S-Adenosylmethionine Decarboxylase Inhibitors: New Aryl and Heteroaryl Analogues of Methylglyoxal Bis(guanylhydrazone)

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A series of 3-acylbenzamidine (amidino)hydrazones **7a-h**, the corresponding (hetero)aromatic congeners **7i-p**, and 3,3'-bis-amidino-biaryls **25a-e** were synthesized. The hydrazones **7a-p** were prepared by conversion of the corresponding acyl nitriles **1a,c-d,i,n-p** to the imido esters **3a,c-d,i** and the amidines **5a,c-d,h-i**, followed by a reaction with aminoguanidine, or vice versa. Similarly, the biaryl 3,3'-dinitriles **23a-e** were converted, via the imino esters **24a-c** or the imino thioesters **27d-e**, to the diamidines **25a-e**. These new products are conformationally constrained analogues of methylglyoxal bis(guanylhydrazone) (MGBG). They are up to 100 times more potent as inhibitors of rat liver *S*-adenosylmethionine decarboxylase (SAMDC) and generally less potent inhibitors of rat small intestine diamine oxidase (DAO) than MGBG. Some of these SAMDC inhibitors, e.g., compounds **7a, 7e, 7i, 25a,** and **25d**, have shown antiproliferative effects against T24 human bladder carcinoma cells. These products, whose structure-activity relationships are discussed, are of interest as potential anticancer agents and drugs for the treatment of protozoal and *Pneumocystis carinii* infections.

Introduction

The diamine putrescine (PUT) and the polyamines spermidine (SPD) and spermine (SPM) play an important role in cell growth.[1,2] The biosynthetic pathway to SPD and SPM is controlled by *S*-adenosylmethionine decarboxylase (SAMDC), a rate-limiting enzyme of polyamine (PA) biosynthesis. From decarboxylated *S*-adenosylmethionine, the aminopropyl moiety is transferred to one or both amino groups of PUT, affording SPD or SPM. The activity of SAMDC is controlled by natural PAs[3-5] and is dependent on the metabolic status of the appropriate tissue. The enzyme activity is elevated in response to proliferative stimuli, and is generally increased in rapidly growing and neoplastic cells.[6] Depending on the biological system, the inhibition of intracellular SAMDC leads to arrest of cell proliferation in consequence of the depletion of SPD and SPM pools, or due to aberrant methylation by accumulated *S*-adenosylmethionine.[7]

Interest in SAMDC as a therapeutic target developed after the discovery that the antileukemic drug methylglyoxal bis(guanylhydrazone) (MGBG) inhibits SAMDC.[8] MGBG is a nonselective SAMDC inhibitor[9] that also inhibits diamine oxidase (DAO) and thereby prevents degradation of PUT.[10] MGBG is the first SAMDC inhibitor to show clinical efficacy;[11-12] however, owing to its severe toxicity it has not become established as an antitumor agent.[13-14] To a certain extent, the toxicity is thought to result from inhibition of pyruvate oxidation and the damage to mitochondrial structural integrity, and to be unrelated to interference with polyamine metabolism.[15-17]

In an attempt to find more selective SAMDC inhibitors as potential chemotherapeutic agents, several aliphatic congeners of MGBG were synthesized. Some of these

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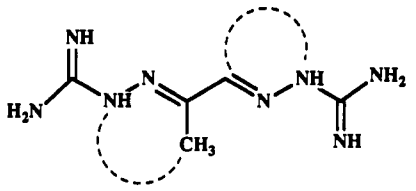


Figure 1. Proposed structural variations of MGBG.

products proved to have a more potent SAMDC inhibitory effect, greater selectivity for DAO and reduced toxicity.[18-20] An interesting feature of their structure–activity relations emerged from crystal structure analyses of MGBG and the inactive trifluoromethyl analogue. It was concluded that only compounds with an extended all-trans conformation and delocalized π electrons could inhibit SAMDC.[21-23] Proceeding from this hypothesis, we have developed new analogues of MGBG with partially fixed all-trans conformations by integrating parts of the chain into aromatic rings (Figure 1). Since, meanwhile, several antiparasitic agents, e.g. berenil or pentamidine, have been shown to inhibit SAMDC of mammalian as well as of parasitic origin, and vice versa, our SAMDC inhibitors may not only be of interest as potential anticancer agents, but also as new drugs for the treatment of, e.g., trypanosomiasis and of *Pneumocystis carinii* pneumonia.[24-27]

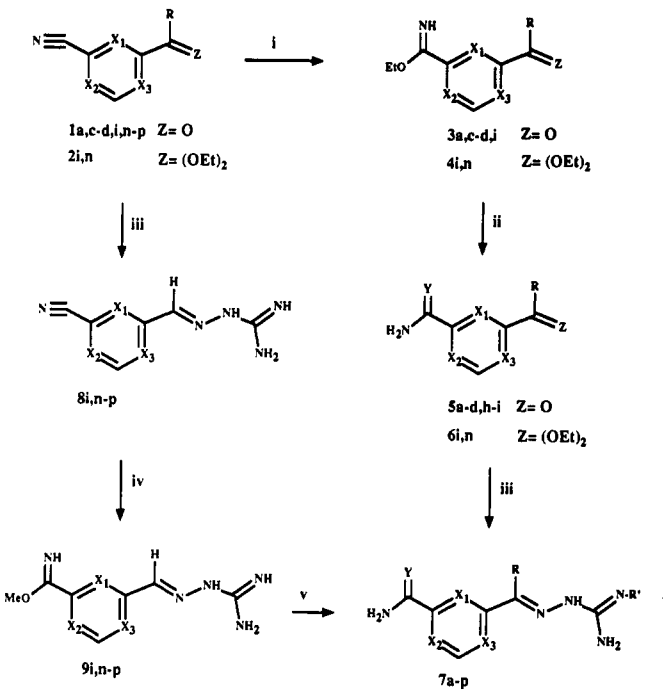
Chemistry

The synthesis of novel amidinoarylidene and (hetero)arylidene guanylhydrazones has been achieved using standard synthetic methods[28] and following the routes described in Scheme I. *m*-Acylbenzonitriles **1a,c–d** and 2-cyano-6-formylpyridine (**1i**)[29] were subjected to the Pinner reaction with ethanolic HCl, followed by reaction with ammonia or 1-butylamine, to give, via the imido esters **3a,c–d,i**, the amidines **5a,c–d,h–i**. Similarly, starting from cyanoformylpyridine diethylacetals **2i** and **2n**, the imido esters **4i** and **4n** were obtained, which upon treatment with ammonia gave the amidines **6i** and **6n**. The amidines **5a,c–d,h–i** and **6i,n**, and 3-formylbenzamide (**5b**) were then treated under slightly acidic conditions with aminoguanidine, yielding the corresponding hydrazones **7a–d,h–i,n**. Similarly, **5a** reacted with 1-amino-3-hydroxyguanidine and 1-amino-3-*n*-butylguanidine to give the guanylhydrazones **7e** and **7g**, respectively, whereas with the first reagent **5i** gave the product **7j**. Alternatively, the acylnitriles **1i,n–p** were treated first with aminoguanidine to obtain the guanylhydrazones **8i,n–p**. These were further converted by a base-catalyzed Pinner reaction to the imido esters **9i,n–p** which, without isolation and after treatment with ammonium chloride gave the amidines **7i,n–p**. The product **9i** was also treated with hydroxylamine hydrochloride and hydrazine hydrochloride, yielding **7k** and **7m**, respectively.

Treatment of **5a** and **5i** with 1,3-diaminoguanidine (**10**) led to the reaction of both amino groups of **10**, affording, besides the aminoguanylhydrazones **7f** and **7l**, the bis(hydrazone)s **11** and **12**, respectively (Scheme II).

