4-phenyl-5-((pyrimidin-2-ylthio)methyl)-1,2,4-triazole-3-thiol as a platform for the synthesis of unsymmetrical disulfides

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

The article discusses effective methods for the synthesis of unsymmetrical disulfides, which are important in the development of new bioactive molecules, pharmaceuticals, and materials for bioconjugation and systemic release.

The aim of the work is to develop a method for the synthesis of unsymmetrical disulfides of 4-phenyl-5-((pyrimidin-2-ylthio)methyl)-1,2,4-triazole-3-thiols under mild conditions, with high selectivity and wide tolerance to functional groups, including the disulfide fragment as a promising structural framework for the development of new active pharmaceutical ingredients.

Materials and methods. ^{1}H NMR spectra were recorded on a Bruker AC-500 spectrometer (500 MHz) in DMSO-d_6. Elemental analysis (C, H, N, S) was performed using an ELEMENTAR vario EL cube. Melting points were determined using the capillary method.

Results. A new strategy for the synthesis of unsymmetrical disulfides of 4-phenyl-5-((pyrimidin-2-ylthio)methyl)-1,2,4-triazole-3-thiols using 1-chlorobenzotriazole has been developed, providing high product yields and selective transformation. This method does not require low temperatures and demonstrates high compatibility with various functional groups, allowing easy modification of molecules and further research potential. Spectral data confirm the structure of the obtained compounds and indicate the formation of stable disulfide bonds.

Conclusions. The synthesis of new unsymmetrical disulfides of 4-phenyl-5-((pyrimidin-2-ylthio)methyl)-1,2,4-triazole-3-thiols was successfully carried out under mild conditions using 1-chlorobenzotriazole as a selective reagent, resulting in high yields and good tolerance to functional groups. Their structural similarity to natural antioxidants such as cystine and allicin makes them promising model compounds for further studies of the mechanisms of redox activity and the development of new drugs to regulate oxidative stress.

Keywords: 1,2,4-triazole, disulfides, mild conditions, high selectivity, antimicrobial activity, antioxidant activity.

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4-феніл-5-((піримідин-2-ілтіо)метил)-1,2,4-тріазол-3-тіол як платформа для синтезу несиметричних дисульфідів

Ю. В. Карпенко, М. О. Панасенко

Описано ефективні методи синтезу несиметричних дисульфідів, що мають значення під час розробки нових біоактивних молекул, фармацевтичних препаратів і матеріалів для біокон'югацій і систем контрольованого вивільнення.

Мета роботи – розробка методу синтезу несиметричних дисульфідів 4-феніл-5-((піримідин-2-ілтіо)метил)-1,2,4-тріазол-3-тіолів за м'яких умов із високою селективністю та широкою толерантністю до функціональних груп, включаючи дисульфідний фрагмент як перспективний структурний каркас для розроблення нових активних фармацевтичних інгредієнтів.

Матеріали і методи. Спектри ^{1}H ЯМР записано на спектрометрі Bruker AC-500 (500 МГц відповідно) у ДМСО-d_6. Елементний аналіз (C, H, N, S) здійснили на приладі ELEMENTAR vario EL cube. Температури плавлення визначали капілярним методом.

Результати. Розроблено нову стратегію синтезу несиметричних дисульфідів 4-феніл-5-((піримідин-2-ілтіо)метил)-1,2,4-тріазол-3-тіолів за допомогою 1-хлоробензотріазолу, що забезпечує високий вихід продуктів і селективність перетворення. Цей метод не потребує низьких температур і характеризується високою сумісністю з різноманітними функціональними групами, що дає змогу легко модифікувати молекули, підвищує їхній потенціал для наступних досліджень. Спектральні дані підтверджують структуру отриманих сполук і вказують на утворення стабільних дисульфідних зв'язків.

Висновки. Синтез нових несиметричних дисульфідів 4-феніл-5-((піримідин-2-ілтіо)метил)-1,2,4-тріазол-3-тіолів успішно здійснено за м'яких умов із використанням 1-хлоробензотріазолу як селективного реагенту, що забезпечує високий вихід і значну толерантність до функціональних груп. Структурна подібність до природних антиоксидантів, зокрема цистину й аліцину, робить їх перспективни-

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ми модельними сполуками для наступних досліджень механізмів окисно-відновної активності та розроблення нових препаратів, спрямованих на регуляцію оксидативного стресу.

Ключові слова: 1,2,4-тріазол, несиметричні дисульфіди, м'які умови, висока селективність, протимікробна активність, антиоксидантна активність.

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The synthesis of unsymmetrical disulfides has garnered significant attention in recent years due to their relevance in developing novel bioactive molecules, pharmaceuticals, bioconjugation materials, and controlled release systems [1]. Unlike symmetrical disulfides, the synthesis of unsymmetrical disulfides requires strict control of reaction conditions to prevent the formation of homodisulfides, thereby posing a significant challenge to achieve high chemical selectivity and efficient preparation. Disulfide bonds play a crucial role in stabilizing the three-dimensional structure of proteins, maintaining intracellular redox homeostasis, and enabling the design of compounds with either prooxidant or antioxidant properties. In this context, the development of efficient, selective, and environmentally friendly methods for the synthesis of unsymmetrical disulfides remains a highly relevant and timely objective.

