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Antileukemic Activity of Tetrazole Analogs of Phenylalanine Derivatives

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Received February 18, 1981, from the *Auburn University School of Pharmacy, Department of Pharmacal Sciences, Auburn, AL 36849.* Accepted for publication June 9, 1981.

Abstract □ Eleven tetrazole analogs of substituted phenylalanines were prepared and tested for antitumor activity using P-388 lymphocytic leukemia cells in mice. None of the compounds exhibited significant activity (T/C %, < 125).

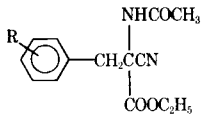
Keyphrases □ Antileukemic activity—tetrazole analogs of substituted phenylalanines against P-388 lymphocytic leukemia □ Phenylalanines, substituted—tetrazole analogs, preparation and screening for antileukemic activity □ Tetrazole—analogs of substituted phenylalanines, preparation and screening for antileukemic activity

The demand for nutrients differs between tumor cells and normal cells (1). Since rapidly proliferating cancer cells take up nutrients more rapidly, they may be selectively "starved" by substituting nonfunctional amino acid derivatives for the normal substrate. *N*-Chloroacetyl derivatives of *para*-substituted phenylalanines were reported (2) to have significant growth inhibitory activity in a microbial antitumor prescreen.

Recent reviews (3, 4) indicated that no reports have appeared for testing tetrazole analogs of amino acids for antineoplastic activity. Studies of the biological activity of 5-substituted tetrazoles have been prompted by a close similarity between the acidity of the tetrazole group and the carboxylic acid group, and the fact that the tetrazole function appears to be metabolically more stable (3).

The chemically similar tetrazole ring system (5) has been used to replace the carboxyl group in several amino acids (6–8). These *in vitro* studies suggested that the tetrazole analog may serve as substrate inhibitor of the respective amino acid in certain enzymatic reactions. The purpose of this study was to determine whether tetrazole analogs of certain phenylalanine derivatives would exhibit antileukemic action in mice.

Table I—Aryl-Substituted Ethyl 2-Acetamido-2-cyano-3-phenylpropanoates



Compound	R	Melting Point	Yield, %	Formula	Analysis, %	Calc.	Found
					C	60.43	61.07
I	2F	94–96°	65.8	$C_{14}H_{15}FN_2O_3$	H	5.43	5.51
					N	10.07	10.07
					C	58.35	58.46
II[a]	2Cl	145–8°	28.6	$C_{15}H_{17}ClN_2O_3$	H	5.55	5.61
					N	9.07	9.04
					C	49.57	49.63
III	2Br	150–152°	68.7	$C_{14}H_{15}BrN_2O_3$	H	4.46	4.51
					N	8.26	8.24
					C	43.54	43.61
IV	2I	174–176°	67.5	$C_{14}H_{15}IN_2O_3$	H	3.92	3.96
					N	7.25	7.23
					C	62.06	62.12
V	$2OCH_3$	142–144°	92.8	$C_{15}H_{18}N_2O_4$	H	6.25	6.31
					N	9.65	9.67
					C	62.06	62.01
VI	$4OCH_3$	164–167°	57.1	$C_{15}H_{18}N_2O_4$	H	6.25	6.29
					N	9.65	9.63
					C	49.57	49.41
VII	4Br	165–168°	62.7	$C_{14}H_{15}BrN_2O_3$	H	4.46	4.52
					N	8.26	8.24

[a] Isopropyl ester (the analytical sample was inadvertently recrystallized from isopropyl alcohol resulting in transesterification).

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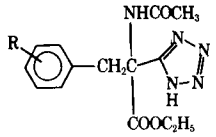
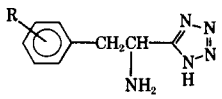
Table II—Aryl-Substituted Ethyl 1-Acetamido-1-tetrazol-5-yl-2-phenylpropanoates



Compound	R	Melting Point	Yield, %	Formula	Analysis, %	
					Calc.	Found
VIII	2F	131–133°	9.6	$C_{14}H_{16}FN_5O_3 \cdot \frac{1}{2}H_2O$	C 50.91 H 5.19 N 21.20	51.20 4.99 21.35
IX	2Cl	65–68°	79.3	$C_{14}H_{16}ClN_5O_3$	C 49.78 H 4.77 N 20.73	49.71 4.94 20.53
X	2Br	71–74°	63.3	$C_{14}H_{16}BrN_5O_3$	C 43.99 H 4.22 N 18.32	43.74 5.25 16.13
XI	2I	163–165°	52.2	$C_{14}H_{16}IN_5O_3$	C 39.18 H 3.76 N 16.32	38.50 3.83 16.18
XII	$2OCH_3$	95–100°	30.0	$C_{15}H_{19}N_5O_4$	C 54.05 H 5.75 N 21.01	53.29 5.97 20.76
XIII	$4OCH_3$	100–102°	17.2	$C_{15}H_{19}N_5O_4 \cdot H_2O$	C 51.28 H 6.02 N 19.93	51.19 6.00 20.16
XIV	4Br	90–92°	63.7	$C_{14}H_{16}BrN_5O_3 \cdot H_2O$	C 42.01 H 4.53 N 17.50	41.87 4.57 17.52

