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Synthesis, Identification and Biological Activity Study of Some New Spiro-Isatin Derivatives

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Abstract

Spiro-isatin derivatives have been the subject of extensive research due to their diverse pharmacological properties and potential therapeutic applications. These molecules are characterized by a spirocyclic structure that includes an isatin moiety and one or more heterocyclic rings, resulting in a unique and versatile molecular architecture. In this work, a series of new spiro-isatin derivatives **B1-12** were synthesized over two steps. The first step included a condensation reaction between isatin and various aromatic amines, affording the corresponding Schiff bases **A1-12** in high yields (up to 96%). In the second step, **A1-12** underwent a reaction with glycine, resulting in the formation of **B1-12** with yields varying between 65 and 92%. The structures of the compounds were confirmed by FT-IR and ^{1}H NMR spectroscopy. A few of the produced compounds were examined for antibacterial and antioxidant properties.

Keywords: Antioxidant activity, Isatin, Imidazolidone ring, Spiro-compounds.

تحضير، تشخيص و دراسة الفعالية البايولوجية لمجموعة من مشتقات سبايرو–ايستن الجديدة

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الخلاصة

كانت مشتقات السبايرو–ايستن موضوع بحث مكثف بسبب خصائصها الدوائية المتنوعة و التطبيقات العلاجية المحتملة. تتميز هذه الجزيئات بهيكل حلزوني تشتمل على جزء ايستن و واحدة أو أكثر من الحلقات الحلقية غير المتجانسة، مما ينتج عنه بنية جزيئية فريدة و متعددة الاستخدامات. في هذا العمل، تم تحضير سلسلة من مشتقات سبايرو–ايستن الجديدة **B1–12** على خطوتين. تضمنت الخطوة الاولى تكاثف بين الايستن و امينات اروماتية مختلفة، منح قواعد شيف **A1–12** بمنتوجات عالية (تصل الى 96%). في الخطوة الثانية، خضعت المركبات **A1–12** للتفاعل مع الكلايسين، مما ادى الى تكوين المركبات **B1–12** بمنتوجات تتراوح بين 65 و 92%. تم تأكيد تراكيب المركبات بواسطة مطيافية FT–IR و ^{1}H NMR. تم اختبار عدد قليل من المركبات المحضرة لخصائصها المضادة للبكتيريا و مضادات الأكسدة.

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1. Introduction

Isatin, alternatively referred to as indole-1H-2,3-dione or indoline-2,3-dione, is a compound composed of a combination of five and six-membered rings. Positioned at 1 is a nitrogen atom, and at positions 2 and 3 are two carbonyl groups. This molecule can be found in several potent synthetic pharmaceutical drugs. Progesterone receptor modulators [1], anticancer [2], and antimalarial [3] are a few examples (Figure 1, A). Isatin can also be found in a variety of natural alkaloid products, including elacomine [4], (±)-horsfiline [5], and (±)-coerulescine [6] (Figure 1, B). In the medical and pharmaceutical industries, Schiff bases are a significant class of chemicals that have demonstrated a wide range of biological activities, including antifungal [7], antibacterial [8], anti-inflammatory [9], anticancer [10], antimicrobial [11,12], and antioxidant effects [13]. Additionally, they are employed as catalysts, dyes, corrosion inhibitors [14-16], and stabilizing agents [17]. On the other hand, spiro-compounds, formally known as bicyclic organic compounds, comprise rings connected through just one atom that present several unique characteristics, such as 3D structural properties related to their inherent rigidity. Notably, these compounds display a broad range of biological activities. Recently, spiro-compounds have attracted increasing attention as scaffolds in modern drug discovery. The intrinsic complexity and, more importantly, the rigidity of these scaffolds offer several advantages to drug discovery programs [18]. Our project objective involves a two-step process for producing spiro-compounds from isatin. The first step comprises combining isatin with various aromatic amines to create corresponding imines through condensation. The second step involves a cycloaddition reaction between the imines and glycine to yield the targeted spiro compounds.

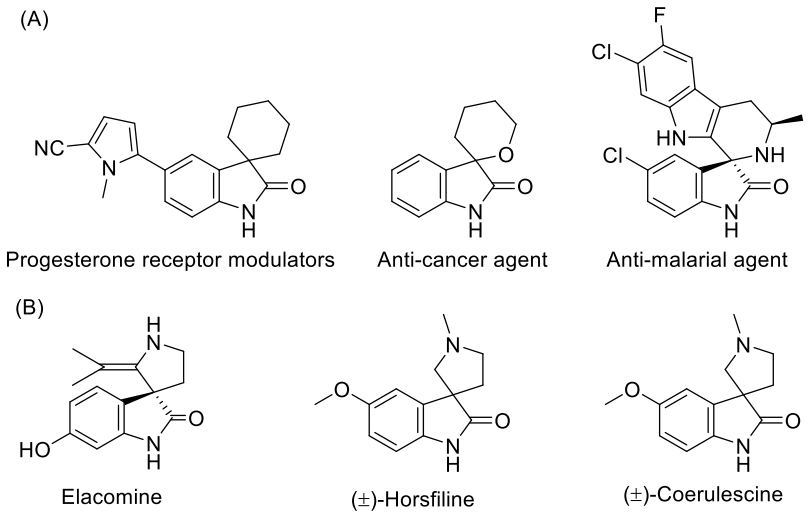


Figure 1: Some compounds containing isatin in their structures. (A) Synthetic pharmaceutical drugs. (B) Natural products.

