7c, 139276-22-5; 7d, 108837-12-3; resorcinol, 108-46-3; chloroacetonitrile, 107-14-2; p-chlorobenzaldehyde, 104-88-1; p-bromobenzaldehyde, 1122-91-4; p-fluorobenzaldehyde, 459-57-4; benzaldehyde, 100-52-7; 4-[2-(dimethylamino)ethoxy]phenyl bromide, 2474-07-9; 4-[2-pyrrolidinoethoxy]phenyl bromide, 1081-73-8; 4-[2-piperidinoethoxy]phenyl bromide, 836-58-8; 4-[2-morpholinoethoxy]phenyl bromide, 836-59-9.

Supplementary Material Available: Analytical, ^1H NMR, and mass spectral data for 2-benzylidene-6-hydroxy-3(2*H*)-benzofuranones (**6b–d**), 2-benzylidene-6-methoxy-3(2*H*)-benzofuranones (**6f–h**), 2-benzyl-6-methoxy-3(2*H*)-benzofuranones (**7b–d**), and 2-(*p*-chlorobenzyl)-3-aryl-6-methoxybenzofurans (**3c–d**), and ^{13}C NMR spectral data for **6b–d,f–h** (1 page). Ordering information is given on any current masthead page.

2-Substituted 3-(Aminooxy)propanamines as Inhibitors of Ornithine Decarboxylase: Synthesis and Biological Activity

Jaroslav Stanek,* Jörg Frei, Helmut Mett, Peter Schneider, and Urs Regenass

Research Laboratories, Pharmaceuticals Division, Ciba-Geigy AG., CH-4002 Basel, Switzerland. *Received June 27, 1991*

1-Amino-3-(aminooxy)-2-propanol (**6a**) has been synthesized and found to inhibit rat liver ornithine decarboxylase (ODC) with an IC_{50} in the nanomolar range. Compound **6a** served as a basis for the design of new enzyme inhibitors, which led to the identification of 3-(aminooxy)-2-fluoropropanamine (**15**) as a new powerful enzyme blocker. Compound **15** inhibited ODC at 3 times lower concentrations than **6a** and 3-(aminooxy)propanamine (APA), and it was superior to APA as an antiproliferative agent in inhibiting the growth of human T_{24} bladder carcinoma cells in vitro.

Introduction

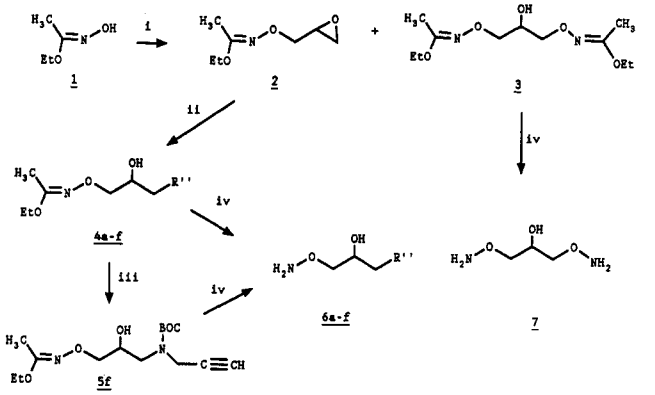
Ornithine decarboxylase (ODC) is one of the rate-limiting enzymes of polyamine biosynthesis.[1] It is present in every mammalian cell and is responsible for the conversion of L-ornithine to putrescine, a precursor of the higher polyamines spermidine and spermine.[2] ODC activity is elevated in rapidly proliferating and neoplastic tissues and is therefore a possible target for inhibition of polyamine metabolism.[3] In an attempt to control polyamine biosynthesis, several substrate and putrescine-based ODC inhibitors were synthesized.[4-6] Furthermore, 3-(aminooxy)propanamine (APA), previously reported as a chemical intermediate for the synthesis of bacteriostatic products,[7] was identified in 1985 as the most potent substrate-competitive inhibitor of ODC in vitro.[8] APA is more potent than the homologous 4-(aminooxy)butylamine[8] or 2-aminooxyethylamine[9] and appears not to inhibit ODC by interaction with the coenzyme pyridoxal phosphate (PP).[10]

The potent biological activity and the simple structure of APA prompted us to synthesize a series of new ODC inhibitors based on 2-substituted 3-(aminooxy)-1-propanamines and evaluate their biological activity. The aim of our study was to elucidate the structural requirements for selective ODC inhibition and to find new, more potent drugs. In the present article, we describe the work leading to the identification and first characterization of 3-(aminooxy)-2-fluoropropanamine (**15**) as a new lead compound.

Chemistry

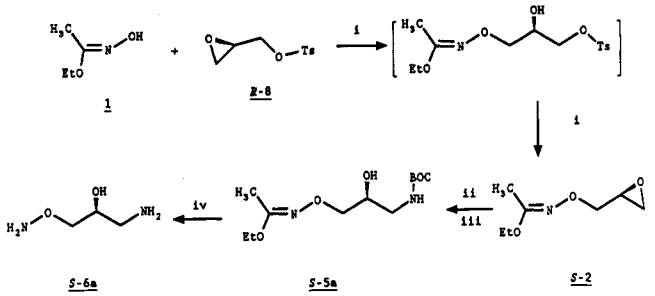
The racemic 3-(aminooxy)-2-propanols **6a–f** and the achiral 1,3-bis(aminooxy)-2-propanol **7** were synthesized, with some modifications, according to the published procedure for **6a**, **6d**, and **7**[11] (Scheme I). Instead of treating the dry sodium salt of **1** with a large excess of boiling epichlorohydrin, **1** was alkylated in acetone solution with 2 equiv of epichlorohydrin, during slow addition of 10 N NaOH, to give an easily separable mixture of **2** and **3**. Our procedure is easy to perform and affords pure **2** in a satisfactory 46% yield. Compound **2** reacted with concentrated ammonium hydroxide and different amines, leading to the 2-propanolamines **4a–f**. The intermediate **4a** was isolated by crystallization of its hydrochloride salt, whereas

