Potent CYP19 (Aromatase) 1-[(Benzofuran-2-yl)(phenylmethyl)pyridine, -imidazole, and -triazole Inhibitors: Synthesis and Biological Evaluation

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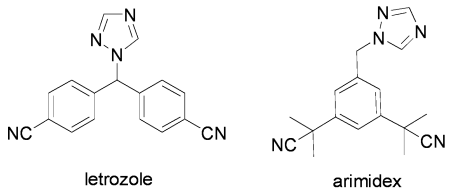
The synthesis of a series of novel 1-[(benzofuran-2-yl)phenylmethyl]-pyridine, -imidazole, and -triazole derivatives is described. All the compounds were evaluated in vitro for inhibitory activity against aromatase ($P450_{AROM}$, CYP19), using human placental microsomes. The 6-methoxy- and 6-hydroxy-substituted benzofuran derivatives were shown to be potent CYP19 inhibitors ($IC_{50} = 0.01-1.46$ μM) with activity greater than that observed for the unsubstituted parent compounds and inhibitory activity comparable with or greater than the reference compound arimidex ($IC_{50} = 0.6$ μM). Six of the benzofuran derivatives were subjected to in vitro cytotoxicity assays, using rat liver hepatocytes with cytotoxicity determined from alteration in cell morphology and lactate dehydrogenase enzyme retention over a period of 24 h, and selectivity (CYP17, 17β-HSD types 1 and 3, CYP24, and CYP26) determination; negligible inhibitory activity was observed, suggesting a good selectivity for CYP19. The pyridine benzofuran **4a** containing the 4-fluorophenyl group was the most promising ($IC_{50} = 44$ nM; $LC_{50} > 100$ μM) compared with arimidex ($IC_{50} = 600$ nM; $LC_{50} > 200$ μM).

Introduction

Breast cancer is the most commonly diagnosed cancer among women and continues to be a major cause of cancer deaths.[1] The two most important identified risk factors for breast cancer are gender and age,[2] and more than 70% of women over the age of 50 with breast cancer do not have any other remarkable risk factor.[2] A high proportion of breast tumors in postmenopausal women are dependent on estrogen for growth and after surgery estrogen deprivation strategies are used to prevent development of metastases.[3] Aromatase inhibitors, which were first reported in the 1970s, have been used in the clinic as second line drugs following relapse on tamoxifen therapy for this purpose.[4,5]

The third generation nonsteroidal aromatase inhibitors are triazole derivatives, e.g. letrozole and anastrozole (arimidex) (Figure 1), and have shown considerable advances in the treatment of hormone-dependent breast cancer.[6] In vitro studies showed that letrozole significantly suppressed the endogenous aromatase-induced proliferation of MCF-7 cells.[7] The "ATAC" (Arimidex, Tamoxifen, Alone or in Combination) trial suggested that aromatase inhibitors, specifically arimidex, may well be superior to tamoxifen (the current first-line therapy of choice) for the treatment of hormone-dependent breast cancer.[8,9] Studies have also indicated the use of aromatase inhibitors for preventive therapeutics, owing to their ability to reduce estrogen levels; a high level of plasma estrogen has been shown to be a high risk factor for subsequent breast cancer.[10]

1-[(Benzofuran-2-yl)phenylmethyl]imidazoles[11] and -triazoles[12] are potent $P450_{AROM}$ inhibitors, with the pyridine derivatives displaying moderate activity (Figure 1).[13] These inhibitors bind to the active site of $P450_{AROM}$ through coordination of a heterocyclic nitrogen lone pair of electrons with the



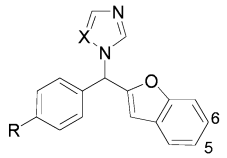


Figure 1. Potent nonsteroidal CYP19 inhibitors.

Fe^{3+} of the heme in the active site of the enzyme; therefore, the coordination potential of the heterocyclic nitrogen is of importance.[13,14] For the triazole derivatives, substitution in the 5-position of benzofuran ring was shown to be detrimental to inhibitory activity compared with the unsubstituted parent benzofuran.[12] Molecular modeling studies indicated that introduction of a hydrogen donor group, e.g. OH or OCH_3, in the 6-position of the benzofuran ring, which would also more closely mimic the natural substrate, androstenedione, might enhance binding interaction at the enzyme active site. We report here the synthesis and biological evaluation of these novel aromatase inhibitors.

Chemistry. The synthesis of the 6-methoxybenzofuran-2-ylphenylmethanone derivatives **3** involved reaction of commercially available 4-methoxysalicylaldehyde **1** with the appropriate phenacyl bromide **2**, which was either obtained commercially or prepared by conventional methodology by bromination of the precursor acetophenone with bromine/$AlCl_3$ as previously described.[15] The Rap−Stoermer reaction[16,17] produced the required benzofuran ketones **3a**−**h** in yields ranging from 37 to 80% after purification by recrystallization.

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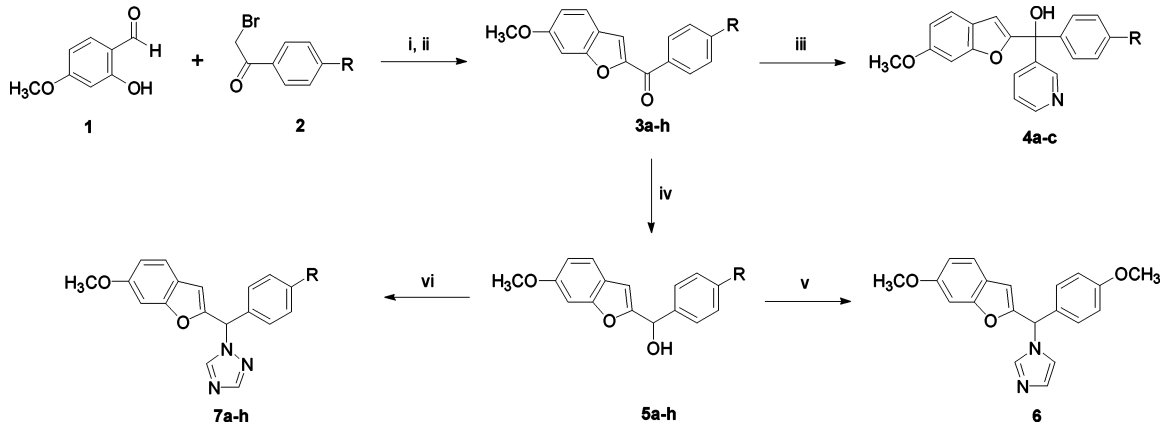
Scheme 1[a]



[a] Reagents and conditions: i. **1**, NaH, DMF, 10 min, then **2**, 80 °C, 2 h; ii. NaOMe, 80 °C, 1 h; iii.Mg, I_2, THF, 3-bromopyridine, 70 °C, 2 h, then **3**, THF, 70 °C, 1 h; iv. $NaBH_4$, dioxane, rt, 2 h; v. $SOCl_2$, imidazole, CH_3CN, −10 °C, 1 h, then **5**, CH_3CN, K_2CO_3, rt, 4 days; vi. $SOCl_2$, triazole, CH_3CN, −10 °C, 1 h, then **5**, CH_3CN, K_2CO_3, rt, 4 days. [**a**, R = F; **b**, R = Cl; **c**, R = OCH_3; **d**, R = CN; **e**, R = NO_2; **f**, R = CH_3; **g**, R = CF_3; **h**, R = CH_2CH_3].

