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Synthesis and CYP24A1 inhibitory activity of *N*-(2-(1*H*-imidazol-1-yl)-2-phenylethyl)arylamides

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ABSTRACT

A series of *N*-(2-(1*H*-imidazol-1-yl)-2-phenylethyl)arylamides were prepared, using an efficient three- to five-step synthesis, and evaluated for their inhibitory activity against human cytochrome P450C24A1 (CYP24A1) hydroxylase. Inhibition ranged from IC_{50} 0.3–72 μM compared with the standard ketoconazole IC_{50} 0.52 μM, with the styryl derivative (**11c**) displaying enhanced activity (IC_{50} = 0.3 μM) compared with the standard, providing a useful preliminary lead for drug development.

1. Introduction

Calcitriol (1α,25-$(OH)_2D_3$), the biologically active metabolite of vitamin D binds to the vitamin D receptor (VDR), which mediates tissue-specific effects including enhancement of intestinal calcium transport, maintenance of skeletal health and epidermal integrity and immune cell regulation (e.g., macrophages).[1] Replacement calcitriol therapy has been used in chronic kidney disease, hyperproliferative skin diseases and osteoporosis, with clinical trials for various forms of cancer and autoimmune conditions ongoing.[2,3] Although the potential of vitamin D as an antiproliferative drug has been realised in psoriasis and parathyroid cell hyperplasia, an anticancer treatment incorporating vitamin D remains a challenge owing to increased drug resistance.[3] Evidence is growing that this resistance is caused by up-regulation of the cytochrome P450 enzyme, CYP24A1, in tumours resulting in accelerated metabolism of calcitriol and derivatives.[4,5] Inhibitors of CYP24A1 are expected to extend the half-life of calcitriol thereby enhancing endogenous levels of calcitriol, or vitamin D analogues, which may result in stabilized CYP24A1 and enhanced stability of vitamin D compounds. Small molecule inhibitors of CYP24A1 have been described by our group[6,7] and others,[8–11] with the non-specific P450 inhibitor ketoconazole[8] (Fig. 1) used as a standard for comparison. The most promising CYP24A1 inhibitors described to date are VID-400[9] (IC_{50} = 15.1 nM compared with ketoconazole IC_{50} = 126 nM in human epidermal keratinocytes) and the vitamin D derived sulfoximes[10] (IC_{50} = 7.4 nM compared with ketoconazole IC_{50} = 312 nM in V79-CYP24A1 recombinant cell line expressing human CYP24A1), which are based on the structure of the natural substrate, calcitriol.

Here we report the synthesis and inhibitory activity of a series of *N*-(2-(1*H*-imidazol-1-yl)-2-phenylethyl)arylamides. The scaffold of the benzofuran series (**7**) were based on VID-400, while the phenylmethyl (**11b**) and styryl (**11c**) derivatives combined pharmacophores of VID-400 and calcitriol.

2. Chemistry

The synthesis of *N*2-[2-phenyl-2-(1*H*-imidazoyl)ethyl]-benzo[*b*]furan-2-carboxamide derivatives (**7a–e**), was achieved using a five-step reaction (Scheme 1) and involved a modification of the procedure described by Schuster and Egger[12] and Moenius et al.[13]

The substituted ethyl benzo[*b*]furan-2-carboxylates (**2**) were prepared by the reaction of the appropriate salicylaldehyde (**1**) with ethyl bromoacetate in anhydrous DMF at 130 °C for 6 h according to the methodology previously described.[14] After purification by flash chromatography or recrystallisation with petroleum ether, the

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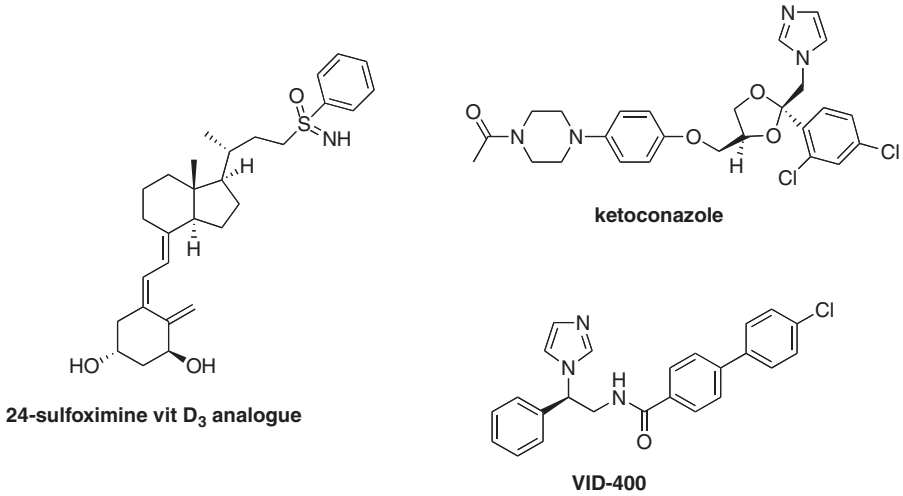
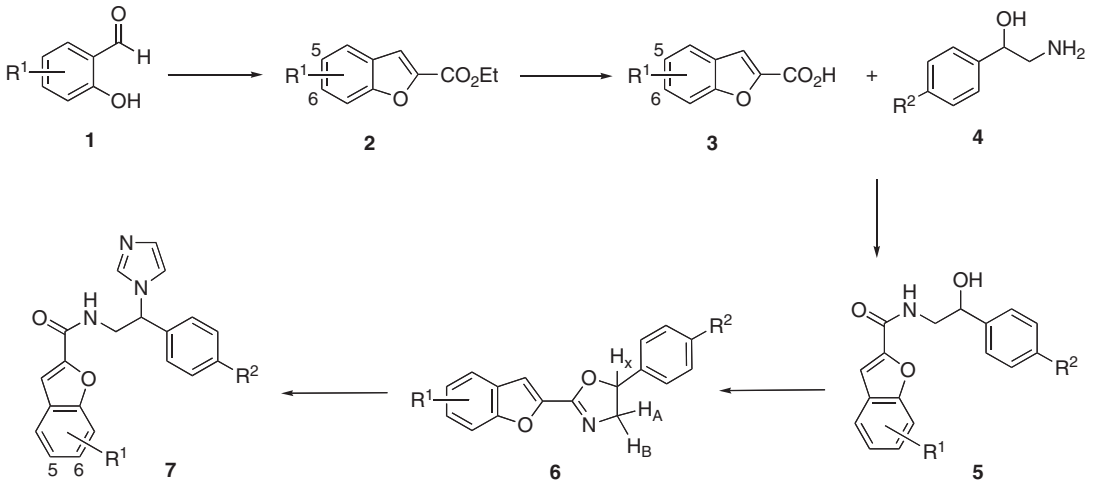


Figure 1. Inhibitors of CYP24A1.



Scheme 1. Reagents and conditions: (i) ethyl bromoacetate, K_2CO_3, DMF, 130 °C, 6–7 h, 22–53%; (ii) 2 M NaOH (aq), CH_3OH, rt, 20 min, 85–98%; (iii) (a) CDI, DMF, rt, 1 h; (b) substituted 2-amino-1-phenyl-ethanol (**4**), rt, 12 h, 72–88%; (iv) $(CH_3SO_2)_2O$, Et_3N, 0 °C, 24 h, 16–67%; (v) imidazole, isopropyl acetate, 125 °C, 24 h, 28–72%.

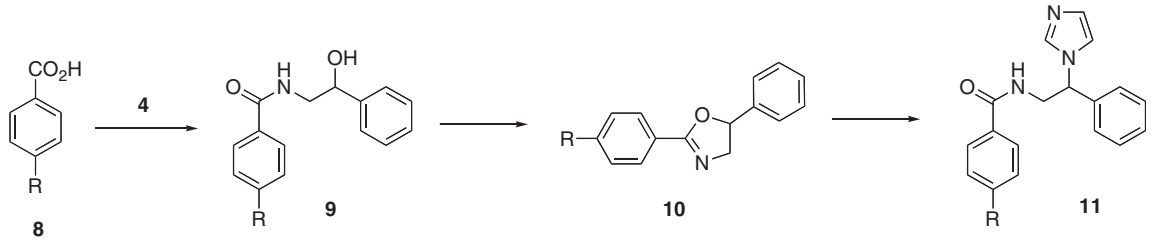
products (**2**) were obtained in low to moderate yields (22–53%). Base hydrolysis of the ethyl benzo[*b*]furan-2-carboxylates (**2**) gave the substituted benzo[*b*]furan-2-carboxylic acid compounds (**3**) in good yields (88–98%). The synthesis of the substituted *N*2-(2-hydroxy-2-phenylethyl)-benzo[*b*]furan-2-carboxamides (**5**) was carried out using 1,1′-carbonyldiimidazole (CDI) with the corresponding substituted 2-amino-1-phenyl-ethanol (**4**) (Scheme 1). The products were recrystallised from ethanol and ice water to give good purity and yield (72–88%).