Scheme I[a]



[a] (i) Epichlorohydrin, 10 N NaOH, acetone, 60 °C, 20 h; (ii) concentrated NH_4OH or amines, 20–85 °C, 5–18 h; (iii) $(BOC)_2O$, CH_2Cl_2; (iv) 2 N HCl, reflux, 1 h.

Scheme II[a]



[a] (i) 10 N NaOH, acetone, 60 °C, 20 h; (ii) concentrated NH_4OH, room temperature, 18 h; (iii) $(BOC)_2O$, Na_2CO_3, THF, H_2O, 6 h; (iv) 2 N HCl, reflux, 1 h.

4f was purified as the *N*-BOC derivative **5f**. BOC cleavage and deprotection of the aminooxy groups afforded racemic

* Address correspondence to Dr. J. Stanek, Research Laboratories, Pharmaceuticals Division, Ciba-Geigy AG., K-136.4.83, CH-4002 Basel, Switzerland.

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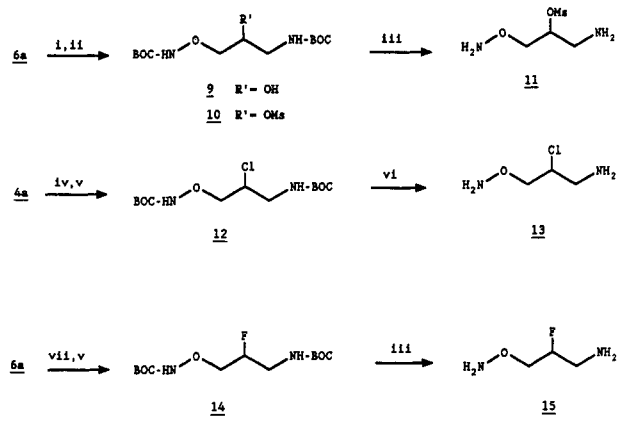
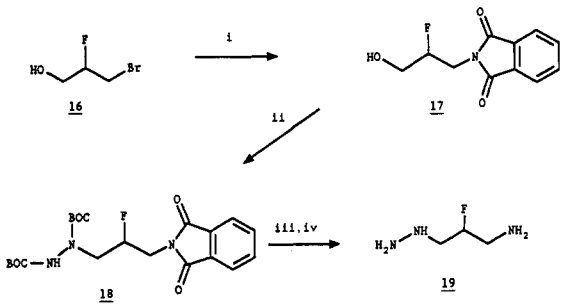
Scheme III[a]



[a] (i) $(BOC)_2O$, Na_2CO_3, THF, room temperature, 24 h; (ii) MsCl/py, room temperature, 16 h; (iii) 5.5 N HCl/EtOH, room temperature, 24 h; (iv) PCl_5, CH_2Cl_2, room temperature, 1 h; (v) $(BOC)_2O$, $NaHCO_3$, THF, room temperature, 24 h; (vi) HCl/EtOH, room temperature, 24 h; (vii) SF_4, liquid HF, −78 °C, 3 h and room temperature, 16 h.

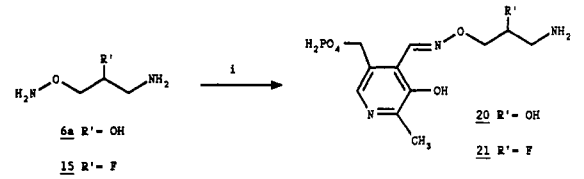
6a–f. Similarly, **3** was hydrolyzed to give achiral **7**. In order to direct the biological activity of **6a**, which will be discussed later, to one of its enantiomers, we prepared these in pure form. They were synthesized similarly to the racemic compound, using optically pure glycidyl tosylates (*R*)-(−)-**8** and (*S*)-(+)-**8**, respectively,[12] instead of epichlorohydrin. As shown by McClure, these chiral building blocks are first substituted at the oxirane ring; this is followed in the second phase of the reaction by elimination of the tosyl residue and consequently inversion of the absolute configuration on C-2.[13] Hence, starting from (*R*)-(−)-**8** and (*S*)-(+)-**8**, the products (*S*)-(+)-**6a** (Scheme II) and (*R*)-(−)-**6a** were obtained, respectively.

Scheme IV[a]



[a] (i) Potassium phthalimide, DMF, 100 °C, 6 h; (ii) BOC-N=N-BOC, $P(Ph_3)$, THF, room temperature, 20 h; (iii) $NH_2NH_2{\cdot}H_2O$, room temperature, 1 h; (iv) 2 N HCl, 60 °C, 2 h.

Scheme V[a]



[a] (i) Pyridoxal phosphate, 0.1 N NaOH, room temperature, 1 h.