2. Experimental part

2.1. Materials and instruments

All chemicals were obtained from commercial sources and used directly, unless stated otherwise. Merck silica gel 60 F_{254} was visualized using a UV lamp and aqueous, alkaline potassium permanganate in TLC. Using Stuart Scientific SMP3, melting points were measured in open capillary tubes without correction. A Shimadzu 8400 FT-IR spectrometer was used to record the infrared spectral data, and a Bruker AV400 spectrometer was used to record the ^{1}H NMR spectral data. Chemical shifts in ^{1}H NMR are measured in ppm downfield from either deuterated DMSO (δ_H 2.50 ppm) or tetramethylsilane (TMS), which serves as an internal standard and reference. A UV-1800 spectrophotometer made by Shimadzu was used to collect the data on antioxidant activity.

2.2. Chemistry

*2.2.1. General procedure for the synthesis of isatin Schiff's bases **A1-12** [19,20]*

A mixture of EtOH:DMF (3:1, 20 mL) was used to dissolve isatin (500 mg, 3.4 mmol, 1.0 eq.), and then glacial acetic acid (4-5 drops) was added to the solution. The mixture was stirred at room temperature for 30 minutes before a dropwise addition of a solution of the corresponding amine (3.4 mmol, 1.0 eq.) in EtOH (5-10 mL). After the completion of the addition, the mixture was heated at 70-80 °C for 2-18 hours. The reaction was monitored by TLC (eluent with petroleum ether/ethyl acetate) until no starting material remained. The reaction mixture was then poured on ice, filtered, washed with water, and dried. The desired products **A1-12** were obtained with 75-96% yields. The physical properties of these compounds (**A1-12**) are shown in Table 1.

*2.2.2. General procedure for the synthesis of spiro-compounds derived from isatin **B1-12***

In a 50-mL round-bottom flask, Schiff's bases **A1-12** (1.2 mmol, 1.0 eq.) were dissolved in EtOH (20 mL) and stirred for 5 minutes. A solution of glycine (90 mg, 1.2 mmol, 1.0 eq.) in EtOH (5 mL) and water (some drops) was then added dropwise to the reaction mixture before heating to reflux for 12-24 hours. The completion of the reaction was determined by TLC (eluent with petroleum ether/ethyl acetate). The precipitate was collected after the solvent had evaporated, filtered, and washed with an appropriate solvent. The physical properties of these compounds (**B1-12**) are shown in Table 2.

2.3. Biological activity

2.3.1. Antibacterial activity test

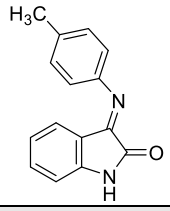
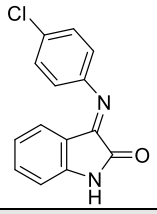
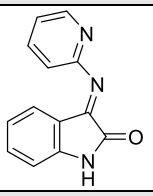
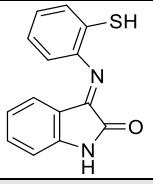
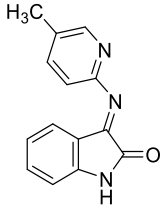
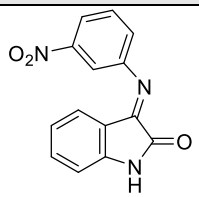
The agar well-diffusion method was used for the *in vitro* antibacterial activity assay of some samples against two strains of bacteria [21]: *Escherichia coli* and *Staphylococcus aureus*. The antibiotic Amikacin was used as a standard reference. A 1 mg/mL solution of DMSO was used to prepare test samples and standard references. The microorganism suspension (1 mL/100 mL of medium) was added to sterilized and liquefied agar, and the mixture was poured into a Petri dish to a depth of approximately 3 mm. The test samples and references were placed on the wells, the wells were established in solidified medium, and the resulting plates were refrigerated for 1 hour at 5 °C and then incubated at 37 °C for 18 hours.

2.3.2. Antioxidant activity test

The synthesized compounds' ability to scavenge free radicals was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [22]. To conduct the assay, the compound was prepared at various concentrations (25, 50, and 100 ppm), and 1 mL of each concentration was mixed with 1 mL of DPPH solution (400 mg in 100 mL). Then, the mixture was incubated in the dark for 30 minutes at room temperature. Finally, the absorbance of each sample was measured at 517 nm using a spectrophotometer. The potential to scavenge DPPH was calculated using the following equation, where ascorbic acid was used as a standard.

