Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 4, 2013

Research Article

SYNTHESIS AND EVALUATION OF NEW BIS-ISATIN DERIVATIVES FOR ANTIOXIDANT ACTIVITY

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Received: 08 Jul 2013, Revised and Accepted: 15 Aug 2013

ABSTRACT

The objective of the present work is to synthesize a new series of *N*'1, *N'2*-bis [2-oxoindolin-3-ylidene] phthalohydrazides) for antioxidant activity. All the compounds were screened by DPPH method. The results of this study revealed that all synthesized compounds significantly scavenged DPPH free radicals in a concentration dependent manner. The IC50 values of all test compounds were found to be between 41.22 and 73.36 mM. All the synthesized compounds have shown moderate antioxidant activity.

Keywords: Bis-isatin, antioxidant, DPPH, *N*'1, *N'2*-bis [2-oxoindolin-3-ylidene] phthalohydrazides)

INTRODUCTION

It is interesting to note from the literature that the various derivatives with isatin moiety have got CNS activities like anticonvulsant, antipsychotic and other activities like antioxidant, anticancer, antihypertensive etc. In continuation of such investigations and in a search for less toxic pharmacologically and more potential derivatives, we have taken up the synthesis and pharmacological evaluation of some new isatin derivatives.Melting points of all synthesized compounds were determined by open capillary tubes using Toshniwal & Cintex melting point apparatus. Expressed in 0C and are uncorrected. The IR spectra KBr pellets) were recorded on Elmer Spectrum BX-1 spectrometer for the compounds.1H NMR spectra were recorded for compounds on AV 300MHz NMR Spectrometer, using TMS as an internal standard. The Mass spectra were recorded on LCQ ion Mass spectrometer. The purity of the compounds were checked by Thin Layer Chromatography TLC) on Merck Silica gel 60 F254 pre coated sheet using Petroleum Ether and Ethyl acetate in 1:1 %v/v.

Chemistry

Synthesis of Dimethyl phthalate II)

Phthalic acid was refluxed in methanol for about 2hours. Few drops of conc.H2SO4 were added to progress the reaction. Dimethyl phthalate was formed and the reaction is known as esterification. As esterification was a reversible reaction, methanol was taken in more quantity approximately double the quantity of phthalic acid). All the chemicals used were of AR grade Sigma-Aldrich, Hi-media).

Synthesis of phthalohydrazide III)

To the compound II, hydrazine hydrate was added in 1:5 ratios and was refluxed for about 2 hours in methanol as a solvent. The solvent was evaporated, the product thus obtained was filtered and washed with water and dried.

Synthesis of Indole-2,3-diones Isatins)

a) Isonitrosoacetanilide – General Procedure

In a 5 lit. R.B. flask were placed chloral hydrate 0.54 mol) and 1200 ml of water. To this solution, were then added crystallized sodium sulphate 1300gm) followed by a solution of an appropriate aromatic amine in 300ml of water and concentrated hydrochloric acid 0.52mol). Finally, a solution of hydroxylamine HCl 1.58 mol) in 500 ml of water was added. The contents of the flask were heated over a wire-gauge by a Mecker burner so that vigorous boiling begins in about 45 minutes. After 1 to 2 minutes of vigorous boiling the reaction was completed. During the heating period itself the crystals of isonitrosoacetanilide started separating out. On cooling under the current of water, the entire product was solidified. It was filtered under suction, air dried and purified by recrystallization from suitable solvents).

b) Indole-2,3-diones – General Procedure

Sulphuric acid 600g, d:1.84, 326 ml) was warmed at 50oC in a one litre RB flask fitted with an efficient mechanical stirrer and to this, finely powdered appropriate isonitrosoacetanilide 0.46 mol) was added at such a rate so as to maintain the temperature between 60oC to 70oC but not higher. External cooling was applied at this stage so that the reaction could be carried out more rapidly. After the addition of isonitroso compound was completed the temperature of the solution was raised to 80oC and maintained at that temperature for 10 minutes to complete the reaction. Then the reaction mixture was cooled to room temperature and poured onto crushed ice 2.5 kg) while stirring. After standing for about half-an-hour, the product separated was filtered, washed several times with small portions of cold water and dried. Purification of the compound was effected by the recrystallization from methanol. Various derivatives of Indole-2, 3-diones were prepared by using different aromatic amines and were confirmed by TLC.

