

Synthesis and analgesic activity of 1-(1-((substituted methyl)-5- methoxy-2-oxoindolin-3-ylidene)-4-(substituted pyridin-2-yl)thiosemicarbazide

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ABSTRACT

Some novel Mannich base isatin derivatives were synthesized by reacting 1-(5-methoxy-2-oxoindolin-3-ylidene)-4-(substituted pyridin-2-yl)thiosemicarbazide with formaldehyde and several secondary amines. Their chemical structure was elucidated by means of spectral (FT-IR, ¹H NMR and mass) analysis. Investigation of analgesic activity of synthesized compounds was done by acetic acid induced writhing reflex method using diclofenac sodium as standard drug. The synthesized compounds showed significant analgesic activity.

Key words: Isatin, Schiff base, Mannich base, analgesic activity.

INTRODUCTION

Isatin (1H-indole-2, 3-dione) was first obtained by Erdman and Laurent in 1841 as a product from the oxidation of indigo by nitric and chromic acids. Isatin is an endogenous compound identified in human that possesses wide range of biological activities. Isatin has anxiogenic¹, anticonvulsant², anticancer² activity and act as a potent antagonist on atrial natriuretic peptide receptors *in vitro*³. Isatin derivatives of Mannich bases have antibacterial⁴, antifungal⁵, antiviral⁶, anti HIV⁷⁻⁹ and antiallergic¹⁰ activity. In addition pyridines are associated with diverse biological activities^{11,12}. The N-Mannich bases of isatin derivatives were synthesized by condensing the Schiff base of isatin derivative with formaldehyde and various secondary amines (Scheme 1). The structures, yields and melting points of the synthesized compounds are given in Table 1. All the compounds gave satisfactory elemental analysis. IR, ¹H NMR and mass spectra were consistent with the assigned structures. All the synthesized compounds were screened for analgesic activity.

MATERIAL AND METHODS

All chemicals used in the study were supplied by E. Merck and SD fine. Melting points were taken in a Thomas Hoover capillary melting point apparatus and are uncorrected. The purity of the synthesized compounds was routinely checked by TLC on silica gel G. IR spectra on a Shimadzu FT 8300 infrared spectrophotometer (ν_{\max} cm⁻¹), ¹H NMR spectra were recorded on JEOL GSX 400 spectrometer using TMS as an internal standard (chemical shifts in δ , ppm) and mass spectra on a JEOL MSMATE spectrometer.

EXPERIMENTAL

Synthesis of 5-methoxy isatin (1)

To 65ml of concentrated sulphuric acid at 50°C was added 15 g of dry isonitrosoacetanilide derivative. The solution was heated to 80°C and was kept at this temperature for about 10 minutes. Then it was cooled to room temperature and poured upon cracked ice. After 90 minutes, the 5-methoxy isatin was filtered, washed several times with cold water to remove sulphuric acid and then dried in air.

Synthesis of 4-(substituted pyridin-2-yl) thiosemicarbazide (2)

To a solution of 2-amino-3-methylpyridine (0.01 mol) in DMF (10 ml) was added sodium hydroxide (0.01 mol) and carbon disulphide (0.75 ml). The mixture was stirred at 15-20°C for 1 hour, to the stirred mixture was added hydrazine hydrate (0.01 mol) and stirring continued at 60°C for 1 hour. On adding water a pale yellow solid separated out which is recrystallized from DMF and ethanol. Similarly other derivative was synthesized.

