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Research Article

**SYNTHESIS, ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF
NEW BISISATIN MALONOHYDRAZIDES**

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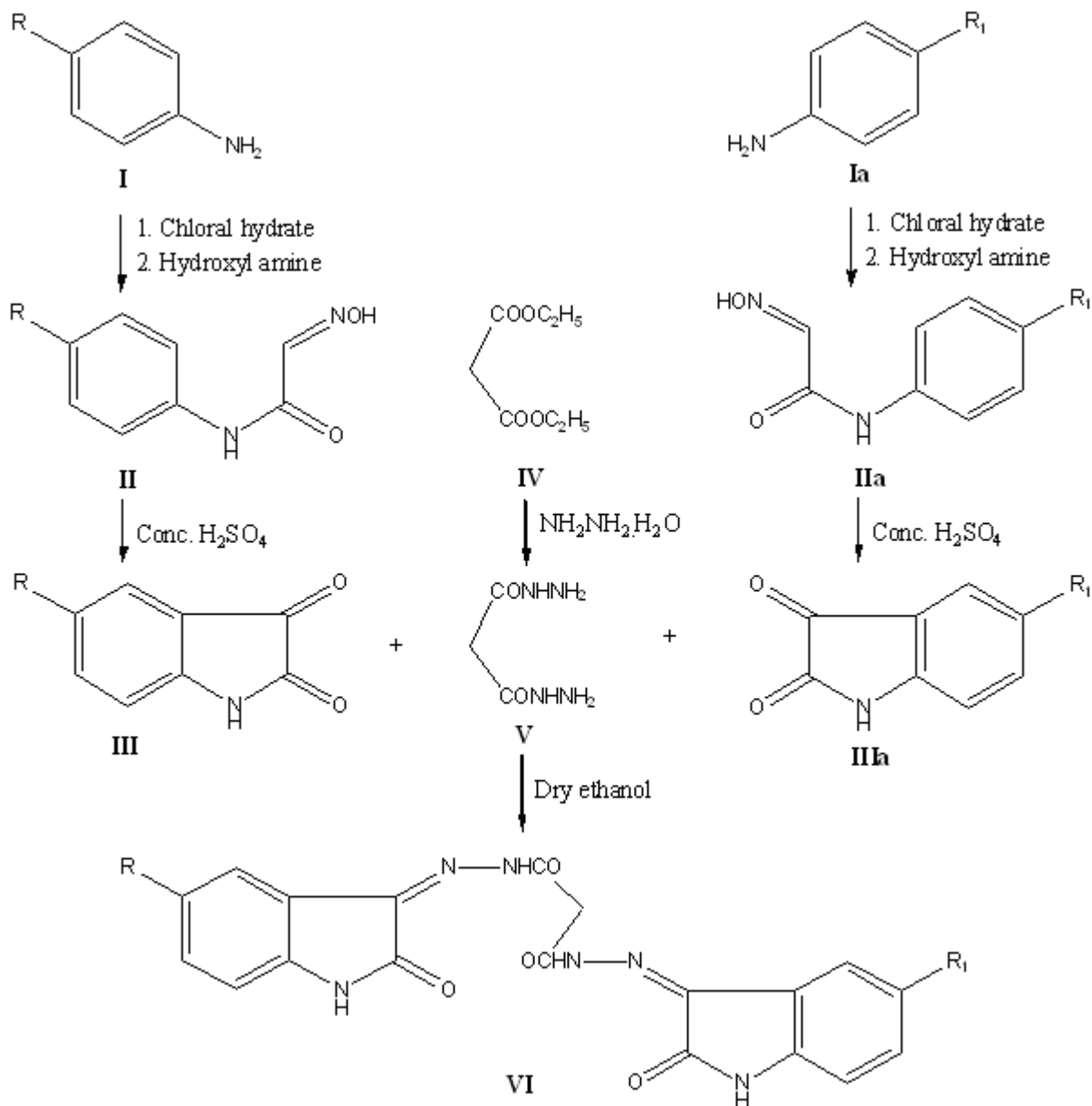
Abstract:

N¹,N³-bis(2-oxoindolin-3-ylidene)malonohydrazides (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 diethylmalonate (IV) with hydrazine hydrate. All the title compounds (VI) were screened for Acute toxicity, analgesic, anti-inflammatory and ulcerogenic activity. Ibuprofen and indomethacin were employed as standard. The structures of newly synthesized compounds were established on the basis of elemental analysis, IR, ¹H NMR and mass spectral data.

