Ultrasound-Assisted Synthesis and In Silico Modeling of Methanesulfonyl-Piperazine-Based Dithiocarbamates as Potential Anticancer, Thrombolytic, and Hemolytic Structural Motifs

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Abstract: Piperazine-based dithiocarbamates serve as important scaffolds for numerous pharmacologically active drugs. The current study investigates the design and synthesis of a series of dithiocarbamates with a piperazine unit as well as their biological activities. Under ultrasound conditions, the corresponding piperazine-1-carbodithioates were synthesized from monosubstituted piperazine and N-phenylacetamides in the presence of sodium acetate and carbon disulfide in methanol. The structures of the newly synthesized piperazines were confirmed, and their anti-lung carcinoma effects were evaluated. A cytotoxic assay was performed to assess the hemolytic and thrombolytic potential of the synthesized piperazines. The types of substituents on the aryl ring were found to affect the anticancer activity of piperazines. Piperazines containing 2-chlorophenyl and 2,4-dimethylphenyl moieties demonstrated the most potent antiproliferative activity. On the other hand, piperazines containing 3,4-dichlorophenyl and 3,4-dimethylphenyl rings demonstrated the least cytotoxicity. The piperazine with the 2,5-dimethoxyphenyl moiety showed the best thrombolytic effect. To determine the mode of binding, in silico modeling of the most potent piperazine was performed, and the results were in accordance with those of antiproliferation. It exhibits a similar binding affinity to PQ10 and an efficient conformational alignment with the lipophilic site of PDE10A conserved for PQ10A.

Keywords: anticancer; piperazine dithiocarbamates; hemolysis; in silico modeling; lung carcinoma; ultrasound

1. Introduction

Lung carcinoma is one of the most well-known causes of cancer death in many countries [1,2]. The annual death rate from lung carcinoma is double that of all other tumors combined [3,4]. The incomplete development of early detection methods is largely responsible for lung carcinoma’s uncontrolled spread [5]. Smoking is the leading cause of lung carcinoma worldwide [6]. Early diagnosis of other tumors, such as colon carcinoma,
cervical carcinoma, and esophageal carcinoma, increases the survival rate [7]. It is vital to develop novel drug candidates for this type of carcinoma [8].

Structurally modified nitrogen heterocycles have a broad spectrum of biological activities [9]. For example, heterocycles containing the piperazine moiety have a significant role in drug discovery programs [10]. Due to their vast array of pharmaceutical applications, N-substituted piperazines have garnered attention in academia and the pharmaceutical industry. They exhibit antimicrobial [11], antimycobacterial [12], antidepressant [13], and anticancer [14,15] properties. Previous reports revealed that N-substituted piperazines are a crucial structural component of the anticancer effect [16,17]. The presence of polar nitrogen in the piperazine framework is an essential component of the skeletons of numerous biologically active drugs [18]. Figure 1 shows the structures of some common piperazine-based drugs.

![Figure 1. Structures of some common piperazine-based drugs.](image1)

Dithiocarbamates are important intermediates in the synthesis of many pharmacologically active molecules [19,20]. They are active ingredients in numerous drugs (Figure 2), as well as naturally occurring and synthesized anticancer agents [21,22]. Including a dithiocarbamate moiety in the skeleton of different organic compounds elevates the biological profile of the synthesized derivatives [23].

![Figure 2. Structures of dithiocarbamate based drugs.](image2)

The incorporation of two or more biologically active molecules to create new hybrid structures with potential medicinal applications is a useful approach [24]. This strategy is adopted in an effort to enhance the biological profile of newly synthesized hybrids compared to individual molecules included in their skeletons [25,26]. In continuation of our research on hybrid molecules with cytotoxic potential, we now report the design, synthesis, and antiproliferative effect of piperazine-based acetamides against lung carcinoma (A-549).

2. Results and Discussion

2.1. Chemistry

Several piperazine-dithiocarbamate hybrids 5a–5j were synthesized, as shown in Scheme 1. The treatment of excess piperazine (1) with methane sulfonyl chloride at 0 °C in dichloromethane (DCM) produced monosubstituted piperazine 2 in an 80% yield [27]. Treating aryl amines 3a–3j in a basic medium with bromoacetyl bromide afforded the corresponding N-phenyl acetamides 4a–4j [28,29]. Reactions of 2 and 4a–4j in the presence of sodium acetate (AcONa) and carbon disulfide (CS₂) in methanol (MeOH) for 12–18 h at
room temperature produced 5a–5j in 55–65% yields. Piperazine-1-carbodithioates 5a–5j were obtained in excellent yields (80–90%) when reactions of 2 and 4a–4j were carried out under ultrasonic conditions at 70 °C for 30 min.

