Journal of Enzyme Inhibition and Medicinal Chemistry, December 2003 Vol. 18 (6), pp. 511–528

Taylor & Francis
healthsciences

Some Aryl Substituted 2-(4-Nitrophenyl)-4-oxo-4-phenylbutanoates and 3-(4-Nitrophenyl)-1-phenyl-1,4-butanediols and Related Compounds as Inhibitors of Rat Liver Microsomal Retinoic Acid Metabolising Enzymes

PETER MASON, VALERIE P. GREER, ANDREW J. KIRBY, CLAIRE SIMONS, PAUL J. NICHOLLS and H. JOHN SMITH*

Welsh School of Pharmacy, Cardiff University, Cathays Park, Cardiff, CF10 3XF, UK.

(Received 27 June 2003; In final form 11 August 2003)

Some aryl substituted methyl 2-(4-nitrophenyl)-4-oxo-4-phenylbutanoates generally had poor to moderate inhibitory potency (4–73%) towards rat liver microsomal retinoic acid metabolising enzymes compared with ketoconazole (80%). Conversion to the corresponding 3-(4-nitrophenyl)-1-aryl-1,4-butanediols considerably increased potency (29–78%). The 4-iodophenyl analogue, (30) and the 4-iodo- (45) and 4-methoxyphenyl (46) analogues, were the most potent in both series respectively. The corresponding 5-membered lactones, in the three instances examined, were also potent (52%, 67%, 69%) as were the *cis*- and *trans*-isomers of the 5-membered tetrahydrofuran (77%, 65% respectively). Beckmann rearrangement of the oxime methyl 4-(2,4-dichlorophenyl)-4-hydroxyimino-2-(4-nitrophenyl)butanoate (54) gave the expected products (55) and (56), which were potent inhibitors (75%, 74% respectively) of the enzyme whereas the oxime was an activator.

Keywords: Retinoic acid metabolising enzymes; Retinoic acid; Inhibitors; RA; RAMBAs

INTRODUCTION

All-trans-retinoic acid (RA) is a naturally occurring retinoid responsible for growth and differentiation of mammalian epithelial tissues[1] and exerts activity by binding to transcription-regulatory factors in the cell nucleus known as RAR (retinoic acid receptor) and RXR (retinoid X receptor), each having subtypes α, β and δ.[2] Retinoic acid has been used in a number of clinical situations, especially oncology and dermatology. In oncology, RA has shown spectacular success in the treatment of acute promyelocytic leukaemia,[3–5] although any remission seen is followed by relapse within 4–6 months; this appears to be due to increased RA-metabolism as a result of RA-induction so leading to decreased clinical efficacy. RA may improve the efficacy of other treatments such as radiation, cisplatin and interferon therapies.[6,7] Retinoids have been used for some time in the treatment of psoriasis, cystic acne, cutaneous malignancies due to hyperkeratinisation as well as in the treatment of photo-damaged skin.[8–10]

Vitamin A (retinol) is oxidised through retinal by dehydrogenases in the cytoplasm of target cells in low yields to all-trans-retinoic acid, which is at least 100 fold more active than retinol and accounts for its biological action. RA has a short half life (ca. 1 h) and its potency is reduced when it is administered systemically, due to metabolism by human liver and intestine cytochrome P450s to the inactive 4-hydroxy-RA and thence by dehydrogenases to the partially active 4-keto-RA and inactive polar metabolites.[11]

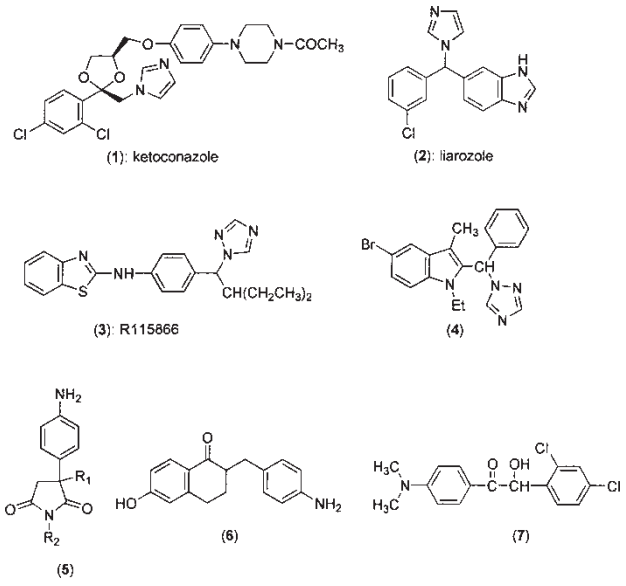
The specific P450s (P450-RA) responsible for 4-hydroxylation of RA in the human liver are CYP2C8[12] as a major contributor as well as 3A7, 3A5, 3A4, 2C9 and 1A1.[13,14] Several CYP isozymes from different rat cadaverous tissues have been shown *in vitro* to be capable of metabolising RA via 4-hydroxylation[15] with RA metabolism by rat liver microsomes being mainly by the 1A1/2,2A6 and 3A4 forms. However, in living tissues, RA administration induces another RA-metabolising enzyme,

*Corresponding author. Tel.: +44-2920-845830. Fax: +44-2920-874149. E-mail: smithhj1@cardiff.ac.uk

ISSN 1475-6366 print/ISSN 1475-6374 online © 2003 Taylor & Francis Ltd
DOI: 10.1080/1475636032000141881

CYP26[16–18] which recognises only RA as its substrate, and the expression of this isozyme can be induced by RA both *in vitro* and *in vivo*.[19]

A drug which can prolong and intensify the action of endogenous RA on epidermal cells by inhibiting P450-RA metabolising enzymes would have potential as a clinical agent in the treatment of certain skin conditions and as an anti-cancer agent. The imidazoles, ketoconazole (**1**) and liarozole (**2**), were reported as inhibitors of RA-metabolising enzymes whilst being studied as inhibitors of 17α-hydroxylase: 17,20-lyase (P450 17α) as agents for the treatment of androgen-dependent prostatic cancer by lowering testosterone levels.[11] Ketoconazole is not a suitable oral agent as an RA-mimetic for sex hormone-independent cancers since it inhibits several other P450 enzymes on the steroidogenic pathway of androgen synthesis and, furthermore, has a poor pharmacokinetic profile. Liarozole (Liazal®) inhibits testicular (but not adrenal) P450 17 and is a potent inhibitor of aromatase ($P450_{AROM}$), both these targets negate its potential as an oral RA-mimetic for sex hormone-independent cancers despite its effectiveness in clinical trials (oral administration) in psoriasis,[20,21] as well as icthyosis and hormone resistant prostate cancer.[22–26] Fluconazole, a triazole antifungal, can reverse the decline in RA plasma levels in leukaemia patients.[27]



The triazole R115866 (**3**) has been described as a novel inhibitor of CYP26 which, *in vivo* in rats after a single oral dose, increases endogenous tissue RA levels and mimics RA in several other of its biological actions.[19] However, it is not clear that oral administration of R115866 producing skin effects is necessarily a direct effect of RA-induction of CYP26 and its subsequent inhibition in skin since recent work with cultured epidermal keratinocytes suggests that CYP26 is not RA-induced in this tissue.[28] However, R115866 has shown beneficial effects when administered topically to skin.[29] Perhaps systemic absorption of this highly potent compound ($IC_{50} = 4\,nM$) with plasma RA distribution from internal tissues accounts for the beneficial skin effects observed with R115866.[19]

Some 3-azoylmethyl-1*H*-indoles and 2, 3 or 5-(α-azolylbenzyl)-1*H*-indoles have recently been described as inhibitors of rat liver microsomal RA-metabolising enzymes; **4** was the most potent and comparable in activity with ketoconazole.[30] We have recently described some substituted 3-(4-aminophenyl)pyrrolidine-2,5-diones (**5**) as inhibitors of rat liver microsomal RA-metabolism[31] and the *N*-cyclohexyl analogue (**5**) in other cadaverous systems (pig brain, human placenta and human liver microsomes; rat and human skin homogenates) as well as RA-induced cell cultures (human male genital fibroblasts and HaCat cells).[31] In general (**5**) was much less active than ketoconazole in the cadaverous *in vitro* systems and very weakly active in the cell culture systems where ketoconazole was a potent inhibitor. A further phenylamine, compound (**6**) based on the tetralin structure, as an RA-metabolising enzyme inhibitor in these test systems was more potent than or equipotent with ketoconazole in the cadaverous systems but less active towards RA-induced cell culture systems.[32] Examination of the data suggests that RA-induction generates metabolising enzymes not present in the cadaverous systems, which were more susceptible to inhibition by ketoconazole than (**6**). We have further examined some 1,2-ethandiones, 2-hydroxyethanones and 1-ethylenedioxyethanones based on aryl-substituted 1,2-diphenylethane.[33] The 2-hydroxy ethanone (**7**) with a 1-(4-dimethylaminophenyl) substituent was overall the most potent compound for rat liver microsomal enzyme ($IC_{50} = 52.1\,\mu M$; ketoconazole, $2.8\,\mu M$) and the RA-induced enzyme ($100\,\mu M$, 66% inhibition; ketoconazole, $20\,\mu M$, 75%).

Here we describe the synthesis and testing of some aryl substituted 1,2-diphenylpropanes *e.g.* 2-(4-nitrophenyl)-4-oxo-4-phenylbutanoates and 3-(4-nitrophenyl)-1-phenyl-1,4-butanediols as inhibitors of rat microsomal RA-metabolising enzymes.

MATERIALS

^1H and ^{13}C NMR spectra were recorded with a Brucker Avance DPX300 spectrometer operating at 300 and 75 MHz respectively, in $CDCl_3$, unless stated otherwise, with tetramethylsilane (TMS) as internal standard. Chemical shifts are expressed in parts per million downfield from TMS, all coupling constants, J, are reported in Hz. Nuclear Overhauser enhancements were measured by the difference

method using standard Brucker software. A relaxation delay of 30 ms followed a low intensity presaturation pulse of 50 ms and a shorter delay of 3 μs was applied before each acquisition. A sequence of 10 acquisitions irradiated at a nearby blank position was repeated 50 times. The summed irradiation and blank free induction decays were subtracted and the result transformed after application of a line broadening factor of 0.30 Hz. Irradiation power level was 67 dB. High resolution Mass spectroscopy was performed at the EPSRC Mass Spectroscopy Centre, Swansea University under CI or EI conditions. Flash column chromatography was performed with Acros silica gel (pore size 0.035–0.07 nm) and TLC was carried out using Merck silica gel 60 F_{254} pre-coated aluminium plates, which were visualised with UV light or iodine. Melting points were measured with a Gallenkamp Melting Point Apparatus and are reported uncorrected. IR spectra were obtained as KBr disks, as solids via a diffuse reflectance accessory using a KBr matrix, or between NaCl plates using a Perkin Elmer 1600 series FTIR.

All reagents and solvents were general purpose grade, where necessary reactions requiring anhydrous conditions were performed in flame or oven dried apparatus under a nitrogen apparatus.

A Milton Roy LC system was used consisting of a Model 3000 Constametric pump, a Rheodyne injection unit and a model 3100 variable wavelength spectromonitor. A model CL-4100 computing integrator was used to process the data. The HPLC column used was an amylose coated silica gel column (Chiralpak AD; 0.46 cm I.D. × 5 cm, Diacel Chemical Ltd.) using a precolumn (0.46 cm I.D. × 5 cm) both packed with identical material [amylose tris (3,5-dimethyl phenyl carbamate)]. Injection on the column was achieved using a Hamilton syringe (50 μL) into a Rheodyne 20 μL loop.

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

METHODS

Chemistry

2-(4′-Nitrophenyl)butan-1,4-dioic Acid

Phenylsuccinic acid (61.9 g, 0.32 mol) was added portionwise to fuming nitric acid (250 mL) at −40°C over a period of 2.5 h. The mixture was stirred for a further 30 min, then added to ice/water (500 mL) to give a white precipitate. The solid was filtered, washed with ice/water and dried to give the butan-1,4-dioic acid as a white solid (64.2 g, 84%). m.p. 214–216°C. υmax KBr/cm^{-1} 2921 and 2858 (COOH), 1711 (C═O), 1606 (Ph-H), 1524 and 1349 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.2 (d, J = 9 Hz,2H, Ph-*H*), 7.6 (d, J = 9 Hz, 2H, Ph-*H*), 4.2 (dd, J_{XB} = 9 Hz, J_{XA} = 6 Hz, 1H, C*H*$_x$CH$_A$H$_B$), 3.0 (dd, J_{BA} = 17 Hz, J_{BX} = 9 Hz, 1H, CH$_x$CH$_A$*H*$_B$), 2.7 (dd, J_{AB} = 17 Hz, J_{AX} = 6 Hz, 1H, CH$_x$C*H*$_A$H$_B$).

2-Bromo-1-phenyl-1-ethanone Derivatives 8–19

2-Bromo-1-phenyl-1-ethanone **8**

To a solution of acetophenone (1.0 g, 8.3 mmol) in diethyl ether (50 mL), was added a catalytic amount of aluminium chloride (0.1 g), and the mixture stirred at 0°C. Bromine (1.33 g, 0.4 mL, 8.3 mmol) was added dropwise to the solution over a period of 20 min and the solution stirred for a further 30 min. The reaction mixture was quenched with water (10 mL) and stirred for 10 min. The water layer was separated from the ether and the organic layer dried ($MgSO_4$), filtered and evaporated to give the phenyl-1-ethanone as white crystals. Recrystallised (CH_3OH) (1.33 g, 81%). m.p. 46.5–48.7°C (lit. 49–51°C[1]). υmax KBr/cm^{-1} 1701 (C═O). ^{1}H NMR δ 8.05 (d, J = 7.1 Hz, 2H, 3,5-Ph-*H*), 7.66 (dt, J = 7.4 and 1.1 Hz, 1H, 2,6-Ph-*H*), 7.55 (t, J = 7.3 Hz, 2H, Ph-*H*), 4.52 (s, 2H, C*H*$_2$).

The following analogues of **8** were prepared using the same general method detailed above. All had satisfactory IR and ^{1}H NMR data.

2-Bromo-1-(2-chlorophenyl)-1-ethanone **9**

With 2-chloroacetophenone a dark red oil was obtained. Purification by column chromatography on silica gel with CH_2Cl_2:petroleum ether 4:1 v/v gave a yellow oil, (62%).

2-Bromo-1-(3-chlorophenyl)-1-ethanone **10**

With 3-chloroacetophenone a yellow solid was obtained (68%). m.p. 35.6–37.1°C.

2-Bromo-1-(4-chlorophenyl)-1-ethanone **11**

With 4-chloroacetophenone a white solid was obtained (71%). m.p. 93.7–94.2°C.

2-Bromo-1-(2,4-dichlorophenyl)-1-ethanone **12**

With 2,4-dichloroacetophenone a yellow oil was obtained. Purification by column chromatography on silica gel with CH_2Cl_2:petroleum ether 4:1 v/v gave a clear oil, (82%). R_f = 0.62.

2-Bromo-1-(2,5-dichlorophenyl)-1-ethanone **13**

With 2,5-dichloroacetophenone green crystals were obtained. Recrystallised (CH_3OH) (80%). m.p. 34.0–35.1°C.

2-Bromo-1-(3,4-dichlorophenyl)-1-ethanone **14**

With 3,4-dichloroacetophenone green crystals were obtained. Recrystallised (Et_2O) (75%). m.p. 51.7–52.6°C.

2-Bromo-1-(4-ethylphenyl)-1-ethanone **15**

With 4-ethylacetophenone and recrystallisation from CH_3OH gave brown crystals (80%).

2-Bromo-1-(4-fluorophenyl)-1-ethanone **16**

With 4-fluoroacetophenone dark red crystals were obtained. Recrystallised (CH_3OH) (49%). m.p. 46.7–47.8°C.

2-Bromo-1-(4-iodophenyl)-1-ethanone **17**

With 4-iodoacetophenone red crystals were obtained. Recrystallised (CH_3OH) (76%). m.p. 110.5–111.0°C.