$I\% = (Abs\ blank - Abs\ sample) / Abs\ blank \times 100$ [23]

Table 1: Physical properties of compounds **A1-12**

No.	Compound structure	Compound formula	Reaction time (hours)	M.Wt (g/mol)	m.p. (°C)	Color	Yield (%)
A1		$C_{15}H_{12}ON_2$	18	236.27	218-220	Yellow	90
A1 - 3-(*p*-tolylimino)indolin-2-one							
A2		$C_{14}H_9OClN_2$	15	256.69	258-260	Pale orange	85
A2 - 3-((4-chlorophenyl)imino)indolin-2-one							
A3		$C_{13}H_9ON_3$	12	223.24	264-266	Orange	84
A3 - 3-(pyridin-2-ylimino)indolin-2-one							
A4		$C_{14}H_{10}N_2OS$	10	254.30	120-122	Red	75
A4 - 3-((2-mercaptophenyl)imino)indolin-2-one							
A5		$C_{14}H_{11}ON_3$	8	237.26	178-180	Deep orange	96
A5 - 3-((5-methylpyridin-2-yl)imino)indolin-2-one							
A6		$C_{14}H_9O_3N_3$	18	267.24	229-231	Pale orange	80
A6 - 3-((3-nitrophenyl)imino)indolin-2-one							

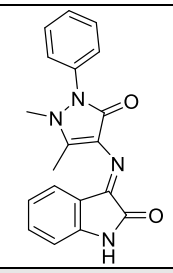
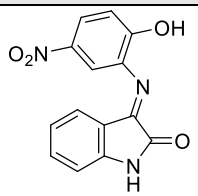
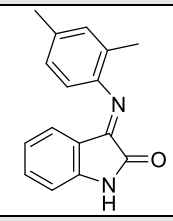
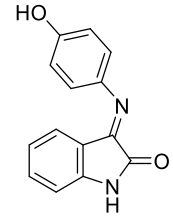
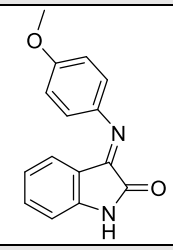
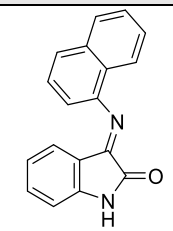
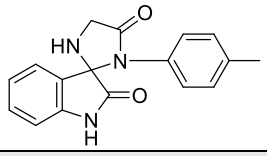
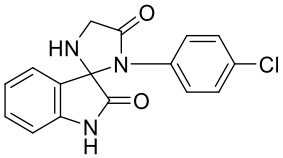
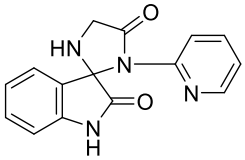
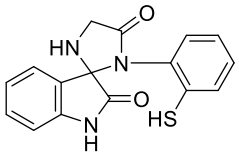
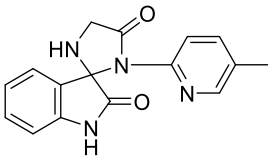
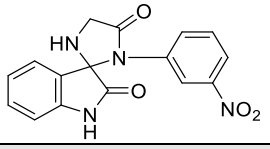
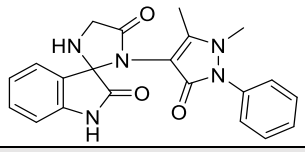
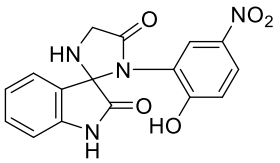
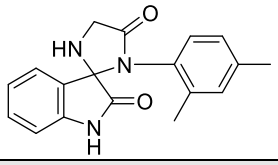
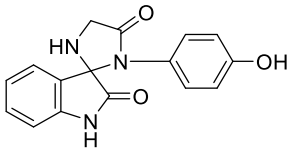
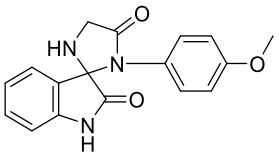
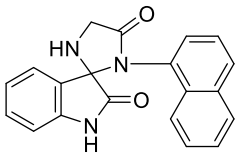
A7		$C_{19}H_{16}N_4O_2$	14	332.36	150-152	Orange	88
A7 - 3-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)imino)indolin-2-one							
A8		$C_{14}H_9O_4N_3$	8	283.24	116-118	Red	92
A8 - 3-((2-hydroxy-5-nitrophenyl)imino)indolin-2-one							
A 9		$C_{16}H_{14}ON_2$	16	250.30	105-107	Pale yellow	90
A9 - 3-((2,4-dimethylphenyl)imino)indolin-2-one							
A10		$C_{14}H_{10}O_2N_2$	8	238.25	292-294	Orange	92
A10 - 3-((4-hydroxyphenyl)imino)indolin-2-one							
A11		$C_{15}H_{12}O_2N_2$	2	252.72	234-236	Yellow	95
A11 - 3-((4-methoxyphenyl)imino)indolin-2-one							
A12		$C_{18}H_{12}ON_2$	11	272.31	234-236	Brown	80
A12 - 3-(naphthalen-1-ylimino)indolin-2-one							