The structures of piperazine-1-carbodithioates 5a–5j were established using a variety of spectroscopic techniques, and their purity was confirmed by elemental analyses (see the experimental section for details). The NMR spectra of 5a–5j are shown in Figures S1–S20. The HRMS confirms the chemical formula of each derivative’s molecular ion peak. The FTIR spectra showed the presence of absorption bands corresponding to the NH (3230–3330 cm\(^{-1}\)), C=O (1670–1660 cm\(^{-1}\)), C=C (1530–1520 cm\(^{-1}\)), and C=S (1225–1220 cm\(^{-1}\)) groups. The NMR spectra of 5a–5j contained all of the expected protons and carbons. At high fields, the \(^1\)HNMR spectra of 5a–5j revealed the presence of methyl protons of the sulfonyl group (2.85–2.79 ppm) and methylene protons (4.36–4.20 ppm), as well as piperazine and aryl protons. At very low fields, the \(^{13}\)CNMR spectra revealed the presence of C=S (197.4–196.6 ppm) and C=O (167.0–163.9 ppm) carbons.

2.2. Antiproliferative Potential

The anticancer potential of newly synthesized piperazine-1-carbodithioates 5a–5j against the human lung cancer cell line (A-549) was investigated using the MTT assay [30]. The cell viability of 5a–5j varied depending on the aryl ring substituents (Table 1).

| Piperazine | Ar                  | Cell Viability A-549 \(\pm\) SD | Hemolysis (%) | Thrombolysis (%) |
|-----------|---------------------|---------------------------------|---------------|------------------|
| 5a        | Phenyl              | 61.35 ± 2.29                    | 3.1           | 58.9             |
| 5b        | 2-Chlorophenyl      | 25.11 ± 2.49                    | 1.27          | 54.4             |
| 5c        | 4-Chlorophenyl      | 60.29 ± 5.96                    | 10            | 47.8             |
| 5d        | 3,4-Dichlorophenyl  | 38.08 ± 2.85                    | 0.1           | 56.03            |
| 5e        | 2-Fluorophenyl      | 68.94 ± 6.64                    | 0.3           | 53.5             |
| 5f        | 4-Fluorophenyl      | 41.01 ± 3.73                    | 2.8           | 50.3             |
| 5g        | 4-Methoxyphenyl     | 43.36 ± 4.42                    | 1.3           | 50.9             |
| 5h        | 2,5-Dimethoxyphenyl | 40.25 ± 3.34                    | 7.1           | 60.2             |
| 5i        | 2,4-Dimethoxyphenyl | 25.31 ± 3.62                    | 2.8           | 57.4             |
| 5j        | 3,4-Dimethoxyphenyl | 47.94 ± 1.57                    | 0.1           | 58.7             |
| Standard  |                     | 100 ± 0                         | 0.01          | 0.57             |
| DMSO      |                     |                                 | 95.9          | 80               |
| ABTS      |                     |                                 |               |                  |

\(\text{a Cell viability: (Mean ± SD) in triplicate with a concentration of 100 \(\mu\)g/mL. DMSO and ABTS were used as negative and positive controls, respectively.}\)
The SAR analysis of 5a–5j was carried out to determine the role of various substituents on the aryl ring. Compounds 5b and 5i produced the best results, as shown in Table 1. The most potent derivative was compound 5b, which contains a chlorine substituent at the ortho-position of the aryl ring and has a cell viability of 25.11 ± 2.49. The cell viability of compound 5i, which contains two methyl groups at the ortho and para positions of the aryl ring, was comparable to that of compound 5b, at 25.31 ± 3.62. Compounds 5d, 5f, and 5h exhibited a significant effect, but it was lower than 5b and 5i. Compound 5e, with a fluorine atom at the ortho-position of the aryl ring, showed the lowest potency and highest cell viability (68.94 ± 6.64), followed by 5c (60.29 ± 5.96), which has a chlorine substituent at the para-position of the aryl ring.

The cell viability of 5b and 5i was investigated further using various concentrations (0.3–200 µg) to test the dose-response relationship. Figures 3 and 4 show that 5b and 5i produced the best results at a dose of 200 µg.

2.3. Hemolytic Potential

The cytotoxic activity of 5a–5j was investigated using a reported methodology [31]. The age hemolysis (%) shown in Table 1 indicated that 5a–5j had a low cytotoxic profile. The age hemolysis (%) for compound 5c was 10%, demonstrating its binding affinity for
hemoglobin, followed by 5h (7%). Compounds 5a (3.1%), 5b (1.27%), 5f (2.8%), 5g (1.3%), and 5i (2.8%) exhibited low toxicity. Compounds 5e (0.3%), 5d, and 5j (0.1%) had the lowest cytotoxicity when compared to ABTS (95.9%).

2.4. Thrombolytic Potential

Cerebral venous sinus thrombosis (CVST) is a common central nervous system disorder caused by thrombophilia syndrome. Due to its anticoagulant profile, heparin is regarded as an effective treatment for CVST. Many thrombolytic agents are now used to treat thrombophilia syndrome. A reported methodology was used to evaluate compounds 5a–5j for thrombolysis [31]. Compound 5h, which contains a methoxy group at the ortho-position of the aryl ring, showed the highest potential (60.2%). Compounds 5a (58.9%), 5i (57.4%), and 5j (58.7%) showed moderate thrombolytic potential. Compounds 5c (47.8%), 5f (50.3%), and 5g (50.9%) had a mild effect compared to the positive control (i.e., ABTS; 80%).

