Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 4752–4756

Synthesis, biochemical evaluation and rationalisation of the inhibitory activity of a range of 4-substituted phenyl alkyl imidazole-based inhibitors of the enzyme complex 17α-hydroxylase/17,20-lyase ($P450_{17\alpha}$)

Chirag H. Patel,[a] Sachin Dhanani,[b] Caroline P. Owen[a] and Sabbir Ahmed[a,*]

[a]*Department of Pharmacy, School of Pharmacy and Chemistry, Kingston University, Penrhyn Road, Kingston upon Thames, Surrey, KT1 2EE, UK*
[b]*School of Life Sciences, Kingston University, Penrhyn Road, Kingston upon Thames, Surrey, KT1 2EE, UK*

Received 22 May 2006; revised 30 June 2006; accepted 30 June 2006
Available online 25 July 2006

Abstract—We report the preliminary results of the synthesis, biochemical evaluation and rationalisation of the inhibitory activity of a number of phenyl alkyl imidazole-based compounds as inhibitors of the two components of 17α-hydroxylase/17,20-lyase ($P450_{17\alpha}$), that is, 17α-hydroxylase (17α-OHase) and 17,20-lyase (lyase). The results show that *N*-3-(4-bromophenyl) propyl imidazole (**12**) (IC_{50} = 2.95 μM against 17α-OHase and IC_{50} = 0.33 μM against lyase) is the most potent compound within the current study, in comparison to ketoconazole (KTZ) (IC_{50} = 3.76 μM against 17α-OHase and IC_{50} = 1.66 μM against lyase). Modelling of these compounds suggests that the length of the alkyl chain enhances the interaction between the inhibitor and the area of the active site corresponding to the C(3) area of the steroid backbone, thereby increasing potency.

The enzyme complex 17α-hydroxylase/17,20-lyase ($P450_{17\alpha}$) is a pivotal enzyme in the conversion of progestins (such as progesterone and pregnenolone) to the androgens[1] (androstenedione and dehydroepiandrosterone, respectively, Fig. 1) and requires both NADPH and oxygen in the sequential oxidative steps.[2]

The enzyme catalyses both the hydroxylation of the progestin backbone, via 17α-hydroxylase (17α-OHase), followed by the cleavage of the C(17) and C(20) bond, via 17,20-lyase (lyase), both of which are believed to be undertaken within an active site that possesses two binding sites.[3] However, little specific information is known about the active site of this enzyme as no crystal structure for it exists, although workers have utilised homology modelling in an attempt to discover detailed information regarding the active site.[3] $P450_{17\alpha}$ is therefore responsible for the second step in the steroidal cascade leading to the biosynthesis of the sex hormones, glucocorticoids and mineralocorticoids, the latter two series of compounds being produced directly from the 17α-hydroxy progestins. This enzyme has become the focus of attention as it may have a role to play in the treatment of hormone-dependent prostate cancer through the overall reduction in the biosynthesis of androgens. Here, we report: the synthesis of a range of imidazole-based compounds (which have previously been evaluated as potential inhibitors of the enzyme aromatase by Ahmed et al;[4] as well as potential hypolipidemic agents[5]); their biochemical evaluation [in comparison to ketoconazole (KTZ)], and the rationalisation of their inhibitory activity using the substrate–haem complex (SHC) approach.

The general approach for the construction of the SHC has been described previously[6–9] and will therefore not be detailed here. In general, however, the structures of progesterone, 17α-hydroxyprogesterone, the haem and potential hydrogen bonding groups were all constructed and refined by performing a pre-optimization calculation in mechanics using Augmented MM2 as implemented in the molecular modelling program CaChe.[10]

Keywords: Imidazole; 17α-Hydroxylase/17,20-lyase; Inhibitors; Prostate cancer.
* Corresponding author. Tel.: +44 20 8547 2000; fax: +44 20 8547 7562; e-mail: S.AHMED@KINGSTON.AC.UK

