

SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITIES OF SOME INDOLO [2-3-B] QUINOXALINE DERIVED FROM 3,4-DIAMINOTOLUENE OR 3,4-DIAMINOBENZOIC ACID WITH ISATIN

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ABSTRACT : In this research, many substituted o-phenylenediamine derivatives were synthesized. The reaction of isatin with 3,4-diaminobenzoic acid or 3,4-diaminotoluene in the aqueous sodium bicarbonate solution produce the compounds [O₁] and [O₂], respectively. Then the compound 2-methyl-6H-indolo [2-3-b] quinoxaline [O₂] react with phenyl isothiocyanate to give a new compound [O₃], as well as react with benzyl chloride to synthesize compound [O₄]. New compounds have been created [O₉-O₁₂] from the reaction of various compounds synthesized N-alkyl - isatin [O₅-O₈] with 3,4-diaminobenzoic acid. The spectral methods [FT-IR, ¹H-NMR] were used to prove the chemical structure for compounds. Also, the biological activity of some compounds evaluated against two types of bacteria (*E.colias*) and (*S. aureus*).

Key words : Isatin, Indolo [2-3-b] quinoxaline, alkyl halides, antibacterial activities.

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INTRODUCTION

The N-substituted isatin has been frequently used as intermediates and synthetic precursors for the preparation of a wide variety of heterocyclic compounds. In addition, properly functionalized N-alkyl isatin presents different biological activities (Vine *et al*, 2007). Indole is an important heterocyclic system because, it is built into proteins in the form of amino acid tryptophan, because it is the basis of drugs like indomethacin and because it provides the skeleton of indole alkaloids (Sharma *et al*, 2010). The indole derivative has been reported to possess a wide variety of biological activities, namely, anti-inflammatory, anticonvulsant, cardiovascular (Walmik, 2021), antibacterial, cyclooxygenase-2 inhibitor and antiviral. The significant contribution of many derivatives of indole in the development of medicinal chemistry is well recognized. Serotonin, known for its vasoconstrictor principle, plays a vital role as a neurotransmitter and in psychosis. Quinoline derivatives constitute an important class of compounds for new drug development. The quinoline ring system is an important structural unit widely existing in alkaloids, therapeutics and synthetic analogues

with exciting biological activities (Orhan *et al*, 2013). Also used antitumor (Ajani, 2014), antimalarial (Husain *et al*, 2011; Mustafa *et al*, 2018; Arkan *et al*, 2021; Huda *et al*, 2021) antiparasitic and insecticidal, antiviral (Senerovic *et al*, 2019), antiplatelet (Ali *et al*, 2017), anti-inflammatory (Asif, 2017), herbicidal, and antioxidant (Shang *et al*, 2018). Indole and quinoxaline derivatives display diverse pharmacological activities (Olayiwola *et al*, 2007).

MATERIALS AND METHODS

All chemicals and solvents used during synthesis compounds were purchased from a number of different companies such as Merck, BDH, Sigma Aldrich and Fulka. They were used as obtained without further purification. The purity of the synthesized compounds was checked by TLC sheet and the chemical structures were characterized by FT-IR, ¹H-NMR. The melting points of compounds were determined on Gallenkamp (MFB-600) melting point apparatus and are uncorrected. FT-IR spectra of compounds were recorded PERKIN ELMER SPECTUM-65 within the range [4000-400] using KBr disc in the Chemistry Department, University of Diyala. The ¹H-NMR spectra was recorded by Bruker 400 MHz

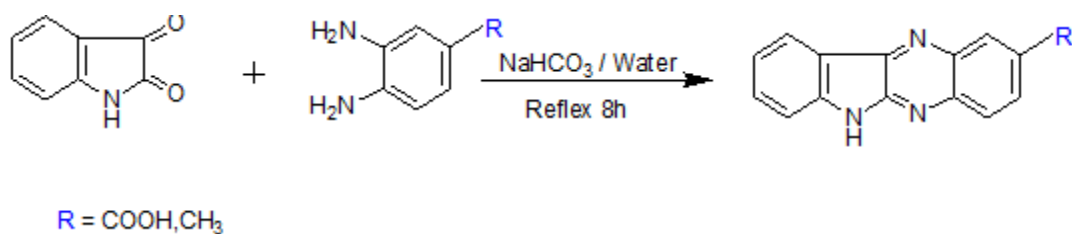


Fig. I :

