

Article

Synthesis, Characterization, and Biological Evaluation of Some Novel Pyrazolo[5,1-*b*]thiazole Derivatives as Potential Antimicrobial and Anticancer Agents

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Abstract: The pharmacological activities of thiazole and pyrazole moieties as antimicrobial and anticancer agents have been thoroughly described in many literature reviews. In this study, a convenient synthesis of novel pyrazolo[5,1-*b*]thiazole-based heterocycles was carried out. The synthesized compounds were characterized by IR, ¹H and ¹³C NMR spectroscopy and mass spectrometry. Some selected examples were screened and evaluated for their antimicrobial and anticancer activities and showed promising results. These products could serve as leading compounds in the future design of new drug molecules.

Keywords: pyrazolo[5,1-*b*]thiazole; X-ray crystallography; antibacterial activity; antifungal activity; anticancer activity

1. Introduction

Antibiotics saved millions of lives during the twentieth century by eliminating the deadly threat of infection. In recent years, the overuse of antimicrobial agents has played a significant role in creating more resistant strains of bacteria [1], thus causing an increase in morbidity and mortality [2]. Therefore, safer, cheaper, and more effective antimicrobial agents with a new mode of action are needed [3]. Although cancer is considered the second leading cause of death, taking the lives of 9.6 million people every year [4,5], many cancers are curable if detected early and treated promptly [6]. Chemotherapy is a treatment that uses medications to destroy cancer cells. It typically works by preventing cancer cells from developing, dividing, or proliferating. However, chemotherapy has several disadvantages, one of which is a lack of selectivity leading to extreme side effects and minimal efficacy. Another is the emergence of drug resistance [7]. Therefore, there is an urgent need to design and synthesize potent and highly selective anticancer molecules that offer little-to-zero toxicity to normal cells [8]. Thiazole derivatives demonstrate many pharmacological activities [9–17]. The thiazole ring can be traced in several well-established drugs such as the non-steroidal anti-inflammatory drug meloxicam, the anti-ulcer drug famotidine, the antibacterial sulfathiazole, the antiviral ritonavir, the antiparasitic thiabendazole, and

many anticancer medicines including dasatinib, dabrafenib, and epothilones. Figure 1 shows some of the most effective drugs containing a thiazole ring [18].

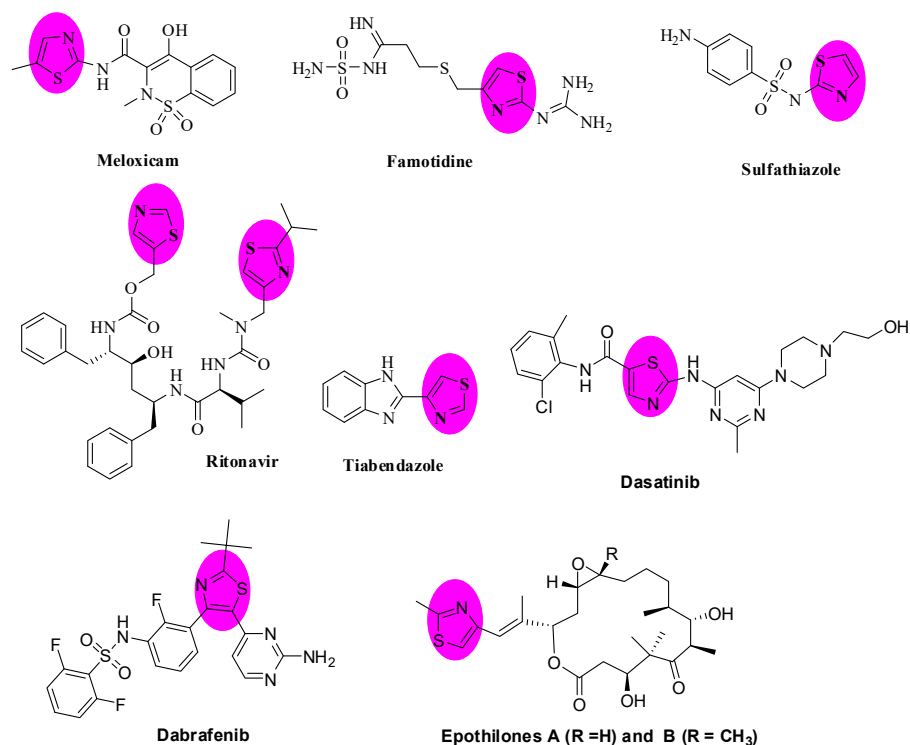


Figure 1. Commercial drugs containing a thiazole ring.

Pyrazole derivatives have been reported as antimicrobial [19], analgesic [20], anti-inflammatory [21], and anticancer agents [22]. Additionally, many pharmaceutical drugs contain the pyrazole moiety, such as the antidepressant fezolamine and the anti-inflammatories celecoxib, mepirizole, and lonazolac. Moreover, the pyrazole derivative pyrazofurin has been reported to have antiviral [23,24] and anticancer activities [24,25]. Figure 2 depicts some of the most potent drugs containing a pyrazole ring.

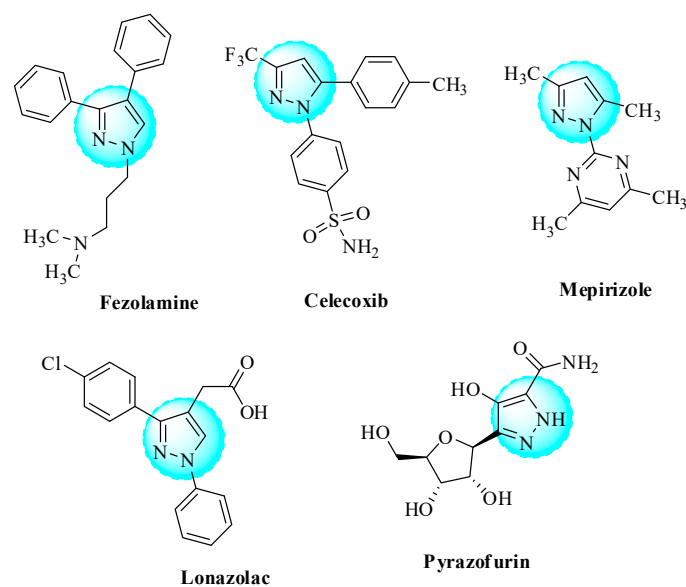


Figure 2. Commercial drug molecules containing pyrazole rings.

Many literature reviews suggest that the pharmacophore hybrids may have enhanced efficacy, fewer drug–drug interactions, and less potential to induce drug resistance [26]. In light of the significance of pyrazoles and thiazoles, numerous studies have been conducted on the synthesis and biological evaluations of new hybrid pharmacophores containing pyrazole and thiazole moieties [27–30].

Figure 3 presents three examples of pharmacologically active pyrazolo[5,1-*b*]thiazole derivatives: pyrazolo[5,1-*b*]thiazole derivative (A) (a protein kinase inhibitor for treating cancer and other diseases) [31], pyrazolothiazole (B) (a potent corticotropin-releasing factor 1 (CRF1) receptor antagonists) [32], and pyrazolo[5,1-*b*]thiazole derivative (C) (possessing a strong suppressant function against the H37Ra strain) [33] (Figure 3).

