Design, synthesis, and evaluation of the antimycobacterial activity of 3-mercapto-1,2,4-triazole–pyrrole hybrids

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Abstract: A series of 3-mercapto-1,2,4-triazole–pyrrole hybrids was designed as antimycobacterial agents by employing 5-(4-(1H-pyrrol-1-yl)phenyl)-4H-1,2,4-triazole-3-thiol as the scaffold onto which several types of moieties were introduced in the triazole ring at N-4 and N-2 and as substituents of the mercapto function. The aforementioned moieties are an allyl or a phenyl moiety at N-4; an aminomethyl group at N-2; or methyl, substituted benzyl, ethoxycarbonylmethyl, or substituted phenacyl at sulfur. Investigation of the compounds in the resulting library as growth inhibitors of Mycobacterium smegmatis showed that their minimum inhibitory concentration was higher than 64 mg/L.

Key words: 1,2,4-Triazole, pyrrole, S-alkylation, aminomethylation, antimycobacterial

1. Introduction

Tuberculosis is a major contagious disease that affects tens of millions of people, especially in poor countries in Africa and Southeast Asia, and, with approximately 1.7 million deaths in 2016, is the leading cause of death from infectious disease worldwide, ranking above HIV/AIDS, according to the WHO Global Tuberculosis Report 2017 (www.who.int/entity/tb/publications/global_report/gtbr2017_main_text.pdf). Despite the fact that several drugs are available for keeping tuberculosis under control or even curing it, the rate of successful treatments in Directly Observed Treatment Short-course (DOTS), the multidrug therapy program developed by the World Health Organization, remains in the low eighties, as stated in the same WHO Global Tuberculosis Report 2017. The most common cause of treatment failure using first-line antitubercular drugs in DOTS (isoniazid, rifampicin, pyrazinamide, and ethambutol) is often poor patient compliance due to the lengthy period of treatment (6 months) and sometimes to the side effects (e.g., toxicity of isoniazid) of the drugs used in therapy. The consequences associated with this failure include the emergence of multidrug-resistant strains of M. tuberculosis leading to high rates of recurrence, appearance of extensively drug-resistant strains, and eventually mortality. The discovery of novel drugs that could improve treatment of both sensitive and resistant tuberculosis is highly needed, and several drug candidates have been developed and have reached early stages in clinical trials in recent years.1–4

Chemical modification of a known antituberculosis drug, target-based drug design, combinatorial synthesis, in silico design, and high-throughput screening represent a few of the approaches that are commonly employed in the discovery of novel drug candidates for treatment of tuberculosis. Among them, high-throughput screening is essential for the identification of novel lead compounds, preferably acting on hitherto unknown tar-
gets, in a relatively short time. The Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) Program has been established specifically to provide reliable screening of chemical libraries with the view to identify novel scaffolds of interest, and make the data publicly accessible to the tuberculosis research community.\(^5\) As a part of this program, a chemical library containing 100,997 compounds, obtained from the ChemBridge Corporation and selected for diversity and drug-likeness using the Lipinski criteria, has been screened against *M. tuberculosis* strain H\textsubscript{37}Rv using the microdilution Alamar blue assay adapted for high-throughput screening in 384-well plate format.\(^6\) The screening uncovered several scaffolds and clusters of active compounds, which appear to be novel classes of compounds that exhibit significant antitubercular activity, and may therefore serve as leads in antitubercular drug discovery and development. One of these clusters of compounds with antitubercular activity included a small number of S-alkylated derivatives of 3-mercaptop-1,2,4-triazole (particularly esters or amides of acetic acid), which had IC\textsubscript{90} values lower than 5.0 µg/mL. This finding is confirmed by the numerous literature examples of mercaptotriazole derivatives that have been recently successfully investigated as antimycobacterial agents.\(^7\)–\(^12\) On the other hand, pyrrole has emerged as a privileged scaffold in the design of

\[\text{Figure 1. Examples of chemical entities that incorporate a pyrrole motif in their structure and have been evaluated as antitubercular agents.}\]
antitubercular agents in the last 15 years. The discovery of the antimycobacterial activity of BM212 1 (Figure 1) and its analogues\textsuperscript{13–16} and the evaluation of Lupin Ltd.’s drug candidate LL-3858 (Sudoterb) 2 (Figure 1) in clinical trials in India\textsuperscript{17} have ushered in a new direction in the development of antitubercular agents, and many papers reporting the evaluation of antitubercular activity of various structures that incorporate a pyrrole motif have been published in the last decade. For example, a series of inhibitors 1–5 that target essential enzymes in the mycolic acid biosynthetic pathway have been based on the pyrrole scaffold (Figure 1).\textsuperscript{18–21} Joshi et al. have also reported in a series of papers the screening for antimycobacterial activity against \textit{M. tuberculosis} H\textsubscript{37}Rv of pyrrole-containing compounds 6–9 (Figure 1).\textsuperscript{22–24} Additional types of pyrrole derivatives that have been tested against \textit{M. tuberculosis} H\textsubscript{37}Rv include pyrrol-1-ylacetic acids 10 and related amides,\textsuperscript{25} diesters 11 of carbazole-substituted pyrrole-2,3-dicarboxylic acids,\textsuperscript{26} or hydrazones 12 from pyrrole-3-carboxaldehydes and diversely substituted thiazole-2-hydrazines\textsuperscript{27} (Figure 1).

Molecular hybridization is a rational strategy in drug design that has been extensively employed in recent years.\textsuperscript{28,29} This approach identifies two or more structural features that are pharmacologically relevant for the desired biological activity in different molecules, and subsequently develops hybrid molecules by merging these pharmacophores through an adequate linker. In the field of antitubercular agents, this concept has been successfully applied to the design of hybrid molecules derived from analogues of BM212 and either antibacterial oxazolidinones\textsuperscript{30} or antitubercular adamantane-diamine SQ109.\textsuperscript{31} In the current study, the previously identified 3-mercapto-1,2,4-triazole and pyrrole pharmacophores were combined through a 1,4-phenylene linker to provide a hybrid scaffold 13 (Figure 2) that could be subsequently modified at the marked reactive sites using suitable chemistry. To the best of our knowledge, compounds 14 (R = H or CH\textsubscript{3}) (Figure 2) are the only candidates based on scaffold 13 that have been evaluated so far against \textit{M. tuberculosis} H\textsubscript{37}Rv, and these investigations led to mixed results. Thus, the minimum inhibitory concentration (MIC) was 62.5 µg/mL when R = H,\textsuperscript{32} whereas when R = CH\textsubscript{3} MIC was as low as 4 µg/mL.\textsuperscript{33} The present study expands the structural diversity of such valuable candidates for antimycobacterial testing by creating a small library of compounds obtained through chemical modifications of the mercapto triazole fragment of scaffold 13.

\textbf{Figure 2.} Structure of the hybrid scaffold 13 employed in the development of the novel potential antitubercular agents in this paper and the general structure of candidates 14 featuring this scaffold that have been evaluated as tuberculostatics so far.

