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DERIVATIVES OF 4-PHENYL-3-(THIOPHEN-2-YLMETHYL)-1*H*-1,2,4-TRIAZOLE-5(4*H*)-THIONE

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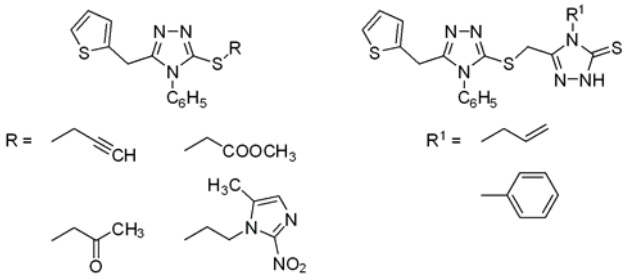
4-Phenyl-3-(thiophen-2-ylmethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione was *S*-alkylated with propargyl bromide, chloroacetone, methyl bromoacetate and 1-(2-chloroethyl)-2-methyl-5-nitro-1*H*-imidazole in the presence of a base to give the corresponding thioethers in good yield. Methyl 2-(4-phenyl-5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazol-3-ylthio)acetate was subsequently employed as starting material in a multi-step reaction comprising its sequential transformation into the corresponding hydrazide, two intermediate thiosemicarbazides, and finally into the target compounds having two 1,2,4-triazole systems linked via a sulfanylmethyl group.



INTRODUCTION

The development of azole-based antimycotics, which has revolutionized the clinical treatment of fungal infections, was stimulated by the discovery of 1,2,4-triazole derivatives such as fluconazole, itraconazole or posaconazole in the 1980s. Nowadays, the usefulness of 1,2,4-triazole as scaffold in drug discovery extends beyond its well-known importance as pharmacophore in antifungal drugs. 1,2,4-Triazole can be found as the core structure in antibacterial, antimycobacterial and antimalarial agents,[1] or compounds with analgesic, anti-inflammatory and anticonvulsant activities.[2] The potential as antiviral agents of candidates featuring variously substituted 1,2,4-triazoles has been reviewed,[3] and the significance of 1,2,4-triazole scaffold for a wide range of other biological activities has been recently surveyed as well.[4,5] Close inspection of the information reported in these literature reviews reveals the particular interest for 1,2,4-triazole-5-thiones, a class of compounds that is readily accessible from easily available materials through several versatile synthetic approaches that allow substituent diversity at both *N*-4 and *C*-3, and which can be further converted into a variety of derivatives through functionalization at *N*-1 (in the thione form) or at the exocyclic sulfur atom (as the thiol tautomer).[6] Amongst these 1,2,4-triazole-5-thiones, several derivatives of thiophene-substituted analogues have been synthesized with a view to investigate their biological activities. The evaluation of 4-substituted 3-(thiophen-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thiones as antibacterial, antifungal and antioxidant agents showed their modest action compared to well-established drugs, but substitution with various aminomethyl moieties at *N*-1 has led to derivatives with significantly improved activities,[7] and hybrids of thiophene-substituted 1,2,4-triazole-5-thione with nitroimidazoles have also been found to be

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efficient against both bacteria and fungi.[8] However, insertion of a methylene group between the thiophene ring and the 1,2,4-triazole-5-thione scaffold resulted in candidates showing no growth inhibition of Gram-positive or Gram-negative bacteria up to 500 μg/mL (although the growth of *Staphylococcus* strains was marginally affected at the highest concentration), whereas the growth of *Trychophyton spp.* dermatophytes was totally or at least partially inhibited by these compounds.[9] Furthermore, replacement of exocyclic sulfur in the 1,2,4-triazole-5-thione scaffold with oxygen led to a series of 3-(thiophen-2-ylmethyl)-substituted 1,2,4-triazole-5-ones whose evaluation against several types of aerobic bacteria has showed that they possess moderate activity towards *Streptococcus pyogenes, Pseudomonas aeruginosa* and *Staphylococcus aureus*.[10] In addition, antimicrobial and anticancer activities of analogues of 3-(thiophen-2-ylmethyl)-substituted 1,2,4-triazole-5-ones having a 4-(arylideneamino) substituent have been investigated, and while a few candidates had promising anti-*Candida* activity, none were cytotoxic against the cancer lines in the panel.[11]