2-Bromo-1-(4-methoxyphenyl)-1-ethanone **18**

With 4-methoxyacetophenone and recrystallisation from CH_3OH gave brown crystals (69%).

2-Bromo-1-(4-methylphenyl)-1-ethanone **19**

With 4-methylacetophenone green crystals were obtained. Recrystallised (Et_2O) (67%). m.p. 45.3–47.1°C.

Methyl 2-(4-nitrophenyl)-4-oxo-4-phenylbutanoate Derivatives 20–33

Methyl 2-(4-nitrophenyl)-4-oxo-4-phenylbutanoate **20**

Anhydrous potassium carbonate (5.53 g, 40 mmol) in dry acetone (100 mL) was stirred with methyl 4-nitrophenyl acetate (1.95 g, 9.9 mmol) for 30 min at reflux and 2-bromo-1-phenyl-1-ethanone (**5**) (2.0 g, 10.1 mmol) added dropwise over a period of 30 min. The solution was refluxed for a further 3 h, after which time the resulting dark brown mixture was filtered. The filtrate was concentrated under reduced pressure to leave a dark brown oil which crystallised when mixed with diethyl ether. The crystals were triturated with diethyl ether and filtered to give the 4-phenylbutanoate as pale green crystals (2.54 g, 72%). m.p. 165.1–165.9°C. υmax KBr disc/cm^{-1} 1728 (CO_2CH_3), 1681 (C═O), 1519 and 1347 (NO_2). ^{1}H NMR (Acetone-d_6) δ 8.18 (d, J = 8.9 Hz, 2H, 3″-Ph-*H*), 8.0 (d, J = 7.1 Hz, 2H, 2′, 6′-h-*H*), 7.67 (d, J = 8.8 Hz, 2H, 2″-Ph-*H*), 7.59 (t, J = 7.2 Hz, 1H, 4′-Ph-*H*), 7.46 (t, J = 7.6 Hz, 2H, 3′,5′-Ph-*H*), 4.40 (dd, J_{XA} = 4.3 Hz, J_{XB} = 10.1 Hz, 1H, CH_xCH_AH$_B$), 3.98 (dd, J_{BX} = 10.1 Hz, J_{BA} = 18.3 Hz,1H, CH$_x$CH$_A$-*H*$_B$), 3.61 (s, 3H, CH_3), 3.48 (dd, J_{AX} = 4.3 Hz, J_{AB} = 18.2 Hz, 1H, CH$_x$C*H*$_A$H$_B$). ^{13}C NMR (Acetone-d_6) δ 196.15 (C═O, C-4), 172.99 (C═O, Ac), 147.80, 145.81, 135.13 and 132.48 (Ph-C), 132.48, 130.00, 129.39, 129.32, 124.54 and 122.67 (CH, Ph-H), 53.21 (CH, C-2), 46.53 (CH_3, Ac), 42.51 (CH_2, C-3). Found: C, 53.59; H, 3.44; N, 3.57. $C_{17}H_{15}NO_5$ (313.0950) requires: C, 53.42; H, 3.42; N, 3.66%.

The following analogues of **20** were prepared using the same general method detailed above.

Methyl 4-(4-bromophenyl)-2-(4-nitrophenyl)-4-oxobutanoate **21**

With 2-bromo-1-(4-bromophenyl)-1-ethanone a yellow solid was formed (65%). m.p. 113.9–115.3°C. υmax KBr disc/cm^{-1} 1726 (CO_2CH_3), 1672 (C═O), 1516 and 1346 (NO_2). ^{1}H NMR δ 8.23 (d, J = 7.9 Hz, 2H, 3″-Ph-*H*), 7.85 (d, J = 7.7 Hz, 2H, 2′,6′-Ph-*H*), 7.65 (d, J = 7.8 Hz, 2H, 3′,5′-Ph-*H*), 7.57 (d, J = 8.2 Hz, 2H, 2″-Ph-*H*), 4.48 (dd, J_{XA} = 4.6 Hz, J_{XB} = 9.4 Hz, 1H, CH_xCH$_A$H$_B$), 3.95 (dd, J_{BX} = 9.4 Hz, J_{BA} = 18.0 Hz, 1H, CH$_x$CH$_A$*H*$_B$), 3.75 (s, 3H, CH_3), 3.34 (dd, J_{AX} = 4.6 Hz, J_{AB} = 18.0 Hz, 1H, CH$_x$C*H*$_A$H$_B$). ^{13}C NMR δ 196.15 (C═O, C-4), 172.99 (C═O, Ac), 160.98, 147.80, 145.81, and 135.13 (Ph-C), 132.48, 130.00, 129.39 and 124.54 (CH, Ph-H), 53.21 (CH, C-2), 46.53 (CH_3, Ac), 42.51 (CH_2, C-3). Found: C, 52.27; H, 3.71; N, 3.39. $C_{17}H_{14}BrNO_5$ (392.2053) requires: C, 52.06; H, 3.59; N, 3.57%.

Methyl 4-(2-chlorophenyl)-2-(4-nitrophenyl)-4-oxobutanoate **22**

With 2-bromo-1-(2-chlorophenyl)-1-ethanone, yellow crystals were obtained (22%). m.p. 85.4–86.2°C. υmax KBr disc/cm^{-1} 1727 (CO_2CH_3), 1694 (C═O), 1514 and 1344 (NO_2). ^{1}H NMR δ 8.22 (d, J = 8.7 Hz, 2H, 3″-Ph-*H*), 7.57 (d, J = 7.4 Hz, 1H, 6′-Ph-*H*), 7.55 (d, J = 8.8 Hz, 2H, 2″-Ph-*H*), 7.45 (d, J = 7.3 Hz, 2H, 3′,5′-Ph-*H*), 7.33 (m, 1H, 4′-Ph-*H*), 4.48 (dd, J_{XA} = 4.9 Hz, J_{XB} = 9.5 Hz, 1H, CH_xCH$_A$H$_B$), 3.94 (dd, J_{BX} = 9.5 Hz, J_{BA} = 18.1 Hz, 1H, CH$_x$CH$_A$-*H*$_B$), 3.75 (s, 3H, CH_3), 3.40 (dd, J_{AX} = 4.9 Hz, J_{AB} = 18.1 Hz, 1H, CH$_x$C*H*$_A$H$_B$). ^{13}C NMR δ 199.88 (C═O, C-4), 172.83 (C═O, Ac), 147.79, 145.61, 138.42 and 131.56 (Ph-C), 132.78, 131.16, 129.83, 129.45, 127.50 and 124.50 (CH, Ph-H), 53.20 (CH, C-2), 46.89 (CH_3, Ac), 46.47 (CH_2, C-3). Found: C, 58.59; H, 3.94; N, 3.87. $C_{17}H_{14}ClNO_5$ (347.7543) requires: C, 58.71; H, 4.06; N, 4.03%.

Methyl 4-(3-chlorophenyl)-2-(4-nitrophenyl)-4-oxobutanoate **23**

With 2-bromo-1-(3-chlorophenyl)-1-ethanone, yellow crystals were obtained (41%). m.p. 117.3–118.2°C. υmax KBr disc/cm^{-1} 1737 (CO_2CH_3), 1680 (C═O), 1518 and 1351 (NO_2). ^{1}H NMR δ 8.23 (d, J = 8.4 Hz,2H, 3″-Ph-*H*), 7.94 (s, 1H, 2′-Ph-*H*), 7.85 (d, J = 7.4 Hz, 1H, 6′-Ph-*H*), 7.57 (d, J = 8.1 Hz, 3H, 2″- and 4′-Ph-*H*), 7.43 (t, J = 7.7 Hz, 1H, 5′-Ph-*H*), 4.44 (dd, J_{XA} = 4.5 Hz, J_{XB} = 9.1 Hz, 1H, CH_xCH$_A$H$_B$),3.97 (dd, J_{BX} = 9.4 Hz, J_{BA} = 18.0 Hz, 1H, CH$_x$CH$_A$*H*$_B$), 3.73 (s, 3H, CH_3), 3.35 (dd, J_{AX} = 4.5 Hz, J_{AB} = 18.1 Hz, 1H, CH$_x$C*H*$_A$H$_B$). ^{13}C NMR δ 195.95 (C═O, C-4), 172.94 (C═O, Ac), 147.78, 145.76, 137.90 and 135.45 (Ph-C), 133.95, 130.53, 129.34, 128.56, 126.60 and 124.52

(CH, Ph-H), 53.12 (CH, C-2), 42.45 (CH_3, Ac), 42.62 (CH_2, C-3). Found: C, 58.62; H, 4.00; N, 4.16. $C_{17}H_{14}ClNO_5$ (347.7543) requires: C, 58.71; H, 4.06; N, 4.03%.

Methyl 4-(4-chlorophenyl)-2-(4-nitrophenyl)-4-oxobutanoate **24**

With 2-bromo-1-(4-chlorophenyl)-1-ethanone, yellow crystals were obtained (56%). m.p. 107.5–108.6°C. υmax KBr disc/cm^{-1} 1726 (CO_2CH_3), 1673 (C═O), 1517 and 1346 (NO_2). ^{1}H NMR δ 8.22 (d, J = 8.6 Hz, 2H, 3″-Ph-*H*), 8.06 (d, J = 1.8 Hz, 1H, 2′-Ph-*H*), 7.82 (dd, J = 1.8, 8.3 Hz, 1H, 6′-Ph-*H*), 7.57 (d, J = 1.8 Hz, 2H, 2″-Ph-*H*), 7.57 (d, J = 8.8 Hz, 2H, 2″-Ph-*H*), 4.45 (dd, J_{XA} = 4.6 Hz, J_{XB} = 9.5 Hz, 1H, CH_xCH_AH$_B$), 3.95 (dd, J_{BX} = 9.6 Hz, J_{BA} = 18.1 Hz, 1H, CH$_x$C$H_A$$H_B$), 3.75 (s, 3H, CH_3), 3.33 (dd, J_{AX} = 4.7 Hz, J_{AB} = 18.1 Hz, 1H, CH$_x$CH_AH$_B$). ^{13}C NMR δ 195.94 (C═O, C-4), 173.00 (C═O, Ac), 147.80, 145.84, 140.58 and 134.74 (Ph-C), 129.92, 129.48, 129.38, and 124.52 (CH, Ph-H), 53.19 (CH, C-2), 46.58 (CH_3, Ac), 42.53 (CH_2, C-3). Found: C, 58.59; H, 4.00; N, 4.15. $C_{17}H_{14}ClNO_5$ (347.7543) requires: C, 58.71; H, 4.06; N, 4.03%.

Methyl 4-(2,4-dichlorophenyl)-2-(4-nitrophenyl)-4-oxobutanoate **25**

With 2-bromo-1-(2,4-dichlorophenyl)-1-ethanone pale yellow crystals were obtained (60%). m.p. 118.3–118.8°C. υmax KBr disc/cm^{-1} 1728 (CO_2CH_3), 1691 (C═O), 1519 and 1347 (NO_2). ^{1}H NMR δ 8.20 (d, J = 8.7 Hz, 2H, 3″-Ph-*H*), 7.55 (d, J = 8.4 Hz,1H, 6′-Ph-*H*), 7.50 (d, J = 8.8 Hz, 2H, 2″-Ph-*H*), 7.45 (d, J = 1.9 Hz, 1H, 3′-Ph-*H*), 7.33 (dd, J = 8.3 Hz, 1.9 Hz, 1H, 5′-Ph-*H*), 4.44 (dd, J_{XA} = 4.8 Hz, J_{XB} = 9.7 Hz, 1H, CH_xCH_AH$_B$), 3.86 (dd, J_{BX} = 9.7 Hz, J_{BA} = 18.1 Hz, 1H, CH$_x$C$H_A$$H_B$), 3.70 (s, 3H, CH_3), 3.32 (dd, J_{AX} = 4.8 Hz, J_{AB} = 18.1 Hz, 1H, CH$_x$CH_AH$_B$). ^{13}C NMR δ 198.59 (C═O, C-4), 172.78 (C═O, Ac), 147.84, 145.43, 138.56, 136.53 and 132.79 (Ph-C), 131.13, 131.01, 129.41, 127.93 and 124.56 (CH, Ph-H), 53.25 (CH, C-2), 46.92 (CH_3, Ac), 42.44 (CH_2, C-3). Found: C, 53.39; H, 3.44; N, 3.57. $C_{17}H_{13}Cl_2NO_5$ (382.1994) requires: C, 53.42; H, 3.42; N, 3.66%.

Methyl 4-(2,5-dichlorophenyl)-2-(4-nitrophenyl)-4-oxobutanoate **26**

With 2-bromo-1-(2,5-dichlorophenyl)-1-ethanone yellow crystals were obtained (32%). m.p. 97.0–97.5°C. υmax KBr diffusion/cm^{-1} 1732 (CO_2CH_3), 1686 (C═O), 1517 and 1347 (NO_2). ^{1}H NMR δ 8.23 (d, J = 8.7 Hz, 2H, 3″-Ph-*H*), 7.54 (d, J = 8.7 Hz, 2H, 2″-Ph-*H*), 7.54 (s, 1H, 6′-Ph-*H*), 7.40 (m, 2H, 3′,4′-Ph-*H*), 4.47 (dd, J_{XA} = 4.7 Hz, J_{XB} = 9.7 Hz, 1H, CH_x-CH$_A$H$_B$), 3.91 (dd, J_{BX} = 9.7 Hz, J_{BA} = 18.3 Hz, 1H, CH$_x$CH$_A$$H_B$), 3.76 (s, 3H, CH_3), 3.35 (dd, J_{AX} = 4.7 Hz, J_{AB} = 18.3 Hz, 1H, CH$_x$CH_AH$_B$). ^{13}C NMR δ 198.61 (C═O, C-4), 172.71 (C═O, Ac), 147.85, 145.33, 139.54, 133.69 and 129.80 (Ph-C), 132.66, 132.37, 129.71, 129.42 and 124.56 (CH, Ph-H), 53.28 (CH, C-2), 46.82 (CH_3, Ac), 42.41 (CH_2, C-3). Found: C, 53.59; H, 3.44; N, 3.57. $C_{17}H_{13}Cl_2NO_5$ (382.1994) requires: C, 53.42; H, 3.42; N, 3.66%.

Methyl 4-(3,4-dichlorophenyl)-2-(4-nitrophenyl)-4-oxobutanoate **27**

With 2-bromo-1-(3,4-dichlorophenyl)-1-ethanone yellow crystals were obtained (47%). m.p. 114.6–115.7°C. υmax KBr diffusion/cm^{-1} 1731 (CO_2CH_3), 1692 (C═O), 1519 and 1347 (NO_2). ^{1}H NMR δ 8.22 (d, J = 8.6 Hz, 2H, 3″-Ph-*H*), 8.06 (d, J = 1.8 Hz, 1H, 2′-Ph-*H*), 7.82 (dd, J = 1.8 Hz, J = 8.3 Hz, 1H, 6′-Ph-*H*), 7.57 (d, J = 7.8 Hz, 1H, 5′-Ph-*H*), 7.57 (d, J = 8.8 Hz, 2H, 2″-Ph-*H*), 4.45 (dd, J_{XA} = 4.6 Hz, J_{XB} = 9.5 Hz, 1H, CH_xCH$_A$H$_B$), 3.95 (dd, J_{BX} = 9.6 Hz, J_{BA} = 18.1 Hz, 1H, CH$_x$CH$_A$$H_B$), 3.75 (s, 3H, CH_3), 3.33 (dd, J_{AX} = 4.7 Hz, J_{AB} = 18.1 Hz, 1H, CH$_x$CH_AH$_B$). ^{13}C NMR δ 195.06 (C═O, C-4), 172.87 (C═O, Ac), 147.80, 145.62, 138.62, 135.92 and 133.83 (Ph-C), 131.23, 130.48, 129.39, 127.53 and 124.54 (CH, Ph-H), 53.24 (CH, C-2), 46.47 (CH_3, Ac), 42.51 (CH_2, C-3). Found: C, 53.59; H, 3.44; N, 3.57. $C_{17}H_{13}Cl_2NO_5$ (382.1994) requires: C, 53.42; H, 3.42; N, 3.66%.

