Chemical Sciences Journal
ISSN: 2150-3494

Shalof et al., Chem Sci J 2018, 9:3
DOI: 10.4172/2150-3494.1000196

Research Article Open Access

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

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Received date: September 22, 2018; **Accepted date:** October 15, 2018; **Published date:** October 30, 2018

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 (^{1}H-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 ^{1}H-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 (^{1}H-indole-2,3-Dione) and its derivatives exhibit various biological activities such as anticancer [4], anticonvulsant [5], anti-inflammatory [6], antimicrobial, antiviral [7] and anti neo-plastic activities [8]. These compounds are versatile building blocks for the synthesis of a large variety of heterocyclic compounds such as indoles, isotopic anhydride, quinolines, spirooxin-doles, 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 substituted phenyl ring moieties, heterocyclic rings and aliphatic system. Isatin (^{1}H-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 satin 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 νcm^{-1} (KBr) were recorded in Perkin Elmer Infrared Spectrophotometer Model 157, Grating. The ^{1}H-NMR spectra were run on Varian Spectrophotometer at 300 and 75 MHz, respectively, using Tetramethylsilane (TMS) as an internal reference and DMSO-d_6 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 mixture was stirred for 30 min, phenyl isothiocyanate (0.01 mol) was added to the resulting mixture. Stirring was continued at room temperature for 12 hrs. The reaction mixture was acidified with cold dilute HCl. The solid product that separated was filtered, washed with water and recrystallized from ethanol to give **(3)** [10].

3-(argiothio)-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-chloroacetylchloride, ethyl 2-bromoacetate, 2-chloroacetone, 2-chloroacetonitrile and phenyl bromide (0.01 mol) was added portion wisely over a period of 30 min. After the addition was complete, the reaction mixture was stirred for 24 hrs the intermediates **(4(a-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 **(4(a-e))**.

2-((2-cyano-3-oxo-3-(2-(2-oxoindolin-3-ylidene)hydrazinyl)-1(phenylamino)-prop-1-en-1-yl)thio)acetylchloride (4a): Brown powder; 180-182°C; (85%); IR (KBr) ν_{max} cm^{-1}: 3566 (NH), 3447 (NH), 3061 (NH), 2198 (CN), 1700, 1669 and 1621 (3C=O); ^{1}H-NMR (DMSO-d_6, δ, ppm): 4.45 (s, 2H, CH_2), 6.96-7.46 (m, 9H, ArH), 10.03 (s, ^{1}H, NH-amid), 10.44 (s, ^{1}H, NH), 12.133 (s, ^{1}H, NH-hydrazid). MS m/z (%): 439 (M^+ +1, 100), 440.10 (58.0%), 436.05 (51.6%), 403.05 (26.9%).

Anal. for: $C_{20}H_{14}ClN_5O_3S$ (M. wt. 439): Calc.: C, 54.61; H, 3.21; N, 15.92. Found: C, 54.87; H, 3.23; N, 15.94%.

Ethyl-2-((2-cyano-3-oxo-3-(2-(2-oxoindolin-3-ylidene)hydrazinyl)-1(phenylamino)prop-1-en-1-yl)thio)acetate (4b): Brown crystals; 165-167°C; (85%); IR (KBr) ν_{max} cm^{-1}: 3316, 3190 (2NH), 3109 (NH), 2218 (CN), 1729, 1693 and 1650 (3C=O); ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.5 (t, 3H, CH_3), 3.98 (s, 2H, CH_2) 4,18 (q, 2H, CH_2), 6.96-7.46 (m, 9H, ArH), 10.03 (s, ^{1}H, NH-amid), 10.44 (s, ^{1}H, NH), 11.133 (s, ^{1}H, NH-hydrazid). MS m/z (%): 449 (M^+ +1, 100), 450.12 (23.8%), 451.11 (4.5%), 451.12 (2.7%). Anal. for: $C_{22}H_{19}N_5O_4S$ (M. wt. 449): Calc.: C, 58.79; H, 4.26; N, 15.58. Found: C, 58.81; H, 4.28; N, 15.60%.

