mixture was purified by flash column chromatography [(1) $CHCl_3$, (2) $CHCl_3$–MeOH, 95:5]; the main reaction product was isolated in 44% yield (400 mg, 0.64 mmol). After it was dissolved in 20 mL of toluene, nitrogen was bubbled through the solution. A catalytic amount of AIBN and 800 μL of *n*-tributyltin hydride (2.6 mmol) were added. The mixture was heated for 2 h at 100 °C under a N_2 atmosphere. After cooling, the solvent was evaporated and the residual oil was purified by column chromatography: 240 mg (70%) of 1-[5-*O*-trityl-2-(1,2,4-triazol-1-yl)-2,3-dideoxy-β-D-*erythro*-pentofuranosyl]thymine (**15**) was obtained as a foam. UV (MeOH) λ_{max} 266 nm; ^1H NMR ($CDCl_3$) δ 1.54 (s, 3 H, CH_3), 2.59 (m, 2 H, H-3′), 3.55 (m, 2 H, H-5′), 4.56 (m, 1 H, H-4′), 5.16 (m, 1 H, H-2′), 5.87 (d, 1 H, H-1′), 6.82–7.65 (m, 15 H, trityl), 7.77 (s, 1 H, H-6), 7.97 (s, 1 H, H-3″), 8.50 (s, 1 H, H-5″), 9.90 (br s, 1 H, NH) ppm. The trityl group of **15** was removed by heating the product (240 mg, 0.45 mmol) for 1 h at 100 °C in a mixture of HOac–MeOH (8:2). After evaporation and chromatographic purification, 106 mg (82%) of the title product **17** was obtained (overall yield 25% from **12c**). The product was taken up in MeOH and precipitated by addition of Et_2O. UV (MeOH) λ_{max} 2.66 nm, log ε 3.95; MS *m/e* 293 (M^+); ^1H NMR δ 1.81 (d, 3 H, CH_3), 2.30–3.19 (m, H-3′ partially hidden by DMSO), 3.69 (m, 2 H, H-5′), 4.57 (m, 1 H, H-4′), 5.01–5.57 (m, 2 H, H-2′ and 5′-OH), 5.98 (d, 1 H, J = 4.0 Hz, H-1′), 7.86 (d, 1 H, H-6), 8.06 (s, 1 H, H-3″), 8.59 (s, 1 H, H-5″), 11.30 (br s, 1 H, NH) ppm; ^{13}C NMR δ 12.5 (CH_3), 31.9 (C-3′), 62.1 (C-5′), 63.1 (C-2′), 81.2 (C-4′), 89.3 (C-1′), 109.9 (C-5), 136.3 (C-6), 144.4 (C-5″), 150.7 (C-3″), 152.3 (C-2), 164.2 (C-4) ppm. Anal. ($C_{12}H_{15}N_5O_4 \cdot H_2O$) C, H, N.

1-(3-Imidazol-1-yl-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine (16b). Compound **16b** was synthesized according to the general procedure described for **16c**. Starting from 1.120 g (2 mmol) of **11b**, 70 mg of **16b** (12% overall yield) was obtained. The compound was crystallized from MeOH–Et_2O: mp 248–250 °C; UV (MeOH) λ_{max} 266 nm, log ε 3.98; MS *m/e* 292 (M^+); ^1H NMR δ 1.82 (s, 3 H, CH_3), 2.68 (m, 2 H, H-2′), 3.51 (m, 2 H, H-5′), 4.11 (m, 1 H, H-4′), 5.23 (t, 1 H, OH), 7.78 (m, 2 H, H-6 and H-2″), 11.34 (br s, 1 H, NH) ppm; ^{13}C NMR δ 12.3 (CH_3), 109.3 (C-5), 136.3 (C-6), 150.4 (C-2), 163.7 (C-4) ppm. Anal. ($C_{13}H_{16}N_4O_4 \cdot {}^1/_2H_2O$) C, H, N.

1-(3-Pyrazol-1-yl-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)thymine (16a). The procedure for the synthesis of **16c** was followed. Starting from 1.00 g (1.8 mmol) of **11a**, 78 mg of **16a** (15% overall yield) was obtained. The compound was crystallized from acetone: mp 162–164 °C; UV (MeOH) λ_{max} 267 nm, log ε 4.00; MS *m/e* 292 (M^+); ^1H NMR δ 1.80 (s, 3 H, CH_3), 2.39–2.71 (m, H-2′ partially hidden by DMSO), 3.65 (m, 2 H, H-5′), 4.11 (m, 1 H, H-4′), 5.13 (t, 1 H, OH), 7.83 (m, 2 H, H-6 and H-5″), 11.03 (br s, 1 H, NH) ppm; ^{13}C NMR δ 12.4 (CH_3), 109.9 (C-5), 136.5 (C-6), 150.6 (C-2), 164.0 (C-4) ppm. Anal. ($C_{13}H_{16}N_4O_4$) C, H, N.

Acknowledgment. A. Van Aerschot is a fellow of the Janssen Research Foundation. Dr. P. Herdewijn is a research associate of the Belgian "Nationaal Fonds voor Wetenschappelijk Onderzoek". This work was supported by grants from the Belgian F.G.W.O. (Fonds voor Geneeskundig Wetenschappelijk Onderzoek, Project No. 3.0040.83, 3.009.87, and 3.0040.87). We are indebted to Luk Kerremans, Ann Absillis, and Lizette van Berckelaer for excellent technical assistance and Christiane Callebaut and Laurent Palmaerts for fine editorial help.

