Synthesis, Characterization, Docking Study and Biological Activates of New 3-Aminorhodanine Derivatives

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Abstract :

Abstract: Increase in fatalities among cancer patients is one of untreated facts and today cancer remains one of the main health issues. The most common cause of cancer death is lung cancer. Rhodanine has been recognized as a privileged scaffold in medicinal chemistry due to its well-known ability to demonstrate a broad range of biological activities, and most of its derivatives exhibited remarkable anticancer activity in the (µg/mL) concentration range, while causing negligible cytotoxicity to normal cells. New *N*- and 5-disubstituted aminorhodanine derivatives were synthesized by Schiff base and Knoevenagel condensation reactions over two consecutive steps. Human cancer cells and Hdfn (human dermal fibroblasts isolated from neonatal foreskin) cells line, were used to evaluate the synthesized compounds' activity by MTT assay. The new compounds revealed higher anticancer activity against A549 lung cells cancer when compared with reference drug Erlotinib (anticancer drug) and determined no toxicity or safety on normal cell. Among the tested compounds, **2b2** compound show potent anticancer activity which have IC_{50} about (55.8 µg/mL).

Key Words: 3- aminorhodanine, lung cancer, EGFR mutation.

تخليق وتشخيص وألارساء ألجزيئي وألفعالية ألبايلوجية للمشتقات ألجديده ل 5, 3 أمينورودانين

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*****مركز أبحاث وتطبيقات المواد الطبية الحيوية والمغناطيسية وأشباه الموصلات, جامعة سكاريا, اسطنبول.***

الخلاصة:

تعد ألزيادة المستمره في ألوفيات بين مرضى ألسرطان أحد ألحقائق التي لم يتم معالجتها لحد الان، حيث ان سرطان ألرئة يعد من أكثر أنواع ألسرطان ألمسببة للوفيات عالمياً. ان مركب ألرودانين يعتبر أحد المركبات المميزه في ألكيمياء ألطبية نظرا لفعاليته البايلوجية ألواسعه ألتي أظهرتها مشتقاته، بعض هذه المشتقات أظهرت نشاطاً مضادًا للسرطان في نطاق تركيز (ميكروغرام / مل). تم تصنيع مركبات جديده ل 5- , ن- أمينورودانين بوساطة خطوتين متتاليتين و هما : تفاعل تكوين قواعد شف و تفاعل تكاثف نوفونجيل. لقد قيمت فعالية ألمركبات ألمحضرة الجديده على ألخلايا ألسرطانيه للرئة من نوع A549 و ألخلايا ألطبيعيه ألبشريه من نوع HdFn بأستخدام تقنيه أل MTT. أظهرت المركبات نشاط مضاد للسرطان

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على ألخلايا ألسرطانية للرئة عند مقارنتها بألعقار Erlotinib (عقار مضاد للسرطان) وسلامه عالية على ألخلايا البشريه ألطبيعيه. المركب **2b1** أبدى فعالية جيدة كمضاد سرطاني حيث أن IC_{50}لهذا المركب كانت بمقدار 55.8 مايكروغرام / مل.

ألكلمات ألمفتاحية: 3-أمينورودانين، سرطان ألرئة, طفرة EGFR.

Introduction

Despite unending attempts to create novel therapeutics for various forms of cancer, cancer remains one of the major causes of human mortality globally [1]. According to the WHO, lung, bronchus and trachea malignancies were the sixth leading cause of death around the world in 2019 [2,3]. Non-small cell lung cancer (NSCLC) accounts for 80% to 85% of all lung cancers [4–9].

The epidermal growth factor receptor (EGFR) is a crucial target in the therapy of NSCLC. It is a tyrosine kinase receptor that can be inhibited reversibly by first-generation TKIs, such as Gefitinib and Erlotinib. However, resistance to Gefitinib/Erlotinib due to mutations has been linked to low sensitivity to therapeutically effective doses. Thus, the primary issue in the modern world is still the development of novel substances that could specifically target cancer cells.

Heterocycles have become important in developing and producing broad variety of organic compounds in medical science, heterocyclic compounds that have nitrogen atoms are the most important family of chemical substances [9]. Also, heterocyclic compounds that have sulfur atom in their structures are commonly employed in biochemical experiments. These two families of compounds have been associated with a wide range of biological effects [10], such as anti-cancer [11], anti-microbial [12], anti-diabetic [13], anti-inflammatory [14], anti-viral [15], anti-fungal [16], and anti-hypertension [17].

Chemical molecules with five membered rings such as rhodanine and its derivatives 2, 4-thiazolidinedione (TZD) analogues (that have a thiazolidine core and a carbonyl group located on the fourth carbon) are commonly used in drug discovery approaches, **Figure (1)**. These substances with rhodanine cores have several biological activities such as anti-bacterial [18, 19], anti-HIV [20], anti-tuberculosis [21], hypnotic [22], anti-parasitic [23], anti-fungal [24], anti-inflammatory [25], carbonic anhydrase inhibitor [26] and anti-diabetic [27].