2.5. Structure-Activity Relationship (SAR) Study

The effect of aryl ring substituents in compounds 5a–5j on anticancer activity against A-549 was investigated. Compound 5b, with Cl at the ortho-position of the aryl ring, exhibited the lowest cell viability and thus proved to be a potent cytotoxic agent. The replacement of Cl in 5b (cell viability = 25.11 ± 2.49) with hydrogen (i.e., 5a; cell viability = 61.35 ± 2.29) or fluorine (i.e., 5e; cell viability = 68.94 ± 6.64) resulted in a significant decrease in cytotoxic potential. However, the substitution of Cl in 5b with a fluorine atom at the para-position of the aryl ring (i.e., 5f; cell viability = 41.01 ± 3.73) or a chlorine atom at the ortho-position (i.e., 5c; cell viability = 60.29 ± 5.96) led to a sharp decrease in anticancer activity. In contrast, the insertion of two Cl at the meta and para-positions of the aryl ring (i.e., 5d; cell viability = 38.08 ± 2.85) enhances anticancer activity. Inserting a methoxy group at the para-position of the aryl ring (i.e., 5g; cell viability = 43.36 ± 4.42), on the other hand, led to a significant change in anticancer activity. The two methoxy substituents at the ortho and meta-positions in compound 5h (cell viability = 40.25 ± 3.34) are responsible for the good antiproliferative potential when compared to 5e, which has a methoxy group at the ortho-position of the aryl ring. However, the replacement of the Cl in 5b with two methoxy groups at the ortho and para-positions of the aryl ring (i.e., 5i; cell viability = 25.31 ± 3.62) resulted in better activity when compared to the case where two methyl groups were present at the meta and para-positions of the aryl ring (i.e., 5j; cell viability = 47.94 ± 1.57).

The trend for SAR studies related to hemolysis (Table 1) suggested that the presence of the chlorine atom at the para-position of the aryl ring (i.e., 5c) showed the highest cytotoxic potential (10%) among the others. Compounds 5d and 5j exhibited the lowest cytotoxic potential (0.1%) for hemolysis, implying that the Cl and OMe substituents at the ortho and para-positions of the aryl ring have no affinity for hemoglobin. When compared to 5d and 5j, the two methoxy substituents at the ortho and meta-positions of the aryl ring (i.e., 5h; 7.1%) increased the cytotoxic activity. However, the unsubstituted phenyl ring (i.e., 5a) has 30 times more cytotoxic activity (3.1%).

Based on the data from the SAR study of piperazine-1-carbodithioates 5a–5j, it is concluded that the presence of the Cl motif at the ortho-position of the aryl ring played a significant role in the antiproliferative activity. The presence of chlorine, dimethyl, and methoxy substituents improves anticancer activity (Figure 5).
2.6. In Silico Modeling

The mode of action of compound 5b was investigated further in silico. The SwissTargetPrediction identified phosphodiesterase 10A (PDE10A) as a potential target for 5b with the highest (~1600) active similarity and 0.105 probability. The IFD method was validated by cognate re-docking, which resulted in a conformation with 0.2519 Å RMSD compared to the co-crystallized native conformation (Figure 6).

The IFD was carried out to investigate the binding of 5b with its potential therapeutic target and simulate its binding conformation into the catalytic site. The binding free energy analysis revealed that compound 5b binds at the catalytic site of PDE10A with $\Delta G$ of $-8.30 \text{ kcal/mol}$ which is comparable to $-8.97 \text{ kcal/mol}$ for the PQ-10 as a standard for PDE10A (Table 2).
Table 2. The IFD parameters of 5b and PQ-10 simulated in the active site of PDE10A.

| Ligand | ΔG (kcal/mol) | Interacting Residues | Interaction Type |
|--------|---------------|-----------------------|------------------|
| 5b     | −8.30         | TYR514, PHE686, TYR683, VAL712, PRO702, MET703, GLU711, SER667 | H–bonding, sulfur–X, π–σ, π–π stacked, amide–π stacked, alkyl, π–alkyl |
| PQ-10  | −8.97         | GLU711, PRO702, TYR683, VAL712, GLN716, ILE682, ALA679, TYR514, ASP664, PHE716, MET703 | H–bonding, sulfur–X, π–σ, π–π stacked, amide–π stacked, alkyl, π–alkyl |

The conformational analysis of compound 5b highlighted its efficiency in attaining a binding pose that established the diverse interactions with the vital residues within the catalytic pocket of PDE10A (Figure 7). Interestingly, 5b shares similar interaction patterns to PQ-10. It is efficiently penetrated and anchored into the lipophilic pocket of PDE10A within the catalytic site which is inaccessible in all other isoforms of PDE. Therefore, the comparable binding behavior and affinity may suggest that 5b exhibits a similar activity compared to PQ-10.