Key words: Isatin, acute toxicity, analgesic, anti-inflammatory and ulcerogenic activity.

Introduction: Isatin hydrazones belong to an important class of heterocyclic compounds in medicinal chemistry associated with wide range of biological activities^[1-3] such as antimicrobial activity, antiviral activity, antineoplastic activity, CNS activity. Analgesic and anti-inflammatory activity. The biological importance of the compounds inspired us to synthesize some new bisisatin hydrazones to get more potent compounds and screen for Analgesic^[4] and anti-inflammatory activity^[5-7]. Synthesis of the title compounds was affected as shown in Scheme1.

Scheme 1: Synthetic route of new bisatin malonohydrazides.



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, FT IR spectrophotometer using KBr discs. PMR spectra were recorded on Bruker 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. Synthesis of the title compounds was shown in the scheme I. The required istains were prepared by using the method available in literature ^[8].

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 C₃H₈N₄O₂: C, 27.27; H, 6.10; N, 42.41; O, 24.22

N¹, N³-bis (2-oxoindolin-3-ylidene) malonohydrazide (VI) by following method, 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.

Table I: Physical data of new bisisatin malonohydrazides

Compound	R	R ₁	Mol. Formula	Melting Point (⁰ C)	Yield (%)
VIa	H	H	C ₁₉ H ₁₄ N ₆ O ₄	265-268	72
VIb	F	F	C ₁₉ H ₁₂ F ₂ N ₆ O ₄	272-276	68
VIc	Cl	Cl	C ₁₉ H ₁₂ Cl ₂ N ₆ O ₄	228-232	74
VI d	Br	Br	C ₁₉ H ₁₂ Br ₂ N ₆ O ₄	280-283	78
VIe	CH ₃	CH ₃	C ₂₁ H ₁₈ N ₆ O ₄	269-273	66
VI f	NO ₂	NO ₂	C ₁₉ H ₁₂ N ₈ O ₈	231-234	58
VIg	OH	OH	C ₁₉ H ₁₄ N ₆ O ₆	221-223	66
VIh	F	Cl	C ₁₉ H ₁₂ ClFN ₆ O ₄	268-271	72
VIi	F	Br	C ₁₉ H ₁₂ BrFN ₆ O ₄	278-282	61
VIj	Cl	Br	C ₁₉ H ₁₂ BrClN ₆ O ₄	241-243	63

N¹,N³-bis(2-oxoindolin-3-ylidene)malonohydrazide (VIa)

IR (KBr) (cm⁻¹): 1545 (C=N), 1690 (C=O), 3198 (NH). ¹ H-NMR (DMSO-d₆, 400 MHz), δ (ppm): 3.22 (s, 2H, CH₂), 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).

N¹,N³-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-d₆, 400 MHz), δ (ppm): 3.22 (s, 2H, CH₂), 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).

N¹,N³-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-d₆, 400 MHz), δ (ppm): 3.22 (s, 2H, CH₂), 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).

N¹,N³-bis(5-bromo-2-oxoindolin-3-ylidene)malonohydrazide (VIId)

IR (KBr) (cm⁻¹): 1550 (C=N), 1694 (C=O), 3184 (NH). ¹ H-NMR (DMSO-d₆, 400 MHz), δ (ppm): 3.22 (s, 2H, CH₂), 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).

N¹,N³-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-d₆, 400 MHz), δ (ppm): 2.5 (s, 6H, CH₃), 3.22 (s, 2H, CH₂), 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).

N¹,N³-bis(5-nitro-2-oxoindolin-3-ylidene)malonohydrazide (VIIf)

IR (KBr) (cm⁻¹): 1339 (NO₂), 1556 (C=N), 1702 (C=O), 3227 (NH). ¹ H-NMR (DMSO-d₆, 400 MHz), δ (ppm): 3.22 (s, 2H, CH₂), 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).

N¹,N³-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-d₆, 400 MHz), δ (ppm): 3.22 (s, 2H, CH₂), 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).