Table 1. IC_{50} Data for Inhibitory Activity of Benzofuran Pyridine (**4**), Imidazole (**6**), and Triazole Derivatives (**7** and **11**) vs Human Placental CYP19[a]

compound	R	yield (%)	IC_{50} (μM)[a]	compound	R	yield (%)	IC_{50} (μM)[a]
4a	F	80	0.044	**7e**	NO_2	30	0.6
4b	Cl	80	0.049	**7f**	CH_3	27	0.1
4c	OCH_3	55	0.16	**7g**	CF_3	28	0.13
6	OCH_3	85	0.13	**7h**	CH_2CH_3	77	1.23
7a	F	83	0.049	**11b**	Cl	76	1.46
7b	Cl	80	0.044	**11d**	CN	85	0.02
7c	OCH_3	85	0.13	**11e**	NO_2	86	0.06
7d	CN	73	0.01	arimidex	-	-	0.6

[a] IC_{50} values are the average (±5%) of two experiments.

Preparation of the benzofuran-2-yl-(phenyl)-3-pyridylmethanols **4a**−**c** was achieved by reaction of the corresponding ketones **3a**−**c** with the Grignard reagent pyridine-3-magnesium bromide, which was generated in situ from commercially available pyridin-3-yl bromide and magnesium in the usual manner (Scheme 1).[13] The racemic pyridylmethanols were obtained in moderate to good yields, after purification by column chromatography and subsequent recrystallization.

Sodium borohydride reduction of the ketones **3** gave the carbinols **5** in almost quantitative yields, which were converted into the target imidazole **6** and triazoles **7** by the described methodology[12,17] (Scheme 1): the benzofuran alcohols **5** and activated potassium carbonate in anhydrous acetonitrile were added to a solution of azole (imidazole or triazole) and thionyl chloride in anhydrous acetonitrile at 10 °C, and the reactions were left stirring at 10 °C for 1 h and at room temperature for 4 days (Scheme 1).

The yields for this final reaction were variable (Table 1), and in many cases, the reaction failed to reach completion. Low yields of product were obtained together with recovered starting material. After purification by flash column chromatography, the products were confirmed by ^{1}H NMR by the disappearance of the OH group at approximately δ 3.7 and the presence of two triazole protons at δ 8.2 and 8.1, respectively. In a few reactions the 1,3,4-triazole isomer was observed by TLC running a more polar CH_2Cl_2−MeOH eluent and characterized by a singlet integrating for 2 protons at δ 8.25. The ratio of the 1,2,4-triazole:1,3,4-triazole was found to be approximately 10:1. Owing to the very small quantities of the isolated 1,3,4-triazole product, only the major 1,2,4-triazole derivatives were fully characterized and presented here.

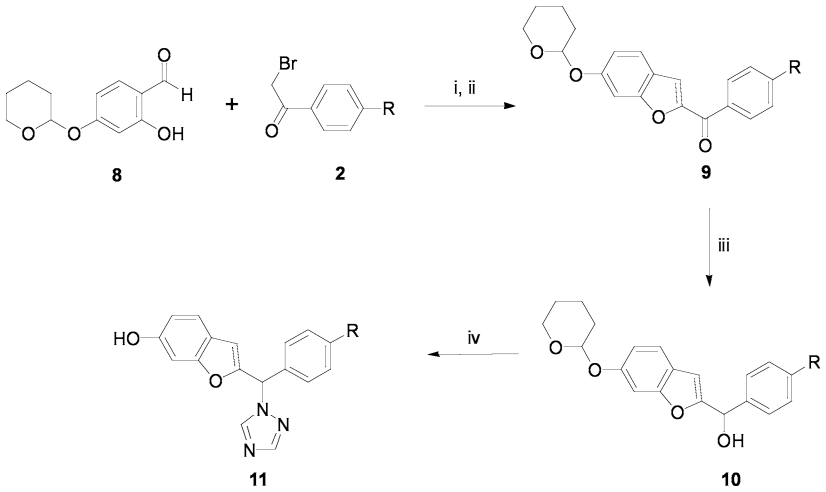
It was hypothesized that the 6-hydroxy derivative might have enhanced binding interaction (H-bonding) at the enzyme active site; therefore, attempts were made to prepare the corresponding 6-hydroxy derivatives. This initially involved investigating deprotection of the parent 6-methoxy derivative; however, this proved unsatisfactory with either no reaction occurring or decomposition, depending on the method applied. A different protecting group strategy was then explored. Although 2,4-dihydroxybenzaldehye has two hydroxy groups, only one was selectively protected in the presence of 3,4-dihydropyran and *p*-toluenesulfonic acid in diethyl ether. The reaction yielded 48% of the 6-*O*-pyran-protected product **8**, owing to incomplete reaction with starting material recovered (Scheme 2).

Compound **8** was further reacted with phenacyl bromides **2b**, **2d**, and **2e** to give required benzofurans **9** in moderate yields. Reduction of the benzofuran ketones gave almost quantitative yields of alcohols **10** (Scheme 2). It was anticipated that two more steps were required to produce our target compounds, but when the alcohols were reacted with triazole in the presence of thionyl chloride and potassium carbonate, the pyran protecting group was also cleaved in the process as indicated NMR. Compounds **11b**, **11d**, and **11e** were synthesized in excellent yields.

CYP19 Inhibitory Activity. The pyridine (**4a**−**c**), imidazole (**6**), and triazole (**7a**−**h**, **11b**, **11d**, and **11e**) benzofuran derivatives were evaluated for CYP19 inhibitory activity using human placental microsomes,[18] using radiolabeled [1,2,6,7-^{3}H]-androstenedione as the substrate and arimidex as the standard for comparison (Table 1).

Arimidex was determined to have an IC_{50} value of 0.6 μM. The pyridine benzofuran derivatives **4** were all potent inhibitors of CYP19, with optimal activity obtained with the halogen-substituted derivatives **4a** and **4b** (IC_{50}: **4a** = 0.044 μM; **4b** = 0.049 μM). The more bulky methoxy-substituted pyridine derivative **4c** was less active and was comparable with the

Scheme 2[a]



[a] Reagents and conditions: i. **8**, NaH, DMF, 10 min, then **2**, 80 °C, 2 h; ii. NaOMe, 80 °C, 1 h; iii. $NaBH_4$, dioxane, rt, 2 h; v. $SOCl_2$, triazole, CH_3CN, −10 °C, 1 h, then **10**, CH_3CN, K_2CO_3, rt, 4 days.

