I (1.2 mM) and [γ-^{32}P]ATP (50 μM) as described previously for the routine assay of the p40 protein-tyrosine kinase[42] except that reactions contained 8% DMSO, which was used as a carrier for the inhibitors. Control reactions run in the absence of inhibitor also contained 8% DMSO. Angiotensin I was prepared by the Purdue Peptide Synthesis Facility. p56^{lck} was partially purified from bovine thymus by sequential chromatography on columns of DEAE-cellulose and heparin-agarose exactly as described for the purification of p40.[42] The first peak of tyrosine kinase activity to elute from the heparin-agarose column, which contains p56^{lck}, was further fractionated by chromatography on butyl-agarose.[42]

The catalytic subunit of cAMP-dependent protein kinase was isolated from bovine heart by method I of Bechtel et al.[43] Kinase activity was measured in 25-μL reactions containing 0.5 mM Leu-Arg-Ala-Ser-Leu-Gly (Kemptide, Sigma Chemical Co.), 180 mM Hepes, pH 7.4, 10 mM $MgCl_2$, 50 μM [γ-^{32}P]ATP, 5 mM *p*-nitrophenyl phosphate, and 8% DMSO. Protein kinase C was kindly provided by Dr. Curtis Ashendel, Purdue University. Activity was measured in 125-μL reactions containing 0.24 mg/mL phosphatidylserine, 1 nM 12-*O*-tetradecanoylphorbol-13-acetate, 4% glycerol, 20 μM [γ-^{32}P]ATP, 5 mM *p*-nitrophenyl phosphate, and 1.6% DMSO.

Acknowledgment. This investigation was made possible by Grant RO1 CA47476, awarded by the National Cancer Institute, DHHS.

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(43) Bechtel, P. J.; Beavo, J. A.; Krebs, E. G. *J. Biol. Chem.* **1977**, *252*, 2691.

Dihydropyrimidine Calcium Channel Blockers. 3.[1] 3-Carbamoyl-4-aryl-1,2,3,4-tetrahydro-6-methyl-5-pyrimidinecarboxylic Acid Esters as Orally Effective Antihypertensive Agents

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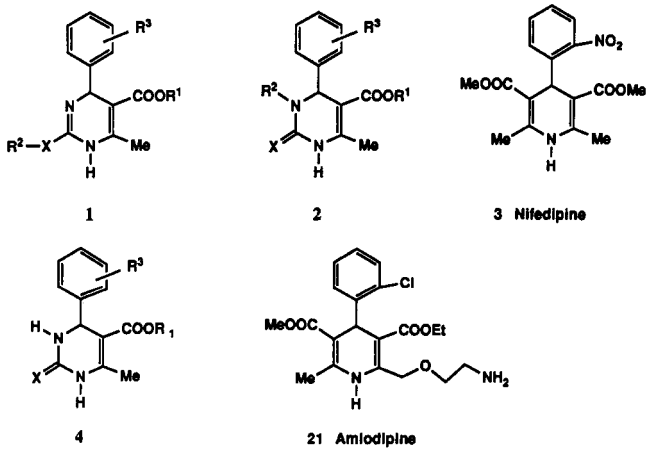
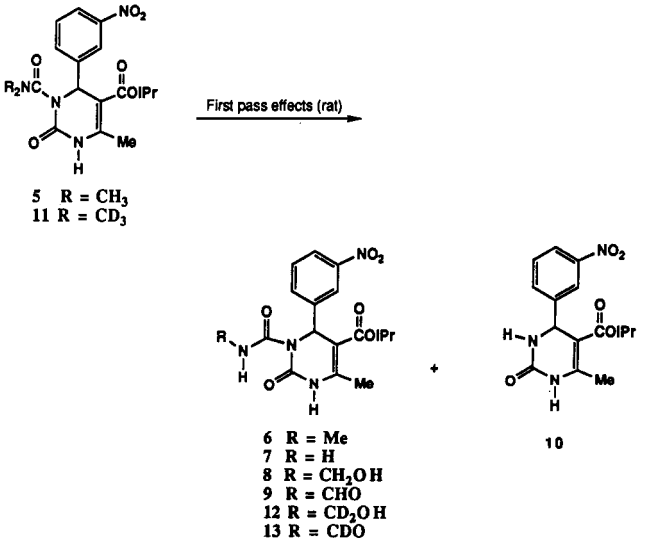
In order to explain the potent antihypertensive activity of the modestly active (IC_{50} = 3.2 μM) dihydropyrimidine calcium channel blocker **5**, we carried out drug metabolism studies in the rat and found **5** is metabolized to compounds **6–10**. Two of the metabolites, **6** (IC_{50} = 16 nM) and **7** (IC_{50} = 12 nM), were found to be responsible for the antihypertensive activity of compound **5**. Potential metabolism of **6** into **7** in vivo precluded our interest in pursuing compounds related to **6**. Structure–activity studies aimed at identifying additional aryl-substituted analogues of **7** led to **17g,j,p** with comparable potential in vivo, though these compounds were less potent than **7** in vitro. To investigate the effects of absolute stereochemistry on potency, we resolved **7** via diastereomeric ureas **19a,b**, prepared from **18** by treatment with (*R*)-α-methylbenzylamine. Our results demonstrate that the active *R*-(−)-enantiomer **20a** of **7** is both more potent and longer acting than nifedipine (**1**) as an antihypertensive agent in the SHR. The in vivo potency and duration of **20a** is comparable to the long-acting dihydropyridine amlodipine. The superior oral antihypertensive activity of **20a** compared to that of previously described carbamates **2** (R^2 = COOEt) could be explained by its improved oral bioavailability, possibly resulting from increased stability of the urea functionality.