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.06.092

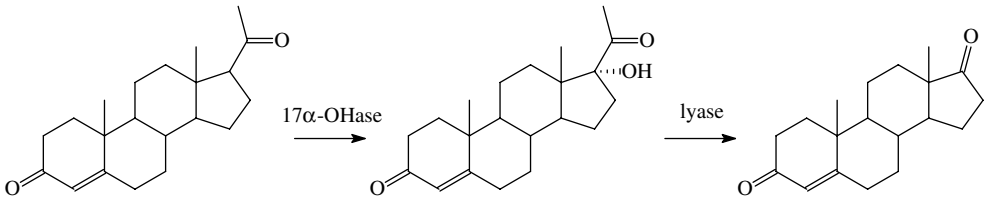
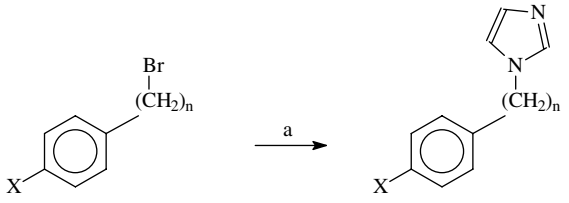


Figure 1. Action of the overall enzyme complex on progesterone resulting in the subsequent biosynthesis of androstenedione.

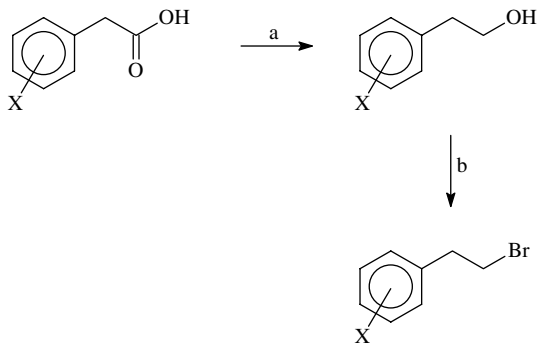
The atoms of the SHC were then 'locked' and the proposed inhibitors attached to the SHC representing the active site of the overall enzyme complex resulting in the production of the enzyme–inhibitor complex, in the attachment of the inhibitors, interactions at both the haem (N–Fe dative covalent bond) and potential polar–polar interactions with groups at the active site were mimicked. The enzyme–inhibitor complex was minimised and optimised so as to produce the final binding conformer of the inhibitor within the representation of the overall active site of $P450_{17\alpha}$.

In the synthesis of the proposed inhibitors, the azole functionality was reacted with a phenyl alkyl halide in the presence of a suitable base (Scheme 1); the synthesis of *N*-1-phenylethyl-imidazole (**2**) is given as an example.[11] In general, the compounds were synthesised in good yield (ranging from 45% to 80% yield) and without any major problems.

However, in the synthesis of the majority of the long chain-containing compounds ($n > 1$; X = F, Cl, and Br), the 4-substituted phenyl alkyl bromides were not readily available, and as such, were required to be synthesised. For example, 2-(4-bromophenyl)-ethyl bromide was synthesised from 2-(4-bromophenyl)-acetic acid (Scheme 2).



Scheme 1. Synthesis of potential inhibitors of $P450_{17\alpha}$. Reagents: (a) imidazole/K_2CO_3/THF/Δ. (n = 1–3; X = H, F, Cl and Br).



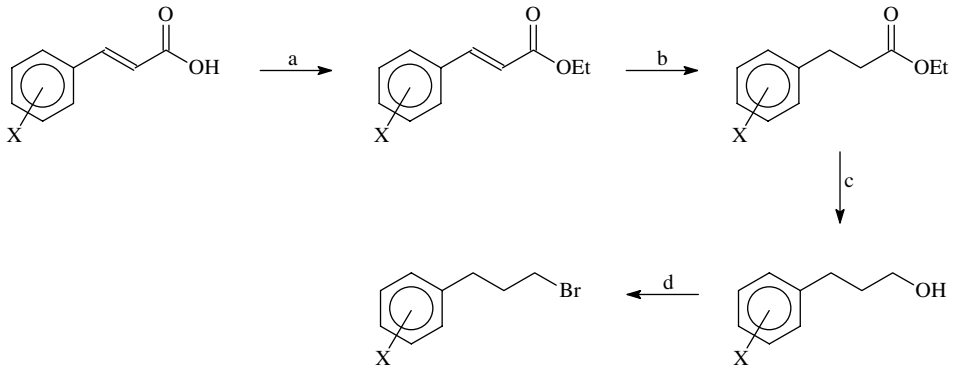
Scheme 2. Synthesis of 2-(4-substituted phenyl)-ethyl bromide (X = F, Cl and Br). Reagents: (a) $LiAlH_4$/THF/Δ; (b) PBr_3/THF/Δ.

