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Indolizine Derivatives with Biological Activity I: *N'*-Substituted Hydrazides of Indolizine-2-carboxylic Acid

MARIO CARDELLINI ˣ, FRANCESCO CLAUDI, MARIO GRIFANTINI, UGO GULINI, and SANTE MARTELLI

Abstract □ The synthesis and antimonoamine oxidase activity of some *N'*-substituted hydrazides of indolizine-2-carboxylic acid are described. They all inhibit monoamine oxidase and are more active than iproniazid. The structure–activity relationships also are discussed.

Keyphrases □ Indolizine-2-carboxylic acid hydrazides—synthesized, screened for effect on monoamine oxidase activity □ Hydrazides, *N'*-substituted—of indolizine-2-carboxylic acid synthesized, screened for effect on monoamine oxidase activity □ Monoamine oxidase activity—effect of various *N'*-substituted hydrazides of indolizine-2-carboxylic acid □ Structure–activity relationships—*N'*-substituted hydrazides of indolizine-2-carboxylic acid screened for effect on monoamine oxidase activity

Numerous *N'*-substituted hydrazides of indolecarboxylic acids have been synthesized in recent years as potential psychotherapeutic agents, and their inhibiting activity on monoamine oxidase has been tested *in vitro* (1, 2). The fundamental characteristic of such derivatives, some of which have proved to be intensely active, is the association in a single compound of a functional group with known antimonoamine oxidase activity, such as the hydrazide group, and the indole system. The indole system is present in various natural and synthetic compounds distinguished by psychotropic properties. The present paper reports the synthesis and study of the antimonoamine oxidase activity of a series of *N'*-substituted hydrazides of indolizine-2-carboxylic acid, which are analogs of the indole derivatives.

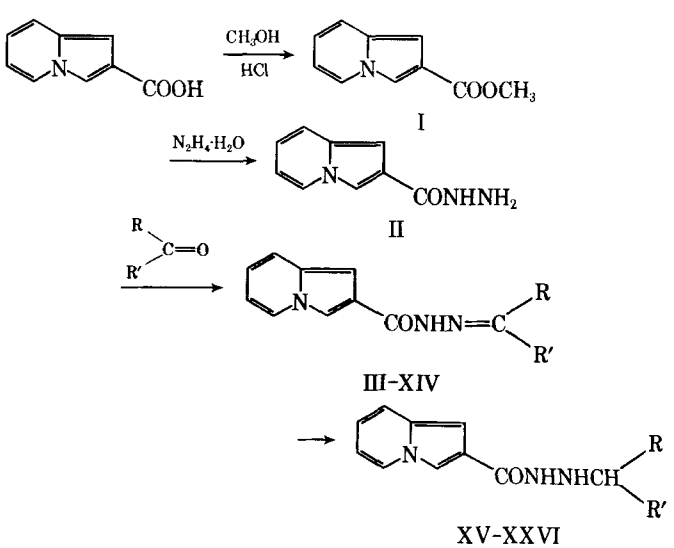
There are few literature reports on analogs of biologically active indole derivatives in which indole is replaced by indolizine. 1-Indolizinylalanine (3) has been prepared as a potential tryptophan antimetabolite; 1-indolizineacetic acid (4), an analog of 3-indoleacetic acid, has shown moderate auxinic activity; and numerous alkylindolizines (5), arylindolizines (6), and alkylaminoalkylindolizines (7, 8) have been synthesized and shown to influence the central nervous system.

Some 1-indolizineacetic acids (9, 10) possess analgesic and anti-inflammatory activity. Several indolizine analogs of indoxole (11, 12), a well-known anti-inflammatory agent, were synthesized recently.

CHEMISTRY

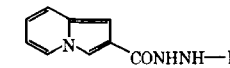
The *N'*-substituted hydrazides of indolizine-2-carboxylic acid (XV–XXVI) were synthesized according to Scheme I. On treatment with hydrazine hydrate, methyl indolizine-2-carboxylate (I), obtained by esterification of the corresponding acid (13) with methanol, gave indolizine-2-carboxylic acid hydrazide (II). The latter, by reaction with aldehydes and ketones, yielded the corresponding hydrazones, which were reduced with sodium borohydride.

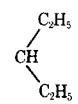
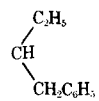
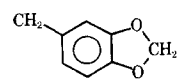
It is not possible to perform the reduction of benzylidenehydrazide (IX) by this method. It was performed with lithium aluminum hydride or with hydrogen in the presence of palladium-on-charcoal and traces of acetic acid. At the end of each reduction, the IR spectrum of the product obtained indicated that the characteristic band of the C=N group of the initial hydrazones had disappeared.



