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Novel retinoic acid 4-hydroxylase (CYP26) inhibitors based on a 3-(1*H*-imidazol- and triazol-1-yl)-2,2-dimethyl-3-(4-(phenylamino)phenyl)propyl scaffold

Mohamed S. Gomaa [a,†], Caroline E. Bridgens [b], Nicola A. Illingworth [b], Gareth J. Veal [b], Christopher P. F. Redfern [b], Andrea Brancale [a], Jane L. Armstrong [c,*], Claire Simons [a,*]

[a] Medicinal Chemistry, Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff CF10 3NB, UK
[b] Northern Institute for Cancer Research, Paul O'Gorman Building, Medical School, Framlington Place, Newcastle University, Newcastle Upon Tyne NE2 4HH, UK
[c] Institute of Cellular Medicine, Framlington Place, Newcastle University, Newcastle upon Tyne NE2 4HH, UK

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ABSTRACT

Retinoic acid (RA), the biologically active metabolite of vitamin A, is used medicinally for the treatment of hyperproliferative diseases including dermatological conditions and cancer. The antiproliferative effects of RA have been well documented as well as the limitations owing to toxicity and the development of resistance to RA therapy. RA metabolism inhibitors (RAMBAs or CYP26 inhibitors) are attracting increasing interest as an alternative method for enhancing endogenous levels of retinoic acid in the treatment of hyperproliferative disease. Here the synthesis and inhibitory activity of novel 3-(1*H*-imidazol- and triazol-1-yl)-2,2-dimethyl-3-(4-(phenylamino)phenyl)propyl derivatives in a MCF-7 CYP26A1 microsomal assay are described. The most promising inhibitor methyl 2,2-dimethyl-3-(4-(phenylamino)phenyl)-3-(1*H*-1,2,4-triazol-1-yl)propanoate (**6**) exhibited an IC_{50} of 13 nM (compared with standards Liarozole IC_{50} 540 nM and R116010 IC_{50} 10 nM) and was further evaluated for CYP selectivity using a panel of CYP with >100-fold selectivity for CYP26 compared with CYP1A2, 2C9 and 2D6 observed and 15-fold selectivity compared with CYP3A4. The results demonstrate the potential for further development of these potent inhibitors.

1. Introduction

Retinoic acid (RA) is one of several biologically active forms of vitamin A[1] and is the natural substrate to the three known retinoic acid receptors RARα, RARβ and RARγ.[2] These receptors are ligand-dependent transcription factors which regulate gene expression as a result of binding to retinoic acid response elements (RAREs) within the DNA of retinoic acid-regulated genes. Once stimulated, retinoic acid receptors induce the production of proteins that play roles in the regulation of cell apoptosis, differentiation and proliferation.[3] Therefore, alteration of intracellular RA concentration can influence signalling in these pathways and have important consequences for cell function and survival.[4]

RA and other retinoids have long had a variety of clinical applications, including their utilisation for the treatment of hyperkeratinisation disorders such as acne and psoriasis.[5–8] As a result of alteration in gene expression, RA inhibits keratinocytes differentiation and alters lipid production.[9] Furthermore, RA induces the apoptosis of defective cells leading to the alleviation of symptoms.[2] RA also has an important role in the treatment of several types of cancer, including acute promyelocytic leukaemia (APL)[10,11] and neuroblastoma.[12] In a high-risk neuroblastoma setting, the introduction of 13-*cis* retinoic acid has led to a marked increase in 3 year event-free survival (EFS) rates when utilised after intensive chemotherapy and myeloablative therapy.[12]

Unfortunately, retinoid use has limitations; due to the pleiotropic actions of RA high pharmacological concentrations can lead to serious side effects, such as multi-organ failure and hyperleukocytosis.[13,14] Additionally, retinoids cause auto-induction of their own metabolism, through negative feedback mechanisms, leading to reduced plasma concentrations and a subsequent failure to respond to therapy.[1,15] These problems can limit the use of retinoids and prevent their application in new areas.

RA metabolism inhibitors (RAMBAs) are attracting increasing interest as an alternative method for enhancing endogenous levels of retinoic acid in the treatment of hyperproliferative diseases with notable success in the treatment of dermatological diseases.[3,16] Two RAMBAs are currently licenced; liarozole[17,18] for the treatment of ichthyosis and talarozole (R115866) for psoriasis and acne

* Corresponding authors. Tel.: +44 (0) 2920 876307; fax: +44 (0) 2920 874149 (C.S.); tel.: +44 (0) 191 222 5644; fax: +44 (0) 191 222 7179 (J.L.A.).
E-mail addresses: j.l.armstrong@newcastle.ac.uk (J.L. Armstrong), SimonsC@Cardiff.ac.uk (C. Simons).
† Present address: Pharmaceutical Chemistry Department, Faculty of Pharmacy, Suez Canal University, Egypt.

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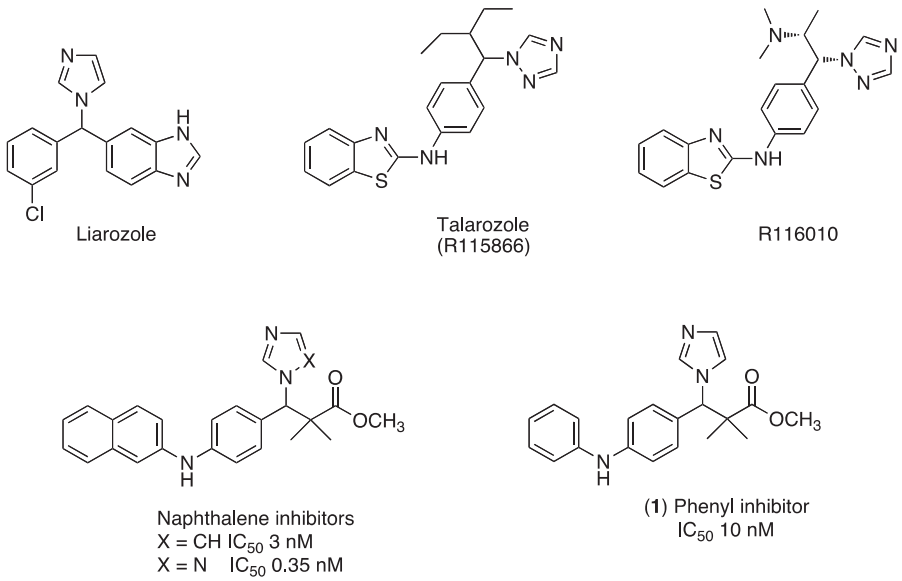


Figure 1. Retinoic acid metabolism blocking agents (RAMBAs).

(Fig. 1).[19–21] Several promising RAMBAs have emerged including *N*-(4-((1*R*,2*R*)-2-(dimethylamino)-1-(1*H*-triazol-1-yl)propyl)phenyl)-benzo[*d*]thiazol-2-amine (R116010) (Fig. 1),[22] a potent and selective inhibitor of RA metabolism, which inhibits all-*trans*-RA (ATRA) metabolism in neuroblastoma both in vitro and in vivo.[23] We have previously described potent naphthalene derivatives which display low to sub nM activity (Fig. 1).[24,25] Here we report studies on the comparative phenyl derivatives based on the lead phenyl CYP26 inhibitor (**1**) (Fig. 1), exploring the effect of modifying the heterocycle, dimethyl propylester chain and substitution of the phenyl ring to determine optimal structure for CYP26 inhibition.

