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SYNTHESIS, CHARACTERIZATION AND INVESTIGATION OF ANTIBACTERIAL ACTIVITY FOR SOME NEW FUNCTIONALIZED LUMINOL DERIVATIVES

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ABSTRACT. The present study describes synthesis of some new luminol derivatives, which might play an important role in biological active agents. These new synthesized compounds are functionalized based on luminol (LM) with different carboxylic drugs such as mefenamic acid, ibuprofen, diclofenac sodium, and ampicillin). Synthesis processes was conducted by converting carboxylic group in the investigated drugs into acid chloride group by reacting with $SOCl_2$. Then the synthesized chloride drug derivatives were reacted with luminol in presences of DMSO and TEA to yield the final target molecules. These compounds were characterized using FTIR, NMR and CHNS techniques. Besides that, their physical properties and solubility were also investigated. Biological activity of the derivatives (TH1-TH4) was investigated using a pathogenic bacterium, *Staphylococcus aureus* (gram +ve), and *Escherichia (E. coli)* (gram -ve). The obtained results for antibacterial activity showed that TH1-TH4 derivatives have higher antibacterial activity against these types of bacteria in comparison with pure LM compound and the investigated drugs.

KEY WORDS: Luminol, Mefenamic acid, Ibuprofen, Diclofenac sodium, Ampicillin

INTRODUCTION

Luminol (LM) (5-amino-2,3-dihydrophthalazine-1,4-dione) is a chemical compound that displays chemiluminescence properties with a blue glow when it is mixed with an appropriate oxidizing agent. Luminol compound is a white-to-pale-yellow crystalline solid that is soluble in most polar organic solvents, but it is insoluble in water. LM is one of the most widely studied chemiluminescent molecules. Besides that, it is synthesized, and spectroscopic properties were significantly investigated. The emission of light requires both H_2O_2 and the oxidant enhancer to produce excited 3-aminophthalate [1]. A free-radical mechanism has been proposed [2] but the mechanism of oxidant enhancement, increased luminescence in the presence of the oxidant is still not fully comprehended yet. ferric enzymes such as horseradish, peroxidase and luciferin enhance luminescence [3, 4]. The mechanism of the luminol light-emitting process has appeared in (Figure 1).

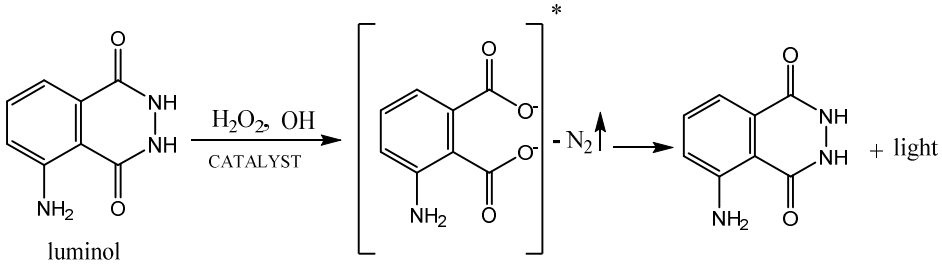

Figure 1. The luminol light-emitting mechanism.

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In this context, many applications were reported in different fields such as detection of ion concentrations in aqueous solutions [5-7], monitoring levels of hydrogen peroxide dependent reactions [8], detection of blood at crime scenes [9] and as biological active agent [10].

Recently, Ewies *et al.* [11] reacted of luminol with various phosphorus and thiating reagents indicated the creation of distinct compounds depending on the type of the reagents. The less steric carbonyl group of luminol is the favored attack site. The antibacterial activity of the produced compounds were tested. Most of the novel chemicals' antibacterial action was highly selective against fungus.

Fahad *et al.* [12, 13] reported that using acetylacetone to create the two new azo dyes luminol and procaine. To create complexes of metals(II) with a general stoichiometry, CuL_2 and NiL_2, these new dyes were combined in a 1:2 molar ratio with copper and nickel ions. Both gram positive and gram-negative bacteria were tested, and both the ligand and their complexes were confirmed to exhibit antibacterial action.

The present work would involve synthesis of some luminol derivatives. These derivatives of LM with different carboxylic drugs. Antibacterial activity of these derivatives was investigated against both gram positive and gram-negative bacteria.

EXPERIMENTAL

Materials and instrumentation

Chemicals used in this work were of high purity, they were analytical grade, and all of them were purchased from Sigma Aldrich and Fluka Company. Samarra Company for drug production (Iraq), supplied mefenamic acid and other drugs. Gallenkamp MFB-600-Melting point Stuart apparatus was used for investigating melting points for the synthesized compounds. FTIR spectra were recorded using Bruker spectrophotometer in the Chemistry Department, College of Science, University of Babylon. ^{1}H NMR spectra were recorded using Bruker AC 400 NMR spectrometer set to 500 MHz for ^{1}HNMR, chemical shifts (δ) were expressed in parts per million (ppm) relative to tetramethylsilane (TMS) as a default (δ = 0.0 ppm) in the University of Tehran's Central lab. Euro EA3000 Elemental Analyzes in the central laboratory of the University of Tehran, the analysis of the microelements carbon, hydrogen, nitrogen, and sulfur was carried out.

