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Synthesis and Evaluation of 1,3 Di-Substituted Schiff, Mannich Bases and Spiro Isatin Derivatives

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ABSTRACT

Schiff bases of isatin with aminothiazole, its N-mannich bases and Spiro isatin derivatives were synthesized. Their chemical structures were confirmed by Infrared, 1H-Nuclear Magnetic Resonance data and elemental analysis. Antimicrobial evaluation was performed by the agar diffusion method against four pathogenic bacteria and two pathogenic fungi. Anti-inflammatory activity was tested by carragenin-induced rat paw edema and compounds were evaluated for analgesic action by the acetic acid-induced writhing method; Compounds Aa, Ab and A5, A6 were found to be active against bacteria and fungi. The compounds A3, A6, Aa and Ab showed anti-inflammatory activity, having a percentage protection value of 34.69, 32.65, 38.77 and 36.73 as compared with that of indomethacin, with % protection of 46.93. Similarly, the compounds Aa, Ab and A6 showed analgesic activity, with % protection of 67.51, 64.78 and 49.81 as compared with the standard with % protection of 79.56.

Key words: Analgesic, antipyretic, anti-inflammatory, isatin, Spiro compounds

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INTRODUCTION

Isatin (1-H indole 2-3 dione) are synthetically versatile substances that are employed for the synthesis of a large variety of heterocyclic compounds, including some drugs. Over the years, the molecules with isatin show diversified biological activities, e.g. antibacterial,[1] anticonvulsants,[2] antiviral[3] and antitubercular[4] activities. Schiff and mannich bases of isatin have shown good antimicrobial activities against gram-negative bacteria and fungi.[3] A number of Spiro compounds derived from isatin are important pharmacophores and exhibit promising biological activities,[5] such as analgesic, fungicidal and anti-inflammatory activity. Thiazole and its derivatives are reported to show good antibacterial, anti-inflammatory and analgesic[6] activity. Hence, it was thought worthwhile to synthesize new Schiff, mannich and Spiro isatin derivatives and investigate them for biological and pharmacological activities. The key starting materials, 1-H-indole 2-3 dione and 2-aminothiazole, were refluxed in the presence of alcohol and glacial acetic acid to get Schiff base 3 (1'-3'thiazole 2-yl imino) 1-3 dihydro 2-H-indole-2-one (A); mannich bases are prepared with different secondary amines in the presence of formaldehyde (A_1-A_6). Meanwhile, Spiro isatin derivatives are prepared with Azetidine (A_a) and thiazolidone (A_b) in the presence of chloroacetyl chloride (Cl-CH_2-CO-Cl) and thioglycolic acid (SH-CH_2-COOH).

MATERIALS AND METHODS

Melting points are recorded in open capillary tubes and are uncorrected. The IR spectra (KBr) were recorded on a

SHIMADZU FTIR-8300, spectrophotometer, Japan. The H-NMR spectra were recorded on a Bruker Advance-400 MHz spectrometer, USA. Purity of the compounds was checked by Thin layer chromatography For analgesic, anti-inflammatory activity studies, adult healthy albino rats (Swiss strain) of either sex weighing 80–120 g were used. All the animals were maintained under standard conditions and had access to pelleted animal feed and water. The study protocol was approved by the Institutional Animal Ethics Committee.

Synthesis[7] of 3(1'-3'Thiazole-2-yl Imino) 1-3 Dihydro-2H Indole-2-One (A)

Equimolar quantity of isatin and 2-amino thiazole was taken in a round bottom flask, dissolving the content with a sufficient amount of ethanol and two to three drops of glacial acetic acid was added. The content was refluxed for 4–5 h and then poured into ice cold water. The solid obtained was filtered, dried and recrystallized from ethanol.

Synthesis[7] of 1[(Diphenyl Amino) Methyl]-3-(1'-3' Thiazole-2-yl Imino) 1-3 Dihydro-2H Indole-2-One (A_1)

To a slurry containing A (0.003 mol), ethanol (5 ml) and 37% formalin (1 ml), diphenylamine (0.003 mol) was added slowly with good stirring. The reaction mixture was cooled and allowed to stand at room temperature for 1 h, with occasional shaking. Then, it was warmed on a steam bath for 15 min, cooled and the product was recovered. The product was recrystallized from chloroform–methanol (1:1) mixture.