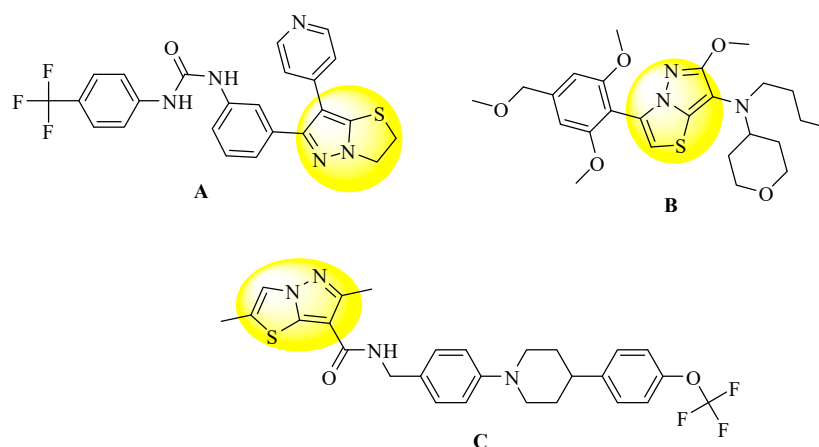


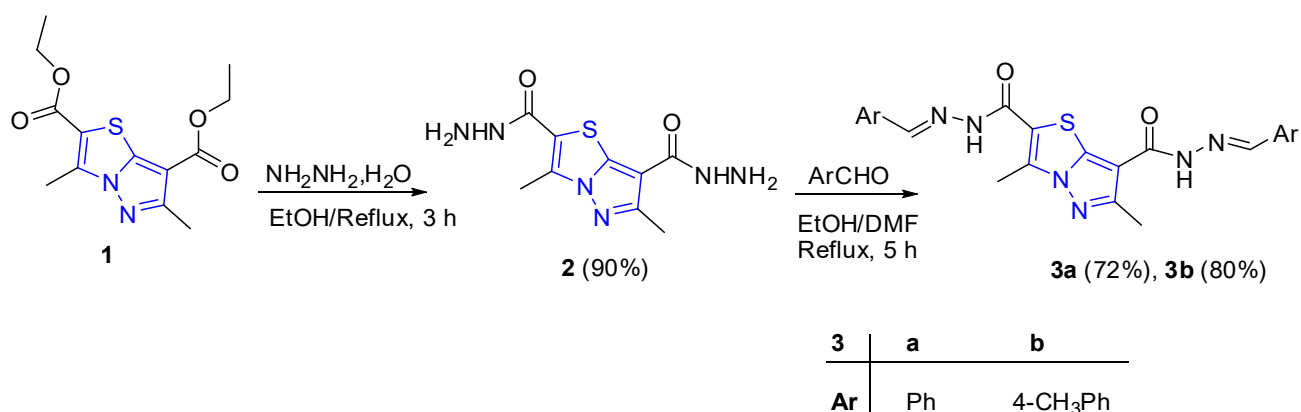
Figure 3. Pharmacologically active pyrazolo[5,1-*b*]thiazole derivatives.

Hydrazides are an important class of biologically active compounds [34–38]. Hydrazides and their condensation products have been reported to possess a wide range of pharmacological and biological activities, including antibacterial [34], tuberculostatic [35], HIV inhibitory [36], pesticidal [37], and antifungal [38] activities. Some of them are used as monoamine oxidase (MAO) inhibitors and serotonin antagonists in psychopharmacology [39]. Furthermore, isonicotinoyl hydrazide (isoniazid) is an excellent antituberculosis drug [40–42]. A variety of methods have been used to form hydrazides [43]. The hydrazinolysis of carboxylic acid esters in alcohol solutions is a convenient method for preparing carbohydrazides [44]. In light of this, and as part of our ongoing research on pharmacologically potent molecules [45–50], new hydrazide–hydrazones attached to pyrazolothiazole were synthesized and evaluated for their antimicrobial and anticancer activities.

2. Results

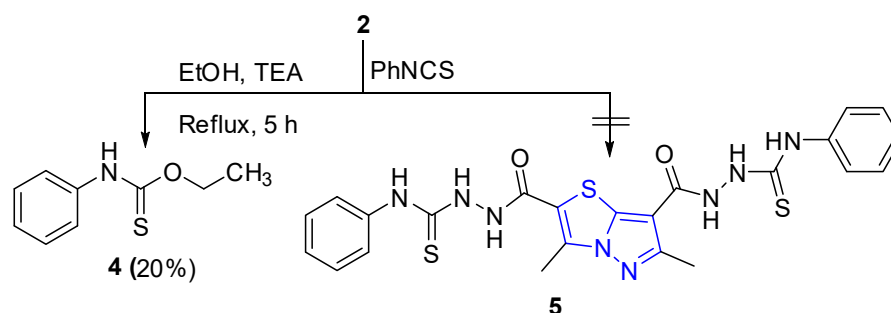
2.1. Chemistry

Hydrazide (2) was synthesized by treating diethyl 3,6-dimethylpyrazolo[5,1-*b*]thiazole-2,7-dicarboxylate (1) [51] with hydrazine hydrate (Scheme 1). The molecular structure was confirmed using IR, MS, and NMR analyses. Its IR spectrum showed the absence of C=O in the ester group, and the presence of absorption bands due to C=O in the amide and NHH₂ functions (see Experimental section). Another perfect confirmation of the structure formation obtained from NMR (¹H and ¹³C) revealed the absence of any signals due to ethoxy protons and carbons. Additionally, the mass spectrum demonstrated the molecular ion peak at the expected *m/z* value of 268 (41%). Compound 2 was reacted with the appropriate aromatic aldehyde to afford the corresponding hydrazones 3a,b (Scheme 1). Their ¹H NMR spectra revealed the absence of amino signals, which also confirms the presence of a signal at (6.78–6.81 ppm) for N=CH (imine group) in the hydrazone compounds.



Scheme 1. Synthesis of pyrazolothiazoles **2** and **3a,b**.

Hydrazide **2** was reacted with phenyl isothiocyanate in ethanol and in the presence of a catalytic amount of triethylamine to afford *O*-ethyl *N*-phenylcarbamothioate (**4**) [52], rather than the expected product **5** (Scheme 2). The structure was confirmed using spectral and X-ray analysis (Figure 4). CCDC 2075096 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Additional information relating to compound **4** is provided in Table 1.



Scheme 2. Unexpected synthesis of *O*-Ethyl *N*-phenylcarbamothioate (**4**).

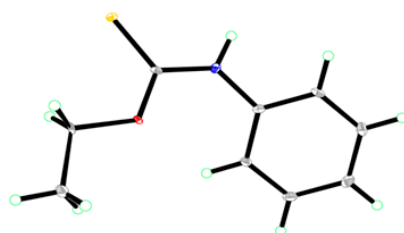
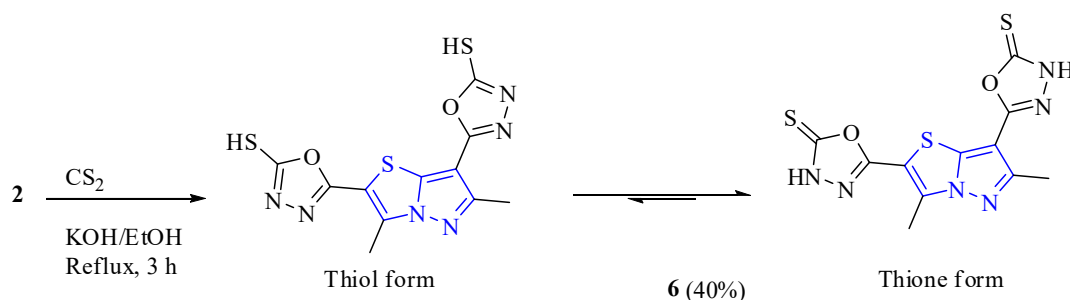


Figure 4. ORTEP diagram of compound **4**. Displacement ellipsoids are plotted at the 40% probability level for non-H atoms.