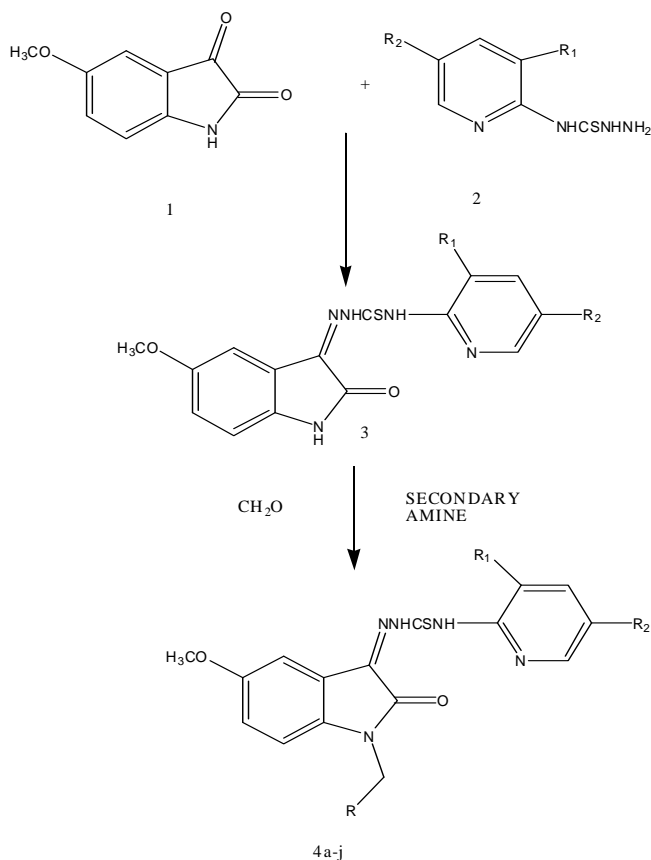
Synthesis of 1-(5-methoxy-2-oxoindolin-3-ylidene)-4-(substitutedpyridin-2-yl) thiosemicarbazide (3)

Equimolar quantities of 5-methoxy isatin and 4-(substituted pyridin-2-yl) thiosemicarbazide were dissolved in warm ethanol (50 ml) containing 1 ml of glacial acetic acid. The reaction mixture was refluxed for 10 hours and set aside. The resultant solid was washed with dilute ethanol, dried and recrystallized from ethanol and chloroform mixture.

Synthesis of compound (4a-4j): A slurry consisting of 1-(5-methoxy-2-oxoindolin-3-ylidene)-4-(substituted pyridin-2-yl) thiosemicarbazide (0.002 mol), THF (3 ml) and 37% formalin (1 ml) was made. To this secondary amine (0.003 mol) was added drop wise with cooling and shaking. The reaction mixture was allowed to stand at room temperature for 1 hour with occasional shaking after, which it was warmed on a steam bath for 15 minutes. At the end of the period the contents were cooled and the product obtained was recrystallized from chloroform and petroleum ether.

1-((dimethylamino)methyl)-5-methoxy-2-oxoindolin-3-ylidene)-4-(3-methylpyridin-2-yl) thiosemicarbazide (4a)

The sample was recrystallized using chloroform and petroleum ether. IR(KBr): 3365, 2850, 1690, 1645, 1280, 1105 cm^{-1} ; $^1\text{H NMR}$ (DMSO d_6): δ 8.09 (d, H), 7.6 (d, H), 7.38 (d, H), 7.1 (d, H), 7.0 (s, H), 6.8 (m, H), 6.51 (m, H), 4.03 (s, 2H), 4.0



Scheme 1

(s, H), 3.73 (s, 3H), 2.32 (s, 3H), 2.27 (m, 6H); MS (relative intensity): m/z value 398.15 (23); Calcd. for $C_{19}H_{22}N_6O_2S$ % C 57.27 H 5.56 N21.09; found C 57.25 H 5.54 N 21.06

1-(1-((diethylamino) methyl)-5-methoxy-2-oxoindolin-3-ylidene)-4-(3-methyl pyridin-2-yl) thiosemicarbazide (4b)

