Synthesis and Characterization of Some New Analogues of Luciferin, Calculating Their Stokes Shift and Energy Band Gaps by the Tauc Plot Method

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Abstract: Luciferin is a chemical compound found in some living organisms. It is responsible for producing light. Due to the importance of luciferin in various applications, in this study, a series of new luciferin analogues are synthesised and characterized using different techniques, including: proton nuclear magnetic resonance (^{1}H-NMR), carbon-13 nuclear magnetic resonance (^{13}C-NMR), Correlation spectroscopy (COSY), Heteronuclear single quantum coherence (HSQC), Electron impact (EI) mass spectra, and Fourier transform infrared spectroscopy (FTIR). These compounds are synthesized by reacting cysteine monohydrate hydrochloride with aryl nitrile derivatives through the click reaction, using sodium bicarbonate and reflux for two days, with high yields and purity. The thiazole motif is also prepared by reacting the aldehyde with 2-aminothiophenol using urea nitrate as a catalyst with high yield, rapid, easy, quick isolation, and solvent-free. The Stokes shift for their analogues was studied and calculated. The Tauc formalism calculated the energy band gaps. The Stokes shift and the energy band gaps were almost the same value for the thiazoline motifs C3, C4, C5, and C6, which is attributed to the same chemical structures for them. Still, there is a difference for benzothiazole motifs T4, T6, as a consequence of different substitutions.

Keywords: Thiazoline, Benzothiazole, Luciferin, Photophysical properties, Tauc plot.

Introduction

Heterocycles containing sulfur and nitrogen, such as thiazolines and thiazole scaffolds, have garnered significant attention due to their diverse properties and potential applications in medicinal chemistry. These sulfur- and nitrogen-containing heterocycles exhibit a wide range of biological activities, making them crucial targets for the development of new drugs. On the other hand, the compound luciferin is the best example of a natural compound that exhibits bioluminescent properties. This compound has sulfur and nitrogen atoms in its structure [1]. Firefly luciferase is an example of a bioluminescent source that produces light from the oxidation reaction of D-luciferin (D-LH2) [2]. The light-producing process requires four components present in the system to be successful, which are the luciferase enzyme, the luciferin substrate, Magnesium (II), Adenosine triphosphate (ATP), and Oxygen, as shown in Scheme (1) [3,4]. This process will produce light, which is known as bioluminescence, without the need for an external light source to produce fluorescence [5]. Firefly luciferase and luciferin combination characterized by specificity and high sensitivity for ATP; therefore, it is suitable for the detection of ATP and biological studies with luciferase as a gene reporter [6]. Depending on a bioengineering study, many serious of luciferases producing various wavelengths were synthesized. Among several luciferases besides their mutants, the range of wavelengths of light emission is between 534 nm to 623 nm [7]. In 1960, the composition of luciferin was described and synthesized, and after that, several adjusted luciferin analogues like 6-amino-luciferin were synthesized, and some of them work as light-emitting substrates [8]. Photoluminescence is a process of emitting light from a substance after exposure to ultraviolet radiation [9]. There are several factors that make compounds suitable for photoluminescence studies, like π-conjugated systems, planarity, rigidity, electron-donating/electron-withdrawing groups, and intramolecular charge transfer [10]. Depending on the structure of luciferin, most synthetic luciferin analogues form from two main units, which are benzothiazole and 4-carboxythiazolin-2-yl rings Fig (1).

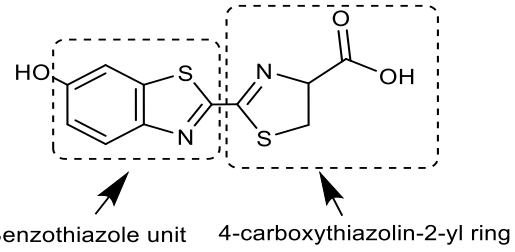

Fig. 1: The main structure of D-Luciferin.

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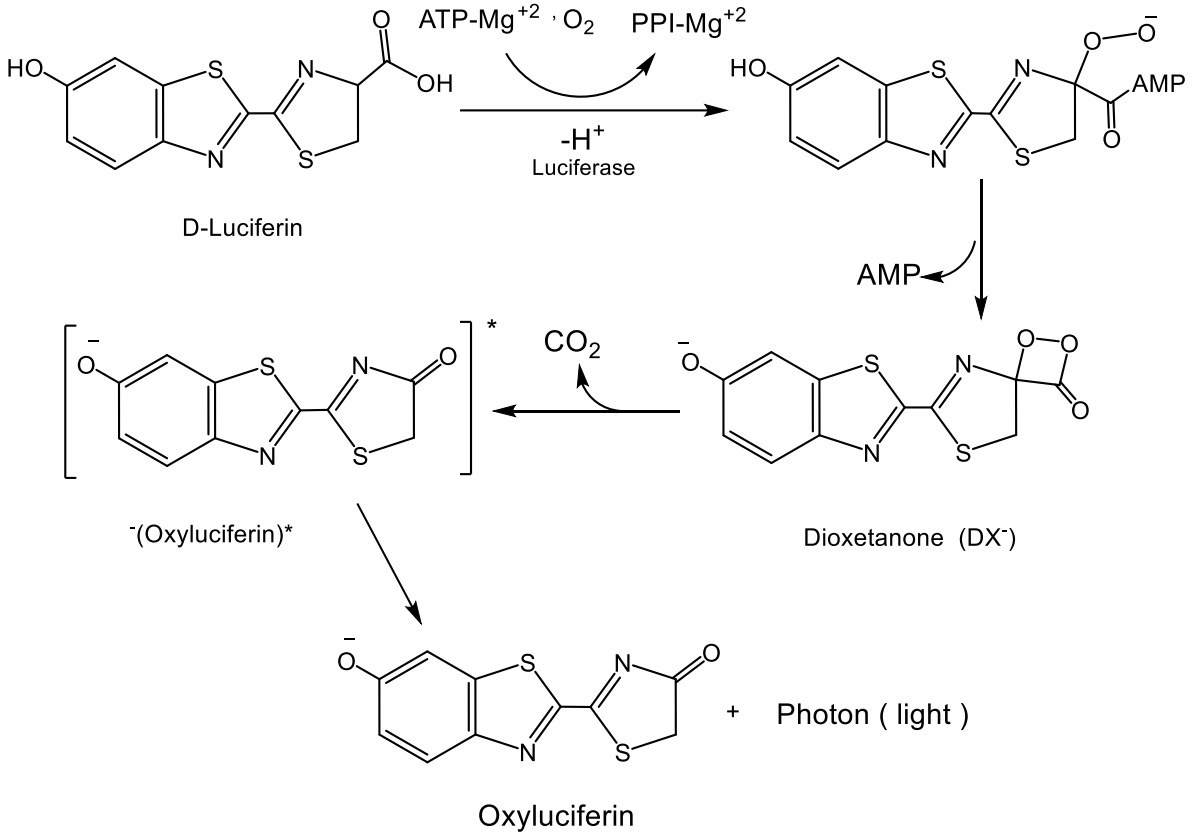


Scheme-1: mechanism of D-luciferin bioluminescence.

In our study, we have prepared two series of luciferin analogues with synthetic easy, the first serious was substitution benzothiazole with a simplified aromatic structure (T4, T6) by urea nitrate catalyst and the other was the substitution 4-carboxythiazolin-2-yl rings with a simplified aromatic structure (C3, C4, C5, C6) the letter series have a potential of a biological application like vivo imaging due to thiazoline ring and carboxylic group in their structure as described in Fig (2).

