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Biologically active indolylmethyl-1,3,4-oxadiazoles, 1,3,4-thiadiazoles, 4*H*-1,3,4-triazoles and 1,2,4-triazines*

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Introduction

Indole derivatives have yielded several biologically active compounds [1, 2]. Several of these derivatives possess potent central nervous system (CNS) as well as antiinflammatory properties [3–6]. Indolyl quinazolones possess potent anti-inflammatory as well as CNS activities [7, 8]. It has been reported that substitution by heterocyclic moieties, ie, oxadiazole, triazine or triazole, at positions 1 and 3 of the indole nucleus enhances these activities [9]. To this end we have synthesized indole congeners whose skeleton contains the above essential features. The compounds were studied for their antiinflammatory properties, CNS activity and acute toxicity.

Chemistry

The reaction of indole-3-acetohydrazide **2** with carbon disulfide in alkaline medium afforded, after acidic treatment, 5-(indol-3-ylmethyl)-1,3,4-oxadiazole-2-thiol **2a** (scheme 1). The same hydrazide was transformed into 3-(indol-3-yl methyl)-6-phenyl-1,2,4-triazine **2b** by condensation with phenacyl bromide and dimethylformamide. Lastly condensation with chloroacetamide led to 3-(indol-3-ylmethyl)-1,2,5,6-tetrahydro-1,2,triazine-5-one **2c**. The oxadiazole **2a** could be converted into the corresponding 4-amino-1,2,4- triazole **2d** by hydrazinolysis.

Hydrazide **2** gave the thiosemicarbazide **3**, which upon treatment in alkaline medium, afforded the 3-mercapto-4*H*-1,2,4-triazole **3a**; in acidic medium (H_3PO_4) the 2-amino-1,3,4-thiadiazole **3b** was obtained. Condensation of **3** with hydrazine afforded 3,4-diamino-4*H*-1,3,4-triazole **3c**.

Starting from (3-formylindol-1-yl)acetohydrazide **4**, we carried out the same heterocyclization reactions leading to **4a–d** (scheme 2).

Physicochemical data for the compounds are given in table I.

Table I. Physicochemical data of the compounds **2a–d**, **3a–c**, and **4a–d**.

Compound	*Mp (°C)*	*Recrystallization solvent*	*Yield (%)*	*Molecular formula*
2a	179	Ethanol/water	52	$C_{11}H_9N_3OS$
2b	242	DMF/water	20	$C_{18}H_{14}N_4$
2c	238	DMF/water	52	$C_{12}H_{11}N_4O$
2d	201	Ethanol/water	56	$C_{11}H_{11}N_5S$
3a	182	Ethanol/water	41	$C_{11}H_9N_4S$
3b	182	Ethanol/water	47	$C_{11}H_{10}N_4S$
3c	152	Ethanol/water	38	$C_{11}H_{12}N_6$
4a	192	Ethanol/water	39	$C_{12}H_0N_3O_2S$
4b	242	DMF/water	20	$C_{19}H_{14}N_4O$
4c	253	DMF/water	11	$C_{13}H_{11}N_4O_2$
4d	184	Ethanol/water	23	$C_{12}H_{11}N_5OS$

*Part of this work was presented at the XII International Congress of Pharmacology held in July 1994 at Montreal, Canada.

