Long-acting Dihydropyridine Calcium Antagonists. Part 8.[1] A Comparison of the Pharmacological and Pharmacokinetic Properties of Amlodipine with its Carba and Thio-bioisosteres

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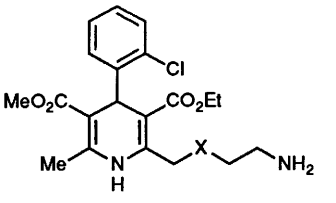
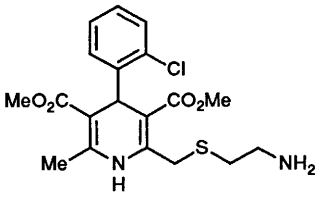
In order to evaluate the contribution to the overall pharmacokinetic and pharmacological profile of amlodipine made by the side-chain ether oxygen atom and the intramolecular hydrogen bond to the DHP ring NH proton, the profile of amlodipine was compared with that of its carba and thio bioisosteres. Replacing the side-chain oxygen by carbon dramatically reduces *in vitro* calcium antagonist potency, an effect which may be attributed to the loss of a through-bond inductive effect on the DHP ring NH proton, while both the thio and carba analogues show lower *in vitro* selectivity than amlodipine for vascular over cardiac tissue. On intravenous administration to anaesthetised dogs, compounds **2** and **3** both exhibit marked depression of myocardial contractility at doses equal or close to their ED_{50} for reduction of coronary vascular resistance. The plasma clearances of amlodipine and analogues **3** and **4** are similar, suggesting that the conformation adopted by the 2-sidechain has little influence on this parameter although bulk and polarity are important. However, compounds **3** and **4** have markedly lower volumes of distribution (6 and 8 dm^3 kg^{-1}, respectively) than amlodipine (25 dm^3 kg^{-1}) and consequently shorter half-lives; this may be a consequence of their inability to form an intramolecular hydrogen bond.

Calcium antagonists are a well established class of pharmacological agents which are finding increasing use in the treatment of various cardiovascular diseases.[2] Although there are a number of distinct structural classes with this mode of action,[3] the 1,4-dihydropyridines (DHPs) have attracted most synthetic effort and new agents regularly appear in the literature.[4] At the start of our work, we elected to carry out a systematic modification of the 2-position of the DHP ring with the objective of improving bioavailability and duration of action. This work demonstrated for the first time that extended 2-substituents bearing basic functionality were well tolerated at the DHP receptor and led to the identification of amlodipine **1**[5] which is now marketed for the once-daily treatment of angina and hypertension.

The major structural difference between amlodipine and other DHPs is the basic 2-aminoethoxymethyl group on the 2-position of the DHP ring. Thus, we were keen to determine which individual structural features in this extended substituent influenced the unique combination of pharmacological and pharmacokinetic properties exhibited by amlodipine.[6] For example, X-ray[7] and NMR[8] studies have shown that there is an intramolecular hydrogen bond between the ring NH and the side-chain oxygen in amlodipine which could influence receptor affinity and susceptibility to metabolic oxidation by cytochrome P-450 enzymes. In addition, it has been suggested[7] that the location of amlodipine in biological membranes may differ from that of other DHPs due to an ionic interaction between the protonated amino function and the negatively charged phospholipid head-group. In order to address these issues we have prepared analogues of amlodipine in which the side-chain oxygen atom is replaced by sulfur or methylene and we have compared their calcium antagonist activities and pharmacokinetics with the parent molecule. In these bioisosteres, the basic centre and overall molecular dimensions of amlodipine are maintained, although intramolecular hydrogen bonding between the side chain and the DHP NH function is obviously not possible.



