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Design and Synthesis of Novel Bis Thiazolo[4,5-*c*]Isoxazolines Bearing 1,2,4-triazole Ring Derived From the Related 4-thiazolidinons as Antimicrobial Agents

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Abstract

Design and synthesis of novel poly heterocycles together using same heterocyclic compound is the main task of the present paper. The target compounds entitled 4,4'-[benzene-1,4-diylbis[ethylidenehydrazine-2-ylidene]bis[4-[3,5-di(5-substitutedpyridin-2-yl)-3,3a-dihydro[1,3]thiazolo[4,5-c][1,2]oxazol-6(5H)-yl]-4H-3-yl-1,2,4-triazole-3-thiol] have been synthesized starting from the reaction of 1,4-diacetylphenyl and carbohydrazide to give Schiff base derivatives then 1,2,4- triazole derivatives from the reaction with CS_2 and an excess of hydrazine hydrate. The same applies for the condensing of these newly heterocyclic amines with different pyridine-2-carbaldehydes, which resulted in the synthesis of some new Schiff bases, which were then cyclized to develop new thiazolidinones by thioglycolic acid. Additionally, by fusing thiazolidinones with various pyridine-2-carbaldehydes, a novel chalcone was formed. To produce the desired fused isoxazolines, the later compounds underwent cyclization with hydroxylamine hydrochloride and potassium hydroxide. From the examination of the (CHN), FTIR, ^{1}H NMR, and ^{13}C NMR spectra, the structural identities of all recently synthesized compounds were determined. The newly synthesized chemicals were also successfully tested as an antibacterial agent.

Keywords: Thiazolo[4,5-*c*] isoxazoline, 4-Thiazolidinones, Pyridine-2-carbaldehyde, Thioglycolic acid, Carbohydrazide

تصميم وتحضير مشتقات جديدة من ثنائي ثايازولو *[4,5-c]* ايزواوكسازولين التي تحمل حلقة *1,2,4* -ترايازول مشتقة من 4-ثايازوليدينون التابعة لها كعوامل مضادة للمايكروبات

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الخلاصة

تصميم وتحضير حلقات غير متجانسة متعددة جديدة مجتمعة في نفس النظام كان الهدف الرئيسي لهذا البحث. المركبات المنشودة والمعنونة 4,4/-(بنزين-1،4-داي ايل ثنائي (اثيليدين هيدرازين-2-يليدين ثنائي(4(3،5-داي(5- مشتق البيريدين-2-ايل)3-3a- داي هيدرو(3،1)ثايازولو [4,5-c] [2,1] اوكسازول-6-(5H) ايل-(4H) -3-ايل-4،2،1-ترايازول-5-ثايول قد تم تحضيرها ابتداءا من مفاعلة 4،1-داي اسيتايل فنيل مع الكاربوهيدرازايد لينتج قواعد شيف بعدها مشتقات 4،2،1-تريازول بتفاعلها مع CS2

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وزيادة من الهيدرازين هيدريت. بالمقابل، هذه الامينات الحلقية غير المتجانسة الجديدة تم تكثيفها مع مشتقات البيريدين الديهايد المختلفة لتنتج قواعد شيف التي بدورها تم تحولقها بواسطة حامض الثايوكلايكولك لتعطي ثايوزوليدينونات جديدة. واكثر من ذلك، تم الحصول على جالكونات من صهر هذه الثايوزوليدينونات مع مشتقات البيريدين الديهايد. المركبات الاخيرة تم تحولقها بواسطة الهيدروكسيل امين هيدروكلورايد مع هيدروكسيد البوتاسيوم لتنتج الايزواوكسازولينات الملتحمة المستهدفة. التركيب الكيميائي لكل المركبات الجديدة المحضرة تم استنتاجه من خلال التحليل الدقيق للعناصر C.H.N واطياف الاشعة تحت الحمراء FTIR واطياف الرنين النووي المغناطيسي ^{1}H NMR و ^{13}C NMR. بالاظافة الى ذلك، تم فحص المركبات الجديدة كعوامل مضادة للميكروبات وبنتائج ممتازة

1. Introduction

Due to microbial resistance to the majority of existing antimicrobial drugs, the treatment of microbial illnesses is the most exciting subject. A challenging scenario has arisen as a result of antibiotic resistance in microorganisms, necessitating the rapid design and development of novel antibiotics [1]. Nevertheless, the five-membered heterocyclic compounds such as thiazoles and oxazoles are well-known and have a range of biological functions [2,3]. Additionally, the thiazole nucleus is a fundamental component of all penicillin-based medications, which have revolutionized the way bacterial illnesses are treated [4]. Additionally, isoxazolines are a significant class and have been shown to have potent antibacterial [2,3], anti-diabetic [5], anthelmintic [6], diuretic [7,8] properties. Thus, the 1,3-thiazolidien-4-one ring system's chemistry is of importance since it serves as a fundamental component in several synthetic pharmaceuticals and has outstanding value for both their synthetic and biological features [9]. Numerous thiazolidinone compounds have been synthesized and have shown to exhibit exceptional bioactivities, including antibacterial [10], antidiarrhoeal [11], anticancer [12]. Due to their major function in organic and medicinal chemistry, there has been a surge in interest in the synthesis of novel heterocycles including isoxazole/isoxazoline derivatives in recent years. Following a review of the literature, many novel isoxazoline derivatives were synthesized using various techniques. However, novel isoxazoline compounds were produced by 1,3-dipolar cycloaddition of nitrile oxides with dialkyl maleate [13,14]. In contrast, chalcones have been used to produce a number of novel mono- and twin-fused pyrazolone (indazol-3-one) and thioxopyrimidine derivatives [15]. Similarly, hydroxylamine hydrochloride was used to react with chalcone derivatives to produce methylene-bis-tetrahydro[1,3]thiazolo[4,5-c]isoxazoles [10]. In addition, novel isoxazoline compounds have been synthesized by reacting chalcones with hydroxyl amine hydrochloride in the presence of sodium hydroxide as a base [16]. The condensation reaction of N-phenylhydroxylamine with various aldehydes was recently used to produce several novel isoxazolines under various circumstances [17]. Only a few reports of the synthesis of an isoxazoline core fused with a thiazolo moiety were made as a result of these discoveries. Our overall approach in this study is to develop and synthesize novel triazolo[4,5-c]isoxazolines heterocycles and evaluate them for antibacterial properties in order to address this problem.

2. Materials and Methods

Without additional purification, all chemicals and reagents were used as they were acquired from their suppliers. By employing pre-coated metal plates with silica gel, thin layer chromatography was used to track the reaction's progress. On the Electrothermal SMP30 melting point equipment, melting points were recorded (and are uncorrected). The Fison 1108 (CHN) Elemental Analyzer was used to calculate percentages of (CHN) elements. For FTIR spectra, an 8400s FTFTIR-Shimadzu spectrophotometer was used with a (KBr) disc. Bruker spectrometer (300 MHz and 150 MHz, respectively) was used to outline ^{1}H NMR and ^{13}C NMR

spectra in DMSO-d_6 using TMS as internal standard reference. The chemical structures were drawn with CS Chem Draw Ultra (6.0) software.

Preparation of N,N\ -[benzene-1,4-diyldieth-1-yl-1- ylidene] dicarbohydrazide, 2*:*

Ten mL of ethanol, 0.001 mole of 1,4-diacetylphenyl, and 0.002 mole of carbohydrazide were mixed, and the mixture was then refluxed for 2 hours. It was allowed for the mixture to cool at room temperature. The material that had precipitated was forced into crushed ice, filtered, and then crystallized again using DMF solvent [18].

FTIR (cm^{-1}, KBr); 3447–3218 (NH), 3061–2964 (CH),1686 (C=O), 1614 (C=N). ^{1}H NMR (δ ppm, DMSO-d_6); 7.93–7.74 (s, 4H, CONH), 7.38–7.30 (m, 4H, ArH), 2.51 (s, 4H, NH_2), 1.87 (s, 6H, CH_3).

Preparation of 5,5'-[benzene-1,4-diylbis[(eth-1-yl-1-ylidene hydrazin-1-yl-2-yl] bis(4-amino-3-mercapto-4H- 1,2,4-triazole-), 3*:*

A 0.06 mole of CS2 was added to a solution of 0.01 mole of 2 and 0.025 mole of KOH diluted in (15 mL) of ethanol. The mixture was stirred continuously for 24 hours at 25 °C. After that, 30 mL of petroleum ether was added, and the mixture was agitated for three hours. The resulting solid was separated by filtration, cleaned, and dried to produce potassium hydrazine salt (and was not isolated), and it was then heated at 50°C for 6 hours in order to react with the excess hydrazine hydrate. The mixture was then acidified by adding 5 mL of 10% HCl. The solid product was filtered, washed, and crystallized usimg ethyl acetate [17].
FTIR (KBr, cm^{-1}); 3371–3195 (NH), 3080–2880 (CH), 1658 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm); 7.91-7.89 (s, 2H, C-NH-N), 7.47–6.52 (m, 4H, ArH), 4.29 (s, 4H, NH_2), 2.51(s, 2H, ArSH), 1.32 (s, 6H, CH_3).

