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Bioorganic & Medicinal Chemistry 16 (2008) 8301–8313
Bioorganic & Medicinal Chemistry

Novel azolyl-(phenylmethyl)]aryl/heteroarylamines: Potent CYP26 inhibitors and enhancers of all-*trans* retinoic acid activity in neuroblastoma cells

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Received 30 March 2007; revised 19 June 2007; accepted 26 June 2007
Available online 29 June 2007

Abstract—The synthesis and potent inhibitory activity of novel 4-[(imidazol-1-yl and triazol-1-yl)(phenyl)methyl]aryl-and heteroaryl amines versus a MCF-7 CYP26A1 cell assay is described. Biaryl imidazole ([4-(imidazol-1-yl-phenyl-methyl)-phenyl]-naphthalen-2-yl-amine (**8**), IC_{50} = 0.5 μM; [4-(imidazol-1-yl-phenyl-methyl)-phenyl]-indan-5-yl-amine (**9**), IC_{50} = 1.0 μM) and heteroaryl imidazole derivatives ((1*H*-benzoimidazol-2-yl)-{4-[(5*H*-imidazol-1-yl)-phenyl-methyl]-phenyl}-amine (**15**), IC_{50} = 2.5 μM; benzooxazol-2-yl-{4-[(5*H*-imidazol-1-yl)-phenyl-methyl]-phenyl}-amine (**16**), IC_{50} = 0.9 μM; benzothiazol-2-yl-{4-[(5*H*-imidazol-1-yl)-phenyl-methyl]-phenyl}-amine (**17**), IC_{50} = 1.5 μM) were the most potent CYP26 inhibitors. Using a CYP26A1 homology model differences in activity were investigated. Incubation of SH-SY5Y human neuroblastoma cells with the imidazole aryl derivative **8**, and the imidazole heteroaryl derivatives **16** and **17** potentiated the atRA-induced expression of CYP26B1. These data suggest that further structure–function studies leading to clinical development are warranted.

1. Introduction

Retinoic acid (RA) is a naturally occurring retinoid responsible for growth and differentiation of mammalian epithelial tissues[1] which exerts activity by binding to transcription-regulatory factors in the cell nucleus known as RAR (retinoic acid receptor) and RXR (retinoid X receptor), each having subtypes α, β and γ.[2] RA controls transcription by binding to RAR/RXR dimers, which locally modifies chromatin structure (Fig. 1). Synthesis and degradation of RAR and RXR by proteasomes control amount and timing of the retinoid responses and also modulates phosphorylations.[3]

The main function of RA is to modulate gene transcription by liganding nuclear receptors that bind to their DNA response element motifs in promoters of target genes.[4] RXRs are the heterodimeric partners of approximately 1/3 of the human members of the nuclear hormone receptor subfamily,[5] for example, the nuclear receptors VDR and TR, and the orphan nuclear receptors PPAR, LXR, FXR and NURR1, many of these heterodimers are activated by RXR agonists including the endogenous ligand 9-*cis*-RA (Fig. 1). As such RA (all-*trans*-RA and 9-*cis*-RA) has been used in a number of clinical situations, especially oncology (acute promyelocytic leukaemia[6,7]) and dermatology (acne, psoriasis[8,9]). RA may improve the efficacy of other treatments such as radiation, cisplatin and interferon therapies.[10,11] Biochemical results also support lower retinoid supply, synthesis, impaired transport, and hypofunction as contributing factors to late onset Alzheimer's disease (LOAD)[12] suggesting that increasing endogenous levels of RA may modify memory performance via the promotion of both adult neurogenesis and adult synaptic plasticity.[13]

Abbreviations: atRA, all-*trans*-retinoic acid; RA, retinoic acid; RAMBAs, retinoic acid metabolism blocking agents.

Keywords: 4-[(Imidazol-1-yl and triazol-1-yl)(phenyl)methyl]aryl- and heteroaryl amines; IC_{50} enzyme inhibition; Retinoic acid metabolism blocking agents (RAMBAs); MCF-7; CYP26A1; Molecular modelling; Neuroblastoma.

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doi:10.1016/j.bmc.2007.06.048

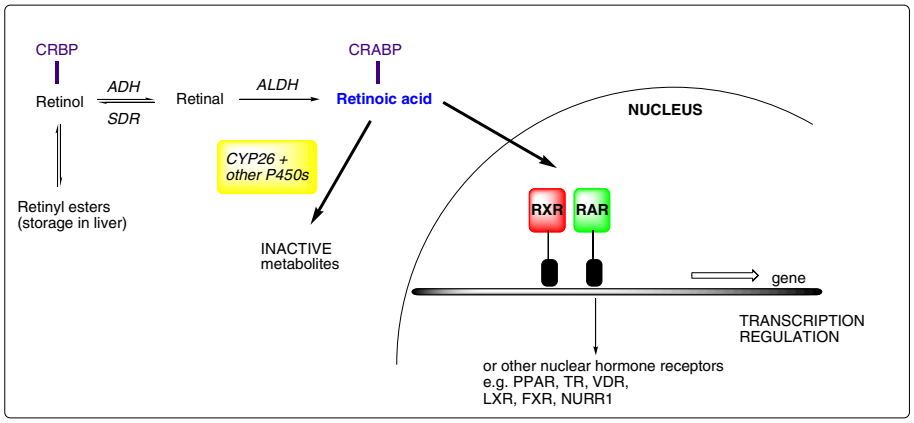


Figure 1. Biosynthetic pathway of retinol and receptor activation by retinoic acid. Alcohol dehydrogenases (ADH); short-chain dehydrogenase/reductase (SDR); aldehyde dehydrogenases (ALDH); cellular retinol-binding protein (CRBP); cellular retinoic acid-binding protein (CRABP); retinoic acid receptor (RAR); retinoid X receptor (RXR); peroxisome-proliferator-activator receptors (PPARs), thyroid hormone receptor (TRs), vitamin D receptor (VDR), liver X receptors (LXRs), farnesoid X receptors (FXRs) and NURR1.

atRA has a short half life and its potency is reduced when administered systemically, owing to metabolism by several human liver and intestine cytochrome P450s to the inactive 4-hydroxy-RA and then by dehydrogenases to the partially active 4-keto-RA and inactive polar metabolites.[14] Indeed, a relationship between induction of atRA metabolism and drug resistance has been demonstrated in acute promyelocytic leukaemia patients.[15] In living tissues, all-*trans*-RA administration induces another enzyme CYP26[16] which recognises only RA as its substrate, and the expression of this isozyme can be induced by atRA both in vitro and in vivo. Three members of the CYP26 family have now been identified: CYP26A1[16] and CYP26B1,[17] which metabolise atRA in the embryo and adult and, more recently,[18] CYP26C1 that may have a role in the specific metabolism of both all-*trans* and 9-*cis* isomers of RA. The dose-dependent induction of CYP26 mRNA has been used as a sensitive marker of retinoid response: in SH-SY5Y neuroblastoma cells CYP26 mRNA is normally undetectable but treatment with atRA results in the dose-dependent induction of CYP26A1 and CYP26B1 mRNA and increased metabolism of atRA within 24 h.[19]

An inhibitor of the metabolism of endogenous RA would be expected to have a beneficial effect on epithelial differentiation and proliferation as a RA-mimetic, with considerable therapeutic potential. A number of retinoic acid metabolism blocking agents (RAMBAs) have been described (Fig. 2) and recently reviewed by Njar et al.[20] The Janssen triazoles R115866[21] and R116010[22] are potent inhibitors of retinoic acid metabolism, and R115866, in vivo in rats after a single oral dose, increases endogenous tissue RA levels and mimics RA in several other of its biological actions.[21] Selective naphthyl derivatives have also been described with IC_{50} = 3.3 nM for the most potent compound (OSI Pharma).[23] The majority of CYP26 inhibitors contain an azolyl group, either an imidazole or triazole heterocycle, which coordinates with the Fe^{3+} of the porphyrin haem. Non-azoyl CYP26 inhibitors have also been described such as the tetralone derivatives,[24] designed using a computer generated homology model of CYP26A1.[25]

We have recently described a series of benzofuran-2-yl-[(4-alkyl/aryl-phenyl)methyl]triazole derivatives with modest CYP26 inhibitory activity (Fig. 2).[26] Using SAR derived from this series with the amine moiety common in the very potent CYP26 inhibitors described by Janssen and OSI Pharma, a series of novel 4-[(imidazol-1-yl and triazol-1-yl)(phenyl)methyl]aryl- and heteroaryl amines (Fig. 2) were designed, using our CYP26A1 model,[25] and synthesized. These compounds were evaluated for their ability to inhibit CYP26 activity and their ability to enhance the biological activity of atRA in inducing gene expression in human SH-SY5Y neuroblastoma cells.

2. Chemistry

The *N*-aryl substituted triazol- and imidazol-1-ylmethylphenylamines were prepared via a three step procedure involving a Suzuki coupling to give the 4-aminophenyl ketones (**1**) via route A or B (Scheme 1), subsequent reduction to the alcohols (**2**) and finally addition of the aza ring (triazole (**3**–**4**) or imidazole (**5**–**9**)).

The *N*-arylation using the Suzuki reaction followed described methodology[27,28] employing a stoichiometric amount of copper and a tertiary amine base, pyridine in this reaction series. The reaction was performed in air allowing oxygen uptake and therefore more efficient oxidation of a reduced copper intermediate.[29] This was done by using vigorous stirring in flasks with a large volume relative to that of the solvent volume. This is more important during the first few hours of the reaction since it was found that the reaction proceeded smoothly to 35–45% conversion in the first 4–5 h, and catalytic activity rapidly diminished over the next 20 h.[30] The coupled products were confirmed by the presence of an NH singlet peak at approximately δ_H 5.5 (Scheme 1).

The 4-aminophenyl ketones were efficiently reduced to their corresponding **4**-aminophenylmethanol derivatives (**2**) by treatment with $NaBH_4$ in MeOH or MeOH/dioxane depending on solubility.

