Synthesis, Spectroscopy, Computational and Anticancer Evaluation of Some Novel Isatin Derivatives

Rabab T Shalof*, Eman H Tawfik and Fadda AA
Department of Chemistry, Faculty of Science, Mansoura University, Mansoura, Egypt

*Corresponding author: Rabab T Shalof, Department of Chemistry, Faculty of Science, Mansoura University, Mansoura, 35561, Egypt, Tel: 002-050-2242388; E-mail: rababtawfik2009@yahoo.com

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Abstract

A series of Isatin derivatives was synthesized using potassium 2-cyano-3-oxo-3-(2-(2-oxoindolin-3-ylidene)hydrazinyl)-1-(phenylamino)prop-1-ene-1-thiolate (2) as starting material. Compound (2) reacts with various reagents under different conditions to give the corresponding thiazol, thiophen and pyridine derivatives, which were characterized by elemental analysis, spectroscopy (1H-NMR, IR and Mass spectra). The anticancer activities of the newly synthesized compounds were studied against colon carcinoma cells by using the Minimum Inhibition Concentration (MIC) method. Compounds belonging to 8, 12 and 13 series produced a high anti-cancer reactivity.

Keywords: Isatin; Heterocyclic; Thiazol; Thiophen; Pyridine; Antimicrobial activity

Introduction

Isatin or 1H-indole-2,3-dione is an indole derivative. The compound was first obtained [1,2] as a product from the oxidation of indigo dye by nitric acid and chromic acid. Isatin forms a blue dye if it is mixed with sulfuric acid and crude benzene. The formation of the blue indophenin was long believed to be a reaction with benzene. Victor Meyer was able to isolate the substance responsible for this reaction from benzene. This new heterocyclic compound was thiophene [3]. Isatin is exerting a broad spectrum of biological activity like antipyretic activity, analgesic effect anticonvulsant activity; few compounds were also reported as psychotropic agents and Monoamine Oxidase (MAO) inhibitors [3].

Isatins are an important group of heterocyclic compounds which are biologically active and of significant importance in medicinal chemistry. A literature survey identified several Isatin derivatives in the development phase as potential new drugs. Isatin (1H-indole-2,3-Dione) and its derivatives exhibit various biological activities such as antitumor [4], anticonvulsant [5], anti-inflammatory [6], antimicrobial, antiviral [7] and ant neo-plastic activities [8]. These compounds are versatile building blocks for the synthesis of a large variety of heterocyclic compounds such as indoles, isothiocyanate, quinolines, spirooxindoles, and etc. The unique structural array of these compounds has made them attractive synthetic targets in chemistry. Isatins are capable of crossing the blood-brain-barrier [9].

Isatin, a heterocyclic compound was identified in animals as a major component of the endogenous MAO inhibitors. The various substituents at 3rd position of the Isatin which were reported various substituited phenyl ring moieties, heterocyclic rings and aliphatic system. Isatin (1H-Indole-2,3-dione) is one of the most promising new class of heterocyclic molecules having many interesting activity profiles and well-tolerated in human subjects. As a continuation of our efforts [10-19] to identify new condition that may be of value in designing new, potent, selective, less toxic antimicrobial agents, we report here the synthesis of some new heterocycles incorporating an indole moiety starting from cyanoacetohydrazide and Isatin, we found that 2-cyano-N’-(2-oxoindolin-3-ylidene) acetohydrazide (1) [20] is a highly active against tumor cells and useful building for the synthesis of a variety of phenylthiazolidin, phenylthiazol and thiophene derivatives incorporating sati moiety of potential biological activity.

Materials and Methods

Instruments

All melting points (m.p.) are recorded in Gallenkamp electric m.p. apparatus and are uncorrected. The IR spectra vcm-1 (KBr) were recorded in Perkin Elmer Infrared Spectrophotometer Model 157, Grating. The 1H-NMR spectra were run on Varian Spectrophotometer at 300 and 75 MHz, respectively, using Tetramethylsilane (TMS) as an internal reference and DMSO-d6 as solvent. The mass spectra (EI) were recorded on 70 eV with Kratos MS equipment and/or a Varian MAT 311 A Spectrometer at Micro analytical Unit, Faculty of Science, Cairo University and Al-Azhar University, Cairo, Egypt. Elemental analyses (C, H and N) were carried out at the micro analytical center of Cairo University, Giza, Egypt, the results were found to in good agreement (± 0.3%) with the calculated values. Antimicrobial screening for the selected new compounds was carried out in the regional center for mycology and biochemistry, Al-Azhar University, Cairo, Egypt.