Synthesis of *N*'1, *N*'2-bis [2-oxoindolin-3-ylidene] phthalohydrazides IV)

Each of the isatin has been condensed with the compound III in methanol and traces of glacial acetic acid for about 3-4 hours to get their respective *N*'1, *N*'2-bis [2-oxoindolin-3-ylidene] phthalohydrazides IV). Compound IV was characterized by physical data, TLC, melting point, IR spectra, Mass and NMR spectra.

Ten compounds have been prepared adopting the above method, and the physical data is presented in Table-1.

Spectral data

Compound IVa (R=H): **IR (cm^{-1})** NH (stret) 3256.03, NH (bend) 1560.06,

–C=O (stret) 1685.21,–C=O (stret) 1729.09, –C=N (stret) 1654.33, N-N (stret) 1023.41. **^{1}H NMR (400MHz, DMSO):** ∂ **[ppm]:** Aromatic protons (12) 7.02-7.69(m), Amide NH(s) 10.00, Cyclic amide NH(s) 14.00. **Mass** Spectrum ([M+1]$^+$ 453, M$^+$ 452.

Compound IVb (R=7-Cl): **IR (cm^{-1})** NH (stret) 3266.13, NH (bend) 1566.26,

–C=O (stret) 1695.11, –C=O (stret) 1732.09, –C=N (stret) 1659.33, N-N (stret) 1026.41. **^{1}H NMR (400MHz, DMSO):** ∂ **[ppm]:** Aromatic protons (10) 7.04-7.72(m), Amide NH(s) 10.40, Cyclic amide NH(s) 14.30. **Mass** Spectrum ([M+1]$^+$ 522, M$^+$ 521.

Compound IVc (R=5-Br): **IR (cm^{-1})** NH (stret) 3267.04, NH (bend) 1567.16,

–C=O (stret) 1688.22, –C=O (stret) 1733.06, –C=N (stret) 1658.23,

N-N (stret) 1022.44.**^{1}H NMR (400MHz, DMSO):** ∂ **[ppm]:** Aromatic protons (10) 7.32-7.99(m), Amide NH(s) 10.30, Cyclic amide NH(s) 14.35**. Mass** Spectrum [M+1]$^+$ 611, M$^+$ 610.

Compound IVd (R=7-F): **IR (cm^{-1})** NH (stret) 3257.63, NH (bend) 1562.66,

–C=O(stret) 1688.21,–C=O(stret) 1730.19, –C=N(stret) 1655.63, N-N(stret)1023.21 **^{1}H NMR (400MHz, DMSO):** ∂ **[ppm]:**Aromatic

protons(10) 7.08-7.75(m),Amide NH(s)10.40,Cyclic amide NH(s) 14.30**. Mass** Spectrum ([M+1]$^+$ 489, M$^+$ 488.

Compound IVe (R=5-Cl): **IR (cm^{-1})** NH (stret) 3255.07, NH (bend) 1561.03,

–C=O(stret) 1684.61,–C=O(stret) 1731.89, –C=N(stret) 1655.33, N-N(stret)1022.41 **^{1}H NMR (400MHz, DMSO):** *∂* **[ppm]:** Aromatic protons(10)7.04-7.72(m),Amide NH(s)10.40,Cyclic amide NH(s) 14.30:**Mass** Spectrum([M+1]$^+$ 522, M$^+$ 521.

Compound IVf (R=5-F): **IR (cm^{-1})** NH (stret) 3259.33, NH (bend) 1560.16,

–C=O(stret) 1686.22,–C=O(stret) 1729.49, –C=N(stret) 1654.36, N-N(stret)1021.31 **^{1}H NMR (400MHz, DMSO):** *∂* **[ppm]:** Aromatic protons(10) 7.08-7.75(m),Amide NH(s)10.40,Cyclic amide NH(s) 14.30. **Mass** Spectrum [M+1]$^+$ 489, M$^+$ 488.

