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SYNTHESIS AND STRUCTURAL INVESTIGATION OF NEW DERIVATE OF 5-MERCAPTO-3-PHENYL-1,3,4-THIADIAZOL-2-THIONE

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Abstract. 1,3,4-Thiadiazole derivatives are a significant class of heterocyclic compounds with diverse biological activities, making them valuable in pharmaceutical research. In this study, a novel derivative, 5,5'-(methylenebis(sulfanediyl))bis(3-phenyl-1,3,4-thiadiazole-2(3H)-thione), was synthesized using dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) catalysts in the presence of methylene chloride. The compound's molecular structure was described by NMR monitoring**.** The compound's molecular structure was elucidated by X-ray diffraction, revealing a planar thiadiazole-thione core stabilized by intermolecular hydrogen bonding and van der Waals forces. Hirschfeld surface analysis identified significant H…H (26.3%) and S…H (23.3%) interactions contributing to crystal packing. A molecular docking and molecular dynamic studies have been carried out to determine possible anticancer activity of new product relative to 5-mercapto-3-phenyl-1,3,4-thiadiazol-2-thione.

Keywords: *1,3,4-Thiadiazole derivative, synthesis, X-ray crystallography, Hirschfeld surface analysis, molecular docking, biological activity.*

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1. Introduction

The development of highly effective and low-toxicity drugs remains a cornerstone of pharmaceutical research. The synthesis of novel compounds with diverse biological activities is vital for improving therapeutic outcomes and minimizing adverse effects. One of the most promising areas in this field is the design and synthesis of heterocyclic compounds, particularly derivatives of 1,3,4-thiadiazole, which exhibit a wide range of pharmacological activities.

Heterocyclic compounds containing the 1,3,4-thiadiazole moiety have been extensively studied due to their unique structural and physicochemical properties. The sulfur atom in the thiadiazole core imparts significant lipophilic characteristics, enabling these compounds to penetrate biological membranes effectively and interact with cellular targets (Song *et al*., 1999). Mechanistic insights suggest that thiadiazole fragments may participate in biochemical reactions through binding with G-protein-coupled receptors or enzymes at active cysteine residues. This dual functionality, acting as both a hydrogen-

bond donor and an electron donor, enhances their ability to engage in diverse biological interactions and contributes to their broad pharmacological potential.

1,3,4-Thiadiazole derivatives have been shown to possess a wide array of biological activities, including fungicidal, insecticidal (Holla *et al*., 2002; Togaev *et al*., 2024), bactericidal, herbicidal, antitumor, anti-inflammatory and central nervous system (CNS) stimulant properties (Tilyabaev *et al*., 2017). These activities make them versatile scaffolds for drug discovery in various therapeutic areas. For instance, their fungicidal and insecticidal activities have significant applications in agriculture, while their antitumor and anti-inflammatory properties make them promising candidates for cancer and autoimmune disease therapies. The structural adaptability of the thiadiazole core allows for functional modifications, further expanding its utility in medicinal chemistry.

Sulfur-containing compounds, including thiadiazoles, have garnered particular interest due to their antibacterial efficacy and reduced toxicity compared to other chemical classes (Kulikov *et al*., 2004). Their ability to selectively target microbial systems while minimizing harm to host organisms underscores their potential as safer therapeutic agents. Moreover, the involvement of thiadiazole derivatives in key biochemical processes, such as enzyme inhibition and receptor modulation, highlights their relevance as electrophilic “target sites” in drug design (Sharma *et al*., 2013).

Obtaining new derivatives of thiadiazoles derivatives is relevant and timely, due to the wide range of biological activity of such compounds. As is known, the biggest problem is the resistance of existing drugs.

Despite the extensive studies on 1,3,4-thiadiazole derivatives, there is a continuous demand for novel compounds with improved pharmacological profiles. The structural diversity of thiadiazoles and their ability to accommodate various functional groups offer opportunities to discover new compounds with enhanced selectivity and efficacy. Furthermore, the exploration of their interaction mechanisms at the molecular level can provide valuable insights into their biological activities and therapeutic applications.

In this study, we aim to synthesize a novel 1,3,4-thiadiazole derivative, 5,5'-(methylenebis(sulfanediyl))bis(3-phenyl-1,3,4-thiadiazole-2(3H)-thione) and investigate its structural and biological properties. The compound was characterized using X-ray diffraction and Hirshfeld surface analysis to understand its structural features and intermolecular interactions. We performed docking analysis of the new compound to predict its potential anticancer activity.These findings contribute to the growing body of knowledge on thiadiazole derivatives and pave the way for further research into their therapeutic applications.

2. Methods

Chemical and Solvent Purification

All chemicals and solvents used in this study were of analytical grade and were purified prior to use. Purification was performed using standard techniques, including distillation and recrystallization, to ensure high purity. Thin-layer chromatography (TLC) was employed to monitor reaction progress. Silica gel plates (Silufol) were used with a mobile phase of ethyl acetate:hexane (1:5, v/v) as the eluent. Visualization of compounds on the TLC plates was achieved through iodine vapor exposure.

NMR monitoring of intermediates

^{1}H NMR spectra were recorded on JNM-ECZ600R spectrometer (JEOL, Japan) under the operating frequency of 600 MHz for ^{1}H in $CDCl_3$ solution. TMS (0 ppm) was used as an internal standard in ^{1}H NMR spectra. $CDCl_3$ was used as an internal standard.

X-ray Crystallography

The single-crystal X-ray diffraction data were collected using an 'XtaLAB Synergy, HyPix3000' CCD diffractometer equipped with a CuKα radiation source (λ = 1.54184 Å). The ω-scan mode and a graphite monochromator were employed to optimize data quality. Measurements were performed at 293 K. The crystal structure was solved using the direct methods provided in the SHELXS-97 program and refined by full-matrix least-squares methods on F^2 using SHELXL-2018 (Rigaki, 2020; Sheldrick, 2015). All non-hydrogen atoms were refined anisotropically, while hydrogen atoms were positioned geometrically and refined with isotropic displacement parameters. Molecular illustrations and structural diagrams were generated using the MERCURY program package (Macrae *et al*., 2008).

Crystallographic Data Deposition

The crystallographic data for the synthesized compound have been deposited with the Cambridge Crystallographic Data Centre (CCDC) under Deposition Number 2255753. These data are available free of charge via the CCDC website (http://www.ccdc.cam.ac.uk/conts/retrieving.html) or can be requested by contacting the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223-336-033; email: deposit@ccdc.cam.ac.uk).