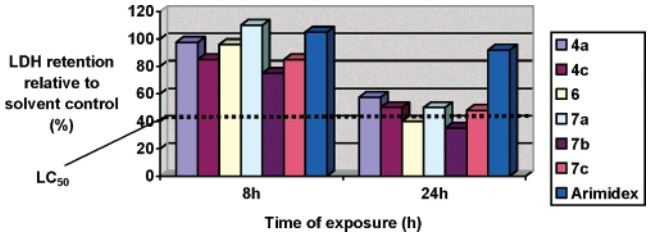


Figure 2. Cytotoxicity of the tested compounds (100 μM) determined from LDH retention data.

methoxy-substituted imidazole **6** and triazole **7c** benzofuran derivatives (IC_{50}: **4c** = 0.16 μM; **6** = 0.13 μM; **7c** = 0.13 μM).

In the triazole benzofuran series the nitrile derivative **7d** was the most potent (IC_{50} = 0.01 μM) with the halogen-substituted derivatives **7a** and **7b** showing potency comparable with their pyridine counterparts (IC_{50}: **7a** = 0.049 μM; **7b** = 0.044 μM). The alkyl-substituted triazole derivatives were less potent with a substantial reduction in activity on progressing from the methyl or trifluoromethyl (IC_{50}: **7f** = 0.1 μM; **7g** = 0.13 μM) to the ethyl (IC_{50}: **7h** = 1.23 μM) group. The nitro-substituted triazole derivative (**7e**) was found to have inhibitory activity of the same order as the standard arimidex (IC_{50}: **7e** = arimidex = 0.6 μM). In the 6-hydroxy triazole benzofuran series, the nitrile derivative **11d** showed activity comparable with the 6-methoxy derivative (IC_{50}: **11d** = 0.02 μM; **7d** = 0.01 μM), the nitro derivative **11e** displayed enhanced inhibitory activity compared with the 6-methoxy series (IC_{50}: **11e** = 0.06 μM; **7e** = 0.6 μM), whereas the chloro derivative **11b** showed a loss in potency (IC_{50}: **11b** = 1.46 μM; **7b** = 0.044 μM).

Six of the inhibitor compounds, the pyridine derivatives **4a** and **4c**, the imidazole **6**, and the triazoles **7a**, **7b**, and **7c**, were evaluated further for CYP19 selectivity and cytotoxicity (see Toxicology, Figure 2).

The compounds were evaluated for inhibition of steroidogenic enzymes P450 17 (CYP17, 17,20-lyase) and 17β-HSD types 1 and 3. Using 2 μM concentrations of the most potent inhibitors, imidazole **6** and triazoles **7a**−**c**, negligible inhibition of CYP17 was observed. No inhibition of 17β-HSD type 1 and 17β-HSD type 3 using inhibitor concentrations of 20 μM was observed with any of the inhibitors. The compounds were also evaluated against the P450 enzymes CYP24 and CYP26 involved in differentiation and proliferation, with no inhibition at concentrations up to 20 μM observed. The enzyme preparation and assays have been described by us previously.[19−22]

Toxicology. Toxicology evaluation was undertaken with six of the 6-methoxy benzofuran inhibitor compounds, chosen to reflect the varying heterocycle, and the reference standard arimidex, using rat liver hepatocytes. Cytotoxicity was determined using two criteria: alteration in cell morphology and marker enzyme retention. Drug concentrations of 100, 50, 25, 10, 5, 1, 0.5, and 0.1 μM (diluted using DMSO) were used, with 2,4-dinitrophenol (DNP), a known hepatotoxin, used as a positive control. In this study, samples were taken at 0, 4, 8, and 24 h. None of the compounds caused any changes in cell morphology or caused cell death, as determined from lactate dehydrogenase (LDH) enzyme retention, over an 8 h period at any of the concentrations tested (Figure 2).

However, after 24 h exposure of cells to these substances at the highest concentration (100 μM), cell death and changes in morphology were observed. Morphological evaluation indicated a gradation in the cellular perturbation seen at 100 μM of the test compounds. In order of being most disruptive: **6** > **7a** > **7b** and **7c** > **4a** > **4c** > arimidex. Cytotoxicity as determined by LDH retention was in the order: **6** and **7b** > **7c** > **4c** and **7a** > **4a** > arimidex (Figure 2). The LC_{50} data for the benzofuran inhibitor compounds and arimidex are shown in Table 2. Arimidex was not found to be cytotoxic at a concentration of 200 μM (24 h).

Discussion

The benzofuran inhibitors described are very potent using the human placental aromatase assay, compared with the reference compound arimidex (IC_{50} = 0.6 μM), with nanomolar inhibitory concentrations (Table 1). The preferred substitutions in the 4-position of the phenyl ring were previously determined[12] to be limited to small substituents/groups, and this was reflected in this series with the larger OCH_3, CH_3, CF_3, and CH_2CH_3 substituents resulting in reduced inhibitory activity compared with the F, Cl, NO_2, and CN substituted derivatives. Introduction of the 6-methoxy or 6-hydroxy substituent into the benzofuran ring greatly enhanced CYP19 inhibitory activity, and this was further investigated by molecular modeling to probe for potential bonding interactions with the enzyme active site.

Ligand docking of the inhibitor compounds with the CYP19 theoretical model (PDB 1TQA[23,24]) was performed using both SYBYL FlexX[25] and MOE-Dock[26] software. The inhibitor

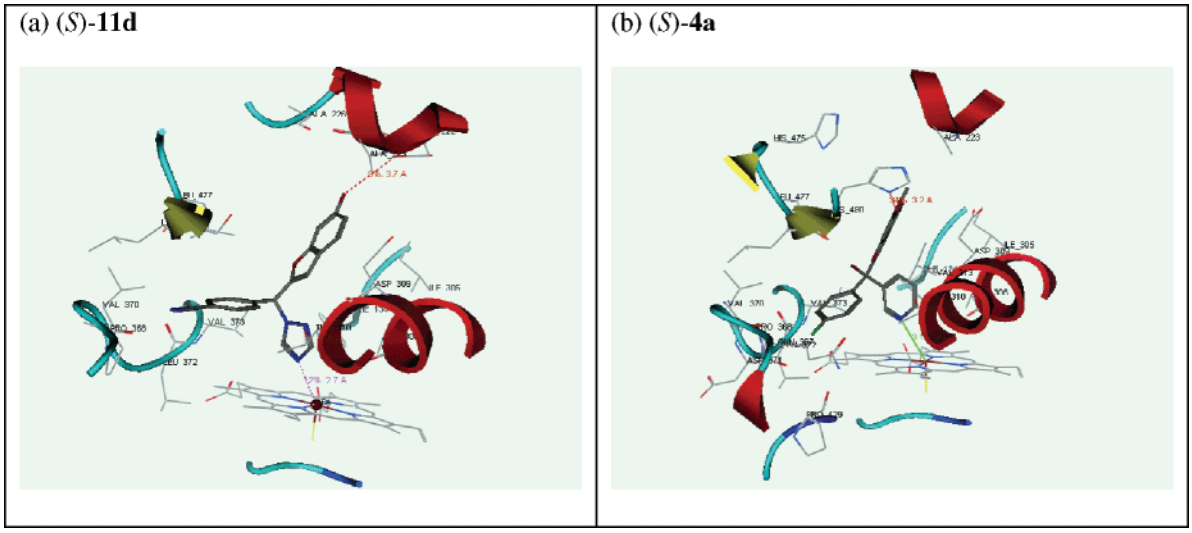
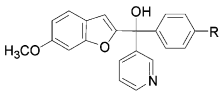
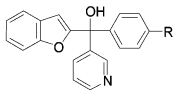
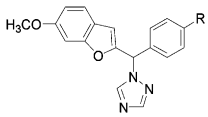
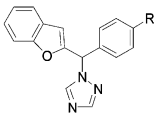


