Registry No. **5**, 94-52-0; **6**, 70264-94-7; **7a**, 107754-55-2; **7b**, 127973-84-6; **8**, 107754-54-1; **9a**, 107754-53-0; **9b**, 107786-75-4; **10a**, 107754-56-3; **10b**, 107786-76-5; **11a**, 107786-78-7; **11b**, 107786-77-6; **13a**, 107786-81-2; **13b**, 127973-85-7; **13c**, 127973-86-8; **14**, 107786-80-1; **15**, 107753-88-8; **16**, 107753-89-9; **17**, 107753-90-2; **18**, 6361-21-3; **19**, 20699-86-9; **20**, 4965-26-8; **21a**, 107754-50-7; **22**, 107754-49-4; **23a**, 107786-72-1; **23b**, 127973-87-9; **24a**, 107786-73-2; **24b**, 127973-88-0; **25a**, 107786-74-3; **25b**, 127973-89-1; **25c**, 127973-90-4; **26**, 19727-83-4; **27**, 127973-91-5; **28**, 104447-63-4; **29**, 104447-64-5; **30**, 104448-19-3; **31**, 104437-13-0; **32**, 28226-22-4; **33**, 107754-42-7; **34**, 107754-41-6; **35a**, 107754-44-9; **35b**, 107786-69-6; **36a**, 107754-45-0; **36b**, 107786-70-9; **37a**, 107754-48-3; **37b**, 107786-71-0; **37c**, 107786-89-0; **39**, 107786-92-5; **40**, 127973-92-6; **41**, 127973-93-7; **42**, 127973-94-8; **45**, 127973-95-9; **46**, 127973-96-0; **47**, 127973-97-1; **48**, 127973-98-2; cyclopentyl chloroformate, 50715-28-1; cyclopentylacetic acid, 1123-00-8; 5-nitrobenzotriazole, 2338-12-7; methyl thioglycolate, 2365-48-2; 3-aminophenol, 591-27-5.

Dihydropyrimidine Calcium Channel Blockers. 2.[1] 3-Substituted-4-aryl-1,4-dihydro-6-methyl-5-pyrimidinecarboxylic Acid Esters as Potent Mimics of Dihydropyridines

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To enhance the intrinsic potency of dihydropyrimidine calcium channel blockers, we have modified the structure of previously described 2-heteroalkyl-1,4-dihydropyrimidines **2** to 3-substituted 1,4-dihydropyrimidines **3**. Structure–activity studies using potassium-depolarized rabbit aorta show that ortho,meta-disubstituted aryl derivatives are more potent than either ortho- or meta-monosubstituted compounds. While vasorelaxant activity was critically dependent on the size of the C5 ester group, isopropyl ester being the best, a variety of substituents (carbamate, acyl, sulfonyl, alkyl) were tolerated at N3. Our results show dihydropyrimidines **3** are significantly more potent than corresponding 2-heteroalkyl-1,4-dihydropyrimidines **2** and only slightly less potent than similarly substituted 2-heteroalkyl-1,4-dihydropyridines **4** and **5**. Whereas dihydropyridine enantiomers usually show 10–15-fold difference in activity, the enantiomers of dihydropyrimidine **3j** show more than a 1000-fold difference in activity. These results strengthen the requirement of an enamino ester for binding to the dihydropyridine receptor and indicate a nonspecific role for the N3-substituent.

Introduction

Calcium channel blocking agents are widely used in the management of angina pectoris and hypertension.[2] Within this class of cardiovascular agents, the dihydropyridines (e.g., nitrendipine, **1**) have found widespread use in the clinic and have served as important tools for the study of calcium channel structure and function.[3-5] Although the effects on potency resulting from modifications of every substituent on the dihydropyridine ring have been reported,[6] efforts involving the modification of a dihydropyridine ring have been limited.[7] Metabolic oxidation to form the inactive pyridine derivative frequently results in short duration of action of these drugs.

We have reported that 2-heteroalkyl-1,4-dihydropyrimidines **2** mimic the biological effects of dihydropyridines.[1] Although some analogues of **2** show potent vasorelaxant activity in vitro, these compounds generally demonstrate lower affinity for the dihydropyridine receptor than similarly substituted 2-heteroalkyl-1,4-dihydropyridines **4** and **5**. To enhance the potency of 2-heteroalkyl-1,4-dihydropyrimidines **2**, we have further modified their structure to 3-substituted 1,4-dihydropyrimidines **3**. In this publication we demonstrate that this modification results in an increase in calcium channel blocking potency relative to 2-hetero-1,4-dihydropyrimidines **2**.

When the esters at C3/C5 and the alkyl groups at C2/C6 are equivalent, dihydropyridines are C_s symmetric. Nonidentical esters and/or C2/C6 substituents impart chirality to the molecule and, generally, result in 5–100-fold dif-

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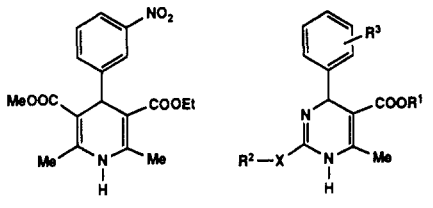
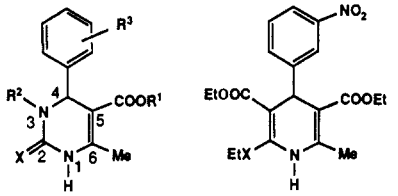


