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Synthesis and CYP26A1 inhibitory activity of novel methyl 3-[4-(arylamino)phenyl]-3-(azole)-2,2-dimethylpropanoates

Mohamed S. Gomaa [a,†], Andrew S. T. Lim [a], S. C. Wilson Lau [a], Ann-Marie Watts [a], Nicola A. Illingworth [b], Caroline E. Bridgens [b], Gareth J. Veal [b], Christopher P. F. Redfern [b], Andrea Brancale [a], Jane L. Armstrong [c], Claire Simons [a,*]

[a] Medicinal Chemistry Division, 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

The role of all-*trans*-retinoic acid (ATRA) in the development and maintenance of many epithelial and neural tissues has raised great interest in the potential of ATRA and related compounds (retinoids) as pharmacological agents, particularly for the treatment of cancer, skin, neurodegenerative and autoimmune diseases. The use of ATRA or prodrugs as pharmacological agents is limited by a short half-life in vivo resulting from the activity of specific ATRA hydroxylases, CYP26 enzymes, induced by ATRA in liver and target tissues. For this reason retinoic acid metabolism blocking agents (RAMBAs) have been developed for treating cancer and a wide range of other diseases.

The synthesis, CYP26A1 inhibitory activity and molecular modeling studies of novel methyl 3-[4-(arylamino)phenyl]-3-(azole)-2,2-dimethylpropanoates are presented. From this series of compounds clear SAR can be derived for 4-substitution of the phenyl ring with electron-donating groups more favourable for inhibitory activity. Both the methylenedioxyphenyl imidazole (**17**, IC_{50} = 8 nM) and triazole (**18**, IC_{50} = 6.7 nM) derivatives were potent inhibitors with additional binding interactions between the methylenedioxy moiety and the CYP26 active site likely to be the main factor. The 6-bromo-3-pyridine imidazole **15** (IC_{50} = 5.7 nM) was the most active from this series compared with the standards liarozole (IC_{50} = 540 nM) and R116010 (IC_{50} = 10 nM).

1. Introduction

Differentiation and maintenance of many epithelial and neural tissues is dependent on intracellular formation of retinoic acid and its delivery to ligand-dependent transcription factors (retinoic acid receptors or RARs) in the cell nucleus.[1,2] Activity of this signalling pathway in relation to differentiation is exquisitely controlled by the expression of dehydrogenases and binding proteins to generate all-*trans* retinoic acid (ATRA) from retinol, and subsequent negative feedback by the induction of metabolism to limit and reduce intracellular ATRA levels.[3–5] These tight developmental controls have raised great interest in the potential of ATRA and related compounds (retinoids) as pharmacological agents, particularly for the treatment of cancer, and skin, neurodegenerative and autoimmune diseases.

The development of retinoids as pharmacological agents has progressed through two main routes—the design of synthetic retinoids which mimic ATRA by binding to and activating RARs, but which have greater biological stability, and the administration of ATRA, or pro-drugs such as 13-*cis* retinoic acid, at pharmacological doses.[6] While synthetic retinoids have been successful in certain contexts, some have suffered from high toxicity in vivo;[7] moreover, the design of these compounds needs to take into consideration the ability to bind to intracellular transport proteins (e.g., CRABPS) and to permit the ligand-dependent conformational changes required for RARs to function as transcriptional regulators.[8] Conversely, the use of ATRA or prodrugs as pharmacological agents is limited by a short ATRA half-life of <1 h in vivo[9] resulting from the activity of specific ATRA hydroxylases induced by ATRA in target tissues.[10] Although ATRA can be metabolised by several promiscuous cytochrome p450 enzymes (CYP), of which CYP 3A4 and 2C8 may be the most important, specific RA hydroxylases of the CYP26 family (CYP26A1, 26B1 and 26C1) are central to regulating intracellular ATRA concentrations as part of precise developmental schedules.[11] Compounds that inhibit CYP26 have been developed; liarozole (6-[(3-chlorophenyl)-imidazol-1-ylmethyl]-1*H*-benzimidazole)

* Corresponding author. Tel.: +44 (0) 2920 876307; fax: +44 (0) 2920 874149.
E-mail address: SimonsC@Cardiff.ac.uk (C. Simons).
URL: http://www.cardiff.ac.uk/phrmy/staffinfo/CS/homepage/CS.html (C. Simons).
† Present address: Pharmaceutical Chemistry Department, Faculty of Pharmacy, Suez Canal University, Egypt.

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and talarozole (*N*-[4-(2-ethyl-1-[1,2,4-triazol-1-yl]butyl)phenyl]-1,3-benzothiazol-2-amine) (Fig. 1) have shown promise in clinical trials for the treatment of dermatological disease.[12,13] However, as a strong aromatase inhibitor, liarozole lacks specificity leading to unwanted side effects; talarozole is 750 times more potent than liarozole and has greater specificity towards CYP26.[12] In view of the potential of retinoic acid metabolism blocking agents (RAMBAs) for treating cancer and other diseases, identifying novel agents with greater potency, selectivity for CYP26 and good pharmacological profiles is a high priority.

We have recently synthesised and characterised a novel series of arylamine CYP26 inhibitors, with a naphthyl imidazole ([4-(imidazol-1-yl-phenyl-methyl)-phenyl]-naphthalen-2-yl-amine) (MCC147, Fig. 1) being the most potent with an IC_{50} against CYP26 of 0.5 μM.[14] Replacement of the phenyl ring with a flexible C3 chain resulted in a compound with a CYP26 IC_{50} of 3 nM (MCC154, Fig. 1), comparable with that of talarozole; retention of the 2-naphthyl and NH linker is important for maximal activity.[15] Although showing good selectivity for CYP26, this compound showed some activity against CYP3A4, a relatively promiscuous xenobiotic metabolising CYP.[15] Selectivity to CYP26 was increased from <33 to 1100-fold by replacing the imidazole with a triazole, with a concomitant increase in potency to an IC_{50} of 0.35 nM (MCC219, Fig. 1).[16] Further studies to assess the potency and selectivity of triazole and imidazole derivatives containing modifications to the flexible side-chain and phenyl substituents in place of the 2-naphthyl led to the identification of new potent CYP26 inhibitors.[17] Therefore, we have now explored the possibility that 4-substituted and 3,4,5-substituted phenyl derivatives in combination with the imidazole, triazole and 2-methylimidazole would improve activity and CYP26 selectivity while optimising the drug-like properties of these compounds.

2. Results and discussion

2.1. Chemistry

The N-arylation of the amine (**2**)[15] using the Chan–Lam coupling reaction, followed described methodology[18,19] employing a stoichiometric amount of copper and a tertiary amine base, pyridine in this 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).

Introduction of the N-heterocycle involved reaction of the alcohol precursor (**3**) with 1,1′-carbonyldiimidazole (CDI) and imidazole in acetonitrile[16] to give the imidazole derivatives (**1**, **4–12**) (Scheme 1).

To explore additional hydrogen bonding interactions within the CYP26 active site, the phenyl ring was replaced with a 6-bromo-3-pyridine ring and a methylenedioxyphenyl ring. Using the same Chan–Lam coupling methodology as described above, the alcohol precursors **13** and **14** were prepared (Scheme 2). Introduction of either imidazole or triazole ring, using CDI/imidazole or carbonylditriazole (CDT)/triazole,[16] gave the 6-bromo-3-pyridine derivatives (**15**, **16**) and the methylenedioxyphenyl derivatives (**17**, **18**).

Exchanging the imidazole haem binding moiety for 2-methylimidazole has been shown to improve CYP selectivity,[20,21] therefore the unsubstituted phenyl 2-methylimidazole derivative (**19**) and the methylenedioxyphenyl 2-methylimidazole derivative (**20**) were prepared by reaction of the precursor alcohols (**3a** and **14**, respectively) with 2-methyl imidazole and 1,1′-carbonylbis(2-methylimidazole) (Scheme 3).