The substituted 2-benzo[*b*]furan-2-yl-5-phenyl-4,5-dihydro-1,3-oxazoles (**6**) were prepared by the reaction of the amide-containing compound (**5**) with methanesulfonic anhydride and a base (triethylamine).[15,16] The presence of the 4,5-dihydro-1,3-oxazole ring structure was observed on the ^{1}H NMR spectra as an ABX system, which showed the signals of the three carbon-bound protons of the 4,5-dihydro-1,3-oxazole system. The geminal pair, H_A and H_B, are diastereotopic; they are coupled to each other with the largest of the three coupling constants (~15 Hz) and they are each coupled to H_X, with different coupling constants, one large (~10 Hz) and one small (~8 Hz). The H_X signal (~5.8 ppm) was downfield from the H_A and H_B signals (~4.5 and 4.0 ppm).

Overall, the synthesis of the oxazole compounds (**6**) gave moderate yields after purification by column chromatography or recrystallisation from ethanol. The isolated yield of compound **6c** was very low (16%), which may be owing to the presence of the electron withdrawing nitro group affecting the ring closure.

In the last step of the reaction scheme, heating the oxazole compound (**6**) in the presence of imidazole opened the oxazole ring by nucleophilic displacement (Scheme 1)[17] A reasonable yield of compound (**7a**–**e**) was produced after purification by column chromatography and/or recrystallisation from ethanol/water (2:1 v/v).

In a similar manner 4-bromo-*N*-(2-(1*H*-imidazol-1-yl)-2-phenylethyl)benzamide (**11a**), 4-benzyl-*N*-(2-(1*H*-imidazol-1-yl)-2-phenylethyl)benzamide (**11b**), and (*E*)-4-*N*-(2-(1*H*-imidazol-1-yl)-2-phenylethyl)styrylbenzamide (**11c**) were prepared (Scheme 2), commencing from either commercially available or readily prepared carboxylic acids (**8**), which on coupling with 2-amino-1-phenyl–ethanol (**4**) gave the amides (**9**). The oxazoles (**10**) and final imidazole products (**11**) were prepared as previously described for the benzofuran series with good yields obtained for the oxazoles (61–82%) and low to moderate yields obtained for the imidazoles (16–41%).



Scheme 2. Reagents and conditions: (i) (a) CDI, DMF, rt, 1 h; (b) 2-amino-1-phenyl-ethanol (**4**), rt, 12 h, 39–86%; (ii) $(CH_3SO_2)_2O$, Et_3N, 0 °C, 24 h, 71–82%; (iii) imidazole, isopropyl acetate, 125 °C, 24 h, 16–41%.

3. CYP24A1 inhibitory activity

The imidazole derivatives **7a–e** and **11a–c** were evaluated for their calcitriol metabolism (CYP24A1) inhibitory activity using a cell based assay employing a recombinant cell line expressing human CYP24A1 enzyme (V79-CYP24),[18] using radiolabelled [^{3}H-1β]-calcitriol as the substrate and ketoconazole as the standard for comparison. In the benzofuran series (**7**), the 4-fluorophenyl (**7d**) and unsubstituted (**7a**) derivatives were the most potent (IC_{50} = 2.8 and 4.0 μM, respectively), with introduction of the larger chloro group (**7e**) resulting in a small reduction in activity (Table 1). Incorporation of substituents in the benzofuran moiety was less favourable (IC_{50} = 6.4 μM (5-NO_2, **7c**) and 36.9 μM (6-OCH_3, **7b**)). However all the benzofuran derivatives (**7**) were less active than the standard ketoconazole (IC_{50} = 0.52 μM) (Table 1). Replacing the benzofuran group with either 4-bromobenzene (**11a**) or methylenedibenzene (**11b**) resulted in a substantial loss of activity, however the introduction of the styryl moiety (**11c**) resulted in a potent inhibitor (IC_{50} = 0.3 μM).

4. Docking

To investigate the possible binding mode of this series of compounds, we have performed a set of molecular docking simulations, using our model of the CYP24A1.[19] In the benzofuran analogues series (Fig. 2) it is possible to observe how the imidazole N3 complexes the Haem–Fe, while the phenyl ring is placed in a small hydrophobic pocked created by Ile242, Val391, Thr330, and Ala326. The benzofuran ring is placed in a hydrophobic channel and it establishes a series of interactions with Ile149, Pro392, and Trp134. From these results it also appears that the amide function does not have a specific role in the binding, but acts just as a linker between the two aromatic parts of the compounds.

The styryl analogue **11c** docks in a very similar pose as **7a** (Fig. 3), but in this case the styryl group extends further in the hydrophobic channel, establishing a contact with Phe104. Finally, it should be noted that compound **11b** does not dock successfully in the CYP24A1 model, probably because of the steric hindrance of the side chain, which cannot be properly placed in the narrow hydrophobic channel.

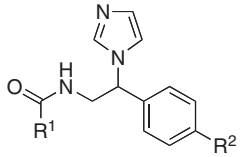
5. Experimental

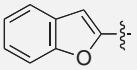
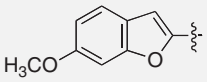
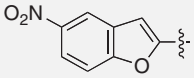
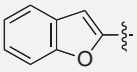
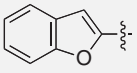
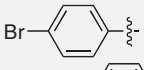
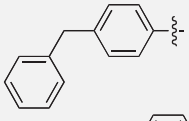
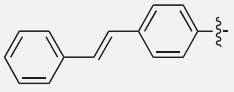
5.1. Materials and methods: chemistry

^{1}H 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 (230–400 mesh) (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. Compounds were visualised by illumination under UV light (254 nm) or by the use of vanillin stain followed by charring on a hotplate. All solvents were dried prior to use as described by the handbook Purification of Laboratory Chemicals[20] and stored over 4 Å molecular sieves, under nitrogen.

The ethyl benzo[*b*]furan-2-carboxylic acid derivatives (**3**) were prepared according to the general procedure described by Suzuki et al,[14] compounds **2** and **3** (R1 = H) were previously reported.[14] The 2-amino-1-phenyl-ethanol derivatives (**4**) were either available commercially (R = H) or prepared (R = F, Cl) according to the method described by Cho et al.[21]

Table 1
IC_{50} data for the novel *N*-(2-(1*H*-imidazol-1-yl)-2-phenylethyl)aryl amides **7** and **11**



Compound	R^1	R^2	IC_{50}[a] (μM)
7a		H	4.0
7b		H	36.9
7c		H	6.4
7d		F	2.8
7e		Cl	10.6
11a		H	23
11b		H	72
11c		H	0.3
Ketoconazole	—	—	0.52

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

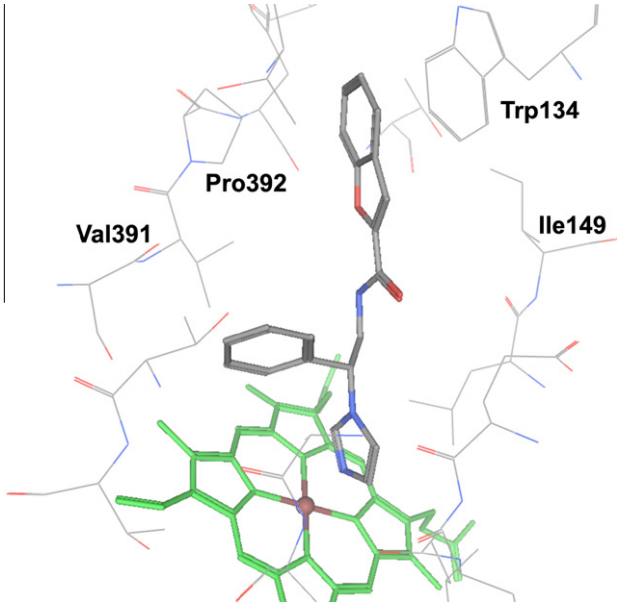


Figure 2. Proposed binding mode of compound **7a**.