Table III—Aryl-Substituted 5-(1-Amino-2-phenylethyl)tetrazoles



Compound[a]	R	Melting[b] Point	Yield, %	Formula	Analysis, %	
					Calc.	Found
XV	2F	270°	46.5	$C_9H_{10}FN_5$	C 52.17 H 4.86 N 33.80	51.91 4.92 33.68
XVI	2Cl	284°	34.7	$C_9H_{10}ClN_5$	C 48.33 H 4.51 N 31.31	48.34 4.53 31.26
XVII	2Br	273°	62.3	$C_9H_{10}BrN_5$	C 40.32 H 3.76 N 26.12	40.51 3.43 26.26
XVIII	2I	270°	62.2	$C_9H_{10}IN_5$	C 34.30 H 3.20 N 22.22	34.46 2.92 22.27
XIX	$2OCH_3$	280°	26.1	$C_{10}H_{13}N_5O$	C 54.78 H 5.98 N 31.94	54.69 5.99 31.91
XX	$4OCH_3$	280°	26.3	$C_{10}H_{13}N_5O$	C 54.78 H 5.98 N 31.94	54.81 5.63 32.15
XXI	4OH	290°	76.6	$C_9H_{11}N_5O \cdot \frac{1}{2}H_2O$	C 50.46 H 5.65 N 32.69	49.91 5.43 32.58
XXII	4Br	269°	70.7	$C_9H_{10}BrN_5$	C 40.32 H 3.76 N 26.12	40.19 3.79 26.16
XXIII	3F[c]				—	—
XXIV	4Cl[c]				—	—
XXV	$4CH_3$[c]				—	—

[a] All racemates. [b] All compounds melted with decomposition. [c] Reference 6.

EXPERIMENTAL[1]

Aryl-substituted Ethyl 2-Acetamido-2-cyano-3-phenylpropanoates (I–VII)—A previously reported method (6) was used to prepare intermediates I–VII. Physical and chemical data are shown in Table I.

NMR data agreed with the proposed structures. The NMR spectra (dimethyl sulfoxide-d_6, tetramethylsilane internal standard) showed the following common absorption peaks: δ 1.00–1.20 (t, 3H, CCH_3), 1.90–2.10 (s, 3H, $COCH_3$), 3.35–3.55 (s, 2H, aryl CH_2C), 4.00–4.20 (q, 2H, OCH_2C), and 7.10–7.90 (4H, the pattern varied depending on the ring substituent) ppm. Each spectrum integrated for the correct number of protons. The spectra of V and VI exhibited an additional absorption peak at δ 3.70 (s, 3H, OCH_3). The isopropyl ester of II showed absorption peaks for isopropyl at δ 1.10 [m, 6H, $C(CH_3)_2$] and 4.90 (m, 1H, CH) ppm.

Aryl-substituted Ethyl 1-Acetamido-1-tetrazol-5-yl-2-phenylpropanoates (VIII–XIV)—Intermediates VIII–XIV were prepared

[1] Elemental analyses were performed by Atlantic Microlab, Atlanta, Ga. NMR data were recorded on a Varian T-60A spectrophotometer. Melting points were taken in open capillary tubes and are uncorrected.

using a previous procedure (6) and Table II lists their physical and chemical data.

In agreement with the proposed structures, the NMR data exhibited the common absorption peaks (dimethyl sulfoxide-d_6, tetramethylsilane internal standard): δ 1.10–1.20 (t, 3H, CCH_3), 1.90–2.00 (s, 3H, $COCH_3$), 3.65–3.85 (s, 2H, aryl CH_2C), 4.10–4.20 (q, 2H, OCH_2C), and 6.80–7.30 (m,4H, aromatic) ppm. The spectra of XII and XIII showed a singlet at δ 3.60 ppm that integrated for 5 protons (aryl CH_2 and aryl OCH_3).

Aryl-substituted 5-(1-Amino-2-phenylethyl)tetrazoles XV–XXII)—Compounds XV–XX and XXII were prepared by a previously reported method (6). Compound XXI was prepared by modification of a previously reported method (9). A mixture of compound XX (3.0 g, 0.014 mole) in 48% hydrogen bromide (50 ml) was heated at reflux under nitrogen for 4 hr. The mixture was concentrated under reduced pressure. The residue was dissolved in distilled water and the pH was adjusted to 7 with concentrated ammonium hydroxide. After standing at room temperature for 12 hr, the product was filtered, air dried, and recrystallized from *N,N*-dimethylformamide, 2.2 g (76.6%), mp 290° dec. Table III contains physical and chemical data for XV–XXII.