Despite notable advances in this area, most existing methods still suffer from several significant limitations. In particular, many rely on highly toxic reagents such as bromine (Br_2), thionyl chloride ($SOCl_2$), or sulfuryl chloride (SO_2Cl_2), which greatly complicates their practical use – especially in the context of bio-oriented synthesis. Additionally, these approaches often require harsh reaction conditions, multi-step procedures, and prior activation of thiol groups through their conversion into sulfonyl derivatives or similar intermediates. Such factors reduce chemical selectivity, lower yields of target compounds, and ultimately diminish the overall efficiency of the synthetic process [2].

In medicinal chemistry, the disulfide bond is of particular interest as a pharmacophoric element capable of modulating the biological activity of organic molecules. The incorporation of a disulfide moiety into a compound's structure can not only stabilize its three-dimensional conformation but also serve as a functional group that imparts specific biological properties, particularly antimicrobial activity. In this context, the development of new antimicrobial agents based on unsymmetrical disulfides represents a promising research direction, especially in addressing the growing challenge of multidrug-resistant microorganisms.

This hypothesis is supported by natural organosulfur compounds found in garlic (*Allium sativum*), such as S-alkylcysteine sulfoxides, diallyl disulfides, and allicin. Allicin, in particular, has attracted considerable scientific interest due to its broad spectrum of biological activities, including antibacterial, antifungal, and antiviral effects. According to a recent study [3], allicin's high reactivity, short intracellular metabolic pathway, and lack of specificity for protein targets are key factors that hinder the development of bacterial resistance. The study's authors highlight that this nonspecific yet potent mode of action may serve as a foundation for designing new therapeutics aimed at combating multidrug-resistant pathogens.

Thus, the disulfide moiety is regarded as a promising structural motif for the development of novel drug molecules capable of addressing one of the most critical challenges in modern pharmacology – antibiotic resistance.

Another promising avenue for the development of novel unsymmetrical disulfide-containing compounds is the design of molecules with pronounced antioxidant potential. In light of the growing interest in therapeutic agents capable of effectively neutralizing reactive oxygen species (ROS) and preventing oxidative stress, the targeted synthesis of antioxidant-active structures represents a key objective in medicinal chemistry. One of the most effective domestic antioxidant drugs, Thiotriazolin®, is known for its strong cardio- and hepatoprotective effects, which are attributed to its ability to stabilize cell membranes, inhibit lipid peroxidation, and modulate the activity of antioxidant enzymes [4,5].

In this context, the proposed compounds for the synthesis incorporate a functionally significant 1,2,4-triazole-3(2*H*)-thione fragment [6] – a key structural motif found in Thiotriazolin® and various other biologically active derivatives *(Fig. 1)*. It is anticipated that the combination of this heterocyclic core with a disulfide bridge will enhance antioxidant properties, owing to the potential involvement of the disulfide group in reversible redox transformations.

Additionally, the structural analogy with natural amino acids, particularly cystine, which contains a disulfide bond and plays a crucial role in maintaining redox homeostasis in the human body, is noteworthy. Cystine, a dimeric form of cysteine, serves as a substrate for the biosynthesis of glutathione, one of the primary low-molecular antioxidants in cells. Glutathione exists in both oxidized (GSSG) and reduced (GSH) forms, and its functions include neutralizing ROS, maintaining the reduced state of thiol groups in proteins, and detoxifying xenobiotics [7]. Thus, the structural mimicry or functional replication of the behavior of natural redox-active compounds offers promising prospects for the development of novel synthetic antioxidants with potential pharmacological activity.

The interconversion between the oxidized and reduced forms of glutathione is catalyzed in the body by the enzyme glutathione S-transferase. This enzyme belongs to the oxidoreductase family and exhibits specific activity towards the donor thiol group and the acceptor disulfide group.

Considering the above, a pressing challenge is to develop new methods for the synthesis of unsymmetrical disulfides that adhere to the principles of green chemistry, offer mild reaction conditions, high selectivity, and compatibility with a broad range of functional groups.

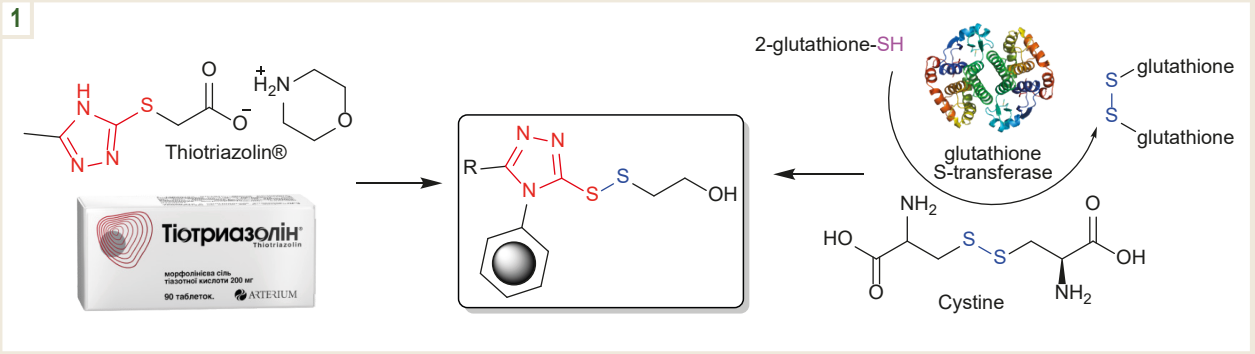

Fig. 1. Strategy for identifying antioxidant activity of disulfide derivatives of 4-phenyl-4*H*-1,2,4-triazoles.