(2-)2-cyano-N'-(2-oxoindolin-3-ylidene)-3-((2-oxopropyl)thio)-3(phenylamino)- acrylohydrazide (4c): Light brown crystals; 190-192°C; (85%); IR (KBr) ν_{max} cm^{-1}:3423(H), 3327 (NH), 3193 (NH), 2179 (CN), 1709, 1651, 1592 (3C=O); ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.28 (s, 3H, CH_3), 4.03 (s, 2H, CH_2), 6.96-7.46 (m, 9H, ArH), 10.03 (s, ^{1}H, NH-amid), 10.44 (s, ^{1}H, NH), 12.13 (s, ^{1}H, NH-hydrazid). MS m/z (%): 419 (M^+ +1, 100), 420.11 (22.7%), 421.10 (4.5%), 421.11 (2.5%), 420.10 (4.8%). Anal. for: $C_{21}H_{17}N_5O_3S$ (M. wt. 419): Calc.: C, 60.13; H, 4.09; N, 16.70. Found: C, 60.15; H, 4.11; N, 16.72%.

(2-)2-cyano-3-((cyanomethyl)thio)-N'-(2-oxoindolin-3-ylidene)-3(phenylamino)-acrylohydrazide (4d): Brown crystals; 210-212°C; (85%); IR (KBr) ν_{max} cm^{-1}: 3450 (NH), 3327 (NH), 3193 (NH), 2228 (CN) 1729, 1693, (2C=O); ^{1}H-NMR (DMSO-d_6, δ, ppm): 4.38 (s, 2H, CH_2), 6.96-7.46 (m, 9H, ArH), 10.03 (s, ^{1}H, NH-amid), 10.34 (s, ^{1}H, NH), 12.133 (s, ^{1}H, NH-hydrazid). MS m/z (%): 402 (M^+ +1, 100), 403.09 (21.6%), 402.09 (4.5%), 382.10 (2.2%). Anal. for: $C_{20}H_{14}N_6O_2S$ (M. wt. 402): Calc.: C, 59.69; H, 3.51; N, 20.88. Found: C, 59.71; H, 3.53; N, 20.90%.

2-cyano-3-((2-oxo-2-phenylethyl)thio)-N'-(2-oxoindolin-3-ylidene)-3-(phenylamino)- acrylohydrazide (4e): Dark brown crystals; 211-213°C; (85%); IR (KBr) ν_{max} cm^{-1}: 3450 (NH), 3327 (NH), 3193 (NH), 2218 (CN) 1729, 1693 (2C=O); ^{1}H-NMR (DMSO-d_6, δ, ppm): 4.03 (s, 2H, CH_2), 6.96-7.46 (m, 9H, ArH), 10.03 (S, ^{1}H, NH-amid), 10.34 (s, ^{1}H, NH), 12.133 (s, ^{1}H, NH-hydrazid). MS m/z (%): 481 (M^+ +1, 100), 482.12 (28.1%), 484.12 (4.5%), 483.13 (2.7%). Anal. for: $C_{26}H_{19}N_5O_3S$ (M. wt. 481): Calc.: C, 64.85; H, 3.98; N, 14.54. Found: C, 64.87; H, 4.00; N, 14.56%.

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-chloroacetylchloride (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 filtered off, washed with ethanol, dried and crystallized from an ethanol\DMF mixture to give the compound **(5)**; dark brown crystals; 250-252°C; (85%); IR (KBr) ν_{max} cm^{-1}: 3327 (NH), 3193 (NH), 2218 (CN) 1729, 1693 (2C=O); ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.22 (s, 2H, CH_2), 6.96-7.46 (m, 9H, ArH), 10.03 (S, ^{1}H, NH-amid), 11.133 (s, ^{1}H, NH-hydrazid). MS m/z (%): 401.09 (M^+ +1, 100), 404.08 (21.6%), 405.07 (4.5%), 405.08 (2.2%). Anal. for: $C_{20}H_{13}N_5O_3S$ (M. wt. 403): Calc.: C, 59.55; H, 3.25; N, 17.36%. Found: C, 59.57; H, 3.27; N, 17.38%.

2-(4-aryl-3-phenylthiazol-2(3H)-ylidene)-2-cyano-N'-(2-oxoindolin-3-ylidene)- acetohydrazide (6,7): A stirring of 2 (0.01 mol) in 1,4-dioxan for 6 hrs at room temperature, then added appropriate 2-chloroacetone and 2-chloroacetonitrile (0.01 mol). After the addition, the reaction mixture was stirred for 24 hrs at room temperature was formed **(4(c,d))**. After 24 hrs the reaction mixture was heated under reflux for 5 hrs. Then poured onto crushed ice containing hydrochloric acid. The formed solid product was filtered off, washed with ethanol, dried and crystallized from an ethanol\DMF mixture to give the compounds 6, 7.