Figure 3. Active site region of CYP19 model showing the inhibitor ligand (a) *S*-**11d** and (b) *S*-**4a** enantiomers as a ball-and-stick form. Hydrogen bonding interactions are shown as red dashed lines, and distance from the heme is indicated with a green line, and transition metal interaction with a purple line. Amino acid residues identified are involved in hydrophobic interactions.

Table 2. Comparison of 6-Methoxy and Parent Benzofuran Inhibitors

IC_{50} (μM)		IC_{50} (μM)	
6-OCH_3 derivative	**Parent**[a]	**6-OCH_3 derivative**	**Parent**[b]
			
4a (R = F) 0.044	7.0	**7a** (R = F) 0.049	0.19
4b (R = Cl) 0.049	4.3	**7b** (R = Cl) 0.044	0.20
4c (R = OCH_3) 0.16	1.3	**7d** (R = CN) 0.13	0.21

[a] Reference 13. [b] Reference 12.

compounds are all racemic; therefore, both enantiomers of all the compounds were docked to determine any preference for (*R*)- or (*S*)-configuration; no preference was observed, with both enantiomers exhibiting a good 'fit' in the active site with the nitrogen of the heterocyclic ring coordinating with the Fe^{3+} of the haem. Therefore, for comparison, Figure 3 shows the (*S*)-enantiomers of the 4-methoxybenzofuran **4a** and the 6-hydroxy-benzofuran **11d**.

Ligand docking indicated that the 6-hydroxy group may form hydrogen bonding interactions with ALA223 (Figure 3a), allowing enhanced binding; however, this has not resulted in substantially enhanced activity with the 6-hydroxy triazole benzofuran series showing similar or, in the case of the 4-chloro derivative **11b**, reduced CYP19 inhibitory activity compared with the 6-methoxy derivatives.

The 6-methoxy substituent of the pyridine series may form hydrogen bonding interactions with HIS480, as determined from docking studies. The pyridine substituent may allow optimal orientation at the enzyme active site and maximize hydrophobic interactions (Figure 3b), resulting in the enhanced inhibitory activity observed compared with the unsubstituted parent benzofuran (e.g. Table 2). This increase in inhibitory potency is particularly noted in the pyridine series (**4a**−**c**).

Initial toxicology data (rat hepatocytes) is promising, with the pyridine derivatives showing the most favorable profile (general trend **4** > **7** > **6**). Comparison of the six compounds with arimidex cannot be conclusive, as the in vivo concentration of the benzofuran inhibitor compounds required to produce the same as or greater in vivo activity compared with arimidex would be anticipated to be less, owing to their enhanced potency.

Table 3. LC_{50}/IC_{50} Ratio

compound	IC_{50} (μM)	LC_{50} (μM) 24h	LC_{50}/IC_{50}
4a	0.044	>100	>2273
4c	0.16	100	625
6	0.13	50	385
7a	0.049	100	2041
7b	0.044	50	1136
7c	0.13	90	692
arimidex	0.6	>200	>333

A comparison of LC_{50}/IC_{50} data is shown (Table 3) to provide an indication of in vitro selectivity.

All the benzofuran inhibitors described are racemic; therefore, further research would involve chiral resolution of the enantiomers (chiral HPLC) for CYP19 inhibitory and cytotoxicity assays. Likewise, further toxicology evaluation of the most potent CYP19 inhibitors **7d** and **11d** (IC_{50} 0.01 and 0.02 μM, respectively) is warranted, in particular to consider the difference, if any, between the 6-methoxy and 6-hydroxy substituent on cytotoxicity.

Experimental Section

General Procedures. ^{1}H and ^{13}C NMR spectra were recorded with a Brucker Avance DPX300 spectrometer operating at 300 and 75 MHz, with Me_4Si as internal standard. Mass spectra were determined by the EPSRC mass spectrometry center (Swansea, UK). Microanalyses were determined by Medac Ltd (Surrey, UK). Flash column chromatography was performed with silica gel 60 (230−400mesh) (Merck), and TLC was carried out on precoated silica plates (kiesel gel 60 F_{254}, BDH). Compounds were visualized by illumination under UV light (254 nm) or by the use of vanillin stain followed by charring on a hotplate. Melting points were determined on an electrothermal instrument and are uncorrected. All solvents were dried prior to use as described by the handbook *Purification of Laboratory Chemicals*[27] and stored over 4 Å molecular sieves, under nitrogen. [1,2,6,7-^{3}H]Androstenedione (86.4 Ci/mmol, 37 MBq/mL) was purchased from NEN-Dupont UK (Stevenage, Herts). Scintillation fluid was Optiphase Hisafe from Fisons Chemicals (Loughborough, UK). The scintillation counter used was a LKB Wallac, 1217, Rack-beta. Toxicology evaluation, cell morphology, and marker enzyme retention assays were performed by PrimaCell (Cardiff University). The numbering of compounds for ^{1}H and ^{13}C NMR characterization and assignment of ^{1}H and ^{13}C NMR for all compounds is provided in the Supporting Information.

General Method for the Preparation of the Ketones 3 and 9. To a solution of sodium hydride (60%, 11 mmol) in dry *N*,*N*-dimethylformamide (10 mL) was added a solution of the methoxyaldehyde (**1** or **8**, 10 mmol) in dry *N*,*N*-dimethylformamide (6 mL) dropwise. Hydrogen gas was liberated to give a yellow solution of the sodium salt. A solution of the phenacyl bromide (**2**,10 mmol) in dry *N*,*N*-dimethylformamide (10 mL) was then added dropwise and the reaction heated under nitrogen at 80 °C for 1.5 h. Sodium methoxide (2.5 mmol) was added to the mixture and heating continued for another 1 h (TLC system: petroleum ether−ethyl acetate 4:1 v/v). After cooling, the reaction was evaporated to about a third of its volume, diluted with dichloromethane (100 mL), washed with water (100 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The resulting ketone **3** or **9** was purified by recrystallization. Analytical data is provided in Supporting Information.