The structure of **6a** allowed further chemical modification of the 2-hydroxy group with a view to increase the lipophilic character of this drug (Scheme III). Compound **6a** was protected on both amino functions by using di-*tert*-butyl dicarbonate. The resultant di-*N*-BOC derivative **9** was treated with mesyl chloride in pyridine and gave, after removal of the *tert*-butyloxycarbonyl groups, the target compound **11**. The 2-propanol derivative **4a** was halogenated with phosphorus pentachloride in dichloromethane. This reaction was accompanied by concomitant loss of the aminooxy protecting group during the workup. The intermediate obtained was treated again with di-*tert*-butyl dicarbonate to give **12**, which was deprotected to afford the pure 2-chloro derivative **13**. For the synthesis of the fluoro derivative **15**, **6a** was subjected to sulfur tetrafluoride in liquid hydrogen fluoride. The product was isolated again as a di-BOC derivative **14** and converted to a pure, crystalline and nonhygroscopic dihydrochloride salt of **15** (method H).

For comparison with **15**, we were also interested in the biological properties of the corresponding hydrazino derivative **19**. It was synthesized according to Scheme IV; potassium phthalimide was alkylated with **16**[14] to give the derivative **17**. Treatment of **17** with azodicarboxylic acid di-*tert*-butyl ester under modified Mitsunobu conditions afforded **18**, from which, after removal of the protecting groups, the desired compound **19** was obtained. Although **15** and **19** can be considered structural isosteres, comparison of their biological data (see below) demonstrates the eminent importance of the aminooxy group for inhibition of ODC.

Since O-alkylated hydroxylamines are known to react readily with carbonyl compounds, especially aldehydes, **6a** and **15** could react with the ODC coenzyme pyridoxal phosphate (PP) in situ. Therefore, we investigated the

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Table I. Physical Properties of Products 2–21 ($RCH_2CHR'CH_2R''$)

compd	R	R′	R″	yield, % (method)	mp, °C or bp/mbar	recryst[a] solvent	mol wt	formula	anal.[b]
2	$CH_3C(OEt)=NO$	O		45.9 (A)	78/18		159.2	$C_7H_{13}NO_3$	C,H,N
(*R*)-**2**	$CH_3C(OEt)=NO$	O		34.0 (A)	89/20		159.2	$C_7H_{13}NO_3$	C,H,N
(*S*)-**2**	$CH_3C(OEt)=NO$	O		38.0 (A)	84/19		159.2	$C_7H_{13}NO_3$	*c*
3	$CH_3C(OEt)=NO$	OH	$ON=C(OEt)CH_3$	8.7 (A)	107/0.04		262.3	$C_{11}H_{22}N_2O_5$	C,H,N
4a	$CH_3C(OEt)=NO$	OH	NH_2	51.0 (B)	105–8	A	212.7	$C_7H_{16}N_2O_3 \cdot 2HCl$	C,H,N,Cl
4b	$CH_3C(OEt)=NO$	OH	$NHCH_3$	75.8 (C)	oil		190.2	$C_8H_{18}N_2O_3$	*c*
4c	$CH_3C(OEt)=NO$	OH	$N(CH_3)_2$	99.0 (C)	oil		204.3	$C_9H_{20}N_2O_3$	*c*
4d	$CH_3C(OEt)=NO$	OH	$NHCH(CH_3)_2$	90.0 (C)	oil		218.3	$C_{10}H_{22}N_2O_3$	*c*
4e	$CH_3C(OEt)=NO$	OH	$NHCH_2CH=CH_2$	73.0 (C)	oil		216.3	$C_{10}H_{20}N_2O_3$	C,H,N
(*R*)-**5a**	$CH_3C(OEt)=NO$	*R*-OH	NH-BOC	72.8 (D)	oil		276.3	$C_{12}H_{24}N_2O_5$	C,H,N
(*S*)-**5a**	$CH_3C(OEt)=NO$	*S*-OH	NH-BOC	61.0 (D)	oil		276.3	$C_{12}H_{24}N_2O_5$	C,H,N
5f	$CH_3C(OEt)=NO$	OH	$N(CH_2C{\equiv}CH)BOC$	21.5 (D)	oil		314.4	$C_{15}H_{26}N_2O_5$	C,H,N
6a	NH_2O	OH	NH_2	74.4 (E)	153–6	B	179.1	$C_3H_{10}N_2O_2 \cdot HCl$	C,H,N,Cl
(*R*)-**6a**	NH_2O	*R*-OH	NH_2	81.0 (E)	157–8	B	179.1	$C_3H_{10}N_2O_2 \cdot HCl$	C,H,N,Cl
(*S*)-**6a**	NH_2O	*S*-OH	NH_2	33.5 (E)	150–5	B	179.1	$C_3H_{10}N_2O_2 \cdot HCl$	C,H,N,Cl
6b	NH_2O	OH	$NHCH_3$	91.0 (E)	150–4	B	193.1	$C_4H_{12}N_2O_2 \cdot HCl$	C,H,N
6c	NH_2O	OH	$N(CH_3)_2$	46.0 (E)	117–23	B	269.2	$C_5H_{14}N_2O_2$[d]	C,H,N
6d	NH_2O	OH	$NHCH(CH_3)_2$	98.0 (E)	156–60	B	221.1	$C_6H_{16}N_2O_2 \cdot HCl$	C,H,N,Cl
6e	NH_2O	OH	$NHCH_2CH=CH_2$	54.4 (E)	oil		219.1	$C_6H_{14}N_2O_2 \cdot HCl$	C,H,N
6f	NH_2O	OH	$NHCH_2C{\equiv}CH$	74.6 (E)	amorph		217.1	$C_6H_{12}N_2O_2$[e]	C,H,N,Cl
7	NH_2O	OH	ONH_2	35.0 (E)	amorph		195.1	$C_3H_{10}N_2O_3 \cdot HCl$[f]	C,H,N,Cl
9	BOC-NHO	OH	NH-BOC	53.2 (F)	106–8	C/D	306.4	$C_{13}H_{26}N_2O_6$	C,H,N
10	BOC-NHO	OMs	NH-BOC	98.0 (G)	oil		384.4	$C_{14}H_{28}N_2O_8S$	C,H,N
11	NH_2O	OMs	NH_2	79.2 (J)	amorph	C/E	256.9	$C_4H_{12}N_2O_4S \cdot HCl$	C,H,N,S
12	BOC-NHO	Cl	NH-BOC	33.0 (H)	111–3	E/D	324.8	$C_{13}H_{25}ClN_2O_5$	C,H,N,Cl
13	NH_2O	Cl	NH_2	59.8 (J)	amorph	C	197.5	$C_3H_9ClN_2O \cdot HCl$[f]	C,H,N,Cl
14	BOC-NHO	F	NH-BOC	45.0 (I)	67–69	E/D	308.4	$C_{13}H_{25}FN_2O_5$	C,H,N
15	NH_2O	F	NH_2	42.0 (J)	201–3	C/D	181.0	$C_3H_9FN_2O \cdot HCl$	C,H,N,Cl,F
17	HO	F	N=Phth	62.8 (K)	101–3	C/D	223.2	$C_{11}H_{10}FNO_3$	C,H,N
18	BOC-NHN(BOC)	F	N=Phth	15.7 (L)	153–4	C/D	437.5	$C_{21}H_{28}FN_3O_6$	*c*
19	NH_2NH	F	NH_2	55.1 (M)	170–3	B/E	180.1	$C_3H_{10}FN_3 \cdot HCl$	C,H,N
20	P-Pyridox=NO[g]	OH	NH_2	91.0 (N)	lyoph		448.1	$C_{11}H_{18}N_3O_7P$[h]	C,H,N,Cl
21	P-Pyridox=NO[g]	F	NH_2	99.0 (N)	lyoph		450.2	$C_{11}H_{17}FN_3O_6P$[h]	C,H,N,Cl,F,P