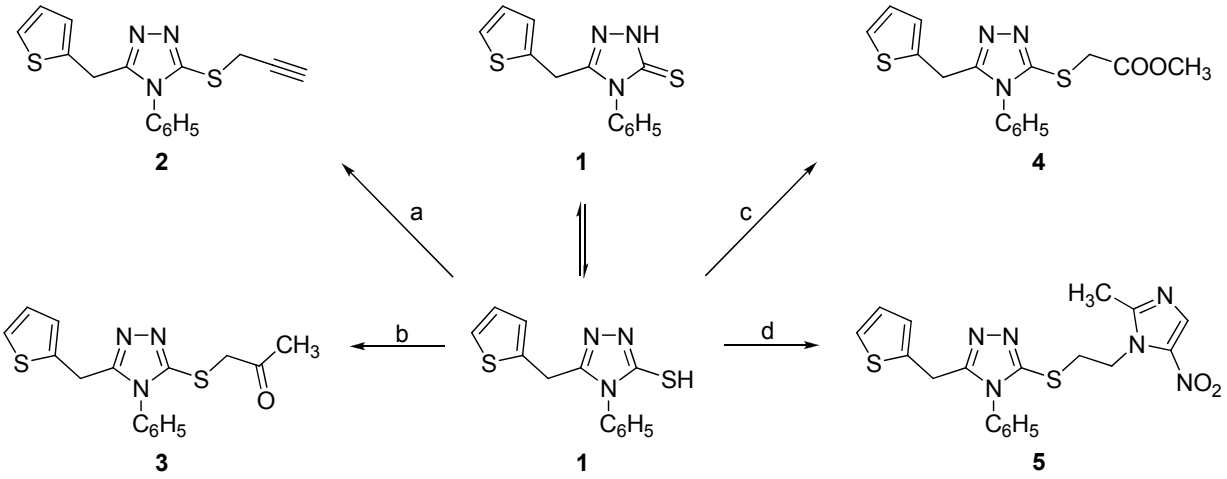
In continuation of our previous studies on the preparation of 1,2,4-triazole-5-thiones and 1,2,4-triazole-5-ones substituted at position 3 with a thiophen-2-ylmethyl moiety,[12,13] the present paper reports the synthesis of novel derivatives of 4-phenyl-3-(thiophen-2-ylmethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione with potential biological activity.

RESULTS AND DISCUSSION

In the first part of this paper, a series of derivatives of the previously reported[13] 4-phenyl-3-(thiophen-2-ylmethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione **1** were obtained through the *S*-alkylation of its thiol form with a few selected halogenated derivatives in the presence of a base. These halogenated derivatives have been chosen either because they have a second function amenable to subsequent chemical modification (propargyl bromide, chloroacetone and methyl bromoacetate), or because they retain in their structure a well-established biologically active moiety (1-(2-chloroethyl)-2-methyl-5-nitro-1*H*-imidazole).

Propargyl halides have been scarcely used as alkylating agents for 1,2,4-triazole-5-thiones. Literature search showed that this type of reaction has been conducted in ethanol at reflux temperature for a short period of time either in the presence of triethylamine[14,15] or in the presence of sodium ethoxide[16] to yield *S*-propargylated 1,2,4-triazole-5-thiols useful for the subsequent preparation of alkyne Mannich bases with potential biological activity. Harsher reaction conditions (heating in toluene at 90 °C for 3 to 8 h) have also been employed for the one-pot reaction of several 4-amino-5-substituted-1*H*-1,2,4-triazole-5(4*H*)-thiones, isobenzofuran-1,3-dione and propargyl bromide, which led to the expected prop-2-ynyl thioethers.[17] However, previous reports have disclosed that thermal isomerisation of the propargyl moiety to allenyl may occur sometimes in the presence of a strong base such as KOH.[18] Therefore, *S*-alkylation of substrate **1** was performed in a mixture of tetrahydrofuran–water in the presence of the stoechiometric amount of KOH at 0 °C and afforded the desired thioether **2** in good yield after column chromatography (Scheme 1). The presence of the propargyl moiety in the structure of sulfide **2** was confirmed in the ^{1}H NMR spectrum by the poorly-defined triplet at 2.21 ppm associated with the proton at the terminal alkyne carbon atom, and in the ^{13}C NMR spectrum by the peaks at 72.5 and 78.2 ppm corresponding to the carbon atoms involved in the triple bond.

Although phenacyl halides have been extensively used as alkylating agents for 1,2,4-triazole-5-thiones, there are only a handful of reports describing similar reactions employing chloroacetone or bromoacetone instead. The yields reported in the literature range from modest when the reaction was conducted in *N*,*N*-dimethylformamide in the presence of K_2CO_3 at 90 °C for 2 h[19] to moderate when the reactants were heated at reflux temperature in ethanol in the presence of sodium acetate for 7 h,[20] or even very good when the reactants were kept at room temperature in a methanolic solution of sodium methoxide for 12 h.[21] We decided, however, to explore a different set of conditions, and the *S*-alkylation of substrate **1** with an equimolar amount of chloroacetone was performed in ethanol in the presence of KOH at reflux temperature. Because the TLC analysis showed the presence of unreacted 1,2,4-triazole-5-thione **1** after 2 h, additional chloroacetone (50% molar excess) was added, and the reaction mixture was further heated at reflux temperature for 1 h, when a second TLC analysis confirmed that almost complete conversion of the substrate into a reaction product had occurred. With a view to avoid the chromatographic separation, the semi-solid residue obtained after the removal of the solvent was stirred with dilute aqueous KOH in order to remove the small amounts of unreacted 1,2,4-triazole-5-thione **1** as the corresponding water-soluble potassium salt. The crude reaction product was isolated as a