2.2. CYP26A1 enzyme inhibition

The imidazole and triazole derivatives were evaluated for their ability to inhibit CYP26-mediated retinoic acid metabolism (CYP26) inhibitory activity using a cell-free microsomal assay as previously described,[14,22] with radiolabelled [11,12-^{3}H] all-*trans* retinoic acid as the substrate. Liarozole (a non-selective CYP26 inhibitor[23,24]) and R116010[25] (Fig. 1) were included in all experiments as comparative standards.

Introduction of an electron-withdrawing group in the 4-position of the phenyl ring for example F (**4**), Cl (**5**), CN (**9**) or NO_2 (**10**) reduced inhibitory activity (IC_{50} range from 250 nM to >1 μM) compared with the unsusbtituted phenyl derivative (**1**, IC_{50} = 10 nM), whereas introduction of an electron-donating group, for example CH_3 (**6**), CF_3 (**7**) retained activity compared with unsubstituted phenyl (**1**), with the exception the larger 4-methoxy derivative (**8**) for which a large loss in activity was observed. Multiple substitutions were well tolerated with comparable inhibitory activity observed for the 3,4,5-trimethoxy substituted phenyl derivative (**11**, IC_{50} = 45 nM) and the 3,5-dimethyl-4-methoxy

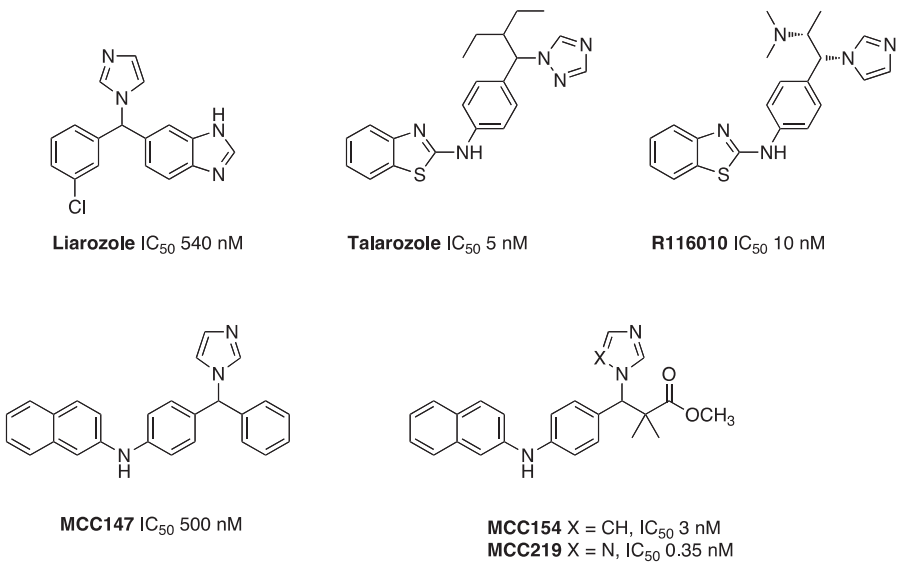
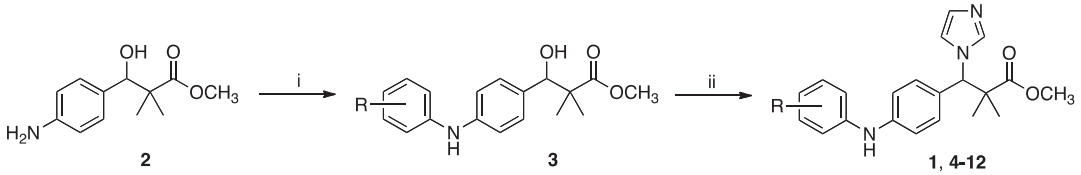
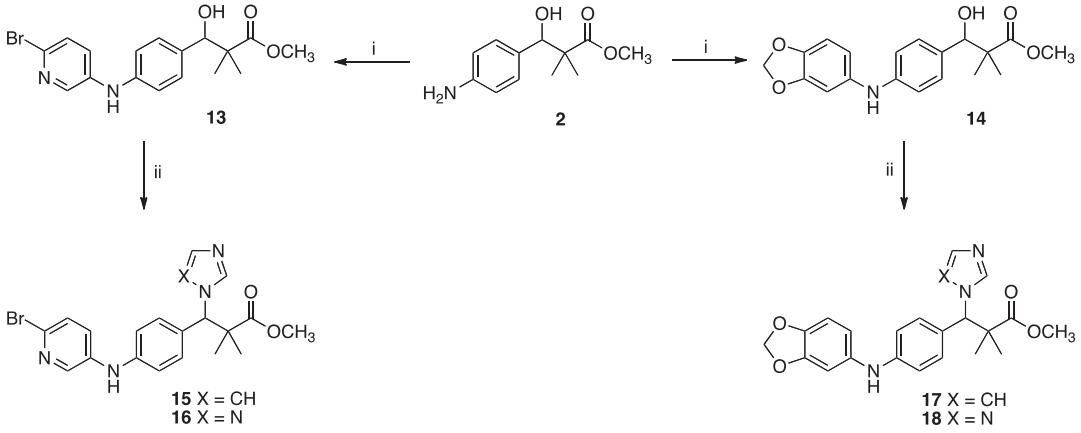


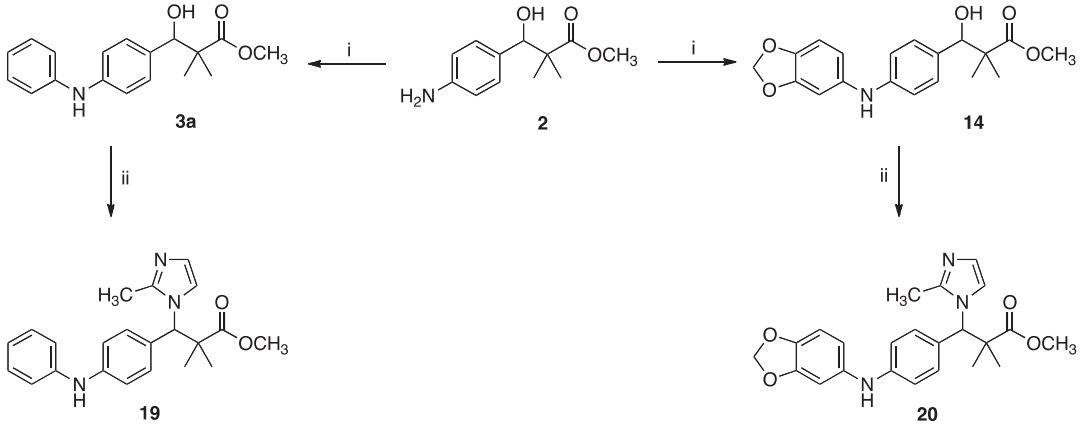
Figure 1. Examples of reported CYP26 inhibitors and inhibitory activity.



Scheme 1. Reagents and conditions: (i) aryl boronic acid, CuOAc, pyridine, 4 Å molecular sieves, CH_2Cl_2, rt, 2 days; (ii) 1,1′-carbonyldiimidazole, imidazole, CH_3CN, reflux, 24–48 h.



Scheme 2. Reagents and conditions: (i) 6-bromo-3-pyridinyl boronic acid or 3,4-(methylenedioxy)phenyl boronic acid, CuOAc, pyridine, 4 Å molecular sieves, CH_2Cl_2, rt, 2 days; (ii) 1,1′-carbonyldiimidazole, imidazole, CH_3CN, reflux, 24–48 h; (iii) 1,1′-carbonyditriazole, triazole, CH_3CN, reflux, 24–48 h.



Scheme 3. Reagents and conditions: (i) phenyl boronic acid or 3,4-(methylenedioxy)phenyl boronic acid, CuOAc, pyridine, 4 Å molecular sieves, CH_2Cl_2, rt, 2 days; (ii) 2-methylimidazole, 1,1′-carbonylbis(2-methylimidazole), CH_3CN, reflux, 24–48 h.

substituted phenyl derivative (**12**, IC_{50} = 14 nM) (Table 1). The most interesting results were observed with the 6-bromo-3-pyridine and methylenedioxyphenyl derivatives with improved CYP26 inhibitory activity observed for both imidazole derivatives (**15**, IC_{50} = 5.7 nM and **17**, IC_{50} = 8 nM, respectively). Activity was lost for the 6-bromo-3-pyridine triazole derivative (**16**, IC_{50} = 500 nM) but retained for the methylenedioxyphenyl triazole derivative (**18**, IC_{50} = 6.7 nM) (Table 1). Reduction in inhibitory activity was observed for the 2-methylimidazole derivatives (**19** and **20**) activity compared with the unsubstituted phenyl imidazole (**1**) (Table 1).

