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Synthesis and Evaluation of Guanazole Prodrugs as Antineoplastic Agents

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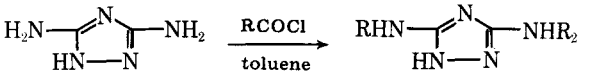
Received January 22, 1981, from the **Section of Biomedicinal Chemistry, School of Pharmacy, and the* ‡ *Department of Pathology, School of Medicine, University of Southern California, Los Angeles, CA 90033.* Accepted for publication April 16, 1981.

Abstract □ Nine amide derivatives of guanazole were synthesized, and eight of them were tested for antineoplastic activity *versus* L1210 cells *in vitro*. Several of these compounds exhibited activity comparable to or greater than that of guanazole.

Keyphrases □ Guanazole—synthesis and evaluation of prodrugs as antineoplastic agents □ Prodrugs—of guanazole, synthesis and evaluation as antineoplastic agents □ Antineoplastic agents—synthesis and evaluation of guanazole prodrugs

Guanazole (3,5-diamino-1,2,4-triazole, NSC 1895) was synthesized by Pellizzari (1) and discovered in a general screen by the National Cancer Institute. Subsequently, Brockman *et al.* (2) reported that guanazole inhibited DNA synthesis by inhibiting ribonucleotide reductase. Antitumor activity against various animal leukemias and tumors (3) and remissions in some patients with actute myelocytic leukemia (4) were reported. However, extensive clinical utilization of this drug has been limited by its high polarity and water solubility, low molecular weight, and relatively low potency, which warrants continuous intravenous infusion at frequent intervals (5).

Structure–activity relationship analysis of past and current work on guanazole derivatives established the importance of both amino groups for antineoplastic activity (2, 3). Thus, to circumvent the problem of the high elimination rate and to increase the efficacy of guanazole as an antieoplastic agent, amide derivatives with decreased polarity, water solubility, and increased molecular weight were synthesized (Scheme I). These compounds were then tested for anticancer activity *versus* L1210 cells *in vitro*.



R = CH_3, C_2H_5, C_3H_7, C_6H_5, CF_3, C_2F_5, C_3F_7, p-$NO_2C_6H_4$

Scheme I

The compounds and their physical properties are outlined in Table I.

EXPERIMENTAL

Chemistry—All solvents were analytical grade. All melting points were taken in open capillary tubes[1] and are corrected. Elemental analysis[2] was within ±0.4% of the theoretical values. All proton NMR spectra were recorded on a 60 MHz spectrometer[3] with tetramethylsilane as the internal standard and solvents such as deuterochloroform, dimethyl sulfoxide-d_6, and trifluoroacetic acid. IR spectra were recorded on a spectrophotometer[4] as potassium bromide pellets or Fluorolube mulls. UV spectra were recorded on a double-beam spectrophotometer[5]. TLC was performed using Eastman-type 6060 chromatogram sheets (silica gel), and the sheets were developed in an iodine chamber to check the purity of the amides.

Most of these derivatives were synthesized by a general procedure for the acylation of amines. The appropriate acid chloride or anhydride was condensed with guanazole, usually in the presence of a nonpolar solvent. Two typical reactions for preparing the amides are described using III and VI as examples.

One gram (0.01 *M*) of guanazole and 4.2 g (0.02 *M*) of trifluoroacetic anhydride were refluxed in dry toluene for 8 hr. The white precipitate

[1] Thomas Hoover melting-point apparatus.
[2] Performed by the Analytical Laboratory, California Institute of Technology, Pasadena, Calif.
[3] Hitachi Perkin-Elmer R-24.
[4] Beckman IR-4240.
[5] Perkin-Elmer Coleman 124.

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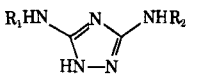
Table I—Physical Properties of Guanazole Amides



