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Synthesis of New Isatin-3-[N^2-(3-Arylideneamino-4-hydroxybenzoyl)]hydrazones as Antioxidant and Cytotoxic Agents

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In an effort to establish twenty five new isatin-3-[N^2-(3-arylideneaimino-4-hydroxybenzoyl)]hydrazones **(4a-y)**, isatin-3-[N^2-(3-amino-4-hydroxybenzoyl)]hydrazones **(3a-e)** were condensed with five different aromatic aldehydes *viz.*, benzaldehyde, 4-chlorobenzaldehyde, salicylaldehyde, anisaldehyde and veratraldehyde. The compounds, isatin-3-[N^2-(3-amino-4-hydroxybenzoyl)]hydrazones **(3a-e)** were prepared from 3-amino-4-hydroxy benzoic acid hydrazide (**2**) by reacting with different isatins *viz.*, isatin, 5 chloroisatin, 5-methylisatin, 5-bromoisatin and 5-flouroisatin in presence of ethanol. The compounds **(4a-y)** were screened for their *in vitro* antioxidant activity by 1,1-diphenyl-2-picrylhydrazil (DPPH) method and the cytotoxic activity by 3-(4,5-dimethyl thiazol-2,5-diphenyl)-tetrazolium bromide (MTT) assay method using HBL-100 cell lines and HeLa cell lines. All the 25 compounds showed antioxidant activity with IC_{50} values in the range of 7.38 to 12.47 μM. Most significant of them was found to be the compound **4r** (R = Br, R' = OH) showed highest percentage of free radical scavenging activity at 7.38 μM. Compound **4q** (R = Br, R'' = Cl) was found to be relatively more cytotoxic agent against HeLa cell lines and HBL-100 cell lines with IC_{50} values of 192.31 and 198.18 μM, respectively.

Key Words: Isatin, Cytotoxic, Antioxidant.

INTRODUCTION

Isatin (1*H*-indole-2,3-dione) is found in plant of genus isatis and in humans as a metabolic derivative of adrenaline. Over the years, the molecules with isatin have shown diversified biological activities, *e.g.* antimicrobial, antiviral, anticonvulsant, antihistaminic and antitubercular[1-6]. In present investigation, involving reactions of isatins with a view to synthesize some biologically

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active compounds, it has been felt worthwhile to study the condensation reaction of isatin-3-[N^2-(3-amino-4-hydroxybenzoyl)]hydrazones with different arylaldehydes and to evaluate the title products for antioxidant and cytotoxic activities by the DPPH and MTT assay methods.

EXPERIMENTAL

All melting points were determined in open-glass capillaries on a Toshniwall melting point apparatus and are uncorrected. The purity of compounds was ascertained by TLC on silica gel-G plates (Merck). The IR spectra were recorded using KBr (cm^{-1}) discs on a Perkin-Elmer infrared spectrophotometer. ^{1}H NMR Spectra were recorded on a Bruker spectrospin 400 MHz spectrophotometer using TMS as an internal standard. MS of the compounds were recorded by the direct inlet method on Tandam-Mass-Quantam AP1400H mass spectrophotometer.

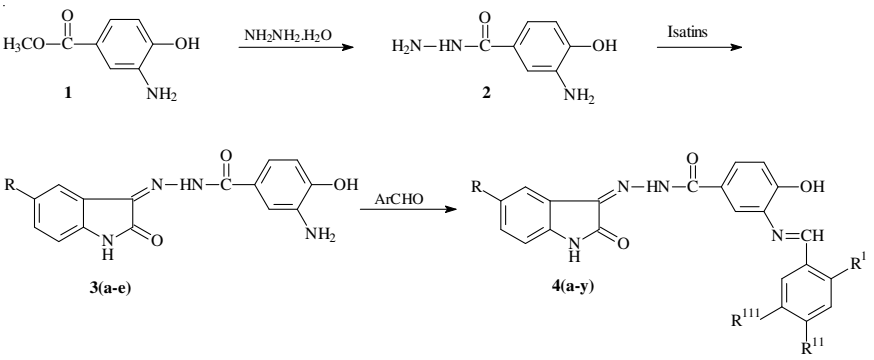
Isatin-3-[N^2-(3-amino-4-hydroxybenzoyl)]hydrazones (3a-e): An appropriate isatin (0.01 mol) was heated under reflux in alcohol (50 mL) with 3-amino-4-hydroxy benzoic acid hydrazide (2, 0.01 mol) for 2 h and the product thus separated was filtered and purified by recrystallization from suitable solvents. **3a** (R = H), yield 85 % and m.p. 278 ºC. IR (KBr, ν_{max}, cm^{-1}): 3650 (OH, phenolic) 3390 (NH_2), 3349 (NH), 1683 (C=O, lactam), 1654 (C=O, acid hydrazide) and 1559 (C=N). ^{1}H NMR spectrum in DMSO-d_6/TMS/400 MHz (δ ppm). 4.87 (s, 2H, NH_2), 6.74-7.56 (m, 7H, Ar-H), 9.8 (s, 1H, CONH), 11.3 (s, 1H, NH, lactam) and 13.77 (s, 1H, OH phenolic). Its MS exhibited molecular ion (M^+) peak at m/z 296 and base peak at 136. **3b** (R = Cl), yield 90 % and m.p. 296 ºC. IR (KBr, ν_{max}, cm^{-1}): 3664 (OH, phenolic), 3385 (NH_2), 3324 (NH), 1680 (C=O, lactam), 1645 (C=O, acid hydrazide) and 1590 (C=N). ^{1}H NMR spectrum in DMSO-d_6/TMS/400 MHz (δ ppm) 4.96 (s, 2H, NH_2), 6.20-7.43 (m, 7H, Ar-H), 10.2 (s, 1H, CONH), 11.25 (s, 1H, NH, lactam) and 13.64 (s, 1H, OH, phenolic). Its MS exhibited molecular ion (M^+) peak at m/z 330 and base peak at 152.