Methyl 4-(4-ethylphenyl)-2-(4-nitrophenyl)-4-oxobutanoate **28**

With 2-bromo-1-(4-ethylphenyl)-1-ethanone yellow crystals were formed (21%). m.p. 88.7–89.5°C. υmax KBr diffusion/cm^{-1} 1731 (CO_2CH_3), 1674 (C═O), 1519 and 1345 (NO_2). ^{1}H NMR δ 8.20 (d, J = 8.4 Hz, 2H, 3″-Ph-*H*), 7.92 (d, J = 6.8 Hz, 2H, 2′,6′-Ph-*H*), 7.56 (d, J = 7.2 Hz, 2H, 2″-Ph-*H*), 7.30 (d, J = 6.3 Hz, 2H, 3′,5′-Ph-*H*), 4.47 (m, 1H, CH_xCH$_A$H$_B$), 3.95 (dd, J_{BX} = 9.1 Hz, J_{BA} = 17.7 Hz, 1H, CH$_x$CH$_A$-H_B), 3.74 (s, 3H, CH_3), 3.38 (dd, J_{AX} = 4.8 Hz, J_{AB} = 18.1 Hz, 1H, CH$_x$CH_AH$_B$), 2.71 (m, 2H, CH_2CH$_3$), 1.26 (t, J = 7.4 Hz, 3H, CH$_2$CH_3). ^{13}C NMR δ 197.01 (C═O, C-4), 173.45 (C═O, Ac), 151.41, 148.00, 146.46 and 134.47 (Ph-C), 129.71, 129.01, 128.92 and 124.42 (CH, Ph-H), 53.39 (CH, C-2), 46.93 (CH_3, Ac), 42.77 (CH_2, C-3), 29.66 (CH_2), 15.88 (CH_3). Found: C, 66.60; H, 5.62; N, 3.97. $C_{19}H_{19}NO_5$ (341.3628) requires: C, 66.85; H, 5.61; N, 4.10%.

Methyl 4-(4-fluorophenyl)-2-(4-nitrophenyl)-4-oxobutanoate **29**

With 2-bromo-1-(4-fluorophenyl)-1-ethanone, yellow crystals were obtained (41%). m.p. 117.5–120.9°C. υmax KBr diffusion/cm^{-1} 1725 (CO_2CH_3), 1680 (C═O), 1519 and 1346 (NO_2). ^{1}H NMR δ 8.23 (d, J = 8.7 Hz, 2H, 3″-Ph-*H*), 8.01 (dd, J_{FH} = 5.9 Hz, J = 8.5 Hz, 2H, 2′,6′-Ph-*H*), 7.58 (d, J = 8.4 Hz, 2H, 2″-Ph-*H*), 7.45 (t, J = 8.5 Hz, 2H, 3′,5′-Ph-*H*), 4.47 (dd, J_{XA} = 4.7 Hz, J_{XB} = 9.5 Hz, 1H, CH_xCH$_A$H$_B$), 3.97 (dd, J_{BX} = 9.5 Hz, J_{BA} = 18.0 Hz, 1H, CH$_x$CH$_A$ H_B), 3.75 (s, 3H, CH_3), 3.35 (dd, J_{AX} = 4.7 Hz, J_{AB} = 18.0 Hz, 1H, CH$_x$CH_AH$_B$). Found: C, 61.80; H, 4.40;

N, 3.99. $C_{17}H_{14}FNO_5$ (331.2997) requires: C, 61.63; H, 4.26; N, 4.23%.

Methyl 4-(4-iodophenyl)-2-(4-nitrophenyl)-4-oxobutanoate **30**

With 2-bromo-1-(4-iodophenyl)-1-ethanone, yellow crystals were obtained (66%). m.p. 159.9–161.9°C. υmax KBr disc/cm^{-1} 1724 (CO_2CH_3), 1693 (C═O), 1519 and 1348 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.15 (d, J = 8.6 Hz, 2H, 3″-Ph-*H*), 7.85 (d, J = 8.3 Hz, 2H, 2′,6′-Ph-*H*), 7.72 (d, J = 8.4 Hz, 2H, 3′,5′-Ph-*H*), 7.63 (d, J = 8.6 Hz, 2H, 2″-Ph-*H*), 4.35 (dd, J_{XA} = 3.9 Hz, J_{XB} = 9.9 Hz, 1H, C*H*$_x$CH$_A$H$_B$), 3.90 (dd, J_{BX} = 10.1 Hz, J_{BA} = 18.4 Hz, 1H, CH$_x$CH$_A$*H*$_B$), 3.56 (s, 3H, CH_3), 3.45 (dd, J_{AX} = 4.0 Hz, J_{AB} = 18.4 Hz, 1H, CH$_x$C*H*$_A$H$_B$). ^{13}C NMR δ 197.77 (C═O, C-4), 173.24 (C═O, Ac), 147.64, 146.66, 135.95 and 133.28 (Ph-C), 138.54, 130.61, 130.29, and 124.59 (CH, Ph-H), 53.14 (CH, C-2), 46.23 (CH_3, Ac), 42.25 (CH_2, C-3). Found: C, 46.13; H, 3.44; N, 3.39. $C_{17}H_{14}INO_5$ (439.2058) requires: C, 46.49; H, 3.21; N, 3.19%.

Methyl 4-(4-methoxyphenyl)-2-(4-nitrophenyl)-4-oxobutanoate **31**

With 2-bromo-1-(4-methoxyphenyl)-1-ethanone, yellow crystals were obtained (43%). m.p. 113.6–114.7°C. υmax KBr diffusion/cm^{-1} 1732 (CO_2CH_3), 1680 (C═O), 1520 and 1344 (NO_2). ^{1}H NMR δ 8.21 (d, J = 8.6 Hz, 2H, 3″-Ph-*H*), 7.95 (d, J = 8.8 Hz, 2H, 2′,6′-Ph-*H*), 7.57 (d, J = 8.6 Hz, 2H, 2″-Ph-*H*), 6.95 (d, J = 8.8 Hz, 2H, 3′,5′-Ph-*H*), 4.46 (dd, J_{XA} = 4.8 Hz, J_{XB} = 9.2 Hz, 1H, C*H*$_x$CH$_A$H$_B$), 3.95 (dd, J_{BX} = 9.4 Hz, J_{BA} = 18.0 Hz, 1H, CH$_x$CH$_A$*H*$_B$), 3.90 (s, 3H, OCH_3), 3.75 (s, 3H, CO_2CH_3), 3.35 (dd, J_{AX} = 4.8 Hz, J_{AB} = 17.8 Hz, 1H, CH$_x$C*H*$_A$H$_B$). ^{13}C NMR δ 195.53 (C═O, C-4), 173.23 (C═O, Ac), 164.29, 147.72, 146.22 and 129.50 (Ph-C), 130.80, 129.41, 124.46, and 124.27 (CH, Ph-H), 55.94 (OCH_3), 53.11 (CH, C-2), 46.69 (CH_3, Ac), 42.24 (CH_2, C-3). Found: C, 62.82; H, 5.07; N, 3.99. $C_{18}H_{17}NO_6$ (343.3354) requires: C, 62.69; H, 4.99; N, 4.08%.

Methyl 4-(4-methylphenyl)-2-(4-nitrophenyl)-4-oxobutanoate **32**

With 2-bromo-1-(4-methylphenyl)-1-ethanone, yellow crystals were obtained (21%). m.p. 95.4–96.8°C. υmax KBr diffusion/cm^{-1} 1730 (CO_2CH_3), 1672 (C═O), 1514 and 1344 (NO_2). ^{1}H NMR δ 8.20 (d, J = 8.6 Hz, 2H, 3″-Ph-*H*), 7.88 (d, J = 8.1 Hz, 2H, 2′,6′-Ph-*H*), 7.56 (d, J = 8.6 Hz, 2H, 2″-Ph-*H*), 7.27 (d, J = 8.0 Hz, 2H, 3′,5′-Ph-*H*), 4.45 (dd, J_{XA} = 4.8 Hz, J_{XB} = 9.3 Hz, 1H, C*H*$_x$CH$_A$H$_B$), 3.95 (dd, J_{BX} = 9.4 Hz, J_{BA} = 18.0 Hz, 1H, CH$_x$CH$_A$*H*$_B$), 3.74 (s, 3H, CH_3), 3.38 (dd, J_{AX} = 4.8 Hz, J_{AB} = 18.0 Hz, 1H, CH$_x$C*H*$_A$H$_B$), 2.42 (s, 3H, Ph-CH_3). ^{13}C NMR δ 196.70 (C═O, C-4), 173.17 (C═O, Ac), 147.71, 146.16, 144.97 and 133.96 (Ph-C), 129.81, 129.42, 128.61, and 124.45 (CH, Ph-H), 53.11 (CH, C-2), 46.63 (CH_3, Ac), 42.46 (CH_2, C-3), 22.10 (CH_3). Found: C, 66.09; H, 5.33; N, 4.02. $C_{18}H_{17}NO_5$ (327.336) requires: C, 66.08; H, 5.24; N, 4.28%.

Methyl 4-(4-nitrophenyl)-2-(4-nitrophenyl)-4-oxobutanoate **33**

With 2-bromo-1-(4-nitrophenyl)-1-ethanone, yellow crystals were obtained (28%). m.p. 121.8–123.7°C. υmax KBr diffusion/cm^{-1} 1730 (CO_2CH_3), 1693 (C═O), 1530 and 1346 (NO_2). ^{1}H NMR δ 8.32 (d, J = 8.8 Hz, 2H, 3′,5′-Ph-*H*), 8.22 (d, J = 8.8 Hz, 2H, 3″-Ph-*H*), 8.16 (d, J = 8.8 Hz, 2H, 2′,6′-Ph-*H*), 7.60 (d, J = 8.8 Hz, 2H, 2″-Ph-*H*), 4.48 (dd, J_{XA} = 4.5 Hz, J_{XB} = 9.6 Hz, 1H, C*H*$_x$CH$_A$H$_B$), 4.05 (dd, J_{BX} = 9.6 Hz, J_{BA} = 18.3 Hz, 1H, CH$_x$CH$_A$*H*$_B$), 3.76 (s, 3H, CH_3), 3.42 (dd, J_{AX} = 4.5 Hz, J_{AB} = 18.3 Hz, 1H, CH$_x$C*H*$_A$H$_B$). ^{13}C NMR δ 195.88 (C═O, C-4), 172.82 (C═O, Ac), 150.94, 147.82, 145.50 and 140.79 (4 × Ph-C), 129.62, 129.40, 124.58, and 124.37 (4 × CH, Ph-H), 53.31 (CH, C-2), 46.48 (CH_3, Ac), 43.04 (CH_2, C-3). Found: C, 56.69; H, 3.74; N, 8.01. $C_{17}H_{14}N_2O_7$ (358.3068) requires: C, 56.98; H, 3.94; N, 7.82%.

Dimethyl 2,3-di(4-nitrophenyl)succinate **34**

This was separated from the reaction mixture of methyl 4-(2,4-dichlorophenyl)-2-(4-nitrophenyl)-4-oxobutanoate by column chromatography on silica, with EtOAc:petroleum ether (3:7 v/v) as eluent to give green crystals (18%). R_f = 0.22. m.p. 150.2–152.7°C. υmax KBr disc/cm^{-1} 1736 (CO_2CH_3), 1525 and 1350 (NO_2). ^{1}H NMR δ 8.02 (dd, J = 8.8 Hz, 4H, 3,5-Ph-*H*), 7.21 (d, J = 8.8 Hz, 4H, 2,6-Ph-*H*), 4.45 (s, 2H, C*H*), 3.74 (s, 6H, C*H*$_3$). ^{13}C NMR δ 172.28 (C═O, Ac), 147.96 and 142.43 (Ph-C), 129.69 and 124.53 (CH, Ph-H), 54.39 (CH, C-2), 53.53 (CH_3, Ac). Found: C, 55.46; H, 4.15; N, 7.08. $C_{18}H_{16}N_2O_8$ (388.333) requires: C, 55.67; H, 4.12; N, 7.21%.

3-(4-Nitrophenyl)-1-phenyl-1,4-butanediol Derivatives 35–48

3-(4-Nitrophenyl)-1-phenyl-1,4-butanediol **35**

To a solution of methyl 2-(4-nitrophenyl)-4-oxo-4-phenylbutanoate (1.01 g, 3.2 mmol) in diglyme (50 mL) was added sodium borohydride (0.5 g, 13 mmol) and the reaction stirred overnight at room temperature. The purple solution was added to ice water (50 mL), ethyl acetate (50 mL) and then acidified (2 M HCl). The organic layer was separated and washed with water (2 × 50 mL), saturated sodium bicarbonate (50 mL), water (50 mL), then dried ($MgSO_4$). The organic solvent was removed to leave a brown oil which crystallised when mixed with diethyl ether to give the phenyl-1,4-butanediol as yellow crystals (0.57 g, 62%). m.p. 142.9–143.9°C. υmax KBr disc/cm^{-1}

3342 and 3269 (OH), 1517 and 1343 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.20 (d, J = 8.7 Hz, 2H, 3″-Ph-*H*), 7.59 (d, J = 8.7 Hz, 2H, 2″-Ph-*H*), 7.25 (m, 5H, Ph-*H*), 5.27 (d, J = 4.5 Hz, 1H, CH-O*H*), 4.78 (t, J = 5.4 Hz, 1H, CH_2OH), 4.18 (m, 1H, CH_cOH), 3.60 (m, 2H, CH_2OH), 3.25 (dt, J = 6.3 Hz, $J_{BX} = J_{BY} = 10.5$ Hz, 1H, CH_2-$CH_BCH_xH_Y$), 2.01 (dt, $J_{XC} = 4.0$ Hz, $J_{XB} = 10.0$ Hz, $J_{XY} = 14.0$ Hz, 1H, CH_BCH_X-H_YCH_C), 1.85 (dt, $J_{YC} = 2.6$ Hz, $J_{YB} = 10.8$ Hz, $J_{YX} = 13.5$ Hz, 1H, $CH_BCH_XH_YCH_C$). ^{13}C NMR (DMSO-d_6) δ 152.93, 147.41 and 146.83 (Ph-C), 130.49, 128.83, 127.48, 126.34 and 124.03 (CH, Ph-H), 70.69 (CH, C-1), 66.62 (CH_2, C-4), 45.96 (CH, C-3), 42.79 (CH_2, C-2). Found: C, 66.77; H, 5.99; N, 4.76. $C_{16}H_{17}NO_4$ (287.3146) requires: C, 66.88; H, 5.96; N, 4.87%.

The following analogues of **35** were prepared using the same general method detailed above.

1-(4-Bromophenyl)-3-(4-nitrophenyl)-1,4-butanediol **36**

With methyl 4-(4-bromophenyl)-2-(4-nitrophenyl)-4-oxobutanoate, a yellow oil was obtained. Column chromatography with EtOAc:petroleum ether (4:1 v/v) as eluent gave green crystals (79%). m.p. 127.2–128.4°C. υmax KBr disc/cm^{-1} 3519 and 3395 (OH), 1510 and 1353 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.20 (d, J = 8.2 Hz, 2H, 3″-Ph-*H*), 7.56 (d, J = 8.4 Hz, 2H, 2″-Ph-*H*), 7.45 (d, J = 8.0 Hz, 2H, 2′,6′-Ph-*H*), 7.20 (d, J = 8.1 Hz, 2H, 3′,5′-Ph-*H*), 5.36 (d, J = 4.5 Hz, 1H, CH-O*H*), 4.79 (t, J = 5.5 Hz, 1H, CH_2OH), 4.18 (m, 1H, CH_cOH), 3.59 (m, 2H, CH_2OH), 3.21 (dt, J = 6.1 Hz, $J_{BX} = J_{BY} = 10.0$ Hz, 1H, CH_2-$CH_BCH_xH_Y$), 1.99 (dt, $J_{XC} = 3.8$ Hz, $J_{XB} = 10.2$ Hz, $J_{XY} = 14.3$ Hz, 1H, $CH_BCH_XH_YCH_C$), 1.85 (dt, $J_{YC} = 2.5$ Hz, $J_{YB} = 10.9$ Hz, $J_{YX} = 14.3$ Hz, 1H, $CH_BCH_XH_YCH_C$). ^{13}C NMR (DMSO-d_6) δ 152.47, 145.83, 145.90 and 145.03 (Ph-C), 130.75, 129.50, 127.70 and 123.10 (CH, Ph-H), 69.19 (CH, C-1), 65.65 (CH_2, C-4), 44.94 (CH, C-3), 41.59 (CH_2, C-2). Found: C, 52.40; H, 4.20; N, 3.59. $C_{16}H_{16}BrNO_4$ (366.2107) requires: C, 52.47; H, 4.40; N, 3.82%.

