100 µl) and 50 µl was injected into a HPLC machine equipped with a 10 µm C18 µBondapak column (Waters), connected to a β-RAM online scintillation detector, connected to a Compaq PC running Laura data acquisition and analysis software (LabLogic Ltd). A Milton–Roy pump was used, at a flow rate of 1.90 ml/min. The mobile phase was acetonitrile/1% ammonium acetate in water/formic acid (75:25:0.1). The scintillation fluid was Optiflow Safe 1 from Fisher.

Metabolites were measured in terms of percentage activity relative to the total radioactivity (i.e. metabolite peak plus retinoic acid peak). Using a control with ethanol instead of inhibitor, these results were expressed as "percentage inhibition relative to control" = 100 − [(% metabolites with inhibitor/% metabolites control) × 100]. Ketoconazole was used as a standard inhibitor. Due to the photosensitivity of retinoic acid all the above assays were carried out in a dark room under yellow light.

Rat Liver Microsomes

These were prepared as described previously[26] and the general assay method was followed using a protein concentration of 0.25 mg ml^{-1}.

Fibroblast Cell Line

Human male genital epidermal fibroblasts were generously donated by Dr. B. Evans, Heath Hospital, Cardiff. The assay method differed from the general method in that the metabolising enzymes were induced by prior incubation of the fibroblasts with RA.

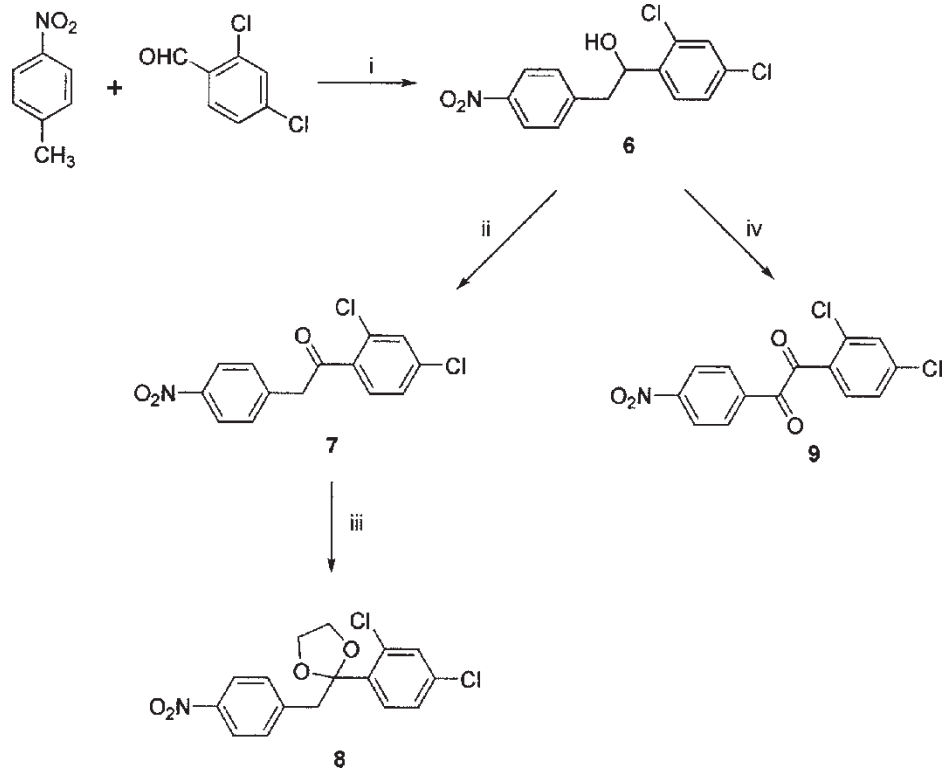
Cold retinoic acid (RA) was added to Dulbecco's Modifield Eagle's medium (DMEM) to a final concentration of 2.4 µM. 500 µl of this RA-medium