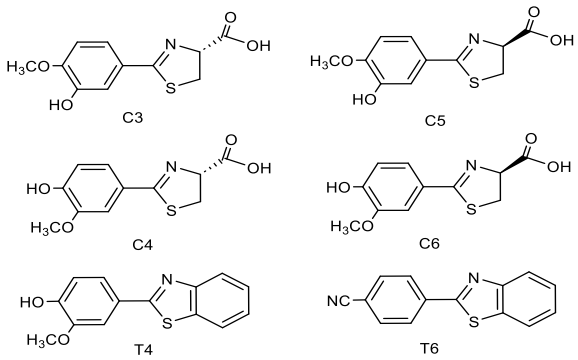


Fig. 2: The structures of synthesized compounds.

Experimental

Materials and physical Instruments.

N-hexane (99% purity, SRL, India). Ethyl acetate (99% purity, GCC, United Kingdom). Chloroform (99% purity, MERCK, Germany). Vanillin (99% purity, MERCK, Germany). Isovanillin (95% purity, Aldrich, American). Hydroxylamine hydrochloride (96% purity, VWR, American). Acetic acid (98% purity, Honeywell, Germany). Sodium hydroxide (97% purity, VWR, American). Toluene (99% purity, VWR, American). D-cysteine hydrochloride monohydrate (98% purity, Aldrich, American). L-cysteine hydrochloride monohydrate (98% purity, Aldrich, American). Sodium bicarbonate (99% purity, VWR, American). Hydrochloric acid (37% purity, BAKER, American). Ethanol absolute (99% purity, VWR, American). 2-Aminothiophenol (98% purity, Aldrich, American). Nitric acid (95% purity, MERCK, Germany). P-cyanobenzaldehyde (98% purity, Aldrich, American). Diethyl ether (99% purity, Scharlau, Spain). Urea (99% purity, Fluka, United Kingdom). Open capillaries and the Thermo Fisher thermal point apparatus (England) are used to

determine the melting point. 12 UV flashlight model TT-FL001 from the Taotronics company to note the emission from solutions. The ^{1}H NMR spectra and C13 NMR spectra from the broker company with 400 MHz and 125 MHz, respectively. FT-IR spectra were recorded on a Bruker FTIR ALPHA II. UV spectra by the SHIMADZU company type UV-1800, fluorescence spectra by fluo time 300 using λ_{abs} max of testing compounds to excitation it. Mass spectra from AB Sciex 3200 q Trap. Polarmeter from the E.HARTNACK company, made in Germany. In thin layer chromatography (TLC) analysis, the plates were coated with silica gel G (suspended in pet ether-ethyl acetate), and UV light or potassium permanganate solution was used as a visualizing agent.

Method of work

Synthesis of Luciferin Analogues from Aryl Nitrile Derivatives

Preparation of vanillin **(V1)**

The mixture of vanillin (3.02 g, 20.0 mmol) and hydroxylamine hydrochloride (2.09 g, 30.0 mmol) in acetic acid (16 mL) was refluxed for three hours. The solution was allowed to cool and poured into 50 mL of diethyl ether in a beaker and washed three times first with distilled water (25 mL) and twice with (25 mL) of 5% NaOH. The aqueous layers were assembled and extracted with 25 mL of Diethyl ether, and the ether layers were collected and evaporated to provide a crude solid product. Recrystallization from toluene to give 2.1 g of white solid product with a yield 70% and a melting point of 83 °C [11]. FTIR (cm^{-1}) 3378, 3024, 2950, 2223, 1283. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.27 (s, 1H, OH phenolic), 7.36 (d, J = 1.9 Hz, 1H, aromatic), 7.27 (dd, J = 8.1, 1.9 Hz, 1H, aromatic), 6.90 (d, J = 8.2 Hz, 1H, aromatic), 3.81 (s, 3H, OCH_3).

Preparation of isovanillin nitrile **(V2)**

The same procedure as above was followed using isovanillin. Recrystallization from toluene to give white solid 1.9 g with yield 64% and melting point 123 °C. FTIR (cm^{-1}) 3312, 3000, 2939, 2222, 1256. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (dd, J = 8.37, 2.0 Hz, 1H, aromatic), 7.17 (d, J = 2.03 Hz, 1H, aromatic), 6.89 (d, J = 8.35 Hz, 1H, aromatic), 5.89 (s, 1H, OH phenolic), 3.96 (s, 3H, OCH_3).

Synthesis of R-2-(4-Methoxy-3-hydroxy phenyl)-4,5-dihydrothiazole-4-carboxylic Acid as luciferin analogue **(C3)**

The mixture of Isovanillonitrile (V2) (0,148g -1mmol), L-cysteine hydrochloride monohydrate (0,35g - 2mmol) and sodium bicarbonate (0,336g - 4mmol) in absolute ethanol (11ml) was stirred at 80 °C for 48 hours. The color of the mixture was converted to green and was evaporated under reduced pressure. 35 mL of water was added to the residual green solid and completely dissolved. The solution was cooled and acidified to pH 2 (indicator paper) by using 2 M of hydrochloric acid. The precipitated solid was collected by filtration and washed with a small amount of water [12]. The crude product was recrystallization by mixture (ethyl acetate/n-hexane) (3-1) v/v to afford the desired product L-2-(4-Methoxy-3-hydroxy phenyl)-4,5-dihydrothiazole-4-carboxylic acid. White powder 0.2g with yield 79%, mp 209 °C. Thin layer chromatography TLC by using a mixture of 1/1 of (THF/n-hexane), Rf = 0,53. FTIR (cm^{-1}) 3290, 2969, 1698, 1580, 1265, 648 .^{1}H NMR (400 MHz, DMSO) δ 12.97 (s, 1H, OH of carboxylic acid), 9.45 (s, 1H), 7.30 (d, J = 2.2 Hz, 1H, aromatic), 7.20 (dd, J = 8.3, 2.1 Hz, 1H, aromatic), 7.00 (d, J = 8.4 Hz, 1H, aromatic), 5.23 (t, J = 8.7 Hz, 1H, CH aliphatic), 3.82 (s, 3H, OCH_3), 3.66 (dd, J = 11.2, 9.4 Hz, 1H, aliphatic), 3.55 (dd, J = 11.1, 8.1 Hz, 1H, aliphatic). 13C NMR (101 MHz, DMSO) δ 172.49, 168.10, 151.21, 146.86, 125.60, 120.91, 114.94, 112.09, 78.63, 56.07, 40.58, 40.37, 40.16, 40.04, 39.95, 39.74, 39.53, 39.32, 35.29. H-COSY NMR 3.66 (dd, 1H, CH2 aliphatic), 3.55 (dd, 1H, CH2 aliphatic). FROM HSQC NMR 9.45 (1H, OH phenolic).[α] L = -98 [DMSO, 0,002], MS (m/z): 254.120 [M+H] (calc. 253.04).

Synthesis of S-2-(4-Methoxy-3-hydroxyphenyl)-4,5-dihydrothiazole-4-carboxylic Acid as luciferin analogue **(C5)**

The same procedure of compound (C3) was followed using D-cysteine hydrochloride monohydrate instead of L-cysteine hydrochloride monohydrate to produce d-2-(4-Methoxy-3-hydroxyphenyl)-4,5-dihydrothiazole-4-carboxylic acid. White powder 0.17g with yield 67%, mp 206 °C. FTIR (cm^{-1}) 3176, 2941, 1595, 1566, 1273, 634. ^{1}H NMR (400 MHz, DMSO) δ 12.97 (s, 1H, OH of carboxylic acid), 9.46 (s, 1H,), 7.30 (d, *J* = 2.2 Hz, 1H, aromatic), 7.20 (dd, *J* = 8.4, 2.1 Hz, 1H, aromatic), 7.00 (d, *J* = 8.3 Hz, 1H, aromatic), 5.23 (t, *J* = 8.7 Hz, 1H, CH aliphatic), 3.82 (s, 3H, OCH_3), 3.66 (dd, *J* = 11.2, 9.4 Hz, 1H, aliphatic), 3.56 (dd, *J* = 11.1, 8.1 Hz, 1H, aliphatic). C13-NMR (101 MHz, DMSO) δ 172.47, 168.08, 151.21, 146.86, 125.60, 120.89, 114.95, 112.11, 78.64, 56.08, 35.29. [α] D = +98 [THF, 0,002], MS (m/z): 254.129 [M+H] (calc. 253.04).