Table 2: Physical properties of compounds **B1-12**

No.	Compound structure	Molecular formula	Reaction time (hours)	M.Wt (g/mol)	m.p. (°C)	Color	Yield (%)
B1		$C_{17}H_{15}N_3O_2$	16	293.33	82-84	Brown	80
B1 - 1-(*p*-tolyl)spiro[imidazolidine-2,3'-indoline]-2',5-dione							
B2		$C_{16}H_{12}ClN_3O_2$	22	313.74	126-128	Yellow brown	65
B2 - 1-(4-chlorophenyl)spiro[imidazolidine-2,3'-indoline]-2',5-dione							
B3		$C_{15}H_{12}N_4O_2$	17	280.29	120-122	Light brown	88
B3 - 1-(pyridin-2-yl)spiro[imidazolidine-2,3'-indoline]-2',5-dione							
B4		$C_{16}H_{13}N_3O_2S$	24	311.36	90-92	Brown	90
B4 - 1-(2-mercaptophenyl)spiro[imidazolidine-2,3'-indoline]-2',5-dione							
B5		$C_{16}H_{14}N_4O_2$	22	294.31	128-130	Light brown	81
B5 - 1-(5-methylpyridin-2-yl)spiro[imidazolidine-2,3'-indoline]-2',5-dione							
B6		$C_{16}H_{12}N_4O4$	20	324.30	120-122	Dark brown	84
B6 - 1-(3-nitrophenyl)spiro[imidazolidine-2,3'-indoline]-2',5-dione							
B7		$C_{21}H_{19}N_5O_3$	20	398.42	152-154	Red	76
B7 - 1-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)spiro[imidazolidine-2,3'-indoline]-2',5-dione							
B8		$C_{16}H_{12}N_4O_5$	14	340.30	120-122	Dark brown	83
B8 - 1-(2-hydroxy-5-nitrophenyl)spiro[imidazolidine-2,3'-indoline]-2',5-dione							

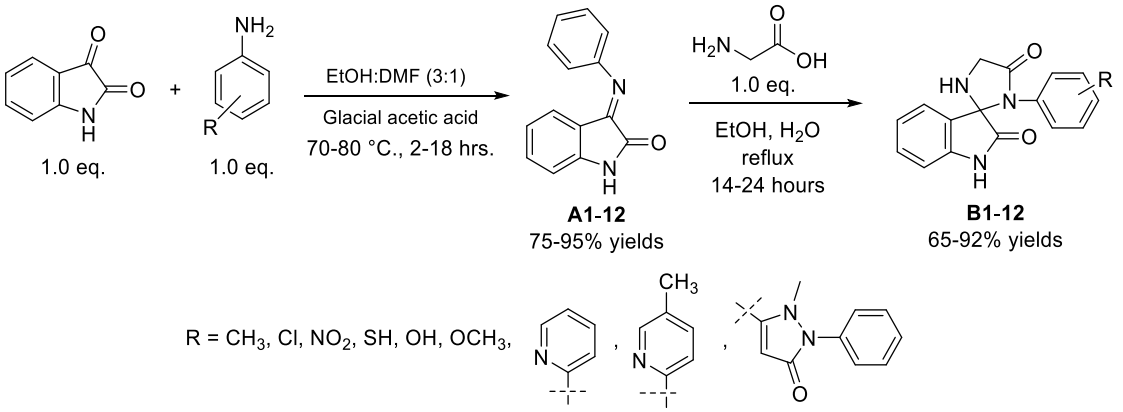
B9		$C_{18}H_{17}N_3O_2$	16	307.35	118-120	Deep yellow	92
B9 - 1-(2,4-dimethylphenyl)spiro[imidazolidine-2,3'-indoline]-2',5-dione							
B10		$C_{16}H_{13}O_3N_3$	18	295.30	93-95	Red brown	83
B10 -1-(4-hydroxyphenyl)spiro[imidazolidine-2,3'-indoline]-2',5-dione							
B11		$C_{17}H_{15}O_3N_3$	12	309.33	100-102	Red brown	90
B11 - 1-(4-methoxyphenyl)spiro[imidazolidine-2,3'-indoline]-2',5-dione							
B12		$C_{20}H_{15}O_2N_3$	19	329.36	152-154	Brown	92
B12 - 1-(naphthalen-1-yl)spiro[imidazolidine-2,3'-indoline]-2',5-dione							