\section{Results and discussion}

\subsection{Design}

Depending on the substituent at positions 2 and 5 of the pyrrole ring (either hydrogen or methyl), the candidates in the present study belong to two subsets of structures. In addition, the compounds that were designed and synthesized for this small library feature either a phenyl or an allyl moiety at N-4 of the triazole ring. Starting
from these four parent compounds 23–26 (Figure 3), the remaining members of the library were designed to generate a broader chemical diversity through chemical modifications of the mercaptotriazole fragment of scaffold 13 either at the sulfur atom or at N-1 of the triazole ring. Because the S-alkylated mercaptotriazoles that have been identified as a result of screening of the ChemBridge Corporation library for antitubercular hit compounds have either an acetic acid ester or an acetamide moiety attached to the sulfur atom, this particular modification is well represented in the library through compounds 27–30. Further S-alkylation of the parent mercaptotriazoles was performed with the view to investigate the effect on the antitubercular activity of the replacement of the carboxy group in some of esters 27–30. Thus, formal replacement of the carboxy group with hydrogen led to compound 31, while candidates 32 and 33 were designed by replacing the carboxy group with aryl moieties. In addition, in order to evaluate the effect that the replacement of the ethoxy group in esters 29 and 30 with an aryl moiety has on antitubercular activity, two other S-alkylated mercaptotriazoles, namely compounds 34 and 35, were included in this library. Finally, in order to glean an insight into the significance upon antitubercular activity of modification of mercaptotriazole in scaffold 13 at different reactive sites, substitution of this fragment with an aminomethyl group at N-2 was also undertaken to provide candidates 36–40.

2.2. Chemistry

The synthesis of the candidates in this library employs methyl 4-aminobenzoate and either 2,5-dimethoxycyclotetrahydrofuran or hexane-2,5-dione as starting materials to generate intermediate esters 15 and 16, respectively, which are next converted into the corresponding hydrazides 17 and 18 (Figure 3). Reaction of these two hydrazides with either allyl isothiocyanate or phenyl isothiocyanate affords the related $N^1,N^4$-disubstituted thiosemicarbazides 19–22, which are subsequently converted into the parent mercaptotriazole–pyrrole hybrids 23–26. These four key compounds are S-alkylated with ethyl bromoacetate to give candidates 27–30, with methyl iodide to afford thioether 31, and with two chloro-substituted benzyl halides to provide thioethers 32 and 33. The use of phenacyl bromides in the S-alkylation of two selected mercaptotriazole–pyrrole hybrids led to thioethers 34 and 35. Reaction of hybrids 25 and 26 with formaldehyde and morpholine, piperidine, or 4-(pyridin-2-yl)piperazine under the conditions of the Mannich reaction afforded the N-2-aminomethylated triazolethiones 36–40.

The structure of the synthesized compounds was analyzed using NMR spectroscopy. Pyrrole ring closure was confirmed through the presence of signals in the $^1$H NMR spectra of compounds 15 and 16 that can be attributed to protons in the pyrrole ring (6.39 and 7.16 ppm for 15, and 5.84 ppm for 16). The formation of the hydrazides 17 and 18 was established through the presence in their $^1$H NMR spectra of a singlet at approximately 4.5 ppm integrating for two protons and of a second singlet at approximately 9.8 ppm integrating for one proton; the integral value of both signals decreases significantly upon addition of deuterated water to the NMR sample, which is indicative of the presence of mobile protons in the structure of compounds 17 and 18. Conversion of hydrazides 17 and 18 into thiosemicarbazides 19–22 can be correlated with the presence of three singlets integrating for one proton in the 8–10.7 ppm range of each proton spectrum of intermediates 19–22; these singlets are associated with the three exchangeable protons discernible in the structure of thiosemicarbazides 19–22 owing to their facile replacement by deuterium following the addition of deuterated water to the NMR sample. Evidence for the successful ring closure of thiosemicarbazides 19–22 to mercaptotriazoles 23–26 is provided by the absence in the proton spectra of these compounds of the three singlets noticed in the spectra of thiosemicarbazides, whereas a novel singlet that is located above 14 ppm, integrates for one proton, and is
Figure 3. Synthesis of variously substituted 3-mercapto-1,2,4-triazole–pyrrole hybrids. Reaction conditions: a) methyl 4-aminobenzoate, gl. acetic acid, reflux, 1 h; b) methyl 4-aminobenzoate, 96% ethanol, reflux, 6 h; c) hydrazine, 96% ethanol, reflux, 3–18 h; d) allyl (or phenyl) isothiocyanate, 96% ethanol, reflux, 1 h; e) KOH, water, reflux, 3 h; f) CH$_3$I, KOH, 96% ethanol, reflux, 1 h; g) ethyl bromoacetate, KOH, 96% ethanol, reflux, 1 h; h) ethyl bromoacetate, K$_2$CO$_3$, 2-butanone, reflux, 6 h; i) cyclic secondary amine, 37% aq. formaldehyde, 96% ethanol, reflux, 1 h; j) substituted benzyl halide (or substituted phenacyl bromide), KOH, 96% ethanol, reflux, 1 h.

\[ R = \text{H or CH$_3$} \]
\[ R^1 = -\text{CH}_2\text{CH=CH}_2 \text{ or C}_6\text{H}_5 \]
exchangeable with deuterium can be attributed to the proton at N-1 in the triazolethione tautomer of compounds 23–26. The outcome of the S-alkylation of these mercaptotriazoles with various reactive halogenated derivatives can be also evaluated using NMR spectroscopy by showing the particular spectral features that can be associated with the appendages grafted onto the mercaptotriazole fragment. Thus, the presence of an acetic acid ester moiety in the structure of candidates 27–30 is supported by the identification of a triplet in the 1.1–1.2 ppm range, a quartet centered at approximately 4.2 ppm and a singlet close to 4.1 ppm corresponding to methyl and methylene protons on the ester function, and to methylene protons directly linked to sulfur, respectively. A sharp singlet at 2.6 ppm in the 1H NMR spectrum of methyl thioether 31 confirms the S-methylation of mercaptotriazole 24, whereas the singlet at approximately 4.4 ppm in the proton spectra of benzyl thioethers 32 and 33 is indicative of the S-benzylation of mercaptotriazole–pyrrole hybrids 25 and 26. The attachment of a phenacyl moiety at the sulfur in mercaptotriazole–pyrrole hybrids 23 and 26 is demonstrated by the presence of a singlet at approximately 4.7–5.0 ppm in the proton spectra of compounds 34 and 35. As far as the aminomethyl derivatives 36–40 are concerned, the correct number and integration of the signals corresponding to the protons in the amine moiety in each case, corroborated with the presence of a singlet at 5.2–5.4 ppm in the 1H NMR spectrum of these compounds, which is associated with the protons of the methylene group adjacent to N-2 of the triazole ring, provide sufficient experimental evidence to validate the formation of these Mannich bases.

2.3. Biological evaluation
The antimycobacterial activity of compounds 23–40 was evaluated against Mycobacterium smegmatis using the broth dilution method. The candidates were tested at ten different concentrations: 0.125 mg/L, 0.25 mg/L, 0.5 mg/L, 1 mg/L, 2 mg/L, 4 mg/L, 8 mg/L, 16 mg/L, 32 mg/L, and 64 mg/L. No growth inhibition of the mycobacterium was noted for any of the candidates, even at the highest concentration. Under the same experimental conditions, a MIC value of 2 mg/L was determined for the standard drug rifampicin. Because the MIC values for the first-line antitubercular drugs are so much lower than the value of the highest concentration used in this study (64 mg/L), no further determination of a MIC value for individual compounds was pursued, and the experimental evidence was interpreted as a lack of any significant activity against M. smegmatis type strain for the candidates under evaluation.