1 Nitrendipine 2



3

4 X = S
5 X = O

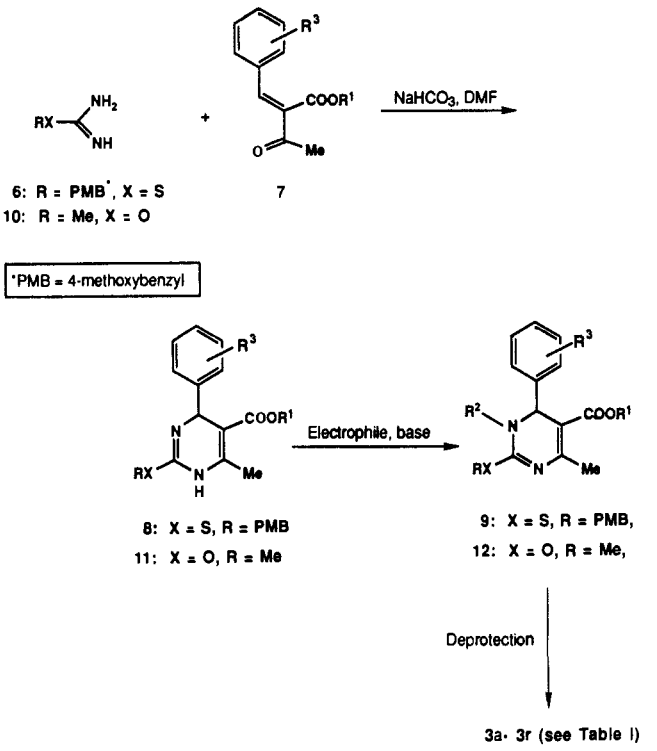
ference in potency of two enantiomers.[8,9] Being inherently asymmetric, dihydropyrimidines **3** provide an additional opportunity to study the effect of chirality on biological activity. Accordingly, we resolved one of the most potent dihydropyrimidine analogues (**3j**) into its enantiomers. Their biological activity and its implication to the structural requirement for binding to the dihydropyridine receptor are also described in this paper.

Chemistry

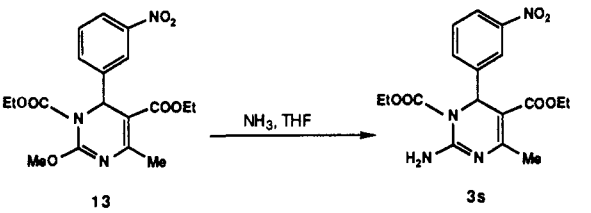
The details of the methodology for the preparation of 1,4-dihydropyrimidines **3** (X = S, O, and NH_2) have been described.[10] The analogues prepared are listed in Table I along with their biological activity. The synthesis of dihydropyrimidinethiones **3** (X = S) begins with the condensation of 2-(4-methoxybenzyl)-2-thiopseudourea (**6**) with α-benzylidene-β-keto ester **7** to give 2-(4-methoxybenzyl)-1,4-dihydropyrimidine **8**. Treatment of **8** with electrophiles provided **9**, which, on deprotection with trifluoroacetic acid/ethanethiol, gave **3** (X = S) in 41–72% overall yield (Scheme I). The process for the preparation of 1,4-dihydropyrimidinones **3** (X = O) is very similar to that of pyrimidinethiones **3** (X = S) except that 2-(4-methoxybenzyl)-2-thiopseudourea (**6**) was replaced with *O*-methylisourea (**10**). The deprotection of the functionalized intermediate **12** was accomplished by treatment with aqueous acid. This methodology avoids the troublesome functionalization of unsubstituted pyrimidines **3** (R^2 = H), the outcome of which depends on the nature and position of the aryl substituent.[10] Aminopyrimidine **3s** was obtained by exchange of ammonia for the methoxy group in **13** (Scheme II).

The preparation of enantiomers **19a** and **19b** of **3j** is outlined in Scheme III. The previously described 2-methoxy-1,4-dihydropyrimidine **14**[10] was converted to the diastereomeric ureas **16a** and **16b** by a two-step procedure involving treatment of **14** with 4-nitrophenyl chloroformate followed by reaction of the resulting intermediate **15** with (*R*)-(−)-α-methylbenzylamine. The diastereomers **16a** and **16b** were separated by crystallization, and the urea was cleaved by treatment with DBU to yield enantiomers **17a** and **17b** of 2-methoxy-1,4-dihydropyrimidine **14**. For the conversion of **17a**/**17b** into **19a**/**19b**, the 2-methoxy group

Scheme I



Scheme II



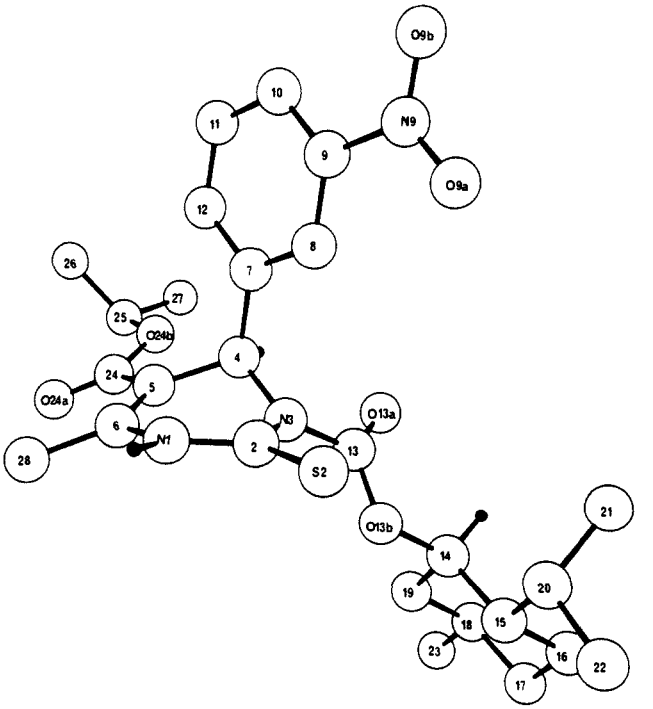
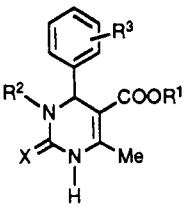


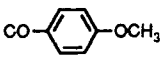
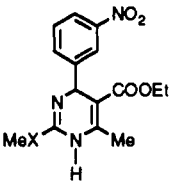
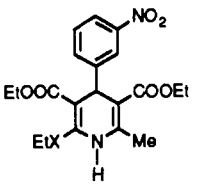
Figure 1. The solid-state structure of (*R*)-3,6-dihydro-4-methyl-6-(3-nitrophenyl)-2-thioxo-1,5(2*H*)-pyrimidinedicarboxylic acid, 5-(1-methylethyl) 1-[[1*S*-[1α,2α,3β]]-5-methyl-2-(1-methylethyl)cyclohexyl] diester (**20a**).

was exchanged by 4-methoxybenzyl mercaptan and the resulting dihydropyrimidines **18a**/**18b** were treated se-

(8) Tamazawa, T.; Arima, H.; Kojima, T.; Isomura, Y.; Okada, M.; Fujita, S.; Furuya, T.; Takenata, T.; Inagaki, O.; Terai, M. *J. Med. Chem.* **1986**, *29*, 2504.
(9) Twart, R.; Wehinger, E.; Meyer, H. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1981**, *317*, 183.
(10) Atwal, K. S.; Rovnyak, G. C.; O'Reilly, B. C.; Schwartz, J. *J. Org. Chem.* **1989**, *54*, 5898.

