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Synthesis and Evaluation of Some New Isatin Hydrazones for Their Antibacterial, Antioxidant and Cytotoxic Activities

J. VENKATESHWAR RAO* and M. SARANGAPANI†
Talla Padmavathi College of Pharmacy, Urus, Kareemabad, Warangal-506 009, India
Tel: (91)(870)2434057; E-mail: drsarangapani@yahoo.co.in

Twenty new isatin-3-[N^2-(N,N-dialkylamino/diphenylamino/heterylamino)acetyl]hydrazones **IV(a-t)** were synthesized from five different isatin-3-[N^2-(chloroacetyl)]hydrazones **III(a-e)** by reacting with dimethyl amine, diethylamine, N-methylpiperazine, morpholine and diphenylamine in presence of anhydrous potassium carbonate and dry acetone. The intermediates were obtained from isatin hydrazones on condensation with chloroacetyl chloride. The title compounds were characterized by their spectral data and evaluated for *in vitro* antibacterial, antioxidant and cytotoxic activities. Some of the compounds showed promising biological activity against *B. subtilis* and *S. aureus*.

Key Words: Isatin, Antibacterial, Cytotoxic, Antioxidant.

INTRODUCTION

Isatin derivatives are known to exhibit varied biological and pharmacological properties *viz.*, antimicrobial[1], antiviral[2], antineoplastic[3], analgesic[4] and CNS activities[5,6]. An interesting observation one could make from a careful survey of the literature is absence of any report on isatin derivatives containing different secondary amines in the side chain at third position. So, it has been felt worthwhile to undertake the present work of synthesizing such compounds for the first time by appropriate synthetic routes, with a view to evaluate for antibacterial, antioxidant and cytotoxic activities.

For this purpose, the required isatins (**I**) and their hydrazones (**II**) were prepared by the standard methods available in literature[7]. Isatin hydrazones on treatment with chloroacetyl chloride afforded their respective isatin-3-[N^2-(chloroacetyl)]hydrazones (**III**). Each of these compounds was reacted with different secondary amines in presence of dry acetone with an anhydrous potassium carbonate to yield the title compounds (**IV**), which were purified by recrystallization and characterized by analytical and spectral data.

†Medicinal Chemistry Laboratory, University College of Pharmaceutical Sciences, Kakatiya University, Warangal-506 009, India.

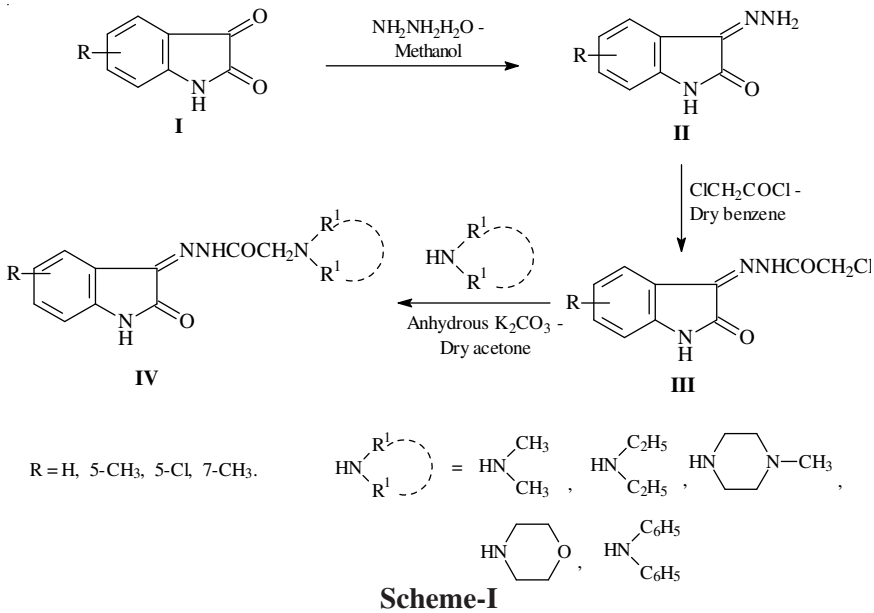
EXPERIMENTAL

Melting points of the compounds were determined in open glass capillaries using Toshniwal melting point apparatus and are uncorrected. The purity of the compounds was ascertained by TLC on silica gel-G (Merck) plates. The IR spectra were recorded in KBr using Perkin-Elmer spectrophotometer. ^{1}H NMR spectra was recorded on a Bruker Spectrospin 400 MHz instrument using TMS as an internal standard. Mass spectra were recorded by the direct inlet-method on Tandom-Mass-Quantam API 400 H mass spectrophotometer.

Synthesis of isatin-3-[N^2-(chloroacetyl)]hydrazones (III): An appropriate isatin hydrazone (**II**, 0.01 mol) was heated under reflux with chloroacetyl chloride (0.01 mol) in dry benzene under anhydrous condition using calcium chloride guard tube for 2 h. The product thus formed was filtered and washed with small portions of benzene and dried. It was purified by recrystallization from acetone.