Similarly, in the synthesis of 4-halogen-substituted phenyl propyl bromide, only the appropriate cinnamic acid derivatives were commercially available and the reactions outlined in Scheme 3 were undertaken in the synthesis of the range of 4-halogen substituted phenyl propyl bromides. No substituted phenyl alkyl bromides (or derivatives) were found to be commercially available for compounds containing spacer groups (between the substituted phenyl ring and the azole moiety) greater than C_3, and the synthesis of these compounds is currently under investigation within our laboratories.

The synthesised compounds were then initially screened and IC_{50} values determined against both 17α-OHase and lyase using modified literature methods,[12–15] where a modified mobile phase to that used by Li et al.[12] in the separation and identification of the radiolabelled substrate from the radiolabelled products was used.

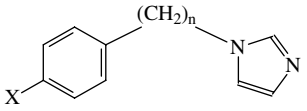
Table 1 shows the IC_{50} values obtained for the inhibition of both 17α-OHase and lyase by the synthesised compounds; in general, most of the compounds possessed weaker inhibitory activity than KTZ. In particular, consideration of the inhibitory data shows that the compounds based upon the propyl chain possessed good inhibitory activity in comparison to the shorter chain-containing compounds, such as those based on the benzyl backbone (e.g., **1**, **4**, **7** and **10**). The most potent inhibitor was found to be 4-bromophenyl propyl imidazole (**12**) (IC_{50} = 2.95 μM against 17α-OHase and IC_{50} = 0.33 μM against lyase) which is slightly more potent against 17α-OHase and ~5 times more potent against lyase, in comparison to the standard compound KTZ (IC_{50} = 3.76 μM against 17α-OHase and IC_{50} = 1.66 μM against lyase).

Further consideration of the inhibitory activity (Table 1) shows that within the series of compounds considered, a trend is observed where the IC_{50} value decreases with an increase in the alkyl spacer group. Consideration of the fluoro derivatives shows that compound **4** (n = 1, Table 1) is found to possess IC_{50} values of 96.46 and 11.26 μM against 17α-OHase and lyase, respectively, whereas compound **6** (n = 3, Table 1) has IC_{50} values of 27.81 and 1.96 μM against 17α-OHase and lyase, respectively. On modelling these compounds within the SHC, we observed that the increase in chain length of the spacer group allows for increased interaction between the substituted phenyl moiety and the area of the enzyme active site corresponding to the C(3) area of the steroid backbone (Fig. 2), thereby presumably leading to the formation of a more stable enzyme–inhibitor complex, and more potent inhibitory activity.



Scheme 3. Synthesis of 3-(4-substituted phenyl)-propyl bromide (X = H, F, Cl and Br). Reagents: (a) EtOH/Δ/H^+; (b) H_2/Pd/C; (c) $LiAlH_4$/THF/Δ; (d) PBr_3/THF/Δ.

Table 1. Table to show the percentage inhibition and the IC_{50} values obtained for a range of imidazole-based compounds against 17α-OHase and lyase (the values are means of three determinations, $n = 9$; ND = not determined)