Table I. Calcium Channel Blocking Activity of 1,4-Dihydropyrimidines and 1,4-Dihydropyridines



no.	X	R^1	R^2	R^3	IC_{50}, nM (95% C.I.)
3a	S	Et	COOEt	3-NO_2	17 (16, 19.6)
3b	S	Et	COOEt	2-NO_2	3.1 (1.9, 5.1)
3c	S	Et	COOEt	3-Cl	88 (39, 200)
3d	S	Et	COOEt	2-Cl	17 (9.2, 32)
3e	S	Et	COOEt	3-CF_3	280 (170, 440)
3f	S	Et	COOEt	2-CF_3	26 (11, 62)
3g	S	Et	COOEt	2,3-Cl	1.5 (0.62, 3.6)
3h	S	Et	COOEt	2-Cl, 3-NO_2	2.7 (1.1, 6.4)
3i	S	Me	COOEt	3-NO_2	100 (56, 200)
3j	S	iPr	COOEt	3-NO_2	1.7 (0.85, 3.4)
3k	S	Et	COOMe	3-NO_2	3.6 (2.7, 4.9)
3l	S	Et	COOiPr	3-NO_2	14 (9.3, 21)
3m	S	Et		3-NO_2	11 (5.2, 24)
3n	S	Et	$CONMe_2$	3-NO_2	270 (140, 520)
3o	S	Et	SO_2Ph	3-NO_2	5.2 (3.4, 8.0)
3p	S	Et	$CH_2CH_2CH_3$	3-NO_2	50 (28, 91)
3q	O	Et	COOEt	3-NO_2	140 (60, 300)
3r	O	iPr	COOEt	3-NO_2	2.6 (1.2, 5.3)
3s	NH	Et	COOEt	3-NO_2	160 (72, 360)
19a (*R*)	S	iPr	COOEt	3-NO_2	2.0 (1.3, 3.2)
19b (*S*)	S	iPr	COOEt	3-NO_2	1500 (960, 2400)
21a	S	Et	H	3-NO_2	25% relax. (1 μM)
21b	O	Et	H	3-NO_2	1% relax. (1 μM)
					
2a	S				130 (58, 280)
2b	O				710 (240, 2100)
					
4	S				4 (2, 7)
5	O				26 (13, 53)
1 (nitrendipine)					1 (0.2, 3.0)

quentially with ethyl chloroformate/pyridine and trifluoroacetic acid/ethanethiol.[10]

The enantiomeric purity of **19a** and **19b** was determined by ^{1}H NMR analysis of the diastereomeric ureas **16a** and **16b**, respectively. The signals due to C4-H in **16a** (δ 6.6 ppm) and **16b** (δ 6.7 ppm) show baseline separation and could be easily integrated. The enantiomeric purity (**19a**, 95 ± 2%; **19b**, 97 ± 2%) was further confirmed by integration of the ^{1}H NMR signal due to C4 proton of menthyl carbamates **20a** (δ 6.4 ppm) and **20b** (δ 6.3 ppm), which were readily prepared from dihydropyrimidines **18a** and **18b**, respectively, by standard methodology.[10] The absolute stereochemistry of **19a** was proven by single-crystal X-ray analysis (Figure 1) of menthyl carbamate **20a**.

Biological Results and Discussion

The IC_{50} values for vasorelaxant activity shown in Table I were determined with potassium-depolarized rabbit thoracic aorta and has been described previously.[11] In agreement with previous investigators,[12] we have shown that there is a good correlation between the IC_{50} values for relaxation of rabbit aorta and the K_d values for displacement of ^{3}[H]nitrendipine.[1]

For an initial evaluation of dihydropyrimidines, we decided to look at pyrimidinethiones **3** (X = S); the choice of the thiono group was based on the similarity of sulfur to the carbon of alkyl groups at C2/C6 of dihydropyridines.[13] The carbamate group was chosen as an

(11) Atwal, K. S.; Bergey, J. L.; Hedberg, A.; Moreland, S. *J. Med. Chem.* **1987**, *30*, 635.

(12) Rovnyak, G.; Anderson, N.; Gougoutas, J.; Hedberg, A.; Kimball, S. D.; Malley, M.; Moreland, S.; Porubcan, M.; Pudzianowski, A. *J. Med. Chem.* **1988**, *31*, 936.