General procedure for the synthesis of 4,4'-[benzene-1,4-diylbis[eth-1-yl-1-ylidene hydrazin-1-yl-2-ylidene]]bis[5-(4-[(5-substitutedpyridin-2-ylmethylidene)-amino-3-mercapto]-4H-1,2,4-triazole, 4a-4d:

A mixture of 0.005 mole of compound **3** and 0.01 mole of pyridine carbaldehyde was acidified with two drops of GAA in 20 mL of absolute ethanol and refluxed for 4h. After cooling, the solid was filtered, washed with water, dried, and crystallized from ethyl acetate [18].

4a)4,4'-[benzene-1,4-diylbis(ethyl-1-yl-hydrazin-1-yl-2-ylidene)]bis[5-(4-[(pyridinmethyl-idene)-amino-3-mercapto]-4H-1,2,4-triazole: FTIR (KBr, cm^{-1}); 3216 (NH), 3068,2988 (CH), 1642 (C=N), 1592 (C=$C_{arom.}$). ^{1}H NMR (DMSO-d_6, δ ppm); 8.59-7. 00 (m, 2H, CH =N,12H, H_{arom}), 6.55 (s, 2H, NH), 3.75 (s, 2H, ArSH), 1.26 (s, 6H, CH_3). ^{13}C NMR (DMSO-d_6, δ ppm); 156.91 (C-C=N, imine); 138.15 (N-C=N, triazole ring); 131.68 -125. 89 (Ar-Carbons); 22.97 (-CH_3).

4b)4,4'-[benzene-1,4-diylbis[(ethyl-1-ylidene hydrazinyl-2-yl)]bis[5-(4-[(5-methylpyridin-methylidene)-amino-3-mercapto]-4H-1,2,4-triazole-: FTIR (KBr, cm^{-1}); 3236 (NH), 3082,2993 (CH), 1674 (C=N), 1598 (C=C). ^{1}H NMR (DMSO-d_6, δ ppm); 8.74-7. 37 (m, 2H, CH =N 10H, H_{arom}), 6.23 (s, 2H, NH), 3.65 (s, 2H, Ar-SH), 2.84 (s, 6H, Ar-CH_3), 1.18 (s, 6H, CH_3). ^{13}C NMR (DMSO-d_6, δ ppm); 159.24 (C-C=N, imine); 143.10 (N-C=N, triazole ring); 132.88 -122. 14 (Ar-Carbons); 31.06 (ArCH_3); 22.97 (-CH_3).

4c)4,4'-[benzene-1,4-diylbis[ethyl-1-ylidenehydrazin-1-yl-2-ylidene]]bis[5-(4-[(5-methoxypyridinmethylidene)-amino-3-mercapto]-4H-1,2,4-triazole: FTIR (KBr, cm^{-1}); 3310

(NH), 3066,2884 (C-H), 1642 (C=N), 1584 (C=C). ^{1}H NMR (DMSO-d_6, δ ppm); 8.74-7. 37 (m, 2H, CH=N, 10H, H_{arom}), 6.23 (s, 2H, NH), 3.65 (s, 2H, Ar-SH), 3.96 (s, 6H, $ArOCH_3$), 1.18 (s, 6H, CH_3). ^{13}C NMR (DMSO-d_6, δ ppm); 160.28 (C-C=N, imine); 141.24 (N-C=N, triazole ring); 134.17 -123. 20 (Ar-Carbons); 61.5 ($ArOCH_3$); 24.17 (-CH_3).

4d)4,4'-[benzene-1,4-diylbis[ethyl-1-ylidenehydrazin-1-yl-2-ylidene]]bis[5-(4-[(5-chloropyridinmethylidene)-amino-3-mercapto]-4H-1,2,4-triazole: FTIR (KBr, cm^{-1}); 3294 (NH), 3064,2998 (CH), 1670 (C=N), 1594 (C=C). ^{1}H NMR (DMSO-d_6, δ ppm); 8.10-7. 28 (m, 2H, CH=N, 10H, $H_{arom.}$), 5.84 (s, 2H, NH), 3.62 (s, 2H, ArSH), 1.36 (s, 6H, CH_3). ^{13}C NMR (DMSO-d_6, δ ppm); 158.05, 157.44 (C-C=N, imine); 145.10 (N-C=N, triazole ring); 132.09 -113.52 (Ar-Carbons and Ar-C-Cl); 32.73 (-CH_3).

General synthetic procedure for the synthesis of 4,4'-[benzene-1,4-diylbis[ethyl-1-ylidenehydrazin-2-ylidene]bis[5-mercapto-4H-1,2,4-triazol-4-yl]-2-(5-substituted-pyridin-2-yl)-4-thiazolidinone], 5a-d:

Thioglygolic acid, 4a–4d, and a trace amount of anhydrous ZnCl2 were combined with 15 mL of dry benzene, mixed, and heated at 45 °C for 19 hours. After cooling, the mixture's solid component was removed and crystallized from the DMF-H2O solution (1:1).

5a)4,4'-[benzene-1,4-diylbis[ethyl-1-ylidenehydrazin-1-yl-2-ylidene]bis[5-sulfanyl-4H-1,2,4-triazol-4-yl]-2-(pyridin-2-yl)-4-thiazolidinone FTIR (KBr, cm^{-1}); 3334 (NH), 3048,2984 (CH), 1689 (C=O), 1644 (C=N). ^{1}HNMR (DMSO-d_6, δ ppm); 8.06-7.27 (m, 2H, CH=N, 12H, H_{arom}), 5.91(s, 2H, CH thiazolidinone), 5.40 (s, 4H, CH_2 thiazolidinone), 3.47 (s, 2H, Ar-SH), 1.22 (s, 6H, CH_3). ^{13}C NMR (DMSO-d_6, δ ppm); 164.11 (N-C=O); 131.94 (C-C=N, imine); 129.00 -124.38 (Ar-Carbons); 51.56 (CH thiazolidinone); 27.50 (CH_2 thiazolidinone); 18.91 (CH_3).

5b)4,4'-[benzene-1,4-diylbis[ethyl-1-ylidenehydrazin-1-yl-2-ylidene]bis[5-sulfanyl-4H-1,2,4-triazol-4-yl]-2-(5-methylpyridin-2-yl)-4-thiazolidinone]: FTIR (KBr, cm^{-1}); 3362 (NH), 3036,2984 (C-H), 1708 (C=O), 1667 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm); 8.24-7.47 (m, 2H, CH=N, 10H, $H_{arom.}$), 5.85 (s, 2H, CH thiazolidinone), 5.29 (s, 4H, CH_2 thiazolidinone), 3.77 (s, 2H, Ar-SH), 2.94 (s, 6H, Ar-CH_3), 1.02 (s, 6H, CH_3). ^{13}C NMR (DMSO-d_6, δ ppm); 165.25 (N-C=O); 138.94 (C-C=N, imine); 132.08 -126.32 (Ar-Carbons); 57.56 (CH thiazolidinone); 29.87 (CH_2 thiazolidinone); 33.42 (Ar-CH_3); 21.47 (CH_3).

5c)4,4'-[benzene-1,4-diylbis[(ethyl-1-ylidenehydrazin-1-yl-2-ylidene]bis[5-sulfanyl-4H-1,2,4-triazol-4-yl]-2-(5-methoxypyridin-2-yl)-4-thiazolidinone]: FTIR (KBr, cm^{-1}); 3383 (NH), 3072, 2985 (CH), 1694 (C=O), 1642 (C=N). ^{1}H NMR (DMSO-d_6, δ ppm); 8.24-7.47 (m, 2H, CH=N, 10H, $H_{arom.}$), 5.85 (s, 2H, CH thiazolidinone), 5.29 (s, 4H, CH_2 thiazolidinone), 3.74 (s, 2H, ArSH), 3.26 (s, 6H, $ArOCH_3$), 1.07 (s, 6H, CH_3). ^{13}C NMR (DMSO-d_6, δ ppm); 168.48 (N-C=O); 141.04 (C-C=N, imine); 139.14 -126.882 (Ar-Carbons); 69.5 (Ar-OCH_3); 58.21 (CH thiazolidinone); 39.87 (CH_2 thiazolidinone); 20.08 (CH_3).

5d)4,4'-[benzene-1,4-diylbis[ethyl-1-ylidenehydrazin-1-yl-2-ylidene]bis[5-sulfanyl-4H-1,2,4-triazol-4-yl]-2-(5-chloropyridin-2-yl)-4-thiazolidinone]: FTIR (KBr, cm^{-1}); 3295 (NH), 3077,2982 (CH), 1692 (C=O), 1643 (C=N). ^{1}HNMR (DMSO-d_6, δ ppm); 7.98-7.28 (m, 2H, CH=N, 10H, $H_{arom.}$), 6.04 (s, 2H, CH thiazolidinone), 5.26 (s, 4H, CH_2 thiazolidinone), 4.15 (s, 2H, Ar-SH), 1.23 (s, 6H, CH_3). ^{13}C NMR (DMSO-d_6, δ ppm); 169.42 (N-C=O); 140.73 (C-C=N, imine); 138.24 -120.82 (Ar-Carbons and Ar-C-Cl); 58.55 (CH thiazolidinone); 38.24 (CH_2 thiazolidinone); 21.04 (CH_3).