Scheme 1 – *S*-Alkylation of 4-phenyl-3-(thiophen-2-ylmethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione **1** with selected halogenated derivatives. a) propargyl bromide, KOH, THF–water, rt, 1 h; b) chloroacetone, KOH, ethanol, reflux, 3 h; c) methyl bromoacetate, K_2CO_3, acetone, reflux, 6 h; d) 1-(2-chloroethyl)-2-methyl-5-nitro-1*H*-imidazole, KOH, ethanol, reflux, 4 h.

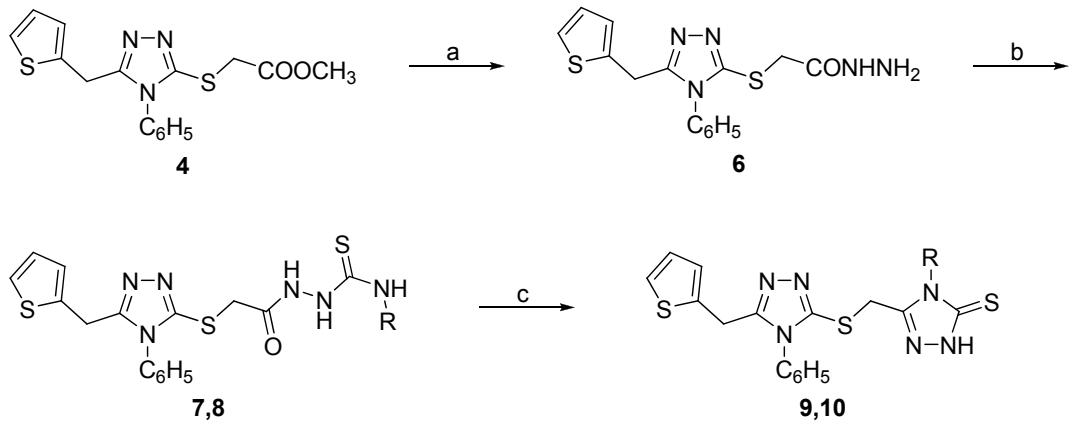
crystalline solid, which was subsequently purified through recrystallization from ethanol to give thioether **3**. The presence of two singlets (one at 2.33 ppm for the protons in the methyl group, and the second partially covered by the signal of the methylene group bridging the thiophene and the 1,2,4-triazole rings) in the ^{1}H NMR spectrum of compound **3** proved the successful grafting of the acetonyl moiety onto 1,2,4-triazole-5-thione **1**. Examination of the ^{13}C NMR spectrum further confirmed the structure of compound **3** as the 2-oxopropyl thioether of 1,2,4-triazole-5-thione **1** through the peaks at 29.2 ppm (–CH_3), 43.0 ppm (–SCH_2–), and 201.6 ppm (>C=O) associated with the carbon atoms in the acetonyl moiety.

S-Alkylation of 1,2,4-triazole-5-thiones with esters of haloacetic acids is one of the best represented reaction in the chemistry of this type of substrate, and has been often used to generate candidates with potential biological activity[22,23] or intermediates in the preparation of a wide range of structurally diverse derivatives of 1,2,4-triazole-5-thiones.[24,25] Reaction of 1,2,4-triazole-5-thione **1** with methyl bromoacetate was conducted in refluxing acetone in the presence of anhydrous K_2CO_3 for 6 h to give a good yield of ester **4**, whose structure was supported by NMR analysis. The presence of two singlets in the proton spectra was associated with the signals of the protons in the methylene adjacent to sulfur (4.04 ppm) and the protons of the methyl group in the ester function (3.73 ppm) of the methoxycarbonylmethyl moiety introduced through *S*-alkylation. Also, the peaks at 34.4, 53.0 and 168.9 ppm in the ^{13}C NMR spectrum of **4** have been assigned to the carbon atoms in the methoxycarbonylmethyl moiety.