Molecular docking, MD simulations and gmx-MMGBSA/MMPBSA analyses

Molecular docking studies have been performed by using CB-Dock2 server. The target protein of CDK2 (PDB ID: 1B38) was obtained from Protein Data Bank (PDB, https://www.rcsb.org/). Protein molecule cleaned from extra molecules (water and native ligands) by using Biovia DS program package and it is used to visualize obtained results. MD simulations have been carried out by using Gromacs-2025.2 program package with AMBER99SB-ILDN force field. Topology files of ligands have been obtained by using ACPYPE server.

3. Synthesis and Crystallization

To synthesize the target compound, 5-mercapto-3-phenyl-1,3,4-thiadiazol-2-thione (0.95 mmol) was reacted with equimolar amounts of dicyclohexylcarbodiimide (DCC, 0.95 mmol) and 4-dimethylaminopyridine (DMAP, 0.95 mmol) in methylene chloride (CH_2Cl_2) as the solvent. The reaction mixture was heated to reflux with constant stirring for 8 hours. During the initial 4 hours, the reaction was conducted under an inert nitrogen atmosphere to prevent oxidation or other unwanted side reactions. Upon completion, the reaction solution was diluted with 10 mL of methylene chloride, followed by the addition of 30% acetic acid dropwise until the solution reached a pH of 5-6. The organic layer was separated and the resulting organic extracts were washed thoroughly with a saturated sodium chloride (NaCl) solution to remove residual water-soluble impurities. The washed extracts were dried over anhydrous sodium sulfate (Na_2SO_4) and the solvent was removed under reduced pressure using a rotary evaporator. The crude product was obtained with a yield of 85%. For purification, the residue was recrystallized by slow evaporation of a

methylene chloride solution of the crude product at ambient temperature. This process resulted in the formation of high-quality single crystals, suitable for X-ray crystallographic analysis.

^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 5.08 (2H, s, CH_2), 7.43 (2H, t, J=7.4 Hz, Ar-H), 7.50 (4H, m, Ar-H), 7.70 (2H, d, J=7.2 Hz, Ar-H), 7.76 (2H, d, J=7.3 Hz, Ar-H).

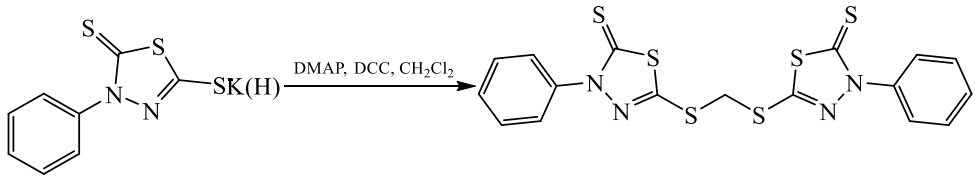
4. Results and Discussion

Synthesis of 5-Mercapto-3-Phenyl-1,3,4-Thiadiazol-2-Thione Derivative

The synthesis of novel heterocyclic derivatives with potential biological activity is a vital step in exploring new pharmacological compounds. In this study, we synthesized a new derivative of 5-mercapto-3-phenyl-1,3,4-thiadiazol-2-thione, a compound known for its diverse biological properties, using a well-established methodology involving dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) as catalytic agents (Sheikh *et al*., 2010). Although the DCC/DMAP catalytic system is widely used, the innovation of this study lies in obtaining a new methylene-bridged disulfide derivative with unique structural and biological properties, which has not been previously reported. The reaction was performed in excess methylene chloride, which served both as a solvent and a facilitator for the reaction dynamics. Compared with previously described thiadiazole derivatives, the synthesized compound [5,5'-(methylenebis(sulfanediyl))bis(3-phenyl-1,3,4-thiadiazole-2(3H)-thione)] exhibits distinct structural and functional features. Unlike the earlier disulfide-linked derivative [5,5'-(disulfanediyl)bis(3-phenyl-1,3,4-thiadiazole-2(3H)-thione)], which was primarily investigated for its anti-inflammatory properties, the present compound incorporates a methylene-bridged disulfide linkage. This modification results in enhanced intermolecular interactions, as demonstrated by X-ray diffraction and Hirshfeld surface analyses, which contribute to improved structural stability. Moreover, molecular docking and MD simulations revealed that the new derivative exhibits significantly higher binding affinity toward CDK2 compared to the parent compound 5-mercapto-3-phenyl-1,3,4-thiadiazol-2-thione, suggesting stronger anticancer potential. These differences highlight the structural novelty, broadened biological relevance and expanded scope of potential applications for the methylene-bridged derivative in pharmaceutical development. The product, 5,5'-(methylenebis(sulfanediyl))bis(3-phenyl-1,3,4-thiadiazole-2(3*H*)-thione), was obtained from the 5-mercapto-3-phenyl-1,3,4-thiadiazol-2-thione potassium salt under the described reaction conditions. The selection of methylene chloride in excess was deliberate, as it not only functioned as a solvent but also promoted the effective coordination of the potassium cation with the chloride ion. This interaction likely facilitated the formation of a methylene bridge, enabling the final structural arrangement of the target compound. The final product was analyzed by ^{1}H NMR spectroscopy. According to the ^{1}H NMR results, in the middle of the ^{1}H NMR spectrum of the compound, a two-proton singlet signal was observed at δ_H 5.08 ppm from the methylene group at the heteroatom (S-CH_2-S) and in the aromatic part of the spectrum were resonated signals characteristic of two monosubstituted benzene rings at δ_H H 7.43 (2H, t, J=7.4 Hz), 7.50 (4H, m), 7.70 (2H, d, J = 7.2 Hz), 7.76 (2H, d, J = 7.3 Hz).

Reaction Mechanism and Structural Insights

The reaction mechanism is postulated to involve nucleophilic substitution, where the thiol group of the starting material interacts with the methylene chloride under the catalytic influence of DCC and DMAP. The use of DCC as a dehydrating agent likely activated the thiol group, while DMAP acted as a nucleophilic catalyst, ensuring the smooth progression of the reaction. The formation of the methylene bridge is attributed to the synergistic action of the reagents, which stabilized the intermediate and facilitated its conversion into the desired product.



Scheme 1.

The high yield of the product demonstrates the efficiency of this reaction setup. Further, the crystalline nature of the product highlights its purity and suitability for detailed structural analysis.