1 X = O (amlodipine)
2 X = S
3 X = CH_2



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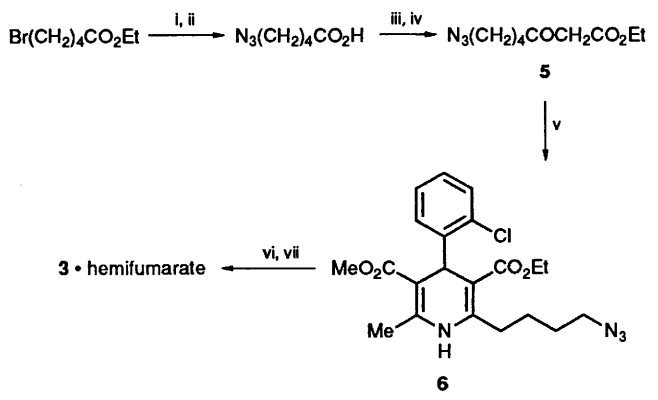
Results and Discussion

The 2-(2-aminoethylthio)methyl compounds **2**[9] and **4**[10] were prepared as described in the literature while the 4-aminobutyl analogue **3** was obtained as shown in Scheme 1. Thus, reaction of $Br(CH_2)_4CO_2Et$ with tetramethylguanidinium azide[11] followed by saponification afforded 5-azidopentanoic acid which was converted to the β-ketoester **5** *via* the Meldrum's acid adduct.[12] Hantzsch condensation of **5** with methyl 3-aminocrotonate and 2-chlorobenzaldehyde afforded the azide **6** which was converted directly to the desired amine **3** by hydrogenation in the presence of Pd on $CaCO_3$ catalyst.

In vitro vascular calcium antagonist activity (expressed as an IC_{50}) was assessed as the concentration of the compound required to inhibit the calcium-induced contraction of potassium depolarised rat aorta by 50% while negative inotropy



Scheme 1 *Reagents:* i, tetramethylguanidinium azide, $CHCl_3$, reflux, 9 h; ii, NaOH, H_2O, dioxane, room temperature, 16 h; iii, 2,2-dimethyl-1,3-dioxane-4,6-dione, pyridine, room temperature, 20 h; iv, EtOH, reflux, 5 h; v, 2-chlorobenzaldehyde, methyl 3-aminocrotonate, EtOH, reflux, 3 h; vi, 5% Pd on $CaCO_3$ (catalytic), H_2, EtOH, room temperature; vii, fumaric acid, Et_2O

Table 1 Comparison of the *in vitro* and *in vivo* calcium antagonist activity

	Ca[a]	Negative inotropy[b]	CVR[c]
Compound	pIC_{50}	pIC_{25}	ED_{50}/μg kg^{-1}
1	8.1	7.2	103
2	7.8	7.5	94
3	7.1	6.6	300–400[d]
4	7.6	7.2	NT[e]

[a] Negative logarithm of the molar concentration required to block Ca^{2+}-induced contraction of K^+-depolarised rat aorta by 50%. Nifedipine was used as the standard compound, standard deviation ±0.01. [b] Negative logarithm of the molar concentration required to depress contraction in the Langendorff-perfused guinea pig heart by 25%. Nifedipine was used as the standard compound, standard deviation ±0.26. [c] Intravenous dose required to reduce coronary vascular resistance in anaesthetised dogs by 50%, $n = 2$. [d] $n = 1$. [e] Not tested.

(expressed as an IC_{25}) was determined *in vitro* using a Langendorff-perfused guinea pig heart preparation. As can be seen from the data in Table 1, compounds **2** and **4**, in which the oxygen atom in the side-chain is replaced by a sulfur atom, are 2–3 fold less potent than amlodipine. In contrast, the methylene analogue **3** is some ten-fold weaker which suggests that the side-chain heteroatoms in compound **1**, **2** and **4** exert a positive effect on affinity for the DHP receptor. Thus, it is possible that the through-bond inductive effect of the side-chain oxygen and sulfur atoms may reduce electron density on the proton on N1 of the DHP ring thereby increasing its electropositive character. Such a situation would enhance the strength of a hydrogen bond between the DHP ring NH proton and an acceptor atom in the DHP receptor resulting in increased *in vitro* affinity. By contrast the pK_a of the terminal amino group does not appear to influence calcium antagonist activity. Data in Table 1 also show that the selectivity of amlodipine for vascular over cardiac tissue is superior to that seen for **2**–**4**. Thus the presence of the side-chain oxygen atom in amlodipine is preferred over the carba and thio analogues with respect to both *in vitro* calcium antagonist activity and tissue selectivity.

