Innovations in Pharmaceuticals and Pharmacotherapy

www.innpharmacotherapy.com

eISSN: 2321–323X
pISSN: 2395-0781

Research article

Synthesis, characterization and biological evaluation some novel schiff base and mannich base of isatin and its derivatives with benzimidazole

Faruk Alam*, Biplab. Kr. Dey, Arpita Chakraborty, Mrinmoy Basak
Department of Pharmacy, Assam Down Town University, Panikhaiti, Guwahati, Assam, India

Abstract

Isatin is a synthetically versatile substrate, where it can be used for the synthesis of a large variety of heterocyclic compounds and as a raw material for drug synthesis. In the past few decades, Isatin and its derivatives have received much attention due to their chemotherapeutic values. This review covers updated information on the most active isatin derivatives that have been reported to show considerable pharmacological actions such as antimicrobial, anticancer, antiviral, anticonvulsant, anti-inflammatory and analgesic. From these results, ideas for future molecular modifications leading to compounds with greater favorable pharmacological properties may be derived. Isatin have been reacted with 2-(1H-benzimidazol-2-yl) aniline to form Schiff bases and the N-Mannich bases of the compounds were synthesized by reacting them with formaldehyde and several secondary amines. Investigation of antimicrobial activity of the compounds was made by the agar dilution method. The compounds are significantly active against bacteria and fungi.

Keywords: Schiff-bases, *N-Mannich* bases, Isatin, Antimicrobial activity, Anti-inflammatory activity.

***Corresponding author: Mr. Faruk Alam,** Assistant Professor, Department of Pharmacy, Assam down town University Gandhinagar, Panikhaiti, Dispur, Assam-781026, India. Email: faruk_2007a@rediffmail.com

1. Introduction

Inflammation is a local reaction of the vascular and supporting elements of a tissue to injury resulting in the formation of a protein-rich exudates; it is a protective response of the non spe- cific immune system that serves to localize, neutralize, or to destroy an injurious agent in preparation for the process of healing. The cardinal signs of inflammation are rubor (redness), calor (heat), dolor (pain), tumor (swelling), and functio laesa (loss of function). Cause of inflammation includes physical agents, chemical agents, immunological reactions, and infection by pathogenic organism [1]. Inflammation is divided into acute and chronic patterns. The characteristics of acute inflammation are the exudation of fluid and plasma proteins (oedema) and the emigration of leukocytes, predominantly neutrophils. Chronic inflammation is considered to be inflammation of prolonged duration (weeks or months) in which active inflammation, tissue destruction, and attempts at repair are proceeding simultaneously. Chronic inflammation includes some of the most common and disabling human diseases, such as rheu- matoid arthritis, atherosclerosis, tuberculosis, and chronic lung diseases [2]. Isatin has been known for about 150 years and has been recently found, like oxindole and Isatin has also been found in mammalian tissues, and its function as a modulator of biochemical processes. Endogenous polyfunctional heterocyclic compounds,to exhibit biological activity in mammals. Isatin (1H-indole-2, 3-dione, Fig.1) was first obtained by Erdman and Laurent in 1841 as a product from the oxidation of indigo by nitric and chromic acids. The synthetic versatility of

isatin has led to the extensive use of this compound in organic synthesis. Three reviews have been published regarding the chemistry of this compound: the first by Sumpter, in 1954 [3],a second by Popp in 1975 [4], and the third on the utility of isatin as a precursor for the synthesis of other heterocyclic compounds [5]. Isatin also synthetically versatile substrate that can be used to prepare the large variety of heterocyclic compounds, such as indoles and as a raw material for drug synthesis. It is evident from literature,that isatin derivatives are known to be associated with broad spectrum of biological activity like antimicrobial [6-10], anti-inflammatoryand analgesic [11-13],antioxidant activity [14-15], anticonvulsant[16-17] and anticancer activity [19-20] activities. Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used for the choice treatment in various inflammatory diseases such as arthritis, rheumatisms as well as to relieve the aches and pain of everyday life. Classical NSAIDs exhibit their action by restricting the biosynthesis of prostaglandin, some of which are pro-inflam- matory. This is essentially brought about by inhibiting the rate limiting cyclooxygenase (COX) enzyme involved in the inflamma- tory cascade. Among different types of NSAIDs, imidazole and fused imidazole with six-membered rings [21], occupy central position among those compounds that are used as analgesic and anti-inflammatory agents. Fused imidazole derivatives have occupied a prominent place in medicinal chemistry because of their significant properties as therapeutics in clinical applications [22–24]. Thus, benzimidazole is being explored in the pharmaceutical industries and the substituted benzimidazole derivatives have also been found in the diverse therapeutic applications [25, 26]. Because of the versatile core contained in several substances of benzimidazole derivatives are possess a broad spectrum of pharmacological activities [27–30]. In particular, it has been an important pharmacophore and privi- leged structure in medicinal chemistry [31, 32], encompassing a diverse range of biological activities including antimicrobial [33-35], antioxidant [36], antiviral [37, 38], antihypertensive [39], antiprotozoal [40], anti-inflammatory [41] and molluscicidal [42] agents. Furthermore, benzimidazoles showed anticancer activity against DNA topoisomerase I [43, 44] and colon cancer cell lines [45]. So in the present work the attempted has been made for synthsis of 2-(2-amino phenyl) benzimidazole from mixture of *o*-phenylenediamine and anthranilic acid. It was condensed with isatin and its derivative to form Schiff bases. The *N*-Mannich bases of the above Schiff bases were synthesized by condensing the acidic imino group of isatin with formaldehyde and secondary amines (Scheme). Purity of the compounds was ascertained by the thin layer chromatography (TLC), all compounds gave satisfactory elemental analysis. IR and ^{1}H NMR spectra were consistent with the assigned structures. The synthesized scaffold was screened for antimicrobial activity, anti-Inflammatory activity by the agar dilution and Carrageenan-induced rat paws oedema method. In view of these facts and as a continuation of search of newer antimicrobial as well as anti-inflammatory agents, Schiff and Mannich bases of Isatin and its derivatives with benzimidazole are synthesized in the present work.