Scheme III

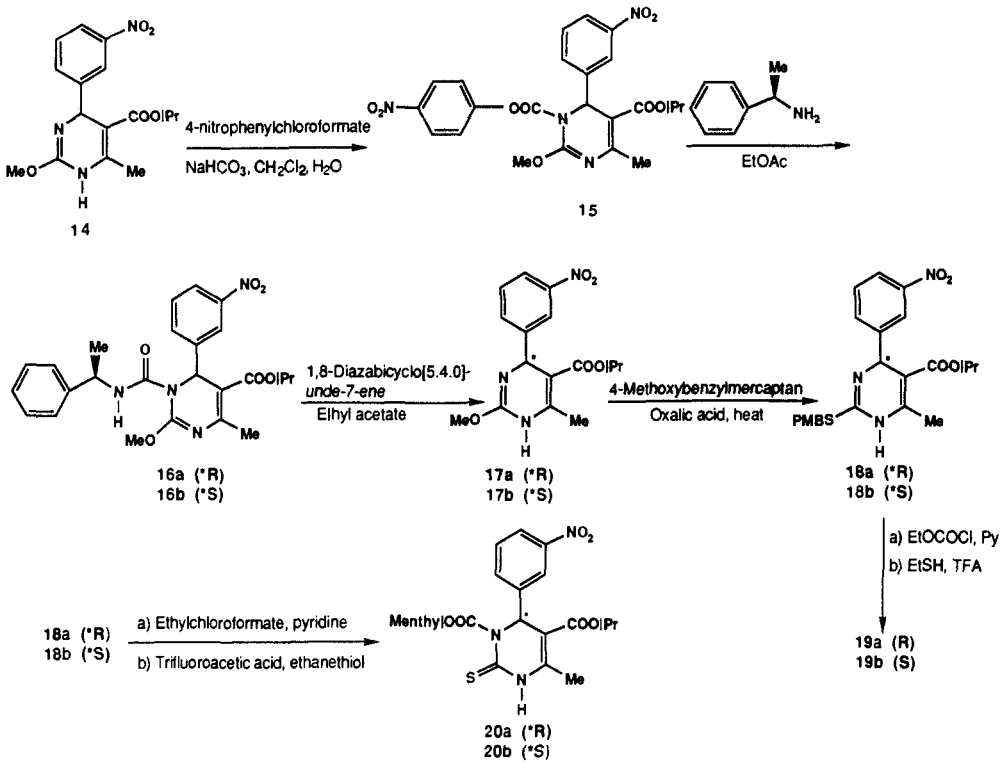


Table II. Physical Properties of 1,4-Dihydropyrimidines

no.	mol formula (microanalysis)	mp, °C (solvent)	% yield
3a	$C_{17}H_{19}N_3O_6S$ (C,H,N,S)	a	a
3b	$C_{17}H_{19}N_3O_6S$ (C,H,N,S)	130–132 (C)	71
3c	$C_{17}H_{19}N_2ClO_4S$ (C,H,N,Cl,S)	95–97 (A)	60
3d	$C_{17}H_{19}N_2ClO_4S$ (C,H,N,Cl,S)	126–128 (D)	42
3e	$C_{18}H_{19}N_2F_3O_4S$ (C,H,N,F,S)	98–100 (B)	62
3f	$C_{18}H_{19}N_2F_3O_4S$ (S C,H,N,F,S)	a	a
3g	$C_{17}H_{18}N_2Cl_2O_4S$ (S C,H,N,Cl,S)	155–157 (A)	75
3h	$C_{17}H_{18}N_3ClO_6S$ (S C,H,N,Cl,S)	136–138 (A)	56
3i	$C_{16}H_{17}N_3O_6S$ (C,H,N,S)	163–165 (F)	67
3j	$C_{18}H_{21}N_3O_6S$ (C,H,N,S)	163.5–165 (B)	81
3k	$C_{16}H_{17}N_3O_6S$ (C,H,N,S)	130.5–131 (B)	70
3l	$C_{18}H_{21}N_3O_6S$ (C,H,N,S)	150–152 (A)	70
3m	$C_{22}H_{21}N_3O_6S$ (C,H,N,S)	130–132 (A)	62
3n	$C_{17}H_{20}N_4O_5S$ (C,H,N,S)	a	a
3o	$C_{20}H_{19}N_3O_6S_2$ (C,H,N,S)	162–164 (A)	48
3p	$C_{17}H_{21}N_3O_4S$ (C,H,N,S)	a	a
3q	$C_{17}H_{19}N_3O_7$ (C,H,N,S)	a	a
3r	$C_{18}H_{21}N_3O_7$ (C,H,N,S)	156–157 (E)	48
3s	$C_{17}H_{20}N_4O_6$ (C,H,N,S)	a	a
19b (R)	$C_{18}H_{21}N_3O_6S$ (C,H,N,S)	120–122 (B)	b
19b (S)	$C_{18}H_{21}N_3O_6S$ (C,H,N,S)	120–121 (B)	b

[a] See ref 10. [b] See the Experimental Section: A, isopropyl ether; B, ethyl acetate/hexanes, C, isopropyl ether/hexanes; D, dichloromethane/ethyl acetate; E, dichloromethane/isopropyl ether; F, acetone/isopropyl ether, G, 2-propanol.

isosteric replacement for one of the C3/C5 esters. Dihydropyrimidinethione **3a** (IC_{50} = 17 nM) was considerably less potent than the reference dihydropyridine nitrendipine (IC_{50} = 1 nM) in vitro. In general we found that effects of aromatic substitution on potency in vitro was ortho,-meta-disubstituted (**3g**, **3h**) > ortho-substituted (**3b**, **3d**, **3f**) > meta-substituted (**3a**, **3c**, **3e**). Among individual substituents at the ortho or meta position, the nitro derivatives (**3a**, **3b**) were more potent than either the chloro (**3c**, **3d**) or the trifluoromethyl (**3e**, **3f**) analogues.

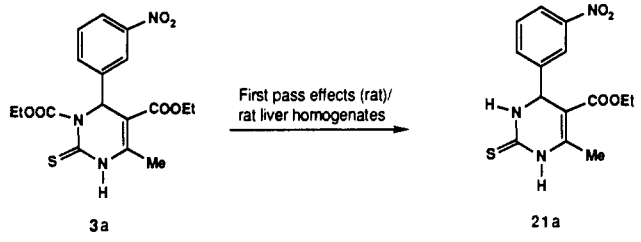
As described previously[1] for 2-hetero-1,4-dihydropyrimidines **2**, the ester group of N3-substituted derivatives **3** was found to be the most critical area for optimization of potency. The isopropyl ester **3j** (IC_{50} = 1.7 nM) was 10-fold more potent than the ethyl ester **3a** (IC_{50} = 17 nM) and 60-fold more potent than the methyl ester **3i** (IC_{50} = 100 nM). As shown by comparison of **3a**, **3k**, and **3l**, the effect of size of the carbamate group was less pronounced. When compared directly with 2-oxo (**3q**) and 2-amino (**3s**) dihydropyrimidines, 2-thiono compound **3a** was nearly 1 order of magnitude more potent. However, changing ethyl (**3q**) to an isopropyl ester (**3r**) dramatically increased the potency of the oxo compound.

Examination of compounds **3m–p** demonstrates that acyl (**3m**) and sulfonate (**3o**) groups are tolerated at N3. In view of these results, we suggest that the N3-substituent may exert a nonspecific effect and does not, itself, directly participate in receptor binding. Nevertheless, the importance of the N3-substituent cannot be underestimated, as the corresponding unsubstituted analogues **21a** and **21b** are devoid of activity.

When the C3 and C5 esters of dihydropyridine calcium channel blockers are ethyl and methyl, the individual enantiomers usually show a small difference (5–10-fold) in activity.[9] However, in the case where dihydropyridine esters are methyl and isopropyl, a 100-fold difference in potency has been observed between the enantiomers.[9] Since dihydropyrimidines **3** are inherently asymmetric, we resolved one of the most potent analogues **3j**. The enantiomers **19a** and **19b** showed more than a 1000-fold difference in vasorelaxant potency. The absolute stereochemistry (*R*) of the more potent enantiomer **19a** corresponds to the reported *S* stereochemistry of the more potent enantiomer **22** of dihydropyridine with methyl and isopropyl esters.[9] These results are consistent with the requirement of an enamino ester of dihydropyrimidines, e.g., **19a** (N1–C6–C5–C4–C7) and of dihydropyridines such

(13) For a discussion on bioisosterism in drug design, see, for example: Thornber, C. W. *Chem. Soc. Rev.* **1979**, *8*, 563 and references therein.