Synthesis of R-2-(4-hydroxy-3-methoxyphenyl)-4,5-dihydrothiazole-4-carboxylic Acid as luciferin analogue **(C4)**

The same procedure of compound (C3) was followed using vanillonitrile (V1) (0,148g -1mmol), instead of isovanillnitrle and after acidified solution, The product was extracted by Dichloromethane (30 ×3), the organic layer was collected ad evaporated to produce a green-yellow solid, then recrystallization by a mixture of (ethyl acetate/n-hexane) (3-1) v/v to afford the desired product L-2-(4-hydroxy-3-methoxy phenyl)-4,5-dihydrothiazole-4-carboxylic Acid. Pale yellow powder 0.12 g with yield 47% and melting point 178 °C. Thin layer chromatography TLC by using a mixture of 1/1 of (THF/n-hexane), Rf = 0,59. FTIR (cm^{-1}) 3290, 2969, 1698, 1580, 1265, 648. ^{1}H NMR (400 MHz, DMSO) δ 12.97 (s, 1H, OH of carboxylic acid), 9.82 (s, 1H), 7.36 (d, *J* = 2.0 Hz, 1H, aromatic), 7.19 (dd, *J* = 8.2, 2.0 Hz, 1H, aromatic), 6.85 (d, *J* = 8.2 Hz, 1H, aromatic), 5.21 (t, *J* = 8.7 Hz, 1H, CH aliphatic), 3.81 (s, 3H, OCH_3), 3.66 (dd, *J* = 11.2, 9.3 Hz, 1H, aliphatic), 3.55 (dd, *J* = 11.1, 8.2 Hz, 1H, aliphatic). 13C NMR (101 MHz, DMSO) δ 172.54, 168.33, 150.64, 148.02, 124.24, 123.14, 115.68, 111.20, 78.56, 56.04, 40.53, 40.32, 40.24, 40.11, 39.90, 39.70, 39.49, 39.28, 35. 34. HSQC-NMR 9.82 (s, 1H, OH phenolic). H-COSY 3.66 (dd, 1H, CH2 aliphatic), 3.55 (dd, 1H, CH2 aliphatic)**.** [α] L = -52 [THF, 0,002], MS (m/z): 254.001 [M+H] (calc. 253.04).

Synthesis of S-2-(4-hydroxy-3-methoxy phenyl)-4,5-dihydrothiazole-4-carboxylic Acid as luciferin analogue **(C6)**

The same procedure of compound (C4) was followed using D-cysteine hydrochloride monohydrate instead of L-cysteine hydrochloride monohydrate, and after acidification, the product was extracted by Dichloromethane (30 ×3), the organic layer was collected and evaporated to produce d-2-(4-hydroxy-3-methoxy phenyl)-4,5-dihydrothiazole-4-carboxylic acid (C6). Pale yellow powder 0.09 g with yield 35%, mp 175 °C. FTIR (cm^{-1}) 3075, 2933, 1722, 1578, 1284, 660. ^{1}H NMR (400 MHz, DMSO) δ 13.00 (s, 1H, OH of carboxylic acid), 9.85 (s, 1H), 7.36 (d, *J* = 2.1 Hz, 1H, aromatic), 7.19 (dd, *J* = 8.2, 2.1 Hz, 1H, aromatic), 6.85 (d, *J* = 8.1 Hz, 1H, aromatic), 5.21 (t, *J* = 8.7 Hz, 1H, CH aliphatic), 3.81 (s, 3H, OCH_3), 3.66 (dd, *J* = 11.2, 9.4 Hz, 1H, aliphatic), 3.55 (dd, *J* = 11.1, 8.2 Hz, 1H, aliphatic). C13-NMR (101 MHz, DMSO) δ 172.56, 168.33, 150.64, 148.02, 124.22, 123.15, 115.67, 111.15, 78.55, 56.02, 40.52, 40.32, 40.11, 39.90, 39.69, 39.48, 39.27, 35.34. [α] D = +52 [THF, 0,002], MS (m/z): 254.119 [M+H] (calc. 253.04).

Synthesis of benzothiazole derivatives as luciferin analogues

Preparation of urea nitrate catalyst

In the beaker, 20 mL of concentrated. HNO_3 was added to 5 g urea, and this mixture was dissolved completely on heating with stirring. The solution was allowed to cool, and white crystals, which are crystals of urea nitrate, appeared. The crystals were filtered and stored in air-tight containers [13].

Synthesis of 2-(4-Hydroxy-3-methoxyphenyl) benzothiazole as luciferin analogue **(T4)**

The mixture of vanillin (VA) (2.0 mmol, 0.304 g) with catalytic of $NH_2CONH_2.HNO_3$ (1.5 wt % of aldehydes) in a test tube, and added to this mixture a dropwise of 2-Aminothiophenol (2.0 mmol - 0,21 ml) with trituration by the glass rod. This mixture undergoes an instant exothermic reaction, and the completion of the reaction will take 10 minutes at the maximum, and the color of the mixture will turn yellowish orange [13]. The mixture was recrystallization directly by 50% methanol to produce white powder, mp = 175 °C. TLC 4\1 (ethyl acetate—n-hexane) 0.39. FTIR (cm^{-1}) 3051, 2930, 1590, 1524, 1364, 1277, 753 .^{1}H NMR (400 MHz, DMSO) δ 9.90 (s, 1H), 8.09 (dd, *J* = 8.1, 1.3 Hz, 1H), 8.04 – 7.97 (m, 1H), 7.64 (d, *J* = 2.1 Hz, 1H), 7.55 – 7.47 (m, 2H), 7.41 (ddd, *J* = 8.2, 7.2, 1.2 Hz, 1H), 6.95 (d, *J* = 8.3 Hz, 1H), 3.90 (s, 3H, OCH3). 13C NMR (101 MHz, DMSO) δ 168.02, 154.10, 150.49, 148.56, 134.63, 126.94, 125.43, 124.77, 122.78, 122.59, 121.75, 116.36, 110.45, 56.15. HSQC-NMR δ 9.90(s, 1H, OH phenolic), 7.52 (2H) for two equivalent protons on different carbon atoms. MS (m/z): 258.061 [M+H] (calc. 257.05).

Synthesis of 2-(4-cyanophenyl) benzothiazole as luciferin analogue **(T6)**

The same procedure above for compound (T4) was followed using 0,262 g of 4-cyanobenzaldehyde (CA) instead of vanillin. The product was recrystallization by 70% methanol to produce white powder, mp = 170 °C. TLC 7\1 (ethyl acetate—n-hexane) 0.51. FTIR (cm^{-1}) 3062, 2224, 1580 ,834, 762. ^{1}H NMR (400 MHz, DMSO) δ 8.31 – 8.25 (m, 2H), 8.22 (dd, J = 8.0, 1.3 Hz, 1H), 8.17 – 8.10 (m, 1H), 8.08 – 8.01 (m, 2H), 7.61 (ddd, J = 8.4, 7.2, 1.4 Hz, 1H), 7.53 (ddd, J = 8.4, 7.2, 1.3 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 165.81, 153.89, 137.14, 135.39, 133.81, 128.32, 127.51, 126.72,

123.86, 123.11, 118.80, 113.83. MS (m/z): 237.210 [M+H] (calc. 236.04).