Chemistry

Potassium-2-cyano-3-oxo-3-(2-(2-oxoindolin-3-ylidene)hydrazinyl)-1(phenylamino)prop-1-ene-1-thiolate (2): The reaction of 2-cyano-N’-(2-oxoindolin-3-ylidene) acetohydrazide (1) with phenyl isothiocyanate in the presence of potassium hydroxide in Dimethylformamide (DMF) to give a potassium 2-cyano-3-Oxo-3-(2-(2-oxoindolin-3-ylidene)hydrazinyl)-1-(phenylamino)prop-1-ene-1-thiolate (2) was prepared as previously described [21].

2-cyano-3-oxo-3-(2-(2-oxoindolin-3-ylidene)hydrazinyl)-N-phenylpropanethioamide (3): To a stirred solution of potassium hydroxide (0.01 mol) in N,N-DMF (20 ml) was added compound (1)
(0.01 mol). After the reaction mixture was stirred for 30 min, the solid product was filtered off, washed with water and recrystallized from ethanol to give the compound (3) [10].

3-(arythio)-2-cyano-N'-(2-oxoindolin-3-ylidene)-3-(phenylamino)acrylohydrazide (4a-e): Equal molar amounts of (2) (0.01 mol) in DMF (20 ml) were stirring, then added appropriate 2-chloroacetyl chloride, ethyl 2-bromoacetate, 2-chloroacetone, 2-chloroacetonitrile and phenyl bromide (0.01 mol) was added portion wise over a period of 30 min. After the addition was complete, the reaction mixture was stirred for 24 hrs the intermediates (4a-e) was formed. Then poured onto crushed ice containing hydrochloric acid, the solid product was filtered off, washed with ethanol, dried and crystallized from an ethanol/DMF mixture to give the compound (4a-e).

2-(2-cyano-3-oxo-3-(2-oxoindolin-3-ylidene)hydrazinyl)-1(phenylimino)-prop-1-en-1-yl)acetyl chloride (4a): Brown powder; 180-182°C; (85%); IR (KBr) ν̇: 3566 (NH), 3447 (NH), 3061 (NH), 2198 (CN), 1700, 1669 and 1621 (3C=O); 1H-NMR (DMSO-d6, δ, ppm): 4.45 (s, 2H, CH2), 6.96-7.46 (m, 9H, ArH), 10.03 (s, 1H, NH-amid), 10.44 (s, 1H, NH), 11.133 (s, 1H, NH-hydrazid). MS m/z (%): 439 (M+ +1, 100), 440.10 (58.0%), 456.05 (51.6%), 403.05 (26.9%).


2-cyano-3-((2-oxo-2-phenylethyl)thio)-N'-(2-oxoindolin-3-ylidene)-3-(phenylamino)acrylohydrazide (4f): Dark brown crystals; 211-213°C; (85%); IR (KBr) ν̇max cm⁻¹: 3450 (NH), 3327 (NH), 3193 (NH), 2218 (CN) 1729, 1693 (2C=O); 1H-NMR (DMSO-d6, δ, ppm): (3 s, 2H, CH2), 6.96-7.46 (m, 9H, ArH), 10.03 (s, 1H, NH-amid), 10.34 (s, 1H, NH), 12.133 (s, 1H, NH-hydrazid). MS m/z (%): 481 (M+ +1, 100), 482.12 (28.1%), 484.12 (4.5%), 483.13 (2.7%).

2-cyano-2-(4-oxo-3-phenylthiazolidin-2-ylidene)-N'-(2-oxoindolin-3-ylidene)-acetohydrazide (5): Equal molar amounts of (2) (0.01 mol) in DMF (20 ml) were stirring, then added appropriate 2-chloroacetyl chloride (0.01 mol) and ethyl 2-bromoacetate (0.01 mol) the reaction mixture was stirred for 24 hrs the intermediates was formed (4(a,b)). After 24 hrs the reaction mixture was heated under reflux for 5 hrs and then poured onto crushed ice containing hydrochloric acid. The formed solid product was followed off, washed with ethanol, dried and crystallized from an ethanol/DMF mixture to give the compound (5).