Registry No. 1a, 30516-87-1; 1c, 106060-78-0; 2a, 52450-18-7; 2b, 124355-25-5; 2b acetate, 124355-26-6; 2c, 124355-23-3; 2c·HCl, 124355-43-7; 3, 124355-24-4; 4, 123533-11-9; 5, 124355-28-8; *cis*-6, 13269-48-2; *trans*-6, 13269-49-3; 7, 628-36-4; 8, 124355-27-7; 9, 115913-84-3; 10a, 288-13-1; 10b, 288-32-4; 10c, 288-88-0; 11a, 124355-29-9; 11b, 124355-30-2; 11c, 124355-32-4; 11c phenoxythionocarbonyl derivative, 124355-36-8; 12c, 124355-33-5; 13a, 124379-59-5; 13b, 124355-31-3; 13c, 124355-34-6; 14, 124355-35-7; 15, 124355-39-1; 16a, 124355-42-6; 16b, 124355-41-5; 16c, 124355-38-0; 16c trityl derivative, 124355-37-9; 17, 124355-40-4; 18, 122370-58-5.

Studies on Ca^{2+} Channel Antagonists. A 2-Diazo-3,3,3-trifluoropropionamide Derivative Related to Verapamil as a Potential Photoaffinity Probe

Louis J. Theodore,† Wendel L. Nelson,*,† Bharti Dave,‡ and John C. Giacomini*,‡

Department of Medicinal Chemistry, School of Pharmacy, University of Washington, Seattle, Washington 98195, and Stanford University School of Medicine and the Veterans Administration Medical Center Hospital, Palo Alto, California 94305. *Received May 15, 1989*

2-(3,4-Dimethoxyphenyl)-2-isopropyl-5-[*N*-[4-(*N*-methyl-2-diazo-3,3,3-trifluoropropionamido)phenethyl]methylamino]valeronitrile (**3**), a potential photoaffinity probe for Ca^{2+} channels related to verapamil (**1**), was prepared from *N*-methyl-4-nitrophenethylamine (**7**) and 2-(3,4-dimethoxyphenyl)-2-isopropyl-5-(methanesulfonoxy)valeronitrile (**12**). Compound **3** showed concentration-dependent negative inotropic effects in rat right myocardial ventricular strips, EC_{50} = (1.05 ± 0.33) × 10^{-7} M (mean ± SD), being slightly less potent than gallopamil (**2**), EC_{50} = (2.18 ± 0.66) × 10^{-8} M. It displaced [^3H]gallopamil in myocardial membranes, K_i = (3.76 ± 1.55) × 10^{-8} M, compared to **2**, K_i = (1.55 ± 0.16) × 10^{-8} M. Photoactivation at 265 nm reduced the recoverable binding of [^3H]gallopamil to 26% compared to no effect on **2**. This agent may be a useful photoaffinity probe to aid in further characterization of Ca^{2+} channels.

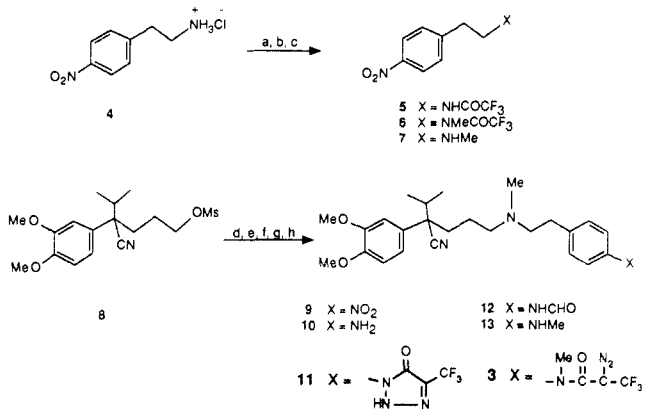
Influx of extracellular Ca^{2+} through ion channels is an important process in the excitation coupling of contraction of cardiac, smooth, and skeletal muscle.[1-4] Structurally diverse Ca^{2+} channel antagonists like verapamil (**1**) and gallopamil (**2**), nifedipine, diltiazem, prenylamine, and their congeners are used in the treatment of a variety of cardiovascular diseases.[5,6] These compounds also have been studied widely in efforts to characterize these ion channels in various tissues. Evidence has been accumulated showing separate binding sites for different chemical classes of ligands, and the use of photoaffinity ligands related to nifedipine and verapamil (**1**), and other techniques have provided useful information about the polypeptides associated with Ca^{2+} ion channels.[7-15]

† University of Washington.
‡ Stanford University School of Medicine and the Veterans Administration Medical Center Hospital.

(1) Catterall, W. A. *Science* **1988**, *242*, 50–61.
(2) Vaghy, P. L.; McKenna, E.; Itagaki, K.; Schwartz, A. *Trends Pharmacol. Sci.* **1988**, *9*, 398–402.
(3) Miller, R. J. **1987**, *235*, 46–52.
(4) Glossmann, H.; Ferry, D. R.; Striessnig, J.; Goll, A.; Moosburger, K. *Trends Pharmacol. Sci.* **1987**, *8*, 95–100.
(5) Janis, R. A.; Triggle, D. J. *J. Med. Chem.* **1983**, *26*, 776–785.
(6) *Calcium Blockers—Mechanism of Action and Clinical Implications*; Flaim, S. F., Zelis, R., Eds.; Urban and Schwarzenberg: Baltimore, MD, 1982.

0022-2623/90/1833-0873$02.50/0

Scheme I[a]



[a] Reagents: (a) Et_3N, trifluoroacetic anhydride, CH_2Cl_2; (b) NaH, CH_3I, THF; (c) CH_3OH, aqueous NaOH; (d) 7, Et_3N, DME; (e) 10% Pd/C, concentrated HCl, THF; (f) ethyl formate; (g) BH_3, THF; (h) $ClCOC(N_2)CF_3$, Et_3N, CH_2Cl_2.

Because structure–activity relationship studies on analogues of the phenylalkylamine Ca^{2+} channel blocker verapamil (1) have shown that a great variety of small structural changes, including changes in the aromatic rings, may be made in these molecules while they still retain high potency and because the 2-diazo-3,3,3-trifluoropropionyl substituent has provided useful photoaffinity probes in other systems,[16-18] the incorporation of this substituent into an analogue related to 1 was attempted. In this paper we report the synthesis of analogue 3 in which this potentially photolabile group is incorporated into the 4′-position of the aromatic ring of the phenethyl side chain. Initial work showing that 3 interacts with Ca^{2+} channels in heart membranes and is photoactivated in this preparation is reported.

