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Synthesis and Biological Evaluation of 3-(Prop-2-enyl)- and 3-(Prop-2-ynyl)pyrrolidine-2,5-dione Derivatives as Potential Aromatase Inhibitors

K. J. BARRELL, W. L. WOO, M. AHMADI, H. J. SMITH* AND P. J. NICHOLLS

Welsh School of Pharmacy, University of Wales, College of Cardiff, King Edward VII Avenue, Cardiff CF1 3XF, UK

Abstract

3-(4'-Aminophenyl)pyrrolidine-2,5-dione (WSP3), a known reversible inhibitor of P450 aromatase, was modified using molecular graphics and our model of reversible inhibitor and substrate binding to resemble 10β-prop-2-ynylestr-4-ene-3,17-dione (PED), a mechanism-based inactivator of the enzyme.

The analogues prepared were 3-substituted 3-(prop-2-enyl) or 3-(prop-2-ynyl) pyrrolidine-2,5-diones and their *N*-alkyl derivatives.

The reported compounds demonstrated no irreversible (time-dependent) inhibition of the human placental P450 aromatase enzyme. However, some reversible activity was seen in several of the 3-(prop-2-ynyl) compounds.

The aromatase enzyme ($P450_{AROM}$) catalyses the final step in the oestrogen biosynthesis cascade and it has been a target for the design of inhibitors as agents for the treatment of post-menopausal women with breast cancer (Brodie 1994). Inhibition of this enzyme has been shown to reduce plasma oestrogen levels and subsequently the stimulus to the growth of metastases.

Aminoglutethimide is a well established reversible $P450_{AROM}$ inhibitor used clinically but it has several undesirable clinical features (Shaw et al 1988; Santen 1990), and other more potent non-steroidal inhibitors devoid of side-effects are in clinical trials, e.g. formestane (CGS 16949A) (Steele et al 1987), CGS 20267 (Iverson et al 1993), Vorozole (R 76713) (Krekels et al 1990). Several mechanism-based irreversible inhibitors of the enzyme have been described and 10β-prop-2-ynylestr-4-ene-3,17-dione (PED) (Metcalf et al 1981; Johnston et al 1984) and 4-hydroxyandrostenedione (4-OHA) (Brodie et al 1977) have entered the clinic. The irreversible inhibitors have the potential advantage of less frequent dosing but a possible disadvantage is that they may be metabolized to agents with oestrogenic activity. Non-steroidal irreversible inhibitors of the enzyme are not known but would not be expected to possess inherent oestrogenic activity or be metabolized to such agents.

We have described a model (Banting et al 1988) based on non-steroidal reversible inhibitor-substrate (androstenedione) superimpositions using molecular graphics which accommodates liganding of the basic nitrogen to the Fe^{3+} of the haem (positioned above the steroidal substrate C-10 methyl group) and binding of the remainder of the molecule to specific positions of the ring with any additional hydrophobic group extending along the steroidal skeleton binding site. Aminoglutethimide and the analogue 3-(4-aminophenyl)pyrrolidine-2,5-dione (WSP3) are postulated to bind to the 3-carbonyl steroidal binding site through their 6-, and 5-carbonyl groups so placing the 3-phenylamine function of the active (R)-+-form above the A ring to ligand with the Fe^{3+} haem (Fig. 1).

The steroidal agent PED is a mechanism-based inactivator of $P450_{AROM}$ due to oxidation of the prop-2-ynyl group to an electrophilic species (Metcalf et al 1981). We have designed potential irreversible inhibitors where the skeleton of a known reversible inhibitor (Daly et al 1986) of the enzyme, WSP3 has been modified by replacement of the 3-aminophenyl substituent with the oxidisable prop-2-enyl or prop-2-ynyl function and additional substitution at the 3-position with a bulky hydrophobic substituent. Some of the compounds were also alkylated at the N-1 position since this has been shown to considerably increase potency (Whomsley et al 1993).

Molecular graphics using the designed 3-alkyl substituted compounds-PED superimpositions show that the specific binding to the steroidal A ring of the substrate postulated in the model, places the oxidizable 3-prop-2-enyl or 3-prop-2-ynyl function in the same position as that in PED and the other 3-substituent runs along the steroidal skeleton binding site and extends into a hydrophobic region (Li & Brueggemeier 1990) adjoining the 7-position of the steroid (Fig. 2).

Materials and Methods

Materials

D-Glucose-6-phosphate (mono-phosphate salt) and NADP (mono-sodium salt) were purchased from Sigma Chemicals Co. D-Glucose-6-phosphate dehydrogenase was from Boehringer-Mannheim. [1β,2β-^{3}H]Androstenedione (40·00 Ci mmol^{-1}, 1 mCi (37 MBq) in 1 mL ethanol) was purchased from Dupont (UK) Ltd. All unlabelled laboratory reagents

Correspondence: H. J. Smith, Welsh School of Pharmacy, University of Wales, College of Cardiff, King Edward VII Avenue, Cardiff CF1 3XF, UK.