Introduction

In previous papers we described the synthesis and structure–activity studies with 1,4-dihydropyrimidines such as **1** and **2**.[1,2] Similar to the structurally analogous dihydropyridines **3**, the dihydropyrimidines possess potent calcium channel blocking activity. However, throughout our early investigations with the dihydropyrimidines, we observed that the potency in vitro was not accompanied by antihypertensive activity when the compounds were orally administered to spontaneously hypertensive rats



Scheme I



(1) For part 2, see: Atwal, K. S.; Rovnyak, G. C.; Kimball, S. D.; Floyd, D. F.; Moreland, S.; Swanson, B. N.; Gougoutas, J. Z.; Schwartz, J.; Smillie, K. M.; Malley, M. F. *J. Med. Chem.* **1990**, *33*, 2629.

(2) Atwal, K. S.; Rovnyak, G. C.; Schwartz, J.; Moreland, S.; Hedberg, A.; Gougoutas, J. Z.; Malley, M. F.; Floyd, D. M. *J. Med. Chem.* **1990**, *33*, 1510.

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(SHR). At least for compounds containing an N3 acyl substituent, i.e., 2 (R^2 = COOEt, X = S, O); this observation was explained by metabolism studies both in vitro and in vivo demonstrating rapid deacylation yielding 4 (X = S, O), which possess greatly attenuated potency as calcium channel blocking agents.[1]

In this paper we describe the antithetical situation in which we observed potent antihypertensive activity in SHR upon oral dosing of the N3 aminocarbonyl (carbamoyl) derivative 5 despite its low level of potency in effecting the relaxation of potassium-contracted rabbit aorta. By examining the extract of livers obtained from rats dosed with 5 we were able to identify both the monodesmethyl analogue 6 and the primary ureido derivative 7 as major metabolites (Scheme I). Subsequent synthesis and testing in vitro of these materials demonstrated both to be potent calcium channel blocking agents. The results of these studies explained our initial observations with 5 and served to define a new series of compounds for study.

Initial Metabolism Studies

During the course of structure–activity studies with N3 acylated dihydropyrimidines (2) we observed that the (*N*,*N*-dimethylamino)carbonyl derivative 5 was significantly less potent than the corresponding ester 2 (X = O, R^1 = *i*Pr, R^2 = COO*i*Pr, R^3 = 3-NO_2). We were, however, surprised to see that upon oral administration to SHR, 5 demonstrated a marked antihypertensive effect that presisted for the entire 24 h of the test. In an attempt to understand this observation we focused on an investigation of the metabolism of 5 in the rat to ascertain the nature of the materials that might account for the potent antihypertensive activity.

In this study, Sprague–Dawley rats were given two 60 mg/kg doses of 5 2 h apart. One hour after the second dose, the animals were sacrificed and their livers were removed and processed as described in the Experimental Section. Analysis of the liver extracts by HPLC showed the presence of four major (6–9) products and one minor (10) product in addition to the parent compound 5. On the basis of the mass spectral analysis of each of the collected fractions noted in Figure 1, we assigned the structures 6 and 7 to two of the major metabolites arising from sequential N-demethylation of the *N*,*N*-dimethyl group of 5. The minor metabolite was identified by mass spectral analysis as 10, which, in analogy to our earlier experience, was expected to be a major metabolic product.[1] These structural assignments were easily verified by synthesis (see below) of the known materials and direct comparison of HPLC retention times and mass spectra to those of the fractions isolated from the metabolism study.

Attempted synthesis of the suspected major metabolites 8 and 9 was not successful. To obtain additional support for these structural assignments we prepared the deuterated analogue 11, in which the *N*,*N*-dimethyl groups of 5 were perdeuterated, and dosed this material to rats as described above. After isolation of the peaks corresponding to the previously observed metabolites 8 and 9, the materials were subjected to mass spectral analysis. We observed that the peak corresponding to 8 in the previous study, was 2 mass units higher in molecular weight, which is consistent with structure 12. Analogously, the peak corresponding to 9 in the previous study demonstrated a molecular mass one unit higher and was assigned structure 13. This study demonstrates that for both 8 and 9, one of the *N*-methyl groups of 5 is retained and that 8 and 9 differ from the *N*-methyl metabolite 6, and from each other, only in the oxidation state of the remaining *N*-methyl group.

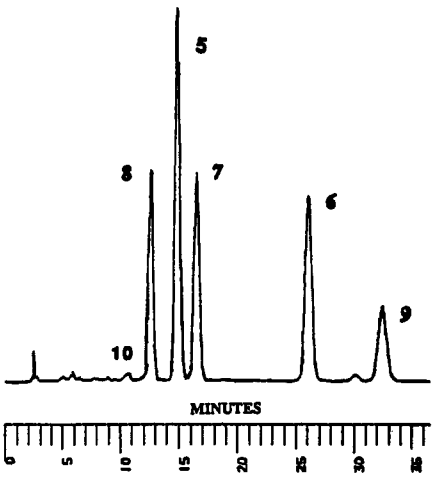


Figure 1. HPLC chromatogram of an extract of liver 90 min after oral administration of 5.

