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Research Article

Synthesis of some 1H-indole-2, 3-dione Derivatives as Antibacterial, Analgesic and Anti-inflammatory Agents

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Abstract

The starting compound 1H-indole-2,3-dione on reaction with different substituted anilines formed the Schiff bases. The corresponding N-Mannich bases have been prepared by the reaction of the Schiff bases with diphenyl amine in the presence of formaldehyde. The chemical structures of the title compounds have been confirmed and elucidated by means of their physical and spectral data respectively. The synthesized Mannich bases were screened for their antibacterial, analgesic and anti-inflammatory activities by the standard methods. Among the tested compounds, the compound containing chloro group showed the most favorable activity.

Keywords: Isatin, Schiff bases, Mannich bases, antibacterial, analgesic, anti-inflammatory drugs.

Introduction

The well documented biological profile of isatin (1H-indole-2,3-dione) derivatives have attracted much attention over the years. A number of Schiff and Mannich bases of isatin are well known for their broad spectrum of pharmacological activities such as antibacterial,[1-4] antifungal,[5-6] antiviral,[7-8] anti-HIV,[9-12] antidepressant,[13] anticonvulsant,[14-16] analgesic[17] and anti-inflammatory[18] activities. The good biological profile of isatin derivatives prompted us to synthesize some Mannich bases of isatin. The present communication intends to report the synthesis, characterization, antibacterial, analgesic and anti-inflammatory study of some N-Mannich bases of isatin. The Schiff bases were prepared by reacting isatin with the primary aromatic amines. The corresponding N-Mannich bases were synthesized by reacting them with the secondary amine and formaldehyde. The chemical structures of the synthesized compounds were confirmed by means of their physical, IR, ^{1}H-NMR and Mass spectral data. The N-Mannich bases were evaluated for their antibacterial, analgesic and anti-inflammatory activity by cup plate, acetic acid induced writhing response and carrageenan induced paw edema method respectively.

Materials and Methods

Materials

Commercially available reagent grade chemicals were used for the synthesis work. All the chemicals were purchased from Loba Chemie Ind Co., Mumbai, India, and S. D. Fine-chemicals limited, Mumbai, India.

Analytical techniques

The melting points were determined in open capillaries by using a Thomas Hoover melting point apparatus, expressed in ^{0}C and are uncorrected. The IR spectra of the compounds were recorded on Shimadzu IR Affinity series-1 in KBr and the values are expressed in cm^{-1}. The ^{1}H-NMR spectra of the compounds were recorded on a Bruker Advance II 400 MHz spectrophotometer and the values were expressed in δ ppm. The mass spectra of the compounds were recorded on Micromass Q-Tof Micro; in *m/z.* The purity of the compounds was checked by thin layer chromatography on silica gel G coated plates.

Synthesis of Schiff bases of isatin

Equimolar (0.01 mol) quantity of isatin and substituted anilines were dissolved in sufficient amount of ethanol and refluxed for 3 h in presence of glacial acetic acid. After standing for approximately 24 h at room temperature, the products were separated by filtration, dried under vacuum and recrystallized from warm ethanol.

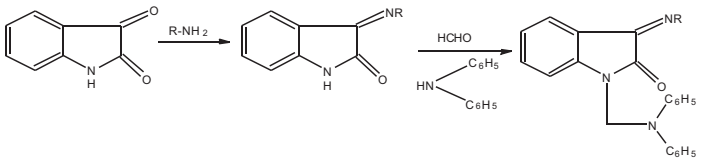
Synthesis of Mannich bases of isatin

Equimolar quantity of diphenylamine (0.004mol) in 10 ml of ethanol was added to the solution containing Schiff bases and formaldehyde (37% v/v). The reaction mixture was stirred for 2 h at room temperature and kept under refrigeration for 24 h. The products were separated by suction filtration, dried under vacuum

and recrystallized from ethanol. The molecular formula, molecular weight, melting point, yield, R_f and spectral data were presented in Table1-2. TLC was monitored by using solvent system benzene: chloroform (55:45) and the spots were identified by placing the dried plate in iodine chamber.

