Research Article

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Synthesis and Biological Activity Evaluation of Some New Fused Pyrano [2,3-b] Pyridine Derivatives

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Abstract: Some fused Pyrano[2,3-b]Pyridines derivatives have been synthesized by the cyclo condensation reaction of amino cyano spiro pyrano derivative (3'-Acetyl-6'-amino-2'-methyl-2-oxospiro[indoline-3,4'- pyran]-5'-carbonitrile) with some arylidene malononitrile derivatives. The synthesized compounds have been characterized by FT-IR, NMR spectroscopy, and elemental analyses. Newly synthesized compounds were evaluated for antimicrobial activity against filamentous and unicellular fungi *Aspergillus fumigatus* and *Candida albicans*, gram-negative bacteria *Pseudomonas aeruginosa*, and gram-positive bacteria *Staphylococcus aureus*. The synthesized compounds promise to possess high selectivity and limited side effects. All compounds exhibited effective to moderate action against all tested microorganisms thanks to spiro heterocycles, which have a vital part in biological processes, pharmacological and therapeutic activities, and a combination of Pyran, isatin, and pyridine moieties which exhibit fungicidal, antibacterial properties, and unique, versatile molecular structure. Compound **8** was the most potent and active compound against the tested bacteria; particularly, against Staphylococcus aureus as a gram-positive bacteria with an impact (up to 90%).

Keywords: Pyrano[2,3-b] Pyridines, spiro pyran and antimicrobial activity.

1. Introduction

The pharmaceutical industry has traditionally placed a high emphasis on the research of aromatic six-membered *N*-heterocyclic rings because they possess a bioisosteric component that has significant implications for both theory and practice. They can be found in highly toxic alkaloids like nicotine as well as in the essential vitamins pyridoxine (vitamin B6) [1], numerous synthetic medications, alkaloids, vital amino acids, hemoglobin, hormones, and colors [2]. *N*-heterocyclic compounds are used as antibiotics, anthelmintics, antivirals, antidepressants, and anti-inflammatory medications [3-5]. They are also vital structural elements for medicinal chemists. Pyran derivatives are used as antitumor [6], anti-microbial [7], anticancer [8], antiviral [9], antimalarial [10], anti-HIV [11], anti dyslipidemic, anti-hyperglycemic [12], anti-inflammatory [13], antifungal [14], antituberculosis [15] and cytotoxic activities [16, 17]. Isatin is found in a lot of potent synthetic pharmaceutical drugs and natural alkaloid products [18]. One of the key components of biological activities is the asymmetric chiral spiro carbon link between the isatin and pyran rings in the molecule [18,19]. Our work aims to create antifungal drugs that are more selective and have fewer side effects by combining a spiro pyran ring with a pyridine scaffold.

2. Materials and methods

Melting points were determined using a Melt-Temp-II instrument. The IR spectra were recorded using potassium bromide discs with a Shimaduz 408 instrument. The ^{1}H NMR (400 MHz) and ^{13}C NMR (101 MHz) spectra were measured using a Brucker Bio spin AG-400 spectrometer in DMSO-d6 as the solvent and the internal standard TMS. Elemental analysis was performed using a PerkinElmer 240C Micro Analyzer. Thin layer chromatography, UV light, and silica plates (Merck Fertigplatten Kieselgel 60 F254) followed the reaction's progress.

2.1 Synthesis of 3'-Acetyl-6'-amino-2'-methyl-2-oxospiro[indoline-3,4'- pyran]-5'-carbonitrile (3)

Isatin (1 mmol, 0.15gm), malononitrile (2 mmol, 0.13ml), and acetylacetone (1 mmol, 0.1ml) were mixed in a beaker with a solution of DAHP (10 mol %) in H_2O(10ml): EtOH(5 ml) and exposed to M.W at 900 W for 5-7 minutes. After completion of the reaction, the solid product was filtered and purified by washing with ethanol [20].
Yellow powder; Yield 80%, m.p : 270-275 ^{o}C ; IR (KBr, cm^{-1}): 3369, 3295 and 3195(NH_2, NH); 2193 (CN), 1716, 1661(CO); 1H-NMR (400 MHz, DMSO, d6): δ 10.39 (s, 1H, NH), 7.19 (t, 1H, CH-Ar), 7.13 (s, 2H, NH_2), 6.99 (d, 1H, CH-Ar), 6.93 (t, 1H, CH-Ar), 6.79 (d, 1H, CH-Ar), 2.29 (s, 3H, CH_3-acetyl), 2.09 (s, 3H, CH_3-pyran).