General Method for the Preparation of Benzofuran-2-yl-(phenyl)-3-pyridylmethanols 4a−c. To a solution of pyridine-3-magnesium bromide (20 mmol) in THF (30 mL), generated in situ by the reaction of pyridin-3-yl bromide (20 mmol) and dry magnesium turnings (20 mmol) in THF (30 mL) under reflux for 1 h, was added the ketone **3** (5 mmol) and the reaction heated at 70 °C for 1 h. The solvent was removed under reduced pressure and the resulting residue dissolved in CH_2Cl_2 (50 mL), washed with H_2O (30 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The crude product was purified by flash column chromatography (petroleum ether−ethyl acetate 3:2 increasing to 2:3 v/v) to give the target compound.

6-Methoxybenzofuran-2-yl-(4-fluorophenyl)-3-pyridylmethanol (4a). White solid. Yield: 80%; mp 66−68 °C; R_f 0.14 (petroleum ether/ethyl acetate 3:1). ^{1}H NMR ($CDCl_3$): δ 8.64 (d, 1, J = 2.3 Hz, Ar), 8.60 (dd, 1, J = 1.6, 4.6 Hz, Ar), 7.78 (dt, 1, J = 2.0, 8.1 Hz, Ar), 7.38 (m, 4, Ar), 7.10 (m, 2, Ar), 7.02 (d, 1, J = 1.9 Hz, Ar), 6.93 (dd, 1, J = 2.2, 8.6 Hz, Ar), 6.28 (d, 1, J = 0.7 Hz, Ar), 3.89 (s, 3, OCH_3), 3.74 (s, 1, OH). ^{13}C NMR ($CDCl_3$): δ 160.4 (C), 158.0 (C), 156.0 (C), 149.5 (CH), 149.3 (CH), 148.0 (C), 141.5 (C), 135.3 (CH), 129.6 (CH), 126.2 (CH), 123.4 (CH), 122.0 (CH), 120.5 (C), 115.8 (CH), 115.5 (CH), 112.8 (CH), 107.5 (CH), 96.4 (CH, C-3), 77.9 (CH, H-1), 56.1 (CH_3). Anal. ($C_{21}H_{16}FNO_3$) C, H, N.

6-Methoxybenzofuran-2-yl-(4-chlorophenyl)-3-pyridylmethanol (4b). White solid. Yield: 80%; mp 68−70 °C; R_f 0.23 (petroleum ether/ethyl acetate 3:1). ^{1}H NMR ($CDCl_3$): δ 8.58 (m, 2, Ar), 7.76 (dt, 1, J = 2.0, 8.0 Hz, Ar), 7.37 (m, 7, Ar), 7.02 (s, 1, Ar), 6.93 (dd, 1, J = 2.2, 8.5 Hz, Ar), 6.29 (d, 1, J = 0.7 Hz, Ar), 3.88 (s, 3, OCH_3), 3.74 (s, 1, OH). ^{13}C NMR ($CDCl_3$): δ 161.4 (C), 161.0 (C), 158.7 (C), 148.2 (CH), 147.3 (CH), 143.7 (C), 141.1 (C), 135.7 (C), 134.4 (CH), 130.5 (CH), 129.4 (CH), 125.0 (C), 123.5 (CH), 122.8 (CH), 106.2 (CH), 106.0 (CH), 96.2 (CH, C-3), 78.0 (CH, H-1), 56.9 (CH_3). Anal. ($C_{21}H_{16}ClNO_3$) C, H, N.

(6-Methoxybenzofuran-2-yl)-(4-methoxyphenyl)-3-pyridylmethanol (4c). White crystalline solid. Yield: 55%; mp 39−43 °C; R_f 0.22 (petroleum ether/ethyl acetate 2:1). ^{1}H NMR ($CDCl_3$): δ 8.58 (ψt, 1, J = 1.7 Hz, Ar), 8.45 (dd, 1, J = 1.6, 4.8 Hz, Ar), 7.79 (m, 1, Ar), 7.38 (d, 1, J = 8.5 Hz, Ar), 7.27 (m, 3, Ar), 6.96 (d, 1, J = 2.0 Hz, Ar), 6.88 (m, 3, Ar), 6.28 (d, 1, J = 0.8 Hz, H-3), 5.08 (s, 1, OH), 3.84 (s, 3, OCH_3), 3.73 (s, 3, OCH_3). ^{13}C NMR ($CDCl_3$): δ 159.7 (C), 159.3 (C), 158.5 (C), 156.7 (C), 149.1 (CH), 148.8 (CH), 140.8 (C), 136.7 (C), 135.6 (CH), 129.0 (CH), 123.3 (CH), 121.8 (CH), 121.4 (C), 114.0 (CH), 112.5 (CH), 106.7 (CH), 96.5 (CH, C-3), 76.9 (CH, H-1), 56.1 (CH_3), 55.7 (CH_3). Anal. ($C_{22}H_{19}NO_4$) C, H, N.

General Method for the Preparation of the Carbinols 5 and 10. To a suspension of the ketone (**3** or **9**, 3 mmol) in dry dioxane (7 mL) was added sodium borohydride (3 mmol) and the reaction stirred at room temperature under nitrogen for 2 h. The reaction was concentrated under reduced pressure and 2 M aqueous hydrochloric acid (approximately 10 mL) added to the resulting syrup. This solution was extracted into diethyl ether (100 mL), washed with water (2 × 25 mL), dried ($MgSO_4$), and concentrated under reduced pressure to give the required product in quantitative yield which was used in the next reaction without any further purification. Analytical data is provided in Supporting Information.

General Procedure for the Preparation of the 1-[(6-Methoxybenzofuran-2-yl)-(4-substituted phenyl)methyl]-1*H*-imidazole (6), -1*H*-1,2,4-triazoles (7), and 6-Hydroxybenzofuran-1*H*-1,2,4-triazole Derivatives (11). To a cooled (0 °C) solution of imidazole or 1,2,4-triazole (12.15 mmol) in anhydrous acetonitrile (5 mL) was added thionyl chloride (0.36 g, 3.04 mmol) in anhydrous acetonitrile (5 mL) and the reaction stirred at 10 °C for 1 h. Potassium carbonate (3.0 mmol) was then added followed by a solution of the carbinol (**4** or **10**, 3.0 mmol) in anhydrous acetonitrile (5 mL) and the reaction allowed to stir at room temperature for 5 days. The suspension was filtered and the solution concentrated under reduced pressure to produce a residue which was extracted with dichloromethane (2 × 50 mL) and water (2 × 25 mL). The organic layers were combined and dried ($MgSO_4$), and the solvent was evaporated to give a yellow syrup. Purification by flash column chromatography (petroleum ether−ethyl acetate 9:1 to 6:4 v/v) gave the required product.