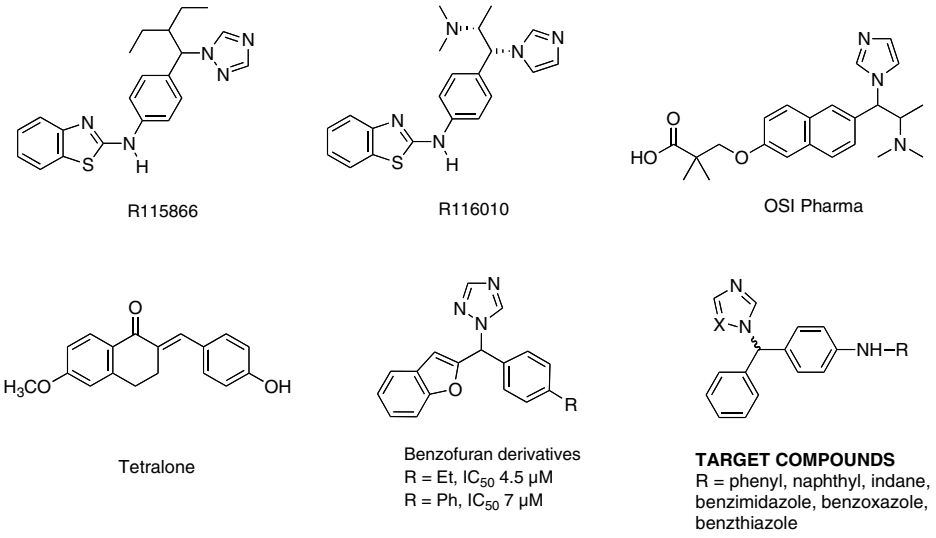
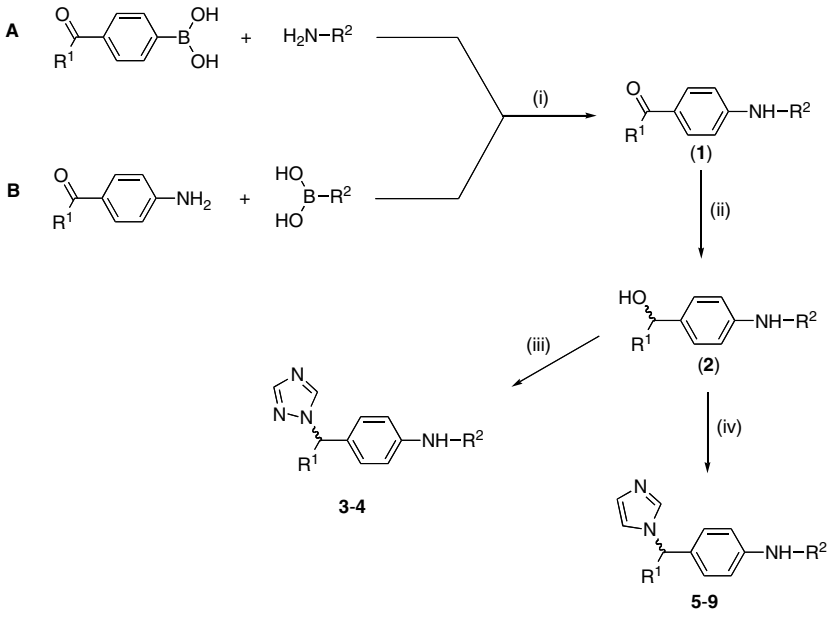


Figure 2. Retinoic acid metabolism blocking agents (RAMBAs) and target compounds.

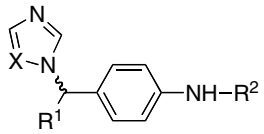


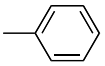
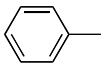
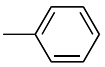
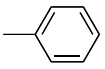
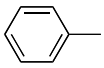
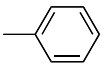
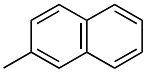
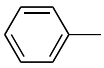
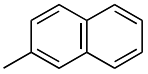
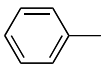
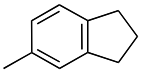
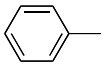
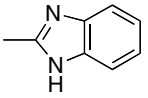
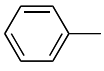
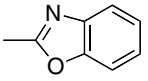
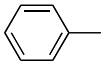
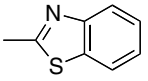
Scheme 1. Reagents and conditions: (i) CuOAc, pyridine, 4 Å molecular sieves, CH_2Cl_2, rt, 3 days; (ii) $NaBH_4$, MeOH/dioxane, rt, 1–18 h; (iii) (triazol-1-yl)$_2$SO, K_2CO_3, CH_3CN, rt, 4 days; (iv) CDI, imidazole, CH_3CN, 65 °C, 1 h.

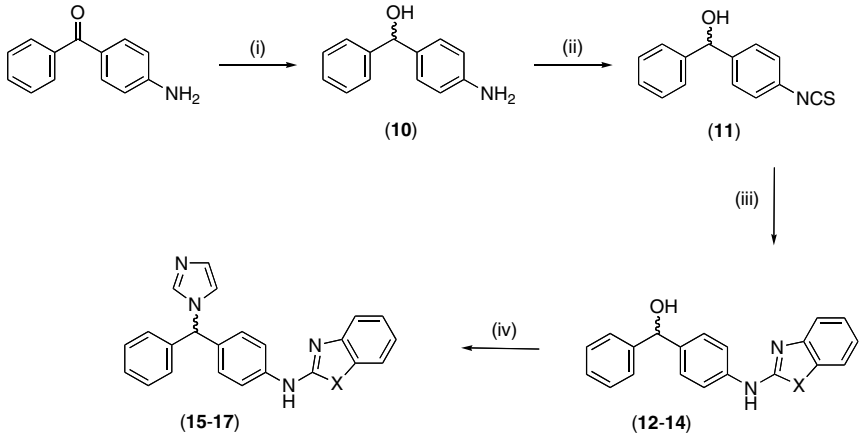
The synthesis of the triazole compounds (**3**–**4**) involved the reaction of the aminophenylmethanol derivatives (**2**) with the in situ prepared *N*,*N*′-di(1*H*-1,2,4-triazol-1-yl)sulfoxide.[31] After purification by column chromatography, the products were confirmed by ^{1}H NMR with the disappearance of the OH group at approximately δ 1.5–2.0 and the presence of the two singlet protons of the triazole at approximately δ 8.1 and 8.2. The imidazole compounds (**5**–**9**) were prepared by direct reaction of the aminophenylmethanol derivatives (**2**) with 1,1′-carbonyldiimidazole in the presence of excess imidazole as previously reported.[23,32] Therefore, the need for the in situ preparation of the reactive species *N*,*N*′-di(1*H*-imidazol-1-yl)sulfoxide was eliminated. The yields for the final triazole and imidazole derivatives (**3**–**9**) are given in Table 1.

The *N*-heteroaryl substituted imidazol-1-ylmethyl-phenylamines (**15**–**17**) were prepared via a four-step procedure in good yields (Table 1). Aminobenzophenone was reduced to the corresponding carbinol (**10**) by reaction with $NaBH_4$ in methanol, reaction of **10** with thiophosgene as described in the literature[33] produced the isothiocyanate (**11**),[34] which was then cyclized using mercury(II) oxide in the presence of a catalytic amount of sulphur[35,36] to give the cyclized products (**12**–**14**). Treatment of **12**–**14** with 1,1′-carbonyldiimidazole in the presence of excess imidazole gave the imidazole derivatives (**15**–**17**) (Scheme 2).

Table 1. Substituents, yields and mps for triazole (X = N, **3**–**4**) and imidazole (X = CH, **5**–**9**, **15**–**17**) products



Compound	R^1	R^2	Yield (%)	mp (°C)
3	CH_3		72	108–110
4			69	138–140
5	CH_3		84	110–112
6			89	208–210
7	CH_3		84	170–172
8			88	94–96
9			83	88–90
15			76	274–276
16			71	214–216
17			73	258–260



Scheme 2. Reagents and conditions: (i) $NaBH_4$, MeOH, rt, 1 h; (ii) $CSCl_2$, CH_2Cl_2, ice/H_2O, 0 °C, 2 h, refrigerator o/n; (iii) 2-substituted aminobenzene, EtOH, HgO, S (cat.), reflux, 2 h; (iv) CDI, imidazole, CH_3CN, 65 °C, 1 h.

3. CYP26A1 inhibitory activity

The imidazole and triazole derivatives were evaluated for their retinoic acid metabolism inhibitory activity using a MCF-7 cell assay,[24] using radiolabelled [11,12-^{3}H] all-*trans* retinoic acid as the substrate and liarozole (a non-selective CYP26 inhibitor[20]) and R115866 as standards for comparison. With the exception of the methyl derivatives (**3**, **5** and **7**, IC_{50} = >50, 35 and 15 μM, respectively), the novel 4-[(imidazol-1-yl and triazol-1-yl)(phenyl)methyl]aryl- and heteroaryl amines were all potent inhibitors of CYP26A1 (Table 2). The biaryl imidazole (naphthyl **8**, IC_{50} = 0.5 μM; indane **9**, IC_{50} = 1.0 μM) and bicyclic heteroaryl imidazole derivatives (benzimidazole **15**, IC_{50} = 2.5 μM; benzoxazole **16**, IC_{50} = 0.9 μM; benzothiazole **17**, IC_{50} = 1.5 μM) were the most potent CYP26 inhibitors. Considerable differences in activity were observed with $R^1 = CH_3$ (**3** and **5**, IC_{50} = 50 μM) and R^1 = Ph (**4** and **6**, IC_{50} = 5 μM). Likewise a difference in activity was observed for R^2 = Ph (**4**, IC_{50} = 5 μM) and R^2 = biaryl/biheteroaryl (e.g. **8**, IC_{50} = 0.5 μM and **15**, IC_{50} = 2.5 μM).

4. Enhancement of retinoic acid effects

The induction of CYP26B1 mRNA was used to evaluate the ability of **8**, **16** and **17** to enhance the biological effects of atRA. CYP26B1 mRNA was chosen as it is a more-responsive marker: we see a 15–20 fold induction of B1 compared with 10-fold of A1 with the same concentration of atRA.[19] None of the CYP26 inhibitors induced CYP26 when used alone. R116010 (IC_{50} 8.7 nM[22]) at a concentration of 1 μM co-incubated with 0.01 μM atRA induced CYP26B1 mRNA 5–6 fold compared with 0.01 μM atRA alone; this level of induction was comparable with treatment with a 10-fold higher of atRA (0.1 μM) (Fig. 3). Co-incubation of 0.01 μM atRA with **8**, **16** or **17** (0.1–10 μM) for 24 h also substantially increased expression of CYP26B1 compared with 0.01 μM atRA alone. The imidazole **8** at 10 μM had an activity comparable to 1 μM R116010. The bicyclic heteroaryl imidazole derivatives **16** and **17** were less effective, reaching maximal activity of 2- to 4-fold induction of CYP26B1 for **17** and **16**, respectively, over the concentration range 0.1–10 μM (Fig. 3). The order of CYP26B1 induction activity for these inhibitors at a concentration of 10 μM (inhibitor 8 > 16 > 17) correlated with the IC_{50} values determined in the MCF-7 cell assay. Compound **4** was only tested at 1 μM but at this concentration, unlike **8**, **16** and **17**, it was ineffective at increasing CYP26B1 expression in the presence of 0.01 μM atRA.