Scheme IV



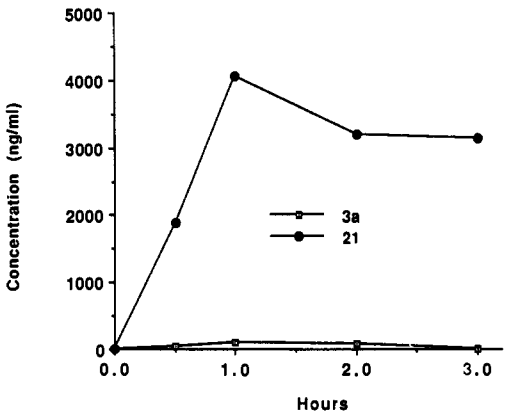
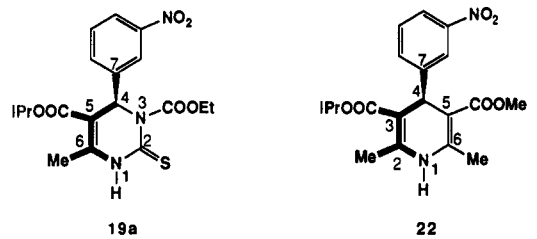


Figure 2. Mean concentrations of **3a** and its metabolite **21** in rat plasma after 53 mg/kg oral dose of **3a** (n = 3).

as **22** (N1–C2–C3–C4–C7) for binding to the receptor, as indicated by heavy lines in the formulae **19a** and **22**.

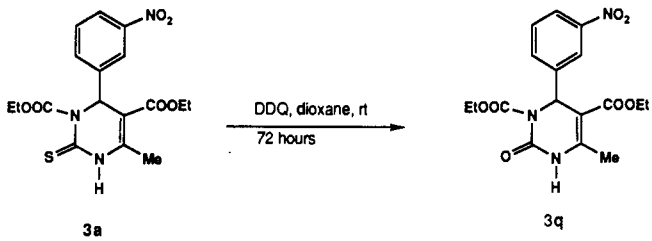


As shown by the comparison of **3a** with **2a** and **3q** with **2b**, 3-substituted dihydropyrimidines **3** show 5–7-fold more potent vasorelaxation than previously described[1] 2-heteroalkyl-1,4-dihydropyrimidines **2**. To separate the effects due to the heteroatom substitution from those due to the pyrimidine nitrogen, dihydropyrimidines **3** were compared with 2-heteroalkyl-1,4-dihydropyridines **4** and **5**.[1] As shown in Table I, the dihydropyridines **4** and **5** are 3–5-fold more potent in vitro than analogous dihydropyrimidines **3a** and **3q**, respectively.

These results show that there is some loss of activity on substitution of the trigonal nitrogen for an sp^2 carbon of dihydropyridines (**3a** vs **1** and **4**). However, this loss can be recovered by modification of the ester group or by changing the aromatic substitution. In fact, some of the dihydropyrimidines (e.g., **3g**, **3j**, **3r**) are as potent as the reference agent nitrendipine (**1**).

Although potent calcium channel blocking agents in vitro, dihydropyrimidines **3** are devoid of antihypertensive activity when administered orally to spontaneously hypertensive rats. The lack of oral activity in some of them appears to be related to their rapid metabolism. Drug metabolism studies in rats show that carbamate **3a** suffers first-pass effects to yield the biologically inactive dihydropyrimidine **21a** (Scheme IV). As shown in Figure 2, peak plasma concentration of **3a** averaged only 50 ng/mL 1 h after dosing. The peak concentration of its major metabolite **21a**, which eluted faster than **3a** in reverse-phase chromatography systems, averaged 4000 ng/mL, also 1 h after dosing. Although significant levels of **21a** were maintained in the plasma, the concentration of **3a** was too low to be detected after 2 h (Figure 2). From these studies we conclude plasma levels of **3a** were too low to express biological activity in vivo.

Scheme V



When incubated with rat liver homogenates containing the soluble enzyme fraction (100000g supernatant), dihydropyrimidine **3a** underwent deacylation to give **21a**. After incubation for 1 h, 60% of **3a** was converted to **21a**. These results show that dihydropyrimidine **3a** is metabolically labile and its deacylation takes place in the liver. Interestingly, isopropyl carbamate **3l** and pyrimidinone **3q** analogues were more stable to rat liver homogenates (less than 5% deacylation after 1 h) but failed to express activity in vivo. Dihydropyrimidines with N3-substituents other than carbamates (e.g., sulfonyl, **3o**; alkyl, **3p**) also lacked oral antihypertensive activity. The reasons for their lack of oral activity are not clear at the present time.

Dihydropyridine calcium channel blockers suffer rapid first-pass effects due to their oxidation into the inactive pyridines which parallels their chemical oxidation with oxidizing agents such as dichlorodicyanoquinone (DDQ). We carried out simple experiments to compare qualitatively the oxidation potential of dihydropyrimidines and dihydropyridines. When dihydropyrimidine carbamates **3a** and **3q** were subjected to reaction with DDQ in dioxane, we observed no oxidation of the dihydropyrimidine ring. Only slow oxidation of pyrimidinethione **3a** into pyrimidinone **3q** was observed (Scheme V). Nitrendipine, on the other hand, underwent rapid oxidation with DDQ to yield the corresponding pyridine derivative. These results support previous findings that dihydropyrimidines have a higher oxidation potential than dihydropyridines.[14] Further, major metabolites isolated after oral administration of **3a** to rats contained the intact dihydropyrimidine ring. Therefore, substitution of trigonal nitrogen for an sp^2 carbon (dihydropyrimidine vs dihydropyridines) appears to prevent, as far as we can detect, both chemical and biological oxidation to inactive aromatic products.

