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Synthesis of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic Acid and Theoretical Evaluation of Its Biological Activity

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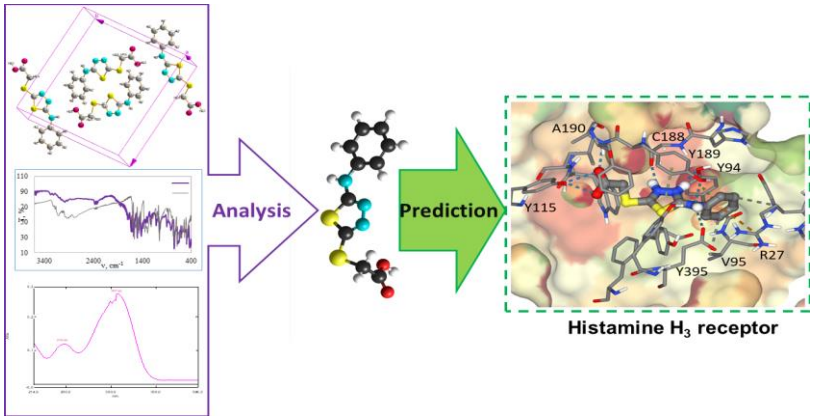
2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic acid exhibits a variety of biological activities. Consequently, the synthesis, structure, and biological activity of this compound were investigated. The synthetic procedure is described in detail on a step-by-step basis, with the compound's composition and structure confirmed by FTIR, UV–Vis spectroscopy, and X-ray crystallography. Additionally, pharmacokinetic and toxicological properties were predicted using the ADMETPred program. The biological potential was further evaluated through the 3DStarPred program and molecular docking studies, which revealed high affinity for the human histamine H_3 receptor, suggesting a potential role as an antagonist/inverse agonist in the central nervous system. Overall, these findings indicate that the synthesised compound represents a promising candidate for further medicinal development.

Graphical abstract



Keywords

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1. Introduction

Thiadiazoles are an important class of heterocycles containing nitrogen and sulphur. These structural motifs are found in various biologically significant molecules and are widely employed as valuable scaffolds in medicinal chemistry. Numerous 1,3,4-thiadiazole derivatives have been reported to exhibit diverse pharmacological properties, including antibacterial, antifungal, analgesic, anticancer, anticonvulsant, anxiolytic, antidiabetic, and anti-inflammatory activities. Consequently, extensive research has focused on synthesising organic compounds containing the thiadiazole ring.

For instance, relatively safe and efficient methods for

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synthesising 5-arylamino-1,3,4-thiadiazol-2-yl-acetamides have been reported, demonstrating good yields and minimal environmental impact [1, 2]. Similarly, the synthesis of 2-((4-phenyl-5-((5-phenylamino-1,3,4-thiadiazol-2-yl)thio)methyl)-1,2,4-triazol-3-yl)thio)ethanoic acid has been optimised, with water serving as an environmentally benign reaction medium. This aqueous synthetic route not only reduces the use of hazardous organic solvents but also facilitates product isolation and purification. Appropriate conditions for obtaining both organic and inorganic salts of this acid have been systematically investigated and established. The structural characteristics of these derivatives were confirmed through spectroscopic techniques, while their physicochemical properties, including solubility, stability, and thermal behaviour, were comprehensively characterised. The biological potential of the synthesised compounds was preliminary evaluated through molecular docking studies, which revealed promising binding affinities toward specific biological targets [3].

Primarily, the thiadiazole ring exists in four isomeric forms: 1,3,4-thiadiazole, 1,2,3-thiadiazole. 1,2,4-thiadiazole, and 1,2,5-thiadiazole. Among these, 1,3,4-thiadiazole derivatives have been shown to possess the most significant therapeutic potential and have therefore attracted considerable attention in medicinal chemistry. They demonstrate a remarkably wide range of biological activities, including antimicrobial, antifungal, antimycobacterial, analgesic, antiinflammatory, antipsychotic, antidepressant, anticonvulsant and antileishmanial effects. Substantial evidence also supports the anticancer activity of 1,3,4-thiadiazole derivatives [4].

Additionally, derivatives of 1,3,4-oxadiazole, 1,3,4-thiadiazole and 1,2,4-triazole have been structurally modified by introducing thio-groups into the heterocyclic rings, by substitution at the 5-position, and through the incorporation of aliphatic chains, aromatic carbocyclic groups and heterocyclic moieties [5]. In this study, the antibacterial potential of several 1,3,4-thiadiazole derivatives was evaluated.

Moreover, investigations have shown that thiadiazoles affect the central nervous system, and among all the thiadiazole derivatives, 1,3,4-thiadiazoles are recognised as key pharmacophores in drug design. Several studies have confirmed their wide-ranging effects on the CNS, including antidepressant, anxiolytic and analgesic activities. Furthermore, various thiadiazole derivatives have been reported to exhibit anticonvulsant properties. Notably, acetazolamide, a clinically used anticonvulsant drug, contains a 1,3,4-thiadiazole nucleus [6–8].

With the aim of imparting synergistic activity with the 4(3H)-quinazolinone core, the 1,3,4-thiadiazole ring was introduced and additional chemical modifications of the 4(3H)-quinazolinone structure were carried out. As a result, new 3-(1,3,4-thiadiazol-2-yl)-2-styrylquinazolin-4(3H)-ones were synthesised, and their anticonvulsant, neurotoxic, and central nervous system depressant properties were investigated [9].

It has been observed that replacing the benzene ring with heteroaromatic analogues can preserve comparable biological activity in pharmacological agents. This principle of bioisosterism is highly significant in drug design, where heterocycles such as pyridine, furan and thiophene are widely utilised as bioisosteric equivalents of benzene [10–12].