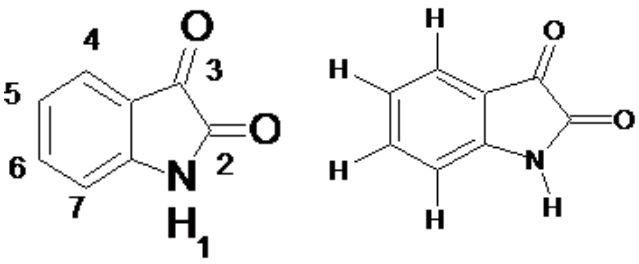


Figure 1**:** Structure of Isatin (IUPAC Name: 1*H*-indole-2, 3 dione

1.1. Structure Activity Relationship:

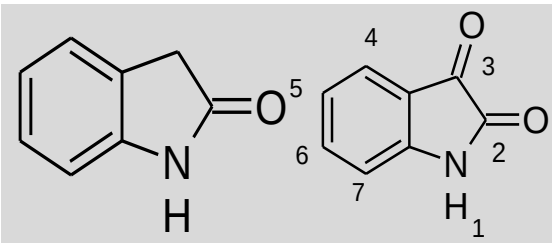


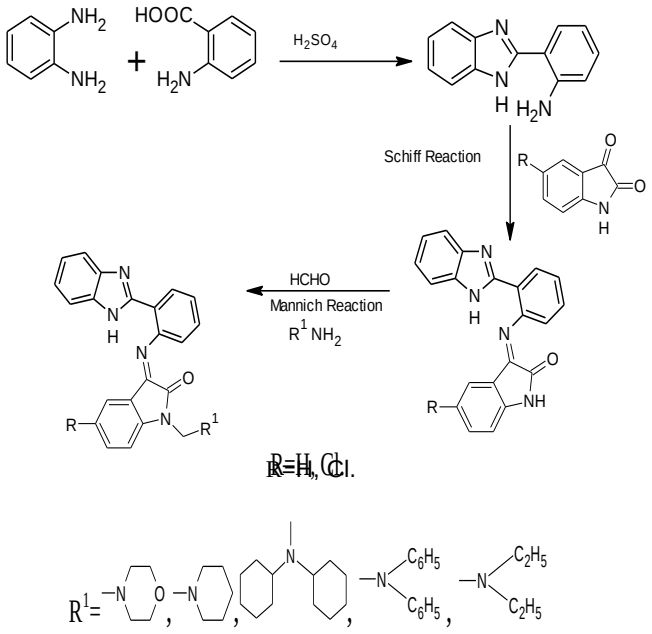
Figure 2**:**2-indolinone (I) and 2, 3-indolindione (II)

i. Bond acceptor at the position (3)
ii. Free rotation bond O-H
iii. Bond donor (1)
iv. Polar surface area-37.38

v**.** C5, C6and C7 substitution generally enhanced CNS activity with some di and tri halogenated isatin (Fig.2).

In the above structure it was found that a little variation at position 3 of 2- indolinone (I) and 2, 3-indolindione (II) produce different degree of biological activity.

Scheme: Synthetic protocol of the compounds



2. Experimental

All the chemicals and reagents were of synthetic grade and commercially procured from S.D. Fine Chem. Ltd. (Mumbai, India). The melting points were determined using open capillary tubes and are uncorrected. Purity of the all synthesized compounds was checked by thin layer chromatography technique (0.2 mm thickness of silica gel G plates) and iodine was used as visualizing agent.IR spectra were recorded on FT-IR8400S, Fourier Transform (Shimadzu) Infrared spectrophotometer using KBr disk method. ^{1}H NMR spectra were recorded on JEOL (JNM-ECS400, 400 MHz) in dimethyl sulfoxide (DMSO-*d6*) using tetramethylsilane (TMS) as an internal standard. The mass spectra were recorded on Shimadzu LC–MS 2010A Mass Spectrometer and CHN elemental analysis was performed on Perkin Elmer Autosystem XL analyzer.

2.1. General procedure for the preparation of 2-(2-amino phenyl) benzimidazole [46]:

Heated together mixture of *o*-phenylenediamine (0.03 mole), 20 mL of water, anthranilic acid (0.09 mole) under reflux for 45 minutes. Made the cooled reaction mixture distinctly basic by the gradual addition of the concentrated ammonia solution, the precipitated product was collected and recrystallized from ethanol gave white crystals, Yield: 86.55%. m.p 248-250 ^{0}C (Found C: 74.62, H: 5.30, N: 20.08: $C_{13}H_{11}N_3$) , Rf value: 0.250, IR: 3370 (N-H stretching), 3080 (aromatic C-H stretching), 1632 (C=N stretching), 1396 (C-N stretching), 3461(NH_2), 894 (Ar-H). ^{1}H NMR: δ 7.0-7.94 (13H, m, Ar-H), 11.8 (1H, s, imidazole ring NH), Mass spectra *m/z*, 209.04$(M)^+$.