2.2 General Procedure for the Synthesis of Compounds (5-8)

Compound 3 (1 mmol, 0.3gm), arylidene malononitrile derivatives(1 mmol, **4a**;0.18g, **4b**;0.19g, **4c**;0.15g, **4d**;0.17g) were combined in EtOH (20 ml) as the solvent and piperidine (0.5 ml) as the catalyst. The reaction mixture was allowed to reflux for 20 hours. The reaction was monitored by TLC analysis. The solid products were precipitated on cold, and crystalized by ethanol.

3'-acetyl-5'-amino-7'-(4-methoxyphenyl)-2'-methyl-2-oxospiro[indoline-3,4'-pyrano[2,3-b]pyridine]-6'-carbonitrile (5)

Color; Orange , m.p: 240 ºC , Yield; 50 %, IR(KBr,cm^{-1}); 3335, 3208, 3141 (NH_2, NH) , 3008 (C-H aromatic), 2934, 2838 (C-H aliphatic), 2215 (CN), 1697 (CO) ; ^{1}H NMR (400 MHz, DMSO) δ 12.16 (s, 1H, NH), 7.70-6.69 (m, 10H, 8Haromatic, 2H, NH_2), 3.83 (S, 3H, OCH_3), 2.36 (s, 3H,CH_3–acetyl), 2.28 (s,3H,CH_3); ^{13}C NMR (101 MHz, DMSO) δ 165.7, 161.6, 161.1, 160.7, 148.5, 138.0, 135.6, 132.3, 130.3, 129.3, 128.2, 127.6, 126.7, 121.5, 120.3, 119.2, 116.8, 104.7, 104.0, 102.0, 101.2, 55.7, 29.4, 21.2. Chemical Formula: $C_{26}H_{20}N_4O_4$ (452.46); Elemental Analysis: C, 69.02; H, 4.46; N, 12.38; Found: C, 69.01; H, 4.45; N, 12.36.

3'-acetyl-5'-amino-7'-(4-chlorophenyl)-2'-methyl-2-oxospiro[indoline-3,4'-pyrano[2,3-b]pyridine]-6'-carbonitrile (6)

Color; Yellow powder, m.p>300 ºC, Yield;50 %, IR(KBr,cm^{-1}); 3359, 3294, 3139, (NH_2, NH) , 3007 (C-H aromatic), 2962, 2906 (C-H aliphatic), 2219 (CN), 1683 (CO); ^{1}H NMR (400 MHz, DMSO) δ 12.28 (s, 1H,NH), 7.72-6.63 (m, 10H, 8Haromatic, 2H, NH_2), 2.38 (s, 3H,CH_3-acetyl), 2.29 (s, 3H,CH_3); ^{13}C NMR (101 MHz, DMSO) δ 161.8, 161.3, 160.4, 155.2, 151.5, 147.9, 138.2, 138.1, 136.2, 134.9, 134.5, 129.8, 129.6, 123.5, 121.2, 116.5, 116.3, 115.1, 107.5, 101.8, 101.1, 97.8, 21.2, 19.6. Chemical Formula: $C_{25}H_{17}ClN_4O_3$ (456.88);Elemental Analysis: C, 65.72; H, 3.75; Cl, 7.76; N, 12.26;Found: C, 65.70; H, 3.72; Cl, 7.74; N, 12.23 .

