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Synthesis and Biological Evaluation of Isatin Derivatives as Antibacterial Inhibitors

Mohamed G. Badrey, [a, b], Abd El-Naby I. Essawy [a], Ibrahim A. Hassan [a,**], Asmaa K. Mourad [a]

[a] *Department of Chemistry, Faculty of Science, Fayoum University,63514 Fayoum, Egypt.*

[b] *Department of Chemistry, Faculty of Science and Arts-Almandaq, Al-Baha University, Al-Baha 65515, Saudia Arabia*

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ABSTRACT

Herein, 2'-amino-6'-(ethylthio)-2-oxo-1'*H*-spiro[indoline-3,4'-pyridine]-3',5'-dicarbo-nitrile **I** and 2-(2-oxoindolin-3-ylidene)hydrazine-1-carbox-imidamide **II** were served as prestarting intermediates for the production of polyfunctional substituted heterocycles like; pyrazole, pyridine, imidazole, and pyrimidine. Subsequently, representative chemicals of the manufactured series were verified and assessed as antibacterial inhibitors. Spiro-indoline pyridine **I** was prepared to be used as a beginning material for manufacturing assorted novel heterocyclic compounds containing an isatin moiety. Derivative **I** was obtained *via* the reaction of isatylidene malononitrile with malononitrile and ethyl thiol in the presence of ammonium acetate. The starting compound **I** was permitted to combine with carbon disulfide in boiling pyridine to afford the carbamodithioic acid derivative **III**. Also, the reaction of compound **I** with triethyl orthoformate, maleic anhydride, dimethylformamide, dimethyl acetal, hydrazine hydrate, and diethyl malonate yielded compounds **IV**, **V**, **VI**, **VII**, and **VIII** respectively. Moreover, the reaction of the ketonic carbonyl group of isatin with aminoguanidine salt in aqueous alcohol when there was a catalytic amount of sodium bicarbonate yielded compound **II**. Likewise, carboximidamide derivative **II** reacted with active methylene compounds such as malononitrile and its derivatives; namely, ethoxy methylene malononitrile and chloroacetonitrile to afford compounds **XI**, **XII**, and **IX**, respectively. On the other hand, compound **II** interaction with dimethyl acetylenedicarboxylate allowed a heterocyclic imidazole derivative to be obtained.

1. Introduction

Isatin derivatives have a long history of applications in the pharmaceutical industry as active pharmacophores. Heterocyclic compounds with an isatin nucleus have assorted biological merits, including anti-inflammatory, analgesic [1-5], anticancer [6-11], antiglycation [12], antimalarial [13-17], antioxidant [18-21], anthelmintic [22], and antianxiety activities [23]. Moreover, they work as anticonvulsants [24, 25], anti-HIV [26-28], antiviral [29], antibacterial [30-34], anti-fungal [35], and anti-tubercular agents [36-39]. Isatin derivatives also have various industrial applications, they are used as corrosion inhibitors [40-42], fluorescent sensors [43-46], and also in the dye industry [47, 48].

2. Materials and Methods

Oxoindoline hydrazine carboximidamide **II** was synthesized in accordance with literature methods [Krátký *et al*, 2021] [63]. All chemicals and initial materials were collected from commercial providers and were applied without any additional purification.

* Corresponding author.
E-mail address: iah13@fayoum.edu.eg (I. A. Hassan) Tel.: +201011350536
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2.1. 2'-Amino-6'-(ethylthio)-2-oxo-1'*H*-spiro[indoline-3,4'-pyridine]-3',5'-dicarbo-nitrile (I)

To a well-stirred mixture of carbonyl compounds "such as isatin" (0.147 g, 1.0 mmol), active methylene compounds "such as Malononitrile" (0.123 g, 2.0 mmol), and mercaptan "such as ethyl thiol" (0.072 mL, 1.0 mmol) in ethanol, after adding the basic catalyst "diethylamine" (0.1 mL), stirring was carried out for 180 minutes. As soon as the reaction is finished (showed by TLC), ethanol (5.0 mL) was added, and After another 5 minutes of stirring, the reaction was finished. The resulting white crude was congregated by filtration and subsequently rinsed with a blend of cyclohexane and chloroform. (80:20, v/v) and then dried. The resultant crude was recrystallized from alcohol "such as EtOH". Yield: 40%, m.p.: 272 °C, IR (KBr, *ύ*/ cm^{-1}): 3446, 3309, 3242, 3212 (NH_2, 2NH), 3088 (C-H, aromatic), 2951 (C-H, aliphatic), 2215, 2176 (2CN), 1707 (C=O, amide), 1626 (C=C, aliphatic), 1606 (C=C, aromatic); ^{1}H NMR (DMSO-*d6*, 300 MHz, ppm): δ/ppm = 1.27 (t, 3H, C*H*$_3$), 3.03 (q, 2H, C*H*$_2$), 6.16 (s, 2H, N*H*$_2$), 6.84-7.28 (m, 4H, Ar-*H*), 9.46 (s, 1H, N*H*), 10.51 (s, 1H, N*H*); MS(EI) ($C_{16}H_{13}N_5OS$, M.wt.= 323): *m/z* (%) = 327 (M^+- 4, 7.7%), 281 (41.63%), 221 (3.46%), 207 (100%, $C_{12}H_5N_3O$), 191 (16.93%), 165 (6.23%), 105 (4.82%), 77 (7.02%), 75 (3.23%), 73 (60.05%). Math. Calcd for $C_{16}H_{13}N_5OS$: C: 59.43, H: 4.05, N: 21.66, S: 9.91. Found: C: 57.89, H: 3.82, N: 21.03, S: 9.54.