Conducting the experiment under catalytic conditions allowed to significantly reduce the time of organic synthesis and let to obtaining a completely new structure of 5-mercapto-3-phenyl-1,3,4-thiadiazol-2-thione.

The efficiency of synthesis is confirmed by a sufficiently high 85% yield of the reaction product, which emphasizes the innovative significance of the chosen synthetic way of reaction.

The new modification of the thiadiazole derivative differs from the existing derivatives by the presence of a disulfide bridge separated by a methylene fragment. This design, on the one hand, allows the compound to easily penetrate the cell membrane by interacting with the -thiol groups of proteins, with subsequent cytostatic action. On the other hand, binding of the 1.2.4-thiadiazole fragment of the heterocyclic derivative as an electrophilic “target site” interacts with the cysteine residues of proteins and such supposes discussing by some autors (Sharma *et al*., 2013).

Previously, we (Shakirzyanova *et al*., 2024) obtained the thiadiazole derivative - 5,5-(Disulfanediyl)bis[3-phenyl-1,3,4-thiadiazole-2(*3H*)-thione. The anti-inflammatory activity of the synthesized disulfide was studied for antiexudative and anti-inflammatory activity (Shakirzyanova *et al*., 2024).

X-ray structure analysis and refinement

Crystal data of the title compound: $C_{17}H_{12}N_4S_6$, orthorhombic, Fdd2 (No.43): a = 16.457(2), b = 32.008(5), c = 7.6778(12)Å, V = 4044.3(10)Å^3, Z =8, M = 464.67, d_{calc} = 1.526 g/cm^3, 2519 reflections were collected at 273 K and 1387 independent reflections [R_{int} =0.064] were used in the refinement procedure, which converged to wR2 = 0.2592 calculated for F2hkl (GOF = 1.056, R1 = 0.0907 calculated for F_{hkl} using 1152 reflections with I > 2σ(I)). Flack x = -0.01(11).

The crystallographic data were deposited at the Cambridge Crystallographic Data Centre (Deposite Number 2255753). The data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/ retrieving.html or from the Cambridge

Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk.

Description of molecular crystal structure

5,5'-(Methylenebis(sulfanediyl))bis(3-phenyl-1,3,4-thiadiazole-2(3*H*)-thione) crystallized in the non-centrosymmetric orthorhombic space group Fdd2 with a half-molecule in the asymmetric unit; a two-fold rotation axis, which passes through the C9 atom, generates the other half of the molecule (Figure 1). The 1,3,4-thiadiazole-2-thione unit was planar, with an r.m.s. deviation of 0.019 Å from the corresponding square plane defined by the seven constituent atoms. Benzene and thiadiazole rings are not coplanar (corresponding C2-C1-N2-N1 torsion angles are 32.4 (12)°).

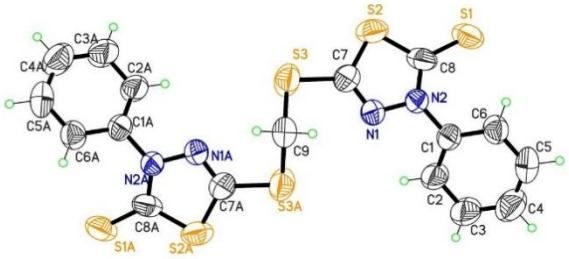


Figure 1. Structure of molecule 5,5'-(methylenebis(sulfanediyl))bis(3-phenyl-1,3,4-thiadiazole-2(3*H*)-thione) with numbering of the nonhydrogen atoms and the thermal ellipsoids drawn at 50% probability

The crystal packing is stabilized by intramolecular H-bonds C6-H6A …S1 (2.86, 3.241(14)Å, 106.00°), close intermolecular S…S contacts in the range of 3.416 Å and van der Waals interactions. Molecules are combined into tree-dimensional construction.

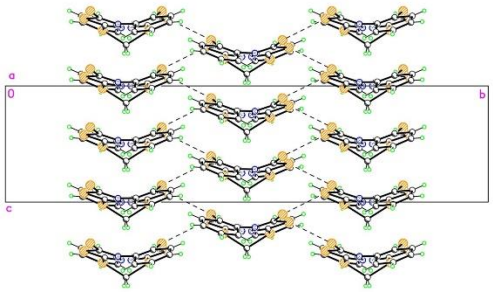


Figure 2. Crystal structure of 5,5'-(methylenebis(sulfanediyl))bis(3-phenyl-1,3,4-thiadiazole-2(3*H*)-thione). Dots show close intermolecular S…S contacts

Hirshfeld surface analysis

Additional insights into intermolecular interactions were obtained from the analysis of the Hirschfeld surface (HS) (Spackman & Jayatilaka, 2009) and two-dimensional fingerprint plots (McKinnon *et al*., 2007).

The CrystalExplorer program (Turner *et al*., 2017) was used to generate Hirschfeld surfaces mapped over d_{norm} and the electrostatic potential of the title compound. The function d_{norm} is the ratio of the distances of any surface point to the nearest interior (d_i) and exterior (d_e) atoms and the van der Waals (vdW) radii of the atoms. Figure 3 shows the Hirschfeld surfaces mapped over d_{norm} in the range -0.2247 (red) to 1.3787 (blue) a.u.

