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SYNTHESIS AND EVALUATION OF SOME NOVEL SCHIFF`S AND MANNICH BASES OF INDOLE-2,3-DIONES AS POTENTIAL ANTIBACTERIAL, ANALGESIC AND ANTIINFLAMMATORY AGENTS

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Schiff's bases of indole-2,3-diones (isatin) and 5-methyl indole-2,3- diones (methyl isatin) with anthranilic acid have been synthesized by a novel methodology. Mannich bases of these Schiff's bases were also synthesized. The compounds synthesized were evaluated for antibacterial, analgesic and antiinflammatory activities. Some of the compounds synthesized were found to possess analgesic and antiinflammatory activities more than the standard diclofenac sodium.

Keywords: *Anthranilic acid; Isatin; Schiff's base; Mannich base; Indole*

Introduction

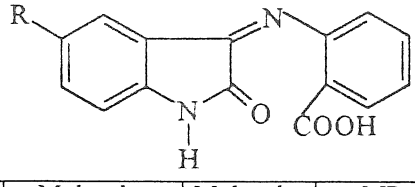
The indole derivative indomethacin and the derivatives of anthranilic acid like mefenamic acid, flufenamic acid and meclofenamate sodium are used as analgesic and antiinflammatory agents. In order to search for the lead compound with potent analgesic and antiinflammatory activities at lower dose, in the present study Schiff`s bases of indole-2,3-dione and 5-methylindole-2,3-dione with anthranilic acid was aimed to be synthesized. However the preparation of Mannich bases of these Schiff`s bases will also increase the lipophilicity and may enhance the pharmacological activity. Literature survey also reveals a potent antibacterial activity in Schiff`s and Mannich bases of isatin. (1-6)

Hence the following series of compounds 3-(2`-carboxyanilino)5-substituted indole-2-one-(**I,II**) and 3-(2`-carboxyanilino)1-(N,N-dialky aminomet-hyl) – 5 - substituted indole-2-one (**III-X**) were aimed to be synthesized as novel antibacterial, analgesic and antiinflammatory agents.

Materials and Methods

Schiff`s base of indole-2,3-diones(**I**) and 5-methylindole-2,3-diones (**II**) were prepared by fusing anthranilic acid with isatin and methyl isatin respectively. A series of Mannich bases of these Schiff`s bases were synthesized by treating the concerned Schiff`s base with formaldehyde and the desired secondary amines to afford the final products(**III-X**). All the compounds were obtained in good yield. The compounds synthesized were characterized by spectral data

Table 1. Physical data for 3-(2'-carboxy anilino) indole-2-ones



Compd. No.	R	Molecular formula	Molecular weight	MP °C	Yield %	Rf. value
I	-H	$C_{15}H_{10}N_2O_3$	266	215-18	81	0.50
II	$-CH_3$	$C_{16}H_{12}N_2O_3$	280	165-66	79	0.52