2.3. Molecular modelling

A series of molecular docking simulations were performed on this series of compounds with ligands docked within the active site of the CYP26A1 homology model,[26] using the methodology reported previously.[15] Results obtained for the phenyl derivatives **4**–**12** and methylenedioxyphenyl derivatives **17** and **18** showed a putative binding mode similar to the one observed previously for the corresponding phenyl imidazole analogue **1**. In particular, binding was dominated by a series of hydrophobic interactions: for the methylenedioxyphenyl inhibitor **18** the methylenedioxyphenyl group lay in a channel formed, among others, by Pro338, Phe53 and Phe343 while the ester moiety was in contact with Phe268 (Fig. 2). Furthermore, Ser192 is within hydrogen bond distance to the methylenedioxyphenyl ring.

The 6-bromo-3-pyridine imidazole derivative **15** was also positioned within the hydrophobic pocket (Fig. 3) with an additional hydrogen bond interaction between Ser84 and the pyridine nitrogen.

It should also be noted that the 2-methylimidazole compounds **19** and **20** did not dock that favourably, as the substitution induces a change in the compound conformations that do not allow coordination between the imidazole ring and the Fe atom (Fig. 4).

Table 1
IC_{50} values for imidazole, triazole and 3-methylimidazole derivatives

Compd	R_1	X	[a]IC_{50} (nM)
1	H	—	10
4	4-F	—	250
5	4-Cl	—	450
6	4-CH_3	—	16
7	4-CF_3	—	9.4
8	4-OCH_3	—	700
9	4-CN	—	>1 μM
10	4-NO_2	—	100
11	3,4,5-triOCH_3	—	45
12	3,5-diCH_3, 4-OCH_3	—	14
15	—	CH	5.7
16	—	N	500
17	—	CH	8
18	—	N	6.7
19	—	—	90
20	—	—	>10 nM
Liarozole	—	—	540
R116010	—	—	10

[a] IC50 values derived from the best fit of a 4 point dose–response curve.

3. Discussion

A clear SAR was observed for the 4-substituted and 3,4,5-substituted phenyl derivatives **4–12** with electron-withdrawing groups detrimental to inhibitory activity whereas electron-donating groups generally resulted in inhibitory activity comparable with the unsubstituted phenyl imidazole **1**. The methylenedioxyphenyl inhibitors **18** and **19** were able to interact within the active site in a manner similar to the phenylimidazole **1** with additional hydrogen bonding interaction between the methylenedioxy ring and active site residues favourable for inhibitory activity. Both the imidazole and triazole displayed potent CYP26 inhibitory activity (**18**, IC_{50} = 8 nM; **19**, IC_{50} = 6.7 nM) comparable with the phenylimidazole **1** (IC_{50} = 10 nM) and the R116010 standard (IC_{50} = 10 nM) and was considerably more active than Liarozole (IC_{50} = 540 nM). The 6-bromo-3-pyridine imidazole derivative **15** was the most potent CYP26 inhibitor from this series (IC_{50} = 5.7 nM) however replacing the imidazole group with a triazole to give compound **16** resulted in a substantial loss in inhibitory activity. From our

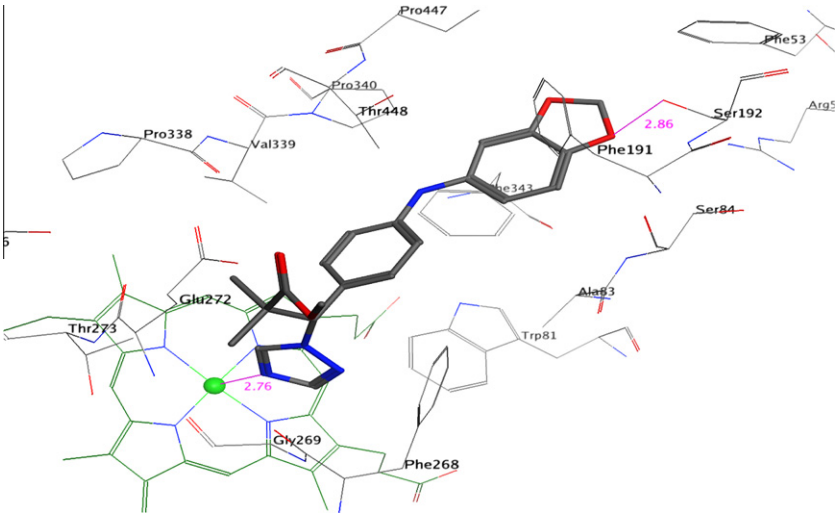


Figure 2. Putative binding mode for **18**. The Fe atom of the heme is shown as a green sphere.

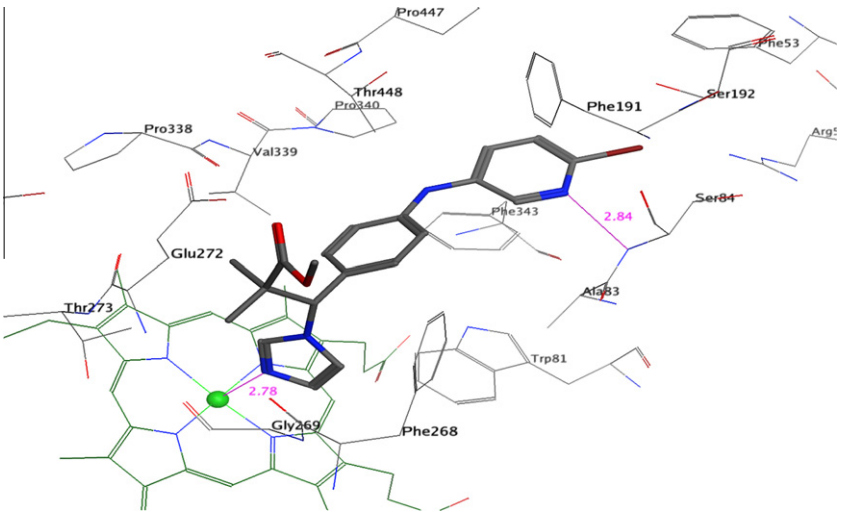


Figure 3. Putative binding mode for **15**. The Fe atom of the heme is shown as a green sphere.

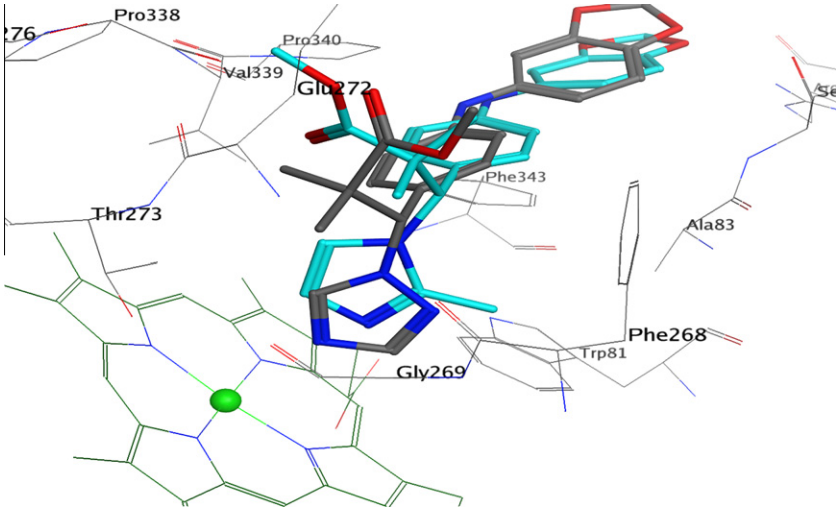


Figure 4. Different binding mode between **18** (gray) and **20** (cyan). The Fe atom of the heme is shown as a green sphere.

previous studies changing the imidazole for a triazole results in comparable[15,17] or improved[16] CYP26 inhibitory activity. At this stage, molecular modelling studies do not suggest any specific structural justification for this difference in activity. Finally, for both the phenyl and methylenedioxyphenyl derivates replacing the imidazole with 2-methylimidazole was unfavourable as a result of a different orientation of the imidazole ring in the binding pocket (Fig. 4).

