International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol. 3, No.2, pp 772-777, April-June 2011

Synthesis and Biological Evaluation of some New 2-(2-Methyl-5-Nitro-1*H*-Imidazol-1-yl)-*N*'-[(3*Z*)-2-Oxo-1, 2-Dihydro-3*H*-Indol-3-ylidene]Acetohydrazide derivatives.

Judy Jays[1]*, Amit Kumar[1], C H S Venketaramana[1], B V Suma[a], V. Madhavan[2]

**[1]Department of Pharmaceutical Chemistry,[2]Department of Pharmacognosy,
M.S. Ramaiah College of Pharmacy ,Bangalore – 560054,India.**

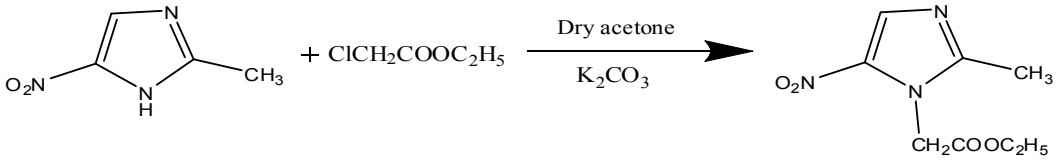
Corres.author: judyjays4@gmail.com
Tel: +9180 2360 8942, Fax: +9180 23603947

Abstract: Research on Isatin(1H-indole-2,3-dione) and their synthetic analogs have revealed that they posses anti-inflammatory and antibacterial activities along with anthelminthic, amoebicidal, antifungal, antifertility, analgesic and sedative activities.
A series of 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-*N*'-[(3*Z*)-2-oxo-1,2-dihydro-3*H*-indol-3- ylidene]acetohydrazide derivatives (3A-3H) have been synthesized and their structures were confirmed by the elemental analysis and spectral data (IR, ^{1}H NMR , MS). These new derivatives were screened for antimicrobial activity and *in vitro* anti-inflammatory activity.
Keywords: Isatin , Imidazole, Antimicrobial activity, *In vitro* anti-inflammatory activity.

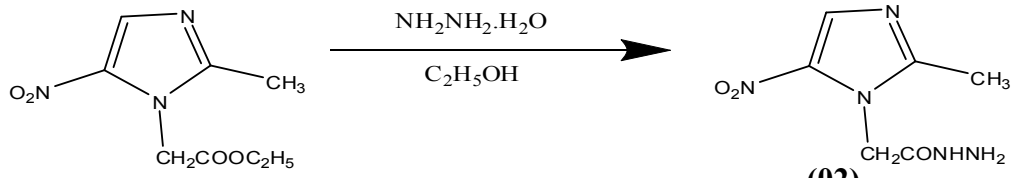
Introduction

Scientific effort for the design and synthesis of novel heterocyclic compounds has been focused continuously because of their wide range of pharmacological utility. Isatin is an endogenous compound identified in humans and their derivatives possess a wide range of biological activities such as antibacterial, anthelmintic, amoebicidal, antifungal, antifertility, anti-HIV, CNS-depressant, analgesic, anti-inflammatory, anxiogenic, sedative and also acts as a potent antagonist on atrial natriuretic peptide receptors *in vitro*.
A series of p-substituted isatin semicarbazones have shown anticonvulsant activity. Various isatin-N-Mannich bases of isatin-3-thiosemicarbazones have shown antiviral activity. Methisazone is an effective compound against variola and vaccinia viruses. Isatins also find use as fibrinolytic, muscle relaxant, antiallergic, immunosuppressant, antithrombotic, hypotensive, respiratory depressent, antidiuertic and showed cardio inhibitory effect on frog's heart.[1,2,3] Isatins have been used as valuable synthetic intermediates in both the pharmaceutical and dye industries for many decades.[4]
In recent years, interest in the synthesis and pharmacological evaluation of numerous Isatin(1H-indole-2,3-dione) has grown as they have shown kinase inhibitory properties against three serine/threonine kinases namely CDK1/cyclin B, CDK5/p25 and GSK3α/β and in vitro antitumor properties against MCF7(breast), NCl-H460(lung) and SF268(CNS) cancer cell lines.[5] In view of these observations, we would like to report synthesis of new Isatin(1H-indole-2,3-dione) derivatives (Scheme-I) as potential antimicrobial and anti-inflammatory agents.

