J. Pharm. Pharmacol. 1999, 51: 427–433
Received September 21, 1998
Accepted November 25, 1998

Inhibition of Aromatase ($P450_{Arom}$) by some 1-(Benzofuran-2-ylmethyl)imidazoles

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Abstract

Studies of a series of 1-(benzofuran-2-ylmethyl)imidazoles, **1**–**5**, previously proposed as potential agents for prostatic cancer by their inhibition of 17β-hydroxylase:17,20-lyase (P450 17), have been extended to their selectivity against placental microsomal aromatase ($P450_{Arom}$) in man.

The compounds were 3–7-fold more potent than aminoglutethimide and had some selectivity for P450 17 as expressed by the ratio (IC50 $P450_{Arom}$)/(IC50 P450) 17)/17·0 (**2**), 10·3 (**3**), 34·6 (**4**) and 42·0 (**5**), where IC50 is the concentration resulting in 50% inhibition. The lower potency of **1**–**5** towards $P450_{Arom}$ compared with the racemic α-phenyl-substituted compounds (**6**, 80–1000 × aminoglutethimide) and some racemic α-methyl (8·5 and 12·2 × aminoglutethimide) and α-ethyl (12·1 and 32·9 × aminoglutethimide) analogues has been rationalized.

This work selectively extends studies of the P450 17 inhibitor **5**, a potential prostatic cancer agent, towards other cytochrome P450 enzymes in the steroidogenic pathway and provides a general method for determining the relative influence of chemical manipulation of a parent inhibitor towards two enzymes in the pathway using additional literature data.

Aromatase ($P450_{Arom}$) catalyses the final step in the steroidogenesis pathway of oestrogens from cholesterol. In postmenopausal women androstenedione synthesized in the adrenals is converted by peripheral and breast $P450_{Arom}$ to oestrone and then to the potent oestradiol by the action of 17β-hydroxysteroid dehydrogenase. Aromatase has been the target for the design of inhibitors as agents in the treatment of breast cancer in postmenopausal women (Brodie 1994). Inhibition of the enzyme reduces plasma oestrogen levels and the stimulus to growth of metastases.

The first-generation reversible inhibitor, still used clinically, was aminoglutethimide (Shaw at al 1988; Santen 1990); this has been replaced by other more selective and potent non-steroidal inhibitors (Miller 1996). Irreversible steroidal inhibitors which are mechanism-based inactivators of the enzyme have also entered the clinic (Lombardi 1995; Miller 1996). Recently, we have described some non-steroidal mechanism-based inactivators (Saeed et al 1997).

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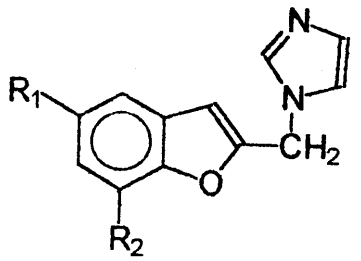
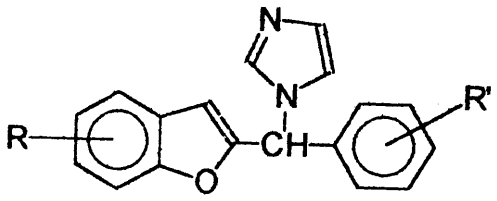
Substituted 1-[(benzofuran-2-yl)phenylmethyl]-imidazoles, **6**, are very potent, in-vitro reversible inhibitors of $P450_{Arom}$ (80–1000 × aminoglutethimide) (Whomsley et al 1993) but are non-selective because of high potency towards other cytochrome P450 enzymes in the steroidogenic pathway, i.e. 17α-hydroxylase:17,20-lyase (P450 17) and 11β-hydroxylase (P450 11β) (Al Hamrouni 1996; Al Hamrouni et al 1997). This lack of selectivity would make them unsuitable as agents for the treatment of breast cancer as cortisol and aldosterone production in-vivo would be affected. Modification of **6** by removal of the phenyl group gives the 1-(benzofuran-2-ylmethyl)imidazoles **1**–**5** which have been examined as selective inhibitors of human testicular and bovine adrenal microsomal P450 17 with a view to their use as potential agents for prostatic cancer (Bahshwan et al 1998).

Here we describe the synthesis of **1**–**5** and extend the selectivity studies to inhibition of $P450_{Arom}$ to determine the effect of the phenyl group on the potency and selectivity of the compounds as inhibitors of $P450_{Arom}$ and P450 17.

The structures of compounds **1**–**6** are given in Figure 1.



1 R_1 = H, R_2 = H
2 R_1 = Cl, R_2 = H
3 R_1 = Br, R_2 = H
4 R_1 = Cl, R_2 = Cl
5 R_1 = Br, R_2 = Br



6

Figure 1. The structures of compounds **1**–**6**.

Materials and Methods

Chemistry

The reagents used were either general purpose or analytical grade and were obtained from either Aldrich (Gillingham, Dorset, UK), BDH (Poole, Dorset, UK) or Lancaster Synthesis (Morecombe, Lancashire, UK).