2. Chemistry

The N-arylation of the amine (**2**)[24] using the Suzuki reaction, followed described methodology[26,27] employing a stoichiometric amount of copper and a tertiary amine base, pyridine in the reaction series. Using this method, reaction with the appropriate aryl boronic acid gave the *N*-aryl products (**3**) in good yields. The coupled products were confirmed by the presence of an NH singlet peak at δ_H 5.7–5.9 in ^{1}H NMR (Scheme 1). The preparation of the acids (**4**, **8** and **9**) was performed through saponification of the esters (**3, 1** and **7** respectively) with LiOH in THF and H_2O.[28,29] The acid (**4**) was obtained in good yield after acidification (pH 3) and extraction with diethyl ether and used without further purification (Scheme 1).

The amide derivatives (**5**) were prepared through the reaction of the acid (**4**) with an amine in the presence of EDC and HOBt in dichloromethane,[28,30] and obtained in good yield after purification by flash column chromatography. Introduction of the *N*-heterocycle involved reaction of the alcohol precursor (**3** or **5**) with either 1,1′-carbonyldiimidazole (CDI) and imidazole or 1,1′-carbonyldi-triazole (CDT) and triazole in acetonitrile[24] to give the imidazole and triazole derivatives (**1, 6**–**14**) (Scheme 1).

3. Biology

3.1. CYP26A1 inhibitory activity

The imidazole and triazole derivatives were evaluated for their retinoic acid metabolism (CYP26) inhibitory activity using a cell-free microsomal assay as previously described,[31,32] with radiolabelled [11,12-^{3}H] all-*trans* retinoic acid as the substrate. Liarozole (a non-selective CYP26 inhibitor[17,18]) and R116010[22] were included in all experiments as comparative standards.

The triazole methyl ester (**6**) showed inhibitory activity (IC_{50} 13 nM) comparable with the imidazole methyl ester lead (**1**, IC_{50} 10 nM) (Table 1), however a reduction was noted for the 4-carboxymethylphenyl derivative (**7**, IC_{50} = 95 nM). A considerable reduction in activity was observed for the imidazole carboxylic acid derivative (**8**, IC_{50} >1 μM) compared with the lead ester (**1**). In contrast, inhibitory activity for the dicarboxylic acid triazole derivative (**9**, IC_{50} = 85 nM) was retained compared with the parent diester (**7**). A reduced activity was observed for both the methyl amide imidazole (**10**, IC_{50} = 200 nM) and ethyl amide imidazole (**11**, IC_{50} = 250 nM) derivatives compared with the ester lead (**1**), with introduction of either methoxy or chloro groups at the 4-position of the phenyl ring resulting in a substantial loss in activity (4-methoxy **12**, IC_{50} >1 μM; 4-chloro **13**, IC_{50} = 1 μM). The triazole methyl amide (**14**, IC_{50} = 1 μM) showed a greater reduction in activity compared with the imidazole methyl amide (**10**, IC_{50} 200 = nM).

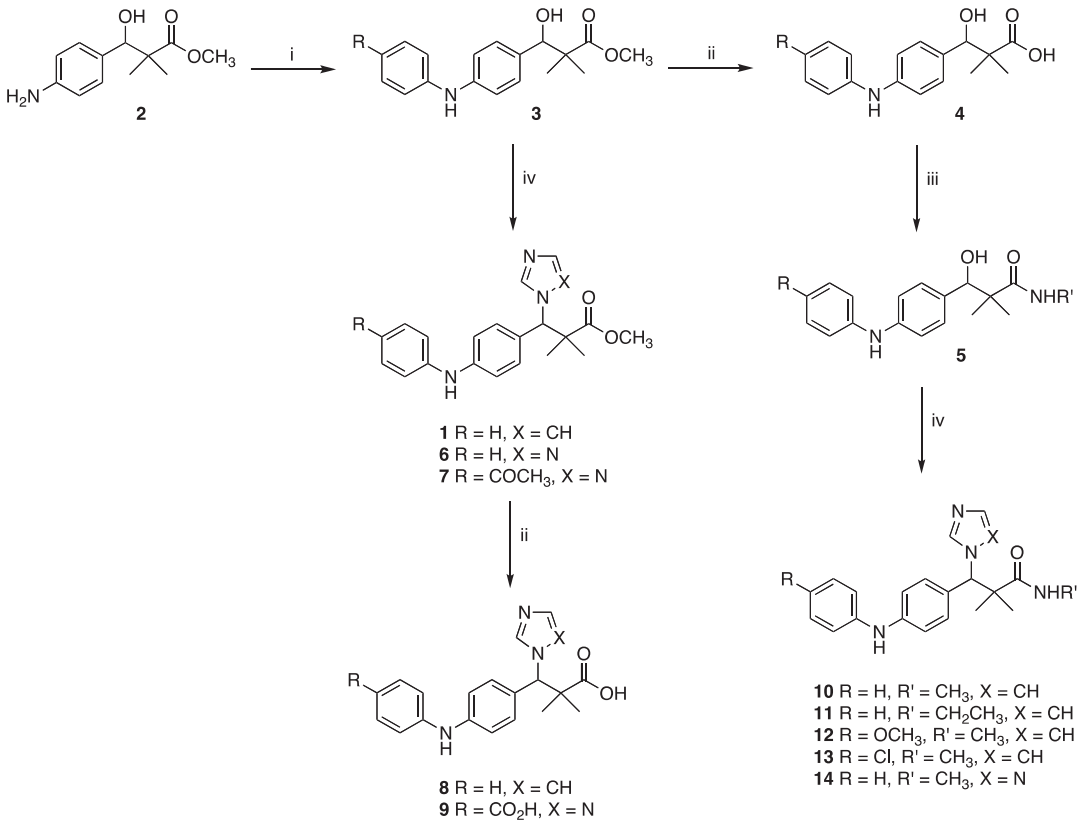
3.2. CYP selectivity

The triazole ester (**6**) and R116010 were evaluated for their inhibitory activity against a panel of P450 isoforms expressed in human liver microsomes (Table 2). The triazole ester (**6**) was borderline against CYP3A4 and inactive against the other four CYP enzymes in the panel. This compared favourably with the standard R116010, which showed high activity for CYP3A4 (Table 2).

To determine the selectivity of the triazole methyl ester (**6**) in comparison with R116010, IC_{50} assays against P450 isoforms expressed in human liver microsomes were performed. Micromolar concentrations of compounds **6** or R116010 were needed to inhibit CYPs 1A2, 2C9, 2C19and 2D6. However, both compounds inhibited CYP3A4 with sub-micromolar activity (triazole **6** IC_{50} 0.2 μM compared with R116010 IC_{50} 0.35 μM) (Table 3).

