4-Amidinoindan-1-one 2'-Amidinohydrazone: A New Potent and Selective Inhibitor of *S*-Adenosylmethionine Decarboxylase

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Two isomeric amidino-2-acetylpyridine amidinohydrazones, 11 and 12, and 4-amidinoindanone amidinohydrazone, 17, have been synthesized and tested for inhibition of *S*-adenosylmethionine decarboxylase (SAMDC) and diamine oxidase and for antiproliferative activity against T24 human bladder carcinoma cells. Compound 11 inhibited SAMDC with an IC_{50} of 10 nM and was 140- and >500-fold more potent than methylglyoxal bis(guanylhydrazone) (MGBG) and 12, respectively. The difference in potency between 11 and 12 was interpreted with the help of molecular modeling and appeared to be associated with two different low-energy conformations of the compounds. Compound 17 which represents a conformationally constrained analogue of 11, was superior to the latter and MGBG with respect to selective inhibition of SAMDC and antiproliferative activity, and is of interest as a potential anticancer agent and a drug for the treatment of protozoal and *Pneumocystis carinii* infections.

Introduction

S-Adenosylmethionine decarboxylase (SAMDC) is a rate-limiting enzyme of polyamine (PA) biosynthesis which controls the conversion of the diamine putrescine to the higher polyamines spermidine and spermine.[1] SAMDC represents a therapeutic target for rapidly proliferating cells which have up to 3 orders of magnitude higher specific activity of PA biosynthetic enzymes and larger polyamine pools and are generally more sensitive to modulation of PA biosynthesis than non-proliferating cells.[2] Consequently, inhibition of SAMDC leads to accumulation of putrescine and *S*-adenosylmethionine,[3] depletion of spermidine and spermine, and finally inhibition of cell proliferation.[4,5] Putrescine may be degraded by diamine oxidase (DAO), whereas *S*-adenosylmethionine is also used intracellularly as a methyl donor.[3,6] Today two classes of inhibitors of SAMDC are preclinically studied as potential antitumor agents and drugs for the treatment of protozoal and *Pneumocystis carinii* infections: Analogues of methylglyoxal bis(guanylhydrazone) (MGBG), which are competitive enzyme inhibitors and more selective than the parent drug,[7,8] as well as analogues of *S*-adenosylmethionine, which are irreversible enzyme inhibitors binding covalently to the pyruvate residue at the active site of SAMDC.[9,10] Since the three-dimensional structure of SAMDC is not known, new inhibitors may significantly contribute to an understanding of events at the enzyme's catalytic site. Recently we published the synthesis and biological activity of a series of conformationally constrained analogues of MGBG, and showed that products, in which the linear chain of MGBG has been partly replaced by bulky but conformationally constrained 3-amidinoaryl residues, selectively inhibit SAMDC.[11,12] In addition, potent SAMDC inhibitors appear to have two bidentate basic groups and a fully extended planar low-energy conformation, which is superimposable on the crystal structure of MGBG.[11] In continuation of our investigations, we now report the synthesis and biological characterization of two isomeric amidino-2-acetylpyridine amidinohydrazones, 11 and 12, and the design of 4-amidinoindanone guanylhydrazone, 17, as a novel highly potent and selective SAMDC inhibitor with antiproliferative activity.[13]

Chemistry

The synthesis of the amidino-2-acetylpyridine guanylhydrazones 11 and 12 has been achieved using standard synthetic methods as previously described[11] and following the routes described in Scheme I. The dioxolanylpyridine 1[14] was cyanated in analogy to the Reissert-Henze reaction[15,16] by oxidation with MCPBA to give the *N*-oxide 2, which was O-alkylated with dimethyl sulfate and treated with NaCN to give the cyanopyridine 4. This intermediate reacted with hydrogen sulfide in pyridine in the presence of triethylamine to give the thioamide 6, which was alkylated with methyl iodide to form the imino thioester 7 and further converted to the amidine 8. The amidine 8 was finally treated under slightly acidic conditions with aminoguanidine to give the target hydrazone 11. The isomeric 4-amidino-2-acetylpyridine guanylhydrazone (12) was prepared in an alternative way starting from 4-cyano-2-acetylpyridine (5).[17] Compound 5 was condensed with aminoguanidine hydrogen carbonate in presence of dilute hydrochloric acid to give the guanylhydrazone 9. The cyano function of 9 was then converted via intermediate 10 to the amidine group in 12 by sodium methoxide-catalyzed Pinner reaction followed by the treatment with ammonium chloride.