General synthetic procedure for the synthesis of 4,4'-[benzene-1,4-diylbis[ethyl-1-ylidenehydrazin-1-yl-2-ylidene]bis[-3-(5-sulfanyl-4H-1,2,4-triazol-4-yl)-2-(5-substitutedpyridin-2-yl)-5-(pyridin-2-ylmethylidene)-1,3-thiazolidin-4-one], 6a-d:

A mixture of 0.001mole of pyridine-2-carbaldehydes and 0.0005 mole of **5a-5d** was heated for 3h in presence of 0.3 mL of piperidine. The crude product was left to cool at room temperature. Then the solid was filtered-off, washed, and crystallized using acetone.

6a)4,4'-[benzene-1,4-diylbis[ethyl-1-ylidenehydrazin-1-yl-2-ylidene] bis[3-(5-sulfanyl-4H-1,2,4-triazol-4-yl)-2-(pyridin-2-yl)-5-(pyridin-2-ylmethylidene)-1,3-thiazolidin-4-one FTIR (KBr, cm^{-1}); 3323 (NH), 3053,2884 (C-H), 1704 (C=O), 1664 (C=N), 1598 (C=C). ^{1}H NMR (DMSO-d_6, δ ppm); 8.88-7.61 (m, 2H, NH and 20H, ArH), 6.88 (s, 2H, C=CH Chalcone), 5.62 (s, 2H, CH thiazolidinone), 4.98 (s, 2H, ArSH), 1.25 (s, 6H, CH_3). ^{13}C NMR (DMSO-d_6, δ ppm); 166.30 (N-C=O); 155.5 (C-C=N, imine); 133.13 (C=C Chalcone); 129.92 -126.26 (Ar-Carbons); 76.78 (CH thiazolidinone); 23.50 (CH_3).

6b)4,4'-[benzene-1,4-diylbis[ethyl-1-ylidenehydrazin-1-yl-2-ylidene]bis[3-(5-sulfanyl-4H-1,2,4-triazol-4-yl)-2-(5-methylpyridin-2-yl)-5-(pyridin-2-ylmethylidene)-1,3-thiazolidin-4-one]: FTIR (KBr, cm^{-1}); 3320 (NH), 3061,2899 (CH), 1689 (C=O), 1645 (C=N), 1592 (C=C). ^{1}H NMR (DMSO-d_6, δ ppm); 7.92-7.24 (m, 2H, NH and 18H, ArH), 6.82(s, 2H, C=CH Chalcone), 5.77 (s, 2H, CH thiazolidinone), 4.38 (s, 2H, Ar-SH), 3.12 (s, 6H, Ar-CH_3), 1.05 (s, 6H, CH_3). ^{13}C NMR (DMSO-d_6, δ ppm); 167.72 (N-C=O); 156.55 (C-C=N, imine); 142.03 (C=C Chalcone); 131.12 -124.24 (Ar-Carbons); 71.18 (CH thiazolidinone); 26.27 (Ar-CH3); 23.50 (CH_3).

6c)4,4'-[benzene-1,4-diylbis[ethyl-1-ylidenehydrazin-1-yl-2-ylidene]bis[3-(5-sulfanyl-4H-1,2,4-triazol-4-yl)-2-(5-methoxypyridin-2-yl)-5-(pyridin-2-ylmethylidene)-1,3-thiazolidin-4-one]: FTIR (KBr, cm^{-1}); 3298 (NH), 3072,2992 (C-H), 1710 (C=O), 1664 (C=N), 1590 (C=C). ^{1}H NMR (DMSO-d_6, δ ppm); 9.12-7.57 (m, 2H, NH and 18H, ArH), 6.39(s, 2H, C=CH Chalcone), 5.16 (s, 2H, CH thiazolidinone), 4.16 (s, 2H, ArSH), 3.32 (s, 6H, ArOCH_3), 1.35 (s, 6H, CH_3). ^{13}C NMR (DMSO-d_6, δ ppm); 167.78 (N-C=O); 157.08 (C-C=N, imine); 140.35 (C=C Chalcone); 131.72 -123.08 (Ar-Carbons); 71.20 (CH thiazolidinone); 63.5 (ArOCH_3); 21.44 (CH_3).

6d)4,4'-[benzene-1,4-diylbis[ethyl-1-ylidenehydrazin-1-yl-2-ylidene]bis[3-(5-sulfanyl-4H-1,2,4-triazol-4-yl)-2-(5-chloropyridin-2-yl)-5-(pyridin-2-ylmethylidene)-1,3-thiazolidin-4-one]: FTIR (KBr, cm^{-1}); 3312 (NH), 3058,2898 (CH), 1708 (C=O), 1663 (C=N), 1587 (C=C). ^{1}H NMR (DMSO-d_6, δ ppm); 7.93-7.22 (m, 2H, NH, 18H, ArH), 6.16 (s, 2H, C=CH Chalcone), 5.21 (s, 2H, CH thiazolidinone), 4.37 (s, 2H, Ar-SH), 1.26 (s, 6H, CH_3). ^{13}C NMR (DMSO-d_6, δ ppm); 167.75 (N-C=O); 155.83 (C-C=N, imine); 149.15 (C=C Chalcone); 123.76 -110.59 (Ar-Carbons and Ar-C-Cl); 55.91; (CH thiazolidinone); 29.61 (CH_3).

General procedure for the synthesis of 4,4'-[benzene-1,4-diylbis[ethyl-1-ylidenehydrazin-2-ylidene]bis[4-[3,5-di(5-substitutedpyridin-2-yl)-3,3a-dih-ydro[1,3]thiazolo[4,5-c][1,2]oxazol-6(5H)-yl]-4H-3-yl-3-mercapto-1,2,4-triazole],7a-7d*:*

To a mixture of 0.001mole of **6a-6d** and 0.0025 mole of KOH dissolved in 10 mL of ethanol, 0.002 mole of hydroxylamine hydrochloride was added and heated under reflux for 18h. After cooling, the solid was washed with ice- water, filtered, and crystallized using DMF.

7a)4,4'-[benzene-1,4-diylbis[ethyl-1-ylidenehydrazin-2-ylidene]bis[4-[3,5-di(pyridin-2-yl)-3,3a-dih-ydro[1,3]thiazolo[4,5-c][1,2]oxazol-6(5H)-yl]-4H-3-yl-3-mercapto-1,2,4-triazole]: FTIR (KBr, cm^{-1}); 3224 (NH), 3062,2988 (CH), 1646 (C=N), 1593 (C=$C_{arom.}$), 1267 (C-N), 1046 (C-O). ^{1}HNMR (DMSO-d_6, δ ppm); 7.98-7.15 (m, 2H, NH, 20H, ArH), 5.75 (s, 2H, O-CH, fused oxazole), 4.83 (s, 2H, S-CH, fused thiazole), 4.56 (s, 2H, Ar-SH), 1.25 (s, 6H, CH_3). ^{13}C NMR (DMSO-d_6, δ ppm); 167.33 (O-N=C, oxazole); 155.88 (N-C=N); 121.76 -120.48 (Ar-Carbons); 79.62 (O-C-Ar, oxazole); 76.78 (N-C-S, thiazole); 55.91, 44.05 (S-CH-C, fused thiazole); 29.61 (CH3).

7b)4,4'-[benzene-1,4-diylbis[ethyl-1-ylidenehydrazin-2-ylidene]]bis[4-[3,5-di(5-methylpyridin-2-yl)-3,3a-dih-ydro[1,3]thiazolo[4,5-c][1,2]oxazol-6(5H)-yl]-4H-3-yl-3-mercapto-1,2,4-triazole]: FTIR (KBr, cm^{-1}); 3224 (NH), 3062,2988 (CH), 1646 (C=N), 1593 (C=$C_{arom.}$), 1267 (C-N), 1046 (C-O). ^{1}H NMR (DMSO-d_6, δ ppm); 7.96-7.12 (m, 2H, NH and 18H, ArH), 5.98 (s, 2H, O-CH, fused oxazole), 5.26 (s, 2H, S-CH, fused thiazole), 4.77 (s, 2H, Ar-SH), 3.34 (s, 6H, Ar-CH_3); 1.22 (s, 6H, CH_3). ^{13}C NMR (DMSO-d_6, δ ppm); 166.68 (O-N=C, oxazole); 159.28 (N-C=N); 128.08 -120.18 (Ar-Carbons); 75.68 (O-C-Ar, oxazole); 72.25 (N-C-S, thiazole); 54.94, 44.21 (S-CH-C, fused thiazole); 28.54 (Ar-CH_3); 24.06 (CH_3).