Melting points were determined with an Electrothermal instrument and infrared spectra were determined with a Perkin-Elmer 681 infrared spectrophotometer. Thin-layer chromatography was performed on aluminium-backed silica gel plates with fluorescent indicator. ^{1}H Nuclear magnetic resonance (NMR) spectra were recorded, with TMS as an internal standard, on a Perkin-Elmer R32 (90 MHz) spectrophotometer. Mass spectra were acquired by the EPSRC National Mass Spectrometry Service Centre, University of Wales, Swansea. Elemental analyses were determined by the School of Pharmacy, University of London.

Synthesis

The imidazoles **1**–**5** were synthesized by the general method shown in Figure 2.

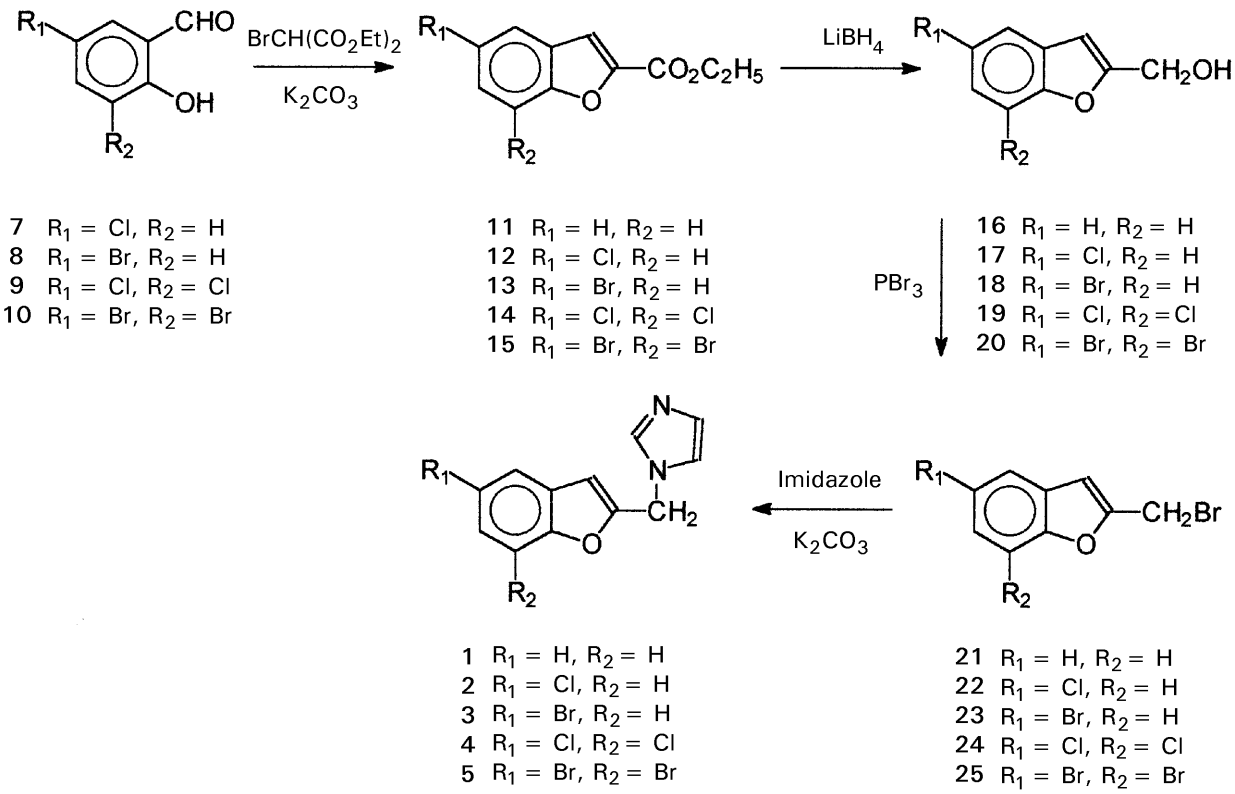


Figure 2. The synthesis of 1-(benzofuran-2-ylmethyl)imidazoles.

1-(Benzofuran-2-y methyl)imidazole, ***1***

2-Hydroxymethylbenzofuran ***16***. Methyl benzofuran-2-carboxylate (**11**) (2 g, 0·011 mol) was dissolved in tetrahydrofuran (THF) (30 mL) and to this was added dropwise, lithium borohydride (2·0 M solution in THF; 11·5 mL, 0·022 mol). The reaction mixture was heated under reflux for 6 h, the THF removed under vacuum and water (100 mL) added. The mixture was carefully acidified (HCl) and extracted with dichloromethane (2 × 50 mL). The combined organic layers were washed with water (3 × 100 mL) and dried ($MgSO_4$). The solvent was removed under vacuum to give 2-hydroxymethylbenzofuran as a pale yellow oil (yield 1·6 g, 95%). Found: C, 73·08; H, 5·39%; M^+ 148·052. $C_9H_8O_2$ requires C, 72·95; H, 5·45%; M^+ 148·052. ν_{max} (neat): 3350 (br. OH), 3035 (Ar, C−H), 2940, 2885 (C−H), 1605 (Ar, C=C) cm^{-1}. δ_H ($CDCl_3$): 7·3 (4H, m, Ar, CH), 6·55 (H, s, Ar, CH), 4·65 (2H, s, C*H*$_2$−OH), 3·17 (H, br. s, CH_2−O*H*).

