Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 267–273
Bioorganic & Medicinal Chemistry Letters

Synthesis, biological evaluation and molecular modelling studies of novel ACD- and ABD-ring steroidomimetics as inhibitors of CYP17

Mariano A. E. Pinto-Bazurco Mendieta,[a] Matthias Negri,[a] Carsten Jagusch,[a] Ulrike E. Hille,[a] Ursula Müller-Vieira,[b] Dirk Schmidt,[c] Klaus Hansen[c] 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 AG, Alfred-Nobel-Str. 10, D-40789 Monheim, Germany*

Received 4 September 2007; revised 22 October 2007; accepted 25 October 2007
Available online 30 October 2007

Abstract—Two novel classes of non-steroidal substrate mimetics were synthesised and examined for their potency as inhibitors of human CYP17. Selected compounds were tested for inhibition of hepatic CYP enzymes 3A4, 1A2, 2C9 and 2C19. The most promising compound **15** showed a good inhibition of the target enzyme (31% and 66% at 0.2 and 2 μM, respectively), and little inhibition of the most important hepatic enzyme CYP3A4 (6% and 19% inhibition at 0.2 and 2 μM, respectively) and the key enzyme of glucocorticoid biosynthesis CYP11B1 (3% and 23% inhibition at 0.2 and 2 μM, respectively). Docking studies revealed that this compound does not assume the same binding mode as steroidal ligands.

Prostate cancer is the most common disease and age-related cause of death in elder men worldwide.[1] Since it is in over 80% of the cases androgen dependent, the standard treatment is orchiectomy or its medicinal equivalent the chemical castration by gonadotropin-releasing hormone analogues, which reduce the testicular androgen production.[2] Because these treatments do not affect adrenal androgen production, they are frequently combined with androgen receptor antagonists (flutamide, cyproterone acetate) to reduce the stimulatory effects of the remaining androgens.[3] However, due to mutations in the androgen receptor, anti-androgens might be recognized as agonists,[4,5] making this so-called 'combined androgen blockade' therapy not suitable for all patients.

The antimycotic ketoconazole has proven itself clinically as a good adjuvant therapy by reducing testosterone biosynthesis through inhibition of CYP17.[6,7] Nevertheless, the toxicity drawbacks it showed have forced to suspend it from use.[8] On the other hand, the steroidal CYP17 inhibitor abiraterone (Fig. 1) passed phase II clinical trials showing high activity in post-docetaxel castration refractory PC patients and seems to have no dose-limiting toxicity.[9]

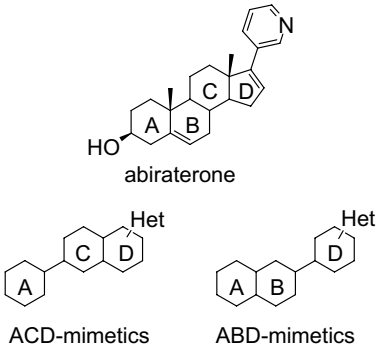


Figure 1. The steroidal CYP17 inhibitor abiraterone and ACD- and ABD-ring mimetics. Het: N-containing heterocycle.

Keywords: Prostate cancer; 17α-hydroxylase-17,20-lyase (CYP17) inhibitors; Steroidomimetics; Hepatic CYPs; Docking studies.
* Corresponding author. Tel.: +49 681 302 2424; e-mail: rwh@mx.uni-saarland.de

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.10.079

All this makes CYP17 an interesting target, since it catalyses both the 17α-hydroxylation of pregnenolone and progesterone and the subsequent 17,20-lyase reaction cleaving the C17–C20 bond to yield the 17-keto androgens androstendione and dehydroandrostendione, the precursors of testosterone (Fig. 2).[10]

We also developed highly active steroidal inhibitors, which showed up to 3-fold higher activities against human CYP17 than abiraterone in vitro.[11] In order to selectively inhibit CYP17 without the potential side effects of steroidal drugs[12] non-steroidal substrate mimetics have been prepared before.[12–16] In this work, in order to find a new core structure, two different classes of substrate analogues were synthesised (Fig. 3) which mimic the A-, C- and D-rings (compounds **1**–**11**), and the A-, B- and D-rings of the substrate with benzene nuclei (compounds **12**–**17**). Different nitrogen bearing heterocycles were introduced at different positions, since the heme complexation by an aromatic nitrogen is an important prerequisite for a high binding affinity.[17] From previous work,[14–16] it was known that the introduction of a fluorine in the A-ring strongly contributed to a better inhibition of our target enzyme. Hydroxyl groups were introduced, too, in order to mimic the oxygen functionality of the steroidal substrates. In the following, the synthesis, biological activities and molecular modelling studies are presented. Beside the CYP17 activity, inhibition of other CYP enzymes was examined, that is, selectivity towards hepatic CYP enzymes and CYP11B1 was determined, since the latter is the key enzyme in glucocorticoid biosynthesis. The most promising structure was docked into our protein model, and the key interactions with the enzyme were elucidated.

