ELSEVIER

Il Farmaco 53 (1998) 244–247

IL FARMACO

Short communication

Synthesis and preliminary antileukemic studies of cyclic mitoguazone analogues

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Received 31 July 1997; accepted 7 January 1998

Abstract

Analogues of mitoguazone bearing a terminal amidino group as a part of the seven-membered ring of 1,3-diazepine and six-membered ring of pyrimidine were prepared in order to evaluate in vivo antileukemic action towards L1210 leukemia in mice. Preliminary pharmacological screening showed that the investigated compounds increase the life span (T/C%) of the treated mice in comparison with the untreated animals. The strongest antineoplastic effect was exhibited by compound **8**.

Keywords: Antileukemic studies; Mitoguazone analogues

1. Introduction

Many drugs and compounds containing tautomeric amidinohydrazone groups possess a wide spectrum of pharmacological activities. They include, for example, guanofuracin (chemotherapeutic activity), thiogin (tuberculostatic activity), guanabenz and guanoxan (hypotensive activity) [1]. Antineoplastic effect is exhibited by bisanthrene [2], ambazone [3] (possessing also bacteriostatic activity) and mitoguazone [4] [2,2′-(1-methyl-1,2-ethanediylidene)-bis(hydrazinecarboximidamide)]. The latter, long since known as an antileukemic drug, is a powerful inhibitor of SAMDC (*S*-adenosylmethionine decarboxylase), a key enzyme of natural polyamines biosynthetic pathway, such as spermidine and spermine. The concentration of these amines is particularly high in rapidly multiplying cells. Mitoguazone, the activity mechanism of which is not yet known, shows a number of undesirable side effects, and in the therapy of hyperplastic diseases is used in combination with other drugs [5]. It seems therefore advisable to synthesise new mitoguazone analogues of more favourable pharmacological properties, which might possibly give new data concerning the mechanism of the drug action.

The present study is a part of more comprehensive investigations on the structure–activity relationship in a group of derivatives of 1,3-diazaheterocycles (imidazole, pyrimidine and 1,3-diazepine). Its aim was the synthesis and preliminary evaluation of antileukemic activity of new bicyclic analogues of mitoguazone, derivatives of glyoxals. Their terminal amidine groups constitute part of the seven-membered 1,3-diazepine ring (it was found earlier that some smaller ring derivatives of glyoxals, such as 2-imidazolinyl and 2-tetrahydropyrimidinyl showed no antileukemic effect) [6]. The study concerns also the monocyclic derivatives, whose amidine groups are embodied in six- or seven-membered rings. The structural elements of 1,3-diazepine and tetrahydropyrimidine are, respectively, 1,4-butanediamine and 1,3-propanediamine. Molecules of these amines form spermine and spermidine. Perhaps the mitoguazone analogues modified in this way may by taken up more effectively by mammalian cells via the polyamine transport system and not display the profound antimitochondrial activity typical of mitoguazone [7,8].

In a previous paper [9] we described a new class of derivatives of cyclic 1,3-diamines, chloroacylhydrazones and their alkylating properties towards 4-(4′-nitrobenzyl)pyridine [10,11] (NBP, the reagent used in the search of compounds with potential antitumor activity). We have shown that the

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0014-827X/98/$19.00
PII S0014-827X(98)00011-1