5. Discussion

In order to rationalise the results obtained, FlexX[37] docking studies of all the inhibitors were performed using a human CYP26A1 model,[25] built using the

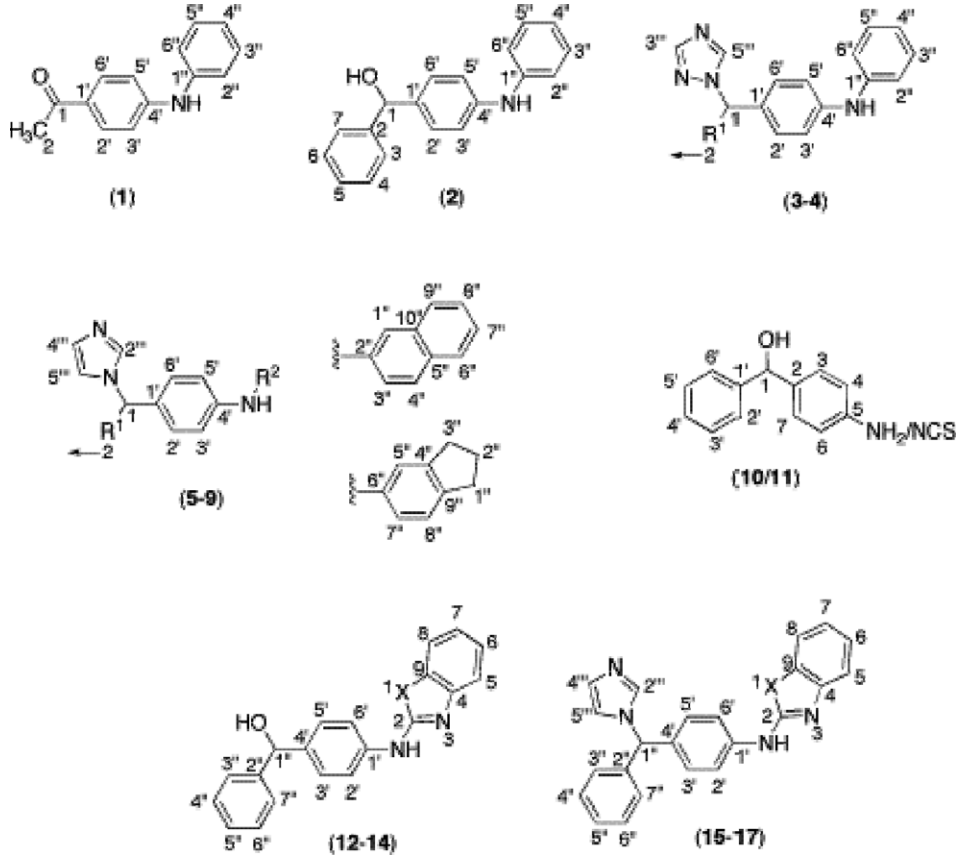
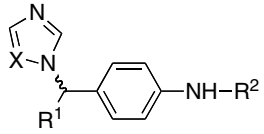
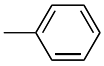
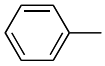
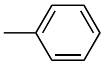
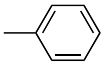
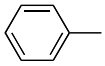
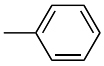
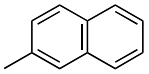
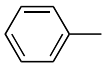
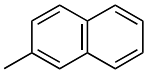
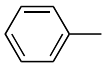
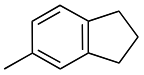
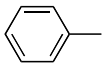
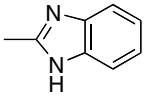
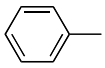
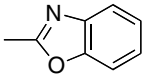
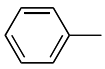
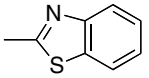


Table 2. IC_{50} data for the novel triazole (X=N, **3**–**4**) and imidazole (X=CH, **5**–**9** and **15**–**17**) products using MCF-7 assay



Compound	R^1	R^2	MCF-7 cells IC_{50}[a] (μM)
3	CH_3		>50
4			5
5	CH_3		35
6			5
7	CH_3		15
8			0.5
9			1
15			2.5
16			0.9
17			1.5
Liarozole	—	—	7
R115866	—	—	0.005

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

recently crystallised human CYP3A4 as the template. The results of the docking were only analysed visually due to the lack of correlation between the FlexX scoring functions and the visual compound–active site interaction. The position of the triazole ring from the haem was first evaluated. In the well-docked compounds, the distance between the imidazole or triazole N and the iron of the haem should be 3 Å or less thereby allowing the coordination bond to be formed. Second, the side chain R^1 and R^2 were assessed for their ability to form hydrogen and/or hydrophobic bonds with different residues at the active site.

Compounds having $R^1 = CH_3$ (**3**, **5** and **7**) are relatively small and have been found to move freely in the active site with no conformation that favours the coordination of the imidazole or the triazole N with the iron of the haem and this correlated well with

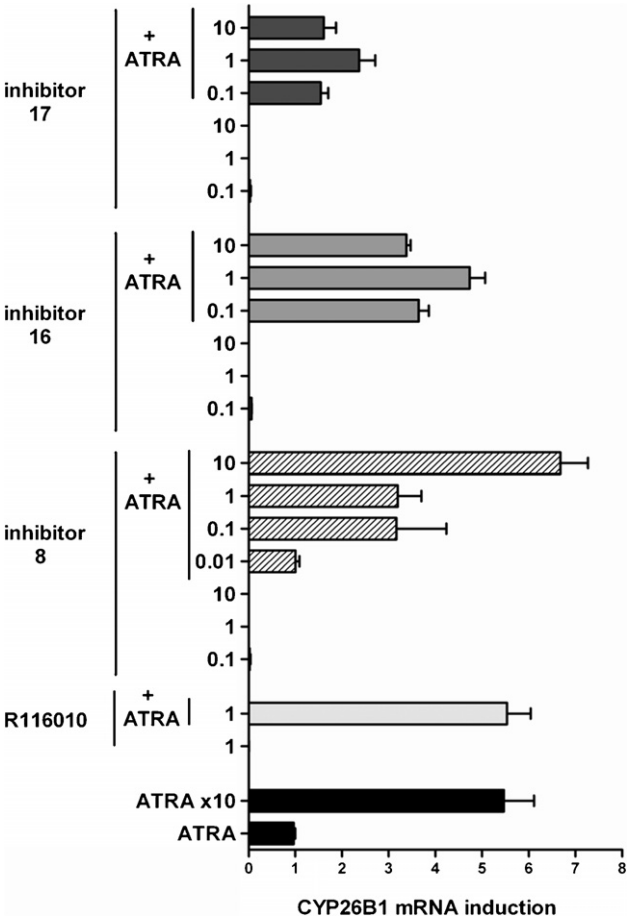


Figure 3. Real-time PCR analysis of CYP26B1 mRNA expression after treatment with atRA in combination with **8**, **16** and **17**. SH-SY5Y cells were treated with inhibitor (1 μM) either alone or in combination with atRA (0.01 μM) for 24 h. Total RNA was isolated, reverse transcribed, and subjected to real-time PCR using TaqMan probes for CYP26B1 and β-actin. Values are normalised for β-actin levels and expressed as fold increase relative to CYP26B1 expression in SH-SY5Y cells treated with 0.01 μM ATRA. Data are mean values ±S.E.M. ($n \geqslant 3$).

their IC_{50} values in the MCF7 assay. Compounds having R^1 = Ph and R^2 = biaryl/biheteroaryl (**8**, **9**, **15**, **16** and **17**) docked well in the active site establishing multiple hydrophobic interactions with different residues at the active site, in particular TRP112, PHE374, PHE84, PHE299, VAL116 and PRO371, thereby holding the inhibitors tightly in the active site resulting in greater IC_{50} values in the MCF7 assay and activity in the CYP26B1 induction assay (Fig. 4).

Compounds having R^1 = Ph and R^2 = Ph (**4** and **6**) were less active as the reduced size, compared with the biaryl/biheteroaryl derivatives, resulted in fewer hydrophobic interaction with residues at the active site. It is worth noting that only the (*S*)-conformation of these compounds docked in the active site while the (*R*)-conformation did not dock favourably, suggesting that the pure (*S*)-enantiomer may be more active than the racemic compound, this is currently under investigation.

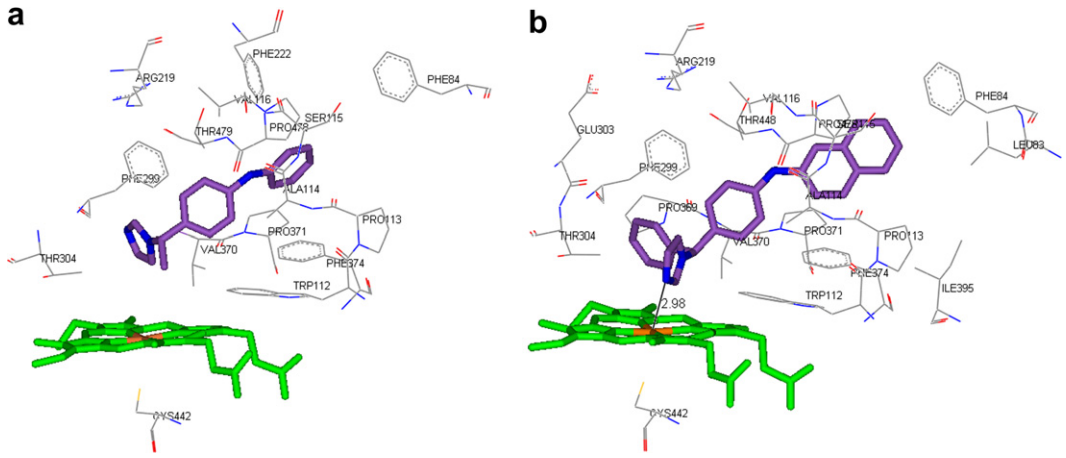


Figure 4. Active site region of CYP26A1 model showing (a) (*S*)—**5** and (b) (*S*)—**8** in stick form. Transition metal interaction with the haem (green stick form with iron colored orange) is indicated with a black line. Amino acid residues identified are involved in hydrophobic interactions.

In addition to their ability to inhibit CYP26-mediated atRA metabolism in the MCF-7 assay, compounds used in the SH-SY5Y cell assays enhanced the biological activity of exogenous atRA. The R^1 = Ph and R^2 = biaryl compound **8** had a peak activity comparable with R116010. When used at relatively low doses (0.1 μM), compound **8** and the *N*-heteroaryl-substituted benzothiazole **16** were effective at enhancing the biological activity of atRA, suggesting that in vivo studies with a view to clinical development are warranted.

