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Bioorganic & Medicinal Chemistry 16 (2008) 1992–2010
Bioorganic & Medicinal Chemistry

Synthesis, biological evaluation and molecular modelling studies of methyleneimidazole substituted biaryls as inhibitors of human 17α-hydroxylase-17,20-lyase (CYP17). Part I: Heterocyclic modifications of the core structure

Carsten Jagusch,[a] Matthias Negri,[a] Ulrike E. Hille,[a] Qingzhong Hu,[a] Marc Bartels,[a] Kerstin Jahn-Hoffmann,[a] Mariano A. E. Pinto-Bazurco Mendieta,[a] Barbara Rodenwaldt,[b] Ursula Müller-Vieira,[b] Dirk Schmidt,[c] Thomas Lauterbach,[c] Maurizio Recanatini,[d] Andrea Cavalli[d] and Rolf W. Hartmann[a,*]

[a]*Pharmaceutical and Medicinal Chemistry, Saarland University, PO Box 151150, D-66041 Saarbrücken, Germany*
[b]*Pharmacelsus CRO, Science Park 2, D-66123 Saarbrücken, Germany*
[c]*Schwarz Pharma, Alfred-Nobel-Str. 10, D-40789 Monheim, Germany*
[d]*Department of Pharmaceutical Sciences, University of Bologna, Via Belmeloro 6, I-40126 Bologna, Italy*

Received 20 August 2007; revised 22 October 2007; accepted 30 October 2007
Available online 4 November 2007

Abstract—Novel chemical entities were prepared via Suzuki and S_N reaction as AC-ring substrate mimetics of CYP17. The synthesised compounds **1**–**31** were tested for activity using human CYP17 expressed in *Escherichia coli*. Promising compounds were tested for selectivity against hepatic CYP enzymes (3A4, 2D6, 1A2, 2C9, 2C19, 2B6). Two potent inhibitors (**27**, IC_{50} = 373 nM/**28**, IC_{50} = 953 nM) were further examined in rats regarding their effects on plasma testosterone levels and their pharmacokinetic properties. Compound **28** was similarly active as abiraterone and showed better pharmacokinetic properties (higher bioavailability, $t_{1/2}$ 9.5 h vs 1.6 h). Docking studies revealed two new binding modes different from the one of the substrates and steroidal inhibitors.

1. Introduction

Prostate cancer (PC) is currently the most common malignancy and age-related cause of death in elder men worldwide. In the US about 220,000 new cases of PC are expected to be newly diagnosed in the year 2007 and about 30,000 men will die of this disease.[1] Approximately 80% of human prostatic tumours are androgen dependent. Consequently the standard treatment of PC is orchidectomy or its medicinal equivalent the chemical castration by gonadotropin-releasing hormone (GnRH) analogues, which reduce the testicular androgen production. A major disadvantage of these treatments is that they do not affect the adrenal androgen production. Therefore it is frequently combined with androgen receptor antagonists (flutamide, cyproterone acetate) to reduce the stimulatory effects of the remaining androgens. However, this so-called 'combined androgen blockade' is not effective in all patients as due to mutations in the androgen receptor, anti-androgens might act as agonists.[2,3] Thus, the inhibition of CYP17 (P45017, 17α-hydroxylase-C17,20-lyase) is a promising alternative to the combination of anti-androgens and GnRH analogues because testicular and adrenal androgen biosynthesis will both be reduced.

CYP17, the cytochrome b_5 modulated key enzyme in androgen biosynthesis,[4] catalyses both the 17α-hydroxylation of pregnenolone and progesterone and the subsequent 17,20-lyase reaction cleaving the C17–C20

Keywords: Prostate cancer; 17α-Hydroxylase-17,20-lyase (CYP17) inhibitors; Steroidomimetics; Hepatic CYPs; Pharmacokinetic studies; Testosterone plasma concentrations; Docking studies; Molecular electrostatic potential maps.
* Corresponding author. Tel.: +49 681 302 2424; fax: +49 681 302 4386; e-mail: rwh@mx.uni-saarland.de

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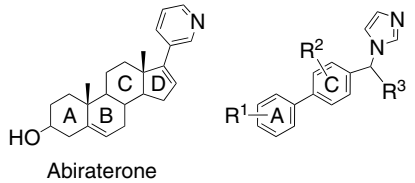
doi:10.1016/j.bmc.2007.10.094

bond to yield the 17-keto androgens androstendione and dehydroandrostendione (DHEA), the precursors of testosterone.[5]

Until now the antimycotic ketoconazole was the only CYP17 inhibitor used clinically for the treatment of advanced PC to reduce testosterone biosynthesis.[6,7] However, this drug is not a very potent inhibitor of CYP17 and relatively nonselective regarding the inhibition of other CYP enzymes. Besides it shows a number of notable side effects including liver damage. These drawbacks motivated us and others to look for more active and selective CYP17 inhibitors (for reviews, see[8–13]).

Recently, the steroidal CYP17 inhibitor abiraterone passed phase II clinical trials showing high activity in post-docetaxel castration refractory PC patients and seems to have no dose-limiting toxicity.[14] We also developed highly active steroidal inhibitors, which showed up to threefold higher activities against human CYP17 than abiraterone in vitro.[15] Because of the fact that steroidal drugs are known to show side effects which rely on their scaffold, we also developed nonsteroidal inhibitors.[16–18] Important for the mode of action of steroidal and nonsteroidal inhibitors is a nitrogen bearing heterocycle which is capable of complexing the haeme iron of the enzyme. In the class of imidazole-methyl substituted biphenyls potent inhibitors were discovered.[17,18] The biphenyl moiety of these inhibitors should mimic the A- and the C-ring of the substrates (Chart 1). Different substituents were introduced in the A-ring trying to achieve similar interactions as the oxygen at C3 of the substrates. In this study, we exchanged the A- or the C-phenyl nuclei by different heterocycles in order to improve the potency of these AC-ring mimetics. In some compounds (**27**–**31**) methyl and ethyl substituents were introduced in the methylene bridge between imidazole ring and biphenyl moiety as we have seen that methyl substituents as well as the rigidified indane compounds are appropriate to increase CYP17 inhibition in some cases.[17,18]

This approach was chosen to study other possible interactions between active site and ligand. In the following, the synthesis of the compounds shown in Scheme 1 and the determination of their inhibitory potency towards human CYP17 are described. Selected compounds were tested for their inhibition of hepatic CYP enzymes (3A4, 1A2, 2C9, 2C19, 2B6 and 2D6). Two potent inhibitors, compounds **27** and **28**, were examined for their potential



Abiraterone

Chart 1. Schematic representation of abiraterone and the scaffold of our biaryl inhibitors.

of reducing plasma testosterone levels in rats and their pharmacokinetic properties. Docking studies and molecular electrostatic potential (MEP) calculations were performed in order to elucidate some interesting structure–activity relationships.

2. Chemistry

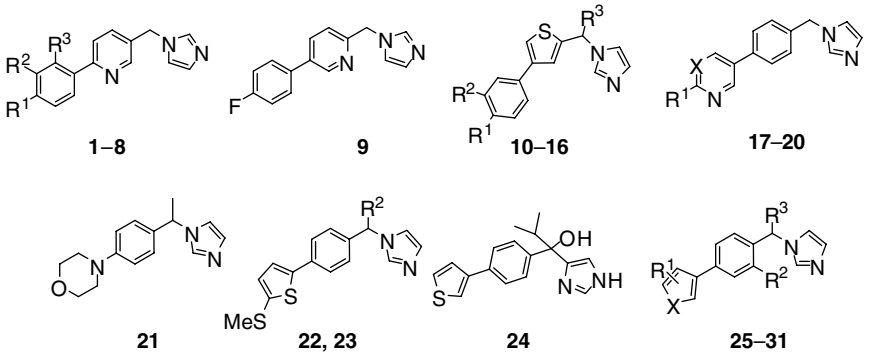
The syntheses of compounds **1**–**31** are shown in Schemes 2–8. After an eventual derivatisation of the A- or C-ring, both rings were coupled by means of a palladium catalysed Suzuki cross-coupling reaction (Method B), and a nitrogen containing heterocycle was attached subsequently. The derivatisations carried out on the rings were mostly brominations and reductions of the carbonyl compounds to the corresponding alcohols, since the Suzuki coupling is described to give higher yields when using carbonyl substituted compounds instead of alcohols.[19] For the synthesis of some compounds, the introduction of the imidazole moiety before the coupling was possible (Schemes 4 and 5), allowing a wider range of substitution patterns. Two different reaction types for the introduction of the *1H*-imidazole were used resulting in comparable yields, an S_N2 reaction between imidazole and the corresponding bromide (Schemes 2, 5; Method A), and an S_Nt reaction between 1,1-carbonyl diimidazole (CDI) and the corresponding alcohol[20] (Schemes 3, 4, 6 and 8; Method E). The carbonyl compound was generally reduced with $NaBH_4$ (Schemes 3, 4, 6 and 8; Method C). For the synthesis of compounds **14**, **30** and **31** the introduction of the alkyl substituent at the methylene bridge was performed via Grignard reaction (Schemes 3, 8; Method D). In order to introduce the C–C linked imidazole, a protection with trityl chloride was necessary before submitting the compound to a lithium-halogen exchange (Scheme 7) like reported previously by Tasaka and Kaku.[21]

3. Results

3.1. Biological results

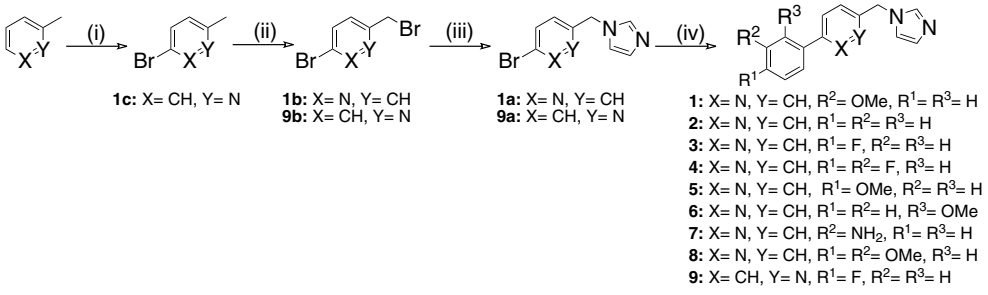
Inhibition of CYP17 was evaluated using human enzyme expressed in *Escherichia coli*.[22] The percent inhibition values of the compounds were determined with the 50,000 g sediment of the *E. coli* homogenate, progesterone (25 μM) as substrate and the inhibitors at a concentration of 0.2 and 2.0 μM. Separation of substrate and product was accomplished by HPLC using UV detection.[23a]

The inhibitory activities of the C-ring modified compounds **1**–**16** towards human CYP17 are shown in Table 1. It becomes apparent that the C-ring pyridyl compounds **1**–**9** are inactive, independent from their substitution pattern at the A-ring. On the contrary, the C-ring thiophenyl compounds **10**–**16** show low to moderate activity. In case of the methylene bridge unsubstituted compounds (**10**–**14**, **16**), it can be observed that the introduction of fluorine substituents and the 3-methoxy



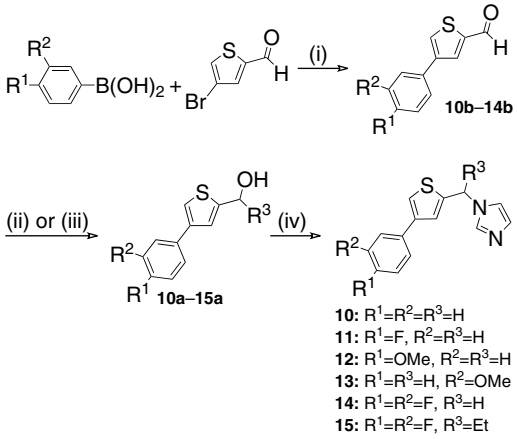
Compound	R^1	R^2	R^3	X
1	H	OMe	H	
2	H	H	H	
3	F	H	H	
4	F	F	H	
5	OMe	H	H	
6	H	H	OMe	
7	H	NH_2	H	
8	OMe	OMe	H	
10	H	H	H	
11	F	H	H	
12	OMe	H	H	
13	H	OMe	H	
14	F	F	H	
15	F	F	Et	
16	OMe	OMe	H	
17	H			CH
18	F			CH
19	CH_2Cl			CH
20	H			N
22		H		
23		Et		
25	H	H	H	O
26	4-Me	H	H	S
27	H	H	Et	S
28	H	H	Me	S
29	4-Me	H	Et	S
30	4-Me	F	Et	S
31	2-Cl	H	Et	S

Scheme 1. List of synthesised compounds **1**–**31**.



Scheme 2. Reagents and conditions: (i) $AlCl_3$, Br_2, 100 °C, 2.5 h; (ii) NBS, DBPO, CCl_4, 90 °C, 12 h; (iii) Method A: imidazole, K_2CO_3, acetonitrile, 90 °C, 12 h; (iv) Method B: Aryl-B(OH)2, Na_2CO_3, toluene/MeOH/H_2O, reflux, 5 h.

group in the A-ring does not alter the activity in regard to the unsubstituted compound whereas the 4-methoxy compounds (**12** and **16**) exhibit reduced activity. Interestingly, the introduction of an ethyl group at the meth-

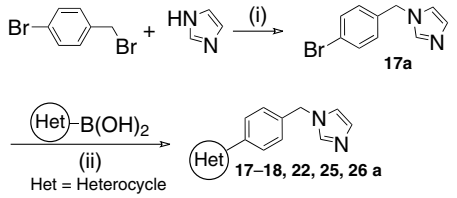


Scheme 3. Reagents and conditions: (i) Method B: $Pd(PPh_3)_4$, Na_2CO_3, toluene/EtOH/H_2O, reflux, 16 h; (ii) Method C: **10b**–**14b**: $NaBH_4$, MeOH, 2 h, rt; (iii) Method D: **14b**: EtMgBr, THF, 16 h, rt; (iv) Method E: CDI, acetonitrile, reflux, 8 h.

ylene bridge (compound **15**) leads to a strong increase in activity for compound **14**.

In Table 2 the inhibition values of the A-ring modified compounds **17**–**31** are presented in comparison to ketoconazole and abiraterone. Neither the substitution by a pyridine (**17**–**19**) nor a pyrimidine (**20**) or a morpholine (**21**) leads to moderate activity. On the other hand, in the class of the thiophenyl compounds **22**–**24** and **26**–**31** highly active inhibitors were obtained. The most active compounds bear an alkyl (methyl or ethyl) substituent at the methylene bridge. The ethyl compound **27** (IC_{50} = 373 nM) has a threefold higher activity compared to the methyl compound **28** (IC_{50} = 953 nM). The methyl group in 4-position of the thiophene in compound **29** (IC_{50} = 584 nM) slightly reduces the activity of compound **27**. Both the insertion of a fluorine atom at the C-ring (compound **30**, IC_{50} = 236 nM) and the exchange of the 4-methyl group by a 2-chloro substituent at the thiophene (compound **31**, IC_{50} = 263 nM) increased the potency and led to the most active inhibitors of this study.

The selectivity of selected compounds towards hepatic CYP enzymes was investigated in regard of their important role in drug metabolism. The most critical one is CYP3A4 as it is involved in the metabolism of 50% of the commercial drugs. Inhibition of this enzyme can result in severe side effects because of a known genetic polymorphism. The compounds tested showed inhibition of this enzyme (Table 2). However, it was significantly lower than that for ketoconazole which is used clinically as an antimycotic. Besides 3A4 five other hepatic enzymes



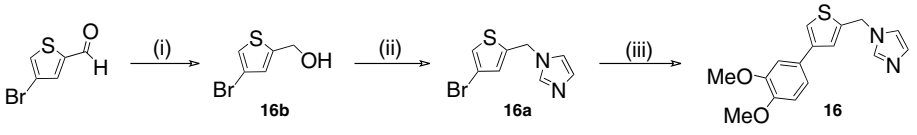
Scheme 5. Reagents and conditions: (i) Method A: K_2CO_3, 18-crown-6, acetone, reflux, 2.5 h; (ii) Method B: $Pd(PPh_3)_4$, Na_2CO_3, toluene/EtOH/H_2O, reflux, 16 h.

1A2, 2C9, 2C19, 2B6 and 2D6 are also important for drug metabolism; for CYP2D6 a genetic polymorphism is described too. In contrast to ketoconazole and abiraterone our compounds showed strong inhibitory activities at concentrations of 10 μM (Table 3).