Ethyl-2-(2-cyano-3-oxo-3-(2-oxoindolin-3-ylidene)hydrazinyl)-1(phenylimino)-prop-1-en-1-yl)acetoacetate (4b): Brown crystals; 165-167°C; (85%); IR (KBr) ν̇max cm⁻¹: 3316, 3190 (2NH), 3109 (NH), 2218 (CN), 1729, 1693 and 1650 (3C=O); 1H-NMR (DMSO-d6, δ, ppm): 1.5 (t, 3H, CH3), 3.98 (s, 2H, CH2), 4.18 (q, 2H, CH2), 6.96-7.46 (m, 9H, ArH), 10.03 (s, 1H, NH-amid), 10.44 (s, 1H, NH), 11.133 (s, 1H, NH-hydrazid). MS m/z (%): 449 (M+ +1, 100), 450.12 (23.8%), 451.11 (4.5%), 451.12 (2.7%).

Anal. for: C2H12ClN2O3S (M. wt. 493): Calc.: C, 58.79; H, 4.32; N, 15.38. Found: C, 58.81; H, 4.11; N, 15.60%.

2-(2-cyano-3-N'-(2-oxoindolin-3-ylidene)-3-(2-oxopropyl)thio)-1(phenylimino)-prop-1-en-1-yl)acetyl chloride (4c): Light brown crystals; 190-192°C; (85%); IR (KBr) ν̇max cm⁻¹: 3422(H), 3327 (NH), 3193 (NH), 2179 (CN), 1709, 1651, 1592 (3C=O); 1H-NMR (DMSO-d6, δ, ppm): 2.28 (s, 3H, CH3), 4.03 (s, 2H, CH2), 6.96-7.46 (m, 9H, ArH), 10.03 (s, 1H, NH-amid), 10.44 (s, 1H, NH), 12.13 (s, 1H, NH-hydrazid). MS m/z (%): 419 (M+ +1, 100), 420.11 (22.7%), 421.10 (4.5%), 421.12 (2.5%), 420.10 (4.8%).

Anal. for: C2H12N3O3S (M. wt. 419): Calc.: C, 60.13; H, 4.09; N, 16.70. Found: C, 60.15; H, 4.11; N, 16.72%.

2-cyano-3-((cynamethythio)-N'-(2-oxoindolin-3-ylidene)-3(phenylamino)acrylohydrazide (4d): Brown crystals; 210-212°C; (85%); IR (KBr) ν̇max cm⁻¹: 3450 (NH), 3327 (NH), 3193 (NH), 2228 (CN) 1729, 1693 (2C=O); 1H-NMR (DMSO-d6, δ, ppm): 4.38 (s, 2H, CH2), 6.96-7.46 (m, 9H, ArH), 10.03 (s, 1H, NH-amid), 10.34 (s, 1H, NH), 12.133 (s, 1H, NH-hydrazid). MS m/z (%): 402 (M+ +1, 100), 403.09 (21.6%), 402.09 (4.5%), 382.10 (2.2%).

the crude product was precipitated, collected by filtration and crystallized from ethanol/DMF. Yield 45% as dark brown m.p. 290-292°C, FT-IR (KBr, cm⁻¹): 3566 (NH), 3591(NH), 3421(NH) and 1706 (C=O), 1617(C=O); 1H-NMR (DMF-d6, δ, ppm): 6.91(s, 2H, NH2), 6.95-7.59(m, 9H, ArH), 10.25(s, 1H, NH-amiid), 10.91(NH) and 11.13(s, 1H, NH-hydradiz). MS m/z (%): 481 (M⁺ + 1, 100), 482.12 (28.1%), 483.12 (4.5%), 485.12 (2.7%), 482.12 (1.8%), 481.12 (1.3%), 483.12 (1.1%). Anal. for C2H2N3O2S (M. wt. 481): Calc.: C, 66.67; H, 3.84; N, 19.24. Found: C, 66.87; H, 4.00; N, 19.14.