7c)4,4'-[benzene-1,4-diylbis[ethyl-1-ylidenehydrazin-2-ylidene]]bis[4-[3,5-di(5-methoxypyridin-2-yl)-3,3a-dih-ydro[1,3]thiazolo[4,5-c][1,2]oxazol-6(5H)-yl]-4H-3-yl-3-mercapto-1,2,4-triazole]: FTIR (KBr, cm^{-1}); 3305 (NH), 3086,2998 (CH), 1649 (C=N), 1598 (C=$C_{arom.}$), 1260 (C-N), 1035 (C-O).^{1}H NMR (DMSO-d_6, δ ppm); 7.93-7.52 (m, 2H, NH and 18H, ArH), 5.96 (s, 2H, O-CH, fused oxazole), 5.26 (s, 2H, S-CH, fused thiazole), 4.23 (s, 2H, Ar-SH), 3.74 (s, 6H, Ar-OCH_3); 1.18 (s, 6H, CH_3). ^{13}C NMR (DMSO-d_6, δ ppm); 168.62 (O-N=C, oxazole); 161.44 (N-C=N); 129.10 -120.14 (Ar-Carbons); 77.75 (O-C-Ar, oxazole); 72.55 (N-C-S, thiazole); 57.06, 49.76 (S-CH-C, fused thiazole); 31.84 (Ar-OCH_3); 25.08 (CH_3).

7d)4,4'-[benzene-1,4-diylbis[ethyl-1-ylidenehydrazin-2-ylidene]]bis[4-[3,5-di(5-chloropyridin-2-yl)-3,3a-dih-ydro[1,3]thiazolo[4,5-c][1,2]oxazol-6(5H)-yl]-4H-3-yl-3-mercapto-1,2,4-triazole]: FTIR (KBr, cm^{-1}); 3274 (NH), 3065,2898 (CH), 1654 (C=N), 1597 (C=$C_{arom.}$), 1268 (C-N), 1074 (C-O). ^{1}H NMR (DMSO-d_6, δ ppm); 7.62-8.05 (m, 2H, NH and 18H, ArH), 4.49 (s, 2H, O-CH, fused oxazole), 4.35 (s, 2H, S-CH, fused thiazole), 3.73 (s, 2H, Ar-SH), 1.62 (s, 6H, CH_3). ^{13}C NMR (DMSO-d_6, δ ppm); 166.77 (O-N=C, oxazole); 156.23 (N-C=N); 139.87 -126.32 (Ar-Carbons and Ar-C-Cl); 75.65 (O-C-Ar, oxazole); 72.25 (N-C-S, thiazole); 52.27 (S-CH-C, fused thiazole); 30.83 (CH_3).

3. Results and Discussion

3.1. Chemistry

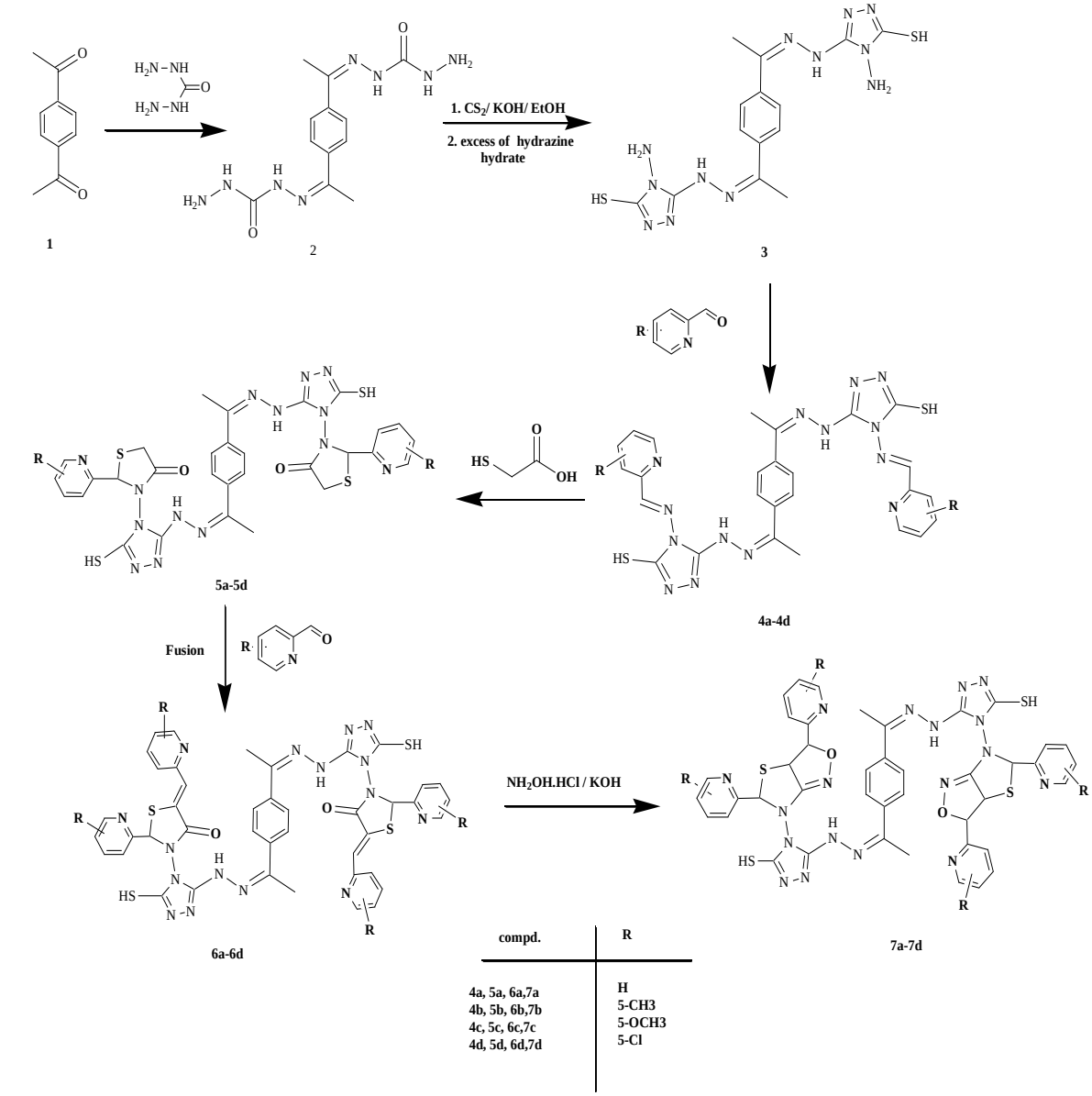
Following the instructions in Scheme 1, a number of new fused heterocyclic compounds of bis thiazolo[4,5-c]isoxazoline derivatives carrying 1,2,4-triazole moieties have been synthesized. First, in an acidic solution, carbohydrazide and 1,4-diacetylphenyl 1 were condensed to produce dicarbohydrazide, 2. FTIR spectrum of 2, Figure 1 showed a new band at 1614 (C=N) stretching.Whereas, ^{1}H NMR reveals a singlet signals at 7.93–7.74 which corresponding to the four protons of (CONH) group, Figure 2. The appropriate salt was then produced by treating 2 with CS2 in alcoholic KOH (and was not isolated), and by hydrazinolyzing 3 with too much hydrazine hydrate, new derivatives of bis (4-amino-4H-3-mercapto-1,2,4-triazole) were formed. The disappearance of carbonyl group vibration in the FTIR spectrum of 3, as well as, a singlet signal due to aromatic-SH at 2.51has been appeared in proton NMR spectrum, Figures 3 and 4. Additionally, new Schiff base derivatives 4a–4d

were produced by the direct condensation reaction of 3 with pyridine-2–carbaldehydes in the presence of glacial acetic acid as a catalyst. A coincidence structural confirmation for compounds 4a-4d were deduced from their spectroscopic data, especially by disappearance of primary amines vibrations as shown in Figures 5-8. These derivatives were then cyclized to produce new 4-thiazolidinone derivatives 5a–5d by reacting with thioglyolic acid under reflux conditions. ^{1}H and ^{13}C NMR Figures 10 and 11 showed a singlet signals at 6.88 ppm and 5.62 corresponding to C=CH Chalcone and CH thiazolidinone, respectively. Moreover, chalcones **6a-6d** were produced by fusion reaction of **5a-5d** with different pyridine-2-carbaldehyde in presence of piperidine as a base. Finally, the cyclization of **6a-6d** with hydroxylamine hydrochloride and KOH in refluxing ethanol gave the target thiazolo[4,5-c]isoxazoline derivatives **7a-7d**. Although the suggested mechanism of the preparation of isoxazolines remains to be clarified [21], nevertheless the proposed mechanism of the target compound could be explained as outlined in Scheme 2. Meanwhile, the intermediates B and C are produced as the initial stage of the Michael addition reaction between the 5-arylidine-4-thiazolidinone derivatives A and hydroxylamine hydrochloride, which is then further rearranged to create the intermediates D and E. Then, intramolecular cyclization and dehydration processes might be used to produce the final thiazolo[4,5-c]isoxazoline derivatives F. From the FTIR spectroscopy data (See experimental section), as well as from the physiochemical and microanalysis of C.H.N elements (Table 1), it has been determined that all compounds have coincidental structural confirmation, Figures 12-15.