The sample was recrystallized using chloroform and petroleum ether. IR(KBr): 3370, 2854, 1700, 1640, 1284, 1110 cm^{-1} ; 1H NMR (DMSO d_6): δ 8.09 (m, H), 7.6 (d, H), 7.38 (d, H), 7.1 (d, H), 7.0 (s, H), 6.8 (t, H), 6.51 (d, H), 4.03 (s, 2H), 4.0 (s, H), 3.73 (s, 3H), 2.40 (m, 4H), 2.32 (s, 3H), 1.00 (m, 6H); MS (relative intensity): m/z value 428.18(25) ; Calcd. for $C_{21}H_{26}N_6O_2S$ % C 59.13 H 6.14 N 19.70; found C 59.11 H 6.12 N 19.67

1-(1-((diphenylamino)methyl)-5-methoxy-2-oxoindolin-3-ylidene)-4-(3-methylpyridin-2-yl) thiosemicarbazide (4c)

The sample was recrystallized using chloroform and petroleum ether. IR(KBr): 3372, 2867, 1702, 1620, 1292, 1075 cm^{-1} ; 1H NMR (DMSO d_6): δ 8.09 (m, H), 7.6 (d, H), 7.38 (d, H), 7.1 (d, H), 7.04 (m, 4H), 7.0 (s, H), 6.8 (t, H), 6.58 (m, 2H), 6.51 (d, H), 6.43 (m, 4H), 4.73 (s, 2H), 4.0 (s, H), 3.73 (s, 3H), 2.32 (s, 3H); MS (relative intensity): m/z value 522.18 (12); Calcd. for $C_{29}H_{26}N_6O_2S$ % C 66.65 H 5.01 N 16.08; found C 66.64 H 5.00 N 16.05

1-(5-methoxy-2-oxo-1-(pyrrolidin-1-ylmethyl)indolin-3-ylidene)-4-(3-methylpyridin-2-yl) thiosemicarbazide (4d)

The sample was recrystallized using chloroform and petroleum ether. IR(KBr): 3358, 2848, 1695, 1620, 1288, 1045 cm^{-1} ; 1H NMR (DMSO d_6): δ 8.09(m, H), 7.6 (d, H), 7.38 (d, H), 7.1 (d, H), 7.0(s, H), 6.8 (t, H), 6.51 (d, H), 4.03 (s, 2H), 4.0 (s, H), 3.73 (d, H), 2.32 (s, 3H), 2.25(m, 4H), 1.59 (m, 4H); MS (relative intensity): m/z value 424.17 (27); Calcd. for $C_{21}H_{24}N_6O_2S$ % C 59.41 H 5.70 N 19.80; found C 59.39 H 5.67 N 19.78

1-(5-methoxy-2-oxo-1-(piperidin-1-ylmethyl)indolin-3-ylidene)-4-(3-methylpyridin-2-yl) thiosemicarbazide (4e)

The sample was recrystallized using chloroform and petroleum ether. IR(KBr): 3352,

2843, 1692, 1627, 1286, 1037 cm^{-1} ; 1H NMR (DMSO d_6): δ 8.09 (m, H), 7.6 (d, H), 7.38 (d, H), 7.1 (d, H), 7.0 (s, H), 6.8(t, H), 6.51 (d, H), 4.03 (s, 2H), 4.0 (s, H), 3.73 (s, 3H), 2.32 (s, 3H), 2.24 (m, 4H), 1.50 (m, 6H); MS (relative intensity): m/z value 438.18 (32); Calcd. for $C_{22}H_{26}N_6O_2S$ % C 60.25 H 5.98 N 19.16; found C 60.23 H 5.97 N 19.13

4-(5-chloropyridin-2-yl)-1-(1-((dimethyl amino)methyl)-5-methoxy-2-oxoindolin-3-ylidene) thiosemicarbazide 4f

The sample was recrystallized using chloroform and petroleum ether. IR(KBr): 3365, 2851, 1679, 1617, 1289, 1086 620 cm^{-1} ; 1H NMR (DMSO d_6): δ 8.31(d, H), 7.68 (t, H) 7.6 (d, H), 7.1 (d, H), 7.0(s, H), 6.89 (d, H), 6.8 (t, H), 4.03 (s, 2H), 4.0 (s, H), 3.73 (s, 3H), 2.27 (d, 6H); MS (relative intensity): m/z value 418.1 (32); Calcd. for $C_{18}H_{19}ClN_6O_2S$ % C 51.61 H 4.57 N 20.06; found C 51.59 H 4.54 N 20.05