In recent years, many classes of thiadiazole derivatives have been reported to exhibit valuable biological properties, including antimicrobial, antitubercular and anti-inflammatory activities. The use of 5-nitroheterocycles as antibacterial, antiprotozoal and anticancer agents is well established. Several new compounds containing 1,3,4-thiadiazole rings substituted at the C2 position with 5-nitroheterocycles have been synthesised and evaluated for their anti-mycobacterial activity against Mycobacterium tuberculosis [13].

N-allyl-N-{4-[3-[(2,4-dichlorobenzyl)thio]-4-methyl-4H-1,2,4-triazol-5-yl]phenyl}thiourea was found to exhibit strong inhibitory activity against *Mycobacterium tuberculosis* H37Rv, with a minimum inhibitory concentration (MIC) value reported to be 6.25 μg/ml [6]. In addition, the synthesis and antitubercular activity of derivatives of 2-(1-methyl-5-nitro-1H-imidazol-2-yl)-1,3,4-thiadiazole and 2-(5-nitro-2-furyl)-1,3,4-thiadiazole were investigated, and these compounds demonstrated significant antitubercular activity. Furthermore, the synthesis and antitubercular properties of 2-(5-nitro-2-furyl)-5-alkylthio-1,3,4-thiadiazole derivatives were reported. Among them, the methyl-, ethyl-, and 4-nitrobenzylthio-substituted compounds showed good antitubercular activity [14].

The antimicrobial properties of 1,3,4-thiadiazole derivatives have been extensively investigated. Conjugation of the thiadiazole ring with various heterocyclic moieties often enhances biological activity, with the magnitude of improvement strongly dependent on the nature of the substituents and linkers. Owing to these favourable structural features, nitroaryl-substituted 1,3,4-thiadiazoles have attracted considerable research interest, and numerous analogues have been synthesised and evaluated.

For instance, several novel 5-(nitroaryl)-1,3,4-thiadiazoles were prepared by introducing ethylsulphonylethyl and ethylthioethyl fragments into the side chain, which resulted in improved antibacterial activity against Helicobacter pylori [15]. In addition, the synthesis of 2-[5-(aryl)-1,3,4-oxadiazol-2-ylsulfanyl]alkanoic acids has been reported, and these compounds were assessed for their in vitro antibacterial potential. Collectively, these studies demonstrate that the strategic modification of heterocyclic scaffolds—particularly through nitroaryl substitution and sulfur-containing linkers—plays a crucial role in enhancing antimicrobial efficacy [16].

It was observed that acetylated triazole derivatives exhibited stronger activity against *Staphylococcus aureus* compared with their non-acetylated analogues. Based on this finding, acetylation strategies were pursued to enhance the antibacterial potential of the synthesised 1,3,4-thiadiazole derivatives against *S. aureus*. As a result, eighteen new thiadiazole-containing compounds were prepared and subsequently evaluated for their anticonvulsant, antibacterial, and antifungal activities.

Furthermore, nifurtimox, a clinically used 5-nitrofuran derivative substituted at the 2-position, remains an important therapeutic agent for the treatment of Chagas disease, a chronic parasitic infection prevalent in Latin America. In line with the pharmacological significance of nitroheterocycles, several studies have focused on the synthesis and biological evaluation of novel hybrid molecules that integrate the 1,3,4-thiadiazole scaffold with various 5-nitroheterocyclic systems. These hybrid structures have been investigated for their potential antitubercular activity and represent a promising direction in the development of new agents against infectious diseases [17].

The anticancer, antibacterial, and antifungal activities of 1,3,4-thiadiazole derivatives have been reported in the literature. The “N–C–S” moiety within the 1,3,4-thiadiazole structure can act as an active centre, enabling chelation of

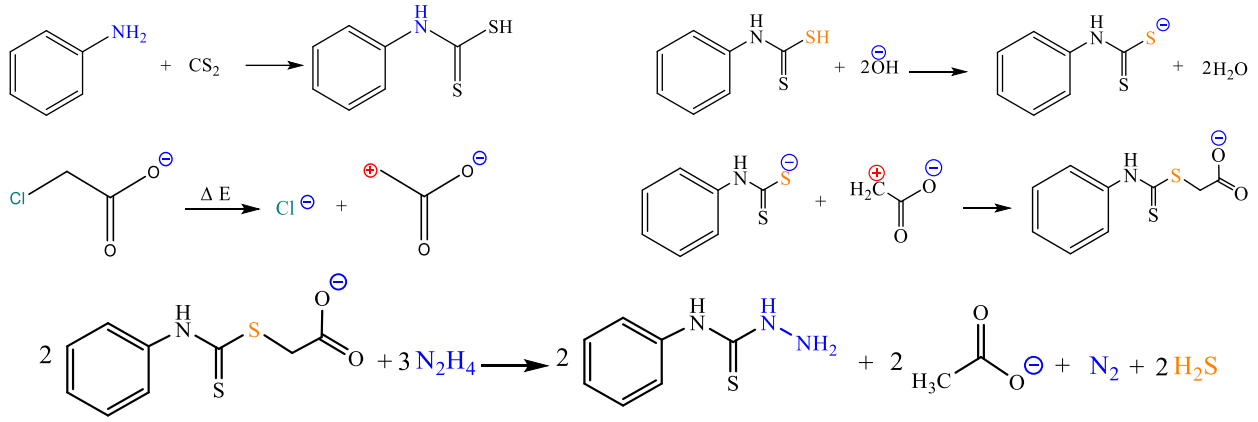
biologically relevant metal ions and facilitating tissue penetration. Furthermore, the incorporation of various heterocyclic groups into the 1,3,4-thiadiazole core has been reported to modulate its biological activity [18, 19].