[a] A, ethanol; B, methanol; C, ethyl acetate; D, hexane; E, ether; F, 2-propanol. [b] Analytical results were within 0.4% of the theoretical value. [c] Microanalysis was not performed. [d] Sesquioxalate. [e] 1.8-Hydrochloride. [f] 0.2-Hydrate. [g] P-Pyridox = 3-hydroxy-5-[(phosphonooxy)methyl]-2-methylpyridyl-4-methylene. [h] Dihydrochloride, contains 1 NaCl and 1 H_2O.

Table II. Optical Activities of Enantiomers of **2**, **5a**, and **6a**

compd	$[\alpha]^{20}_D$, deg	solvent	concn, %
(*S*)-**2**	−4.8	$CHCl_3$	1.018
(*R*)-**2**	+4.9	$CHCl_3$	1.133
(*S*)-**5a**	+2.1	$CHCl_3$	1.013
(*R*)-**5a**	−3.9	$CHCl_3$	1.028
(*S*)-**6a**	+3.1	H_2O	1.044
(*R*)-**6a**	−3.0	H_2O	1.039

interactions of **6a** and **15** with the cofactor and the corresponding changes in their enzyme-inhibiting effects. For this purpose we treated **6a** and **15** with PP (Scheme V) and obtained the conjugates **20** and **21** as freeze-dried products containing 1 equiv of NaCl. All newly synthesized compounds and their physical properties are listed in Tables I and II.

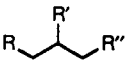
Pharmacological Results and Discussion

The newly synthesized substituted 1-oxaputrescine analogues were tested for inhibition of rat liver ODC by measuring the release of labeled CO_2 from [1-^{14}C]ornithine and for antiproliferative activity against human T_{24} bladder carcinoma cells. The respective IC_{50} values, i.e., the concentrations inhibiting CO_2 release by 50% or inhibiting cell growth by 50% as compared to control cultures, were determined (Table III). Moreover, to verify their selectivity, the new products were also tested for inhibition of rat liver *S*-adenosylmethionine decarboxylase (SAMDC), but at concentrations of 50–500 μM none of the products inhibited this enzyme.

The data (see Table III) show that compound **6a** (IC_{50} 0.039 μM) was as effective an inhibitor of ODC as APA (IC_{50} 0.035 μM). The enantiomers (*R*)-(−)-**6a** and (*S*)-(+)-**6a** showed no significant differences in ODC inhibitory activity (IC_{50} 0.049 μM or 0.033 μM, respectively). The corresponding achiral product **7**, with a second aminooxy group in place of the primary amino group (R,R″ = O–NH_2), was substantially less active (IC_{50} 670 μM). The *N*-methyl derivative **6b** inhibited ODC with an IC_{50} of 1.9 μM, and the potency of other *N*-alkyl derivatives decreased with increasing size of the R″ substituent (methyl in **6b** < propargyl **6f** < allyl **6e** < isopropyl **6d**). Secondary amines were more potent than the tertiary amine **6c**. Among this series of compounds, **6a** resulted as a new, potent inhibitor of ODC, which when tested in cell culture, proved similar in potency to APA as an antiproliferative agent against T_{24} bladder carcinoma cells (IC_{50} 24.4 μM and 8.34 μM). These results confirm that, apart from the importance of the optimal distance between the aminooxy and the amino groups, the N-substituent is crucial for efficient ODC inhibition. The 2-hydroxy group apparently is not involved in any specific interaction, because both enantiomers of **6a** show the same enzyme-inhibitory effect.