![Figure 7](image_url)
interact with GLN726 conserved in other PDE isoforms, resulting in greater selectivity for PDE10A.

Figure 8. The Interaction behavior of ligands within the active site of PDE10A; (a) a 2D representation of PQ10 and (b) compound 5b interacting with the PDE10A catalytic residues colored by type of interactions.

Compound 5b exhibits a binding affinity comparable to that of PQ10 and an efficient conformational alignment with the lipophilic site of PDE10A conserved for PQ10A. In addition, 5b establishes interactions with the conserved PQ10 residues, but with a distinct bonding pattern, implying potential differences in activity and selectivity. This study provides valuable insight into the potential therapeutic activity of 5b against PDE10A.

3. Materials and Methods

3.1. General

Chemicals, solvents, and reagents were purchased from Merck (Gillingham, UK) and used without further purification. Melting points were recorded on a Gallenkamp instrument (Fisons; Uckfield, UK). The $^1$H (400 MHz) and $^{13}$C NMR (100 MHz) spectra were recorded in deuterated chloroform (CDCl$_3$) using a Bruker DPX spectrophotometer
(Bruker; Zürich, Switzerland). The chemical shifts were recorded in ppm related to either tetramethylsilane or CDCl₃. Thin-layer chromatography (DCM/MeOH) was utilized in conjunction with a Spectroline E-Series UV lamp to monitor the progression of chemical reactions (Merck; Gillingham, UK). Compound 2 [27] and N-phenyl acetamides 4a–4j [28,29] were produced based on reported procedures.

3.2. General Procedures for the Synthesis of Piperazine-1-carbodithioates 5a–5j

3.2.1. Conventional Method

A solution of appropriate N-phenylacetamides 4a–4j (0.6 mmol) in dry MeOH (0.3 mL) was added to a stirred mixture of 2 (0.10 g, 0.6 mmol), anhydrous AcONa (0.36 g, 0.60 mmol), and CS₂ (0.39 g, 0.6 mmol) in dry MeOH (0.8 mL). The mixture was stirred at 20 °C for 12–18 h according to the TLC. The produced solid was collected, washed with H₂O, dried, and recrystallized from EtOH to give pure 5a–5j as white solids in moderate to good yields (55–65%).

3.2.2. Ultrasound-Assisted Method

The procedure was identical to that described in Section 3.2.1. with the exception that the mixture was sonicated at 70 °C for 30 min. Following crystallization, pure 5a–5j was obtained in excellent yields (80–90%) as white solids.

3.2.3. 2-Oxo-2-(phenylamino)ethyl 4-(methylsulfonyl)piperazine-1-carbodithioate (5a)

Yield 90%, m.p. 188–189 °C. IR: ν (cm⁻¹): 3230 (NH), 1664 (C=O), 1524 (C=C), 1473 (CH₂), 1222 (C=S). ¹H NMR (δ): 8.79 (s, 1H, NH), 7.48 (d, 2H, J = 8.0 Hz, Ar), 7.29 (t, 2H, J = 8.0 Hz, Ar), 7.08 (t, 1H, J = 8.0 Hz, Ar), 4.48 (br s, 2H, piperazinyl), 4.22 (s, 2H, SCH₂), 4.10 (br s, 2H, piperazinyl), 3.36 (t, 4H, J = 4.0 Hz, piperazinyl), 2.79 (s, 3H, Me). ¹³C NMR (δ): 197.4 (C=S), 166.8 (C=O), 137.8, 129.6, 124.7, 120.1, 45.7 (NCH₂), 45.4 (NCH₂), 40.7 (SMe), 35.5 (SCH₂). HRMS: m/z [M⁺] calculated for C₁₄H₁₉N₃O₃S₃: 373.0589; found: 373.0582. Analysis calculated for C₁₄H₁₉N₃O₃S₃: C, 45.02; H, 5.13; N, 11.25; found: C, 45.07; H, 5.17; N, 11.28%.

3.2.4. 2-((2-Chlorophenyl)amino)-2-oxoethyl 4-(methylsulfonyl)piperazine-1-carbodithioate (5b)