2-Bromomethylbenzofuran, ***21***. 2-Hydroxymethylbenzofuran (**16**) (1·5 g, 0·01 mol) in anhydrous diethyl ether (150 mL) was cooled to 0°C. Phosphorous tribromide (1·5 mL, 0·016 mol) was added dropwise at a rate such that the temperature was kept constant, and DMF (1 mL) was then added. The resulting solution was stirred for 12 h at room temperature and was then poured cautiously on to ice. The ether layer was washed sequentially with water (3 × 100 mL), sodium bicarbonate solution (1 × 100 mL), and water (3 × 100 mL). The ether layer was dried ($MgSO_4$) and the ether removed under vacuum to give a yellow oil of 2-bromomethylbenzofuran (yield 1·3 g, 61%). Found: M^+ 209·9680. C_9H_7OBr requires M^+ 209·9680. ν_{max} (neat): 3020 (Ar C−H), 2880 (C−H), 1590 (Ar, C=C), 660 (C−Br) cm^{-1}. δ_H ($CDCl_3$): 7·35 (4H, m, Ar, CH), 6·75 (1H, s, Ar, C−H), 4·55 (2H, s, CH_2−Br).

1-(Benzofuran-2-ylmethyl)imidazole, ***1***. Imidazole (0·41 g, 0·006 mol) was added to anhydrous potassium carbonate (0·35 g, 0·0025 mol) and dry acetone (100 mL). The mixture was stirred at room temperature for 10 min and then 2-bromomethylbenzofuran (**21**) (1 g, 0·0047 mol) was added and stirring continued at room temperature for a further 24 h. After filtration, the acetone was removed under vacuum to leave a clear yellow oil which was dissolved in dichloromethane (50 mL) and extracted with hydrochloric acid (0·1 M, 3 × 50 mL). The combined acid layers were made alkaline with saturated sodium bicarbonate and then extracted with dichloromethane (2 × 50 mL). The combined dichloromethane layers were washed with water (2 × 50 mL), dried ($MgSO_4$) and filtered. Removal of the dichloromethane gave 1-(benzofuran-2-ylmethyl)imidazole as a pale buff solid. Crystallization (petroleum ether) gave off-white crystals (yield 550 mg, 59%), mp 77·8–79·8°C. Found: C, 72·29; H, 5·11; N, 14·02%; M^+ 198·0793. $C_{12}H_{10}ON_2$ requires C, 72·71; H, 5·08; N, 14·13%; M^+ 198·0793. ν_{max} (KBr): 3020 (Ar, C−H), 2970 (C−H), 1610 (Ar, C=C) cm^{-1}. δ_H ($CDCl_3$): 7·4 (7H, br. m, Ar, Im, C−H), 6·7 (1H, s, Ar, C−H), 5·3 (2H, s, CH_2).

1-(5-Chloro-benzofuran-2-ylmethyl)imidazole, ***2***

Ethyl 5-chlorobenzofuran-2-carboxylate, ***12***. 5-Chlorosalicylaldehyde (**7**) gave a pale yellow solid of ethyl 5-chlorobenzofuran-2-carboxylate which on crystallization (ethanol) gave pale-cream crystals (yield 66%); mp 49·5–50·7°C (Kurdukar & Subba Rao (1963); mp 65°C). Found: C, 58·55; H, 4·25%; M^+ 224·0240. $C_{11}H_9O_3Cl$ requires C, 58·8; H, 4·14%; M^+ 224·0240. ν_{max} (KBr): 3010 (Ar, C−H), 2990, 2950 (C−H), 1730 (C=O), 1590 (Ar, C=C), 1110 (C−Cl) cm^{-1}. δ_H ($CDCl_3$): 7·6 (4H, m, Ar, C−H), 4·55 (2H, q, J = 7 Hz, C*H*$_2$−CH_3), 1·55 (3H, t, J = 7 Hz, CH_2−C*H*$_3$).

5-Chloro-2-hydroxymethylbenzofuran, ***17***. Ethyl 5-chlorobenzofuran-2-carboxylate (**12**) gave 5-chloro-2-hydroxymethylbenzofuran as a pale yellow solid. Crystallization and recrystallization (petroleum ether) gave pale yellow crystals (90%), mp 80·5–82°C. Found: C, 59·72; H, 3·92%; M^+ 182·0135. $C_9H_7O_2Cl$ requires C, 59·34; H, 3·88%; M^+ 182·0135. ν_{max} (KBr): 3350 (br. OH), 2950 (C−H), 1610 (Ar, C=C), 1115 (Ar, C−Cl) cm^{-1}. δ_H ($CDCl_3$): 7·4 (3H, m, Ar, C−H), 6·62 (1H, s, Ar, C−H), 4·78 (2H, s, C*H*$_2$−OH), 2·05 (1H, br. s, CH_2−O*H*).