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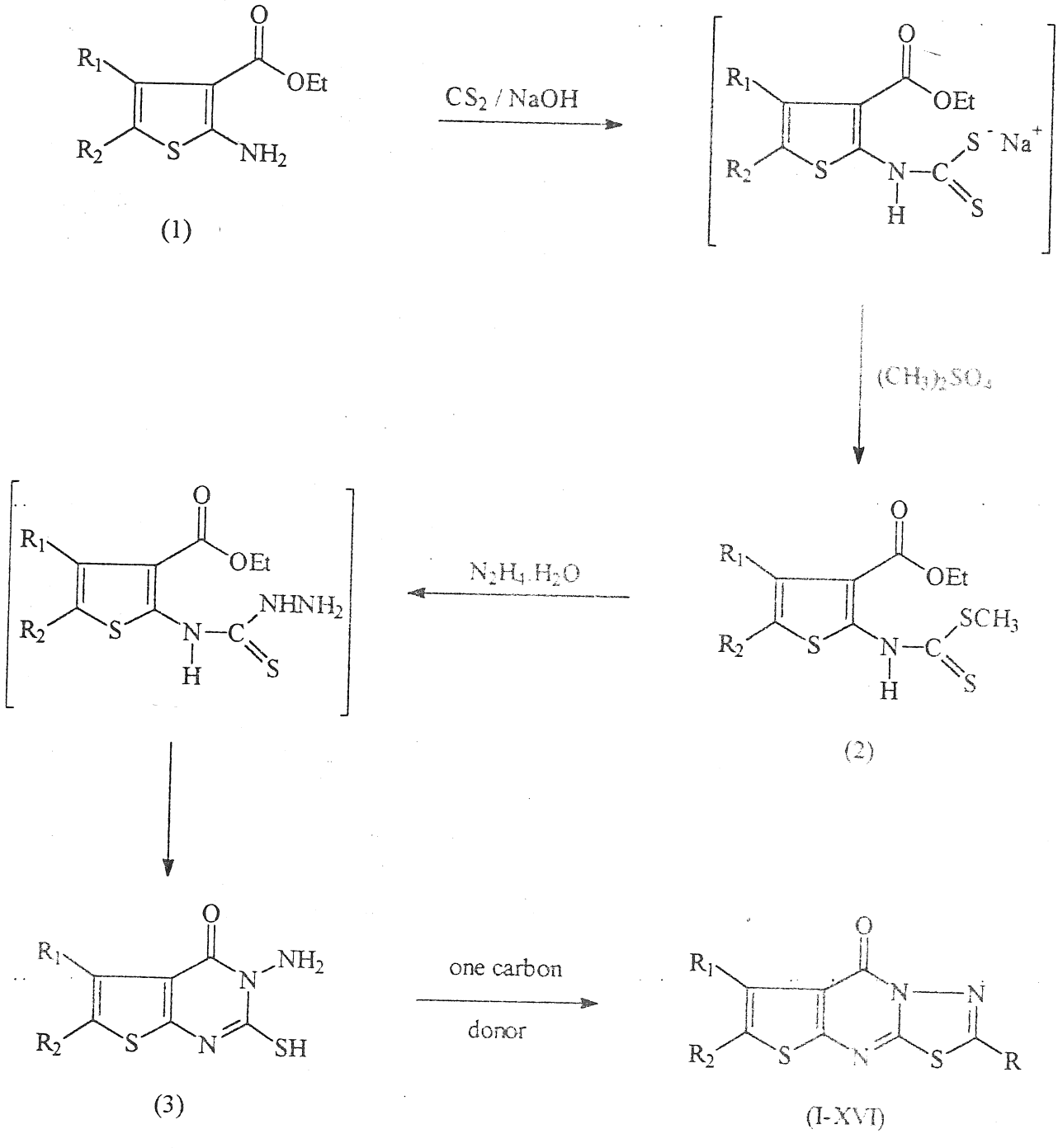
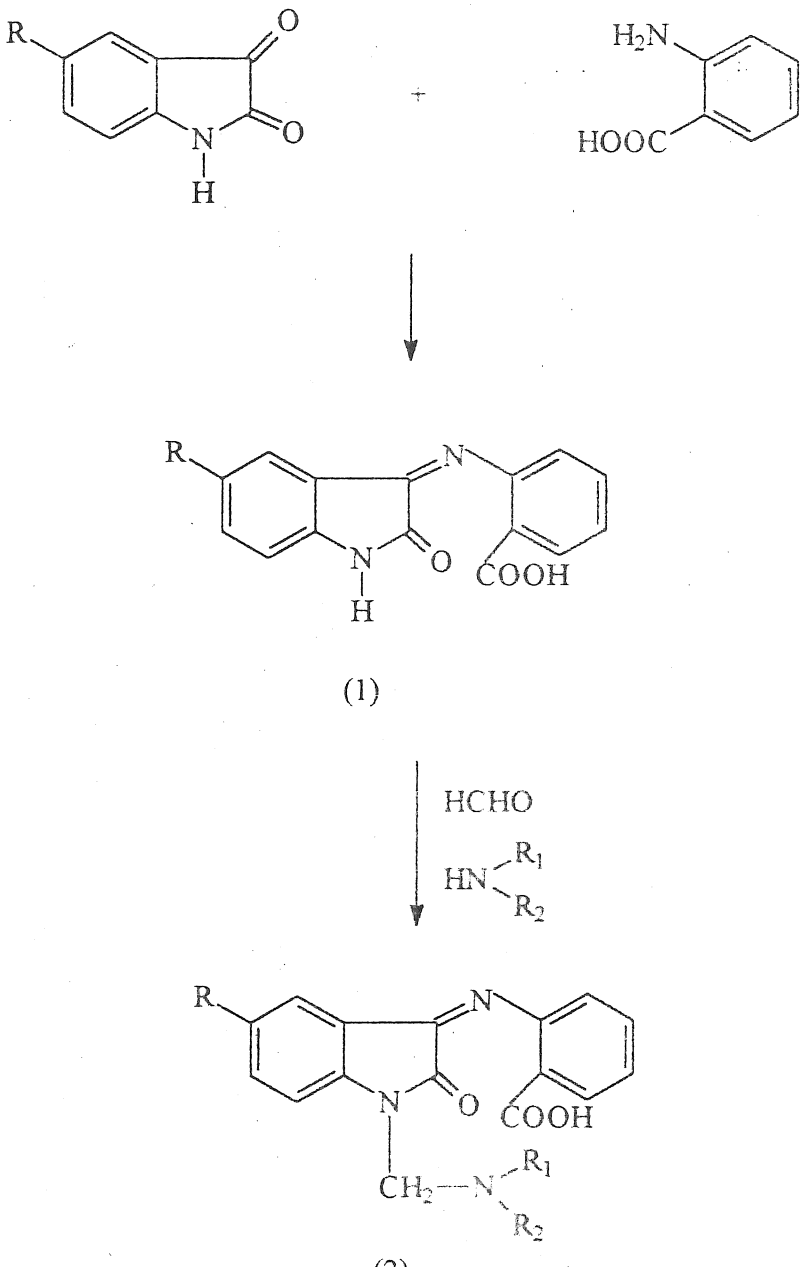