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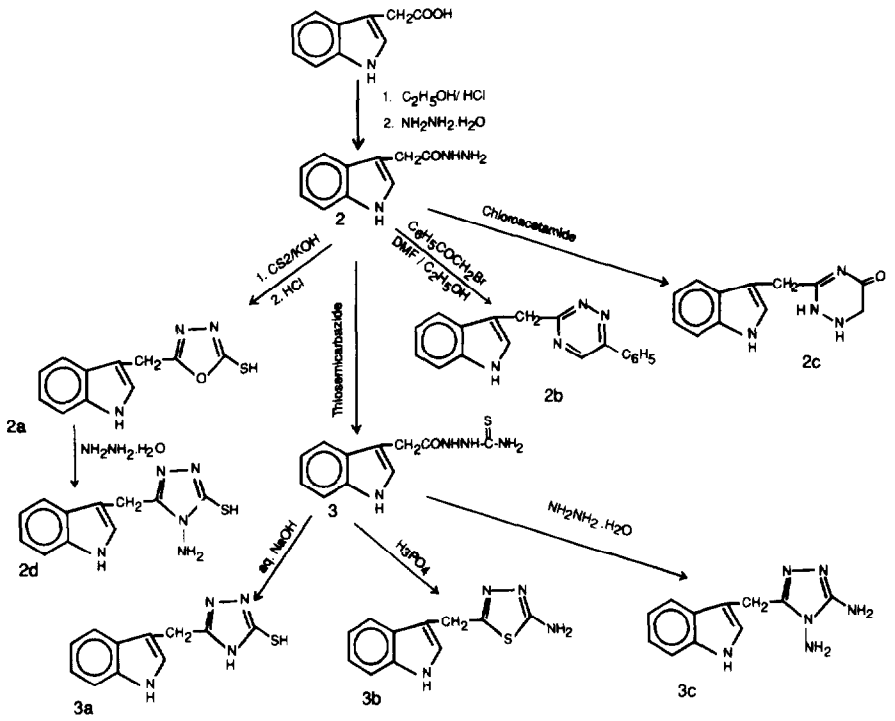
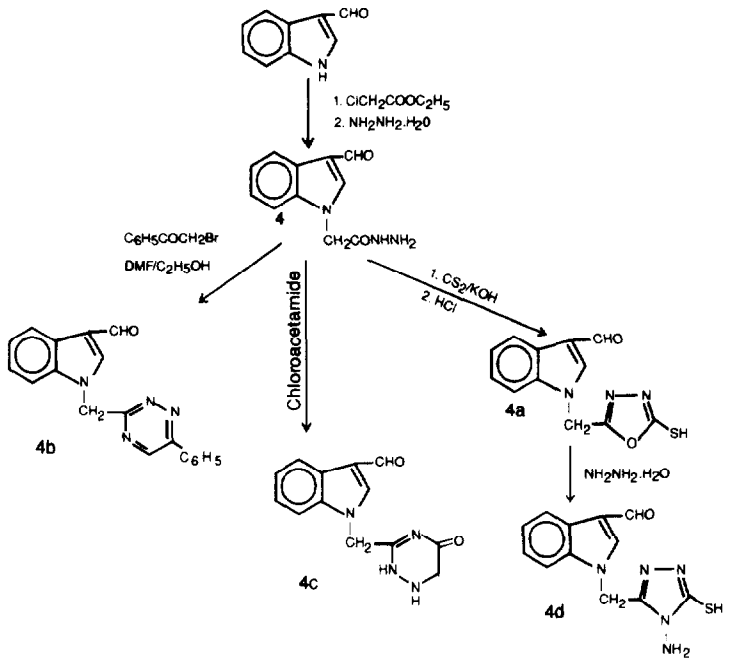


Scheme 1.



Scheme 2.

Table II. Anti-inflammatory activity of the compounds **2a–d**, **3a–c** and **4a–d**.

Compound no	*Dose (mg/kg po)*	*Mean difference in paw volume ± SE*	*Mean % inhibition ± SE*
2a	50	0.196 ± 0.027	31.00 ± 7.10
2b	50	0.094 ± 0.013	31.90 ± 6.96[a]
2c	50	0.230 ± 0.040	39.40 ± 5.10[a]
2d	50	0.094 ± 0.013	41.00 ± 6.42[a]
3a	50	0.152 ± 0.031	46.50 ± 7.14[a]
3b	50	0.160 ± 0.27	43.70 ± 7.21
3c	50	0.176 ± 0.24	38.10 ± 1030
4a	50	0.136 ± 0.023	62.00 ± 5.13[a]
4b	50	0.092 ± 0.026	74.20 ± 6.23[a]
4c	50	0.132 ± 0.071	63.00 ± 5.63[a]
4d	50	0.184 ± 0.037	48.40 ± 6.51[a]
Control	–	0.382 ± 0.071	–
Indomethacin	50	0.084 ± 0.015	74.00 ± 6.29

[a] $p < 0.05$.