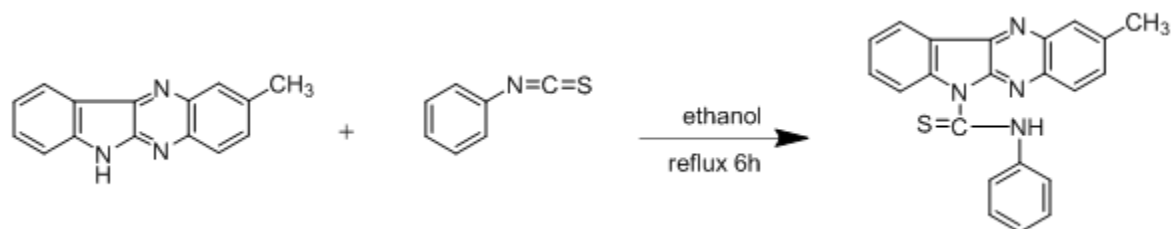


Fig. II :

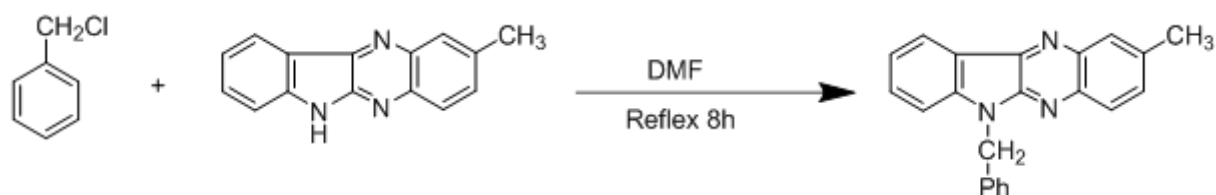


Fig. III :

spectrophotometer with TMS as internal standard and deuterated DMSO was used as a solvent in University of Science and technology, College of science, Irbid city, Jordan.

Synthesis methods

Synthesis and identification of 6H-indolo[2,3-b]quinoxaline-2-carboxylic acid [O_1] and 2-methyl-6H-indolo[2,3-b]quinoxaline [O_2] (Fig. I)

Isatin (1.71g, 11.6 mmol) was dissolved in the aqueous solution of refluxed sodium bicarbonate (2.38 g, 28.3 mmol in 80 ml water). 3,4-diamino benzoic acid or 3,4-diaminotoluene (13.2 mmol) was added and refluxed the mixture 8 h. The completion of the reaction was checked by using T.L.C, mobile phase (ethyl acetate:hexane 1:3). After cooling to room temperature, acetic acid was used to acidify the solution and allowed to remain overnight. The precipitate appeared was filtered, washed with water, dried and recrystallized from ethanol (Ismail *et al*, 2020). The physical properties of compounds (O_1 and O_2) are mentioned in Table (1).

Synthesis of 2-methyl-indolo[2,3-b]quinoxaline-6-carbothioic acid phenylamide [O_3] (Fig. II)

A mixture of (0.2g ,0.85 mmol) of 2-methyl-6H-indolo[2,3-b] quinoxaline [O_2] and (0.11g , 0.85 mmol) of phenyl isothiocyanate in absolute ethanol 20 ml was refluxed at 78°C for 6 h in water a bath. The solvent evaporated under the reduced pressure, and the precipitate was filtered off, washed with hexane and dried

in an oven to give pure light brown precipitate. The physical properties of compound are listed in Table 1.

Synthesis of 6-Benzyl-2-methyl-6H-indolo[2,3-b]quinoxaline [O_4] (Fig. III)

A mixture of 0.2g 0.81 mmol 2-methyl-6H-indolo[2,3-b]quinoxaline dissolved in 15 ml DMF. Then added potassium carbonate (0.27 g, 2 mmol) and (0.11g, 0.88 mmol) of benzyl chloride that dissolved in 15 ml DMF. The mixture was refluxing for 8 h in oil bath. The solvent evaporated under the reduced pressure, and the brown residue was filtered off, washed with hexane, and dried in an oven to give precipitate pure brown-black (Hassan *et al*, 2011). The physical properties of compounds are registered in Table 1.