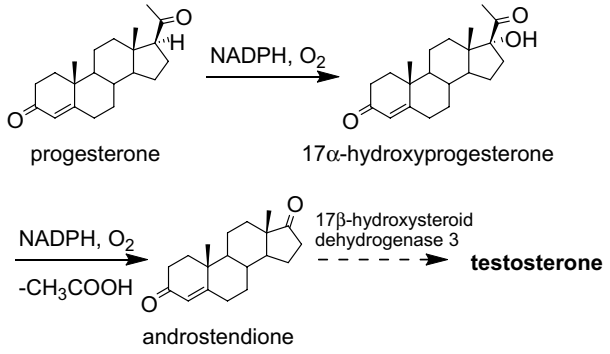
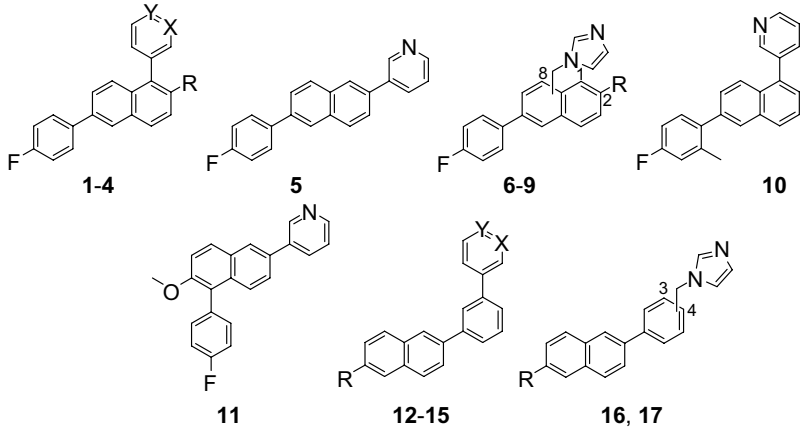


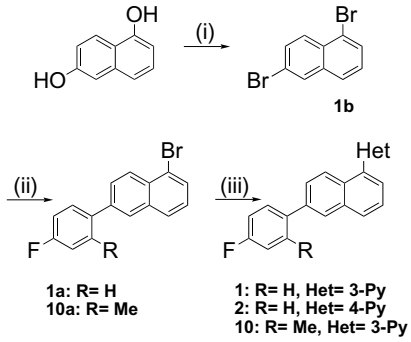
Figure 2. The role of CYP17 in androgen biosynthesis.

The syntheses of compounds **1**–**17** are shown in Schemes 1–9. In our aim to find the correct pattern for

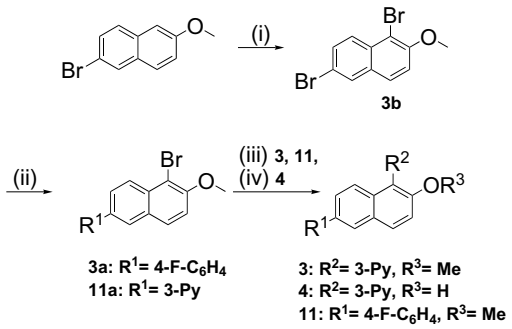


Compound	R	X	Y	subst. pos.
1	H	N	CH	
2	H	CH	N	
3	OMe	N	CH	
4	OH	N	CH	
6	H			1
7	OMe			1
8				2
9	H			8
12	OMe	N	CH	
13	OMe	CH	N	
14	OH	N	CH	
15	OH	CH	N	
16	OMe			3
17	OH			4

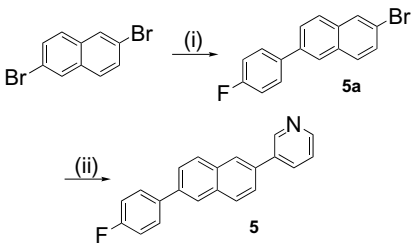
Figure 3. List of synthesised compounds **1**–**17**.