Another biological property of thiadiazoles is that the heterocyclic ring is considered a bioisostere of pyrimidine. Pyrimidine constitutes the structural framework of three nucleic bases. Therefore, 1,3,4-thiadiazole derivatives possess the ability to disrupt processes associated with DNA replication. This enables them to inhibit the replication of bacterial and cancer cells [20].

Given their diverse pharmacological profiles, including antibacterial, antifungal, analgesic, anticancer, anticonvulsant, anxiolytic, antidiabetic, and anti-inflammatory properties of 1,3,4-thiadiazole derivatives, continue to attract considerable interest for further research. 1,3,4-thiadiazole derivatives bearing an aryl group are known to exhibit biological activity, whereas the activities of compounds containing a thioacetic moiety have been less extensively studied. Therefore, we conducted work on the synthesis of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic acid, and studied its composition and biological activity.

2. Results and Discussion

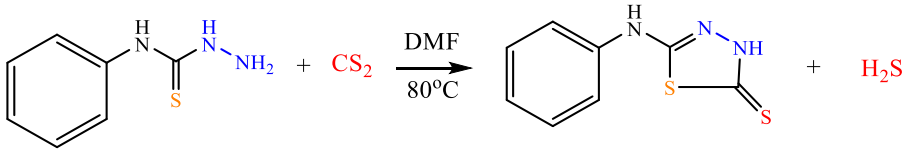
In the first step of the synthesis, aniline reacts with carbon(IV) sulphide in the presence of ammonium hydroxide, resulting in the formation of the phenylcarbamodithioate anion. Upon the addition of potassium monochloroacetate, the reagent undergoes thermal dissociation and subsequently reacts with the phenylcarbamodithioate anion (Scheme 1).



Scheme 1. First-stage synthesis reactions.

Upon the addition of hydrazine to this reaction system, N-phenylhydrazinecarbothioamide is formed. As carbon(IV) sulphide gas is released during the reaction, all experiments must be carried out in a fume hood.

In the second step of the synthesis, carbon(IV) sulphide is added to the DMF solution of N-phenylhydrazinecarbothioamide, and the reaction is conducted at 80 °C to yield 5-(phenylamino)-1,3,4-thiadiazole-2(3H)-thione (Scheme 2).



Scheme 2. Second-stage synthesis: Synthesis of 5-(phenylamino)-1,3,4-thiadiazole-2(3H)-thione.

In the third step of the synthesis, the reaction proceeds according to the scheme shown in Scheme 3.

In the third step of the synthesis, the addition of potassium hydroxide to 5-(phenylamino)-1,3,4-thiadiazole-2(3H)-thione affords potassium 5-(phenylamino)-1,3,4-thiadiazole-2-thiolate. During the reaction, the 5-(phenylamino)-1,3,4-thiadiazole-2-thiolate anion reacts with potassium monochloroacetate to yield potassium 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetate, along with KCl. Upon the addition of hydrochloric acid to the resulting potassium salt, a white precipitate of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic acid is formed. The precipitate is purified by washing with cold water to remove residual salts and impurities, and is subsequently recrystallised from ethanol.

The melting points of the compounds obtained during the synthesis were determined (Table 1).

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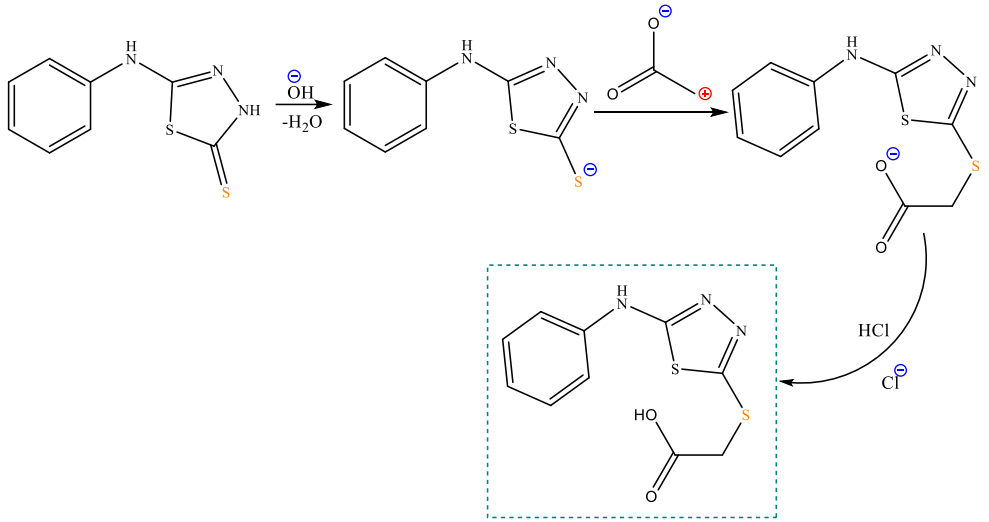

Scheme 3. Third-stage synthesis reaction: Synthesis of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic acid.

Table 1. Melting points of the synthesised compounds.

Compounds	Melting points
N-phenylhydrazine-carbothioamide	223°C
5-(phenylamino)-1,3,4-thiadiazole-2(3H)-thione	204-205°C
2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic acid	194°C

FTIR and UV-Vis spectroscopy as well as X-ray crystallography, were employed to study the composition and structure of the synthesised 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic acid.