Scheme 1(1)

(IR, N.M.R and mass spectra) and the purity was ascertained by TLC and micro analysis.

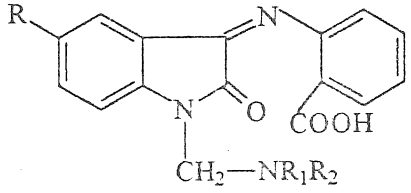
The following route depicted in Scheme 1 outlines the synthetic part of the present work. Physical data of the compounds synthesized are shown in Table 1&2.



Scheme 1(2)

Synthesis of 3-(2'carboxyanilino)indole-2-one(I)
A mixture of anthranilic acid (0.006 moles) and isatin (0.006 moles) were fused at 145°C with continuous stirring for one hour. The product obtained was recrystallized from ethanol to yield 1.29 %g (81%) of compound (I);mp 215-218°C; IR (KBr) (cm^{-1}) 3450-3370 (-OH of COOH), 3300 (-NH), 3050 (-CH), 1690 (-C=O of COOH), 1630 (-C=O), 1680 (C=N). NMR ($CDCl_3$) δ ppm-[(4,5,6,7H)(3',4',5',6'H)] 6.4-7.1, (4 NH) 9.5, (2'-COOH) 10.23, Anal ($C_{15}H_{10}N_2O_3$) C, H, N.

Table 2. Physical data for 3-(2'-carboxy anilino) 1-dialkylamino methyl-5-substituted-indole-2-ones



Compd. No.	R	R_1,R_2	Molecular formula	Molecular weight	MP °C	Yield %	Rf. Value
III	-H	$(CH_3)_2$	$-C_{18}H_{17}N_3O_3$	323	105-7	71	0.60
IV	-H	$(C_2H_5)_2$	$-C_{20}H_{21}N_3O_3$	351	101-3	70	0.61
V	-H	-N⬚	$-C_{20}H_{19}N_3O_3$	349	189-190	75	0.53
VI	-H	N⬚O	$-C_{20}H_{19}N_3O_4$	365	146-148	69	0.62
VII	$-CH_3$	$(CH_3)_2$	$-C_{19}H_{19}N_3O_3$	337	110-111	72	0.63
VIII	$-CH_3$	$(C_2H_5)_2$	$C_{21}H_{23}N_3O_3$	365	105-106	69	0.66
IX	$-CH_3$	N⬚	$C_{21}H_{21}N_3O_3$	363	178-179	70	0.59
X	$-CH_3$	N⬚O	$-C_{21}H_{22}N_3O_4$	379	139-140	63	0.65

