

Short Note

***N*"-[(3*Z*)-1-Acetyl-5-chloro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]thiocarbohydrazide**

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Abstract: A novel synthetic methodology for preparation of thiocarbohydrazone by reacting thiocarbohydrazide with 1-acetyl-5-chloroisatin is described. The title compound was prepared by condensation of thiocarbohydrazide and substituted isatin in aqueous ethanol. The newly synthesized compound was characterized using ¹H-NMR, ¹³C-NMR, FT-IR and mass spectrometry.

Keywords: chloroisatin; thiocarbohydrazide; carbonyl-amine condensation

1. Introduction

Isatin (indoline-2,3-dione), possessing an indole nucleus with two chemically distinct cyclic carbonyl groups, keto and lactam, has provoked tremendous interest due to its numerous biological and pharmacological activities. The growing importance of substituted isatins in the field of medicinal chemistry as potential chemotherapeutic agents and their implication for pro-drug design have been previously reported [1–9].

Particular attention has been paid to the structural perturbations caused by 5-substitution. Antimicrobial activity studies revealed that substitution in the 5th position of isatin with strong electron-donating atoms/groups, such as chlorine, bromine or fluorine, produced more active compounds in a series compared to the parent molecule [10–12]. This observation encouraged us to further study the reactions of 5-halo substituted isatin heterocycles with carbohydrazide and thiocarbohydrazide in order to obtain new derivatives [13].

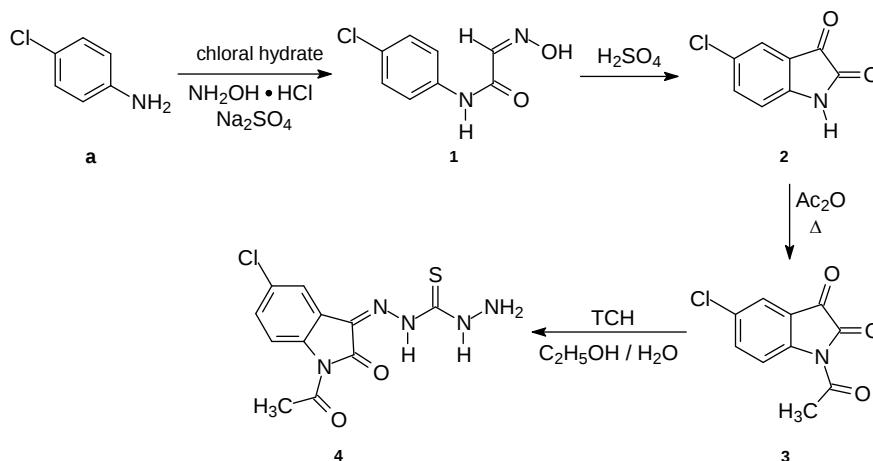
In the last decades several isatin heterocycles with hydrazine moiety have been prepared that exhibited remarkable biological activity including antimicrobial, antitubercular, antiviral, anti-inflammatory, anticonvulsant, antihypertensive, hypoglycemic, cytotoxic, anticancer activity and enzymatic inhibition [14]. The Schiff-bases of the isatin analogs also showed inhibitory activity against eLF2 kinase activator [15], TNF- α , CDK2 [16] and SARS protease [17]. In addition, isatin- β -thiosemicarbazone derivatives possess a range of chemotherapeutical activities [18,19].

Considering the fact that the thiocarbohydrazone group is necessary for antimicrobial activity [20], we have investigated the carbonyl-amine reactions of some isatin derivatives and we have observed that *N*-ethylisatin- β -thiocarbohydrazone has cytostatic activity towards malignant melanoma cells [9]. Herein we report the synthesis and spectral characterization of the novel derivative *N*'-[(3*Z*)-1-acetyl-5-chloro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]thiocarbonohydrazide (**4**).

