Tetrazole Analogs of Phenylalanine Derivatives as Tyrosine Hydroxylase Inhibitors

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Abstract □ Eight new tetrazole analogs of phenylalanine derivatives in which the carboxyl group was replaced by a tetrazole ring were synthesized. At a concentration of 2.5×10^{-5} *M*, each compound exhibited greater than 50% inhibition of tyrosine hydroxylase activity.

Keyphrases □ Tetrazole analogs of phenylalanine derivatives—synthesized, effect on tyrosine hydroxylase activity □ Phenylalanine derivatives—various tetrazole analogs synthesized, effect on tyrosine hydroxylase activity □ Tyrosine hydroxylase activity—effect of various tetrazole analogs of phenylalanine derivatives □ Enzymes—tyrosine hydroxylase activity, effect of various tetrazole analogs of phenylalanine derivatives □ Structure–activity relationships—various tetrazole analogs of phenylalanine derivatives, effect on tyrosine hydroxylase activity

Because it catalyzes the rate-determining step in catecholamine biosynthesis (1), tyrosine hydroxylase has been the target for the synthesis of numerous agents. The majority of these agents may be considered derivatives of phenylalanine and tyrosine, with attention focused on the aromatic portion of the structure.

BACKGROUND

With an intact amino acid side chain, derivatives of phenylalanine and tyrosine potentially can be metabolized to catecholamines, which may modify the overall pharmacological action. The α-methylated analog, α-methyltyrosine, a potent competitive inhibitor of tyrosine hydroxylase (2), is metabolized to methyldopa, α-methyldopamine, and α-methylnorepinephrine (3). These metabolites exhibit weak sympathomimetic action, which led to the false transmitter hypothesis for the antihypertensive action of methyldopa (4). However, if the carboxyl group is replaced by a tetrazole ring, the derivatives are not likely to be metabolized to catecholamines.

The chemically similar tetrazole ring system (5) has been used to replace a carboxyl group. Elwood *et al.* (6) reported weak inhibition of glutamic acid dehydrogenase by tetrazole analogs of glutamic acid. They suggested that the low degree of binding of the tetrazole analogs to the receptor was not due to poor steric fit or the degree of ionization but to the much greater area available within the tetrazole ring for the distribution of charge in the ionized form. The preparation of tetrazole analogs of other amino acids was reported (7).

The purpose of this study was to determine whether tetrazole analogs of phenylalanine derivatives would inhibit tyrosine hydroxylase. Several analogs in which the carboxyl group was replaced by a tetrazole ring were evaluated.

The aryl-substituted tetrazole analogs of phenylalanine were synthesized by the route outlined in Scheme I.

EXPERIMENTAL[1]

Aryl-Substituted Ethyl 2-Acetamido-2-cyano-3-phenylpropanoates (I–IX)—Intermediates I–IX were prepared by a modification of the method of Matsumoto (8). A mixture of ethyl acetamidocyanoacetate (68.0 g, 0.40 mole), sodium iodide (1.0 g), and anhydrous potassium carbonate (30.0 g, 0.22 mole) in dry acetone (300 ml) was heated at reflux while the appropriately substituted benzyl halide (0.30 mole) was added dropwise during 15 min. After heating at reflux for 7 hr, the mixture was poured over ice and filtered. The residue was recrystallized from the appropriate solvent (Table I) and activated charcoal.

NMR data agreed with the proposed structures. The NMR spectra (dimethyl sulfoxide-d_6, tetramethylsilane internal standard) showed the following common absorption peaks: δ 1.10 (t, 3H, CCH_3), 1.99 (s, 3H, $COCH_3$), 3.35 (s, 2H, Ar-CH_2C), and 4.10 (q, 2H, OCH_2C) ppm. Depending on the ring substituents, the absorption in the aromatic region ranged from δ 7.10 to 7.90 ppm and the pattern varied from singlet to multiplet. Each spectrum integrated for the correct number of protons. The NMR spectrum of VII showed an absorption peak at δ 2.25 ppm while that of VIII showed one at δ 2.30 ppm, each of which integrated for three protons corresponding to an aromatic methyl substituent.