Conclusion

Our results show that 3-substituted 1,4-dihydropyrimidines **3** are potent calcium channel blocking agents in vitro. These compounds are significantly more potent than previously described 2-hetero-1,4-dihydropyrimidines **2**. Comparison of **3k** (IC_{50} = 3.6 nM) with nitrendipine (IC_{50} = 1 nM) shows that dihydropyrimidine derivatives described herein are only slightly less potent than classical dihydropyridine calcium channel blockers. As reported for 2-hetero-1,4-dihydropyrimidines **2**, the calcium channel blocking activity of dihydropyrimidines **3** is most sensitive to the size of the alkyl ester, the isopropyl ester being preferred. This result, combined with the observed

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1000-fold difference in the biological activity of enantiomers (**19a**, **19b**) of dihydropyridine **3j**, strongly suggests that an enamino ester is required for binding to the dihydropyridine receptor. Although required for maximal potency, the structural requirements for N3-substituent are more flexible and a variety of groups are tolerated at this position. The dihydropyrimidines described herein lacked oral activity. The metabolic instability of some of them could partly account for their lack of activity in vivo.

Experimental Section

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. 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.

2-Methoxy-4-methyl-6-(3-nitrophenyl)-1,5(6*H*)-pyrimidinedicarboxylic Acid, 5-(1-Methylethyl) 1-(4-Nitrophenyl) Diester (15). A solution of 1,4-dihydro-2-methoxy-6-methyl-4-(3-nitrophenyl)-5-pyrimidine carboxylic acid 1-methylethyl ester (**14**)[10] (1.0 g, 3.0 mmol) in dichloromethane (5.0 mL) and water (5.0 mL) was treated with sodium bicarbonate (1.0 g, 12.0 mmol) and 4-nitrophenyl chloroformate (690 mg, 3.3 mmol). After stirring of the two-phase reaction at room temperature for 6 h more, 4-nitrophenyl chloroformate (200 mg, 0.96 mmol) was added and the reaction was stirred for 10 h more. The layers were separated, and the aqueous layer was reextracted with dichloromethane. The combined organic extracts were washed with 5% sodium carbonate, water, and brine. The dried (anhydrous magnesium sulfate) solution was evaporated under reduced pressure and the residue was triturated with isopropyl ether to give a colorless solid **15** (1.42 g, 95%): mp 160–162 °C; ^{1}H NMR ($CDCl_3$) δ 8.3 (d, J = 9.5 Hz, 2 H), 8.2 (s, 1 H), 8.17 (d, J = 7.3 Hz, 1 H), 7.67 (d, J = 7.9 Hz, 1 H), 7.52 (t, J = 7.9 Hz, 1 H), 7.38 (d, J = 9.0 Hz, 2 H), 6.35 (s, 1 H), 5.07 (m, 1 H), 4.0 (s, 3 H), 1.28, 1.16 (d, J = 7.3 Hz, 3 H each).

[1(*R*)]-1,6-Dihydro-2-methoxy-4-methyl-6(*R)-(4-nitrophenyl)-1-[[(1-phenylethyl)amino]carbonyl]-5-pyrimidinecarboxylic Acid, 1-Methylethyl Ester (16a) and [1(*R*)]-1,6-Dihydro-2-methoxy-4-methyl-6(*S**)-(4-nitrophenyl)-1-[[(1-phenylethyl)amino]carbonyl]-5-pyrimidinecarboxylic Acid, 1-Methylethyl Ester (16b).** A solution of pyrimidine **15** (25.0 g, 0.05 mol) in ethyl acetate (125 mL) was heated to flux temperature to effect solution and then cooled to room temperature, resulting in a fine precipitate. To this mixture was added (*R*)-(+)-α-methylbenzylamine (6.0 g, 0.05 mol). The yellow mixture, stirred for 16 h while slowly becoming homogeneous, was washed with 10% potassium dihydrogen phosphate, 1 N sodium hydroxide, water, and brine. The dried (anhydrous magnesium sulfate) organic fraction was concentrated to give a mixture of diastereomers **16a** and **16b** (24.8 g) (TLC, silica gel, toluene/ethyl acetate, 10:3, R_f = 0.50, 0.55). A portion of this mixture (10 g) was flash chromatographed on 1500 mL of LPS-1 silica gel and eluted with toluene/ethyl acetate (20:1). The faster moving isomer (FMI; 3.2 g) was crystallized from isopropyl ether to give **16a** (2.6 g, 54%): mp 105–107 °C; ^{1}H NMR ($CDCl_3$) δ 8.16 (s, 1 H), 8.10 (d, J = 7.7 Hz, 1 H), 7.68 (d, J = 8.0 Hz, 1 H), 7.45 (dd, J = 8.0, 8.0 Hz, 1 H), 7.22–7.40 (m, 5 H), 6.88 (d, J = 7.0 Hz, 1 H), 6.60 (s, 1 H), 4.92–5.06 (m, 2 H), 4.01 (s, 3 H), 2.41 (s, 3 H), 1.51 (d, J = 7.0 Hz, 3 H), 1.22 (d, J = 6.2 Hz, 3 H), 1.07 (d, J = 6.2 Hz, 3 H); $[\alpha]_D$ = +330.5° (c = 1, $CHCl_3$). The slower moving isomer (SMI) was rechromatographed to give 2.3 g (47%) of homogeneous product **16b** as an oil: ^{1}H NMR ($CDCl_3$) δ 8.09 (d, J = 1.1 Hz, 1 H), 8.07 (d, J = 8.0 Hz, 1 H), 7.60 (d, J = 7.7 Hz, 1 H), 7.40 (dd, J = 8.0, 8.0 Hz, 1 H), 7.20–7.38 (m, 5 H), 6.87 (d, J = 6.9 Hz, 1 H), 6.70 (s, 1 H), 4.95–5.10 (m, 2 H), 3.97 (s, 3 H), 2.44 (s, 3 H), 1.52 (d, J = 7.0 Hz, 3 H), 1.26 (d, J = 6.2 Hz, 3 H), 1.11 (d, J = 6.2 Hz, 3 H); $[\alpha]_D$ = −146.7° (c = 1, $CHCl_3$).