3. Results and discussion

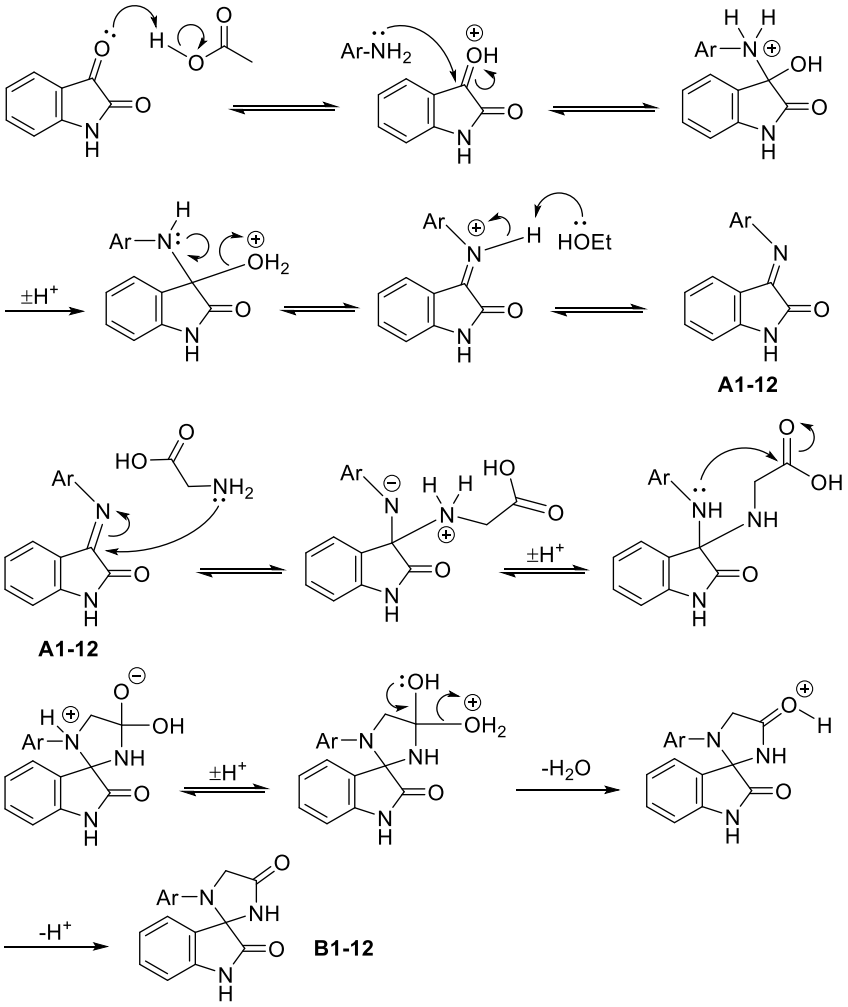
3.1. Chemistry

The first part of the spiro-compound synthesis was the preparation of the Schiff bases by the reaction of ketone (isatin) and primary substituted aromatic amines, which involved nucleophilic addition. The second part is the cyclization of Schiff bases **A1-12** with glycine to give spiro-imdazolidone derivatives **B1-12** (Scheme 1). Each product of **B1-12** is likely to be a mixture of two enantiomers. The mechanism of spiro-compounds **B1-12** formation starting from isatin is hypothesized in Scheme 2. The FT-IR spectral data of **A1-12** showed the absence of the carbonyl group of ketone at isatin (1747 cm^{-1}) and showed new absorptions at 1678-1641 cm^{-1} due to the C=N frequency. Other bands in the spectra included 3313-3188 cm^{-1} for N-H stretching, 3100-3026 cm^{-1} for aromatic C-H stretching, 2984-2835 cm^{-1} for aliphatic C-H stretching, and 1749-1709 cm^{-1} for amide carbonyl groups [24]. Two absorption bands at 1526 and 1348 cm^{-1} observed in product 3 for the asymmetric and symmetric stretching vibrations of the NO_2 group, respectively [25]. Table 3 displays all FT-IR spectral data for compounds **A1-12**. The FT-IR spectra of the spiro-compounds **B1-12** revealed the disappearance of the absorption bands at 1678-1641 cm^{-1} for C=N and the appearance of new absorption bands at 3369-3221 cm^{-1}, 1686-1639 cm^{-1}, and 1199-1053 cm^{-1}, which belong to the stretching vibrations of N-H, C=O, and C-N, respectively, at the imidazolidone ring [24,26]. Table 4 contains all of the FT-IR spectral data for compounds **A1-12**. The ^1H NMR spectral data of compound **B1** showed a singlet signal at 11.0 ppm due to the N-H proton of the oxoindole ring. Multiple signals between 7.95 and 6.33 ppm are for the N-H of the imidazolidone ring and aromatic protons [24]. Multiple signals at 3.53-3.23 ppm belong to the CH_2 protons of the imidazolidone ring. A singlet signal at 2.36 ppm is attributed to the CH_3 protons. The ^1H NMR spectral data of compound **B4** showed a singlet signal at 11.05 ppm for the N-H proton of the oxoindole ring and a singlet signal at 10.37 ppm due to the S-H proton. There are multiple signals between 8.41 and 6.42 (8 protons) for the N-H of the imidazolidone ring and aromatic protons. Multiple signals belonging to the CH_2 protons were observed at

3.53-3.23 ppm. A singlet signal at 2.36 ppm due to the CH_3 protons. All the ^1H NMR data are shown in Table 5.

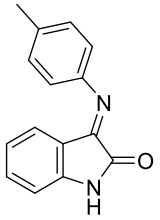
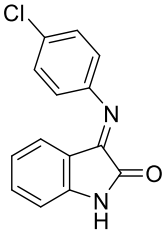
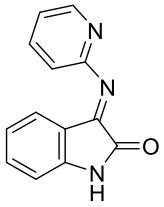
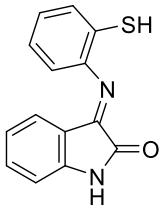
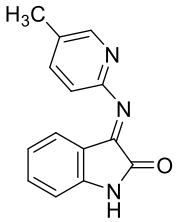
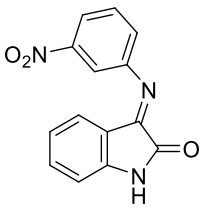


Scheme 1: Synthesis of compounds **B1-12** over two steps



Scheme 2: Proposed mechanism for the generation of spiro-compounds **B1-12**, commencing from isatin