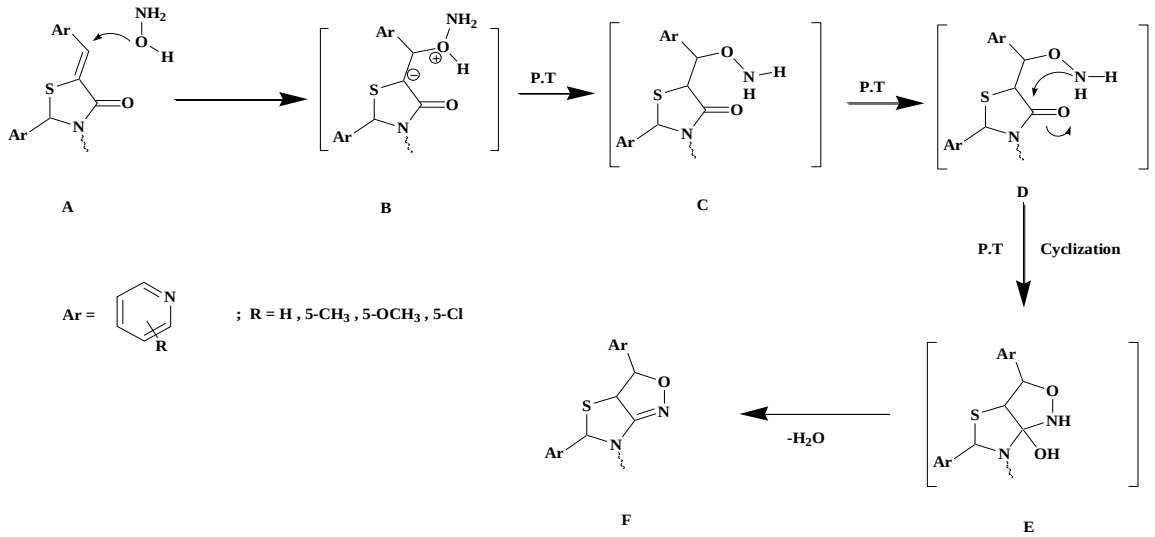
Table 1: Physicochemical and microanalysis properties of the designed compounds

Compd.	R	Yield (%)	mp (°C)	M. wt. (g/mol)	Emperical Formula	Anal. found (calcd.) (%)
2		87	125-126	306.32	$C_{12}H_{18}N_8O_2$	C, 47.08(47.05); H, 5.94 (5.92); N, 36.61 (36.58)
3		71	149–152	418.51	$C_{14}H_{18}N_{12}$	C, 40.22(40.18); H, 4.36(4.34); N, 40.20 (40.16)
4a	H	74	166-168	596.70	$C_{26}H_{24}N_{14}S_2$	C, 53.08(52.33); H, 4.63(4.05); N, 33.06 (32.86)
4b	CH_3	55	187-190	624.75	$C_{28}H_{28}N_{14}S_2$	C, 54.32(53.83); H, 4.88(4.52); N, 31.98 (31.39)
4c	OCH_3	49	157-159	656.75	$C_{28}H_{28}N_{14}O_2S_2$	C, 52.15(51.21); H, 4.84(4.30); N, 30.14 (29.86)
4d	Cl	75	162-164	665.58	$C_{26}H_{22}Cl_2N_{14}S_2$	C, 47.58(46.92); H, 3.72(3.33); N, 30.06 (29.46)
5a	H	61	186-188	744.90	$C_{30}H_{28}N_{14}O_2S_4$	C, 48.92(48.37); H, 4.04(3.79); N, 26.14 (26.32)
5b	CH_3	66	198-201	772.95	$C_{32}H_{32}N_{14}O_2S_4$	C, 50.11(49.72); H, 4.38(4.17); N, 25.11 (25.37)
5c	OCH_3	46	175-179	804.95	$C_{32}H_{32}N_{14}O_4S_4$	C, 47.08(47.75); H, 3.98(4.01); N, 24.15 (24.36)
5d	Cl	73	192-194	813.79	$C_{30}H_{26}Cl_2N_{14}O_2S_4$	C, 45.79(44.28); H, 3.72(3.22); N, 24.85 (24.10)
6a	H	67	187-190	923.09	$C_{42}H_{34}N_{16}O_2S_4$	C, 56.02(54.65); H, 4.28(3.71); N, 23.04 (24.28)
6b	CH_3	59	188-189	979.20	$C_{42}H_{42}N_{16}O_2S_4$	C, 55.26(56.24); H, 3.93(4.32); N, 23.03 (22.89)
6c	OCH_3	55	179-181	1043.19	$C_{46}H_{42}N_{16}O_6S_4$	C, 53.28(52.96); H, 4.29(4.06); N, 21.88 (21.48)
6d	Cl	42	191-193	1060.87	$C_{42}H_{30}Cl_4N_{16}O_2S_4$	C, 47.02(47.55); H, 2.41(2.85); N, 20.86 (21.12)
7a	H	49	199-202	953.12	$C_{42}H_{36}N_{18}O_2S_4$	C, 52.16(52.93); H, 3.44(3.81); N, 23.96 (26.45)

7b	CH_3	51	192-195	1009.23	$C_{46}H_{44}N_{18}O_2S_4$	C, 55.02(54.74); H, 4.71(4.39); N, 25.10 (24.98)
7c	OCH_3	57	204-206	1073.22	$C_{46}H_{44}N_{18}O_6S_4$	C, 52.08(51.48); H, 4.92(4.13); N, 24.16 (23.49)
7d	Cl	68	221-223	1090.90	$C_{42}H_{32}Cl_4N_{18}O_2S_4$	C, 46.48(46.24); H, 3.14(2.96); N, 23.80 (23.11)



Scheme 1**:** General synthetic pathway for designed compounds



Scheme 2**:** The suggested mechanism for the synthesis of thiazolo[4,5-c]isoxazolines

3.2. Antimicrobial Investigation

Antimicrobial activity of **7a-7d** and their related 4-thiazolidinones **5a-5d, 6a-6d** were investigated for their biological properties against *B. subtilis, S. Sciuri* and *E. coli*, *P. aeruginosa* strains. Furthermore, these compounds were examined versus *A. flavus* and *C. albicans* as fungal strains. Diffusion method was applied for this investigation [22]. In order to compare the studied compounds to the standard references of ciprofloxacin and ketoconazole, the examined compounds were dissolved in DMSO solvent to get (100 µg/mL). The plates were incubated for 24 hours at 37 °C for the bacterial culture. While the fungal culture was incubated at 25 °C and checked after 72 hours. Table 2 lists the satisfactory findings that were obtained.

Table 2: Antimicrobial activities of 7a-7d compounds and their 4-thiazolidinones 5a-5d,6a-6d.

Compd.	Inhibition Zone (mm)					
	S. Sciuri	*B. Subtilis*	*E. coli*	*P. Aeruginosa*	*A. Flavus*	*C. Albicans*
5a	10	08	11	14	17	13
5b	13	10	09	12	16	15
5c	11	16	10	14	16	15
5d	15	13	14	12	22	16
6a	12	12	11	12	16	11
6b	12	13	12	11	17	12
6c	14	16	16	15	16	11
6d	16	17	11	13	19	18
7a	17	20	15	18	21	20
7b	19	19	18	18	19	24
7c	20	16	17	17	18	23
7d	22	19	20	19	24	25
Ciprofloxacin	18	18	22	22		
Ketoconazole					20	20

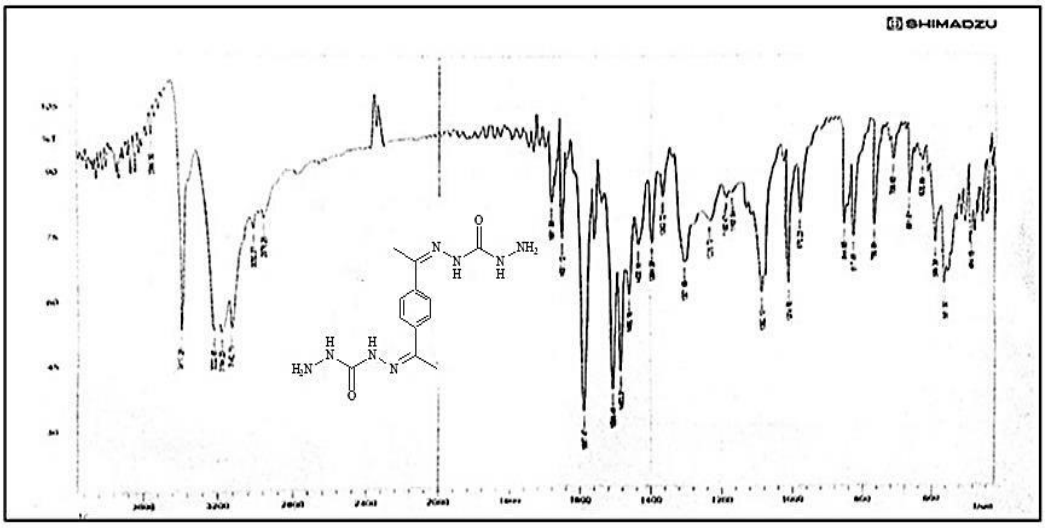


Figure 1: FTIR spectrum of compound 2.

