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SYNTHESIS, ANTICANCER AND ANTIBACTERIAL ACTIVITY OF MALONIC ACID BISISATIN HYDRAZONES

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ABSTRACT

Malonic Acid Bisisatin Hydrazones (VIa-i) have been synthesized by the condensation of malonohydrazide (V) with corresponding isatin derivatives (III) in alcohol. The intermediate malonohydrazide (V) was prepared by the reaction of diethylamalonate (IV) with hydrazine hydrate. All the title compounds (VI) were screened for anticancer activity using HBL-100 cell lines by MTT method and antibacterial activity against *B. subtiliis*, *S.aureus*, *E.coli* and *P.vulgaris*. The structures of newly synthesized compounds were established on the basis of elemental analysis, IR, ^{1}H NMR and mass spectral data.

Key words: Isatin, Anticancer Activity, Antibacterial Activity.

INTRODUCTION

Isatin hydrazones belong to an important class of heterocyclic compounds in medicinal chemistry associated with wide range of biological activities such as antimicrobial activity (Pandeya *et al*., 2005), antiviral activity (Beauchard *et al*., 2006), antineoplastic activity and CNS activity (Knockaert *et al*., 2002). The biological importance of the compounds inspired us to synthesize some new bisisatin hydrazones to get more potent compounds and screen for anticancer activity by the MTT method (Krief *et al*., 2005) and antibacterial activity by cup plate method (Seely *et al*., 1975). Synthesis of malonic acid bisisatin hydrazones (VIa-j) is shown in Scheme 1.

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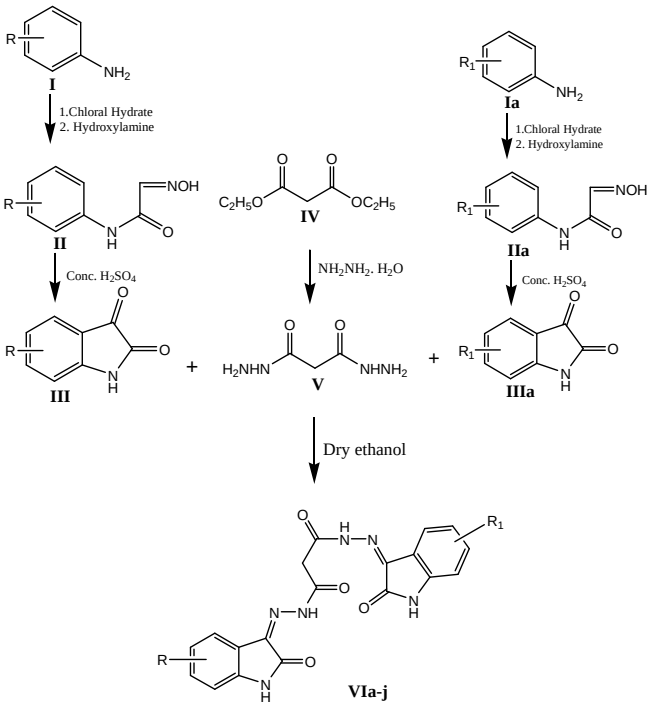
MATERIALS AND METHODS

Melting points were determined in open capillary tubes, using Toshniwal melting point apparatus and are uncorrected. IR spectra were recorded on Perkin – Elmer spectrum BX-I series, FTIR spectrophotometer using KBr discs. PMR spectra were recorded on Brucker spectrospin 400 MHz spectrophotometer using TMS as an internal standard. Purity was checked by TLC using TLC aluminum sheets silica gel 60, supplied by E. Merk, Mumbai, India. The spots were located by keeping the plate in iodine vapor and 2,4,5-trichlorobenzamine was supplied by S. D. Fine Chem Ltd, Mumbai, India.

Malonohydrazide (V) was prepared by refluxing, diethylmalonate (IV) in alcohol with hydrazine hydrate for 15min. The progress of reaction and purity were routinely checked on TLC. The resultant white crystalline solid was filtered, washed with cold alcohol. The product was dried and recrystallized from ethanol (90%). m.p. 153°C and Yield 90%. Elemental Analysis found: C, 27.21; H, 6.12; N, 42.44; O, 24.23. Calculated for $C_3H_8N_4O_2$: C, 27.27; H, 6.10; N, 42.41; O, 24.22

N^1,N^3-bis(2-oxoindolin-3-ylidene)malonohydrazide (VI) by following method (Joaquim *et al*., 2001), the malonohydrazide (V, 0.01 mol) was added to an appropriate isatin (III, 0.02 mol) in ethanol (95%. 20 ml), and refluxed for 3-4 hours. The product obtained was filtered and washed repeatedly, with small portions of cold ethanol to remove the un-reacted istains and hydrazide. The product was dried and purified by using column chromatography. The purity of the compound was checked by TLC. The compounds thus obtained were characterized as bisisatin malonohydrazide (VI) by their physical (Table 1) and spectral data.