(4*R*)-1,4-Dihydro-2-methoxy-6-methyl-4-(3-nitrophenyl)-5-pyrimidinecarboxylic Acid, 1-Methylethyl Ester (17a). A solution of **16a** (6.2 g, 0.013 mol) in 100 mL of dichloromethane was treated with diazabicycloundecene (1.96 g, 0.013 mol) and heated at reflux temperature for 4 h. Volatiles were stripped in vacuo and the residue was flash chromatographed on 500 mL of LPS-1 silica gel, eluting with ethyl acetate/hexane (1:2) to give the product as a viscous oil (3.46 g, 81%): ^{1}H NMR ($CDCl_3$) δ 8.03–8.23 (m, 2 H), 7.68 (d, J = 8.0 Hz, 1 H), 7.45 (dd, J = 8.0, 8.0 Hz, 1 H), 6.04, 5.57 (s, d, J = 3.0 Hz, 1 H), 5.70, 5.34 (s, d, J = 3.0 Hz, 1 H), 4.90–5.07 (m, 2 H), 3.73, 3.87 (2 s, 3 H), 2.34, 2.40 (2 s, 3 H), 1.23 (d, J = 6.2 Hz, 3 H), 1.08 (d, J = 6.2 Hz, 3 H); some resonances doubled because of pyrimidine tautomers; $[\alpha]_D$ = +42.5° (c = 2.2, $CHCl_3$); this value is variable because of the existence of both 1,4- and 3,4-dihydropyrimidine tautomers in solution.

(4*S*)-1,4-Dihydro-2-methoxy-6-methyl-4-(3-nitrophenyl)-5-pyrimidinecarboxylic Acid 1-Methylethyl Ester (17b). Using the procedure described for **17a**, **16b** (1.58 g, 0.032 mol) was converted to **17b** (0.61 g, 56%): $[\alpha]_D$ = −33.5° (c = 1.82, $CHCl_3$); this value is variable because of the existence of both 1,4- and 3,4-dihydropyrimidine tautomers in solution.

(4*R*)-1,4-Dihydro-2-[[(4-methoxyphenyl)methyl]thio]-6-methyl-4-(3-nitrophenyl)-5-pyrimidinecarboxylic Acid, 1-Methylethyl Ester (18a). A mixture of **17a** (3.3 g, 0.01 mol) and p-methoxy-α-toluenethiol (4.6 g, 0.03 mol) was treated with citric acid (0.1 g, 0.5 mmol) and heated in vacuo at 95 °C for 16 h (gentle reflux). The reaction mixture, dissolved in ethyl acetate, was washed with saturated sodium bicarbonate, water, and brine, dried (anhydrous magnesium sulfate), and concentrated to give an oil (6.6 g). Flash chromatography on 800 mL of LPS-1 silica gel and elution with toluene/ethyl acetate (10:1) afforded 3.08 g (68%) of **18a**: ^{1}H NMR ($CDCl_3$) δ 8.13 (s, 1 H), 8.08 (d, J = 8.2 Hz, 1 H), 7.61 (d, J = 7.7 Hz, 1 H), 7.42 (dd, J = 7.6, 7.2 Hz, 1 H), 7.13 (d, J = 8.8 Hz, 2 H), 6.69 (d, J = 8.8 Hz, 2 H), 6.21 (s, 1 H), 5.82 (s, 1 H), 5.00 (m, 1 H), 4.27 (d, J = 13.5 Hz, 1 H), 4.07 (d, J = 13.5 Hz, 1 H), 3.75 (s, 3 H), 2.31 (s, 3 H), 1.24 (d, J = 6.5 Hz, 3 H), 1.11 (d, J = 6.5 Hz, 3 H); $[\alpha]_D$ = −127.4° (c = 0.99, MeOH).

With use of the procedure described for **18a**, **18b** was prepared from **17b**.

3,6-Dihydro-4-methyl-6-(3-nitrophenyl)-2-thioxo-1,5-(2*H*)-pyrimidinecarboxylic Acid, 1-Ethyl 5-(1-Methylethyl) Diester (19a/19b). With use of the previously described procedure,[10] **18a/18b** was converted to **19a/19b**. **19a**: mp 120–122 °C (ethyl acetate/hexanes); $[\alpha]_D$ = −57.7° (c = 1.24, $CDCl_3$); IR (KBr) 1701, 1231, 1089 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.2 (m, 2 H), 8.12 (d, J = 8 Hz, 1 H), 7.75 (d, J = 8 Hz, 1 H), 7.5 (t, J = 8 Hz, 1 H), 6.37 (s, 1 H), 5.1 (pent, H = 7 Hz, 1 H), 4.35 (m, 2 H), 2.35 (s, 3 H), 1.3 (m, 9 H). **19b**: mp 120–121 °C (ethyl acetate/hexanes); $[\alpha]_D$ = +56.8° (c = 1, $CDCl_3$).

X-ray Analysis of 20a: a = 15.490 (2) Å, b = 7.278 (1) Å, c = 13.521 (2) Å, β = 110.39 (1)°, V = 1428.8 (6) Å^3, D_{calc} = 1.20 g cm^{-3} for $C_{26}H_{35}N_3O_6S$, Z = 2, space group $P2_1$. A total of 2871 reflections were measured on a SYNTEX $P2_1$ diffractometer at 23 °C with the θ–2θ variable-scan technique (λ = 1.5481 Å) and corrected only for Lorentz and polarization factors. The structure was solved by direct methods and refined by full-matrix least-squares analysis on the basis of 2075 "observed" reflections with $I \geq 3\sigma(I)$. Although most hydrogen positions were evident on difference maps, they were introduced in idealized positions and their scattering was taken into account in the later stages of refinement. The least squares weight, $\omega = \sigma^{-2}(F_o)$ were calculated with the assumption that $\sigma^2(I) = \epsilon^2 + (rp)^2$, where ϵ is a statistical counting error and p = 0.04. The refinements, assuming anisotropic motion for all non-hydrogen atoms, converged at R = 0.041, R_w = 0.047. The final difference maps contained no significant features. Tables of atomic coordinates, bond distances and angles, and thermal parameters are available as supplemental material.

Pharmacology. Vasorelaxant Potency. Vasorelaxant potency was determined with rabbit thoracic aorta. The experimental protocol is very similar to the one previously described in the literature.[1] IC_{50} values were determined with a quadratic fit to the logit transformation of the concentration–response curves.