Starting from the antibacterial metronidazole, the synthesis and evaluation against various biological targets of a series of structurally diverse derivatives of this drug have been reported recently.[26–30] To the best of our knowledge, only a limited number of metronidazole–1,2,4-triazole-5-thione conjugates have been synthesized up-to-date.[8,31] Reaction of the potassium salt of 1,2,4-triazole-5-thione **1** with 1-(2-chloroethyl)-2-methyl-5-nitro-1*H*-imidazole[26] in ethanol at reflux temperature for 4 h led to the desired conjugate **5** in good yield. After unreacted 1,2,4-triazole-5-thione **1** from the crude semi-solid residue obtained after work-up had been removed using dilute aqueous KOH, the solid isolated was analyzed using TLC and ^{1}H NMR spectroscopy. Both investigations showed that only a small amount of unreacted metronidazole-derived alkylating agent and a single reaction product were present in the isolated solid. When a number of structurally related 1,2,4-triazole-5-thiones underwent a similar reaction under slightly different conditions (metronidazole tosylate as alkylating agent, DMF, K_2CO_3, 75–80 °C, overnight), the *S*-alkylated derivatives were identified as major products in the reaction mixture along with the corresponding N^2-alkylated 1,2,4-triazole-5-thiones as minor components.[31] In our case, the single reaction product was shown to be the *S*-alkylated derivative **5**, as demonstrated by the chemical shift values of the protons in the ethylene bridging the triazole and imidazole rings

(3.53 and 4.81 ppm), which are very similar to those recorded for other analogous *S*-alkylated 1,2,4-triazole-5-thiones.[31] In addition, the chemical shift values of the carbon atoms in the same ethylene moiety (31.1 and 45.3 ppm) clearly showed that one of these atoms was directly linked to sulfur, while the other was adjacent to a nitrogen atom. Furthermore, these chemical shift values were also similar to those reported for *S*-alkylated 1,2,4-triazole-5-thiones in the aforementioned paper.[31]

A series of studies have recently reported the synthesis and pharmacological evaluation of compounds having two 1,2,4-triazole systems linked through a sulfanylmethyl group. Two 4-substituted 3-((4-phenyl-4*H*-1,2,4-triazol-3-ylsulfanyl)methyl)-1*H*-1,2,4-triazole-5(4*H*)-thiones showed interesting antinociceptive properties in mice,[32] while two 4-substituted 3-((5-methyl-4-phenyl-4*H*-1,2,4-triazol-3-ylsulfanyl)methyl)-1*H*-1,2,4-triazole-5(4*H*)-thiones had good activity against pathogenic (*S. aureus*) or opportunistic (*Staphylococcus epidermidis, Micrococcus luteus, Bacillus subtilis*) bacteria.[33] In contrast, MIC values ranging between 500 μg/mL and 1 mg/mL were determined for the most promising 4-substituted 3-((4,5-diphenyl-4*H*-1,2,4-triazol-3-ylsulfanyl)methyl)-1*H*-1,2,4-triazole-5(4*H*)-thiones[34] and 4-substituted 5-((4-phenyl-5-(pyridin-3-yl)-4*H*-1,2,4-triazol-3-ylsulfanyl)methyl)-1*H*-1,2,4-triazole-5(4*H*)-thiones[35] that have been tested for antibacterial activity. The synthesis of these compounds having two 1,2,4-triazole systems bridged by a sulfanylmethyl group usually employs esters of (1,2,4-triazol-3-yl)sulfanylacetic acids as starting materials in a reaction sequence comprising conversion of the ester into the corresponding hydrazide, addition of an isothiocyanate to the hydrazide, and finally the base-catalyzed ring closure to generate the second 1,2,4-triazole-5-thione moiety. We used a similar approach to obtain compounds **9** and **10** starting from ester **4** (Scheme 2). Its reaction with a threefold excess of hydrazine hydrate in refluxing ethanol gave a good yield of hydrazide **6**, which was identified through the presence in its proton spectrum of the signals associated with the hydrazide function (a dublet integrating for two protons at 4.27 ppm, and a second singlet integrating for one proton at 9.33 ppm). Thiosemicarbazides **7** and **8** were obtained in excellent yields from the reaction of hydrazide **6** with phenyl isothiocyante and allyl isothiocyanate, respectively, in refluxing ethanol. N^4-Phenyl-substituted thiosemicarbazide **7** was poorly soluble in most common organic solvents, and only a small sample was recrystallized from a large volume of ethanol prior to analysis. Although the solubility of N^4-allyl-substituted thiosemicarbazide **8** in common organic solvents was marginally superior to that of its N^4-phenyl-substituted analogue **7**, the recrystallization of an analytical sample of **8** rather than the purification of the entire batch produced in the synthesis has been preferred in this case as well. The structure of these intermediates in the synthesis of the target compounds having two 1,2,4-triazole systems was confirmed by the presence in their proton spectrum of three well-defined singlets in the 9.7–10.5 ppm range for thiosemicarbazide **7** derived from the aromatic isothiocyanate, and in the 8.4–10.3 ppm range for thiosemicarbazide **8** derived from the aliphatic isothiocyanate. In addition, the peak corresponding to the carbon atom of the thiocarbonyl function in the newly formed thiosemicarbazide moiety has been identified in the ^{13}C NMR spectra of compounds **7** and **8** at approximately 181 ppm.[32–35] Ring closure of crude thiosemicarbazides **7** and **8** to 1,2,4-triazole-5-thiones **9** and **10**, respectively, has been performed in refluxing aqueous KOH, from which the target compounds were then isolated by treatment with dilute acetic acid until the pH of the mixture became slightly acidic. Compound **9** separated as a solid, and was filtered and air-dried. A small amount of unreacted thiosemicarbazide **7** was subsequently removed by stirring the crude 1,2,4-triazole-5-thione **9** with little chloroform, followed by filtration of the insoluble part. The solid recovered from the chloroform solution was finally recrystallized from ethanol to afford pure 1,2,4-triazole-5-thione **9**. On the other hand, the neutralization of the reaction mixture containing 1,2,4-triazole-5-thione **10** led to the separation of a semi-solid that did not solidify when kept at room temperature for two days or in a refrigerator for one day. The crude 1,2,4-triazole-5-thione **10** was then extracted into ethyl acetate, and purified from its mixture with unreacted thiosemicarbazide **8** using flash column chromatography to yield the pure compound **10** as a slightly yellowish oil which gradually solidified to a colorless solid. The successful ring closure to 1,2,4-triazole-5-thiones **9** and **10** was confirmed by the disappearance in the proton spectra of these compounds of the three aforementioned singlets which are typical for thiosemicarbazides, and by the presence of a singlet associated with the proton of the thiolactam function in the 1,2,4-triazole-5-thione ring system in the off-set of their ^{1}H NMR spectra.