Absorbance and Fluorescence measurements

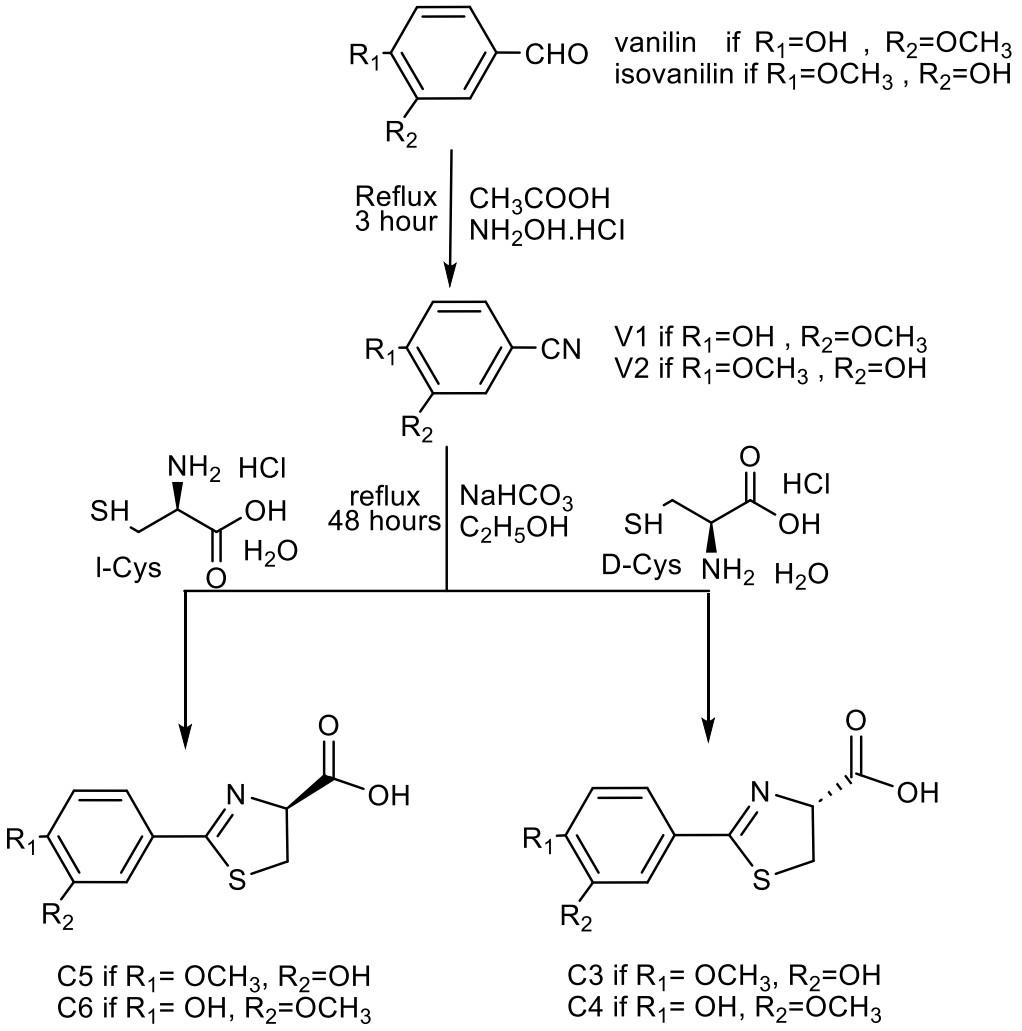
The solutions of compounds (C3, C4, C5, C6) in DMSO (2 x 10^{-4} M at 25 °C) and the solutions of compounds (T4, T6) in methanol (2 x 10^{-4} M at 25 °C) were studied for the optical properties of UV-visible and emission spectra. All solutions emitted color light from green to weak blue when exposed to a UV flashlight. The Tauc plot method was used to calculate the direct energy band gap (Eg) according to the data from Ultraviolet-visible absorbance spectra for all compounds [14].

Results and Discussion.

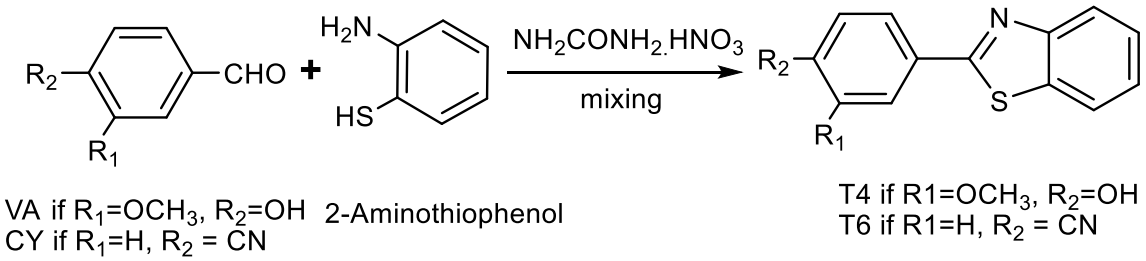
Synthesis discussion

Substitution benzonitrile (V1, V2) was prepared from some derivatives of Benzaldehyde after that a luciferin analogues (C3, C4, C5, C6) were synthesized depending on 4-carboxythiazolin-2-yl rings as a main unit by reaction of (V1, V2) with two isomers (L and D) of cysteine hydrochloride monohydrate as described in the Scheme (3).

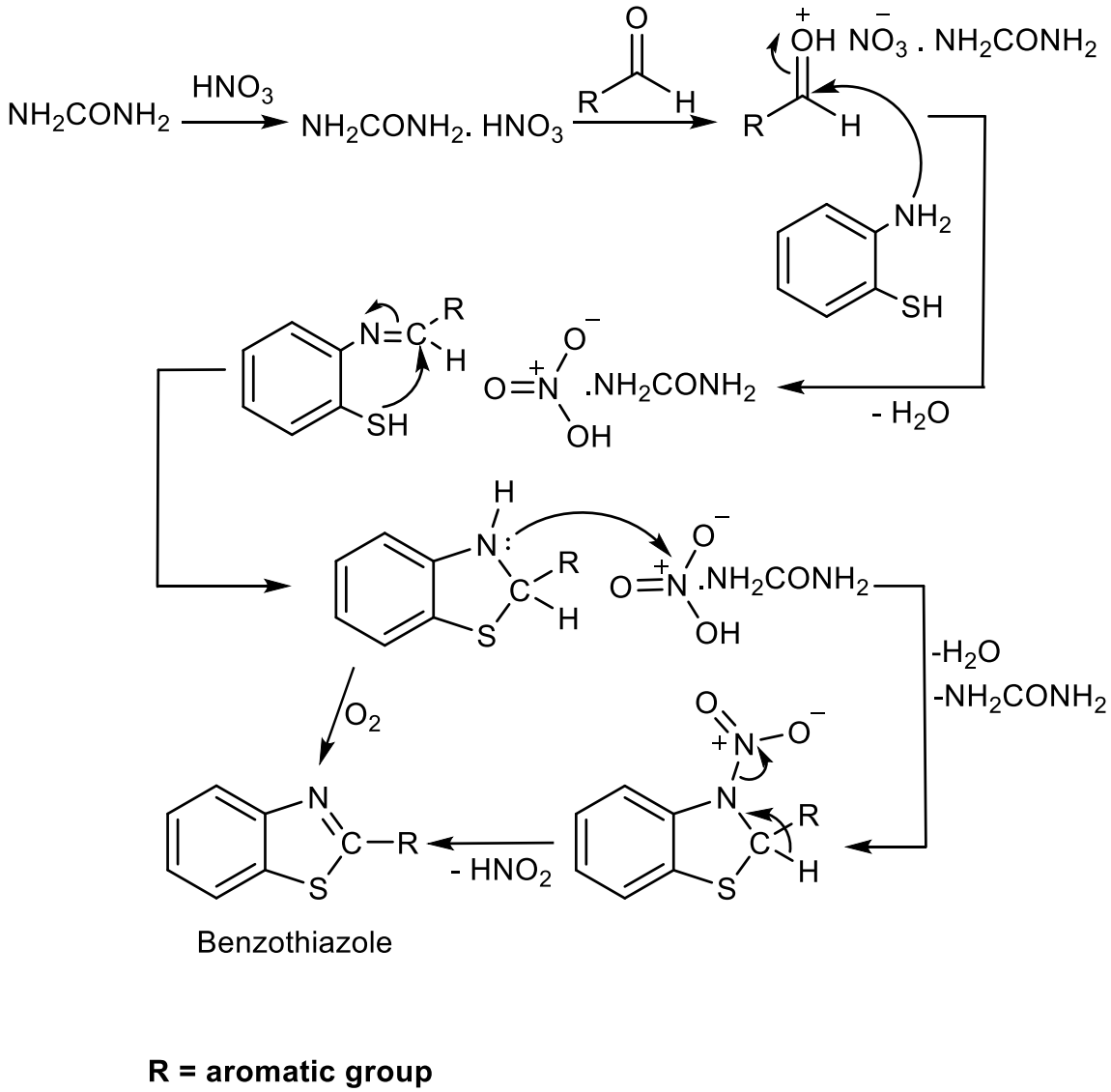
Another series of luciferin analogues depending on the benzothiazole as a main unit by condensation of 2-aminothiophenol with derivatives of benzaldehyde (VA, CY) Scheme (4) with urea nitrate as a catalyst without solvent, which is described mechanism below in Scheme (5) [13,15].