The cyanopyridines **2i,n** and the pyrimidinecarbonitriles **1o** and **1p** used as starting materials have not been previously described in the literature, and a new synthesis for these intermediates had to be developed. The intermediates **2i** and **2n** were prepared by cyanation of appropriately substituted pyridine 1-oxides by analogy with the Reissert–Henze reaction.[30,31] The *N*-oxides

Scheme I [a]



[a] Reagents: (i) EtOH/HCl, Et_2O, 0 °C, 24–72 h; (ii) NH_3 or $NH_2C_4H_9$ in EtOH, 70 °C, 2–3 h; (iii) $NH_2NH(C{=}NH)NH_2{\cdot}H_2CO_3$, $NH_2NH(C{=}NOH)NH_2{\cdot}TsOH$, $NH_2NH(C{=}NH)NHNH_2{\cdot}HCl$, or $NH_2NH(C{=}NC_4H_9)NH_2{\cdot}HCl$, HCl, H_2O, 40–80 °C, 0.5–3 h; (iv) NaOMe, MeOH, 50 °C, 2–5 h; (v) NH_4Cl, $NH_2OH{\cdot}HCl$, $NH_2NH_2{\cdot}HCl$ or $NH_2C_4H_9{\cdot}HCl$, MeOH, 60 °C, 2–5 h.

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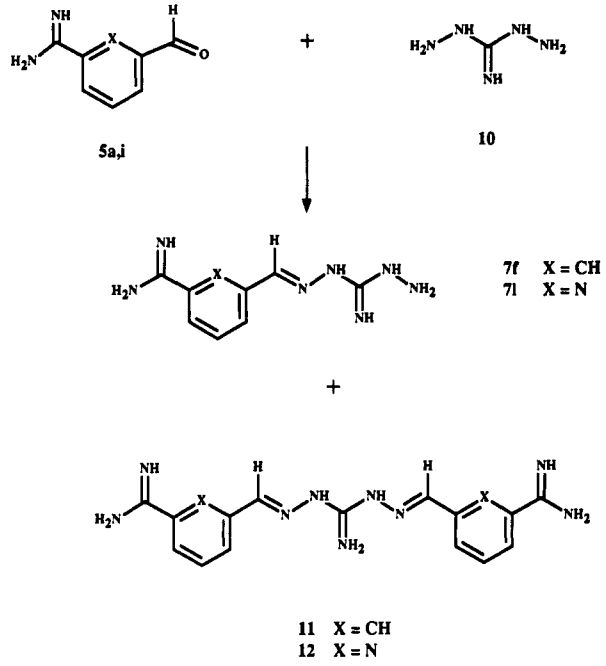
Scheme II

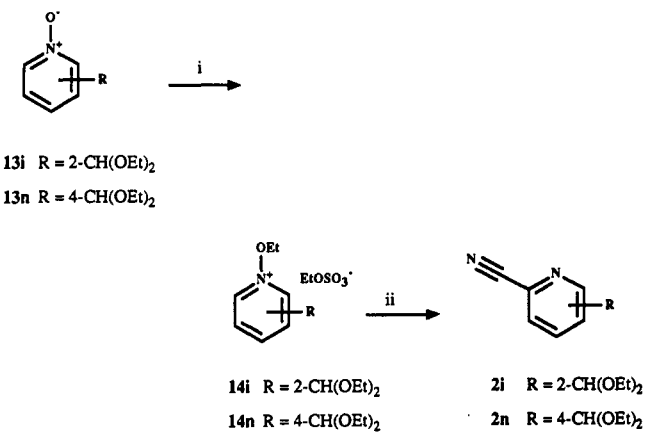


5a,i 10

7f X = CH
7l X = N

11 X = CH
12 X = N

Scheme III [a]



13i R = 2-CH(OEt)$_2$
13n R = 4-CH(OEt)$_2$

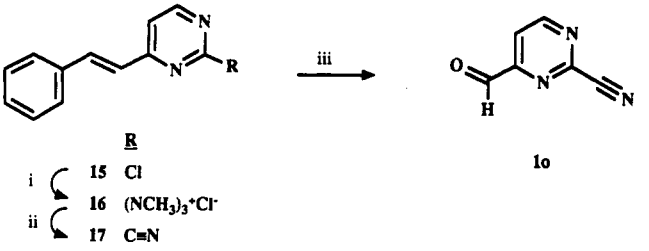
14i R = 2-CH(OEt)$_2$ 2i R = 2-CH(OEt)$_2$
14n R = 4-CH(OEt)$_2$ 2n R = 4-CH(OEt)$_2$

[a] Reagents: (i) $(C_2H_5O)_2SO_4$, CH_2Cl_2, room temperature, 6 h; (ii) NaCN, H_2O, room temperature, 8 h.

13i,n[32] were O-alkylated with diethyl sulfate and treated with NaCN to give **2i** and **2n** (Scheme III).

The formylpyrimidinecarbonitriles **1o** and **1p** were obtained via ozonolysis of the corresponding styrylpyrimidines **17** and **22**, respectively (Schemes IV and V). Compound **15**[33] reacted with trimethylamine in benzene, affording the quaternary ammonium salt **16**, which was sufficiently reactive to couple with NaCN to give **17**. This was then subjected to ozonolysis to give the formylpyrimidine **1o**. The isomeric 4-cyano-2-formylpyrimidine (**1p**) was obtained starting from the 4-hydroxy-6-methylpyrimidine 18[34] (Scheme V). Heating of **18** with phosphoryl chloride gave the chloro derivative **19**. The chlorine atom was removed by hydrogenation with Lindlar catalyst, and

Scheme IV [a]

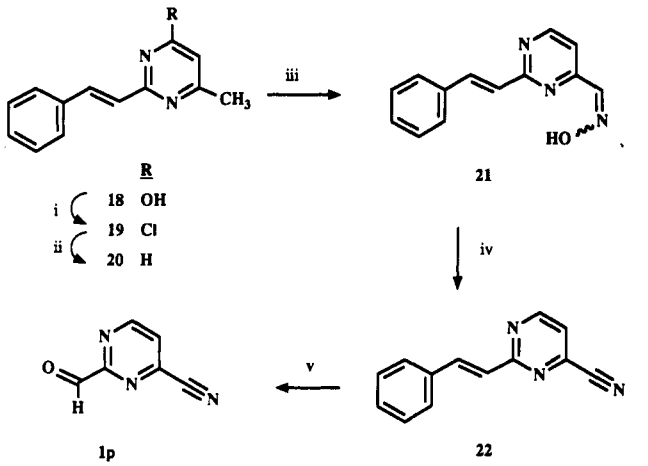


R
15 Cl
16 $(NCH_3)_3^+Cl^-$
17 C≡N

1o

[a] Reagents: (i) NMe_3, benzene; (ii) NEt_4CN, CH_2Cl_2, room temperature; (iii) O_3, MeOH/CH_2Cl_2 2:1, −70 °C, 0.5 h, then Me_2S.

Scheme V [a]



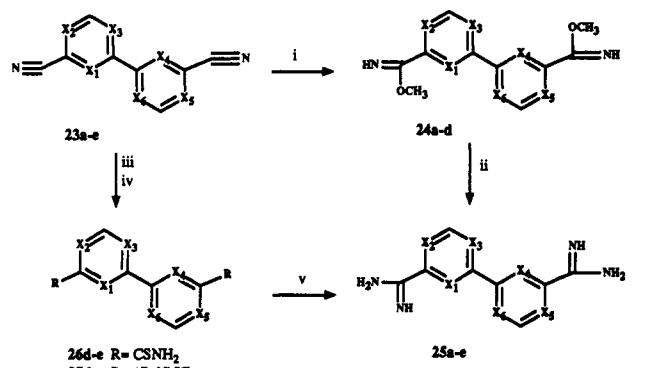
R
18 OH
19 Cl
20 H

21

1p 22

[a] Reagents: (i) $POCl_3$, reflux, 35 min; (ii) H_2/Lindlar catalyst, EtOH, NEt_3, room temperature, normal pressure, 7.5 h; (iii) Na/liquid NH_3, $FeCl_3$, *n*-butyl nitrite, −20 °C, 1 h, then NH_4Cl; (iv) $POCl_3$, reflux, 1 h; (v) O_3, MeOH/CH_2Cl_2 2:1, −70 °C, 0.5 h, and then Me_2S.

Scheme VI [a]



23a-e 24a-d

26d-e R= $CSNH_2$
27d-e R= (C=N)SEt

25a-e

[a] Reagents: (i) MeONa, absolute MeOH, room temperature to reflux, 5–48 h; (ii) NH_4Cl, NH_3/EtOH, reflux 1 h; (iii) H_2S, NEt_3, pyridine, 40 °C, 5 h; (iv) $Et_3O(BF_4)$, K_2CO_3, CH_2Cl_2, room temperature; (v) NH_4Cl, EtOH, reflux.

the product **20** was treated with 1-butyl nitrite to give a mixture of the oximes **21**. Compound **21** was then refluxed with phosphoryl chloride, followed by ozonolysis, yielding the formylpyrimidine **1p**.

