Synthesis of 1-(*N*-Piperidinoacetyl)-4-arylthiosemicarbazides as Possible Anticonvulsants

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Several 1-(*N*-piperidinoacetyl)-4-arylthiosemicarbazides were synthesized from *N*-piperidinoacetyl hydrazide, which was obtained by the reaction of hydrazine hydrate with ethyl *N*-piperidinoacetate. The anticonvulsant activity of these substituted thiosemicarbazides was reflected by their ability to provide 10-50% protection against pentylenetetrazol-induced convulsions in mice. All substituted thiosemicarbazides (0.3 m*M*) inhibited *in vitro* monoamine oxidase activity of rat brain homogenates and provided 21-86% protection against hypoosmotic hemolysis at a final concentration of 0.1 m*M*.

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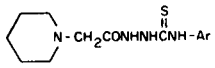
In several literture reports hydrazine derivatives (3), semicarbazides (4) and thiosemicarbazides (5) have been shown to inhibit monoamine oxidase activity. Monoamine oxidase inhibitors have also been reported to possess anticonvulsant property (6). Earlier studies have proposed that the stabilization of the red blood cell membrane could presumbaly account for the pharmacological properties of various central nervous system depressants, tranquilizers, and biogenic amines (7-10). These observations led to the synthesis of 1-(*N*-piperidinoacetyl)-4-arylthiosemicarbazides which were evaluated for their anticonvulsant activity. All substituted thiosemicarbazides inhibited monoamine oxidase activity and possessed membrane-stabilizing property which, however, were found to be unrelated with their anticonvulsant activity.

Ethyl *N*-piperidinoacetate, obtained by the reaction of ethylchloroacetate and piperidine, was refluxed with hydrazine hydrate in absolute ethanol to obtain *N*-piperidinoacetylhydrazide. This hydrazide on treatment with the appropriate arylisothiocyanates yielded the corresponding 1-(*N*-piperidinoacetyl)-4-arylthiosemicarbazides (**1-8**).

All substituted thiosemicarbazides (**1-8**) possessed anticolvulsant activity (100 mg./kg., i.p.). The degree of protection ranged from 10-50% where maximum protection was observed with 1-(*N*-piperidinoacetyl)-4(4-chlorophenyl)-thiosemicarbazide (**2**). All substituted thiosemicarbazides inhibited the oxidative deamination of kynuramine at a final concentration of 0.3 m*M* using rat brain homogenate as the source of the enzyme monoamine oxidase. The maximum inhibition of 81% was observed with 1-(*N*-piperidinoacetyl)-4(1-napthyl)thiosemicarbazide (**8**) while minimum inhibition (44%) was observed with 1-(*N*-piperidinoacetyl)-4-(4-methoxyphenyl)thiosemicarbazide (**7**). The inhibition of the enzyme monoamine oxidase by these compounds was found to be concentration dependent. All compounds (**1-8**) exhibited membrane stabilizing property which was reflected by their ability to provide protection against hypoosmotic hemolysis of canine red blood cells. The degree of protection ranged from 21-80% at a final concentration of 0.1 m*M* of the test compounds (**1-8**). The minimum antihemolytic activity was observed with 1-(*N*-piperidinoacetyl)-4-(1-napthyl)thiosemicarbazide (**8**)