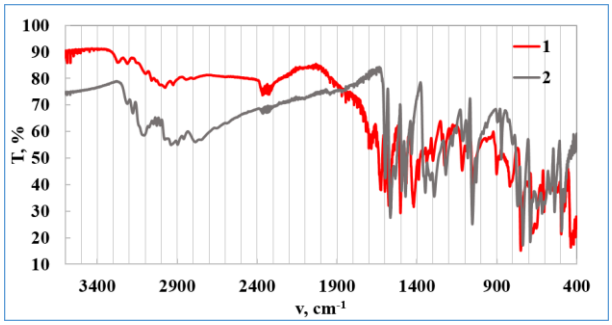

Fig. 1. FTIR spectra of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic acid and 5-(phenylamino)-1,3,4-thiadiazole-2(3H)-thione.

The broad absorption peak at around 3300 cm^{-1} in the FTIR spectrum of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic acid is characteristic of the O–H stretching vibration of the carboxyl group. The absorption at 3057.7 cm^{-1} corresponds to the C–H stretching of the benzene ring, while the peaks at 1420, 1470, and 2979 cm^{-1} are attributed to the C–H stretching vibrations of the methylene group. The strong absorption at 1696 cm^{-1} is characteristic of the C=O stretching vibration of the carboxyl group. The absorptions observed in the 1570–1630 cm^{-1} region are associated with the C=C stretching vibrations of the aromatic ring (Figure 1).

The absorption at 1227 cm^{-1} is attributed to the C–O stretching vibration of the carboxyl group, whereas the peak at 1116.5 cm^{-1} may correspond to C–N or C–S stretching vibrations. The peak at 1050 cm^{-1} is characteristic of C=S stretching, while the absorptions at 680 and 750 cm^{-1} are related to out-of-plane C–H bending vibrations of the benzene ring.

Based on the FTIR spectral data, the successful synthesis of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic acid is confirmed to a considerable extent. In addition to the IR spectrum, the UV–Vis spectrum of the compound was also examined.

The alkali metal salts of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic acid are readily soluble in both water and alcohol, whereas the free acid is poorly soluble in water but readily soluble in alcohol. Therefore, its UV–Vis spectrum was recorded in ethanol (Figure 2). The spectrum shows two principal absorption peaks at 248 and 307 nm.

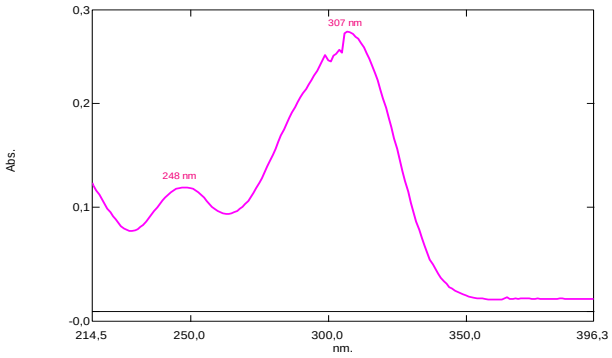

Fig. 2. UV-Vis spectrum of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic acid.

The absorption regions observed at 248 and 307 nm in the UV–Vis spectrum correspond to π→π* and n→π* electronic transitions within the molecule. The absorption at 248 nm is characteristic of conjugated systems or aromatic rings, whereas the band at 307 nm is likely associated with the presence of carbonyl groups.

Single crystals of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic acid were grown from its ethanolic solution. The structure of the compound was investigated using X-ray crystallography on the obtained single crystals. The analysis confirmed both the composition and the molecular structure of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic acid (Figure 3).

The crystal structure of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic acid exhibits a monoclinic lattice, indicating that the molecules are ordered but possess symmetry along only one axis. The compound crystallises in the space group P121/n1, which is among the most common

monoclinic groups. The 4 value of Z corresponds to the number of molecules present in the unit cell and reflects the packing density.

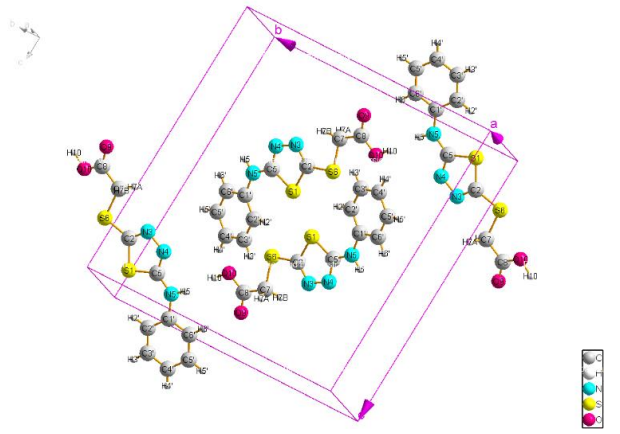

Fig. 3. Unit cell structure of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic acid.

The unit-cell parameters are as follows: a = 4.3285(5) Å, b = 16.2780(17) Å, c = 16.2584(17) Å, α = γ = 90°, β = 90.979(6)°, unit-cell volume V = 1145.39(20) Å^3.

Close intermolecular contacts within the unit cell were examined in detail. The shortest distance was observed between H10 of the carboxyl group of one molecule and H3' of the phenyl group of a neighbouring molecule, measuring 2.9507 Å. This proximity results in strong intermolecular interactions and contributes to the spatial orientation of the molecules.

Furthermore, intermolecular hydrogen bonds were identified between molecules beyond the unit cell. Hydrogen bonding occurs between the carboxyl groups, the nitrogen atoms of the 1,3,4-thiadiazole ring, and the –NH– groups (Figure 4). The hydrogen-bond distance between the carbonyl group (C=O) of the carboxyl moiety and –NH– is 1.9297 Å, while the shortest hydrogen bond, observed between an azole nitrogen and the –OH of the carboxyl group, measures 1.5699 Å.