The novel biaryl-3,3′-diamidines were synthesized following the routes described in Scheme VI. Stirring of a methanolic solution of the biaryl 3,3′-dinitriles **23a** and **23b**[35] with a catalytic amount of sodium methoxide for 2 days at room temperature, or in the case of **23c** refluxing

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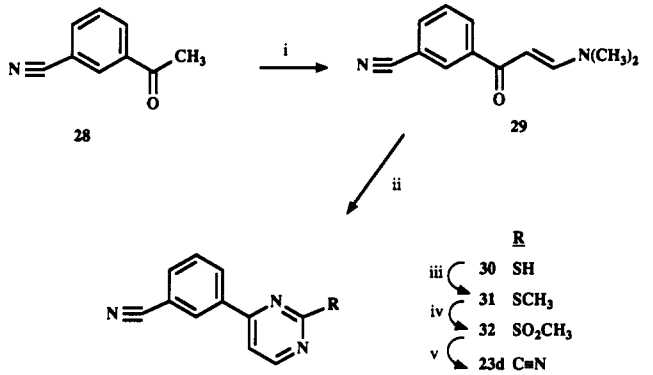
Scheme VII [a]



[a] Reagents: (i) $(CH_3)_2N[CH(OCH_3)_2]$, 100 °C, 1 h; (ii) thiourea, KOH, EtOH, 90 °C, 4 h; (iii) $(CH_3O)_2SO_2$, 2 N NaOH, H_2O, room temperature, 16 h; (iv) *m*-CPBA, CH_2Cl_2, room temperature, 1 h; (vi) KCN, DMF, 100 °C, 2.5 h.

for 5 h, gave the corresponding bis-imino esters **24a–c**. Treatment of **24a–c** with dry ammonia and ammonium chloride in refluxing EtOH gave the diamidines **25a–c**. Compounds **23d** and **23e**[36] were converted to the corresponding bis-amidines **25d–e** using an alternative procedure via the imino thioesters **27d–e**. Hydrogen sulfide was passed through a pyridine solution of **23d–e** to give the thioamides **26d–e**, which were S-alkylated and converted to the desired diamidines as described above.

Compound **23a** as a starting material was synthesized by O-methylation of 2,2′-bipyridine 1,1′-dioxide[37] followed by reaction with sodium cyanide, whereas **23c** was obtained by dehydration of 4,4′-dicarbamoyl-2,2′-bipyridine[38] with thionyl chloride. The synthesis of **23d** was carried out as outlined in Scheme VII. The phenyl–pyrimidine moiety was constructed by starting with cyanoacetophenone **28** and reacting with *N,N′*-dimethylformamide dimethylacetal, followed by the ring closure of **29** with thiourea. After S-methylation of **30** the 2-methylthio group of **31** was oxidized with *m*-CPBA and replaced by the reaction with potassium cyanide to give the target dinitrile **23d**.

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

Pharmacological Results and Discussion

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

As shown in Table IV, the hydrazone **7a** inhibited SAMDC with an IC_{50} of 36 nM and was about 25 times more potent than MGBG (IC_{50} 1 μM). In addition, compound **7a** inhibited DAO with an IC_{50} of 5.6 μM and had a 100-fold higher selectivity index (IC_{50} DAO/IC_{50} SAMDC) than MGBG. The identification of this product, in which one part of the chain of MGBG had been replaced by a bulky but conformationally constrained 3-amidinophenyl residue, and whose molecular structure was superimposable on the crystal structure of MGBG, led us to further investigate the effects of structural variations of **7a** on SAMDC inhibitory activity.

Replacement of the aromatic amidino group by a carbamoyl group in **7b**, or alkyl substitution of the amidino group in **7h**, resulted in a significant reduction of SAMDC inhibitory potency. The SAMDC inhibition was retained after introduction of an alkyl substituent R, e.g., in **7c,d** (IC_{50} 66–88 nM), and after R′ substitution of the amidinohydrazone, e.g., in **7e–g** (IC_{50} 92–210 nM). All these products inhibited DAO at higher concentrations than MGBG and had higher selectivity indices.

To test the role of the nitrogen atom in position X_1, as in MGBG, the 3-amidinophenyl residue was replaced by a 2-(6-amidinopyridyl) residue, e.g., in **7i–m**. Compound **7i** inhibited SAMDC 10 times more potently than MGBG and was similar in potency to MGBG in its effect on DAO. The *N*-amino-substituted amidines **7l** and **7m** inhibited SAMDC with IC_{50}s of 0.67 and 0.084 μM, respectively, whereas the *N*-hydroxyamidino hydrazone **7j** was 100 times as potent as the amide oxime **7k**. Two side products, the bis(arylidene)aminoguanidines **11** and **12**, were formed along with the (*N*-amino-guanyl)hydrazones **7f** and **7l**. These showed high SAMDC inhibitory activity (IC_{50} 0.061 and 0.12 μM) and excellent selectivity indices.

Movement of the ring nitrogen from position X_1 into X_2, e.g., in 4-(2-amidinopyridyl) derivative **7n**, or the introduction of a second ring nitrogen in position X_2, as in **7o**, resulted in a 3–5-fold reduction of SAMDC inhibitory activity as compared to **7i**. The pyrimidine derivative **7o** was 20 times more potent in inhibiting SAMDC than product **7p**, which bears ring nitrogens in the position X_1 and X_3. These results, together with the results of molecular modeling studies, suggest that low-energy conformations of **7i** and **7o** represent a coplanar arrangement of the ring and the side chain with an *anti*periplanar orientation of the lone pairs of the nitrogen X_1 and the imine hydrazone nitrogen. The weaker activity of **7p** seems to be mainly due to an unfavorable repulsive electrostatic interaction between the lone pairs of the nitrogen X_3 and the imine hydrazone nitrogen, forcing the side chain from the coplanar conformation with the ring. A similar observation has been made with the trifluoromethyl analogue of MGBG, in which an intramolecular hydrogen bond let to an unfavorable conformation.[22]

All tested compounds were weaker inhibitors of cell proliferation than MGBG; the most potent products **7a**, **7e**, and **7i** showed 50% growth inhibition of T24 cells at 10 times higher concentrations than MGBG. Reduced cellular uptake rates, as well as lower levels of intracellular accumulation of the new inhibitors as compared to MGBG may contribute to this apparent discrepancy between enzyme inhibitory and antiproliferative potency of certain compounds. In addition, variations among these compounds with respect to metabolic stability may affect their antiproliferative potency. To understand these complex correlations, a detailed experimental analysis is needed for every single compound. The pronounced cytotoxicity of MGBG, as mentioned before, is believed to be, at least partly, due to its polyamine-unrelated effects on mitochondrial structure and function.[17] Such polyamine-unrelated effects may add another parameter of complexity to the correlation between inhibition of SAMDC and antiproliferative effects.

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(37) Fielden, R.; Summers, L. A. Chemical Constitution and Activity of Bipyridylium Herbicides. Part IX. N-Alkoxy Quaternary Salts of 4,4′-Bipyridyl. *J. Heterocycl. Chem.* **1974**, *11*, 299–301.