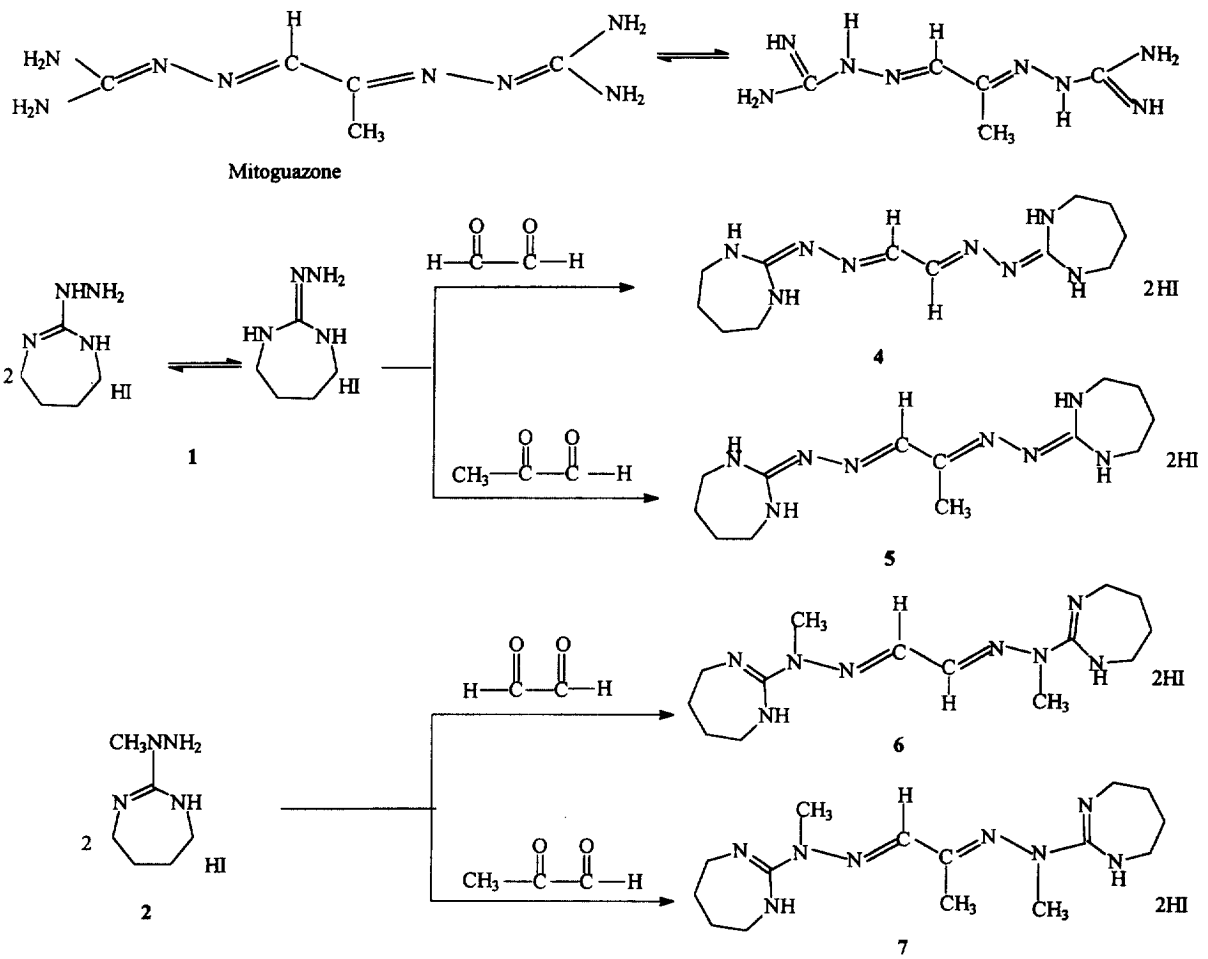
alkylating properties increase in a significant manner with the size of the 1,3-diamine ring.