2.2. General procedure for the preparation of isatin [47]:

(A) Synthesis of Isonitrosoacetanilide:
In a 500 mL round-bottomed flask are placed (0.1 mole) of chloral hydrate and 220 mL of water. To this solution are then added, in order: 238 g of crystallized sodium sulfate; a solution of (0.1 mole) of aniline/ 4-chloro aniline in 60 mL of water to which of concentrated HCl (8.2 mL, 0.1 mole) has been added to dissolve the amine and finally a solution of (0.31 mole) of hydroxylamine hydrochloride in 99 mL of water. The flask is heated over wire gauze by a burner so that vigorous boiling begins in about forty to forty-five minutes. After one to two minutes of vigorous boiling the reaction is complete. During the heating period, some crystals of Isonitroso-acetanilide separate. On cooling the solution in running water the remainder crystallizes, is filtered with suction, and air-dried. The yield of the crude product was 91% and mp 173-175^{0}C.

(B) Synthesis of Isatin:

About 70 mL of concentrated sulphuric acid is warmed to 50^{0}C in a 250 mL round-bottomed flask fitted with an efficient mechanical stirrer, and, to this, 0.1 mole of dry isonitroso-acetanilide is added at such a rate as to keep the temperature between 60^{0}C and 70 ^{0}C but not higher. External cooling should be applied at this stage so that the reaction can be carried out more rapidly. After the addition of the

substituted Isonitroso-acetanilide, the solution is heated to 80^0C and kept at this temperature for about ten minutes to complete the reaction. Then the reaction mixture is cooled to room temperature and poured upon ten to twelve times its volume of cracked ice. After standing for about one-half hour, the Isatin is filtered with suction, washed several times with cold water to remove the excess acid, and then dried in the air and recrystallized from rectified spirit. The yield of crude product was 78% and mp.188 –190^0C. IR (cm^{-1}): 1620, 1740 (C=O str); 3190(N-H str); 1365 (C-N); 1470, 1595 (C=C aromatic).

2.3. Synthesis of Schiff Bases:

Equimolar quantities (0.01mol) of substituted-isatin and 2-(2-amino phenyl) benzimidazole (0.01mol) were dissolved in 20 mL of dry ethanol. To it was added 1-2 drops of concentrated sulfuric acid and heated at reflux for 2-3 h. After standing for approximately 24 h at room temperature, the end product were separated by filtration, dried and recrystallized from alcohol.

2.4. Synthesis of Mannich Bases:

Equimolar quantity (0.02 mole) of secondary amine was added in to slurry containing the appropriate Schiff base and (37%) formalin (1mL) solution dissolved in 10 mL of DMSO (Dimethyl sulphoxide). The reaction mixture was stirred for 1 h at room temperature and refrigerated for 24 h. The products were separated, dried and purified by recrystallization from chloroform. All the physical and spectral data are shown in table I and II.

3. Pharmacological evaluation:

3.1. *In-vitro* antimicrobial activity [48]:

Evaluation of antibacterial and antifungal activity is done by the agar dilution method. All bacteria were grown on Mueller–Hinton agar (Hi-media) plates (37^0C, 24 h) and fungi were grown on Sabouraud dextrose agar (Hi-media) plates (26^0C, 48–72 h). The synthesized compounds were subjected to antimicrobial screening by cup plate method for zone of inhibition. The antibacterial activity was tested against various gram positive and gram negative bacteria and antifungal activity against various fungal stains compared with standard drug (Amoxycilline and Ketoconazole) using solvent control. Results have been given in Table III.

3.2. Experimental animals:

Adult Swiss albino mice (20–25 g) and albino rats weighing (150–200 g) of either sex were used as experimental animals. All the animals were housed in groups of 4–8 per cage at a temperature of 25 ± 1^0C and a relative humidity of 45–55%. A 12 h dark and 12 h light cycle was followed during the experiments. Animals were allowed free access to food and water *ad libitum*. During the study period, guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Institutional Animals Ethics Committee (IAEC) were followed for the maintenance of animals.

3.2.1. Acute toxicity studies

The acute toxicity studies were carried out in groups of six Swiss albino mice, weighing 20–25 g which were fasted over- night and treated orally with the test compounds. The dosage was varied from 100–1000 mg/kg body weight orally. All the animal experiments were performed with the approval of Institutional Animal Ethics Committee, Himalayan Pharmacy Institute, East-Sikkim, India.

3.2.2. Anti-inflammatory activity by Carrageenan-induced rat paws edema method [49]:

The anti-inflammatory activity of the test compounds was evaluated by carrageenan induced rat paw edema model of Winter et al. (1962). Rats of either sex were treated with benzimidazole derivatives (100 mg/kg p.o.) and standard drug Diclofenac sodium (100 mg/kg p.o.), one hour prior to the 1% w/v solution injection of 0.1 mL carrageenan into the plantar region of left hind paw. The marking was just made beyond the tibia-tarsal junction of (knee joint) left hind paw in each animal of all groups. Paw volume was measured by Plethysmometer (Model 520, IITC, Life sciences, USA) at 0 h, 1 h, 2 h, 3 h and 4 h after carrageenan injection. The difference between the paw volume at 4th h and 0 h measurement was calculated and

taken as edema volume. Percentage inhibition in the paw was calculated by using the formula,
Percentage inhibition = 100 (1-Vt/Vc)
Where,
Vt = mean increase in paw volume of test, and
Vc = mean increase in paw volume with the control.
Results have been given in Table IV.

4. Results and Discussion:

4.1. Screening of anti-microbial activity:

All the synthesized final compounds were first purified by successive recrystallization using appropriate solvents. The purity of the synthesized compounds was checked by performing thin layer chromatography and determining melting points. Then the synthesized compounds were subjected to spectral analysis such as IR, ^{1}HNMR and Mass Spectra to confirm the structures. All the analytical details show satisfactory results (Table II).