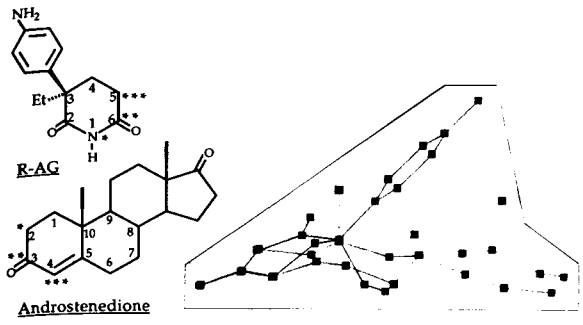


FIG. 1. Superimposition of (+)-R-AG onto androstenedione.

were Analar grade and obtained from BDH, Poole, Dorset. Radioactivity was determined on a KLB Wallac 1217 Rackbeta liquid scintillation counter. Scintillation fluid for the $P450_{AROM}$ assay was Optiphase Hisafe 3, purchased from FSA Laboratory Supplies, Loughborough, Leics.

Molecular modelling

All molecular display and manipulations were performed using Chem-X software (1988 version) (Chemical Design Ltd, Oxford) running on a VAX VMS computer. The structure of androstenedione was retrieved from the Cambridge Structural Databank (CSSR) available from the Chemical Databank System (CDS) at Daresbury Laboratory. Both 10β-2-propenyl- and 10β-2-propynylandrostenedione were constructed from the structure of androstenedione by substitution of the C-19 methyl with the appropriate functional group, minimized and optimized.

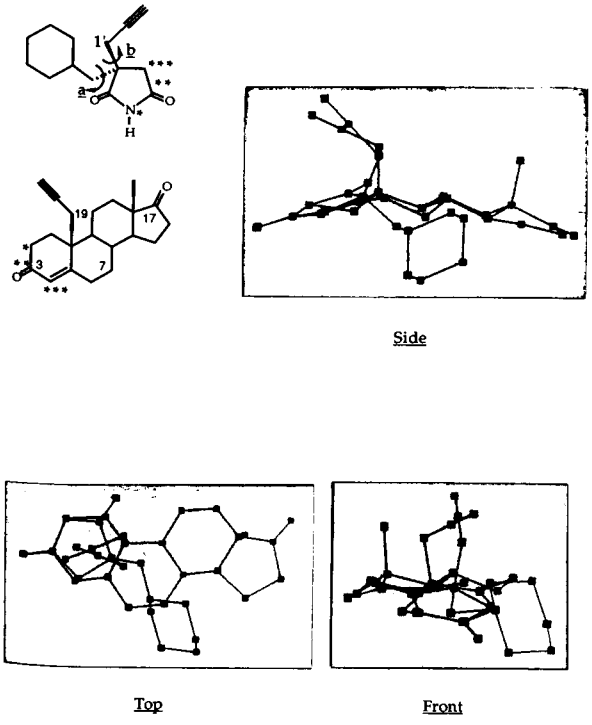


FIG. 2. Superimposition of compound **9** onto 10β-2-propynylandrostenedione with viewing of the matched pair from different positions. Conformational analysis strategy: a (24 steps) and b (360 steps) simultaneously.

The C-10–C-19 bond was then rotated at C-10 through 360° with an increment of 0.5° (steps 720) and the conformer with the lowest energy was minimized and optimized.

The structure of each synthesized compound to be studied was constructed using the Chem-X software, minimized and optimized. Conformational analysis was performed where freely rotatable bonds, either as a pair or individually were selected and rotated in a stepwise sequence through 360° (Fig. 2). The low-energy conformation was then superimposed onto either 10β-2-propenyl- or 10β-2-propynylandrostenedione in the manner set out in Fig. 1. The matched pair was viewed at different positions ie. front, top and side elevations.

Biochemical studies

Preparation of the enzyme. $P450_{AROM}$ was prepared from human term placental tissue using the general method reported by Thompson & Siiteri (1974) .

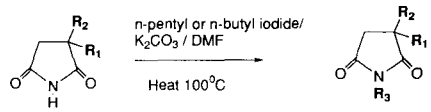
Assays. $P450_{AROM}$ activity was determined by the measurement of ^3H_2O released from [1β,2β-^{3}H]androstenedione (0·6 μM) using the general method of Graves & Salhanick (1974). The results in Table 1 are expressed as a percentage inhibition of the enzyme by the inhibitor (200 μM) compared with a control in the absence of the inhibitor. The values quoted are only for the reversible inhibition of the enzyme since on pre-incubation of the inhibitor with the enzyme for 1 h at 30°C followed by removal of the inhibitor with charcoal, enzyme activity was unaffected. Aminoglutethimide (100 μM) was included in the tests for comparative purposes and inhibited the enzyme by 74·5%.

Chemistry. Melting points were determined on an Electrothermal instrument and are uncorrected. Infrared spectra on KBr discs were recorded on a Perkin Elmer 681 spectrophotometer. ^{1}H NMR spectra were recorded in $CDCl_3$, unless otherwise stated, on a Perkin Elmer R32 (90 MHz) and JEOL-FX90Q (90 MHz) spectrometer in ppm relative to tetramethylsilane as an internal reference. Mass spectra were determined by the SERC Mass Spectrometry Centre at Swansea. Elemental analyses were determined at the School of Pharmacy, London.

Synthesis. The parent 3-octyl and 3-cyclohexylmethyl-3-prop-2-enyl- pyrrolidine-2,5-diones (**1** and **4**) and their 3-prop-2-ynyl analogues (**7** and **9**) as well as the 3-carboxy-3-(prop-2-ynyl) pyrrolidine-2,5-dione (**10**) and its 3-(*t*-butoxycarbonyl) ester (**15**) have been described previously

Table 1. Inhibitory activity (%) for some 3-substituted amide and ester derivatives of 3-carboxy-3-(prop-2-ynyl)pyrrolidine-2,5,-diones.