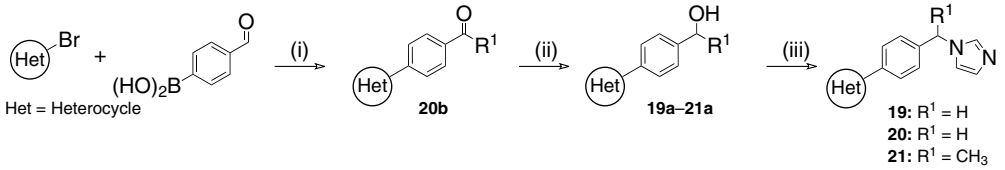
After it has been demonstrated that compounds **27** and **28** also inhibited the rat enzyme (Table 2), they were chosen for in vivo studies to investigate the influence of the methyl substituent versus the ethyl substituent at the methylene bridge. Both were evaluated for reduction of the plasma testosterone concentration (Table 4) and for pharmacokinetic parameters (Table 5) in male Wistar rats using abiraterone as a reference compound. All compounds reduced significantly the plasma testosterone levels. Compounds **27** and **28** showed strong effects already after 1 h. Abiraterone which was administered as a prodrug (acetate) exhibited maximum activity after 2 h. At 6 and 8 h abiraterone and compound **28** still showed high activity whereas compound **27** showed decreased effects. It is surprising that abiraterone is not more active than **28** since it is a stronger inhibitor of rat CYP17 (IC_{50} = 220 nM, Table 2). This finding can be explained by the pharmacokinetic properties of the compounds which differ strongly. From Table 5 it becomes apparent that abiraterone exhibits a low bioavailability. The plasma half-lives of compound **28** (9.5 h) and abiraterone (1.6 h) are also different. The comparatively long duration of activity seen after application of abiraterone acetate is caused by the fact that the ester cleavage gradually takes place.

3.2. Molecular modelling studies

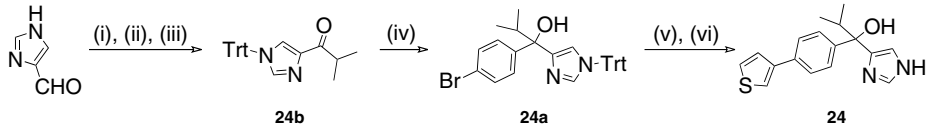
No crystal structure for CYP17 is currently available, for that reason we built a homology model based on the crystal structure of human CYP2C9. Based on the different activities of compounds shown in Tables 1 and 2, docking simulations were carried out by means of the GOLD v3.0.1 software.[24] The nonchiral compound **22**and both enantiomers of compounds **23** and



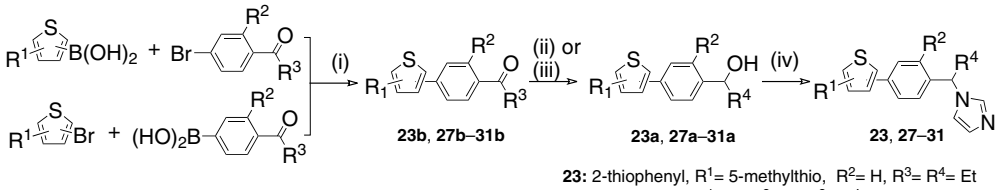
Scheme 4. Reagents and conditions: (i) Method C: $NaBH_4$, MeOH, 2 h, rt; (ii) Method E: CDI, acetonitrile, reflux, 8 h; (iii) Method B: Ar-$B(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, toluene/EtOH/H_2O, reflux, 16 h.



Scheme 6. Reagents and conditions: (i) Method B: $Pd(PPh_3)_4$, Na_2CO_3, toluene/EtOH/H_2O, reflux, 16 h; (ii) Method C: $NaBH_4$, MeOH, 2 h, rt; (iii) Method E: CDI, acetonitrile, reflux, 8 h.

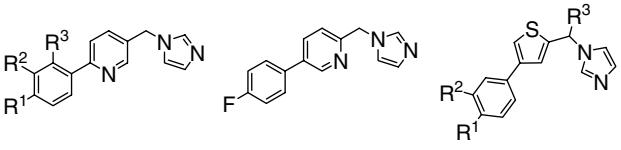


Scheme 7. Reagents: (i) NH_2OH_xHCl, acetanhydride, pyridine; (ii) 1—*i*-PrMgCl, THF/Et_2O; 2—H_2SO_4/H_2O; (iii) TrtCl, Et_3N, DCM; (iv) 1—*n*-BuLi, THF/Et_2O; 2—1,4-dibromobenzene; (v) Method B: 3-thiophenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME; (vi) pyridinex HCl, MeOH.



Scheme 8. Reagents and conditions: (i) Method B: $Pd(PPh_3)_4$, Na_2CO_3, reflux 6 h; (ii) Method C: **23b**, **27b**–**29b**: $NaBH_4$, THF, MeOH; (iii) Method D: **30b**–**31b**: EtMgCl, THF; (iv) Method E: CDI, NMP, reflux, 3h.

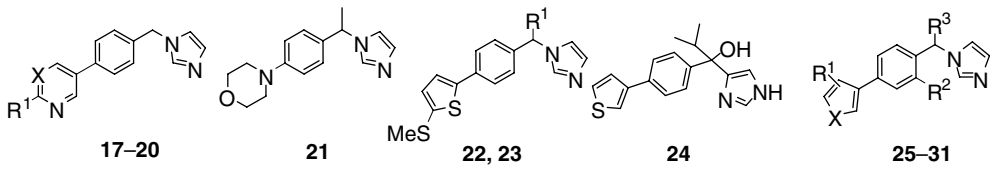
Table 1. Inhibition of CYP17 by compounds **1**–**16**



Compound	Structures			CYP17 % Inhibition[a]	
	R^1	R^2	R^3	0.2 μM	2 μM
1	H	OMe	H	0	1
2	H	H	H	0	4
3	F	H	H	5	5
4	F	F	H	0	0
5	OMe	H	H	0	0
6	H	H	OMe	0	5
7	H	NH_2	H	2	3
8	OMe	OMe	H	1	2
9				0	2
10	H	H	H	0	21
11	F	H	H	0	20
12	OMe	H	H	0	13
13	H	OMe	H	0	24
14	F	F	H	7	25
15	F	F	Et	17	68
16	OMe	OMe	H	0	14

[a] Concentration of progesterone (substrate): 25 μM; standard deviations were within <±5%.

Table 2. Inhibition of CYP17 and CYP3A4 by compounds **17**–**31**



Compound	Structures				Human CYP17[c]			Rat CYP17[c]	CYP3A4	
	R^1	R^2	R^3	X	% Inhibition		IC_{50} (nM)[d]	IC_{50} (nM)[d]	% Inhibition	
					0.2 μM	2 μM			1 μM	10 μM
17	H			CH	2	14				
18	F			CH	4	13				
19	CH_2Cl			CH	9	24				
20	H			N	5	3				
21					5	12				
22	H				3	35				
23	Et				2	3				
24					7	49				
25	H	H	H	O	3	29				
26	4-Me	H	H	S	0	27				
27	H	H	Et	S	28	83	373	562	88	92
28	H	H	Me	S	21	67	953	676	80	94
29	4-Me	H	Et	S	27	84	584			
30	4-Me	F	Et	S	41	92	236		88	97
31	2-Cl	H	Et	S	40	85	263		87	97
KTZ[a]						19[b]	2780		96	98
ABT[a]							72	220[e]	4	25

[a] KTZ, ketoconazole; ABT, abiraterone.
[b] % Inhibition at 1.0 μM.
[c] Concentration of progesterone (substrate): 25 μM; standard deviations were within <±5%.
[d] Concentration of inhibitors required to give 50% inhibition. The given values are mean values of at least three experiments. The deviations were within ±10%.
[e] Ref. 15.

Table 3. Inhibition of hepatic CYP enzymes by compounds **15**, **27** and **28**

Compound	CYP1A2 % Inhibition[b]		CYP2C9 % Inhibition[b]		CYP2C19 % Inhibition[b]		CYP2B6 % Inhibition[b]		CYP2D6 % Inhibition[b]	
	1 μM	10 μM	1 μM	10 μM	1 μM	10 μM	1 μM	10 μM	1 μM	10 μM
15	93	96	94	100	95	97				
27	98	100	81	74	95	100	60	87	24	77
28	97	100	75	89	87	99	49	87	17	68
KTZ[a]	8	38	21	75	24	79	11	57	1	4
ABT[a]	36	53	17	51	3	21	2	11	7	7

[a] KTZ, ketoconazole; ABT, abiraterone.
[b] Standard deviations were within <±5%.

27–**31** were docked to explore the active site of the enzyme and to investigate their binding mode. Based on the suggestion that our compounds are substrate mimetics, their binding mode was presumed to be similar to the one of the steroidal substrates and the steroidal inhibitors abiraterone and Sa40.[15] Abiraterone was docked into our protein model and the same binding mode as described for the substrates was found.[25,26] The lone pair of the sp^2 hybridised nitrogen pointed perpendicular towards the haeme iron. The steroidal scaffold was oriented almost parallel to the haeme plane in the direction of the BC-loop. This pose was stabilised by hydrophobic interactions with Ile371, Ile112, Ala113 and Phe114.[25,26] Additionally, the highly conserved Arg96 which is important for substrate binding and recognition, as shown by site-directed mutagenesis,[27] showed interactions of the same kind with the steroidal A-ring. Another important interaction was the H-bond between the hydroxy group in C3 position and the backbone carbonyl group of Gln98.

Table 4. Reduction of the plasma testosterone concentrations in rats by compounds **27** and **28**[a]

Compound	Relative plasma testosterone level[b] (%)				
	1 h	2 h	4 h	6 h	8 h
Control	143.1 ± 13.3	76.4 ±13.3	81.4 ± 24.6	109.6 ± 31.7	90.6 ± 22.8
27	37.6 ± 14.8[c]	30.5 ± 13.9[c]	26.9 ± 12.1[d]	62.6 ± 44.6	63.0 ± 26.4
28	30.9 ± 7.2[e]	n.d.	25.9 ± 8.1[e]	29.7 ± 12.6[c]	34.1 ± 13.5[d]
Abiraterone acetate	92.5 ± 43.1	44.0 ± 14.7 [c]	43.5 ± 12.4[d]	43.3 ±12.8[c]	35.6 ± 9.7[d]

n.d., not determined.

[a] Compounds **27** and **28** were applied at a dose of 50 mg/kg body weight, abiraterone acetate at a dose of 56.02 mg/kg (corresponding to 50 mg/kg abiraterone).

[b] The plasma testosterone concentrations at pre-treatment time points (−1, −0.5 and 0 h) were averaged and set to 100%. The values shown above are the relative levels compared to the pre-treatment value.

[c] $P < 0.05$.

[d] $P < 0.01$.

[e] $P < 0.001$.

Table 5. Pharmacokinetic properties of compounds **27** and **28**[a]

Compound	$t_{1/2\ z}$[b] (h)	t_{max}[b] (h)	C_{max}[b] (ng/mL)	$AUC_{0-\infty}$[b] (ng h/mL)	CL[b] (l/kg/h)
27	3.8	1.0	1745	6700	7.48
28	9.5	1.0	1277	24900	2.01
Abiraterone	1.6	2.0	592	4015	11.21

[a] **27** and **28** were applied at a dose of 50 mg/kg body weight, abiraterone acetate at a dose of 56.02 mg/kg (corresponding to 50 mg/kg abiraterone).

[b] $t_{1/2\ z}$, terminal half-life; t_{max}, time of maximal concentration; C_{max}, maximal concentration; $AUC_{0-\infty}$, area under the curve; CL, total body clearance (during elimination phase; assumes $F = 1$).

Based on the analysis of the docking results of both enantiomers of our chiral compounds we could identify the eutomers (scoring values and deviations of docked pose of ligand to its global energetic minimum were considered). The poses of compound **22** and of the eutomers of **23** and **27**–**31** indicated two different orientations compared to that of the steroidal inhibitors, a primary binding mode 1 (BM1: **22**, **23 R**, GoldScore values: in a range from 48.35 to 53.45; **29 R**, **30 R**, **31 R**, GoldScore values: in a range from 67.68 to 72.02), preferred from the statistical and interaction pattern points of view, and an alternative binding mode 2 (BM2: **27 S**, **28 S**, GoldScore values: in a range from 64.85 to 69.89) (Fig. 1). Compounds **22**–**23** and **27** showed poses in only one of the binding modes, while the other compounds were found in both ones with a clear preference for the binding mode as described above.

Starting point for these docking studies was the complexation of the haeme iron by the sp^2 hybridised imidazole nitrogen, as it is experimentally shown that CYP inhibitors with a sterically accessible aromatic nitrogen coordinate in this way.[15] Both binding modes have in common the anchoring of the alkyl substituent in a hydrophobic pocket (Fig. 1A and B) next to the haeme, which is delimitated in its extent by the alkyl side-chains of Ile371, Val366, Ala367 and Thr306 (Fig. 1A). The fact that compounds without methyl or ethyl substituents show no or little activity (Tables 1 and 2) indicates that interactions with this hydrophobic pocket are very important for inhibitory activity. Decisive for the different binding modes is the absence or presence of an *ortho*-substituent at the thiophenyl ring leading to planar, thin (**27** and **28**) or twisted, thicker (**29**–**31**) biaryl cores of the ligands. To better understand the influence of electron-withdrawing or -releasing substituents on both A- and C-rings on the binding mode, molecular electrostatic potential (MEP) maps were plotted with GaussView 3.0[42] in a range of ±12.5 kcal/mol.

3.3. Binding mode 2 (BM2)

Striking in this binding mode are the sterical limitations for the biaryl moiety. This is the reason why only ligands with a planar conjugated π-system (**27** and **28**) bind in this mode. The described anchoring of the alkyl substituent in the hydrophobic pocket causes the biaryl core of compounds **27** and **28** to cross the I-helix at the kink.[28] The thiophene–sulphur points towards the peptide bonds of Gln199 and Asn200 (F-helix), and undergoes electrostatic interactions with these amino acids. The thiophenyl ring forms hydrophobic interactions with the alkyl-groups of Gln199, Asn202, Glu203 and Ile206. The C-ring interacts with the hydrophobic alkyl side-chains of Val304 and Glu305 (Fig. 1).