All the mass spectral data were shown the molecular ion peaks for their respective molecular weights apart from fragmentation profile. Since our titled compounds are known to possess antimicrobial activity, the compounds were screened for their antibacterial and antifungal activity by cup-plate method. Gram positive bacteria such as *Staphylococcus aureus* (NTCC-6571) and *Bacillus subtilis* (B_2) two gram negative bacteria such as *Escherichia coli* (TG_1)4 and *Streptococcus typhi* and two fungal species such as *Aspergillus niger* and *Candida albicans* are tested for the activities. The concentration of 100 µg/ml of our titled compounds has been used. Amoxicillin and Ketoconazole have been used as standards. DMSO is used as solvent control. The results are summarized in Table III. The tested compounds exhibited mild to moderate antibacterial activity against all four strains of bacteria. The compounds, III, VI, and X tested against *S. aureus*, showed highest activity. The antimicrobial study revealed that substitution in the 5 position of isatin with chlorine, produced more active compounds in a series. It has also been observed that compounds, VI and VII showed activity against *B. subtilis.* The antifungal activity of the compounds was studied for the two pathogenic fungi, *A. niger* and *C. albicans.* It was observed that compounds VI, VII and VIII had highest activity against *A. niger* and VI showed most promising activity against *C. albicans.* Replacing the substituent H in the phenyl group at position 5 of the isatine moiety in compounds III with electron withdrawing groups, e.g., X = Cl as in VI leads to a increased in the antifungal activity. Among the evaluated compounds, VI [3-{[2-(1*H*-benzimidazol-2-yl) phenyl] imino}-5-chloro-1-(morpholinomethyl)-1H-inden-2(3H)-one] was found to be the most active against all bacteria and fungi, as it could inhibit the microbial growth at concentration of 100 µg/mL with zone of inhibition ranging from 5-28 mm. When a comparison is made between the compounds VI,VII and VIII, it appears that compounds VI with morpholino group is more active than the compounds having only piperidino and cyclohexyl substituent at position 1 of the isatin moiety. This was further confirmed by comparing the data for compounds X and VI. Compound X was less active due to the presence of alkyl side chain at position 1 of the isatin moiety. Thus, it is obvious from the structure-activity profile of substituted mannich base derivatives; a small structural variation may induce an effect on antibacterial activity.

4.2. Screening of anti-inflammatory activity:

From the Table IV, it was found that most of the tested compounds showed significant results in comparison with standard. Amongst all the compounds VI, VII, and VIII showed potent anti-inflammatory activity and the rest of the compounds showed moderate activity. The results of tested compounds as well as reference standard were measured before administration the carrageenan. After the administration of carrageenan inflammation was induced in rats, the effect was measured in the intervals of 30, 60, 120, 180 and 240 min. The percent oedema inhibition was calculated as a regard to saline control group, as depicted in Table IV. Most of the tested compounds have shown good results in comparison with standard diclofenac sodium standard drug. Amongst all the compounds, compound VI and VII have shown potent anti-inflammatory activity. From a view of structure–activity relationship (SAR) studies, chloro substituted isatine along with morpholine and piperidine have shown potent anti-inflammatory activity when compared with other substituted isatine

moiety with standard drug diclofenac sodium. This has resulted new path in the synthesis of new class of benzimidazole with isatine derivatives.

4.3. Statistical analysis

In analgesic and anti-inflammatory study, data are expressed as Means±SEM. Differences between vehicle control and treatment groups were tested using one-way ANOVA followed by Turkey– Krammer Multiple comparison test. A probability value less than 0.05 was considered as statistically significant.

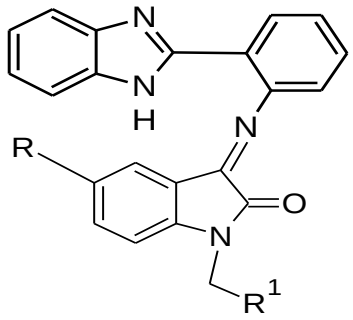


Table 1: Physical data of the synthesized compounds:

Compd	Mol. formula	Mol.Wt.	m.p. ^{0}C	Yield %	Rf	Elemental analysis					
						Calculated			Found		
						C	H	N	C	H	N
I	$C_{26}H_{23}N_5O_2$	437.49	138-140	64	0.78	71.38	5.30	16.01	71.45	5.27	16.07
II	$C_{27}H_{25}N_5O$	435.52	165-167	60	0.88	74.46	5.79	16.08	74.51	5.60	16.12
III	$C_{34}H_{37}N_5O$	531.69	149-152	65	0.84	76.80	7.01	13.17	76.83	7.04	13.20
IV	$C_{29}H_{23}N_5O$	457.52	130-132	66	0.66	76.13	5.07	15.31	76.16	5.06	15.39
V	$C_{25}H_{23}N_5O$	409.48	144-146	69	0.69	73.33	5.66	17.10	73.41	5.33	17.14
VI	$C_{26}H_{22}ClN_5O_2$	471.93	100-102	78	0.81	66.17	4.70	14.84	66.14	3.98	14.91
VII	$C_{27}H_{24}ClN_5O$	469.96	206-207	72	0.59	69.00	5.15	14.90	69.12	5.20	15.00
VIII	$C_{34}H_{36}ClN_5O$	566.13	160-163	73	0.79	72.13	6.41	12.37	72.17	6.31	12.43
IX	$C_{29}H_{22}ClN_5O$	491.97	122-125	70	0.55	70.80	4.51	14.24	70.79	3.92	14.28