Results and Discussion

Synthesis of 3 commenced from *N*-methyl-4-nitrophenethylamine hydrochloride (4, Scheme I). Treatment with trifluoroacetic anhydride afforded secondary amide 5, which was then converted to tertiary amide 6 by reaction with NaH and iodomethane. Hydrolysis of 6 with aqueous 5 N NaOH in methanol gave secondary amine 7 in 50% overall yield from 5. Nucleophilic displacement of mesylate 8[19] by 7 in refluxing dimethoxyethane (DME) gave 9, which was then converted to aniline 9 by catalytic hydrogenation over 10% Pd/C in THF, the overall yield being 58% from 8.

Initial attempts to convert 10 to a 2-diazo-3,3,3-trifluoropropionamide by reaction with 2-diazo-3,3,3-trifluoropropionyl chloride afforded a compound which, by FAB mass spectrometry, proved to be isomeric to the expected secondary amide having MH^+ m/z 546. In its CI-MS spectrum, expected fragments at m/z 436 (formation of the isothiocyanate by loss of CF_3CHN_2) and m/z 303 (α-cleavage) were observed. The solution IR spectrum of this isomer did not exhibit either the characteristic diazo band (2240 cm^{-1}) or the amide bond (1690 cm^{-1}), but instead showed an absorbance at 1767 cm^{-1}, indicating that base-catalyzed rearrangement of the desired amide to triazole derivative 11 had occurred.[20,21]

Due to these results and because previous reports of

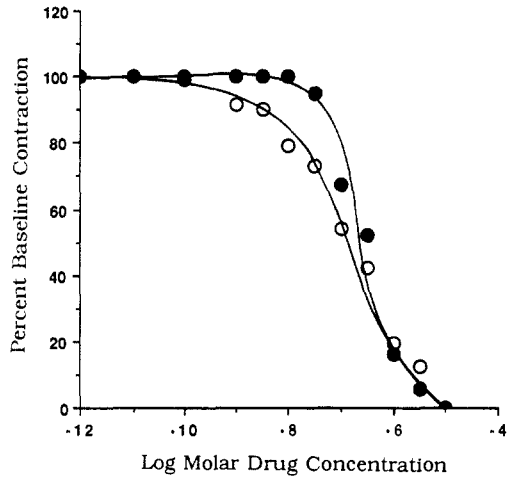


Figure 1. Inhibition of electrically stimulated contraction of rat right ventricular strips by gallopamil (2) (○) and 3 (●). Data are from representative experiments. Each point represents the mean of triplicate determinations.

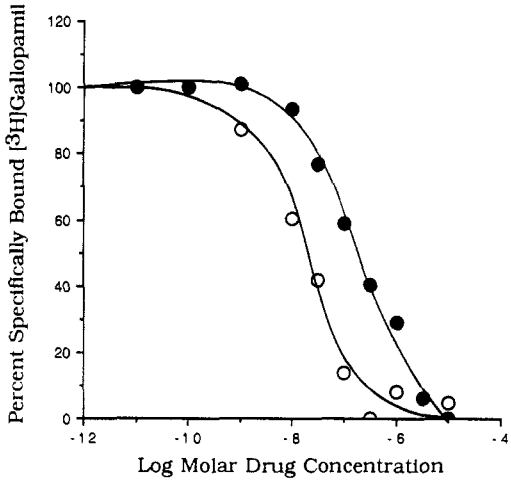


Figure 2. Displacement of [^{3}H]gallopamil from specific binding sites on rat myocardial membrane particulates by unlabeled gallopamil (2) (○) and by 3 (●). Data are representative experiments. Each point represents the mean of triplicate determinations.

(7) Striessnig, J.; Knaus, H.-G.; Grabner, M.; Moosburger, K.; Seitz, W.; Lutz, H.; Glossmann, H. *FEBS Lett.* **1987**, *212*, 247–253.
(8) Striessnig, J.; Moosburger, A.; Goll, A.; Ferry, D. R.; Glossmann, H. *Eur. J. Biochem.* **1986**, *161*, 603–609.
(9) Chang, F. C.; Hosey, M. M. *J. Biol. Chem.* **1988**, *263*, 18929–18937.
(10) Curtis, B. M.; Catterall, W. A. *Biochemistry* **1984**, *23*, 2113–2118.
(11) Vaghy, P. L.; Striessnig, J.; Miwa, K.; Knaus, H.-G.; Itagaki, K.; McKenna, E.; Glossmann, H.; Schwartz, S. *J. Biol. Chem.* **1987**, *262*, 14337–14342.
(12) Catterall, W. A.; Seager, M. J.; Takahashi, M. *J. Biol. Chem.* **1988**, *263*, 3535–3538.
(13) Zobrist, R. H.; Giacomini, K. M.; Giacomini, J. C.; *J. Mol. Cell. Cardiol.* **1986**, *18*, 963–974.
(14) Ferry, D. R.; Rombusch, M.; Goll, A.; Glossmann, H. *FEBS Lett.* **1984**, *169*, 112–118.
(15) Campbell, K. P.; Lipschutz, G. M.; Denney, G. H. *J. Biol. Chem.* **1984**, *259*, 5384–5387.
(16) Chowdhry, V.; Vaughan, R.; Westheimer, F. H. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 1406–1408.
(17) Gupta, C. M.; Radhakrishnan, R.; Gerber, G. E.; Olsen, W. L.; Quay, S. C.; Khorana, H. G. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 2595–2599.
(18) Nagarajan, S.; Ganem, B. *J. Org. Chem.* **1985**, *50*, 5735–5737.
(19) Theodore, L. J.; Nelson, W. L. *J. Labelled Compd. Radiopharm.* **1989**, *XXVII*, 491–501.
(20) Looker, J. H.; Carpenter, J. W. *Can. J. Chem.* **1967**, *45*, 1727–1734.
(21) Boger, J. H. In *Heterocyclic Compounds*; John Wiley and Sons: New York, 1961, Vol. 7, p 392.

