Asian Journal of Chemistry Vol. 20, No. 3 (2008), 1665-1669

Synthesis and *in vitro* Cytotoxic Activity of Isatin Derivatives

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The objective of the study was to synthesize a novel series of 3-substituted isatin derivative by reacting substituted isatin with amino derivatives of lamotrigine. The Schiff base is further condensed with secondary amine and formaldehyde (mannich reaction). N-acetyl derivatives of isatin were prepared using acetic anhydride. The structure of synthesized compounds was elucidated by spectral analysis. The synthesized compounds were studied for its *in vitro* cytotoxic potential against two cancerous cell lines *viz.*, HEp-2 and DLA cells by standard procedures. All the compounds exhibited cytotoxic properties and compound **5c** exhibited comparatively more cytotoxic activity. The results obtained from the present study show the compound are cytotoxic in nature and may posses antitumor activity.

Key Words: Isatin derivatives, *in vitro* Cytotoxicity, HEp-2, Dalton's lymphoma ascites.

INTRODUCTION

(1*H*-Indole-2,3-dione) is found in plant of *Genus isatis* and in human as a metabolic derivative of adrenaline. 3-Substituted isatin derivatives have been evaluated for a wide spectrum biological activity such as anticonvulsant[1], antibacterial[2], antiviral[3] and antituberculor[4]. The objective of the study was to synthesize a novel series of 3-substituted isatin derivatives.

EXPERIMENTAL

Schiff bases have been synthesized by isatin/5-bromo isatin/5-chloro isatin with amino derivatives of lamotrigine. The Schiff bases further condensed with secondary amine and formaldehyde (mannich base[5]). N-acetyl derivatives of isatin were prepared by using acetic anhydride[6] (**Scheme-I**).

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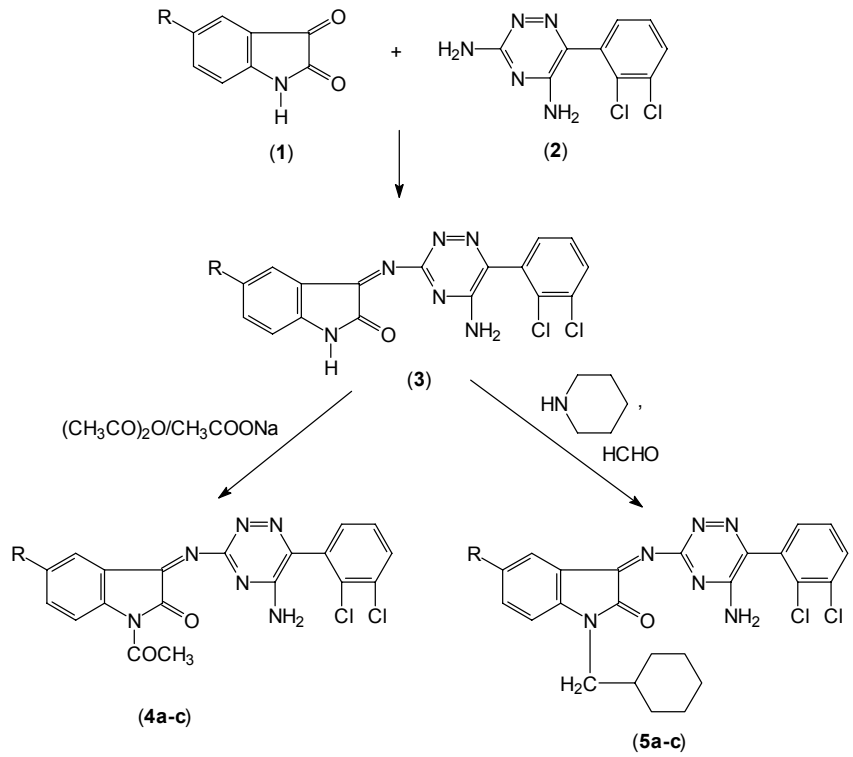


Scheme-I

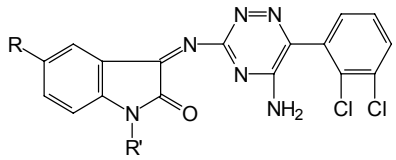
Melting points were determined by using Thomas melting point apparatus and are uncorrected. The purity was checked by TLC by using silica gel-G as stationary phase. The structure of synthesized compounds were elucidated by using Perkin Elmer FT-IR in KBr disc and PMR was taken on a Bruker AMX-(400 MHz) FT-NMR using DMSO-d_6 as a solvent and TMS as an internal standard.