Table II: Spectral data of the synthesized compounds

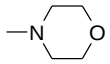
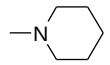
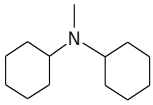
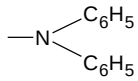
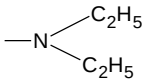
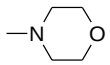
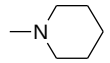
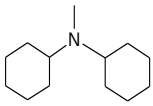
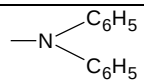
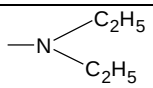
Compd.	R	R1	Spectral data IR(cm-1):1H NMR δ(ppm):m/z
I	H		IR (cm^{-1}): 3042 (C-H Ar); 2918,2828 (CH_2alkane);1700,1716 (C=O);1315 (C-N); 1596 (C=N); 1120 (C-O-C); 1480, 1500 (C=C aromatic). ^{1}H NMR δ (ppm): 7.8-8.5 (m, 4H, benzimidazole ring), 12.60 (s, H, NH), 7.2-7.5 (m, 4H, ArH); 4.28(s,2H,CH_2); 7.50 (m, 4H, indolyl),2.37 (d, 4H,morpholine); 3.67 (d, 4H, morpholine). m/z: $(M)^+$ 437; $(M+1)^+$ 438
II	H		IR (cm^{-1}): 3056 (C-H Ar);2922, 2865 (CH_2alkane);1315 (C-N); 1500 (C=N); 1500,1480(C=C aromatic). ^{1}H NMR δ (ppm): 6.71-8.2(m, 4H, benzimidazole ring),12.48 (s, H, NH), 7.2-7.5 (m, 4H, ArH);4.18(s,2H,CH_2); 7.63 (m, 4H, indolyl),1.5 to 2.37 (m, 10H, piperidine). m/z:-$(M)^+$ 435
III	H		IR (cm^{-1}): 3056 (C-H str. Ar); 2934,2843(CH_2alkane); 1728 (C=O str); 1588 (C=C aromatic); 1353 (C-N str.); 1532 (C=N). ^{1}H NMR δ (ppm): 7.5-8.6 (m, 4H, benzimidazole ring), 12.42 (s, H, NH), 7.1-7.3 (m, 4H, ArH);4.57 (s,2H,CH_2);6.95 (m, 4H, indolyl),1.5 to 2.6 (m, 20H, dicyclohexane); m/z: - $(M)^+$ 531; $(M-1)^+$ 530
IV	H		IR (cm^{-1}): 3032(C-HAr);2927,2854,2837(CH_2alkane);1698 (C=O str); 1509,1458(C=C aromatic); 1296(C-N);1605(C=N);741 (C-Cl). m/z: -,$(M)^+$457
V	H		IR (cm^{-1}): 3028,3005(C-H Ar);2794,2842, 2935 (CH_2alkane); 1487(C=C aromatic),1735 (C=O str); 1306(C-N);1605(C=N);738 (C-Cl). ^{1}H NMR δ (ppm): 7.6-8.1(m, 4H, benzimidazole ring), 12.10 (s, H, NH), 7.3 to 7.6 (m, 4H,Ar-H), 4.79 (s,2H,CH_2)8.12 (s, 1H, indolyl),3.6-3.8(m,10H diethylamine). m/z: -,$(M)^+$ 409;$(M-1)^+$408
VI	Cl		IR (cm^{-1}): 3054 (C-H Ar), 2906, 2848 (CH_2alkane),740 (C-Cl),1311 (C-N), 1109 (C-O-C Six membered ring). ^{1}H NMR δ (ppm) : 6.1-8.6 (m, 4H, benzimidazole ring), 12.45 (s, H, NH), 7.5-7.8 (m, 4H, ArH); 4.83(s,2H,CH2);7.25(m, 3H, indolyl),2.37 (d, 4H, morpholine); 3.67 (d, 4H, morpholine) m/z : $(M+1)^+$ 472
VII	Cl		IR (cm^{-1}): -3051 (C-HAr), 2920, 2859(CH_2alkane), 1316 (C-N),748 (C-Cl) ^{1}H NMR δ (ppm) : 7.9-8.7 (m, 4H, benzimidazole ring), 12.36 (s, H, NH), 4.52 (s,2H,CH2);6.69 to 8.1 (m, 4H,Ar-H); 6.90 (m, 3H, indolyl),1.5 to 2.37 (m, 10H,piperidine)m/z:-$(M)^+$469;$(M+1)^+$ 470
VIII	Cl		IR (cm^{-1}):3056 (C-H Ar), 2934, 2843 (CH_2alkane) 1722 (C=O), 1588 (C=C), 1353(C-N),763 (C-Cl) ^{1}H NMR δ (ppm):7.9-8.5 (m, 4H, benzimidazole ring), 12.35 (s, H, NH), 4.83 (s,2H,CH_2);6.8 to 8.9 (m, Ar-H), 6.55 (m, 4H, indolyl), 1.7 to 2.5 (m, 20H,dicyclohexane). m/z: - $(M)^+$566;$(M+1)^+$567
IX	Cl		IR (cm^{-1}): 3055.7(C-HAr); 2829 (CH_2alkane)1690 (C=O);1605 (C=N), 1422 (C-N);1509,1458(C=C aromatic);747 (C-Cl). m/z: - $(M)^+$491;$(M+1)^+$492
X	Cl		IR(cm^{-1}): 3038(C-HAr), 2948(CH_2alkane), 1615(C=N),1437 (C-N),1712(C=O); 742(C-Cl). m/z: - $(M)^+$ 443;$(M+1)^+$444