Pharmacological results and discussion

Inhibition of inflammation

Compounds **2a–d** showed 31.0 ± 7.10% to 41.0 ± 6.42% inhibition, while compounds **3a-c** exhibited 38.10 ± 10.30% to 46.50 ± 7.14% inhibition of inflammation after oral administration to rats. The compounds **3a** and **3b**, which possess respectively a triazole and thiadiazole nucleus, showed better activity than compounds **2a–d** (table II).

The compounds **4a–d** showed inhibition ranging from 48.40 ± 6.51% to 74.20 ± 6.23%. In this series the compound **4b**, which showed the greatest anti-inflammatory activity (74.20%), was studied at the graded doses of 12.5 and 25 mg/kg po. At these doses the inhibition was 42.0 ± 0.021% and 58.40 ± 0.053%, respectively. Its ED_{50} was found to be 36 ± 0.021% mg/kg and its ALD_{50} was over 1000 mg/kg in albino mice. The ED_{50} of the active compounds was calculated by regression analysis.

Comparison of the activities of compounds **2d** and **2b** with those of the previously synthesized corresponding derivatives in the coumaryl series [6] demonstrates the positive influence of the indole moiety. Compound **2d** exhibited moderate antiinflammatory activity at a dose of 50 mg/kg, whereas 4-amino-5-(2-oxo-2*H*-1-benzopyran-3-yl)-1,2,4-triazole-3-thiole showed practically no activity (10.6%), even at 100 mg/kg dose. In the same way, compound **3b** was more active than 5-(2-oxo-2*H*-1-benzopyran-

Table III. Antidepressant activity of the compounds **2a–d** and **3a–c** administered at a dose of 100 mg/kg ip.

Compound	*General behaviour (in mice)*		*Stereotypy*	*Piloerection*	*Ptosis mean score*	*ALD_{50} (mg/kg ip) (in mice)*
	Awareness	*Gait*				
2a	2+	Ab	1+	1+	8.0	>1000
2b	1+	Ab	1+	3+	8.0	>500
2c	1+	N	2+	1+	8.0	>1000
2d	1+	N	1+	1+	8.0	>1000
3a	2+	N	1+	1+	3.0	>1000
3b	1+	N	3+	3+	4.0	>1000
3c	2+	N	3+	3+	4.0	>1000
Control	1+	N	–	1+	4.0	–

Ab = abnormal; N = normal.

Table IV. CNS profile of compounds **2a–d** and **3a–c**.

Compound	*Reserpine reversal test*		*Swimming despair test*		*L-Dopa potentiation test*	
	Control[a] *mean ± SE*	*Hypokinesia mean ± SE*	*Control*[a] *mean ± SE*	*Immobility time mean ± SE*	*Control*[a] *mean ± SE*	*Locomotor activity mean ± SE*
2a	35 ± 1.85	29.2 ± 5.84	2.82 ± 0.25	2.37 ± 0.30	36.0 ± 1.85	15.4 ± 5.40
2b	10.8 ± 5.10	25 ± 1.44	2.35 ± 0.48	2.92 ± 0.29	42.0 ± 8.20	18.4 ± 5.71
2c	9.4 ± 4.23	6.5 ± 5.76	1.85 ± 0.33	2.29 ± 0.45	44.0 ± 8.20	27 ± 10.84
2d	38 ± 7.40	27.8 ± 6.19	2.51 ± 0.37	2.10 ± 0.36	24.0 ± 10.83	15.2 ± 6.48
3a	31.8 ± 7.61	30.4 ± 4.40	2.41 ± 0.40	2.27 ± 0.18	31.8± 7.61	33.2 ± 9.18
3b	42.2 ± 8.84	20.2 ± 3.20[b]	2.89 ± 0.39	1.48 ± 0.20[c]	15.2 ± 5.85	86 ± 1.46[d]
3c	83 ± 12.24	46 ± 0.56[b]	3.33 ± 0.18	1.80 ± 0.12[c]	40.0 ± 4.25	96 ± 0.14[d]

[a]Indicates the values of hypokinesia, immobility time and locomotor activity, respectively, before treatment with the test compound; [b]reserpine reversal test, $p < 0.001$; [c]swimming despair test, $p < \delta\ 0.01$; [d]L-Dopa potentiation test, $p < 0.05$.