2. Results and Discussion

The title compound **4** was obtained via three steps as described in Scheme 1. The first step involved synthesis of isatin derivative 5-chloroindoline-2,3-dione (**2**) using a modified Sandmeyer methodology [21]. It was prepared with dehydrative cyclization of the precursor 4-chloroisitonitrosoacetanilide (**1**) with concentrated H₂SO₄ at controlled temperature. Compound **1** was synthesized in very good yield by reaction of the respective aromatic amine 4-chloroaniline (**a**) with chloral hydrate and hydroxylamine hydrochloride.

Scheme 1. Synthesis of *N*'-[(3*Z*)-1-acetyl-5-chloro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene]thiocarbonohydrazide (**4**).



In order to hinder the formation of isatin-like centrosymmetric dimers in the carbonyl-amine reaction of **2** with thiocarbohydrazone, the second step was *N*-substitution of 5-chloroindoline-2,3-dione (**2**) to obtain the corresponding 1-acetyl derivative (**3**). 1-Acetyl-5-chloroindoline-2,3-dione (**3**) was prepared by adapting a literature method [22].

Finally, in the carbonyl-amine condensation of the disubstituted isatin (**3**) with thiocarbohydrazone (TCH), synthesized according to the Taguchi method [23], **4** was obtained. In order to improve the yield of **4**, the effect of the mole ratio of reactants and the amount of solvents was investigated. It was

found that the corresponding isatin product was predominantly obtained by using equimolar amounts of **3** and TCH, in ethanol/water (3:1, v/v) as a solvent system.

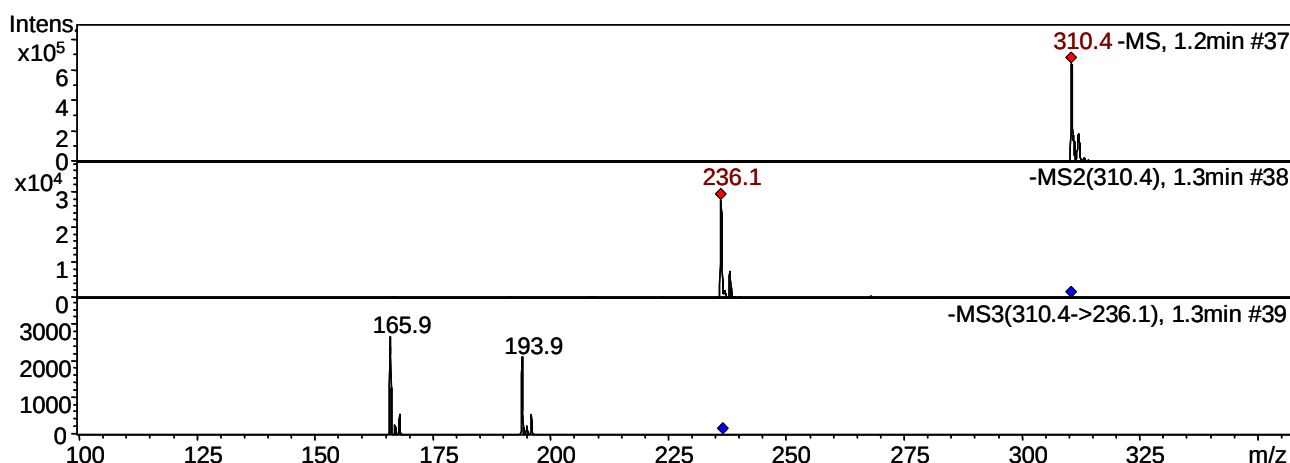
The structure of the title compound (**4**) was confirmed using FT-IR, ^1H -NMR, ^{13}C -NMR and MS^n .

Complex bands dominate in the $3,500\text{--}2,800\text{ cm}^{-1}$ spectral region of $N''\text{--}[(3Z)\text{-}1\text{-acetyl-}5\text{-chloro-}2\text{-oxo-}1,2\text{-dihydro-}3H\text{-indol-}3\text{-ylidene}]\text{thiocarbonohydrazide}$. The band at around $3,315\text{ cm}^{-1}$ can be attributed with great certainty to $\nu(\text{NH})$. The most prominent bands in the spectrum of the synthesized compound (**4**) are the ones due to the carbonyl stretchings $\nu(\text{C=O})$ of the keto and lactam group in the region around $1,700\text{ cm}^{-1}$. The absence of carbonyl (C=O) peak at around $1,750\text{ cm}^{-1}$ characteristic for the keto group in (**3**) could explain the formation of a thiocarbohydrazone.