2-Bromomethyl-5-chlorobenzofuran, ***22***. 5-Chloro-2-hydroxymethylbenzofuran (**17**) gave 2-bromomethyl-5-chlorobenzofuran as a white solid. Crystallization (methanol) furnished white crystals (yield, 1·1 g, 82%), mp 82·9–84·3°C. Found M^+ 245·9270. C_9H_6OBrCl requires M^+ 245·9270. ν_{max} (KBr): 3055 (Ar C−H), 2975 (C−H), 1590 (Ar, C=C), 1060 (C−Cl), 695 (C−Br) cm^{-1}. δ_H ($CDCl_3$): 7·35 (3H, m, Ar, C−H), 6·71 (1H, s, Ar, C−H), 4·58 (2H, s, CH_2−Br).

1-(5-Chlorobenzofuran-2-ylmethyl)imidazole, ***2***. 2-Bromomethyl-5-chlorobenzofuran (**22**) gave 1-(5-

chloro-benzofuran-2-ylmethyl)imidazole as a cream solid. Crystallization and recrystallization (ethanol) gave clear crystals (yield 500 mg, 53%), mp 113·6–114·8°C. Found: C, 61·68, H, 3·97, N, 12·23%; M^+ 232·0403. $C_{12}H_9OClN_2$ requires C, 61·93; H, 3·90; N, 12·04%; M^+ 232·0403. ν_{max} (KBr): 3110 (Ar, C–H), 2980 (C–H), 1605 (Ar, C=C) cm^{-1}. δ_H ($CDCl_3$): 7·35 (6H, br. m, Ar, Im, C–H), 6·58 (1H, s, Ar, C–H), 5·22 (2H, s, CH_2–Im).

1-(5-Bromobenzofuran-2-ylmethyl)imidazole, ***3***

Ethyl 5-bromobenzofuran-2-carboxylate, ***13***. 5-Bromosalicylaldehyde (**8**) gave ethyl 5-bromobenzofuran-2-carboxylate as pale brown crystals (yield 60%) from ethanol, mp 53·5–54·8°C (Kurdukar & Subba Rao (1963); mp 90°C). Found: C, 49·56; H, 3·44%; M^+ 267·9735. $C_{11}H_9O_3Br$ requires C, 49·26; H, 3·38%; M^+ 267·9735. ν_{max} (KBr): 3100, 3070 (Ar, C–H), 2990 (C–H), 1740 (C=O), 1610 (Ar, C=C) cm^{-1}. δ_H ($CDCl_3$): 7·5 (4H, m, Ar, C–H), 4·5 (2H, q, J = 7 Hz, CH_2–CH_3), 1·48 (3H, t, J = 7 Hz, CH_2–CH_3).

5-Bromo-2-hydroxymethylbenzofuran, ***18***. Ethyl 5-bromobenzofuran-2-carboxylate (**13**) gave 5-bromo-2-hydroxymethylbenzofuran as a white solid (yield, 94%), mp 95·1–97·0°C (Dann et al (1982); mp 99–103°C). Found M^+ 225·9629. $C_9H_7O_2Br$ requires M^+ 225·9629. ν_{max} (Nujol): 3300 (br. OH), 1610 (Ar, C=C) cm^{-1}. δ_H ($CDCl_3$): 7·38 (3H, m, Ar, C–H), 6·84 (1H, s, Ar, C–H), 4·78 (2H, s, CH_2–OH), 2·18 (1H, br. s, CH_2–O*H*).

5-Bromo-2-bromomethylbenzofuran, ***23***. 5-Bromo-2-hydroxymethylbenzofuran (**18**) gave 5-bromo-2-bromomethylbenzofuran as a pale cream solid (yield 78%), mp 166·7–167·4°C (Dann et al (1982); mp 84–85°C) Found: M^+ 287·8786. $C_9H_6Br_2O$ requires M^+ 287·8786. ν_{max} (Nujol): 1610 (Ar, C=C), 670 (C–Br) cm^{-1}. δ_H ($CDCl_3$): 7·4 (3H, m, Ar, C–H), 6·85 (1H, s, Ar, C–H), 6·30 (2H, s, CH_2–Br).

1-(5-Bromobenzofuran-2-ylmethyl)imidazole, ***3***. 5-Bromo-2-bromomethylbenzofuran (**23**) gave 1-(5-bromobenzofuran-2-ylmethyl)imidazole as a pale cream solid. Crystallization (petroleum ether) gave off-white crystals, (yield 56%), mp 124–125·2°C. Found: C, 48·69; H, 3·68; N, 9·53%; M^+ 276·9898. $C_{12}H_9OBrN_2.H_2O$ requires C, 48·99; H, 3·76; N, 9·52%, M^+ 276·9898). ν_{max} (KBr): 3100 (Ar, C–H), 2990, 2930 (C–H), 1605 (Ar, C=C) cm^{-1}. δ_H ($CDCl_3$): 7·3 (6H, br. m, Ar, Im, CH), 6·61 (1H, s, Ar, C–H), 5·25 (2H, s, CH_2–Im).

