Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry 14 (2006) 3643–3653
Bioorganic & Medicinal Chemistry

Synthesis and CYP26A1 inhibitory activity of 1-[benzofuran-2-yl-(4-alkyl/aryl-phenyl)-methyl]-1*H*-triazoles

Stephane Pautus,[a] Sook Wah Yee,[a] Martyn Jayne,[a] Michael P. Coogan[b] and Claire Simons[a,*]

[a]*Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff CF10 3XF, UK*
[b]*School of Chemistry, Cardiff University, Cardiff CF10 3TB, UK*

Received 29 November 2005; revised 9 January 2006; accepted 10 January 2006
Available online 3 February 2006

Abstract—Methodology previously described by our group was applied to the preparation of a series of 4-alkyl/aryl-substituted 1-[benzofuran-2-yl-phenylmethyl]-1*H*-triazoles. The [1,2,4]-triazole derivatives were prepared for a range of alkyl and aryl substituents, and for the 4-methyl, 4-ethyl, 4-*i*propyl, 4-*t*butyl, 4-phenyl and 4-chlorophenyl derivatives, the minor [1,3,4]-triazole isomer also isolated. All the triazole derivatives were evaluated for CYP26A1 inhibitory activity using a MCF-7 cell-based assay. The 4-ethyl and 4-phenyl-1,2,4-triazole derivatives displayed inhibitory activity (IC_{50} 4.5 and 7 μM, respectively) comparable with that of the CYP26 inhibitor liarozole (IC_{50} 7 μM). Using a CYP26A1 homology model (based on CYP3A4) template, docking experiments were performed with MOE with multiple hydrophobic interactions observed in addition to coordination between the triazole nitrogen and the haem transition metal.

1. Introduction

In humans, CYP26A1 is expressed in the liver, heart, pituitary gland, adrenal gland, testis, brain and placenta,[1] and has been mapped to chromosome 10q23-q24.[2] It is thought that the principal role of CYP26A1 is homeostatic, that is, the regulation of intracellular ATRA steady-state levels via a negative feedback loop similar to that of CYP24 in the metabolism of cholecalciferol.[3] The enzyme therefore may have a protective function, as an important regulator of differentiation and a possible modulator of disease states indirectly by controlling ATRA and other retinoid concentrations.[4]

Retinoic acid has been used in a number of clinical situations, especially oncology and dermatology. In oncology, ATRA has shown spectacular success in the treatment of acute promyelocytic leukaemia,[5,6] although remission seen is followed by relapse within 4–6 months; this appears to be due to increased RA metabolism as a result of RA induction so leading to decreased clinical efficacy. Although other CYPs are also induced by ATRA and catalyse its metabolism, it is thought that CYP26A1 is likely to be the most important enzyme involved in its degradation[7] (Fig. 1).

ATRA may improve the efficacy of other treatments such as radiation, cisplatin and interferon therapies.[8,9] Retinoids have been used for some time in the treatment of psoriasis, cystic acne, cutaneous malignancies due to hyperkeratinisation as well as in the treatment of photodamaged skin.[10,11] As CYP26A1 appears to be so important in the management of intracellular levels of ATRA and the development of resistance to its effects in cancer, it presents a useful target in combating the development of resistance. Inhibition of CYP26A1 would elevate normal tissue levels of ATRA or maintain high therapeutic levels of ATRA preventing the resistance that develops to treatment. This might provide a leap forward in the prevention and treatment of a number of diseases including hormone refractory prostate cancer and psoriasis.

A number of inhibitors of ATRA metabolism have been developed over the last fifteen years.[12–15] The discovery of the imidazoles as inhibitors led to further study into this class of compounds, resulting in the synthesis of

Keywords: 1-[Benzofuran-2-yl-(4-alkyl/aryl-phenyl)-methyl]-1*H*-triazoles; CYP26A1; Enzyme inhibition; MCF-7; Molecular modelling.
* Corresponding author. Tel.: +44 02920 876307; fax: +44 02920 874149; e-mail: SimonsC@Cardiff.ac.uk

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.01.018

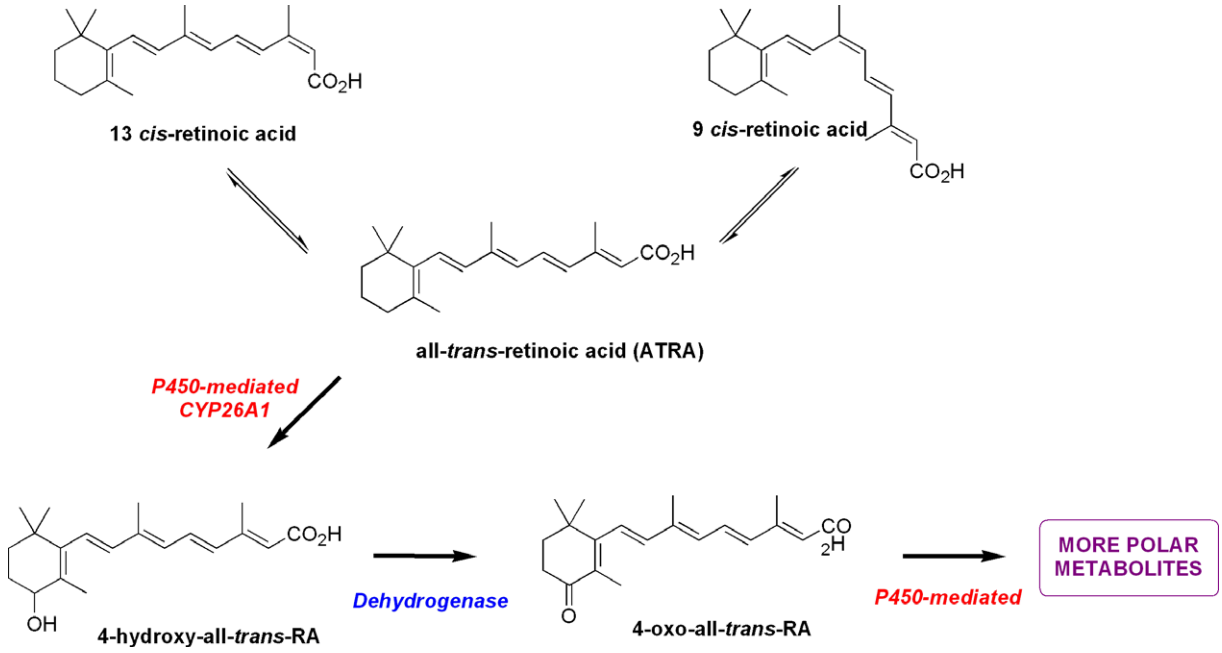


Figure 1. Metabolism of *all-trans* retinoic acid (RA = retinoic acid).

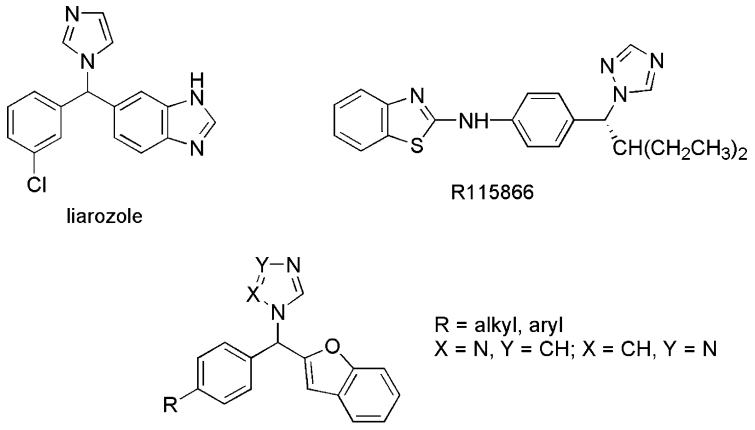


Figure 2. CYP26A1 metabolism inhibitors and target structures.

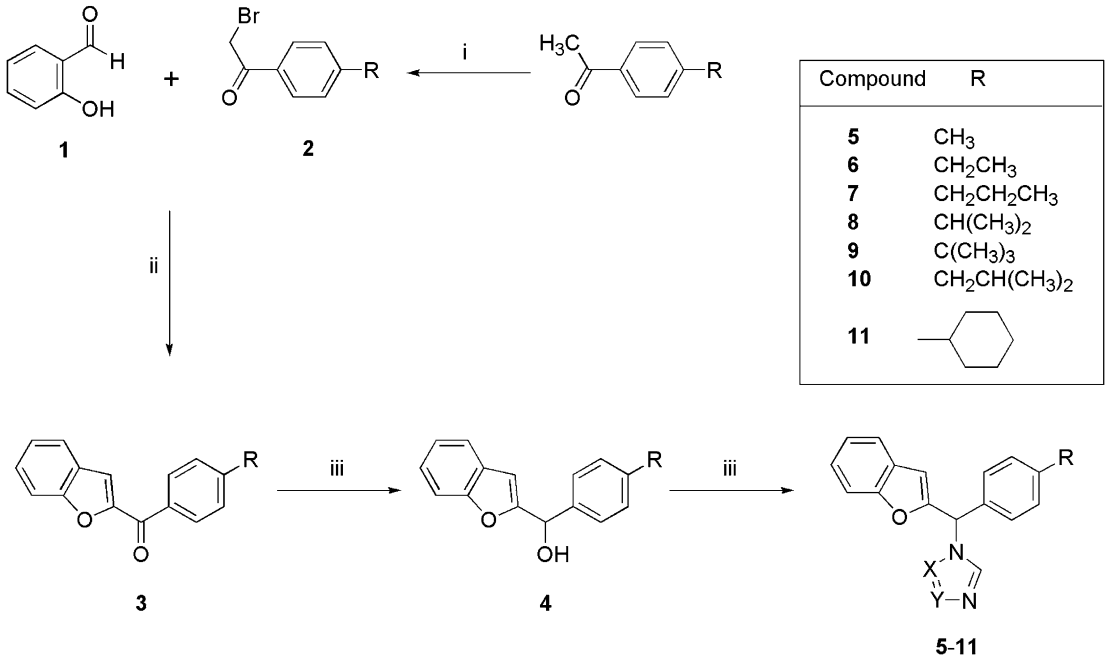
liarozole, which became the most studied inhibitor of ATRA metabolism.[16–18] Though more specific and effective than the other imidazoles, liarozole is still a potent inhibitor of aromatase, preventing its use as an oral retinoic acid-mimetic for sex hormone-independent cancers, therefore clinical development of liarozole was discontinued.[3] Further development has led to the discovery of the 1*H*-triazole derivative, R115866, a potent and selective inhibitor of ATRA metabolism (Fig. 2).[19]

As part of our ongoing research into the development of CYP26A1 inhibitors, we evaluated a number of compound classes for their inhibitory activity. The 4-alkylbenzofuran derivatives were promising candidates owing to their structural similarity with liarozole as confirmed by molecular modelling. The synthesis, CYP26A1 inhibitory activity and bonding interactions at the CYP26A1 active site are described.