Isatin-3-[(N^2-(3-arylideneamino-4-hydroxybenzoyl)]hydrazones (4a-y): A mixture of **3a-e** (0.01 mol) and an appropriate aromatic aldehyde (0.01 mol) in alcohol (20 mL) containing 2 or 3 drops of glacial acetic acid was heated under reflux on a water bath for 3 h. The solvent was removed to the possible extent by distillation under reduced pressure. The product thus obtained was filtered, washed with water and purified by recrystallization from suitable solvents. For example, the product obtained on condensation of **3a** with benzaldehyde (R^1 = R^{11} = R^{111} = H) and few drops of glacial acetic acid in ethanol afforded a yellow crystalline solid, yield 80 %, m.p. 240 ºC. The compound obtained was characterized by physical and spectral data. Its IR spectrum (KBr) showed characteristic bands (cm^{-1}) at

3450 ν(OH, phenolic) 3239 ν(NH), 1670 ν(C=O, lactam), 1623 ν(C=O, acid hydrazide) and 1596 ν(C=N). ^{1}H NMR spectrum (DMSO-d_6) showed characteristic signals (δ ppm) at 6.96-8.77 (m, 12H, Ar-H), 8.77 (s, 1H, N=CH), 10.15 (s, 1H, NHCO), 11.37 (s, 1H, NH, lactam) and 13.92 (s, 1H, OH, phenolic). Its MS exhibited molecular ion (M^+) peak at m/z 384 and basepeak at 383. **4b** (R^1 and R^{111} = H, R^{11} = Cl), yield 85 %, mp 270 ºC. IR (KBr, ν_{max}, cm^{-1}), 3510 (OH, phenolic), 3244 (NH), 1680 (C=O, lactam), 1649 (C=O, acid hydrazide) and 1585 (C=N). ^{1}H NMR spectrum (DMSO-d_6, in δ ppm): 6.45-8.46 (m, 11H, Ar-H), 8.8 (s, 1H, N=CH), 10.24 (s, 1H, NHCO), 11.29 (s, 1H, NH, lactam) and 13.89 (s, 1H, OH, phenolic). Its MS exhibited molecular ion (M^+) peak at m/z 418 and base peak at 417.

RESULTS AND DISCUSSION

The reaction sequence used in the synthesis of the target compounds **4a-y** is depicted in **Scheme-I**. The compound **2** was obtained by reacting methyl-3-amino-4-hydroxybenzoate (**1**) with hydrazine hydrate (99 %). Different isatins were synthesized by the literature methods[7] and were refluxed with 3-amino-4-hydroxybenzoic acid hydrazide (**2**) in presence of ethanol for 2 h to yieldisatin-3-[N^2-(3-amino-4-hydroxybenzoyl)]hydrazones (**3a-e**).