Yield 82%, m.p. 180–181 °C. IR: ν (cm⁻¹): 3330 (NH), 1662 (C=O), 1524 (C=C), 1470 (CH₂), 1220 (C=S). ¹H NMR (δ): 8.80 (s, 1H, NH), 8.36 (d, 1H, J = 8.0 Hz, Ar), 7.37 (d, 1H, J = 8.0 Hz, Ar), 7.08 (t, 2H, J = 8.0 Hz, Ar), 7.06 (t, 1H, J = 8.0 Hz, Ar), 4.55 (br s, 2H, piperazinyl), 4.32 (s, 2H, SCH₂), 4.24 (br s, 2H, piperazinyl), 3.14 (t, 4H, J = 4.0 Hz, piperazinyl), 2.84 (s, 3H, SMe). ¹³C NMR (δ): 197.4 (C=S), 166.7 (C=O), 134.9, 128.3, 125.2, 123.5, 121.8, 50.0 (NCH₂), 45.3 (NCH₂), 40.9 (SMe), 35.3 (SCH₂). HRMS: m/z [M⁺] calculated for C₁₄H₁₉ClN₃O₃S₃: 407.0199; found: 407.0209. Analysis calculated for C₁₄H₁₉ClN₃O₃S₃: C, 41.22; H, 4.45; N, 10.30; found: C, 41.26; H, 4.48; N, 10.35%.
3.2.5. 2-((4-Chlorophenyl)amino)-2-oxoethyl 4-(methylsulfonyl) piperazine-1-carbodithioate (5c)

![Chemical Structure Image]

Yield 87%, m.p. 200–201 °C. IR: ν (cm⁻¹): 3230 (NH), 1662 (C=O), 1525 (C=C), 1470 (CH₂), 1225 (C=S). ¹H NMR (δ): 8.89 (s, 1H, NH), 7.43 (d, 2H, J = 8.0 Hz, Ar), 7.26 (d, 2H, J = 8.0 Hz, Ar), 4.49 (br s, 2H, piperazinyl), 4.21 (s, 2H, SCH₂), 4.97 (br s, 2H, piperazinyl), 3.38 (t, 4H, J = 4.0 Hz, piperazinyl), 2.84 (s, 3H, SMe). ¹³C NMR (δ): 197.4 (C=S), 167.0 (C=O), 136.1, 129.6, 129.3, 121.4, 119.2, 45.6 (NCH₂), 45.3 (NCH₂), 40.73 (SMe), 35.4 (SCH₂). HRMS: m/z [M]+ calculated for C_{14}H_{18}ClN_{3}O_{3}S_{3}: 407.0199; found: 407.0209. Analysis calculated for C_{14}H_{18}ClN_{3}O_{3}S_{3}: C, 41.22; H, 4.45; N, 10.30; found: C, 41.25; H, 4.46; N, 10.33%.

3.2.6. 2-((3,4-Dichlorophenyl)amino)-2-oxoethyl 4-(methylsulfonyl) piperazine-1-carbodithioate (5d)

![Chemical Structure Image]

Yield 86%, m.p. 162–163 °C. IR: ν (cm⁻¹): 3235 (NH), 1667 (C=O), 1528 (C=C), 1470 (CH₂), 1222 (C=S). ¹H NMR (δ): 8.89 (s, 1H, NH), 7.69 (s, 1H, Ar), 7.33 (d, 1H, J = 8.0 Hz, Ar), 7.24 (d, 1H, J = 8.0 Hz, Ar), 4.48 (br s, 2H, piperazinyl), 4.20 (s, 2H, SCH₂), 4.12 (br s, 2H, piperazinyl), 3.37 (t, 4H, J = 4.0 Hz, piperazinyl), 2.81 (s, 3H, SMe). ¹³C NMR (δ): 197.4 (C=S), 166.7 (C=O), 137.4, 132.9, 130.6, 127.7, 121.5, 119.2, 45.6 (NCH₂), 45.4 (NCH₂), 40.4 (SMe), 35.6 (SCH₂). HRMS: m/z [M]+ calculated for C_{14}H_{17}Cl_{2}N_{3}O_{3}S_{3}: 440.9809; found: 440.9812. Analysis calculated for C_{14}H_{17}Cl_{2}N_{3}O_{3}S_{3}: C, 38.01; H, 3.87; N, 9.50; found: C, 38.05; H, 3.87; N, 9.54%.

3.2.7. 2-((2-Fluorophenyl)amino)-2-oxoethyl 4-(methylsulfonyl) piperazine-1-carbodithioate (5e)

![Chemical Structure Image]

Yield 84%, m.p. 182–183 °C. IR: ν (cm⁻¹): 3250 (NH), 1670 (C=O), 1530 (C=C), 1475 (CH₂), 1225 (C=S). ¹H NMR (δ): 8.80 (s, 1H, NH), 8.27 (d, 1H, J = 8.0 Hz, Ar), 7.04 (d, 1H, J = 8.0 Hz, Ar), 4.46 (br s, 2H, piperazinyl), 4.27 (s, 2H, SCH₂), 4.10 (br s, 2H, piperazinyl), 3.37 (t, 4H, J = 4.0 Hz, piperazinyl), 2.80 (s, 3H, SMe). ¹³C NMR (δ): 197.4 (C=S), 166.4 (C=O), 154.2 (d, J = 243 Hz), 151.41, 126.3 (d, J = 10 Hz), 124.8 (d, J = 3 Hz), 121.8, 115.0 (d, J = 8 Hz), 45.6 (NCH₂), 45.4 (NCH₂), 40.8 (SMe), 35.4 (SCH₂). HRMS: m/z [M]+ calculated for C_{14}H_{18}FN_{3}O_{3}S_{3}: 391.0494; found: 391.0496. Analysis calculated for C_{14}H_{18}FN_{3}O_{3}S_{3}: C, 42.95; H, 4.63; N, 10.73; found: C, 42.92; H, 4.67; N, 10.74%.
3.2.8. 2-((4-Fluorophenyl)amino)-2-oxoethyl 4-(methylsulfonyl) piperazine-1-carbodithioate (5f)