2. Chemistry

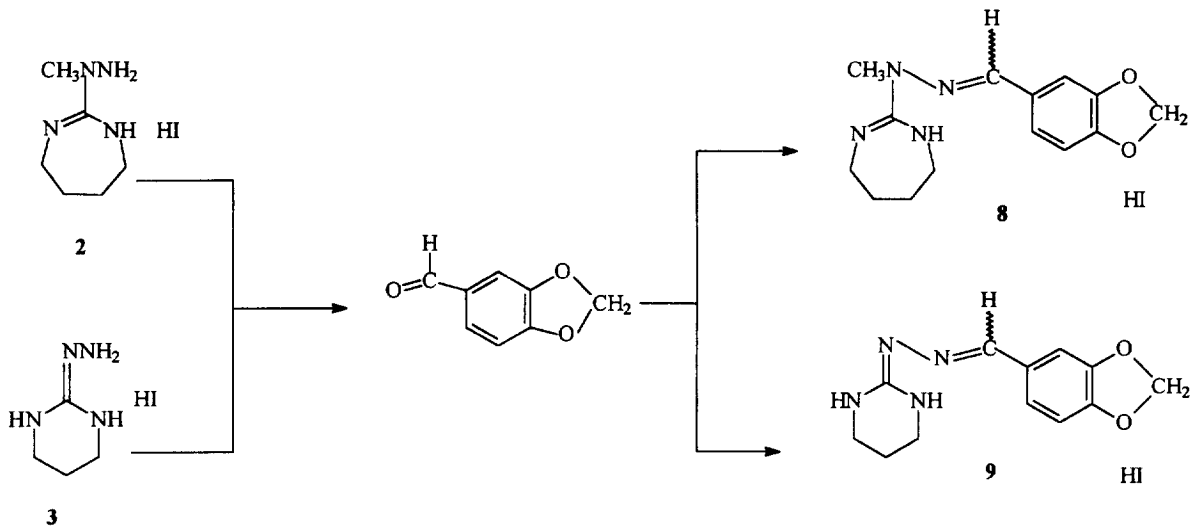
Starting compounds **1** and **2** were obtained by the reaction of hydrazine and methylhydrazine with 2-methylthio-4,5,6,7-tetrahydro-1*H*-1,3-diazepine hydroiodide and **3** by the reaction of hydrazine with 2-methylthio-1,4,5,6-tetrahydropyrimidine hydroiodide. New bicyclic analogues **4–7** of mitoguazone were obtained by the reactions of glyoxal and methylglyoxal with 1,3,4,5,6,7-hexahydro-2*H*-1,3-diazepin-2-one hydrazone hydroiodide (**1**) and *N*-[2-(4,5,6,7-tetrahydro-1*H*-1,3-diazepin-2-yl)]-*N*-methylhydrazine (**2**) in acetic acid (Scheme 1).

Considering the presence of imine and guanidine groups in the molecules of the investigated compounds, it is worthwhile to take into account the isomerism about the C=N bond and tautomerism in the guanidino moiety. Compounds **4** and **6** can exist as three, and compounds **5** and **7** as four geometrical isomers. Stereochemical configurations of compounds with –CH=N– grouping (e.g. imines, hydrazones, oximes, etc.) can be very often defined on the basis of ^{1}H NMR spectra analysis. Imine protons give sharp singlets, and the chemical shifts of the *E*- and *Z*-isomers differ [12]. In the ^{1}H NMR spectra of compounds **4–7** in DMSO-d_6, imine protons were observed as individual sharp singlets in the range 7.75–7.95 ppm. These data suggest that compounds **4–7** probably exist as single geometrical isomers, like that of mitoguazone with *E*-configuration –CH=N– bonds and s-*trans* (single bond *trans*) conformation [13].

In the ^{1}H NMR spectra of derivatives of glyoxal **4–7**, the presence of broadened eight-proton singlets of the $-CH_2N-$ groups in the range 3.45–3.62 ppm was observed. In the spectrum of **4** slightly broadened (exchangeable with D_2O) six-proton singlets corresponding to the protons of the –N*H*– groups were present. This suggests that the exocyclic guanidine nitrogen atom was protonated and that the protons bound to the nitrogen atoms are equivalent and **4** exists as one tautomer. In the spectrum of **6** the presence of a broadened four-proton singlet suggests that the amidine nitrogen atoms of the rings have been protonated and therefore all protons bound to the nitrogen atoms are equivalent. Thus the derivative **6** exists as one tautomer also. In the spectra of the methylglyoxal derivatives **5** and **7**, the protons of the –N*H*– groups (unlike in glyoxal derivatives **4** and **6**) as two singlets differing inconsiderably in chemical shift were observed.



Scheme 1.



Scheme 2.

This is possibly due to the asymmetry of the structure of the molecule (the presence of a methyl group at the carbon atom) rather than their occurring as a mixture of tautomers.

Monocyclic amidinohydrazones **8** and **9** were prepared by the reactions of **2** and **3** with 3,4-(methylenedioxy)-benzaldehyde (Scheme 2).

The 3,4-methylenedioxyphenyl moiety appears in antimitotic agents, such as cornigerine, podophyllotoxin and steganacin [14]. The structure of **8** and **9** was determined by both analytical and spectroscopic data. ^{1}H NMR data of **8** and **9** of one sharp signal at 8.16 ppm for **8** and 8.26 ppm for **9** and the picture of the protons of the $-CH_2H-$ and $-NH-$ groups allow it to be assumed that these compounds are the single geometrical isomers and exist as the single tautomers. Due to a closer structural affinity to mitoguazone, the preliminary pharmacological studies focused on methylglyoxal derivatives **5**, **7** and monocyclic compounds **8** and **9**.