Compound IVg (R=5-CH_3): **IR (cm^{-1})** NH (stret) 3254.01, NH (bend) 1558.06,

–C=O (stret) 1684.21,–C=O (stret) 1727.49, –C=N (stret) 1652.23, N-N (stret) 1021.21. **^{1}H NMR (400MHz, DMSO):** *∂* **[ppm]:** Aromatic protons (10) 7.02-7.59(m), Amide NH(s) 9.98, Cyclic amide NH(s) 13.98, Methyl Protons(s) 1.98. **Mass** Spectrum [M+1]$^+$ 479, M$^+$ 480.

Compound IVh (R=7-NO_2): **IR (cm^{-1})** –NH (stret) 3257.03, NH (bend) 1562.06,

–C=O(stret) 1688.21,–C=O(stret) 1725.09, –C=N(stret) 1654.33, N-N(stret)1023.11 **^{1}H NMR (400MHz, DMSO):** *∂* **[ppm]:** Aromatic protons (10) 7.02-7.64(m),Amide NH(s)9.96,Cyclic amide NH(s) 13.96. **Mass** Spectrum [M+1]$^+$ 541, M$^+$ 542.

Compound IVi (R=5-F,6-Cl): **IR (cm^{-1})** –NH (stret) 3257.03, NH (bend) 1562.06,

–C=O(stret) 1688.21,–C=O(stret) 1725.09, –C=N(stret) 1654.33, N-N(stret)1023.11 **^{1}H NMR (400MHz, DMSO):** *∂* **[ppm]:** Aromatic protons (9) 7.02-7.64(m),Amide NH(s)9.96,Cyclic amide NH(s) 13.96. **Mass** Spectrum [M+1]$^+$ 557.M$^+$ 558.

Compound IVj (R=6-Br): **IR (cm^{-1})** NH (stret) 3260.06, NH (bend) 1568.16,

–C=O (stret) 1684.31,–C=O (stret) 1731.19, –C=N (stret) 1664.13, N-N (stret) 1023.61. **^{1}H NMR (400MHz, DMSO):** *∂* **[ppm]:** Aromatic protons (10) 7.32-7.99(m), Amide NH(s) 10.30, Cyclic amide NH(s) 14.35. **Mass** Spectrum [M+1]$^+$ 611, M$^+$ 610.

MATERIALS &METHODS

Chemicals: All solvents, reagents, and catalysts used are of AR grade. DPPH (α,α, -diphenyl-β-picrylhydrazyl) was purchased from Sigma Chemical Company (St. Louis, MO, USA).

Test compounds

Ten newly synthesized series of (*N*'^{1},*N*'^{2}-bis[2-oxoindolin-3-ylidene] phthalohydrazides) derivatives were used for the evaluation of antioxidant activity. These10 different novel series of (*N*'^{1}, *N'2*-bis [2-oxoindolin-3-ylidene] phthalohydrazides) derivatives were prepared by treating phthalohydrazide with different isatin derivatives. The structures and physical data of the compounds were shown in Table 1.