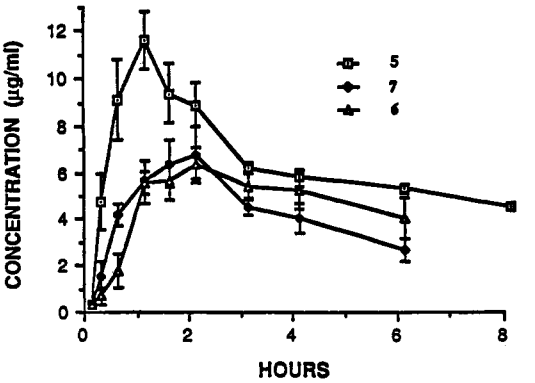
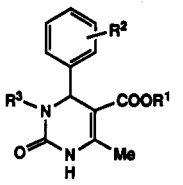


Figure 2. Mean concentrations of 5 and its metabolites 6 and 7 in the rat plasma after oral administration of 5 at a dose of 53 mg/kg (n = 3).

Unlike the parent compound 5 (IC_{50} = 3200 nM), which was relatively inactive in the rabbit aorta primary screen (see Table I), the metabolites 6 (IC_{50} = 16 nM) and 7 (IC_{50} = 12 nM) were potent calcium channel blocking agents in vitro. Estimation of the circulating concentrations of 5–7 in rat plasma by HPLC (see the Experimental Section) showed a peak mean concentration of 11.3 μg/mL for 5 (Figure 2) 1 h after a single 53 mg/kg oral dose. The peak plasma concentration of 6 and 7 averaged 6 μg/mL 2 h after the oral dose. These observations demonstrate that oral administration of 5 is followed by metabolism to 6 and 7, which are potent calcium channel blocking agents, and that the high circulating concentrations of these agents are responsible for most of the antihypertensive activity of 5. Furthermore, oral dosing of either 6 or 7 also results in a marked antihypertensive effect in the SHR (Table I).

In addition to explaining our initial observation of potent antihypertensive activity with a compound (5) that lacked significant activity in vitro, these studies indicated that the N3 (aminocarbonyl)dihydropyrimidones were highly resistant to N-deacylation. This contrasts with the previously described N3 alkoxycarbonyl derivatives 2 (R^2 = COOEt, X = S, O) where deacylation apparently resulted in extremely low oral bioavailability and explained the lack of antihypertensive activity of these compounds upon oral dosing in SHR.[1] With the carbamoyldihydropyrimidines, formation of the inactive deacylated metabolite 10 is a minor process which explains the observed potent antihypertensive activity in SHR of these derivatives. This is also in line with the observation that 5–7 all demonstrate a long duration of antihypertensive effect (>24 h) when

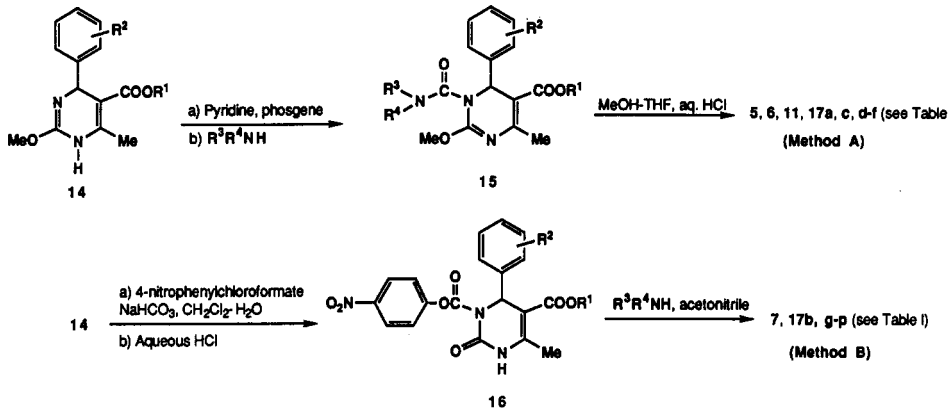
Table I. Biological Activity of 1,4-Dihydropyrimidine Analogues, Nifedipine, and Amlodipine



compd	R^1	R^2	R^3	IC_{50}, nM (95% CI)	antihypertensive activity[c] 0–6 h	6–18 h
5	*i*Pr	3-NO_2	$CONMe_2$	3200 (3080, 3390)	39	34
6	*i*Pr	3-NO_2	$CONHMe_2$	16 (15, 18)	30	38
7	*i*Pr	3-NO_2	$CONH_2$	12 (8, 17)	32	30
10	*i*Pr	3-NO_2	H	1580 (1370, 2280)	7	16[a]
17a	Et	3-NO_2	$CONH_2$	39 (28, 54)	15	9
17b	Me	3-NO_2	$CONH_2$	880 (600, 1200)	17	16
17c	*i*Pr	3-NO_2	CONHEt	13 (12, 14)	18	14[a]
17d	*i*Pr	3-NO_2	CONHiPr	60 (55, 73)	40	34
17e	*i*Pr	3-NO_2	$CONHCH_2Ph$	3 (2.8, 4)	3	7b
17f	*i*Pr	3-NO_2	$CH_2CH_2N(Me)Bn$	2 (1.5, 3)	30	25
17g	*i*Pr	2-NO_2	$CONH_2$	20 (13, 31)	34	27
17h	*i*Pr	3-CF_3	$CONH_2$	1670 (697, 4000)	18	19
17i	*i*Pr	2-CF_3	$CONH_2$	44 (31, 64)	32	22[a]
17j	*i*Pr	3-Cl	$CONH_2$	40 (23, 59)	30	25
17k	*i*Pr	2-Cl	$CONH_2$	34 (20, 58)	20	17
17l	*i*Pr	3-Br	$CONH_2$	55 (38, 80)	18	13
17m	*i*Pr	2-Br	$CONH_2$	31 (20, 48)	21	19
17n	*i*Pr	2,6-Cl_2	$CONH_2$	83 (57, 120)	12	13
17o	*i*Pr	2,3-Cl_2	$CONH_2$	43 (31, 61)	12	12
17p	*i*Pr	2,3-oxadiazolyl	$CONH_2$	82 (57, 119)	28	25
20a (*R*)	*i*Pr	3-NO_2	$CONH_2$	8.5 (7, 11)	–[b]	
20b (*S*)	*i*Pr	3-NO_2	$CONH_2$	3790 (2140, 6730)	5	4
		1 (nifedipine)		2.5 (1.5, 40)	–[b]	
		21 (amlodipine)		2.5 (2, 3.3)	–[b]	