2. Chemistry

The required intermediates for the preparation of the 1-[(benzofuran-2-yl)phenylmethyl] triazoles were the corresponding ketones, benzo[*b*]furan-2-yl-phenylmethanones (**3**). The synthesis of the ketones was achieved by the Rap–Stoermer reaction,[20] and involved reaction of salicylaldehyde (**1**) with the α-bromoketones (**2**),[21] generated by reaction of the appropriate acetophenone with bromine and aluminium trichloride according to literature methodology[22] (Scheme 1).

The ketones were then reduced to the respective racemic carbinols (**4**) in quantitative yields by reaction with sodium borohydride. The target triazole derivatives (**5**–**11**) were then obtained by reaction with *N*,*N*′-thionylditriazole, which was prepared by the reaction of 1,2,4-1*H*-triazole with thionyl chloride at 10 °C for 1 h, for 4 days.



Scheme 1. Reagents and conditions: (i) Br_2, $AlCl_3$, Et_2O, 1 h; (ii) NaH, DMF, 80 °C, 2 h then NaOMe, 80 °C, 1 h; (iii) $NaBH_4$, dioxane, 2 h; (iv) *N*,*N*′-thionylditriazole, K_2CO_3, CH_3CN, 4 days.

In addition to the 1,2,4-triazole isomer, the minor 1,3,4-isomer was obtained for some of the 1-[(benzofuran-2-yl)phenylmethyl] triazoles. For the 1,2,4-isomers, the triazole protons were observed as two singlets, each integrating for one proton, at ~8.2 and 8.1 ppm. The structure of the 1,3,4-triazole isomers was confirmed by ^{1}H NMR, which gave a singlet, integrating for two protons, at ~8.30 ppm for the symmetrical triazole protons.

The 4-aryl ketone derivatives (**13**) were prepared from the 4-bromoketone (**12**) derivative using a Suzuki coupling[23] with the appropriate phenyl boronic acid. Subsequent reduction gave the alcohols (**14**), which were reacted with *N*,*N*′-thionylditriazole to generate the 1,2,4- and 1,3,4-triazole isomers of the phenyl (**15**) and 4-chlorophenyl (**16**) benzofurans (Scheme 2).

3. CYP26A1 inhibitory activity

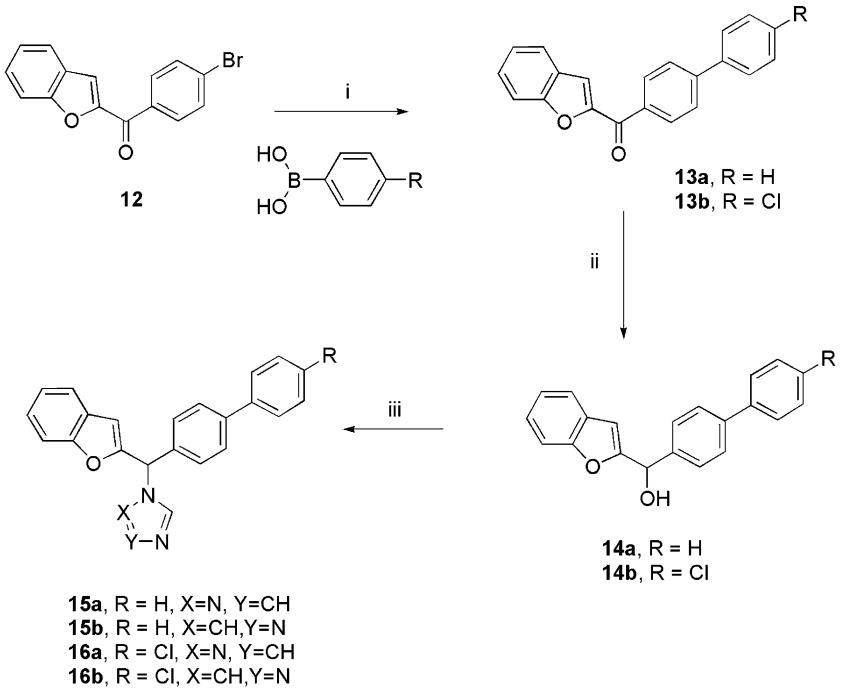
The 1-[benzofuran-2-yl-(4-alkyl/aryl-phenyl)-methyl]-1*H*-triazoles (**5**–**11**, **15** and **16**) were evaluated for their retinoic acid metabolism inhibitory activity using a MCF-7 cell assay,[13] using radiolabelled [11,12-^{3}H] all-*trans* retinoic acid as the substrate and liarozole and R115866 as standards for comparison. The 4-ethyl (**6a**, IC_{50} = 4.5 μM; **6b**, IC_{50} = 7 μM) and 4-phenyl (**15a**, IC_{50} = 7 μM; **15b**, IC_{50} = 9 μM) derivatives displayed inhibitory activity of CYP26A1 comparable with that of liarozole (IC_{50} = 7 μM), and the cyclohexyl derivative (**11**, IC_{50} = 5–15 μM) displayed moderate activity, the remaining alkyl/aryl derivatives all poor CYP26A1 inhibitors of retinoic acid metabolism (IC_{50} > 20 μM) (Table 1). All the 1-[benzofuran-2-yl-(4-alkyl/aryl-phenyl)-methyl]-1*H*-triazoles were considerably less active than R115866 (IC_{50} = 5 nM).

The 4-methyl derivative (**5**) has previously been evaluated as an aromatase (CYP19) inhibitor (IC_{50} = 0.59 μM, cf. anastrazole = 0.6 μM).[21] The 4-ethyl (**6**) and 4-phenyl (**15**) derivatives were also screened against CYP19 to determine selectivity, both having an inhibitory activity IC_{50} > 100 μM. The negligible CYP19 inhibitory activity of **6** and **15** was expected from SAR studies[21] which had shown that only small substituents, for example, F, Cl, CH_3 and CN were tolerated at the 4-position of the phenyl ring for CYP19 inhibitors.

4. Molecular modelling

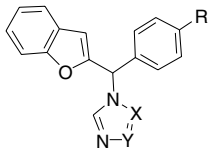
In order to rationalise the results obtained, MOE (Molecular Operating Environment)[24] docking studies of the inhibitors were performed using a human CYP26A1 model,[25] built using the recently crystallised human CYP3A4[26] as a template. Docking interactions at the enzyme active site were shown to be comparable with those of the CYP26 inhibitor liarozole with the benzofurans positioned above the haem with multiple hydrophobic interactions (Fig. 3). The inhibitor 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 similar '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-ethyl (**6a**) and the 4-phenyl (**15a**) 1,2,4-triazole benzofuran derivatives.

Docking of the 4-alkyl derivatives with a chain length of >2 indicated that steric hindrance was encountered preventing close (2.5–3.5 Å) interaction with the haem.



Scheme 2. Reagents and conditions: (i) $Pd(PPh_3)_4$, $Na_2CO_{3(aq)}$ toluene, 100 °C, 5 h, then H_2O_2, rt, 1 h (60–70 %); (ii) $NaBH_4$, dioxane, 2–6 h; (iii) *N*,*N*′-thionylditriazole, K_2CO_3, CH_3CN, 4 days.

Table 1. IC_{50} data for the novel benzofuran derivatives using CYP26A1 MCF-7 assays



Compound	R	X	Y	IC_{50} (μM)
5a	CH_3	N	CH	>40
5b	CH_3	CH	N	>40
6a	CH_2CH_3	N	CH	4.5
6b	CH_2CH_3	CH	N	5
7	$CH_2CH_2CH_3$	N	CH	50–100
8a	$CH(CH_3)_2$	N	CH	20–40
8b	$CH(CH_3)_2$	CH	N	50–100
9	$C(CH_3)_3$	N	CH	10–25
10	$CH_2CH(CH_3)_2$	N	CH	40–50
11	Cyclohexyl	N	CH	5–15
15a	C_6H_5	N	CH	7
15b	C_6H_5	CH	N	9
16a	*p*-Cl–C_6H_5	N	CH	20–40
16b	*p*-Cl–C_6H_5	CH	N	20–40
Ketoconazole	—	—	—	12
Liarozole	—	—	—	7
R115866	—	—	—	0.005

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

The phenyl derivative (**15**, IC_{50} = 7–9 μM) has a greater inhibitory activity despite its size owing to favourable conformation and enhanced hydrophobic bonding. This may strengthen the interaction of the phenyl derivative increasing its potency as an inhibitor of the enzyme; however, the unsubstituted phenyl was the limit with the introduction of the chloro substituent (**16**, IC_{50} = 20–40 μM) resulting in a reduction of inhibitory activity owing to steric/electronic factors.