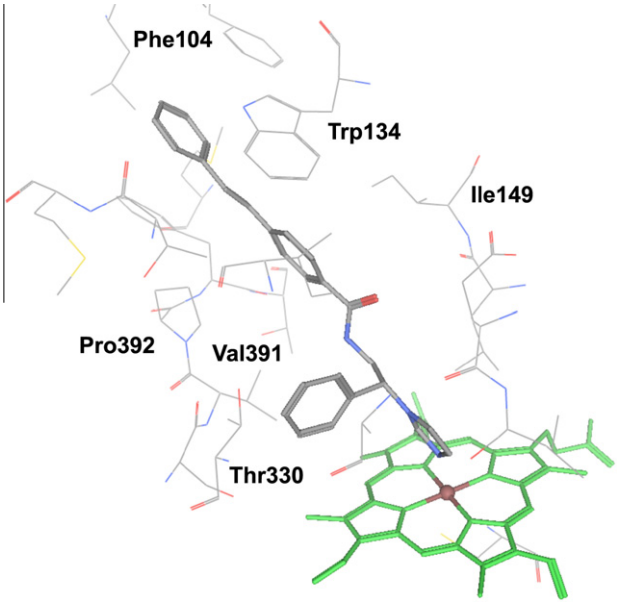


Figure 3. Proposed binding mode of compound **11c**.

5.1.1. General procedure for the synthesis of ethyl benzo[*b*]furan-2-carboxylate derivatives (2)

Activated potassium carbonate (16.55 g, 120 mmol) was added to a stirred solution of salicylaldehyde (**1**) (60 mmol) in anhydrous DMF (15 mL). Ethyl bromoacetate (10.0 g, 60 mmol) in anhydrous DMF (20 mL) was then added to the above mixture and the reaction was stirred at 130 °C for 6 h. The solvent was then evaporated to about 1/3 its volume to give a brown syrup. The crude product was extracted with CH_2Cl_2 (200 mL) and water (3 × 100 mL). The organic layer was dried ($MgSO_4$), filtered and reduced in vacuo and the resulting residue purified by flash column chromatography (petroleum ether/ethyl acetate 100:0 v/v increasing to 95:5 v/v)

5.1.1.1. Ethyl 6-methoxybenzo[*b*]furan-2-carboxylate (2, R = 6-OMe). A brown solid was obtained. Yield: 53%, R_f 0.55 (petroleum ether/EtOAc 3:1 v/v); mp 66–68 °C; ^{1}H NMR: δ 7.63 (d, J = 8.7 Hz, 1H, H-4), 7.57 (d, J = 0.9 Hz, 1H, H-3), 7.17 (d, J = 1.8 Hz, 1H, H-7), 7.03 (dd, J = 2.2, 8.7 Hz, 1H, H-5), 4.53 (q, J = 7.1 Hz, 2H, CH_2), 3.97 (s, 3H, OCH_3), 1.52 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C NMR: δ 160.9 (C, C-7a), 160.0 (C-6), 157.5 (C, C=O), 145.4 (C, C-2), 123.4 (CH, CH-4), 120.7 (C, C-3a), 114.5 (CH, CH-3), 114.4 (CH, CH-5), 96.2 (CH, CH-7), 61.7 (CH_2), 56.1 (CH_3, –OCH_3), 14.8 (CH_3). Anal. Calcd for $C_{12}H_{12}O_4$ (220.223): C, 65.45; H, 5.49. Found: C, 65.32; H, 5.53.

5.1.1.2. Ethyl 5-nitrobenzo[*b*]furan-2-carboxylate (2, R = 5-NO$_2$). A yellow solid was obtained. Yield: 22%, R_f 0.60 (petroleum ether/EtOAc 4:1 v/v); mp 139–141 °C; ^{1}H NMR: δ 8.69 (d, J = 2.3 Hz, 1H, H-4), 8.42 (dd, J = 2.3, 9.1 Hz, 1H, H-6), 7.75 (d, J = 9.1 Hz, 1H, H-7), 7.70 (s, 1H, H-3), 4.53 (q, 2H, J = 7.1 Hz, CH_2), 1.50 (t, 3H, J = 7.1 Hz, CH_3). ^{13}C NMR: δ 159.0 (C, C-7a), 158.4 (C=O), 149.1 (C, C-2), 145.2 (C, C-5), 127.7 (C, C-3a), 123.3 (CH, CH-6), 119.9 (CH, CH-4), 114.3 (CH, CH-3), 113.4 (CH, CH-7), 62.6 (CH_2), 14.7 (CH_3). Anal. Calcd for $C_{11}H_9NO_5$ (235.194): C, 56.17; H, 3.86; N, 5.95. Found: C, 56.38; H, 3.90; N, 5.88.

5.1.2. General procedure for the synthesis of ethyl benzo[*b*]furan-2-carboxylic acid derivatives (3)

A solution of ethyl benzo[*b*]furan-2-carboxylate (**2**) (32 mmol) in methanol (100 mL) was treated with 2 M aqueous sodium hydroxide (50 mL) and warmed gently on a steam bath. After hydrolysis was complete (ca. 30 min.), the reaction solution was cooled and acidified to pH 1 by the dropwise addition of concentrated HCl. The solution was then extracted with diethyl ether (3 × 150 mL) and the organic layer dried ($MgSO_4$), filtered, and reduced in vacuo to give a crude yellow solid. The crude yellow solid was washed with water, collected and dried under vacuum with phosphorus pentoxide

5.1.2.1. 6-Methoxybenzo[*b*]furan-2-carboxylic acid (3, R = 6-OMe). Yield: 88%, R_f 0.0 (petroleum ether/EtOAc 1:1 v/v); mp 194–196 °C; ^{1}H NMR (DMSO-d_6): δ 13.36 (s, 1H, COO*H*), 7.67 (d, J = 8.7 Hz, 1H, H-4), 7.61 (s, 1H, H-3), 7.31 (s, 1H, H-7), 6.99 (split d, J = 2.1, 8.6 Hz, H-5), 3.85 (s, 3H, OCH_3). ^{13}C NMR (DMSO-d_6): δ 160.4 (C=O and C, C-7a), 156.8 (C, C-6), 145.6 (C, C-2), 123.7 (CH, CH-4), 120.3 (C, C3a), 114.2 (CH, CH-3), 114.1 (CH, CH-5), 96.2 (CH, CH-7), 56.1 (CH_3, –OCH_3). Anal. Calcd for $C_{10}H_8O_4$ (192.169): C, 62.50; H, 4.20. Found: C, 62.49; H, 4.19.

5.1.2.2. 5-Nitrobenzo[*b*]furan-2-carboxylic acid (3, R = 5-NO$_2$). Yield: 95%, R_f 0.0 (petroleum ether/EtOAc 1:1 v/v); mp 268–270 °C; ^{1}H NMR (DMSO-d_6): δ 14.00 (s, 1H, COO*H*), 8.75 (d, J = 2.2 Hz, 1H, H-4), 8.36 (dd, J = 2.2, 9.2 Hz, 1H, H-4), 7.97 (d, J = 9.2 Hz, 1H, H-7), 7.85 (s, 1H, H-3). ^{13}C NMR (DMSO-d_6): δ 159.8 (C, C-7a), 157.9 (C=O), 149.4 (C, C-5), 144.5 (C, C-2), 127.8 (C, C-3a), 123.1 (CH, CH-6), 120.2 (CH, CH-4), 114.5 (CH, CH-3), 113.6 (CH, CH-7). Anal. Calcd for $C_9H_5NO_5$ (207.140): C, 52.19; H, 2.43; N, 6.76. Found: C, 52.30; H, 2.43; N, 6.68.