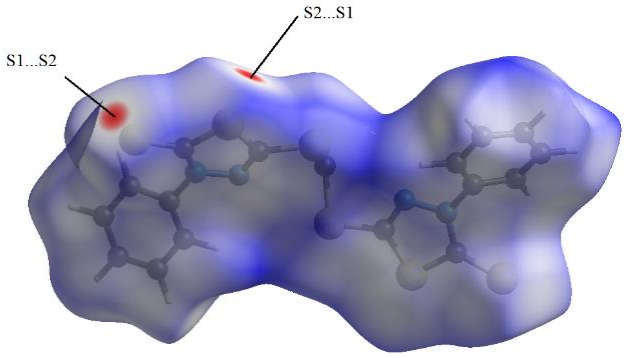
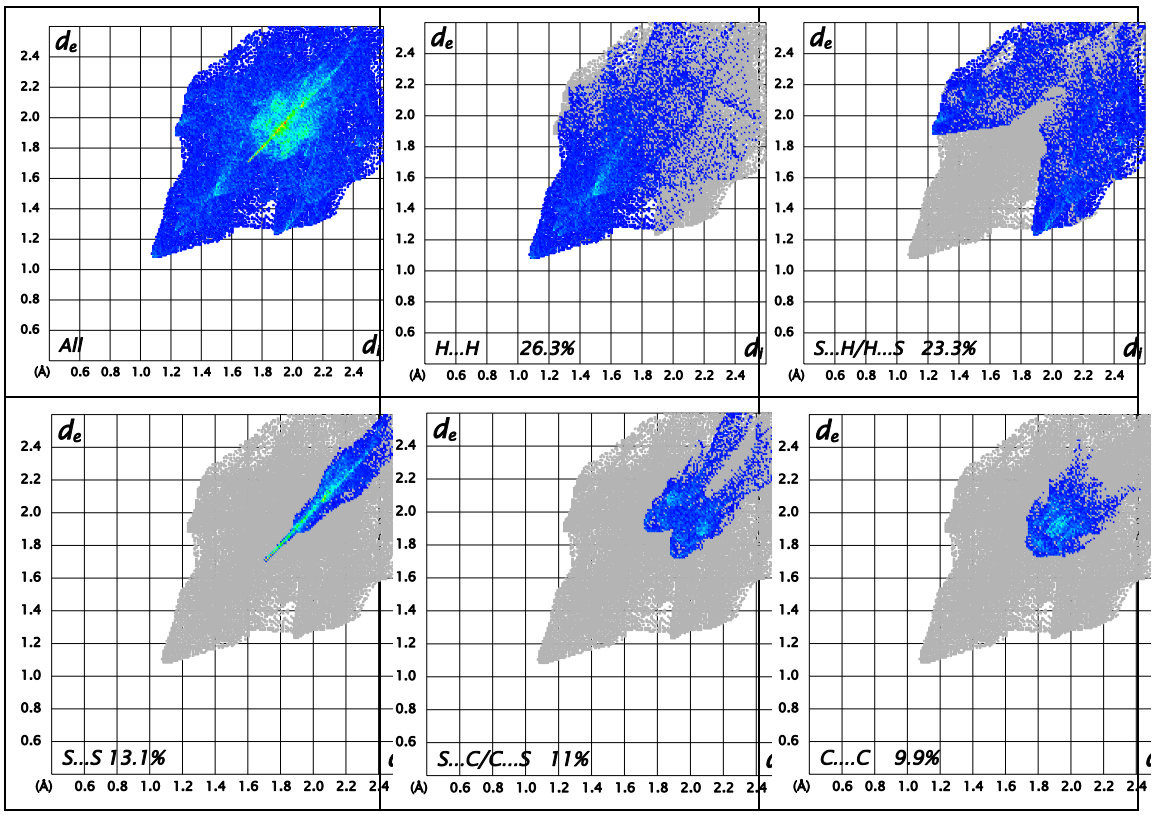


Figure 3. Hirshfeld surface displayed for the asymmetric unit of 5,5'-(methylenebis(sulfanediyl))bis(3-phenyl-1,3,4-thiadiazole-2(3H)-thione) mapped over dnorm in the range −0.2247 to 1.3787 a.u. The color scheme reflects intermolecular contact distances relative to van der Waals radii: red regions indicate contacts shorter than the sum of van der Waals radii, white regions indicate contacts approximately equal to van der Waals separations and blue regions correspond to longer contacts. These visualizations highlight the key interaction sites responsible for stabilizing the crystal packing

The overall two-dimensional fingerprint plot (Figure 4) and those delineated into H…H, S…H/H…S, S…S, S…C/C…S, C…C, C…H/H…C, N…S/S…N and N…N contacts are illustrated together with their relative contributions to the Hirschfeld surface.

Hirschfeld surface analysis indicated that the most important contributions to the crystal packing were H…H (26.3%) and S…H/H…S (23.3%) – weak H-bonds and van der Waals interactions. The S…S (13.1%), S…C/C…S (11%), C…C (9.9%) and C…H/H…C (5.5%) interactions contributed less and the S…N/N…S (1.9%) and N…N (0.4%) interactions contributed minor significance to the overall HS.



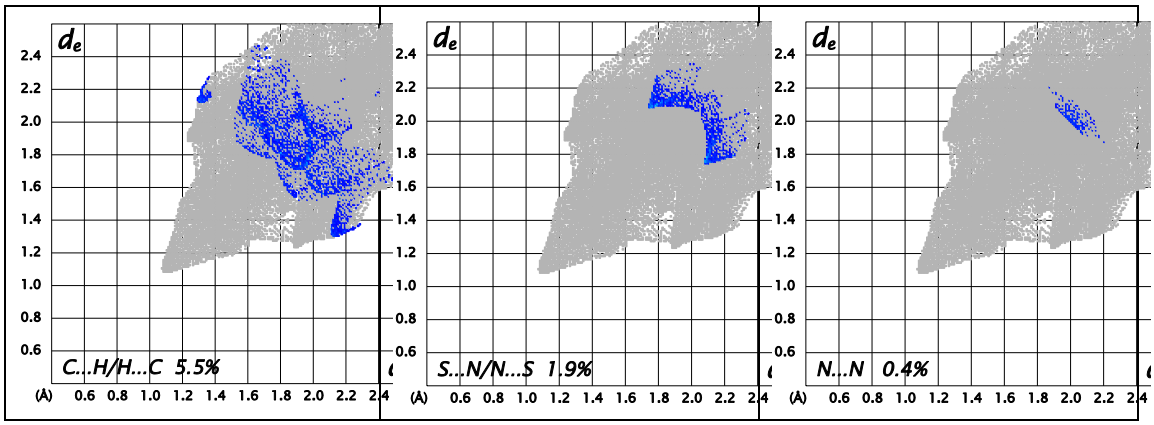


Figure 4. Two-dimensional fingerprint plots for the asymmetric unit of 5,5'-(methylenebis(sulfanediyl))bis(3-phenyl-1,3,4-thiadiazole-2(3H)-thione), showing the relative contributions of different types of intermolecular interactions to the Hirshfeld surface. Major contributions arise from H…H (26.3%) and S…H/H…S (23.3%) contacts, which represent weak hydrogen bonds and van der Waals forces. Additional contributions include S…S (13.1%), S…C/C…S (11%), C…C (9.9%) and C…H/H…C (5.5%), while S…N/N…S (1.9%) and N…N (0.4%) interactions play only a minor role. These results emphasize the predominance of hydrogen and sulfur-related interactions in defining the compound's crystal structure and packing stability

Molecular docking investigations

5-mercapto-3-phenyl-1,3,4-thiadiazol-2-thione and its derivatives exhibit broad biological activities, including antimicrobial, anti-inflammatory, antifungal, anticancer, antiviral and antioxidant properties. Studies have explored its potential as a therapeutic agent for various diseases and as a plant growth regulator (Song *et al.*, 1999; Holla *et al.*, 2002). In this paper, we present molecular docking studies only for anticancer activity.