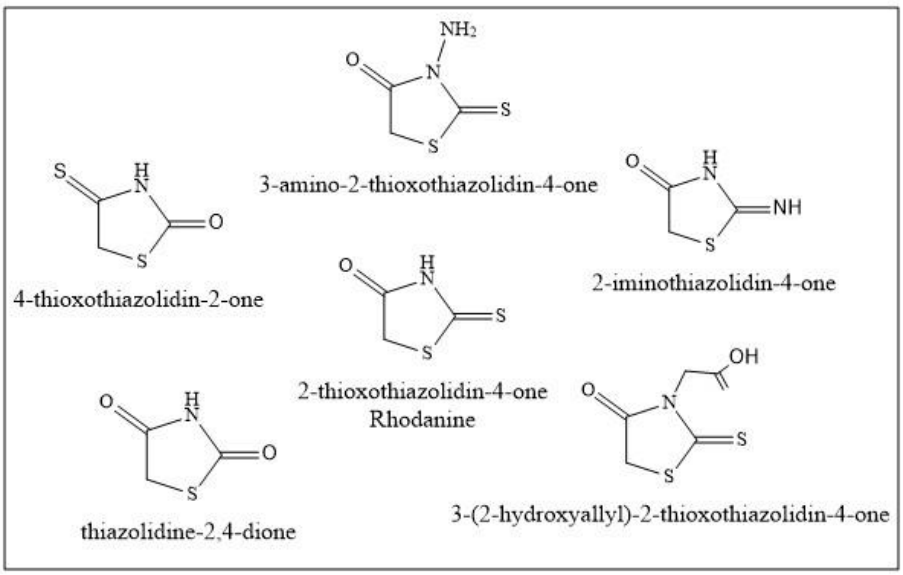

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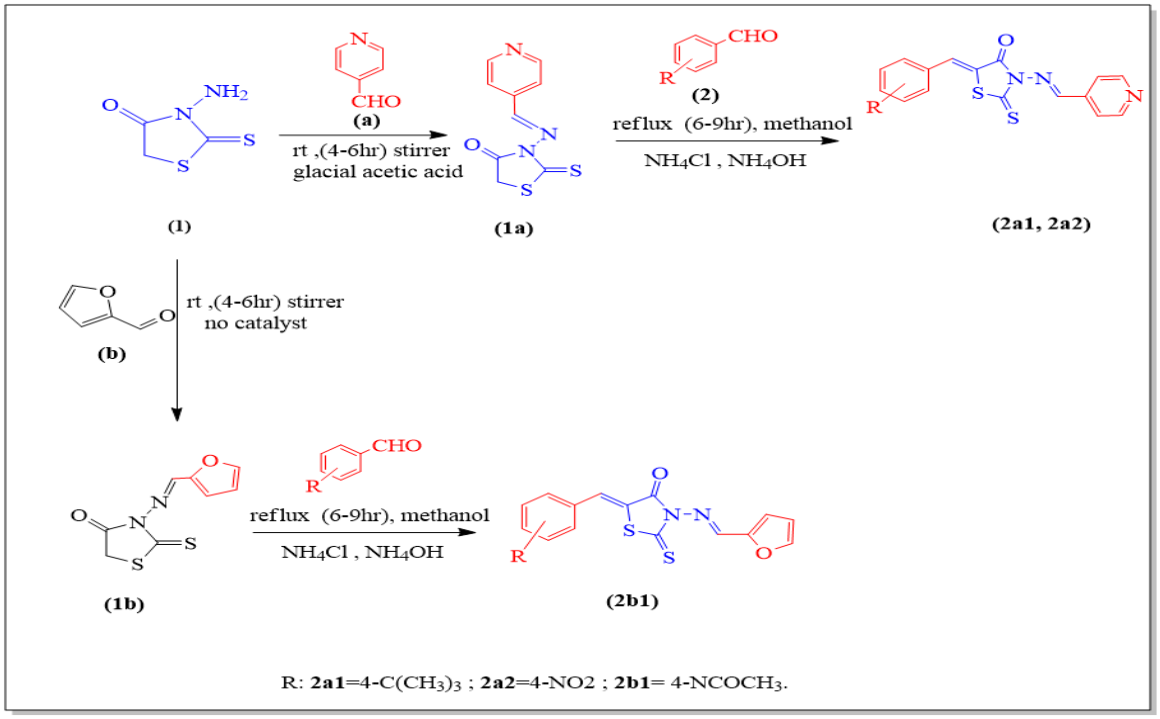
Figure (1): rhodanine and its 2, 4-thiazolidinedione (TZD) analogues.

It is relatively common to synthesize rhodanine derivatives and evaluate their possible effects, but synthesis of amino-rhodanine derivatives has undergone very little research. For this reason, in the present study, we developed new *N*-substituted rhodanine derivatives (which exhibit distinct natures) and assess their toxic effects on the A549 lung cancer cell line and on the HdFn human normal cell.

1. Materials and methods:

All chemicals and solvents were commercially available from Sigma-Aldrich or Merck. Infrared spectra were measured by KBr/ Shimadzu spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were measured on 300 (75)-MHz Bruker spectrometer. The melting points were determined without correction using an electro-thermal melting point apparatus. The reaction progress was monitored by thin-layer chromatography (TLC, 0.25 mm-thick precoated Merck silica plates).