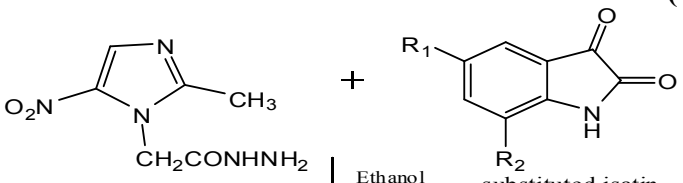
Scheme for Synthetic Methodology:

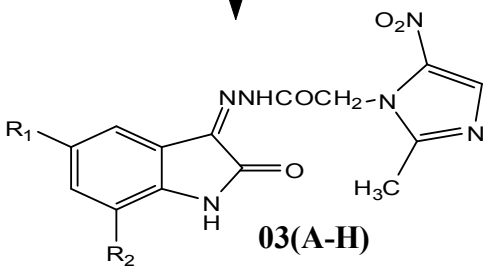


2-methyl-5-nitro-1H-imidazole **(01)**



(02)





03(A-H)

R_1- H, NO_2, Cl, I, CH_3, F, Br
R_2-H, F

S.No.	Compound	R_1	R_2
1	03A	H	H
2	03B	CH_3	H
3	03C	Br	H
4	03D	I	H
5	03E	Cl	H
6	03F	F	H
7	03G	H	F
8	03H	NO_2	H

Experimental

The chemicals and solvents were of reagent grade. Melting points were determined by open capillary method and are uncorrected. The IR spectra were recorded on a Fourier Transform IR spectrometer (8400S, Shimadzu) at M.S. Ramaiah college of pharmacy, Bangalore. 1H NMR spectra were recorded on NMR spectrometer (AMX-400, Bruker) at Indian Institute of Science Bangalore using DMSO and chemical shifts (δ) are reported in parts per million downfield from internal reference Tetramethylsilane (TMS). Elemental analysis reports were provided by Uwin Global Services, Bangalore, which were recorded on elemental analyzer (Flash EA 1112 series Thermo finnigan). Mass spectra were provided by Uwin Global Services, Bangalore, which were

recorded on Mass spectrometer (LCMS-2010 A, Shimadzu).

(a) Procedure for synthesis of (2-methyl-5-nitro-imidazole-1-yl) acetic acid ethyl ester (01): [6]

A solution of 2-methyl-5-nitro-1H-imidazole (12.7g, 0.10mol) in 100ml dry acetone was heated with ethyl chloro acetate (12.3ml, 0.11mol) on a water bath for 3hrs in presence of anhydrous potassium carbonate (7g, 0.10mol). The reaction mixture was cooled and filtered to separate potassium chloride and unreacted potassium carbonate. Acetone was removed under vaccum and the product isolated was recrystallized from methanol: water (7:3).

(2-methyl-5-nitro-imidazole-1-yl) acetic acid ethyl ester (01): white needle shaped crystals; mp. 102-106^{o} C; % yield 85.96 %; Rf 0.34 Chloroform : Ethylacetate :: (9 : 1); IR (KBr) ν (Ar, C-H str) at 2987 cm^{-1}, (alkanes, C-H str) at 2923cm^{-1}, (ester, C=O str) at 1730 cm^{-1}, (nitro, N=O str) at 1539 cm^{-1},1330 cm^{-1}

(b) Procedure for synthesis of (2-methyl-5-nitro-imidazole-1-yl) acetic acid hydrazide (02):[6]

A mixture of compound (01) (2.13g, 0.01mol) and 99% hydrazine hydrate (0.5ml, 0.015mol) in ethanol (20ml) was refluxed for about 3hrs. The reaction mixture was then allowed to cool to room temp. The separated white coloured crystalline solid was filtered, washed with ethanol and recrystallised from ethanol.

(2-methyl-5-nitro-imidazole-1-yl) acetic acid hydrazide (02): white crystalline solid; mp. 189-193^{o}C; % yield 75.75 %; Rf 0.42 Chloroform : Ethylacetate :: (9 : 1); IR (KBr) ν (1^{o} amine, N-H str) at 3240 cm^{-1}, (2^{o} amine, N-H str) at 3336 cm^{-1}, (Ar, C-H str) at 3008 cm^{-1},(alkene C-H str) at 2964 cm^{-1}, (>C=O str of hydrazide) at 1689 cm^{-1}, (nitro, N=O str) at 1539 cm^{-1},1330 cm^{-1}.