The ring closure reaction of acid hydrazide **2** with carbon disulfide in ethanolic KOH afforded the target compound **6** (Scheme 3). It was observed that 1,3,4-oxadiazole-2-thione derivatives exist in the thione form in solution, rather than in the thiol form [53,54]. Additionally, the thione tautomer is more stable than the thiol in the solution [54,55]. The equilibrium is even more favored towards the thione as it is better solvated than the thiol form [54]. In the ¹H NMR spectrum of compound **6** (Scheme 3), a signal at δ 12.9 of the NH proton was recorded.

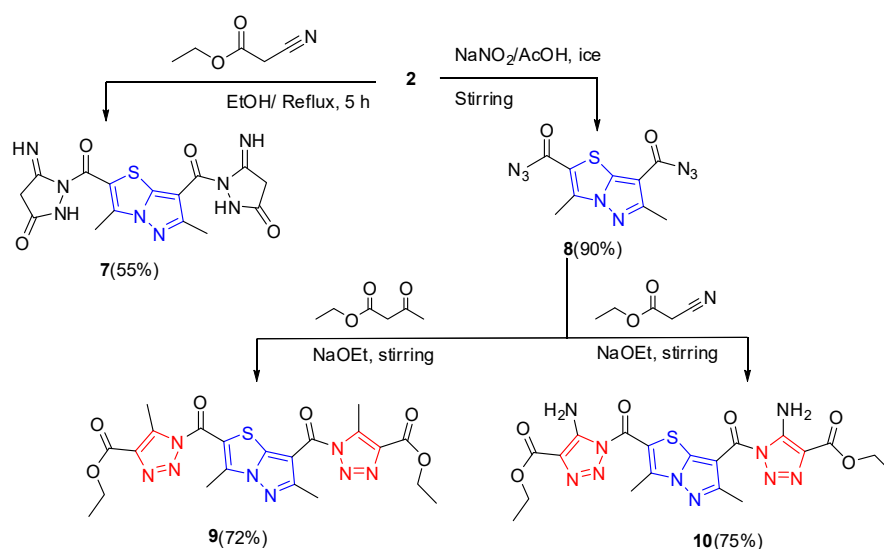
Table 1. Experimental details of compound 4.

Crystal Data	
Chemical formula	C ₉ H ₁₁ NOS
Mr	181.25
Crystal system, space group	Triclinic, P-1
Temperature (K)	293
a, b, c (Å)	9.6587 (4), 11.7585 (5), 12.1212 (5)
β (°)	88.807 (2), 84.858 (2), 84.314 (2)
V (Å ³)	1364.24 (10)
Z	6
Radiation type	Cu Kα
μ (mm ⁻¹)	2.76
Crystal size (mm)	0.47 × 0.27 × 0.15
Data collection	
Diffractometer	Bruker APEX-II CCD
Absorption correction	Multi-scan SADABS Bruker 2018
Tmin, Tmax	0.932, 0.959
No. of measured, independent and observed [I > 2σ(I)] reflections	12,396, 4728, 4213
Rint	0.055
Refinement	
R[F ₂ > 2σ(F ₂)], wR(F ₂), S	0.045, 0.118, 1.06
No. of reflections	4728
No. of parameters	341
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement
Δρmax, Δρmin (e Å ⁻³)	0.36, -0.56

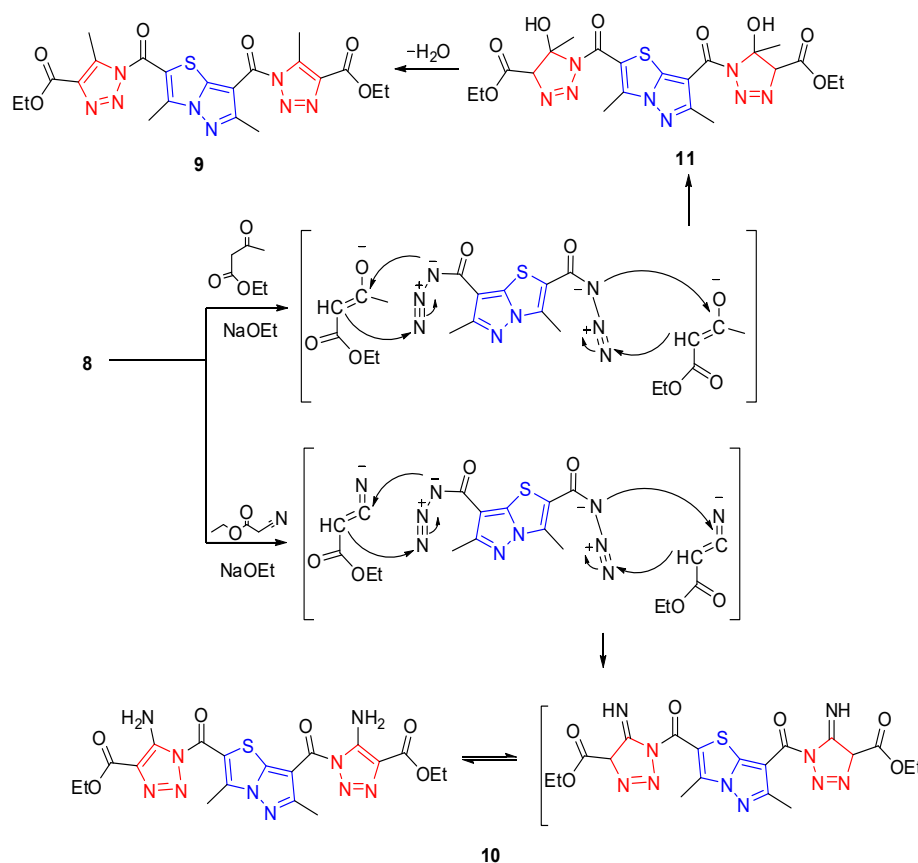
**Scheme 3.** Synthesis of 5-(2-(5-mercapto-1,3,4-oxadiazol-2-yl)-3,6-dimethylpyrazolo[5,1-*b*]thiazol-7-yl)-1,3,4-oxadiazole-2(3*H*)-thione (6).

The reaction of hydrazide 2 with ethyl cyanoacetate in absolute ethanol resulted in compound 7 as the sole product (Scheme 4). Its IR spectrum showed the absence of any absorption band due to the cyano group and the presence of the stretching bands at 3164, 1726, and 1651 cm⁻¹, corresponding to NH and two C=O groups, respectively. Its mass spectrum demonstrated the molecular ion peak at an *m/z* value of 402. On the other hand, hydrazide 2 was converted to acyl azide 8 in the presence of sodium nitrite and acetic acid (Scheme 4). The reaction between compound 8 and ethyl acetoacetate or ethyl cyanoacetate

afforded the target compounds **9** and **10**, respectively (Scheme 4). The structures of compounds **8–10** were confirmed through analytical data and spectral analysis (See Experiment section). The suggested mechanism for the selective synthesis of compounds **9** and **10** via the reaction of hydrazide **8**, ethyl acetoacetate, or ethyl cyanoacetate in the presence of sodium ethoxide is outlined in Scheme 5 [56]. The reaction was assumed to proceed through a concerted [3+2]-cycloaddition reaction. The non-isolable intermediate **11** was further transformed into stable 1,2,3-triazole derivative **9** through rapid elimination of two water molecules induced by sodium ethoxide.



Scheme 4. Synthesis of bis(1,2,3-triazole) derivatives **7–10**.



Scheme 5. A suggested reaction mechanism for the synthesis of triazoles **9** and **10**.

2.2. Biological Activity Evaluation

2.2.1. Anticancer Screening of the Synthesized Compounds

The in vitro anti-tumor activity of the synthesized compounds was assessed against two human cancer cell lines: human hepatocellular carcinoma cell line (HepG-2) and colon carcinoma cell line (HCT-116), using the MTT assay [57]. Their activity was compared to the reference drug Doxorubicin. In addition, calculations of the tested compounds' concentrations needed to inhibit 50% of the cancerous cell population (IC_{50}) were implemented. These are presented in Tables 2 and 3.