2.2. (3',5'-Dicyano-2'-(ethylthio)-2-oxo-1'*H*-spiro[indoline-3,4'-pyridin]-6'-yl)carbamodithioic acid (III).

Carbon disulfide (0.06 mL, 1.0 mmol) was added drop-wise with product **I** (0.323 g, 10 mmol) into pyridine (5.0 mL). The blend was heated for 12 hours. After cooling, the product solution was neutralized by dilute-cooled hydrochloric acid to obtain a dark brown precipitate. ethyl acetate was used for the purification of the resultant solid. Yield: 76%, m.p.: 267 °C, IR (KBr, *ύ*/ cm^{-1}): 3316 (NH), 3216 (NH), 2198 (CN), 2179 (CN), 1712 (C=O, amide), 1625 (C=C), 1214 (C=S); MS(EI) ($C_{17}H_{13}N_5OS_3$, M.wt.= 399): *m/z* (%) = 399 (M^+, 2.17%), 336 (2.17%), 295 (100%, $C_{14}H_7N_4S_2$), 281 (15.73%), 140 (6.65%), 116 (3.16%), 90 (6.44%), 64 (50.72%).

2.3. Ethyl-*N*-(3',5'-dicyano-2'-(ethylthio)-2-oxo-1'*H*-spiro[indoline-3,4'-pyridin]-6'-yl)formimidate (IV).

In acetic anhydride (5.0 mL), a blend of spiro[indoline-pyridine]-dicarbonitrile derivative **I** (0.323 g, 1.0 mmol) and triethyl orthoformate (0.166 mL, 1.0 mmol) was heated for five hours. After refluxing, the blend was left to evaporate to form a brown resin. With the addition of diethyl ether, the resultant brown resin was solidified and the brown solid product was gotten. The product was separated by filtration, desiccated, and purified from EtOH. Yield: 44 %, m.p.: 165°C, IR (KBr, *ύ*/ cm^{-1}): 3316 (NH), 3266 (NH), 2200 (CN), 2179 (CN), 1712 (C=O, amide), 1635 (C=C); ^{1}H NMR (DMSO-*d6*, 300 MHz, ppm): δ/ppm= 1.09 (t, 3H, C*H*$_3$), 1.22 (t, 3H, C*H*$_3$), 3.11 (q, 2H, C*H*$_2$), 3.38 (q, 2H, C*H*$_2$), 4.33 (s, 1H, C*H*), 6.8-7.5 (m, 4H, Ar-*H*), 9.45 (s, 1H, N*H*), 12.28 (s, 1H, N*H*).

2.4. 4-((3',5'-Dicyano-2'-(ethylthio)-2-oxo-1'*H*-spiro[indoline-3,4'-pyridin]-6'-yl)amino)-4-oxo-but-2-enoic acid (V).

In acid " glacial CH_3COOH" (5.0 mL), a blend of product **I** (0.323 g, 1.0 mmol) and maleic anhydride (0.098 g, 1 mmol) was included, boiled for 2 hours, following that was placed into ice to produce a pale brown crude solid. The brown precipitate was purified from alcohol "such as EtOH". Yield: 66%, m.p.: 272 °C, IR (KBr, *ύ*/ cm^{-1}): 3448 (NH), 3360 (NH), 3251 (NH), 3000-3600 (OH, Acid), 2202 (CN), 2183 (CN), 1722 (C=O, acid), 1712 (C=O, amide), 1639 (C=C); ^{1}H NMR (DMSO-*d6*, 300 MHz, ppm): δ/ppm= 1.27 (t, 3H, C*H*$_3$), 3.09 (q, 2H, C*H*$_2$), 5.78 (s, 1H, C*H*), 5.42 (s, 1H, C*H*), 6.99 (d, 1H, Ar-*H*), 7.17 (t, 1H, Ar-*H*), 7.38 (t, 1H, Ar-*H*), 7.48 (d, 1H, Ar-*H*), 11.05 (s, 1H, N*H*), 11.27 (s, 1H, N*H*), 11.36 (s, 1H, N*H*), 11.61 (s, 1H, O*H*). Math. Calcd for $C_{20}H_{15}N_5O_4S$: C: 57.00, H: 3.59, N: 16.62, S: 7.61. Found: C: 56.32, H: 3.01, N: 15.79, S: 7.21.

2.5. N'-(3',5'-dicyano-2'-(ethylthio)-2-oxo-1'*H*-spiro[indoline–3,4'–pyridine]-6'–yl)-*N*,*N*-dimethylformimidamide (VI).

To a blend of product **I** (0.323 g, 1.0 mmol) in dioxane (5 mL), *N, N*-dimethylformamide dimethyl acetal (DMF-DMA) (0.132 mL, 1.0 mmol) was included. The blend was heated for four hours. The yellow substance was created and separated by filtering after cooling and purified from ester "such as ethyl acetate". Yield: 60%, m.p.: 243 °C, IR (KBr, *ύ*/ cm^{-1}): 3347, 3228 (2NH), 2210, 2208 (2CN), 1716(C=O, amide), 1627 (C=N), 1619 (C=C); ^{1}H NMR (DMSO-*d6*, 300 MHz, ppm): δ/ppm= 1.38 (t, 3H, C*H*$_3$), 3.15 (q, 2H, C*H*$_2$), 3.29 (s, 6H, 2C*H*$_3$), 5.24 (s, 1H, N*H*), 6.62 (t, 1H, Ar-*H*), 6.76 (d, 1H, Ar-*H*), 6.96 (d, 1H, Ar-*H*), 7.17 (t, 1H, Ar-*H*), 8.82 (s, 1H, C*H*).