3. Experimental

3.1. Chemistry

Melting points are uncorrected and were recorded on a Boetius apparatus. ^{1}H NMR spectra were made on a Varian EM = 360 (60 MHz). Chemical shifts (δ) are given in parts per million (ppm) relative to tetramethylsilane as internal standard. All compounds were analysed for C, H, N. Analytical results were $\pm 0.3\%$ of the theoretical values.

3.1.1. General procedure for the synthesis of compounds (4–9)

An equimolar mixture (5 mmol) of glyoxal (40% solution in H_2O), methylglyoxal, 3,4-(methylenedioxy)benzaldehyde and the appropriate hydrazine derivate in acetic acid (3 ml) was stirred at room temperature. After about 10 min the substrates (**1**, **2**, 3) were dissolved and a new solid separated. Stirring was continued for an additional 1 h. Then ethyl ether (10 ml) was added to the reaction mixture which was allowed to stand for 20 h at room temperature. The precipitate was filtered off and washed with acetone.

3.1.2. Ethanedial bis(perhydro-1,3-diazepin-2-ylidene)hydrazone dihydroiodide (4)

Compound **4** was obtained in 58% yield as a white powder, m.p. 253–256°C, from ethanol/ethyl ether. *Anal.* ($C_{12}H_{24}I_2N_8$): C, H, N. ^{1}H NMR: 1.77(8H, bs, $2CH_2CH_2$), 3.45(8H, bs, $2NCH_2CH_2N$), 7.93(2H, s, 2CH=N), 8.36-(6H, bs, 4HN, $2N^+H$).

3.1.3. 2-Oxo-propanal bis(perhydro-1,3-diazepin-2-ylidene)hydrazone dihydroiodide (5)

Compound **5** was obtained in 52% yield as light-yellow needles, m.p. 230–232°C, from ethanol/ethyl ether. *Anal.* ($C_{13}H_{26}I_2N_8$): C, H, N. ^{1}H NMR: 1.82(8H, bs, $2CH_2CH_2$), 2.28(3H, s, CH_3), 3.49(8H, bs, $2NCH_2CH_2N$), 7,9 (1H, s, CH=N), 8.04(4H, bs, 4HN), 8.28(2H, s, N^+H).

3.1.4. Ethanedial bis(4,5,6,7-tetrahydro-1H-1,3-diazepin-2-yl)methylhydrazone dihydroiodide (6)

Compound **6** was obtained in 49% yield as light-yellow powder, m.p. 220–223°C, from ethanol/acetone. *Anal.* ($C_{14}H_{28}I_2N_8$): C, H, N. ^{1}H NMR: 1.88(8H, bs, $2CH_2CH_2$), 3.38(6H, s, $2NCH_3$), 3.62(8H, bs, $2NCH_2CH_2N$), 7.95(2H, s, 2CH=N), 8.85(4H, s, 2NH, $2N^+H$).

3.1.5. 2-Oxo-propanal bis(4,5,6,7-tetrahydro-1H-1,3-diazepin-2-yl)methylhydrazone dihydroiodide (7)

Compound **7** was obtained in 47% yield as yellow powder, m.p. 210–212°C, from ethanol/acetone. *Anal.* ($C_{15}H_{30}I_2N_8$): C, H, N. ^{1}H NMR: 1.62(8H, bs, $2CH_2CH_2$), 2.32(3H, s, CH_3), 3.28, 3.45(6H, 2s, $2NCH_3$), 3.86(8H, bs, $2NCH_2CH_2N$), 7.75(1H, s, CH=N), 8.05(2H, s, 2NH), 8.65(2H, s, $2N^+H$).