Table III. Antimicrobial screening of the synthesize scaffold

Compund code	*Zone of inhibition in (mm)					
	Bacteria				Fungi	
	S. aureus	*E. coli*	*S. typhi*	*B.subtilis*	*A. niger*	*C.albicans.*
I	15	16	08	05	09	07
II	12	15	10	18	05	08
III	20	20	10	18	15	10
IV	15	18	13	14	10	08
V	16	10	11	13	09	07
VI	20	28	09	22	18	21
VII	23	26	08	21	17	17
VIII	15	13	07	10	16	11
IX	15	16	09	11	10	09
X	20	18	10	21	06	09
Amoxycilline	31	39	33	30	NA	NA
Ketoconazole	NA	NA	NA	NA	20	25
DMSO	----------	----------	---------	----------	----------	-------

Table IV. Anti-inflammatory activity of synthesized compounds on carrageenan-induced acute paw edema in rats

Comp. code	Tail withdrawing time in second (Mean±SEM) % Inhibition					
	0 h	1 h	2 h	3 h	4h	4h
Control	0.15± 0.01	0.22 ±0.01	0.23±0.02–	0.25± 0.01	0.25±0.01	------
Std	0.15± 0.01	0.12 ±0.01	0.10±0.01	0.08± 0.01	0.08±0.01	68
I	0.13± 0.01	0.10± 0.01	0.11± 0.01	0.12± 0.01	0.12±0.01	52
II	0.12± 0.01	0.12± 0.01	0.12± 0.01	0.14± 0.01	0.15±0.01	40
III	0.14± 0.01	0.15± 0.01	0.17± 0.01	0.18± 0.01	0.17±0.01	32
IV	0.11± 0.01	0.15± 0.01	0.11± 0.01	0.13± 0.01	0.13±0.01	48
V	0.18± 0.01	0.15± 0.01	0.16± 0.01	0.19± 0.01	0.19±0.01	24
VI	0.12± 0.01	0.12± 0.01	0.13± 0.01	0.13± 0.01	0.10±0.01	60
VII	0.10± 0.01	0.10± 0.01	0.08± 0.01	0.09± 0.01	0.09±0.01	64
VIII	0.13± 0.01	0.12± 0.01	0.12± 0.01	0.11± 0.01	0.11±0.01	56
IX	0.16± 0.01	0.15± 0.01	0.15± 0.01	0.15± 0.01	0.16±0.01	36
X	0.13± 0.01	0.13± 0.01	0.13± 0.01	0.14± 0.01	0.14±0.01	44

n=6 animals in each group.
All synthesized compounds tested at a dose of 100 mg/kg p.o. body weight, Std- diclofenac sodium (3.9 mg/kg i.p)., Control-vehicle (0.5% CMC).
* $p<0.05$ vs control.
** $p<0.01$ vs control.

5. Conclusion

In conclusion, we have described a simple protocol for the synthesis of 3-{[2-(1*H*-benzimidazol-2-yl) phenyl] imino}-5-chloro-1, 3-dihydro-2*H*-indol-2-one and 3-{[2-(1*H*-benzimidazol-2-yl) phenyl] imino}-1, 3-dihydro-2*H*-indol-2-one derivatives with remarkable yields. All the synthesized compounds have been screened for their in-vitro antimicrobial and *in-vivo* anti-inflammatory activities. In the newly synthesized compounds, it is cleared

that the highest antimicrobial activity in compound (VI) against all the bacteria as well as fungi and anti-inflammatory activity in compound (VI) and (VII) were observed. Apart from compound (VII), remaining compounds have shown good analgesic activity almost equal to standard nimesulide drug. rest of the compounds have shown moderate anti-inflammatory activity.

The preliminary in-vivo studies of these compounds evidenced that, the chloro group in the 5th position, morpholino and piperidino group in the 1st position in the isatine moiety enhances the anti-inflammatory activities, which might serve as new templates in the synthesis and development of potent therapeutics.

Therefore, it can be concluded that such compounds exert their pharmacological effects. This has resulted good impact on chemists and biochemists for further investigations in the field of medicinal chemistry for search of antimicrobial and anti-inflammatory agents containing halo and morpholino/ piperidino methyl functional groups. Furthermore, an extensive toxicological study of these derivatives are highly recommended to assess the safety and pharmacological efficacy of the compounds studied.

6. Acknowledgments:

The authors are grateful to the Chancellor, Dr. N. N. Dutta Down town University, Panikhaiti, Guwahati, Assam, 781026, India, for providing the laboratory facilities.

7. References:

[1]. Wilson LM, 2003, Pathophysiology: Clinical Concepts of Disease Processes, in: Price SA, Wilson LM (Eds.), Mosby, *St. Louis, Missouri,* 44–61.

[2]. Collins T, 2001, Robbins Pathologic Basis of Disease, in: Cotran RS, Kumar V, Collins T (Eds.), *Harcourt India Pvt. Ltd.,* New Delhi, 50–88.

[3]. Sumpter WC, 1954, The chemistry of isatin, *Chem. Rev,* 34; 393-407.

[4]. Popp FD, 1975, The chemistry of isatin, *Adv. Heterocycl. Chem,* 18; 1-58.

[5]. Shvekhgeimer MGA, 1996, Synthesis of heterocyclic compounds by the cyclization of isatin and its derivatives (review), Chem. Heterocycl. *Compd. Engl. Transl,* 32; 249.

[6]. Nathani BR, Pandya KS, Jeni MM, and Patel MR, 2011, Synthesis and antimicrobial activity of some new is atins derivatives , *Der Pharma Chemica*, 3(4); 367-372.

[7]. Pandeya SN, Srivastava M, Pathak AK and Nath GJ, 2010, Synthesis of 6-methyl-2-oxo-4-phenyl (substituted)- 1,2,3,4-tetrahydro-pyrimidine-5- carboxylic acid (2-oxo-1,2-dihydro (1,5-sustituted) -indol-3-ylidene)-hydrazide and their biological activity, *Pharm. Res,* 3(9); 2270-2272.