IR spectrum of the compound (**IIIa**, R-H) exhibited characteristic absorption frequencies (cm^{-1}) at : 3238 ν(NH), 1700 ν(C=O, lactam), 1666 ν(C=O, acid hydrazide) 1624 ν(C=C, aromatic) and 1533 ν(C=N). Its PMR spectrum (DMSO-d_6) showed characteristic signals (δ ppm) at: 4.91 (s, 2H, $COCH_2$), 6.9-7.8 (m, 4H, Ar-H), 9.82 (s, 1H, CONH), 11.95 (s, 1H, lactam). The mass spectrum showed a molecular ion peak at: m/z 237 (45 %) and a base peak at m/z 160.

Synthesis of isatin-3-[N^2-(N,N-dialkylamino/diphenylamino/heteryl amino)acetyl]hydrazones (IV): A mixture of an appropriate isatin-3-[N^2-chloroacetyl)]hydrazone (**III**, 0.01 mol) and N,N-dialkylamine/diphenylamine (or) heterylamine (0.01 mol) in dry acetone (20 mL) and anhydrous potassium carbonate, heated under reflux on water bath for 4 h. The solvent was evaporated and the residue was poured into crushed ice. The product thus formed was filtered, washed with cold water and dried. The compound was purified by recrystallization from suitable solvents and characterized by spectral data. For example, the product obtained on condensation of isatin-3-[N^2-(chloroacetyl)]hydrazone (**III**, R = H) with dimethyl amine ($R^1 = CH_3$) and anhydrous potassium carbonate in dry acetone was yellow crystalline solid, m.p. 201 ºC. It was characterized as isatin-3-[N^2-(dimethylamino)acetyl] hydrazone **IV(a)** (**Scheme-I**). Its IR spectrum (cm^{-1}) at 3158 ν(NH), 1700 ν(C=O, lactam), 1678 ν(C=O, acid hydride) and 1618 ν(C=N). Its PMR spectrum (DMSO-d_6) showed characteristic signals (δ ppm) at 13.73 (s, 1H, lactam), 10.9 (s, 1H, NH), 6.92-7.5 (m, 4H, Ar-H), 3.23 (s, 2H, CH_2), 2.50 (s, 6H, $(CH_3)_2$). The mass spectrum showed a molecular ion peak at m/z 246 and a base peak at m/z 245. The physical data of the synthesized compounds is presented in Table-1.



Scheme-I

TABLE-1
PHYSICAL DATA OF ISATIN-3-[N^2-(N,N-DIALKYLAMINO/ DIPHENYLAMINO/HETERYLAMINO)ACETYL]HYDRAZONES (**IV**)

Compd.	R	R'	m.p. (ºC)	Yield (%)	m.f.
IVa	H	$-N(CH_3)_2$	201	72	$C_{12}H_{14}N_4O_2$
IVb	H	$-N(C_2H_5)_2$	175	65	$C_{14}H_{18}N_4O_2$
IVc	H	N-methylpiperazino	221	82	$C_{15}H_{15}N_5O_2$
IVd	H	Morpholino	208	75	$C_{14}H_{12}N_4O_3$
IVe	H	$-N(C_6H_5)_2$	210	85	$C_{22}H_{18}N_4O_2$
IVf	5-CH_3	$-N(CH_3)_2$	176	75	$C_{13}H_{16}N_4O_2$
IVg	5-CH_3	$-N(C_2H_5)_2$	152	70	$C_{15}H_{20}N_4O_2$
IVh	5-CH_3	N-methylpiperazino	165	85	$C_{16}H_{17}N_5O_2$
IVi	5-CH_3	Morpholino	155	70	$C_{15}H_{14}N_4O_3$
IVj	5-CH_3	$-N(C_6H_5)_2$	198	90	$C_{23}H_{20}N_4O_2$
IVk	5-Cl	$-N(CH_3)_2$	149	85	$C_{12}H_{13}N_4O_2Cl$
IVl	5-Cl	$-N(C_2H_5)_2$	188	75	$C_{14}H_{17}N_4O_2Cl$
IVm	5-Cl	N-methylpiperazino	212	85	$C_{15}H_{14}N_5O_2Cl$
IVn	5-Cl	Morpholino	189	70	$C_{14}H_{11}N_4O_3Cl$
IVo	5-Cl	$-N(C_6H_5)_2$	228	85	$C_{22}H_{17}N_4O_2Cl$
IVp	7-CH_3	$-N(CH_3)_2$	188	76	$C_{13}H_{16}N_4O_2$
IVq	7-CH_3	$-N(C_2H_5)_2$	154	70	$C_{15}H_{20}N_4O_2$
IVr	7-CH_3	N-methylpiperazino	175	80	$C_{16}H_{17}N_5O_2$
IVs	7-CH_3	Morpholino	145	72	$C_{15}H_{14}N_4O_3$
IVt	7-CH_3	$-N(C_6H_5)_2$	238	80	$C_{23}H_{20}N_4O_2$