3.1.6. 3,4-(Methylenedioxy)benzaldehyde (4,5,6,7-tetrahydro-1H-1,3-diazepin-2-yl)methylhydrazone hydroiodide (8)

Compound **8** was obtained in 72% yield as yellow needles, m.p. 270–272°C, from ethanol. *Anal.* ($C_{14}H_{19}IN_4O_2$): C, H, N. ^{1}H NMR: 1.81(4H, bs, CH_2CH_2), 3.41–3.59(7H, m, NCH_2CH_2N, NCH_3), 6.21(2H, s, OCH_2O), 7.12, 7.51, 7.72(3H, 3m, Ar), 8.15(1H, s, CH=N), 8.45(2H, bs, NH, N^+H).

3.1.7. 3,4-(Methylenedioxy)benzaldehyde (hexahydropyrimidin-2-ylidene)hydrazone hydroiodide (9)

Compound **9** was obtained in 68% yield as yellow powder, m.p. 245–247°C, from ethanol. *Anal.* ($C_{12}H_{15}IN_4O_2$): C, H, N. ^{1}H NMR: 1.91(4H, m, CH_2CH_2), 3.76(4H, m, NCH_2CH_2N), 6.11(2H, s, OCH_2O), 7.17, 7.67, 7.87(3H, 3m, Ar), 8.26(1H, s, CH=N), 8.52(3H, s, 2NH, N^+H).

3.2. Pharmacology

Antineoplastic effect of compounds **5**, **7**, **8**, **9** on L1210 leukemia development in vivo was examined by Geran et al. (NCI) [15]. Acute toxicity of the compounds (LD_{50}) was calculated by the Deichmann and Le Blanck [16] method to choose doses for investigating antineoplastic activity. L1210 leukemia was propagated in DBA/2 mice, and transplanted into BDF_1 mice. All the animals were inoculated intraperitoneally with 3×10^5 cell/mouse. They were divided into 5 groups, each of 5 mice: group 1, untreated, was the control group; groups 2–4 were given the investigated compounds, **5**, **7**, **8**, **9**. Tested samples were suspended in 1% methyl cellulose and administered intraperitoneally.

The following schemes of injection of investigated compounds were planned:

1. injection in the dose LD_{50}
2. injection in the dose 0.5 LD_{50}
3. injection in the dose 0.1 LD_{50}

4. Results and discussion

The compounds were injected i.p. 24 h after neoplasm implantation. The increase in the life span (T/C%) of the treated mice in comparison with untreated animals was assumed as the antineoplastic activity. T/C% of at least 125% testifies the antineoplastic activity of the dose of the investigated compound.

Results are shown in Table 1.

The longest survivals, adequate T/C%, were observed in mice treated with 0.1 LD_{50} of **5** (130%), 0.1 LD_{50} of **7** (138%), 1.0 LD_{50} of **8** (154%), 0.1 LD_{50} of **9** (130).

The tested compounds exhibited a variable toxicity ranging from 0.073 g/kg for **7** to 0.38 g/kg for **9**, and among them the strongest antineoplastic activity was exhibited by **8**. Further investigations will be performed in order to compare our antineoplastic results with the activity of the model drug mitoguazone.

Of the two investigated bicyclic derivatives of mitoguazone analogues **5** and **7**, compound **7** appeared to be more active, but also more toxic. Therefore, the replacement of hydrogen atoms bound to nitrogen atoms by the methyl group results in the increase of its antileukemic activity, but also toxicity. Based on the earlier literature [6] data and the presented results of our study, it can be concluded that out of the bicyclic analogues of mitoguazone including 1,3-diazaheterocyclic rings only the derivatives which contain the seven-membered 1,3-diazepine ring possess the antileukemic effect. Of the monocyclic amidinohydrazones **8** and **9** containing 3,4-methylendioxyphenyl, the derivative of 1,3-diazepine **8** shows a distinctly higher antileukemic activity than the derivative of pyrimidine **9**. In conclusion, bi- and monocyclic amidinohydrazones of 1,3-diazaheterocycles show some antileukemic activity.

Table 1
The antineoplastic activity of the compounds **5**, **7–9**

Compound	LD_{50} (g/kg)	Doses in part of LD_{50}	T/C (%)	Effect according to Geran et al. (NCI) [15]
5	0.25	1.0	107	–
		0.5	107	–
		0.1	130	+
9	0.38	1.0	31	–
		0.5	123	–
		0.1	130	+
7	0.073	1.0	107	–
		0.5	107	–
		0.1	138	+
8	0.17	1.0	154	+
		0.5	123	–
		0.1	123	–

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