Bioorganic & Medicinal Chemistry Letters 20 (2010) 5345–5348

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biochemical evaluation of a range of (4-substituted phenyl)sulfonate derivatives of 4-hydroxybenzyl imidazole-based compounds as potent inhibitors of 17α-hydroxylase/17,20-lyase ($P450_{17\alpha}$) derived from rat testicular microsomes

Caroline P. Owen [a], Imran Shahid [b], Wai-Yee Lee [b], Sabbir Ahmed [a,*]

[a] School of Science, Faculty of Science and Technology, University of the West of Scotland, High Street, Paisley, PA1 2BE, Scotland, UK
[b] Kingston University, Penrhyn Road, Kingston, UK

ARTICLE INFO

Article history:
Received 2 December 2009
Revised 24 February 2010
Accepted 26 February 2010
Available online 3 March 2010

Keywords:
Synthesis
Inhibition
Enzyme
17α-Hydroxylase
17,20-Lyase

ABSTRACT

We report the synthesis, biochemical evaluation and rationalisation of the inhibitory activity of a range of (4-substituted phenyl)sulfonate derivatives of 4-hydroxybenzyl imidazole against the two components of 17α-hydroxylase/17,20-lyase ($P450_{17\alpha}$), namely, 17α-hydroxylase (17α-OHase) and 17,20-lyase (lyase). The results show the compounds to be highly potent inhibitors with limited selectivity towards the lyase component [e.g., toluene-4-sulfonic acid 4-imidazol-1-ylmethyl-phenyl ester (**4**) possessed an IC_{50} value of 40 nM against 17α-OHase and 30 nM against lyase].

The cytochrome P450 enzyme 17α-hydroxylase/17,20-lyase ($P450_{17\alpha}$) is involved in the production of the C_{19} containing androgen precursors from the C_{21} containing steroids such as the pregnanes and progestins (Fig. 1). Steroids such as androstenedione (AD) and dehydroepiandrosterone (DHEA) are therefore synthesised as a result of the action of $P450_{17\alpha}$ on progesterone and pregnenolone, respectively,[1] involving an initial 17α-hydroxylation [via 17α-hydroxylase (17α-OHase)], followed by the cleavage of the C(17)–C(20) bond [via 17,20-lyase (lyase)]—both hydroxylation and C–C cleavage steps require NADPH and oxygen.[2,3]

$P450_{17\alpha}$ is a potential biological target in the treatment of hormone-dependent diseases such as prostate cancer which has been shown to be androgen-dependent in its early stages. A number of compounds has therefore been investigated, including the antimycotic ketoconazole (KTZ) and abiraterone acetate (Fig. 2). The latter compound is currently in clinical trials, however, KTZ, a known azole-based $P450_{17\alpha}$ inhibitor (albeit an unselective inhibitor of steroid biosynthesis and which has been shown to inhibit other steroidogenic enzymes whilst possessing weak activity towards $P450_{17\alpha}$), has been used previously in the treatment of prostate cancer, however, was withdrawn for this use due to serious adverse effects. In more recent studies, however, KTZ has been evaluated against hormone-refractory prostate cancer in lower doses.[4]

Previously, we have outlined the synthesis and biochemical evaluation of halogenated derivatives of benzyl azole-based compounds which have been shown to be good inhibitors of $P450_{17\alpha}$ in comparison to the standard KTZ;[5–9] recently we reported a range of (4-substituted phenyl)sulfonate derivatives of 4-hydroxybenzyl imidazole (**1**) which were found to possess good inhibitory activity against $P450_{17\alpha}$.[10] Indeed, compounds based on the biphenyl backbone have also been developed and have also shown good level of potentcy.[11–13] Here, we report: the synthesis of a range of (4-alkyl-substituted phenyl)sulfonate derivatives of **1** and their biochemical evaluation (in comparison to KTZ) against both components of $P450_{17\alpha}$ in an attempt to discover potent and highly specific inhibitors of $P450_{17\alpha}$. In an effort to compare the results of our previous studies, we also undertook the evaluation of the compounds previously reported by us as inhibitors of $P450_{17\alpha}$, in particular, benzyl imidazole (**2**) and 4-iodobenzyl imidazole (**3**) (the latter compound was shown to possess the highest inhibitory activity within the halogen derivatives of benzyl imidazole[8]) as well as three previously reported sulfonate derivatives,[10] namely, compounds **4**, **9** and **10** (Table 1).

In the synthesis of the (4-alkylsubstituted phenyl)sulfonate derivatives of **1** (compounds **4**–**11**), the reactions outlined in Scheme 1 were undertaken—we have reported previously the

* Corresponding author. Tel.: +44 141 848 3000.
E-mail address: sabbir.ahmed@uws.ac.uk (S. Ahmed).