Antibacterial study

The synthesized compounds were screened *in-vitro* for their antibacterial activity against *Staphylococcus aureus* (MTCC-87), *Escherichia coli (*MTCC-40), *Staphylococcus epidermidis* (MTCC-2639), *Pseudomonas aeruginosa* (MTCC-424) and *Proteus vulgaris* (MTCC 426) using cup plate method.[19] The compounds were tested at 500 μg concentration in DMSO, using nutrient agar as the medium. After 24 h of incubation at 37°C, the zone of inhibition formed were measured in mm against standard drug tetracycline and the data were presented in Table 3.



Analgesic activity

The analgesic activity of the synthesized compounds was studied using acetic acid induced writhing response.[20] In this model the animals (Swiss albino mice) were divided into different groups (n=6). Group I served as control (1% Carboxy Methyl Cellulose as vehicle, 1ml/kg, p.o.), group II served as standard (Indomethacin, 10 mg/kg, p.o.) and other groups were served as test groups and received the test compounds each at the dose of 200 mg/kg/p.o. The vehicle, standard and test compounds were administered in the suspension form in Carboxy Methyl Cellulose to the respective groups, 30 min before the induction of pain by acetic acid.

Acetic acid induced writhing method

Healthy Swiss albino mice (20-30 g) were placed into individual restraining cages. The animals were then allowed to adapt in the cages for 30 min before testing. Writhing was induced in mice by administration of 0.6% acetic acid (10 ml/kg body weight, i.p.). The number of writhes was calculated over the period of 20 min after acetic acid injection. A writh is indicated by an abdominal constriction followed by full extension of hind limb. The data represent the total numbers of writhes observed over the 20 min period.

Table 1: Physical data of the synthesized compounds

Compounds	R	Molecular Formula	Molecular Weight	Melting Point (°C)	Yield (%)	R_f
5A	Phenyl	$C_{27}H_{21}N_3O$	403.47	178-179	83.12	0.812
5B	2-nitrophenyl	$C_{27}H_{20}N_4O_3$	448.47	234-235	78.26	0.791
5C	3-nitrophenyl	$C_{27}H_{20}N_4O_3$	448.47	241-242	72.51	0.811
5D	4-nitrophenyl	$C_{27}H_{20}N_4O_3$	448.47	247-248	88.41	0.910
5E	3-chlorophenyl	$C_{27}H_{20}ClN_3O$	437.92	238-239	68.50	0.819
5F	4-chlorophenyl	$C_{27}H_{20}ClN_3O$	437.92	251-252	65.23	0.921
5G	4-bromophenyl	$C_{27}H_{20}BrN_3O$	482.37	255-256	76.35	0.771
5H	4-fluorophenyl	$C_{27}H_{20}FN_3O$	421.46	220-221	69.46	0.761
5I	2,6-dichlorophenyl	$C_{27}H_{19}Cl_2N_3O$	472.36	231-232	77.71	0.687
5J	3,4-dichlorophenyl	$C_{27}H_{19}Cl_2N_3O$	472.36	242-243	84.33	0.813
5K	3-chloro-4-fluorophenyl	$C_{27}H_{19}ClFN_3O$	455.91	256-257	68.21	0.744
5L	2,4-dinitrophenyl	$C_{27}H_{19}N_5O_5$	493.47	232-233	75.18	0.766
5M	4-chloro-2-nitrophenyl	$C_{27}H_{19}ClN_4O_3$	482.91	250-251	85.22	0.860
5N	2-chloro-4-nitrophenyl	$C_{27}H_{19}ClN_4O_3$	482.91	207-208	69.20	0.642

Anti-inflammatory study

The anti-inflammatory activity was determined by carrageenan induced paw edema method[21] in Wistar rats by using digital plethysmometer (Panlab LE 7500). Wistar rats of either sex (180-250 g) were selected and housed under standard laboratory conditions, given standard rat pellet and tap water ad libitum and maintained under standard environmental conditions throughout the period of experimentation. The animals were housed in cages under standard laboratory condition. They had free access to standard diet and water. The animals were divided into different groups of six animals each and fasted for 12h before the experiment. Group I served as control and received vehicle, group II served as standard group and received indomethacin (10 mg/kg, p.o.) and other groups are served as test groups and received the test compounds (200 mg/kg/p.o.) one hour prior to carrageenan injection. The initial right hind paw volume of the rats were measured using a digital plethysmometer and then 0.1 ml of 1% w/v carrageen solution in normal saline was injected into the sub plantar region of the right hind paw. The volume of right hind paw was measured at 1, 2, 3, 4 and 5 h after carrageenan injection by using digital plethysmometer. The data were expressed is paw volume (ml), compared with the initial hind paw volume of each rat.