1-(2-Chlorophenyl)-3-(4-nitrophenyl)-1,4-butanediol **37**

With methyl 4-(2-chlorophenyl)-2-(4-nitrophenyl)-4-oxobutanoate and column chromatography with EtOAc:petroleum ether (3:2 v/v) (30%). m.p. 123.5–124.7°C. υmax KBr disc/cm^{-1} 3276 and 3196 (OH), 1519 and 1353 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.20 (d, J = 8.1 Hz, 2H, 3″-Ph-*H*), 7.61 (d, J = 7.8 Hz, 3H, 2″-Ph-*H*, Ph-*H*), 7.35 (t, J = 7.3 Hz, 1H, Ph-*H*), 7.30 (d, J = 7.6 Hz, 1H, Ph-*H*), 7.23 (t, J = 7.1 Hz, 1H, Ph-*H*), 5.44 (d, J = 4.6 Hz, 1H, CH-O*H*), 4.79 (t, J = 5.1 Hz, 1H, CH_2OH), 4.48 (m, 1H, CH_cOH), 3.59 (t, J = 5.8 Hz, 2H, CH_2OH), 3.32 (m, 1H, CH_2-$CH_BCH_xH_Y$), 1.85 (m, 2H, $CH_BCH_XH_YCH_C$). ^{13}C NMR (DMSO-d_6) δ 152.29, 146.95, 144.81 and 130.97 (Ph-C), 130.62, 129.64, 129.06, 128.15, 128.04 and 123.88 (CH, Ph-H), 67.25 (CH, C-1), 66.61 (CH_2, C-4), 45.95 (CH, C-3), 41.11 (CH_2, C-2). Found: C, 59.59; H, 5.24; N, 4.57. $C_{16}H_{16}ClNO_4$ (321.7597) requires: C, 59.72; H, 5.01; N, 4.35%.

1-(3-Chlorophenyl)-3-(4-nitrophenyl)-1,4-butanediol **38**

With methyl 4-(3-chlorophenyl)-2-(4-nitrophenyl)-4-oxobutanoate yellow crystals were obtained (34%). m.p. 114.4–115.0°C. υmax KBr disc/cm^{-1} 3330 and 3260 (OH), 1514 and 1353 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.17 (d, J = 8.4 Hz, 2H, 3″-Ph-*H*), 7.59 (d, J = 8.5 Hz, 2H, 2″-Ph-*H*), 7.29 (m, 4H, 2′,4′,5′ and 6′-Ph-*H*), 5.42 (d, J = 4.7 Hz, 1H, CH-O*H*), 4.78 (t, J = 5.2 Hz, 1H, CH_2OH), 4.18 (m, 1H, CH_cOH), 3.59 (m, 2H, CH_2OH), 3.23 (m, 1H, CH_2-$CH_BCH_xH_Y$), 1.90 (m, 2H, $CH_BCH_XH_YCH_C$). ^{13}C NMR (DMSO-d_6) δ 152.62, 150.01, 146.85 and 133.67 (Ph-C), 130.73, 130.45, 127.37, 126.63, 125.06 and 124.02 (CH, Ph-H), 70.17 (CH, C-1), 66.63 (CH_2, C-4), 45.90 (CH, C-3), 42.42 (CH_2, C-2). Found: C, 59.59; H, 5.04; N, 4.47. $C_{16}H_{16}ClNO_4$ (321.7597) requires: C, 59.72; H, 5.01; N, 4.35%.

1-(4-Chlorophenyl)-3-(4-nitrophenyl)-1,4-butanediol **39**

With methyl 4-(4-chlorophenyl)-2-(4-nitrophenyl)-4-oxobutanoate a yellow solid was obtained (79%). m.p. 124.8–125.3°C. υmax KBr disc/cm^{-1} 3526 and 3357 (OH), 1507 and 1353 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.17 (d, J = 8.3 Hz, 2H, 3″-Ph-*H*), 7.55 (d, J = 8.3 Hz, 2H, 2″-Ph-*H*), 7.33 (d, J = 8.7 Hz, 2H, 2′,6′-Ph-*H*), 7.26 (d, J = 8.0 Hz, 2H, 3′,5′-Ph-*H*), 5.37 (d, J = 3.4 Hz, 1H, CH-O*H*), 4.79 (t, J = 4.5 Hz, 1H, CH_2OH), 4.19 (m, 1H, CH_cOH), 3.59 (brs, 2H, CH_2OH), 3.21 (m, 1H, CH_2-$CH_BCH_xH_Y$), 2.00 (m, 1H, $CH_BCH_XH_YCH_C$), 1.83 (m, 1H, $CH_BCH_XH_Y$-CH_C). ^{13}C NMR (DMSO-d_6) δ 152.72, 146.83, 146.34 and 131.94 (Ph-C), 130.43, 128.83, 128.23 and 124.02 (CH, Ph-H), 70.08 (CH, C-1), 66.59 (CH_2, C-4), 45.88 (CH, C-3), 42.56 (CH_2, C-2). Found: C, 59.72; H, 5.02; N, 4.49. $C_{16}H_{16}ClNO_4$ (321.7597) requires: C, 59.72; H, 5.01; N, 4.35%.

1-(2,4-Dichlorophenyl)-3-(4-nitrophenyl)-1,4-butanediol **40**

With methyl 4-(2,4-dichlorophenyl)-2-(4-nitrophenyl)-4-oxobutanoate yellow crystals were obtained (86%). m.p. 126.8–128.5°C. υmax KBr disc/cm^{-1} 3469 and 3364 (OH), 1516 and 1347 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.20 (d, J = 8.7 Hz, 2H, 3″-Ph-*H*), 7.60 (d, J = 8.3 Hz, 1H, 6′-Ph-*H*), 7.58 (d, J = 8.7 Hz, 2H, 2″-Ph-*H*), 7.47 (d, J = 2.0 Hz, 1H, 3′-Ph-*H*), 7.42 (dd, J = 2.0 Hz, J = 8.4 Hz, 1H, 5′-Ph-*H*), 5.51 (d, J = 4.7 Hz, 1H, CH-O*H*), 4.78 (t, J = 5.4 Hz, 1H, CH_2OH), 4.42 (m, 1H, CH_cOH), 3.57 (t, J = 5.9 Hz, 2H, CH_2OH), 3.30 (m, 1H, CH_2-$CH_BCH_xH_Y$), 1.84 (m, 2H, $CH_BCH_XH_YCH_C$). ^{13}C NMR (DMSO-d_6) δ 150.00, 144.84 and 141.80 (Ph-C),

128.49, 127.52, 126.91, 126.17 and 121.78 (CH, Ph-H), 64.87 (CH, C-1), 64.39 (CH_2, C-4), 43.74 (CH, C-3), 38.78 (CH_2, C-2). Found: C, 54.12; H, 4.23; N, 4.00. $C_{16}H_{15}Cl_2NO_4$ (356.1988) requires: C, 54.08; H, 4.22; N, 3.94%.

1-(2,5-Dichlorophenyl)-3-(4-nitrophenyl)-1,4-butanediol **41**

With methyl 4-(2,5-dichlorophenyl)-2-(4-nitrophenyl)-4-oxobutanoate a yellow solid was isolated (32%). m.p. 144.2–145.3°C. υmax KBr disc/cm^{-1} 3404 and 3301 (OH), 1517 and 1346 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.23 (d, J = 8.7 Hz, 2H, 3″-Ph-*H*), 7.63 (d, J = 8.9 Hz, 2H, 2″-Ph-*H*), 7.60 (d, J = 3.3 Hz, 1H, 6′-Ph-*H*), 7.38 (d, J = 8.5 Hz, 1H, 3′-Ph-*H*), 7.32 (dd, J = 2.5 Hz, J = 8.5 Hz, 1H, 4′-Ph-*H*), 5.61 (d, J = 4.7 Hz, 1H, CH-O*H*), 4.81 (t, J = 5.4 Hz, 1H, CH_2O*H*), 4.43 (m, 1H, CH_cOH), 3.60 (t, J = 6.2 Hz, 2H, CH_2OH), 3.31 (m, 1H, CH_2-C$H_B$$CH_xH_Y$), 1.87 (m, 2H, CH_BC$H_X$$H_Y$$CH_C$). ^{13}C NMR (DMSO-$d_6$) δ 159.9, 151.5, 146.5, 132.4 and 129.1 (Ph-C), 131.0, 130.1, 128.5, 127.4 and 123.4 (CH, Ph-H), 66.8 (CH, C-1), 66.0 (CH_2, C-4), 45.4 (CH, C-3), 39.0 (CH_2, C-2). Found: C, 54.00; H, 4.27; N, 3.99. $C_{16}H_{15}Cl_2NO_4$ (356.1988) requires: C, 54.08; H, 4.22; N, 3.94%.

1-(3,4-Dichlorophenyl)-3-(4-nitrophenyl)-1,4-butanediol **42**

With methyl 4-(3,4-dichlorophenyl)-2-(4-nitrophenyl)-4-oxobutanoate a yellow solid was isolated (28%). m.p. 126.2–126.9°C. υmax KBr disc/cm^{-1} 3518 and 3362 (OH), 1516 and 1348 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.15 (d, J = 8.3 Hz, 2H, 3″-Ph-*H*), 7.55 (d, J = 8.4 Hz, 2H, 2″-Ph-*H*), 7.50 (d, J = 8.1 Hz, 1H, 5′-Ph-*H*), 7.46 (d, J = 1.3 Hz, 1H, 2′-Ph-*H*), 7.23 (dd, J = 1.1 Hz, J = 8.2 Hz, 1H, 6′-Ph-*H*), 5.47 (d, J = 4.8 Hz, 1H, CH-O*H*), 4.78 (t, J = 5.3 Hz, 1H, CH_2O*H*), 4.18 (m, 1H, CH_cOH), 3.55 (m, 2H, CH_2OH), 3.19 (m, 1H, CH_2-C$H_B$$CH_xH_Y$), 1.93 (m, 2H, CH_BC$H_X$$H_Y$$CH_C$). ^{13}C NMR (DMSO-$d_6$) δ 152.47, 148.52, 146.81, 131.60 and 131.5 (Ph-C), 131.02, 130.46, 128.45, 126.78 and 123.98 (CH, Ph-H), 69.69 (CH, C-1), 66.60 (CH_2, C-4), 45.83 (CH, C-3), 42.13 (CH_2, C-2). Found: C, 53.99; H, 4.07; N, 4.01. $C_{16}H_{15}Cl_2NO_4$ (356.1988) requires: C, 54.08; H, 4.22; N, 3.94%.

1-(4-Ethylphenyl)-3-(4-nitrophenyl)-1,4-butanediol **43**

With methyl 4-(4-ethylphenyl)-2-(4-nitrophenyl)-4-oxobutanoate (50%). m.p. 105.2–106.1°C. υmax KBr disc/cm^{-1} 3582 (OH), 1530 and 1352 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.17 (d, J = 8.0 Hz, 2H, 3″-Ph-*H*), 7.52 (d, J = 8.3 Hz, 2H, 2″-Ph-*H*), 7.11 (d, J = 11.0 Hz, 4H, 2′,3′,5′,6′-Ph-*H*), 5.14 (d, J = 4.4 Hz, 1H, CH-O*H*), 4.73 (t, J = 5.2 Hz, 1H, CH_2O*H*), 4.12 (m, 1H, CH_cOH), 3.55 (m, 2H, CH_2OH), 3.18 (dt, J = 4.1 Hz, J_{BX} = J_{BY} = 10.3 Hz, 1H, CH_2-C$H_B$$CH_xH_Y$), 2.53 (q, 2H, C$H_2$$CH_3$), 1.99 (dt, J_{XC} = 4.5 Hz, J_{XC} = 4.5 Hz, J_{XB} = 13.3 Hz, J_{XY} = 17.75 Hz, 1H, CH_BC$H_X$$CH_Y$$CH_C$), 1.79 (dt, J_{YC} = J_{YB} = 2.8 Hz, J_{YX} = 13.6 Hz, 1H, $CH_B$$CH_X$-$H_Y$$CH_C$), 1.13 (t, 3H, CH_2CH_3). ^{13}C NMR (DMSO-d_6) δ 152.97, 146.78, 144.62 and 142.93 (Ph-C), 130.43, 128.17, 126.37 and 123.99 (CH, Ph-H), 70.56 (CH, C-1), 66.60 (CH_2, C-4), 45.98 (CH, C-3), 41.49 (CH_2, C-2), 28.68 (CH_2), 16.65 (CH_3). Found: C, 58.57; H, 6.74; N, 4.57. $C_{18}H_{21}NO_4$ (315.3682) requires: C, 58.55; H, 6.71; N, 4.44%.

1-(4-Fluorophenyl)-3-(4-nitrophenyl)-1,4-butanediol **44**

With methyl 4-(4-fluorophenyl)-2-(4-nitrophenyl)-4-oxobutanoate (79%). m.p. 88.0–89.4°C. υmax KBr disc/cm^{-1} 3520 and 3397 (OH), 1515 and 1350 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.20 (d, J = 8.2 Hz, 2H, 3″-Ph-*H*), 8.08 (dd, J_{AF} = 5.3 Hz, J_{AB} = 8.8 Hz, 2H, Ph-H_A), 7.56 (d, J = 8.4 Hz, 2H, 2″-Ph-*H*), 7.22 (t, J_{BF} = J_{BA} = 8.6 Hz, 2H, Ph-H_B), 5.35 (d, J = 4.4 Hz, 1H, CH-O*H*), 4.78 (t, J = 5.5 Hz, 1H, CH_2O*H*), 4.16 (m, 1H, CH_cOH), 3.62 (m, 2H, CH_2OH), 3.20 (m, 1H, CH_2-C$H_B$$CH_xH_Y$), 1.99 (dt, J_{XC} = 4.7 Hz, J_{XB} = 13.1 Hz, J_{XY} = 17.7 Hz, 1H, CH_BC$H_X$$H_Y$$CH_C$), 1.79 (m, 1H, $CH_B$$CH_X$$H_Y$-$CH_C$). Found: C, 62.77; H, 5.20; N, 4.33. $C_{16}H_{16}FNO_4$ (305.3051) requires: C, 62.94; H, 5.28; N, 4.59%.

1-(4-Iodophenyl)-3-(4-nitrophenyl)-1,4-butanediol **45**

With methyl 4-(4-iodophenyl)-2-(4-nitrophenyl)-4-oxobutanoate a yellow solid was obtained (40%). m.p. 145.5–147.1°C. υmax KBr disc/cm^{-1} 3526 and 3396 (OH), 1510 and 1353 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.20 (d, J = 8.5 Hz, 2H, 3″-Ph-*H*), 7.65 (d, J = 8.2 Hz, 2H, 2′,6′-Ph-*H*), 7.56 (d, J = 8.5 Hz, 2H, 2″-Ph-*H*), 7.09 (d, J = 8.1 Hz, 2H, 3′,5′-Ph-*H*), 5.36 (d, J = 4.5 Hz, 1H, CH-O*H*), 4.77 (t, J = 5.2 Hz, 1H, CH_2O*H*), 4.17 (m, 1H, CH_cOH), 3.60 (m, 2H, CH_2OH), 3.21 (m, 1H, CH_2-C$H_B$$CH_xH_Y$), 2.00 (dt, J_{XC} = 3.7 Hz, J_{XB} = 11.8 Hz, J_{XY} = 13.6 Hz, 1H, CH_BC$H_X$$H_Y$$CH_C$), 1.85 (t, J_{YC} = J_{YB} = 10.9 Hz, 1H, $CH_B$$CH_X$$H_Y$$CH_C$). ^{13}C NMR (DMSO-$d_6$) δ 152.26, 146.72, 146.36 and 145.9 (Ph-C), 137.09, 129.99, 128.38 and 123.58 (CH, Ph-H), 69.76 (CH, C-1), 66.11 (CH_2, C-4), 45.40 (CH, C-3), 42.02 (CH_2, C-2). Found: C, 46.60; H, 3.74; N, 3.51. $C_{16}H_{16}INO_4$ (413.2112) requires: C, 46.50; H, 3.90; N, 3.39%.