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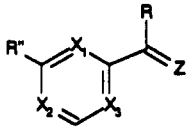
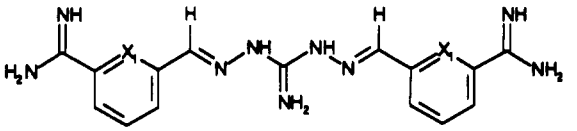
Table I. Structure and Properties of New Compounds



1-9



11-12

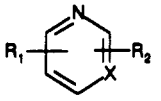
comp	R″	X_1	X_2	X_3	R	Z[a]	yield % (method)[b]	mp, °C	mol wt	formula	anal.[c]
3a	C(=NH)OEt	CH	CH	CH	H	O	62 (A)	126–8	213.7	$C_{10}H_{11}NO_2$[d]	C,H,N,Cl[e]
5a	$C(=NH)NH_2$	CH	CH	CH	H	O	93 (B)	152–4	184.6	$C_8H_8N_2O$[d]	nd
7a	$C(=NH)NH_2$	CH	CH	CH	H	GH	34 (C)	206–7	284.4	$C_9H_{12}N_6$[f,g]	C,H,N,Cl
7b	$C(=O)NH_2$	CH	CH	CH	H	GH	51 (C)	270	272.3	$C_9H_{11}N_5O$[h,i]	C,H,N,S
3c	C(=NH)OEt	CH	CH	CH	CH_3	O	57 (A)	110	227.8	$C_{11}H_{13}NO_2$[d]	C,H,N,Cl
5c	$C(=NH)NH_2$	CH	CH	CH	CH_3	O	75 (B)	oil	198.7	$C_9H_{10}N_2O$[d]	nd
7c	$C(=NH)NH_2$	CH	CH	CH	CH_3	GH	59 (C)	185	304.5	$C_{10}H_{14}N_6$[f,j]	C,H,N,Cl
3d	C(=NH)OEt	CH	CH	CH	C_2H_5	O	66 (A)	118–20	241.7	$C_{12}H_{15}NO_2$[d]	C,H,N,Cl
5d	$C(=NH)NH_2$	CH	CH	CH	C_2H_5	O	95 (B)	oil	212.7	$C_{10}H_{12}N_2O$[d]	nd
7d	$C(=NH)NH_2$	CH	CH	CH	C_2H_5	GH	75 (C)	amorph	383.7	$C_{11}H_{16}N_6$[k,i]	C,H,N,S
7e	$C(=NH)NH_2$	CH	CH	CH	H	G–OH	50 (C)	248–50	293.2	$C_9H_{12}N_6O$[f,l]	C,H,N,Cl
7f	$C(=NH)NH_2$	CH	CH	CH	H	G–NH_2	25 (G)	160	319.2	$C_9H_{13}N_7$[f,m]	C,H,N
7g	$C(=NH)NH_2$	CH	CH	CH	H	G–C_4H_9	30 (C)	140	351.0	$C_{13}H_{20}N_6$[f,n]	C,H,N,Cl
5h	$C(=NC_4H_9)NH_2$	CH	CH	CH	H	O	ni (B)	nd	240.7	$C_{12}H_{16}N_2O$[d]	nd
7h	$C(=NC_4H_9)NH_2$	CH	CH	CH	H	GH	42 (C)	230	351.3	$C_{13}H_{20}N_6$[f,i]	C,H,N
3i	C(=NH)OEt	N	CH	CH	H	O	100 (A)	oil	214.7	$C_9H_{10}N_2O_2$[d]	nd
5i	$C(=NH)NH_2$	N	CH	CH	H	O	94 (B)	oil	185.6	$C_7H_7N_3O$[d]	nd
4i	C(=NH)OEt	N	CH	CH	H	$(OEt)_2$	100 (A)	oil	288.8	$C_{13}H_{20}N_2O_3$[d]	nd
6i	$C(=NH)NH_2$	N	CH	CH	H	$(OEt)_2$	92 (B)	oil	259.7	$C_{11}H_{17}N_3O_2$[d]	nd
7i	$C(=NH)NH_2$	N	CH	CH	H	GH	41 (C)	160	296.2	$C_8H_{11}N_7$[f,i]	C,H,N,Cl
8i	C≡N	N	CH	CH	H	GH	63 (E)	295–8	224.7	$C_8H_8N_6$[d]	C,H,N,Cl
9i	C(=NH)OMe	N	CH	CH	H	GH	ni (F)	nd	220.2	$C_9H_{12}N_6O$	nd
7j	$C(=NH)NH_2$	N	CH	CH	H	G–OH	34 (D)	230–5	565.0	$C_8H_{11}N_7O$[o]	C,H,N
7k	$C(=NOH)NH_2$	N	CH	CH	H	GH	63 (F)	260–3	312.2	$C_8H_{11}N_7O$[f,i]	C,H,N,Cl
7l	$C(=NH)NH_2$	N	CH	CH	H	G–NH_2	25 (G)	250–3	298.4	$C_8H_{12}N_8$[f,p]	C,H,N
7m	$C(=NNH_2)NH_2$	N	CH	CH	H	GH	42 (F)	245–8	312.2	$C_8H_{12}N_8$[f,i]	C,H,N,Cl
2n	C≡N	CH	N	CH	H	$(OEt)_2$	76	61	206.3	$C_{11}H_{14}N_2O_2$	C,H,N
4n	C(=NH)OEt	CH	N	CH	H	$(OEt)_2$	83 (A)	95–7	252.8	$C_{13}H_{20}N_2O_3$	C,H,N
6n	$C(=NH)NH_2$	CH	N	CH	H	$(OEt)_2$	ni (B)	nd	259.7	$C_{11}H_{17}N_3O_2$[d]	nd
7n	$C(=NH)NH_2$	CH	N	CH	H	GH	52 (D)	290	298.2	$C_8H_{11}N_7$[f,m]	C,H,N,Cl
1o	C≡N	N	N	CH	H	O	50	oil	133.1	$C_6H_3N_3O$	nd
8o	C≡N	N	N	CH	H	GH	66 (E)	>240	225.6	$C_7H_7N_7$[d]	C,H,N,Cl
9o	C(=NH)OMe	N	N	CH	H	GH	ni (F)	nd	221.2	$C_8H_{11}N_7O$	nd
7o	$C(=NH)NH_2$	N	N	CH	H	GH	40 (F)	200	297.1	$C_7H_{10}N_8$[f,i]	C,H,N,Cl
1p	C≡N	N	CH	N	H	O	82	oil	133.1	$C_6H_3N_3O$	nd
8p	C≡N	N	CH	N	H	GH	60 (E)	200	244.4	$C_7H_7N_7$[d,i]	C,H,N,Cl
9p	C(=NH)OMe	N	CH	N	H	GH	ni (F)	nd	221.2	$C_8H_{11}N_7O$	nd
7p	$C(=NH)NH_2$	N	CH	N	H	GH	78 (F)	>240	297.1	$C_7H_{10}N_8$[f,i]	C,H,N,Cl
11		CH					27 (G)	230	471.3	$C_{17}H_{19}N_9$[q,i]	C,H,N,Cl
12		N					16 (G)	260–5	478.5	$C_{15}H_{17}N_{11}$[r,i]	C,H,N,Cl

[a] GH = =NNH(C=NH)NH_2, G–OH = =NNH(C=NOH)NH_2, G–NH_2 = =NNH(C=NNH_2)NH_2, G–C_4H_9 = =NNH(C=NC_4H_9)NH_2. [b] ni = not isolated. [c] Analytical results were within ±0.4% of the theoretical value; nd = not determined. [d] Hydrochloride. [e] Cl: calcd, 16.56; found, 15.8. [f] Dihydrochloride. [g] 0.4 Hydrate. [h] Hemisulfate. [i] Hydrate. [j] 0.75 Hydrate. [k] 1.1 Sulfate–0.7 hydrochloride. [l] 0.11 Hydrate. [m] 1.1 Hydrate. [n] 0.9 Hydrate. [o] Di-*p*-toluenesulfonate. [p] 0.3 Hydrate. [q] 2.85 Hydrochloride. [r] Trihydrochloride.

On the basis of the results discussed above, we replaced the guanylhydrazone side chain of **7a** by a second amidino(hetero)aryl residue and prepared a series of the biaryldiamidines **25a–e** (Table V). The biphenyl derivative **25e** inhibited SAMDC at a concentration (IC_{50} 0.6 μM) similar to MGBG. A 3-fold increase in SAMDC inhibitory activity as compared to **25e** was observed with the bipyridyl derivative **25b** (X_2, X_5 = N), whereas a similar decrease in activity was found with **25c** (X_3, X_6 = N). Interestingly, the bipyridine **25a** (X_1, X_4 = N) and the phenylpyrimidine derivative **25d** (X_4, X_5 = N) were 100 times more potent than **25e** and highly selective. Compound **25a** was equal to MGBG in its inhibitory effect on T24 cell proliferation.