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doi:10.1016/j.bmcl.2010.02.100

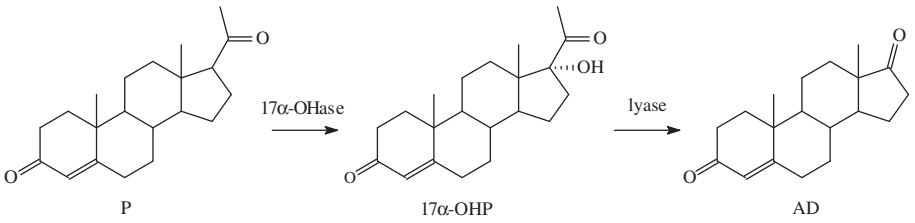


Figure 1. Reaction catalysed by $P450_{17\alpha}$ in the conversion of progesterone (P) to AD via 17α-hydroxyprogesterone (17α-OHP).

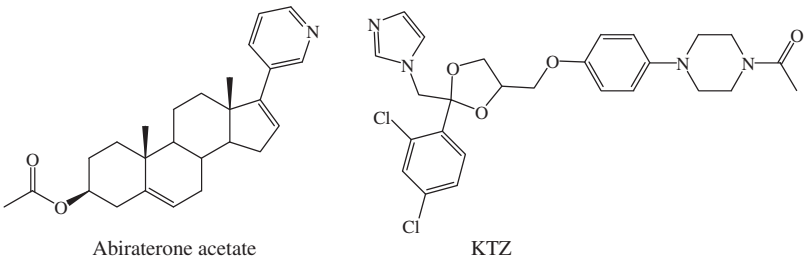
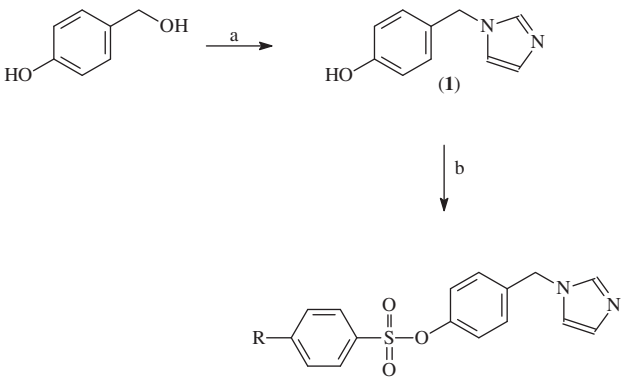


Figure 2. Structures of two KTZ and abiraterone acetate.

synthesis of other derivatives of compound **1** where we have successfully utilised the reactions outlined in Scheme 1,[10] as such, no specific methodology has been discussed within the current report.

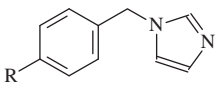
The biochemical evaluation of the synthesised compounds against both 17α-OHase and lyase components was undertaken using the method of Owen et al.[14] and involved the use of thin layer chromatography (TLC) in the separation and identification of the radiolabelled substrate from the incubation mixture. Table 1 shows the IC_{50} values obtained for the compounds considered within the current study against both 17α-OHase and lyase. $P450_{17\alpha}$ is also indirectly involved in the biosynthesis of the glucocorticoids and mineralocorticoids, in particular, the latter is produced directly from the 17α-hydroxylated derivatives of the progestins, as such, inhibitors of $P450_{17\alpha}$ should preferentially inhibit the C–C bond cleavage reaction in comparison to the hydroxylation step. This is therefore expected to have minimal effect on the hydroxylase reaction and therefore glucocorticoid biosynthesis.[15]

Initial consideration of the IC_{50} values show that compounds **4** to **11** are more potent than KTZ (which was found to possess an IC_{50} value of 2660 nM and 206 nM against the 17α-OHase and lyase components, respectively). Furthermore, with the exception of compound **8**, all the compounds appear to possess greater potency against the lyase component in comparison to the 17α-OHase component; this was also observed with our own standard (compound **3**) which was found to possess an IC_{50} value of 730 nM and 164 nM against the 17α-OHase and lyase components, respec-



Scheme 1. Synthesis of a number of sulfonate derivatives of **1** and its derivatives (a = imidazole/Δ; b = 4-substituted phenyl sulfonyl chloride/DCM/Δ; R = various substituents, e.g., CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, CF_3, F and Ph).