5.1.3. General procedure for the synthesis of *N*2-(2-hydroxy-2-phenylethyl)aryl-2-carboxamide derivatives (5) and (9)

To a suspension of carboxylic acid (**3** or **8**) (28 mmol) in anhydrous DMF (30 mL) was added 1,1′-carbonyldiimidazole (28 mmol) and the reaction stirred at room temperature for 1 h. The reaction was cooled to 0 °C and subsequently combined with a solution of 2-amino-1-phenyl-ethanol (**4**) (28 mmol) in DMF (10 mL). The mixture was stirred at room temperature for 8 h. After the reaction was complete, an aliquot amount of ice was poured into the flask to precipitate out the product, which was then filtered and washed with 10 mL of ethanol and dried under vacuum with phosphorus pentoxide.

5.1.3.1. *N*2-(2-Hydroxy-2-phenylethyl)benzo[*b*]furan-2-carboxamide (5a). A white solid was obtained. Yield: 72%, R_f 0.45 (petroleum ether/EtOAc 3:2 v/v); mp 140–142 °C; ^{1}H NMR (DMSO-d_6): δ 8.67 (t, J = 5.6 Hz, 1H, NH), 7.78 (d, J = 7.7 Hz, 1H, Ar), 7.67 (d, J = 8.3 Hz, 1H, Ar), 7.56 (s, 1H, H-3), 7.47 (m, 1H, Ar), 7.41–7.24 (m, 5H, Ar), 5.60 (d, J = 4.4 Hz, 1H, OH), 4.83 (m, 1H, H-1), 3.58–3.34 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6): δ 158.5 (C=O), 154.5 (C), 149.5 (C), 143.9 (C), 128.4, 128.4 (2 × CH), 127.5 (C), 127.4, 127.1, 126.4, 126.4, 124.0, 123.1, 112.1, 109.7 (8 × CH), 71.3 (*C*H–OH), 47.4 (CH_2). Anal. Calcd for $C_{17}H_{15}NO_3$ (281.309): C, 72.58; H, 5.37; N, 4.98. Found: C, 72.44; H, 5.32; N, 4.88.

5.1.3.2. *N*2-(2-Hydroxy-2-phenylethyl)-6-methoxybenzo[*b*]furan-2-carboxamide (5b). A light brown powder was obtained. Yield: 88%, R_f 0.40 (petroleum ether/EtOAc 3:2 v/v); mp 147–149 °C; ^{1}H NMR (DMSO-d_6): δ 8.45 (t, J = 5.7 Hz, 1H, NH), 7.62 (d, J = 8.7 Hz, 1H, Ar), 7.46 (d, J = 0.8 Hz, 1H, H-3), 7.39–7.30 (m, 4H, Ar), 7.27–7.20 (m, 2H, Ar), 6.95 (dd, J = 2.2, 8.6 Hz, 1H, Ar), 5.58 (d, J = 4.4 Hz, 1H, OH), 4.79 (m, 1H, H-1), 3.82 (s, 3H, O*C*H$_3$), 3.55–3.30 (m, 2H, C*H*$_2$). ^{13}C NMR (DMSO-d_6): δ 159.8 (C), 158.6 (C=O), 155.9 (C), 148.7 (C), 143.9 (C), 128.4, 128.4, 127.5, 126.4, 126.4, 123.3 (6 × CH), 120.7 (C), 113.5 (CH), 109.8 (CH), 96.3 (CH), 71.5 (*C*H–OH), 56.1 (O*C*H$_3$), 47.3 (CH_2). Anal. Calcd for $C_{18}H_{17}NO_4$ (311.335): C, 69.44; H, 5.50; N, 4.50. Found: C, 69.47; H, 5.46; N, 4.37.

5.1.3.3. *N*2-(2-Hydroxy-2-phenylethyl)-5-nitrobenzo[*b*]furan-2-carboxamide (5c). A light yellow solid was obtained. Yield: 88%, R_f 0.59 (petroleum ether/EtOAc 3:2 v/v); mp 174–176 °C; ^{1}H NMR (DMSO-d_6): δ 8.92 (t, J = 5.6 Hz, 1H, NH), 8.80 (d, J = 2.1 Hz, 1H, H-4), 8.35 (dd, J = 2.0, 9.1 Hz, 1H, H-6), 7.93 (d, J = 9.1 Hz, 1H, H-7), 7.79 (s, 1H, H-3), 7.44–7.26 (m, 5H, Ar), 5.62 (d, J = 2.9 Hz, 1H, OH), 4.85 (m, 1H, H-1), 3.59–3.37 (m, 2H, C*H*$_2$). ^{13}C NMR (DMSO-d_6): δ 157.8 (C, C=O), 157.2 (C), 152.3 (C), 144.5 (C), 143.8 (C), 128.5 (2 × CH), 128.1 (C), 127.5, 126.4, 126.4, 122.4, 119.9, 113.2, 110.4 (7 × CH), 71.3 (*C*H–OH), 47.5 (CH_2). Anal. Calcd for $C_{17}H_{14}N_2O_5$ (326.306): C, 62.57; H, 4.32; N, 8.58. Found: C, 62.42; H, 4.21; N, 8.59.

5.1.3.4. *N*2-[2-(4-Fluorophenyl)-2-hydroxyethyl]benzo[*b*]furan-2-carboxamide (5d). A white solid was obtained. Yield: 85%, R_f 0.41 (petroleum ether/EtOAc 3:2 v/v); mp 172–174 °C; ^{1}H NMR (DMSO-d_6): δ 8.69 (t, J = 5.7 Hz, 1H, NH), 7.79 (d, J = 7.7 Hz, 1H, Ar), 7.68 (d, J = 8.3 Hz, 1H, Ar), 7.58 (s, 1H, Ar), 7.51–7.27 (m, 4H, Ar), 7.18 (t, J = 8.9 Hz, 2H, Ar), 5.68 (d, J = 4.5 Hz, 1H, OH), 4.85 (m, 1H, C*H*-OH), 3.57–3.37 (m, 2H, C*H*$_2$). ^{13}C NMR (DMSO-d_6): δ 163.3 (C=O), 160.1 (C), 158.6 (C–F), 154.6 (C), 149.5 (C), 140.1 (C), 128.4 (CH), 128.3 (CH), 127.5 (C), 127.1, 124.05, 123.1, 115.3, 115.0, 112.2, 109.7 (7 × CH), 70.8 (*C*H–OH), 47.2 (CH_2). Anal. Calcd for $C_{17}H_{14}FNO_3$ (299.300): C, 68.22; H, 4.71; N, 4.68. Found: C, 68.27; H, 4.67; N, 4.66.

5.1.3.5. *N*2-[2-(4-Chlorophenyl)-2-hydroxyethyl]benzo[*b*]furan-2-carboxamide (5e). A white solid was obtained. Yield: 84%, R_f 0.47 (petroleum ether/EtOAc 3:2 v/v); mp 189–191 °C; ^{1}H NMR (DMSO-d_6): δ 8.69 (t, J = 5.7 Hz, 1H, NH), 7.79 (d, J = 7.8 Hz, 1H, Ar), 7.68 (d, J = 8.3 Hz, 1H, Ar), 7.57 (s, 1H, Ar), 7.51–7.33 (m, 6H, Ar), 5.71 (d, J = 4.5 Hz, 1H, OH), 4.84 (ddd, J = 4.9, 7.2, 12.1 Hz, 1H, C*H*-OH), 3.56–3.37 (m, 2H, C*H*$_2$). ^{13}C NMR (DMSO-d_6): δ 158.6 (C=O), 154.6 (C), 149.5 (C), 142.9 (C), 128.4, 128.4, 128.3, 128.3 (4 × CH), 127.5 (C), 127.2, 124.1, 123.1, 112.2, 109.8 (5 × CH), 70.8 (*C*H–OH), 47.1 (CH_2). Anal. Calcd for $C_{17}H_{14}ClNO_3$ (315.754): C, 64.67; H, 4.47; N, 4.43. Found: C, 64.58; H, 4.51; N, 4.44.