[8]. Prakash CR, Raja1 S, Saravanan G, Kumar DP and Selvam TP, 2011, Synthesis and Evaluation of Antioxidant Activities of Some Novel Isatin derivatives and Analogs, *Asian J. Res. Pharm. Sci,* 1(4); 140-143

[9]. Saini L, Jain A and Bhadoriya U, 2011, Synthesis and anti-microbial activities of some novel schiff bases derivatives of 5-nitro isatin, *Asian J. Pharm. Life Sci,* 1 (3); 232-238.

[10]. Madhu, Priyanka B, Maharaj, Krishnaveni J, Brahmeshwari G, Sarangapani M and Sammaiah GJ, 2011, *A. Pharm. Sci, 1*(1); 20-33.

[11]. Prasad BD, VasanthiR, Kanth BC, Prabhakar D and Mohan MR, 2012, Synthesis, Characterization And Anti-Inflammatory Activity Of Isatin Derivatives, *Int. J. Bio. Pharm Res,* 3 (1); 182-187.

[12]. Panneerselvam P, Reddy RS, Murali K and Kumar NR, 2010, Synthesis, analgesic, anti-inflammatory and antimicrobial activities of some novel Schiff's bases of 5-subsituted Isatin, *Der Pharm. Chem,* 2(1); 28-37.

[13]. Ramachandran S and Uma Maheswari V, 2010, Synthesis, analgesic and ulcerogenic evaluation of some novel schiff and mannich bases of isatin derivatives, *Int. J. Pharm. & Bio Sci,* 2(1); 251-260.

[14]. Prakash, CR, Raja S, Selvam TP, Saravanan G, Karthick V and Kumar PD, 2009, Synthesis and Antimicrobial Activities of Some Novel Schiff Bases of 5-Substituted Isatin Derivatives, *Rasayan Journal of Chemistry,* 2; 960-968.

[15]. Naik N, Kumar HV and Vidyashree PB, 2011, Synthesis and evaluation of antioxidant potential of novel isatin analogues, *J. Pharma Res,* 4(8); 2686-2689.

[16]. Burnelli S, Andreani A, Granaiola M and Leoni A, 2010, New isatin derivatives with antioxidant activity, *Euro. J. Med. Chem*, 45(4); 1374-137.

[17]. Prakash CR, Raja S and Saravanan GI, 2010, J. Pharm & Pharmaceutical Sci, 2(4); 177-181. Ragavendran JV, Sriram D, Patel SK, Reddy IV and Bhathwajan N, 2007, Design and synthesis of anticonvulsants from a combined phthalimide–GABA–anilide

and hydrazone pharmacophore, *Euro. J. Med. Chem,* 42 (2); 146-151.

[18]. Krishnegowda G, Gowda ASP, Tagaram HRS, Carroll KFSO, Irby RB, Sharma AK and Amin S, 2011, Synthesis and biological evaluation of a novel class of isatin analogs as dual inhibitors of tubulin polymerization and Akt pathway, *Bio org &Med. Chem,* 19(20); 6006-6014.

[19]. Matesic L, Locke JM, Vine KL, Ranson M, Bremner JB and Skropeta D, 2011, Synthesis and hydrolytic evaluation of acid-labile imine-linked cytotoxic isatin model systems, *Bio org & Med. Chem*, 19(5); 1771-1778.

[20]. Vicini P, Incerti M, Amoretti L, Ballabeni V, Ognolini M and Barocelli E, 2002, Synthesis and pharmacological properties of benzisothiazole/benzimidazole derivatives with acidic groups" Il Farmaco, 57, 363–367.

[21]. Terioglu N and Gursoy A, 2003, Synthesis and anticancer evaluation of some new hydrazone derivatives of 2,6-dimethylimidazo[2,1-b][1,3,4]thiadiazole-5-carbohydrazide, *Eur. J. Med. Chem*, 38 (7-8); 781–786.

[22]. Andreani A, Granaiola M, Leoni A, Locatelli A, Morigi R, Rambaldi, Linaz G, Bergamini C and Farruggia G, 2005, Potential Antitumor Agents-37. Synthesis and Antitumor Activity of Guanylhydrazones from Imidazo [2,1-b] thiazoles and from the New Heterocyclic System Thiazolo [2',3':2,3] imidazo [4,5-c] quinoline, *J. Med. Chem,* 48(8); 3085–3089.

[23]. Andreani A, Leoni A, Locatelli A, Morigi R, Rambaldi M, Recanatini M and Garaliene V, 2000, Potential antitumor agents. part 29[1]: synthesis and potential coanthracyclinic activity of Imidazo[2,1-b]thiazole guanylhydrazones, *Bioorg. Med. Chem*, 8(9); 2359–2366.

[24]. Sheehan DJ, Hitchcock CA and Sibley CM, 1999, Current and Emerging Azole Antifungal Agents, *Clin. Microbiol. Rev*, 12(1) ; 40–79.

[25]. Lu J, Yang B and Bai Y, 2002, Microwave irradiation synthesis of 2-substituted benzimidazoles using ppa as a catalyst under solvent-free conditions, *Synthetic. Commun,* 32(24); 3703–3709.

[26]. Velyk J , Baliharova V, Fink-Gremmels J, Bull S, Lamka J and Skalova L, 2004, Benzimidazole drugs and modulation of biotransformation enzymes, Res. Veter. Sci, 76(2); 95–108.

[27]. Liu JF, Lee J, Dalton AM, Bi G, Yu L, Baldino CM, McElory E and Brown M, 2005, Microwave-assisted one-pot synthesis of 2,3-disubstituted 3H-quinazolin-4-ones, *Tet. Lett,* 46(8) ; 1241–1244.