1-(5,7-Dibromobenzofuran-2-ylmethyl)imidazole, ***4***

Ethyl 5,7-dichlorobenzofuran-2-carboxylate, ***14***. 3,5-Dichloro-2-hydroxybenzaldehyde (**9**) gave ethyl 5,7-dichlorobenzofuran-2-carboxylate as pale buff crystals (yield, 48%) from ethanol, mp 78·9–80·7°C (Kurdukar & Subba Rao (1963); mp 123–124°C). Found: C, 50·86; H, 3·12%; M^+ 257·9850. $C_{11}H_8O_3Cl_2$ requires C, 50·97; H, 3·11%; M^+ 257·9851. ν_{max} (KBr): 3090, 3010 (Ar, C–H), 2980, 2920 (C–H), 1725 (C=O), 1590 (Ar, C=C) cm^{-1}. δ_H($CDCl_3$): 7·55 (1H, d, J = 2 Hz, Ar, C–H), 7·45 (1H, d, J = 2 Hz, Ar, C–H), 7·28 (1H, s, Ar, C–H), 4·46 (2H, q, J = 7 Hz, $-CH_2CH_3$), 1·42 (3H, t, J = 7 Hz, $-CH_2CH_3$).

5,7-Dichloro-2-hydroxymethylbenzofuran, ***19***. Ethyl 5,7-dichlorobenzofuran-2-carboxylate (**14**) gave 2-hydroxymethyl-5,7-dichlorobenzofuran as a white solid. Crystallization (petroleum ether) furnished white crystals (yield 95%), mp 113·5–114·5°C. Found: C, 50·21; H, 2·77%; M^+ 215·9745. $C_9H_6Cl_2O_2$ requires C, 50·00; H, 2·80%; M^+ 215·9745. ν_{max} (KBr): 3250 (br. OH), 3100 (Ar, C–H), 2970, 2880 (C–H), 1610 (Ar, C=C) cm^{-1}. δ_H ($CDCl_3$): 7·36 (1H, d, J = 2 Hz, Ar, C–H), 7·29 (1H, d, J = 2 Hz, Ar, C–H), 6·66 (1H, s, Ar, C–H), 4·82 (2H, s, CH_2OH), 2·2 (1H, br. s, CH_2O*H*).

2-Bromomethyl-5,7-dichlorobenzofuran, ***24***. 5,7-Dichloro-2-hydroxymethylbenzofuran (**19**) gave 2-bromomethyl-5,7-dichlorobenzofuran as a white solid. Crystallization (ethanol) furnished white crystals (yield 71%), mp 67·1–68·5°C. Found: C, 38·54; H, 1·78%; M^+ 279·8880. $C_9H_5OBrCl_2$ requires C, 38·59; H, 1·80%; M^+ 279·8881. ν_{max} (KBr): 3040 (Ar, C–H), 2980 (C–H), 1595 (Ar, C=C) cm^{-1}. δ_H ($CDCl_3$): 7·43 (1H, d, J = 2 Hz, Ar, C–H), 7·34 (1H, d, J = 2 Hz, Ar, C–H), 6·78 (1H, s, Ar, C–H), 4·57 (2H, s, CH_2–Br).

1-(5,7-Dichlorobenzofuran-2-ylmethyl)imidazole, ***4***. 2-Bromomethyl-5,7-dichlorobenzofuran (**24**) gave 1-(5,7-dichlorobenzofuran-2-ylmethyl)imidazole as a white solid. Crystallization (diethyl ether) gave white needles (yield 42%), mp 82·8–83·7°C. Found: C, 52·89; H, 3·22; N, 9·97%; M^+ 266·0014. $C_{12}H_8OCl_2N_2.5H_2O$ requires C, 52·38; H, 3·29; N, 10·15%; M^+ 266·0014. ν_{max} (KBr):

3100 (Ar, C−H), 1610 (Ar, C=C) cm^{-1}. δ_H ($CDCl_3$): 7·65 (1H, br. s, Im, C−H), 7·42 (1H, d, J = 2 Hz, Ar, C−H), 7·32 (1H, d, J = 2 Hz, Ar, C−H), 7·1 (2H, m, Im, C−H), 6·59 (1H, s, Ar, C−H), 5·28 (2H, s, CH_2-Im).

1-(5,7-Dibromobenzofuran-2-ylmethyl)imidazole, ***5***

Ethyl 5,7-dibromobenzofuran-2-carboxylate, ***15***. 3,5-Dibromosalicylaldehyde (**10**) gave ethyl 5,7-dibromobenzofuran-2-carboxylate as pale cream crystals (yield 2·4 g, 66%) from ethanol, mp 106·9–108°C (Kurdukar & Subba Rao (1963); mp 102–103°C). Found M^+ 347·8820. $C_{11}H_8O_3Br_2$ requires M^+ 347·8820. ν_{max} (Nujol): 3040 (Ar, C−H), 1720 (C=O), 1590 (Ar, C=C) cm^{-1}. δ_H ($CDCl_3$): 7·75 (2H, m, Ar, C−H), 7·51 (1H, s, Ar, C−H), 4·5 (2H, q, J = 7 Hz, CH_2−CH_3), 1·46 (3H, t, J = 7 Hz, CH_2−CH_3).

5,7-Dibromo-2-hydroxymethylbenzofuran, ***20***. Ethyl 5,7-dibromobenzofuran-2-carboxylate (**15**) gave 5,7-dibromo-2-hydroxymethylbenzofuran as a white solid. Crystallization (petroleum ether) furnished white crystals (yield 94%), mp 130·2–131·5°C. Found M^+ 305·8714. $C_9H_6O_2Br_2$ requires M^+ 305·8714. ν_{max} (KBr): 3210 (br. OH), 3080 (Ar, C−H), 2945 (C−H), 1600 (Ar, C=C) cm^{-1}. δ_H ($CDCl_3$): 7·65 (2H, m, Ar, C−H), 6·72 (1H, s, Ar, C−H), 4·85 (2H, s, CH_2−OH), 2·0 (1H, br. s, CH_2−O*H*).