Biological activity

For each of the synthesized compounds (TH1–TH4), biological activity was investigated using diffusion agar method for each of positive gram bacteria and negative gram bacteria. The antibacterial activity of these materials was performed using agar diffusion method. Both pathologic isolates *E. coli* and *Staphylococcus aureus* were employed in the current study and provided by biology department, University of Babylon, Iraq. The method used to estimate the inhibitory effect of prepared compounds on these types of bacteria is agar diffusion method; it includes the work of three drilling in the dishes planted with bacteria to put the prepared derivatives in the excavation of cultivars planted with bacteria in three concentrations (1 mg/mL). The dishes were put in an incubator at temperature of (37 °C) for 24 hours, and then the inhibition zone was measured for each case [14].

Luminol synthesis

LM synthesis was conducted in a series of reactions as shown in Figure 2. In this approach, a solution of 2 mL of 8% an aqueous solution of hydrazine was added to 1.0 g of 3-nitrophthalic acid, and the resulting mixture was heated until the solid was dissolved. Then clamp the tube vertically over a bunsen burner and add 2 mL of diethylene glycol (DEG), the solution was heated

at a temperature between 110 and 130 °C to remove all residual water. The temperature was allowed to increase quickly to around 215 °C during the next 3-4 min. Then heating was stopped with monitoring the time and maintaining a temperature around 215-220 °C for 2 min with intermittent moderate heating. After that, the tube was cooled to about 100 °C, then 15 mL of hot water was added, the mixture was chilled under running water, and the resulting light yellow granular nitro compound was collected. Next, to the solution, 5 mL of 3 M NaOH solution was added, stirred by a glasses rod, and then 3 g of Sodium dithionite ($Na_2S_2O_4$) was added to the resulting deep brown-red solution. The edges of the tube were washed with a little amount of water. After boiling for 5 min and 8. 2 mL of acetic acid was added. The resultant was precipitated filtered, and washed with ethanol to afforded yellow luminol precipiatae in 73%, the molecular formula of luminol is $C_8H_7N_3O_2$ [15].

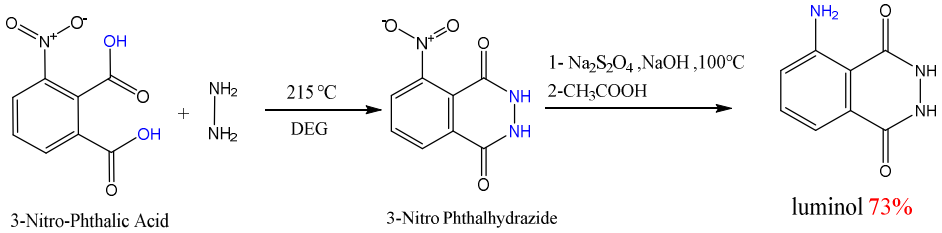

Figure 2. Synthetic routes of LM compound.

Synthesis of luminol derivatives (TH1-TH4)

Synthesis of LM derivatives TH1-TH4 were synthesized as follows, in a beaker 100 mL, an excess of thionyl chloride was added to (1.0 mmol) of carboxylic drugs [(0.34 g) ampicillin, (0.24 g) mefenamic acid, diclofenac sodium (0.29 g), and ibuprofen (0.21 g), respectively]. The mixture was left at room temperature for 30 min with stirring, then the obtained product was dissolved in 10 mL of DMSO and then it was added to a mixture of LM (0.1g, 1.0 mmol) dissolved in 10 mL of DMSO. The new mixture was heated at 60 ℃ for three hours. After that, (0.087 g, 3.0 mmol) of triethylamine was added to the mixture and was kept under these conditions for 30 min. Then, the mixture was cooled by ice bath until the precipitate appeared then they were filtered and dried to afford the products [TH1-TH4] (Figure 3, Table 1) [15, 16]. The molecular formulas for these synthesized derivatives are $C_{21}H_{23}N_3O_3$, $C_{22}H_{16}C_{l2}N_4O_3$, $C_{23}H_{20}N_4O_3$, and $C_{24}H_{24}N_6O_5S$, respectively.

RESULTS AND DISCUSSION

All synthesized compounds structure were characterized and confirmed by FTIR, ^{1}H NMR,^{13}C NMR spectra and CHNS microelemental analysis. Some of the physical properties and solubility of (TH1-TH4) compounds are represented in Table 1 and 2.

FTIR spectrum for TH1 shows the following values (ν_{max}, cm^{-1}): 3454.62 (NH), 3014.84-3161.43 (CH Ar.), 2918.40-2962.76 (CH, str.), 1654.98 (C=O amide), 1496.81 (C=C Ar.), 1296.21-1323.21 (C-N). ^{1}H NMR (500 MH, δ ppm) spectrum for the TH1 derivative shows the following chemical shifts: 0.84-1.78 (9H, CH_3), 2.38 (2H, CH aliphatic), 2.49 (DMSO), 3.6 (H_2O, moisture), 6.87-8.05 (7H, CH benzene), 10.02 (2H, NH amide), 11.31 (1H, amide). ^{13}C NMR spectrum for TH1 shows the following chemical shifts, (125 MH, δ ppm): 15.4-22.8 (CH_3), 29.0-42.5 (CH), 40.3 (DMSO), 44.5 (CH_2), 119.3-140.3 (benzene), 167.3-172.2 (C=O amide) (Figure 4). The elemental analysis (calc.) found: C% (69.02) 68.10; H% (6.34) 6.11; N% (11.50) 10.2.