Aim

The aim of this work is to develop a synthetic method for unsymmetrical disulfides of 4-phenyl-5-((pyrimidin-2-ylthio)methyl)-1,2,4-triazole-3-thiols under mild conditions, with high selectivity and broad functional group tolerance, incorporating a disulfide moiety as a promising structural scaffold for the development of novel active pharmaceutical ingredients.

Materials and methods

^{1}H NMR spectra were recorded on a Bruker AC-500 spectrometer (500 MHz, respectively) in DMSO-d_6, using TMS as the internal standard (Agilent Technologies, Santa Clara, California, USA). Elemental analysis (C, H, N, S) was carried out on an ELEMENTAR vario EL cube, with sulfanilamide as the standard. Melting points were determined using the capillary method in a Stanford Research Systems Melting Point Apparatus 100 (SRS, USA). The reagents used were purchased from Sigma-Aldrich (Merck).

The compounds were synthesized using a known method [8], and the constants obtained corresponded to the literature data.

Method for obtaining 1-chlorobenzotriazole. Treat benzotriazole with sodium hypochlorite in 50 % aq acetic acid. The reagent quickly precipitates and is obtained in nearly quantitative yield after recrystallization from CH_2Cl_2 / petroleum ether.

Method for obtaining 3-(R-disulfanyl)-5-(4-R')-4-phenyl-4H-1,2,4-triazoles. A solution of 1-chlorobenzotriazole (0.61 g, 4 mmol), 1,2,3-benzotriazole (0.32 g, 0.7 mmol) and triethylamine (0.03 g, 0.3 mmol) in dichloromethane was cooled to -25 °C using an insulated ice bath (66 g NaBr + 100 g ice). To this solution, a solution of 5-R-4-phenyl-4*H*-1,2,4-triazole-3-thiol (2.7 mmol) in 2 ml of dichloromethane was added dropwise. The reaction mixture was stirred for 2 hours while gradually warming to 10 C. Subsequently, a second thiol derivative (4 mmol) in dichloromethane was added dropwise, and stirring was continued at 0 °C for an additional 30 minutes. The reaction was quenched by the simultaneous addition of 10 ml of an aqueous sodium thiosulfate solution (0.45 g) and 10 ml of saturated aqueous sodium bicarbonate under vigorous stirring for 20 minutes. After completion, the mixture was subjected to liquid-liquid extraction with dichloromethane (3 × 100 ml). The combined organic layers were dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The crude products were purified by recrystallization from hexane.

2-((5-(2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-4-phenyl-4H-1,2,4-triazol-3-yl)disulfaneyl)acetic acid (**4a**). Yield 70 %, yellow crystalline compound, mp 196–198 °C.^{1}H NMR(DMSO-d_6, 500 MHz): δ (ppm) 3.73 (s, 2H), 6.15 (s, 1H), 7.37–7.43 (m, 2H), 7.43–7.49 (m, 2H), 7.52–7.59 (m, 1H), 11.29 (s, 1H), 11.83 (s, 1H), 11.89 (s, 1H). Found, %: C 44.51; H 2.96; N 18.51; S 17.04. $C_{14}H_{11}N_5O_4S_2$. Calculated, %: C 44.56; H 2.94; N 18.56; S 16.99.

6-(5-((2-hydroxyethyl)disulfaneyl)-4-phenyl-4H-1,2,4-triazol-3-yl)pyrimidine-2,4(1H,3H)-dione (**4b**). Yield 95 %, orange amorphous substance, mp 184–185 °C. ^{1}H NMR(DMSO-d_6, 500 MHz): δ (ppm) 2.94 (t, J = 4.1 Hz, 2H), 3.65 (dt, J = 5.4, 4.2 Hz, 2H), 5.33 (t, J = 5.4 Hz, 1H), 6.15 (s, 1H), 7.37–7.43 (m, 2H), 7.43–7.48 (m, 2H), 7.53–7.59 (m, 1H), 11.29 (s, 1H), 11.83 (s, 1H). Found, %: C 46.21; H 3.74; N 19.33; S 17.69. $C_{14}H_{13}N_5O_3S_2$. Calculated, %: C 46.27; H 3.61; N 19.27; S 17.64.