Scheme-3: Reaction synthesis of luciferin analogues.



Scheme-4: Reaction synthesis of benzothiazoles.



Scheme-5: Mechanism of synthesis of benzothiazole by urea nitrite.

Table-1: ^{1}HNMR data for compounds **C3-C6**.

Sym.	OH of carboxylic acid (A)	OH of phenolic group (B)	OCH_3 (D)	CH Aliphatic (C)	CH Aliphatic (E)	CH Aliphatic (F)
C3	12.97	9.45	3.82	5.23	3.66	3.55
C4	12.97	9.82	3.81	5.21	3.66	3.55
C5	12.97	9.46	3.82	5.23	3.66	3.56
C6	13	9.85	3.81	5.21	3.66	3.55

FT-IR analysis.

The FT-IR of aryl nitrile compounds (V1, V2) shows a sharp peak at 2220 cm^{-1} for stretching vibration of the nitrile group [11]. The FT-IR of compounds (C3, C4, C5, C6) show one peak at range (1564-1722) cm^{-1} for stretching vibration of carbonyl of carboxylic groups. The FT-IR spectra of benzothiazole derivative compounds T4 and T6 show one peak at 2224 cm^{-1} for T6 belongs to the stretching vibration of the nitrile group, and one peak at 1277 cm^{-1} for T4 belongs to the stretching vibration of the ether bond (C-OCH_3) [16, 17]. All spectra above show one peak at the range 616-685 cm^{-1} for the stretching vibration of the carbon-sulfur bond (C-S).

NMR analysis

The ^{1}HNMR spectra of compounds V1 and V2 show one singlet signal at 10.27 ppm and 5.89 ppm, respectively, for the phenolic proton and one singlet signal at 3.81 ppm and 3.96 ppm, respectively, due to protons of the methoxy group [11,18,19]. The most distinguish peaks for thiazoline motifs (C3, C4, C5, C6) were mentioned in Table (1) at ppm with symbolized proton according to the Fig (3).

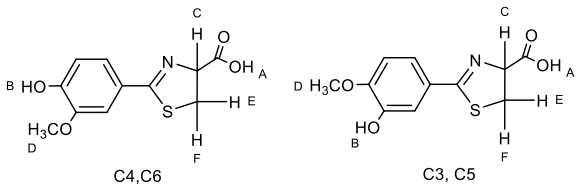


Fig 3: Structures of luciferin analogues with symbolized the most distinguished protons

The ^{13}C-NMR spectra of compound (C3, C4, C5, C6) show proper peaks with proper intensity that correspond to different environments of carbon for thiazoline motifs [12]. The COSY spectra of compounds C3 and C4 have great benefit in determining precisely the position of coupling protons, which is the coupling of aliphatic protons CH (C) and CH_2 (F, E) that belong to the thiazoline ring which confirm diastereotopic effect. The HSQC spectra of compounds C3 and C4 have a great benefit in determining the precise position of aliphatic carbon, which is at (78, 35) ppm [20]. The ^{1}HNMR spectra of compound (T4, T6) show several signals with different multiplicity in the range (8.09- 6.96) ppm for aromatic protons besides two singlet signals at 9.91 ppm and 3.92 ppm for the proton of the hydroxyl group and methoxy group of compound T4, respectively. The ^{13}C-NMR spectra of compounds T4, T6 show proper peaks with proper intensity that correspond with different environments of carbon for benzothiazole motifs [13]. The COSY spectra of compound T4 have great benefit in determining the precise position of aromatic protons. The HSQC spectra of compound T4 have great benefit in determining the precise position of aromatic carbons that are bonded with protons [20].

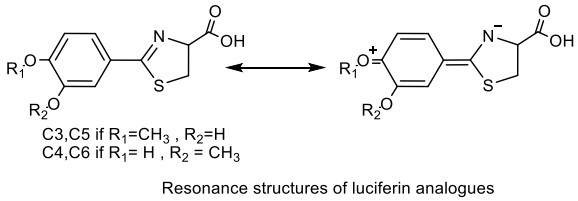
EI-Mass analysis

The results obtained from the mass spectra for the synthesis compounds (C3, C4, C5, C6, T4, T6) with molecular ion peaks are consistent with the proposed molecular formula of these compounds [20].

Absorbance and fluorescence analysis, and Stokes shift calculation

The basic requirement for a molecule to be fluorescent is the existence of a small value of energy band gap between the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) more precisely between the energy of the excited state and the ground state. Common ways to attain a small value of band gap are the extension of π-conjugated systems or the conjugation of electron-donating groups with electron-withdrawing groups (donor-acceptor) [21]. Heterocyclic molecules like thiazole and thiazoline are characterized by a five-membered heterocyclic ring, which possesses an electron-donating group in the form of a sulfur atom with sp^2 hybridization and an electron-accepting group in the form of a carbon-nitrogen double bond (C=N) [22]. In our work thiazoline ring in luciferin analogues has a donor-acceptor system besides carboxylic group substitution, which increases the drawing effect for the thiazoline ring and extends π-conjugated through aryl group substitution at position 2 and the resonance effect as shown in Scheme (6). All synthesis luciferin analogues (C3, C4, C5, C6) have the same value of λ_{abs} max= 300±5 which causes from π-π* transition by carbon carbon double bond through the aromaticity which is likely happened in fluorescence in contrast of the transition n-

π* which is likely in phosphorescence and blue-green color emission (λ_{PL} max = 470±5) as shown in Figs (S.42-45) of absorbance and emission spectrum and chart (2) belong to the electronic transition from excited state of molecule to the ground state. All luciferin analogues seem to have the same value of λ max for both absorbance and emission spectra which is attributed to the same structure of these analogues where all analogues having donor substitution which are methoxy and hydroxy groups on aryl ring and both of pairs of C3, C5 and C4, C6 is enanshomers so as a result having the same stokes shift ~172 as shown in Fig A (4) for compound C3 as example and mention in chart (2) where the stokes shift is define as a difference between the maximum value of emission wavelenght to the maximum value of absorbance wavelength in photoluminescence process [23].