[a] Test dose 135 μmol/kg. [b] See Figures 3 and 4. [c] Percent maximum reduction (±5%) in blood pressure in SHR at 45 μmol/kg po (n = 5).

Scheme II



given orally to SHR. Thus, the results of these studies demonstrated the primary ureido analogue **7** as a new lead compound.

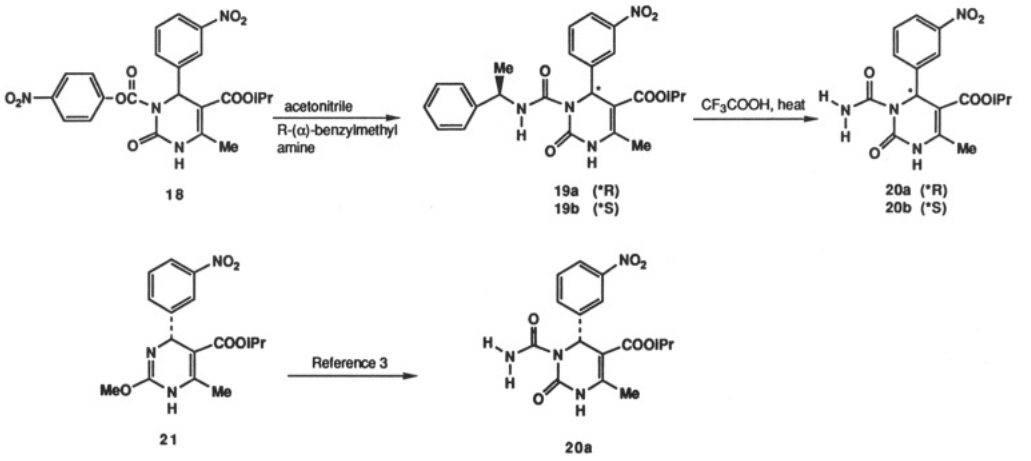
Synthesis

The synthesis of the carbamoyldihydropyrimidines has been described previously.[3] For the compounds described in this study, two methods were used to elaborate 2-methoxydihydropyrimidinone **14**. Method A in Table II employed the reaction of **14** with phosgene to generate an intermediate that was treated in situ with an amine to give **15** (Scheme II). Hydrolysis of **15** in aqueous acid gave the desired products. In method B referred to in Table II, the intermediate *p*-nitrophenyl carbonate **16** was prepared from **14** and, in an additional step, converted to the desired products.

For the synthesis of enantiomers **20a,b** of **7**, 4-nitrophenyl carbamate **18**[3] was treated with (*R*)-α-methylbenzylamine to give diastereomeric ureas **19a** (*RR*) and **19b** (*RS*) (Scheme III). The *RR* diastereomer **19a** was separated (33%) from the mixture by crystallization from isopropyl ether and the *RS* diastereomer **19b** was purified (26%) from the mother liquor by flash chromatography. The absolute stereochemistry assigned to **19a** and **19b** is based on their conversion to **20a** and **20b**, respectively, carried out by heating them in trifluoroacetic acid (Scheme III). The *R* stereochemistry of the active enantiomer **20a** is based on its correlation with the same material derived from the optically active methoxypyrimidine **21** (Scheme III), the absolute stereochemistry of which was established by single crystal X-ray analysis.[1] The enantiomeric purity

(3) Atwal, K. S.; Rovnyak, G. C.; O'Reilly, B. C.; Schwartz, J. *J. Org. Chem.* **1989**, *54*, 5898.

Scheme III



(98 ± 1%) of **20a,b** was determined by ^{1}H NMR integration of the C4 proton signal which shows a baseline separation in **19a** (δ 6.8 ppm) and **19b** (δ 6.7 ppm). It was further confirmed by HPLC analysis of **20a,b** on a Pirkle-type Bakerbond chiral phase [(*R*)-*N*-(3,5-dinitrobenzoyl)phenylglycine] analytical column using hexanes–dioxane (72:28) as the mobile phase.