Assignments of the common NMR absorption peaks are (10% sodium deuteroxide): δ 2.95–3.10 (m, 2H, aryl CH_2), 4.30–4.50 (m, 1H, CCH), and 6.70–7.20 (m, 4H, aromatic) ppm. There was an additional absorption peak for XIX and XX at δ 3.40 (s, 3H, aryl OCH_3) ppm.

Anticancer Screening—The tetrazole analogs of substituted phenylalanines were screened for anticancer activity[2] using P-388 lymphocytic leukemia cells in mice of either sex. On day zero, mice were inoculated intraperitoneally with 10^6 leukemic cells. Twenty-four hours later, a test compound was administered intraperitoneally once daily for the first 9 days or in three injections on every 4th day (XVI, XXIII). The test results were recorded on the 30th day.

[2] Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Silver Spring, MD 20910.

RESULTS AND DISCUSSIONS

All compounds were tested for antileukemic activity at doses of 25, 50, 100, and 200 mg/kg (some in triplicate). For compounds XVI, XVIII, and XIX, the T/C% was near or greater than 125. For all other compounds, the T/C% was < 125 (all mice died when compounds XX and XXIII were administered at 200 mg/kg). Compounds XVI, XVIII, and XIX were retested at 400 mg/kg (in duplicate) and exhibited a T/C% < 125. None of the compounds exhibited significant antileukemic activity.

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Analysis of Commercial Pilocarpine Preparations by High-Performance Liquid Chromatography

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Abstract □ Pilocarpine, isopilocarpine, pilocarpic acid, and isopilocarpic acid can be measured effectively by high-performance liquid chromatography (HPLC). Previous reports have differed on the degree of contamination of commercial pilocarpine preparations with isopilocarpine and pilocarpic acid. This report describes a study of commercial pilocarpine in which no significant contamination was found.

Keyphrases □ Pilocarpine—analysis of commercial preparations by high-performance liquid chromatography □ High-performance liquid chromatography—analysis of commercial pilocarpine □ Ocular agents—pilocarpine, analysis by high-performance liquid chromatography

Several assays have been reported for pilocarpine, isopilocarpine, pilocarpic acid, and isopilocarpic acid using high-pressure liquid chromatography (HPLC) (1–4). The earliest of these reports includes the results of a study done on commercial pilocarpine samples, which showed that in one case, contamination with isopilocarpine was as high as 25% (1). An effort to corroborate this study failed, due to technical difficulties (2). Modifications of the original HPLC method were used in a recent study of commercial pilocarpine preparations and no significant contamination with the isopilocarpine isomer or the degradation products, pilocarpic acid or isopilocarpic acid, was found (5). The present report describes a similar study.

EXPERIMENTAL

Each locally available commercial pilocarpine preparation was obtained through prescription from a retail pharmacy. Letters were sent to all other manufacturers[1] of pilocarpine asking for a 1% sample of their product to be used for animal experimentation. Nine fresh samples of pilocarpine were obtained. Each sample was diluted with HPLC grade water to a concentration of 0.10%. Pilocarpine hydrochloride[2] and isopilocarpine hydrochloride[3] standards were obtained commercially in powder form. Pilocarpic acid and isopilocarpic acid were prepared by the hydrolysis of pilocarpine and isopilocarpine in 0.1 *N* NaOH, respectively. These were diluted to make 0.10% standard solutions.

The mobile phase was prepared by dissolving 50 g of monobasic potassium phosphate in a solution of 900 ml of water and 30 ml of methanol. The solution pH was adjusted to 2.5 with 85% phosphoric acid, and the total volume was brought to 1 liter with water. Separation was achieved by isocratic reversed-phase chromatography[4] on an RP-C18 10μm column[5], (flow rate 1.5 ml/min) at ambient temperature. Detection was by optical absorbance at 216 nm[6]. Peak heights of standard preparations were compared with peak heights of the unknown commercial prepara-

[1] Listed in the "Pharmaceutical Drug Topic Redbook" (6) as of January 1979.
[2] Mallinckrodt, St. Louis, Mo.
[3] Aldrich, Milwaukee, Wis.
[4] Model 310 high-performance liquid chromatograph, Altex Scientific, Berkeley, Calif.
[5] Lichrosorb RP-C18 (10 μm) in 4.6 × 250-mm column, Altex Scientific, Berkeley, Calif.
[6] Model 785 variable-wavelength detector, Micromeritics Instrument Corp., Norcross, Va.

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