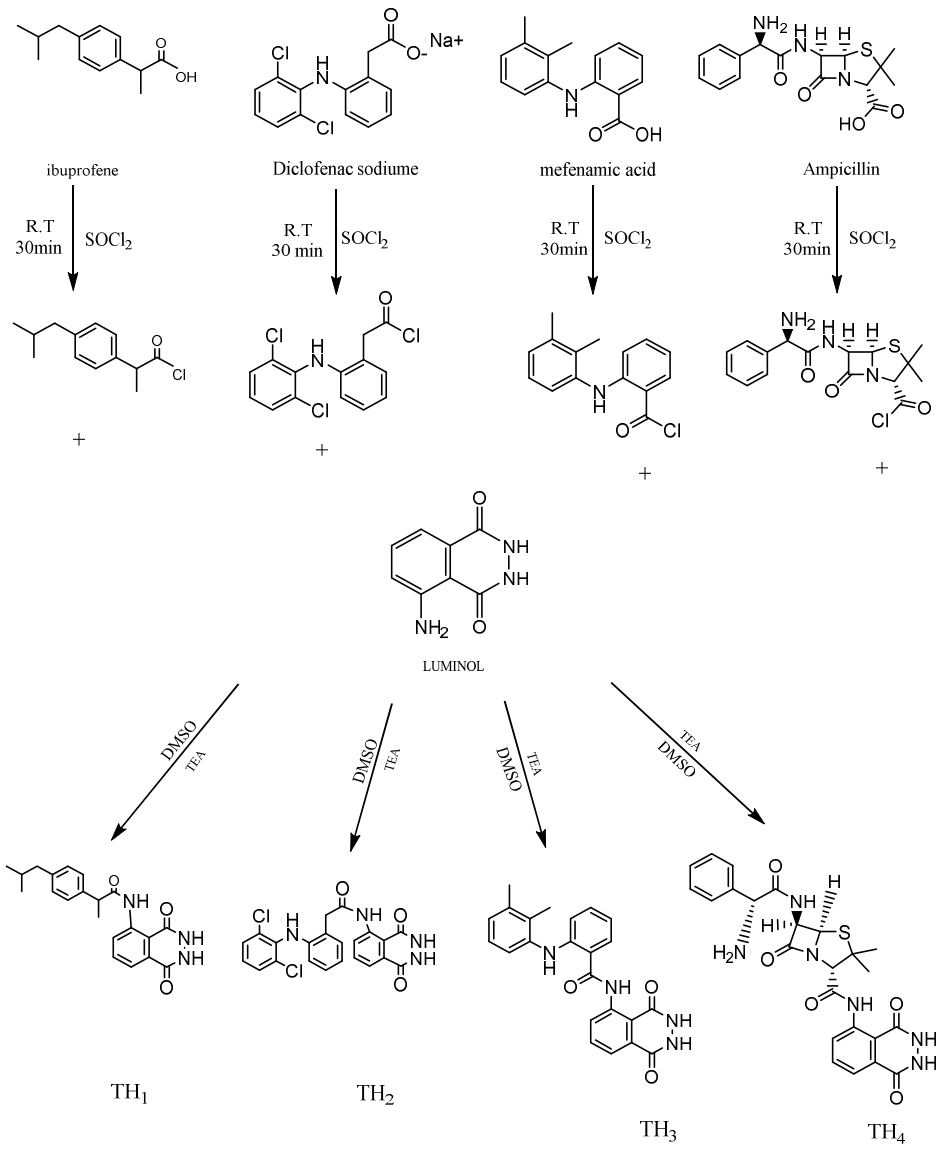

Figure 3. Synthesis of luminol derivatives (TH1-TH4).

FTIR spectrum for TH2 derivative shows the following values ($\nu_{max.}$, cm^{-1}): 3340.82-3471.98 (NH, amide), 3028.34-3167.22 (CH Ar.), 2918.40-2968.55 (CH str.), 1654.98 (C=O str.), 1492.95 (C=C Ar.), 1240.27-1321.28 (C-N str.), 609.53 (C-Cl). ^{1}H NMR and ^{13}C NMR for the derivative TH2 are shown in figure 5. ^{1}H NMR (500 MH, δ ppm) for the derivative TH2 shows the following chemical shifts: 11.63, 10.33 (2H, NH, amide), 9.22 (2H, NH, amide), 7.73-6.37 (10H, CH benzene), 3.86 (2H, CH_2), 2.49 (DMSO). ^{13}C NMR spectrum for the derivative TH2 shows the

following chemical shifts: (125 MH, δ ppm): 167.88-163.02 (C=O, amide), 148.46 (C-N), 143.32 (C-Cl, benzene), 134.89-108.95 (benzene), 39.89 (DMSO), 35.53(CH_2). The elemental analysis (calc.), found: C% (58.04) 56.50; H% (3.54) 3.33; N% (12.31) 11.1.