2.4. Conclusions
Based on a scaffold that incorporates two fragments, namely pyrrole and 3-mercaptop-1,2,4-triazole, which have been previously identified as pharmacophores in compounds with antmycobacterial activity, a library of candidates was designed as potential antitubercular agents using the hybridization approach. Aiming at molecular diversity within the library, three active sites in the mercaptotriazole fragment of the scaffold were chemically modified with structurally diverse moieties. The candidates in the library were screened against M. smegmatis, and their MICs were found to be higher than 64 mg/L. Because the compounds in the collection are practically devoid of any useful antmycobacterial activity in spite of the diverse substitution pattern within the library, it appears that the core structure in the mercaptotriazole–pyrrole hybrids is largely responsible for the lack of biological activity in these series of candidates. Therefore, the association between 3-mercaptop-1,2,4-triazole and pyrrole pharmacophores through a 1,4-phenylene linker does not seem to be conducive to the manifestation of antmycobacterial activity as it was rationalized in the working hypothesis presented in the Introduction.
3. Experimental

3.1. Materials and methods

All chemical reagents and solvents were obtained from Sigma–Aldrich (Schnelldorf, Germany). Mueller Hinton broth was provided by Merck (Darmstadt, Germany). Standard antibiotic rifampicin was a product of Sigma–Aldrich (Schnelldorf, Germany). Column chromatography was performed on silica gel (230–400 mesh, 60 Å) (Sigma–Aldrich, Schnelldorf, Germany). Analytical TLC was performed on Merck glass-backed precoated silica gel 60 F254 plates, and the compounds were visualized by UV illumination (254 nm). Melting points were recorded on a Mel-Temp II apparatus and are uncorrected. NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer. The signals from residual protons in deuterated solvents were used as internal standards for the $^1$H NMR spectra. Chemical shifts for the carbon atoms are given relative to CDCl$_3$ ($\delta = 77.16$ ppm) or DMSO-$d_6$ ($\delta = 39.52$ ppm). Elemental analysis was conducted on a PerkinElmer 2400 Series II CHNS/O system.

3.2. Synthesis

3.2.1. Methyl 4-(1$H$-pyrrol-1-yl)benzoate (15)

A mixture of methyl 4-aminobenzoate (1.51 g, 10 mmol) and 2,5-dimethoxytetrahydrofuran (1.36 g, 10 mmol) in glacial acetic acid (5 mL) was heated at reflux temperature for 1 h. The dark brown solution was diluted with water (50 mL) while still hot, and then allowed to cool to room temperature. The precipitate was filtered, washed thoroughly with water, and air dried. One recrystallization from 2-propanol yielded tan crystals (1.61 g, 80%, mp 124–125 °C) pure enough for the next step. Flash column chromatography (silicagel, hexanes–ethyl acetate 9:1 v/v) afforded the analytical sample as colorless plates, mp 125–126 °C (lit.$^{34}$ mp 125–127 °C), $R_f$ 0.38 (hexanes–ethyl acetate 9:1 v/v); $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 3.93 (s, 3H), 6.39 (t, $J = 2.2$ Hz, 2H), 7.16 (t, $J = 2.2$ Hz, 2H), 7.45 (d, $J = 8.8$ Hz, 2H), 8.10 (d, $J = 8.8$ Hz, 2H); $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 52.3, 111.6, 119.2, 119.4, 127.1, 131.4, 144.1, 166.5. Anal. Calcd. for C$_{12}$H$_{11}$NO$_2$: C, 71.63; H, 5.51; N, 6.96. Found: C, 71.40; H, 5.57; N, 7.03.

3.2.2. Methyl 4-(2,5-dimethyl-1$H$-pyrrol-1-yl)benzoate (16)

A solution of methyl 4-aminobenzoate (3.02 g, 20 mmol), 2,5-hexanedione (2.28 g, 20 mmol), and glacial acetic acid (0.5 mL) in 96% ethanol (20 mL) was heated at reflux temperature for 6 h, and then the solvent was partially removed under reduced pressure. The solid that separated on cooling was filtered and recrystallized from 2-propanol yielded tan crystals (1.61 g, 80%, mp 124–125 °C) pure enough for the next step. Flash column chromatography (silicagel, hexanes–ethyl acetate 9:1 v/v) afforded the analytical sample as colorless plates, mp 125–126 °C (lit.$^{35}$ mp 125–127 °C), $R_f$ 0.38 (hexanes–ethyl acetate 9:1 v/v); $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 3.93 (s, 3H), 6.39 (t, $J = 2.2$ Hz, 2H), 7.16 (t, $J = 2.2$ Hz, 2H), 7.45 (d, $J = 8.8$ Hz, 2H), 8.10 (d, $J = 8.8$ Hz, 2H); $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 52.3, 111.6, 119.2, 119.4, 127.1, 131.4, 144.1, 166.5. Anal. Calcd. for C$_{12}$H$_{11}$NO$_2$: C, 71.63; H, 5.51; N, 6.96. Found: C, 71.40; H, 5.57; N, 7.03.

3.2.3. 4-(1$H$-Pyrrol-1-yl)benzoic acid hydrazide (17)

A solution of methyl 4-(1$H$-pyrrol-1-yl)benzoate (15) (1.2 g, 6 mmol) and anhydrous hydrazine (5 mL) in 96% ethanol (20 mL) was heated at reflux temperature for 3 h. The solvent was removed under reduced pressure and the residue was recrystallized from a small volume of 2-propanol to give colorless crystals (1 g, 83%), mp 179–181 °C (lit.$^{32}$ mp 180–182 °C; lit.$^{36}$ mp 180–181 °C); $^1$H NMR (DMSO-$d_6$, 400 MHz): $\delta$ 4.50 (br s, 2H,
exchangeable with D), 6.29 (t, J = 2.0 Hz, 2H), 7.47 (t, J = 2.0 Hz, 2H), 7.68 (d, J = 8.8 Hz, 2H), 7.92 (d, J = 8.8 Hz, 2H), 9.81 (s, 1H, exchangeable with D); 13C NMR (DMSO-d6, 100 MHz): δ 111.1, 118.5, 119.0, 128.6, 129.6, 141.8, 165.1. Anal. Calcd. for C11H11N3O: C, 65.66; H, 5.51; N, 20.88. Found: C, 65.84; H, 5.42; N, 21.02.

3.2.4. 4-(2,5-Dimethyl-1H-pyrrol-1-yl)benzoic acid hydrazide (18)
A mixture of methyl 4-(2,5-dimethyl-1H-pyrrol-1-yl)benzoate (16) (2.29 g, 10 mmol) and hydrazine hydrate (2.25 g, 45 mmol) in 96% ethanol (10 mL) was heated at reflux temperature overnight. The solid that separated on cooling was filtered and recrystallized to give colorless crystals (1.95 g, 85%), mp 164–165 °C (ethanol) (lit. mp 148 °C); 1H NMR (DMSO-d6, 400 MHz): 1.97 (s, 6H), 4.54 (s, 2H, exchangeable with D), 5.81 (s, 2H), 7.34 (d, J = 8.4 Hz, 2H), 7.94 (d, J = 8.4 Hz, 2H), 9.89 (s, 1H, exchangeable with D); 13C NMR (DMSO-d6, 100 MHz): δ 12.9, 106.4, 127.5, 127.8, 128.0, 132.4, 140.6, 165.2. Anal. Calcd. for C13H15N3O: C, 68.10; H, 6.59; N, 18.33. Found: C, 68.25; H, 6.71; N, 18.48.