Both van der Waals forces and hydrogen bonding play significant roles in the formation and stabilisation of the crystal structure.

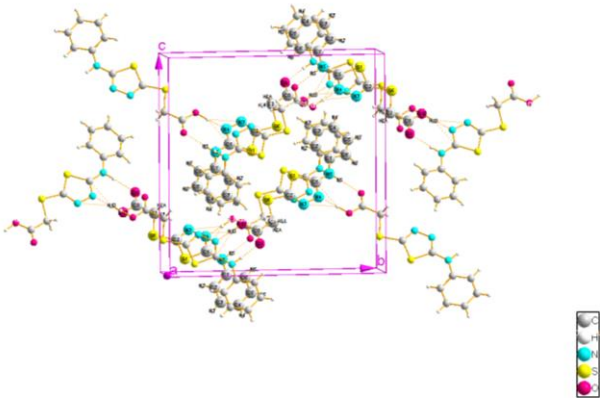

Fig. 4. Hydrogen-bonding interactions between molecules of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic acid located in adjacent unit cells. The figure illustrates the network formed by intermolecular hydrogen bonds involving the carboxyl group, –NH–, and the nitrogen atoms of the 1,3,4-thiadiazole ring, which collectively contribute to the stabilisation of the crystal lattice.

The results of the physical analyses confirm that the composition and molecular structure of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic acid have been successfully elucidated, thereby verifying the complete synthesis of the compound. Numerous literature reports indicate that a variety of biologically active compounds have been synthesised on the basis of the thiadiazole ring, providing extensive information regarding their applications [13–18].

The biological activity and pharmacokinetic properties of the synthesised 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic acid were evaluated theoretically. Its absorption and toxicity within the body were predicted using the ADMETPred platform [21], allowing a preliminary assessment of its potential as a bioactive agent.

Table 2. Predicted absorption parameters of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetate.

Caco2	pampa	hia	bioavailability	pgp	lipophilicity	solubility	freesolv
0.390	1	1	1	0	3.084	-3.378	-7.078

Table 2 presents the pharmacokinetic properties and physicochemical parameters of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetate.

The Caco-2 permeability value predicted by ADMETPred is 0.390 × 10^{-6} cm/s, indicating relatively low absorption through intestinal epithelial cells, as values ≥ 0.5 × 10^{-6} cm/s are generally considered favourable. The Human Intestinal Absorption (HIA) value is 1, suggesting good intestinal uptake. A bioavailability value of 1 also implies potential suitability for oral administration. The P-gp value of 0 indicates that the compound is not a P-glycoprotein substrate and is therefore unlikely to be actively effluxed from cells. Taken together, these parameters suggest that the compound has potential as a drug candidate.

The lipophilicity value (LogP/LogD = 3.084) indicates optimal lipophilicity according to Lipinski's rule of five (LogP ≤ 5), implying good membrane permeability. Higher lipophilicity may also favour intestinal absorption and may enable penetration into the brain (via the blood–brain barrier) or other tissues. The predicted water-solubility parameter is moderate (–3.378), indicating low aqueous solubility; however, experimental observations show that the potassium salt of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetate is readily soluble in water.

The predicted toxicity parameters of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetate were also assessed, and the results are summarised in Table 3.

Taking into account that both the hERG_blockers and hERG_Karim values are 0, 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetate can be regarded as unlikely to induce cardiac arrhythmia. The alternative hERG model (hERG_Karim) similarly does not indicate any cardiac risk. According to the ADMETPred prediction, the compound presents no mutagenic risk. A skin sensitisation value of 0

suggests a low likelihood of dermal allergic reactions, while a carcinogen value of 0 indicates that the compound is non-carcinogenic. However, a DILI (Drug-Induced Liver Injury) value of 1 points to a potential risk of hepatotoxicity, and therefore, caution is advised during use.

Table 3. Predicted toxicity parameters of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetate.

LD_{50} (oral, rat)	2.202
hERG blockers	0
hERG Karim	0
AMES (mutagenicity)	0
Skin sensitization	0
DILI (Drug-Induced Liver Injury)	1
Carcinogens	0
ClinTox	0

ADMETPred identified only a single toxic liability. To reduce or eliminate this predicted toxicity, further structural optimisation studies — for example, the introduction of different functional groups into the 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic acid will be necessary.

Since the compound is highly likely to exist in its anion form in vivo, an in silico comparison was performed to determine which enzymes or protein targets it may interact with in that ionic state. For this purpose, the 3DStarPred tool within the AI-DrugIP platform was used. The results indicated a high probability of binding between 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetate and the human Histamine H_3 receptor (Table 4). Additionally, other derivatives of 1,3,4-thiadiazole [22], as well as 1-(3-(4-tert-butylphenoxy)propyl)piperidine (ABT-239), the most selective antagonist for the Histamine H_3 receptor, were compared.

Table 4. Predicted activity parameters of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetate and other compounds for the Histamine H_3 receptor

Chemical compound	Pref_name	Organism	Max_similarity	Max_activity (nM)	overall
2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetate	Histamine H_3 receptor	*Homo Sapiens*	0.78216	5000	4.14623
2-((5-(p-tolylamino)-1,3,4-thiadiazol-2-yl)thio)acetate	Histamine H_3 receptor	*Homo Sapiens*	0.76213	5700	3.99668
2-((5-morpholino-1,3,4-thiadiazol-2-yl)thio)acetate	Histamine H_3 receptor	*Homo Sapiens*	0.70213	8400	3.56382
1-(3-(4-tert-butylphenoxy)propyl)piperidine (ABT-239)	Histamine H_3 receptor	*Homo Sapiens*	0.9132	0.26	8.75306

From the results presented in Table 4, it is evident that ABT-239 has a very high likelihood of acting on the Histamine H_3 receptor. However, an evaluation of the toxicological properties of ABT-239 revealed that it may exhibit toxicity and potentially hazardous effects. The predictions of its toxicological characteristics provided the following results (Table 5).