3'-acetyl-5'-amino-2'-methyl-2-oxo-7'-phenylspiro[indoline-3,4'-pyrano[2,3-b]pyridine]-6'-carbonitrile (7)

Color: Yellow, m.p:295 ºC, Yield; 45 %, IR(KBr,cm^{-1}); 3361, 3296, 3138 (NH_2, NH), 3009 (C-H aromatic), 2914, 2860 (C-H aliphatic), 2218 (CN), 1685 (CO); ^{1}H NMR (400 MHz, DMSO): δ 12.26 (s, 1H, NH), 7.85-6.66 (m, 11H, 9Haromatic, 2H, NH_2), 2.38 (s, 3H, CH_3-acetyl), 2.23 (s, 3H, CH_3).^{13}C NMR (101 MHz, DMSO) δ 163.6, 161.5, 161.1, 160.5, 148.4, 137.7, 135.6, 132.4, 130.5, 130.3, 129.3, 128.2, 127.6, 120.5, 120.3, 116.7, 109.5, 107.9, 106.9, 101.0, 100.8, 29.3, 21.2. Chemical Formula: $C_{25}H_{18}N_4O_3$ (422.44); Elemental Analysis: C, 71.08; H, 4.29; N, 13.26; Found: C, 71.06; H, 4.28; N, 13.23.

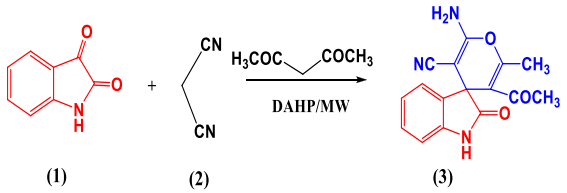
3'-acetyl-5'-amino-2'-methyl-2-oxo-7'-p-tolylspiro[indoline-3,4'-pyrano[2,3-b]pyridine]-6'-carbonitrile (8)

Color; Yellow, m.p>300 ºC, Yield; 60 %, IR (KBr, cm^{-1}); 3361, 3281, 3144 (NH_2, NH), 3079 (C-H aromatic), 2955, 2922 (C-H aliphatic), 2217 (CN), 1686 (CO). ^{1}H NMR (400 MHz, DMSO): δ 12.20 (s, 1H, NH), 7.68-6.64 (m, 10H, 8 H aromatic, 2H, NH_2), 2.36 (s, 3H,CH_3-acetyl), 2.23 (s, 3H,CH_3), 1.58 (s, 3H,CH_3) ; ^{13}C NMR (101 MHz, DMSO) δ 162.1, 161.7, 161.5, 161.3, 150.1, 140.2, 137.7, 135.2, 133.1, 132.9, 130.3, 128.3, 127.9, 124.7, 121.5, 119.1, 116.7, 114.6, 110.3, 106.9,100.6, 31.3, 21.5, 21.4. Chemical Formula: $C_{26}H_{20}N_4O_3$ (436.46); Elemental Analysis: C, 71.55; H, 4.62; N, 12.84; Found: C, 71.53; H, 4.60; N, 12.81.