3.2.5. General procedure for the synthesis of thiosemicarbazides 19–22
A mixture of hydrazide 17 or 18 (5 mmol) and the corresponding isothiocyanate (5 mmol) in 96% ethanol (25 mL) was heated at reflux temperature for 60 min. The solid that separated was filtered, washed with ethanol (2 × 10 mL), and air dried.

3.2.5.1. 4-Allyl-1-[4-(1H-pyrrol-1-yl)benzoyl]thiosemicarbazide (19)
This compound was obtained from 4-(1H-pyrrol-1-yl)benzoic acid hydrazide (17) and allyl isothiocyanate. Yield: 1485 mg (99%). Recrystallization of a sample from this solid (300 mg) afforded yellowish crystals (285 mg, 95%), mp 199–200 °C (methanol); 1H NMR (DMSO-d6, 400 MHz): δ 4.11 (t, J = 5.2 Hz, 2H), 5.04 (dd, J = 1.2 and 10.0 Hz, 1H), 5.14 (dd, J = 1.2 and 17.2 Hz, 1H), 5.77–5.90 (m, 1H), 6.31 (t, J = 8.4 Hz, 2H), 7.51 (t, J = 2.2 Hz, 2H), 7.74 (t, J = 8.8 Hz, 2H), 8.01 (t, J = 8.8 Hz, 2H), 8.32 (br s, 1H, exchangeable with D), 9.38 (s, 1H, exchangeable with D), 10.38 (s, 1H, exchangeable with D); 13C NMR (DMSO-d6, 100 MHz): δ 45.9, 111.2, 115.2, 118.1, 119.0, 128.8, 129.6, 135.1, 142.3, 165.2, 181.8. Anal. Calcd. for C15H16N4OS: C, 59.98; H, 5.37; N, 18.65. Found: C, 60.16; H, 5.22; N, 18.84.

3.2.5.2. 4-Phenyl-1-[4-(1H-pyrrol-1-yl)benzoyl]thiosemicarbazide (20)
This compound was obtained from 4-(1H-pyrrol-1-yl)benzoic acid hydrazide (17) and phenyl isothiocyanate. Yield: 1615 mg (96%). Recrystallization of a sample from this solid (300 mg) afforded yellowish crystals (210 mg, 70%), mp 199–200 °C (acetone–methanol); 1H NMR (DMSO-d6, 400 MHz): δ 6.32 (t, J = 2.0 Hz, 2H), 7.16 (t, J = 7.4 Hz, 1H), 7.33 (t, J = 7.6 Hz, 2H), 7.45 (br s, 2H), 7.52 (t, J = 2.2 Hz, 2H), 7.75 (d, J = 8.4 Hz, 2H), 8.05 (d, J = 8.8 Hz, 2H), 9.73 (s, 1H, exchangeable with D), 9.85 (br s, 1H, exchangeable with D), 10.57 (s, 1H, exchangeable with D); 13C NMR (DMSO-d6, 100 MHz): δ 45.9, 111.2, 115.2, 118.1, 119.0, 128.8, 129.6, 135.1, 142.3, 165.2, 181.8. Anal. Calcd. for C18H16N4OS: C, 64.27; H, 4.79; N, 16.65. Found: C, 64.13; H, 4.86; N, 16.77.
3.2.5.3. 4-Allyl-1-[4-(2,5-dimethyl-1\textit{H}-pyrrol-1-yl)benzoyl]thiosemicarbazide (21)

This compound was obtained from 4-(2,5-dimethyl-1\textit{H}-pyrrol-1-yl)benzoic acid hydrazide (18) and allyl isothiocyanate. Recrystallization afforded yellowish crystals (1360 mg, 83%), mp 194–195 °C (ethanol); \textit{\textsuperscript{1}H NMR} (DMSO-\textit{d_6}, 400 MHz): \(\delta\) 1.99 (s, 6H), 4.12 (t, \(J = 5.2\) Hz, 2H), 5.05 (dd, \(J = 1.6\) and 10.4 Hz, 1H), 5.15 (dd, \(J = 1.6\) and 17.2 Hz, 1H), 5.77–5.89 (m, 1H), 5.83 (s, 2H), 7.40 (d, \(J = 8.4\) Hz, 2H), 8.05 (d, \(J = 8.4\) Hz, 2H), 8.35 (br s, 1H, exchangeable with D), 9.41 (s, 1H, exchangeable with D), 10.47 (s, 1H, exchangeable with D); \textit{\textsuperscript{13}C NMR} (DMSO-\textit{d_6}, 100 MHz): 12.9, 45.9, 106.5, 115.2, 127.5, 127.7, 128.9, 131.5, 135.0, 141.3, 165.2, 181.9. \textit{Anal.} Calcd. for C\textsubscript{17}H\textsubscript{20}N\textsubscript{4}O\textsubscript{s}: C, 62.17; H, 6.14; N, 17.06. F ound: C, 62.11; H, 6.22; N, 16.90.

3.2.5.4. 1-[4-(2,5-Dimethyl-1\textit{H}-pyrrol-1-yl)benzoyl]-4-phenylthiosemicarbazide (22)

This compound was obtained from 4-(2,5-dimethyl-1\textit{H}-pyrrol-1-yl)benzoic acid hydrazide (18) and phenyl isothiocyanate. Recrystallization gave yellowish crystals (1200 mg, 66%), mp 180–181 °C (ethanol); \textit{\textsuperscript{1}H NMR} (DMSO-\textit{d_6}, 400 MHz): \(\delta\) 2.00 (s, 6H), 5.84 (s, 2H), 7.12–7.21 (m, 1H), 7.30–7.37 (m, 2H), 7.38–7.53 (m, 4H), 8.08 (d, \(J = 8.4\) Hz, 2H), 9.77 (s, 1H, exchangeable with D), 9.87 (s, 1H, exchangeable with D), 10.67 (s, 1H, exchangeable with D); \textit{\textsuperscript{13}C NMR} (DMSO-\textit{d_6}, 100 MHz): 12.9, 106.5, 125.1, 126.1, 127.5, 127.8, 128.0, 129.0, 131.6, 139.3, 141.3, 165.3, 181.2. \textit{Anal.} Calcd. for C\textsubscript{20}H\textsubscript{20}N\textsubscript{4}O\textsubscript{s}: C, 65.91; H, 5.53; N, 15.37. F ound: C, 66.03; H, 5.45; N, 15.51.

3.2.6. General procedure for the synthesis of mercaptotriazoles 23–26

A mixture of thiosemicarbazide 19–22 (3 mmol) and KOH (396 mg, 6 mmol, 85% purity) in water (30 mL) was refluxed for 3 h, and then the cooled solution was brought to pH 6 by dropwise addition of 10% acetic acid. The separated solid was filtered, washed thoroughly with water, and air dried.