These results are in agreement with those obtained with the monocyclic derivatives discussed above. Similarly, as in the case of **7i** and **7o**, the symmetrical compound **25a** has one extended and low-energy conformation. Its molecular structure is essentially planar, having torsional angles of about 14° between the rings, as well as between the amidino groups and the rings. The structures of the other diamidines are superimposable, except that, due to steric interactions of the hydrogen atoms in positions X_1 and X_6 and positions X_3 and X_4, respectively, the torsional angle of the rings of **25b** and **25e** is approximately 40°. The data obtained with **25c**, however, suggest that the planar character of a structure alone is insufficient for strong inhibition of SAMDC. Some additional factors, e.g., electrostatic potential on the surface or the presence of at least one ring nitrogen atom in position X_1 or X_4, may be of importance for biochemical activity. Such nitrogen atoms are present not only in **25a** and **25d**, but also in the side chain of the guanylhydrazones described above. The corresponding nitrogen in analogues of MGBG was previously reported to enter hydrogen bridges,[22] suggesting that, besides the two amidino groups placed in

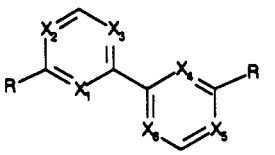
Table II. Structures and Physical Properties of Intermediate Compounds



compd	R_1	X	R_2	yield %	mp, °C	mol wt	formula	anal.[a]
14i	1-OEt	CH	2-CH(OEt)$_2$	100	oil	351.4	$C_{12}H_{20}NO_3^+ \cdot C_2H_5SO_4^-$	nd
2i	2-CN	CH	6-CH(OEt)$_2$	89	oil	206.3	$C_{11}H_{14}N_2O_2$	C,H,N
14n	1-OEt	CH	4-CH(OEt)$_2$	100	oil	351.4	$C_{12}H_{20}NO_3^+ \cdot C_2H_5SO_4^-$	nd
2n	2-CN	CH	4-CH(OEt)$_2$	76	61	206.3	$C_{11}H_{14}N_2O_2$	C,H,N
16	2-$NMe_3^+Cl^-$	N	4-CH=CHPh	100	amorph	275.8	$C_{15}H_{18}ClN_3$	C,H,N
17	2-CN	N	4-CH=CHPh	28	190	207.2	$C_{13}H_9N_3$	C,H,N
1o	2-CN	N	4-CHO	50	oil	133.1	$C_6H_3N_3O$	nd
19	4-Cl-6-Me	N	2-CH=CHPh	51	71–73[b]	230.7	$C_{13}H_{11}ClN_2$	C,H,N
20	4-Me	N	2-CH=CHPh	70	65–67	196.3	$C_{13}H_{12}N_2$	C,H,N
21	4-CH=NOH	N	2-CH=CHPh	46	192–5	225.3	$C_{13}H_{11}N_3O$	C,H,N
22	4-CN	N	2-CH=CHPh	78	147–50	207.2	$C_{13}H_9N_3$	C,H,N
1p	4-CN	N	2-CHO	82	oil	133.1	$C_6H_3N_3O$	nd

[a] Analytical results were within ±0.4% of the theoretical value. [b] bp 118–22 °C (0.1 mbar).

Table III. Structures and Physical Properties of New Biaryl Derivatives



compd	R	X_1	X_2	X_3	X_4	X_5	X_6	R′	yield % (method)[a]	mp, °C	mol wt	formula	anal.[b]
23a	C≡N	N	CH	CH	N	CH	CH	C≡N	91	255–8	206.2	$C_{12}H_6N_4$	C,H,N
24a	C(=NH)OMe	N	CH	CH	N	CH	CH	C(=NH)OMe	ni (H)	nd	286.3	$C_{15}H_{18}N_4O_2$	nd
25a	C(=NH)NH_2	N	CH	CH	N	CH	CH	C(=NH)NH_2	48 (H)	>300	316.8	$C_{12}H_{12}N_6$[c,d]	C,H,N,Cl
24b	C(=NH)OMe	CH	N	CH	CH	N	CH	C(=NH)OMe	ni (H)	nd	286.3	$C_{15}H_{18}N_4O_2$	nd
25b	C(=NH)NH_2	CH	N	CH	CH	N	CH	C(=NH)NH_2	34 (H)	>280	327.6	$C_{12}H_{12}N_6$[c,e]	C,H,N,Cl
23c	C≡N	CH	CH	N	CH	CH	N	C≡N	56	238–40	206.2	$C_{12}H_6N_4$	C,H,N
24c	C(=NH)OMe	CH	CH	N	CH	CH	N	C(=NH)OMe	ni (H)	nd	286.3	$C_{15}H_{18}N_4O_2$	nd
25c	C(=NH)NH_2	CH	CH	N	CH	CH	N	C(=NH)NH_2	36 (H)	>280	334.8	$C_{12}H_{12}N_6$[c,f]	C,H,N,Cl
23d	C≡N	CH	CH	CH	N	N	CH	C≡N	61	185	206.2	$C_{12}H_6N_4$	C,H,N
26d	C(=S)NH_2	CH	CH	CH	N	N	CH	C(=S)NH_2	91 (I)	210	274.4	$C_{12}H_{10}N_4S_2$	C,H,N
27d	C(=NH)SEt	CH	CH	CH	N	N	CH	C(=NH)SEt	98 (J)	amorph	330.3	$C_{16}H_{18}N_4S_2$	nd
25d	C(=NH)NH_2	CH	CH	CH	N	N	CH	C(=NH)NH_2	52 (J)	>230	315.0	$C_{12}H_{12}N_6$[c,g]	C,H,N,Cl
26e	C(=S)NH_2	CH	CH	CH	CH	CH	CH	C(=S)NH_2	76 (I)	188–90	272.4	$C_{14}H_{12}N_2S_2$	C,H,N,S
27e	C(=NH)SEt	CH	CH	CH	CH	CH	CH	C(=NH)SEt	88 (J)	amorph	344.5	$C_{19}H_{24}N_2S_2$	nd
25e	C(=NH)NH_2	CH	CH	CH	CH	CH	CH	C(=NH)NH_2	38 (J)	230–5	347.3	$C_{14}H_{14}N_4$[c,h]	C,H,N,Cl
30	C≡N	CH	CH	CH	N	N	CH	SH	74	250	251.4	$C_{11}H_6KN_3S$	nd
31	C≡N	CH	CH	CH	N	N	CH	SCH_3	73	117–8	227.3	$C_{12}H_9N_3S$	C,H,N
32	C≡N	CH	CH	CH	N	N	CH	SO_2CH_3	80	165–7	259.3	$C_{12}H_9N_3O_2S$	C,H,N

[a] ni = not isolated. [b] Analytical results were within ±0.4% of the theoretical value; nd = not determined. [c] Dihydrochloride. [d] 0.2 Hydrate. [e] 0.8 Hydrate. [f] 1.2 Hydrate. [g] 0.1 Hydrate. [h] Dihydrate.

an appropriate geometrical arrangement, this particular nitrogen atom may also act as a hydrogen-bond acceptor in inhibitors of SAMDC.

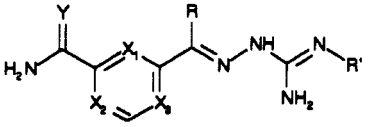
In conclusion, we have developed new conformationally constrained (hetero)aryl analogues of MGBG that were up to 100 times more potent SAMDC inhibitors than MGBG and showed better SAMDC/DAO selectivity indices. The products **7a**, **7e**, **7i**, and **25a** inhibited the growth of human T24 bladder carcinoma cells in low micromolar concentration and are of interest as potential anticancer agents. Some of the novel drugs may be also useful for the treatment of protozoal infections, e.g. by Trypanosoma or *Pneumocystis carinii*. Moreover, the results of our study have shown that the most potent SAMDC inhibitors from both classes of compounds, guanylhydrazones and biaryldiamidines, have two bidentate, and in the case of guanylhydrazones, different, basic groups, have planar low-energy conformations, and are superimposable on the extended conformation of MGBG. The structure–activity relationships of biaryl diamidines further suggest that the ring nitrogen corresponding to the sp_2 nitrogen of the hydrazones, may act as a hydrogen-bond acceptor.[39]

Experimental Section

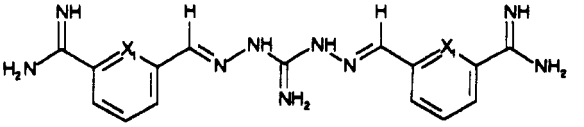
The reference compound MGBG and the educts **1a** and **1c** were purchased from commercial sources (Aldrich, Fluka). Melting points were determined in open capillary tubes and are uncorrected. TLC of each compound was performed on Merck F 254 silica gel or Antec OPTI-UPC$_{12}$ F254 plates and column 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), ^{1}H NMR spectra (Varian HA-100D or Bruker WM-250), 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).[40]

(39) Portions of this work were presented at the XIIth International Symposium on Medicinal Chemistry, Basel, Switzerland, September 1992, Abstract OC-05.5.