1.1. Synthesis of organic compounds



Scheme (1) Synthesis of *N*- 5 disubstituted aminorhodanine derivatives.

a) General synthesis of *N*-substituted-derivatives (1a and 1b)

To a solution of 3-amino-2-thioxothiazolidin-4-one 1mmol (148.2 mg) in 2.5 mL methanol, aldehydes (a or b) 1.2 mmol in 1.5 mL methanol were added slowly to the solution. The reaction mixtures were stirred at room temperature with glacial acetic acid as a catalyst (2 drops) for compound **1a** and without any catalyst for compound **1b** with stirring for 4 to 6 hrs as shown in scheme (1).

The reaction mixture was monitored by TLC. After that, the two mixtures products were recrystallized from methanol. After

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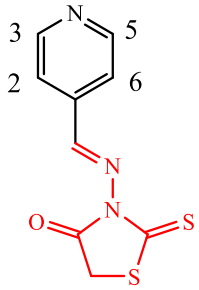
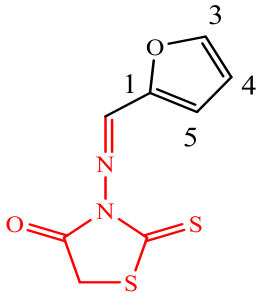
recrystallization, *N*-substituted-rhodanine derivatives were obtained as follows:

3-((pyridin-4-ylmethylene) amino)-2-thioxothiazolidin-4-one (1a)



The product was obtained as off-white powder (200 mg, 84% yield); m.p: 168°C-170°C; R_f: 0.25 in hexane: ethyl acetate (1:1) solvent system. Chemical Formula: $C_9H_7N_3OS_2$. FTIR (KBr, cm^{-1}) *v*max: 3296 (=**C-H** sp^2 stretching), 2814 (-**C-H** sp^3 stretching), 1721 (**C=O** stretching), 1605 (**C=N** stretching), 1418 (**C=C** aromatic stretching), 1293 (**C=S** stretching), 1208 (=**C-N** stretching), 1104 (**C=S** stretching), 1063 (-**C-N** stretching), 869 (**C-S** stretching), 784 (**C-H** aromatic out of plane bending). ^{1}H-NMR (300 MHz, DMSO-*d6*) δ ppm: 4.24 (s, 2H, C**H**$_2$), 7.59 (d, 2H, **H-2**, **6**), 7.83 (s, 1H, C**H**=N), 8.75 (d, 2H, **H-3**, **5**). ^{13}C-NMR (75 MHz, DMSO-*d6*): 33.7 (**C**H$_2$), 124.2 (**C-2**, **6**), 140.3 (**C-1**), 151.1(**C-3, 5**), 158.2 (**C**H=N), 164.0 (**C**=O), 188.1 (**C**=S).

3-((furan-2-ylmethylene) amino)-2-thioxothiazolidin-4-one (1b)



The product was obtained as yellow glittery crystal (165 mg, 73% yield); m.p: 130°C-132°C; R_f: 0.7 in hexane: ethyl acetate (1:1) solvent system.Chemical Formula: $C_8H_6N_2O_2S_2$. FTIR (KBr, cm^{-1}) *v*max: 3128 (=**C-H** sp^2 stretching), 2928 (-**C-H** sp^3 stretching), 1737 (**C=O** stretching), 1614 (**C=N** stretching), 1545 and 1467 (**C=C** aromatic stretching), 1390 (**C=S** stretching), 1228 (**C-O** stretching), 1084 (**C=S** stretching), 1016 (**C-N** stretching), 825 (**C-S** stretching), 767 (**C-H** aromatic out of plane bending). ^{1}H-NMR (300MHz, DMSO-*d6*) δ ppm: 4.37 (s, 2H, C**H**$_2$), 6.81 (dd, 1H, **H-4**), 7.39 (d, 1H, **H-5**), 8.10 (d, 1H, **H-3**) 8.58 (s, 1H, C**H**=N). ^{13}C-NMR (75 MHz, DMSO-*d6*): 35.2 (**C**H$_2$), 113.6 (**C-4**), 121.8 (**C-5**), 147.5 (**C**H=N), 148.8 (**C-3**), 158.7 (**C-1**), 170.1 (**C**=O), 197.0 (**C**=S).

b) Synthesis of *5*-substituted rhodanine derivatives 2a1, 2a2 and 2b1 (final compounds)

The target compounds were synthesized following the method previously reported by Petlichnaya L.I (1967) [28]. To a warm solution of 1 mmol of compound (**1a** or **1b**) in 3 mL methanol, benzaldehyde derivatives (1.2 mmol) which dissolved in 2 mL methanol mixed with 0.2 mL of conc. NH_4OH and 0.1g of NH_4Cl which dissolved in 0.2 mL water were added slowly and heated at 50°C with stirring for 6-9 hrs as shown in scheme (1). The reaction mixture was monitored by TLC. The products of 5-substituted rhodanine derivatives recrystallized using mixture of water and methanol and were obtained as follows:

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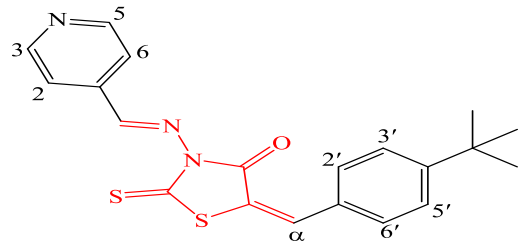
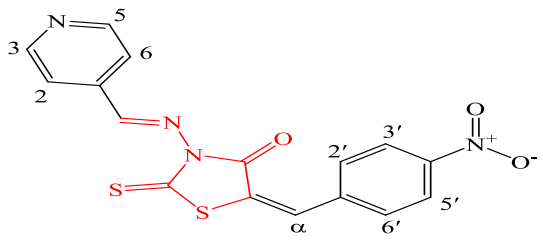
5-(4-(tert-butyl) benzylidene)-3-((*E*)-(pyridin-4-ylmethylene) amino)-2-thioxothiazolidin-4-one (2a1).



