Journal of Enzyme Inhibition and Medicinal Chemistry, February 2004 Vol. 19 (1), pp. 17–32

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by Malmo Hogskola on 12/23/11
For personal use only.

Synthesis of Hydroxy Derivatives of Highly Potent Non-steroidal CYP 17 Inhibitors as Potential Metabolites and Evaluation of their Activity by a Non Cellular Assay using Recombinant Human Enzyme

TILMAN U. HUTSCHENREUTER, PETER B. EHMER and ROLF W. HARTMANN*

8.5 Pharmaceutical and Medicinal Chemistry, Saarland University, P.O. Box 151150, D-66041 Saarbrücken, Germany

(Received 25 July 2003; In final form 25 September 2003)

Inhibition of CYP 17 is a promising strategy for the treatment of prostate cancer. Recently two non-steroidal compounds with high *in vitro* activity were synthesized in our group (BW19 and BW95). However, after a few hours they showed *in vivo* a strong decrease in their activity. This might be due to a fast biodegradation. Potential hydroxy and epoxy metabolites were synthesized and their inhibitory activities were tested by a new non-cellular assay using recombinant enzyme. As source, membrane fractions of *E. coli* pJL17/OR coexpressing human CYP 17 and rat NADPH-P450-reductase were used. Showing a high and constant CYP 17 activity and a fast and easy isolation procedure the new method was advantageous compared with the microsomal assay. Interestingly, all the new synthesized hydroxy and epoxy compounds except one showed a lower inhibition of CYP 17 than the parent compounds. Thus, the loss of *in vivo* activity may be partly explained.

Keywords: Prostate cancer; CYP 17; 17α-Hydroxylase-C17, 20-lyase; Non-steroidal inhibitors; Non-cellular assay; Metabolites

INTRODUCTION

A modern treatment option for hormone dependent prostate cancer aims at the reduction of circulating androgens. The best target for this strategy is CYP 17, which catalyzes the last step in androgen biosynthesis: 17α-hydroxylation of pregnenolone or progesterone followed by a C17-20-lyase step.[1]

Several classes of steroidal[2–5] and non-steroidal[6–10] inhibitors of CYP 17 have been synthesized recently. The potencies of these compounds have been evaluated by us and others using microsomal preparations from human and rat testes.[8,11] The availability of human tissue, however, is limited and the corresponding enzyme preparations do not result in constant activity values, which usually are at a low level. This caused us to look for a new enzyme source for inhibitor testing.

In the present paper we describe the isolation of recombinantly expressed CYP 17 (coexpressed with NADPH-P450-reductase) from *E. coli* yielding a high activity enzyme preparation and the development of a non-cellular assay for CYP 17 inhibitors. The inhibitory data of two important reference compounds in this system are compared to the results of the testicular microsomal assay and a recently established whole cell test.[12] Further we describe the synthesis of hydroxy and methoxy derivatives of two *in vivo* active CYP 17 inhibitors **BW19**[10] and **BW95**.[8] Especially compound **BW95**, which was *in vitro* almost as potent as **BW19**, but showed in rats a complete loss of activity after 6 hours. This could be due to a fast biodegradation of **BW95**.[8] Alternatively the drug candidate **BW19** reached the clinical trial phase I.[10] To determine the metabolic fate of the two parent compounds the hydroxy metabolites were synthesized and their inhibitory activities towards CYP 17 and CYP 19 were evaluated and compared with the activities of the parent compounds.

*Corresponding author. Tel.: +49 681 302 2424. Fax: +49 681 302 4386. E-mail: rwh@mx.uni-saarland.de

ISSN 1475-6366 print/ISSN 1475-6374 online © 2004 Taylor & Francis Ltd
DOI: 10.1080/14756360310001640913

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by Malmo Hogskola on 12/23/11
For personal use only.

MATERIALS AND METHODS

General

Materials were obtained from commercial sources and checked using refraction index for liquids and melting points for solids. Anhydrous acetone was dried over molecular sieves (0.3 nm) and distilled. Reactions at low temperatures were performed using cold baths: water / ice at 0°C and acetone / dry ice at −78°C. Melting point ranges (mp) are measured with a Stuart Melting Point Apparatus SMP3. They were reproducible after resolidification and are uncorrected. Column chromatography was performed on silica gel 60 F_{254} (Merck, Darmstadt, Germany). IR spectra were measured using a Vector 33 (Bruker, Karlsruhe, Germany) with a Zn/Se ATR-unit (ATR Harrick MVP, Bruker, Karlsruhe, Germany). ^{1}H-NMR and ^{13}C-NMR spectra were measured on a DRX 500 (Bruker, Karlsruhe, Germany) at 500 MHz or 125 MHz in d_6-DMSO; chemical shift δ in ppm are relative to tetramethylsilane ($\delta = 0.00$) or relative to deuterated solvent. Elementary analyses indicated by the symbols of the elements were within ± 0.4% of the theoretical values and were performed by the Institute of Inorganic Chemistry, Saarland University, Saarbrücken, Germany.

Synthesis

Method A

4-(6-Methoxy-1-methyl-3,4-dihydro-naphthalen-2-ylmethyl)-1-trityl-1H-imidazole (**1a**)

BW19 (0.5 g, 2.0 mmol) and trityl chloride (0.68 g, 2.2 mmol) were dissolved in anhydrous acetone (50 mL). Anhydrous K_2CO_3 (0.8 g, 5.8 mmol) and a few crystals of 18-crown-6 (18C6) were added and the mixture was stirred for 4 h under a nitrogen atmosphere at 30°C. After reaction was complete the suspension was filtered and the filtrate evaporated. The crude product was purified by recrystallization from diethyl ether–hexane (1:1, v/v). Yield: 83%, white solid, mp: 180–182°C. ^{1}H-NMR (d_6-DMSO) δ 2.02 (s, 3H, —CH_3), 2.16 (t, $^{3}J = 7.3$ Hz, 2H, Ar—CH_2—CH_2—), 2.61 (t, $^{3}J = 7.3$ Hz, 2H, Ar—CH_2—), 3.45 (s, 2H, —CH_2—Im), 3.72 (s, 3H, —OCH_3), 6.70–6.75 (m, $^{3}J = 8.2$ Hz, 2H, Ar H5, H7), 6.77 (s, 1H, Im H5), 7.10 (m, 9H, Tr H2, H4, H6), 7.17 (m, 7H, Ar H8, Tr H3, H5), 7.60 (s, 1H, Im H2). Anal. ($C_{35}H_{32}N_2O$) C, H, N.

Compounds **2a**, **3a**, **3a′**, and **15a** were synthesized as described for to **1a**.

6-Methoxy-2-(1-trityl-1H-imidazol-4-ylmethyl)-7-trityloxy-3,4-dihydro-2H-naphthalen-1-one (**2a**)

With **14** a white solid was obtained. Yield: 90%, mp: 188–190°C. ^{1}H-NMR (d_6-DMSO) δ 1.65–2.02 (m, 2H, Ar—CH_2—CH_2—), 2.58–2.74 (m, 2H, Ar—CH_2—), 2.68 (m, 2H,—CH_2—Im), 3.37 (m, 1H, >CH—), 3.71 (s, 3H, —OCH_3), 6.45 (s, 1H, Im H5), 6.58 (s, 1H, Ar H5), 7.08 (m, 6H, -N-Tr H3, H4), 7.19 (m, 15H, -O-Tr H2, H3, H4, H5, H6), 7.38 (m, 9H, -N-Tr H2, H4, H6), 7.47 (s, 1H, Ar H8), 8.50 (s, 1H, Im H2). Anal. ($C_{53}H_{44}N_2O_3$) C, H, N.

4-(6,7-Dimethoxy-1-methyl-3,4-dihydro-naphthalen-2-ylmethyl)-1-trityl-1H-imidazole (**3a**)

With **15** a white solid was obtained. Yield: 86%, mp: 190–193°C. ^{1}H-NMR (d_6-DMSO) δ 2.05 (s, 3H, —CH_3), 2.16 (t, $^{3}J = 7.3$ Hz, 2H, Ar—CH_2—CH_2—), 2.61 (t, $^{3}J = 7.3$ Hz, 2H, Ar—CH_2—), 3.25 (s, 2H, —CH_2—Im), 3.72 (s, 3H, —OCH_3), 6.55 (s, 1H, Ar H5), 6.65 (s, 1H, Ar H8), 7.08 (m, 10H, Im H2, Tr H2, H4, H6), 7.17 (m, 6H, Tr H3, H5), 7.40 (s, 1H, Im H2). Anal. ($C_{36}H_{34}N_2O_2$) C, H, N.

2-(1-Trityl-1H-imidazol-4-ylmethyl)-6,7-bis-trityloxy-3,4-dihydro-2H-naphthalen-1-one (**3a′**)

With **16** a white solid was obtained. Yield: 83%, mp: 211–213°C. ^{1}H-NMR (d_6-DMSO) δ 1.60–2.05 (m, 2H, Ar—CH_2—CH_2—), 2.20–2.50 (m, 2H, Ar—CH_2—), 2.70 (s, 2H, —CH_2—Im), 3.27 (m, 1H, >CH—), 6.65 (s, 1H, Ar H5), 6.74 (s, 1H, Im H5), 7.05 (m, 9H, N-Tr H2, H4, H6), 7.17 (m, 39H, N-Tr H2, H4, H6; 6-O-Tr & 7-O-Tr H2, H3, H4, H5, H6), 7.33 (d, $^{3}J = 8.2$ Hz, 1H, Ar H8), 7.59 (s, 1H, Im H2). Anal. ($C_{71}H_{56}N_2O_3$) C, H, N.

6,7-Dimethoxy-2-(1-trityl-1H-imidazol-4-ylmethyl)-3,4-dihydro-2H-naphthalen-1-one (**15a**)

With **13** a white solid was obtained. Yield: 93%, mp: 214–216°C. ^{1}H-NMR (d_6-DMSO) δ 1.65–2.02 (m, 2H, Ar—CH_2—CH_2—), 2.53–2.74 (m, 2H, Ar—CH_2—), 2.88 (m, 2H, —CH_2—Im), 3.31 (m, 1H, >CH—), 3.77 (s, 3H, 7-OCH_3), 3.83 (s, 3H, 6-OCH_3), 6.78 (s, 1H, Im H5), 6.87 (s, 1H, Ar H5), 7.08 (m, 9H, Tr H2, H4, H6), 7.27 (s, 1H, Im H2), 7.38 (m, 7H, Ar H8, Tr H3, H5), 7.52 (s, 1H, Im H2). Anal. ($C_{34}H_{34}N_2O_2$) C, H, N.