Synthesis of 3-(2'-carboxy anilino)1-(N,N dimethylaminomethyl) indole-2-one (III)

To a slurry of I (0.004 mole) in alcohol 10 ml was added the mixture 37% formalin (1ml) and dimethyl amine (0.004 mole) dropwise with stirring. The reaction mixture was allowed to stand at room temperature for 30 minutes with occassional stirring. Then the mixture was stirred at ice-cold condition for 15 minutes. The product obtained was dried and recrystallized from equal mixture of ethanol-chloroform to yield 0.92 g. (71.4%) of compound (**III**); mp 105-107°C; IR (KBr) (cm^{-1}) –3430-3360 (-OH of COOH), 2860 (-CH of CH_2), 1690 (-CO of COOH), 1620 (-C=O), 1670 (-C=N). NMR ($CDCl_3$) δ ppm (1-CH_2), 1.9 (1-2CH_3), 2.1[(4,5,6,7H) (3',4',5',6'H)] 6.6-7.2, -(2'-COOH)9.12, Anal ($C_{18}H_{17}N_3O_3$)C, H, N.

Antimicrobial activity

All the compounds synthesized (**I-X**) were screened for their antibacterial activity by the agar cup-plate method(7) at a concentration of 300 µg/ml using DMF as a solvent against the following organisms; *Salmonella typhi, E.coli, Vibrio cholerae, Stap. epidermitis, Klebsiella pneumoniae, Pseudomonas aeroginosa, Shigella flexnari, Citrobacter ferundi.* The zone of inhibition of each strain are recorded in Table 3. The activity was compared with standard drug Ciprofloxacin at 10 µg/ml concentration.

Analgesic activity

Test for analgesic activity was performed by using the "Tail flick technique"(8,9).

Table 3. The zone of inhibition (in C.M) of compounds (I-X)

Micro organisms	Compounds [300 µg/ml]										Standard [10µg/ml]
	I	II	III	IV	V	VI	VII	VIII	IX	X	
Salmonella typhi	3.0	2.6	2.5	2.8	1.9	2.7	3.0	2.9	2.7	3.1	2.6
Escherchia coli	1.7	1.2	1.9	1.7	1.4	1.8	2.0	2.2	1.5	2.1	2.3
Vibro cholerae	1.6	1.2	1.7	1.5	1.6	1.9	1.9	2.9	1.9	1.9	2.3
Stap. epidermitis	1.9	1.9	1.9	2.0	2.2	2.2	2.3	24.	2.1	2.4	2.4
Klebs.pneumoniae	1.6	1.7	2.2	1.9	1.8	2.2	2.2	2.5	1.9	2.5	2.5
Pseud.aeruginosa	1.8	1.8	1.7	1.7	1.6	1.7	1.9	2.1	1.7	1.8	2.3
Shigella flexnari	1.7	1.9	1.9	1.9	1.7	1.9	2.1	2.3	1.6	1.8	2.4
Citrobacter ferundi	1.7	1.8	1.8	1.7	1.6	1.7	2.0	2.1	1.5	1.6	2.5

Mice(30-35 g) were divided into three groups, each of six animals. One group of mice were treated orally with 20 mg/kg body weight of the aqueous suspension (with Na, CMC) of the test compounds. Another group was administered orally with 20 mg/kg body weight of aqueous suspension (with 1% Na CMC) of diclofenac sodium (standard) and the third group (control group) was fed with the same volume of distilled water. The reaction times were recorded at 15 mins, 30 mins, 1 hr, 2 hrs, and 3 hrs, after the drug administration. The percent analgesic activity (PAA) was calculated using the following formula;

$$PAA = \frac{T_2}{T_1} \times 100$$

Where;

T1= Reaction time (sec) before administration of the test compounds

T2= Reaction time (sec) after administration of the test compounds

The percent analgesic activity of the compounds (**I-X**) are shown in Table 4.