was added to each well of the 12 well culture plate containing fibroblasts. The plates were wrapped in aluminium foil and incubated at 37° for 24 h. The RA medium was then removed and replaced with medium (with no added RA) for a further 24 h. The medium in each well was then removed and replaced with 500 μl of DMEM with ^{3}H-RA to a final concentration of 20 nM. 10 μl of test inhibitors (in acetonitrile) at different concentrations were added. Controls on each plate consisted of acetonitrile 10 μl. The plates were foil wrapped and incubated at 37°C for 6 h. 500 μl of 1% formic acid was then added to each well, and the medium was removed into separate tubes. 500 μL distilled water was added to each well and the cells scraped off with a rubber tipped glass rod and the contents added to the appropriate tube. This procedure was repeated with a further 500 μl of water but without scraping. 2 ml of ethyl acetate containing 0.05% butylated hydroxyanisole was added to each tube. After vortexing, the tubes were spun down at 4000 rpm for 5 min. The organic phase was removed to other tubes, evaporated off and the general assay procedure was then followed.

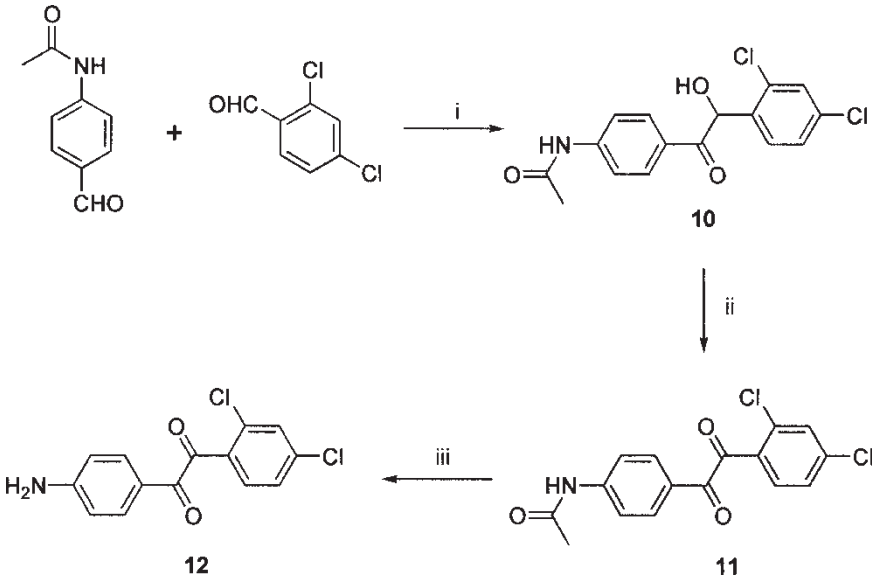
Cytotoxicity of Compounds to Human Genital Fibroblast Cultures

The assay is based on the measurement of incorporation of ^{3}H-leucine into cell protein[28] and is a measure of the biochemical activity of the cells. Lack of radioactivity in the cell layer in the presence of test compound indicates a severely compromised cell and therefore toxicity.

Cells were grown to confluence in 12 well culture plates. 500 μl of fresh, pre-equilibrated medium was added to each well (Dulbecco's Modified Eagle Medium, with 10% foetal bovine serum, 2 mM L-glutamine, 100 U penicillin, 0.1 mg/ml streptomycin, and 1% amphotericin B solution), followed by 10 μL of a known concentration of the compounds in DMSO (20 or 100 μM final concentration) or DMSO alone. The plates were incubated at 37°C for 3 h. 50 μL of ^{3}H-leucine (185 kBq/ml NEN) was then added to each well and the plates were incubated for a further 3 h. The medium was then removed and the monolayer washed (X3) in phosphate buffered saline (PBS) (500 μL). PBS (100 μL) and methanol (100 μL) were then added to each well, removed,



SCHEME 1 i. NaOH, DMF; ii. PCC, CH_2Cl_2; iii. Ethylene glycol, *p*-TsOH, toluene; iv. conc. HNO_3.