2.6 3,5-Diamino-7,8-dihydro-1*H*-spiro[dipyrazolo[3,4-b:4',3'-*e*]pyridine-4,3'-indolin]-2'-one (VII).

To a mixture of compound **I** (0.323 g, 1.0 mmol) in absolute EtOH (10.0 mL), diamino compound "such as hydrazine hydrate" (0.1 mL) was included. The blend was simmered for two hours. The reaction time was finished when the ethyl thiol and ammonia evolution was stopped, as indicated by HCl and lead acetate roads. After overnight cooling, the yellow crude was formed, isolated by filtration, and purification from alcohol "such as EtOH". Yield: 45%, m.p.: > 300 °C, IR (KBr, *ύ*/ cm^{-1}): 3405 (NH), 3255, 3336 (NH_2), 1704 (C=O, amide), 1627 (C=C); MS(EI) ($C_{14}H_{12}N_8O$, M.wt.= 308): *m/z* (%) = 308 (M+, 4.99%), 252 (1.59%), 196 (2.88%), 76 (25.76%), 44 (100%, CH2NO). Math. Calcd for $C_{14}H_{12}N_8O$: C: 54.54, H: 3.92, N: 36.35. Found: C: 42.83, H: 3.78, N: 35.99.

2.7. Ethyl-5'-amino-3'-cyano-2'-(ethylthio)-2,7'-dioxo-7',8'-dihydro-1'*H*-spiro [indoline-3,4'-[1,8]naphthyridine]-6'-carboxylate (VIII).

To a blend of compound **I** (0.323 g, 1.0 mmol) and active methylene compound "such as diethyl malonate" (0.125 mL, 1.0 mmol) in glacial CH_3COOH (10.0 mL), CH_3COONH_4 (0.231 g, 3.0 mmol) was included. The mixture was boiled for 12 hours. After cooling, the blend was tipped onto crushed ice. The pale brown needle was designed, isolated by filtration, and purified by recrystallization from dioxane. Yield: 35%, m.p.: 245 °C, IR (KBr, *ύ*/ cm^{-1}): 3536:3224 (NH_2, 3NH), 2202 (CN), 1708 (C=O, ester),1650 (C=O, amide), 1616 (C=C); ^{1}H NMR (DMSO-*d6*, 300 MHz, ppm): δ/ppm= 0.87 (t, 3H, C*H*$_3$), 1.29 (t, 3H, C*H*$_3$), 1.35 (q, 2H, C*H*$_2$), 1.56 (q, 2H, C*H*$_2$), 6.21 (s, 1H, N*H*), 6.86 (d, 1H, Ar-*H*), 6.99 (t, 1H, Ar-*H*), 7.23 (t, 1H, Ar-*H*), 7.6 (d, 1H, Ar-*H*), 7.27 (s, 2H, 2N*H*), 10.51 (s, 2H, N*H*$_2$). Math. Calcd for $C_{21}H_{19}N_5O_4S$: C: 57.66, H: 4.38, N: 16.01, S: 7.33. Found: C: 55.86, H: 3.98, N: 14.99, S: 7.02.

2.8. 3-(2-(5-Amino-4*H*-imidazol-2-yl)hydrazono)indolin-2-one (IX).

A blend of 2-indolone derivative **II** (0.203 g, 1.0 mmol), chloro compound "such as Chloroacetonitrile" (0.063 mL, 1.0 mmol), and catalytic droplets of base "like piperidine" in alcohol "such as ethanol" (5.0 mL) was boiled for 8 hours. Red precipitate was segregated through reflux. The resultant solid was separated by filtration and purification from alcohol "such as EtOH" as red needles. Yield: 42%, m.p.: over 360 °C, IR (KBr, $\acute{\upsilon}$ /cm^{-1}): 3413, 3390, 3346, 3295 (NH_2, 2 NH), 2923 (CH, aliphatic), 1712 (C=O, amide), 1666 (C=N), 1616 (C=C); MS(EI) ($C_{11}H_{10}N_6O$, M.wt= 242): *m/z* (%) = 242 (M^+, 2.43 %), 262 (2.81%), 185 (30.38%), 159 (2.15%), 157 (21.96%), 145 (2.52%), 129 (59.44%), 103 (77.79%), 77 (25.47%), 52 (55.89%), 43 (100%, CHNO).

2.9. Methyl-2-(5-oxo-2-(2-(2-oxoindolin-3-ylidene)hydrazinyl)-1,5-di-hydro-4*H*-imidazol-4-ylidene)acetate (X).

To a mixture of 2-indolone derivative **II** (0.203 g, 1.0 mmol) and a few droplets of base catalyst "such as piperidine" in alcohol "such as ethanol" as solvent (5.0 mL), dimethyl acetylene dicarboxylate (0.122 mL, 1.0 mmol) was included. The time of reaction was finished by refluxing for four hours. The resultant pale brown crude was designed on hot the crude precipitate was separated by filtration and purified *via* recrystallization from EtOH. Yield: 45 %, m.p.: over 300 °C, IR (KBr, $\acute{\upsilon}$/ cm^{-1}): 3351, 3309, 3193 (3NH), 2962 (CH, aliph.) 1731 (C=O, ester), 1666 (C=O, amide), 1612 (C=C); MS(EI) ($C_{14}H_{14}N_5O_4$, M.wt= 313): *m/z* (%) = 313 (M^+, 2.23 %), 282 (2.25%), 254 (2.63%), 199 (2.34%), 186 (28.56%), 156 (24.04%), 129 (82.1%), 103 (100%, C_7H_5N), 76 (57.45%), 52 (22.46%). Math. Calcd for $C_{14}H_{14}N_5O_4$: C: 51.07, H: 3.37, N: 21.27. Found: C: 50.81, H: 3.07, N: 20.93.