3-yl)-1,3,4-thiadiazole-2-amine: 43.7% at 50 mg/kg and 37.2% at 100 mg/kg.

CNS activities

The compounds were further studied for their CNS activity to assess behavioural effects of these compounds. The CNS activity profiles of the compounds are given in tables III and IV. An increase in awareness was found with compounds **2a**, **3a** and **3c**. Gait remained normal with all the compounds except **2a** and **2b**. From a δ-reserpine reversal test it was observed that compounds **3b** and **3c** antagonized the reserpine-induced effect by blocking the reserpine-induced ptosis, showing similarity with imipramine. Compounds **3b** and **3c** were found to potentiate L-Dopa effects and decrease immobility time in the swimming despair test, thus showing anti-depressant activity. Compounds **3b** and **3c**, with heterocyclic thiadiazole and triazole moieties, possess greater anti-depressant activity compared to the rest of the compounds.

The ALD_{50} values were found to be over 1000 mg/kg (maximum dose tested) for all compounds except for **2b**.

Experimental protocols

Chemistry

Melting points were measured in open capillary tubes and are uncorrected. Thin layer chromatography (TLC) was performed on silica-gel G plates. Proton magnetic resonance (^{1}H NMR) spectra in $CDCl_3$ were recorded on an EM-360 spectrophotometer (Perkin Elmer 3R-32) using tetramethylsilane (TMS) as internal standard (chemical shift in δ ppm). IR spectra in KBr were recorded on a Perkin Elmer infracord 137 instrument (υ_{max} in cm^{-1}) and mass spectra on a JMSD 300 instrument (Jeol D-300) fitted with a JMS-2000 data system, at 70 eV. Ethyl indole-3-acetate [10], ethyl (3-formylindol-1-yl)acetate [11], indole-3-acetylhydrazide [12] and indole-3-acetic acid thiosemicarbazone [12] were prepred by reported methods.

5-(Indol-3-ylmethyl)-1,3,4- oxadiazole-2-thiol ***2a***

A solution of KOH (0.15 mol), compound **2** (0.10 mol) and CS_2 (0.15 mol) in absolute ethanol (100 mL) was refluxed for 11 h. Then it was cooled to room temperature and diluted with dry ether (100 mL). The precipitate thus obtained was filtered and washed with ether and vacuum dried. A suspension of this solid (0.10 mol) in aqueous KOH (0.20 mol) was again refluxed until the evolution of H_2S ceased. Dilution with water (100 mL) and acidification with HCl yielded a white solid, which was filtered, washed with water and recrystallized from ethanol/water. IR (KBr) cm^{-1}: 2318 (SH), 1635 (C=N; cyclic), 3400 (NH), 2950 (CH_2). Mass spectrum exhibited molecular ion peak at *m/z* 231 [M^+]; other important fragments were observed at 198, 157, 156, 131, 116 (base peak, 100%) and 89. ^{1}H NMR ($CDCl_3$, δ ppm): 8.1–8.4 (bs, 1H, NH indole), 7.0–7.7 (m, 4H, H-4, H-5, H-6, H-7 indolic protons), 5.0–5.8 (d, 1H, CH (H-2 of indolic proton), 1.6–1.8 (t, 2H, CH_2), 1.2–1.3 (s, 1H, SH).

3-(Indol-3-ylmethyl)-6-phenyl-1,2,4-triazine ***2b***

Indole-3-acetohydrazide (0.20 mol) and phenacylbromide (0.10 mol) were refluxed in ethanol in the presence of dimethylformamide (DMF) for 20 h. The excess solvent was distilled off and the reaction mixture yielded yellow crystals upon cooling. It was filtered and recrystallized from DMF/water. IR (KBr) cm^{-1}: 3250 (NH, indole), 1590 (C=N, cyclic), 2950 (CH_2). Mass spectrum exhibited molecular ion peak at *m/z* 286 [M^+]; other important fragments were observed at 172, 171, 132, 116, 102 and 40. ^{1}H NMR ($CDCl_3$, δ ppm): 8.3–8.5 (bs,

1H, NH indole), 6.5–7.0 (m, 5H, phenyl ring), 7.1–7.8 (m, 4H, H-4, H-5, H-6 and H-7 indolic protons), 5.1–5.9 (d, 1H, CH (H-2 indolic proton), 1.6–1.9 (t, 2H, CH_2).