SCHEME 2 i. KCN, EtOH/H_2O; ii. $CuSO_4$, H_2O; iii. conc. HCl, MeOH.

and then methanol (300 μL) was added and the cells left for 30 min at room temperature. The methanol was removed and the plates were air-dried. The plates were placed on ice, and the fixed cell monolayer was then washed (×3) in ice-cold trichloracetic acid (10%, 500 μL) for 5 min. After further washing (×3) with methanol (750 μL) the monolayer was air-dried, sodium hydroxide added (100 μl, 1 M) and the cells were left overnight at room temperature to solubilise protein. The sodium hydroxide was transferred to a vial and the well was washed (×2) with water (750 μL), the washings were added to the vial. Optiphase HiSafe III scintillant (2 ml) was added to the vial with vortexing and the ^3H count determined with a Wallac Rackbeta liquid scintillation counter. The % cytotoxicity for each compound was determined from: 100 − [(^3H−leucine count in sample/^3H−leucine count in control) × 100].

RESULTS AND DISCUSSION

The 1,2-diphenyl derivatives were designed as RA-metabolising enzyme inhibitors based on the skeleton of the weak inhibitor 1-cyclohexyl-3-(4-aminophenyl) pyrrolidine-2,5-dione (**3**)[26] where the 4-aminophenyl and aryl rings are separated by 2–3 atoms carrying oxygen atoms. In preliminary work *p*-nitrophenyl-containing compounds were found to be inhibitors of rat liver microsomal enzyme without prior conversion to the P450 haem Fe^{3+}-binding amino group and this function was adopted to avoid difficulties in synthesis of the amino derivative in some instances.

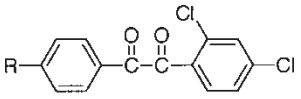
Chemistry

Compound (**6**) was obtained by the condensation of 4-nitrotoluene with 2,4-dichlorobenzaldehyde (Scheme 1) using an excess of the former to push the equilibrium to the right and a catalytic amount of base to reduce the potential for disproportionation of the benzaldehyde (Cannizzaro reaction).

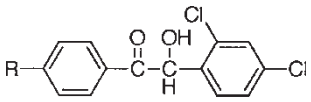
Oxidation of (**6**) with Corey's reagent gave the ketone (**7**) and the benzil (**9**) on oxidation with concentrated nitric acid. The ethylenedioxy derivative (**8**) was obtained from (**7**) by reaction with ethylene glycol and *p*-toluenesulphonic acid.

A benzoin condensation between *p*-acetylaminobenzaldehyde and 2,4-dichlorobenzaldehyde (Scheme 2) using catalytic amounts of potassium cyanide gave the unsymmetrical benzoin (**10**). Protection of the amino group was necessary to prevent condensation to the benzanilide. The mass spectrum showed a major peak at m/e 162 (100%) and a secondary peak at m/e 163 (10%) confirming the structure as (**10**); the isomeric hydroxyketone has theoretical m/e values of 164 (100%) and 165 (10%) respectively. Oxidation of (**10**) with copper sulphate in pyridine (cf Fenton's reagent)

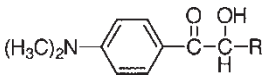
gave the diketone (**11**) which was deprotected in acid to the benzil (**12**).

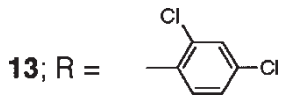


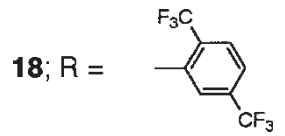
9; R = NO_2
12; R = NH_2
14; R = $N(CH_3)_2$

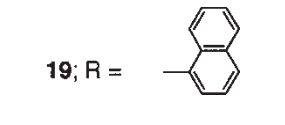


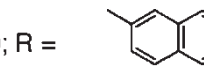
13; R = $(CH_3)_2N$
15; R = $(C_2H_5)_2N$
16; R = $(CH_3(CH_2)_3)_2N$
17; R = cycloC_4H_8-N