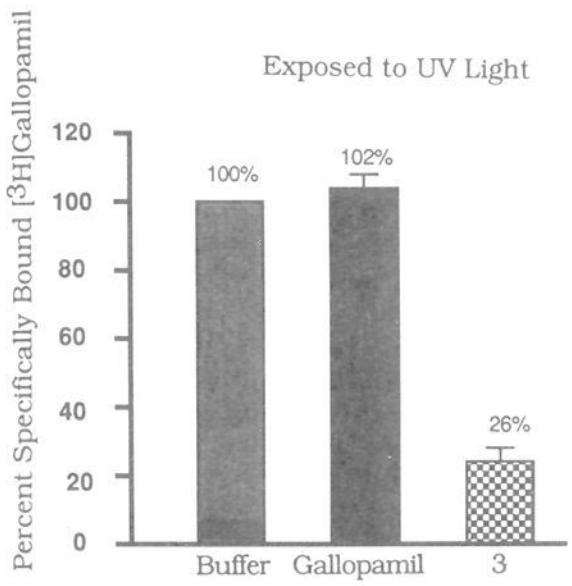


Figure 3. Effect of photoactivation in rat myocardial membrane particulates incubated with control buffer, unlabeled gallopamil (**2**), and **3**. Following light exposure, the membranes were extensively washed (seven wash–dissociation cycles) and then incubated in 0.4 nM [^{3}H]gallopamil. Whereas the binding of specifically bound unlabeled gallopamil was completely reversible, **3** remained tightly bound to the receptor.

2-diazo-3,3,3-trifluoropropionamide photoaffinity probes have been of tertiary amides,[18] we decided to prepare the corresponding secondary *N*-methylamine and to convert it to the corresponding tertiary amide. Amine **10** was converted to **13** by formation of the intermediate formamide (**12**) and subsequent borane reduction. Formation of the 2-diazo-3,3,3-trifluoroacetamide (**3**) occurred in 57% *overall yield from* **10**.

In pharmacologic studies, **3** and gallopamil (**2**) were approximately equipotent in inhibiting contractility of electrically stimulated rat ventricular strips. The EC_{50} for **3** was $(1.05 \pm 0.33) \times 10^{-7}$ M ($n = 11$) compared to $(2.18 \pm 0.66) \times 10^{-8}$ M ($n = 7$) for unlabeled gallopamil (**2**) (Figure 1).

Compound **3** displaced bound [^{3}H]gallopamil (0.4 nM) in rat myocardial membranes. The K_i for **3** was $(3.76 \pm 1.55) \times 10^{-8}$ M ($n = 3$) compared to $(1.55 \pm 0.16) \times 10^{-8}$ M ($n = 3$) for gallopamil (Figure 2). The binding was reversible when not exposed to light with recovery of 95–105% of [^{3}H]gallopamil binding in preparations with **3** or gallopamil after seven washes, each with a 20-min dissociation period (data not shown). Photoactivation (265 nm, 3 min, 0 °C) using 10 μM **3** reduced recoverable bound [^{3}H]gallopamil to 26% of the control (Figure 3). Light exposure had no effect when unlabeled gallopamil (**2**) was incubated and photoactivated at the same concentration.

The results clearly indicate an irreversible effect of photoactivated **3**, which may result from covalent interaction of a reactive species from **3**, likely a carbene, with a spatially nearby part of the Ca^{2+} channel. This ligand could be a useful addition to those photoaffinity probes currently being used to characterize the proteins associated with Ca^{2+} channels.[22-24] The utility of this and other related agents in further characterizing Ca^{2+} channels in different tissues is under study.

Experimental Section

General Methods. High-field proton and carbon NMR spectra were obtained at 300 and 75 MHz on a Varian VXR-300 spectrometer. Chemical shifts are expressed in δ downfield from internal tetramethylsilane (δ 0.0). Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. High-resolution mass spectra were obtained on a VG-7070 mass spectrometer by direct-insertion probe. Infrared spectra were recorded on a Perkin-Elmer 283 infrared spectrophotometer. Elemental combustion analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Analytical thin-layer chromatography (TLC) was carried out on Analtech silica gel HLF TLC plates (0.25-mm thickness), and the spots were detected with a UV lamp (254 nm). Preparative TLC was carried out on Merck precoated silica gel 60 F-254 TLC plates (2-mm thickness). Merck silica gel 60 (230–400 mesh ASTM) was used for flash chromatography.[25] Unless otherwise specified, concentration of reaction mixtures or extracts was carried out (after drying with $MgSO_4$) on a Büchi rotary evaporator at water-aspirator pressure.

Tetrahydrofuran (THF) was distilled under argon from sodium metal with benzophenone as an indicator. Methylene chloride was distilled from phosphorus pentoxide. Triethylamine was distilled under argon from powdered NaH. All solvents used for extraction were reagent grade. Unless otherwise indicated, all other reagents or solvents were purchased from Aldrich Chemical Co. and used without further purification. Prepurified argon was dried by passing through a 2-ft column packed with indicating Drierite and KOH. All glassware was either oven dried for a minimum of 2 h at 140 °C and then purged with argon or flame dried under a continuous flow of argon. All chemical reactions were carried out under an argon atmosphere.