Table 5. Predicted toxicity parameters of ABT-239.

LD_{50} (oral, rat)	3.555
hERG blockers	1
hERG Karim	1
AMES (mutagenicity)	1
Skin sensitization	0
DILI (Drug-Induced Liver Injury)	0
Carcinogens	0
ClinTox	1

Based on the analysis results, the compound has been identified as a hERG blocker (1). The inhibition of hERG channels significantly affects cardiac electrophysiological activity and may lead to potentially life-threatening adverse effects, such as arrhythmias and Torsades de Pointes. Therefore, this parameter represents a serious limitation for the further clinical development of the compound.

In addition, a positive outcome in the ADMETPred test indicates that ABT-239 may possess genotoxic and mutagenic potential. The ADMETPred test is considered a primary screening criterion for identifying molecules that affect genomic stability, and a positive result requires further confirmation through additional in vitro and in vivo studies. Interestingly, although the mutagenicity parameter shows a positive result, the carcinogenicity value (0) is negative, suggesting that the compound may be mutagenic, but its carcinogenic potential has not yet been confirmed. This situation demonstrates that there is no direct association between genotoxicity and carcinogenicity and highlights the need for further experimental validation.

Other toxicological parameters, including DILI (0) and skin sensitisation (0), indicate that the compound is unlikely to induce liver toxicity or skin sensitisation, suggesting a relatively safe profile within certain biological systems. Finally, the ClinTox value of 1 implies a potential risk of clinical adverse effects.

Due to its cardiotoxic (hERG blocking) and genotoxic (positive ADMETPred test) characteristics, ABT-239 presents a high safety risk and therefore is not recommended for direct clinical application. To advance ABT-239 as a drug candidate, structural optimisation, pharmacophore analysis, toxicity-reducing modifications, and comprehensive preclinical evaluation are required.

Some toxicological aspects of ABT-239 have been investigated in preclinical studies. ABT-239 is a highly selective H_3 receptor antagonist and has demonstrated favourable pharmacokinetic properties (bioavailability, half-life, etc.) in various animal models. However, one source reports that QT prolongation (cardiac rhythm extension) was observed in monkeys, which represents a clinically limiting safety concern [23].

In stress-based models, long-term administration of ABT-239 in mice has been shown to influence memory and cognitive function — in some cases producing positive effects (in stressed animals), while in others (unstressed animals), potential adverse impacts have been reported. Additionally, when tested in combination with nicotine, ABT-239 was found to enhance hyperactivity in animals. Since ABT-239 and other antagonists of the H_3 receptor can improve cognitive function, their effects on central nervous system activity remain a point of consideration [24].

The low toxicity profile of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetate ions increases their significance and potential value. The similarity score of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetate to known ligands binding the histamine H_3 receptor was predicted to be 0.782 (78.2%), which represents a relatively high value. This indicates a strong likelihood of interaction with the histamine H_3 receptor. The most structurally similar reference ligand demonstrates an experimental activity of 5000 nM, corresponding to a moderate-to-weak binding affinity.

The overall predicted activity score was calculated as 4.14623, which is considered favourable, as values above 4.0 are typically regarded as strong indicators of biological relevance. Therefore, 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetate is likely to exhibit biological activity; however, it may also present a potential risk of off-target interactions or side effects. Consequently, further detailed experimental validation is required.

Molecular docking simulations were performed using AutoDock Vina to investigate the binding interactions of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic acid and its analogues with the histamine H_3 receptor. The calculations were performed using the validated model of the histamine H_3 receptor based on X-ray analysis (PDB ID: 7F61). The receptor–ligand search space was defined using a grid box centred at coordinates (x = 0 Å, y = 0 Å, z = 0 Å). The grid box dimensions were set to 50 × 107 × 50 Å, covering the entire binding region of the receptor. The docking exhaustiveness parameter was adjusted to 31 to ensure sufficient sampling of ligand conformations, and the maximum number of generated binding poses (modes) was set to 20. The energy range parameter was set to 0–1 kcal/mol, and the grid spacing was configured as 1 Å. Among the generated conformations, only the lowest binding affinity poses were selected for subsequent interaction analysis. The calculations identified key amino acid residues within the histamine H_3 receptor that participate in ligand binding (Figure 5). The Gibbs free energy values for the most favourable binding conformations were −8.0, −7.7, −6.9, and −8.6 kcal/mol for 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetate, 2-((5-(p-tolylamino)-1,3,4-thiadiazol-2-yl)thio)acetate, 2-((5-morpholino-1,3,4-thiadiazol-2-yl)thio)acetate, and 1-(3-(4-tert-butylphenoxy)propyl)piperidine, respectively.