3. Results and Discussion:

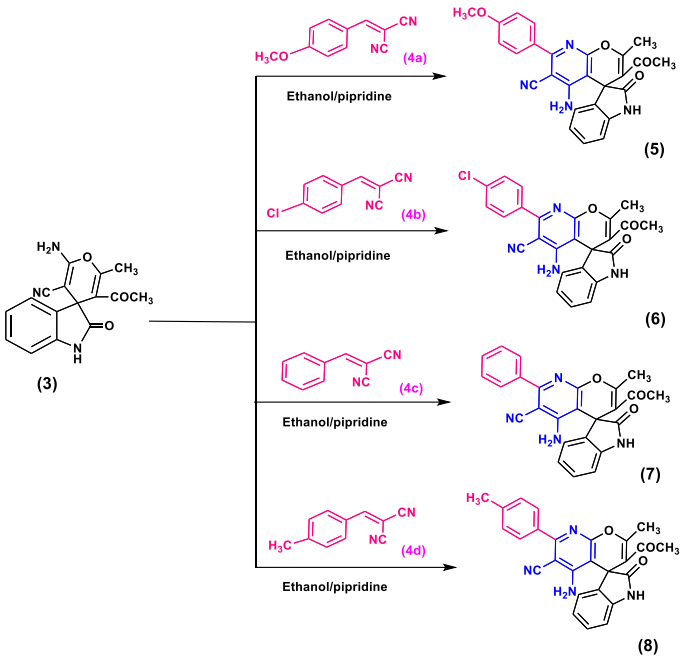
3.1. Chemistry

A spiro compound (3) has been synthesized by reaction of isatin, malononitrile and acetyl acetone in one-pot efficient procedure. The spiro Isatin pyran system was prepared by amount of Di-ammonium Hydrogen Phosphate (DAHP) as a catalyst in aqueous condition under the effect of MW irradiations (Scheme 1). [20]

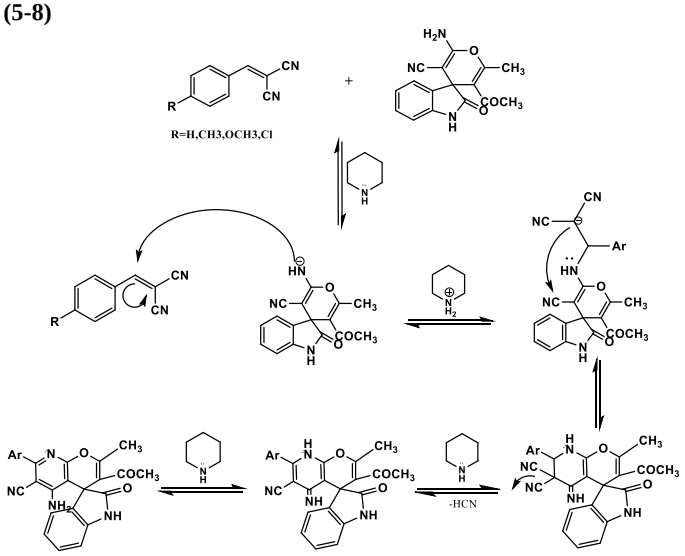


Scheme 1: 3'-Acetyl-6'-amino-2'-methyl-2-oxospiro[indoline-3,4'-pyran]-5'-carbonitrile synthesis (3)

Compounds (**5-8**) were synthesized by cyclo condensation of 3'-Acetyl-6'-amino-2'-methyl-2-oxospiro[indoline-3,4'-pyran]-5'-carbonitrile 3 with some 2-arylidene malononitrile derivatives (synthesized by reaction of some aldehydes with malononitrile) [21-25] in refluxing ethanol containing a catalytic amount of piperidine. The reaction was left for cold precipitation and afforded 3′-acetyl-5'-amino-2'-methyl-2-oxospiro[indoline-3,4'-pyrano[2,3-b]pyridine]-6'-carbonitrile derivatives (**5-8**) in yields ranging from 45%–60%. The suggested mechanism for spiro pyrano[2,3-b]pyridine derivatives formation is explained in Scheme 3. The chemical structures of synthesized derivatives (**5-8**) were demonstrated based on analytical and spectral data. IR Spectroscopy revealed presence of absorption peaks at 3425- 3361 cm^{-1} for NH_2, and 2214-2219 cm^{-1} for CN group, ^{1}HNMR Spectrum showed new signals corresponding to aromatic ring in compounds (**5-8**) at 7-8 ppm, CH_3 for compound 8 at 1.58 ppm, and OCH_3 at 3.83 ppm for compound 5, ^{13}CNMR spectra show increasing carbon signals corresponding to the formation of phenyl and pyridine ring.



Scheme 2: Synthesis of fused spiro pyrano[2,3-b]pyridine derivatives **(5-8)**



Scheme 3: The suggested mechanistic pathway for synthesis of spiro pyrano[2,3-b]pyridine derivatives.