6. Experimental

6.1. Materials and methods: Chemistry

[11,12-^{3}H] All *trans*-retinoic acid (37 MBq/mL) was purchased from Amersham (UK). Acetic acid, ammonium acetate and Optisafe 3 scintillation fluid were obtained from Fisher Scientific (UK). All solvents used for chromatography were HPLC grade from Fisher Scientific (UK).

^{1}H and ^{13}C NMR spectra were recorded with a Brucker Avance DPX300 spectrometer operating at 300 and 75 MHz, with Me_4Si as internal standard. Mass spectra were determined by the EPSRC mass spectrometry 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). Compounds were visualised by illumination under UV light (254 nm) or by the use of vanillin stain followed by charring on a hotplate. Melting points were determined on an electrothermal instrument and are uncorrected. All solvents were dried prior to use as described by the handbook Purification of Laboratory Chemicals[38] and stored over 4 Å molecular sieves, under nitrogen.

The numbering of compounds for ^{1}H and ^{13}C NMR characterisation is as follows:

6.1.1. General procedure for the Suzuki coupling preparation of the 4-aminophenyl ketones (1). To aryl boronic acid (30 mmol), 4-aminoacetophenone or 4-aminobenzophenone or 5-aminoindane (10 mmol), anhydrous cupric acetate (3.63 g, 20 mmol), pyridine (1.58 g, 30 mmol) and 250 mg activated 4 Å molecular sieves under an atmosphere of air was added dichloromethane (25 mL) and the reaction mixture stirred under air atmosphere at ambient temperature for 3 days. The product was isolated by direct flash column chromatography of the crude reaction mixture.

6.1.1.1. 1-(4-Phenyl)-ethanone (1a, $R^1 = CH_3$, R^2 = Ph)[27]. Purification by column chromatography (petroleum ether–ethyl acetate, 100:0 v/v increasing to 80:20 v/v) gave 1-(4-Phenylamino-phenyl)-ethanone as a pale yellow crystalline solid. Yield, 1.35 g (64%); mp 118–120 °C; R_f = 0.57 (petroleum ether–ethyl acetate, 4:1). ^{1}H NMR: δ ($CDCl_3$): 2.57 (s, 3H, H-2), 6.58 (s, 1H, H–NH), 7.03 (d, J = 8.7 Hz, 2H, Ar), 7.10 (t, J = 7.3 Hz, 1H, H-4″), 7.21 (d, J = 7.7 Hz, 2H, Ar), 7.36 (m, 2H, Ar), 7.89 (d, J = 8.7 Hz, 2H, Ar). ^{13}C NMR: δ ($CDCl_3$): 26.14 (CH_3, C-2), 114.46 (CH, C-3′, C-5′), 120.73 (CH, C-2″, C-6″), 123.40 (CH, C-4″), 129.15 (C, C-1′), 129.55 (CH, C-3″, C-5″), 130.62 (CH, C-2′, C-6′), 140.64 (C, C-1″), 148.36 (C, C-4′), 196.31 (C, C-1).

6.1.1.2. Phenyl-(4-phenylamino-phenyl)-methanone (1b, $R^1 = R^2$ = Ph). Purification by column chromatography (petroleum ether–ethyl acetate, 100:0 v/v increasing to 80:20 v/v) gave phenyl-(4-phenylamino-phenyl)-methanone as a shiny yellow crystalline solid. Yield, 1.79 g (65%); mp 140–142 °C; R_f = 0.67 (petroleum ether–ethyl acetate, 4:1). ^{1}H NMR: δ ($CDCl_3$): 6.12 (s, 1H, H–NH), 6.93 (d, J = 8.5 Hz, 2H, Ar), 7.00 (t, J = 7.3 Hz, 1H, Ar), 7.12 (d, J = 8.0, 2H, Ar), 7.26 (t, J = 7.8 Hz, 2H, Ar), 7.38 (t, J = 7.6 Hz, 2H, Ar), 7.46 (t, J = 7.3 Hz, 1H, Ar), 7.67 (d, J = 7.4 Hz, 2H, Ar), 7.69 (d, J = 8.6 Hz, 2H, Ar). ^{13}C NMR: δ ($CDCl_3$): 114.36 (CH, C-3′, C-5′), 120.72 (CH, C-2″, C-6″), 123.37 (CH, C-4″), 128.15 (CH, C-4, C-6), 128.68 (C, C-1′), 129.56 (CH, C-3′, C-5′), 129.62 (CH, C-3, C-7), 131.59 (CH, C-5), 132.76 (CH, C-2′, C-6′), 138.72 (C, C-2), 140.66 (C, C-1″), 148.24 (C, C-4′), 195.21 (C, C-1). Anal. ($C_{19}H_{15}NO$) C, H, N. Anal. Calcd for $C_{19}H_{15}NO$ (273.33): C, 83.49; H, 5.53; N, 5.12. Found: C, 83.26; H, 5.61; N, 5.04.

6.1.1.3. 1-[4-(Naphthalen-2-ylamino)-phenyl]-ethanone (1c, $R^1 = CH_3$, R^2 = naphthyl). Purification by column chromatography (petroleum ether–ethyl acetate, 100:0 v/v increasing to 80:20 v/v) gave 1-[4-(Naphthalen-2-ylamino)-phenyl]-ethanone as a yellowish brown crystalline solid. Yield, 1.65 g (63%); mp 154–156 °C; $R_f = 0.61$ (petroleum ether–ethyl acetate, 4:1). ^{1}H NMR: δ ($CDCl_3$): 2.57 (s, 3H, H-2), 6.37 (s, 1H, H–NH), 6.98 (d, $J = 8.8$ Hz, 2H, H-3′, H-5′), 7.22 (dd, $J_1 = 2.2$ Hz, $J_2 = 8.5$ Hz, 1H, H-3″), 7.3 (m, 1H, H-7″), 7.37 (m, 1H, H-8″), 7.49 (d, $J = 2.1$ Hz, 1H, H-1″), 7.62 (d, $J = 8.4$ Hz, 1H, 4″), 7.72 (t, $J = 8.7$ Hz, 2H, H-6″, H-9″), 7.81 (d, $J = 8.8$ Hz, 2H, H-2′, H-6′). ^{13}C NMR: δ ($CDCl_3$): 26.20 (CH, C-2), 114.82 (CH, C-3′, C-5′), 115.97 (CH, C-1″), 121.37 (CH, C-3″), 124.67 (CH, C-7″), 126.72 (CH, C-9″), 126.91 (CH, C-8″), 127.75 (CH, C-6″), 129.36 (C, C-5″), 129.46 (CH, C-4″), 130.26 (C, C-1′), 130.68 (CH, C-2′, C-6′), 134.30 (C, C-10″), 138.30 (C, C-2″), 148.25 (C, C-4′), 196.44 (C, C-1). Anal. Calcd for $C_{18}H_{15}NO{\cdot}0.1H_2O$ (279.33): C, 82.17; H, 5.75; N, 5.32. Found: C, 82.25; H, 5.78; N, 5.17.

6.1.1.4. [4-(Naphthalen-2-ylamino)-phenyl]-phenyl-methanone (1d, R^1 = Ph, R^2 = naphthyl). Purification by column chromatography (petroleum ether–ethyl acetate, 100:0 v/v increasing to 80:20 v/v) gave [4-(naphthalen-2-ylamino)-phenyl]-phenyl-methanone as a yellowish brown crystalline solid. Yield, 1.98 g (61%); mp 140–142 °C; $R_f = 0.69$ (petroleum ether–ethyl acetate, 4:1). ^{1}H NMR: δ ($CDCl_3$): 6.31 (s, 1H, H–NH), 7.0 (d, $J = 8.7$ Hz, 2H, H-3′, H-5′), 7.22 (dd, $J_1 = 2.2$ Hz, $J_2 = 8.5$ Hz, 1H, H-3″), 7.3 (m, 1H, H-7″), 7.37 (m, 3H, H-4, H-6, H-8″), 7.45 (m, 1H, H-2′), 7.49 (d, $J = 2.2$ Hz, 1H, H-1″), 7.62 (d, $J = 7.9$ Hz, 1H, H-4″), 7.70 (m, 6H, H-3, H-5, H-7, H-6′, H-6″, H-9″). ^{13}C NMR: δ ($CDCl_3$): 114.75 (CH, C-3′, C-5′), 115.95 (CH, C-1″), 123.39 (CH, C-3″), 124.65 (CH, C-7″), 126.71 (CH, C-9″), 126.93 (CH, C-8″), 127.75 (CH, C-6″), 128.18 (CH, C-4, C-6), 128.98 (C, C-5″), 129.45 (CH, C-4″), 129.65 (CH, C-3, C-7), 130.26 (C, C-1′), 131.66 (CH, C-5), 132.79 (CH, C-2′, C-6′), 134.32 (C, C-10″), 138.33 (C, C-2), 138.69 (C, C-2″), 148.10 (C, C-4′), 195.29 (C, C-1). Anal. Calcd for $C_{23}H_{17}NO{\cdot}0.2H_2O$ (326.99): C, 84.48; H, 5.24; N, 4.28. Found: C, 84.54; H, 5.26; N, 4.04.

6.1.1.5. [4-(Indan-5-ylamino)-phenyl]-phenyl-methanone (1e, R^1 = Ph, R^2 = indanyl). Purification by column chromatography (petroleum ether–ethyl acetate, 100:0 v/v increasing to 80:20 v/v) gave [4-(indan-5-ylamino)-phenyl]-phenyl-methanone (**13**) as a shiny yellow crystalline solid. Yield, 2.1 g (67%); mp 96–98 °C; $R_f = 0.73$ (petroleum ether–ethyl acetate, 4:1). ^{1}H NMR: δ ($CDCl_3$): 2.0 (m, 2H, H-2″), 2.82 (m, 4H, H-1″, H-3″), 6.00 (s, 1H, H–NH), 6.86 (d, $J = 8.8$, 2H, H-3′, H-5′), 6.90 (m, 1H, Ar), 7.00 (s, 1H, H-5″), 7.10 (d, $J = 8.0$ Hz, 1H, H-8″), 7.37 (m, 2H, H-4, H-6), 7.44 (m, 1H, H-5), 7.67 (m, 4H, Ar). ^{13}C NMR: δ ($CDCl_3$): 25.69 (C, C-2″), 32.36 (C, C-1″), 33.03 (C, C-3″), 113.75 (CH, C-3′, C-5′), 117.93 (CH, C-7″), 119.86 (CH, C-5″), 125.07 (CH, C-8″), 128.01 (CH, C-4, C-6), 128.01 (C, C-1′), 129.56 (CH, C-3, C-7), 131.44 (CH, C-5), 132.81 (CH, C-2′, C-6′), 138.60 (C, C-9″), 138.90 (C, C-2), 139.95 (C, C-4″), 145.82 (C, C-6″), 149.23 (C, C-4′), 195.13 (C, C-1). Anal. Calcd for $C_{22}H_{19}NO$ (313.39): C, 84.32; H, 6.11; N, 4.47. Found: C, 84.12; H, 6.08; N, 4.33.