Compound	Inhibition (%)
11	25·0
12	18·1
13	44·6
14	48·0
15	<10
16	<10
17	13·0
22	70·0



(1) $R_1 = C_8H_{17}$; $R_2 = -CH_2-CH=CH_2$

(2) $R_1 = C_8H_{17}$; $R_2 = -CH_2-CH=CH_2$; $R_3 = C_4H_9$

(3) $R_1 = C_8H_{17}$; $R_2 = -CH_2-CH=CH_2$; $R_3 = C_5H_{11}$

(4) $R_1 = CH_2$-cycloC_6H_{11} ; $R_2 = -CH_2-CH=CH_2$

(5) $R_1 = CH_2$-cycloC_6H_{11} ; $R_2 = -CH_2-CH=CH_2$; $R_3 = C_4H_9$

(6) $R_1 = CH_2$-cycloC_6H_{11} ; $R_2 = -CH_2-CH=CH_2$; $R_3 = C_5H_{11}$

(7) $R_1 = C_8H_{17}$; $R_2 = -CH_2-C\equiv CH$

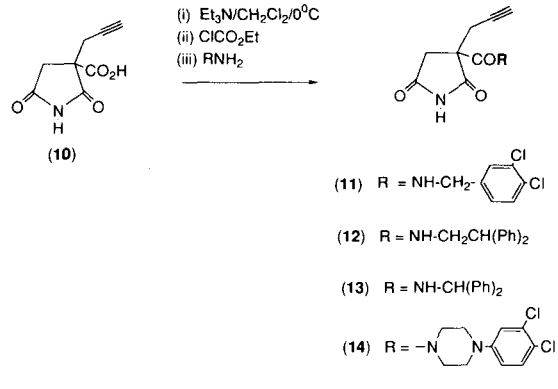
(8) $R_1 = C_8H_{17}$; $R_2 = -CH_2-C\equiv CH$ $R_3 = C_4H_9$

(9) $R_1 = CH_2$-cycloC_6H_{11} ; $R_2 = -CH_2-C\equiv CH$

Scheme 1. Synthesis of *N*-substituted prop-2-enyl and prop-2-ynyl derivatives.

(Woo et al 1993). The *N*-alkylated, amide and ester derivatives were synthesized as shown in Schemes 1–3 respectively.

(RS)-1-Butyl-3-octyl-3-(prop-2-enyl)pyrrolidine-2,5-dione (**2**). The dione (**1**) (1·50 g, 5·97 mmol), 1-iodobutane (1·65 g, 8·9 mmol), anhydrous potassium carbonate (4·1 g, 29·8 mmol, freshly dried at 110°C for 3 h) and dimethylformamide (30 mL) were stirred and heated at 100°C (internal temperature) for 2 h. The suspension was then cooled to room temperature, filtered and the cake washed with petroleum ether 40–60° (100 mL). Water (150 mL) was added to the combined filtrates and the aqueous layer separated and washed with petroleum ether 40–60° (4 × 25 mL). The combined organic extracts were washed with water (4 × 25 mL), dried ($MgSO_4$) and evaporated under reduced pressure to give a yellow oil (1·6 g) which was fractionated on a column of dry silica with petroleum ether 40–60°/ether (2 : 1). Evaporation of the eluent of the second fraction gave the dione (**2**) (1.5 g, 81·8%) as a pale yellow oil. Found: 74·31; H, 10·89; N, 4·39. $C_{19}H_{33}O_2N$ requires C, 74·22;H, 10·81; N, 4·56%; $\upsilon_{max\ (film)}$ 3100 (=C-H), 2980, 2950 and 2870 (C-H), 1780 and 1710 (C=O of imide) and 1650 (C=C) cm^{-1}; δ_H ($CDCl_3$) 0·70–2·00 (24H, m,-CH_2 of butyl and octyl, -CH_3 of butyl and octyl), 2·10–2·80 (4H) [2·28 (dd, $J_{BA} = 13$ Hz and $J_{XA} = 8$ Hz, $C=CH_X$-CH_AH$_B$), 2·45 (d,



(10)

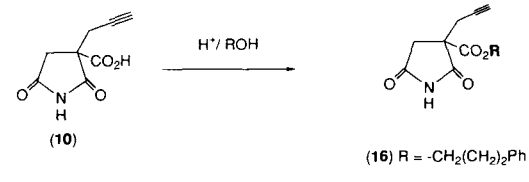
(11) R = NH-CH_2-

(12) R = NH-$CH_2CH(Ph)_2$

(13) R = NH-$CH(Ph)_2$

(14) R = -N N-

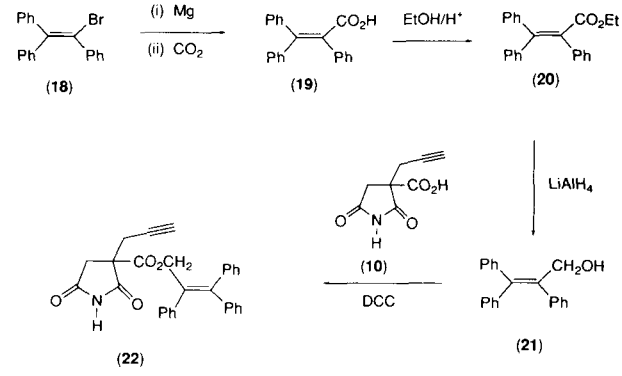
Scheme 2. Synthesis of amido derivatives of 3-carboxy-3-(prop-2-ynyl) pyrrolidine-2,5-dione.