The docking studies however fail to shed any light on the poor results obtained for the methyl compound (**5**,

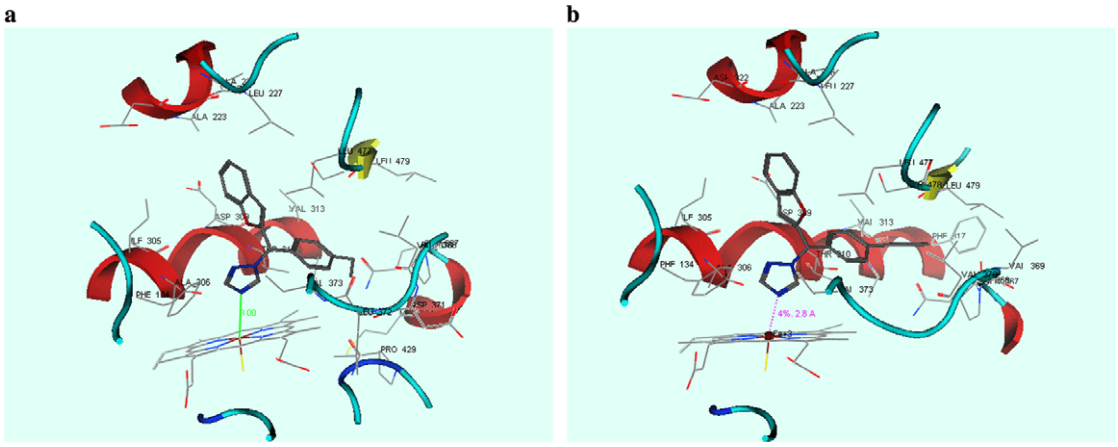


Figure 3. Active site region of CYP26A1 model showing (a) *S*-**6a** and (b) *S*-**15a** in ball and stick form. The distance from the haem is indicated with a green line, transition metal interaction with a purple line. Amino acid residues identified are involved in hydrophobic interactions.

$IC_{50} > 40$ μM), which was expected to possess potency similar to that of the ethyl derivative (**6**, $IC_{50} = 4.5$–5 μM).

A series of 4-alkyl/aryl benzofuran phenyl triazole derivatives has been prepared with the ethyl and phenyl derivatives shown to possess comparable inhibitory activity with that of the known CYP26 inhibitor liarozole.

5. Experimental

5.1. Materials and methods: chemistry

^1H and ^{13}C NMR spectra were recorded with a Brucker Avance DPX500 spectrometer operating at 500 and 125 MHz, with Me_4Si as internal standard. Mass spectra were determined by the EPSRC Mass Spectrometry Centre (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). Melting points were determined on an electrothermal instrument and are uncorrected.

5.1.1. General method for the preparation of the ketones 3. To a solution of sodium hydride (60%, 11 mmol) in dry *N*,*N*-dimethylformamide (10 mL) was added a solution of salicylaldehyde (**1**, 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 α-bromoketone **2** (10 mmol) in dry *N*,*N*-dimethylformamide (10 mL) was then added dropwise and the reaction mixture was heated under nitrogen at 80 °C for 1.5 h. Sodium methoxide (2.5 mmol) was added to the mixture and heating was continued for another hour (TLC system: petroleum ether/ethyl acetate 4:1 v/v). After cooling, the reaction mixture 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.

5.1.1.1. Benzofuran-2-yl-(4-ethyl-phenyl)-methanone (3a, R = CH_2CH_3). Brown solid (34%) after recrystallisation from methanol, mp 60–62 °C. ^1H NMR ($CDCl_3$): δ 8.05 (d, $J = 8.1$ Hz, 2H, Ar), 7.78 (d, $J = 7.7$ Hz, 1H, Ar), 7.70 (d, $J = 7.9$, 1H, Ar), 7.55 (m, 2H, Ar), 7.37 (m, 3H, Ar), 2.81 (q, $J = 7.6$ Hz, 2H, CH_2), 1.36 (t, $J = 7.6$ Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 184.56 (C=O), 156.35 (C, C-7a), 152.83 (C, C-4′), 150.43 (C, C-1′), 135.22 (C, C-2), 130.19 (2× CH, CH-2′ and CH-6′), 128.66 (CH, CH-6), 128.52 (2× CH, CH-3′ and CH-5′), 127.48 (C, C-3a), 124.36 (CH, Ar), 123.70 (CH, Ar), 116.62 (CH, CH-3), 112.98 (CH, CH-7), 29.46 (CH_2), 15.71 (CH_3). Anal. Calcd for $C_{17}H_{14}O_2$ (250.296): C, 81.58; H, 5.64. Found: C, 81.44; H, 5.84.

5.1.1.2. Benzofuran-2-yl-(4-propyl-phenyl)-methanone (3b, R = $CH_2CH_2CH_3$). Cream crystalline solid (42%) after recrystallisation from methanol, mp 64–66 °C. ^1H NMR ($CDCl_3$): δ 7.92 (d, $J = 8.2$ Hz, 2H, Ar), 7.65 (d, $J = 7.9$ Hz, 1H, Ar), 7.57 (dd, $J = 0.40$, 8.5 Hz, 1H, Ar), 7.46 (d, $J = 0.6$ Hz, 1H, Ar), 7.42 (m, 1H, Ar), 7.26 (m, 3H, Ar), 2.62 (m, CH_2, propyl), 1.64 (dt, $J = 7.4$, 8.5 Hz, CH_2, propyl), 0.91 (t, $J = 7.3$ Hz, CH_3, propyl). ^{13}C NMR ($CDCl_3$): δ 184.09 (C=O), 155.95 (C, C-7a), 152.51 (C, C-4′), 148.50 (C, C-1′), 134.84 (C, C-2), 129.67 (2× CH, CH-2′ and CH-6′) and 128.68 (2× CH, CH-3′ and CH-5′), 128.19 (CH, Ar), 127.08 (C, C-3a), 123.92 (CH, Ar), 116.07 (CH, CH-3), 112.56 (CH, CH-7), 38.12 (CH_2), 24.25 (CH_2), 13.75 (CH_3). HRMS (EI^+) *m*/*z* Calcd for $C_{18}H_{16}O_2$ $(M+H)^+$ 265.1223. Found 265.1224.

5.1.1.3. Benzofuran-2-yl-(4-isopropyl-phenyl)-methanone (3c, R = $CH(CH_3)_2$). Pale orange solid (44%) after recrystallisation from methanol, mp 93–95 °C. ^1H NMR ($CDCl_3$): δ 7.85 (m, 2H, Ar), 7.53 (m, 2H, Ar), 7.35 (m, 2H, Ar), 7.34 (s, 1H, H-7), 7.32 (m, 2H, Ar) 3.05 (septet, 1H, CH, isopropyl), 1.32 (s, 6H, isopropyl). ^{13}C NMR ($CDCl_3$): δ 186.36 (C=O), 155.81 (C, C-7a), 154.60 (C, C-2), 154.39 (C, C-4′), 152.39 (C, C-1′), 134.89 (C, C-3a), 131.15 (2× CH, CH-2′ and CH-6′), 129.74 (2× CH, CH-3′ and CH-5′), 126.62 (CH, Ar), 124.22 (CH, Ar), 123.27 (CH, Ar), 115.76 (CH, CH-3), 111.20 (CH,

CH-7), 34.24 (CH, isopropyl), 23.82 (CH_3, isopropyl). Anal. Calcd for $C_{18}H_{16}O_2$ (264.318): C, 81.79; H, 6.10. Found: C, 81.58; H, 6.07.

5.1.1.4. Benzofuran-2-yl-(4-*tert*-butyl-phenyl)-methanone (3d, R = $C(CH_3)_3$). Yellow solid (32%) after recrystallisation from methanol, mp 103–105 °C. ^{1}H NMR ($CDCl_3$): δ 7.87 (m, 2H, Ar), 7.67 (m, 2H, Ar), 7.53 (m, 2H, Ar), 7.34 (s, 1H, H-7), 7.32 (m, 2H, Ar), 1.35 (s, 9H, ^{t}butyl). ^{13}C NMR ($CDCl_3$): δ 184.08 (C=O), 167.76 (C, C-7a), 156.77 (C, C-2), 155.95 (C, C-4′), 155.95 (C, C-1′), 134.54 (C, C-3a), 130.89 (2× CH, CH-2′ and CH-6′), 129.51 (2× CH, CH-3′ and CH-5′), 127.09 (CH, Ar), 125.55 (CH, Ar), 123.92 (CH, Ar), 116.14 (CH, CH-3), 112.57 (CH, CH-7), 68.17 (C, ^{t}butyl), 31.15 (CH_3, ^{t}butyl). Anal. Calcd for $C_{19}H_{18}O_2{\cdot}0.6H_2O$ (289.159): C, 78.92; H, 6.69. Found: C, 79.17; H, 7.06.

5.1.1.5. Benzofuran-2-yl-(4-isobutyl-phenyl)-methanone (3e, R = $CH_2CH(CH_3)_2$). White solid (50%) after recrystallisation from methanol, mp 62–63 °C. ^{1}H NMR ($CDCl_3$): δ 7.90 (m, 2H, Ar), 7.59 (m, 2H, Ar), 7.52 (m, 2H, Ar), 7.34 (m, 2H, Ar), 7.32 (s, 1H, H-7), 2.56 (d, 2H, H-16), 1.96 (m, 1H, H-17), 1.01 (d, 6H, H-18, H-19). ^{13}C NMR ($CDCl_3$): δ 184.08 (C=O), 155.95 (C, C-7a), 152.50 (C, C-2), 147.55 (C, C-4′), 134.86 (2× C, C-1′ and C-3a), 129.63 (2× CH, CH-2′ and CH-6′), 129.30 (2× CH, CH-3′ and CH-5′), 128.19 (CH, Ar), 127.08 (CH, Ar), 123.92 (CH, Ar), 123.25 (CH, Ar), 116.09 (CH, CH-3), 112.55 (CH, CH-7), 45.48 (CH_2, isobutyl), 30.16 (CH, isobutyl), 22.39 (CH_3, isobutyl). Anal. Calcd for $C_{19}H_{18}O_2$ (278.345): C, 81.99; H, 6.52. Found: C, 81.55; H, 6.45.

5.1.1.6. Benzofuran-2-yl-(4-cyclohexyl-phenyl)-methanone (3f, R = $^c C_6H_{12}$).[27] Light yellow solid (29%) after recrystallisation from ethyl acetate. Mp 92–94 °C. ^{1}H NMR ($CDCl_3$): δ 7.93 (m, 2H, Ar), 7.24 (m, 2H, Ar), 7.19 (m, 4H, Ar), 6.62 (s, 1H, H-7), 2.51 (quintet, 1H, C*H*-cyclohexyl), and 1.56 (m, 10H, cyclohexyl).