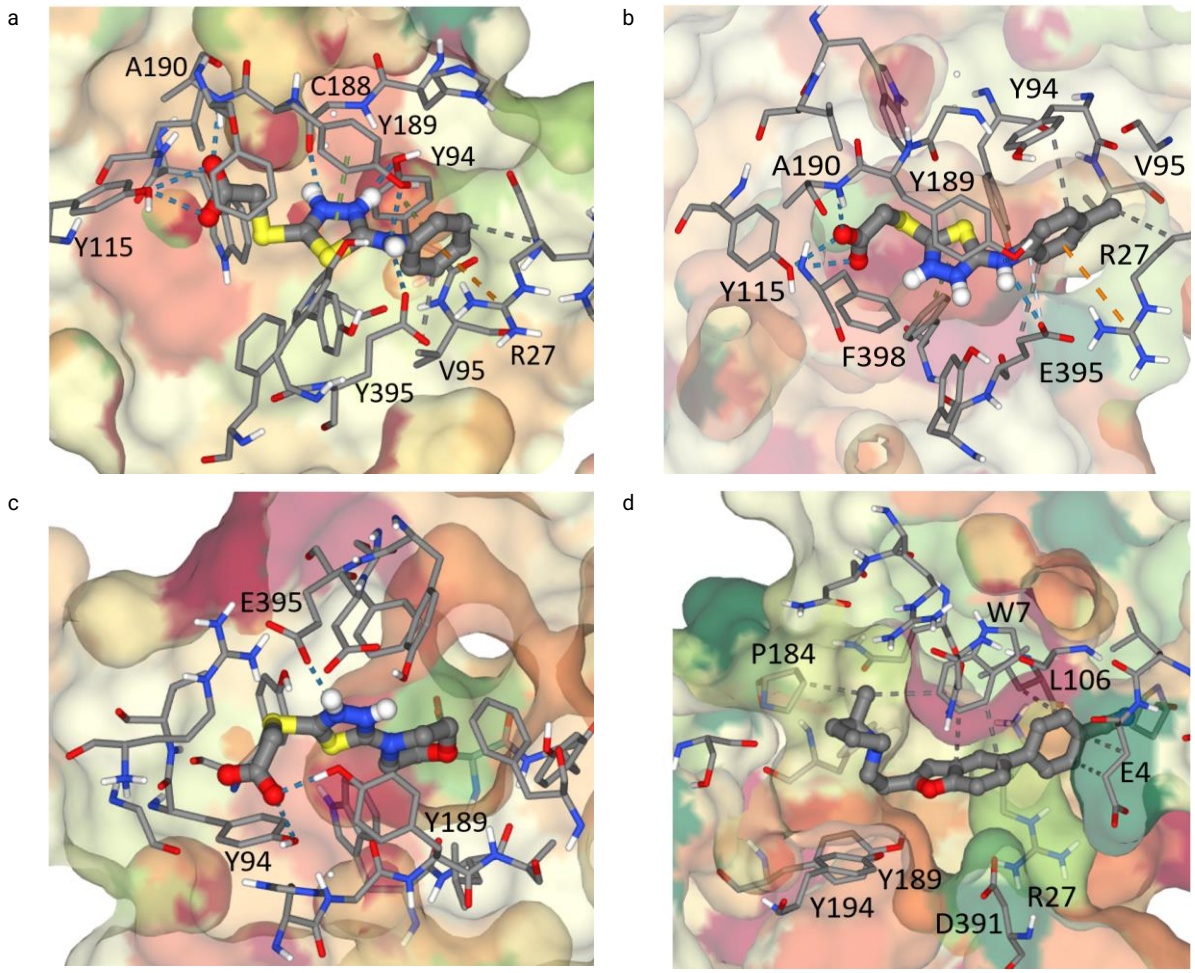

Fig. 5. Binding interactions of different antagonists with the histamine H_3 receptor. Binding of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic (a), 2-((5-(p-tolylamino)-1,3,4-thiadiazol-2-yl)thio)acetate (b), 2-((5-morpholino-1,3,4-thiadiazol-2-yl)thio)acetate (c) and 1-(3-(4-tert-butylphenoxy)propyl)piperidine (d).

Based on the molecular docking results, the ligands with the lowest Gibbs free energy in binding to the histamine H_3 receptor are 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetate (-8.0 kcal/mol) and ABT-239 (-8.6 kcal/mol). In this case, ABT-239 is expected to have a higher binding affinity to the receptor. However, the clinical application of ABT-239 is limited due to its reported side effects and toxic properties. In contrast, the lower probability of adverse effects and

reduced toxicity of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetate is of significant importance. These findings indicate that 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetate and its derivatives may serve as promising candidates for future compound development.

The histamine H_3 receptor functions as an autoreceptor for histamine in the central nervous system. When agonists (such as histamine) bind to the receptor, it becomes activated and inhibits the release of neurotransmitters (including acetylcholine, dopamine and others). In contrast, antagonists stabilise the receptor in its inactive conformation, thereby blocking the effects of agonists and subsequently increasing neurotransmitter release. This mechanism is exploited therapeutically in conditions such as Alzheimer's disease, schizophrenia, and sleep disorders.

The interactions of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetate with the receptor are characteristic of binding to the orthosteric site (binding pocket) of the histamine H_3 receptor, a feature observed in many known antagonists.

The binding pocket of H_3R is formed by transmembrane helices (TM2, TM3, TM5, TM6, TM7) and extracellular loop 2 (ECL2). Residues Y115, A190 (ECL2), C188, Y94, and E395 stabilise the ligand within the pocket and regulate the conformation of the receptor. These interactions anchor the polar functional groups of the ligand (e.g., amino or carbonyl groups).

Residues Y189 (ECL2) and Y94 form a "sandwich" arrangement around aromatic rings, stabilising the ligand in the upper region of the pocket. Y189 engages in π–π stacking with phenyl groups, enhancing antagonist affinity.

For 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetate, cationic interactions with R27 (such as with a protonated amine group) have been observed, contributing to closing of the pocket.