N¹-(5-chloro-2-oxoindolin-3-ylidene)-N³-(5-fluoro-2-oxoindolin-3-ylidene)malonohydrazide (VIh)

IR (KBr) (cm⁻¹): 1560 (C=N), 1706 (C=O), 3205 (NH). ¹ H-NMR (DMSO-d₆, 400 MHz), δ (ppm): 3.22 (s, 2H, CH₂), 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).

N¹-(5-bromo-2-oxoindolin-3-ylidene)-N³-(5-fluoro-2-oxoindolin-3-ylidene)malonohydrazide (VIi)

IR (KBr) (cm⁻¹): 1545 (C=N), 1656 (C=O), 3180 (NH). ¹ H-NMR (DMSO-d₆, 400 MHz), δ (ppm): 3.22 (s, 2H, CH₂), 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).

N¹-(5-bromo-2-oxoindolin-3-ylidene)-N³-(5-chloro-2-oxoindolin-3-ylidene) malonohydrazide (VIj)

IR (KBr) (cm⁻¹): 1515 (C=N), 1676 (C=O), 3181 (NH). ¹ H-NMR (DMSO-d₆, 400 MHz), δ (ppm): 3.22 (s, 2H, CH₂), 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).

Biological Evaluation

Acute toxicity

The test compounds **VIa-i** were tested in mice (500 mg/kg, 1000 mg/kg, 2000 mg/kg,p.o.) after 14 days of administration for their safety as per OECD guidelines^[9]. The compounds did not produce any significant changes in the body weight, food, water intake and other behavioural patterns. There was also no change in the haematological parameters and organ weights of treated animals compared to control. No mortality was observed in the control and compound treated groups. Histopathological examination of *viz* heart, liver, brain, lung, kidney, spleen, stomach, testis and ovary in animals treated with compounds were studied. Results of the histopathological studies revealed that all the compounds **VIa-i** (500 mg/kg, 1000 mg/kg, 2000 mg/kg) and control did not show any pathological changes.

Analgesic activity

The analgesic activity of the compounds was determined by the tail immersion method^[10,11]. In this investigation, it has been observed that all the tested compounds show significant analgesic activity without ulcerogenic liability. Among these, compounds **VIb, VIi and VIj** (284.4-306% analgesic activity) showed enhanced activity and comparable to those of ibuprofen, which is used

as a reference standard (309% analgesic activity). Compounds **VIb** and **VIh**(310.5% and 414% activity) were found to be more potent than the standard drug ibuprofen while compounds **VIa**, **VId**, **VIe** **VIj** and **VIg** gave moderate results(**Table 1I**).

Table II: Analgesic activity of new bisisatin malonohydrazides.

Compd	Mean Tail Withdrawal latencies(sec) \pm SE				%Analgesic activity			
	0 min	30 min	1 hr	2hr	0 min	30 min	1hr	2 hr
Control	2.4 \pm 0.055*	2.38 \pm 0.071*	2.3 \pm 0.114*	2.4 \pm 0.1414*	-	-	-	-
VIa	3.08 \pm 0.04*	5.58 \pm 0.04*	7.20 \pm 0.071*	7.38 \pm 0.021*	181.16	233.77	239.61	243.50
VIb	2.0 \pm 0.10*	3.8 \pm 0.114*	6.0 \pm 0.23*	8.36 \pm 0.11*	190.0	300.0	418.0	414.0
VIc	2.34 \pm 0.0341*	5.52 \pm 0.09*	5.54 \pm 0.14*	6.22 \pm 0.06*	235.9	236.75	265.81	305.9
VId	3.2 \pm 0.071*	5.22 \pm 0.06*	7.30 \pm 0.045*	7.18 \pm 0.0735*	163.12	228.12	224.37	233.12
VIe	3.16 \pm 0.07*	5.22 \pm 0.06*	6.90 \pm 0.084*	6.96 \pm 0.06*	165.19	218.34	220.25	227.84
VIj	3.16 \pm 0.07*	5.20 \pm 0.04*	6.98 \pm 0.081*	6.92 \pm 0.05*	164.19	217.34	221.25	226.84
VIg	2.3 \pm 0.07*	6.10 \pm 0.08*	7.0 \pm 0.07*	6.40 \pm 0.04*	265.82	300.35	280.74	282.35
VIh	2.28 \pm 0.06*	5.36 \pm 0.04*	6.36 \pm 0.051*	6.74 \pm 0.051*	235.09	278.95	295.61	310.52
VIi	2.14 \pm 0.065*	5.16 \pm 0.068*	5.52 \pm 0.086*	6.22 \pm 0.06*	241.12	257.94	290.65	303.74
VIj	2.3 \pm 0.071*	6.16 \pm 0.087*	7.0 \pm 0.071*	6.48 \pm 0.04*	267.82	304.35	281.74	284.35
Ibuprofen	2.44 \pm 0.07*	4.56 \pm 0.04*	5.46 \pm 0.0245*	7.26 \pm 0.051*	186.88	223.77	297.54	309.02