5.1.2. General method for the preparation of the biphenyl ketones 13. Two molar aqueous Na_2CO_3 (11.65 mL) was added to a solution of benzo[*b*]furan-2-yl(4-bromophenyl)methanone[21] (1.0 g, 3.32 mmol) in toluene (20 mL). The mixture was bubbled with nitrogen for one minute and then $Pd(PPh_3)_4$ (0.20 g, 0.166 mmol) was added to the mixture. Phenyl- or 4-phenyl-boronic acid (6.64 mmol) in ethanol (5 mL) was added to the above mixture and the reaction was refluxed at 100 °C for 4 h. After the reaction was complete, the residual borane was oxidised by the addition of H_2O_2 (30%, 2.5 mL) at room temperature for 1 h. The crude product was extracted with CH_2Cl_2 (100 mL) and water (3× 100 mL). The organic layer was dried with $MgSO_4$, filtered and reduced in vacuo to give a light yellow oily residue. Purification by flash column chromatography (petroleum ether/ethyl acetate 95:5 v/v increasing to 80:20 v/v) gave the product, which was recrystallised from petroleum ether.

5.1.2.1. Benzo[*b*]furan-2-yl-biphenyl-4-yl-methanone (13a). Light yellow fine crystals (58%), mp 150–152 °C (lit.[28] 153–155 °C). TLC system: petroleum ether/ethyl acetate, 1:1 v/v, R_f: 0.63. ^{1}H NMR ($CDCl_3$): δ 8.21 (dd, J = 1.6, 8.2 Hz, 2H, Ar), 7.82 (m, 3H, Ar), 7.73 (m, 3H, Ar), 7.65 (s, 1H, H-3), 7.46 (m, 5H, Ar). ^{13}C NMR ($CDCl_3$): δ 184.33 (C=O), 156.43 (C, C-7a), 152.80 (C, C-4′), 146.16 (C, C-1″), 140.29 (C, C-1′), 136.31 (C, C-2), 130.58 (2× CH, CH-2′ and CH-6′), 129.46 (2× CH, CH-3″ and CH-5″), 128.83 (2× CH, CH-3′ and CH–5′), 128.76 (CH, Ar), 127.77 (2× CH, CH-2″ and CH-6″), 127.66 (CH, Ar), 127.48 (C, C-3a), 124.46 (CH, Ar), 123.78 (CH, Ar), 116.83 (CH, CH-3), 113.03 (CH, CH-7).

5.1.2.2. Benzo[*b*]furan-2-yl(4′-chlorobiphenyl-4-yl)methanone (13b). Light yellow solid (73%), mp 76–78 °C. TLC system: petroleum ether/ethyl acetate, 4:1 v/v, R_f: 0.50. ^{1}H NMR ($CDCl_3$): δ 8.25 (dd, J = 1.7, 6.6 Hz, 2H, Ar), 7.81 (m, 4H, Ar), 7.69 (m, 2H, Ar), 7.52 (m, 5H, Ar). ^{13}C NMR ($CDCl_3$): δ 184.18 (C=O), 156.44 (C, C-7a), 152.75 (C, C-4′), 144.82 (C, C-1′), 138.71 (C, C-2), 136.57 (C, C-1″), 134.96 (C, C-4″), 130.67 (2× CH, CH-2′ and CH-6′), 129.64 (2× CH, CH-3″ and CH-5″), 128.89 (2× CH, CH-3′ and CH-5′), 128.65 (CH, CH-6), 127.49 (2× CH, CH-2″ and CH-6″), 127.44 (C, C-3a), 124.49 (CH, Ar), 123.79 (CH, Ar), 116.87 (CH, CH-3), 113.02 (CH, CH-7). Anal. Calcd for $C_{21}H_{13}ClO_2$ (332.785): C, 75.79; H, 3.94. Found: C, 76.14; H, 4.00.

5.1.3. General method for the preparation of the alcohols 4 and 14. To a cooled (0 °C) solution of benzofuran-2-yl-(4-alkyl/aryl-phenyl)-methanone (5 mmol) in anhydrous dioxan (15 mL) was added sodium borohydride (5 mmol) and then the mixture was allowed to stir at room temperature under nitrogen for 2 h. The solvent was concentrated under reduced pressure and aqueous hydrochloric acid (1 M, 10 mL) was added to the residue. The oil formed was extracted with diethyl ether (2× 50 mL) and washed with water (2× 25 mL), the organic layers were combined and dried with $MgSO_4$ and the solvent concentrated under reduced pressure.

5.1.3.1. Benzofuran-2-yl-(4-ethyl-phenyl)-methanol (4a, R = CH_2CH_3). White solid (83%), mp 45–47 °C. TLC system: petroleum ether/ethyl acetate, 3:2 v/v, R_f: 0.64. ^{1}H NMR ($CDCl_3$): 7.60 (m, 1H, Ar), 7.50 (m, 3H, Ar), 7.32 (m, 4H, Ar), 6.61 (s, 1H, H-3), 5.96 (d, J = 4.6 Hz, 1H, C*H*-OH), 2.91 (d, J = 4.6 Hz, 1H, OH), 2.76, (q, J = 7.6 Hz, 2H, CH_2), 1.34 (t, J = 7.6 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 160.31 (C, C-2), 159.21 (C, C-7a), 155.54 (C, C-4′), 144.97 (C, C-1′), 138.12 (C, C-3a), 128.58 (2× CH, CH-3′ and CH-5′), 127.34 (2× CH, CH-2′ and CH-6′), 124.67 (CH, Ar), 123.25 (CH, Ar), 121.58 (CH, Ar), 111.81 (CH, CH-7), 104.35 (CH, CH-3), 71.02 (CH, *C*H-OH), 29.08 (CH_2), 16.05 (CH_3). LRMS (EI$^+$) *m*/*z*: 252.1 (M^+). Anal. Calcd for $C_{17}H_{16}O_2$ (252.311): C, 80.93; H, 6.39. Found: C, 80.73; H, 6.41.

5.1.3.2. Benzofuran-2-yl-(4-propyl-phenyl)-methanol (4b, R = $CH_2CH_2CH_3$). Yellow syrup (60%). TLC system: petroleum ether/ethyl acetate, 4:1 v/v, R_f: 0.64. ^{1}H NMR ($CDCl_3$): δ 7.50 (m, 4H, Ar), 7.26 (m, 4H, Ar), 5.70 (d, J = 9.5 Hz, 1H, C*H*-OH), 2.64 (m, CH_2, pro-

pyl), 2.50 (s, 1H, OH), 1.69 (m, CH_2, propyl), 0.10 (dt, $J = 3.5$, 7.3 Hz, CH_3, propyl). ^{13}C NMR ($CDCl_3$): δ 157.20 (C, C-2), 155.25 (C-7a), 142.95 (C, C-4′), 135.72 (C, C-1′), 128.76 (2× CH, CH-3′ and CH-5′), 128.15 (2× CH, CH-2′ and CH-6′), 127.57 (C, C-3a), 126.78 (CH, Ar), 124.25 (CH, Ar), 122.82 (CH, Ar), 121.13 (CH, Ar), 111.48 (CH, CH-7), 105.09 (CH, CH-3), 75.22 (CH, *C*H-OH), 37.85 (CH_2), 24.51 (CH_2), 13.93 (CH_3). Anal. Calcd for $C_{18}H_{18}O_2$ (266.339): C, 81.17; H, 6.81. Found: C, 80.87; H, 6.92.

5.1.3.3. Benzofuran-2-yl-(4-isopropyl-phenyl)-methanol (4c, R = $CH(CH_3)_2$). White solid (96%), mp 69–70 °C. TLC system: petroleum ether/ethyl acetate, 4:1 v/v, R_f: 0.74. ^1H NMR ($CDCl_3$): δ 7.42 (m, 2H, Ar), 7.34 (m, 2H, Ar), 7.16 (m, 2H, Ar), 7.11 (s, 2H, Ar), 6.45 (s, 1H, H-7), 5.81 (s, 1H, C*H*-OH), 2.82 (m, 1H, isopropyl), 2.59 (s, 1H, OH), 1.18 (s, 6H, isopropyl). ^{13}C NMR ($CDCl_3$): δ 157.64 (C, C-2), 154.03 (C, C-7a), 148.10 (C, C-4′), 136.71 (2× CH, CH-3′ and CH-5′), 127.03 (2× CH, CH-2′ and CH-6′), 125.81 (CH, Ar), 125.65 (CH, Ar), 123.16 (CH, Ar), 110.28 (CH, CH-7), 102.82 (CH, CH-3), 69.54 (CH, *C*H-OH), 32.84 (CH, isopropyl), 22.93 (CH_3, isopropyl). Anal. Calcd for $C_{18}H_{19}O_2 \cdot 0.5H_2O$ (276.3545): C, 78.23; H, 7.29. Found: C, 78.14; H, 7.05.

5.1.3.4. Benzofuran-2-yl-(4-*tert*-butyl-phenyl)-methanol (4d, R = $C(CH_3)_3$). White solid (99%), mp 87–88 °C. TLC system: petroleum ether/ethyl acetate, 4:1 v/v, R_f: 0.72. ^1H NMR ($CDCl_3$): δ 7.39 (m, 2H, Ar), 7.34 (m, 2H, Ar), 7.30 (m, 2H, Ar), 7.12 (m, 2H, Ar), 6.41 (s, 1H, H-7), 5.78 (s, 1H, C*H*-OH), 2.58 (s, 1H, OH), 1.21 (s, 9H, ^{t}butyl). ^{13}C NMR ($CDCl_3$): δ 158.74 (C, C-2), 155.14 (C, C-7a), 151.24 (C, C-4′), 130.72 (2× CH, CH-3′ and CH-5′), 129.36 (2× CH, CH-2′ and CH-6′), 128.15 (CH, Ar), 126.64 (CH, Ar), 125.93 (CH, Ar), 111.39 (CH, CH-7), 103.92 (CH, CH-3), 70.57 (CH, *C*H-OH), 34.66 (C, ^{t}butyl), 31.39 (CH_3, ^{t}butyl). Anal. Calcd for $C_{19}H_{20}O_2$ (280.361): C, 81.40; H, 7.19. Found: C, 81.22; H, 7.01.