(10)

(16) R = -$CH_2(CH_2)_2Ph$

(17) R = -$C(Ph)_3$



(18) (19) (20) (21) (22)

Scheme 3. Synthesis of ester derivatives of 3-carboxy-3-(prop-2-ynyl)pyrrolidine-2,5-dione.

$J_{BA} = 18$ Hz, -CH_AH$_B$-CO-N-CO), 2·64 (dd, $J_{AB} = 13$ Hz and $J_{XB} = 7$ Hz, $C=CH_X$-CH_AH$_B$), 2·68 (d, $J_{AB} = 18$ Hz, -CH_AH$_B$-CO-N-CO], 3·50 (2H, t, J = 7 Hz, N-CH_2), 5·00–5·30 (2 H, m, CH=CH_2) and 5·40–5·95 (1 H, m, -CH=CH_2).

(RS)-1-Pentyl-3-octyl-3-(prop-2-enyl)pyrrolidine-2,5-dione (**3**). This dione was obtained via the method described for the synthesis of **2** using 1-iodopentane as a pale yellow oil (1·1 g, 57·4%). Found: 74·56; H, 10·91; N, 4·16. $C_{20}H_{35}O_2N$ requires C, 74·71;H, 10·97; N, 4·36%; $\nu_{max\ (film)}$ 3090 (=C-H), 2970, 2930, and 2870, (C-H), 1780 and 1705 (C=O of imide) and 1670 (C=C) cm^{-1}; δ_H ($CDCl_3$) 0·57–2·07 (26 H, m,-CH_2 of pentyl and octyl, -CH_3 of pentyl and octyl), 2·07–2·77 (4H) [2·27 (dd, $J_{BA} = 14$ Hz and $J_{XA} = 7·5$ Hz, $C=CH_X$-CH_AH$_B$), 2·42 (d, $J_{BA} = 18$ Hz, -CH_AH$_B$-CO-N-CO), 2·52 (dd, $J_{AB} = 14$ Hz and $J_{XB} = 7$ Hz, $C=CH_X$-CH_AH$_B$), 2·75 (d, $J_{AB} = 18$ Hz, -CH_AH$_B$-CO-N-CO], 4·48 (2H, t, J = 7 Hz, N-CH_2), 4·99–6·00 (2H, m, CH=CH_2) and 5·40–5·93 (1H, m, -CH=CH_2).

(RS)-1-Butyl-3-cyclohexylmethyl-3-(prop-2-enyl)pyrrolidine-2,5-dione (**5**). The dione was obtained from 4 (1·0 g, 4·25 mmol) via the method described for the synthesis of **2** using 1-iodobutane (1·17 g, 6·37 mmol) and a pale yellow oil (0·57 g, 46%) was obtained. Found: 73·71; H, 9·95; N, 4·99. $C_{18}H_{29}O_2N$ requires C, 74·18;H, 10·03; N, 4·81%; $\upsilon_{max\ (film)}$ 3080 (=C-H), 2930 and 2860 (C-H), 1775 and 1705 (C=O of imide) and 1640 (C=C) cm^{-1}; δ_H ($CDCl_3$) 0·76–1·86 (20H, m, -CH_2 and C-H of cyclohexylmethyl, -CH_2 and -CH_3 of butyl), 2·06–2·51 (2H) [2·21 (dd, $J_{BA} = 14$ Hz and $J_{XA} = 7$ Hz, C=CH_X-CH_AH$_B$), 2·49 , (dd, $J_{AB} = 14$ Hz and

J_{XB} = 7 Hz, C=CH_X-CH_AH_B)], 2·58 (2H, s, -CH_2-CO-N-CO), 3·49 (2H, t, J = 7 Hz, N-CH_2), 5·00-5·36 (2H, m, CH=CH_2) and 5·41–5·94 (1H, m, -CH=CH_2).

(RS)-1-Pentyl-3-cyclohexylmethyl-3-(prop-2-enyl)pyrrolidine-2,5-dione (**6**). The dione was obtained similarly from **4** (1·0 g, 4·25 mmol) via the method described for the synthesis of **2** using 1-iodopentane (1·26 g, 6·4 mmol) and a yellow oil (1·15 g, 88·6%) was obtained. Found: 74·59; H, 10·41; N, 4·48. $C_{19}H_{31}O_2N$ requires C, 74·71;H, 10·23; N, 4·59%; υ_{max} (film) 3080 (=C-H), 2930 and 2860 (C-H), 1780 and 1710 (C=O of imide) and 1645 (C=C) cm^{-1}; δH ($CDCl_3$) 0·45–2·00 (22H, m, -CH_2 and C-H of cyclohexylmethyl, -CH_2 and CH_3 of pentyl), 2·02–2·70 (4H) [2·20 (dd, J_{BA} = 14 Hz and J_{XA} = 7 Hz, C=CH_X-CH_AH_B), 2·46 , (dd, J_{AB} = 14 Hz and J_{XB} = 7 Hz, C=CH_X-CH_AH_B), 2·55 (s, -CH_2-CO-N-CO)], 3·43 (2H, t, J = 7 Hz, N-CH_2), 4·92–5·25 (2H, m, CH=CH_2) and 5·32–5·85 (1H, m, -CH=CH_2).