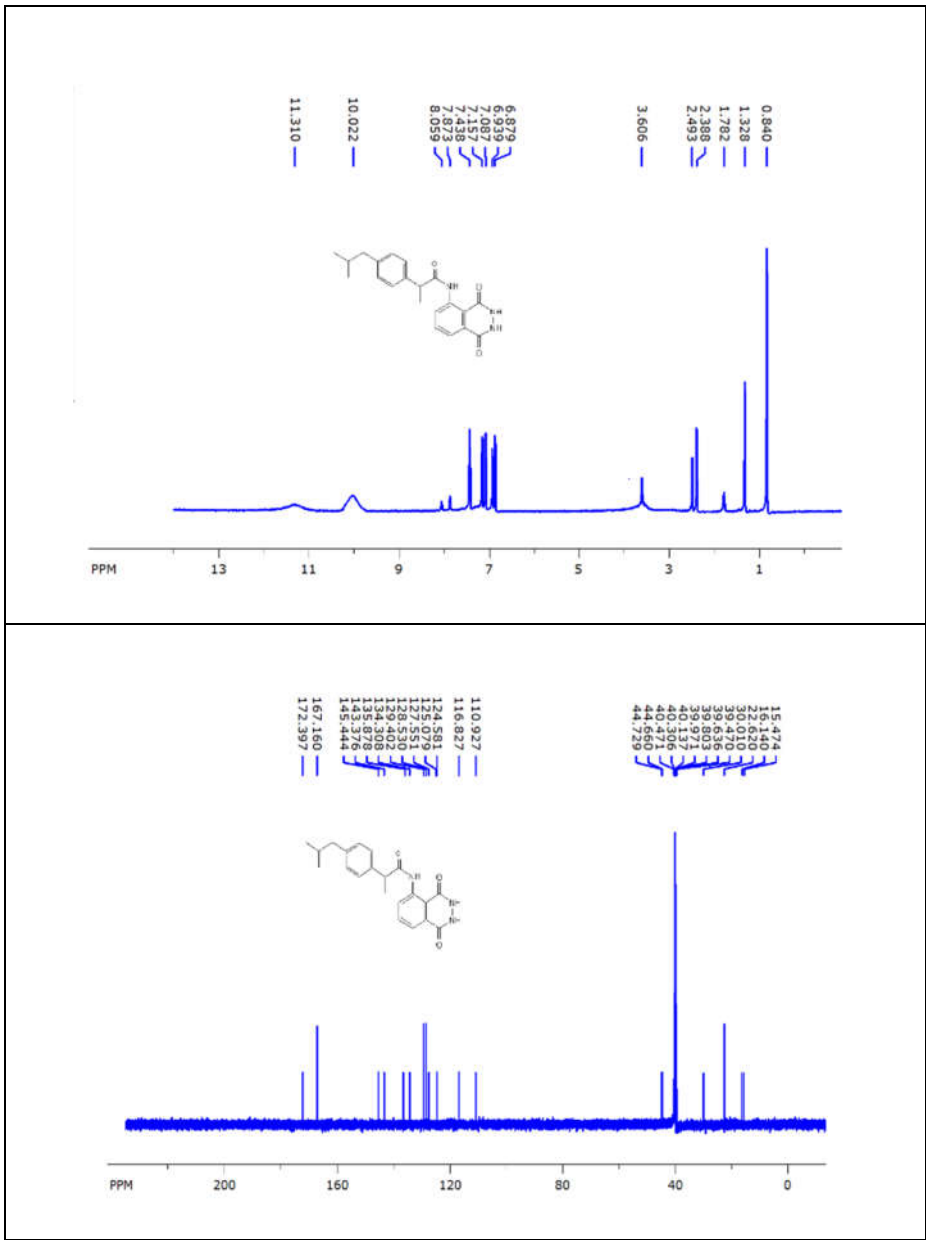

Figure 4. ^{1}H NMR and ^{13}C NMR for the derivative TH1.