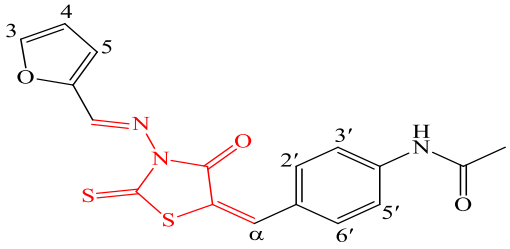
The product was obtained as brown solid (300 mg, 78% yield), m.p: 82°C-84°C, R_f: 0.8 in hexane: ethyl acetate (3:1) solvent system. Chemical Formula: $C_{20}H_{19}N_3OS_2$. FTIR (KBr, cm^{-1}) *v*max: 2960 (=C-H sp^2 stretching), 2866 (-C-H sp^3 stretching), 1735 (C=O stretching), 1602(C=N stretching), 1514 (C=C aromatic stretching), 1410 (C=S stretching), 1103 (C-N stretching), 827 (C-S stretching). ^{1}H-NMR (300 MHz, DMSO-*d6*) δ ppm: 1.28 (s, 9H, 3×C**H**$_3$), 7.14 (s, 1H, **H-α**), 7.42 (d, 2H, **H-3', 5'**), 7.51 (d, 2H, **H-2', 6'**), 7.71 (d, 2H, **H-2**, **6)**, 8.03 (s, 1H, C**H**=N), 8.61 (d, 2H, **H-3**, **5**). ^{13}C-NMR (75 MHz, DMSO-*d6*) 31.2 (3×**C**H_3), 35.2 (**C** $(CH_3)_3$), 115.1 (-**C**- thioxothiazolidin ring), 123.2 (C-**2, 6**), 125.8 (**C-3', 5'**), 129.6 (**C-2'**, **6'**), 129.9 (**C-1'**), 142.7 (**C-α**), 147.5 (**C-1**), 150.3 (**C-3, 5**), 150.8 (**C-4'**), 158.8 (**C=N**), 169.4 (-**C=O**), 193.1 (**C=S**).

5-(4-nitrobenzylidene)-3-((*E*)-(pyridin-4-ylmethylene) amino)-2-thioxothiazolidin-4-one (2a2).



The product was obtained as dark brown solid (300 mg, 81% yield) m.p:100°C102°C, R_f: 0.75 in hexane: ethyl acetate (3:1) solvent system. Chemical Formula: $C_{16}H_{10}N_4O_3S_2$. FTIR (KBr, cm^{-1}) *v*max: 2928 (=C-H sp^2 stretching), 1735 (C=O stretching), 1595 (C=N stretching), 1344 (C=S stretching), 1097 (C-N stretching), 825 (C-S stretching). ^{1}H-NMR (300 MHz, DMSO-*d6*) δ ppm: 7.16 (s, 1H, **H-α**), 8.22 (d, 2H, **H-2**, **6**), 8.27 (d, 2H, **H-2', 6'),** 8.34 (d, 2H, **H-3', 5'**), 8.37 (d, 2H, **H-3, 5**), 8.43 (s, 1H, C**H**=N). ^{13}C-NMR (75MHz, DMSO-*d6*): 120.8 (**C**= thioxothiazolidin ring), 124.3 (**C-2**, **6**), 124.6 (**C-3'**, **5'**), 129.6 (**C-2'**, **6'**), 139.6 (**C-1'**), 142.5 (**C-α**), 144.8 (**C-1**), 145.9 (**C-4'**), 148.0 (**C-3, 5**), 157.2 (**C**=N), 169.2 (**C**=O), 197.5 (**C=S**).