Table 2. Viability values and IC_{50} of some selected samples against hepatocellular carcinoma cell line (HepG-2).

IC_{50} ($\mu\text{g/mL}$)	Viability %								Sample
	Sample Concentration ($\mu\text{g/mL}$)								
	500	250	125	62.5	31.25	15.6	7.8	3.9	
0.36	2.08	3.36	4.86	6.51	11.04	19.38	24.82	28.86	DOX
93.2	20.88	31.76	42.63	57.12	72.36	86.04	92.37	97.48	3a
30.5	8.71	16.38	24.92	37.65	49.43	62.04	78.19	89.28	3b
6.9	4.37	9.46	16.76	23.66	34.15	40.72	46.58	61.43	6
114	24.53	39.15	47.32	61.98	78.43	89.04	95.17	98.76	7
12.6	5.14	11.89	20.97	31.76	38.69	45.38	57.43	72.96	8

DOX (Doxorubicin).

Table 3. Viability values and IC_{50} of evaluated compounds against colon carcinoma cell line (HCT-116).

IC_{50} ($\mu\text{g/mL}$)	Viability %								Sample
	Sample Concentration ($\mu\text{g/mL}$)								
	500	250	125	62.5	31.25	15.6	7.8	3.9	
0.49	2.08	3.36	4.86	6.51	11.04	19.38	24.82	28.86	DOX
86.9	16.08	25.43	36.81	58.19	74.26	88.43	96.51	99.48	3b
13.6	6.91	10.85	17.44	25.28	36.59	43.87	67.34	84.73	6
28.9	9.76	17.34	26.69	35.42	46.94	63.79	76.45	84.76	8

DOX (Doxorubicin).

Of all the tested compounds, 1,3,4-oxadiazole derivative **6** exhibited the highest activity against the two tested cell lines; HepG-2 and HCT-116, with an IC_{50} = 6.9 and 13.6 $\mu\text{g/mL}$, respectively. Having azide moiety, pyrazolothiazole derivative **8** revealed high activity against HepG-2 and HCT-116, with an IC_{50} = 12.6 and 28.9 $\mu\text{g/mL}$, respectively.

These results support previously published results indicating that compound structures containing an 1,3,4-oxadiazole ring [58] or azide moiety [59] have potent antitumor activities.

2.2.2. The In Vitro Antimicrobial Assessments

Assessments of the antimicrobial activities of the synthesized compounds were performed using the inhibition zone technique [60] against six species: two fungal species (*Aspergillus fumigatus* (RCMB 002008 (4) and *Candida albicans* (RCMB 05036)), two Gram-positive bacteria (*Staphylococcus aureus* (RCMB010010 and *Bacillus subtilis* (RCMB 010067)), and two Gram-negative bacteria (*Salmonella SP.* (RCMB 010043) and *Escherichia coli* (RCMB 010052)). The standard drugs used for comparison were Amphotericin B, Gentamicin, and Ampicillin. The inhibition zone diameter (IZD) was used as the criterion for the antimicrobial activity and all results are summarized in Table 4.

Table 4. The in vitro antimicrobial assessment of the synthesized compounds tested at 5 mg/mL using the inhibition zone assay (inhibition zone diameter in millimeters (mm)).

Sample	Microorganisms					
	Fungi		Gram-Positive Bacteria		Gram-Negative Bacteria	
	AF	CA	SA	BS	SSP	EC
3a	11	15	13	12	13	14
3b	13	15	10	NA	12	14
4	17	16	15	14	15	17
6	18	15	14	15	14	15
7	NA	10	12	9	12	12
8	12	NA	14	12	13	14
10	14	12	12	12	12	14
Amphotericin B.	23	25				
Ampicillin	-	-	23	32		
Gentamycin	-	-	-	-	17	19

NA: no activity. Results of the antimicrobial evaluation are expressed as the mean of inhibition zone diameter (mm) for different compounds tested in triplicate: *Aspergillus fumigatus* (RCMB 002008 (4) (AF), *Candida albicans* (RCMB 05036) (CA), *Staphylococcus aureus* (RCMB010010) (SA), *Bacillus subtilis* (RCMB 010067) (BS), *Salmonella SP.* (RCMB 010043) (SSP), *Escherichia coli* (RCMB 010052) (EC).

The results of Table 4 illustrate the following points:

- All the tested compounds except compound 7 showed excellent activity against *Aspergillus fumigatus*. Compounds 4 and 6 were especially effective.
- All tested compounds except compound 8 showed high antifungal activity against *Candida albicans*.
- Compounds 3b, 7, and 8 were found to be more active against *Staphylococcus aureus* than against *Bacillus subtilis*.
- The best antibacterial activity was observed for compounds 4 and 6: their inhibitory effect appears to be equipotent to Gentamycin against *Salmonella SP* and *Escherichia coli*.

Many thiocarbamate derivatives such as tolinaftate, tolciclate, and piritetrate are commonly used as fungicidal agents, and this explains the highest antifungal activity of compound 4 [61–65].

3. Materials and Methods

3.1. Chemistry

3.1.1. Materials and Equipment

See Supplementary Materials.

3.1.2. Synthesis of 3,6-Dimethylpyrazolo[5,1-b]thiazole-2,7-dicarbohydrazide (2)

A mixture of diethyl 3,6-dimethylpyrazolo[5,1-*b*]thiazole-2,7-dicarboxylate (1) [51] (1.48g, 5 mmol), and hydrazine hydrate (80%, 15 mmol) in ethanol (15 mL) were refluxed for 3 h. Excess ethanol was evaporated under reduced pressure and the solid product was filtered, dried, and recrystallized from ethanol/DMF to afford target compound 2 at 90% yield; mp: 210–211 °C; IR (KBr) ν_{\max} 3338–3201(NH₂+NH), 1717 (C=O) cm⁻¹; ¹H NMR (CDCl₃): δ 2.04 (s, 3 H, CH₃), 2.16 (s, 3 H, CH₃), 3.31 (s, 4H, NH₂), 11.58 (s, 2 H, 2NH); ¹³C NMR (CDCl₃): δ 13.52 (CH₃), 15.68 (CH₃), 128.55, 129.71, 137.77, 137.86, 147.46, 186.80 (C=O); MS *m/z* (%) 268 (M⁺, 41%), 251 (100%). Anal. Calcd. for C₉H₁₂N₆O₂S (268.30): C, 40.29; H, 4.51; N, 31.32. Found: C, 40.33; H, 4.62; N, 31.25.

3.1.3. Synthesis of Hydrazones 3a,b

A mixture of hydrazide **2** (0.536g, 2 mmol) and appropriate aldehydes (4.2 mmol) in absolute ethanol/DMF (20mL) were refluxed for 5 h. The resulting precipitate was filtered off, washed, dried, and recrystallized by DMF/ethanol to afford the corresponding hydrazones **3a,b**.

3a: Yield (72%), mp. > 300 °C; IR (KBr) ν_{\max} 3274 (NH), 1714 (C=O), 1609 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3): δ 1.92 (s, 3H, CH_3), 2.20 (s, 3H, CH_3), 6.78 (s, 2H, 2CH), 7.23–7.85 (m, 10H, ArH), 10.78 (s, 1H, NH), 11.75 (s, 1H, NH); $^{13}\text{C-NMR}$: δ 11.02 (CH_3), 14.0 (CH_3), 111.12, 128.5, 129.8, 130.3, 133.1, 135.7, 136.0, 146.2, 151.4, 163.0, 167.2 (C=O). Anal. Calcd. for $\text{C}_{23}\text{H}_{20}\text{N}_6\text{O}_2\text{S}$ (444.51): C, 62.15; H, 4.54; N, 18.91. Found: C, 62.22; H, 4.42; N, 18.77.