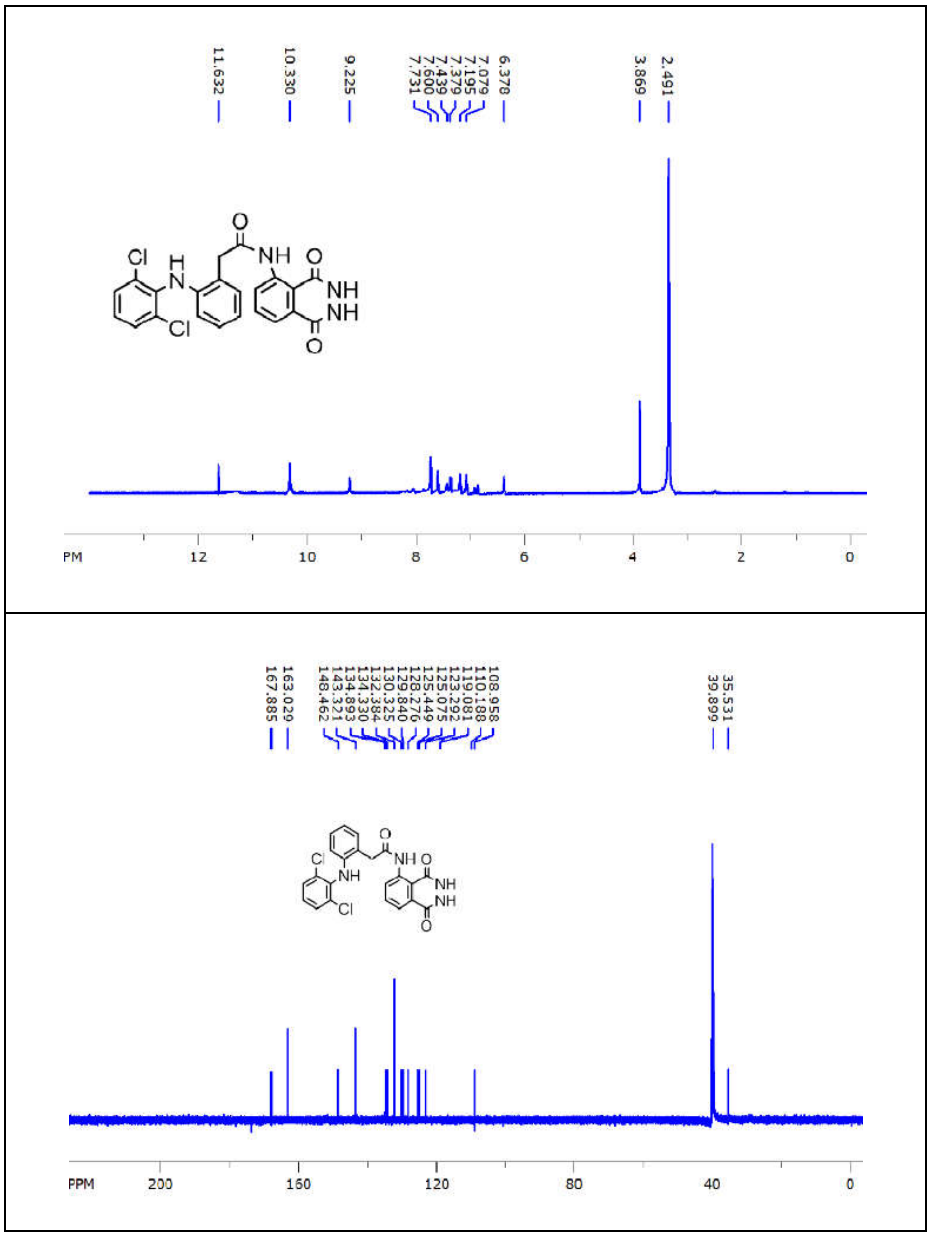

Figure 5. ^{1}H NMR and ^{13}C NMR for the derivative TH2.

FTIR spectrum for the derivative TH3 shows the following absorption peaks ($\nu_{max.}$, cm^{-1}): 3163.36-3443.05 (NH, amide), 3012.91 (CH, Ar.), 1656.91 (C=O, amide), 1498.74 (C=C aromatic), 1323.21 (CH_3), 1240.27 (C-N str.). ^{1}H NMR and ^{13}C NMR for the derivative TH3 are

shown in Figure 6. ^{1}H NMR (500 MHz, δ ppm) for the derivative TH3 shows the following chemical shifts: 11.83-11.51 (2H, NH, amide), 9.83 (2H, NH amide), 8.92-6.69 (10H, CH benzene), 2.49 (DMSO), 2.09-1.19 (6H, CH_3). ^{13}C NMR spectrum for the derivative TH3 shows the following chemical shifts: (125 MHz, δ ppm):168.64-167.18 (C=O, amide), 155.90-144.41 (C-N) 143.39-109.95 (benzene), 39.94 (DMSO), 21.27-15.68 (CH_3). The elemental analysis (calc.), found: C% (68.99) 67.66; H% (5.03) 4.88; N% (13.99) 13.77.