5.1.3.5. Benzofuran-2-yl-(4-isobutyl-phenyl)-methanol (4e, R = $CH_2CH(CH_3)_2$). Yellow syrup (99%), mp 87–88 °C. TLC system: petroleum ether/ethyl acetate, 4:1 v/v, R_f: 0.73. ^1H NMR ($CDCl_3$): δ 7.53 (m, 1H, Ar), 7.48 (m, 1H, Ar), 7.41 (m, 2H, Ar), 7.20 (m, 4H, Ar), 6.57 (s, 1H, H-7), 5.95 (s, 1H, C*H*-OH), 3.72 (s, 1H, OH), 2.52 (s, 2H, isobutyl), 1.91 (m, 1H, isobutyl), 0.96 (s, 6H, isobutyl). ^{13}C NMR ($CDCl_3$): δ 158.74 (C, C-2), 155.12 (C, C-7a), 142.05 (C, C-4′), 137.66 (C, C-1′), 129.38 (2× CH, CH-3′ and CH-5′), 128.10 (CH, Ar), 126.63 (2× CH, CH-2′ and CH-6′), 124.23 (CH, Ar), 122.81 (CH, Ar), 121.12 (CH, Ar), 111.34 (CH, CH-7), 103.89 (CH, CH-3), 70.66 (CH, *C*H-OH), 45.16 (CH_2, isobutyl), 30.21 (CH, isobutyl), 22.38 (CH_3, isobutyl). Anal. Calcd for $C_{19}H_{20}O_2$ (280.361): C, 81.40; H, 7.19. Found: C, 81.07; H, 7.43.

5.1.3.6. Benzofuran-2-yl-(4-cyclohexyl-phenyl)-methanol (4f, R = $^cC_6H_{12}$). White solid (98%), mp 67–68 °C. TLC system: petroleum ether/ethyl acetate, 4:1 v/v, R_f: 0.63. ^1H NMR ($CDCl_3$): δ 7.59 (m, 1H, Ar), 7.48 (m, 3H, Ar), 7.25 (m, 4H, Ar), 6.58 (s, 1H, H-7), 5.89 (s, 1H, C*H*-OH), 3.72 (s, 1H, OH), 2.61 (m, 1H, cyclohexyl), 1.58 (m, 10H, cyclohexyl). ^{13}C NMR ($CDCl_3$): δ 159.28 (C, C-2), 155.46 (C, C-7a), 144.09 (C, C-4′), 134.90 (C, C-1′), 128.72 (2× CH, CH-3′ and CH-5′), 128.59 (2× CH, CH-2′ and CH-6′), 124.00 (CH, Ar), 122.77(CH, Ar), 120.91 (CH, Ar), 111.25 (CH, Ar), 103.24 (CH, Ar), 67.63 (CH, *C*H-OH), 45.00 (CH, C*H*-cyclohexyl), 35.40 (CH_2, cyclohexyl), 27.65 (CH_2, cyclohexyl), 26.95 (CH_2, cyclohexyl). HRMS (EI^+) *m/z* Calcd for $C_{21}H_{22}O_2$ $(M+H)^+$ 358.1914. Found 358.1910.

5.1.3.7. Benzo[*b*]furan-2-yl-biphenyl-4-yl-methanol (14a, R = phenyl). White solid (90%), mp 121–123 °C (lit.[28] 123–125 °C). TLC system: petroleum ether/ethyl acetate, 1:1 v/v, R_f: 0.63. ^1H NMR ($CDCl_3$): δ 7.63 (m, 6H, Ar), 7.46 (m, 4H, Ar), 7.30 (m, 3H, Ar), 6.64 (d, $J = 0.6$ Hz, 1H, H-3), 6.04 (d, $J = 3.1$ Hz, 1H, C*H*-OH), 2.73, (d, $J = 3.9$ Hz, 1H, OH). ^{13}C NMR ($CDCl_3$): δ 158.84 (C, C-2), 155.57 (C, C-7a), 141.76 (C, C-1′), 141.10 (C, C-1″), 139.69 (C, C-4′), 129.28 (2× CH, CH-3″ and CH-5″), 128.47 (C, C-3a), 127.92 (2× CH, CH-2′ and CH-6′), 127.83 (2× CH, CH-3′ and CH-5′), 127.72 (2× CH, CH-2″ and CH-6″), 127.60 (CH, Ar), 124.82 (CH, Ar), 123.34 (CH, Ar), 121.64 (CH, Ar), 111.83 (CH, CH-7), 104.57 (CH, CH-3), 70.91 (*C*H-OH).

5.1.3.8. Benzo[*b*]furan-2-yl-(4′-chlorobiphenyl-4-yl)methanol (14b, R = 4-chlorophenyl). White solid (75%), mp 76–78 °C. TLC system: petroleum ether/ethyl acetate, 1:1 v/v, R_f: 0.60. ^1H NMR ($CDCl_3$): δ 7.54 (m, 7H, Ar), 7.44 (m, 3H, Ar), 7.25 (m, 2H, Ar), 6.59 (s, 1H, H-3), 6.00 (d, $J = 4.5$ Hz, 1H, C*H*-OH), 2.65 (d, $J = 4.6$ Hz, 1H, OH). ^{13}C NMR ($CDCl_3$): δ 158.69 (C, C-2), 155.55 (C, C-7a), 140.48 (C, C-1′), 140.31 (C, C-4′), 139.51 (C, C-1″), 134.02 (C, C-4″), 129.42 (2× CH, CH-3″ and CH-5″), 128.81 (2× CH, CH-2″ and CH-6″), 128.41 (C, C-3a), 127.80 (2× CH, CH-2′ and CH-6′), 127.64 (2× CH, CH-3′ and CH-5′), 124.87 (CH, Ar), 123.36 (CH, Ar), 121.65 (CH, Ar), 111.82 (CH, CH-7), 104.59 (CH, CH-3), 70.82 (*C*H-OH). Anal. Calcd for $C_{21}H_{15}ClO_2$ (334.800): C, 75.20; H, 4.45. Found: C, 75.34; H, 4.52.

5.1.4. General method for the preparation of the triazole compounds 5–11, 15 and 16. Thionyl chloride (4 mmol) in anhydrous acetonitrile (10.0 mL) was added dropwise to a stirred solution of 1,2,4-triazole (16 mmol) in anhydrous acetonitrile (10.0 mL) at a temperature of 10 °C. The white suspension formed was stirred for 1 h at 10 °C. A solution of benzo[*b*]furan-2-yl-(4-alkyl/arylphenyl)methanol (**4a–f**, **14**) (1.0 g, 4 mmol) in anhydrous acetonitrile (10.0 mL) was added to the mixture followed by activated potassium carbonate (1.10 g, 8 mmol). The suspension was stirred under nitrogen at room temperature for 4 days. The resulting suspension was filtered and the filtrate was evaporated in vacuo to yield a light brown oil. The oil was extracted with CH_2Cl_2 (150 mL) and water (3× 100 mL). The organic layer was dried with $MgSO_4$, filtered and reduced in vacuo.

5.1.4.1. 1-[Benzo[*b*]furan-2-yl-(4-methylphenyl)methyl]-1*H*-[1,2,4]triazole (5a) and 4-[benzo[*b*] furan-2-yl-(4-methylphenyl)methyl]-4*H*-[1,3,4]triazole (5b). Purified by column chromatography (petroleum ether/ethyl acetate, 1:1 v/v) to give the [1,2,4]triazole **5a**, further elution (dichloromethane/methanol, 95:5 v/v) gave the [1,3,4]triazole **5b**.

Data for ***5a***: orange crystals (28%), mp 106–108 °C. TLC system: petroleum ether/ethyl acetate, 4:1 v/v, R_f: 0.14. ^{1}H NMR ($CDCl_3$): δ 8.14 (s, 1H, H-3‴), 8.05 (s, 1H, H-5‴), 7.56 (d, J = 7.7 Hz, 1H, Ar), 7.48 (d, J = 8.3 Hz, 1H, Ar), 7.34 (m, 1H, Ar), 7.25 (m, 5H, Ar), 6.84 (s, 1H, H-1), 6.59 (s, 1H, H-3), 2.40 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 155.34 (C, C-2), 152.93 (C, C-7a), 152.21 (CH, CH-5‴), 143.21 (CH, CH-3‴), 139.24 (C, C-3a), 132.61 (C, C-1′), 129.80 and 127.67 (CH, Ar), 127.55 (C, C-4′), 125.17, 123.30 and 121.49 (CH, Ar), 111.54 (CH, CH-7), 107.58 (CH, CH-3), 62.10 (CH, CH-1), 21.19 (CH_3).

Data for ***5b***: white solid (12%), mp 64–66 °C. TLC system: dichloromethane/methanol, 9:1 v/v, R_f: 0.27. ^{1}H NMR ($CDCl_3$): δ 8.21 (s, 2H, H-2‴ and H-5‴), 7.57 (d, J = 7.7 Hz, 1H, Ar), 7.48 (d, J = 8.3 Hz, 1H, Ar), 7.36 (m, 1H, Ar), 7.28 (m, 3H, Ar), 7.17 (d, J = 8.1 Hz, 2H, Ar), 6.64 (s, 1H, H-1), 6.59 (s, 1H, H-3), 2.41 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 155.39 (C, C-2), 152.42 (C, C-7a), 142.43 (2× CH, CH-2‴ and CH-5‴), 139.72 (C, C-1′), 132.27 (C, C-4′),130.08 and 127.38 (CH, Ar), 127.22 (C, C-3a), 125.56, 123.56 and 121.57 (CH, Ar), 111.62 (CH, CH-7), 107.60 (CH, CH-3), 58.00 (CH, CH-1), 21.18 (CH_3). HRMS (EI^+) m/z Calcd for $C_{18}H_{15}N_3O$ $(M+H)^+$ 290.1288. Found 290.1287.

5.1.4.2. 1-[Benzo[*b*]furan-2-yl-(4-ethylphenyl)methyl]-1*H*-[1,2,4]triazole (6a) and 4-[benzo[*b*] furan-2-yl-(4-ethylphenyl)methyl]-4*H*-[1,3,4]triazole (6b). Purified by column chromatography (petroleum ether/ethyl acetate, 7:3 v/v) to give the [1,2,4]triazole **6a**, further elution (dichloromethane/methanol, 99:1 v/v) gave the [1,3,4]triazole **6b**.