3.4. Binding mode 1 (BM1)

Ligands which fit in this orientation carry an *ortho*-substituent (**29**–**31**). The latter disrupts the conjugated π-system, thus twisting the angle between both rings and demanding more space. This hydrophobic, thicker biaryl core comes to lie almost parallel to the I-helix (Fig. 1). The lipophilic region delimitated by the kink (Gly301-Ala302-Gly303) allows good π–π-stacking with the C-ring. As MEP maps have shown, an electron withdrawing group

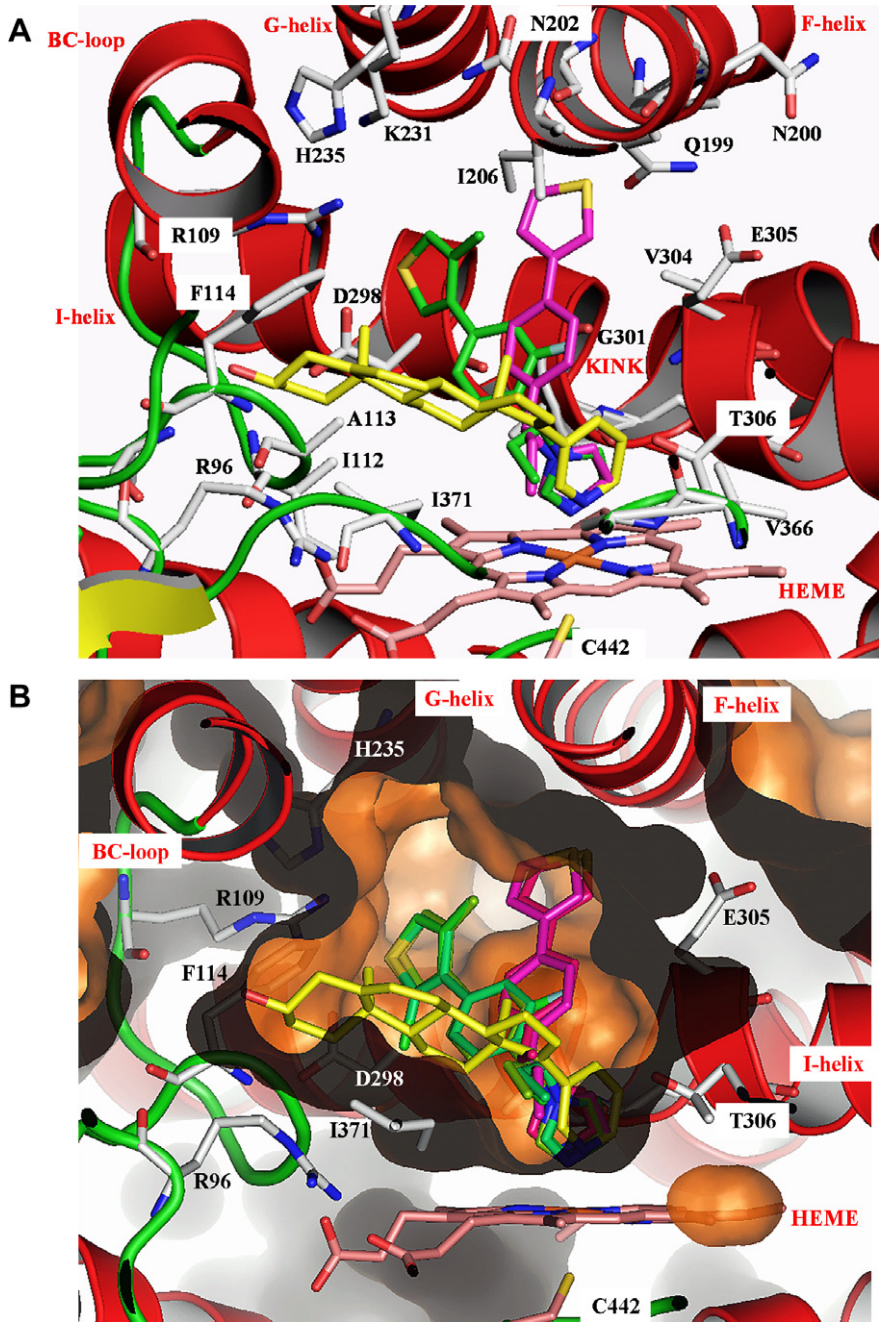


Figure 1. (A) Docking complexes between CYP17 and compounds **30** (green), **27** (magenta) and abiraterone (yellow). Further, haeme, interacting residues and ribbon rendered tertiary structure of the active site are shown. Figures were generated with Pymol (http://www.pymol.org). (B) A cross-section of the solvent accessible surface of CYP17 is shown in orange, revealing the active-site cavity with docked abiraterone (yellow) and all compounds in BM1 (**29**–**31**; green) and BM2 (**27**–**28** magenta).

at the C-ring, like fluorine (**30**), or in *ortho*-position on the thiophene, like chlorine (**31**), leads to a lower electron density on the C-ring (see Fig. 2). As a consequence of this, an improvement of the π–π interactions between the π-system of this ring and the protein environment, namely the I-helix, could be observed. As a further evidence of this observation, compounds **1**–**9**, bearing a pyridine as C-ring, highly increasing the electron density there (MEP map not shown), show a total loss of activity.

Additionally, the electronegative fluorine atom can form electrostatic interactions with the backbone atoms of Gly301, Ala302 and Val304, which is in agreement with the improved activity of compound **30**.

Further, in this binding mode the sulphur of the 3-thiophene points towards the polar side-chain of Arg109, allowing electrostatic interactions between the free electron pair (and perhaps d-orbitals) of the sulphur and the polar groups of Arg109 and Asp298. In addition the aromatic system of the thiophene ring undergoes T-shaped (edge-to-face) packing with Phe114, which is stabilised by Arg109, forming a very strong complex. The sulphur atom of the 2-thiophenyls (**22** and **23**) is located

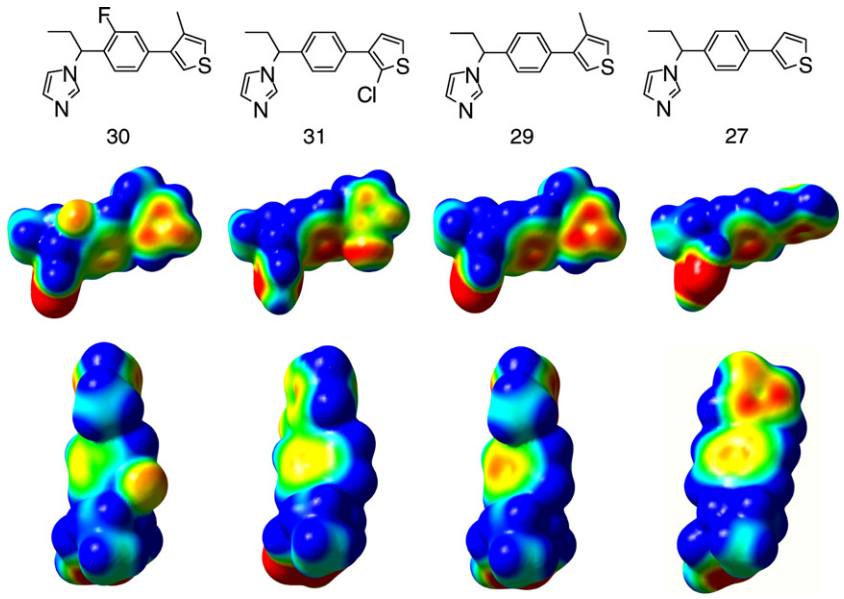


Figure 2. MEP of compounds **27**, **29**, **30** and **31**. The electrostatic potential surfaces were plotted with GaussView 3.0 in a range of ±12.5 kcal/mol.

in a different region, thus being unable to undergo these electrostatic interactions. This might be one reason for the lack of activity of these compounds. It is striking that in this binding mode there seems to be space for the annelation of additional rings at the A- or C-ring of the biaryl moiety.

Both, the electron donating 5-*ortho*-methyl (**29** and **30**) and the electron withdrawing 2-*ortho*-chlorine (**31**) substituents decrease the electron density on the thiophene and enhance its dipole moment (negatively charged on the sulphur–C2-bond and positively on the methyl group; see MEP Fig. 2). The resulting relatively weak electronegativity of sulphur confers a strong aromatic character on the thiophene ring, leading to better π–π-stacking with the enzyme.

Even more, the 5-Me group points away from the haeme and could form hydrophobic interactions with Ile206. In contrast, the chlorine in **31** points towards the haeme and seems to be capable of both hydrophobic (with Ile112, Ala113 and the hydrophobic CH_2-groups of Asp298) and electrostatic interactions (with the carboxylate group of Asp298, involved in a charge-cluster with Arg109 and His235).

4. Discussion and conclusion

From the data shown in this paper it becomes apparent that we succeeded in finding new lead compounds as inhibitors of CYP17. They are very potent towards the target enzyme and show less inhibition of CYP3A4 compared to ketoconazole which is used clinically. However, their inhibition of some other hepatic CYP enzymes is still too high and needs to be further improved.

The structure–activity relationships obtained in this study demonstrate that an alkyl substituent at the methylene bridge fitting into the hydrophobic pocket near the haeme strongly contributes to the inhibitory potency. It also seems that electron withdrawing groups like chlorine and fluorine at the A- or C-ring are appropriate for increasing inhibition and show influence on the binding mode.

Very important is the finding that compounds of this series (**27** and **28**) showed strong in vivo activities and pharmacokinetic properties in the rat which were similar to those of abiraterone.

Interestingly, in the molecular modelling studies two distinct binding modes of our biaryl compounds were discovered, differing from the binding of the steroidal substrates and inhibitors observed by us and others.[25,26] From these findings the design of modified ligands which should show a stronger interaction with the active site could be possible. On the one hand, enlargement of the ligands binding in BM1 might be envisaged by annelation of additional rings to the A- or C-ring. On the other hand, it could also be examined to design compounds which fill both binding cavities. Compounds modified in that way should be good candidates for showing less inhibition of hepatic CYP enzymes than the compounds of the present study. Those compounds should clinically be superior to the GnRH analogues which are presently used for the treatment of androgen dependent prostate cancer as they additionally block adrenal androgen formation thus decreasing androgen levels below those of castration.

5. Experimental

5.1. CYP17 preparation and assay

Human CYP17 was expressed in *E. coli*[22] (coexpressing human CYP17 and NADPH-P450 reductase) and the assay was performed as previously described.[23a]

Rat testicular CYP17 was obtained from adult male Sprague–Dawley rats and the assay was performed as previously described.[23b]

5.2. Inhibition of hepatic CYP enzymes

The recombinantly expressed enzymes from baculovirus-infected insect microsomes (Supersomes) were used and the manufacturer's instructions (www.gentest.com) were followed.

5.3. In vivo study

The in vivo tests were performed in intact adult male Wistar rats (Harlan Winkelmann, Germany), 5–6 for each compound. These rats were cannulated with silicone tubing via the right jugular vein. The compounds were applied po at 50 mg/kg body weight. The concentrations of testosterone in the rat plasma were determined using the Testosterone ELISA (EIA-1559) from DRG Instruments according to the manufacturer's instructions. The plasma drug levels were measured by LC–MS. Noncompartmental pharmacokinetic analysis of concentration vs time data was performed for each compound on the mean plasma level using a validated computer program (PK solution 2 software; Summit Research Services, Montrose, USA). Plasma concentrations below the limit of detection were assigned a value of zero.

5.4. Chemistry

5.4.1. General. Melting points were determined on a Mettler FP1 melting point apparatus and are uncorrected. IR spectra were recorded neat on a Bruker Vector 33FT-infrared spectrometer. ^{1}H NMR spectra were measured on a Bruker DRX-500 (500 MHz). Chemical shifts are given in parts per million (ppm), and TMS was used as an internal standard for spectra obtained in $CDCl_3$. All coupling constants (J) are given in Hz. ESI (electrospray ionization) mass spectra were determined on a TSQ quantum (Thermo Electron Corporation) instrument. Elemental analyses were performed at the Department of Instrumental Analysis and Bioanalysis, Saarland University. Column chromatography was performed using silica gel 60 (50–200 μm), and reaction progress was determined by TLC analysis on Alugram® SIL G/UV$_{254}$ (Macherey–Nagel). Boronic acids and bromoaryls used as starting materials were commercially obtained (CombiBlocks, Chempur, Aldrich, Acros).

5.4.1.1. 5-Bromo-2-picoline (1c). 2-Picoline (46.60 g, 0.50 mol) was added under nitrogen to mechanically stirred aluminium chloride (200.00 g, 1.50 mol). This slurry was heated under stirring to 100 °C, and bromine (40.00 g, 0.25 mol) was added over a period of 1 h. The heating was continued at 100 °C for 0.5 h. The reaction mixture was poured into 2 L of ice water containing 75 mL of concentrated HCl. Additional concentrated HCl was added until the mixture became acidic (pH 3). Excess $NaHSO_3$ (solid) was added, and the mixture was left overnight at room temperature, filtered and extracted with methylene chloride (3× 150 mL). The aqueous phase was alkalised with 50% aq NaOH solution and extracted with ether (4× 150 mL). The combined organic extracts were washed with brine (100 mL) and dried. Solvent removal gave a residue, which was chromatographed on silica gel using a mixture of EtOAc/MeOH (95:5) as solvent; yield: 4.56 g (26.7 mmol, 11% of 0.25 mol bromine used); colourless solid; R_f = 0.68 EtOAc/MeOH (95:5); δ_H ($CDCl_3$, 500 MHz) 2.51 (s, 3H), 7.05 (d, J = 8.2 Hz, 1H), 7.68 (dd, J = 2.5, 8.2 Hz, 1H), 8.55 (d, J = 2.5 Hz, 1H); δ_C ($CDCl_3$, 75 MHz) 23.8 (CH_3), 117.6 (C_q), 124.6 (CH), 138.8 (CH), 150.1 (CH), 156.9 (C_q); MS (ESI): m/z = 172 [M^++H].

5.4.1.2. 2-Bromo-5-(bromomethyl)pyridine (1b). 2-Bromo-5-methylpyridine (3.00 g, 17.40 mmol) was dissolved in 40 mL of dry carbon tetrachloride. To this solution were added *N*-bromosuccinimide (NBS) (3.41 g, 19.20 mmol) and benzoyl peroxide (0.23 g, 0.80 mmol) and the mixture was refluxed overnight. After cooling, the succinimide was removed by filtration and the filtrate was concentrated under vacuum. The crude product was further purified by flash column chromatography on silica gel using a mixture of petroleum ether/EtOAc (95:5) as eluent; yield: 2.56 g (59%); lachrymatory yellow needles; IR (ATR) υ (cm^{-1}) 3030 (m), 2923 (w), 2365 (w), 1994 (w), 1577 (s), 1556 (m), 1470 (s), 1451 (s), 1396 (m), 1379 (s), 1294 (s), 1097 (s), 920 (m), 838 (s), 810 (m), 727 (s), 645 (s), 623 (m); δ_H ($CDCl_3$, 500 MHz) 4.14 (s, 2H), 7.47 (d, J = 8.2 Hz, 1H), 7.59 (d, J = 8.2 Hz, 1H), 8.38 (s, 1H); MS (ESI): m/z = 252 [M^++H].

5.4.1.3. 5-Bromo-2-(bromomethyl)pyridine (9b). Compound **1c** (3.00 g, 17.40 mmol) was dissolved in 40 mL of dry carbon tetrachloride. To this solution were added NBS (3.41 g, 19.20 mmol) and benzoyl peroxide (0.24 g, 0.80 mmol), and the mixture was refluxed overnight. After cooling, the succinimide was removed by filtration and the filtrate was concentrated under vacuum. The crude product was further purified by flash column chromatography on silica gel using petroleum ether/EtOAc (95:5) as eluent; yield: 1.70 g (39%); lachrymatory lilac oil; IR (ATR) υ (cm^{-1}) 3038 (m), 3006 (m), 2919 (w), 2361 (w), 1983 (w), 1575 (s), 1468 (s), 1448 (s), 1374 (s), 1290 (s), 1090 (s), 1009 (s), 915 (m), 828 (s), 806 (m), 718 (s), 635 (s), 616 (m) δ_H ($CDCl_3$, 500 MHz) 4.24 (s, 2H), 7.16 (d, J = 8.2 Hz, 1H), 7.78 (dd, J = 2.5, 8.2 Hz, 1 H), 8.65 (d, J = 2.5 Hz, 1H); δ_C ($CDCl_3$, 75 MHz) 24.1 (CH_2), 118.4 (C_q), 126.5 (CH), 138.8 (CH), 151.2 (CH), 157.8 (C_q); MS (ESI): m/z = 252 [M^++H].

5.4.2. Method A: Nucleophilic substitution with imidazole. The α-brominated compound (**1b**, **9b**), imidazole (2 equiv), a catalytical amount of 18-crown-6 and anhydrous K_2CO_3 (1.5 equiv) in dry acetonitrile were heated under reflux overnight. After the solution was cooled down, the solvent was removed under reduced pressure. The residue was dissolved with water (10 mL/equiv) and extracted three times with CH_2Cl_2 (15 mL/equiv). The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated.

The crude material was purified by flash chromatography on silica gel, using 5% MeOH in CH_2Cl_2.

5.4.2.1. 5-((1*H*-Imidazol-1-yl)methyl)-2-bromopyridine (1a). Synthesised according to Method A using **1b** (1.32 g, 5.26 mmol), imidazole (0.75 g, 11.00 mmol), K_2CO_3 (1.13 g, 8.16 mmol) and 18-crown-6; yield: 0.75 g (60%); yellow solid; $R_f = 0.33$ (EtOAc/MeOH, 9:1); δ_H ($CDCl_3$, 500 MHz) 5.12 (s, 2H), 6.88 (t, $J = 1.2$ Hz, 1H), 7.13 (s, 1H), 7.28 (d, $J = 2.5$ Hz, 1H), 7.48 (d, $J = 8.5$ Hz, 1H), 7.56 (s, 1H), 8.28 (d, $J = 2.5$ Hz, 1H); MS (ESI): $m/z = 239$ [M^++H].

5.4.2.2. 2-((1*H*-Imidazol-1-yl)methyl)-5-bromopyridine (9a). Synthesised according to Method A using **9b** (1.18 g, 4.70 mmol), imidazole (0.72 g, 9.60 mmol), K_2CO_3 (1.00 g, 7.24 mmol) and 18-crown-6; yield: 0.81 g (72%); yellow solid; $R_f = 0.30$ (EtOAc/MeOH, 9:1); δ_H ($CDCl_3$, 500 MHz) 5.19 (s, 2H), 6.83 (d, $J = 8.5$ Hz, 1H), 6.96 (br s, 1H), 7.10 (s, 1H), 7.59 (s, 1H), 7.76 (dd, $J = 2.1$, 8.5 Hz, 1H), 8.62 (d, $J = 2.1$ Hz, 1H); MS (ESI): $m/z = 239$ [M^++H].