General method for the synthesis of the compounds [O_5 – O_8] (Fig. IV)

Isatin (1.03g, 7 mmol) was dissolved in the aqueous solution of refluxed potassium carbonate (1.38 g, 10 mmol) in DMF 15 ml, the corresponding alkyl halide (7.7mmol) in DMF 15 ml was added to this solution and the mixture was refluxed in an oil bath at appropriate temperature for 8-10 h and monitored by TLC. When the reaction was completed, the reaction mixture was poured into ice-water. If the product crystallized, the resulting solid was filtered, washed with water and purified by recrystallization with appropriate solvent. If not, the suspension was extracted with chloroform and the organic layer was washed by using water and then dried. Most

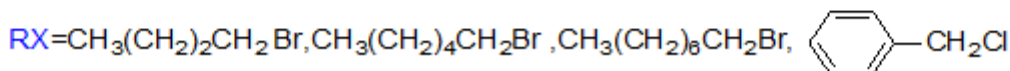
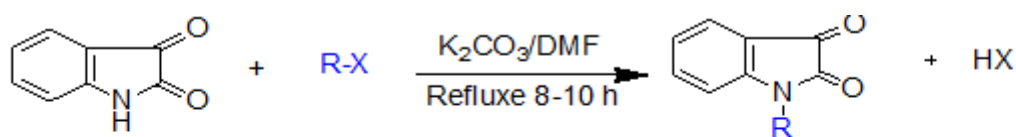


Fig. IV :

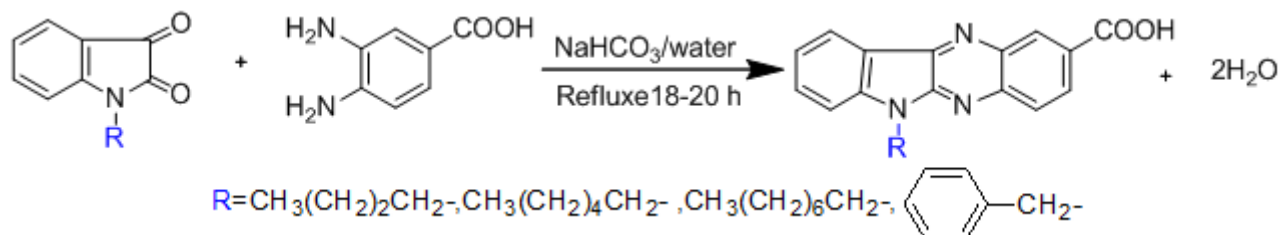


Fig. V :

of the resulting compounds were oily (Shmidt *et al*, 2008). Table 1 explain the physical properties of compounds (O_5 - O_8).

General method for the synthesis of the compounds [O_9 - O_{12}] (Fig. V)

A mixture of O_5 - O_8 (N-alkyl isatin) (11 mmol) was dissolved in refluxing aqueous sodium bicarbonate solution (2.38 g, 28.3 mmol in 80 ml water) with (13mmol) of 3,4-diaminobenzoic acid was added and the mixture was refluxed for 18-20 h. The completion of the reaction was checked by using T.L.C, mobile phase (ethyl acetate:hexane 1:3). After cooling, the solution was acidified with acetic acid and left to stay overnight. The solution was filtered, and the precipitate formed washed with distilled water, and desiccated, also recrystallized using absolute ethanol. Table 1 explains the physical properties of compounds (O_9 - O_{12}).

Biological activity

The synthesized compounds (O_3 , O_{10} and O_{12}) were screened for their antibacterial activities toward two strains of bacteria (*E. coli*) as a gram-negative and (*S. aureus*) as a gram-positive bacteria by using mueller hinton agar diffusion method (66) in nutrient agar medium. These sterilized agar media were poured into petri dishes and allowed to solidify. The microbial suspensions were spread on the surface of the media by sterilized triangular loop. A sterilized stainless-steel cylinder of 8mm was used to make cavities and the solution of the test compounds in DMSO 200 mg/ml were added into each cavity with the help of micropipette and allowed to spread for 1 hour, the plates were then incubated for 24 h at 37°C. The zones of inhibition appeared around the cups were measured in mm. STREPTOMTCIN used as standard

antibiotics. The isolates used in this study were multidrug resistance to antibiotics.