Table 1
IC_{50} and log *P* values for compounds under consideration within the current study (log *P* was calculated using Project Leader from Fujitsu)



Compound number	R	IC_{50} (nM) against lyase	IC_{50} (nM) against 17α-OHase	Calculated log *P*
1	OH	1470 ± 1	2496 ± 300	1.03
2	H	>3000	>3000	—
3	I	164 ± 50	730 ± 80	—
4	CH_3–$C_6H_4SO_2O$–	30 ± 7	40 ± 8	2.50
5	C_2H_5–$C_6H_4SO_2O$–	20 ± 4	110 ± 1	2.90
6	C_3H_7–$C_6H_4SO_2O$–	30 ± 1	1210 ± 20	3.30
7	C_4H_9–$C_6H_4SO_2O$–	40 ± 0.1	70 ± 9	3.69
8	C_5H_{11}–$C_6H_4SO_2O$–	160 ± 14	130 ± 30	4.09
9	CF_3–$C_6H_4SO_2O$–	34 ± 5	1040 ± 61	2.92
10	F–$C_6H_4SO_2O$–	14 ± 0.9	200 ± 10	2.18
11	Ph–$C_6H_4SO_2O$–	10 ± 0.04	100 ± 1	3.72
KTZ	—	206 ± 33	2660 ± 10	3.18

tively. It should be noted that the previously reported values for these compounds appear to be inconsistent with the current study, however, this would be expected as different rat testicular tissues was utilised in the previous study when compared to the current study, as such, consistent data would not be expected.

The most potent compounds within the current series of compounds were therefore **4**, **5**, **6**, **9**, **10** and **11**, with compound **6** possessing the best selectivity towards the lyase reaction in comparison to the hydroxylase reaction, indeed, this compound was found to possess an IC_{50} value of 1210 nM against the 17α-OHase component and 30 nM against the lyase component. Compound **11** showed the most potent inhibitory activity with an IC_{50} value of 100 nM against the 17α-OHase component and 10nM against the lyase component. In comparison, KTZ was found to possess good selectivity but weaker inhibitory activity in comparison to compounds **3** to **11**.

A detailed consideration of the IC_{50} values shows that compounds **4** to **8** possess potent inhibitory activity against the lyase component with an optimum activity being possessed by the ethyl (compound **5**) and phenyl (compound **11**) derivatives, whereas the butyl and the pentyl derivatives appear to possess weaker inhibitory activity.

Since no crystal structure exists for $P450_{17\alpha}$, we developed a novel technique to model this enzyme using the substrate–haem complex approach. The methodology for the derivation of the substrate–haem complex has been previously described,[16–18] as such, no detailed discussion is given here. In general, however, the determination of the overall representation of the $P450_{17\alpha}$ active site initially involved the derivation of the binding site corresponding to the 17α-OHase component using pregnenolone bound to the haem moiety—the substrate was allowed to minimise to give the approximate position of the hydrogen bonding group which would be expected to bind to the C(3) moiety within the substrate. The structure was 'locked' after the removal of the pregnenolone and the location of the second binding site (corresponding to the lyase component) was then determined using 17α-hydroxypregnenolone as the substrate and the structure again minimised. The haem and the two hydrogen bonding groups at the active site were then locked and 17α-hydroxypregnenolone removed to give the overall substrate–haem complex for $P450_{17\alpha}$. That the active site consists of two lobes where the two substrates for this enzyme bind to (at different times) was previously proposed by Laughton et al.[19] who found that the two 'lobes' were orientated in such a manner so as to approximate to the L-shape with the lyase binding site being larger than the 17α-OHase binding site. The substrate–haem complex approach also shows the lyase binding site to be the larger of the two and that the overall orientation of the two hydrogen bonding groups appears to be of a similar orientation as reported by Laughton et al. (with respect to the haem).

Inhibitors of $P450_{17\alpha}$ can also utilise these two hydrogen bonding groups and we have previously reported the use of both groups by inhibitors (such as 3,5-dichlorobenzyl imidazole[6]) which resulted in the di-substituted compounds possessing potent inhibitory activity in comparison to mono-halogenated compounds. Modelling of compound **8** using the substrate–haem complex approach shows that this compound is able to utilise either of the two potential hydrogen bonding groups, albeit one at a time (Fig. 3). However, from the consideration of the conformational analysis of the alkyl moiety within **8** shows that conformers exist which allow the alkyl chain to undergo steric interaction with the hydrogen bonding group at the active site. We therefore propose that the increase in the steric interactions results in an overall reduction in inhibitory activity when compared to compounds containing smaller alkyl chains, for example, compound **7**.