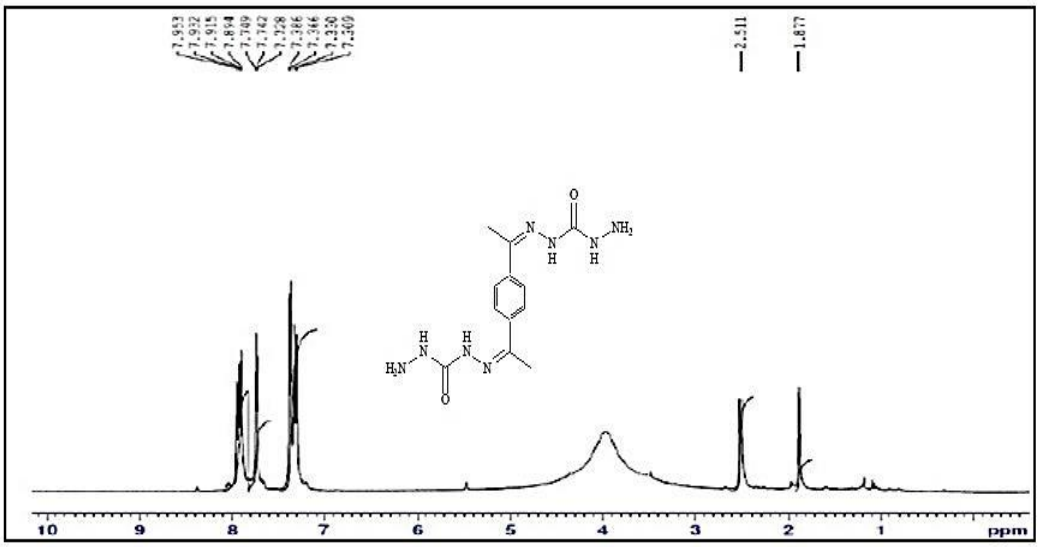


Figure 2: [1]HNMR spectrum of compound 2.

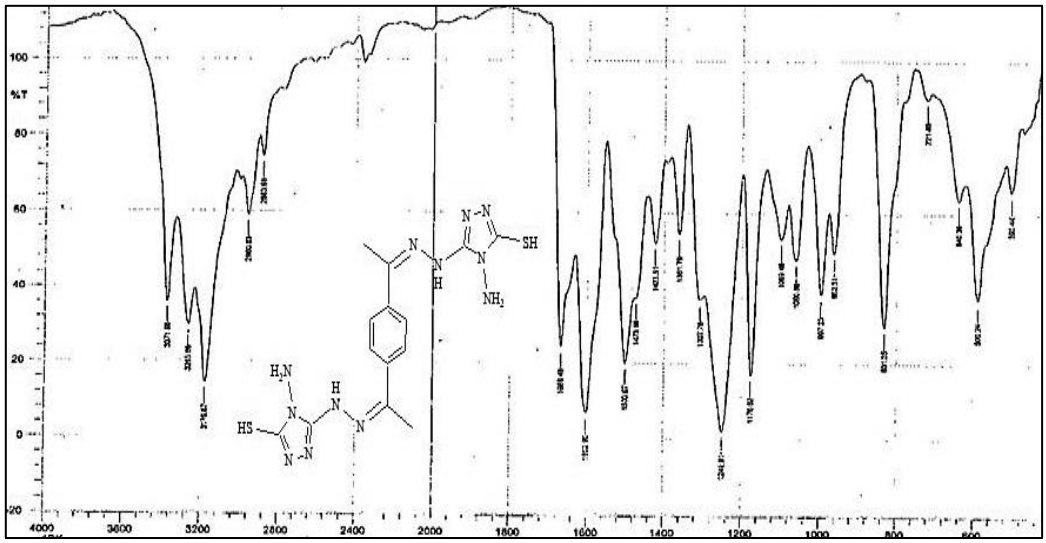


Figure 3: FTIR spectrum of compound 3

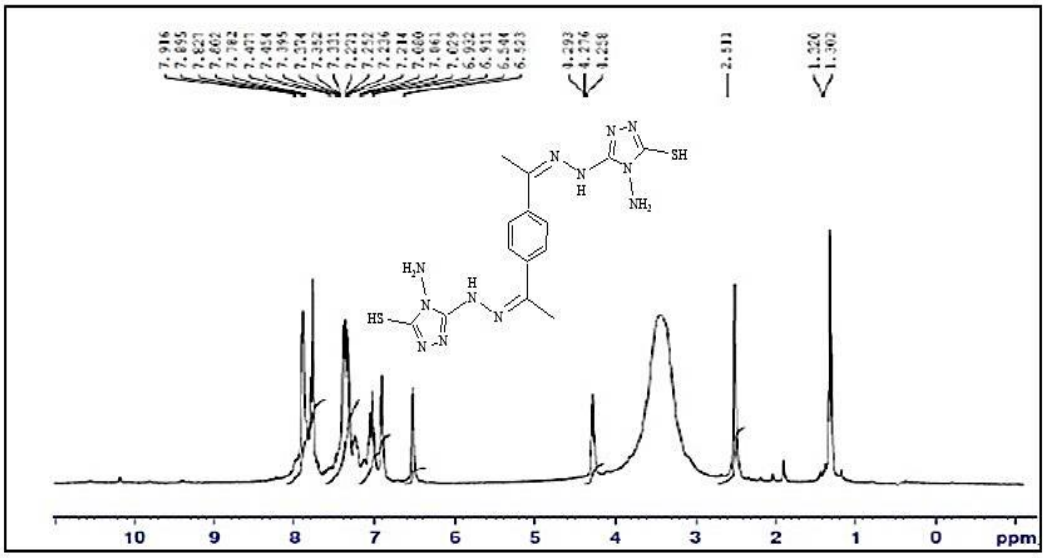


Figure 4: [1]HNMR spectrum of compound 3.