Table 3: FT-IR spectral data (υ, cm^{-1}) of compounds **A1-12**

No.	Compound Structure	N-H Amide	C-H Aromatic	C-H Aliphatic	C=O Amide	C=N Imine	C=C Aromatic	Other bands
A1		3302	3098	2978	1732	1666	1576	-
A2		3198	3086	-	1728	1647	1587	C-Cl 1101
A3		3198	3026	-	1728	1643	1538	-
A4		3273	3080	-	1715	1666	1522	S-H 2368
A5		3192	3059	2984	1730	1678	1576	-
A6		3202	3099	-	1717	1651	1576	NO_2 Asymmetric 1526 Symmetric 1348

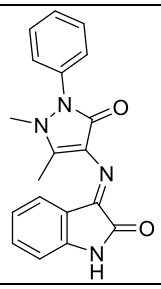
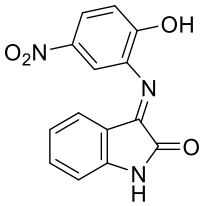
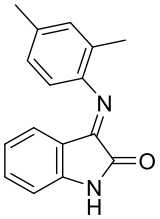
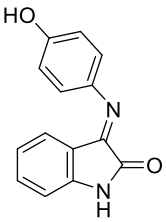
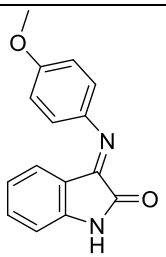
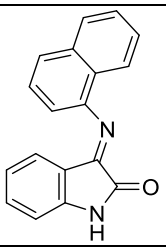
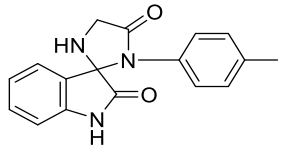
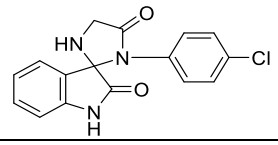
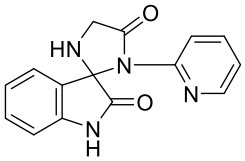
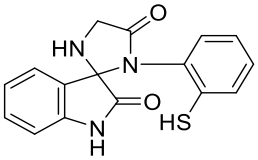
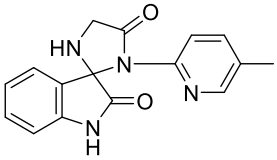
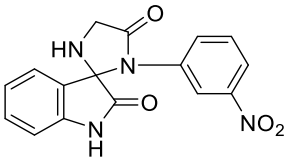
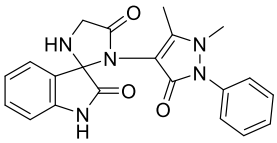
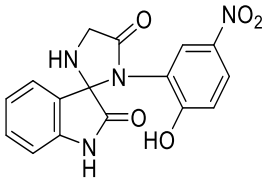
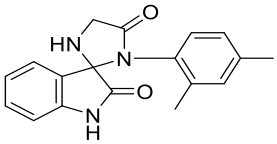
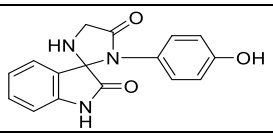
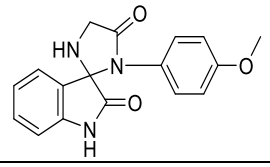
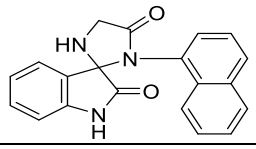
A7		3313	3074	-	1709	1651	1576	-
A8		3269	3076	-	1713	1668	1580	NO_2 Asymmetric 1522 Symmetric 1344
A9		3250	3067	2972	1749	1664	1562	-
A10		3188	3060	-	1713	1641	1597	O-H 3285
A11		3236	3100	2835	1740	1641	1572	C-O-C 1244
A12		3204	3055	-	1730	1655	1576	-

Table 4: FT-IR spectral data (υ, cm^{-1}) of compounds **B1-12**

No.	Compound Structure	N-H Amide and Imidazolidone	C-H Aromatic	C-H Aliphatic	C=O Isatin and Imidazolidone	C=C Aromatic	Other bands
B1		3369 3205	3099 3007	2922 2814	1722 1672	1518	-
B2		3319 3286	3182	-	1707 1641	1560	C-Cl 1020
B3		3367 3259	3196 3061	-	1718 1651	1574	C=N 1668
B4		3248 3171	3072 3003	-	1728 1639	1574	S-H 2359
B5		3333 3277	3198 3034	2885 2748	1717 1676	1555	-
B6		3221 3178	3070	-	1730 1686	1580	NO_2 Asym. 1508 Sym. 1331
B7		3377 3261	3261 3165	2981	1720 1649	1576	-
B8		3368 3248	3090	-	1717 1666	1580	NO_2 Asym. 1526 Sym. 1339 OH 3420
B9		3302 3200	3061 3003	2976 2880	1728 1664	1578	-
B10		3240 3142	3061 3013	-	1745 1649	1572	OH 3445