3.2.6.1. 4-Allyl-3-[4-(1\textit{H}-pyrrol-1-yl)phenyl]-1\textit{H}-1,2,4-triazole-5-thione (23)

This compound was obtained from 4-allyl-1-[4-(1\textit{H}-pyrrol-1-yl)benzoyl]thiosemicarbazide (19). Recrystallization afforded colorless crystals (805 mg, 95%), mp 199–200 °C (methanol); \textit{\textsuperscript{1}H NMR} (DMSO-\textit{d_6}, 400 MHz): \(\delta\) 4.70–4.77 (m, 2H), 4.89 (dd, \(J = 1.0\) and 17.4 Hz, 1H), 5.16 (dd, \(J = 1.0\) and 10.6 Hz, 1H), 5.80–5.93 (m, 1H), 6.31 (t, \(J = 2.2\) Hz, 2H), 7.49 (t, \(J = 2.0\) Hz, 2H), 7.71–7.80 (m, 4H), 14.02 (s, 1H, exchangeable with D); \textit{\textsuperscript{13}C NMR} (DMSO-\textit{d_6}, 100 MHz): \(\delta\) 46.0, 111.2, 117.2, 118.9, 119.1, 122.4, 129.8, 131.9, 141.3, 150.8, 167.6. \textit{Anal.} Calcd. for C\textsubscript{15}H\textsubscript{14}N\textsubscript{4}S: C, 63.81; H, 5.00; N, 19.84. F ound: C, 63.66; H, 5.45; N, 19.97.

3.2.6.2. 4-Phenyl-3-[4-(1\textit{H}-pyrrol-1-yl)phenyl]-1\textit{H}-1,2,4-triazole-5-thione (24)

This compound was obtained from 4-phenyl-1-[4-(1\textit{H}-pyrrol-1-yl)benzoyl]thiosemicarbazide (20). Yield: 915 mg (96%). Recrystallization of a sample from this solid (200 mg) afforded colorless crystals (186 mg, 93%), mp 309–311 °C (ethanol); \textit{\textsuperscript{1}H NMR} (DMSO-\textit{d_6}, 400 MHz): \(\delta\) 4.60 (t, \(J = 2.0\) Hz, 2H), 7.35 (d, \(J = 8.8\) Hz, 2H), 7.37–7.43 (m, 4H), 7.48–7.55 (m, 3H), 7.58 (d, \(J = 8.8\) Hz, 2H), 14.13 (br s, 1H, exchangeable with D); \textit{\textsuperscript{13}C NMR} (DMSO-\textit{d_6}, 100 MHz): \(\delta\) 111.2, 118.6, 118.9, 122.2, 128.8, 129.4, 129.5, 129.6, 134.6, 140.9, 150.0, 168.6. \textit{Anal.} Calcd. for C\textsubscript{18}H\textsubscript{14}N\textsubscript{4}S: C, 67.90; H, 4.43; N, 17.60. F ound: C, 68.03; H, 4.49; N, 17.41.

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3.2.6.3. 4-Allyl-3-[4-(2,5-dimethyl-1\text{H}-pyrrol-1-yl)phenyl]-1\text{H}-1,2,4-triazole-5-thione (25)

This compound was obtained from 4-allyl-1-[4-(2,5-dimethyl-1\text{H}-pyrrol-1-yl)benzoyl]thiosemicarbazide (21). Recrystallization yielded colorless crystals (845 mg, 91%), mp 177–178 °C (ethanol); $^1$H NMR (DMSO-$d_6$, 400 MHz): $\delta$ 2.00 (s, 6H), 4.71–4.80 (m, 2H), 4.89 (dd, $J = 1.2$ and 17.2 Hz, 1H), 5.15 (dd, $J = 1.2$ and 10.4 Hz, 1H), 5.81–5.92 (m, 1H), 5.83 (s, 2H), 7.44 (d, $J = 8.4$ Hz, 2H), 7.80 (d, $J = 8.4$ Hz, 2H), 14.07 (br s, 1H, exchangeable with D); $^{13}$C NMR (DMSO-$d_6$, 100 MHz): 12.9, 46.1, 106.5, 117.4, 125.1, 127.6, 128.5, 131.8, 140.3, 150.7, 167.7.

Anal. Calcd. for C$_{17}$H$_{18}$N$_4$S: C, 65.78; H, 5.85; N, 18.05. Found: C, 65.64; H, 5.80; N, 18.21.

3.2.6.4. 3-[4-(2,5-Dimethyl-1\text{H}-pyrrol-1-yl)phenyl]-4-phenyl-1\text{H}-1,2,4-triazole-5-thione (26)

This compound was obtained from 1-[4-(2,5-dimethyl-1\text{H}-pyrrol-1-yl)benzoyl]-4-phenylthiosemicarbazide (22). Recrystallization gave colorless crystals (760 mg, 73%), mp 260–262 °C (ethanol); $^1$H NMR (DMSO-$d_6$, 400 MHz): $\delta$ 1.91 (s, 6H), 5.78 (s, 2H), 7.24 (d, $J = 8.4$ Hz, 2H), 7.38–7.46 (m, 4H), 7.49–7.55 (m, 3H), 14.19 (s, 1H, exchangeable with D); $^{13}$C NMR (DMSO-$d_6$, 100 MHz): 12.8, 106.4, 124.9, 127.5, 128.0, 128.8, 129.0, 129.4, 129.6, 134.9, 139.9, 149.9, 168.7. Anal. Calcd. for C$_{20}$H$_{18}$N$_4$S: C, 69.34; H, 5.24; N, 16.17. Found: C, 69.43; H, 5.21; N, 16.30.

3.2.7. General procedure for the synthesis of esters 27 and 28

To the solution obtained from mercaptotriazoles 23 or 24 (1 mmol) and KOH (66 mg, 1 mmol, 85% purity) in 96% ethanol (10 mL) was added ethyl bromoacetate (167 mg, 1 mmol) and the mixture was heated at reflux temperature for 1 h. The cooled reaction mixture was gradually diluted with water (20 mL) and the solid that separated was filtered, washed with a mixture of ethanol–water (10 mL, 1:2 v/v), and air dried.

3.2.7.1. Ethyl 2-{{5-[4-(1\text{H}-pyrrol-1-yl)phenyl]-4-allyl-4\text{H}-1,2,4-triazol-3-yl}thio}acetate (27)

This compound was obtained from 4-allyl-3-[4-(1\text{H}-pyrrol-1-yl)phenyl]-1\text{H}-1,2,4-triazole-5-thione (23). The solid was dissolved in refluxing 96% ethanol (5 mL), the solution was cooled at approximately 40 °C, and then it was diluted with $n$-hexane (7 mL) and refrigerated overnight. The resulting precipitate was filtered, washed with a mixture of ethanol–$n$-hexane (10 mL, 1:4 v/v), and air dried to give yellowish crystals (200 mg, 54%), mp 108–109 °C; $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 1.28 (t, $J = 7.2$ Hz, 3H), 4.12 (s, 2H), 4.22 (q, $J = 7.2$ Hz, 2H), 4.64–4.70 (m, 2H), 5.06 (dt, $J = 2.0$ and 17.2 Hz, 1H), 5.37 (d, $J = 10.4$ Hz, 1H), 5.92–6.05 (m, 1H), 6.39 (t, $J = 2.2$ Hz, 2H), 7.15 (t, $J = 2.2$ Hz, 2H), 7.51 (d, $J = 8.8$ Hz, 2H), 7.71 (d, $J = 8.8$ Hz, 2H); $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 14.2, 35.7, 47.0, 62.2, 111.4, 118.5, 119.1, 120.3, 123.9, 129.9, 131.6, 142.1, 150.9, 155.5, 168.5. Anal. Calcd. for C$_{19}$H$_{20}$N$_4$O$_2$S: C, 61.94; H, 5.47; N, 15.21. Found: C, 61.84; H, 5.40; N, 15.33.