Table 2: IR, ^{1}H-NMR and Mass spectral data of the synthesized compounds

Compounds	IR (KBr), ^{1}H NMR (400 MHz, DMSO-d_6), *M/Z*
5A	IR (cm^{-1}): 1724(C=O), 1622(C=N), 1458(C=C)
	^{1}H NMR δ (ppm): 4.66(s, 2H, CH_2), 7.04-8.11(m, 19H, Ar-H). *m/z*(M^+ 404)
5B	IR(cm^{-1}): 1730(C=O), 1620(C=N), 1504 & 1344(NO_2), 1460(C=C)
	^{1}H NMR δ (ppm): 4.62(s, 2H, CH_2), 7.10-8.15(m, 18H, Ar-H)
5C	IR(cm^{-1}): 1716(C=O), 1616(C=N), 1529 & 1354 (NO_2), 1456 (C=C)
	^{1}H NMR δ (ppm): 4.62(s, 2H, CH_2), 7.10-8.15(m, 18H, Ar-H). *m/z*(M^+ 449)
5D	IR(cm^{-1}): 1751(C=O), 1602(C=N), 1529 & 1369(NO_2), 1469(C=C)
	^{1}H NMR δ (ppm): 4.66(s, 2H, CH_2), 7.08-8.13(m, 18H, Ar-H)
5E	IR(cm^{-1}): 1720(C=O), 1614(C=N), 1462(C=C), 748(C-Cl)
	^{1}H NMR δ (ppm): 4.64(s, 2H, CH_2), 7.10-8.19(m, 18H, Ar-H)
5F	IR(cm^{-1}): 1716(C=O), 1608(C=N), 1458(C=C), 744(C-Cl)
	^{1}H NMR δ (ppm): 4.67(s, 2H, CH_2), 6.56-7.45(m, 18H, Ar-H). *m/z*(M^+ 422)
5G	IR (cm^{-1}): 1730(C=O), 1606(C=N), 582(C-Br), 1462 (C=C)
	^{1}H-NMR δ ppm: 5.01(s, 2H, CH_2), 6.31-7.67(m, 18H, Ar-H)
5H	IR(cm^{-1}): 1716(C=O), 1614(C=N), 1458(C=C), 1097(C-F)
	^{1}H NMR δ (ppm): 4.42(s, 2H, CH_2), 6.55-7.45(m, 18H, Ar-H). *m/z*(M^+ 421)
5I	IR(cm^{-1}): 1710(C=O), 1608(C=N), 1451(C=C), 742(C-Cl)
	^{1}H NMR δ (ppm): 4.62(s, 2H, CH_2), 6.71-7.84(m, 17H, Ar-H)
5J	IR(cm^{-1}): 1714(C=O), 1614(C=N), 1463(C=C), 744(C-Cl)
	^{1}H NMR δ (ppm): 4.66(s, 2H, CH_2), 6.87-7.88(m, 17H, Ar-H)

Compounds	IR (KBr), ^{1}H NMR (400 MHz, DMSO-d_6), *M/Z*
5K	IR(cm^{-1}): 1714(C=O), 1614(C=N), 1460(C=C), 1055(C-F), 611(C-Cl)
	^{1}H NMR δ ppm: 4.69(s, 2H, CH_2), 7.11-8.12(m, 17H, Ar-H)
5L	IR(cm^{-1}): 1721(C=O), 1606(C=N), 1521 & 1367(NO_2), 1465(C=C)
	^{1}H NMR δ (ppm): 4.52(s, 2H, CH_2), 7.13-8.09(m, 17H, Ar-H). *m/z*(M^+ 494)
5M	IR(cm^{-1}): 1757(C=O),1627(C=N),1463(C=C),1508&1334(NO_2),631(C-Cl)
	^{1}H NMR δ (ppm): 4.64(s, 2H, CH_2), 7.15-8.08(m, 17H, Ar-H)
5N	IR(cm^{-1}): 1757(C=O),1589(C=N),1462(C=C),1502&1336(NO_2),746(C-Cl)
	^{1}H NMR δ (ppm): 4.68(s, 2H, CH_2), 7.16-8.05(m, 18H, Ar-H)

