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Monoamine Oxidase Inhibitory and Anticonvulsant Activities of Some Substituted Isatin Semicarbazones

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Abstract: ***Substituted isatin-semicarbazones were synthesized by condensation reaction of appropriate N-substituted-5-(un) substituted isatin with corresponding phenyl semicarbazide and studied for monoamine oxidase (MAO) inhibitory and anticonvulsant activities. In vitro MAO-inhibiting activity was determined by radiometric enzyme assay method in rat liver tissue, and anticonvulsant activity was performed using standard maximal electroshock (MES) induced seizures method in mice. . All of these compounds exhibited MAO inhibiting and anticonvulsant activities, however, there appeared no parallel correlation ship between the activities of these compounds. Thus, these results indicate that MAO inhibiting property does not prove the biochemical basis for the anticonvulsant activity of these compounds. Acute toxicity studies indicate that the compounds have a wide margin of safety.***

Keywords: Isatin semicarbazones, synthesis, monoamine oxidase activity, anticonvulsant activity, Acute toxicity

1. Introduction

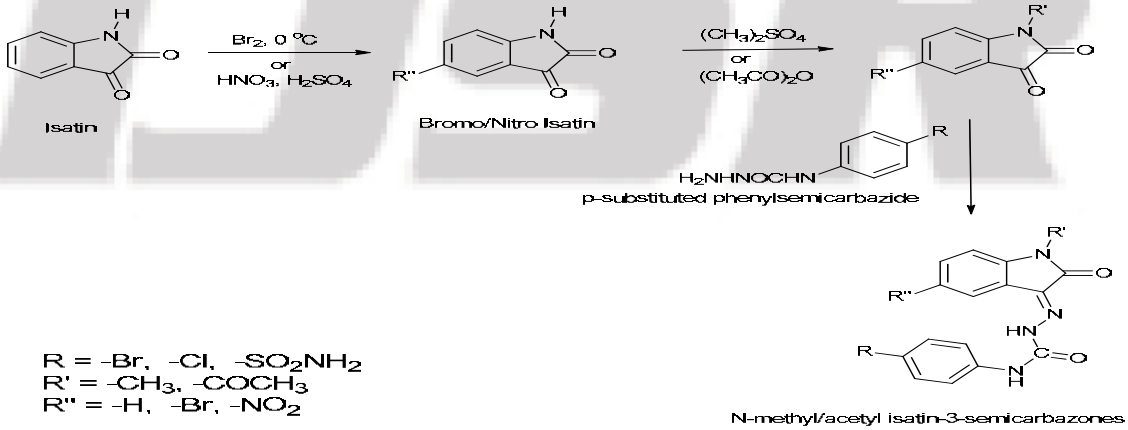
Monoamine oxidase inhibitors are known to exhibit significant anticonvulsant activity against experimentally induced seizures and a parallel correlation between these two properties has been reported although reports to the contrary also exist [1-5] .Several isatin , semicarbazones and isatin-semicarbazones have been reported to possess significant anticonvulsant activity as well as many other biological activities [6-12]. In an attempt to find a better MAO inhibitor, some isatin semicarbazones were synthesized. In the present investigation, there *in vitro* MAO inhibiting property and anticonvulsant activity were studied. Attempts were made also to correlate the *in vitro* MAO inhibitory effectiveness with their anticonvulsant property as a function of their chemical structure.

2. Methodology

2.1. Chemistry

The melting points determined with Thomas Hoover Apparatus in open capillary method were uncorrected. The purity of the compounds was checked by thin layer chromatography (TLC) using silica gel G as stationary phase and chloroform and ethanol (9:1) as the eluant. Elemental analysis was performed on Perkin Elmer Model 240 C Analyser (USA) and was within 0.4% limit of the calculated values. UV spectra were obtained on Cintra 10 UV Visible Spectrometer. IR spectra were recorded on JASCO FT IR-5300 spectrometer using KBr pellets. and ^{1}H NMR spectra were determined at MHz 90 300-40 MHz on JEOL FX 90 Q FT-NMR Spectrometer using tetramethyl silane as internal standard. Synthesis of substituted semicarbazones (compounds 1-6) was carried out according to the Scheme-1 by the condensation reaction of equimolar quantities of appropriate substituted isatin compound with the corresponding semicarbazide [13] .

a) Synthesis of N-methyl/acetylisatin-5-(un)- substituted isatin-3- semicarbazones



Scheme 1

b) Condensation of N-methyl/acetyl isatin and its 5-(un)-substituted isatin with para substituted phenyl semicarbazides.