Scheme I

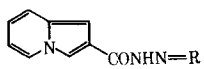
Table I—*N'*-Alkyl- and *N'*-Arylalkylhydrazides of Indolizine-2-carboxylic Acid and Their Monoamine Oxidase Inhibitory Activity

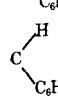
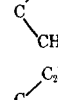


Compound	R	Reaction Solvent[a]	Extraction Solvent[a]	Melting Point, Recrystallization Solvent	Yield, %	Melting Point, Hydrochloride	I_{50}	X[b]	Molecular Formula	Analysis, %		Analysis[c], Hydrochloride, %	
										Calc.	Found	Calc.	Found
Iproniazid[d]		—	—	—	—	—	7.24×10^{-4}	1	—	—		—	
II	H	—	—	167–168°, E	74	228–230°	1.24×10^{-5}	58.2	$C_9H_9N_3O$	C 61.70 H 5.18 N 23.99	61.92 5.42 23.71	C 51.07 H 4.76 N 19.85	51.02 4.61 19.91
XV	CH_2CH_3	E–D (3:1)	G	128–130°, B	57	224–226°	1.44×10^{-4}	5	$C_{11}H_{13}N_3O$	C 65.00 H 6.45 N 20.68	65.07 6.63 20.95	C 55.15 H 5.88 N 17.53	55.38 6.09 17.62
XVI	$CH_2CH_2CH_3$	H	A	107–109°, E	70	218–220°	1.77×10^{-4}	4	$C_{12}H_{15}N_3O$	C 66.34 H 6.96 N 19.34	66.57 7.07 19.68	C 56.80 H 6.35 N 16.56	57.02 6.51 16.82
XVII	$CH_2CH_2CH_2CH_3$	H	A	96–98°, E	70	208–210°	2.18×10^{-4}	3.3	$C_{13}H_{17}N_3O$	C 67.50 H 7.41 N 18.17	67.71 7.16 18.47	C 58.31 H 6.77 N 15.69	58.59 6.52 15.81
XVIII		H	G	114–115°, E	60	228–230°	1.60×10^{-4}	4.5	$C_{13}H_{17}N_3O$	C 67.50 H 7.41 N 18.17	67.64 7.42 17.98	C 58.31 H 6.77 N 15.69	58.56 6.83 15.63
XIX		E–H (1:1)	G	151–152°, F	90	228–230°	1.52×10^{-4}	4.7	$C_{14}H_{17}N_3O$	C 69.11 H 7.04 N 17.27	69.13 7.10 17.32	C 60.1. H 6.48 N 15.02	60.05 6.42 15.06
XX		H	G	127–128°, E	80	230–231°	2.5×10^{-4}	2.89	$C_{14}H_{19}N_3O$	C 68.54 H 7.81 N 17.13	68.83 7.64 17.28	C 59.67 H 7.15 N 12.58	59.82 7.21 12.72
XXI	$CH_2C_6H_5$	—	—	150–152°, E	54	224–226°	4.6×10^{-5}	15.7	$C_{16}H_{15}N_3O$	C 72.43 H 5.70 N 15.84	72.73 5.72 15.86	C 63.67 H 5.34 N 13.92	63.54 5.41 13.79
XXII		E–H (1:1)	G	176–177°, E	73	230–232°	1.73×10^{-4}	4.17	$C_{17}H_{17}N_3O$	C 73.09 H 6.13 N 15.04	72.74 6.18 14.96	C 64.65 H 5.75 N 13.30	64.62 6.01 13.06
XXIII	$CH_2C_6H_4p\text{-}CH_3$	E–H (1:1)	A	176–178°, E	55	222–224°	9.82×10^{-5}	7.37	$C_{17}H_{17}N_3O$	C 73.09 H 6.13 N 15.04	73.29 6.38 15.24	C 64.65 H 5.75 N 13.30	64.41 5.93 13.19
XXIV		H–D (14:1)	G	133–134°, E	75	208–210°	1.27×10^{-4}	5.67	$C_{18}H_{19}N_3O$	C 73.69 H 6.53 N 14.33	73.83 6.55 14.48	C 65.54 H 6.11 N 12.74	65.42 6.02 12.88
XXV		H–D (7:1)	G	131–132°, E	80	215–218°	1.13×10^{-4}	6.39	$C_{19}H_{21}N_3O$	C 74.24 H 6.89 N 13.67	74.65 6.79 13.94	C 66.36 H 6.44 N 12.22	66.28 6.19 12.03
XXVI		H–E (1:1)	C	155–158°, E	35	216–218°	1.77×10^{-5}	40.8	$C_{17}H_{15}N_3O_3$	C 66.01 H 4.89 N 13.59	66.15 5.04 13.72	C 59.04 H 4.66 N 12.15	59.15 4.85 11.81

[a] A = ethyl acetate, B = benzene, C = methylene chloride, D = dioxane, E = ethanol, F = anhydrous ethanol, G = ethyl ether, and H = methanol. [b] X = I_{50}iproniazid/I_{50}compound. [c] Monohydrochlorides. [d] *N'*-Isonicotinoyl-*N'*-isopropylhydrazine.