2.10. 3-(2-(4,6-Diaminopyrimidin-2-yl)hydrazono)indolin-2-one (XI).

A blend of 2-indolone derivative **II** (0.203 g, 1.0 mmol), active methylene compound "such as malononitrile" (0.066 g, 1.0 mmol), and 3 drops of piperidine in dimethyl formamide (5.0 mL) was boiled for 6 hours. The built dark black crude was obtained by filtration and then cleaned by washing with hot dimethyl formamide. The resultant dark needle was pure enough for making analysis. Yield: 77 %, m.p.: over 300 °C, IR (KBr, $\acute{\upsilon}$/ cm^{-1}): 3395, 3351, 3302, 3201 ($2NH_2$, 2NH), 1720 (C=O, amide), 1644 (C=N), 1616 (C=C); MS(EI) ($C_{12}H_{11}N_7O$, M.wt= 269): *m/z* (%) = 269 (M^+, 23.14 %), 230 (11.38%), 188 (17.14%), 173 (18.17%), 157 (74.55%), 129 (100%, $C_8H_5N_2$), 103 (47.51%), 77 (28.38%), 51 (18.88%). Math. Calcd for $C_{12}H_{11}N_7O$: C: 53.53, H: 4.12, N: 36.41. Found: C: 52.77, H: 3.78, N: 34.96.

2.11. 4-Amino-2-(2-(2-oxoindolin-3-ylidene)hydrazinyl)pyrimidine-5-carbonitrile (XII).

Oxindole derivative **II** (0.203 g, 1.0 mmol) was dissolved in alcohol "such as EtOH" (10.0 mL). Ethoxymethylene malononitrile (0.122 g, 1 mmol) was included in the blend. The blend was boiled under heating for four hours. During reflux, a reddish-brown precipitate was formed then isolated by filtration and purified *via* recrystallization from alcohol "such as ethanol". Yield: 50 %, m.p.: over 300 °C, IR (KBr, $\acute{\upsilon}$/ cm^{-1}): 3368, 3320 (2NH), 3120:3193 (NH_2), 2217 (CN), 1666 (C=O, amide), 1612 (C=N), 1600 (C=C); MS(EI) ($C_{13}H_9N_7O$, M.wt= 279): *m/z* (%) = 279 (M^+, 26.60 %), 252 (2.8%), 250 (22.94%), 186 (11.47%), 185 (62.65%), 129 (62.95%), 102 (100%, C_7H_4N), 76 (62.26%), 52 (39.61%).

2.12. Antimicrobial assay

The antimicrobial activity of products under research was estimated against Gram-positive bacteria "such as *Bacillus subtilis* and *Staphylococcus aureus*" and Gram-negative bacteria "like *Escherichia coli* and *Pseudomonas aeruginosa*". Drug chloramphenicol was applied as a controller standard for *in vitro* antibacterial activity. Antimicrobial activity of recently-manufactured samples, as opposed to multi-pathological strains, was indicated as inhibition diameter zones in millimeters (mm) as follows in Table (1).

Table 1: Examined compounds' *in vitro* antibacterial effects.

Entry	Compound	Gram (+Ve) bacteria				Gram (-Ve) bacteria			
		Staphylococcus aureus		*Bacillus subtilis*		*Escherichia coli*		*Pseudomonas aeruginosa*	
		I.Z.±S.D.*	% Activity index	I.Z.±S.D.*	% Activity index	I.Z.±S.D.*	% Activity index	I.Z.±S.D.*	% Activity index
1	I	[a]NA	-	14±3.39	51.8	18±2.88	51.7	NA	-
2	II	20±1.33	111.1	32±2.03	118.5	34±1.93	117.2	30±1.83	120
3	III	19±1.03	105.5	29±1.13	107.4	27±0.17	93.1	28±1.23	112
4	IV	NA	-	13±3.69	48.1	26±0.47	89.6	21±0.87	84
5	V	25±2.84	138.8	32±2.03	118.5	33±1.63	113.8	32±2.43	128
6	VI	20±1.33	111.1	30±1.43	111.1	31±1.03	106.9	31±2.13	124
7	VII	28±3.74	155.5	30±1.43	111.1	25±0.77	86.2	34±3.04	136
8	VIII	27±3.44	150	28±0.83	103.7	28±0.17	96.5	32±2.43	128
9	IX	26±3.14	144.4	29±1.13	107.4	25±0.77	86.2	33±2.74	132
10	X	22±1.93	122.2	29±1.13	107.4	27±0.17	93.1	29±1.53	116
11	XI	NA	-	20±1.58	74	29±0.42	100	17±2.08	68
12	XII	NA	-	17±2.48	62.9	21±1.98	72.41	NA	-
13	Control	18	100	27	100	29	100	25	100

*I.Z. Inhibition diameter regions expressed in millimeters (mm); S.D. Standard deviation. aNA: antimicrobial inactivity sensed