(RS)-1-Butyl-3-octyl-3-(prop-2-ynyl)pyrrolidine-2,5-dione (**8**). The dione was obtained from 7 (0.6 g, 2.4 mmol) via the method described for the synthesis of **2** using 1-iodobutane (0.66 g, 3.55 mmol) and a yellow oil (0.45 g, 61.2%) was obtained. Found: 74.42; H, 10.32; N, 4.51. $C_{19}H_{31}O_2N$ requires C, 74.71; H, 10.23; N, 4.59%; υ_{max} (film) 3310 (≡C-H), 2970, 2940 and 2870 (C-H), 2130 (C≡C), 1780 and 1710 (C = O of imide) cm^{-1}; δ_H ($CDCl_3$) 0.93 (6H, t, J = 7Hz, -CH_3 of butyl and octyl), 1.07-1.93 (18H, s,-CH_2) of butyl and octyl), 2.01 (1H,t, J = 2.5Hz, H-C≡C-CH_2), 2.23-3.10 (4H) [2.39 (dd, J_{BA} = 17Hz and J_{XA} = 3Hz, H_X-C≡C-CH_AH_B), 2.53 (d, J_{BA} = 18Hz, -CH_AH_B-CONHCO), 2.65, (dd, J_{AB} = 17Hz and J_{XB} = 3Hz, H_X-C≡C-CH_AH_B), 2.85 (d, J_{AB} = 18Hz, -CH_AH_B-CONHCO)] and 3.52 (2H, t, J = 7Hz, N-CH_2).

(RS) - 3-[N-(3', 4'-Dichlorobenzyl)carboxamido]-3-(prop-2-ynyl)pyrrolidine-2,5-dione (**11**). Triethylamine (0·9 mL, 6·5 mmol) was added dropwise to the crude 3-carboxy-3-(prop-2-ynyl)pyrrolidine-2,5-dione (**10**) (0·59 g, 3·3 mmol) and dichloromethane (6 mL) was added to the resulting solution. After cooling the mixture with stirring to 0°C, ethyl chloroformate (96·5%, 0·32 mL, 3·3 mmol) was added dropwise and the resulting mixture was stirred for an additional 15 min at this temperature. 3,4-Dichlorobenzylamine (1·17 g, 6·5 mmol) in dichloromethane (2 mL) was then added in portions and the mixture was warmed gradually to room temperature and stirred overnight. The reaction mixture was then evaporated and the residue was diluted with ether (50 mL). The resultant was then washed with dilute sulphuric acid (2 × 25 mL) and then water (3 × 50 mL). The ethereal layer was dried ($MgSO_4$) and evaporated to give a yellowish brown oil (0·8 g) which was then dissolved in hot dichloromethane. Upon cooling, the dione (**11**) (0·14 g, 12·7%) was obtained as colourless crystals, m.p. 205·0–206·8°C. Found: 52·89; H, 3·58; N, 7·79. $C_{15}H_{12}O_3N_2Cl_2$ requires C, 53·12; H, 3·57; N, 8·26%; υ_{max} (KBr) 3400, 3160 and 3080 (N-H of imide and 2^0 amide), 3305 (≡C-H), 2980 2940 (C-H), 1780 and 1710 (C=O of imide), 1680 (C=O of 2^0 amide), 1550 (C_6H_5) cm^{-1} ; δ_H (acetone-d_6/DMSO-d_6, ca. 20 : 1) 2·65–2·94 (2H) [2·76 (t, J = 3 Hz, H-C≡C-CH_2), 2·78 (d, J_{BA} = 18 Hz, -CH_AH_B-CONHCO)],3·04(2H, d, J = 2 Hz, H-C:C-CH_2), 3·36 (1H, d, J_{AB} = 18 Hz, -CH_AH_B-CONHCO), 4·40 (2H, d, J = 5 Hz, -CONHCH_2), 7·18–7·68 (3H, m, H of $C_6H_3Cl_2$) and 8·43–8·78 (1H, br t, J = 6 Hz, -CO-NH-CH_2).

(RS)-3-[N-(2', 2'-Diphenylethyl)carboxamido]-3-(prop-2-ynyl)pyrrolidine-2,5-dione (**12**). The dione was obtained from **10** via the method described for the synthesis of **11** using 2,2-diphenylethylamine and colourless crystals (34·2%) were obtained, m.p. 171·3–172·8°C. Found: 73·30; H, 5·53; N, 7·73. $C_{22}H_{20}O_3N_2$ requires C, 73·31; H, 5·59; N, 7·77%); υ_{max} (KBr) 3380 and 3120 (N-H of imide and 2^0 amide), 3305 (≡C-H), 3090, 3070 3040 (aromatic C-H), 2940 and 2890 (aliphatic C-H), 1795 and 1710 (C=O of imide), 1675 (C=O of 2^0 amide) and 1545 , 1500 and 1450 (C=C of C_6H_5) cm^{-1}; δ_H ($CDCl_3$/DMSO-d_6, ca. 20 : 1) 2·30 (1H, t, J = 3 Hz, H-C≡C-CH_2), 2·50–3·00 (3H) [2·51(dd, J_{BA} = 16 Hz and J_{XA} = 3 Hz, H_X-C≡C-CH_AH_B) 2·67 (d, J_{BA} = 18 Hz, -CH_AH_B-CONHCO), 2·72 (dd, J_{AB} = 16 Hz and J_{XB} = 3 Hz, H_X-C≡C-CH_AH_B)], 3·00–3·50 (2H) [3·21 (d, J_{AB} = 18 Hz, -CH_AH_B.CONHCO),3·25 (br s, -CO-NH-CH_2)], 3·60–4·10 (2H, m,-CONH-CH_2-CH), 4·29 [1H, t , J = 7 Hz, -CH_2-CH(C_6H_5)$_2$], 7·20 (10H, s, C-H of 2 × C_6H_5) and 11·10–11·80 (1H, br s, -CONHCO).