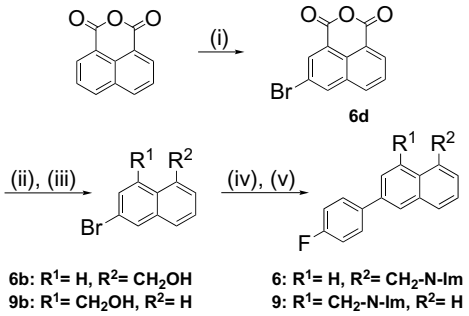
Scheme 1. Reagents and conditions: (i) Br_2, PPh_3, acetonitrile, μw, 240 °C, 10 min; (ii) Method A: **1a**: 4-fluorophenylboronic acid (**10a**: 2-methyl^{-4}-fluorophenylboronic acid), Na_2CO_3, $Pd(PPh_3)_4$, toluene, 110 °C, 16 h; (iii) Method A: 1,10: 3-pyridylboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, toluene, 110 °C, 16 h, for 2: 4-pyridylboronic acid, $NaHCO_3$, $Pd(PPh_3)_4$, DMF, H_2O, μw, 150 °C, 15 min.



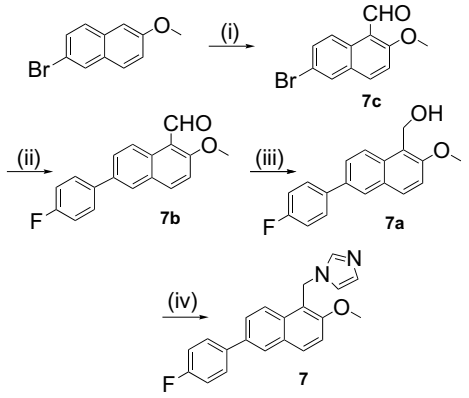
Scheme 2. Reagents and conditions: (i) NBS, THF, 75 °C, 2 h; (ii) Method A: 3a: 4-fluorophenylboronic acid (**11a**: 3-pyridylboronic-acid), Na_2CO_3, $Pd(PPh_3)_4$, toluene, 110 °C, 16 h; (iii) Method A: **3**: 3-pyridylboronicacid (11: 4-fluorophenylboronic acid), Na_2CO_3, $Pd(PPh_3)_4$, toluene, 110 °C, 16 h; (iv) Method B: BBr_3, DCM, −78–0 °C, 16 h.



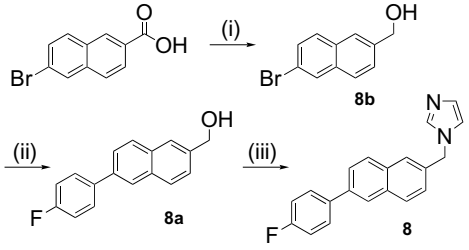
Scheme 3. Reagents and conditions: (i) Method A: 4-fluorophenylboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, toluene, 110 °C, 16 h; (ii) Method A: 3-pyridylboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, toluene, 110 °C, 16 h.



Scheme 4. Reagents and conditions: (i) Br_2, Ag_2SO_4, H_2SO_4, 65 °C, 6 h; (ii) NaOH, HgO, acetic acid, H_2O, 100 °C, 4d; (iii) Method C: $LiAlH_4$, THF, 75 °C, 2 h; (iv) Method A: 4-fluorophenylboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, toluene, 110 °C, 16 h; (v) Method D: CDI, imidazole, NMP, 170 °C, 3 h.

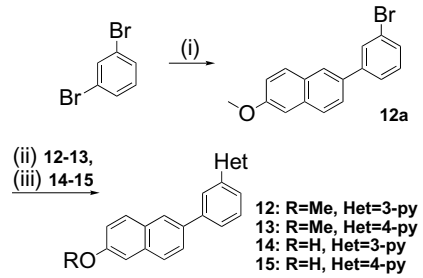


Scheme 5. Reagents and conditions: (i) $TiCl_4$, 1,1-dichloromethylmethylether, DCM, 40 °C, 2 h; (ii) Method A: 4-fluorophenylboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, toluene, 110 °C, 16 h; (iii) Method E: $NaBH_4$, MeOH, THF, rt, 1 h; (iv) Method D: CDI, acetonitrile, 85 °C, 2d.