(standard)

Statistical analysis was carried out by using one-way Anova(F-test) followed by Dunnett's test.

*Significantly different from the control value at p<0.001.

Anti-inflammatory activity

The anti-inflammatory activity of the prepared compounds was determined by the carrageenan induced paw oedema standard method in rats¹². In this investigation it has been observed that all the tested compounds show significant anti-inflammatory activity without ulcerogenic liability.

From the obtained data (**Table III**), compounds **VIb** and **VIh** showed enhanced anti-inflammatory activity. While remaining compounds gave moderate results.

Table III-Anti-inflammatory activity of new bisisatin malonohydrazides

Compound	Mean values(±SE)of oedema Volume at different intervals				Percentage of anti-inflammatory activity at different intervals			
	60 min	120 min	180 min	240 min	60 min	120 min	180min	240 min
Control	1.634± 0.003*	1.874± 0.022*	1.83± 0.005*	1.77± 0.009*	-	-	-	-
VIa	1.328± 0.03*	1.344± 0.026*	1.092± 0.031*	1.01± 0.009*	18.73	28.28	40.46	42.94
VIb	1.196± 0.008*	1.158± 0.019*	0.95± 0.017*	0.894± 0.01*	26.80	38.21	48.20	49.49
VIc	1.348± 0.024*	1.138± 0.019*	0.936± 0.016*	0.904± 0.014*	17.50	39.27	48.96	48.92
VI d	1.30± 0.01*	1.228± 0.032*	1.152± 0.005*	1.122± 0.01*	20.44	34.47	37.19	36.61
VIe	1.43± 0.013*	1.206± 0.058*	1.186± 0.009*	1.056± 0.012*	12.48	36.65	35.33	40.34
VI f	1.29± 0.04*	1.19± 0.02*	1.19± 0.16*	1.048± 0.012*	21.68	37.39	37.60	41.79
VI g	1.43± 0.01*	1.20± 0.08*	1.18± 0.009*	1.05± 0.012*	12.48	35.65	34.33	39.34
VI h	1.202± 0.03*	1.184± 0.023*	0.956± 0.022*	0.896± 0.013*	26.44	36.82	47.87	49.38
VI i	1.296± 0.04*	1.192± 0.013*	1.126± 0.012*	1.048± 0.014*	20.68	36.39	38.60	40.79
VI j	1.264± 0.019*	1.198± 0.02*	1.156± 0.005*	1.11± 0.01*	22.64	36.07	36.97	37.29
Indomethacin (standard)	1.14± 0.017*	1.005± 0.023*	0.744± 0.012*	0.4462± 0.023*	30.23	43.85	59.43	74.7

Statistical analysis was carried out by using one-way Anova(F-test) followed by Dunnett's test.

*Significantly different from the control value at $p < 0.001$.

Ulcerogenic activity

The ulcerogenic liability of the prepared compounds **VIa-I** were determined in wistar rats following the previously reported standard method^[12]. Histopathological examination of stomachs of mice treated with compounds did not show any pathological changes in acute toxicity study.

Results and Discussion

The title compounds were obtained in good yields and purity. All the compounds were found to be safe even at 2000 mg/kg dose levels. The results obtained clearly indicate that the compounds discussed here showed significant analgesic (227.8-414.0% analgesic activity) and anti-inflammatory activities (40.3-52.9% inhibition of oedema) without an ulcerogenic activity. Compounds **VIb** and **VIh** appear to be the most active derivative in our series.

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