Table II—*N'*-Alkylidene- and *N'*-Arylalkylidenehydrazines of Indolizine-2-carboxylic Acid



Compound	R	Recrystallization Solvent	Melting Point	Reaction Time (hr)/ Reaction Temperature	Yield, %	Molecular Formula	Analysis, %	
							Calc.	Found
III	$CHCH_3$	Methanol	167–169°	4/20°	87	$C_{11}H_{11}N_3O$	C 65.67 H 5.51 N 20.88	65.85 5.65 21.08
IV	$CHCH_2CH_3$	Ethanol	150–153°	4/40°	70	$C_{12}H_{13}N_3O$	C 66.95 H 6.09 N 19.52	67.01 6.19 19.75
V	$CHCH_2CH_2CH_3$	Ethanol	146–148°	2/50°	89	$C_{13}H_{15}N_3O$	C 68.10 H 6.59 N 18.33	67.95 6.65 18.29
VI		Ethanol	141–142°	5/45°	89	$C_{13}H_{15}N_3O$	C 68.10 H 6.59 N 18.33	68.44 6.75 18.29
VII		Ethanol	160–161°	4/Reflux	80	$C_{14}H_{15}N_3O$	C 69.69 H 6.27 N 17.42	69.46 6.24 17.23
VIII		Ethanol	131–132°	5/Reflux	77	$C_{14}H_{17}N_3O$	C 69.11 H 7.04 N 17.27	69.00 7.01 17.23
IX		Ethanol	224–226°	1/20°	75	$C_{16}H_{13}N_3O$	C 72.98 H 4.98 N 15.96	72.71 4.87 16.21
X		Tetrahydrofuran	210–212°	3/20°	82	$C_{17}H_{15}N_3O$	C 73.63 H 5.45 N 15.15	73.84 5.44 15.15
XI		Ethanol	210–212°	5/20°	77	$C_{17}H_{15}N_3O$	C 73.63 H 5.45 N 15.15	73.82 5.45 15.30
XII		Ethanol	176–177°	15/20°	75	$C_{18}H_{17}N_3O$	C 74.20 H 5.88 N 14.42	74.28 5.73 14.48
XIII		Ethanol	141–142°	1/Reflux	92	$C_{19}H_{19}N_3O$	C 74.73 H 6.27 N 13.76	75.03 6.45 14.05
XIV		Ethanol	196–197°	3/20°	75	$C_{17}H_{13}N_3O_3$	C 66.44 H 4.26 N 13.68	66.09 4.32 13.48

EXPERIMENTAL[1]

Methyl Indolizine-2-carboxylate (I)—Indolizine-2-carboxylic acid (0.2 mole) (13) was added to 500 ml of methanol saturated with dry hydrogen chloride gas. The mixture was refluxed for 6 hr, and hydrogen chloride gas was bubbled through the solution. The solvent was then eliminated under vacuum, and the residue was extracted with hot *n*-hexane. Evaporation of the *n*-hexane gave a solid, which was crystallized from ethanol, yielding 80%, mp 96–97° [lit. (13) mp 97–99°].

Hydrazide of Indolizine-2-carboxylic Acid (II)—Hydrazine hydrate (85%) (1.06 moles) was added to a solution of 0.14 mole of I in 260 ml of methanol, and the mixture was refluxed for 6 hr. Cooling led to the precipitation of a solid which was washed with water and crystallized; IR (ν_{max}): 1660, 1620 (C=O), 3290, and 3200 (NH) cm^{-1} (Table I).

***N'*-Alkylidene- and *N'*-Arylalkylidenehydrazides of Indolizine-2-carboxylic Acid (III–XIV)**—The appropriate aldehyde or ketone (0.006 mole) was added to a solution of 0.005 mole of II in 40 ml of methanol. The mixture was stirred at room temperature or was heated for various times. With 3,4-methylenedioxybenzaldehyde and the ketones, the reaction was promoted by the addition of 0.005 mole of acetic acid. The products precipitated directly on cooling or by concentration of the solution and were subsequently crystallized; IR (ν_{max}): 3120–3390 (NH), 1630–1655 (C=O), and 1600–1630 (C=N) cm^{-1} (Table II).

***N'*-Alkyl- and *N'*-Arylalkylhydrazides of Indolizine-2-carboxylic Acid (XV–XXVI)**—With stirring at 0°, 0.020–0.10 mole of sodium borohydride was slowly added to 0.021 mole of one compound, III–XIV, dissolved in the minimum amount of solvent. At the end of the addition, the mixture was stirred for 5 hr and the solvent was evaporated under vacuum. The residue was treated with water and extracted with a suitable solvent. The organic solution was dried over sodium sulfate, and the solvent was eliminated under reduced pressure. The residue was crystallized; IR (ν_{max}): 3200–3290 (NH) and 1620–1660 (C=O) cm^{-1} (Table I).