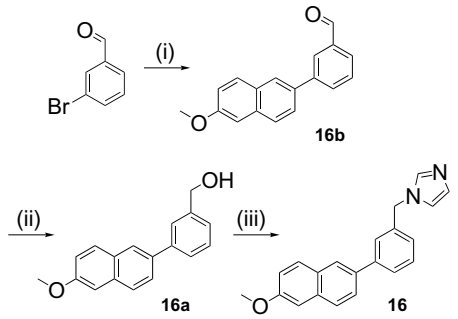


Scheme 6. Reagents and conditions: (i) Method C: $LiAlH_4$, Et_2O, 35 °C, 4 h; (ii) Method A: 4-fluorophenylboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, toluene, 110 °C, 16 h; (iii) Method D: CDI, imidazole, NMP, 180 °C, 16 h.

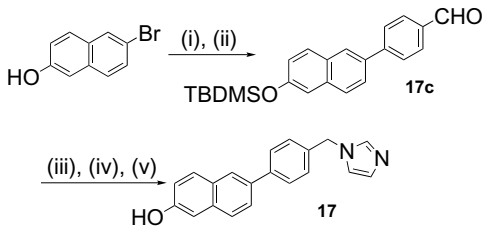
progesterone mimetics, different core structure alignments were synthetised, hence the diverse synthetical pathways. The substances can be divided in pyridyl (Schemes 1, 2, 3, 7) and imidazolyl (Schemes 4, 5, 6, 8, 9) compounds. The introduction of the pyridine moiety was achieved by means of Suzuki coupling[18] (Method A), as well as the coupling of the naphthalenes and the phenyl rings. When the necessary bromides for the couplings were not commercially available, they were prepared either by bromination using NBS (Scheme 2) or with triphenylphosphine dibromide (Scheme 1). The imidazoles were introduced by performing a S_Nt reaction with 1,1-carbonyl diimidazole (CDI) and the corresponding alcohol[19] (Method D) in the last step. The alcohols were obtained from either the carboxylic acids (Method C) or from the aldehydes (Method E). In some cases the carbonyl group had first to be introduced (Scheme 5) or modified (Scheme 4) before reducing it



Scheme 7. Reagents and conditions: (i) Method A: 6-methoxynaphthalen^{-2}-ylboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, toluene, 110 °C, 16 h; (ii) Method A: **12**: 3-pyridylboronic acid (**13**: 4-pyridylboronic acid), Na_2CO_3, $Pd(PPh_3)_4$, toluene, 110 °C, 16 h; (iii) Method B: BBr_3, DCM, −78–0 °C, 16 h.



Scheme 8. Reagents and conditions: (i) Method A: 6-methoxynaphthalen^{-2}-ylboronic acid, Na_2CO_3, $Pd(PPh_3)_4$, toluene, 110 °C, 16 h; (ii) Method E: $NaBH_4$, MeOH, THF, rt, 1 h; (iii) Method D: CDI, acetonitrile, 85 °C, 16 h.



Scheme 9. Reagents and conditions: (i) TBDMSCl, imidazole, DCM, rt, 16 h; (ii) Method A: Na_2CO_3, $Pd(OAc)_2$, TBAB, toluene, EtOH, H_2O, 110 °C, 16 h; (iii) Method E: $NaBH_4$, MeOH, rt, 16 h; (iv) Method D: CDI, NMP, 170 °C, 6 h; (v) TBAF, THF, rt, 16 h.

to the corresponding alcohol. In some cases the methoxy-substituted compounds were submitted to an ether cleavage (Method B). For the preparation of compound **17**, the hydroxyl group on the naphthalene had to be protected before the Suzuki coupling due to otherwise very low yields.[18]

Inhibition of human CYP17 was determined by performing our previously described assay[20] at inhibitor concentrations of 0.2 and 2 μM. As source of human CYP17, our *Escherichia coli* system[21] (coexpressing human CYP17 and NADPH-P450 reductase) stably expressing human CYP17 was used. After homogenisation the 50,000*g* sediment was incubated with progesterone and NADPH as previously described.[22] Separation of the product was performed by HPLC using UV-detection.

The 3- and the 4-pyridyl-substituted ACD mimetics (**1**–**5**) showed no or little inhibition, respectively, in contrast to the reference compounds ketoconazole and abiraterone (Table 1). Amongst our methylene-imidazolyl-substituted compounds (**6**–**9**), the 8-substituted one showed the best result (**9**). It can also be observed that the introduction of a 2-methoxy group in compound **6** leads to a higher activity (**7**). Neither the introduction of a methyl substituent in the A-ring of **1** (**10**), nor the switch of the position of the A-ring from 6- to 5- (**11**) resulted in an active compound.