1-(4-Methoxyphenyl)-3-(4-nitrophenyl)-1,4-butanediol **46**

With methyl 4-(4-methoxyphenyl)-2-(4-nitrophenyl)-4-oxobutanoate a yellow solid was obtained (48%). m.p. 103.2–104.4°C. υmax KBr disc/cm^{-1} 3520 and 3371 (OH), 1511 and 1338 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.16 (d, J = 7.4 Hz, 2H, 3″-Ph-*H*), 7.52 (d, J = 8.2 Hz, 2H, 2″-Ph-*H*), 7.12 (d, J = 8.3 Hz, 2H, 2′,6′-Ph-*H*), 6.82 (d, J = 8.5 Hz, 2H, 3′,5′-Ph-*H*), 5.12 (d, J = 4.4 Hz, 1H, CH-O*H*), 4.74 (t, J = 5.0 Hz, 1H, CH_2O*H*), 4.10 (m, 1H, CH_cOH), 3.70 (s, 3H, OCH_3), 3.56 (m, 2H, CH_2OH), 3.17 (m, 1H, CH_2-C$H_B$$CH_xH_Y$), 1.98 (dt, J_{XC} = 3.7 Hz, J_{XB} = 12.1 Hz, J_{XY} = 17.7 Hz,

1H, $CH_BCH_XH_YCH_C$), 1.77 (dt, $J_{YC} = J_{YB} = 9.8$ Hz, $J_{YX} = 14.3$ Hz, 1H, $CH_BCH_XH_YCH_C$). ^{13}C NMR (DMSO-d_6) δ 156.48, 150.63, 144.37 and 143.93 (Ph-C), 128.01, 125.10, 121.59 and 111.77 (CH, Ph-H), 67.83 (CH, C-1), 64.14 (CH_2, C-4), 53.42 (OCH_3), 43.53 (CH, C-3), 40.31 (CH_2, C-2). Found: C, 64.53; H, 6.04; N, 4.44. $C_{17}H_{19}NO_5$ (317.3408) requires: C, 64.34; H, 6.03; N, 4.41%.

1-(4-Methylphenyl)-3-(4-nitrophenyl)-1,4-butanediol **47**

With methyl 4-(4-methylphenyl)-2-(4-nitrophenyl)-4-oxobutanoate (38%). m.p. 125.9–127.2°C. υmax KBr disc/cm^{-1} 3517 and 3358 (OH), 1510 and 1337 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.17 (d, J = 7.1 Hz, 2H, 3″-Ph-*H*), 7.53 (d, J = 7.1 Hz, 2H, 2″-Ph-*H*), 7.11 (d, J = 7.1 Hz, 2H, 2′,6′-Ph-*H*), 7.08 (d, J = 3.6 Hz, 2H, 3′,5′-Ph-*H*), 5.16 (d, J = 3.6 Hz, 1H, CH-O*H*), 4.74 (t, J = 5.1 Hz, 1H, CH_2OH), 4.13 (m, 1H, CH_cOH), 3.58 (m, 2H, CH_2OH), 3.21 (m, 1H, CH_2-$CH_BCH_xH_Y$), 2.24 (s, 3H, CH_3), 1.97 (dt, $J_{XC} = 4.5$ Hz, $J_{XB} = 10.5$ Hz, $J_{XY} = 17.7$ Hz, 1H, $CH_BCH_XH_YCH_C$), 1.80 (t, $J_{YX} = 11.9$ Hz, 1H, $CH_BCH_XH_YCH_C$). ^{13}C NMR (DMSO-d_6) δ 152.43, 146.22, 143.81 and 135.85 (Ph-C), 129.86, 128.79, 125.71 and 123.44 (CH, Ph-H), 69.89 (CH, C-1), 65.99 (CH_2, C-4), 45.35 (CH, C-3), 41.59 (CH_2, C-2), 30.91 (CH_3),. Found: C, 67.70; H, 6.28; N, 4.46. $C_{17}H_{19}NO_4$ (301.3414) requires: C, 67.76; H, 6.35; N, 4.65%.

1-(4-Nitrophenyl)-3-(4-nitrophenyl)-1,4-butanediol **48**

With methyl 4-(4-nitrophenyl)-2-(4-nitrophenyl)-4-oxobutanoate (40%). m.p. 121.8–123.7°C. υmax KBr disc/cm^{-1} 3519 and 3395 (OH), 1510 and 1353 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.20 (d, J = 8.2 Hz, 2H, 3″-Ph-*H*), 8.18 (d, J = 8.2 Hz, 2H, 3′-Ph-*H*), 7.62 (d, J = 8.4 Hz, 2H, 2′-Ph-*H*), 7.45 (d, J = 8.0 Hz, 2H, 2″-Ph-*H*), 5.36 (d, J = 4.5 Hz, 1H, CH-O*H*), 4.79 (t, J = 5.5 Hz, 1H, CH_2OH), 4.18 (m, 1H, CH_cOH), 3.59 (m, 2H, CH_2OH), 3.21 (m, 1H, CH_2-$CH_BCH_xH_Y$), 1.99 (m, 1H, $CH_BCH_XH_YCH_C$), 1.85 (m, 1H, $CH_BCH_XH_YCH_C$). Found: C, 57.89; H, 4.59; N, 8.50. $C_{16}H_{16}N_2O_6$ (332.3122) requires: C, 57.82; H, 4.85; N, 8.43%.

3-(4-Nitrophenyl)-5-phenyltetrahydro-2-furanone Derivatives

Synthesis of 3-(4-nitrophenyl)-5-phenyltetrahydro-2-furanone **49**

Methyl 2-(4-nitrophenyl)-4-oxo-4-phenylbutanoate (1.38 g, 4.40 mmol) in 70% ethanol/water (50 mL) was stirred at room temperature with sodium borohydride (0.2 g, 7.4 mmol) for 8 h. The resulting purple reaction mixture was added to ice water (50 mL) and ethyl acetate (50 mL) and then acidified (2 M HCl). The organic layer was separated and washed with water (2 × 50 mL), saturated sodium bicarbonate (50 mL), water (50 mL), and then dried ($MgSO_4$). The volatile components were removed to leave a brown oil. The final product was purified by column chromatography with ethyl acetate:petroleum ether (3:7 v/v) to give a pale yellow solid (1.06 g, 85%). m.p. 96.2–98.3°C. υmax KBr disc/cm^{-1} 1773 (–CO_2CH–), 1518 and 1347 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.24 (d, J = 8.8 Hz, 2H, 3″-Ph-*H*), 7.56 (d, J = 8.7 Hz, 2H, 2″-Ph-*H*), 7.45 (m, 5H, Ph-*H*), 5.62 (dd, $J_{XA} = 5.45$ Hz, $J_{XB} = 10.7$ Hz, 1H, $CH_XCH_AH_B$), 4.24 (dd, $J_{YA} = 8.3$ Hz, $J_{YB} = 12.8$ Hz, 1H, $CH_AH_BCH_Y$), 3.19 (ddd, $J_{AX} = 5.3$ Hz, $J_{AY} = 8.2$ Hz, $J_{AB} = 12.8$ Hz, 1H, $CH_XCH_AH_BCH_Y$), 2.45 (m, $J_{BX} = 10.7$ Hz, $J_{BY} = 12.8$ Hz, $J_{BA} = 12.8$ Hz, 1H, $CH_XCH_AH_BCH_Y$). ^{13}C NMR (DMSO-d_6) δ 175.59 (C═O, C-2), 147.79, 143.70 and 134.43 (Ph-C), 129.65, 129.38, 127.48, 126.02 and 124.55 (CH, Ph-H), 79.88 (CH, C-3), 47.63 (CH, C-5), 40.32 (CH_2, C-4). Found: C, 67.56; H, 4.78; N, 4.90. $C_{16}H_{13}NO_4$ (283.283) requires: C, 67.83; H, 4.62; N, 4.94%.

The following analogues of **49** were prepared using the same general method detailed above.

5-(4-Bromophenyl)-3-(4-nitrophenyl)tetrahydro-2-furanone **50**

With methyl 4-(4-bromophenyl)-2-(4-nitrophenyl)-4-oxobutanoate yellow crystals were obtained which were triturated with ether to give the tetrahydro-2-furanone as yellow crystals (25%). υmax KBr disc/cm^{-1} 1774 (–CO_2CH–), 1519 and 1350 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.16 (d, J = 8.6 Hz, 2H, 3″-Ph-*H*), 7.49 (t, J = 8.7 Hz, 4H, 2″ and 2′,6′-Ph-*H*), 7.24 (d, J = 8.4 Hz, 2H, 3′,5′-Ph-*H*), 5.46 (dd, $J_{XA} = 5.5$ Hz, $J_{XB} = 10.7$ Hz, 1H, $CH_XCH_AH_B$), 4.15 (dd, $J_{YA} = 8.3$ Hz, $J_{YB} = 12.8$ Hz, 1H, $CH_AH_BCH_Y$), 3.10 (m, $J_{AX} = 5.6$ Hz, $J_{AY} = 7.8$ Hz, $J_{AB} = 12.7$ Hz, 1H, $CH_XCH_AH_BCH_Y$), 2.32 (m, $J_{BX} = 10.7$ Hz, $J_{BY} = 12.8$ Hz, $J_{BA} = 12.7$ Hz, 1H, $CH_XCH_AH_BCH_Y$). ^{13}C NMR (DMSO-d_6) δ 175.22 (C═O, C-2), 147.84, 143.31, 137.44 and 123.36 (Ph-C), 132.54, 129.60, 127.66 and 124.46 (CH, Ph-H), 79.06 (CH, C-3), 47.90 (CH, C-5), 40.19 (CH_2, C-4). Found: C, 53.02; H, 3.22; N, 3.99. $C_{16}H_{12}BrNO_4$ (362.1791) requires: C, 53.06; H, 3.34; N, 3.87%.

5-(2,4-Dichlorophenyl)-3-(4-nitrophenyl)tetrahydro-2-furanone **51**

With methyl 4-(2,4-dichlorophenyl)-2-(4-nitrophenyl)-4-oxobutanoate, a pale yellow oil was obtained. Column chromatography with ethyl acetate:petroleum ether (3:7 v/v) gave pale yellow crystals (48%). m.p. 148.7–153.9°C. υmax KBr disc/cm^{-1} 1780 (–CO_2CH–), 1519 and 1347 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.28 (d, J = 8.7 Hz, 2H, 3″-Ph-*H*), 7.54 (d, J = 8.5 Hz, 1H, 5′-Ph-*H*), 7.53 (d, J = 8.7 Hz, 2H, 2″-Ph-*H*), 7.48 (d, J = 2.0 Hz, 1H, 3′-Ph-*H*), 7.40 (dd, J = 2.0 Hz, J = 8.5 Hz, 1H, 5′-Ph-*H*), 5.62 (dd, $J_{XA} = 5.6$ Hz, $J_{XB} = 10.3$ Hz, 1H, $CH_XCH_AH_B$), 4.24

(dd, $J_{YA} = 8.6$ Hz, $J_{YB} = 12.3$ Hz, 1H, $CH_AH_BCH_Y$), 3.41 (m, $J_{AX} = 5.7$ Hz, $J_{AY} = 8.5$ Hz, $J_{AB} = 12.9$ Hz, 1H, $CH_XCH_AH_BCH_Y$), 2.26 (m, $J_{BX} = 10.3$ Hz, $J_{BY} = 12.3$ Hz, $J_{BA} = 12.9$ Hz,1H, $CH_XCH_AH_BCH_Y$). ^{13}C NMR (DMSO-d_6) δ 174.84 (C═O, C-2), 147.96, 143.03, 135.53, 135.24 and 132.31 (Ph-C), 130.17, 129.50, 128.42, 127.43 and 124.67 (CH, Ph-H), 76.52 (CH, C-3), 47.15 (CH, C-5), 38.81 (CH_2, C-4). Found: C, 54.39; H, 3.32; N, 3.71. $C_{16}H_{11}Cl_2NO_4$ (352.1732) requires: C, 54.56; H, 3.15; N, 3.98%.

4-(4-Nitrophenyl)-2-phenyltetrahydrofuran Derivatives

Synthesis of 4-(4-nitrophenyl)-2-phenyltetrahydrofuran **52**

3-(4-Nitrophenyl)-1-phenyl-1,4-butanediol (1.03 g, 3.58 mmol) was stirred with *p*-toluenesulphonyl chloride (1.0 g, 5.25 mmol) in pyridine (10 mL) for 12 h at room temperature. The mixture was poured into an ice water mixture (30 mL), and extracted with ether (3 × 30 mL). The combined organic extracts were washed with 2 M HCl (3 × 20 mL) and brine (2 × 5 mL). The organic layer was then dried ($MgSO_4$) and concentrated to give a light brown oil. The oil was dissolved in methanol (15 mL), sodium methoxide (0.27 g, 0.3 mL) was added and the mixture was refluxed for 1 h. The cooled reaction mixture was added to ice water (20 mL) and extracted with ether (3 × 30 mL). The combined organic extract phase was dried ($MgSO_4$) and concentrated to give a pale yellow oil (0.83 g, 89%). Diastereoisomers of this compound were separated by column chromatography with ethyl acetate:petroleum ether (1:4 v/v) as eluent.

Fraction 1. Green crystals. m.p. 75.9–77.6°C. υmax KBr disc/cm^{-1} 1518 and 1347 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.28 (d, J = 8.7 Hz, 2H, 3″-Ph-*H*), 7.55 (d, J = 8.7 Hz, 2H, 2″-Ph-*H*), 7.48 (d, J = 4.3 Hz, 4H, 2′,3′,5′,6′-Ph-*H*), 7.39 (m, 1H, 4′-Ph-*H*), 5.35 (t, $J_{YA} = J_{YB} = 6.8$ Hz, 1H, $CH_AH_BCH_Y$), 4.59 (t, $J_{CX} = J_{CD} = 7.3$ Hz, 1H, $CH_XCH_CH_D$), 4.07 (t, $J_{DX} = J_{DC} = 7.3$ Hz, 1H, $CH_XCH_CH_D$), 3.75 (quin, J = 7.3 Hz 1H, $CH_CH_DCH_XCH_AH_B$), 2.55 (m, 2H, $CH_XCH_AH_BCH_Y$). ^{13}C NMR (DMSO-d_6) δ 151.68, 148.30 and 144.54 (Ph-C), 130.08, 129.81, 127.02, 127.03 and 125.38 (CH, Ph-H), 82.09 (CH, C-2), 76.13 (CH_2, C-5), 46.08 (CH, C-4), 44.14 (CH_2, C-3). Found: C, 71.02; H, 5.91; N, 4.91. $C_{16}H_{15}NO_3$ (269.2994) requires: C, 71.35; H, 5.61; N, 5.20%.