3-(Indol-3-ylmethyl)-1,2,5,6-tetrahydro-1,2,4-triazin-5-one ***2c***
To indole-3-acetylhydrazide **2** (0.10 mol) was added chloroacetamide (0.10 mol) and DMF (80 mL) and the reaction mixture was refluxed for 25 h. It was then concentrated and cooled; whereupon a solid separated out which was filtered, washed with EtOH and recrystallized from DMF/water. IR (KBr) cm^{-1}: 3245 (NH, indole), 1725 (C=O, cyclic), 2950 (CH_2), 1590 (C=N, cyclic). Mass spectrum exhibited molecular ion peak at *m/z* 228 [M⁺]; other important fragments were observed at 130, 116, 105, 102, 77 (base peak, 100%) and 62. ^1H NMR ($CDCl_3$, δ ppm): 8.1–8.4 (bs, 1H, NH indole), 6.9–7.4 (m, 4H, H-4, H-5, H-6 and H-7 indolic protons), 5.6–5.9 (bs, 2H, NH triazinone), 5.2–5.8 (d, 1H, CH (H-2 of indolic proton), 2.5–2.8 (s, 2H, CH_2) of triazinone).

4-Amino-5-(indol-3-yl methyl)-4H-1,3,4-triazole-3-thiol ***2d***
A suspension of the compound **2a** (0.10 mol) in hydrazine hydrate (0.20 mol, 99%) was refluxed for 5 h. It was then diluted with water and acidified with HCl, and a white solid separated out. This was filtered, washed with water and recrystallized from EtOH/water. IR (KBr) cm^{-1}: 2310 (SH), 3210 (NH, indole). Mass spectrum exhibited molecular ion peak at *m/z* 245 [M⁺]; other important fragments were observed at 130, 127, 115, 102, 91, 87 and 77 (base peak, 99%). ^1H NMR ($CDCl_3$, δ ppm): 8.1–8.6 (bs, 1H, NH indole), 6.5–7.0 (m, 4H, H-4, H-5, H-6, H-7 indolic protons), 5.8–6.2 (d, 1H, CH (H-2 indolic proton), 3.4–3.6 (s, 2H, NH_2 triazole ring), 1.1–1.2 (s, 1H, SH of triazole ring), 1.5–1.7 (t, 2H, CH_2).

5-(Indol-3-ylmethyl)-1,2,4-triazole-3-thiol ***3a***
Indol-3-thiosemicarbazide **3** (0.2 mol) was dissolved in 1 M aqueous sodium hydroxide (0.2 mol) and refluxed for 2 h. The reaction mixture was cooled and acidified with acetic acid, whereupon a solid separated out. The solid formed was filtered and recrystallized from ethanol/water. IR (KBr) cm^{-1}: 2310 (SH), 3310 (NH, indole), 1590 (C=N, cyclic). Mass spectrum exhibited molecular ion peak at *m/z* 229 [M⁺]; other important fragments were observed at 196, 130 (base peak 100%), 131, 91, and 76. ^1H NMR ($CDCl_3$, δ ppm): 8.1–8.3 (1H, bs, NH indole), 6.9–7.4 (m, 4H, H-4, H-5, H-6, H-7 indolic protons), 5.6–6.2 (d, 1H, CH (H-2 inolic proton), 4.6–5.2 (bs, 1H, NH of triazole nucleus), 1.4–1.6 (t, 2H, CH_2), 1.1–1.2 (s, 1H, SH).