Regarding the ABD mimetics (Table 2) it is striking that the class of the 4-pyridyl compounds (**13**, **15**) showed again activity while the 3-pyridyl compounds (**12**, **14**) were inactive. The cleavage of their methyl ether led to an enhancement of inhibition (**14**, **15**). An increase in activity for compound **13** with 23–66% inhibition at 2 μM (**15**) could be observed, leading to the most active compound of this study. Analogous to the work done in the ACD class, the heterocycle was replaced by a methylene-imidazole moiety, and moderate activities for compounds **16** and **17** were observed.

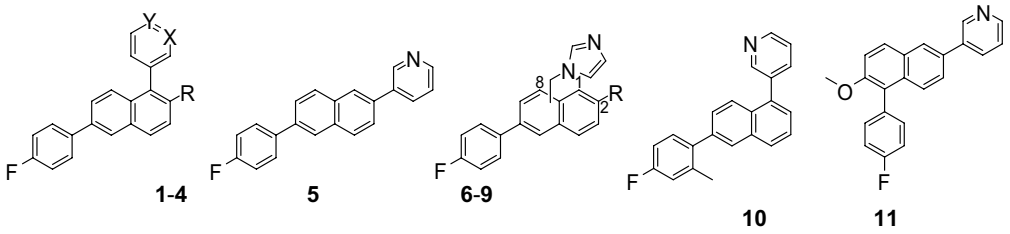
Since CYP3A4 is the hepatic enzyme responsible for the metabolism of lipophilic substances and therefore of about 50% of current prescription drugs,[23] a broader spectrum of our compounds were studied for their effects on this enzyme. Critical is the fact that this enzyme together with CYP2D6 and CYP1A2 shows pronounced genetic polymorphism.[24] Selected compounds (**3**, **7**, **15**, **16**) showed moderate to high activity towards the mentioned hepatic CYP enzymes (Table 3). Since compound **15** exhibited a very low activity of 19% inhibition at 10 μM towards CYP3A4[25] it was further tested on 2D6. At this enzyme, it showed inhibitions of 80% at 1 μM and 85% at 10 μM.

In further terms of selectivity, compound **15** was additionally tested on the steroidogenic CYP enzyme CYP11B1 which is involved in the glucocorticoid biosynthesis. For the assay,[26] V79MZh11B1 cells expressing human CYP11B1 were used. Compound **15** showed very low activities of 3% at 0.2 μM and 23% at 2 μM.

Since there is no crystal structure of CYP17 available we built a homology model of CYP17 using the X-ray structure of human CYP2C9 (PDB code 1r9o) as template, as described before by us.[28] Docking simulations were carried out by means of the GOLD v3.0.1 software. For the docking studies, our homology model was used running Linux Suse 10.1 on Intel(R) P4 CPU 3.00 GHz, and the energy minimisation was also performed as previously described.[28] Compound **15** and the steroidal inhibitor abiraterone were docked as shown in Figure 4.

Beside the already described interaction between the sp^2 hybridised nitrogen and the heme iron, the key

Table 1. Inhibition of CYP17 by steroidal ACD-ring mimetics (compounds **1**–**11**)



Compound	R	X	Y	subst. pos.	CYP17% Inhibition[a,b]	
					0.2 μM	2 μM
1	H	N	CH		2	3
2	H	CH	N		1	28
3	OMe	N	CH		6	7
4	OH	N	CH		0	0
5					0	22
6	H			1	2	19
7	OMe			1	13	45
8				2	3	14
9	H			8	9	50
10					3	3
11					0	1

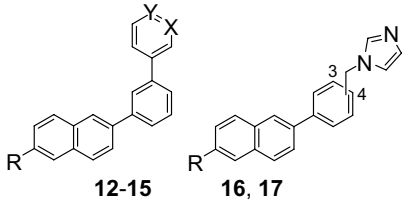
[a] Ketoconazole (IC_{50} = 2780 nM); abiraterone (IC_{50} = 72 nM).
[b] Data shown are means of at least one independent test in duplicate. Concentration of progesterone (substrate): 25 μM. The deviations were within < ± 5%.

interaction for compound **15** seems to be the H-bond net of the hydroxyl group on the naphthalene with the amino acids Arg109, His235, Lys231 and Asn202 in the active site of our target enzyme. Further, hydrophobic interactions between the aromatic core and Ile206, Gly301, Ala302, Glu305, Val366 and Val482 were observed.