5.4.2.3. 1-(4-Bromobenzyl)-1*H*-imidazole (17a). Synthesised according to Method A using 1-bromo-4-(bromomethyl)benzene (6.25 g, 25.00 mmol), imidazole (6.80 g, 0.10 mol), K_2CO_3 (28.00 g, 0.20 mol) and 18-crown-6; yield: 5.06 g (85%); $R_f = 0.19$ (EtOAc); IR (ATR) υ (cm^{-1}) 3111 (w), 1508 (m), 1489 (m), 1232 (m), 1072 (s), 1012 (m), 802 (s), 744 (s), 662 (s), 607 (m); δ_H ($CDCl_3$, 500 MHz) 5.00 (s, 2H), 6.81 (s, 1H), 6.94 (d, $J = 8.4$ Hz, 2H), 7.02 (s, 1H), 7.40 (d, $J = 8.4$ Hz, 2H), 7.46 (s, 1H); δ_C ($CDCl_3$, 125 MHz) 52.9 (CH_2), 118.2 (CH), 121.3 (C_q), 127.9 (CH), 129.0 (CH), 131.1 (CH), 134.2 (C_q), 136.3 (CH); MS (ESI): $m/z = 239/237$ [M^++H].

5.4.3. Method B: Suzuki-coupling. The corresponding brominated aromatic compound (1 equiv) was dissolved in toluene (7 mL/mmol), an aqueous 2.0 M Na_2CO_3solution (3.2 mL/mmol) and an ethanolic solution (3.2 mL/mmol) of the corresponding boronic acid (1.5–2.0 equiv) were added. The mixture was deoxygenated under reduced pressure and flushed with nitrogen. After repeating this cycle several times $Pd(PPh_3)_4$ (4 mol%) was added and the resulting suspension was heated under reflux for 8 h. After cooling ethyl acetate (10 mL) and water (10 mL) were added and the organic phase was separated. The water phase was extracted with ethyl acetate (2× 10 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered over a short plug of Celite® and evaporated under reduced pressure. The compounds were purified by flash chromatography on silica gel.

5.4.3.1. 5-((1*H*-Imidazol-1-yl)methyl)-2-(3-methoxyphenyl)pyridine (1). Synthesised according to Method B using compound **1a** (0.20 g, 0.84 mmol) and 3-methoxyphenylboronic acid (0.19 g, 1.26 mmol); yield: 0.05 g (18%); yellow solid: mp 99 °C; $R_f = 0.31$ (CH_2Cl_2/MeOH, 95:5); IR (ATR) υ (cm^{-1}) 2962 (w), 2926 (w), 1565 (m), 1477 (m), 1261 (m), 1072 (m), 1023 (s), 799 (m), 766 (s), 665 (m); δ_H ($CDCl_3$, 500 MHz) 3.85 (s, 3H), 5.13 (s, 2H), 6.89 (s, 1H), 6.94 (ddd, $J = 0.9$, 2.5, 8.2 Hz, 1H), 7.09 (s, 1H), 7.34 (t, $J = 8.2$ Hz, 1H), 7.44 (dd, $J = 2.5$, 8.2 Hz, 1H), 7.49 (m, 1H), 7.54 (m, 1H), 7.66 (d, $J = 8.2$ Hz, 1H), 8.53 (d, $J = 2.2$ Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 48.0 (CH_2), 55.3 (CH_3), 112.1 (CH), 115.3 (CH), 119,0 (CH), 119.2 (CH), 120.6 (CH), 129.8 (CH), 130.1 (C_q), 130.2 (CH), 135.7 (CH), 137.2 (CH), 140.0 (C_q), 148.4 (CH), 157.3 (C_q), 160.0 (C_q); MS (ESI): $m/z = 252$ [M^++H].

5.4.3.2. 5-((1*H*-Imidazol-1-yl)methyl)-2-phenylpyridine (2). Synthesised according to Method B using compound **1a** (0.20 g, 0.84 mmol) and phenylboronic acid (0.20 g, 1.68 mmol); yield: 0.10 g (57%); yellow solid: mp 99 °C; $R_f = 0.35$ (CH_2Cl_2/MeOH 95:5); IR (ATR) υ (cm^{-1}) 2925 (s), 1503 (m), 1476 (s), 1344 (m), 1290 (m), 1110 (m), 1081 (s), 908 (m), 839 (s), 731 (s), 625 (m); δ_H ($CDCl_3$, 500 MHz) 5.19 (s, 2H), 6.94 (t, $J = 1.3$ Hz, 1H), 7.13 (s, 1H), 7.48–7.50 (m, 4H), 7.60 (s, 1H), 7.72 (d, $J = 8.2$ Hz, 1H), 7.97–7.99 (m, 1H); δ_C ($CDCl_3$, 125 MHz) 48.1 (CH_2), 119.0 (CH), 120.6 (CH), 126.9 (CH), 128.8 (CH), 130.0 (CH), 130.4 (C_q), 135.7 (CH), 137.3 (CH), 138.6 (C_q), 148.6 (CH) 157.7 (C_q); MS (ESI): $m/z = 236$ [M^++H].

5.4.3.3. 5-((1*H*-Imidazol-1-yl)methyl)-2-(4-fluorophenyl)pyridine (3). Synthesised according to Method B using compound **1a** (0.20 g, 0.84 mmol) and 4-fluorophenylboronic acid (0.23 g, 1.68 mmol); yield: 0.16 g (76%); yellow solid: mp 95 °C; $R_f = 0.15$ (EtOAc/MeOH, 9:1); IR (ATR) υ (cm^{-1}) 3101 (w), 1598 (m), 1511 (m), 1478 (m), 1390 (w), 1231 (m), 1083 (m), 1026 (w), 910 (w), 824 (w), 770 (m), 737 (s), 663 (m); δ_H ($CDCl_3$, 500 MHz) 5.18 (s, 2H), 6.92 (s, 1H), 7.12 (br s, 1H), 7.15 (t, $J = 8.8$ Hz, 2H), 7.48 (dd, $J = 2.2$, 8.2 Hz, 1H), 7.60 (s, 1H), 7.67 (d, $J = 8.2$ Hz, 1H), 7.97 (dd, $J = 5.4$, 8.8 Hz, 2H), 8.57 (d, $J = 1.9$ Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 48.1 (CH_2), 115.7 (d, $^2J_{CF} = 22.1$ Hz, CH), 119.0 (CH), 120.2 (CH), 128.7 (d, $^3J_{CF} = 8.6$ Hz, CH), 130.0 (C_q), 130.4 (CH), 134.7 (d, $^4J_{CF} = 3.9$ Hz, C_q), 135.8 (CH), 137.3 (CH), 148.6 (CH), 156.6 (C_q), 163.7 (d, $^1J_{CF} = 249.5$ Hz, C_q); MS (ESI): $m/z = 254$ [M^++H].

5.4.3.4. 5-((1*H*-Imidazol-1-yl)methyl)-2-(3,4-difluorophenyl)pyridine (4). Synthesised according to Method B using compound **1a** (0.20 g, 0.84 mmol) and 3,4-difluorophenylboronic acid (0.27 g, 1.68 mmol); yield: 0.21 g (91 %); yellow solid: mp 100 °C; $R_f = 0.16$ (EtOAc/MeOH, 9:1); IR (ATR) υ (cm^{-1}) 3106 (w), 2930 (w), 2856 (w), 1599 (m), 1567 (m), 1523 (s), 1507 (s), 1481 (s), 784 (s); δ_H ($CDCl_3$, 500 MHz) 5.18 (s, 2H), 6.92 (t, $J = 1.3$ Hz, 1H), 7.13 (t, $J = 1.3$ Hz, 1H), 7.25 (ddd, $J = 8.2$, 8.5, 9.8 Hz, 1H), 7.49 (dd, $J = 2.5$, 8.2 Hz, 1H), 7.59 (s, 1H), 7.66 (dd, $J = 0.6$, 8.3 Hz, 1H), 7.69–7.73 (m, 1H), 7.86 (ddd, $J = 2.2$, 7.6, 11.4 Hz, 1 H), 8.56 (dd, $J = 0.6$, 2.3 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 48.0 (CH_2), 116.0 (d, $^2J_{CF} = 18.2$ Hz, CH), 117.6 (d, $^2J_{CF} = 17.3$ Hz, CH), 119.0 (CH), 120.2 (CH), 122.8 (dd, $^4J_{CF} = 3.8$ Hz, $^3J_{CF} = 6.7$ Hz, CH), 130.4 (CH), 130.6 (C_q), 135.6 (dd, $^4J_{CF} = 3.8$ Hz, $^3J_{CF} = 5.8$ Hz, C_q), 135.9 (CH), 137.3 (CH), 148.6 (CH), 150.8 (dd, $^2J_{CF} = 20.2$ Hz, $^1J_{CF} = 255.3$ Hz, C_q),

151.3 (dd, $^2J_{CF}$ = 15.4 Hz, $^1J_{CF}$ = 254.3 Hz, C_q), 155.3 (C_q); MS (ESI): *m/z* = 272 [M^++H].

5.4.3.5. 5-((1*H*-Imidazol-1-yl)methyl)-2-(4-methoxyphenyl)pyridine (5). Synthesised according to Method B using compound **1a** (0.40 g, 1.68 mmol) and 4-methoxyphenylboronic acid (0.38 g, 3.35 mmol); yield: 0.40 g (89%); yellow solid: mp 138 °C; R_f = 0.15 (EtOAc/MeOH, 9:1); IR (ATR) υ (cm^{-1}) 2962 (w), 2928 (w), 1597 (m), 1478 (m), 1249 (s), 1174 (m), 1023 (m), 828 (s), 814 (s), 762 (s); δ_H ($CDCl_3$, 500 MHz) 3.86 (s, 3H), 5.16 (s, 2H), 6.92 (t, *J* = 1.3 Hz, 1H), 6.99 (d, *J* = 9.1 Hz, 2H), 7.11 (s, 1H), 7.46 (dd, *J* = 2.5, 8.2 Hz, 1H), 7.59 (s, 1H), 7.66 (d,*J* = 8.2 Hz, 1H), 7.94 (d, *J* = 9.1 Hz, 2H), 8.54 (d, *J* = 2.5 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 48.1 (CH_2), 55.4 (CH_3), 114.2 (CH), 119.0 (CH), 119.8 (CH), 128.2 (CH), 129.2 (C_q), 130.3 (CH), 131.2 (C_q), 135.7 (CH), 137.3 (CH), 148.5 (CH), 157.4 (C_q), 160.8 (C_q); MS (ESI): *m/z* = 266 [M^++H].

5.4.3.6. 5-((1*H*-Imidazol-1-yl)methyl)-2-(2-methoxyphenyl)pyridine (6). Synthesised according to Method B using compound **1a** (0.20 g, 0.84 mmol) and 2-methoxyphenylboronic acid (0.19 g, 1.68 mmol); yield: 0.21 g (92%); yellow solid: mp 103 °C; R_f = 0.15 (EtOAc/MeOH, 9:1); IR (ATR) υ (cm^{-1}) 2924 (m), 2854 (m), 1598 (m), 1477 (s), 1395 (m), 1261 (m), 1237 (s), 1079 (m), 1022 (s), 835 (m), 795 (s), 665 (s); δ_H ($CDCl_3$, 500 MHz) 3.85 (s, 3H), 5.17 (s, 2H), 6.95 (br s, 1H), 7.00 (dd, *J* = 0.6, 8.5 Hz, 1H), 7.08 (dt, *J* = 1.1, 7.6 Hz, 1H), 7.12 (s, 1H), 7.38 (ddd, *J* = 1.9, 7.6, 8.5 Hz, 1H), 7.44 (dd, *J* = 2.2, 8.5 Hz, 1H), 7.60 (s, 1H), 7.77 (dd, *J* = 1.9, 7.6 Hz, 1H), 7.83 (d, *J* = 8.2 Hz, 1H), 8.60 (d, *J* = 1.6 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 48.2 (CH_2), 55.6 (CH_3), 111.4 (CH), 119.1 (CH), 121.1 (CH), 125.2 (CH), 128.3 (C_q), 129.5 (C_q), 130.2 (CH), 130.3 (CH), 131.1 (CH), 134.6 (CH), 137.3 (CH), 148.2 (CH), 156.4 (C_q), 157.0 (C_q); MS (ESI): *m/z* = 266 [M^++H].

5.4.3.7. 3-(5-((1*H*-Imidazol-1-yl)methyl)pyridin-2-yl)benzeneamine (7). Synthesised according to Method B using compound **1a** (0.20 g, 0.84 mmol) and 2-methoxyphenylboronic acid (0.29 g, 1.68 mmol); yield: 0.14 g (67%); orange solid: mp 145 °C; R_f = 0.11 (EtOAc/MeOH, 9:1); IR (ATR) υ (cm^{-1}) 3406 (w), 3408 (w), 3323 (w), 3212 (w), 2957 (m), 2924 (m), 2854 (m), 1630 (m), 1600 (s), 1476 (s), 1235 (m), 1105 (s), 1073 (s), 1026 (s), 787 (s), 758 (s); δ_H ($CDCl_3$, 500 MHz) 5.11 (s, 2H), 6.72 (ddd, *J* = 0.9, 2.4, 7.6 Hz, 1H), 6.89 (br s, 1H), 7.08 (br s, 1H), 7.21 (t, *J* = 7.7 Hz, 1H), 7.28 (dt, *J* = 1.3, 7.8 Hz, 1H), 7.33 (t, *J* = 2.2 Hz, 1H), 7.41 (dd, *J* = 2.2, 8.2 Hz, 1H), 7.55 (br s, 1H), 7.62 (d, *J* = 8.2 Hz, 1H), 8.50 (d, *J* = 2.2 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 48.0 (CH_2), 113.3 (CH), 116.0 (CH), 117.0 (CH), 119.0 (CH), 120.5 (CH), 129.6 (CH), 129.9 (C_q), 130.1 (CH), 135.6 (CH), 137.2 (CH), 139.5 (C_q), 146.9 (C_q), 148.3 (CH) 157.6 (C_q); MS (ESI): *m/z* = 251 [M^++H].

5.4.3.8. 5-((1*H*-Imidazol-1-yl)methyl)-2-(3,4-dimethoxyphenyl)pyridine (8). Synthesised according to Method B using compound **1a** (0.40 g, 1.68 mmol) and 3,4-dimethoxyphenylboronic acid (0.61 g, 3.36 mmol); yield: 0.47 g (94%); yellow solid: mp 114 °C; R_f = 0.16 (EtOAc/MeOH 9:1); IR (ATR) υ (cm^{-1}) 3110 (w), 3086 (w), 3002 (w), 2960 (w), 2926 (w), 2855 (w), 1596 (m), 1519 (m), 1477 (m), 1280 (m), 1150 (m), 1022 (s), 808 (s), 764 (s), 666 (m); δ_H ($CDCl_3$, 500 MHz) 3.93 (s, 3H), 3.98 (s, 3H), 5.16 (s, 2H), 6.92 (br s, 1H), 6.94 (d, *J* = 8.5 Hz, 1H), 7.11 (br s, 1H), 7.46 (dd, *J* = 2.2, 8.5 Hz, 1H), 7.47 (dd, *J* = 2.2, 8.5 Hz, 1H), 7.58 (br s, 1H), 7.65 (d, *J* = 2.2 Hz, 1H), 7.67 (d, *J* = 8.5 Hz, 1H), 8.54 (d, *J* = 2.2 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 48.1 (CH_2), 56.0 (CH_3), 56.0 (CH_3), 113.3 (CH), 110.0 (CH), 111.1 (CH), 119.0 (CH), 119.5 (CH), 120.0 (CH), 129.4 (C_q), 130.3 (CH), 131.4 (C_q) 135.7 (CH), 148.4 (CH), 149.4 (C_q), 150.3 (C_q), 157.3 (C_q); MS (ESI): *m/z* = 296 [M^++H].