TABLE-2
ANTIBACTERIAL, ANTIOXIDANT AND CYTOTOXIC ACTIVITY OF ISATIN-3-[N^2-(N,N-DIALKYLAMINO/ DIPHENYLAMINO/HETERYLAMINO)ACETYL]HYDRAZONES (IV)

Compd.	R	R'	Antibacterial activity Zone of inhibition (mm)*				Antioxidant activity IC_{50} value (µm)	Cytotoxicity HBL-100 cell lines IC_{50} values (µm)	Cytotoxicity HeLa cell lines IC_{50} values (µm)
			B. subtilis	*S. aureus*	*E. coli*	*P. vulgaris*			
IVa	H	$-N(CH_3)_2$					26.72	NA	NA
IVb	H	$-N(C_2H_5)_2$	12	10	–	–	25.49	NA	NA
IVc	H	N-methylpiperazino	14	12	10	–	24.67	510.3	NA
IVd	H	Morpholino	10	10	8	–	24.55	NA	469.15
IVe	H	$-N(C_6H_5)_2$	11	8	–	–	26.97	NA	NA
IVf	5-CH_3	$-N(CH_3)_2$	10	–	–	–	27.93	NA	NA
IVg	5-CH_3	$-N(C_2H_5)_2$	15	12	11	–	27.43	NA	NA
IVh	5-CH_3	N-methylpiperazino	12	–	–	–	26.97	497.35	NA
IVi	5-CH_3	Morpholino	9	–	–	–	25.42	NA	NA
IVj	5-CH_3	$-N(C_6H_5)_2$	–	–	–	–	27.84	NA	NA
IVk	5-Cl	$-N(CH_3)_2$	12	10	12	–	23.12	382.46	364.17
IVl	5-Cl	$-N(C_2H_5)_2$	11	9	10	–	21.39	395.83	379.45
IVm	5-Cl	N-methylpiperazino	12	10	12	9	22.03	415.39	406.32
IVn	5-Cl	Morpholino	10	–	–	–	21.43	399.05	375.26
IVo	5-Cl	$-N(C_6H_5)_2$	–	–	–	–	22.15	NA	NA
IVp	7-CH_3	$-N(CH_3)_2$	10	–	–	–	25.46	NA	NA
IVq	7-CH_3	$-N(C_2H_5)_2$	12	10	–	–	24.79	494.79	NA
IVr	7-CH_3	N-methylpiperazino	11	–	–	–	24.32	NA	NA
IVs	7-CH_3	Morpholino	12	10	–	–	25.06	480.39	488.13
IVt	7-CH_3	$-N(C_6H_5)_2$	–	–	–	–	26.72	NA	NA
Ampicillin			22	20	18	17			
Ascorbic acid							5.87		
Cisplatin								25.00	29.00

*Concentration of the test compound 100 µg/cup.

Antibacterial activity: Antibacterial activity of the compounds was assayed against *Bacillus subtilis, Sataphylococcus aureus* (gram-positive), *Escherichia coli* and *Proteus vulgaris* (gram-negative) by the cup-plate method[8] using ampicillin as standard drug and results are presented in Table-2. However, the compounds exhibited a marginal antibacterial activity against *B. subtilis* and *S. aureus*. Few of the compounds showed mild activity against *E. coli* where as only one compound **IVm** was mildly active against *P. vulgaris*. The compound **IVg** with a 5-CH_3 substituent in indolinone and a terminal diethyl group showed relatively better antibacterial profile against *B. subtilis* and *S. aureus*.

Antioxidant activity: Antioxidant activity was carried out by using 1,1-diphenyl-2-picrylhydrazil (DPPH) method[9]. Ascorbic acid was used as reference compound (IC_{50} = 5.87 μM). All the compounds exhibited antioxidant activity in the range of 21.39 μM to 27.93 μM. Compound **IVl** with a 5-chlorosubstituent in indolinone and a terminal diethylamino group showed relatively better antioxidant activity with an IC_{50} value of 21.39 μM among the twenty compounds tested in this series. This was closely followed by the compound **IVn** with 5-chloro substituent in indolinone and terminal morpholine group (IC_{50} 21.43 μM).

Cytotoxic activity: Cytotoxic activity of the test compounds was determined by 3-(4,5-dimethylthiazol-2,5-diphenyl)tetrazoliumbromide (MTT) method[10]. *cis*-Platin was used as reference compound. Eight compounds exhibited cytotoxic activity at a concentration range with an IC_{50} value of 382.46 to 510.3 μM against HBL-100 cell lines and six compounds of this series exhibited cytotoxic activity at a concentration range of 364.17 to 488.13 mM against HeLa cell line. Compound **IVk** was found to be superior in cytotoxic activity among this series of compounds.

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