N-methyl/acetyl isatin and its 5-(un)-substituted derivatives were condensed with corresponding substituted phenyl semicarbazides in the presence of glacial acetic acid or sodium acetate.

R'' = H, -Br, -NO_2; R' =-CH_3, -$COCH_3$; R= -Cl, -Br, -SO_2NH_2.

Synthesis of some representative compounds is as follows:

c) Synthesis of N-methyl isatin-3-para bromophenyl semicarbazone (1)

N-methyl isatin (0.002 M, 0.322 g) and p-bromophenyl semicarbazide (0.002 M., 0.46 g) were dissolved in 15 mL of ethanol and 1 mL of glacial acetic acid was added. The content was refluxed for 40 minutes and kept in ice. The yellow product formed was filtered, dried and re-crystallized from ethanol.

Yield: 76% ; M. p.: 248°C.
UV (λ_{max}, nm) : 245.5
IR (KBr), cm^{-1}: 3425 (2^0NH), 3406 (amide -NH), 2953 (-CH_3), 1653 (amide C=O), 1610 (C-H), 1590 (C=N), 1460, 869 cm^{-1}.
^{1}+ 1 0 5 ' 0 62 ' SSP V + 1 &+ $_3$), 5.8 (s, 1H -CONH, D_2O exchangeable) 7-7.7 (m, 8H, Ar-H) 8.6 (s, 1H, =N-NH, D_2O exchangeable)
Anal: $C_{16}H_{13}N_4O_2Br$ (C, H, N)(Table-2).

d) Synthesis of N-acetyl-isatin-3-para chlorophenyl semicarbazone (2)

Equimolar quantities of N-acetyl isatin (0.003 M, 0.569 gm) and para chlorophenyl semicarbazide (0.003 M., 0.555 gm) was dissolved in 10 mL of ethanol containing few drops of glacial acetic acid. The reaction mixture was refluxed for 45 minutes and kept in ice. The resultant solid was dried and re-crystallized from ethanol.

Yield: 72% ; M.p. :143°C
UV (λ_{max}, nm) : 243, 285.5
IR (KBr), cm^{-1}: 3423 (2^0NH), 3314 (amide -NH), 2928 (CH_3), 1728 (acetyl C=O), 1612 (amide C=O), 1589 (C=N), 1460, 821.
^{1}H-NMR (DMSO-D_6 SSP V + 1 &2 &+ $_3$), 5.8(s, 1H, -CONH, D_2O exchangeable), 6.8-7.8 (m, 8H, Ar-H), 8.8 (s, 1H, =N-NH, D_2O exchangeable).
Anal: $C_{17}H_{13}N_4O_3Cl$ (C, H, N)(Table-2).

e) Synthesis of 5-Bromo-N-acetyl isatin-3-para sulfamoylphenyl semicarbazone (4)

To a solution of p-sulfamoyl phenylsemi carbazide (0.01 M., 2.30 gm) in ethanol, 1-2 mL of glacial acetic acid added to maintain pH between 5-6. To this solution an equimolar quantity of 5-bromo-N-acetyl isatin (0.01 M., 2.68 gm) in ethanol was added. The reaction mixture was refluxed for 45 minutes. The resulting product obtained after cooling was filtered and re-crystallized from ethanol.