6-amino-2-oxo-1-(2-oxoindolin-3-ylidine)amino)-4-aryl-1,2-dihydropyridine-3,5-dicarbonitrile 9: Yellow crystals, m.p. 270-272°C, yield 45%; FT-IR (KBr, cm⁻¹); 3571 (NH), 3345 (NH2), 2204 (CN) and 1721 (C=O); 1H-NMR (DMF-d6, δ, ppm): 4.6 (s, H, NH), 5.98 (2s, 2H, NH2), 6.91-7.59(m, 9H, ArH), 10.40 (s, 1H, NH-amiid), MS m/z: 380.10(100.0%), 381.11 (22.7%), 382.11 (2.5%), 381.12 (2.2%). (M⁺ + 1, 100). Anal. for C12H14N3O4S (M. wt: 381): Calc.: C, 63.64; H, 4.72; N, 21.20. Found: C, 63.33; H, 4.72; N, 21.25.

6-amino-4-(4-hydroxyphenyl)-2-oxo-1-(2-oxoindolin-3-ylidine)amino)-1,2-dihydropyridine-3,5-dicarbonitrile 10: Orange crystals, m.p. 270-272°C, yield 45%; FT-IR (KBr, cm⁻¹); 3577 (NH), 3328 (OH), 2200 (CN) and 1721 (C=O); 1H-NMR (DMF-d6, δ, ppm): 4.6 (s, H, NH), 5.98 (2s, 2H, NH2), 6.84-7.36 (m, 9H, ArH), 9.60(s, 1H, OH), 10.40 (s, 1H, NH-amiid), MS m/z: 396.10 (100.0%), 397.10 (22.7%), 398.10 (2.5%), 397.09 (2.2%)(M⁺ + 1, 100). Anal. for C12H14N3O5S (M. wt: 396): Calc.: C, 64.87; H, 4.00; N, 14.56.

6-amino-4-(4-hydroxy-3-methoxyphenyl)-2-oxo-1-((2-oxoindolin-3-ylidene)amino)-4-aryl-1,2-dihydropyridine-3,5-dicarbonitrile 11: Green crystals, m.p. 270-272°C, yield 45%; FT-IR (KBr, cm⁻¹): 3677 (NH), 3328 (OH), 2200 (CN) and 1721 (C=O); 1H-NMR (DMF-d6, δ, ppm): 3.83(s, 3H, OCH3), 5.98 (2s, 2H, NH2), 6.84-7.36 (m, 9H, ArH), 9.60(s, 1H, OH), 10.32 (s, 1H, NH-amiid), MS m/z: 427.11 (23.8%), 428.11 (2.7%), 427.10 (2.2%). (M⁺ + 1, 100). Anal. for C12H14N3O5S (M. wt: 427): Calc.: C, 63.64; H, 3.84; N, 21.20. Found: C, 63.66; H, 3.07; N, 21.25.

6-amino-4-(4-chlorophenyl)-2-oxo-1-(2-oxoindolin-3-ylidine)amino)-1,2-dihydropyridine-3,5-dicarbonitrile 12: Red crystals, m.p. 270-272°C, yield 45%; FT-IR (KBr, cm⁻¹): 3677 (NH), 2214 (CN) and 1721 (C=O); 1H-NMR (DMF-d6, δ, ppm): 5.6 (s, H, NH), 6.91-7.59(m, 9H, ArH), 10.40 (s, 1H, NH-amiid), m/z: 414.06 (100.0%), 416.06 (32.0%), 415.07 (22.7%), 417.06 (7.3%), 416.07 (2.5%), 415.06 (2.2%). (M⁺ + 1, 100). Anal. for C12H14ClN3O5S (M. wt: 414): Calc.: C, 60.81; H, 2.67; N, 20.26. Found: C, 60.83; H, 2.69; N, 20.28.

Anticancer evaluation of cytotoxicity against HCT cell line

**Antitumor activity assay:** Human colon carcinoma (HCT-116) cell line was obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 µg/ml gentamycin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO₂ and were subcultured two to three times a week. For antitumor assays, the tumor cell lines were suspended in medium at concentration 5 x 10⁴ cell/well in Corning® 96-well tissue culture plates, then incubated for 24 hrs.

The tested compounds were then added in 96-well plates (six replicates) to achieve eight concentrations of each compound. Six vehicle controls with media or 0.5% DMSO were run for each 96 well plate as a control. After incubating for 24 hrs, the numbers of viable cells were determined by the MTT test. Briefly, the media was removed from the 96 well plate and replaced with 100 µl of fresh culture RPMI 1640 medium without phenol red, then 10 µl of the 12 mm MTT stock solution (5 mg of MTT in 1 ml of PBS) to each well including the untreated controls (Figure 1). The 96 well plates were then incubated at 37°C and 5% CO₂ for 4 hrs. An 85 µl aliquot of the media was removed from the wells, and 50 µl of DMSO was added to each well and mixed thoroughly with the pipette and incubated at 37°C for 10 min. Then, the optical density was measured at 590 nm with the micro plate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [1-(ODt/ODc)]x 100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells [22-24].