Resonance structures of luciferin analogues

Scheme-6: Resonance structures of luciferin analogues

Benzothiazole is a representative class of sulfur-containing heterocycles and involves a benzene ring fused to a thiazole ring, so it's suitable for photoluminescence studies [24,25]. Our benzothiazole compounds T4 and T6 have aromaticity extended through the skeleton of the molecule, as shown in Scheme (7). The spectra of absorbance and emission for compound T4 shows a value of λ_{abs} max= 328 nm which comes from π-π* transition by carbon carbon double bond through the aromaticity which is likely happened in fluorescence in contrast of the transition n-π* which is likely in phosphorescence and blue color emission (λ_{PL} max = 468 nm) which is attributed to the system of donor-acceptor, aromaticity and resonance effect of 2-(4-Hydroxy-3-methoxyphenyl) benzothiazole molecule. The absorpance and emission spectra of compound T6 shows a value of λ_{abs} = 296 nm which comes from π-π* transition and weak blue color emission (λ_{PL} = 472) nm which is attributed to the system of donor-acceptor, aromaticity and resonance effect of 2-(4-cyanophenyl) benzothiazole molecule and drawing effect of cyano group which drawing the electron density from benzene ring so will decrease the system of donor-acceptor between aryl ring and thiazole ring. The Stokes shift for compounds T4 and T6 was calculated and mentioned in chart (2) based on data from UV-visible and fluorescence spectra as shown in Fig B (4) for compound T6 as an example. All synthesis analogues have smaller values of wavelengths for absorbance and emission and Stokes shift compared with D-luciferin, which is (λ_{abs} = 327, λ_{PL} = 530, stokes shift =203) nm due to the absence of the thiazole ring and as a result decreasing in the resonance effective and as a consequence decreasing in the wavelength [26].

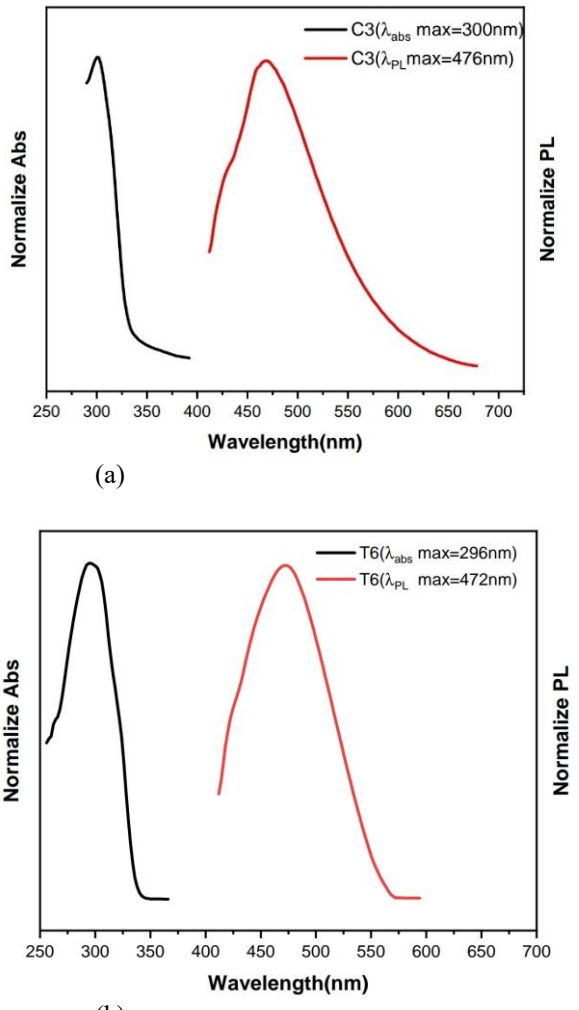


Fig. 4: Absorbance and emission spectrum of compounds C3 and T6.

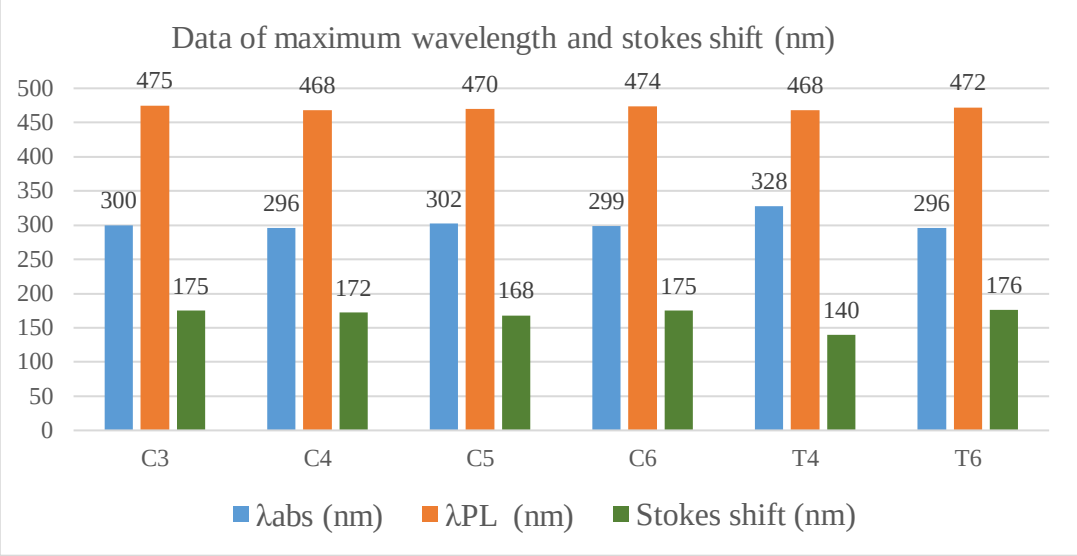
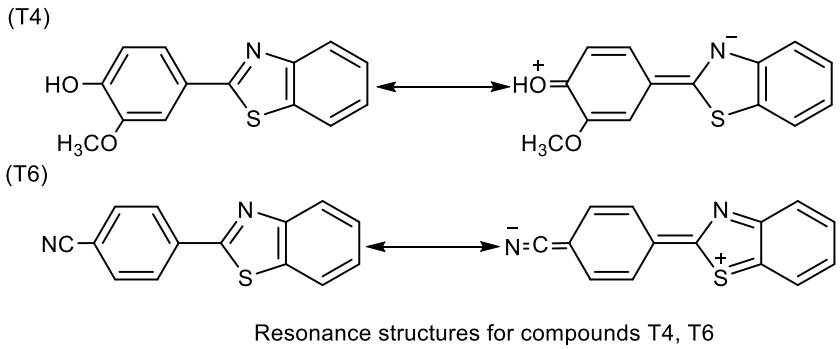


Chart 1-Data on the maximum wavelength and Stokes shift of synthesized compounds.



Scheme-7: Resonance structures of synthesised benzothiazoles.

Direct Energy band gap calculation by the Tauc plot method

The following equations are the principle of the Tauc plot method.

$$(\alpha \cdot h\upsilon)^{1/n} = B\,(h\nu - Eg) \qquad (1)$$

where h is the Planck constant, ν is the photon frequency, Eg is the band gap energy, B is a constant, α is the absorbance coefficient, and n depends on the type of electronic transition, which is equal to 1/2 for a direct transition (allowed transition) and 2 for an indirect transition (forbidden transition). The absorbance coefficient is

calculated by equation (2)

$$\alpha = \ln(10) \cdot A/L \qquad (2)$$

where A is absorbance and L is the thickness of the material in centimeters, which in our case is 1 cm (path of incident light through the quartz cell. So the absorption coefficient will be calculated according to the next equation (Eq. 3)

$$\text{So } \alpha = 2.302\,A \qquad (3)$$

The Tauc plot assumed that the absorption coefficient follows the power law dependence on the photon energy, which might not be correct for all materials. The Tauc plot is very sensitive to the choice of correct n, defects, quality, and experimental methods [27]. The determination of band gap energy according to the Tauc plot is done by plotting the value of $(\alpha h\nu)^{1/n}$ on the y-axis versus hν (energy) on the x-axis, then followed by taking the extrapolation in the linear area across the energy axis on the corresponding graph. The intersection with the energy-axis is the estimation of the corresponding energy band gap as

described in Figs (2-7) [28, 29]. Plotting the $(\alpha h\nu)^{1/n}$ versus (hν) is a matter of testing n values (1/2, 2) to find which provides the best fit linear region and thus. Identifies the correct transition type, which is, in our case, the best value of n is half to calculate the direct energy band gap [30]. According to n = 1/2 for the direct energy band, the tauc plot will be between $(\alpha h\nu)^2$ on the y-axis and hν (energy) on the x-axis. The energy band gap of luciferin analogues (C3, C4, C5, C6) as shows in Fig (5-8) and mention in chart (2) is almost the same value (~3.8 eV) because they have the same structures but the analogues (C3, C5) have a lower energy band gap than (C4, C6) which may be attributed to the methoxy group at a para position for compounds (C3, C5) which is more donating groups than hydroxy group for compounds (C4, C6) so the intramolecular charge transfer will be increased from benzene ring to thiazoline ring which increase donor-acceptor system and stability. The energy band gap of compound T4 (Eg=3.52) eV is lower than the energy band gap of compound T6 (Eg=3.75) eV as show in Figs (9, 10) and mention in chart (2) which is attributed to the effect of methoxy and hydroxy group at the aryl ring by increasing the electron density and resonance effect which result in more stability for compound T4 than compound T6 that have nitrile (with-drawing group) on aryl ring. All synthesis analogues have larger energy band gaps than the Eg of D-luciferin, which is (3.22 eV) due to the decrease of resonance as a consequence of the absence of the thiazoline ring [31].