Yield: 74%; M.p. : 173°C
UV (λ_{max}, nm) : 271, 279, 326.5
IR (KBr), cm^{-1}: 3381 (2^0NH), 3232 (amide -NH), 2922 (-CH_3), 1728 (acetyl C=O), 1690 (amide C=O), 1590 (C=N), 1460, 1160 (S=O), 804.
^{1}H-NMR (DMSO-D_6 SSP V + 1 &2 &+ $_3$), 5.6(s, 1H, -CONH, D_2O exchangeable), 6.4-7.8 (m, 7H, Ar-H), 9.8 (s, 1H, = N-NH, D_2O exchangeable), (bs, 2H, SO_2NH_2, D_2O exchangeable).
Anal: $C_{17}H_{14}N_5O_5BrS$ (C, H, N)(Table-2).

f) Synthesis of 5-Nitro-N-acetyl isatin-3-para chlorophenyl semicarbazone (5)

To an equimolar solution of 5-Nitro-N-acetyl isatin (0.01 M, 2.35 gm) and p-chlorophenyl semicarbazide (0.01 M, 1.85 gm) in ethanol, 10 drops of glacial acetic acid were added. The reaction mixture was refluxed for 40 mins. The resultant product was cooled, filtered, dried and re-crystallized from ethanol.

Yield: 68%; M.p. : 150°C
UV (λ_{max}, nm) : 242, 247, 294
IR (KBr), cm^{-1} : 3429 (2^0NH), 3314 (amide -NH), 2922 (-CH_3), 1735 (C=O), 16550 (C=O), 1548 (C=N), 1462, 1332 (C-NO_2), 821 cm^{-1}.
^{1}H-NMR (DMSO-D_6 SSP V + 1 &2 &+ $_3$), 5.8(s, 1H, -CONH, D_2O exchangeable), 6.8-7.8 (m, 7H, Ar-H), 8.7 (s, 1H, = N-NH, D_2O exchangeable).
Anal: $C_{17}H_{12}N_5O_5Cl$ (C, H, N)(Table-2).

2.2. Pharmacology

a) Monoamine oxidase inhibitory activity

MAO inhibitory activity was determined by radiometric enzyme assay method [2]. The enzyme source was rat liver homogenate and the substrate used was ^{14}C-tyramine. The test drugs were dissolved in propylene glycol, and MAO inhibition afforded by graded concentrations of the compounds was determined with respect to controls, where equivalent amount of propylene glycol was added to the reaction mixture. The ID_{50} values of test compounds were calculated as the concentration required to induce 50% inhibition of enzyme activity.

b) Anticonvulsant activity

Anticonvulsant activity of the compounds was assessed in albino mice (20 to 26 g) of either sex against maximal electroshock –induced seizures [14]. The compounds suspended in 2% Tween 80 were administerd intraperitoneally (i.p.) in graded doses to group of 10 mice each. The anticonvulsant activity was determined and the ED_{50} dose was calculated.

c) Acute toxicity

Acute toxicity studies were conducted in albino mice (20-30g), of either sex. Graded doses (200, 500, 1000mg/kg, i.p.) of the compounds, suspended in 2% Tween 80, were administered to group of 10 mice for each dose. Mortality and the presence of any overt toxic sign were determined over a 24 h period.

3. Results and Discussion

The physical and chemical data of synthesized compounds are given in Table 1 & 2. The UV spectra of compound (1) revealed absorption peak at 245.5 nm (λ_{max}). IR spectrum showed the presence of secondary -NH group and amide -NH absorption at 3425 cm^{-1}, respectively. Absorption band at 1653 cm^{-1} occurs due to amide C=O stretching. The band at 1630 cm^{-1} showed the presence of C=N group. The -CH (aromatic) stretching appeared at 1610 cm^{-1}. The adsorption band at 869 cm^{-1} showed the presence of the phenyl ring with para substitution. The ^{1}H-NMR spectrum of N-methyl-(p-bromophenyl) isatin-3-semicarbazone (1) in DMSO-d_6 showed a singlet at δ 3.2 and confirmed the N-CH_3 protons. A D_2O exchangeable singlet at δ 5.80 confirmed the 1H of CONH protons. A multiplet at a range of δ 7-7.7 appeared due to aryl protons. Another D_2O exchangeable singlet for 1H at δ 8.6 confirmed the =N-NH proton.