The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC₅₀), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. The synthesized compounds (5-13) showed the greater selectivity towards colon carcinoma cells (Table 1). The most active compound shown antiproliferative at low micro molar concentration and also activated the effector caspases in a dose dependent manner. It is interesting that the compound (8), (12) and (13) showed significant growth inhibitory activity on the HCT-116 c, while (5) and (6) showed the lowest effect on the same tumor cell line (Figure 1).
isothiocyanate in KOH/DMF followed by spectrum, which showed band in the region 3316, 3190 and 3109 cm$^{-1}$ due to methyl group and showed singlet at δ 3.98 due to methylene (4a), was established based on IR spectrum, which showed band in the region 3327-3193 cm$^{-1}$ due to aromatic and also, showed quarterly at δ 4.18 due to methylene in addition to aromatic multiple in the region δ 6.91-7.46 and three single’s at δ 10.03, 10.44 and 12.133 due to NH-amid and NH-hydrazid.

The intermediate (3) was obtained in good yield upon treatment of (1) with phenyl isothiocyanate in DMF, in the presence of catalytic amount of triethylamine at room temperature yielded the corresponding C-condensation product (1). Compound (3) was obtained in good yield upon treatment of (1) with phenyl isothiocyanate in KOH/DMF followed by acidification with dilute HCl.

The intermediate (2) with phenyl isothiocyanate in DMF, in the presence of potassium hydroxide give the intermediate (2) and also, showed a singlet at δ 4.03 due to methylene in addition to aromatic multiple in the region δ 6.91-7.46 and three single’s at δ 10.03, 10.44 and 12.133 due to NH-amid, -NH and NH-hydrazid.

The intermediate (4d) (Scheme 2). The IR spectrum of (4d) lacked an absorption band due to nitrile function and revealed absorption bands at 3423, 3327 and 3193 cm$^{-1}$ due to three (NH) groups, a strong, sharp band at 2179 cm$^{-1}$ due to nitrile function and strong absorption band at 1709 cm$^{-1}$ due to the carbonyl group (C=O). Moreover, its $^1$H-NMR showed singlet at δ 2.28 due to methyl group and also, showed a singlet at δ 3.98 due to methylene in addition to aromatic multiple in the region δ 6.91-7.46 and three single’s at δ 10.03, 10.44 and 12.133 due to NH-amid and NH-hydrazid.

Treatment of the Isatin (1) with phenyl isothiocyanate in DMF, in the presence of potassium hydroxide give the intermediate (2) and also, an equal molar amount of 2-chloroacetylchloride and ethyl 2-bromoacetate reacted with intermediate (2) give intermediate (4(a,b)) furnished, in each case give the same product (5). The intermediate (4a), was established based on IR spectrum, which showed band in the region 3566,3447 and 3061 cm$^{-1}$ due to three (NH) groups, a strong, sharp band at 2179 cm$^{-1}$ due to nitrile function and strong absorption band at 1709 cm$^{-1}$ due to the carbonyl group (C=O), Moreover, its $^1$H-NMR showed singlet at δ 4.45 due to methylene in addition to aromatic multiple in the region δ 6.91-7.46 and three single’s at δ 10.03, 10.44 and 12.133 due to NH-amid, NH group and NH-hydrazid (Scheme 1). Also, the intermediate (4b) was established based on IR spectrum, which showed band in the region 3316, 3190 and 3109 cm$^{-1}$ due to three (NH) groups, a strong, sharp band at 2218 cm$^{-1}$ due to nitrile function and strong absorption band at 1729 cm$^{-1}$ due to the carbonyl group (C=O). Moreover, its $^1$H-NMR showed triplet at δ 1.5 due to methyl group and showed singlet at δ 3.98 due to methylene and also, showed quarterly at δ 4.18 due to methylene in addition to aromatic multiple in the region δ 6.91-7.46 and three single’s at δ 10.03, 10.44 and 12.133 due to NH-amid and NH-hydrazid. The reaction products were identified as (2-cyano-2-(4-oxo-3-phenylthiazolidin-2-ylidene)-N’-(2-oxoindolin-3-ylidene) acetohydrazide (6). The intermediate (4c) which showed on IR spectrum a strong band in the region 3423, 3327 and 3193 cm$^{-1}$ due to three (NH) groups, a strong, sharp band at 2179 cm$^{-1}$ due to nitrile function and strong absorption band at 1709 cm$^{-1}$ due to the carbonyl group (C=O). Moreover, its $^1$H-NMR showed singlet at δ 4.03 due to methylene in addition to aromatic multiple in the region δ 6.91-7.46 and three single’s at δ 10.03, 10.44 and 12.133 due to NH-amid and NH-hydrazid.