***N*-Methyl-4-nitrophenethylamine (7).** To a mixture of 5.01 g (24.7 mmol) of 4-nitrophenethylamine hydrochloride (**4**) in 100 mL of dry CH_2Cl_2 was added 5.16 g (51.0 mmol, 2.06 equiv) of dry triethylamine followed by 10.4 g (49.5 mmol, 2.00 equiv) of trifluoroacetic anhydride at 0 °C. The mixture was stirred at 0 °C for 2 h and then concentrated. The mixture was diluted with 100 mL of CH_2Cl_2 and washed with 5% v/v aqueous HCl (2 × 75 mL) followed by saturated aqueous sodium bicarbonate (2 × 75 mL). The organic phase was dried, filtered, and concentrated to afford **5** as a reddish-brown oil: ^{1}H NMR ($CDCl_3$) δ 8.18 (2 H, d, J = 8.6 Hz), 7.37 (2 H, d, J = 8.6 Hz), 6.55 (1 H, m), 3.68 (2 H, q, J = 6.8 Hz), 3.03 (2 H, t, J = 6.8 Hz). To a stirred solution of **5** in 100 mL of dry THF was added 8.75 g (61.6 mmol) of iodomethane. Then, 2.00 g (83.33 mmol) of sodium hydride was added in small portions over a 15-min period. The mixture was stirred at room temperature for 2 h and then filtered through a layer of Celite. The filtrate was concentrated, and the residue was diluted with 100 mL of CH_2Cl_2 and washed with 5% v/v aqueous HCl (2 × 75 mL). The organic phase was dried, filtered, and concentrated. The residue was chromatographed on 150 g of silica gel, eluting first with 25% EtOAc/hexanes and then with 50% EtOAc/hexanes, to afford 5.07 g of tertiary amide **6** as a reddish-brown oil: 74% from **4**. To a stirred solution of the oil in 100 mL of methanol was added 10 mL of aqueous 5 N NaOH (50 mmol). The mixture was stirred at room temperature for 30 min and then concentrated. The residue was diluted with 50 mL of H_2O and extracted with CH_2Cl_2 (2 × 100 mL). The organic extracts were combined, dried, filtered, and concentrated to afford 3.30 g of **7** as a reddish-brown oil: 70% from **6**; ^{1}H NMR ($CDCl_3$) δ 8.13 (2 H, d, J = 8.6 Hz), 7.38 (2 H, d, J = 8.6 Hz), 2.91 (4 H, s), 2.49 (3 H, s), 1.84 (1 H, m); ^{13}C NMR ($CDCl_3$) 148.6, 146.9, 129.9, 124.0, 53.1, 36.9, 36.7.

2-(3,4-Dimethoxyphenyl)-2-isopropyl-5-[*N*-(4-nitrophenethyl)methylamino]valeronitrile (9). A stirred solution of 3.50 g (9.85 mmol) of mesylate **8**,[19] 3.04 g (16.7 mmol, 1.69 equiv) of amine **7**, and 1.50 g (14.8 mmol, 1.50 equiv) of triethylamine in 40 mL of dry dimethoxyethane was refluxed for 16 h under argon. The mixture was then concentrated, diluted with 150 mL of EtOAc, and washed with 100 mL of 5% aqueous NaOH. The

(22) Catterall, W. A.; Seagar, M. J.; Takahashi, M.; Nunoki, K. In *Calcium Channels: Structure and Function*; Wray, D. W., Norman, R. I., Hess, P., Eds.; *Ann. N. Y. Acad. Sci.* **1989**, *560*, 1–14.

(23) Barhanin, J.; Borsotto, M.; Coppola, T.; Fosset, M.; Hosey, M. M.; Mourre, C.; Pauron, D.; Qar, J.; Romey, G.; Schmid, A.; Vandaele, G.; Van Renterghem, C.; Lazdunski, M. In *Calcium Channels: Structure and Function*; Wray, D. W., Norman, R. I., Hess, P., Eds.; *Ann. N. Y. Acad. Sci.* **1989**, *560*, 15–26.

(24) Hosey, M. M.; Chang, F. C.; O'Callahan, C. M.; Ptasienski, J. In *Calcium Channels: Structure and Function*; Wray, D. W., Norman, R. I., Hess, P., Eds.; *Ann. N. Y. Acad. Sci.* **1989**, *560*, 27–38.

(25) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.

organic phase was dried, filtered, and concentrated. The residue was chromatographed on 120 g of silica gel, eluting first with EtOAc and then with 10% MeOH/EtOAc, to afford 3.30 g of the product (**9**) as a colorless oil: 76%; ^{1}H NMR ($CDCl_3$) δ 8.15 (2 H, d, J = 8.7 Hz), 7.34 (2 H, d, J = 8.7 Hz), 6.92 (1 H, dd, J = 8.5, 2.0 Hz), 6.87 (1 H, d, J = 2.0 Hz), 6.86 (1 H, d, J = 8.5 Hz), 3.91 (6 H, s), 2.84 (2 H, t, J = 7.3 Hz), 2.58 (2 H, t, J = 7.3 Hz), 2.37 (2 H, m), 2.21 (3 H, s), 2.07 (2 H, m), 1.79 (1 H, td, J = 12.8, 4.4 Hz), 1.54 (1 H, m), 1.18 (4 H, d on m, J = 6.7 Hz), 0.80 (3 H, d, J = 6.7 Hz).