The biological activity of 11 which will be discussed later encouraged us to integrate the methyl group of the side chain into an annelated ring and to prepare new products with constrained structures. The most potent SAMDC inhibitor 17 was synthesized starting from 4-cyanoindanone 13.[18] This was converted as described above to a crystalline thioamide 14, which was S-alkylated with triethyloxonium tetrafluoroborate and, upon reaction with ammonium chloride, yielded the amidine 16. Intermediate 16 reacted then smoothly with aminoguanidine in dilute hydrochloric acid to give crystalline and nonhygroscopic dihydrochloride salt of the 4-amidinoindanone derivative 17 (Scheme II).

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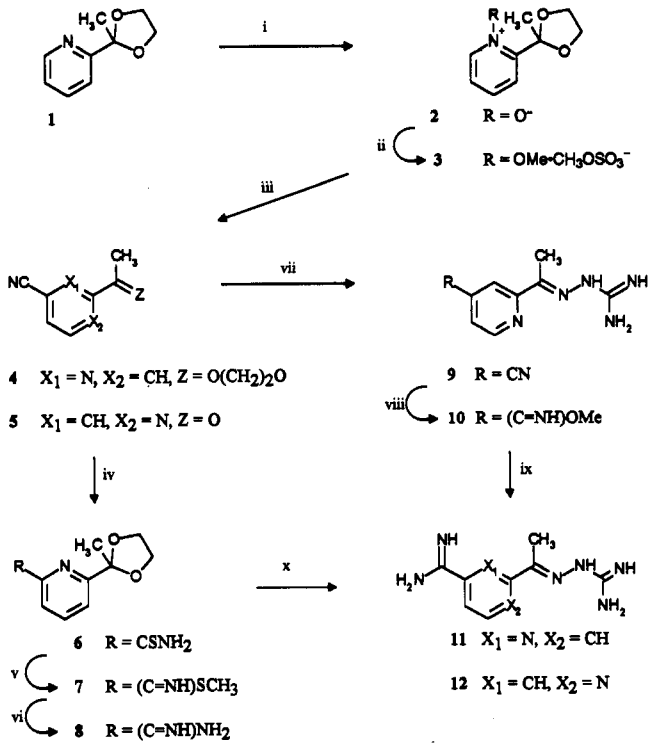
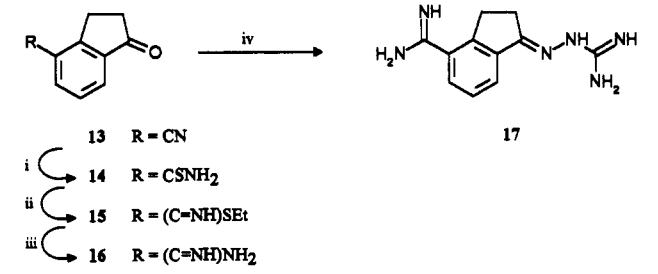
Scheme I [a]



[a] (i) MCPBA, CH_2Cl_2, 0–30 °C, 7 h; (ii) $(CH_3O)_2SO_2$, 75 °C, 1 h; (iii) NaCN, H_2O, 0 °C, 3 h; (iv) H_2S, pyridine/NEt_3, room temperature, 5 h; (v) CH_3I, acetone, room temperature, 36 h; (vi) NH_4Cl, EtOH, reflux, 3 h; (vii) $NH_2NH(C{=}NH)NH_2{\cdot}H_2CO_3$, 2 N HCl, H_2O, MeOH, THF, room temperature, 0.5 h; (viii) NaOMe, absolute MeOH, room temperature, 16 h; (ix) NH_4Cl, absolute MeOH, room temperature, 18 h; (x) $NH_2NH(C{=}NH)NH_2{\cdot}H_2CO_3$, 2 N HCl, H_2O, reflux, 2 h.