RESULTS AND DISCUSSION

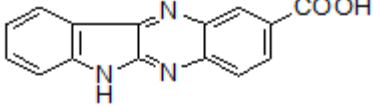
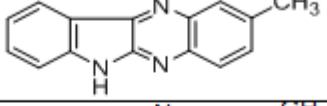
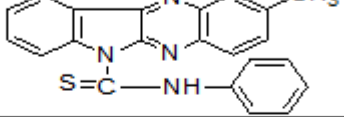
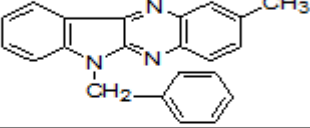
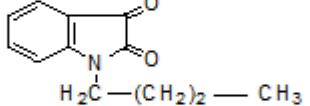
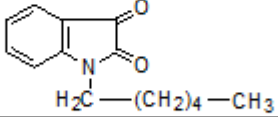
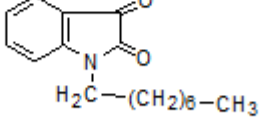
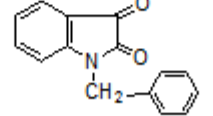
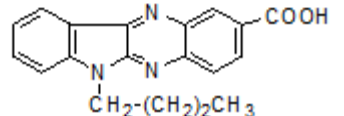
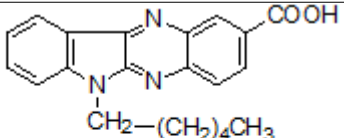
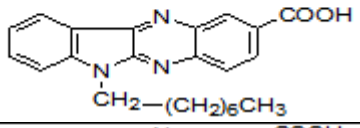
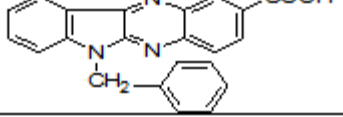
FT-IR spectroscopy for the prepared compounds

The FT-IR spectrum of compound O_1 shows absorption band at 3365 cm^{-1} was attributed to O-H group (broad). The new clear absorption band at 1564 cm^{-1} was due to C=N stretching vibration. Bond absorption at 1667 cm^{-1} was due to stretching vibration of C=O. The spectrum of compound O_3 indicated the absorption band at 3211 cm^{-1} was attributed to stretching vibration of N-H. The absorption bands at 3027 cm^{-1} and at 2981 cm^{-1} were due to CH aromatic and CH aliphatic respectively. Also, the band at 1655 cm^{-1} was due to C=N stretching vibration. And the spectrum of compound O_4 shows absorption bands at 3027 cm^{-1} was due to C-H aromatic, absorption band at $2919\text{-}2847\text{ cm}^{-1}$ was due to C-H aliphatic, and at 1607 cm^{-1} was due to C=N stretching vibration. Whereas the spectrum of compound O_5 showed the band at 1715 cm^{-1} was assigned to stretching vibration of C=O. As stretching bands of aliphatic C-H appeared at $2961\text{-}2871$. Finally, the FT-IR spectra of compound O_{10} showed the appearance absorption band at 3471 cm^{-1} was due to O-H. The band at 3081 cm^{-1} was due to C-H aromatic. Whereas stretching bands of aliphatic CH appeared at $2958, 2856$. Bond absorption at 1636 cm^{-1} was assigned to C=N stretching vibration. The bands at 1777 cm^{-1} due to the stretching vibration of C=O (carboxylic acid) [15]. The FT-IR spectral data of compounds O_1 - O_{12} are listed in Table 2.