Fraction 2. Green crystals. m.p. 52.2–53.5°C. υmax KBr disc/cm^{-1} 1518 and 1347 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.17 (d, J = 8.8 Hz, 2H, 3″-Ph-*H*), 7.44 (d, J = 8.7 Hz, 2H, 2″-Ph-*H*), 7.38 (dd, J = 1.8 Hz, J = 8.7 Hz, 4H, 2′,3′,5′,6′-Ph-*H*), 7.29 (m, 1H, 4′-Ph-*H*), 5.08 (dd, $J_{YA} = 5.9$ Hz, $J_{YB} = 9.9$ Hz, 1H, $CH_AH_BCH_Y$), 4.37 (t, $J_{CX} = J_{CD} = 8.4$ Hz, 1H, $CH_XCH_CH_D$), 4.07 (t, $J_{DX} = J_{DC} = 8.3$ Hz, 1H, $CH_XCH_CH_D$), 3.73 (quin, J = 7.8 Hz 1H, $CH_CH_DCH_XCH_AH_B$), 2.85 (ddd, $J_{AY} = 6.0$ Hz, $J_{AX} = 7.3$ Hz, $J_{AB} = 12.5$ Hz, 1H, $CH_CH_DCH_XCH_AH_BCH_Y$), 1.97 (dd, $J_{BX} = J_{BY} = 9.9$ Hz, $J_{BA} = 12.5$ Hz, 1H, $CH_XCH_AH_BCH_Y$). ^{13}C NMR (DMSO-d_6) δ 150.90, 147.14 and 142.27 (Ph-C), 128.99, 128.56, 128.11, 126.11 and 124.35 (CH, Ph-H), 82.28 (CH, C-2), 75.07 (CH_2, C-5), 46.15 (CH, C-4), 44.19 (CH_2, C-3). Found: C, 71.44; H, 5.79; N, 4.99. $C_{16}H_{15}NO_3$ (269.2994) requires: C, 71.35; H, 5.61; N, 5.20%.

NOE Fraction 1. Selective irradiation of H_6 ($\delta = 5.35$) resulted in an enhancement (5.4%) of half of the multiplet at $\delta = 2.55$ (H_4 or H_5) and, as expected, enhancement (5.9%) of the aromatic protons (δ = 7.39 to 8.28). However, no enhancement of H_3 was observed which would suggest that H_3 and H_6 are on opposite sides of the molecule, that is, fraction 1 contains the enantiomeric *trans* isomers (*RR* and *SS*).

NOE Fraction 2. Selective irradiation of H_6 ($\delta = 5.08$) resulted in an enhancement (4.6%) of H_5 ($\delta = 4.37$), the aromatic signals (δ = 7.28 to 8.14) (5.5%) and importantly H_3 ($\delta = 3.73$) (3.8%). A weak enhancement (1.6%) was also observed for H_1 ($\delta = 2.97$). the enhancement observed for H_3 would indicate that fraction 2 contains the enantiomeric *cis* isomers (*SR* and *RS*).

2-(2,4-Dichlorophenyl)-4-(4-nitrophenyl)tetrahydrofuran **53**

With 1-(2,4-dichlorophenyl)-3-(4-nitrophenyl)- 1,4-butanediol a yellow oil was obtained. Column chromatography with ethyl acetate:petroleum ether (3:17 v/v) as eluent gave a yellow solid (83%). m.p. 75.9–77.6°C. υmax KBr disc/cm^{-1} 1519 and 1347 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.14 (d, J = 8.8 Hz, 2H, 3″-Ph-*H*), 7.59 (d, J = 8.5 Hz, 2H, 6′-Ph-*H*), 7.38 (d, J = 8.6 Hz, 2H, 2″-Ph-*H*), 7.35 (d, J = 2.1 Hz, 1H, 3′-Ph-*H*), 7.28 (dd, J = 2.2 Hz, J = 8.6 Hz, 1H, 5′-Ph-*H*), 5.28 (dd, $J_{YA} = 6.2$ Hz, $J_{YB} = 9.6$ Hz, 1H, $CH_AH_BCH_Y$), 4.37 (t, $J_{CX} = J_{CD} = 8.3$ Hz, 1H, $CH_XCH_CH_D$), 4.05 (t, $J_{DX} = 7.6$ Hz, $J_{DC} = 8.0$ Hz, 1H, $CH_XCH_CH_D$), 3.73 (quin, J = 7.7 Hz, 1H, $CH_CH_DCH_XCH_AH_B$), 3.09 (m, $J_{AY} = 6.2$ Hz, $J_{AX} = 7.5$ Hz, $J_{AB} = 12.7$ Hz, 1H, $CH_XCH_AH_BCH_Y$), 1.73 (m, $J_{BX} = J_{BY} = 9.6$ Hz, $J_{BA} = 12.7$ Hz, 1H, $CH_XCH_AH_BCH_Y$). ^{13}C NMR (DMSO-d_6) δ 149.63, 149.00, 146.81, 139.61 and 138.72 (Ph-C), 129.23, 128.06, 127.24, 127.01 and 123.94 (CH, Ph-H), 77.64 (CH, C-2), 74.39 (CH_2, C-5), 45.65 (CH, C-4), 41.83 (CH_2, C-3). Found: C, 56.64; H, 3.96; N, 4.04. $C_{16}H_{13}Cl_2NO_3$ (338.1896) requires: C, 56.82; H, 3.87; N, 4.14%.

Methyl 4-(2,4-dichlorophenyl)-4-hydroxyimino-2-(4-nitrophenyl)butanoate 54

A mixture of methyl 4-(2,4-dichlorophenyl)-2-(4-nitrophenyl)-4-oxobutanoate (2.02 g, 5.09 mmol) and

ethanol (50 mL), and a mixture of hydroxylamine hydrochloride (1.53 g, 22.0 mmol) and sodium acetate (1.80 g, 22.0 mmol) in water (50 mL) were combined and refluxed with stirring for 6 h. The reaction mixture was left to cool and then poured into an ice water mixture (50 mL) and left at 4°C overnight. A waxy green solid formed, and the water/ethanol was decanted. The remaining slush was taken up in ethyl acetate (50 mL), dried ($MgSO_4$) and concentrated to leave a brown oil (1.23 g, 61%). Purified by preparative TLC with CH_2Cl_2:MeOH (19:1 v/v) as eluent. υmax KBr disc/cm^{-1} 3354 (OH), 1737 (CO_2CH_3), 1522 and 1347 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.1 (d, J = 8.7 Hz, 2H, Ph-*H*), 7.36 (d, J = 1.9 Hz, 1H, Ph-*H*), 7.34 (d, J = 9.1 Hz, 2H, Ph-*H*), 7.0 (dd, J = 1.9 Hz, J = 8.2 Hz, 1H, Ph-*H*), 6.74 (d, J = 8.1 Hz, 1H, Ph-*H*), 4.10 (m, 1H, CH_XCH_AH$_B$), 3.65 (s, 3H, OCH_3), 3.50 (dd, J_{AX} = 7.9 Hz, J_{AB} = 14.7 Hz, 1H, CH$_X$CH_AH$_B$), 3.33 (dd, J_{BX} = 8.0 Hz, J_{BA} = 14.8 Hz, 1H, CH$_X$CH$_A$$H_B$). Found: C, 51.65; H, 3.67; N, 6.88. $C_{17}H_{14}Cl_2N_2O_5$ (397.214) requires: C, 51.40; H, 3.55; N, 7.05%.

Methyl 4-(2,4-dichlorophenylamino)-4-oxo-2-(4-nitrophenyl)butanoate (**55**) and Methyl 3-(2,4-dichlorophenyloxoamino)-2-(4-nitrophenyl)-propanoate (**56**)

Methyl 4-(2,4-dichlorophenyl)-4-hydroxyimino-2-(4-nitrophenyl)butanoate (**54**) (2.67 g, 6.72 mmol) was stirred with phosphorus pentachloride (3 g) in ether (20 mL) for 3 h. The volatile components were removed and water (25 mL) added. The mixture was boiled for 10 min and water was decanted to leave a yellow paste which was purified by column chromatography with ethyl acetate: petroleum ether (3:7 v/v) to give two main fractions. Fraction 1 (**55**). (0.15 g, 5.6 %). m.p. 124.3–125.7°C. υmax KBr disc/cm^{-1} 3291 (NH), 1730 (CO_2CH_3), 1644 (C═O), 1513 and 1349 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.23 (d, J = 8.7 Hz, 2H, 3″-Ph-*H*), 7.59 (d, J = 8.3 Hz, 1H, 6′-Ph-*H*), 7.57 (d, J = 8.7 Hz, 2H, 2″-Ph-*H*), 7.42 (d, J = 1.9 Hz, 1H, 3′-Ph-*H*), 7.26 (dd, J = 1.9 Hz, J = 8.3 Hz, 1H, 5′-Ph-*H*), 4.27 (dd, J_{XA} = 5.8 Hz, J_{XB} = 8.7 Hz, 1H, CH_XCH$_A$H$_B$), 3.96 (m, 2H, CH$_X$C$H_A$$H_B$), 3.70 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6) δ 172.64 (C═O, C-1), 166.07 (C═O, C-4), 148.00, 143.61, 137.55, 133.10 and 131.80 (Ph-C), 131.65, 130.52, 129.55, 128.04 and 124.57 (CH, Ph-H), 53.18 (OCH_3), 50.95 (CH, C-2), 42.87 (CH_2, C-3). Found: C, 51.62; H, 3.58; N, 6.90. $C_{17}H_{14}Cl_2N_2O_5$ (397.214) requires: C, 51.40; H, 3.55; N, 7.05%. Fraction 2 (**56**). (1.04 g, 39 %). m.p. 118.6–120.3°C. υmax KBr disc/cm^{-1} 3373 (NH), 1726 (CO_2CH_3), 1697 (C═O), 1516 and 1349 (NO_2). ^{1}H NMR (DMSO-d_6) δ 8.18 (d, J = 7.0 Hz, 2H, 3″-Ph-*H*), 7.68 (brs, 1H, 6′-Ph-*H*), 7.47 (d, J = 8.7 Hz, 2H, 2″-Ph-*H*), 7.32 (d, J = 2.3 Hz, 1H, 3′-Ph-*H*), 7.18 (dd, J = 2.3 Hz, J = 8.8 Hz, 1H, 5′-Ph-*H*), 4.37 (dd, J_{XA} = 5.2 Hz, J_{XB} = 9.5 Hz, 1H, CH_XCH$_A$H$_B$), 3.68 (s, 3H, OCH_3), 3.33 (dd, J_{BX} = 9.5 Hz, J_{BA} = 15.6 Hz, 1H, CH$_X$CH$_A$$H_B$), 2.78 (dd, J_{AX} = 5.2 Hz, J_{AB} = 15.6 Hz, 1H, CH$_X$CH_AH$_B$). ^{13}C NMR (DMSO-d_6) δ 171.46 (C═O, C-1), 167.05 (C═O, C-4), 146.48, 143.84, 131.87, 128.47 and 122.36 (Ph-C), 127.90, 127.78, 126.87, 123.18 and 121.49 (CH, Ph-H), 51.92 (OCH_3), 45.97 (CH, C-2), 39.51 (CH_2, C-3). Found: C, 51.29; H, 3.60; N, 7.11. $C_{17}H_{14}Cl_2N_2O_5$ (397.214) requires: C, 51.40; H, 3.55; N, 7.05%.

3-(4-Aminophenyl)-5-(2,4-dichlorophenyl)-2-pyrrolidinone (**57**)

A mixture of methyl 4-(2,4-dichlorophenyl)-4-hydroxyimino-2-(4-nitrophenyl)butanoate (**54**) (2.04 g, 5.14 mmol), acetic acid (30 mL) and water (2 mL) was stirred with a mechanical stirrer and zinc dust (10 g) added carefully. The mixture was stirred and refluxed for 1 h, then water (10 mL) was added and the solution basified with NaOH (6 M). The organic components were extracted with ethyl aetate (3 × 50 mL), dried ($MgSO_4$) and concentrated to give green crystals which were recrystallised from benzene (0.1 g, 6%). m.p. 215.0–218.0°C. υmax KBr disc/cm^{-1} 3342 and 3220 (NH_2), 1695 (CH_2CONH–). ^{1}H NMR (DMSO-d_6) δ 8.24 (s, 1H, NH), 7.62 (d, J = 1.8 Hz, 1H, 3′-Ph-*H*), 7.55 (d, J = 8.4 Hz, 1H, 6′-Ph-*H*), 7.50 (dd, J = 1.9 Hz, J = 8.4 Hz, 1H, 5′-Ph-*H*), 6.85 (d, J = 8.4 Hz, 2H, 3″-Ph-*H*), 6.49 (d, J = 8.4 Hz, 2H, 2″-Ph-*H*), 4.97 (m, 3H, CH_XCH$_A$H$_B$ and NH_2), 3.59 (dd, J_{YB} = 9.1 Hz, J_{YA} = 10.4 Hz, 1H, CH$_A$H$_B$CH_Y), 2.98 (m, J_{BX} = 7.1 Hz, J_{BY} = 8.7 Hz, J_{BA} = 12.5 Hz, 1H, CH$_X$CH$_A$$H_BCH_Y$), 1.60 (m, J_{AX} = 8.7 Hz, J_{AY} = 10.7 Hz, J_{AB} = 12.4 Hz, 1H, CH$_X$CH_AH$_B$CH$_Y$). HRMS m/z: 320.0483 $(M - H)^+$.

Biochemistry

Rat Liver Microsomes

5 Male Wistar rats, 2 months old (250–300 g) were killed by stunning (concussion) and the liver was removed. Livers were rapidly placed in ice cold tris buffer (0.1 M, pH 7.4, 6°C) containing 0.25 M sucrose. The buffer was drained off, the tissue weighed, quickly cut into smaller pieces using scissors and then added to four times its own weight in buffer. The suspension was homogenised with an Elvejhm-Potter homogeniser, ensuring that the tissue remained cool by immersing the homogenising tube in an ice bucket.

The homogenate was centrifuged for 20 min at 10,000 g, 4°C. Pellets were discarded and the supernatant spun for 60 min at 105,000 g, 4°C. The supernatant was discarded and the final pellet was rinsed with 3 × 5 mL washes with tris/sucrose, then resuspended in tris buffer pH 7.4 (no sucrose). The suspension was homogenised on ice using an Elvejhm-Potter homogeniser, distributed into 1.5 mL capped vials, frozen in liquid N_2 and stored at −80°C until use.

Screening of Potential RAMBAs

10 μL of retinoic acid, a mixture of unlabelled (Sigma) and tritiated (NEN) RA to a concentration of 120 μM, was added to a tube containing rat liver microsomes (1.5 mg mL^{-1}), 20 μL, test inhibitor (100 μM final concentration) in acetonitrile (Fisher, HPLC grade) (or solvent as control, ketoconazole as positive control), 10 μL, and phosphate buffer 50 mM, pH 7.4 at 37°C, to 350 μL. The reaction was initiated by the addition of 50 μL of NADPH (Sigma) to a final concentration of 2 mM. The reaction mixture was incubated in a shaking water bath at 37°C. After 40 mins, the enzyme action was arrested by addition of 100 μL 1% formic acid, and the retinoids (retinoic acid + metabolites) were extracted from the reaction mixture using ethyl acetate (Fisher) containing 0.02% butylated hydroxy anisole (antioxidant, Sigma). The organic layer was removed from each tube, transferred to another set of tubes and the ethyl acetate extracts were evaporated using a Christ centrifuge connected to a vacuum pump and a multitrap at −80°C. The residue was reconstituted in methanol and analysed by HPLC. HPLC was carried out using 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 v/v/v). The scintillation fluid was Optiflow Safe 1 (Fisher).

Metabolites[15] 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]. Due to the photosensitivity of retinoic acid all the above assays were carried out in a dark room under yellow light.