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



7a-p

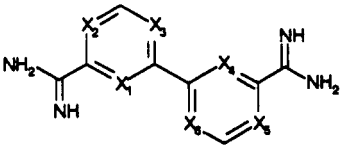


11-12

compd	Y	X_1	X_2	X_3	R	R'	IC_{50}, μM[d] SAMDC[a]	DAO[b]	T24[c]
7a	NH	CH	CH	CH	H	H	0.036	5.6	9.1
7b	O	CH	CH	CH	H	H	20.0	>100	>184
7c	NH	CH	CH	CH	CH_3	H	0.066	2.7	117.9
7d	NH	CH	CH	CH	C_2H_5	H	0.088	15	>152
7e	NH	CH	CH	CH	H	OH	0.092	20	14.1
7f	NH	CH	CH	CH	H	NH_2	0.21	0.87	25.0
7g	NH	CH	CH	CH	H	C_4H_9	0.16	2.8	>26.8
7h	NC_4H_9	CH	CH	CH	H	H	>50	5	>28.5
7i	NH	N	CH	CH	H	H	0.09	0.87	10.1
7j	NH	N	CH	CH	H	OH	0.24	7.5	32.9
7k	N-OH	N	CH	CH	H	H	25.0	>100	>119
7l	NH	N	CH	CH	H	NH_2	0.67	1.66	83.6
7m	$N-NH_2$	N	CH	CH	H	H	0.084	19	60.5
7n	NH	CH	N	CH	H	H	0.33	3.2	>180
7o	NH	N	N	CH	H	H	0.53	8.05	>168
7p	NH	N	CH	N	H	H	11.0	3.4	>126
11		CH					0.061	0.83	>101
12		N					0.12	0.76	49.8
MGBG							1.0	1.5	1.1

[a] *S*-Adenosylmethionine decarboxylase from rat liver. [b] Diamine oxidase from rat small intestine. [c] Human T24 bladder carcinoma cells. [d] The data are presented as the mean of at least three independent determinations; nd = not determined.

Table V. Inhibition of Enzymatic Activities and T24 Cell Growth by Diamidines



compd	X_1	X_2	X_3	X_4	X_5	X_6	IC_{50}, μM[d] SAMDC[a]	DAO[b]	T24[c]
25a	N	CH	CH	N	CH	CH	0.006	240	1.1
25b	CH	N	CH	CH	N	CH	0.18	12.9	>151
25c	CH	CH	N	CH	CH	N	3.1	nd	>143
25d	CH	CH	CH	N	N	CH	0.006	10	68.2
25e	CH	CH	CH	CH	CH	CH	0.6	nd	>160
MGBG							1.0	1.5	1.1

[a-d] Refer to Table IV.

General Method for the Pinner Synthesis of Imino Esters (Method A). Ethyl-3-formylbenzimidate Hydrochloride (3a). A solution of 86.7 g (0.662 mol) of **1a** in 530 mL of dry ether and 59.7 mL (1.025 mol) of absolute ethanol was cooled to 0 °C, saturated with dry HCl gas, and left to stand for 6 days at 0 °C. After filtration of a fine precipitate, 1 L of ether was added to the reaction solution, affording 86.0 g (61.5%) of crystalline **3a**: mp 126–128 °C (with foaming); ^{1}H NMR (250 MHz, DMSO-d_6) δ 1.5 (t, 3 H), 4.68 (q, 2 H), 7.88 (t, 1 H), 8.28–8.65 (m, 3 H), 10.12 (s, 1 H). Anal. ($C_{10}H_{11}NO_2$·HCl) C, H, N; Cl: calcd, 16.56; found, 15.8.

The same procedure was used for the preparation of compounds **3c–d,i** and **4i,n**. In contrast to the reported instability of benzaldehyde acetals during the Pinner synthesis of imino esters,[29] no cleavage of acetals was observed by the synthesis of **4i** and **4n**.

General Method for the Pinner Synthesis of Amidines (Method B). 3-Formylbenzamidine Hydrochloride (5a). A solution of 21.3 g (0.1 mol) of **3a** in 250 mL of absolute ethanol was treated with 250 mL of a saturated ethanolic ammonia solution, and the whole was heated at 70 °C for 3 h. After cooling, the ethanol was removed by evaporation. The residue was crystallized from ethanol/ether, affording 17.1 g (93%) of crude **5a**. The product contained some ammonium chloride and was used without further purification.

The same procedure, using also 1-butylamine (2 equiv) instead of ammonia, was applied for the preparation of compounds **5c–d,h–i** and **6i,n**.

3-Formylbenzamidine 2′-Amidinohydrazone Dihydrochloride (7a). (Method C). A solution of 18.5 g (0.1 mol) of crude **5a** in 200 mL of ethanol was added to a mixture of 17.2 g (0.1 mol) of aminoguanidine sulfate, 60 mL of water, and a few drops of concentrated sulfuric acid, and the whole was boiled under reflux for 15 min. Upon cooling, 20 g of **7a** crystallized out in the form of the sulfate salt. This was added to a solution of 6.3 g (0.15 mol) of NaOH in 400 mL of ethanol and heated at 70 °C for 2.5 h. After cooling, undissolved material was removed by filtration, and the filtrate was concentrated by evaporation. The residue was dissolved in a small amount of ethanol and acidified with 10% ethanolic hydrochloric acid, affording 9.7 g (34%) of **7a**, mp 206–207 °C. Anal. ($C_9H_{12}N_6$·2HCl·0.4H_2O) C, H, N, Cl.

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The same procedure, starting from **5b**,[41] **c–d,h–i** and using 1-hydroxy-3-aminoguanidine *p*-toluenesulfonate,[42] 1-amino-3-(1-butyl)guanidine hydrochloride,[43] or aminoguanidine hydrogencarbonate in dilute HCl instead of aminoguanidine sulfate, was applied for the preparation of compounds **7b–e,g–i**.

1-Hydroxy-3-[[(2′-amidino-6′-pyridyl)methylene]amino]guanidine Di-*p*-toluenesulfonate (7j). (Method D). A solution of 1.99 g (7.6 mmol) of 1-amino-3-hydroxyguanidine toluenesulfonate in 4 mL of water was added to a solution of 2.2 g (7.6 mmol) of **6i** in 15 mL of methanol, and after the addition of 3.7 mL of 2 N HCl, the reaction mixture was heated at 70 °C for 1 h. After cooling, the reaction mixture was evaporated. The residue was purified by chromatography over Amberlite XAD 1180 (water as eluent) and subsequently crystallized from ethanol/ether, affording 1.48 g (34%) of pure **7j**, mp 230–235 °C dec. Anal. ($C_8H_{11}N_7O \cdot 2C_7H_8O_3S$) C, H, N.

The same procedure was used for the preparation of **7n**.

4-Cyano-2-formylpyrimidine 2′-Amidinohydrazone Hydrochloride (8p). (Method E). To a solution of 207 mg (1.49 mmol) of aminoguanidine hydrogen carbonate in 1.65 mL of 2 N HCl was added a solution of 200 mg (1.5 mmol) of **1p** in 1.5 mL of methanol. The mixture was heated to 70 °C for 45 min and evaporated. The residue was crystallized from dilute ethanol, affording 0.20 g (60%) of **8p**: mp 200 °C dec; ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.16 (s, 1 H), 8.3 (s, 1 H), 9.23 (d, 1 H), 7.97 (bs, 1 H), 12.65 (bs, 1 H). Anal. ($C_7H_7N_7 \cdot HCl \cdot H_2O$) C, H, N, Cl.