3b: Yield (80%), mp. 260 °C; IR (KBr) ν_{\max} 3515 (NH), 1686 (C=O), 1595 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3): δ , 1.90 (s, 3H, CH_3), 2.20 (s, 3H, CH_3), 2.45 (s, 6H, 2 CH_3), 6.81 (s, 2H, 2CH), 7.28–7.84 (m, 8H, ArH), 11.25 (s, 2H, 2NH); $^{13}\text{C-NMR}$: δ 11.50 (CH_3), 14.31 (CH_3), 18.95 (2 CH_3), 111.30, 128.1, 129.7, 131.20, 133.1, 136.12, 136.7, 148, 151.0, 156.0, 163.0, 165.61 (C=O). Anal. Calcd. for $\text{C}_{25}\text{H}_{24}\text{N}_6\text{O}_2\text{S}$ (472.56): C, 63.54; H, 5.12; N, 17.78. Found: C, 63.34; H, 5.22; N, 17.87.

3.1.4. Synthesis of O-Ethyl N-phenylcarbamothioate (4)

A mixture of hydrazide (**2**) (0.268g, 1 mmol) and phenyl isothiocyanate (0.27 g, 0.24 mL, 2 mmol) in EtOH (10 mL), in the presence of a few drops of triethylamine as a catalyst, was heated under reflux for 5 h and then allowed to cool to room temperature. The precipitated solid was filtered off, dried, and recrystallized from methanol to afford compound **4** [52] at 20% yield; mp: 55–56 °C; IR (KBr) ν_{\max} 3212 (NH), 3039, 2982 (CH) cm^{-1} ; $^1\text{H NMR}$ (CDCl_3): δ 1.23 (t, 3 H, CH_3), 4.65 (q, 2 H, CH_2), 7.20–7.46 (m, 5H, ArH), 9.12 (s, 1 H, NH); $^{13}\text{C NMR}$ (CDCl_3): δ 14.90, 68.50, 121.10, 121.10, 128.40, 129.57, 129.57, 137.85 (Ar-C), 188.50 (C=S). Anal. Calcd. for $\text{C}_9\text{H}_{11}\text{NOS}$ (181.25): C, 59.64; H, 6.12; N, 7.73. Found: C, 59.75; H, 6.07; N, 7.88.

3.1.5. Synthesis of Bis(1,3,4-oxadiazole) 6

Hydrazide **2** (3.75 g, 14 mmol) was dissolved in absolute ethanol (50 mL). CS_2 (2.7g, 2.1 mL, 35 mmol) was then added to the solution, followed by the addition of a KOH solution (1.6 g, 28 mmol) in water (20 mL). The reaction mixture was thoroughly stirred and refluxed for 3 h until the evolution of H_2S ceased. After completion of the reaction, excess ethanol was removed under reduced pressure. The mixture was poured into a mixture of H_2O /ice and acidified with concentrated HCl. The precipitated solid was filtered off and recrystallized from ethanol/DMF, resulting in thione **6**. Yield (40%); mp. 250 °C; IR (KBr) ν_{\max} 3313 (NH), and 1116 (C=S) cm^{-1} ; $^1\text{H NMR}$ (CDCl_3): δ 1.31 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 12.90 (s, 2H, NH); $^{13}\text{C-NMR}$ δ 11.50 (CH_3), 14.01(CH_3), 104.02, 107.30, 135.11, 142.90, 156.74, 162.21, 177.77 (C=S). Anal. Calcd. for $\text{C}_{11}\text{H}_8\text{N}_6\text{O}_2\text{S}_3$ (352.42): C, 37.49; H, 2.29; N, 23.85; Found: C, 37.38; H, 2.18; N, 23.99.

3.1.6. Synthesis of Bis(pyrazole) Derivative 7

A mixture of hydrazide **2** (1.34 g, 5 mmol) and ethyl cyanoacetate (2.26 mL, 20 mmol) in ethanol (10 mL) was heated under reflux for 5 h. The precipitated solid product was filtered and recrystallized from ethanol, resulting in **7** at 55% yield; mp. 250–251 °C; IR (KBr) ν_{\max} 3164 (NH), 2982 (CH aliphatic), 1726, 1651 (2C=O), 1560 (C=N) cm^{-1} ; MS m/z (%) 402 (M^+ , 4%), 400 (52%), 399 (35%), 45 (100%). Anal. Calcd. for $\text{C}_{15}\text{H}_{14}\text{N}_8\text{O}_4\text{S}$ (402.39): Calc.: C, 44.77; H, 3.51; N, 27.85. Found: C, 44.65; H, 3.42; N, 27.93.

3.1.7. Synthesis 3,6-Dimethylpyrazolo[5,1-b]thiazole-2,7-dicarbonyl Azide (8)

To a suspension of hydrazide **2** (2.68 g, 10 mmol) in 30 mL of H_2O , 3.5 g (50 mmol) of sodium nitrite was added. The mixture was then cooled in ice and treated portion-wise with 3 mL (50 mmol) of acetic acid. After stirring at room temperature for 3 h, the resulting precipitate **8** was filtered, washed with H_2O , and dried: yield 90%; mp 120–121 °C; IR (KBr)

ν_{\max} 2982 (CH), 2167 (N=N), 1724 (C=O), 1686 (C=O) cm^{-1} ; MS m/z (%) 290 (M^+ , 14%), 40 (100%). Anal. Calcd for $\text{C}_9\text{H}_6\text{N}_8\text{O}_2\text{S}$ (290.26): C, 37.24; H, 2.08; N, 38.60. Found: C, 37.35; H, 2.16; N, 38.49.

3.1.8. Synthesis of Bis(1,2,3-triazole) Derivatives 9,10

Compound 8 (0.290 g, 1 mmol) was added to a stirred solution of sodium metal (0.10 g) in ethanol (20 mL), and the mixture was left to stir at room temperature for 20 min. Either ethyl acetoacetate or ethyl cyanoacetate (2 mmol) was added while stirring. The reaction mixture was then left to stir for a further 24 h. The formed solid product was filtered off, washed with water, dried, and recrystallized from EtOH to afford the corresponding bis(1,2,3-triazole) derivatives 9 and 10, respectively.

9. Yield (72%), mp. 300 °C; IR (KBr) ν_{\max} 1694 (2C=O), 1594 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3): δ 1.31 (s, 6H, 2 CH_3), 2.26 (s, 6H, 2 CH_3), 2.38 (s, 6H, 2 CH_3), 4.32 (q, 4H, 2 CH_2); $^{13}\text{C-NMR}$: δ 13.40, 14.3, 15.10, 18.10, 60.75, 111.10, 130.0, 134.0, 137.20, 138.0, 145.00, 151.15, 164.93, 169.34 (C=O). Anal. Calcd. for $\text{C}_{21}\text{H}_{22}\text{N}_8\text{O}_6\text{S}$ (514.51): C, 49.02; H, 4.31; N, 21.78. Found: C, 49.13; H, 4.37; N, 21.88.

10. Yield (75%), mp. 210–211 °C; IR (KBr) ν_{\max} 1697 (2C=O), 1597 (C=N) cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3): δ 1.19 (s, 6H, 2 CH_3), 2.46 (s, 3H, CH_3), 3.30 (s, 3H, CH_3), 4.28 (q, 4H, 2 CH_2), 7.71 (s, 4H, NH_2); $^{13}\text{C-NMR}$: δ 12.40, 15.10, 19.44, 60.23, 61.75, 111.30, 131.20, 133.89, 137.20, 138.20, 146.00, 151.02, 165.93, 169.20 (C=O). Anal. Calcd. for $\text{C}_{19}\text{H}_{20}\text{N}_{10}\text{O}_6\text{S}$ (516.49): C, 44.18; H, 3.90; N, 27.12. Found: C, 44.22; H, 3.84; N, 27.23.