Structure–Activity Studies

The effect of structural modifications on potency in vitro and antihypertensive activity with a series of carbamoylpyrimidines is summarized in Table I. Similar to our previous observations[1,2] with dihydropyrimidines, potency in vitro was optimized in isopropyl ester **7** (IC_{50} = 12 nM) with both ethyl (**17a**; IC_5 = 39 nM) and methyl (**17b**; IC_{50} = 880 nM) ester analogues being considerably less potent. For the carbamoyl substituent, vasorelaxant potency followed the order benzyl (**17e**) > NH_2 (**7**), Me (**6**), Et (**17c**) > isopropyl (**17d**). The lower potency of **17d** is indictive of a deleterious effect on potency resulting from branching in both the ester and carbamoyl alkyl groups. While isopropyl analogue **17d** showed antihypertensive activity comparable to those of **6** and **7**, **17c,e** were considerably less potent in vivo. (Aminoalkyl)carbamoyl derivative **17f**, analogous to the dihydropyridine nicardipine,[5] demonstrated good potency in vitro (IC_5 = 2 nM) and in vivo.

We also examined structure–activity associated with changing the aromatic substituent of **7**; our observations indicating that alkylureas (e.g., **6**) are converted in vivo to primary urea **7** precluded our interest in making analogues of those compounds.[4] In this study, no clear trends emerged. Although both ortho (**17g,i,k,m**) and meta (**17h,j,l**) substituted analogues showed lower vasorelaxant potency in vitro than **7**, the 2-nitro (**17g**) and 3-chloro (**17j**) analogues possessed antihypertensive activity comparable to that of **7**. With the exception of 2,3-oxadiazolyl derivative **17p**, which was equipotent to **7** as an antihypertensive agent, disubstituted compounds **17n–p** in general were less potent than **7** in vitro as well as in vivo. From these studies, we identified three analogues, **17g** (IC_{50} = 20 nM), **17j** (IC_{50} = 40 nM), and **17p** (IC_{50} = 82 nM), with antihypertensive activity comparable to that of **7**, though these compounds were less potent than **7** (IC_{50} = 12 nM) in vitro.

We prepared the enantiomers of racemate **7** and found biological activity resides in the *R*-(−)-enantiomer **20a** (IC_{50} = 8 nM). The corresponding *S*-(+)-enantiomer **20b** (IC_{50} = 3790 nM) was more than 400-fold less potent in vitro and was devoid of antihypertensive activity. The *R* stereochemistry of **20a** is consistent with our previous findings with dihydropyrimidine carbamates.[1] The optically active dihydropyrimidine **20a** is 3-fold less potent in vitro than the reference dihydropyridines, nifedipine (**1**; IC_{50} = 2.5 nM) and amlodipine (**21**; IC_{50} = 2.5 nM).

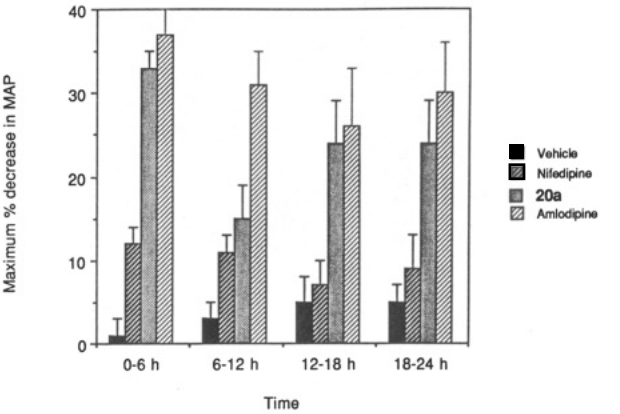


Figure 3. Comparison of antihypertensive effects of **20a**, nifedipine, and amlodipine in the SHR at a dose of 15 μmol/kg (po) (n = 7).

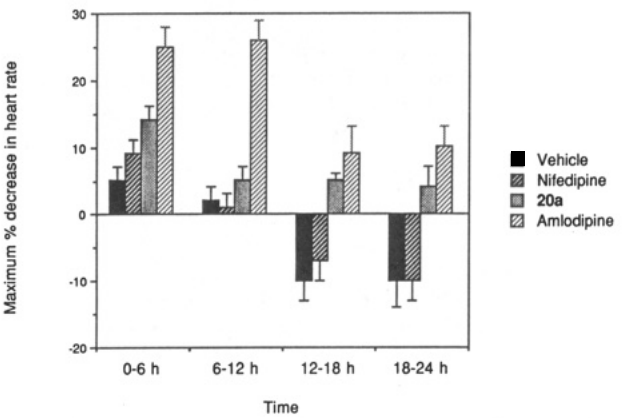


Figure 4. Comparison of heart rates after oral administration of **20a**, nifedipine, and amlodipine in the SHR at a dose of 15 μmol/kg (po) (n = 7).

We compared **20a** with nifedipine (**1**) and amlodipine (**21**) for its effect on blood pressure in the SHR at an oral dose of 15 μmol/kg. The results are summarized in Figures 3 and 4. Our results show that **20a** is more potent than nifedipine as a blood pressure lowering agent and importantly, the effect on blood pressure with **20a** lasts longer

(4) Swanson, B. N.; Weinstein, S. H., unpublished results.
(5) Wynsen, J. C.; Shimsak, T. M.; Preuss, K. C.; Hardman, H. F.; Warltier, D. C. *J. Cardiovas. Pharm.* **1987**, *10*, 37.