B11		3283 3194	3065 3001	2966 2833	1718 1639	1612 1576	C-O-C 1244
B12		3254 3215	3063	-	1725 1647	1620 1576	-

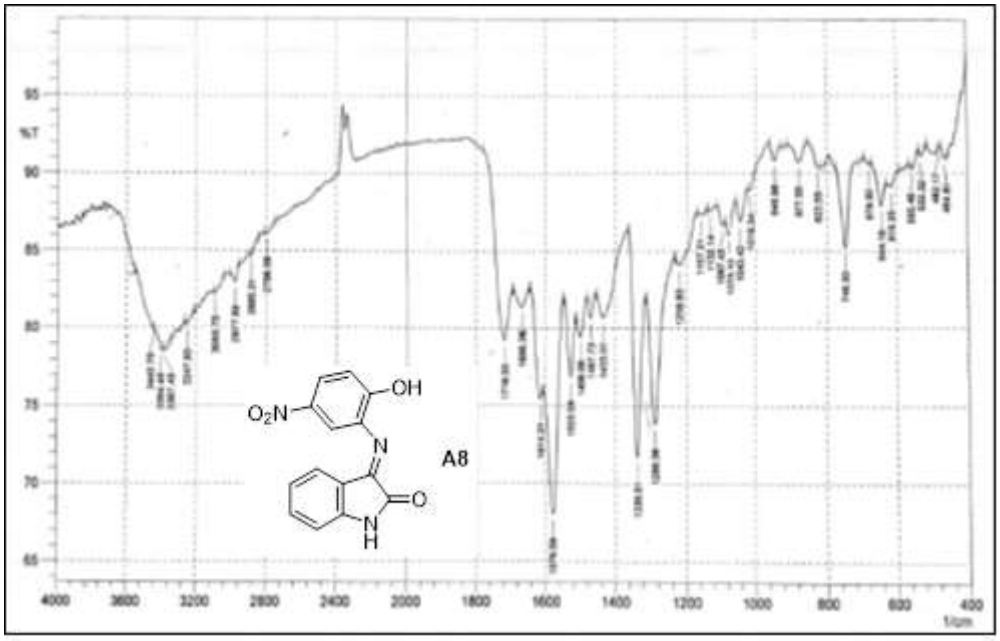


Figure 2: FT-IR spectrum of compound **A8**

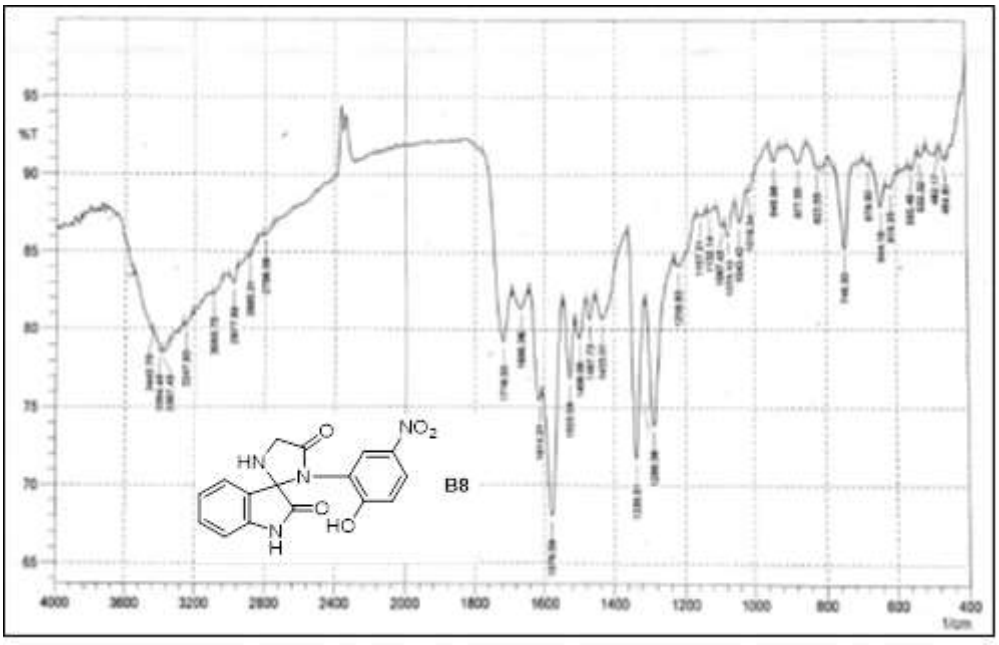
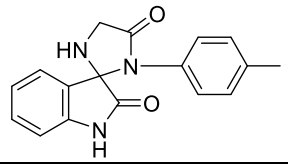
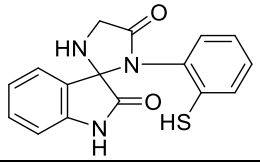
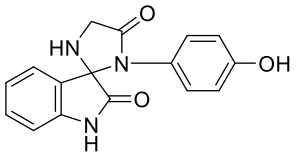


Figure 3: FT-IR spectrum of compound **B8**

Table 5: ^{1}H NMR spectral data (δ, ppm) of compounds **B1**, **B9**, and **B10**