6-(5-((3-hydroxypropyl)disulfaneyl)-4-phenyl-4H-1,2,4-triazol-3-yl)pyrimidine-2,4(1H,3H)-dione (**4c**). Yield 91 %, yellow crystalline substance, mp 172–173 °C. ^{1}H NMR(DMSO-d_6, 500 MHz): δ (ppm) 1.73 (p, J = 5.5 Hz, 2H), 2.86 (t, J = 5.6 Hz, 2H), 3.57 (q, J = 5.4 Hz, 2H), 3.93 (t, J = 5.6 Hz, 1H), 6.15 (s, 1H), 7.37–7.43 (m, 2H), 7.43–7.48 (m, 2H), 7.53–7.59 (m, 1H), 11.29 (s, 1H), 11.83 (s, 1H). Found, %: C 47.65; H 4.13; N 18.51; S 16.86. $C_{15}H_{15}N_5O_3S_2$. Calculated, %: C 47.73; H 4.01; N 18.56; S 16.99.

6-(5-((2-hydroxypropyl)disulfaneyl)-4-phenyl-4H-1,2,4-triazol-3-yl)pyrimidine-2,4(1H,3H)-dione (**4d**). Yield 85 %, yellow crystalline substance, mp 154–155 °C. ^{1}H NMR(DMSO-d_6, 500 MHz): δ (ppm) 1.32 (d, J = 6.1 Hz, 3H), 2.76 (dd, J = 12.8, 4.0 Hz, 1H), 3.03 (dd, J = 12.8, 4.2 Hz, 1H), 4.07 (qdt, J = 6.0, 4.8, 4.2 Hz, 1H), 4.22 (d, J = 4.8 Hz, 1H), 6.15 (s, 1H), 7.37–7.43 (m, 2H), 7.43–7.48 (m, 2H), 7.53–7.59 (m, 1H), 11.29 (s, 1H), 11.83 (s, 1H). Found, %: C 47.71; H 4.11; N 18.45; S 16.73. $C_{15}H_{15}N_5O_3S_2$. Calculated, %: C 47.73; H 4.01; N 18.56; S 16.99.

6-(5-((4,5-dihydrothiazol-2-yl)disulfaneyl)-4-phenyl-4H-1,2,4-triazol-3-yl)pyrimidine-2,4(1H,3H)-dione (**4e**). Yield 93 %, white crystalline powder, mp 228–230 °C. ^{1}H NMR(DMSO-d_6, 500 MHz): δ (ppm) 3.39 (dd, J = 4.6, 3.9 Hz, 2H), 3.90–3.94 (m, 2H), 6.15 (s, 1H), 7.37–7.43 (m, 2H), 7.43–7.48 (m, 2H), 7.53–7.59 (m, 1H), 11.29 (s, 1H), 11.83 (s, 1H). Found, %: C 44.46; H 2.91; N 20.73; S 23.71. $C_{15}H_{12}N_6O_2S_3$. Calculated, %: C 44.54; H 2.99; N 20.78; S 23.78.

2-((4-phenyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazol-3-yl)disulfaneyl)acetic acid (**4f**). Yield 74 %, yellow crystalline substance, mp 225–227 °C.^{1}H NMR(DMSO-d_6, 500 MHz): δ (ppm) 3.73 (s, 2H), 4.64 (s, 2H), 7.20 (t, J = 3.7 Hz, 1H), 7.37–7.44 (m, 2H), 7.41 (s, 2H), 7.52–7.60 (m, 1H), 8.52 (d, J = 3.7 Hz, 2H), 11.89 (s, 1H). Found, %: C 46.12; H 3.31; N 17.93; S 24.31. $C_{15}H_{13}N_5O_2S_3$. Calculated, %: C 46.02; H 3.35; N 17.89; S 24.57.

2-((4-phenyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazol-3-yl)disulfaneyl)ethan-1-ol (**4g**). Yield 86 %, yellow crystalline substance, mp 168–170 °C. ^{1}H NMR(DMSO-d_6, 500 MHz): δ (ppm) 2.94 (t, J = 4.1 Hz, 2H), 3.65 (dt, J = 5.4, 4.2 Hz, 2H), 4.64 (s, 2H), 5.33 (t, J = 5.4 Hz, 1H), 7.20 (t, J = 3.7 Hz, 1H), 7.37–7.44 (m, 4H), 7.52–7.60 (m, 1H), 8.52 (d, J = 3.7 Hz, 2H). Found, %: C 47.64; H 4.22; N 18.51; S 25.37. $C_{15}H_{15}N_5OS_3$. Calculated, %: C 47.73; H 4.01; N 18.55; S 25.48.

4-((4-phenyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazol-3-yl)disulfaneyl)butan-1-ol (**4h**). Yield 90 %, yellow crystalline substance, mp 218–220 °C.^{1}H NMR(DMSO-d_6, 500 MHz): δ (ppm) 1.60 (ttd, J = 7.5, 5.5, 1.0 Hz, 2H), 1.67–1.76 (m, 2H), 2.79 (t, J = 5.1 Hz, 2H), 3.58 (q, J = 5.6 Hz, 2H), 3.77–3.83 (m, 1H), 4.64 (s, 1H), 7.20 (t, J = 3.7 Hz, 1H), 7.37–7.44 (m, 2H), 7.41 (s, 2H), 7.52–7.60 (m, 1H), 8.52 (d, J = 3.7 Hz, 2H). Found, %: C 50.28; H 4.51; N 17.43; S 23.78. $C_{17}H_{19}N_5OS_3$. Calculated, %: C 50.35; H 4.72; N 17.27; S 23.72.