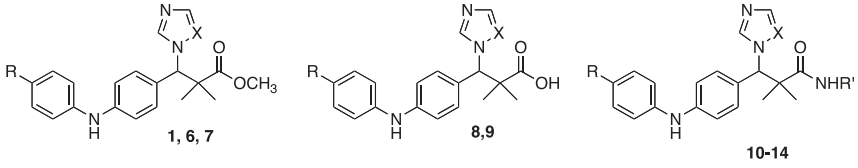
4. Discussion

Evaluation of CYP26 inhibitory activity revealed that the triazole methyl ester (**6**, IC_{50} = 13 nM) displayed comparable activity with the lead imidazole methyl ester (**1**, IC_{50} = 10 nM), both of



Scheme 1. Reagents and conditions: (i) Aryl boronic acid, CuOAc, pyridine, 4 Å molecular sieves, CH_2Cl_2, rt, 2 days; (ii) LiOH, THF, H_2O, 2 h, reflux then aqueous 1 N HCl; (iii) EDC, HOBt, CH_2Cl_2, 0.5 h, rt then $R'NH_2$, rt, overnight; (iv) 1,1′-carbonyldiimidazole, imidazole, CH_3CN, reflux or 1,1′-carbonylditriazole, triazole, CH_3CN, reflux, 24–48 h.

Table 1
IC_{50} values for different R substituents



Compd	R	R′	X	CYP26 IC_{50} (μM)[a]
1	H	—	CH	0.01
6	H	—	N	0.013
7	$COCH_3$	—	N	0.095
8	—	—	CH	>1.0
9	CO_2H	—	N	0.085
10	H	CH_3	CH	0.20
11	H	CH_2CH_3	CH	0.25
12	OCH_3	CH_3	CH	>1.0
13	Cl	CH_3	CH	1.0
14	H	CH_3	N	1.0
Liarozole	—	—	—	0.54
R116010	—	—	—	0.01

[a] IC_{50} values derived from the best fit of a 4 point dose–response curve.

which exhibited equal potency to the standard R116010 (IC_{50} = 10 nM). This contrasted with the comparable naphthalene imidazole and triazole inhibitors (Fig. 1) where an enhancement in activity was observed on going from the imidazole (IC_{50} = 3 nM) to the triazole derivative (IC_{50} = 0.3 nM). Replacement of the methyl ester group with a methyl amide isostere (**10**, IC_{50} = 200 nM) resulted in reduced activity for the imidazole derivative. Although the amides (**10** and **11**) were less active than the lead compound (**1**) they were more active than Liarozole (IC_{50} = 540 nM). This reduction in activity suggests that a H-bond donor is less favourable for CYP26 inhibition and this finding is magnified for the triazole methyl amide (**14**, IC_{50} = 1 μM), with a substantial reduction in inhibitory activity. To explore the effect of substitution at the 4-position of the phenyl ring, compounds

Table 2
Percent inhibition data against CYP panel at 0.4 μM

Compound	1A2	3A4	2C9	2C19	2D6
6	10	47	19	19	13
R116010	6	83	3	17	27

Inactive | Borderline | Highly active

Table 3
CYP IC_{50} (μM) profile of triazole **6** compared with R116010

Compd	1A2	2C9	2C19	3A4	2D6	26A1
6	8.5	9.1	4.2	0.20	1.3	0.013
R116010	8.3	11.0	5.9	0.35	3.9	0.01

with an electron donating group (methoxy **12**) and electron withdrawing group (chloro **13**) were prepared. The resulting enzyme inhibition data (Table 1) showed that neither substituent was tolerated, with a marked loss in activity observed. The carboxylic acid derivative (**8**) showed a loss in activity (**8**, IC_{50} >1 μM) compared with the ester lead inhibitor (**1**), potentially related to reduced uptake. However, the dicarboxylic acid triazole derivative (**9**) retained activity compared to the parent ester (**7**), suggesting that binding interaction rather than uptake is likely to account for the differences observed.

The triazole ester (**6**) was chosen for CYP selectivity as previous research[24] has shown improved CYP selectivity for triazole compared with imidazole derivatives. Direct comparison of CYP selectivity profiles for compound **6** and R116010 revealed a similar profile, with decreased activity against CYPs 1A2, 2C9, 2C19 and 2D6 as compared with CYP26. However, both compounds exhibited activity against the known RA hydroxylase CYP3A4, with IC_{50} values of 0.2 μM for compound **6** and 0.35 μM for R116010. CYP3A4 liability would discount the development of these inhibitors for systemic use. However, as seen with Liarozole and R116010, this is less likely to be an issue for topical administration in combination with RA, suggesting that compound **6** represents an appropriate candidate for further evaluation and development for use in the treatment of dermatological diseases.

5. Experimental

5.1. Materials and methods: chemistry

[11,12-^{3}H] All *trans*-retinoic acid (37 MBq/mL) and Ultima Flo M scintillation fluid were purchased from Perkin Elmer (UK). Acetic acid and ammonium acetate were obtained from Fisher Scientific (UK). All solvents used for chromatography were HPLC grade from Fisher Scientific (UK). Liarozole was kindly donated by Janssen (High Wycombe, UK) and R116010 was kindly donated by Barrier Therapeutics Inc. (Princeton, NJ, USA)

^{1}H and ^{13}C NMR spectra were recorded with a Bruker 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). 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 and stored over 4Å molecular sieves, under nitrogen. All compounds were more than 95% pure.

5.1.1. General procedure for the Suzuki coupling preparation of compounds 3a–3d

To the appropriate aryl boronic acid (4.0 mmol), 3-(4-aminophenyl)-3-hydroxy-2,2-dimethylpropionic acid methyl ester (**2**) (2.2 mmol), anhydrous Cu(II)$(OAc)_2$ (3.0 mmol), pyridine (4.0 mmol) and 250 mg activated 4 Å molecular sieves under an atmosphere of air was added CH_2Cl_2 (15 mL) and the reaction stirred under air atmosphere at ambient temperature for 2 days. The product was isolated by direct flash column chromatography of the crude reaction mixture (petroleum ether/EtOAc 70:30 v/v).

5.1.1.1. 3-Hydroxy-2,2-dimethyl-3-(4-phenylaminophenyl)propionic acid methyl ester (3a, R = H). Prepared from the reaction of phenylboronic acid and (**2**) in 79% yield as a light brown oil. TLC (2:1 Petroleum ether/EtOAc, R_f = 0.53). ^{1}H NMR ($CDCl_3$): δ 1.17 (s, 3H, CH_3), 1.22 (s, 3H, CH_3), 3.21 (s, 1H, OH), 3.75 (s, 3H, $COOCH_3$), 4.86 (s, 1H, CH-OH), 5.91 (s, 1H, NH), 6.96 (t, J = 7.2 Hz, 1H, Ar), 7.03 (d, J = 7.8 Hz, 2H, Ar), 7.09 (d, J = 7.9 Hz, 2H, Ar), 7.21 (d, J = 7.8 Hz, 2H, Ar), 7.29 (t, J = 7.4 Hz, 2H, Ar). ^{13}C NMR ($CDCl_3$): δ 19.19 (CH_3), 22.95 (CH_3), 47.96 (C, C-dimethyl), 52.08 (CH_3-ester), 78.48 (CH, CH-OH), 116.56 (CH, Ar), 117.95 (CH, Ar), 121.04 (CH, Ar), 128.84 (CH, Ar), 129.56 (CH, Ar), 132.39 (C, Ar), 143.06 (C, Ar), 143.25 (C, Ar), 178.29 (C, $COOCH_3$). HRMS (EI) *m/z* Calcd for $C_{18}H_{21}NO_3$ $(M)^+$ 299.1516. Found 299.1521.