Method B

6-(3H-Imidazol-4-ylmethyl)-5-methyl-7,8-dihydro-naphthalen-2-ol HCl (**1**)

A solution of **1a** (1.0 g, 2.0 mmol) in anhydrous CH_2Cl_2 (80 mL) was cooled to −78°C. Under an atmosphere of nitrogen, boron tribromide (0.43 mL, 4.2 mmol) was added slowly. After 30 min of stirring at −78°C and 3 h at 25°C, methanol (2.3 mL) was added dropwise. The solution was evaporated to dryness and the resulting residue suspended in 1 M aqueous sulfuric acid (20 mL). The mixture was stirred at 25°C for 15 min until the trityl group was cleaved, then was washed several times with diethyl ether (10 mL) and neutralized with saturated $NaHCO_3$ (25 mL). The precipitating crude product

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by Malmo Hogskola on 12/23/11
For personal use only.

was filtered and purified by column chromatography using ethyl acetate–ethanol (9: 1, v/v) and recrystallized from *i*-propanol–ethyl acetate (1: 1, v/v). The resulting compound was dissolved in diethyl ether/methanol (4: 1, v/v). 2 M HCl in diethyl ether was added until the product crystallized. Yield: 48%, white crystals, mp: 160°C. IR (Zn/Se-ATR): $\nu = 3140, 1611, 1442, 1242, 824, 616\,cm^{-1}$. ^{1}H-NMR ($d_6$-DMSO) δ 1.99 (s, 3H, —$CH_3$), 2.12 (t, $^3J = 7.2$ Hz, 2H, Ar—CH_2—), 2.54 (t, $^3J = 7.2$ Hz, 2H, Ar—CH_2—CH_2—), 3.42 (s, 2H, —CH_2—Im), 6.51 (d, 1H, Ar H5), 6.56 (dd, 1H, Ar H7), 6.73 (s, 1H, Im H5), 7.04 (d, 1H, Ar H8), 7.53 (s, 1H, Im H2), 9.21 (bs, 1H, —OH, exchangeable with D_2O). ^{13}C—NMR (d_6—DMSO) δ 17.5 (—CH_3), 26.7 (—CH_2—Im), 30.5 (C4, Ar—CH_2—), 31.4 (C3, Ar—CH_2—CH_2—), 65.2 (C1), 77.3 (C2), 112.6 (C7), 114.7 (C5), 116.0 (Im C5), 127.1 (C8), 132.6 (C8a) 134.3 (Im C4), 135.3 (Im C2), 137.7 (C4a), 154.1 (Ar C6). Anal. ($C_{15}H_{16}N_2O \cdot HCl \cdot H_2O$) C, H, N.

Compounds **3** and **8** were synthesized as described for **1a**.

7-(1H-Imidazol-4-ylmethyl)-8-methyl-5,6-dihydro-naphthalen-2,3-diol (**3**)

With **15** a white solid was obtained. Yield: 27%, mp: 178–180°C. IR (Zn/Se-ATR): $\nu = 3420, 2972, 2901, 1622, 1605, 1067, 700, 568, 535\,cm^{-1}$. ^{1}H-NMR ($d_6$-DMSO) δ 2.02 (s, 3H, —$CH_3$), 2.19 (t, $^3J = 7.3$ Hz, 2H, Ar—CH_2—CH_2—), 2.59 (t, $^3J = 7.3$ Hz, 2H, Ar—CH_2—), 3.33 (s, 2H, —CH_2—Im), 4.20 (bs, 2H, 6-OH, 7—OH; exchangeable with D_2O), 6.48 (d, 2H, Ar H5, Ar H8), 6.81 (s, 1H, Im H5), 7.60 (s, 1H, Im H2). Anal. ($C_{15}H_{16}N_2O_2$) C, H, N.

4′-Imidazol-1-ylmethyl-biphenyl-3,4,5-triol (**8**)

With **20** yellow needles were obtained. Yield: 81%, mp: 119–120°C. IR (Zn/Se-ATR) $\nu = 3129, 1577, 1487, 1400, 1282, 1070, 1012, 800, 743, 646, 620, 608\,cm^{-1}$. ^{1}H-NMR ($d_6$-DMSO) δ 5.46 (s, 2H, —$CH_2$—), 6.79 (d, 2H, Ar H2′, H6′), 7.41 (d, $^3J =$ 8.35 Hz, 2H, Ar H3, H5), 7.62 (d, $^3J = 8.35$ Hz, 2H, Ar H2, H6), 7.73 (t, 1H, Im H4), 7.83 (t, 1H, Im H5), 9.40 (s, 1H, Im H2), 14.41 (bs, 3H, 3′-OH, 4′-OH, 5′-OH). Anal. ($C_{19}H_{20}N_2O_3$ H_2O) C, H, N.

Method C

6-(3H-Imidazol-4-ylmethyl)-5-methyl-naphthalen-2-ol (**9**)

Compound **BW19** (0.30 g, 1.2 mmol) was stirred in aqueous HBr (48%, 15 mL) under an atmosphere of N_2 for 2 h at 120°C, and then cooled to 25°C and poured onto ice (100 g). The solution was washed twice with ethyl acetate (15 mL × 2) and adjusted to pH 8 with saturated Na_2CO_3 (30 mL). The solution was extracted with a 4: 1 (v/v) mixture of ethyl acetate and methanol (20 mL × 3). The combined organic layers were washed with brine (15 mL), dried over $MgSO_4$ and the solvent was removed. The resulting crude product was filtered through silica gel using a mixture of CH_2Cl_2/ethanol (9: 1, v/v, NH_4OH saturated) as solvent. Yield: 73%, yellow solid, mp: 154–155°C. IR (Zn/Se-ATR): $\nu = 3100, 2937, 2737, 2675, 2491, 1607, 1476, 1433, 1389, 1209, 1169, 1035, 958, 867, 848, 808, 643, 633, cm^{-1}$. ^{1}H-NMR ($d_6$-DMSO): δ 2.55 (s, 1H, —$CH_3$), 4.06 (s, 2H, —$CH_2$—Im), 6.91 (s, 1H, Im H5), 7.09 (m, 2H, Ar H3, H4), 7.23 (d, $^3J = 8.45$ Hz, 1H, Ar H5), 7.48 (d, $^3J = 8.45$ Hz, 1H, Ar H7), 7.92 (d, 1H, Ar H8), 8.23 (s, 1H, Im H2), 9.75 (bs, 1H, 6-OH, exchangeable with D_2O). Anal. ($C_{15}H_{14}N_2O \cdot H_2O$) C, H, N.

Method D

4-(5-Methoxy-7b-methyl-3,7b-dihydro-2H-1-oxa-cyclopropa[a]naphthalen-1a-ylmethyl)-1H-imidazol (**4**)

Compound **BW19** (250 mg, 0.98 mmol) dissolved in CH_2Cl_2/acetonitrile (2: 1, v/v, 10 mL) was slowly added to a solution of meta-chloroperoxybenzoic acid (m-CPBA, 70%, 250 mg, 1 mmol) in CH_2Cl_2 (10 mL) at 0°C. The solution was stirred for 12 h at 0°C then a further 12 h at 25°C. After completion the solution was washed twice with a mixture (2: 1, v/v, 10 mL) of saturated $NaHCO_3$ and $NaHSO_3$. The solvents were evaporated and the crude product purified by preparative HPLC (Jasco 887-PU, Groß-Zimmern; Germany) with a diode array (Perkin Elmer LC-DES 480, Rodgau-Jügesheim, Germany, $\lambda = 254$ nm) and a Gilson 203 Micro Fraction Collector with 80 tubes (Gilson, Bad Camberg, Germany). A mixture of water/acetonitrile / triethyl amine (59.9: 39.9: 0.2, v/v/v) with a flow of 13.5 mL/min was used to elute the fractions from the EP 250/16 Nucleosil 100-7 C_{18} HD-column (Macherey Nagel, Düren, Germany). The solvents were evaporated and the product was dissolved in ethyl acetate, dried over $MgSO_4$ and crystallized from ethyl acetate. Yield: 87%, white crystals, mp: 122°C. IR (Zn/Se-ATR): $\nu = 3047, 2893, 2364, 1659, 1470, 1435, 1193, 1115, 840, 801, 723, 667\,cm^{-1}$. ^{1}H-NMR ($d_6$-DMSO) δ 1.83 (s, 3H, —$CH_3$), 1.91 (m, 2H, Ar—$CH_2$—$CH_2$—), 2.79–3.02 (m, 4H, Ar—CH_2—, —CH_2—Im), 6.61 (s, 1H, Im H5), 6.71 (d, $^4J = 2.65$ Hz, 1H, Ar H5), 6.87 (dd, $^4J = 2.65$ Hz, $^3J = 8.85$ Hz, 1H, Ar H7), 7.56 (d, $^3J = 8.85$ Hz, 1H, Ar H8), 7.92 (s, 1H, Im H2). ^{13}C-NMR (d_6-DMSO) δ 17.5 (—CH_3), 26.7 (—CH_2—Im), 30.5 (C4), 31.4 (C3), 65.2 (C1), 77.3 (C2), 112.6 (C7), 114.7 (C5), 116.0 (Im C5), 127.1 (C8), 132.6 (C8a), 134.3 (Im C4), 135.3 (Im C2), 137.7 (C4a), 154.1 (C6). Anal. ($C_{16}H_{18}N_2O_2$) C, H, N.

Method E1

6-Hydroxytetralone (**10a**)[10]

6-Methoxytetralone (5.0 g, 28 mmol) dissolved in toluene (250 mL) was stirred with $AlCl_3$

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by Malmo Hogskola on 12/23/11
For personal use only.

(8.0 g, 60 mmol) at 110°C for 2.5 h. The solvent was removed and the brownish residue was poured onto ice water (200 mL). The precipitated crude product was filtered, recrystallized from water, dried over P_2O_5 and again recrystallized using CH_2Cl_2. Yield: 92%, slightly yellow crystals; mp: 154–157°C. ^{1}H-NMR (d_6-DMSO) δ 1.97 (m, 2H, Ar—CH_2—*CH_2*—), 2.49 (m, 2H, Ar—CH_2—), 2.83 (m, 2H, O═C—CH_2—), 6.67 (s, 1H, Ar H5), 6.74 (dd, 1H, Ar H7), 7.77 (d, 1H, Ar H8), 10.31 (s, 1H, 6-OH). Anal. ($C_{10}H_{10}O_2$) C, H.

Compound **12b** was prepared in the same way as described for compound **10a**.