[28]. Liu JF, Wilson CJ, Ye P, Sprague K, Sargent K, Si Y, Beletski G , Yohannes D and Ng SC, 2006, Privileged structure-based quinazolinone natural product-templated libraries: Identification of novel tubulin polymerization inhibitors*, Biorg. Med. Chem*. Lett, 16(3) ; 686–690.

[29]. Liu JF, Kaselj M, Isome Y, Ye P, Sargent K, Sprague K, Cherrak D, Wilson CJ, Si Y, Yohannes D and Ng SC, 2006, Design and Synthesis of a Quinazolinone Natural Product-Templated Library with Cytotoxic Activity, J. Comb. Chem, 8(1); 7–10.

[30]. Preston PN, 1980, In the Chemistry of Heterocyclic Compounds, Benzimidazoles and Congeneric Tricyclic Compounds*, John Wiley & Son, New York*, 40.

[31]. Evans BE , Rittle KE, Bock MG , Dipardo RM, Freidinger RM, Whittel WL, Lundell GF, Veber DF, Anderson PS, Chang RSL, Lotti VJ, Cerino DJ, Chen TV, Kling PJ, Kunkel KA, Springer JP and Hirshfield J, 1988, *J. Med. Chem*, 31; 2235–2246.

[32]. Goker H, Kus C, Boykin DW, Yildiz S and Altanlar N, 2002, Synthesis of some new 2-substituted 1H-benzimidazole-5-carbonitrile and their potent activity against candida species, *Bioorg. Med. Chem,* 10; 2589-2596.

[33]. Ozden S, Atabey D, Yildiz S and Goker H, 2005, Synthesis and potent anti microbial acivity of some methyl or ethyl 1H-benzimidazole-5-carboxylate derivatives carrying amide or amidine groups, *Bioorg. Med. Chem*, 13; 1587-1597.

[34]. Nofal ZM, Fahmy HH and Mohamed HS, 2002, Synthesis and antimicrobial activity of new substituted anilinobenzimidazoles, *Arch. Pharm. Res,* 25; 250-257.

[35]. Kus C, Ayhan-Kilcigil G, Eke BC and Iscan M, 2004, Synthesis and antioxidant activities of some novel benzimidazole derivatives on lipid peroxidation on the rat liver*, Arch. Pharm. Res,* 27; 156-163.

[36]. Porcari AR, Devivar RV, Kucera LS, Drach JC and Townsend LB, 1998, Design, synthesis, and antiviral evaluations of 1-(substituted benzyl)-2-substituted-5, 6-dichlorobenzimidazoles as nonnucleoside analogues of 2,5,6- trichloro-1-(beta-Dribofuranosyl)benzimidazole, *J. Med. Chem,* 41; 1252-1262.

[37]. Tewari AK and Mishra A, 2006, Synthesis and antiviral activities of N-substituted-2-substituted-benzimidazole derivatives, *Ind. J. Chem. Sect,* B 45; 489-493.

[38]. Kumar JR, Jawahar JL and Pathak DP, 2006, Synthesis and pharmacological evaluation of benzimidazole derivatives, *Eur. J. Chem,* 3; 278.

[39]. Achar KS, Hosamani KM and Seetharam HR, 2010, *In-vivo* analgesic and anti-inflammatory activities of newly synthesized benzimidazole derivatives, *Eur. J. Med. Chem,* 45 ; 2048-2054.

[40]. Nofal ZM, Fahmy HH and Mohamed HS, 2001, Synthesis, antimicrobial and moluscicidal activities of new benzimidazole derivatives, *Arch. Pharm. Res*, 25; 28-38.

[41]. Selcen AA, Sevil Z, Istvan Z, Gunes C, Borbala R, Semih GH and Zeki T, 2009, Biological activity of bis-benzimidazole derivatives on DNA topoisomerase I and HeLa, MCF7 and A431 cells, *J. Enz. Inhib. Med. Chem,* 24(3); 844-849.

[42]. Alper S, Arpaci OT, Aki ES and Yalcin I, 2003, Some new bi- and ter-benzimidazole derivatives as topoisomerase Inhibitors*, Il Farmaco*, 58; 497-507.

[43]. Abdel-Aziz HA, Tamer S, Saleh TS and El-Zahabi HA, 2010, Facile Synthesis and In-Vitro Antitumor Activity of Some Pyrazolo [3,4-b] pyridines and Pyrazolo [1,5-a] pyrimidines Linked to a Thiazolo[3,2-a] benzimidazole Moiety*, Arch. Pharm. Chem. Life Sci,* 343; 24-30.

[44]. Thompson RL, Price ML and Miaton SA, 1951, Protection of mice against vaccinia virus by administration of benzyldehydethiosem icarbazone, *Proc. Soc. Exptl. Bio. Med*, 84; 496.

[45]. Furniss BS, Hannaford AJ, Smith PWG and Patchel IR ,1996, Vogel's Textbook of practical Organic Chemistry. *Singapore: Pearson Education Pvt. Ltd*.

[46]. Pandey VK, Gupta VD, Upadhyway M, Singh VK and Tandon M, 2005, Synthesis, characterization and biological activity of 1, 3, 4-substituted 2-azetidinones, *Ind. J. Chem***,** 44(B); 158.

[47]. Barry AL, 1999, Antimicrobial susceptibility test, Principle and Practice, *4th edition, ELBS: London*,180.

[48]. Furniss BS, Hannaford AJ, Rogers V, Smith PWG and Tatchell AR, 1989, Vogel's Textbook of Practical Organic Chemistry*, 5th eds. New York.*