3.2.7.2. Ethyl 2-{{5-[4-(1\text{H}-pyrrol-1-yl)phenyl]-4-phenyl-4\text{H}-1,2,4-triazol-3-yl}thio}acetate (28)

This compound was obtained from 4-phenyl-3-[4-(1\text{H}-pyrrol-1-yl)phenyl]-1\text{H}-1,2,4-triazole-5-thione (24). Recrystallization afforded yellowish crystals (137 mg, 34%), mp 164–165 °C (ethanol); $^1$H NMR (DMSO-$d_6$, 400 MHz): $\delta$ 1.20 (t, $J = 7.2$ Hz, 3H), 4.10 (s, 2H), 4.13 (q, $J = 7.2$ Hz, 2H), 6.26 (t, $J = 2.0$ Hz, 2H), 7.38–7.50 (m, 6H), 7.56–7.63 (m, 5H); $^{13}$C NMR (DMSO-$d_6$, 100 MHz): $\delta$ 14.0, 33.9, 61.3, 111.0, 118.7, 118.8, 123.0,
127.6, 129.1, 130.1, 130.2, 133.7, 151.0, 153.8, 168.0. Anal. Calcd. for C_{22}H_{20}N_{4}O_{2}S: C, 65.33; H, 4.98; N, 13.85. Found: C, 65.47; H, 5.07; N, 13.98.

3.2.8. General procedure for the synthesis of esters 29 and 30
A mixture of mercaptotriazole 25 or 26 (2 mmol), ethyl bromoacetate (334 mg, 2 mmol), and anh. K_{2}CO_{3} (552 mg, 4 mmol) in 2-butanone (10 mL) was heated at reflux temperature for 6 h. The mixture was cooled to room temperature, and the solid was filtered and washed with 2-butanone (2 \times 10 mL). The combined filtrate was processed as described in each case.

3.2.8.1. Ethyl 2-{{4-allyl-5-[4-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl]-4H-1,2,4-triazol-3-yl}thio}acetate (29)
This compound was obtained from 4-allyl-3-[4-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl]-1H-1,2,4-triazole-5-thione (25). The solvent in the combined filtrate was removed under reduced pressure to give a brown oil, which was subjected to flash chromatography (hexanes-ethyl acetate 2:1 v/v) to yield the title compound as a golden oil, R_{f} 0.23 (hexanes–ethyl acetate 2:1 v/v), that slowly solidifies into a yellow solid (720 mg, 91%), mp 84–86 °C; \textsuperscript{1}H NMR (CDCl_{3}, 400 MHz): \delta 1.29 (t, J = 7.2 Hz, 3H), 2.06 (s, 6H), 4.14 (s, 2H), 4.21 (q, J = 7.2 Hz, 2H), 4.67–4.76 (m, 2H), 5.07 (dt, J = 1.8 and 17.2 Hz, 1H), 5.39 (dt, J = 1.8 and 10.8 Hz, 1H), 5.93 (s, 2H), 5.95–6.06 (m, 1H), 7.31–7.36 (m, 2H), 7.72–7.78 (m, 2H); \textsuperscript{13}C NMR (CDCl_{3}, 100 MHz): 13.2, 14.2, 35.7, 47.1, 55.9, 118.6, 126.4, 128.8, 128.9, 129.4, 131.6, 141.0, 151.2, 155.5, 168.5. Anal. Calcd. for C_{21}H_{24}N_{4}O_{2}S: C, 63.61; H, 6.10; N, 14.13. Found: C, 63.88; H, 6.03; N, 13.93.

3.2.8.2. Ethyl 2-{{5-[4-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl]-4-phenyl-4H-1,2,4-triazol-3-yl}thio}acetate (30)
This compound was obtained from 3-[4-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl]-4-phenyl-1H-1,2,4-triazole-5-thione (26). The filtrate was evaporated under reduced pressure to give a residue, whose recrystallization from ethanol afforded yellow crystals (683 mg, 79%), mp 138–139 °C; \textsuperscript{1}H NMR (CDCl_{3}, 400 MHz): \delta 1.29 (t, J = 7.2 Hz, 3H), 1.98 (s, 6H), 4.13 (s, 2H), 4.23 (q, J = 7.2 Hz, 2H), 5.07 (dt, J = 1.8 and 17.2 Hz, 1H), 5.39 (dt, J = 1.8 and 10.8 Hz, 1H), 5.93 (s, 2H), 5.95–6.06 (m, 1H), 7.31–7.36 (m, 2H), 7.72–7.78 (m, 2H); \textsuperscript{13}C NMR (CDCl_{3}, 100 MHz): \delta 13.1, 14.2, 35.7, 47.1, 62.2, 106.6, 118.6, 126.4, 128.8, 128.9, 129.4, 131.6, 141.0, 151.2, 155.5, 168.5. Anal. Calcd. for C_{25}H_{24}N_{4}O_{2}S: C, 66.64; H, 5.59; N, 12.95. Found: C, 66.48; H, 5.64; N, 13.09.

3.2.8.3. 5-Methylthio-4-phenyl-3-[4-(1H-pyrrol-1-yl)phenyl]-4H-1,2,4-triazole (31)
A mixture of 4-phenyl-3-[4-(1H-pyrrol-1-yl)phenyl]-1H-1,2,4-triazole-5-thione (24) (318 mg, 1 mmol) and KOH (99 mg, 1.5 mmol, 85% purity) in 96% ethanol (8 mL) was heated at reflux temperature for 10 min; then iodomethane (568 mg, 4 mmol) in 96% ethanol (2 mL) was added and the resulting mixture was heated at reflux temperature for 1 h. The cold reaction mixture was gradually diluted with water (40 mL) under efficient stirring. The separated solid was filtered, washed with water, air dried, and recrystallized from 2-butanone to give yellow crystals (232 mg, 70%), mp 228–229 °C; \textsuperscript{1}H NMR (DMSO-d_{6}, 400 MHz): \delta 2.62 (s, 3H), 6.26 (t, J = 2.0 Hz, 2H), 7.37–7.49 (m, 6H), 7.54–7.61 (m, 5H); \textsuperscript{13}C NMR (DMSO-d_{6}, 100 MHz): \delta 14.4, 111.0, 118.8,
3.2.9. General procedure for the synthesis of thioethers 32–35

To the solution obtained from mercaptotriazole (1 mmol) and KOH (66 mg, 1 mmol, 85% purity) in 96% ethanol (5 mL) was added the alkylation agent (1 mmol) and the mixture was heated at reflux temperature for 1 h. The cooled reaction mixture was gradually diluted with water (40 mL) under efficient stirring.