5.1.1.2. 3-Hydroxy-3-[4-(4-methoxy-phenylamino)phenyl]-2,2-dimethyl-propionic acid methyl ester (3b, R = OCH_3). Prepared from the reaction of 4-methoxyphenylboronic acid and (**2**) in 81% yield as a light brown oil. TLC (2:1 Petroleum ether/EtOAc, R_f = 0.45). ^{1}H NMR ($CDCl_3$): δ 1.13 (s, 3H, CH_3), 1.22 (s, 3H, CH_3), 3.25 (s, 1H, OH), 3.75 (s, 3H, $COOCH_3$), 3.81 (s, 3H, OCH_3), 4.79 (s, 1H, CH-OH), 5.88 (s, 1H, NH), 6.87 (m, 4H, Ar), 7.05 (d, J = 7.8 Hz, 2H, Ar), 7.11 (d, J = 8.0 Hz, 2H, Ar). ^{13}C NMR ($CDCl_3$): δ 19.13 (CH_3), 22.87 (CH_3), 47.97 (C, C-dimethyl), 52.00 (CH_3-ester), 55.54 (CH_3, OCH_3), 78.62 (CH, CH-OH), 114.44 (CH, Ar), 117.87 (CH, Ar), 122.40 (CH, Ar), 128.33 (CH, Ar), 131.00 (C, Ar), 144.78 (C, Ar), 155.17 (C, Ar), 178.26 (C, $COOCH_3$). HRMS (EI) *m/z* Calcd for $C_{19}H_{24}NO_4$ $(M+H)^+$ 330.1700. Found 330.1700.

5.1.1.3. 3-Hydroxy-3-[4-(4-chloro-phenylamino)phenyl]-2,2-dimethyl-propionic acid methyl ester (3c, R = Cl). Prepared from the reaction of 4-chlorophenylboronic acid and (**2**) in 70% yield as a brown oil. TLC (2:1 Petroleum ether/EtOAc, R_f = 0.58). ^{1}H NMR ($CDCl_3$): δ 1.09 (s, 3H, CH_3), 1.16 (s, 3H, CH_3), 3.22 (s, 1H, OH), 3.72 (s, 3H, $COOCH_3$), 4.83 (s, 1H, CH-OH), 5.71 (s, 1H, NH), 6.97 (d, J = 7.4 Hz, 4H, Ar), 7.23 (d, J = 7.3 Hz, 4H, Ar). ^{13}C NMR ($CDCl_3$): δ 19.42 (CH_3), 22.90 (CH_3), 47.89 (C, C-dimethyl), 52.08 (CH_3-ester), 78.82 (CH, CH-OH), 116.89 (CH, Ar), 118.22 (CH, Ar), 122.87 (C, Ar), 128.55 (CH, Ar), 129.01 (CH, Ar), 132.80 (C, Ar), 141.54 (C, Ar), 142.34 (C, Ar), 178.27 (C, $COOCH_3$). HRMS (EI) *m/z* Calcd for $C_{18}H_{21}ClNO_3$ $(M+H)^+$ 334.1204. Found 334.1205.

5.1.1.4. 3-Hydroxy-3-[4-(4-methoxycarbonyl-phenylamino)-phenyl]-2,2-dimethyl-propionic acid methyl ester (3d, R = CO_2CH_3). Prepared from reaction of 4-methoxycarbonyl-phenylboronic acid and (**2**) in 73% yield as a brown oil. TLC (2:1 Petroleum ether/EtOAc, R_f = 0.51). ^{1}H NMR ($CDCl_3$): δ 1.08 (s, 3H, CH_3), 1.16 (s, 3H, CH_3), 3.21 (s, 1H, OH), 3.65 (s, 3H, $COOCH_3$), 3.82 (s, 3H, $COOCH_3$-Ar), 4.83 (s, 1H, CH-OH), 5.72 (s, 1H, NH), 6.93 (d, J = 7.9 Hz, 2H, Ar), 7.07 (d, J = 7.8 Hz, 2H, Ar), 7.21 (d, J = 7.8 Hz, 2H, Ar), 7.88 (d, J = 7.6 Hz, 2H, Ar). ^{13}C NMR ($CDCl_3$): δ 19.16 (CH, CH_3), 22.69 (CH, CH_3), 47.88 (C, C-dimethyl), 51.88 (CH, CH_3-ester), 52.06 (CH, CH_3-ester-Ar), 78.23 (CH, CH-OH), 115.21 (CH, Ar), 119.23 (CH, Ar), 120.74 (C, Ar), 128.65 (CH, Ar), 130.82 (CH, Ar), 143.41 (C, Ar), 148.13 (C, Ar), 152.10 (C, Ar), 167.05 (C, $COOCH_3$-Ar), 178.09 (C, $COOCH_3$). HRMS (EI) m/z Calcd for $C_{20}H_{24}NO_5$ $(M+H)^+$ 357.1576. Found 357.1578.

5.1.2. General method for ester hydrolysis

To a solution of (**1** or **3**) (2 mmol) in THF (5 mL) was added H_2O (5 mL) containing LiOH (4 mmol) and the mixture was homogenised with methanol and then heated under gentle reflux for 2 h. The solvent was removed under reduced pressure and H_2O (100 mL) was added and the unreacted starting materials were extracted with diethyl ether (3 × 50 mL). The H_2O layer was acidified with 1 N HCl till pH 3 and extracted with diethyl ether (100 mL). The organic layer was dried ($MgSO_4$) filtered and the solvent removed under reduced pressure. The product was used crude in the following reaction for preparation of the amides (**5**).

5.1.2.1. 3-Hydroxy-2,2-dimethyl-3-(4-phenylaminophenyl)-propionic acid (4a, R = H). Prepared from (**3a**) in 76% yield as a brown oil. TLC (97:3 CH_2Cl_2/MeOH, R_f = 0.32). ^{1}H NMR (DMSO-d_6): δ 0.87 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 4.72 (s, 1H, CH-OH), 5.77 (s, 1H, OH), 5.91 (s, 1H, NH), 6.76–6.79 (m, 1H, Ar), 7.03–7.07 (m, 4H, Ar), 7.22–7.27 (m, 4H, Ar), 8.08 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 18.75 (CH_3), 23.32 (CH_3), 47.68 (C, C-dimethyl), 78.34 (CH, CH-OH), 116.81 (CH, Ar), 117.68 (CH, Ar), 121.29 (CH, Ar), 128.72 (CH, Ar), 129.67 (CH, Ar), 131.79 (C, Ar), 142.82 (C, Ar), 143.05 (C, Ar), 183.19 (C, COOH).