X	*n*	Compound	% inhibition ([*I*] = 10 μM)	17α-OHase IC_{50} values (μM)	% inhibition ([*I*] = 10 μM)	Lyase IC_{50} values (μM)
H	1	**1**	13.51 ± 0.65	154.20 ± 7.93	12.64 ± 0.65	50.90 ± 0.86
H	2	**2**	10.56 ± 1.76	ND	6.36 ± 0.65	ND
H	3	**3**	23.35 ± 0.97	30.95 ± 0.68	39.95 ± 0.89	6.14 ± 1.21
F	1	**4**	20.47 ± 1.79	96.46 ± 0.20	50.98 ± 1.36	11.26 ± 0.22
F	2	**5**	22.48 ± 1.84	120.20 ± 7.74	45.07 ± 1.21	ND
F	3	**6**	40.77 ± 0.53	27.81 ± 1.44	73.15 ± 0.33	1.96 ± 0.01
Cl	1	**7**	45.70 ± 0.98	29.84 ± 0.27	73.65 ± 0.99	4.94 ± 0.17
Cl	2	**8**	28.42 ± 2.92	49.64 ± 1.48	57.38 ± 2.50	ND
Cl	3	**9**	71.00 ± 0.53	5.85 ± 0.19	80.85 ± 1.28	0.55 ± 0.07
Br	1	**10**	49.29 ± 1.97	16.55 ± 0.23	76.25 ± 0.65	2.85 ± 0.08
Br	2	11	37.54 ± 0.60	30.66 ± 0.13	61.52 ± 2.49	ND
Br	3	**12**	70.15 ± 0.94	2.95 ± 0.03	86.29 ± 0.3	0.33 ± 0.02
—	—	KTZ	61.54 ± 1.53	3.76 ± 0.01	78.69 ± 2.44	1.66 ± 0.15

With regard to the ethyl spacer group-containing compounds, it should be noted that these compounds were synthesised and fully characterised [the synthesis of *N*-1-phenylethyl-imidazole (**2**) is given as an example[11]], however, upon evaluating these compounds for inhibitory activity, we discovered that they possessed weaker inhibitory activity than the compounds based on the benzyl backbone. We undertook analysis of the compounds (involving GC–MS and thin-layer chromatography) and discovered that these compounds, although initially showing good purity (as evidenced by GC–MS analysis) prior to initial screening, had undergone decomposition prior to the determination of their IC_{50} values. As such, the IC_{50} values for compounds **2**, **5**, **8** and **11** against the lyase component were not determined.

From the inhibitory activity data outlined in Table 1, it can be seen that within the substituted series of compounds, the bromo-derivatives are more potent than the chloro-derivatives, which are in turn more potent than the fluoro-derivatives and the non-substituted derivatives. We hypothesise that the trend observed is presumably due to the ability of the substituted atom being able to undergo polar–polar interaction with a hydrogen-bonding group at the active site [which would normally undergo H-bond interaction with the C(3)=O moiety within the natural substrate]. This potential interaction between the heteroatom on the phenyl ring and groups within the active site of $P450_{17\alpha}$ has previously been suggested by other workers[16,17] and by Ahmed (involving the molecular modelling study of a range of imidazole-based antimycotic compounds as inhibitors of $P450_{17\alpha}$).[18] We therefore hypothesise that similar interaction is possible, however, we propose that the bromine atom is able to interact with the group(s) at the active site more effectively than either Cl or F due to the increased electronegativity of the latter two atoms, thereby resulting in weaker binding of the inhibitor within the active site of the enzyme and poorer inhibitory activity. As such, compound **12** is found to possess not only the appropriate alkyl spacer group ($n = 3$) but also a 4-bromo-substituted aromatic ring system allowing this compound to interact favourably within the enzyme active site. As such, these compounds are excellent lead compounds in the search for more potent and specific inhibitors of $P450_{17\alpha}$. Furthermore, the ability of these compounds to inhibit the lyase step in preference to the hydroxylation step is also a major advantage since the inhibition of the 17α-OHase activity may result in