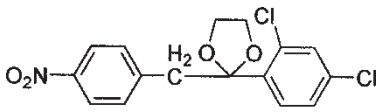












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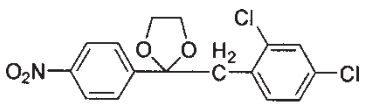

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Similarly, reaction of 4-dimethylaminobenzaldehyde and 2,4-dichlorobenzaldehyde (Scheme 3) gave the benzoin (**13**) subsequently oxidised to the benzil (**14**). Other benzoins, (**15**), (**16**) and (**17**) carrying different substituents (diethyl, dibutyl and cyclobutyl respectively) on the 4-amino group were similarly synthesised from the respective 4-dialkylamino-benzaldehyde. Reaction between 4-dimethylaminobenzaldehyde and other substituted aryl aldehydes (2,5-difluoromethylphenyl, 1-naphthyl and 2-naphthyl) gave the benzoins (**18**), (**19**) and (**20**) respectively.

The isomeric desoxybenzoin to the previously mentioned (**7**), namely (**23**), was prepared by Kröhnke and Vogt's method[29] where the anion of 4-nitrobenzyl pyridinium bromide was reacted to give a single stereoisomer of the styryl salt (**22**) ($\delta_H = 8.12$) which in weak basic media gave (**23**) (Scheme 4). The ethylenedioxy derivative (**24**) was prepared in the same manner from the ketone as the isomeric (**8**).

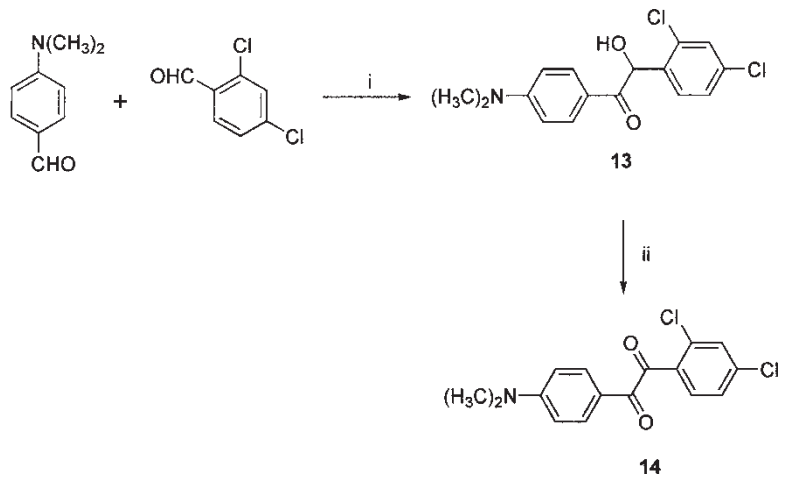
Biochemistry

In the 1,2-diketone series the 4-nitro-, 4-amino- and 4-dimethylaminophenyl derivatives, (**9**), (**12**) and (**14**) were weak inhibitors of rat liver microsomal RA-metabolising enzyme showing (at 100 μM) 56.1, 56.7 and 41.5% inhibition respectively compared with the standard inhibitor ketoconazole (82.0%) (Table I).

In the α-hydroxyketone series potency was not appreciably increased, the 4-dimethylamino phenyl derivatives (**13**) having 54.6% inhibition (Table I). The one stage synthesis of (**13**) from the two relevant aldehydes was successfully applied to the synthesis of analogues modified in either of the aryl rings to increase the hydrophobic nature of the parent compound, (**13**) (Tables I and II). Replacement of the 4-dimethylamino function in (**13**) with diethylamino- (**15**), dibutylamino- (**16**) or cyclobutylamino- (**17**) reduced potency (32.6–39.5% inhibition). Replacement of the 2,4-dichlorophenyl ring in (**13**) with 2,5-trifluoromethylphenyl- (**18**), α-naphthyl- (**19**) and β-naphthyl (**20**) also reduced potency (31.0–45.3%). Taken together these results show that increasing the hydrophobic nature of (**13**) does not increase potency. The ethylenedioxy ethers, (**8**) and (**24**), of the respective monoketones had little inhibitory activity, 19.4 and 15.3% respectively.