The studied chemicals presented differences in their antibacterial activities (Table). Samples **II**, **V**, and **VI** were active against all studied bacteria (Table; entries 2, 5, and 6, respectively). Compounds **I**, **IV**, **XI**, and **XII** were not active against *Staphylococcus aureus* (Table; entries 1, 4, 11, and 12, respectively) while samples **I** and **XII** were inactive against *Pseudomonas aeruginosa* (Table; entries 1 and 12, respectively). Compounds **III**, **VII**, **VIII**, **IX**, and **X** were active against all tested pathogens except *Escherichia coli* (Table; entries 3 and 7-10, respectively). Compound **VII** gave the highest activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria in this test (Table; entry 7). In addition, compound **II** gave the highest activity against *Bacillus subtilis* and *Escherichia coli* (Table; entry 2).

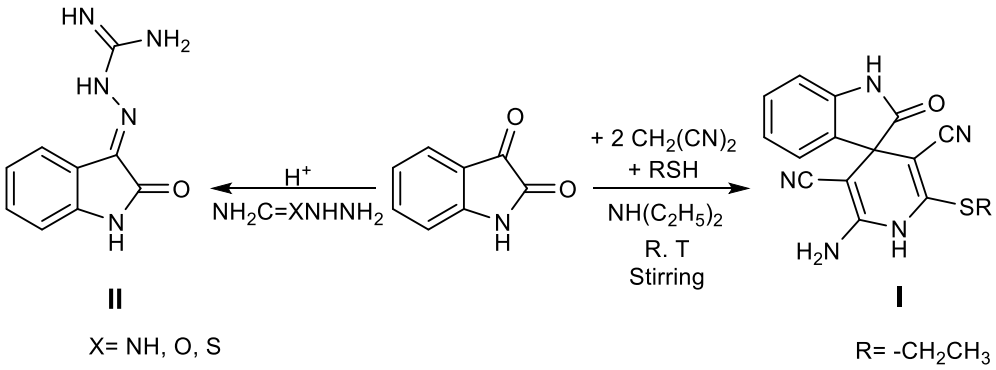
2.13. Antimicrobial activity assay.

According to techniques described in the literature, the antibacterial effect of the chemicals under study was detected *via* a specific bacteria [Russell *et al*, 1977]. [64]. Also, the common medication chloramphenicol had its antibacterial properties tested under the same parameters.

3. Results and discussion

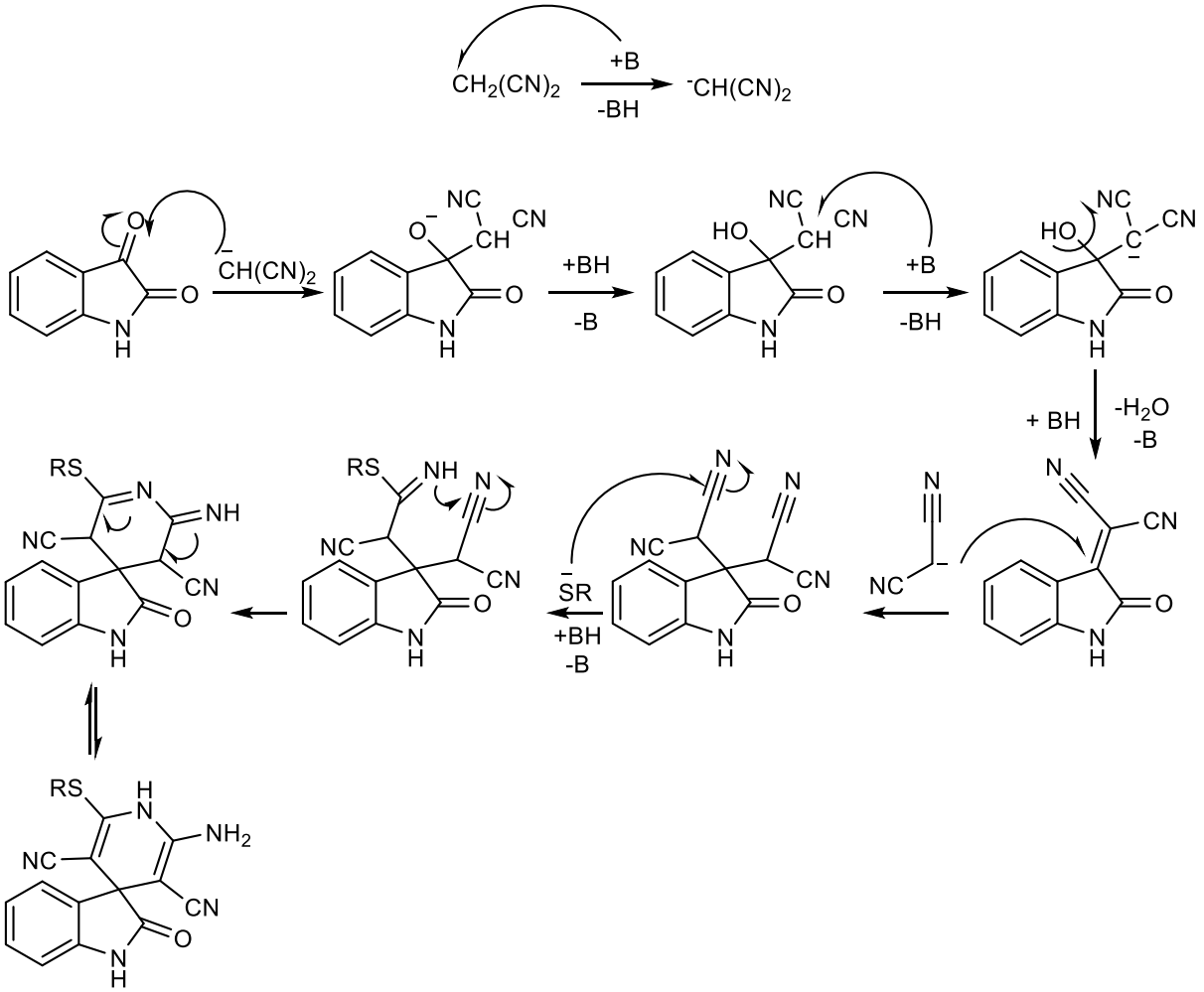
That is generally known pyridine-3,5-dicarbonitriles could be assembled through the condensation of carbonyl derivatives with active methylene compounds and aliphatic thiols. In this work, ethyl thiol was used along with isatin as a carbonyl compound and malononitrile as an active methylene-containing reactant to have access to the product (**I**). In such a reaction, various reaction conditions, including the utilization of assorted bases [49-54], acids [55], amino acids [56], and catalytic ionic salts were explored [57-61]. However, many of the above-mentioned conditions possess considerable disadvantages, such as the creation of unavoidable side products, Low yields, difficult reaction conditions, prolonged reaction times, time-consuming workup, and the employment of costly and naturally harmful catalysts and solvents. As a result, we have tried to overcome these flaws and find effective procedures for these points using less toxic and less expensive pathways.