5.1.3.6. 4-Bromo-*N*-(2-hydroxy-2-phenylethyl)benzamide (9a). A white solid was obtained after recrystallisation from aqueous ethanol. Yield: 86%, R_f 0.8 (petroleum ether/EtOAc 3:2 v/v); mp 188–190 °C; ^{1}H NMR (DMSO-d_6): δ 8.61 (t, J = 5.2 Hz, 1H, NH), 7.79 (dd, J = 2.0, 8.5 Hz, 2H, Ar), 7.36 (m, 2H, Ar), 7.26 (m, 5H, Ar), 5.51 (d, J = 4.5 Hz, 1H, OH), 4.78 (ddd, J = 4.6, 6.6, 1H, C*H*-OH), 3.48 (ddd partially obscured, J = 5.8, 10.9 Hz, 2H, C*H*$_2$). ^{13}C NMR (DMSO-d_6): δ 165.4 (C=O), 143.7 (C), 133.7 (C), 131.2, 131.2, 129.3, 129.3, 128.0, 128.0, 127.0, 125.49, 125.9 (9 × CH, Ar), 124.8 (C), 71.0 (*C*H–OH), 47.7 (CH_2). Anal. Calcd for $C_{15}H_{14}BrNO_2$ (320.18): C, 56.27; H, 4.41; N, 4.37. Found: C, 56.26; H, 4.38; N, 4.31.

5.1.3.7. 4-Benzyl-*N*-(2-hydroxy-2-phenylethyl)benzamide (9b). A white solid was obtained after recrystallisation from aqueous ethanol. Yield: 39%, R_f 0.8 (petroleum ether/EtOAc 1:1 v/v); mp 98–99 °C; ^{1}H NMR (DMSO-d_6): δ 8.28 (t, J = 5.5 Hz, 1H, NH), 7.15–7.39 (m, 14H, Ar), 5.48 (d, J = 4.5 Hz, 1H, OH), 4.76 (dd, J = 5.2, 11.9 Hz, 1H, C*H*-OH), 4.03 (d, J = 2.2 Hz, 2H, Ar-C*H*$_2$-Ar), 4.38 (m, 1H, C*H*$_2$CH(OH)). ^{13}C NMR (DMSO-d_6): δ 169.3 (C=O), 143.5, 141.0, 138.9, 136.8 (4 × C), 130.1, 129.4, 128.9, 128.9, 128.4, 128.4, 128.2, 128.2, 128.0, 127.2, 127.1, 126.1, 125.8, 125.8 (14 × CH, Ar), 71.25 (CH, *C*H-OH), 47.05 (CH_2), 37.7 (CH_2). Anal. Calcd for $C_{21}H_{19}NO_2$ (317.38): C, 79.73; H, 6.39; N, 4.22. Found: C, 79.67; H, 6.41; N, 4.28.

5.1.3.8. *N* -(2-Hydroxy-2-phenylethyl)-4-styrylbenzamide (9c). A white solid was obtained after recrystallisation from ethanol. Yield: 71%, R_f 0.31 (petroleum ether/EtOAc 1:1 v/v); mp 218–219 °C; ^{1}H NMR (DMSO-d_6): δ 8.52 (s, 1H, NH), 7.88 (d, J = 8.0 Hz, 2H, Ar), 7.69 (d, J = 8.0 Hz, 2H, Ar), 7.64 (d, J = 7.5 Hz, 2H, Ar, 7.42–7.25 (m, 10H, Ar), 5.53 (d, J = 4.2 Hz, 1H, OH), 4.82 (m, 1H, C*H*-OH), 3.52 (m, 1H, CH_2), 3.37 (m, 1H, CH_2). ^{13}C NMR (DMSO-d_6): δ 166.0 (C=O), 143.8, 139.7, 136.8, 133.3 (4 × C), 130.1, 128.7, 128.7, 128.0, 128.0, 127.7, 127.7, 127.5, 127.5, 127.0, 126.7, 126.7, 126.2, 126.2, 126.0, 126.0 (16 × CH, Ar), 71.2 (CH, *C*H-OH), 47.7 (CH_2). Anal. Calcd for $C_{23}H_{21}NO_2$ (343.42): C, 80.44; H, 6.16; N, 4.08. Found: C, 80.27; H, 6.21; N, 3.88.

5.1.4. General procedure for the synthesis of 4,5-dihydro-1,3-oxazole derivatives (6) and (10)

To a solution of *N*-(2-hydroxy-2-phenylethyl)benzo[*b*]furan-2-carboxamide (**5**) (7.11 mmol) in anhydrous THF (15 mL) was added methanesulfonic anhydride (10.66 mmol) dissolved in anhydrous THF (5 mL). The homogenous mixture was stirred at 0 °C for 15 min, then triethylamine (3.0 mL, 21.33 mmol) was added dropwise to the above mixture. After keeping the homogenous mixture in the fridge at 0 °C for 24 h the reaction went to completion. The mixture was quenched by the addition of aqueous ammonia solution (28%, 1 mL). After stirring at room temperature for 15 min the mixture was concentrated in vacuo and finally distributed between ethyl acetate (150 mL) and aqueous $NaHCO_3$ solution (2 × 100 mL). After repeated extraction the organic phases were dried and evaporated in vacuo.

5.1.4.1. 2-Benzo[*b*]furan-2-yl-5-phenyl-4,5-dihydro-1,3-oxazole (6a). A yellow solid was obtained after recrystallisation from ethanol. Yield: 54%, R_f 0.57 (petroleum ether/EtOAc 1:1 v/v); mp 79–81 °C; ^{1}H NMR: δ 7.71 (d, J = 7.8 Hz, 1H, Ar), 7.45 (d, J = 8.4 Hz, 1H, Ar), 7.49–7.32 (m, 8H, Ar), 5.75 (dd, $J_{x,a}$ = 8.1 Hz, $J_{x,b}$ = 10.0 Hz, 1H, CH_x), 4.60 (dd, $J_{b,x}$ = 10.1 Hz, $J_{b,a}$ = 15.1 Hz, 1H, CH_b), 4.14 (dd, $J_{a,x}$ = 8.0 Hz, $J_{a,b}$ = 15.1 Hz, 1H, CH_a). ^{13}C NMR: δ 157.3, 156.2, 144.6, 140.7 (4 × C), 129.3 (CH), 129.3 (CH), 129.0 (CH), 127.8 (C), 127.2, 126.3, 126.3, 124.0, 122.7, 112.5, 111.2, 82.1 (8 × CH), 63.60 (CH_2). LRMS (ES$^+$) m/z: 286.1 (M+Na)$^+$. Anal. Calcd for $C_{17}H_{13}NO_2 \cdot 0.1H_2O$ (265.097): C, 77.02; H, 5.02; N, 5.28. Found: C, 77.05; H, 4.95; N, 5.25.

5.1.4.2. 2-(6-Methoxybenzo[*b*]furan-2-yl)-5-phenyl-4,5-dihydro-1,3-oxazole (6b). A light yellow solid was obtained after purification by flash column chromatography (petroleum ether/ethyl acetate 90:10 v/v increasing to 50:50 v/v). Yield: 67%, R_f 0.30 (petroleum ether/EtOAc 3:2 v/v); mp116–118 °C; ^{1}H NMR: δ 7.65 (d, J = 8.7 Hz, 1H, Ar), 7.50 (d, J = 0.5 Hz, 1H, Ar), 7.47–7.33 (m, 6H, Ar), 7.00 (d, J = 2.2, 8.7 Hz, 1H, Ar), 5.82 (dd, $J_{x,a}$ = 7.5 Hz, $J_{x,b}$ = 10.0 Hz, 1H, CH_x), 4.49 (dd, $J_{b,x}$ = 10.0 Hz, $J_{b,a}$ = 15.1 Hz, 1H, CH_b), 3.89 (dd, $J_{a,x}$ = 7.5 Hz, $J_{a,b}$ = 15.1 Hz, 1H, CH_a), 3.86 (s, 3H, OCH_3). ^{13}C NMR: δ 159.9, 156.7, 155.7, 143.4, 141.1 (5 × C), 129.2, 129.2, 128.7, 126.2, 126.2, 123.2 (6 × CH), 120.5 (C), 113.8 (CH), 111.4 (CH), 96.3 (CH), 80.6 (CH), 62.9 (CH_2), 56.1 (CH_3). Anal. Calcd for $C_{18}H_{15}NO_3$ (293.320): C, 73.71; H, 5.15; N, 4.77. Found: C, 73.52; H, 5.03; N, 4.72.