For abiraterone, the same binding mode as described for the substrates was found.[29,30] The lone pair of the sp^2 hybridised nitrogen pointed perpendicular towards the heme iron. The steroidal scaffold was oriented almost parallel to the heme plane in the direction of the BC-loop. This pose was stabilized by hydrophobic interactions with Ile371, Ile112, Ala113 and Phe114.[29,30] Additionally, the highly conserved Arg96 which is important for substrate binding and recognition, as shown by site-directed mutagenesis,[31] presented interactions of the same kind with the steroidal A-ring. Another important interaction was the H-bond between the hydroxyl group in C3 position and the backbone carbonyl group of Gln98.

Relying on our molecular modelling studies, the inhibitory activity of compound **15** is supposed to be increased by introducing other polar groups like an H-bond donor

Table 2. Inhibition of CYP17 by steroidal ABD-ring mimetics (compounds **12**–**17**)



Compound	R	X	Y	subst. pos.	CYP17% Inhibition[a,b]	
					0.2 μM	2.0 μM
12	OMe	N	CH		3	4
13	OMe	CH	N		4	23
14	OH	N	CH		0	8
15	OH	CH	N		31	66
16	OMe			3	0	28
17	OH			4	24	61

[a] Ketoconazole (IC_{50} = 2780 nM); abiraterone (IC_{50} = 72 nM).
[b] Data shown are means of at least one independent test in duplicate. Concentration of progesterone (substrate): 25 μM. The deviations were within < ± 5%.

Table 3. Inhibition of hepatic CYP enzymes (1A2, 2C9, 2C19, 3A4) by compounds **3**, **7**, **15**, **16**

Compound	CYP1A2% Inhibition[a]		CYP2C9% Inhibition[a]		CYP2C19% Inhibition[a]		CYP3A4% Inhibition[a]	
	1.0 μM	10.0 μM	1.0 μM	10.0 μM	1.0 μM	10.0 μM	1.0 μM	10.0 μM
3	40	87	66	54	68	28	64	86
7	80	85	100	100	93	96	93	95
15	98	98	94	97	86	89	6	19
16	96	97	97	100	95	97	91	96
KTZ[b]	8	38	21	75	24	1	1	4
ABT[b]	36	53	17	51	3	7	7	7

[a] Data shown are means of three independent tests.
[b] KTZ, ketoconazole; ABT, abiraterone. The deviations were within < ± 5%.

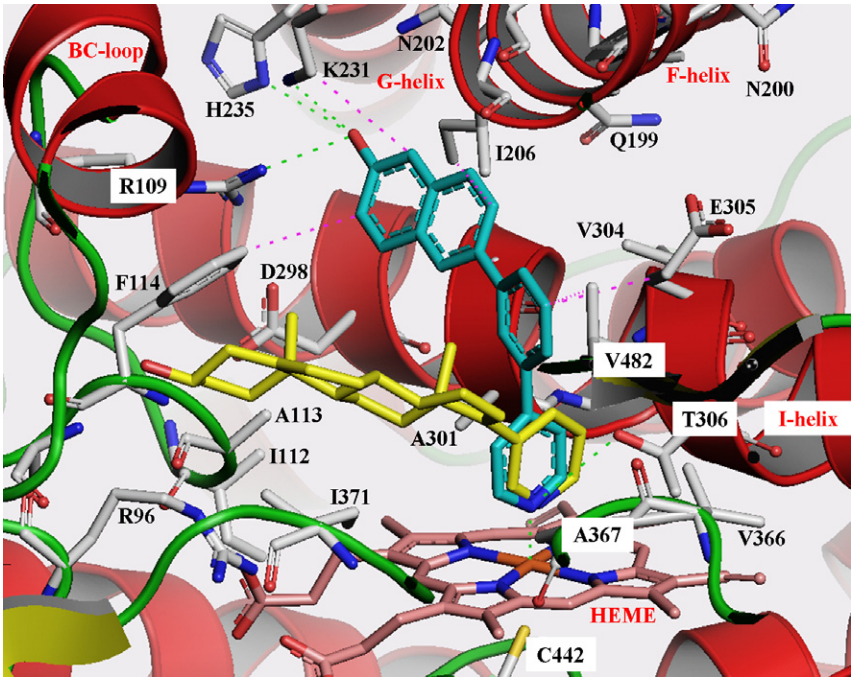


Figure 4. Docking complex between CYP17 and compounds **15** (cyan) and abiraterone (yellow). Heme, interacting residues and ribbon rendered tertiary structure of the active site are shown. Figure was generated with Pymol (http://www.pymol.org).

in 7 position of the naphthalene, which could interact with Asp298. Other possible substitutions to increase activity and perhaps also selectivity towards hepatic CYP enzymes are chlorine or fluorine at the 1 position of the naphthalene, which should improve the hydrophobic interactions with the I-helix. Also a substituent at 3 position capable of undergoing H-bonds could interact with Glu204.