1-((4-phenyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazol-3-yl)disulfaneyl)propan-2-ol (**4i**). Yield 88 %, white crystalline substance, mp 192–193 °C. ^{1}H NMR(DMSO-d_6, 500 MHz): δ (ppm) 1.32 (d, J = 6.1 Hz, 3H), 2.76 (dd, J = 12.8, 4.0 Hz, 1H), 3.03 (dd, J = 12.8, 4.2 Hz, 1H), 4.08 (qdt, J = 6.0, 4.8, 4.2 Hz, 1H), 4.22 (d, J = 4.8 Hz, 1H), 4.64 (s, 2H), 7.20 (t, J = 3.7 Hz, 1H), 7.37–7.44 (m, 4H), 7.52–7.60 (m, 1H), 8.52 (d, J = 3.7 Hz, 2H). Found, %: C 49.13; H 4.24; N 17.62; S 24.51. $C_{16}H_{17}N_5OS_3$. Calculated, %: C 49.08; H 4.38; N 17.89; S 24.57.

2-((4-phenyl-5-((pyrimidin-2-ylthio)methyl)-4H-1,2,4-triazol-3-yl)disulfaneyl)-4,5-dihydrothiazole (**4j**). Yield 96 %, white crystalline powder, mp 164–166 °C. ^{1}H NMR(DMSO-d_6, 500 MHz): δ (ppm) 3.39 (dd, J = 4.6, 3.9 Hz, 2H), 3.90–3.95 (m, 2H), 4.64 (s, 2H), 7.20 (t, J = 3.7 Hz, 1H), 7.37–7.44 (m, 4H), 7.52–7.60 (m, 1H), 8.52 (d, J = 3.7 Hz, 2H). Found, %: C 45.83; H 3.32; N 20.36; S 30.32. $C_{16}H_{14}N_6S_4$. Calculated, %: C 45.91; H 3.37; N 20.08; S 30.64.

Results

Considering the aforementioned, it became necessary to design a rational and selective synthetic strategy for accessing this class of potentially bioactive disulfide-containing compounds [9]. To this end, we analyzed and built upon the methodologies described by Abe [10] and Hunter [11], which explore thiol activation pathways suitable for disulfide bond formation. Specifically, Abe demonstrated that *N*-chlorosuccinimide (NCS) can convert thiols into sulfenyl chlorides, which, in the presence of triethylamine, undergo further reaction with succinimide – a by-product of the initial chlorination step – yielding *N*-sulfenylsuccinimide intermediates. These intermediates can then react with a second thiol to form disulfides. However, this approach suffers from a critical drawback: the highly reactive sulfenyl chloride intermediate can also undergo direct, non-selective coupling with a second equivalent of the starting thiol, leading to the undesired formation of symmetrical disulfides as major by-products.

To overcome this limitation, Hunter and co-workers proposed the use of 1-chlorobenzotriazole as a more chemoselective chlorinating agent. This reagent allows for the direct conversion of thiols into *N*-sulfenylbenzotriazole intermediates, bypassing the formation of unstable sulfenyl chlorides. More importantly, under carefully controlled conditions, it becomes possible to selectively generate the *N*-sulfenyl intermediate without promoting side reactions that would lead to homodimer formation. Once formed, this intermediate exhibits sufficient electrophilicity to undergo efficient coupling with a structurally distinct thiol, thus providing a viable route to the targeted unsymmetrical disulfides with improved selectivity and synthetic reliability. This strategy therefore lays the groundwork for the efficient and scalable synthesis of novel disulfide-based scaffolds of pharmacological relevance.

During the course of our investigation, we established that the synthesis of the target disulfide derivatives of 4-phenyl-4*H*-1,2,4-triazoles does not require extremely low reaction temperatures, as previously assumed. Moreover, the efficiency and reproducibility of the transformation were significantly improved by the addition of a small amount of triethylamine, which effectively scavenges the hydrogen chloride released during the activation step. This adjustment not only simplified the experimental procedure but also enhanced the overall yield of the desired products.

The general synthetic strategy employed *(Fig. 2)* relies on the *in situ* generation of 1-chlorobenzotriazole (BtCl) via the oxidation of 1,2,3-benzotriazole with sodium hypochlorite. This reagent serves as a selective electrophilic chlorinating agent that reacts smoothly with the thiol precursor – a 4-phenyl-5-((pyrimidin-2-ylthio)methyl)-1,2,4-triazole-3-thiol – to afford a key sulfenylated intermediate, namely 1-((5-R-4-phenyl-4*H*-1,2,4-triazol-3-yl)thio)-1*H*-benzo[d][1,2,3]triazole. The resulting intermediate is sufficiently stable under the reaction conditions and undergoes a chemoselective nucleophilic substitution upon treatment with a second, structurally distinct thiol. This final step yields the unsymmetrical disulfide product in good yield and with high regioselectivity.