(RS)-3-[N-(Diphenylmethyl)carboxamido]-3-(prop-2-ynyl) pyrrolidine-2,5-dione (**13**). The dione was obtained from **10** via the method described for the synthesis of **11** using diphenylmethylamine and colourless crystals (21·9%) were obtained, m.p. 126·0–127·1°C. Found: 72·56; H, 5·32; N, 8·17. $C_{21}H_{18}O_3N_2$ requires C, 72·82; H, 5·24 N, 8·09%; υ_{max} (KBr) 3480, 3440 and 3220 (N-H of imide and 2^0 amide), 3300 (≡C-H), 3090, 3070 3040 (aromatic C-H), 2130 (C≡C),1780, 1730 and 1715 (C=O of imide),1650 (C=O of 2^0 amide) and 1535, 1500 and 1450 (C=C of C_6H_5) cm^{-1}; δ_H ($CDCl_3$) 2·11 (1H t, J = 2 Hz, H-C≡C-CH_2), 2·62–3·10 (3H) [2·74 (dd, J_{BA} = 16 Hz and J_{XA} = 3 Hz, H_X-C≡C-CH_AH_B), 2·78 (d, J_{BA} = 19 Hz, -CH_AH_B-CONHCO), 2·96 (dd, J_{AB} = 16 Hz and J_{XB} = 3 Hz, H_X-C≡C-$CH_AC H_B$], 3·51(1H, d, J_{AB} = 19 Hz, -CH_AH_B-CONHCO), 6·21(1H, d, J = 8 Hz, -CONH-CH(C_6H_5)$_2$), 7·26 and 7·28 (10H, two s, C-H of 2 × C_6H_5), 7·87 (1H. d, J = 8 Hz, -CONH-CH(C_6H_5)$_2$) and 9·03–9·40 (1H, br s, -CONHCO).

(RS)-3-[4'-(m,p-Dichlorophenyl)-1'-piperazinecarboxamido]-3-(prop-2-ynyl) pyrrolidine-2,5-dione (**14**). The dione was obtained from **10** via the method described for the synthesis of **11** using *N*-(*m,p*-dichlorophenyl)piperazine and colourless crystals (14·4%) were obtained, m.p. 173·5–174·8°C. Found: 54·59; H, 4·41; N, 10·65. $C_{18}H_{17}O_3N_3Cl_2$ requires C, 54·84; H, 4·34 N, 10·66%; υ_{max} (KBr) 3300 (≡C-H), 3180 3100 (N-H), 1790 and 1725 (C=O of imide), 1630 (C=O of amide) and 1600 (C_6H_5) cm^{-1}; δ_H (acetone-d_6) 2·56 (1H, t, J = 2 Hz, H-C≡C-CH_2), 2·67–4·17 (12H) {2·80 (dd, J_{BA} = 17 Hz and J_{XA} = 2 Hz, H_X-C≡C-CH_AH_B), 2·93 (d, J_{BA} = 18 Hz, -CH_AH_B-CONHCO), 3·12 (dd, J_{AB} = 17 Hz and J_{XB} = 2 Hz, H_X-C≡C-CH_AH_B), 3·27 (d, J_{AB} = 18 Hz, -CH_AH_B-CONHCO) and 3·21, 3·27, 3·32, 3·65, 3·69, 3·75 and 3·79 [m, -CH_2-N(C=O)-CH_2 and -CH_2-N($C_6H_3Cl_2$)-CH_2]}, 6·88 (1H, dd, $J_{m',o'}$ = 9Hz, $J_{o,o'}$ = 2Hz, C_6H_3-H_o·), 7·06 (1H, d, $J_{o',o}$ = 2 Hz, $C_6H_3H_o$), 7·32 (1H, d, $J_{o',m'}$ = 9 Hz, C_6H_3-$H_{m'}$) and 10·12–10·87 (1H, br s,-CONHCO).

RS-3(3'-phenylpropoxycarbonyl)-3(prop-2-ynyl)pyrrolidine-2,5-dione (**16**). 3-Carboxy-3-(prop-2-ynyl) pyrrolidine-2,5-dione (**10**) (0·67 g; 3·7 mmol) and 3-phenyl propanol (1·20 g; 8·8 mmol) were refluxed in dry benzene (50 mL) and the water removed using a Dean and Stark separator. The solvent was removed at the pump and the residue fractionated on a column of dry silica using cyclohexane and acetone as the eluent (5 : 1) to yield the ester as a pale yellow oil (0·43 g 39%) Found : C,67·8 ; H,5·8 ; N,4·6%. $C_{17}H_{17}NO_4$ requires C,68·22 ; H,5·72 ; N,4·68%). υ_{max} (film)/cm^{-1} 3280–3240 (≡CH and NH unresolved), 1780 (C=O,imide); 1735 (-CO_2CH_2-) and 1720 (C=O),imide; δ_H ($CDCl_3$) 2·01 (2H, m, J = 7 Hz, -$CH_2CH_2C_6H_5$), 2·10 (1H, t, J = 3 Hz, *H*-C≡C-CH_2), 2·72 (2H, t, J = 7 Hz, $CH_2C_6H_5$), 2·92 (1H, dd, J_{BA} = 12 Hz and J_{XA} = 3 Hz, CH_AH_B-C≡CH_X), 2·97 (1H, dd, J_{AB} = 12 Hz and J_{XB} = 3 Hz, CH_AH_B-C≡CH_X), 2·99 (1H, d, J_{BA} = 18 Hz, CH_AH_BCONHCO-), 3·14 (1H, d, J_{AB} = 18 Hz, CH_AH_BCONHCO-), 4·25 (2H, t, J = 7 Hz,-$CO_2CH_2CH_2CH_2C_6H_5$),7·00–7·50 (5H,m,aromatic), 8·90 (1H, br s,-CONHCO-).