The ^1H -NMR spectra of (**4**) displayed three separate singlets at 14.15, 9.54 and 6.62 ppm which can be attributed to C=NN-H , $\text{C=NNH}(\text{CS})\text{N-H}$ and $\text{C=NNH}(\text{CS})\text{NH-NH}_2$ from the thiocarbohydrazone moiety, respectively. The assignment of the peak at δ 14.15 as C=NN-H is supported by its location at a lower field (indicative of an intramolecularly hydrogen-bonded proton) [24]. According to the spectral data analysis, the synthesized monothiocarbohydrazone is a *Z*-geometrical isomer.

The mass spectrum (MS) of (**4**) was obtained by electrospray ionization in the negative mode and it demonstrated a molecular ion $[\text{M-H}]^-$ at m/z 310 as a base peak in MS^1 with the corresponding $\text{M}+2$ peak at m/z 312 due to the ^{37}Cl (Figure 1). The base peak in MS^2 was observed at m/z 236 with isotopic peaks at 237 and 238, which results from cleavage of the $-\text{CSNHNH}_2$ part of the molecule. Fragments at m/z 208, 194 and 166 with low intensity (1%–2%) were also observed in MS^2 . Further fragmentation of the ion at m/z 236 in MS^3 led to two main fragments at m/z 194 and 166 followed by their corresponding isotopic peaks. The peak at m/z 194 is due to a loss of an acetyl group, and the peak at 166 to a further loss of CO, which occurs in amides as *Claisen* rearrangement [25].

Figure 1. ESI MS spectra of $N''\text{--}[(3Z)\text{-}1\text{-acetyl-}5\text{-chloro-}2\text{-oxo-}1,2\text{-dihydro-}3H\text{-indol-}3\text{-ylidene}]\text{thiocarbonohydrazide}$ (**4**).



3. Experimental

3.1. General

Melting points were determined using Koffler apparatus and were uncorrected. C, H elemental analysis was carried out with Coleman Model 33. N elemental analysis was carried out by the Dumas

method. Infrared spectra (KBr pellets) were measured on a Perkin-Elmer System 2000 FT-IR. NMR spectra were recorded on Gemini NMR Spectrometer (200 MHz) determined in either DMSO- d_6 or $CDCl_3$ as solvent and using tetramethylsilane as internal standard. The mass spectrometer was a G2449A Ion-Trap Mass Spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with an electrospray ionisation (ESI) system and controlled by LCMSD software v.6.1. A syringe pump (kdScientific) was used for introducing the sample in the MS with a flow rate of 1.5 mL/h. Nitrogen was used as a nebulizing gas at pressure of 12 psi and the flow was adjusted to 5 L·min⁻¹. The heated capillary and the voltage were maintained at 350 °C and 3.5 kV, respectively. MS data were acquired in the negative ionization mode. The full scan covered the mass range m/z 100–1,000. Collision-induced fragmentation experiments were performed in the ion trap using helium as collision gas, with voltage ramping cycle from 0.3 up to 2 V. Maximum accumulation time of the ion trap and the number of MS repetitions to obtain the MS average spectra were set at 300 ms and 5, respectively.

Preparative flash chromatography was performed using Merck silica gel 60 (230–400 mesh) and thin layer chromatography (TLC) was carried out on aluminum sheets with silica gel with fluorescent indicator (254 nm), obtained from Sigma-Aldrich. Spots were visualized using either UV-lamp at 254 nm or iodine.