RESULTS AND DISCUSSION

Chemistry

Discovery of 1- or 3-alkyl substituted 3-(4-aminophenyl)pyrrolidine-2,5-diones (**5**) as weak inhibitors of rat liver microsomal P450 RA-metabolising enzymes but without an effect on RA-induced metabolising enzymes in human epidermal fibroblast cultured systems and HaCat cells[31] led to the screening of other phenylamines in our chemical library. 2-(4-Aminophenylmethyl)-6-hydroxy-3,4-dihydronaphthalene -1(2*H*)-one (**6**) was found to be two-fold more potent than the standard inhibitor ketoconazole against the rat liver microsomal enzymes but an order less potent in the RA-induced cultured cellular systems.[32]

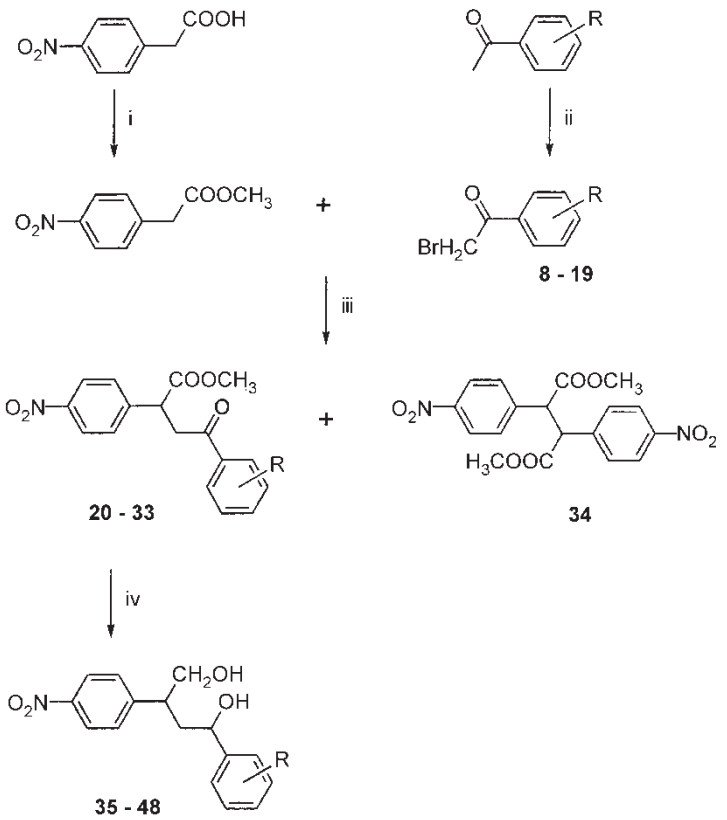
Further development of a hydrophilic area linking suitably substituted aryl functions, as evident in ketoconazole, was based on 1,2-diphenylethane. Several compounds were weak inhibitors of the rat liver microsomal enzyme and moderate inhibitors of the RA-induced cultural cellular systems; 2-(2′,4′-dichlorophenyl)-1-(4-dimethylaminophenyl)-2-hydroxy-1-ethanone (**7**) was the most potent compound being about 20-fold and 5-fold weaker than ketoconazole in these systems respectively. Here this work has been extended using the 1,3-diphenylpropane skeleton.

Results

4-Nitrophenylsuccinic acid was readily prepared in good yield by nitration of phenylsuccinic acid at low temperature (−40°C, fuming HNO_3) which gave relatively pure 4-substitution as shown by Jones and Nazareth.[34] The methyl ester was reacted with a series of ω-bromoacetophenones (**8**–**19**), prepared by bromination of the acetophenone in the presence of a catalytic amount of Al_2Cl_6 in 49–82% yield, in the presence of K_2CO_3 as base to give the 2-(4-nitrophenyl)-4-oxo-4-phenylbutanoates (**20**–**33**) in 21–72% yield (Scheme 1).

TLC of the reaction mixtures showed that a side product was formed, which was present in the reaction mixture of each analogue synthesised. The product had a R_f of 0.22 and was isolated from the reaction for the formation of methyl 4-(2,4-dichlorophenyl)-2-(4-nitrophenyl)-4-oxobutanoate (**25**) by column chromatography with ethyl acetate:petroleum ether (3:7 v/v) as eluent, to give a green solid in a yield of 18%. This compound was dimethyl 2,3-di(4-nitrophenyl)succinate (**34**) formed by self condensation of methyl 4-nitrophenyl acetate, as shown by elemental analysis, high resolution mass spectroscopy and ^{1}H NMR. In a control experiment, methyl 4-nitrophenyl acetate was refluxed with potassium carbonate in acetone for 24 h and the reaction mixture purified by column chromatography. The product of the reaction was dimethyl 2,3-di(4-nitrophenyl)succinate (**34**) in a yield of 8% which was separated from unreacted starting material.

Reduction of the methyl 2-(4-nitrophenyl)-4-oxo-4-phenylbutanoates (**20**–**33**) with sodium borohydride in diglyme gave the respective 3-(4-nitrophenyl)-1-phenyl-1,4-butanediols (**35**–**48**) in 28–86% yield. In the ^{1}H NMR spectrum of (**35**) the secondary and primary hydroxyl groups showed a doublet at δ 5.27 (adjacent to CH) and a triplet at δ 4.78 (adjacent to CH_2) respectively. In the system $-{}^1CH(OH)-{}^2CH_2-{}^3CH(Ph)-{}^4CH_2$-OH the benzylic proton of C-1 had a shift of δ 4.18



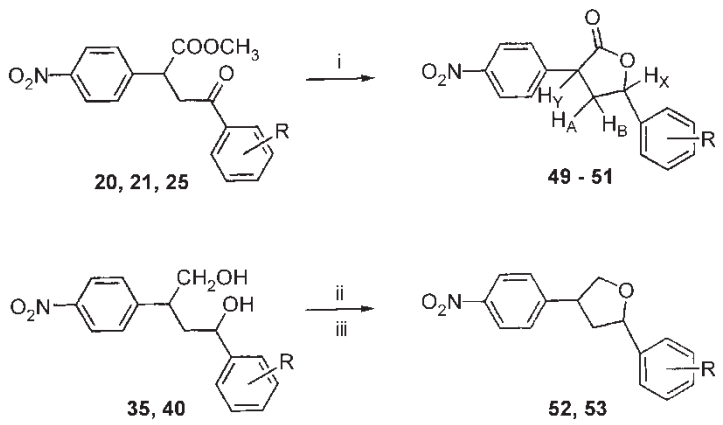
SCHEME 1 Synthesis of some 1-phenyl-3-(4-nitrophenyl)-1,4-butanediols. (i) Trimethylorthoformate, MeOH, HCl, reflux, 3 h; (ii) Br_2, $AlCl_3$, Et_2O, 0°C, 30 min; (iii) K_2CO_3, acetone, reflux, 3.5 h; (iv) $NaBH_4$, diglyme, r.t., 12 h.

and was split into a multiplet by the CH_2 of C-2 and the secondary hydroxyl proton. The mutiplet at δ 3.60 is attributable to the C-4 CH_2 group which is deshielded by the primary hydroxyl and coupled with this hydroxyl and the neighbouring chiral centre of C-3. The chiral proton at C-3 has a chemical shift of δ 3.25 and was split into a doublet of triplets with a coupling constant $J = 6.3$ Hz for the interaction with the C-4 CH_2 and $J_{BX} = J_{BY} = 10.5$ Hz for the interaction with the C-2 CH_2. The protons of C-2 CH_2 (δ 2.01 and 1.85) were split by the CH of C-1, CH of C-3 and its companion proton of C-2, into doublet triplets ($J_{CX} = 4.0$ Hz, $J_{BX} = 10.0$ Hz and $J_{YX} = 14.0$ Hz for $CH_BC\mathit{H}_XH_YCH_C$ and $J_{CY} = 2.6$ Hz, $J_{BY} = 10.8$ Hz and $J_{XY} = 13.5$ Hz for $CH_BCH_X\mathit{H}_YCH_C$).

The ^{1}H NMR spectrum of 1-(2,4-dichlorophenyl)-3-(4-nitrophenyl)-1,4-butanediol showed a duplication of peaks in the aromatic region. Since the sample was judged pure this effect was almost certainly caused by the presence of isomers in a ration of 10:3. The ^{13}C NMR also showed the presence of isomers since the chiral CHOH group and the non chiral CH_2OH group showed small adjacent single minor peaks although this was not evident for the other chiral CH group. The formation of the diastereomeric excess may have been caused by chiral induction from the enantiomeric centre present in the starting material. The methyl 2-(4-nitrophenyl)-4-oxo-4-phenylbutanoate (**20**) analogues contain a chiral centre which may cause steric hindrance when the nearby pro-chiral carbonyl group is reduced by the borohydride complex where preferential nucleophilic attack occurs from the less hindered face of the molecule. This may lead to a slight excess of one of the diastereoisomers. Additionally, diastereoisomers have different physical proeprties and this partial resolution may be a result of the purification process, which for this compound was column chromatography, with the partial loss of one of the diastereoisomers.

Reduction of selected methyl 2-(4-nitrophenyl)-4-oxo-4-phenylbutanoates (**20**, **21** and **25**) with sodium borohydride in an alternative solvent to the previously used diglyme, 70% ethanol/water, led to the formation of the respective lactones (**49**–**51**) in 25–85% yield (Scheme 2). ^{1}H NMR showed the presence of two pairs of diastereoisomers in a ratio of ~ 1:3 confirming the presence of a newly generated chiral centre. The stereochemistry of the lactones was not pursued.

Two 3-(4-nitrophenyl)-1-phenyl-1,4-butanediols (**35** and **40**) were selectively tosylated on the primary alcohol with toluene sulphonic acid in pyridine[35] and cyclised with sodium methoxide as base to

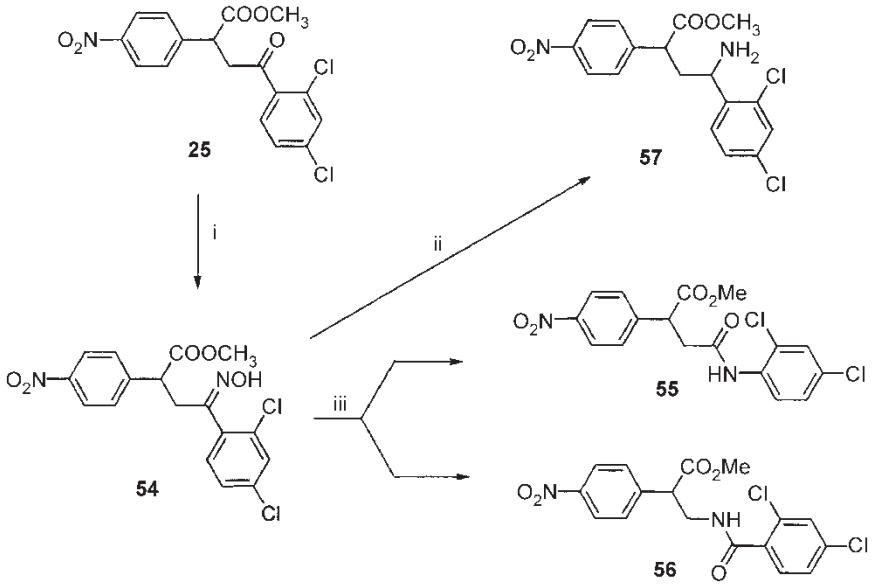


SCHEME 2 Synthesis of some 3-(4-nitrophenyl)-5-phenyltetrahydro-2-furanones (**49**–**51**) and 4-(4-nitrophenyl)-5-phenyltetrahydrofurans (**52**–**53**). (i) $NaBH_4$, EtOH–H_2O, 8 h; (ii) *p*-TsCl, pyridine, r.t., 12 h; (iii) NaOMe, MeOH, reflux, 1 h.

the corresponding 2-phenyltetrahydrofurans (**52** and **53**) in high yield. The crude product **52** was fractioned by silica gel chromatography into the constituent (*RS*/*SR*) *cis*- and (*RR*/*SS*)-*trans*-diastereoisomers. The two forms showed notable differences in the ^{1}H NMR spectra in that H_1 (on C1) and H_6 (benzylic C4) were more upfield in fraction 1 than fraction 2 and additionally, there was an overlap of H_4 and H_5 (–CH–CH_2–CH– on C3) in fraction 1 (δ 2.55, integral = 2) but resolution of these protons occurred in fraction 2 (δ 2.85, integral = 1; δ 1.97, integral = 1). In NOE experiments selective irradiation of H_6 (δ 5.08) in fraction 1 gave no enhancement of H_3 (4-nitrobenzylic C2) suggesting that H_3 and H_6 had a *trans* arrangement but similar treatment of fraction 2 resulted in a 3.8% enhancement of H_3 suggesting that this was the *cis*-isomer.

Reaction of methyl 4-(2,4-dichlorophenyl)-2-(4-nitrophenyl)-4-oxobutanoate (**25**) with hydroxylamine hydrochloride and sodium acetate in ethanol gave the required oxime (**54**) as a light brown oil in 61% yield following column chromatography. The ^{1}H NMR showed a complex mixture of isomers due to (*E*)-anti and (*Z*)-syn forms of the oxime. The ratio of the major:minor isomers by NMR was ~ 2:3 (based on CH_3–O).

In the Beckmann rearrangement, the group which migrates is normally the group which is anti to the hydroxyl group. However the nature of the migration is actually determined by many factors. There are no clear cut indicators to the nature of migrating group aptitudes. In general, aryl migration > alkyl migration[36] and electron withdrawing substituents on aryl groups have a retarding effect on migration



SCHEME 3 Reactions of methyl 4-(2,4-dichlorophenyl)-4-hydroxyimino-2-(4-nitrophenyl)butanoate (**54**). (i) $NH_2OH.HCl$, CH_3COONa, EtOH, reflux, 6 h; (ii) Zn dust, CH_3COOH–H_2O, reflux, 1 h, NaOH; (iii) PCl_5, Et_2O, 3 h.

whilst electron donating groups in the *ortho* and *para* positions have a reinforcing effect.

The reaction of methyl 4-(2,4-dichlorophenyl)-4-hydroxyimino-2-(4-nitrophenyl)butanoate (**54**) with phosphorus pentachloride gave two products which were separated by column chromatography. Fraction 1 (5.6%) and fraction 2 (39%) were the isomers of the Beckmann rearrangement as shown by elemental analysis, and had similar ^{1}H NMR spectra. Mass spectrscopy (CI) showed a mass ion of 189.1 in the spectrum of fraction 2, corresponding to a McLafferty rearrangement on **56**, which was absent in fraction 1 which therefore corresponds to **55**. This shows that the substituted propanoate residue migrated more readily than the 2,4-dichlorophenyl residue and that the major oxime formed was anti with respect to the propanoate residue (Scheme 3).

The lactam (**57**) was derived by reduction of the oxime of (**54**) with zinc dust in acetic acid.

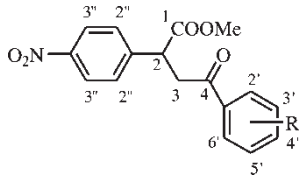
Biochemistry

An inhibitor of the metabolism of endogenous RA would be expected to have a beneficial effect on epithelial differentiation and proliferation as a RA-mimetic, with potential use as an agent for non hormone-dependent cancers and various skin conditions. The target enzymes involved in RA metabolism are a wide group of non-specific liver CYPs, among which in humans CYP2C8 is a major contributor,[12–14] and the RA-induced specific enzyme CYP26 (P450RAI).[16–18] It seems likely that both the non-specific group and CYP26 would need to be targeted since without initial RA build-up due to non-specific enzyme action, RA would not be at a sufficiently high level to induce CYP26. Consequently these *a priori* considerations require that a prospective inhibitor as an RA-mimetic requires at least good activity against the liver microsomal CYP enzymes. On this basis we have examined our compounds against the more readily available rat liver microsomal enzymes as a preliminary screen for further cellular studies with the RA-induced CYP26.

Results

As inhibitors of liver microsomal retinoic acid metabolising enzymes the substituted 4-aryl-2-(4-nitrophenyl)-4-oxobutanoates showed a range of activities; inert or little activity (4–17%, **21**, **23**, **28**, **29**, **31**, **32**), some activity (32–56%, **22**, **24**, **26**) moderate activity (61%, **27**, **33**) and high activity (73%, **30**) compared with the standard inhibitor ketoconazole (80%) at 100 μM (Table I). The best inhibitor was the 4-iodo substituted compound (**30**, 73%). Conversion to the substituted 3-(4-nitrophenyl)-1-aryl-1,4-butanediols almost without exception increased activity so that most compounds showed either moderate (59–67%, **38**, **39**, **41**) or high activity (70–78%, **36**, **42**, **45**, **46**, **48**) (Table II). The most potent inhibitors were the 4-iodo (73%) and the 4-methoxy (78%) substituted compounds. Notable changes in activity on reduction of the oxobutanoates to the butanediols were seen for **31** → **46** (9% → 78%), **32** → **47** (8% → 53%), **29** → **44** (4% → 51%) and **21** → **36** (10% → 72%). The differences in the activity

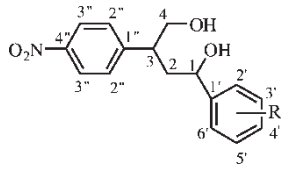
TABLE I Screening of some methyl 2-(4-nitrophenyl)-4-oxo-4-phenylbutanoate derivatives as inhibitors of rat liver microsomal retinoic acid metabolising enzymes



Compound**	R	% Inhibition*
21	4-Br	10
22	2-Cl	32
23	3-Cl	17
24	4-Cl	56
26	2,5-DiCl	52
27	3,4-DiCl	61
28	4-Et	9
29	4-F	4
30	4-I	73
31	4-OCH_3	9
32	4-CH_3	8
33	4-NO_2	61
Ketoconazole		80

*3 μM RA. **100 μM. The results are the means (spread < ±10%) of duplicates in a single run.