Since our efforts to increase the lipophilic character of **6a** by alkyl substitution of the amino group did not result in enhanced biological activity, we concentrated on variations of R′. The potency of the 2-substituted analogues increased from the mesyloxy derivative **11** (IC_{50} 1.1 μM) to the chloro derivative **13** (IC_{50} 0.093 μM), reaching an IC_{50} of 0.014 μM with the fluoro analogue **15**. Structural modification of **15**, consisting in replacement of the aminooxy group by a hydrazino group of comparable chemical reactivity, as in **19** (IC_{50} 3.3 μM), resulted in a 200-fold reduction of ODC-inhibitory potency. The enzyme-inhibitory activities of **11**, **13**, and **15** correlated well with their antiproliferative effects (IC_{50} 111.1 μM, 6.3 μM, and 2.38

Table III. Inhibition of ODC and Antiproliferarive Effects of New Compounds



compd	R	R′	R″	ODC:[a] IC_{50},[c] μM	T_{24}:[b] IC_{50},[c] μM
6a	NH_2O	OH	NH_2	0.039	24.4
(*S*)-**6a**	NH_2O	*S*-OH	NH_2	0.033	16.3
(*R*)-**6a**	NH_2O	*R*-OH	NH_2	0.049	20.5
6b	NH_2O	OH	$NHCH_3$	1.9	>33.3
6c	NH_2O	OH	$N(CH_3)_2$	>50	>36.6
6d	NH_2O	OH	$NHCHMe_2$	66	>45.2
6e	NH_2O	OH	$NHCH_2CH{=}CH_2$	21	>230
6f	NH_2O	OH	$NHCH_2C{\equiv}CH$	8.4	>230
7	NH_2O	OH	ONH_2	670	181
11	NH_2O	OMs	NH_2	1.1	111.1
13	NH_2O	Cl	NH_2	0.093	6.3
15	NH_2O	F	NH_2	0.014	2.38
19	NH_2NH	F	NH_2	3.3	163
20	P-Pyridox=NO[d]	OH	NH_2	24	>112
21	P-Pyridox=NO[d]	F	NH_2	5.2	0.60
APA	NH_2O	H	NH_2	0.035	8.34

[a] Rat liver ornithine decarboxylase. [b] Human T_{24} bladder carcinoma cells. [c] The data are presented as the mean of at least three independent determinations. [d] P-Pyridox = 3-hydroxy-5-[(phosphonooxy)methyl]-2-methyl-pyridyl-4-methylene.

μM) and were inversely proportional to the size of the substituent. These results indicate that **15** is superior to **6a** and APA not only as an inhibitor of ODC (2–3×) but also as an antiproliferative agent (10× and 4×, respectively). The data for **15** and **19** further demonstrate the importance of the aminooxy group in 3-aminooxypropanamines for inhibition of ODC.

Aminooxy groups are known as powerful nucleophiles. This property raises a question, whether **6a** and **15** block the enzyme per se or only after condensation with the coenzyme PP. We preincubated **6a** and **15** in the presence or absence of PP for up to 60 min at 37 °C followed by addition of the enzyme and substrate and observed time-dependent reduction of inhibitory potency in PP-pretreated samples (data not shown). In addition, the PP conjugates **20** and **21** inhibit ODC at >500 times higher concentration (IC_{50} 24 μM and 5.2 μM, respectively) than the parent drugs. These results suggest that **6a** and **15** bind per se to the enzyme and are not trapped by the free PP in vitro. The high antiproliferative activity of **21** (IC_{50} 0.6 μM) may point to a hitherto unknown, possibly polyamine-unrelated effect and is not necessarily a result of inhibition of polyamine biosynthesis.

In conclusion, we have identified the 2-hydroxy analogue **6a** of 3-(aminooxy)propanamine (APA) as a highly potent ODC inhibitor. Further structural modifications led to the synthesis of the corresponding 2-fluoro derivative **15**, which exerts greater enzyme-inhibitory activity and a more potent antiproliferative effect against human T_{24} bladder carcinoma cells in vitro than the reference compound APA. Compound **15** readily reacts under preparative conditions with PP to yield the corresponding coenzyme-inhibitor conjugate **21**. On the other hand, the biological results suggest that ODC is not inhibited via in situ-produced **21**, but with the genuine drug **15**. More detailed biological evaluation of **15** is in progress and will be reported elsewhere.

Experimental Section

Melting points were determined in open capillary tubes and are uncorrected. TLC of each compound was performed on Merck F 254 silica gel plates, and flash column chromatography was performed, if necessary, on Merck silica gel 60 (230–400 mesh). R_f values for characterization of oily compounds were determined with the same solvent systems as for the corresponding preparative chromatography. Gas chromatography was performed with Carlo Erba GC 6000, Vega Series 2. Elemental analyses were within 0.4% of the theoretical values, except where indicated. The structure of all compounds were confirmed by their IR (Perkin-Elmer 1310 and 298 spectrophotometers) and ^1H NMR spectra (Varian HA-100D or Bruker WM-250). The optical rotations were recorded at 20 °C using a Perkin-Elmer 241 polarimeter with a 10-cm cuvette.