Our hypothesis is further supported by the inhibitory activity observed within compounds **9** and **10**, which contain CF_3 and F as the substituents, respectively, rather than the alkyl chain. These two compounds are hypothesised to occupy reduced conformational space, as such, they do not undergo the level steric interactions observed within compounds **5** to **8**. Modelling of compound **9**

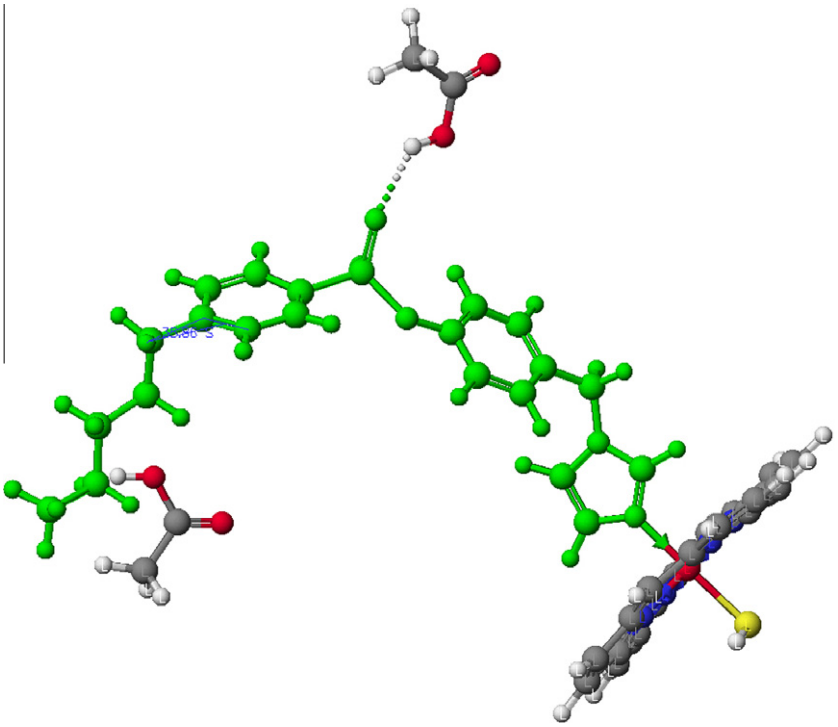


Figure 3. Conformer of compound **8** bound to the haem moiety within the overall substrate–haem complex representation of $P450_{17\alpha}$ showing the potential steric interaction between the pentyl moiety and the hydrogen bonding group at the active site.

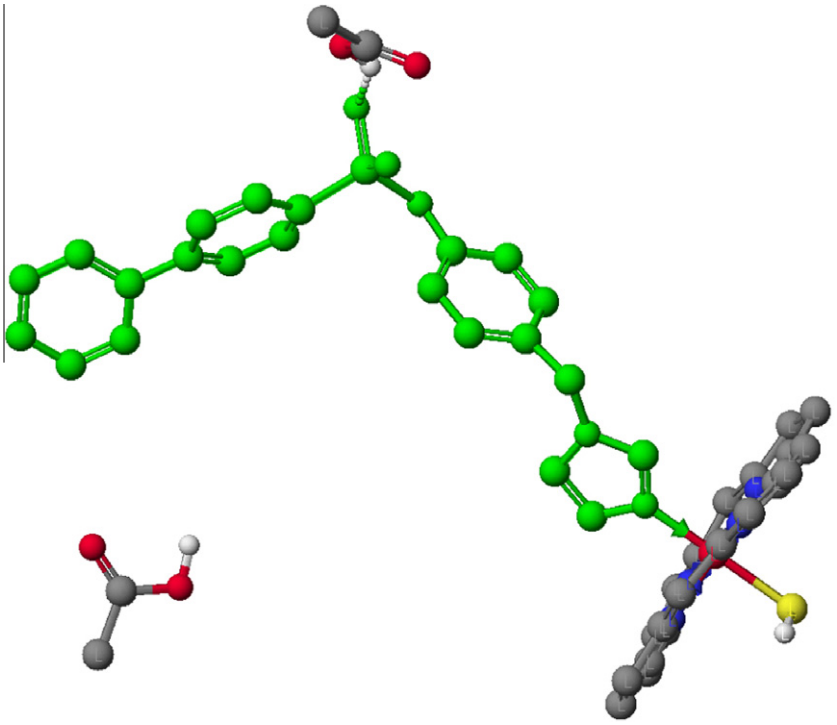


Figure 4. Compound **11** bound to the haem moiety within the overall substrate–haem complex representation of $P450_{17\alpha}$.

within the overall enzyme complex shows that the CF_3 moiety is far removed from the second hydrogen bonding group at the active site to undergo any steric interaction, indeed, the nearest atom is 5.7 Å away from the hydrogen bonding group. Modelling the more bulky derivative of **9** (more specifically compound **11**, Fig. 4) within the substrate–haem complex shows that as a result of the greatly reduced conformational flexibility of this inhibitor, the additional phenyl moiety is unable to undergo any steric interaction with the active site and therefore possesses greater inhibitory activity in comparison to **9**.