***N*-(4-(3-((*E*)-(furan-2-ylmethylene) amino)-4-oxo-2-thioxothiazolidin-5-ylidene) methyl) phenyl) acetamide (2b1)**



The product was obtained as dark brown solid (300 mg, 78% yield), m.p: 85°C-87°C, R_f: 0.3 in hexane: ethyl acetate (3:1) solvent system. Chemical Formula: $C_{17}H_{13}N_3O_3S_2$. FTIR (KBr, cm^{-1}) *v*max: 2929 (=C-H sp^2 stretching), 2956 (-C-H sp^3 stretching), 1728 (C=O stretching), 1678(N-C=O stretching), 1597 (C=N stretching), 1519(C=C aromatic stretching), 1410 (C=S stretching), 1253(C-O stretching), 1093 (C-N stretching), 833 (C-S stretching). ^{1}H-NMR (300 MHz, DMSO-*d6*) δ ppm: 2.06 (s, 3H, CH_3), 5.93 (s, 1H, N-H), 6.62 (dd, 1H, **H-4**), 6.98 (d, 1H, **H-5**), 7.58 (d, 2H, **H-2',6'**), 7.63 (s,1H, **H-α**), 7.73 (d, 2H, **H-3',5'),** 7.81 (s,1H, N=**CH**), 7.98 (d,1H, **H-3**). ^{13}C-NMR (75 MHz, DMSO-*d6*): 24.5 (CH3), 112 .7 (**C-4**), 114.5 (**C**-thioxothiazolidine ring), 117.4 (**C-5**), 119.1 (**C-3', 5'**), 128.4 (**C-2', 6'**), 129.2 (**C-1'**), 132.9 (**C**=N), 139.9 (**C-4'**), 142.5 (**C-α**), 143.5 (**C-3**), 149.8 (**C-1**), 169.6 (**C=O**, acetamid), 168.9 (C=O), 188.9 (**C**=S).

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1.2 Molecular docking studies

Determining the binding energies of the new compounds to the protein (EGFR) with the (GOLD) (v.5.7.1) Suite is the final result of the docking investigation [29,30]. The compounds' 2D structures were drawn, then their 3D structures were established, energy was computed and reduced using MM2 minimize and MM2 dynamic options. EGFR (PDB ID: 4HJO) protein was downloaded from Protein Data Bank receptor. Research Collaboratory for Structural Bioinformatics (RCSB) was used for protein preparation and binding site and prediction X-ray crystal structure of receptor EGFR (PDB ID: 4HJO). The protein binding site was defined as being within (10 A°) of the reference ligand, with the removal of all ligands and water molecules that were not involved in the binding location. Structures were chosen for their high resolution and high percentile assessments. Moreover, the structures have a ligand which can be used for docking validation test in the catalytic active site. The receptors were compiled before the docking process by adding polar hydrogen atoms to achieve specific ionization and tautomeric positions of amino acid residues. plp fitness scores have been determined to indicate that anticipated binding sites demonstrated catalytic active site features and the anti-tumor activity of these drugs with the reference medication Erlotinib.

1.3 Biochemical studies

The synthesized chemical compounds were evaluated *in vitro* for their potential activity to suppress tumor cell growth in the A549 lung cancer cell line and for their safety by assessing their cytotoxicity on human normal cell HdFn. MTT (3-(dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay was used to determine the cytotoxic effects caused by various concentrations (25, 50, 100, 200, and 400 µg/mL) of new compounds. By measuring at a 575 nm wavelength with an ELISA reader, for each cell line, the optical density data was statistically analyzed to determine the concentration of chemicals needed to decline cell viability by 50%.

2. Results and discussion

We started with (3-amino-2-thioxothiazolidin-4-one) and aldehydes. Room temperature, using methanol as a solvent and glacial acetic acid as a catalyst were the perfect parameters for the reaction to obtain **1a** while yield of **1b** compound obtained without using catalyst, the reaction was regio-selective at position 3 of 3-aminorhodanine which involve Schiff base formation [28].

Chemical spectral analyses for the structures of the final compounds using FTIR, ^{1}H-NMR and ^{13}C-NMR done to prove the formation of the new synthesized compounds. Compounds **1a** and **1b's** FTIR spectra revealed different absorption bands at 1721 and 1737 cm^{-1}, respectively, owing to the C=O group of the thioxothiazolidin ring. The C=N stretching bands have been found at 1605 cm^{-1} for compound **1a** and 1614 cm^{-1} for compound **1b**. Another bands at 1418 cm^{-1} for **1a** while 1545 and 1467 cm^{-1} for **1b**, were ascribed to C=C stretching aromatic. Furthermore, for **1a** and **1b** compounds, the C=S group occurred at 1104 and 1084 cm^{-1}, respectively.

The ^{1}H-NMR spectra revealed a proton resonated as a singlet at 7.83 ppm for **1a** and 8.58 ppm for **1b** compound for azomethine group (CH=N), confirming the production of the imine group. Most significant singlets at 4.24 and 4.37 ppm for compounds **1a** and **1b,** respectively, matches to the 2H proton at C5 of the thioxothiazolidine ring which explains the reaction at the 3-amino position of the 3-aminothioxothiazolidine core and the production of Schiff bases. Compound doublets signals were detected in **1a** at 7.59 ppm for H-2, 6 and 8.75 ppm for H-3, 5 of the pyridine ring. The ^{1}H-NMR spectra of **1b** also revealed furan ring

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signals as doublates of doublat for H-4, doublat for H-5, and doublat for H-3 at 6.81, 7.39, and 8.10 ppm, respectively.

The ^{13}C-NMR analysis identified Schiff base carbon which appeared at 158.2 and 147.5 ppm for **1a** and **1b** compounds, respectively. The thioxothiazolidine ring **C5** resonated at 33.7 ppm in **1a** and 35.6 ppm in **1b**, and the carbonyl groups appeared at 164.0 and 170.1 ppm for **1a** and **1b**, respectively.