6.1.2. General procedure of the reduction reaction for the preparation of the carbinol compounds (2) and (10). To a cooled solution of 4-aminophenyl ketones (**1**) or 4-aminobenzophenone (5 mmol) in anhydrous methanol (10 mL) or a mixture of anhydrous methanol (5 mL) and anhydrous dioxane (5 mL) was added sodium borohydride (10 mmol) and the reaction mixture stirred under nitrogen at room temperature for the specified time. The solvent was evaporated in vacuo and acetone (1 mL) was added to the residue in order to quench any excess reducing agent. The oil that separated was extracted with dichloromethane (2× 150 mL), washed with water (3× 100 mL), then the organic layer was dried with $MgSO_4$, filtered and reduced in vacuo. The residue was then purified by flash column chromatography.

6.1.2.1. 1-(4-Phenylamino-phenyl)-ethanol (2a, $R^1 = CH_3$, R^2 = Ph). Stirring for 2 h in anhydrous methanol and purification by column chromatography (petroleum ether–ethyl acetate, 95:5 v/v increasing to 50:50 v/v) gave 1-(4-phenylamino-phenyl)-ethanol as a white solid. Yield, 1.07 g (88%); mp 66–68 °C; $R_f = 0.51$ (petroleum ether–ethyl acetate, 1:1). ^{1}H NMR: δ ($CDCl_3$): 1.41 (d, $J = 6.4$ Hz, 3H, H-2), 1.73 (s, 1H, H–OH), 4.76 (q, $J = 6.3$ Hz, 1H, H–CH), 5.63 (s, 1H, H–NH), 6.84 (t, $J = 7.3$ Hz, 1H, H-4″), 6.97 (m, 4H, Ar), 7.18 (m, 4H, Ar). ^{13}C NMR: δ ($CDCl_3$): 24.94 (CH_3, C-2), 70.12 (CH, C-1), 117.79 (CH, C-2″, C-6″), 117.84 (CH, C-3′, C-5′), 121.04 (CH, C-4″), 126.62 (CH, C-2′, C-6′), 129.38 (CH, C-3″, C-5″), 138.37 (C, C-1′), 142.55 (C, C-4′), 143.15 (C, C-1″). Anal. Calcd for $C_{14}H_{15}NO{\cdot}0.1H_2O$ (215.08): C, 78.18; H, 7.12; N, 6.51. Found: C, 78.19; H, 7.16; N, 6.46.

6.1.2.2. Phenyl-(4-phenylamino-phenyl)-methanol (2b, $R^1 = R^2$ = Ph). Stirring for 3 h in anhydrous methanol/anhydrous dioxane and purification by column chromatography (petroleum ether–ethyl acetate, 95:5 v/v increasing to 50:50 v/v) gave phenyl-(4-phenylamino-phenyl)-methanol as a brown oil. Yield, 0.95 g (86%); $R_f = 0.58$ (petroleum ether–ethyl acetate, 1:1). ^{1}H NMR: δ ($CDCl_3$): 2.94 (s, 1H, H–OH), 5.81 (s, 1H, H-1), 5.87 (s, 1H, H–NH), 7.04 (t, $J = 7.3$ Hz, 1H, Ar), 7.08 (d, $J = 8.5$ Hz, 2H, Ar), 7.13 (d, $J = 7.6$ Hz, 2H, Ar), 7.31 (d, $J = 8.4$ Hz, 2H, Ar), 7.36 (m, 3H, Ar), 7.44 (t, $J = 7.3$ Hz, 2H, Ar), 7.49 (d, $J = 7.3$ Hz, 2H, Ar). ^{13}C NMR: δ ($CDCl_3$): 75.76 (CH, C-1), 117.44 (CH, C-2″, C-6″), 117.70 (CH, C-3′, C-5′), 121.34 (CH, C-4″), 126.37 (CH, C-3, C-7), 127.48 (CH, C-5), 128.16 (CH, C-4, C-6), 128.87 (CH, C-2′, C-6′), 129.82 (CH, C-3″, C-5″), 136.51 (C, C-1′), 142.69 (C, C-4′), 143.15 (C, C-2), 144.24 (C, C-1″). HRMS (ES^+) *m/z* Calcd for $C_{19}H_{17}NO$ $(M + H)^+$ 275.1305. Found: 275.1309.

6.1.2.3. 1-[4-(Naphthalen-2-ylamino)-phenyl]-ethanol (2c, $R^1 = CH_3$, R^2 = naphthyl). Stirring for 3 h in anhydrous methanol/anhydrous dioxane and purification by column chromatography (petroleum ether–ethyl acetate, 95:5 v/v increasing to 50:50 v/v) gave 1-[4-

(naphthalen-2-ylamino)-phenyl]-ethanol as a brown solid. Yield, 1.34 g (89%); mp 98–100; R_f = 0.57 (petroleum ether–ethyl acetate, 2:1).). ^{1}H NMR: δ ($CDCl_3$): 1.42 (d, J = 6.5 Hz, 1H, H-2), 1.76 (s, 1H, H–OH), 4.78 (q, J = 6.5 Hz, 1H, H-1), 5.81 (s, 1H, H–NH), 7.04 (d, J = 8.5 Hz, 2H, H-3′, H5′), 7.11 (dd, J_1 = 2.3 Hz, J_2 = 8.7 Hz, 1H, H-3″), 7.2 (d, J = 1.2 Hz, 1H, H-1″), 7.22 (d, J = 8.3 Hz, 2H, H-2′, H-6′), 7.31 (m, 2H, H-7″, H-8″), 7.54 (d, J = 8.2 Hz, 1H, H-4″), 7.64 (d, J = 8.8 Hz, 2H, H-6″, H-9″). ^{13}C NMR: δ ($CDCl_3$): 24.98 (CH, C-2), 70.13 (CH, C-1), 111.57 (CH, C-1″), 118.32 (CH, C-3′, C-5′), 120.02 (CH, C-3″), 123.54 (CH, C-7″), 126.50 (CH, C-6″, C-9″), 126.70 (CH, C-2′, C-6′), 127.03 (C, C-5″), 127.68 (CH, C-8″), 129.23 (CH, C-4″), 134.66 (C, C-1′), 138.79 (C, C-10″), 140.90 (C, C-4′), 142.35 (C, C-2″). Anal. Calcd for $C_{18}H_{17}NO{\cdot}0.3H_2O$ (268.73): C, 80.45; H, 6.38; N, 5.21. Found: C, 80.80; H, 6.44; N, 5.13.

6.1.2.4. [4-(Naphthalen-2-ylamino)-phenyl]-phenyl-methanol (2d, R^1 = Ph, R^2 = naphthyl). Stirring for 1 h in anhydrous methanol/anhydrous dioxane and purification by column chromatography (petroleum ether–ethyl acetate, 95:5 v/v increasing to 50:50 v/v) gave [4-(naphthalen-2-ylamino)-phenyl]-phenyl-methanol as a red-brown oil. Yield, 1.38 g (92%); R_f = 0.69 (petroleum ether–ethyl acetate, 2:1). ^{1}H NMR: δ ($CDCl_3$): 3.24 (s, 1H, H–OH), 5.81 (s, 1H, H-1), 6.01 (s, 1H, H–NH), 7.15 (d, J = 8.6 Hz, 2H, H-3′, H-5′), 7.23 (dd, J_1 = 2.2 Hz, J_2 = 8.7 Hz, 1H, H-3″), 7.35 (d, J = 8.7 Hz, 2H, H-2′, H-6′), 7.39 (m, 1H, H-7″), 7.41 (d, J = 2.2 Hz, 1H, H-1″), 7.47 (m, 6H, H-3, H-4, H-5, H-6, H-7, H-8″), 7.70 (d, J = 8.2 Hz, 1H, H-4″), 7.78 (d, J = 8.8 Hz, 1H, H-9″), 7.82 (d, J = 8.1 Hz, 1H, H-6″). ^{13}C NMR: δ ($CDCl_3$): 76.26 (CH, C-1), 117.84 (CH, C-1″), 118.43 (CH, C-3′, C-5′), 120.50 (CH, C-3″), 123.37 (CH, C-7″), 126.95 (CH, C-9″), 127.19 (CH, C-3, C-7), 127.79 (CH, C-8″), 128.00 (CH, C-6″), 128.33 (CH, C-4, C-6), 128.78 (CH, C-2′, C-6′), 128.88 (CH, C-5), 129.25 (CH, C-4″), 129.28 (C, C-5″), 134.75 (C, C-1′), 136.99 (C, C-10″), 140.92 (C, C-4′), 142.46 (C, C-2″), 144.37 (C, C-2). HRMS (ES^+) m/z Calcd for $C_{23}H_{19}NO$ $(M + H)^+$ 326.1539. Found: 326.1538.

6.1.2.5. [4-(Indan-5-ylamino)-phenyl]-phenyl-methanol (2e, R^1 = Ph, R^2 = indanyl). Stirring for 7 h in anhydrous methanol/anhydrous dioxane and purification by column chromatography (petroleum ether–ethyl acetate, 95:5 v/v increasing to 50:50 v/v) gave [4-(indan-5-ylamino)-phenyl]-phenyl-methanol as a brown oil. Yield, 1.36 g (91%); R_f = 0.71 (petroleum ether–ethyl acetate, 2:1). ^{1}H NMR: δ ($CDCl_3$): 1.98 (m, 2H, H-2″), 2.12 (s, 1H, H–OH), 2.77 (t, J = 7.4Hz, 4H, H-1″, H-3″), 5.51 (s, 1H, H–NH), 5.21 (s, 1H, H-1), 6.76 (dd, J_1 = 2.0 Hz, J_2 = 8.5 Hz, 1H, H-7″), 6.87 (m, 3H, H-3, H-7, H-5″), 7.02 (d, J = 8.00 Hz, 1H, H-8″), 7.11 (d, J = 8.5 Hz, 2H, H-2′, H-6′), 7.17 (m, 1H, H-5), 7.25 (m, 2H, H-4, H-6), 7.30 (d, J = 7.5 Hz, 2H, H-3′, H-5′). ^{13}C NMR: δ ($CDCl_3$): 25.71 (C, C-2″), 32.38 (C, C-1″), 33.08 (C, C-3″), 76.00 (CH, C-1), 115.28 (CH, C-7″), 116.70 (CH, C-3′, C-5′), 117.35 (CH, C-5″), 124.89 (CH, C-5), 126.60 (CH, C-3, C-7), 127.86 (CH, C-8″), 128.11 (CH, C-4, C-6), 128.42 (CH, C-2′, C-6′), 135.62 (C, C-9″), 137.64 (C, C-1′), 140.99 (C, C-4″), 143.78 (C, C-6″), 144.12 (C, C-4′), 145.59 (C, C-2). HRMS (ES^+) m/z Calcd for $C_{23}H_{19}NO$ $(M + H)^+$ 316.1696. Found: 316.1695.