The same procedure was used for the preparation of compounds **8i,n–o**.

2-Amidino-4-formylpyrimidine 2′-Amidinohydrazone Dihydrochloride (7o). (Method F). A solution of 200 mg (0.8 mmol) of **8o** in 10 mL of methanol was stirred with 300 mg of molecular sieve 3 Å for 1 h at room temperature, followed by the addition of 0.97 mL of 1.9 M sodium methoxide and continued stirring for a further 2.5 h at 50 °C. The reaction mixture of **9o** was then treated with 64.1 mg of dry ammonium chloride and was stirred for 7 h at 50 °C. After cooling, 1 N HCl was added until pH 2–3, and the reaction mixture was filtered and evaporated. The residue was purified by reversed-phase chromatography on Opti-UP-C_{12} silica gel (Antec) with water. The product was then crystallized from methanol and ether, affording 95 mg (40%) of **7o**: mp 200 °C dec; FAB (MS): $(M + H)^+ = 207$; ^{1}H NMR (DMSO-d_6) δ 9.6–10.0 (bm), 9.16 (d), 8.62 (d), 8.0–8.2 (bm). Anal. ($C_7H_{10}N_8 \cdot 2HCl \cdot H_2O$) C, H, N, Cl.

The same procedure, also using hydroxylamine hydrochloride or hydrazine hydrochloride instead of ammonium chloride, was used for the preparation of compounds **7i, 7k, 7m–n**, and **7p**.

3-Amino-1-[(3′-amidinobenzylidene)amino]guanidine Dihydrochloride (7f) and 1,3-Bis[(3′-amidinobenzylidene)amino]guanidine Trihydrochloride (11). (Method G). To a solution of 1.25 g (0.01 mol) of 1,3-diaminoguanidine hydrochloride (**10**) in 10 mL of water a solution of 1.84 g (0.01 mol) of **5a** in 10 mL of methanol was added and the mixture was stirred for 16 h at room temperature. After evaporation the residue was chromatographed over Amberlite XAD 1180 with water as eluent, affording first 0.79 g (25%) of **7f**, mp 160 °C dec. Anal. ($C_9H_{13}N_7 \cdot 2HCl \cdot 1.5H_2O$) C, H, N.

In the second fraction 1.48 g (30%) of **11** was obtained: mp 230 °C dec; FAB(MS): $(M + H)^+$: = 350. Anal. ($C_{17}H_{16}N_9 \cdot 2.85HCl \cdot H_2O$) C, H, N, Cl.

The same procedure was used for the preparation of compounds **7l** and **12**.

2,2′-Diamidino-4,4′-bipyridine Dihydrochloride (25b). (Method H). 2.5 g (12.4 mmol) of **23b**[35] was added to a solution of 28 mg (1.2 mg atom) of sodium in 15 mL of absolute methanol and stirred for 2 days at room temperature. Then, 1.53 g (28.6 mmol) of ammonium chloride and 20 mL of saturated ethanolic ammonia solution were added, and the mixture was heated for 1 h at 70 °C and evaporated. The residue was crystallized from dilute HCl, affording 1.3 g (34%) of **25b**, mp >280 °C. Anal. ($C_{12}H_{12}N_6 \cdot 2HCl \cdot 0.8H_2O$) C, H, N, Cl.

The same procedure, except that the dinitrile **23c** was treated with sodium methoxide during 5 h at reflux, was used for the preparation of compounds **25a,c**.

General Method for the Preparation of Thioamides (Method I). 3,3′-Dithiocarbamoylbiphenyl (26e). Into a solution of 1.0 g (4.9 mmol) of **23e**[36] in 22 mL of pyridine and 1.4 mL (9.8 mmol) of triethylamine was introduced dry hydrogen sulfide for 7 h at 40 °C and the reaction mixture was kept for a further 15 h at the same temperature. The reaction mixture was then evaporated, affording after two crystallizations from dichloromethane 1.0 g (76%) of **26e**, mp 188–190 °C. Anal. ($C_{14}H_{12}N_2S_2$) C, H, N, S.

The same procedure was used for the preparation of **26d**.

General Method for the Preparation of Amidines via Imino Thioesters (Method J). 3,3′-Diamidinobiphenyl Dihydrochloride (25e). A mixture of 1.0 g (3.6 mmol) of **26e** and 1.4 g (7.3 mmol) of triethyloxonium tetrafluoroborate in 45 mL of dichloromethane was stirred for 2 h at room temperature. After the addition of 348 mg (2.5 mmol) of potassium carbonate and 0.35 mL of water, the reaction mixture was filtered, washed with water, dried and evaporated, affording 1.07 g (88%) of the crude diimino dithioester **27e**. A total of 530 mg (1.6 mmol) of the crude **27e** was dissolved in 5 mL of ethanol, 0.203 g (3.8 mmol) of ammonium chloride was added and the reaction mixture was heated under reflux for 6 h. After evaporation, the residue was purified by chromatography on Amberlite XAD 1180 (water as eluent) and crystallized from water, affording 0.19 g (38%) of **25e**, mp 230–235 °C. Anal. ($C_{14}H_{14}N_4 \cdot 2HCl \cdot 2H_2O$) C, H, N, Cl.

The same procedure was used for the preparation of **25d**.

Synthesis of Starting Materials. 2-Cyano-4-formylpyrimidine (1o). A solution of 1.211 g (5.9 mmol) of 2-cyano-4-(β-styryl)pyrimidine (**17**) in 85 mL of methanol and 55 mL of dichloromethane was cooled to −70 °C. Ozone was passed through until a blue solution was produced. Then nitrogen was passed through the solution until the excess of ozone had been removed, and 1.95 mL of dimethyl sulfide was added to the reaction mixture. The solution was allowed to warm up to room temperature and was evaporated. The residue was purified over silica gel (eluent, *n*-hexane/ethyl acetate 1:1), affording 0.64 g (82%) of **1o**, ^{1}H NMR (DMSO-d_6) δ 9.94 (s), 9.32 (d), 8.18 (d). The product was used directly in the next step.

Product **1p** was prepared similarly.

2-Formyl-1-ethoxypyridine Diethylacetal Ethyl Sulfate (14i). 4.93 g (0.025 mol) of 2-formylpyridine *N*-oxide diethylacetal (**13i**) was treated dropwise at 70–75 °C with 3.85 g (0.025 mol) of diethyl sulfate. The mixture was stirred at 75–80 °C for 1 h and cooled, affording 8.7 g (100%) of **14i**. The product was used immediately, without further purification, in the following reaction step.

The same procedure was used for the preparation of compound **14n**.

2-Cyano-6-formylpyridine Diethylacetal (2i). To a solution of 3.67 g (75 mmol) of sodium cyanide in 14 mL of water was slowly added at 0 °C a solution of 8.7 g (25 mmol) of the crude **14i** in 6 mL of water over a period of 1 h. The resulting suspension was stirred at 0 °C for 2 h and extracted with ethyl acetate. The organic layer was dried and evaporated, and the residue was purified by chromatography with dichloromethane/ethyl acetate, 95:5, as eluent, to give 3.7 g (76%) of pure **2i** as a colorless oil. Anal. ($C_{11}H_{14}N_2O_2$) C, H, N.

The same procedure was used for the preparation of compound **2n**.

4-(β-Styryl)-2-(trimethylammonio)pyrimidine Chloride (16). 5.3 g (24.4 mmol) of 2-chloro-4-(β-styryl)pyrimidine (**15**)[33] was dissolved in 100 mL of 1.92 M trimethylamine in benzene and stirred for 24 h at room temperature under argon. Then 400 mL ether was added, and the separated compound was filtered off, affording 6.73 g (100%) of **16**, ^{1}H NMR (DMSO-d_6) δ 9.02 (d), 8.18 (d), 7.89 (d), 3.65 (s). Anal. ($C_{15}H_{18}ClN_3$) C, H, N.