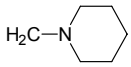
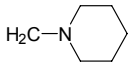
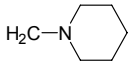
General procedure for the preparation of 3-[5-amino-6-(2,3 dichlorophenyl)-[1,2,4] triazin-3yl] imino 5-substituted isatin (3): An equimolar (0.01 mol) mixture of 5-substituted isatin and lamotrigine was refluxed for 4 h in glacial acetic acid and the mixture was cooled to room temperature and poured into crushed ice. The solid obtained was recrystallized from ethanol. m.p. 223 ºC, yield 76 %; IR (KBr, ν_{max}, cm^{-1}): 3245 (NH), 1708 (C=O), 1430 (N=N), PMR (DMSO-d_6): δ ppm, 7.05 (s,H, C_4-H), 8.46 (m, 1H, C_5-H), 7.80 (m, 1H, C_6-H), 9.05 (m, 1H, C_7-H), 7.12-7.62 (m, 8H, Ar-H).

1-Piperidinomethyl/1-acetyl 3-[5-amino-6-(2,3 dichlorophenyl)-[1,2,4] triazin-3yl]imino-5-substituted isatin (4,5): Compound 3 (0.01 mol), cyclic secondary amine (0.01 mol), formaldehyde (0.03 mol) were refluxed for 2 h in the presence of ethanol, after completion of reaction the

mixture was poured into crushed ice. The precipitated solid was filtered and recrystallized from methanol. Similarly 1-acetyl derivatives were prepared by using acetic anhydride in presence of sodium acetate and the characterization data indicated in Table-1.

TABLE-1



Compd.	R	R^1	m.p. (°C)	Yield (%)
4a	H	$COCH_3$	194	78
4b	Cl	$COCH_3$	225	72
4c	Br	$COCH_3$	206	62
5a	H		232	76
5b	Cl		245	71
5c	Br		214	79

4a: IR (KBr, ν_{max}, cm^{-1}): 3300 (NH), 1674 (C=O), 1583 (C=C), 1510 (C=N), 1438 (N=N); PMR (DMSO-d_6) δ ppm: 1.3 (s, 3H, $-CH_3$), 7.08 (s, H, C_4-H), 8.02 (m, 1H, C_5-H), 7.84 (m, 1H, C_6-H). 9.12 (m, 1H, C_7-H), 7.16-7.74 (m, Ar-H).

4b: IR (KBr, ν_{max}, cm^{-1}): 3311 (NH), 1678 (C=O), 1581 (C=C), 1509 (C=N), 1442 (N=N); PMR (DMSO-d_6) δ ppm: 1.2 (t, 3H, $-CH_3$), 4.5 (d, 2H, $-NH_2$), 7 (t, 1H, C_4-H),7.6 (t, 1H, C_6-H), 8.6 (d, 1H, C_7-H), 6.96-7.69 (m,Ar-H).

4c: IR (KBr, ν_{max}, cm^{-1}): 3297 (NH), 1665 (C=O), 1607 (C=C), 1508 (C=N), 1432 (N=N); PMR (DMSO-d_6) δ ppm, 4.5 (d, 2H, $-NH_2$), 1.6 (t,3H, $-CH_3$), 7.0 (t, 1H, C_4-H), 7.6 (t, 1H, C_6-H), 8.1 (d, 1H, C_7-H), 7.0-7.4 (m, Ar-H).

5a: IR (KBr, ν_{max}, cm^{-1}): 3216 (NH), 1698 (C=O), 1556 (C=N), 1440 (N=N), PMR (DMSO-d_6) δ ppm, 2.54 (m, 4H, $-CH_2$-N--CH_2-), 4.49 (s, 2H, -N-CH_2-N-), 7.05 (s, 1H, C_4-H), 8.50 (m, 1H, C_5-H), 7.82 (m, 1H, C_6-H), 9.0 (m, 1H, C_7-H), 7.12-7.65 (m,Ar-H).

5b: IR (KBr, ν_{max}, cm^{-1}): 3245 (NH), 1672 (C=O), 1508 (C=N), 1442 (N=N), PMR (DMSO-d_6) δ ppm, 2.59 (t, 4H, $-CH_2$-N-CH_2-), 4.39 (s, 2H, -

N-CH_2-N-), 8.09 (s, 1H, C_4-H), 7.80 (m, -NH, C_6-H), 9.10 (m, 1H, C7-H), 6.96-7.60 (m, Ar-H).