6,7-Dihydroxy-1-tetralone (**12b**)

With 6,7-dimethoxy-1-tetralone slightly yellow crystals were obtained. Yield: 90%, mp: 192–195°C. ^{1}H-NMR (d_6-DMSO) δ 1.95 (m, 2H, Ar—CH_2—*CH_2*—), 2.44 (m, 2H, Ar—CH_2—), 2.74 (m, 2H, O═C—CH_2—), 6.65 (s, 1H, Ar H5), 7.29 (s, 1H, Ar H8), 9.49 (bs, 2H, 6-OH, 7-OH). Anal. ($C_{10}H_{10}O_3$) C, H.

Method E2

4′-Imidazol-1-ylmethyl-biphenyl-2,5-diol HCl (**5**)

$AlCl_3$ (2.3 g, 17 mmol) was added to a solution of compound **17** (1.5 g, 5.0 mmol) in toluene (100 mL) and stirred at 110°C for 2.5 h. The solution was cooled to 25°C and the solvent was evaporated. The brownish residue was poured onto a mixture of ice with sodium citrate (2 g) until the crude product precipitated. The crude product was filtered and dried over P_2O_5. The filtrate was adjusted to pH 1 with aqueous H_2SO_4 (5%) and washed with diethyl ether (20 mL × 2). The remaining product was then extracted with ethyl acetate/methanol (4: 1, v/v, 20 mL × 3) at pH 8 (adjusted with saturated Na_2CO_3), washed with brine (20 mL) and dried over $MgSO_4$. The solvents were evaporated. The combined crude products were purified by filtration through silica gel using CH_2Cl_2/ethanol (9: 1, v/v, NH_4OH saturated) as eluent and crystallized from ethyl acetate–hexane (2. 1, v/v, 15 mL). The purified compound was dissolved in diethyl ether/methanol (4: 1, v/v) and 2 M HCl in diethyl ether was added until crystallization. Yield: 83%, white crystals; mp: 194–195°C. IR (Zn/Se-ATR): ν = 3203, 1504, 1439, 1346, 1207, 1183, 839, 809, 779, 744, 649, 632, cm^{-1}. ^{1}H-NMR (d_6-DMSO) δ 5.49 (s, 2H, —CH_2—), 6.61 (dd, ^{4}J = 3.20 Hz, ^{3}J = 8.68 Hz, 1H, Ar H4′), 6.70 (d, ^{4}J = 3.20 Hz, 1H, Ar H3′), 6.81 (d, ^{3}J = 8.22 Hz, 1H, Ar H6′), 7.44 (d, ^{3}J = 8.22 Hz, 2H, Ar H3, H5), 7.55 (d, ^{3}J = 8.22 Hz, 2H, Ar H2, H6), 7.71 (s, 1H, Im H4), 7.86 (s, 1H, Im H5), 8.85–9.25 (bs, 2H, 2′-OH, 5′-OH), 9.44 (s, 1H, Im H2). Anal. ($C_{16}H_{14}N_2O_2$ · HCl) C, H, N.

Compounds **6** and **7** were prepared in a similar way to that described for compound **5**.

4′-Imidazol-1-ylmethyl-biphenyl-3,4-diol HCl (**6**)

With **18** white crystals were obtained. Yield: 96%, mp: 201–203°C. IR (Zn/Se-ATR): ν = 3152, 2988, 1576, 1494, 1298, 1270, 1243, 1080, 907, 808, 758, 625 cm^{-1}. ^{1}H-NMR (d_6-DMSO) δ 5.46 (s, 2H, —CH_2—), 6.83 (d, ^{3}J = 8.00 Hz, 1H, Ar H5′), 6.93 (dd, ^{4}J = 2.28 Hz, ^{3}J = 8.22 Hz, 1H, Ar H2′), 7.08 (d, ^{4}J = 2.28 Hz, ^{3}J = 8.22 Hz, 1H, Ar H6′), 7.45 (d, ^{3}J = 8.22 Hz, 2H, Ar H3, H5), 7.56 (d, ^{3}J = 8.22 Hz, 2H, Ar H2, H6), 7.69 (s, 1H, Im H4), 7.82 (s, 1H, Im H5), 9.00–9.30 (bs, 2H, 2′—OH, 5′—OH), 9.38 (s, 1H, Im H2). Anal. ($C_{16}H_{14}N_2O_2$ · HCl) C, H, N.

4′-Imidazol-1-ylmethyl-biphenyl-2,3,4-triol HCl (**7**)

With **19** white crystals were obtained. Yield: 21%, mp: 213–215°C. IR (Zn/Se-ATR): ν = 3139, 1577, 1503, 1468, 1449, 1366, 1311, 1168, 1079, 1000, 796, 671, 645 cm^{-1}. ^{1}H-NMR (d_6-DMSO) δ 5.43 (s, 2H, —CH_2—), 6.39 (d, 1H, ^{3}J = 8.55 Hz, Ar H5′), 6.58 (d, 1H, ^{3}J = 8.55 Hz, Ar H6′), 7.38 (d, ^{3}J = 8.24 Hz, 2H, Ar H3, H5), 7.42 (d, ^{3}J = 8.24 Hz, 2H, Ar H2, H6), 7.63 (s, 1H, Im H5), 7.78 (s, 1H, Im H4), 9.24 (s, 1H, Im H2). Anal. ($C_{16}H_{14}N_2O_3$ · HCl · H_2O) C, H, N.

6,7-Dihydroxy-2-(1H-imidazol-4-ylmethyl)-3,4-dihydro-2H-naphthalen-1-one (**16**)

This compound was synthesized starting from **13** in a similar way to that described for compounds **5–7** with the exception of precipitation of compound **16** by addition of sodium chloride to a solution of the compound at pH 6.

Yield: 79%, white solid; mp: 260°C. IR (Zn/Se-ATR): ν = 3438, 2970, 1614, 1395, 1279, 1074, 861, 665 cm^{-1}. ^{1}H-NMR (d_6-DMSO) δ 1.60–2.04 (m, 2H, Ar—CH_2—*CH_2*—), 2.21–2.44 (m, 2H, Ar—CH_2—), 2.77 (s, 2H, —CH_2—Im), 3.17 (m, 1H, >CH—), 4.20 (bs, 2H, 6-OH, 7-OH), 6.65 (s, 1H, Ar H5), 6.84 (s, 1H, Im H5), 7.33 (d, ^{3}J = 8.2 Hz, 1H, Ar H8), 7.70 (s, 1H, Im H2). Anal. ($C_{14}H_{14}N_2O_3$ · 2 H_2O) C, H, N.

Method F

7-Hydroxy-6-methoxy-3,4-dihydro-2H-naphthalen-1-one (**12a**)

6,7-Dihydroxy-1-tetralone (3.8 g, 21.9 mmol, **12b**) was dissolved in a mixture of 200 mL saturated $NaHCO_3$ and 100 mL methanol. After addition of dimethyl sulfate (6.6 mL, 39.5 mmol) the solution was stirred for 10 h at 65°C under an atmosphere of N_2. To remove the non-converted reagent dimethyl sulfate, sodium tartrate (5 g) dissolved in water (75 mL) was added and the mixture was heated again to 65°C. Then the organic solvent was evaporated. The remaining aqueous solution was extracted with ethyl acetate (20 mL × 3), the solvent evaporated and the crude product recrystallized using dichloromethane. Yield: 76%, white crystals, mp: 105°C. IR (Zn/Se-ATR): ν = 3198, 1650, 1592,

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by Malmo Hogskola on 12/23/11
For personal use only.

1507, 1432, 1270, 1214, 1177, 1136, 1028, 805 cm^{-1}. ^{1}H-NMR (d_6-DMSO) 2.02 (m, 2H, Ar—CH_2—CH_2—), 2.47 (m, 2H, Ar—CH_2—), 2.82 (m, 2H, O=C—CH_2—), 3.86 (s, 3H, 6-OCH_3), 6.76 (s, 1H, Ar H5), 7.29 (s, 1H, Ar H8), 9.12 (bs, 1H, 7-OH). Anal. ($C_{11}H_{12}O_3$) C, H.

Method G

6-Hydroxy-2-(1H-imidazol-4-ylmethylene)-3,4-dihydro-2H-naphthalen-1-one (**10**)

A mixture of 6-hydroxytetralone (3.9 g, 24 mmol), 4(5)-imidazolecarboxaldehyde (3.17 g, 33 mmol), and phosphoric acid (85%, 75 mL) was stirred for 10 h at 80°C. Subsequently it was cooled to 25°C, poured onto ice (200 g) and ice-cold saturated Na_2CO_3 (100 mL) was added until the crude product precipitated. The red solid was filtered and dried over P_2O_5. The filtrate was extracted with CH_2Cl_2/*i*-propanol (5:1, v/v, 20 mL × 3), the organic layers dried (Na_2SO_4) and the solvent removed. The combined crude products were recrystallized using ethyl acetate. Yield: 13%, yellow solid, mp: 240°C. IR (Zn/Se-ATR): $\nu = 2951$, 1613, 1565, 1466, 1407, 1296, 1246, 1100, 898, 852, 807, 656 cm^{-1}. ^{1}H-NMR (d_6-DMSO) δ 2.85 (t, 2H, Ar—CH_2—CH_2—), 3.34 (t, 2H, Ar—CH_2—), 6.68 (s, 1H, Ar H5), 6.75 (dd, 1H, Ar H7), 7.51 (s, 1H, Im H5), 7.57 (s, 1H, vinyl), 7.82 (t, 2H, Im H2, Ar H8). Anal. ($C_{14}H_{14}N_2O_2$) C, H, N.

Compounds **11** und **12** were prepared in a similar way to that described for compound **10**.

2-(1H-Imidazol-4-ylmethylene)-6,7-dimethoxy-3,4-dihydro-2H-naphthalen-1-one (**11**)

With **11a** a slightly yellow solid was obtained. Yield: 79%, mp: 240°C. IR (ZnSe-ATR): $\nu = 3298$, 1666, 1592, 1509, 1361, 1274, 1231, 1200, 1152, 1101, 1013, 970, 901, 871, 752, 625, 578 cm^{-1}. ^{1}H-NMR (d_6-DMSO) δ 2.89 (t, 2H, Ar—CH_2—CH_2—), 3.32 (t, 2H, Ar—CH_2—), 3.80 (s, 3H, 7-OCH_3), 3.86 (s, 3H, 6-OCH_3), 6.94 (s, 1H, Ar H5), 7.43 (s, 1H, Ar H8), 7.52 (s, 1H, Im H5), 7.59 (s, 1H, vinyl), 7.82 (s, 1H, Im H2). Anal. ($C_{16}H_{16}N_2O_3$) C, H, N.