***N*-(Oxiranylmethoxy)ethanimidic Acid Ethyl Ester (2) and 1,3-Bis[[(1-ethoxyethylidene)amino]oxy]-2-propanol (3). Method A.** A mixture of 103.1 g (1 mol) of **1** and 156.8 mL (2 mol) of epichlorohydrin in 150 mL of acetone was treated at 60 °C dropwise during 16 h with 220 mL of 10 N NaOH. After a further 4 h, no epichlorohydrin could be detected by GC. The reaction mixture was cooled and diluted with 750 mL of diethyl ether and 100 mL of water. The organic layer was separated, washed with brine, dried, and evaporated. The residual oil was distilled, affording 73 g (45.9%) of **2**: bp 78 °C/18 mbar; GC: 96% pure; ^1H NMR (250 MHz, $CDCl_3$) δ 1.28 (t, 3 H), 1.97 (s, 3 H), 2.62 (q, 1 H), 2.86 (t, 1 H), 3.24 (m, 1 H), 3.8–4.12 (m, 4 H). Anal. ($C_7H_{13}NO_3$) C, H, N.

On continuation of the distillation, 22.8 g (8.7%) of **3** was obtained: bp 107 °C/0.04 mbar; GC: 95.9% pure; ^1H NMR (250 MHz, $CDCl_3$) δ 1.3 (t, 6 H), 1.95 (s, 6 H), 3.9–4.15 (m, 5 H). Anal. ($C_{11}H_{22}N_2O_5$) C, H, N.

***N*-(3-Amino-2-hydroxypropoxy)ethanimidic Acid Ethyl Ester Hydrochloride (4a). Method B.** A mixture of 119 g (0.75 mol) of **2** in 1.6 L of concentrated NH_4OH was stirred in a closed flask for 18 h and evaporated. The resulting oil was dissolved in 300 mL of ethanol and brought to pH 6 with 1.05 N hydrogen chloride in ethanol (ca. 650 mL). About 250 mL of ethanol was distilled off, and the mixture was cooled in an ice bath. The separated crystals were collected by filtration, washed with ether, and dried, affording 81.4 g (51%) of **4a** as the hydrochloride salt: mp 105–108 °C; ^1H NMR (100 MHz, $CDCl_3$, free base) δ 1.3 (t, 3 H), 1.98 (s, 3 H), 2.5–2.9 (m, 5 H), 3.75–4.25 (m, 5 H). Anal. ($C_7H_{16}N_2O_3{\cdot}HCl$) C, H, N, Cl.

***N*-[2-Hydroxy-3-(methylamino)propoxy]ethanimidic Acid Ethyl Ester (4b). Method C.** A mixture of 8.0 g (0.05 mol) of **2** in 100 mL of 2-propanol was treated with 50 mL of a 33% solution of methylamine in ethanol and stirred for 5 h at 85 °C. The solution was evaporated, and the residue was chromatographed on silica gel (250 g) with CH_2Cl_2/MeOH/concentrated NH_4OH (300:50:1), affording 7.2 g (75.8%) of **4b** as yellow oil: R_f = 0.11; ^1H NMR (100 MHz, $CDCl_3$) δ 1.31 (t, 3 H), 1.92 (s, 3 H), 2.44 (s, 3 H), 2.68 (d, 2 H), 3.44 (s, 2 H), 3.7–4.2 (m, 5 H). This was used directly in the next step.

***N*-[2-Hydroxy-3-[*N*-[(*tert*-butyloxy)carbonyl]-propargylamino]propoxy]ethanimidic Acid Ethyl Ester (5f). Method D.** A mixture of 2.12 g (0.013 mol) of **2** and 8.5 mL (0.133 mol) of propargylamine in 10.5 mL of 2-propanol was kept for

96 h at 0 °C. The reaction mixture was evaporated, and the resulting yellow oil was dissolved in 30 mL of dichloromethane and treated with an excess of di-*tert*-butyl dicarbonate. The reaction mixture was stirred for 6 h at room temperature and diluted with 100 mL of ether. The ether layer was separated, washed with water, dried, and evaporated. The residue was chromatographed on silica gel (150 g) with CH_2Cl_2/EtOAc (85:15), affording 0.9 g (21.5%) of **5f** as yellow oil (R_f = 0.36): ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.23 (t, 3 H), 1.46 (s, 9 H), 1.94 (s, 3 H), 2.25 (t, 1 H), 3.58 (m, 2 H), 3.74 (m, 3 H), 3.9–4.2 (m, 4 H), 4.52 (s, 1 H). Anal. ($C_{15}H_{26}N_2O_5$) C, H, N.

1-Amino-3-(aminooxy)-2-propanol Dihydrochloride (6a). Method E. A solution of 88.8 g (0.4 mol) of **4a** in 1 L of 2 N HCl was refluxed for 1 h and evaporated. The residue was treated with 200 mL of ethanol, evaporated again, and afforded, after crystallization from 500 mL of methanol, 53.5 g (74.4%) of **6a**: mp 153–156 °C (lit.[11] mp 155–156 °C). Anal. ($C_3H_{10}N_2O_2$·2HCl) C, H, N, Cl.