FTIR spectral analysis for compound **2a1** express of bands at regions 2960, 1735, 1602 and 1410 cm^{-1} for (=C-H sp^2 stretching), (C=O), (C=N) and (C=S), as shown in **Figure (2)**. Also, compound **2a2** FTIR spectrum resulted bands at 2928, 1735, 1595 and 1344 cm^{-1} area for the same chemical bonds, while compound **2b1** show many distinctive bands for many chemical bonds appeared at regions 2929, 1728, 1678, 1597, 1519, 1410 and 1253 cm^{-1} for (=C-H sp^2 stretching), (C=O), (N-C=O), (C=N), (C=C aromatic), (C=S) and (C-O) stretching bonds, respectively.

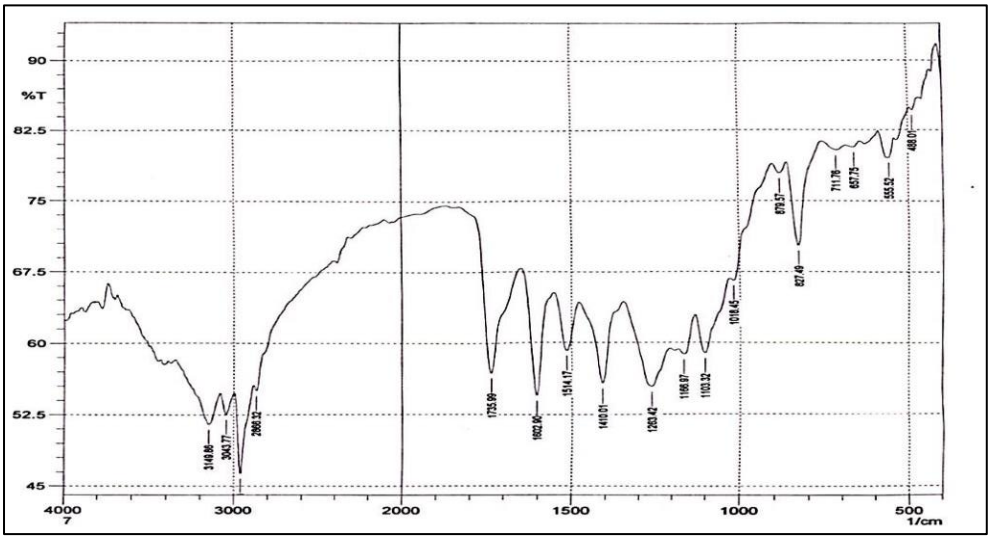

Figure (2): Compound **(2a1)** FT-IR spectra.

The ^{1}H-NMR spectrum signifies the most important peaks of a protons resonated as singlet in 7.14 and 8.03 ppm in **2a1** which refer to α-hydrogen and CH=N imine group formation. Also, the ^{1}H-NMR of **2a2** show these peaks for the same groups in 7.16 and 8.43 ppm, while in compound **2b1** 7.63 ppm represent α-hydrogen and 7.81 belong to Schiff base. These two peaks in the new three compounds confirm the formation of them.

The same derivatives ^{13}C-NMR spectra showed signals at 142.7 and 158.8 ppm for C-α and imine group, respectively, for **2a1** compound, while these two peaks appear at 142.5 and 157.2 ppm in **2a2** derivative. Also, 142.5 and 132.9 ppm in ^{13}C-NMR spectra related to C-α and C=N, respectively, in **2b1** compound. **Figure (3)** and **Figure (4)** show the ^{1}H-NMR and ^{13}C-NMR of compound **2a1.**

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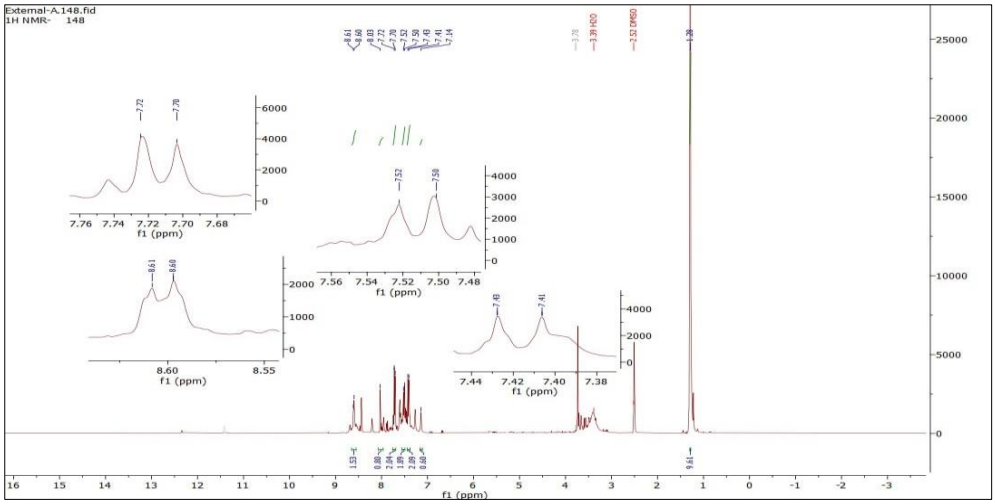

Figure (3): ^{1}H-NMR spectrum of **(2a1)** compound.