Screening of the compounds against the RA-induced enzymes in human male genital fibroblast cultures showed that three of the compounds (**12**), (**13**) and (**24**), at 100 μM, inhibited the induced enzymes by 57.0, 65.9 and 66.1% respectively compared with 75.0% inhibition by ketoconazole at 20 μM.

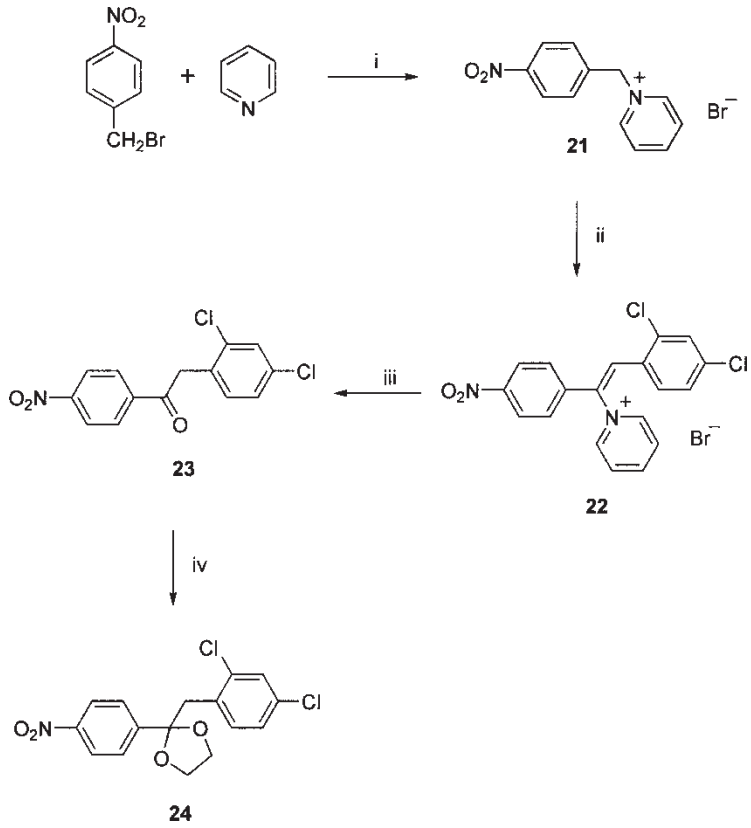
In previous studies we have shown that the pyrrolidinedione (**3**)[26] was a weak inhibitor of rat



SCHEME 3 i. KCN, EtOH/H_2O; ii. PCC, CH_2Cl_2.

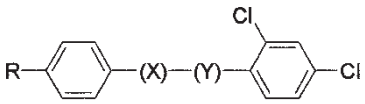
liver and other microsomal RA-metabolising tissues and RA-induced human genital fibroblast and HaCat cultured systems. The tetralone (**5**)[27] was comparable in activity with ketoconazole in microsomal systems and more active in skin homogenates and although showing moderate activity in cultured systems was much less active than ketoconazole. Analysis of the relative activity of (**5**) and ketoconazole for rat liver microsomal enyme compared to that for the fibroblast culture system suggested that the two inhibitors were targetted to a different mix of RA-metabolising enzymes.[27] This may be due to the appearance in RA-induced culture systems of the specific RA-metabolising enzyme CYP26,[15–17] a known RA-inducible enzyme, which is inducible in fibroblasts and HaCat cells.