In order to assess the effect of the ether oxygen atom in amlodipine on *in vivo* activity, amlodipine and compounds **2** and **3** were evaluated in instrumented anaesthetised dogs. All compounds were administered intravenously and the effects on coronary vascular resistance (CVR) were measured (see Table 1). As might be expected from their similar *in vitro* potencies, compound **2** was approximately equipotent with amlodipine in reducing CVR. However, compound **2** also caused depression of myocardial contractility which was particularly marked at doses of 400 μg kg^{-1} and above. In contrast, amlodipine had no adverse effects on cardiac electrical or mechanical function although, as expected, reflex stimulation of cardiac output and myocardial contractility in response to peripheral vasodilation were observed.[13] Preliminary data on the effects of **3** in the anaesthetised dog are even more striking. As might be expected from its *in vitro* profile, **3** is weaker than amlodipine in reducing CVR while at all doses profound myocardial depression is observed. These data confirm the *in vitro* observations that the presence of the side-chain oxygen atom in amlodipine is a crucial determinant of selective calcium antagonist activity. In particular, the presence of the intramolecular hydrogen bond in amlodipine may be responsible for the absence of those adverse cardiac effects seen for **2** and **3**. Preliminary molecular modelling studies have shown[14] that, in contrast to amlodipine, a coplanar arrangement of the side-chain CH_2S atoms and the NH bond in the DHP ring is not lowest in energy for **3** and that the preferred conformation is one in which the sulfur atom is twisted away from the plane of the DHP ring. Although it has been proposed[15] that the apparent inherent selectivity of DHPs for vascular over cardiac tissue arises because they bind preferentially[16,17] to the inactivated state of the voltage-dependent calcium channel, we have shown[18,19] that structural features can significantly alter tissue selectivity. One hypothesis which accounts for the differences seen in tissue selectivity is that the environments of the calcium channels in vascular and cardiac tissue are in some way different. Alternatively, since DHP binding to the inactivated state of the channel is not exclusive,[16,17] the presence of certain structural features in DHPs may alter their relative binding to the open and inactivated states of the DHP channel, thereby causing differences in tissue selectivity. Whichever explanation is correct, it is possible that the alternative preferred conformation for **2** (and, by analogy, presumably also **3**) compared to amlodipine leads to lower vascular over cardiac tissue selectivity thereby accounting for the depression of myocardial contractility seen for **2** and **3** and not amlodipine.

In view of the long plasma half-life of amlodipine in animals and man, we explored the effect on its pharmacokinetic parameters of replacing the side-chain oxygen by methylene and sulfur (Table 2). The plasma clearance values for amlodipine and compounds **3** and **4** are very similar and are substantially lower than that for felodipine, a DHP lacking basic functionality. Since it has been reported that there is a direct relationship between rate of metabolism and increasing lipophilicity for DHPs,[20] the lower clearance of these compounds relative to felodipine may reflect the increased polarity which results from protonation of the basic function at physiological pH (as can be seen from the data in Table 2, the log *D* of felodipine is some three orders of magnitude higher than those of amlodipine, **3**, and **4**). Alternatively, it has been suggested[18,21] that polar but not necessarily basic substituents in the 2-position of the DHP ring hinder binding to the cytochrome P450 enzyme(s) responsible for metabolic oxidation of DHPs to the corresponding pyridines. The similarity of the plasma clearance values for amlodipine, **3** and **4** suggests that restriction of the side-chain by formation of an intramolecular hydrogen bond does not influence metabolic stability. Thus, the conformation adopted by the 2-substituent has little impact on clearance, while bulk and polarity do appear to be important. However, the conformation of the side-chain does appear to have a major influence on volume of distribution. Both **3** and **4** have lower volumes of distribution than amlodipine and this is reflected in a 3–4 fold decrease in their plasma half-life values. It has been shown[7] that amlodipine binds in phospholipid