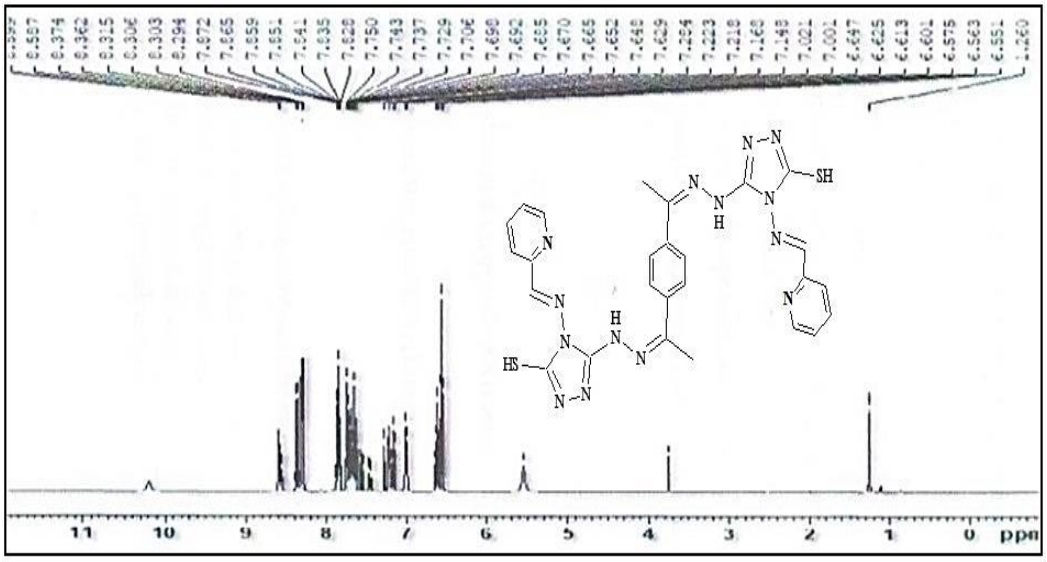


Figure 5: [1]HNMR spectrum of compound 4a

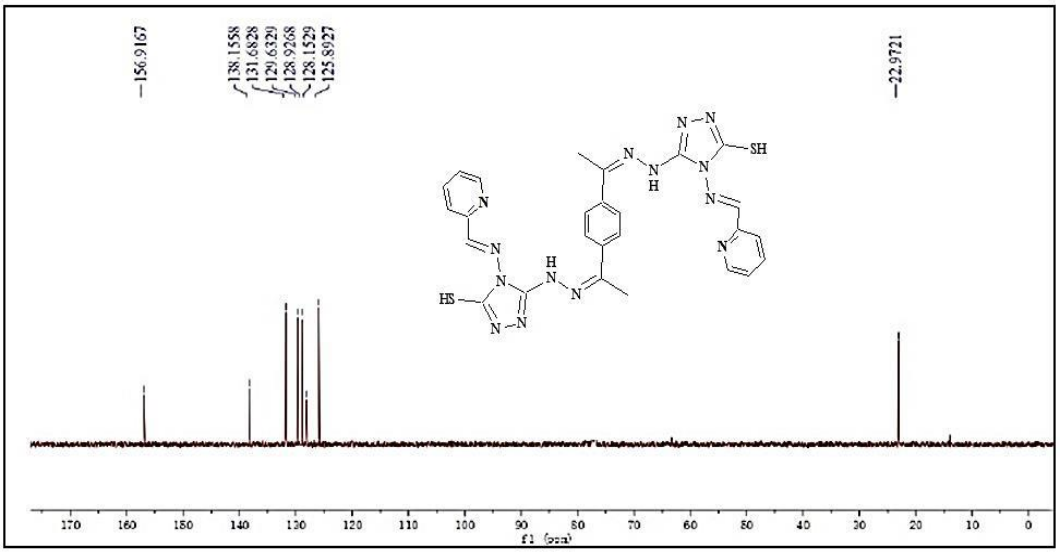


Figure 6: [13]C NMR spectrum of compound 4a

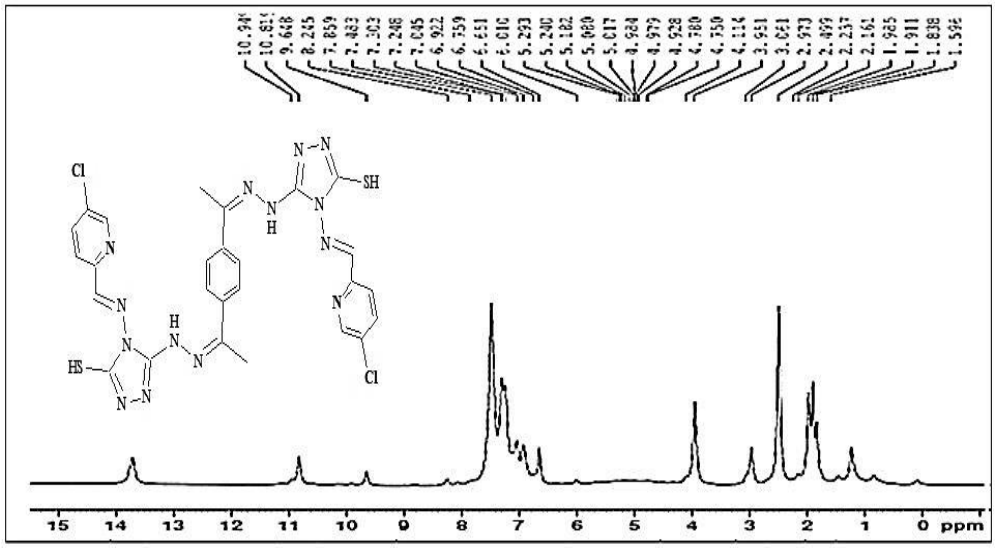


Figure 7: [1]HNMR spectrum of compound 4d

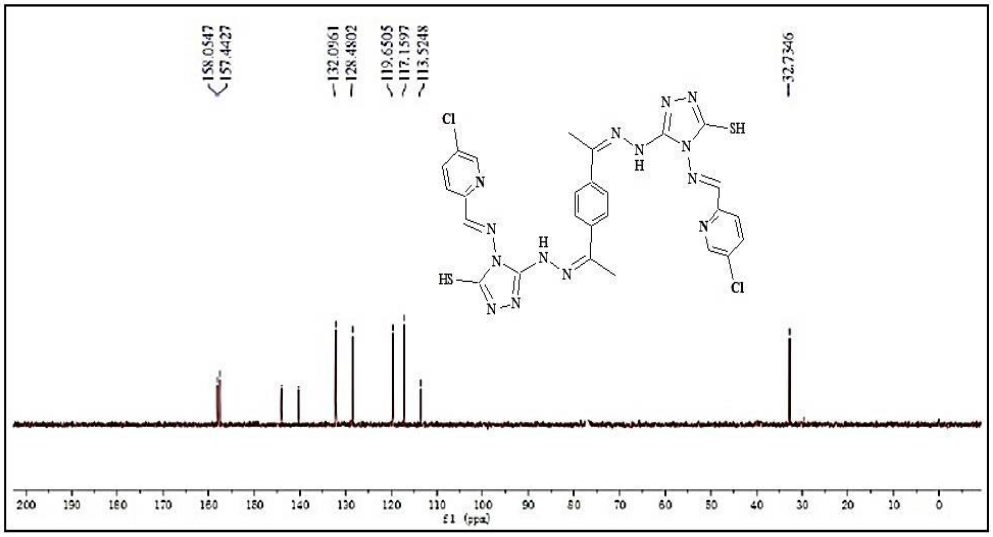


Figure 8: [13]C NMR spectrum of compound 4d

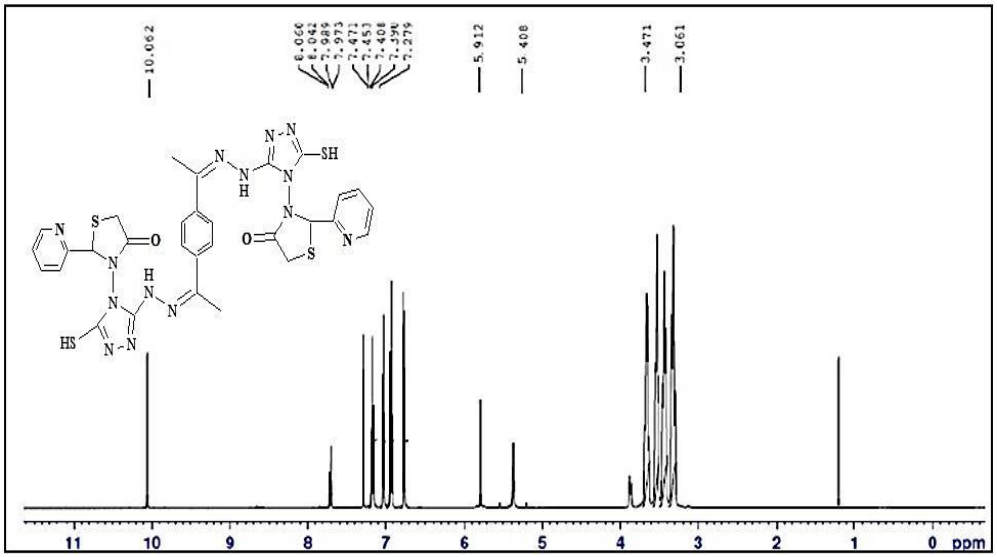


Figure 9: [1]HNMR spectrum of compound 5a

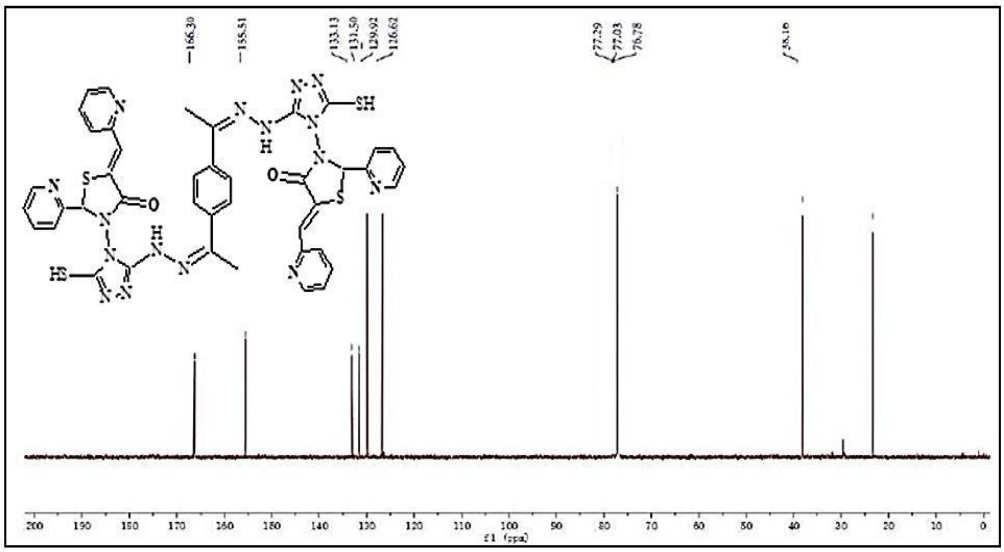


Figure 10: ^{13}C NMR spectrum of compound 6a

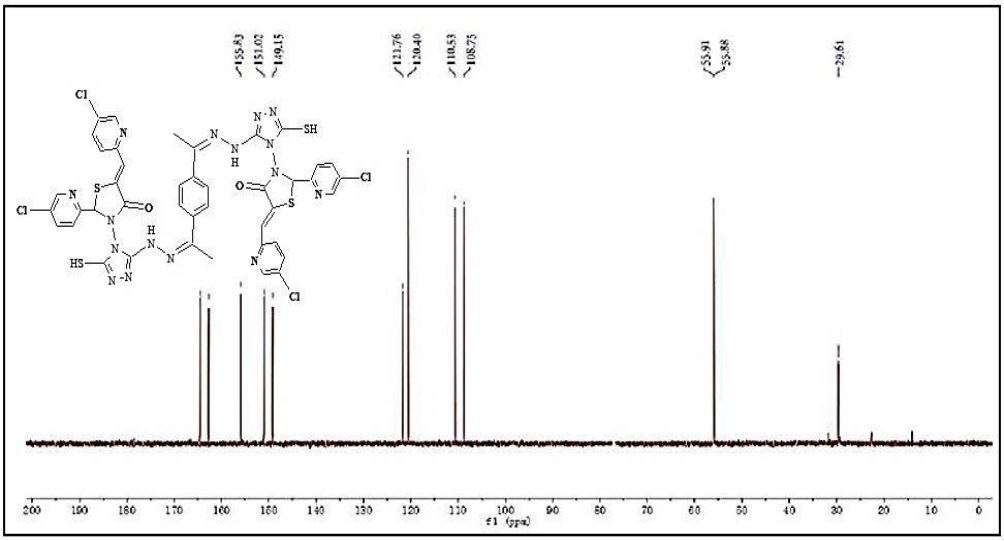


Figure 11: ^{13}C NMR spectrum of compound 6d

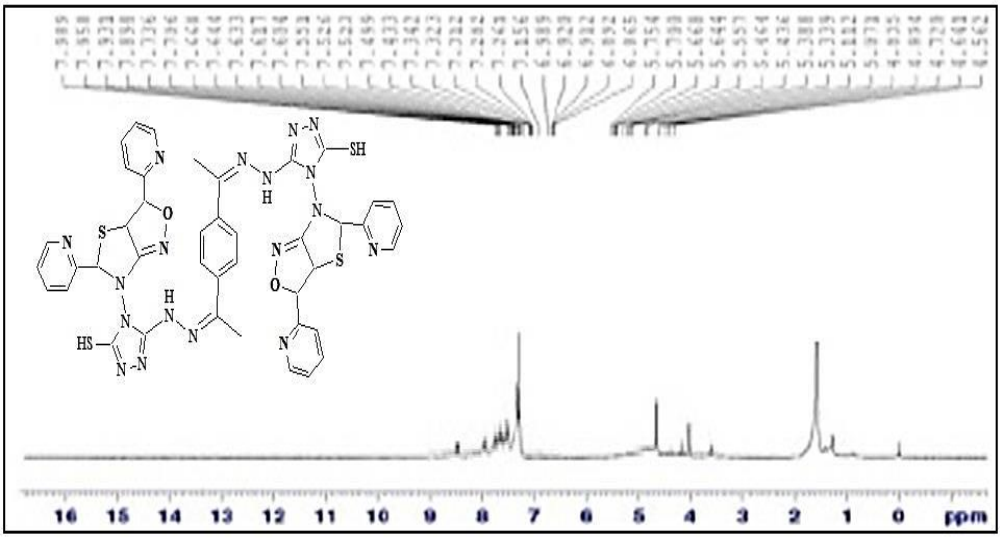


Figure 12: ^{1}HNMR spectrum of compound 7a

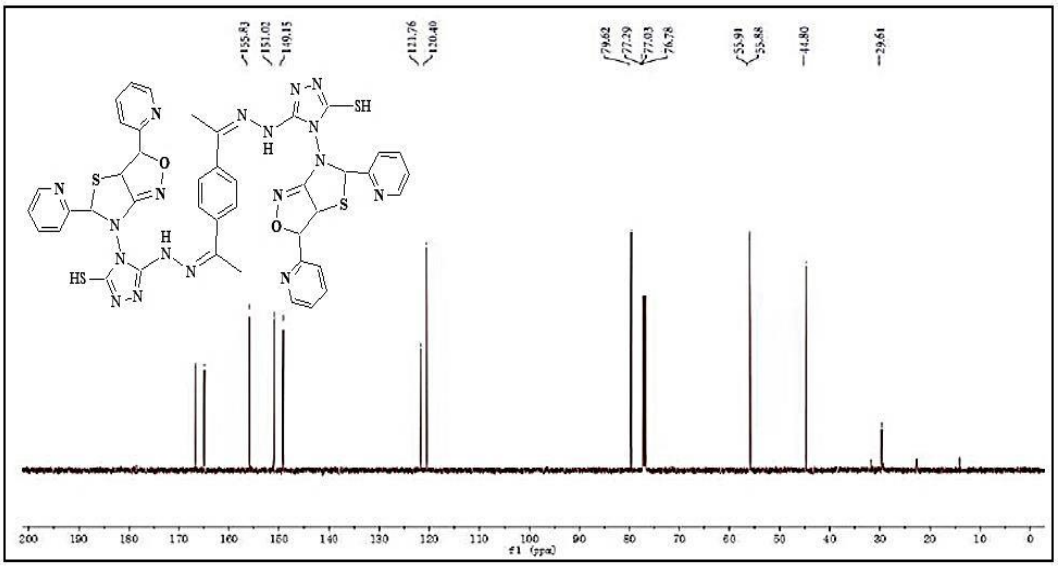


Figure 13: ^{13}C NMR spectrum of compound 7a

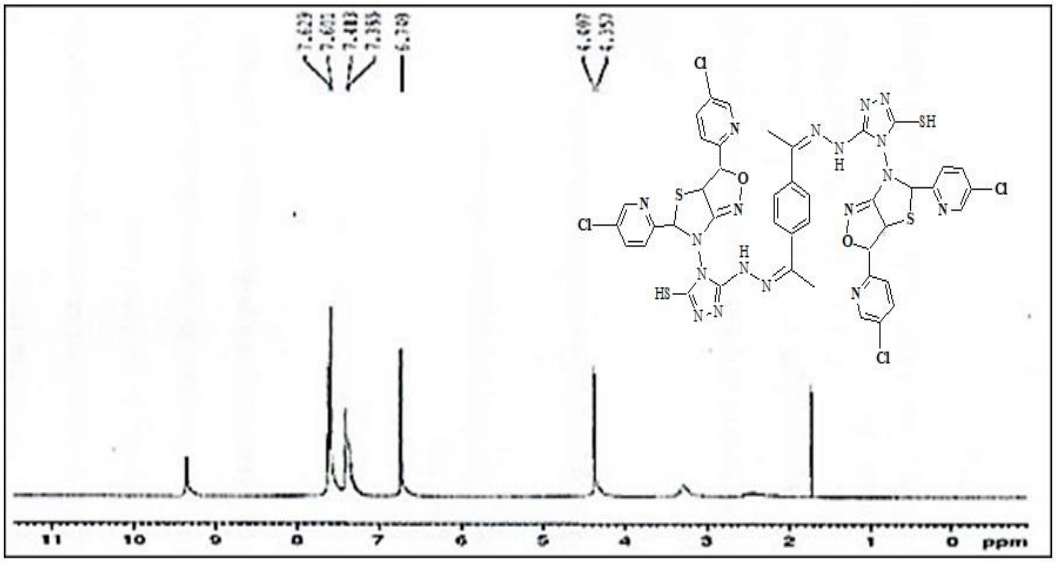