A molecular docking and molecular dynamic studies have been carried out to determine possible anticancer activity of new product relative to 5-mercapto-3-phenyl-1,3,4-thiadiazol-2-thione. For this purpose a cyclin-dependent kinase 2 protein (PDB ID: 1B38) was obtained from Protein Data Bank (PDB (Berman *et al.*, 2000), https://www.rcsb.org/) as target for anticancer agents (Tadesse *et al.*, 2020). Protein molecule cleaned from extra molecules (water and native ligands) by using Biovia DS program package (Biovia, 2021). An interaction of ligand molecules with the target protein were conducted by using CB-Dock2 server (Liu *et al.*, 2022). As a result of molecular docking studies the best binding affinity found for new product (GA_{bind}=-8.4 kcal/mol) relative to 5-mercapto-3-phenyl-1,3,4-thiadiazol-2-thione (GA_{bind}=-6.1 kcal/mol).

The initial compound and new product, 5,5'-(methylenebis(sulfanediyl))bis(3-phenyl-1,3,4-thiadiazole-2(3H)-thione) fits well in the active site of the 1B38 protein (Figure 5). Title compound form H-bond with LYS33 through nitrogen atom and at the time new product form H-bond with LYS129 amino acid residue through its sulfur atom of a thione group (Figure 6).

Using docking alone to assess the potential activity of a ligand can lead to suboptimal choices, as the complex may be unstable and allow the ligand to escape from the protein's active site. Therefore, the obtained protein-ligand complexes have been used for molecular dynamics simulations to study their behavior in solvent medium.

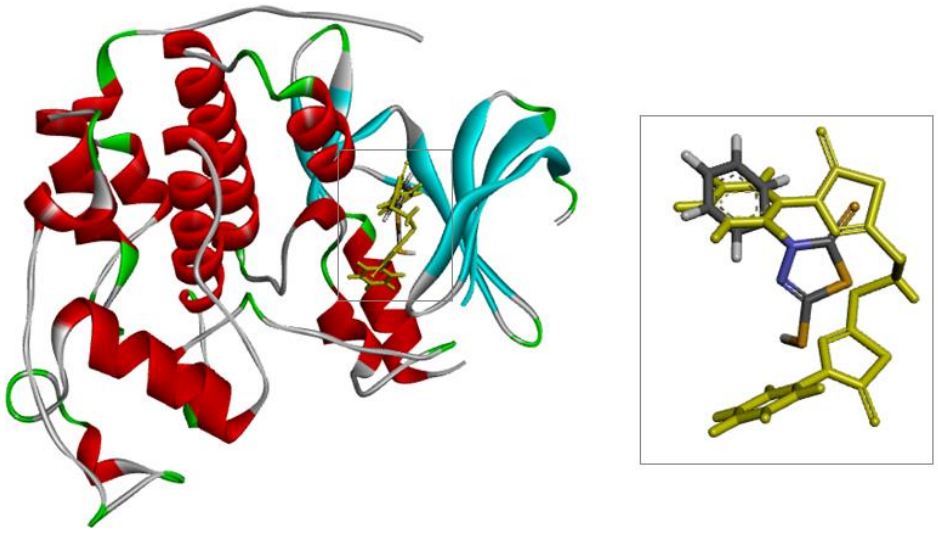


Figure 5. The localization of the initial and product (yellow colored) molecules in the active site of the 1B38 protein

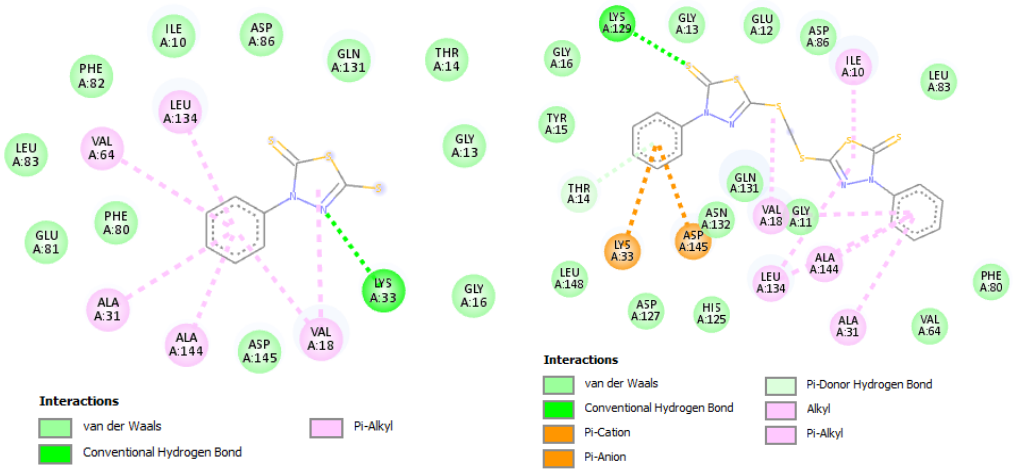


Figure 6. Contacting residues for the initial compound (left) and the product (right) in the active site of the protein 1B38

Molecular dynamics and gmx-MMGBSA/MMPBSA studies

MD simulations have been carried out by the way described in (Boltayeva *et al*., 2025), where Gromacs (Abraham *et al*., 2015, 2025) program package and AMBER99SB-ILDN (Lindorff-Larsen *et al*., 2010) force field fruitfully used. Topology files of ligands have been obtained by using ACPYPE server (Sousa da Silva & Vranken, 2012). All system Protein-Ligand complex, water molecules (SPC model) and ions (Na^+, Cl^-) in a dodecahedral box have been minimized. The equilibrium of the system in the NVT and NPT ensemble was carried out. Temperature and pressure stability were maintained using a V-rescale thermostat (Lemkul, 2018) and a Berendsen barostat (Bussi *et al*., 2007), respectively. The MD simulation phase (by applying a V-rescale thermostat (Berendsen *et al*., 1984) and a Parrinello-Rahman barostat (Parrinello & Rahman, 1981)) was carried out with a simulation time of 200 ns at 310 K and 1 bar using an integration time of 2 fs. To evaluate the simulation results, RMSD plots were obtained.