No.	Compound structure	^{1}H NMR and ^{13}C NMR spectral data (δ, ppm)
B1		11.0 (1H, s, N-H), 7.95-6.33 (9H, m, N-H and Ar-H), 3.53-3.23 (2H, m, CH_2), 2.36 (3H, s, CH_3)
B4		11.05 (1H, s, N-H), 10.37 (1H, s, S-H), 8.41-6.42 (9H, m, N-H and Ar-H), 3.47-3.26 (2H, m, CH_2)
B10		10.8 (1H, s, N-H), 8.30-6.30 (10H, m, N-H, O-H and Ar-H), 3.52-3.10 (2H, m, CH_2)

3.2. Biological activity

3.2.1. Antibacterial activity

The tested compounds were found to have a moderately strong inhibitory effect, with compounds **B3** and **B4** showing the highest level of activity against gram-positive bacteria while having relatively weaker activities against gram-negative bacteria. The effect of compound **B9**, on the other hand, was comparable for both gram-positive and gram-negative bacteria. Compounds **B8** and **B11** inhibited only the gram-negative bacteria and had no effect on the gram-negative bacteria. The antibiotic Amikacin was used as a reference, and DMSO was used as a solvent (Table 6).

Table 6: Antibacterial activity of the tested prepared compounds

Compound number	Negative-gram bacteria (*Escherichia coli*)	Positive-gram bacteria (*Staphylococcus aura*)
B3	10	16
B4	16	18
B8	12	8
B9	14	14
B11	10	8
Amikacin	30	38
DMSO	8	8

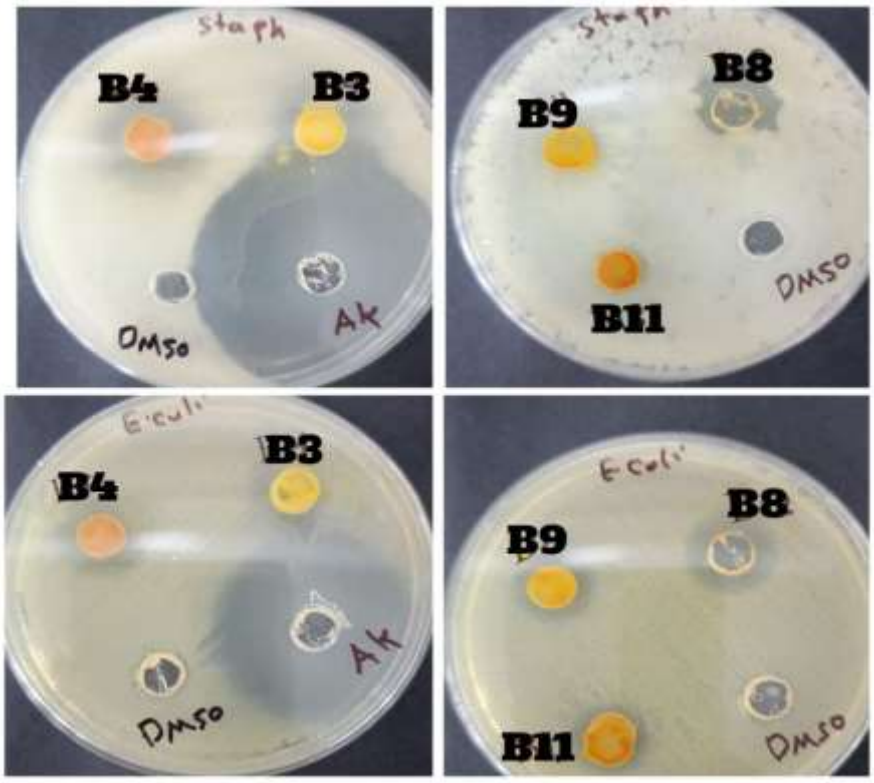


Figure 4: Inhibition zone of compounds **B3**, **B4**, **B8**, **B9**, and **B11** on gram-negative and gram-positive bacteria

3.2.2. Antioxidant activity

The *in vitro* antioxidant activity of compounds **B1**, **B3**, **B4**, **B7**, **B8**, **B9**, **B10**, and **B11** was determined spectrophotometrically using DPPH assay radicals in three concentrations (25, 50, and 100 mg/mL). Compound **B8** gave the best result compared to ascorbic acid, and all the results are in Table 7.

Table 7: Free radical-scavenging activity (%) for some of the prepared compounds

Compound number	**Inhibition (%) for the concentrations (mg/mL)**		
	100%	**50%**	**25%**
B1	26	44	24
B3	17	16	23
B4	84	55	32
B7	70	81	52
B8	88	90	86
B9	33	32	36
B10	11	24	31
B11	64	48	40
Ascorbic acid	93.54	89.25	80.95

4. Conclusion

With a favorable yield, a set of novel spiro-isatin analogues was produced in two steps, and they were identified using a variety of spectrophotometric techniques. The synthetic compounds displayed a wide variety of extremely promising antibacterial and antioxidant properties. Compounds **B3**, **B4**, and **B9** in particular demonstrated potent inhibitory activity against gram-positive bacteria, while **B8**, **B9**, and **B11** demonstrated potent inhibitory effects against gram-negative bacteria. Furthermore, the antioxidant activity of the compounds was assessed, and the results were remarkably significant and outperformed ascorbic acid. With a concentration of 50%, compound **B8** showed the strongest scavenging effect against the tested free radicals, while compounds **B4** and **B8** showed good scavenging activity at a concentration of 100%.

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