2-(3,4-Dimethoxyphenyl)-2-isopropyl-5-[*N*-(4-aminophenethyl)methylamino]valeronitrile (10). A mixture of 2.30 g (5.23 mmol) of **9**, 1.55 g (15.7 mmol, 3.01 equiv) of aqueous 37% HCl, and 200 mg of 10% Pd/C in 100 mL of THF was shaken in a 500-mL bottle on a Parr hydrogenator under 50 psi of hydrogen at room temperature for 24 h. The mixture was then filtered through a layer of Celite. The filtrate was washed with 50 mL of aqueous 5% NaOH. The aqueous phase was extracted with EtOAc (2 × 75 mL). The organic extracts were combined, dried, filtered, and concentrated. The residue was chromatographed on 130 g of silica gel, eluting with 50% MeOH/EtOAc, to afford 1.78 g of aniline **10** as a pale yellow oil: 83%; ^{1}H NMR ($CDCl_3$) δ 6.93 (2 H, d, J = 8.3 Hz), 6.91 (1 H, dd, J = 8.1, 2.1 Hz), 6.85 (1 H, d, J = 2.1 Hz), 6.83 (1 H, d, J = 8.1 Hz), 6.60 (2 H, d, J = 8.3 Hz), 3.88 (6 H, s), 3.55 (2 H, m), 2.59 (2 H, m), 2.44 (2 H, m), 2.32 (2 H, m), 2.16 (3 H, s), 2.06 (2 H, m), 1.83 (1 H, td, J = 12.8, 4.4 Hz), 1.52 (1 H, m), 1.18 (4 H, d on m, J = 6.6 Hz), 0.79 (3 H, d, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$) δ 149.4, 148.7, 145.0, 131.2, 130.7, 129.9, 122.0, 119.2, 115.6, 111.6, 110.1, 60.2, 57.4, 56.6, 56.5, 53.9, 42.6, 38.5, 36.2, 33.3, 24.0, 19.6, 19.3.

2-(3,4-Dimethoxyphenyl)-2-isopropyl-5-[*N*-[4-(methylamino)phenethyl]methylamino]valeronitrile (12). A solution of 620 mg of aniline **10** in 10 mL of ethyl formate was stirred at 55 °C for 24 h. The mixture was then cooled and concentrated. The residue was diluted with 50 mL of CH_2Cl_2 and washed with 25 mL of saturated aqueous $NaHCO_3$. The organic phase was dried, filtered, and concentrated to afford 606 mg of formamide **12** as a yellow oil. To a stirred solution of 595 mg (1.40 mmol) of this oil in 4 mL of dry THF was added 3.77 mL of 1 M borane (3.77 mmol, 2.69 equiv) in THF. The mixture was stirred at room temperature for 18 h and at 60 °C for 2 h. The mixture was cooled to room temperature, treated with 10 mL of concentrated HCl, and stirred at 60 °C for 6 h. The mixture was then cooled to room temperature, treated with 20 mL of aqueous 6 N NaOH, and extracted with CH_2Cl_2 (3 × 75 mL). The organic extracts were dried, filtered, and concentrated. The residue was chromatographed on a preparative TLC plate, eluting with 40% MeOH/EtOAc, to afford 401 mg of the product (**12**) as a nearly colorless oil: 70%; ^{1}H NMR ($CDCl_3$) δ 6.97 (2 H, d, J = 8.5 Hz), 6.92 (1 H, dd, J = 8.4, 2.1 Hz), 6.86 (1 H, d, J = 2.1 Hz), 6.84 (1 H, d, J = 8.4 Hz), 6.55 (2 H, d, J = 8.5 Hz), 3.89 (3 H, s), 3.88 (3 H, s), 2.81 (3 H, s), 2.61 (2 H, m), 2.47 (2 H, m), 2.35 (2 H, m), 2.18 (3 H, s), 2.07 (2 H, m), 1.85 (1 H, td, J = 12.8, 4.4 Hz), 1.53 (1 H, m), 1.19 (4 H, d on m, J = 6.7 Hz), 0.80 (3 H, d, J = 6.7 Hz); ^{13}C NMR ($CDCl_3$) δ 149.4, 148.7, 148.1, 131.2, 129.8, 122.0, 119.2, 113.0, 111.6, 110.1, 60.3, 57.5, 56.6, 56.5, 54.0, 42.6, 38.5, 36.3, 33.3, 31.6, 24.0, 19.6, 19.3.

2-(3,4-Dimethoxyphenyl)-2-isopropyl-5-[*N*-[4-(*N*-methyl-2-diazo-3,3,3-trifluoropropionamido)phenethyl]methylamino]valeronitrile (3). To a stirred solution of 118 mg (0.287 mmol) of aniline **12** and 45 mg (0.45 mmol, 1.6 equiv) of dry triethylamine in 2 mL of dry CH_2Cl_2 was added 100 mg (0.580 mmol, 2.0 equiv) of 2-diazo-3,3,3-trifluoropropionyl chloride in 1 mL of dry CH_2Cl_2. The mixture was stirred in the dark at room temperature for 1 h and then concentrated. The residue was chromatographed on a preparative TLC plate, eluting with EtOAc, to afford 127 mg of the product (**3**) as a yellow oil: 81%; ^{1}H NMR ($CDCl_3$) δ 7.24 (2 H, d, J = 8.4 Hz), 7.17 (2 H, d, J = 8.4 Hz), 6.95 (1 H, dd, J = 8.4, 2.1 Hz), 6.88 (1 H, d, J = 2.1 Hz), 6.87 (1 H, d, J = 8.4 Hz), 3.91 (6 H, s), 3.35 (3 H, s), 2.76 (2 H, t, J = 7.3 Hz), 2.54 (2 H, t, J = 7.3 Hz), 2.83 (2 H, m), 2.21 (3 H, s), 2.11 (2 H, m), 1.87 (1 H, td, J = 12.8, 4.4 Hz), 1.58 (1 H, m), 1.21 (4 H, d on m, J = 6.6 Hz), 0.82 (3 H, d, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$) δ 159.1, 148.8, 148.1, 141.3, 139.8, 130.5, 130.3, 126.5, 123.9 (J_{CF} = 270.1 Hz), 121.2, 118.5, 110.9, 109.4, 59.0, 56.8, 55.9, 55.8, 53.3, 41.8, 38.2, 37.9, 35.6, 33.1, 23.4, 18.9, 18.6; IR (CH_2Cl_2) 2945, 2238, 2110, 1690, 1517, 1467, 1373, 1330, 1148, 1083, 1028, 972 cm^{-1}. Anal. Calcd. for $C_{29}H_{36}N_5O_3F_3$: C, H; N calc. 12.51, found 11.95.