3.2.9.1. 4-Allyl-3-[(3,4-dichlorobenzyl)thio]-5-[4-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl]-4H-1,2,4-triazole (32)

This compound was obtained from 4-allyl-3-[4-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl]-1H-1,2,4-triazole-5-thione (25) and 3,4-dichlorobenzyl chloride. Upon dilution with water, a dense oil separated, which was extracted with ethyl acetate (2 ×30 mL), and the combined organic phases were washed with water and brine and dried over anh. Na₂SO₄. The solvent was removed under reduced pressure to give a residue that was chromatographed (hexanes–ethyl acetate 2:1 v/v) to yield a light yellow oil (390 mg, 83%); R_f 0.22 (hexanes–ethyl acetate 2:1 v/v); ¹H NMR (CDCl₃, 400 MHz): δ 2.04 (s, 6H), 4.46 (s, 2H), 4.49–4.54 (m, 2H), 4.94 (dt, J = 1.8 and 17.2 Hz, 1H), 5.31 (dt, J = 1.8 and 10.8 Hz, 1H), 5.82–5.92 (m, 1H), 5.92 (s, 2H), 7.26 (dd, J = 2.0 and 8.4 Hz, 1H), 7.32 (d, J = 8.4 Hz, 2H), 7.37 (d, J = 8.4 Hz, 1H), 7.49 (d, J = 2.0 Hz, 1H), 7.72 (d, J = 8.4 Hz, 2H); ¹³C NMR (CDCl₃, 100 MHz): δ 13.1, 36.7, 46.9, 106.5, 118.6, 128.5, 128.7, 128.8, 128.9, 129.3, 129.7, 130.0, 131.1, 132.1, 132.7, 137.2, 141.0, 151.4, 155.3. Anal. Calcd. for C₂₄H₂₂Cl₂N₄S: C, 61.41; H, 4.72; N, 11.94. Found: C, 61.62; H, 4.58; N, 11.92.

3.2.9.2. 3-[(4-Chlorobenzyl)thio]-5-[4-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl]-4-phenyl-4H-1,2,4-triazole (33)

This compound was obtained from 3-[4-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl]-4-phenyl-1H-1,2,4-triazole-5-thione (26) and 4-chlorobenzyl bromide. The emulsion obtained after dilution with water was stirred overnight. The solid was filtered, washed with a mixture of ethanol–water (10 mL, 1:1 v/v), air dried, and recrystallized from 96% ethanol to afford yellowish crystals (390 mg, 83%), mp 188–189 °C; ¹H NMR (DMSO-d₆, 400 MHz): δ 1.91 (s, 6H), 4.41 (s, 2H), 5.77 (s, 2H), 7.24 (d, J = 8.4 Hz, 2H), 7.34–7.44 (m, 6H), 7.48 (d, J = 8.0 Hz, 2H), 7.51–7.59 (m, 3H); ¹³C NMR (DMSO-d₆, 100 MHz): δ 12.9, 35.3, 106.4, 125.7, 127.5, 127.7, 128.1, 128.4, 128.5, 130.0, 130.3, 130.9, 132.1, 133.8, 136.4, 139.4, 151.6, 153.8. Anal. Calcd. for C₂₇H₂₂Cl₂N₄S: C, 61.41; H, 4.72; N, 11.94. Found: C, 61.62; H, 4.58; N, 11.92.

3.2.9.3. 1-(2,4-Dichlorophenyl)-2-{{4-allyl-5-[4-(1H-pyrrol-1-yl)phenyl]-4H-1,2,4-triazol-3-yl}thio}ethanone (34)

This compound was obtained from 4-allyl-3-[4-(1H-pyrrol-1-yl)phenyl]-1H-1,2,4-triazole-5-thione (23) and 2-bromo-1-(2,4-dichlorophenyl)ethan-1-one. The solid was filtered, washed with a mixture of ethanol–water (10 mL, 1:1 v/v), air dried, and recrystallized from 96% ethanol to afford yellowish crystals (260 mg, 55%), mp 134–135 °C; ¹H NMR (CDCl₃, 400 MHz): δ 4.60–4.64 (m, 2H), 4.76 (s, 2H), 5.06 (d, J = 17.2 Hz, 1H), 5.38 (d, J = 10.4 Hz, 1H), 5.91–6.03 (m, 1H), 6.39 (t, J = 2.2 Hz, 2H), 7.15 (t, J = 2.2 Hz, 2H), 7.35 (dd, J = 2.0 Hz, 1H).
and 8.4 Hz, 1H), 7.46 (d, J = 2.0 Hz, 1H), 7.51 (d, J = 8.8 Hz, 2H), 7.66–7.73 (m, 3H); $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 43.6, 47.1, 111.4, 118.7, 119.2, 120.4, 123.8, 127.7, 129.9, 130.5, 131.3, 131.5, 132.4, 135.7, 138.4, 142.2, 150.1, 155.6, 194.7. Anal. Calcd. for C$_{23}$H$_{18}$Cl$_2$N$_4$O: C, 58.85; H, 3.87; N, 11.94. Found: C, 58.80; H, 3.92; N, 12.04.

3.2.9.4. 1-(4-Chlorophenyl)-2-{{5-[4-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl]-4-phenyl-1,2,4-triazol-3-yl}thio}ethan-1-one (35)

This compound was obtained from 3-[4-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl]-4-phenyl-1H-1,2,4-triazole-5-thione (26) and 2-bromo-1-(4-chlorophenyl)ethan-1-one. The emulsion obtained after dilution with water was stirred overnight. The solid was filtered, washed with a mixture of ethanol–water (10 mL, 1:1 v/v), air dried, and recrystallized from 96% ethanol to give yellow crystals (335 mg, 67%), mp 215–216 °C; $^1$H NMR (DMSO-$d_6$, 400 MHz): 1.92 (s, 6H), 4.95 (s, 2H), 5.78 (s, 2H), 7.25 (d, $J$ = 8.4 Hz, 2H), 7.44–7.53 (m, 4H), 7.57–7.64 (m, 3H), 7.65 (d, $J$ = 8.8 Hz, 2H), 8.05 (d, $J$ = 8.8 Hz, 2H); $^{13}$C NMR (DMSO-$d_6$, 100 MHz): 12.8, 40.1, 106.4, 125.6, 127.5, 127.6, 128.1, 128.5, 129.0, 130.1, 130.3, 133.7, 134.0, 138.7, 139.4, 151.6, 153.7, 192.2. Anal. Calcd. for C$_{28}$H$_{23}$ClN$_4$O: C, 67.39; H, 4.65; N, 11.23. Found: C, 67.51; H, 4.59; N, 11.29.

3.2.10. General procedure for the synthesis of Mannich bases 36 and 37

A mixture of mercaptotriazole (2 mmol), aq. 37% formaldehyde (240 mg, 3 mmol), and cyclic secondary amine (3 mmol) in 96% ethanol (5 mL) was heated at reflux temperature for 1 h. The mixture was refrigerated for 2 days and the resulting solid was filtered and air dried.

3.2.10.1. 4-Allyl-5-[4-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl]-2-(morpholinomethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (36)

This compound was obtained from 4-allyl-3-[4-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl]-1H-1,2,4-triazole-5-thione (25) and morpholine. The crude solid was dissolved in boiling 96% ethanol (5 mL). Upon cooling at room temperature, a small amount of a sticky solid resulted from the solution, from which the supernatant was removed with a pipette. Refrigeration of the clear solution yielded yellowish crystals (515 mg, 63%), mp 94–96 °C; $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 2.07 (s, 6H), 2.88 (t, $J$ = 4.6 Hz, 4H), 3.72 (t, $J$ = 4.6 Hz, 4H), 4.78–4.85 (m, 2H), 5.08 (d, $J$ = 17.2 Hz, 1H), 5.23 (s, 2H), 5.33 (d, $J$ = 10.4 Hz, 1H), 5.94 (s, 2H), 5.95–6.07 (m, 1H), 7.36 (d, $J$ = 8.4 Hz, 2H), 7.75 (d, $J$ = 8.4 Hz, 2H); $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 13.2, 48.0, 51.0, 67.0, 69.9, 106.8, 118.5, 124.9, 128.7, 129.0, 129.5, 131.2, 141.7, 150.1, 169.7. Anal. Calcd. for C$_{22}$H$_{27}$N$_5$OS: C, 64.52; H, 6.65; N, 17.10. Found: C, 64.63; H, 6.57; N, 17.20.