Scheme 1: Synthesis of malonic acid bisisatin hydrazones

N^1,N^3-bis(2-oxoindolin-3-ylidene)malonohydrazide (VIa)

IR (KBr) (cm^{-1}): 1545 (C=N), 1690 (C=O), 3198 (NH). [1] H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 3.22 (s, 2H, CH_2), 6.8-7.9 (m, 8H, Ar-H), 10.80 (s, 2H, NHCO), 11.4 (s, 2H, NH indole). LC-MS (m/z): 391.11 (M+1).

N^1,N^3-bis(5-fluoro-2-oxoindolin-3-ylidene)malonohydrazide (VIb)

IR (KBr) (cm^{-1}): 1566 (C=N), 1720 (C=O), 3248 (NH). [1] H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 3.22 (s, 2H, CH_2), 6.8-7.8 (m, 6H, Ar-H), 10.80 (s, 2H, NHCO), 11.4 (s, 2H, NH indole). LC-MS (m/z): 427.09 (M+1).

N^1,N^3-bis(5-chloro-2-oxoindolin-3-ylidene)malonohydrazide (VIc)

IR (KBr) (cm^{-1}): 1466 (C=N), 1724 (C=O), 3235 (NH). [1] H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 3.22 (s, 2H, CH_2), 6.8-7.9 (m, 6H, Ar-H), 10.80 (s, 2H, NHCO), 11.4 (s, 2H, NH indole). LC-MS (m/z): 460.03 (M+1).

N^1,N^3-bis(5-bromo-2-oxoindolin-3-ylidene)malonohydrazide (VId)

IR (KBr) (cm^{-1}): 1550 (C=N), 1694 (C=O), 3184 (NH). [1] H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 3.22 (s, 2H, CH_2), 6.8-7.9 (m, 6H, Ar-H), 10.80 (s, 2H, NHCO), 11.4 (s, 2H, NH indole). LC-MS (m/z): 549.92 (M+1).

N^1,N^3-bis(5-methyl-2-oxoindolin-3-ylidene)malonohydrazide (VIe)

IR (KBr) (cm^{-1}): 1530 (C=N), 1690 (C=O), 3198 (NH). [1] H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 2.5 (s, 6H, CH_3), 3.22 (s, 2H, CH_2), 6.8-7.9 (m, 6H, Ar-H), 10.80 (s, 2H, NHCO), 11.4 (s, 2H, NH indole). LC-MS (m/z): 419.14 (M+1).

N^1,N^3-bis(5-nitro-2-oxoindolin-3-ylidene)malonohydrazide (VIf)

IR (KBr) (cm^{-1}): 1339 (NO_2), 1556 (C=N), 1702 (C=O), 3227 (NH). [1] H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 3.22 (s, 2H, CH_2), 6.8-7.9 (m, 6H, Ar-H), 10.80 (s, 2H, NHCO), 11.4 (s, 2H, NH indole). LC-MS (m/z): 481.08 (M+1).

N^1,N^3-bis(5-hydroxy-2-oxoindolin-3-ylidene)malonohydrazide (VIg)

IR (KBr) (cm^{-1}): 2985 (OH), 1632 (C=N), 1675 (C=O), 3168 (NH). [1] H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 3.22 (s, 2H, CH_2), 6.8-7.9 (m, 6H, Ar-H), 9.6 (s, 2H, OH), 10.80 (s, 2H, NHCO), 11.4 (s, 2H, NH indole). LC-MS (m/z): 423.10 (M+1).

N^1-(5-chloro-2-oxoindolin-3-ylidene)-N^3-(5-fluoro-2-oxoindolin-3-ylidene)malonohydrazide (VIh)
IR (KBr) (cm^{-1}): 1560 (C=N), 1706 (C=O), 3205 (NH). ^{1}H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 3.22 (s, 2H, CH_2), 6.8-7.9 (m, 6H, Ar-H), 10.80 (s, 2H, NHCO), 11.4 (s, 2H, NH indole). LC-MS (m/z): 444.06 (M+1).

N^1-(5-bromo-2-oxoindolin-3-ylidene)-N^3-(5-fluoro-2-oxoindolin-3-ylidene)malonohydrazide (VIi)
IR (KBr) (cm^{-1}): 1545 (C=N), 1656 (C=O), 3180 (NH). ^{1}H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 3.22 (s, 2H, CH_2), 6.8-7.9 (m, 6H, Ar-H), 10.80 (s, 2H, NHCO), 11.4 (s, 2H, NH indole). LC-MS (m/z): 488.01 (M+1).