Table 2 Physicochemical properties and pharmacokinetic data in dogs for amlodipine **1**,[a] felodipine,[b] and compounds **3** and **4**

Compound	*c*log *P*	log *D* (pH 7.4)	pK_a	Plasma clearance/ cm^3 min^{-1} kg^{-1}	Volume of distribution/ dm^3 kg^{-1}	Plasma half life/h
Felodipine	4.8	4.8[d]	—	39	3.7	1.1
1 (Amlodipine)	2.9	1.8	8.6	11.0	25	30
3	3.8	1.5[d]	9.7[c]	9.3	5.4	7.0
4	2.8	1.6	8.5[c]	10.3	7.7	8.7

[a] D. A. Stopher, A. P. Beresford, P. V. Macrae and M. J. Humphrey, *J. Cardiovasc. Pharmacol.*, 1988, **12**, suppl. **7**, 555. [b] M. J. Humphrey and D. A. Stopher, unpublished work. [c] Calculated on the basis of the pK_a of amlodipine and the rank order of pK_a values for butylamine, 2-methoxyethylamine and 2-methylthioethylamine (data obtained from MEDCHEM computer program); A. Leo, *J. Pharm. Sci.*, 1987, **76**, 166. [d] Calculated using *c*log *P* and estimated pK_a data according to the method of C. N. Manners, D. W. Payling and D. A. Smith, *Xenobiotica*, 1988, **18**, 331.

bilayers in a location which is consistent with an ionic interaction between its protonated amino function and the negatively charged phospholipid head-group region and this charge–charge interaction may account for the observed large volume of distribution. The hydrogen bond in amlodipine between the ether oxygen and the N1 proton on the DHP ring, which has been shown to be present in the crystal[7] and in chloroform solution,[8] appears to restrict the molecule to a conformation which maximises its interaction with the negative charge in the phosphate headgroup of phospholipid bilayers. The lower volumes of distribution seen for **3** and **4** may therefore be a consequence of their inability to form an intramolecular hydrogen bond and consequently inappropriate positioning of the protonated centre for effective interaction with the negative charge in the phosphate head-group.

Conclusion

We have shown that the ether oxygen in the 2-substituent on the DHP ring in amlodipine is an important determinant of its vascular calcium antagonist potency and selectivity. In contrast to amlodipine, the carba and thio bioisosteres show marked depression of myocardial contractility after intravenous administration to anaesthetised dogs. Furthermore the presence of the intramolecular hydrogen bond in amlodipine is probably responsible for its large volume of distribution while its improved clearance compared to felodipine is a consequence of the presence of the protonated basic function.

Experimental

Pharmacology.—*In vitro* calcium antagonist activity (pIC_{50}) and negative inotropy (pIC_{25}) were measured as previously described.[6] *In vivo* haemodynamic measurements were made in anaesthetised beagle dogs as previously described.[18]

Chemistry.—All melting points are uncorrected. Compound **2** was prepared according to the literature procedure.[9] ^{1}H-NMR spectra were recorded on a General Electric QE300 spectrometer, and all *J* values are given in Hz. All compounds were obtained as racemic mixtures. Pyridinium bromide perbromide was purchased from Aldrich Chemical Co. (technical grade, 90%). Column chromatography was performed on Silica Gel 60, 230–400 mesh (E. Merck).