$^1\text{H-NMR}$ spectroscopy for the prepared compounds

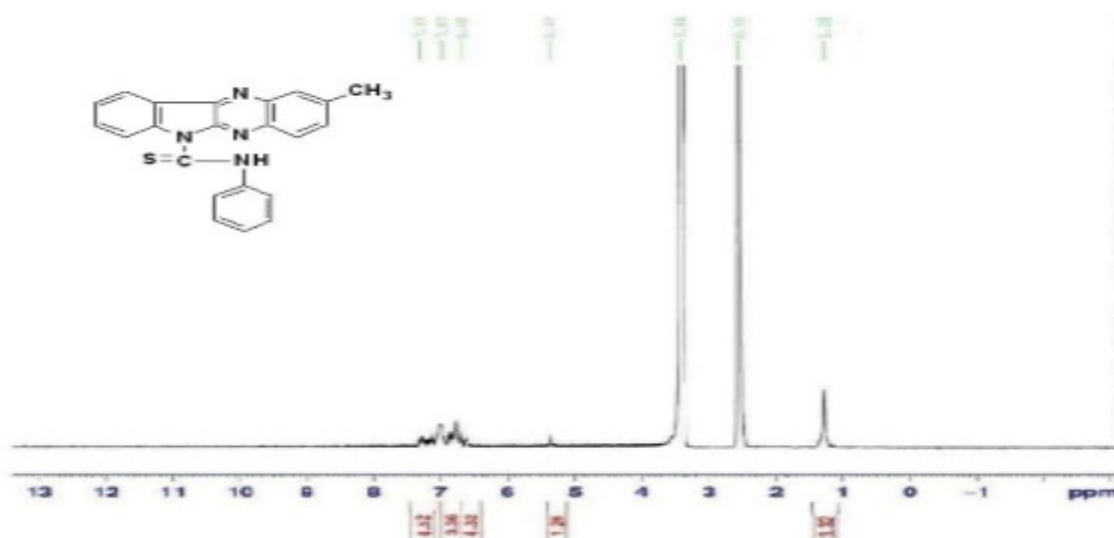
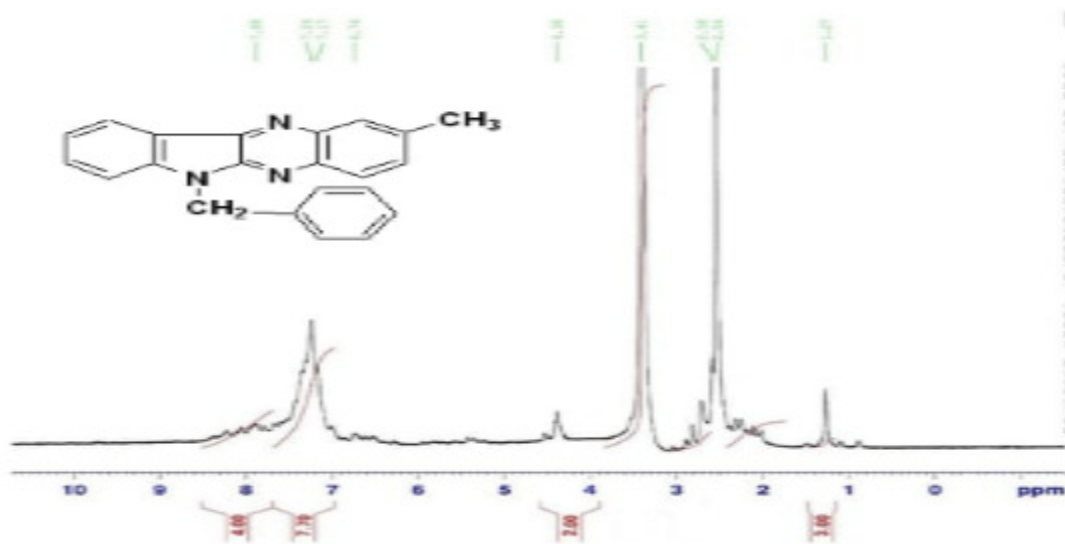
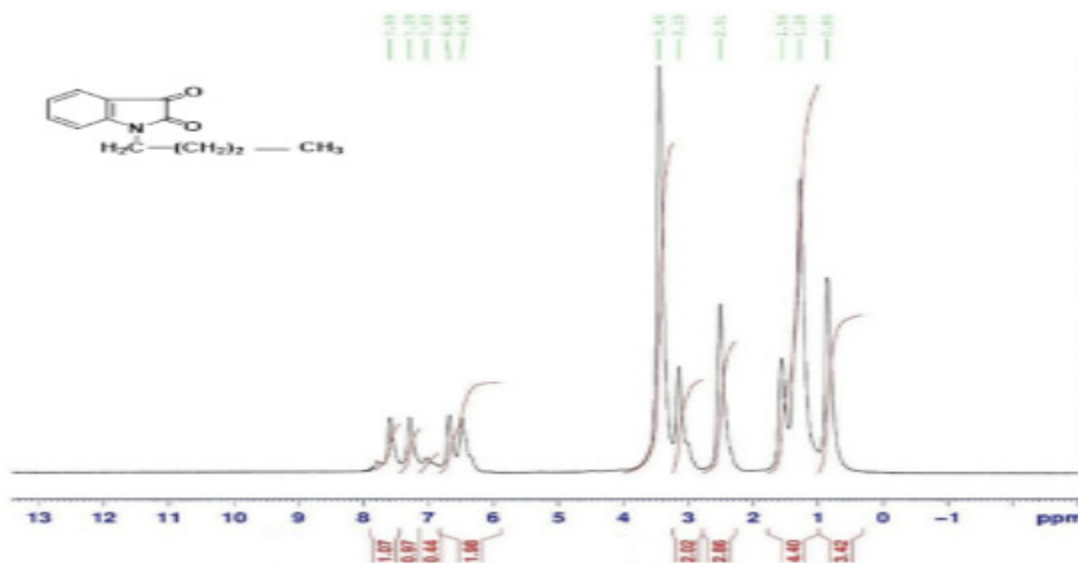
The $^1\text{H-NMR}$ spectra of the compound [O_2] shows the following chemical shifts (DMSO- d_6 , ppm):