Scheme 2 – Ester **4** as starting material in the synthesis of compounds having two 1,2,4-triazole systems linked via a sulfanylmethyl group. a) hydrazine hydrate, ethanol, reflux, 4 h; b) phenyl (or allyl) isothiocyanate, ethanol, reflux, 1 h; c) KOH, water, reflux, 3 h.

EXPERIMENTAL

Materials and methods

All chemical reagents and solvents were purchased from commercial suppliers (Sigma–Aldrich, Alfa Aesar and Merck), and they were used without further purification. Melting points were taken on a Mel-Temp II apparatus and are uncorrected. Elemental analysis was conducted in-house, on a PerkinElmer 2400 Series II CHNS/O system. Analytical thin-layer chromatography was carried out on glass-backed pre-coated plates (Merck silica gel 60, F_{254}) and visualized with UV light. Flash column chromatography was performed on Merck silica gel (230–400 mesh). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400-MHz spectrometer. The signals owing to residual protons in the deuterated solvents were used as internal standards for the ^{1}H NMR spectra. The chemical shifts for the carbon atoms are given relative to $CDCl_3$ (δ = 77.16 ppm) or d_6-DMSO (δ = 39.52 ppm).

4-Phenyl-3-(prop-2-ynylthio)-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole (**2**)

To the solution of 4-phenyl-3-(thiophen-2-ylmethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione **1** (819 mg, 3 mmol) in tetrahydrofuran (7 mL), a solution of KOH (200 mg KOH, 84% purity, 3 mmol) in water (1 mL) was added. The mixture was cooled in an ice bath, and then a solution of propargyl bromide (492 mg, 80% wt. in toluene, 3 mmol) in tetrahydrofuran (3 mL) was added dropwise under efficient stirring. After having been stirred at 0 °C for 1 h, the reaction mixture was diluted with water (40 mL) and extracted with dichloromethane (2× 15 mL). The organic phase was dried over anhydrous Na_2SO_4, and then the solvent was removed under reduced pressure to yield a yellowish oil. Flash column chromatography (silica gel, ethyl acetate–chloroform, 1:1, v/v) afforded a colorless oil that crystallized under vacuum overnight to an off-white solid (720 mg, 77%), R_f 0.5 (ethyl acetate–chloroform, 1:1, v/v), mp 88–90 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.21 (t, ^4J = 2.4 Hz, 1H, ≡CH), 3.92 (d, ^4J = 2.4 Hz, 2H, –SCH_2–), 4.20 (s, 2H, thiophen-2-yl-CH_2–), 6.54 (d, J = 3.2 Hz, 1H), 6.77 (dd, J = 3.6 and 4.8 Hz, 1H), 7.01–7.13 (m, 3H, overlapped), 7.38–7.54 (m, 3H, overlapped); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 21.2, 26.1, 72.5, 78.2, 124.9, 126.4, 126.8, 127.2, 129.9, 130.2, 132.8, 137.3, 150.4, 154.5. *Anal.* Calcd. for $C_{16}H_{13}N_3S_2$: C, 61.71; H, 4.21; N, 13.49. Found: C, 61.88; H, 4.04; N, 13.66.