2-(4-*Aminobutyl*)-4-(2-*chlorophenyl*)-3-*ethoxycarbonyl*-5-*methoxycarbonyl*-6-*methyl*-1,4-*dihydropyridine* (**3**).—A solution of the azidoester **5** (3.5 g, 16 mmol), 2-chlorobenzaldehyde (2.3 g, 16 mmol) and methyl 3-aminocrotonate (1.9 g, 16 mmol) in EtOH (35 cm^3) was heated under reflux for 3 h and evaporated. The residue was purified by chromatography on silica using hexane–Et_2O (3:1) as eluent. Appropriate fractions were combined and evaporated to give 2-(4-azidobutyl)-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine (**6**) (1.30 g, 19%) as a colourless oil. This material was used directly in the preparation of **3** without characterisation.

A solution of the above azide **6** (1.30 g, 3 mmol) in EtOH (40 cm^3) was stirred for 4 h at room temperature under an atmosphere of hydrogen in the presence of 5% Pd on $CaCO_3$ (400 mg), filtered, and evaporated. The residue was purified by chromatography on silica using CH_2Cl_2 plus 0–25% MeOH as eluent. Appropriate fractions were combined and evaporated. The residue was dissolved in Et_2O (50 cm^3) and the solution was treated with an excess of a saturated solution of fumaric acid in Et_2O. The resulting precipitate was collected, washed with Et_2O, and dried to give the hemifumarate and hemihydrate of amine **3** (0.28 g, 23%) as a hygroscopic solid, m.p. 175–185 °C (Found: C, 58.4; H, 6.3; N, 5.7. $C_{21}H_{27}ClN_2O_4 \cdot 0.5C_4H_4O_4 \cdot 0.5H_2O$ requires C, 58.5; H, 6.4; N, 5.9%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3700–3100 (NH_3^+) and 1675 (C=O); ^{1}H NMR δ_H(solvent $CDCl_3$; internal standard Me_4Si) 8.01 (1 H, s, NH), 7.28 (1 H, d, *J* = 8), 7.17 (1 H, d, *J* = 8), 7.06 (1 H, t, *J* = 8) and 6.95 (1 H, t, *J* = 8, C_6H_4), 6.53 (1 H, s, HO_2CCH=), 5.34 (1 H, s, 4-H), 4.2–4.8 (3 H, broad s, NH_3^+), 3.82 (2 H, q, *J* = 7, CH_2CH_3), 3.48 (3 H, s, MeO), 2.80–2.98 (2 H, br s, $CH_2NH_3^+$), 2.45–2.7 (2 H, br s, CH_2–DHP), 2.16 (3 H, s, 6-Me), 1.75–1.4 (4 H, br s, 2-$CH_2CH_2CH_2CH_2NH_3^+$) and 1.00 (3 H, t, *J* = 7, OCH_2CH_3).