4. Conclusions

From this series of compounds clear SAR can be derived for 4-substitution of the phenyl ring, with electron-donating groups more favourable for inhibitory activity. Both the methylenedioxyphenyl imidazole (**17**) and triazole (**18**) derivatives were potent inhibitors with additional binding interactions between the methylenedioxy moiety and the active site likely to be the main factor. The 6-bromo-3-pyridine imidazole **15** was the most active from this series and has the additional potential benefit of improved drug like properties, most notably the option to prepare as the hydrochloride salt to enhance aqueous solubility. Introduction of the pyridine ring does have limitations with replacement of the imidazole ring with a weakly basic azole resulting in loss of activity. Extention and further exploration of the pyridine series is warranted to develop SAR and optimise drug properties.

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).

^{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 4 Å molecular sieves, under nitrogen. All compounds were more than 95% pure.

Compound **3a** (R = H), **3c** (R = Cl), **3f** (R = OCH_3), **14** and **17** were prepared as previously described.[15,17]

5.1.1. General procedure for the Chan–Lam coupling preparation of compounds 3a–3j and 13

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-3-[4-(4-fluoro-phenylamino)-phenyl]-2,2-dimethyl-propionic acid methyl ester (3b, R = 4-F). Prepared from the reaction of 4-fluorophenylboronic acid and (**2**) in 65% yield as a brown oil. TLC (2:1 Petroleum ether/EtOAc, R_f = 0.55). ^{1}H NMR ($CDCl_3$): δ 1.11 (s, 3H, CH_3), 1.17 (s, 3H, CH_3), 3.17 (s, 1H, OH), 3.70 (s, 3H, $COOCH_3$), 4.81 (s, 1H, CH-OH), 5.66 (s, 1H, NH), 6.88 (d, J = 7.0 Hz, 2H, Ar), 6.99 (d, J = 6.9 Hz, 2H, Ar), 7.04–7.08 (m, 2H, Ar), 7.15 (d, J = 7.2 Hz, 2H, Ar). ^{13}C NMR ($CDCl_3$): δ 19.14 (CH, CH_3), 22.92 (CH, CH_3), 47.90 (C, C-dimethyl), 52.06 (CH, CH_3-ester), 78.46 (CH, CH-OH), 115.80 (CH, Ar), 115.98 (CH, Ar), 120.57 (CH, Ar), 128.43 (CH, Ar), 131.94 (C, Ar), 138.49 (C, Ar), 143.60 (C, Ar), 147.58 (C, Ar), 178.29 (C, $COOCH_3$). EI-HRMS $(M+Na)^+$ found 340.1321, calculated for $C_{18}H_{20}FNO_3Na$ 340.1319.

5.1.1.2. 3-Hydroxy-3-[4-(4-methyl-phenylamino)-phenyl]-2,2-dimethyl-propionic acid methyl ester (3d, R = 4-CH_3). Prepared from the reaction of 4-methylphenylboronic acid and (**2**) in 74% yield as a brown oil. TLC (2:1 Petroleum ether/EtOAc, R_f = 0.62). ^{1}H NMR ($CDCl_3$): δ 1.09 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 2.32 (s, 3H, CH_3-Ar), 3.13 (s, 1H, OH), 3.71 (s, 3H, $COOCH_3$), 4.82 (s, 1H, CH-OH), 5.69 (s, 1H, NH), 6.89 (d, J = 7.6 Hz, 2H, Ar), 7.04 (d, J = 7.7 Hz, 2H, Ar), 7.15 (d, J = 7.1 Hz, 2H, Ar), 7.22 (d, J = 7.2 Hz, 2H, Ar). ^{13}C NMR ($CDCl_3$): δ 19.12 (CH, CH_3), 23.04 (CH, CH_3), 26.54 (CH, CH_3-Ar), 47.90 (C, C-dimethyl), 52.07 (CH, CH_3-ester), 78.54 (CH, CH-OH), 115.92 (CH, Ar), 119.04 (CH, Ar), 125.12 (CH,

Ar), 128.61 (CH, Ar), 129.87 (C, Ar), 131.03 (C, Ar), 140.16 (C, Ar), 143.63 (C, Ar), 178.32 (C, $COOCH_3$). EI-HRMS $(M+H)^+$ found 314.1752, calculated for $C_{19}H_{24}NO_3$ 314.1751.

5.1.1.3. 3-Hydroxy-3-[4-(4-trifluoromethyl-phenylamino)-phenyl]-2,2-dimethyl-propionic acid methyl ester (3e, R = CF_3). Prepared from the reaction of 4-trifluoromethylphenylboronic acid and (**2**) in 71% yield as a brown oil. TLC (2:1 Petroleum ether/EtOAc, R_f = 0.58). ^1H NMR ($CDCl_3$): δ 1.06 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 3.16 (s, 1H, OH), 3.71 (s, 3H, $COOCH_3$), 4.89 (s, 1H, CH-OH), 6.04 (s, 1H, NH), 7.06 (d, J = 7.6 Hz, 2H, Ar), 7.14 (d, J = 7.8 Hz, 2H, Ar), 7.38 (d, J = 7.1 Hz, 2H, Ar), 7.51 (d, J = 7.0 Hz, 2H, Ar). ^{13}C NMR ($CDCl_3$): δ19.17 (CH, CH_3), 22.95 (CH, CH_3), 47.83 (C, C-dimethyl), 52.19 (CH, CH_3-ester), 78.38 (CH, CH-OH), 115.78 (CH, Ar), 119.31 (CH, Ar), 125.10 (CH, Ar), 127.84 (CH, Ar), 129.12 (C, Ar), 140.84 (C, Ar), 146.64 (C, Ar), 178.27 (C, $COOCH_3$). EI-HRMS $(M+Na)^+$ found 390.1289, calculated for $C_{19}H_{20}F_3NO_3Na$ 390.1287.

5.1.1.4. 3-Hydroxy-3-[4-(4-cyano-phenylamino)-phenyl]-2,2-dimethyl-propionic acid methyl ester (3g, R = 4-CN). Prepared from the reaction of 4-cyanophenylboronic acid and (**2**) in 62% yield as a reddish brown oil. TLC (2:1 Petroleum ether/EtOAc, R_f = 0.50). ^1H NMR ($CDCl_3$): δ 1.07 (s, 3H, CH_3), 1.17 (s, 3H, CH_3), 3.22 (s, 1H, OH), 3.71 (s, 3H, $COOCH_3$), 4.87 (s, 1H, CH-OH), 6.44 (s, 1H, NH), 6.95 (d, J = 7.6 Hz, 2H, Ar), 7.11 (d, J = 7.6 Hz, 2H, Ar), 7.26 (d, J = 7.5 Hz, 2H, Ar), 7.47(d, J = 7.6 Hz, 2H, Ar). ^{13}C NMR ($CDCl_3$): δ 19.21 (CH, CH_3), 22.87 (CH, CH_3), 47.87 (C, C-dimethyl), 52.14 (CH, CH_3-ester), 78.25 (CH, CH-OH), 101.33 (C, C-CN), 114.75 (CH, Ar), 116.40 (C, CN), 117.49 (CH, Ar), 130.90 (CH, Ar), 135.37 (CH, Ar), 139.71 (C, Ar), 143.23 (C, Ar), 152.18 (C, Ar), 178.17 (C, $COOCH_3$). EI-HRMS $(M-H)^+$ found 323.1396, calculated for $C_{19}H_{19}N_2O_3$ 323.1396.