1-[(6-Methoxybenzofuran-2-yl)-(4-methoxyphenyl)methyl]-1*H*-imidazole (6). Amorphous white solid. Yield: 85%; R_f 0.20 (petroleum ether/ethyl acetate 1:1). ^{1}H NMR ($CDCl_3$): δ 7.66 (s, 1, H-2″), 7.49 (d, 1, J = 8.6 Hz, H-4″), 7.26 (m, 3, Ar), 7.09 (d, 1, J = 2.0 Hz, Ar), 7.01 (m, 4, Ar), 6.63 (s, 1, H-3), 6.53 (s, 1, H-1), 3.95 (s, 3, OCH_3), 3.93 (s, 3, OCH_3). ^{13}C NMR ($CDCl_3$): δ 164.5 (C), 160.4 (C), 159.0 (C), 143.1 (CH), 136.9 (C), 132.0 (CH), 128.7 (CH), 126.9 (C), 124.3 (CH), 120.2 (CH), 116.9 (CH), 107.5 (C), 105.8 (CH), 96.9 (CH), 60.3 (CH), 55.5 (CH_3), 55.0 (CH_3). Anal. ($C_{20}H_{18}N_2O_3$) C, H, N.

1-[(6-Methoxybenzofuran-2-yl)-(4-fluorophenyl)methyl]-1*H*-1,2,4-triazole (7a). Opaque syrup. Yield: 83%; R_f 0.16 (petroleum ether/ethyl acetate 3:1). ^{1}H NMR ($CDCl_3$): δ 8.18 (s, 1, H-3″), 8.08 (s, 1, H-5″), 7.46 (d, 1, J = 8.6 Hz, H-8), 7.33 (m, 2, Ar), 7.15 (m, 2, Ar), 7.04 (d, 1, J = 7.1 Hz, Ar), 6.94 (dd, 1, J = 2.3, 8.6 Hz, H-7), 6.85 (s, 1, H-3), 6.55 (s, 1, H-1), 3.89 (s, 3, OCH_3). ^{13}C NMR ($CDCl_3$): δ 165.0 (C), 159.2 (C), 156.9 (C), 152.7 (CH), 151.5 (C), 143.6 (CH), 132.1 (C), 130.0 (CH), 129.8 (CH), 122.1 (CH), 120.9 (C), 116.7 (CH), 116.4 (CH), 113.1 (CH), 108.4 (CH), 96.3 (CH), 62.0 (CH), 56.1 (CH_3). HRMS (ES^+) Calcd for $C_{18}H_{14}FN_3O_2$ $[M]^+$ 323.325, found 323.100.

1-[(6-Methoxybenzofuran-2-yl)-(4-chlorophenyl)methyl]-1*H*-1,2,4-triazole (7b). Opaque syrup. Yield: 80%; R_f 0.25 (petroleum ether/ethyl acetate 3:2). ^{1}H NMR ($CDCl_3$): δ 8.18 (s, 1, H-3″), 8.08 (s, 1, H-5″), 7.43 (ψt, 1, J = 3.9, 4.6 Hz, Ar), 7.32 (m, 2, Ar), 7.15 (m, 2, Ar), 7.13 (m, 2, Ar), 7.02 (d, 1, J = 1.9 Hz, Ar), 6.92 (dd, 1, J = 2.2, 8.6 Hz, H-7), 6.85 (s, 1, H-3), 6.53 (s, 1,

H-1), 3.85 (s, 3, OCH_3). ^{13}C NMR ($CDCl_3$): δ 161.7 (C), 159.1 (C), 156.9 (C), 152.7 (CH), 151.5 (C), 143.6 (CH), 132.1 (C), 130.0 (CH), 129.9 (CH), 122.1 (CH), 120.9 (C), 116.6 (CH), 116.4 (CH), 113.1 (CH), 108.3 (CH), 96.3 (CH), 61.9 (CH), 56.1 (CH_3). HRMS (ES^+) Calcd for $C_{18}H_{14}ClN_3O_2$ $[M]^+$ 339.770, found 339.770.

1-[(6-Methoxybenzofuran-2-yl)-(4-methoxyphenyl)methyl]-1*H*-1,2,4-triazole (7c). Syrup. Yield: 85%; R_f 0.28 (petroleum ether/ethyl acetate 2:1). ^1H NMR ($CDCl_3$): δ 8.09 (s, 1, H-3″), 8.01 (s, 1, H-5″), 7.38 (d, 1, J = 8.6 Hz, Ar), 7.26 (d, 1, J = 82.1 Hz, Ar), 7.23 (d, 1, J = 2.0 Hz, Ar), 6.97 (d, 1, J = 1.9 Hz, Ar), 6.92 (m, 2, Ar), 6.87 (dd, 1, J = 2.2, 8.6 Hz, H-7), 6.75 (s, 1, H-3), 6.47 (s, 1, H-1), 3.82 (s, 3, OCH_3), 3.81 (s, 3, OCH_3). ^{13}C NMR ($CDCl_3$): δ 165.5 (C), 158.9 (C), 156.8 (C), 152.6 (CH), 152.3 (C), 143.5 (CH), 129.5 (CH), 128.1 (C), 122.0 (CH), 121.1 (C), 114.8 (CH), 112.9 (CH), 107.9 (CH), 96.3 (CH), 62.2 (CH), 56.1 (CH_3), 55.8 (CH_3). Anal. ($C_{19}H_{17}N_3O_3$) C, H, N.

4-[6-Methoxybenzofuran-2-yl)-1*H*-1,2,4-triazol-1-ylmethyl]-benzonitrile (7d). Yellow honeycomb solid. Yield: 73%; mp 57−59 °C; R_f 0.63 (petroleum ether/ethyl acetate 1:1). ^1H NMR ($CDCl_3$): δ 8.24 (s, 1, H-3″), 8.11 (s, 1, H-4″), 7.77 (d, 2, J = 8.3 Hz, H-2′, H-6′), 7.42 (m, 3, H-3′, H-5′, H-4), 7.01 (d, 1, J = 1.9 Hz, 1, H-7), 6.96 (m, 2, H-5, H-3), 6.63 (s, 1, H-1), 3.90 (s, 3, OCH_3). ^{13}C NMR ($CDCl_3$): δ 159.43 (CH), 158.36 (CH), 157.00 (C), 153.04 (C), 149.42 (C), 143.87 (C), 141.42 (C), 133.26 (CH), 128.64 (CH), 122.29 (CH), 120.65 (C), 118.53 (CH), 113.51 (CH), 113.46 (CH), 96.30 (CH), 61.99 (CN), 56.17 (CH_3). Anal. ($C_{19}H_{14}N_4O_2 \cdot 0.2H_2O$) C, H, N.