2-Bromomethyl-5,7-dibromobenzofuran, ***25***. 5,7-Dibromo-2-hydroxymethylbenzofuran (**20**) gave 2-bromomethyl-5,7-dibromobenzofuran as a white solid. Crystallization (ethanol) produced white crystals (yield 500 mg, 83%), mp 91·7–93°C. Found M^+ 367·7870. $C_9H_5OBr_3$ requires M^+ 367·7870. ν_{max} (Nujol): 3030 (Ar, C−H), 1595 (Ar, C=C), 670 (C−Br) cm^{-1}. δ_H ($CDCl_3$): 7·62 (2H, m, Ar, C−H), 6·78 (H, s, Ar, C−H), 4·6 (2H, s, CH_2−Br).

1-(5,7-Dibromobenzofuran-2-ylmethyl)imidazole, ***5***. 2-Bromomethyl-5,7-dibromobenzofuran (**25**) gave 1-(5,7-dibromobenzofuran-2-ylmethyl)imidazole as a pale brown solid. Crystallization (petroleum ether) gave cream crystals (yield 260 mg, 67%), mp 121·7–123·1°C. Found: C, 40·14; H, 2·29; N, 7·76%; M^+ 355·8983. $C_{12}H_8OBr_2N_2$ requires C, 40·45; H, 2·25; N, 7·86%; M^+ 355·8983. ν_{max} (KBr): 3070 (Ar, C−H), 2990 (C−H), 1605 (Ar, C=C) cm^{-1}. δ_H ($CDCl_3$): 7·64 (3H, m, Ar, Im, C−H), 7·15 (2H, b. m, Im, C−H), 6·61 (H, s, Ar, C−H), 5·25 (2H, s, CH_2−Im).

Biochemistry

All non-radioactive steroids, NADPH (mono sodium salt), D-glucose-6-phosphate (mono sodium salt) and protein standards were from Sigma (Poole, Dorset). All unlabelled laboratory reagents were Analar grade and from BDH. D-Glucose-6-phosphate dehydrogenase (suspension in ammonium sulphate) was from Boehringer Mannheim (Mannheim, Germany). [1β,2β-^{3}H]Androstenedione (40 Ci $mmol^{-1}$, in ethanol) was from NEN, Dupont (UK), Stevenage, Hertfordshire, UK. Aminoglutethimide was a gift from Ciba-Geigy, Horsham, Sussex, UK.

Radioactivity was measured with a LKB Wallac 1217 Rackbeta liquid scintillation counter. Scintillation fluid was Optiphase III (Hisafe) from Pharmacia LKB.

Preliminary homogenization of placental tissue was performed with a Kenwood Chef blender. Low-speed centrifugation was performed with a MSE Europa 65M Ultracentrifuge.

The NADPH-generating system consisted of D-glucose-6-phosphate (28·2 mg), NADP (8·6 mg), D-glucose-6-phosphate dehydrogenase (4 international units mL^{-1}, 15 μL) and phosphate buffer pH 7·4 (50 mM, 1 mL).

$P450_{Arom}$ assay

Placental microsomes were prepared by the method of Thompson & Siiteri (1974). The assay followed was that described by Khodarahmi et al (1998). Incubation mixtures (0·5 mL) in triplicate containing [1β,2β-^{3}H]androstenedione, unlabelled substrate (10 μL, 0·5 μM final concentration) and NADPH-generating system (50 μL) in phosphate buffer (50 mM, pH 7·4) were warmed to 37°C in a shaking water-bath. The placental microsomes were thawed and warmed to 37°C before addition (20 μL, 0·452 mg mL^{-1} final concentration) to the incubation mixtures. After incubation for 6 min, a sample (300 μL) from each assay tube was added to 1% activated charcoal (900 μL) and mercuric chloride (1 mM, 300 μL) and mixed thoroughly. After standing on ice for 15 min the tubes were centrifuged for 20 min at 3000 rev min^{-1} (2000 *g*). Samples (500 μL) of the supernatant from each tube were then removed and dispersed in 2 mL scintillation fluid and counted (^{3}H) for 1 min.

Inhibition studies

Inhibitors **1**–**5** in ethanol (10 μL) were included in the assay procedure which was conducted in triplicate. Controls (10 μL, ethanol) were also incubated and aminoglutethimide was used as a

Table 1. Some substituted 1-(benzofuran-2-ylmethyl)imidazoles as inhibitors of placenta microsomal $P450_{Arom}$ and testicular microsomal P450 17[a] in man.