RS-3(triphenylmethoxycarbonyl)-3-(prop-2-ynyl)pyrrolidine-2,5-dione (**17**). This compound was obtained via the method described for the preparation of **16**. The 3-carboxy-3-(prop-2-ynyl) pyrrolidine-2,5-dione (**10**) (0·76 g; 4·2 mmol) and triphenylmethanol (3·30 g; 12·7 mmol) were refluxed in dry benzene (50 mL) and the water removed using a Dean and Stark separator. The solvent was removed at the pump and the residue was fractionated on a column of dry silica using chloroform and methanol as the eluent (9 : 1) to yield the ester as pale yellow crystals (0·47 g, 26%) m.p. 206·0–208·0°C. Found : C,76·1; H,5·1; N,3·2%. $C_{27}H_{21}NO_4$ requires C,76·58 ; H,5·0 ; N,3·31%.) υ_{max} (KBr) cm^{-1} 3280-3240 (≡CH and NH unresolved), 1780 (C=O,imide), 1735 ($CO_2C(C_6H_5)_3$) and 1720 (C=O),imide); δ_H($CDCl_3$) 1·90 (1H, t, J = 4 Hz, *H*-C≡C-CH_2), 2·87 (1H, dd, J_{BA} = 15 Hz and J_{XA} = 5 Hz, CH_AH_B-C≡CH_X), 2·92 (1H, dd, J_{AB} = 15 Hz and J_{XB} = 5 Hz, CH_AH_B-C≡CH_X), 3·88 (1H, d, J_{BA} = 15 Hz, CH_AH_BCONHCO-), 3·96 (1H, d, J_{AB} = 15 Hz, CH_AH_BCONHCO-), 7·20–7·50 (15H, m, aromatic), 7·80 (1H, br s -CON*H*CO).

(RS)-3(2,3,3-triphenyl-prop-2-enoxycarbonyl)-3(prop-2-ynyl) pyrrolidine -2,5-dione (**22**).

2,3,3-Triphenyl-prop-2-enoic acid (**19**). A solution of 1-bromo-1,2,2-triphenylethene (**18**) (10 g, 30 mmol) in anhydrous ether (100 mL) was added dropwise to a vigorously stirred mixture of magnesium turnings (0·8 g, 0·033 atoms) in anhydrous ether (10 mL). The mixture was stirred and refluxed until the reaction was complete, noted by the disappearance of the magnesium. The yellow solution was poured onto solid carbon dioxide and allowed to reach room temperature. Alternatively gaseous carbon dioxide was bubbled into the reaction mixture. The Grignard complex was destroyed with dilute sulphuric acid (3 M, 50 mL) and the organic layer separated and extracted with potassium carbonate (2%, w/v). This was carefully acidified with dilute hydrochloric acid (3 M) to give a white solid, which was filtered and washed with water until the filtrate was neutral to indicator paper. The crude acid was deemed sufficiently pure to be used for the next reaction without further purification. An analytical sample was prepared from glacial acetic acid which separated as colourless crystals (4·0 g; 44%). m.p. 217–219°C (lit 217–218°C (Koelsch 1932)), υ_{max} (KBr)/cm^{-1} 3500–2200 (CO_2H),1685 (C=O).

Ethyl 2,3,3-triphenyl-prop-2-enoate (**20**). A mixture of the acid (**19**) (2 g, 6·7 mmol), ethanol (1·5 mL, 41 mmol) and concentrated sulphuric acid (0·25 mL) was heated under reflux in benzene (100 mL) using the Dean and Stark apparatus. The reaction mixture was cooled and washed with water (3 × 25 mL), saturated sodium bicarbonate solution (3 × 25 mL) and then again with water (3 × 25 mL). The organic layer was dried ($MgSO_4$) and evaporated to give a yellow solid. This was crystallized from ethanol to give a white cystalline solid (1·37 g, 62%), m.p.123–124°C, υ_{max} (KBr)/cm^{-1} 1715 (C=O ester); δ_H ($CDCl_3$) 0·98 (3H, t, J = 8 Hz -CH_2CH_3), 4·05 (2H,q, J = 8 Hz,-OCH_2CH_3), 6·9–7·4 (15 H, m, aromatic).