5.4.3.9. 2-((1*H*-Imidazol-1-yl)methyl)-5-(4-fluorophenyl)pyridine (9). Synthesised according to Method B using compound **9a** (0.20 g, 0.84 mmol) and 4-fluorophenylboronic acid (0.23 g, 1.68 mmol); yield: 0.18 g (83%); yellow solid: mp 90 °C; R_f = 0.11 (EtOAc/MeOH, 9:1); IR (ATR) υ (cm^{-1}) 3116 (w), 3046 (w), 2927 (w), 1064 (m), 1519 (m), 1486 (s), 1393 (w), 1220 (s), 1163 (m), 1070 (s), 906 (w), 828 (s), 817 (s), 769 (s), 684 (s), 660 (s); δ_H ($CDCl_3$, 500 MHz) 5.29 (s, 2H), 7.02 (m, 2H), 7.14 (br s, 1H), 7.17 (t, *J* = 8.8 Hz, 2H), 7.52 (dd, *J* = 5.4, 8.8 Hz, 1H), 7.65 (s, 1H), 7.79 (dd, *J* = 2.2, 8.2 Hz, 2H), 8.76 (d, *J* = 2.2 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 52.3 (CH_2), 116.2 (d, $^2J_{CF}$ = 22.1 Hz, CH), 119.5 (CH), 121.1 (CH), 128.8 (d, $^3J_{CF}$ = 8.6 Hz, CH), 130.0 (CH), 130.4 (CH), 133.2 (d, $^4J_{CF}$ = 3.8 Hz, C_q), 135.2 (C_q), 135.4 (CH), 148.0 (CH), 154.9 (C_q), 163.0 (d, $^1J_{CF}$ = 247.6 Hz, C_q); MS (ESI): *m/z* = 254 [M^++H].

5.4.3.10. 4-Phenylthiophene-2-carbaldehyde (10b). Synthesised according to Method B using phenylboronic acid (0.49 g, 2.00 mmol) and 4-bromothiophen-2-carbaldehyde (0.38 g, 2.00 mmol); yield: 0.35 g (93%); white solid: mp 57–58 °C; R_f = 0.39 (PE/EtOAc, 10:1); IR (ATR) υ (cm^{-1}) 3096 (w), 2838 (w), 1670 (s), 1597 (w), 1430 (m), 1247 (w), 1180 (s), 1048 (w), 755 (s), 695 (m), 660 (s); δ_H ($CDCl_3$, 500 MHz) 7.36 (m, 1H), 7.44 (dd, *J* = 7.3, 7.9 Hz, 2H), 7.59 (dd, *J* = 1.3 Hz, 8.5 Hz, 2H), 7.85 (dd, *J* = 1.3 Hz, 1.6 Hz, 1H), 8.03 (d, *J* = 1.6 Hz, 1H), 9.97 (d, *J* = 1.3 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 115.3 (CH), 126.3 (CH), 128.0 (CH), 129.1 (CH), 129.6 (CH), 134.4 (C_q), 143.7 (C_q), 144.4 (C_q), 183.0 (CH); MS (ESI): *m/z* = 189 [M^++H].

5.4.3.11. 4-(4-Fluorophenyl)-thiophene-2-carbaldehyde (11b). Synthesised according to Method B using 4-fluorophenylboronic acid (0.56 g, 4.00 mmol) and 4-bromothiophen-2-carbaldehyde (0.38 g, 2.00 mmol); yield: 0.38 g (92%); white solid: mp 76–78 °C; R_f = 0.34 (PE/EtOAc, 10:1); IR (ATR) υ (cm^{-1}) 2945 (w), 2839 (w), 1665 (s), 1547 (m), 1507 (s), 1435 (m), 1228 (s), 1216 (s), 1181 (m), 829 (s), 666 (s), 566 (s); δ_H ($CDCl_3$, 500 MHz) 7.13 (dd, *J* = 8.5, 8.8 Hz, 2H), 7.55 (dd, *J* = 5.4, 8.8 Hz, 2H), 7.79 (dd, *J* = 1.3, 1.6 Hz, 1H), 7.98 (d, *J* = 1.6 Hz, 1H), 9.97 (d, *J* = 1.3 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 116.0 (d, $^2J_{CF}$ = 22.1 Hz, CH),

128.0 (d, $^3J_{CF}$ = 8.6 Hz, CH), 129.3 (CH), 130.6 (d, $^4J_{CF}$ = 3.6 Hz, C_q), 134.4 (CH), 142.6 (C_q), 144.6 (C_q), 162.6 (d, $^1J_{CF}$ = 247.6 Hz, C_q), 182.8 (CH); MS (ESI): *m*/*z* = 207 [M^++H].

5.4.3.12. 4-(4-Methoxyphenyl)-thiophene-2-carbaldehyde (12b). Synthesised according to Method B using 4-methoxyphenylboronic acid (0.34 g, 2.25 mmol) and 4-bromothiophene-2-carbaldehyde (0.29 g, 1.50 mmol); yield: 0.25 g (76%); white solid: mp 72 °C; R_f = 0.34 (PE/EtOAc, 10:1); IR (ATR) υ (cm^{-1}) 1512 (m), 1406 (m), 1309 (m), 1246 (m), 1180 (m), 1026 (s), 832 (s), 756 (m), 521 (m); δ_H ($CDCl_3$, 500 MHz) 3.85 (s, 3H), 6.96 (d, *J* = 8.8 Hz, 2H), 7.52 (d, *J* = 8.8 Hz, 2H), 7.75 (dd, *J* = 1.3, 1.6 Hz, 1H), 7.98 (d, *J* = 1.6 Hz, 1H), 9.96 (d, *J* = 1.3 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 55.4 (CH_3), 114.5 (CH), 127.3 (C_q), 127.5 (CH), 128.4 (CH), 134.5 (CH), 143.4 (C_q), 144.3 (C_q), 159.6 (C_q), 183.0 (CH); MS (ESI): *m*/*z* = 219 [M^++H].

5.4.3.13. 4-(3-Methoxyphenyl)-thiophene-2-carbaldehyde (13b). Synthesised according to Method B using 3-methoxyphenylboronic acid (0.61 g, 4.00 mmol) and 4-bromothiophen-2-carbaldehyde (0.38 g, 2.00 mmol); yield: 0.25 g (76%); white solid: mp 225 °C; R_f = 0.41 (PE/EtOAc, 10:1); IR (ATR) υ (cm^{-1}) 2836 (w), 1666 (s), 1600 (m), 1458 (m), 1258 (m), 1161 (s), 1039 (m), 850 (m), 771 (s), 689 (m), 666 (s); δ_H ($CDCl_3$, 500 MHz) 3.87 (s, 3H), 6.90 (ddd, *J* = 1.0, 2.5, 8.5 Hz, 1H), 7.11 (dd, *J* = 1.9, 2.5 Hz, 1H), 7.17 (ddd, *J* = 1.0, 1.9, 7.9 Hz, 1H), 7.36 (dd, *J* = 7.9, 8.5 Hz, 1H), 7.85 (dd, *J* = 1.3, 1.6 Hz, 1H), 8.02 (d, *J* = 1.6 Hz, 1H), 9.97 (d, *J* = 1.3 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 55.3 (CH_3), 112.3 (CH), 113.3 (CH), 118.8 (CH), 129.8 (CH), 130.1 (CH), 134.7 (CH), 135.7 (C_q), 143.5 (C_q), 144.4 (C_q), 160.1 (C_q), 182.9 (CH); MS (ESI): *m*/*z* = 219 [M^++H].

5.4.3.14. 4-(3,4-Difluorophenyl)-thiophene-2-carbaldehyde (14b). Synthesised according to Method B using 3,4-difluorophenylboronic acid (0.63 g, 4.00 mmol) and 4-bromothiophen-2-carbaldehyde (0.38 g, 2.00 mmol); yield: 0.43 g (96%); white solid: mp 115–116 °C; R_f = 0.35 (PE/EtOAc, 10:1); IR (ATR) υ (cm^{-1}) 3051 (w), 1658 (s), 1517 (s), 1447 (m), 1352 (m), 1278 (s), 1190 (s), 852 (m), 816 (s), 772 (s), 670 (s), 601 (s), 574 (s); δ_H ($CDCl_3$, 500 MHz) 7.23 (ddd, *J* = 8.2, 8.5, 10.1 Hz, 1H), 7.29-7.32 (m, 1H), 7.38 (ddd, *J* = 2.2, 7.6, 11.4 Hz, 1H), 7.80 (dd, *J* = 1.3, 1.6 Hz, 1H), 7.95 (d, *J* = 1.6 Hz, 1H), 9.97 (d, *J* = 1.3 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 115.4 (d, $^2J_{CF}$ = 18.2 Hz, CH), 118.0 (d, $^2J_{CF}$ = 18.2 Hz, CH), 122.4 (dd, $^4J_{CF}$ = 3.8 Hz, $^3J_{CF}$ = 6.7 Hz, CH), 129.9 (CH), 131.6 (dd, $^4J_{CF}$ = 3.8 Hz, $^3J_{CF}$ = 6.7 Hz, C_q), 134.1 (CH), 141.5 (C_q), 144.8 (C_q), 150.0 (dd, $^2J_{CF}$ = 58.5 Hz, $^1J_{CF}$ = 249.5 Hz, C_q), 150.1 (dd, $^2J_{CF}$ = 59.5 Hz, $^1J_{CF}$ = 249.5 Hz, C_q), 182.7 (CH); MS (ESI): *m*/*z* = 225 [M^++H].

5.4.3.15. 3-(4-((1*H*-Imidazol-1-yl)methyl)phenyl)pyridine (17). Synthesised according to Method B using compound **17a** (0.24 g, 1.00 mmol) and pyridine-3-boronic acid (0.25 g, 2.00 mmol); yield: 0.17 g (73%); R_f = 0.20 (EtOAc/MeOH, 9:1); IR (ATR) υ (cm^{-1}) 3384 (m), 3115 (w), 1509 (m), 1225 (s), 1081 (s), 1030 (m), 1005 (m), 804 (s), 747 (s), 708 (s), 669 (s); δ_H ($CDCl_3$, 500 MHz) 5.09 (s, 2H), 6.85 (s, 1H), 7.02 (s, 1H), 7.17 (d, *J* = 8.2 Hz, 2H), 7.27 (ddd, *J* = 1.0, 4.7, 8.0 Hz, 1H), 7.47 (d, *J* = 8.2 Hz, 2H), 7.50 (s, 1H), 7.76 (ddd, *J* = 1.6, 2.4, 8.0 Hz, 1H), 8.50 (dd, *J* = 1.6, 4.7 Hz, 1H), 8.73 (d, *J* = 1.6 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 49.4 (CH_2), 118.2, 126.6, 126.7, 128.9, 133.3, 134.8, 135.1, 136.5, 136.8, 147.0, 147.9; MS (ESI): *m*/*z* = 236 [M^++H].

5.4.3.16. 5-(4-((1*H*-Imidazol-1-yl)methyl)phenyl)-2-fluoropyridine (18). Synthesised according to Method B using compound **17a** (0.24 g, 1.00 mmol) and 4-fluoropyridine-3-boronic acid (0.28 g, 2.00 mmol); yield: 0.14 g (56%); R_f = 0.20 (EtOAc); IR (ATR) υ (cm^{-1}) 3108 (w), 1592 (m), 1497 (s), 1250 (s), 1075 (m), 813 (s), 737 (m), 684 (s); δ_H ($CDCl_3$, 500 MHz) 5.10 (s, 2H), 6.85 (s, 1H), 6.91 (dd, *J* = 2.6, 8.6 Hz, 1H), 7.02 (s, 1H), 7.18 (d, *J* = 8.3 Hz, 2H), 7.43 (d, *J* = 8.3 Hz, 2H), 7.50 (s, 1H), 7.86 (ddd, *J* = 2.6, 7.5, 10.1 Hz, 1H), 8.29 (d, *J* = 2.6 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 49.3 (CH_2), 108.6 (d, $^2J_{CF}$ = 38.7 Hz, CH), 118.3 (CH), 126.6 (CH), 127.0 (CH), 129.0 (CH), 133.0 (d, $^4J_{CF}$ = 4.6 Hz, C_q), 135.3 (CH), 135.7 (C_q), 136.4 (C_q), 138.6 (d, $^3J_{CF}$ = 7.7 Hz, CH), 144.7 (d, $^3J_{CF}$ = 15.5 Hz, CH), 162.0 (d, $^1J_{CF}$ = 238.9 Hz, C_q); MS (ESI): *m*/*z* = 254 [M^++H].

5.4.3.17. 1-(4-(5-(Methylthio)thiophen-2-yl)benzyl)-1*H*-imidazole (22). Synthesised according to Method B using compound **17a** (0.12 g, 0.50 mmol) and 5-(methylsulfanyl)-2-thiophenylboronic acid (0.17 g, 1.00 mmol); light brown solid: mp 139–140 °C; R_f = 0.17 (EtOAc/MeOH, 10:1); IR (ATR) υ (cm^{-1}) 1503 (m), 1442 (m), 1226 (m), 1078 (s), 849 (m), 802 (s), 756 (s), 741 (s), 661 (s); δ_H ($CDCl_3$, 500 MHz) 2.50 (d, *J* = 1.0 Hz, 3H), 5.11 (s, 2H), 6.72-6.73 (m, 1H), 6.91 (t, *J* = 1.3 Hz, 1H), 7.09 (d, *J* = 1.6 Hz, 1H), 7.10 (s, 1H), 7.13 (d, *J* = 8.3 Hz, 2H), 7.52 (d, *J* = 8.3 Hz, 2H), 7.57 (s, 1H); δ_C ($CDCl_3$, 125 MHz) 15.4 (CH_3), 50.5 (CH_2), 119.2 (CH), 123.3 (CH), 125.9 (CH), 126.3 (CH), 127.8 (CH), 129.8 (CH), 134.6 (C_q), 134.8 (C_q), 137.4 (CH), 140.0 (C_q), 141.0 (C_q); MS (ESI): *m*/*z* = 255 [M^++H].

5.4.3.18. 1-(4-(5-Methylsulfanyl-thiophen-2-yl)-phenyl)-propan-1-one (23b). Synthesised according to Method B from 5-(methylthio)thiophen-2-yl-2-boronic acid (1.00 g, 6.30 mmol) and 1-(4-bromophenyl) propan-1-one (1.10 g, 5.20 mmol); yield: 1.19 g (88 %); white solid; R_f = 0.63 (Hex/EtOAc, 4:1); IR (ATR) υ (cm^1) 1678 (s), 1597 (s), 1462 (s), 1409 (m), 1349 (m), 1220 (s), 1181 (s), 1112 (m), 1012 (m), 949 (s), 796 (vs); δ_H ($CDCl_3$, 500 MHz) 1.23 (t, *J* = 7.3 Hz, 3H, CH_3), 2.53 (s, 3H, S-CH_3), 2.99 (q, *J* = 7.3 Hz, 2H, CH_2), 6.76–6.77 (m, 1H), 7.22–7.26 (m, 1H), 7.60–7.62 (m, 2H), 7.94–7.95 (m, 2H); δ_C ($CDCl_3$, 125 MHz) 8.3 (CH_3), 15.1 (S-CH_3), 31.7 (CH_2), 124.5, 125.3, 128.3, 134.7, 138.3, 140.5, 141.3, 200.9 (CO); MS (ESI): *m*/*z* = 233 [M^+−Et].

5.4.3.19. 1-(4-Bromophenyl)-2-methyl-1-(1-trityl-1*H*-imidazol-5-yl)propan-1-ol (24a). The preparation of **24a** has been reported previously by Tasaka and Kaku.[21]

5.4.3.20. 1-(1*H*-Imidazol-5-yl)-2-methyl-1-(4-(thiophen-3-yl)phenyl)propan-1-ol (24). Synthesised according to Method B using compound **24a** (0.17 g, 0.31 mmol) and thiophen-3-ylboronic acid (0.08 g, 0.62 mmol), and DME as solvent. The crude was then deprotected with pyridinium hydrochloride (0.05 g, 0.44 mmol) in MeOH (5 ml) at 80 °C for 5 h; yield: 0.03 g (34%); light-yellow solid; $R_f = 0.36$ (DCM/MeOH, 9:1); IR (ATR) υ (cm^{-1}) 3122 (w), 1506 (w), 1457 (w), 1179 (w), 1003 (w), 948 (w), 865(m), 779 (s), 733, 681 (m), 669 (m), 649 (m), 567 (m), 532 (s), 517 (s); δ_H ($CDCl_3$, 500 MHz) 0.77 (d, $J = 6.1$ Hz, 3H), 0.94 (d, $J = 4.8$ Hz, 3H), 2.50–2.60 (m, 1H), 6.91 (s, 1H), 7.33 (s, 2H), 7.38 (s, 1H), 7.40–7.53 (m, 5H); δ_C ($CDCl_3$, 125 MHz) 17.3 (CH_3), 17.3 (CH_3), 30.3 (CH), 37.3 (C_q), 120.1 (CH), 125.2, 125.4, 125.5, 125.8, 125.9, 125.9, 126.1, 126.2, 126.3, 126.4, 126.6, 134.1 (C_q), 141.9 (C_q); MS (ESI): $m/z = [M^+ + H]$.