3.2.10.2. 4-Allyl-5-[4-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl]-2-(piperidin-1-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (37)

This compound was obtained from 4-allyl-3-[4-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl]-1H-1,2,4-triazole-5-thione (25) and piperidine. Recrystallization from 96% ethanol gave a yellowish solid (585 mg, 72%), mp 101–102 °C; $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 1.35–1.46 (m, 2H), 1.55–1.66 (m, 4H), 2.06 (s, 6H), 2.82 (t, $J$ = 5.2 Hz, 4H), 4.78–4.85 (m, 2H), 5.07 (d, $J$ = 17.2 Hz, 1H), 5.22 (s, 2H), 5.33 (d, $J$ = 10.4 Hz, 1H), 5.94 (s, 2H), 5.96–6.08 (m, 1H), 7.35 (d, $J$ = 8.4 Hz, 2H), 7.76 (d, $J$ = 8.4 Hz, 2H); $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 13.2,
3.2.11. General procedure for the synthesis of Mannich bases 38–40

A mixture of mercaptotriazole (1 mmol), aq. 37% formaldehyde (120 mg, 1.5 mmol), and cyclic secondary amine (1.5 mmol) in 96% ethanol (20–25 mL) was heated at reflux temperature for 1 h. The reaction mixture was allowed to reach room temperature before the work-up.

3.2.11.1. 5-[4-(2,5-Dimethyl-1H-pyrrol-1-yl)phenyl]-2-(morpholinomethyl)-4-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (38)

This compound was obtained from 3-[4-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl]-4-phenyl-1H-1,2,4-triazole-5-thione (26) and morpholine. The solid that separated upon cooling was filtered, washed with 96% ethanol, air dried, and recrystallized to yield yellowish crystals (345 mg, 77%), mp 215–216 °C (ethyl acetate); 1H NMR (CDCl₃, 400 MHz): δ 1.99 (s, 6H), 2.96 (t, J = 8.8 Hz, 4H), 3.75 (t, J = 8.8 Hz, 2H), 5.29 (s, 2H), 5.89 (s, 2H), 7.13 (d, J = 8.4 Hz, 2H), 7.32–7.38 (m, 2H), 7.43 (d, J = 8.8 Hz, 2H), 7.51–7.57 (m, 3H); 13C NMR (CDCl₃, 100 MHz): 13.1, 51.0, 67.1, 70.1, 106.6, 124.6, 128.5 (2×), 128.7, 129.0, 130.0, 130.2, 135.2, 141.1, 148.7, 170.9. Anal. Calcd. for C₂₅H₂₇N₅OS: C, 67.39; H, 6.11; N, 15.72. Found: C, 67.27; H, 6.01; N, 15.86.

3.2.11.2 5-[4-(2,5-Dimethyl-1H-pyrrol-1-yl)phenyl]-4-phenyl-2-(piperidin-1-ylmethyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (39)

This compound was obtained from 3-[4-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl]-4-phenyl-1H-1,2,4-triazole-5-thione (26) and piperidine. Partial removal of the solvent at the end of the reaction time gave a solid that was filtered, air dried, and recrystallized from 96% ethanol to afford yellowish crystals (310 mg, 70%), mp 148–149 °C; 1H NMR (CDCl₃, 400 MHz): δ 1.39–1.50 (m, 2H), 1.58–1.69 (m, 4H), 1.99 (s, 6H), 2.90 (t, J = 5.6 Hz, 4H), 5.28 (s, 2H), 5.89 (s, 2H), 7.13 (d, J = 8.0 Hz, 2H), 7.32–7.39 (m, 2H), 7.44 (d, J = 8.0 Hz, 2H), 7.50–7.58 (m, 3H); 13C NMR (CDCl₃, 100 MHz): δ 13.1, 51.0, 67.1, 70.1, 106.6, 124.6, 128.5 (2×), 128.7, 129.0, 130.0, 130.2, 135.2, 141.1, 148.7, 170.7. Anal. Calcd. for C₂₆H₂₉N₅S: C, 70.50; H, 6.66; N, 15.65.

3.2.11.3 5-[4-(2,5-Dimethyl-1H-pyrrol-1-yl)phenyl]-4-phenyl-2-[[4-(pyridin-2-yl)piperazin-1-yl]methyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione (40)

This compound was obtained from 3-[4-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl]-4-phenyl-1H-1,2,4-triazole-5-thione (26) and 4-(pyridin-2-yl)piperazine. Upon cooling, a sticky solid separated. The supernatant was decanted and the solvent was removed under a stream of nitrogen to give a solid that was dried under high vacuum overnight. The solid was redissolved in hot ethanol (5 mL) and water was added dropwise until a slight turbidity was observed. Refrigeration overnight afforded an off-white solid (245 mg, 47%), mp 136–138 °C; 1H NMR (CDCl₃, 400 MHz): δ 1.98 (s, 6H), 3.07 (t, J = 5.2 Hz, 4H), 3.61 (t, J = 5.2 Hz, 4H), 5.38 (s, 2H), 5.88 (s, 2H), 6.61 (dd, J = 5.0 Hz, 1H), 6.64 (d, J = 8.8 Hz, 1H), 7.09–7.15 (m, 2H), 7.29–7.37 (m, 2H), 7.39–7.56 (m, 6H), 8.16–8.20 (m, 1H); 13C NMR (CDCl₃, 100 MHz): δ 13.1, 45.3, 50.6, 70.0, 106.6, 107.2, 113.5, 124.6, 128.5,
128.7, 129.0, 130.0, 130.2, 135.2, 137.6, 141.1, 148.1, 148.7, 159.5, 170.8. Anal. Calcd. for C_{30}H_{31}N_{7}S: C, 69.07; H, 5.99; N, 18.79. Found: C, 69.21; H, 6.05; N, 18.61.

3.3. Biological activity

Evaluation of the antimycobacterial activity was performed according to CLSI M24-A2 standard, a broth microdilution method that allows the determination of MIC for a given substance against different mycobacteria. As testing medium, cation-adjusted Mueller Hinton broth (enriched with 20 mg/L Ca^{2+} and 10 mg/L Mg^{2+}, respectively) was used in order to determine the MICs of candidate compounds. Compounds 23–40 were evaluated for their in vitro antimycobacterial activity against one type strain of nontuberculous mycobacteria, namely M. smegmatis ATCC 14468. The antimicrobial activity of these compounds was compared to that of the standard first-line tuberculostatic antibiotic rifampicin. For each compound, stock solutions of 1280 mg/L were prepared using dimethylsulfoxide as solvent. The final concentrations of testing candidates ranged between 64 and 0.125 mg/L, representing two-fold dilutions according to CLSI recommendations. MICs of the compounds and standard antibiotic were determined in 96-well plates after 40 h incubation at 36 ± 1 °C, considering as endpoint the lowest concentration that completely inhibited the growth.

References

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