1-[(6-Methoxybenzofuran-2-yl)-(4-nitrophenyl)methyl]-1*H*-1,2,4-triazole (7e). Yellow amorphous solid. Yield: 30%; R_f 0.27 (petroleum ether/ethyl acetate 1:1). ^1H NMR ($CDCl_3$): δ 8.33 (s, 1, H-3″), 8.31 (s, 1, H-4″), 8.25 (s, 1, H-2′), 8.12 (s, 1, H-6′), 7.49 (m, 3, H-3′, H-5′, H-4), 7.05 (d, 1, J = 1.8 Hz, H-7), 7.00 (m, 2, H-5, H-3), 6.65 (s, 1, H-1), 3.90 (s, 3, OCH_3). ^{13}C NMR ($CDCl_3$): δ 159.47 (CH), 156.87 (CH), 157.02 (C), 153.10 (C), 149.84 (C), 148.59 (C), 143.86 (C), 128.90 (CH), 124.65 (CH), 122.31 (C), 120.63 (CH), 113.50 (CH), 109.15 (CH), 96.30 (CH), 76.56 (CH), 19.04 (CH_3). Anal. ($C_{18}H_{14}N_4O_4$) C, H, N.

1-[(6-Methoxybenzofuran-2-yl)-*p*-tolylmethyl]-1*H*-1,2,4-triazole (7f). Yellow syrup. Yield: 27%; R_f 0.34 (petroleum ether/ethyl acetate 1:1). ^1H NMR ($CDCl_3$): δ 8.16 (s, 1, H-3″), 8.08 (s, 1, H-4″), 7.46 (d, 1, J = 8.6 Hz, H-4), 7.26 (dd, 4, J = 8.5 Hz, H-2′, H-6′, H-3′, H-5′), 7.04 (d, 1, J = 1.9 Hz, H-7), 6.93 (dd, 1, J = 2.1 Hz, H-5), 6.83 (s,1, H-3), 6.55 (s, 1, H-1), 3.89 (s, 3, OCH_3), 2.43 (s, 3, CH_3). ^{13}C NMR ($CDCl_3$): δ 158.99 (CH), 156.84 (CH), 152.60 (C), 152.13 (C), 143.58 (C), 139.55 (C), 133.19 (C), 130.18 (CH), 127.97 (CH), 122.01 (CH), 121.08 (CH), 112.97 (CH), 108.07 (CH), 96.34 (CH), 62.51 (CH), 56.13 (CH_3), 21.63 (CH_3). Anal. ($C_{19}H_{17}N_3O_2$) C, H, N.

1-[(6-Methoxybenzofuran-2-yl)-(4-trifluoromethylphenyl)-methyl]-1*H*-1,2,4-triazole (7g). Yellow/brown syrup. Yield: 28%; R_f 0.30 (petroleum ether/ethyl acetate 1:1). ^1H NMR ($CDCl_3$): δ 8.22 (s, 1, H-3″), 8.10 (s, 1, H-4″), 7.73 (d, 2, J = 8.1 Hz, H-2′, H-6′), 7.46 (m, 3, H-3′, H-5′, H-4), 7.05 (s, 1, H-7), 6.95 (s, 2, H-5, H-3), 6.60 (s, 1, H-1), 3.90 (s, 3, OCH_3). ^{13}C NMR ($CDCl_3$): δ 159.34 (CH), 156.98 (CH), 152.89 (C), 150.61 (C), 143.76 (C), 140.24 (C), 131.94 (C), 131.50 (C), 128.32 (CH), 126.51 (CH), 122.35 (CH), 122.22 (CH), 120.79 (CH), 113.34 (CH), 108.88 (CH), 96.32 (CF_3), 56.14 (CH_3). Anal. ($C_{19}H_{14}F_3N_3O_2.0.5H_2O$) C, H, N.

1-[(6-Methoxybenzofuran-2-yl)-(4-ethylphenyl)methyl]-1*H*-1,2,4-triazole (7h). Yellow amorphous solid. Yield: 77%; R_f 0.33 (petroleum ether/ethyl acetate 1:1). ^1H NMR ($CDCl_3$): δ 8.33 (s, 1, H-3″), 8.18 (s, 1, H-4″), 7.43 (d, 1, J = 8.6 Hz, H-4), 7.28 (m, 4, H-2′, H-6′, H-3′, H-5′), 7.03 (d, 1, J = 1.9 Hz, H-7), 6.93 (dd, 1, J = 2.2 Hz, H-5), 6.84 (s, 1, H-3), 6.65 (s, 1, H-1), 3.87 (s, 3, OCH_3), 2.72 (dd, 2, J = 7.6 Hz, CH_2), 1.30 (m, 3, CH_3). ^{13}C NMR ($CDCl_3$): δ 159.00 (CH), 156.85 (CH), 152.58 (C), 152.20 (C), 145.76 (C), 143.63 (C), 133.45 (C), 128.99 (CH), 128.05 (CH), 122.02 (CH), 121.12 (C), 112.97 (CH), 108.05 (CH), 96.36 (CH), 62.51 (CH), 56.10 (CH_3), 28.97 (CH_2), 15.82 (CH_3). Anal. ($C_{20}H_{19}N_3O_2$) C, H, N.

2-[(4-Chlorophenyl)-[1,2,4]triazol-1-ylmethyl]benzofuran-6-ol (11b). Yellow amorphous solid. Yield: 76%; R_f 0.55 (petroleum ether/ethyl acetate 1:1). ^1H NMR ($CDCl_3$): δ 9.08 (s, 1, OH), 8.32 (s, 1, H-3″), 8.20 (s, 1, H-4″), 7.49 (m, 3, H-2′, H-6′, H-4), 7.34 (m, 3, H-3′, H-5′, H-7), 7.01 (d, 1, J = 1.7 Hz, H-5), 6.93 (m, 1, H-3), 6.62 (s, 1, H-1). ^{13}C NMR ($CDCl_3$): δ 156.91 (CH), 156.42 (CH), 151.99 (C), 150.15 (C), 143.47 (C), 135.72 (C), 134.48 (C), 131.50 (C), 129.77 (CH), 129.30 (CH), 122.41 (CH), 120.29 (CH), 113.91 (CH), 109.09 (CH), 98.62 (CH). Anal. ($C_{17}H_{12}$ $ClN_3O_2 \cdot 0.2H_2O$) C, H, N.

