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.](image)

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.](image)
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.](image-url)

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 NHNH2 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).

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.

Figure 4. ORTEP diagram of compound 4. Displacement ellipsoids are plotted at the 40% probability level for non-H atoms.
Table 1. Experimental details of compound 4.

| Crystal Data |
|--------------|
| Chemical formula | C_9H_{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 (Å³)       | 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 reflections | 12,396, 4728, 4213 |
| Rint          | 0.055             |

Refinement

| R[F2 > 2σ(F2)], wR(F2), 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 |

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$_{50}$ (µg/mL) | Viability % | Sample Concentration (µg/mL) | Sample |
|------------------|-------------|-------------------------------|--------|
| 500              | 125         | 62.5                          | 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    |

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

| IC$_{50}$ (µg/mL) | Viability % | Sample Concentration (µg/mL) | Sample |
|------------------|-------------|-------------------------------|--------|
| 500              | 125         | 62.5                          | 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 µg/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 µg/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 | 32 |     |
| Ampicillin    | -   | - |    |    | 17 | 19 |
| Gentamycin    | -   | - |    |    |    |    |

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 tolnaftate, tolciclate, and piritetrade 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) v max 3338–3201(NH₂+NH), 1717 (C=O) cm⁻¹; H 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); C 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 (0.536 g, 2 mmol) and appropriate aldehydes (4.2 mmol) in absolute ethanol/DMF (20 mL) 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) vmax 3274 (NH), 1714 (C=O), 1609 (C=N) cm⁻¹; 1H-NMR (CDCl₃): δ 1.92 (s, 3H, CH₃), 2.20 (s, 3H, CH₂), 6.78 (s, 2H, 2CH), 7.23–7.85 (m, 10H, ArH), 10.78 (s, 1H, NH), 11.75 (s, 1H, NH); 13C-NMR: δ 11.02 (CH₃), 14.0 (CH₂), 111.12, 128.5, 129.8, 131.3, 133.1, 136.0, 146.2, 151.4, 163.0, 167.2 (C=O). Anal. Calcd. for C₁₈H₁₈N₂O₂S (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) vmax 3515 (NH), 1686 (C=O), 1595 (C=N) cm⁻¹; 1H-NMR (CDCl₃): δ 1.90 (s, 3H, CH₃), 2.20 (s, 3H, CH₂), 2.45 (s, 6H, 2CH₃), 6.81 (s, 2H, 2CH), 7.28–7.84 (m, 8H, ArH), 11.25 (s, 2H, 2NH); 13C-NMR: δ 11.50 (CH₃), 14.31 (CH₂), 18.95 (2CH₃), 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 C₂₅H₂₉N₄O₂S (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) (0.268 g, 1 mmol) and phenyl isothiocyanate (0.27 g, 0.24 mL, 2 mmol) in EtOH (10 mL) was refluxed under slight agitation to complete the reaction (4 h). After cooling, the mixture was poured into a mixture of cold water and ice. The precipitated solid was filtered off, washed with water, dried, and recrystallized by ethanol to afford compound 4 [52] at 20% yield; mp. 55–56 °C; IR (KBr) vmax 3212 (NH), 3039, 2982 (CH) cm⁻¹; 1H NMR (CDCl₃): δ 1.23 (t, 3 H, CH₂), 4.65 (q, 2 H, CH₂), 7.20–7.46 (m, 5H, ArH), 9.12 (s, 1 H, NH); 13C-NMR (CDCl₃): 114.90, 68.50, 121.10, 121.10, 128.40, 129.57, 129.57, 137.85 (Ar-C), 188.50 (C=S). Anal. Calcd. for C₁₃H₁₁NOS (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). CS₂ (2.7 g, 21 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 H₂S ceased. After completion of the reaction, excess ethanol was removed under reduced pressure. The mixture was poured into a mixture of H₂O/ice and acidified with concentrated HCl. The precipitated solid was filtered off and recrystallized from ethanol/acetone to afford the corresponding bis(1,3,4-oxadiazole) 6. Yield (40%); mp. 250 °C; IR (KBr) vmax 3313 (NH), and 1116 (C=S) cm⁻¹; 1H NMR (CDCl₃): δ 1.23 (t, 3 H, CH₂), 4.65 (q, 2 H, CH₂), 7.20–7.46 (m, 5H, ArH), 9.12 (s, 1 H, NH); 13C NMR (CDCl₃): 114.90, 68.50, 121.10, 121.10, 128.40, 129.57, 129.57, 137.85 (Ar-C), 188.50 (C=S). Anal. Calcd. for C₁₃H₁₁NOS (181.25): C, 59.64; H, 6.12; N, 7.73. Found: C, 59.75; H, 6.07; N, 7.88.

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) vmax 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 C₁₅H₁₃N₄O₄S (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 H₂O, 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 H₂O, and dried: yield 90%; mp 120–121 °C; IR (KBr)
vmax 2982 (CH), 2167 (N=N), 1724 (C=O), 1686 (C=O) cm$^{-1}$; MS m/z (%) 290 (M$^+$, 14%), 40 (100%). Anal. Calcd for C$_{9}$H$_{6}$N$_{8}$O$_{2}$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) vmax 1694 (2C=O), 1594 (C=N) cm$^{-1}$; $^1$H-NMR (CDCl$_3$): $\delta$ 1.31 (s, 6H, 2CH$_3$), 2.26 (s, 6H, 2CH$_3$), 2.38 (s, 6H, 2CH$_3$), 4.32 (q, 4H, 2CH$_2$); $^{13}$C-NMR: $\delta$ 13.40, 14.30, 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 C$_{21}$H$_{22}$N$_{8}$O$_{6}$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) vmax 1697 (2C=O), 1597 (C=N) cm$^{-1}$; $^1$H-NMR (CDCl$_3$): $\delta$ 1.19 (s, 6H, 2CH$_3$), 2.46 (s, 3H, CH$_3$), 3.30 (s, 3H, CH$_3$), 4.28 (q, 4H, 2CH$_2$), 7.71 (s, 4H, NH$_2$); $^{13}$C-NMR: $\delta$ 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 C$_{19}$H$_{20}$N$_{10}$O$_{6}$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, $^1$H-NMR, and $^{13}$C-NMR spectra of compound 1, Figures S5–S7: IR, $^{13}$C-NMR and Mass spectra of compound 2, Figures S8–S10: IR, $^1$H-NMR, and $^{13}$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.

Author Contributions: Data curation, A.A., A.B.M., Y.I.A., F.A.A.-a., H.A.G. and Y.N.M.; Formal analysis, A.A., H.A.G. and Y.N.M.; Funding acquisition, Y.N.M.; Investigation, Y.N.M.; Methodology, Y.N.M.; Project administration, Y.N.M.; Supervision, Y.I.A. and Z.M.A.; Writing—original draft, Y.N.M.; Writing—review & editing, N.A.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by the Deanship of Scientific Research, King Khalid University (Grant No. R.G.P.2/26/ 42).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.
Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The financial support by the Deanship of Scientific Research (Grant No. R.C.P.2/26/42), King Khalid University, Saudi Arabia is gratefully acknowledged.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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

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