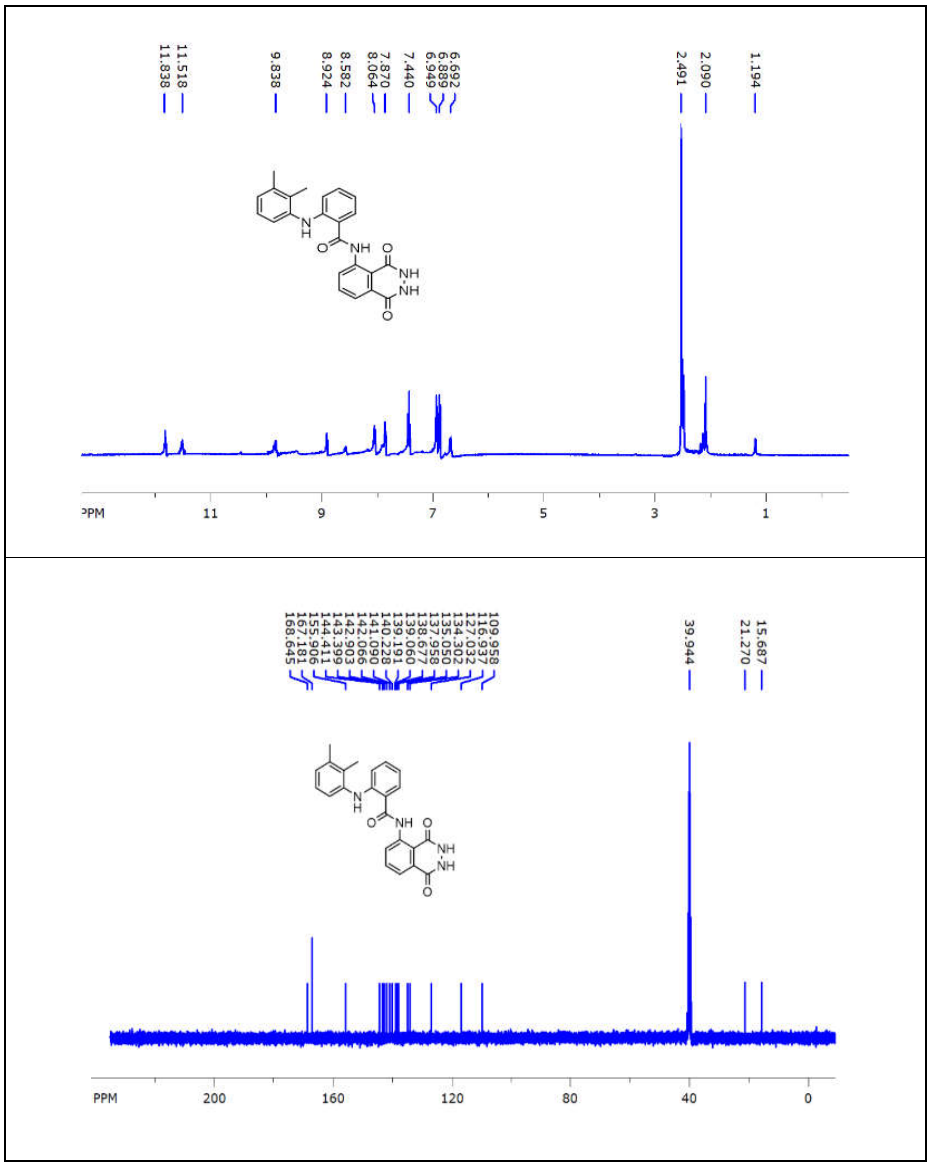

Figure 6. ^{1}H NMR and ^{13}C NMR for the derivative TH3.

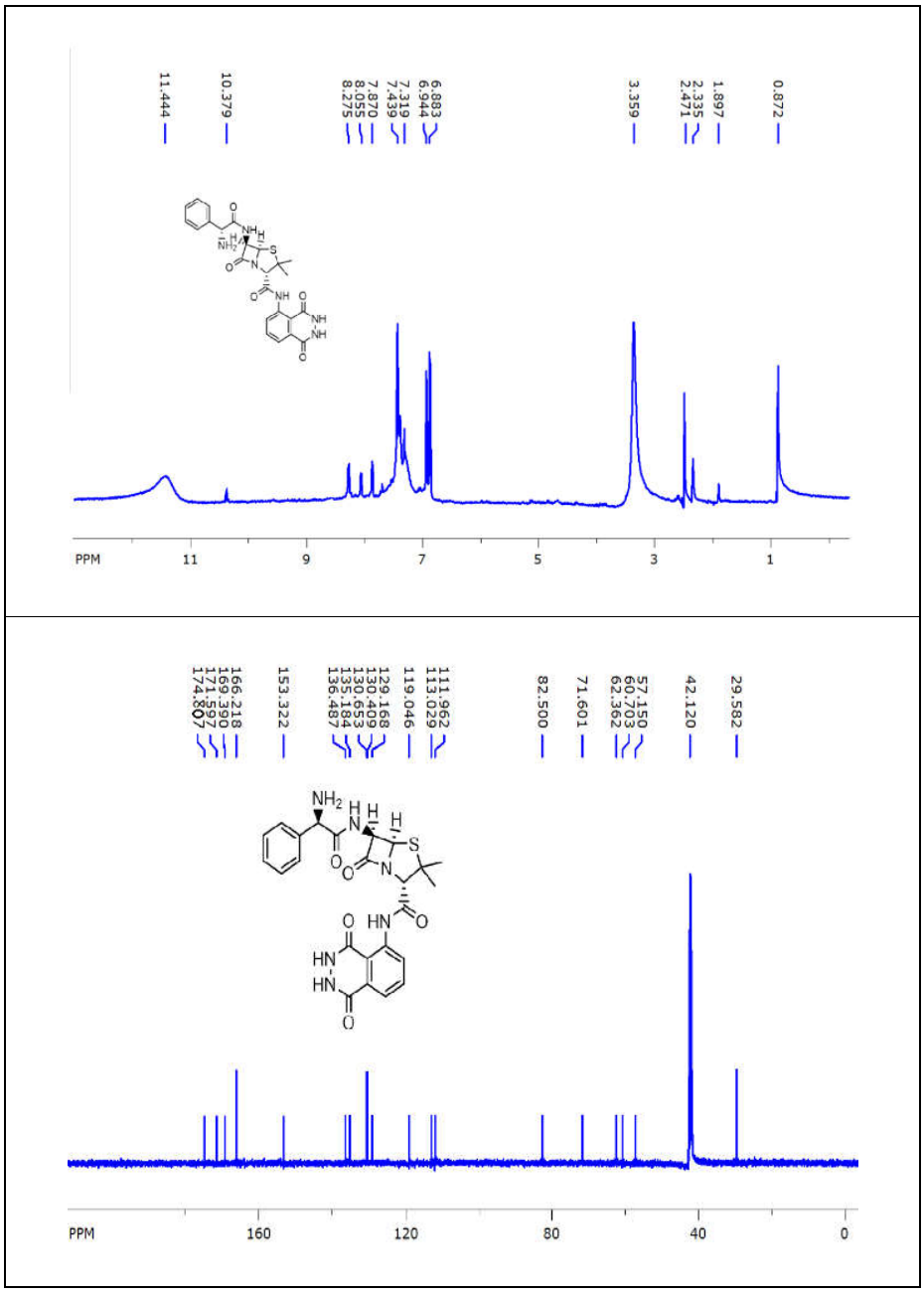

Figure 7. ^{1}H NMR and ^{13}C NMR for the derivative TH4.