5-(Indol-3-ylmethyl)-3,4-thiadiazole-2-amino-1,3,4-thiadiazole ***3b***
To compound **3** (0.2 mol) was gradually added orthrophosphoric acid (100 mL) over 10 min and the reaction mixture was heated in an oil bath at 120 °C for 2 h. The formed slurry was poured into crushed ice, and slowly a solid separated out. This was filtered and washed with cold water. IR (KBr) cm^{-1}: 3210 (NH, indole), 1590 (C=N, cyclic). Mass spectrum exhibited molecular ion peak at *m/z* 230 [M⁺]; other important fragments were observed at 128, 116, 102, 100, 77 (base peak 100%) and 66. ^1H NMR ($CDCl_3$, δ ppm): 7.9–8.4 (bs, 1H, NH indole), 7.0–7.5 (m, 4H, H-4, H-5, H-6, H-7 indolic protons), 6.2–6.8 (d, 1H, CH (H-2 indolic proton), 3.1–3.4 (s, 2H, NH_2, 1.2–1.4 (t, 2H, CH_2).

5-(Indol-3-ylmethyl)-4H-1,2,4 triazole-3,4-diamine ***3c***
A suspension of compound **3** (0.2 mol) and hydrazine hydrate (0.2 mol, 99%) was gently refluxed for 2 h at 60 °C. The reaction mixture was cooled and poured into crushed ice. The solid thus obtained was filtered, washed with water and air-dried to afford the title compound **3c**. IR (KBr) cm^{-1}: 3220 (NH, indole), 1610 (C=N, cyclic). Mass spectrum exhibited molecular ion peak at *m/z* 228 [M⁺]; other important fragments were observed at 213, 197, 185, 159, 131, 105 (100%, base peak) and 77. ^1H NMR ($CDCl_3$, δ ppm): 7.9.2 (bs, 1H, NH indole), 7.1–7.3 (m, 4H, H-4, H-5, H-6, H-7 indolic protons), 6.3–6.7 (d, 1H, CH (H-2 indolic proton), 4.1–4.2 (s, 2H, NH_2 attached to C), 3.1–3.4 (s, 2H, NH_2 attached to N), 1.1–1.3 (t, 2H, CH_2).

(3-Formylindol-1-yl)acetohydrazide ***4***
To solution of indole-3-carboxaldehyde ethyl acetate (0.01 mol) in dry benzene (50 mL) was added hydrazine hydrate (0.2 mol). After 16 h reflux, the reaction mixture was concentrated. On cooling a solid crystallized out, which was filtered, washed with benzene and air-dried to obtain the pure compound. IR (KBr) cm^{-1}: 3250 (NH, indole), 1690 (C=O). ^1H NMR ($CDCl_3$, δ ppm): 7.2–7.8 (m, 4H, H-4, H-5, H-6, H-7 indolic protons), 5.6–5.8 (s, 1H, CH aldehydic group), 4.5–5.1 (d, 1H, CH (H-2 indolic proton), 1.5–1.9 (t, 2H, CH_2), 1.3–1.4 (s, 1H, SH).

1-(5-Mercapto-1,3,4-oxadiazol-2-ylmethyl)indole-3-carbaldehyde ***4a***
A solution of KOH (0.15 mol), compound **4** (0.10 mol) and CS_2 (0.15 mol) in absolute ethanol (100 mL) was refluxed for 11 h, then cooled to room temperature and diluted with dry ether (100 mL). The precipitate thus obtained was filtered, washed with ether and vacuum-dried. A suspension of this solid (0.10 mol) in aqueous KOH (0.20 mol) was again refluxed until the evolution of H_2S ceased, and diluted with water (100 mL). Acidification with HCl yielded a white solid, which was filtered, washed with water, and recrystallized from ethanol/water. IR (KBr) cm^{-1}: 3240 (NH, indole), 1590 (C=N, cyclic). Mass spectrum exhibited a peak at *m/z* 260 [M + 1]; other important fragments were observed at 146, 137, 131, 100 and 77 (base peak, 100%). ^1H NMR ($CDCl_3$, δ ppm); 10.6–10.8 (s, 1H, of CH aldehydic group), 7.2–7.8 (m, 4H, H-4, H-5, H-6, H-7 indolic protons), 4.5–5.1 (d, 1H, CH (H-2 indolic proton), 1.5–1.9 (t, 2H, CH_2), 1.3–1.4 (s, 1H, SH).