Scheme II [a]



[a] (i) H_2S, pyridine/NEt_3, 40 °C, 19 h; (ii) triethyloxonium tetrafluoroborate, CH_2Cl_2, room temperature, 16 h; (iii) NH_4Cl, EtOH, reflux, 20 h; (iv) $NH_2NH(C{=}NH)NH_2{\cdot}H_2CO_3$, H_2O, HCl, room temperature, 20 h.

All newly synthesized compounds and their physical properties are listed in Table I. Their structures were confirmed by IR, NMR, and MS spectral data.

Pharmacological Results and Discussion

The new guanylhydrazones were tested for inhibition of rat liver SAMDC, inhibition of rat duodenal diamine oxidase (DAO), and antiproliferative activity against human T24 bladder carcinoma cells. None of the compounds inhibited rat liver ornithine decarboxylase at concentrations of 50 μM.

As shown in Table II, the hydrazone 11 inhibited SAMDC with an IC_{50} of 10 nM and was 140 times more potent than MGBG (IC_{50} 1.4 μM). It inhibited DAO with an IC_{50} of 5.8 μM and had a >4000-fold higher selectivity index (IC_{50} DAO/IC_{50} SAMDC) than MGBG. Furthermore, compound 11 showed antiproliferative activity against T24 cells with an IC_{50} of 23.2 μM. Reduced cellular uptake rate, lower levels of intracellular accumulation, and a reduced polyamine-unrelated toxicity of 11 as compared to MGBG may contribute to the apparent discrepancy between enzyme inhibitory and antiproliferative potency of this compound.

Movement of the ring nitrogen from position X_1 to X_2 resulted in a significant reduction of SAMDC inhibitory activity. This difference in biological behavior of the acetylpyridine derivatives 11 and 12 suggested that the shift of the ring nitrogen is associated with a more serious structural change than expected. Indeed, molecular-modeling studies revealed that the lowest energy conformation of 11 represents a coplanar arrangement of the ring and the imino ethyl residue of the side chain, with the methyl group being oriented toward the ring nitrogen. The calculated energy of this conformation was 2.2 kcal/mol lower as compared to the conformer with a flipped orientation of the side chain, which seems to be mainly due to an unfavorable repulsive electrostatic interaction between the nitrogen lone pairs in the latter conformation. Accordingly, movement of the ring nitrogen from the position X_1 to X_2 in the 2-(4-amidinopyridyl) derivative 12, which was expected to lead to a bent and unfavorable conformation, resulted in a >500-fold reduction of SAMDC inhibitory activity as compared to 11 (Figure 1). A similar observation with respect to the crystal structure has been made with a trifluoromethyl analogue of MGBG.[19]

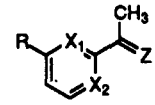
The proposed conformation of 11 and the fact that the methyl group of the side chain positively affected potency and selectivity of the drug as compared to the corresponding formyl derivative[11] encouraged us to incorporate the methyl group in a fused-ring system leading to the indanone derivative 17. Compound 17 inhibited SAMDC with an IC_{50} of 5 nM and was 2 and 280 times more potent than 11 and MGBG, respectively. SAMDC inhibition by 17 caused depletion of intracellular spermidine and spermine pools (data not shown), and the compound showed a pronounced antiproliferative activity (IC_{50} of 0.71 μM). Moreover, compound 17 inhibited DAO with an IC_{50} of 18 μM, thus showing a >2500-fold higher selectivity index (IC_{50} DAO/IC_{50} SAMDC) than MGBG. We feel that with compound 17 we have identified a very potent and selective SAMDC inhibitor, which is a good candidate to be tested in clinical trials.