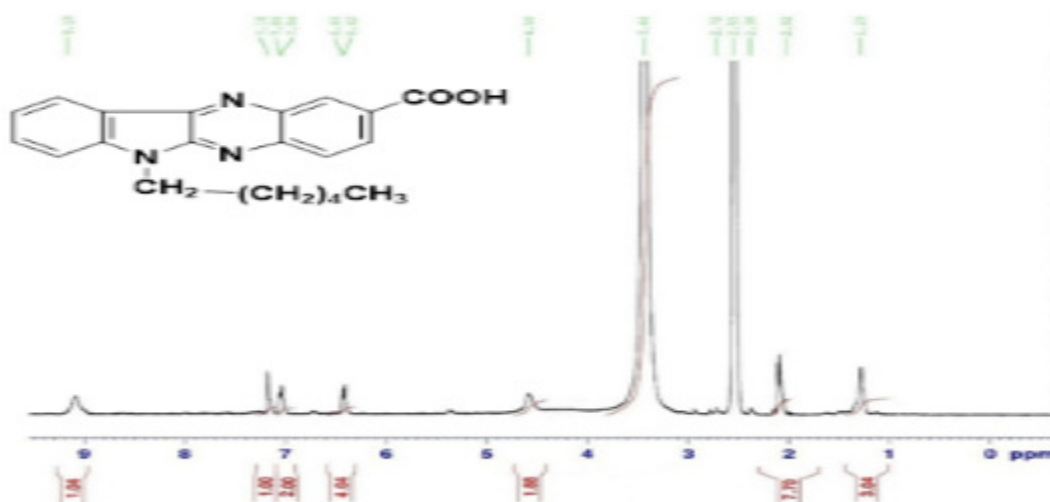
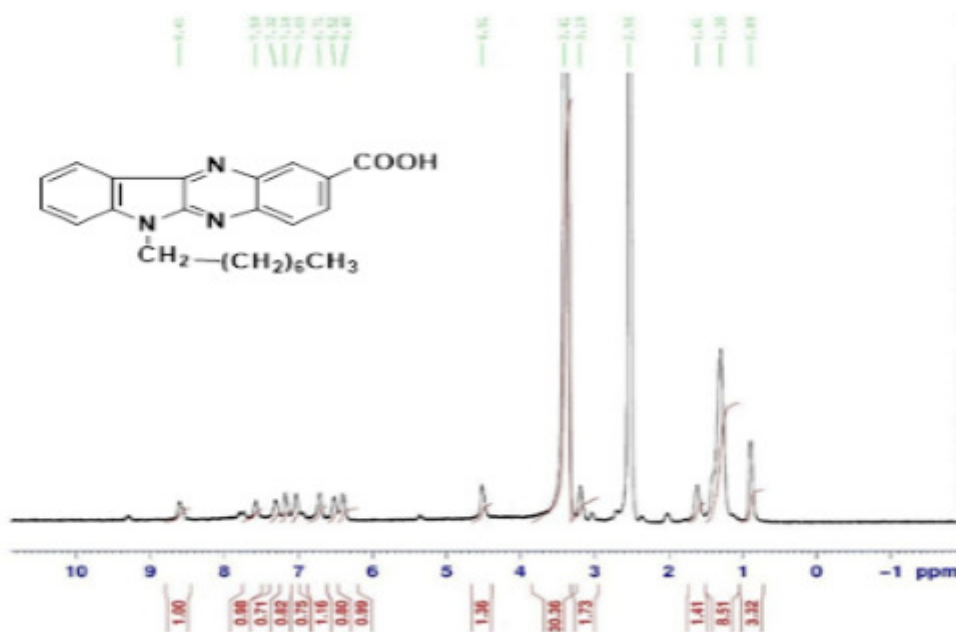
Table 1 : Physical properties of the synthesized compounds [O₁-O₁₂].