Metabolism Studies. Metabolism in the Rat. Compound **3a** was given orally in PEG 400 (25 mg/mL) at a dose of 135 μmol/kg to 300-g male Sprague-Dawley rats (Charles River Labs). Blood was collected from a catheter implanted in the right jugular

vein. Heparinized plasma was prepared, immediately frozen, and stored at -20 °C. Thawed specimens were assayed for the concentrations of **3a** and **21a** by HPLC. Plasma (0.2 mL) was pipetted onto a Bond Elut C2 column (1-mL capacity, Analytichem), followed by the addition of 20 μL of a 10 μg/mL solution of **3l**, which was used as an internal standard. Vacuum was applied, and the columns were washed once with 1.0 mL of water and then once with 1.0 mL of a 20/80 mixture of acetonitrile and water. Compounds **3a**, **3l**, and **21a** were then eluted with 1.0 mL of pure acetonitrile. The acetonitrile eluate was evaporated to dryness with nitrogen gas and the residue was reconstituted to 100 μL of acetonitrile; a 20-μL aliquot was injected onto a Whatman 5μ ODS-3 column (25 cm long × 4.6 mm i.d.). The compounds were eluted isocratically with a mobile phase consisting of 65% acetonitrile and 35% 0.01 M ammonium acetate (pH 7.0) at a flow of 1.0 mL/min, and the UV absorbance was monitored at 335 nm. The ratio of **3a** and **3l** peak heights was directly proportional to the concentration of **3a** in plasma. Similarly, the peak height ratios of **21a** versus **3l** were linearly related to the concentration of **21a**. The recovery of all three substances from plasma was essentially complete.

Biotransformation Studies in Vitro. The test compounds were incubated with rat liver homogenates (1:6 dilution of tissue in buffer) containing the soluble enzyme fraction (100000*g* supernatant) for 1 h at 37 °C at a concentration of 0.1 mmol/L. The concentrations of the test compounds and the hydrolytic products were measured in the incubation mixtures by HPLC. Aliquots from each incubation were diluted with two parts of acetonitrile to precipitate protein and centrifuged at 10000 rpm in an Eppindorf 5413 microcentrifuge. Compounds in the supernatant were separated on a Whatman 5μ ODS 3 column (25 cm length × 4.6 mm i.d.) using a mobile phase consisting of methane/tetrahydrofuran/0.1 M acetic acid, isocratic at 61.7/3.3/35 for 6 min, followed by 6 min gradient up to 76/4/20. The detector was set at 335 nm for **3a**,**l** and at 280 for **3q**. Pure external standards were injected directly on the column to determine absolute concentrations of each compound.

Registry No. **2a**, 125734-74-9; **2b**, 125734-98-7; **3a**, 128114-29-4; **3b**, 128114-30-7; **3c**, 128114-31-8; **3d**, 128114-32-9; **3e**, 128114-33-0; **3f**, 128114-34-1; **3g**, 128114-35-2; **3h**, 128114-36-3; **3i**, 128114-37-4; **3j**, 128114-38-5; **3k**, 128114-39-6; **3l**, 128114-40-9; **3m**, 128114-41-0; **3n**, 128114-42-1; **3o**, 128114-43-2; **3p**, 128114-44-3; **3q**, 128114-45-4; **3r**, 128114-46-5; **3s**, 128114-47-6; **4**, 125735-01-5; **5**, 125735-02-6; **14**, 128114-48-7; **15**, 128114-49-8; **16a**, 113094-77-2; **16b**, 113116-81-7; **17a**, 128162-86-7; **17b**, 125735-03-7; **18a**, 112770-77-1; **18b**, 112770-80-6; **19a**, 108931-39-1; **19b**, 108931-40-4; **20a**, 128114-50-1; **21a**, 128162-87-8; **21b**, 123371-44-8; (*R*)-(+)-α-methylbenzylamine, 3886-69-9; *p*-methoxy-α-toluenethiol, 6258-60-2; 4-nitrophenyl chloroformate, 7693-46-1.

Supplementary Material Available: Tables of atomic coordinates, bond distances and angles, and thermal parameters (7 pages). Ordering information is given on any current masthead page.

1-(Aminoalkyl)-2-phenylindoles as Novel Pure Estrogen Antagonists

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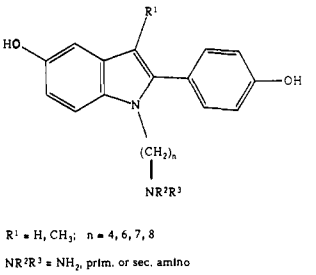
A number of 1-(ω-aminoalkyl)-5-hydroxy-2-(4-hydroxyphenyl)indoles were synthesized and studied for their binding affinities for the calf uterine estrogen receptor and estrogen antagonistic activities. Highest binding affinities were found with derivatives bearing a methyl group in position 3 and a hexamethylene chain between the indole and amino nitrogen atoms. Values for relative binding affinity (RBA) are between 20 and 30 for derivatives **5b**, **5c**, **5f**, and **5h** (17β-estradiol = 100). In the mouse uterine weight test, no significant agonistic (estrogenic) activity was observed up to a daily dose of 125 μg/animal, except for derivatives **5c**, **5j**, and **5l**. 2-Phenylindoles with amino (**5b**), pyrrolidino (**5f**), piperidino (**5h**), and morpholino (**5k**) groups as the amino function completely suppressed estrone-stimulated uterine growth as a dose of 125 μg/animal (100% antagonism). Therefore, these derivatives can be considered as first examples of nonsteroidal pure antiestrogens.

The antiestrogen tamoxifen is widely used in the endocrine therapy of hormone-dependent breast cancer. Unfortunately, about 40% of the patients do not respond to this treatment despite the presence of estrogen receptors in the malignant tissue.[1] The reason for this failure of therapy is not yet known. It might be due to the incomplete antagonism and/or weak agonism of this drug, being only a partial antiestrogen. Tamoxifen exhibits weak estrogenic activity both in man[2] and rat;[3] in the mouse, it acts as pure estrogen.[4] Some of the side effects of tamoxifen during long-term treatment are thought to be a consequence of the agonist (estrogenic) activity of the drug.[5] To our knowledge, all other antiestrogens in clinical use or trials also show estrogenic effects. This observation

Chart I



R^1 = H, CH_3; n = 4, 6, 7, 8

NR^2R^3 = NH_2, prim. or sec. amino

applies not only to triphenylethylene derivatives but also to the 2-phenylindole zindoxifene that we have developed for the treatment of hormone-dependent mammary and prostatic carcinomas.[6,7]

The development of a pure antiestrogen which is entirely free of estrogenic activity should eliminate many of the toxicological problems characteristic of tamoxifen (e.g.

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