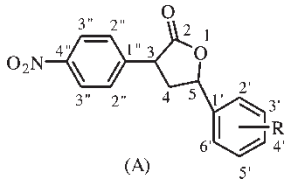
TABLE II Screening of some 3-(4-nitrophenyl)-1-phenyl-1,4-butanediol derivatives as inhibitors of rat liver microsomal retinoic acid metabolising enzymes

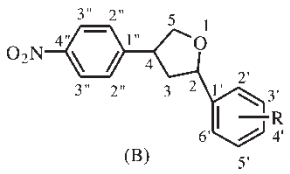


Compound**	R	% Inhibition*
35	H	54
36	4-Br	72
37	2-Cl	49
38	3-Cl	59
39	4-Cl	67
41	2,5-DiCl	63
42	3,4-DiCl	70
43	4-Et	29
44	4-F	51
45	4-I	73
46	4-OCH_3	78
47	4-CH_3	53
48	4-NO_2	70
Ketoconazole		80

*3 μM RA. **100 μM. The results are the means (spread < ±10%) of duplicates in a single run.

TABLE III Screening of some 3-(4-nitrophenyl)-5-phenyltetrahydro-2-furanone (A) and 4-(4-nitrophenyl)-2-phenyltetrahydrofuran (B) derivatives as inhibitors of rat liver microsomal retinoic acid metabolising enzymes





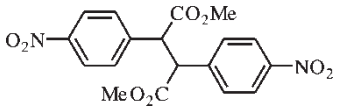
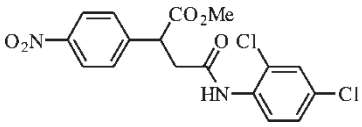
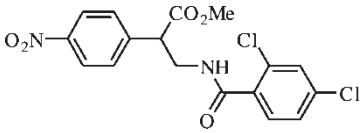
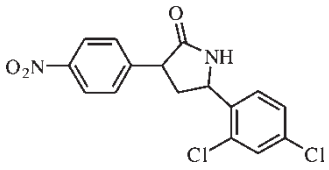
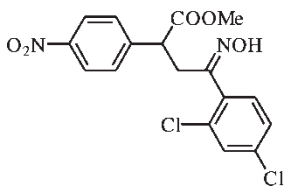
Compound**			
(A)	(B)	R	% Inhibition*
49	–	H	52
50	–	4-Br	67
51	–	2,4-DiCl	69
–	**52** Fraction 1	H	77
–	**52** Fraction 2	H	65
Ketoconazole			80

*3 μM RA. **100 μM. The results are the means (spread $< \pm 10\%$) of duplicates in a single run.

between the two series would suggest that the replacement of two hydrogen bond acceptor carbonyl functions in the butanoates by hydrogen bond donor groups provides additional binding to the enzyme and enhancement of potency. However there were difficulties with this view since in two cases examined the activity of the diols **35** (54%) and **36** (72%) were practically unchanged by comparison with the hydrogen bond acceptor lactones **49** (52%) and **50** (67%) respectively (Table II). Further more the *trans*- and *cis*-isomers of the tetrahydrofuran of **52** (77% and 65% respectively), hydrogen bond acceptors, were more potent than the diol **35** (54%), a hydrogen bond donor (Table III).

The Beckmann rearrangement products of the oxime (**54**), **55** (75%) and **56** (74%) were the most potent of all the compounds studied and have hydrogen bond donor and acceptor functions present (Table IV). The oxime (**54**) itself was unique in that it showed a two fold activation of the enzyme(s) (Table IV).

TABLE IV Screening of some miscellaneous compounds as inhibitors of rat liver microsomal retinoic acid metabolising enzymes

Compound**		% Inhibition*
34		38
55		75
56		74
57		62
54		– 99***
Ketoconazole		80

*3 μM RA. **100 μM. The results are the means (spread ± 10%) of duplicates in a single run. ***activation.

Several of the compounds described here had an inhibitory potency comparable with that of ketoconazole, *viz* **30**, **36**, **45**, **46**, **52**, **55** and **56** and warrant further examination in RA-induced cellular cultured systems for detection of inhibitory action against the specific RA-metabolising enzyme, CYP26.

References

[1] Sporn, M.B., Roberts, A.B. and Goodman, D.S. (1994) *The Retinoids, Biology, Chemistry and Medicine* (Raven Press, New York).

[2] Sonneveld, E., Van den Brink, C.E., Van der Leede, B.-J., Schulkes, R.-K., Petkovich, M., Van der Burg, B. and Van der Saag, P.T. (1998) "Human retinoic acid (RA) 4-hydroxylase (CYP26) is highly specific for all-trans-RA and can be induced through RA receptors in human breast and colon carcinoma cells", *Cell Growth Differ.* **9**, 629–637.

[3] Estey, E.H., Giles, F., Kantarjian, H., O'Brien, S., Cortes, J., Freireich, E., Lopez-Berestein, G. and Keating, M. (1999) "Molecular remissions induced by liposomal-encapsulated all-trans retinoic acid in newly diagnosed acute promyelocytic leukemia", *Blood* **94**, 2230–2235.

[4] Fenaux, P., Chastang, C., Chevret, S., Sanz, M., Dombret, H., Archimbaud, E., Fey, M., Rayon, C., Huguet, F., Sotto, J.-J., Gardin, C., Makhoul, P.C., Travade, P., Solary, E., Fegueux, N., Bordessoule, D., San Miguel, J., Link, H., Desablens, B., Stamatoullas, A., Deconinck, E., Maloisel, F., Castaigne, S., Preudhomme, C. and Degos, L. (1999) "A randomized comparison of all transretinoic acid (ATRA) followed by chemotherapy and ATRA plus chemotherapy and the role of maintenance therapy in newly diagnosed acute promyelocytic leukemia", *Blood* **94**, 1192–1200.

[5] He, L.-Z., Merghoub, T. and Pandolfi, P.P. (1999) "*In vivo* analysis of the molecular pathogenesis of acute promyelocytic leukaemia in the mouse and its therapeutic implications", *Oncogene* **18**, 5278–5292.

[6] Weiss, G.R., Liu, P.Y., Alberts, D.S., Peng, Y.-M., Fisher, E., Min Jian, X., Scudder, S.A., Baker, L.H., Moore, D.F. and Lippman, S. (1998) "13-cis-retinoic acid or All-trans-retinoic acid plus interferon-alpha in recurrent cervical cancer: A Southwest Oncology Group phase II randomized trial", *Gynecol. Oncol.* **71**, 386–390.

[7] Riecken, E.O. and Rosewicz, S. (1999) "Retinoids in pancreatic cancer", *Ann. Oncol.* **10**, S197–S200.

[8] Chaquor, B., Seite, S., Coutant, K., Fourtanier, A., Borel, J.P. and Bellon, G. (1995) "Chronic UVB-induced and all trans retinoic-acid-induced qualitative and quantitative changes in hairless mouse skin", *J. Photochem. Photobiol. B-Biol.* **28**, 125–135.

[9] Fisher, G.J., Wang, Z., Datta, S.C., Varani, J., Kang, S. and Voorhees, J.J. (1997) "Pathophysiology of premature skin aging induced by ultraviolet light", *N. Engl. J. Med.* **337**, 1419–1428.

[10] Fisher, G.J. and Voorhees, J.J. (1998) "Molecular mechanisms of photoaging and its prevention by retinoic acid: Ultraviolet irradiation induces MAP kinase signal transduction cascades that induce Ap-1-regulated matrix metalloproteinases that degrade human skin *in vivo*", *J. Invest. Dermatol. Symp. Proc.* **3**, 61–68.

[11] Smith, H.J., Nicholls, P.J., Simons, C. and LeLain, R. (2001) "Inhibitors of steroidogenesis as agents for the treatment of hormone-dependent cancers", *Exp. Opin. Ther. Patents* **11**, 789–824.

[12] Nadin, L. and Murray, M. (1999) "Participation of CYP2C8 in retinoic acid 4-hydroxylation in human hepatic microsomes", *Biochem. Pharmacol.* **58**, 1201–1208.

[13] Marill, J., Cresteil, T., Lanotte, M. and Chabot, G.G. (2000) "Identification of human cytochrome P450s involved in the formation of all-trans-retinoic acid principal metabolites", *Mol. Pharmacol.* **58**, 1341–1348.

[14] McSorley, L.C. and Daly, A.K. (2000) "Identification of human cytochrome P450 isoforms that contribute to all-trans-retinoic acid 4-hydroxylation", *Biochem. Pharmacol.* **60**, 517–526.

[15] Ahmad, M., Nicholls, P.J., Smith, H.J. and Ahmadi, M. (2000) "Effect of P450 isozyme-selective inhibitors on *in vitro* metabolism of retinoic acid by rat hepatic microsomes", *J. Pharm. Pharmacol.* **52**, 311–314.

[16] Ray, W.J., Bain, G., Yao, M. and Gottlieb, D.I. (1997) "CYP26, a novel mammalian cytochrome P450, is induced by retinoic acid and defines a new family", *J. Biol. Chem.* **272**, 18702–18708.

[17] Haque, M., Andreola, F. and DeLuca, L.M. (1998) "The cloning and characterisation of a novel cytochrome P450 family, CYP26, with specificity toward retinoic acid", *Nutrition Rev.* **56**, 84–85.

[18] Marikar, Y., Wang, Z., Duell, E.A., Petkovich, M., Voorhees, J.J. and Fisher, G.J. (1998) "Retinoic acid receptors regulate expression of retinoic acid 4-hydroxylase that specifically inactivates all-trans retinoic acid in human keratinocyte HaCat cells", *J. Invest. Dermatol.* **111**, 434–439.

[19] Stoppie, P., Borgers, M., Borghgraef, P., Dillen, L., Goossens, J., Sanz, G., Szel, H., Van Hove, C., Van Nyen, G., Nobels, G., Vanden Bossche, H., Venet, M., Willemsens, G. and Van Wauwe, J. (2000) "R115866 inhibits all-trans-retinoic acid metabolism and exerts retinoidal effects in rodents", *J. Pharmacol. Exp. Ther.* **293**, 304–312.

[20] Kuijpers, A., Van Pelt, J., Bergers, M., Boegheim, P., Den Bakker, J., Siegenthaler, G., Van De Kerkhof, P. and Schalkwijk, J. (1998) "The effect of oral liarozole on epidermal proliferation and differentiation in severe plaque psoriasis are comparable with those of acitretin", *Br. J. Dermatol.* **139**, 380–389.

[21] Van Pelt, J., De Jong, E., De Bakker, E. and Van de Kerkhof, P. (1998) "Effects of systemic treatment with liarozole on cutaneous inflammation, epidermal proliferation and differentiation in extensive plaque psoriasis", *Skin Pharmacol. Appl. Skin Physiol.* **11**, 70–79.

[22] Dijkman, G.A., Fernandex del Moral, P., Bruynseels, J., De Porte, P., Denis, L. and Debruyne, F. (1997) "Liarozole (R75251) in hormone-resistant prostate cancer patients", *Prostate* **33**, 26–31.

[23] Debruyne, F., Murray, R., Fradet, Y., Johansson, J.E., Tyrrell, C., Boccardo, F., Denis, L., Marberger, J.M., Brune, D., Rassweiler, J., Vangeneugden, T., Bruynseels, J., Janssens, M. and De Porre, P. (1998) "Liarozole—A novel treatment approach for advanced prostate cancer: Results of a large randomized trial versus cyproterone acetate", *Urology* **52**, 72–81.

[24] Denis, L., Debruyne, F., De Porre, P. and Bruynseels, J. (1998) "Early clinical experience with liarozole (Liazal(TM)) in patients with progressive prostate cancer", *Eur. J. Cancer* **34**, 469–475.

[25] Miller, W. (1998) "The emerging role of retinoids and retinoic acid metabolism blocking agents in the treatment of cancer", *Cancer* **83**, 1471–1482.

[26] O'Byrne, K.J., Han, C., Mitchell, K., Lane, D., Carmichael, J., Harris, A.I. and Talbot, D.C. (1998) "Phase II study of liarozole in advanced non-small cell lung cancer", *Eur. J. Cancer* **34**, 1463–1466.

[27] Schwartz, E.L., Hallam, S., Gallagher, R.E. and Wiernik, P.H. (1995) "Inhibition of all-trans-retinoic acid metabolism by fluconazole *in-vitro* and in patients with acute promyelocytic leukemia", *Biochem. Pharmacol.* **50**, 923–928.

[28] Popa, C., Dicker, A.J., Dahler, A. and Saunders, N.A. (1999) "Cytochrome P450, CYP26A1, is expressed at low levels in human epidermal keratinocytes and is not retinoic acid-inducible", *Br. J. Dermatol.* **141**, 460–468.

[29] Gendimenico, G., Stoppie, P., Van Wauwe, J., Mezick, V., Liebel, J., Mack, W., Mallon, J., Li, W. and Shapiro, S. (2001) "R115866: A selective retinoic acid metabolism inhibitor acts topically as a retinoidal agent in cutaneous model systems", *J. Invest. Dermatol.* **117**, 952.

[30] Le Borgne, M., Marchand, P., Le Baut, G., Ahmadi, M., Smith, H.J. and Nicholls, P.J. (2003) "Retinoic acid metabolism inhibition by 3-azolylmethyl-1*H*-indoles and 2,3 or 5-(α-azolylbenzyl)-1*H*-indoles", *J. Enz. Inhib. Med. Chem.* **18**, 155–158.

[31] Kirby, A.J., LeLain, R., Mason, P., Maharlouie, F., Nicholls, P.J., Smith, H.J. and Simons, C. (2002) "Some 3-(4-aminophenyl)-pyrrolidine-2,5-diones as all-trans-retinoic acid metabolising enzyme inhibitors (RAMBAs)", *J. Enz. Inhib.* **17**, 321–327.

[32] Kirby, A.J., LeLain, R., Maharlouie, F., Mason, P., Nicholls, P.J., Smith, H.J. and Simons, C. (2003) "Inhibition of retinoic acid metabolising enzymes by 2-(4-aminophenylmethyl)-6-hydroxy-3,4-dihydronaphthalen-1(2*H*)-one and related compounds", *J. Enz. Inhib. Med. Chem.* **18**, 27–33.

[33] Greer, V.P., Mason, P., Kirby, Smith, H.J., Nicholls, P.J. and Simons, C. (2003) "Some 1,2-diphenylethane derivatives as inhibitors of retinoic acid—metabolising enzymes", *J. Enz. Inhib. Med. Chem.*, in press.

[34] Nazareth, W. (1988) "Synthesis and evaluation of potential aromatase inhibitors" PhD Thesis, University of Wales (Cardiff).

[35] Escher, S. and Niclass, Y. (1991) "Structure and synthesis of novel C12 terpenoids from quince fruit (cydonia-oblonga mill)", *Helv. Chim. Acta.* **74**, 179–188.

[36] Palmere, R.M., Conley, R.T. and Rabinowitz, J. (1972) "Yhe Scmidt and Beckmann reactions of a-trisubstituted ketones and ketoximes. The synthesis of isotopically labelled aniline", *J. Org. Chem.* **37**, 4095.