Compound	R_1	R_2	Melting Point	Yield %	Formula	Analysis, %	Calc.	Found
I (3-amino-5-acetamido-1,2,4-triazole)	H	$COCH_3$	235–236°	64	$C_4H_7N_5O$	C	34.04	33.61
						H	5.00	5.33
						N	49.62	49.35
II (3,5-diacetamido-1,2,4-triazole)	$COCH_3$	$COCH_3$	>250°	81	$C_6H_9N_5O_2$	C	39.36	39.30
						H	4.92	4.99
						N	38.25	38.20
III (3,5-ditrifluoroacetamido-1,2,4-triazole)	$COCF_3$	$COCF_3$	>300°	78	$C_6H_3F_6N_5O_2$	C	24.74	25.11
						H	1.03	1.17
						N	24.05	24.18
IV (3,5-dipropionamido-1,2,4-triazole)	$COCH_2CH_3$	$COCH_2CH_3$	>250°	48	$C_8H_{13}N_5O_2$	C	45.42	45.99
						H	6.16	6.30
						N	33.18	33.40
V (3,5-dibutyramido-1,2,4-triazole)	COC_3H_7	COC_3H_7	>300°	50	$C_{10}H_{17}N_5O_2$	C	50.21	50.03
						H	7.11	6.79
						N	29.29	29.23
VI (3-amino-5-heptafluorobutyramido-1,2,4-triazole)	H	COC_3F_7	300–301°	91	$C_6H_4F_7N_5O$	C	24.42	24.44
						H	1.37	1.38
						N	23.72	(23.15)
VII (3,5-dipentafluoropropionamido-1,2,4-triazole)	COC_2F_5	COC_2F_5	230–231°	54	$C_8H_3F_{10}N_5O_2$	C	24.56	25.01
						H	0.76	0.76
						N	17.90	18.28
VIII (3,5-dibenzamido-1,2,4-triazole)	COC_6H_5	COC_6H_5	314–315°	71	$C_{16}H_{13}N_5O_2$	C	62.54	62.26
						H	4.23	4.08
						N	22.80	22.92
IX (3-amino-5-*p*-nitrobenzamido-1,2,4-triazole)	H	*p*-$NO_2C_6H_4CO$	>300°	20	$C_9H_8N_6O_3$	C	43.54	43.48
						H	3.22	3.44
						N	33.87	(32.62)
X (3,5-bis-*p*-nitrobenzamido-1,2,4-triazole)	*p*-$NO_2C_6H_4CO$	*p*-$NO_2C_6H_4CO$	>300°	73	$C_{16}H_{11}N_7O_6$	C	48.38	47.97
						H	2.79	2.83
						N	24.68	24.30

Table II—Summary of IR Data for Guanazole Prodrugs

Compound	νN–H, cm^{-1}	νC=O, cm^{-1}	νC–N, cm^{-1}	νC–H/C–F, cm^{-1}
I	3050–3400	1680	1610	2880, 2940
II	3100–3450	1680, 1720	1590	2860, 2960
III	3100–3300	1730, 1760	1630	1150–1230
IV	3200–3460	1690, 1710	1585	2860, 2880 2980, 2940
V	3100–3450	1680, 1720	1585	2840, 2880 2970, 2940
VI	3150–3450	1725	1635	1150–1220
VIII	3050–3400	1670, 1695	1590	3000, 710[a] 735[a]
IX	3100–3460	1610	1610	3010, 708[a] 740[a]
X	3100–3460	1695, 1720	1600	3010, 708[a] 740[a]

[a] δC–H aromatic.

that formed was then washed with ether and dried *in vacuo* over phosphorus pentoxide. Drying afforded 2.26 g (78% yield) of the bis amide (III), mp 300°; IR (KBr): 1730 and 1760 (νC = 0) cm^{-1}.

One gram (0.01 *M*) of guanazole and 6.5 ml (1.58 g, 0.01 *M*) of heptafluorobutyric anhydride were refluxed in 10 ml of dry benzene for 4 hr. After standing overnight, the pale-yellow crystals were collected, washed with 5% ammonia, recrystallized from ethanol, and dried *in vacuo* over phosphorus pentoxide to yield 2.7 g (91% yield) of the product (VI), mp 300–301°; IR (KBr): 1725 (νC = 0) cm^{-1}.

Biological Screening *versus* L1210 Cells *In Vitro*—For routine passage and during dose–response experiments, cells were maintained in asynchronous logarithmic growth at 37° in medium[6] supplemented with 10% fetal calf serum, 1% penicillin, and 1% streptomycin.

The cells were grown in a humidified incubator supplied with 95% air and 5% carbon dioxide at 37°. Stock cells were usually suspended at 6000–9000 cells/ml. The pH of the experimental flasks was adjusted to 7.4 with the addition of carbon dioxide. The drugs were solubilized with 1% dimethyl sulfoxide, diluted in phosphate-buffered saline, and added to the cell culture in 1:10 dilution to achieve the desired drug concentrations. Cell cultures were set up at 5000 cell/ml in duplicate for each drug concentration in 25-cm^2 flasks.

[6] Roswell Park Memorial Institute medium.