4a, R = R^1 = R^{11} = R^{111} = H
4b, R = R^1 = R^{111} = H, R^{11} = Cl
4c, R = R^{11} = R^{111} = H, R^1 = OH
4d, R = R^1 = R^{111} = H, R^{11} = OCH_3
4e, R = R^1 = H, R^{11} = R^{111} = OCH_3
4f, R = Cl, R^1 = R^{11} = R^{111} = H
4g, R = R^{11} = Cl, R^1 = R^{111} = H
4h, R = Cl, R^1 = OH, R^{11} = R^{111} = H
4i, R = Cl, R^1 = R^{111} = H, R^{11} = OCH_3
4j, R = Cl, R^1 = H, R^{11} = R^{111} = OCH_3
4k, R = CH_3, R^1 = R^{11} = R^{111} = H
4l, R = CH_3, R^1 = R^{111} = H, R^{11} = Cl
4m, R = CH_3, R^1 = OH, R^{11} = R^{111} = H
4n, R = CH_3, R^1 = R^{111} = H, R^{11} = OCH_3
4o, R = CH_3, R^1 = H, R^{11} = R^{111} = OCH_3
4p, R = Br, R^1 = R^{11} = R^{111} = H
4q, R = Br, R^1 = R^{111} = H, R^{11} = Cl
4r, R = Br, R^1 = OH, R^{11} = R^{111} = H
4s, R = Br, R^1 = R^{111} = H, R^{11} = OCH_3
4t, R = Br, R^1 = H, R^{11} = R^{111} = OCH_3
4u, R = F, R^1 = R^{11} = R^{111} = H
4v, R = F, R^1 = R^{111} = H, R^{11} = Cl
4w, R = F, R^1 = OH, R11 = R^{111} = H
4x, R = F, R^1 = R^{111} = H, R^{11} = OCH_3
4y, R = F, R^1 = H, R^{11} = R^{111} = OCH_3

Scheme-I

These compounds were condensed with different aromatic aldehydes *viz.*, benzaldehyde, 4-chlorobenzaldehyde, salicyladehyde, anisaldehyde and veratraldehyde in alcohol containing a trace of glacial acetic acid to afford isatin-3-[N^2-(3-arylidieneamino-4-hydroxybenzoyl)]hydrazones (**4a-y**) in good yields and purity. All the newly synthesized compounds were characterized by physical (Table-1) and spectral (IR, PMR and Mass) data.

TABLE-1
PHYSICAL DATA OF ISATIN-3-[N^2-(3-ARYLIDENEAMINO-4-HYDROXYBENZOYL)]HYDRAZONES (**4**)

Compd.	R	R^1	R^{11}	R^{111}	m.f.	m.p. (ºC)
4a	H	H	H	H	$C_{22}H_{16}N_4O_3$	240
4b	H	H	Cl	H	$C_{22}H_{15}N_4O_3Cl$	270
4c	H	OH	H	H	$C_{22}H_{16}N_4O_4$	275
4d	H	H	OCH_3	H	$C_{23}H_{18}N_4O_4$	262
4e	H	H	OCH_3	OCH_3	$C_{24}H_{20}N_4O_5$	290
4f	Cl	H	H	H	$C_{22}H_{15}N_4O_3Cl$	272
4g	Cl	H	Cl	H	$C_{22}H_{14}N_4O_3Cl_2$	>300
4h	Cl	OH	H	H	$C_{22}H_{15}N_4O_4Cl$	295
4i	Cl	H	OCH_3	H	$C_{23}H_{17}N_4O_4Cl$	282
4j	Cl	H	OCH_3	OCH_3	$C_{24}H_{19}N_4O_5Cl$	299
4k	CH_3	H	H	H	$C_{23}H_{18}N_4O_3$	284
4l	CH_3	H	Cl	H	$C_{23}H_{17}N_4O_3Cl$	287
4m	CH_3	OH	H	H	$C_{23}H_{18}N_4O_4$	>300
4n	CH_3	H	OCH_3	H	$C_{24}H_{20}N_4O_4$	295
4o	CH_3	H	OCH_3	OCH_3	$C_{25}H_{22}N_4O_5$	296
4p	Br	H	H	H	$C_{22}H_{15}N_4O_3Br$	282
4q	Br	H	Cl	H	$C_{22}H_{14}N_4O_3BrCl$	297
4r	Br	OH	H	H	$C_{22}H_{15}N_4O_4Br$	294
4s	Br	H	OCH_3	H	$C_{23}H_{17}N_4O_4Br$	244
4t	Br	H	OCH_3	OCH_3	$C_{24}H_{19}N_4O_5Br$	295
4u	F	H	H	H	$C_{22}H_{15}N_4O_3F$	242
4v	F	H	Cl	H	$C_{22}H_{14}N_4O_3ClF$	253
4w	F	OH	H	H	$C_{22}H_{15}N_4O_4F$	>300
4x	F	H	OCH_3	H	$C_{23}H_{17}N_4O_4F$	235
4y	F	H	OCH_3	OCH_3	$C_{24}H_{19}N_4O_5F$	245