Table 3: *In vitro* Antibacterial activity of the synthesized compounds

	Diameter of zone of inhibition (mm)				
Compound	**P. vulgaris**	**P. aeruginosa**	**E. coli**	**S. aureus**	**S. epidermidis**
5A	11.33 ± 0.57	07.33 ± 0.57	13.00 ± 1.00	10.00 ± 1.00	08.33 ± 0.57
5C	15.00 ± 1.00	09.00 ± 1.00	16.33 ± 1.52	12.00 ± 1.00	09.33 ± 0.57
5D	16.00 ± 1.00	06.66 ± 0.57	11.66 ± 0.57	10.66 ± 1.52	11.00 ± 1.00
5E	14.00 ± 1.00	06.33 ± 0.57	14.00 ± 1.00	13.33 ± 1.52	13.00 ± 1.00
5F	15.00 ± 1.00	07.00 ± 1.00	12.00 ± 1.00	11.66 ± 1.52	08.66 ± 1.52
5G	10.00 ± 1.00	09.00 ± 1.00	07.66 ± 0.57	-	-
5H	13.00 ± 1.00	-	06.33 ± 0.57	-	-
5I	10.00± 1.00	09.00 ± 1.00	10.66 ± 1.52	-	11.00 ± 1.00
5J	14.33 ± 0.57	-	16.33 ± 1.52	10.33 ± 0.57	07.00 ± 1.00
5K	11.33 ± 0.57	-	12.33 ± 0.57	12.00 ± 1.00	11.33 ± 0.57
5L	11.00 ± 1.00	09.00 ± 1.00	12.00 ± 1.00	10.00 ± 1.00	
5M	22.00 ± 1.00	15.33 ± 0.57	25.33 ± 1.52	16.66 ± 1.15	15.00 ± 1.00
5N	18.33 ± 1.52	12.00 ± 1.00	22.33 ± 1.52	13.66 ± 1.52	12.33 ± 1.52
Control					
Standard	24.33 ± 0.57	21.66 ± 0.57	32.33 ± 0.57	21.66 ± 0.57	23.00 ± 1.00

Results were expressed as Mean ± S.D. (n = 3), "-" indicates no zone of inhibition

Table 4: Effect of the synthesized compounds on carrageenan induced paw edema in rats

Compound	Change in Paw volume				
	1h	2h	3h	4h	5h
Control	0.38 ± 0.01	0.55 ± 0.02	0.82 ± 0.03	1.00 ± 0.08	1.30 ± 0.07
Standard	0.26 ± 0.02	0.45 ± 0.03*	0.22 ± 0.03***	0.18 ± 0.04***	0.22 ± 0.02***
5A	0.42 ± 0.03	0.55 ± 0.03	0.84 ± 0.04	1.05 ± 0.07	1.26 ± 0.07
5B	0.33 ± 0.01	0.54 ± 0.03	0.75 ± 0.01	0.94 ± 0.04	1.25 ± 0.05
5C	0.29 ± 0.01	0.52 ± 0.03	0.70 ± 0.04	0.89 ± 0.06	1.17 ± 0.07
5D	0.37 ± 0.02	0.52 ± 0.02	0.67 ± 0.06	0.87 ± 0.06	1.16 ± 0.07
5E	0.32 ± 0.02	0.55 ± 0.03	0.74 ± 0.01	0.75 ± 0.02**	1.25 ± 0.04
5F	0.40 ± 0.03	0.54 ± 0.03	0.81 ± 0.03	1.04 ± 0.06**	1.33 ± 0.08
5G	0.36 ± 0.02	0.52 ± 0.02	0.70 ± 0.04	0.84 ± 0.03	1.16 ± 0.06
5I	0.31 ± 0.01	0.52 ± 0.04	0.71 ± 0.01	0.93 ± 0.04	1.25 ± 0.04
5J	0.31 ± 0.01	0.49 ± 0.03	0.73 ± 0.01	0.79 ± 0.05*	1.27 ± 0.04
5K	0.37 ± 0.03	0.53 ± 0.03	0.78 ± 0.03	1.01 ± 0.05	1.28 ± 0.05
5M	0.35 ± 0.03	0.51 ± 0.03	0.71 ± 0.02	0.90 ± 0.02	1.22 ± 0.03