7-Hydroxy-2-(1H-imidazol-4-ylmethylene)-6-methoxy-3,4-dihydro-2H-naphthalen-1-one (**12**)

With **12a** a slightly yellow solid was obtained. Yield: 64%, mp: 220°C. IR (ZnSe-ATR): $\nu = 3303$, 1666, 1593, 1510, 1274, 1200, 753, 625 cm^{-1}. ^{1}H-NMR (d_6-DMSO) δ 2.85 (t, 2H, Ar—CH_2—CH_2—), 3.35 (t, 2H, Ar—CH_2—), 3.86 (s, 3H, 6—OCH_3), 6.88 (s, 1H, Ar H5), 7.36 (s, 1H, Ar H8), 7.50 (s, 1H, Im H5), 7.58 (s, 1H, vinyl), 7.83 (s, 1H, Im H2). Anal. ($C_{15}H_{14}N_2O_3$) C, H, N.

Method H

2-(1H-Imidazol-4-ylmethyl)-6,7-dimethoxy-3,4-dihydro-2H-naphthalen-1-one (**13**)

Palladium on carbon (10%, 0.4 g) was added to a solution of 6,7-dimethoxy-2(4-imidazolylmethylene)-1-tetralone (**11**, 3.0 g, 10.6 mmol) in methanol (150 mL). The flask was evacuated and filled with hydrogen. The hydrogen stream was stopped after 2 hours, the flask was aerated and the catalyst was separated by filtration. The solvent was removed and the crude product was purified by recrystallization using ethyl acetate–diethyl ether.

Yield: 92%, white solid, mp: 194–195°C. IR (ZnSe-ATR): $\nu = 2971$, 2901, 1666, 1580, 1509, 1412, 1368, 1262, 1235, 1146, 1057, 1019, 839, 637 cm^{-1}. ^{1}H-NMR (d_6-DMSO) δ 1.65–2.10 (m, 2H, Ar—CH_2—CH_2—), 2.50–2.71 (m, 2H, Ar—CH_2—), 3.12 (m, 1H, >CH—), 3.33 (m, 2H, —CH_2—Im), 3.77 (s, 3H, 7—OMe), 3.83 (s, 3H, 6—OMe), 6.78 (s, 1H, Im H5), 6.87 (s, 1H, Ar H5), 7.36 (s, 1H, Ar H8), 7.52 (s, 1H, Im H2). Anal. ($C_{16}H_{18}N_2O_3$) C, H, N.

Compound **14** was synthesized in a similar way to that described for **13**.

7-Hydroxy-2-(1H-imidazol-4-ylmethyl)-6-methoxy-3,4-dihydro-2H-naphthalen-1-one (**14**)

With **12** a white solid was obtained. Yield: 92%, mp: 170–171°C. IR (ZnSe-ATR): $\nu = 2978$, 2603, 1617, 1512, 1468, 1440, 1397, 1271, 1246, 1228, 1169, 1109, 1079, 1050, 829, 629 cm^{-1}. ^{1}H-NMR (d_6-DMSO) δ 1.80–2.10 (m, 2H, Ar—CH_2—CH_2—), 2.25–2.58 (m, 2H, Ar—CH_2—), 2.67 (m, 2H, —CH_2—Im), 3.37 (m, 1H, >CH—), 3.69 (s, 3H, 6—OMe), 6.55 (s, 1H, Im H5), 6.83 (s, 1H, Ar H5), 7.31 (s, 1H, Ar H8), 7.39 (s, 1H, Im H2), 10.65 (bs, 1H, 7-OH, exchangeable with D_2O), 14.62 (bs, 1H, NH). Anal. ($C_{15}H_{16}N_2O_3$) C, H, N.

Method I

3,4-Dihydro-2-(4-imidazolyl-6,7-dimethoxy-1-methyl-naphthalene (**15**)

Magnesium turnings (0.24 g, 9.5 mmol) were covered with anhydrous diethyl ether (15 mL) and methyl iodide (0.6 mL, 9.5 mmol) dissolved in 10 mL anhydrous diethyl ether was added drop wise to maintain gentle reflux. The resulting mixture was heated to reflux for 1 h, until the magnesium was fully converted. The suspension was cooled to 25°C and 6-methoxy-2-(1-trityl-*1H*-imidazol-4-ylmethyl)-3,4-dihydro-*2H*-naphthalen-1-one (**15a**, 2.45 g, 5 mmol) dissolved in absolute diethyl ether (70 mL) was added over 15 min with stirring. The resulting suspension was refluxed for 14 h. After cooling to 25°C, water (20 mL) and aqueous H_2SO_4 (25%, 10 mL) were added and the mixture was heated for 2 h at 85°C. The reaction mixture was cooled again to 25°C and the aqueous layer was washed twice with diethyl ether (20 mL × 2) and once with ethyl acetate (20 mL). The aqueous layer were made alkaline with saturated Na_2CO_3 solution (25 mL) and then extracted several times with ethyl acetate/methanol (5: 1, v/v, 20 mL × 3). The organic layers were

washed with brine (20 mL) and dried over Na_2SO_4. The solvents were evaporated and the crude product was purified by filtration through silica gel with ethyl acetate (NH_4OH saturated) as solvent and recrystallized using ethyl acetate–hexane (2: 1, v/v, 20 mL). Yield: 35%, white solid, mp: 220°C. IR (Zn/Se-ATR): $\nu = 2961$, 1509, 1461, 1263, 1207, 1126, 1059, 862, 826, 630 cm^{-1}. ^{1}H-NMR (d_6-DMSO) δ 1.99 (s, 3H, —CH_3), 2.11 (t, $^3J = 7.3$ Hz, 2H, Ar—CH_2-—*CH_2*—), 2.55 (t, $^3J = 7.3$ Hz, 2H, Ar—CH_2—), 3.51 (s, 2H, —CH_2—Im), 3.86 (s, 3H, 7—OCH_3), 3.91 (s, 3H, 6—OCH_3), 6.58 (s, 1H, Ar H5), 6.87 (s, 1H, Im H5), 7.17 (s, 1H, Ar H8), 7.59 (s, 1H, Im H2). Anal. ($C_{17}H_{20}N_2O_2$) C, H, N.

Compounds **2** and **3** were prepared in the same way to that described for compound **15**.

7-(3H-Imidazol-4-ylmethyl)-3-methoxy-8-methyl-5,6-dihydro-naphthalen-2-ol (**2**)

With **14** a white solid was obtained. Yield: 23%, mp: 177°C. IR (Zn/Se-ATR): $\nu = 3307$, 2932, 2358, 1730, 1604, 1526, 1436, 1258, 1220, 1101, 1024, 810, 764, 659 cm^{-1}. ^{1}H-NMR (d_6-DMSO) δ 2.04 (s, 3H, —CH_3), 2.14 (t, $^3J = 7.3$ Hz, 2H, Ar—CH_2—*CH_2*—), 2.60 (t, $^3J = 7.3$ Hz, 2H, Ar—CH_2—), 3.45 (s, 2H, —CH_2—Im), 3.72 (s, 3H, —OCH_3), 6.70–6.75 (m, $^3J = 8.2$ Hz, 2H, Ar H5, H7), 6.77 (s, 1H, Im H5), 7.26 (d, $^3J = 8.2$ Hz, 1H, Ar H8), 7.80 (s, 1H, Im H2), 8.90 (s, 1H, OH). Anal. ($C_{16}H_{18}N_2O_2$) C, H, N.

7-(1H-Imidazol-4-ylmethyl)-8-methyl-5,6-dihydro-naphthalene-2,3-diol (**3**)

With **15** a white solid was obtained. Yield: 35%, mp: 178–180°C. IR (Zn/Se-ATR): $\nu = 3420$, 2972, 1622, 1605, 1103, 1067, 879, 700 cm^{-1}. ^{1}H-NMR (d_6-DMSO) δ 2.02 (s, 3H, —CH_3), 2.19 (t, $^3J = 7.3$ Hz, 2H, Ar—CH_2—*CH_2*—), 2.59 (t, $^3J = 7.3$ Hz, 2H, Ar—CH_2—), 3.33 (s, 2H, —CH_2—Im), 4.20 (bs, 2H, 6-OH, 7-OH), 6.48 (d, 1H, Ar H5, Ar H8), 6.81 (s, 1H, Im H5), 7.60 (s, 1H, Im H2). Anal. ($C_{15}H_{16}N_2O_2$) C, H, N.

Method K

1-(2′,5′-Dimethoxy-biphenyl-4-ylmethyl)-1H-imidazole (**17**)

A 50 mL-flask was charged with $Pd(PPh_3)_4$ (0.076 g, 0.0066 mmol), toluene (20 mL), 1-(4-bromobenzyl)imidazole (**17a**, 0.37 g, 1.6 mmol), and an aqueous solution of Na_2CO_3 (2 M, 5 mL) under an atmosphere of N_2. 2,5-Dimethoxyphenylboronic acid (0.56 g, 3.1 mmol) in ethanol (5 mL) was added and the mixture was refluxed for 2.5 h under vigorous stirring. Subsequently the residual boronic acid was oxidized with 30% H_2O_2 (0.5 mL) at 25°C for 1 h. The product was extracted with CH_2Cl_2/ethanol (9: 1, v/v, 10 mL × 3) and washed with brine. The organic layer was extracted with 0.1 N aqueous HCl and the aqueous layer was washed with diethyl ether (10 mL × 2) and made alkaline with saturated Na_2CO_3 until the product precipitated. The mixture was filtered and the crystals were washed with water and diethyl ether. The crude product was recrystallized using *i*-propanol–hexane (2:1, v/v, 5 mL). Yield: 93%, white to colourless crystals, mp: 97°C. IR (Zn/Se-ATR): $\nu = 2936$, 2833, 1486, 1457, 1438, 1292, 1229, 1190, 1171, 1022, 828, 798, 748, 727, 692, 665 cm^{-1}. ^{1}H-NMR (d_6-DMSO) δ 3.68 (s, 3H, 2′—OCH_3), 3.73 (s, 3H, 5′—OCH_3), 5.22 (s, 2H, —CH_2—), 6.87 (s, $^4J = 2.74$ Hz, 1H, Ar H4′), 6.91 (dd, $^4J = 2.74$ Hz, $^3J = 9.14$ Hz, 1H, Ar H5′), 6.96 (s, 1H, Im H5), 7.01 (dd, $^3J = 9.14$ Hz, 1H, Ar H2′), 7.22 (s, 1H, Im H4), 7.29 (d, $^3J = 8.22$ Hz, 2H, Ar H3, H5), 7.48 (d, $^3J = 8.22$ Hz, 2H, Ar H2, H6), 7.81 (s, 1H, Im H2). Anal. ($C_{18}H_{18}N_2O_2$) C, H, N.

Compounds **18** and **19** were prepared in the same way to that described for compound **17**.