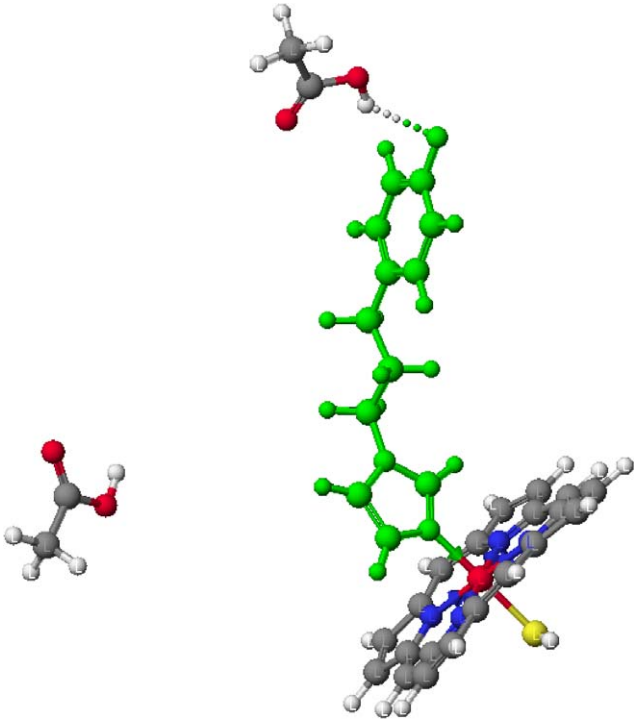


Figure 2. Binding of compound **6** to the overall SHC for $P450_{17\alpha}$.

significant side-effects due to an effect on corticosteroid biosynthesis.[19]

In conclusion, the SHC approach has allowed the design and subsequent synthesis of a small range of potent inhibitors of this enzyme complex. Whilst the compounds within the current study have been shown, in general, to possess weaker inhibitory activity against the lyase components of the overall enzyme complex of $P450_{17\alpha}$ in comparison to the standard compound KTZ, all are significantly weaker inhibitors against the 17α-OHase component. The greater selectivity is seen for compounds **6** and **9** which show over ~14- and ~10-fold differences, respectively, in activity between the two components. They are therefore extremely good lead compounds in the design and synthesis of more potent inhibitors of $P450_{17\alpha}$.

Acknowledgments

The authors thank the EPSRC National Mass Spectrometry service at the University of Wales College Swansea (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. Robichaud, P.; Wright, J. N.; Akhtar, M. *J. Chem. Soc., Chem. Commun* **1994**, *12*, 1501.
3. Laughton, C. A.; Neidle, S.; Zvelebil, M. J. J. M.; Sternberg, M. J. E. A. *Biochem. Biophys. Res. Commun.* **1990**, *171*, 1160.
4. Ahmed, S.; Smith, J. H.; Nicholls, P. J.; Whomsley, R.; Cariuk, P. *Drug Des. Discovery* **1995**, *13*, 27.
5. Baggaley, K. H.; Heald, M.; Hindley, R. M.; Morgan, B.; Tee, J. L.; Green, J. *J. Med. Chem.* **1975**, *18*, 833.
6. Ahmed, S. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2795.
7. Ahmed, S.; Davis, P. J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2789.
8. Ahmed, S. *J. Enzyme Inhib.* **1997**, *12*, 59.
9. Ahmed, S. *Biochem. Biophys. Res. Commun.* **2000**, *275*, 75.
10. CaChe is a trademark of Oxford Molecular Ltd, Oxford Science Park, Oxford, UK.
11. *N*-1-Phenylethyl-imidazole (**2**): imidazole (2 g, 29.4 mmol) was added to anhydrous potassium carbonate (K_2CO_3) (1.02 g, 7.34 mmol) and anhydrous tetrahydrofuran (THF) (50 mL). The mixture was stirred at room temperature for 10 min prior to the addition of phenylethyl bromide (2.72 g, 14.7 mmol). The mixture was then stirred under reflux for 24 h. After filtration, the THF was removed under vacuum to leave a yellow solid which was dissolved in dichloromethane (DCM) (40 mL) and washed with water (3× 50 mL). The organic layer was then extracted using hydrochloric acid (HCl) (2 M, 3× 30 mL) followed by water (2× 50 mL). The combined acid layer was neutralised with solid saturated sodium bicarbonate ($NaHCO_3$) and then extracted into DCM (2× 40 mL). The combined DCM layer was washed with water (3× 50 mL), dried over anhydrous magnesium sulfate ($MgSO_4$) and filtered. Removal of DCM under vacuum gave **2** as a yellow oil (0.99 g, 39%). $\nu_{(max)}$ (film) cm^{-1}: 3387.3 (NCN imidazole), 3031.7 (CH aromatic), 1605.1 (C=C aromatic); δ_H (300 MHz, $CDCl_3$): 7.31 (1H, s, NCHN imidazole), 7.21 (5H, m, Ph-H), 6.82 (1H, s, NCH imidazole), 4.16 (2H, t, $J = 7$ Hz, Ph-CH_2), 3.13 (2H, t, $J = 7$Hz, NCH_2); δ_C (75 MHz, $CDCl_3$): 137.45 (NCN), 135.00 (ImC), 129.43, 128.77, 128.59, 126.99, (ArC), 118.77 (ImC), 48.52 (Ph-CH_2), 37.86 (NCH_2); GCMS t_R 8.21 min *m/z* 172 (M^+), 54 (base peak).
12. Li, J. S.; Li, Y.; Son, C.; Brodie, A. M. H. *J. Med. Chem.* **1996**, *39*, 4335.
13. *17α-OHase assay.* Rat testicular microsomal suspension was thawed under cold running water and vortexed. The final incubation assay mixture (1 mL) consisted of sodium phosphate buffer (50mM, pH 7.4, 905 μl), radiolabelled progesterone as substrate (1.5 and 15 μL), NADPH generating system (50 μL) and inhibitor (10 and 20 μL). Tubes were warmed to 37 °C for 5 min and the assay initiated by the addition of microsomal enzyme (final concentration 0.16 mg/mL, 10 μL). The assay mixture was incubated for 15 min. The reaction was quenched by the addition of ether (2 mL), vortexed and placed on ice. The organic layer was then placed into a separate tube. The assay mixture was further extracted with ether (2× 2 mL), and the organic layers were combined. The solvent was removed under a stream of nitrogen, acetone (30 μL) was added to each tube and the solution spotted onto silica-based TLC plates along with carrier steroids (progesterone, 17α-hydroxyprogesterone, testosterone and androstenedione, 5 mg/mL). The TLC plates were developed using the mobile phase DCM/ethylacetate (7:3). The separated spots were identified under UV light and each spot cut out and placed into scintillation vials. Acetone (1 mL) and scintillation fluid (HiSafe) (3 mL) were added to each vial, vortexed and counted for 3 min for ^3H.
14. *Lyase assay.* Rat testicular microsomal suspension was thawed under cold running water, and vortexed. The final incubation assay mixture (1 mL) consisted of sodium