The RMSD plot for original compound [5-mercapto-3-phenyl-1,3,4-thiadiazol-2-thione] (Figure 7, blue-line) shows significant fluctuations. During the initial phase (0-20 ns), the RMSD increases from approximately 0.2 nm to around 0.5 nm, indicating a relaxation of the system from its initial conformation. From 20 to 60 ns, the trajectory exhibits more pronounced fluctuations, with peaks reaching about 0.8-0.9 nm, suggesting structural changes. After approximately 60 ns, the RMSD stabilizes around 0.6-0.7 nm, though it still exhibits noticeable fluctuations within a range of about 0.1-0.15 nm. Once equilibrium is reached, the complex does not revert to the lower RMSD values but instead maintains a stable higher level (~0.6-0.7 nm), indicating that the system has achieved a new conformational state. But, new compound [5,5'-(methylenebis(sulfanediyl))bis(3-phenyl-1,3,4-thiadiazole-2(3H)-thione)] quickly reached the equilibrium phase after an initial relaxation period (Figure 7, orange-line). The initial relaxation period lasted up to 47 ns. Starting from 47 ns, the system tends to stabilize, achieving stability during the simulation period from 50 to 200 ns. This indicates that the product remained stably bound to the target protein throughout the simulation. However, from Figures 8 and 9 one can notice that a changing of initial positions of both compounds during the MD simulations and new types of interactions were formed compared to the original geometry, for example, relative to the docked geometry.

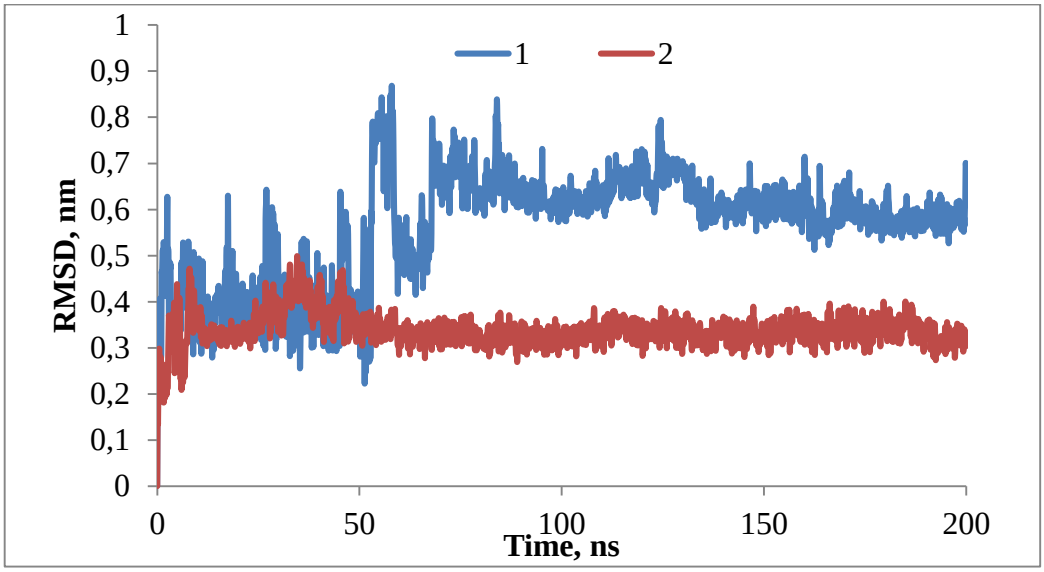


Figure 7. RMSD graph of the complexes as a function of time

The last 10 ns of the 200 ns MD simulations were subjected to analysis of binding free energy and energy decomposition analysis by using gmxMMPBSA tool (Valdés-Tresanco *et al*., 2021).

The gmxMMGBSA program allows one to estimate the binding free energies of protein-ligand interactions by solving the Poisson-Boltzmann equation (PB, MMPBSA method) or using the generalized Born method (GB, MMGBSA method) (Valdés-Tresanco *et al*., 2021; Al-Bustany *et al*., 2022). The former method provides a more thorough analysis of protein-ligand interactions than the latter (Valdés-Tresanco *et al*., 2021).

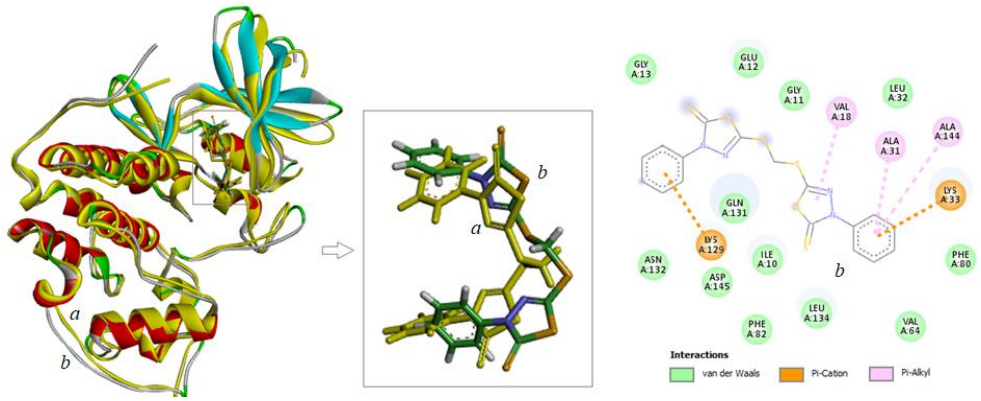


Figure 8. Overlay of the geometry of the initial complex (*a*, yellow colored) which taken for MD simulation and the MD simulated complex's geometry (at 200 ns) of the 1B38-1 complex. A 2D view shows the interactions between the final geometry of the 1 and the amino acid residues of 1B38