3.2. Antimicrobial Activity

The biological activity of the newly synthesized compounds was investigated using the disc diffusion assay method, which used discs of disinfected filter paper (6 mm diameter). The tested compounds were dissolved in DMSO and placed onto the discs at a concentration of 5 mg/ml. The discs were then placed in Petri dishes that had previously been loaded with various bacterial strains: *Pseudomonas aeruginosa* (ATCC 47085) as -Ve bacteria, *Staphylococcus aureus* (ATCC 25923) as +Ve bacteria, and *Aspergillus fumigatus* (ON428521) and *Candida albicans* (RCMB 05036) as filamentous and unicellular fungi. The results of the biological activity are shown in Table (1).

Table 1: The antimicrobial action of synthesized compounds (diameter of the inhibitory zone in millimeters)

Tested microorganisms	+Ve Bacteria *	-Ve Bacteria *	Fungi *	
Stand. / Compds.	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Aspergillus fumigatus*	*Candida albicans*
	Ampicillin	Gentamicin	Amphotericin B	
	23.8 ± 0.2	17.3 ± 0.1	20.2 ± 0.1	21.9 ± 0.1
3	13.2 ± 0.3 (55.46%)	09.5 ± 0.3 (54.91%)	09.9 ± 0.1 (49.01%)	10.1 ± 0.2 (46.12%)
5	19.8 ± 0.2 (83.19%)	13.1 ± 0.2 (75.72%)	17.1 ± 0.3 (84.65%)	18.9 ± 0.2 (86.30%)
6	17.1 ± 0.3 (71.85%)	12.0 ± 0.1 (61.85%)	14.3 ± 0.2 (70.79%)	15.4 ± 0.2 (70.32 %)
7	15.3 ± 0.1 (64.29%)	11.7 ± 0.5 (67.63%)	12.7 ± 0.2 (62.87%)	13.8 ± 0.2 (63.01%)
8	21.9 ± 0.1 (92.02%)	13.3 ± 0.5 (76.88%)	16.8 ± 0.5 (83.17%)	19.2 ± 0.2 (87.67%)

+Ve: gram positive, -Ve: gram negative; * Inhibition zone diameter in mm and (%) value

The results in **Table 1** reveal that compound 8 has potent activity against +Ve bacteria, whereas compounds 5 and 8 have good activity against +Ve and -Ve bacteria, respectively. Compounds 5 and 8 were the most effective against *Aspergillus fumigatus* and *Candida albicans*, respectively.

4. Conclusion

Some fused Pyrano[2,3-b]Pyridines derivatives have been synthesized. The newly synthesized compounds were tested for antibacterial activity against *Aspergillus fumigatus*, *Candida albicans*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. All of the compounds evaluated had an effective to moderate effect on the bacteria. Compound 8 demonstrated the most powerful inhibitory effect against gram-positive bacteria, with an impact of more than 90%; compounds 5, 8 are the most active against *Aspergillus fumigatus* and *Candida albicans*.

CRediT authorship contribution statement:

Conceptualization, validation, investigation, project administration, visualization, writing—original draft preparation Mahmoud Abd El Aleem Ali Ali El-Remaily; writing—review and editing, resources, Ahmed.M.M.Soliman; methodology, data curation Ahmed E Abdel-Aziz, Moumen S. Kamel; software, writing—review and editing, writing—original draft preparation, data curation Alaa El-Araby; supervision E. Kh. Shokr. All authors have read and agreed to the published version of the manuscript.

Data availability statement

Supplementary material and the published article are available on the publisher's website.

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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References