6.1.2.6. (4-Amino-phenyl)-phenyl-methanol (10). Stirring for 1 h in anhydrous methanol gave (4-amino-phenyl)-phenyl-methanol (**10**) as a white solid without further purification. Yield, 1.92 g (95%); mp 116–118 °C; R_f = 0.39 (petroleum ether–ethyl acetate, 2:1). ^{1}H NMR: δ ($CDCl_3$): 3.21 (s, 1H, H–OH), 4.83 (s, 2H, H–NH), 5.62 (s, 1H, H-1), 6.59 (dd, J_1 = 1.65 Hz, J_2 = 1.71 Hz, 2H, H-4, H-6), 7.00 (dd, J_1 = 1.55 Hz, J_2 = 1.55 Hz, 2H, H-3, H-7), 7.12 (m, 2H, H-3′, H-5′), 7.20 (m, 1H, H-4′), 7.25 (m, 2H, H-2′, H-6′). ^{13}C NMR: δ ($CDCl_3$): 76.86 (CH, C-1), 116.42 (CH, C-4, C-6), 127.58 (CH, C-2′, C-6′), 127.94 (CH, C-4′), 128.93 (CH, C-3′, C-5′), 129.13 (CH, C-3, C-7), 135.63 (C, C-2), 146.36 (C, C-1′), 147.88 (C, C-5). Anal. Calcd for $C_{13}H_{13}NO{\cdot}0.3H_2O$ (204.653): C, 76.30; H, 6.40; N, 6.84. Found: C, 76.36; H, 6.43; N, 6.80.

6.1.3. (4-Isothiocyanato-phenyl)-phenyl-methanol (11). To a solution of **10** (1.4 g, 7.03 mmol) in CH_2Cl_2 (20 mL) was added a mixture of ice (2 g) and water (1 mL) and subsequently thiophosgene (0.64 mL, 8.4 mmol) dropwise with vigorous stirring. The mixture was stirred 2 h at 0 °C and kept overnight in a refrigerator. The organic layer was separated and extracted successively with water (2× 50 mL), 10% $NaHCO_3$ aq. (50 mL) and water again (50 mL), dried ($MgSO_4$) and evaporated to obtain the pure product as a yellow oil. Yield, 1.34 g (79%); R_f = 0.73 (petroleum ether–ethyl acetate, 1:1). ^{1}H NMR: δ ($CDCl_3$): 2.69 (s, 1H, H–OH), 5.59 (s, 1H, H-1), 7.00 (d, J = 8.3 Hz, 2H, H-4, H-6), 7.18 (m, 7H, H-3, H-7, H-2′, H-3′, H-4′, H-5′, H-6′). ^{13}C NMR: δ ($CDCl_3$): 75.58 (CH, C-1), 125.50 (CH, C-4, C-6), 126.08 (CH, C-2′, C-6′), 127.46 (CH, C-4′), 127.89 (CH, C-3′, C-5′), 129.02 (CH, C-3, C-7), 130.29 (C, C-5), 135.45 (C, C-2), 143.06 (C, C-1′), 143.25 (C, C-NCS). HRMS (ES^+) m/z Calcd for $C_{14}H_{11}NOS$ $(M + H)^+$ 241.0556. Found: 241.0557.

6.1.4. General procedure for the preparation of 2-substituted aminobenzoheterocycles. 2-Substituted aminobenzene (1.7 mmol) was added to a solution of **11** (1.7 mmol) in absolute ethanol (10 mL) and the mixture stirred overnight at room temperature. To the same flask HgO (3.3 mmol) and S (0.33 mmol) was added and the reaction mixture refluxed for 2 h then filtered through Celite. The solvent was evaporated in *vacuo* to give an oil, which was purified by column chromatography.

6.1.4.1. [4-(1*H*-Benzoimidazol-2-ylamino)-phenyl]-phenyl-methanol (12). Purification by column chromatography (dichloromethane–methanol, 100:0 v/v to 95:5 v/v) gave [4-(1H-benzoimidazol-2-ylamino)-phenyl]-phenyl-methanol (**12**) as a yellowish white solid. Yield, 0.42 g (81%); mp 190–192 °C; R_f = 0.54 (dichloromethane–methanol, 95:5). ^{1}H NMR: δ (DMSO): 5.67 (s, 1H, H-1″), 5.78 (s, 1H, H–OH), 6.98 (s, 2H, Ar), 7.18 (m, 1H, H-5″), 7.29 (m, 6H, Ar), 7.38 (d, J = 7.4 Hz, 2H, H-3′,

H-5′), 7.66 (d, J = 8.3 Hz, 2H, H-5, C-8), 9.41 (s, 1H, NH), 10.87 (s, 1H, NH). ^{13}C NMR: δ (DMSO): 76.73 (CH, C-1″), 113.65 (CH, C-5″), 119.54 (CH, C-5, C-8), 122.05 (CH, C-2′, C-6′), 127.71 (CH, C-6, C-7), 128.20 (CH, C-3″, C-7″), 129.08 (CH, C-4″, C-6″), 129.54 (CH, C-3′, C-5′), 138.78 (C, C-4′), 139.97 (C, C-4, C-9), 140.66 (C, C-2), 146.06 (C, C-1′), 152.81 (C, C-2″). Anal. Calcd for $C_{20}H_{16}N_3O{\cdot}0.7H_2O$ (326.973): C, 73.47; H, 5.36; N, 12.85. Found: C, 73.36; H, 5.35; N, 12.63.

6.1.4.2. [4-(Benzooxazol-2-ylamino)-phenyl]-phenyl-methanol (13). Purification by column chromatography (petroleum ether/ethyl acetate 100:0 v/v to 75:25 v/v) gave [4-(benzooxazol-2-ylamino)-phenyl]-phenyl-methanol (**13**) as a yellow solid. Yield, 0.4 g (77%); mp 160–162 °C; R_f = 0.57 (petroleum ether–ethyl acetate, 2:1). ^{1}H NMR: δ (DMSO): 5.71 (s, 1H, H-1″), 5.85 (s, 1H, H–OH), 7.15 (m, 1H, H-5″), 7.25 (m, 2H, H-2′, H-6′), 7.34 (m, 2H, Ar), 7.41 (m, 4H, Ar), 7.49 (dd, J_1 = 7.5 Hz, J_2 = 7.8 Hz, 2H, H-3′, C-5′), 7.72 (d, J = 8.4 Hz, 2H, H-5, C-8), 10.59 (s, 1H, NH). ^{13}C NMR: δ (DMSO): 73.92 (CH, C-1″), 108.87 (CH, C-5), 116.51 (CH, C-8), 117.39 (CH, C-2′, C-6′), 121.54 (CH, C-6), 123.94 (CH, C-7), 126.17 (CH, C-3″, C-7″), 126.58 (CH, C-5″), 126.87 (CH, C-4″, C-6″), 127.99 (CH, C-3′, C-5′), 137.31 (C, C-4′), 139.59 (C, C-9), 142.44 (C, C-2″), 145.84 (C, C-1′), 147.00 (C, C-4), 158.04 (C, C-2). HRMS (ES$^+$) m/z Calcd for $C_{20}H_{16}N_2O_2$ $(M + H)^+$ 317.1285. Found: 317.1287.

6.1.4.3. [4-(Benzothiazol-2-ylamino)-phenyl]-phenyl-methanol (14). Purification by column chromatography (petroleum ether/ethyl acetate 100:0 v/v to 75:25 v/v) gave [4-(benzothiazol-2-ylamino)-phenyl]-phenyl-methanol (**14**) as a greyish white solid. Yield, 0.45 g (82%); mp 138–140 °C; R_f = 0.54 (petroleum ether–ethyl acetate, 2:1). ^{1}H NMR: δ (DMSO): 5.68 (s, 1H, H-1″), 5.81 (s, 1H, H–OH), 7.15 (m, 1H, Ar), 7.21 (m, 1H, Ar), 7.35 (m, 7H, Ar), 7.58 (d, J = 7.9 Hz, 1H, Ar), 7.70 (d, J = 8.3 Hz, 2H, H-3′, C-5′), 7.79 (d, J = 7.7 Hz, 2H, H-5, C-8), 10.51 (s, 1H, NH). ^{13}C NMR: δ (DMSO): 73.94 (CH, C-1″), 117.65 (CH, C-2′, C-6′), 119.10 (CH, C-5), 120.98 (CH, C-8), 122.14 (CH, C-6), 125.80 (CH, C-7), 126.16 (CH, C-3″, C-7″), 126.57 (CH, C-5″), 126.88 (CH, C-4″, C-6″), 127.99 (CH, C-3′, C-5′), 129.96 (C, C-9), 139.25 (C, C-4′), 139.54 (C, C-1′), 145.85 (C, C-2″), 152.10 (C, C-4), 161.64 (C, C-2). Anal. Calcd for $C_{20}H_{16}N_2OS{\cdot}0.4H_2O$ (339.618): Calcd C, 70.73; H, 4.99; N, 8.24. Found: C, 70.87; H, 5.08; N, 7.84.

6.1.5. General procedure for the addition of the triazole ring. Thionyl chloride (2 mmol) in anhydrous acetonitrile (10 mL) was added dropwise to a stirred solution of 1,2,4-triazole (4 mmol) in anhydrous acetonitrile (10 mL) at a temperature of 10 °C. The white suspension formed was stirred for 1 h at 10 °C. A solution of the carbinol compound (**2**) (1 mmol) in anhydrous acetonitrile (10 mL) was added to the mixture followed by activated potassium carbonate (2 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 ethyl acetate (150 mL) and water (3× 100 mL). The organic layer was dried with $MgSO_4$, filtered and reduced in vacuo. The residue was then purified by flash column chromatography.