4-[(6-Hydroxybenzofuran-2-yl)-[1,2,4]triazol-1-ylmethyl]benzonitrile (11d). Orange amorphous solid. Yield: 85%; R_f 0.57 (petroleum ether/ethyl acetate 1:1). ^1H NMR ($CDCl_3$): δ 8.29 (s, 1, OH), 8.22 (s, 1, H-3″), 8.17 (s, 1, H-4″), 7.76 (d, 2, J = 8.3 Hz, H-2′, H-6′), 7.43 (dd, 3, J = 3.2 Hz, H-3′, H-5′, H-4), 6.93 (m, 3, H-7, H-5, H-3), 6.61 (s, 1, H-1). ^{13}C NMR ($CDCl_3$): δ 156.95 (CH), 156.40 (CH), 152.38 (C), 149.25 (C), 143.69 (C), 141.12 (C), 133.28 (CH), 128.62 (CH), 131.36 (C), 122.58 (CH), 120.27 (C), 118.43 (CH), 114.05 (CH), 113.63 (CH), 109.50 (CH). Anal. ($C_{18}H_{12}N_4O_2 \cdot 0.5H_2O$) C, H, N.

2-[(4-Nitrophenyl)-[1,2,4]triazol-1-ylmethyl]benzofuran-6-ol (11e). Pale yellow amorphous solid. Yield: 86%; R_f 0.20 (petroleum ether/ethyl acetate 1:1). ^1H NMR ($CDCl_3$): δ 8.43 (s, 1, OH), 8.32 (m, 3, H-3″, H-4″, H-2′), 8.20 (s, 1, H-6′), 7.43 (m, 3, H-3′, H-5′, H-4), 6.95 (m, 3, H-7, H-5, H-3), 6.65 (s, 1, H-1). ^{13}C NMR ($CDCl_3$): δ 156.98 (CH), 156.47 (CH), 152.42 (C), 149.08 (C), 148.67 (C), 143.73 (C), 142.85 (C), 132.68 (C), 128.89 (CH), 124.68 (CH), 122.60 (CH), 129.43 (CH), 123.33 (CH), 121.47 (CH), 114.09 (CH). Anal. ($C_{17}H_{12}N_4O_4 \cdot 0.2H_2O$) C, H, N.

Aromatase Assay. The classical ^3H_2O assay[18] was used to measure the effect of the inhibitor compounds on aromatase activity using human placental microsomes. A solution of [1,2,6,7-^{3}H]-androstenedione and androstenedione (0.5 μM final concentration) was incubated in test tubes at 37 °C for 15 min with the human placental microsomal preparation (8.24 mg/mL, 30 μL), phosphate buffer (400 μL, 50 mM, pH 7.4), and NADPH (50 μL, 16 mM) in the presence of inhibitor (10 μL, 1 or 5 mmol, 20 or 100 μM final concentration, respectively) in EtOH. Control experiments were run with EtOH (10 μL) in place of the inhibitor. The reaction was quenched by the addition of aqueous $HgCl_2$ (30 μL, 1 mM) followed by an aqueous suspension of charcoal (1 mL, 1 wt %). The test tubes were centrifuged (15 min, 3000 rpm), and then the supernatant liquid was placed in scintillation vial to which 1 mL of scintillation fluid was added. The ^3H_2O contained in each vial was then determined using a LKB Wallac, 1217, Rack-beta scintillation counter.

For IC_{50} values the general method described for determination of percentage inhibition was followed except that a range of concentrations of inhibitor were used. Calculation of IC_{50} was determined by plotting % inhibition versus Log [I] using Cricket Graph III 1.5f software.[28]

Toxicology. Hepatocytes were prepared and cultured from male Sprague Dawley rats as described previously.[29,30] Cells were cultured in 96-well plates (8-well strip format, Fisher Scientific) on an adsorbed collagen layer (type III). The coated wells were prepared by covering the bottom of each well with sterile collagen (2 mg/mL) in 0.1% acetic acid. The plates were left for 1 h, washed three times with sterile water, and placed in a 37 °C incubator for 48 h before use. The plating medium was Leibovitz L-15 medium pH 7.4 supplemented with glucose (8.3 mM), HEPES (25 mM), insulin (0.8 μg/mL), dexamethasone (1 μM), 10% (v/v) heat-inactivated (56 °C for 30 min) new born calf serum, and gentamycin (50 μg/mL). Hepatocytes (4.5×10^4) were plated out in 0.2 mL. The plates were placed in a 37 °C humidified incubator to allow cell attachment. After 2 h the plates were removed from the incubator and the medium aspirated off. Each well was washed twice with 0.2 mL of serum free medium, and 0.2 mL of serum free medium containing the appropriate concentration of the test compounds was added to the wells. The plates were returned to the 37 °C humidified incubator for the times indicated in the figure legends.

Stock solutions (5 mM) of the test compounds were prepared in DMSO. They were diluted to the appropriate concentrations by serial dilution with serum free medium. All the compounds were soluble at 100 μM, after incubating at 37 °C. Diluted stocks were filter sterilized. Triplicate samples were taken at each time point along with the appropriate DMSO solvent controls.

Cell morphology in the presence of test compounds was monitored with an Olympus inverted microscope fitted with a Nikon digital camera. Cell viability was determined using a CytoTox 96 nonradioactive cytotoxicity assay kit (Promega). Cells on compound treated and control well strips were removed from the incubator at 0, 4, 8, and 24 h. The culture medium was aspirated away; the cells were lysed and diluted with buffer pH 7.4, containing sucrose (0.25 M), HEPES (5 mM), and EDTA (1 mM), until linear rates of lactate dehydrogenase were recorded. The cytotoxicity assay was carried out at 37 °C, in 96-well dishes, using a Tecan GENios plate reader. The cytotoxicity of the test compounds was determined by comparing with the appropriate DMSO solvent controls.

Molecular Docking. All molecular modeling studies were performed on an RM Innovator Pentium IV 2.4GHz running either Linux Fedora Core 3 or Windows XP using Molecular Operating Environment (MOE) 2004.03[26] and FlexX module in SYBYL 7.0[25] molecular modeling software. All the minimizations were performed with MOE until RMSD gradient of 0.05 kcal mol^{-1} $Å^{-1}$ with the force field specified, and the partial charges were automatically calculated.

Ligands were docked within the active site of the homology model PDN 1TQA[23] using both the FlexX docking program of SYBYL and MOE-Dock. For MOE-Dock, simulated annealing was used as the search protocol with a total of 5 runs, 10 cycles per run, 8000 steps per cycle, and an initial temperature of 1000 K. Docking with FlexX was performed with the default values. Molecular dynamics was performed with MOE using the NVT environment for 100 ps and constant temperature of 300 K using the MMFF94X force field with all other default settings in MOE-dynamics chosen. The lowest energy conformation was selected and subjected to an energy minimization using the MMFF94X force field. The output of FlexX docking was visualized in MOE and the scoring.svl script was used to identify interaction types between ligand and protein.

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Supporting Information Available: The numbering of compounds for ^{1}H and ^{13}C NMR characterization and assignment of ^{1}H and ^{13}C NMR spectra for ketones **3** and **9**, carbinols **4** and **10**, and 2-hydroxy-4-(tetrahydropyran-2-yloxy)benzaldehyde (**8**). This material is available free of charge via the Internet at http://pubs.acs.org.

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