The UV spectra of compound (4) revealed the (λ_{max}) at 326.5, 279 and 271 nm. These latter two bands are characteristic of *para* substituted phenyl ring. IR spectrum of compound (4) showed the absorption band at 3232 cm^{-1} and 3381 cm^{-1} of -NH stretching. The adsorption band of amide C=O stretching and C=N stretching appeared at 1690 cm^{-1} and 1590 cm^{-1}, respectively. The absorption bands at 1460 cm^{-1} and 1160 cm^{-1} were due to S=O stretching. The absorption band of aromatic group has been observed at 804 cm^{-1}. ^{1}H-NMR spectra of compound (4) revealed a singlet at δ 2.6 of 3H of the N-acetyl substituent. The singlet at δ 5.6 of 1H was due to amide -CONH proton, which has been D_2O exchangeable. A singlet was observed at δ 9.8 for 1H of -N-NH protons that has been D_2O exchangeable. A singlet at δ 10.7 of 2H proton confirmed the sulfamoyl group.

Similarly, structures of all other synthesized compounds were established on the basis of spectral data and other physical and chemical data and the purity of the compounds was assessed by TLC and R_f values (Table 1 & 2). MAO inhibiting and anticonvulsant activity data are summarized in Table 1. All the synthesized compounds exhibited concentration dependent MAO inhibitory activity and their I_{50} values were determined. Compound 4 exhibited maximum monoamine oxidase inhibitory and anticonvulsant activities. However, the comparative observation of MAO inhibiting and anticonvulsant properties of each of the compounds does not correlate well. It is concluded that monoamine oxidase inhibitory activity does not represent the biochemical basis for the anticonvulsant activity of these compounds. Most of the compounds produced sign of sedation in animals. The animals showed reduction in spontaneous motor activity and ptosis, grooming, irritability and startle response was markedly inhibited. There was no discernible effect on respiration, urination or defecation. Acute toxicity studies indicate that the compounds have a wide margin of safety. The compounds did not induce any overt toxicity or death up to dose of 1000mg/kg, ip, over a 24 h observation time.

Table 1: Physical data, MAO inhibiting and anticonvulsant properties of N-methyl/acetyl-5-(un)-substituted isatin-3-semicarbazones.

Co-mpd	*R*	*R″*	*R′*	*Yield (%)*	*M.P. (°C)*	*Molecular formula*	*Mol.wt.*	R_f[a]	*MAO-inhibiting activity ($I_{50}X10^4M$)*	*Anticonvulsant activity (ED_{50},mg/kg,i.p.)*
1	Br	H	CH_3	76	248	$C_{16}H_{13}N_4O_2$ Br	373.04	0.581	1.31	92
2	Cl	H	$COCH_3$	72	143	$C_{17}H_{13}N_4O_3Cl$	356.59	0.619	1.27	75
3	SO_2NH_2	H	$COCH_3$	66	139	$C_{17}H_{15}N_5O_5S$	401.04	0.666	1.61	157
4	SO_2NH_2	Br	$COCH_3$	74	173	$C_{17}H_{14}N_5O_5BrS$	480.06	0.471	1.52	28
5	Cl	NO_2	$COCH_3$	68	150	$C_{17}H_{12}N_5O_5Cl$	401.57	0.510	1.29	66
6	SO_2NH_2	NO_2	$COCH_3$	60	185	$C_{17}H_{14}N_6O_7S$	446.16	0.482	1.69	138

[a] The solvent system used for TLC was chloroform : methanol (9:1) for all the compounds.

Table 2: Chemical data of representative N-methyl/acetyl-5-(un)-substituted isatin-3- semicarbazones