5.1.2.2. 3-Hydroxy-3-[4-(4-methoxy-phenylamino)phenyl]-2,2-dimethyl-propionic acid (4b, R = OCH_3). Prepared from (**3b**) in 71% yield as a brown oil. TLC (95:5 CH_2Cl_2/MeOH, R_f = 0.39). ^{1}H NMR ($CDCl_3$): δ 1.11 (s, 6H, CH_3), 3.35 (s, 1H, OH), 3.82 (s, 3H, OCH_3), 4.88 (s, 1H, CH-OH), 6.21 (s, 1H, NH), 6.86–6.90 (m, 4H, Ar), 7.06 (d, J = 7.7 Hz, 2H, Ar), 7.14 (d, J = 7.6 Hz, 2H, Ar). ^{13}C NMR ($CDCl_3$): δ 18.79 (CH_3), 23.38 (CH_3), 47.63 (C, C-dimethyl), 55.60 (CH_3, OCH_3), 78.47 (CH, CH-OH), 114.73 (CH, Ar), 114.81 (CH, Ar), 115.54 (CH, Ar), 121.81 (CH, Ar), 122.46 (CH, Ar), 128.69 (CH, Ar), 129.67 (CH, Ar), 130.40 (C, Ar), 135.45 (C, Ar), 145.08 (C, Ar), 155.45 (C, Ar), 182.73 (C, COOH).

5.1.2.3. 3-Hydroxy-3-[4-(4-chloro-phenylamino)phenyl]-2,2-dimethyl-propionic acid (4c, R = Cl). Prepared from (**3c**) in 61% yield as a brown oil. TLC (95:5 CH_2Cl_2/MeOH, R_f = 0.40). ^{1}H NMR ($CDCl_3$): δ .16 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 3.25 (s, 1H, OH), 4.90 (s, 1H, CH-OH), 6.19 (s, 1H, NH), 6.97–7.01 (m, 4H, Ar), 7.22–7.26 (m, 4H, Ar). ^{13}C NMR ($CDCl_3$): δ 18.76 (CH_3), 23.33 (CH_3), 47.65 (C, C-dimethyl), 78.28 (CH, CH-OH), 117.97 (CH, Ar), 119.10 (CH, Ar), 125.77 (C, Ar), 128.81 (CH, Ar), 129.32 (CH, Ar), 132.25 (C, Ar), 141.51 (C, Ar), 142.55 (C, Ar), 183.10 (C, COOH).

5.1.2.4. 3-Imidazol-1-yl-2,2-dimethyl-3-(4-phenylaminophenyl)propionic acid (8, R = H, X = CH). Prepared from (**1**) [24] in 62% yield as a brown oil. TLC (95:5 CH_2Cl_2/MeOH, R_f = 0.29). ^{1}H NMR (DMSO-d_6): δ 1.22 (s, 6H, CH_3), 5.88 (s, 1H, CH-OH), 6.82–6.85 (m, 1H, Ar), 7.03–7.08 (m, 5H, Ar), 7.23–7.26 (m, 2H, Ar), 7.33–7.37 (m, 2H, Ar), 7.68 (s, 1H, Ar), 8.02 (s, 1H, Ar), 9.41 (s, 1H, NH), 15.05 (s, 1H, COOH). ^{13}C NMR (DMSO-d_6): δ 22.62 (CH_3), 23.26 (CH_3), 46.35 (C, C-dimethyl), 68.67 (CH, CH-imd), 115.54 (CH, Ar), 117.64 (CH, Ar), 120.46 (CH, Ar), 124.90 (C, Ar), 129.17 (CH, Ar), 129.93 (CH, Ar), 142.49 (C, Ar), 144.20 (C, Ar), 176.01 (C, COOH). HRMS (EI) m/z Calcd for $C_{20}H_{21}N_3O_2$ $(M+H)^+$ 336.1712. Found 336.1710.

5.1.2.5. 4-(4-(2-Carboxy-2-methyl-1-(1*H*-1,2,4-triazol-1-yl)propyl)phenylamino)benzoic acid (9, R = CO_2H, X = N). Prepared from (**7**) in 69% yield as a yellow solid; mp 136–140 °C. TLC (97:3 EtOAC/MeOH, R_f = 0.31). ^{1}H NMR (DMSO): δ 1.16 (s, 3H, CH_3), 1.27 (s, 3H, CH_3), 5.99 (s, 1H, CH-tri), 7.02 (d, J = 8.0 Hz, 2H, Ar), 7.13 (d, J = 7.9 Hz, 2H, Ar), 7.48 (d, J = 7.9 Hz, 2H, Ar), 7.79 (d, J = 7.8 Hz, 2H, Ar), 8.03 (s, 1H, NH), 8.64 (s, 1H, Ar), 8.75 (s, 1H, Ar). ^{13}C NMR (DMSO): δ 21.80 (CH, CH_3), 22.79 (CH, CH_3), 47.16 (C, C-dimethyl), 67.00 (CH, CH-tri), 114.36 (CH, Ar), 117.93 (CH, Ar), 120.63 (C, Ar), 128.82 (C, Ar), 130.13 (CH, Ar), 131.12 (CH, Ar), 141.35 (C, Ar), 147.64 (C, Ar), 151.39 (CH, Ar), 167.06 (C, COOH-Ar), 176.43 (C, COOH). EI-HRMS $(M-H)^+$ found 379.1405, Calcd for $C_{20}H_{19}N_4O_4$ 379.1403.

5.1.3. General method for the synthesis of the amides (5)

EDC (0.18 mmol), (**4**) (0.18 mmol) and HOBt (0.18 mmol) in CH_2Cl_2 (10 mL) were stirred at rt under N_2 for 0.5 h. Amine (0.56 mmol) was added and the reaction mixture was stirred overnight at rt EtOAc (70 mL) was added and the organic layer was washed with 1 M HCl (30 mL), saturated aqueous $NaHCO_3$ (30 mL), brine (30 mL) and H_2O (30 mL). The organic layer was dried ($MgSO_4$) filtered and reduced in vacuo. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH 100:0 v/v increasing to 98:2 v/v).

5.1.3.1. 3-Hydroxy-2,2,N-trimethyl-3-(4-phenylaminophenyl)-propionamide (5a, R = H, R′ = CH_3). Prepared from the reaction of (**4a**) and 33 % methylamine in absolute EtOH in 69% yield as a brown oil. TLC (97:3 CH_2Cl_2/MeOH, R_f = 0.49). ^{1}H NMR ($CDCl_3$): δ 1.05 (s, 3H, CH_3), 1.22 (s, 3H, CH_3), 2.76 (d, J = 6.2 Hz, 3H, CH_3-amide), 4.40 (s, 1H, OH), 4.52 (s, 1H, CH-OH), 5.88 (s, 1H, NH), 6.23 (s, 1H, NH-amide), 6.91 (t, J = 7.1 Hz, 1H, Ar), 7.00 (d, J = 7.6 Hz, 2H, Ar), 7.07 (d, J = 7.8 Hz, 2H, Ar), 7.16 (d, J = 7.8 Hz, 2H, Ar), 7.27 (t, J = 7.5 Hz, 2H, Ar). ^{13}C NMR ($CDCl_3$): δ 20.66 (CH_3), 24.44 (CH_3), 26.40 (CH_3-amide), 47.24 (C, C-dimethyl), 79.80 (CH, CH-OH), 116.90 (CH, Ar), 117.84 (CH, Ar), 121.31 (CH, Ar), 128.75 (CH, Ar), 129.66 (CH, Ar), 133.20 (C, Ar), 142.70 (C, Ar), 143.04 (C, Ar), 178.58 (C, CONH). EI-HRMS $(M + Na)^+$ found 321.1575, Calcd for $C_{18}H_{22}N_2O_2Na$ 321.1573.