***N'*-Benzylhydrazide of Indolizine-2-carboxylic Acid (XXI)**—*Method A*—The benzylidenehydrazide IX (0.0046 mole) in 50 ml of anhydrous tetrahydrofuran was added to a suspension of 0.021 mole of lithium aluminum hydride in 20 ml of anhydrous tetrahydrofuran. The mixture was refluxed for 2.5 hr and then stirred overnight at room temperature. Excess lithium aluminum hydride was destroyed with water-saturated ether. The mixture was filtered, the solvent was evaporated under vacuum, and the residue was crystallized.

Method B—A suspension of 0.0038 mole of IX in 200 ml of absolute ethanol and 30 ml of dioxane containing 0.1 g of 10% palladium-on-charcoal and a few drops of acetic acid was hydrogenated at 1.5 atm and 60°. When the theoretical amount of hydrogen had been consumed, the suspension was filtered while hot. The solvent was evaporated under vacuum, and the residue was crystallized, yielding 45%.

Hydrochlorides of *N'*-Alkyl- and *N'*-Arylalkylhydrazides of Indolizine-2-carboxylic Acid—Dry hydrochloric acid was bubbled through a solution of 0.005 mole of one of the hydrazides, XV–XXVI, in the minimum amount of absolute ethanol. The hydrochlorides precipitated directly or on the addition of anhydrous ether and were crystallized from absolute ethanol (Table I).

Antimonoamine Oxidase Activity *In Vitro*—The enzyme was prepared from pig plasma by a reported method (14). For the tests of

[1] Melting points were determined with a Büchi apparatus and are not corrected. IR spectra were taken in mineral oil using a Perkin-Elmer 257 spectrophotometer.

enzyme activity and inhibition, the protein extract derived from stage II (precipitation with 35–55% ammonium sulfate) was used. The monoamine oxidase activity of the enzyme, determined by the method of Zeller *et al.* (15), was followed at 253 nm[2] at 37° and pH 7.2, and the values of ΔE/min during the first 10 min of the reaction were calculated. The reaction mixture consisted of 40 μl of 4.29×10^{-3} *M* 3-iodobenzylamine, 20 μl of protein solution (1.6 mg), 1500 μl of 0.1 *M* sodium phosphate buffer (pH 7.2), and double-distilled water to a final volume of 2000 μl. The same reaction mixture not containing the substrate was used as the reference sample.

The inhibition tests were performed (15) using the described mixture and keeping the concentrations of the components and the final volume constant while varying only the inhibitor concentration, both in the reference solution and the test solution. The I_{50} values (inhibitor molar concentration producing 50% inhibition) were calculated graphically.

Some water-insoluble compounds were added to the reaction mixture in dioxane solution. The dioxane concentration in the reaction mixture was less than 3%. To determine the I_{50} values, the inhibition due to the dioxane was taken into account, since this solvent has a weak inhibiting effect on enzymatic activity. Compounds XV–XVIII were added to the reaction mixture as hydrochlorides, while the others were used in the form of the free bases dissolved in dioxane because of the low solubility of the hydrochlorides in the aqueous solution and in dioxane.

RESULTS AND DISCUSSION

All tested compounds proved to be about three to 58 times more active than iproniazid in the inhibition of monoamine oxidase (Table I). In addition, their activity was distinctly greater than that of the corresponding hydrazides of indole-2-carboxylic and indole-3-carboxylic acids tested on an enzyme from calf liver with iproniazid as the standard. Hence, the displacement of the nitrogen from the 1-position of the indole to the bridge 4-position of indolizine leads, at least in the cases studied, to an enhancement of activity.

The unsubstituted hydrazide was most active. In general, the activity was greater in the series containing arylalkyl groups on the *N'*-nitrogen of the hydrazide chain than in the series with simple alkyl chains.

In the first series, one of the most active compounds was the benzyl derivative (XXI). Compounds XXII and XXIII, with a methyl substituent on the benzyl methylene group or on the benzene ring, were less active than the parent compound; XXVI, with a methylbenzodioxole substituent, was more active. The α-methyl- and α-ethyl-β-phenylethyl homologs (XXIV and XXV) were also more active than the α-methylbenzyl derivative (XXII).

In the second series, the activity decreased with an increase in the length of the chain (XV–XVII). However, with the same number of carbon atoms, branching (XVII *versus* XVIII) and replacement of the alkyl group by the corresponding cycloalkyl group (XIX *versus* XX) appeared to have a positive influence.

[2] Zeiss PMQ II spectrophotometer.

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