The selectivity against CYP1A2 and CYP2D6 could also be enhanced through different medicinal chemistry strategies. It is described that planar, lipophilic structures[32] like compound **15** have a great affinity towards CYP1A2. This planarity could be abolished by either dearomatising one of the benzene rings, or by specific ortho-substitutions. Regarding CYP2D6, its high inhibition is not surprising, since compound **15** bears three important pharmacophores responsible for its inhibition: the aromatic nitrogen, the H-bond donor and the lipophilic core structure.[33] Since CYP2D6 has a relatively small binding site, most of the changes or substitutions made on compound **15** might increase its selectivity against this enzyme.

Summarising, we have discovered compound **15** which shows good inhibition values, a good selectivity towards the steroidogenic CYP11B1 and a modest selectivity against CYP3A4, the most relevant hepatic enzyme in xenobiotic metabolism. Compound **15** might therefore be a good candidate for further structure optimisation. Using our protein model, we made some suggestions of how to further increase activity and selectivity towards hepatic CYP enzymes.

Acknowledgments

We thank the Fonds der Chemischen Industrie for financial support. U. E. H. is grateful to the European Postgraduate School 532 (DFG) for a scholarship. We also thank Dr. J. J. R. Hermans, Cardiovascular Research Institute, University of Maastricht (the Nether-

lands), for providing us with V79 cells expressing human CYP11B1.

Supplementary data

Supporting information regarding the molecular modelling studies and the detailed synthetical procedures and characterisation of the compounds is available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.10.079.