The compounds 1(piperazin-1-yl) methyl-3-(1'-3' thiazole-2-yl imino) 1-3 dihydro-2H indole-2-one (A_2), 1[(dicyclohex-yl) methyl]-3-(1'-3' thiazole-2-yl imino) 1-3 dihydro-2H indole-2 one (A_3), 1[(dimethylamino) methyl]-3-(1'-3' thiazole-2-yl imino) 1-3 dihydro-2H indole-2 one (A_4), 1-[(diethylamino) methyl]-3-(1'-3' thiazole-2-yl imino) 1-3 dihydro-2H indole-2 one (A_5) and 1-[(morpholin-4''yl) methyl]-3-(1'-3' thiazole-2-yl imino) 1-3 dihydro-2H indole-2 one (A_6) were prepared by following a similar procedure.

Synthesis of 3'Chloro-1-(1''-3''Thiazo-2-yl) 4'-H-Spiro [Azetidine-2, 3'Indole] 2'4-(1-H) Di One (A_a)[8]

Compound 3(1'-3'thiazole-2-yl imino) 1-3dihydro-2H indole-2-one (A) (0.01mol) was dissolved in 1-4 dioxan (20 ml) in a 100 ml beaker and trimethyl amine (0.001 mol) was added to it. Then, the contents of the beaker were stirred and chloroacetyl chloride (0.001 mol) was added slowly, continuing stirring of contents of the beaker for 8 h. The resulting solution was poured into ice cold water, filtered, dried, recrystallized from chloroform–methanol (1:1) and added in a round bottom flask. The content of the flask was refluxed for 12–14 h, the product was recovered in ice cold water, filtered, dried and finally recrystallized from chloroform–methanol (1:1) mixture.

Synthesis of 3'Chloro-1-(1''-3''Thiazo-2-yl) 4'-H-Spiro [Indole-3,2-(1,3) Thiazolidine] 2'4-(1-H) Di One (A_B)[5]

Equimolar quantity of compound A and thioglycollic acid was dissolved in a round bottom flask. Then, 1,4-dioxane and a pinch of zinc chloride was added to it. The contents of the mixture were refluxed for 12–14 hr, concentrating the content of the flask, and poured into ice cold water. The solid mass was collected and dried. The product was recrystallized from chloroform–methanol mixture (1:1).

Antimicrobial activity

The *in vitro* antimicrobial activity[9] was carried out against 24-h-old cultures of microorganisms by the cup–plate method. Compound A_1-A_6 and A_a, A_b were tested against four pathogenic bacteria, *Pseudomonas mirebelis*, *Pseudomonas auroginosa*, *Escherichia* coli and *Staphylococcus aureus*. The antifungal activity was tested against *Aspergillus niger* and *Candida albicans*. Ampicillin and cotrimoxazole was taken as an internal standard for antibacterial and antifungal activity, respectively. The compound was tested at a concentration of 100 μ/ml in DMF against all organisms. The zone of inhibition was compared with the standard drug after 24-h incubation at 37°C for antibacterial and 48 h at 25°C for antifungal activity.

Analgesic activity

The analgesic activity[10] was evaluated by the acetic acid-induced writhing test[6] using adult Swiss albino mice of either sex. In this method, mice are made to writhe by a simple intraperitonial injection of 0.6% v/v aqueous acetic acid (0.1 ml/kg). Test substances were administered 30 min before the injection of acetic acid. The number of writhes (full extension of hind paws) was recorded.

Anti-inflammatory activity

The anti-inflammatory activity[10] was evaluated by rat paw edema. Edema represents the early phase of inflammation and carragenin-induced paw edema is the simplest and most widely used method[8] for studying the anti-inflammatory activity of the chemical compounds. This method is based on the plethysmographic measurement

Table 1: Characterization data of the synthesized compounds

Compounds	M.P (°C)	Yield%	Molecular formula	Found (calculated) %		
				C	H	N
A_1	108–110	59	$C_{24}H_{18}N_4OS$	58.52 (58.72)	5.03 (5.23)	21.12 (21.39)
A_2	238–240	68	$C_{16}H_{17}N_5OS$	69.90 (70.22)	4.21 (4.41)	13.40 (13.65)
A_3	110–114	72	$C_{24}H_{30}N_4OS$	67.01 (68.21)	6.89 (7.16)	12.89 (13.28)
A_4	220–223	72	$C_{14}H_{14}H_4OS$	58.60 (58.72)	4.81 (4.93)	19.40 (19.57)
A_5	252–254	61	$C_{16}H_{18}N_4OS$	61.01 (61.12)	5.72 (5.77)	17.81 (17.82)
A_6	248–250	65	$C_{16}H_{16}N_4O_2S$	58.71 (58.82)	4.80 (4.91)	16.99 (17.06)
A_a	340–342	65	$C_{13}H_8N_3SO_2Cl$	50.88 (51.47)	2.47 (2.64)	13.53 (13.74)
A_b	343–344	62	$C_{13}H_9N_3S_2O$	51.12 (51.47)	2.83 (2.99)	3.63 (13.85)