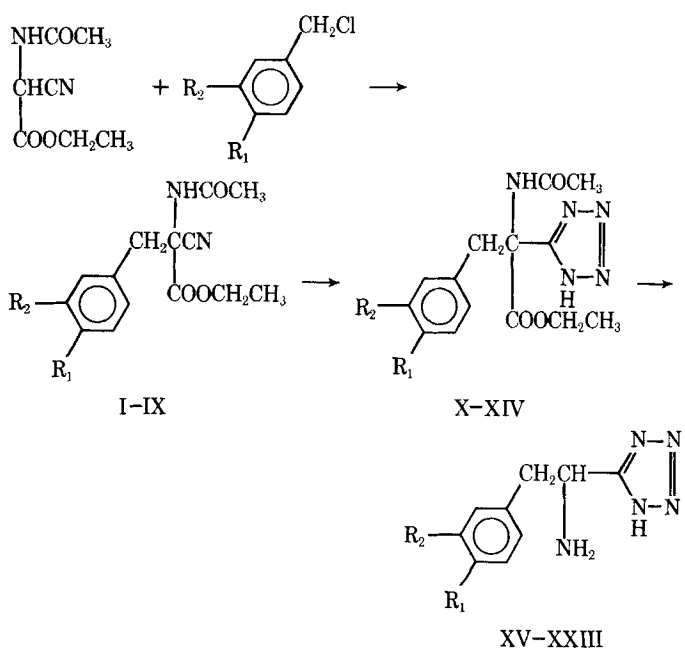
Aryl-Substituted Ethyl 1-Acetamido-1-tetrazol-5-yl-2-phenylpropanoates (X–XIV)—These compounds were synthesized by a modification of the method of Finnegan *et al.* (9), which is exemplified by the procedure for X.

A mixture of II (14.5 g, 0.052 mole), sodium azide (6.8 g, 0.104 mole), and ammonium chloride (5.5 g, 0.104 mole) in dry dimethylformamide (125 ml) was heated at 88–95° for 28 hr. The hot mixture was filtered, and the filtrate was concentrated *in vacuo*. The residue was dissolved in distilled water, and the solution was adjusted to pH 2 with dilute hydrochloric acid. The resulting precipitate was washed with cold water and recrystallized twice from distilled water and activated charcoal, 8.4 g (50.3%), mp 147–149°. Table II contains physical and chemical data for X–XIV.

NMR data agreed with the proposed structures. Assignments of the common absorption peaks are (dimethyl sulfoxide-d_6): δ 1.10–1.15 (t, 3H, CCH_3), 1.90–1.99 (s, 3H, $COCH_3$), 3.65–3.75 (s, 2H, Ar-CH_2C), 4.10–4.15 (q, 2H, CH_2C), and 6.85–7.25 (m, 3H or 4H, aromatic) ppm. There was an additional absorption peak for XIII at δ 2.20 (s, 3H, Ar-CH_3) ppm and for XIV at δ 2.25 (s, 3H, Ar-CH_3) ppm.

Aryl-Substituted 5-(1-Amino-2-phenylethyl)tetrazoles (XV–XXIII)—These compounds were prepared by two different methods (Table III).

Method A—The procedure is a modification of the method of McManus and Herbst (7). Typically, a mixture of the appropriately substituted cyano ester (I, III, IV, or IX) (0.10 mole), sodium azide (19.5 g, 0.30 mole), and anhydrous aluminum chloride (13.3 g, 0.10 mole) in dry tetrahydrofuran (250 ml) was heated at reflux for 24 hr. After cooling, the solvent was displaced with an equal volume of water on a continuous flow flash evaporator. The solids were filtered and air dried.



Scheme I

[1] Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, Ga. NMR data were recorded on a Varian Associates T-60A spectrophotometer.

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

Compound	R_1	R_2	Melting Point[a]	Crude Yield, %	Recrystallization[b] Solvent	Formula	Analysis, % Calc.	Found
I	H	H	130–132°[c]	92	A	—	—	—
II	H	F	88–90°	90	B	$C_{14}H_{15}FN_2O_3$	C 60.43	60.42
							H 5.43	5.50
							N 10.07	9.92
III	F	H	162–163°[d]	90	B	—	—	—
IV	H	Cl	80–82°	95	A	$C_{14}H_{15}ClN_2O_3$	C 57.05	57.12
							H 5.13	5.17
							N 9.50	9.57
V	Cl	H	161–163°[e]	96	B	—	—	—
VI	Cl	Cl	119–121°	71	A	$C_{14}H_{14}Cl_2N_2O_3$	C 51.08	51.25
							H 4.29	4.32
							N 8.51	8.33
VII	H	CH_3	84–86°	90	A	$C_{15}H_{18}N_2O_3$	C 65.68	65.63
							H 6.61	6.71
							N 10.21	10.29
VIII	CH_3	H	152–154°	92	A	$C_{15}H_{18}N_2O_3$	C 65.68	65.42
							H 6.61	6.69
							N 10.21	10.28
IX	NO_2	H	183–185°[f]	98	A	—	—	—