2-(2-*Aminoethylthio*)*methyl*-3,5-*bis*(*methoxycarbonyl*)-4-(2-*chlorophenyl*)-6-*methyl*-1,4-*dihydropyridine* (**4**).—Pyridinium bromide perbromide (200 g, 5 mmol) was added in one portion to a stirred, ice-cooled solution of 3,5-bis(methoxycarbonyl)-4-(2-chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine[22] (1.68 g, 5.0 mmol) and pyridine (0.80 cm^3) in $CHCl_3$ (50 cm^3). The ice-cooled mixture was stirred for 30 min, washed twice with ice-cold HCl (0.25 mol dm^{-3}), dried over Na_2SO_4 and evaporated without external heating. The residue was immediately dissolved in dioxane (10 cm^3) and the solution was added to a mixture of $HS[CH_2]_2NH_2 \cdot HCl$ (2.26 g, 20 mmol) and K_2CO_3 (5.54 g) in dioxane (50 cm^3) and DMF (20 cm^3). The mixture was stirred at room temperature for 20 h and evaporated. The residue was partitioned between EtOAc and water and the organic layer was washed with water, dried over Na_2SO_4, and evaporated. The residue was purified by chromatography on SiO_2 using CH_2Cl_2 plus 0–10% MeOH as eluent. Appropriate fractions were combined and evaporated. The residue was dissolved in EtOH and the solution was treated with excess of a saturated solution of fumaric acid in Et_2O. The resulting precipitate was collected, washed with Et_2O, and dried to give the hemifumarate of amine **4** (0.32 g, 14%) as a colourless solid, m.p. 180–185 °C (Found: C, 53.9; H, 5.5; N, 6.1. $C_{19}H_{23}ClN_2O_4 \cdot 0.5C_4H_4O_4$ requires C, 53.8; H, 5.3; N, 6.0%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3800–2500 (NH_3^+) and 1695 (C=O); ^{1}H NMR δ_H(solvent $CDCl_3$; internal standard Me_4Si) 7.28 (1 H, d, *J* = 8), 7.15 (1 H, d, *J* = 8), 7.04 (1 H, t, *J* = 8) and 6.90 (1 H, t, *J* =

8, C_6H_4) (1 H, s, NH), 6.58 (1 H, s, HO_2CCH=), 5.34 (1 H, s, 4-H), 4.2–4.8 (3 H, br s, NH_3^+), 3.98 (2 H, s) and 2.80 (2 H, s) ($SCH_2CH_2NH_3^+$) and 2.22 (3 H, s, 6-Me).

Ethyl 7-Azido-3-oxoheptanoate (**5**).—A mixture of ethyl 5-bromopentanoate (20.9 g, 0.10 mol) and tetramethylguanidinium azide[11] (17.4 g, 0.11 mol) (**CAUTION: Potentially Explosive!**) in $CHCl_3$ (200 cm^3) was heated under reflux for 9 h, washed with water, and evaporated. The residue was dissolved in dioxane (100 cm^3) and the solution was treated with aqueous NaOH solution (100 cm^3; 6 mol dm^{-3}), stirred at room temperature for 16 h, and evaporated. The residue was partitioned between water and $CHCl_3$ and the aqueous layer was washed with $CHCl_3$, acidified with conc. HCl, and extracted into CH_2Cl_2. The organic extracts were dried over $MgSO_4$ and evaporated to give 5-azidopentanoic acid (12.3 g, 86%) as a colourless oil; $\nu_{max}(CHCl_3)/cm^{-1}$ 2095 (N_3) and 1710 (C=O); ^{1}H-NMR δ_H(solvent $CDCl_3$; internal standard Me_4Si) 3.30 (2 H, t, J = 7, CH_2N_3), 2.42 (2 H, t, J = 7, CH_2CO) and 1.50–1.95 (4 H, m, 3-H_2, 4-H_2). Carbonyl diimidazole (14.5 g, 90 mmol) was added portionwise over 30 min to a solution of 5-azidopentanoic acid (12.0 g, 84 mmol) in CH_2Cl_2 (20 cm^3) and the mixture was stirred at room temperature for 1 h. The resulting solution was added slowly over 30 min to a solution of 2,2-dimethyl-1,3-dioxane-4,6-dione (12.9 g, 90 mmol) and pyridine (7.1 g, 90 mmol) in CH_2Cl_2 (200 cm^3) and the mixture was stirred at room temperature for 20 h, washed with HCl (2 mol dm^{-3}), dried over $MgSO_4$, and evaporated. The residue was dissolved in EtOH (300 cm^3) and the solution was heated under reflux for 5 h and evaporated. The residue was dissolved in Et_2O and the solution was extracted into 10% aqueous Na_2CO_3 solution. The basic extracts were washed with Et_2O, acidified with conc. HCl, and extracted into CH_2Cl_2. The organic extracts were dried over $MgSO_4$ and evaporated to give the azido ester **5** (6.0 g, 34%) as a colourless oil; $\nu_{max}(CHCl_3)/cm^{-1}$ 2100 (N_3) and 1740 and 1715 (2 × C=O); ^{1}H-NMR δ_H(solvent $CDCl_3$; internal standard Me_4Si) 4.25 (2 H, q, J = 7, CH_2CH_3), 3.46 (2 H, s, CH_2CO_2Et), 3.33 (2 H, t, J = 7, CH_2N_3), 2.64 (2 H, t, J = 7, CH_2CO), 1.50–1.85 (4 H, m, 5-H_2, 6-H_2) and 1.32 (3 H, t, J = 7, CH_2CH_3).