2,3,3-Triphenyl-prop-2-en-1-ol (**21**). Lithium aluminium hydride (1 M solution in THF 3 mL, 3 mmol)was added dropwise to a solution of the ester (**20**) (1·35 g, 4·1 mmol) in anhydrous ether (25 mL). The mixture was stirred and refluxed for 2 h and upon cooling the excess lithium aluminium hydride destroyed by the dropwise addition of dilute hydrochloric acid (1 M). The organic fraction was washed with water (3 × 10 mL), dried ($MgSO_4$) and evaporated under reduced pressure to give an orange solid (1·11 g). This was crystallized from dichloromethane/petroleum ether 40–60°C (1 : 1) by vapour diffusion to give a white crystalline solid (0·7 g, 60%) m.p. 125·5–127°C, R_f = 0·6 (ether/petroleum ether 40–60° (1 : 1)), υ_{max} (KBr)/cm^{-1} 3560 and 3450 (OH); δ_H ($CDCl_3$) 1·47 (1H, br s, CH_2OH), 4·50(2H, s, -CH_2OH), 6·80–7·45 (15 H, m, aromatic).

A solution of the carboxylic acid (**10**) (0·18 g, 1·0 mmol) *N,N*-dicyclohexyl-carbodiimide (0·25 g, 1·2 mmol), the alcohol (**21**) (0·30 g, 1 mmol) and 4-pyrrolidinopyridine (41 mg, 0·3 mmol) in dichloromethane (25 mL) was stirred overnight at room temperature. The completion of the reaction was noted by thin-layer chromatography and the presence of the white precipitate of *N,N*-dicyclohexylurea. This was filtered and the filtrate washed with water (3 × 15 mL), 5% acetic acid (3 × 15 mL) and again with water (3 × 15 mL), dried ($MgSO_4$) and the solvent evaporated in-vacuo to give the crude ester. This was fractionated on a column of dry silica using petroleum ether 40–60°C/ether (1 : 1) as eluent. The third fraction was evaporated to give the pure ester (**22**) as a white crystalline solid (260 mg, 58%) m.p. 143–146°C, R_f = 0·15 (ether/petroleum ether (1 : 1)), 0·5 (chloroform/methanol (49 : 1)). Found : C,77·3; H,5·2; N,3·25%. $C_{29}H_{23}O_4N$ requires C, 77·49; H 5·12; N,3·12%). υ_{max} (KBr) cm^{-1} 3305 (≡CH), 3240 (NH), 1750 (C=O, imide),1735 (CO_2CH_2), 1715 (C=O, imide); δ_H ($CDCl_3$)1·98 (1H, t, J = 3 Hz, *H*C≡C-CH_2), 2·56 (1H, d, J_{BA} = 18 Hz, CH_A, $H_BCONHCO$-), 2·59 (1H, dd, J_{AB} = 17 Hz and J_{XB} = 3 Hz, CH_AH_B-C≡CH_X), 2·82 (1H, d, J_{AB} = 18 Hz, CH_AH_B-CONHCO-), 2·97 (1H, dd, J_{BA} = 17 Hz and J_{XA} = 3 Hz, CH_AH_B-C≡CHX), 4·96 (1H, d, J_{BA} = 12 Hz, $CO_2CH_AH_B$-), 5·05 (1H, d, J_{AB} = 12 Hz, $CO_2CH_AH_B$-), 6·80–7·50 (15 H, m, aromatic), 8·40 (1H, br s, -CON*H*CO-).

Results and Discussion

Our attempts to design an irreversible non-steroidal inhibitor of $P450_{AROM}$ were unsuccessful; however, reversible inhibitory activity was seen in some of the compounds. In the 3-alkyl-substituted compounds, **4** and **9** showed marginal inhibitory activity (18 and 15%, respectively), whereas of the remainder **1-3**, **5-6** and **7-8** were inactive (<10% inhibition). In the 3- substituted amide series, **11**, **13** and **14** exhibited potency (25, 45 and 48% inhibition, respectively) whereas the ester, **22**, had about half the potency of aminoglutethimide (Table 1).

It would seem that removal of the 4-aminophenyl sustituent (Fe^{3+}-coordinating domain) from the structure, decreased the binding properties to the active site. However, the binding profile was improved by the incorporation of a bulky hydrophobic moiety at the C-3 position (**13**, **14** and **22**), compound **22** being the most active.

Molecular modeling studies showed that the propynyl (propenyl) function in the low-energy conformation of the 3-alkyl-substituted compounds, as the R-enantiomer, and PED were juxta positioned on superimposition of the two molecules (Fig. 2), whereas in the 3-amide series, as the S-enantiomer, the propynyl (propenyl) function was somewhat displaced and overlay the B ring due to the interaction with the 3-carbonyl moiety. Consequently the lack of irreversible inhibitory action could be due to ineffective presentation of the reactive function to the haem iron in the reversibly bound amides and esters or transient (weak) binding to the active site of the 3-alkyl-substituted compound possessing the correct orientation for the oxidizable function. However an alternative view is that strong hydrophobic binding by the 3- substitutent in the amides and esters leads to more favourable overall non-specific binding with an associated displacement from the 5-carbonyl binding site and unfavourable orientation of the oxidizable function. If this is a correct prediction it seems unlikely that the correct balance between these binding moieties will be obtained in the type of molecule studied here to produce an irreversible inhibitor. It follows that future studies on the design of potential irreversible (mechanism based) non-steroidal inhibitors must pay attention to not only a strong binding parameter but use of the $P450_{AROM}$ substrate recognition sites to position the reactive function of the inhibitor precisely near the haem atom of the enzyme where the required metabolism takes place.

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