Yield 83%, m.p. 185–186 °C. IR: ν (cm⁻¹): 3330 (NH), 1660 (C=O), 1520 (C=C), 1470 (CH₂), 1222 (C=S). ¹H NMR (δ): 8.80 (s, 1H, NH), 7.50 (m, 2H, Ar), 7.02 (m, 2H, Ar), 4.52 (br s, 2H, piperazinyl), 4.25 (s, 2H, SCH₂), 4.09 (br s, 2H, piperazinyl), 3.40 (t, 4H, J = 4.0 Hz, piperazinyl), 2.85 (s, 3H, SMe). ¹³C NMR (δ): 197.4 (C=S), 168.3 (C=O), 159.4 (d, J = 240 Hz), 133.9, 121.6, 116.5, 50.0 (NCH₂), 45.7 (NCH₂), 40.3 (SMe), 35.3 (SCH₂). HRMS: m/z [M⁺] calculated for C₁₄H₁₈FN₃O₃S₃: 391.0494; found: 391.0496. Analysis calculated for C₁₄H₁₈FN₃O₃S₃: C, 42.95; H, 4.63; N, 10.73; found: C, 42.97; H, 4.65; N, 10.76%.

3.2.9. 2-((4-Methoxyphenyl)amino)-2-oxoethyl 4-(methylsulfonyl) piperazine-1-carbodithioate (5g)

Yield 80%, m.p. 190 °C. IR: ν (cm⁻¹): 3330 (NH), 1665 (C=O), 1528 (C=C), 1470 (CH₂), 1222 (C=S). ¹H NMR (δ): 8.63 (s, 1H, NH), 7.40 (d, 2H, J = 8.0 Hz, Ar), 6.83 (d, 2H, J = 8.0 Hz, Ar), 4.46 (br s, 2H, piperazinyl), 4.20 (s, 2H, SCH₂), 4.09 (br s, 2H, piperazinyl), 3.76 (s, 3H, OMe), 3.36 (t, 4H, J = 4.0 Hz, piperazinyl), 2.80 (s, 3H, SMe). ¹³C NMR (δ): 197.4 (C=S), 166.1 (C=O), 157.0, 131.2, 122.3, 114.6, 56.0 (OMe), 50.2 (NCH₂), 45.4 (NCH₂), 40.7 (SMe), 35.8 (SCH₂). HRMS: m/z [M⁺] calculated for C₁₅H₂₁N₃O₄S₃: 403.0694; found: 403.0697. Analysis calculated for C₁₅H₂₁N₃O₄S₃: C, 44.65; H, 5.25; N, 10.41; found: C, 44.67; H, 5.28; N, 10.45%.

3.2.10. 2-((2,5-Dimethoxyphenyl)amino)-2-oxoethyl 4-(methylsulfonyl) piperazine-1-carbodithioate (5h)

Yield 81%, m.p. 150 °C. IR: ν (cm⁻¹): 3270 (NH), 1668 (C=O), 1524 (C=C), 1470 (CH₂), 1222 (C=S). ¹H NMR (δ): 8.87 (s, 1H, NH), 8.04 (s, 1H, Ar), 6.75 (d, 1H, J = 8.0 Hz, Ar), 6.54 (d, 1H, J = 8.0 Hz, Ar), 4.36 (br s, 2H, piperazinyl), 4.26 (s, 2H, SCH₂), 4.16 (br s, 2H, piperazinyl), 3.81 (s, 3H, OMe), 3.75 (s, 3H, OMe), 3.35 (t, 4H, J = 4.0 Hz, SMe), 2.79 (s, 3H, SMe). ¹³C NMR (δ): 196.6 (C=S), 165.9 (C=O), 153.9, 142.5, 128.3, 111.2, 109.0, 106.1, 56.6 (OMe), 55.8 (OMe), 45.3 (NCH₂), 45.2 (NCH₂), 41.3 (SMe), 35.2 (SCH₂). HRMS: m/z [M⁺] calculated for C₁₆H₂₃N₃O₅S₃: 433.0800; found: 433.0805. Analysis calculated for C₁₆H₂₃N₃O₅S₃: C, 44.32; H, 5.35; N, 9.69; found: C, 44.35; H, 5.39; N, 9.73.
3.2.11. 2-((2,4-Dimethylphenyl)amino)-2-oxoethyl 4-(methylsulfonyl)piperazine-1-carbodithioate (5i)