1-(3′,4′-Dimethoxy-biphenyl-4-ylmethyl)-1H-imidazole (**18**)

With 2,5-dimethoxyphenylboronic acid white crystals were obtained. Yield: 89%, mp: 137°C. IR (Zn/Se-ATR): $\nu = 2966$, 2833, 1502, 1438, 1250, 1140, 1018, 806, 759, 745, 675 cm^{-1}. ^{1}H-NMR (d_6-DMSO) δ 3.78 (s, 3H, 3′—OCH_3), 3.83 (s, 3H, 4′—OCH_3), 5.22 (s, 2H, —CH_2—), 6.93 (s, 1H, Ar H6′), 7.02 (d, 1H, Im H5), 7.18 (dd, $^4J = 1.83$ Hz, 1H, Im H4), 7.21 (d, $^4J = 1.83$ Hz, 2H, Ar H2′, H6′), 7.31 (d, $^3J = 8.22$ Hz, 2H, Ar H3, H5), 7.62 (d, $^3J = 8.22$ Hz, 2H, Ar H2, H6), 7.78 (s, 1H, Im H2). Anal. ($C_{18}H_{18}N_2O_2$) C, H, N.

1-[(2′,3′,4′-trimethoxy-1,1′-biphenyl-4-yl)methyl]-1H-imidazole HCl (**19**)

Compound **19** did not crystallize. Therefore it was extracted with CH_2Cl_2/ethanol (5:1, v/v) from the alkaline solution and purified by column chromatography (CH_2Cl_2/ethanol, 9: 1, v/v, NH_4OH saturated). The resulting oil was dissolved in diethyl ether/methanol (4: 1, v/v) and 2 M hydrogen chloride in diethyl ether was added until crystallization. Yield: 71%, white crystals, mp: 223–225°C. IR (Zn/Se-ATR): $\nu = 2937$, 2833, 1486, 1460, 1278, 1229, 1111, 1079, 1019, 1004, 805, 759, 692 cm^{-1}. ^{1}H-NMR (d_6-DMSO) δ 3.60 (s, 3H, 2′-OCH_3), 3.78 (s, 3H, 3′—OCH_3), 3.83 (s, 3H, 4′-OCH_3), 5.49 (s, 2H, CH_2—Im), 6.87 (d, 1H, $^3J = 8.74$ Hz, Ar H5′), 6.93 (s, 1H, Im H5), 7.00 (d, 2H, $^3J = 8.74$ Hz, Ar H6′), 7.23 (s, 1H, Im H4), 7.28 (d, $^3J = 8.35$ Hz, 2H, Ar H3, H5), 7.42 (d, $^3J = 8.35$ Hz, 2H, Ar H2, H6), 7.78 (s, 1H, Im H2). Anal. ($C_{19}H_{21}N_2O_3Cl$) C, H, N.

Method L

5-Iodo-1,2,3-trimethoxy-benzene (**20a**)

3,4,5-Trimethoxyaniline (5.0 g, 27.3 mmol) was dissolved in diluted H_2SO_4 (25%, 100 mL) and cooled below 5°C in an ice bath. During the addition of

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by Malmo Hogskola on 12/23/11
For personal use only.

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by Malmo Hogskola on 12/23/11
For personal use only.

$NaNO_2$ (5.66 g, 82 mmol, dissolved in 40 mL water) the internal temperature was kept below 5°C. The reaction was stopped, when NO_2^- was detectable on a iodine starch strip (Merck, Darmstadt, Germany) in the mixture for more than 5 min. The residual reagent was removed using urea (1 g). KI (13.6 g, 82 mmol, dissolved in 30 mL water) was added and the solution was heated for at least 1 h until bubbling stopped. Then the reaction mixture was extracted with ethyl acetate (20 mL × 3). The crude product was purified by filtering through silica gel using CH_2Cl_2 and recrystallization from hexane. Yield: 58%, white to colorless needles, mp: 86°C. IR (Zn/Se-ATR): $\nu = 1577$, 1495, 1458, 1433, 1397, 1296, 1230, 1171, 1120, 762, 728, 580, 525 cm^{-1}. ^{1}H-NMR (d_6-DMSO) δ 3.82 (s, 3H, 4-MeO), 3.84 (s, 6H, 3-MeO, 5-MeO), 6.89 (s, 2H, Ar H2, H6). Anal. ($C_9H_{11}O_3I$) C, H.

Method M

1-(3′,4′,5′-Trimethoxy-biphenyl-4-ylmethyl)-1H-imidazole (**20**)

To a solution of **20a** (1.47 g, 5 mmol) in dry THF (70 mL) under an atmosphere of N_2 at −78°C was added t-BuLi (6.25 mL of a 1.6 M solution in THF, 10 mmol) dropwise. The solution was warmed to 25°C over 0.5 h and was stirred for an additional 30 min at 25°C. After cooling again to −78°C anhydrous $ZnCl_2$ (20 mL of a 0.5 M solution in THF, 10 mmol) was added and then the mixture allowed to reach rt. $Pd(PPh_3)_4$ (0.25 g, 0.22 mmol) and **17a** (0.71 g, 3 mmol) were added and the mixture was refluxed for 2.5 h, then cooled to 0°C and quenched with water (50 mL) and 1N aqueous HCl (5 mL) and washed with diethyl ether (20 mL × 2). Subsequently the solution was made alkaline with saturated Na_2CO_3 and extracted with ethyl acetate/methanol (4: 1, v/v, 20 mL × 3). The combined organic layers were washed with brine (20 mL), dried (Na_2SO_4) and evaporated. The crude product was purified by recrystallization from *i*-propanol–hexane (2: 1, v/v). Yield: 68%, white crystals, mp: 265°C. IR (Zn/Se-ATR): $\nu =$ 3395, 1631, 1501, 1402, 1239, 1128, 1080 cm^{-1}. ^{1}H-NMR (d_6-DMSO) δ 3.68 (s, 3H, 4′-OCH_3), 3.84 (s, 6H, 3′-OCH_3, 5′-OCH_3), 5.23 (s, 2H, —CH_2—), 6.90 (d, 3H, Ar H2′, H6′, Im H4), 7.21 (s, 1H, Im H5), 7.33 (d, $^3J = 8.22$ Hz, 2H, Ar H3, H5), 7.66 (d, $^3J =$ 8.22 Hz, 2H, Ar H2, H6), 7.78 (s, 1H, Im H2). Anal. ($C_{19}H_{20}N_2O_3$) C, H, N.

Biological Tests

Enzyme Preparation of CYP 17

Recombinant *E. coli* pJL17/OR coexpressing human CYP 17 and rat NADPH-P450-reductase were grown and stored as described previously.[12] For isolation of membrane fractions, 5 mL of bacterial suspension with an OD_{578} of 50 were washed using phosphate buffer (0.05 M, pH 7.4, 1 mM $MgCl_2$, 0.1 mM EDTA and 0.1 mM dithiothreitol). Bacteria were harvested by centrifugation (2,000 × g) and the pellet was resuspended in 10 mL of ice-cold TES buffer (0.1 M tris-acetate, pH 7.8, 0.5 mM EDTA, 0.5 M sucrose). Lysozyme was added with 10 mL of ice-cold water resulting in a concentration of 0.2 mg/mL followed by incubation for 30 min on ice with continuous shaking. Spheroplasts were harvested by centrifugation (12,000 × g for 10 min), and resuspended in 4 mL of ice-cold phosphate buffer (the same as described above plus 0.5 mM phenylmethylsulfonyl fluoride (PMSF)).

After freezing and thawing, samples were sonicated on ice (pulse 20 s on, 30 s off, five times), using a sonicator Sonopuls HD60 (Bandelin, Berlin, Germany) at maximum power. Unbroken cells and debris were pelleted at 3,000 × g for 7 min, and the supernatant was centrifuged at 50,000 × g for 20 min at 4°C. The membrane pellet was resuspended in 2 mL of phosphate buffer (the same as described above) with 20% glycerol using an ultra-turrax T25 (IKA-Labortechnik, Staufen, Germany). Protein content was determined by the method of Lowry.[13] Aliquots of this preparation, which generally had a content of about 5 mg protein per mL, were stored at −70°C until used.

Determination of the Inhibitory Activity Towards CYP 17

The 17α-hydroxylase activity of CYP 17 was determined by measuring the conversion of progesterone into 17α-hydroxyprogesterone and the byproduct 16α-hydroxyprogesterone. The assay was performed as follows: A solution of 6.25 nmol progesterone (in 5 μL methanol) in 140 μL phosphate buffer (0.05 M, pH 7.4, 1 mM $MgCl_2$, 0.1 mM EDTA and 0.1 mM dithiothreitol), 50 μL NADPH generating system (in phosphate buffer with 10 mM $NADP^+$, 100 mM glucose-6-phosphate, and 2.5 units of glucose-6-phosphate dehydrogenase) and inhibitor (in 5 μL DMSO) was preincubated at 37°C for 5 min. Control cups were supplemented with 5 μL DMSO without inhibitor. The reaction was started by adding 50 μL of a 1:5 diluted membrane suspension in phosphate buffer (0.8–1 mg protein per mL). After mixing, incubation was performed for 30 min at 37°C. Subsequently the reaction was stopped with 50 μL 1 N HCl.

Extraction of steroids was performed by addition of 1.0 mL ethyl acetate and vigorous shaking for 1 min. After a centrifugation step (5 min, 2,500 × g) the organic phase (0.9 mL) was transferred into a cup containing 0.25 mL of incubation buffer and

50 μL 1 N HCl and mixed again. After centrifugation, 0.8 mL ethyl acetate solution was evaporated to dryness in a fresh cup. Samples were dissolved in 50 μL water + methanol (1: 1) and analyzed by HPLC.

HPLC was performed using an Agilent 1100 HPLC system with PDA detector (Waldbronn, Germany) and a Nucleodur C18ec column (120-3, 3 × 12.5 mm, Macherey Nagel, Düren, Germany),[14] which was run at 40°C. The steroids were eluted under isocratic conditions using 10 mM sodium acetate buffer (adjusted to pH 4.0 with acetic acid): methanol (30: 70; V/V) at a flow rate of 0.70 mL/min and a pressure of about 25 MPa. For each analysis 25 μL were injected by an Agilent 1100 autosampler (Waldbronn, Germany), which was thermostated at 4°C. UV absorbance was monitored at 240 and 254 nm. The products 17α-hydroxyprogesterone and 16α-hydroxyprogesterone eluted with retention times of 1.8 and 2.8 min, respectively. Elution time for the substrate progesterone was 4.7 min. Peak areas were determined by integration of the resulting chromatograms using ChemStations software (Agilent, Waldbronn, Germany). Substrate conversion was determined by product versus substrate peak areas. The inhibitory potencies were calculated using the diminished substrate conversion caused by the inhibitors.