Experimental Section

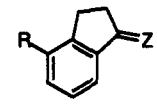
Melting points were determined in open capillary tubes and are uncorrected. TLC of each compound was performed on Merck F 254 silica gel or Antec OPTI-UPC_{12} F254 plates, and column chromatography on Merck silica gel 60 (230–400 mesh), Amberlite XAD 1180, or Antec OPTI-UPC_{12}. Gas chromatography was performed with a Carlo Erba GC 6000, Vega Series 2. Elemental analyses were within ±4% of the theoretical values, except where indicated. The structures of all compounds were confirmed by their IR spectra (Perkin-Elmer 1310 or 298 spectrophotometers), ^1H NMR spectra (Varian Gemini 200 or 300), and fast-atom bombardment mass spectra FAB-MS (VG-Manchester). The conformations of products were generated and energy minimized (AMBER force field) in MacroModel (version 2.0).[20] The reference compound MGBG was purchased from a commercial source (Aldrich).

2-(2-Methyldioxolan-2-yl)pyridine 1-Oxide (2). A solution of 17.2 g (55 mmol) of MCPBA (55%) in 100 mL of CH_2Cl_2 was dried over Na_2SO_4 and added dropwise to an ice-cooled solution of 9.1 g (55 mmol) of 1[14] in 50 mL of CH_2Cl_2. Stirring was continued for 1 h at 0 °C, for 5.5 h at room temperature, and 0.5 h at 30 °C. The reaction mixture was then washed with 10%

Table I. Structures and Physical Properties of New Compounds



2-12



14-17

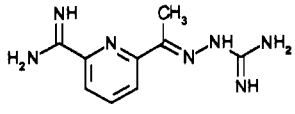
compd	X_1	X_2	R	Z	yield	mp, °C	mol wt	formula	anal.[a]
2	N^+O^-	CH	H	$O(CH_2)_2O$	50	oil	181.2	$C_9H_{11}NO_3$	nd[b]
3	N^+OMe	CH	H	$O(CH_2)_2O$	100	oil	307.3	$C_{11}H_{17}NO_7S$	nd
4	N	CH	CN	$O(CH_2)_2O$	76	oil	190.2	$C_{10}H_{10}N_2O_2$	C,H,N
6	N	CH	$CSNH_2$	$O(CH_2)_2O$	93	140–3	224.3	$C_{10}H_{12}N_2O_2S$	C,H,N,S
7	N	CH	(C=NH)SMe	$O(CH_2)_2O$	100	oil	238.3	$C_{11}H_{14}N_2O_2S$	nd
8	N	CH	$(C=NH)NH_2$	$O(CH_2)_2O$	78	193–5	243.7	$C_{10}H_{13}N_3O_2 \cdot HCl$	C,H,N
9	CH	N	CN	GH[c]	67.8	218–20	249.5	$C_9H_{10}N_6 \cdot HCl \cdot 0.6H_2O$	C,H,N,Cl,H_2O
10	CH	N	(C=NH)OMe	GH	ni[d]	nd	234.3	$C_{10}H_{14}N_6O$	nd
11	N	CH	$(C=NH)NH_2$	GH	64	d ~310[e]	299.4	$C_9H_{13}N_7 \cdot 2HCl \cdot 0.4H_2O$	C,H,H,Cl
12	CH	N	$(C=NH)NH_2$	GH	5[f]	>250	296.7	$C_9H_{13}N_7 \cdot 2HCl \cdot 0.25H_2O$	C,H,N,Cl,H_2O[g]
14			$CSNH_2$	O	66.6	d 197	191.3	$C_{10}H_9NOS$	C,H,N
15			(C=NH)SEt	O	98.7	amorph	219.3	$C_{12}H_{13}NOS$	nd
16			$(C=NH)NH_2$	O	49.7	d 215–8	228.7	$C_{10}H_{10}N_2O \cdot HCl \cdot H_2O$	C,H,N[h]
17			$(C=NH)NH_2$	GH	72	>330	321.9	$C_{11}H_{14}N_6 \cdot 2HCl \cdot H_2O$	C,H,N,Cl

[a] Analytical results were within ±0.4% of the theoretical value. [b] nd = not determined. [c] GH = =NNH(C=NH)NH_2. [d] ni = not isolated. [e] d = decomposition. [f] One experiment, not optimized. [g] N: calcd, 33.05; found, 32.6. [h] C: calcd, 52.52; found, 52.0.