4-(5-chloropyridin-2-yl)-1-(1-((diethylamino) methyl)-5-methoxy-2-oxoindolin-3-ylidene) thiosemicarbazide (4g)

The sample was recrystallized using chloroform and petroleum ether. IR(KBr): 3382 2856, 1683, 1637, 1281, 1078, 628 cm^{-1} ; 1H NMR (DMSO d_6): δ 8.31(d, H), 7.68(t, H) 7.6 (d, H), 7.1 (d, H), 7.0(s, H), 6.89(d, H), 6.8 (t, H), 4.03(s, 2H), 4.0 (s, H), 3.73(s, 3H), 2.40 (m, 4H), 1.00(m, 6H); MS (relative intensity): m/z value 446.13 (35); Calcd. for $C_{20}H_{23}ClN_6O_2S$ % C 53.74 H 5.19 N 18.80; found C 53.71 H 5.17 N 18.78

4-(5-chloro pyridin-2-yl)-1-(1-((diphenyl amino) methyl)-5-methoxy-2-oxoindolin-3-ylidene) thiosemicarbazide (4h)

The sample was recrystallized using chloroform and petroleum ether. IR(KBr): 3374, 2854, 1693, 1632, 1284, 1058 and 624 cm^{-1} ; 1H NMR (DMSO d_6): δ 8.31 (d, H), 7.68 (t, H) 7.6 (d, H), 7.1 (d, H), 7.04 (m, 4H), 7.0 (s, H), 6.89 (d, H), 6.8 (t, H), 6.58 (m, 2H), 6.43(m, 4H), 4.73 (s, 2H), 4.0 (s, H), 3.71 (s, 3H); MS (relative intensity): m/z value 542.13 (21); Calcd. for $C_{28}H_{23}ClN_6O_2S$ % C 61.93 H 4.27 N 15.48; found C 61.91 H 4.25 N 15.46

4-(5-chloropyridin-2-yl)-1-(5-methoxy-2-oxo-1-(pyrrolidin-1-ylmethyl)indolin-3-ylidene) thiosemicarbazide (4i)

Table 1: Structures, yields and melting points of the synthesized compounds

Compounds	R	R ₁	R ₂	Yield (%)	m.p (°C)	Crystallization solvents (1:1)
4a		CH ₃	H	68	160	chloroform and petroleum ether
4b		CH ₃	H	74	172	chloroform and petroleum ether
4c		CH ₃	H	71	182	chloroform and petroleum ether
4d		CH ₃	H	78	164	chloroform and petroleum ether
4e		CH ₃	H	81	150	chloroform and petroleum ether
4f		H	Cl	71	162	chloroform and petroleum ether
4g		H	Cl	77	166	chloroform and petroleum ether
4h		H	Cl	69	170	chloroform and petroleum ether
4i		H	Cl	73	168	chloroform and petroleum ether
4j		H	Cl	80	158	chloroform and petroleum ether

The sample was recrystallized using chloroform and petroleum ether. IR(KBr): 3388, 2850, 1682, 1615, 1280, 1023, 629 cm^{-1} ; ^1H NMR (DMSO d_6): δ 8.31(d, H), 7.68 (t, H) 7.6 (d, H), 7.1 (d, H), 7.0 (s, H), 6.89 (d, H), 6.8 (t, H), 4.03 (s, 2H), 4.0 (s, H), 3.74 (s, 3H) 2.25 (m, 4H), 1.59 (m, 4H) ; MS (relative intensity): m/z value 444.11 (16); Calcd. for $\text{C}_{20}\text{H}_{21}\text{ClN}_6\text{O}_2\text{S}$ % C 53.99 H 4.76 N 18.89; found C 53.97 H 4.73 N 18.87