Several procedures have been adopted to obtain the final product **I**. The most appropriate and high-yield pathway was a one-pot four-component procedure, including the stirring of an ethanolic solution of one mole of isatin, two moles of malononitrile, and one mole of ethyl mercaptan in a basic catalytic amount of diethyl amine at normal temp. (Scheme 1). In comparison to other methods, this one not only utilized available catalysts and solvents but also the reaction was accomplished in a shorter time with a cleaner profile.



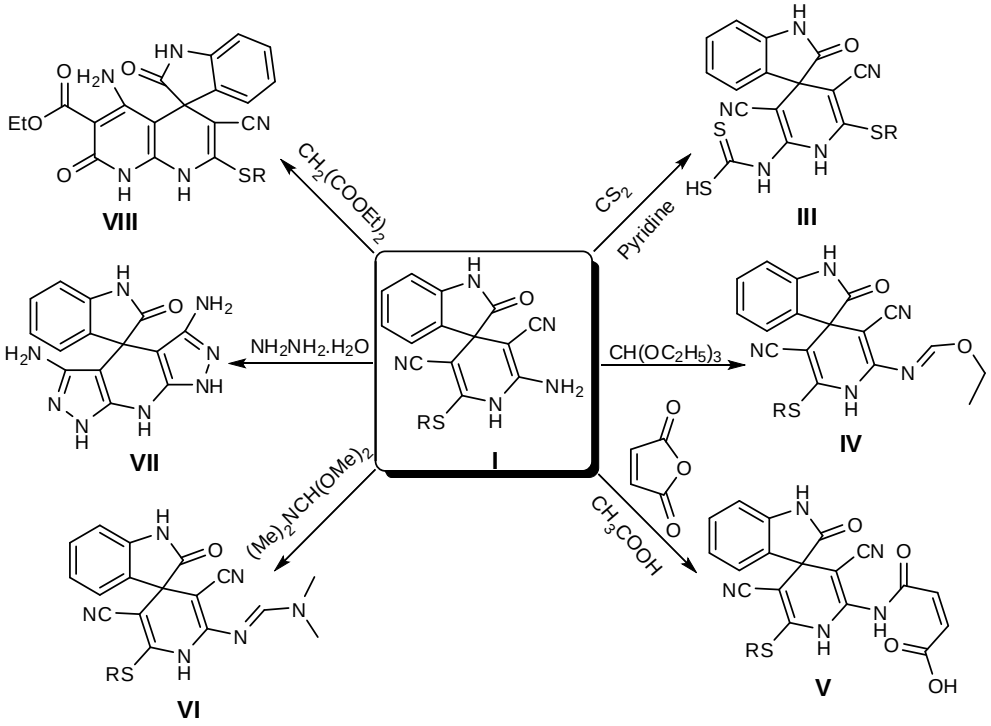
Scheme 1, Production of compounds **I** and **II**.

The reaction possibly took place *via* the following mechanism [50]:



Scheme 2, Proposed mechanism for compound **I** formation.

The configuration of compound **I** was established from their chemical analysis and spectroscopy information. The IR chart of start **I** indicated sturdy absorption bands for the primary amine group at *ύ* 3309 and 3242, for the secondary amino groups at *ύ* 3446 and 3212 cm^{-1}, according to the cyano group at *ύ* 2215 and 2176 cm^{-1}, and because of the (C=O) carbonyl group of an amide at *ύ* 1707 cm^{-1}. Furthermore, the ^{1}H NMR (DMSO-d_6) spectrum of the product **I** presented a singlet signal at δ 10.51 ppm appointed to (N*H*) proton of isatin, singlet signals around δ 9.46 ppm of the (dihydropyridine-N*H*) one proton, signals around δ 6.84-7.28 ppm assigned to the four protons of isatin aromatic ring, a singlet signal around δ 6.16 ppm of (NH_2) two protons, a quartet signal around δ 3.03 ppm for the two protons of (CH_2) group, and a triplet signal around 1.27 ppm for the 3 protons of the terminal (CH_3) group. Also, the mass spectroscopy spectrum presented the peak of molecular ion at *m/z* = 327 (M^++4), which corresponds with the chemical structure that has been proposed. [62]. Compound **I** reacted with different substances such as carbon disulfide, triethyl orthoformate, *N*, *N*-dimethylformamide dimethyl acetal, and maleic anhydride to afford several valuable heterocyclic derivatives (Scheme 3).