5.1.4.3. 2-(5-Nitrobenzo[*b*]furan-2-yl)-5-phenyl-4,5-dihydro-1,3-oxazole (6c). A white solid was obtained after purification by flash column chromatography (petroleum ether/ethyl acetate 90:10 v/v increasing to 60:40 v/v). Yield: 16%, R_f 0.30 (petroleum ether/EtOAc 3:2 v/v); mp159–161 °C; ^{1}H NMR (DMSO-d_6): δ 8.75 (d, J = 2.3 Hz, H-4), 8.36 (dd, J = 2.4, 9.1 Hz, H-6), 7.99 (d, J = 9.1 Hz, 1H, H-7), 7.79 (s, 1H, H-3), 7.48–7.36 (m, 5H, Ar), 5.89 (dd, $J_{x,a}$ = 7.8 Hz, $J_{x,b}$ = 10.0 Hz, 1H, CH_x), 4.55 (dd, $J_{b,x}$ = 10.0 Hz, $J_{b,a}$ = 15.5 Hz, 1H, CH_b), 3.96 (dd, $J_{a,x}$ = 7.8 Hz, $J_{a,b}$ = 15.5 Hz, 1H, CH_a). ^{13}C NMR (DMSO-d_6): δ 157.9, 155.0, 147.1, 144.6, 140.6 (5 × C), 129.2 (CH), 129.2 (CH), 128.8 (CH), 128.0 (C), 126.3, 126.3, 122.6, 119.6, 113.3, 112.0, 81.2 (7 × CH), 62.9 (CH_2). Anal. Calcd for $C_{17}H_{12}N_2O_4$ (308.91): C, 66.23; H, 3.92; N, 9.08. Found: C, 66.09; H, 3.89; N, 9.06.

5.1.4.4. 2-Benzo[*b*]furan-2-yl-5-(4-fluorophenyl)-4,5-dihydro-1,3-oxazole (6d). A yellow solid was obtained after recrystallisation from ethanol. Yield: 66%, R_f 0.74 (petroleum ether/EtOAc 3:2 v/v); mp 88–90 °C; ^{1}H NMR (DMSO-d_6): δ 7.78 (d, J = 7.8 Hz, 1H, Ar), 7.72 (d, J = 8.3 Hz, 1H, Ar), 7.59 (s, 1H, Ar), 7.52–7.45 (m, 3H, Ar), 7.39–7.33 (m, 1H, Ar), 7.27 (t, J = 8.9 Hz, 2H), 5.86 (dd, $J_{x,a}$ = 7.8 Hz, $J_{x,b}$ = 9.9 Hz, 1H, CH_x), 4.50 (dd, $J_{b,x}$ = 9.9 Hz, $J_{b,a}$ = 15.3 Hz, 1H, CH_b), 3.92 (dd, $J_{a,x}$ = 7.8 Hz, $J_{a,b}$ = 15.3 Hz, 1H, CH_a). ^{13}C NMR (DMSO-d_6): δ 164.0, 160.7, 155.6, 155.3, 144.2, 137.2, 137.1 (7 × C), 128.6 (CH), 128.6 (CH), 128.5 (CH), 128.5 (CH), 127.4 (C), 127.3, 124.2, 122.9, 116.2, 116.2, 115.9, 115.9, 112.1, 111.3, 80.2 (10 × CH), 62.9 (CH_2). Anal. Calcd for $C_{17}H_{12}FNO_2$ (281.285): C, 72.59; H, 4.30; N, 4.98. Found: C, 72.40; H, 4.28; N, 4.90.

5.1.4.5. 2-Benzo[*b*]furan-2-yl-5-(4-chlorophenyl)-4,5-dihydro-1,3-oxazole (6e). A light yellow solid was obtained by recrystallisation from ethanol. Yield: 65%, R_f 0.62 (petroleum ether/EtOAc 3:2 v/v); mp 103–105 °C; ^{1}H NMR (DMSO-d_6): δ 7.70 (d, J = 7.7 Hz, 1H, Ar), 7.74 (d, J = 8.3 Hz, 1H, Ar), 7.62 (s, 1H, Ar), 7.54–7.36 (m, 6H, Ar), 5.88 (dd, $J_{x,a}$ = 7.5 Hz, $J_{x,b}$ = 10.0 Hz, 1H, CH_x), 4.53 (dd, $J_{b,x}$ = 10.0 Hz, $J_{b,a}$ = 15.3 Hz, 1H, CH_b), 3.92 (dd, $J_{a,x}$ = 7.5 Hz, $J_{a,b}$ = 15.3 Hz, 1H, CH_a). ^{13}C NMR (DMSO-d_6): δ 155.6, 155.3, 144.1, 140.0, 133.3 (5 × C), 129.2 (CH), 129.2 (CH), 128.1 (CH), 128.1 (CH), 127.4 (C), 127.3, 124.2, 123.0, 112.1, 111.3, 80.0 (6 × CH), 62.9 (CH_2). Anal. Calcd for $C_{17}H_{12}ClNO_2$ (297.740): C, 68.58; H, 4.06; N, 4.70. Found: C, 68.56; H, 3.97; N, 4.72.

5.1.4.6. 2-(4-Bromophenyl)-5-phenyl-4,5-dihydro-1,3-oxazole (10a). A yellow syrup was obtained. Yield: 82%, R_f 0.8 (petroleum ether/EtOAc 1:1 v/v); ^{1}H NMR (DMSO-d_6): δ 7.91 (d, J = 8.5 Hz, 2H, Ar), 7.80 (d, J = 6.8 Hz, 2H, Ar), 7.43 (m, 5H, Ar), 5.83 (dd, $J_{x,a}$ = 7.5 Hz, $J_{x,b}$ = 10.1 Hz, 1H, CH_x), 4.50 (dd, $J_{b,x}$ = 10.1 Hz, $J_{b,a}$ = 15.1 Hz, 1H, CH_b), 3.88 (dd, $J_{a,x}$ = 7.5 Hz, $J_{a,b}$ = 15.1 Hz, 1H, CH_a). ^{13}C NMR (DMSO-d_6): δ 161.7 (C), 141.0 (C), 131.8, 131.8, 129.8, 129.8, 128.8, 128.8, 128.2 (7 × CH), 126.5 (C), 125.6 (CH), 125.6 (CH), 125.3 (C), 80.4 (CH), 62.5 (CH_2). Anal. Calcd for $C_{18}H_{16}BrN_3O{\cdot}0.3H_2O$ (375.65): C, 57.55; H, 4.45; N, 11.19. Found: C, 57.38; H, 4.42; N, 11.22.

5.1.4.7. 2-(4-Benzylphenyl)-5-phenyl-4,5-dihydro-1,3-oxazole (10b). A yellow syrup was obtained. Yield: 80%, R_f 0.8 (petroleum ether/EtOAc 1:1 v/v); ^{1}H NMR (DMSO-d_6): δ 7.83 (d, J = 7.9 Hz, 1H, Ar), 7.48 (dd, J = 1.4, 7.6 Hz, 1H, Ar), 7.36–7.19 (m, 15H, Ar), 5.68 (dd, $J_{x,a}$ = 7.8 Hz, $J_{x,b}$ = 10.2 Hz, 1H, CH_x), 4.50 (d, J = 14.5 Hz, 2H, Ar-CH_2-Ar), 4.47 (dd, $J_{b,x}$ = 10.2 Hz, $J_{b,a}$ = 14.9 Hz, 1H, CH_b), 3.85 (dd, $J_{a,x}$ = 7.8 Hz, $J_{a,b}$ = 14.9 Hz, 1H, CH_a). ^{13}C NMR (DMSO-d_6): δ 162.5 (C), 141.2 (C), 141.2 (C), 141.1 (C), 131.2, 130.8, 129.7, 128.9, 128.7, 128.7, 128.5, 128.2, 128.1, 128.0 (10 × CH), 126.8 (C), 126.4, 126.3, 125.8, 125.7, 79.2 (5 × CH), 63.1 (CH_2), 38.5 (CH_2). Anal. Calcd for $C_{25}H_{23}N_3O{\cdot}0.1H_2O$ (383.28): C, 78.34; H, 6.10; N, 10.96. Found: C, 78.39; H, 6.12; N, 11.10.