Table 2: Results of antimicrobial activity

Compounds	Zone of inhibition* (mm)					
	Antibacterial activity			Antifungal activity		
	P. mirebelis	*P. auroginosa*	*E. coli*	*S. aureus*	*A. niger*	*C. albicans*
A_1	12	14.2	10	9.5	22.2	17.3
A_2	13	12.9	10	8.3	21	16.5
A_3	10	10.3	11.2	9.8	23	20
A_4	11.1	11	10	8.8	17	17.8
A_5	11	11.2	13.6	9.3	21	15.3
A_6	12.2	12.5	14	9.1	22.9	19
A_a	15.2	17	20	10	5	21.2
A_b	13.5	16.1	17.5	10	24.3	20
Ampicillin	23	22.5	24	26	27.6	28
CotrimoxazoleDMF	-		-	-	-	-

*average of three readings.

of carragenin-induced acute rat paw edema. For this study, Wister rats of either sex, weighing between 80 and 100 g were divided into 10 groups of five animals each. Group1 serves as a solvent control, and received 0.5% Carboxy Methyl Cellulose in normal saline orally. Group 2 received indomethacin (10 mg/kg) in solvent as a standard. Groups 3–10 received test drugs at a dose of 100 mg/kg orally. These drugs were administered 1 h before the injection of an irritant, carragenin. After 1 h, all the animals were injected subcutaneously with a suspension of carragenin in CMC solution (0.1 ml) to the left hind paw in the subplantar region and the paw volume was measured immediately. After 3 h, the paw volume was measured for the control, standard and test groups. Percentage inhibition of paw volume was calculated.

RESULTS AND DISCUSSION

The structures of newly synthesized compounds were elucidated by IR, ^{1}H-NMR and elemental analysis and are reported as follows:

The IR spectrum of **A** in KBr (in cm^{-1}): 3446 (NH str), 1728 (C=O), 1610.95 (C=N), 1469.01 (CH=CH of Ar), 1096.66 (C-S-C).

^{1}H-NMR of **A** (δ values)**:** 11.04 (S, 1H, NH), 6.89–7.61 (m, Ar-H), 3.39 (1H,CH), 3.16 (1H, CH).

Table 3: Analgesic activity of the compounds

Compound code	Dose (mg/kg)	Number of writhing movements	% of protection
0.5% CMC	-	58.8 ± 0.95	-79.5632.1128.46
Indomethacin	10	11.2 ± 4.32**	43.7933.2127.91
A_1	100	37.2 ± 3.21*	49.8167.5164.78
A_2	100	39.2 ± 3.48	
A_3	100	30.8 ± 2.23*	
A_4	100	36.6 ± 2.97*	
A_5	100	39.5 ± 3.27	
A_6	100	27.5±2.85**	
A_a	100	17.8 ± 2.19**	
A_b	100	19.3 ± 2.98**	

Values are expressed as mean ± SE (n = 6). *P<0.05, **P<0.01 and ***P<0.001 compared with vehicle control (ANOVA followed by Dunnet's t-test).

IR of $\mathbf{A_1}$ (in cm^{-1}): 2890 (CH_2), 1730 (C=O), 1670 (C=N), 1459.37 (CH=CH), 1200 (C-N), 1090 (C-S-C), 766.888 (C=C aromatic hydrocarbon).

^{1}H-NMR of A_1 (δ values): 6.89–7.09 (m, Ar-H), 2.50 (S.2H, N-CH_2), 3.38 (S, 2H, CH of thiazole).

IR of $\mathbf{A_2}$ (in cm^{-1}): 2832 (CH_2), 1720 (C=O), 1609 (C=N), 1480 (CH=CH), 1363 (C-N), 1164.30 (C-S-C str), 3305 (N-H).

^{1}H-NMR of $\mathbf{A_2}$ (δ values): 6.89–7.92 (m, Ar-H), 4.38 (S, 1H, N-H), 3.06–3.38 (δ, 1H, CH), 1.23 (t, 2H, CH_2 piperazine).