5.1.1.5. 3-Hydroxy-3-[4-(4-nitro-phenylamino)-phenyl]-2,2-dimethyl-propionic acid methyl ester (3h, R = 4-NO_2). Prepared from the reaction of 4-nitrophenylboronic acid and (**2**) in 72% yield as a reddish brown oil. TLC (2:1 Petroleum ether/EtOAc, R_f = 0.53). ^1H NMR ($CDCl_3$): δ 1.19 (s, 3H, CH_3), 1.20 (s, 3H, CH_3), 3.15 (s, 1H, OH), 3.72 (s, 3H, $COOCH_3$), 4.85 (s, 1H, CH-OH), 6.73 (s, 1H, NH), 6.93 (d, J = 7.8 Hz, 2H, Ar), 7.13 (d, J = 7.4 Hz, 2H, Ar), 7.28 (d, J = 7.1 Hz, 2H, Ar), 8.09 (d, J = 7.0 Hz, 2H, Ar). ^{13}C NMR ($CDCl_3$): δ 19.23 (CH, CH_3), 22.87 (CH, CH_3), 47.86 (C, C-dimethyl), 52.35 (CH, CH_3-ester), 78.32 (CH, CH-OH), 113.77 (CH, Ar), 121.14 (CH, Ar), 126.20 (CH, Ar), 128.41 (CH, Ar), 136.04 (C, Ar), 139.23 (C, Ar), 139.56 (C, Ar), 150.22 (C, Ar), 178.22 (C, $COOCH_3$). EI-HRMS $(M-H)^+$ found 343.1291, calculated for $C_{18}H_{19}N_2O_5$ 343.1299.

5.1.1.6. 3-Hydroxy-3-[4-(3,4,5-trimethoxy-phenylamino)-phenyl]-2,2-dimethyl-propionic acid methyl ester (3i, R = 3,4,5-tri-OCH_3). Prepared from the reaction of 3,4,5-trimethoxyphenylboronic acid and (**2**) in 69% yield as a brown oil. TLC (2:1 Petroleum ether/EtOAc, R_f = 0.41). ^1H NMR ($CDCl_3$): δ 1.07 (s, 3H, CH_3), 1.15 (s, 3H, CH_3), 3.24 (s, 1H, OH), 3.59 (s, 3H, $COOCH_3$), 3.72 (s, 9H, OCH_3), 4.82 (s, 1H, CH-OH), 5.82 (s, 1H, NH), 6.37 (s, 2H, Ar), 6.95 (d, J = 7.5 Hz, 2H, Ar), 7.13 (d, J = 7.4 Hz, 2H, Ar). ^{13}C NMR ($CDCl_3$): δ19.16 (CH, CH_3), 22.88 (CH, CH_3), 47.88 (C, C-dimethyl), 52.01 (CH, CH_3-ester), 56.03 (CH, OCH_3), 56.43 (CH, OCH_3), 60.98 (CH, OCH_3), 78.40 (CH, CH-OH), 107.34 (CH, Ar), 116.39 (CH, Ar), 119.94 (CH, Ar), 128.92 (CH, Ar), 132.07 (C, Ar), 139.16 (C, Ar), 143.26 (C, Ar), 153.78 (C, Ar), 178.22 (C, $COOCH_3$). EI-HRMS $(M+H)^+$ found 390.1912, calculated for $C_{21}H_{28}NO_6$ 390.1911.

5.1.1.7. 3-Hydroxy-3-[4-(3,5-dimethyl-4-methoxy-phenylamino)-phenyl]-2,2-dimethyl-propionic acid methyl ester (3j, R = 3,5-di-OCH_3, 4-CH_3). Prepared from the reaction of 3,5-dimethyl-4-methoxyphenylboronic acid and (**2**) in 70% yield as a reddish brown oil. TLC (2:1 Petroleum ether/EtOAc, R_f = 0.53). ^1H NMR ($CDCl_3$): δ 1.08 (s, 3H, CH_3), 1.14 (s, 3H, CH_3), 2.25 (s, 6H, CH_3-Ar), 3.21 (s, 1H, OH), 3.70-3.74 (m, 6H, OCH_3-$COOCH_3$), 4.79 (s, 1H, CH-OH), 5.66 (s, 1H, NH), 6.72 (s, 2H, Ar), 6.91 (d, J = 7.6 Hz, 2H, Ar), 7.15 (d, J = 7.8 Hz, 2H, Ar). ^{13}C NMR ($CDCl_3$): δ16.30 (CH, CH_3-Ar), 16.50 (CH, CH_3-Ar), 19.23 (CH, CH_3), 22.91 (CH, CH_3), 47.93 (C, C-dimethyl), 52.11 (CH, CH_3-ester), 59.93 (CH, OCH_3), 78.81 (CH, CH-OH), 116.27 (CH, Ar), 119.63 (CH, Ar), 126.80 (CH, Ar), 131.52 (C, Ar), 137.80 (C, Ar), 143.82 (C, Ar), 157.91 (C, Ar), 178.26 (C, $COOCH_3$). EI-HRMS $(M+H)^+$ found 358.2013, calculated for $C_{21}H_{28}NO_4$ 358.2013.

5.1.1.8. Methyl 3-(4-(6-bromopyridin-3ylamino)phenyl)-3-hydroxy-2,2-dimethylpropanoate (13). Prepared from the reaction of 6-bromo-3-pyridinylboronic acid and (**2**) in 58% yield as pale orange crystals. Mp 120–124 °C. TLC (2:1 Petroleum ether/EtOAc, R_f = 0.55). ^1H NMR (DMSO-d_6): δ 0.94 (s, 3H, CH_3), 1.06 (s, 3H, CH_3), 3.61 (s, 3H, OCH_3), 4.77 (d, J = 4.4 Hz, 1H, CH-OH), 5.43 (d, J = 4.5 Hz, 1H, CH-OH), 7.06 (d, J = 8.4 Hz, 2H, Ar), 7.20 (d, J = 8.4 Hz, 2H, Ar), 7.41 (s, 2H, Py), 8.11 (d, J = 0.9 Hz, 1H, Py), 8.48 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ19.54 (CH_3, C-4), 21.38 (CH_3, C-5), 47.80 (C-dimethyl, C-3), 51.45 (OCH_3, C-1), 76.38 (CH(OH), C-6), 116.72 (2 × C, Ar), 125.42 (CH, Py), 127.82 (CH, Py), 128.96 (C, Py/Ar), 134.53 (C, Py/Ar), 138.24 (CH, Py), 140.21 (C, Py/Ar), 140.49 (C, Py/Ar), 176.60 (CO, C-2). EI-HRMS $(M+H)^+$ found 379.0654, calculated for $C_{17}H_{20}BrN_2O_3$ 379.0657.

5.1.2. General method for addition of the N-heterocyclic ring to prepare compounds 1, 6, 8–12, 15, 16, 18–20

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) or 2-methylimidazole (4.5 mmol) and 1,1′-carbonylbis(2-methylimidazole) (2.3 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.

5.1.2.1. Methyl 3-(1*H*-imidazol-1-yl)-3-(4-((4-fluorophenyl) amino)phenyl)-2,2-dimethylpropanoate (4). Prepared by the reaction of **3b** with CDI and imidazole. After 48 h reflux column chromatography (EtOAc–MeOH 100:0 v/v increasing to 99:1 v/v) gave this product in 44% yield as a yellow oil. TLC (99:1 EtOAc/MeOH, R_f = 0.58). ^1H NMR ($CDCl_3$): δ 1.23 (s, 3H, CH_3), 1.25 (s, 3H, CH_3), 3.63 (s, 3H, $COOCH_3$), 5.49 (s, 1H, CH-imid), 5.96 (s, 1H, NH), 6.88 (d, J = 7.8 Hz, 2H, Ar), 6.95–7.00 (m, 2H, Ar), 7.03–7.09 (m, 4H, Ar), 7.13 (d, J = 7.9 Hz, 2H, Ar), 7.62 (s, 1H, Ar). ^{13}C NMR ($CDCl_3$): δ 22.89 (CH_3), 23.41 (CH_3), 47.63 (C, C-dimethyl), 52.46 (CH_3-ester), 67.52 (CH-imid), 115.54 (CH, Ar), 115.95 (CH, Ar), 116.13 (CH, Ar), 121.55 (CH, Ar), 127.54 (C, Ar), 129.65 (CH, Ar), 138.00 (C, Ar), 138.02 (C, Ar), 144.39 (C, Ar), 176.27 (C, $COOCH_3$). EI-HRMS $(M)^+$ found 368.1768, calculated for $C_{21}H_{22}N_3O_2F$ 368.1769.