In conclusion, we have produced some highly potent inhibitors of $P450_{17\alpha}$ in comparison to the standard compound KTZ. Also, due to the limited specificity of these compounds against lyase in comparison to the 17α-OHase component, these compounds would be expected to have a major impact on corticosteroid biosynthesis. Furthermore, through the consideration of the modelling of these inhibitors, we have provided an insight into the conformational space available about the area of the active site. Although the compounds have shown high levels of potency, the biochemical evaluation of similar benzyl imidazole-based compounds suggests that the use of these compounds as potential drug substances is limited due to their ability to inhibit other cytochrome P450 enzymes such as cholesterol side chain cleavage[5] or indeed aromatase,[5,20] and which therefore suggests a lack of selectivity.

Acknowledgements

The authors thank the Mass Spectrometry service at the Kings College London (UK) and the elemental analysis service at the School of Pharmacy, University of London (UK) for the provision of high resolution and elemental analysis data, respectively.

References and notes

1. Ortiz de Montellano, P. R. In *Cytochrome P-450: Structure Mechanism and Biochemistry*; Ortiz de Montellano, P. R., Ed.; Plenum Press: New York, 1986; pp 217–272.
2. Ahmed, S.; Owen, C. P. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1023.
3. Robichaud, P.; Wright, J. N.; Akhtar, M. *J. Chem. Soc., Chem. Commun.* **1994**, *12*, 1501.
4. Nakabayashi, M.; Xie, W.; Regan, M. M.; Jackman, D. M.; Kantoff, P. W.; Oh, W. K. *Cancer* **2006**, *107*, 975.
5. Ahmed, S.; Smith, H. J.; Nicholls, P. J.; Whomsley, R.; Cariuk, P. *Drug Des. Discovery* **1995**, *13*, 27.
6. Owen, C. P.; Dhanani, S.; Patel, C. H.; Shahid, I.; Ahmed, S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4011.
7. Patel, C. H.; Dhanani, S.; Owen, C. P.; Ahmed, S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4752.
8. Shahid, I.; Patel, C. H.; Dhanani, S.; Owen, C. P.; Ahmed, S. *J. Steroid Biochem. Mol. Biol.* **2008**, *110*, 18.
9. Owen, C. P.; Shahid, I.; Olusanjo, M. S.; Patel, C. H.; Dhanani, S.; Ahmed, S. *J. Steroid Biochem. Mol. Biol.* **2008**, *111*, 117.
10. Ahmed, S.; Shahid, I.; Dhanani, S.; Owen, C. P. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4698.
11. Hu, Q.; Negri, M.; Jahn-Hoffmann, K.; Zhuang, Y.; Olgen, S.; Bartels, M.; Müller-Vieira, U.; Lauterbach, T.; Hartmann, R. W. *Bioorg. Med. Chem.* **2008**, *16*, 7715.
12. Zhuang, Y.; Wachall, B. G.; Hartmann, R. W. *Bioorg. Med. Chem.* **2000**, *8*, 1245.
13. Hille, U. E.; Hu, Q.; Vock, C.; Negri, M.; Bartels, M.; Muller-Vieira, U.; Lauterbach, T.; Hartmann, R. W. *Eur. J. Med. Chem.* **2009**, *44*, 2765.
14. Owen, C. P.; Nicholls, P. J.; Smith, H. J.; Whomsley, R. *J. Pharm. Pharmacol.* **1999**, *51*, 427.
15. Njar, V. C. O.; Brodie, A. M. H. *Curr. Pharm. Des.* **1999**, *5*, 163.
16. Ahmed, S. *Drug Des. Discovery* **1994**, *12*, 77.
17. Ahmed, S. *Biochem. Biophys. Res. Commun.* **2004**, *316*, 595.
18. Ahmed, S. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2795.
19. Laughton, C. A.; Neidle, S.; Zvelebil, M. J. J. M.; Sternberg, M. J. E. A. *Biochem. Biophys. Res. Commun.* **1990**, *171*, 1160.
20. Adat, S.; Owen, C. P.; Ahmed, S. *Lett. Drug Des. Discovery* **2007**, *4*, 545.