Antiinflammatory activity

Antiinflammatory activity was measured using the carrageenan induced paw oedema test in rats(10). Albino rats (100-135 g) were divided into three groups of five rats each. One group was treated orally with 20 mg/kg body weight of aqueous suspension (with 1% NaCMC) of the test compounds. Another group was administered orally 20 mg/kg body weight of aqueous suspension (with 1% NaCMC) of diclofenac sodium (standard) and the third group (control) was fed with the same volume of distilled water. After 30 minutes the animals were injected 0.1 ml of the 1% carrageenan (as NaCMC suspension) in the right hind paw planter apponeurosis. The measurements of the paw volume were made using the mercury displacement technique with the help of a plethysmometer immediately before and 15mins, 30mins, 1hr, 2hrs, 3hrs, 4hrs, 5hrs after the carrageenan injection. The percent inhibition of inflammation was calculated by using the following formula;

$$\text{Percent inhibition } I = 100 \left(1 - \frac{(a-x)}{(b-y)} \right)$$

Where;

x= Mean paw volume of rats before the administration of carrageenan and test compounds or standard compounds

a = Mean paw volume of rats after the administration of carrageenan injection in the control group

y = Mean paw volume of rats before the administration of carrageenan injection in the control group.

b = Mean paw volume of rats after the administration of carrageenan injection in the control group.

The percent inhibition of paw volume of compounds (**I-X**) are shown in Table 5.

Results and Discussion

The results of the antibacterial, analgesic and antiinflammatory activities are presented in Tables 3,4 and 5 respectively.

From the Table 1 it is observed that all the compounds exhibit good antibacterial activity and most of the compounds show comparable antibacterial activity (at 300 μg/ml) when compared to standard ciprofloxacin (at 10 μg/ml) against all the pathogenic microorganisms tested. Compounds **I, IV, VI, VII, VIII, IX, X** show more antibaterial activity (at 300 μg/ml) against *Salmonella typhi*, when compared to ciprofloxacin (at 10 μg/ml). The Mannich bases showed increased antibacterial activity because of the increased lipophilicity.

From the results of the analgesic activity (Table 4), it has been observed that all the compounds synthesized were found to possess analgesic activity and the compounds **III, IV, V** exhibit more analgesic activity than the standard diclofenac sodium at the same dose level while the compounds **VIII** and **IX** exhibited comparable analgesic activity when compared with the standard.

From the screening results of the compounds **I-X** for antiinflammatory activity (Table 5), it has been observed that all the compounds were found to

Table 4. Percent analgesic activity of compounds **I-X**

Compound no	15 min	30 min	1 hr	2 hr	3 hr
I	90.85	197.82	112.83	81.82	42.70
II	71.56	162.53	92.00	62.34	33.12
III	100.81	382.60	196.60	141.98	107.60
IV	92.54	250.33	156.57	131.00	36.32
V	112.55	342.22	181.27	133.74	94.86
VI	39.85	160.60	119.27	98.41	39.31
VII	112.21	206.86	169.54	95.00	56.10
VIII	90.15	239.62	216.00	112.97	62.10
IX	107.33	236.40	166.62	112.32	36.12
X	86.15	181.80	155.90	120.90	25.62
Standard	110.02	275.10	195.34	132.12	44.32

Table 5. Percent inhibition of paw volume of compounds **I-X**

Compound No	15 min	30 min	1 hr	2 hr	3 hr	4 hr	5 hr
I	24.69	61.20	67.93	65.50	60.40	45.12	26.21
II	39.75	65.18	69.93	63.50	83.50	39.51	28.23
III	43.60	67.87	69.87	67.50	50.00	32.22	24.14
IV	25.21	59.01	68.59	69.01	32.10	22.14	19.15
V	26.00	57.12	61.00	35.02	22.52	19.51	15.11
VI	25.00	25.00	50.00	33.44	32.44	26.11	18.84
VII	29.53	59.12	67.20	62.21	45.20	25.21	19.21
VIII	29.10	55.10	65.22	66.12	29.10	17.17	11.19
IX	30.10	61.52	65.22	39.21	22.10	16.19	14.51
X	34.74	55.01	59.93	32.50	35.25	15.12	11.28
Standard	35.13	43.60	59.63	53.50	50.00	39.12	25.20

possess antiinflammatory activity. Compounds **I, II, III, VII** were found to exhibit more antiinflammatory activity when compared to standard diclofenac sodium at the same dose level. However, the rest of the compounds exhibited comparable antiinflammatory activity when compared to standard.

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