1-(4-Phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)propan-2-one (**3**)

4-Phenyl-3-(thiophen-2-ylmethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione **1** (819 mg, 3 mmol) was added to a solution of KOH (200 mg, 84% purity, 3 mmol) in ethanol (10 mL), followed by chloroacetone (278 mg, 3 mmol). The mixture was heated at reflux temperature for 2 h, then additional chloroacetone (139 mg, 1.5 mmol) was added, and the mixture was further heated at reflux temperature for 1 h. The solvent was removed under reduced pressure, and then the semi-solid residue was stirred with water (30 mL) while being dropwise treated with 5% KOH until pH 9. The solid that resulted was filtered, washed thoroughly with water and air-dried. Recrystallization from ethanol gave yellowish crystals (690 mg, 70%), mp 107–108 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.33 (s, 3H, –$COCH_3$), 4.18 (s, 2H), 4.19 (s, 2H), 6.55 (d, J = 2.8 Hz, 1H), 6.79 (dd, J = 3.6 and 4.8 Hz, 1H), 7.05–7.14 (m, 3H, overlapped), 7.41–7.54 (m, 3H, overlapped); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 26.1, 29.2, 43.0, 125.0, 126.5, 126.9, 127.2, 130.0, 130.4, 132.8, 137.3, 151.2, 154.5, 201.6. *Anal.* Calcd. for $C_{16}H_{15}N_3OS_2$: C, 58.33; H, 4.59; N, 12.76. Found: C, 58.50; H, 4.46; N, 12.59.

Methyl 2-(4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetate (**4**)

A mixture of 4-phenyl-3-(thiophen-2-ylmethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione **1** (1092 mg, 4 mmol), methyl bromoacetate (918 mg, 6 mmol), anhydrous K_2CO_3 (828 mg, 6 mmol) in acetone (15 mL) was heated at reflux temperature for 6 h. After the solvent had been removed under reduced pressure, gradual addition of water (50 mL) to the residue under efficient stirring yielded a solid, which was filtered, washed thoroughly with water and air-dried. Recrystallization from a small volume of ethanol afforded light brown crystals (895 mg, 65%), mp 110–111 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.73 (s, 3H, –$COOCH_3$), 4.04 (s, 2H, –SCH_2–), 4.19 (s, 2H, thiophen-2-yl-CH_2–), 6.55 (dd, J = 0.8 and 3.6 Hz, 1H), 6.79 (dd, J = 3.6 and 5.2 Hz, 1H), 7.05–7.14 (m, 3H, overlapped), 7.42–7.53 (m, 3H, overlapped); ^{13}C NMR ($CDCl_3$, 100 MHz):

δ 26.2, 34.4, 53.0, 124.9, 126.4, 126.9, 127.3, 130.0, 130.3, 132.8, 137.4, 150.8, 154.5, 168.9; *Anal.* Calcd. for $C_{16}H_{15}N_3O_2S_2$: C, 55.63; H, 4.38; N, 12.16. Found: C, 55.81; H, 4.54; N, 12.40.

3-(2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethylthio)-4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazole (**5**)

To a solution of KOH (200 mg, 84% purity, 3 mmol) in ethanol (10 mL), 4-phenyl-3-(thiophen-2-ylmethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione **1** (819 mg, 3 mmol) was added, and the mixture was stirred at room temperature for 15 min. 1-(2-Chloroethyl)-2-methyl-5-nitro-1*H*-imidazole[26] (569 mg, 3 mmol) was then added, and the reaction mixture was heated at reflux temperature for 4 h. The solvent was removed under reduced pressure, and then the semi-solid residue was stirred with water (30 mL) while being dropwise treated with 5% KOH until pH 9. The solid that resulted was filtered, washed thoroughly with water and air-dried. Recrystallization from a small volume of ethanol gave light brown crystals (690 mg, 54%), mp 144–145 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.59 (s, 3H, $-CH_3$), 3.53 (t, J = 6.8 Hz, 2H, $-SCH_2-$), 4.21 (s, 2H thiophen-2-yl-CH_2-), 4.81 (t, J = 6.8 Hz, 2H, $-CH_2N-$), 6.57 (d, J = 2.8 Hz, 1H), 6.81 (dd, J = 3.6 and 5.2 Hz, 1H), 7.02–7.08 (m, 3H, overlapped), 7.10 (dd, J = 1.2 and 5.2 Hz, 1H), 7.43–7.54 (m, 3H, overlapped), 7.92 (s, 1H, H-4 in imidazole); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.6, 26.2, 31.1, 45.3, 125.1, 126.5, 127.0, 127.1, 130.2, 130.5, 132.5, 133.4, 137.2, 138.4, 151.0, 151.6, 154.8; *Anal.* Calcd. for $C_{19}H_{18}N_6O_2S_2$: C, 53.50; H, 4.25; N, 19.70. Found: C, 53.73; H, 4.08; N, 19.48.