Preparation of Aromatase (CYP 19)

The enzyme was obtained from the microsomal fraction of freshly delivered human term placental tissue according to the procedure of Thompson and Siiteri.[15] The isolated microsomes were suspended in a minimum volume of phosphate buffer (0.05 M, pH 7.4, 20% glycerol). Additionally, DTT (dithiothreitol, 10 mM) and EDTA (1 mM) was added to protect the enzyme from degradation. Protein concentration was determined by the method of Lowry *et al.* and usually was about 35 mg/mL.

Inhibition of Aromatase

This assay was performed similar to the described methods,[16,17] monitoring enzyme activity by measuring the ^3H_2O formed from [1β- ^{3}H]androstenedione during aromatization. Each incubation tube contained 15 nM [1β- ^{3}H]androstenedione (0.08 μCi), 485 nM unlabeled androstenedione, 2 mM NADPH, 20 mM glucose-6-phosphate, 0.4 units of glucose-6-phosphate dehydrogenase, and inhibitor (0–100 μM) in phosphate buffer (0.05 M, pH 7.4). The test compounds were dissolved in DMSO and diluted with buffer. The final DMSO concentration in the control and inhibitor incubation was 2%. Each tube was preincubated for 5 min at 30°C in a water bath. Microsomal protein was added to start the reaction (0.1 mg). The total volume for each incubation was 0.2 mL. The reaction was terminated by the addition of 200 μL of a cold 1 mM $HgCl_2$ solution. After addition of 200 μL of an aqueous dextran-coated charcoal (DCC) suspension (2%), the vials were shaken for 20 min and centrifuged at 1500 × g for 5 min to separate the charcoal-absorbed steroids. The supernatant was assayed for ^3H_2O by counting in a scintillation mixture using a LKB-Wallac β-counter. The calculation of the IC_{50} values was performed by plotting the percent inhibition *vs.* the concentration of inhibitor on a semi-log plot. From this the molar concentration causing 50% inhibition was calculated.

RESULTS AND DISCUSSION

Development of a New Test System for the Evaluation of CYP 17 Inhibitors

Hydroxylation of Progesterone by Isolated Membrane Fractions Containing Human CYP 17 and NADPH-P450-reductase

Bacterial membranes containing CYP 17 and NADPH-P450-reductase were isolated and tested for activity as described above. Supplied with NADPH generating system, the enzyme was active showing that the isolation procedure had resulted in an active membrane bound monooxygenase system. The specific activity was determined to be about 1040 pmol/min/mg of protein. The enzyme activity of this preparation was constant for an incubation time of more than 30 min as shown in Figure 1.

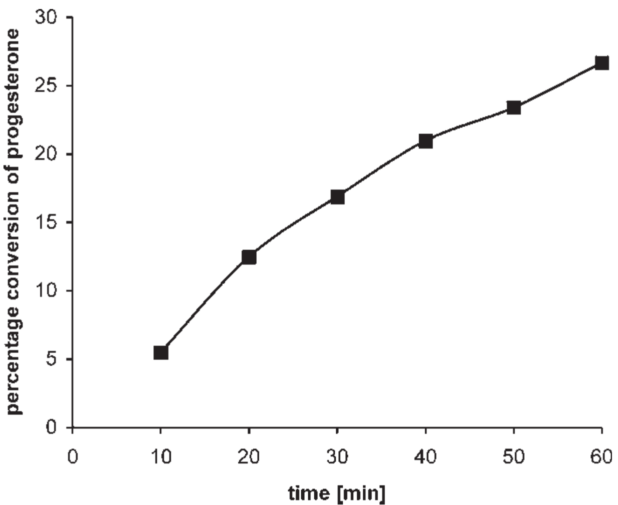


FIGURE 1 Progesterone conversion by isolated bacterial membrane fractions from *E. coli* pJL17/OR in assay buffer pH 7.4 at 37°C with a protein content of 0.2 mg/mL. Substrate was 25 μM progesterone. Conversion of progesterone was quantified by HPLC.

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by Malmo Hogskola on 12/23/11
For personal use only.

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by Malmo Hogskola on 12/23/11
For personal use only.

These results show that, although a rather vigorous extraction method has been applied, the enzyme activity was surprisingly high. Compared with the whole cell assay, for which a specific activity of about 175 pmol/min/mg of protein[12] has been determined, the enzyme activity was much higher. This can be explained by the fact that cytosolic and other non-membrane bound bacterial proteins have been separated and by the presence of inclusion bodies in the intact cells which contain over-expressed proteins in an inactive form. By using the ultrasonic procedure, the proteins might be released from the inclusion bodies and some of them might be integrated into the membrane fragments resulting in functional enzyme complexes. Compared with human testicular tissue preparations, which showed a specific activity in the low range of 10–30 pmol/min/mg of protein, the superiority of the new preparation is even more obvious. The membrane fractions can easily be prepared in large amounts from the previously described bacteria providing sufficient enzyme with a high and constant activity. As the enzyme preparations can be stored at −70°C for several months without decrease in activity, this procedure is much more convenient and effective than the previous one using human testes.

Evaluation of CYP 17 Inhibitors

An assay procedure was set up as described in 'Materials and Methods'. In comparison to the testicular microsomal assay the incubation time was reduced from 40 to 30 min and a NADPH generating system was used instead of direct addition of high amounts of NADPH. Because of the high specific activity of the new enzyme preparation, the protein amount was reduced by a factor of 16. As reference compounds **BW19**[10] and **Sa40**[5] have been used. As described in Table I the IC_{50} values of these compounds were determined and compared to the data from the cellular test and the testicular microsomal assay.

The inhibitory data show that **BW19** and **Sa40** exhibit very similar inhibition values in the three assays. The fact that both non-cellular assays lead to similar results demonstrates that the new assay is appropriate for inhibitor testing. As **BW19** and **Sa40** also show no decrease in their inhibitory activity when tested in the whole cell assay, it can be concluded that the compounds can penetrate the bacterial membrane very easily.

Using the Nucleodur C18 column material the analysis time could be reduced by more than 60 percent. The separation of the steroids is shown in Figure 2. The linear relations of the substrate and product concentrations were demonstrated between 3 and 150 μM (data not shown).

Using this new procedure we are now able to measure more samples within the same time, which is the basic requirement for a high throughput assay. Besides, the acidic buffer eluent leads to an early elution of basic compounds such as the P450 inhibitor tested in the assay. Thus, less disturbing impurities are co-eluted with the progesterone derivatives. A clogging of the column is reduced because the acidic buffer is keeping protein traces solved and the end capped and metal ion-free material is leading to fewer interactions between proteins and solid phase. As a consequence of using the new material and the optimized buffer conditions the durability of the columns is greatly increased.

In summary, the new non-cellular CYP 17 inhibitor assay provides a rapid and time saving method for accurately measuring CYP 17 inhibitory potencies. The isolation procedure used for the preparation of human CYP 17 from recombinant *E. coli* results in a highly active new enzyme source. The new assay does not depend on human tissue and contains less metabolizing enzymes, which could interfere with compound evaluation. By the application of a new high performance liquid chromatography method, which is based on end capped column material and optimized buffer conditions, a fast and robust method could be set up for the evaluation of CYP 17 inhibitors.

Chemistry

For each of the parent compounds **BW19** and **BW95** four metabolites could be isolated in a metabolic study (Figures 3 and 4).[18] These compounds were synthesized to determine their biological activity.

Compound **1** was obtained in a two step reaction (Scheme 1). In the first step the proton of the imidazole was substituted by a trityl group.[19] Subsequently the ether was cleaved by boron tribromide.[20] Finally the trityl group was removed by adding diluted acid and stirring at room temperature for 15 min. Using this strategy the yield of the ether cleaving reaction was increased two-fold.

Additional attempts to synthesize compound **1** by using aqueous hydrogen bromide (48%) also led to an aromatization of the dihydronaphthalene structure resulting in **9**. Compound **4** was synthesized by epoxidation of the double bond of **BW19** using 3-chloroperbenzoic acid (m-CPBA) at low temperature.

Compounds **2** and **3** were synthesized starting from the 6,7-dimethoxytetralone **11a**. To obtain the 6-methoxy-5-hydroxy compound **12a**, both methoxy groups were cleaved with aluminum chloride followed by selective ether formation in the 6-position[21] (Scheme 2). Compounds **11a** and **12a** were reacted with 4(5)-imidazolecarboxaldehyde

TABLE I IC_{50} values of reference compounds in different CYP 17 assays

Compound	Human testicular microsomes[a] IC_{50} [nM]	Recombinant human CYP 17 from *E. coli*, membrane fractions[a] IC_{50} [nM]	Recombinant human CYP 17 from *E. coli*, whole cell assay[a] IC_{50} [nM]
BW19[10]	110	150	110
Sa40[5]	24	36	33

[a]: substrate progesterone 25 μM; [b]: standard deviation less than ± 15%.

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by Malmo Hogskola on 12/23/11
For personal use only.

under acidic conditions using the recently described method[6] to yield compounds **11** and **12** (Scheme 3). Hydrogenation at normal pressure led to compounds **13** and **14**. Both substances were treated with trityl chloride, sodium carbonate and 18C6 to enhance the solubility in diethyl ether and THF which were used in the following Grignard reaction with methyl magnesium bromide resulting in **2** and **15**. Compound **15** was again protected with trityl chloride and the ether groups were cleaved under the same conditions as used in the synthesis of **1** resulting in **3**.