Table I

Physical Constants of 1-(*N*-Piperidinoacetyl)-4-arylthiosemicarbazides



Compound	Ar	Melting Point °C	Yield %	Molecular Formula	Analysis % Calculated C	H	N	Found C	H	N
1	C_6H_5	176	72	$C_{14}H_{20}N_4OS$	57.53	6.85	19.18	57.16	6.97	18.85
2	4-ClC_6H_4	172	76	$C_{14}H_{19}ClN_4OS$	51.45	5.81	17.15	51.18	5.98	16.98
3	4-BrC_6H_4	172	78	$C_{14}H_{19}BrN_4OS$	45.28	5.12	15.09	45.60	4.94	14.88
4	4-IC_6H_4	181	80	$C_{14}H_{19}IN_4OS$	40.19	4.54	13.39	40.52	4.43	13.62
5	3-$CH_3C_6H_4$	147	68	$C_{15}H_{22}N_4OS$	58.82	7.19	18.30	58.53	7.28	18.12
6	4-$CH_3C_6H_4$	158	77	$C_{15}H_{22}N_4OS$	58.82	7.19	18.30	59.10	6.92	18.54
7	4-$OCH_3C_6H_4$	110	74	$C_{15}H_{22}N_4O_2S$	55.90	6.83	17.39	56.18	6.54	17.65
8	(1-$C_{10}H_7$)	183	70	$C_{18}H_{22}N_4OS$	63.16	6.43	16.37	62.90	6.56	16.14

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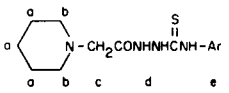
Table II

Spectral Data of 1-(*N*-Piperidinoacetyl)-4-arylthiosemicarbazides (a)



Compound No.	Ar	Infrared Absorption (cm^{-1})				Pmr Chemical Shift (δ)				
		NH	C=O	C=C	C=S	a	b	c	d	e
1	C_6H_5	3220, 1630	1675	1575	1120	1.30 (6H, b)	2.30 (4H, b)	2.86 (2H, s)	9.30 (1H, b)	7.00-7.40 (5H, m)
2	4-ClC_6H_4	3220, 1625	1675	1570	1120	1.30 (6H, b)	2.30 (4H, b)	2.90 (2H, s)	9.30 (1H, b)	7.20 (2H, d, J = 3Hz) 7.35 (2H, d, J = 3Hz)
3	4-BrC_6H_4	3220, 1630	1670	1560	1120	1.45 (6H, b)	2.50 (4H, b)	3.00 (2H, s)	9.50 (1H, b)	7.43 (4H, s)
4	4-IC_6H_4	3230, 1640	1680	1560	1130	1.40 (6H, b)	2.40 (4H, b)	3.05 (2H, s)	9.45 (1H, b)	7.30 (2H, d, J = 3 Hz) 7.60 (2H, d, J = 3 Hz)
5	3-$CH_3C_6H_4$	3220, 1625	1670	1560	1130	1.45 (6H, b)	2.50 (4H, b)	3.00 (2H, s)	9.40 (1H, b)	2.30 (3H, s), 7.25 (1H, s), 6.70-7.40 (3H, m)
6	4-$CH_3C_6H_4$	3220, 1625	1670	1565	1120	1.45 (6H, b)	2.50 (4H, b)	3.03 (2H, s)	9.40 (1H, b)	2.30 (3H, s), 7.13 (2H, d, J = 3 Hz), 7.27 (2H, d, J = 3 Hz)
7	4-$OCH_3C_6H_4$	3200, 1630	1665	1570	1160	1.45 (6H, b)	2.40 (4H, b)	3.03 (2H, s)	9.40 (1H, b)	3.76 (3H, s), 6.90 (2H, d, J = 4 Hz), 7.30 (2H, d, J = 4 Hz)
8	(1-$C_{10}H_7$)	3200, 1630	1670	1560	1115	1.45 (6H, b)	2.40 (4H, b)	3.50 (2H, s)	9.70 (1H, b)	7.35-7.70 (4H, m) 7.80-8.15 (3H, m)

(a) Abbreviations: s = singlet; d = doublet; m = multiplet; b = broad.

while 1-(*N*-piperidinoacetyl)-4-phenylthiosemicarbazide (**1**),provided maximum protection against hypoosmotic hemolysis. The antihemolytic activity of these compounds was concentration dependent and their ED_{50} values ranged from 0.04-0.37 m*M*.

EXPERIMENTAL

All 1-(*N*-piperidinoacetyl)-4-arylthiosemicarbazides were analyzed for their carbon, hydrogen and nitrogen contents. Melting points were taken in open capillary tubes with an immersion thermometer and are corrected. The infrared spectra of these compounds were recorded on Beckman IR-33 spectrometer in nujol mull suspension. The proton magnetic resonance spectra of these thiosemicarbazides were obtained in deuterodimethylsulphoxide solution on Varian EM-390 instrument using tetramethylsilane as an internal reference.