The microanalytical and spectroscopic data were in agreement with the proposed structure (3) [10].

The reaction of Intermediate (2) with chloroacetone containing the nitrile function and strong absorption band at 1729 cm$^{-1}$ due to the carbonyl group (C=O). Moreover, its $^1$H-NMR showed singlet at δ 1.5 due to methyl group and also, showed a singlet at δ 3.98 due to methylene in addition to aromatic multiple in the region δ 6.91-7.46 and three single’s at δ 10.03, 10.44 and 12.133 due to NH-amid, -NH and NH-hydrazid.

The intermediate (4d) (Scheme 2). The IR spectrum of (4d) lacked an absorption band due to nitrile function and revealed absorption bands at 3423, 3327, 3193, 2218 and 1709 cm$^{-1}$ characteristic of three NH, C=N and C=O functions, respectively. The $^1$H-NMR spectrum of (4d) showed a singlet at δ 4.38 due to methylene in addition to aromatic multiple in the region δ 6.91-7.46 and three single’s at δ 10.03, 10.44 and 12.43 due to CH-thiazole, NH-amid and NH-hydrazid (Scheme 1).

### Table 1: Compounds 5-13 selectivity towards colon carcinoma cells.

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### Results and Discussion

**Chemistry**

It was now that the reaction of Isatin with cyanoacetohydrazide in the presence of catalytic amount of triethylamine at room temperature yielded the corresponding C-condensation product (1). Compound (3) was obtained in good yield upon treatment of (1) with phenyl isothiocyanate in KOH/DMF followed by acidification with dilute HCl. The IR spectrum of the product (6) while revealed the presence of 3369, 3193 cm$^{-1}$ due to carbonyl group and the $^1$H-NMR spectrum of compound (6) showed three singlet at δ 5.56, 10.03 and 11.133 due to CH-thiazole, NH-amid and NH-hydrazid (Scheme 1).

2-chloroacetonitrile reacted with intermediate (2) to afford a product identifier 2-(4-amino-3-phenylthiazol-2(3H)-ylidene)-2-cyano-N’-(2-oxoindolin-3-ylidene) acetohydrazide 7 through the intermediate (4d) (Scheme 2). The IR spectrum of (4d) lacked an absorption band due to nitrile function and revealed absorption bands at 3423, 3327, 3193, 2218 and 1709 cm$^{-1}$ characteristic of three NH, C=N and C=O functions, respectively. The $^1$H-NMR spectrum of (4d) showed a singlet at δ 4.38 due to methylene in addition to aromatic multiple in the region δ 6.91-7.46 and three single’s at δ 10.03, 10.44 and 12.133 due to CH-thiazole, NH-amid and NH-hydrazid (Scheme 1).
and 12.133 due to NH-amid, -NH and NH-hydrazid. The IR spectrum of the product (7) exhibits, band at 3680, 3747, 3419, 2202 and 1706 cm\(^{-1}\) due to two (NH) groups, (NH\(_2\)), (CN) group and carbonyl group, respectively. Its \(^1\)H-NMR spectrum revealed a single signal at \(\delta\) 5.023 due to NH\(_2\) and strong band at 85.50 due to CH-thiazole protons and D2O -exchangeable signal at \(\delta\) 7.95 and 8.66 due to NH-amid and NH-hydrazid. Phencyl bromide reacted with intermediate (2) to afford a product identified as 4-amino-5-benzoyl-N’-(2-oxoindolin-3-ylidene)-2-(phenylimino)-2,5-dihydrothiophene-3-carbohydrazide (8) through the intermediate (6e) which showed on IR spectrum a strong band in the region 3423, 3327 and 3193 cm\(^{-1}\) due to three (NH) groups, a strong, sharp band at 2179 cm\(^{-1}\) due to nitrile function and strong absorption band at 1709 cm\(^{-1}\) due to the carbonyl group (C=O). Moreover, its \(^1\)H-NMR showed singlet at \(\delta\) 2.28 due to methyl group and also, showed a singlet at \(\delta\) 4.03 due to methylene in addition to aromatic multiple in the region \(\delta\) 6.91-7.46 and three singlet’s at \(\delta\) 10.03, 10.44 and 12.133 due to NH-amid, -NH and NH-hydrazid. The IR spectrum of compound (8) showed the absence of Nitrile band and strong band at 2179 cm\(^{-1}\) due to secondary amine and strong absorption band at 3377 cm\(^{-1}\) due to (NH), a presence of 2214 cm\(^{-1}\) due to carbonyl group (C=O). Moreover, its \(^1\)H-NMR showed singlet at \(\delta\) 6.91 due to NH\(_2\) in addition to aromatic multiple in the region \(\delta\) 6.91-7.59 and two single’s at \(\delta\) 8.501, 10.03 and 10.91 due to NH-thiophene, NH-amid and NH-hydrazid (Scheme 2).