4-(5-chloropyridin-2-yl)-1-(5-methoxy-2-oxo-1-(piperidin-1-ylmethyl)indolin-3-ylidene)thiosemicarbazide (4j)

The sample was recrystallized using chloroform and petroleum ether IR(KBr): 3390,

2851, 1692, 1634, 1292, 1071, 632 cm^{-1} ; ^1H NMR (DMSO d_6): δ 8.31(d, H), 7.68 (t, H) 7.6 (d, H), 7.1 (d, H), 7.0 (s, H), 6.89 (d, H), 6.8 (t, H), 4.03 (s, 2H), 4.0 (s, H), 3.71 (s, 3H), 2.24 (m, 4H), 1.50 (m, 6H); MS (relative intensity): m/z value 458.13 (15); Calcd. for $\text{C}_{21}\text{H}_{23}\text{ClN}_6\text{O}_2\text{S}$ % C 54.96 H 5.05 N 18.31; found C 54.94 H 5.03 N 18.29

Analgesic activity

The analgesic activity of isatin derivatives 4a-j were assessed by acetic acid induced writhing reflex in mice. Groups of 6 mice of either sex with body weight of 22 to 41 gm were used. Appropriate volume of acetic acid solution was administered to the first group (which serves as control), placed them individually under glass jar for observation.

Table 2 : Analgesic effect of synthesized compound by writhing reflex method

Treatment	Dose (mg/kg)	Mean writhing \pm S.E.M	Percentage protection
Control	Saline	32 \pm 0.7303	-
Diclofenac sodium	45mg/kg	8.3 \pm 0.33**	73.96
4a	100mg/kg	30.8 \pm 0.4773 ^{NS}	6.25
4b	100mg/kg	26.5 \pm 0.4282**	17.18
4c	100mg/kg	30 \pm 0.730 ^{NS}	6.25
4d	100mg/kg	27.3 \pm 0.494 [*]	14.59
4e	100mg/kg	26.6 \pm 0.4216**	16.58
4f	100mg/kg	29.5 \pm 0.6708 [*]	7.8
4g	100mg/kg	26.8 \pm 0.307 [*]	16.25
4h	100mg/kg	27 \pm 0.3651**	15.62
4i	100mg/kg	27.81 \pm 0.3073 [*]	13.09
4j	100mg/kg	26.16 \pm 0.3073**	18.25

**P<0.01 by Dunnet 't' test (multiple comparison test) compared with control. Values are expressed in mean \pm SEM (n = 6)

Onset on wriths were noted, the number of abdominal contractions were recorded, trunk twist response and extension of hind limbs as well as the number of animals showing such response during a period of 10 minutes were also recorded. To the second group of animals diclofenac sodium 45mg/kg were administered. Fifteen minutes later, to these animals acetic acid solution were

administered. The onset and severity of writhing response were noted. The mean writhing scores in control and diclofenac sodium treated groups were calculated (Table 2).

Statistical analysis

Results are expressed as mean \pm SEM. n represents the number of animals. Data obtained

from pharmacological experiments were analyzed by one way (ANOVA) followed by Dunnet's "t" test and used to evaluate the results, employing Pharmacologic Calculation System Version 4.1.

RESULTS AND DISCUSSION

The structures, yields and melting points of the compounds are listed in Table 1. The Schiff bases of isatin derivatives were synthesized by condensation of the keto group of isatin with various thiosemicarbazides and further with secondary amines to get Mannich bases. All spectral data are in accordance with assumed structures. All the synthesized compounds were tested for analgesic activity. The synthesized compounds 4b, 4e, 4g and 4j show significant analgesic activity when compared to standard diclofenac sodium (45mg/kg).

CONCLUSION

The synthesized compounds shows significant analgesic activity, but generally, the synthesized compounds 4b, 4e, 4g and 4j ($P < 0.01$) possess remarkable analgesic activity. Therefore, they seem to be really promising compounds for their analgesic activity. The synthetic studies should be continued concerning this group of compounds followed with further *in vivo* studies.

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