5.4.3.21. 1-(4-(Furan-3-yl)benzyl)-1*H*-imidazole (25). Synthesised according to Method B using compound **17a** (0.24 g, 1.00 mmol) and furan-3-ylboronic acid (0.22 g, 2.00 mmol); yield: 0.16 g (73%); $R_f = 0.17$ (EtOAc); IR (ATR) υ (cm^{-1}) 3102 (w), 1518 (m), 1439 (m), 1242 (m), 1161 (m), 1071 (m), 872 (m), 784 (s), 751 (s), 662 (s), 597 (s); δ_H ($CDCl_3$, 500 MHz) 5.05 (s, 2H), 6.61 (dd, $J = 1.8$, 0.9 Hz, 1H), 6.84 (t, $J = 1.2$ Hz, 1H), 7.03 (s, 1H), 7.09 (d, $J = 8.2$ Hz, 2H), 7.39 (d, $J = 8.2$ Hz, 2H), 7.41 (m, 1H), 7.49 (s, 1H), 7.66 (s, 1H); δ_C ($CDCl_3$, 125 MHz) 49.5 (CH_2), 107.7 (CH), 118.2 (CH), 124.8 (C_q), 125.4 (CH), 126.8 (CH), 128.9 (CH), 131.6 (C_q), 133.7 (C_q), 136.4 (CH), 137.7 (CH), 142.8 (CH); MS (ESI): $m/z = 225$ $[M^+ + H]$.

5.4.3.22. 1-(4-(4-Methylthiophen-3-yl)benzyl)-1*H*-imidazole (bf 26). Synthesised according to Method B using compound **17a** (0.24 g, 1.00 mmol) and furan-2-ylboronic acid (0.28 g, 2.00 mmol); red oil: bp >200 °C; yield: 0.17 g (68%); $R_f = 0.35$ (EtOAc/MeOH, 9:1); IR (ATR) υ (cm^{-1}) 1505 (m), 1230 (m), 1075 (m), 792 (s), 729 (m), 662 (s); δ_H ($CDCl_3$, 500 MHz) 2.25 (s, 3H), 5.16 (s, 2H), 6.95 (br s, 1H), 7.02–7.03 (m, 1H), 7.12 (br s, 1H), 7.18–7.20 (m, 3H), 7.38 (d, $J = 8.2$ Hz. 2H), 7.60 (br s, 1H); δ_C ($CDCl_3$, 125 MHz) 15.5 (CH_3), 50.6 (CH_2), 119.3 (CH), 122.2 (CH), 123.3 (CH), 127.2 (CH), 129.1 (CH), 129.7 (CH), 134.8 (C_q), 136.0 (C_q), 137.2 (C_q), 137.4 (CH), 142.2 (C_q).

5.4.3.23. 1-(4-(4-Methylthiophen-3-yl)phenyl)propan-1-one (29b). Synthesised according to Method B from 4-methylthiophen-3-yl-3-boronic acid (0.80 g, 6.30 mmol) and 1-(4-bromophenyl)propan-1-one (1.36 g, 6.40 mmol); yield: 0.96 g (80%); white solid; $R_f = 0.68$ (Hex/EtOAc, 4:1); δ_H ($CDCl_3$, 500 MHz) 1.25 (t, $J = 7.3$ Hz, 3H, CH_3), 2.29 (s, 3H, CH_3), 3.01–3.06 (q, 2H, CH_2), 7.05–7.06 (m, 1H), 7.26–7.27 (m, 1H,), 7.48–7.50 (m, 2H), 8.00–8.02 (m, 2H); δ_C (CDCl3, 125 MHz) 8.3 (CH_3), 31.8 (CH_2), 122.6, 123.9, 128.1, 128.6, 135.4, 135.9, 141.6, 142.0, 200.3 (C = O); MS (ESI): $m/z = 231$ $[M^+ + H]$.

5.4.3.24. 2-Fluoro-4-(4-methylthiophen-3-yl)benzaldehyde (30b). Synthesised according to Method B from 4-bromo-2-fluorobenzaldehyde (1.36 g, 6.40 mmol) and 4-methylthiophen-3-yl-3-boronic acid (1.42 g, 7.10 mmol). This compound was used directly in the next step without further purification.

5.4.3.25. 4-(2-Chlorothiophen-3-yl)-benzaldehyde (31b). Synthesised according to Method B from 3-bromo-2-chlorothiophene (0.20 g, 1.00 mmol) and 4-formylphenylboronic acid (0.30 g, 2.00 mmol); yield: 0.14 g (67%); white solid; $R_f = 0.37$ (PE/EtOAc, 10:1); δ_H ($CDCl_3$, 500 MHz) 7.10 (d, $J = 5.7$ Hz, 1H), 7.20 (d, $J = 5.7$ Hz, 1H), 7.75 (d, $J = 8.5$ Hz, 2H), 7.95 (d, $J = 8.2$ Hz, 2H), 10.06 (s, 1H, CHO); δ_C ($CDCl_3$, 125 MHz) 123.3, 126.5, 128.2, 129.0, 129.9, 135.3, 136.9, 140.2, 191.7 (CO); MS (ESI): $m/z = 223$ $[M^+ + H]$.

5.4.4. Method C: Reduction with $NaBH_4$. To an ice-cooled solution of the corresponding aldehyde or ketone (1 equiv) in methanol (5 mL/mmol) was added $NaBH_4$ (2 equiv). Then the resulting mixture was heated to reflux for 30 min. After cooling to ambient temperature, the solvent was distilled off under reduced pressure. Then water (10 mL) was added, and the resulting mixture was extracted with ethyl acetate (3× 10 mL). The combined organic phases were washed with brine, dried over $MgSO_4$ and evaporated under reduced pressure. Then the desired product was purified by chromatography on silica gel.

5.4.4.1. 4-Phenyl-2-thiophenemethanol (10a). Synthesised according to Method C using **10b** (0.31 g, 1.60 mmol) and $NaBH_4$ (0.11 g, 2.90 mmol); yield: 0.27 g (88%); white solid: mp 109–110 °C; $R_f = 0.13$ (PE/EtOAc, 10:1); IR (ATR) υ (cm^{-1}) 3290 (m), 1596 (w), 1500 (w), 1449 (m), 1195 (m), 1158 (m), 1018 (s), 837 (m), 733 (s), 688 (s); δ_H ($CDCl_3$, 500 MHz) 1.89 (br s, 1H, OH), 4.86 (s, 2H, CH_2), 7.28-7.32 (m, 2H), 7.39 (d, $J = 1.6$ Hz, 1H), 7.40 (dd, $J = 7.3$, 8.0 Hz, 2H), 7.57 (dd, $J = 1.6$, 8.0 Hz, 2H); δ_C ($CDCl_3$, 125 MHz) 60.3 (CH_2), 120.3 (CH), 124.7 (CH), 126.3 (CH), 127.2 (CH), 128.8 (CH), 135.8 (C_q), 142.1 (C_q), 144.8 (C_q); MS (ESI): $m/z = 191$ $[M^+ + H]$.

5.4.4.2. 4-(4-Fluorophenyl)-2-thiophenemethanol (11a). Synthesised according to Method C using **11b** (0.31 g, 1.50 mmol) and $NaBH_4$ (0.10 g, 2.70 mmol); yield: 0.27 g (87%); white solid: mp 120–121 °C; $R_f = 0.09$ (PE/EtOAc, 10:1); IR (ATR) υ (cm^{-1}) 3354 (m), 1602 (m), 1511 (s), 1419 (m), 1246 (s), 1151 (m), 1026 (s), 839 (s), 752 (s), 566 (s); δ_H ($CDCl_3$, 500 MHz) 1.90 (br s, 1H, OH), 4.86 (s, 2H), 7.08 (dd, $J = 8.5$, 8.8 Hz, 2H), 7.24–7.25 (m, 1H), 7.32 (d, $J = 1.6$ Hz, 1H), 7.51 (dd, $J = 5.4$, 8.8 Hz, 2H); δ_C ($CDCl_3$, 125 MHz) 60.2 (CH_2), 115.6 (d, $^2J_{CF} = 21.1$ Hz, CH), 120.0 (CH), 124.6 (CH), 127.8 (d, $^3J_{CF} = 7.7$ Hz, CH), 132.0 (d, $^4J_{CF} = 3.8$ Hz, C_q), 141.1 (C_q), 145.0 (C_q), 162.2 (d, $^1J_{CF} = 246.6$ Hz, C_q); MS (ESI): $m/z = 209$ $[M^+ + H]$.

5.4.4.3. 4-(4-Methoxyphenyl)-2-thiophenemethanol (12a). Synthesised according to Method C using **12b** (0.22 g, 1.00 mmol) and $NaBH_4$ (0.07 g, 1.80 mmol); yield: 0.19 g (84%); white solid: mp 138 °C (lit.[29] 136.5–138 °C); $R_f = 0.14$ (PE/EtOAc, 10:1); IR (ATR)

υ (cm^{-1}) 1512 (m), 1406 (m), 1309 (m), 1246 (m), 1180 (m), 1026 (s), 832 (s), 756 (m), 521 (m); δ_H ($CDCl_3$, 500 MHz) 3.77 (s, 3H), 4.64 (d, J = 5.4 Hz, 2H), 5.47 (t, J = 5.4 Hz, 1H), 6.95 (d, J = 8.8 Hz, 2H), 7.31–7.32 (m, 1H), 7.57 (d, J = 1.6 Hz, 1H), 7.59 (d, J = 8.8 Hz, 2H); δ_C ($CDCl_3$, 125 MHz) 55.0 (CH_3), 58.3 (CH_2), 114.1 (CH), 117.9 (CH), 122.7 (CH), 126.9 (CH), 128.0 (C_q), 140.3 (C_q), 147.0 (C_q), 158.3 (C_q); MS (ESI): m/z = 221 [M^++H].

5.4.4.4. 4-(3-Methoxyphenyl)-2-thiophenemethanol (13a). Synthesised according to Method C using **13b** (0.31 g, 1.40 mmol) and $NaBH_4$ (0.10 g, 2.50 mmol); yield: 0.29 g (95%); colourless oil; R_f = 0.10 (PE/EtOAc, 10:1); IR (ATR) υ (cm^{-1}) 3354 (m), 1600 (s), 1460 (m), 1285 (m), 1148 (s), 1038 (s), 841 (m), 784 (s), 753 (s), 688 (m); δ_H ($CDCl_3$, 500 MHz) 1.90 (br s, 1H), 3.85 (s, 3H), 4.86 (d, J = 3.5 Hz, 2H), 6.85 (ddd, J = 0.6, 2.5 Hz, J = 8.2 Hz, 1H), 7.10 (dd, J = 0.6, 2.5 Hz, 1H), 7.16 (ddd, J = 1.0, 1.6 Hz, 7.6 Hz, 1H), 7.29 (dd, J = 0.6, 1.3 Hz, 1H), 7.31 (dd, J = 7.6, 8.2 Hz, 1H), 7.38 (d, J = 1.6 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 55.3 (CH_3), 60.2 (CH_2), 112.1 (CH), 112.6 (CH), 118.9 (CH), 120.6 (CH), 124.8 (CH), 129.8 (CH), 137.1 (C_q), 142.0 (C_q), 144.7 (C_q), 160.0 (C_q); MS (ESI): m/z = 203 [M^+−OH].

5.4.4.5. 4-(3,4-Difluorophenyl)-2-thiophenemethanol (14a). Synthesised according to Method C using **14b** (0.34 g, 1.50 mmol) and $NaBH_4$ (0.10 g, 2.70 mmol); yield: 0.27 g (80%); white solid: mp 66 °C; R_f = 0.11 (PE/EtOAc, 10:1); IR (ATR) υ (cm^{-1}) 3264 (m), 1605 (m), 1515 (s), 1279 (s), 1224 (m), 1170 (m), 1118 (m), 1013 (s), 816 (s), 759 (s), 581 (s); δ_H ($CDCl_3$, 500 MHz) 1.93 (br s, 1H), 4.85 (s, 2H), 7.16 (ddd, J = 1.6, 8.2, 8.5 Hz, 1H), 7.21–7.22 (m, 1H), 7.24–7.28 (m, 1H), 7.33 (d, J = 1.6 Hz, 1H), 7.34 (ddd, J = 2.2, 7.6, 11.4 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 115.1 (d, $^2J_{CF}$ = 18.2 Hz, CH), 117.5 (d, $^2J_{CF}$ = 18.2 Hz, CH), 120.8 (CH), 122.3 (dd, $^4J_{CF}$ = 2.9 Hz,presup3J_{CF} = 5.8 Hz, CH), 124.3 (CH), 132.9 (dd, $^4J_{CF}$ = 3.8 Hz, $^3J_{CF}$ = 6.7 Hz, C_q), 140.0 (d, $^5J_{CF}$ = 1.9 Hz, C_q), 145.4 (C_q), 149.0 (dd, $^2J_{CF}$ = 12.5 Hz, $^1J_{CF}$ = 248.5 Hz, C_q), 150.0 (dd, $^2J_{CF}$ = 13.4 Hz, $^1J_{CF}$ = 248.5 Hz, C_q); MS (ESI): m/z = 209 [M^+−OH].

5.4.4.6. (4-Bromothiophen-2-yl)methanol (16b). Synthesised according to Method C using 4-bromothiophene-2-carbaldehyde (1.00 g, 5.20 mmol) and $NaBH_4$ (0.36 g, 9.40 mmol); yield: 0.99 g (98%); colourless oil; R_f = 0.13 (PE/EtOAc, 10:1); IR (ATR) υ (cm^{-1}) 3318 (m), 1528 (w), 1343 (w), 1147 (m), 1007 (s), 818 (s), 734 (s), 580 (s); δ_H ($CDCl_3$, 500 MHz) 2.04 (br s, 1H), 4.78 (s, 2H), 6.92 (m, 1H), 7.17 (d, J = 1.6 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 59.8 (CH_2), 109.3 (C_q), 122.6 (CH), 127.7 (CH), 145.3 (C_q); MS (ESI): m/z = 177/175 [M^+−OH].

5.4.4.7. 1-(4-(5-Methylsulfanylthiophen-2-yl)phenyl)-propan-1-ol (23a). Synthesised according to Method C from **23b** (0.79 g, 3.00 mmol); yield: 0.71 g (89%); white solid; R_f = 0.58 (DCM/MeOH, 98:2); IR (ATR) υ (cm^{-1}) 3317 (br), 1513 (m), 1465 (w), 1416 (w), 1261 (m), 1210 (w), 1008 (m), 970 (w), 946 (w), 823 (s), 803 (s); δ_H ($CDCl_3$, 500 MHz) 0.95 (t, J = 7.3 Hz, 3H, CH_3), 1.82 (m, 2H, CH_2), 1.88 (d, J = 3.1 Hz, 1H, OH), 2.51 (s, 3H, S-CH_3), 4.58–4.61 (m, 1H, CH), 6.72–6.73 (m, 1H), 7.10–7.13 (m, 1H), 7.31–7.33 (m, 2H), 7.52–7.54 (m, 2H); δ_C ($CDCl_3$, 125 MHz) 10.1 (CH_3), 15.4 (S-CH_3), 31.8 (CH_2), 75.7 (C–OH), 122.8, 125.4, 126.1, 126.4, 133.9, 139.4, 141.7, 143.3; MS (ESI): m/z = 217 [M^+−SMe].

5.4.4.8. 1-(4-Thiophen-3-ylphenyl)propan-1-one (27b). This compound is commercially available.

5.4.4.9. 1-(4-Thiophen-3-ylphenyl)propan-1-ol (27a). Synthesised according to Method C from **27b** (0.42 g, 1.90 mmol); yield: 0.41 g (91%); white solid; R_f = 0.33 (DCM/MeOH, 98:2); IR (ATR) υ (cm^{-1}) 3307 (br), 2928 (w), 1425 (w), 1261 (w), 1092 (m), 1044 (m), 973 (m), 865 (m), 844 (s), 728 (m), 690 (m); δ_H (DMSO, 500 MHz) 0.83 (t, J = 7.3 Hz, 3H, CH_3), 1.63–1.65 (m, 2H, CH_2), 4.45–4.46 (m, 1H, CH–OH), 7.35–7.37 (m, 2H), 7.53–7.55 (dd,J = 1.6, 3.4 Hz, 1H), 7.63–7.64 (m, 1H), 7.66–7.68 (m, 2H), 7.81 (d, J = 1.3 Hz, 1.6 Hz, 1H); δ_C (DMSO, 125 MHz) 9.9 (CH_3), 31.8 (CH_2), 73.2 (C–OH), 120.3, 122.5, 126.0, 126.2, 126.8, 133.4, 141.3, 144.9; MS (ESI): m/z = 201 [M^+-H_2O].