2-cyano-N'-(2-oxoindolin-3-ylidene)-2-(thiazol-2(5H)-ylidene)acetohydrazide 6: Dark brown crystals, m.p. 270-272°C, yield 45% ; FT-IR (KBr, cm^{-1}): 3369 (NH), 3193 (NH), 2199 (CN) and 1700 (C=O); ^{1}H-NMR (DMSO-d_6, δ, ppm): 2 (s, 3H, CH_3), 5.56 (s, ^{1}H, CH), 6.91-7.59(m, 9H, ArH), 10.03 (s, ^{1}H, NH-amid), 11.133 (s, ^{1}H, NH-hydrazid). MS m/z (%): 403 (M^+ +1, 100), 402.10 (22.7%), 403.09 (4.5%), 403.10 (2.5%), 402.09 (1.8%). Anal. for $C_{21}H_{15}N_5O_2S$: (M. wt. 401): Calc.: C, 62.83; H, 3.77; N, 17.45%. Found: C, 62.85; H, 3.79; N, 17.47%.

2-(4-amino-3-phenylthiazol-2(3H)-ylidene)-2-cyano-N'-(2-oxoindolin-3-ylidene)- acetohydrazide 7: Dark brown crystal, m.p. 255-257°C, yield 45%; FT-IR (KBr, cm^{-1}): 3747 (NH), 3680(NH), 3419(NH_2), 2202 (CN) and 1706 (C=O); ^{1}H-NMR (DMSO-d_6, δ, ppm): 5.023(s, 2H, NH_2),5.51(s, ^{1}H, CH), 6.91-7.59(m, 9H, ArH), 9.80 (S, ^{1}H, NH-amid), 12.2 (s, ^{1}H, NH-hydrazid). MS m/z (%): 402 (M^+ +1, 100), 403.09 (21.6%), 404.09 (4.5%), 404.10 (2.2%), 403.09 (2.2%). Anal. for $C_{20}H_{14}N_6O_2S$: (M. wt. 402): Calc.: C, 62.83; H, 3.51; N, 20.88%. Found: C, 62.85; H, 3.53; N, 20.90%.

4-amino-5-benzoyl-N'-(2-oxoindolin-3-ylidene)-2-(phenylimino)-2,5-dihydrothiophene-3-carbohydrazide 8: To a solution of **(2)** in DMF and an equal molar amount of phencyl bromide a mixture was stirred with heat for 5 hrs **(4e)** was formed. After cooling, the reaction mixture was acidified by hydrochloric acid and

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_2) and 1706 (C=O), 1617(C=O); ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.91(s, 2H, NH_2), 6.95-7.59(m, 9H, ArH), 10.25 (S, ^{1}H, NH-amid), 10.91(NH) and 11.133 (s, ^{1}H, NH-hydrazid). MS m/z (%): 481 (M^+ +1, 100), 482.12 (28.1%), 483.12 (4.5%), 483.13 (2.7%), 482.12 (1.8%), 484.12 (1.3%), 483.13 (1.1%). Anal. for $C_{20}H_{14}N_6O_2S$: (M. wt. 481): Calc.: C, 64.85; H, 3.98; N, 14.54%. Found: C, 64.87; H, 4.00; N, 14, 56%.

6-amino-2-oxo-1-((2-oxoindolin-3-ylidene)amino)-4-aryl-1,2-dihydropyridine-3,5-dicarbonitrile 9-13: Treatment of a compound of **(1)** (0.01 mol) in absolute ethanol (20 ml) containing a few drops of piperidine either different arylidene malononitrile (0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 5 hrs, then cooled and neutralized by pouring onto ice/water mixture containing a few drops of hydrochloric acid. The solid products formed, in each case, was filtered off and crystallized from ethanol.

6-amino-2-oxo-1-((2-oxoindolin-3-ylidene)amino)-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile 9: Yellow crystals, m.p. 270-272°C, yield 45% ; FT-IR (KBr, cm^{-1}): 3571 (NH), 3345 (NH_2), 2204 (CN) and 1721 (C=O); ^{1}H-NMR (DMSO-d_6, δ, ppm): 4.6 (s, H, NH), 5.98 (s, 2H, NH2), 6.91-7.59(m, 9H, ArH), 10.40 (s, ^{1}H, NH-amid), MS m/z : 380.10 (100.0%), 381.11 (22.7%), 382.11 (2.5%), 381.10 (2.2%). (M^+ +1, 100). Anal. for $C_{21}H_{12}N_6O_2$: (M. wt. 380): Calc.: C, 66.31; H, 3.18; N, 22.09; O, 8.41. Found: C, 66.33; H, 3.20; N, 22.11; O, 8.43.