This approach thus provides a concise and operationally simple method for accessing a variety of functionalized unsymmetrical disulfides, bypassing the common pitfalls

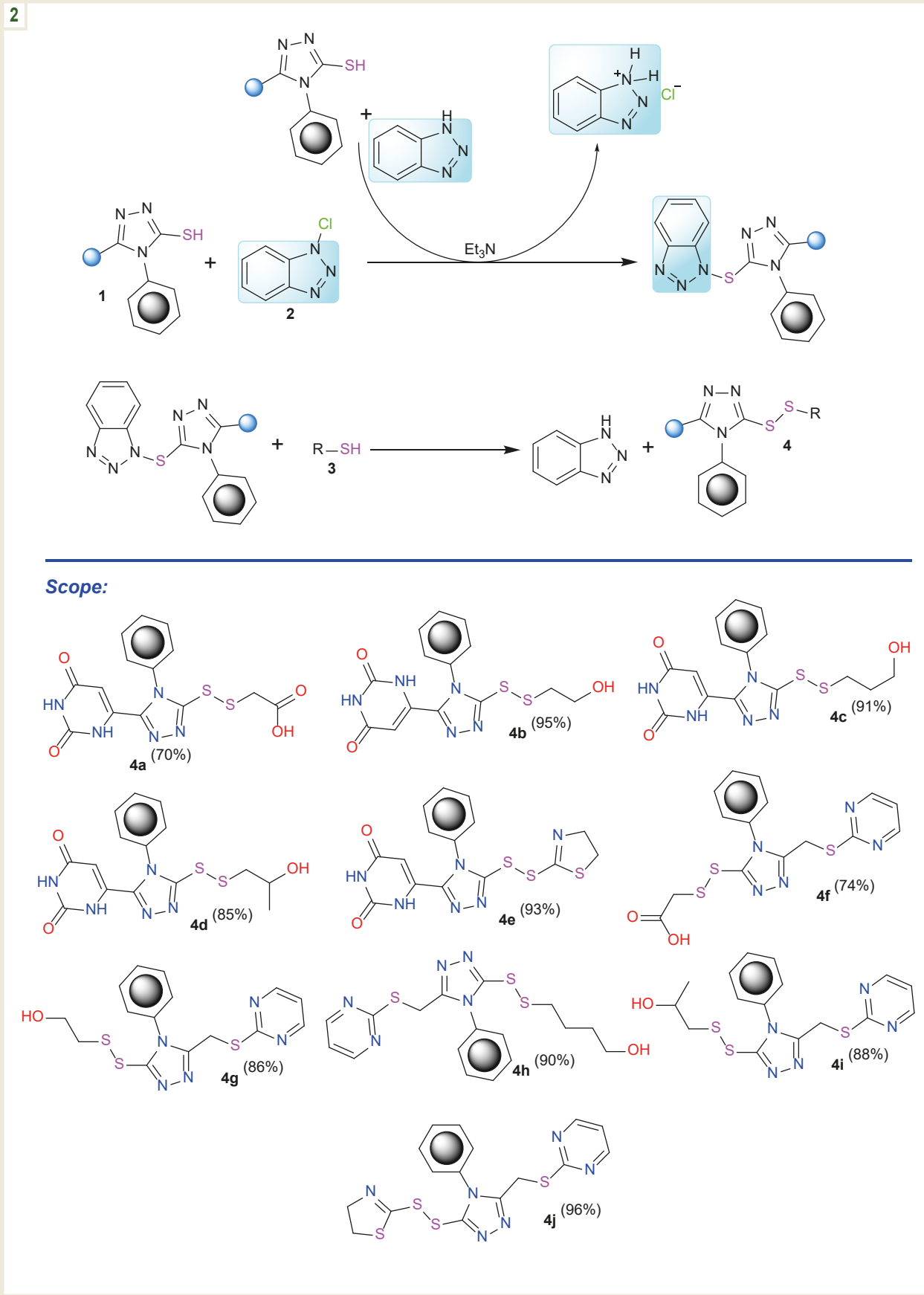

Fig. 2. Scope of 4-phenyl-5-((pyrimidin-2-ylthio)methyl)-1,2,4-triazole-3-thiol derivatives.