Table II. Physical Properties of 3-Carbamoyl-1,4-dihydropyrimidines

no.	mol. form. (microanal.)	mp, °C (solvent[a])	% yield	method
5	$C_{18}H_{22}N_4O_6$(C, H, N)	–[b]	–	A
6	$C_{17}H_{20}N_4O_6$ (C, H, N)	205–206 (A)	79	A
7	$C_{16}H_{18}N_4O_6$ (C, H, N)	–[b]	–	B
10	$C_{15}H_{17}N_3O_5$ (C, H, N)	183–184 (B)	81	–[c]
11	$C_{18}D_6H_{16}N_4O_6$ (C, H, N)	165–166 (C)	43	A
17a	$C_{15}H_{16}N_4O_6$ (C, H, N)	213–215 (C)	33	A
17b	$C_{14}H_{14}N_4O_5$ (C, H, N)	208–210 (D)	63	B
17c	$C_{18}H_{22}N_4O_6$ (C, H, N)	153–155 (C)	52	A
17d	$C_{19}H_{24}N_4O_6$ (C, H, N)	145–146 (C)	69	A
17e	$C_{23}H_{24}N_4O_6$ (C, H, N)	184–185 (E)	63	A
17f	$C_{26}H_{31}N_5O_6$ (C, H, N)	133–135 (E)	54	A
17g	$C_{16}H_{18}N_4O_6$ (C, H, N)	209-211 (F)	78	B
17h	$C_{17}H_{18}F_3N_3O_4$ (C, H, N, F)	203–205 (G)	81	B
17i	$C_{17}H_{18}F_3N_3O_4$ (C, H, N, F)	116–118 (H)	43	B
17j	$C_{16}H_{18}ClN_3O_4$ (C, H, N, Cl)	201–203 (C)	53	B
17k	$C_{16}H_{18}ClN_3O_4$ (C, H, N, Cl)	194–195 (H)	71	B
17l	$C_{16}H_{18}BrN_3O_4$ (C, H, N)	206–208 (H)	88	B
17m	$C_{16}H_{18}BrN_3O_4$ (C, H, N)	108–111 (H)	89	B
17n	$C_{16}H_{17}Cl_2N_3O_4$ (C, H, N)	198–199 (H)	50	B
17o	$C_{16}H_{17}Cl_2N_3O_4$ (C, H, N)	203–205 (H)	90	B
17p	$C_{16}H_{17}N_5O_5$ (C, H, N)	207–208 (I)	28	B
20a	$C_{16}H_{18}N_4O_6$ (C, H, N)	160–161 (C)	–[d]	
20b	$C_{16}H_{18}N_4O_6$ (C, H, N)	160–161 (C)	–[d]	

[a] Solvent for crystallization: A, ethyl acetate–hexanes; B, 2-propanol–isopropyl ether; C, dichloromethane–isopropyl ether; D, methanol; E, ether–isopropyl ether; F, isopropyl ether; G, dichloromethane–isopropyl ether; H, ether; I, chloroform–2-propanol; J, chloroform–isopropyl ether. [b] See ref 3. [c] See ref 8. [d] See the Experimental Section.

than that with nifedipine (Figure 3). Whereas the blood pressure of nifedipine-treated animals returned to the control level within 12 h, the blood pressure of animals treated with **20a** was lower than that of the control group even at the end of the 24-h recording period. Our results also show that **20a** is similar in potency and duration of effect to the long-acting dihydropyridine, amlodipine (**21**) (Figure 3).[6] The decrease in blood pressure by all compounds was associated with an increase in heart rate (Figure 4), presumably reflexogenic in nature, which seemed to normalize within 6 h for **20a** and nifedipine but persisted beyond 12 h for amlodipine.

Conclusion

We have shown that 3-(dimethylcarbamoyl)-1,4-dihydropyrimidine **5** is metabolized in vivo to compounds **6–10**. Whereas the structures of metabolites **6**, **7**, and **10** were confirmed by comparison with authentic samples, the structures of **8** and **9** were confirmed by studying the metabolism of deuterated analogue **11**. Our results demonstrate most of the antihypertensive activity of **5** is due to its metabolism in vivo to the potent calcium channel blocking agents **6** and **7**. We resolved **7** and found that biological activity resides in the *R*-(–)-enantiomer **20a**. Though 3-fold less potent in vitro than nifedipine and amlodipine, **20a** is a long-acting antihypertensive agent and, similar to amlodipine, offers the potential for a single daily dosing regimen for the treatment of hypertension. Additionally, being a single enantiomer, **20a** may demonstrate an improved side-effect profile and possess superior pharmacokinetic properties compared to racemic dihydropyridines.[7]

(6) Arrowsmith, J. E.; Campbell, S. F.; Cross, P. E.; Stubbs, J. K.; Burges, R. A.; Gardiner, D. G.; Blackman, K. J. *J. Med. Chem.* **1986**, *29*, 1696.

(7) Smith, R. L.; Caldwell, J. *Trends Pharm. Sci.* **1988**, *9*, 75 and references therein.