6-amino-4-(4-hydroxyphenyl)-2-oxo-1-((2-oxoindolin-3-ylidene)amino)-1,2-dihydropyridine-3,5-dicarbonitrile 10: Orange crystals, m.p. 270-272°C, yield 45% ; FT-IR (KBr, cm^{-1}): 3677 (NH), 3328 (OH), 2200 (CN) and 1721 (C=O); ^{1}H-NMR (DMSO-d_6, δ, ppm): 4.6 (s, H, NH), 5.98 (s, 2H, NH_2), 6.91-7.59(m, 9H, ArH), 9.60(s, ^{1}H, OH),10.40 (s, ^{1}H, NH-amid), MS m/z: 396.10 (100.0%), 397.10 (22.7%), 398.10 (2.5%), 397.09 (2.2%)(M^+ +1, 100). Anal. for $C_{21}H_{12}N_6O_3$: (M. wt. 396): Calc.: C, 63.64; H, 3.05; N, 21.20. Found: C, 63.66; H, 3.07; N, 21.25%.

6-amino-4-(4-hydroxy-3-methoxyphenyl)-2-oxo-1-((2-oxoindolin-3-ylidene)amino)-1,2-dihydropyridine-3,5-dicarbonitrile 11: Green crystals, m.p. 270-272°C, yield 45% ; FT-IR (KBr, cm^{-1}): 3677 (NH), 3448(OH), 2214 (C N) and 1721 (C=O); ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.83(s, 3H, OCH_3), 5.98 (s, 2H, NH_2), 6.84-7.36 (m, 9H, ArH), 9.60(s, ^{1}H, OH), 10.32 (s, ^{1}H, NH-amid), m/z: 426.11 (100.0%), 427.11 (23.8%), 428.11 (2.7%), 427.10 (2.2%). (M^+ +1, 100). Anal. for $C_{22}H_{14}N_6O_4$: (M. wt. 426): Calc.: C, 61.97; H, 3.31; N, 19.71; O, 15.0. Found: C, 61.99; H, 3.67; N, 19.72; O, 15.33.

6-amino-4-(4-chlorophenyl)-2-oxo-1-((2-oxoindolin-3-ylidene)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); ^{1}H-NMR (DMSO-d_6, δ, ppm): 5.6 (s, H, NH_2), 6.91-7.59(m, 9H, ArH), 10.40 (s, ^{1}H, NH-amid), 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 $C_{21}H_{11}ClN_6O_2$: (M. wt. 414) Calc.: C, 60.81; H, 2.67; N, 20.26. Found: C, 60.83; H, 2.69; N, 20.28%.

6-amino-4-(1-hydroxynaphthalen-2-yl)-2-oxo-1-((2-oxoindolin-3-ylidene)amino)-1,2-dihydropyridine-3,5-dicarbonitrile 13: Dark brown crystals, m.p. 270-272°C, yield 45% ; FT-IR (KBr, cm^{-1}): 3677 (NH), 2214 (CN) and 1721 (C=O); ^{1}H-NMR (DMSO-d_6, δ, ppm): 6.49 (s, H, NH_2), 7.45-8.25 (m, 9H, ArH), 10.03 (s, ^{1}H, NH-amid) and 11.30 (s, H, OH), m/z: 446.11 (100.0%), 447.12 (27.0%), 448.12 (2.7%), 447.11 (2.2%). (M^+ +1, 100). Anal. for $C_{25}H_{14}N_6O_3$: (M. wt. 446) Calc.: C, 67.26; H, 3.16; N, 18.83. Found: C, 67.29; H, 3.18; N, 18.85%.

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% CO_2 and were subculture two to three times a week. For antitumor assays, the tumor cell lines were suspended in medium at concentration 5×10^4 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% CO_2 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].

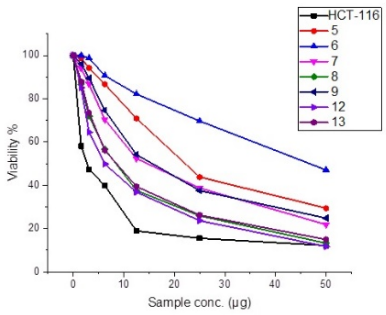


Figure 1: Activity of compound towards colon carcinoma cells.

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 (IC_{50}), 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 capsases in a dose dependent manner. It is interesting that the compound **(8)**, **(12)** and **(13)** showed signification growth inhibitory activity on the HCT-116 c, while **(5)** and **(6)** showed the lowest effect on the same tumor cell line (Figure 1).