(c) General procedure for synthesis of substituted 2-(2-methyl-5-nitro-1*H*-imidazol- 1-yl)-*N*'-[(3*Z*)-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]acetohydrazide [3A-3H].

A mixture of compound (02)(0.01mol) and isatin (0.01) in ethanol (50ml) containing 3-4 drops of glacial acetic acid was refluxed for (3-4)hrs and left over night at room temperature . The solid obtained was dried. The dried crude product was recrystallized with DMF: Water (7:3).

2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-*N*'-[(3*Z*)-2-oxo-1, 2-dihydro-3*H*-indol-3-ylidene] acetohydrazide [3A]:

Yellow powder; mp. 301-303º C; % yield 82.26%; Rf 0.70 Chloroform:Methanol :: (9 : 1);IR (KBr) ν (2^{o} amine, N-H str) at 3130 cm^{-1}, (>C=O str of isatin) at 1718 cm^{-1} , 1689 (>C=O str of hydrazide) at1689 cm^{-1}, (imine, C=N str) at 1622 cm^{-1} (Ar, C-H str) at 3082 cm^{-1}, (alkanes, C-H str) at 2962 cm^{-1}, (nitro, N=O str) at 1552 cm^{-1}, 1330 cm^{-1}, also the absence of (1^{o} amine, N-H str) at 3240 cm^{-1}. 1H NMR (400 MHz, DMSO) δ 2.3 (3H, CH_3), 7.0-7.9 (5H, Ar), 5.5 (2H, CH_2), 11.3 (1H, NH), 12.7 (1H, NH). Anal. Calcd for $C_{14}H_{12}N_6O_4$: C, 51.22; H, 3.68; N, 25.60. Found: C, 51.26; H, 3.72; N, 25.65% .MS (APCI +) *m/z* 328 $(M)^+$.

2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-*N*'-[(3*Z*)-5-methyl-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene] acetohydrazide [3B]:

Yellow powder; mp. 280-282º C; % yield 73.39%; Rf 0.42 Chloroform : Ethylacetate :: (9 : 1);IR (KBr) ν (2^{o} amine, N-H str) at 3137 cm^{-1}, (>C=O str of isatin) at 1706 cm^{-1} , (>C=O str of hydrazide) at 1627 cm^{-1}, (imine, C=N str) at 1622 cm^{-1} (Ar, C-H str) at 3068 cm^{-1}, (alkanes, C-H str) at 2989 cm^{-1}, (nitro, N=O str) at 1533 cm^{-1}, 1325 cm^{-1}. 1H NMR (400 MHz, DMSO) δ 2.1 (3H, CH_3), 2.3 (3H, CH_3), 6.9-8.1 (4H, Ar), 5.6 (2H, CH_2), 12.0 (1H, NH), 12.9 (1H, NH). Anal. Calcd for $C_{15}H_{14}N_6O_4$: C, 52.63; H, 4.12; N, 24.55. Found: C, 52.65; H, 4.16; N, 24.61%. MS (APCI +) *m/z* 342 $(M)^+$.

2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-*N*'-[(3*Z*)-5-bromo-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene] acetohydrazide [3C]:

Yellow powder; mp. 250-253º C; % yield 84.72%; Rf 0.44 Chloroform : Ethylacetate :: (9 : 1);IR (KBr) ν (2^{o} amine, N-H str) at 3139 cm^{-1}, (>C=O str of isatin) at 1685 cm^{-1} , (>C=O str of hydrazide) at 1658 cm^{-1}, (imine, C=N str) at 1620 cm^{-1} (Ar, C-H str) at 3068 cm^{-1}, (alkanes, C-H str) at 2989 cm^{-1}, (nitro, N=O str) at 1537 cm^{-1}, 1328 cm^{-1}, (bromo, C-Br str) at 583 cm^{-1}. 1H NMR (400 MHz, DMSO) δ 2.2 (3H, CH_3), 7.3-8.2 (4H, Ar), 5.6 (2H, CH_2), 12.0 (1H, NH), 12.9 (1H, NH). Anal. Calcd for $C_{14}H_{11}BrN_6O_4$: C, 41.30; H, 2.72; N, 20.64. Found: C, 41.35; H, 2.77; N, 20.70%. MS (APCI +) *m/z* 409 $(M+2)^+$.