1-(5-Phenyl-1,2,4-triazin-3-ylmethyl)indole-3-carbaldehyde) ***4b***
Compound **4** (0.20 mol) and phenacylbromide (0.10 mol) were refluxed in ethanol in the presence of a few drops of DMF, for 20 h. The excess solvent was distilled off and the reaction mixture, upon cooling, yielded yellow crystals. These were filtered and washed with ethanol and recrystallized from DMF/water. IR (KBr) cm^{-1}: 3250 (NH, indole), 1610 (C=N, cyclic). Mass spectrum exhibited molecular ion peak at *m/z* 315 [M⁺]; other important fragments were observed at 144, 171 two fragments, 145 and 130, 129 (base peak, 100%) and 117. ^1H NMR ($CDCl_3$, δ ppm); 10.6–10.8 (s, 1H, CH aldehydic group), 1.5–1.9 (s, 2H, CH_2), 6.8–7.2 (m, 5H, phenyl ring), 7.2–7.7 (m, 4H, H-4, H-5, H-6, H-7 indolic protons), 5.2–5.9 (d, 1H, CH (H-2 indolic proton).

1-(5-Oxo-1,2,5,6-tetrahydro-1,2,4-triazin-3-ylmethyl)indole-3-carbaldehyde ***4c***
To compound **4** (0.10 mol) ws added chloroacetamide (0.10 mol) in DMF (80 mL); the reaction mixture was refluxed for 25 h, concentrated and cooled, whereupon a solid separated out which was filtered, washed with ethanol and recrystallized from DMF/water. IR (KBr) cm^{-1}: 1590 (C=N), 1600 (C=O), 3240 (NH, indole). Mass spectrum exhibited molecular ion peak at *m/z* 256 [M⁺]; other important fragments were observed at 144, 112 (two fragments), 145, 116, 89, 113, 85, 70 and 55. ^1H NMR ($CDCl_3$, δ ppm): 10.6–10.8 (s, 1H, CH aldehydic

group), 6.8–7.4 (m, 4H, H-4, H-5, H-6, H-7 indolic protons), 5.3–5.9 (d, 1H, CH (H-2 indolic proton), 5.6–5.9 (bs, 2H, NH aromatic), δ 2.6–2.9 (s, 2H, CH_2 of triazinone).

1-(4-Amino-5-mercapto-4H-1,2,4-triazol-3yl)indole-3-carbaldehyde ***4d***

A suspension of compound **4b** (0.10 mol) in hydrazine hydrate (0.20 mol, 99%) was refluxed for 5 h and then diluted with water and acidified with HCl. A white solid separated out which was filtered, washed with water, and recrystallized from ethanol/water. IR (KBr) cm^{-1}: 3240 (NH, indole), 1540 (C=N). Mass spectrum exhibited molecular ion peak at *m/z* 276 [M^+]; other important fragments were observed at 225, 194, 185, 159, 131, 106 (100%, base peak) and 77. ^1H NMR ($CDCl_3$, δ ppm): 10.0–10.5 (s, 1H, CH aldehydic group), 6.4–7.1 (m, 4H, H-4, H-5, H-6, H-7 indolic protons), 5.9–6.0 (d, 1H, CH (H-2 indolic proton), 3.3–3.6 (s, 1H, NH of triazole ring), 1.2–1.3 (s, 1H, SH of triazole ring), 1.6–1.8, (t, 2H, CH_2).

Pharmacology

CNS activities

The present study was carried out in mice of either sex weighing 20–30 g. The animals were allowed food and water ad libitum. Test compounds were administered in doses of 20 mg/kg (ip) as an aqueous suspension in gum acacia and results were compared with those of imipramine. Gross behaviour of the compounds on motor sensory and autonomic systems was studied in mice [13]. Compounds were screened for effects on gross behaviour and anti-depressant activity.

Behavioural effects

Spontaneous motor activity (SMA), awareness, posture, gait, reflexes and autonomic symptoms (respiration, lachrymation, salivation, etc) were observed before and 3 h after administration of test compounds.

Antidepressant actitivy

Reserpine reversal test

Antidepressant effect was observed by testing the compounds by the reserpine reversal test [14]. Reserpine (5 mg/kg ip) was administered 15 min prior to the compounds to be tested and the following parameters were observed for 2 h after test compound administration.