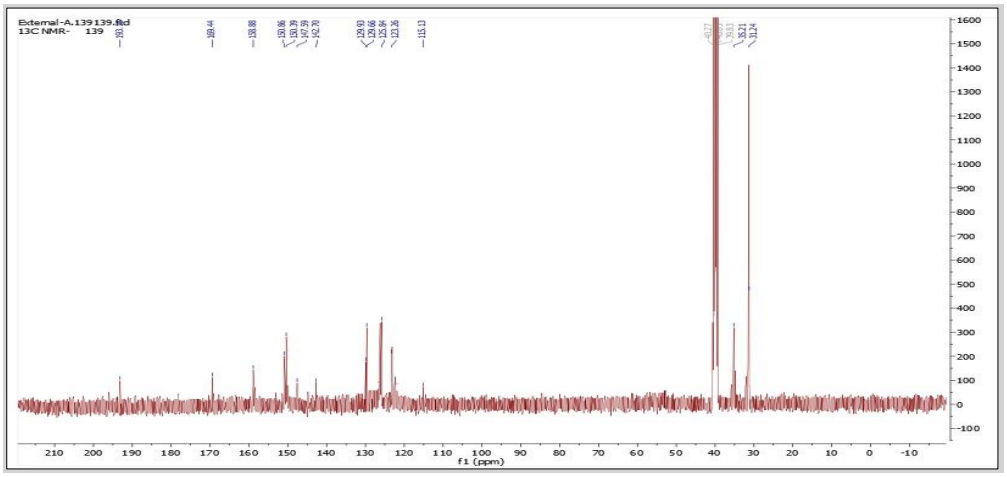

Figure (4): ^{13}C-NMR spectrum of (**2a1)** compound.

We employed a human EGFR homology model to display that compounds **2a1-2** and **2b1** efficiently interact with it and illustrate anticancer activity before looking into the inhibitory actions of these compounds in a greater extent. In order to do this, we used GOLD (v. 5.6.2) Suite to build a human EGFR homology model using the human EGFR-related protein (PDB ID: 4HJO). **Figure (5)** illustrates the binding interactions of Erlotinib and newly generated substances with the receptor.

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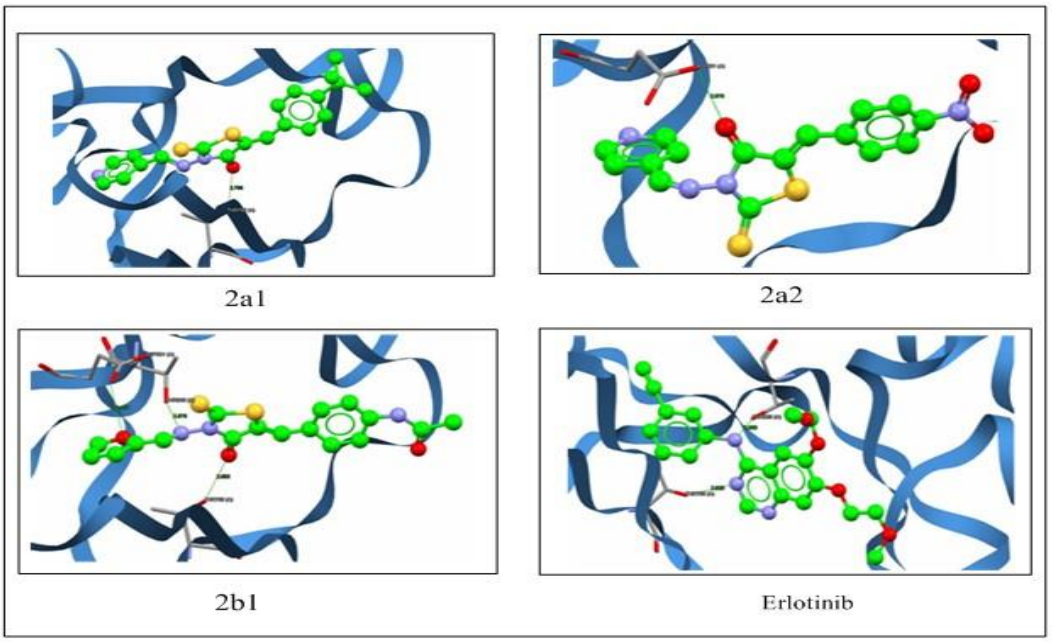

Figure (5): docking investigations using the GOLD Suite on compounds 2a1–2, 2b1, and Erlotinib as a reference. Docking simulations were performed using the EGFR (PDB ID: 4HJO). Erlotinib, 2a1-2 and 2b1 pharmacophore data are shown in 3D form.

The plp fitness (Binding Energy) measured for **2a1** and **2a2** and they were about 69.47 and 69.03 Kcal/mol, respectively, these values are higher than those of standard Erlotinib, which have 8.78 Kcal/moL plp fitness, as shown in **table (1)**. Molecular docking results have demonstrated a strong binding of **2a1** and **2a2** ligands with THR766 and ASP831. Indeed, the **2b1** molecule's affinity towards the EGFR which bind to THR830 amino acid is about 68.88 Kcal/mol with one hydrogen bond link **table (1).** So, the results obtained suggested that the aminorhodanine scaffold was tolerable in the highly active regions of (PDB ID: 4HJO), EGFR-related proteins.