5.1.4.8. (*E*)-5-Phenyl-2-(4-styrylphenyl)-4,5-dihydro-1,3-oxazole (10c). A white solid was obtained by recrystallisation from ethanol. Yield: 61%, R_f 0.57 (petroleum ether/EtOAc 1:1 v/v); mp 126–127 °C; ^{1}H NMR (DMSO-d_6): δ 7.97 (d, J = 8.3 Hz, 2H, Ar), 7.6 (d, J = 8.3 Hz, 2H, Ar), 7.67 (d, J = 7.5 Hz, 2H, Ar), 7.46–7.33 (m, 10H, Ar), 5.80 (dd, $J_{x,a}$ = 7.7 Hz, $J_{x,b}$ = 9.9 Hz, 1H, CH_x), 4.48 (dd, $J_{b,x}$ = 10.1 Hz, $J_{b,a}$ = 14.9 Hz, 1H, CH_b), 3.85 (dd, $J_{a,x}$ = 7.5 Hz, $J_{a,b}$ = 14.9 Hz, 1H, CH_a). ^{13}C NMR (DMSO-d_6): δ 162.2 (C), 141.3 (C), 140.1 (C), 136.7 (C), 130.4, 128.7, 128.7, 128.7, 128.2, 128.2, 128.1, 128.05, 127.5, 127.5, 126.7, 126.7, 126.6, 126.6, 125.6, 125.6 (16 × CH, Ar), 126.2 (C), 80.1 (CH), 62.6 (CH_2). Anal. Calcd for $C_{23}H_{19}NO$ (325.40): C, 84.89; H, 5.88; N, 4.30. Found: C, 84.70; H, 5.94; N, 4.11.

5.1.5. General procedure for the synthesis of *N*2-(2-phenyl-2-(1*H*-1-imidazoyl)ethyl)-arylamide derivatives (7) and (11)

A mixture of oxazole (**6**) or (**10**) (1.25 mmol) and imidazole (25 mmol) dissolved in isopropyl acetate (3 mL) was heated at 125 °C for 24 h. After completion of the reaction the mixture was partitioned between water (100 mL) and ethyl acetate (150 mL). The organic layer was washed three times with water (3 × 100 mL), dried over $MgSO_4$ and concentrated in vacuo.

5.1.5.1. *N*2-(2-Phenyl-2-(1*H*-1-imidazoyl)ethyl)-benzo[*b*]furan-2-carboxamide (7a). A white solid was obtained after recrystallisation from ethanol. Yield: 28%, R_f 0.74 (CH_2Cl_2/MeOH 9:1 v/v); mp 180–182 °C; ^{1}H NMR (DMSO-d_6): δ 9.04 (t, J = 5.4 Hz, 1H, NH), 7.90 (s, 1H, imid), 7.79 (d, J = 7.7 Hz, 1H, Ar), 7.66 (d, J = 8.3 Hz, 1H, Ar), 7.56 (s, 1H, H-3), 7.52–7.33 (m, 8H, Ar and imid), 6.95 (s, 1H, H-imidazole), 5.75 (dd, J = 5.6, 9.3 Hz, 1H, H-1), 4.23–3.98 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6): δ 158.8 (C═O), 154.5 (C), 149.0 (C), 139.5 (CH), 137.1 (C), 129.1, 129.1, 128.9, 128.5 (4 × CH), 127.4 (C), 127.3, 127.2, 127.2, 124.1, 123.2, 118.7, 112.1, 110.2, 59.8 (9 × CH), 43.2 (CH_2). LRMS (ES^+) m/z: 332.13 $(M+H)^+$. Anal. Calcd for $C_{20}H_{17}N_3O_2{\cdot}0.4H_2O$ (338.579): C, 70.95; H, 5.30; N, 12.41. Found: C, 71.08; H, 5.40; N, 12.21.

5.1.5.2. *N*2-[2-Phenyl-2-(1*H*-1-imidazoyl)ethyl]-6-methoxybenzo[*b*]furan-2-carboxamide (7b). A brown solid was obtained after recrystallisation from ethanol/water (2:1 v/v). Yield: 66%, R_f 0.63 (CH_2Cl_2/MeOH 9:1 v/v); mp 110–112 °C; ^{1}H NMR (DMSO-d_6): δ 8.83 (t, J = 5.5 Hz, 1H, NH), 7.87 (s, 1H, imid), 7.62 (d, J = 8.6 Hz, 1H, Ar), 7.45 (s, 1H, H-3), 7.38–7.29 (m, 6H, Ar and imid), 7.16 (d, J = 1.8 Hz, 1H), 6.96 (dd, J = 1.8, 8.7 Hz, 1H, Ar), 6.92 (s, 1H, imid), 5.70 (dd, J = 5.7, 9.4 Hz, 1H, H-1), 4.17–3.94 (m, 2H, CH_2), 3.82 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 158.8 (C═O), 154.5 (C), 149.0 (C), 139.5 (CH), 137.14 (C), 129.1, 129.1, 128.9, 128.5 (4 × CH), 127.4 (C), 127.3, 127.2, 127.2, 124.1, 123.2,

118.7, 112.1, 110.2, 59.8 (9 × CH), 43.24 (CH_2). Anal. Calcd for $C_{21}H_{19}N_3O_3 \cdot 0.1H_2O$ (363.201): C, 69.45; H, 5.33; N, 11.60. Found: C, 69.43; H, 5.41; N, 11.54.

5.1.5.3. *N*2-[2-Phenyl-2-(1*H*-1-imidazoyl)ethyl)-5-nitrobenzo[*b*]furan-2-carboxamide (7c). A brown solid was obtained after recrystallisation from ethanol/water (2:1 v/v). Further purification by flash column chromatography (CH_2Cl_2/MeOH 100:0 v/v increasing to 95:5 v/v) gave **7c** as a white solid. Yield: 33%, R_f 0.23 (CH_2Cl_2/MeOH 9:1 v/v); mp 204–206 °C; ^{1}H NMR (DMSO-d_6): δ 9.23 (t, J = 5.6 Hz, 1H, NH), 8.79 (d, J = 2.4 Hz, 1H, Ar), 8.33 (dd, J = 2.4, 9.1 Hz, 1H, Ar), 7.90 (d, J = 8.6 Hz, 2H, Ar), 7.75 (s, 1H, imid), 7.42–7.33 (m, 6H, Ar and imid), 6.93 (s, 1H, imid), 5.71 (dd, J = 5.6, 9.3 Hz, 1H, Ar), 4.22–3.98 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6): δ 158.1 (C=O), 157.2, 151.7, 144.6, 139.4 (4 × C), 137.2, 129.1, 129.1, 129.0, 129.0, 128.5 (6 × CH), 128.0 (C), 127.1, 122.6, 120.0, 118.7, 113.3, 111.0, 59.8 (7 × CH), 43.35 (CH_2). Anal. Calcd for $C_{20}H_{16}N_4O_4 \cdot 0.3H_2O$ (381.775): C, 62.92; H, 4.38; N, 14.68. Found: C, 62.72; H, 4.13; N, 14.50.

5.1.5.4. *N*2-[2-(4-Fluorophenyl)-2-(1*H*-1-imidazoyl)ethyl]-benzo[*b*]furan-2-carboxamide (7d). A brown solid was obtained after recrystallisation from ethanol/water (2:1 v/v). Yield: 73%, R_f 0.38 (CH_2Cl_2/MeOH 9:1 v/v); mp 198–200 °C; ^{1}H NMR (DMSO-d_6): δ 9.00 (t, J = 5.5 Hz, 1H, NH), 7.88 (s, 1H, imid), 7.78 (d, J = 7.7 Hz, 1H, Ar), 7.54 (s, 1H, Ar), 7.65 (d, J = 8.3 Hz, 1H, Ar), 7.48 (m, 3H, Ar), 7.35 (m, 2H, Ar), 7.23 (t, J = 8.8 Hz, 2H, Ar), 6.93 (s, 1H, imid), 5.73 (dd, J = 6.2, 8.9 Hz, 1H, H-1), 4.18–3.96 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6): δ 163.8 (C=O), 160.5, 158.8, 154.6, 149.0 (4 × C), 137.1 (CH), 135.8 (C), 135.5 (C), 129.5 (CH), 129.4 (CH), 129.0 (CH), 127.4 (C), 127.3, 124.1, 123.2, 118.6, 116.1, 115.8, 112.1, 110.3, 59.1 (9 × CH), 43.20 (CH_2). Anal. Calcd for $C_{20}H_{16}FN_3O_2$ (349.363): C, 68.76; H, 4.62; N, 12.02. Found: C, 68.56; H, 4.62; N, 12.11.