FTIR spectrum for the derivative TH4 shows the following values ($\nu_{max.}$, cm^{-1}): 3161.43, 3329.25 (NH amine and amide), 3010.98 (CH Ar.), 2964.69 (CH aliphatic), 2582.77 (S-H str., mercaptan), 1647.26 (C=O str., amide), 1600.97 (C=C str., aromatic), 1379.15 (CH_3), 1321.28 (C-N str.). ^{1}H NMR and ^{13}C NMR for the derivative TH4 are shows in Figure 7. ^{1}H NMR (500 MH, δ ppm) for the derivative TH4 shows the following chemical shifts: 11.44, 10.37, 8.05 (4H, NH amide), 8.27 (2H, NH amine), 7.87-6.88 (8H, benzene), 3.35 (2H, propiolactam), 2.47 (DMSO), 2.33-1.89 (1H methyl groups), 0.87 (6H, CH_3). ^{13}C NMR (125 MH, δ ppm) spectrum for the derivative TH4 shows the following chemical shifts: 174.80-166.21 (C=O amide), 153.32 (C-N), 136.48-111.96 (benzene), 82.50-71.60 (CH aliphatic), 62.36-57.15 (CH aliphatic), 40-42.12 (DMSO), 29.58 (CH_3). The elemental analysis (calc.), found: C% (56.68) 56.15; H% (4.76) 4.44; N% (16.53) 15.62; S% (6.30) 6.13.

Solubility of synthesized compounds

The synthesized compounds were insoluble in diethyl ether, petroleum ether, acetone and hexane but having a good solubility in DMSO and DMF. Some of them having partial solubility in water, ethanol and ethyl acetate. Solubility properties of the prepared compounds in different solvents (H_2O, ethanol, CH_2Cl_2, ether, petroleum ether, DMSO, hexane, DMF, ethyl acetate and acetone) are listed in Table 2.

Table 1. Some of physical properties of (TH1-TH4) compounds

Comp.	Color and phys. state	Chem. formula	M.W.	M.P. °C	Yield	R_f	TLC Solvent system
TH1	Green solid	$C_{21}H_{23}N_3O_3$	365.43	283-286	87%	0.78	Hexane-acetone (3-7)
TH2	Brown solid	$C_{22}H_{16}C_{l2}N_4O_3$	455.30	264-268	92%	0.71	Hexane-acetone (2-8)
TH3	Dark brown solid	$C_{23}H_{20}N_4O_3$	400.44	215-217	87%	0.46	Hexane-acetone (1-9)
TH4	Brown solid	$C_{24}H_{24}N_6O_5S$	508.55	249-252	83%	0.63	Hexane-acetone (1-9)

Table 2. Solubility of synthesized compounds in different solvents.

Comp.	DMSO	DMF	DCM	Pet. ether	Ethyl acetate	Acetone	Diethyl ether	H_2O	Hexane	EtOH
TH1	+	+	-	-	Partial	-	-	+	Partial	+
TH2	+	+	+	-	+	-	-	Partial	-	Partial
TH3	+	+	-	-	-	-	-	+	-	+
TH4	+	+	-	-	Partial	-	-	Partial	-	Partial

Antibacterial activity

The antibacterial activity results for new compounds are shown in Table 3. (TH1-TH4) compounds are more effective in limiting the spread of bacteria than luminol and the drugs used in the preparation of these new derivatives (TH1-TH4). These compounds were dissolved in dimethylsulfoxide. The biological activity against the two types of bacteria, *Staphylococcus aureus* (gram +ve), and *Escherichia* (*E. coli*) (gram -ve) was assessed using the agar disc diffusion technique.

Table 3. Anti-bacterial activity for prepared compounds and other drugs that are used in preparations at 1 mg/mL concentration.

Compound	Gram +ve (*Staphylococcus aureus*) (mm)	Gram –ve (*E. coli*) (mm)	Pure drug	Gram +ve (*Staphylococcus aureus*)	Gram -ve (*E. coli*)
TH1	6	15	Ibuprofen	6	14
TH2	5	6	Diclofenac sodium	6	0
TH3	5	20	Mefenamic acid	0	8
TH4	30	20	Ampicillin	35	26
LUMINOL	5	10			
DMSO	0	0			

The obtained results showed the effect of these materials on the negative bacteria of the Gram stain, represented by the intestinal bacteria *E. coli* were almost more compared to the Gram-positive bacteria represented by *S. aureus*. *E. coli* was recorded the highest inhibition zone (20 mm) with TH4 is followed by TH1 with (15 mm), the results showed less inhibition areas [19].

TH4 has higher biological activity than other compounds; this might be because luminol incorporates with Ampicillin which is used to treat bacterial infections such as meningitis and infections of the sinuses, throat, lungs, urinary system, reproductive organs, and gastrointestinal tract. Ampicillin is a class of penicillins antibiotic family.

In general, the ability of these derivatives to inhibit growth of bacteria species is probably due to its high affinity of cellular proteins components of these bacteria towards these derivatives. This effect can result in either direct dying of bacteria or at least stop its growth and effectiveness [17, 18]. The bonding between the derivatives and cell membrane of these bacteria leads to change structure of bacteria membrane and formation of irregular cell surfaces, which results in increasing in membrane permeability. This leads to membrane leakage which results in cell death or at least stop cell growth.

In addition to that, the obtained results showed that inhibition zone for *E. coli* bacteria was in almost more than that for *S. aureus* bacteria applying same inhibitor conditions for each case. This can be related to the nature of structure of *S. aureus* bacteria that makes it more resistance and less affinity to bind with these materials under the applied conditions [17-19].