Experimental Chemistry

Chemistry. All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The infrared spectra were recorded with Perkin-Elmer 983 spectrophotometer in KBr pellet. ^{1}H NMR spectra were measured on JOEL-GX-400 and FX-270 spectrometers using Me_4Si as internal standard. Mass spectral analysis was performed by Finnigan TSQ-46 and VG ZAB HF spectrometers. Flash chromatography was run with Whatman LPS-1 silica gel. Spectral data of only key intermediates and final compounds are included. Microanalyses of all crystalline compounds were in agreement with the structures assigned. The details of the methodology for the preparation of carbamoylpyrimidines are described elsewhere.[3]

[1(*R*),6*R*]-1,2,3,6-Tetrahydro-4-methyl-6-(3-nitrophenyl)-2-oxo-1-[[(1-phenylethyl)amino]carbonyl]-5-pyrimidinecarboxylic Acid, 1-Methylethyl Ester (19a), and [1-(*R*),6*S*]-1,2,3,6-Tetrahydro-4-methyl-6-(3-nitrophenyl)-2-oxo-1-[[(1-phenylethyl)amino]carbonyl]-5-pyrimidinecarboxylic Acid, 1-Methylethyl Ester (19b). To a suspension of 3,6-dihydro-4-methyl-6-(3-nitrophenyl)-2-oxo-1,5(2*H*)-pyrimidinedicarboxylic acid 5-(1-methylethyl) 1-(4-nitrophenyl) diester (18;[3] 10.0 g, 20.66 mmol) in acetonitrile (10 mL) was added (*R*)-(+)-α-methylbenzylamine (2.6 g, 21.0 mmol). The reaction, which turned yellow instantaneously, was allowed to stir at room temperature for 15 min. Some solid precipitated out of the reaction. The solvent was evaporated and the residue in ethyl acetate was washed with 5% aqueous sodium carbonate (until colorless washings were obtained) and brine and was dried over anhydrous magnesium sulfate. The solvent was evaporated and the residue was crystallized from isopropyl ether–dichloromethane to yield a colorless solid (4.7 g) which was recrystallized from the same solvent mixture to yield **19a** (3.16 g, 32.7%): mp 186 °C; $[\alpha]_D$ = –116.3° (*c* = 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 9.12 (d, *J* = 6.9 Hz, 1 H), 8.15 (d, *J* = 1.5 Hz, 1 H), 8.10 (d, *J* = 7.9 Hz, 1 H), 7.65 (d, *J* = 7.9 Hz, 1 H), 7.4 (t, *J* = 7.9 Hz, 1 H), 7.25 (m, 5 H), 6.8 (s, 1 H), 5.0 (m, 2 H), 2.4 (s, 3 H), 1.5 (d, *J* = 6.9 Hz, 3 H), 1.3 (d, *J* = 6.3 Hz, 3 H), 1.16 (d, *J* = 6.33 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 163.8, 153.4, 152.0, 148.4, 145.0, 143.35, 143.3, 133.5, 129.5, 128.7, 127.4, 125.8, 123.0, 122.2, 104.3, 68.4, 53.9, 50.8, 22.5, 21.9, 21.7, 17.7; IR (KBr) 1718, 1670, 1643, 1532 cm^{-1}. The mother liquour was allowed to stand at room temperature and the racemic material that crystallized out was removed by filtration. The filtrate was concentrated and recrystallized from ether–hexanes to provide **19b** (2.41 g, 26%): mp 153–155 °C; $[\alpha]_D$ = +246° (*c* = 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 9.16 (d, *J* = 7.2 Hz, 1 H), 8.24 (d, *J* = 1.5 Hz, 1 H), 8.13 (d, *J* = 8.4 Hz, 1 H), 7.94 (s, 1 H), 7.76 (d, *J* = 7.4 Hz, 1 H), 7.5 (t, *J* = 7.9 Hz, 1 H), 7.3 (m, 5 H), 6.7 (s, 1 H), 5.0 (m, 2 H), 2.33 (s, 3 H), 1.5 (d, *J* = 6.9 Hz, 3 H), 1.25 (d, *J* = 6.3 Hz, 3 H), 1.10 (d, *J* = 6.33 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 163.9, 153.4, 152.05, 148.3, 145.35, 143.1, 142.9, 133.2, 129.5, 128.7, 127.4, 125.8, 122.9, 121.9, 104.3, 68.5, 53.6, 50.9, 22.65, 21.95, 21.7, 17.7; IR (KBr) 1716, 1671, 1646, 1533 cm^{-1}.

[6*R*]-1-(Aminocarbonyl)-1,2,3,6-tetrahydro-4-methyl-6-(3-nitrophenyl)-2-oxo-5-pyrimidinecarboxylic Acid, 1-Methylethyl Ester (20a), and [6*S*]-1-(Aminocarbonyl)-1,2,3,6-tetrahydro-4-methyl-6-(3-nitrophenyl)-2-oxo-5-pyrimidinecarboxylic Acid, 1-Methylethyl Ester (20b). A solution of **19a** (3.0 g, 6.43 mmol) in trifluoroacetic acid (10 mL) was heated at 75 °C under argon for 4 h. The solvent was evaporated under reduced pressure, residue was taken up in ethyl acetate and washed with water, saturated sodium bicarbonate solution, and brine. After drying over anhydrous magnesium sulfate, the solvent was evaporated and the residue was crystallized from isopropyl ether–dichloromethane to yield a colorless solid (1.71 g). The mother liquor was concentrated and crystallized from the same solvent to give a second crop (470 mg). The combined product was recrystallized from isopropyl ether–dichloromethane to yield [6*R*]-1-(aminocarbonyl)-1,2,3,6-tetrahydro-4-methyl-6-(3-nitrophenyl)-2-oxo-5-pyrimidinecarboxylic acid, 1-methylethyl ester (**20a**; 1.80 g, 77.3%): mp 160–161 °C; $[\alpha]_D$ = –147.4° (*c* = 1, MeOH): ^{1}H NMR ($CDCl_3$) δ 8.5 (br s, 1 H), 8.25 (br s, 1 H), 8.22 (s, 1 H), 8.10 (d, *J* = 8.4 Hz, 1 H), 7.72 (d, *J* = 7.91 Hz, 1 H), 7.48 (t, *J* = 7.91 Hz, 1 H), 6.72 (s, 1 H), 6.0 (br s, 1 H), 5.05 (m, *J* =