3.2. Biological Tests

3.2.1. Evaluation of Antitumor Activity

The MTT assay was used to investigate the *in vitro* antitumor activity of the synthesized compounds against two human cancer cell lines: human hepatocellular carcinoma cell line (HepG-2) and colon carcinoma cell line (HCT-116) [57].

3.2.2. Antimicrobial Evaluation

The inhibition zone technique [60] was used to evaluate the antimicrobial activity of the synthesized compounds against six pathogens. Amphotericin B, Gentamicin, and Ampicillin were the standard medications utilized for comparison. The antimicrobial activity was measured using the inhibition zone diameter (IZD).

4. Conclusions

New pyrazolo[5,1-*b*]thiazole derivatives, synthesized using simple synthetic methods, can be used as leading compounds in the development of future, novel drug molecules.

Supplementary Materials: The following are available online, Online supplementary information includes detailed methods of the antitumor and antimicrobial evaluations, Figures S1–S4: IR, Mass, $^1\text{H-NMR}$, and $^{13}\text{C-NMR}$ spectra of compound 1, Figures S5–S7: IR, $^{13}\text{C-NMR}$ and Mass spectra of compound 2, Figures S8–S10: IR, $^1\text{H-NMR}$, and $^{13}\text{C-NMR}$ spectra of compound 4, Figures S11,S12: IR, and Mass spectra of compound 7, Figures S13,S14: IR, and Mass spectra of compound 8.

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Sample Availability: Samples of the compounds are available from the authors.

References

1. Ventola, C.L. The antibiotic resistance crisis: Part 1: Causes and threats. *Pharm. Ther.* **2015**, *40*, 277–283.
2. Chandrakantha, B.; Shetty, P.; Nambiyar, V.; Isloor, N.; Isloor, A.M. Synthesis, characterization and biological activity of some new 1,3,4-oxadiazole bearing 2-fluoro-4-methoxy phenyl moiety. *Eur. J. Med. Chem.* **2010**, *45*, 1206–1210. [[CrossRef](#)]
3. Vijesh, A.M.; Isloor, A.M.; Shetty, P.; Sundershan, S.; Fun, H.K. New pyrazole derivatives containing 1,2,4-triazoles and benzoxazoles as potent antimicrobial and analgesic agents. *Eur. J. Med. Chem.* **2013**, *62*, 410–415. [[CrossRef](#)]
4. Farooqi, S.I.; Arshad, N.; Channar, P.A.; Perveen, F.; Saeed, A.; Larik, F.A.; Javeed, A. Synthesis, theoretical, spectroscopic and electrochemical DNA binding investigations of 1,3,4-thiadiazole derivatives of ibuprofen and ciprofloxacin: Cancer cell line studies. *J. Photochem. Photobiol.* **2018**, *189*, 104–118. [[CrossRef](#)] [[PubMed](#)]
5. Nagai, H.; Kim, Y. Cancer prevention from the perspective of global cancer burden patterns. *J. Thorac. Dis.* **2017**, *9*, 448–451. [[CrossRef](#)]
6. Molina, J.R.; Yang, P.; Cassivi, S.D.; Schild, S.E.; Adjei, A.A. Non-small cell lung cancer: Epidemiology, risk factors, treatment, and survivorship. *Mayo Clin. Proc.* **2008**, *83*, 584–594. [[CrossRef](#)]
7. Bhatt, P.; Kumar, M.; Jha, A. Design, Synthesis and Anticancer Evaluation of Oxa/Thiadiazolyhydrazones of Barbituric and Thiobarbituric Acid: A Collective In Vitro and In Silico Approach. *Chem. Select.* **2018**, *3*, 7060–7065. [[CrossRef](#)]
8. Rashid, M.; Husain, A.; Mishra, R.; Karim, S.; Khan, S.; Ahmad, M.; Khan, S.A. Design and synthesis of benzimidazoles containing substituted oxadiazole, thiadiazole and triazolo-thiadiazines as a source of new anticancer agents. *Arab. J. Chem.* **2015**, *12*, 3202–3224. [[CrossRef](#)]
9. Carter, J.S.; Kramer, S.; Talley, J.J.; Penning, T.; Collins, P.; Graneto, M.J.; Seibert, K.; Koboldt, C.; Masferrer, J.; Zweifel, B. Synthesis and activity of sulfonamide-substituted 4,5-diaryl thiazoles as selective cyclooxygenase-2 inhibitors. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1171–1174. [[CrossRef](#)]
10. Hargrave, K.D.; Hess, F.K.; Oliver, J.T. N-(4-substituted-thiazolyl)oxamic acid derivatives, a new series of potent, orally active antiallergy agents. *J. Med. Chem.* **1983**, *26*, 1158–1163. [[CrossRef](#)] [[PubMed](#)]
11. Bondock, S.; Khalifa, W.; Fadda, A.A. Synthesis and antimicrobial evaluation of some new thiazole, thiazolidinone and thiazoline derivatives starting from 1-chloro-3,4-dihydronaphthalene-2-carboxaldehyde. *Eur. J. Med. Chem.* **2007**, *42*, 948–954. [[CrossRef](#)] [[PubMed](#)]
12. Patt, W.C.; Hamilton, H.W.; Taylor, M.D.; Ryan, M.J.; Taylor, D.G., Jr.; Connolly, C.J.C.; Doherty, A.M.; Klutchko, S.R.; Sircar, I.; Steinbaugh, B.A.; et al. Structure-activity relationships of a Series of 2-Amino-4-thiazole Containing Renin Inhibitors. *J. Med. Chem.* **1992**, *35*, 2562–2572. [[CrossRef](#)]
13. Sharma, P.K.; Sawhney, S.N.; Gupta, A.; Singh, G.B.; Bani, S. Synthesis and antiinflammatory activity of some 3-(2-thiazolyl)-1,2-benzisothiazoles. *Indian J. Chem.* **1998**, *37B*, 376–381.
14. Jaen, J.C.; Wise, L.D.; Caprathe, B.W.; Teclé, H.; Bergmeier, S.; Humblet, C.C.; Heffner, T.G.; Meltzner, L.T.; Pugsley, T.A. 4-(1,2,5,6-Tetrahydro-1-alkyl-3-pyridinyl)-2-thiazolamines: A novel class of compounds with central dopamine agonist properties. *J. Med. Chem.* **1990**, *33*, 311–317. [[CrossRef](#)]
15. Ergenc, N.; Capan, G.; Gunay, N.S.; Ozkirimli, S.; Gungor, M.; Ozbey, S.; Kendi, E. Synthesis and hypnotic activity of new 4-thiazolidinone and 2-thioxo-4,5-imidazolidinedione derivatives. *Arch. Pharm. Pharm. Med. Chem.* **1999**, *332*, 343–347. [[CrossRef](#)]
16. Tsuji, K.; Ishikawa, H. Synthesis and anti-pseudomonal activity of new 2-isocephems with a dihydroxypyridone moiety at C-7. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1601–1606. [[CrossRef](#)]
17. Bell, F.W.; Cantrell, A.S.; Hogberg, M.; Jaskunas, S.R.; Johansson, N.G.; Jordon, C.L.; Kinnick, M.D.; Lind, P.; Morin, J.M., Jr.; Noreen, R.; et al. Phenethylthiazolethiourea (PETT) Compounds, a New Class of HIV-1 Reverse Transcriptase Inhibitors. 1. Synthesis and Basic Structure-Activity Relationship Studies of PETT Analogs. *J. Med. Chem.* **1995**, *38*, 4929–4936. [[CrossRef](#)] [[PubMed](#)]
18. Sharma, P.C.; Bansal, K.K.; Sharma, A.; Sharma, D.; Deep, A. Thiazole-containing compounds as therapeutic targets for cancer therapy. *Eur. J. Med. Chem.* **2020**, *188*, 112016. [[CrossRef](#)] [[PubMed](#)]
19. Isloor, A.M.; Kalluraya, B.; Shetty, P. Regioselective reaction: Synthesis, characterization and pharmacological studies of some new Mannich bases derived from 1,2,4-triazoles. *Eur. J. Med. Chem.* **2009**, *44*, 3784–3787. [[CrossRef](#)]
20. Isloor, A.M.; Kalluraya, B.; Rao, M. Sydnone derivatives: Part IV; synthesis of 3-aryl-4-(substituted pyrazolidene hydrazine-4-thiazolyl) sydnones as possible analgesic and anticonvulsant agents. *J. Saudi Chem. Soc.* **2000**, *4*, 265–270.