[a] Melting points were taken in open capillary tubes and are uncorrected. [b] A = 50% ethanol; B = toluene. [c] Lit. (8) mp 132–134°. [d] Lit. (8) mp 162–163°. [e] Lit. (8) mp 163–164°. [f] Lit. (8) mp 185–186°.

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

Compound	R_1	R_2	Melting Point[a]	Yield, %	Formula	Analysis, % Calc.	Found
X	H	F	147–149°	50.3	$C_{14}H_{16}FN_5O_3$	C 52.33	52.44
						H 5.02	5.02
						N 21.80	21.86
XI	Cl	H	138–140°	17.2	$C_{14}H_{16}ClN_5O_3$	C 49.78	49.37
						H 4.77	4.88
						N 20.73	20.87
XII	Cl	Cl	183–185°	45.1	$C_{14}H_{15}Cl_2N_5O_3$	C 45.18	45.29
						H 4.06	4.11
						N 18.82	18.69
XIII	H	CH_3	130–132°	23.8	$C_{15}H_{19}N_5O_3$	C 56.77	56.80
						H 6.03	6.06
						N 22.07	22.11
XIV	CH_3	H	101–105°	43.2	$C_{15}H_{19}N_5O_3 \cdot H_2O$	C 53.72	53.72
						H 6.31	6.33
						N 20.88	20.97

[a] Melting points were taken in open capillary tubes and are uncorrected.

The dried residue was suspended in concentrated hydrochloric acid (300 ml), and the mixture was heated at reflux for 3 hr. The mixture was concentrated *in vacuo*, and the residue was added to a solution of pyridine (30 ml) and 95% ethanol (250 ml). After refrigeration, the product was filtered and recrystallized from water–activated charcoal. Table III lists physical and chemical data for XV–XXIII.

Method B—The tetrazole ester (from Table II) (0.05 mole) was added to concentrated hydrochloric acid (100 ml) and heated at reflux overnight. After the mixture was concentrated *in vacuo*, the residue was dissolved in distilled water and the solution was adjusted to pH 8 with ammonium hydroxide solution. After decanting, the gummy residue was recrystallized from water and activated charcoal. Table III lists physical and chemical data for XV–XXIII.

NMR data agreed with the proposed structures. Assignments of the common absorption peaks are (10% sodium deuteroxide): δ 2.90–2.95 (m, 2H, Ar-CH_2), 4.25–4.35 (m, 1H, CCH), and 6.75–6.90 (m, 3H or 4H, aromatic) ppm. There was an additional absorption peak for XXI at δ 2.00 (s, 3H, Ar-CH_3) ppm and for XXII at δ 1.93 (s, 3H, Ar-CH_3) ppm.

Tyrosine Hydroxylase Inhibition—Tyrosine hydroxylase was isolated from beef adrenals[2] by the method of Nagatsu *et al.* (10). Active enzyme was precipitated by the addition of ammonium sulfate, resuspended in 10^{-3} *M* mercaptoethanol, and stored at −20°. Tetrazole derivatives, dissolved in dilute hydrochloric acid and measured at final concentrations of 2.5×10^{-5} *M*, were incubated at 37° for 15 min with shaking in the following medium: 200 μmoles of 1 *M* acetate buffer (pH 6.0), 0.1 μmole of *l*-tyrosine, 1.5–2.5 × 10^5 cpm of 3,5-ditritiotyrosine, 1.0 μmole of 2-amino-4-hydroxy-6,7-dimethyltetrahydropteridin in 0.1 ml of 0.1 *M* phosphate buffer (pH 7.4), 100 μmoles of mercaptoethanol, 0.5 μmole of ferrous sulfate, 0.2 ml of enzyme preparation, and water to a total volume of 1.0 ml. The medium alone was used as the control.