[1] E.H. El-Sayed, A.A. Fadda, *Journal of Heterocyclic Chemistry* 55(10) (2018) 2251-2260.
[2] A.R. Bhat, R.S. Dongre, F.A. Almalki, M. Berredjem, M. Aissaoui, R. Touzani, T.B. Hadda, M.S. Akhter, *Bioorganic Chemistry* 106 (2021) 104480.
[3] P. Ramalingam, S. Ganapaty, C.B. Rao, *Bioorganic & medicinal chemistry letters* 20(1) (2010) 406-408.
[4] A.H. Shamroukh, M.E. Zaki, E.M. Morsy, F.M. Abdel-Motti, F.M. Abdel-Megeid, *Archiv der Pharmazie: An International Journal Pharmaceutical and Medicinal Chemistry* 340(5) (2007) 236-243.
[5] A.K. Mansour, M.M. Eid, N.S. Khalil, *Molecules* 8(10) (2003) 744-755.
[6] R.A. Azzam, R.M. Mohareb, *Chemical and Pharmaceutical Bulletin* 63(12) (2015) 1055-1064.
[7] G.H. Sayed, M.E. Azab, K.E. Anwer, *Journal of heterocyclic chemistry* 56(8) (2019) 2121-2133.
[8] U.D. Nimbalkar, J.A. Seijas, M.P. Vazquez-Tato, M.G. Damale, J.N. Sangshetti, A.P.G. Nikalje, *Molecules* 22(10) (2017) 1628.
[9] P.N. Kalaria, S.P. Satasia, D.K. Raval, *New Journal of Chemistry* 38(4) (2014) 1512-1521.
[10] A. Kumar, R.A. Maurya, S. Sharma, P. Ahmad, A. Singh, G. Bhatia, A.K. Srivastava, *Bioorganic & medicinal chemistry letters* 19(22) (2009) 6447-6451.
[11] J. RAJAN, S. KRISHNAN, V.M. SRUTHI, M.Z. SUBIN, G. RAJASREE, M. Surya, M. JAVAHAR, N. GEORGE, *Asian Journal of Pharmaceutical and Clinical Research* 10(7) (2017) 408-413.
[12] P.K. Paliwal, S.R. Jetti, S. Jain, *Medicinal Chemistry Research* 22 (2013) 2984-2990.
[13] H. Xiao, M. Lu, T.Y. Lin, Z. Chen, G. Chen, W.-C. Wang, T. Marin, T.-p. Shentu, L. Wen, B. Gongol, *Circulation* 128(6) (2013) 632-642.
[14] R.M. Mohareb, N.Y.M. Abdo, *Chemical and Pharmaceutical Bulletin* 63(9) (2015) 678-687.
[15] S. ABDULLAH EL-ASSIERY, G. HOSNI SAYED, A. Fouda, *Acta Pharmaceutica* 54(2) (2004) 143-150.
[16] S. Said Fatahala, S. Hasabelnaby, A. Goudah, G.I. Mahmoud, R. Helmy Abd-El Hameed, *Molecules* 22(3) (2017) 461.
[17] W.S. Shehab, A.A. Ghoneim, *Arabian Journal of Chemistry* 9 (2016) S966-S970.
[18] F.N. Faisal, R.S. Dawood, *Iraqi Journal of Science* (2024) 1839-1854.
[19] A. Mumtaz, T. Mahmud, M. Elsegood, G. Weaver, J.I. Qazi, F. Deeba, *Farmacia* 67(1) (2019).
[20] F. F Abdel-Latif, M. A. Ameen, O. M Elhady, M. S Kamel, *Journal of Pharmaceutical and Applied Chemistry* 1(2) (2015) 24-27.
[21] M.S. Mohamed, R. Kamel, S.S. Fathallah, *Archiv der Pharmazie* 344(12) (2011) 830-839.
[22] M. Mohamed, S. Ali, D. Abdelaziz, S.S. Fathallah, *BioMed research international* 2014(1) (2014) 249780.
[23] M.S. Mohamed, R. Kamel, R.H. Abd El-Hameed, *Medicinal Chemistry Research* 22 (2013) 2244-2252.
[24] G.Z. Zheng, Y. Mao, C.-H. Lee, J.K. Pratt, J.R. Koenig, R.J. Perner, M.D. Cowart, G.A. Gfesser, S. McGaraughty, K.L. Chu, *Bioorganic & medicinal chemistry letters* 13(18) (2003) 3041-3044.
[25] G.Z. Zheng, C.-H. Lee, J.K. Pratt, R.J. Perner, M.Q. Jiang, A. Gomtsyan, M.A. Matulenko, Y. Mao, J.R. Koenig, K.H. Kim, *Bioorganic & medicinal chemistry letters* 11(16) (2001) 2071-2074.