S. No.	Compd.	Analysis%		UV (λ_{max}) nm (ethanol)	IR (ν_{max} cm^{-1}) (KBr)	+ 1 0 5 SSP ' 0 62 ' $_6$)
		Calcd.	Found			
1.	1	C:51.51 H: 3.48 N : 15.01	C : 51.54 H : 3.54 N : 15.05	245.5	3425 (2^oNH) 3406 (amide NH) 2953 (CH_3), 1653 (amide C=O) 1610 (C-H), 1590 (C=N), 1460, 869 cm^{-1}	3.2 (s, 3H, N-CH_3) 5.8 (s, 1H -CONH, D_2O exchangeable), 7-7.7 (m, 8H, Ar-H), 8.6 (s, 1H, = N-NH, D_2O exchangeable)
2.	2.	C:57.25 H: 3.64 N : 15.70	C:57.29 H: 3.68 N :15.74	243, 285.5	3423 (2^oNH) 3314 (amide NH) 2928 (CH_3), 1728 (amide C=O) 1612 (C-H), 1589 (C=N), 1460, 821 cm^{-1}	3.5 (s, 3H, N-$COCH_3$) (J_i-J_j = 20 Hz, J_i-J_k = 22 Hz, J_j-J_k =20 Hz), 5.8 (s, 1H -CONH, D_2O exchangeable), (J_m-J_l = 9Hz), 6.8-7.8 (m, 8H, Ar-H), (J_a-J_b = 7 Hz, J_d-J_c= 6Hz, J_c-J_d= 8Hz, J_a-J_c= 2Hz, J_a-J_d = 1 Hz, J_b-J_d= 3Hz) (J_c-J_f = 8Hz, J_f-J_g = 4 Hz, J_e-J_g = 1Hz, J_e-J_h= 3Hz, J_g-J_h- 8Hz) 8.8 (s, 1H, = N-NH, D_2O exchangeable)
3.	3	C:50.91 H: 3.74 N:17.45	C:50.95 H : 3.78 N:17.49	226, 325	3479 (N-H) 3375 (N-H) 3275 (SO_2NH_2, N-H), 2922 (-CH_3), 1728 (acetyl C=O), 1630 (amide C=O), 1595 (C=N), 1315, 1147 (S=O), 825 cm^{-1}	3.5 (s, 3H, N-$COCH_3$), 5.8 (s, 1H -CONH, D_2O exchangeable), 7.0-7.8 (m, 8H, Ar-H), 7.65 (bs, 2H, SO_2NH_2, D_2O exchangeable) 8.6 (s, 1H, = N-NH, D_2O exchangeable)
4.	4	C:42.53 H: 2.94 N:14.58	C:42.57 H : 2.90 N:14.53	271, 279, 326.5	3381 (2^oN-H) 3232 (N-H) 2922 (CH_3), 1728 (acetyl C=O), 1690 (amide C=O), 1590 (C=N), 1460, 1160 (S=O), 1466, 804 cm^{-1}	2.6 (s, 3H, N-$COCH_3$), 5.6 (s, 1H -CONH, D_2O exchangeable), 6.4-7.8 (m, 7H, Ar-H), 9.8 (s, 1H, = N-NH, D_2O exchangeable), 10.7 (bs, 2H, SO_2NH_2, D_2O exchangeable).
5.	5	C:50.84 H: 2.98 N:17.43	C:50.88 H: 3.02 N:17.47	242, 247, 294	3429 (2^oN-H), 3314 (N-H) 2922 (-CH_3), 1735 (acetyl C=O), 1690 (amide C=O), 1548 (C=N), 1462, 1332 (C-NO_2), 821 cm^{-1}	3.5 (s, 3H, N-$COCH_3$), 5.8 (s, 1H -CONH, D_2O exchangeable), 6.8-7.8 (m, 7H, Ar-H), 8.7 (s, 1H, = N-NH, D_2O exchangeable).
6.	6	C:45.75 H: 3.13 N:18.82	C:45.79 H: 3.17 N:18.86	242, 247, 295	3470 (2^oN-H), 3366 (N-H) 2924 (-CH_3), 1747 (acetyl C=O), 1620 (amide C=O), 1595 (C=N), 1310, 1128 (S=O), 804 cm^{-1}	3.6 (s, 3H, N-$COCH_3$), 5.7 (s, 1H -CONH, D_2O exchangeable), 7.0-7.8 (m, 7H, Ar-H), 8.8 (bs, 2H, SO_2 NH_2, D_2O exchangeable).

4. Conclusion

The present investigation revealed the synthesis, characterization and pharmacological evaluation of some substituted isatin-semicarbazones. Compounds were synthesized by condensation reaction of appropriate N-substituted-5-(un) substituted isatin with corresponding phenyl semicarbazide and studied for monoamine oxidase inhibitory and anticonvulsant activities. All of these compounds exhibited dose dependant MAO inhibiting and anticonvulsant effects and wide margin of safety but there appeared no parallel correlation ship between these two effects of the compounds. It concludes that MAO inhibiting property does not prove the biochemical basis for the anticonvulsant activity of these compounds .These results warrant further detailed investigation of structurally similar compounds of their potential pharmacological actions.

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