Scheme 3, Reactions of Compound **I**

(3',5'-Dicyano-2'-(ethylthio)-2-oxo-1'*H*-spiro[indoline-3,4'-pyridin]-6'-yl)carbamo-dithioic acid **III** was produced by reacting compound **I** with carbon disulfide in pyridine under reflux conditions for 12 hours (Scheme 3). The construction of compound **III** was examined by chemical investigation and spectroscopy results. The IR chart spectrum of sample **III** recorded the absence of the amino group (NH_2) absorption band and the presence of a new sturdy absorption band at *ύ* 1214 cm^{-1} equivalent to (C=S) group. furthermore, The predicted chemical formula was confirmed by the mass spectrum, which revealed the peak of molecular ion at *m/z* = 399.

To further search the synthetic potential of derivative **I**, its mixture with triethyl orthoformate was observed. Thus, refluxing compound **I** with triethyl orthoformate in acetic anhydride or ethanol solution gave compound **IV** (Scheme 3). The construction of the last compound was asserted given basic chemical analysis and spectroscopy information. The IR spectrum of chemical **IV** presented the absence of the amino group (NH_2) absorption band. The ^{1}H NMR (DMSO-d_6) results supplied still more support for compound IV's proposed structure., which discovered three new peaks at δ 1.22 ppm according to the three protons of (CH_3) group, a quartet signal around δ 3.38 ppm for the two protons of (CH_2) group, and at δ 4.33 ppm confirming the assert of (C*H*) proton.

Additionally, the reaction of sample **I** with maleic anhydride in acid "such as CH_3COOH" afforded the maleic acid derivative **V**. Spectroscopic and analytical information were used to verify the compound's **V** configuration. Thus, IR spectra of compound **V** exhibited a broad absorption band at *ύ* 3000-3600 cm^{-1} corresponding to (OH) group. Another piece of suggestion for the proposed construction of compound **V** was gained from the ^{1}H NMR (DMSO-*d6*) chart, which revealed three singlet resonances at δ 5.78, 5.42, and 11.61 ppm, approving the existence of 2(C*H*) and (O*H*) groups, respectively.

Moreover, *N*, *N*-dimethyl formimidamide derivative **VI** could be afforded *via* the reaction of compound **I** with *N*, *N*-dimethylformamide dimethyl acetal in refluxing dioxane (Scheme 3). The construction of product **VI** was verified founded on its investigative and spectroscopic information, as the infrared spectrum presented an absorption band at *ύ* 1627 cm^{-1} equivalent to (C=N) groups. Further confirmation for the proposed formation of the product **V** was gained from the ^{1}H NMR (DMSO-*d*6) data, which revealed two singlet resonances at δ 3.29 ppm integrating for six protons of two terminal methyl groups and at δ 8.82 ppm confirming the existence of (C*H*) proton.

The synthetic scheme for developing a pyrazole ring converged with the pyridine moiety of compound **I** was based on its reaction with hydrazine hydrate in ethanol under refluxing conditions followed by a subsequent cyclization reaction of the product to give spiro[dipyrazolo]pyridine derivative **VII** (Scheme 3). The product's **VII** IR spectra revealed the absence of the two cyano groups (CN) absorption bands. Furthermore, the mass spectra revealed a molecular ion peak at *m/z* = 308 which corresponds to the calculated molecular composition.