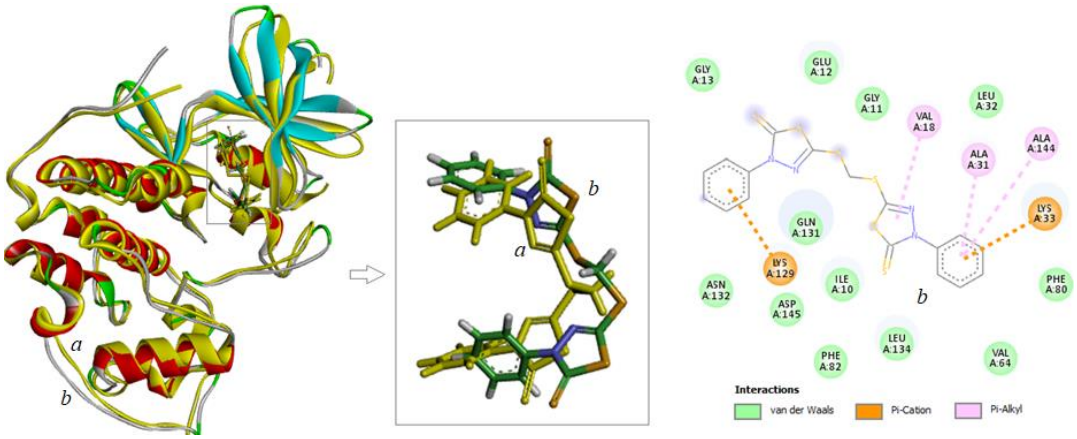


Figure 9. Overlay of the geometry of the initial compound (*a*, yellow colored) which taken for MD simulation and the MD simulated complex's geometry (at 200 ns) of the 1B38-new compound. A 2D view shows the interactions between the final geometry of the 2 and the amino acid residues of 1B38

When estimating the binding free energy, the MMPBSA and MMGBSA methods use well-balanced systems of small molecule complexes with receptors and explicit molecular dynamics (MD) simulations. As a result, free energy values are obtained that are comparable with experimental data (Genheden & Ryde, 2015; Wang *et al*., 2018).

The binding free energies of the initial compound and the new product to 1B38 were estimated using both methods. The average binding energy of each ligand to 1B38 is shown in Table 1. This table lists the enthalpy (ΔH) and entropy (ΔS) changes during ligand binding to the protein and also determines the binding energy (ΔGbind = ΔH - TΔS) based on them. According to the authors of (Valdés-Tresanco *et al*., 2021), energies below -7 kcal/mol indicate very strong interactions of ligands with proteins and indicate the strength of the protein-ligand complex.

According to per-residue decomposition analysis using MMPBSA, the following amino acid residues contribute to the stabilization of the 1B38-1 complex: GLY11, VAL18, GLU81, ASN132 and LEU134 (Figure 10). In the case of complex 1B38-2, the following amino acid residues contribute to the stabilization of the complex: ILE10, GLY13, TYR15, VAL18, TYR19, ALA31, LEU32, PHE80, PHE82, ASN132 and LEU134. However, the amino acid residues LYS33, GLU81, ASP86 and ASP145 contribute to the destabilization of the complex (Figure 11).

Table 1. Average values of ΔH, TΔS and ΔGbind (kcal/mol) determined based on the interaction of ligands with 1B38 using the MMGBSA and MMPBSA methods

Parameter	MMGBSA	MMPBSA
	1B38-initial compound	
ΔH	-18.66 ± 1.3	-11.93 ± 2.39
-TΔS	1.57 ± 0.04	1.57 ± 0.04
ΔG_{bind}	-17.08 ± 1.3	-10.35 ± 2.39
	1B38 – new product	
ΔH	-44.65 ± 1.92	-26.44 ± 3.04
-TΔS	2.76 ± 0.04	2.76 ± 0.04
ΔG_{bind}	-41.89 ± 1.92	-23.69 ± 3.04

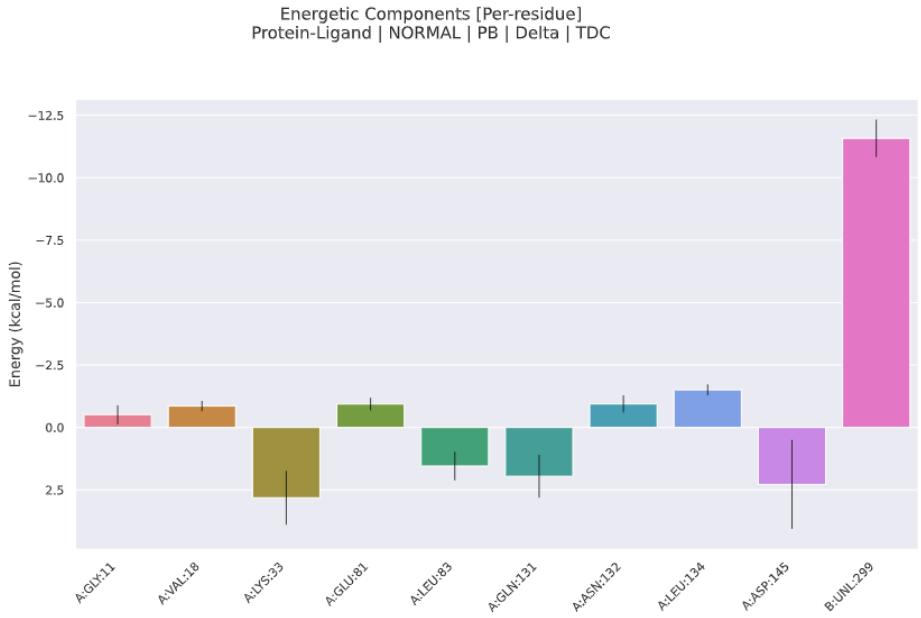


Figure 10. Per-residue (TDC) decomposition analysis for 1B38 - ini by MMPBSA

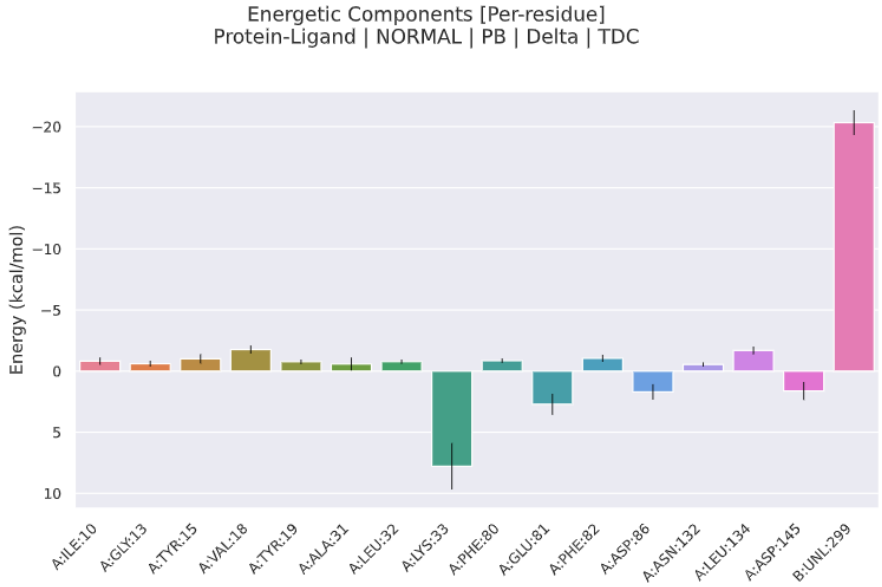


Figure 11. Per-residue (TDC) decomposition analysis for 1B38 - new product (2) complex by MMPBSA