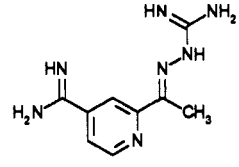
Table II. Inhibition of Enzymatic Activities and T24 Cell Growth by Guanylhydrazones

	IC_{50}, μM		
compd	SAMDC[a]	DAO[b]	T24[c]
11	0.01 ± 0.0006	5.8 ± 0.7	23.2 ± 10
12	5.1 ± 0.036	8.5 ± 0.07	≥152 ± 23
17	0.005 ± 0.0005	18.0 ± 0.3	0.71 ± 0.2
MGBG	1.4 ± 0.59	1.9 ± 0.3	1.13 ± 0.3

[a] *S*-Adenosylmethionine decarboxylase from rat liver. [b] Diamine oxidase from rat small intestine. [c] Human T24 bladder carcinoma cells. The data are presented as the mean of at least three independent determinations.



11



12

Figure 1. Proposed low-energy conformations of the SAMDC inhibitors 11 and 12.

$NaHSO_3$ solution and saturated $NaHCO_3$ solution, was dried over Na_2SO_4, and evaporated, affording 4.54 g (50%) of **2**: ^1H NMR (200 MHz, $CDCl_3$) δ 8.21–8.31 (m, 1 H), 7.55–7.67 (m, 1 H), 7.15–7.30 (m, 2 H), 4.04–4.20 (m, 2 H), 3.81–3.98 (m, 2 H), 1.97 (s, 3 H). The product was used in the next experiment.

1-Methoxy-2-(2-methyldioxolan-2-yl)pyridine (3). 4.54 g (25 mmol) of **2** was treated dropwise at 70–75 °C with 3.15 g (25 mmol) of dimethyl sulfate. The mixture was stirred at 75–80 °C for 1 h and cooled, affording 7.7 g (100%) of **3**. The product was used immediately, without further purification, in the following reaction step.

2-Cyano-6-(2-methyldioxolan-2-yl)pyridine (4). To a solution of 3.67 g (75 mmol) of NaCN in 14 mL of H_2O was slowly added at 0 °C a solution of 7.67 g (25 mmol) of crude **3** in 6 mL of H_2O over a period of 1 h. The resulting suspension was stirred at 0 °C for 2 h and then extracted with EtOAc. The organic layer was dried over Na_2SO_4 and the solvent removed under vacuum. Flash chromatography with CH_2Cl_2/EtOAc (95:5) as eluent gave 3.6 g (76%) of pure **4** as a colorless oil: ^1H NMR (300 MHz, $CDCl_3$) δ 7.92 (t, 1 H), 7.83 (dd, 1 H), 7.69 (dd, 1 H), 4.09–4.20 (m, 2 H), 3.88–3.98 (m, 2 H), 1.73 (s, 3 H); IR (CH_2Cl_2) 2990, 2890, 2240, 1585, 1373, 1199, 1084, 1040, 817 cm^{-1}. Anal. ($C_{10}H_{10}N_2O_2$) C, H, N.

2-Thiocarbamoyl-6-(2-methyldioxolan-2-yl)pyridine (6). A solution of 2.9 g (15 mmol) of **4** in 30 mL of pyridine and 2.1 mL (15 mmol) of Et_3N was treated with dry H_2S for 5 h at room temperature. The resulting red-brown solution was evaporated and the residue taken up twice in EtOH and evaporated again. Recrystallization of the crude product from MeOH/H_2O gave 3.1 g (93%) of **6**: mp 140–143 °C; ^1H NMR (200 MHz, DMSO-d_6) δ 10.10–10.40 (broad, 1 H), 9.65–9.89 (broad, 1 H), 8.46 (d, J = 9.5 Hz, 1 H), 8.00 (t, J = 9.5 Hz, 1 H), 7.74 (d, J = 9.5 Hz, 1 H), 3.80–4.13 (m, 4 H), 1.73 (s, 3 H); IR (CH_2Cl_2) 3480, 3340, 1568, 1201, 1038, 825 cm^{-1}. Anal. ($C_{10}H_{12}N_2O_2S$) C, H, N, S.