Data for ***6a***: yellow syrup (67%). TLC system: petroleum ether/ethyl acetate, 1:1 v/v, R_f: 0.42. ^{1}H NMR ($CDCl_3$): δ 8.19 (s, 1H, H-3‴), 8.09 (s, 1H, H-5‴), 7.58 (d, J = 8.4 Hz, 1H, Ar), 7.51 (d, J = 9.3 Hz, 1H, Ar), 7.39–7.27 (m, 6H, Ar), 6.89 (s, 1H, H-1), 6.63 (s, 1H, H-3), 2.73 (q, J = 7.6 Hz, 2H, CH_2), 1.30 (t, J = 7.6 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 155.75 (C, C-2), 153.342 (C, C-7a), 152.65 (CH, CH-5‴), 145.91 (C, C-4′), 143.67 (CH, CH-3‴), 133.20 (C, C-1′), 129.06 (2× CH, CH-2′ and CH-6′), 128.16 (2× CH, CH-3′ and CH-5′), 127.96 (C, C-3a), 125.59 (CH, Ar), 123.73 (CH, Ar), 121.93 (CH, Ar), 111.97 (CH, CH-7), 108.06 (CH, CH-3), 62.51 (CH, CH-1), 28.99 (CH_2), 15.85 (CH_3). HRMS (EI^+) m/z Calcd for $C_{19}H_{17}N_3O$ $(M+H)^+$ 304.1444. Found 304.1442.

Data for ***6b***: White solid (13%), mp 126–128 °C. TLC system: dichloromethane/methanol, 9:1 v/v, R_f: 0.31. ^{1}H NMR ($CDCl_3$): δ 8.25 (s, 2H, H-2‴ and H-5‴), 7.57 (d, J = 6.8 Hz, 1H, Ar), 7.46 (d, J = 8.4 Hz, 1H, Ar), 7.37–7.19 (m, 6H, Ar), 6.75 (s, 1H, H-1), 6.62 (s, 1H, H-3), 2.70 (q, J = 7.6 Hz, 2H, CH_2), 1.26 (t, J = 7.6 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ 155.76 (C, C-2), 152.90 (C, C-7a), 146.26 (C, C-4′), 142.95 (2× CH, CH-2‴ and CH-5‴), 132.98 (C, C-1′), 129.27 (2× CH, CH-2′ and CH-6′), 127.89 (2× CH, CH-3′ and CH-5′), 127.67 (C, C-3a), 125.91 (CH, Ar), 123.94 (CH, Ar), 122.01 (CH, Ar), 112.01 (CH, CH-7), 108.04 (CH, CH-3), 58.28 (CH, CH-1), 28.95 (CH_2), 15.83 (CH_3). Anal. Calcd for $C_{19}H_{17}N_3O$ (303.362): C, 75.23; H, 5.65; N, 13.84. Found: C, 75.19; H, 5.74; N, 13.61.

5.1.4.3. 1-[Benzo[*b*]furan-2-yl-(4-propylphenyl)methyl]-1*H*-[1,2,4]triazole (7). Purified by column chromatography (petroleum ether/ethyl acetate, 1:1 v/v) to give the [1,2,4]triazole **7** as a yellow syrup (2%). TLC system: petroleum ether/ethyl acetate, 4:1 v/v, R_f: 0.25. ^{1}H NMR ($CDCl_3$): δ 8.03 (s, 1H, H-3‴), 7.94 (s, 1H, H-5‴), 7.46 (d, J = 7.7 Hz, 1H, Ar), 7.38 (d, J = 8.3 Hz, 1H, Ar), 7.23 (m, 1H, Ar), 7.16 (m, 5H, Ar), 6.74 (s, 1H, H-1), 6.49 (s, 1H, H-3), 2.53 (t, J = 7.6 Hz, CH_2, propyl), 1.57 (dt, J = 7.5, 15 Hz, CH_2, propyl), 0.87 (t, J = 7.3 Hz, CH_3, propyl). ^{13}C NMR ($CDCl_3$): δ 154.32 (C, C-2), 151.92 (C, C-7a), 151.17 (CH, CH-5‴), 142.96 (C, C-3a), 131.77 (C, C-1′), 128.17 (2× CH, CH-2′ and CH-6′), 126.61 (2× CH, CH-3′ and CH-5′), 126.53 (C, C-4′), 124.13, 122.27 and 120.46 (CH, Ar), 110.52 (CH, CH-7), 106.55 (CH, CH-3), 61.11 (CH, CH-1), 36.68 (CH_2), 23.34 (CH_2), 12.79 (CH_3). HRMS (EI^+) m/z Calcd for $C_{20}H_{19}N_3O$ $(M+H)^+$ 318.1601. Found 318.1601.

5.1.4.4. 1-[Benzo[*b*]furan-2-yl-(4-isopropylphenyl)-methyl]-1*H*-[1,2,4]triazole (8a) and 4-[benzo[*b*] furan-2-yl-(4-isopropylphenyl)methyl]-4*H*-[1,3,4]triazole (8b). Purified by column chromatography (hexane/ethyl acetate, 9:1 v/v) to give the [1,2,4]triazole **8a**, further elution (dichloromethane/methanol, 9:1 v/v) gave the [1,3,4]triazole **8b**.

Data for ***8a***: yellow syrup (78%). TLC system: petroleum ether/ethyl acetate, 1:1 v/v, R_f: 0.72. ^{1}H NMR ($CDCl_3$): δ 8.42 (s, 1H, H-3‴), 8.33 (s, 1H, H-5‴), 7.31 (m, 1H, Ar), 7.24 (m, 2H, Ar), 7.06 (m, 5H, Ar), 6.62 (s, 1H, H-1), 6.37 (s, 1H, H-3), 2.73 (septet, 1H, isopropyl), 1.05 (d, 6H, isopropyl). ^{13}C NMR ($CDCl_3$): δ 155.25 (C, C-2), 153.15 (CH, C-7a), 152.11 (CH, CH-5‴), 149.79 (C, C-3a), 143.35 (C, C-1′), 133.09 (C, C-4′), 127.76 (2× CH, CH-2′ and CH-6′), 126.84 (2× CH, CH-3′ and CH-5′), 125.04 (CH, Ar), 123.22 (CH, Ar), 112.44 (CH, Ar), 111.42 (CH, CH-7), 107.07 (CH, CH-3), 62.03 (CH, CH-1), 34.47 (C, isopropyl), 24.16 (CH_3, isopropyl). HRMS (EI^+) m/z Calcd for $C_{20}H_{19}N_3O$ $(M)^+$ 318.1601. Found 318.1601.

Data for ***8b***: yellow syrup (7%). TLC system: petroleum ether/ethyl acetate, 1:1 v/v, R_f: 0.11. ^{1}H NMR ($CDCl_3$): δ 8.12 (s, 2H, H-2‴ and H-5‴), 7.47 (m, 1H, Ar), 7.39 (m, 1H, Ar), 7.27 (m, 1H, Ar), 7.20 (m, 4H, Ar), 7.13 (m, 3H, Ar), 6.56 (s, 1H, H-1), 6.49 (s, 1H, H-3), 2.75 (septet, 1H, isopropyl), 1.18 (d, 6H, isopropyl). ^{13}C

NMR ($CDCl_3$): δ 155.39 (C, C-2), 152.30 (C, C-7a), 150.63 (2× CH, CH-2‴ and CH-5‴), 142.47 (C, C-4′), 132.46 (C, C-1′), 127.72 (C, C-3a), 127.52 (2× CH, CH-2′ and CH-6′), 127.21 (2× CH, CH-3′ and CH-5′), 125.58 (CH, Ar), 123.58 (CH, Ar), 121.59 (CH, Ar), 111.64 (CH, CH-7), 107.67 (CH, CH-3), 58.08 (CH, CH-1), 33.89 (C, isopropyl), 23.85 (CH_3, isopropyl). HRMS (EI^+) *m/z* Calcd for $C_{20}H_{19}N_3O$ $(M+H)^+$ 318.1601. Found 318.1603.

5.1.4.5. 1-[Benzo[*b*]furan-2-yl-(4-*tert*-butyl-phenyl)-methyl]-1*H*-[1,2,4]triazole (9). Purified by column chromatography (hexane/ethyl acetate, 9:1 v/v) to give the [1,2,4]triazole **9**, as a yellow syrup (62%). TLC system: petroleum ether/ethyl acetate, 1:1 v/v, R_f: 0.61. ^1H NMR ($CDCl_3$): δ 8.15 (s, 1H, 1H, H-3‴), 8.01 (s, 1H, H-5‴), 7.49 (m, 1H, Ar), 7.40 (m, 3H, Ar), 7.25 (m, 3H, Ar), 7.19 (m, 3H, Ar), 6.76 (s, 1H, H-1), 6.57 (s, 1H, H-3), 1.32 (s, 9H, ^{t}butyl). ^{13}C NMR ($CDCl_3$): δ 156.98 (C, C-2), 154.16 (CH, CH-5‴), 150.30 (C, C-7a), 148.52 (CH, CH-3‴), 136.47 (C, C-4′), 132.56 (2× CH, CH-2′ and CH-6′), 129.94 (C, C-1′), 129.08 (C, C-3a), 127.92 (2× CH, CH-3′ and CH-5′), 123.37 (CH, Ar), 122.14 (CH, Ar), 120.55 (CH, Ar), 110.82 (CH, CH-7), 101.37 (CH, CH-3), 61.90 (CH, CH-1), 34.80 (C, ^{t}butyl), 31.41 (CH_3, ^{t}butyl). HRMS (EI^+) *m/z* Calcd for $C_{21}H_{21}N_3O$ $(M)^+$ 332.1757. Found 332.1761.