5.4.4.10. 1-(4-Thiophen-3-yl-phenyl)-ethanone (28b). This compound has been reported by Reuben.[30]

5.4.4.11. 1-(4-Thiophen-3-ylphenyl)ethanol (28a). Synthesised according to Method C from **28b** (1.02 g, 5.10 mmol); yield: 0.86 (83%); white solid; R_f = 0.4 (DCM); IR (ATR) υ (cm^{-1}) 3290 (br), 1362 (w), 1290 (w), 1200 (w), 1072 (m), 892 (m), 864 (m), 835 (m), 779 (s), 731 (m); δ_H (DMSO, 500 MHz) 1.35 (d, J = 6.6 Hz, 3H, CH_3), 4.72–7.43 (m, 1H, CH–OH), 7.36–7.37 (m, 2H), 7.53 (dd, J = 1.6, 3.4 Hz 1H), 7.63–7.65 (m, 1H), 7.67–7.68 (m, 2H), 7.82 (dd, J = 1.3, 1.6 Hz, 1H); δ_C (DMSO, 125 MHz) 25.8 (CH_3), 67.9 (CH–OH), 120.4, 125.8, 126.2, 127.0,133.4, 141.5, 146.3; MS (ESI): m/z = 187 [M^+-H_2O].

5.4.4.12. 1-(4-(4-Methylthiophen-3-yl)-phenyl)propan-1-ol (29a). Synthesised according to Method C from **29b** (1.23 g, 5.30 mmol); yield: 1.03 g (83%); white solid; R_f = 0.55 (DCM/MeOH, 98:2); δ_H ($CDCl_3$, 500 MHz) 0.97 (t, J = 7.6 Hz, 3H, CH_3), 1.81–1.83 (m, 2H, CH_2), 2.27 (s, 3H, CH_3), 4.63–4.66 (m, 1H, CH), 7.02–7.03 (m, 1H), 7.19–7.20 (m, 1H), 7.38–7.42 (m, 4H); δ_C ($CDCl_3$, 125 MHz) 9.9 (CH_3), 15.3 (CH_3), 31.6 (CH_2), 121.8, 122.7, 125.7, 128.4, 135.9, 136.1, 142.6, 143.0; MS (ESI): m/z = 233 [M^++H].

5.4.5. Method D: Grignard reaction. Under exclusion of air and moisture a 1.0 M EtMgBr (1.2 equiv) solution in THF was added dropwise to a solution of the aldehyde or ketone (1 equiv) in THF (12 mL/mmol). The mixture was stirred overnight at room temperature. Then ethyl acetate (10 mL) and water (10 mL) were added and the organic phase was separated. The organic phase was extracted with water and brine, dried over Na_2SO_4 and

evaporated under reduced pressure. The crude products were purified by flash chromatography on silica gel.

5.4.5.1. 1-(4-(3,4-Difluorophenyl)-2-thiophen-1-yl)-1-propanol (15a). Synthesised according to Method D using **14b** (0.20 g, 0.89 mmol) and 1.0 M EtMgBr (0.98 mL, 0.98 mmol); yield: 0.18 g (79%); colourless oil; R_f = 0.14 (PE/EtOAc, 10:1); δ_H ($CDCl_3$, 500 MHz) 0.93 (t, J = 7.3 Hz, 3H, CH_3), 1.80–1.88 (m, 2H, CH_2), 1.96 (br s, 1H, OH), 4.79 (t, J = 6.6 Hz, 1H, C*H*CH_2), 7.09–7.13 (m, 1H), 7.16–7.18 (m, 1H), 7.19–7.22 (m, 1H), 7.23 (d, J = 1.6 Hz, 1H), 7.27 (ddd, J = 1.6, 2.2, 7.6 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 10.0 (CH_3), 32.2 (CH_2), 71.8 (CH), 115.0 (CH), 117.4 (CH), 117.6 (CH), 119.8 (CH), 122.1 (CH), 122.6 (CH), 133.0 (C_q), 139.7 (C_q), 150.0 (C_q), 150.6 (C_q); MS (ESI): m/z = 255 [M^++H].

5.4.5.2. 1-(2-Fluoro-4-(4-methylthiophen-3-yl)-phenyl)propan-1-ol (30a). Synthesised according to Method D from **30b** (2.00 g, 4.90 mmol); yield: 0.73 g (30%); white solid; R_f = 0.32 (Hex/EtOAc, 4:1); δ_H ($CDCl_3$, 500 MHz) 0.98 (t, J = 7.6 Hz, 3H, CH_3), 1.85–1.87 (m, 2H, CH_2), 2.52 (s, 3H, CH_3), 4.96 (t, J = 6.3 Hz, 1H, CH), 7.03–7.04 (m, 1H), 7.05–7.06 (m, 1H), 7.18–7.20 (m, 2H), 7.49–7.50 (m, 1H); δ_C ($CDCl_3$, 125 MHz) 10.0 (CH_3), 15.5 (CH_3), 30.9 (CH_2), 70.0 (CHOH), 115.2, 122.3, 123.4, 124.5, 127.2, 130.0, 135.9, 138.0, 160.7; MS (ESI): m/z = 251 [M^++H].

5.4.5.3. 1-(4-(2-Chlorothiophen-3-yl)phenyl)propan-1-ol (31a). Synthesised according to Method D from **31b** (1.32 g, 6.50 mmol); yield: 1.48 g (95%); white solid; R_f = 0.30 (PE/EtOAc, 10:1); δ_H ($CDCl_3$, 500 MHz) 0.81 (t, J = 7.6 Hz, 3H, CH_3), 1.69–1.71 (m, 2H, CH_2), 2.07 (br, 1H, OH), 4.45 (t, J = 6.6 Hz, 1H, CH), 6.61–6.62 (m, 1H), 6.98–6.99 (m, 1H), 7.18–7.20 (m, 2H), 7.40–7.41 (m, 2H); δ_C ($CDCl_3$, 125 MHz) 10.2 (CH_3), 31.8 (CH_2), 75.7 (CH), 122.8, 125.5, 126.1, 126.5, 127.1, 128.8, 133.9, 139.4, 141.7, 143.4; MS (ESI): m/z = 253 [M^++H].

5.4.6. Method E: CDI reaction. To a solution of the corresponding alcohol (1 equiv) in NMP or acetonitrile (10 mL/mmol) was added CDI (5 equiv). Then the solution was heated to reflux for 4–18 h. After cooling to ambient temperature, it was diluted with water (30 mL) and extracted with ethyl acetate (3× 10 mL). The combined organic phases were washed with brine, dried over $MgSO_4$ and evaporated under reduced pressure. Then the desired product was purified by chromatography on silica gel.

5.4.6.1. 1-((4-Phenylthiophen-2-yl)methyl)-1*H*-imidazole (10). Synthesised according to Method E using **10a** (0.19 g, 1.00 mmol) and CDI (0.24 g, 1.50 mmol); yield: 0.09 g (38%); light brown solid: mp 112–113 °C; R_f = 0.22 (EtOAc); IR (ATR) υ (cm^{-1}) 3110 (w), 1504 (m), 1430 (m), 1358 (w), 1230 (m), 1084 (m), 817 (m), 744 (s), 660 (s); δ_H ($CDCl_3$, 500 MHz) 5.29 (s, 2 H), 7.00 (t, J = 1.3 Hz, 1H), 7.10 (br s, 1H), 7.24–7.25 (m, 1H), 7.30 (tt, J = 1.3, 7.5 Hz, 1H), 7.38 (d, J = 1.6 Hz, 1H), 7.39 (dd, J = 7.5, 7.8 Hz, 2H), 7.53 (dd, J = 1.3, 7.8 Hz, 2H), 7.60 (s, 1H); δ_C ($CDCl_3$, 125 MHz) 45.7 (CH_2), 118.9 (CH), 120.8 (CH), 126.0 (CH), 126.3 (CH), 127.5 (CH), 128.9 (CH), 130.0 (CH), 135.2 (C_q), 137.0 (CH), 139.2 (C_q), 142.4 (C_q); MS (ESI): m/z = 241 [M^++H].

5.4.6.2. 1-((4-(4-Fluorophenyl)thiophen-2-yl)methyl)-1*H*-imidazole (11). Synthesised according to Method E using **11a** (0.21 g, 1.00 mmol) and CDI (0.24 g, 1.50 mmol); yield: 0.12 g (49%); brown oil; R_f = 0.20 (EtOAc); IR (ATR) υ (cm^{-1}) 3088 (w), 1511 (s), 1224 (s), 1160 (m), 1075 (m), 830 (s), 761 (m), 661 (m), 565 (m); δ_H ($CDCl_3$, 500 MHz) 5.28 (s, 2H), 6.99 (t, J = 1.2 Hz, 1H), 7.07 (dd, J = 8.5, 8.8 Hz, 2H), 7.09 (br s, 1H), 7.17-7.18 (m, 1H), 7.31 (d, J = 1.6 Hz, 1H), 7.48 (dd, J = 5.4 Hz, J = 8.8 Hz, 2H), 7.59 (br s, 1H); δ_C ($CDCl_3$, 125 MHz) 45.6 (CH_2), 115.7 (d, $^2J_{CF}$ = 21.1 Hz, CH), 118.9 (CH), 120.5 (CH), 125.9 (CH), 127.9 (d, $^3J_{CF}$ = 8.6 Hz, CH), 129.9 (CH), 131.4 (d, $^4J_{CF}$ = 3.8 Hz, C_q), 137.0 (CH), 139.4 (C_q), 141.4 (C_q), 162.3 (d, $^1J_{CF}$ = 246.6 Hz, C_q); MS (ESI): m/z = 259 [M^++H].

5.4.6.3. 1-((4-(4-Methoxyphenyl)thiophen-2-yl)methyl)-1*H*-imidazole (12). Synthesised according to Method E using **12a** (0.16 g, 0.70 mmol) and CDI (0.16 g, 1.00 mmol); yield: 0.08 g (44%); light brown solid: mp 140 °C; R_f = 0.20 (EtOAc); IR (ATR) υ (cm^{-1}) 2961 (w), 1517 (m), 1254 (m), 1077 (m), 1020 (s), 828 (s), 758 (s), 660 (m); δ_H ($CDCl_3$, 500 MHz) 3.83 (s, 3H), 5.28 (s, 2H), 6.92 (d, J = 8.8 Hz, 2H), 6.99 (br s, 1H), 7.19 (br s, 1H), 7.27 (d, J = 1.6 Hz, 2H), 7.45 (d, J = 8.8 Hz, 2H), 7.59 (br s, 1H); δ_C ($CDCl_3$, 125 MHz) 45.7 (CH_2), 55.3 (CH_3), 114.3 (CH), 118.9 (CH), 119.4 (CH), 126.0 (CH), 127.4 (CH), 128.1 (C_q), 129.9 (CH), 137.0 (CH), 139.0 (C_q), 142.1 (C_q), 159.1 (C_q); MS (ESI): m/z = 271 [M^++H].

5.4.6.4. 1-((4-(3-Methoxyphenyl)thiophen-2-yl)methyl)-1*H*-imidazole (13). Synthesised according to Method E using **13a** (0.28 g, 1.30 mmol) and CDI (0.31 g, 1.90 mmol); yield: 0.11 g (31%); brown solid: mp 48 °C; R_f = 0.27 (EtOAc); IR (ATR) υ (cm^{-1}) 3076 (w), 1591 (m), 1496 (m), 1261 (m), 1222 (m), 1175 (m), 1031 (m), 784 (s), 744 (s), 659 (s); δ_H ($CDCl_3$, 500 MHz) 3.84 (s, 3H), 5.27 (s, 2H), 6.85 (ddd, J = 1.0, 2.5, 8.2 Hz, 1H), 6.98 (t, J = 1.3 Hz, 1H), 7.05 (dd, J = 1.6, 2.5 Hz, 1H), 7.09 (dd, J = 1.0, 1.3 Hz, 1H), 7.11 (ddd,J = 1.0, 1.6, 7.6 Hz, 1H), 7.22–7.23 (m, 1H), 7.30 (dd, J = 7.6, 8.2 Hz, 1H), 7.37 (d, J = 1.6 Hz, 1H), 7.58 (br s, 1H); δ_C ($CDCl_3$, 125 MHz) 45.6 (CH_2), 55.3 (CH_3), 112.1 (CH), 112.7 (CH), 118.8 (CH), 118.9 (CH), 121.0 (CH), 126.1 (CH), 129.6 (C_q), 129.9 (CH), 136.6 (C_q), 136.9 (CH), 139.1 (CH), 142.2 (C_q), 160.0 (C_q); MS (ESI): m/z = 271 [M^++H].

5.4.6.5. 1-((4-(3,4-Difluorophenyl)thiophen-2-yl) methyl)-1*H*-imidazole (14). Synthesised according to Method E using **14a** (0.24 g, 1.10 mmol) and CDI (0.24 g, 1.50 mmol); yield: 0.12 g (41%); light brown solid: mp 83 °C; R_f = 0.25 (EtOAc); IR (ATR) υ (cm^{-1}) 3092 (w), 1605 (m), 1515 (s), 1439 (m), 1268 (m), 1226 (m), 1070 (m), 819 (s), 766 (s), 664 (m), 575

(m); δ_H ($CDCl_3$, 500 MHz) 5.19 (s, 2H), 6.90 (t, J = 1.3 Hz, 1H), 7.00 (dd, J = 1.0, 1.3 Hz, 1H), 7.06 (dt, J = 8.2, 10.9 Hz, 1H), 7.05–7.06 (m, 1H), 7.12–7.15 (m, 1H), 7.21 (ddd, J = 1.9, 7.3, 11.4 Hz, 1H), 7.23 (d, J = 1.6 Hz, 1H), 7.50 (s, 1H); δ_C ($CDCl_3$, 125 MHz) 45.4 (CH_2), 115.1 (d, $^2J_{CF}$ = 18.2 Hz, CH), 117.5 (d, $^2J_{CF}$ = 17.3 Hz, CH), 118.8 (CH), 121.2 (CH), 122.1 (dd, $^3J_{CF}$ = 5.8 Hz, $^4J_{CF}$ = 2.9 Hz, CH), 125.5 (CH), 129.8 (CH), 132.3 (dd, $^3J_{CF}$ = 5.8 Hz, $^4J_{CF}$ = 3.8 Hz, C_q), 136.9 (CH), 139.8 (Cq), 140.1 (Cq), 149.7 (dd, $^1J_{CF}$ = 249.5 Hz, $^2J_{CF}$ = 12.5 Hz, C_q), 150.3 (dd, $^1J_{CF}$ = 248.6 Hz, $^2J_{CF}$ = 13.5 Hz, C_q); MS (ESI): m/z = 277 [M^++H].

5.4.6.6. 1-(1-(4-(4-Fluorophenyl)thiophen-2-yl)propyl)-1*H*-imidazole (15). Synthesised according to Method E using **15a** (0.15 g, 0.60 mmol) and CDI (0.29 g, 1.80 mmol); yield: 0.08 g (45%); brown oil; R_f = 0.29 (DCM/MeOH, 98:2); IR (ATR) υ (cm^{-1}) 1606 cm^{-1} (w), 1516 (s), 1277 (m), 1222 (m), 1117 (w), 1072 (w), 815 (s), 770 (s), 662 (m); δ_H ($CDCl_3$, 500 MHz) 0.97 (t, J = 7.4 Hz, 3H), 2.22–2.34 (m, 2H), 5.26 (dd, J = 6.3, 8.8 Hz, 1H), 7.01 (br s, 1H), 7.09–7.10 (m, 1H), 7.11 (br s, 1H), 7.15 (ddd, J = 8.0, 8.5, 9.9 Hz, 1H), 7.20–7.23 (m, 1H), 7.30 (ddd, J = 2.2, 7.5, 11.5 Hz, 1H), 7.31 (d, J = 1.3 Hz, 1H), 7.65 (br s, 1H); δ_C ($CDCl_3$, 125 MHz) 10.9 (CH_3), 30.0 (CH_2), 58.9 (CH), 115.2 (d, $^2J_{CF}$ = 18.2 Hz, CH), 117.2 (CH), 117.6 (d, $^2J_{CF}$ = 17.3 Hz, CH), 120.5 (CH), 122.2 (dd, $^3J_{CF}$ = 6.7 Hz, $^4J_{CF}$ = 3.8 Hz, CH), 124.1 (CH), 129.8 (CH), 132.5 (dd, $^3J_{CF}$ = 6.7 Hz, $^4J_{CF}$ = 3.8 Hz, C_q), 136.4 (CH), 140.0 (C_q), 145.2 (C_q), 149.7 (dd, $^1J_{CF}$ = 247.5 Hz, $^2J_{CF}$ = 12.5 Hz, C_q), 150.5 (dd, $^1J_{CF}$ = 249.5 Hz, $^2J_{CF}$ = 13.5 Hz, C_q); MS (ESI): m/z = 305 [M^++H].