V95 maintains the aromatic portion of the ligand in a hydrophobic environment, thus lowering binding energy. Hydrogen bonding/π-π interactions with Y115 and Y94, along with π-π/hydrophobic contacts with Y189 and A190, effectively “lock” the ligand inside the pocket.

These interactions indicate that 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetate occupies the orthosteric binding site (where histamine binds), thereby preventing agonist entry. For example, hydrogen bonding with Y115 and E395 positions the ligand near D114 and E206, residues that bind the protonated amine group of histamine. However, instead of activating the receptor, the ligand functions as an antagonist.

Stabilisation of the inactive conformation is achieved through π-π (Y189, Y94) and cation-π (R27) interactions, which position ECL2 as a “lid”, closing the binding site and maintaining inward orientation of the TM helices.

The observed hydrophobic (V95) and hydrogen bonding (A190, C188) interactions contribute significantly to strong ligand binding and enhanced inhibition. Based on these interactions, the compound behaves as an antagonist, as it prevents conformational changes associated with receptor activation by agonists.

3. Material and Methods

The following reagents were used for the syntheses: carbon(IV) sulphide (99 %, KK International, West Bengal), aniline (Alfa Aesar by Thermo Fisher Scientific), ammonia (25 %, Merck KGaA, Darmstadt, Germany), potassium monochloroacetate (99.9 %, Nature of Business, India), bidistilled water, ethanol (99.99 %), dimethylformamide (99.99 %, Malaysia Bio Lab), hydrazine (80 %, M/s Shalibhadra Speciality Chemical), and potassium hydroxide (99.9 %, Nature of Business, India).

The following equipment was used: magnetic stirrer (DLab, MS7-H550-S), two-neck round-bottom flask (100 ml), Liebig condenser, conical flask (250 ml), beakers (50, 100, and 500 ml), filter paper, Büchner funnel, Bunsen flask, vacuum pump, 2 Petri dishes, analytical balance (Aczel CY 224 C), 2 silica gel papers, UV-Vis spectrophotometer (UV-1800 Shimadzu), FTIR spectrometer (JASCO FT/IR-4600), pipettes (1, 5, and 10 ml), graduated cylinders (25 and 50 ml), diffractometer D8 VENTURE PHOTON III (Bruker), glass capillaries for melting point measurement (internal diameter 1 mm), and a melting point apparatus (SMP 10).

3.1. Synthesis of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic acid

The synthesis was performed using the following steps.

First stage: Synthesis of N-phenylhydrazinecarbothioamide

A total of 0.02 mol of aniline was dissolved in ethanol. To the resulting solution, 0.06 mol of concentrated aqueous ammonia was added. While stirring continuously, 0.025 mol of carbon (IV) sulphide was introduced dropwise. The reaction temperature was maintained below 30 °C throughout.

After the carbon (IV) sulphide had completely dissolved, the reaction mixture was left to stand for 1 hour and then poured into a pre-prepared solution containing 0.02 mol of the potassium salt of monochloroacetic acid dissolved in 25 ml of water. The mixture was subsequently heated at 60–70 °C until a clear solution was obtained. Without cooling, 10 ml of 50 % hydrazine was added, resulting in the formation of a yellow solution. After standing, colourless crystals of N-phenylhydrazinecarbothioamide began to precipitate.

The reaction mixture was filtered, and the filtrate was concentrated to half of its initial volume by heating. Additional N-phenylhydrazinecarbothioamide precipitated and was collected by filtration and washed with water.

Second stage: Synthesis of 5-(phenylamino)-1,3,4-thiadiazole-2(3H)-thione

A total of 0.01 mol of N-phenylhydrazinecarbothioamide and 0.012 mol of carbon (IV) sulphide were dissolved in 15 ml of dimethylformamide and maintained at 80 °C for 1.5 hours. The resulting reaction mixture was then poured into water at ten times its volume, leading to the formation of a white precipitate. The solid product was collected by filtration and washed with distilled water.

Third stage: Synthesis of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic acid

A total of 30 ml of ethanol was placed in a 100 ml round-bottom flask, and 0.05 mol of 5-(phenylamino)-1,3,4-thiadiazole-2(3H)-thione was dissolved in it at 75 °C under continuous stirring. 0.05 mol of solid KOH was then added gradually, and the mixture was stirred until complete dissolution was achieved.

Subsequently, 20 ml of a 2.5 M potassium monochloroacetate solution in ethanol was added dropwise, and the reaction to proceed for 5 hours. The resulting precipitate was treated with 20 ml of 2.5 M hydrochloric acid solution, collected by filtration, and washed with cold water to remove residual KCl. The crude product was then recrystallised from ethanol. The product was obtained in 87% yield.

The composition and structure of the obtained 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic acid crystals were characterised using physicochemical methods.

4. Conclusions

The synthesis of 2-((5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio)acetic acid was successfully achieved, and its composition and structure were confirmed using standard physicochemical methods. In silico pharmacokinetic and toxicological assessments suggested that the compound possesses favourable drug-like properties; however, the predicted risk of hepatotoxicity requires further investigation. Molecular docking studies demonstrated that the compound exhibits strong binding affinity toward the Histamine H_3 receptor, indicating a potential influence on central nervous system targets. Overall, these findings suggest that the compound may serve as a promising scaffold for the development of neuroactive agents. Experimental biological validation and further pharmacological studies are recommended for future work.

Author Contributions

Otaniyoz Ataniyazov- conceptualisation, investigation, methodology, writing - original draft; Khudaybergan Polvonov - conceptualisation, methodology, data curation, supervision; Khushnudbek Eshchanov - formal analysis, Investigation, methodology, writing - review & editing; Rasul Okmanov - validation, resources; Nurbek Razzokberdiev - resources, validation.

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