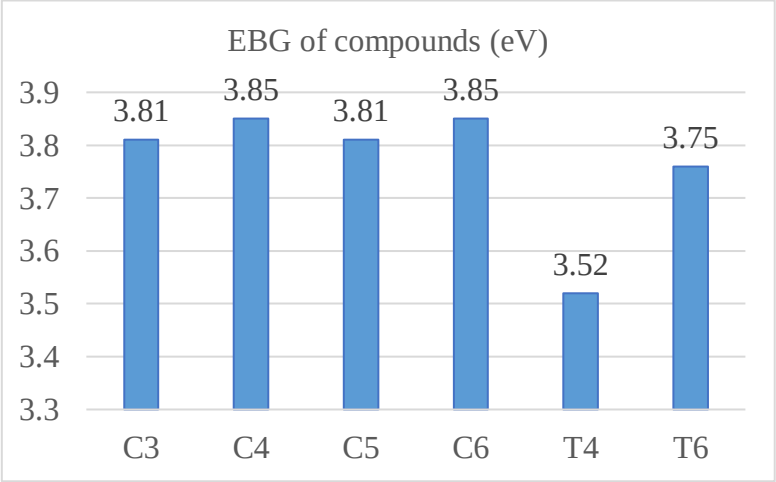


Chart-2: Data on the maximum wavelength and Stokes shift of synthesized compounds.

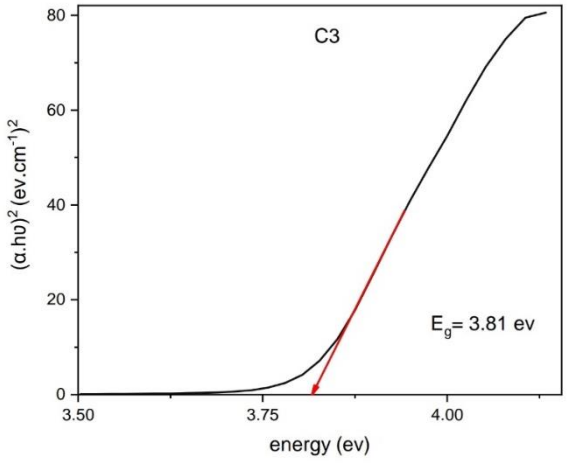


Fig. 5: Energy band gap for compound C3 by Tauc plot.

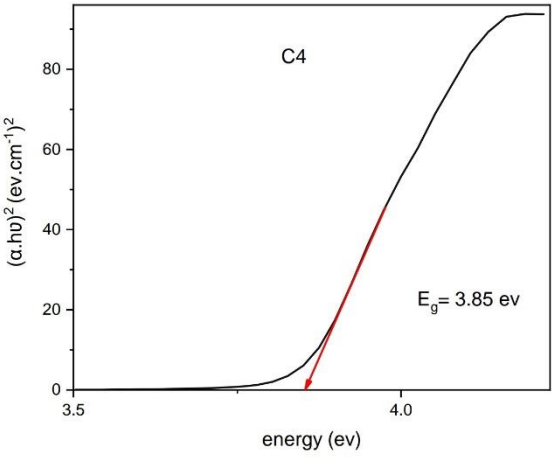


Fig. 6: Energy band gap for compound C4 by Tauc plot.

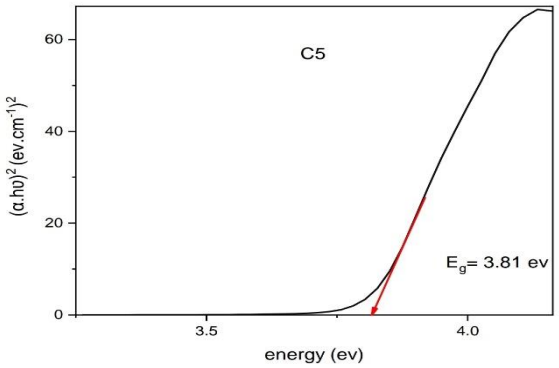


Fig. 7: Energy band gap for compound C5 by Tauc plot. Tauc plot.

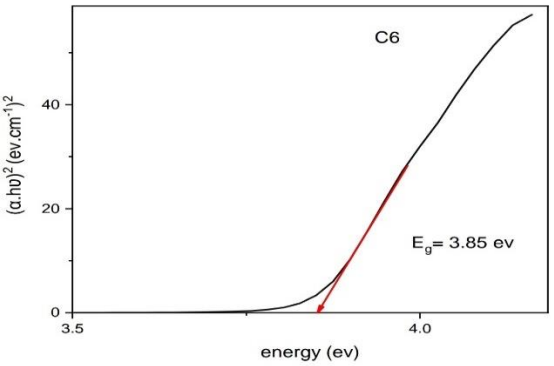


Fig. 8: Energy band gap for compound C6 by

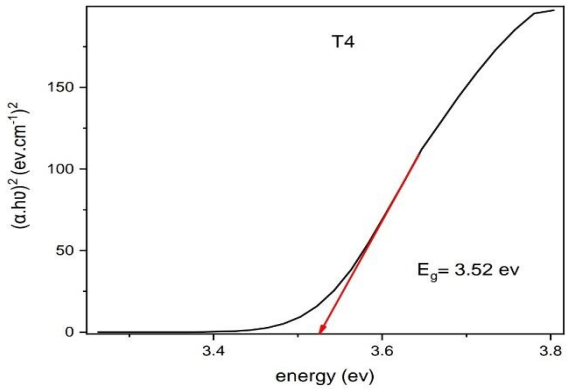


Fig. 9: Energy band gap for compound T4 by Tauc plot.

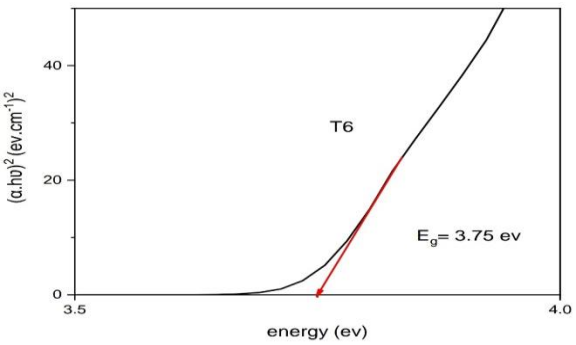


Fig. 10: Energy band gap for compound T6 by Tauc plot.

Conclusions

Novel compounds of 2-phenyl substitution-4,5-dihydrothiazole-4-carboxylic Acid (C3, C4, C5, C6) derivatives were successfully synthesized and characterized. The compound of benzothiazole derivative (T4, T6) was successfully synthesized and characterized. The absorbance and fluorescence spectra for solution compounds (C3, C4, C5, C6) in DMSO and (T4, T6) in methanol were studied and gave weak blue-green color emission for compounds (C3, C4, C5, C6) and blue color emission for T4 compound and weak blue color emission for compound T6 and The Stokes shift were calculated for all compounds. The energy band gaps (Eg) were calculated for all synthesis compounds by the Tauc plot method.