IR of $\mathbf{A_3}$ (in cm^{-1}): 2946 (CH), 2854 (C-H), 1718.84 (C=O),

Table 4: Anti-inflammatory activity of the compounds

Compound code	Group	Volume of mercury displaced (ml)		
		1 h (% of protection)	2 h (% of protection)	3 h (% of protection)
0.5% CMC	1	0.49 ± 0.013	0.55 ± 0.033	0.59 ± 0.022
Indomethacin	2	0.26 ± 0.012 (46.93)	0.37 ± 0.022 (32.72)	0.39 ± 0.012 (33.89)
A_1	3	0.41 ± 0.025* (16.32)	0.54 ± 0.029 (1.81)	0.45 ± 0.022** (23.72)
A_2	4	0.48 ± 0.041 (2.02)	0.54 ± 0.012 (1.81)	0.57 ± 0.032 (3.38)
A_3	5	0.32 ± 0.041** (34.69)	0.53 ± 0.034 (3.36)	0.46±0.030** (22.03)
A_4	6	0.47 ± 0.033 (4.08)	0.52 ± 0.036 (5.54)	0.43±0.032** (27.11)
A_5	7	0.46 ± 0.043 (6.12)	0.54 ± 0.012 (1.81)	0.56 ± 0.032 (1.69)
A_6	8	0.33 ± 0.032** (32.65)	0.39±0.031* (29.09)	0.46±0.016** (22.03)
A_a	9	0.30 ± 0.043** (38.77)	0.37±0.013** (32.72)	0.45±0.041*** (23.72)
A_b	10	0.31 ± 0.041** (36.73)	0.38±0.033** (30.90)	0.46±0.061** (22.03)

Values are expressed as mean ± SE (n = 6). *P<0.05, **P<0.01 and ***P<0.001 compared with vehicle control (ANOVA followed by Dunnet's t-test).

1647.58 (C-N), 1517 (C=N), 1458 (CH=CH of Ar), 1105 (C-S-C).

IR of $\mathbf{A_5}$ (in cm^{-1}): 2942 (CH_2), 1735 (C=O), 1676 (C=N), 1654 (C-N), 1542 (CH=CH of Ar), 1042 (C-S-C str), 758 (C=C).

IR of $\mathbf{A_6}$ (in cm^{-1}): 2396 (CH_2 str), 1718 (C=O), 1560 (C-N str), 1654 (C=N), 1542 (CH=CH of Ar), 1108 (C-S-C str), 1005 (C-O-C str), 717 (C=C Ar).

IR of $\mathbf{A_a}$ (in cm^{-1}): 3445 (NH str), 1720 (C=O), 1710 (C=O), 1640 (C-N), 649 (C-Cl).

^{1}H-NMR $\mathbf{A_a}$: 11.08 (s, 1H, NH), 6.93–7.79 (m-Ar-H), 3.39 (δ,1H, CH of thiazole), 2.27 (s, 1H, CH, CH_2Cl).

IR of $\mathbf{A_b}$ (in cm^{-1}): 3442 (NH str), 1724 (C=O), 1708 (C=O), 1645 (C-N), 1090 (C-S-C).

^{1}H-NMR of $\mathbf{A_b}$ (δ values): 11.08 (s, 1H, NH), 6.71–7.78 (m-Ar-H), 3.39 (δ, 1H, CH of thiazole), 2.50 (s, 2H, CH_2).

The characterization of synthesized compounds is given in Table 1. Analytical data of the compounds supports the proposed structures.

Results of antimicrobial activity, analgesic activity and anti-inflammatory activity are shown in Tables 2–4, respectively. The results of the antimicrobial activity revealed that the compounds A_3, A_5, A_6, A_a and A_b were found to be active against bacteria and fungi. The compounds A_3, A_6, A_a and A_b showed significant analgesic activity, with percentage of inhibition of 43.69, 49.81, 67.51, 64.78, respectively, as compared to indomethacin, with percentage inhibition of 79.56. The compounds A_a, A_6, A_b, showed considerable anti-inflammatory activity, having percentage protection of 38.77, 32.65 and 36.73 as compared with indomethacin, having percentage protection value of 46.93.

Thus, it is observed that the synthesized compounds showed promising antimicrobial, analgesic and anti-inflammatory activities. Further studies are needed to discover new novel compounds of this class with profound activities.

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