N^1-(5-bromo-2-oxoindolin-3-ylidene)-N^3-(5-chloro-2-oxoindolin-3-ylidene) malonohydrazide (VIj)
IR (KBr) (cm^{-1}): 1515 (C=N), 1676 (C=O), 3181 (NH). ^{1}H-NMR (DMSO-d_6, 400 MHz), δ (ppm): 3.22 (s, 2H, CH_2), 6.8-7.9 (m, 6H, Ar-H), 10.80 (s, 2H, NHCO), 11.4 (s, 2H, NH indole). LC-MS (m/z): 504.98 (M+1).

Antimicrobial Activity

The antimicrobial activity of all the newly synthesized compounds were determined by well plate method in nutrient agar (Hi-Media) was used for antibacterial activity. The antibacterial activity of the test compounds was assayed against *Bacillus subtilis*, *Staphylococcus aureus* (gram – positive) and *Escherichia coli* and *Proteus vulgaris* (gram – negative) by CUP-plate method.

The compounds were tested at a concentration of a 100 µg/ml were prepared in dimethylformamide (DMF). The Petri dishes used for antibacterial screening were incubated at 37 ± 1º for 24 h; the diameters of zone of inhibition (mm) surrounding each of the wells were recorded. The results were compared with Ampicillin of a 50 µg/ml concentration and the screening results were presented in Table 2.

Anticancer Activity

Malonic acid bisisatin hydrazones were subjected to *in vitro* MTT [3-(4,5-Dimethylthiazol-2-yl) -2,5-diphenyltetrazolium Bromide] assay to detect cytotoxic antitumor property and *in vivo* test using tumor mouse model to detect noncytotoxic antitumor property were used. MTT assay was used for *in vitro* cytotoxicity test and was performed as per the method of Alley *et al*. Cells were harvested from experimental-phase maintenance cultures. Four hundred cells were counted by trypan blue exclusion and dispensed within triplicate 96-well culture plates in 100 µl volumes for each venom concentration (Alley *et al*., 1988). The assay at each concentration was repeated twice. The cell proliferation activity was qualified on HBL-100 (ICLC NO. HTL 00004) - breast myoepithelial tumor cell line, by using Cisplatin as a standard. The results are represented in Table.2.

Table 1: Physical data of malonic acid bisisatin hydrazones

Compound	R	R_1	Mol. Formula	Melting Point (^{0}C)	Yield (%)
VIa	H	H	$C_{19}H_{14}N_6O_4$	265-268	72
VIb	F	F	$C_{19}H_{12}F_2N_6O_4$	272-276	68
VIc	Cl	Cl	$C_{19}H_{12}Cl_2N_6O_4$	228-232	74
VId	Br	Br	$C_{19}H_{12}Br_2N_6O_4$	280-283	78
VIe	CH_3	CH_3	$C_{21}H_{18}N_6O_4$	269-273	66
VIf	NO_2	NO_2	$C_{19}H_{12}N_8O_8$	231-234	58
VIg	OH	OH	$C_{19}H_{14}N_6O_6$	221-223	66
VIh	F	Cl	$C_{19}H_{12}ClFN_6O_4$	268-271	72
VIi	F	Br	$C_{19}H_{12}BrFN_6O_4$	278-282	61
VIj	Cl	Br	$C_{19}H_{12}BrClN_6O_4$	241-243	63

Table 2: Anticancer and antibacterial activity of malonic acid bisisatin Hydrazones (VI)

Compound	Cytotoxic activity IC_{50} (μM)	Antibacterial activity (Zone of inhibition in mm)			
		B. Subtilis	***S. aureus***	***E. coli***	***P. vulgaris***
VIa	78	16	10	--	06
VIb	101	08	12	10	11
VIc	42	20	18	16	15
VId	56	15	08	12	10
VIe	31	10	10	11	09
VIf	152	15	17	14	12
VIg	66	17	18	15	14
VIh	96	11	12	11	--
VIi	171	06	10	10	08
VIj	135	--	08	02	06
Cisplatin	25	NA	NA	NA	NA
Ampicillin	NA	22	20	18	17

RESULTS AND DISCUSSION

The title compounds were obtained in good yields and purity. All the test compounds at the conc. of 20 μg/ml, 80 μg/ml, 100 μg/ml and 200 μg/ml were taken to evaluate the anticancer activity against HBL-100 cell lines and the results are presented as IC_{50} values. All the compounds showed anticancer activity in the range of 31 μM to 171 μM. The structure activity studies reveal that among the test compounds, the compound VIe with methyl substitution at C-5 position on indolinone miety showed relatively high degree of anticancer activity with IC_{50} of 31 μM. The compounds, VIc, VId, VIg was next in the order of anticancer activity with IC_{50} values of 42 μM and 56 μM, 66 μM respectively. The results are statistically significant and the activity of the compounds is compared with the standard Cisplatin.

The test compounds showed mild antibacterial activity at the concentration of 100 μg/disc against gram-positive organism (*B. subtilis*, *S. aureus*) and gram negative (*E. coli*, *P. vulgaris*) organisms. The compound VIc was more active among all the test compounds followed by compound Vic, VIg, VId.

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