References and notes

1. Jemal, A.; Siegel, R.; Ward, E.; Murray, T.; Xu, J.; Thun, M. J. *CA Cancer J. Clin.* **2007**, *57*, 43.
2. Huhtaniemi, I.; Nikula, H.; Parvinen, M.; Rannikko, S. *Am. J. Clin. Oncol.* **1988**, *11*, 11.
3. Labrie, F.; Dupont, A.; Belanger, A.; Lefebvre, F. A.; Cusan, L.; Monfette, G.; Laberge, J. G.; Emond, J. P.; Raynaud, J. P.; Husson, J. M.; Fazekas, A. T. *J. Steroid Biochem.* **1983**, *19*, 999.
4. Nnane, I. P.; Long, B. J.; Ling, Y.-Z.; Grigoryev, D. N.; Brodie, A. M. *Br. J. Cancer* **2000**, *83*, 74.
5. Schuurmans, A. L. G.; Bolt, J.; Veldscholte, J.; Mulder, E. *J. Steroid Biochem. Mol. Biol.* **1990**, *37*, 849.
6. Harris, K. A.; Weinberg, V.; Bok, R. A.; Kakefuda, M.; Small, E. J. *J. Urol.* **2002**, *168*, 542.
7. Eklund, J.; Kozloff, M.; Vlamakis, J.; Starr, A.; Mariott, M.; Gallot, L.; Jovanovic, B.; Schilder, L.; Robin, E.; Pins, M.; Bergan, R. C. *Cancer* **2006**, *106*, 2459.
8. Small, E. J.; Halabi, S.; Dawson, N. A.; Stadler, W. M.; Rini, B. I.; Picus, J.; Gable, P.; Torti, F. M.; Kaplan, E.; Vogelzang, N. J. *J. Clin. Oncol.* **2004**, *22*, 1025.
9. Cougar Biotechnology, Inc. http://www.cougarbiotechnology.com/docs/052107CougarPhaseIIAUAAnnualMeeting2007.pdf accessed: 05/31/2007.
10. Kolar, N. W.; Swart, A. C.; Mason, J. I.; Swart, P. J. *J. Biotechnol.* **2007**, *129*, 635.
11. Samer, H.; Ehmer, P. B.; Barassin, S.; Batzl-Hartmann, C.; Hartmann, R. W. *J. Steroid Biochem. Mol. Biol.* **2003**, *84*, 555.
12. Haidar, S.; Hartmann, R. W. In *Enzymes and their Inhibition, Drug Development*; Smith, H. J., Simons, C., Eds.; CRC Press: Boca Raton, 2005; p 241.
13. Matsunaga, N.; Kaku, T.; Itoh, F.; Tanaka, T.; Hara, T.; Miki, H.; Iwasaki, M.; Aono, T.; Yamaoka, M.; Kusaka, M. *Bioorg. Med. Chem.* **2004**, *12*, 2251.
14. Hartmann, R. W.; Wachall, B.; Yoshihama, M.; Nakakoshi, M.; Nomoto, S.; Ikeda, Y. WO018075, 1999.
15. (a) Wachall, B. G.; Hector, M.; Zhuang, Y.; Hartmann, R. W. *Bioorg. Med. Chem.* **1999**, *7*, 1913; (b) Zhuang, Y.; Wachall, B. G.; Hartmann, R. W. *Bioorg. Med. Chem.* **2000**, *8*, 1245.
16. Leroux, F.; Hutschenreuter, T. U.; Charrière, C.; Scopelliti, R.; Hartmann, R. W. *Helv. Chim. Acta* **2003**, *86*, 2671.
17. Schenkman, J. B.; Sligar, S. G.; Cinti, D. L. *Pharmacol. Ther.* **1981**, *12*, 43.
18. Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
19. Tang, Y.; Dong, Y.; Vennerstrom, J. L. *Synthesis* **2004**, *15*, 2540.
20. Hutschenreuter, T. U.; Ehmer, P. B.; Hartmann, R. W. J. *Enzyme Inhib. Med. Chem.* **2004**, *18*, 17.
21. Ehmer, P. B.; Jose, J.; Hartmann, R. W. *J. Steroid Biochem. Mol. Biol.* **2000**, *75*, 57.
22. Sergejew, T. F.; Hartmann, R. W. *J. Enzyme Inhib.* **1994**, *8*, 113.
23. Manga, N.; Duhhy, J. C.; Rowe, P. H.; Cronin, M. T. D. *SAR QSAR Environ. Res.* **2005**, *16*, 43.
24. Hodgson, J. *Nat. Biotech.* **2001**, *19*, 722.
25. For hepatic CYP inhibition data the recombinantly expressed enzymes from baculovirus-infected insect microsomes (Supersomes) were used and the manufacturer's instructions (www.gentest.com) were followed.
26. V79MZh11B1 CYP11B1 cells expressing the human enzyme were used and our assay procedure using [4-^{14}C]-11-deoxycorticosterone was applied (Ref.27).
27. Ulmschneider, S.; Muller-Vieira, U.; Mitrenga, M.; Hartmann, R. W.; Oberwinkler-Marchais, S.; Klein, C. D.; Bureik, M.; Bernhardt, R.; Antes, I.; Lengauer, T. *J. Med. Chem.* **2005**, *48*, 1796.
28. Jagusch, C.; Negri, M.; Hille, U. E.; Hu, Q.; Bartels, M.; Jahn-Hoffmann, K.; Pinto-Bazurco Mendieta, M. A. E.; Biemel, K.; Rodenwaldt, B.; Müller-Vieira, U.; Schmidt, D.; Lauterbach, T.; Recanatini, M.; Cavalli, A.; Hartmann, R. W. *Bioorg. Med. Chem.* **2008**, *18*, 228.
29. (a) Lin, D.; Zhang, L.; Chiao, E.; Miller, L. W. *Mol. Endocrinol.*. **1994**, *8*, 391; (b) Auchus, R. J.; Miller, W. L. *Mol. Endocrinol.* **1999**, *13*, 1169.
30. Mathieu, A. P.; LeHoux, J.-G.; Auchus, R. J. *Biochim. Biophys. Acta* **2003**, *1619*, 291.
31. Brooke, A. M.; Taylor, N. F.; Shepherd, J. H.; Gore, M. E.; Ahmad, T.; Lin, L.; Rumsby, G.; Papari-Zareei, M.; Auchus, R. J.; Achermann, J. C.; Monson, J. P. *J. Clin. Endocrinol. Metab.* **2006**, *91*, 2428.
32. Lewis, D. F.; Lake, B. G.; Dickins, M. *J. Enzyme Inhib. Med. Chem.* **2007**, *22*, 1.
33. Vaz, R. J.; Nayeem, A.; Santone, K.; Chandrasena, G.; Gavai, A. V. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3816.