3.2. Preparation Procedures

All chemicals used for synthesis and purification were of p.a. grade (Merck). Commercial isatin was recrystallized twice from ethanol. Compounds **1**, **2** and **3** are not commercially available and they were prepared by adapting reported methods [2,21,22]. The Sandmeyer methodology was modified as described below. **4-chloroisnitrosoacetanilide (1)**. The reaction mixture was refluxed for 30 min and the desired product was obtained by recrystallization from ethanol (86%) m.p. 160 °C (lit. [2] rec. EtOAc, 170–172 °C). **5-chloroindoline-2,3-dione (2)**. 4-Chloroisnitrosoacetanilide (**1**) was added to a pre-warmed concentrated sulfuric acid (70–75 °C), at such a rate as to maintain the reaction temperature between 85 and 90 °C. After the addition, the reaction mixture was heated at 95–100 °C for 15 min. The red product **2** was obtained via recrystallization from 50% aqueous acetic acid (92%). M.p. 250 °C (lit. [2] rec. EtOAc, 230–232 °C). The spectroscopic data matched those reported in the literature [2].

N''-[(3Z)-1-Acetyl-5-chloro-2-oxo-1,2-dihydro-3H-indol-3-ylidene]thiocarbonohydrazide. A solution of thiocarbonohydrazide (0.53 g, 5 mmol) in 10 mL hot water was added in small aliquots to 1-acetyl-5-chloroindoline-2,3-dione (1.12 g, 5 mmol) in 30 mL of ethanol. The mixture was refluxed for five minutes and then was stirred overnight at room temperature. The resulting yellow precipitate was collected by vacuum filtration, washed with ethanol/water solution (3:1, v/v) and air dried. Bright yellow solid was obtained after recrystallization from ethanol in typical yield of 70%.

M.p.: 252–253 °C

FTIR (KBr, cm⁻¹): ν (NH) 3319, 3192, 3174, 3140, ν (CO) 1672, ν (CO) lactam 1605.

^1H -NMR (200 MHz, DMSO- d_6): δ /ppm 14.15 (s, 1H, NH, C=N-NH-CS), 9.54 (s, 1H, NH CSNHNH $_2$), 6.62 (s, 2H, CSNHNH $_2$), 7.9–7.47 (m, 3H, Ar-H), 2.01 (s, 3H, CH $_3$ CO).

^{13}C -NMR (200 MHz, DMSO- d_6): δ /ppm 182.87 (C=S), 168.68 (C=O, NCOCH $_3$), 148.64 (C=O, lactam), 143.34 (C=N), 136.38 (C $_{\text{Ar}}$), 130.71 (C $_{\text{Ar}}$), 130.18 (C $_{\text{Ar}}$), 127.59 (C $_{\text{Ar}}$), 125.63 (C $_{\text{Ar}}$), 125.00 (C $_{\text{Ar}}$), 24.15 (NCOCH $_3$).

MS m/z : $[\text{M}-\text{H}]^-$ at 310 (100%) represents the molecular ion with formula C $_{11}$ H $_{10}$ ^{35}Cl N $_5$ O $_2$ S (minus one H due to the negative ionization mode). The corresponding isotopic peak (for ^{37}Cl) was observed at m/z 312 (26.1%).

MS 2 m/z : 236 (100%), 237 (7.5%), 238 (28.0%), 208 (1.5%), 194 (1%), 166 (1.5%).

MS 3 m/z : 194 (91.1%), 195 (10.2%), 196 (20.5%), 166 (100%), 167 (11.7%), 168 (22.6%).

Anal. Calcd. for C $_{11}$ H $_{10}$ ClN $_5$ O $_2$ S (311.75): C, 42.38; H, 3.23; N, 22.46. Found: C, 42.22; H, 3.16; N, 22.25.

4. Conclusions

The isatin derivatives are very useful synthetic intermediates and can function as suitable building blocks for preparation of other biologically active compounds. The N'' -[(3Z)-1-acetyl-5-chloro-2-oxo-1,2-dihydro-3H-indol-3-ylidene]thiocarbonohydrazide was synthesized in good yield and its identity was confirmed using FT-IR, ^1H -NMR, ^{13}C -NMR and MS n . The biological properties and the crystal structure of the newly synthesized **4** will be thoroughly investigated.

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