The reaction of (1) to 2-benzylidenemalononitrile in absolute ethanol gave 6-amino-2-Oxo-1-((2-oxoindolin-3-ylidene)amino)-4-phenyl-1,2-dihydropridine-3,5-dicarbonitrile (9) (Scheme 3).

The IR spectrum of 6-amino-2-Oxo-1-((2-oxoindolin-3-ylidene)amino)-4-phenyl-1,2-dihydropridine-3,5-dicarbonitrile (9) revealed 2CN stretching bands at 2024 cm\(^{-1}\). And a characteristic C=O stretching at 1721 cm\(^{-1}\) due to carbonyl group (C=O). Moreover, its \(^1\)H-NMR showed singlet at \(\delta\) 9.53 due to OH (Scheme 3). Also,6-amino-4-(1-hydroxynaphthalen-2-yl)-2-oxo-1-((2-oxoindolin-3-ylidene)amino)-1,2-dihydropyridine-3,5-dicarbonitrile (13) was obtained by the addition of 2-((2-hydroxynaphthalen-1-yl)methylene)malononitrile to (1) in ethanol.

The IR spectrum showed bands at 3677, 2938 and 2214 cm\(^{-1}\) due to aromatic multiple in the region \(\delta\) 6.84-7.36 and two single’s at \(\delta\) 5.98, 10.32 due to NH\(_2\) and NH-amid and at \(\delta\) 6.53 due to OH (Scheme 3).

Reflexing of (1) with 2-(3-chlorobenzylidene) malononitrile in ethanol afforded 6-amino-4-(4-chlorophenyl)-2-oxo-1-((2-oxoindolin-3-ylidene)amino)-1,2-dihydropyridine-3,5-dicarbonitrile (13) was obtained by the addition of 2-((2-hydroxynaphthalen-1-yl)methylene)malononitrile to (1) in ethanol.

The IR spectrum showed bands at 3677, 2938 and 2214 cm\(^{-1}\) due to and hydroxyl group, NH\(_2\) and nitrile group, a strong absorption band at 1706 cm\(^{-1}\) due to the carbonyl group (C=O). Moreover, its \(^1\)H-NMR showed singlet at \(\delta\) 3.83 due to the methoxy group in addition to aromatic multiple in the region \(\delta\) 6.84-7.36 and two single’s at \(\delta\) 5.98, 10.32 due to NH\(_2\) and NH-amid and at \(\delta\) 6.53 due to OH (Scheme 3).

Conclusion

Isatin (1H-indole-2,3-dione) are synthetically versatile substrates, where they can be used for the synthesis of a large variety of...
heterocyclic compounds, and as raw material for drug synthesis. The advances in the use of Isatin for organic synthesis during the last 25 years, as well as a survey of its biological and pharmacological properties are reported in this review and in the accompanying supplementary information. The survey of the literature revealed that, Isatin is a versatile lead molecule for designing potential bioactive agents, and its derivatives were reported to possess broad-spectrum anticonvulsant, anxiety activities and against cancer of colon and give the unexpected result. Further we can conclude that many other derivatives of Isatin can be synthesized which will be expected to show potent pharmacological activities.

References