***N*,*N*′-Bis[(*tert*-butyloxy)carbonyl]-1-amino-3-(aminooxy)-2-propanol (9). Method F.** To a solution of 18.0 g (0.1 mol) of **6a** and 21.2 g (0.2 mol) of sodium carbonate in 200 mL of water/THF (1:1) was added a solution of 48.0 g (0.22 mol) of di-*tert*-butyl dicarbonate in 100 mL of THF. After 4 h, THF was distilled off, and the reaction mixture was extracted with ether. The organic layer was washed with water, dried, and evaporated. The residue was crystallized from ether and hexane, affording 16.3 g (53.2%) of **9**: mp 106–108 °C. Anal. ($C_{13}H_{26}N_2O_6$) C, H, N.

***N*,*N*′-Bis[(*tert*-butyloxy)carbonyl]-3-(aminooxy)-2-(mesyloxy)propanamine (10). Method G.** A solution of 2.75 g (0.009 mol) of **9** in 10 mL of pyridine was treated dropwise with 1.26 g (0.011 mol) of methanesulfonyl chloride and stirred for 16 h at room temperature. Ice–water (20 mL) was added, and the reaction mixture was evaporated. The residue was dissolved in ether, and the solution was washed with water, dried, and evaporated. The product was chromatographed on silica gel (150 g) with hexane/EtOAc (1:1), affording 3.4 g (98%) of **10** as yellow oil: R_f = 0.28; ^{1}H NMR (100 MHz, CCl_4) δ 1.5 (s, 18 H), 3.16 (s, 3 H), 3.48 (m, 2 H), 4.12 (m, 2 H), 4.8–5.3 (m, 2 H), 7.8 (s, 1 H). Anal. ($C_{14}H_{28}N_2O_8S$) C, H, N.

***N*,*N*′-Bis[(*tert*-butyloxy)carbonyl]-3-(aminooxy)-2-chloropropanamine (12). Method H.** To a suspension of 2.1 g (10 mmol) of **4a** in 20 mL of dichloromethane 2.7 g (13 mmol) of phosphorus pentachloride was added, and the mixture was stirred for 1 h at room temperature. Then 100 mL ice–water was added, and the reaction mixture was carefully neutralized with 10 g of solid sodium hydrogen carbonate and treated with a solution of 2.5 g of di-*tert*-butyl dicarbonate in 50 mL of THF as described above (method F). The crude product was chromatographed on silica gel (100 g) with hexane/EtOAc (2:1), affording, after crystallization from ether/hexane, 1.1 g (33% (2 steps)) of **12**: mp 111–113 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.48 (s, 3 H), 1.51 (s, 3 H), 3.53 (m, 2 H), 4.04 (m, 2 H), 4.16 (m, 1 H), 5.25 (m, 1 H), 7.42 (s, 1 H). Anal. ($C_{13}H_{25}ClN_2O_5$) C, H, N, Cl.

***N*,*N*′-Bis[(*tert*-butyloxy)carbonyl]-3-(aminooxy)-2-fluoropropanamine (14). Method I.** In a 300-mL Teflon reactor 4.48 g (25 mmol) of **6a** was dissolved at −78 °C in 40 g of liquid HF, and 5.4 g (50 mmol) of sulfur tetrafluoride was added. The reactor was closed and kept for 3 h at −78 °C and a further 16 h at 0 °C. The reaction mixture was then evaporated, and the residue was dissolved in 50 mL of 2 N HCl. The solution was filtered, and the filtrate was diluted with 100 mL of THF, neutralized with an excess of solid sodium hydrogen carbonate, and treated with a solution of 13 g of di-*tert*-butyl dicarbonate in 70 mL of THF as described above (method F). The crude product was chromatographed on silica gel (450 g) with hexane/EtOAc (2:1), affording, after crystallization from ether/hexane, 3.47 g (45% (2 steps)) of **14**: mp 67–69 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.44 (s, 3 H), 1.48 (s, 3 H), 3.3–3.6 (m, 2 H), 3.98–4.1 (m, 2 H), 4.62–4.75 and 4.82–4.95 (2 m, 1 H), 5.0–5.12 (m, 1 H), 7.33 (s, 1 H). Anal. ($C_{13}H_{25}FN_2O_5$) C, H, N.

3-(Aminooxy)-2-fluoropropanamine Dihydrochloride (15). Method J. A mixture of 3.4 g (11 mmol) of **14** in 40 mL of ethyl acetate was treated with 40 mL of 2.2 N hydrogen chloride in ethyl acetate. After 24 h, the crystalline product was collected, washed with ether, and dried, affording 1.9 g (42%) of **15**: mp 201–203 °C; ^{1}H NMR (250 MHz, D_2O) δ 3.3–3.6 (m, 2 H), 4.27–4.51 (m, 2 H), 5.1 and 5.25 (2 m, 1 H). Anal. ($C_3H_9FN_2O$·2HCl) C, H, N, F, Cl.

2-(2-Fluoro-3-hydroxypropyl)phthalimide (17). Method K. A mixture of 2.5 g (15.1 mmol) of **16** (95% pure),[14] 3.245 g (17.5 mmol) of potassium phthalimide, and 10 mL of DMF was stirred for 6 h at 100 °C. The reaction mixture was then evaporated, and the residue was partitioned between dichloromethane and water. The organic phase was separated, dried, and concentrated in vacuo. The residue crystallized from ethyl acetate/hexane, affording 2.23 g (62.8%) of **17**: mp 101–103 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.5–3.83 (m, 3 H), 3.88–4.02 (m, 1 H), 4.65 and 4.82 (2 m, 1 H), 5.1 (t, 1 H), 7.84–7.95 (m, 4 H). Anal. ($C_{11}H_{10}FNO_3$) C, H, N.