After 24, 48, and 72 hr of continuous drug exposure, the cells were harvested and counted using a Coulter counter. A control, 1% dimethyl sulfoxide-treated set of cultures, was included for each separate dose–response experiment. From the data obtained, a dose–response curve was drawn and the ID_{50} value was calculated according to a reported method

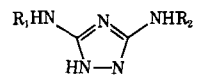


Table III—NMR Chemical Shift of Prodrugs

Compound	R_1	R_2	Shift, (δ), ppm[a]
I[b]	H	$COCH_3$	1.83 (s)
II[c]	$COCH_3$	$COCH_3$	2.37 (s)
IV[c]	COC_2H_5	COC_2H_5	3.00 (q), 1.15 (t)
V[d]	COC_3H_7	COC_3H_7	1.00 (t), 1.69 (m), 2.86
VIII[b]	CO–C_6H_5	COC_6H_5	7.40 (m), 7.80 (m)
IX[c]	H	CO–C_6H_4-*p*-NO_2	5.55 (s), 7.90, 8.10 (m)
X[c]	CO–C_6H_4-*p*-NO_2	CO–C_6H_4-*p*-NO_2	8.20, 8.30 (m)
Guanazole[c]	H	H	5.15 (s)

[a] *S* = singlet, *t* = triplet, q = quartet, and m = multiplet. [b] In trifluoroacetic acid. [c] In dimethylsulfoxide-d_6. [d] In deuterochloroform.

Table IV—UV Absorption Spectra[a]

Compound	λ_{max}, nm	ϵ, M^{-1} cm^{-1}
I	273	6,400
II	236	8,100
III	249	13,500
IV	236	9,200
V	236	2,200
VI	264	11,000
VIII	256	17,300
IX	260	16,000
X	264	43,000
Guanazole	200	500

[a] In Roswell Park Memorial Institute medium.

Table V—First-Order Rate Constants of Hydrolysis and Half-Lives of Guanazole Prodrugs

Compound	k, hr^{-1}	$t_{1/2}$, hr
I	0.0128	54.0
II	0.0022	315.0
III	0.0087	79.0
IV	0.0030	231.0
V	0.0078	88.0
VI	0.0059	117.0
VIII	0.0050	138.0
IX	0.0113	61.0
X	0.0042	165.0

(6, 7). The ID_{50} value represents the concentration of drug that halves the growth rate relative to untreated controls. Compound VII was not tested against L1210 cells *in vitro* due to insufficient quantity. The ID_{50} value of VI could not be calculated due to negligible activity after 48 hr.

RESULTS AND DISCUSSION

The amide derivatives were synthesized, and their structures were established by elemental analysis and IR, NMR, UV, and mass spectra (Tables II–IV). The hydrolysis rates of these amides are listed in Table V, and Table VI lists the calculated ID_{50} values.

An assessment of the results *versus* L1210 cells revealed that, on the 1st day, VIII was substantially active. Solvolysis of this prodrug to an appreciable amount of guanazole by the medium was eliminated on the basis of the low hydrolysis rate ($t_{1/2}$ = 138 hr). Thus, VIII apparently shows activity *per se*, possibly due to its increased lipophilicity, which facilitates its transport into the cells where subsequent cytoplasmic hydrolysis may occur. However, increased interactions with the accessory binding site on the enzyme ribonucleotide reductase cannot be ruled out.

Compounds I and III showed activity comparable to that of guanazole against L1210 cells *in vitro*. Compound I, the most water soluble of the prodrugs, has a relatively short half-life ($t_{1/2}$ = 54 hr), which accounts for its activity after 48 hr. Compound III also has a short solvolytic half-life ($t_{1/2}$ = 79 hr), but its enhanced activity could be attributed to the release of trifluoroacetic acid, a well-known poison (8), as well as guanazole. The other prodrugs with extended half-lives may not have released guanazole in adequate amounts to exert the observed response. Some of the prodrugs such as VI, VIII, and IX might act as excellent depots of guanazole *in vivo*. *In vivo* testing of some of these compounds is in process.

Table VI—*In Vitro* Inhibition of L1210 by Guanazole Prodrugs

Compound	ID_{50}, M
Guanazole	2.08×10^{-4}
I	2.40×10^{-4}
II	4.35×10^{-3}
III	1.90×10^{-4}
IV	4.67×10^{-3}
V	3.82×10^{-2}
VIII	9.26×10^{-4}
IX	2.32×10^{-3}
X	3.66×10^{-3}

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