6.1.5.1. Phenyl-[4-(1-[1,2,4]triazol-1-yl-ethyl)-phenyl]-amine (3). Purification by column chromatography (petroleum ether–ethyl acetate, 90:10 v/v increasing to 10:90 v/v) gave [phenyl-[4-(1-[1,2,4]triazol-1-yl-ethyl)-phenyl]-amine (**3**) as a light brown solid. Yield, 0.19 g (72%); mp 108–110 °C; R_f = 0.32 (petroleum ether–ethyl acetate, 1:1). ^{1}H NMR: δ ($CDCl_3$): 1.93 (d, J = 7.0 Hz, 3H, H-2), 5.50 (q, J = 7.0 Hz, 1H, H-1), 5.89 (s, 1H, H–NH), 6.98 (t, J = 7.3 Hz, 1H, Ar), 7.05 (d, J = 8.5 Hz, 2H, Ar), 7.10 (d, J = 7.9 Hz, 2H, Ar), 7.19 (d, J = 8.5 Hz, 2H, Ar), 7.30 (t, J = 7.6 Hz, 2H, Ar), 7.98 (s, 1H, H-3‴), 8.03 (s, 1H, H-5‴). ^{13}C NMR: δ ($CDCl_3$): 21.26 (CH_3, C-2), 59.32 (CH, C-1), 117.26 (CH, C-3′, C-5′), 118.58 (CH, C-2″, C-6″), 121.70 (CH, C-4″), 127.87 (CH, C-2′, C-6′), 129.42 (CH, C-3″, C-5″), 131.45 (C, C-1′), 141.83 (CH, C-3‴), 142.39 (C, C-4′), 143.75 (C, C-1″), 151.81 (C, C-5‴). Anal. Calcd for $C_{16}H_{16}N_4{\cdot}0.3H_2O$ (269.734): C, 71.24; H, 5.97; N, 20.77. Found: C, 71.44; H, 6.03; N, 20.37.

6.1.5.2. Phenyl-[4-(phenyl-[1,2,4]triazol-1-yl-methyl)-phenyl]-amine (4). Purification by column chromatography (petroleum ether–ethyl acetate, 90:10 v/v increasing to 10:90 v/v) gave phenyl-[4-(phenyl-[1,2,4]triazol-1-yl-methyl)-phenyl]-amine (**4**) as a light brown solid. Yield, 0.41 g (69%); mp 138–140 °C; R_f = 0.42 (petroleum ether–ethyl acetate, 1:1). ^{1}H NMR: δ ($CDCl_3$): 5.91 (s, 1H, H–NH), 6.72 (s, 1H, H-1), 7.04 (m, 4H, Ar), 7.13 (m, 4H, Ar), 7.31 (m, 3H, Ar), 7.39 (m, 3H, Ar), 7.97 (s, 1H, H-3‴), 8.06 (s, 1H, H-5‴). ^{13}C NMR: δ ($CDCl_3$): 67.56 (CH, C-1), 116.95 (CH, C-2″, C-6″), 118.83 (CH, C-3′, C-5′), 121.90 (CH, C-4″), 127.77 (CH, C-3, C-7), 128.39 (CH, C-5), 128.89 (CH, C-4, C-6), 129.40 (C, C-1′), 129.43 (CH, C-2′, C-6′), 129.57 (CH, C-3″, C-5″), 138.52 (C, C-4′), 142.16 (C, C-2), 143.51 (CH, C-3‴), 143.93 (C, C-1″), 152.27 (CH, C-5‴). Anal. Calcd for $C_{21}H_{18}N_4$ (326.40): C, 77.28; H, 5.56; N, 17.16. Found: C, 76.90; H, 5.54; N, 17.04.

6.1.5.3. General procedure for the addition of the imidazole ring. To a solution of the carbinol compound (**2**) (1.8 mmol) in acetonitrile (20 mL) were added imidazole (5.5 mmol) and 1,1′-carbonydiimidazole (3.5 mmol). The mixture was then heated under reflux for 1 h. The reaction mixture was allowed to cool and then extracted with ethyl acetate (150 mL) and water (3× 100 mL). The organic layer was dried with $MgSO_4$, filtered and reduced in vacuo. The residue was then purified by flash column chromatography.

6.1.5.4. Phenyl-[4-(1-imidazol-1-yl-ethyl)-phenyl]-amine (5). Purification by column chromatography (dichloromethane–methanol, 100:0 v/v increasing to 90:10 v/v) gave phenyl-[4-(1-imidazol-1-yl-ethyl)-phenyl]-amine (**5**) as a light brown solid. Yield, 0.52 g (84%); mp 110–112 °C; R_f = 0.37 (dichloromethane–methanol, 90:10). ^{1}H NMR: δ ($CDCl_3$): 1.85 (d, J = 7.0 Hz, 3H, H-2), 5.30 (q, J = 7.0 Hz, 1H, H-1), 6.02 (s, 1H, H–NH), 6.97 (m, 2H, Ar), 7.07 (m, 7H, Ar), 7.29 (m, 2H, Ar), 7.60 (s, 1H, H-2‴). ^{13}C NMR: δ ($CDCl_3$): 22.07 (CH_3, C-2), 56.15 (CH, C-1), 117.35 (CH, C-3′, C-5′), 117.89 (CH, C-4‴), 118.35

(CH, C-2″, C-6″), 121.47 (CH, C-4″, C-5‴), 127.24 (CH, C-2′, C-6′), 129.40 (CH, C-3″, C-5″), 133.28 (C, C-1′), 136.05 (CH, C-2‴), 142.63 (C, C-4′), 143.35 (C, C-1″). Anal. Calcd for $C_{17}H_{17}N_3 \cdot 0.1H_2O$ (265.141): C, 77.01; H, 6.46; N, 15.85. Found: C, 76.89; H, 6.46; N, 15.87.

6.1.5.5. Phenyl-[4-(phenyl-imidazol-1-yl-methyl)-phenyl]-amine (6). Purification by column chromatography (dichloromethane–methanol, 100:0 v/v increasing to 90:10 v/v) gave [phenyl-[4-(phenyl-imidazol-1-yl-methyl)-phenyl]-amine (**6**) as a white solid. Yield, 0.53 g (89%); mp 208–210 °C; $R_f = 0.41$ (dichloromethane–methanol, 90:10). ^{1}H NMR: δ ($CDCl_3$): 5.80 (s, 1H, H–NH), 6.48 (s, 1H, H-1), 7.01 (m, 4H, Ar), 7.17 (m, 4H, Ar), 7.30 (m, 4H, Ar), 7.38 (m, 3H, Ar), 7.46 (s, 1H, H-2‴). ^{13}C NMR: δ ($CDCl_3$): 64.83 (CH, C-1), 116.79 (CH, C-2″, C-6″), 117.06 (CH, C-4″), 118.42 (CH, C-3′, C-5′), 121.92 (CH, C-4‴), 126.93 (CH, C-5, C-5‴), 128.27 (CH, C-3, C-7), 128.87 (CH, C-4, C-6), 129.30 (CH, C-2′, C-6′), 129.40 (CH, C-3″, C-5″), 129.75 (C, C-1′), 132.54 (CH, C-2‴), 139.55 (C, C-4′), 143.06 (C, C-2), 143.91 (C, C-1″). Anal. Calcd for $C_{22}H_{19}N_3 \cdot 0.3H_2O$ (330.815): C, 79.87; H, 5.78; N, 12.70. Found: C, 79.72; H, 5.86; N, 12.56.

6.1.5.6. [4-(1-Imidazol-1-yl-ethyl)-phenyl]-naphthalen-2-yl-amine (7). Purification by column chromatography (dichloromethane–methanol, 100:0 v/v increasing to 95:5 v/v) gave 4-(1-imidazol-1-yl-ethyl)-phenyl]-naphthalen-2-yl-amine (**7**) as a white solid. Yield, 0.5 g (84%); mp 170–172 °C; $R_f = 0.33$ (dichloromethane–methanol, 90:10). ^{1}H NMR: δ ($CDCl_3$): 1.78 (d, $J = 6.4$ Hz, 1H, H-2), 5.19 (q, $J = 6.4$ Hz, 1H, H-1), 5.87 (s, 1H, H–NH), 6.91 (d, $J = 1.7$ Hz, 1H, H-1″), 7.05 (d, $J = 8.5$ Hz, 4H, H-2′, H-3′, H-5′, H-6′), 7.15 (dd, $J_1 = 2.1$ Hz, $J_2 = 8.6$ Hz, 1H, H-3″), 7.19 (m, 1H, H-7″), 7.32 (m, 1H, H-8″), 7.38 (d, $J = 7.7$ Hz, 2H, H-4‴, H-5‴), 7.51 (s, 1H, H-2‴), 7.59 (d, $J = 8.4$ Hz, 1H, H-4″), 7.71 (d, $J = 8.8$ Hz, 2H, H-6″, H-9″). ^{13}C NMR: δ ($CDCl_3$): 21.05 (CH, C-1), 52.40 (CH, C-2), 111.45 (CH, C-1″), 116.79 (CH, C-3′, C-5′), 119.22 (CH, C-3″), 122.78 (CH, C-4‴, C-7″), 125.53 (CH, C-8″, C-9″), 126.31 (CH, C-5‴, C-6″), 126.65 (C, C-5″), 128.30 (CH, C-2′, C-6′), 128.42 (C, C-4″), 132.75 (C, C-1′), 133.52 (C, C-10″), 135.03 (CH, C-2‴), 139.18 (C, C-4′), 142.06 (C, C-2″). HRMS (ES^+) *m/z* Calcd for $C_{21}H_{19}N_3$ $(M + H)^+$ 314.1652. Found: 314.1654.

6.1.5.7. [4-(Imidazol-1-yl-phenyl-methyl)-phenyl]-naphthalen-2-yl-amine (8). Purification by column chromatography (dichloromethane–methanol, 100:0 v/v increasing to 95:5 v/v) gave [4-(imidazol-1-yl-phenyl-methyl)-phenyl]-naphthalen-2-yl-amine (**8**) as a pink solid. Yield, 0.51 g (88%); mp 94–96 °C; $R_f = 0.40$ (dichloromethane–methanol, 90:10). ^{1}H NMR: δ ($CDCl_3$): 6.06 (s, 1H, H–NH), 6.38 (s, 1H, H-1), 6.81 (s, 1H, H-1″), 6.94 (d, $J = 8.6$ Hz, 2H, H-3′, H-5′), 7.03 (m, 5H, Ar), 7.15 (dd, $J_1 = 2.3$ Hz, $J_2 = 8.5$ Hz, 1H, H-3″), 7.23 (m, 4H, Ar), 7.32 (m, 1H, Ar), 7.38 (m, 2H, Ar), 7.57 (d, $J = 8.2$ Hz, 1H, H-4″), 7.66 (d, $J = 8.8$ Hz, 2H, H-6″, H-9″). ^{13}C NMR: δ ($CDCl_3$): 64.73 (CH, C-1), 112.80 (CH, C-1″), 117.36 (CH, C-3′, C-5′), 120.40 (CH, C-3″), 123.87 (CH, C-7″), 126.56 (CH, C-9″), 126.60 (CH, C-8″, C-4‴), 127.69 (CH, C-5, C-5‴), 127.80 (CH, C-3′, C-7), 128.23 (CH, C-6″), 128.85 (C, C-2′, C-6′), 129.29 (CH, C-2‴), 129.45 (C, C-4″), 129.51 (CH, C-4, C-6), 129.51 (C, C-5″), 131.10 (C, C-1′), 134.53 (C, C-10″), 139.67 (C, C-4′), 140.00 (C, C-2″), 143.45 (C, C-2). Anal. Calcd for $C_{26}H_{21}N_3 \cdot 0.3H_2O$ (380.87): C, 81.99; H, 5.56; N, 11.03. Found: C, 81.86; H, 5.59; N, 10.82.