Pharmacological Experiments. Isolated myocardial strips were dissected from the right ventricle of male Sprague–Dawley rats (300–400 g) and cut into longitudinal segments approximately 2 mm wide and 5 mm in length. Preparations were suspended in a 100-mL multichambered muscle chamber containing aerated (O_2–CO_2, 95:5) Krebs–Henseleit buffer at 30 °C. The salt solution had the following composition (mM): NaCl, 118; KCl, 4.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25; $CaCl_2$, 2.5; glucose, 10.0. The pH was adjusted to 7.4 and continuously monitored. Muscles were isometrically contracted against a working tension of 1 g. Contractions were induced by short square stimuli (2 ms) at a rate of 1 pulse/s and a voltage just sufficient to induce contractions.

Cumulative dose–response curves were generated after an equilibration period of 1–1.5 h. Drug was then added and effects measured at conditions of equilibrium, which was considered attained after a 40-min exposure time. Steady-state contraction amplitudes in the absence of drug were set as 100%. The depression of this steady-state contraction amplitude occurring under the influence of increasing drug concentrations was compared with the control contraction amplitudes and expressed in percentage.

Binding Experiments. Male Sprague–Dawley rats (200–300 g) were sacrificed by cervical dislocation. The heart was immediately removed and perfused through the aorta with ice-cold 10 mM Tris/HCl buffer pH 7.4. The ventricles were dissected away from the atria, finely minced with scissors, and homogenized at a tissue concentration of 100 mg of original wet tissue weight per mL of buffer. The resulting homogenate was stirred for 15 min on ice in the pressure of an equal volume of aqueous 1 M KCl to remove contractile proteins and then filtered through four layers of cheese cloth. Tissue homogenates were washed three times by centrifugation at 50,000g for 10 min, after which the final pellet was resuspended in fresh buffer at a concentration of 50 mg of original ventricular weight per mL of buffer.

Tissue homogenate (100 μL) was incubated with 0.4 nM [^{3}H]gallopamil and either verapamil or compound **3** in a total incubation of 1 mL for 60 min at room temperature. Incubations were terminated by rapid vacuum filtration over Whatman GF/C filters presoaked at least 30 min in 1% polyethyleneimine. The filters were rinsed with 4 × 2.5-mL aliquots of ice-cold buffer, and the amount of trapped radioactivity was determined by liquid-scintillation spectrometry. Binding in the presence of 10 μM gallopamil (**2**) was defined as nonspecific. Protein was determined by the method of Lowry et al.[26] with bovine serum albumin as standard.

In order to determine the binding of ligand **3** to the putative phenylalkylamine binding site, myocardial membrane particulates were prepared as described above with the final tissue pellet being resuspended at a concentration of 50 mg of wet ventricular weight per mL of Tris buffer. A 4-mL aliquot of this final tissue homogenate was incubated in the presence of either gallopamil (**2**), buffer, or **3** in a total volume of 5 mL for 60 min at 0–4 °C. The resulting pellet was resuspended in 25 mL of fresh ice-cold buffer and the homogenate was allowed to stand for 20 min on ice to allow the dissociation of membrane-bound drug. The dissociation half-life for gallopamil under similar conditions was previously determined to be approximately 10 min (unpublished data). At the end of the 20-min dissociation period, the homogenate was again centrifuged, and the entire procedure was repeated again. The wash and dissociation cycles were repeated seven times. The final pellet was resuspended in 3.3 mL of buffer and used in standard competition assays. A 0.1-mL aliquot (0.150 μg of protein/tube) of the final homogenate was incubated in the presence of 0.4 nM [^{3}H]gallopamil as above in a 1-mL total assay volume. The remaining procedures were as described above.

For photoaffinity labeling experiments, myocardial membrane particulates were prepared as above at a tissue concentration of 50 mg/mL. A 4-mL aliquot of this final tissue homogenate was incubated in the presence of either gallopamil, buffer, or **3** at a concentration of 10 μM. A similar set of above compounds was

(26) Lowry, O. H.; Rosenbrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265–275.

also prepared and treated the same way. This set acted as a control. Samples of one set were transferred to plastic petri dishes (8.5-cm diameter) on ice and photolyzed for 3 min with a ultraviolet lamp (115 volts, 60 Hz, 0.16 amp, Ultraviolet Products, Inc. San Gabriel, CA) at a distance of 15 cm (wavelength 265 nm). Controls were not exposed to UV light. After UV-light exposure, samples of both the sets were centrifuged at 50,000g for 10 min after adding 20 mL of buffer. The wash-dissociation cycles were repeated seven times, and binding of ligand to the receptor was determined as described above.

Acknowledgment. We acknowledge support of this work by the National Heart, Lung, and Blood Institute through research Grant HL-34052 to W. L. Nelson and by the Veterans Administration through a Merit Review grant to J. C. Giacomini.

Design and Synthesis of 14α-Methyl-15-aza-D-homosterols as Novel Antimycotics

Roland E. Dolle,* H. S. Allaudeen,† and Lawrence I. Kruse

Department of Medicinal Chemistry, Smith Kline & French Research Limited, The Frythe, Welwyn, Hertfordshire, AL6 9AR U.K. *Received October 3, 1988*