Table (1) Docking estimates and binding energies of conjugated Erlotinib (anticancer drugs) and identified rhodanine derivatives to EGFR active site.

Compounds	plp Fitness (BindingEnergy) Kcal/mol	No. of the H- bindings are included.	Amino Acids included	No. of bonding	Power of bonding
Erlotinib	68.78	2	THR766	1	2.92
			THR830	1	3.06
2a1	69.47	1	THR766	1	2.708
2a2	69.03	1	ASP831	1	2.978
2b1	68.88	1	THR830	1	2.947

The epidermal growth factor receptor (EGFR) is known to be essential in the development of non-small lung cancer (NSLC) and lung cancer, based on clinical research. In order diminish the consequences of metastatic spread and cell proliferation, so suppression of this would be beneficial. When compared to the control and clinically used drug Erlotinib (antitumor), which is used in our study as a

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reference standard, the new **2b1** compound with an IC_{50} value of 55.8µg/mL was found. Additionally, the results of the cytotoxicity of new drugs **2a1**, **2a2** and **2b1** on HdFn humon normal cell, showed 155.6, 95.9 and 110.4 µg/mL respectively, as shown in **Table (3)** and **Figure (6).** So, our new synthesized compounds have no effect or with high safety on normal cells in comparing with the standard Erlotinib which was showed 74.23µg/mL cytotoxic effects on human normal cell. Finally, about becoming drug candidates with acceptable near values as antitumor drugs and with better safety in comparison to Erlotinib as a conventional clinically utilized antitumor treatment, it can be stated that the new **2b1** molecule has produced acceptable outcomes.

Table (2): Compounds (2a1-2) and (2b1) cytotoxicity on the lung cancer cell line (A549) after 72 hours of incubation at 37 ℃.

Compound	cytotoxicity of compounds after incubation for 72 hours at 37 ℃				
	25 (µg/mL)	**50 (µg/mL)**	**100 (µg/mL)**	**200 (µg/mL)**	**400 (µg/mL)**
2a1	79.32±2.2	65.20±0.9	44.36±2.26	30.63±1.38	22.33±2.58
2a2	64.85±2.54	56.7±2.47	44.79±2.8	36.26±1.44	22.53±2.14
2b1	55.67±4.46	43.63±1.2	33.37±1.8	24.46±1.8	15.66±2.31
Erlotinib	57.40±1.10	43.98±2.19	33.48±1.51	21.18±1.2	20.29±2.2

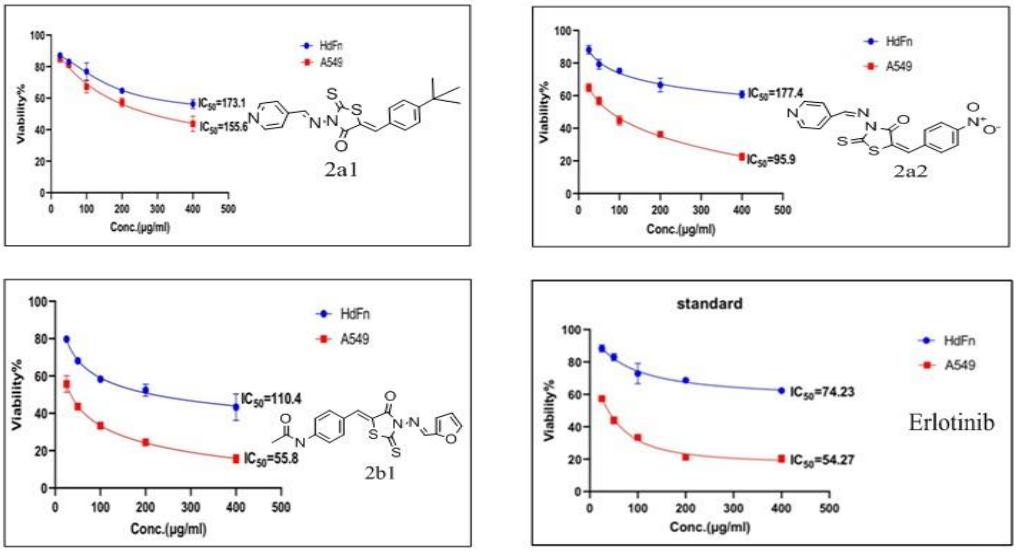

Figure (6): analysis of IC_{50} graphs for highly efficient A549 lung cancer inhibition and cytotoxicity of drug on HdFn human normal cell.

3. Conclusion

A new series of minorhodanine derivatives were designed and synthesized in a two-step pathway, beginning with a condensation reaction between 3-aminorhodanine and various aldehydes, the resulting compounds were then treated to a further Knoevenagel condensation at position 5 with a various benzaldehyde and ending with an exocyclic double bond formation. These new compounds have

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been investigated for cytotoxicity in vitro against A549 lung cancer cell lines.
Compound **2b1**with a good cytotoxic activity against cancer cell line, which express IC_{50} values near IC_{50} ranges of the clinically used anticancer drug Erlotinib. Molecular docking studies of the all newly synthesized compound have showed a binding with EGFR protein in different strong. Importantly, all synthesized compounds have no effect and with high safety on human normal cells. These results suggest that aminorhodanine scaffold offers a template for the design of antitumor drugs.

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