For compound **3** an alternative route was used. Starting from compound **13** the two ether groups were cleaved with anhydrous aluminum chloride resulting in **16**, which was converted to **3** using the procedure described above. Attempts to synthesize compound **1** analogously to **2** and **3** were not

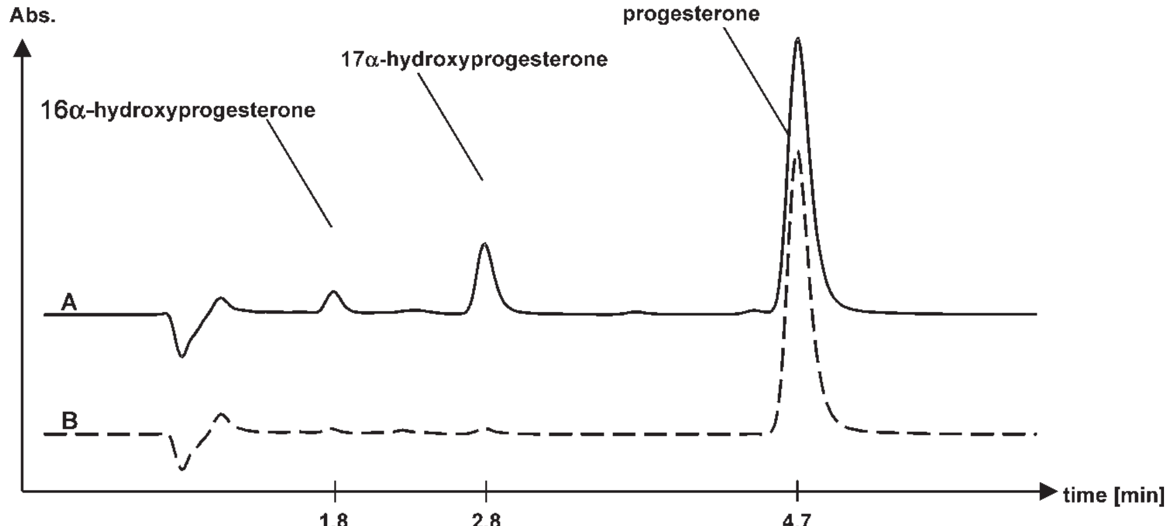


FIGURE 2 Typical chromatograms of the substrate progesterone and the products 16α-hydroxyprogesterone and 17α-hydroxyprogesterone after conversion; A: control incubation; B: incubation with 2.5 μM inhibitor (**BW19**).

FIGURE 3 Metabolites of the *in vivo* active CYP 17 inhibitor **BW19**.

successful, since the extremely water soluble dipolar ion **10** could only be isolated in low amounts and the subsequent hydrogenation was not sufficient.

The starting material **17a** for the biphenyl compounds was synthesized as described from Baggaley *et al.*[22,23] In a Suzuki reaction **17a** was coupled with the corresponding di- or trimethoxy phenylboronic acid and triphenylphosphine palladium (0) ($(PPh_3)_4Pd(0)$) as catalyst resulting in compounds **17**, **18** and **19**[24] (Scheme 4). Sodium carbonate (2 M, aqueous solution) was used as basic component and toluene as solvent.[25] Compounds **5–7** were obtained by cleaving the ether groups with anhydrous aluminum chloride.

Compound **8** was synthesized starting from 3,4,5-trimethoxyaniline (Scheme 5). In a Sandmeyer reaction the aniline was converted to the corresponding iodide **20a**, which was reacted in a Negishi cross coupling[26,27] with **17a** to compound **20**. The methoxy groups were cleaved with boron tribromide resulting in **8**.

Biological Results

The inhibition of the target enzyme CYP 17 of the two series was determined using the membrane fraction of recombinant *E. coli* pJL17/OR coexpressing human CYP 17 and rat NADPH-P450-reductase

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by Malmo Hogskola on 12/23/11
For personal use only.

FIGURE 4 The *in vivo* active CYP 17 inhibitor **BW95** and its metabolites.

FIGURE 5 **BW57**.[10]

as described in 'Materials and Methods'. Additionally the inhibition of CYP 19 was determined. For the inhibitory activities exceeding 75% inhibition at 2.5 μM and 25 μM respectively the IC_{50} values were determined (Tables II and III).

Compounds with a imidazolylmethylene and imidazolylmethyl structure like **GW93** and **GW113** are known as inhibitors of CYP 19.[6] Therefore it is not surprising that **10–14** showed a high CYP 19 inhibitory activity and a low inhibition of CYP 17. However, compound **16** was nearly inactive. This might be due to the fact that the compound is ionized and as a consequence of this is very hydrophilic.

Comparing the biological activity of the parent compound **BW19** with the activity of the metabolites (**1–4**) a strong decrease of activity is observed. Compound **4** is not active at all. This might be due to the polar oxirane ring. The dihydroxy compound **3** is also not active which also might be due to the hydrophilic groups. This view is confirmed by the higher inhibitory activity of the corresponding dimethoxy compound **15**; with an IC_{50} value of 0.49 μM **15** is a much more potent CYP 17 inhibitor than **3**. However, this compound is less active than the two corresponding monomethoxy compounds **BW19** and **BW57**.

The 7-hydroxy-6-methoxy compound **2** showed a reduced activity compared to **15**. Similar results are already described for the cleavage of the methyl ether group of 4-pyridyl substituted dihydronaphthalene derivates.[1] All 6-hydroxy compounds are less active as CYP 17 inhibitors than the parent compound **BW19**. In contrast to these results the recently synthesized analogous 1-imidazolyl substituted methyldihydronaphthalene derivatives showed for hydroxy and methoxy compounds comparable activities.[10]

The decrease in activity after aromatisation of the dihydronaphthalfene structure like in compound **9** has already been described for correspondingly substituted 4-pyridyl compounds.[1]

As shown in Table III on the other hand, the inhibitory activities of the biphenyl derivatives increased significantly after ether cleavage.[8]

However, in comparison to the parent compound **BW95**, most of the synthesized hydroxy metabolites showed a lower inhibition of CYP 17. Only compound **6** was a better inhibitor than its parent compound.

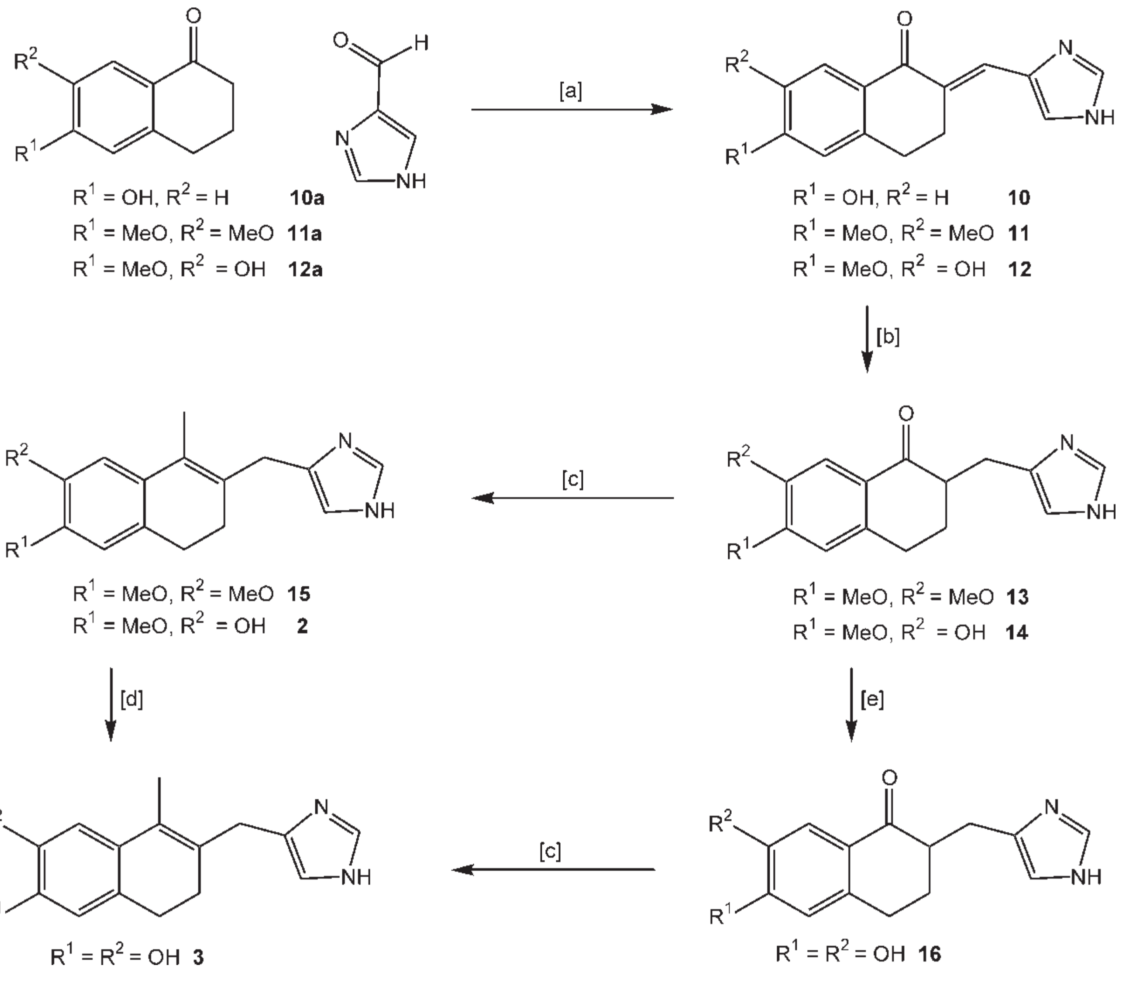
SCHEME 1 Synthesis of compounds **1**, **4** and **9**. Reagents and conditions: [a] (1) trityl chloride, K_2CO_3, 18C6, anhydrous acetone, 30°C, 4 h, N_2 (method A); (2) BBr_3, CH_2Cl_2, −78°C, 0.5 h, then 25°C, 3 h, N_2 (method B); [b] HBr 48%, 120°C, 2 h, N_2 (method C) [c] m-CPBA, MeCN / CH_2Cl_2 (1: 2), 0°C, 12 h, then 25°C, 12 h (method D).

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by Malmo Hogskola on 12/23/11
For personal use only.

10b, 11a → [a] → 10a, 12b → [b] → 12a

	R^1	R^2
10a	OH	H
10b	OMe	H
11a	OMe	OMe
12a	OMe	OH
12b	OH	OH

SCHEME 2 Synthesis of compounds **10a**, **12a** and **12b**. Reagents and conditions: [a] $AlCl_3$, toluene, 110° C, 2.5 h (method E1); [b] $(CH_3)_2SO_4$, saturated $NaHCO_3$/MeOH (1:2), 80°C, 10 h, N_2 (method F).



SCHEME 3 Synthesis of compounds **2**, **3** and **10**–**16**. Reagents and conditions: [a] phosphoric acid (H_3PO_4) 85%, 80°C, 10 h (method G); [b] H_2 / Pd(C), 1 bar, 25°C, 4 h (method H); [c] (1) trityl chloride, K_2CO_3, 18C6, anhydrous acetone, 30°C, 4 h, N_2 (method A); (2) CH_3I, Mg, 35°C, 12 h (method I); [d] (1) trityl chloride, K_2CO_3, 18C6, anhydrous acetone, 30°C, 4 h, N_2 (method A); (2) BBr_3, CH_2Cl_2, −78°C, then to 25°C, 3 h, N_2 (method B); [e] $AlCl_3$, toluene, 110° C, 2.5 h (method E2).

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by Malmo Hogskola on 12/23/11
For personal use only.

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by Malmo Hogskola on 12/23/11
For personal use only.