5.1.5.5. *N*2-[2-(4-Chlorophenyl)-2-(1*H*-1-imidazoyl)ethyl]-benzo[*b*]furan-2-carboxamide (7e). A brown residue was obtained after recrystallisation from ethanol/water (2:1 v/v). Further purification by flash column chromatography (dichloromethane/methanol 100:0 v/v increasing to 96:4 v/v) gave a light yellow solid. Yield: 72%, R_f 0.16 (petroleum ether/EtOAc 3:2 v/v); mp 76–78 °C; ^{1}H NMR (DMSO-d_6): δ 9.00 (t, J = 5.4 Hz, 1H, NH), 7.88 (s, 1H, imid), 7.76 (d, J = 7.7 Hz, 1H, Ar), 7.63 (d, J = 8.3 Hz, 1H, Ar), 7.53 (s, 1H, Ar), 7.49–7.31 (m, 7H, Ar and imid), 6.91 (s, 1H, imid), 5.73 (dd, J = 6.2, 8.8 Hz, 1H, H-1), 4.16–3.95 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6): δ 158.8 (C=O), 154.6 (C), 149.0 (C), 138.5 (C), 137.2 (CH), 133.2 (C), 129.2, 129.2, 129.1, 129.1 (4 × CH), 127.4 (C), 127.3, 124.1, 123.2, 123.2, 118.7, 112.1, 110.3 (7 × CH, Ar), 59.1 (CH), 43.10 (CH_2). Anal. Calcd for $C_{20}H_{16}ClN_3O_2 \cdot 0.2H_2O$ (369.421): C, 65.03; H, 4.47; N, 11.37. Found: C, 64.93; H, 4.35; N, 11.36.

5.1.5.6. 4-Bromo-*N*-(2-(1*H*-imidazol-1-yl)-2-phenylethyl)benzamide (11a). A pale yellow solid was obtained after recrystallisation from ethanol. Yield: 41%, R_f 0.5 (CH_2Cl_2/MeOH 9:1 v/v); mp 180–181 °C; ^{1}H NMR (DMSO-d_6): δ 8.86 (t, J = 5.4 Hz, 1H, NH), 7.87 (s, 1H, imid), 7.74 (m, 4H, Ar), 7.38 (m, 6H, Ar), 6.95 (s, 1H, imid), 5.70 (dd, J = 9.3 Hz, 1H, CH_x), 4.11 (m, 1H, CH_b), 4.01 (m, 1H, CH_a). ^{13}C NMR (DMSO-d_6): δ 165.79 (C=O), 139.18 (C), 133.16 (C), 125.08 (C), 136.75, 131.31, 129.26, 128.69, 128.52, 128.06, 126.92, 119.60, 118.30 (9 × CH, Ar), 59.34 (CH), 43.48 (CH_2). Anal. Calcd for $C_{18}H_{16}BrN_3O$ (370.24): C, 58.39; H, 4.36; N, 11.34. Found: C, 58.67; H, 4.36; N, 11.56.

5.1.5.7. 4-Benzyl-*N*-(2-(1*H*-imidazol-1-yl)-2-phenylethyl)benzamide (11b). A pale brown solid was obtained after recrystallisation from ethanol. Yield: 40%, R_f 0.55 (CH_2Cl_2/MeOH 9:1 v/v); mp 40–42 °C; ^{1}H NMR (DMSO-d_6): δ 8.67 (t, J = 5.5 Hz, 1H, NH), 7.88 (s, 1H, imid), 7.06–7.88 (m, 15H, Ar), 6.95 (s, 1H, Ar), 5.67 (dd, $J_{x,b}$ = 9.3 Hz, $J_{x,a}$ = 6.0 Hz, 1H, CH_x), 4.09 (m, 1H, CH_b), 4.01 (m, 3H, Ar-CH_2-Ar and CH_a). ^{13}C NMR (DMSO-d_6): δ 169.46 (C=O), 140.90 (C), 139.15 (C), 138.97 (C), 136.40 (C), 136.75, 130.05, 129.50, 128.94, 128.87, 128.67, 128.52, 128.19, 128.17, 128.06, 127.19, 126.9, 126.9, 125.8, 125.7, 125.3, 118.3 (17 × CH, Ar), 59.39 (CH), 43.02 (CH_2), 30.6 (CH_2). Anal. Calcd for $C_{25}H_{23}N_3O$ (381.48): C, 78.71; H, 6.08; N, 11.02. Found: C, 78.91; H, 6.13; N, 11.20.

5.1.5.8. (*E*)-4-*N*-(2-(1*H*-imidazol-1-yl)-2-phenylethyl)-4-styrylbenzamide (11c). Purification by flash column chromatography (CH_2Cl_2/MeOH 95:5 v/v) gave a white solid. Yield: 16%, R_f 0.40 (CH_2Cl_2/MeOH 9:1 v/v); mp 190–192 °C; ^{1}H NMR (DMSO-d_6): δ 8.77 (s, 1H, NH), 7.89 (s, 1H, imid), 7.81 (d, J = 8.0 Hz, 2H, Ar), 7.71 (d, J = 8.0 Hz, 2H, Ar), 7.66 (d, J = 7.4 Hz, 2H, Ar), 7.43–7.32 (m, 11H, Ar), 6.96 (s, 1H, imid), 5.67 (dd, $J_{x,b}$ = 8.7 Hz, $J_{x,a}$ = 5.8 Hz, 1H, CH_x), 4.12 (m, 1H, CH_b), 4.03 (m, 1H, CH_a). ^{13}C NMR (DMSO-d_6): δ 166.3 (C=O), 140.0, 139.3, 136.7, 132.8 (4 × C), 130.3, 128.7, 128.7, 128.6, 128.5, 128.5, 128.1, 128.0, 128.0, 127.6, 127.6, 127.4, 127.4, 126.8, 126.8, 126.7, 126.7, 126.2, 126.2 (19 × CH, Ar), 59.4 (CH), 43.5 (CH_2). Anal. Calcd for $C_{26}H_{23}N_3O$ (393.48): C, 79.36; H, 5.89; N, 10.67. Found: C, 79.10; H, 5.80; N, 10.43.

5.2. Materials and methods: CYP24A1 assay

A recombinant cell line expressing human CYP24A1 enzyme (V79-CYP24) was used to measure the inhibitory properties of imidazoles **7** and **11** on the enzyme activity as described before.[18,22] Briefly, the inhibitors were dissolved in 100% ethanol or 100% DMSO to form a 0.02 M stock solution. All stock solutions were diluted with 100% ethanol to obtain the range of inhibitor solutions. Ketoconazole (Sigma) was weighed in a darkened room immediately prior to incubation and dissolved in 0.05 M HCl to form a 1.9 mM solution, which was diluted to 190 μM using 0.05 M HCl.

Each millilitre of incubation media contained 1 or 2 μL *N*′,*N*-diphenyl-*p*-phenylenediamine (DPPD) (Sigma), substrate, 5 μL of inhibitor or carrier or enough 190 μM ketoconazole to give the correct final concentration, and the same medium used for cell culture of that cell type but containing 1% BSA (Boehringer Mannheim, Laval, PQ, Canada) instead of FCS. The substrate for CYP24A1 cells was 250,000 cpm of [1β-3*H*]1α,25-$(OH)_2D_3$ dissolved in ethanol per mL incubation media. Dead cell controls were prepared by microwaving plates for 3 min. A 1 mL volume of incubation media was used for each well of a 6-well plate, whereas 2 mL of incubation media was used for each 60-mm plate. All conditions were conducted in triplicate.

The reaction was terminated by the addition of 500 μL of methanol and transferred to a glass tube. The aqueous phase was extracted by standard Bligh–Dyer extraction in which we substituted dichloromethane for chloroform.[23] Samples were then spun at 4000 rpm for 5 min. Triplicate 100 μL aliquots of aqueous fraction containing water-soluble CYP24A1 products were mixed with 600 μL of scintillation fluid and the radioactivity was measured by use of a scintillation counter. All values were normalized for background.

The log of the inhibitor concentration was plotted versus the quantification property using GraphPad Prism and the curve was analysed using sigmoidal dose–response analysis to calculate an IC_{50} value for the inhibitor.

5.3. Molecular docking

All molecular modelling studies were performed on a MacPro dual 2.66 GHz Xeon running Ubuntu 8. Docking simulations were performed using PLANTS[24] and the ligands were built with Molec-

ular Operating Environment (MOE).[25] The docking results were visualized with MOE.

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