phosphate buffer (50 mM, pH 7.4, 905 μL), radiolabelled 17α-hydroxyprogesterone as substrate (1 and 10 μL), NADPH generating system (50 μL) and inhibitor (10 and 20 μL). Tubes were warmed to 37 °C for 5 min and the assay initiated by the addition of microsomal enzyme (final concentration 0.23 mg/mL, 15 μL). The assay mixture was incubated for 30 min. The reaction was quenched by the addition of ether (2 mL), vortexed and placed on ice. The organic layer was then removed and placed into a separate tube. The assay mixture was further extracted with ether (2× 2mL), and the organic layers combined. The solvent was removed under a stream of nitrogen, acetone (30 μL) was added to each tube and the solution spotted onto silica-based TLC plates along with carrier steroids (17α-hydroxyprogesterone, testosterone and androstenedione, 5 mg/mL). The TLC plates were developed using the mobile phase DCM/ethylacetate (4:1). The separated spots were identified under UV light and each spot cut out and placed into scintillation vials. Acetone (1 mL) and scintillation fluid (Cocktail T) (3 mL) were added to each vial, vortexed and counted for 3 min for ^{3}H.

15. *IC_{50} determination.* In determining the IC_{50} values for the most potent compounds, the inhibitory activity was determined using the method outlined above, however, for each compound, five or more inhibitor concentrations were used and the inhibitory activity determined at each concentration (in triplicate); the IC_{50} was then determined from a graph (using linear regression analysis) of the inhibitory activity versus log[*I*].

16. Wachall, B. G.; Hector, M.; Zhuang, Y.; Hartmann, R. W. *Bioorg. Med. Chem.* **1999**, *7*, 1913.
17. Leroux, F.; Hutschenreuter, T. U.; Charriere, C.; Scopelliti, R.; Hartmann, R. W. *Helv. Chim. Acta* **2003**, *86*, 2671.
18. Ahmed, S. *Drug Des. Discovery* **1994**, *12*, 77.
19. Njar, V. C. O.; Brodie, A. M. H. *Curr. Pharm. Des.* **1999**, *5*, 163.