Sample conc. (µg)	Viability (%)							
	HCT	5	6	7	8	9	12	13
50	12.16	29.46	47.06	21.84	13.27	24.83	11.74	14.98
25	15.54	43.82	69.73	38.72	25.89	37.56	23.65	26.23
12.5	18.92	70.95	82.17	52.39	37.64	54.21	36.89	39.48
6.25	39.86	86.73	90.68	70.43	56.75	74.62	49.86	56.17
3.125	47.30	94.18	98.76	86.71	71.92	89.49	64.52	73.46
1.56	58.11	98.74	100	94.08	86.73	96.13	85.13	87.95

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

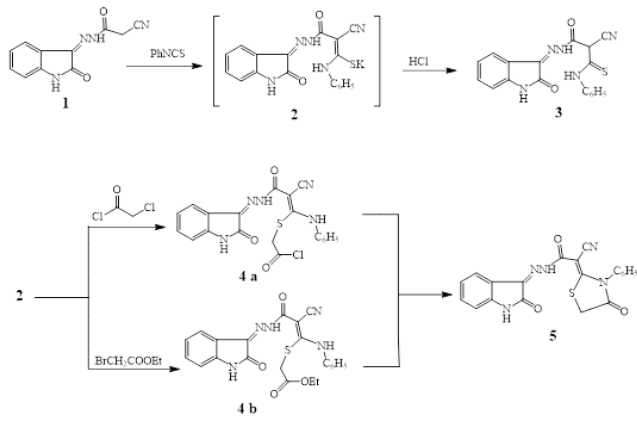
Results and Discussion

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 micro analytical and spectroscopic data were in agreement with the proposed structure **(3)** [10].

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 2198 cm^{-1} due to nitrile function and strong absorption band at 1700 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 **(5)**. The structures were established based on IR spectrum, which showed band in the region 3327- 3193 cm^{-1} due to two (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 singlet at δ 3.32 due to methylene in addition to aromatic multiple in the region δ 6.91-7.46 and two single's at δ 10.03 and 11.133 due to NH-amid and NH-hydrazid (Scheme 1).

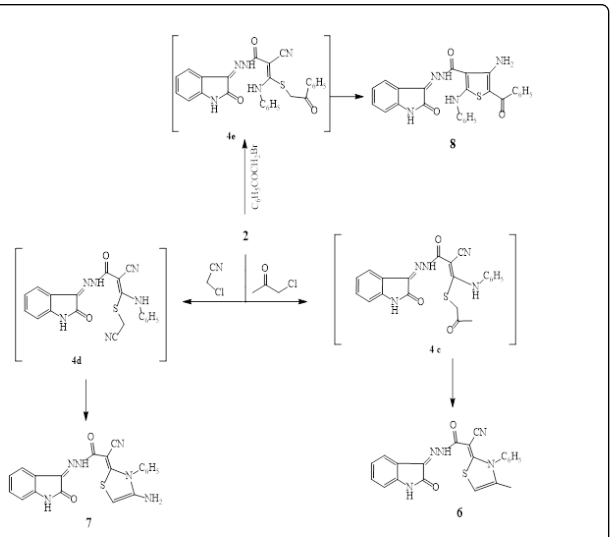
Reaction of intermediate **(2)** with chloroacetone containing the intermediate **(4c)** which the final isolable product 2-cyano-2-(4-methyl-3-phenylthiazol-2(3H)-ylidene)-N'-(2-oxoindolin-3-ylidene)acetohydrazide **(6)**. The intermediate **(4c)** which showed on IR spectrum a strong band in the region 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 2.28 due to methyl group 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 IR spectrum of the product **(6)** while revealed the presence of 3369, 3193 cm^{-1} due to two (NH) groups, a presence of 2199 cm^{-1} due to nitrile function and strong absorption band at 1700 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-hydrazide (Scheme 1).



Scheme 1: Synthesis and reactions of Thiazole derivatives.

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 singlets at δ 10.03, 10.44

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 δ 5.023 due to NH_2 and strong band at δ5.50 due to CH-thiazole protons and D2O -exchangeable signal at δ 7.95 and 8.66 due to NH-amid and NH-hydrazide. 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 **(4e)** 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 2.28 due to methyl group 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 IR spectrum of compound **(8)** showed the absence of Nitrile band and appearance band in the region 3566-3591 cm^{-1} due to two (NH) groups, 3421 cm^{-1} due to secondary amine and strong absorption band at 1706 cm^{-1} due to the carbonyl group (C=O). Moreover, its ^{1}H-NMR showed singlet at δ 6.91 due to NH_2 in addition to aromatic multiple in the region δ 6.91-7.59 and two single's at δ 8.501, 10.03 and 10.91 due to NH-thiophene, NH-amid and NH-hydrazid (Scheme 2).