The same procedure was used for preparation of **4-thiocarbamoylindan-1-one (14)**: yield 66.6%; mp 197 °C dec.; ^1H NMR (200 MHz, DMSO) δ 10.12 (s, 1 H), 9.62 (s, 1 H), 7.71 (m, 2 H), 7.47 (t, 1 H), 3.24 (t, 2 H), 2.66 (t, 2 H). Anal. ($C_{10}H_9NOS$) C, H, N.

2-Amidino-6-(2-methyldioxolan-2-yl)pyridine Hydrochloride (8). To 1.5 g (6.6 mmol) of **6** in 15 mL of acetone was added 0.95 g (6.7 mmol) of methyl iodide, and the reaction mixture was stirred for 36 h. The precipitate was filtered and partitioned between CH_2Cl_2 and saturated $NaHCO_3$ solution at 0 °C. The aqueous layer was extracted with CH_2Cl_2, and the combined organic extracts were washed with saturated NaCl solution, dried, and evaporated. The crude imino thioester **7** [1.3 g (~100%)] was dissolved in 18 mL of absolute EtOH and treated with 0.34 g (6.3 mmol) of NH_4Cl. The mixture was heated under reflux for 3 h, cooled to room temperature, filtered, and evaporated. The residue was recrystallized from CH_3CN, affording 1.0 g (78%) of **8**: mp 193–195 °C; FAB (MS) $(M + H)^+$ 208; ^1H NMR (200 MHz, DMSO-d_6) δ 8.4–9.4 (broad, ~5 H), 8.34 (d, J = 9.5 Hz, 1 H), 8.19 (t, J = 9.5 Hz, 1 H), 7.91 (d, J = 9.5 Hz, 1 H), 3.84–4.16 (m, 4 H), 1.75 (s, 3H). Anal. ($C_{10}H_{13}N_3O_2 \cdot HCl$) C, H, N.

2-Amidino-6-acetylpyridine 2′-Amidinohydrazone Dihydrochloride (11). To a solution of 0.55 g (4 mmol) of aminoguanidine hydrogen carbonate in 20 mL of H_2O and 2.1 mL of 2 N HCl was added 0.9 g (3.7 mmol) of **8** in 5 mL of H_2O, and the reaction mixture was heated under reflux for 2 h. The slightly yellow solution was cooled and evaporated to a small volume. After addition of EtOH the precipitate was filtered and washed with a small amount of EtOH, affording 0.7 g (64%) of **11** containing 0.4 mol of H_2O: mp ~310 °C dec; FAB (MS) $(M + H)^+$ 220; ^1H NMR (200 MHz, DMSO-d_6) δ 11.57–11.80 (broad, 1 H), 9.41–9.85 (m, 4 H), 8.86 (d, J = 9.5 Hz, 1 H), 8.41 (d, J = 9.5 Hz, 1 H), 7.92–8.35 (m, 5 H), 2.56 (s, 3 H). Anal. ($C_9H_{13}N_7 \cdot 2HCl \cdot 0.4H_2O$) C, H, N, Cl.

4-Cyano-2-acetylpyridine 2′-Amidinohydrazone Hydrochloride (9). A solution of 4.64 g (34 mmol) of aminoguanidine hydrogen carbonate in 10 mL of H_2O and 34.2 mL of 2 N HCl was added to a solution of 4.97 g (34 mmol) of **5**[17] in 75 mL of MeOH and 35 mL of THF. The reaction mixture was stirred for 0.5 h at room temperature, and the resulting suspension was cooled in ice bath. Precipitated solid was removed by filtration affording 5.62 g (68.7%) of crude **9** (mp 203–206 °C). An

analytical sample was prepared by crystallization from EtOH: mp 218–220 °C dec; ^{1}H NMR (200 MHz, DMSO-d_6) δ 11.58 (s, 1 H), 9.00 (d, 1 H), 8.84 (d, 1 H), 8.08 (bs, 4 H), 7.88 (dd, 1 H), 2.44 (s, 3 H). Anal. ($C_9H_{10}N_6$·HCl·0.6H_2O) C, H, N, Cl, H_2O.