Biological activity

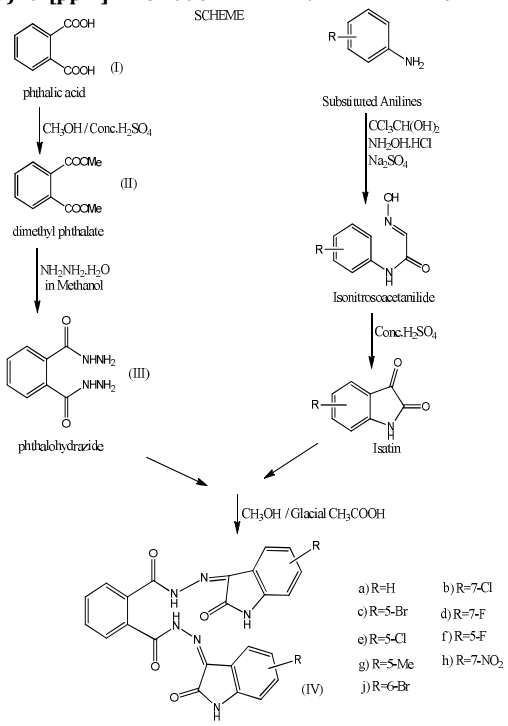
Evaluation of antioxidant activity

α,α-Diphenyl picrylhydrazyl (DPPH 1ml of 0.135mM in methanol), a stable free radical was used for the evaluation of antioxidant activity of the test compounds (Liyana-Pathiana and Shahidi 2005). To 1ml of the test compound (at different concentrations), 1ml of DPPH solution were added, mixed thoroughly and absorbance (OD) read at 517nm against blank. The percentage reduction of free radical concentration (OD) with different concentrations of test compounds was calculated and compared with standard, ascorbic acid. Results were expressed as IC_{50} values (concentration of test required to scavenge 50 % free radicals.)

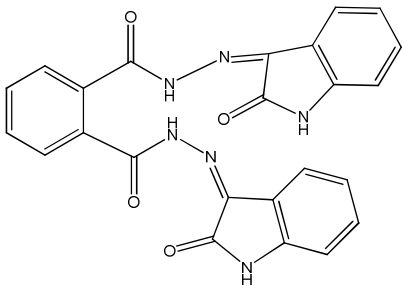
RESULTS AND DISCUSSIONS

The free radical scavenging or *in vitro* antioxidant activity of all synthesized compounds was evaluated using DPPH as a free radical and results were shown in Table 2. The results of this study revealed that all synthesized compounds significantly scavenged DPPH free radicals in a concentration-dependant manner. The IC_{50} of all the test compounds were found between 41.22 and 73.36 mM. Among all compounds IVa (R=H), IVj (R=6-Br), IV b (7-Cl) displayed more potent antioxidant activity. This may be due to increased lipophilicity of molecules because of substitution with electronegative atoms such as chloro/bromo in the aromatic ring (Hari et al.2008).

SCHEME



Physical data of *N*'^{1}, *N*'^{2}-bis [2-oxoindolin-3-ylidene] phthalohydrazides (IV a-i)



(IV)

Compound	Substituents (R)	Mol.Formula	Colour	m.r (ºC)	Yield (%)	Mol.Wt
IVa	H	$C_{24}H_{16}N_6O_4$	Red	280-285	90	452
IVb	7-Cl	$C_{24}H_{14}N_6O_4Cl_2$	Red	254-258	92	521
IVc	5-Br	$C_{24}H_{14}N_6O_4Br_2$	Red	272-276	95	610
IVd	7-F	$C_{24}H_{14}N_6O_4F_2$	Orange	238-240	85	488
IVe	5-Cl	$C_{24}H_{14}N_6O_4Cl_2$	Grey	325-328	87	521
IVf	5-F	$C_{24}H_{14}N_6O_4F_2$	Yellow	268-273	70	488
IVg	5-CH_3	$C_{26}H_{20}N_6O_4$	Orange	201-203	75	480
IVh	7-NO_2	$C_{24}H_{14}N_8O_8$	Yellow	255-260	83	542
IVi	5-F,6-Cl	$C_{24}H_{12}N_6O_4Cl_2F_2$	Brown	330-335	45	577
IVj	6-Br	$C_{24}H_{14}N_6O_4Br_2$	Brown	250-256	40	610

m.r.: Melting Range

Antioxidant activity of ***N'1, N'2*-bis [2-oxoindolin-3ylidene] phthalohydrazides (IV a-j)**