*Results were expressed as Mean ± SEM (n = 6), *p < 0.05, **p < 0.01, ***p < 0.001 as compared to control.*

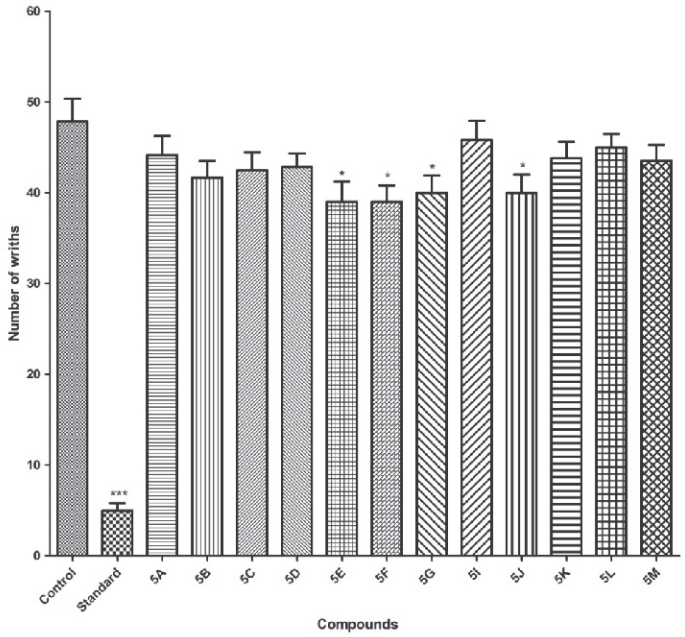


Fig. 1: Effects of the synthesized compounds on acetic acid induced writhing in mice. Results were expressed as mean ± SEM (n=6). *p < 0.05, ***p < 0.001 compared to control.

Statistical analysis

The results of statistical analysis for animal experiments were expressed as mean ± SEM. The number of animals in each group were six (n=6). The results were statistically analyzed by two way ANOVA followed by Bonferroni post tests in case of anti-inflammatory study and in acetic acid induced writhing model the data were analyzed by one way ANOVA followed by Dunnet's multiple comparison test by using GraphPad Prism software, v 5.0 (trial), (GraphPad Inc, USA). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to control were considered to be statistically significant.

Results and Discussion

The spectral and physical data proved the structure and purity of the synthesized compounds. The synthesized compounds were evaluated for *in-vitro* antibacterial activity by cup plate method against five different bacterial strains. The results were summarized in Table 3 including the activity of standard. Among the tested compounds the compound 5M exhibited highest

activity against all the test organisms. Other test compounds of the series also exhibited moderate antibacterial activity but the activity was less than the standard drug Tetracycline in this test concentration.

The analgesic activity of the synthesized compounds was evaluated by using chemical method. Acetic acid induced writhing test used for detecting both central and peripheral analgesia. Intraperitoneal administration of acetic acid releases prostaglandins and mediators like PGE_2 and $PGF_{2\alpha}$ and their levels were increased in the peritoneal fluid of the acetic acid induced mice.

Some of the tested compounds showed significant analgesic activity. The compounds 5E, 5F, 5G and 5J were found to be significant ($p < 0.05$) in reducing the number of wriths whereas other compounds of this series were found to be non-significant. The standard drug Indomethacin was found to be more potent than the test compounds (Fig.1).

Carrageenan induced paw edema is a multimediated phenomenon that liberates diversity of mediators. It is believed to be biphasic, the first phase (1h) involves the release of serotonin and histamine while the second phase (over 1h) is mediated by prostaglandins, the cyclooxygenase products and the continuity between the two phases is provided by kinins. The result of this study shows that some of the synthesized compounds have significant anti-inflammatory activity. Among these, the compounds 5E and 5F showed significant anti-inflammatory activity at fourth ($p < 0.01$) hour of the study. Similarly the compound 5J was also found to be significant at fourth ($p < 0.05$) hour of the study only whereas other compounds of the series were found to be non-significant in this test concentration. Although some of the synthesized compounds showed significant anti-inflammatory activity, but they were not remarkably comparable to that obtained by the standard drug Indomethacin (Table 4).

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