5.1.3.2. N-Ethyl-3-hydroxy-2,2-dimethyl-3-(4-phenylaminophenyl)propionamide (5b, R = H, R′ = CH_2CH_3). Prepared from the reaction of (**4a**) and 2 M ethylamine in THF in 66% yield as a brown oil. TLC (97:3 CH_2Cl_2/MeOH, R_f = 0.51). ^{1}H NMR ($CDCl_3$): δ 1.04 (s, 3H, CH_3), 1.13 (t, J = 6.1 Hz, 3H, CH_3-amide), 1.27 (s, 3H, CH_3), 3.21–3.27 (m, 2H, CH_2-amide), 4.39 (s, 1H, OH), 4.53 (s, 1H, CH-OH), 5.31 (s, 1H, NH), 6.11 (s, 1H, NH-amide), 6.90 (t, J = 7.2 Hz, 1H, Ar), 7.03 (d, J = 7.8 Hz, 2H, Ar), 7.09 (d, J = 7.8 Hz, 2H, Ar), 7.17 (d, J = 7.7 Hz, 2H, Ar), 7.27 (t, J = 7.6 Hz, 2H, Ar). ^{13}C NMR ($CDCl_3$): δ 14.80 (CH_3-amide), 20.76 (CH_3), 24.50 (CH_3), 34.58 (CH_2-amide), 46.06 (C, C-dimethyl), 79.88 (CH, CH-OH), 116.61 (CH, Ar), 117.80 (CH, Ar), 121.33 (CH, Ar), 128.55 (CH, Ar), 129.64 (CH, Ar), 133.34 (C, Ar), 142.63 (C, Ar), 143.08 (C, Ar), 177.78 (C, CONH). EI-HRMS $(M + Na)^+$ found 335.1532, Calcd for $C_{19}H_{24}N_2O_2Na$ 321.1573.

5.1.3.3. 3-Hydroxy-3-[4-(4-methoxy-phenylamino)phenyl]-2,2, N-trimethyl-propionamide (5c, R = OCH_3, R′ = CH_3). Prepared from the reaction of (**4b**) and 33 % methylamine in absolute EtOH in 67% yield as a brown oil. TLC (97:3 CH_2Cl_2/MeOH, R_f = 0.44). ^{1}H NMR ($CDCl_3$): δ 1.03 (s, 3H, CH_3), 1.23 (s, 3H, CH_3), 2.77 (d, *J* = 6.4 Hz, 3H, CH_3-amide), 3.81 (s, 3H, OCH_3), 4.41 (s, 1H, OH), 4.52 (s, 1H, CH-OH), 5.58 (s, 1H, NH), 6.23 (s, 1H, NH-amide), 6.84–6.90 (m, 4H, Ar), 7.02–7.07 (m, 4H, Ar). ^{13}C NMR ($CDCl_3$): δ 20.62 (CH_3), 24.47 (CH_3), 26.41 (CH_3-amide), 46.25 (C, C-dimethyl), 55.59 (CH_3, OCH_3), 79.86 (CH, CH-OH), 114.66 (CH, Ar), 114.85 (CH, Ar), 122.11 (CH, Ar), 128.48 (CH, Ar), 131.79 (C, Ar), 135.65 (C, Ar), 144.66 (C, Ar), 155.22 (C, Ar), 178.60 (C, CONH). EI-HRMS (M + Na)$^+$ found 351.1681, Calcd for $C_{19}H_{24}N_2O_3Na$ 351.1682.

5.1.3.4. 3-Hydroxy-3-[4-(4-chloro-phenylamino)phenyl]-2,2,N-trimethyl-propionamide (5d, R = Cl, R′ = CH_3). Prepared from the reaction of (**4c**) and 33 % methylamine in absolute EtOH in 60% yield as a brown oil. TLC (97:3 CH_2Cl_2/MeOH, R_f = 0.48). ^{1}H NMR ($CDCl_3$): δ 1.07 (s, 3H, CH_3), 1.29 (s, 3H, CH_3), 2.82 (d, *J* = 6.6 Hz, 3H, CH_3-amide), 4.47 (s, 1H, OH), 4.63 (s, 1H, CH-OH), 5.74 (s, 1H, NH), 6.03 (s, 1H, NH-amide), 6.94–6.99 (m, 4H, Ar), 7.12–7.20 (m, 4H, Ar). ^{13}C NMR ($CDCl_3$): δ 20.60 (CH_3), 24.44 (CH_3), 26.43 (CH_3-amide), 46.19 (C, C-dimethyl), 79.82 (CH, CH-OH), 117.19 (CH, Ar), 118.85 (CH, Ar), 125.55 (C, Ar), 128.61 (CH, Ar), 129.27 (CH, Ar), 133.74 (C, Ar), 141.69 (C, Ar), 142.19 (C, Ar), 178.49 (C, CONH). EI-HRMS (M + Na)$^+$ found 355.1186, Calcd for $C_{18}H_{21}ClN_2O_2Na$ 355.1186.

5.1.4. General method for addition of the *N*-heterocyclic ring to prepare compounds 1, 6, 8–12

To a solution of alcohol (**3** or **5**) (1.5 mmol) in anhydrous CH_3CN (20 mL) was added imidazole (4.5 mmol) and CDI (2.25 mmol) or triazole (6 mmol) and CDT (3 mmol). The mixture was then heated under reflux for 24–48 h. The reaction mixture was allowed to cool and then extracted with EtOAc (150 mL) and H_2O (3 × 100 mL). The organic layer was dried ($MgSO_4$) filtered and reduced in vacuo. The product was purified by flash column chromatography.

5.1.4.1. Methyl 2,2-dimethyl-3-(4-(phenylamino)phenyl)-3-(1*H*-1,2,4-triazol-1-yl)propanoate (6, R = H, X = N). Prepared by the reaction of **3a** with CDT and triazole. After 24 h reflux, column chromatography (Petroleum Ether/EtOAc 70:30 v/v increasing to 50:50 v/v) gave this product in 68 % yield as a pale yellow oil. TLC (50:50 petroleum ether/EtOAc, R_f = 0.58). ^{1}H NMR ($CDCl_3$): δ 1.22 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 3.63 (s, 3H, $COOCH_3$), 5.71 (s, 1H, CH-tri), 6.10 (s, 1H, NH), 6.91–7.01 (m, 3H, Ar), 7.11 (d, *J* = 8.0 Hz, 2H, Ar), 7.29–7.33 (m, 2H, Ar),7.38 (d, *J* = 7.9 Hz, 2H, Ar), 7.93 (s, 1H, Ar), 8.15 (s, 1H, Ar). ^{13}C NMR ($CDCl_3$): δ 20.99 (CH_3), 23.95 (CH_3), 48.06 (C, C-dimethyl), 52.30 (CH_3-ester), 68.88 (CH, CH-tri), 116.23 (CH, Ar), 118.78 (CH, Ar), 121.76 (CH, Ar), 126.57 (C, Ar), 129.39 (CH, Ar), 130.44 (CH, Ar), 142.23 (C, Ar), 143.94 (C, Ar), 144.40 (CH, Ar), 151.46 (CH, Ar), 176.35 (C, $COOCH_3$). EI-HRMS (M+H)$^+$ found 351.1815, Calcd for $C_{20}H_{23}N_5O_2$ 351.1816.