***N*-(2-Fluoro-3-phthalimidopropyl)-*N*,*N*′-bis(*tert*-butoxycarbonyl)hydrazine (18). Method L.** To a solution of 1.30 g (5.82 mmol) of **17** and 1.53 g (5.83 mmol) of triphenylphosphine in 10 mL of THF was added dropwise at 20–30 °C (slight exotherm) a solution of di-*tert*-butyl azodicarboxylate in 5 mL of THF. The reaction mixture was left at room temperature overnight and then evaporated under reduced pressure to dryness. Flash chromatography of the residue on silica gel (EtOAc/hexane: 1:3, 1:2, 1:1) yielded crude product, which was crystallized from ethyl acetate hexane, affording 0.40 g (15.7%) of **18**: mp 153–154 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (bs, 18 H), 3.6–3.95 (m, 3 H), 3.97–4.14 (m, 1 H), 4.9 and 5.07 (2 m, 1 H), 6.5 (s, 1 H), 7.74 (m, 2 H), 7.88 (m, 2 H). This was used directly in the next step.

(3-Amino-2-fluoropropyl)hydrazine Dihydrochloride (19). Method M. A mixture of 0.36 g (0.823 mmol) of **18** and 3 mL (61.7 mmol) of hydrazine hydrate was stirred for 1 h at room temperature. After addition of 10 mL of ether and continued stirring for a further 2 h, the ether layer was separated, and the hydrazine hydrate phase was extracted with ether (3 × 10 mL). The combined organic fractions were washed successively with water (10 mL) and brine (5 mL) and dried (Na_2SO_4), and the solvent was removed under reduced pressure to leave 0.28 g of a yellowish oil. The crude intermediate (0.24 g, 0.706 mmol) was immediately taken up in 5 mL of 2 N HCl, and after being stirred for 2 h at 60 °C, the mixture was evaporated at reduced pressure. The residue was crystallized from methanol/ether, affording 0.07 g (55.1%) of **19**: mp 170–173 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.0–3.25 (m, 4 H), 4.91 and 5.08 (2 m, 1 H), 8.14 (bs, 7 H). Anal. ($C_3H_{10}FN_3$·2HCl) C, H, N.

2-Fluoro-3-[[[[3-hydroxy-2-methyl-5-[(phosphonooxy)methyl]-4-pyridyl]methylidene]amino]oxy]propanamine Hydrochloride (21). Method N. A mixture of 362 mg (2 mmol) of **15** and 530 mg (2 mmol) of pyridoxal-5′-phosphate monohydrate was dissolved in 20 mL of 0.1 N NaOH. After 1 h, the solution was filtered and freeze-dried, affording 0.9 g of **21** (containing 1 equiv of NaCl): TLC system methyl ethyl ketone/EtOH/concentrated NH_4OH/H_2O (15:5:5:5), R_f = 0.17, pyridoxal phosphate R_f = 0.31; ^{1}H NMR (250 MHz, D_2O) δ 2.68 (s, 3 H), 3.35–3.58 (m, 2 H), 4.53–4.92 (m, 2 H), 5.14 (d, 2 H), 5.14 and 5.34 (2 m, 1 H), 8.22 (s, 1 H), 8.78 (s, 1 H); UV λ_{max} (ε × 10^{-3}) H_2O, 224 nm (12.64), 342 (5.72). Anal. ($C_{11}H_{17}FN_3O_6P$·HCl·NaCl·H_2O) C, H, N, F, Cl, P.

Enzyme Preparation and Assay for ODC Activity. Rat liver ODC was prepared essentially as published by Hayashi.[15] The protein content, as determined by Bradford assay,[16] was 34 mg/mL. The ODC assay contained, in a total volume of 250 μL, 100 mM Tris-HCl pH 7, 4 mM EDTA, 4 mM dithiothreitol, 0.21 mM L-ornithine (including 0.2 μCi L-[1-^{14}C]ornithine, specific activity 50 Ci/mol), 0.4 mM pyridoxal phosphate, and up to 100 μL of the ODC preparation. The reaction mixture was incubated for up to 1 h at 37 °C. The released CO_2 was absorbed from the gas phase on an alkali-humidified filter paper (Whatman 3MM, 15 × 25 mm, humidified with 25 μL Soluene-100, Packard). Enzyme reaction was stopped by addition of 0.17 mL of 2 N HCl, which expelled residual CO_2 from the assay liquid. After another

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20-min incubation at 37 °C, filters were removed and counted with 5 mL of Irgascint in a liquid scintillation counter (Rack Beta type 1215, LKB). Inhibitors were dissolved in Tris-HCl buffer (pH 7, 10 mM) and added simultaneously with the substrate, or the coenzyme was preincubated with the inhibitor prior to substrate and enzyme addition. IC_{50} values (50% inhibitory concentration) were calculated by linear regression analysis from plots of log inhibitor concentration versus % ODC activity (control = 100%). Only activities between 95 and 5% were included in the calculations.

Antiproliferative Activity of ODC Inhibitors. Antiproliferative effects on human T_{24} bladder carcinoma cells were determined as described previously.[17] Cell numbers were measured by staining with methylene blue.

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