4-Amidino-2-acetylpyridine 2'-Amidinohydrazone Dihydrochloride (12). To a stirred suspension of 2.26 g (9.5 mmol) of **9** in 30 mL of anhydrous MeOH was added 3.5 mL (18.9 mmol) of an approximately 5.4 N NaOMe solution, and the mixture was stirred at room temperature overnight. To the solution of methyl imino ester **10** thus formed was added 1.52 g (28.4 mmol) of NH_4Cl, and the stirring was continued for additional 18 h. The reaction mixture was filtered, and the filtrate was concentrated to approximately half of its volume under reduced pressure. The separated product was removed by filtration and washed with MeOH affording 0.14 g (5.0%) of **12** as an off-white solid: mp >250 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 11.67 (bs, 1 H), 9.90 (bs, 2 H), 9.55 (bs, 2 H), 8.90 (s, 1 H), 8.87 (d, 1 H), 7.84 (dd, 1 H), 2.47 (s, 3 H). Anal. ($C_9H_{13}N_7$·2HCl·0.25H_2O) C, H, Cl, H_2O; N: calcd, 33.05; found, 32.6.

4-Amidinoindan-1-one Hydrochloride (16). A mixture of 9.8 g (51.3 mmol) of **14** and 10.8 g (54 mmol) of triethyloxonium tetrafluoroborate in 500 mL of CH_2Cl_2 was stirred for 16 h at room temperature. After addition of 4.2 g (30.4 mmol) of K_2CO_3 and 4.2 mL of H_2O, the reaction mixture was filtered, washed with H_2O, dried, and evaporated, affording 11.1 g (98.7%) of a crude imino thioester **15**. The residue (50.6 mmol) was dissolved in 160 mL of EtOH, 3.3 g (60 mmol) of NH_4Cl was added, and the reaction mixture was heated under reflux for 20 h. After evaporation, the residue was purified by chromatography on Amberlite XAD 1180 (H_2O as eluent) and crystallized from EtOH and Et_2O, affording 5.3 g (49.7%) of **16** in form of a monohydrate: mp 215–218 °C dec; FAB (MS) $(M + H)^+$ 175; ^{1}H NMR (200 MHz, D_2O) δ 7.92 (m, 2 H), 7.58 (t, 1 H), 3.27 (t, 2 H), 2.78 (t, 2 H). Anal. ($C_{10}H_{10}N_2O$·HCl·H_2O) H, N; C: calcd, 52.52; found, 52.0.

4-Amidinoindan-1-one 2'-Amidinohydrazone Dihydrochloride (17). To a solution of 3.8 g (27.9 mmol) of aminoguanidine hydrogen carbonate and 14.7 mL of 2 N HCl in 200 mL of H_2O was added 5.85 g (27.8 mmol) of **16**, and the reaction mixture was stirred for 20 h at room temperature. The separated product was collected and recrystallized from H_2O, affording 6.44 g (72%) of **17** as monohydrate: mp >330 °C; FAB (MS) $(M + H)^+$ 231; ^{1}H NMR (200 MHz, D_2O) δ 8.01 (d, 1 H), 7.66 (m, 1 H), 7.51 (t, 1 H), 3.30 (m, 2 H), 2.91 (m, 2 H). Anal. ($C_{11}H_{14}N_6$·2HCl·H_2O) C, H, N, Cl.

Enzyme Preparations and Assays for Biological Activity. SAMDC from homogenized livers of MGBG-pretreated rats, diamine oxidase from rat small intestine, and ornithine decarboxylase from rat liver were prepared and assayed as described before.[12]

Antiproliferative Activity of SAMDC Inhibitors. Antiproliferative effects on human T24 bladder carcinoma cells were determined as described previously.[12,21] Cell proliferation was quantitated by staining with methylene blue.

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