Compound	$P450_{Arom}$ IC50 (μM)[b]	P450 17 IC50 (μM)[a]
Aminoglutethimide	28·69 ± 1·65	–
Compound **1**	7·32 ± 0·12	ND
Compound **2**	3·9 ± 0·02	0·23 ± 0·043
Compound **3**	3·91 ± 0·08	0·38 ± 0·04
Compound **4**	6·25 ± 0·3	0·18 ± 0·033
Compound **5**	8·06 ± 0·1	0·185 ± 0·021
Ketoconazole	–	0·029 ± 0·003

Values are means ± s.d. of results from three determinations, each in triplicate. [a]From Bahshwan et al (1998). [b]Androstenedione, 0·5 μM. IC50 is the concentration resulting in 50% inhibition. ND = not determined.

standard for comparative purposes. The percentage conversion of androstenedione to oestrone and oestradiol was calculated and the percentage inhibition determined by comparing the conversion in the presence of inhibitors with that of the controls. IC50 values (amounts resulting in 50% inhibition) were determined by use of a range of inhibitor concentrations, from a plot of percentage activity remaining against log [inhibitor], using Cricket Graph (Table 1). All points were means of three determinations, each conducted in triplicate and r^2 was in the range 0·987–0·995.

Results and Discussion

We have found that a series of 16 substituted 1-[(benzofuran-2-yl)phenylmethyl]imidazoles **6** (Whomsley et al 1993) were potent inhibitors of microsomal testicular and bovine adrenal P450 17 from man (Al Hamrouni et al 1997). We have modified the general structure (**6**) by removal of the phenyl group to give the 1-(benzofuran-2-ylmethyl)imidazoles, **2**–**5**, which as inhibitors of P450 17 were evaluated as potential anti-prostatic agents. Their specificity towards other steroidogenic and liver enzymes was compared with that of ketoconazole (Bahshwan et al 1998). All four compounds were inhibitors of the testicular enzyme (**4**, IC50 = 0·18 μM; **5**, 0·185 μM) but less potent than ketoconazole (0·03 μM). Towards bovine adrenal enzyme, **4** and **5** were 35- and 31-fold, respectively, more potent than ketoconazole (IC50 = 39·8 μM). Compound **5** was considered a useful lead compound; although less potent than ketoconazole towards P450scc and P45011β, but not P450c21, at the enhanced dose required for equivalent effects in-vivo on P450 17 it is likely that cortisol and aldosterone production will be affected to a greater extent than with ketoconazole. The 1-[(benzofuran-2-yl)phenylmethyl]imidazoles **6** were also potent inhibitors of $P450_{Arom}$ (IC50 = 7·3–91·8 nM), 80–100-fold more potent than aminoglutethimide (Whomsley et al 1993). Here the imidazoles **1**–**5** have been examined for their additional selectivity towards the target P450 17 enzyme by evaluation of their potency towards $P450_{Arom}$.

The 1-(benzofuran-2-ylmethyl)imidazoles **1**–**5** were 3–7-fold (approx.) more potent than aminoglutethimide (Table 1) towards aromatase, the 5-chloro- and 5-bromo-substituted compounds, **2** and **3**, respectively, being the most potent with IC50 values of 3·9 μM (aminoglutethimide, 28·7 μM).

The in-vitro selectivity of **2**–**5**, as inhibitors of testicular P450 17 in man (Table 1; Bahshwan et al (1998)), towards $P450_{Arom}$ can be expressed by the ratio (IC50 $P450_{Arom}$) / (IC50 P450 17) although this is not an absolute value because IC50 values are substrate-dependent. Values of 17·0 (**2**), 10·3 (**3**), 34·6 (**4**) and 42·0 (**5**) were obtained, implying reasonable selectivity for **4** and **5**. In the context of the role of **5** as a potential prostatic cancer agent targetting P450 17, the level of selectivity towards $P450_{Arom}$ is considered unimportant. This is because inhibition of the low level of $P450_{Arom}$ activity present in males is unlikely to enhance conversion of androstenedione (breaking through because of incomplete inhibition of P450 17) by alternative pathways to its main androgen metabolites, testosterone and dihydrotestosterone, with an associated stimulus to prostatic growth.

Within the two series of related compounds, **6** and **1**–**5**, as inhibitors of $P450_{Arom}$ and P450 17, the overall effect of removal of the α-phenyl group was determined by normalizing the potency in each series to aminoglutethimide and ketoconazole, respectively. Comparisons of the ratio (relative potency to aminoglutethimide)/(relative potency to ketoconazole) for **1**–**5** gave values of 22·8–96·2 whereas for **6** (4-Cl-, 4-F-, 2-CH_3-, 4-CH_3- and 2-OCH_3 phenyl derivatives; (Whomsley et al 1993; Al Hamrouni et al 1997)), the values were 566–9150. These different ratios indicate that removal of the α-phenyl group in **6**, to give **2**–**5**, leads to a reduction in potency towards $P450_{Arom}$ relative to P450 17. Direct comparison of IC50 values for either series with either enzyme was not possible because different workers used different substrate concentrations, and this affects measurements of IC50 values. The considerable reduction in potency towards $P450_{Arom}$ observed on removal of the phenyl group in the imidazoles **1**–**5** can be explained on the basis of the mode of binding of **6** to the active site (Khodarahmi 1996) of a model of aromatase (Laughton et al 1993). The imidazole ring is coordinated to the haem-Fe^{3+} through N3,

and the phenyl and benzofuran rings bind in the hydrophobic steroidal substrate backbone binding cavity and the hydrophobic cavity below the A ring of the steroid (Khodarahmi 1996). The positions taken by the two ring structures are interchangeable as suggested by the high potency for both enantiomers of the 4′-chloro- and 4′-fluoro (phenyl ring) compounds with IC50 values of 8·4 nM ((+) form), 8·4 nM ((−) form) and 5·3 nM ((+) form), 65·0 nM ((−) form), respectively (Khodarahmi 1996). The 1-(benzofuran-2-ylmethyl)imidazoles **1**–**5** lacking the phenyl substituent of **6** could adopt either of the positions occupied by the benzofuran group in the enantiomers of **6** but with considerably reduced binding and potency.