2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-*N*'-[(3*Z*)-5-iodo-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene] aceto hydrazide [3D]:

Yellowish brown powder; mp. 137-139º C; % yield 76.37%; Rf 0.33 Chloroform : Ethylacetate :: (9 : 1); IR (KBr) ν (2^{o} amine, N-H str) at 3143 cm^{-1}, (>C=O str of isatin) at 1706 cm^{-1} , (>C=O str of hydrazide) at 1693 cm^{-1}, (imine, C=N str) at 1610 cm^{-1} (Ar, C-H str)

at 3068 cm^{-1}, (alkanes, C-H str) at 2989 cm^{-1}, (nitro, N=O str) at 1550 cm^{-1}, 1326 cm^{-1}, (iodo, C-I str) at 570 cm^{-1}. 1H NMR (400 MHz, DMSO) δ 2.3 (3H, CH_3), 7.2-8.0 (4H, Ar), 5.5 (2H, CH_2), 11.9 (1H, NH), 12.7 (1H, NH). Anal. Calcd for $C_{14}H_{11}IN_6O_4$: C, 37.02; H, 2.44; N, 18.50. Found: C, 37.07; H, 2.51; N, 18.55%. MS (APCI +) *m/z* 454 $(M)^+$.

2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-*N*'-[(3*Z*)-5-chloro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]aceto hydrazide [3E]:

Yellow powder; mp. 263-265º C; % yield 86.74%; Rf 0.80 Chloroform : Methanol :: (9 : 1); IR (KBr) ν (2º amine, N-H str) at 3112 cm^{-1}, (>C=O str of isatin) at 1704 cm^{-1} , (>C=O str of hydrazide) at 1670 cm^{-1}, (imine, C=N str) at 1623 cm^{-1} (Ar, C-H str) at 3043 cm^{-1}, (alkanes, C-H str) at 2956 cm^{-1}, (nitro, N=O str) at 1550 cm^{-1}, 1326 cm^{-1}, (chloro, C-Cl str) at 729 cm^{-1}. 1H NMR (400 MHz, DMSO) δ 2.2 (3H, CH_3), 7.3-8.2 (4H, Ar), 5.6 (2H, CH_2), 12.0 (1H, NH), 12.9 (1H, NH). Anal. Calcd for $C_{14}H_{11}ClN_6O_4$: C, 46.36; H, 3.06; N, 23.17. Found: C, 46.42; H, 3.12; N, 23.20%. MS (APCI +) *m/z* 364 $(M+2)^+$.

2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-*N*'-[(3*Z*)-5-fluoro-2-oxo-1, 2-dihydro-3*H*-indol-3-ylidene] aceto hydrazide [3F]:

Yellow powder; mp. 266-268º C; % yield 74.20%; Rf 0.42 Chloroform : Methanol :: (9 : 1); IR (KBr) ν (2º amine, N-H str) at 3143 cm^{-1}, (>C=O str of isatin) at 1718 cm^{-1} , (>C=O str of hydrazide) at 1687 cm^{-1}, (imine, C=N str) at 1602 cm^{-1} (Ar, C-H str) at 3074 cm^{-1}, (alkanes, C-H str) at 2989 cm^{-1}, (nitro, N=O str) at 1552 cm^{-1}, 1326 cm^{-1}, (fluoro, C-F str) at 1000 cm^{-1}. 1H NMR (400 MHz, DMSO) δ 2.3 (3H, CH_3), 6.9-7.4 (4H, Ar), 5.5 (2H, CH_2), 11.3(1H, NH), 12.7 (1H, NH). Anal. Calcd for $C_{14}H_{11}FN_6O_4$: C, 48.56; H, 3.20; N, 24.27. Found: C, 48.63; H, 3.23; N, 24.32%. MS (APCI +) *m/z* 346 $(M)^+$.

2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-*N*'-[(3*Z*)-7-fluoro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]acetohydrazide [3G]:

Yellow powder; 296-298º C; % yield 68.11%; Rf 0.58 Chloroform: Ethylacetate :: (9 : 1); IR (KBr) ν (2º amine, N-H str) at 3137 cm^{-1}, (>C=O str of isatin) at 1720 cm^{-1} , (>C=O str of hydrazide) at 1689 cm^{-1}, (imine, C=N str) at 1596 cm^{-1} (Ar, C-H str) at 3080 cm^{-1}, (alkanes, C-H str) at 2964 cm^{-1}, (nitro, N=O str) at 1564 cm^{-1}, 1340 cm^{-1}, (fluoro, C-F str) at 1006 cm^{-1}. 1H NMR (400 MHz, DMSO) δ 2.3 (3H, CH_3), 7.1-7.5 (4H, Ar), 5.6 (2H, CH_2), 11.8(1H, NH), 12.7 (1H, NH). Anal. Calcd for $C_{14}H_{11}FN_6O_4$: C, 48.56; H, 3.20; N, 24.27. Found: C, 48.61; H, 3.25; N, 24.30%. MS (APCI +) *m/z* 346 $(M)^+$.