5.1.2.2. Methyl 3-(1*H*-imidazol-1-yl)-3-(4-((4-chlorophenyl) amino)phenyl)-2,2-dimethylpropanoate (5). Prepared by the reaction of **3c** with CDI and imidazole. After 48 h reflux column chromatography (EtOAc–MeOH 100:0 v/v increasing to 99:1 v/v) gave this product in 44% yield as a yellow oil. TLC (99:1 EtOAc/MeOH, R_f = 0.59). ^1H NMR ($CDCl_3$): δ 1.23 (s, 3H, CH_3), 1.25 (s, 3H, CH_3), 3.63 (s, 3H, $COOCH_3$), 5.48 (s, 1H, CH-imid), 5.93 (s, 1H, NH), 6.95 (d, J = 7.9 Hz, 2H, Ar), 6.99 (d, J = 7.7 Hz, 2H, Ar), 7.04 (s, 1H, Ar), 7.07 (s, 1H, Ar), 7.17 (d, J = 7.8 Hz, 2H, Ar), 7.22 (d, J = 7.8 Hz, 2H, Ar), 7.63 (s, 1H, Ar). ^{13}C NMR ($CDCl_3$): δ 22.97 (CH_3), 23.41 (CH_3), 47.64 (C, C-dimethyl), 52.41 (CH_3-ester),

67.56 (CH-imid), 116.78 (CH, Ar), 119.78 (CH, Ar), 126.39 (C, Ar), 128.56 (C, Ar), 129.36 (CH, Ar), 129.66 (CH, Ar), 140.91 (C, Ar), 143.19 (C, Ar), 176.18 (C, $COOCH_3$). EI-HRMS $(M)^+$ found 384.1471, calculated for $C_{21}H_{22}N_3O_2Cl$ 384.1470.

5.1.2.3. Methyl 3-(1*H*-imidazol-1-yl)-3-(4-((4-methylphenyl)amino)phenyl)-2,2-dimethylpropanoate (6). Prepared by the reaction of **3d** with CDI and imidazole. After 48 h reflux column chromatography (EtOAc–MeOH 100:0 v/v increasing to 99:1 v/v) gave this product in 56% yield as a yellow oil. TLC (99:1 EtOAc/MeOH, R_f = 0.70). ^{1}H NMR ($CDCl_3$): δ 1.23 (s, 3H, CH_3), 1.26 (s, 3H, CH_3), 2.32 (s, 3H, CH_3-Ar), 3.65 (s, 3H, $COOCH_3$), 5.51 (s, 1H, CH-imid), 5.90 (s, 1H, NH), 6.87 (d, J = 7.8 Hz, 2H, Ar), 7.03 (d, J = 7.9 Hz, 2H, Ar), 7.11–7.18 (m, 6H, Ar), 7.72 (s, 1H, Ar). ^{13}C NMR ($CDCl_3$): δ 20.78 (CH_3-Ar), 22.80 (CH_3), 23.59 (CH_3), 47.61 (C, C-dimethyl), 52.50 (CH_3-ester), 67.77 (CH-imid), 115.61 (CH, Ar), 119.80 (CH, Ar), 116.92 (C, Ar), 129.62 (CH, Ar), 129.94 (CH, Ar), 131.81 (C, Ar), 139.25 (C, Ar), 144.47 (C, Ar), 176.27 (C, $COOCH_3$). EI-HRMS $(M)^+$ found 364.2022, calculated for $C_{22}H_{25}N_3O_2$ 364.2020.

5.1.2.4. Methyl 3-(1*H*-imidazol-1-yl)-3-(4-((4-trifluoromethylphenyl)amino)phenyl)-2,2-dimethylpropanoate (7). Prepared by the reaction of **3e** with CDI and imidazole. After 48 h reflux column chromatography (EtOAc–MeOH 100:0 v/v increasing to 99:1 v/v) gave this product in 40% yield as a yellow oil. TLC (99:1 EtOAc/MeOH, R_f = 0.68). ^{1}H NMR ($CDCl_3$): δ 1.27 (s, 6H, CH_3), 3.62 (s, 3H, $COOCH_3$), 5.55 (s, 1H, CH-imid), 6.48 (s, 1H, NH), 7.05 (d, J = 7.9 Hz, 6H, Ar), 7.17 (d, J = 7.8 Hz, 2H, Ar), 7.44 (d, J = 7.8 Hz, 2H, Ar), 7.68 (s, 1H, Ar). ^{13}C NMR ($CDCl_3$): δ 23.04 (CH_3), 23.36 (CH_3), 47.61 (C, C-dimethyl), 52.43 (CH_3-ester), 67.56 (CH-imid), 116.15 (CH, Ar), 118.58 (CH, Ar), 125.71 (CH, Ar), 129.67 (CH, Ar), 129.97 (C, Ar), 141.78 (C, Ar), 145.94 (C, Ar), 176.12 (C, $COOCH_3$). EI-HRMS $(M)^+$ found 418.1734, calculated for $C_{22}H_{22}N_3O_2F_3$ 418.1734.

5.1.2.5. Methyl 3-(1*H*-imidazol-1-yl)-3-(4-((4-methoxyphenyl)amino)phenyl)-2,2-dimethylpropanoate (8). Prepared by the reaction of **3f** with CDI and imidazole. After 48 h reflux column chromatography (EtOAc–MeOH 100:0 v/v increasing to 99:1 v/v) gave this product in 53% yield as a yellow oil. TLC (99:1 EtOAc/MeOH, R_f = 0.55). ^{1}H NMR ($CDCl_3$): δ 1.23 (s, 3H, CH_3), 1.25 (s, 3H, CH_3), 3.66 (s, 3H, $COOCH_3$), 3.81 (s, 3H, OCH_3), 5.43 (s, 1H, CH-imid), 5.79 (s, 1H, NH), 6.83 (d, J = 7.8 Hz, 2H, Ar), 6.87 (d, J = 7.8 Hz, 2H, Ar), 7.01–7.09 (m, 6H, Ar), 7.61 (s, 1H, Ar). ^{13}C NMR ($CDCl_3$): δ 22.83 (CH_3), 23.29 (CH_3), 47.68 (C, C-dimethyl), 52.35 (CH_3-ester), 55.55 (OCH_3), 67.61 (CH, CH-imid), 114.71 (CH, Ar), 123.03 (CH, Ar), 125.71 (CH, Ar), 126.64 (C, Ar), 127.68 (CH, Ar), 129.59 (CH, Ar), 134.82 (C, Ar), 145.57 (C, Ar), 155.76 (C, Ar), 176.28 (C, $COOCH_3$). EI-HRMS $(M)^+$ found 380.1964, calculated for $C_{22}H_{25}N_3O_3$ 380.1960.

5.1.2.6. Methyl 3-(1*H*-imidazol-1-yl)-3-(4-((4-cyanophenyl)amino)phenyl)-2,2-dimethylpropanoate (9). Prepared by the reaction of **3g** with CDI and imidazole. After 48 h reflux column chromatography (EtOAc–MeOH 100:0 v/v increasing to 99:1 v/v) gave this product in 40% yield as a pale yellow oil. TLC (99:1 EtOAc/MeOH, R_f = 0.39). ^{1}H NMR ($CDCl_3$): δ 1.31 (s, 6H, CH_3), 3.65 (s, 3H, $COOCH_3$), 5.54 (s, 1H, CH-imid), 6.54 (s, 1H, NH), 7.02 (d, J = 7.9 Hz, 6H, Ar), 7.08–7.14 (m, 4H, Ar), 7.22 (d, J = 7.8 Hz, 2H, Ar), 7.50 (d, J = 7.9 Hz, 2H, Ar), 7.69 (s, 1H, Ar). ^{13}C NMR ($CDCl_3$): δ 23.12 (CH_3), 23.34 (CH_3), 47.57 (C, C-dimethyl), 52.49 (CH_3-ester), 67.49 (CH-imid), 102.34 (C, Ar), 115.60 (CH, Ar), 119.66 (C, Ar), 119.90 (CH, Ar), 129.74 (CH, Ar), 131.19 (C, Ar), 133.78 (CH, Ar), 140.59 (C, Ar), 147.15 (C, Ar), 176.03 (C, $COOCH_3$). EI-HRMS $(M)^+$ found 375.1812, calculated for $C_{22}H_{22}N_4O_2$ 375.1816.