5.1.4.6. 1-[Benzo[*b*]furan-2-yl-(4-isobutylphenyl)methyl]-1*H*-[1,2,4]triazole (10). Purified by column chromatography (hexane/ethyl acetate, 9:1 v/v) to give the [1,2,4]triazole **10** as a yellow syrup (56%). TLC system: petroleum ether/ethyl acetate, 1:1 v/v, R_f: 0.63. ^1H NMR ($CDCl_3$): δ 8.28 (s, 1H, 1H, H-3‴), 8.22 (s, 1H, 1H, H-5‴), 7.60 (m, 2H, Ar), 7.40 (m, 2H, Ar), 7.31 (m, 2H, Ar), 7.23 (m, 2H, Ar), 6.82 (s, 1H, H-1), 6.54 (s, 1H, H-3), 2.54 (d, 2H, CH_2, isobutyl), 1.95 (m, 1H, CH, isobutyl), 1.02 (s, 6H, isobutyl). ^{13}C NMR ($CDCl_3$): δ 155.27 (C, C-2), 153.21 (CH, CH-5‴), 152.11 (CH, CH-3‴), 136.47 (C, C-7a), 132.51 (2× CH, CH-2′ and CH-6′), 131.47 (2× CH, CH-3′ and CH-5′), 131.36 (C, C-4′), 129.94 (C, C-1′), 128.88 (C, C-3a), 123.37 (CH, Ar), 122.14 (CH, Ar), 120.55 (CH, Ar), 110.89 (CH, CH-7), 101.37 (CH, CH-3), 56.50 (CH, CH-1), 45.54 (CH_2, isobutyl), 27.57 (CH, isobutyl), 22.23 (CH_3, isobutyl). Anal. Calcd for $C_{21}H_{21}N_3O{\cdot}0.3H_2O$ (336.821): C, 74.89; H, 6.46; N, 12.48. Found: C, 74.87; H, 6.45; N, 12.15.

5.1.4.7. 1-[Benzo[*b*]furan-2-yl-(4-cyclohexylphenyl)-methyl]-1*H*-[1,2,4]triazole (11). Purified by column chromatography (hexane/ethyl acetate, 9:1 v/v) to give the [1,2,4]triazole **11** as a yellow syrup (14%). TLC system: petroleum ether/ethyl acetate, 1:1 v/v, R_f: 0.70. ^1H NMR ($CDCl_3$): δ 8.28 (s, 1H, 1H, H-3‴), 8.22 (s, 1H, 1H, H-5‴), 7.60 (m, 2H, Ar), 7.40 (m, 2H, Ar), 7.31 (m, 2H, Ar), 7.23 (m, 2H, Ar), 6.82 (s, 1H, H-1), 6.54 (s, 1H, H-3), 2.54 (m, 1H, CH, cyclohexyl), 1.48 (m, 10H, cyclohexyl). ^{13}C NMR ($CDCl_3$): δ 156.98 (C, C-2), 154.16 (CH, C-7a), 148.52 (CH, CH-5‴), 143.74 (CH, CH-3‴), 136.47 (C, C-4′), 131.56 (2× CH, CH-2′ and CH-6′), 129.81 (2× CH, CH-3′ and CH-5′), 128.64 (C, C-3a), 123.37 (CH, Ar), 122.14 (CH, Ar), 120.55 (CH, Ar), 110.89 (CH, CH-7), 101.37 (CH, CH-3), 56.50 (CH, CH-1), 45.00 (CH, cyclohexyl), 35.40 (2× CH_2, cyclohexyl), 27.65 (2× CH_2, cyclohexyl), 26.95 (1× CH_2, cyclohexyl). HRMS (EI^+) *m/z* Calcd for $C_{23}H_{23}N_3O_2$ $(M+H)^+$ 358.1914. Found 358.1910.

5.1.4.8. 1-(Benzo[*b*]furan-2-yl-biphenyl-4-yl-methyl)-1*H*-[1,2,4]triazole (15a) and 4-(benzo[*b*]furan-2-yl-biphenyl-4-yl-methyl)-4*H*-[1,2,4]triazole (15b). Purified by column chromatography (petroleum/ethyl acetate, 9:1 v/v increasing to 65:35 v/v) to give the [1,2,4]triazole **15a**, further elution (dichloromethane/methanol, 99:1 v/v) gave the [1,3,4]triazole **15b**.

*Data for **15a***: White solid (53%), mp 130–132 °C. TLC system: petroleum ether/ethyl acetate, 1:1 v/v, R_f: 0.41. ^1H NMR ($CDCl_3$): δ 8.24 (s, 1H, H-3‴), 8.12 (s, 1H, H-5‴), 7.68 (d, J = 8.3 Hz, 2H, Ar), 7.63 (m, 3H, Ar), 7.53 (m, 3H, Ar), 7.45–7.31 (m, 5H, Ar), 6.96 (s, 1H, H-1), 6.69 (s, 1H, H-3). ^{13}C NMR ($CDCl_3$): δ 155.80 (C, C-2), 152.99 (C, C-7a), 152.78 (CH, CH-5‴), 143.76 (CH, CH-3‴), 142.6.1 (C, C-1″), 140.55 (C, C-1′), 134.92 (C, C-4′), 129.36 (2× CH, CH-2′ and CH-6′), 128.58 (2× CH, CH-3″ and CH-5″), 128.27 (2× CH, CH-2″ and CH-6″), 128.23 (2× CH, CH-3′ and CH-5′), 127.92 (C, C-3a), 127.60 (CH, CH-4″), 125.75 (CH, Ar), 123.83 (CH, Ar), 122.02 (CH, Ar), 112.02 (CH, CH-7), 108.34 (CH, CH-3), 62.41 (CH, CH-1). Anal. Calcd for $C_{23}H_{17}N_3O$ (351.406): C, 78.61; H, 4.88; N, 11.95. Found: C, 78.38; H, 4.74; N, 11.89.

*Data for **15b***: Light yellow solid (6%), mp 56–58 °C. TLC system: dichloromethane/methanol, 9:1 v/v, R_f: 0.31. ^1H NMR ($CDCl_3$): δ 8.41 (s, 2H, H-2‴ and H-5‴), 7.79 (d, J = 8.4 Hz, 2H, Ar), 7.72 (m, 3H, Ar), 7.61 (m, 3H, Ar), 7.58–7.40 (m, 5H, Ar), 6.87 (s, 1H, H-1), 6.79 (s, 1H, H-3). ^{13}C NMR ($CDCl_3$): δ 155.85 (C, C-2), 152.44 (C, C-7a), 143.02 (C, C-1″), 142.93 (2× CH-2‴ and CH-5‴), 140.23 (C, C-1′), 134.50 (C, C-4′), 129.42 (2× CH, CH-2′ and CH-6′), 128.51 (2× CH, CH-3″ and CH-5″), 128.41 (2× CH, CH-2″ and CH-6″), 128.31 (CH, CH-4″), 127.59 (2× CH, CH-3′ and CH-5′ and C, C-3a), 126.12 (CH, Ar), 124.07 (CH, Ar), 122.09 (CH, Ar), 112.10 (CH, CH-7), 108.34 (CH, CH-3), 58.33 (CH, CH-1). Anal. Calcd for $C_{23}H_{17}N_3O$ (351.406): C, 78.61; H, 4.88; N, 11.95. Found: C, 78.77; H, 4.62; N, 11.79.

5.1.4.9. 1-[Benzo[*b*]furan-2-yl-(4′-chloro-biphenyl-4-yl)-methyl]-1*H*-[1,2,4]triazole (16a) and 4-[benzo[*b*]furan-2-yl-(4′chloro-biphenyl-4-yl)-methyl]-4*H*-[1,2,4]triazole (16b). Purified by column chromatography (petroleum/ethyl acetate, 9:1 v/v increasing to 65:35 v/v) to give the [1,2,4]triazole **16a**, further elution (dichloromethane/methanol, 99:1 v/v) gave the [1,3,4]triazole **16b**.

*Data for **16a***: white solid (67%), mp 34–36 °C. TLC system: petroleum ether/ethyl acetate, 1:1 v/v, R_f: 0.26. ^1H NMR ($CDCl_3$): δ 8.24 (s, 1H, H-3‴), 8.11 (s, 1H, H-5‴), 7.64 (d, J = 8.3 Hz, 2H, Ar), 7.60–7.52 (m, 3H, Ar), 7.49–7.29 (m, 7H, Ar), 6.95 (s, 1H, H-1), 6.69 (s, 1H, H-3). ^{13}C NMR ($CDCl_3$): δ 155.80 (C, C-2), 152.80 (CH, CH-5‴ and C, C-7a), 143.74 (CH, CH-

3‴), 141.36 (C, C-1′), 138.97 (C, C-1″), 135.32 (C, C-4′), 134.38 (C, C-4″), 129.52 (2× CH, CH-2′ and CH-6′), 128.83 (2× CH, CH-3″ and CH-5″), 128.67 (2× CH, CH-2″ and CH-6″), 128.10 (2× CH, CH-3′ and CH-5′), 127.87 (C, C-3a), 125.80 (CH, Ar), 123.86 (CH, Ar), 122.02 (CH, Ar), 112.02 (CH, CH-7), 108.40 (CH, CH-3), 62.35 (CH, CH-1). Anal. Calcd for $C_{23}H_{16}ClN_3O$ (385.852): C, 71.60; H, 4.18; N, 10.89. Found: C, 71.45; H, 4.10; N, 10.62.

Data for ***16b***: Light yellow solid (13%), mp 68–70 °C. TLC system: dichloromethane/methanol, 9:1 v/v, R_f: 0.50. ^{1}H NMR ($CDCl_3$): δ 8.31 (s, 2H, H-2‴ and H-5‴), 7.65 (d, $J = 8.3$ Hz, 2H, Ar), 7.61–7.43 (m, 6H, Ar), 7.41–7.31 (m, 4H, Ar), 6.79 (s, 1H, H-1), 6.70 (s, 1H, H-3). ^{13}C NMR ($CDCl_3$): δ 155.85 (C, C-2), 152.29 (C, C-7a), 142.89 (2× CH, CH-2‴ and CH-5‴), 141.75 (C, C-1′), 138.66 (C, C-1″), 134.93 (C, C-4′), 134.58 (C, C-4″), 129.59 (2× CH, CH-2′ and CH-6′), 128.82 (2× CH, CH-3″ and CH-5″), 128.42 (2× CH, CH-2″ and CH-6″), 128.35 (2× CH, CH-3′ and CH-5′), 127.56 (C, C-3a), 126.16 (CH, Ar), 124.10 (CH, Ar), 122.10 (CH, Ar), 112.10 (CH, CH-7), 108.37 (CH, CH-3), 58.26 (CH, CH-1). Anal. Calcd for $C_{23}H_{16}ClN_3O{\cdot}0.1H_2O$ (387.653): C, 71.26; H, 4.21; N, 10.83. Found: C, 71.05; H, 4.09; N, 10.75.