Comp. No.	Comp. Structure	Molecular Formula	M.P. °C	Yield%
O ₁		C ₁₅ H ₉ N ₃ O ₂	166-167	93
O ₂		C ₁₅ H ₁₁ N ₃	77-79	90
O ₃		C ₂₂ H ₁₆ N ₄ S	150-151	81
O ₄		C ₂₂ H ₁₇ N ₃	180-182	90
O ₅		C ₁₂ H ₁₃ NO ₂	36-38	62
O ₆		C ₁₄ H ₁₇ NO ₂	66-68	64
O ₇		C ₁₆ H ₂₁ NO ₂	77-72	60
O ₈		C ₁₅ H ₁₁ NO ₂	130-132	69
O ₉		C ₁₉ H ₁₇ N ₃ O ₂	102-104	78
O ₁₀		C ₂₁ H ₂₁ N ₃ O ₂	190-193	60
O ₁₁		C ₂₃ H ₂₅ N ₃ O ₂	117-118	72
O ₁₂		C ₂₂ H ₁₅ N ₃ O ₂	156-157	78

10.04(s,1H, N-H), 6.5-8.04 (m, 7H, Ar-H), 1.21 (s, 3H, CH₃). Whereas the ¹H-NMR spectrum of compound [O₃] showed the chemical shifts: 5.37 ppm (s, 1H, N-H), 6.60-7.37 ppm (m, 12H, Ar-H) and 1.28 ppm (s, 3H, CH₃).

Fig. 1 explain that. The ¹H-NMR results for compound [O₄]. Fig. 2 displayed the chemical shifts in ppm: 6.74-7.88(m, 12 H, Ar-H), 4.38(s, 2H, CH₂) and 1.27 (s, 3H, CH₃). While the data of spectra for the compound [O₅].


 Fig. 1 : ¹H-NMR spectrum of compound [O₃].

 Fig. 2 : ¹H-NMR spectrum of compound [O₄].

 Fig. 3 : ¹H-NMR spectrum of compound [O₅].

Fig. 4 : ^1H -NMR spectrum of compound $[\text{O}_{10}]$.Fig. 5 : ^1H -NMR spectrum of compound $[\text{O}_{11}]$.Table 2 : FT-IR absorption spectra data (cm^{-1}) of the prepared compounds.

Comp. no.	ν OH	ν C-H aromatic	ν C-H aliphatic	ν C=O	ν C=N	ν C=C aromatic	Others
O_1	3365	3041	-	1667	1564	1401	
O_2	-	3023	2919	-	1610	1513-1434	ν (NH)3327
O_3	-	3027	2981	-	1655	1527-1442	ν (NH)3211
O_4	-	3027	2919,2847	-	1607	1510-1495	
O_5	-	3081	2961,2871	1715	-	1519-1453	
O_6	-	3077	2927,2864	1714	-	1521-1458	
O_7	-	3347	2931,2865	1735	-	1514-1460	
O_8	-	3183	2921	1699	-	1504-1405	
O_9	3471	3068	2972-2882	1695	1632	1596-1452	
O_{10}	3471	3081	2958,2856	1777	1636	1426	
O_{11}	3341	3055	2929	1734	1667	1457	
O_{12}	3435	3152	2982	1706	1643	1552-1414	

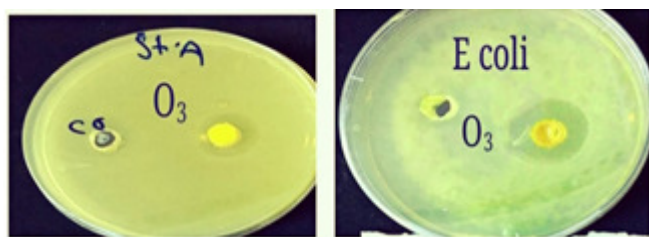


Fig. 6 : Zones of inhibition of the compounds O_3 .