2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)-*N*'-[(3*Z*)-5-nitro-2-oxo-1, 2-dihydro-3*H*-indol-3-ylidene] aceto hydrazide [3H]:

Yellow powder; 273-275º C; % yield 82.52%; Rf 0.40 Chloroform : Methanol :: (9 : 1); IR (KBr) ν (2º amine, N-H str) at 3105 cm^{-1}, (>C=O str of isatin) at 1706 cm^{-1} , (>C=O str of hydrazide) at 1664 cm^{-1}, (imine, C=N str) at 1625 cm^{-1} (Ar, C-H str) at 3072 cm^{-1}, (alkanes, C-H str) at 2937 cm^{-1}, (nitro, N=O str) at 1548 cm^{-1}, 1340 cm^{-1}. 1H NMR (400 MHz, DMSO) δ 2.3 (3H, CH_3), 7.7-8.4 (4H, Ar), 5.6 (2H, CH_2), 11.9(1H, NH), 12.5 (1H, NH). Anal. Calcd for $C_{14}H_{11}N_7O_6$: C, 45.05; H, 2.97; N, 26.27. Found: C, 45.10; H, 2.99; N, 26.32%. MS (APCI +) *m/z* 374 $(M+1)^+$.

d) Antibacterial activity

The antibacterial activity of newly synthesized Isatin derivatives was carried out by agar diffusion method against *Staphylococcus aureus and Bacillus Subtilis (*gram-positive) and *Klebsiella and Proteus Vulgaris (gram-negative)* using **:** Amoxicillin and Ciprofloxacin as standard reference drugs. The results are presented in Table-IA.

All compounds have shown antibacterial activity against the gram-positive and gram-negative bacteria tested.
The order of the antibacterial activity for the synthesized compounds is as follows.

a) Against *Staphylococcus aureus*
3C (22mm) > 3F (20mm) > 3B, 3E (17mm) > 3G (16mm) > 3A, 3D, 3H (15mm).

b) Against *Bacillus Subtilis*
3C (20mm) > 3A, 3E, 3H (19mm) > 3F (18mm) > 3D,3G (17mm) > 3B (16mm).

c) Against *Klebsiella*
3G (25mm) > 3D (24mm) > 3C, 3E (23mm) > 3A (22mm) > 3H,3B,3F (20mm).

d) Against *Proteus Vulgaris*
3G (29mm) > 3B,3H (24mm) > 3C,3F (23mm) > 3D (22mm) > 3A,3E (20mm).

e) Antifungal activity
The antifungal activity was evaluated against *Aspergillus niger* and *Candida Albicans* by agar

Table-I (A): Results of Antimicrobial activity

Compounds	R^1	R^2	Antibacterial activity Zone of Inhibition (mm)				Antifungal activity Zone of Inhibition (mm)	
			S.aureus	*B.Subtilis (Gram +ve)*	*Klebsiella (Gram -ve)*	*Proteus Vulgaris (Gram -ve)*	*Aspergillus niger*	*Candida Albicans*
3A	H	H	15	19	22	20	12	11
3B	CH_3	H	17	16	20	24	17	15
3C	Br	H	22	20	23	23	14	09
3D	I	H	15	17	24	22	17	06
3E	Cl	H	17	19	23	20	20	18
3F	F	H	20	18	20	23	12	12
3G	H	F	16	17	25	29	10	12
3H	NO_2	H	15	19	20	24	17	20
Ciprofloxacin			35	41	34	35	-	-
Amoxycillin			40	38	32	38	-	-
Fluconazole			-	-	-	-	30	28
Amphotericin B			-	-	-	-	25	24
Control (DMF)			NI	NI	NI	NI	NI	NI

NOTE: - Average zone diameter of triplicates in mm., NI : - No inhibition

diffusion method. The standards used are Fluconazole and Amphotericin B. The results are presented in Table-IA.