21. Kalluraya, B.; Isloor, A.M.; Shenoy, S. Synthesis and biological activity of 6-substituted-3-[4-(3-substituted pyrazolidene) hydrazino-4-thiazolyl] coumarins. *Indian J. Heterocycl. Chem.* **2001**, *11*, 159–162.
22. Sunil, D.; Isloor, A.M.; Shetty, P. Synthesis, characterization and anticancer activity of 1,2,4-Triazolo [3,4-b]-1,3,4-thiadiazoles on Hep G2 cell lines. *Der. Pharma Chem.* **2009**, *1*, 19–26.
23. Canonico, P.G.; Jahrling, P.B.; Pannier, W.L. Antiviral efficacy of pyrazofurin against selected RNA viruses. *Antivir. Res.* **1982**, *2*, 331–337. [[CrossRef](#)]
24. Westhead, J.E.; Price, H.D. Quantitative assay of pyrazofurin a new antiviral, antitumor antibiotic. *Antimicrob. Agents Chemother.* **1974**, *5*, 90–91. [[CrossRef](#)]
25. Ardiansah, B.A.Y.U. Recent reports on pyrazole-based bioactive compounds as candidate for anticancer agents. *Asian J. Pharm. Clin. Res.* **2017**, *12*, 45. [[CrossRef](#)]
26. Pulici, M.; Marchionni, C.; Piutti, C.; Gasparri, F. Bicyclic pyrazoles as protein kinase inhibitors. WO 2010070060 (2010). *Chem. Abstr.* **2010**, *153*, 87782.
27. Desai, N.; Trivedi, A.; Pandit, U.; Dodiya, A.; Rao, V.K.; Desai, P. Hybrid Bioactive Heterocycles as Potential Antimicrobial Agents: A Review. *Mini Rev. Med. Chem.* **2016**, *16*, 1500–1526. [[CrossRef](#)]
28. Mahajan, N.S.; Pattana, S.R.; Jadhav, R.L.; Pimpodhar, N.V.; Manikrao, A.M. Synthesis of some thiazole compounds of biological interest containing mercapta group. *Int. J. Chem. Sci.* **2008**, *6*, 800–806.
29. Rizk, H.; El-Borai, M.A.; Ragab, A.; Ibrahim, S.A. Design, synthesis, biological evaluation and molecular docking study based on novel fused pyrazolothiazole scaffold. *J. Iran. Chem. Soc.* **2020**, *17*, 2493–2505. [[CrossRef](#)]
30. Chandanshive, J.Z.; Bonini, B.F.; Tiznado, W.; Escobar, C.A.; Caballero, J.; Femoni, C.; Fochi, M.; Franchini, M.C. 1,3-Dipolar Cycloaddition of Nitrile Imines with Cyclic α - β -Unsaturated Ketones: A Regiochemical Route to Ring-Fused Pyrazoles. *Eur. J. Org. Chem.* **2011**, *25*, 4806–4813. [[CrossRef](#)]
31. Abdel-Wahab, B.F.; Mohamed, H.A. Pyrazolothiazoles: Synthesis and Applications. *Phosphorus Sulfur Silicon Relat. Elem.* **2013**, *188*, 1680–1693. [[CrossRef](#)]
32. Takahashi, Y.; Hashizume, M.; Shin, K.; Terauchi, T.; Takeda, K.; Hibi, S.; Murata-Tai, K.; Fujisawa, M.; Shikata, K.; Taguchi, R.; et al. Design, synthesis, and structure-activity relationships of novel pyrazolo[5,1-b]thiazole derivatives as potent and orally active corticotropin-releasing factor 1 receptor antagonists. *J. Med. Chem.* **2012**, *55*, 8450–8463. [[CrossRef](#)]
33. Lu, X.; Tang, J.; Liu, Z.; Li, M.; Zhang, T.; Zhang, X.; Ding, K. Discovery of new chemical entities as potential leads against Mycobacterium tuberculosis. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 5916–5919. [[CrossRef](#)]
34. Rollas, S.; Gulerman, N.; Erdeniz, H. Synthesis and antimicrobial activity of some new hydrazones of 4-fluorobenzoic acid hydrazide and 3-acetyl-2,5-disubstituted-1,3,4-oxadiazolines. *Farmaco* **2002**, *57*, 171–174. [[CrossRef](#)]
35. Pavan, F.R.; Maia, P.I.D.S.; Leite, S.R.; Deflon, V.M.; Batista, A.A.; Sato, D.N.; Leite, C.Q. Thiosemicarbazones, semicarbazones, dithiocarbazates and hydrazide/hydrazones: Anti-Mycobacterium tuberculosis activity and cytotoxicity. *Eur. J. Med. Chem.* **2010**, *45*, 1898–1905. [[CrossRef](#)]
36. Marastoni, M.; Baldisserotto, A.; Trapella, C.; McDonald, J.; Bortolotti, F.; Tomatis, R. HIV protease inhibitors: Synthesis and activity of N-aryl-N'-hydroxyalkyl hydrazide pseudopeptides. *Eur. J. Med. Chem.* **2005**, *5*, 445–451. [[CrossRef](#)] [[PubMed](#)]
37. Tabanca, N.; Ali, A.; Bernier, U.R.; Khan, I.A.; Kocyigit-Kaymakcioglu, B.; Oruç-Emre, E.E.; Unsalan, S.; Rollas, S. Biting deterrence and insecticidal activity of hydrazide-hydrazones and their corresponding 3-acetyl-2,5-disubstituted 2,3-dihydro-1,3,4-oxadiazoles against Aedes aegypti. *Pest. Manag. Sci.* **2013**, *69*, 703–708. [[CrossRef](#)]
38. Turan-Zitouni, G.; Altintop, M.D.; Özdemir, A.; Demirci, F.; Mohsen, U.A.; Kaplancıklı, Z.A. Synthesis and antifungal activity of new hydrazide derivatives. *J. Enzyme Inhib. Med. Chem.* **2013**, *28*, 1211–1216. [[CrossRef](#)]
39. Vardanyan, R.S.; Hruby, V.J. Antidepressants. In *Synthesis of Essential Drugs*; Elsevier: Amsterdam, The Netherlands, 2006; pp. 103–116.
40. Patole, J.; Sandbhor, U.; Padhye, S.; Deobagkar, D.N.; Ansonc, C.E.; Powell, A. Structural chemistry and in vitro antitubercular activity of acetylpyridine benzoyl hydrazone and its copper complex against Mycobacterium smegmatis. *Bioorg. Med. Chem. Lett.* **2004**, *13*, 51–55. [[CrossRef](#)]
41. Maccari, R.; Ottana, R.; Vigorita, M.G. In vitro advanced antimycobacterial screening of isoniazid-related hydrazones, hydrazides and cyanoboranes: Part 14. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2509–2513. [[CrossRef](#)]
42. Bottari, B.; Maccari, R.; Monforte, F.; Ottana, R.; Vigorita, M.G.; Bruno, G.; Nicolo, F.; Rotondo, A.; Rotondo, E. Nickel(II) 2,6-diacetylpyridine bis(isonicotinoylhydrazonate) and bis(benzoylhydrazonate) complexes: Structure and antimycobacterial evaluation. Part XI. *Bioorg. Med. Chem.* **2001**, *9*, 2203–2211. [[CrossRef](#)]
43. Al-Ajely, M.S.; Yaseen, A.N. Synthesis and Characterization of Some New Hydrazides and Their Derivatives. *Ibn AL-Haitham J. For. Pure Appl. Sci.* **2017**, *28*, 103–112.
44. Elshaarawy, R.F.; Janiak, C. 2-Thiophenecarbohydrazides: A novel efficient method for the synthesis of 2-thiophenecarbohydrazide. *Z. Nat. B* **2011**, *66*, 1202–1208.
45. Khodair, A.I.; Ahmed, A.; Emam, D.R.; Kheder, N.A.; Elmalki, F.; Hadda, T.B. Synthesis, Antiviral, DFT and Molecular Docking Studies of Some Novel 1,2,4-Triazine Nucleosides as Potential Bioactive Compounds. *Carbohydr. Res.* **2021**, *500*, 108246. [[CrossRef](#)] [[PubMed](#)]