5.1.2.7. Methyl 3-(1*H*-imidazol-1-yl)-3-(4-((4-nitrophenyl)amino)phenyl)-2,2-dimethylpropanoate (10). Prepared by the reaction of **3h** with CDI and imidazole. After 48 h reflux column chromatography (EtOAc–MeOH 100:0 v/v increasing to 98:2 v/v) gave this product in 36% yield as a yellow oil. TLC (99:1 EtOAc/MeOH, R_f = 0.35). ^{1}H NMR ($CDCl_3$): δ 1.32 (s, 6H, CH_3), 3.63 (s, 3H, $COOCH_3$), 5.54 (s, 1H, CH-imid), 6.54 (s, 1H, NH), 6.95 (d, J = 7.9 Hz, 2H, Ar), 7.04 (s, 1H, Ar), 7.09 (s, 1H, Ar), 7.16 (d, J = 7.9 Hz, 2H, Ar), 7.22 (d, J = 7.9 Hz, 2H, Ar), 7.70 (s, 1H, Ar), 8.12 (d, J = 7.7 Hz, 2H, Ar). ^{13}C NMR ($CDCl_3$): δ 23.13 (CH_3), 23.36 (CH_3), 47.56 (C, C-dimethyl), 52.52 (CH_3-ester), 67.51 (CH, CH-imid), 114.30 (CH, Ar), 120.55 (CH, Ar), 126.16 (CH, Ar), 129.77 (CH, Ar), 131.78 (C, Ar), 140.13 (C, Ar), 140.24 (CH, Ar), 149.45 (C, Ar), 176.00 (C, $COOCH_3$). EI-HRMS $(M)^+$ found 395.1709, calculated for $C_{21}H_{22}N_4O_4$ 395.1714.

5.1.2.8. Methyl 3-(1*H*-imidazol-1-yl)-3-(4-((3,4,5-trimethoxyphenyl)amino)phenyl)-2,2-dimethylpropanoate (11). Prepared by the reaction of **3i** with CDI and imidazole. After 48 h reflux column chromatography (EtOAc–MeOH 100:0 v/v increasing to 98:2 v/v) gave this product in 39% yield as a colourless oil. TLC (99:1 EtOAc/MeOH, R_f = 0.51). ^{1}H NMR ($CDCl_3$): δ 1.25 (s, 3H, CH_3), 1.27 (s, 3H, CH_3), 3.64 (s, 3H, $COOCH_3$), 3.81–3.86 (m, 9H, OCH_3), 5.52 (s, 1H, CH-imid), 5.84 (s, 1H, NH), 6.32 (s, 2H, Ar), 6.93 (d, J = 8.1 Hz, 2H, Ar), 7.02 (s, 1H, Ar), 7.08 (s, 1H, Ar), 7.13 (d, J = 8.0 Hz, 2H, Ar), 7.60 (s, 1H, Ar). ^{13}C NMR ($CDCl_3$): δ 22.95 (CH_3), 23.39 (CH_3), 47.69 (C, C-dimethyl), 52.37 (CH_3-ester), 56.13 (OCH_3), 61.02 (OCH_3), 67.56 (CH, CH-imid), 97.55 (CH, Ar), 116.02 (CH, Ar), 127.77 (C, Ar), 129.67 (CH, Ar), 133.56 (C, Ar), 138.14 (C, Ar), 144.19 (C, Ar), 153.87 (C, Ar), 176.21 (C, $COOCH_3$). EI-HRMS $(M)^+$ found 440.2180, calculated for $C_{24}H_{29}N_3O_5$ 440.2180.

5.1.2.9. Methyl 3-(1*H*-imidazol-1-yl)-3-(4-((3,5-dimethyl-4-methoxyphenyl)amino)phenyl)-2,2-dimethylpropanoate (12). Prepared by the reaction of **3j** with CDI and imidazole. After 48 h reflux column chromatography (EtOAc–MeOH 100:0 v/v increasing to 99:1 v/v) gave this product in 42% yield as a yellow oil. TLC (99:1 EtOAc/MeOH, R_f = 0.57). ^{1}H NMR ($CDCl_3$): δ 1.23 (s, 3H, CH_3), 1.26 (s, 3H, CH_3), 2.22 (s, 6H, CH_3-Ar), 3.61 (s, 3H, $COOCH_3$), 3.71 (s, 3H, OCH_3), 5.52 (s, 1H, CH-imid), 5.71 (s, 1H, NH), 6.74 (s, 2H, Ar), 6.91 (d, J = 8.0 Hz, 2H, Ar), 7.02 (s, 1H, Ar), 7.10 (s, 1H, Ar), 7.13 (d, J = 8.0 Hz, 2H, Ar), 7.61 (s, 1H, Ar). ^{13}C NMR ($CDCl_3$): δ 16.19 (CH, CH_3-Ar), 22.89 (CH_3), 23.44 (CH_3), 47.69 (C, C-dimethyl), 52.36 (CH, CH_3-ester), 59.88 (OCH_3), 67.55 (CH, CH-imid), 115.56 (CH, Ar), 120.27 (CH, Ar), 127.22 (C, Ar), 129.57 (CH, Ar), 131.75 (C, Ar), 137.40 (C, Ar), 144.62 (C, Ar), 152.35 (C, Ar), 176.30 (C, $COOCH_3$). EI-HRMS $(M)^+$ found 408.2275, calculated for $C_{24}H_{29}N_3O_3$ 408.2282.

5.1.2.10. Methyl 3-(4-(6-bromopyridin-3-ylamino)phenyl)-3-(1*H*-imidazol-1-yl)-2,2-dimethylpropanoate (15). Prepared by the reaction of **13** with CDI and imidazole. After 48 h reflux column chromatography (Petroleum ether–EtOAc 100:0 v/v increasing to CH_2Cl_2–MeOH 90:10 v/v) gave this product in 30% yield as a yellow oil. TLC (EtOAc, R_f = 0.15). ^{1}H NMR (DMSO-d_6): 1.20 (s, 6H, 2 × CH_3), 3.54 (s, 3H, OCH_3), 5.64 (s, 1H, CH-imid), 7.07 (d, J = 8.3 Hz, 2H, Ar), 7.34 (d, J = 8.4 Hz, 2H, Ar), 7.59 (m, 4H, 2Ar/2Py), 8.13 (d, J = 02.3 Hz, 1H, Py), 8.31 (s, 1H, Ar), 8.60 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 22.74 (CH_3), 22.86 (CH_3), 47.24 (C, C-dimethyl), 51.93 (OCH_3), 116.69 (2 × CH, Ar), 126.23 (CH, Py), 127.83 (CH, Py), 129.10 (C, Ar/Py), 129.67 (C, Ar/Py), 129.92 (2CH, Ar), 138.98 (CH, Py), 139.69 (C, Ar/Py), 141.71 (C, Ar/Py), 176.07 ($COOCH_3$). EI-HRMS $(M + H)^+$ found 429.0924, calculated for $C_{20}H_{22}BrN_4O_2$ 429.0921.