Scheme 2: Synthesis and reactions of Thiazole derivatives and Thiophen derivatives.

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-dihydropyridine-3,5-dicarbonitrile **(9)** (Scheme 3).

The IR spectrum of 6-amino-2-Oxo-1-((2-oxoindolin-3-ylidene)amino)-4-phenyl-1,2-dihydropyridine-3,5-dicarbonitrile **(9)** revealed 2CN stretching bands at 2204 cm^{-1}. And a characteristic C=O stretching at 1721 cm^{-1}, revealed the presence of NH_2 at 3345 cm^{-1} and NH at 3571 cm^{-1}. Moreover, the ^{1}H-NMR spectrum of the obtained product revealed the presence of the NH_2 group at 5.981 ppm, NH-amide at 10.400 ppm and NH at 4.465 ppm.

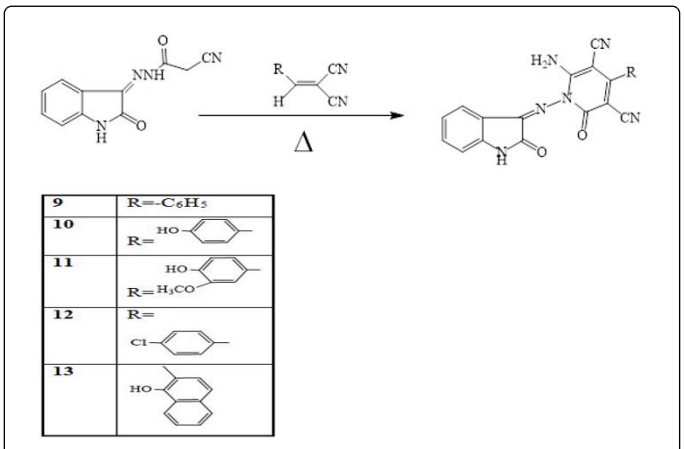
6-amino-4-(4-hydroxyphenyl)-2-oxo-1-((2-oxoindolin-3-ylidene)amino)-1,2-dihydropyridine-3,5-dicarbonitrile **(10)** was obtained by the addition of 2-(4-hydroxybenzylidene)malononitrile to 1 in ethanol in the presence of piperidine. The IR spectrum of compound **(10)** showed a hydroxyl group at 3328 and nitrile group at 2200 cm^{-1}. The ^{1}H-NMR spectrum of the obtained product revealed the presence of the OH group at 9.60 ppm, NH at 10.03 ppm and NH_2 at 5.98 ppm.

Treatment of 2-(4-hydroxy-3-methoxybenzylidene) malononitrile with **(1)** in ethanol in the presence of piperidine gave 6-amino-4-(4-hydroxy-3-methoxyphenyl)-2-oxo-1-((2-oxoindolin-3-ylidene)amino)-1,2-dihydropyridine-3,5-dicarbonitrile **(11)**. The IR spectrum showed bands at 3677and 3448 cm^{-1} due to NH, hydroxyl group and nitrile group at 2214 cm^{-1}. And strong absorption band at 1721 cm^{-1} due to carbonyl group (C=O). Moreover, its ^{1}H-NMR showed singlet at δ 3.83 due to The methoxy group in addition to aromatic multiple in the region δ 6.84-7.36 and two single's at δ 5.98, 10.32 due to NH_2 and NH-amid and at δ 9.53 due to OH (Scheme 3).

Refluxing 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 **(12)** was established based on IR spectrum while revealed the presence of 3377 cm^{-1} due to (NH), a presence of 2214 cm^{-1} due to nitrile function and strong absorption band at 1721 cm^{-1} due to carbonyl group and the ^{1}H-NMR spectrum of compound **(12)** showed singlet at δ 5.6 and 10.40 due to NH_2 and NH-amid.

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 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 δ 3.83 due to the methoxy group in addition to aromatic multiple in the region δ 6.84-7.59 and two single's at δ 6.49, 10.03 due to NH_2 and NH-amid and eat δ 11.30 due to OH (Scheme 3).



Scheme 3: Synthesis and reactions of pyridine derivatives.

Conclusion

Isatin (^{1}H-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.

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