References

1. D. Pansare, M. Shaikh, P. Chavan, B. Dhotre, R. Shelke, S. Thopate, K. Ameta and R. Pawar, Synthesis and Biological Evaluation of Thiazoline, Thiophene and Thiazole Scaffolds, Eds. Ameta, L K , Springer, Singapore. pp. 105 (2024).
2. A. Syed and J. Anderson, Applications of bioluminescence in biotechnology and beyond, *Chem. Soc. Rev*. 50, pp. 5668(2021).
3. B. Haixiu, C. Wang, W. Wenxiao, M. Zhao, Z. Huateng, J. Tianyu, Z. Tianchao, Z. Yubin, D. Lupei, S. Yuemao and L. Minyong, Discovery of a series of 2-phenyl naphthalenes as firefly luciferase inhibitors, *RSC Advances*, 5, pp. 63450 (2015).
4. N. Ugarovaa and Y. G. Lomakina, Emitter as an Intramolecular Probe in a Luciferase Active Center, *Moscow Univ. Chem. Bull*, 75, pp. 15 (2020).
5. A. Dagli and O. Arihan, A Light To Our Darkness: Bioluminescence And Its Uses In Medical Research, *Eastern J Med*, 28, pp. 68 (2023).
6. H. Kim, O. Jung, S. Lee and Y. Lee, Bioluminescent Systems for Theranostic Applications, *Int. J. Mol. Sci, 25*, 7563(2024).
7. B. Branching, D. Ablamsky, A. Davis, T, Butler B. Southworth, F. Fan, A. Jathoul and M. Pule, Red-emitting luciferases for bioluminescence reporter

and imaging applications, *Anal Biochem*, 396, pp. 290 (2010).
8. C. Woodroof, J. Shultz, M. Wood, J. Osterman, J, Cali, W. Daily, L. Meisenheimer and D. Klaubert *Biochemistry,* 47, pp. 10383 (2008).
9. N. Kalyani, H. Swart and S. Dhoble, Luminescence: Basic Principles and Applications, Eds. Kalyani N, Swart H, and Dhoble J Woodhead Publishing. pp. 1-37(2017).
10. J. Lakowicz, Introduction to Fluorescence, Eds. Lakowicz, R. J., Springer, Boston, MA. pp. 1–26(2006).
11. I. Ilic, K. Leus, J. Schmidt, J. Hwang, M. Maranska, S, Eigler and C. Liedel, Polymerization in Carbone: A Novel Method for the Synthesis of More Sustainable Electrodes and Their Application as Cathodes for Lithium–Organic Energy Storage Materials Based On Vanillin, *ACS Sustainable Chem. Eng*, 8, pp. 3055 (2020).
12. O. Maltsev, V. Walter, M. Brandl and L. Hintermann, Medium buffer effects on the condensation of l-cysteine and aryl nitriles to (R)-2-Aryl-4,5-dihydrothiazole-4-carboxylic acids, *Synthesis*, 45, pp. 2763 (2020).
13. P. Kumar, R. Bhatia, R. Khanna, A. Dalal, D. Kumar, P. Surain and R. Kamboj, Synthesis of some benzothiazoles by developing a new protocol using urea nitrate as a catalyst and their antimicrobial activities, *Journal of Sulfur Chemistry*, 38, pp. 585 (2017).
14. M. Hussein, A. Dannoun, B. Aziz, A. Brza, T. Abdulwahid, A. Hussen, S. Rostam, T. Mustafa and S. Muhammad, Steps Toward the Band Gap Identification in Polystyrene Based Solid Polymer Nanocomposites Integrated with Tin Titanate Nanoparticles. *Polymers*, 12, 2320(2020).
15. V. Juthiga, M. Boddapati, M. Balha and R. Tamminana, Comprehensive review on green methods: Synthesis of benzothiazoles. *Results in Chemistry*, 15, 102212(2025).
16. M. Thompson, Infrared Spectroscopy (1st eds.). Jenny Stanford Publishing (2018).
17. A. Walaa, B. Mohammed, S. Basil and A. Gamal, Synthesis, characterization, and fluorescence properties of new polyethers derived from curcumin analogs, *J. Chem. Res*, 47, pp. 1-10 (2023).
18. O. Bonjour, H. Nederstedt, V. Arcos-Hernandez, S. Laanesoo, L. Vares and P. Jannasch, Lignin-Inspired Polymers with High Glass Transition Temperature and Solvent Resistance from 4-Hydroxybenzonitrile, Vanillonitrile, and Syringonitrile Methacrylates. *ACS sustainable chemistry & engineering*, *9*, pp. 16874 (2021).
19. J. Raghad, A. Faris, A. Tahseen and L. Mohd, Synthesis, Thermal Analysis, and Thermodynamic Properties Study of New Quinoline Derivative and Their V(IV), Co(II), and Cu(II) Complexes, *Indones. J. Chem*, 22, pp. 1376 (2022).
20. R. Silverstein, F. Webster, D. Kiemle and D. Bryce, Spectrometric Identification of Organic Compounds. 8th Edition, John Wiley & Sons, Ltd. Chi- chester. pp. 230 (2015).
21. S. Schramm and D. Weiss, Fluorescent heterocycles: Recent trends and new developments, Eds. Scriven, V. F. E., and Ramsden, A. C., Academic Press Inc. pp. 103 (2019).
22. S. Kumar, A. Arora, S. Sapra, R. Kumar, R. Singh and S. Singh, Recent advances in the synthesis and utility of thiazoline and its derivatives, *RSC Advances*, 2, pp. 902 (2024).
23. F. Ghasem, N. Fahimi-Kashani, A. Bigdeli, H. Alshatteri, S. Abbasi-Moayed, H. Al-Jaf, Y. Merry, M. Omer and R. Hormozi-Nezhad, Paper-based optical nanosensors - A review, *Anal Chim Acta*, 15, 340640(2023).
24. X. Gao, J. Liu, X. Zuo, X. Feng and Y. Gao, Recent Advances in Synthesis of Benzothiazole Compounds Related to Green Chemistry. *Molecules*, 25, 1675(2020).
25. M. Paczkowski, L. Coelho and F. Campo, 2, 1, 3-Benzothiadiazole dyes conjugated with benzothiazole and benzoxazole: Synthesis, solvatochromism and solid-state properties. *Journal of Molecular Liquids*, *319*, 114277(2020).
26. P. Asare, M. Zniber, M. Zouheir, L. Wang, X. Wang and P. Huynh, Luciferin-based fluorescent hydrogel as a pH sensor. *MRS Communications*, *12*, pp. 90 (2022).
27. J. Klein, L. Kampermann, B. Mockenhaupt, M. Behrens, J. Strunk and C. Bacher, Limitations of the Tauc plot method. *Advanced Functional Materials*, *33*, 2304523(2023).
28. V. Mursyalaat, V. Variani, W. Arsyad and M. Firihu, The development of the program for calculating the band gap energy of semiconductor material based on UV-Vis spectrum using delphi 7.0, *J. Phys.: Conf. Ser*. 2498(2023).
29. S. Abbas, S. Al-Luaibi and B. Saeed, Experimental and DFT Study of Structural and Electronic Properties of Polyfuran@nanoCuO Hybrid Polymer, *SEEJPH*. pp. 496 (2024).
30. B. Coulter and P. Birnie, Assessing Tauc plot slope quantification: ZnO thin films as a model system. *Physica status solidi (b)*, *255*, 1700393(2018).
31. H. Chang, M. Fontaine, S. Gómez, R. Branchini and C. Anderson, Synthesis and Bioluminescence of 'V'-Shaped Firefly Luciferin Analogues Based on A Novel Benzobisthiazole Core. *Chemistry–A European Journal*. *29*, pp. e202302204(2023).