Ethyl *N*-Piperidinoacetate.

Following the earlier reported method (11) a solution of ethyl chloroacetate (0.2 mole) in 100 ml. of dry benzene was added slowly to a solution of piperidine (0.4 mole) in 200 ml. of dry benzene. The reaction mixture was stirred during the addition of ethylchloroacetate and after that, refluxed for 1 hour on a steam bath. The precipitated piperidine hydrochloride was filtered off and the filtrate on concentration under reduced pressure gave ethyl *N*-piperidinoacetate which was distilled at 215° as a colorless liquid. The hydrochloride of *N*-piperidinoacetate melted at 130° (reported m.p. 130-131°) and was characterized by its spectral analysis; ir (carbon tetrachloride): $COOCH_2CH_3$ (1750 cm^{-1}); nmr (carbon tetrachloride): 4.03 ($COOCH_2CH_3$, 1q), 3.00 ($N\text{-}CH_2COO$-, 1s), 2.50 (-CH_2-N-CH_2-, t), 1.50 (-CH_2-CH_2-CH_2-, m) and 1.23 ($COOCH_2CH_3$, t).

N-Piperidinoacetylhydrazide.

A mixture of ethyl *N*-piperidinoacetate (0.15 mole) and hydrazine hydrate (0.3 mole; 99-100%) in 150 ml. of absolute ethanol was refluxed on water bath for 8 hours. The excess of ethanol was removed under reduced pressure and the fraction distilling at 180°/25 mm was collected, (reported b.p. 130°/2 mm) (12). The colorless liquid, which on crystallization with cyclohexane melted at 38-40°, was characterized by its spectral analysis; ir (carbon tetrachloride): $CONHNH_2$ (3350 and 1630 cm^{-1}), $CONHNH_2$ (3220) and $CONHNH_2$ (1690); nmr (carbon tetrachloride): 7.80 ($CONHNH_2$, b), 3.60 ($CONHNH_2$, b), 2.90 ($N\text{-}CH_2COO$-, 2), 2.40 (-CH_2-N-CH_2-, t) and 1.53 (-CH_2-CH_2-CH_2-, m).

1-(*N*-Piperidinoacetyl)-4-arylthiosemicarbazides (**1-8**).

A mixture of *N*-piperidinoacetylhydrazide (0.005 mole) and the suitable arylisothiocyanate (0.005 mole) in 25 ml. of dry benzene was refluxed on a steam bath for 2-3 hours. The excess of benzene was removed by distillation under reduced pressure. The crude product which precipitated out was washed with ether, dilute hydrochloric acid and finally several times with cold water. All 1-(*N*-piperidinoacetyl)-4-arylthiosemicarbazides (**1-8**) were recrystallized from ethanol and characterized by their sharp melting points and elemental (Table I) and spectral analyses (Table II).

Anticolvulsant Activity.

The anticolvulsant activity of these substituted thiosemicarbazides was determined (13) in albino mice of either sex weighing 25-30 g. A suspension of test compound in 5% aqueous gum acacia was injected intraperitoneally at a does of 100 mg./kg. to evaluate their ability to provide protection against subcutaneous injection of pentylenetetrazol-induced convulsions (90 mg./kg.).

Monoamine Oxidase Activity.

Spectrophotofluorometric method (13) was used for the determination of monoamine oxidase activity of rat brain homogenate using kynuramine as the substrate. The various substituted thiosemicarbazides were used at a final concentration of 0.3 m*M*.

Hypoosmotic Hemolysis.

The assay of hypoosmotic hemolysis of canine red blood cells was carried out by following the method reported earlier (13). The membrane stabilizing property of these 1-(*N*-piperidinoacetyl)-4-arylthiosemicarbazides at a final concentration of 0.1 m*M* was studied by determining the decrease in the degree of hypoosmotic hemolysis.

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