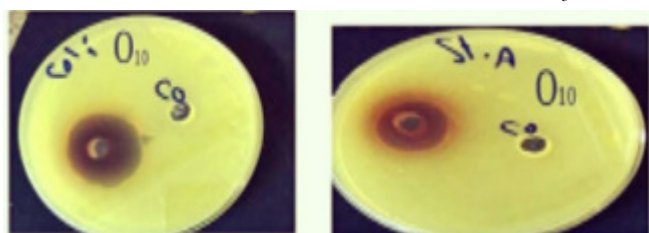


Fig. 7 : Zones of inhibition of the compounds O_{10} .

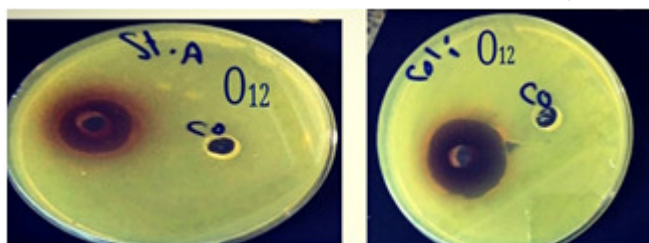


Fig. 8 : Zones of inhibition of the compounds O_{12} .

Table 3 : The inhibition zones of the compounds.

Comp. no.	Concentration (mg/ml)	Zone of inhibition (in mm)	
		Gram-positive <i>S. aureus</i>	Gram-negative <i>E. coli</i>
O_3	200	5	16
O_{10}	200	14	33
O_{12}	200	25	26

Fig. 3 showed the chemical shifts (ppm): 6.49-7.59 (m, 4H, Ar-H), 3.15 (t, 2H, N-CH₂), 1.28-1.56 (m, 4H, 2CH₂), and 0.85 (t, 3H, CH₃), whereas ¹H-NMR spectra of compound [O₉] showed the shifts (ppm): 9.31 (s, 1H, COOH), 6.40-7.17 (m, 7 H, Ar-H), 4.55 (t, 2H, N-CH₂), 1.13-1.28 (m, 4H, 2CH₂), and 0.91 (t, 3H, CH₃) and the spectrum of compound [O₁₀] showed the following chemical shifts: 9.19 (s, 1H, COOH), 6.42-7.18 (m, 7 H, Ar-H), 4.55 (t, 2H, N-CH₂), 2.02 (m, 8H, 4CH₂) and 1.29 (t, 3H, CH₃) as illustrated in Fig. 4. In addition to that, the spectrum for compound [O₁₁]. Fig. 5 showed the chemical shifts in ppm: 8.61 (s, 1H, COOH), 6.40-7.59 (m, 7 H, Ar-H), 4.51 (t, 2H, N-CH₂), 3.19 (m, 2H, NCH₂CH₂), 1.30-1.61 (m, 10H, 5CH₂) and 0.89 (t, 3H, CH₃). Finally, the ¹H-NMR spectrum of compound [O₁₂] showed the chemical shifts: 9.0 ppm (s, 1H, COOH), 6.46-7.20 ppm (m, 12 H, Ar-H), and 1.73 ppm (s, 2H, N-CH₂).

Biological activity

Some of the synthesized compounds were screened

for their antibacterial activities toward two strains of bacteria (*E. coli*) and (*S. aureus*). The result indicated that the activity against (*S. aureus*) bacteria was high for (O_{12}), moderate for (O_{10}) and low for compound (O_3), also the data proved the activity against (*E. coli*) bacteria for compounds (O_{10} , O_{12}) was highest than (O_3). Zones of inhibition Figures (6-8) caused by the compounds were measured in (mm). The results of antibacterial activity of the synthesized compounds are listed in Table 3.

CONCLUSION

In the current work, synthesized new derivatives of indolo[2-3-b]quinoxaline compounds categorized O_1 - O_{12} . These compounds were characterized utilizing diverse spectroscopic methods like FT-IR, ¹H-NMR, In addition to measurement some of their physical properties. Evaluated antibacterial activities for some prepared compounds such as O_3 , O_{10} and O_{12} and exhibited good to acceptable antibacterial activity against two types of bacteria used (*E. coli*) a gram-negative and (*S. aureus*) is a gram-positive bacteria.

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