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References

1 Part 7, D. Alker and P. E. Cross, *Eur. J. Med. Chem.*, 1991, **26**, 619.
2 J. D. Horowitz and A. C. Powell, *Med. J. Aust.*, 1989, **150**, 591; D. Waters, *Can. J. Cardiol.*, 1989, **5**, 181; D. A. Weiner, *Med. Clin. North Amer.*, 1988, **72**, 83.
3 P. M. Vanhoutte and R. Paoletti, *Trends Pharmacol. Sci.*, 1987, **8**, 4.
4 *Drugs Future*, 1989, **14**, 317 and references therein.
5 J. E. Arrowsmith, S. F. Campbell, P. E. Cross, J. K. Stubbs, R. A. Burges, D. G. Gardiner and K. J. Blackburn, *J. Med. Chem.*, 1986, **30**, 1696.
6 R. A. Burges, M. G. Dodd and D. G. Gardiner, *Amer. J. Cardiol.*, 1989, **64**, 10I; R. A. Burges and M. G. Dodd, *Cardiovasc. Drug Rev.*, 1990, **8**, 25.
7 R. P. Mason, S. F. Campbell, S. Wang and L. G. Herbette, *Mol. Pharmacol.*, 1989, **36**, 634.
8 D. Alker, D. V. Bowen, M. E. Gerrard and A. G. Swanson, unpublished work.
9 D. Alker and S. M. Denton, *Tetrahedron*, 1990, **46**, 3693.
10 D. Alker and A. G. Swanson, *Tetrahedron Lett.*, 1990, **31**, 1479.
11 A. J. Papa, *J. Org. Chem.*, 1966, **31**, 1426.
12 Y. Oikawa, K. Sugano and O. Yonemitsu, *J. Org. Chem.*, 1978, **43**, 2087.
13 M. G. Dodd, D. G. Gardiner, A. J. Carter, M. R. Sutton and R. A. Burges, *Cardiovasc. Drugs and Therapy*, 1989, **3**, 545.
14 M. S. Tute, unpublished work.
15 T. Godfraind, N. Morel and J. Wibo, *Trends Pharmacol. Sci.*, 1988, **8**, 37.
16 M. C. Sanguinetti and R. A. Kass, *Circ. Res.*, 1984, **55**, 336.
17 B. P. Bean, *Proc. Natl. Acad. Sci. USA*, 1984, **81**, 6388.
18 D. Alker, S. F. Campbell, P. E. Cross, R. A. Burges, A. J. Carter and D. G. Gardiner, *J. Med. Chem.*, 1990, **33**, 585.
19 D. Alker, S. F. Campbell, P. E. Cross, R. A. Burges, A. J. Carter and D. G. Gardiner, *J. Med. Chem.*, 1990, **30**, 1805.
20 M. J. Humphrey, in *Xenobiotic Metabolism and Disposition*, eds. R. Kato, R. W. Estabrook and M. N. Cayen, Taylor and Francis, London, 1989, p. 245.
21 D. Alker, S. F. Campbell and P. E. Cross, *J. Med. Chem.*, 1991, **34**, 19.
22 J. Aritoni, S. Ueda and H. Nishimura, *Chem. Pharm. Bull.*, 1980, **28**, 3163.

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