5c: IR (KBr, ν_{max}, cm^{-1}): 3222 (NH), 1541 (C=N), 1655 (C=O), 1443 (N=N), PMR (DMSO-d_6) δ ppm, 2.48 (t, 4H, -CH_2-N-CH_2-), 4.50 (s, 2H, -N-CH_2-N-), 7.98 (s, 1H, C_4-H), 8.13 (m, 1H, C_6-H), 9.05 (m, 1H, C_7-H), 7.01-7.67 (m, Ar-H).

***in vitro* Cytotoxic activity[7]:** Each synthesized compounds were separately dissolved in distilled dimethyl sulphoxide and the volume was made up to 10 mL with Dulbecco's Modified Eagle's medium (DMEM), pH 7.4, supplemented with 2 % inactivated new born calf serum (Maintenance medium, PAA Laboratories, Austria), to obtain a stock solution of 1 mg/mL concentration, sterilized by filtration and stored at -20 °C till use.

Hep-2 (Caucasian male larynx epithelium carcinoma) cell line was obtained from the Pasteur Institute of India, Coonoor, India. Dalton's lymphoma ascites (DLA) cells were obtained from JSS College of Pharmacy were propagated in DMEM, pH 7.4 supplemented with 10 % inactivated newborn calf serum, penicillin (100 IU/mL), streptomycin (100 µg/mL) and amphotericin B (5 µg/mL) and maintained in a humidified atmosphere of 5 % CO_2 at 37 °C until confluent. The cells were dissociated with 0.2 % trypsin, 0.02 % EDTA in phosphate buffer saline solution (TPVG). The stock was grown in 25 cm^2 tissue culture flasks and all cytotoxicity experiments were carried out in 96 well micro titre plates. DLA cells used were propagated and maintained in the peritoneal cavity of Swiss albino rats.

Cell lines in exponential growth phase were washed, trypsinized and resuspended DMEM medium with 10 % inactivated newborn calf serum. Cells were plated at 10000 cells/well in 96 well microtitre plate and incubated for 24 h at 37 °C, 5 % CO_2 in a humidified atmosphere during which period a partial monolayer was formed. The cells were then exposed to different concentrations (1000-15.6 µg/mL) prepared by serial twofold dilution using maintenance medium from the stock solution) of the test extracts in quadruplicate. Control wells received only maintenance medium, the cells were incubated at 37 °C in a humidified incubator with 5 % CO_2 for a period of 72 h. Morphological changes of cell cultures were examined using an inverted tissue culture microscope at 24 h time intervals and compared with the control. At the end of 72 h, cellular viability was determined using standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and sulphorhodamine B (SRB) assays[8]. The CTC50 value (concentration of the sample required to kill 50 % of the cells) was calculated (Table-2).

RESULTS AND DISCUSSION

Of the six synthesized compounds, the **5c** exhibited comparatively more cytotoxic activity against all the two cell lines tested, the Hep-2 was found to be more susceptible with a CTC50 value of 49.2-56.5 mg/mL of solution. The other five compounds showed less activity as indicated by the relatively high CTC50 value.

The results obtained from the present study show that the bromo substituted isatin derivative is cytotoxic in nature and may posses antitumor activity.

TABLE-2
CYTOTOXIC ACTIVITY OF ISATIN DERIVATIVES ON DIFFERENT CELL LINES BY MTT AND SRB ASSAYS

Compd.	CTC50 (μg/mL)			
	HEp-2		DLA	
	MTT	SRB	MTT	SRB
4a	494.17 ± 14.63	446.27 ± 11.42	494.17 ± 14.63	450.56 ± 2.67
4b	360.32 ± 13.18	212.56 ± 5.49	360.32 ± 13.18	436.18 ± 8.85
4c	244.63 ± 4.98	452.35 ± 13.67	244.63 ± 4.98	436.87 ± 8.48
5a	244.44 ± 4.91	452.41 ± 14.21	244.44 ± 4.91	> 500
5b	446.91 ± 14.13	> 500	446.91 ± 14.13	> 500
5c	49.2 ± 56.5	121.14 ± 6.42	127.58 ± 2.85	221.18 ± 3.94

Average of three independent determination, 4 replicates, values are ± SEM

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(*Received*: 27 March 2006; *Accepted*: 15 October 2007) AJC-6026