6.1.5.8. [4-(Imidazol-1-yl-phenyl-methyl)-phenyl]-indan-5-yl-amine (9). Purification by column chromatography (dichloromethane–methanol, 100:0 v/v increasing to 95:5 v/v) gave [4-(imidazol-1-yl-phenyl-methyl)-phenyl]-indan-5-yl-amine (**9**) as a white solid. Yield, 0.48 g (83%); mp 88–90 °C; $R_f = 0.43$ (dichloromethane–methanol, 90:10). ^{1}H NMR: δ ($CDCl_3$): 1.98 (m, 2H, H-2″), 2.76 (m, 4H, H-1″, H-3″), 5.78 (s, 1H, H–NH), 6.35 (s, 1H, H-1), 6.79 (m, 2H, Ar), 6.87 (m, 4H, Ar), 6.92 (d, $J = 1.5$ Hz, 1H, H-5″), 7.01 (m, 3H, Ar), 7.04 (d, $J = 8.0$ Hz, 1H, H-8″), 7.25 (m, 3H, Ar), 7.34 (s, 1H, H-2‴). ^{13}C NMR: δ ($CDCl_3$): 25.69 (C, C-2″), 32.31 (C, C-1″), 33.05 (C, C-3″), 64.73 (CH, C-1), 115.98 (CH, C-7″), 116.09 (CH, C-3′, C-5′), 118.02 (CH, C-5″), 124.57 (CH, C-4‴), 127.63 (CH, C-5, C-5‴), 127.97 (CH, C-8″), 128.67 (CH, C-3, C-7), 129.02 (CH, C-4, C-6), 129.19 (CH, C-2′, C-6′), 129.38 (C, C-9″), 132.81 (CH, C-2‴), 138.25 (C, C-1′), 139.83 (C, C-4″), 140.30 (C, C-6″), 145.19 (C, C-4′), 145.64 (C, C-2). Anal. Calcd for $C_{25}H_{23}N_3 \cdot 0.7H_2O$ (378.08): C, 79.42; H, 6.13; N, 11.11. Found: C, 79.37; H, 6.48; N, 11.32.

6.1.5.9. (1*H*-Benzoimidazol-2-yl)-{4-[(5*H*-imidazol-1-yl)-phenyl-methyl]-phenyl}-amine (15). Purification by column chromatography (dichloromethane–methanol, 100:0 v/v increasing to 95:5 v/v) gave (1*H*-benzoimidazol-2-yl)-{4-[(5*H*-imidazol-1-yl)-phenyl-methyl]-phenyl}-amine (**15**) as a yellowish white solid. Yield, 0.28 g (76%); mp 274–276 °C; $R_f = 0.17$ (dichloromethane–methanol, 90:10). ^{1}H NMR: δ (DMSO): 6.80 (s, 1H, H-1″), 6.99 (m, 3H, Ar), 7.13 (m, 5H, Ar), 7.35 (m, 5H, Ar), 7.6 (s, 1H, H-2‴), 7.78 (d, $J = 7.4$ Hz, 2H, H-5, H-8), 9.54 (s, 1H, NH), 10.68 (s, 1H, NH). ^{13}C NMR: δ (DMSO): 63.13 (CH, C-1″), 117.14 (CH, C-5, C-8), 119.18 (CH, C-5″), 120.08 (CH, C-6, C-7), 127.48 (CH, C-3″, C-7″), 127.73 (CH, C-5‴), 128.51 (CH, C-3′, C-5′, C-4″, C-6″), 128.78 (CH, C-2‴, C-4‴), 131.58 (C, C-4′), 140.48 (C, C-4, C-9), 140.66 (C, C-2), 150.35 (C, C-1′, C-2″). HRMS (ES^+) *m/z* Calcd for $C_{23}H_{19}N_5$ $(M + H)^+$ 316.1713. Found: 316.1715.

6.1.5.10. Benzooxazol-2-yl-{4-[(5*H*-imidazol-1-yl)-phenyl-methyl]-phenyl}-amine (16). Purification by column chromatography (petroleum ether–ethyl acetate, 80:20 v/v increasing to 0:100 v/v) gave benzooxazol-2-yl-{4-[(5H-imidazol-1-yl)-phenyl-methyl]-phenyl}-amine (**16**) as a yellow solid. Yield, 0.19 g (71%); mp 214–216 °C; $R_f = 0.37$ (ethyl acetate, 100%). ^{1}H NMR: δ (DMSO): 6.84 (s, 1H, H-1″), 6.98 (s, 1H, Ar), 7.14 (m, 4H, Ar), 7.21 (m, 3H, Ar), 7.35 (m, 1H, H-5″), 7.39 (m, 3H, Ar), 7.49 (d, $J = 7.9$ Hz, 1H, H-4‴), 7.66 (s, 1H, H-2‴), 7.77 (d, $J = 8.0$ Hz, 2H, H-5, H-8), 10.78 (s, 1H, NH). ^{13}C NMR: δ (DMSO): 62.99 (CH, C-1″), 108.97 (CH, C-8), 116.64 (CH, C-5), 117.75 (CH, C-2′, C-6′), 119.15 (CH, C-6), 121.74 (CH, C-7), 124.01 (CH, C-5″), 127.54 (CH, C-3″, C-7″), 127.81 (CH, C-5‴), 128.65 (CH, C-4″, C-6″,

C-3′, C-5′), 128.74 (CH, C-2″, C-4″), 133.39 (C, C-4′), 138.44 (C, C-4), 140.27 (C, C-2″), 142.29 (C, C-1′), 147.00 (C, C-9), 157.86 (C, C-2). HRMS (ES^+) *m/z* Calcd for $C_{23}H_{18}N_4O$ $(M + H)^+$ 367.1553. Found: 367.1555.

6.1.5.11. Benzothiazol-2-yl-{4-[(5*H*-imidazol-1-yl)-phenyl-methyl]-phenyl}-amine (17). After reflux for 1 h, the reaction mixture was allowed to cool and the precipitate that formed was filtered and washed with hot CH_3CN (2× 5 mL) and dried to give benzothiazol-2-yl-{4-[(5*H*-imidazol-1-yl)-phenyl-methyl]-phenyl}-amine (**17**) as a yellowish white solid. Yield, 0.29 g (73%); mp 258–260 °C; $R_f = 0.26$ (ethyl acetate, 100%). ^1H NMR: δ (DMSO): 6.83 (s, 1H, H-1″), 6.97 (s, 1H, Ar), 7.15 (m, 6H, Ar), 7.34 (m, 2H, Ar), 7.40 (m, 2H, Ar), 7.59 (d, $J = 7.9$ Hz, 1H, H-4‴), 7.65 (s, 1H, H-2‴), 7.80 (m, 3H, Ar), 10.59 (s, 1H, NH). ^{13}C NMR: δ (DMSO): 63.01 (CH, C-1″), 117.88 (CH, C-2′, C-6′), 119.15 (CH, C-5), 119.25 (CH, C-8), 121.05 (CH, C-6), 122.34 (CH, C-7), 125.86 (CH, C-5″), 127.56 (CH, C-3″, C-7″), 127.81 (CH, C-5‴), 127.99 (CH, C-4″, C-6″), 128.62 (CH, C-3′, C-5′), 128.71 (CH, C-2‴, C-5‴), 130.02 (C, C-9), 133.21 (C, C-4′), 137.14 (C, C-2″), 140.29 (C, C-1′), 151.96 (C, C-4), 161.43 (C, C-2). Anal. Calcd for $C_{23}H_{18}N_4S{\cdot}0.3H_2O$ (387.886): Calcd C, 71.22; H, 4.68; N, 14.44. Found: C, 71.01; H, 4.73; N, 14.18.

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

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 Buffer Saline (PBS) and replaced by fresh medium plus 10 μL inhibitor/solvent (acetonitrile) and 10 μL of atRA (to give 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 was removed into separate glass tubes. Distilled water (200 μL) was added to each well and the cells 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.

6.3. 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 beta-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. The Ecoscint™ was used as the flow scintillation fluid.

6.4. Neuroblastoma cell assays

6.4.1. Cell lines and retinoid treatments. SH-SY5Y neuroblastoma cells were cultured at 37 °C in RPMI 1640 medium containing foetal calf serum (10%) and L-glutamine (2 mM) in a humidified atmosphere of 5% CO_2 in air. atRA was dissolved in dimethyl sulfoxide and added to the culture medium as described by Armstrong et al.[19]

6.4.2. Real-time PCR. RNA was reverse-transcribed from random hexamer primers and real-time PCR performed on 20 ng cDNA using TaqMan Gene Expression products for human CYP26B1 in combination with the TaqMan Universal PCR master mix (Applied Biosystems, Warrington, UK) on a GeneAmp 5700 Sequence Detection System as described previously for CYP26A1.[19] Appropriate controls for non-specific amplification and contamination were included and β-actin was measured simultaneously using the endogenous control assay provided by Applied Biosystems as an internal standard. The thermocycling program consisted of one cycle at 50 °C for 2 min followed by 95 °C for 10 min and 40 cycles at 95 °C (15 s) and 60 °C (1 min). The comparative C_t method ($2^{-\Delta\Delta C_t}$) was used for relative quantification of gene expression.

6.5. Molecular docking

Ligands were docked within the active site of the CYP26A1 homology model[25] using the FlexX docking program of SYBYL,[38] performed with the default values. The active site was defined by all the amino acid residues within a 6.5 Å distance from TRP112, VAL116, THR304, VAL370 and GLY373, including the haem in a heteroatom file. Subsequent manipulation and interaction evaluation was performed with MOE (Molecular Operating Environment) software.[39]

Acknowledgments

For MG we acknowledge the Embassy of the Arab Republic of Egypt for the award of a PhD scholarship. We also acknowledge the technical staff of the Tenovus Cancer Centre for the preparation of MCF-7 cells and media, and the EPSRC Mass Spectrometry Centre, Swansea, UK, for mass spectroscopy data. Studies on SH-SY5Y cells were supported by SPARKS and Cancer Research UK.

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