17	**2,5-DiMeO**
18	**3,4-DiMeO**
19	**2,3,4-TriMeO**
5	**2,5-DiOH**
6	**3,4-DiOH**
7	**2,3,4-DiOH**

SCHEME 4 Synthesis of compounds **5**–**7** and **17**–**19**. Reagents and conditions: [a] $Pd(PPh_3)_4$, 2 M Na_2CO_3, toluene, 110°C, reflux, 2.5 h, N_2 (method K); [b] $AlCl_3$, toluene, 110°C, reflux, 2.5 h (method E2).

SCHEME 5 Synthesis of compounds **8** and **20**. Reagents and conditions: [a] (1) aqueous $NaNO_2$, diluted H_2SO_4 (25%), 0–5°C; (2) urea, 25°C; (3) KI, 80°C, 1 h (method L); [b] (1) t-BuLi, −78°C, then 25°C, N_2; (2) $ZnCl_2$, −78°C, then 25°C, N_2 (3) **17a**, RT (method M); [c] BBr_3, CH_2Cl_2, −78°C, then 25°C, 3 h (method B).

TABLE II Inhibitory activities of the naphthalene and dihydronaphthalene derivatives towards CYP 17 and CYP 19

Compound	Substituent(s) (R, R′)	% Inhibition of CYP 17 (IC_{50} [μM])[a]	% Inhibition of CYP 19 (IC_{50} [μM])[b]
GW93[6]	6-OMe	n. i.	(1.5)[c]
GW113[6]	6-OMe	14 ± 3	(3.5) [c]
BW19[10]	6-OMe	(0.11)[c]	(1.6)[c]
1	6-OH	65 ± 3	(2.3)
2	6-OMe, 7-OH	(1.1)[c]	(1.7)[c]
3	6,7-DiOH	n. i.	14 ± 0.5
4	6-OMe	10 ± 2	n. i.
9	6-OH	57 ± 3	(3.9)[c]
10	6-OH	28 ± 2	(2.1)[c]
11	6,7-DiOMe	n. i.	(2.2)[c]
12	6-OMe, 7-OH	n. i.	(2.5)[c]
13	6,7-DiOMe	19 ± 3	(4.7)[c]
14	6-OMe, 7-OH	33 ± 2	(6.4)[c]
15	6,7-DiOMe	(0.49)c	(6.7)[c]
16	6,7-DiOH	25 ± 0.2	35 ± 3

n. i.: no inhibition; [a]: non-cellular assay: 25 μM progesterone, 10 mM glucose-6-phosphate; 1 mM $NADP^+$; 2 U/mL glucose-6-phosphate dehydrogenase; 2.5 μM inhibitor; [b]: 15 nM [1β^{3}H]-androstenedione, 485 nM androstenedione, 10 mM glucose-6-phosphate; 1 mM $NADP^+$; 2 U/mL glucose-6-phosphate dehydrogenase; 25 μM inhibitor; [c]: standard deviation less than ±15%.

TABLE III : Inhibitory activities of the methyl imidazole biphenyl derivatives towards CYP 17 and CYP 19

Compound	Substituent(s) (R)	% Inhibition of CYP 17 (IC_{50} [μM])[a]	% Inhibition of CYP 19 (IC_{50} [μM])[b]
BW94[9]	3-OMe	30 ± 3	52 ± 2
BW95[9]	3-OH	67 ± 5	65 ± 2
5	2,5-diOH	13 ± 2	61 ± 3
6	3,4-diOH	(0.12)[c]	(1.8)[c]
7	2,3,4-triOH	30 ± 2	58 ± 3
8	3,4,5-triOH	8 ± 1	(1.2)[c]
17	2,5-diOMe	n. i.	42 ± 3
18	3,4-diOMe	37 ± 3	52 ± 3
19	2,3,4-triOMe	5 ± 0.2	(7.7)[c]
20	3,4,5-triOMe	8 ± 1	35 ± 3

n. i.: no inhibition; [a]: non-cellular assay: 25 μM progesterone, 10 mM glucose-6-phosphate; 1 mM $NADP^+$; 2 U/mL glucose-6-phosphate dehydrogenase; 2.5 μM inhibitor; [b]: 15 nM [1ß^{3}H]-androstenedione, 485 nM androstenedione, 10 mM glucose-6-phosphate; 1 mM $NADP^+$; 2 U/mL glucose-6-phosphate dehydrogenase; 25 μM inhibitor; [c]: standard deviation less than ±15%.

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by Malmo Hogskola on 12/23/11
For personal use only.

Surprisingly the inhibitory activity of **BW95** in the testicular microsomal assay (IC_{50}: 0.13 μM) differed from the one in the assay using recombinant enzyme (IC_{50}: 0.6 μM). In contrast to this the inhibitory activities of **6** in the two different assays were similar (human testicular microsomal assay IC_{50}: 0.087 μM; human recombinant enzyme assay IC_{50}: 0.12 μM). These results may be due to a hydroxylation of **BW95** catalyzed by metabolizing enzymes in the microsomal assay resulting in the more active inhibitor **6**. This hypothesis also might be true for the varying inhibitory activities of **BW94** in the two assays (human microsomal assay: 68% inhibition; human recombined assay: 30% inhibition). By metabolic cleavage of the methoxy group in the human testicular microsomal assay the more active inhibitor **BW95** may be produced.

The inhibition of CYP 19 is only marginally influenced by the number and the position of hydroxy as well as methoxy groups.

CONCLUSION

The new established CYP 17 assay as it is described here is in comparison to the previous assay based on microsomal fractions much easier to perform, faster and independent of human tissue. For further evaluation of a compound, for which *in vivo* tests are considered, the cellular assay should be performed subsequently to elucidate the potency of the compound in intact cells. A compound showing strong activity and selectivity should be tested *in vivo*.

The polar metabolites **3** and **4** of **BW19** were nearly inactive. Compounds **1** and **2** showed activity, which, however, was decreased by approximately one order of magnitude.

The activities of most of the metabolites of **BW95** presented were even lower. Only compound **6** was more potent than the parent compound. However, hydroxylation of the catechol structure leads to a complete loss of inhibitory activity. These results may be one reason for the lower half-life of **BW95** in rats.

It can be concluded from our findings that it should be possible to structurally modify **BW95** to increase its metabolic stability as we have recently shown.[28]

Acknowledgements

Thanks are due to Anja Palusczak for technical assistance and to the Fonds der Chemischen Industrie for financial support.

References

[1] Hartmann, R.W., Ehmer, P.B., Haidar, S., Hector, M., Klein, C.D.P., Seidel, S., Sergejew, T.F., Wachall, B.G., Wächter, G.A. and Zhuang, Y. (2002) *Arch. Pharm. Pharm. Med. Chem.* **4**, 119–128.
[2] Njar, V.C.O., Hector, M. and Hartmann, R.W. (1996) *Bioorg. Med. Chem.* **4**, 1447–1453.
[3] Hartmann, R.W., Hector, M., Wachall, B.G., Palusczak, A., Palzer, M., Huch, V. and Veith, M. (2000) *J. Med. Chem.* **43**, 4437–4445.
[4] Hartmann, R.W., Hector, M., Haidar, S., Ehmer, P.B., Reichert, W. and Jose, J. (2000) *J. Med. Chem.* **43**, 4266–4277.
[5] Haidar, S., Ehmer, P.B. and Hartmann, R.W. (2001) *Arch. Pharm. Pharm. Med. Chem.* **334**, 373–374.
[6] Wächter, G.A., Hartmann, R.W., Sergejew, T., Grün, G.L. and Ledergerber, D. (1996) *J. Med. Chem.* **39**, 834–841.
[7] Zhuang, Y. and Hartmann, R.W. (1998) *Arch. Pharm. Pharm. Med. Chem.* **331**, 36–40.
[8] Wachall, B.G., Hector, M., Zhuang, Y. and Hartmann, R.W. (1999) *Bioorg. Med. Chem.* **7**, 1913–1924.
[9] Zhuang, Y., Wachall, B.G. and Hartmann, R.W. (2000) *Bioorg. Med. Chem.* **8**, 1245–1252.
[10] Hartmann, R.W., Wachall, B.G., Yoshihama, M., Nakakoshi, M., Nomoto, S. and Ikeda, Y. (1999) *U.S. Pat. Appl.*, US 2002/0032211 A1 (Chem. Abstr. 1999 (**130**), 252360).
[11] Sergejew, T. and Hartmann, R.W. (1994) *J. Enz. Inhib.* **8**, 113–122.
[12] Ehmer, P.B., Jose, J. and Hartmann, R.W. (2000) *J. Steroid Biochem. Mol. Biol.* **75**, 57–63.
[13] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randell, R.H. (1951) *J. Biol. Chem.* **193**, 265–275.
[14] Rieger, D. and Pfeiffer, J. (2002) *Lab. Prax.* **26**(2), 20–21.
[15] Thompson, E.A. and Siiteri, P.K. (1974) *J. Biol Chem.* **249**, 5364–5372.
[16] Foster, A.B., Jarman, M., Leung, C.S., Rowlands, M.G. and Taylor, G.N. (1983) *J. Med. Chem.* **26**, 50–54.
[17] Graves, P.E. and Salhanick, H.A. (1979) *Endocrinology* **105**, 52–57.
[18] Hutschenreuter, T.U. and Hartmann, R.W. (2003) and *unpublished results*.
[19] Bernabé, M. and Burger, A. (1971) *J. Med. Chem.* **14**, 883–884.
[20] McOmie, J.F.W. and West, D.E. (1969) *Organic Syntheses*, 412–414.
[21] Bertram, J. (1892) Patent DE 63007.
[22] Baggaley, K.H., Heald, M., Hindley, R.M., Morgan, B., Tee, J.L. and Green, J. (1975) *J. Med. Chem.* **18**, 833–836.
[23] Welcome Foundation, Patent DE 2903653, (Chem. Abstr. 1979, (**92**), 58775).
[24] Miyaura, N. and Suzuki, A. (1995) *Chem. Rev.* **95**, 2457–2483.
[25] Miyaura, N., Yanagi, T. and Suzuki, A. (1981) *Synth. Comm.* **11**, 513–519.
[26] Negishi, E., King, A.O. and Okukado, N. (1977) *J. Org. Chem.* **42**, 1821–1823.
[27] Minatto, A., Tamao, K., Hayashi, T., Suzuki, K. and Kumada, M. (1980) *Tetrahedron Lett.* **21**, 845–848.
[28] Leroux, F., Hutschenreuter, T.U., Charièrre, C., Scopelliti, R. and Hartmann, R.W. (2003) *Helv. Chim. Acta* **86**, 2671–2686.

Journal of Enzyme Inhibition and Medicinal Chemistry Downloaded from informahealthcare.com by Malmo Hogskola on 12/23/11
For personal use only.