Yield 87%, m.p. 186–187 °C. IR: ν (cm⁻¹): 3330 (NH), 1665 (C=O), 1525 (C=C), 1475 (CH₂), 1220 (C=S). ¹H NMR (δ): 8.30 (s, 1H, NH), 7.69 (d, 1H, J = 8.0 Hz, Ar), 6.99 (s, 1H, Ar), 6.96 (d, 1H, J = 8.0 Hz, Ar), 4.47 (br s, 2H, piperazinyl), 4.27 (s, 2H, SCH₂), 4.13 (br s, 2H, piperazinyl), 3.35 (t, 4H, J = 4.0 Hz, piperazinyl), 2.80 (s, 3H, SMe), 2.26 (s, 3H, Me), 2.23 (s, 3H, Me). ¹³C NMR (δ): 197.4 (C=S), 166.6 (C=O), 135.0, 133.2, 131.3, 129.2, 127.3, 122.8, 45.0 (NCH₂), 45.4 (NCH₂), 40.6 (SMe), 35.4 (SCH₂), 21.0 (Me), 18.4 (Me). HRMS: m/z [M]+ calculated for C₁₆H₂₃N₃O₃S₃: 401.0902; found: 401.0906. Analysis calculated for C₁₆H₂₃N₃O₃S₃: C, 47.86; H, 5.77; N, 10.46%; found: C, 47.90; H, 5.79; N, 10.48%.

3.2.12. 2-((3,4-Dimethylphenyl)amino)-2-oxoethyl-4-(methylsulfonyl)piperazine-1-carbodithioate (5j)

Yield 82%, m.p. 170–171 °C. IR: ν (cm⁻¹): 3235 (NH), 1660 (C=O), 1525 (C=C), 1470 (CH₂), 1220 (C=S). ¹H NMR (δ): 8.65 (s, 1H, NH), 7.28 (s, 1H, Ar), 7.26 (d, 1H, J = 8.0 Hz, Ar), 7.08 (d, 1H, J = 8.0 Hz, Ar), 4.53 (br s, 2H, piperazinyl), 4.26 (s, 2H, SCH₂), 4.15 (br s, 2H, piperazinyl), 3.39 (t, 4H, J = 4.0 Hz, piperazinyl), 2.84 (s, 3H, SMe), 2.26 (s, 3H, Me), 2.23 (s, 3H, Me). ¹³C NMR (δ): 197.4 (C=S), 163.9 (C=O), 137.9, 135.9, 133.2, 129.9, 117.1, 50.0 (NCH₂), 49.6 (NCH₂), 40.9 (SMe), 35.2 (SCH₂), 19.9 (Me), 19.2 (Me). HRMS: m/z [M]+ calculated for C₁₆H₂₃N₃O₃S₃: 401.0902; found: 401.0906. Analysis calculated for C₁₆H₂₃N₃O₃S₃: C, 47.86; H, 5.77; N, 10.46%; found: C, 47.89; H, 5.77; N, 10.50%.

3.3. Experimental Procedures for Antiproliferative, Hemolytic, and Thrombolytic Activity

3.3.1. Culture and Treatment of Cell Lines

The activity of piperazin-1-carbodithioates 5a–5j against human liver carcinoma (A-549) was investigated. Dulbecco’s Modified Eagles Medium (DMEM) was used for the cultural growth of A-549. The DMEM was enriched with fetal bovine serum (10%), streptomycin (100 units/mL), and penicillin (100 µg/mL). The inoculation was carried out in a moistened atmosphere containing carbon dioxide (5%). Compounds 5a–5j were dissolved in dimethyl sulfoxide (DMSO). The cell cultures (0.05%) were added to DMSO and employed as a negative control [32,33].

3.3.2. Evaluation of Cell Viability

A-549 cells (100 µL) were cultured overnight in 96-well plates, treated with compounds 5a–5j, and incubated (48 h at 37 °C). The MTT reagent (10 µL, 5 mg/mL) was added to each plate and incubated (4 h at 37 °C). In the final step, each cell plate was diluted with DMSO (150 µL), and the absorbance was estimated at 490 nm using a microplate-reader to calculate the cell viability percentage.
3.3.3. Hemolytic Potential

A blood sample (5 mL) from albino mice was centrifuged (1000 rpm for 5 min). The pellet of red blood cells (RBCs) was isolated and washed four times with chilled phosphate buffer saline (pH 7.4). A solution of 5a–5j (20 µL; 10 mg/mL) was added to the RBCs pellet (180 µL). The sample was incubated (30 min at 37 °C); removed from the incubator; cooled in an ice bath for 5 min; and centrifuged (13,000 rpm for 5 min). Ice-cold phosphate buffer saline was added to the supernatant (100 µL) in each tube. The DMSO was designated as a negative control, while 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS)) served as a positive control. The absorbance of the sample was measured at 517 nm, and the age hemolysis (%) was calculated using Equation (1) [34].