5.1.4.2. Methyl 4-((4-(3-methoxy-2,2-dimethyl-3-oxo-1-(1*H*-1,2,4-triazol-1-yl)propyl)phenyl)amino)benzoate (7, R = CO_2CH_3, X = N). Prepared by the reaction of **3d** with CDT and triazole. After 24 h reflux, column chromatography (EtOAC/MeOH 100:0 v/v increasing to 98:2 v/v) gave this product in 63 % yield as a brown oil. TLC (99:1 EtOAC/MeOH, R_f = 0.48). ^{1}H NMR ($CDCl_3$): δ 1.23 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 3.64 (s, 3H, $COOCH_3$), 5.73 (s, 1H, CH-imd), 6.52 (s, 1H, NH), 6.96 (d, *J* = 8.0 Hz, 2H, Ar), 7.11 (d, *J* = 7.8 Hz, 2H, Ar), 7.43 (d, *J* = 7.7 Hz, 2H, Ar), 7.92 (d, *J* = 7.8 Hz, 2H, Ar), 7.95 (s, 1H, Ar), 8.11 (s, 1H, Ar). ^{13}C NMR ($CDCl_3$): δ 21.62 (CH, CH_3), 23.77 (CH, CH_3), 48.08 (C, C-dimethyl), 51.75 (CH, CH_3-ester-Ar), 52.33 (CH, CH_3-ester), 68.72 (CH, CH-imd), 115.28 (CH, Ar), 118.75 (CH, Ar), 121.66 (C, Ar), 128.79 (C, Ar), 130.51 (CH, Ar), 131.76 (CH, Ar), 141.67 (C, Ar), 147.26 (C, Ar), 166.87 (C, $COOCH_3$-Ar), 176.16 (C, $COOCH_3$). EI-HRMS (M+H)$^+$ found 409.1872, Calcd for $C_{22}H_{25}N_4O_4$ 409.1870.

5.1.4.3. 3-(1*H* -Imidazol-1-yl)-N,2,2-trimethyl-3-(4-(phenylamino)phenyl)propanamide (10). Prepared by the reaction of **5a** with CDI and imidazole. After 48 h reflux column chromatography (EtOAc/MeOH 100:0 v/v increasing to 90:10 v/v) gave this product in 46 % yield as a colourless oil. TLC (95:5 EtOAC/MeOH, R_f = 0.41). ^{1}H NMR ($CDCl_3$): δ 1.22 (s, 3H, CH_3), 1.26 (s, 3H, CH_3), 2.71 (d, *J* = 6.0 Hz, 3H, CH_3-amide), 5.53 (s, 1H, NH), 5.62 (s, 1H, CH-imid), 5.89 (s, 1H, NH-amide), 6.94–7.01 (m, 4H, Ar), 7.12 (d, *J* = 7.7 Hz, 2H, Ar), 7.19 (s, 1H, Ar), 7.26 (d, *J* = 7.8 Hz, 2H, Ar), 7.29–7.32 (m, 2H, Ar), 7.71 (s, 1H, Ar). ^{13}C NMR ($CDCl_3$): δ 22.37 (CH_3), 23.60 (CH_3), 26.80 (CH_3-amide), 47.82 (C, C-dimethyl), 68.04 (CH, CH-imid), 116.52 (CH, Ar), 118.75 (CH, Ar), 121.83 (CH, Ar), 127.93 (C, Ar), 129.41 (CH, Ar), 129.87 (CH, Ar), 142.18 (C, Ar), 143.65 (C, Ar), 176.04 (C, CONH). EI-HRMS (M+H)$^+$ found 349.2028, Calcd for $C_{21}H_{25}N_4O$ 349.2023.

5.1.4.4. *N*-Ethyl-3-(1*H*-imidazol-1-yl)-2,2-dimethyl-3-(4-(phenylamino)phenyl)propanamide (11). Prepared by the reaction of **5b** with CDI and imidazole. After 48 h reflux column chromatography (EtOAc/MeOH 100:0 v/v increasing to 90:10 v/v) gave this product in 38 % yield as a yellow oil. TLC (95:5 EtOAc/MeOH, R_f = 0.42). ^{1}H NMR ($CDCl_3$): δ 1.25–1.35 (m, 9H, CH_3, CH_3-amide), 3.26 (q, *J* = 7.2 Hz, 2H, CH_2-amide), 5.51 (s, 1H, NH), 5.62 (s, 1H, CH-imid), 5.84 (s, 1H, NH-amide), 6.95–7.01 (m, 3H, Ar), 7.11 (d, *J* = 7.8 Hz, 2H, Ar), 7.22 (s, 1H, Ar), 7.29 (d, *J* = 7.8 Hz, 2H, Ar), 7.29–7.35 (m, 3H, Ar), 7.72 (s, 1H, Ar). ^{13}C NMR ($CDCl_3$): δ 14.49 (CH_3-amide), 22.50 (CH_3), 23.50 (CH_3), 34.81 (CH_2-amide), 47.78 (C, C-dimethyl), 67.90 (CH, CH-imid), 116.62 (CH, Ar), 118.66 (CH, Ar), 121.80 (CH, Ar), 128.22 (C, Ar), 129.42 (CH, Ar), 129.93 (CH, Ar), 142.19 (C, Ar), 143.49 (C, Ar), 175.11 (C, CONH). EI-HRMS (M+H)$^+$ found 363.2184, Calcd for $C_{22}H_{27}N_4O$ 363.2184.

5.1.4.5. 3-(4-(4-Methoxyphenylamino)phenyl)-3-(1*H*-imidazol-1-yl)-N,2,2-trimethylpropanamide (12). Prepared by the reaction of **5c** with CDI and imidazole. After 48 h reflux column chromatography (EtOAc/MeOH 100:0 v/v increasing to 90:10 v/v) gave this product in 40 % yield as a yellow oil. TLC (95:5 EtOAc/MeOH, R_f = 0.38). ^{1}H NMR ($CDCl_3$): δ 1.27 (s, 3H, CH_3), 1.29 (s, 3H, CH_3), 2.89 (s, 3H, CH_3-amide), 4.79 (s, 1H, CH-imid), 5.52 (s, 1H, NH), 5.83 (s, 1H, NH-amide), 6.63 (d, *J* = 7.9 Hz, 2H, Ar), 6.81–6.90 (m, 3H, Ar), 7.05–7.11 (m, 1H, Ar), 7.28 (s, 3H, Ar). ^{13}C NMR ($CDCl_3$): δ 19.01 (CH_3), 23.08 (CH_3), 26.45 (CH_3-amide), 46.27 (C, C-dimethyl), 55.55 (CH_3, OCH_3), 67.88 (CH, CH-imid), 114.43 (CH, Ar), 114.67 (CH, Ar), 114.72 (CH, Ar), 123.11 (CH, Ar), 128.63 (CH, Ar), 131.88 (C, Ar), 134.72 (C, Ar), 144.15 (C, Ar), 155.16 (C, Ar), 177.99 (C, CONH). EI-HRMS (M+H)$^+$ found 379.2133, Calcd for $C_{22}H_{27}N_4O_2$ 379.2134.