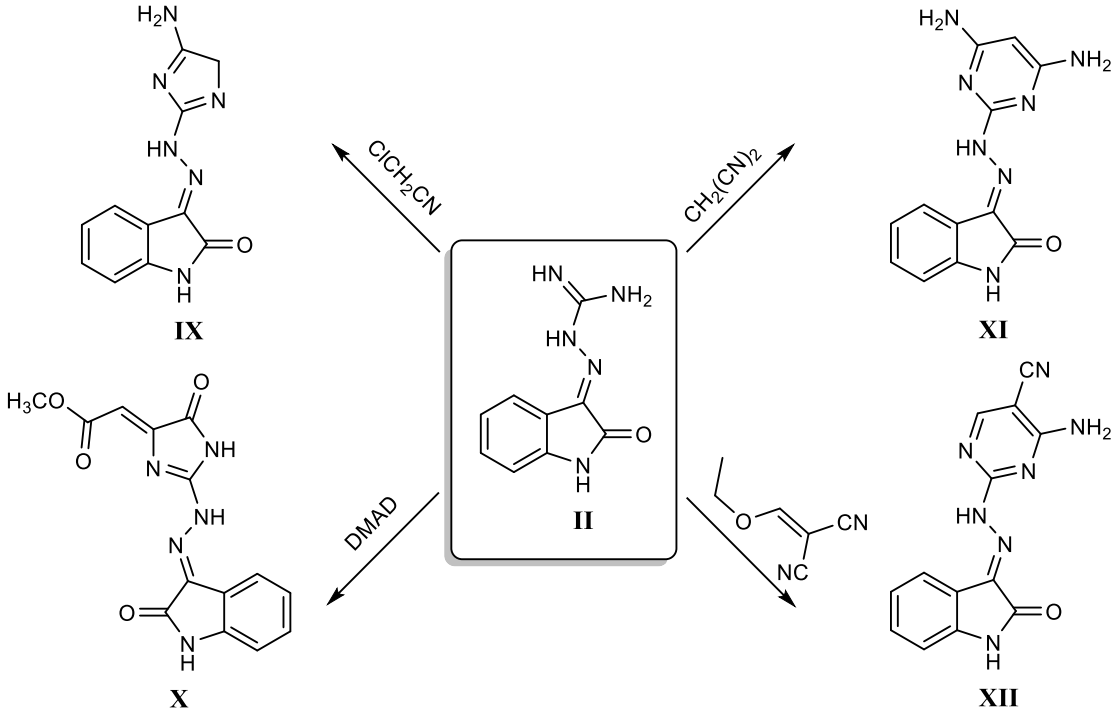
An additional synthetic method for the manufacturing of ethyl-5'-amino-3'-cyano-2'-(ethylthio)-2,7'-dioxo-7',8'-dihydro-1'*H*-spiro[indoline-3,4'-[1,8]naphtha-yridine]-6'-carboxylate (**VIII**) was completed through refluxing compound **I** and diethyl malonate in glacial acetic acid for 12 hours (Scheme 3). The compound's **VIII** IR spectra displayed the absence of the cyano group (CN) absorption band at *ύ* 2176 cm^{-1} and the appearance of a strong absorption band for ester (C=O) at *ύ* 1708 cm^{-1}. A strong verification for the suggested structure of product **VIII** was won from the ^{1}H NMR (DMSO-d_6) records, which indicated the triplet-quartet design of the ethoxy group at δ 1.29 and δ 1.56 ppm substantiates the presence of (CH_3) and (CH_2) groups, respectively.

Moreover, building up an imidazole ring was available through the alkylation reaction of compound (**II**) with chloro reagent "such as chloroacetonitrile", followed by a subsequent cyclization reaction of the alkylated produce in a basic condition. The alkylation reaction occurred in ethanol having drops of piperidine to have 5-amino-4*H*-imidazole derivative **IX** (Scheme 4). The construction of compound **IX** was proved from spectroscopic figures such as IR, which exhibited the presence of a new absorption band at *ύ* 2923 cm^{-1} for aliphatic (CH). Also, The molecular ion peak at *m/z*= 242 which corresponds to the molecular composition $C_{11}H_{10}N_6O$ was identified by the mass spectroscopy of compound IX.

Furthermore, a novel imidazole ring connected to oxindole derivative **II** was available through the reaction of dimethyl acetylene dicarboxylate with 2-indolone derivative **II** in ethanol under reflux conditions to give methyl-2-(5-oxo-2-(2-(2-oxoindolin-3-ylidene)hydrazinyl)-1,5-dihydro-4*H*-imidazol-4-ylidene)acetate (**X**) (Scheme 4). The configuration of compound **X** was proved *via* spectral data. IR presented no absorption bands for the amino group (NH_2) but exposed the appearance of two new absorption bands at *ύ* 2962 cm^{-1} for aliphatic (CH) and at *ύ* 1731 cm^{-1} for ester (C=O). The mass spectral data for compound **X** indicated the molecular ion peak at *m/z* = 313 according to the molecular installation $C_{14}H_{11}N_5O_4$.

Moreover, 4,6-diaminopyrimidine derivative **XI** could be manufactured *via* the condensation of 2-indolone derivative **II** with an active methylene compound "like malononitrile" in refluxing DMF containing droplets of piperidine (Scheme 4). The configuration of the construction of product **XI** was depend on Fundamental analysis and spectroscopic information. Thus, the IR chart discovered the appearance of a new absorption band for (NH_2) group. Additionally, the mass spectroscopy spectrum of product **XI** indicated the molecular ion peak at *m/z* = 269 analogous to the molecular installation $C_{12}H_{11}N_7O$.

Finally, 4-amino-pyrimidine-5-carbonitrile derivative **XII** was obtained by boiling 2-indolone derivative **II** with ethoxy methylene malononitrile in ethanol (Scheme 4). Fundamental analysis and data from spectroscopy were used to confirm the manufacturing of product **XII**. The IR spectrum of product **XII** revealed the Existence of a different absorption band at *ύ* 2217 cm^{-1} for (CN) group. Finally, The mass spectral data for sample **XII** presented the molecular ion peak at *m/z* = 279, which matched the chemical formula that was suggested.



Scheme 4, Reactions of compound **II**.

4. Conclusions

To prepare spiro[indoline-pyridine]dicarbonitrile derivative (I), we find that it is one of the best ways to obtain high efficiency and less reaction time with the use of available, cheap, and energy-saving chemicals, as the reaction takes place between isatin, active methylene "such as malononitrile", and mercaptan when there was diethylamine as a basic catalyst in cold alcohol. In addition to the ease of forming derivatives with different open chains and heterocyclic compounds by interacting with different reagents, for example, carbon disulfide, maleic anhydride, triethyl orthoformate, hydrazine hydrate, diethyl malonate, and dimethyl formamide dimethyl acetal.

Similarly, indolinylidene hydrazine derivative (II) is also highly active when interacting with different reagents to form heterocyclic compounds.

All synthesized compounds were tried *in vitro* opposite four types of bacteria to study their antibacterial activity.

Author Contribution

All authors have contributed equally.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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