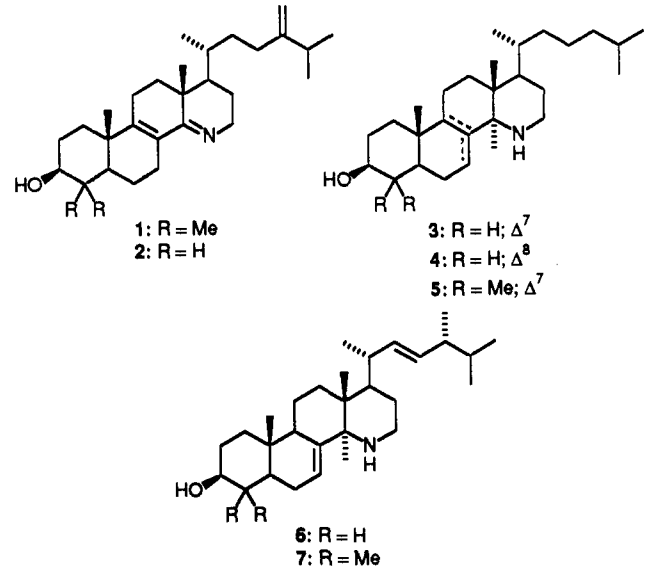
A novel series of 14α-methyl-15-aza-*D*-homosterols **3–7** has been synthesized. These compounds display significant antimycotic activity in vitro (MIC = 0.8–3.1 μg/mL) that compares quite favorably to the activity observed for fluconazole (MIC = 0.8 μg/mL). Azasterols **3** and **4** were active in vivo as reflected in the increased survival time of *Candida albicans* infected mice. The antimycotic activity of **3–7** is hypothesized to be a consequence of the inhibition of fungal 14,15-sterol reductase.

Over the past three decades an extensive amount of research has been devoted to the discovery of agents useful for the treatment of fungal infections in man.[1a] Particularly severe are the ever-increasing incidences of systemic mycoses, caused by various species of *Candida*, which occur in immuno-compromised and AIDS patients.[1bc] There is a great need for selective, orally active antimycotic agents that have a high therapeutic index.

Antimycotics either in clinical use or undergoing extensive clinical evaluation can be broadly grouped into two distinct classes: (1) those agents that disrupt membrane function (e.g. the ionophore antibiotics)[2] and (2) those agents that interrupt essential fungal enzyme processes and consequently are specific in their mode of action (e.g. the azoles).[1,2] The design and synthesis of fungal enzyme inhibitors presents itself as an appealing and tractable approach to the discovery of novel antimycotics. Such an approach is being explored in a number of laboratories as evidenced by the continued description of new inhibitors for a variety of fungal enzymes. Enzymes recently targeted for inhibition include: 14α-demethylase,[1,2] chitin synthetase,[3a] squalene epoxidase,[3b-d] squalene cyclase,[3e] and *S*-adenosylmethionine-Δ[24]-methyl transferase (24-SMT).[3f]

We have been interested in the enzyme 14,15-sterol reductase as a potential therapeutic target.[4] This enzyme, a salient member of the multienzyme cascade that is responsible for ergosterol biosynthesis in fungi and yeasts,[5a,b] has received little attention.[5c,d] Potent, naturally occurring inhibitors of the enzyme are known where A25822 factors A (**1**) and B (**2**) are representatives of this novel structural class of antimycotics.[6] The inhibition constant (K_i) for **2** against the enzyme is 2.0 nM, and both **1** and **2** exhibit impressive in vitro and in vivo antimycotic activity.[6b,d]

14,15-Sterol reductase is an NADPH-dependent enzyme that catalyzes the formal trans addition of hydrogen across the 14,15-π bond in 8,14-sterol dienes.[5c] Presumably, allylic carbocation (i) (Figure 1), a high-energy intermediate, is generated in the enzyme active site and is tightly bound to enzyme during catalysis. We have postulated that the high affinity of **1** and **2** for the enzyme is due to the structural similarity of protonated azasterols ii ↔ iii with intermediate i which is tightly bound to enzyme during



catalysis.[4] We reasoned that other sterols possessing a 15-aza-*D*-homo steroid nucleus may also be enzymatically

† Department of Immunology, Research and Development Division, Smith Kline & French, Swedeland, PA 19479.

(1) (a) Gravestock, M. B.; Ryley, J. F. In *Annual Reports in Medicinal Chemistry*; Bailey, D. M., Ed.; Academic: Orlando, 1984; pp 127–136 and references cited therein. (b) Zuger, A.; Louie, E.; Holzman, R. S.; Simberkoff, M. S.; Rahal, J. J. *Ann. Intern. Med.* **1986**, *104*, 234. (c) Kovacs, J. A.; Kovacs, A. A.; Polis, M.; Wright, W. C.; Gill, V. J.; Tuazon, C. U.; Gelman, E. P.; Lane, H. C.; Longfield, R.; Overturf, G.; Macher, A. M.; Fauci, A. S.; Parrillo, J. E.; Bennett, J. E.; Masur, H. *Ann. Intern. Med.* **1985**, *103*, 533.

(2) (a) Rieth, H. *Pharm. Unserer Zeit* **1980**, *9*, 1. (b) Cartwright, R. Y. *Ther. Med.* **1981**, 173. (c) Chmel, H.; Louria, D. B. In *Antibiotics in Laboratory Medicine*; Lorain, V., Ed.; Williams & Williams Co.: Baltimore, 1980.

(3) (A) Shenbagamurthi, P.; Smith, H. A.; Becker, J. M.; Naider, F. *J. Med. Chem.* **1986**, *29*, 802. (b) Sandifer, R. M.; Thompson, M. D.; Gaugham, R. G.; Poulter, C. D. *J. Am. Chem. Soc.* **1982**, *104*, 9376. (c) Stutz, A.; Georgepoulos, A.; Granitzer, W.; Petranyi, G.; Berney, D. *J. Med. Chem.* **1986**, *29*, 112. (d) Ryder, N. S. *Pestic. Sci.* **1987**, *21*, 281. (e) Delprino, L.; Balliano, G.; Cattel, L.; Benveniste, P.; Bouvien, P. *J. Chem. Soc., Chem. Commun.* **1983**, 381. (f) Narula, A. S.; Rahien, A.; Benveniste, P.; Schuben, F. *J. Am. Chem. Soc.* **1981**, *103*, 2408.

(4) Dolle, R. E.; Kruse, L. I. *J. Chem. Soc., Chem. Commun.* **1988**, 133.

0022-2623/90/1833-0877$02.50/0