2-(4-Phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio) acetohydrazide (**6**)

A mixture of methyl 2-(4-phenyl-5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazol-3-ylthio)acetate **4** (690 mg, 2 mmol) and hydrazine hydrate (300 mg, 6 mmol) in ethanol (6 mL) was heated at reflux temperature for 4 h. The reaction mixture was cooled to room temperature and gradually diluted with water (40 mL). The solid that precipitated was filtered, washed thoroughly with water, and air-dried. Recrystallization from a small volume of ethanol afforded colorless crystals (435 mg, 63%), mp 128–129 °C; ^{1}H NMR (d_6-DMSO, 400 MHz): δ 3.84 (s, 2H), 4.21 (s, 2H), 4.27 (d, J = 3.2 Hz, 2H, $-NH_2$), 6.55 (d, J = 3.2 Hz, 1H), 6.82 (dd, J = 3.2 and 4.8 Hz, 1H), 7.27–7.37 (m, 3H, overlapped), 7.49–7.59 (m, 3H, overlapped), 9.33 (s, 1H, –CONH–); ^{13}C NMR (d_6-DMSO, 100 MHz): δ 25.5, 34.2, 125.3, 126.3, 126.8, 127.2, 129.9, 130.1, 132.7, 137.5, 150.2, 153.9, 166.1; *Anal.* Calcd. for $C_{15}H_{15}N_5OS_2$: C, 52.15; H, 4.38; N, 20.27. Found: C, 52.33; H, 4.24; N, 20.05.

N-Phenyl-2-(2-(4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetyl)hydrazinecarbothioamide (**7**)

A mixture of 2-(4-phenyl-5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazol-3-ylthio)acetohydrazide **6** (690 mg, 2 mmol) and phenyl isothiocyanate (270 mg, 2 mmol) in ethanol (10 mL) was heated at reflux temperature for 1 h. The product that separated towards the end of the reaction time was filtered, washed with ethanol, and air-dried to give a colorless solid (935 mg, 97%). Recrystallization of the analytical sample (100 mg) from methanol (30 mL) afforded colorless crystals, mp 190–191 °C; ^{1}H NMR (d_6-DMSO, 400 MHz): δ 3.93 (s, 2H), 4.21 (s, 2H), 6.55 (d, J = 2.4 Hz, 1H), 6.83 (dd, J = 3.6 and 4.8 Hz, 1H), 7.12–7.22 (m, 1H), 7.28–7.42 (m, 5H, overlapped), 7.49–7.62 (m, 5H, overlapped), 9.72 (s, 1H), 9.76 (s, 1H), 10.42 (s, 1H); ^{13}C NMR (d_6-DMSO, 100 MHz): δ 25.4, 34.4, 125.1, 125.3, 125.7, 126.3, 126.8, 127.2, 128.0, 129.9, 130.2, 132.6, 137.3, 138.9, 150.5, 153.9, 166.9, 180.7; *Anal.* Calcd. for $C_{22}H_{20}N_6OS_3$: C, 54.98; H, 4.19; N, 17.49. Found: C, 54.82; H, 4.37; N, 17.72.

N-Allyl-2-(2-(4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)acetyl)hydrazinecarbothioamide (**8**)

A mixture of 2-(4-phenyl-5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazol-3-ylthio)acetohydrazide **6** (1035 mg, 3 mmol) and allyl isothiocyanate (297 mg, 3 mmol) in ethanol (15 mL) was heated at reflux temperature for 1 h. The product that separated towards the end of the reaction time was filtered, washed with ethanol, and air-dried to give a colorless solid (1265 mg, 95%). Recrystallization of the analytical sample (200 mg) from methanol (15 mL) afforded colorless crystals, mp 181–182 °C; ^{1}H NMR (d_6-DMSO, 400 MHz): δ 3.89 (s, 2H), 4.16 (s, 2H), 4.22 (s, 2H), 5.00 (d, J_{cis} = 10.4 Hz, 1H, $=CH_2$), 5.10 (d, J_{trans} = 17.2 Hz, 1H, $=CH_2$), 5.77–5.91 (m, 1H, –CH=), 6.57 (d, J = 3.2 Hz, 1H), 6.83 (dd, J = 4.0 and 5.2 Hz, 1H), 7.28–7.40 (m, 3H, overlapped), 7.52–7.62 (m, 3H, overlapped), 8.45 (s, 1H), 9.43 (s, 1H), 10.29 (s, 1H); ^{13}C NMR (d_6-DMSO, 100 MHz): δ 25.4, 33.8, 45.8, 115.3, 125.3, 126.3, 126.8, 127.1, 130.0, 130.2, 132.4, 134.8, 137.3, 150.7, 154.0, 166.7, 181.7; *Anal.* Calcd. for $C_{19}H_{20}N_6OS_3$: C, 51.33; H, 4.53; N, 18.90. Found: C, 51.54; H, 4.41; N, 18.69.