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6.33 Hz, 1 H), 2.44 (s, 3 H), 1.29 (d, J = 5.80 Hz, 3 H), 1.16 (d, J = 6.33 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 163.96, 154.08, 153.0, 148.28, 145.46, 142.95, 133.19, 129.50, 123.00, 122.22, 104.20, 68.49, 53.84, 21.92, 21.73, 17.82; IR (KBr) 1648, 1716 cm^{-1}. With the same procedure, [6*S*]-1-(aminocarbonyl)-1,2,3,6-tetrahydro-4-methyl-6-(3-nitrophenyl)-2-oxo-5-pyrimidinecarboxylic acid, 1-methylethyl ester (**19b**; 1.7 g, 3.65 mmol), was converted to [6*S*]-1-(aminocarbonyl)-1,2,3,6-tetrahydro-4-methyl-6-(3-nitrophenyl)-2-oxo-5-pyrimidinecarboxylic acid, 1-methylethyl ester (**20b**; 870 mg, 72%): mp 160–161 °C; $[\alpha]_D$ = +153° (c = 1, MeOH).

Drug Metabolism Studies. Isolation of Metabolites. Two male Sprague–Dawley rats were given two 60 mg/kg oral doses of **5** (50 mg/mL in PEG-400) 2 h apart. The liver was removed from each rat 1 h after the second dose and homogenized in two volumes of ice-cold, distilled water with a Tekmar Tissumizer. The combined homogenates were mixed with equal volumes of acetonitrile and then centrifuged at 10000g for 20 min at 4 °C. The clear supernatant was diluted with an equal volume of distilled water and passed through Bond Elut C2 columns. Compound **5** and its metabolites were retained on Bond Elut columns under these conditions. These compounds were then eluted with acetonitrile, and eluates were pooled and evaporated to dryness under a stream of nitrogen. A portion of the residue was analyzed by thermospray mass spectrometry. The remainder of the residue was reconstituted in a small volume of acetonitrile and chromatographed on a Whatman ODS 3 (5 μm particle size, 25 cm long × 4.6 mm i.d.) column using a Hewlett-Packard 1090A HPLC, UV detection (Hewlett-Packard diode-array detector) at 273 nm, and a mobile phase consisting of 40% acetonitrile and 60% 0.01 M ammonium acetate (pH 3.0) at a flow rate of 1.0 mL/min. The eluates were monitored at 273 nm, and fractions corresponding to chromatographic peaks were collected up to 45 min. Each HPLC fraction was diluted with an equal volume of distilled water and passed through Bond Elut C2 columns; **5** and its metabolites were eluted with acetonitrile, concentrated under a stream of nitrogen, and subjected to mass spectral analysis by chemical ionization (CI), fast atom bombardment (FAB), mass spectrometry/mass spectrometry (MS/MS), and high-resolution mass spectral analysis.

Oral Absorption. Male Sprague–Dawley rats were each given single, 53 mg/kg oral doses of **5**. The dosing solution was prepared by dissolving **5** in polyethylene glycol 400 (20 mg/mL) and administered by gavage via a 3.5 in. × 20 G blunt needle. Blood samples (0.4 mL/sample) were collected from a Silastic catheter situated in the right external jugular vein for up to 24 h after dosing. Plasma specimens (0.15 mL) were loaded by vacuum onto a Bond Elut C2 column (1 mL capacity, Analytichem) which had been previously washed with 1 mL of acetonitrile and then 1 mL of water. Plasma proteins and other substances endogenous to plasma were eluted and discarded by first washing the column with 1 mL of water and then with 1 mL of 20% acetonitrile in water. Compound **5** and its metabolites were then eluted with 1 mL of acetonitrile. The acetonitrile eluate was evaporated to dryness under nitrogen gas, and the residue was reconstituted in 0.1 mL of acetonitrile. A portion of each reconstituted sample was analyzed by high-performance liquid chromatography (HPLC) using a Whatman ODS 3 (5 μm particle size, 25 cm long × 4.6 mm i.d.) column, UV detection at 273 nm, and a mobile phase consisting of 60% acetonitrile and 40% 0.01 M ammonium acetate (pH 6.0) at a flow rate of 1.0 mL/min. Drug standards were prepared by adding **5** to rat plasma at concentrations ranging between 0.1 and 10 μg/mL. Standard curves were linear over this concentration range. Extraction efficiencies and UV extinction coefficients of the biotransformation products were assumed to be the same as that of **5**.

Pharmacology. Vasorelaxant potency was determined in rabbit thoracic aorta using our previously described protocol.[9] IC_{50} values were determined with a quadratic fit to the logit transformation of the concentration–response curves. For determination of antihypertensive activity, male SHR were prepared surgically according to the method of Weeks and Jones.[10] The test compounds were administered as a suspension in agar at a single oral dose of 45 μmol/kg and blood pressure was recorded by using the method described by Laffin et al.[11] Antihypertensive activity is expressed as percent maximum decrease in blood pressure from 0–6 h and 6–18 h after dosing. These figures give a good estimate of both potency and duration of action of the test compound.

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