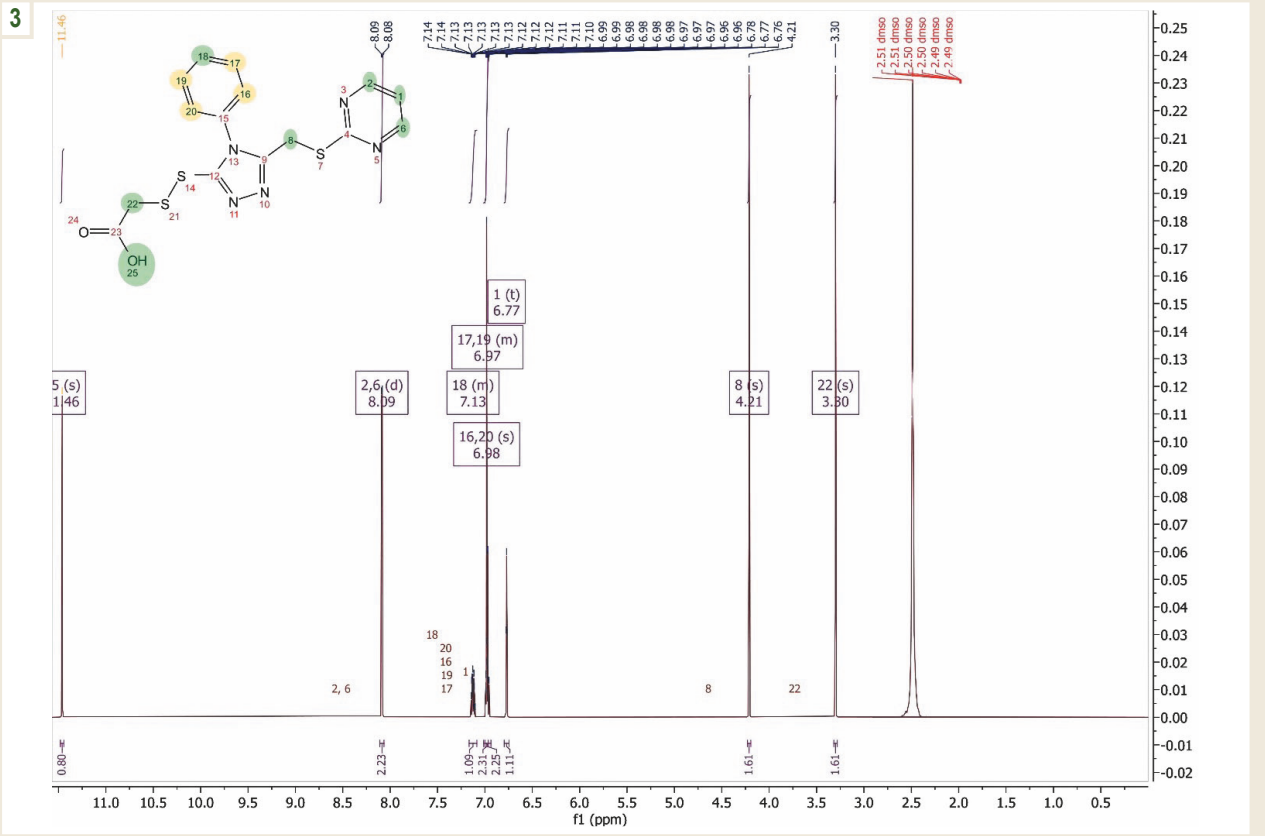

Fig. 3. ^{1}H NMR spectrum 2-((5-(2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-4-phenyl-4*H*-1,2,4-triazol-3-yl)disulfaneyl)acetic acid (**4a**).

of homocoupling and overoxidation, and is particularly well-suited for structurally diverse thiol partners.

To ensure high selectivity toward the formation of the target unsymmetrical disulfide, the reaction was quenched at the appropriate stage by kinetic termination with an aqueous solution of sodium thiosulfate, combined with saturated sodium bicarbonate. This step effectively neutralized any residual electrophilic intermediates, thereby suppressing unwanted side reactions.

Discussion

The substances are individual crystalline compounds in the form of white, light yellow and red colors, insoluble in water, soluble in organic solvents. The structure of the compounds has been proven using spectral analysis methods, and their individuality – chromatographically.

A noteworthy feature of the developed synthetic approach is the high selectivity observed for the formation of unsymmetrical disulfide products, with no significant traces of homodimeric disulfides detected. This conclusion was supported by thin-layer chromatography (TLC) analysis, which showed the absence of additional non-target byproducts corresponding to homocoupled species. TLC monitoring of the reaction progress revealed complete consumption of the initial 5-R-4-phenyl-4*H*-1,2,4-triazole-3-thiol at the first stage, accompanied by the appearance of two closely migrating polar intermediates. These species are attributed to regioisomeric sulfenylated adducts formed via substitution at the N-1 and N-2 positions of the benzotriazole ring, respectively.

An additional highlight of this synthetic protocol is the critical role of triethylamine, which facilitates the *N*-sulfenylation step by scavenging generated hydrogen chloride and stabilizing the sulfenyl intermediate. Moreover, from a sustainability perspective, the procedure offers a valuable advantage: the 1-chlorobenzotriazole used as an activating agent can be readily hydrolyzed under basic conditions to regenerate 1,2,3-benzotriazole. This recyclability underscores the catalytic-like behavior of the heterocycle in the overall transformation and aligns well with the principles of green chemistry.

The successful formation of the desired disulfide derivatives was further confirmed by ^{1}H NMR spectroscopy *(Fig. 3)*, which clearly demonstrated the disappearance of the thiol proton signal and the appearance of diagnostic signals corresponding to the newly formed disulfide linkage, thereby verifying the structural integrity of the products.

The ^{1}H NMR spectrum of the obtained compound in DMSO-d_6 (500 MHz) confirms the structure of the target disulfide derivative and reveals a well-resolved set of characteristic signals corresponding to all expected proton environments.