The racemic dichloro (**4**) and dibromo (**5**) compounds were 4·8- and 3·5-fold more potent than aminoglutethimide but less active than the corresponding racemic α-methyl analogues (12·2 and 8·5 × aminoglutethimide, respectively) and α-ethyl analogues (32·9 and 12·1 × aminoglutethimide, respectively) (Khodarahmi et al 1998). These differences in potency reflect the additional binding as a result of the hydrophobic methyl and ethyl residues.

Overall this work extends selectively studies of the P450 17 inhibitor **5**, a potential prostatic cancer agent, toward other cytochrome P450 enzymes in the steroidogenic pathway. It also provides a general method for determining the relative influence of chemical manipulation of a parent inhibitor towards two enzymes in the pathway using additional literature data.

Acknowledgements

We wish to thank the Cancer Research Campaign for financial support and the EPSRC National Mass Spectrometry Service Centre, University of Wales, Swansea, for the mass spectra determinations.

References

Al Hamrouni, A. M. (1996) Studies on Tissue Selectivity of inhibitors of P450 17. MPhil. Thesis, University of Wales

Al Hamrouni, A., Ahmadi, M., Nicholls, P. J., Smith, H. J., Lombardi, P., Pestellini, V. (1997) 1-[(Benzofuran-2-yl)phenylmethyl]imidazoles as inhibitors of 17α-hydroxylase:17,20-lyase (P450 17): species and tissues differences. Pharm. Sci. 3: 259–263

Bahshwan, S. A., Owen, C. P., Nicholls, P. J., Smith, H. J., Ahmadi, M. (1998) Some 1-[(benzofuran-2yl)methyl]imidazoles as inhibitors of 17α-hydroxylase:17,20-lyase (P450 17) and their specificity patterns. J. Pharm. Pharmacol. 50: 1109–1116

Brodie, A. (1994) Aromatase inhibitors. In: Sandler, M., Smith, H. J. (eds) Design of Enzyme Inhibitors as Drugs. Vol. 2. Oxford University Press, Oxford, pp 424–438

Dann, O., Char, H., Greissmeir, H. (1982) Synthesis of biscationic trypanocidal 1-benzofuran compounds. Liebigs Ann. Chem. Part 10. 1836–1869

Khodarahmi, G. A. (1996) Synthesis, Chirality and Molecular Modelling Studies on Some Aromatase Inhibitors as Potential Agents for the Treatment of Breast Cancer. PhD. Thesis, University of Wales

Khodarahmi, G. A., Smith, H. J., Nicholls, P. J., Ahmadi, M. (1998) Enantioselectivity of some 1-(benzofuran-2-yl)-1-(1-*H*-imidazol-1-yl) alkanes as inhibitors of $P450_{Arom}$. J. Pharm. Pharmacol. 50: 1321–1330

Kurdukar, R., Subba Rao, N. V. (1963) Physiologically active compounds. VII. Synthesis of halo and nitro coumarones. Proc. Indian Acad. Sci. Sect. A 58: 336–342

Laughton, A. C., Zvelebil, M., Neidle, S. (1993) A detailed molecular model for human aromatase. J. Steroid Biochem. Mol. Biol. 44: 399–407

Lombardi, P. (1995) The irreversible inhibition of aromatase (oestrogen sythetase) by steroidal compounds. Curr. Pharm. Des. 1: 23–50

Miller, W. R. (1996) Aromatase inhibitors. Endocr. Rel. Cancers 3: 65–79

Saeed, G., Khodarahmi, A., Fradgley, S. J., Smith, H. J., Nicholls, P. J., King, M., Ahmadi, M. (1997) Non-steroidal irreversible inhibitors of aromatase based on *N*-pentyl-3-phenylpyrrolidine-2,5-dione. Pharm. Sci. 3: 265–277

Santen, R. J. (1990) Clinical use of aromatase inhibitors: current data and future perspectives. J. Enzyme Inhib. 4: 79–99

Shaw, M. A., Nicholls, P. J., Smith, H. J. (1988) Aminoglutethimide and ketoconazole: historical perspectives and future prospects. J. Steroid Biochem. 31: 137–146

Thompson, E. A., Siiteri, P. K. (1974) The involvement of human placental microsomal cytochrome P-450 in aromatization. J. Biol. Chem. 249: 5373–5378

Whomsley, R., Fernandez, E., Nicholls, P. J., Smith. H. J., Lombardi, P., Pestellini, V. (1993) Substituted 1-[(benzofuran-2-yl)phenylmethyl]imidazoles as potent inhibitors of aromatase in vitro and in female rats in vivo. J. Steroid Biochem. Mol. Biol. 44: 675–676