Antioxidant and cytotoxic assays: Antioxidant activity was carried out by using DPPH[8] method, which is based on the decrease in absorbance of DPPH radical due to the donation of hydrogen by the test compound. It is visually noticeable as a discolouration from purple to yellow colour. 0.2 mL of DPPH (0.05 mM) solution was added to 2.8 mL of test compounds in a test tube wrapped with aluminium foil and its absorbance was read at 517 nm using UV-Visible double beam spectrophotometer. The results were

plotted on a graph and IC_{50} values of the test compounds were determined. Ascorbic acid was used as reference compound (IC_{50} 5.87 μM). Cytotoxic activity of the test compounds was determined by MTT method[9]. The principle of MTT assay is based on the metabolic reduction of MTT to water insoluble formazan crystals with mitochondrial dehydrogenase enzyme which gives direct correlation of viable cells. The confluent monolayers of HBL-100 cell lines and HeLa cell lines were exposed to different concentrations of the test compounds by adding it to 96 well plates. The plates were incubated with 5 % CO_2 at 37º C for 72 h. During the incubation period, viable cells convert MTT to water insoluble purple formazan dye which is quantified with ELISA reader at 562 nm. Cisplatin was used as reference compound and results are presented in Table-2.

TABLE-2
PHARMACOLOGICAL DATA OF ISATIN-3-[N^2-(3-ARYLIDENEAMINO-4-HYDROXYBENZOYL)]HYDRAZONES (**4**) BY DPPH AND MTT METHOD

Compd.	R	R^1	R^{11}	R^{111}	Antioxidant activity IC_{50} value (μM)	Cytotoxic activity	
						HBL-100 cell lines IC_{50} value (μM)	HeLa cell lines IC_{50} value (μM)
4a	H	H	H	H	12.47	NA	291.63
4b	H	H	Cl	H	9.48	261.96	266.53
4c	H	OH	H	H	7.90	288.10	287.70
4d	H	H	OCH_3	H	11.30	247.60	236.62
4e	H	H	OCH_3	OCH_3	9.71	273.64	261.39
4f	Cl	H	H	H	8.48	245.09	258.31
4g	Cl	H	Cl	H	8.26	204.73	217.74
4h	Cl	OH	H	H	8.91	229.35	233.19
4i	Cl	H	OCH_3	H	8.73	238.22	240.26
4j	Cl	H	OCH_3	OCH_3	8.84	241.43	243.84
4k	CH_3	H	H	H	8.93	NA	307.84
4l	CH_3	H	Cl	H	8.86	251.29	240.55
4m	CH_3	OH	H	H	8.98	313.17	305.65
4n	CH_3	H	OCH_3	H	9.12	307.38	301.89
4o	CH_3	H	OCH_3	OCH_3	9.31	NA	290.95
4p	Br	H	H	H	8.92	252.37	255.81
4q	Br	H	Cl	H	7.72	198.18	192.31
4r	Br	OH	H	H	7.38	220.50	214.06
4s	Br	H	OCH_3	H	7.59	212.08	213.90
4t	Br	H	OCH_3	OCH_3	7.64	203.15	209.22
4u	F	H	H	H	9.98	NA	NA
4v	F	H	Cl	H	9.49	NA	293.17
4w	F	OH	H	H	10.28	NA	NA
4x	F	H	OCH_3	H	10.21	NA	NA
4y	F	H	OCH_3	OCH_3	10.53	NA	NA
Ascorbic acid					5.87		
Cisplatin						25.00	29.00

NA = Not active

All the 25 compounds showed very good antioxidant activity with IC_{50} values in the range of 7.83 to 12.47 μM. Most significant of them was found to be compound **4r** (R = Br, R' = OH) showed highest percentage of free radical scavenging activity with IC_{50} values of 7.38 μM. But, however, it is interesting to note that a few of this series of compounds **4s**, **4t**, **4q** and **4c** showed relatively high percentage of inhibition with IC_{50} value of 7.59, 7.64, 7.72 and 7.90 μM, respectively and rest of the compounds showed moderate percentage of free radical scavenging activity.

Cytotoxic activity data of isatin-3-[N^2-(3-arylideneamino-4-hydroxybenzoyl)]hydrazones (**4a-y**) indicates that all the compounds except compounds **4k**, **4o**, **4u-4y** showed a noticeable degree of cytotoxic activity against HBL-100 cell lines where as the compounds **4a-t** and **4r**, showed activity against HeLa cell lines. But however, amongst them, compound **4q** was found to be relatively more effective against HeLa cell lines and HBL-100 cell lines with IC_{50} values of 192.31 and 198.18 μM, respectively. This was followed by compound **4t** with significant cytotoxic activity against HBL-100 cell lines and HeLa cell lines with IC_{50} values of 203.15 and 209.22 μM. It is interesting to note that a few of compounds of this series **4g**, **4h**, **4r** and **4s** showed relatively good cytotoxic activity and rest of the compounds showed moderate cytotoxic activity against HBL-100 cell lines and HeLa cell lines. But, none of the new compounds was comparable in cytotoxicity with that of the standard cisplatin employed.

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