Figure 14: ^{1}HNMR spectrum of compound 7d

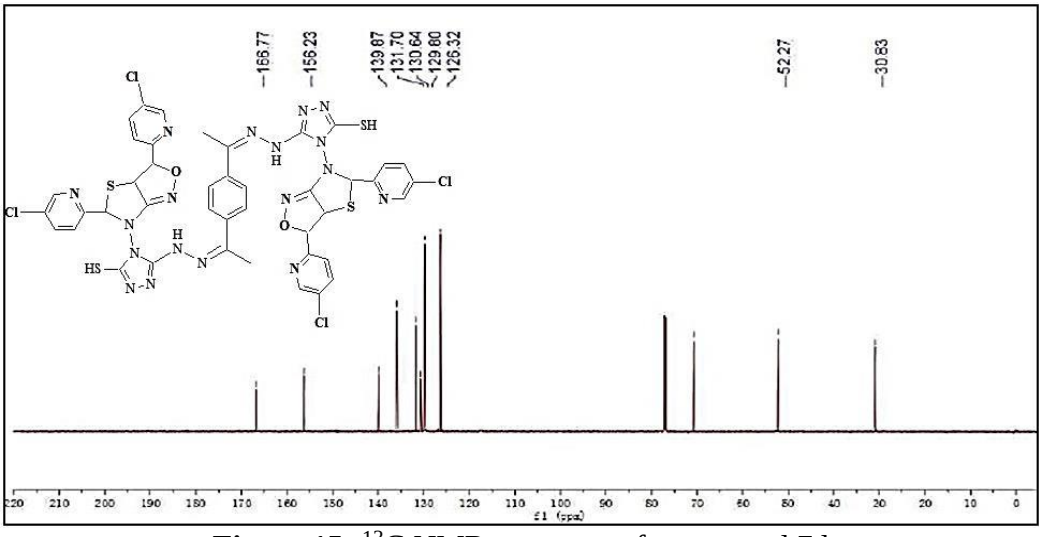


Figure 15: ^{13}C NMR spectrum of compound 7d

Conclusion

As a result, several novel poly heterocycles carrying 1,2,4-triazole moieties have been produced in the same systems of thiozolo[4,5-c]isoxazolines using standard and affordable techniques. These reactions were based on their 4-thiazolidinones. From the physiochemical and spectroscopic data of newly synthesized compounds, the structures of such molecules have been determined. Additionally, the majority of developed compounds show promising outcomes as antibacterial agents. The **7c** and **7d** showed good antibacterial properties among the investigated substances.

5. Disclosure and conflict of interest

The authors declare that they have no conflicts of interest.

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