CONCLUSION

In this work, some of new luminol compound derivatives were synthesized and termed as TH1-TH4. These synthesized new functional compounds based on luminol with different carboxylic drugs such as mefenamic acid, ibuprofen, diclofenac sodium, and ampicillin). Biological activity of the derivatives (TH1-TH4) against bacteria, *Staphylococcus aureus* (gram +ve), and *Escherichia (E. coli)* (gram -ve) was investigated. The results show the inhibited bacteria by the new luminol derivatives in comparison with pure luminol and used drugs.

REFERENCES

1. White, E.H.; Zafiriou, O.; Kagi, H.H.; Hill, J.H.M. Chemilunimescence of luminol: The chemcial reaction. *J. Am. Chem. Soc.* **1964**, 86, 940–941.
2. Lind, J.; Merenyi, G.; Eriksen, T.E. Chemiluminescence mechanism of cyclic hydrazides such as luminol in aqueous solutions. *J. Am. Chem. Soc.* **1983**, 105, 7655–7661.
3. Whitehead, T.P.; Thorpe, G.H.G.; Carter, T.J.N.; Groucutt, C.; Kricka, L.J. Enhanced luminescence procedure for sensitive determination of peroxidase-labelled conjugates in

immunoassay. *Nature* **1983**, 305, 158–159.
4. Easton, P.M.; Simmonds, A.C.; Rakishev, A.; Egorov, A.M.; Candeias, L.P. Quantitative model of the enhancement of peroxidase-induced luminol luminescence. *J. Am. Chem. Soc.* **1996**, 118, 6619–6624.
5. Kurihara, M.; Hasebe, T.; Kawashima, T. Chemiluminescent methods in analytical chemistry (review). *Bunseki Kagaku* **2002**, 51, 205–233.
6. Meseguer-Lloret, S.; Campíns-Falcó, P.; Tortajada-Genaro, L.A.; Blasco-Gómez, F. A guide to avoid method bias of chromium (III, VI) chemiluminescence determination by luminol-hydrogen peroxide reaction-application to water samples. *Int. J. Environ. Anal. Chem.* **2003**, 83, 405–416.
7. Birks, J.W. *Chemiluminescence and Photochemical Reaction Detection in Chromatography*, VCH Publishers: New York; **1989**.
8. Yuan, J.; Shiller, A.M. Determination of subnanomolar levels of hydrogen peroxide in seawater by reagent-injection chemiluminescence detection. *Anal. Chem.* **1999**, 71, 1975–1980.
9. Kent, E.J.M.; Elliot, D.A.; Miskelly, G.M. Inhibition of bleach-induced luminol chemiluminescence. *J. Forensic Sci.* **2003**, 48, 1–4.
10. Josef, B.; Wolfgang, B.; Astrid, K.; Beate, L.; Gerrit, M.; Thomas, M.; Wolfgang, M.; Michael, N. Crystalline forms for 5-amino-2,3-dihydrophthalazine-1,4-dione sodium salt, pharmaceutical preparations containing the same and method for the production of said forms. Patent Application Publication, United States, US 2012/0329801 A1, **2012**.
11. Ewies, F.E.; Naglaa, F.E.; Marwa, E.; Mohamed, S.A. Synthesis and antimicrobial evaluation of new 5-amino-2,3-dihydrophthalazine-1,4-dione derivatives. *Egypt. J. Chem.* **2021**, 64, 7165–7173 .
12. Noor, A.; Fahad, T.A.; Asaad, A.A. Synthesis, spectroscopic, and thermal studies of azo compounds from luminol and procaine with acetylacetone and their complexes. *J. Phys.: Conf. Ser.* **2021**, 2063, 012016.
13. Ali, A.A.; Fahad,T.A.; Al-muhsin, A.A. Preparation and pectroanalytical studies of two new azo dyes based on luminol. *IOP Conf. Ser. Mater. Sci Eng.* **2020**, 928. 52007.
14. Mounyr, B.; Moulay, S.; Saad, K.I. Methods for in vitro evaluating antimicrobial activity: A review. *J. Pharm. Anal.* **2016**, 6, 71–79.
15. Hamad, H.K.; Luma, M.A.; Kareem, M.M. Facile synthesis of spinel $CoCr_2O_4$ and its nanocomposite with ZrO_2: Employing in photo-catalytic decolorization of Fe(II)-(luminol-tyrosine) complex. *Egypt. J. Chem.* **2022**, 65, 481–488.
16. Mohammed, I.A.; Kareem, M.M. Synthesis, characterization and study of some of new mefenamic acid derivatives as cytotoxic agents. *J. Physics: Conference Series* **2020**, 1664, 012081.
17. Almamoori, A.M.J.; Salih, H.K.; Abbas, J.L.; Kareem, M.M. Biological activity of cefotaxime sodium and their complexes with iron and copper metals. *J. Glob. Pharm. Technol.* **2017**, 10, 257–261.
18. Yanping, X.; Yiping, H.; Peter, L.I.; Tony, J.; Xianming, S. Antibacterial activity and mechanism of action of zinc oxide nanoparticles against *Campylobacter jejuni*. *Appl. Environ. Microbiol.* **2011**, 77, 2325–2331.
19. Zhang, L.L.; Jiang, Y.H.; Ding, Y.L.; Povey, M.; York, D. Investigation into the antibacterial behavior of suspensions of ZnO nanoparticles (ZnO nanofluids). *J. Nanoparticle Res.* **2007**, 9, 479–489.