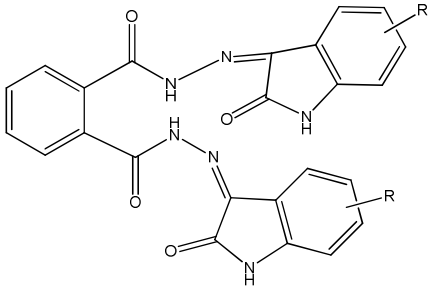
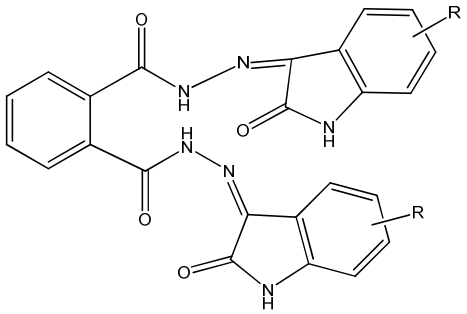
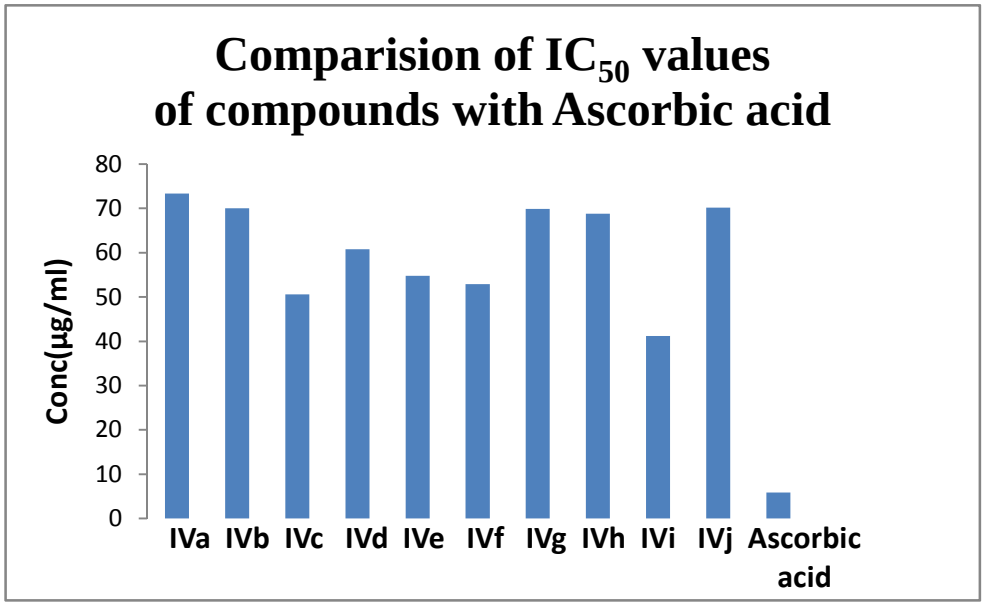


Table 2:

S. No.	Compound	R	IC_{50} (µg/ml)
1	IVa	H	73.36
2	IVb	7-Cl	70.01
3	IVc	5-Br	50.61
4	IVd	7-F	60.75
5	IVe	5-Cl	54.80
6	IVf	5-F	52.88
7	IVg	5-CH_3	69.91
8	IVh	7-NO_2	68.77
9	IVi	5-F,6-Cl	41.22
10	IVj	6-Br	70.16
11	standard	Ascorbic acid	5.84

Antioxidant activity of ***N'1, N'2*-bis [2-oxoindolin-3ylidene] phthalohydrazides (IV a-j):**





CONCLUSIONS

The present study results indicate the antioxidant profile of series of (N'[1], N'2bis [2-oxoindolin-3-ylidene] phthalohydrazides) derivatives. The results of the antioxidant activity revealed the compounds to possess good spectrum of antioxidant activity by incorporation of electron withdrawing substituents.

ACKNOWLEDGEMENT

The authors are very grateful to the Principal, University College of Pharmaceutical Sciences for providing facilities to perform the work. The authors are also thankful to Central Instrumentation Centre, Kakatiya university, Warangal and IICT Hyderabad for providing the spectral data.

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