The aliphatic region displays two distinct signals: a triplet at $\delta = 2.94$ ppm ($J = 4.1$ Hz, 2H) and a doublet of triplets at $\delta = 3.65$ ppm ($J = 5.4$, 4.2 Hz, 2H), which are assignable to

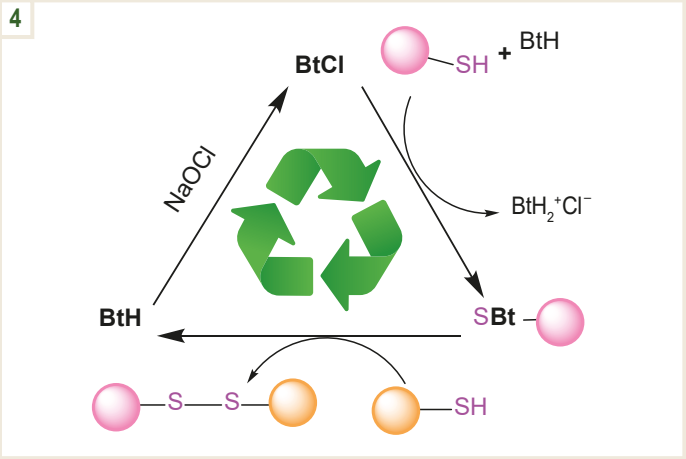

Fig. 4. Unsymmetrical disulfide process cycle using BtCl.

the methylene protons of a –CH_2–CH_2– fragment adjacent to a heteroatom (most likely sulfur or nitrogen). The triplet at δ = 5.33 ppm (J = 5.4 Hz, 1H) corresponds to a methine proton, presumably located between two heteroatoms, indicating the presence of a –CH–S– linkage within a substituted triazole ring or adjacent to a disulfide bridge.

A singlet at δ = 6.15 ppm (1H) likely represents the proton at position 5 of the 1,2,4-triazole ring, as it appears in the aromatic region yet is slightly shielded due to the electronic environment of the heterocycle.

The aromatic region consists of a multiplet between δ = 7.37 ppm and 7.59 ppm integrating for five protons, which is consistent with a monosubstituted phenyl ring. The chemical shift and multiplicity are typical for phenyl protons that are electronically decoupled from strongly withdrawing or donating substituents.

Two downfield singlets at δ = 11.29 ppm and 11.83 ppm (1H each) are indicative of labile NH protons, most likely belonging to amide-like or thiol-like environments, possibly due to hydrogen bonding interactions with the solvent (DMSO). Their distinct chemical shifts suggest non-equivalent environments, supporting the presence of multiple heterocyclic or polar functional groups within the molecule.

Overall, the spectral data are in full agreement with the proposed structure of the unsymmetrical disulfide bearing a substituted 1,2,4-triazole core and confirm the successful formation of the expected product.

In conclusion, the proposed synthetic methodology offers several compelling advantages in terms of sustainability, cost-efficiency, and operational simplicity. A key innovation lies in the use of household bleach (sodium hypochlorite) as a mild and readily available oxidizing agent, in combination with benzotriazole (BtH), which acts not only as a ligand precursor but also as a recyclable mediator of oxidative transformations.

The environmental appeal of this approach is further enhanced by the recyclability of BtH, which can be recovered in high yield using either column chromatography (up to 96 % recovery) or a more accessible acid–base extraction protocol. In the latter case, post-reaction acidification of the aqueous layer followed by vigorous stirring in the presence of dichloromethane for 1 hour enabled efficient partitioning of benzotriazole into the aqueous phase as its hydrochloride salt, achieving up to 99 % recovery. However, under these conditions, a modest reduction in the isolated yield of the disulfide product to approximately 70 % was observed, as detailed in the Experimental Section. Notably, shorter mixing times resulted in incomplete extraction of benzotriazole, thereby diminishing overall recovery efficiency and complicating downstream processing.

This one-pot protocol is also notable for its operational simplicity, avoiding the need for multiple isolation steps and minimizing the use of hazardous reagents. The core strategic features of this disulfide-forming transformation are summarized schematically in *Fig. 4*.

Further investigations are underway to expand the scope of this methodology, including detailed mechanistic studies and its application to the preparation of structurally diverse disulfide-containing bioconjugates.

Conclusions

1. The synthesis of new unsymmetrical disulfides of 4-phenyl-5-((pyrimidin-2-ylthio)methyl)-1,2,4-triazole-3-thiols was successfully implemented under mild conditions using 1-chlorobenzotriazole as a selective reagent, which ensured a high yield of target compounds and good tolerance to various functional groups.

2. The obtained disulfide derivatives form pharmacophore active fragments – a disulfide bridge and a 1,2,4-triazole core – which increases their prospects as discovered biologically active compounds with antioxidant and antimicrobial effects, in particular in the context of combating antibiotic-resistant pathogens.

3. The structural similarity of the synthesized compounds to natural antioxidants, such as cystine and allicin, allows us to consider them as model compounds for further studies of the mechanisms of redox activity and the development of new drugs aimed at regulating oxidative stress.

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