5.1.4.6. 3-(4-(4-Chlorophenylamino)phenyl)-3-(1*H*-imidazol-1-yl)-N,2,2-trimethylpropanamide (13). Prepared by the reaction of **5d** with CDI and imidazole. After 48 h reflux column chromatography (EtOAc/MeOH 100:0 v/v increasing to 90:10 v/v) gave this product in 35 % yield as a yellow oil. TLC (95:5 EtOAc/MeOH, R_f = 0.44). ^{1}H NMR ($CDCl_3$): δ 1.25 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 2.79 (d, *J* = 6.1 Hz, 3H, CH_3-amide), 5.56 (s, 1H, NH), 5.65 (s, 1H, CH-imid), 5.79 (s, 1H, NH-amide), 6.93–6.99 (m, 3H, Ar), 7.05 (s, 1H, Ar), 7.11 (d, *J* = 7.9 Hz, 2H, Ar), 7.19–7.24 (m, 2H, Ar), 7.26–7.31 (m, 3H, Ar). ^{13}C NMR ($CDCl_3$): δ 19.25 (CH_3), 23.61 (CH_3), 26.48 (CH_3-amide), 46.19 (C, C-dimethyl), 67.34 (CH, CH-imid), 114.43 (CH, Ar), 114.89 (CH, Ar), 119.72 (CH, Ar), 123.25

(CH, Ar), 125.38 (C, Ar), 128.72 (CH, Ar), 133.76 (C, Ar), 142.28 (C, Ar), 143.05 (C, Ar), 177.83 (C, CONH). EI-HRMS $(M+H)^+$ found 383.1636, Calcd for $C_{21}H_{24}N_4OCl$ 383.1638.

5.1.4.7. N,2,2-trimethyl-3-(4-(phenylamino)phenyl)-3-(1*H*-1,2,4-triazol-1-yl)propanamide (14). Prepared by the reaction of **5a** with CDT and triazole. After 24 h reflux column chromatography (EtOAc/MeOH 100:0 v/v increasing to 99:1 v/v) gave this product in 67 % yield as a white solid; mp 106–110 °C. TLC (99:1 EtOAc/MeOH, $R_f = 0.37$). ^1H NMR ($CDCl_3$): δ 1.23 (s, 3H, CH_3), 1.25 (s, 3H, CH_3), 2.66 (d, $J = 6.0$ Hz, 3H, CH_3-amide), 5.80 (s, 1H, CH-tri), 5.83 (s, 1H, NH), 5.97 (s, 1H, NH-amide), 6.90–6.99 (m, 3H, Ar), 7.09 (d, $J = 8.0$ Hz, 2H, Ar), 7.25–7.29 (m, 2H, Ar), 7.46 (d, $J = 7.9$ Hz, 2H, Ar), 7.92 (s, 1H, Ar), 8.17 (s, 1H, Ar). ^{13}C NMR ($CDCl_3$): δ 21.33 (CH_3), 23.36 (CH_3), 26.62 (CH_3-amide), 48.41 (C, C-dimethyl), 68.66 (CH, CH-tri), 116.26 (CH, Ar), 118.58 (CH, Ar), 121.65 (CH, Ar), 127.04 (C, Ar), 129.38 (CH, Ar), 130.36 (CH, Ar), 142.34 (C, Ar), 143.54 (C, Ar), 145.06 (CH, Ar), 151.66 (CH, Ar), 176.07 (C, CONH). Anal. C, H, N. EI-HRMS $(M+H)^+$ found 350.1980, Calcd for $C_{20}H_{24}N_5O$ 350.1980.

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

MCF-7 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 sulphoxide and added to the culture medium as described by Armstrong et al.[23] Liarozole, R116010 and imidazole derivatives were dissolved in ethanol and diluted in cell culture medium. The final concentration of ethanol in all experiments never exceeded 0.8 %.

MCF-7 cells were pretreated for 24 h with 1 μM RA to induce CYP26 expression. Microsomes were prepared as described by Han and Choi.[32] Briefly, cells were homogenised in Buffer A (10 mM Tris, pH 7.4, 1 mM EDTA, 0.5 M sucrose, and Complete Protease Inhibitor Cocktail (Roche, UK)) using a Dounce homogeniser, diluted with an equal volume of Tris/EDTA, and the diluted homogenate laid over a volume of Buffer A equal to the original volume. Microsomes were then isolated by differential centrifugation (9000*g*, 10 min, 4 °C; 100,000*g*, 60 min, 4 °C). The microsomal pellet was suspended in Buffer B (10 mM Tris, pH 7.4, 1 mM EDTA, 0.25 M sucrose, Complete Protease Inhibitor Cocktail) and stored at −70 °C. Cytochrome *c* reductase activity was calculated at 5–15 U cyt *c*/μg protein, using the cytochrome c reductase (NADPH) kit (Sigma) according to the manufacturer's instructions. For ATRA metabolism, 50 μg microsomal protein was incubated in Assay buffer (50 mM Tris, pH 7.4, 150 mM KCl, 10 mM $MgCl_2$, 0.02 % w/v BSA, 2 mM NADPH, 10 nM ATRA, 0.1 μCi ^3H ATRA) in amber eppendorfs in the absence or presence of CYP26 inhibitor (1–1000 nM) in a final volume of 200 μL for 1 h at 37 °C with shaking. The reaction was quenched with acetonitrile, mixed, then centrifuged (18,000*g*, 5 min, 4 °C). Resolution of retinoids was performed with a Luna C18(2) column (3 μm, 50 × 2 mm) using a Waters 2690 Separations Module and subsequent Radiomatic Series 500TR Flow Scintillation Analyzer (Packard Biosciences), with Empower 2 Chromatography Data Software and Flow-ONE software respectively for data acquisition. ^3H ATRA and ^3H metabolites were separated by gradient reversed-phase chromatography, using mobile phase A (50% acetonitrile, 50% (0.2%) acetic acid, w/w) and mobile phase B (acetonitrile, 0.1% acetic acid, w/w). A flow rate of 0.3 mL/min was used with linear gradients employed between the specified times as follows: 0, 100% A; 5 min, 100% A; 5.5 min, 40% A, 60% B; 12 min, 40% A, 60% B; 12.5 min, 20% A, 80% B; 17.5 min, 20% A, 80% B; 18 min, 100%A; 25 min, 100% A. Scintillant flow rate was 1 mL/min. CYP26 inhibition was calculated as the percentage ^3H ATRA metabolite peak area formation (activity ^3H metabolite(s)/total activity) compared to metabolite formation in the absence of inhibitor. IC_{50} values were calculated by non-linear regression analysis in SigmaPlot (Systat Software Inc., USA) using an inhibition curve constructed from a minimum of four data points.

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