5.2. MCF-7 (CYP26A1) assay for inhibition of metabolism of atRA

Human MCF-7 breast cancer cells were cultured in phenol red free RPMI 1640 medium supplemented with 5 % (v/v) charcoal free foetal calf serum, antibiotics (penicillin and streptomycin) and fungizone at the same concentration of 10 iU/mL. Cells were grown in a humidified incubator (5% CO_2, 95% air) at 37 °C. MCF-7 cells were seeded in 12-well cell culture plates (Cornings Inc., New York, USA) at 2.5×10^5 cells per well in a total volume of 1.5 mL. Cells were allowed to adhere to the well for 24 h. After 24 h, the medium from each well was removed, washed once with phosphate-buffered saline (PBS) and replaced by fresh medium plus 10 μL inhibitor/solvent (acetonitrile) and 10 μL of atRA (to give a final concentration of 1×10^{-7} M atRA and 0.1 μCi [11,12-^{3}H] *all-trans* retinoic acid). The plates were foil wrapped and incubated at 37 °C for 9 h. Each treatment was performed in duplicate. The incubation was stopped by addition of 1% acetic acid (100 μL/well), the medium removed into separate glass tubes. Two hundred microlitres of distilled water was added to each well and the cells were scrapped off and the contents added to the appropriate glass tube. This procedure was repeated with a further 400 μL water but without scraping. Ethyl acetate containing 0.05% (w/v) butylated hydroxyanisole (2× 2 mL) was added to each tube. After vortexing for 15 s, the tubes were spun down at 3000 rpm for 15 min. The organic layer was then evaporated using a Christ centrifuge connected to a vacuum pump and a multitrap at −80 °C.

5.3. High performance liquid chromatography (HPLC)

The HPLC system was equipped with a high pressure pump (Milton-Roy pump), injector with a 50 μL loop connected to a β-RAM radioactivity detector, connected to a Compaq™ computer running Laura® data acquisition and analysis software. This enabled on-line detection and quantification of radioactive peaks. The HPLC column (10 μM C_{18} μBondapak™ 3.9 × 300 mm HPLC column from Waters, UK) operating at ambient temperature was used to separate the metabolites which were eluted with acetonitrile/1% ammonium acetate in water/acetic acid (75:25:0.1 v/v/v) at a flow rate of 1.9 mL/min. Ecoscint™ was used as the flow scintillation fluid.

5.4. Molecular docking

All molecular modelling studies were performed on a RM Innovator Pentium IV 2.4 GHz running either Linux Fedora Core 3 or Windows XP using Molecular Operating Environment (MOE) 2004.03[24] software. All the minimisations were performed with MOE a until a RMSD gradient of 0.05 Kcal mol^{-1} Å^{-1} with the forcefield specified and the partial charges were automatically calculated.

Ligands were docked within the active site of the CYP26A1 homology model[25] using the MOE-Dock with simulated annealing 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. Molecular dynamics was performed with MOE using the NVT environment for 100 ps and constant temperature of 300 K using the MMFF94X forcefield with all other default settings in MOE-dynamics chosen. The lowest energy conformation was selected and subjected to an energy minimisation using the MMFF94X forcefield. The scoring.svl script was used to identify interaction types between ligand and protein.

Acknowledgments

One of us (S.W.Y.) would like to acknowledge the ORS Awards Scheme for a United Kingdom Scholarship. We also acknowledge the technical staff of the Tenovus Cancer Centre for the preparation of cells and media, and the EPSRC Mass Spectrometry Centre, Swansea, UK, for mass spectroscopy data.

References and notes

1. Ray, W. J.; Bain, G.; Yao, M.; Gottlieb, D. I. *J. Biol. Chem.* **1997**, *272*, 18702–18708.
2. White, J. A.; Beckett, B.; Scherer, S. W.; Herbrick, J. A. *Genomics* **1998**, *48*, 270–272.
3. Njar, V. C. O. *Mini-Rev. Med. Chem.* **2002**, *2*, 261–269.
4. Marill, J.; Idres, N.; Capron, C. C.; Nguyen, E.; Chabot, G. G. *Curr. Drug Metab.* **2003**, *4*, 1–10.
5. Estey, E. H.; Giles, F.; Kantarjian, H.; O'Brien, S.; Cortes, J.; Freireich, E.; Lopez-Berestein, G.; Keating, M. *Blood* **1999**, *94*, 2230–2235.
6. Fenaux, P.; Chastang, C.; Chevret, S.; Sanz, M.; Dombret, H.; Archimbaud, E.; Fey, M.; Rayon, C.; Huguet, F.; Sotto, J. J.; Gardin, C.; Makhoul, P. C.; Travade, P.; Solary, E.; Fegueux, N.; Bordessoule, D.; San Miguel, J.;

Link, H.; Desablens, B.; Stamatoullas, A.; Deconinck, E.; Maloisel, F.; Castaigne, S.; Preudhomme, C.; Degos, L. A. *Blood* **1999**, *94*, 1192–1200.
7. Marill, J.; Cresteil, T.; Lanotte, M.; Chabot, G. G. *Mol. Pharmacol.* **2000**, *58*, 1341–1348.
8. Weiss, G. R.; Liu, P. Y.; Alberts, D. S.; Peng, Y. M.; Fisher, E.; Xu, M. J.; Scudder, S. A.; Baker, L. H.; Moore, D. F.; Lippman, S. M. *Gynecol. Oncol.* **1998**, *71*, 386–390.
9. Pettersson, F.; Colston, K. W.; Dalgleish, A. G. *Pancreas* **2001**, *23*, 273–279.
10. Ahmad, N.; Mukhtar, H. *J. Invest. Dermatol.* **2004**, *123*, 417–425.
11. Brecher, A. R.; Orlow, S. J. *J. Am. Acad. Dermatol.* **2003**, *49*, 171–182.
12. Smith, H. J.; Nicholls, P. J.; Simons, C.; LeLain, R. *Exp. Opin. Ther. Patents* **2001**, *11*, 789–824.
13. Yee, S. W.; Jarno, L.; Gomaa, M. S.; Elford, C.; Ooi, L.-L.; Coogan, M. P.; McClelland, R.; Nicholson, R. I.; Evans, B. A. J.; Brancale, A.; Simons, C. *J. Med. Chem.* **2005**, *48*, 7123–7131.
14. Patel, J. B.; Huynh, C. K.; Handratta, V. D.; Gediya, L. K.; Brodie, A. M. H.; Goloubeva, O. G.; Clement, O. O.; Nanne, N. P.; Soprano, D. R.; Njar, V. C. O. *J. Med. Chem.* **2004**, *47*, 6716–6729.
15. Mulvihill, M. J.; Kan, J. L. C.; Beck, P.; Bittner, M.; Cesario, C.; Cooke, A.; Keane, D. M.; Nigro, A. I.; Nillson, C.; Smith, V.; Srebernak, M.; Sun, F. L.; Vrkjan, M.; Winski, S. L.; Castelhano, A. L.; Emerson, D.; Gibson, N. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1669–1673.
16. Kuijpers, A. L. A.; Van Pelt, J. P. A.; Bergers, M.; Boegheim, P. J.; Den Bakker, J. E. N.; Siegenthaler, G.; Van de Kerkhof, P. C. M.; Schalkwijk, J. *Br. J. Dermatol.* **1998**, *139*, 380–389.
17. Lucker, G. P. H.; Heremans, A. M. C.; Boegheim, P. J.; Van de Kerkhof, P. C. M.; Steijlen, P. M. *Br. J. Dermatol.* **1997**, *136*, 71–75.
18. Denis, L.; Debruyne, F.; De Porre, P. *Eur. J. Cancer* **1998**, *34*, 469–475.
19. Stoppie, P.; Borgers, M.; Borghgraef, P.; Dillen, L.; Goossens, J.; Sanz, G.; Szel, H.; Van Hove, C.; Van Nyen, G.; Nobels, G.; Vanden Bossche, H.; Venet, M.; Willemsens, G.; Van Wauwe, J. *J. Pharmacol. Exp. Ther.* **2000**, *293*, 304–312.
20. Stoermer, R. *Annalen der Chemie* **1900**, *312*, 237.
21. Vinh, T. K.; Yee, S. W.; Kirby, A. J.; Nicholls, P. J. *Anticancer Drug Des.* **2001**, *16*, 217–225.
22. Cowper, R. M.; Davidson, L. H. *Organic Syntheses Collective Volumes*. 1943, Vol. 1, p 480.
23. Miyaura, N.; Yanagi, T.; Suzuki, A. *Synth. Commun.* **1981**, *11*, 513–519.
24. Molecular Operating Environment (MOE). Chemical Computing Group, Inc. Montreal, Quebec, Canada. http://www.chemcomp.com. Code 'scoring svl' obtained from SVL Exchange website http://svl.chemcomp.com., Chemical Computing Group, Inc. Montreal, Canada.
25. Gomaa, M.; Yee, S. W.; Milbourne, C. E.; Chiara, M. B.; Simons, C.; Brancale, A. Submitted for publication.
26. Yano, J. K.; Wester, M. R.; Schoch, G. A.; Griffin, K. J.; Stout, D.; Johnson, E. F. *J. Biol. Chem.* **2004**, *279*, 38091–38094.
27. Richter, P. H.; Elsner, H.; Vogt, B. Preparation of benzo[*b*]furan-derived ketone amidinohydrazome class III antiarrhythmic agents. *Eur. Pat. Appl.*, 1997, 32 pp.
28. Pestellini, V.; Giolitti, A.; Pasqui, F.; Abelli, L.; Cutrufo, C.; De Salvia, G.; Evangelista, S.; Meli, A. *Eur. J. Med. Chem.* **1988**, *23*, 203–206.