\[
\text{age hemolysis (\%)} = \frac{\text{Absorbance of sample} - \text{Absorbance of negative control}}{\text{Absorbance of positive control}} \times 100 \quad (1)
\]

3.3.4. Thrombolytic Potential

A blood sample (1 mL) was taken from albino mice and transferred to various rinsed and weighed Eppendorf tubes. The Eppendorf tube sample was incubated (1 h at 37 °C) to form clots. The serum was discarded, and the Eppendorf tubes were weighed to calculate the initial weight of the clot. A solution (100 µL) of 5a–5j in DMSO was added separately to the Eppendorf tubes and incubated (3 h at 37 °C). The ABTS was used as a positive control, while water was used as a negative control. The serum was removed again, and the Eppendorf tubes were weighed to measure the clot lysis (%) using Equation (2) [35].

\[
\text{Clot lysis (\%)} = \frac{\text{Initial clot weight} - \text{Final clot weight}}{\text{Initial weight of the clot}} \times 100 \quad (2)
\]

3.4. Computational Study

Compound 5b was investigated for its in silico-modeled anticancer activity and potential mechanism of action. A potential target of compound 5b was predicted using the SwissTargetPrediction tool. It employs a ligand-based approach and derives the probability of prediction using 2D and 3D similarity to chemical compounds with known targets [36]. Compound 5b was molecularly docked on a predicted target to investigate its binding affinity, mode of action, and interactions using the induced fit docking (IFD) protocol in the Molecular Operating Environment (MOE) 2015.10. The IFD protocol was validated by cognate redocking of the co-crystallized ligand within the active site, with the root-mean-square deviation (RMSD) serving as the measure of validation. The PubChem CID: 11704101 (PQ-10) is an inhibitor of PDE10A in lung carcinoma and is modeled as a standard molecule in this simulation. The 3D X-ray crystalized structure of phosphodiesterase 10A (PDB ID: 3HR1; 1.53 Å) was retrieved from the RSCB Protein Data Bank (http://www.rscb.org; accessed on 2 February 2022). The inherent structural problems of macromolecules were corrected in the QuickPrep module of MOE. The structure was further protonated and minimized under the Amber 10:EHT forcefield to optimize the molecular mechanics’ refinements and tether restraints in docked poses. The Site Finder module defined the binding pocket in the vicinity of the co-crystallized ligand at the active site. The Dock module was used to dock Compound 5b using the triangular matcher placement method and the London dG scoring function. The docked poses were further refined using the IFD method and the GBVI/WSA dG scoring function. A pose with the lowest binding free energy (ΔG) was investigated further for potential binding affinity, binding pose, and interactions using Discovery Studio Visualizer v17.2 (San Diego, CA, USA).

4. Conclusions

The structures of a new class of N-methylsulfonyl-piperazine-based acetamides have been determined. Their anticancer potential against human lung carcinoma (A-549) was investigated. The types of substituents on the aryl ring within the synthesized piperazines
affect the anticancer activity. For example, piperazines containing 2-chlorophenyl and 2,4-dichlorophenyl moieties showed the most potent antiproliferative activity. Piperazines with 2,4-dichlorophenyl and 3,4-dichlorophenyl rings showed the lowest cytotoxicity. The cytotoxic effect of the synthesized piperazines was investigated through hemolysis and thrombolysis. The piperazine with a 2,5-dimethoxyphenyl moiety had the greatest thrombolytic effect. The anticancer activity of the most potent piperazine was investigated further using in silico studies to delineate its potential mechanism of action. More structural modifications to piperazine aryl rings are required to create more potent and selective antiproliferative and cytotoxic candidates.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules27154776/s1, Figure S1: $^1$H NMR spectrum of 5a, Figure S2: $^{13}$C NMR spectrum of 5a, Figure S3: $^1$H NMR spectrum of 5b, Figure S4: $^{13}$C NMR spectrum of 5b, Figure S5: $^1$H NMR spectrum of 5c, Figure S6: $^{13}$C NMR spectrum of 5c, Figure S7: $^1$H NMR spectrum of 5d, Figure S8: $^{13}$C NMR spectrum of 5d, Figure S9: $^1$H NMR spectrum of 5e, Figure S10: $^{13}$C NMR spectrum of 5e, Figure S11: $^1$H NMR spectrum of 5f, Figure S12: $^{13}$C NMR spectrum of 5f, Figure S13: $^1$H NMR spectrum of 5g, Figure S14: $^{13}$C NMR spectrum of 5g, Figure S15: $^1$H NMR spectrum of 5h, Figure S16: $^{13}$C NMR spectrum of 5h, Figure S17: $^1$H NMR spectrum of 5i, Figure S18: $^{13}$C NMR spectrum of 5i, Figure S19: $^1$H NMR spectrum of 5j, and Figure S20: $^{13}$C NMR spectrum of 5j.

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