Locomotor activity. Locomotor activity was measured by placing each mouse in a photoactometer for 5 min and the total count was recorded.

Ptosis. Intensity of ptosis was graded according to the method of Rubin et al (1957) [15].

L-Dopa potentiation test. The L-dopa potentiation test [16] was performed by placing each mouse in a photoactometer and observing locomotor activity.

Swimming despair test. Each mouse was subjected to this test 24 h prior to (control) and 2 h after drug treatment. The swimming despair test was conducted according to the method of Porsolt et al [17].

Toxicity studies

The active compounds were investigated for their acute neurological toxicity and approximate lethal dose (ALD_{50}). Mice (either sex) weighing 20–25 g were used for the study. ALD_{50} values were determined by observing mortality within 24 h after drug administration [18].

Anti-inflammatory activity

Rats (either sex) weighing 80–120 g were divided into groups of five animals each. A freshly prepared suspension of carrageenin (1.0% in 0.9% saline) 0.05 mL, was injected under the plantar aponeurosis of the right paw of the rat by the method of Winter et al [19]. One group was kept as control and the animals of the other groups were pretreated with the test drugs, suspended in gum acacia, given orally, 1 h before the carageenin injection. The volume of the paw was measured before and 3 h after carrageenin treatment by the micropipette method as described by Buttle et al [20]. The mean increase in paw volume in each group was measured and percentage-inflammatory activity was calculated by the formula [1–dt/dc] × 100, where dt = drug treated and dc = control. The ED_{50} of the most active compound was calculated using regression analysis.

Statistical calculation

Data are expressed as mean ± SE. The Student's *t* test was applied to determine the significance of the difference between the control and the treated groups.

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References

1 Rapport MM, Green AA, Page IH, (1948) *Science* 108, 329–333
2 Azima H, Arthur A, Silver A, Azima FJ, (1962) *Am J Psychiatry* 119, 537–540
3 Archer S, Wylie DW, Harris LS et al (1962) *J Am Chem Soc* 84, 1306–1308
4 Shen TY, Winter CA, Simmonds AB (1977) *Advances in Drug Res* 12, Academic, NY, 89
5 Shen TY (1965) Non-steroidal anti-inflammatory drugs. *Experta Medica Found*, New York, 13
6 Bhalla M, Hitkari A, Gujrati VR, Bhalla TN, Shanker K (1994) *Eur J Med Chem* 29 (9), 713–717
7 Plasica Q (1986) *J Med Chem* 20, 291–295
8 Singh IP, Saxena AK, Sinha JN, Bhargawa KP, Shanker K (1984) *Indian J Chem* 23B, 592–594
9 Bhalla M, Srivastava VK, Bhalla TN, Shanker K (1993) *Arzneim Forsch* 43, 5, 595–600
10 Bullock MW, Fox SW (1951) *J Am Chem Soc* 73, 5155–5157
11 Shanker K, Agarwal VK, Selveraj RJ, Parmar SS (1969) *J Med Chem* 12, 324–327
12 Sathi G, Gujrati VR, Nath C, Shanker K (1983) *Arzneim Forsch* 33, 1218–1221
13 Nodine JH, Eigel RF (1914) *Animal and Clinical Pharmacological Techniques in Drug Evaluation*, Medical Publishers Inc, Chicago
14 Chessin ME, Kramer ER, Scott CC (1957) *J Pharmacol* 119, 453–456
15 Rubin B, Malone MH, Wangh MH, Burki JC (1957) *J Pharmacol Exp Ther* 120, 125–128
16 Everette GM, Darwin JC, Tomen JEP (1959) *Fed Proc* 18, 338–340
17 Porsolt RD, Asten G, Blaret N, Jaltreen M (1978) *Eur J Pharmacol* 23, 378–380
18 Swinyard EA, Brown WC, Goodman LS (1962) *J Pharmacol Exp Ther* 106, 319–322
19 Winter CA, Risley EA, Nuss GW (1962) *Proc Soc Exp Biol Med* 111, 534–537
20 Buttle CAN, Arcy PFW, Howard EM, Kettel DM (1969) *Nature (London)* 179, 629–637