2-Cyano-4-(β-styryl)pyrimidine (17). **16** (7.0 g, 24.4 mmol) was dissolved in 55 mL of dichloromethane, and a solution of 4.433 g (24.4 mol) of tetraethylammonium cyanide in 55 mL of dichloromethane was added. The reaction mixture was stirred for 1 h at room temperature, and then washed with water and

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dilute brine, dried, and evaporated. The residue was purified by chromatography on silica gel with *n*-hexane/ethyl acetate, 1:1, affording 1.42 g (28%) of **17**: mp 190 °C dec; ^{1}H NMR (DMSO-d_6) δ 8.93 (d), 8.02 (d), 7.91 (d), 7.7–7.85 (m), 7.35–7.55 (m), 7.39 (d). Anal. ($C_{13}H_9N_3$) C, H, N.

4-Chloro-6-methyl-2-(β-styryl)pyrimidine (19). A mixture of 212 mg (1 mmol) of **18**[34] in 1 mL of phosphoryl chloride was heated under reflux for 35 min. After cooling, the reaction mixture was poured onto ice, and the solution was neutralized with NaOH and extracted with ether. The dried organic phase was evaporated, and the residue was distilled, affording 117.6 mg (51%) of **19**: bp 118–22 °C (0.1 mbar); mp 71–73 °C. Anal. ($C_{13}H_{11}ClN_2$) C, H, N.

4-Methyl-2-(β-styryl)pyrimidine (20). A mixture of 13.65 g (59.17 mmol) of **19**, 9.02 mL of triethylamine and 1.36 g of Lindlar catalyst in 220 mL of ethanol was hydrogenated at room temperature under normal pressure. The reaction was filtered and evaporated, and the residue was purified by chromatography on silica gel with hexane/ethyl acetate, 20:1 and 5:1, affording 8.13 g (70%) of **20**, mp 65–67 °C. Anal. ($C_{13}H_{12}N_2$) C, H, N.

4-[(*N*-Hydroxyimino)methyl]-2-(β-styryl)pyrimidine (21). 2.91 g (126.7 mmol) of metallic sodium and 100 mg of anhydrous $FeCl_3$ were dissolved in 416 mL of liquid ammonia. The reaction mixture was stirred for 20 min at −20 °C, followed by the addition of 8.16 g (41.16 mmol) of **20**. After a further 1 h, 9.7 mL of *n*-butyl nitrite were added dropwise over a period of 10 min, and the mixture was stirred for a further 1 h. Then, 6.93 g (129 mmol) of ammonium chloride was added, and the ammonia was allowed to evaporate. The residue was extracted with acetone, affording, after chromatography on silica gel with CH_2Cl_2/acetone, 20:1, 4.26 g (46%) of a mixture of the oximes **21**: mp 192–5 °C; ^{1}H NMR (DMSO-d_6) δ 12.65 and 12.29 (s, 1 H), 8.94 and 8.80 (d, 1 H), 8.15 and 7.61 (d, 1 H), 8.09 and 7.57 (s, 1 H), 7.97 and 7.96 (d, 1 H), 7.31 and 7.29 (d, 1 H). Anal. ($C_{13}H_{11}N_3O$) C, H, N.

4-Cyano-2-(β-styryl)pyrimidine (22). A mixture of 425 mg (1.88 mmol) of **21** in 4.25 mL of phosphoryl chloride was heated for 1 h at 120 °C. The solution was poured onto 50 g of ice and 22 mL of 28% ammonia solution, was saturated with solid potassium carbonate, and extracted with ethyl acetate. The organic phase was washed neutral, dried, and evaporated, affording after recrystallization from chloroform/ether 303.8 mg (78%) of **22**, mp 147–150 °C. Anal. ($C_{13}H_9N_3$) C, H, N.

6,6′-Dicyano-2,2′-bipyridine (23a). A mixture of 11.1 g (0.06 mol) of 2,2′-bipyridine-1,1′-dioxide[37] and of 22.8 mL (0.24 mol) of dimethyl sulfate was stirred at 80 °C for 15 min. After cooling, the reaction mixture was evaporated under a high vacuum, and the residue was dissolved in 30 mL of water. This solution was added dropwise to an ice-cooled solution of 17.4 g (0.33 mol) of sodium cyanide in 66 mL of water. The separated product was filtered off and washed with water and ethanol, affording 11.22 g (91%) of **23a**, mp 255–258 °C. Anal. ($C_{12}H_6N_4$) C, H, N.

4,4′-Dicyano-2,2′-bipyridine (23c). A suspension of 4.73 g (19.5 mmol) of 4,4′-dicarbamoyl-2,2′-bipyridine[38] and 5.67 g (47.6 mmol) of thionyl chloride in 30 mL of pyridine was stirred for 16 h at 100 °C. After cooling, the reaction mixture was diluted with 100 mL of dichloromethane, filtered, and concentrated to dryness. The residue was crystallized from ethyl acetate, affording 2.26 g (56.2%) of **23c**, mp 238–240 °C. Anal. ($C_{12}H_6N_4$) C, H, N.

4-(3-Cyanophenyl)-2-cyanopyrimidine (23d). A solution of 13.2 g (51 mmol) of **32** in 200 mL of DMF was treated with 8.3 g (128 mmol) of potassium cyanide, stirred for 2.5 h at 100 °C and evaporated. The residue was extracted with dichloromethane, and the extract was washed with water, dried, and evaporated. The product was crystallized from ethyl acetate, affording 6.4 g (61%) of **23d**, mp 185 °C. Anal. ($C_{12}H_6N_4$) C, H, N.

1-(3-Cyanophenyl)-3-(dimethylamino)acrolein (29). A mixture of 14.5 g (0.1 mol) of 3-cyanoacetophenone (**28**) and 26.5 g (0.2 mol) dimethylformamide dimethylacetal was heated for 1 h at 110 °C, affording after crystallization from ether/hexane 11.3 g (56.5%) of **29** as orange crystals, mp 114–117 °C. Anal. ($C_{12}H_{12}N_2O$) C, H, N.

Potassium 4-(3-Cyanophenyl)-2-mercaptopyrimidine (30). A mixture of 22.0 g (0.11 mol) of **29** and 16.5 g (0.22 mol) of thiourea in 275 mL of ethanol and 110 mL of 1 N KOH in ethanol was heated on reflux for 4 h. After cooling, the separated orange-yellow crystals were collected, affording 17.2 g (73.5%) of **30**, mp >250 °C. The product was used directly in the next step.

4-(3-Cyanophenyl)-2-(methylthio)pyrimidine (31). **30** (17.3 g, 81.1 mmol) was dissolved in 260 mL of water followed by the addition of 15.9 mL (168 mmol) of dimethyl sulfate and 120 mL of 2 N NaOH. The reaction mixture was stirred for 12 h at room temperature and extracted with 1.5 L of ether. The ether layer was separated, washed with water, dried, and evaporated. The residue was crystallized from ether/hexane, affording 13.5 g (73.2%) of **31**: mp 117–8 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 2.68 (s, 3 H), 7.37 (d, 1 H), 7.66 (t, 1 H), 7.8 (m, 1 H), 8.3 (m, 1 H), 8.42 (s, 1 H), 8.63 (d, 1 H). Anal. ($C_{12}H_9N_3S$) C, H, N.

4-(3-Cyanophenyl)-2-(methylsulfonyl)pyrimidine (32). A solution of 13.5 g (59.4 mmol) of **31** in 300 mL of dichloromethane was treated with a solution of 27.9 g of *m*-chloroperbenzoic acid in 300 mL of dichloromethane. After 12 h the reaction mixture was filtered, and the filtrate was washed with water, a 5% sodium bisulfite solution, and a dilute sodium carbonate solution and water, then dried, and evaporated. The residue was crystallized from methanol, affording 12.35 g (80.2%) of **32**, mp 165–7 °C. Anal. ($C_{12}H_9N_3O_2S$) C, H, N.

Enzyme Preparation and Assay for SAMDC Activity. SAMDC was prepared from homogenized livers of MGBG-pretreated rats and assayed as described by Pegg and Pösö.[44]

Enzyme Preparation and Assay for Diamine Oxidase Activity. Diamine oxidase from rat small intestine was prepared and assayed as described by Seppänen et al.[45]

Enzyme Preparation and Assay for ODC Activity. Rat liver ODC was prepared and assayed as described by Hayashi.[46]

Antiproliferative Activity of SAMDC Inhibitors. Antiproliferative effects on human T24 bladder carcinoma cells were determined as described previously.[47] Cell proliferation was monitored by staining dishes with methylene blue.

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