5.4.6.7. 1-((4-Bromothiophen-2-yl)methyl)-1*H*-imidazole (16a). Synthesised according to Method E using **16b** (0.74 g, 3.81 mmol) and CDI (1.24 g, 7.67 mmol); yield: 0.72 g (78%); colourless oil; R_f = 0.17 (EtOAc); δ_H (CDCltextsubscript3, 500 MHz) 5.23 (s, 2H), 6.88–6.89 (m, 1H), 6.94 (s, 1H), 7.09 (s, 1H), 7.18 (d, J = 1.6 Hz, 1H), 7.55 (s, 1H); δ_C ($CDCl_3$, 125 MHz) 45.2 (CH_2), 109.8 (C_q), 118.8 (CH), 123.3 (CH), 129.2 (CH), 130.2 (CH), 137.0 (CH), 139.9 (C_q); MS (ESI): m/z = 245/243 [M^++H].

5.4.6.8. 2-(4-((1*H*-Imidazol-1-yl)methyl)phenyl)-5-(chloromethyl)pyridine (19). Synthesised according to Method E using compound **19a** (0.49 g, 2.10 mmol) and CDI (0.68 g, 4.20 mmol); yield: 0.22 g (37%); R_f = 0.15 (EtOAc/MeOH, 95:5); IR (ATR) υ (cm^{-1}) 1507 (m), 1458 (s), 1384 (m), 1231 (m), 1104 (s), 1024 (m), 814 (m), 739 (s), 662 (s); δ_H ($CDCl_3$, 500 MHz) 3.94 (s, 2H), 5.09 (s, 2H), 6.89 (t, J = 1.3 Hz, 1H), 7.08 (t, J = 1.3 Hz, 1H), 7.09 (d, J = 8.5 Hz, 2H), 7.14 (d, J = 8.5 Hz, 2H), 7.23 (d, J = 8.2 Hz, 1H), 7.40 (dd, J = 2.5, 8.2 Hz, 1H), 7.53 (s, 1H), 8.24 (d, J = 2.2 Hz, 1H); δ_C ($CDCl_3$, 125 MHz) 37.8 (CH_2), 50.5 (CH_2), 119.2 (CH), 124.1 (CH), 127.8 (CH), 129.4 (CH), 129.7 (CH), 134.7 (C_q), 135.0 (CH), 137.3 (CH), 139.1 (CH), 139.5 (C_q), 149.6 (C_q), 149.7 (C_q); MS (ESI): m/z = 284 [M^++H].

5.4.6.9. 5-(4-((1*H*-Imidazol-1-yl)methyl)phenyl)pyrimidine (20). Synthesised according to Method E using compound **20a** (0.11 g, 0.60 mmol) and CDI (0.28 g, 1.10 mmol); yield: 0.08 g (57%); white solid: mp 135–138 °C; R_f = 0.29 (EtOAc/MeOH, 10:1); δ_H ($CDCl_3$, 500 MHz) 5.18 (s, 2H), 6.92 (s, 1H), 7.10 (s, 1H), 7.28 (d, J = 8.1 Hz, 2H), 7.55 (d, J = 8.1 Hz, 2H), 7.57 (br s, 1H), 8.91 (s, 2H), 9.19 (s, 1H); δ_C ($CDCl_3$, 125 MHz) 50.3 (CH_2), 119.2 (CH), 127.5 (CH), 128.2 (CH), 130.0 (CH), 133.5 (C_q), 134.3 (CH), 137.2 (C_q), 137.4 (C_q), 154.8 (CH), 157.7 (CH); MS (ESI): m/z = 237 [M^++H].

5.4.6.10. 4-(4-((1*H*-Imidazol-1-yl)methyl)phenyl)morpholine (21). Synthesised according to Method E using compound **21a** (0.33 g, 1.60 mmol) and CDI (0.52 g, 3.20 mmol); light yellow oil: bp >200 °C; R_f = 0.22 (EtOAc); IR (ATR) υ (cm^{-1}) 2854 cm^{-1} (w), 1613 (m), 1518 (m), 1450 (m), 1230 (s), 1117 (s), 926 (s), 826 (s), 664 (s); δ_H ($CDCl_3$, 500 MHz) 1.82 (d, J = 7.0 Hz, 3H), 3.15 (m, 4H), 3.85 (m, 4H), 5.27 (q, J = 7.0 Hz, 1H), 6.86 (d, J = 8.6 Hz, 2H), 6.90 (br s, 1H), 7.05 (br s, 1H), 7.07 (d, J = 8.6 Hz, 2H), 7.56 (br s, 1H); δ_C ($CDCl_3$, 125 MHz) 22.0 (CH_3), 49.0 (CH_2), 56.0 (CH), 66.8 (CH_2), 115.6 (CH), 117.8 (CH), 127.1 (CH), 129.3 (CH), 132.4 (C_q), 136.0 (CH), 151.0 (C_q); MS (ESI): m/z = 257 [M^++H].

5.4.6.11. 1-(1-(4-(5-Methylsulfanyl-thiophen-2-yl)-phenyl)-propyl)-1*H*-imidazole (23). Synthesised according to Method E from **23a** (0.60 g, 2.30 mmol); yield: 0.40 g (56%); white solid: mp 86 °C; R_f = 0.48 (DCM/MeOH, 98:2); IR (ATR) υ (cm^{-1}) 3119 (w), 2967 (w), 1684 (w), 1512 (m), 1461 (m), 1232 (s), 1071 (s), 1022 (m), 908 (s), 836 (s), 786 (s), 741 (s), 665 (s); δ_H (DMSO, 500 MHz) 0.93 (t, J = 7.3 Hz, 3H, CH_3), 2.16-2.18 (m, 2H, CH_2), 2.42 (s, 3H, S-CH_3), 4.92 (t, J = 7.6 Hz, 1H, CH), 6.63–6.64 (m, 1H), 6.87–6.88 (m, 1H), 7.01–7.03 (m, 2H), 7.09–7.11 (m, 2H), 7.42–7.44 (m, 2H), 7.53 (s, 1H); δ_C (DMSO, 125 MHz) 11.1 (CH_3), 15.4 (S-CH_3), 28.5 (CH_2), 63.0 (C–OH), 117.6, 123.2, 125.8, 126.3, 127.0, 129.6, 134.6, 136.3, 138.9, 139.9, 141.0; MS (ESI): m/z = 285 [M^+− Et].

5.4.6.12. 1-(1-(4-Thiophen-3-yl-phenyl)propyl)-1*H*-imidazole (27). Synthesised according to Method E from **27a** (0.32 g, 1.40 mmol); yield: 0.37 g (87%); white solid: mp 128 °C; R_f = 0.55 (DCM/MeOH, 98:2); IR (ATR) υ (cm^{-1}) 3134 (w), 2930 (w), 1798 (vs), 1528 (w), 1479 (m), 1401 (vs), 1321 (s), 1265 (vs), 837 (m); δ_H (DMSO, 500 MHz) 0.91 (t, J = 7.3 Hz, 3H, CH_3), 1.95 (m, 1H, CH_2), 2.05–2.7 (m, 1H, CH_2), 5.83–5.85 (m, 1H, CH), 7.09–7.10 (m, 1H), 7.52–7.53 (m, 2H), 7.55 (d, J = 8.2 Hz, 1H), 7.63–7.65 (m, 1H), 7.67 (t, J = 1.6 Hz, 1H), 7.73–7.75 (m, 2H), 7.88–7.89 (m, 1H), 8.37–8.38 (m, 1H); δ_C (DMSO, 125 MHz) 9.4 (CH_3), 28.5 (CH_2), 117.4, 121.2, 126.0, 126.8, 127.0, 130.3, 135.1, 137.2, 137.6, 140.8, 147.6; MS (ESI): m/z = 269 [M^++H].

5.4.6.13. 1-(1-(4-Thiophen-3-ylphenyl)ethyl)-1*H*-imidazole (28). Synthesised according to Method E from **28a** (0.50 g, 2.45 mmol); yield: 0.63 g (87%); white solid: mp 153 °C; R_f = 0.57 (DCM/MeOH, 98:2); IR (ATR) υ

(cm^{-1}) 3136 (w), 1745 (vs), 1476 (m), 1392 (s), 1259 (m), 836 (m); δ_H (DMSO, 500 MHz) 1.67 (d, J = 6.3 Hz, 3H, CH_3), 6.03–6.06 (q, 1H, CH), 7.08–7.09 (m, 1H), 7.53–7.55 (m, 3H), 7.63–7.64 (m, 2H), 7.75–7.77 (m, 2H), 7.88–7.89 (m, 1H), 8.34–8.36 (m, 1H); δ_C (DMSO, 125 MHz) 27.6 (CH_3), 121.5, 126.1, 127.6, 134.9, 137.3, 141.3, 147.5; MS (ESI): m/z = 255 [M^++H].

5.4.6.14. 1-(1-(4-(4-Methylthiophen-3-yl)phenyl)propyl)-1*H*-imidazole (29). Synthesised according to Method E from **29a** (0.90 g, 3.90 mmol); yield: 0.23 (21%); white solid; R_f = 0.35 (DCM/MeOH, 95:5); IR (ATR) υ (cm^{-1}) 2969 (w), 2932 (w), 1494 (m), 1455 (w), 1223 (s), 1072 (s), 906 (m), 790 (vs), 734 (s), 663 (vs); δ_H ($CDCl_3$, 500 MHz) 0.99 (t, J = 7.3 Hz, 3H, CH_3), 2.22–2.25 (m, 3H), 5.06 (t, J = 7.6 Hz, 1H, CH), 6.99–7.01 (m, 1H), 7.02–7.03 (m, 1H), 7.10–7.11 (m, 1H), 7.17–7.18 (m, 1H), 7.21–7.23 (m, 2H), 7.35–7.37 (m, 2H), 7.62–7.63 (m, 1H); δ_C ($CDCl_3$, 125 MHz) 11.1 (CH_3), 15.5 (CH_3), 28.6 (CH_2), 63.1 (CH), 117.7, 120.2, 122.2, 123.2, 126.5, 128.9, 129.6, 136.0, 136.4, 137.0, 139.0, 142.3; MS (ESI): m/z = 283 [M^++H].

5.4.6.15. 1-(1-(2-Fluoro-4-(4-methylthiophen-3-yl)-phenyl)propyl)-1 *H*-imidazole (30). Synthesised according to Method E from **30a** (0.73 g, 2.90 mmol); yield: 0.12 g (22%); white solid; R_f = (PE/EtOAc, 10:1); IR (ATR) υ (cm^{-1}) 2970 (w), 1624 (m), 1572 (m), 1493 (m), 1446 (w), 1407 (w), 1280 (m), 1223 (s), 1111 (m), 1073 (m), 906 (m), 865 (m), 791 (vs), 732 (s); δ_H ($CDCl_3$, 500 MHz) 0.96 (t, J = 7.3 Hz, 3H, CH_3), 2.25 (m, 5H), 5.37 (t, J = 6.9 Hz, 1H, CH), 7.03–7.05 (m, 2H), 7.11–7.13 (m, 2H), 7.20–7.22 (m, 3H), 7.65–7.66 (m, 1H); δ_C ($CDCl_3$, 125 MHz) 11.1 (CH_3), 15.4 (CH_3), 27.7 (CH_2), 56.6 (CH), 115.9, 117.8, 122.6, 123.8, 124.8, 125.9, 127.1, 129.3, 135.7, 136.4, 139.1, 141.0, 158.9, 160.9; MS (ESI): m/z = 301 [M^++H].

5.4.6.16. 1-(1-(4-(2-Chlorothiophen-3-yl)-phenyl)propyl)-1*H*-imidazole (31). Synthesised according to Method E from **31a** (0.81 g, 3.20 mmol); yield: 0.32 g (33%); yellow oil; R_f = 0.36 (DCM/MeOH, 20:1); IR (ATR) υ (cm^{-1}) 2967 (w), 1495 (m), 1410 (w), 1260 (m), 1224 (m), 1109 (m), 1072 (s), 1024 (s), 906 (m), 876 (s), 812 (vs), 719 (vs), 663 (vs), 622 (vs), 549 (m); δ_H ($CDCl_3$, 500 MHz) 0.96 (t, J = 7.3 Hz, 3H, CH_3), 2.25 (q, J = 7.3, 7.6 Hz, 2H, CH_2), 5.04 (t, J = 7.6 Hz, 1H, CH), 6.97 (s, 1H), 7.00 (d, J = 6.0 Hz), 7.09 (s, 1H), 7.13 (d, J = 5.7 Hz, 1H), 7.23 (d, J = 8.2 Hz, 2H), 7.53 (d, J = 8.2 Hz, 2H), 7.61 (s, 1H); δ_C ($CDCl_3$, 125 MHz) 11.1 (CH_3), 28.5 (CH_2), 62.9 (CH), 117.6, 122.8, 125.0, 126.5, 128.2, 128.8, 129.5, 133.9, 136.3, 137.2, 139.5; MS (ESI): m/z = 303 [M^++H], 235 [M^+−Im].

5.5. Docking studies

5.5.1. Ligands. All molecular modelling studies were performed on Intel(R) P4 CPU 3.00 GHz running Linux Suse 10.1. The structures of the inhibitors were built with SYBYL 7.3.2 (Sybyl, Tripos Inc., St. Louis, Missouri, USA) and energy-minimized in MMFF94s force-field[31] as implemented in Sybyl. The resulting geometries for our compounds were then subjected to ab initio calculation employing the B3LYP functional[32,33] in combination with a 6-31G* basis set using the package Gaussian03 (Gaussian, Inc., Pittsburgh, PA, 2003).

5.5.2. Homology modelling. A homology model was built with MODELLER v8.0,[34,35] using the X-ray structure of human CYP2C9 (PDB (http://www.rcsb.org) code 1R9O, Res. 1.9 Å) as template. The two amino acid sequences were globally aligned with T-COFFEE[36] and then manually refined considering the predicted secondary structure of CYP17 (secondary structure prediction by PSIPRED[37] to give an overall sequence identity percentage of approximately 30%). The haeme group was included in model building.

The resulting best homology model was first checked with the BIOPOLYMER module of SYBYL and AMBER charges were loaded. Hydrogens at histidine residues were positioned at the ε-nitrogen. Serine residues were considered to be neutral, whereas all basic and acidic residues were considered protonated and deprotonated, respectively. This structure was minimized for 200 steps with the steepest descent minimizer as implemented in SYBYL with the backbone atoms kept at fixed positions in order to achieve a low energy conformation and a satisfactory protein geometry containing no conformationally disallowed regions. Finally the chosen homology model was validated using different online tools (PROCHECK[38] and WHATIF[39]).

5.5.3. Docking. Molecular docking calculations were performed for various inhibitors of Tables 1 and 2. Since the GOLD docking program allows flexible docking of the compounds, no conformational search was employed to the ligand structures. GOLD gave the best poses by a genetic algorithm (GA) search strategy, and then various molecular features were encoded as a chromosome.

Ligands were docked in 50 independent genetic algorithm (GA) runs using GOLD. Haeme iron was chosen as active-site origin, while the radius was set equal to 19 Å. The automatic active-site detection was switched on. A distance constraint of a minimum of 1.9 and a maximum of 2.5 Å between the sp^2-hybridised nitrogen of the imidazole and the iron was set. Further, some of the GOLDSCORE parameters were modified to improve the weight of hydrophobic interaction and of the coordination between iron and nitrogen. The genetic algorithm default parameters were set as suggested by the GOLD authors. On the other hand, the annealing parameters of fitness function were set at 3.5 Å for hydrogen bonding and 6.5 Å for van der Waals interactions.

All 50 poses for each compound were clustered with ACIAP[40,41] and the representative structure of each significant cluster was selected. The quality of the docked representative poses was evaluated based on visual inspection of the putative binding modes of the ligands, as outcome of docking simulations and cluster analysis.

5.5.4. Mep. For each docked compound geometry optimization was performed at the B3LYP/6-31G* level by means of the Gaussian03 software and the molecular electrostatics potential map (MEP) was plotted using GaussView3, the 3D molecular graphics package of Gaussian.[42] These electrostatic potential surfaces were generated by mapping 6-31G* electrostatic potentials onto surfaces of molecular electron density (isovalue = 0.002 e/Å).[43]

Acknowledgments

We thank the Fonds der Chemischen Industrie for financial support. U. E. H. is grateful to the European Postgraduate School 532 (D.F.G.) for a scholarship.

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