In this study with 1,2-diarylethandiones, 1,2-diaryl-2-hydroxyethanones and 1,2-diaryl-1-ethanone cyclic ethers weak inhibitory activity was seen against the rat liver RA-metabolisng enzyme but several compounds, (**12**), (**13**) and (**24**) exhibited moderate activity in the RA-induced fibroblast



SCHEME 4 i. Pyridine, CH_2Cl_2; ii. KOAc, AcOH, Ac_2O; iii. Piperidine, pyridine, H_2O; iv. Ethylene glycol, *p*-TsOH, toluene.

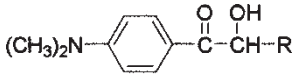
TABLE I Inhibition of RA-metabolising enzymes from rat liver microsomes and RA-induced genital fibroblast cultures by some 1,2-diphenyl ethane derivatives

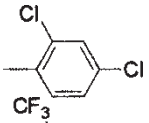
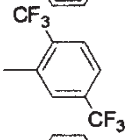
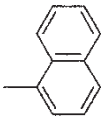
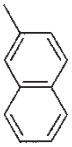


Compound	R	X	Y	Rat liver microsomes %*	Fibroblasts %
9	O_2N			56.1 ± 10.9	−0.6 ± 9.0†
12	H_2N			56.7 ± 12.9	57.0 ± 2.1†
14	$(CH_3)_2N$			41.5 ± 15.7	0.7‡
13	$(CH_3)_2N$			54.6 ± 8.7	65.9 ± 7.2†
15	$(C_2H_5)_2N$			39.0 ± 6.9	6.5‡
16	$(C_4H_9)_2N$			32.6 ± 20.2	4.07‡
17	Cyclo C_4H_8N			39.5 ± 14.8	ND
8	O_2N	$-CH_2-$		19.4	ND
24	O_2N		CH_2	15.3 ± 22.6	66.1 ± 5.3†
Ketoconazole				82.0 ± 8.6	75.0‡

*Compound = 100 μM, RA = 3 μM, Mean ± S.D.; n = 3–4, usually with duplicate determinations. †Compound = 100 μM, RA = 20 nM, n = 1 with triplicates. ‡Compound = 20 μM, RA = 20 nM, n = 1 with duplicates.

TABLE II Inhibition of RA-metabolising enzymes by some analogues of (**13**) where the 2,4-dichlorophenyl group has been modified



Compound	R	Inhibition Rat liver microsomes %*	Fibroblasts %
13		54.6 ± 8.7	65.9 ± 7.2†
18		32.2 ± 9.33	4.49 ± 3.4‡
19		31.0 ± 8.8	ND
20		45.3 ± 15.8	ND
Ketoconazole		82.0 ± 8.6	77.2 ± 6.4‡

*Compound = 100 μM, RA = 3 μM, Mean ± S.D.; n = 3–4, usually with duplicate determinations. †Compound = 100 μM, RA = 20 nM, n = 3 with duplicates. ‡Compound = 20 μM, RA = 20 nM, n = 1 with duplicates.

TABLE III Cytotoxicity of some 1,2-diphenylethane derivates towards human genital fibroblasts

Compound	% Cytotoxicity*
9	75.9 ± 13.0†
12	34.5 ± 24.4†
14	1.8‡
15	81.5‡
16	55.3‡
13	39.3 ± 17.6†
24	31.7 ± 16.4†
18	−16.57
Ketoconazole	79.7 ± 14.8†

*Compound = 100 μM. †n = 5, triplicate determinations. ‡n = 1, duplicate determinations.

cultures although this was at least an order less than that shown by ketoconazole.

The cytotoxicity of the studied compounds on the growth of the fibroblast cultures was determined using the incorporation of ^{3}H-leucine into cell protein as a measure of the biochemical activity of the cell (Table III).[29] At 100 μM the inhibitors (**12**), (**13**) and (**24**) (34.5%, 39.3% and 31.7% cytotoxicity respectively) were less cytotoxic than ketoconazole (79.7%). A direct relationship between cytotoxicity and inhibition was not apparent (Tables II and III). This reduced the possibility that the presence of the compounds decreased cellular growth and enzyme expression compared with controls so introducing a false inhibition effect.

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