4-Phenyl-3-((4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)methyl)-1H-1,2,4-triazole-5(4H)-thione (**9**)

A mixture of *N*-phenyl-2-(2-(4-phenyl-5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazol-3-ylthio)acetyl)hydrazinecarbothioamide **7** (480 mg, 1 mmol) and KOH (100 mg, 84% purity, 1.5 mmol) in water (10 mL) was heated at reflux temperature for 3 h. The solution was then cooled in an ice bath, diluted with water (50 mL), and treated dropwise with 20% aqueous acetic acid, under efficient stirring, until pH 5–6. The solid that separated was filtered, washed thoroughly with water, and air-dried. The solid was then stirred with cold chloroform (15 mL), the resulting mixture was filtered, and the solvent was removed from the filtrate under reduced pressure. Recrystallization of the residue from ethanol afforded colorless crystals (380 mg, 79%), mp 199–200 °C; ^{1}H NMR (d_6-DMSO, 400 MHz): δ 4.06 (s, 2H), 4.18 (s, 2H), 6.54 (d, J = 3.2 Hz, 1H), 6.84 (dd, J = 4.0 and 4.8 Hz, 1H), 7.15 (d, J = 7.2 Hz, 2H), 7.20–7.28 (m, 2H), 7.31 (d, J = 4.8 Hz, 1H), 7.45–7.60 (m, 6H, overlapped), 13.87 (s, 1H, –NH); ^{13}C NMR (d_6-DMSO, 100 MHz): δ 25.5, 27.6, 125.3, 126.3, 126.8, 127.1, 128.2, 129.3, 129.6, 129.8, 130.0, 132.6, 133.1, 137.3, 148.1, 148.2, 154.4, 168.3; *Anal.* Calcd. for $C_{22}H_{18}N_6S_3$: C, 57.12; H, 3.92; N, 18.17. Found: C, 56.90; H, 4.05; N, 17.99.

4-Allyl-3-((4-phenyl-5-(thiophen-2-ylmethyl)-4H-1,2,4-triazol-3-ylthio)methyl)-1H-1,2,4-triazole-5(4H)-thione (**10**)

A mixture of *N*-allyl-2-(2-(4-phenyl-5-(thiophen-2-ylmethyl)-4*H*-1,2,4-triazol-3-ylthio)acetyl)hydrazinecarbothioamide **8** (888 mg, 2 mmol) and KOH (200 mg, 84% purity, 3 mmol) in water (20 mL) was heated at reflux temperature for 3 h. The solution was then cooled in an ice bath, diluted with water (50 mL), and treated dropwise with 20% aqueous acetic acid, under efficient stirring, until pH 5–6. The semi-solid that separated was extracted into ethyl acetate (2× 20 mL), then the organic phase was washed with water (2× 20 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give a semi-solid residue that was subjected to flash column chromatography (silica gel, ethyl acetate). The resulting oil gradually solidified to colorless

crystals (545 mg, 64%), R_f 0.52 (ethyl acetate), mp 143–144 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 4.23 (s, 2H), 4.40 (s, 2H), 4.75 (d, J = 5.6 Hz, 2H, –SCH_2–), 5.07 (d, J_{trans} = 17.2 Hz, 1H, =CH_2), 5.22 (d, J_{cis} = 10.4 Hz, 1H, =CH_2), 5.77–5.92 (m, 1H, –CH=), 6.55 (d, J = 2.4 Hz, 1H), 6.79 (dd, J = 3.6 and 4.8 Hz, 1H), 7.01 (d, J = 7.2 Hz, 2H), 7.07 (dd, J = 0.8 and 5.2 Hz, 1H), 7.41–7.56 (m, 3H, overlapped), 12.10 (s, 1H, –NH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 26.2, 26.8, 46.2, 118.8, 125.1, 126.6, 127.0, 127.1, 130.1, 130.5, 130.6, 132.5, 137.0, 148.4, 149.6, 155.2, 168.2; *Anal.* Calcd. for $C_{19}H_{18}N_6S_3$: C, 53.50; H, 4.25; N, 19.70. Found: C, 53.42; H, 4.38; N, 19.53.

CONCLUSIONS

S-Alkylation of 4-phenyl-3-(thiophen-2-yl-methyl)-1*H*-1,2,4-triazole-5(4*H*)-thione **1** with propargyl bromide, chloroacetone and methyl bromoacetate led to novel derivatives which have a function that allows subsequent development of libraries of compounds with potential biological activity. This concept has been illustrated in this paper with the synthesis of two compounds having two 1,2,4-triazole systems linked via a sulfanylmethyl group that employs the ester prepared by the *S*-alkylation of the initial 1,2,4-triazole-5-thione with methyl bromoacetate as starting material in a multi-step reaction comprising its sequential transformation into the corresponding hydrazide, the intermediate thiosemicarbazides and the final target compounds. In addition, *S*-alkylation of 4-phenyl-3-(thiophen-2-ylmethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione **1** with 1-(2-chloroethyl)-2-methyl-5-nitro-1*H*-imidazole afforded a metronidazole–1,2,4-triazole-5-thione conjugate.

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