46. Gomha, S.M.; Edrees, M.M.; Muhammad, Z.A.; Kheder, N.A.; Abu-Melha, S.; Saad, A.M. Synthesis, Characterization, and Antimicrobial Evaluation of Some New 1,4-Dihydropyridines-1,2,4-Triazole Hybrid Compounds. *Polycycl. Aromat. Compd.* **2020**. [[CrossRef](#)]
47. Hadda, T.B.; Deniz, F.S.S.; Orhan, I.E.; Zgou, H.; Rauf, A.; Mabkhot, Y.N.; Bennani, B.; Emam, D.R.; Kheder, N.A.; Asayari, A.; et al. Spiro Heterocyclic Compounds as Potential Anti-Alzheimer agents (Part 2): Their Metal Chelation Capacity, POM Analyses and DFT Studies. *Med. Chem.* **2021**, *17*. [[CrossRef](#)]
48. Mabkhot, Y.; Algarni, H.; Alsayari, A.; bin Muhsinah, A.; Kheder, N.A.; Almarhoon, Z.; Al-Aizari, F. Synthesis, X-ray Analysis, Biological Evaluation and Molecular Docking Study of New Thiazoline Derivatives. *Molecules* **2019**, *24*, 1654. [[CrossRef](#)]
49. Mabkhot, Y.; Alharbi, M.; Al-Showiman, S.; Soliman, S.; Kheder, N.A.; Frey, W.; Asayari, A.; Bin Muhsinah, A.; Algarni, H. A novel synthesis, X-ray analysis and computational studies of (Z)-ethyl 2-((Z)-5-((dimethylamino)methylene)-4-oxo-3-phenylthiazolidin-2-ylidene)acetate as a potential anticancer agent. *BMC Chem.* **2019**, *13*, 35. [[CrossRef](#)]
50. Abu-Melha, S.; Edrees, M.M.; Salem, H.H.; Kheder, N.A.; Gomha, S.M.; Abdelaziz, M.R. Synthesis and biological evaluation of some novel thiazole-based heterocycles as potential anticancer and antimicrobial agents. *Molecules* **2019**, *24*, 539. [[CrossRef](#)] [[PubMed](#)]
51. Al-aizari, F.A. Synthesis of Some Thioxothiazole Derivatives and Evaluation of their Biological Activity. Ph.D. Thesis, King Saud University, Riyadh, Saudi Arabia, 2017.
52. Sahoo, S.K.; Chakraborty, S.; Patel, B.K. A one-pot conversion of di-substituted thiourea to O-organyl arylthiocarbamate using FeCl₃. *J. Sulphur Chem.* **2012**, *33*, 143–153. [[CrossRef](#)]
53. Horning, D.E.; Muchowski, J.M. Five-membered Heterocyclic Thiones. Part I. 1,3,4-Oxadiazole-2-thione. *Can. J. Chem.* **1972**, *50*, 3079–3082. [[CrossRef](#)]
54. Tomi, I.H.; Al-Qaisi, A.H.; Al-Qaisi, Z.H. Synthesis, characterization and effect of bis-1,3,4-oxadiazole rings containing glycine moiety on the activity of some transferase enzymes. *J. King Saud Univ. Sci.* **2011**, *23*, 23–33. [[CrossRef](#)]
55. Thurston, D.E.; Pysz, I. *Chemistry and Pharmacology of Anticancer Drugs*, 2nd ed.; CRC Press: Boca Raton, FL, USA, 2021. [[CrossRef](#)]
56. Ramachary, D.B.; Gujral, J.; Peraka, S.; Reddy, G.S. Triazabicyclodecene as an Organocatalyst for the Regiospecific Synthesis of 1,4,5-Trisubstituted N-Vinyl-1,2,3-triazoles. *Eur. J. Org. Chem.* **2017**, *3*, 459–464. [[CrossRef](#)]
57. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55–63. [[CrossRef](#)]
58. Glomb, T.; Szymankiewicz, K.; Świątek, P. Anti-cancer activity of derivatives of 1,3,4-oxadiazole. *Molecules* **2018**, *23*, 3361. [[CrossRef](#)]
59. Fu, D.J.; Fu, L.; Liu, Y.C.; Wang, J.W.; Wang, Y.Q.; Han, B.K.; Li, X.R.; Zhang, C.; Li, F.; Song, J.; et al. Structure-activity relationship studies of β-lactam-azide analogues as orally active antitumor agents targeting the tubulin colchicine site. *Sci. Rep.* **2017**, *7*, 12788. [[CrossRef](#)]
60. Ghorab, M.M.; Alsaied, M.S.; El-Gaby, M.S.; Safwat, N.A.; Elaasser, M.M.; Soliman, A.M. Biological evaluation of some new N-(2,6-dimethoxypyrimidinyl)thioureido benzenesulfonamide derivatives as potential antimicrobial and anticancer agents. *Eur. J. Med. Chem.* **2016**, *124*, 299–310. [[CrossRef](#)] [[PubMed](#)]
61. Petranyi, G.; Ryer, N.S.; Stutz, A. Allylamine derivatives: New class of synthetic antifungal agents inhibiting fungal squalene epoxidase. *Science* **1984**, *224*, 1239–1241. [[CrossRef](#)]
62. Ryder, N.S.; Dupont, M.C. Inhibition of squalene epoxidase by allylamine antimycotic compounds. A comparative study of the fungal and mammalian enzymes. *Biochem. J.* **1985**, *230*, 765–770.
63. Ryder, N.S.; Frank, I.; Dupont, M.C. Ergosterol biosynthesis inhibition by the thiocarbamate antifungal agents tolinaftate and tolciclate. *Antimicrob. Agents Chemother.* **1986**, *29*, 858–860. [[CrossRef](#)] [[PubMed](#)]
64. Morita, T.; Iwata, K.; Nozawa, Y. Inhibitory effect of a new mycotic agent, piritetrate on ergosterol biosynthesis in pathogenic fungi. *Med. Vet. Mycol.* **1989**, *27*, 17–25. [[CrossRef](#)] [[PubMed](#)]
65. Iwatani, W.; Arika, T.; Yamaguchi, H. Two mechanisms of butenafine action in *Candida albicans*. *Antimicrob. Agents Chemother.* **1993**, *37*, 785–788. [[CrossRef](#)] [[PubMed](#)]