All compounds have shown **antifungal activity** and the order of activity is as follows.

a)* Against *Aspergillus niger

3E (20mm) > 3B,3D,3H (17mm) > 3C (14mm) > 3A,3F (12mm) > 3G (10mm).

b)* Against *Candida Albicans

3H (20mm) > 3E (18mm) > 3B (15mm) > 3F,3G (12mm) > 3A (11mm) > 3C (9mm) > 3D(6mm).

f) *in vitro* Anti-inflammatory activity

The *in vitro* anti-inflammatory activity was performed by adopting the inhibition of bovine serum albumin denaturation method. The standard used was ibuprofen.

The results of *in vitro* anti-inflammatory screening (Table-IB) revealed that all the eight compounds have exhibited significant inhibition of albumin denaturation when compared with standard ibuprofen. The order of potency of the newly synthesized compounds in terms of their ability to denature serum albumin is as follows.

[3C] > [3D] > [3G] > [3E] > [3A] > [3B] > [3H] > [3F].

Table-I (B): Results of *In vitro* Anti-inflammatory activity

Inhibition of Bovine Serum Albumin Denaturation by compounds (3A-3H)

S. No	Compound	R_1	R_2	Conc. (mg/ml)	Blank	0.2	0.4	0.6	0.8	1.0
1	3A	H	H		0	20.4	32.7	43.2	43.8	53.6
2	3B	CH_3	H	Inhibition of denaturation (%)	0	16.4	20.3	25.5	40.7	47.5
3	3C	Br	H		0	23.2	36.7	49.2	56.8	70.1
4	3D	I	H		0	21.3	33.2	43.8	47.6	63.2
5	3E	Cl	H		0	20.2	37.4	37.0	42.7	55.7
6	3F	F	H		0	12.4	27.2	26.4	35.2	47.2
7	3G	H	F		0	18.7	29.3	32.9	50.3	62.4
8	3H	NO_2	H		0	19.6	35.2	45.2	53.7	57.1
9	Ibuprofen (std)				0	24.0	42.0	55.3	65.8	83.0

Results and Discussion

The objective of the present work was to synthesize, purify, characterize and evaluate the antimicrobial and *in vitro* anti-inflammatory activity of the newly synthesized Isatin derivatives.

The yield of the products ranged from 75-86 %. The purity was checked by TLC and Elemental analysis. The structures of the newly synthesized compounds [3A-3H] are characterized and confirmed by spectral data viz. IR, 1H NMR and Mass spectra and all the synthesized compounds [3A-3H] were screened for antimicrobial and *in vitro* anti-inflammatory activity. Some of these derivatives have shown reasonable antimicrobial activity. The *in vito* anti-inflammatory activity of the bromo-derivative[3C] was comparable with the standard.

Acknowledgement

The authors thank Gokula Education Foundation (Medical) for providing the facilities.

References

1 Rastogi N, Kant P, Harrison DA, Sethi R, Tripathi D., Synthesis of 1-aminomethly-5-substituted-3-{4'(2"chlorobenzyloxy)benzoylhydrazono}indoline -2-ones as antifungal agents, .Indian J. Heterocycl. Chem., 2009, Jan-Mar, 18, 263-266.

2 Rastogi N, Harisson DA, Tripathi D, Shukla S., Synthesis and antimicrobial potential of mannich bases of 4-chloro-3-{4-(chlorobenzyloxy)benzoylhydrazono}indoline-2-ones, J. Indian Chem. Soc., 2009, Sept, 86, 991-995.

3 Pandeya SN, Smitha S, Jyoti M, Sridhar SK., Biological activities of isatin and it's derivatives, Acta Pharm., 2005, 55, 27-46.

4 Hewawasam P, Meanwell A., A general method for the synthesis of isatins:preparation of regiospecifically funtionalized isatins from anilines. Tetrahedron Lett., 1994, 35(40), 7303-7306.

5 Abadi AH et al., Synthesis of 3-substituted-2-oxoindole analogues and their evaluation as kinase inhibitors, anticancer and antiangiogenic agents, Eur. J. Med. Chem., 2006, 41, 296-305.

6 Havaldar FH, Patil AR., .Synthesis of biologically active 1-[2-(2-methly-5-nitroimidazole-1-yl)acetyl]-3-substituted phenyl-4-carbaldehyde-1H-pyrazoles. Asian J. Chem., 2008, 20(1), 97-101.

7 Sammaiah G, Sarangapani M., Synthesis and biological activity of phenyl amino acetic acid (2-oxo-1,2-dihydroindol-3-ylidene)hydrazides Asian J. Chem., 2008,20(1), 75-80.

*****