5.1.2.11. Methyl 3-(4-(6-bromopyridin-3-ylamino)phenyl)-3-(1*H*-1,2,4-triazol-1-yl)-2,2-dimethylpropanoate (16). Prepared by the reaction of **13** with CDT and triazole. After 48 h reflux column chromatography (Petroleum ether–EtOAc 100:0 v/v increasing to 100% CH_2Cl_2) gave this product in 54% yield as a yellow oil. TLC (EtOAc, R_f = 0.68). ^{1}H NMR (DMSO-d_6): 1.20 (s, 3H, CH_3), 1.24 (s, 3H, CH_3), 3.55 (s, 3H, OCH_3), 5.92 (s, 1H, CH-triazole), 7.08 (d, J = 8.7 Hz, 2H, Ar), 7.44 (m, 4H, 2Ar/2Py), 8.02 (s, 1H, triazole), 8.13 (dd, J = 0.7 Hz, 2.8 Hz, 1H, Py), 8.59 (s, 1H, NH), 8.64 (s, 1H, triazole). ^{13}C NMR (DMSO-d_6): δ 21.72 (CH_3), 22.71 (CH_3), 47.52 (C-dimethyl), 51.92 (CH), 67.17 (CH), 116.48 (2 × CH, Ar), 126.23 (CH, Py), 127.83 (CH, Py), 129.65 (C, Py/Ar), 130.25 (2 × CH, Ar), 138.97 (CH, Py), 139.71 (CH, Py/Ar), 141.82 (CH, Py/Ar), 151.43 (2 × CH, triazole), 175.03 (CO, C-2). EI-HRMS $(M+H)^+$ found 430.0876, calculated for $C_{19}H_{21}BrN_5O_2$ 430.0873.

5.1.2.12. Methyl 3-(4-benzo[*d*][1,3]dioxol-5-ylamino)phenyl)-3-(1*H*-1,2,4-triazol-1-yl)-2,2-dimethylpropanoate (18). Prepared by the reaction of **14** with CDT and triazole. After 48 h reflux column chromatography (Petroleum ether–EtOAc 100:0 v/v increasing to CH_2Cl_2–MeOH 95:5 v/v) gave this product in 51% yield as a brown oil. TLC (CH_2Cl_2–MeOH 95:5 v/v, R_f = 0.55). ^{1}H NMR (DMSO-d_6): 1.19 (s, 3H, CH_3), 1.23 (s, 3H, CH_3), 3.69 (s, 3H, OCH_3), 5.85 (s, 1H, CH-triazole), 5.96 (s, 2H, methylene CH_2), 6.55 (d, J = 7.2 Hz, 2H, Ar), 6.69 (s, 1H, Ar), 6.81 (d, J = 7.8 Hz, 1H, Ar), 6.90 (d, J = 7.3 Hz, 2H, Ar), 7.33 (d, J = 7.3 Hz, 2H, Ar), 8.00 (s, 1H, triazole), 8.03 (s, 1H, triazole), 8.62 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 21.63 (CH_3), 22.76 (CH_3), 47.56 (C-dimethyl), 51.88 (CH_3), 67.38 (CH), 100.73 (CH_2), 101.34 (CH, Ar), 108.46 (CH, Ar), 111.52 (CH, Ar), 114.20 (2 × CH, Ar), 125.28 (C, Ar), 130.06 (2 × CH, Ar), 137.11 (C, Ar), 141.61 (C, Ar), 144.71 (C, Ar), 147.68 (C, Ar), 151.33 (2 × CH, triaz), 175.13 ($COOCH_3$). EI-HRMS $(M+H)^+$ found 395.1708, calculated for $C_{21}H_{23}N_4O_4$ 395.1714.

5.1.2.13. Methyl 3-((4-phenylamino)phenyl)-3-(2-methyl-1*H*-imidazol-1-yl)-2,2-dimethylpropanoate (19). Prepared by the reaction of **3a** with 1,1′-carbonylbis(2-methylimidazole) and 2-methylimidazole. After 24 h reflux column chromatography (Petroleum ether–EtOAc 100:0 v/v increasing to CH_2Cl_2–MeOH 95:5 v/v) gave this product in 73% yield as a light brown crystalline solid. Mp 66–70 °C. TLC (CH_2Cl_2–MeOH 9:1 v/v, R_f = 0.50). ^{1}H NMR (DMSO-d_6): 1.25 (s, 6H, $C(CH_3)_2$), 2.30 (s, 3H, 2-CH_3-imid), 3.52 (s, 3H, OCH_3), 5.43 (s, 1H, CH), 6.78 (s, 1H, Ar), 6.84 (s, 1H, Ar), 7.05 (m, 4H, Ar), 7.23 (s, 4H, Ar), 7.45 (s, 1H, imid), 8.25 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 13.35 (CH_3, 2-CH_3-imid), 22.07 (CH_3), 24.22 (CH_3), 39.52 (C-dimethyl), 51.93 (CH_3), 64.80 (CH), 115.67 (CH, Ar), 117.22 (CH, Ar), 117.48 (CH, Ar), 120.07 (CH, Ar), 126.60 (CH, imid), 127.19 (2 × C, Ar), 129.13 (CH, Ar), 129.62 (CH, Ar), 142.85 (C, Ar), 143.24 (C, Ar), 175.43 ($COOCH_3$). Anal. Calcd for $C_{22}H_{25}N_3O_2{\cdot}0.7H_2O$ (376.06904): C, 70.26, H, 7.08, N, 11.17. Found: C, 70.57, H, 6.97, N, 10.56. EI-HRMS $(M+H)^+$ found 364.2025, calculated for $C_{22}H_{26}N_3O_2$ 364.2024.

5.1.2.14. Methyl 3-(4-benzo[*d*][1,3]dioxol-5-ylamino)phenyl)-3-(2-methyl-1*H*-imidazol-1-yl)-2,2-dimethylpropanoate (20). Prepared by the reaction of **14** with 1,1′-carbonylbis(2-methylimidazole) and 2-methylimidazole. After 48 h reflux column chromatography (Petroleum ether–EtOAc 100:0 v/v increasing to CH_2Cl_2–MeOH 95:5 v/v) gave this product in 47% yield as a light brown crystalline solid. Mp 62–64 °C. TLC (CH_2Cl_2–MeOH 95:5 v/v, R_f = 0.48). ^{1}H NMR (DMSO-d_6): 1.24 (s, 6H, $C(CH_3)_2$), 2.28 (s, 3H, 2-CH_3-imid), 3.52 (s, 3H, OCH_3), 5.40 (s, 1H, CH), 5.96 (s, 2H, methylene CH_2), 6.55 (d, J = 7.6 Hz, 1H, Ar), 6.69 (s, 1H, Ar), 6.77 (s, 1H, imid), 6.81 (d, J = 8.1 Hz, 1H, Ar), 6.89 (d, J = 7.8 Hz, 2H, Ar), 7.19 (d, J = 7.7 Hz, 2H, Ar), 7.44 (s, 1H, imid), 8.00 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 13.32 (CH_3, 2-CH_3-imid), 22.04 (CH_3), 24.21 (CH_3), 47.26 (C-dimethyl), 51.92 (CH_3), 64.81 (CH), 100.73 (CH_2), 101.32 (CH, Ar), 108.46 (CH, Ar), 111.47 (CH, Ar), 114.44 (2 × CH, Ar), 117.85 (CH, imid), 126.26 (C, Ar), 126.50 (CH, imid), 129.63 (2 × CH, Ar), 137.13 (C, Ar), 141.60 (C, Ar), 144.49 (C, Ar), 144.65 (C, Ar), 147.69 (C, Ar), 175.44 ($COOCH_3$). Anal. Calcd for $C_{23}H_{25}N_3O_4{\cdot}0.5H_2O$ (416.4758): C, 65.73, H, 6.39, N, 10.10. Found: C, 66.33, H, 6.29, N, 10.09. EI-HRMS $(M+H)^+$ found 408.1923, calculated for $C_{23}H_{26}N_3O_4$ 408.1921.

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 sulfoxide and added to the culture medium as described by Armstrong et al.[10] 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.[22] 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 ^{3}H 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 mm × 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. ^{3}H ATRA and ^{3}H 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 ^{3}H ATRA metabolite peak area formation (activity ^{3}H 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.

5.3. Molecular modeling

All molecular modeling studies were performed on a MacPro dual 2.66 GHz Xeon running Ubuntu. Ligand structures were built in MOE[27] minimised using the MMFF94x forcefield until a RMSD gradient of 0.05 kcal mol^{-1} Å^{-1} was reached. Docking simulations

were performed using PLANTS[28] (aco_ants 20; aco_evap 0.15; aco_sigma 5.0), with ligands docked within the active site of the CYP26A1 homology model[26] and the results were visualised in MOE.

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