5. Conclusion

The synthesis and exploration of new pharmaceuticals remain crucial for advancing medical chemistry, with particular emphasis on identifying compounds that exhibit high efficacy and improved tolerability. Among the promising candidates, derivatives of 1,3,4-thiadiazole stand out due to their diverse biological activities and potential therapeutic applications. While the synthesis approach employed is well-established, the originality of our work is demonstrated by the novel methylene-bridged disulfide derivative and its distinct structural and pharmacological features, which expand the chemical space of thiadiazole compounds.

In this study, we successfully synthesized a novel 1,3,4-thiadiazole derivative, 5,5'-(methylenebis(sulfanediyl))bis(3-phenyl-1,3,4-thiadiazole-2(3H)-thione). The synthesis approach, facilitated by the use of catalytic reagents, promoted the formation of a methylene bridge, yielding a compound with distinct structural features. The crystal structure of the derivative was determined through X-ray diffraction analysis, revealing key intermolecular interactions, which were further characterized by Hirschfeld surface analysis.

The conducted MD studies showed that the new compound [5,5'-(methylenebis(sulfanediyl))bis(3-phenyl-1,3,4-thiadiazole-2(3H)-thione)] binds strongly to amino acid residues then original compound [5-mercapto-3-phenyl-1,3,4-thiadiazol-2-thione] and the MM-GBSA and MM-PBSA methods determined an approximately twice higher binding affinity for new compound (-23.69/-41.89) compared to (-10.35/-17.08). The per-residue decomposition analysis by MMPBSA shows that, the ILE10, GLY13, TYR15, VAL18, TYR19, ALA31, LEU32, PHE80, PHE82, ASN132 and LEU134 amino acid residues contribute to the stabilization of the 1B38-2 complex. Based on the conducted studies, new compound 5,5'-(methylenebis(sulfanediyl))bis(3-phenyl-1,3,4-thiadiazole-2(3H)-thione) can be recommended for anticancer pharmacological studies. Although the present study is primarily oriented toward medicinal chemistry, the structural features of the methylene-bridged disulfide derivative - such as strong S…S contacts, enhanced intermolecular interactions and crystallographic stability - suggest that it may also have potential in materials-related fields. Future investigations could explore its electronic, optical or catalytic properties, thereby expanding the scope of applications beyond biological systems.

The findings presented herein contribute to the expanding knowledge of 1,3,4-thiadiazoles as versatile scaffolds in drug discovery, offering new avenues for developing highly effective and selective pharmaceutical agents.

Author Contributions

Conceptualization: Omon Kholbekov and Bakhrom Babaev; methodology: Bakhtiyar Ibragimov; synthesis: Gulnara Shakirzyanova and Omon Kholbekov; X-ray analysis: Lidiya Izotova; writing: Gulnara Shakirzyanova, Lidiya Izotova and Ulugbek Togaev, molecular docking: Alisher Eshimbetov. All authors have read and agreed to the proposed version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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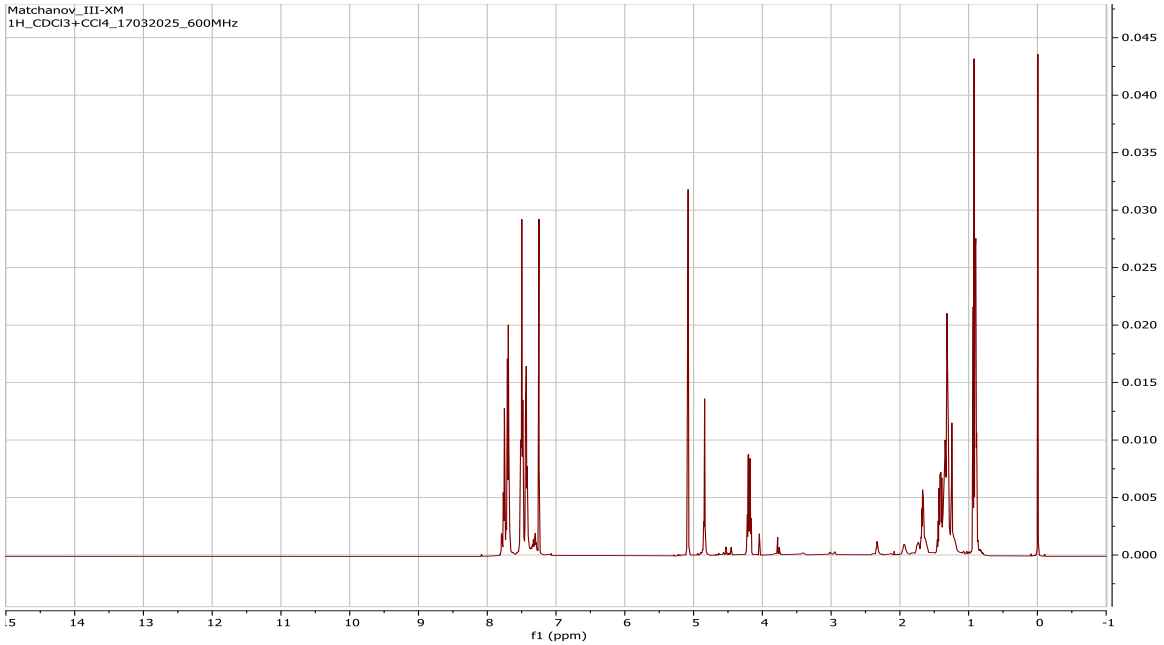
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