The reaction was stopped by the addition of 0.05 ml of acetic acid. The mixture was centrifuged, and the supernate was placed on an ion-exchange column[3] and washed once with 1.0 ml of water. The original supernate and washing were collected in a liquid scintillation vial, scintillation fluid[4] was added, and the solution was counted[5]. Data as nanomoles of tyrosine oxidized were calculated by measuring tritiated water released following the conversion of 3,5-ditritiotyrosine to 5-tritiodihydroxyphenylalanine. Enzyme activity in the presence of tetrazoles is presented in Table III.

RESULTS AND DISCUSSION

These preliminary results (Table III) indicate that replacement of the carboxyl group of phenylalanine derivatives with a tetrazole ring does not abolish the inhibitory properties of the respective parent compounds. Although no statistically significant differences (Duncan multiple range test) (11) in percent inhibition were found among the tetrazoles, statistical analysis by Dunnett's test (12) did reveal that all tetrazole analogs induced significant reduction ($p < 0.01$) of tyrosine hydroxylase activity compared to control enzyme activity.

Counsell *et al.* (13) reported 0, 35, 26, and 30% inhibition of tyrosine hydroxylase activity for 3-fluoro-, 4-fluoro-, 3-chloro-, and 4-chlorophenylalanine, respectively, at a concentration of 2×10^{-4} *M*. This result may be compared to the greater percent inhibition at a lower concentration by the corresponding tetrazole analogs, XVI, XVII, XVIII, and XIX (Table III).

[2] Meats Laboratory, Auburn University.

[3] Dowex 5 H^+.

[4] Riafluor, New England Nuclear, Boston, Mass.

[5] Beckman LS-150 liquid scintillation system.

Table III—Tetrazole Analogs of Phenylalanine and Their Inhibition of Tyrosine Hydroxylase

Compound[a]	R_1	R_2	Melting Point[b]	Method	Yield, %[c]	Formula	Analysis, % Calc.	Analysis, % Found	Enzyme Activity[d]	Inhibition, % ± *SEM*
Control									18.29 (10)	
XV	H	H	273°[e]	A	19	$C_9H_{11}N_5$	—	—	9.27 (6)	49.3 ± 5.2
XVI	H	F	288°	B	69.2	$C_9H_{10}FN_5$	C 52.17	52.14	7.84 (5)	57.1 ± 4.8
							H 4.86	4.70		
							N 33.80	34.12		
XVII	F	H	283°	A	17	$C_9H_{10}FN_5$	C 52.17	52.04	6.61 (7)	63.6 ± 5.7
							H 4.86	4.82		
							N 33.80	33.76		
XVIII	H	Cl	274°	A	11	$C_9H_{10}ClN_5$	C 48.33	48.26	8.51 (7)	53.6 ± 4.3
							H 4.51	4.49		
							N 31.31	31.26		
XIX	Cl	H	286°	B	50.1	$C_9H_{10}ClN_5$	C 48.33	48.18	7.57 (7)	58.7 ± 8.6
							H 4.51	4.54		
							N 31.31	31.20		
XX	Cl	Cl	282°	B	43.5	$C_9H_9Cl_2N_5$	C 41.88	41.79	6.87 (8)	62.5 ± 4.9
							H 3.51	3.55		
							N 27.13	27.17		
XXI	H	CH_3	284°	B	70.5	$C_{10}H_{13}N_5$	C 59.10	59.06	5.73 (6)	68.6 ± 1.4
							H 6.45	6.20		
							N 34.46	34.65		
XXII	CH_3	H	288°	B	44.2	$C_{10}H_{13}N_5$	C 59.10	58.96	6.09 (8)	66.9 ± 1.3
							H 6.45	6.26		
							N 34.46	34.61		
XXIII	NO_2	H	256°	A	17	$C_9H_{10}N_6O_2$	C 46.15	46.26	6.63 (6)	63.8 ± 2.8
							H 4.30	3.89		
							N 35.88	36.07		

[a] All racemates. [b] All compounds melted with decomposition. Melting points were taken in open capillary tubes and are uncorrected. [c] Yields in Method A are calculated from the cyano derivative; in Method B, they are calculated from the tetrazole ester. [d] Nanomoles of *l*-tyrosine oxidized in presence of inhibitor (2.5×10^{-5} *M*). Parentheses indicate the number of replicates. [e] Lit. (7) mp 270.5–271.5°.

This preliminary study indicates that tetrazole analogs of phenylalanine derivatives inhibit tyrosine hydroxylase *in vitro*. This study is being utilized as the basis for the design of additional compounds and more detailed pharmacological studies.

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